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A study of type of *Pluteus inocybecystis* (Pluteaceae, Agaricales) described from Siberia and its synonimization under *Homophron spadiceum* (Psathyrellaceae, Agaricales)

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Keywords: Basidiomycota, ITS phylogeny, holotype, revision, taxonomy.

Summary. The type collection of *Pluteus inocybecystis* A. Petrov deposited in the Mycological herbarium of the Komarov Botanical Institute RAS (LE F) was re-examined. Our morphological observations have revealed that the specimen represents a species from the genus *Homophron*. Phylogenetically, the sequence of the nrDNA internal transcribed spacer (ITS) of the *P. inocybecystis* holotype is nested within *Homophron spadiceum* clade and matches ITS sequences of *H. spadiceum* with 99.8–100.0 % similarity. Thus, on the basis of morphological and molecular studies, *Pluteus inocybecystis* is considered as a synonym of *Homophron spadiceum*.

Изучение типового образца вида *Pluteus inocybecystis* (Pluteaceae, Agaricales), описанного из Сибири, и сведение его в синонимы *Homophron spadiceum* (Psathyrellaceae, Agaricales)

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Ключевые слова: голотип, ревизия, таксономия, Basidiomycota, ITS филогения.

Аннотация. Проведено изучение типовой коллекции *Pluteus inocybecystis* A. Petrov, хранящейся в Микологическом гербарии Ботанического института им. В. Л. Комарова РАН (LE F). Наши морфологические наблюдения выявили, что образец представляет собой вид из рода *Homophron*. Филогенетический анализ показал, что ITS последовательность, полученная из голотипа *P. inocybecystis,* попадает в кладу *Homophron spadiceum* и совпадает с ITS последовательностями *H. spadiceum* на 99,8-100,0 %. Таким образом, на основании морфологических и молекулярных данных, *Pluteus inocybecystis* следует рассматривать как синоним *Homophron spadiceum*.

Introduction

The genus *Pluteus* Fr. is the largest genus in the Pluteaceae. It is characterized by basidiocarps without volva, free lamellae, pink or pinkish brown spore print, smooth, colourless and inamyloid basidiospores, and inverse hymenophoral trama. The species of this genus are widely distributed all over the

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world. They often grow on wood of deciduous and coniferous trees of various degrees of decomposition and soil. Currently, there are about 500 documented species of the genus *Pluteus* worldwide (He et al., 2019). However, some species still remain poorly known.

Pluteus inocybecystis A. Petrov was originally described from the southeastern Siberia (Petrov, 1983). The author pointed out *P. inocybecystis* had "sporae elongate-ellipticae ad subcilindricas, saepe inaequilaterales vel ad apicem angustatae" (spores elongate-elliptical and subcylindrical, often unequal or tapered at the apex) and "cystidia fusiformia, metuloidea, apice excretionibus crystallicis notata; metuloidis *Inocybe* analoga" (cystidia fusiform, metuloid, apex with crystal excretions; analogous to metuloids of *Inocybe*). These characteristics are not typical for members of the genus *Pluteus*.

Subsequently, the taxon was mentioned in the literature, including the Red Data Books of Siberian regions – Irkutsk Region and Krasnoyarsk Territory (Petrov, 1986, 2008; Kutafyeva, Kosheleva, 2012; Petrov, 2020), but no additional morphological information was provided. The conflicting descriptions and illustrations given in the Red Data Books make the identity of the taxon even more confusing than the description given in the protologue.

Therefore, the aim of our study was to re-examine the holotype specimen of *P. inocybecystis* to resolve the problem of its taxonomic identity and provide new morphological and molecular data to confirm our conclusions.

