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ORIGINAL ARTICLE

Type studies of two *Tricholomopsis* species described by Peck

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Abstract A study of the types of two species of *Tricholomopsis* described by C. H. Peck, *T. flavescens* and *T. sulphureoides*, revealed that their ITS sequences differ clearly from each other and from previously studied European species. The species *T. osiliensis* described from Estonia is a synonym of T. *sulphureoides*. Because both type collections were mixed, lectotypes were designated for each.

Keywords Molecular phylogeny · Fungal taxonomy · Lectotype

Introduction

Charles Horton Peck (1872) described a lignicolous mushroom, *Agaricus sulphureoides*, with a sulphur-yellow, subsquamulose, or smooth pileus from the Catskill Mountains, USA. The next year he reported another lignicolous species from Bethlehem and North Greenbush, USA, *Agaricus (Tricholoma) flavescens*, with a white or pale yellow and smooth pileus (Peck 1873, 1874). That epithet was illegitimate because it had been used on two previous occasions, first by Scopioli in 1772. Peck (1891) was valid in then describing it as *Tricholoma flavescens*, referring to his 1874 description of *Agaricus flavescens*. Subsequently, both these species have been transferred to the genus *Tricholomopsis* Singer.

Murrill (1914) transferred *A. flavescens* Peck (1873) to *Melanoleuca* Pat. as *M. thompsoniana*. As his publication shows, this was merely a case of giving Peck's species a legitimate name, not the description of a new species. He referred directly to the species described by Peck (1873) and mentioned Peck's type locality without referring to a type or other collections of his own. Presumably he was not aware of Peck's 1891 nomen novum for his original species, and therefore, created the synonym.

After Singer created the genus *Tricholomopsis* (Singer 1939), he transferred *Agaricus sulphureoides* Peck there as *Tricholomopsis sulphureoides* (Singer 1943), and *Agaricus* (*Tricholoma*) *flavescens* Peck as *Tricholomopsis flavescens* (Peck) Singer (1951). Smith (1960) reviewed the genus *Tricholomopsis*, and transferred *Melanoleuca thompsoniana* there as *Tricholomopsis thompsoniana*, for which he listed *Tricholomopsis flavescens* (Peck) Singer as a synonym. He obviously overlooked Peck's 1891 report, where *Tricholoma flavescens* was legitimately renamed.

Vauras (2009) described a new European species, *Tricholomopsis osiliensis*, from Estonia, characterised by a pale brownish yellow and fibrillose to smooth pileus. Subsequently the species has also been collected from Slovakia (Holec 2012; Holec and Kolařík 2012). In a recent phylogenetic overview of the genus *Tricholomopsis* in Europe, Holec and Kolařík (2012) included *T. flammula* Métrod ex Holec, *T. decora* (Fr.: Fr.) Singer, *T. osiliensis* Vauras, and *T. rutilans* (Schaeff.: Fr.) Singer. *Tricholomopsis ornata* (Fr.) Singer was considered as a doubtful, poorly documented taxon.

A review of *Tricholomopsis* in the Canadian province of Newfoundland and Labrador (NL) noted that *T. sulphureoides* in that province bore a marked resemblance to *T. osiliensis*

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described from Europe (Voitk 2011). Subsequent comparison of the ITS sequences of *T. sulphureoides* from NL with that of the holotype of *T. osiliensis* revealed them to be conspecific (Vauras et al. 2012). However, conspecificity was proven with collections from NL assumed to be *T. sulphureoides*, not with Peck's type collection (Peck 1872). The ITS sequence from a sample of one fruit body in Peck's type collection of *Tricholoma sulphureoides* showed it to be conspecific with *Tricholomopsis decora* (Saar, unpublished data).

The similarity of *T. sulphureoides* and *T. flavescens* raised the possibility of conspecificity. Peck's type collection of *T. sulphureoides* contains at least 17 relatively intact fruit bodies in addition to several fragments. We have not encountered *T. sulphureoides* in such quantities from single collections, and considered it highly likely that it is a mixed collection. The type collection of *T. flavescens* contains 19 intact fruit bodies and is marked as consisting of two separate collections, again making mixed species possible.

