

FUNGI — ГРИБЫ

First record of rare boreal-alpine waxcap *Hygrocybe calciphila* (Hygrophoraceae, Agaricales) in Southeastern Europe

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Abstract. *Hygrocybe calciphila*, an uncommon species of the genus was found for the first time in Southeastern Europe in forests of *Pinus heldreichii* in Bulgaria. Description and illustrations of the finding are presented. The European distribution of the species is summarized and briefly discussed, with emphasis on the scarce previous findings in Southern Europe.

Keywords: *Hygrocybe calciphila*, Hygrophoraceae, relict habitats, southern refugia, Balkan Peninsula, Bulgaria.

Первая находка редкого бореально-альпийского вида *Hygrocybe calciphila* (Hygrophoraceae, Agaricales) в Юго-Восточной Европе

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Резюме. *Hygrocybe calciphila* — редкий вид этого рода был впервые обнаружен в Юго-Восточной Европе в лесах *Pinus heldreichii* в Болгарии. Приведены описание и иллюстрации находки, а также обобщение и краткое обсуждение европейского распространения этого вида с акцентом на немногочисленные предыдущие находки в Южной Европе.

Ключевые слова: *Hygrocybe calciphila*, Hygrophoraceae, реликтовые местообитания, южные рефугиумы, Балканский полуостров, Болгария.

The genus *Hygrocybe* (Fr.) P. Kumm. is a fungus genus attracting the attention of mycologists by the lavish colors, and also due to their indicator value for habitat quality and the rarity of some of its members (Griffith *et al.*, 2002; Ruthsatz, Boertmann, 2011). Colloquially known as “waxcaps”, a number of their representatives have been recorded from the Balkan Peninsula so far (cf. e. g. Zervakis *et al.*, 1998; Mešić,

Tkalčec, 2002; Denchev, Assyov, 2010; Karadelev *et al.*, 2018). Nevertheless, the genus is worthy of further attention as its inventory in the area seems far from comprehensive. Due course of work on the fungal diversity of endemic pine forests in Bulgaria, the authors recovered an older collection made by one of them in such habitats, which turned out to be *Hygrocybe calciphila* Arnolds, an interesting and rare species, not yet documented from Southeastern Europe. This finding is described herein.

Material and Methods

The specimen was documented in the fresh state and desiccated for permanent preservation in the Mycological Collection of the Institute of Biodiversity and Ecosystem Research of the Bulgarian Academy of Sciences (SOMF). The colors in the macroscopic description match as closely as possible the color standards in the “Flora of British Fungi Colour Identification Chart” (Colour..., 1969). Microscopic study was held on dried samples, using 5% KOH with the addition of Congo Red in ammonia to highlight the studied micromorphological features. Microscopic measurements were read by Piximètre v. 5.10 (Piximètre..., 2021) from calibrated digital images. In the description below, the dimensions of basidiospores are presented in the form ‘minimum–(average±standard deviation)–maximum’; “n =” denotes the number of spores measured; “Q” refers to the spore quotient (length/width ratio). The remaining microscopic structures are presented with their minimum and maximum values. Drawings of microscopic features were prepared by tracing objects from microphotographs onto semi-transparent paper.

For the preparation of the distribution map in Fig. 3, the background relief map was generated by a free online utility (Maps..., 2021). For the purposes of the map the term “perimediterranean” is understood as including the Mediterranean biome as outlined in Olson *et al.* (2001) and closely situated territories north of its borders in Europe, but excluding the Alpine province as defined in Rivas-Martínez *et al.* (2004).

Results

Hygrocybe calciphila Arnolds, 1985, *Persoonia* 12(4): 475. (Figs. 1, 2)

Pileus up to 15 mm across, hemispherical, with slightly depressed center, dry, almost smooth, except for the center where finely pruinose or slightly scaly (lens), in young basidiomata scarlet to red and with pale, yellow colored margin, in mature fruitbodies gradually and unevenly discoloring to orange, saffron, yellow, luteous or straw, with somewhat darker, radial, fibrillose-like streaks; margin slightly translucently striate, more or less uneven, straight, at maturity usually wavy, often distinctly colored (bright yellow) than the rest of pileus. Lamellae broadly adnate or notched, thick, distant, broad, occasionally bifurcate, cream when young, then yellowish and gradually becoming somewhat salmon colored from the base towards the edge. Stipe up to 25 × 3 mm, cylindrical, dry, glabrous, smooth, somewhat rubbery on lip test, in young basidiomata concolorous with the pileus, sometimes with a yellowish uppermost portion, then discoloring towards peach color or salmon, sometimes in places straw. Context thin, yellow in pileus, darker