Materials and methods

The *P. inocybecystis* holotype deposited in the Mycological herbarium of the Komarov Botanical Institute RAS (LE F) was studied. Standard methods for microscopic observations were used; material was revived in water (to prevent dissolving apical crystals on hymenial cystidia) or in a drop of 5 % KOH. Terminology follows Kits van Waveren (1985). A total of 30 basidiospores were measured. Basidiospore dimensions are given following the form (a)b–c(d), with b–c containing at least 90 % of all values and the extremes (a, d) enclosed in parentheses, and L_{av} and W_{av} are the mean value of length and width of the total basidiospores measured. Q indicates the basidiospore length/width ratio, Q_{av} represents the mean length/width quotient of the total basidiospores measured.

DNA isolation, PCR, and sequencing of ITS nrDNA followed the protocols published by Malysheva et al. (2023). Sequencing was performed with an ABI model 3500 Genetic Analyzer (Applied Biosystems, CA, USA). Raw data were edited and assembled in MEGA 11 (Tamura et al., 2021). Newly generated sequence was submitted to GenBank with corresponding accession number.

For phylogenetic analysis, in addition to newly generated, 11 ITS sequences of taxa from the genus *Homophron* (Britzelm.) Örstadius et E. Larss. and two sequences of *Lacrymaria lacrymabunda* (Bull.) Pat. (as an outgroup) were retrieved from the GenBank database (www.ncbi.nlm.nih.gov/genbank/) using the BLAST application and taxonomic considerations (Örstadius et al., 2015; Eberhardt et al., 2022). The taxonomic identities of these sequences and collection information for taxa are given in the phylogenetic tree (Fig. 1). The alignment was carried out in MAFFT vs. 7 (MAFFT version 7. URL: https://mafft.cbrc.jp/alignment/server/index.html) with the FFT-NS-i option and improved where necessary using MEGA11.

Phylogenetic reconstruction was performed with Maximum Likelihood (ML) analysis for ITS dataset. Before the analysis, the best-fit substitution model was estimated using FindModel web server (http://www.hiv.lanl.gov/content/sequence/findmodel/findmodel.html) under the Akaike information criterion. The HKY+G model was chosen. ML analysis was run on IQ-Tree web server (http://iqtree.cibiv.univie.ac.at/; Trifinopoulos et al., 2016) with standard settings and 1000 rapid bootstrap replicates. Branches with bootstrap support (BS) values greater than or equal to 70 % were considered significantly supported (Hillis, Bull, 1993). The tree was then edited and visualized in iTOL (Letunic, Bork, 2019).

All microscopic and molecular studies of specimens were carried out at the Center for collective use of scientific equipment "Cellular and molecular technology of studying plants and fungi" (Komarov Botanical Institute of the Russian Academy of Sciences, St. Petersburg).

Results

Phylogeny

The final alignment of ITS sequences for members of *Homophron* contained 640 characters, including gaps. The results of ML analysis are depicted in Fig. 1.

Four species of the genus *Homophron* involved into analysis form independent monophyletic clades with high statistical support. According to the phylogenetic analysis, ITS sequence from the *P. inocybecystis* holotype is nested within *Homophron spadiceum* s. str. clade (BS = 79 %) and matches sequences of *H. spadiceum*, including its epitype, with 99.8–100.0 % similarity (GenBank AM712288, AM712289, AM712290, DQ389729).

Thus, the type collection of *P. inocybecystis* studied here was resolved on the phylogenetic tree as *Homophron spadiceum*.

