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# New collection, iconography and molecular evidence for *Tylopilus neofelleus* (Boletaceae, Boletoidae) from southwestern China and the taxonomic status of *T. plumbeoviolaceoides* and *T. microsporus*

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## Highlights

- A full description of the rare *Tylopilus neofelleus* is provided.
  - The holotype collection of *T. neofelleus* is revised.
  - Molecular (ITS and LSU analyses) confirmation of *T. neofelleus* from China.
  - The holotype of *T. microsporus* (a Chinese taxon) is a posterior synonym of *T. neofelleus*.
  - *T. plumbeoviolaceoides* (holotype sequenced), another taxon recently described from China, is a distinct species.
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## Abstract

*Tylopilus neofelleus*, a bolete belonging to *Tylopilus* s. str. and originally reported from Japan, is extensively revisited and illustrated. Based on a recent fresh collection from southwestern China, an exhaustive macro- and micromorphological description is provided and supported by molecular analyses of the nuclear ribosomal internal transcribed spacer (ITS) and nuclear ribosomal large subunit (LSU) sequences. Color pictures of both fresh basidiomes in habitat and dried material along with photomicrographs and line drawings of the main anatomical features and a comprehensive phylogram are provided, supported by comparative notes on lookalike and closely related species. Original materials of the Chinese *T. microsporus* and *T. plumbeoviolaceoides* were successfully sequenced and a type revision of *T. microsporus* and *T. neofelleus* is presented. Based

on molecular inference the holotype of *T. microsporus* is conspecific with Japanese samples of *T. neofelleus* and must be considered a posterior synonym. Phylogenetic data also demonstrate evidence for the presence of *T. neofelleus* in China and for a separation from the allied Chinese taxon *T. plumbeoviolaceoides*. Furthermore, morphological differences separate *T. neofelleus* from all other unsequenced species of the same consortium.

## Keywords

- Boletales;
- Chinese ectomycorrhizal fungi;
- Molecular phylogeny;
- *Tylopilus griseipurpureus*;
- *Tylopilus plumbeoviolaceus* complex

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## 1. Introduction

The pinkish-flesh or reddish to vinaceous-brown or brownish-purple spored taxa belonging to the family Boletaceae appear to be quite well represented in eastern and south-eastern Asia ([Chiu, 1948](#), [Chiu, 1957](#), [Hongo, 1960](#), [Corner, 1972](#), [Zang, 1986](#), [Imazeki et al., 1988](#), [Nagasawa, 1997](#), [Li and Song, 2000](#), [Zhuang, 2001](#), [Mao, 2009](#), [Horak, 2011](#) and [Wu et al., 2011](#)). More than 40 species with a pinkish hymenophore and smooth spores have so far been reported from China ([Li and Yang 2011](#)), most of which are presently included in *Tylopilus* P. Karst. ([Fu et al. 2006](#)), a large ectomycorrhizal genus, typified by *T. felleus* (Bull.) P. Karst., that has proven definitely heterogeneous and polyphyletic ([Binder and Bresinsky, 2002](#), [Binder and Hibbett, 2006](#), [Drehmel et al., 2008](#), [Nuhn et al., 2013](#), [Li et al., 2014](#) and [Wu et al., 2014](#)).

A number of new genera have recently been split from *Tylopilus* s.l. based on both morphological and molecular evidence ([Li et al., 2011](#), [Li et al., 2014](#), [Halling et al., 2012a](#) and [Halling et al., 2012b](#)), yet many groups remain to be phylogenetically re-evaluated using modern molecular tools. One of these species-complex is that of *T. plumbeoviolaceus* (Snell & E.A. Dick) Singer, a challenging consortium encompassing several cosmopolitan but biogeographically disjunct boletoid taxa, unified by fleshy basidiomes, purple-violaceous to reddish-brown colored pileus and stipe, pinkish to violaceous hymenophore, not reticulate stipital surface or only finely so at the very apex, whitish basal mycelium, extremely bitter-tasting context, smooth spores and cystidia with dextrinoid content.

Based on such traits and in agreement with [Baroni and Both \(1998\)](#), this group was ascribed to sect. *Tylopilus* as broadly defined by [Singer \(1986\)](#), in stirps *Rubrobrunneus* ([Smith and Thiers 1971](#)). According to the most recent comprehensive multilocus analyses carried out by [Nuhn et al. \(2013\)](#) and [Wu et al. \(2014\)](#), species of the *T. plumbeoviolaceus* complex are closely related to the generic type *T. felleus* and should therefore be considered members of *Tylopilus* s. str. (= *Tylopilus* clade in [Nuhn et al. 2013](#)) in the subfamily Boletoidae ([Wu et al. 2014](#)).

Due to the unsavoury organoleptic properties, species belonging to this group are unanimously considered inedible and thus rejected from consumption, although *T. griseipurpureus* (Corner) E. Horak, a species associated with introduced exotic plants, appears to be routinely eaten and found for sale in free markets of northeastern Thailand ([Aungaudchariya et al. 2012](#); Arora, pers. comm.). However, American and Australasian species have till now been investigated mostly from the

morphological viewpoint and urgently require molecular adjustment ( [Fu et al., 2006](#) and [Sitta et al., 2007](#) ).

During a recent field expedition carried out in north-western Yunnan (China), the first author recorded several specimens of a *Tylopilus* species related to *T. plumbeoviolaceus* and allied taxa, which later revealed to be *T. neofelleus* Hongo. The aim of the present study is to bring new insights to the knowledge of this interesting species and to elucidate its relationships and taxonomic placement within *Tylopilus* s. str.

## 2. Materials and methods

### 2.1. Collection site and sampling

Material was collected by the first author from Jinshan, Lijiang County, Yunnan Province, China, on September 2012 in a pine-trees woodland. Specimens examined in this study were deposited in Fungal Herbaria of the Guangdong Institute of Microbiology (GDGM), the State Key Laboratory of Mycology, Beijing Institute of Microbiology (HMAS) and the National Museum of Nature and Science, Tsukuba (TNS) as well as in the private herbarium of MG, MZ and YT. Herbarium acronyms follow [Thiers \(2014\)](#) except “MG”, “MZ” and “YT” that refer to the personal herbaria of Matteo Gelardi, Ming Zhang and Yuichi Taneyama, respectively.

### 2.2. Morphological studies

Macroscopical descriptions, macrochemical reactions, habitat notations and associated plant communities were based upon detailed field notes from fresh basidiomes. Colors were recorded under daylight and described in general terms only. Macro-photograph in habitat was taken by a Nikon D3100 camera. Micromorphological features were observed from dried material; sections were either rehydrated in water, 5% potassium hydroxide (KOH) or in ammoniacal Congo Red. Observation of structures and measurements of anatomical features were performed by mounting preparations in ammoniacal Congo Red, 30% NH<sub>4</sub>OH and Phloxine B. Colors and amount of pigmentation was described after examination in water and 5% KOH. Measurements were made at 1000× with a calibrated ocular micrometer (Nikon Eclipse E200 optical light microscope). Spores were measured from the hymenophore of mature basidiomes, values are given as (minimum) average ± standard deviation (maximum),  $Q$  = average length/width ratio ± standard deviation with minimum and maximum values in parentheses, while average spore volume was approximately estimated as a rotation ellipsoid ( $V = 4/3 * (\text{length}/2) * ((\text{width}/2) * \text{width}) * \pi/2 \pm \text{standard deviation}$ ). The notation [n/m/p] indicates that measurements were made on “n” randomly selected spores from “m” basidiomes of “p” collections. Metachromatic, cyanophilic and iodine reactions were tested by staining the spores and the other microscopic elements in Brilliant Cresyl blue, Cotton blue and Melzer's reagent, respectively. Line-drawings of microstructures were made free hand from rehydrated material and based on photomicrographs.

### 2.3. DNA extraction, PCR amplification and sequencing

Total genomic DNA of 13 *Tylopilus* collections ( [Table 1](#) ) was extracted from dried specimens using the Sangon Fungus Genomic DNA Extraction kit (Sangon Biotech Co., Ltd., Shanghai, China) according to the manufacturer's instructions. The internal transcribed spacer (ITS/5.8S rRNA) was amplified with primers ITS1F and ITS4 ([White et al. 1990](#)). A portion of the nuclear ribosomal large subunit (LSU) was amplified with primers LROR and LR5 ([Vilgalys and Hester 1990](#)). PCR was performed in a total volume of 20 µl containing 10 µl PCR mix, 0.5 µl per primer

(10 µM), 0.5 µl DNA template. PCR reactions were performed with 4 min initial denaturation at 95 °C, followed by 34 cycles of denaturation at 94 °C for 40 s, annealing at 53 °C for 60 s, extension at 72 °C for 80 s and a final extension at 72 °C for 7 min. The amplified products were determined by electrophoresis on 1% agarose gels with known standard DNA marker and directly sequenced in Beijing Genomic Institute (BGI). The sequences are deposited in GenBank (<http://www.ncbi.nlm.nih.gov/Genbank/>) under the accession numbers given in [Table 1](#) and [Fig. 1](#) and [Fig. 2](#).