yellow to saffron in the stipe. Odor inconspicuous, taste not noted. Basidiospores in lateral view $6.5-(7.9\pm 0.6)-9.2 \times 4.8-(5.8\pm 0.4)-6.5 \mu\text{m}$, $Q = 1.2-(1.4\pm 0.1)-1.6$ ($n = 30$), broadly ellipsoid, ellipsoid or ovoid, similar in frontal view, not wider towards the base and not constricted, thin-walled. Basidia $42-67 \times 7.5-12 \mu\text{m}$, 4–9 times longer than wide, elongate-clavate, thin-walled, predominantly 4-spored, rarely 2-spored; sterigmata $5-7 \mu\text{m}$; clamps seen. Cystidia absent. Lamellar trama subregular, elements cylindrical or somewhat inflated, $29-125 \times 5-13 \mu\text{m}$, on average $83.4 \times 8 \mu\text{m}$, clamps not seen. Pileipellis a trichodermium, not differentiated into layers, of septate, up to $12 \mu\text{m}$ broad, clamped, repent hyphae, bundles of hyphal ends protruding in places from the main surface.



Fig. 1. *Hygrocybe calciphila* – basidiomata *in situ* (SOMF 30344).
Scale bar: 1 cm.

Specimen examined: Bulgaria, Pirin Mts, in the vicinity of Banderitsa chalet, $41^{\circ}46'05.2''\text{N}$, $23^{\circ}25'33.9''\text{E}$, 1820 m a. s. l., marble outcrops in an opening in a forest of *Pinus heldreichii* H. Christ, 22 VIII 2002, B. Assyov, SOMF 30344.

Discussion

Hygrocybe calciphila is morphologically most similar to *H. miniata* (Fr. : Fr.) P. Kumm., a species sparingly found in the Bulgarian high mountains (Denchev,

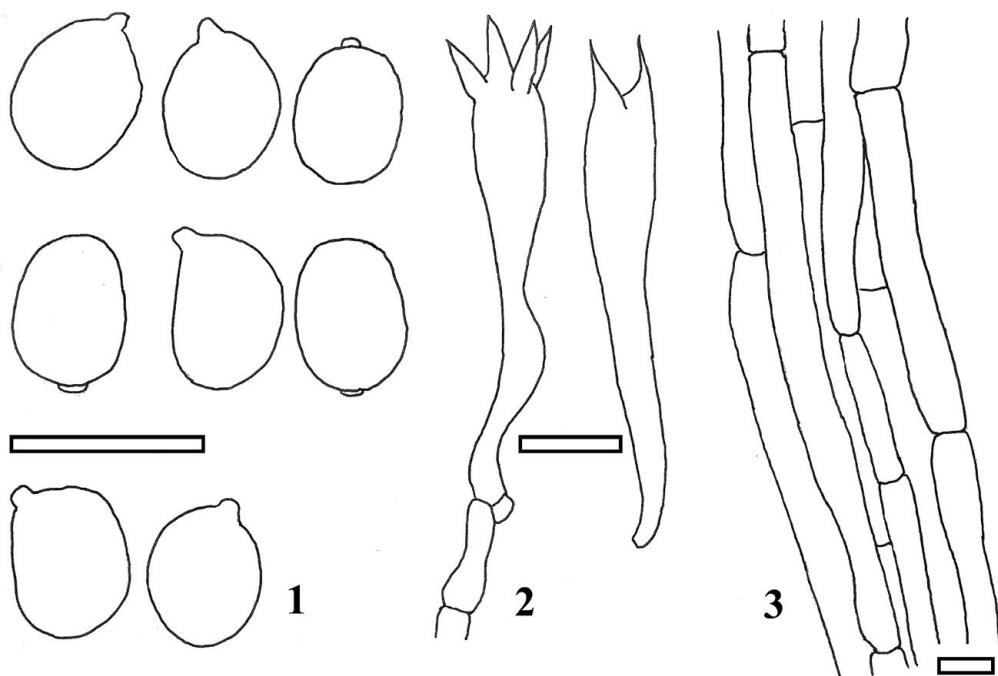


Fig. 2. Microscopic features of *Hygrocybe calciphila* (SOMF 30344).

1 – basidiospores; 2 – basidia; 3 – hymenial trama.

Scale bars: 10 μ m.

