Botanica SERBICA

(2021) 45 (1): 143-148

DOI: https://doi.org/10.2298/BOTSERB2101143A
journal homepage: botanicaserbica.bio.bg.ac.rs

Original Scientific Report

A contribution to *Amanita alseides,* a recently described European species in the section *Vaginatae*

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ABSTRACT:
The authors present a contribution to *Amanita alseides,* so far known from its type specimens from France and Italy. A detailed description is included, based on molecularly supported collections from Bulgaria and Greece, expanding the knowledge on the morphological variability and the distribution of the species.

Keywords:
Amanitaceae, *Amanitopsis,* Balkan mycota, biogeography, taxonomy

UDC: 582.284.51:581.41+581.95

Received: 05 September 2020
Revision accepted: 18 February 2021

The genus *Amanita* Pers. has been extensively studied in Europe by means of the morphological approach, resulting in a number of dedicated treatments (see e. g. Gilbert 1918, 1941; Seyot 1930; Bas 1962a, b, 1969; Merlo & Traverso 1983; Persson 1992; Fraiture 1993; Traverso 1998; Galli 2001; Contu 2003; Krieglsteiner 2003; Neville & Poumarat 2004, 2009; Vesterholt 2008; Kibby 2012). Molecular phylogenetic studies involving European members of the genus date back to the end of the 20th century and the genus has recently attracted considerable attention (see e. g. Weiss *et al.* 1998; Moreno *et al.* 2008; Justo *et al.* 2010; Vizzini *et al.* 2012, 2020; Loizides *et al.* 2018). Nevertheless, some groups of *Amanita* pose considerable taxonomic problems. The most prominent example of this is probably the section *Vaginatae* Quél., where to the best of our knowledge, numerous collections from Southeastern Europe could not be satisfactorily tied to a species, despite the existence of numerous recent monographs, atlases and keys tackling the section on this continent (Persson 1992; Fraiture 1993; Traverso 1998; Galli 2001; Contu 2003; Krieglsteiner 2003; Rune 2006; Vesterholt 2008; Neville & Poumarat 2009; Kibby 2012). The systematic exploration of the European *Vaginatae* by molecular tools started very recently (Vizzini *et al.* 2016; Loizides *et al.* 2018; Hanss & Moreau 2020), so far revealing astonishing morphological variability and synonymy, as well as the existence of critical groups and undescribed species.

Amanita alseides Hanss was described just recently based on materials from France and Italy (Hanss & Moreau 2020). Having embarked on a project focusing on the diversity of the genus *Amanita* in Bulgaria and Turkey, during the assessment of their samples, the authors revealed Balkan collections belonging to this species. As some of the examined specimens show notable colour variation, they took advantage of the opportunity to add further data on the morphological variability and the extent of occurrence of this still lesser-known species.

The specimens were photographed at the time of collection and the relevant salient features were dutifully noted. They were deposited in an air-dried state in the Mycological Collection of the Institute of Biodiversity and Ecosystem Research (SOMF). The microscopic study was carried out with an AmScope T360B light microscope,

equipped with an AmScope MU900 digital camera. The microscopic observations were conducted on preparations from dried specimens after rehydration with 5% KOH. Congo red in ammonia was added to all of the preparations with the exception of the spores, which were measured solely in the above solution. A total of 30 random, normally developed, mature basidiospores were assessed from each basidioma, also following the recommendations for spore preparations in Hanss & Moreau (2020). In the description, the spore measurements are presented 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}); where the abbreviations "n=", "m=" and "p=" denote the number of basidiospores, basidiomata and collections from which the spore data originate respectively. The description is based on two sequenced specimens, as well as on one which did not yield any sequence, but was collected in the immediate vicinity of another sequenced collection and safely assigned to *A. alseides* according to its morphological features, matching the original description.