The aims of the study were: (1) to settle the possible conspecificity of *Tricholomopsis sulphureoides* and *T. osiliensis*; (2) to examine the possible conspecificity of *T. sulphueroides* and *T. flavescens*; (3) to clarify the nomenclatural status of *T. flavescens*; (4) to determine whether Peck's type collections of *T. sulphureoides* and *T. flavescens* were a mix of different species.

Materials and methods

Fungal material

The microscopic investigations were carried out using a Leica DM750 light microscope at magnification x1000. The measurements were made in 3% KOH solution. The spore measurements are based on 30 randomly taken basidiospores from each sample. The abbreviations of fungaria are used according to the Index Herbariorum (Thiers 2015).

The intact fruit bodies from both type specimens were numbered for further identification. For DNA extraction, samples from selected numbered fruit bodies were removed, from which an approximately 5×5 mm piece from lamellae was used for molecular analyses. Five of 17 fruit bodies of *Tricholomopsis sulphureoides* and four of 19 of *T. flavescens* were sampled.

Molecular methods

Genomic DNA from the Peck's types was extracted with a high pure PCR template preparation kit (Roche Applied Science, Mannheim, Germany) following the manufacturer's protocol. PCR amplification was performed with primers ITSOFt (5′ -acttggtcatttagaggaagt- 3′) and ITS4B (5′ -

Table 1 Material sequenced for this study (ITS sequences)

Species	Locality	Fungarium code	UNITE code
Amanita muscaria	Estonia	TU106037	UDB011252
Pluteus romellii	Estonia	TU118096	UDB015327
Tricholomopsis decora	Estonia	TU106634	UDB011838
Tricholomopsis decora	Estonia	TU106836	UDB015633
Tricholomopsis decora (as *T. sulphureoides*)	New York State, USA	NYSf3116.A holotype	UDB018405
Tricholomopsis flammula	Estonia	TU118217	UDB015402
Tricholomopsis flammula	Estonia	TU118261	UDB015434
Tricholomopsis flavescens	New York State, USA	NYSf1195.1 * syntype	UDB022699
Tricholomopsis flavescens	New York State, USA	NYSf1195.2 * syntype	UDB022700
Tricholomopsis flavescens	New York State, USA	NYSf1195.4 * syntype	UDB022702
Tricholomopsis rutilans	Estonia	TU106244	UDB011443
Tricholomopsis sulphureoides	Newfoundland, Canada	TU101674	UDB015071
Tricholomopsis sulphureoides	Newfoundland, Canada	TU101675	UDB015072
Tricholomopsis sulphureoides (as *T. osiliensis*, holotype)	Estonia	JV26540F (TUR-A)	UDB011101
Tricholomopsis sulphureoides (as *T. osiliensis*)	Estonia	TU101571	UDB015070
Tricholomopsis sulphureoides (as *T. osiliensis*)	Estonia	TU118828	UDB019502
Tricholomopsis sulphureoides	New York State, USA	NYSf3116.1 * holotype	UDB023122
Tricholomopsis sulphureoides	New York State, USA	NYSf3116.3 * holotype	UDB023123
Tricholomopsis sulphureoides	New York State, USA	NYSf3116.6 * holotype	UDB023124
Tricholomopsis sulphureoides	New York State, USA	NYSf3116.10 * holotype	UDB023125
Tricholomopsis sulphureoides (as *T. flavescens*)	New York State, USA	NYSf1195.3 syntype	UDB022701

The lectotypes designated in this work are marked with asterisks (*). JV=Jukka Vauras

caggagacttgtacacggtccag- 3′) for the ITS region, using puReTaq Ready-To-Go ™ PCR Beads (GE Healthcare, Freiburg, Germany) with 0.8 μM of each primer and 23 μl of DNA solution. In case of a negative PCR product, the second amplifications were done in two shorter parts using primer pairs ITSOFt –ITS2 (5′ -gctgcgttcttcatcgatgc- 3′) and 58SF (5′ -atgcatcgatgaagaacgc- 3′) – ITS4 (5′ -tcctccgcttattgatatgc- 3′). The PCR amplification program was as follows: an initial denaturation at 95 °C for 15 min, followed by 35 cycles at 95 °C for 30 sec, at 55 °C for 30 sec, at 72 °C for 1 min, and a final extension at 72 °C for 10 min. The PCR products were purified with enzymes (10 u Exonuclease I – 1 u FastAP TM Thermosensitive Alkaline Phosphatase; Thermo Fisher Scientific Inc., Waltham, MA USA), according to the manufacturer's instructions. Sequences were performed by Macrogen Inc. (Seoul, Korea) using primers ITS5 (5′ -ggaagtaaaagtcgtaacaagg- 3′), ITS2, ITS3 (5′ -gcatcgatgaagaacgcagc- 3′) and ITS4.

