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Tylopilus griseiolivaceus sp. nov. and *T. leucomycelinus* (Boletaceae) revisited from the Dominican Republic within a comprehensive phylogeny of *Tylopilus* s. str.

Matteo Gelardi[1] · Claudio Angelini[2,3] · Federica Costanzo[1] · Francesco Dovana[4] · Beatriz Ortiz-Santana[5] · Alfredo Vizzini[4]

Abstract

The genus *Tylopilus* s. str. in the Dominican Republic is investigated. *Tylopilus griseiolivaceus* is introduced as a novel species based on material recorded in a neotropical montane pine woodland but also known to occur in temperate coniferous forests in southeastern USA. The Central American species *T. leucomycelinus* is also re-evaluated and its geographical range expanded. A detailed morphological description is provided for both species and accompanied by colour images of fresh basidiomes in habitat, line drawings of the main anatomical features and molecular support relying on a multilocus phylogenetic inference (nrITS, nrLSU (28S), *rpb2* datasets). In addition, DNA was successfully isolated from the type material of *T. leucomycelinus* and compared with the genetic material obtained from additional neotropical samples. The taxonomic placement, ecological requirements and distribution patterns of *T. griseiolivaceus* and *T. leucomycelinus* are investigated/reviewed and their relationships with closely allied taxa in *Tylopilus* s. str. are elucidated and discussed. Epitypification of *T. leucomycelinus* is proposed herein.

Introduction

Tylopilus P. Karst., typified by *T. felleus* (Bull.) P. Karst., is a cosmopolitan and ubiquitous genus that is mainly characterized by pileate-stipitate basidiomes with non-glutinous surfaces, pinkish flesh to vinaceous brown spore print, smooth basidiospores and ectomycorrhizal (ECM) nutritional mode (Horak 1968; Pegler and Young 1981; Singer 1986; Watling

Editorial Responsibility: Zhu-Liang Yang

✉ Alfredo Vizzini
alfredo.vizzini@unito.it

[1] Anguillara Sabazia, Italy

[2] Pordenone, Italy

[3] Dr. Rafael Ma. Moscoso National Botanical Garden, Av. República de Colombia, Santo Domingo, Dominican Republic

[4] Department of Life Science and Systems Biology, University of Turin, Viale P.A. Mattioli 25, I-10125 Torino, Italy

[5] USDA Forest Service, Northern Research Station, Center for Forest Mycology Research, One Gifford Pinchot Drive, Madison, WI 53726, USA

2008). It was one of the earlier boletoid genera to be separated from *Boletus* Fr. s.l. as intended in the Friesian system and its establishment is dated back to the second half of the nineteenth century (Karsten 1881). Up to very recent times, *Tylopilus* has been conceived in a very broad sense, including a very large array of species with a wide range of different morphological, chemical and ecological features (Singer 1947, 1986; Heinemann 1951; Hongo 1960; Horak 1968, 2011; Snell and Dick 1970; Smith and Thiers 1971; Corner 1972; Thiers 1975; Grund and Harrison 1976; Wolfe Jr 1979, 1986; Singer et al. 1983, 1991; Imazeki et al. 1988; Both 1993; Wolfe Jr and Bougher 1993; Watling and Turnbull 1994; Watling and Li 1999; Bessette et al. 2000; Mao 2009). However, molecular phylogenetic approaches carried out in the last 20 years have clearly and repeatedly demonstrated that such an assemblage of species into a single genus should be disregarded, since *Tylopilus* s.l. appears to be definitely polyphyletic, consisting of several independent phylogenetic lineages (Binder and Hibbett 2006; Drehmel et al. 2008; Li et al. 2011, 2014; Halling et al. 2012a, b; Nuhn et al. 2013; Wu et al. 2014, 2016; Magnago et al. 2017). A number of different genera have thus been erected to accommodate species previously placed in this genus, such as *Zangia* Yan C. Li & Zhu L.

Yang, *Sutorius* Halling, Nuhn & Fechner, *Australopilus* Halling & Fechner, *Harrya* Halling, Nuhn & Osmundson, *Pseudoaustroboletus* Yan C. Li & Zhu L. Yang and *Chiua* Yan C. Li & Zhu L. Yang (Li et al. 2011, 2014; Halling et al. 2012a, b; Wu et al. 2016). Currently, the circumscription of *Tylopilus* s.str. as a monophyletic lineage belonging to the subfamily *Boletoideae* Singer (Wu et al. 2014, 2016) is restricted to sect. *Tylopilus* (= sect. *Fellei* Singer) and sect. *Oxydabiles* Singer pro parte as outlined by Singer (1986) and Singer et al. (1991), encompassing species featuring brownish, brown-olive to violet-purple colours, reticulate or smooth stipe, white context and basal mycelium, tissues unchangeable or reddening-darkening, occasionally greenish blue to sea green on bruising, bitter to rarely mild taste and dextrinoid cystidia (Binder and Hibbett 2006; Nuhn et al. 2013; Wu et al. 2014, 2016; Gelardi et al. 2015; Chakraborty et al. 2018; Liang et al. 2018; Chai et al. 2019). Furthermore, two additional morphologically homogeneous and well-delimited species groups, the *T. balloui* (Peck) Singer complex (characterized by yellow-orange to orange-red or reddish brown colours, smooth stipe, negative reaction with ammonia, mild taste, short phaseoliform-allantoid to subovoid basidiospores and trichoderm to ixotrichoderm pileipellis) and *T. potamogeton* Singer complex (sect. *Potamogetones* Singer) (sharing tomentose pileus and stipe, non-staining context, bluing surfaces with ammonia, bitter taste and ECM association with caesalpinoid legumes) should also be retained within *Tylopilus* s. str. (Osmundson 2009; Nuhn et al. 2013; Wu et al. 2014, 2016; Gelardi et al. 2015; Magnago et al. 2017; Chakraborty et al. 2018). However, due to the considerable morphological plasticity of *Tylopilus*, its taxonomic and phylogenetic boundaries have only partially been assessed and the infrageneric classification still remains unclear.

In America, the diversity of *Tylopilus* s.l. has a long tradition of study, having been widely explored over the past two centuries, especially in the temperate belt of southeastern Canada and eastern USA (Frost 1874; Peck 1889; Murrill 1909, 1914, 1948; Coker and Beers 1943; Singer 1947; Snell and Dick 1970; Smith and Thiers 1971; Grund and Harrison 1976; Wolfe Jr 1981, 1983, 1986; Wolfe Jr and Halling 1989; Both 1993; Baroni and Both 1998; Bessette et al. 2000, 2016) as well as in the adjacent neotropical forests of mainland Central America and Northern South America (Singer 1978, 1989; Singer et al. 1983, 1991; Halling 1989; Wolfe Jr and Bougher 1993; Flores Arzù and Simonini 2000; Henkel 1999, 2001; Halling and Mueller 2001, 2005; Amtoft et al. 2002; Fulgenzi et al. 2007; Ortiz-Santana et al. 2007; Fulgenzi 2009; Osmundson and Halling 2010), owing to the large number of species being distributed in these regions. By contrast, the genus is apparently very scarcely represented along the Pacific Northwest with just a few species occurring (Thiers 1975; Arora 1986; Siegel and Schwarz 2016) and

similarly only a limited number of taxa have till now been reported from the underexplored areas of central and southern South America (Palfner 2005; Barbosa-Silva et al. 2017; Magnago et al. 2017). The situation appears to be not much dissimilar in the islands of the Caribbean, where the genus is nearly absent. As a matter of fact, only a couple of *Tylopilus* species have been reported to occur in the Dominican Republic to date, viz. *T. chromapes* (Frost) A.H. Sm. & Thiers and *T. balloui* (Peck) Singer (Ortiz-Santana et al. 2007). However, the former has recently been reassigned to a separate genus *Harrya* (Halling et al. 2012b) within the subfamily *Zangioideae* G. Wu, Y.C. Li & Zhu L. Yang (Wu et al. 2014) based on morphological and molecular phylogenetic evidence, whereas the latter resulted from a misidentification of *T. leucomycelinus* (Singer & M.H. Ivory) R. Flores & Simonini with the North American taxon (see below).

As part of an ongoing project aiming at recording and analysing the boletes of the Dominican Republic, *T. griseiolivaceus* is presented as a new species and a modern re-description and epitypification of *T. leucomycelinus* is provided. In addition, DNA was successfully extracted from the type material of *T. leucomycelinus* and inferred to match with sequences obtained from samples recently recorded in Belize, Guatemala and the Dominican Republic.

Materials and methods

Collection site and sampling

Specimens examined were collected in Belize, Dominican Republic and Honduras, and are deposited in BRH, CFMR, F and JBSD (acronyms from Thiers 2019), while "ANGE" and "MG" refer to the personal herbarium of Claudio Angelini and Matteo Gelardi, respectively. Herbarium numbers are cited for all collections from which morphological features were examined. Author citations follow the Index Fungorum, Authors of Fungal Names (www.indexfungorum.org/authorsoffungalnames.htm). Novel names and typifications are registered with MycoBank (http://www.mycobank.org/).

Morphological studies

Macroscopic descriptions and ecological information such as habitat notations, time of fruiting and associated plant communities accompanied the detailed field notes of the fresh basidiomes. In the field, latitude, longitude, and elevation were determined with a Global Positioning System (GPS) receiver. Colour terms in capital letters (e.g. White, Plate LIII) are from Ridgway (1912). Photographs of collections were taken in the natural habitat using a Nikon Coolpix 8400 camera. Microscopic anatomical features were observed and recorded from revived dried material; sections were

rehydrated either in water, 5% potassium hydroxide (KOH) or in anionic solution saturated with Congo red. All anatomical structures were measured from preparations in anionic Congo red. Colours and pigments were described after examination in water and 5% KOH. Measurements were made at × 1000 using a calibrated ocular micrometre (Nikon Eclipse E200 optical light microscope). Basidiospores were measured directly from the hymenophore of mature basidiomes, dimensions are given as (minimum) average ± standard deviation (maximum), Q = length/width ratio with the extreme values in parentheses, Qm = average quotient (length/width ratio) ± standard deviation and average spore volume was approximated as a rotation ellipsoid ($V = (\pi \cdot L \cdot W2)/6 \pm$ standard deviation). The notation (n/m/p) indicates that measurements were made on "n" randomly selected basidiospores from "m" basidiomes of "p" collections. The width of each basidium was measured at the widest part, and the length was measured from the apex (sterigmata excluded) to the basal septum. Radial and/or vertical sections of the pileipellis were taken midway between the center and margin of the pileus. Sections of the stipitipellis were taken from the middle part along the longitudinal axis of the stipe. Metachromatic, cyanophilic and iodine reactions were tested by staining the basidiospores in Brilliant Cresyl blue, Cotton blue and Melzer's reagent, respectively. Line drawings of microstructures were traced in free hand based on digital photomicrographs of rehydrated material.

