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Six New Species of *Agaricus* (Agaricaceae, Agaricales) from Northeast China

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Abstract: *Agaricus* belongs to Agaricaceae, and is one of the most well-known macrofungi, with many edible species. More than 300 *Agaricus* specimens were collected during a three-year macrofungal resource field investigation in Northeast China. Based on morphological observations and multi-gene (ITS + nrLSU + *tef1-a*) phylogenetic analyses, six new *Agaricus* species, i.e., *Agaricus aurantipileatus*, *A. daqinggouensis*, *A. floccularis*, *A. griseopileatus*, *A. sinoagrocyboides*, and *A. velutinosus*, were discovered. These new species belong to four sections within different subgenera: *A.* (subg. *Agaricus*) sect. *Agaricus*, *A.* (subg. *Flavoagaricus*) sect. *Arvenses*, *A.* (subg. *Minores*) sect. *Minores*, and *A.* (subg. *Pseudochitonia*) sect. *Xanthodermatei*. Morphological descriptions, line illustrations, and basidiomata photographs of these new species are provided, and their differences from similar species are discussed.

Keywords: morphology; multi-gene phylogeny; taxonomy; new species

1. Introduction

Agaricus L. is a large genus of the family Agaricaceae, with *Agaricus campestris* L. as the type; it could be distinguished from other genera by its unique characteristics, which include small-sized to large-sized fleshy basidiomata, free lamellae which are white or pink when young but at maturity become brown to dark brown, presence of an annulus on the stipe, brown basidiospores, brown spore prints, and pileipellis a cutis of cylindrical hyphae [1–5]. The species of *Agaricus* are saprophytic, distributed on all continents except Antarctica, and commonly found in forests, pastures, or grasslands [6–7].

Agaricus was first described by Linnaeus in 1753; Linnaeus collectively referred to all the large agaric mushrooms found at the time as '*Agaricus*' [5], however the current defined genus *Agaricus* was formally established by Karsten (1879) [8]. The taxonomic study of *Agaricus* has a long history, and different taxonomists hold different taxonomy views on the taxonomy system of *Agaricus*. Before the application of molecular systematics, the taxonomy system proposed by Parra (2008) was widely accepted based on morphology [9]. Parra (2008) summarized the previous studies and divided *Agaricus* into three subgenera: *A.* subg. *Agaricus*, *A.* subg. *Conioagaricus* and *A.* subg. *Lanagaricus* and eight sections, viz., *A.* sect. *Agaricus*, *A.* sect. *Arvensis*, *A.* sect. *Bivelares*, *A.* sect. *Chitonioedes*, *A.* sect. *Minores*, *A.* sect. *Sanguinolenti*, *A.* sect. *Spissicaulles*, and *A.* sect. *Xanthodermatei*, based on its macroscopic and microscopic characteristics [1].

In recent years, with the continuous development of biotechnology and scientific means, the taxonomy system of *Agaricus* has also tended to be stable. Based on the molecular identification of *Agaricus* species, Zhao et al. (2011) added sequences of specimens from tropical and temperate regions, and molecular phylogenetic results verified the known eight sections proposed by Parra (2008) and found that there were 11 independent clades [7]. Additionally, Zhao et al. (2016) proposed a new taxonomic system that

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classified *Agaricus* into five subgenera and 20 sections based on divergence times [2]. In subsequent studies, new subgenera and sections were established based on the new taxonomic system; presently, *Agaricus* comprises six subgenera and 27 sections [6, 10–14].

There is a seventh subgenus, *A.* subg. *Conioagaricus*, containing three sections: *A.* sect. *intermedii*, *A.* sect. *Pulverotecti*, and *A.* sect. *Striati* [15]. However, due to the members of *A.* subg. *Coniagaricus* have an epithelium pileipellis which is completely different from species of the other subgenera, *A.* subg. *Coniagaricus* is suggested to be relocated to other genera [2, 16]. Unfortunately, in the absence of available molecular data, *A.* subg. *Conioagaricus* has not been studied molecularly within the framework of the new taxonomic system proposed by Zhao et al. (2016) [2]. *A.* subg. *Lanagaricus* [15] has been proved a heterotypic synonym of *A.* subg. *Pseudochitonia* [2].

This study involves four sections of four subgenera, viz., *A.* sect. *Agaricus* in *A.* subg. *Agaricus*, *A.* sect. *Arvenses* in *A.* subg. *Flavoagaricus*, *A.* sect. *Minores* in *A.* subg. *Minores* and *A.* sect. *Xanthodermatei* in *A.* subg. *Pseudochitonia*, in the system by Zhao et al. (2016).

Agaricus species are abundant; so far, more than 500 species have been recorded [1–3, 7, 17–20]. At present, over 116 Agaricus species supported by molecular phylogenetic and morphological studies have been reported in China [9, 21–27]. However, the richness of *Agaricus* species diversity in some regions of China still needs to be studied and explored, such as Northeast China. The Northeast China is mainly north temperate zone, has a temperate monsoon climate with four distinct seasons [28] and abundant plant resources [29], which provides the basis for fungal diversity.

From 2021 to 2023, we collected more than 300 *Agaricus* specimens in Northeast China. Among those, after morphological study and phylogenetic analyses, 34 species were identified, including six new species. In this paper, we describe these new species of *Agaricus* to better understand the species diversity of *Agaricus* in this region.

2. Material and Methods

2.1. Specimens and Morphological observations

The examined specimens were mainly collected from Northeast China, including Inner Mongolia Autonomous Region, Jilin Province and Heilongjiang Province, and some were studied from the Herbarium of Mycology of Jilin Agricultural University (HMJAU). The voucher specimens are deposited in the Herbarium of Mycology of Jilin Agricultural University. The macromorphological characteristics are based on field records and photographs of fresh basidiomata. The color description of the fresh basidiomata is referenced to Kornerup and Wanscher (1978) [30]. Microscopic features were observed based on dry specimens. The corresponding structures were taken and prepared freehand, floated in 5% KOH solution or sterile water, stained with 1% Congo red solution if necessary, and observed through a light microscope (OLYMPUS BX50). The microscopic characteristics of each structure were based on at least 20 measurements. The symbol '(a) b–c (d)' is used to describe the size of basidiospores, where the 'b–c' range represents 90 % of the measured values, while the 'a' and 'd' are extreme values. '[Xav = e × f]' indicates the average size of basidiospores. 'Q' refers to the ratio of length to width of a single basidiospore from the side view, and 'Qav' refers to the average value of 'Q' of all specimens. Other microstructural measurements include the range between the extreme of length and width measurements.

2.2. DNA extraction, PCR amplification, and sequencing

Genomic DNA was extracted using the NuClean Plant Genomic DNA kit (CWBIO, Beijing, China) in strict accordance with the instruction manual. The primer pairs ITS1F/ITS4 [31], LR0R/LR5 [32] and EF1-983F/EF1-1567R [33] were used to amplify the sequences of three DNA regions, ITS, nrLSU and *tef1-a*, respectively. The polymerase chain reaction (PCR) procedure was based on the protocol described by Mou and Bau (2021) [34]. The PCR products were detected by 1 % agarose gel electrophoresis, and the

qualified products were sent to Bioengineering (Shanghai) Co., Ltd., China, for sequencing with the same primers.

2.3. Sequence alignment and phylogenetic analyses

The chromatograms were checked in BioEdit v.7.1.3.0 [35] to ensure that each sequence had good quality, then a BLAST search was carried in the National Center of Biotechnology Information (NCBI) database (https://www.ncbi.nlm.nih.gov/) to confirm the sequencing results, and finally the sequences were submitted to GenBank (Table 1 in bold). Based on the BLAST search results, sequences corresponding to the subgenera of the studied species were downloaded for phylogenetic analyses. Subsequently, the multigene phylogenetic trees of these subgenera were constructed separately. Particularly, species (Table 1) falling within the clades of the species described in this paper were selected and integrated to construct new multi-gene phylogenetic trees. *Heinemannomyces* sp. ZRL185 was used as outgroup [2, 13].

