

Macroscopic variability of *Rubroboletus legaliae* with special regard to *Boletus spinarii*

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The paper deals with the macroscopic variability of *Rubroboletus legaliae*. A detailed macroscopic description of this species is presented, based on collections from the region of the type locality in Central Bohemia. An epitype is selected because of the age and insufficient representativeness of the holotype. The authors point out that *Boletus spinarii*, a species described by Hlaváček from South Bohemia as a member of the *B. regius* complex (genus *Butyriboletus* at present), is conspecific with the previously described *Boletus legaliae* (genus *Rubroboletus* at present); therefore the name *B. spinarii* is a synonymous name. The alleged distinguishing character of *B. spinarii* – orange, cinnabar to brick-red pores when young, soon changing colour to orange-yellow or yellow – is occasional according to our long-term field observations, caused possibly by external factors and not fixed within individual mycelia of *R. legaliae*. The taxonomic value of this deviation is not important enough to consider a separate taxon. Comparison of ITS rDNA sequences supports the conspecificity of both species. Although the name *B. spinarii* was validly published, the holotype was not deposited in the herbarium designated in the protologue. Therefore, a neotype is designated here.

Key words: *Boletus legaliae* f. *spinarii*, ITS sequence data, neotype, epitype, Czech Republic.

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Článek se zabývá makroskopickou variabilitou hříbu Le Galové – *Rubroboletus legaliae*. Je prezentován jeho podrobný makroskopický popis sestavený podle sběrů pocházejících z oblasti typové lokality ve středních Čechách. S ohledem na stáří a nedostatečnou reprezentativnost holotypu je stanoven epityp. Autoři článku poukazují na fakt, že hřib Špinarův – *Boletus spinarii*, popsáný Hlaváč-

kem z jižních Čech jako druh z příbuzenstva *B. regius* (dnešní rod *Butyriboletus*), je totožný s dříve popsáním hříbem Le Galové; jméno *B. spinarii* je proto synonymum. Údajný charakteristický znak hříbu Špinarova – v mládí oranžově, rumělkové až cihlově červené póry rourek brzy měnící barvu do oranžovožluta až žluta – je podle našich dlouhodobých pozorování v terénu nahodilý, způsobený pravděpodobně vnějšími podmínkami a není pevně zafixován v rámci jednotlivých mycelií *R. legaliae*. Taxonomická hodnota této odchylky proto není natolik významná, aby mohla být hodnocena jako samostatný taxon. Totožnost obou druhů je podpořena porovnáním sekvencí ITS rDNA. Ačkoli bylo jméno *B. spinarii* platně publikováno, holotyp nebyl uložen do herbáře uvedeného v protologu. Je proto stanoven neotyp.

INTRODUCTION

During his long-term regular survey in Luční National Nature Monument (before 1999), Pavel Špinar observed an interesting feature on a bolete which he knew under the name *Boletus legaliae* Pilát & Dermek (previously also named *Boletus splendidus* subsp. *splendidus* C. Martín, see e.g. Beran & Špinar 1996). The initially orange-red pores of tubes in young and middle-aged fruit bodies of this bolete discoloured with time and finally changed to almost or completely yellow at maturity. He reported this feature and showed fruit bodies coloured this way (Fig. 12) to Jiří Hlaváček in the course of their joint visit to the site of Luční on 15 September 1999. Three days later they visited the locality again and collected material of 12 fruit bodies in various developmental stages, which Hlaváček studied in detail (Hlaváček 2000). He considered this bolete to be a new, undescribed species, but mentioned by some French authors under various names in the past. For the first time, this bolete was presented by Peltereau (1926: tab. 17), titled „*Boletus regius* Krombh. forme (*Boletus torosus*)“. Konrad & Maublanc (1935: no. 402) classified it as a member of a wider species concept of *Boletus appendiculatus* – in the rank of subspecies (*B. appendiculatus* subsp. *torosus*). Blum (1965, 1970) followed Peltereau and named this bolete *Boletus regius* var. *peltereaui*. Based on this, Hlaváček decided to describe it as a new species closely related to *B. regius* Krombh. [= *Butyriboletus regius* (Krombh.) D. Arora & J.L. Frank], therefore as a member of the butter bolete group, labelled as subsection *Duri* within the genus *Boletus* s.l. (for his overall systemisation, see Hlaváček 1992).

Unfortunately, Hlaváček did not state any characters important for delimitation of the species from the most similar *Rubroboletus legaliae* (Pilát & Dermek) Della Maggiora & Trassin. in the protologue. As we considered this fact a basic insufficiency of his publication, we decided to clarify all the aspects associated with *B. spinarii*. Furthermore, the aim of this paper is to present results of a comparison of both species morphologically and molecularly (ITS rDNA sequences) and to show the variability of *R. legaliae* by means of a supplement of colour photographs.

MATERIAL AND METHODS

Macrocharacters of the fruit bodies were studied on fresh material collected in the Czech Republic during about last 15 years and documented with photographs. The herbarium specimens have been deposited in the Mycological Department, National Museum, Prague (PRM). Abbreviations of public herbaria follow Thiers (on-line).

Ponds mentioned in this paper are man-made, created by intentional damming of streams for fishery purposes (despite the fact that larger ones may be referred to as lakes, the term is unified as ponds regardless their size).

