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First record of *Neolecta vitellina* (Bres.) Korf & J. K. Rogers and *Sowerbyella fagicola* J. Moravec in Serbia

Original Article

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Abstract:

The rare ascomycetes *Neolecta vitellina* and *Sowerbyella fagicola* have been recently registered in Serbia as new for the country. Morphological description based on found specimens, along with macro- and microscopic characteristics of fruiting bodies and attributes on their habitat, ecology and distribution in Serbia are given.

Key words:

Neolecta vitellina, Sowerbyella fagicola, first record, Serbia

Apstract:

Prvi nalaz vrsta *Neolecta vitelina* (Bres.) Korf. & J. K. Rogers and *Sowerbyella fagicola* J. Moravec in Serbia

Retke askomicete *Neolecta vitellina* i *Sowerbyella fagicola* registrovane su po prvi put u Srbiji. U radu je predstavljen morfološki opis zasnovan na makro- i mirokarakteristikama plodonosnih tela zabeleženih primeraka uz opis staništa i podatke o ekologiji i distribuciji navedenih askomiceta u Srbiji.

Ključne reči:

Neolecta vitellina, Sowerbyella fagicola, prvi nalaz, Srbija

Introduction

During fungal diversity field studies conducted from 2016-2018, in selected regions in southern and central Serbia, the rare ascomycetes *Neolecta vitellina* and *Sowerbyella fagicola* have been registered. These specimens represent the first documented records of the species in Serbia. Both taxa are already taken into consideration as "relevant for assessment" in the European Red List candidates of endangered fungi which is still under preparation, and only few countries (Austria, Germany, Norway and Sweden) have included these species in their National Red List of fungi (ECCF, c1998-2019). Fungal conservation is a complex and challenging issue due to high species diversity, incomplete knowledge of degree of rarity or risk status, large number of little known species, poor understanding of their trophic or habitat requirements (Molina et al., 2011). Reports of *Neolecta vitellina* and *Sowerbyella fagicola* from Europe are scarce, therefore biogeographical studies with data on local populations and habitat are necessary to better understand the nature of its distribution and further conservation approaches. This study aimed to present identification of these two rare species based on morphological description and examination of the present collections, as well as data on their habitats, ecological notes and distribution in Serbia.

Materials and methods

Specimens have been collected during the period 2016-2018 in two selected regions in southern (Suva Planina Mountain - Suva Planina Mt.) and central (Kopaonik National Park - Kopaonik NP) Serbia (**Fig. 1**). A fresh material with substratum was observed for macro- and micromorphological characterisation and exsiccates were deposited in the private fungal collection of the Mycological Society of Niš (M.S.N.). Part specimens were also stored in the officially unregistered fungarium of Fruška Gora National Park. Microscopic characteristics and measurements were made on multiple preparations primarily of fresh material, to obtain vital taxonomic characterisation (Baral, 1992), and for comparison with dry material, using Optika N-400M and Leica DM 1000 light microscopes. Examination and meas-

