





https://doi.org/10.11646/phytotaxa.482.2.4

Amanita vladimirii (Amanitaceae, Agaricales), a new European species in section Vaginatae

HANA ŠEVČÍKOVÁ^{1,4*}, JEAN-MICHEL HANSS^{2,5} & PIERRE-ARTHUR MOREAU^{3,6}

¹ Department of Botany, Moravian Museum, Zelný trh 6, CZ-659 37 Brno, Czech Republic

² 341, rue de Montjustin, F–70200 Arpenans, France

³ULR 4515 – LGCgE, Faculté de pharmacie, Univ. Lille, F-59000 Lille, France

⁴ shanyzka@mail.muni.cz; ⁶ https://orcid.org/0000-0001-8674-0410

⁵ hanjm@sfr:fr; ⁶ https://orcid.org/0000-0001-9390-4284

⁶ spierre-arthur.moreau@univ-lille.fr; ⁶ https://orcid.org/0000-0003-4783-3643

* Corresponding author

Abstract

Amanita vladimirii, a new species in sect. *Vaginatae*, is described and illustrated based on collections from the Czech Republic and France. The distinctive sequences of the LSU, ITS and tef1- α genetic markers, support the status of *A. vladimirii* as a new species. *Amanita vladimirii* is characterised by a greyish pileus striate at margin, a whitish stipe; and a relatively thin, but firm, membranous volva which is externally white with brownish yellow tinges where touched and internally is white-beige or greyish beige. Microscopically, the universal veil is composed of 3 layers; globose basidiospores; a ramose subhymenium; lamellar trama reduced to a mediostratum composed of parallel elements and a pileipellis without a differentiated subpellis.

Keywords: Amanitopsis, taxonomy, phylogeny

Introduction

Amanitas without an annulus (*Amanita* sect. Vaginatae (Fr.) Quél., Cui et al. 2018) represent the most diverse lineage in the genus worldwide, with 302 valid binomials, and 97 provisional names currently recorded by Tulloss & Yang (www. amanitaceae.org 2019, continuously updated). This diversity was already supposed by the extensive type revision by Tulloss (1993) and the description of eight new species from Eastern Asia by Yang (1997). Romagnesi (1992) was the first European author proposing a detailed identification key, with 14 species recognized in Europe and North Africa; later Contu (2003), compiling many previous publications, proposed the recognition of 44 species in Europe. Finally, Neville & Poumarat (2009) introduced ecology as a specific feature for most species including new taxa, i.e. hypothesizing that the host is a driver of evolution in Vaginatae. On the other hand, the respective value given to each feature remained the opinion of each author, and Fraiture (1993) developed a relatively conservative taxonomy with only 23 species recognized in Europe.

In the two last decades, DNA studies revealed the unexpected diversity of this section worldwide, with a cascade of publications focused on Eastern Russia (Malysheva & Kovalenko 2015), Canada (Lambert *et al.* 2018), Korea (Kim *et al.* 2013), China (Yang 2005, 2015; Liu *et al.* 2017; Cui *et al.* 2018), Thailand (Thongbai *et al.* 2018), India (Mehmood *et al.* 2016), Pakistan (Jabeen *et al.* 2017; Kiran *et al.* 2018; Saba *et al.* 2019, Ullah *et al.* 2019), Australia (Davison *et al.* 2015), Brazil (Wartchow *et al.* 2009), tropical Africa and Madagascar (Tang *et al.* 2015). North American taxa are mostly recorded without formal binomials by Tulloss & Yang (www.amanitaceae.org, continuously updated). The oldest names published in sect. *Vaginatae* are from Europe, but before 2020 only two European species *viz., A. simulans* Contu (1999: 11) and *A. lividopallescens* (Gillet 1888: tab. 23) Bigeard & H. Guillem. in Bigeard & Guillemin (1913: 752) had been re-assessed molecularly (see Vizzini *et al.* 2015). Also, various European collections were sequenced and included in several publications (Weiß *et al.* 1998; Zhang *et al.* 2004; Cui *et al.* 2018), however, without sequencing type collections and typifications of modern collections, the identity of these European species remains unresolved. Hanss & Moreau (2020), produced the first comprehensive account of *Amanita* sect. *Vaginatae*

by focusing on grey-coloured species, in an effort to molecularly document all species with available type collections described from Finland, France, Great Britain, Italy and Germany. In their work, 28 phylogenetic species based on ITS sequences, to which a Latin binomial could be reliably applied, were recorded from Europe. However, some collections sequenced for this work could not be assigned any binomial and remain unpublished, waiting for confirmation by more sampling. One of them, not shown by Hanss & Moreau (2020), was documented by only one specimen collected in Northern France (P.-A. Moreau, unpublished data). The only ITS sequence obtained from this specimen was positioned apart from all European species, with weak statistical support. However, four collections from the Czech Republic were analyzed for a project of revision of Czech species of *Amanita* sect. *Vaginatae* at the same time and revealed to be conspecific with this French one. This species displays a unique combination of features which justifies its isolated position within European lineages of sect. *Vaginatae*, and is described here as a new species based on detailed macroscopic and microscopic studies as well as phylogenetic analyzes (ITS, LSU, and $tef1-\alpha$).

Materials and methods

Morphological studies

Macroscopic descriptions were based on fresh basidiomata. The types of subhymenium and structures of the volva follow Bas (1969); the types of volva follow Fraiture (1993). Colour abbreviations follow Kornerup and Wanscher (1978). An Olympus BX-50 light microscope and a Nikon Eclipse 220 with a magnification of $100\times$, $400\times$ and $1000\times$ were used to examine the microscopic features from dried material mounted in water, and Congo Red; mounted in KOH 5 %; rehydrated in KOH 3 % and mounted in aqueous Congo Red; rehydrated in "ramollisseur GDS" and mounted in SDS Congo Red (after Clémençon 1999); rehydrated and mounted in ammoniacal Congo Red + phloxin. The sections were made with a Ranvier's microtome. Herbarium abbreviations follow Thiers (2014). Terminology follows Bas (1969). Abbreviations: L = number of entire lamellae; l = number of lamellulae between each pair of entire lamellae; avl = average of basidiospore length; avw = average of basidiospore width; Q = quotient of length and width in any one basidiospore; avQ = average of basidiospore Q values. At least 30 spores, 20 basidia and 30 other elements in each of the studied basidiomata were measured.