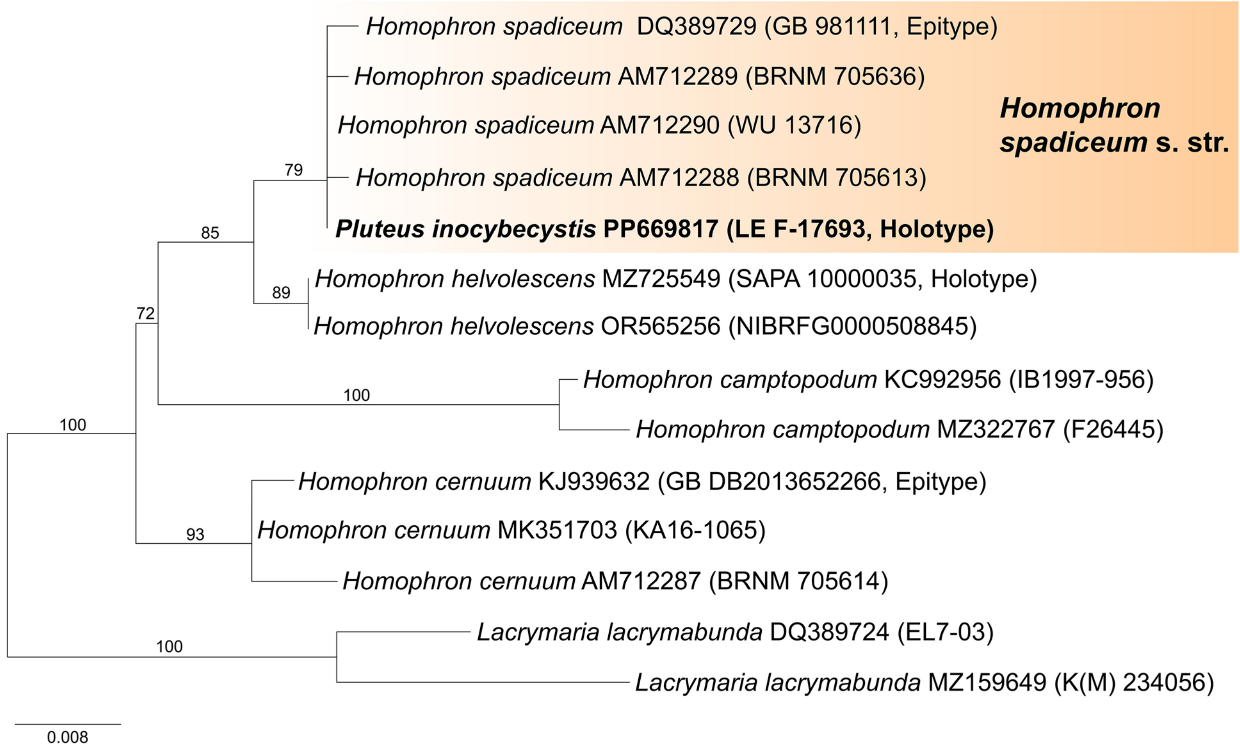


Fig. 1. Phylogenetic tree inferred from a Maximum Likelihood analysis of the ITS dataset for *Homophron* species, with *Lacrymaria lacrymabunda* as outgroup. Bootstrap support values (BS) are given above the branches. All tips are labelled with taxon name, GenBank accession number and voucher number. For taxon in bold the sequence is generated in present study.

