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## БИОРАЗНООБРАЗИЕ, СИСТЕМАТИКА, ЭКОЛОГИЯ

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# *AMANITA PHALLOIDES* IN NORTHWEST EUROPEAN RUSSIA

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The article reviews available data on the "death cap" (*Amanita phalloides*) occurrences in the North-West of the European Russia (Leningrad, Novgorod, Pskov Oblasts and St. Petersburg City). The literature data are analyzed, the review of ecological preferences of the species in the studied area is carried out. It has been suggested that the species can be both native and imported and was introduced with broad-leaved tree seedlings during the foundation of numerous manor parks in the XVIII–XIX centuries. Molecular-genetic analysis was carried out, showing that ITS sequences of *A. phalloides* samples from Leningrad Oblast form a common clade with the sequences of collections from Central Russia as well as from Northern and Central Europe.

*Keywords: Amanitaceae*, biodiversity, death cap, fungi, poisonous species

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## INTRODUCTION

*Amanita phalloides* (Vaill. ex Fr.) Link is an iconic species, "death cap" that is responsible for numerous human poisonings. It was assumed earlier, that the species is rare or even absent in the Northwestern part of European Russia. During the last twenty years, new findings of the species on the territory were made by both mycologists and amateurs. Present paper summarizes all existing collections of the species to highlight the presence of this deadly poisonous species in the region.

## MATERIALS AND METHODS

**Sampling and identification.** For the literature review information from database of agaricoid fungi distribution was used (Bolshakov et al., 2021). For the existing specimens we checked Mycological Herbarium of the Komarov Botanical Institute RAS (LE). During survey of the mycobiota of Northwest Russia, several new collections of the species were obtained and new localities were revealed. Due to characteristic appearance, identification was performed in the field.

**DNA techniques and phylogenetic analysis.** DNA was extracted from small pieces of dried basidiomata using the FitoSORB DNA extraction kit (Syntol, Russia) according to the manufacturer's instructions. PCR reactions were performed in 20 μL of reaction mixtures containing 10 μL of iQ Supermix (BioRad), 0.2 μL of each PCR primer, 4.6 μL of deionized $H_2O$, and 5 μL of template DNA. The ribosomal ITS1–5.8S–ITS2 region was amplified with the primers ITS1F/ITS4B (Gardes, Bruns, 1993). PCR products were visualized using agarose gel electrophoresis and GelRed staining, and subsequently purified with the Fermentas PCR Purification Kit (Thermo Fisher Scientific, Lithuania). Purified PCR products were sequenced on an ABI model 3500 Genetic Analyzer (Applied Biosystems, USA). Raw data were edited and assembled using Molecular Evolutionary Genetics Analysis Version 6.0 (MEGA6) software (Tamura et al., 2012). Newly generated sequences were deposited in the GenBank.

**Table 1.** Sequences and strains of *Amanita* species included in molecular analysis

| Species | GenBank accession number | ID (specimen number, strain) | Country | References |
|---|---|---|---|---|
| *Amanita pantherina* | MK327260 | RET 403-8 | Czech Republic | –** |
| | MH508488 | MB-102863 | Germany | Cui at al. (2018) |
| *A. phalloides* | AJ308097 | FVORO-0023 | Russia | – |
| | AY325834 | O Gulden 49/94, Norway | Norway | – |
| | EU909444 | Am.pha.PV02.1 | France | Pringle et al. (2009) |
| | MK512068 | A54 | Czech Republic | – |
| | MW036159 | LE-BIN 4016 (strain) | Russia | – |
| | **MZ647956** | **Khovpachev-A3b** | Russia | **current study** |
| | **MZ647957** | **LE 332058** | Russia | **current study** |
| | NOBAS5223-18* | O-F-21495 | Norway | – |
| | AJ889921 | KF02-19 | Denmark | – |
| *A. virosa* | **MZ647955** | **Khovpachev-A1b** | **Russia** | **current study** |
| | KY924845 | RET 291-3 | France | – |

Note. The newly generated sequences are given in boldface. *The BOLD Systems accession number.
**Sequences marked with "–" are unpublished.

