

# First encounters of *Boletus subappendiculatus* (*Boletaceae*) in Bulgaria

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**Abstract.** *Boletus subappendiculatus* is recorded for the first time in Bulgaria. The species is described and illustrated on the basis of Bulgarian specimens. The differences with the similar *B. appendiculatus* are briefly discussed.

**Key words:** *Boletales*, *Boletus* sect. *Appendiculati*, Bulgarian mycota, fungal diversity

## Introduction

The section *Appendiculati* Konrad & Maubl. ex Lannoy & Estadès of the genus *Boletus* comprises five species in Europe (Muñoz 2005) and so far four of them have been reported to occur in Bulgaria: *B. appendiculatus* Schaeff. : Fr., *B. fechtneri* Velen., *B. pseudoregius* (Huber) Estadès, and *B. regius* Krombh. (Assyov & Denchev 2004, Denchev & Assyov 2010). The fifth member of the section, *B. subappendiculatus* Dermek, Lazebn. & J. Veselský, has been recorded in some neighboring Balkan countries. During the first author's studies into the diversity of genus *Boletus* in Bulgaria, *B. subappendiculatus* was found for the first time in the country. It is presumably a rare species for many states of Europe and yet not well known, and therefore a detailed description and illustrations are presented in this paper.

## Material and methods

Air-dried specimens of the fungus are preserved in the Mycological Collection at the Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences (SOMF). Specimens of *Boletus appendiculatus* kept in the same collection were used for comparison. A list of all examined specimens is provided at the end of the paper.

The samples are documented with color photographs (partly shown on Fig. 1) and concise description. Colour notations in the description below refer to Kornerup & Wanscher (1978), or the British Fungus Flora Colour Chart (Anonymous 1969), the later abbreviated as BFF. Microscopic features are observed and measured in tap water under Amplival LM, with magnification  $\times 1000$ . Measurement values for basidiospores are presented below as follows: (min–) mean $\pm\sigma$  (–max). Spore volume ( $V_m$ ) is calculated according to the formula  $V_m = 4/3\pi \cdot (1/2Sw)2.1/2Sl$ ; Sl – spore length, Sw – spore width, and the result is estimated to an integer number (Breitenbach & Kränzlin 1991). Iodine reaction was tested by Melzer's solution (Kirk & al. 2001) on dried samples, following the procedure described by Ladurner & Simonini (2003).

The abbreviations of the authors of fungal names follow Kirk & Ansell (2004). The distribution of *Boletus subappendiculatus* is shown on a UTM-grid map (Fig. 2).

## Description of the species

***Boletus subappendiculatus*** Dermek, Lazebn. & J. Veselský, in Dermek, Fungorum Rar. Icon. Color., 9: 13, 1979 (Fig. 1 a–f)

Icons: Engel & al. (1983: Tab. 18), Breitenbach & Kränzlin (1991: 65, Fig. 24), Galli (1998: 179), Muñoz (2005: 343, 692–694, 849–850, 893), Šutara & al. (2009: 123).



Fig. 1. Basidiomata of *Boletus subappendiculatus* at different stages of development *in situ* (photos: a, e – M. Gyosheva; b, f – B. Assyov; c, d – I. Assyova).

**Pileus** up to 8 cm in diameter, initially hemispherical, subsequently convex to flat-convex, seldom flat or slightly depressed, dry or occasionally slightly viscid when old, smooth, fibrillose or finely cracked, brownish-orange, sunburn, cinnamon, light-brown (5C3-4, 6D4-5, 7D4-6), buff or clay buff (BFF 32, 52); surface

unchanging when bruised; margin appendiculate. **Stipe** up to 13 × 5.5 cm, initially subspherical or ovoid, subsequently club-shaped or occasionally cylindrical, sometimes rooting, yellowish-white or pastel-yellow (1A2-4, 2A2-3), seldom discolored in places or entirely to whitish or brownish, occasionally

