First record of *Boletus ichnusanus* (*Boletaceae*) in Bulgaria

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Abstract. The paper provides information about the first finding of *Boletus ichnusanus* (≡ *Xerocomus ichnusanus*) in Bulgaria. Description and illustrations, including SEM microphotograph, are presented on the basis of Bulgarian materials. Some observations are discussed in support of the microscopic separation of *B. ichnusanus* from *B. roseoalbidus*.

Key words: Balkan mycota, *Boletales,* Mediterranean fungi, xerocomoid boletes

Introduction

Boletes have been extensively studied in many parts of Europe but the distribution of some of them is still largely unknown and there is a number of less studied species. In the last few years, the surveys in Southeast Europe have produced a number of interesting records of species earlier thought to be restricted to the Mediterranean area (Assyov 2005; Karadelev & al. 2006; Perić & Perić 2006; Assyov & Denchev 2009; Konstantinidis 2009; Lukić 2009). During field studies in 2009, one rare southern bolete, namely *Boletus ichnusanus* (Alessio, Galli & Littini) Oolbekkink, was discovered for the first time in Bulgaria and for the second time on the Balkan Peninsula. This record is described and illustrated herein.

Material and methods

The fresh collection was photographed and described with colour notations, according to the *British Fungus Flora Colour Chart* (Anonymous 1969). Microscopic features were observed in water and KOH and measured in water. Measurement values are presented below as follows: (min–) mean±1σ (–max). Spore volume (Vm) is calculated according to the formula $Vm=4/3\pi.(1/2Sw)^2.1/2Sl$; Sl – spore length, Sw – spore width, and the result is estimated to an integer number (Breitenbach & Kränzlin 1991). Iodine reaction was tested with Melzer's solution (recipe from Kirk & al. 2001) on dried samples, following the procedure described by Ladurner & Simonini (2003). The surface structures of the basidiospores were studied and photographed with JEOL JSM-6390 scanning electron microscope at 10 kV. Spores for the SEM-preparation were obtained from spore deposits on the stipe surface. They were mounted on metal stubs with double-sided adhesive tape and sputter-coated with gold. Air-dried specimens, together with colour photographs of the fresh collection and microphotographs are preserved in the Mycological Collection of the Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences (SOMF).

Description of the species

***Boletus ichnusanus* (Alessio, Galli & Littini) Oolbekkink**, Persoonia **14**(3): 269, 1991. (Figs 1-2, Tables 1-2)

Fig. 1. *Boletus ichnusanus* – basidiomata *in situ.*

Table 1. Comparison of the mean±1σ values of the length and width of basidiospores in the Bulgarian collections of *Boletus ichnusanus* (this paper) and *B. roseoalbidus* (from Assyov & Denchev 2009) by means of Welch's t-test.

Species	Sample size	Spore length / width (mean±1σ)	T-value	P
Spore length				
B. roseoalbidus	100	14.1±1.3	15.52	p < 0.0001
B. ichnusanus	200	11.9± 0.8		
Spore width				
B. roseoalbidus	100	6.3±0.5	16.57	p < 0.0001
B. ichnusanus	200	5.4±0.3		

Table 2. Comparison of the mean±1σ values of the length and width of basidiospores in extralimital collections of *Boletus ichnusanus* and *B. roseoalbidus* (from Ladurner & Simonini 2003) by means of Welch's t-test.

Species	Sample size	Spore length / width (mean±1σ)	T-value	P
Spore length				
B. roseoalbidus	155	13.9±1.17	8.11	p< 0.0001
B. ichnusanus	62	12.8±0.77		
Spore width				
B. roseoalbidus	155	6.7±0.39	24.83	p< 0.0001
B. ichnusanus	62	5.5±0.29		