Additionally, 10 ITS sequences were retrieved from GenBank and the BOLD Systems (Ratnasingham, Hebert, 2007) (Table 1). Sequences were aligned with the MAFFT version 7 web tool (Katoh et al., 2019) using the E-INS-1 option. Maximum Likelihood (ML) analysis was performed in the IQ-TREE Web Server (Trifinopoulos et al., 2016) with 1000 ultrafast bootstrap replicates.

## RESULTS

### Literature review

**XIX–XX centuries.** In 1828 Weinmann published the list of fungi that were found in Pavlovsk manor (Weinmann, 1828). He mentioned "*Agaricus phalloides* pileo *flavo*" with reference to Fries. Fries in his Systema Mycologicum (Fries, 1821) accepted broad concept of the species and cited five "infraspecific" taxa differing in pileus color. Iconography given to the form "b. pileo *flavo*" depicts rather *Amanita citrina* Pers. in modern concept (Fig. 1). As Weinmann followed Fries, it can be assumed that he probably found not *A. phalloides*, but *A. citrina*. Later, in subsequent summarizing work on flora of "agro Petropolitano" (Weinmann, 1837) he also mentioned five forms, simultaneously citing iconography. Fungi depicted on illustrations cited fit quite well the modern concept of *A. phalloides* (Fig. 2). No precise localities are given, but it is known that Weinmann mostly worked in Tsarskoye Selo (suburban area of modern St. Petersburg) and Gatchina (modern Leningrad Oblast). To our knowledge, no specimen of *A. phalloides* collected by Weinmann exists. In 1892 and 1894 Thesleff found the species during his investigations on the territory of modern Vyborgsky District (Thesleff, 1920) with mention "ekskog" (oak forests). According to the list of localities, he visited oak forests in three localities: around Vyborg Bay ("kring Viborgska viken i Viborgs socken"), Vesennii Island ("Luuri-holmen i St Johannes socken") and Malyi Beryozovyi Island ("holmen Vasikkasaari i Finska viken, Björkö socken"). Thus, up to 2000 there were only literature data based on century-old observations of the species from the territory of North-Western Russia with no specimens deposited in collections.

**New findings (2000 – nowadays).** First data on *A. phalloides* findings from the territory of St. Petersburg confirmed by specimens belongs to the 2010s when the species was found in Primorsky and Kurortny districts of St. Petersburg. Since 2011, death cap basidiomata collected from the territory of St. Petersburg and Leningrad Oblast were regularly exposed during the annual autumn mushroom exhibition organized by St. Petersburg Mycological Society (Fig. 2, g). In 2018 the species was included in Red Data Book of St. Petersburg (Arslanov, 2018). In 2018, 2020, and 2021 authors of present paper found new localities of the species in Leningrad Oblast (Kingiseppsky and Luzhsky districts).

In Pskov Oblast the species is included in the Red Data Book (Sudnitsyna, 2014) as protected on the territory of Sebezhsky National Park with reference to Kovalenko et al. (2003). Nevertheless, in the latter paper the species is not mentioned and its' presence in the protected area is not confirmed. Later it was found in Loknyansky district (Popov et al., 2013), Polistovsky Nature Reserve (Kalinina, 2021) and in the Pushkin Museum-Reserve (Morozova et al., 2022). Notewor-