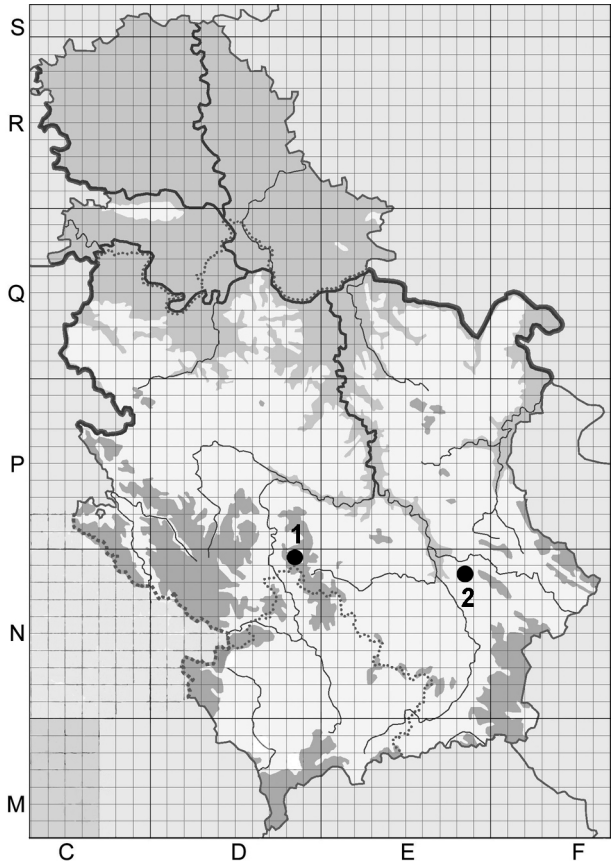

Fig 1. Map of investigated areas: 1) Kopaonik NP, Crvene Bare nature reserve - *Neolecta vitellina* site; 2) Suva Planina Mt., above Gadžin Han town - *Sowerbyella fagicola* site.

uring of approximately 30 spores released from the ascus and other elements of fresh and dry material were made first in water, later in Lugol's solution (IKI) and the ornamentation of the cells was observed in Congo red and Cotton blue. Macro- and microscopic descriptions are based only on the collected specimens. The species names follow Index Fungorum (Kirk, 2018) and MycoBank (Stalpers & Cock, 2018) and the following works were used as resources for identification of the species: Moravec (1973, 1985, 1988), Redhead (1977, 1979), Roffler (1999), Hansen & Knudsen (2000), Klofac & Herrman (2003), Landvik et al. (2003), Benkert (2005), Van Vooren (2007), Perić (2008), Galán et al. (2010), Chinan & Hewitt (2012), Friebes (2015), Wieschollek (2015), Ribes et al. (2016).

Results

***Neolecta vitellina* (Bres.) Korf & J.K. Rogers**, Phytologia 21(4): 204 (1971). **Figs. 2 – 4**.

Basionym: *Geoglossum vitellinum* Bres., Revue Mycologique 4: 212 (1882).

Description of studied specimen:

Ascomata 12-38 x 3-7 mm in diameter, slender, cylindrical, club-shaped or irregularly clavate, spathuliform or somewhat truncate, elongate or lanceolate, some flattened, smooth or wrinkled, often with longitudinal furrows, bright yellow; flesh elastic. Stipe 4-25 x 2-4 mm, central, cylindric, gradually merges with the fertile part, tomentose, white-coloured. Asci 50-75(120) x 4-7 µm, cylindrical or narrowly clavate, 8-spored, mostly uniseriate, apex obtuse and not staining blue in iodine, aporynchous ascus base. Paraphyses absent. Ascospores 4-6(-9) x 2.5-4 µm, smooth, hyaline, thick-walled, elliptical, subglobose, amygdaliform or reniform, usually contains small globose oil drops and a nucleus most visible in Lugol's solution and Congo red. Excipulum of *textura intricata*, with septate hyaline hyphae (5)10-20(30) µm.

Habitat: Fruiting bodies were found in a slope, scattered among the humid fallen needles of *Picea abies* (**Fig. 2a, b**).

Fig 2. *In situ* photographs of *Neolecta vitellina*; a) ascomata growing from spruce rootlets, photo I. Kajevska, b) fruiting bodies at different stages of maturity. Photo: M. Blagojević.

Specimen examined:

Kopaonik NP (**Fig. 1**), Crvene Bare nature reserve, 1600 m, *P. abies* forest, on litter, among *P. abies* fallen needles, attached on roots, very wet place,

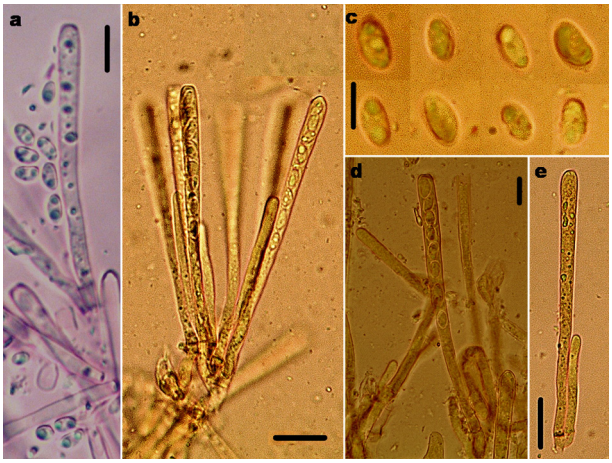

Fig. 3. Micrographs of *Neolecta vitellina*, a) ascus with spores in Congo red; b) asci with spores in IKI; c) ascospores in IKI; d) ascus with spores in IKI; e) young ascus. Bars: a, b, e = 10 µm; c,d = 5 µm.

