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Volvopluteus asiaticus (Agaricales, Basidiomycota) – the second finding in the world

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Summary. The specimens collected in the Russian Far East were identified as *Volvopluteus asiaticus*, based on morphology and nrITS+nrLSU sequences analyses. The detailed description and illustration of the studied collection are provided. This is the first record of the species in Russia and the second find in the world.

Volvopluteus asiaticus (Agaricales, Basidiomycota) – вторая находка в мире

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Ключевые слова: Дальний Восток, микобиота, таксономия, филогения, Pluteaceae.

Аннотация. Образцы, собранные на Дальнем Востоке России, были идентифицированы как *Volvopluteus asiaticus* на основе морфологии и анализа последовательностей nrITS+nrLSU. Приводится подробное описание и иллюстрация изученной коллекции. Это первая регистрация вида в России и вторая находка в мире.

Introduction

The genus *Volvopluteus* Vizzini, Contu et Justo was established in 2011 (Justo et al., 2011b) with *Volvopluteus gloiocephalus* (DC.) Vizzini, Contu et Justo as the type species. The genus comprises four species described using polyphasic approach (*V. gloiocephalus, V. earlei* (Murrill) Vizzini, Contu et Justo, *V. michiganensis* (A.H. Sm.) Justo et Minnis and *V. asiaticus* Justo et Minnis). According to the Index Fungorum (Index Fungorum. URL: https://indexfungorum.org/), there are two more species of *Volvopluteus* (namely *V. diversisporus* M. Kaur et Yadw. Singh and *V. shafferi* M. Kaur et Yadw. Singh), but latter were described based on morphology only, and additional molecular investigations of the type specimens are needed to confirm their identity. Representatives of the genus are characterized by medium or even large-sized basidiomata with volva at stipe base, viscid pileus, numerous free lamellae and pink or pinkish brown spore print. *Volvopluteus asiaticus* was described based on molecular data and micromorphological differences of the dried specimen that was previously identified as *Volvariella gloiocephala* (Justo et al., 2011a).

During the mycobiota survey of the Far East, we found specimens of *Volvopluteus* sp. with conspicuously brown pileus that turned out to be *Volvopluteus asiaticus*. This collection represents second finding of the species in the world after the type specimen

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according to GBIF (Global Biodiversity Information Facility. URL: https://www.gbif.org). Here we offer an updated description of the species based on fresh material and accompanied with photos of basidiomata and micromorphological characters as well as with supporting evidence based on nrITS and nrLSU sequences analyses.

Material and Methods

Study site

The studied specimens were collected in vicinities of Gorno-Taezhnoye settlement, Primorye Territory (43°42'N, 132°09'E), on gentle northern slope in wet deciduous forest with *Tilia* spp., *Quercus mongolica*, *Acer mono*, *Juglans mandshurica,* and *Fraxinus* spp., on soil in proximity to fallen and decayed trunk of unidentifiable deciduous tree.

Sampling and morphological study

Basidiomata were photographed *in situ* and transported to the field station, where they were examined according to standard techniques applied to fungal taxonomy (Clémençon, 2009). Microscopic observations were made from dried material mounted in 5 % KOH and 10 % Congo Red in NH_4OH using a Zeiss Axio Scope.A1 and Axio Imager.A1 light microscope with differential interference contrast (DIC). Basidiospore dimensions were based on at least 30 basidiospores from each basidioma; (n = 60, s = 2) indicates measurements based on 60 basidiospores from two basidiomata in one collection. Spore dimensions (without hilar appendage) are provided as (a–)b–c(–d), with b–c containing at least 90 % of all values and a and d representing extreme values. Q indicates the basidiospore length/width ratio, Q* represents the mean length/width quotient of the total basidiospores measured. The measurements of the other morphological structures are based on at least 20 elements.

The studied collection was deposited in the Mycological Herbarium of the Komarov Botanical Institute (LE).