Table 1.

*Tylopilus* samples sequenced for the present study.

Species	GenBank accession no.		Source and country
	ITS	LSU	
<i>Tylopilus griseipurpureus</i>	KM975484	KM975493	MG521a, Nan'ao island, Guangdong, China
<i>Tylopilus microsporus</i>	KM975485	KM975494	HMAS84730, <b>holotype</b> , Yunnan, China
<i>Tylopilus neofelleus</i>	KM975486	–	MG475a dupl. in GDGM42626, Jinshan, Yunnan, China
<i>Tylopilus neofelleus</i>	KF000100	KF000101	MZ-2013, China
<i>Tylopilus neofelleus</i>	KM975487	KM975495	YT20120811, Karasawa, Suwa, Nagano, Japan
<i>Tylopilus neofelleus</i>	KM975488	KM975496	YT20121007, Aga-cho, Higashikanbaragun, Niigata, Japan
<i>Tylopilus neofelleus</i>	KM975489	KM975497	YT20090720, Ranjou-no-mori, Tonami, Toyama, Japan
<i>Tylopilus plumbeoviolaceoides</i>	KC984859	KC984858	GDGM20311, <b>holotype</b> , north suburbs, Guangzhou, Guangdong, China
<i>Tylopilus plumbeoviolaceoides</i>	–	KM975498	GDGM42624, <b>topotype</b> , north suburbs, Guangzhou, Guangdong, China
<i>Tylopilus plumbeoviolaceoides</i>	KF000102	–	GDGM42625, Mount Baiyun, Guangzhou, Guangdong, China
<i>Tylopilus plumbeoviolaceoides</i>	KM975490	–	GDGM43255, Mount Baiyun, Guangzhou, Guangdong, China
<i>Tylopilus plumbeoviolaceoides</i>	KM975491	–	GDGM32630, Mount Baiyun, Guangzhou, Guangdong, China
<i>Tylopilus</i> sp.	KM975492	KM975499	MG509a, Dinghushan, Guangdong, China

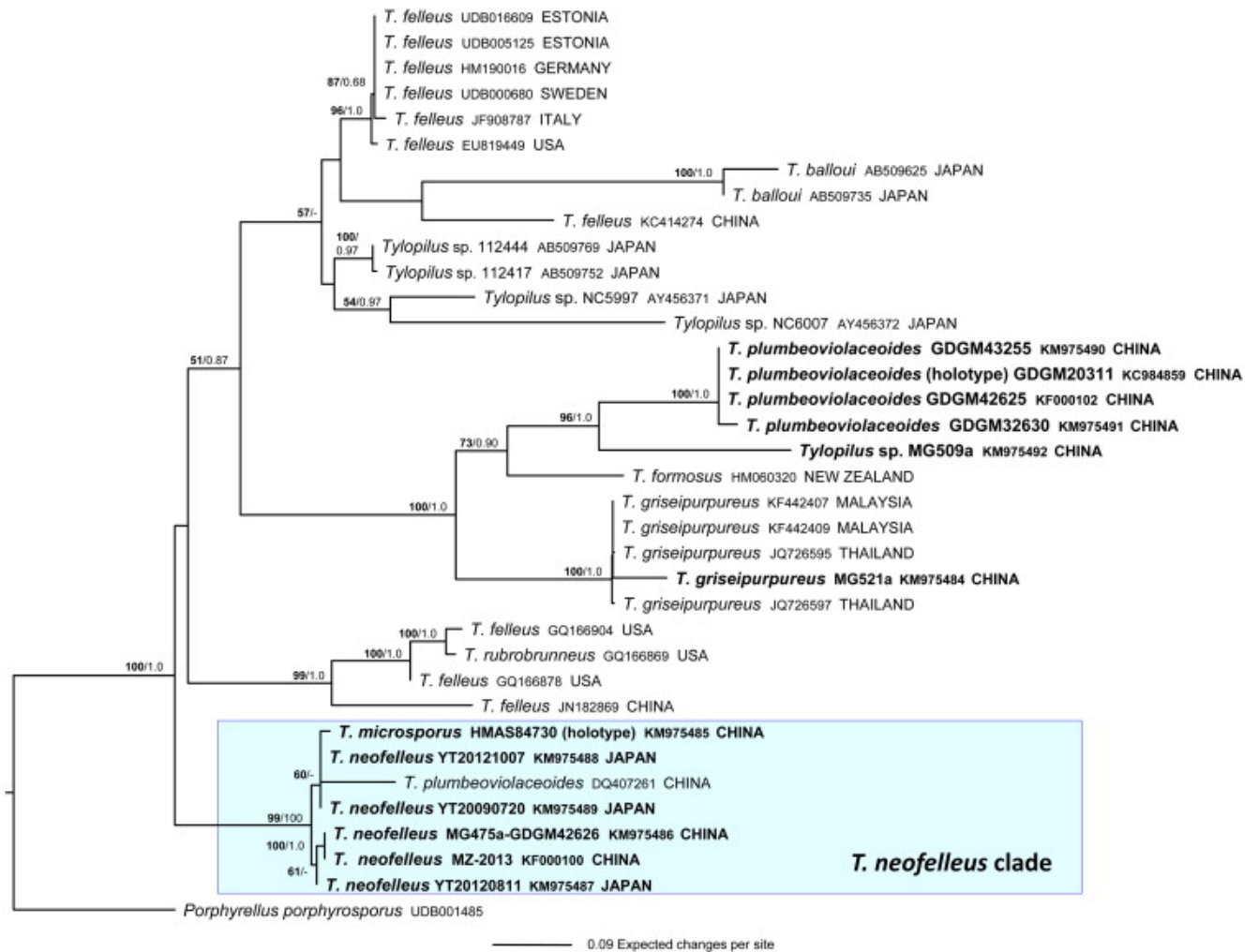


Fig. 1.

Maximum likelihood phylogram obtained from the general ITS sequence alignment of *Tylopilus* s. str. species. *Porphyrellus porphyrosporus* is used as outgroup taxon. Support values in either the maximum likelihood [ML Bootstrap percentage (MLB)] or Bayesian [Posterior probabilities values (BPP)] analyses are indicated. Only MLB values over 50 (in bold) and BPP values over 0.75 are given above clade branches. Newly sequenced collections are in bold.



