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First modern findings of red-listed fungus *Battarrea phalloides* (*Agaricales*, *Basidiomycota*) in the Republic of Dagestan, Russia

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Abstract
Aim. The monitoring of known as well as the search for new localities of rare and protected species is a requirement for Red Data Books and conservation efforts as a whole. The aim of this paper is to describe the finding of basidiomycete fungus *Battarrea phalloides*, a rare and protected species in the Republic of Dagestan, and to discuss features of its phylogenetic diversity, distribution and ecology.
Material and Methods. Primary identification of the material was carried out by light microscopy. Phylogenetic analysis was performed based on the Maximum Likelihood method.
Results. New locality of *B. phalloides* has been revealed in Gunibsky district of the Republic of Dagestan. A detailed description of macro- and micromorphology of the collected basidioma, as well as peculiarities of new ecotope occupied by the species is presented. The complete ITS1–5.8S–ITS2 nrDNA sequence has been obtained and deposited in the NCBI GenBank database. In phylogenetic analysis the specimen studied formed a separate clade among other individuals of *B. phalloides* with different geographical origins.
Conclusion. The new *B. phalloides* finding recorded in Dagestan allowed us to expand the knowledge on its ecology and distribution, as well as on intraspecific phylogenetic structure of the species red-listed in the region and little-known in the Caucasus.

Key Words
Biodiversity, DNA barcoding, fungal conservation, gasteromycetes, North-Eastern Caucasus, phylogeny, Red Data Book, sandy stiltball.

Первые современные находки краснокнижного гриба *Battarrea phalloides* (*Agaricales*, *Basidiomycota*) в Республике Дагестан, Россия

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Резюме

Цель. Мониторинг известных, а также поиск новых местонахождений редких и охраняемых видов является необходимым условием ведения Красных книг и природоохранной деятельности в целом. Цель данной работы – представить описание находки базидиомицета *Battarrea phalloides*, редкого и охраняемого вида в Республике Дагестан, а также обсудить особенности его филогенетического разнообразия, распространения и экологии.

Материал и методы. Первичная идентификация материала выполнена методами световой микроскопии. Филогенетический анализ проведен на основе метода максимального правдоподобия.

Результаты. Выявлено новое местонахождение *B. phalloides* на территории Гунибского района Республики Дагестан. Приведено подробное описание макро- и микроморфологии собранной базидиомы, а также особенностей занимаемого данным видом нового экотопа. Получена и депонирована в международную базу NCBI GenBank полная нуклеотидная ITS1–5.8S–ITS2 области ярДНК. По итогам филогенетического анализа изученный образец сформировал отдельную кладу среди других представителей вида *B. phalloides*, имеющих различное географическое происхождение.

Заключение. Новая находка *B. phalloides*, зарегистрированная в Дагестане, позволила расширить знания об экологии и распространении, а также о внутривидовой филогенетической структуре краснокнижного в регионе и малоизвестного на Кавказе вида.

Ключевые слова
биоразнообразие, ДНК-штрихкодирование, охрана грибов, гастеромицеты, макромицеты, Северо-Восточный Кавказ, филогения, Красная книга, баттаррея весёлковая.

INTRODUCTION

Fungi play a crucial role in terrestrial ecosystem functioning as saprotrophs, parasites and mycorrhizae-forming agents. They are main drivers of carbon cycle owing to the ability to decompose a wide range of plant remnants – from large-size coarse woody debris to modified lignocellulose-containing fragments of litter [1–3]. The unique enzymatic complexes and rather high adaptability to abiotic environmental factors predict the significant diversity of habitats and areas occupied by fungi [4–6].

At the same time, most fungal species, along with animals and plants, are also affected by loss and degradation of habitat, pollution, pressure from invasive species, and direct and indirect impacts of climate change [7]. The identification and documentation of those species most in need of conservation attention if global or regional extinction rates are to be reduced is the aim of red-lists [8].

