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Chlorophyllum sphaerosporum (Agaricales, Basidiomycota) – the second observation worldwide

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Summary. A specimen of *Chlorophyllum sphaerosporum* was collected in the Republic of Altai, Russia, and was confirmed both by morphology and nrITS sequence analysis. The record represents the first finding of this species in Russia and the second observation worldwide. The paper provides morphological description, ecological features of *Chlorophyllum sphaerosporum* and presents molecular phylogenetic analysis of sequenced specimen. Comparisons were made with the type specimen and morphologically similar species.

Chlorophyllum sphaerosporum (Agaricales, Basidiomycota) – вторая находка в мире

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Ключевые слова: Западная Сибирь, микобиота, Республика Алтай, Россия, фунга, Agaricaceae, nrITS.

Аннотация. Образцы, собранные в России на территории Республики Алтай, были идентифицированы как *Chlorophyllum sphaerosporum* на основе морфологии и анализа последовательностей nrITS. Это первая регистрация вида в России и вторая находка в мире. В статье представлены морфологические и экологические особенности *Chlorophyllum sphaerosporum*, а также проведен молекулярно-филогенетический анализ секвенированного образца. Сравнения проводились с типовым экземпляром и морфологически близкими видами.

Introduction

The genus *Chlorophyllum* Massee (Agaricaceae, Agaricales) has complex systematics (Vellinga, 2002, 2004; Vellinga, Kok, 2002; Vellinga et al., 2003; Ge et al., 2018; Dutta at al., 2020; Loizides et al., 2020) and remains insufficiently studied worldwide. Modern phylogenetic results confirm the presence of about 19 species within the genus (Loizides et al., 2020), which are characterized by various types of basidiomes – agaricoid, secotioid, and gasteroid, with scaly caps, smooth stems (if present), and basidiospores of different colors with or without germ pore. Species of this genus are saprotrophs anddistribu-

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ted worldwide. Recent taxonomic and phylogenetic studies of the genus *Chlorophyllum* (Ge et al., 2018) have revealed six well-supported clades (*Chlorophyllum, Ellipsoidospororum, Endoptychorum, Rhacodium, Parvispororum*, and *Sphaerospororum*), each includes from one to four species. In this study, the section *Sphaerospororum* Z. W. Ge (Ge et al., 2018) included two species with gasteroid fruiting bodies and one agaricoid species, *Chlorophyllum sphaerosporum* Z. W. Ge and Zhu L. Yang, described from northern China (Ge, Yang, 2006). M. Loizides with coauthors (Loizides et al., 2020) revised the descriptions of the sections *Chlorophyllum*, *Endoptychorum* and *Sphaerospororum*. According to this work, the section *Sphaerospororum* now includes four species, three of which have angiocarpic basidiomes: *Chlorophyllum levantinum* Loizides, P. Alvarado et Konstantinou, *C. lusitanicum* G. Moreno, Muñ.-Moh., Manjón, Carlavilla et Altés., *C. arizonicum* (Shear et Griffiths) G. Moreno et Altés, and *C. sphaerosporum* – a species with an agaricoid phenotype. These species share several common features: pale-colored basidiomes, flesh that does not redden upon injury and colorless, generally spherical spores with thick walls lacking a germ pore. Until now, *C. sphaerosporum* has only been recorded in Northern China, and no other locations of this species have been reported worldwide (Ge, Yang, 2006). The authors explained this by the ecological characteristics of this species. Its habitats in China include forests dominated by *Xantoceras* Bunge and *Keteleeria* Carrière, distributed exclusively in Inner Mongolia region in China.

Morphological and molecular studies of specimens collected in the Republic of Altai confirmed the first record of *Chlorophyllum sphaerosporum* in Russia. The paper provides morphological description, ecological features and presents molecular phylogenetic analysis of sequenced specimen. Comparisons were made with the type specimen and morphologically similar species.