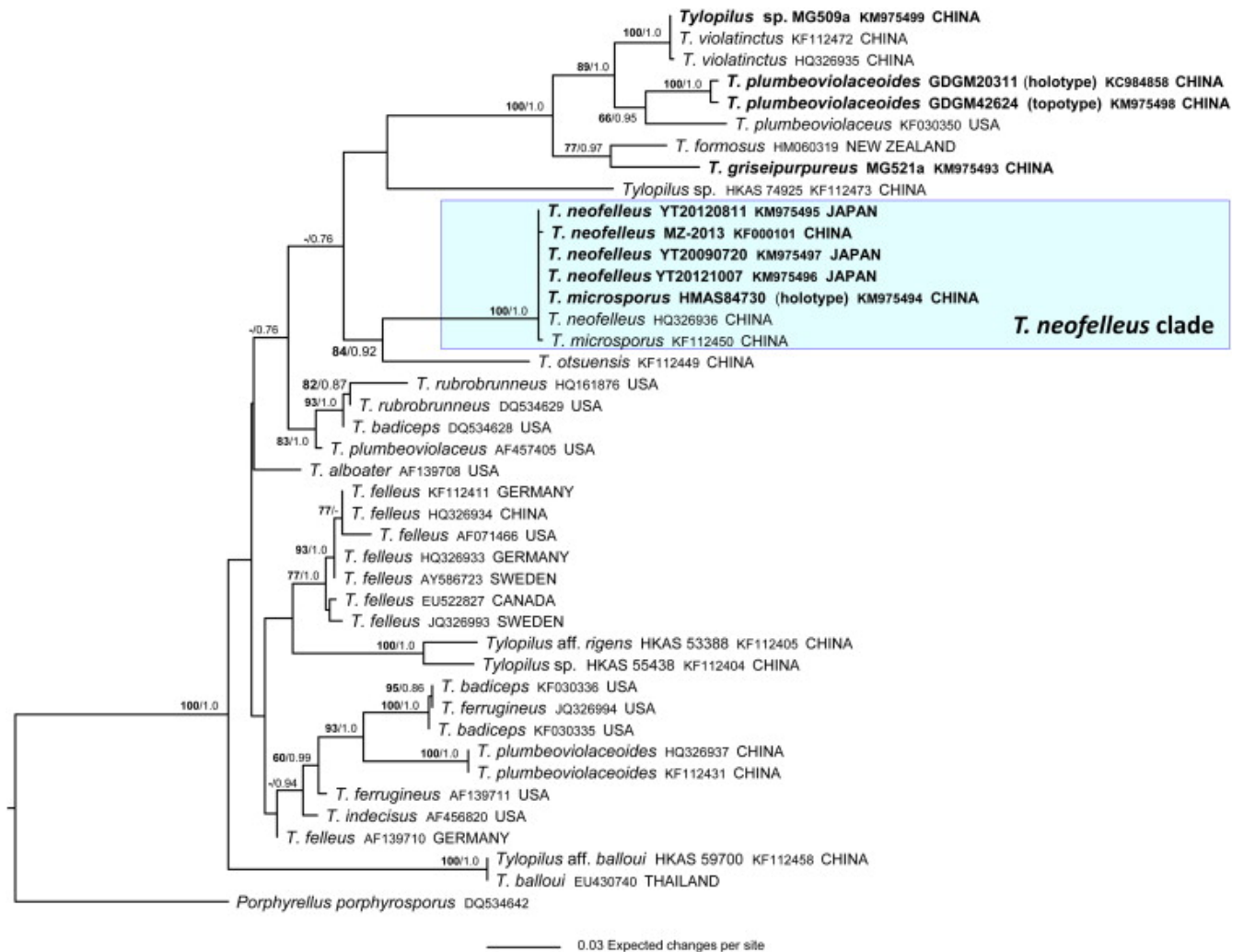


Fig. 2.

Maximum likelihood phylogram obtained from the general LSU sequence alignment of *Tylopilus* s. str. species. *Porphyrellus porphyrosporus* is used as outgroup taxon. Support values in either the maximum likelihood [ML Bootstrap percentage (MLB)] or Bayesian [Posterior probabilities values (BPP)] analyses are indicated. Only MLB values over 50 (in bold) and BPP values over 0.75 are given above clade branches. Newly sequenced collections are in bold.

## 2.4. Sequence alignment, data set assembly and phylogenetic analysis

Based on BLASTn results, preliminary phylogenetic analysis and outcomes of recent molecular studies on the Boletaceae (Binder and Hibbett, 2006, Nuhn et al., 2013 and Wu et al., 2014), sequences were retrieved from GenBank and UNITE (unite.ut.ee/) databases for comprehensive analysis. Two separate analyses of ITS and LSU sequences were carried out. Alignments were generated for each ITS and LSU data set using MAFFT (Katoh et al. 2002) with default conditions for gap openings and gap extension penalties. Alignments were then imported into MEGA 5.10 (Tamura et al. 2011) for manual adjustment. The best-fit substitution model for each alignment was estimated by both the Akaike information criterion (AIC) and the Bayesian information criterion (BIC) with jModelTest 0.1.1 (Posada 2008) to provide a substitution model for the alignment. GTR +  $\Gamma$  model was chosen for the ITS alignment, while TrNef +  $\Gamma$  was selected for LSU alignment. Based on the results of Nuhn et al. (2013) and Wu et al. (2014), *Porphyrellus*



*porphyrosporus* (Fr. & Hök) E.-J. Gilbert (UDB001485, DQ534642) was chosen as outgroup taxon for both data set.

Phylogenetic hypotheses were constructed using maximum likelihood (ML) and Bayesian inference (BI) criteria. ML estimation was performed with RAxML v.7.3.2 ([Stamatakis 2006](#)) with 1000 bootstrap replicates ([Felsenstein 1985](#)) using the GTRGAMMA algorithm to perform a tree inference and search for optimal topology. Support values from bootstrapping runs (MLB) were mapped on the globally best tree using the “-f a” option of RAxML and “-x 12345” as a random seed to invoke the novel rapid bootstrapping algorithm. The BI was performed with MrBayes 3.1.2 ([Huelsenbeck and Ronquist 2001](#)) with four incrementally heated simultaneous Monte Carlo Markov Chains (MCMC) run for 10 million generations, under the selected evolutionary model. 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 Probabilities (BPP).

Only MLB values over 50% and BPP values over 0.75 are reported in the resulting trees ([Fig. 1](#) and [Fig. 2](#)). Branch lengths were estimated as mean values over the sampled trees. Pairwise % identity values of ITS sequences were calculated using MEGA 5.10 ([Tamura et al. 2011](#)). Alignments and phylogenetic trees are available at TreeBASE ([www.treebase.org](http://www.treebase.org), submission number 16028).

### 3. Results

#### 3.1. Molecular results

Both maximum likelihood and Bayesian analyses produced the same topology; therefore, only the ML trees with both MLB and BPP values are shown ([Fig. 1](#) and [Fig. 2](#)). The ITS data matrix comprised a total of 36 sequences (including 20 from GenBank and 4 from UNITE databases); the alignment comprised 994 characters, and contains 601 variable sites. The LSU matrix consisted of 42 sequences (including 33 from GenBank); the alignment comprised 914 characters, and contains 259 variable sites.

In both the ITS and LSU analyses ([Fig. 1](#) and [Fig. 2](#)), collections of *T. neofelleus* from China and Japan cluster with *T. microsporus* S.Z. Fu, Q.B. Wang & Y.J. Yao (holotype included) forming a well-supported *T. neofelleus* clade (MLB = 100%; BPP = 1). The ITS sequences of the *T. neofelleus* clade share a pairwise % identity value of 98.5. The ITS sequence (DQ407261) from the Chinese collection identified as “*T. plumbeoviolaceoides*” represents *T. neofelleus*. In the LSU phylogram ([Fig. 2](#)) *T. otsuensis* Hongo is sister to the *T. neofelleus* clade. According to both ITS and LSU data, *T. plumbeoviolaceoides* T.H. Li, B. Song & Y.H. Shen is a clearly circumscribed species. Finally, the ITS analysis recognized that multiple species are hidden under the name *T. felleus*.

### 3.2. Taxonomy

*Tylopilus neofelleus* Hongo, Journal of Japanese Botany 42: 154. 1967. [Fig. 3](#), [Fig. 4](#), [Fig. 5](#) and [Fig. 6](#).

= *Tylopilus microsporus* S.Z. Fu, Q.B. Wang & Y.J. Yao, Mycotaxon 96: 42. 2006.

= *T. felleus* (Bull.) P. Karst. ss. Kawamura, Japanese Fungi 68. 1929.

= *T. felleus* (Bull.) P. Karst. ss. Kawamura, Icones of Japanese Fungi vol. II: 272 and plate 244. 1954.

= *T. plumbeoviolaceus* (Snell) Snell ss. Hongo, Acta Phytotaxonomica et Geobotanica 18: 108. 1960.

= *T. plumbeoviolaceoides* T.H. Li, B. Song & Y.H. Shen ss. Wang et al., Color Atlas of Wild Commercial Mushrooms in Yunnan 125. 2004.

