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Agaricus bernardii and *A. maleolens* (section Duploannulatae, Agaricaceae) in the Bulgarian mycobiota: diversity, distribution, morphology and ecology

Maria Lacheva

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

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Abstract

This paper summarizes the available data of *Agaricus bernardii* and *A. maleolens* in Bulgaria. Review of the available previous records is made and new findings are reported. The article brings closer taxonomic profile, ecological requirements and distribution pattern of the both species. New additions are included, together with a critical review of data reported in literature for the Bulgarian territory. The fungus appeared to be widespread, but not common. Detailed macro- and microscopic descriptions are given. The species are illustrated with color photographs on the basis of collected Bulgarian specimens. Totally 21 localities of these species are currently known. An UTM-grid map is appended. An identification key for the species is given.

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

Introduction

Mushrooms in the genus *Agaricus* have a worldwide distribution. There are *c*. 200 described *Agaricus* species worldwide and approximately 60 of them occuring in Europe (Cappelli, 1984, 1985; Bas, 1991; Calvo-Bado *et al.*, 1999; Kirk *et al.*, 2001, 2008).

The genus *Agaricus* includes as sections including only edible and economically important species (sections *Flavescentes* (J. Shaeff. & F.H. Møller) Wasser) or only inedible and less toxic (e.g. all taxa in section *Xanthodermatei* Singer) as well and sections including as edible and inedible species (Cappelli, 1984, 1985; Bas, 1991; Calvo-Bado *et al.*, 1999; Kirk *et al.*, 2001, 2008). This is section *Duploannulatae* Wasser which include edible species (e.g. *A. bisporus, A. bitorquis, A. subfloccosus* etc.), excepting *Agaricus bernardii* (Quél.) Quél. and *A. maleolens* F.H. Møller are known to possess unpleasant odours and taste (Wasser, 1980, 1985; Cappelli, 1984; Łuszczyński, 2008; Lacheva, 2006).

The taxonomic status of the *A. maleolens* F.H. Møller is formulated in various ways. In relation with this, many authors consulted (Parra, 2005; Denchev and Assyov, 2010; Kirk, 2013) consider the species is synonym for *A. bernardii* (Quél.) Quél., while according to other authors (Bohus, 1961; Alessio, 1975; Wasser, 1980; Cappelli, 1984, 1985; Gerault, 2005; Lacheva, 2006), *Agaricus bernardii* and *A. maleolens* are two separate species represented in Europe. According to Lacheva (2006, 2011), *Agaricus bernardii* and *A. maleolens* are distributed in Bulgaria. In the latest Bulgarian mycological literature (Denchev and Assyov, 2010), *A. maleolens* not reported for the Bulgarian mycota.

The aim of the paper is confirm the presence and distribution of *Agaricus bernardii* and *A. maleolens* as two separate species in the Bulgaria's mycobiota.

This paper provides: (a) a taxonomic revision of *Agaricus bernardii* and *A. maleolens* in Bulgaria; (b) descriptions of basidiomata development in the field;

(c) a discussion and an expanded description for these species;(d) a discussion of the morphological characters previously used to segregated species, and(e) summarize the knowledge about the distribution of these interesting fungal species in Bulgaria.

Materials and methods

Collection and keeping of the samples

Distribution of the taxa is given according to the floristic regions adopted in the *Flora of the PR Bulgaria* (Jordanov, 1966). The chorological map of the occurrence of each of the species in the country, have been depicted using the program software dSOA (Stoyanov, 2003). Air-dried studied specimens of the fungus are kept in the mycological section of the Herbarium of the University of Agriculture - Plovdiv (SOA).

Macro- and microscopic processing methods

The fruiting bodies were identified according to the keys and descriptions published by Cappelli (1984, 1985), Wasser (1980, 2002), Parra (2005), Łuszczyński (2008).

Description of morphological characters of fruiting bodies 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. Size of spores, basidia, cheilocystidia and pleurocystidia were examined in Melzer's reagent and were taken from the spore print for 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±1 σ) – max.; of the basidia and cheilocistidia: min-max.

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.

