

New data on two Mediterranean species of *Amanita* (*Agaricales*, *Amanitaceae*) in Bulgaria

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Abstract. The paper presents two new species of the genus *Amanita* from Bulgaria: *A. boudieri* and *A. curtipes*. Occurrence of *A. crocea* in the country has been confirmed.

Key words: *Amanita*, *Amidella*, *Aspidella*, Balkan mycota, new data

Introduction

Genus *Amanita* Pers. forms a large group within the family *Amanitaceae* Pouzar or *Pluteaceae* Kotl. & Pouzar (Kirk & Cannon 2007; Kirk & al. 2008) and is among the most typical genera within *Agaricales* Clem. This genus is species-rich in Europe and, in spite of extensive studies at morphological and molecular level, still some undescribed species have been found to occur in the Mediterranean area (Loizides & al. 2018). The present knowledge on Bulgarian members is still incomplete, although there are numerous records in the national mycological literature (Denchev & al. 2006; Denchev & Assyov 2010) and some species have been recently reported from the country (Assyov & Stoykov 2011; Gyosheva & al. 2017; Gyosheva & Stoykov 2019; Assyov & al. 2021).

Material and methods

The studied specimens were deposited in the Mycological Collection of the Institute of Biodiversity and Ecosystem Research of the Bulgarian Academy

of Sciences (SOMF). Macroscopic features of the collections were examined in a fresh state before air-drying. Species descriptions are prepared on the base of field notes and colour photographs of the specimens taken in the initial place of collection. The microscopic study was conducted on dried samples in water, 3 % KOH, and ammonia solution of Congo red. Spore measurements used in the descriptions were taken in water. They are presented below in the following format: (min–) mean±standard deviation (–max), n, Q (min–) mean±standard deviation (–max), where ‘n’ – denotes the number of spores measured, and ‘Q’ – length/width coefficient. For the remaining microscopic structures only minimum and maximum values are noted. Microphotographs of the velar tissues, basidia and spores were taken with Canon PS A460 and Olympus E330 digital cameras on Boeco BM-180/T-SP and Olympus BX-41 LM from mounts in a mixture of 1 % Congo red in ammonia and 3 % KOH (1:1). Identification of the taxa principally relied on the works of Bas (1969), Fraiture (1993), Breitenbach & Kränzlin (1995), Galli (2001), Contu (2003), Krieglsteiner (2003), Neville & Poumarat (2004), and Vesterholt (2008).

Survey of the taxa

***Amanita boudieri* Barla**, Bull. Soc. Mycol. Fr. 3: 193 (1887), Plate I, Figs 1-3; Plate II, Figs 1-3.

Pileus up to 8 cm in diameter, initially hemispherical, subsequently convex to aplanate, white to off-white; surface with small off-white to whitish-grey pyramidal verrucae; margin with overhanging velar remnants. **Stipe** up to 10 cm long, white, subcylindrical, more or less swollen and with squamulose velar residues at the bulbous base in one to three vague circles, below the bulb tapering and deeply buried in the substrate; ring fugace. **Gills** crowded, free, white. Context white. Smell agreeable, taste unrecorded.

Basidiospores (10.5–) 12.1 ± 1.2 (–16) \times (5–) 6.5 ± 0.8 (–8) μm , $n=40$, Q (1.6–) 1.9 ± 0.2 (–2.4), oblong to cylindrical, hyaline, amyloid. **Basidia** 50–75 \times 10–11.5 (–12) μm , 4-spored, elongate clavate, without clamp connections; sterigmata up to 5 μm long. **Velar tissue** of the volva composed of filamentous, branched, septate hyphae up to 6 μm wide, intermixed with abundant ovoid, ellipsoid to elongate clavate inflated elements.

Specimen examined: Southern part of the Valley of River Strouma, Petrich distr., Belasitsa Nature Park, after an old frontier post, Tumbite locality, 41.397663° N, 23.271717° E, alt. ca 250 m, on soil, 08.04.2013, leg. R. Natcheva & D. Ivanova, det. M. Gyosheva & D. Stoykov (SOMF 28529).