In the collection of *Boletus spinarii*, nuclear DNA was extracted from a dry piece of fungal biomass using the NucleoSpin® Plant II extraction kit (Macherey-Nagel, Düren, Germany) according to the manufacturer's instructions. In other collections (Tab. 1), the NaOH extraction method was used (Osmundson et al. 2013). The ITS rDNA region (ITS1, 5.8S, and ITS2 sequences) and the D1-D2 domain at the 5' end of the nuclear LSU rRNA gene were amplified with primer pairs ITS1F-ITS4B and NLI-NL4, respectively, under PCR conditions described in Borovička et al. (2011). In *B. spinarii*, ITS rDNA sequencing was based on a seminested PCR product obtained with primer pair ITS1F-ITS4 in the second step. Amplicons were purified by isopropanol precipitation. Both strands were sequenced at Macrogen Europe (Amsterdam, the Netherlands). Sequences were edited in BioEdit (Hall 1999) and submitted to EMBL-Bank.

Tab. 1. Sequenced collections of *Boletus spinarii* and *Rubroboletus legaliae*.

Species	Sample ID	Herbarium specimen	EMBL-Bank accession numbers
<i>Boletus spinarii</i> , neotype	B 37	PRM 894134	LT797165, LT797166
<i>Rubroboletus legaliae</i>	B 30	PRM 924881	LT797162
<i>Rubroboletus legaliae</i>	B 31	PRM 945070	LT797163
<i>Rubroboletus legaliae</i> , epitype	B 32	PRM 945076	LT797164, LT838330

Evolutionary analysis was conducted in MEGA7 (Kumar et al. 2016), using the Maximum likelihood method based on the Kimura 2-parameter model (Kimura 1980). Taxon sampling was based on species included in the ITS rDNA phylogeny tree published in Zhao et al. (2014: Table 2). Corresponding sequences were downloaded from public databases GenBank and UNITE (UDB accession numbers) and aligned using Kalign (Lassmann & Sonnhammer 2005) at <http://www.ebi.ac.uk/Tools/msa/kalign/>. The tree with the highest log likelihood (-1541.7495) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial trees for the heuristic search

were obtained automatically by applying the Neighbor-join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum composite likelihood (MCL) approach, and then selecting the topology with the superior log likelihood value. A discrete Gamma distribution was used to model evolutionary rate differences between sites [5 categories (+G, parameter = 0.3433)]. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 23 nucleotide sequences. All positions containing gaps and missing data were eliminated. There were a total of 432 positions in the final dataset. *Caloboletus calopus* (Pers.) Vizzini (KJ605655) was used as outgroup.

RESULTS AND DISCUSSION

MOLECULAR STUDY

The ITS rDNA sequence obtained in *Boletus spinarii* (B 37) matches the sequences of *Rubroboletus legaliae* (B 30–32), which supports our hypothesis of conspecificity of both taxa. According to the phylogenetic analysis (Fig. 1), both taxa are placed in the *Rubroboletus* clade, closely related to *R. sinicus* (W.F. Chiu Kuan Zhao & Zhu L. Yang, which is the type species of the genus *Rubroboletus* Kuan Zhao & Zhu L. Yang.

TAXONOMY

Boletus spinarii Hlaváček, Mykol. Sborn. 77(2): 59, 2000

H o l o t y p e. None deposited. In the protologue, Hlaváček (2000) stated that a holotype specimen is deposited in the herbarium of the Mycological Department of the National Museum in Prague and provided details on locality, date and collectors as follows: „Luční“ (i.e. area of Luční National Nature Monument, Tábor District, South Bohemia), 18 Sept. 1999, leg. P. Špinar et J. Hlaváček (however, without voucher number). We looked through all of Hlaváček’s material deposited here in the period from 1999 to 2002 (year of the author’s death). Unfortunately no collection with the given date was found. This finding is somewhat bizarre from the nomenclatural point of view – the name was validly published according to the ICN (McNeill et al. 2012), although in reality the holotype specimen was not deposited by the author at the indicated herbarium.

O t h e r o r i g i n a l m a t e r i a l. No collection found. Four colour photos of fruit bodies collected by P. Špinar (erroneously stated as “J. Špinar”) and J. Hlaváček at the type locality of „Luční“ from September 1999 have been published as a part of the protologue (Hlaváček 2000). Pavel Špinar confirmed that 18 Sept. 1999 is the date of these photos. Hlaváček’s macro- and microscopic description based on collected fruit bodies at the type locality of „Luční“ on 18 Sept. 1999 labelled “*Boletus spinarii* nom. prov.” (handwritten inheritance; six pages of both descriptions and drawings in notebook) is deposited in the archive of the Mycological Department of the National Museum in Prague.

N e o t y p e (designated here, MycoBank MBT375043). Czech Republic, South Bohemia, Turovec near Sezimovo Ústí, on dike of Luční Pond (Luční National Nature Monument), alt. 417–419 m, under *Quercus robur*, 17 Aug. 2000, leg. J. Hlaváček et P. Špinar, det. J. Hlaváček. Neotype deposited in the National Museum, Prague (PRM 894134). EMBL-Bank: LT797165, LT797166. This voucher represents

