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***Pholiota gallica* (Fungi, Strophariaceae): first Balkan collection of a little-known dark-spored agaric**

Abstract

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The author reports the first Balkan collection of the scarcely featured in the mycological literature *Pholiota gallica* based on material collected in Bulgaria. Description of the macro-morphology and microscopic features of the studied specimens is included, supplemented with illustrations.

Key words: Balkan mycota, biogeography, *Pholiota lubrica* var. *obscura*, Strophariaceae, taxonomy.

Introduction

The genus *Pholiota* (Fr.) P. Kumm. is one of the genera of the family Strophariaceae Singer & A.H. Sm., comprising saprobic species, mostly occurring on woody substrates. The genus attracted the attention of mycologists in Europe for a long time and has been subject of several monographic treatments (Jacobsson 1991, 1992, 2008; Noordeloos 1999, 2011; Holec 2001) and new species have been recognized (Holec & Niemelä 2000; Holec & al. 2014). Among these, the name *Pholiota gallica* Holec & M. Kolařík was recently proposed to accommodate *P. lubrica* var. *obscura* Bon & Chevassut, when elevated to species rank (Holec & Kolařík 2014). It was later inferred that *P. highlandensis* (Peck) Singer var. *citrinosquamulosa* Maire ex Bidaud & Borgarino is a synonym (Holec & al. 2016), confirming the variability of the species. Nevertheless, *P. gallica* still remains one of the least known species of the genus in Europe. In 2021 the author had the opportunity to observe and collect *P. gallica* in Bulgaria. Description and illustrations of the taxon are reported here.

Materials and Methods

The basidiomata were photographed *in situ* and the relevant features were noted. The voucher specimen is deposited in air-dried state in the Mycological Collection of the

Institute of Biodiversity and Ecosystem Research (SOMF). The microscopic features were observed under an AmScope T360B light microscope, equipped with AmScope MU900 digital camera. The study of the microscopic characters was performed on preparations from dried specimens after rehydration with 5% KOH. Congo red in ammonia was added to the preparations except for the observation of spores, which were measured solely in KOH solution. Melzer's reagent was employed in addition to assess the iodine reactions. The assessment of the size of the microscopic features was performed on pre-calibrated digital photographs with the aid of Piximètre ver. 5.10 (©A. Henriot & J.-L. Cheype). The spore measurements are presented below by the minimum and maximum values of length, width and quotient (Q), followed by the average values for spore length (L_{av}), width (W_{av}) and quotient (Q_{av}); the spore data refer to sample of 30 basidiospores. For the remaining microscopic features minimum and maximum values are included. The colour terms refer to the 'Flora of British Fungi Colour Identification Chart' (Royal Botanic Garden Edinburgh 1969) followed in parenthesis by a colour code beginning with the abbreviation "BFF". The remaining colour terms are vernacular names rather than chart entries. The colours in the description of the microscopic structures refer to slides in KOH.

Results

Pholiota gallica Holec & M. Kolařík, Mycotaxon 127: 165 (2014); \equiv *Pholiota lubrica* var. *obscura* Bon & Chevassut, Docums Mycol. 19(fasc. 75): 44 (1989); = *Pholiota highlandensis* var. *citrosquamulosa* Maire ex Bidaud & Borgarino, Bull. Sem. Féd. Assoc. Mycol. Médit. 30: 6 (2006). (Figs 1, 2).