Material and methods

The material consisted of specimens of the genus *Chlorophyllum* collected in steppe vegetation communities of the Republic of Altai in August 2011, near the village of Kulada (1010 m above sea level) in the territory of the Ongudai district. Samples were photographed, described, and dried using standard methods (Clémençon, 2009). Morphological description of basidiomes was based on the study of fresh and dried material. Dried specimens and a standard set of reagents (5 % KOH, 1 % Congo Red solution in NH_4OH, Melzer's reagent) were used for microstructure examination under a Zeiss AxioStar plus microscope. Spore dimensions were calculated for 26 basidiospores and are given as minimum minus maximum of length × minimum minus maximum of width (average values). Q is given as minimum minus maximum of ratio length/width (average values). The voucher specimen stored at the M. G. Popov Herbarium of the Central Siberian Botanical Garden SB RAS (NSK, Novosibirsk) and the duplicate stored at the Fungarium of Yugra State University (YSU, Khanty-Mansiysk). Microstructure photographs were taken at the Laboratory of the Yugra State University and the plates were created using InkScape software (https://inkscape.org/). Information about the specimens has been entered into the Specify collection information system (http://specify.ugrasu.ru). Collection data is available for searching and downloading, including specimen photographs and obtained sequences, through GBIF (Gorbunova, Filippova, 2024).

The PCR was made using the TransDirect® Plant Tissue PCR Kit without DNA extraction. For amplification of the ITS region the primers ITS1-F (Gardes, Bruns, 1993) and ITS4 (White et al., 1990) were used. PCR and sequence reaction products were purified using CleanMag DNA (Evrogen, Russia) and Dynabeads™ Sequencing Clean-Up kits. Sequencing was performed with BrilliantDye™ Terminator (v3.1) Cycle Sequencing kit (NimaGen, Netherlands) using Applied Biosystems® Sanger Sequencing 3500 Series Genetic Analyzer.

Raw data were edited and assembled using Molecular Evolutionary Genetics Analysis Version 11 (MEGA11) software (Koichiro et al., 2021). Newly generated sequences were deposited in the GenBank. Additionally, 48 ITS sequences were retrieved from GenBank (including 22 sequences from the section *Sphaerospororum*) (Table 1). Sequences were aligned with the MAFFT version 7 web tool (Kazutaka et al., 2019) using the E-INS-i option. We chose E-INS-I option to improve of progressive alignment by the iterative refinement method (according to MAFFT algorithms and parameters guide: https://mafft.cbrc.jp/alignment/software/algorithms/algorithms.html#GLE). The best-fit substitution model (HKY+F+G4) was estimated based on the Bayesian Information Criterion (BIC) using ModelFinder (Kalyaanamoorthy et al., 2017). Maximum likelihood (ML) phylogenetic reconstruction was performed in IQTREE 1.6.12 (Nguyen et al., 2015). The support was tested by 1000 ultrafast bootstraps (BS) (Hoang et al., 2017), as well as the Shimodaira-Hasegawalike approximate likelihood-ratio test (Guindon et al., 2010). BS consensus tree was constructed from 1000 bootstrap trees (Nguyen et al., 2015). The final technical formatting of the resulting tree was made in FigTree (Rambaut, 2008–2016) and InkScape (https://inkscape.org) software. Similarity percentage of the sequences was calculated in Basic Local Alignment Search Tool (BLAST) at NCBI portal (https://blast.ncbi.nlm.nih.gov/Blast.cgi) (Altschul et al., 1990).

Table 1

The dataset of all sequences of the section *Sphaerospororum* included in the molecular phylogenetic analysis

Species	*Accession number*	*Country*	*Reference*
C. arizonicum	MN161875	USA	Loizides et al., 2020
C. arizonicum	KR233490	Mexico	Unpublished
C. arizonicum	MN161873	USA	Loizides et al., 2020
C. arizonicum HOLOTYPE	NR171848	USA	Loizides et al., 2020
C. arizonicum	MN161876	USA	Loizides et al., 2020
C. arizonicum	MN161874	USA	Loizides et al., 2020
C. levantinum HOLOTYPE	NR169974	Cyprus	Loizides et al., 2020
C. levantinum	MN161878	Cyprus	Loizides et al., 2020
C. levantinum	MN161879	Cyprus	Loizides et al., 2020
C. lusitanicum HOLOTYPE	NR158317	Spain	Crous et al., 2015
C. lusitanicum	MN161880	Greece	Loizides et al., 2020
C. lusitanicum	KR233482	Spain	Crous et al., 2015
C. lusitanicum	KR233483	Spain	Crous et al., 2015
C. lusitanicum	KR233484	Spain	Crous et al., 2015
C. lusitanicum	KY828146	Spain	Unpublished
C. lusitanicum	KY828147	Spain	Unpublished
C. lusitanicum	KY828148	Spain	Unpublished
C. lusitanicum	MH368354	Spain	Carlavilla, Moreno, 2018
C. sphaerosporum	PP277302	Russia	This study
C. sphaerosporum HOLOTYPE	MG742011	China	Ge et al., 2018
C. sphaerosporum	MG742010	China	Ge et al., 2018
C. sphaerosporum	MG742012	China	Ge et al., 2018