Red-lists are one of key sources of information for setting conservation priorities along with other conservation values. In particular, an analysis of habitat and substratum requirements of red-listed organisms makes it possible to determine environmental deficiencies and helps to identify appropriate habitat qualities and land-management requirements for red-listed species [9].

The monitoring of known localities of rare and protected species, as well as the search for new occurrences, is a mandatory requirement for conservation activities and the maintenance of the Red Data Books. The Red Data Book of the Republic of Dagestan includes ten species of non-lichenized fungi, among which ascomycetes (one species), aphyllophoroid (six species), agaricoid (one species) and gasteroid (two species) basidiomycetes are represented. The latter group contains the species *Battarrea phalloides*, which is protected in the Republic of Dagestan with category 3 (VU), vulnerable species [10].

In 2022, a new locality of basidiomycete fungus *Battarrea phalloides* was discovered during the mycological survey on the territory of Gunibsky district of the Republic of Dagestan. This study is aimed to describe the new finding of *Battarrea phalloides*, a rare and protected species in the Republic of Dagestan, and to discuss features of its phylogenetic diversity, distribution and ecology.

MATERIAL AND METHODS

Specimen of *Battarrea phalloides* presented by a single mature basidioma was collected in September 2022 on soil under living shrub of *Berberis* sp. in scrubland with *Cornus australis*, *Hippophaë rhamnoides*, *Prunus cerasifera*, *Rosa* sp. and *Asparagus* sp. (Fig. 1) in a north-east-facing slope, in 6 km from Salta settlement along the road to Gunibsky hydroelectric power station, Gunibsky District, Republic of Dagestan. The geographical coordinates of sampling area are 42.42513° N, 47.00704° E, altitude is 940 m a.s.l. The voucher specimen is deposited in the Mycological Herbarium of the Komarov Botanical Institute, Saint Petersburg (LE) under the collection number LE F-342520.

Figure 1. The habitat of *Battarrea phalloides* revealed in Gunibsky district, Republic of Dagestan. Photo S.V. Volobuev
Рисунок 1. Местообитание *Battarrea phalloides*, выявленное в Гунибском районе Республики Дагестан. Фото С.В. Волобуева

Identification of specimen was performed following Pegler et al. [11].

The description of macromorphological characteristics is based on the study a single basidioma of *Battarrea phalloides* collected by the authors. Microscopic features were studied by light microscopy technique using a LOMO Mikmed-6 microscope (Russia) and a Carl Zeiss AxioScope A1 microscope (Germany) with magnification up to 1000× and an AxioCam MRc5 digital camera (Germany). Small pieces of gleba were mounted in a 5% aqueous solution of KOH. A total of 30 basidiospores with ornamentation were measured. The Q value means an average length/width ratio of all measured individual basidiospores.

SPECIMENS STUDIED: *Battarrea phalloides* (Dicks.) Pers. — Russian Soviet Federative Socialist Republic, Terskaya Guberniya, Kizlyarskii Uezd, on the road from Bolshaya Areshevka to Marenovka [current locality – Russia, Republic of Dagestan, Kizlyarsky District, vicinity of Novaya Serebryakovka], on solonchak, 18.10.1922, coll. and det. A. I. Lobik (LE 1983, as *Battarrea stevenii* (Libosch.) Fr.) (Fig. 2). — Russian Soviet Federative Socialist Republic, Terskaya Guberniya, Kizlyarskii Uezd, on the road between Bolshaya Areshevka and Marenovka [current locality – Russia, Republic of Dagestan, Kizlyarsky District, vicinity of Novaya Serebryakovka], on solonchak, 18.10.1922, coll. and det. A. I. Lobik (LE 1984, as *Battarrea stevenii* (Libosch.) Fr.). — Russia, Republic of Dagestan, Gunibsky District, northern bank of the Bakdakuli river, north-east-facing slope, in 6 km from Salta settlement along the road to Gunibskaya hydroelectric power station, 42.42513° N, 47.00704° E, 940 m a.s.l., scrubland (*Berberis*, *Cornus australis*, *Hippophaë rhamnoides*, *Prunus cerasifera*, *Rosa*), on soil, 23.09.2022, coll. and det. S. V. Volobuev and N. V. Shakhova (LE F-342520).