Pileus up to 5 cm broad, convex or convex-applanate, sometimes slightly irregular, almost smooth, radially fibrillose to finely rugulose, with somewhat greasy shine and slightly sticky, rusty tawny (BFF14) to brick-red (BFF15), sometimes discolouring in places to cinnamon (BFF10), along the margin yellowish buff (BFF52) to straw (BFF50); pileal edge appendiculate and usually with brownish remnants of veil. Stipe up to 1 cm wide, cylindrical, sometimes curved and often widened at the base, buff (BFF52), downwards brick (BFF15) to rusty tawny (BFF14), in the upper part with brownish fibrillose annular zone and with straw to yellowish floccose girdles below, in section in the upper half hollow; mycelial strands off-white to lemon yellow. Lamellae more or less crowded, narrowly adnate to adnexed, pale buff (BFF52) to pale vinaceous buff (BFF31), occasionally rusty spotted, edge concolorous, often uneven; lamellulae present, 1–3 between lamellae. Context whitish in the pileus and upper stipe, downwards yellowish, brownish towards the stipe base. Odour and taste inconspicuous. Exsiccata in UV 365 nm produce yellowish green fluorescence, located at lamellar edges. Basidiospores $6.3\text{--}7.5 \times 4\text{--}4.8 \mu\text{m}$, $Q = 1.4\text{--}1.7$; $L_{av} = 6.9$, $W_{av} = 4.5 \mu\text{m}$, $Q_{av} = 1.5$, ellipsoid, ellipsoid-ovoid to ovoid, some of them flattened on adaxial side, yellow-brown, with a distinct brown wall up to $0.7 \mu\text{m}$ thick and a germ pore. Basidia predominantly 4-spored, occasionally 2-spored, $18.3\text{--}23.9 \times 5.5\text{--}7.2 \mu\text{m}$, cylindrical to narrowly clavate; sterigmata $2\text{--}2.5 \mu\text{m}$ long. Leptocystidia on lamellar edge and faces, partly or entirely with distinctive yellowish, non-refractive content. Cheilocystidia $30.8\text{--}49.9 \times 9.6\text{--}16.4 \mu\text{m}$, fusiform, utriform or lageniform. Pleurocystidia $45.7\text{--}68.3 \times 8.2\text{--}16.2 \mu\text{m}$, lageniform, narrowly lageniform or occasionally clavate.



Fig. 1. Macromorphological features of *Pholiota gallica*. Scale bar = 1 cm.

Chrysocystidia absent. Lamellar trama of 5.3–15.8 μm wide, cylindrical, septate, hyaline hyphae. Pileipellis an ixotrichodermium, 3-layered: outer layer composed of 1.5–4.5 μm wide, encrusted by yellow brown pigment, loosely to densely arranged, septate and branching hyphae; medium layer of 2–5 μm broad, encrusted by yellow brown pigment hyphae; inner layer of somewhat inflated, 3.9–9.7 μm wide, sub-parallel, septate hyphae with reddish brown membrane pigment; pileocystidia absent. Stipitipellis a cutis-like, of running parallel to stipe axis, 2–5.7 μm broad, septate hyphae with yellowish to brownish yellow encrusting pigment; caulocystidia not seen; stipe flocci made of densely interwoven, 2.5–7.6 μm broad, septate hyphae with yellow outline and encrusting pigments. No amyloid or dextrinoid microstructures detected. Clamp connections present in all tissues.

Specimen examined. Bulgaria, Blagoevgrad Province, Strumyani Municipality, between Mikrevo and Kamenitsa villages, 41°38'12.4"N, 23°10'27.2"E, ca 190 m, in grasslands close to *Quercus coccifera* L, on non-calcareous, sandy soils, 19 Dec 2021, leg. B. Assyov (SOMF 30431).

Discussion

The new collection of *P. gallica* matches the morphological description of the type specimen and other findings of the species as reported by Holec & Kolařík (2014) and Holec & al. (2016). Basidiomata in the Bulgarian specimen feature darker colours in comparison

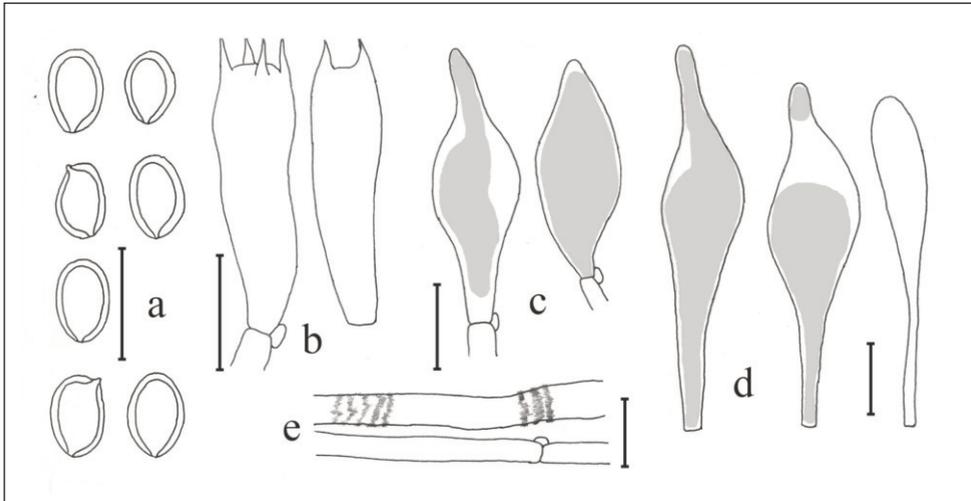


Fig. 2. Microscopic characters of *Pholiota gallica*: a. basidiospores, b. basidia, c. cheilocystidia, d. pleurocystidia, e. pileipellis hyphae (encrusting pigments shown partly). Scale bars = 10 μm .