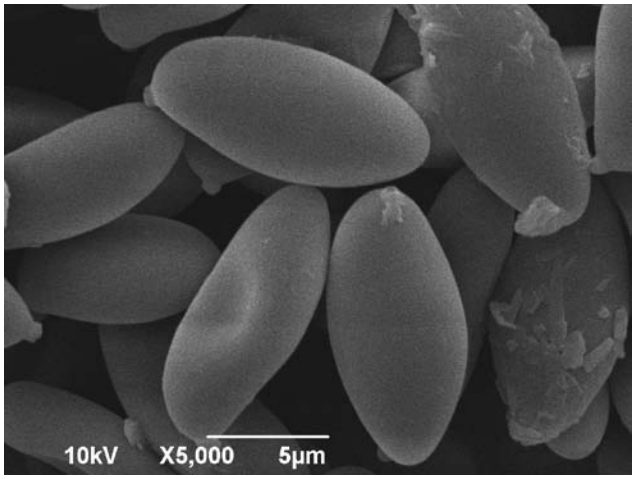

Fig. 2. SEM microphotograph of basidiospores of *Boletus ichnusanus.*

≡ *Xerocomus ichnusanus* Alessio, Galli & Littini, in Alessio, Boll. Gruppo Micol. 'G. Bresadola' (Trento) 27(3–4): 170, 1984.

Basidiomata single, or often clustered in groups (solitary and scattered in the Bulgarian finding). **Pileus** up to 7 cm in diameter, initially hemispherical, then convex, finally flat-convex, flat or slightly depressed, glabrous or somewhat fibrillose, occasionally finely cracked, clay-buff or fawn, later purplish-date or date-brown, occasionally spotted purplish-chestnut, bay or cigar-brown; surface unchanging when bruised, margin often undulate. **Stipe** up to 6×2 cm, tapering or spindle-shaped, somewhat rooting; in the upper portion straw to buff coloured, downwards rusty, pale-fulvous, snuff-brown or cigar-brown, occasionally almost black at the same base, slightly blueing after rough handling; stipe surface with a distinct reticulum and usually with a ring-like pattern of coarse granules in the upper portion, downwards finely granulate. **Context** pale lemon-yellow in the stipe, whitish in the cap, vinaceous to vinaceous-brown in the stipe base, blueing when exposed to the air. **Tubes** up to 1.5 cm long, adnate or subdecurrent, lemon-yellow when young, then with somewhat olivaceous tint, blueing when injured. **Pores** angular, lemon-yellow when young, later with olivaceous tint and often rusty spotted with age, blueing when bruised. **Smell** not distinctive. **Taste** distinctly acid. **Basidiospores** broadly ellipsoid, (10–) 11.9±0.8 (–14.5) × (4.5–) 5.4±0.3 (–7) µm (*n*=200), ratio (1.9–) 2.3±0.1 (–2.7), spore volume (113–) 180±32 (–338) µm^3, with 1–3 large guttules. **Basidia** inconspicuous, clavate, hyaline in KOH, generally 4-spored (2- and 3-spored basidia also occur), (25–) 31.3±2.4 (–37.5) × (10–) 10.9±1.2 (–12.5) µm (*n*=60). **Cystidia**

ventricose-fusiform, with yellowish content in KOH, (37.5–) 53.3±5.7 (–67.5) × (10–) 12.8±1.5 (–15) µm (*n*=60). Hymenophoral trama parallel '*Xerocomus*-typ' (Singer, 1965). **Pileipellis** a trichoderm of interwoven branched septate thin-walled hyphae, terminal cells mostly cylindrical with rounded apex, (25–) 41.9±13.1 (–75) × (7.5–) 8±1 (–10) µm (*n*=30), ratio (3–) 5.3±1.6 (–10). **Stipitipellis** consisting of interwoven septate hyphae, in the reticulum and in the 'ring-zone' with numerous spore-bearing basidia. **Macrochemical and microchemical reactions**: weak 'fleeting-amyloid' reaction observed with Melzer's solution with the hymenophore; hyphae of the stipe base inamyloid, no other macro- or microchemical reactions are noted.

Habitat. Thermophilous broadleaved forests, on poor dry soils, under *Quercus* spp.