The sequences were inspected and assembled using Sequencher 5 software (Gene Codes, Ann Arbor, USA). The DNA sequences were uploaded into the PlutoF cloud database (Abarenkov et al. 2010b) and can be reached through public web output UNITE (Abarenkov et al. 2010a). Unpublished ITS sequences of Estonian *Tricholomopsis* specimens, previously uploaded into UNITE database by the first author, were also used in the phylogenetic analyses (Table 1).

Additionally, sequences of *Tricholomopsis* species from GenBank were added into the data matrix. Because

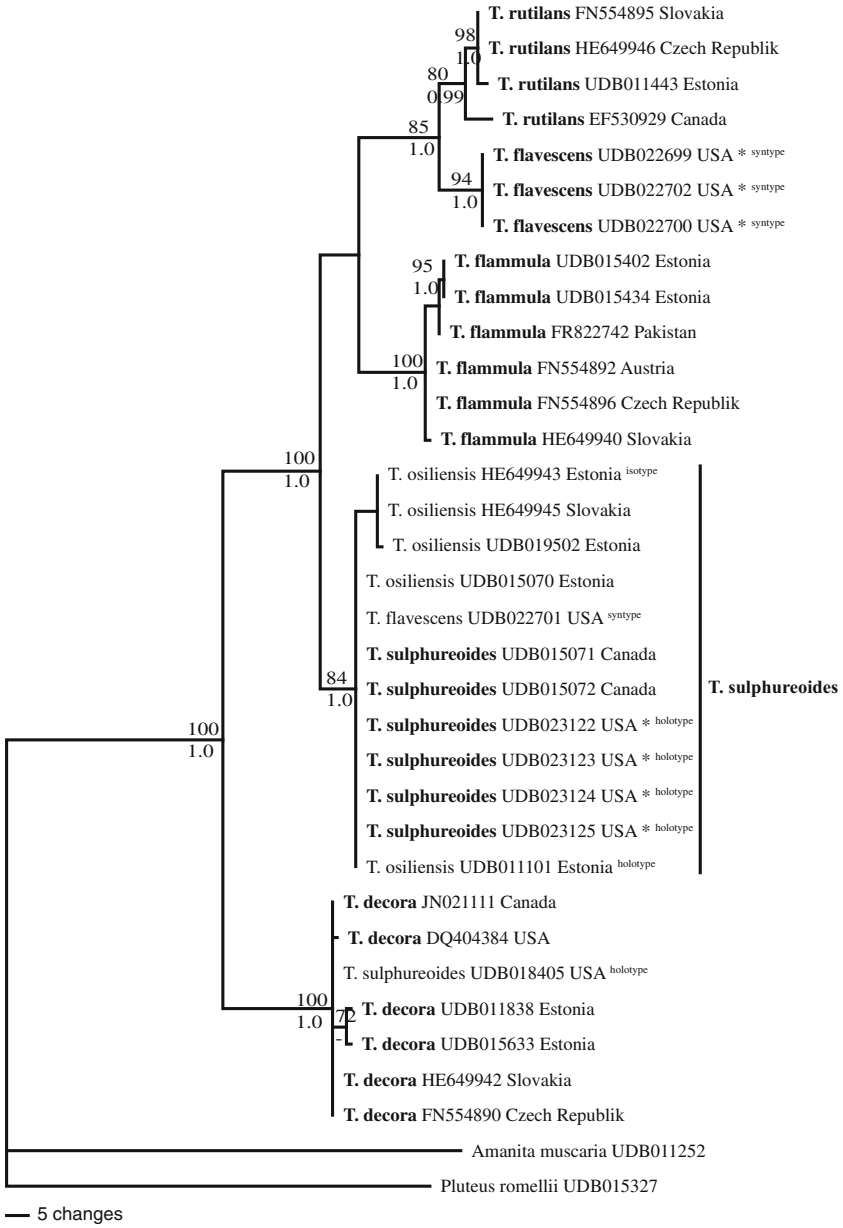

Fig. 1 The phylogeny of ITS sequence data for *Tricholomopsis* taxa inferred by MP analysis. Bootstrap support ≥70% and the posterior probabilities ≥95% are shown above and below the branches (bs/pp), respectively. The lectotypes designated in this work are marked with asterisks (*)

Tricholomopsis decora was related to Amanitaceae in the Pluteoid clade (Matheny et al. 2006), the sequences of *Amanita muscaria* and *Pluteus romellii* were chosen as outgroup members.