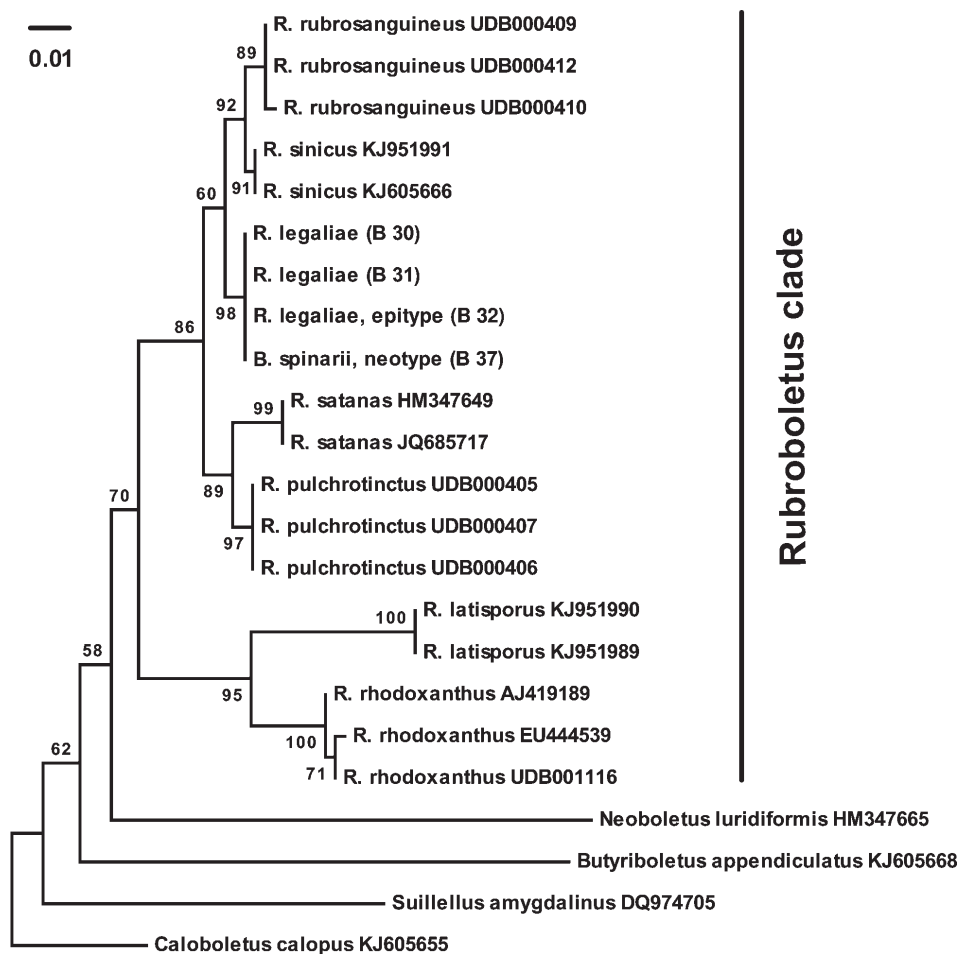


Fig. 1. Maximum likelihood phylogenetic tree generated from ITS rDNA sequences. Bootstrap support values are indicated at the branches.

the only collection deposited by Hlaváček under the name *Boletus spinarii* in PRM. All data on this voucher correspond with those in the protologue except for the later date. We therefore selected this collection as a neotype.

Etymology. Named after Pavel Špinar, Czech mycologist, currently voluntary ranger of Luční National Nature Monument in South Bohemia.

Selected illustrations. Hlaváček (2000): colour supplement of Mykol. Sborn. 77(2); Papoušek (2004): p. 722.

Original description. Pileus – 12 cm diam., primo haemisphaericus – convexus, crasse carnosus, laevi, glabro, albido griseolus, roseo inhalatus, deinde roseus – roseocarmineus-purpureus. Stipes – 9/5 cm, ovato ventricosus – claviformis, crassus, superne rubellus, basi luteo subbrunescenti.

Superne sanguineo reticulatus. Caro flavida, caerulescens, odore gratis B. variegati similis. Tubuli lutei, subcaerulescens, pori minutis, junior modo coccineis, deinde aurantio luteis, tactu caerulescentis. Sporis longitudine elipsoideis, subluteis (10)11–13,5(15) × 4,5–5,5 µm. Basidia tetrasporis, – 10 µm latis. Cystidia clavi- fusiformis, – 9 µm latis. In silvis frondosis, in quercetis et fagetis, rarius. Holotypus exsiccatus in mycoherbario Musei Nationalis Pragensis depositus est. Holotypus in loc. “Luční” 18. 9. 1999 leg. Špinar et Hlaváček.

Notes. Hlaváček’s publication of the new species *Boletus spinarii* has caused embarrassment, since characters distinguishing this species from similar taxa, especially *B. legaliae*, are missing. Moreover, we could not consult our doubts concerning *B. spinarii* with Hlaváček himself because of his sudden death in 2002. For these reasons we have decided to explore *B. spinarii* in detail. Our conclusions are as follows.

Red to orange-red pores when young (after pileus opening) or middle-aged obviously indicate a taxon belonging to a genus created for species formerly united in section *Luridi* of genus *Boletus* s.l. (e.g. Muñoz 2005, Šutara et al. 2009). All studied fruit bodies showed clearly opened pores at all stages – indicating a taxon belonging to the group of boletes without a conspicuous coherent layer of cheilocystidia (see Šutara 2014), therefore the genus *Butyriboletus* is excluded. The results of our observations and also our subsequent monitoring of the type locality of *Boletus spinarii* convinced us that this taxon is conspecific with *B. legaliae*, nowadays validly recombined as *Rubroboletus legaliae* (Pilát & Dermek) Della Maggiora & Trassin.¹ Moreover, a test of the edibility of “*B. spinarii*” performed by Pavel Špinar (pers. comm.) resulted in strong gastrointestinal problems, which is typically caused by *Rubroboletus* species when eaten raw (a small piece of fruit body collected on 18 Sept. 1999 was sufficient!).