DNA extraction, PCR amplification, and sequencing

We performed DNA extraction from small part of dried basidioma using FitoSORB DNA extraction kit according to the manufacturer's protocol (Syntol, Russia). The following primers were used for amplification and sequencing: ITS1F-ITS4B (Gardes, Bruns, 1993) for internal transcribed spacer (ITS1-5.8S-ITS2); LROR-LR5 (Vilgalys, Hester, 1990; White et al., 1990) for part of the nrLSU region. We visualized product of PCR reaction using agarose gel electrophoresis and SYBR Green staining and subsequently purified it with CleanMag DNA kit (Evrogen, Russia). Sequencing was performed with an ABI model 3500 Genetic Analyzer (Applied Biosystems, CA, USA). Raw data were edited and assembled in MEGA X (Kumar et al., 2018).

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, Russian Academy of Sciences, St. Petersburg).

Sequence alignment and phylogenetic analyses

For this study one new nrITS and one nrLSU sequences were generated. In addition, 21 nrITS and 14 nrLSU sequences, including an outgroup, were retrieved from the GenBank database (www.ncbi.nlm.nih.gov/genbank/), using the BLAST application and taxonomic considerations (Justo et al., 2011a; Kaygusuz et al., 2021). The taxonomic identities of these sequences with GenBank accession numbers are given in Table. The sequences were aligned with the Muscle tool (Edgar, 2004) incorporated into MEGA X program separately for each genetic marker and then combined into a single dataset.

Phylogenetic reconstructions were performed with Maximum Likelihood (ML) and Bayesian Inference (BI) analyses. Before the analyses, the best-fit substitution model was estimated based on the Akaike Information Criterion (AIC) using FindModel web server (http://www.hiv.lanl.gov/content/sequence/findmodel/findmodel.html). For each dataset (nrITS and nrLSU) the model turned out to be the same, thereby GTR+G model was chosen for concatenated dataset (nrITS+nrLSU) and used for further analyses. Maximum likelihood analysis was run on RAxML servers, v.1.0.0 (https://raxml-ng.vital-it.ch/#/) with one hundred rapid bootstrap replicates. BI analysis was performed with MrBayes 3.2.5 software (Ronquist et al., 2012), for two independent runs, each with 10 million generations under described model and four chains with sampling every 100 generations. To check for convergence of MCMC analyses and to get estimates of the posterior distribution of parameter values, Tracer v1.7.1 was used (Rambaut et al., 2018). We accepted the result where the ESS (Effective Sample Size) was above 200 and the PSRF (Potential Scale Reduction Factor) was close to 1.

Newly generated sequences were deposited in GenBank with corresponding accession numbers (Table).

Table

Sequences used for molecular analyses. Newly generated sequences are marked with *, sequences from type specimens are given in bold

No	Taxon	Voucher	GenBank number		Country
			ITS	LSU	
1	*Pluteus heteromarginatus*	AJ	HM562058	HM562249	USA
2	*Volvopluteus asiaticus*	**TNSF15191**	**HM562206**	–	Japan
3	*V. asiaticus*	LE F-332246	OP862868*	OP862780*	Russia
4	*V. earlei*	MA22816	HM562204	HM562253	Spain
5	*V. earlei*	TO AV133	HM246496	HM246480.1	Italy
6	*V. earlei*	TOHG2001	HM246498	HM246477.1	Italy
7	*V. earlei*	OKA-TR649	MW033389.1	MW029820.1	Turkey
8	*V. earlei*	OKA-TR654	MW033394.1	MW029825.1	Turkey
9	*V. earlei*	WV-3	JN086658.1	–	India
10	*V. earlei*	TNS:F-70247	MH021868	–	Japan
11	*V. earlei*	Mamet7	HM562205.1	MK278661.1	Congo
12	*V. michiganensis*	LE 311991	MK049912.1	–	Russia
13	*V. michiganensis*	**Smith32-590**	**HM562195.1**	–	USA
14	*V. michiganensis*	UBC F-32158	MF954699.1	–	Canada
15	*V. gloiocephalus*	TO AV136	HM246495.1	HM246478.1	Italy
16	*V. gloiocephalus*	TO AV135	HM246490.1	HM246476.1	Italy
17	*V. gloiocephalus*	OKA-TR659	MW033399.1	MW029830.1	Turkey
18	*V. gloiocephalus*	OKA-TR660	MW033400.1	MW029831.1	Turkey
19	*V. gloiocephalus*	AJ239	HM562202.1	MK278662.1	Spain
20	*V. gloiocephalus*	AFTOL-ID 890	DQ494701.1	AY745710	USA
21	*V. gloiocephalus*	PDD_103792	MN738645.1	MN738593.1	New Zealand
22	*V. gloiocephalus*	LOU18619	HM562207.1	–	Portugal