Molecular phylogeny

Total genomic DNA was extracted from ca 2–4 mg of exsiccate by the CTAB method (Doyle & Doyle 1987). The ITS locus was amplified and sequenced using primers ITS1F (Gardes & Bruns 1993) and ITS4 (White *et al.* 1990), and domains D1 to D3 of 28S rDNA (LSU) with primers LR0R and LR6 (Vilgalys & Hester 1990). All PCRs were performed in 20µL reaction mixtures with Kapa polymerase (Kapa Biosystems, Massachusetts, USA) and a touchdown protocol with annealing temperature of 61–56 °C in the first six cycles and 56 °C in the following 34 cycles. The *tef*1- α locus was amplified and sequenced using primers EF1-983F and EF1–1567R (Rehner & Buckley 2005). PCRs were performed in 20µL reaction mixtures with Kapa polymerase (Kapa Biosystems) following a standard protocol with 37 cycles and an annealing temperature of 54° C. The PCR products were purified by precipitation with polyethylene glycol (10% PEG 6000 and 1.25 M NaCl in the precipitation mixture) and sequenced by the Sanger method at Macrogen Europe (The Netherlands).

For each marker (LSU, ITS and *tef*1- α) the datasets consisting of 2 to 3 sequences from separate collections of the new species, and sequences selected amongst BLAST results in the GenBank database were analysed separately (Figs. 1–3). Multiple sequence alignment was carried out with MUSCLE 3.7 (Edgar 2004) and edited manually. Phylogenetic analyses were all performed online at www.phylogeny.lirmm.fr (Dereeper *et al.* 2008). Maximum Likelihood (ML) phylogenetic analyses were performed with PhyML 3.0 (Guindon *et al.* 2010), using the GTR + I + Γ model of evolution. Branch support was assessed using the non-parametric, Shimodaira-Hasegawa version of the approximate likelihood-ratio test (SH-aLRT; Anisimova *et al.* 2011, Bellanger *et al.* 2015). Trees were built using TreeDyn 198.3 at www.phylogeny.lirmm.fr and edited with Inkscape 0.91 (https://inkscape.org).



FIGURE 1. Partial phylogenetic reconstruction of *Amanita* sect. *Vaginatae* (Maximum likelihood) from ITS marker. Sequences of *A. magnivolvata* and *A. pachyvolvata* ("*Clade 1*", *stirps Magnivolvata*) were selected as outgroups. *: non-typified taxa interpreted following Hanss & Moreau (2020). Legends follow Hanss & Moreau (2020).

Results

Phylogeny

Amanita vladimirii sp. nov. is well-supported by the phylogenetic analyses of all three genes used (LSU, ITS and *tef*1- α). All sequences generated from the four collections studied are identical in the ITS, except for one substitution (C/T) on the ITS2 region of the collection labelled PAM05100302. The BLAST analysis of the newly generated ITS sequences in GenBank (*https://blast.ncbi.nlm.nih.gov*) also suggested an isolated position in sect. *Vaginatae*, as the best match was a sequence of an unpublished American species "*Amanita pahasapaensis* Tulloss, Kudzma, Gerenday



FIGURE 2. Partial phylogenetic reconstruction of *Amanita* sect. *Vaginatae* (Maximum likelihood) from LSU marker. Sequences of *A. alboflavescens, A. huijsmanii* and *A. ovalispora* ("Clade 2", Stirps *Argentea*) were designated as outgroup following Hanss & Moreau (2020). Legends follow Hanss & Moreau (2020).



FIGURE 3. Phylogenetic reconstruction of *Amanita* sect. *Vaginatae* (Maximum likelihood) from *tef*1-α marker. Sequences of various *Amanita* species outside *Vaginatae* (sect. *Amanita* and *Caesareae*) were designated as outgroups following Cui *et al.* (2018). Legends follow Hanss & Moreau (2020).

& A. E. State" (in Tulloss & Yang 2019, continuously updated; sequence KP313578.1) with only 89.74 % similarity to the new species.

Based on phylogenetic analyses, the *tef*1-a marker (Fig. 3) indicates *Amanita vladimirii* clusters in a small but well-supported clade comprising sequences of *A. albidostipes* Y.Y. Cui *et al.* (2018: 70), *A. brunneoprocera* Thongbai *et al.* (2018: 471) and *A. orienticrocea* Zhu L. Yang *et al.* in *Cui et al.* (2018: 90). The analyses based on the ITS and LSU markers (Figs. 1, 2), for which more taxa are documented, support this "Clade 6" with more available sequences. This clade encompasses the unpublished species "OR-1", "N-63", "MO-06", "T-49", "*elongatior*", "*bablerensis*" (ITS and LSU), as well as *A. albidostipes*, *A. spadicea ss.* Hanss & Moreau (2020) (ITS), and various other unpublished taxa so far only documented by ITS or LSU (Figs. 1, 2), but do not confirm the inclusion of *A. orientifulva* (Fig. 2). In all these analyses, *Amanita vladimirii* occupies a singular position, distinct from the other subclades of "Clade 6".

According to available ITS sequences, Clade 6 (Fig. 1) encompasses *Amanita vladimirii*, *A. albidostipes* Y.-Y. Cui *et al.* (2018: 70), *A. brunneoprocera* Thongbai *et al.* (2018: 471), the Chinese species *A. lignitincta* Zhu L. Yang ex Y.Y. Cui *et al.* (2018: 82), *A. spadicea ss. auct.*, and the unpublished or informally published North American species MO06, N63, OR01, T49, *A. "elongatior"* Lamoureux, *nom. prov.* (in Lamoureux 2006; Tulloss & Yang 2019), and *A. "bablerensis"* of Tulloss & Yang (2019, continuously updated).

