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Tricholoma smithii, a new species in the *Pardinicutis* complex from New Mexico and Colorado

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Abstract: A new species of *Tricholoma*, *T. smithii*, is described from New Mexico and Colorado. It is reminiscent of and belongs in the same clade as *Tricholoma pardinum*. It has the same stature as *T. pardinum* but differs by the browner pileal coloration and by the presence of pleurocystidia. A detailed description, drawings and images of the new species are provided as well as a phylogenetic analysis based on ITS sequences.

Key words: Agaricomycetes, fleshy fungi, Rocky Mountains, new species

Introduction: In this paper we describe a new species of *Tricholoma* from the southern Rocky Mountain area. The species is related to *Tricholoma pardinum* (Pers.) Quél. from

Slovenia and to North American "*T. pardinum*", a fairly well-known taxon in temperate North America especially in the Pacific Northwest. The new species is distinct from *T. pardinum* by its

browner pileal coloration and by the presence of pleurocystidia. We provide both morphological and molecular support for describing the species as new.

Materials and Methods

Macroscopic and microscopic descriptions.

Macroscopic descriptions are based on field notes recorded by the first author and those recorded by Alexander H. Smith. The color term in parentheses is from Ridgway (1912). Microscopic notes are based on sections made from dried collections and mounted in 3% KOH. Spore data are based on mostly 15 measurements per collection and Q indicates length/width ratio.

Molecular methods. DNA extraction and PCR protocols were performed as described in Mata et al. (2007). Primers ITS1F or ITS5 coupled with ITS4 (White et al. 1990) were used to amplify the nuclear ribosomal RNA internal transcribed region (ITS, fungal barcode). Dideoxy sequencing was carried out by the University of Tennessee Molecular Biology Facility. Sequence files were edited manually and in GCG (2000); outgroup sequences were selected from BLAST searches in GenBank. Maximum likelihood trees were calculated with PhyML (Guindon and Gascuel 2008) in Geneious 11.0.3 with 1000 bootstrap replicates.

Species delineation metrics. Delineation of species was analyzed using the Species Delimitation plugin in Geneious v11.0.3 (Masters et al. 2011). Species trees and gene trees (trees based on a single DNA sequence) may differ for a number of reasons and relationships based on a single DNA sequence may not reflect an overall species divergence pattern. The Geneious plugin computes Rosenberg's P_{AB} statistic (Rosenberg 2007) and P_{ID} statistics (Ross et al. 2008). Rosenberg's P_{AB} statistic is the probability that a putative taxon will be monophyletic relative to a sister clade containing 'b' taxa under the null model of random coalescence (Rosenberg 2007).

The P_{ID} statistic is the mean probability of making a correct identification under either strict or relaxed cladistic criteria ($P_{ID}[Strict]$ or $P_{ID}[Liberal]$) which is predicted from the *Intra/Inter* ratio (Intra=within taxon distance; Inter=distance to the nearest taxon) and is considered a better predictor of species than the barcode gap (Hebert et al. 2003; Ross et al. 2008). The P_{ID} strict criterion requires the query sequence to fall within a monophyletic clade for an identification to be made. The P_{ID} liberal criterion requires the query sequence to fall within, or sister to a monophyletic clade.

Results

Divergence estimates. Results of a PhyML phylogeny based on the nuclear ribosomal ITS region are given in Figure 3. A single collection from Slovenia established as European *Tricholoma pardinum* by Christensen and Heilmann-Clausen (Heilmann-Clausen et al., 2017) was selected as representative of authentic *T. pardinum*. The ITS region of this collection differs from a homogeneous western North American clade consisting of collections variously identified as *T. pardinum*, *T. venenatum* G. F. Atk. or *T. huronense* A. H. Smith (difference is 5 bp/543bp or 0.92%). The *T. smithii* clade differs from both European and western North American collections by five unique apomorphies. A sixth apomorphy is shared with a single haplotype in a collection from California (divergence is 6bp/543bp or 1.1%).