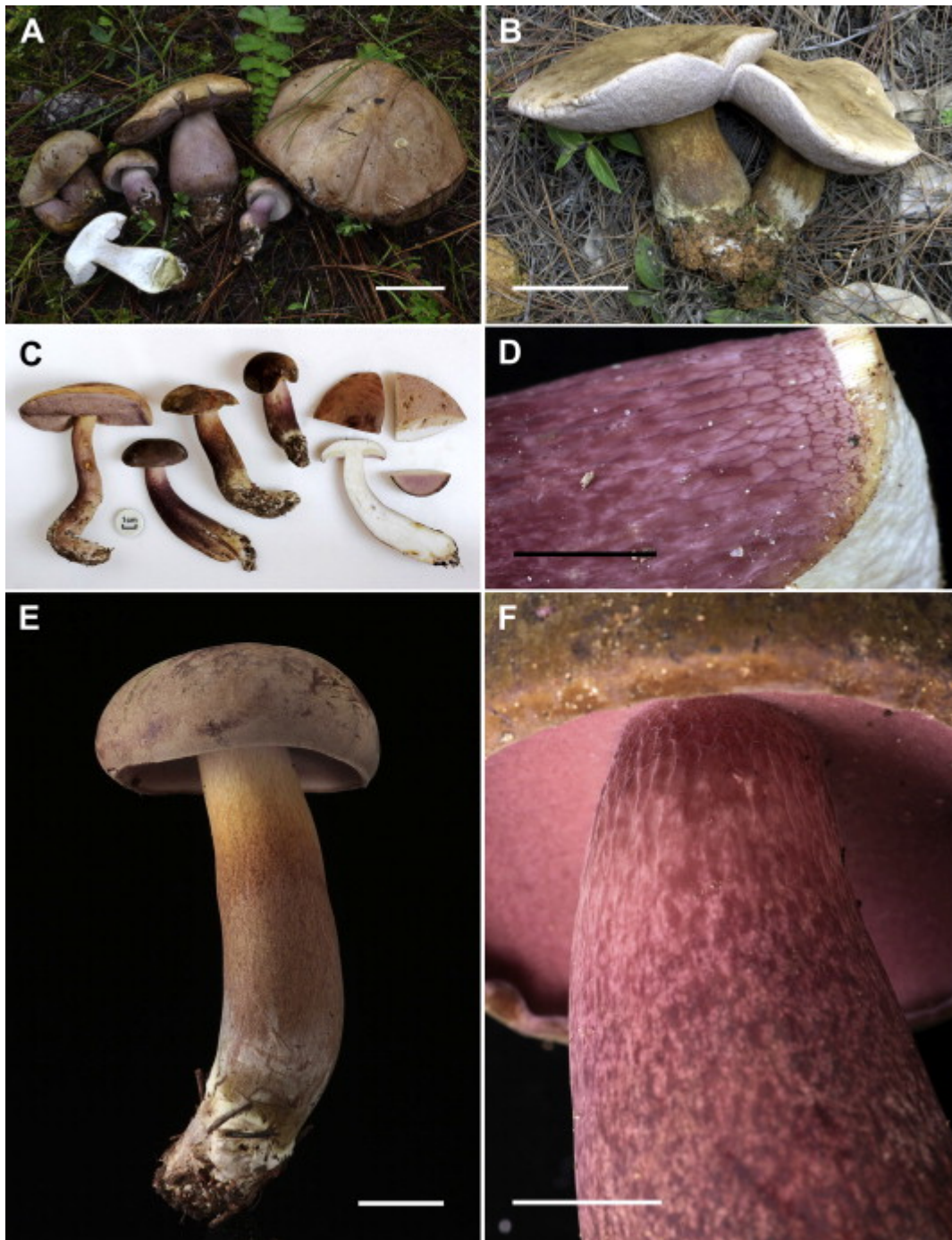


Fig. 3.

*Tylopilus neofelleus*. A–C, E: Basidiomes. D, F: Details of the stipe surface with reticulation restricted to the apex. A: GDGM 42626, MG475a, photo by M. Gelardi. B: HKAS50319, photo by Y.-C. Li. C: YT20120820, photo by A. Koyama. D: YT20110807-L, photo by Y. Taneyama. E: YT20120811, photo by Y. Taneyama. F: YT20110807-S, photo by Y. Taneyama. Bars: A, B 5 cm; D–F 1 cm.



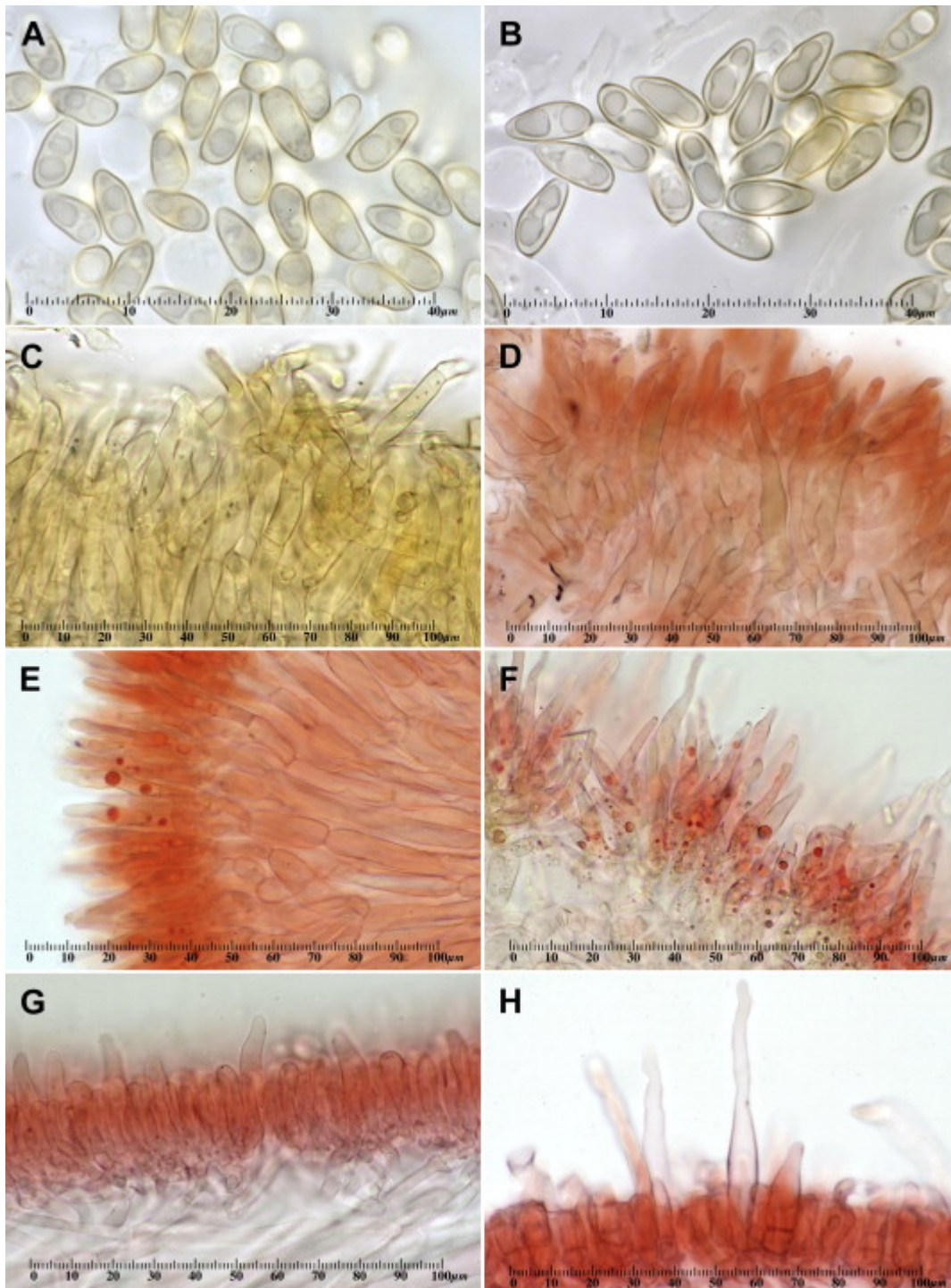


Fig. 4.

*Tylopilus neofelleus*. Microscopic features (in Congo Red). A, B: Basidiospores (YT20090720, YT20120820). C, D: Elements of pileipellis (YT20121007, YT20110807-L). E, F: Cheilocystidia (YT 20110807-L, YT20120811). G: Pleurocystidia (YT20120820). H: Caulocystidia (YT20120811). All photos by Y. Taneyama. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