Specimen examined. Bulgaria. Mt Ograzhden: Petrich distr., between the villages of Strumeshnitsa and Borovichene (41°24' 20.1" N, 23°02'14.7" E), at the edge of a thermophilous oak woodland by the road, on sandy soil, alt. *ca* 300 m, under *Quercus pubescens* Willd., 24.09.2009, leg. *I. Assyova, B. Assyov* & *D. Stoykov* (SOMF 27945; Fig. 3).

Fig. 3. Map of the locality of *Boletus ichnusanus.*

Discussion

Boletus ichnusanus is one of the most easily recognized European xerocomoid boletes. Macroscopically it is distinguished by the colours of the basidiomata, the well developed reticulum on the stipe, as well as by its usually (but not always) clustered habit. Another peculiar feature of this taxon is the specific (most likely secondarily angiocarpic) development of the basidiomata, leading to formation of a characteristic 'ring zone' at the stipe surface, where the cap margin has been initially attached. Among the other European boletes, this type of development and the ring-like zone are encountered only in *B. roseoalbidus* (Alessio & Littini) Moreno & Heykoop. This peculiarity has been emphasized by Ladurner & Simonini (2003), who placed both species in a separate, yet unnamed section. Old and discoloured basidiomata of *B. roseoalbidus* may sometimes resemble *B. ichnusanus*. Nonetheless, they do always retain some pinkish tint on the pileal surface, as well as the characteristic pink context in the cap. Also the reticulum, so typical for *B. ichnusanus*, is rather unusual for *B. roseoalbidus*, where only by exception a reticulum-like pattern may be present in the uppermost part of the stipe. Ladurner & Simonini (2003) have pointed out as important microscopic character the thin-walled pileipellis hyphae in *B. ichnusanus* vs. the thick-walled hyphae in *B. roseoalbidus*. However, *B. ichnusanus* is microscopically clearly distinguished from *B. roseoalbidus* also by its shorter and narrower basidiospores, with both Bulgarian and extralimital specimens showing differences of high statistical significance, when Welch's t-test is employed (Tables 1–2). Instead of the spore dimensions, the abundant, bright coloured hymenial cystidia in KOH in *B. ichnusanus* (vs. the hyaline cystidia of *B. roseoalbidus*) will most likely also prove a useful character for microscopic delimitation.

The Bulgarian collection corresponds both macro- and microscopically to the descriptions, available in the literature (Alessio 1984, 1985; Galli 1998; Ladurner & Simonini 2003; Gelardi 2007). Some discrepancies are to be noted though between the original and the later descriptions. Alessio (1984) gives the spore size as 12–18(–23–28) × 5–7(–7.5) µm (cited later by Alessio 1985; Oolbekkink 1991, and Engel & al. 1996), while Ladurner & Simonini (2003) recorded (11–) 12.8±0.77 (–14.3) × (5–) 5.5±0.29 (–6.3). Although slightly different, the measurements of the Bulgarian specimens are close to this last figure. This difference could be probably due to the different sampling sizes – 200 basidiospores (note, however, that these were obtained from a single collection) in this paper and 62 measured by Ladurner & Simonini (2003), but it could be also due to simply individual variability caused by specific environmental conditions, genetic variability, etc. The extreme values in the original description may include aberrant basidiospores that are occasionally seen in mounts.

When studying the spores of xerocomoid boletes under SEM, Oolbekkink (1991) encountered some difficulties in resolving the ornamentation of the basidiospores of *B. ichnusanus* due to adhering substance, presumed to be of foreign origin. However, Oolbekkink considered the spores to be smooth. The SEM examination of the Bulgarian collection clearly shows smooth spores (Fig. 2).

Boletus ichnusanus is apparently a rare southern species with a limited distribution, previously known to be restricted to the Mediterranean area, namely France, Greece (Mainland), Italy and Spain. Therefore, its occurrence in Bulgaria is very interesting from mycogeographical point of view, because it extends its known distribution to the east, which points out to the possibility of its existence in some other Balkan countries, especially in Macedonia, as the new locality is situated close to the borders with this state. It may also be looked for in suitable habitats in Turkey.

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