Notes. *Amanita boudieri* is known from Mediterranean Europe (France, Greece, Italy, Republic of North Macedonia, Portugal, and Spain). The habitat preferences of the species tend to open poor and sandy soils in broadleaf oak (*Quercus rotundifolia* Lam. and *Q. suber* L. with *Cistus ladanifer* L.), mixed broadleaf communities (*Carpinus orientalis* Mill. with *Q. cerris* L., *Q. coccifera* L. or *Q. frainetto* Ten.), and secondary Mediterranean Pine plantations with oaks (Brottu & Colomo 2009; Karadelev & al. 2011, etc.). It is a rare species, known on the Balkan Peninsula with collections in Greece (Konstantinidis 2009) and in the Republic of North Macedonia (Karadelev & al. 2011). The amyloid basidiospores of *A. boudieri* with an average Q coefficient, usually higher than 1.8, are a characteristic feature of this species. When measuring the image jpeg files of basidiospores, taken with Canon PS A460 from mounts in solution of 1% Congo red in 3% ammonia, by specialized software

for digital images Carnoy 2.0 (Schols & al. 2002), the basidiospores have been found to be slightly shorter in length than those measured in water, (9.5–) 11.3 ± 0.8 (–13.5) \times (6.0–) 6.6 ± 0.6 (–8.0) μm , with Q coefficient (1.5–) 1.72 ± 0.12 (–2.1), $n=120$. The morphologically similar *Amanita gracilior* Bas & Honrubia is distinguished by the persistent membranous ring and the presence of clamp connections (Bas & Honrubia 1982; Neville & Poumarat 2004).

***Amanita crocea* (Qué.) Singer**, Lilloa 22: 386 (1951), Fig. 1; Plate II, Figs 4–6.

Pileus up to 7 cm in diameter, initially campanulate or conical, becoming convex to flattened, with low broad umbo, yellowish orange, pale orange or apricot, darker in the center; surface free of velar remnants; margin distinctly striate. **Stipe** up to 15 cm long, cylindrical or thickening downwards, hollow, cream to pale yellowish orange and usually darkening with time, with more or less pronounced zigzag-like pattern; ring absent; volva persistent, thick, whitish and occasionally darker spotted outside, cream to somewhat orange inside. **Gills** free to adnexed, crowded, white to pale cream. **Context** thin, whitish, often pale orange below the cap cuticle. Smell agreeable, taste unrecorded.

Basidiospores (9–) 11.1 ± 1.0 (–12.5) \times (9–) 10.5 ± 0.9 (–12.5) μm , $n=60$, Q (1–) 1.1 ± 0.1 (–1.3), spherical or subspherical, hyaline, inamyloid. **Basidia** 37–60 \times 10–16 μm , 4-spored, clavate to elongate clavate, without clamp connections; sterigmata up to 5 μm long. **Marginal cells** pyriform, 20–40 \times 17–25 μm . **Velar tissue** of the volva constituted of branched, septate filamentous hyphae; spherical elements unseen.

Specimens and material examined: Stara Planina Mts (Western), on soil, above Voynyagovtsi village, in the vicinity of conifer trees mixed with oaks, 42°49'05"N, 023°26'40.1"E, alt. ca 775 m, 24.06.2007, D. Stoykov (photographs); idem., on a forest dirt road, in mixed coniferous plantation with *Quercus rubra* L., 20.07.2013, leg. D. Stoykov, det. M. Gyosheva & D. Stoykov; Central Rhodopi Mts, under Perelik chalet, on soil, in a spruce forest, 41.621638°N, 24.608117° E, alt. ca 1843 m, 07.08.2020, leg. & det. M. Gyosheva (SOMF 30454).

Notes. *Amanita crocea* was first reported in Bulgaria by Kuthan & Kotlaba (1981, 1989), from localities in Central Stara Planina and Strandzha mountains. In Great Britain and Northern Ireland, *A. crocea* was reported in mycorrhiza with hardwood

Plate I.