The protocol used for DNA extraction, amplification and sequencing is described in detail in Bozok *et al.* (2020). The phylogenetic analysis was done with Mega7.0 and BioEdit Sequence Alignment Editor Software. All of the sequences were aligned and edited by using the BioEdit Sequence Alignment Editor and Sequencher version 5.4.5 (Gene Codes, Ann Arbor, MI, USA). The phylogenetic tree was created by using the Maximum Likelihood (ML) method based on the Tamura-Nei model in Mega7.0 software (Kumar *et al.* 2016). The support values near the branches were calculated by using bootstrap analysis (1000 replicates).

Assessment of ITS sequences. The ITS rDNA phylogenetic tree (Fig. 1) was constructed by using Maximum Likelihood (ML) analysis in Mega7.0 software. The highest log likelihood value of the phylogenetic tree was -1790.38. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then by selecting the topology with the superior log likelihood value. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 17 nucleotide sequences. The final dataset included a total of 750 positions.

The two ITS rDNA sequences of *A. alseides* obtained in the present study were compared with sequences from the GenBank database (Fig. 1).They showed similarity rates of 99.12–100% to the reference sequences. In the obtained phylogenetic tree our sequences clustered together with four sequences from the holotype and other type materials of *A. alseides* (MN490649, MN490678, MN490697, MN490662), forming a well-supported sister clade to *A. griseoumbonata* Y.Y. Cui, Q. Cai & Zhu L. Yang. The topology of the tree including our sequences corresponds to a large extent with that presented in the protologue of *A. alseides*. The exception was the clade of *A. albogrisescens* Contu, where a subclade was resolved, albeit with low support. A comparison of the sequences in the alignment revealed that *A. shenongjiana* (NR159595) differs by one substitution and three indels and has similarity rate of 98.48% with MN490665, and 99.15-99.26% with the other sequences (KX834249, KX834251, MN490648, MN490654). Given the apparent lack of consistent difference in the three sequences comprising this subclade and the remainder in the clade of *A. albogrisescens,* it may be concluded that the subclade in question may represent a byproduct of unaligned regions due to different sequence lengths. There is, however, a consistent difference in terms of two indel positions and one substitution between the three publicly available ITS sequences of *A. shennongjiana* (NR159595, MH508590, MH508591), generated by Cui *et al.* (2018), and the sequences from European materials (consult also Hanss & Moreau 2020). This may hint at the existence of a certain geographic pattern, although it is rather subtle and in favour of the conspecificity between *A. shennongjiana* and *A. albogrisescens* as already suggested by Hanss & Moreau (2020). While the genetic variability within the clade of *A. albogrisescens* is outside the scope of this paper, this difference may be further assessed, when more ITS sequences of different geographic origins become available.

Description of the Balkan collections. *Amanita alseides* Hanss, in Hanss & Moreau, Bull. Soc. Mycol. France 133: 101 (2020); Fig. 2.

Pileus up to 9 cm across, smoke grey or in different shades of milky coffee, buff, clay buff, fawn and pale date brown, striate to 1/5–1/3 of the pileal radius; striation deeper in overmature and desiccated basidiomata; pileal surface discolouring with age at the cap margin, particularly in the interlamellar spaces; velar remnants present on the pileal surface scarce to abundant, initially whitish, then brownish, greyish-brown or greyish. Lamellae white, sometimes becoming pale salmon on desiccation; lamellar edge slightly serrate or nearly smooth, concolorous with lamellar faces and remaining so with age. Stipe up to 8 × 1 cm, up to 1.5 times longer than pileal diameter, subcylindrical to narrowly clavate, finely flocculose at first, then somewhat fibrillose to nearly smooth; surface white or off-white, becoming greyish ochraceous on handling; volva vaginate, friable (type Ib or III), initially whitish, then greying with age, especially on the inner surface. Basidiospores 8.9–13.2 × 7.7–12.2 µm, Q=1.01–1.31, L_{av}=10.1–11.1 µm, W_{av}=8.7–9.9 µm, Q_{av}=1.13–1.16 (n=120, m=4, p=2; Bulgarian collections); 11.1–16.0 × 9.3–14.2 µm, Q=1.08–1.33, L_{av}=14.0 µm, W_{av}=11.9 µm,