The nomenclature for fungi is according to Cappelli (1984, 1985), Wasser (1980, 2002), Parra (2005), Kirk *et al.* (2008) and Index fungorum (www.speciesfungorum.org: accessed 1 March 2010). The abbreviations of the authors of fungal names follow Kirk and Ansell (2004). Schäeffer reaction was tested by aniline and 65% HNO₃ acid (Schäeffer and Møller, 1938) on dried samples.

Abbreviations are used throughout the text for the collectors' names as follows: GS - G. Stoichev; ML - M. Lacheva; MD - M. Dimova.

Results and discussion

The perennial field studies confirm the *Agaricus bernardii* and *A. maleolens* for the territory of Bulgaria. The present study provides data for a clear delimitation of both species in the Bulgarian mycota. Their characters are described below.

The review of the literature reports, the revision of the herbarium specimens, together with the unpublished collections of the author revealed 23 known records (9 for *Agaricus bernardii* and 14 for *Agaricus maleolens*) of the species, corresponding to 19 UTM-Grid squares. They are all listed below and presented on Fig. 11a-b.

Description of the taxa

Agaricus bernardii (Quél.) Quél., Clavis Syn. Hymen. Europ., p. 89, 1878. (Figs 1–5)

Basionym: *Psalliota bernardii* Quel.,Syll. Fung., 5: 999, 1887.

Icons: Møller (1950: Figs 4, 7), Essette (1964: Tab. 7), Pilát (1972: Tab. 82), Wasser & Soldatova (1977: Fig. 19), Wasser (1979: Pl. 76), Wasser (1980: Tab. 13), Cappelli (1984: Fig. 2, Tab. 2), Lacheva (2006: Tablo 23). Pileus up to 4-7 cm in diameter, thick-fleshy, initially hemispherical, subsequently applanate or slightly umbonate to flat, with or without a hollow in the center, white, cream-white, subsequently lightbrownish, hazelnut-brownish in the center, surface cracked with ochre brown, tileform scales, larger and thicker in the center (up to 1.5 mm in diameter), to edge gradually disappears, slightly brownish at touch. Margin initially involute, then straight, fibrous or with smaller cream white scales, 5 mm thick, at young fruiting bodies almost knitted with the top of the stipe, sometimes with fragments of the partial veil. consisting of whitish thick-walled Pileipellis cylindrical hyphae, with clamps, 5 µm in diameter. Gills free, thin, crowded, initially gravish pink, subsequently pink red to dark brown, with light, sterile edge. Hymenophoral trama in young carpophores initially regular, subsequently irregular, consisting of cylindric, thin-walled hyphae, 5-10 µm in diameter. Stipe 4,5-8,5 x 2,5-3.5 cm, central, cylindrical, evenly thick, sometimes narrowed at the base, glabrous to silky-fibrillose, white with grayish tint, pinkish at touch, staining pink to reddish brown where cutting. Ring in the upper part of the stipe, sometimes in the middle, thick, narrow, patent, sometimes double layer, white, with or without a silky-fibrillose coating, with time disappears. Flesh thick, firm, white, initially orange red at the stipe base when cut, subsequently red-brown to dark wine red in the whole context. Context in pileus and stipe quite fragile, whitish. Smell of uric acid. Taste distinct. Spores $5-(6.5 \pm 0.02)-7.7 \times 4.5-(5.3 \pm 0.02)-6 \mu m$, N=50, broadly-ovate, brown, not ornamented, mostly with a fluorescent spots, with an apical germ pore. Spore print dark brown. Basidia $20.5-40.5 \times 6.5-10$ μ m, N=30, clavate, hyaline, with 4 sterigmata. Sterigmata 3.5-4 µm long. Cheilocystidia 15.5-38.5 \times 7–20.5 µm, N=30, numerous, clavate, cylindrical to fusiform, with a blunt hemispherical tip, erect or arching ascendant, thin-walled, hyaline or slightly brownish, on gill edges. Pleurocystidia absent. Macrochemical reactions: Cross reaction with Schaeffer's reagent negative.