Sequences alignment using online MAFFT tool [36] (https://mafft.cbrc.jp/alignment/software/, accessed on 8–17 October 2023) to align sequences independently for each region, then manually adjusted in BioEdit v.7.1.3.0. The ITS, nrLSU, and *tef1-a* were assembled in Phylosuite v1.2.2 [37]. The multi-locus dataset (ITS + nrLSU + *tef1-a*) of *Agaricus* had an aligned length of 2107 (ITS subset: 1–730 bp; nrLSU subset: 731–1594 bp; *tef1-a* subset: 1595–2107 bp) total characters including gaps. The alignment was submitted to Figshare (https://doi.org/10.6084/m9.figshare.24631926.v2, accessed on 23 December 2023).

Maximum likelihood (ML) phylogenies were inferred using IQ-TREE [38] under the TIM2+I+G4+F model (ModelFinder [39] was used to select the best-fit model using BIC criterion) for 5000 ultrafast bootstraps [40], as well as the Shimodaira–Hasegawa–like approximate likelihood-ratio test. Bayesian Inference (BI) phylogenies were inferred using MrBayes 3.2.6 [41] under partition model (2 parallel runs, 743,400 generations), in which the initial 25% of sampled data were discarded as burn-in. ModelFinder was again used to select the best-fit partition model (Edge-linked) using BIC criterion, and best-fit model according to BIC was HKY+F+G4 for ITS, HKY+F+I for nrLSU and K2P+I for *tef1-a*. The final trees were visualized using iTOL [42] and edited using Adobe Illustrator 2021 (Adobe).

Table 1. Sequences used in the phylogenetic analysis. 'T' refers to holotype. Bold refers to the sequences produced from this study. Green font refers to the new species. '–' means no relevant genetic information.

Taxon	Voucher ID	Origin	ITS	nrLSU	*tef1-α*	References
A. sect. *Agaricus*						
Agaricus albovariabilis	TBGT18462	India	ON555770	–	–	[43]
A. albovariabilis	TBGT18487 T	India	ON555779	–	–	[43]
A. argyropotamicus	RWK2017	The USA	KJ877748	–	–	[3]
A. argyropotamicus	F2047	France	JF727849	–	–	[24]
A. flavicentrus	MFLU12-0146 T	Thailand	NR151752	–	–	[24]
A. flavicentrus	MFLU12-0149	Thailand	KR025856	–	–	[24]
A. inilleasper	H4452 T	Australia	NR119947	–	–	[24]
A. sinoagrocyboides	**HMJAU 67738 T**	**China**	**OR690292**	**OR690373**	**OR711549**	**In this study**
A. sinoagrocyboides	**HMJAU 46513**	**China**	**OR690290**	**OR690371**	**OR711547**	**In this study**
A. sinoagrocyboides	**HMJAU 46514**	**China**	**OR690291**	**OR690372**	**OR711548**	**In this study**
A. sp.	F2272	France	JF727850	–	–	[7]

A. sect. *Arvenses*

Table 1. *Cont.*

Taxon	Voucher ID	Origin	ITS	nrLSU	*tef1-α*	References
A. abruptibulbus	LAPAG524	Czech Republic	KJ548132	–	–	[17]
A. abruptibulbus	ZRL20161250	China	MK617909	MK617821	MK614417	[25]
A. abruptibulbus	**HMJAU 67833**	**China**	**OR690278**	–	–	**In this study**
A. abruptibulbus	**HMJAU 67834**	**China**	**OR690279**	–	–	**In this study**
A. arvensis	RWK2287	the USA	KJ847460	–	KX198047	[3]
A. arvensis	LAPAG450 T	Spain	KT951328	KP739801	KT951619	[2]
A. aurantipileatus	**HMJAU 67746**	**China**	**OR690304**	**OR690379**	**OR711537**	**In this study**
A. aurantipileatus	**HMJAU 67747 T**	**China**	**OR690305**	**OR690380**	**OR711538**	**In this study**
A. fissuratus	ZRL20170745	China	MK617935	MK617847	MK614441	[25]
A. fissuratus	RWK2285	the USA	KJ859085	–	–	[3]
A. floccularis	**HMJAU 67744 T**	**China**	**OR690297**	**OR690377**	**OR711535**	**In this study**
A. floccularis	**HMJAU 67745**	**China**	**OR690298**	**OR690378**	**OR711536**	**In this study**
A. greuteri	PALGR57140 T	Italy	KF114473	–	–	[17]
A. greuteri	LAPAG399	Spain	MK617859	–	–	[25]
A. griseopileatus	**HMJAU 67838**	**China**	**OR690299**	**OR690381**	**OR711539**	**In this study**
A. griseopileatus	**HMJAU 67841**	**China**	**OR690300**	**OR690382**	**OR711540**	**In this study**
A. griseopileatus	**HMJAU 67848 T**	**China**	**OR690301**	**OR690383**	**OR711541**	**In this study**
A. griseopileatus	**HMJAU 67852**	**China**	**OR690302**	**OR690384**	**OR711542**	**In this study**
A. griseopileatus	**HMJAU 67856**	**China**	**OR690303**	**OR690385**	**OR711543**	**In this study**
A. guizhouensis	HKAS 81081 T	China	KJ755658	–	–	[44]
A. guizhouensis	ZRL20160420	China	MK617906	MK617818	MK614414	[25]
A. guizhouensis	**HMJAU 67830**	**China**	**OR690276**	–	–	**In this study**
A. guizhouensis	**HMJAU 67831**	**China**	**OR690277**	–	–	**In this study**
A. luteopileus	ZRL2012604 T	China	KT951375	KT951515	KT951620	[2]
A. luteopileus	ZRL20120589	China	MK617868	MK617780	MK614377	[25]
A. macrolepis	LAPAG409	Spain	KJ548128	–	–	[17]
A. megacarpus	QL20170178 T	China	MK617861	MK617774	MK614371	[25]
A. megacarpus	ZRL20152608	China	MK617901	MK617813	MK614409	[25]
A. reducibulbus	SFSUF020926 T	the USA	NR_144994	–	–	[3]
A. reducibulbus	RWK2105	the USA	KJ859130	–	–	[3]
A. sp.	ZRL20170661	China	MK617927	MK617839	MK614434	[25]
A. sp.	ZRL2012630	China	KT951379	KT951495	KT951621	[2]
A. sp.	ZRL20162213	China	MK617916	MK617828	MK614423	[25]
A. sp.	SHY2011073117	China	KT951407	KT951459	KT951622	[2]
A. sp.	**HMJAU 67829**	**China**	**OR690281**	–	–	**In this study**
A. sp.	**HMJAU 67828**	**China**	**OR690282**	–	–	**In this study**
A. subumbonatus	ZRL2012030 T	China	KT951364	KT951455	KT951628	[2]
A. subumbonatus	ZRL20130529	China	MK617872	MK617784	MK614380	[25]

