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Boletus kluzakii, a new species related to *Boletus radicans*

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A new species, *Boletus kluzakii*, which was found in southern Bohemia (Czech Republic), is described. Typical characters of this bolete are: pileus when young whitish or pallid, sometimes with a slight pinkish tint, then more or less rose-pink or purplish pink, at times even purple-reddish in some places; pileus surface conspicuously reddening when handled or bruised; hymenophore yellow, bluing when bruised; stipe yellow with a yellow reticulation; context under pileus cuticle red or purplish red but in other parts of the pileus and in upper half of the stipe pale yellowish or vivid yellow, turning blue when injured; taste bitter. Characters distinguishing *Boletus kluzakii* from *Boletus radicans* (the most closely related species) and from other boletes with a pinkish or reddish shade of pileus (*Boletus regius*, *B. fuscoroseus*, *B. speciosus*, *B. pulchrotinctus*, *B. roseoolivaceus*, *B. roseopurpureus* and *B. pulchriceps*) are discussed.

Key words: *Boletus kluzakii* spec. nov., *Boletus* sect. *Calopodes*, *Boletaceae*, taxonomy, Czech Republic.

Šutara J. a Špinar P. (2006): *Boletus kluzakii*, nový druh příbuzný druhu *Boletus radicans*. – Czech Mycol. 58(1–2): 31–42.

Je popsán nový druh *Boletus kluzakii* (hřib Kluzákův), který byl objeven v jižních Čechách. Typické znaky tohoto hřibu jsou: klobouk v mládí bělavý nebo bledý, někdy s lehkým narůžovělým nádechem, potom více nebo méně růžový nebo purpurově růžový, občas dokonce na některých místech purpurově načervenalý; povrch klobouku po ohmatání nebo otlačení nápadně červenající; hymenofor žlutý, po otlačení modrající; třeň žlutý se žlutou síťkou; dužnina pod pokožkou klobouku je červená nebo purpurově červená, ale v ostatních částech klobouku a v horní polovině třeně bledě žlutavá nebo živě žlutá, po poranění modrající; chuť hořká. Diskutovány jsou znaky odlišující druh *Boletus kluzakii* od druhu *Boletus radicans* (který je nejblíže příbuzný) a od jiných hřibů s narůžovělým nebo načervenalým odstínem klobouku (*Boletus regius*, *B. fuscoroseus*, *B. speciosus*, *B. pulchrotinctus*, *B. roseoolivaceus*, *B. roseopurpureus* a *B. pulchriceps*).

INTRODUCTION

The second author of this contribution has, for a long time, engaged in the study of the mycoflora on a dam of fish-pond Luční in southern Bohemia (Czech Republic). This noteworthy mycological locality, which is protected as a 'national nature monument' (abbreviation: NNM Luční), is well-known as a site of many interesting and rare fungi, above all some rare boletes, e.g. *Boletus appendiculatus*

Schaeff., *Boletus gentilis* (Quél.) Big. et Guill., *Boletus impolitus* Fr., *Boletus legaliae* Pilát et Dermek, *Boletus moravicus* Vacek, *Boletus fuscoroseus* Smotlacha [= *Boletus pseudoregius* (Huber) ex Estades], *Boletus radicans* Pers.: Fr. (= *Boletus albidus* Roques), *Boletus rubellus* Krombh. and *Rubinoboletus rubinus* (W. G. Smith) Pilát et Dermek (for detailed information about NNM Luční and lists of fungi found at the locality, see Špinar and Pravda 1990, Valter 1990, Beran and Špinar 1996).

Another very interesting bolete was found by P. Špinar at the locality NNM Luční in 1992. A later, joint observation of both authors showed that this bolete is well-characterised by some distinctive features. Several years' observations confirmed that these features are constant. As this bolete was not yet validly published, the present authors have decided to describe it as a new species.

MATERIALS AND METHODS

Macroscopic characters of the bolete described below were studied in all developmental stages on fresh carpophores growing at the above-mentioned locality in the course of several seasons in the years 1992–2000. Some of the studied carpophores have been deposited in private herbaria of the authors of this paper (abbreviations used: JŠ = herb. J. Šutara, PŠ = herb. P. Špinar) and in the National Museum, Prague (PRM).

Microscopic characters were examined predominantly on dried material. Sections from dried specimens were revived in Melzer's reagent and in a 3–10 % solution of ammonium hydroxide (NH_4OH) with or without Congo Red. Pigmentation of the cuticle and subcuticular layer of pileus was studied in pure water.