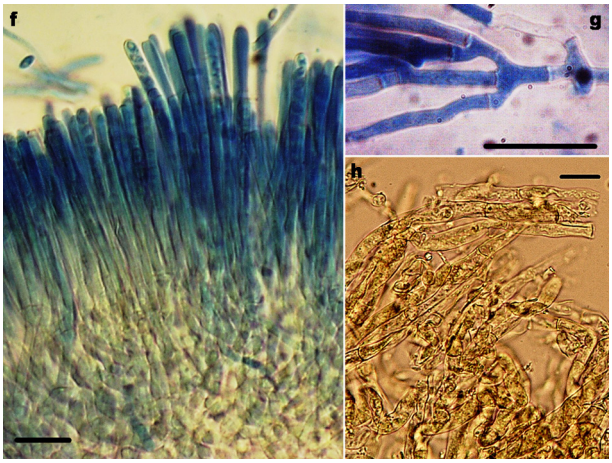

Fig. 4. Micrographs of *Neolecta vitellina*, f) hymenium; g) asci bases; h) excipulum cells. Bars: f =20 µm; g, h = 10 µm.

20/07/2016, I. Kajeveska (legator-leg. A. Trailović), M.S.N. 21/07/16-168; Kopaonik NP, Crvene Bare, cc. 1600 m, N 43°17'35.6", E 20°48'21.81", *P. abies* forest, on litter, among *P. abies* fallen needles, attached on roots, on a slope, 29/07/2017, I. Kajevska, M.S.N. 29/07/17-240.

***Sowerbyella fagicola* J. Moravec**, Česká Mykol., 27 (2), p. 66 (1973). **Figs. 5 – 7**.

Description of studied specimen:

Apothecia 5-23 x 3-9 mm in diameter, shallow cup-shaped, concave to fully extended in maturity; hymenium smooth, bright yellow or orange, young specimens somewhat translucent becoming dark orange in age and orange-brown when dry; excipulum concolorous with the hymenium, finely pubescent with indistinguishable white hairs; margin entire or slightly undulate, somewhat with darker color; flesh thick, very soft, light and fragile, lubricous. Stipe 10-27 x 2-4 mm, central, smooth, delicate, concolourous with the cap, often wrinkled-furrowed or somewhat moniliform, cylindrical but usually with attenuated base. Asci 200-220(270) x 9-13 µm, cylindrical, 8- spored, uniseriate, apex obtuse or truncate and inamyloid but asci walls somewhat blue in Melzer's reagent, ascus base pleurorynchous. Paraphyses 200-240 x 2-3 µm, narrowly cylindrical, slender, septate, with yellow to orange pigments bluish in Melzer's reagent. Ascospores 16-19.5(-22) x (6.5-)7.5-8.5(-9) µm, hyaline, thick-walled, fusiform, usually contains two globose oil drops (2.5-4.5 µm) accompanied with 1-2 smaller drops (< 2 µm) on the ends; ornamentation seemingly smooth but finely verrucose on oil immersion. Medullary excipulum of *textura intricata*, with hyphae 20-45(65) x 5-7(9) µm, hyaline, with thin walls. Ectal excipulum of *textura subglobulosa* to *angularis*, with hyphae of 20-40(50) x (8)15-30(45) µm. Marginal hairs cylindrical, narrow, septate and hyaline to yellowish hairs of 30-150 x 4-7 µm.

Habitat: Investigated specimens were found in the base of a gully with a lot of organic matter accumulated in a mixed deciduous forest (*Carpinus orientalis, Quercus cerris, Cornus mas, Acer campes-*

Fig. 5. In situ photographs of *Sowerbyella fagicola*; a) ascomata arising from litter composed of mixed deciduous forest, b) fruiting bodies at different stages of maturity. Photos: I. Kajevska.