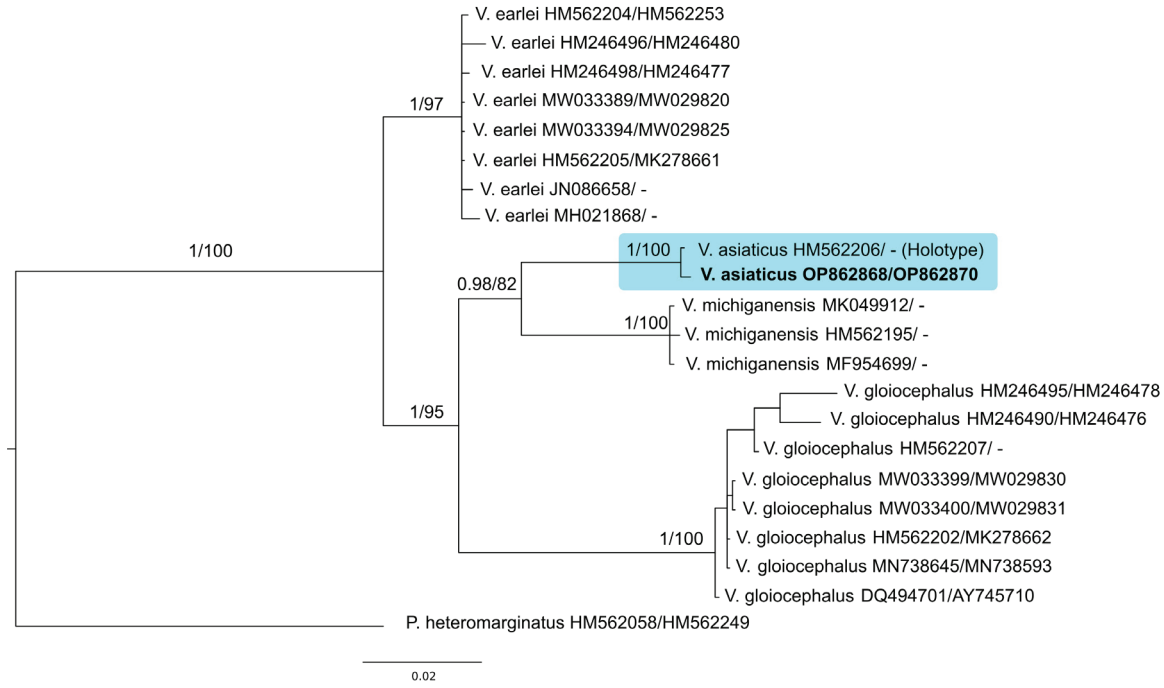

Fig. 1. Phylogenetic tree of *Volvopluteus asiaticus* and allies derived from the nrITS+nrLSU dataset using Bayesian analysis. The Bayesian PP/ML bootstrap support are shown above branches. For all taxa the GenBank accession numbers (nrITS/nrLSU) are presented. The specimen studied for this article is highlighted in bold. Scale bar indicates the mean number of nucleotide substitutions per site.

Results and discussion

Phylogenetic analyses

We choose the available nrITS and nrLSU sequences of all *Volvopluteus* species presented in research dealing with its' taxonomy (Justo et al., 2011b; Kaygusuz et al., 2021) to perform phylogenetic analysis with *Pluteus heteromarginatus* Justo as an outgroup. The final combined nrITS+nrLSU dataset contained 22 nrITS and 15 nrLSU sequences and consisted of 1623 characters (with gaps). Tree topologies were almost congruent while using ML and BI methods for phylogenetic analyses, thus only the BI tree is presented in Fig. 1. Our nrITS+nrLSU phylogeny supports four clades in the genus. These highly supported lineages correspond to four morphological species: *V. gloiocephalus, V. earlei, V. michiganensis,* and *V. asiaticus.* In the tree, *V. asiaticus* is sister to *V. michiganensis*, and those are closer to *V. gloiocephalus* than to *V. earlei*.