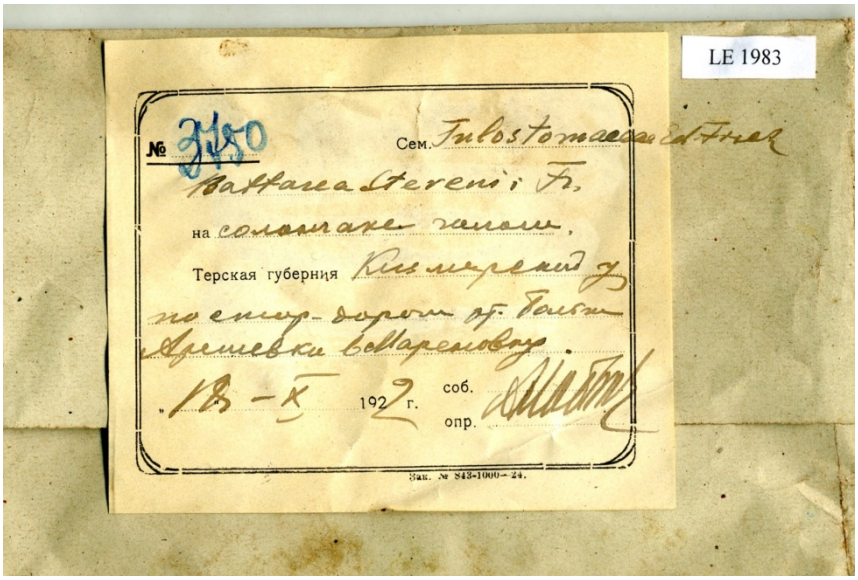

Figure 2. Herbarium envelope with the specimen of *Battarrea stevenii* (LE 1983) collected by A.I. Lobik in 1922
Рисунок 2. Гербарный конверт с образцом *Battarrea stevenii* (LE 1983), собранным А.И. Лобиком в 1922 году

DNA extraction, amplification, and sequencing were performed according to the procedures described in Volobuev and Shakhova [12]. Additionally, 38 sequences of *B. phalloides* and two sequences of *Phellorinia herculeana* (an outgroup) were retrieved from GenBank [13]. A total of 41 ITS sequences were aligned with a MAFFT version 7 web tool [14–15] using the E-INS-i option. Maximum Likelihood (ML) analysis was performed in an IQ-TREE Web Server [16] with 1000 ultrafast bootstrap replicates. Phylogenetic tree was visualized using FigTree 1.4.0 and edited using Adobe Illustrator CS5.1.

RESULTS

Based on macro- and micromorphological analyses, the gasteroid fungus *Battarrea phalloides* has been identified (Fig. 3). The description of Dagestanian specimen is presented below.

Battarrea phalloides (Dicks.) Pers., Syn. Meth. Fung. (Göttingen) 1: XIV, 129 (1801).

Basionym: *Lycoperdon phalloides* Dicks., Fasc. Pl. Crypt. Brit. (London) 1: 24 (1785).

BASIDIOMA 40 cm high, single, mature, epigeous, consisting of a long stipe with an apical spore sac (Fig. 3, A).

STIPE 37 cm high, dry, greyish brown with shaggy surface, covered by cinnamon-brown spore mass, curved to banana-shaped, swollen in a middle part to 3.5 cm, narrowed toward both the apex to 0.8 cm and the base to 2.5 cm, rigid with fibrous texture, hollow inside, with a bunch of white, shiny, silky, transparent hyphae.

EXOPERIDIUM brownish-cream, covered by brown spore mass, membranous, buried in soil and forming a cup-shaped volva at the base of the stipe.