The LSU dataset (Fig. 2) also adds to this clade the unpublished species *A*. "*cattaraugana*", as well as, "DEUT01", "N48", "N62", "MO-06", "T-49" and "N61" (Tulloss & Yang, 2019, continuously updated). The *tef*1-α dataset supports the placement of *A*. *brunneoprocera* and suggests the inclusion of *A*. *orienticrocea* Z.-L. Yang *et al*. in Cui *et al*. (2018: 90) in Clade 6, also compatible with the ITS analysis (Fig. 1), but the LSU analysis does not support this relationship and distinctly places *A*. *orienticrocea* amongst a yet unclassified clade close to Clade 5 (Fig. 2).

Taxonomy

Amanita vladimirii Ševčíková, Hanss & P.-A. Moreau sp. nov. Figs. 4, 5 MycoBank: MB837917

- *Etymology:—"vladimirii*" honours the famous Czech mycologist Vladimír Antonín, collector of the holotype and two additional collections.
- *Diagnosis*:—Differs from all other species of *Amanita* sect. *Vaginatae* by the unique combination of a thick, 3-layered universal membranous veil with predominant yellow-coloured hyphae in the middle and outer layers; a broadly umbonate, grey-coloured pileus; sub-spherical basidiospores, Q=1.0–1.1; an indistinct hymenopodium; thromboplerous hyphae abundant in the mediopellis; and an association with species of *Fagaceae*, or *Pinaceae*.
- Holotype:—CZECH REPUBLIC. Javorníky Mts., Razula National Nature Reserve, Abieto-Fagetum, on soil under Fagus sp., alongside Abies sp. and Picea sp., 4 August 2016 leg. V. Antonín & D. Janda, BRNM 825828, GenBank/EMBL: MW208927 (ITS rDNA); MW208923 (LSU); MW208627 (tef1-α).

Description:—*Pileus* 48–85 mm diam, up to 35 mm high, paraboloid or obtusely conical-campanulate, later applanate, 8–17 mm long striate at margin, smooth, glabrous, slightly viscid when wet; greyish brown (7D–E2, 7E–F3) or greyish yellow (4B3–4) at centre; paler, brownish grey (7D2), yellowish grey (4B2–3), grey (7B1) or white to silvery grey (3A1–7A1) towards margin, becoming silvery metallic reflection with drying. *Lamellae* moderately crowded, L = 106–130, 1 = 0(-1), free, ventricose, up to 4.5 mm broad, white (1A1) to almost cream with white to concolorous edge not darkening with age or when dried. *Stipe* 110–190 × 9–13 mm, cylindrical, slightly broadened towards base (13–30 mm diam), white (1A1), sometimes slightly greyish after bruising, entirely finely whitely floccose to striped. *Volva* at base of stipe 41–60 mm high, up to ca. 30 mm in diam, 0.8–1.2 mm thick, usually type IV (Fraiture 1993), sometimes bilobate, firm, membranous, very finely tomentose and white on exterior, then yellowish on old specimens, ochre brownish (not rusty brown) where bruised, lustrous and white to greyish beige on interior. *Context* white, greyish under pileipellis, hollow in stipe. *Smell* indistinct. *Taste* not recorded. *Spore print* whitish.

Basidiospores globose to subglobose (8)8.5–11(11.5) × (6.7)8–10.1(10.5) μ m, avl = 10.03; avw = 9.58; Q =1.00–1.12(1.18); avQ = 1.046. *Basidia* (45)50–80 × (9)14–20 μ m, tetrasporic, rarely bisporic, clavate to sometimes almost sphaeropedunculate, filled with dense oily content at maturity; sterigmata very thin (less than 1 μ m wide), and up to 5–6 μ m long. *Lamellar trama* 100–125 μ m wide, colourless, reduced to a *mediostratum* composed of parallel, catenulate, inflated physaloidal cells 25–30 × 6–20(30) μ m, occasionally with slender, 3–4 μ m diam, cylindrical,

hyphae, forming compact chains. Hymenopodium not differentiated. Subhymenium composed of hyphae 10-14 × 4-6 µm, mostly ramose (type I of Bas), (sub)cylindrical, rarely inflated or deformed, with very rare subcellular elements only at the base of mature basidia. Lamellar edge sterile, marginal cells (25)28–33(36) \times 10–25 µm, slightly gelatinized, colourless, thin-walled, pyriform to ampullaceous. Pileipellis an ixocutis 400-600 µm wide; upper layer 100–200 µm wide, gelatinized, composed of hyphae 1.5–2.5 µm wide: lower layer faintly gelatinized, composed of dense parallel hyphae 2–3 µm wide, with thick, tortuous, refractive hyphae, dark in Congo Red, of thromboplerous type, becoming more numerous towards the context. The confluence zone with the context consists of the same, but less compact hyphae. Subpellis faintly differentiated, with similar but less compact hyphae than the deep suprapellis. Context of pileus thin, but not measurable, mainly composed of oval physaloidal elements $60-100 \times 10-30 \,\mu\text{m}$, mixed with narrow hyphae. Volva 400–1000 µm thick, distinctly 3-layered: outer layer about 75 µm thick, composed of parallel hyphae strongly twisted and entangled with transversally oriented hyphae $5-6(8) \mu m$ wide; intermediate layer composed of similar but less twisted, more or less intricate hyphae forming a firm structure; inner layer 150-200 µm thick, composed of sphaerocysts (28)30–70(90) \times 25–60(63) µm, less frequently ovoid or broadly clavate physaloidal hyphae $(57)60-130 \times 20-30 \mu m$ (acrophysalids) and filamentous hyphae 4-9 μm wide. In collection BRNM 695616 the lower part of the universal veil has a thick (600 µm) inner stratum and thinner intermediate stratum. Clamps absent from all studied tissues.