The studied specimen from the Russian Far East fits a clade containing *V. asiaticus* holotype with strong support value (100 % ML BS, PP = 1.0).

Morphological description

Pileus 90–110 mm diam., oviform or broadly conical in young specimens, with age applanate, with umbonate center and striate margin, radially fibrillose, glutinous and shiny when wet, dark brown with light olive shade when young changing to somewhat coffee with milk, with darker olive-brown center. Gills numerous, free, ventricose, from white to dusty pink with uneven, serrated edges. Stipe 85–180 × 10–20 mm, cylindrical, white, glabrous, in youth visibly broadening towards the base, in maturity (basidiomata with applanate pileus) with bulbous base enclosed in sac-like volva. Volva white, membranous, woolly at outer surface, with abundant rhizomorphs (Fig. 2). Spore print dirty pink. Basidiospores [63, 2, 1] (9.4)10.2–12.3(13) × (5.7)6.3–7.2(7.4) µm, Q = 1.60–1.80, Q* = 1.7, ellipsoid to oblong. Basidia 29–37 × 11–13 µm, 4-spored, clavate. Pleurocystidia 48–66 × 15–27 µm, narrowly to broadly fusiform, narrowly utriform, commonly rostrate, with one apical excrescence up to 10–13 µm long, hyaline, thin-walled, frequent all over lamellar faces. Cheilocystidia (41.5)48–90 × 20–48 µm,