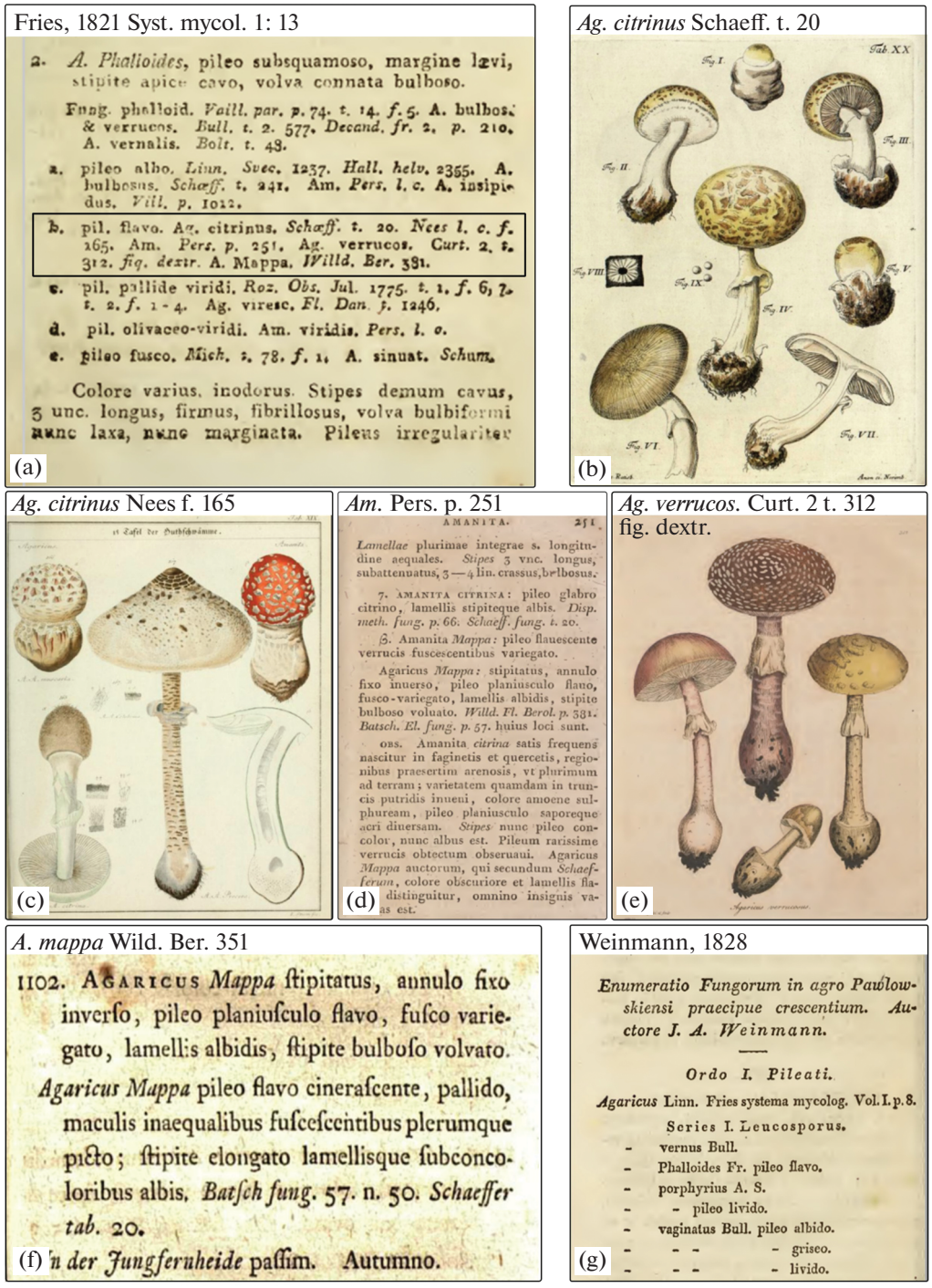


**Fig. 1.** Original descriptions of taxa associated with *Amanita phalloides* concept: a – "forms" of *Agaricus phalloides* from Systema Mycologicum (Fries, 1821). We collect here iconography ascribed to "b. pil. flavo" (encased in rectangle); b – illustration of *Agaricus citrinus* from Schaeffer (1762); c – illustration from Nees von Esenbeck (1817); d – description of *Amanita citrina* by Persoon (1801), there is also reference to Schaeffers' plate; e – illustration of *Agaricus verrucosus* from Curtis (1787); f – description of *Agaricus mappa* from Willdenow, 1787; g – page from Weinmann (1828).

thy, that in 2021 large population of *A. phalloides* was firstly detected in particular site of a forest regularly inspected since 1995.