FIGURE 4. *Amanita vladimirii* holotype BRNM 825828: a basidiospores, b marginal cells, c basidia, d. inner layer of the universal veil, Scale bar = 10 μm. Drawings by H. Ševčíková.

Additional material examined:—CZECH REPUBLIC. Javorníky Mts., Razula National Nature Reserve, *Abieto-Fagetum*, under *Fagus sylvatica*, 13 August 2005, *V. Antonín & D. Janda* (BRNM! 695616, originally as *A. pachyvolvata*). Beskydy Mts., Salajka National Nature Reserve, on soil under *Fagus sp., Abies sp.* and *Picea sp.*, 3 August 2016 *V. Antonín & D. Janda* (BRNM! 825829), GenBank/EMBL: MW208926 (ITS rDNA), MW208921 (LSU); MW208626 (*tef1-a*). Vsetínské vrchy Mts., Karolinka, Smradlavá Nature Monument, *Fagetum* with *Abies alba*, under *Fagus sp.*, 23 July 2018, *H. Ševčíková* (BRNM! 825830), GenBank/EMBL: MW208925 (ITS rDNA), MW208922 (LSU). FRANCE. Pas-de-Calais: Seninghem, Bois Large, mixed deciduous forest on acidic ground, dominated by *Quercus robur, Fagus sylvatica* and *Carpinus betulus*, with *Betula alba* and *Corylus avellana*, on clay soil, 3 October 2005, *P.-A. Moreau & C. Platiau* PAM05100302 (LIP! 0401726), GenBank/EMBL: MW208924 (ITS rDNA).

Habitat:—On soil in Fagetum to Abieto-Fagetum with Picea abies (Czech Republic), or Endymio-Fagetum (France).

Distribution:-So far only known from the Czech Republic and France.

Discussion

The three collections of *A. vladimirii* known to us so far are documented by young to almost mature specimens, two collections are represented by a single, fully mature specimen; one collection from France is represented by two very mature basidiomata. All collections have a grey-coloured pileus, gradually paler from centre to margin, without a darker antemarginal zone. Hanss & Moreau (2020) showed the features of the volva (especially the colour of the outer surface) to be a reliable marker of phylogenetic affinities. Species with foxy brown -to reddish-spotted volva were shown to represent the "Fulva-clade" ("clade 3"), whilst a greying universal veil was characteristic of the "Submembranacea-clade" ("clade 4"). However, species with a usually variable yellow-spotted volva, such as *A. brunneofuliginea* Zhu L. Yang (1997: 96), *A. coryli* Neville & Poumarat (2009: 34) or *A. simulans*, belonged to the same, highly diversified "clade 5" as species with a white volva. *Amanita vladimirii*, which displays a yellowish-spotted volva on mature specimens, resembled "Clade 5" but differs somewhat by its white stipe and lamella edge and is reminiscent of clade 2 whilst most species of clade 5 have a coloured stipe and concolorous edge at least with age.

Microscopically, *Amanita vladimirii* is remarkable for its volva composed of a filamentous outer layer, without physaloidal cells, parallel to the stipe but solidly entangled and an inner layer that has the consistency of the context. *Amanita vladimirii* is also characterized by relatively small, subglobose basidiospores, and a pileipellis without a differentiated subpellis.

Amanita vladimirii differs from all other European species of *Vaginatae* by the microscopy of its volva (see above). Macroscopically, the collections studied by us have grey pilei, white stipes and whitish volvas turning brownish ochre where bruised, and a white lamella edge not darkening with age. It may be compared to *A. argentea* Huijsm. in Huijsman (1959: 14), which mainly differs by a pileus without an umbo, more elongated spores (Qav = 1.27-1.32) and a 1-layered white volva mainly composed of filamentous, strictly parallel hyphae (Hanss & Moreau, 2020). *Amanita argentea* belongs to Clade 2 (Fig. 1).

Amanita vaginata (Bull. in Bulliard 1783: pl. 98) Lam. in Lamarck (1783: 106) is differently interpreted by various authors (*e. g.* Velenovský 1920, Fraiture 1993, Massart 2000, Contu 2003, Kibby 2012, *etc.*), because Fries' (1821) sanctioning description is too vague to apply unequivocally to one of the currently recognized European species. The most mentioned features are a greyish pileus striate at the margin, white lamellae and stipe, a whitish to greyish vaginate volva, an umbonate pileus and globose basidiospores. The tradition (Garcin 1984, Fraiture 1993, Contu 2003) is to consider *Amanita vaginata* as a grey species with a white, membranous volva. To date, no author has typified the name of this taxon. A specimen identified by C. Bas as "*A. vaginata*" (Weiß *et al.* 1998) corresponds to *Amanita simulans* Contu (1999: 11) (see Vizzini *et al.* 2016). The collections of *Amanita vladimirii* cannot represent *A. vaginata* as the volva is not vaginate, and the colour is not uniform enough compared to Bulliard's illustrations. In addition, Bulliard's plates 98 and 512 show less robust basidiomata. *Agaricus plumbeus* Schaeff. in Schaeffer (1774: 37) also growing in "*Abietum*" (*i.e.* coniferous forests) somewhat resembles *A. vladimirii*, but has brownish yellow tinges where the volva is bruised and this is not mentioned in the protologue; this taxon belongs to *A. vaginata* agg., but its taxonomic status needs a re-evaluation.



FIGURE 5. *Amanita vladimirii.* a basidiomata BRNM 825828, b basidioma BRNM 825829, c inner layer of the universal veil, d outer layer of the universal veil, e basidioma BRNM 695616, f basidiospores of LIP 0401726, g lamellar trama of holotype BRNM 825828, h volva of BRNM 825830. Scale bar: a–b, e =1 cm, c–e, f–g 10 μm, h 100 μm. Photos by: a–b, e V. Antonín, c H. Ševčíková, d, g–h J.–M. Hanss, f Pierre-Arthur Moreau.