DNA extraction, PCR amplification and DNA sequencing

Genomic DNA was isolated from 10 mg of herbarium specimens by using the DNeasy PlantMini Kit (Qiagen, Milan Italy) according to the manufacturer's instructions. PCR amplifications were performed with the primers ITS1F/ITS4 for the nrITS region (White et al. 1990; Gardes and Bruns 1993), LR0R and LR5 for the nrLSU region (Vilgalys and Hester 1990) and the reverse complement of bRPB2-6R2 (Matheny et al. 2007) and bRPB2-7.1R2 (5′–CCCATNGCYTGYTTVCCCATDGC–3′) or RPB2-B-F1 and RPB2-B-R (Wu et al. 2014) for partial *rpb2*. Amplification reactions were performed in a PE9700 thermal cycler (Perkin-Elmer, Applied Biosystems) following Vizzini et al. (2015). The PCR products were purified with the AMPure XP kit (Beckman Coulter) and sequenced by MACROGEN Inc. (Seoul, Republic of Korea). Ribosomal sequences of the collections DJL-DR-15/DR 2800 (JBSD, CFMR), BOS-481/BZ 2409 (BRH, CFMR) and BOS-612/BZ 3165 (BRH, CFMR) were generated by B. Ortiz-Santana at CFMR following Palmer et al. (2008). The sequences were submitted to GenBank (http://www.ncbi.nlm.nih.gov/genbank/) and their accession numbers are reported in Table 1.

Sequence alignment, data set assembly and phylogenetic analyses

The sequences obtained in this study were checked and assembled using Geneious v. R 11.1.4 (Kearse et al. 2012) and compared to those available in GenBank database (https://www.ncbi.nlm.nih.gov/genbank/) by using the BLASTN algorithm (Altschul et al. 1990). A general combined maximum likelihood tree including all the Boletaceae sequences present in GenBank database was generated to detect the phylogenetic position of our collections in the major clades of Boletaceae as circumscribed by Wu et al. (2014) (tree not shown). Consequently, phylogenetic analyses were restricted to *Tylopilus* sequences. Our datasets consist of sequences of *Tylopilus* and other sequences with greatest similarity available in GenBank selected based on BLASTN search and previous molecular studies including *Tylopilus* collections (Halling et al. 2008; Gelardi et al. 2015; Wu et al. 2016; Magnago et al. 2017; Chakraborty et al. 2018). Two phylogenetic analyses were performed: the first phylogenetic analysis, based on nrITS sequences, includes two sequences of *Gyroporus castaneus* (KX869874 and GQ166901) as outgroup following Chakraborty et al. (2018); the second phylogenetic analysis based on a combined nrLSU/*rpb2* dataset includes *Xanthoconium sinense* (KT990666: nrLSU and KT990490: *rpb2*) as outgroup following Chakraborty et al. (2018).

Sequences were aligned with MAFFT v. 7.017 (Katoh et al. 2002) and then manually adjusted using Geneious v. R 11.1.4 (Kearse et al. 2012). Following nrITS alignment, gaps and phylogenetically uninformative positions were removed using Gblocks v. 0.91b (Castresana 2000). The introns of *rpb2* were excluded from the analysis. The best-fit substitution model for each alignment was estimated by both the Akaike information criterion (AIC) and the Bayesian information criterion (BIC) with MEGA X (Kumar et al. 2018) to provide a substitution model for the alignment. GTR + G + I model was chosen for the unpartitioned nrITS dataset. For the concatenated dataset, the following partitioned model was used: nrLSU regions were modelled using a GTR + G + I model, TN93 + G + I was selected for the *rpb2* 1st position partition, T92 + G for the *rpb2* 2nd position and K2 + G + I was selected as the best-fit model for *rpb2* 3rd position. The datasets were analysed using Bayesian inference (BI) and maximum likelihood (ML) criteria. The BI was performed with MrBayes v.3.2 (Ronquist et al. 2012) in the CIPRES server with four incrementally heated simultaneous Monte Carlo Markov Chains (MCMC) run for 10 million generations, under the selected evolutionary models. Trees were sampled every 1000 generations, resulting in overall sampling of 10,001 trees; the first 2500 trees were discarded as "burn-in" (25%). For the remaining trees, a majority rule consensus tree showing all compatible partitions was computed to obtain estimates for Bayesian Posterior

Table 1 Samples sequenced for the present study

Species	GenBank acc. number			Source, collector, date and country
	nrITS	**nrLSU (28S)**	***rpb2***	
Tylopilus griseiolivaceus	MN115807	MN115799	MN095208	JBSD127431 (**holotype**), C. Angelini, 22/12/2013, Dominican Republic
Tylopilus griseiolivaceus	MN115808	MN115800	–	JBSD127432, C. Angelini, 07/12/2014, Dominican Republic
Tylopilus griseiolivaceus	MN115809	MN115801	–	JBSD127430, C. Angelini, 28/11/2017, Dominican Republic
Tylopilus griseiolivaceus	MN115810	–	–	JBSD127433, C. Angelini, 23/11/2017, Dominican Republic
Tylopilus leucomycelinus	MN115811	–	–	F-1030852 (**holotype**), M. H. Ivory, 12/10/1976, Honduras
Tylopilus leucomycelinus	MN115812	MN115802	MN095209	JBSD127419 (**epitype**), C. Angelini, 22/12/2013, Dominican Republic
Tylopilus leucomycelinus	MN115813	MN115803	MN095210	JBSD127420, C. Angelini, 07/12/2014, Dominican Republic
Tylopilus leucomycelinus	MN115814	MN115804	–	DJL-DR-15, DR 2800 (JBSD, CFMR), D. J. Lodge, 11/11/2003, Dominican Republic
Tylopilus leucomycelinus	MN115815	MN115805	–	BOS-481, BZ 2409 (BRH, CFMR), Clark L. Ovrebo, 30/11/2002, Belize
Tylopilus oradivensis	MN115816	MN115806	–	BOS-612, BZ 3165 (BRH, CFMR), Roy E. Halling, 13/10/2003, Belize

Probabilities (BPP). The ML was performed with RAxML v.7.2.8. (Stamatakis 2006) and a total of 1000 bootstrap replicates (Felsenstein 1985) were computed to assess the relative robustness of the branches.

Only BPP values ≥ 0.95 and MLB (maximum likelihood bootstrap) values ≥ 70% are shown in the phylogenetic trees (Figs. 1 and 2). Pairwise % identity values of the sequences were calculated using Geneious v. R 11.1.4 (Kearse et al. 2012). Alignments and phylogenetic trees are available at TreeBASE (www.treebase.org, submission number S24614).

Results

Molecular analysis

Ten nrITS, eight nrLSU and three *rpb2* sequences were newly generated for this study (Table 1). Both Bayesian and maximum likelihood analyses produced the same topology; therefore, only the maximum likelihood trees with both MLB and BPP values are shown (Figs. 1 and 2). The nrITS data matrix comprises a total of 107 sequences and 399 characters. The combined nrLSU/*rpb2* data matrix comprised 102 sequences and is 1689-bp long. In the nrITS phylogenetic analysis (Fig. 1), our four sequences of the *T. griseiolivaceus* clustered together with two sequences from North Carolina (USA) (*Tylopilus* sp. AY456372 and uncultured cf. *Xerocomus*

AY456074) forming a well-supported clade (MLB 87; BPP 0.97). The sequences of this clade share a pairwise % identity value of 97.7. In the nrLSU/*rpb2* phylogenetic tree (Fig. 2), the three sequences of *Tylopilus griseiolivaceus* clustered together forming a well-supported clade (MLB 100; BPP 1). In both phylogenetic analysis, the position of *T. griseiolivaceus* within *Tylopilus* remains unresolved.

Tylopilus leucomycelinus is shown to be a distinct species (Figs. 1 and 2) sister to a clade (MLB 99; BPP 1.0) (Fig. 2) consisting of *T. pseudoballoui* (India and China), two collections named *T. balloui* from Australia (T. «balloui» 2) and seven collections named *T. balloui*, *T.* aff. *balloui* or *T.* cf. *balloui* from Asia (T. «balloui» 1). The sequences of the *T. leucomycelinus* clade share a pairwise % identity value of 98.4.

Collections named as *T. balloui*, *T.* aff. *balloui* or *T.* cf. *balloui* form three distinct clades in the nrITS analysis (Fig. 1) and six distinct clades in the combined analysis (Fig. 2). The name *T. balloui* is used here only for the North American collections, following Halling et al. (2008) and Osmundson and Halling (2010).

Taxonomy

Tylopilus griseiolivaceus Angelini, Gelardi, Costanzo & Vizzini, **sp. nov.** Figs. 3 and 4

MYCOBANK MB 831668

Etymology: the specific epithets "*grisei*" (grey) and "*olivaceus*" (olive) are derived from Latin and refers to the grey-olive tints of pileus.

Original diagnosis:

Basidiomes stipitate-pileate with tubular hymenophore characterized by medium-small size, subtomentose to fibrillose-subsquamulose and soon areolate, mouse grey, grey olivaceous to brownish grey pileus surface, whitish projecting sterile margin, white to pinkish lilac hymenophore, smooth stipe, white stipe, context and basal mycelium, unchanging tissues on bruising, bitter taste, ellipsoid-fusiform, smooth, strongly cyanophilic basidiospores measuring (8.0–) 10.8 ± 1.01 (–14.2) × (3.8–)4.4 ± 0.33(–6.0) μm, trichodermal pileipellis consisting of zebra-pattern encrusted, filamentous hyphae and occurrence in temperate and neotropical coniferous forests in ECM association with pine trees (Pinaceae).

Holotype: DOMINICAN REPUBLIC, La Vega Province, Jarabacoa, 19° 11′ 12.5″ N 70° 35′ 25.5″ E, 660 m, 22

Dec 2013, C. Angelini (JBSD127431; *isotypes* ANGE101 and MG744).