RESULTS

Boletus kluzakii Šutara et Špinar spec. nov.

= *Boletus fallax* Kluzák, Sborn. Jihočes. Muz. v Čes. Budějovicích, Přírod. Vědy 28: 39, 1988, illegitimate later homonym of *Boletus fallax* Corner, Boletus in Malaysia, p. 99, 1972.

Diagnosis latina: Pileus 7–15 cm latus, initio hemisphaericus, deinde convexus, ad ultimum pulvinatus, primum albidus, grisello-albidus, ochraceo-albidus vel roseo-albidus, postea plus minusve roseus vel purpureo-roseus, in aliquis locis interdum purpureo-rubellus vel bruneolus. Superficies pilei tactu conspicue rubescens, primum subtomentosa, gradatim glabrescens. Tubuli subadnati

vel subdepressi prope stipitem, 0,7–1,3 cm longi, flavi, fracti caerulescentes, pori concolores, minuti, rotundi, tactu fortiter caerulescentes. Stipes 6–9 × 2–3(–4) cm, cylindraceus vel cylindraceo-clavatus, reticulatus, flavus, basi interdum brunneo maculatus. Caro pallide flavida vel flava, fracta caerulescens; stratum carnis sub cute pilei rubrum vel purpureo-rubrum. Sapor amarus. Sporae leaves, subfusoideae, (10–)11,5–14(–16) × (4,5–)4,8–6(–6,5) µm.

Affinis *Boleti radicantis* Pers.: Fr., sed stratum carnis sub cute pilei rubrum vel purpureo-rubrum, superficies pilei tactu conspicue rubescens et pileus mox plus minusve roseus, purpureo-roseus, qui etiam interdum partim purpureo-rubellus.

Holotypus: Bohemia meridionalis, prope Sezimovo Ústí, in aggere piscinae Luční, in graminosis sub *Quercu robore*, 21.IX.1997, leg. P. Špinar et al. Holotypus in herbario Musaei Nationalis Pragae (PRM 857093) et isotypus in herbario privato J. Šutara asservatur (JŠ 3889).

Pileus at first hemispherical, then convex with an incurved margin, finally pulvinate, 7–15 cm in diameter, when young whitish or pallid with a slight greyish, cream-coloured or pinkish tint, then more or less rose-pink or purplish pink, at times even purple-reddish (rarely also brownish) in some places. Pileus surface immediately stains red when handled or bruised. After many hours the colour of the bruised places slowly changes from red to pale brownish or (when the pileus surface was bruised more strongly) to deep brown. Pileus cuticle subtomentose, gradually glabrescent, dry, matt but somewhat tacky (subviscid) when moist. Subcuticular layer of context red or purplish red. A hint of the reddish tinge of the subcuticular layer persists even in some dried specimens. Other parts of the pileus context pale yellowish or, above tubes, vivid yellow, when cut turning blue. Taste bitter, smell of mushrooms but not very distinct.

Tubes almost adnate or somewhat depressed around the stipe, 0.7–1.3 cm long, lemon-yellow, becoming olive-yellow or brownish yellow with age, bluing when cut. Pores small, roundish, concolorous with tube-sides, when bruised strongly bluing and then (after several hours) very slowly becoming brown.

Stipe 6–9 cm long, 2–3(–4) cm thick, cylindrical or somewhat broadened downwards, vivid yellow above, light yellow below, sometimes with rusty brown spots on base, reticulate nearly over the entire length or at least in upper part, reticulation yellow, darkening on handling. Context vivid yellow at apex, pale yellowish in middle part, ochraceous or light brownish and sometimes also partly wine-red in basal area, when cut changing to blue, particularly in apex. Basal tomentum yellowish or yellow-ochraceous. Partial veil and annulus absent.

Pileus cuticle a trichoderm, when young 400–600(–800) µm thick, composed of filamentous, erect or suberect hyphae, gradually collapsing in later developmental stages, finally forming a collapsed, only 50–100(–150) µm thick layer of hyphae more or less appressed to the pileus surface. In this collapsed state the

trichoderm sometimes looks like a cutis. Trichodermal hyphae (3–)4–7(–10) µm wide, consisting of long elements, in pure water colourless or (on some parts of pileus of older carpophores) pale brownish. Surface of the trichodermal hyphae smooth or covered with a gelatinous matter distinct in microscopic preparations stained with Congo-Red (in Melzer's reagent looking as if covered with an incrustation). Subcuticular layer of tissue reddish pink when mounted in pure water (but this reddish pigmentation disappears in Melzer's reagent or ammonium solution). Thickness of the reddish subcuticular layer (measured in revived herbarium material) 250–500(–700) µm. Pileus trama composed of loosely interwoven, filamentous or somewhat broadened hyphae, (3–)5–14(–18) µm wide, non-amyloid, light-coloured to honey-brownish in Melzer's reagent.