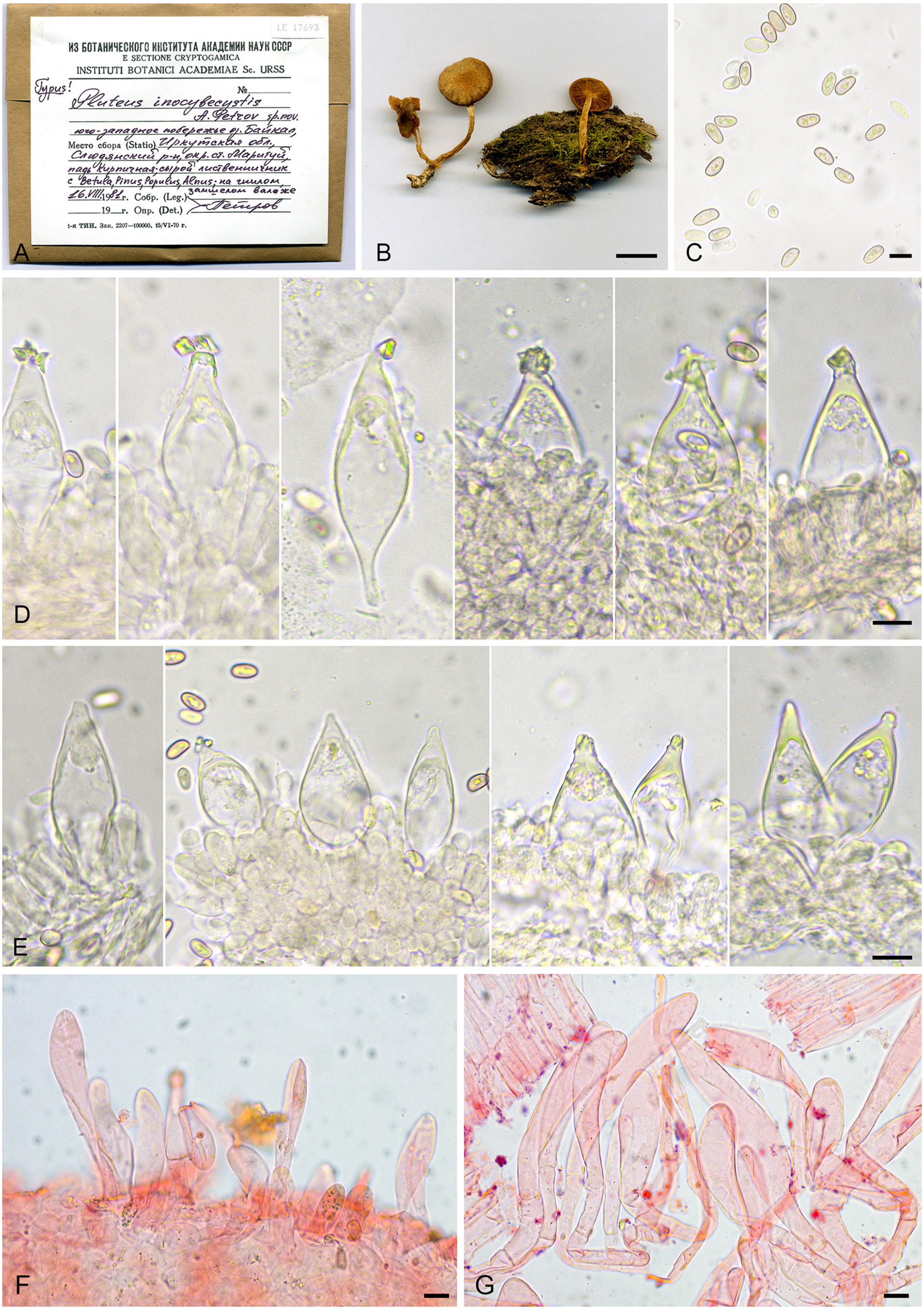
Morphological description of the holotype of *Pluteus inocybecystis*

Here we provide an updated description of microscopic characters only, based on our revision of the herbarium collection.

Basidiospores (7.5)7.9–8.7(10.0) × 4.0–4.7 µm (L_{av} = 8.3 µm, W_{av} = 4.3 µm), Q = 1.7–2.1(2.2), Q_{av} = 1.9, narrowly ellipsoid, ovoid or oblong, in profile often phaseoliform, thin-walled, very pale yellow-brown, germ pore absent. Basidia 4-spored, 18–30 × 7–8 µm, clavate. Pleurocystidia, 57.0–64.5 × 16.5–22.0 µm, inflated fusiform, rostrate, apex covered with crystals, very thick-walled, hyaline or slightly yellowish, rather abundant. Cheilocystidia 47.5–55.5(59.0) × (13.2)15.0–23.5 µm, similar to pleurocystidia, abundant, thick-walled, hyaline or slightly yellowish. Caulocystidia, (54.0)65.0–97.5(117.0) × 14.2–19.5 µm, present at stipe apex, broadly clavate or broadly fusiform, thin- or slightly thick-walled, hyaline. Hairs at pileus margin 53.0–88.0(160.0) × 8.2–13.5 µm, narrowly clavate, cylindrical or fusiform. Pileipellis a hymeniderm with transition to paraderm of 15–40 µm wide cells. Clamps present on stipe hyphae.

Some of the microscopic features described are illustrated in the Fig. 2.

