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First records of *Agaricus annae* (*Agaricales, Agaricomycetes*) for the Bulgarian mycota

Maria Lacheva*

Department of Botany and Agrometeorology, Agricultural University-Plovdiv 12, Mendeleev Str., 4000 Plovdiv, Bulgaria

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Abstract

Agaricus annae Pilát is considered a rare macrofungus throughout Europe and this is the first report of this species in Bulgaria. The article brings closer taxonomic profile, ecological requirements and distribution pattern of the species. It also describes macroscopic and microscopic characteristics of the discovered specimens and presents the specification of habitat the fungus concerned. The differences with the similar *Agaricus silvaticus* are briefly discussed. An UTM-grid map is appended. The species is described and illustrated on the basis of Bulgarian specimens.

* **Corresponding Author:** Maria Lacheva ✉ agaricus@abv.bg

Introduction

The section *Sanguinolenti* (F.H. Møller & Jul. Schäeff.) Singer of the genus *Agaricus* comprises sixteen taxa in Europe (Cappelli, 1984) and so far ten of them have been reported to occur in Bulgaria: *Agaricus altipes* (F.H. Møller) Pilát, *A. bohusii* Bon, *A. deylii*, *A. fuscofibrilosus* (F.H. Møller) Pilát, *A. haemorrhoidarius*, *A. impudicus* (Rea) Pilát, *A. langei* (F.H. Møller) F.H. Møller, *A. mediofuscus*, *A. silvaticus* Schaeff., and *A. squamulifer* (Stoichev and Lacheva, 2002; Lacheva and Stoichev, 2004; Lacheva, 2006, 2008, 2011, 2012). The one member of the section, namely *Agaricus annae* Pilát, has been recorded in some European countries. Some of the species are morphologically very similar and therefore some of them are interpreted differently.

During the author studies into the diversity of genus *Agaricus* in Bulgaria, *A. annae* 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. A detailed macro– and microscopic description is given. We also give the features that distinguish *A. annae* from similar species.

Contemporary knowledge on the diversity of Bulgarian *Agaricus* species is based on years of purposive field and laboratory investigations made by Stoichev and Lacheva (2002); Lacheva and Stoichev (2004); Lacheva (2004, 2006, 2008, 2009, 2011, 2012, 2013). This paper adds a new item to the Bulgarian mycota: *Agaricus annae* Pilát, one of the about seventy "European" species which, according to Cappelli (1983, 1984), belong to *Agaricus* sect. *Sanguinolenti*.

The aim of the present study was to add to the knowledge of Bulgaria's mycota by new macrofungal records.

Material and methods

Collection and keeping of the samples

Air-dried studied specimens of the fungus are kept in the mycological section of the Herbarium of the University of Agriculture - Plovdiv (SOA). The chorological map of the occurrence of each of the species in the country, have been depicted using the program software dSOA (Stoyanov, 2003).

Macro- and microscopic processing methods

The basidiomata were identified according to the keys and descriptions published by Pilát (1951, 1953), Heinemann (1977), Cappelli (1983, 1984) and Saini *et al.* (1997).

The samples are documented with color photographs in their natural habitats and concise description. Fruiting bodies of species were photographed with SONY Cyber-shot 5.1Mpix. in standard JPEG format. Microphotographs were taken on Amplival ML. Drawings were made with the aid of a drawing tube under an oil-immersion objective.

Description of morphological characters of basidiomata are based on fresh and dried specimens. Microscopic features were observed and measured in fresh and in dry fragments of tissues dehydrated in water, under a Amplival ML light microscope, using a 100× objective. Spores were examined in Melzer's reagent and were taken from the spore print for measurements. Size of spores, basidia, cheilocystidia and pleurocystidia, as well as pileipellis elements dimensions were correspondingly based on: 50, 30, 30, 30 and 20 measurements. Drawings were made with the aid of a drawing tube under an oil-immersion objective. Data marks length and width of microstructures are processed statistically variational (Zaitsev, 1984; Lackey, 1990). Measurement values for basidiospores are presented below as follows: min– (mean±1σ) –max.); of the basidia and cheilocistidia: min-max.

Schäeffer reaction was tested by aniline and 65% HNO_3 acid (Schäeffer and Møller, 1938) on dried samples.

Determination and nomenclature of the samples

Terminology of morphological and anatomical elements was adapted from Vellinga (1988). The abbreviations of the authors of fungal names follow

Kirk and Ansell (2004). The taxonomic and nomenclature decisions in the article have been made in conformity with the researches of Cappelli (1983, 1984), Parra (2005), Kirk *et al.* (2008) and Index fungorum (www.speciesfungorum.org: accessed 1 March 2010).