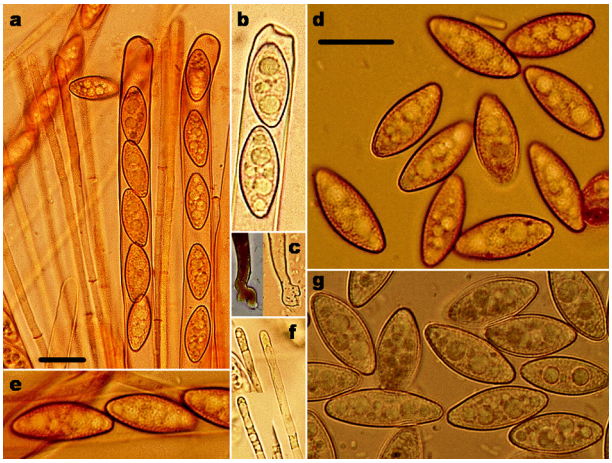

Fig. 6. Micrographs of *Sowerbyella fagicola*, a) asci with spores, paraphyses; b) ascus tip; c) ascus base; d) spores with ornamentation in Congo red; e) spores in ascus, Congo red; f) paraphyses; g) spores in IKI. Bars: a, d, g = 10 µm.

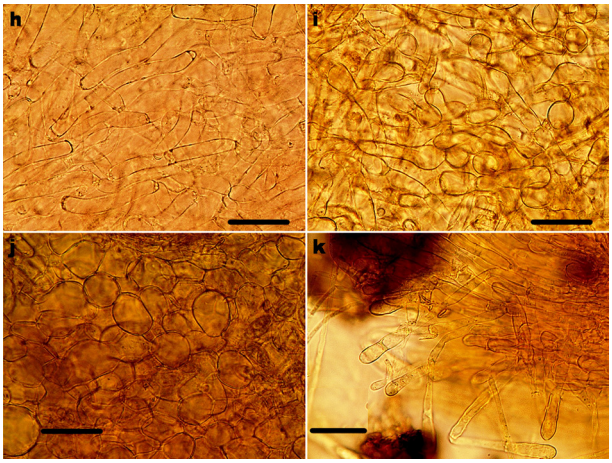

Fig. 7. Micrographs of *Sowerbyella fagicola*, h) cells of medullary excipulum; i) cells of ectal excipulum; j) cells of ectal excipulum; k) hairs. Bars: h, i, j, k = 20 µm.

tre, Robinia pseudoacacia, with one *Fagus sylvatica* tree for about 20 m distance from the collection site), on a shaded place and humid for a long time. Fruiting bodies scattered or gregarious, emerging from the moist forest litter (**Fig. 5a, b**).

Specimen examined:

Suva planina Mt. (**Fig. 1**), above Gadžin Han town (Niš), 347 m, N 43°12'25", E 22°03'06", mixed deciduous forest (*C. orientalis, Q. cerris, C. mas, R. pseudoacacia, A. campestre, F. sylvatica*), on litter, attached on small fallen branches, leafs, tiny pieces of wood, etc., very wet place, 29/05/2016 and 26/05/2018, D. Wieschollek (leg. I. Kajevska), M.S.N. 29/05/16-162; M.S.N. 26/05/18-239.