Basidiomes medium-small. *Pileus* (3.2–)3.6–9.5 cm broad, at first hemispherical then persistently convex and finally broadly pulvinate-flattened, sometimes slightly depressed at centre, regularly to hardly unevenly shaped, moderately fleshy, firm at the beginning but progressively softer with age, flabby in old basidiomes; margin steady to faintly wavy-lobed, initially involute but soon curved downwards and finally completely plane or even uplifted, extending beyond the tubes up to 2 mm; surface matt, subtomentose at the beginning but soon distinctly fibrillose to flattened subsquamulose and minutely cracked, showing the whitish (White, Plate LIII) context beneath; cuticle ranging from pale mouse grey (Light Greyish Olive, Plate XLVI; Light Mouse Grey, Olive Grey, Deep Olive Grey, Plate LI) at the beginning, then grey olivaceous, olive greyish to dull brownish grey (Pale Olive Buff, Plate XL; Olive Lake, Buffy Citrine, Plate XVI;
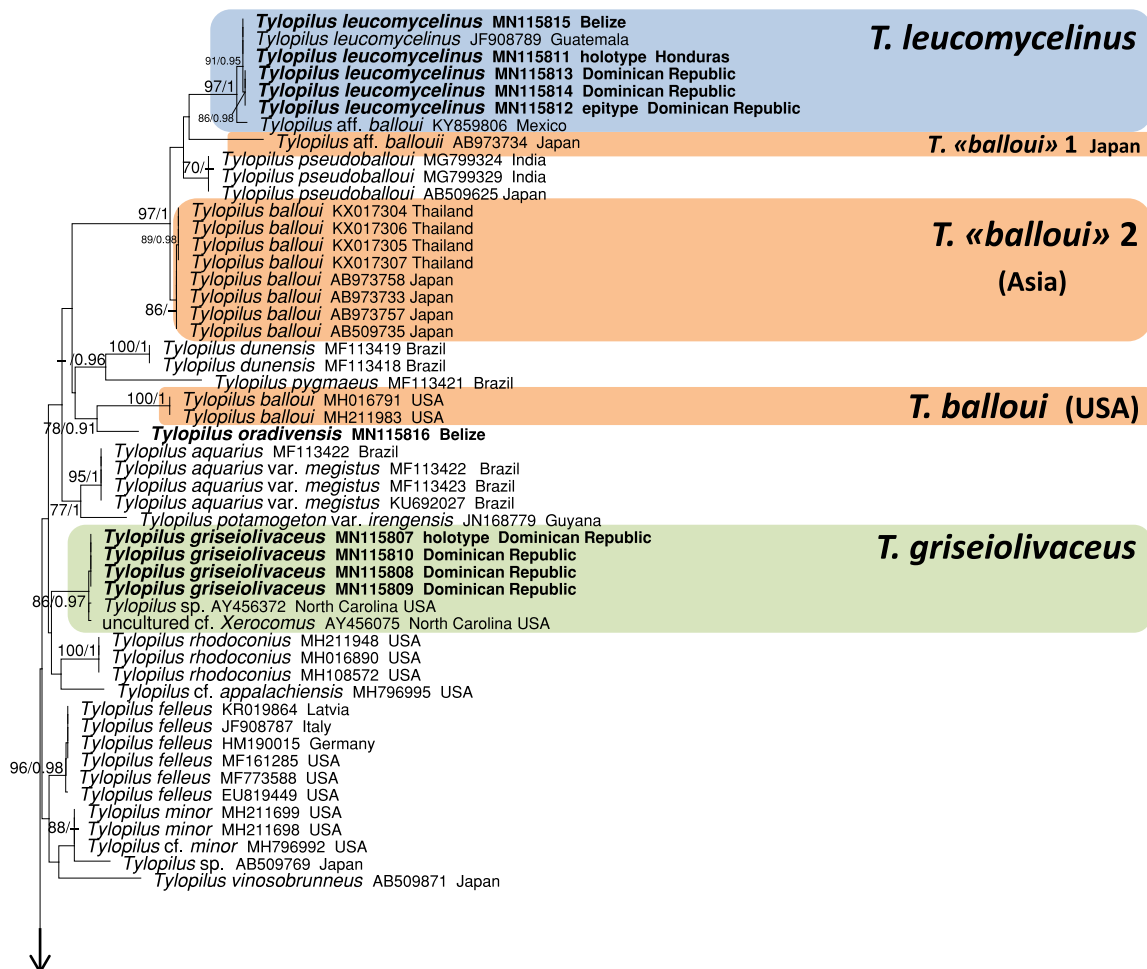

Fig. 1 Bayesian phylogram obtained from the nrITS sequence alignment of *Tylopilus* species. *Gyroporus castaneus* was used as outgroup taxon. Values for clades that are supported in either the Bayesian (posterior probabilities, BPP) and maximum likelihood (ML bootstrap percentage, MLB) analyses are indicated. BPP ≥ 0.95 and MLB ≥ 70% are given above clade branches. Newly sequenced collections are boldfaced. For each collection, the specific epithet (as present in GenBank), GenBank accession number of the nrITS sequence and geographical origin (country) are reported
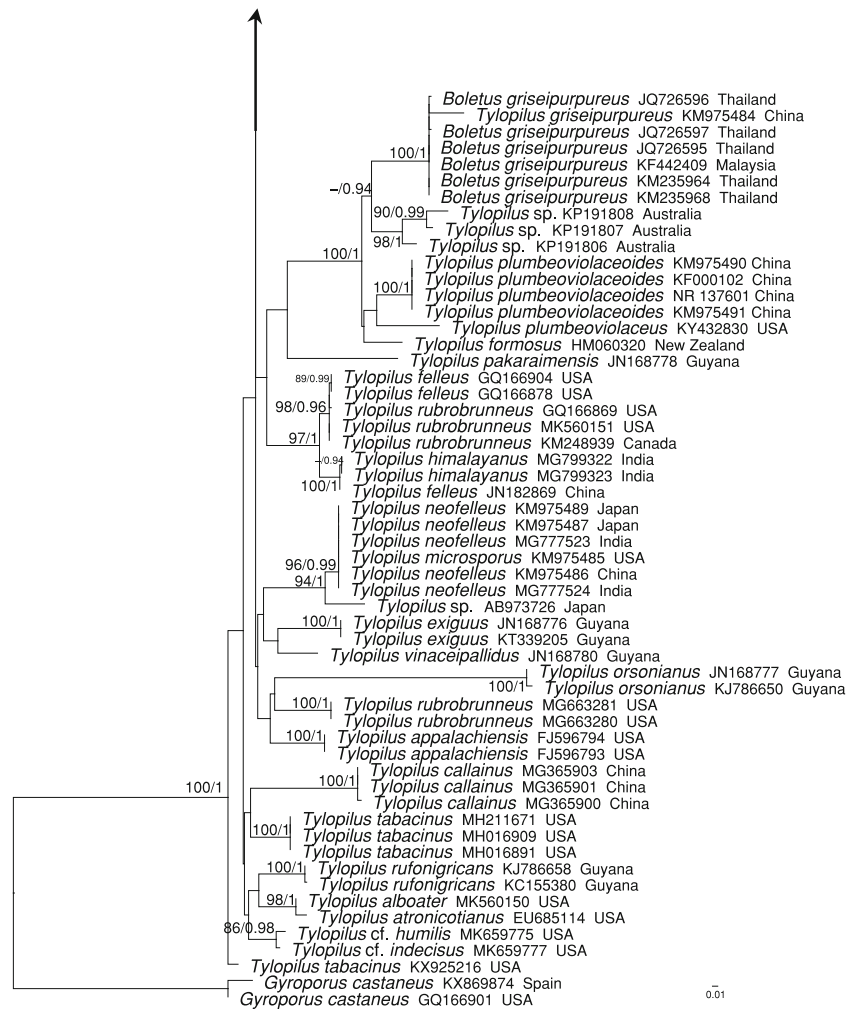
Fig. 1 continued.

Ecru Olive, Light Yellowish Olive, Isabella Colour, Light Brownish Olive, Plate XXX), always paler towards the margin, appendiculate edge whitish (White, Plate LIII); unchangeable on handling or when injured; subcuticular layer white (White, Plate LIII). *Tubes* at first very thin then increasingly broader and shorter than the thickness of the pileus context (up to 1.0-cm long), depressed around the stipe apex and decurrent with a tooth, whitish (White, Plate LIII) at first to pale pinkish flesh (Flesh-Pink, Venetian Pink, Plate XIII; Pale Purple Vinaceous, Plate XXXIX) then pale pinkish lilac (Pale Hortense Violet, Plate XI; Pale Lavander Violet, Plate XXV; Pale Lobelia Violet, Plate XXXVII) at maturity, unchangeable when cut. *Pores* initially forming a slightly concave to flat surface, later more or less convex or ascending, at first very small then gradually wider (up to 1 mm in diam.), simple, roundish to barely angular at maturity, concolourous with the tubes and not staining on bruising or when injured. *Stipe* (5.3–)8.2–9.6(–10.3) × (0.6–)0.8–2.0(–2.2) cm, longer than or as long as the pileus diameter at maturity, central to slightly off-center, solid, firm, dry, straight or curved to

sinuous, often typically creeping parallel to the ground underneath the humus (in some specimens for more than three fourth of its entire length!) and then benting up in the upper portion to support the pileus, subfusiform to more frequently cylindrical but always narrowing towards the apex and the base, not to fairly rooting; surface smooth and glabrous throughout, evelate; evenly white (White, Plate LIII) but frequently with very pale yellowish (Martius Yellow, Plate IV) areas especially downwards, unchangeable when pressed; basal mycelium white (White, Plate LIII). *Context* firm and tough when young, later soft textured and eventually flabby in the pileus (up to 1.6-cm thick in the central zone), a little more fibrous in the stipe, white overall (White, Plate LIII), at times with very pale yellowish (Martius Yellow, Plate IV) areas at the base; unchangeable when exposed to air; whitish (White, Plate LIII) where eaten by slugs; subhymenophoral layer white (White, Plate LIII); exsiccate dull ochraceous (Raw Sienna, Plate III) with olive greyish shades (Deep Olive Buff, Plate XL) on the pileus and dull brick red tones (Sanford's Brown, Plate II) on a somewhat folded-rugose stipe