Amanita pseudovaginata Hongo (1983: 39) is macroscopically similar to *A. vladimirii* by its greyish pileus with a striate margin, a white stipe and a whitish volva, which is whitish with yellowish brown spots on the outer surface and greyish or whitish on the inner surface. However, this species clearly differs by the more membranous volva with the upper part often becoming greyish when mature (Hongo 1983: 39, Yang & Doi 1999). Microscopically, Yang (1997) described Chinese collections of *A. pseudovaginata* (Clade 5; see Fig. 1) as having a filamentous outer layer comparable to that of *A. vladimiri* but with some physaloidal elements, and a gelatinized inner layer. *Amanita pseudovaginata* is cited as widespread in Japan in *Pineto-Quercetum* (Hongo 1983, Yang & Doi 1999) and in *Pinus yunnanensis* forests in China including Tibet (Zhang *et al.* 2004). The current interpretations of *A. pseudovaginata* (Fig. 1) are diverse, but none of them belongs to "Clade 6". Data about holotype are available here http://www.type. kahaku.go.jp/TypeDB/detail?cls=fungi&pkey=fungi-000023.

The pileus of *Amanita brunneofuliginea* f. *ochraceopallida* (Contu 1997: 137) Hanss (in Hanss & Moreau 133: 119) often has an ochraceous grey colour that varies from very light to medium dark ochre brown, or even entirely ochre tones, rarely silvery grey. The pileus is always umbonate, smooth, without veil remnants; the stipe is smooth, the volva is vaginate (type III), white, often ochre-spotted, rarely fully ochraceous, with a slender habit. Microscopically, the species is distinguished by its ramose subhymenium of type Ib which is a remarkable trait; the volva is 1-layered, of mixed type with entangled elements. *Amanita brunneofuliginea* f. *ochraceopallida* grows under conifers and belongs to Clade 5 (Fig. 1).

Amanita albogrisescens f. *subglobispora* Hanss in Hanss & Moreau (133: 107) may be confused with *Amanita vladimirii* by a grey or silver grey or lead grey or (brown, yellow brown or white) pileus, a white or greyish stipe, smooth or not, subglobose basidiospores and a usually vaginate (Type III), but membranous thin volva. The universal veil is usually white but there are exceptions with brown veil fragments on the pileus and grey spots on the volva. Microscopically, the pileipellis of *A. albogrisescens* Contu (1999c) is divided into a distinct suprapellis and subpellis and the volva is of mixed-type in a single layer which is quite different to *A. vladimirii*. It belongs to Clade 5 (Fig. 1). *Amanita lividopallescens* differs from *A. vladimirii* by its predominantly yellowish colors (light grey, ochraceous grey, yellow-cream, ochraceous or straw yellow), by the presence of a partial veil on the stipe (Gillet 1888, Bigeard & Guillemin 1913: 752), by the structure of the volva and by its lamellar trama; Qav of spores can vary from 1 to 1.5. (Vizzini *et al.* 2016; Hanss, personal observation). It belongs to Clade 5 (Fig. 1).

Amanita fulvoides Neville & Poumarat (2009: 44) has a stipe initially smooth and pale, then tinged brownish when mature, the partial veil becoming small brown scales. The membranous volva is of type II or III, white with more or less large brown spots depending on the specimens. However, this species has a brown or grey-brown pileus, sometimes zonate. Microscopically, the most remarkable features are the often subcellular subhymenial cell, the mediostratum and a mixed volva structure with entangled elements in one layer. *Amanita fulvoides* also grows in central and south Europe in forests with *Fagaeceae*, mostly under *Quercus* species. It belongs to Clade 3 (Fig. 1).

The European species Amanita magnivolvata Aalto (1974: 93) and A. pachyvolvata (Bon) Krieglst. (Bon 1978: 36, Krieglsteiner 1984: 191) may macroscopically somewhat resemble A. vladimirii, but these robust species have a firm volva 4–7 mm thick. Amanita pachyvolvata has a thick saccate volva of type IV, slightly to intensely ochre -or brown-spotted, which is distinctive. Microscopically the volva displays three layers like Amanita vladimirii, the outer layer very thin, with longitudinally arranged filamentous hyphae, and the very thick inner layer composed of flexuous, weakly nested hyphal bundles (LIP 0101727). Although, traditionally, Amanita magnivolvata is distinguished from A. pachyvolvata by having more ellipsoidal spores (Aalto 1974, 1978; Bon 1978) further studies are needed to verify if this feature is stable enough to be used to differentiate it from A. vladimirii. Ecologically, these two species differ from A. vladimirii as Amanita pachyvolvata grows under conifers and A. magnivolvata is known from mostly deciduous forests with Corylus avellana, Populus tremula, Betula species, Carpinus betulus etc. (Aalto 1974, Ševčíková 2017). Amanita magnivolvata; and A. pachyvolvata, as interpreted by Hanss & Moreau (2020, type not sequenced) form a basal lineage in sect. Vaginatae ("Clade 1", Fig. 1).

Amanita simulans has a usually grey pileus, which may turn brown with age but rarely before maturity, with or without depressed faces (*i.e.* 'hammered=martelée') (Contu 1999, Vizzini *et al.* 2016). It clearly differs from *A. vladimirii* by its stipe covered by a partial veil reduced to very short and very dense scales—a characteristic of the species; the universal veil is white, often ochre-spotted, not changing with age; the volva is of the mixed type with entangled elements encompassing physaloidal hyphae in significant proportions mixed with very strongly entangled hyphae, covered by a thin cellular second layer. This species grows under *Salicaceae* or with *Helianthemum* species and belongs to Clade 5 (Fig. 1).

The three phylogenetic analyses of ITS, LSU and *tef*1- α markers (Figs. 1–3) place *A. vladimirii* in a clade, not yet characterized in previously published phylogeness of the section. We introduce this lineage as "Clade 6" following

Hanss & Moreau's nomenclature, without a formal name because, except for *A. vladimirii* and *A. spadicea* (*sensu* Hanss & Moreau 2020 and R. Tulloss, pers. comm.), taxa in this clade are only known to us by incomplete bibliographic or genetic data.