Habitat, ecology, phenology: Fruit bodies of *Agaricus bernardii* almost exclusively appears in summer to the middle of the summer (May–August), grows solitary or gregarious, mostly in dry, open, sandy and stony places and dunes, on uncultivated areas; in gardens and parks among grassy vegetation as well as xerothermic or mesothermic lawn; on coasts, 500 m. Humus saprotroph, mostly on basic, clay or sandy soil. There is some evidence that also this species is inedible causing gastrointestinal problems (Wasser, 1980; Cappelli, 1984).

Specimens examined: East Stara Planina: reported by Hinkova and Drumeva (1978), SOMF 9167; Mt Sredna Gora: Sarnegor village, Brezovo municipality, Plovdiv distr., on open stony meadow, 15.07.2003, coll. GS, det. ML (SOA 50168); Krasnovo village, Hissar municipality, Plovdiv distr., Sveta Petka locality, on stony meadow, 23.05.2003, coll. MD, det. ML (SOA 50169); Thracian Plain: Besaparski Hills, Pazardzhik municipality, Plovdiv distr., on dry stony meadow, 23.08.2003, coll. and det. ML, GS (SOA 50170); Shtarkovo village, Pazardzhik municipality, Plovdiv distr., on open dry meadow, 19.08.2003, coll. and det. ML (SOA 50171); reported by Stoichev and Dimcheva (1982), SOMF 14339; Toundzha Hilly Region: Mt Sakar, Sakartsi village, Topolovgrad municipality, Haskovo distr., on open stony meadow, 14.08.2002, coll. and det. ML (SOA 50172); ibid., on sandy soil, 02.09.2010, coll. and det. ML (SOA 60 00370); Dervishka Mogila at Mogilata locality, on stony meadow nearly to mounds, 02.09.2010, coll. and det. ML (SOA 60 00369).

General distribution: In Europe, *A. bernardii* reported from Italy (Cappelli, 1984), France (Moreno and Remondo, 1999; ODONAT (Coord.) 2003; Gerault, 2005), Denmark (Vesterholt, 1998; Vesterholt *et al.*, 2003), Slovakia (Lizoň, 2001), Hungary (Bohus, 1961), the Czech Republic (Pilát, 1961), Spain, Portugal, the Netherlands, Ukraine (Wasser, 1980, 1985), and Bulgaria (Lacheva, 2006); In Asia the species has been recorded in Caucasus, China, Russia, Tajikistan, Uzbekistan, Israel (Wasser, 1980, 2000, 2002), Mongolia (Pilat, 1972; Wasser, 1980), and Turkey (Demirel *et al.*, 2004; Gucin, 1987; Gucin, 1990; Isıloğlu and Oder, 1995; Sesli and Baydar, 1996; Isıloğlu, 1997; Uzun *et al.*, 2004; Sesli and Denchev, 2009); In Africa the species has been recorded in Algeria and Maroko (Wasser, 1980).

Agaricus maleolens F.H. Møller, Friesia, 4: 203, 1952. (Figs 6–10)

Basionym: *Psalliota ingrate* F.H. Møller, Friesia, 4: 17, 1950.

Icons: Møller (1950: Fig. 5, Pl. 8), Essette (1964: Pl.8), Wasser (1980: Fig. 62, Tab. 9), Cappelli (1984: Pl. 3, Fig. 3), Lacheva (2006: Tablo 25).

Pileus up to 5-10 cm in diameter, thick-fleshy, initially hemispherical, subsequently hemispherical convex, applanate or slightly umbonate to flat, with a hollow in the center, glabrous to slightly silkyfibrillose, white-ochre, lighter toward the edge. Margin initially involute, then straight, 2-5 mm thick, draped sometimes with fragments of the partial veil. Pileipellis consisting of whitish thick-walled cylindrical hyphae, with clamps, $5-8 \mu m$ in diameter. Gills free, thin, crowded, initially reddish, subsequently dark brown to chocolate-coloured, with light, sterile edge. Hymenophoral trama in young carpophores initially regular, subsequently irregular, consisting of cylindric, thin-walled hyphae, 5-10 μ m in diameter. Stipe up to 4–8 × 1.5–2.5 cm, central, cylindrical, concolourous with pileus, narrowed at the base, with or without a rod-shaped rhizomorph at the base, reddish at touch, staining pink to light reddish where cutting. Ring in the bottom part of the stipe, sometimes in the middle, thin, spreading, falls down pulling away from the stipe, white, with or without a white-ochre silkyfibrillose coating. Flesh white, ochre-pink on cutting, darkening, darkening particularly quickly in the stipe and becoming brunneous-ochre with a distinctive pink tint. Context in pileus and stipe quite fragile, whitish. Smell of fish, stronger and unpleasant fishy