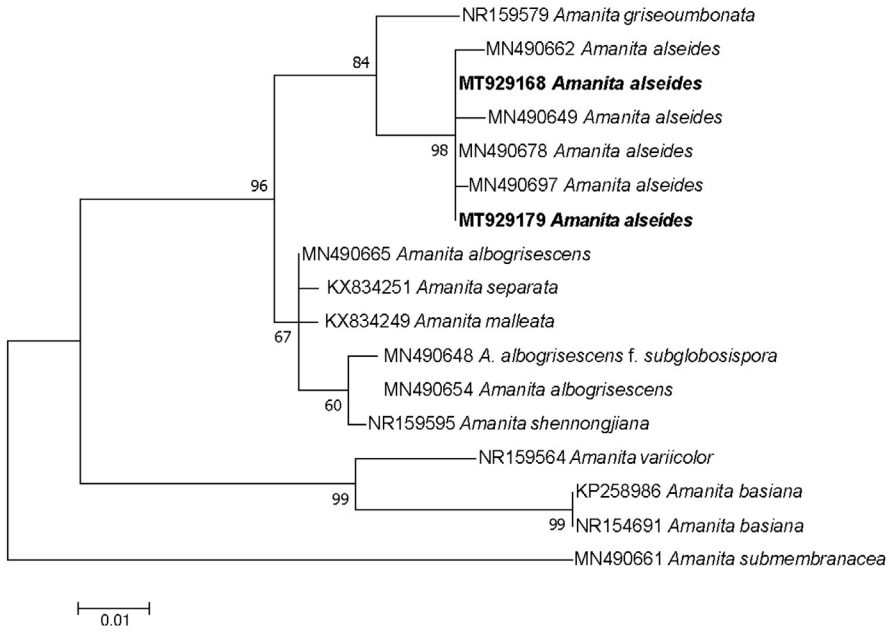

Fig. 1. The ITS phylogenetic tree of *Amanita* species belongs to the stirps *Albogrisescens* of the section *Vaginatae*, obtained with Maximum Likelihood (ML) analysis in Mega7.0 software, with *A. submembranacea* as the outgroup. Bootstrap values (%) above 50% are shown near the branches. The sequences generated in this study are presented in bold.