Janda (in Šutara et al. 2009) proposed the new combination *Boletus legaliae* f. *spinarii*. Although the rank of form is the least important taxonomic level, we

¹ The name *Rubroboletus legaliae* (Pilát & Dermek) Della Maggiora & Trassin. was published on 10 June 2015 by Della Maggiora (via Index Fungorum). The same combination (except for a different epithet orthography) was later also used in a PDF document authored by M. Mikšík, P.-A. Moreau and B. Assyov, unofficially released via academic social network ResearchGate in 2015 allegedly as a paper of Documents Mycologiques. This work entitled “Validation of *Rubroboletus le-galiae*, comb. nov.” was, however, just a provisional manuscript version, finally published in a substantially revised form and under a different title (Mikšík et al. 2016 – published on 2 March 2016, although dated 2015). Curiously enough, all characters of the document in question agree with those of a standard paper published in Documents Mycologiques and the lack of a watermark indicating a manuscript (proof) makes it indistinguishable from the correct final version. However, the nomenclatural consequences of spreading such a work cannot be regarded a relevant matter according to ICN. The publishing of the manuscript seems to indicate desperation of any of the authors motivated by the possibility of publishing nomenclatural novelties instantly via “Index Fungorum e-Publishing”. Notwithstanding this event, the work by Della Maggiora (2015) really represents a paradigmatic example of the Index Fungorum novelties – it contains solely a new combination, furthermore with a corrupted abbreviation of Albert Pilát. For more criticism on this theme, see Janda & Kříž (2016a).

now consider the name *B. spinarii* simply to be a synonym. Unlike the case of e.g. *Rubroboletus satanas* (Lenz) Kuan Zhao & Zhu L. Yang and its xanthoid form *R. satanas* f. *crataegi* (Smotl. ex Antonín & Janda) Janda & Kríž (see Janda & Kríž 2016b), we do not consider the form of *R. legaliae* with yellow pores so markedly different from the typical form that it should be established as a separate taxon. According to our long-term field observations, the character of orange, cinnabar to brick-red pores when young, soon changing colour to orange-yellow or yellow in the case of *R. legaliae*, happens occasionally and is possibly caused by external conditions and not fixed in particular mycelia of *R. legaliae*. This character can even be regarded as a peculiar feature of this species. However, the combination *Rubroboletus legaliae* f. *spinarii* exists, as proposed by “Mikšík” (2015) [Mikšík to be exact].

***Rubroboletus legaliae*²** (Pilát & Dermek) Della Maggiora & Trassin. in Della Maggiora, Index Fungorum 246: 1, 2015 Figs. 2–16

Synonyms:

- ≡ *Boletus legaliae* Pilát & Dermek in Pilát, Houby Československa ve svém životním prostředí, p. 52, 1969 [“*le-galiae*”; basionym].
- = *Boletus spinarii* Hlaváček, Mykol. Sborn. 77(2): 59, 2000.
- = *Boletus legaliae* f. *spinarii* (Hlaváček) Janda in Šutara et al., Hřibovité houby, p. 38, 2009.
- = *Suillellus legaliae* f. *spinarii* (Hlaváček) Blanco-Dios, Index Fungorum 211: 1, 2015.
- = *Rubroboletus legaliae* f. *spinarii* (Hlaváček) Mikšík, Index Fungorum 260: 1, 2015.

Designations not validly published:

- *Boletus purpureus* var. *le-galiae* Pilát in Pilát & Ušák, Naše houby II. Kritické druhy našich hub: number 4, 1959 [no type indication; ICN Art. 40.1].
- *Boletus purpureus* var. *le-galiae* Pilát in Pilát & Ušák, Mushrooms and other fungi: number 4, 1961 [no type indication; ICN Art. 40.1].
- *Boletus legaliae* Pilát in Blum, Rev. Mycol. (Paris) 33(1): 124, 1968 [no type indication; ICN Art. 40.1] [“*Le Galliae*”].
- *Rubroboletus legaliae* (Pilát) Mikšík, Index Fungorum 207: 1, 2014 [invalid basionym; ICN Art. 41.5].
- *Suillellus legaliae* (Pilát) Blanco-Dios, Index Fungorum 211: 1, 2015 [invalid basionym; ICN Art. 41.5].

Holotype. Czech Republic, Central Bohemia, Lysá nad Labem, IX. 1949, leg. A. Lukavec (PRM 647975).

Epitype (designated here, MycoBank MBT375223). Czech Republic, Central Bohemia, Záhornice near Městec Králové, on bank of Jakubský Pond, alt. 205 m, under *Quercus* and *Corylus*, 5 Sept. 2015, leg. & det. V. Janda & T. Pavelka. Epitype deposited in National Museum, Prague (PRM 945076). EMBL-Bank: LT797164, LT838330.

E t y m o l o g y. Named after Marcelle Louise Fernande Le Gal (1895–1979), French mycologist.

S e l e c t e d i l l u s t r a t i o n s. Le Gal (1948): figs. 2–3 (as *Boletus lupinus*); Pilát (1951): p. 467, fig. 57a, p. 468, fig. 60, p. 508, fig. 168 (as *B. purpureus*); Pilát & Ušák (1959, 1961): fig. 4 (as *B. purpureus*

² We did not follow Pilát’s original orthography of the epithet “*le-galiae*”, recently used by Mikšík and his colleagues (see Mikšík et al. 2016). Analogous cases of epithets without the hyphen are e.g. *Clitocybe legaliae* E. Ludw. and *Hypoxyylon vandervekenii* Van der Gucht, Y.M. Ju & J.D. Rogers.

var. *le-galiae*); Phillips (1981): p. 198 (as *B. satanoides*); Papoušek (2004): p. 728, fig. 800 (as *B. legaliae*), p. 731, fig. 803 (as *B. satanoides*); Muñoz (2005): p. 745–747, fig. 69 a–f (as *B. legaliae*); Šutara et al. (2009): p. 181–183 (as *B. legaliae*); Eyssartier & Roux (2011): p. 103 (as *B. legaliae*); Holec et al. (2012): p. 577, fig. 1153 (as *B. legaliae*); Kibby (2012, 2013): p. 31, figs. 38–39 (as *B. legaliae*); Andersson (2013): p. 11–12 (as *B. legaliae*); Hagara (2014): p. 510 (as *B. fuscroseus*), p. 519 (as *B. legaliae*, *B. spinarii*); Halama (2015): figs. 2–3. See also other selected illustrations of *B. spinarii* (above).