Hymenophoral trama true boletoid. Lateral strata divergent, more or less gelatinous, very light-coloured when mounted in Congo-Red, in a fully developed stage (30–)40–50(–60) µm thick, loosely arranged, with hyphae 3–14 µm wide, not touching each other. Distance between hyphae of the lateral stratum in transverse sections (2–)4–8(–12) µm. Mediostratum (8–)12–20(–30) µm, readily staining with Congo-Red, densely arranged, with hyphae touching one another. Subhymenium 7–10(–15) µm. Hymenium 24–30(–34) µm. Basidia clavate, mostly 4-spored, 22–38(–42) × 8–13 µm, usually with a granular and partly globular content. Cheilocystidia smooth– and thin-walled, fusiform or subclavate with somewhat pointed tops, 25–36 × 6–11 µm. Pleurocystidia very sparsely scattered, inconspicuous, smooth– and thin-walled, fusiform or fusiform-rostrate, rarely lageniform, 30–46 × 8–13 µm.

Spores of boletoid shape, in face view fusoid-ellipsoid, in profile inequilateral with suprahilar depression, smooth under a light microscope, (10–)11.5–14(–16) × (4.5–)4.8–6(–6.5) µm, Q (length-breadth ratio) = (1.7–)2.2–2.7(–3.0), yellow or brownish in Melzer's reagent. Spore print not obtained (probably brownish with an olivaceous tint as in other boletes of *Boletus* L. section *Calopodes* Fr.).

Stipe surface fertile, covered by a gradually fragmenting caulohymenium with scattered sporulating caulobasidia. Caulohymenium 25–32(–35) µm. Caulobasidia 24–40 × 8–13 µm, clavate, mostly 4-spored, often with a granular and partly globular content. Caulocystidia fusiform or fusiform-rostrate, 30–50 × 7–12 µm. Lateral stipe stratum of boletoid type, well-developed in upper and middle part of stipe, loosely arranged, with a tendency to gelatinise, (30–)40–90 µm thick, in ridges of the stipe reticulation up to 170 µm (for the meaning of the term 'lateral stipe stratum', see Šutara 2005). Stipe base infertile, covered with a tomentum loosely entangled of filamentous, 3–6(–8) µm wide hyphae. Stipe trama composed of 3–16 µm broad hyphae, densely and more or less regularly arranged, parallel with longitudinal stipe axis, non-amyloid, almost hyaline or pale yellow-brownish in Melzer's reagent. Trama of both pileus and stipe composed of a monomitic