Assyov, 2010) and probably under-recorded. Arnolds (1986) pointed out as the most prominent distinguishing feature the spores, which in *H. miniata* have conceivably higher quotient ratio and are of different spore shape, mostly broadened towards the base in face view and often constricted. In addition, *H. calciphila* is known from the descriptions cited here to be almost exclusively bound to calcareous soils, with merely two findings reported from siliceous ones (Ronikier, Borgen, 2010; Mir, 2020). *Hygrocybe miniata* on the other hand is said to favor acidic soils (see e. g. Arnolds, 1986; Kovalenko, 1989; Bon, 1990; Boertmann, 1995, 2008, 2010). Further on, the similarity between *H. calciphila* and *H. biminiata* Kühner was noted (Borgen, Arnolds, 2004; Borgen, Ohenoja, 2013). This latter species however has been documented as having considerably longer, on average over 8.5 μ m and reaching 13.5 μ m in length, and at least partly constricted spores (Bon, 1990; Borgen, Arnolds, 2004; Borgen, Ohenoja, 2013). By their spore dimensions, those two entities should be thus easily told apart. According to the same authors, *H. biminiata* seems to be a species with arctic-alpine distribution and is said to occur on acidic soils in grasslands, heaths and fens. The recently described *H. fulgens* Fuljer, Kautmanová et Boertm. also appears to share similarity with *H. calciphila* and differs primarily by its considerably larger basidiomata, reaching 65 mm across, as well as by its ellipsoid

to oblong spores (Crous *et al.*, 2021). Further on, from the original description and illustrations it also seems that *H. fulgens* has more numerous lamellae, which lack salmon tint at maturity. As a recently described species, which variability is still less-known, those characters may be worthy of further observations but ought to be taken with due caution for the time being.

The morphological features of the Bulgarian collection seem consistent with the ones in the original diagnosis of *H. calciphila* (Arnolds, 1985), as well as in later authoritative descriptions (Arnolds, 1986, 1990; Bon, 1990; Breitenbach, Kränzlin, 1991; Boertmann, 1995, 2008, 2010; Borgen, Senn-Irlet, 1995; Candusso, 1997). Not all basidiomata in our specimen feature prominent scales on pileus as mentioned in other descriptions and the pileal surface appeared macroscopically almost smooth in some fruitbodies. However, scales are invariably detected (at least sparsely) upon observation under a lens and microscopic examination of pileipellis and in the latter case appear in the form of short bundles of hyphae, protruding from the outer pileal layer. The collection studied by us has predominantly four-spored basidia, but solitary two-spored were also seen. Ronikier, Borgen (2010) described an unusual collection from acidic soils, with somewhat larger spores and two-spored basidia. Similarly to our specimen, occasional two-spored basidia were previously mentioned in the description of Borgen, Senn-Irlet (1995). The basidiospores in the specimen presented here are slightly smaller than in the descriptions in Arnolds (1985, 1986), but nevertheless entirely within the range of variability.

The distribution of *H. calciphila* in Europe appears to be mostly centered on the northern and western parts of the continent, with records in Denmark, France, Ireland, Norway, Russia, Sweden, and the United Kingdom (Arnolds, 1985, 1986, 1990; Boertmann, 1995, 2008, 2010; Candusso, 1997; Rotheroe, 1997; Kovalenko, 1999; Borgen, Ohenoja, 2013; Morozova *et al.*, 2015). While in some of those countries the species is known from more abundant records, most authors nevertheless mention it to be rare. In the rest of Europe, the published findings are scattered and mostly solitary, and appear to be almost exclusively related to the large continental mountain ranges, the Alps, Pyrenees, Apennines and Carpathians in particular. This includes encounters in the countries in Central Europe, namely Austria, Germany, Hungary, Poland, Slovakia, and Switzerland (Arnolds, 1986, 1990; Breitenbach, Kränzlin, 1991; Borgen, Senn-Irlet, 1995; Candusso, 1997; Peintner *et al.*, 1999; Krieglsteiner, 2001; Hausknecht, Klofac, 2002; Kautmanová, 2003; Ronikier, Borgen, 2010; Koszka, 2011). In southern Europe and the Mediterranean area, the records of *H. calciphila* are utmost rare and rather scattered (Fig. 3). In Spain, a few published records exist, mostly confined along the range of the Pyrenees (Candusso, 1997; Vila *et al.*, 1998; Alonso *et al.*, 2001; Moreno, Remondo, 2001; Corriol, 2008; Ribes *et al.*, 2016). An exception is the record from Menorca (Mir, 2020), one of the two so far known from a Mediterranean island, the other one reported recently from Cyprus (Loizides, 2021). In Italy, the species seems to have been found in a handful of localities in the Apennines, as well as in the Alps (Candusso, 1997; Granito, Lunghini, 2011). Closest

to the borders of the Balkan Peninsula come the two records in Croatia (Tkáčec *et al.*, 2008). The species has been recently reported also from Turkey, where a single finding is known from Kop Mountain in the eastern part of the country (Keleş *et al.*, 2017). The macromorphological features of the Turkish collection seem somewhat distinct and it may be interesting to examine in more detail in the future. The same stands for further island collections as exemplified by the already mentioned records from the Balearic Islands and Cyprus, which are interesting due to the presumably long isolation of their populations.