Discussion

The species *Neolecta vitelina* was first described in Italy as *Geoglossum* (*Microglossum*) *vitellinum* Bres. (Bresadola, 1882). In North America, it was reported as *Mitrula vitellina* (Bres.) Sacc. (Durand, 1908), and lately due to the lack of paraphyses as an important distinguishing feature Mains (1955) reports it under the name *Sprague ola vitellina* (Bres.) Nannf. and Imai (1934, 1941) from Japan as *Ascocorynium vitellinum* (Bres.) S. Ito & Imai. Korf (1971) transfers *M. vitellina* into the genus *Neolecta* Speg. Redhead (1977) reports *N. vitellina* in Canada and finely places the genus *Neolecta* within Neolectaceae Redhead due to its unique morphology. Based on morphological and molecular analysis, *Neolecta* has been placed in a separate order, Neolectales Landvik, O. E. Erikss., Gargas & Gustafsson (Landvik et al., 1993). Subsequently, giving full morphological and ultrastructural description as well as molecular data of the genus species, Landvik et al. (1996, 2001, 2003), afterwards Sugiyama et al. (2006) and Healy et al. (2013) confirms the unique position of *Neolecta* (class Neolectomycetes O.E. Erikss. & Winka, subphylum Taphrinomycotina O. E. Erikss. & Winka) as the earliest lineage within Ascomycota. *Neolecta vitelina* so far is the only species of the genus that has been reported from Europe. Available published data on this species from Europe come from the following countries: Italy (type specimen-Bresadola, 1882; Carbone et al., 2015), Poland (Bujakiewicz, 1979), Switzerland (Ryman & Holmåsen, 1992), Germany (Krieglsteiner, 1993), Czech Republic (Svrček, 1978), Fennoscandia-Norway, Sweden and Finland (Eckblad, 1963; Ohenoja, 1975; Hansen & Knudsen 2000), Romania (Chinan & Hewitt, 2012) and recently has been described and illustrated from Austria (Friebes, 2015). Mycological field research held in summer 2016, at Kopaonik NP, revealed the first finding of *N. vitellina* in Serbia. Specimens have been preserved. Another location of this species with numerous fruiting bodies was located the next year in the same area. Although most reports of *N. vitellina* are known from northern Europe, presumably, the precise geographical distribution across Europe cannot be definite yet. Most recent reports of this rare ascomycete like those from Romania, Austria and now from Serbia shift its distribution range towards central and southern Europe. Reports of *N. vitellina* are scarce, therefore more focused research, monitoring and data with insights on local populations of this species are needed to better understand the nature of its distribution. Our collection of *N. vitellina* originates from a forest slope with fruiting bodies emerging from the fallen needles in *P. abies* (L.) H. Karst. forest at an altitude of cc.1600 m. Nu-

merous fruiting bodies have been found at different stages of maturity. Although this rare species has been found exclusively in spruce needle bogs, many questions regarding its morphology, phenology and its trophic affinity remain unclear. Readhead (1979) indicates *N. vitellina* as parasite on spruce rootlets. Fruiting bodies of our collections were also attached to spruce rootlets and while collecting was difficult to separate them from the substrate. Macro- and microscopically, the specimens collected from Serbia correspond well with the descriptions presented in Redhead (1977, 1979), Hansen & Knudsen (2000), Landvik et al. (2003), Chinan & Hewitt (2012) and Friebes (2015). Until now we have only one known collection site, but it may likely occur on other sites especially within the Kopaonik NP where spruce forests prevail. The site where the species is registered is already within a protected area, as it is situated in a nature reserve in the National Park, but it is an area of high recreational use. Accordingly, we anticipate that all recreational activities on this site could be an important threat to the survival of this fungal species.