Species delineation metrics. Both putative North American "*T. pardinum*" and *T. smithii* were monophyletic, allowing calculation of P_{AB} and P_{ID} metrics. The probability that *T. smithii* is monophyletic relative to western North American "*T. pardinum*" under a null model of random coalescence suggests that the null model can be rejected ($P_{AB} = 0.00006$). P_{ID} (strict), the probability of making a correct identification of an unknown specimen was 0.93 (95% confidence intervals 0.81, 1.00). By these criteria, *T. smithii*

is a valid taxon at some rank. Rosenberg's P_{AB} , however, is the most permissive of these estimates and while providing evidence that a clade is not a random coalescence event, it may validate haplotypes rather than a speciation event (Aldrovandi et al. 2015). In contrast, P_{ID} may fail to validate species which are reproductively isolated but have not undergone genetic divergence for the gene(s) being analyzed (Aldrovandi et al. 2015).

Taxonomy

Tricholoma smithii Ovrebo and Hughes sp. nov.
 Figures 1-2

Mycobank: MB 823968

Holotypus. United States: New Mexico: Lincoln Co.: off state highway 37, Big Bonito Trail no. 36 (N 33° 27.805', W 105° 48.278', elevation estimated 7800 ft), 12 Aug 2005, Ovrebo 4510 (DBG; GenBank MG719956).

Diagnosis. Characterized macroscopically by the dry, squamulose, tan to grayish tan pileus. Microscopically, the most distinctive characters are the relatively larger spores, presence of clamp connections and presence of cheilo- and pleurocystidia.

Etymology. The epithet is named in honor of Alexander H. Smith who collected the fungus and called it to the attention of the first author.

Pileus 60-150 mm wide, buttons convex, expanding to broadly convex to plane, often with low rounded umbo, margin often lobed, wavy or uplifted, surface dry, disc glabrous or with fine squamules on or outside the disc, elsewhere radially appressed fibrils to lacerate-fibrillose, and generally with slightly recurved squamules that often extend to the pileal edge, sometimes glabrous in large areas, ground color buff to light ochraceous tan, squamules dull tan to grayish tan, entire pileus taking on a dull tan to grayish

tan color; context 8-14 mm thick, off-white, unchanging, odor and taste farinaceous or rarely not distinctive or with peppery taste.

Lamellae 5-10 mm wide, adnexed or sinuate, buff (Tilleul Buff), not discoloring but becoming dirty grayish tan when older, close, entire, with many tiers of lamellulae.

Stipe 40-120 mm long, 10-30 mm wide, equal to subclavate, base rounded, surface glabrous or with shaggy-loosened fibrils, sometimes with scattered squamules, buff overall; context solid, light buff, but discoloring cinnamon around worm holes.

Chemical Color reactions (from Smith collections): KOH – no reaction; FeSO₄ – pale vinaceous cinnamon, yellowish gray or clay-color in stipe recorded for six collections, no reaction on two collections.

Spore deposit white. **Spores** 8-10 x (5.5)6-7 μm, n = 310, mean length = 9.0 μm, mean width = 6.43 μm, Q=mostly 1.29-1.5, mean Q=1.4, elliptic to broadly elliptic in profile and face view, smooth, thin-walled, hyaline, inamyloid.

Basidia 35-45 x 7-10 μm, 4-sterigmate, clavate, hyaline. **Hymenial cystidia** on sides and edges with cheilocystidia easier to confirm but sometimes rare or level with the hymenium, pleurocystidia rare and mainly collapsed and most evident and non-collapsed on more recent collections, sometimes more visible in squash mounts, both cystidia 30-50 x 12-22 μm, cylindric, clavate, spheropedunculate, smooth, thin-walled, hyaline to light ochre, without visible contents or occasionally with faint granular content. **Hyphae of lamellar trama** 4-12 μm wide, parallel, hyaline. **Hyphae of subhymenium** not distinctive as a layer, hyphae 2-3 μm wide. **Hyphae of pileus surface** cutis-like but tightly interwoven, in places forming interwoven clumps, 3-6 μm wide, smooth, thin-walled, light yellowish ochre, clumped hyphae more strongly pigmented.