The close Eurasian species *A. albidostipes*. is documented by ITS and *tef*1- α sequences (Figs. 1, 3). It differs from *A. vladimirii* by a yellow-brown to brown pileus, lower and more membranous volva differently arranged, moreover with a gelatinized inner surface near the stipe base, a thinner pileipellis and an association with *Pinus* species (Cui *et al.* 2018). The volva is described as "white to dirty white and often with brown tinge" on the outer surface, that is comparable to *A. vladimirii* to some extent.

Amanita brunneoprocera from Northern Thailand is also identified as a member of Clade 6 in our tef1- α tree (see Fig. 3) and is supported as such by the LSU (Fig. 2). This species differs from *A. vladimirii* by a minutely fibrillose pileus surface, a stipe covered with fibrillose squamules, brownish grey on the upper part, paler on the lower part, and darker in bands mostly in the central part, a membranous volva differently arranged with a gelatinized outer surface, and a thinner pileipellis. The volva was also described as "outer surface white, inner surface yellow", which is the opposite to what is shown in Fig. 3 of Thongbai *et al.* (2018), and is to some degree comparable to *A. vladimirii*.

Amanita "elongatior" is described with a white volva and olivaceous tinges (Lamoureux 2006) and does not show any obvious morphological relationships with *A. vladimirii* according to the available descriptions (see also Tulloss & Yang 2019, continuously updated).

Amanita orienticrocea, phylogenetically close to *A. vladimirii* in our *tef*1-a tree (see Fig. 3) but excluded by the LSU analysis (Fig. 2), clearly differs from *A. vladimirii* by its yellow to yellow-brown pileus with greyish lamellae, a dirty white stipe covered with indistinct, dirty white to brownish squamules and volva composed of two layers (Cui *et al.* 2018). All available descriptions for the Asian species *A. orienticrocea* as well as for *A. lignitincta* (Cui *et al.* 2018), which is included in Clade 6 in the ITS tree (Fig. 1), mention a pure white volva inside and out, without brown or ochre stains when bruised (Yang, 1983; Cui *et al.* 2018).

Amanita changtuia Y.-Y. Cui *et al.* (2018: 75) was positioned at the base of Clade 6 in the ITS phylogeny published by Hanss & Moreau (2020). This position is not supported as a member of this clade in the *tef*1- α phylogeny (Fig. 3), and likely represents a separate lineage.

All Czech localities of *Amanita vladimirii* are within a radius of 20 km and it grows in mixed coniferous and deciduous forest with a predominance of *Fagus sp.* and *Abies sp.*, sometimes also with *Picea sp.* On the other hand, a French collection grew in strictly deciduous forest composed of *Quercus sp.*, *Fagus sp.* and *Carpinus sp.*, with some *Betula sp.* and *Corylus sp.* The only tree common to all localities is *Fagus sp.* which could suggest ectomycorrhizal host-specificity. More reports are needed to refine the ecological knowledge about our species.

Acknowledgments

We thank Vladimír Antonín (Moravian Museum, Brno, Czech Republic) for his field data and photos, Clémentine Platiau and Dalibor Janda for their cooperation in the field, Michal Sochor (Crop Research Institute, Olomouc, Czech Republic) and Sylvain Dumez (Université de Lille, France) sequencing the collections of *A. vladimirii*. Pierre-Arthur Moreau acknowledges Rod Tulloss for constructive discussions and advice on Amanitas without an annulus. The studies of Hana Ševčíková appear through the institutional support of the long-term conceptual development of research institutions for the Moravian Museum provided by the Ministry of Culture (ref. MK000094862). Sequences from French collections were granted by the Société Mycologique du Nord de la France through the program "Connaissance de la fonge régionale" (Conseil Régional Hauts-de-France / DREAL Hauts-de-France, 2018-2021). We also thank the two anonymous reviewers and the editor for their corrections and suggestions to improve our work.

References

Aalto, M. (1974) *Amanita magnivolvata* sp. nova (Agaricales). *Karstenia* 14: 93–96. https://doi.org/10.29203/ka.1974.92

Anisimova, M., Gil, M., Dufayard, J.-F., Dessimoz, C. & Gascuel, O. (2011) Survey of branch support methods demonstrates accuracy, power, and robustness of fast likelihood–based approximation schemes. *Systematic Biology* 60: 685–699. https://doi.org/10.1093/sysbio/syr041 Bas, C. (1969) Morphology and subdivision of Amanita and a monograph of its section Lepidella, Persoonia 5 (4), p. 285-579.

- Bas, C. (1977) Species-concept in *Amanita* sect. *Vaginatae*. *In:* Clémençon, H. (Ed.) The species concept in Hymenomycetes. *Proceedings* of a Herbette Symposium. Lausanne, pp. 79–103.
- Bellanger, J.-M., Moreau, P.-A., Corriol, G., Bidaud, A., Chalange, R., Dudova, Z. & Richard, F. (2015) Plunging hands into the mushroom jar: a phylogenetic framework for Lyophyllaceae (Agaricales, Basidiomycota). *Genetica* 143: 169–194. https://doi.org/10.1007/s10709-015-9823-8
- Bon, M. (1978) Amanitopsis pachyvolvata. Documents mycologiques VIII (29). pp. 36.
- Bon, M. (1979) Fungorum Rariorum Icones Coloratae, 11, p. 1-40 + pl. 81-88 + fig. 1-6
- Bon, M. (1985) Novitates Validations de taxons. Documents Mycologiques 16 (61): 16.
- Bulliard, J.B.F. (1783) Herbier de la France 3: 97-144.
- Clémençon, H. (1999) Du (bon) usage du (bon) rouge congo. Schweizerisches Zeitschrift für Pilzkunde 77 (5): 247-252.
- Contu, M. (2003) A revised key to Amanita, section Vaginatae (Fr.) Quél. in Europe. Field Mycology 128-136.