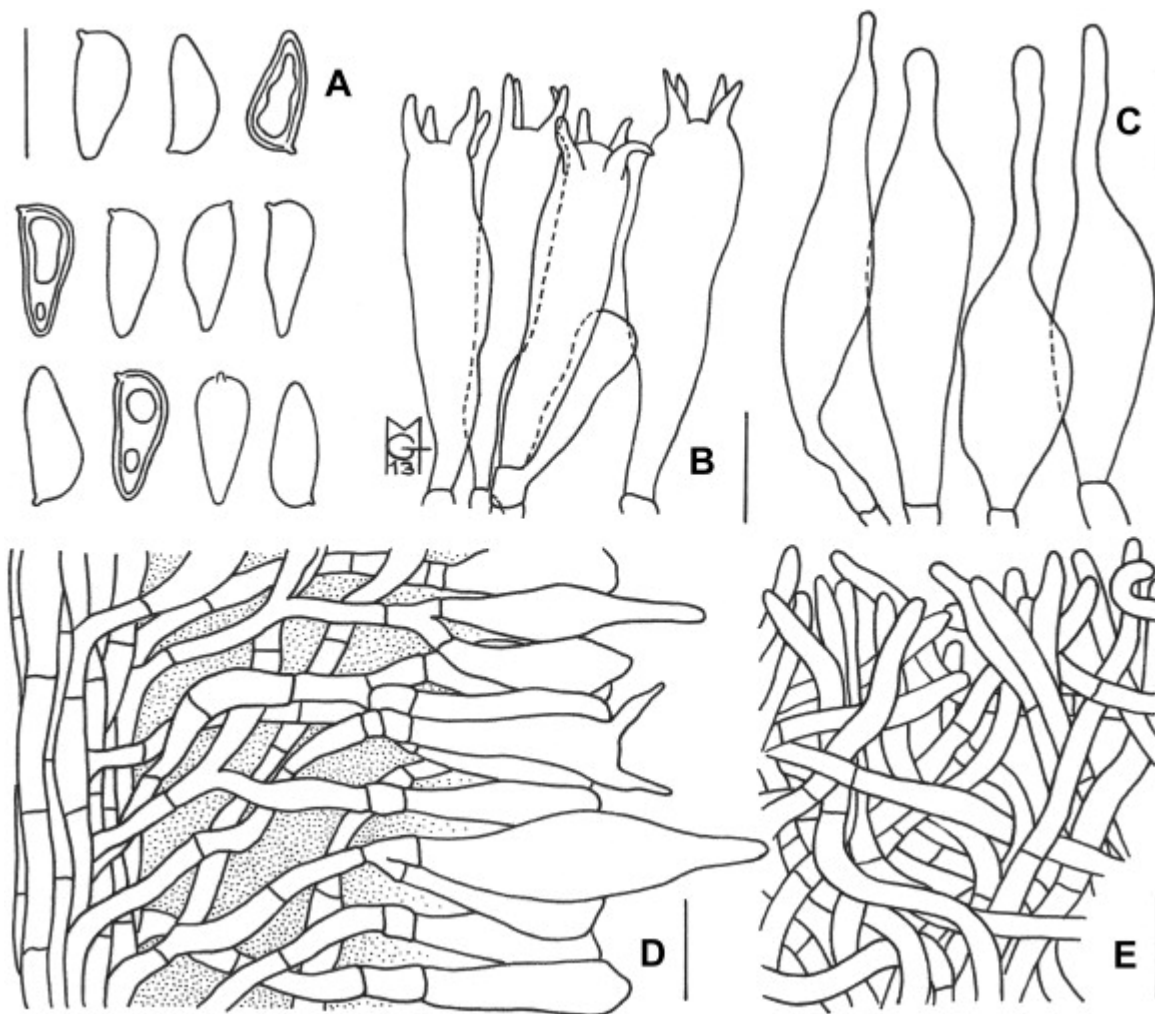


Fig. 5.

*Tylopilus neofelleus*. Microscopic features (GDGM 42626, MG475a). A: Spores. B: Basidia. C: Cheilo- and pleurocystidia. D: Stipitipellis and lateral stipe stratum. E: Pileipellis. Bars: A–C 10  $\mu$ m; D, E 20  $\mu$ m. Drawing by M. Gelardi.



Fig. 6.

A, B: Holotype collection of *Tylopilus neofelleus* (TNS-F-174771), photos by Y. Taneyama. C: Basidiospores of the holotype collection of *Tylopilus neofelleus* (in KOH), photo by Y. Taneyama. D: Holotype collection of *Tylopilus microsporus* (HMAS84730) erroneously labeled as *T. violatinctus*, photo by M. Gelardi. Bars: B 2 cm; D 4 cm.

Basidiomes large-sized. Pileus 5.0–15.5(–17.0) cm broad, at first hemispherical then persistently convex and finally broadly pulvinate-flattened, sometimes slightly depressed at center, fleshy, firm at the beginning but progressively softer with age; margin steady to faintly wavy-lobed, initially involute then curved downwards and finally plane or even uplifted, not or only a little extending beyond the tubes; surface matt, dry but slightly greasy with moist weather, very finely pruinose in the early stage of development but later smooth and glabrous, not cracked; cuticle somewhat variable in color, ranging from brownish to pale ochraceous-brown with olive shades and pale violet to grayish-lilac hues towards the margin, particularly in young specimens, gradually fading with age and becoming beige-ochraceous, especially on the peripheral surface; slowly and almost imperceptibly darkening on handling or when injured; subcuticular layer white. Tubes at first thin then increasingly broader and shorter than the thickness of pileus context (up to 2.5 cm long), adnate but soon depressed around the stipe apex, whitish at first to pale pinkish-flesh, pink-violaceous at maturity and further darkening up to dark violet in old fruiting bodies, unchangeable when cut. Pores initially forming a flat surface, later convex, at first small then gradually wider (up to 1 mm in diam.), simple, roundish to barely angular at maturity, concolorous with tubes and very slowly and faintly darkening on bruising or when injured, sometimes with rusty brown stains at the orifice. Stipe (4.0–)6.0–11.5(–16.0) × (1.0–)1.5–4.0(–4.5) cm, shorter than or as long as the pileus diameter at maturity, central to slightly off-center, solid, firm, dry, straight or curved, at first ovoid to ventricose-fusiform, later cylindrical but swollen towards the base or decidedly clavate, ending with a short taproot at the very base; surface showing an extremely fine pale violet reticulum

restricted to the apex (better visible with a hand lens!), smooth to finely longitudinally fibrillose-ribbed downwards up to half way, always smooth and glabrous in the lower portion, evelate; whitish in the upper third, immediately below pinkish to pinkish-yellow, increasingly more violet to violaceous-brown and mottled downwards, whitish at the very base, unchangeable when pressed; basal mycelium white, rhizomorphs brownish. Context firm and tough when young, later soft textured in the pileus (up to 3.2 cm thick in the central zone), a little more fibrous in the stipe, white throughout, in mature specimens with olivaceous spots or shades at the stipe base, tending to spread over the entire stipe tissues with age; unchangeable when exposed to air; pale ochraceous where eroded by maggots; subhymenophoral layer white; exsiccate brownish to grayish-brown, sometimes brownish-reddish on the stipe. Smell mild to vaguely fruity, agreeable. Taste decidedly bitter, unedible. Spore print not obtained. Macrochemical reactions: 10% KOH: staining very pale yellow on context and hymenophore, no reaction elsewhere.

Basidiospores [50/4/1]  $(8.2-9.8 \pm 1.3(-13.2) \times (3.2-4.0 \pm 0.4(-4.8) \mu\text{m}$ ,  $Q = (2.04-2.47 \pm 0.21(-3.02)$ ,  $V = 85 \pm 34 \mu\text{m}^3$ , fairly variable in dimension, inequilateral, ellipsoid-fusiform to fusiform in side view and progressively tapering towards the apex which is sharply pointed, often with a shallow abaxial depression close to the distal end, ellipsoid to ellipsoid-fusiform in face view, smooth, with a short apiculus and without suprahilar depression, moderately thick-walled ( $0.5-0.7 \mu\text{m}$ ), straw-yellow in water and 5% KOH, having one or two large oil droplets when mature, rarely pluri-guttulate, inamyloid, cyanophilic and with an orthochromatic reaction. Basidia  $(23-27-34(-40) \times 6-10 \mu\text{m}$  ( $n = 13$ ), cylindrical to cylindrical-clavate, moderately thick-walled ( $0.5-0.7 \mu\text{m}$ ), predominantly 4-spored but also 1 or 2-spored, usually bearing relatively long sterigmata ( $3-7 \mu\text{m}$ ), hyaline to 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, about the same size as basidia. Cheilocystidia  $(23-29-50(-58) \times 5-10 \mu\text{m}$  ( $n = 15$ ), very common, moderately slender, projecting straight to sometimes flexuous, irregularly cylindrical or cylindrical-fusiform to ventricose-fusiform or lageniform, showing a narrow and long neck, rarely short mucronate to subclavate, with rounded to subacute tip, smooth, moderately thick-walled ( $0.5-1.0 \mu\text{m}$ ), with a straw-yellow vacuolar pigment in water and 5% KOH, reddish-brown (content strongly dextrinoid) in Melzer's, without epiparietal encrustations. Pleurocystidia  $(35-39-55(-60) \times 7-11 \mu\text{m}$  ( $n = 15$ ), decidedly frequent, shape, size, color and chemical reactions as in cheilocystidia. Pseudocystidia not recorded. Pileipellis a trichoderm consisting of strongly interwoven, elongated, filamentous and sinuous, frequently branched hyphae tending to be repent in the outermost layer and thus turning into a cutis not or only partially embedded in gelatinous matter; terminal elements  $25-83 \times (2-3-8 \mu\text{m}$ , long and slender, cylindrical, apex rounded-obtuse, moderately thick-walled (up to  $1 \mu\text{m}$ ), hyaline to more often straw-yellow in water and 5% KOH, weakly dextrinoid in Melzer's, smooth to occasionally ornamented by a subtle zebra-like epiparietal encrustation; subterminal elements similar in shape, size and color to terminal elements. Stipitipellis a texture of slender, parallel to loosely intermingled and longitudinally running, smooth-walled, adpressed hyphae,  $2-12 \mu\text{m}$  wide, hyaline to yellowish in water and 5% KOH; the stipe apex covered by a well developed caulohymenial layer consisting of sterile caulobasidioles, very sparse, predominantly 2-spored, fertile caulobasidia and abundant projecting caulocystidia similar in shape and color to hymenial cystidia but distinctly longer,  $(31-33-82(-95) \times 6-13(-15) \mu\text{m}$  ( $n = 15$ ), having a wall up to  $1 \mu\text{m}$  thick. Lateral stipe stratum under the caulohymenium present and well differentiated from the stipe trama, of the "boletoid type", at the stipe apex a  $(25-30-60(-80) \mu\text{m}$  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. Stipe trama composed of confusedly and densely arranged, strongly interwoven, filamentous, smooth, inamyloid to barely dextrinoid hyphae,  $2-13(-15) \mu\text{m}$  broad. Hymenophoral trama bilateral divergent of the "*Phylloporus*-type", with very slightly divergent to nearly subparallel and tightly arranged, non-gelatinous hyphae (lateral strata hyphae in transversal section touching or almost



touching each other, 0–3 µm distant from one another, relatively short and inflated, 3–8 µm broad), hyaline in water and 5% KOH, inamyloid in Melzer's; lateral strata (10–)15–25(–30) µm thick, mediostratum 10–35 µm thick, consisting of a tightly adpressed, non-gelatinous bundle of hyphae, 3–6 µm broad; in Congo Red the mediostratum is concolorous with the lateral strata. Rhizomorphs consisting of parallel and densely arranged, unbranched, scarcely septate, filamentous, thick-walled (up to 2 µm), smooth hyphae, (10–)13–20 µm broad, hyaline (but wall straw-yellow) in 5% KOH, inamyloid; hyphae are intermixed with an amorphous, honey yellow to brownish-yellow (5% KOH) granular substance. Clamp connections absent in all tissues. Hyphal system monomitic. Ontogenetic development gymnocarpic.