A. sect. *Minores*

A. fulvoaurantiacus	MFLU16-0980 T	Thailand	KU975107	KX084002	KX198069	[6]

Table 1. *Cont.*

Taxon	Voucher ID	Origin	ITS	nrLSU	*tef1-α*	References
A. fulvoaurantiacus	MFLU16-0974	Thailand	KU975106	KX084001	KX198043	[6]
A. huijsmanii	LAPAG639	Spain	KF447889	KT951444	KT951571	[17]
A. huijsmanii	HMJAU 67712	China	OR159474	–	–	[27]
A. huijsmanii	HMJAU 67713	China	OR159473	–	–	[27]
A. luteofibrillosus	MFLU16-0981	Thailand	KU975108	KX084003	KX198041	[6]
A. luteofibrillosus	ZRL2110	Thailand	KU975109	KX084004	–	[6]
A. ruffifibrillosus	ZRL20151536 T	China	KX684878	KX684893	KX684915	[45]
A. sp.	ZRL2010079	China	KX657046	KX656951	KX684950	[45]
A. sp.	HMJAU 67724	China	OR159498	OR144156	OR166452	[27]
A. sp.	HMJAU 67725	China	OR159497	OR144157	OR166451	[27]
A. velutinosus	**HMJAU 67768 T**	**China**	**OR690296**	**OR690376**	**OR711546**	**In this study**
A. velutinosus	**HMJAU 67763**	**China**	**OR690293**	**OR690374**	**OR711544**	**In this study**
A. velutinosus	**HMJAU 67764**	**China**	**OR690294**	**OR690375**	**OR711545**	**In this study**
A. velutinosus	**HMJAU 67767**	**China**	**OR690295**	**–**	**–**	**In this study**
A. sect. *Xanthodermatei*						
A. atroumbonatus	MM1637 T	Pakistan	MH997905	MK100290	MK169412	[46]
A. atroumbonatus	**HMJAU 67754**	**China**	**OR690273**	**OR690365**	**OR711550**	**In this study**
A. berryessae	RWK2106 T	The USA	KJ609482	–	–	[3]
A. bisporiticus	LD2012111 T	Thailand	KJ575611	KT951507	KT951650	[12]
A. bisporiticus	MCR25	Pakistan	KJ575608	–	–	[12]
A. daliensis	SHY2011071706	China	KM657877	KR006615	KR006643	[47]
A. daqinggouensis	**HMJAU 67756 T**	**China**	**OR690287**	**OR690369**	**OR711554**	**In this study**
A. daqinggouensis	**HMJAU 67758**	**China**	**OR690288**	**–**	**–**	**In this study**
A. daqinggouensis	**HMJAU 67759**	**China**	**OR690289**	**OR690370**	**OR711555**	**In this study**
A. deardorffensis	ecv4230 T	The USA	KJ609494	–	–	[3]
A. moelleroides	LAPAG319 T	Portugal	JN204437	–	–	[17]
A. pseudopratensis	LAPAG259	Spain	DQ182527	MK123324	MK169403	[46]
A. pseudopratensis	**HMJAU 67815**	**China**	**OR690274**	**–**	**–**	**In this study**
A. pseudopratensis	**HMJAU 67816**	**China**	**OR690275**	**–**	**–**	**In this study**
***A.* sp.**	**HMJAU 67739**	**China**	**OR690283**	**OR690366**	**OR711551**	**In this study**
***A.* sp.**	**HMJAU 67740**	**China**	**OR690284**	**OR690367**	**OR711552**	**In this study**
***A.* sp.**	**HMJAU 67741**	**China**	**OR690285**	**OR690368**	**OR711553**	**In this study**
***A.* sp.**	**HMJAU 42077**	**China**	**OR690286**	**–**	**–**	**In this study**
***A.* sp.**	**HMJAU 68021**	**China**	**OR690280**	**–**	**–**	**In this study**
A. tephrolepidus	JBSD123822 T	Dominican Republic	MF511117	–	–	[12]
A. tephrolepidus	**HMJAU 67812**	**China**	**OR690269**	**OR690362**	**OR711556**	**In this study**
A. tephrolepidus	**HMJAU 67813**	**China**	**OR690270**	**OR690363**	**OR711557**	**In this study**
A. tephrolepidus	**HMJAU 67814**	**China**	**OR690271**	**OR690364**	–	**In this study**
A. tephrolepidus	**HMJAU 68018**	**China**	**OR690272**	**–**	**–**	**In this study**

A. tibetensis	ZRL2012585 T	China	KM657895	KR006633	KR006658	[47]

Table 1. *Cont.*

Taxon	Voucher ID	Origin	ITS	nrLSU	*tef1-α*	References
Heinemannomyces						
Heinemannomyces sp.	ZRL185	Thailand	KT951346	KT951527	KT951657	[2]

2.4. Species-Specific ITS Markers

The position of the unique nucleotide (nt) base of a species' ITS sequence in the section to which the species belongs is represented as follows: 'xxxxxXxxxxx @ position', where the capital letter 'X' represents the the exclusive or informative character, '@ position' represents the position of 'X', and 'xxxxx' represents the flanking characters. For the empty characters appearing in the flanking characters, they were not written in this study, but the nucleotides were selected downward. It must be noted that the comparisons are made with the current available sequences of all species in the section and may need to be reassessed when the number of species changes.

3. Results

3.1. Molecular phylogeny

The dataset used for the phylogenetic analysis consisted of 90 specimens (see Table 1), and 84 sequences were generated for this study, including 37 ITS sequences, 24 nrLSU sequences, and 23 *tef1-a* sequences. BI and ML analysis resulted in a same topology, so the ML tree is provided in this paper (Figure 1). Bootstrap support (BS) values ≥50% and Bayesian posterior probability (PP) values ≥0.70 are indicated on clades (BS/PP).

The phylogenetic tree ~~mainly~~ presents four clades, corresponding to four sections within different subgenera, ~~with corresponding names.~~ Six new species are in these four sections. ~~The clade '*A. sect. Xanthodermatei*' was well supported (BS/PP = 98/1);~~ *A. daqinggouensis* and *A. moelleroides* LAPAG319 formed a sister clade with an appreciable support value (BS/PP = 84/0.80). ~~The clade '*A. sect. Agaricus*' was high supported (BS/PP = 93/0.98);~~ *A. sinoagrocyboides* and *A. albovariabilis* TBGT18487/ TBGT18462 formed a sister clade with a high support value (BS/PP = 97/1). ~~The clade '*A. sect. Minores*' was high supported (BS/PP = 99/1);~~ *A. velutinosus* and *A. huijsmanii* LAPAG639/HMJAU67712 /HMJAU67713 formed a sister clade with a ~~high~~ support value (BS/PP = 100/1). ~~The clade '*A. sect. Arvenses*' was high supported (BS/PP = 99/1);~~ *A. floccularis* and *A. fissuratus* ZRL20170745/RWK 2285 formed a sister clade with a ~~high~~ support value (BS/PP = 100/1); *A. aurantipileatus* and *A.* sp. ZRL2012630 formed a sister clade with a ~~high~~ support value (BS/PP = 100 /1), and *A. griseopileatus* also formed a unique clade with a high support value (BS/PP = 90/0.97).