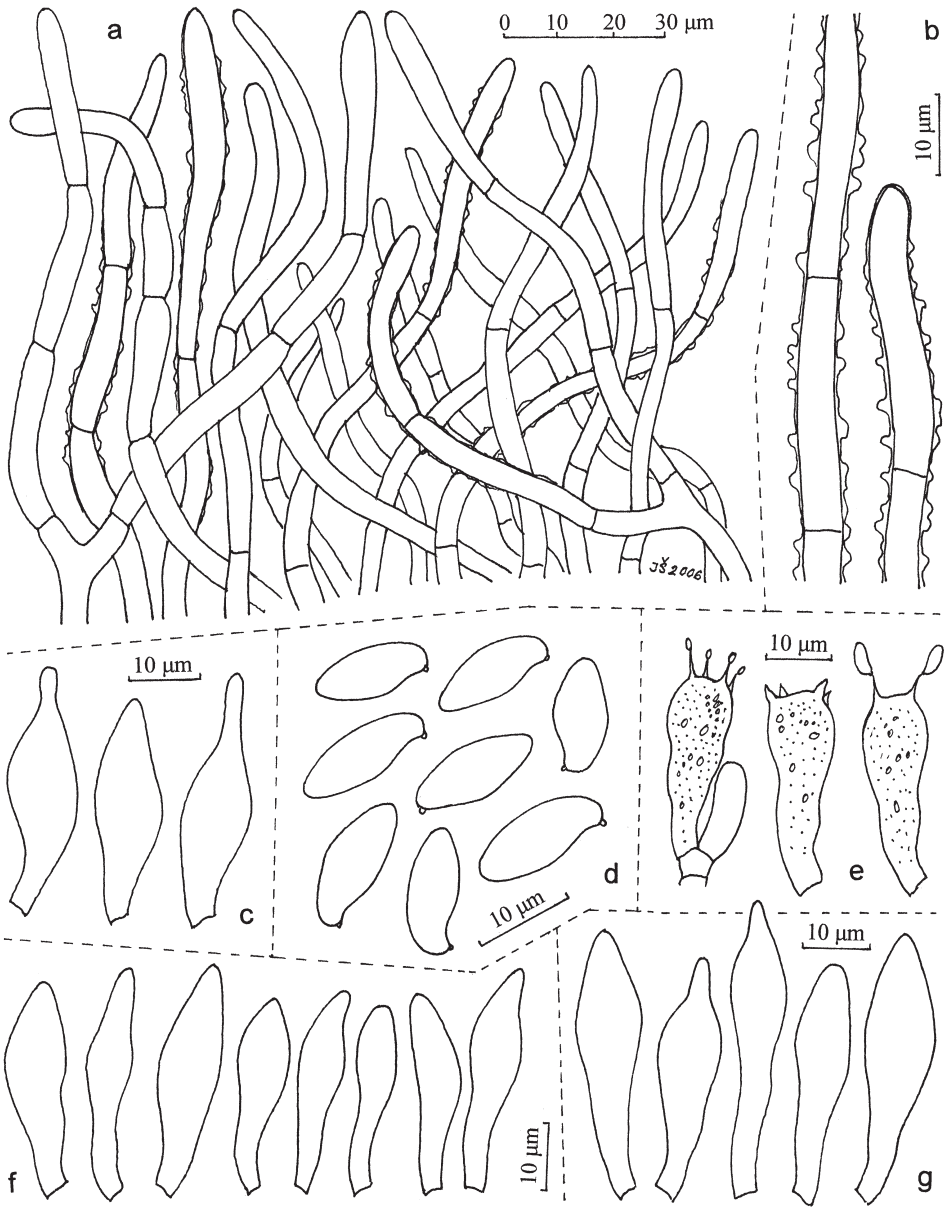

Fig. 1. *Boletus kluzakii* (PRM 857093, holotype). – a: Trichodermal hyphae of the pileus cuticle. – b: Trichodermal hyphae covered with a gelatinous matter in detail. – c: Pleurocystidia. – d: Spores. – e: Caulobasidia. – f: Cheilocystidia. – g: Caulocystidia.

hyphal system with generative, thin-walled hyphae. Clamp connections not found in the carpophore.

Solitary on soil under *Quercus robur* (sometimes also with *Pinus sylvestris* in the vicinity), on dam of fish-pond Luční (national nature monument), not far from Sezimovo Ústí, southern Bohemia, alt. 420 m, leg. P. Špinar et al., 3 Sept., 16 Sept. and 20 Sept. 1992; 9 Sept. 1994; 16 Sept. 1996; 21 Sept. 1997 (PRM 857093, holotype; JŠ 3889, isotype); 18 July, 15 Sept. and 17 Sept. 2000 (JŠ 4215; PŠ 234).

Tab. 1. Numbers of carpophores of *B. kluzakii* found at the locality NNM Luční.

	June	July	August	September	October
1992	–	–	–	4	–
1993	–	–	–	–	–
1994	–	–	–	1	–
1995	–	–	–	–	–
1996	–	–	–	1	–
1997	–	–	–	1	–
1998	–	–	–	–	–
1999	–	–	–	–	–
2000	–	1	–	2	–
In 2001–2005 *B. kluzakii* was not found.					

DISCUSSION

Boletus kluzakii belongs to *Boletus* sect. *Calopodes*. The most typical characters of this species are: pileus when young whitish or pallid, sometimes with a slight pinkish tint, then more or less rose-pink or purplish pink, at times even purple-reddish in some places; pileus surface conspicuously reddening when handled or bruised; hymenophore (both pores and tube-sides) yellow, bluing when bruised; stipe yellow with a yellow reticulation; context under pileus cuticle red or purplish red but in other parts of the pileus and in upper half of the stipe pale yellowish or vivid yellow, turning blue when injured; taste bitter.