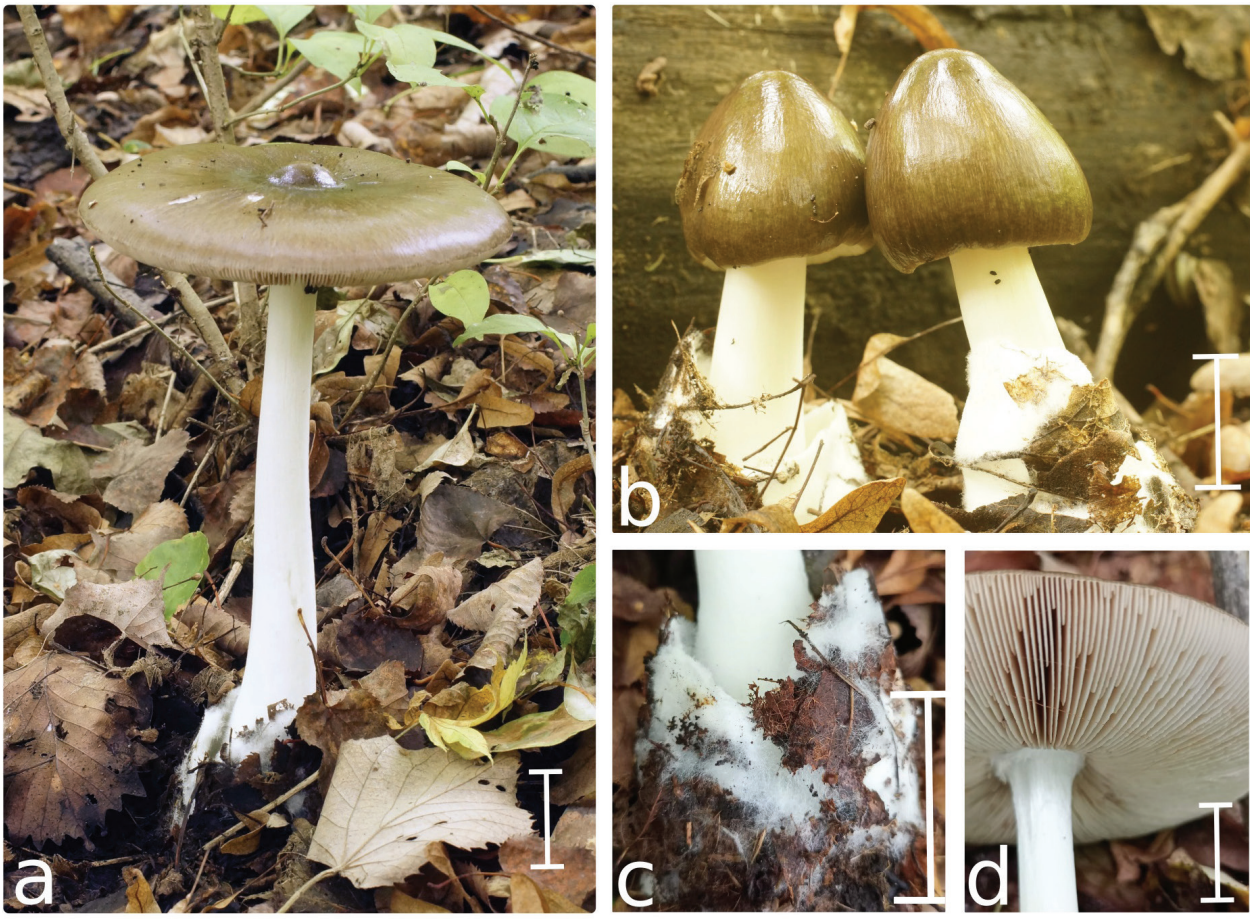

Fig. 2. Basidiomata of *Volvopluteus asiaticus* (LE F-332246) *in situ*: a – mature basidioma with expanded pileus and umbonate center; b – young basidiomata; c – volva with wooly outer surface; d – free numerous lamellae with somewhat serrulate edge. Scale bar 2 cm.

mostly clavate, ovoid or lanceolate, rarely with an apical papilla or excrescence up to 10 µm long, hyaline, thin-walled, crowded, forming a sterile layer at lamellae edge. Pileipellis an ixocutis, composed of hyphae 5–8 µm wide; hyphae cylindrical or irregular in outline, embedded in a 250–450 µm thick gelatinous matrix, hyaline or with pale intracellular brown pigment, thin-walled. Stipitipellis a cutis; hyphae 4–10 µm wide, cylindrical, colorless or with brownish intracellular pigment, with thin, smooth walls. Caulocystidia 70–150 × 10–15 µm, cylindrical or flexuose, some with internal septa or with rare lateral excrescences, thin-walled (Fig. 3). Volva composed of densely interwoven cylindrical hyphae, 8–15 µm wide. Clamp connections absent in all tissues.