No information on *A. phalloides* from Novgorod Oblast is known so far.

***Amanita phalloides* in the North-West of European Russia.** Here we provide list of known occurrences of the species arranged in chronological order (Fig. 3).

St. Petersburg and Leningrad Oblast: Pavlovsk – ?*A. citrina*; no locality given (Weinmann, 1837).

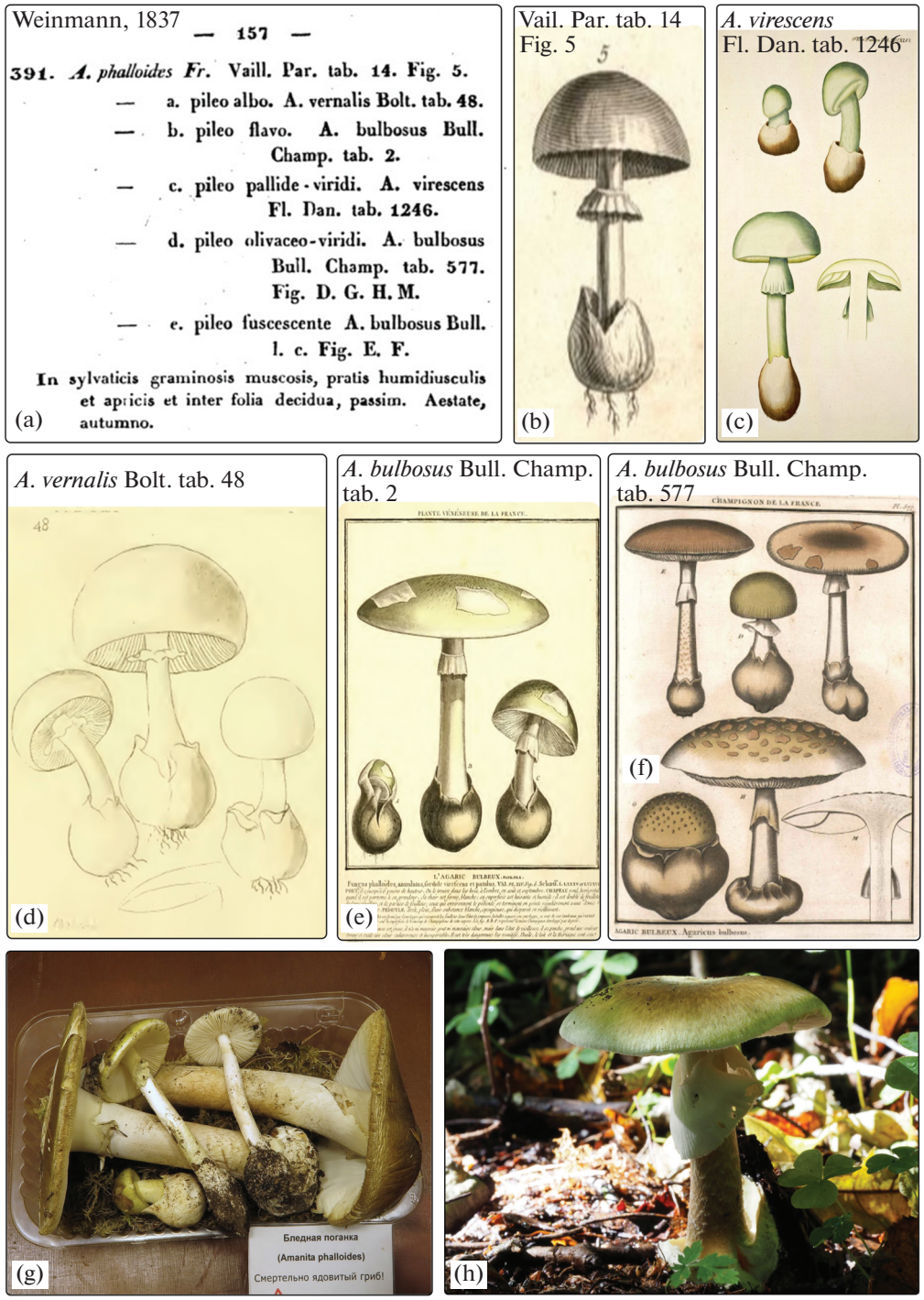


**Fig. 2.** Original descriptions of taxa associated with *Amanita phalloides* and illustration of the species in modern concept: a – "varieties" of *Agaricus phalloides* from Weinmann (1837); b – plate from Vaillant (1727) that was chosen by Weinmann as main "variety"; c – illustration to variety "c. pileo pallide-viridi", *A. virescens* from Vahl (1799); d – to the variety "a. pileo albo", *A. vernalis* from Bolton (1788); e – to the "b. pileo flavo", *A. bulbosus* from Bulliard (1798); f – to the "d. pileo olvaceo-viridi" and "e. pileo fuscescente", *A. bulbosus* from Bulliard (1798); g – basidiomata of *Amanita phalloides* exposed during mushroom exhibition in St. Petersburg in 2018; h – *A. phalloides* in situ.