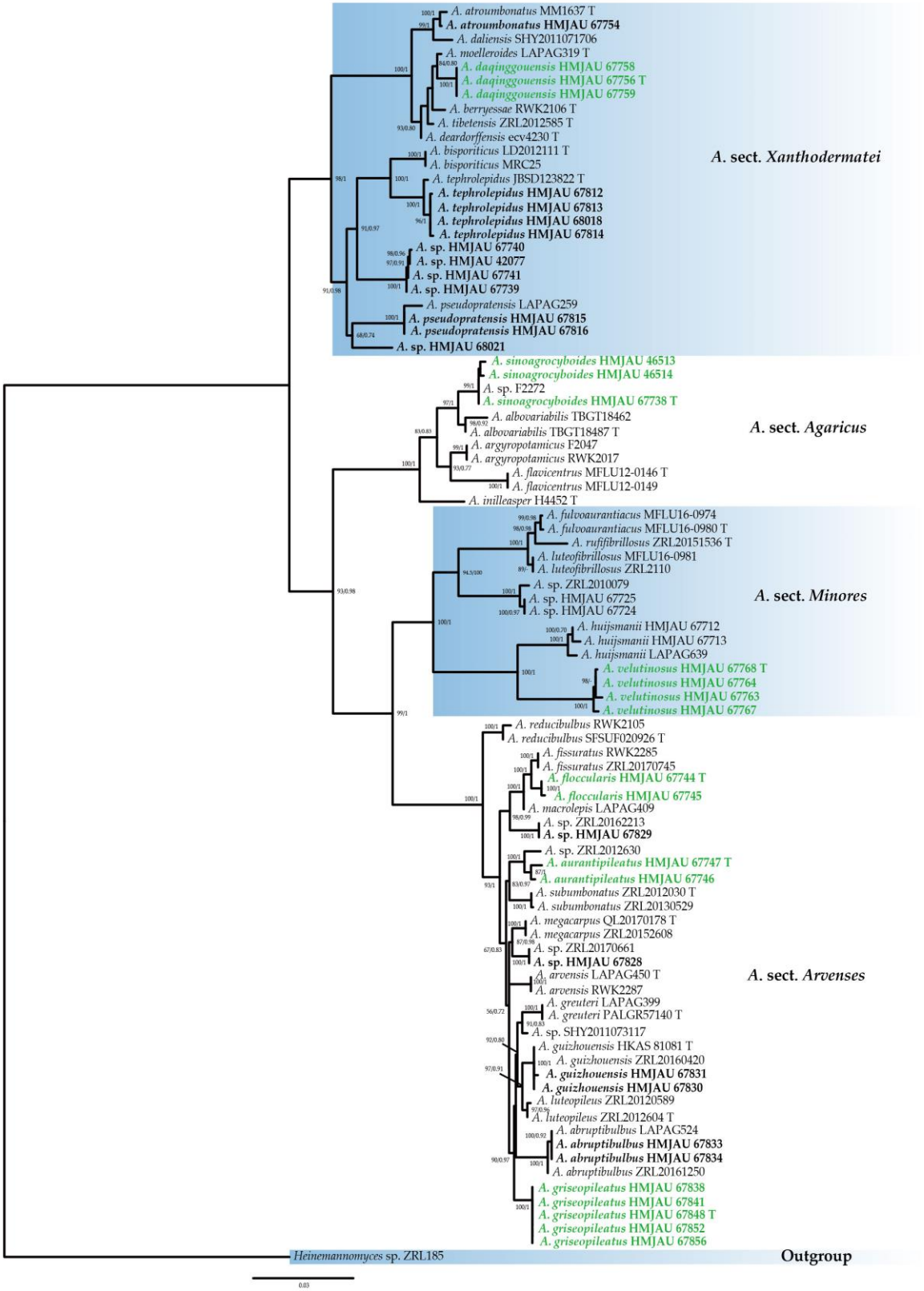

Figure 1. Multi-gene phylogenetic tree of *Agaricus* obtained from the maximum likelihood analysis (ML) based on ITS, nrLSU, and *tef1-a*. *Heinemannomyces* sp. ZRL185 was used as outgroup. Bootstrap support (BS) values ≥50% and Bayesian posterior probability (PP) values ≥0.70 are indicated on ~~clades~~ (BS/PP). 'T' refers to ~~holotype~~. Bold refers to the sequences produced from this study. Green font refers to the new species.



Figure 2. The photographs of fresh basidiomata of *Agaricus* species in this study. (**a–b**) *A. sinoagrocyboides* HMJAU 67738; (**c–d**) *A. aurantipileatus* HMJAU 67747; (**e–f**) *A. floccularis* HMJAU 67744; (**g–h**) *A. griseopileatus* HMJAU 67848; (**i–j**) *A. velutinosus* (**i**) HMJAU 67768, (**j**) HMJAU 67763; (**k–l**) *A. daqinggouensis* (**k**) HMJAU 67756, (**l**) HMJAU 67759. Bars: (**a–l**) =1 cm.

3.2. Taxonomy

Agaricus sinoagrocyboides T. Bau & S.E. Wang, **sp. nov.** (Figures 2 a–b, 3)

MycoBank: MB 851075

Etymology: '*sinoagrocyboides*' refers to the macroscopic characteristics of this Chinese species which are like those of *Agrocybe* species.

Holotype: China, Anhui Province: Feidong county, Hefei City, 117°19′–117°52′ E, 31°34′–32°16′ N, alt. 28 m, 31 July 2022, Tolgor Bau & Hong Cheng, C22073101 (HMJAU 67738).

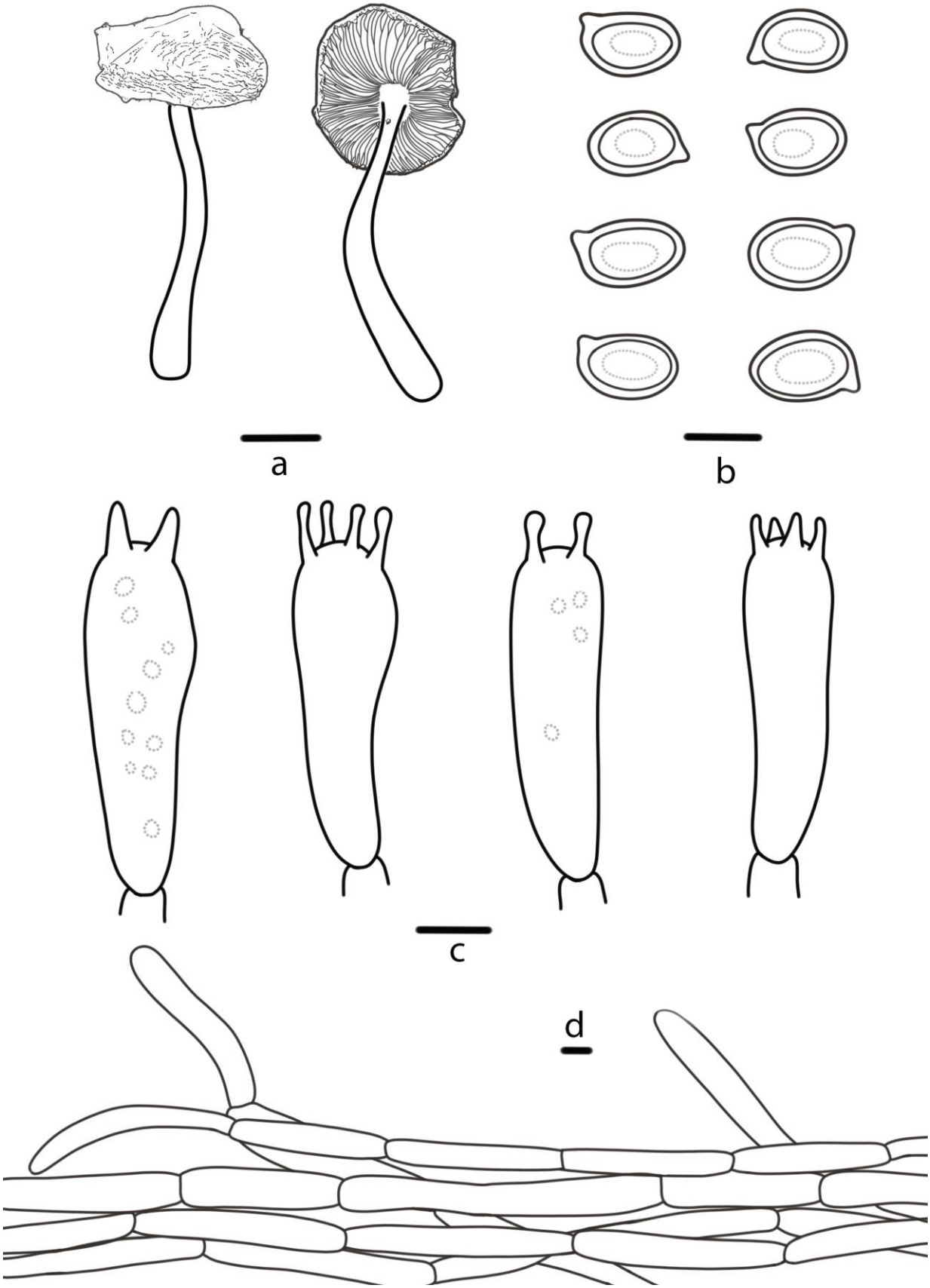

Figure 3. *Agaricus sinoagrocyboides* (HMJAU 46514, HMJAU 67738) (**a**) Basidiomata, (**b**) Basidiospores, (**c**) Basidia, (**d**) Pileipellis. Bars: (**a**) =1 cm, (**b–d**) =5 μm.

Diagnosis: *A. sinoagrocyboides* is characterized by a small to medium sized yellowish white (3A2) basidiomata, pileus with white (2A1) fibrils and cheilocystidia absent.