Ecology: gregarious to scattered, in subtropical montane environment, several specimens (more than 15) in all developmental stages, growing on litter in a mono-dominant stand of *Pinus yunnanensis* on acidic red soil (pH 5.9–6.5).

Material examined: China, Yunnan Province, Lijiang County, Jinshan, 26°57'44" N, 100°22'01" E, 2470 m alt., M. Gelardi, E. Horak, A. Horak, G. Wu, K. Zhao, Q. Zhao and S.-B. Jiao, 16 Sep 2012, GDGM 42626 and MG475a (duplicate).

Distribution: Known from Japan ([Kawamura 1954](#) as "*T. felleus*"; [Hongo 1960](#) as "*T. plumbeoviolaceus*", 1967; [Imazeki et al., 1970](#), [Imazeki et al., 1988](#) and [Takahashi, 1986](#)), southern China (Yunnan, Sichuan, Guizhou, Fujian Provinces) ([Ying and Zang, 1994](#), [Li and Song, 2000](#) and [Wang et al., 2004](#) as "*T. plumbeoviolaceoides*"; [Fu et al. 2006](#) as "*T. microsporus*"; [Wu et al. 2011](#) as "*T. microsporus*"), Russia ([Vasil'jeva 1978](#)) and New Guinea ([Hongo 1973](#)). Most likely widespread throughout eastern and south-eastern Asia.

Additional materials examined: *T. microsporus*: China, Yunnan Province, Simao, Simao free market, 22°48' N, 100°58' E, Q.-B. Wang, 10 Aug 2003, HMAS 84730 (holotype); Sichuan Province, Dechang County, Nanshan, Datianwan Village, 27°25' N, 102°10' E, 1700 m alt., with *Quercus* sp. and *Pinus* sp., S.-Z. Fu, 15 Aug 2003, HMAS 84745. *T. plumbeoviolaceoides*: China, Guangdong Province, Guangzhou, northern suburbs, 200 m alt., with Fagaceae, T.-H. Li, 29 Mar 2001, GDGM 20311 (holotype); Same loc., GDGM 42624 (topotype); Guangdong Province, Guangzhou, Mount Baiyun, 23°10'00" N, 113°17'00" E, 270 m alt., with *Castanopsis fissa* (Champion ex Benth) Rehder & E.H. Wilson, *Castanea henryi* (Skan.) Rehder. & E.H. Wilson and *Lithocarpus* sp., GDGM 43255; same loc., GDGM 42625; same loc., GDGM 32630. *T. neofelleus*: Japan, Toyama Prefecture, Tonami City, Ranjou-no-mori, 36°38'01" N, 137°02'14" E, 100 m alt., with *Pinus densiflora* Siebold & Zucc. and *Quercus serrata* Murray, Kozawa T., 20 Jul 2009, YT20090720; Nagano Prefecture, KitaAzumi-gun, Matsukawa-mura, Barou, 36°25'50" N, 137°49' 24" E, 740 m alt., with *Pinus densiflora*, Y. Taneyama, 07 Aug 2011, YT20110807-L; same loc., Y. Taneyama, 07 Aug 2011, YT20110807-M; same loc., Y. Taneyama, 07 Aug 2011, YT20110807-S; Nagano Prefecture, Suwa City, Karasawa, 36°03'08" N, 138°08'13" E, 970 m alt., with *Pinus densiflora*, Y. Taneyama, 11 Aug 2012, YT20120811; Nagano Prefecture, Suwa city, Kamisuwa, Tateshina-no-umi, 36°04'53" N, 138°07'52" E, 1250 m alt., with *Pinus densiflora*, Koyama A., 20 Aug 2012, YT20120820; Niigata Prefecture, Higashikanbara-gun, Aga-cho, Asunaro-park, 37°35'28" N, 139°25'34" E, 200 m alt., with *Pinus densiflora*, M. Takahashi, 07 Oct 2012, YT20121007; Nagano Prefecture, Chino City, Mt. Kozumi, 35°59'55" N, 138°11'59" E, 1000 m alt., with *Pinus densiflora*, Y. Taneyama, 07 Sep 2014, TNS-F-53608. *T. griseipurpureus*: China, Guangdong Province, Nan'ao island, 23°24'00" N, 117°07'28" E, 10 m alt., with *Casuarina equisetifolia* L., Li P., M. Gelardi, M. Zhang and Li T.-H., 29 Jun 2013, MG521a. *Tylopilus* sp.: China, Guangdong Province, Dinghushan Biosphere Reserve, 23°10'00" N, 112°32'40" E, 66 m alt., with *Castanea henryi* and *Pinus massoniana* Lamb., M. Zhang, M. Gelardi, Xu J. and Li T.-H. 20 Jun 2013, MG509a.

### 3.3. Revision of the holotype material of *T. neofelleus* (TNS-F-174771) ( [Fig. 6](#) )

Basidiospores [174/1/1]  $(7.0\text{--}8.3 \pm 0.6(-10.3) \times (2.0\text{--}3.7 \pm 0.2(-4.4) \mu\text{m}$ ,  $Q = (1.81\text{--}2.22 \pm 0.23(-3.88)$ , moderately thick-walled ( $0.3\text{--}0.7 \mu\text{m}$ ), yellowish-gray in 5% KOH, inamyloid. Basidia clavate, 4-spored,  $20\text{--}27 \times 7\text{--}10 \mu\text{m}$  ( $n = 10$ ), thin-walled. Cheilocystidia collapsed, with dextrinoid content. Pleurocystidia  $(30\text{--})38\text{--}52(-58) \times 6\text{--}11 \mu\text{m}$  ( $n = 48$ ), yellowish in 5% KOH, with dextrinoid content. Hymenophoral trama bilateral divergent of the “*Phylloporus*-type”, lateral stratum hyphae hyaline in 5% KOH, weakly dextrinoid,  $5\text{--}10 \mu\text{m}$  broad; mediostratum hyaline in 5% KOH, dextrinoid, hyphae  $2\text{--}4 \mu\text{m}$  broad. Pileipellis a trichoderm of interwoven hyphae,  $3\text{--}7 \mu\text{m}$  wide, yellowish in 5% KOH, dextrinoid; terminal cells  $23\text{--}56 \times 4\text{--}11 \mu\text{m}$ , moderately thick-walled (up to  $0.9 \mu\text{m}$ ), yellowish in 5% KOH, dextrinoid. Stipitipellis hymeniform, elements with brownish intracellular pigment, dextrinoid. Lateral stipe stratum under caulohymenium present and differentiated from the stipe trama, of the “boletoid type”, at stipe apex a  $30\text{--}70 \mu\text{m}$  thick layer, hyphae  $(2\text{--})3\text{--}6(-9) \mu\text{m}$  wide. Caulocystidia thick-walled (up to  $1.1 \mu\text{m}$ ), at stipe apex  $(20\text{--})27\text{--}60(-86) \times (6\text{--})7\text{--}11(-13) \mu\text{m}$  ( $n = 21$ ); at middle part  $(30\text{--})37\text{--}63(-82) \times (6\text{--})7\text{--}11(-15) \mu\text{m}$  ( $n = 19$ ); at stipe base  $(45\text{--})61\text{--}99(-118) \times (6\text{--})7\text{--}10 \mu\text{m}$  ( $n = 19$ ). Stipe trama composed of filamentous, smooth, thick-walled (up to  $1.3 \mu\text{m}$ ), hyaline in 5% KOH, dextrinoid hyphae,  $(5\text{--})7\text{--}11(-14) \mu\text{m}$  broad. Basal mycelium consisting of filamentous, hyaline in 5% KOH, dextrinoid, thin-walled hyphae. Clamp connections absent.