Misappl. illustrations. Breitenbach & Kränzlin (1991): p. 65, no. 23 (probably *Rubroboletus rubrosanguineus*); Galli (1998): p. 232–233 (probably *R. rubrosanguineus*); Hlaváček (1998): title-page (probably *R. satanas*); Galli (2007): p. 234–235 (probably *R. rubrosanguineus*); Galli (2013): p. 236–237 (probably *R. rubrosanguineus*).

Original description. *Boletus purpureus* Fr. var. *le-galiae* v. n. (Syn. *Boletus lupinus* sensu Bresadola non Fr.) A typo differt pileo obscurius colorato, sordide obscure griseo usque brunneo-olivaceo, ad marginem sordide purpureo, sordide carmineo-vinoso, carne pallidius lutea, sulphurina; stipite pallidius luteo et magis quam typus, sed sordide purpureo. In silvis frondosis locis umbrosis.

Description of macroscopic characters. Pileus 60–140(180) mm broad, hemispherical at first, then convex, broadly convex to pulvinate, at first whitish, dirty whitish or greyish, later becoming grey-pinkish or brownish (mostly in the central part) with pinkish, reddish or purplish tints in places, finally sometimes entirely reddish or purplish, especially in wet conditions. Surface at first finely tomentose (Fig. 8), gradually becoming more or less glabrous with age (Fig. 9), brownish when bruised. Subcuticular layer reddish or red (Fig. 7).

Tubes 5–20 mm long, vivid yellow, later with olive-yellow tint, depressed around stipe apex when mature. Pores minute, roundish, open from an early stage (without coherent layer of cheilocystidia), yellowish (Fig. 4) when young (under closed pileus), but soon (from beginning of the pileus opening) usually becoming orange-red (Fig. 2), rarely almost red (Fig. 5), and then gradually discolouring to orange or orange-yellow, sometimes to pure yellow with age (Figs. 12–16). Tubes and pores blueing when cut, bruised or touched. Red line between tubes and pileus context absent.

Stipe 50–80(120) mm long, 30–50(70) mm thick, fleshy, ovoid to ventricose when young, then cylindrical to clavate, reddish over the entire length when young, then usually bicoloured – light yellow to orange-yellow in upper part and reddish or red-purple in middle and lower parts, sometimes ± entirely red or predominantly yellowish, covered with a less distinct orange to orange-red reticulum in upper and middle parts. Tomentum on stipe base whitish or dirty yellowish.

Context light yellow in pileus and stipe apex, somewhat paler in lower half of stipe, sometimes with dull red or red-brownish spots in stipe base; staining blue over the entire length after cutting when young and middle-aged, later only in pileus and upper part of the stipe.

Taste mild, smell not very distinctive in young fruit bodies, but becoming characteristic, like lovage or Maggi seasoning when drying and in mature fruit bodies.



Fig. 2. *Rubroboletus legaliae*, Jakubský Pond, Záhornice near Městec Králové, Czech Republic, under *Quercus* and *Corylus*, 5 Sept. 2015 (PRM 945076, epitype). Photo V. Janda.



Fig. 3. *Rubroboletus legaliae*, Knězičky Game Preserve, Knězičky, Czech Republic, under *Quercus*, 29 July 2016 (PRM 945070). Photo V. Janda.



Fig. 4. *Rubroboletus legaliae*, Komárovský Pond, Břístev, Czech Republic, under *Quercus*, *Carpinus* and *Tilia*, 27 June 2009 (PRM 945055). Photo V. Janda.



Fig. 5. *Rubroboletus legaliae*, Jakubský Pond, Záhornice near Městec Králové, Czech Republic, under *Quercus* and *Corylus*, 1 July 2016 (PRM 945065). Photo M. Kříž.



Fig. 6 (left). *Rubroboletus legaliae*, Kněžičky Game Preserve, Kněžičky, Czech Republic, under *Quercus*, 18 July 2009 (PRM 945059). Photo V. Janda. **Fig. 7 (right).** *Rubroboletus legaliae*, cross-section – subcuticular purplish red layer of pileus. Jakubský Pond, Záhornice near Městec Králové, Czech Republic, under *Quercus* and *Corylus*, 10 July 2016 (PRM 945066). Photo V. Janda.

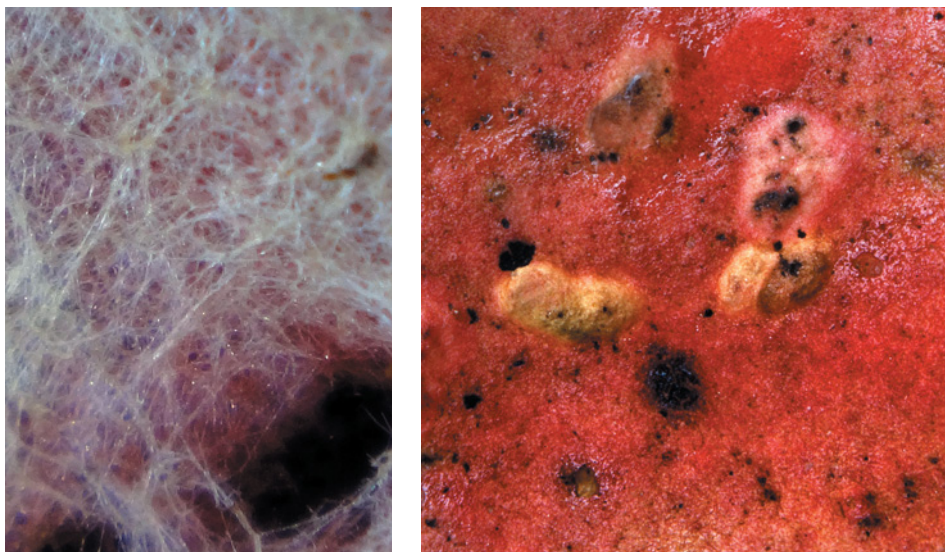


Fig. 8 (left). *Rubroboletus legaliae*, filamentous trichoderm of pileus. Jakubský Pond, Záhornice near Městec Králové, Czech Republic, under *Quercus* and *Corylus*, 10 July 2016 (PRM 945066). Photo V. Janda. **Fig. 9 (right).** *Rubroboletus legaliae*, glabrous surface of pileus after rainfall showing purplish red subcuticular layer. Nový Kravín Pond, Turovec, Czech Republic, under *Quercus*, 18 July 2002 (not documented by voucher). Photo V. Janda.