Sowerbyella fagicola so far is reported only from Europe in the following countries: Czech Republic (type specimen-Moravec, 1973; 1988), Germany (Krieglsteiner, 1979, 1993; Häffner, 1989, 1993; Hohmeyer et al., 1989, Benkert, 2005; Wieschollek, 2015), Austria (Rücker & Wittmann, 1995; Klofac & Voglmayr, 2003), Switzerland (Rofler, 1999), Poland (Chmiel & Ronikier, 2007), France (Van Vooren, 2007; Moyne & Petit, 2010); Montenegro (Perić, 2008); Spain (Galán et al., 2010) and recently from Spain (Ribes et al., 2016), Bosnia and Herzegovina (Jukić & Omerović, 2017) and Bulgaria (Assyov & Slavova, 2017). Recent reports of *S. fagicola* from the abovementioned three Balkan countries have predicted an expected presence of this rare species also on Serbian territory. Thus, in May 2016 and subsequently in 2018, *S. fagicola* has been registered during mycological field research held in the southern part of Serbia, precisely on Suva planina Mt., above Gadžin Han town. Therefore, data on *S. fagicola* published in this paper is new for the country. *Sowerbyella fagicola* was first described by Moravec (1973). The spore ornamentation and macroscopically it reminds *S. imperialis* (Peck) Korf, but this species has smaller spores, and it is found in coniferous habitats (Moravec, 1973, 1985, 1988). Our collection contains fruiting bodies at different stages of maturity and macro- and microscopically, the specimens collected from Serbia correspond well with the descriptions presented in Moravec (1973, 1985, 1988), Roffler (1999), Klofac & Herrman (2003), Benkert (2005), Van Vooren (2007), Perić (2008), Galán et al. (2010), Wieschollek (2015), Ribes et al. (2016) except for slightly differences in dimensions of the excipulum cells. The dimensions of the *textura intricata* cells in Perić (2008) are 23.6-51.7 x 8.6-15.7 µm and are slightly wider than those from our collection (5-7(9)). Also cells of the ectal excipulum from our collection (20-40(50) x (8)15-30(45)) µm) are longer than those (11-23 x 10-22) noted by Van Vooren (2007). Investigated specimens from our collection comprised of small amount of fruiting bodies (cca. 15) have been found in a shaded place, humid for a long time at the base of a gully where lot of organic matter is accumulated. The collection site was situated in a mixed deciduous forest comprised of *C. orientalis* Mill., *Q. cerris* L., *C. mas* L., *R. pseudoacacia* L., *A. campestre* L. and with only one *F. sylvatica* L. tree for about 20 m distance from the collection site. Specimens were found scattered, some gregarious (with max. 3-4 fruiting bodies), barely visible among the moist layer of the forest litter. Stipe base has been attached to fallen leaves, twigs, branches and other small-sized decaying organic matter. We managed to establish only leaf litter from *C. orientalis, Q. cerris, R. pseudoacacia* and *C. mas* but without *Fagus* leaf litter. It might also be worth to mention that the only herbaceous plant species on the collection site were *Galium trifolium* Michx. and *Hedera helix* L. but poorly developed due to little sunlight unable to reach the ground in the dense forest. Regarding the ecology of *S. fagicola*, Moravec (1988) points out the presence of ammonia in the substrate as a possible condition for developing its fruiting bodies. So far this has not been confirmed. And although it is frequently related with *Fagus* it has also been found occurring in other habitats. In our case and in reports from many countries *Fagus* has not been always the predominant tree in the habitat but the most uncommon are the cases from France found in an oak forest (Moyne & Petit, 2010) and Spain found in Mediterranean oak forest (Galán et al., 2010) and from Spain found on hazel litter (Ribes et al., 2016). Thus, the real ecology and substrate preferences of *S. fagicola*, whether it is or not bound to *Fagus*, remains unclear, but it is most likely a saprotrophic species growing on fine litter in its initial phase of decomposition. We also agree with the conviction noted by Wieschollek (2015) that *S. fagicola* grows on a fine litter accumulated predominantly by deciduous trees. Also, although Suva Planina Mt., is also a special nature reserve and a protected area, during our continuous field studies we established various negative anthropogenic activities in this region. In 2017, on this site, we couldn't found developed fruiting bodies of *S. fagicola*. Namely, for the past few years, there were many actions of illegal logging and an increase in illegal landfills. These and similar activities soon

may be possible threat for the persistence of this and other rare species encountered in this small but regarding mycodiversity rich area. Therefore, as soon as possible, this region should be an issue of concern and to be taken into consideration in all available national biodiversity monitoring and protection actions. Regarding the status of *S. fagicola*, we consider it should be included in the Preliminary national red list of fungi and for the future Red Book of fungi deserves a status of a critically endangered (CR) species B2b(iii) according to IUCN categorisation, as well as its only known habitat to be protected. Similar status for *S. fagicola* has been already foreseen in Bosnia and Herzegovina (Jukić & Omerović, 2017).

Conclusion

The rare *Neolecta vitellina* and *Sowerbyella fagicola* have been registered during the period 2016-2018 in two selected regions in southern (Suva Planina Mt.) and central (Kopaonik NP) Serbia. Although protected, both areas where these rare species persist are influenced by harmful anthropogenic activities which may be a possible threat to these ascomycetes. Data on species with such status will increase the insights into better understanding of further fungal conservation approaches. Therefore, morphological description based on found specimens, along with macro- and microscopic images and biogeographical data from Serbia have been fully presented in this paper. Since it is already within a protected area, as it is situated in a nature reserve in the National Park, it is an area of high recreational use. Accordingly, we anticipate that all recreational activities on this site could be an important threat to the survival of this fungal species.

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