https://doi.org/10.1016/S1468-1641(10)60221-6

- Contu, M. (1999a) Appunti sul genere Amanita-VII. Bollettino dell'Accademia Gioenia di Scienze Naturali 32: 5-30.
- Contu, M. (1999b) Appunti sul genere Amanita-VIII. Micologia e Vegetazione Mediterranea 13 (2): 153-166.
- Contu, M. (1999c) Appunti sul genere Amanita-IX. Bollettino dell'Associazione Micologica ed Ecologica Romana 46 (1): 3-22.
- Cui, Y.-Y., Cai, Q., Tang, L.-P., Liu, J.-W. & Yang, Z.-L. (2018) The family *Amanitaceae*: molecular phylogeny, higher-rank taxonomy and the species in China. *Fungal Diversity* 91: 5–230.
 - https://doi.org/10.1007/s13225-018-0405-9
- Davison, E.M., Giustiniano, D., Mcgurk, L.E., Syme, K. & Robinson, R.M. (2015) Amanita drummondii and A. quenda (Basidiomycota), two new species from Western Australia, and an expanded description of A. walpolei. Nuytsia 25: 1–13.
- Dereeper, A., Guignon, V., Blanc, G., Audic, S., Buffet, S., Chevenet, F., Dufayard, J.-F., Guindon, S., Lefort, V., Lescot, M., Claverie, J.-M. & Gascuel, O. (2008) Phylogeny.fr: robust phylogenetic analysis for the non-specialist. *Nucleic Acids Research* 36: W465– W469.

https://doi.org/10.1093/nar/gkn180

Doyle, J.J. & Doyle, J.L. (1987) A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemical Bulletin* 19: 11–15.

https://doi.org/10.12691/jfnr-2-7-6

Edgar, R.C. (2004) MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research* 32: 1792–1797.

https://doi.org/10.1093/nar/gkh340

- Fraiture, A. (1993) Les Amanitopsis d'Europe (genre Amanita, Agaricales, Fungi) Synthèse critique de la littérature. Opera Botanica Belgica 5: 1–128.
- Garcin, R. (1984) Les amanites européennes. Fédération mycologique Dauphiné-Savoie, Marlioz.
- Gillet, C.C. (1888) Les hymenomycètes ou description de tous les champignons (Fungi) qui croissent en France avec l'indication de leurs propriétés utiles ou vénéneuses. Ch. Thomas, Alençon.
- Hanss, J.-M. & Moreau, P.-A. (2020) Une Révision des Amanites « vaginées » (*Amanita* sect. *Vaginatae*) en Europe, 1re partie : quelques Amanites argentées. *Bulletin de la Société mycologique de France* 133 (1–2): 67–141.
- Hongo, T. (1983) Notulae mycologicae (18). Memoirs of Shiga University 33: 37-41.
- Jabeen, S., Kiran, M., Ullah, S., Wilson, A.W., Mueller, G., Fiaz, M. & Khalid, A.N. (2017) Amanita glarea, a new species in section Vaginatae from Pakistan. Phytotaxa 306 (2): 135–145. https://doi.org/10.11646/phytotaxa.306.2.3
- Kibby, G. (2012) The genus Amanita in Great Britain. G. Kibby, UK, 50 pp.
- Kim, C.S., Jo, J.W., Kwag, Y.N., OH, J., Shrestha, B., Sung, G.H. & Han, S.K. (2013) Four newly recorded *Amanita* species in Korea: *Amanita* sect. *Amanita* and sect. *Vaginatae*. *Mycobiology* 41 (3): 131–138. https://doi.org/10.5941/MYCO.2013.41.3.131
- Kiran, M., Khan, J., Sher, H., Pfister, D.H. & Khalid, A.N. (2018) Amanita griseofusca: A new species of Amanita in section Vaginatae from Malam Jabba, Swat, Pakistan. Phytotaxa 364 (2): 181–192. https://doi.org/10.11646/phytotaxa.364.2.5

Kornerup, A. & Wanscher, J.H. (1978) Methuen handbook of colours. 3rd edn. Eyre Methuen, London, UK.

Krieglsteiner, G.J. (1984) Verbreitung und Ökologie ausgewählter Nichtblätterpilze in der BDR. *Beihefte zur Zeitschrift für Mykologie* 5: 69–302.

Lamarck, J.B.A.P. de (1783) Encyclopédie Méthodique, Botanique 1: 1–344.

Lambert, H., Fortin, G., Labbé, R., Labrecque, J., Bérubé, J.A., Landry, J., Ilyukhin, E., Margaritescu, S., Moncalvo, J.-M. & Lamoureux,

Y. (2018) Validation of two *Amanita* species from eastern North America: *A. rhacopus sp. nov.* and *A. variicolor sp. nov. Mycokeys* 38: 47–57.

https://doi.org/10.3897/mycokeys.38.27041

Liu, J.-W., Cai, Q., Cui, Y.-Y. & Yang, Z.-L. (2017) Amanita cingulata, a new annulate species of Amanita sect. Vaginatae from subtropical China. Phytotaxa 326 (1): 41–53.

https://doi.org/10.11646/phytotaxa.326.1.3

- Malysheva, E.F. & Kovalenko, A.E. (2015) Fungi of the Russian Far East. 4. Contribution to the study of *Amanita* sect. *Vaginatae* in the central Sikhote-Alin. *Mikologiya i Fitopatologiya* 149: 151–163.
- Massart, F. (2000) Amanite osservate in Gironda. Parte 1. *Amanitopsis*. *Bolletino del gruppo micologico G. Bresadola–Nuova serie BGMB* 43 (2): 241–259.
- Mehmood, T., Das, K., Hosen, M.D.I., Bhatt, R.P., Uniyal, P. & Singh, U. (2016) Two new species of *Amanita* (Amanitaceae) from Northwestern Himalaya, India. *Phytotaxa* 367 (3): 219–232.

https://doi.org/10.11646/phytotaxa.367.3.2

- Neville, P. & Poumarat, S. (2009) Quelques espèces nouvelles ou mal délimitées d'*Amanita* de la sous-section *Vaginatinae*. *Fungi non Delineati* LI–LII: 1–200.
- Persoon, C.H. (1797) Tentament disponitionis methodicae fungorum in classes, ordines, genera et familias (cum supplemento adjecto). Lipsiae, 76 pp. + 4 plates.

https://doi.org/10.5962/bhl.title.42674

Persoon, C.H. (1801) Synopsis methodicae fungorum, Pars 2–Gottingae. 706 pp.