Fig. 10. *Rubroboletus legaliae*, Bechov, Czech Republic, under *Quercus* and *Carpinus*, 15 July 2006 (PRM 945016, PRM 945017). Photo M. Kříž.



Fig. 11. *Rubroboletus legaliae*, Kněžíčky Game Preserve, Kněžíčky, Czech Republic, under *Quercus*, 11 Aug. 2012 (PRM 924207). Photo V. Janda.



Fig. 12. *Rubroboletus legaliae*, on dike of Luční Pond, Turovec, Czech Republic, under *Quercus*, 15 Sept. 1999 (not documented by voucher). Photo P. Špinar.



Fig. 13. *Rubroboletus legaliae*, Komárovský Pond, Břístev, Czech Republic, under *Quercus*, 6 Sept. 2008 (PRM 945052). Photo V. Janda.



Fig. 14. *Rubroboletus legaliae*, Jakubský Pond, Záhornice near Městec Králové, Czech Republic, under *Quercus* and *Corylus*, 12 Aug. 2014 (not documented by voucher). Photo M. Kříž.



Fig. 15, 16. *Rubroboletus legaliae*, Jakubský Pond, Záhornice near Městec Králové, Czech Republic, under *Quercus* and *Corylus*, 23 Aug. 2012 (PRM 944956). Photo M. Kříž.

Notes. *Rubroboletus legaliae* is a species very variable in colour. The overall appearance of the fruit bodies depends to a large degree on external conditions. The pileus colour is directly conditioned by weather. Presence of pinkish or reddish pileus tints can be observed particularly when wet (especially after rainfalls) (Figs. 6, 9), while brownish tints are the result of dry weather (long-term deficit of moisture also causes cracking of the pileus). On the other hand, the colour of pores and stipe surface is determined by air oxidation – deficient access of air to the surface tissues causes a lower production of the red pigment variegatorubin contained in fruit bodies of this species (Gill & Steglich 1987, cited here as *B. splendidus* subsp. *splendidus*).

The type locality of *R. legaliae* is only indicated by the name of the small town of Lysá nad Labem in Central Bohemia. The locality in question is Semická hůrka hill (Šebek 1979), situated south of the town. Although we don't have any recent find of *R. legaliae* from this site, it is inhabited by a thermophilous fungal community (comprehensively delineated by Šebek and later confirmed by us) containing also other boletes characteristic of relatively warm habitats with a dominance of oaks (for a more detailed description, see Janda et al. 2013). This community is relatively frequent in this region and *R. legaliae* is one of its typical representatives (see frequency of the name of the geomorphological unit "Středolabská tabule plateau" in the section Material examined). As the holotype is almost seventy years old and contains only a small piece of pileus, the voucher specimens selected for our molecular study are recent and come from nearby localities which ecologically correspond to the presumed original site, Semická hůrka hill. We selected one of these representatives and sequenced collections as the epitype (Fig. 2, PRM 945076).

Similar taxa

The most similar European species *Rubroboletus rubrosanguineus* (Cheype) Kuan Zhao & Zhu L. Yang differs from *R. legaliae* by its growth in submontane forests under *Abies*, *Picea* and *Fagus* (never under *Quercus* in thermophytic areas). Often hardly noticeable macroscopic differences include the more saturated red tints on the fruit body in the case of *R. rubrosanguineus* (typically with a purplish to bloody hue) and usually also a more permanent red colour of the pores. In the Czech Republic, *R. rubrosanguineus* is known only from coniferous forests at higher altitudes in eastern Moravia – outside the abovementioned distribution area of *R. legaliae*.

Other similar species are macroscopically rather well distinguishable from *R. legaliae* without having to take into consideration the ecology of the finds: *R. rhodoxanthus* (Krombh.) Kuan Zhao & Zhu L. Yang differs by its conspicuous vivid red reticulum covering almost the entire length of the stipe, and its yellower

context with a fruity smell. *Rubroboletus satanas* (Lenz) Kuan Zhao & Zhu L. Yang differs by its more robust fruit bodies, pale greyish pileus usually without red or purplish tints (due to the absence of a conspicuous red subcuticular layer), and only weak blueing of the whitish coloured context with a characteristic disagreeable smell when mature. *Butyriboletus regius* (Krombh.) Arora & J.L. Frank is well distinguished by its constantly yellow pores, closed when young due to a conspicuous coherent layer of cheilocystidia (see Šutara 2014), and by its non-blueing (or at most only very slow blueing) context having an insignificant smell.

Material examined

Abbreviations. Names of the collectors are abbreviated as follows (in alphabetical order): VJ (Václav Janda), MK (Martin Kříž), ZK (Zdeněk Kučera), LO (Lubomír Opat), TP (Tomáš Pavelka), JR (Jiří Rejsek), JŠ (Josef Šutara).