in the middle or in the upper 1/3 with reddish-white, pale-red or brownish-orange zone (9-10A2-3, 7C7-8), at least in the upper half or most often entirely covered with fine, concolorous, white, yellowish, rusty-orange, orange or orange-red reticulum; stipe surface unchanging when bruised. **Context** lemon- yellow (BFF 54), straw or whitish (BFF 50, 78), but under the pileipellis, above the tubes and under the stipe surface sulphur-yellow, lemon-yellow or lemon-chrome (BFF 53-55), in the stipe base often dirty-salmon (BFF 45) to clay-pink, vinaceous buff or clay buff (BFF 30-32); not blueing when exposed to air. **Tubes** initially lemon-yellow (BFF 54), subsequently yellow with olivaceous tint, unchanging when damaged. **Pores** initially lemon-yellow (BFF 54), subsequently yellow with olivaceous tint, occasionally in old basidiomata rusty spotted, darkening and not blueing when bruised. **Smell** non-distinctive. **Taste** non-distinctive. **Basidiospores**  $9.5-(12.2\pm 1.2)-15 \times 3-(3.8\pm 0.2)-4.5 \mu\text{m}$ , length/width ratio  $2.4-(3.3\pm 0.1)-4.3$ , spore volume  $50-(92\pm 18)-149 \mu\text{m}^3$  ( $n=200$ ). **Basidia** 4-spored, clavate,  $30-39.5 \times 7-10 \mu\text{m}$  ( $n=60$ ). **Cystidia**  $35.5-45 \times 8-10.5 \mu\text{m}$  ( $n=60$ ). **Pileipellis** trichodermium of interwoven, septate, occasionally branched, yellowish, finely incrustated hyphae; most terminal cells cylindrical with rounded apex. **Macrochemical reactions:** NH<sub>4</sub>OH (pileus – BFF 41, context – BFF 30), KOH (pileus – BFF 41, context – BFF 30), FeSO<sub>4</sub> (pileus – 0, context – BFF 65), Melzer's solution (no reaction observed). **Microchemical reactions:** Melzer's solution (no reaction observed).

**Habitats.** Coniferous or mixed mountain forests, mycorrhizal with *Picea abies* (L.) Karst. (Hahn & Raidl 2006) but probably also with *Abies alba* Mill., 1200–1800 m. July–August.

**Distribution in Bulgaria.** Pirin Mts (*Northern*) and Rila Mts (Fig. 2).

**General distribution.** Europe and Asia Minor (apparently within the range of the mycorrhizal hosts, but needs further clarification, as it is probably more widespread and possibly

occasionally confused with *B. appendiculatus*). Described from Slovakia, and further European finds are available in Austria, Czech Republic, France, Germany, Italy, Norway, Poland, Slovenia, Spain, Switzerland and the United Kingdom; on the Balkan Peninsula it is known from Greece, Montenegro, Serbia, and now also from Bulgaria. In Asia the species has been recorded only from Anatolia in Turkey. It has not been found yet in the former Soviet states in Europe, where undoubtedly it should be present and looked for.

## Discussion

The Bulgarian specimens of *B. subappendiculatus* agree macroscopically with the descriptions of Engel & al. (1983), Breitenbach & Kränzlin (1991), Galli (1998), Lannoy & Estadès (2001), Muñoz (2005), Marques & Muñoz (2006), Knudsen & Taylor (2008), and Šutara & al. (2009). Microscopically, the Bulgarian collections show some differences in the spore size from the measurements presented by earlier authors (Table 1). These discrepancies are probably attributable to the different sampling sizes: 98 basidiospores measured by Muñoz (2005), 200 spores in this study, and an unknown number by the other authors.

*B. subappendiculatus* is similar to the more widespread in Europe *B. appendiculatus*, but is well separated morphologically, a distinction confirmed also by recent molecular studies (Eberhardt & Taylor in Muñoz 2005; Marques & Muñoz 2006). It is distinguished pri-