Results and discussion

The paper gives a new contribution to the knowledge of macrofungi from Bulgaria.

The materials for the present study were collected in 2009-2010 during routine field trips in Tracian Lowland floristic region (Figure 1). The floristic region is located in Southern Bulgaria and falls within the Continental-Mediterranean climatic region (Velev, 2002). Summers are warm and winters are mild, without lasting snow cover and with small temperature amplitudes. Plantations from *Robinia pseudoacacia* L., and *Populus canescens* (Ait.) Sm. in particular are very common in the region. The Thracian Lowland and has a high biological diversity ratio. Because of the suitable climate and the type of vegetation, Tracian Lowland floristic region have a rich macromycota, including of the *Agaricus* species (Lacheva, 2006). It is known that studies about Bulgarian mycota are being conducted. However, not all of the fungal diversity in different parts of Bulgaria has been determined.

After the laboratory studies, *Agaricus annae* Pilát were identified and recorded for the first time for the Bulgarian mycota (Lacheva, 2006; Denchev and Assyov, 2010; Lacheva, 2013).

Table 1. Comparison of the spore characteristics of *Agaricus annae* according to different studies.

Authors	Spore length (µm)	Spore width (µm)
Pilat (1951)	9.0–11.0	5–5.5 (–6)
Pilat (1953)	7.8–9.3 (–10)	5.5–6.0
Cappelli (1983)	7.4–9.6	4.3–5.5
Cappelli (1984)	7.5–9.5	4.5–5.5
Gerault (2005)	7.5–10.0	4.0–5.0
This study	7.5–9.0	4.3–5.5

Finally *A. annae* exceed the total number of *Agaricus* species recorded in Bulgaria to fifty-three.

The new records are given below with descriptions, photos, and microscope drawings.

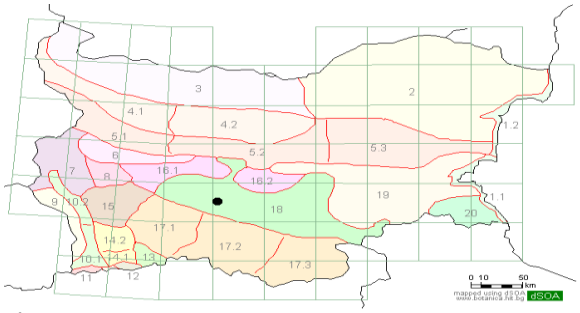

Fig. 1. Map of the distribution of *A. annae* from Thracian Lowland in Bulgaria.

Description of the taxa

Agaricus annae

Pilát, Acta Mus. Nat. Prag., 7B(1): 132, 1951

(Figure 2–7) *Agaricus silvaticus* (Schäffer : Fr.) var. *pallens* Pilát,

Acta Mus. Nat. Prag., 7B(1): 67, 1951. Icones: Pilát (1951: Figs 31-33, 37-40; Tab. 2), Pilát (1953: 21, Tab. 5), Cappelli (1983: 33), Cappelli (1984: Tab. 29).

Pileus up to 5–7 cm in diameter, thick-fleshy, initially hemispherical, subsequently applanate or slightly umbonate to flat, pale orange to ochraceous, orange brown in the center, subsequently reddish-brown, dry and mat, smooth, surface finely radially fibrillose at margin to nearly ochraceous scales in the center. Margin initially involute, thin, with ochraceous fibrillose scales, 2-5 mm thick, rarely with remnants of the veil. *Pileipellis* consisting of whitish thick-walled cylindrical hyphae, with clamps, 5–12 µm in diameter. *Gills* free, thin, initially whitish pink,

subsequently pink, pale red to red-brown, with light, sterile edge. *Hymenophoral trama* in young carpophores initially regular, subsequently irregular, consisting of cylindric, thin-walled hyphae, 5–10 µm in diam. *Stipe* up to 7–10 × 1–1.5 cm, central, cylindrical, slender, ochraceous, whitish above, redish at touch, smooth to silky-fibrillose, staining red where cutting. *Ring* pendent, thin, initially involute, subsequently upright. *Flesh* white, on cutting becoming reddish in the stipe. *Context* in pileus and stipe quite fragile, whitish. *Smell* faint, non-distinctive. *Taste* non-distinctive. *Spores* 7.0– (4.2 ± 0.02) –9.0 × 4.0– (4.1 ± 0.02) –5.5 µm, (n = 50), ellipsoid, brownish, not ornamented, with fluorescent spots with an apical germ pore. Spore print brown. *Basidia* 20–30 × 10–15 µm, clavate, hyaline, with 4 sterigmata. *Sterigmata* 2.3 µm long. *Cheilocystidia* 25–40 × 10–35 µm, numerous, slightly clavate, thin-walled, hyaline. *Macrochemical reactions:* Cross reaction with Schaeffer's reagent negative.