Pileus 2.3–5.8 cm in diameter, convex to plane, obtusely umbonate at the center, surface dry, white (2A1), yellowish white (3A2), yellowish pale (2A3), with white (2A1) fibrils (especially at margin), sometimes wrinkled, with an appendiculate margin by annulus remnants. **Lamellae** 0.2–0.6 cm broad, brownish grey (11F2), then chestnut (6F7) to brownish black (8F8), free, crowded, intercalated with numerous lamellulae. **Stipe** 3.5–5.2 cm long, 0.3–0.9 cm thick, nearly cylindrical with bulbous base, yellowish white (3A2), dull yellow (3B3) or straw yellow (3B4), hollow, nearly smooth, or below the annulus with white (2A1) fibrils. **Annulus** superior, simple, membranous, white (2A1), easy falling out. **Context** white (2A1), thin, no special odor.

Basidiospores (5.8) 6.0–7.4 (7.6) × (3.9) 4.2–5.1 (5.2) µm, [Xav = 6.9 × 4.6], Q = 1.36–1.66, Qav = 1.49, ellipsoid to elongate-ellipsoid, smooth, brown, thick-walled, guttulate. **Basidia** 18–26 × 6–8 µm, clavate, 4(2)-spored, sterigmata 2–3 µm long. **Cheilocystidia** absent. **Pleurocystidia** absent. **Pileipellis** a cutis of cylindrical hyphae, 5–10 µm wide, smooth, hyaline, slightly constricted at the septa.

Habit, habitat, and distribution: Solitary or scattered in grassland in summer. Currently, only known from Anhui and Liaoning Provinces, China.

Species-specific ITS markers in *A.* sect. *Agaricus*: gtcttCggttg@115, gcctgTcgggg@235, and tggcgCgggga@636.

Additional specimens examined: China, Liaoning Province: Economic and Technological Development Zones, Dalian City, 2 July 2017, Tolgor Bau, HMJAU 46513, HMJAU 46514.

Notes: *A. sinoagrocyboides* belongs to *A.* sect. *Agaricus*. Before *A. sinoagrocyboides*, five species of this section have been found in China: *A. argenteus* Braendle ex Peck, *A. aristocratus* Gulden, *A. griseicephalus* Kerrigan, *A. jilinensis* R.L. Zhao & A.Q. Liu, and *A. zhangyensis* R.L. Zhao & A.Q. Liu [24]. *Agaricus argenteus*, *A. aristocratus* and *A. zhangyensis* differs in having a white pileus; *A. griseicephalus* has a smooth, light brown pileus at maturity, and smaller Qav (Qav = 1.3); and *A. jilinensis* in having a dark brown, with reddish-brown fibrille squamules pileus [24].

In the phylogenetic tree (Figure 1), *A. sinoagrocyboides* and *A.* sp. F2272, uploaded by Zhao et al (2011) [7], formed a clade. *A. albovariabilis* C.P. Arya & C.K. Pradeep and *A. sinoagrocyboides* formed a sister clade with a high support value. However, *A. albovariabilis* possesses a white basidiomata, becoming brownish at maturity, larger basidia (16–31 × 7.2–10.4 µm), and clavate to vesiculose clavate cheilocystidia [43].

Agaricus agrocyboides Heinem. & Gooss.-Font. was found in index Fungorum (https://www.speciesfungorum.org/Names/SynSpecies.asp?RecordID=292238). Although no molecular information on *A. agrocyboides* is available, it differs from *A. sinoagrocyboides* in having a brown pileus, slightly pink at margin, with concolor scales, longer stipe (6–8 cm) with small ochre scales below the annulus, narrower basidiospores (5.2–6.6 × 3.2–3.8 µm), smaller basidia (15–18 × 5.5–7 µm) and abundant, pyriform cheilocystidia [15]. According to Zhao et al. [48], *A. agrocyboides* belongs to the tropical species of *A.* sect. *Arvenses*.

Agaricus aurantipileatus T. Bau & S.E. Wang, **sp. nov.** (Figures 2 c–d, 4)

MycoBank: MB 851079

Etymology: '*aurantipileatus*' refers to the orange (5A8), brownish yellow (5C8) floccose squamules on the pileus of this species.

Holotype: China, Jilin Province: Zhuque Mountain, Jilin City, 126°40′ 52″ E, 43°46′ 46″ N, alt. 309.7 m, 19 July 2023, Tolgor Bau & Xia Wang, W23071914 (HMJAU 67747).

Diagnosis: *A. aurantipileatus* is characterized by its pileus with orange (5A8), brownish yellow (5C8), concentrically arranged floccose squamules, context unchanging when cut, lamellae pale red (10A3), reddish brown (8E8), or slightly purplish pink (14A3), and stipe with bulbous base.

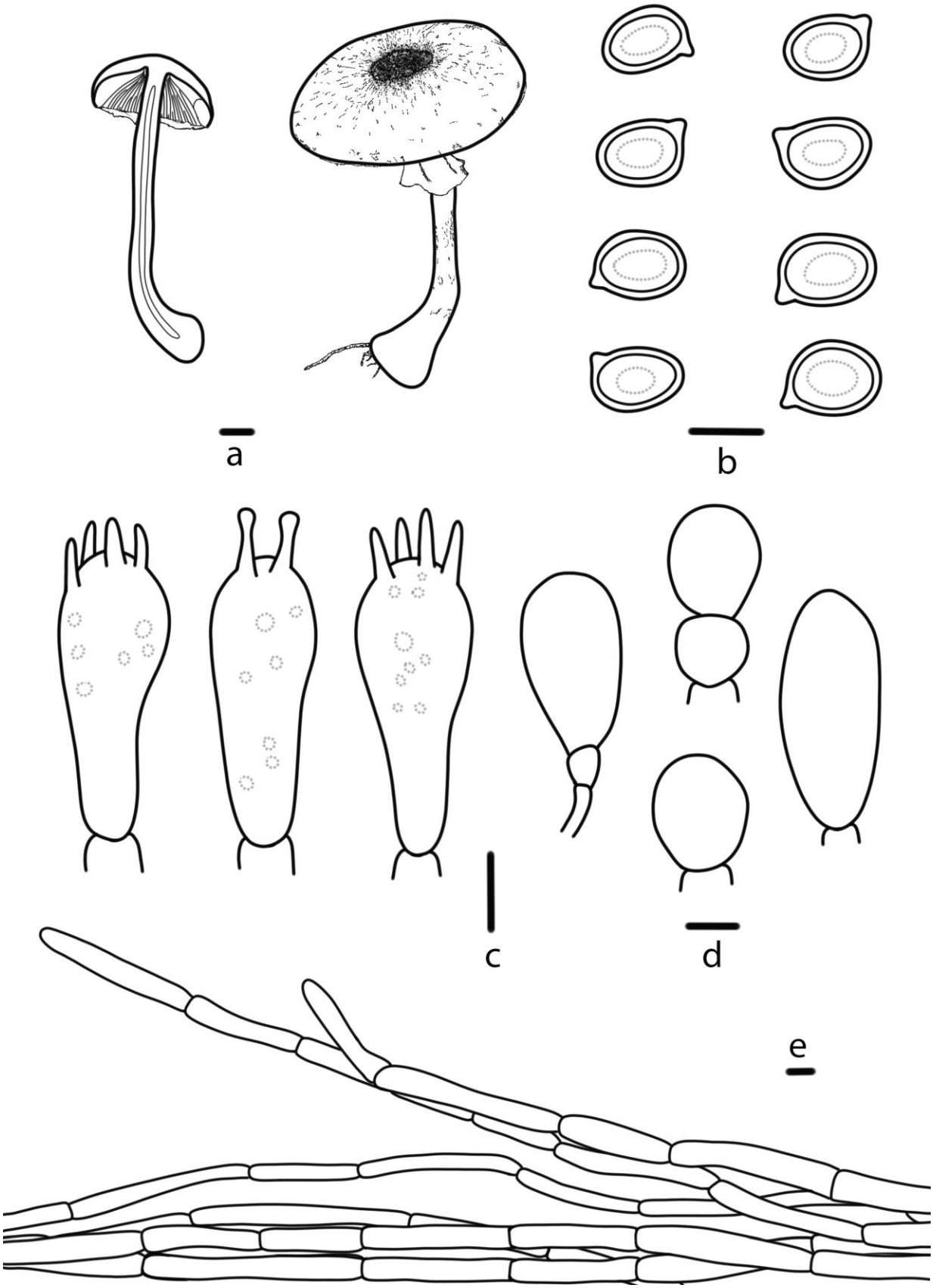

Figure 4. *Agaricus aurantipileatus* (HMJAU 67746, HMJAU 67747) (**a**) Basidiomata, (**b**) Basidiospores, (**c**) Basidia, (**d**) Cheilocystidia, (**e**) Pileipellis. Bars: (**a**) =1 cm, (**b–e**) =5 μm.