Czech Republic. Bohemia. Žďár near Mnichovo Hradiště (Central Bohemia, Jičínská pahorkatina hills), on dike of Hájenský (Zezulák) Pond, under *Quercus*, 20 July 2003, leg. & det. O. Jindřich, J. Sedláček & VJ (PRM 944996, PRM 944997, PRM 944998). – Úhelnice (Central Bohemia, Jičínská pahorkatina hills), Bor forest, on forest track under *Quercus* and *Carpinus*, 6 Sept. 2007, leg. J. Soldát, VJ, MK & ZK, det. MK & VJ (PRM 945031 – photo in Mikšík 2012, p. 16, fig. 11, incorrectly mentioned to be from “East Bohemia”); *ibid.*, 9 Sept. 2007, leg. J. Soldát, VJ, S. Kučerová & M. Kučera, det. VJ (PRM 945032). – Obrubce (Central Bohemia, Jičínská pahorkatina hills), Obrubce forest, under *Quercus*, 7 Aug. 2004, leg. R. Knížek, det. VJ & R. Knížek (PRM 945004); *ibid.*, 28 Aug. 2007, leg. VJ, JR & ZK, det. VJ (PRM 945024 – photo in Šutara et al. 2009, p. 182 below, PRM 945025). – Bechov (Central Bohemia, Jičínská pahorkatina hills), deciduous forest west of the village of Svobodín, under *Quercus* and *Carpinus*, 31 July 2004, leg. R. Knížek & V. Barabáš, det. VJ (PRM 945003); *ibid.*, 15 July 2006, leg. & det. O. Jindřich, J. Sedláček, MK & VJ (PRM 945016, PRM 945017); *ibid.*, 19 July 2007, leg. VJ, TP, JŠ & A. Vít, det. VJ, TP & JŠ (PRM 945023); *ibid.*, 11 Aug. 2007, leg. VJ, JR, MK & ZK, det. VJ & MK (PRM 945027); *ibid.*, 2 Sept. 2016, leg. LO, det. LO & VJ (PRM 945075). – Pěčice (Central Bohemia, Jizerská tabule plateau), on dike of Malopěčický Pond, under *Quercus*, 16 Sept. 2004, leg. & det. MK (PRM 945011); *ibid.*, 1 July 2006, leg. Z. Pelda, det. J. Sedláček (PRM 945013); *ibid.*, 28 Sept. 2014, leg. VJ, TP & LO, det. VJ (PRM 924881). – Libáň, Kozodírky (Central Bohemia, Středolabská tabule plateau), south bank of Stejskal Pond, under *Quercus*, 27 June 2004, leg. Sedláčková, det. VJ & A. Vít (PRM 945001). – Prodašice (Central Bohemia, Středolabská tabule plateau), deciduous forest, under *Quercus* and *Carpinus*, 20 July 2003, leg. & det. O. Jindřich & J. Sedláček (PRM 944999); *ibid.*, 8 July 2006, leg. Horáková, det. J. Sedláček (PRM 945014); *ibid.*, on dike of forest pond, under *Quercus* and *Carpinus*, 4 Aug. 2007, leg. & det. VJ & MK (PRM 945026); *ibid.*, deciduous forest, under *Quercus* and *Carpinus*, 5 Sept. 2015, leg. & det. J. Borovička, B. Bušek & M. Mikšík (PRM 934871). – Brodek (Central Bohemia, Středolabská tabule plateau), Dymokursko – Bahenské louky Nature Monument, eastern bank of Knížek Pond, under *Quercus*, *Carpinus* and *Tilia*, 4 Sept. 2007, leg. & det. JR & ZK (PRM 945030). – Břístev (Central Bohemia, Středolabská tabule plateau), site named “Lichovky”, under *Quercus*, 6 Aug. 2013, leg. & det. JR (PRM 924193). – Břístev (Central Bohemia, Středolabská tabule plateau), northern bank of Komárovský Pond, under *Quercus*, *Carpinus* and *Tilia*, 11 Aug. 2007, leg. VJ, JR & MK, det. VJ & MK (PRM 945028); *ibid.*, under *Quercus*, 6 Sept. 2008, leg. & det. VJ (PRM 945052); *ibid.*, under *Quercus*, *Carpinus* and *Tilia*, 27 June 2009, leg. & det. VJ & JR (PRM 945055); *ibid.*, 4 July 2009, leg. & det. VJ & JR (PRM 945060); *ibid.*, 11 July 2009, leg. & det. JR (PRM 945061); *ibid.*, under *Quercus*, 1 Aug. 2010, leg. & det. VJ, JR & TP (PRM 945064); *ibid.*, under *Quercus*, *Carpinus* and *Tilia*, 30 July 2016, leg. & det. VJ & JR (PRM 945071); *ibid.*, 2 Aug. 2016, leg. & det. VJ, JR & LO (PRM 945072). – Nouzov (Central Bohemia,