Fig. 2 Phylogeny of *Tylopilus* species based on a Bayesian and maximum likelihood inference analysis of a matrix of concatenated sequences from two nuclear gene regions (nrLSU/28S and *rpb2*). *Xanthoconium sinense* was used as outgroup taxon. Values for clades that are supported in either the Bayesian (posterior probabilities, BPP) and maximum likelihood (ML bootstrap percentage, MLB) analyses are indicated. BPP ≥ 0.95 and MLB ≥ 70% are given above clade branches. Newly sequenced collections are boldfaced. For each collection, the specific epithet (as present in GenBank), GenBank accession numbers of the nrLSU/*rpb2* sequences and geographical origin (country) are reported. The /- notation indicates the *rpb2* sequence as missing for that collection
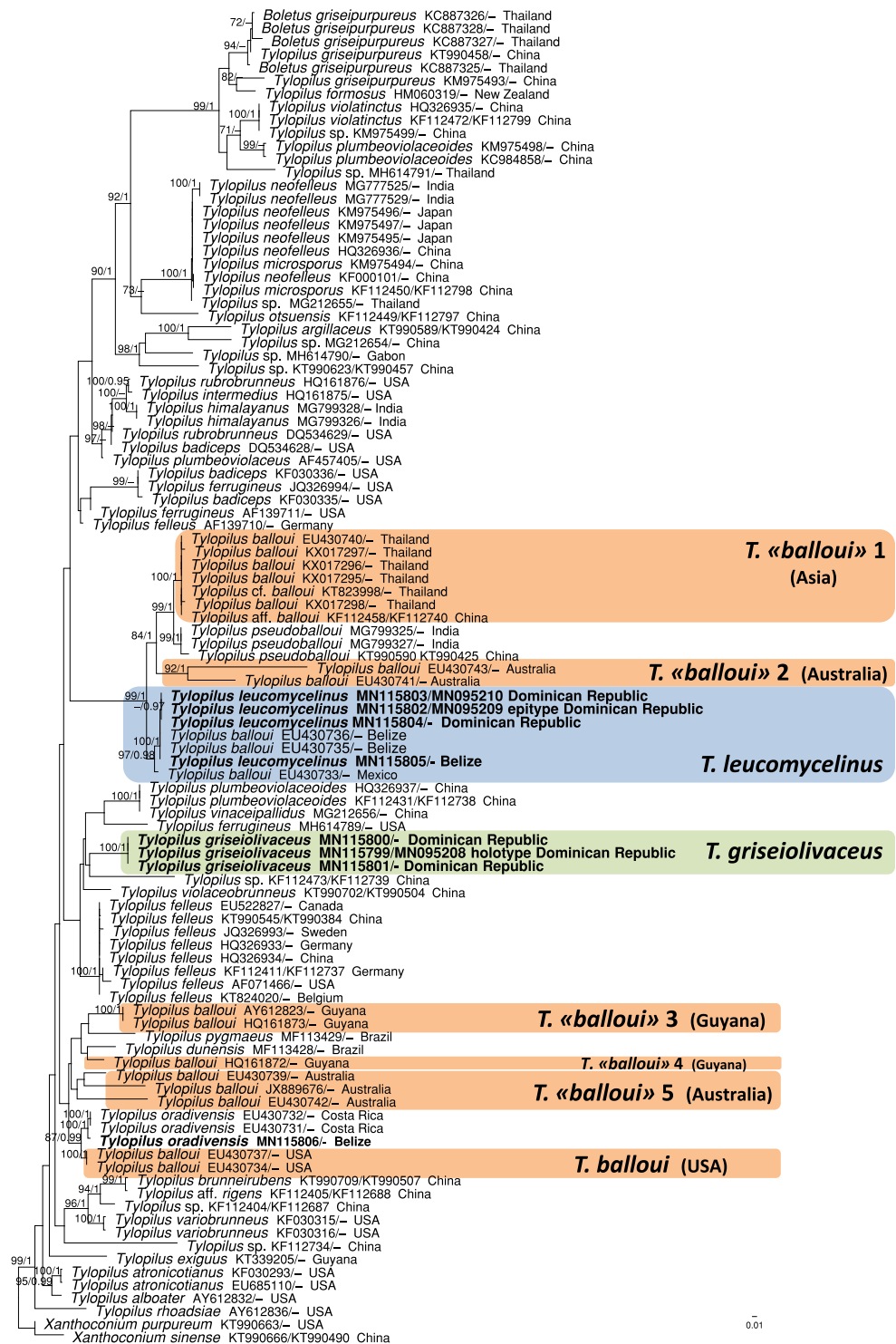
surface. *Odour* indistinct. *Taste* decidedly bitter. *Spore print* deep pinkish-flesh. *Macrochemical spot-test reactions* not tested.

Basidiospores [175/8/5] (8.0−)10.8 ± 1.01(−14.2) × (3.8−)4.4 ± 0.33(−6.0) μm, $Q = (1.95−)2.07−2.83(−2.86)$, $Qm = 2.43 ± 0.16$, $V = 113 ± 27$ μm³, inequilateral, ellipsoid-fusiform to fusiform in side view, ellipsoid-fusiform to ellipsoid in face view, smooth, apex rounded, with a short apiculus and usually with a shallow suprahilar depression or applanation, moderately thick-walled (0.3–0.8 μm), nearly hyaline or with a very faint pinkish hue in water, straw yellow coloured in 5% KOH, having one or two oil droplets when mature, rarely pluriguttulate, inamyloid, strongly cyanophilic and with an ortochromatic reaction. *Basidia* 18–
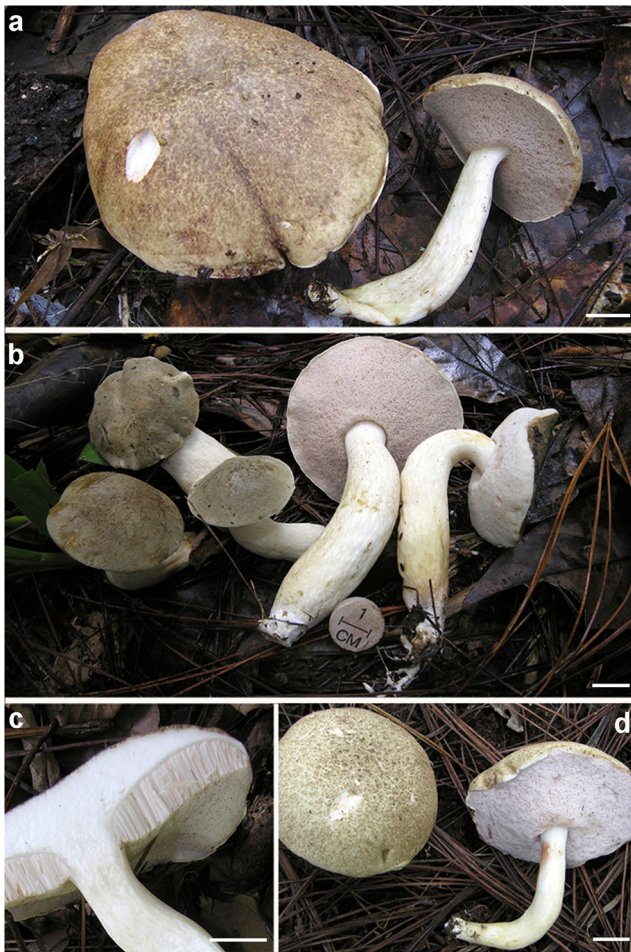
Fig. 3 *Tylopilus griseiolivaceus*. Basidiomes in habitat. **a** JBSD127432. **b** JBSD127431 (holotype). **c** JBSD127434. **d** JBSD127430. Bars = 1 cm. Photos by C. Angelini

31×7–$12\ \mu m$ ($n = 31$), cylindrical-clavate to more commonly clavate, moderately thin-walled (0.3–0.8 μm), predominantly 4-spored but many also 2-spored, usually bearing relatively short sterigmata (2–5 μm), pale yellowish and containing straw-yellow oil guttules in water and 5% KOH, bright yellow (inamyloid) in Melzer's, without basal clamps; basidioles subcylindrical to faintly clavate, similar in size to basidia. *Cheilocystidia* (19–) 23–55 × 5–11(–13) μm ($n = 39$), very common, moderately slender, projecting straight to sometimes flexuous, fusiform to ventricose-fusiform and usually showing a narrow and long neck with sharp, pointed tip, sometimes sublageniform, occasionally subcylindrical, clavate, mucronate, bottle-shaped or peanut-shaped, smooth, relatively thin-walled (0.3–0.8 μm), straw yellow but frequently with a bright golden yellow plasmatic pigment in water and 5% KOH, reddish to reddish-brown (strongly dextrinoid) in Melzer's, without epiparietal encrustations. *Pleurocystidia* (35–)39–67 × 8–13(–15) μm ($n = 21$), common to very common, shape, colour and chemical reactions similar to but slightly larger than cheilocystidia. *Pseudocystidia* not recorded. *Pileipellis* a trichodermium consisting of tufts of

subparallel to moderately interwoven, elongated, filamentous and sinuous, occasionally branched, erect hyphae not embedded in gelatinous matter; terminal elements (24–) 29–98 × 3–13 μm, long and slender, cylindrical, more rarely cystidioid, apex usually slightly attenuated and blunt or pointed, moderately thick-walled (up to 1.0 μm), with a bright golden yellow epiparietal pigment in water and 5% KOH, inamyloid to weakly dextrinoid in Melzer's, mostly heavily ornamented by a pronounced zebra-like epiparietal encrusting brownish pigment, not dissolving in KOH; subterminal elements similar in shape, size and colour to terminal elements. Hyphae of the underlaying layer more or less confusedly arranged and with encrusted walls, although less frequently than those of the suprapellis. *Stipitipellis* a texture of slender, parallel to subparallel and longitudinally running, smooth walled, adpressed hyphae, 2–20-μm wide, wall up to 1-μm thick, hyaline to very pale yellowish in water and 5% KOH; the stipe apex covered by a well-developed caulohymenial layer consisting of sterile clavate caulobasidioles, very rare, predominantly 3- or 2-spored, cylindrical-clavate to clavate fertile *caulobasidia*, 19–37 × 6–11 μm ($n = 3$), sterigmata 2–5-μm long and projecting, fusiform, ventricose-fusiform to sublageniform, rarely short mucronate *caulocystidia* similar in shape and colour to but shorter than hymenial cystidia, 23–37(–45) × 5–11 μm ($n = 13$), having a wall up to 0.8-μm thick. *Lateral stipe stratum* under the caulohymenium occasionally present but not clearly differentiated from the stipe trama, of the "boletoid type", at the stipe apex a 20–25-μm thick layer consisting of divergent, inclined and running towards the external surface, loosely intermingled and branched hyphae remaining separate and embedded in a gelatinous substance. In most specimens the lateral stipe stratum is absent. *Stipe trama* composed of densely arranged, subparallel to moderately interwoven, filamentous, smooth, inamyloid hyphae, 3–14-μm broad, wall up to 1-μm thick. *Hymenophoral trama* bilateral divergent of the "*Boletus*-type", with slightly divergent and loosely arranged, not-branched, restricted at septa, gelatinous hyphae (lateral strata hyphae in transversal section not touching each other, (2–)3–7(–9) μm apart, 2–12-μm broad), hyaline to very pale yellowish in water and 5% KOH, inamyloid in Melzer's; lateral strata (10–)15–20(–25) μm thick, mediostratum (5–)10–20(–25) μm thick, axially arranged, consisting of a tightly adpressed, non-gelatinous bundle of hyphae, 2–10-μm broad; in Congo Red the mediostratum is darker than the lateral strata. In mature specimens, the hymenophoral trama tends to become a homogenous layer 10–60-μm thick, consisting of subparallel to at most slightly divergent and densely arranged, non-gelatinous hyphae more or less touching each other, 0–2 μm apart; at this stage lateral strata and mediostratum are not differentiated. *Oleipherous hyphae* unfrequent, golden yellow in 5% KOH. *Clamp connections* absent in all tissues. *Hyphal system* monomitic. *Ontogenetic development* gymnocarpic.