ENDOPERIDIUM straw-whitish, thin, papyraceous.

GLEBA 8.5 cm in diameter and 2.5 cm high, convex to hemispherical, exposed, powdery, rusty-brown.

CAPILLITIUM of hyaline, thin-walled, smooth, clamped hyphae up to 5 µm wide.

ELATERS 7.0–9.8 µm wide, worm-shaped, beige to pale sandy brown, with smooth, refractive, spiral thickened walls (Fig. 3, B).

BASIDIOSPORES 5.2–7.4 × 4.9–6.9 µm (Q = 0.93 – 1.23, Q_{mean} = 1.06) with ornamentation, globose, subglobose to broad-ellipsoid, yellowish-brown, thick-walled, densely verruculose, with tiny isolated warts (Fig. 3, C).

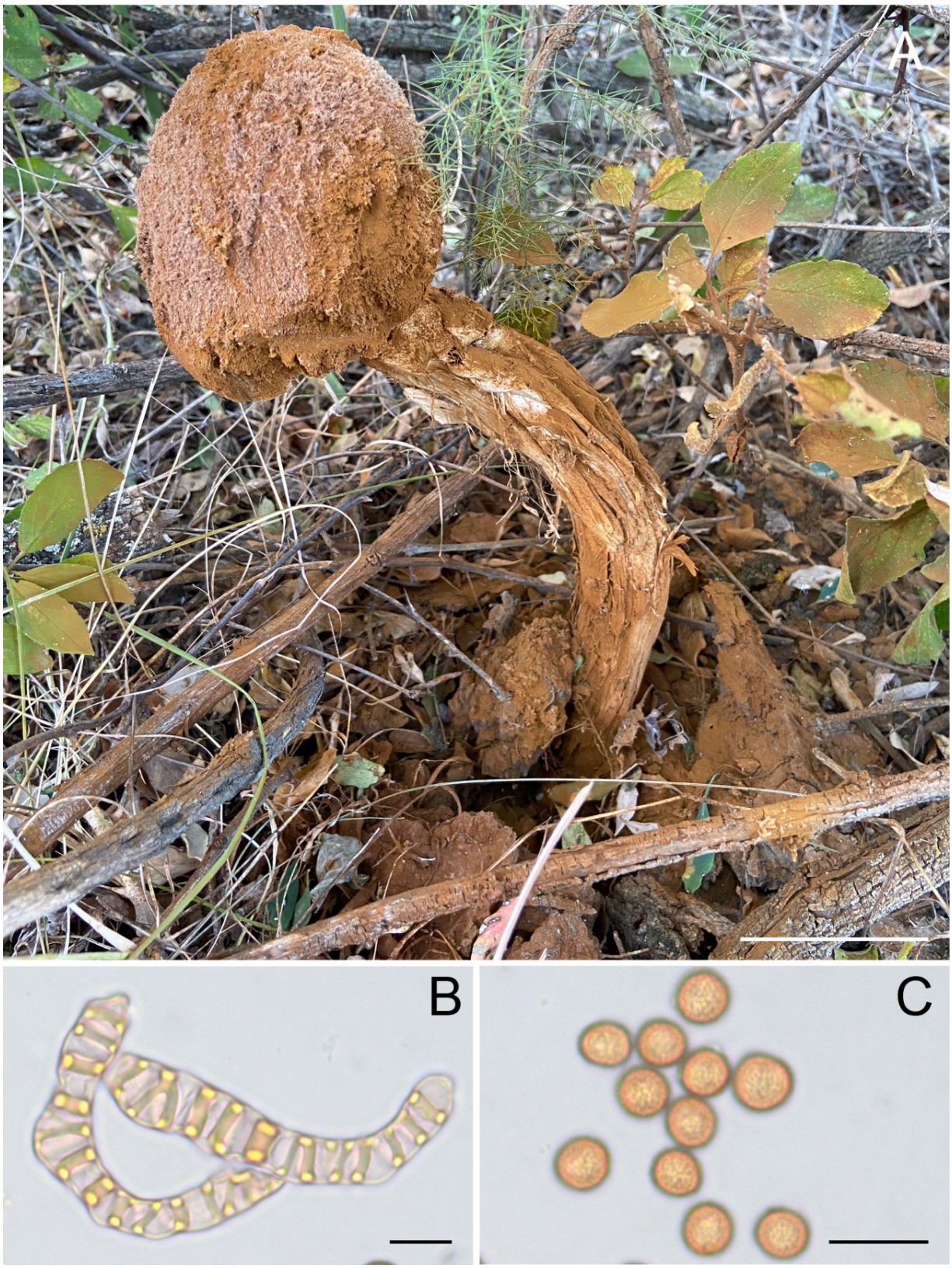

Figure 3. Macro- and micromorphological features of *Battarrea phalloides* basidioma:
A – basidioma, scale – 5 cm, B – elaters, C – basidiospores, scale – 10 µm. Photo S.V. Volobuev
Рисунок 3. Макро- и микроморфологические особенности базидиомы *Battarrea phalloides*:
А – базидиома, масштаб – 5 см, В – элатеры, С – базидиоспоры, масштаб – 10 мкм. Фото С.В. Волобуева

The complete ITS1–5.8S–ITS2 nrDNA sequence of *Battarrea phalloides* has been generated for this study and deposited in the GenBank Nucleotide database (GenBank accession number – OQ581869). The final dataset consisted of 41 ITS specimens belonging to *Battarrea phalloides* (39 sequences from 16 countries of Africa, Asia, Europe, North America, and Oceania) and *Phellorinia herculeana* (two sequences). The MAFFT alignment was 942 bp in length.

ML analisys resulted in the tree rooted with *Phellorinia herculeana* (Fig. 4). Three main strongly

supported clades (A, B, C) are corresponded to previous studies on intraspecific phylogenetic structure of *Battarrea phalloides* [17–20]. Our specimen of *B. phalloides* is nested within clade B, but as a new separate subclade B3, sister to two earlier distinguished subclades – subclade B1 formed by specimens from Spain and a well-supported subclade B2 combined specimens from Burundi, Cyprus, India, Israel, and the United Kingdom.