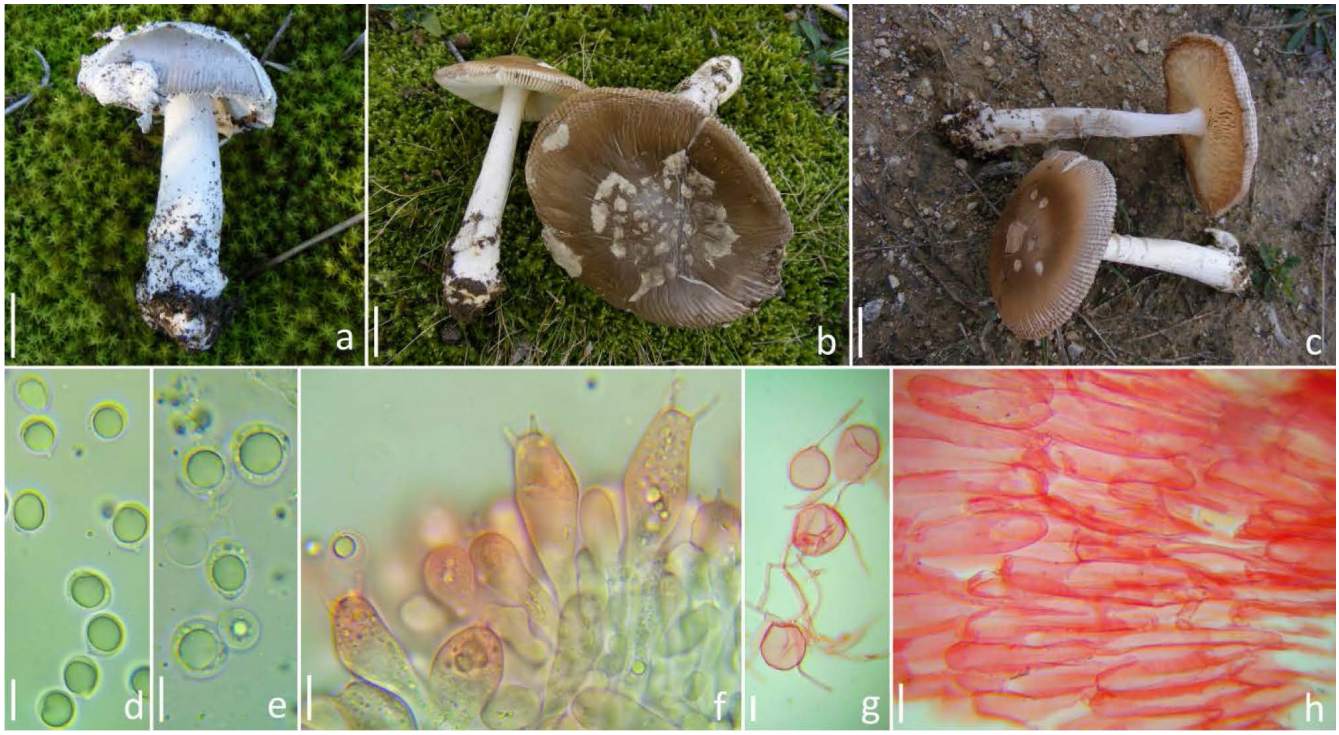

Fig. 2. The macromorphological variability and micromorphological features of the Balkan collections of *Amanita alseides*: a-c – basidiomata *in situ* (a – SOMF 29888, b – SOMF 29986, c – SOMF 29987), d-e – basidiospores (d – SOMF 29986, e – SOMF 29988), f – basidia, g – elements of volva, h – acrophysalides from stipe. Scale bars: a-c = 1 cm, d-g = 10 µm, h = 50 µm.

Q_{av}=1.17 (n=30, m=1, p=1; Greek collection), globose, subglobose or broadly ellipsoid, rarely pruniform, thin-walled and with a large central guttule in KOH. Basidia generally 4-spored, but 1-, 2- and 3-spored basidia also present and particularly abundant in the Greek collection, clavate or rarely ventricose, 39.7–69.1 × 12.4–19 µm; sterigmata 5.5–8 x 1–1.5 µm. Gills edge sterile; marginal cells abundant, ovoid or pyriform, rarely sphaeropedun-

culate, up to 42 × 24 µm, thin-walled or occasionally with wall distally thickened up to 1 µm. Trama bilateral; mediostratum of filamentose hyphae up to 4 µm broad; hymenopode well-developed, composed of mostly ovoid, inflated elements, 67.6–75.2 × 22.3–30 µm; subhymenium ramose. Pileipellis of filamentose hyphae up to 5 µm broad. Universal veil on lower stipe (volva) composed of filamentous undifferentiated hyphae and abundant spherocysts; filamentous hyphae 2.2–6.6 (mostly 2.5–4) µm wide, branching, thin-walled; sphaerocysts 23.8–51.6 × 20–47.6 µm, spherical, ovoid or pyriform, terminal, thin-walled, hyaline; pigment absent. Universal veil remnants of pileus of similar architecture as volva. Stipe context longitudinally acrophysalidic; inflated cells ovoid or clavate, 34.5–71 × 7.8–19.9 µm; trombolerous hyphae present. Clamps not seen in any tissue.

Specimens examined: – BULGARIA, north of the Veselintsi settlement, east of Padesh village, Blagoevgrad distr., N 41°57'16.3", E 23°01'08.8", 23.09.2014, under *Quercus pubescens* Willd., B. Assyov (SOMF 29986; GenBank MT929168); idem, N 41°57'16.2", E 23°01'09.2", 23.09.2014, under *Q. pubescens,* M. Slavova (SOMF 29987); GREECE, between Stavros and Olympiada, Nomos Chalkidikis, N 40°38'03.0", E 23°45'18.3", under *Quercus ilex* L., *Arbutus* sp. and *Erica arborea* L., 15.12.2013, M. Slavova (SOMF 29988; GenBank MT929179).