Fig. 4 *Tylopilus griseiolivaceus*. Micromorphological features (JBSD127431, holotype). **a** Basidiospores. **b** Basidia. **c** Cheilo- and pleurocystidia. **d** Elements of the pileipellis. Bars a–c = 10 μm; d = 20 μm. Drawings by F. Costanzo
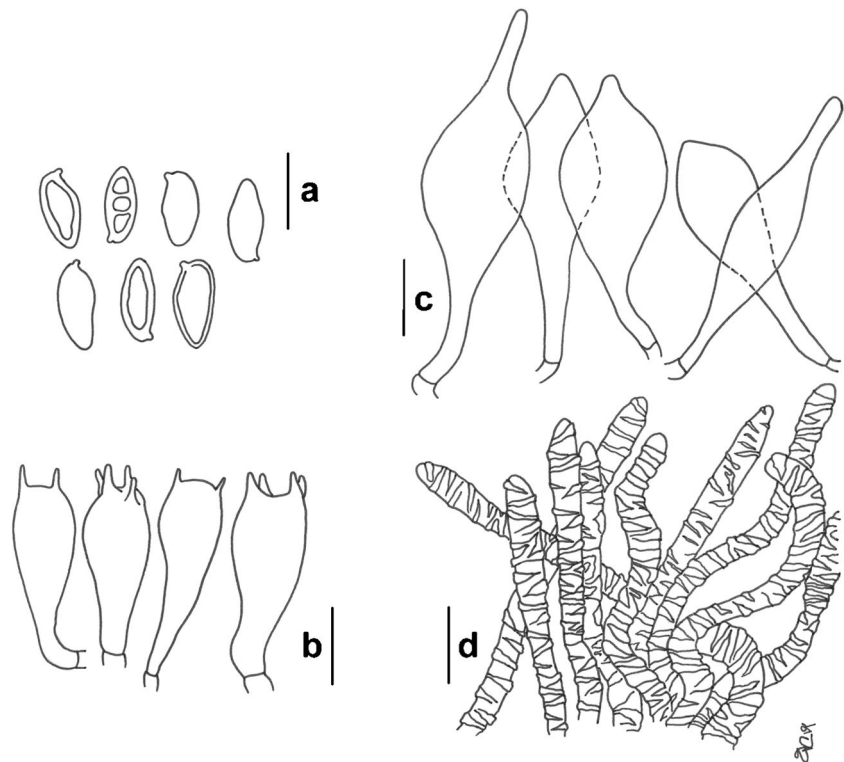
Edibility: to be considered inedible because of the bitter taste.

Ecology: gregarious, growing on soil among litter under *Pinus occidentalis* and *P. taeda*, decidedly common at least in the Dominican Republic.

Known distribution: southeastern USA (North Carolina) and in the Greater Antilles Islands of the Caribbean from the Dominican Republic, in warm temperate to tropical areas, most likely widely distributed throughout southeastern North America and neotropical regions of Central America in cohabitation with pine trees but distribution limits yet to be established.

Examined material: DOMINICAN REPUBLIC, La Vega Province, Jarabacoa, 19° 11′ 12.5″ N 70° 35′ 25.5″ E, 660 m, 22 Dec 2013, several specimens in all developmental stages, C. Angelini (JBSD127431, holotype; ANGE101 and MG744, isotypes); same loc., two middle-aged to mature specimens, 07 Dec 2014, C. Angelini (JBSD127432, ANGE401 and MG745); same loc., two mature specimens, 28 Nov 2017, C. Angelini (JBSD127430, ANGE1006 and MG746); same loc., two young to mature specimens, 23 Nov 2017, C. Angelini (JBSD127433, ANGE1019 and MG747); same loc., two young to middle-aged specimens, 18 Nov 2018, C. Angelini (JBSD127434, ANGE1069 and MG748).

Comments: A unique combination of the following morphological features allows a prompt separation of *T. griseiolivaceus* from its closely allied relatives: fibrillose to flattened subsquamulose, soon areolate, mouse grey to olive greyish or brownish grey pileus surface, whitish projecting sterile margin, flexuous, evenly white coloured stipe that is devoid of any kind of ornamentation and often creeping horizontally underneath the humus, unchangeable tissues on bruising, strongly cyanophilic smooth basidiospores, conspicuously zebra-pattern encrusted pileipellis hyphae and association with pine trees, at least with Hispaniolan pine (*Pinus occidentalis*) and loblolly pine (*P. taeda*). It appears to be quite abundant in mountain five-needled pine woodlands in the Dominican Republic. However, the presence of environmental sequences deposited in GenBank from a pure stand of loblolly pine in North Carolina (Edwards et al. 2004) matching with those obtained in the present study (Fig. 1) indicates that the geographic range of *T. griseiolivaceus* is much wider than it would seem, nevertheless its distribution limits currently remain unknown. Moreover, although not unquestionably associated with pine trees only, it is likely that this species forms obligate ECM with members of both *Pinus* subgen. *Strobus* and subgen. *Pinus*.

Aside from molecular phylogenetic outcomes indicating a clear separation of *T. griseiolivaceus* lineage from those of closely related *Tylopilus* species sequenced to date, a careful examination of the available literature concerning American (Singer 1947; Murrill 1948; Snell and Dick 1970; Smith and Thiers 1971; Singer et al. 1983, 1991; Both 1993; Henkel 1999; Bessette et al. 2000, 2016) and extralimital (Heinemann 1951; Corner 1972; Imazeki et al. 1988;

Watling and Li 1999; Muñoz 2005; Horak 2011; Wu et al. 2016) pinkish-spored and bitter-tasting boletes did not produce any convincing evidence about the identity of *T. griseiolivaceus* with previously described taxa.

As far as the Central American *Tylopilus* species are concerned, *T. hondurensis* Singer & M.H. Ivory seems to be morphologically closest to *T. griseiolivaceus* but is readily separated by the pale hazel brown pileus, yellowish hymenophore, finely reticulate stipe with reddish tones at the base, pungent odour, longer basidiospores [12–13.5 (–14.3) × 3.8–5 (–5.2) µm], smaller cystidia, differently shaped, short cylindrical to fusoid or subclavate to clavate, smooth pileipellis terminal elements and the occurrence apparently restricted to Honduras (Singer et al. 1983, 1991). Another superficially similar species is *T. mitissimus* Singer & L.D. Gómez, described by Singer et al. (1991) from Costa Rica. This species is characterized by a chocolate brown to violet brown pileus, stipe becoming cream greyish with age, context at the stipe base grey to blackish, mild taste, smaller, inamyloid cheilocystidia (21–29 × 3.5–5.5 µm), smooth and narrower pileipellis terminal elements (3.5–6.5-µm wide) and growth in association with oaks (Singer et al. 1991).

The eastern North American bitter-tasting *T. rhoadsiae* (Murrill) Murrill, *T. peralbidus* (Snell & Beardslee) Murrill and *T. intermedius* A.H. Smith & Thiers morphologically approaches *T. griseiolivaceus*, however, they are all distinguished by the initially whitish pileus and stipe surfaces soon developing pale ochraceous pink, pinkish buff or greyish buff to dingy brown tints. The larger basidiome size (pileus up to 15 cm in diam.), the wrinkled pileus, the presence of a faint reticulum on the upper part of the stipe, the tissues slowly staining brown when handled or injured, longer basidiospores (10–15 × 3–5 µm) and a cutis of very thin (2–4.5-µm broad) and smooth pileipellis end elements also separate *T. intermedius* from *T. griseiolivaceus*; *T. rhoadsiae* has a velvety to glabrous pileus surface, a prominent and coarse reticulum on the stipe, slightly longer and narrower basidiospores [(10–)11–13.5 × 3.5–4.5 µm)], smooth and slightly narrower pileipellis hyphae (up to 9-µm broad), narrower hymenial cystidia (6–8.5-µm broad) and larger caulocystidia (26–62 × 9–17 µm), whereas *T. peralbidus* is further separated by the darkening tissues when bruised, a bleach-like odour, suballantoid, narrower basidiospores [(9–)10–12 × 2.3–3.5 µm] and smooth and narrower (2.6–6.5-µm broad) pileipellis terminal elements (Snell 1936; Murrill 1938, 1948; Singer 1947; Snell and Dick 1970; Smith and Thiers 1971; Weber and Smith 1985; Wolfe Jr 1983, 1986; Bessette et al. 2000, 2016); *T. rhodoconius* (Singer) T.J. Baroni, Both & Bessette was originally described as a variety of *T. peralbidus* and exhibits a pale ochraceous, yellow-brown to brown or reddish brown pileus and stipe since the beginning, cream-white context with hyaline marbling turning pinkish on exposure, surfaces quickly staining dark brown

when handled, odour somewhat chlorine, narrower basidiospores [(8.4–) 9.7–13.5(–17.3) × (2–)3–3.5 µm], smaller cheilocystidia (26–32.5 × 5.2–6.5 µm), smooth and somewhat thinner pileipellis end elements (3.9–7.8-µm broad) (Singer 1947; Wolfe Jr 1990; Both 1993; Baroni et al. 1998; Bessette et al. 2000, 2016).

Among the several bolete species described by Murrill from southeastern USA, *T. praeanisatus* Murrill and *T. isabellescens* Murrill are somewhat recalling *T. griseiolivaceus* but appear to be dissimilar on account of the ochraceous to dark brown pileus, tissues bruising brownish on injury, suballantoid, narrower basidiospores (2.5–3.5-µm wide for both species) and pileipellis hyphae slightly narrower (up to 9-µm wide) and devoid of epiparietal encrustations. *Tylopilus isabellescens* also differs in the broader caulocystidia (9–17-µm wide) while *T. praenisatus* also exhibits a strong anise-like odour and lanceolate to fusoid, narrower cheilocystidia (4–6-µm broad) (Murrill 1943, 1946, 1948; Wolfe Jr 1983; Both 1993). The taxonomic status of both species has been questioned over time since *T. isabellescens* was later re-evaluated by the same Murrill (1948) as a synonym of *T. praeanisatus*, although Wolfe Jr (1983) maintained them separated, and the latter species was in turn reduced by Singer (1947) to a simple form of *T. peralbidus*.