Pileus 4.1–9.0 cm in diameter, hemispherical, convex to plane, slightly depressed center, surface dry, white (5A1) or orange white (5A2), covered with orange (5A8) or brownish yellow (5C8) floccose squamules, concentrically arranged, denser at the brownish yellow (5C8) or topaz (5C5) disc, margin appendiculate by annulus remnants. **Lamellae** 0.3–0.5 cm broad, pale red (10A3), reddish brown (8E8), or slightly purplish pink (14A3), free, crowded, intercalated with numerous lamellulae. **Stipe** 9.0–9.5 cm long, 0.6–1.9 cm thick, nearly cylindrical with bulbous base, with short white (5A1) rhizoids, hollow, white (5A1), pale red (10A3), with fibrillose below the annulus. **Annulus** superior, double, curtain-like drooping, membranous, slightly filamentous membranous near the stipe, white (5A1), flocculent squamules on the lower surface, persistent. **Context** of the pileus up to 0.5-0.6 cm thick, white (5A1), when cut unchanging, no special odor.

Basidiospores (5.2) 5.3–6.6 (6.8) × (4.0) 4.3–5.0 (5.1) µm, [Xav = 5.9 × 4.6], Q = 1.17–1.36, Qav = 1.28, broadly-ellipsoid to ellipsoid, smooth, thick-walled, brown, guttulate. **Basidia** 16–22 × 6–8 µm, clavate, 4(2)-spored, sterigmata 2–4 µm long, smooth, hyaline. **Cheilocystidia** abundant, 11–25 × 7–14 µm, nearly globose, oblong or sphaeropedunculate, sometimes catenulate. **Pleurocystidia** absent. **Pileipellis** a cutis of cylindrical hyphae, 4–8 µm wide, light brown, slightly constricted at the septa.

Habit, habitat, and distribution: Gregarious or solitary in broad-leaved forest or coniferous and broad-leaved mixed forest in summer. Currently, only known from Heilongjiang and Jilin Provinces, China.

Species-specific ITS markers in *A*. sect. *Arvenses*: atgggCtgtag@37, tcatcTtgtca@147.

Additional specimens examined: China, Heilongjiang Province: Ussuri River Forest Park, Hulin City, 31 July 2023, Liyang Zhu, Z23073101 (HMJAU 67746).

Notes: *A. aurantipileatus* belongs to *A*. sect. *Arvenses*. *Agaricus aurantipileatus* is similar to *A. guizhouensis* Y. Gui, Zuo Y. Liu & K.D. Hyde of this section. *Agaricus guizhouensis* differs in having a context turning yellow when cut, larger Qav (Qav = 1.68), and smaller cheilocystidia (3.6–16.6 × 3.2–14.3 µm) [44].

In the polygenic tree (Figure 1), *A. aurantipileatus*, *A. subumbonatus* R.L. Zhao & B. Cao and *A.* sp. ZRL2012630 formed a ~~larger~~ clade with a high support value. *Agaricus subumbonatus* possesses a smooth, fibrillose squamulose pileus with age, a cylindrical to slightly clavate stipe and larger basidiospores (Q = 1.3–1.8) [25]. *Agaricus* sp. ZRL2012630 was uploaded by Zhao et al. [2], but no formal description was provided.

Despite some differences in the molecular characters of the specimens HMJAU 67747 and HMJAU 67746, we did not find any obvious morphological differences between them. Therefore, both are here considered conspecific.

Agaricus floccularis T. Bau & S.E. Wang, **sp. nov.** (Figures 2 e–f, 5)

MycoBank: MB 851078

Etymology: '*floccularis*' refers to the pileus of this species with wheat yellow (4B5) to dull yellow (3B3) fibrillose floccose squamules.

Holotype: China, Inner Mongolia Autonomous Region: Horqin Left Middle Banner, Tongliao City, 123°16′ 53″ E, 43°47′ 42″ N, alt. 255.6 m, 21 August 2022, Tolgor Bau & Shi-En Wang, E2208251 (HMJAU 67744).

Diagnosis: Pileus with concentrically arranged, often triangular, fibrillose floccose, wheat yellow (4B5), dull yellow (3B3) squamules, cheilocystidia nearly globose or sphaeropedunculate, sometimes catenulate.

Pileus 5.0–17.5 cm in diameter, truncate conical to plane, with, sometimes, slightly depressed center, white (4A1), yellowish white (4A2), wheat yellow (4B5), with concentrically arranged, often triangular, arranged concentrically, fibrillose floccose squamules, wheat yellow (4B5) or dull yellow (3B3), denser at disc, the squamules sometimes fall off at maturity, becoming nearly smooth, margin brownish beige (6E3), appendiculate by annulus remnants. **Lamellae** 0.3–0.7 cm broad, reddish brown (8E8) to brownish black (8F8), free, crowded, intercalated with numerous lamellulae. **Stipe** 3.5–16.1 cm long, 0.7–3.7 cm thick, nearly cylindrical, or with slightly bulbous base, sometimes with short coarse white (4A1) rhizoids, hollow, white (4A1), yellowish white (4A2), straw yellow (3B4),