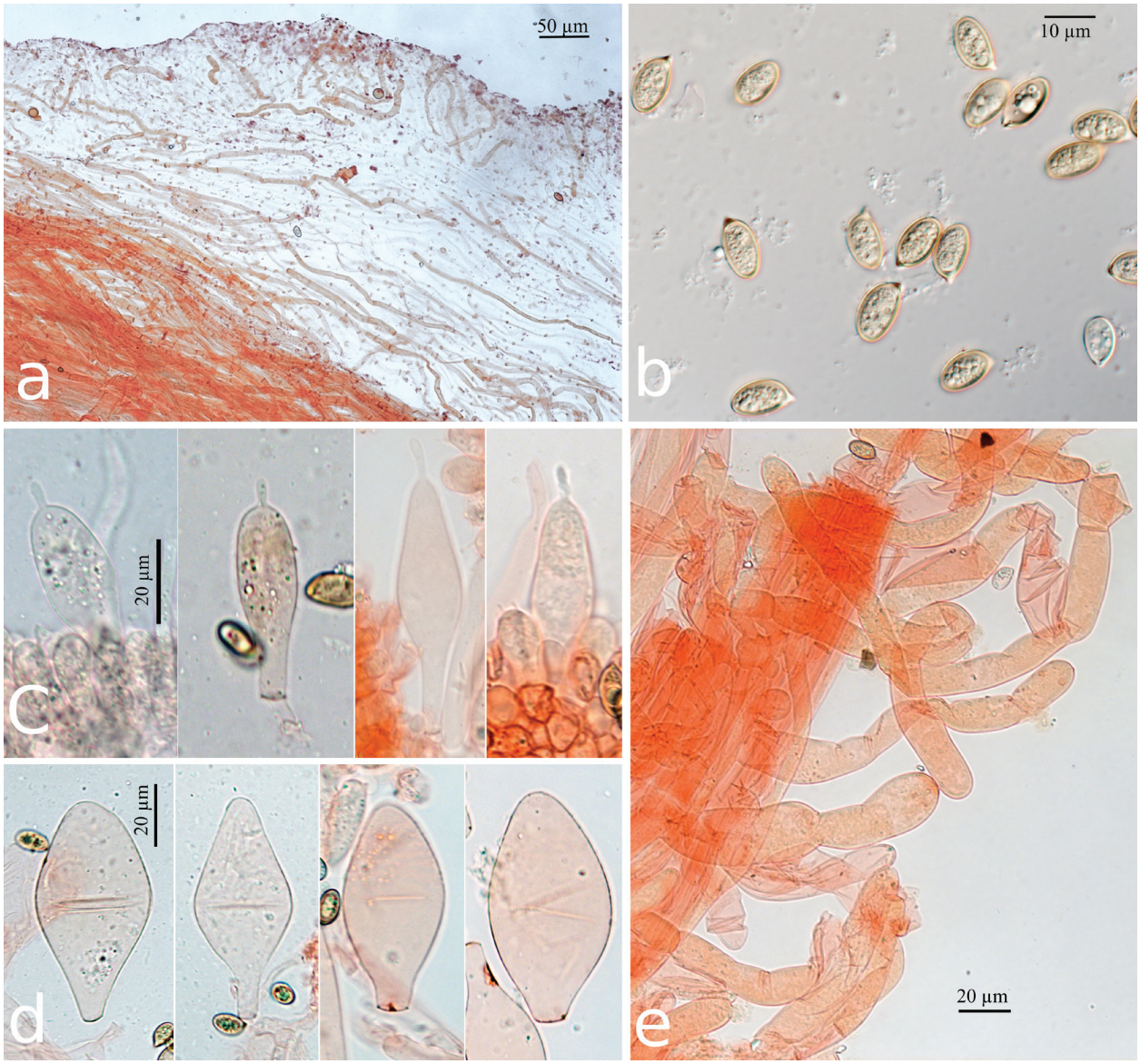

Fig. 3. Microstructures of *Volvopluteus asiaticus* (LE F-332246): a – pileipellis; b – basidiospores; c – rostrate pleurocystidia; d – cheilocystidia; e – caulocystidia.

Specimen examined: "Russia, Primorye Territory, vicinity of Gorno-Taezhnoe settlement, 43°42'11.1"N, 132°09'52.5"E, wet deciduous forest with *Tilia* spp., *Quercus mongolica*, *Acer mono*, *Juglans mandshurica* and *Fraxinus* spp., on soil, 19 IX 2022, L. Kalinina" (LE F-332246, GenBank nrITS – OP862868, nrLSU – OP862780).

Discussion

The pluteoid basidiomata with universal veil remnants present as volva at stipe base, viscid pileus, free and pink lamellae, a stipe that is not viscid, ellipsoid to oblong basidiospores without germ-pore, pileipellis as ixocutis, and terrestrial

habitat support the placement of our collection in *Volvopluteus* (Justo et al., 2011a, 2011b). The species delimitation within a genus is based primarily on colors of basidiomata, basidiospore size and shape, and morphology of hymenial cystidia. *V. asiaticus* is characterized by large basidiomata, with dark colored pileus, white volva, large basidiospores and pleurocystidia with apical excrescence. It differs from the closely related species *V. gloiocephalus* in the presence of rostrate pleurocystidia.

The studied collection from the Russian Far East differs slightly from the type description (Justo et al., 2011b) in the following features: darker pileus coloring with distinct olive shades, slightly smaller spore size (vs 12.0–14.5 × 7.0–8.5(9.0) μm in type collection), and predominance of clavate cheilocystidia. Thus, fresh material allows us to clarify and expand the morphological characteristics of the species.

Volvariella asiaticus was originally described solely on herbarium material (TNSF 15191, from Japan) that was previously identified as common and widely distributed species *Volvariella gloiocephala* (≡ *V. gloiocephalus*). *Volvariella gloiocephalus* is known to be characterized by a variety of coloration of basidiomata, as well as a variety of microscopic characters. The strong variability of morphological features can mislead researchers regarding the boundaries of the species. Therefore, specimens of the latter species with brown pileus collected in the Asian region may turn out to be *V. asiaticus* as a result of further molecular studies.

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