Alignments and phylogenetic analyses

Alignments were performed using L-INS-i strategy as implemented in MAFFT v 7.215 (Katoh and Standley 2013). Minor manual adjustments were performed with Se-Al 2.0a11 (Rambaut 1996).

Maximum parsimony (MP) analyses were conducted in PAUP* 4.0b10 (Swofford 2002) using 1000 heuristic searches with random taxon addition sequences, TBR branch swapping, and the restriction to save ten trees in each replicate applied. The confidence of branching was assessed using bootstrap re-sampling (bs): 1000 replicates, each with ten random taxon addition sequences and MulTrees off. All characters were treated as unordered, equally weighted, treating gaps as missing data.

Bayesian inference of phylogeny was performed with MrBayes 3.1.2 (Ronquist et al. 2012) with default values for prior settings. According to the specified generations, the first 100K generations without reaching a stable likelihood score were discarded, leaving 18,002 trees for computing the consensus trees and Bayesian posterior probability (pp) values.

Results

Phylogenetic analyses

The ITS dataset comprises 34 taxa and has an aligned length of 722 characters, with 462 constant, 130 parsimony-uninformative, and 130 parsimony-informative characters. The Parsimony analysis resulted in 6727 equally most parsimonious trees (length=389, CI=0.871, RI=0.932), from which one of the best trees with the likelihood value (– ln L= 2647.14265) was depicted as a phylogram (Fig. 1).

The phylogenetic analyses of the ITS sequences revealed that the samples from both Peck's type collections (*Agaricus sulphureoides*, *A. flavescens*) contained a mixture of two different species. One (NYSf3116.A) from the five samples of the holotype of *Agaricus sulphureoides* matched the ITS sequences of *Tricholomopsis decora*, while the other four samples formed a well-supported clade (84 bs, 1.0 pp) together with the types of *T. osiliensis* Vauras (Fig. 1). One (NYSf1195.3) from the four samples of the syntype collection of *Agaricus flavescens* clustered with *Tricholomopsis sulphureoides*, while the other three formed a strongly supported clade (94 bs, 1.00 pp). No identical ITS sequences for this clade could be found in public gene depositories.

Taxonomy

Tricholomopsis flavescens (Peck) Singer, Lilloa 22: 196. 1951 [1949].

Basionym: *Tricholoma flavescens* Peck, Annual Report of the Trustees of the State Museum of Natural History 44: 158. 1891.

≡ *Agaricus flavescens* Peck, Bulletin of the Buffalo Society of Natural Sciences 1(2): 42. 1873, **nom. illegit.**, Art. 53.1.

= *Melanoleuca thompsoniana* Murrill, North American Flora 10(1): 14. 1914.

≡ *Tricholoma thompsonianum* (Murrill) Murrill, Mycologia 6(5): 269. 1914.

≡ *Tricholomopsis thompsoniana* (Murrill) A.H. Sm., Brittonia 12(1): 66. 1960.

Type: USA, New York State., Albany & Rensselaer Co., Bethlehem & North Greenbush, Oct., 1872, on old pine stumps, leg. C.H. Peck (NYSf1195, syntype).

Illustration: Peck's original illustration (Fig. 2a).