Hongo (1985) described *T. argillaceus* Hongo from Japan although this species has been shown to have a wider distribution across tropical regions of East Asia (Wu et al. 2016); morphological features such as tomentose to glabrous, pinkish brown to dark reddish brown pileus and stipe surfaces, reddish oxidation reaction of tissues on injury, somewhat smaller basidiospores [7.5–10 × 3.5–4.5(–5) µm], clavate to subfusiform, smooth and narrow pileipellis terminal elements (4–6.5-µm broad) and occurrence under fagaceous trees are in disagreement with those of *T. griseiolivaceus*, moreover the two species have a different geographic distribution and are inferred as phylogenetically distinct.

The generic type *T. felleus* is phylogenetically related although autonomous from *T. griseiolivaceus* and differs significantly from this latter in the large sized basidiomes (pileus up to 20–30 cm in diam.), non-squamulose nor areolate pileus surface, snuff brown, ochraceous brown to brownish yellow or olive-brown coloured pileus and stipe, ventricose-clavate stipe (up to 15 × 9 cm) that is often nodulose at the base and coarsely reticulate lengthwise or at least over the upper three fourth, longer basidiospores [(10–)12–16.5(–18) × (3.5–)4–5(–6) µm], larger caulocystidia (30–60 × 10–17) µm), narrower pileipellis hyphae (3–9-µm broad) without epiparietal encrustations and occurrence with both Fagaceae and Pinaceae, not infrequently on decaying stumps (Singer 1967; Pilát and Dermek 1974; Alessio 1985; Breitenbach and Kränzlin 1991; Lannoy and Estadès 2001; Muñoz 2005; Watling and Hills 2005; Klofac 2007; Knudsen and Taylor

2012; Bessette et al. 2016; Wu et al. 2016; Noordeloos et al. 2018; pers. obs.). Interestingly, *T. felleus* is one of the few boletes with a molecularly confirmed Holarctic distribution being present in the temperate belt of North America, Europe and East Asia (Wu et al. 2016).

Tylopilus leucomycelinus (Singer & M.H. Ivory) R. Flores & Simonini, Rivista di Micologia 43 (2): 132. 2000. Figs. 5 and 6

MYCOBANK MB 467315

≡ *Pulveroboletus leucomycelinus* Singer & M.H. Ivory in Singer, Araujo & Ivory, Beihefte Nova Hedwigia 77: 93. 1983.

Epitype (here selected): DOMINICAN REPUBLIC, La Vega Province, Jarabacoa, 19° 11′ 12.5″ N 70° 35′ 25.5″ E, 660 m, half dozen young to mature specimens, 22 Dec 2013, C. Angelini (JBSD127419), MYCOBANK MBT387722.

Basidiomes medium-small. *Pileus* (3.6–)4.3–7.2(–11.0) cm broad, at first hemispherical then persistenly convex and finally broadly pulvinate-flattened, sometimes slightly depressed at centre when mature, regularly to unevenly shaped, moderately fleshy, firm at the beginning but progressively softer with age, flabby in old basidiomes; margin steady to faintly wavy-lobed, initially curved downwards and finally completely plane to uplifted or even slightly revolute, not or only a little extending beyond the tubes; surface matt, dry but a little greasy with moist weather, very finely subtomentose in the early stage of development but soon smooth and glabrous, not cracked; cuticle somewhat variable in colour, ranging from bright orange, reddish orange to brick red throughout (Scarlet, Brazil Red, Plate I; Grenadine Red, English Red, Plate II; Carmelian Red, Plate XIV; Japan Rose, Terra Cotta, Plate XXVIII) but sometimes with bleached yellowish orange (Light Orange Yellow, Deep Chrome, Plate II) spots or areas especially towards the margin and particularly in young specimens, gradually fading with age and becoming ochraceous red to pale brownish red (Vinaceous-Rufous, Ferruginous, Cinnamon-Rufous, Plate XIV); unchangeable to slowly and almost imperceptibly reddening (Dark Vinaceous, Etruscan Red, Plate XXVII) on handling or when injured; subcuticular layer yellowish orange (Deep Chrome, Plate II). *Tubes* at first thin then increasingly broader and shorter than the thickness of the pileus context (up to 1.0-cm long), deeply depressed around the stipe apex and occasionally decurrent with a tooth, whitish (White, Plate LIII) at first then with pale pinkish flesh to pinkish cream shades (Light Buff, Plate XV; Pale Pinkish Cinnamon, Plate XXIX; Tilleul Buff, Plate XL) at maturity, slowly turning pale dirty brown (Sayal Brown, Plate XXIX) when cut. *Pores* initially forming a shallowly concave to flat surface, later convex, at first small then gradually wider (up to 1.5 mm in diam.), simple, roundish to barely angular at maturity, concolourous with the tubes and slowly becoming pale dirty brown to rusty brown (Sayal Brown, Plate XXIX; Drab,
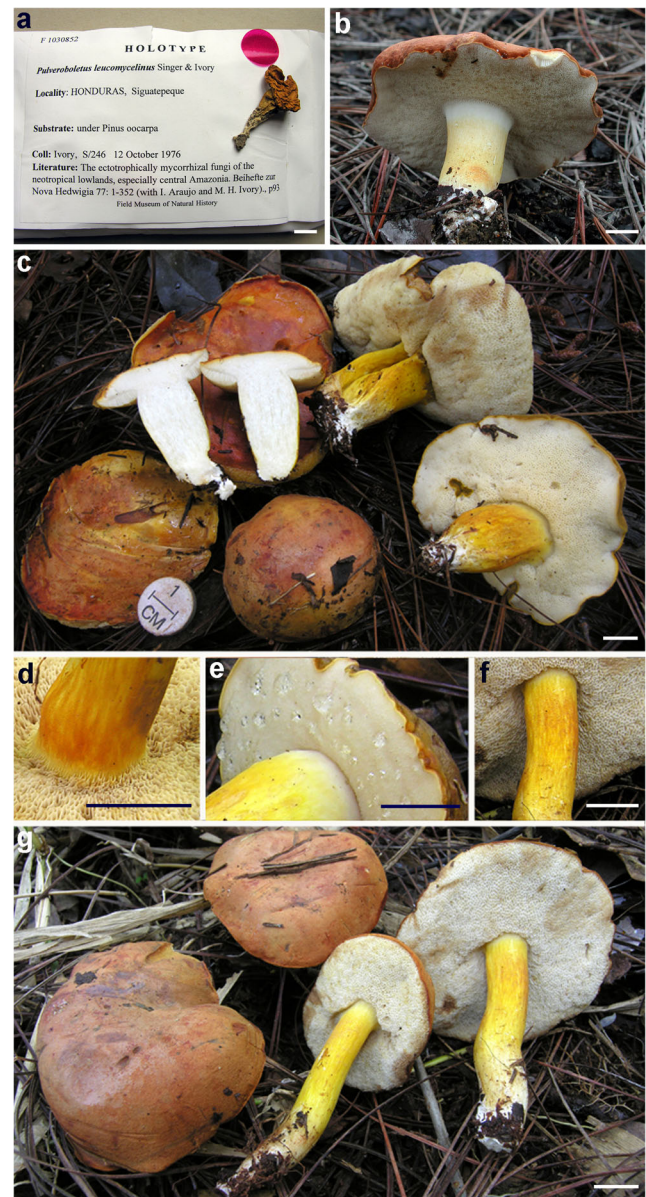

Fig. 5 *Tylopilus leucomycelinus*. Basidiomes. **a** F-1030852 (holotype). **b** BOS-481, BZ 2409 (BRH, CFMR). **c, e** JBSD127419 (epitype). **d, f–g** JBSD127421. Bars = 1 cm. Photos: **a** A. Vizzini, **b** B. Ortiz-Santana, **c–g** C. Angelini

Plate XLVI) on bruising or when injured. The hymenophore of young and fresh specimens exude colourless droplets (Fig. 5e). *Stipe* (3.2–)4.0–6.7(–9.0) × (0.8–)1.0–1.8(–2.3) cm, usually as long as the pileus diameter at maturity, at times slightly shorter or longer, central to slightly off-centre, solid, firm, dry, straight or curved, cylindrical but tapering towards the base to sometimes pinched, ending with a short taproot at the very base; surface smooth to very finely pruinose-fibrillose, rarely showing inconspicuous loosely anastomosing ribs originating at the apex and running down the stipe to the lower third, always smooth in the basal portion, evelate; evenly bright yellow to mustard yellow (Light Orange Yellow,

Fig. 6 *Tylopilus leucomycelinus.* Micromorphological features (JBSD127419, epitype). **a** Basidiospores. **b** Basidia. **c** Cheilo- and pleurocystidia. **d** Elements of the pileipellis. Bars **a**–**b** 10 μm; **c**–**d** 20 μm. Drawings by M. Gelardi
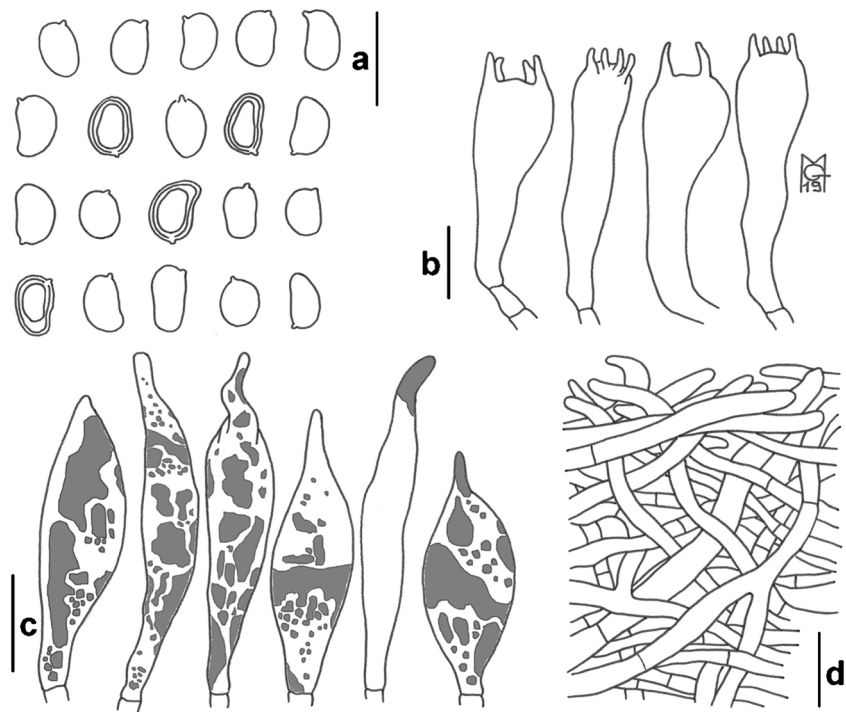
Deep Chrome, Plate II; Primuline Yellow, Plate XVI) but whitish yellow (Martius Yellow, Pale Lemon Yellow, Plate IV) at the very apex and in the lower fourth, white (White, Plate LIII) at the base, unchangeable to slowly turning vinaceous red or purple (Dark Vinaceous, Etruscan Red, Plate XXVII) when pressed; basal mycelium pure white (White, Plate LIII). *Context* firm and tough when young, later soft textured and eventually flabby in the pileus (up to 1.5-cm thick in the central zone), a little more fibrous in the stipe, white throughout (White, Plate LIII), unchangeable when exposed to air; subhymenophoral layer white (White, Plate LIII); exsiccate dull orange (Xanthine Orange, Plate III) on the pileus, ochraceous (Raw Sienna, Plate III) elsewhere. *Odour* indistict. *Taste* mild. *Spore print* not obtained. *Macrochemical spot-test reactions* not tested.