Amanita boudieri, 08.04.2013
(in situ):

Fig. 1. Fruitbody with part of stipe buried in soil;

Fig. 2. Stipe, front view;

Fig. 3. Pileus and stipe, view from above.

trees, either birch and beech, or in communities with larches, pines and spruces (Dennis 1995; Kibby 2012). In Greece, the species was found with *Quercus* sp. and *Q. ilex* L. (Zervakis & al. 1998). The specific epithet 'crocea' refers to the saffron colouring of the cap, which may often vary in hue. *Amanita crocea* is distinguished by a thick white volva, faint white zigzag-like stem markings, and generally without veil remnants attached to the cap surface (Fig. 1). The new finds validate the presence of this species in Bulgaria, and according to its known ecological preferences, it might have wider distribution in the country.

***Amanita curtipes* E.-J. Gilbert**, in Bresadola, Iconogr. Mycol. 27(Suppl. 1): 279 (1941), Plate II, Figs 7-8.

Basidiomata studied in dried state. **Pileus** up to 5 cm in diameter, cream yellow; margin somewhat wavy, striate. **Stipe** up to 5 cm long, yellowish brown, cavernouse; volva off-white to yellowish, deeply into 2-3 lobes; the stipe base with ring-like velar remnants; ring absent. **Gills** moderately crowded, thick, brownish, equal.

Basidiospores (12-) 14.0 ± 1.7 (-16.8) \times (6-) 6.8 ± 0.6 (-8) μm , n=30, Q (1.6-) 2.1 ± 0.3 (-2.7),

Plate II.