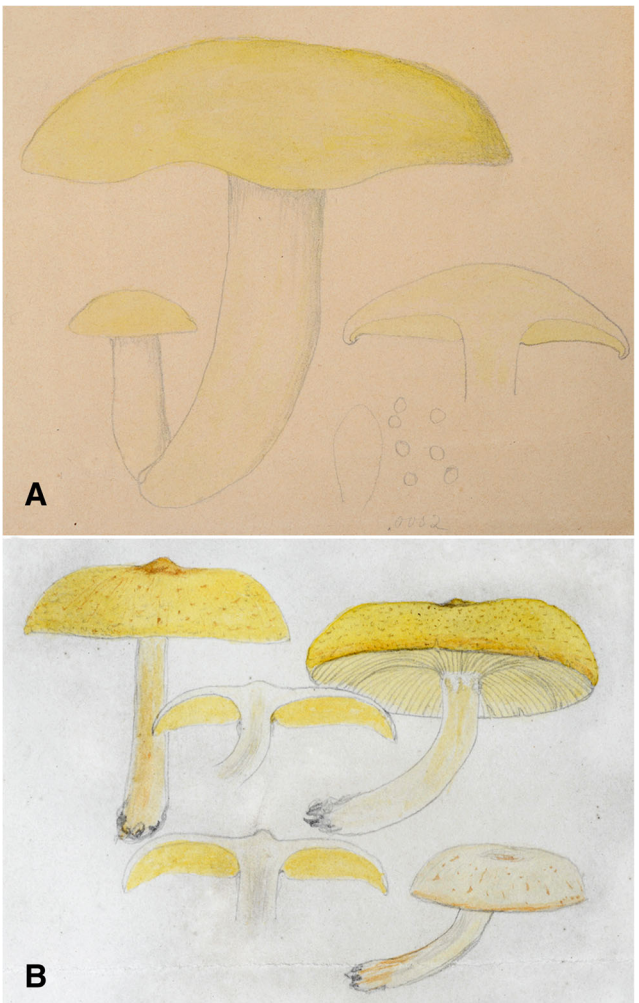

Fig. 2 Peck's original illustrations of the types: **a** *Tricholomopsis flavescens* (NYSf1195); **b** *T. sulphureoides* (NYSf3116). Courtesy New York State Museum, Albany, NY

The lectotype, here designated, consists of the fruit bodies of the syntype numbered NYSf1195.1, NYSf1195.2 and NYSf1195.4 (Fig. 3a).

Remarks: The fruit body NYSf1195.3 was determined to be *T. sulphureoides*.

Melanoleuca thompsoniana Murrill (1914) is a homotypic synonym of *Tricholoma flavescens* Peck (1891), and the latter epithet as the earliest legitimate one should be used. *T. flavescens* is characterised by a smooth, white or pale yellow pileus, and subglobose to broadly ellipsoid, rarely ellipsoid basidiospores 5–7.5×4–6 μm, Q=1.1–1.4 (–1.6) (mean: 1.2). Cheilocystidia 47–84×14–25 μm, clavate, with basal clamp connection (Fig. 4). Pleurocystidia not found.

Tricholomopsis sulphureoides (Peck) Singer, Annales mycologici 41(1/3): 69. 1943.