Basidiospores [105/6/3] (5.1–)6.6 ± 0.47(–8.2) × (3.9–)4.5 ± 0.28(–5.5) μm, $Q = (1.18–)1.23–1.76 (–1.78)$, $Qm = 1.45 ± 0.11$, $V = 71 ± 12$ μm^3, small, inequilateral, allantoid or phaseoliform to ovoid or more rarely subglobose in side view, ovoid to subglobose in face view, sometimes slightly attenuate in the lower half, smooth, apex rounded, with a short apiculus and without a suprahilar depression, thin-walled (0.3–0.5 μm), pale pinkish-yellow coloured in water and pale straw yellow in 5% KOH, having one oil droplet when mature, inamyloid, acyanophilic and with an ortochromatic reaction. *Basidia* 23–38 × 7–11(–13) μm ($n = 30$), cylindrical-clavate to clavate, moderately thick-walled (0.5–1.0 μm), predominantly 4-spored but also 1-, 2- or 3-spored, usually bearing relatively short sterigmata (2–6 μm), hyaline to pale yellowish and containing pale straw-yellow oil guttules in water and 5%

KOH, bright yellow (inamyloid) in Melzer's, without basal clamps; basidioles subcylindrical to faintly clavate, similar in size to basidia. *Cheilocystidia* (38–)42–75(–83) × 7–16 (–19) μm ($n = 29$), very common, moderately slender, projecting straight to sometimes flexuous, fusiform to ventricose-fusiform or lageniform to sublageniform, occasionally bottle-shaped or mucronate, sometimes showing a narrow and long neck, with sharp, pointed tip, smooth, moderately thick-walled (0.3–1.0 μm), hyaline to pale yellowish but very often with a refractive, bright golden yellow oily-foamy vacuolar pigment in water and 5% KOH, bright yellow (inamyloid) in Melzer's, without epiparietal encrustations. *Pleurocystidia* (45–)47–62 (–71) × 8–17 μm ($n = 15$), unfrequent, shape, size, colour and chemical reactions similar to cheilocystidia. *Pseudocystidia* not recorded. *Pileipellis* a cutis consisting of strongly interwoven, elongated, filamentous and sinuous, frequently branched, repent to occasionally erect hyphae not embedded in gelatinous matter; terminal elements 24–115 × 3–8 μm, long and slender, cylindrical, apex usually slightly enlarged and obtuse, moderately thick-walled (up to 0.9 μm), with a refractive, bright golden yellow oily-foamy vacuolar pigment in water and 5% KOH, weakly dextrinoid in Melzer's, smooth; subterminal elements similar in shape, size and colour to terminal elements. *Stipitipellis* a texture of slender, parallel to subparallel and longitudinally running, smooth walled, adpressed hyphae, 2–10-μm wide, hyaline to very pale yellowish in water and 5% KOH; the stipe apex covered by a well-developed caulohymenial layer consisting of sterile clavate caulobasidioles, uncommon, predominantly 3- or 2-spored, cylindrical-clavate to clavate

fertile *caulobasidia*, 31–38 (–49) × 9–10 μm (*n* = 4), sterigmata 3–5-μm long and projecting, fusiform, ventricose-fusiform to subclavate, rarely short mucronate *caulocystidia* similar in shape, size and colour to hymenial cystidia, 40–75(–88) × 7–18(–20) μm (*n* = 14), having a wall up to 0.9-μm thick. *Lateral stipe stratum* under the caulohymenium absent. *Stipe trama* composed of densely arranged, subparallel to moderately interwoven, filamentous, smooth, inamyloid hyphae, 2–14-μm broad. *Hymenophoral trama* bilateral divergent of the "*Boletus*-type", with slightly to strongly divergent, recurved-arcuate and loosely arranged, not-branched, restricted at septa, gelatinous hyphae (lateral strata hyphae in transversal section not touching each other, (3–)4–7(–8) μm apart, 2–13-μm broad), hyaline to very pale yellowish in water and 5% KOH, inamyloid in Melzer's; lateral strata (15–)20–50(–60) μm thick, mediostratum (5–)10–50(–60) μm thick, axially arranged, consisting of a tightly adpressed, non-gelatinous bundle of hyphae, 2–10-μm broad; in Congo Red, the mediostratum is darker than the lateral strata. *Oleipherous hyphae* unfrequent, golden yellow in 5% KOH. *Clamp connections* absent in all tissues. *Hyphal system* monomitic. *Ontogenetic development* gymnocarpic.

Edibility: unknown.

Ecology: solitary to gregarious, growing on soil among litter under *Pinus* species (*P. oocarpa*, *P. caribaea* and *P. occidentalis*), possibly also with *Quercus*.

Known distribution: Reported from Belize, Guatemala and Honduras in mainland Central America and in the Greater Antilles Islands of the Caribbean from the Dominican Republic, apparently widespread throughout mainland and insular neotropical regions at relatively high altitudes and likely extending as far north as southern USA but northern and southern distribution limits yet to be established. Present records from the Dominican Republic and Belize expand the distribution of *T. leucomycelinus*. A sequence (KY859806) from a Mexican collection (Jalisco, Talpa de Allende, Valle del Maple leg. A. Rockefeller, Mushroom Observer 247,674) named *Tylopilus* aff. *balloui*" resulted conspecific with *T. leucomycelinus* (Fig. 1), suggesting its presence also in Mexico.

Examined material: BELIZE, Cayo District, Mountain Pine Ridge Forest Reserve, Five Sisters Lodge, near Lodge, under *P. caribaea*, 17° 2′ 16″ N, 88° 59′ 7.8″ W, 387 m, 30 Nov 2002, C.L. Ovrebo, **BOS-481**, BZ 2409 (BRH, CFMR) (as *T. balloui*). DOMINICAN REPUBLIC, La Vega Province, Jarabacoa, under *P. occidentalis*, 19° 11′ 12.5″ N 70° 35′ 25.5″ E, 660 m, half dozen young to mature specimens, 22 Dec 2013, C. Angelini (JBSD127419, epitype; ANGE110 and MG723, isoepitypes); same loc., three mature specimens, 07 Dec 2014, C. Angelini (JBSD127420, ANGE396 and MG724); same loc., four middle-aged to mature specimens, 28 Nov 2017, C. Angelini (JBSD127421, ANGE1005 and MG725); La Vega Province. Jarabacoa, Jumanuco, under

P. occidentalis, 19° 6′ 40″ N, 70° 42′ W, 11 Nov 2003, D.J. Lodge, **DJL-DR-15**, DR 2800 (JBSD, CFMR) (as *T. balloui*). HONDURAS, Comayagua Dept., Siguatepeque, under *P. oocarpa*, 12 Oct. 1976, M.H. Ivory S/246 (F-1030852, **holotype**).

Comments: *Tylopilus leucomycelinus* is a brightly coloured bolete that is recognizable based on the following key identifying features: medium-small-sized fruiting bodies, reddish orange, brick red, chocolate brown to pale brownish red pileus surface, brown oxydation reaction on the hymenophore when bruised, smooth or finely pruinose-fibrillose, bright yellow to mustard yellow stipe (often with scattered orange shades) that is whitish at the base and with a pure white basal mycelium, white, unchangeable or slightly browning context, mild to bitterish taste, cream ochraceous spore print, short bean-shaped (allantoid) to ovoid, smooth basidiospores, hymenial cystidia and pileipellis hyphae containing a refractive, bright golden yellow oily-foamy vacuolar pigment and cutis pileipellis consisting of smooth filamentous hyphae. As for the previously described species, *T. leucomycelinus* appears to be intimately connected with members of both *Pinus* subgen. *Strobus* and subgen. *Pinus*, although a possible association with oaks cannot be completely excluded (Singer et al. 1983; Flores Arzù and Simonini 2000; Ortiz-Santana et al. 2007 as "*T. balloui*"; present study). The hymenophore of young and fresh specimens exuding a colourless sap (Fig. 5e) seems to have been overlooked by other authors; however, such a feature appears to be shared with some other members of the *T. balloui* complex (Watling et al. 2007).

The identity of the Dominican samples has been primarily confirmed based on molecular investigation by comparing the ITS sequences obtained with that of the original material yielded by Singer in Honduras. Moreover, *T. leucomycelinus* from the Dominican Republic appears to be consistent with the description provided by Flores Arzù and Simonini (2000) from Guatemala and with that of Singer's type material from Honduras (Singer et al. 1983), the only remarkable differences being the non-staining hymenophore and larger cystidia with respect to Singer's protologue (pleurocystidia 28–40 × 8–8.8 μm, cheilocystidia 36–43 × 4.7–7.3 μm) and the absence of dextrinoid pseudocystidia (gloeocystidia sensu Singer 1986) which are reported to occur in the Guatemalan collection (30–80 × 9–13 μm). Unfortunately, as already pointed out by Flores Arzù and Simonini (2000) and according to our own observations, the holotype material of *T. leucomycelinus* consists of a single, immature half basidiome (its characteristics cannot be evaluated with any degree of precision) that is also preserved in very poor conditions, so as to prevent an adequate re-examination of the micromorphological features. Consequently, a recent collection from the Dominican Republic (JBSD127419) genetically and morphologically fitting the protologue and the holotype has been selected here as epitype (see above). *Tylopilus balloui* (Peck) Singer

described from Belize and the Dominican Republic by Ortiz-Santana et al. (2007) is contaxic with *T. leucomycelinus* from the molecular perspective (Figs. 1 and 2). Moreover, morphological features are almost completely matching those of the latter species, even though the context is said to be discolouring brown and cystidia are remarkably small (pleurocystida 24–38.4 × 6.4–8 μm, cheilocystidia 28–47.2 × 10–11.2 μm) (Ortiz-Santana et al. 2007). However, re-examination of the "*T. balloui*"collection BOS-481 (BRH) from Belize cited in Ortiz-Santana et al. (2007) revealed hymenial cystidia measuring 36–72 × 6.3–9 μm, measures that are congruent with other *T. leucomycelinus* specimens cited above.