smell when drying. *Taste* distinct. *Spores* 5.5–(6 \pm 0.01)–7 × 4–(4.5 \pm 0.01)–5 µm, N=50, broadlyelliptical, red-brown to brown, not ornamented, mostly with a fluorescent spots, with an apical germ pore. Spore print dark brown. *Basidia* 30–41 × 7–10 µm, N=30, clavate, hyaline, with 4 sterigmata. *Sterigmata* 3–4 µm long. *Cheilocystidia* 40–70 × 8– 15 µm, N=30, numerous, cylindrical, with a blunt hemispherical tip, erect or arching ascendant, thinwalled, hyaline, on gill edges. *Pleurocystidia* absent. *Macrochemical reactions:* Cross reaction with Schaeffer's reagent negative.

Habitat, ecology, phenology: Fruit bodies of *Agaricus maleolens* almost exclusively appears in summer to early fall season (May–November), grows usually gregarious, mostly on meadow among grasses, in xerothermic or mesothermic lawn; in gardens and parks among grassy vegetation; in urban area, sometimes in plantations from *Robinia pseudoacacia* L., and *Populus nigra* Arn.; possibly under broadleaved trees of *Acer, Carpinus, Fraxinus,* 200-700 m. Humus saprotroph, mostly on basic or sandy soil. There is some evidence that also this species is inedible causing gastrointestinal problems (Wasser, 1980; Cappelli, 1984).

Specimens examined: North Black Sea Coast: nearly Obrochiste village, Balchik municipality, on meadow nearly plantation of Robinia pseudoacacia L., 26.09.2004, coll. and det. ML (SOA 50173); Znepole region: nearly Zemen village, Kyustendil municipality, on meadow, 01.11.2004, coll. and det. GS (SOA 50174); West Frontier Mts: in grasslands, on sandy soil, roadside, nearly Vlahi village, Kresna municipality, Blagoevgrad distr., 16.09.2002, coll. and det. GS (SOA 50175); Mt Sredna Gora: nearly Drangovo village, Plovdiv distr., on meadow under Rubinia pseudoacacia L., nearly to dam, 06.08.2002, coll. and det. ML, GS (SOA 50004); near Hisar town, Plovdiv distr., Dam Momina Banya locality, on open meadow among grasses, 09.06.2004, coll. and det. ML, GS (SOA 50176); ibid., among grasses, 27.09.2010, coll. and det. ML (SOA 60 00368); near Staro Jelezare village, on meadow nearly plantation of Robinia pseudoacacia L., 15.06.2004, coll. and det. ML (SOA 50177); on meadow among grasses over deciduous forest from Quercus cerris L., Quercus frainetto Ten., Carpinus orientalis Miller, above Strelcha town, Pazardzhik municipality, Plovdiv distr., 27.10.2004, coll. GS, det. ML (SOA 50178); Thracian Plain: Stryama village, Plovdiv distr., on meadow to the left of the bridge over the river Stryama, 23.10.2004, coll. and det. ML, GS (SOA 50179); Benkovski village, Plovdiv distr., in Rubinia pseudoacacia culture, 06.10.2004, coll. and det. ML, GS (SOA 50180); on meadow nearly Chernozem village, Plovdiv distr., 05.11.2004, coll. and det. GS (SOA 50181); Toundzha Hilly Region: Mt Sakar, Dobroselets, Topolovgrad municipality, along Haskovo distr., Treskovo kladenche locality, on open meadow nearly plantation of Robinia pseudoacacia L., 14.08.2002, coll. and det. ML (SOA 50182); Lesovo village, on meadow nearly to customs, 26.07.2004, coll. and det. ML (SOA 50183); nearly Balgarska Polyana village, Topolovgrad municipality, Haskovo distr., on meadow, roadside, under thickets in gully with Paliurus spina-christi, 14.10.2004, coll. and det. ML (SOA 50185).