Hyphae of pileus trama 4-10 μm wide, hyaline to pale yellow. **Hyphae of stipe surface** 3-5 μm wide, mainly appressed but in places slightly interwoven, smooth, thin-walled, hyaline to pale yellow. **Hyphae of stipe trama** 4-10 μm wide, hyaline. **Clamp connections** present but not at every septum.

Habitat and Distribution: Scattered, on soil, under mixed conifers including *Pinus*, *Abies*, *Picea*, *Pseudotsuga*, one Smith collection noted *Populus* nearby. Known to date from New Mexico and Colorado at high elevations.

Additional collections studied: Colorado: Clear Creek Co.: Mizpah Campground, N 39° 46.475', W 105° 47.575', elev. 9800 ft, 11 Aug 2012, Ovrebo 5048 (CSU; GenBank MG719954, MG719955); Arapaho National Forest, 11 August 2007, Michael Kuo 08110705 (personal herbarium of Michael Kuo; GenBank MG719953). Gilpin Co.: Peak-to-Peak Highway, 9 Aug 2012, DBG 25191 (DBG). Mineral Co.: Forest Road 633, N 37° 28.04', W 107° 06.866', elevation approximately 8300 ft; 23 August 2013, Ovrebo 5148 (DBG). Pitkin Co.: West Village*, 16 Aug 1975, Smith 85804, 20 Aug 1975, Smith 85897, 22 July 1978, Smith 88681; Elk Camp, Snowmass Village, 4 Aug 1977, Smith 87482, 6 Aug 1977, Smith 87510, 21 Aug 1977, Smith 87777, 25 Aug 1978, Smith 89134, 28 Aug 1980, Smith 90627; Snowmass Village, 16 Aug 1977, Smith 87636, 4 Aug 1978, Smith 88867; Elk Ridge, Snowmass Village, 17 Aug 1977, Smith 87671; site unknown, brought into Rocky Mt. Mushroom Conference, 16 Aug 1981, Smith 91116; Elk Wallow, above Frying Pan River, 8 Aug 1979, DBG 17564 (DBG). New Mexico: Lincoln Co.: off State Hwy 37, South Fork Trail no. 19 (N 33° 26.808', W 105° 45.308', elevation estimated 7600 ft), 13 Aug 2005, Ovrebo 4513 (DBG; GenBank MG719957). Sandoval Co.: west of La Cueva, off of highway 126, Forest Road 604 (N 35° 51.817', W 106° 39.883', elev. 8580 ft), 26 Aug 2006, Ovrebo 4609 (CSU; GenBank MG719958). Taos Co.: off of State Hwy 518, USFS Rd. 442 (N 36° 11.867',

W 105° 32.614', elev. 9894 ft), 27 Aug 2010, Ovrebo 4958 (CSU). All Smith collections are at MICH.

*The notes with the Smith collections indicate a general geographic area and are reported above precisely as worded on his field labels and note cards, so exact coordinates and elevations for the collections that he made can only be approximated. The following are estimates for the general areas where he collected: Elk Camp, Elk Ridge – N 39° 11.267', W 106° 56', the top of Elk Ridge is app. 9800 ft.; Snowmass and West Village areas – W 39° 12', W 106° 57', the base elevation for Snowmass Village is app. 8300 ft.

Discussion: *Tricholoma smithii* resembles *Tricholoma pardinum* (Pers.) Quél. by having a similar robust stature, dry, squamulose pileus, and by the presence of clamp connections. *Tricholoma smithii* differs by a more brownish pileal coloration compared to *paradinum* which forms dark gray to brownish gray squamules giving the pileus a more gray coloration (Christensen and Heilmann-Clausen 2013; Bessette et al 2013; Shanks, 1997; Ovrebo 1980) (Figure 1). *Tricholoma smithii* also forms pleurocystidia, lacking in *T. pardinum* (Figure 2). Pleurocystidia were confirmed in the older Smith collections cited above although they were rare and mainly collapsed. They were more easily confirmed and evident in the more recent Ovrebo collections. So far, *T. smithii* is known only from the southern Rocky Mountains. Whether *T. pardinum* overlaps in distribution with *T. smithii* and how far north *T. smithii* extends needs to be resolved with further collecting. Pileal coloration needs to be noted carefully and lamellar sections need to be scrutinized for the presence of pleurocystidia, in order to distinguish the two species.