St. Petersburg: Primorsky district, vicinity of former station "Morskaya", pine forest, coll. and det. S.N. Arslanov, 30.09.2016, LE 311893, LE 311894; Kurortny district (Arslanov, 2018); Primorsky district, Reserve "Severnoe Poberezhye Nevskoy Guby" (Morozova et al., 2020); Kurortny district, vicinity of Zelenogorsk, 05.09.2020 (A. A. Khovpachev, pers. observ.).

Leningrad Oblast: Vyborgsky District (Thesleff, 1920); Kingiseppsky district, vicinities of Velikino settlement, in oak valley (remnants of manor park) and

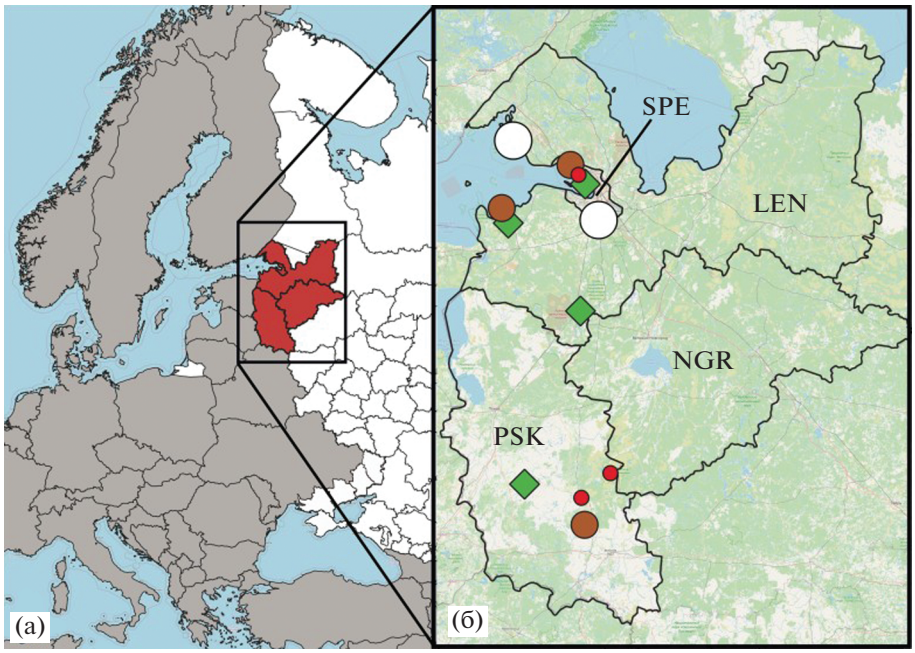


**Fig. 3.** Occurrences of *Amanita phalloides* in the Northwestern European Russia: a – North-West of European Russia (in brown) on the map of Russia; b – Leningrad (LEN), Novgorod (NGR), Pskov (PSK) Oblasts and the city of St. Petersburg (SPE). The white circles represent data from XIX century, brown dots – personal observations, red dots – published data not confirmed by specimens kept in LE and green diamonds represent localities of specimens kept in LE.