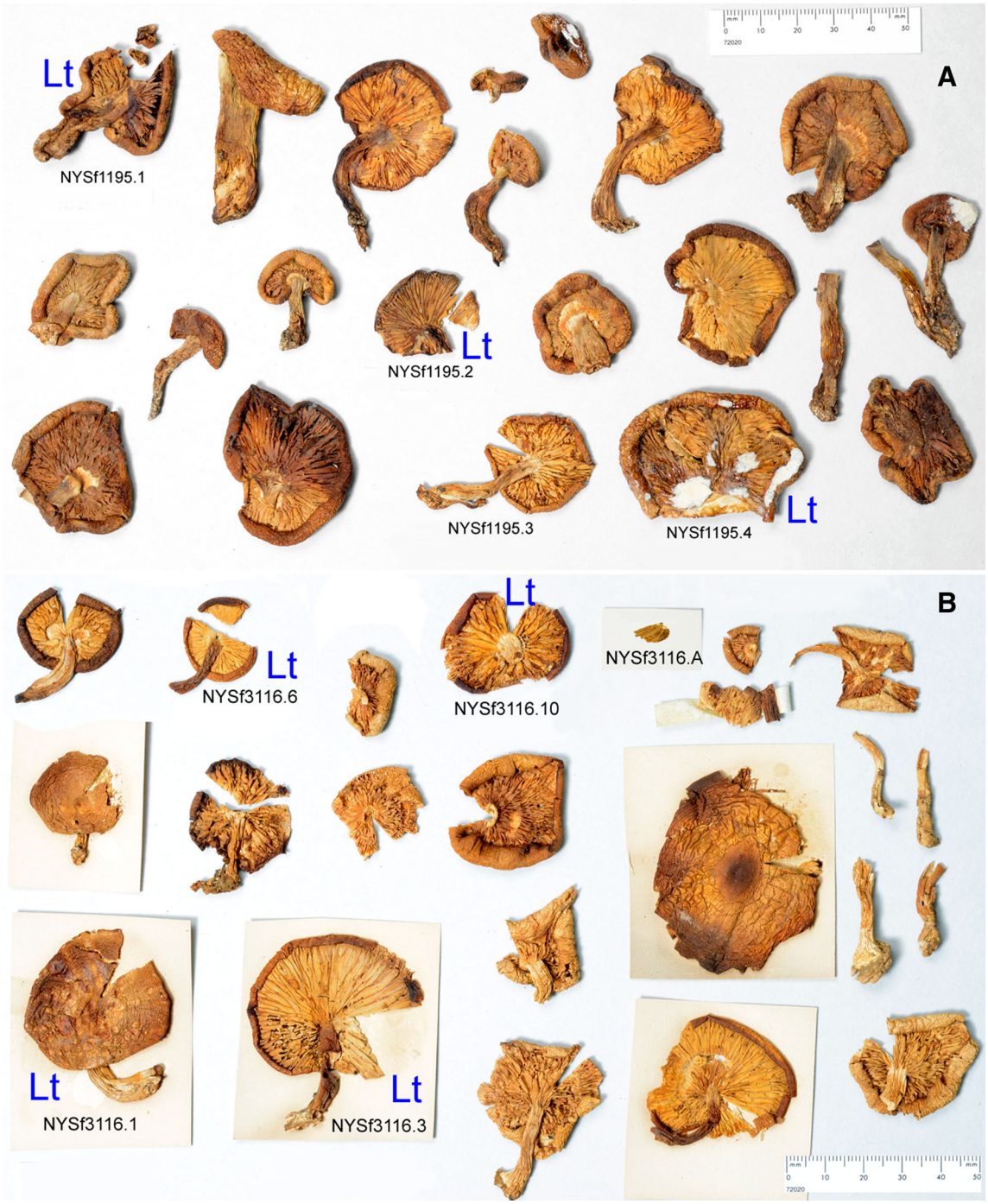

Fig. 3 Photos of the type collections. Sampled fruit bodies are numbered; Lt=lectotype. **a** *Tricholomopsis flavescens* (NYSf1195); **b** *T. sulphureoides* (NYSf3116). Courtesy New York State Museum, Albany, NY

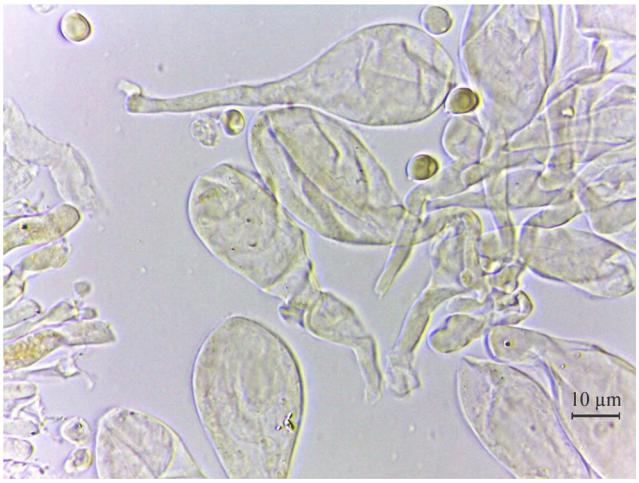

Fig. 4 Cheilocystidia of *Tricholomopsis flavescens* (NYSf1195.4). Morphologically and numerically similar to those of *T. sulphureoides*. Note also the subglobose spores

Basionym: *Agaricus sulphureoides* Peck [as *sulfureoides*], Annual Report of the New York State Museum of Natural History 23: 86. 1872.

≡ *Dendrosarcus sulphureoides* (Peck) Kuntze [as *sulfureodes*], Revisio generum plantarum 3(2): 464. 1898.

≡ *Pleurotus sulphureoides* (Peck) Peck, Annual Report on the New York State Museum of Natural History 39: 60. 1886.

≡ *Tricholomopsis ornata* var. *sulphureoides* (Peck) Singer, Mycologia 35(2): 153. 1943.

= *Tricholomopsis sulphureoides* var. *megaspora* A.H. Sm., Brittonia 12(1): 64. 1960.