The ITS “barcode Gap” (Hebert et al. 2003) by itself is not compelling evidence that *T. smithii* is a distinct species. More compelling is the presence of five distinct base differences

(apomorphies) that are unique to *T. smithii*, coupled with morphological traits that make this entity distinct. These data coupled with good clade support for two distinct North American clades and species delineation metrics which also suggest genetic isolation, justify maintaining *T. smithii* as a species distinct from both the North American and European *T. pardinum* taxa.

At first, one of us (CLO) thought that the fungus was assignable to *T. venenatum*, another taxon in Pardinicutis clade, because of the lighter pileal coloration characteristic of that species. *Tricholoma venenatum* was described from Michigan and collected under hardwoods. Examination of the type (Ovrebo, 1980) revealed that it lacks hymenial cystidia as was also reported by Bessette et al (2013) and Shanks (1997). Whether the west coast fungus which Shanks (1997) reported as occurring under conifers above 1300 m is the same as the species from eastern hardwoods is an issue to be investigated.

We use the epithet *pardinum* cautiously for the North American fungus because a recent treatment of European *Tricholomas* by Christensen and Heilmann-Clausen (2013) indicated that cheilocystidia are “not observed” in the European fungus, whereas cheilocystidia occur in the North American form (Bessette et al 2013; Shanks, 1997; Ovrebo 1980). Furthermore, there are ITS sequence differences between the European *T. pardinum* exemplar and the western North American clade comprising collections with nearly identical ITS sequences under the names *T. pardinum*, *T. venenatum* and *T. huronense*. Christensen and Heilmann-Clausen (2013) state that “the species [*pardinum*], or closely related taxon, does occur on the [North American] continent” but more research is needed concerning European genetic variability before European and North American con-specificity can be determined.

Yang et al. (2017) recently described two new species in the *pardinum* complex, *T. highlandense* Yang, Ding, Kost & Rexer and *T. sinopardinum* Yang, Ding, Kost & Rexer, from eastern Himalaya (China). They provided phylogenetic evidence based on ITS sequences to support differentiation of these species from *T. filamentosum* (Alessio) Alessio and *T. pardinum*. Like *T. smithii*, the Chinese species feature brownish squamules on the pileus. Both species differ from *T. smithii* by stipes that are brownish fibrillose to brownish reflexed squamulose; the stipe of *T. smithii* is glabrous or at most has loosened fibrils. *Tricholoma highlandense* has smaller spores (mean L x W = 7.3 x 5.5 µm) and *T. sinopardinum* has slightly larger spores (mean L x W = 9.3 x 6.9 µm) than *T. smithii*. Both species have cheilocystidia and the authors (Yang et al. 2017) also report that the European collections of *pardinum* that they studied lack cheilocystidia. In addition, *Tricholoma highlandensis* often has mucronate cheilocystidia and both species lack pleurocystidia.

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Figure 1. *Tricholoma smithii*, basidiomes, Ovrebo 5148. Scale line = 3 cm.