- Rehner, SA. & Buckley, E. (2005) A *Beauveria* phylogeny inferred from nuclear ITS and EF1- α sequences: evidence for cryptic diversification and links to *Cordyceps* teleomorphs. *Mycologia* 97 (1): 84–98. https://doi.org/10.3852/mycologia.97.1.84
- Romagnesi, H. (1992) Prodrome à une Flore Analytique des Agaricomycetes. II. Bulletin de la Société mycologique de France 108: 71-86.
- Saba, M., Haelewaters, D., Fiaz, M., Khalid, A.N. & Pfister, D. (2019) Amanita mansehraensis, a new species in section Vaginatae from Pakistan. Phytotaxa 401 (3): 199.

https://doi.org/10.11646/phytotaxa.401.3.5

- Schaeffer, J.C. (1772) Fungorum qui in Bavaria et Palatinu circa Ratisbonam nascuntur Icones Nativis Coloribus Expressae. Volume 3. Erlangae, Apud J.J. Palmium
- Schaeffer, J.C. (1774) Fungorum qui in Bavaria et Palatinu circa Ratisbonam nascuntur Icones Nativis Coloribus Expressae. Volume 4. Erlangae, Apud J.J. Palmium
- Ševčíková, H. (2017) Nepřehlédnutelná, vzácná muchomůrka tlustopochvá-Amanita pachyvolvata. Mykologické listy 136: 12-22.
- Tang, L.P., Cai, Q., Lee, S., Buyck, B., Zhang, P. & Yang, Z.-L. (2015) Taxonomy and phylogenetic position of species of *Amanita* sect. *Vaginatae* s. l. from tropical Africa. *Mycological Progress* 14: 1–15. https://doi.org/10.1007/s11557-015-1061-z
- Thongbai, B., Hyde, K.D., Lumyong, S. & Raspé, O. (2018) High undescribed diversity of *Amanita* section *Vaginatae* in northern Thailand. *Mycosphere* 9 (3): 462–494.

https://doi.org/10.5943/mycosphere/9/3/3

- Thiers, B. (2010) [continuously updated] Index Herbariorum: a global directory of public herbaria and associated staff. Available from: http://sweetgum.nybg.org/ih/ (Retrieved 1 December 2019)
- Tulloss, R. (1994) Type studies in *Amanita* section *Vaginatae* I: some taxa described in this century (studies 1–23) with notes on description of spore and refractive hyphae in *Amanita*. *Myxotaxon* LII: 305–396.
- Tulloss, R.E. & Yang, Z.-L. (Eds.) (2019 [continuously updated]) *Amanitaceae* studies. Available from: http://www.amanitaceae.org (accessed 28 June 2020)
- Ullah, S., Wilson, A.W., Tulloss, R.E., Fiaz, M., Mueller, G.M. & Khalid, A.N. (2019) Amanita cinis and A. olivovaginata (Basidiomycota, Amanitaceae), two new species, and the first record of A. emodotrygon, from Northwestern Pakistan. Turkish Journal of Botany, 43: 1–19.

https://doi.org/10.3906/bot-1903-21

Weiß, M., Yang, Z.-L. & Oberwinkler, F. (1998) Molecular phylogenetic studies in the genus *Amanita*. *Canadian Journal of Botany* 76: 1170–1179.

https://doi.org/10.1139/cjb-76-7-1170

Velenovský, J. (1920) České Houby. Česká Botanická Společnost, Praha, 950 pp.

Venturella, G., Saitta, A. & Contu, M. (2006) A key for the identification for the Amanita species, sect. Vaginatae, subsect. Ovigerae (Basidiomycota, Amanitaceae) from Sicily. Naturalista siciliana S. IV, XXX (3–4): 419–430.

- Vizzini, A., Zotti, M., Traverso, M., Ercole, E., Moreau, P.-A., Kibby, G., Consiglio, G., Cullington, A.P., Moingeon, J.-M. & Peintner, U. (2016) Variability, host range, delimitation and neotypification of *Amanita simulans (Amanita section Vaginatae*): collections associated with *Helianthemum* grasslands, and epitypification of *A. lividopallescens*. *Phytotaxa* 280 (1): 1–22. https://doi.org/10.11646/phytotaxa.280.1.1
- Yang, Z.-L. (1997) Die Amanita-Arten von Südwestchina. Bibliotheca Mycologica 170: 1-240.
- Yang, Z.-L. (2005) Flora fungorum sinicorum. Vol. 27. Amanitaceae. Beijing, Science Press, 258 pp.
- Yang, Z.-L. (2015) Atlas of the Chinese Species of Amanitaceae. Science Press, Beijing, 213 pp.
- Yang, Z.-L. & Doi, Y. (1999) A contribution to the knowledge of Amanita (Amanitaceae, Agaricales) in Japan. Bulletin of the National Science Museum Tokyo 25 (3): 107–130.
- Yang, Z.-L., Li, T.-H. & Wu, X.-L. (2001) Revision of *Amanita* collections made from Hainan, southern China. *Fungal Diversity* 6: 149–165.
- Wartchow, F., Tulloss, R.E. & Cavalcanti, M.A.Q. (2009) Amanita lippiae: a new species from the semi-arid caatinga region of Brazil. Mycologia 101: 864–870.

https://doi.org/10.3852/08-106

Zhang, L.-F., Yang, J.-B. & Yang, Z.-L. (2004) Molecular phylogeny of eastern Asian species of *Amanita (Agaricales*, Basidiomycota): taxonomic and biogeographic implications. *Fungal Diversity* 17: 219–238.