Fig. 3. Presently known published records of *Hygrocybe calciphila* in the perimediterranean parts of Europe and in the Near East. Black dots mark literature records, white dot indicates the new finding, reported here.

The habitats of *H. calciphila* insofar they could be inferred from the literature, appear to be surprisingly diverse. Boertmann (2010) summarized them as “unimproved grasslands, road verges in open woodland and in deciduous scrubland”. The habitat preferences of the species have been analyzed in detail in Norway, where it occurs in seminatural grasslands (the majority of records), dunes, calcareous rocks with shallow soils near the sea, as well as in alpine habitats (Jordal *et al.*, 2016). The species occurs at about the sea level in sand dunes in Britain (Robinson, 2003) and in seaside plantations of *Eucalyptus globulus* L. in Northern Spain (Alonso *et al.*, 2001). The findings in Germany reported by Winterhoff (1995) are from lowlands, in dry calcareous grasslands of *Xerobrometum*, *Trinio-Caricetum humilis* and communities of *Linum tenuifolium* L. and *Carex humilis* Leyss. In the Polish Carpathians the species was found at altitudes above 1350 m and up to 1950 m a. s. l., in alpine meadows and among *Dryas octopetala* L., both on calcareous and siliceous soils (Ronikier, Borgen, 2010). In this country, however, similarly to the states in Northern and Western Europe, lowland collections in dry calcareous grasslands are also known, in particular from communities of the alliance *Festuco-Stipion* (Ślusarczyk, 2009). The records in Slovakia are from grazed calcareous grasslands at moderate elevations, between 730 m and 1100 m a. s. l. (Kautmanová, 2003). In the more southern parts of Europe the species seems to prefer often

somewhat higher elevations, as for example in the Pyrenees, where it occurs up to 2300 m a. s. l., in alpine habitats with *Salix retusa* L. or *D. octopetala* (Vila *et al.*, 1998; Corriol, 2008), similarly to the finds in the Polish Carpathians. Some other encounters in the Spanish mountains occurred between approximately 1200–1400 m a. s. l. (Moreno, Remondo, 2001; Ribes *et al.*, 2016). One of the Italian records is referred to a site with vegetation of *Koelerio splendidis*-*Brometum erectii* at 1400 m a. s. l. (Granito, Lunghini, 2011) and such from the Italian Alps are said to occur in unspecified types of grasslands at 1400–1500 m. Nevertheless, collections from lower-situated sites are known also in Southern Europe, e. g. in Spain at 3 m (Alonso *et al.*, 2001) and in Italy down to 150 m a. s. l. (Candusso, 1997). Notably the two findings from Mediterranean islands, which are the southernmost ones in the Mediterranean Basin, also come from elevations far below 1000 m a. s. l. (Mir, 2020; Loizides, 2021). *Hygrocybe calciphila* seems to mostly adhere to open habitats and reports for occurrence in forests are relatively scarce. Such are known from Spain in mixed forests of *Fagus sylvatica* L. and *Abies alba* Mill. (Ribes *et al.*, 2016), as well as the above mentioned record from *Eucalyptus* plantations (Alonso *et al.*, 2001). The record from the Balearic Islands is from woodlands of *Quercus ilex* L., *Arbutus unedo* L. and *Erica arborea* L. (Mir, 2020) and collections from xeric grasslands in woods of *Cupressus sempervirens* L., *Q. ilex*, *Arbutus unedo*, *Smilax aspera* L. and *Erica arborea* have been mentioned from Italy (Candusso, 1997). The recent specimen from Cyprus (Loizides, 2021) is also from a forested area, with *Pinus brutia* Ten. and plants of the genus *Cistus* L. (Loizides, pers. comm.). The reported here Bulgarian finding came from marble outcrops in an opening in an old forest of the relict Bosnian pine (*Pinus heldreichii*) at 1820 m, which tree habitat generally forms the upper forest limit on marbles in this locality. Some sterile rosettes of plants of the genus *Pilosella* L. were present in the vicinity of the basidiomata of *H. calciphila*. Whether there may be a relation to those plants is not known, but at least in Bulgaria a number of *Hygrocybe* s. l. may occur in habitats where plants of this group are present and it was shown previously that some waxcaps are systemic endophytes in herbaceous plants (Tello *et al.*, 2014).

Our record of *H. calciphila* in Southeastern Europe fills a significant gap in its distribution. The Bulgarian finding, coupled with the extralimital Croatian encounters, strongly suggests that populations of the species may be also present in the high mountains in other countries of the Balkans. The authors believe that considered the general distribution pattern of the species in Europe, its presence in the Balkan Peninsula is putatively relictual, moreover there is evidence that even arctic-alpine members of the genus reach at least as far as the Southern Carpathians (Ronikier, 2010), themselves having links with the high mountain massifs of the Balkans.

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