The colour of the pileus in *A. alseides* is apparently very variable. The Greek collection we studied has the silvery greyish colour described in the specimens used in the original description of the species. On the contrary, the two Bulgarian specimens show distinctive brownish colouration both in the younger and slightly overmature basidiomata. Those two specimens originate from exposed, sunny locations in sparse thermophilous oak woodlands. Similar colour variability is noted by Hanss & Moreau (2020), but whether it is related to environmental or intrinsic factors is yet to be seen. Hanss & Moreau (2020) found that brownish coloured specimens differed by one insertion. The ITS-sequences of our own collections, both greyish and brownish coloured, also have one insertion each, but differ from those in the authentic specimens.

Some of the specimens used in the original description of *A. alseides* were said to show greying of the lamellae, which we have not observed in our own collections. Instead, one of our samples showed a salmon-tinted lamellae on desiccation, even in the field. This is a feature we have observed in several other species of section *Vaginatae,* while not in others, and is worthy of further observations on molecularly characterized collections in order to show whether particular taxonomic value may be attached to it.

The darkening of the volva with age and on handling is a peculiar character of this species (Hanss & Moreau 2020), currently known to be present in only a few European *Vaginatae,* especially in the group of *A. submembranacea* (Bon) Gröger. The distinction of *A. alseides* from the latter is tackled in detail in Hanss & Moreau (2020) and the reader is referred to that work. We should add the prominent volva of type II in *A. submembranacea* to the distinctions between the two species, which we find rather instructive as a field character. Furthermore, the velar remnants on the pileus in our specimens of *A. alseides* become brownish or greyish-brown with age, a colouration which seems distinct from that seen in *A. submembranacea*.

The basidiospores of the Bulgarian collections of *A. alseides* mostly conform with the spore variability reported in Hanss & Moreau (2020), but the average Q-values are somewhat higher in our specimens, expanding the variability of this character in the species tackled here. Surprisingly, the molecularly characterized Greek collection produced considerably higher basidiospore measurements from both those in the original description and the Bulgarian specimens studied here (see the description above). Pruniform basidiospores are rare in the examined Balkan collections. The reason for those findings cannot be explained with any degree of certainty at the moment, but it should be noted here that the Greek specimen consists of a developing basidioma and a small number of mature spores were found in the preparations. Besides, it also has abundant basidia with fewer than 4-spores, the 2-spored being particularly abundant. Such discrepancies are not unknown in *Amanita* (Fraiture 1993; Tulloss & Gminder 2000). Nevertheless, the spore variability of *A. alseides* is apparently worthy of further studies on molecularly supported specimens.

Although *Amanita alseides* is believed to be common (Hanss & Moreau 2020), it was so far known by a handful of collections from mainland France and a single specimen from Italy (Sardinia). Here we expand its range to the Balkan Peninsula, based on molecularly verified collections. Our specimens originate from thermophilous oak forests in southern Bulgaria and Greece, but the variety of its known habitats in France suggests it could be more widespread and may be further sought in other Balkan countries and habitats.

Acknowledgements – The financial support from the Bulgarian Academy of Sciences and the Scientific and Technological Research Council of Turkey (Bilateral grant agreement between BAS and TÜBİTAK, project number 118Z640) is gratefully acknowledged. The authors would also like to extend their thanks to Mr. Marjan Kustera (Niš, Serbia) for the revision of the Serbian abstract.

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REZIME

Botanica SERBICA

Prilog o *Amanita alseides,* nedavno opisanoj evropskoj vrsti sekcije *Vaginatae*

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Autori predstavljaju prilog o *Amanita alseides,* do sada poznatim po tim primercima iz Francuske i Italije. Dat je detaljan opis, baziran na molekularnim analizama kolekcija iz Bugarske i Grčke, što proširuje postojeće znanje o morfološkoj varijabilnosti i distribuciji vrste.

Ključne reči: *Amanitaceae, Amanitopsis,* balkanska mikota, biogeografija, taksonomija