Středolabská tabule plateau), southern bank of Komárovský Pond, under *Quercus*, *Carpinus* and *Tilia*, 26 July 2016, leg. & det. VJ & JR (PRM 945069). – Dymokury (Central Bohemia, Středolabská tabule plateau), deciduous forest on bank of Pustý Pond, under *Quercus* and *Acer campestre*, 14 July 2007, leg. & det. VJ (PRM 945020); *ibid.*, under *Quercus* and *Corylus*, 7 Sept. 2013, leg. & det. VJ (PRM 924173). – Záhornice (Central Bohemia, Středolabská tabule plateau), northern bank of Jakubský Pond, under *Quercus* and *Corylus*, 20 July 2003, leg. & det. VJ (PRM 945000); *ibid.*, 10 July 2004, leg. & det. VJ (PRM 945002); *ibid.*, 15 July 2006, leg. & det. VJ (PRM 945015); *ibid.*, 22 June 2007, leg. VJ, JR & ZK, det. VJ (PRM 945018); *ibid.*, 14 July 2007, leg. & det. VJ (PRM 945019, PRM 945021); *ibid.*, 19 July 2007, leg. VJ, TP, JŠ & A. Vít, det. VJ, TP & JŠ (PRM 945022); *ibid.*, 16 July 2009, leg. TP, det. TP & VJ (PRM 945057); *ibid.*, 23 Aug. 2012, leg. MK & P. Mikuš, det. MK (PRM 944956); *ibid.*, 5 Sept. 2015, leg. & det. VJ & TP (PRM 945076 – epitype); *ibid.*, 1 July 2016, leg. & det. VJ, JR, MK & TP (PRM 945065); *ibid.*, 10 July 2016, leg. & det. VJ, LO, TP & JR (PRM 945066); *ibid.*, 13 July 2016, leg. LO, det. LO & VJ (PRM 945068). – Kněžičky (Central Bohemia, Středolabská tabule plateau), Kněžičky Game Preserve, under *Quercus*, 11 Aug. 2007, leg. VJ, JR, MK & ZK, det. VJ & MK (PRM 945029); *ibid.*, under *Quercus* and *Betula*, 11 June 2008, leg. VJ & ZK, det. VJ (PRM 945033, PRM 945034); *ibid.*, under *Quercus*, 21 June 2008, leg. VJ & JR, det. VJ (PRM 945035, PRM 945036, PRM 945037, PRM 945038); *ibid.*, 10 July 2008, leg. JR, VJ & JŠ, det. VJ & JŠ (PRM 945039); *ibid.*, 16 July 2008, leg. VJ & ZK, det. VJ (PRM 945040); *ibid.*, 18 July 2008, leg. VJ & ZK, det. VJ (PRM 945041, PRM 945042, PRM 945043); *ibid.*, 20 July 2008, leg. VJ, JR & ZK, det. VJ (PRM 945044); *ibid.*, 22 July 2008, leg. VJ & ZK, det. VJ (PRM 945045, PRM 945046, PRM 945047); *ibid.*, 25 July 2008, leg. VJ & JR, det. VJ (PRM 945048, PRM 945049 – photo in Šutara et al. 2009, p. 181 above, PRM 945050); *ibid.*, 2 Sept. 2008, leg. VJ, JR & ZK, det. VJ (PRM 945051); *ibid.*, 13 June 2009, leg. & det. VJ & JR (PRM 945053); *ibid.*, 23 June 2009, leg. & det. VJ & JR (PRM 945054); *ibid.*, 11 July 2009, leg. & det. VJ (PRM 945056); *ibid.*, 18 July 2009, leg. & det. VJ & JR (PRM 945058, PRM 945059); *ibid.*, 23 July 2009, leg. & det. VJ & JR (PRM 945062); *ibid.*, 15 Aug. 2009, leg. & det. VJ & JR (PRM 924212); *ibid.*, 27 July 2010, leg. & det. VJ (PRM 924206); *ibid.*, 31 July 2010, leg. & det. VJ & JR (PRM 924201, PRM 924214, PRM 945063); *ibid.*, 24 Sept. 2011, leg. VJ, JR & N. Melichová, det. VJ (PRM 924213); *ibid.*, 11 Aug. 2012, leg. & det. VJ & JR (PRM 924207); *ibid.*, 29 July 2016, leg. & det. VJ & JR (PRM 945070). – Chlumec nad Cidlinou (East Bohemia, Středolabská tabule plateau), Olešnice Nature Monument, deciduous forest, under *Quercus* and *Tilia*, 13 July 2016, leg. LO, det. LO & VJ (PRM 945067); *ibid.*, 4 Aug. 2016, leg. LO, det. LO & VJ (PRM 945073). – Chlumec nad Cidlinou (East Bohemia, Středolabská tabule plateau), Drábina forest, under *Quercus* and *Carpinus*, 16 Sept. 2015, leg. VJ, LO & TP, det. VJ (PRM 945077); *ibid.*, 14 Aug. 2016, leg. VJ, LO & TP, det. VJ (PRM 945074). – Turovec (South Bohemia, Táborská pahorkatna hills), on dike of Luční Pond, Luční National Nature Monument, under *Quercus*, 12 Aug. 2000, leg. & det. VJ (PRM 894101 – as “*Boletus spinarii* Hlaváček?”); *ibid.*, 17 Aug. 2000, leg. J. Hlaváček & P. Špinar, det. J. Hlaváček (PRM 894134; deposited as “*Boletus spinarii* Hlaváček” – neotype); *ibid.*, 31 Aug. 2004, leg. P. Špinar, N. Melichová & VJ, det. P. Špinar & VJ (PRM 945008). – Turovec (South Bohemia, Táborská pahorkatna hills), on dike of Nový Kravín Pond, under *Quercus*, 31 Aug. 2004, leg. P. Špinar, N. Melichová & VJ, det. P. Špinar & VJ (PRM 945007); *ibid.*, 3 Sept. 2004, leg. N. Melichová & VJ, det. VJ (PRM 945010); *ibid.*, 19 Sept. 2005, leg. & det. VJ (PRM 945012). – Lomnice nad Lužnicí (South Bohemia, Třeboňská pánev basin), on dike of Služebný Pond, under *Quercus*, 29 Aug. 2004, leg. N. Melichová & VJ, det. VJ (PRM 945005); *ibid.*, 2 Sept. 2004, leg. N. Melichová & VJ, det. VJ (PRM 945009). – Lomnice nad Lužnicí (South Bohemia, Třeboňská pánev basin), on dike of Velký Tisý Pond, under *Quercus*, *Tilia* and *Acer*, 29 Aug. 2004, leg. MK, N. Melichová & VJ, det. VJ (PRM 945006).

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