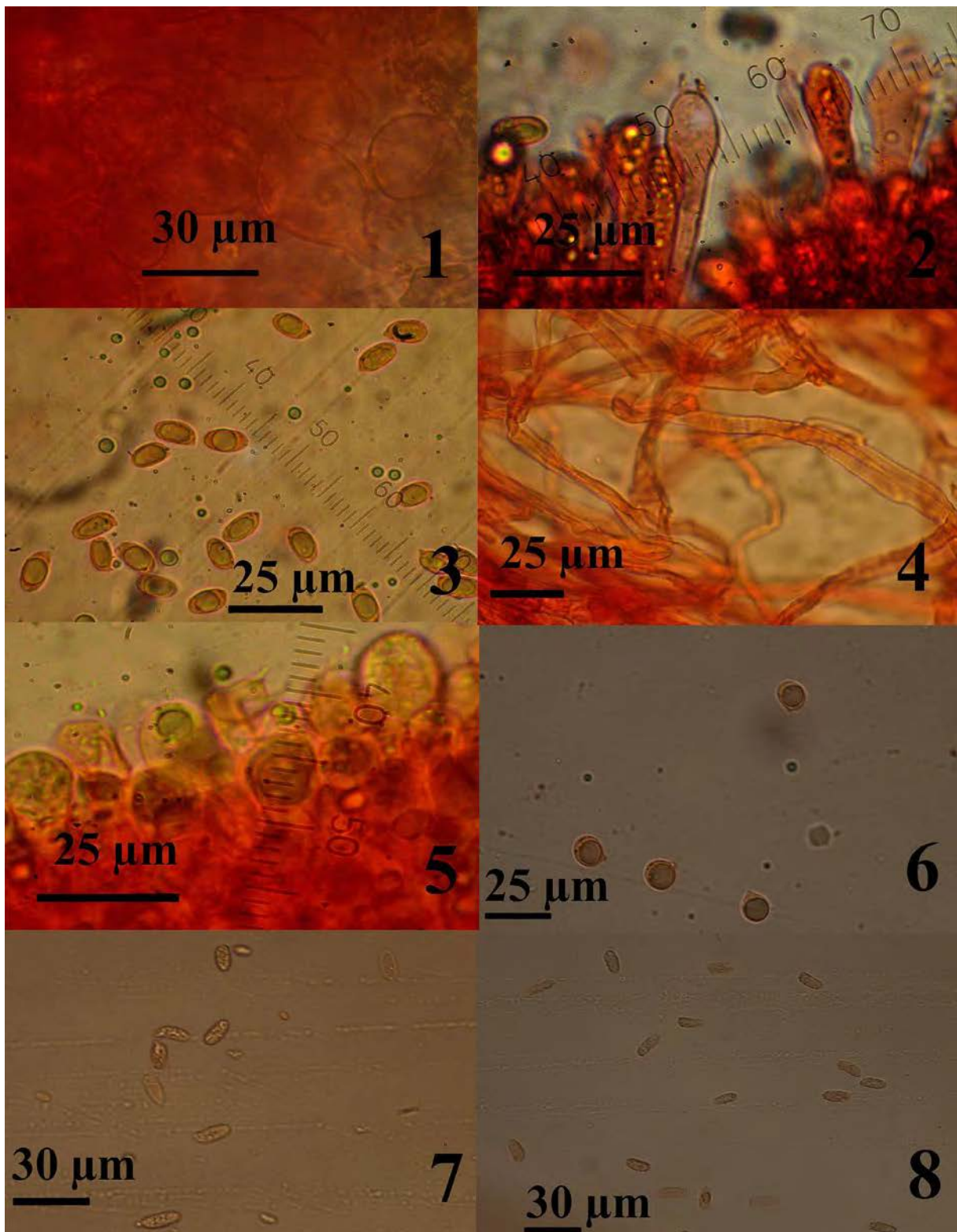


Fig. 1. *Amanita boudieri*, ovoid elements of velar tissue of the volva.
 Fig. 2. *A. boudieri*, basidia.
 Fig. 3. *A. boudieri*, basidiospores.
 Fig. 4. *A. crocea*, hyphae of the velar tissue of the volva.

Fig. 5. *A. crocea*, basidia.
 Fig. 6. *A. crocea*, basidiospores.
 Fig. 7. *A. curtipes*, basidiospores.
 Fig. 8. *A. curtipes*, basidiospores.



Fig. 1. Fruitbodies of *Amanita crocea*, 24.06.2007 (in situ).

ellipsoid to cylindrical, hyaline, amyloid. **Basidia** 55–70 × 10–12, 4-spored, clavate to elongate clavate, without clamp connections; sterigmata up to 5 µm long. **Marginal cells** pyriform, 30–40 × 10 µm. **Velar tissue** on the inner side of the volva generally filamentous, composed of branched septate hyphae up to 7.5 µm wide, with scarce inflated elements; on the outer side of the volva composed of mixed filamentous and abundant inflated elements; hyphae without clamp connections.

Specimen examined: Eastern Rodopi Mts, Makaza Pass, on sandy substrate (inferred from the inspection of basidiomata) in beech forest, 09.07.1977, leg. Cv. Hinkova & M. Drumeva, det. M. Gyosheva (SOMF 13044).

Notes. The studied specimen was originally referred to by its collectors to *A. argentea* Huijsman, and based on the synonymy between *A. argentea* and *A. mairei* Folley suggested by Breitenbach & Kränzlin (1995) and Krieglsteiner (2003) the latter name appeared in the checklists (Denchev & al. 2006; Denchev & Assyov 2010). However, our revision has demonstrated that the specimen in question

undoubtedly belongs to *A. curtipes*, a distinctive fungus with very little in common with either *A. argentea* or *A. mairei*. The above description is based on an exsiccatum (of two basidiomata), and fresh collections of this species are desperately needed to resolve its current variability in Bulgaria. On the Balkan Peninsula, *Amanita curtipes* is known with collections from Greece (Konstantinidis 2009), Republic of North Macedonia (Karadelev & al. 2011), and possibly Croatia (Mešić & Tkalčec 2002). One of the typical features of *A. curtipes* are basidiospores with a mean Q coefficient exceeding 1.8, which it from a similar *A. ponderosa* Malençon & R. Heim. *A. curtipes* has been proven different from *A. ponderosa* not only morphologically, but by a DNA analysis (Moreno & al. 2008). According to the authors, this species is known from South Europe (France, Italy, Corsica, Portugal, Spain), and North Africa (Algeria and Morocco). *Amanita curtipes* belongs to the section *Amidella*, characterized by robust fruitbodies, white flesh, typical white volva with a free membranous margin, amyloid main microscopic features, and ellipsoid to subcylindrical spores.

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