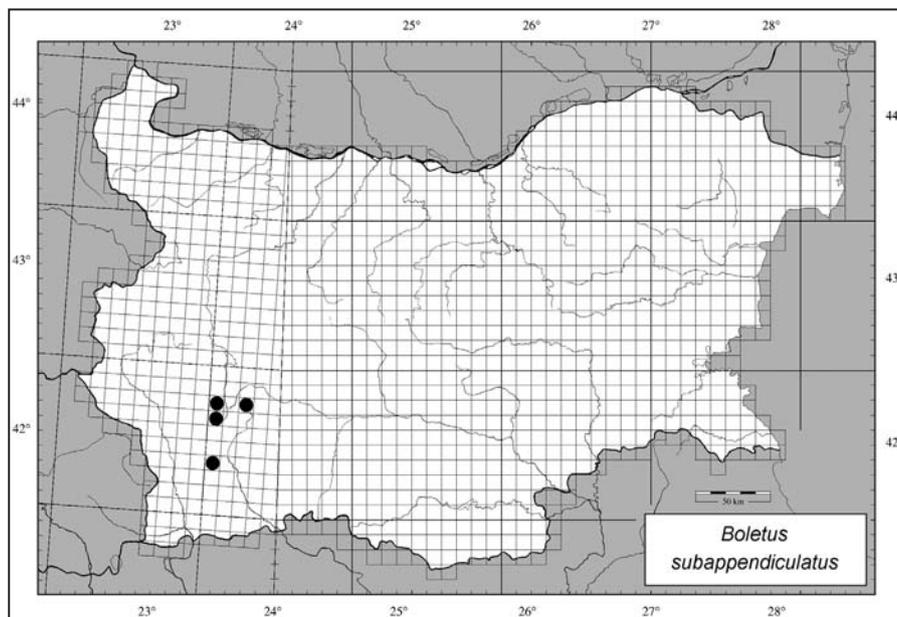


Fig. 2. Distribution of *B. subappendiculatus* in Bulgaria on an UTM-grid.

Table 1. Comparison of the spore characteristics of *B. subappendiculatus* according to different studies.

| Authors                       | Spore length (µm)                  | Spore width (µm)              | Length/width ratio      | Spore volume (µm <sup>3</sup> ) |
|-------------------------------|------------------------------------|-------------------------------|-------------------------|---------------------------------|
| Engel & al. (1983)            | (11.6–)12–17                       | (3.6–)4–5                     | –                       | –                               |
| Breitenbach & Kränzlin (1991) | 12–14.5                            | 3.7–4.5                       | 3.1–3.7                 | mean=110                        |
| Galli (1998)                  | 12–16(–17)                         | 4–5                           | –                       | –                               |
| Muñoz (2005)                  | (10.5–)11.2–13.4(–14)<br>mean=12.7 | (3.8–)4–4.4(–4.8)<br>mean=4.3 | (2.7–)2.8–3.1(–3.2)     | mean=98                         |
| Marques & Muñoz (2006)        | 10.2–15<br>mean=12±0.6             | 3.5–5.6<br>mean=4.1±0.2       | 2.5–3.7<br>mean=3±0.2   | –                               |
| Knudsen & Taylor (2008)       | 12–14.5                            | 3.5–4.5                       | –                       | –                               |
| Šutara & al. (2009)           | (11.5–)12–17                       | (3.5–)4–5                     | –                       | –                               |
| This study                    | 9.5–15<br>mean=12.2±1.2            | 3–4.5<br>mean=3.8±0.2         | 2.4–4.3<br>mean=3.3±0.1 | 50–149<br>mean=92±18            |

marily by the lack of blueing in the context and the generally paler colour of the pileus. Also, the habitats of the two species are different: *B. subappendiculatus* is found under conifers and *B. appendiculatus* under broadleaf trees (*Quercus* spp. or *Fagus sylvatica* L.).

The two species are not easily separated microscopically. The length and width of basidiospores ranges overlap significantly, when the outliers are considered. If the mean±σ ranges are compared, it appears that *B. subappendiculatus* has lower width values and a higher length/width ratio (see Table 2 and Fig. 3a, b). Considering the above explained discrepancies of the spore sizes, these microscopic characters are only secondarily diagnostic.

*Boletus subappendiculatus* is known to be an edible species (Šutara & al. 2009). There is so far no indication in Bulgaria that its edibility is known and presently the fungus is unlikely to be collected for food.