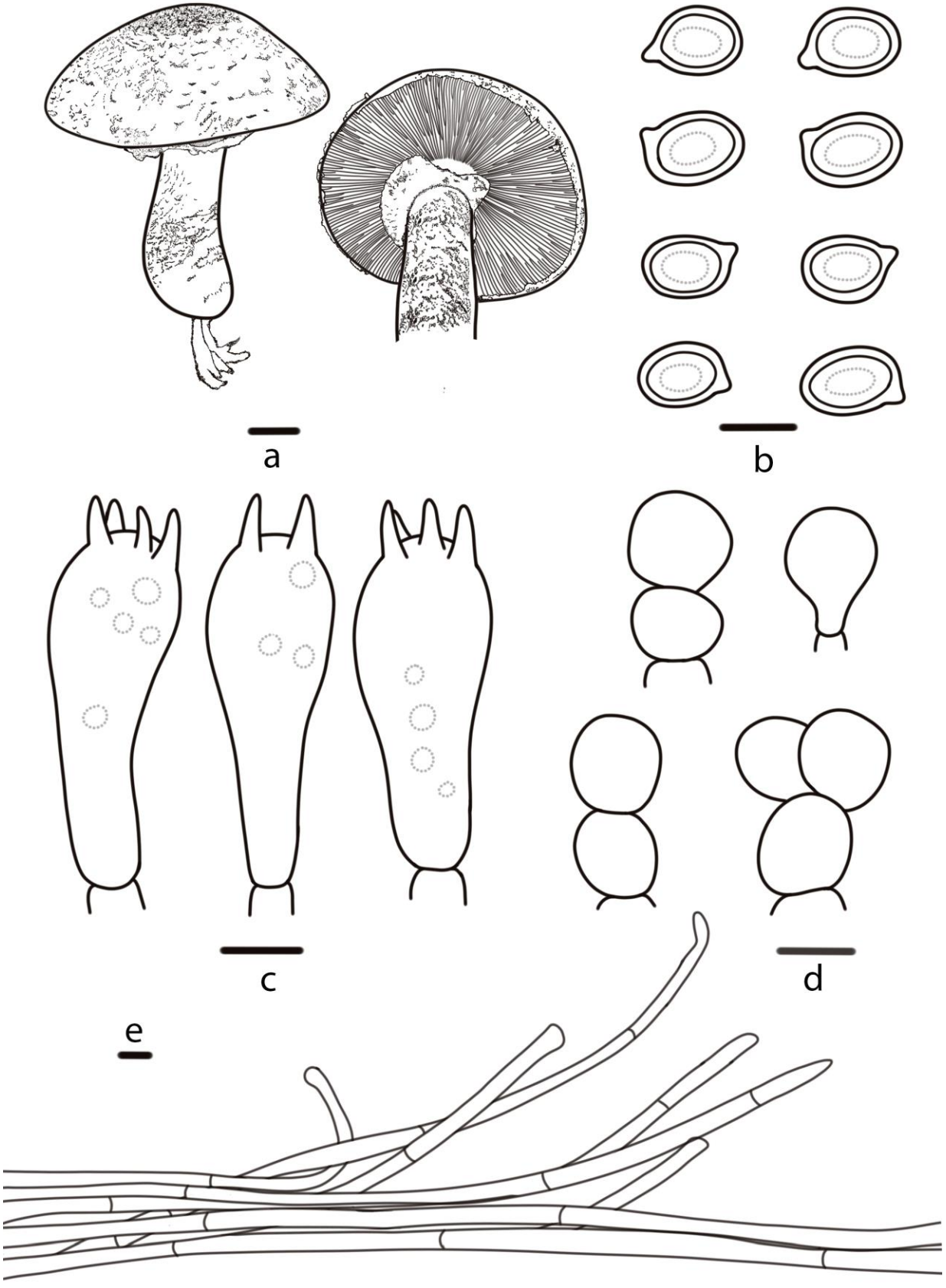

Figure 5. *Agaricus floccularis* (HMJAU 67744, HMJAU 67745) (**a**) Basidiomata, (**b**) Basidiospores, (**c**) Basidia, (**d**) Cheilocystidia, (**e**) Pileipellis. Bars: (**a**) =1 cm, (**b–e**) =5 μm.

dark yellow (4C8) on touching or bruising, smooth above the annulus, woolly-floccose squamules below the annulus. **Annulus** superior, double, membranous, white (4A1), yellowish white (4A2), straw yellow (3B4), fibrillose on the lower surface, persistent. **Context** thick, white (4A1), with no special odor or bitter almond odor.

Basidiospores (6.2) 6.3–7.5 (7.6) × (4.5) 4.8–5.7 (5.9) μm, [Xav = 6.8 × 5.2], Q = 1.17–1.51, Qav = 1.32, broadly-ellipsoid to ellipsoid, smooth, thick-walled, brown, guttulate. **Basidia** 17–24 × 7–9 μm, clavate, 4(2)-spored, sterigmata 2–3 μm long, smooth, hyaline. **Cheilocystidia** abundant, 8–16 × 6–10 μm, nearly globose or sphaeropedunculate, sometimes catenulate. **Pleurocystidia** absent. **Pileipellis** a cutis of cylindrical hyphae, 5–10 μm wide, light brown, not constricted at the septa.

Habit, habitat, and distribution: Solitary in broad-leaved forest in summer and autumn. Currently, only known from Inner Mongolia Autonomous Region and Jilin Province, China.

Species-specific ITS markers in *A*. sect. *Arvenses*: aaatcGctttc@222, gtctcAgtgag@684.

Additional specimens examined: China, Jilin Province: Jilin Agricultural University, Changchun City, 7 September 2022, Shi-En Wang, E2290705 (HMJAU 67745).

Notes: *A. floccularis* belongs to *A*. sect. *Arvenses*. Morphologically *A. floccularis* is similar to *A. greuteri* L.A. Parra, Cappelli & Kerrigan. *Agaricus greuteri* differs in having a smooth or with small squamules stipe provided with numerous white fine mycelial strands at the base, and larger Qav (Qav = 1.48) [17].

In the phylogenetic tree (Figure 1), *A. fissuratus* F.H. Møller, *A. macrolepis* (Pilát & Pouzar) Boisselet & Courtec. and *A. floccularis* formed a larger clade, with a well support value. *Agaricus fissuratus* differs in having an ochre to orange-red, often radially cracked pileus, larger basidiospores (Qav = 1.52) and larger (7–45 × 5–27 μm) spherical, pyriform or broadly clavate ~~cheilocystidia~~ [17]. *Agaricus macrolepis* has a rusted ochre, often fissured pileus and larger (17–40 × 14–32 μm), pyriform, nearly spherical, or broadly clavate cheilocystidia [17].

It is worth mentioning that *A. fissuratus* is considered a synonym for *A. arvensis* Schaeff. in Index fungorum (http://indexfungorum.org/Names/Names.asp). However, the study of Parra showed that *A. fissuratus* is an independent species [17]. Cao et al. [25] and Medel-Ortiz et al. [18] accepted this view. This study agrees with Parra's view.

Agaricus griseopileatus T. Bau & S.E. Wang, **sp. nov.** (Figures 2 g–h, 6)

MycoBank: MB 851080

Etymology: '*griseopileatus*' refers to the pastel grey (2C1) pileus of this species.

Holotype: China, Jilin Province: Jilin Agricultural University, Changchun City, 125°24′ 44″ E, 43°48′ 27″ N, alt. 279.7 m, 22 July 2023, Tolgor Bau & Shi-En Wang, E23072202 (HMJAU 67848).

Diagnosis: *A. griseopileatus* is characterized by its pileus with white (2A1), greyish white (2B1) fibrils when young, nearly smooth at maturity, context unchanging when cut, lamellae first reddish grey (10B2), then reddish brown (8E8) to brownish black (8F8), and stipe with fibrillose below the annulus (especially at the base).

Pileus 2.0–11.1 cm in diameter, hemispherical, convex to plane, white (2A1), pastel grey (2C1), yellowish pale (2A3) at the center, with white (2A1), greyish white (2B1) when fibrils young, nearly smooth at maturity, sometimes cracked, slightly longitudinally striated, and margin appendiculate by annulus remnants. **Lamellae** 0.3–0.9 cm broad, reddish grey (10B2) initially, reddish brown (8E8) to brownish black (8F8), free, crowded, intercalated with numerous lamellulae. **Stipe** 7.5–12.8 cm long, 0.7–2.4 cm thick, nearly cylindrical, or with bulbous base, sometimes with white (2A1) rhizoids, hollow, white (2A1), yellowish white (2A2), fibrillose below the annulus (especially at the base). **Annulus** superior, double, white (2A1), yellowish white (2A2) or brownish black (8F8), membranous, or filamentous towards its insertion to the stipe, with flocculent squamules on the lower surface, cogwheel-like at margin, persistent. **Context** of the pileus up to 0.6–1.0 cm thick, white (2A1), unchanging when cut, no special odor.