coniferous-broadleaved forest, coll. and det. D.A. Tomchin, 14.09.2018, LE F-332058, LE 315730; Luzhsky district, Shalovo-Perechitsky protected area, mixed forest with *Quercus robur*, *Tilia cordata* and *Corylus avellana* in undergrowth, coll. and det. D.A. Tomchin, 25.07.2020, LE 312591; Kingissepky district, vicinity of Vistino village, 25.08.2020 (A.A. Khovpachev, pers. observ.); Kingissepky district, Kotelsky protected area, vicinity of Glubokoe lake, mixed forest with *Quercus robur*, 26.08.2020 (L.Yu. Semyonova, pers. observ.); Kingissepky district, Kurgalsky protected area, mixed forest with *Tilia cordata* and *Corylus avellana* in undergrowth, 12.09.2020 (D.A. Tomchin, pers. observ.); Luzhsky district, Cheremenetsky protected area, mixed forest with *Corylus avellana* in undergrowth, 26.08.2021 (D.A. Tomchin, pers. observ.).

Pskov Oblast: Loknyansky district, vicinity of Bashovo, mixed forest, under oak, 10.08.1998 (Popov et al., 2013); Sebezhsky district – doubtful (Sudnitsyna, 2014); Bezhanitsky district, Polistovsky State Nature Reserve, 17.08.2017 (Kalinina, 2021); Loknyansky district, Polistovsky State Nature Reserve, 04.09.2017 (Kalinina, 2021); Loknyanskiy district, vicinity of Skraby village, under oak, 20.08.2017 (E.S. Popov, pers. observ.); Pushkinogorsky district, museum "Mikhailovskoye", Mikhaylovskoye manor, on the grass under lime and oaks, coll. and det. O.V. Morozova, 11.09.2018, LE 315731 (Morozova et al., 2022); Loknyansky district, vicinity of Maloye Koskovo village, forest with *Quercus robur* and *Corylus avellana* in undergrowth, 14.09.2019 (L.B. Kalinina, pers. observ.); Loknyansky district, Bashovo, forest dominated by *Alnus incana* with admixture of *Quercus robur* and *Fraxinus excelsior* and numerous *Corylus avellana* in undergrowth, under hazel, 21.08.2021 (E.S. Popov, pers. observ.); Loknyansky district, vicinity of Koshnevo village, mixed forest, under oaks, 30.08.2021 (E.S. Popov, pers. observ.).

**Phylogenetic analysis.** Our findings can be considered as conspecific with specimens from Central Russia, Northern and Western Europe as a common clade is formed on the phylogenetic tree derived from ITS nrDNA sequences (Fig. 4).

## DISCUSSION

*A. phalloides*, a conspicuous and attractive mushroom, is responsible for numerous human poisonings due to presence of amatoxins (Wieland, 1968; Wieland, Faulstich, 1991; Gurevich, Zhurkovich, 1995; Khovpachev et al., 2020) and is considered as one of the most feared fungi (Hyde et al., 2018). The species is native to Europe and widespread. In mycological literature of the XX century, the species was reported also from Asia (Imazeki, Hongo, 1987; Teng, 1996), but recent studies showed that Asian lethal amanitas represent several distinct taxa endemic to East Asia (Zhang et al., 2010; Cui et al., 2018). Outside Europe it is known as invasive from North America (Pringl et al., 2009; Wolfe et al., 2010) and from Australia (Trim et al., 1999). The species is easily exported with its symbionts (Tulloss, 2023), and there is evidence that