Boletus kluzakii is closely related to *Boletus radicans*, from which it differs mainly in the red or purplish red subcuticular layer of the pileus. The red coloration of this layer is easily visible both in cross-sections and in scalped places on the pileus surface. The arrangement of the cortical zone of the pileus in two differently coloured layers, viz. the upper, pallid cuticle and the lower, red or purplish red subcuticular layer, is the reason why the pileus surface of *Boletus kluzakii* changes to red on handling or bruising. This colour change, which is usually very conspicuous, can hardly be overlooked during collecting of carpophores. The

Fig. 2. *Boletus kluzakii.* Southern Bohemia, NNM Luční, near Sezimovo Ústí, leg. P. Špinar et al., 21 Sept. 1997 (PRM 857093, holotype). Younger carpophore with a light pinkish shade of the pileus. Photograph by E. Skála.

Fig. 3. *Boletus kluzakii.* Southern Bohemia, NNM Luční, leg. P. Špinar, 17 Sept. 2000 (JŠ 4215). Mature carpophore with a typical, rose-pink to purplish pink coloration of the pileus.

Fig. 4. *Boletus kluzakii.* Southern Bohemia, NNM Luční, leg. P. Špinar, 15 Sept. 2000 (PŠ 234). Pileus of a younger carpophore. On one place of the pallid pileus surface there is a deep red spot which stained red on bruising. Another place, which was injured more strongly, shows blue oxidation of the context. The red colour of the subcuticular layer of the pileus is distinct in several places where the upper, pallid cuticle was removed from the pileus surface by means of a scalp. Photographs 3 and 4 by P. Špinar.

presence of the red subcuticular layer and the reddening of the pileus surface are characters important particularly for the identification of younger carpophores of *B. kluzakii*, which have a whitish or pallid pileus and are, at first sight, rather similar to those of *B. radicans*. In *B. radicans* the layer under the pileus cuticle is pale brownish or grey-brownish (not red) and the pileus surface stains brown or grey-brown when bruised, and blue-brown or blue when more strongly injured.

In later developmental stages, *B. kluzakii* is also usually different from *B. radicans* in the rose-pink or purplish pink pileus, which is sometimes even partly purple-reddish. Typical forms of *B. radicans* have the pileus whitish, white-greyish, pale cream-coloured or pale beige, at times (particularly in older carpophores) pale brownish in some places. A form of *B. radicans* with a partly reddish pileus exists, but is very rare. From the total number of more than 2 thousand carpophores of *B. radicans* examined at 16 localities in the Czech Republic, the authors of this contribution found this partly reddish form only nine times, only at 3 localities. Results of the observations of the present authors can be summarised as follows.

In the partly reddish form of *B. radicans*, the reddish shade on the pileus occurs only in the superficial layer of the cuticle. The lower, subcuticular layer of the pileus is constantly pale brownish or grey-brownish and also the pileus surface stains brown when bruised as in the other forms of *B. radicans*. Moreover, in the reddish form of *B. radicans* the reddish shade is developed relatively soon (already in young carpophores) and only on a smaller part, mostly on the marginal zone of one side of the pileus. In maturing carpophores this reddish shade completely fades away.

In *B. kluzakii*, the reddish coloration develops in another way. The pileus is at first whitish, pallid or only very slightly pinkish, because in young carpophores of *B. kluzakii* the lower, purplish red subcuticular layer is covered by the upper, whitish subtomentose cuticle which is, in this young stage, composed of a well-developed, 400–800 µm thick trichodermal layer of erect or suberect hyphae. During further stages the trichoderm gradually collapses or is partly washed away and slowly exposes the red subcuticular layer, which gradually gives a more and more distinct rose-pink or purplish pink tone to the whole pileus.

The results of the present authors' observations on *B. radicans* agree roughly with descriptions of this species published in the mycological literature. *Boletus radicans* (= *Boletus albidus*) is generally considered a species whose pileus is whitish or pallid, with various pale greyish, pale cream-coloured, pale ochraceous or pale brownish shades (see Alessio 1985, Muñoz 2005, Watling and Hills 2005, and many others). Persoon (1801: 507) described the colour of the pileus in his original diagnosis of *Boletus radicans* in the following way: 'pileo … flavescente-cinereo'. Nevertheless, some authors (e.g. Kallenbach 1926-1942, Pilát and Dermek 1974) noticed that the pileus of this species may also be somewhat red-

dish in rare cases. These authors, however, never mentioned the red colour of the subcuticular layer and the conspicuous reddening of the pileus surface on bruising, which are the most typical characters of *Boletus kluzakii*. In his description of *B. radicans*, Kallenbach (1926-1942: 90) wrote: 'Hut … beim Liegen und bei Druck etwas dunkler bräunlichgrau, zuweilen bei Druck etwas blauend; ... Fleisch ... unter Huthaut etwas graulich bis bräunlich'. According to Pilát and Dermek (1974: 104-105), the characters of the pileus of *B. radicans* are as follows: 'Cuticle … stains bluish grey or blue where bruised … Context … under the pileus cuticle with a greyish or delicate blue tint' (translation from Slovak by Šutara).