= *Tricholomopsis osiliensis* Vauras, Folia Cryptogamica Estonica 45: 87. 2009.

Type: USA, New York State, New York, Catskill Mountains, on old logs in woods, Oct., 1869, leg. C.H. Peck (NYSf3116, holotype).

Illustrations: Peck's original illustration (Fig. 2b); Vauras 2009: 88; Vauras et al. 2012: 10.

The lectotype, here designated, consists of the fruit bodies of the holotype numbered NYSf3116.1, NYSf3116.3, NYSf3116.6 and NYSf3116.10 (Fig. 3b).

Remarks: The fruit body NYSf3116.A was determined to be *T. decora*.

T. sulphureoides is characterised by a subsquamulose or smooth, sulphur-yellow pileus, and broadly ellipsoid to ellipsoid basidiospores 5.5–8.5 (–8.7)×3.5–6 (–6.4) µm, Q=1.2–1.7 (mean: 1.4). Cheilocystidia 39–125×13–38 µm, clavate, with basal clamp connection. Pleurocystidia 56–61×6–8 µm, narrowly cylindrical.

Discussion

Both Peck's species have phylogenetic support, both type collections are mixed, and lectotypes have been designated for each. Because we are not familiar with one of the species, *Tricholomopsis flavescens*, we have not provided detailed descriptions of it. In addition to Peck's original description, *T. flavescens* has been described by Singer (1951) and Smith (1960); we are not aware of more recent detailed descriptions. In addition to the aforementioned, recent descriptions of *T. sulphureoides* can be found by Vauras (2009), Voitk (2011), Holec (2012), Holec and Kolařík (2012), and Vauras et al. (2012); the first, third, and fourth describe it under the name *T. osiliensis*.

According to their descriptions, both these yellow smooth-capped species seem to be rather similar, but because we have not seen any fresh specimens of *T. flavescens*, we are unable to comment further on its macroscopic difference from *T. sulphureoides*. Smith (1960) mentioned that *T. flavescens* has no veil, whereas young specimens of *T. sulphureoides* have a thin veil from which some fibrils remain on the stipe. We have seen some 20 collections of *T. sulphureoides*, including several young fruit bodies, but have not observed a partial veil. Smith (1960) also describes *T. flavescens* as having pallid areas on the cap, lighter gills, and a yellow-staining stipe. The smoothness of their cap makes both quite distinct from the scaly-capped species, such as *T. decora*, *T. flammans*, and *T. rutilans*.

Microscopically they seem to have a very minor difference in spore shape; the spores of *T. flavescens* tend to be more subglobose (mean Q=1.2), whereas those of *T. sulphureoides* are more ellipsoid (mean Q=1.4). The overlap in spore shape is too great to use this micro-character alone as a reliable differentiator between these two species. *T. flavescens* is described to have fewer and less distinct pleurocystidia; our examination did not reveal pleurocystidia in these old specimens, but cheilocystidia were seen (Fig. 4), which were similar for both species in shape and abundance. The spores for these smooth-capped species overlap much of the size range quoted for the scalier species, e.g., *T. decora*, 6.5–9.0×4.5–6.5 µm; *T. flammula*, 5.6–8.0×3.2–4.8 µm; and *T. rutilans*, 5.0–8.5×4.0–6.5 µm (Holec and Kolařík 2012). This conforms to the opinion of Holec and Kolařík (2012) that 'a rather large spore size variability is typical for all European *Tricholomopsis* species.' Although spore morphology "offers exceptionally meager aid" (Smith 1960) in separating these species, fortunately most of the time their macroscopic appearance should serve to set the scalier species apart before they come to microscopy.

According to their ITS sequences, these two morphologically similar Peck's *Tricholomopsis* species, *T. flavescens* and *T. sulphureoides*, are clearly different from each other and from three European species, *T. flammula*, *T. decora*, and *T. rutilans*. The ITS sequences of the type specimens of a fourth European species, *T. osiliensis* (Vauras 2009), are identical to those from the holotype of *T. sulphureoides*. Thus, *T. osiliensis* should be treated as a synonym of *T. sulphureoides*.

The morphological similarity of yellow-coloured species of *Tricholomopsis* and the ease with which they can be confused, as attested by the mixed collections, suggest that type materials for the whole group should be studied to define the species and identify potential synonyms.

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