Specimen examined: Holotype: [Russia] “Irkutsk Region, Slyudyansky district, vicinity of Marituj station, Kirpichnaya pad’, damp larch forest (with *Pinus*, *Betula*, *Populus*, *Alnus*), on the rotten, mossy deadwood. 16 VIII 1981. A. N. Petrov” (G! [LE F-17693]). GenBank PP669817 (ITS).



LE 17693

ИЗ БОТАНИЧЕСКОГО ИНСТИТУТА АКАДЕМИИ НАУК СССР
E SECTIONE CRYPTOGAMICA
INSTITUTI BOTANICI ACADEMIAE Sc. URSS

Typus! №
Pluteus inocybecystis
A. Petrov sp.nov.
юго-западное побережье оз. Байкал,
Место сбора (Statio) Иркутская обл.,
Слюдянский р-н, окр. ст. Маритуй,
падь Кирпичная; сырой лиственничник
с Betula, Pinus, Populus, Alnus; на гнилом
замшелом валёже
16.VIII.1981 г. Собр. (Leg.)
19 г. Опр. (Det.) Петров

1-я ТИН. Зак. 2207—100000. 15/VI-70 г.

Fig. 2. Holotype specimen of *Pluteus inocybecystis* (LE F-17693): A – herbarium envelope with label; B – dried basidiocarps; C – basidiospores; D – pleurocystidia; E – cheilocystidia; F – hairs at pileus margin; G – caulocystidia. Scale bars: = 1 cm (for B), = 10 μm (for B–G).

Notes: Here we provide the specimen label. The label matches all details of the protologue, except for the year. In the protologue, the author of the species made a mistake in indicating the year (1980 is given). In the same article where *P. inocybecystis* was described (Petrov, 1983), other taxa from the same habitat were described with the collection date 1981. Therefore, we believe that this error in the year of collection should be corrected to 1981 (according to article 9.2 ICBN).

Taxonomy

Homophron spadiceum (P. Kumm.) Örstadius et E. Larss., 2015, in Örstadius, Ryberg et Larsson, Mycol. Progr. 14 (no. 25): 35

Basionym: *Psilocybe spadicea* P. Kumm., 1871, Der Führer in die Pilzkunde: 71.

Syn.: *Pluteus inocybecystis* A. Petrov, 1983, Mikol. Fitopatol. 17(1): 42, synonymized here.

Discussion

A unique combination of morphological characters, such as the absence of veil and the presence of cystidia with crystals or incrustations, is a characteristic of the genus *Homophron*, the taxonomic status of which has also been confirmed by phylogenetic analyses based on molecular data (Padamsee et al., 2008; Vašutová et al., 2008; Örstadius et al., 2015). *Homophron spadiceum*, type species of the genus, is characterized by reddish brown, hygrophanous pileus, adnate, reddish brown lamellae, pale yellowish brown, often distinctly phaseoliform basidiospores, thick-walled pleurocystidia with crystals at apex and pileipellis hymeniderm to paraderm.

In the protologue of *P. inocybecystis*, the lamellae were described as “free and pink” (Petrov, 1983). However, our observations differ from the original description: even on the dried specimen (Fig. 2), we can see both with the naked eye and under a stereo microscope that the lamellae are adnate and rusty-brown, which is typical for *H. spadiceum*. Microscopically, the differences between members of the genera *Pluteus* and *Homophron* are even more conspicuous: structure of pileipellis, shape of basidiospores (which are never phaseoliform in the genus *Pluteus*) and pleurocystidia (which never have crystals at apex in the genus *Pluteus*).

Phylogenetic analysis clustered the ITS sequence of *P. inocybecystis* and all four *H. spadiceum* collections into a single clade with high statistical support (Fig. 1).

Judging by the illustrations, observed microscopic features and molecular data, this fungus apparently represents the genus *Homophron*. Thus, *Pluteus inocybecystis* Petrov represents a later synonym of *Homophron spadiceum* (P. Kumm.) Örstadius et E. Larss.

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