## 4. Discussion

Taxa belonging to the *T. plumbeoviolaceus* complex are notoriously difficult to separate from one another due to their morpho-chromatic and organoleptic affinities ( [Sitta et al. 2007](#) ). However, *T. neofelleus* appears to be well characterized amongst other species on account of the following set of distinctive macroscopic and anatomical features: 1) medium-to large-sized basidiomes; 2) pileus displaying somewhat drab and pallid colors, ranging from brownish to ochraceous-brown with olive shades and gradually fading to beige-ochraceous in aged specimens, with scattered grayish-lilac hues which are more pronounced in early developmental stages, without any noticeable discoloration on handling; 3) tubes and pores at first whitish then pinkish and finally violaceous; 4) stipe whitish in the upper third but bright vinaceous-purple, lilac to violet-brown downwards, fading to ochraceous-brown with age, with a very fine violet reticulum at apex; 5) basal mycelium white; 6) context white overall and unchangeable; 7) basidiospores small sized (but see below) and with a peculiar abaxial depression near the distal end; 8) cystidia mostly fusoid with a long, narrow neck, with dextrinoid content; 9) occurrence in mixed woodlands with *Pinus* (*P. yunnanensis*, *P. densiflora*, *P. kesiya* var. *langbianensis*, etc.) and Fagaceae (*Quercus*, *Castanopsis*, *Lithocarpus*). Sometimes, olivaceous tinges in the stipe base context are observable and are also visible in at least one recent Japanese collection (TNS-F-53608). The diagnostic significance of such a feature is yet to be fully understood as the phenomenon is rare in its occurrence. There appear to be some minor morpho-chromatic differences between Chinese and Japanese material referable to *T. neofelleus*, the latter having slightly smaller dimensions, chromatic variability of pileus and stipe and pore surface usually darker than tubes (Japanese specimens nearly always exhibit pores darker than tubes whereas this character is variable in Chinese population); such morphological traits, however, are not supported by molecular analysis and should be accounted as mere infraspecific variation.

Molecular outcomes undoubtedly demonstrate that *T. neofelleus* and *T. microsporus* are contaxic. ITS and LSU sequences obtained from the holotype of *T. microsporus* perfectly match with those obtained from Japanese material of *T. neofelleus* and from the Chinese collection described here, although the holotype of *T. neofelleus* was too old and not suitable for DNA extraction. However, a major discrepancy appears by comparing the spore dimensions of the present collection with those reported for *T. neofelleus* and *T. microsporus* in their respective original descriptions, as in both

cases they appear distinctly shorter [*T. neofelleus* 6.5–9.5 × 3.5–4.5 µm, [Hongo 1967](#); *T. microsporus* (6.5–)7.0–9.0(–10) × 3.0–4.0(–5.0) µm, [Fu et al. 2006](#)]. Critical re-examination of the anatomical characters of type material of *T. microsporus* from Yunnan Province (HMAS 84730) and of an additional sample from Sichuan (HMAS 84745) as well as of the holotype of *T. neofelleus* (TNS-F-174771) from Japan ( [Table 2](#)), clearly revealed that spore values are congruent with those reported in the protologues. Consequently, since there are no other relevant morphological differences, we conclude that the spores may be longer than reported in literature under both names ( [Hongo, 1967](#), [Hongo, 1973](#), [Takahashi, 1986](#), [Imazeki et al., 1988](#) and [Wang et al., 2004](#) as “*T. plumbeoviolaceoides*”; [Fu et al., 2006](#) and [Wu et al., 2011](#)) and, therefore, the circumscription of *T. neofelleus/microsporus* should be adjusted to account for such variation. Additionally, it should be emphasized that the hymenophoral trama bilateral divergent of the “*Phylloporus*-type” (or at most intermediate between the “*Phylloporus*” and “*Boletus*” types) and the presence of the lateral stipe stratum were also observed and confirmed from both the original material of *T. microsporus* and the Japanese samples, including the holotype of *T. neofelleus*. Interestingly, the description of *T. neofelleus* provided in [Chen et al. \(2004\)](#) does not seem to match with that of either the Japanese species or with *T. microsporus* and might indeed be *T. plumbeoviolaceoides* (see discussion below).

Table 2.

Comparison of the spore dimensions from selected samples of *Tylopilus microsporus*/*T. neofelleus*.

Taxon	Voucher	Spore dimensions (µm)	Q
<i>Tylopilus microsporus</i>	HMAS 84730 (holotype)	(6.6–)8.1 ± 0.7(–9.7) × (3.5–)3.9 ± 0.1(–4.3)	2.0 ± 0.1
<i>Tylopilus microsporus</i>	HMAS 84745	(7.2–)8.0 ± 0.4(–9.0) × (3.5–)3.8 ± 0.1(–4.0)	2.1 ± 0.1
<i>Tylopilus neofelleus</i>	TNS-F-174771(holotype)	(7.0–)8.3 ± 0.6(–10.3) × (2.0–)3.7 ± 0.2(–4.4)	2.2 ± 0.2
<i>Tylopilus neofelleus</i>	GDGM 42626, MG475a	(8.2–)9.8 ± 1.3(–13.2) × (3.2–)4.0 ± 0.4(–4.8)	2.4 ± 0.2
<i>Tylopilus neofelleus</i>	YT20090720	(6.4–)7.5 ± 0.5(–9.3) × (3.0–)3.5 ± 0.2(–4.0)	2.2 ± 0.1
<i>Tylopilus neofelleus</i>	YT20120820	(6.3–)8.2 ± 0.6(–9.7) × (3.0–)3.6 ± 0.2(–4.1)	2.3 ± 0.1
<i>Tylopilus neofelleus</i>	TNS-F-53608	(5.6–)8.2 ± 0.6(–10.0) × (3.0–)3.7 ± 0.2(–4.4)	2.2 ± 0.1

[Table options](#)

The LSU sequence analysis ([Fig. 2](#)) suggests a close affinity of *T. neofelleus* with *T. otsuensis*, a species described from Japan ( [Hongo, 1966](#) and [Hongo and Nagasawa, 1975](#)) which differs mainly in having basidiomes without violet tinges, context turning reddish-brown when exposed, taste mild to slightly bitterish, pileipellis an adpressed palisade of erect hyphae consisting of 2–3 shortly fusoid-ventricose to inflated elements [(25.0–)28.3–35.3(–38.6) × (6.5–)8.0–12.3(–15.6) µm ( $n = 19$ ), Y. Taneyama, unpublished data], shorter caulocystidia [(29.5–) 31.1–36.7 (–37.5) × (5.0–) 5.0–8.9 (–11.7) µm ( $n = 10$ ), Y. Taneyama, unpublished data] and largely ellipsoid to ovoid spores with obtuse-rounded apex [7–8 × 4.5–5 µm or 7.5–9 × 5–6.5 µm, according to [Hongo 1966](#); 6–7.5(–

$9) \times 4\text{--}4.5\text{ }\mu\text{m}$ , according to [Hongo and Nagasawa 1975](#);  $(5.7\text{--})6.9 \pm 0.4(-8.2) \times (3.6\text{--})4.3 \pm 0.3(-5.0)\text{ }\mu\text{m}$  ( $n = 116$ ),  $Q = (1.3\text{--})1.6 \pm 0.1(-1.9)$ , Y. Taneyama, unpublished data].

Species morphologically close to *T. neofelleus* are distributed in Asia, America, Oceania and Europe and are discussed below.

*Tylopilus plumbeoviolaceoides*, described from China, closely resembles *T. neofelleus* in the general appearance but is steadily distinguished by the darkly colored pileus and stipe ranging from dark violaceous to brown-vinaceous or chestnut, the context discoloring pinkish to purplish when injured, a more obvious oxidation on pores surface when touched, usually longer and somewhat narrower spores  $[(7.5\text{--})8.5\text{--}10.5(-12) \times (2.5\text{--})3\text{--}3.8(-4.2)\text{ }\mu\text{m}$ ,  $Q$  about 2.8], shorter basidia and the growth in association with fagaceous hosts only; moreover, the phenology is dissimilar as *T. plumbeoviolaceoides* occurs much earlier (in spring season, from late March to May) ([Bi et al. 1997](#) as “*T. plumbeoviolaceus*”; [Li et al., 2002](#), [Li, 2011](#) and [Wu et al., 2011](#)) than *T. neofelleus* (summer to autumn). Molecular analyses further confirm the independent taxonomic position of *T. plumbeoviolaceoides* with respect to *T. microsporus*/*T. neofelleus* ([Fig. 1](#) and [Fig. 2](#)). Two *T. plumbeoviolaceoides* LSU sequences retrieved from GenBank ([HQ326937](#) and [KF112431](#), from Chinese collections) are not phylogenetically related to the holotype collection and probably represent a different taxon ([Fig. 2](#)).

Amongst the Japanese entities, *T. vinosobrunneus* Hongo has noticeably smaller basidiomes (pileus 3–7 cm wide and stipe 3–9  $\times$  0.5–2 cm), vinaceous-brown surfaces, stipe without reticulum, pores staining brown on pressure and context turning reddish or pinkish when cut, slightly larger basidiospores (9–12  $\times$  4–5  $\mu\text{m}$ ), pileipellis a palisadoderm consisting of erect chains of short-cylindrical to inflated elements, 6.5–12.5  $\mu\text{m}$  wide, and is associated with *Quercus* spp. ([Hongo 1979](#)).