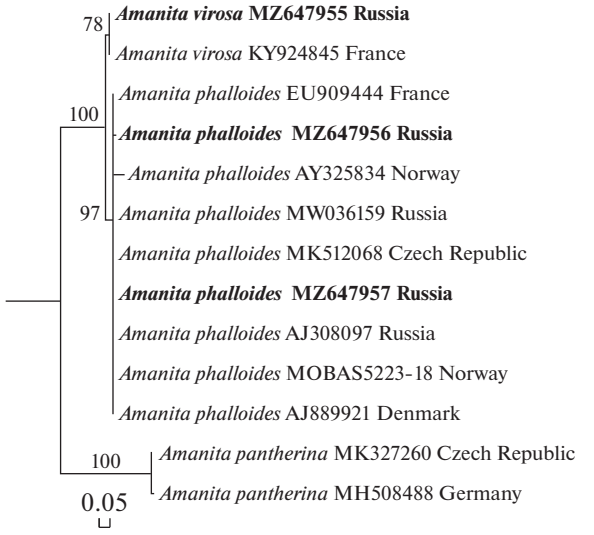


**Fig. 4.** Phylogenetic tree of *Amanita phalloides* reconstructed from an ITS dataset using ML analysis. Newly generated sequences are given in boldface.

the species is able to host shift (Berch et al., 2016). Regarding distribution in Russia, the species is common and widespread in the nemoral zone in the European part (Bolshakov et al., 2021). The most frequent habitats are pine and pine-birch forests, oak and coniferous-broadleaved ones are less frequent. In addition, the species is drawn to anthropogenic habitats such as aspen forests, birch stands with grasses, pine plantings (Kiyashko, 2015).

Habitats of our findings can be divided into two groups – old artificial stands with *Quercus robur* and deciduous or coniferous-broadleaved forest with *Quercus robur*, *Tilia cordata* and/or *Corylus avellana* in undergrowth. Probably, in the artificial stands the species was brought with tree seedlings used for numerous manor parks foundation in XVIII–XIX century. Noteworthy, that during the long-termed survey of genus *Amanita* in Cyprus, *A. phalloides* was found only from a *Corylus avellana* plantation and was absent from native habitats (Loizides et al., 2018). The second group of habitats is presented by deciduous or coniferous-broadleaved forests with *Quercus robur*, *Tilia cordata* and/or *Corylus avellana* in undergrowth. These habitats are not very common and readily accessible, so thorough mycological studies have not been done until recently. Possibly, in such habitats species is native but is not observed. In oak forest situated on the northwest of Izhora Upland Leningrad Oblast (protected area "Oak forests near Velkota village"), *Amanita phalloides* was not found despite the regular surveys since 2012. Interestingly, that in 2021 large population of *A. phalloides* was firstly detected in particular site of a forest (Bashovo, Pskov Oblast) regularly inspected since 1995.

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# *Amanita phalloides* на северо-западе европейской части России

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В статье приведен обзор имеющихся сведений о находках смертельно ядовитой бледной поганки (*Amanita phalloides*) на территории Северо-Запада европейской части России (Санкт-Петербург, Ленинградская, Новгородская и Псковская области). Проанализированы литературные сведения, проведен обзор экологических предпочтений вида на исследуемой территории. Высказано предположение, что вид может являться как аборигенным, так и заносным и был интродуцирован вместе с саженцами широколиственных деревьев при создании многочисленных приусадебных парков в XVIII–XIX вв. Проведен молекулярно-генетический анализ, показавший, что последовательности ITS-образцов *A. phalloides* из Ленинградской обл. образуют единую кладу с последовательностями коллекций из Центральной России, а также Северной и Центральной Европы.

*Ключевые слова:* бледная поганка, биоразнообразие, ядовитые виды, *Amanitaceae*