Results and discussions

Molecular phylogeny

The final nrITS dataset contained 49 sequences, including one sequence generated in this study, 22 sequences from *Sphaerospororum* section representing all available sequences in GenBank (Table 1). Other 26 sequences were added to the analysis (2–3 sequences by each species) to show general topology of other five sections (following Ge et al., 2018). Molecular phylogenetic analysis revealed that the sequence of our specimen nested in *C. sphaerosporum* clade with a holotype and other 3 specimens (Fig. 1). The topology of other sections generally coincided with previous phylogenetic reconstruction of the genus (Ge et al., 2018). The difference with holotype specimen was 1 substitution (99.4 %), while the percentage similarity reached 100 % with other sequences.

Morphological description

Chlorophyllum sphaerosporum Z. W. Ge et Zhu L. Yang, 2006, Mycotaxon 96: 187 (Fig. 2, 3).

Basidiomes agaricoid, pileus 45–55 mm in diameter, convex to plane, with a flat umbo, whitish, with a chocolate-brown central disc and beige-brown appressed or weakly elevated scales throughout the surface; margin uneven, fringed, sometimes torn. Gills free, broad, edge even, frequent, white to yellow-cream when dry. Stipe central, 45–70 × 4–7 mm, cylindrical, slightly enlarged at base, whitish, not changing color upon handling but acquires a lilac hue when dry. Annulus membranous, whitish, movable, compound. Context, unchanging, without any taste or odor. Spore print whitish.

Basidiospores 8.6–12(10) × 7–10.7(8.4) μm, Q 1.1–1.3(1.1) (n = 26 spores); spherical to broadly ellipsoidal, smooth, hyaline, thick-walled (up to 0.3 μm), without germ pore, dextrinoid, apiculus up to 0.8 μm long. Basidia 17–25(20) × 9–10(9.6) μm, clavate, hyaline, thin-walled, 4-spored, rarely 2-spored, sterigmata up to 4 μm long. Pleurocystidia absent. Cheilocystidia 16.8–2(20) × 8.6–12.4(9.7) μm, from clavate to broadly clavate, hyaline, thin-walled. Lamellar trama made by colorless hyphae ranging from 5 to 15 μm in width. Pileipellis hymenodermatous, made by interwoven hyphae and clavate or broadly clavate terminal cells measuring 38–52(43) × 6–9(7) μm. Clamp connections are rare.

Specimen examined: "Russia, Republic of Altai, Ongudai district, vicinity of Kulada village, 50°49'11"N, 85°55'32"E, 1010 m above sea level, in herbaceous steppe, on soil. 7 VIII 2011. Collected by Gorbunova" (NSK 1009543, YSU-F-13811, GenBank PP277302).