General distribution: In Europe, *A. maleolens* is known from Denmark (Hansen and Knudsen, 1992; Vesterholt, 1998; Vesterholt *et al.*, 2003), France (Moreno and Remondo, 1999; Gerault, 2005), Switzerland (Krieglsteiner, 1991), Italy (Cappelli, 1984), Poland (Łuszczyński, 2008); Slovakia (Lizoň, 2001); Hungary, the Czech Republic, Spain, Portugal, the Netherlands, Ukraine (Wasser, 1980, 1985), and Bulgaria (Lacheva, 2006). It occurs also in Africa from Morocco (Malençon and Bertault, 1970).

Identification key

1 Pileus up to 4-7 cm in diameter, cream-white, subsequently light-brownish, hazelnut-brownish in the center, surface cracked with ochre brown, tileform scales; stipe evenly thick, without a rod-shaped rhizomorph at the base; spores $5-7.7 \mu$ m long, 4.5-6

μm wide, broadly-ovate, brown *Agaricus bernardii*

Note: Nearly similar fruiting bodyes produces *A*. *bitorquis* known to be common in Europe, which can be separated with certainty from *A*. *bernardii* in the cap being generally smooth, whitish to off-white, without scales, double ring, length of stipe, colour of flesh when cut as well as mainly on the base of microscopic characters (e.g. size of spores and cheilocystidia) and differences in habitat (terrestrial fungus occurs mainly on manure and enriched soil, in parks and cultures from *Populus nigra L*. and *Robinia pseudoacacia L.*, in fruit and vegetable gardens, in urban areas, streets, cracks in asphalt), sweet fungoid smell and taste (Pilat,1972; Wasser, 1980, 2002; Cappelli, 1984; Lacheva, 2006).

The species *A. maleolens* is close to *A. bernardii* and *A. bitorquis*. Of the three species differ in surface ornamentation, color and form of the cap, type of ring, colour of flesh when cut, smell of fish, as well as mainly on the base of microscopic characters, namely wider spores and wide and curved cheilocystidia (Cappelli, 1984; Gerault, 2005; Lacheva, 2006).

According to some authors (Bohus, 1961; Pilat, 1972; Wasser, 1980; Cappelli, 1984; Isıloğlu and Oder, 1995; Isıloğlu, 1997; Lacheva, 2006), the difference between A. maleolens and A. bernardii is also given by habitat. In 1961, G. Bohus reported A. bernardii for alkaline meadows and fields in Hungary, and Pilat (1972) published it to Mongolia found at 1500 m asl. et some other authors reported the species on mountains, meadows, pastures and parks (Wasser, 1980; Cappelli, 1984; Lacheva, 2006). According to this investigation, the both species should be considered as a species developing among grassy vegetation at lower elevation mostly on basic or sandy soil, as A. maleolens prefers grassy vegetations, sometimes under broadleaved trees of Populus, Robinia, Carpinus, Fraxinus, etc., and very rare under Quercus, until A. bernardii is occurring mainly on sandy soil, in open places and stony meadow (Cappelli, 1984).

The Bulgarian collection of both species corresponds macro- and microscopically to the descriptions, available in the literature (Møller, 1950, 1952; Wasser, 1980; Cappelli, 1984; Gerault, 2005; Lacheva, 2006). Some discrepancies are to be noted though between the original and the later descriptions. Although slightly different, the measurements of the Bulgarian specimens are close to this last fig.. Although slightly different, the measurements of the Bulgarian specimens are close to this last fig.. This difference could be probably due to the different sampling sizes - 50 basidiospores in this paper and 250 measured by Lacheva (2006), but it could be also due to simply individual variability caused by specific environmental conditions, etc. Comparison of the micrometric characteristics of both species according to different studies are outlined in Tables 1-2.