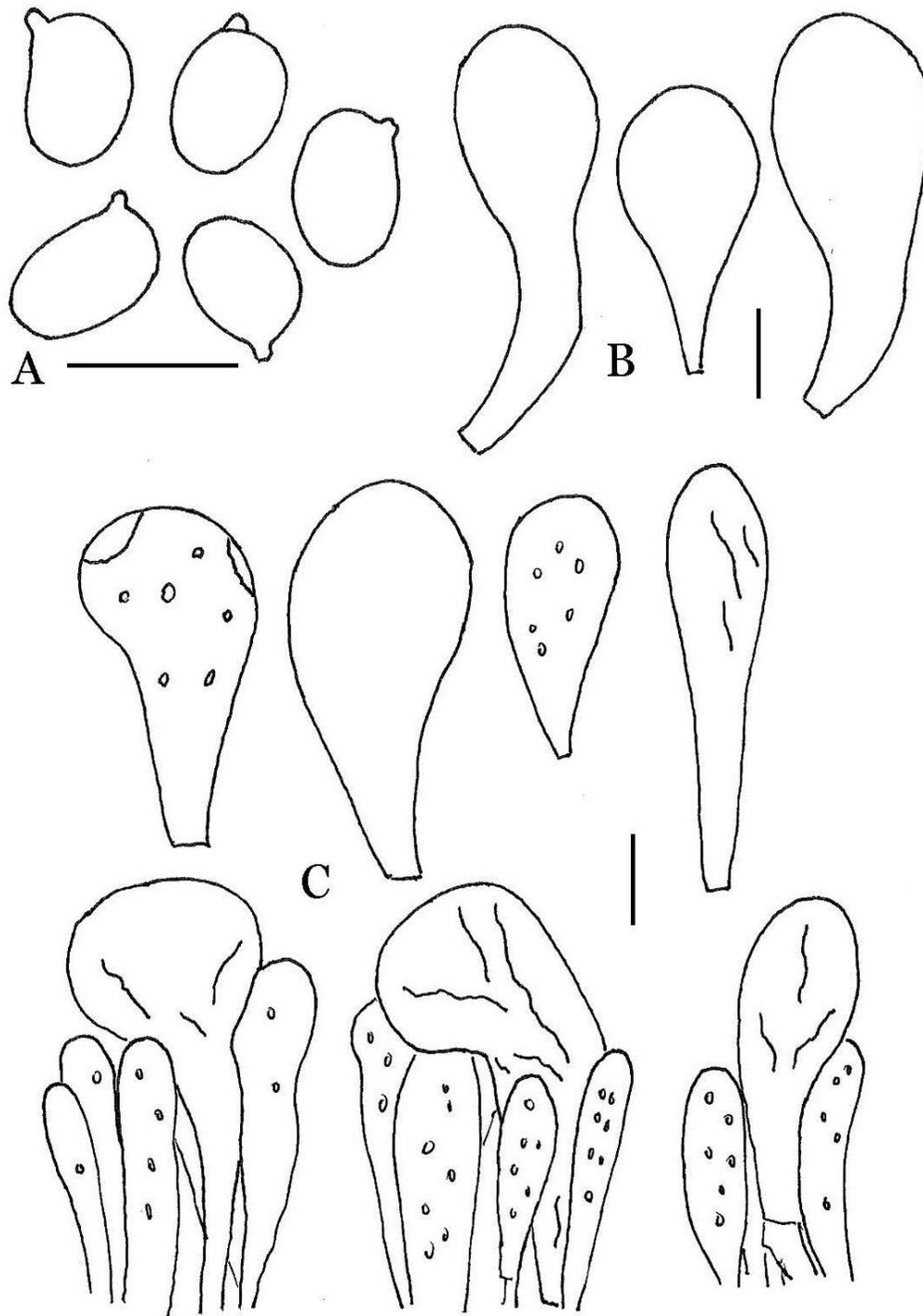


Figure 2. *Tricholoma smithii*, microscopic features. A. Basidiospores, from holotype. B. Cheilocystidia, from holotype. C. Pleurocystidia, middle row from holotype, bottom left from Ovrebo 5148, bottom middle from Smith 88867 and bottom right from Ovrebo 4513. Scale line = 10 μ m.