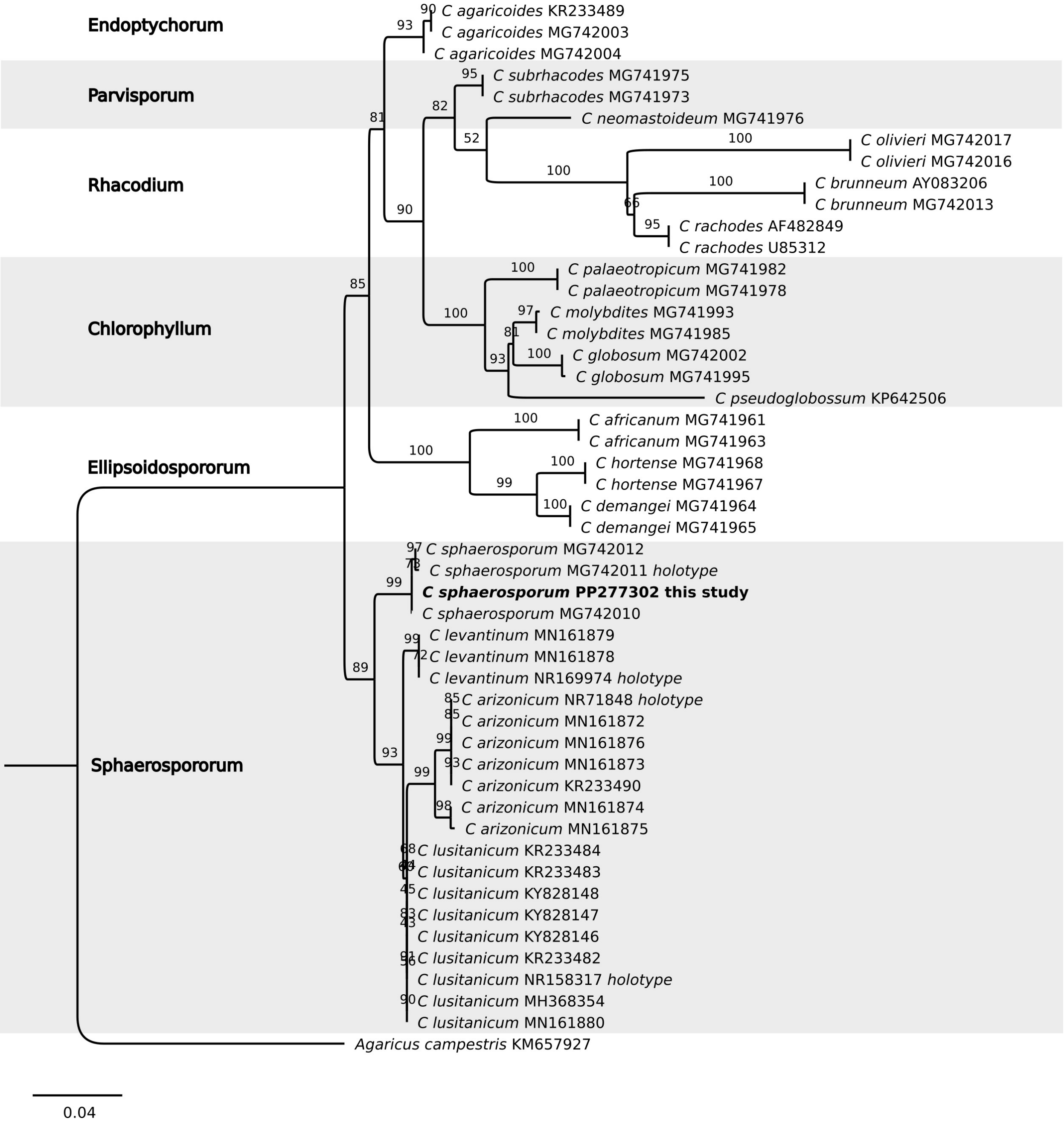

Fig. 1. Maximum Likelihood best tree of selected ITS sequences of *Chlorophyllum* obtained by analysis in IQTREE 1.6.12, the bootstrap percentage is provided above the branches.

Fig. 2. Habitat (A) and macro-morphological features (B) of the new observation of *Chlorophyllum sphaerosporum* in the Republic of Altai, Russia.

Discussion

The genus *Chlorophyllym* includes species with agaricoid, secotioid and gasteroid habitus. *C. sphaerosporum* differs from other agaricoid species of the genus by spherical or nearly spherical thick-walled basidiospores without a germ pore, and by pileipellis of hymenoderm type consisting of loosely arranged club-shaped elements.