In the state when the pileus of *B. kluzakii* is rose-pink, purplish pink or even partly purple-reddish (i.e. on handling or in later stages of development), the carpophores of this species resemble those of *Boletus regius* Krombh. Distinguishing these two species, however, is not difficult because *Boletus regius* has both its hymenophore and context unchanging (not bluing) and the taste of its context is mild and pleasant (not bitter).

Boletus roseoolivaceus Blum, which has a brownish, chamois, beige-greenish pileus with a carmine shade and a bitter context, differs from *B. kluzakii* in the dominant red colour of the stipe (like *Boletus calopus* Pers.: Fr.), positive amyloid reaction of the context and somewhat broader spores (6–7 µm) (see Blum 1970).

Also *Boletus pulchrotinctus* Alessio and *Boletus fuscoroseus* (= *Boletus pseudoregius*) have pink or reddish shades of the pileus. These species, however, have not a bitter context and their stipes are distinctly pink or reddish in the middle or lower part (see Alessio 1985, Estades 1988). *Boletus pulchrotinctus* moreover differs in much larger spores (12–18 × 6–8.5 µm).

Boletus speciosus Frost, which was formerly incorrectly connected with *B. fuscoroseus* (or *B. pseudoregius*), differs from *B. kluzakii* in the mild taste of its context, darker pileus, and red coloration of the lower part of stipe (see Coker and Beers 1943, Smith and Thiers 1971, Both 1993, Bessette et al. 2000, and others). North American material of *Boletus speciosus* examined by the first author was also different from *B. kluzakii* in some microscopic characters, especially in its distinctly narrower spores (3–4.5 µm) and in a large number of conspicuously inflate, subglobose cells in the caulohymenium.

Boletus roseopurpureus Both, A. E. Bessette et Roody, a North American species, has a striking pinkish purple to dark purplish red pileus (not whitish in youth), taste of context very sour like lemon (not bitter), very narrow spores (2.7–3.5 µm) and a white basal part of stipe (see Bessette et al. 2000).

Another North American species, *Boletus pulchriceps* Both, A. E. Bessette et Chapman, has also a pinkish pileus in a certain developmental stage, but its hymenophore and context are unchanging or only very weakly bluing, its taste is not bitter, and its tomentum on the stipe base is white (see Bessette et al. 2000).

In our opinion, *B. kluzakii* is identical with *Boletus fallax* Kluzák, which was found in 1981 under *Quercus robur* and *Tilia cordata* on a dam of fish-pond Naděje near Hrbov, east of Prachatice in southern Bohemia (Kluzák 1988). Unfortunately, *Boletus fallax* Kluzák cannot be considered a correct name because it is a later homonym of *Boletus fallax* Corner. Corner's *Boletus fallax* is a Malaysian species, which is very different from Kluzák's bolete in many characters, above all in longitudinally striate spores (see Corner 1972). *Boletus fallax* Corner was later transferred by Watling (1990) to the genus *Boletellus* Murrill.

Material examined: – Typical forms of *Boletus radicans* without any reddish shade on pileus: JŠ 2120, 2534, 2785, 3143-45, 3876, 3880-86, 4035-37, 4216-33, 4254-56, 4261; PŠ 9, 10, 18-21, 29, 41, 48, 49, 53, 58-61, 68, 206, 208, 300, 321-22, 623, 638, 670, 1001 etc. – Specimens of *Boletus radicans* with a reddish shade on a part of the pileus: JŠ 3661-62; PŠ 113, 115, 118, 181-185, 195. – *Boletus regius*: PRM 614841, 866632; JŠ 044, 3525; PŠ 2000. – *Boletus fuscoroseus*: JŠ 4244; PŠ 86, 89, 95, and CB 1352, 1782 (misidentified as *Boletus speciosus*). – *Boletus speciosus*: PRM 704843, 704844 (material from U.S.A., collected and identified by E. Both). – *Boletus fallax* Kluzák: CB 5001 (holotype).

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