Table 2. Comparison of the mean±σ ranges of spore characteristics of the Bulgarian samples of *B. appendiculatus* and *B. subappendiculatus*.

| Species                     | Sample size | Spore length (µm) | Spore width (µm) | Length/width ratio |
|-----------------------------|-------------|-------------------|------------------|--------------------|
| <i>B. appendiculatus</i>    | 250         | 10.6–11.4–12.3    | 4.3–4.4–4.5      | 2.4–2.6–2.8        |
| <i>B. subappendiculatus</i> | 200         | 11–12.2–13.4      | 3.6–3.8–4        | 3.2–3.3–3.4        |

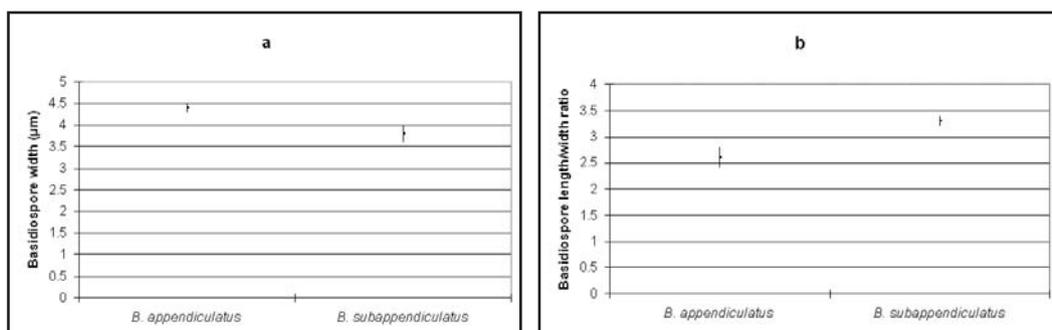


Fig. 3. Comparison of the basidiospore width (a) and length/width ratio (b) of the Bulgarian samples of *B. appendiculatus* and *B. subappendiculatus*.

## Specimens examined

***Boletus subappendiculatus*:** Pirin Mts (Northern): ca. 1 km below Banderishka Polyana locality above Bansko town, ca. 1770 m, under *Abies alba*, 26.08.2009, leg. B. Assyov (SOMF 27904); Rila Mts: Kirilova Polyana locality, along the trail to Suhoto Ezero lake, ca. 1460 m, under *Abies alba*, 28.07.2002, leg. B. Assyov (SOMF 27913); Kirilova Polyana locality, under *Picea abies*, 15.07.2008, leg. A. Tosheva (SOMF 27044); Kirilova Polyana locality, under *Picea abies*, 24.07.2009, leg. B. Assyov & I. Assyova (SOMF 27673 & 27674); Kirilova Polyana locality, 1497 m, 14.07.2009, obs. M. Gyosheva; Preslapa locality, above the Mechit chalet above Govedartsi village, 1647 m, under *Picea abies*, 01.08.2008, leg. M. Gyosheva (SOMF 27045); Generalska Pusiya locality above Raduil village, 1355 m, under *Picea abies* and *Fagus sylvatica*, 04.07.2009, leg. M. Gyosheva (SOMF 27671); Generalska Pusiya locality, 30.07.2008, obs. M. Gyosheva.

***Boletus appendiculatus*:** Black Sea Coast (Southern): along the trail between Perla Estate and cape Maslen Nos, under *Quercus cerris* L., 07.06.2008, leg. B. Assyov (SOMF 27408); Forebalkan (Eastern): in the vicin-

ity of Koman chalet, below Terziisko village (Trojan distr.), under *Quercus frainetto* Ten., 07.06.2002, leg. B. Assyov (SOMF 25371); in the vicinity of Golyama Zhelyazna village (Trojan distr.), under *Quercus* sp., 10.07.2003, leg. D. Stoykov (SOMF 27442); in the vicinity of Golyama Zhelyazna village (Trojan distr.), under *Quercus* sp., 30.06.2004, leg. D. Stoykov (SOMF 27437); Sredna Gora Mt (Eastern): Zelenikovo village (Plovdiv distr.), in a broadleaf woodland (*Carpinus orientalis* Mill., *Fraxinus* sp.), near a sheep pen at the end of the village, 13.08.2002, leg. G. Stoichev & M. Lacheva (SOMF 27240).

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