The nearest relatives of *T. leucomycelinus* are obviously found in the *T. balloui* complex. The North American *T. balloui* is a lookalike of *T. leucomycelinus* and the two species are indeed hardly separable solely relying on morphological and ecological features, the most reliable diagnostic differences being the somewhat longer basidiospores in *T. balloui* [(5–)6.5–10.5(–11) × (3–)3.5–4.8 (–5) μm] and the spheropedunculate to broadly spheropedunculate, wider cheilocystidia (58.5–76.5 × 19.5–39(–60) μm) (Peck 1912; Murrill 1910, 1914 as "*Ceriomyces subsanguineus* (Peck) Murrill"; Coker and Beers 1943 as "*Boletus subsanguineus* Peck"; Singer 1947; Dick 1960; Snell and Dick 1970; Wolfe Jr 1981; Weber and Smith 1985; Singer et al. 1991; Both 1993; Bessette et al. 2000, 2016; Osmundson and Halling 2010]. *Tylopilus balloui* has long been considered a single polymorphic taxon with a very broad disjunct geographic distribution in the western hemisphere, Africa, East and SE Asia and Australasia (Corner 1972, 1974; Heinemann and Rammeloo 1983b; Imazeki and Hongo 1989; Watling and Li 1999; Li and Song 2000; Halling and Mueller 2005; Halling et al. 2007; Watling et al. 2007; Horak 2011; Henkel et al. 2012) but our analyses (Figs. 1 and 2) as well as a number of other recently published molecularly-based works have incontestably highlighted, as formerly predicted by Watling and Gregory (1988), Watling (2001a, b) and Halling et al. (2007), that it rather is a collective morphospecies lumping several independent cryptic species-level lineages in need of urgent taxonomic re-assessment (Halling et al. 2008; Thongklam 2008; Osmundson 2009; Osmundson and Halling 2010; Wu et al. 2016; Magnago et al. 2017; Chakraborty et al. 2018). According to the current state of knowledge, the true *T. balloui* should be restricted to eastern North America and Mexico; however, it is presently unknown whether or not the distribution range of *T. balloui* and *T. leucomycelinus* could be partly overlapping. Records of *T. balloui* from Central America (Lodge et al. 2004) are most likely to be referred to *T. leucomycelinus*.

The generic recognition of *T. balloui* and related species as an extension of either *Gyrodon* Opat., *Rubinoboletus* Pilát & Dermek and *Gyroporus* Quél. as proposed by Snell (1941),

Heinemann and Rammeloo (1983a) and Horak (2011), respectively, based primarily on spore colour and shape must in all cases be considered incorrect, since this species-complex nests within a core *Tylopilus* clade (Binder and Hibbett 2006; Halling et al. 2008; Osmundson 2009; Nuhn et al. 2013; Wu et al. 2014, 2016; Gelardi et al. 2015; Magnago et al. 2017; Chakraborty et al. 2018; this study).

Tylopilus leucomycelinus would key out with *T. oradivensis* Osmundson & Halling which is delimited by smaller size (pileus 3–6-cm broad), generally more brightly reddish orange to tomato red or scarlet red colours of pileus and stipe, subfusiform to fusiform, decidedly longer and slightly narrower basidiospores [(7.6–)8.2–12(–13.6) × (2.6–)3–4(–4.4) μm], larger pleurocystidia [40–92 (−110) × 8–20(−38) μm] and association with *Quercus* in montane forests of Costa Rica (Halling and Mueller 2005 as "*T. balloui*"; Osmundson and Halling 2010). A newly sequenced *Tylopilus* collection from Belize [BOS-612, BZ 3165 (BRH, CFMR), under *P. caribaea* and *Quercus* spp. leg. R.E. Halling] (Table 1) turned out to be conspecific with *T. oradivensis* (Fig. 2), thus allowing an extension of the known geographic range of the species.

Magnago et al. (2017) recently described *T. dunensis* A.C. Magnago & M.A. Neves from the Atlantic forests of northeastern Brazil which bears a close resemblance to *T. leucomycelinus*. Outside of the different geographic range, the Brazilian species displays a whitish to pale cream-yellow stipe surface, cream coloured context, longer and narrower basidiospores (6–9 × 3–4 μm, $Qm = 1.91$), an ixotrichoderm pileipellis and the occurrence in restinga coastal vegetation (Magnago et al. 2017).

The African taxon *Rubinoboletus balloui* var. *luteoporus* Heinem. & Rammeloo appears to be characterized by yellow pores and has been reported under native *Brachystegia* (Caesalpinoideae) from miombo woodland in the Democratic Republic of Congo and Burundi (Heinemann and Rammeloo 1983a).

Among the species attributable to the *T. balloui* complex from the eastern hemisphere, *T. pseudoballoui* appears to be the most recently described (Chakraborty et al. 2018). When compared to *T. leucomycelinus*, it shows a sticky, orange-yellow to brownish yellow pileus surface, slightly longer basidiospores (6.4–9.9 × 3.8–5.7 μm, $Qm = 1.73$), an ixocutis pileipellis and the occurrence under *Quercus* spp. in Indian Himalaya and Japan as well (Chakraborty et al. 2018).

Rubinoboletus monstrosus Har. Takahashi seems to be an additional representative of the *T. balloui* consortium from southern Japan and is characterized by the greyish orange or brownish orange to yellowish brown pileus, greyish orange to yellowish brown, unchanging hymenophore, very short and stout, yellowish brown,

hollow stipe (2.0–4.0 × 1.5–4.5 cm), disagreeable odour recalling that of rotting meat, slightly smaller basidiospores (5–6 × 3.5–4 μm) and the occurrence in evergreen broadleaved forests dominated by *Quercus* and *Castanopsis* (Takahashi 2007; Terashima et al. 2016).

The *T. balloui* complex in the Indomalayan region appears to harbour a significant hidden diversity with several different, taxonomically critical and novel, yet unnamed species (Massee 1909; Patouillard and Baker 1918; Corner 1972, 1974; Watling 2001b; Horak 2011). *Boletus balloui* var. *fuscatus* Corner is a Malaysian entity raised to the specific rank by Horak (2011), differing from *T. leucomycelinus* by the fawn ochraceous to dark brown pileus and stipe, hymenophore and context staining vinaceous to dull purple brown when injured, apparently sterile stipe surface, larger pleurocystidia (70–100 × 16–25 μm) and occurrence with Dipterocarpaceae (Corner 1972; Horak 2011). Outside of peninsular and insular Malaysia (Corner 1974), *B. balloui* var. *fuscatus* has also been reported from Australia under *Eucalyptus* (Myrtaceae) in dry sclerophyll forests (Watling and Li 1999), but it likely represents a different taxon. *Boletochaete bicolor* Singer (= *Boletus bicolor* Massee nom. ill.), *Boletus flavipes* Massee nom. ill. and *Boletus parvulus* Massee nom. ill. are three additional Malaysian species probably orbiting around *T. balloui* or falling within the broad concept of this species as intended by Corner (1972) and Horak (2011). They are all characterized by small dimensions (pileus reaching at most 6 cm diam.). *Boletus parvulus* also has a minutely squamulose pileus surface and brilliant yellow to rust yellow pileus and stipe whereas *B. bicolor* displays radially wide, elongate-hexagonal pores (Massee 1909; Horak 2011). *Boletus viscidulus* Pat. & Baker is characterized by the smaller size (pileus 2.5–4.5-cm broad, stipe 4–5 × 0.5–1.5 cm), smooth and shining pileus surface, pale sordid leather coloured pileus and stipe and slightly longer basidiospores [(7.7–)8–9.5(–10) × 4–5(–5.3) μm] (Patouillard and Baker 1918; Singer 1947; Watling and Lee 1999; Horak 2011). *Tylopilus viscidulus* and *B. balloui* var. *fuscatus* were considered contaxic or putatively contaxic by Corner (1972) and Horak (2011), respectively (in disagreement with Watling and Lee 1999 and Watling and Li 1999, who were inclined to keep them separate), as well as *T. veluticeps* (Pat. & Baker) Singer. This latter taxon, according to the type revision carried out by Singer (1947), has basidiospores measuring 7.5–8.7 × 3.5–4.5 μm and short ventricose to globose pileipellis hyphae. Anyway, all these Malaysian species require much more investigation and a careful taxonomic re-evaluation.

Concerning Australian species, *Rubinoboletus balloui* var. *viscidus* T.H. Li & Watling is discriminated from *T. leucomycelinus* by the smooth, viscid pileus surface, longer basidiospores (7.5–11.0 × 4.0–4.8 μm), ixocutis pileipellis and the occurrence in eastern Australia (New South Wales) (Li and Watling 1999; Watling and Li 1999). Another Australian taxon classified into the *T. balloui* consortium is *Rubinoboletus balloui* f. *persicinus* Watling & T.H. Li nom. prov., which is delimited by the laccate and tacky pileus surface, light pinkish, salmon to peach coloured pileus and stipe, decurrent and unchanging hymenophore on cutting or bruising, ixocutis pileipellis and the occurrence in eastern Australia (Queensland) (Watling and Li 1999). *Rubinoboletus caespitosus* (Cleland) T.H. Li & Watling essentially differs from *T. leucomycelinus* by the larger basidiospores (8.0–8.9 × 5.0–6.0 μm), a caespitose growth and the occurrence in eastern Australia (New South Wales) under Myrtaceae (*Angophora* and *Eucalyptus*) (Cleland 1924, 1935; Watling and Gregory 1988; Grgurinovic 1997; Li and Watling 1999; Watling and Li 1999; Klofac and Krisai-Greilhuber 2006). Finally, *Rubinoboletus phaseolisporus* T.H. Li, R.N. Hilton & Watling possesses a slightly viscid, bright straw yellow then cinnamon coloured pileus, narrower basidiospores (5.5–6.5 × 3–3.5 μm), narrower basidia (5–7-μm broad), ixotrichoderm pileipellis and occurs in Western Australia under *Eucalyptus* (Watling and Li 1999; Klofac and Krisai-Greilhuber 2006).

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