Before present, only two representatives of the genus *Chlorophyllum* were found in the Republic of Altai – *C. agaricoides* (Czern.) Vellinga (*Endoptychorum*), widely distributed in various steppe communities, and *C. rhacodes* (Vittad.) Vellinga (*Rhacodium*), more commonly found in forest communities throughout Russia (Perova, Gorbunova, 2001; Bolshakov et al., 2021). A morphologically similar species *Macrolepiota campestris* Lebedeva ex Samgina was previously noted in the Altai Territory and Kazakhstan (Samgina, 1985). However, despite its external similarity to *C. sphaerosporum*, it has round basidiospores with a germ pore.

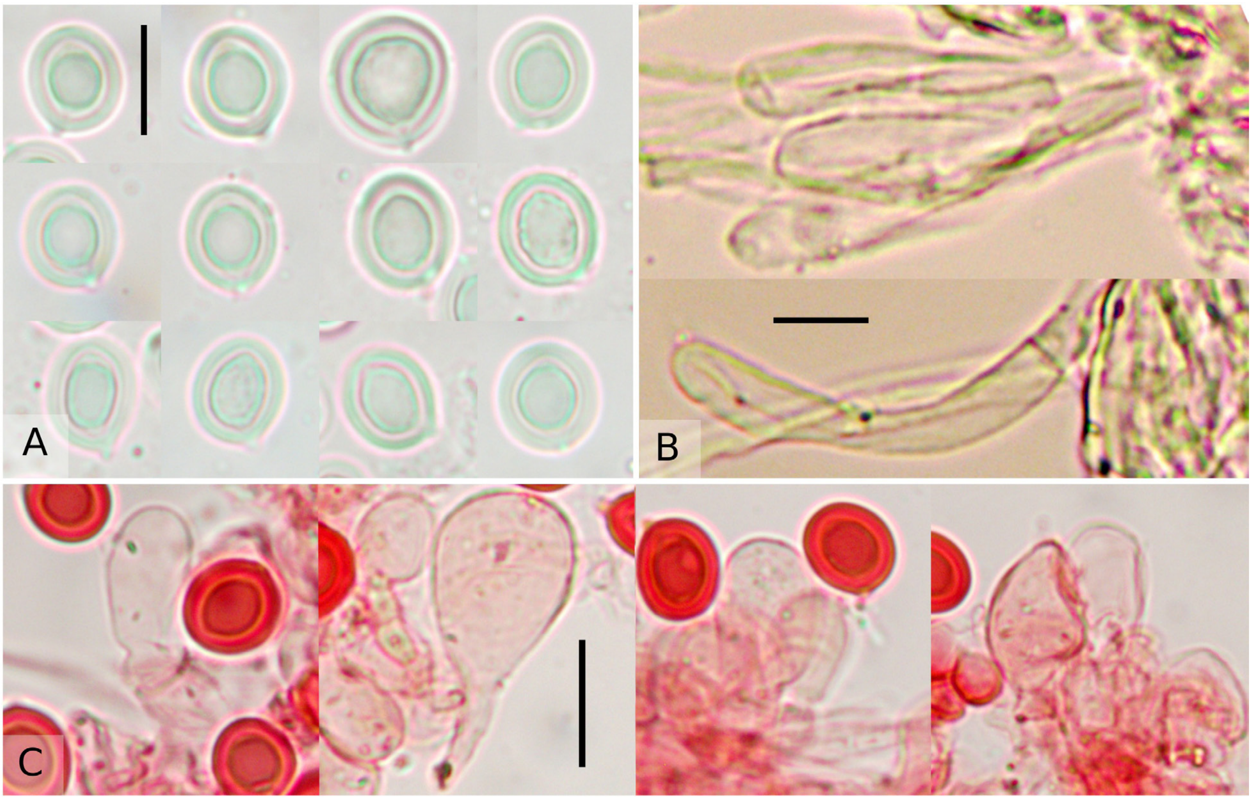

Fig. 3. Micromorphological features of the new observation of *Chlorophyllum sphaerosporum* in the Republic of Altai, Russia: A – basidiospores (KOH); B – terminal elements of pileipellis (KOH); C – cheilocystidia (KOH, stained in Congo Red) (all bars = 10 µm).