Fig. 2–7. Macro- and microscopic features of *Agaricus annae* from Bulgaria: 2, 3 – basidiomata at different stages of development *in situ*, 4 – basidiospores, 5 – basidia, 6 – cheilocistidia, 7 – generative hyphae of the pileipellis with terminal elements (photos and drawn by M. Lacheva). Bar = 5µm.

Habitat, ecology, phenology

Carpophores of *Agaricus annae* almost exclusively appears in summer to early fall season (May–October), solitary or more rarely gregarious, mostly in plantations from *Robinia pseudoacacia* L., and *Populus canescens* (Ait.) Sm., as well as xerothermic or mesothermic broadleaf forests, under broadleaved trees of *Acer*, *Carpinus, Fagus, Fraxinus* and *Quercus* possibly also with coniferous trees, 0-900 m. Humus saprotroph, mostly on basic to neutral soil.

Hardly mentioned in the literature, no sufficient data are available to regard it as surely edible. There is some evidence that also this species has been edible without gastrointestinal problems (Cappelli, 1984).

Specimens examined

Bulgaria. Thracian Lowland, Plovdiv distr., above Benkovski village, on soil, in culture of *Robinia pseudoacacia* L., 18 October 2009, coll. & det. M. Lacheva (SOA 60 00290); ibid., among grasses, in culture of *Populus nigra* Arn., 27 September 2010, coll. & det. M. Lacheva (SOA 60 00291).

General distribution

Rare species in Europe. Described from Czech Republic (Pilát, 1953; Holec and Beran, 2006), Denmark (Vesterholt *et al.,* 2003), France (Heinemann, 1977; Gerault, 2005), Italy (Cappelli, 1983, 1984), Spain (Ruiz, 1997; Moreno and Remondo, 1999); on the Balkan Peninsula reported the first time in Bulgaria.

Note

The first description of *Agaricus annae* was published in 1951 by Pilát, along with the description of *Agaricus silvaticus* (Schäffer : Fr.) var. *pallens* Pilát, as several years later (Pilát, 1953) declared that this species is likely to be conspecific with *A. silvaticus* var. *pallens*, recognizing in effect *A. annae* as valid taxon. Since then the species has been recorded in many European countries, but in some regions it is regarded as rare.

Macroscopically and microscopically, the recorded specimens of *A. annae* agree with the descriptions of Pilát (1951, 1953), as well as of Cappelli (1984). However, basidiospore size was somewhat smaller

than the measurements they presented (Table 1). This discrepancy probably is attributable to difference in sample size. Nevertheless, the cheilo- and pleurocystidia, although differing in appearance, were mostly clavate in shape, confirming the observations of other mycologists (Cappelli, 1983, 1984; Gerault, 2005).

Agaricus annae Pilát is closely with *Agaricus silvaticus* from which differs in the cap being generally pale orange-brown with dark ochraceous center, single collapsed ring, longer stipe, and the slightly greater spores. Moreover, there is an ecological difference between the two species, i.e. *A. annae* grows on periphery of plantations from *Robinia pseudoacacia* L., and *Populus canescens* (Ait.) Sm., as well as xerothermic or mesothermic broadleaf forests, while the latter occurs mostly in coniferous forests (Cappelli, 1983, 1984; Lacheva, 2006).

In Europe this fungus is more frequent only in areas with near-natural to virgin deciduous and mixed forests or in old wooded parks (Pilat, 1951, 1953; Moreno and Remondo, 1999; Gerault, 2005). In addition, Cappelli (1983, 1984) collected *A. annae* from frondose and coniferous woods. According to our investigation, *A. annae* should be considered as a species developing in cultures from *Robinia pseudoacacia* L. and *Populus canescens* (Ait.) Sm., and mixed deciduous forest in lowland areas with low altitude. Recognized as a rare species, *Agaricus annae* has been red-listed in several European countries. This species included in the Red List of fungi in Czech Republic, and Denmark (Vesterholt, 1998; Vesterholt *et al.,* 2003; Holec and Beran, 2006).

Conclusion

The discovery of new records of macrofungi species for Bulgaria, and also rare species throughout in Europe, indicating a great biodiversity that is yet to be discovered in this country moreover, the lack of specific studies in the given area. Given that species is recorded as endangered in many European countries, this could also indicate that the *Red List of fungi in Bulgaria* (Gyosheva *et al.,* 2006) might have new candidates.

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