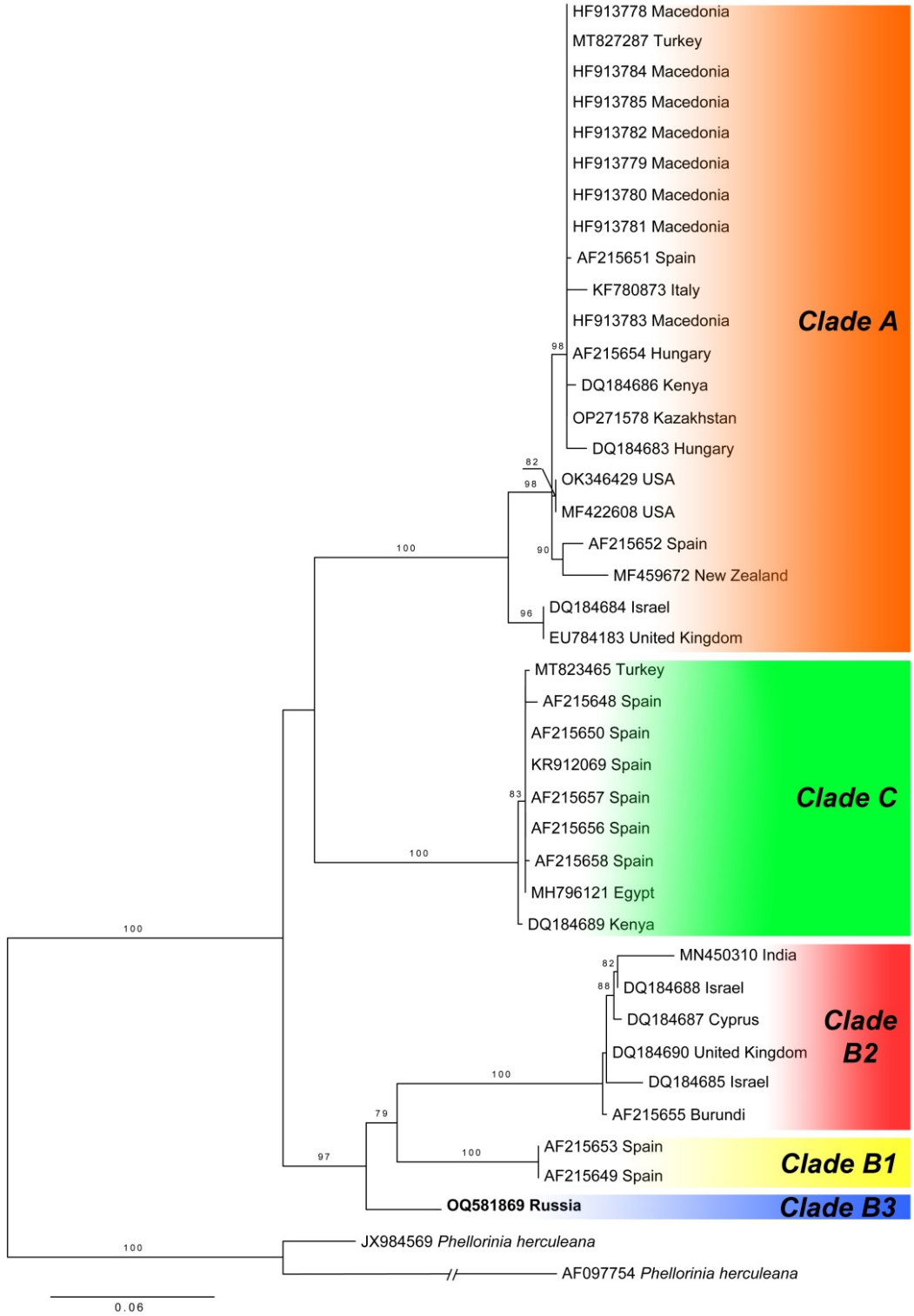

Figure 4. The Maximum Likelihood tree illustrating the phylogeny of *Battarrea phalloides*, based on ITS nrDNA sequences. Ultrafast bootstrap values (%) not less than 75 are shown above the branches. Country name of specimen originated is indicated after the sequence accession number (GenBank). The bold font shows the sequence obtained in this study. Clade names (A, B and C) are corresponding to Martín and Johannesson [17]

Рисунок 4. Дерево, построенное методом максимального правдоподобия и иллюстрирующее филогению *Battarrea phalloides*, на основе ITS-последовательностей ярДНК. Значения ultrafast bootstrap (не менее 75%) показаны над ветвями. Название страны происхождения образца указано после номера последовательности (в базе данных GenBank). Жирным шрифтом выделена последовательность, полученная в данном исследовании. Названия клад (A, B и C) даны по Martín и Johannesson [17]

DISCUSSION

The gasteroid fungus *Battarrea phalloides* is included to the Red Data Book of the Republic of Dagestan [10] as a thermophilic saprotrophic species that grows on a variety of soil types – in steppes (including mountain steppes) and deserts, on areas with sparse vegetation, less frequently in dry forests on litter and strongly destroyed humified wood. The main limiting factor for the species in the region is the restriction of available habitats. Our collection of *B. phalloides* from Gunibsky District cited above is the first modern occurrence of the species in Dagestan. Surprisingly, GBIF database contains the information on additional human observation of this fungus at site close to our collection. Basidioma of *B. phalloides* was registered on 11.08.2022 in Gunibsky district – 42.437554° N, 47.011427° E, on the eastern bank of the Bakdakuli river, in 2.5 km from Gunibskaya hydroelectric power station along the Gunib–Levashi–Buynaksk–Makhachkala road [21]. Both findings of the rare and protected species *B. phalloides* from the inner-mountain part of Dagestan significantly expand the known range of the species in the region. In addition, previous herbarium specimens of this species were collected 100 years ago by A. I. Lobik in the plain part of Dagestan in Kizlyarsky district (Fig. 2).