The specimen of *C. sphaerosporum* from the Republic of Altai closely resembles the type description (Ge, Yang, 2006), characterized by small, thick-walled, nearly spherical, dextrinoid basidiospores without a germ pore, whitish context not changing color, and hymenidermal pileipellis made by brownish clavate elements.

The studied specimen differed from the description of *C. sphaerosporum* by occasional 2-spored basidia, and correspondingly a few larger spores. The elements of the pileipellis were narrower compared to the original description, which may be related to the age of our specimen and the absence of well-preserved tissues at the time of study. As noted by Ge and Yang (2006), *C. sphaerosporum* resembles *C. hortense* but differs by stipe context not becoming red, more spherical spores, and having 4-spored basidia. All of these characteristics were observed in our case except for the basidia (we also observed 2-spored ones). Molecular analysis showed a close similarity between our specimen and the type specimen of *C. sphaerosporum*, while *C. hortense* was placed in a different clade. The authors of the species (Ge, Yang, 2006) also noted a close resemblance of *C. sphaerosporum* to two taxa described from India (*C. shimogaense* and *C. bharatense*). However, the comparison with these species is currently not possible due to the unavailability of the specimens and the absence of sequence data.

The collection of *C. sphaerosporum* in the Republic of Altai was relatively close to the previously known distribution range of the species in Inner Mongolia, China (about 2000 km from our observation).

The habitat of the new record differs from that of the earlier known records. While the type specimen was collected in mountainous forests dominated by *Keteleeria* and *Xantoceras* (Ge, Yang, 2006), in the Republic of Altai the species was found in mountain steppe with admixture of shrubs (*Rhododendron dauricum* L., *Caragana* sp., and *Spiraea alpina* Pall.). The new discovery expands the species distribution range to the northwest and ecological preferences in the Central Asian Mountain province. The majority of *Chlorophyllum* species, including *Sphaerospororum* section, occurs in disturbed or arid habitats (Ge et al., 2018).

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REFERENCES / ЛИТЕРАТУРА

Altschul S. F., Gish W., Miller W., Myers E. W., Lipman D. J. 1990. Basic Local Alignment Search Tool. *J. Mol. Biol.* 215 (3): 403–410. DOI: 10.1016/S0022-2836(05)80360-2

Bolshakov S., Kalinina L., Palomozhnykh E., Potapov K., Ageyev D., Arslanov S., Filippova N., Palamarchuk M., Tomchin D., Voronina E. 2021. Agaricoid and boletoid fungi of Russia: the modern country-scale checklist of scientific names based on literature data. *Biol. Commun.* 66(4): 316–325. DOI: 10.21638/spbu03.2021.404

Carlavilla J. R., Moreno G., Mohedano J. M. 2018. *Chlorophyllum lusitanicum* a rare species from the Iberian Peninsula. *Boletín de la Sociedad Micológica de Madrid* 42: 99–105.

Clémençon H. 2009. *Methods for working with macrofungi. Laboratory cultivation and preparation of large fungi for light microscopy*. Eching: IHW-Verlag. 88 pp.

Crous P. W., Wingfield M. J., Roux J. J. Le, Richardson D. M., Strasberg D., Shivas R. G., et al. 2015. Fungal Planet description sheets: 371–399. *Persoonia – Molecular Phylogeny and Evolution of Fungi* 35(64): 264–327. DOI: 10.3767/003158515X690269

Dutta A. K., Bera S., Paloi S., Rakshit S., Tarafder E., Sherpa A. R., Acharya K. 2020. Lepiotaceous fungi of West Bengal, India: The genus *Chlorophyllum*. *Phytotaxa* 451: 113–131. DOI: 10.11646/phytotaxa.451.2.2

Gardes M., Bruns T. D. 1993. ITS primers with enhanced specificity for basidiomycetes application to the identification of mycorrhizae and rusts. *Molec. Ecol.* 2: 132–118. DOI: 10.1111/j.1365-294x.1993.tb00005.x

Ge Z. W., Jacobs A., Vellinga E. C., Sysouphanthong P., Walt R., Lavorato C., An Y. F., Yang Z. L. 2018. A multigene phylogeny of *Chlorophyllum* (Agaricaceae, Basidiomycota): New species, new combination and infrageneric classification. *MycoKeys* 32: 65–90. DOI: 10.3897/mycokeys.32.23831

Ge Z., Yang Z. L. 2006. The genus *Chlorophyllum* (Basidiomycetes) in China. *Mycotaxon* 96: 181–92.