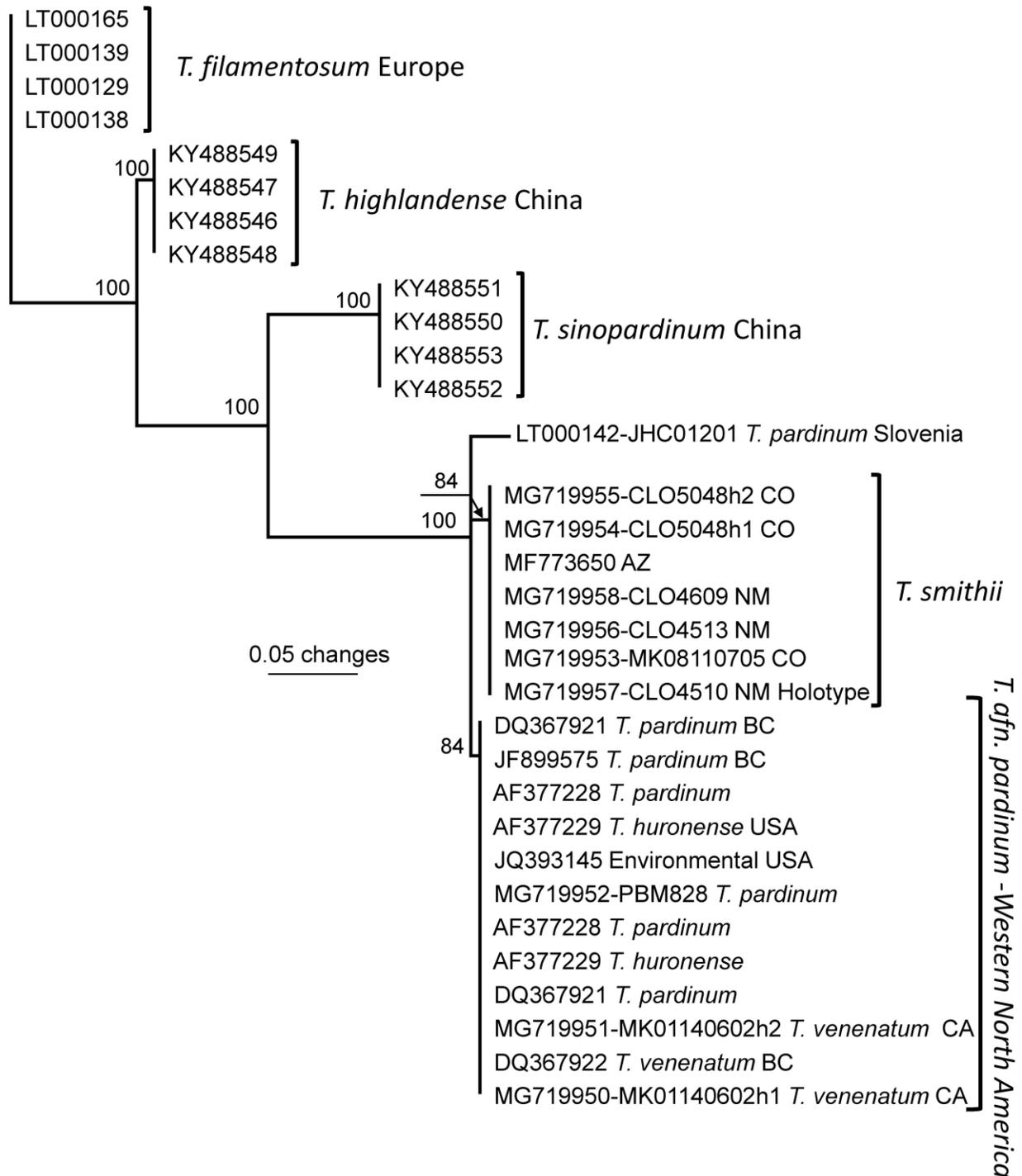


Figure 3. PhyML tree of *Tricholoma pardinum*, *T. smithii* and related taxa based on the nuclear ribosomal ITS (fungal barcode) region. Numbers preceding names are GenBank accession numbers. Collection locations are given as state of province postal code abbreviations. BC is Canada, British Columbia.