Two additional species have recently been reported from Japan, viz. *T. fuligineoviolaceus* Har. Takah. and *T. obscureviolaceus* Har. Takah. The evenly dark purple colored pileus, smaller basidia and the habitat in pure *Quercus*-*Castanopsis* woodlands distinguish both species from *T. neofelleus* ([Takahashi, 2004](#) and [Takahashi, 2007](#)). *Tylopilus obscureviolaceus* is further distinguished by the smaller and differently shaped spores (6–7.2  $\times$  3.3–4  $\mu\text{m}$ ,  $Q = 1.8$ ) ([Takahashi 2004](#)), while *T. fuligineoviolaceus* also differs by the pileus becoming blackish-brown overall at maturity, the brunnescent hymenophore when handled and the smaller cystidia ([Takahashi 2007](#)).

*Tylopilus griseipurpureus* was originally described from Malaysia and differs from *T. neofelleus* by smaller size, dark violet to violet-brownish pileus, slightly longer spores [9–10(–11)  $\times$  3.5–4  $\mu\text{m}$ ], a palisade-like pileipellis consisting of fusoid to cystidioid or clavate terminal cells, 20–45  $\times$  4–9  $\mu\text{m}$ , and the association with Myrtaceae (*Melaleuca*, *Eucalyptus*), Fabaceae (*Acacia*), Casuarinaceae (*Casuarina*) and probably Lecythidaceae (*Gustavia*) ([Corner, 1972](#), [Aungaudchariya et al., 2010](#), [Aungaudchariya et al., 2012](#) and [Horak, 2011](#); present study). The species has been reported from Thailand several times ([Chandrasrikul et al., 2008](#), [Seehanan and Petcharat, 2008](#), [Pukahuta et al., 2009](#), [Aungaudchariya et al., 2010](#) and [Aungaudchariya et al., 2012](#)) whereas the present collection (MG521a) is an additional finding to the sole record from southern China (Hainan Province) to date ([Bi et al., 1997](#) and [Li and Song, 2000](#)). However, it seems to be likely that *T. griseipurpureus* is restricted to the Asian tropical belt.

*Tylopilus atripurpureus* (Corner) E. Horak is another Malaysian species of the same complex, differing from *T. neofelleus* by smaller size, velvety and blackish-violet to dark violet-brown pileus and stipe, slightly longer spores [9–10.5(–12)  $\times$  4–4.5  $\mu\text{m}$ ], longer hymenial cystidia (75–100  $\mu\text{m}$  long) and pileipellis a palisadoderm with short-cylindrical to subconical terminal elements, 15–



25 × 8–10 µm ( [Corner, 1972](#) and [Horak, 2011](#)). *Tylopilus atripurpureus* has also been reported from China ( [Zang 2006](#)).

Concerning the American species, *T. plumbeoviolaceus* has a deep violet-purplish then purple-brown to dull cinnamon-brown pileus which may be overlaid by a hoary bloom, different macrochemical reactions with KOH, larger basidiospores [10–13(–14) × 3–4(–5.5) µm,  $Q = 2.8$ ], a pileipellis consisting of erect hymeniform terminal elements (pileocystidia) and the growth in mixed deciduous woods ( [Snell, 1936](#), [Snell and Dick, 1941](#), [Snell and Dick, 1970](#), [Singer, 1947](#), [Mazzer and Smith, 1967](#), [Smith and Thiers, 1971](#), [Wolfe, 1986](#), [Cetto, 1989](#), [Both, 1993](#), [Bessette et al., 2000](#) and [Kuo, 2005](#)).

Another comparable species, *T. violatinctus* T.J. Baroni & Both, is easily separated by the more brightly colored, bluish-violet to lilac-lavender or purple-grayish pileus, bruising dark rusty-violet when handled, the stipe turning yellowish on bruising, cuticle and context staining yellowish-brown and negative to pinkish-brown with KOH, respectively, differently shaped basidiospores [(5.6–)7–9(–10) × 3–4 µm] and shorter basidia, distinctly smaller caulocystidia and the growth in mixed woodlands possibly with *Quercus*, *Fagus* or *Picea*, in any case not in association with pine trees ( [Baroni and Both, 1998](#), [Bessette et al., 2000](#) and [Ortiz-Santana et al., 2007](#)).

*Tylopilus rubrobrunneus* Mazzer & A.H. Sm. differs from *T. neofelleus* in having larger size (pileus up to 30 cm diam. and stipe up to 20 cm long × 8 cm wide), a deep violet-brown pileus becoming reddish-brown to dingy cinnamon-tan or chocolate-brown in age, the stipe vinaceous-brown without violaceous to lilac shades which are replaced at maturity by the presence of olive or olive-brown tones, longer spores [(10–)12–14 × 3–4.5(–5) µm], shorter basidia, smaller caulocystidia, basidiomes staining reddish-brown overall with KOH and the occurrence mostly, if not exclusively, with hardwoods (especially *Quercus* and *Fagus*) or under Eastern Hemlock (*Tsuga canadensis*) ( [Mazzer and Smith, 1967](#), [Smith and Thiers, 1971](#), [Grund and Harrison, 1976](#), [Both, 1993](#), [Baroni and Both, 1998](#), [Bessette et al., 2000](#), [Roody, 2003](#) and [Kuo, 2005](#)).

*Tylopilus williamsii* Singer & J. García, described from Central America (Mexico), is readily discriminated by the smaller size (pileus 5–9 cm diam., stipe 4.5–6 × 1–1.3 cm), pinkish-gray to dark brown or chocolate pileus (the violet tinge, if at all present, is visible only in the primordia and disappears very early), pores turning ochraceous-yellowish when touched, not reticulated and whitish-yellow stipe with brown areas at maturity, different chemical color reactions, “*Boletus*-type” hymenophoral trama and association with oaks ( [Singer et al., 1991](#) and [Baroni and Both, 1998](#)).

The Costarican *T. bulbosus* Halling & G.M. Muell. differs from *T. neofelleus* based on context and hymenophore discoloring brownish to pinkish-brown on injury or exposure, mild taste, larger spores [10–14 × 4.2–4.9 (–5.6) µm], longer cystidia (50–80 × 8–14 µm) and occurrence in montane neotropical frondose woods dominated by *Quercus* spp. ( [Halling and Mueller 2001](#)).

The Australian *T. austrofelleus* (Cleland) Watling & N.M. Greg. as originally delimited ( [Cleland 1924](#)) is most likely a heterogeneous mixture of different taxa ( [Watling and Gregory 1989](#)), however all samples attributable to this collective species are morpho- and ecologically totally different from *T. neofelleus* ( [Watling and Li 1999](#)).

[Stevenson \(1962\)](#) described *T. formosus* G. Stev. from New Zealand. This species is readily separated from *T. neofelleus* based on the chocolate-brown to dark brownish-black pileus and stipe sometimes with deep purplish tints, the hymenophore becoming pale brownish-orange at maturity due to spores color, longer spores [9.8–14.2(–15) × 4–5.3 µm], broader hymenial cystidia (10–

16 µm wide) and growth in association with *Leptospermum* (Myrtaceae) and *Nothofagus* (Nothofagaceae) ( [Stevenson, 1962](#) and [McNabb, 1968](#)).

*Tylopilus felleus*, described originally from Europe but also present in North America and China (see [Fig. 1](#) and [Fig. 2](#)), differs by the basidiomes without violaceous or purplish tinges in any developmental stage, a usually well developed and pronounced brownish reticulum covering the stipe throughout, the growth often amongst wood debris and longer, fusoid basidiospores, (10.2–)11.7–14.5(–16.2) × (4.1–)4.5–5.3 µm,  $Q = (2.3–)2.5–2.8(–3.0)$  ( [Breitenbach and Kränzlin, 1991](#), [Lannoy and Estadès, 2001](#), [Muñoz, 2005](#) and [Watling and Hills, 2005](#)). According to the ITS analysis ([Fig. 1](#)) it seems that some Asiatic and American collections labeled as “*T. felleus*” could represent distinct and (still) undescribed species.

It is to be noted that several taxonomic relationships within *Tylopilus* s.l. remain unresolved due to the relatively small number of DNA isolates currently available for this large puzzling genus and an evaluation of the species/group limits is still premature.

## Disclosure

The authors declare no conflict of interest.

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## Recommended articles

1.

[Lectotypification, epitypification, and molecular phylogeny of the synnematos hyphomycete \*Pseudogliophragma indicum\*, the second genus in the Wiesneriomycetaceae](#)

2015, Mycoscience

2.

[\*Coprinopsis igarashii\* sp. nov., a coprophilous agaric fungus from Hokkaido, northern Japan](#)

2015, Mycoscience

3.

[Phylogenetic relationships among biological species of \*Armillaria\* from China](#)

2015, Mycoscience