Gorbunova I., Filippova N. 2024. *Fungarium of Gorbunova Irina A.* (Central Siberian Botanical Garden, NSK). Yugra State University Biological Collection (YSU BC). Occurrence dataset DOI: 10.15468/upme2c

Guindon S., Dufayard J.F., Lefort V., Anisimova M., Hordijk W., Gascuel O. 2010. New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. *Syst. Biol.* 59: 307–321.

Hoang D. T., Chernomor O., Haeseler A. V., Minh B. Q., Vinh L. S. 2017. UFBoot2: Improving the ultrafast bootstrap approximation. *Mol. Biol. Evol.* 35(2): 518–522. DOI: 10.1093/molbev/msx281

Kalyaanamoorthy S., Minh B. Q., Wong T. K. F., von Haeseler A., Jermiin L. S. 2017. ModelFinder: Fast model selection for accurate phylogenetic estimates. *Nat. Methods* 14(6): 587–589. DOI: 10.1038/nmeth.4285

Kazutaka K., John R., Kazunori D. Y. 2019. MAFFT online service: multiple sequence alignment, interactive sequence choice and visualization. *Brief. Bioinform.* 20(4): 1160–1166. DOI: 10.1093/bib/bbx108

Koichiro T., Glen S., Sudhir K. 2021. MEGA11: Molecular Evolutionary Genetics Analysis Version 11. *Mol. Biol. Evol.* 38(7): 3022–3027. DOI: 10.1093/molbev/msab120

Nguyen L. T., Schmidt H. A., Haeseler A. V., Minh B. Q. 2015. IQ-TREE: A fast and effective stochastic algorithm for estimating maximum likelihood phylogenies. *Mol. Biol. Evol.* 32: 268–274. DOI: 10.1093/molbev/msu300

Perova N. V., Gorbunova I. A. 2001. *Makromitsety yuga Zapadnoy Sibiri* [*Macromycetes of south of the Western Siberia*]. Novosibirsk: Publishing house SB RAS. 158 pp. [In Russian] (***Перова Н. В., Горбунова И. А.*** Макромицеты юга Западной Сибири. Новосибирск: Изд-во СО РАН, 2001. 158 с.).

Rambaut A. 2006–2018. FigTree v1.4.4. URL: https://github.com/rambaut/figtree/releases/tag/v1.4.4 (Accessed 26 May 2024).

Samgina D. I. 1985. *Flora sporovych rasteniy Kazakhstana. T. XIII, kn. 2. Agaricovyye griby* [*Flora of spore plants of Kazakhstan. Vol. XIII, b. 2. Agaricales*]. Alma-Ata: Nauka. 272 pp. [In Russian] (***Самгина Д. И.*** Флора споровых растений Казахстана. Т. XIII, кн. 2. Агариковые грибы (Agaricales). Алма-Ата: Наука, 1985. 272 с.).

Vellinga E. C. 2002. New combinations in *Chlorophyllum*. *Mycotaxon* 83: 415–417.

Vellinga E. C. 2004. Genera in the family Agaricaceae – Evidence from nrITS and nrLSU sequences. *Mycol. Res.* 108: 354–377. DOI: 10.1017/S0953756204009700

Vellinga E.C., De Kok R. P. J. 2002. Proposal to conserve the name *Chlorophyllum* Massee against *Endoptychum* Czern. (Agaricaceae). *Taxon* 51: 563–564. DOI: 10.2307/1554876

Vellinga E. C., De Kok R. P. J., Bruns T. D. 2003. Phylogeny and taxonomy of *Macrolepiota* (Agaricaceae). *Mycologia* 95(3): 442–456. DOI: 10.2307/3761886

White T. J., Bruns T., Lee S., Taylor J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: M. A. Innis, D. H. Gelfand, J. J. Sninsky, T. J. White (eds.). *PCR Protocols: a guide to methods and applications*. New York: Academic Press. Pp. 315–322.