B. phalloides is distributed in steppe and desert ecosystems all over the world [22]. According to Ivančević et al. [23] the species is recorded in 64 countries on all continents except Antarctica. At the same time, the fungus demonstrates selectivity towards well-warmed habitats, and basidiomata appears at summer to late autumn, preferably on sandy soils of arid and semiarid regions from sea level up to 2.500 m high [24]. As far as the indicator value is concerned, the species indicates sunny dry places in humid areas [25]. Our record of *B. phalloides* in a well-heated and sunny north-east-facing slope of Inner-mountainous Dagestan (Eastern Caucasus) is corresponding to suitable habitat type for this species.

This fungus is included in conservation red-lists at regional or national levels in several countries (Austria, Bulgaria, Czech Republic, France, Germany, Hungary, North Macedonia, Poland, Romania, Russia, Serbia, Slovakia, Spain, and the UK) [25]. Moreover, the species is considered as a candidate for IUCN red-listing under the IUCN Global Fungal Red List Initiative [26]. In the Russian regions adjacent to the Republic of Dagestan, the species is also protected in the Republic of Kalmykia [27].

On the other hand, there currently remains a deficiency of sufficient knowledge on the distribution, occurrence and population trends at the global level, which determines the need for ecological research on the species [28].

The contradictions associated with the ecological preferences of *B. phalloides* are that the species occurs not only in nature, but also in ruderal and anthropogenically transformed habitats, for instance, in disturbed pastures. In addition, based on research carried out in Italy, it has been found that the fungal spores and mycelium are present in soils and soil for sale in flower shops and markets and are capable of developing fruiting bodies in flowerpots containing various ornamental plants (roses, geraniums, etc.) [28].

B. phalloides has a complex taxonomic history. Many independent taxa have been described from different regions and subsequently reduced to synonymy. At the same time, molecular phylogenetic analysis has shown the existence of cryptic species within the *B. phalloides* group [19].

In the ITS phylogeny our specimen from Dagestan has been placed in a separate subclade B3 within clade B previously differentiated in only two subclades [18–20]. The segregation of the new subclade seems likely to be due to the fact that the sequence from the Caucasian specimen was included in the phylogenetic analysis for the first time. Meanwhile, finely verrucose spore ornamentation has been noted for the newly sequenced specimen of *B. phalloides* and is common characteristic for all representatives of clade B [17].

CONCLUSION

The new *B. phalloides* finding recorded in Dagestan expanded the knowledge on the ecology and distribution of the species red-listed in the region. The phylogenetic analysis based on ITS nrDNA sequences allowed us to clarify the intraspecific structure of *B. phalloides* with a distinguishing of new subclade formed only by Caucasian collection. Further taxonomic investigations should be focused on engaging new specimens of *B. phalloides* with different geographical origin as well as more representative datasets from the known localities.

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AUTHOR CONTRIBUTIONS

Both authors collected the material, discussed the results and participated in the writing of the manuscript. Sergey V. Volobuev carried out the microscopic identification of species and the molecular study of the specimen. Both authors are equally responsible for plagiarism, self-plagiarism and other ethical transgressions.

КРИТЕРИИ АВТОРСТВА

Оба автора участвовали в сборе материала, обсуждении результатов и написании рукописи. Сергей В. Волобуев выполнил микроскопическую идентификацию видов и молекулярно-генетическое изучение собранного образца. Оба автора в равной степени несут ответственность за плагиат, самоплагиат и другие неэтические проблемы.

NO CONFLICT OF INTEREST DECLARATION

The authors declare no conflict of interest.

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Авторы заявляют об отсутствии конфликта интересов.

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