with the ones illustrated in Holec & al. (2016) – rusty tawny to brick versus rusty, sienna to cinnamon with somewhat salmon tint in places. The zonate appearance of the pilei, found in the mentioned French collection, is absent. However, from the original description of *P. lubrica* var. *obscura* (Bon & Chevassut 1989) it is evident that at least in some collections pilei may be not zonate and darker. Besides, the stipes in the Bulgarian collection develop brownish tints in the lower parts, which seem to be absent from the studied specimens in Holec & al. (2016). Microscopically the pileipellis is somewhat distinct, in some layers containing reddish brown rather than yellowish brown tinge and this seems to be consistent with the mentioned above darker pileal colours. As far as the remaining microscopic features are concerned, their morphology and dimensions seem to adhere well to the data in Holec & Kolařík (2014) and Holec & al. (2016). The presence of two-spored basidia is noteworthy, while in the previously known specimens only four-spored were observed (Holec & Kolařík 2014; Holec & al. 2016). Notably, the two-spored basidia seem to more often have a cylindrical outline than four-spored ones. It was noted by Holec (2001) that two-spored basidia are present in almost all species of *Pholiota*. As the variability of the *P. gallica* is apparently little-known for the moment, the documented variations are perceived here to be within the expected range.

The closest European ally of *P. gallica* was shown to be *P. chocenensis* Holec & Kolařík (Holec & Kolařík 2014; Holec & al. 2014, 2016). It is a non-pyrophilous taxon, similarly to *P. gallica* lacking caulocystidia (Holec & al. 2014). Macroscopically *P. chocenensis* seems to differ by the colour of floccose remnants on stipe, which are yellow rusty to rusty orange (Holec & al. 2014). In addition, this species was described as having two-layered pileipellis (Holec & al. 2014).

Among the known species, the pyrophyllous *Pholiota brunnescens* A.H. Sm. & Hesler seems to be similar to the species presented here. Originally described from North America, it has been shown recently to have intercontinental distribution occurring also in Central and Eastern Asia, but its presence is so far not confirmed in Europe (Smith & Hesler 1968; Matheny & al. 2018; Tian & Matheny 2021). It seems to differ by microscopic characters and the distinction of *P. gallica* should not be difficult. Smith & Hesler (1968) and Matheny & al. (2018) outlined as the most prominent character of *P. brunnescens* the large caulocystidia, while *P. gallica* does not have caulocystidia (Holec & Kolařík 2014; Holec & al. 2016). Among the distinctive features of *P. brunnescens* Smith & Hesler (1968) to be considered is the presence of numerous forked pleurocystidia, which are not mentioned in the more recent description of Matheny & al. (2018). This character thus needs more observations. The description in Smith & Hesler (1968) also hints at possible subtle differences in the spore dimensions, the width in particular – 4.5 µm. Holec & Kolařík (2014) found broader spores in the holotype of *Pholiota gallica* (up to 5.5 µm), although in the present specimens spore width does not exceed 4.8 µm. Subsequent assessments of additional specimens by Matheny & al. (2018) and Holec & al. (2016) however have shown that the spore dimensions in the two species overlap considerably and are thus unlikely to be a useful character for separating of the two entities.

Pholiota castanea A. H. Sm. & Hessler is another pyrophyllous North American species, phylogenetically related to the cluster of *P. gallica* (Matheny & al. 2018). Apart of its habitat it seems to differ from *P. gallica* by the more delicate stipe, lacking yellow flakes, according to the descriptions of Smith & Hessler (1968) and Matheny & al. (2018). The American species has predominantly cylindrical or subcylindrical caulocystidia and different architecture of the pileipellis (Matheny & al. 2018).

The distribution of *Pholiota gallica* is still fragmentary known. However, it is apparently a taxon with a southern distribution, found so far both in Mediterranean European countries and North Africa. Collections of this species have so far been reported from France, Algeria, Morocco and Cyprus (Holec & Kolařík 2014; Holec & al. 2016, Loizides 2016, 2021). This paper expands its distribution to the Balkan Peninsula and reports the first finding of the species in Bulgaria. The Bulgarian collection confirms the occurrence of *P. gallica* on non-burnt ground in association with Mediterranean vegetation (Holec & Kolařík 2014; Holec & al. 2016; Loizides 2016, 2021), in the present case dry grasslands in close proximity to evergreen oaks.

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