Table 1.	Comp	arison	of the	microm	etric	chara	cteristics	of A	Agaricus	bernai	rdii a	according	to	different	studie	25

Author	Basidiospores (µm)	Basidia (μm)	Sterigmata (µm)	Cheilocystidia (µm)		
	1	2	3	4		
Møller (1950-1952)	5.5–7(–10) × 5–6	26-40 × 7-8.5	2-4	24–56 × 5–16		
Wasser (1980)	$5.5 - 7 \times 5 - 5.5$	26-40 × 7-9	3-4	25-60 × 10-15		
Cappelli (1984)	5.5–7(–10) × 5–5.5	7-9	4	20-60 × 5-15		
Lacheva (2006)	5.1–7.6 × 4.6–6	20–40 × 6.5–10	3-4	15.5–38 × 7–20.5		
Gerault (2005)	$7-9 \times 6-6.5$	_	_	_		
This study	5-7.7 × 4.5-6	20.5–40.5 × 6.5–10	3.5-4	15.5-38.5 × 7-20.5		

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Author	Basidiospores (μm)	Basidia (µm)	Sterigmata (µm)	Cheilocystidia (µm)	
	1	2	3	4	
Møller (1950-1952)	$5.5-7(-8) \times 4.5-5.5(-6)$	26-36 × 7-10	5	30–64 × 6–20	
Wasser (1980)	_	20–41 × 6–10	3-4.5	27-70 × 6-20	
Cappelli (1984)	5.5–7.5 × 4.5–5.5 (–6)	26-36 × 7-20	5	30–64 × 6–20	
Lacheva (2006)	$5-7 \times 4-5.5$	30-41 × 6.8-10	3-4	40-70 × 8-20	
Gerault (2005)	5.5–7 × 4.5–6	—	-	-	
This study	5.5-7 × 4-5	30-41 × 7-10	3-4	40-70 × 8-15	

Table 2. Comparison of the micrometric characteristics of Agaricus maleolens according to different studies.

Conclusion

The both species *Agaricus bernardii* and *A. maleolens* distributed in Europe, occurring in Bulgaria. So far, both species are reported in Bulgaria only from North Black Sea Coast, Znepole region, West Frontier Mts, East Stara Planina, Mt Sredna Gora, Thracian Plain, and Toundzha Hilly Region (Hinkova and Drumeva, 1978; Stoichev and Dimcheva, 1982; Lacheva, 2006, 2012). The present report gives some new records of the *Agaricus* species from Bulgaria. Totally 23 records, corresponding to totally 21 localities of the taxa are currently known.

Although present with a number of records in the country, *Agaricus maleolens* does not seem to be a common fungus in Bulgaria. Observations confirm it is never abundant and is usually present by single or some basidiomata in its localities. It is a rare species, that must be further monitored and conscientiously recorded. Observations point out that the *A. maleolens* should be searched for especially in plains and lowlands regions of the country; mostly among grasses, on sandy soil and open meadow, and only exceptionally found under some deciduous trees of *Populus nigra, Robinia pseudoacacia, Acer* or *Fraxinus*.

Recognized as a rare species, *Agaricus bernardii* and *A. maleolens* has been red-listed in several European countries. This species included in the Red List of fungi in Denmark and Slovakia (Vesterholt, 1998; Vesterholt *et al.*, 2003; Lizoň, 2001). Given that species is recorded as endangered or rare in many European countries, this could also indicate that the *Red List of fungi in Bulgaria* (Gyosheva *et al.*, 2006) might have new candidates.



Figs. 1–5. Macro- and microscopic features of *Agaricus bernardii* from Bulgaria: 1 – fruit bodies at different stages of development *in situ*, 2 – basidiospores, 3 – basidia, 4 – cheilocystidia, 5 – generative hyphae of the pileipellis with terminal elements (photos and drawings by M. Lacheva). Bar = 5μ m.

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Figs. 6–10. Macro- and microscopic features of *Agaricus maleolens* from Bulgaria: 6, 6a – fruit bodies at different stages of development *in situ*, 7 – basidiospores, 8 – basidia, 9 – cheilocystidia, 10 – generative hyphae of the pileipellis with terminal elements (photos and drawings by M. Lacheva). Bar = 5μ m.



Fig. 11. Distribution of the species in Bulgaria: a – *Agaricus bernardii*; b – *Agaricus maleolens*.

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