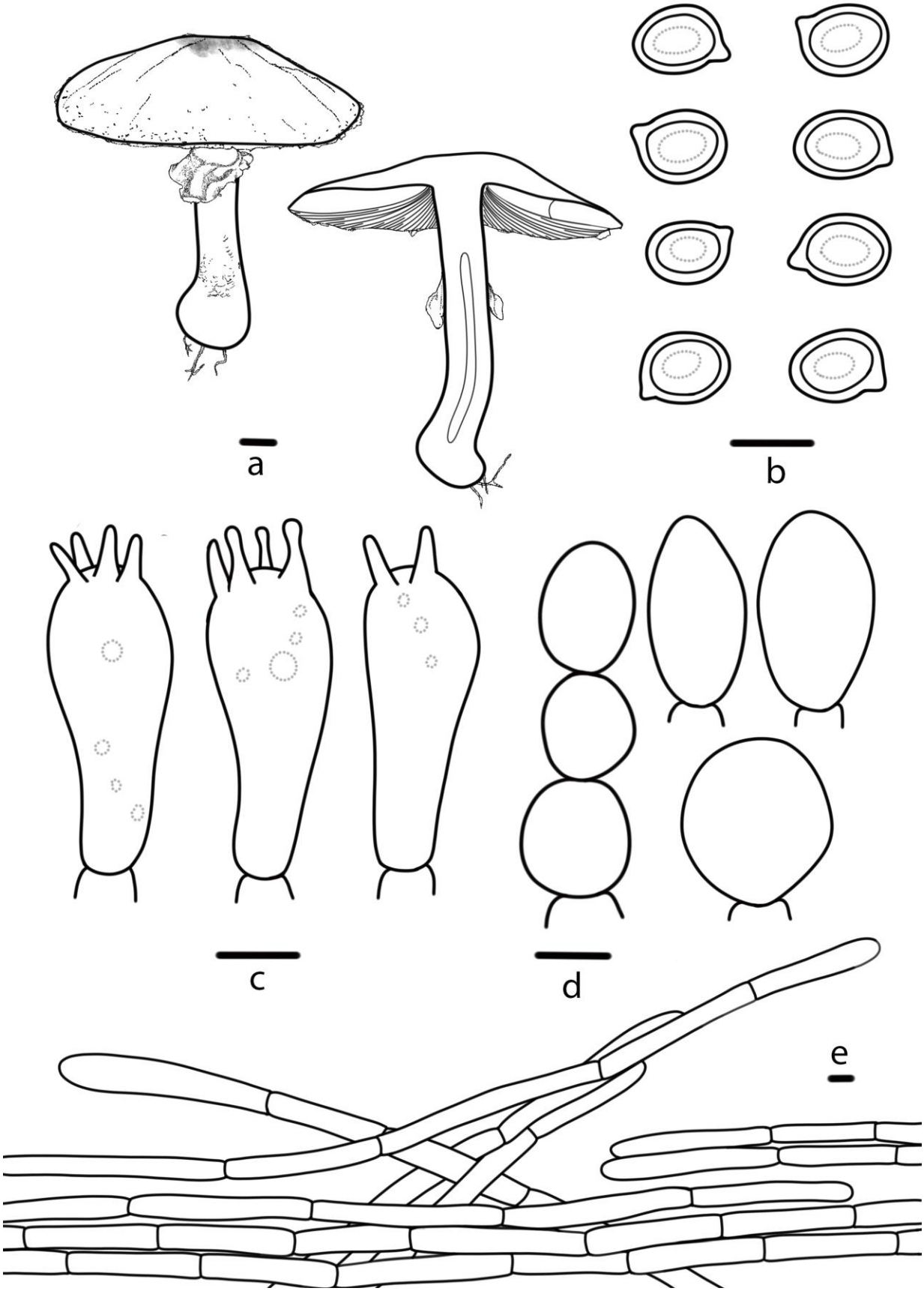

Figure 6. *Agaricus griseopileatus* (HMJAU 67838, HMJAU 67841, HMJAU 67848) (**a**) Basidiomata, (**b**) Basidiospores, (**c**) Basidia, (**d**) Cheilocystidia, (**e**) Pileipellis. Bars: (**a**) =1 cm, (**b–e**) =5 μm.

Basidiospores (5.7) 5.8–7.3 (7.4) × (4.1) 4.2–5.4 (5.6) µm, [Xav = 6.6 × 4.8], Q = 1.24–1.58, Qav = 1.39, broadly-ellipsoid to ellipsoid, smooth, thick-walled, brown, guttulate. **Basidia** 15–24 × 7–9 µm, clavate, 4(2)-spored, sterigmata 2–3 µm long, smooth, hyaline. **Cheilocystidia** abundant, 7–19 × 6–16 µm, nearly globose, oblong, sometimes catenulate or clustered. **Pleurocystidia** absent. **Pileipellis** a cutis of cylindrical hyphae, 5–8 µm wide, hyaline, slightly constricted or not at the septa.

Habit, habitat, and distribution: Gregarious or solitary in broad-leaved forest or coniferous and broad-leaved mixed forest in summer and autumn. Currently, only known from Inner Mongolia Autonomous Region and Jilin Province, China.

Species-specific ITS markers in *A*. sect. *Arvenses*: tttctGaatgg@30, ttgagCtagga@127, and ctactCttgaa@697.

Additional material examined: China, Inner Mongolia Autonomous Region: Tuquan County, Hinggan League, 26 August 2023, Qianru Liu, R238133 (HMJAU 67861). China, Jilin Province: Jingyuetan National Forest Park, Changchun City, 26 September 2020, Qingqing Dong, 21826DQQ4 (HMJAU 67852), same location, 22 August 2022, Shi-En Wang, E2208183 (HMJAU 67853); Tongyu County, Baicheng City, 19 August 2021, Tolgor Bau & Liyang Zhu, Z2181934 (HMJAU 67859); Jilin Agricultural University, Changchun City, 3 July 2022, Shi-En Wang, E22070316 (HMJAU 67838), same location, 11–12 July 2022, Shi-En Wang, E22071108 (HMJAU 67836), E22071204 (HMJAU 67841); Border of Songhua River, Jilin City, 19 July 2023, Xia Wang & Shi-En Wang, E2307159 (HMJAU 67855), W23072009 (HMJAU 67856); Entrepreneurial Reservoir, Taonan City, 26 August 2023, Shi-En Wang, E2308356 (HMJAU 67857).

Notes: *A. griseopileatus* belongs to *A*. sect. *Arvenses*. *Agaricus griseopileatus* is similar to *A. arvensis* Schaeff. of this section. *A. arvensis* has white or grayish white lamellae initially , odour of anise or bitter almonds when the pileus margin is bruised, and context, sometimes, becoming yellow on cutting [17]. Although the macroscopic characteristics of both exhibit minimal differences, discernible disparities exist in their molecular characters.

In the polygenic tree (Figure 1), *A. griseopileatus* formed an independent clade with a high support value. *A. abruptibulbus* Peck is a closely related species of *A. griseopileatus*, but *A. abruptibulbus* possesses a mostly pale-yellow pileus, sometimes with yellow floccose squamules, larger basidiospores Q (Q = 1.29–1.81), and larger (16–34 × 14–26 µm), spherical, pyriform or clavate cheilocystidia [17].

Agaricus velutinosus T. Bau & S.E. Wang, **sp. nov.** (Figures 2 i–j, 7)

MycoBank: MB 851077

Etymology: '*velutinosus*' refers to the fibrillose to minutely floccose white (2A1) pileus and stipe surface of this species velutinous appearance.

Holotype: China, Jilin Province: Qianjin Experimental Forestry Farm, Jiaohe City, 127° 42′18″ E, 43°57′25″ N, alt. 460.2 m, 25 August 2023, Tolgor Bau & Hong Cheng, C2382515 (HMJAU 67768).

Diagnosis: *A. velutinosus* is characterized by small-sized white (2A1) basidiomata, a white (2A1) fibrillose minutely floccose, looking like velutinous, pileus and stipe surfaces, lamellae white (2A1), then pastel grey (2C1), finally brownish grey (8C2), cheilocystidia clavate, broadly clavate, or globose with a long peduncle.

Pileus 1.2–2.4 cm in diameter, convex to plane, obtusely umbonate at the center, white (2A1), yellowish white (2A2) at the center, with white (2A1) looking like velutinous, margin appendiculate by annulus remnants. **Lamellae** 0.1–0.2 cm broad, white (2A1) to pastel grey (2C1) or brownish grey (8C2), free, crowded, intercalated with numerous lamellulae. **Stipe** 1.3–3.9 cm long, 0.2–0.8 cm thick, nearly cylindrical with bulbous base, white (2A1), greyish white (2B1), yellowish white (2A2), hollow, with white (2A1) slender rhizoids, with white (2A1), greyish white (2B1) fibrillose to minutely floccose white squamules below the annulus. **Annulus** superior, simple, white (2A1), greyish white (2B1), membranous, easy to crack radially, easy falling out. **Context** thin, white (2A1), no special odor.

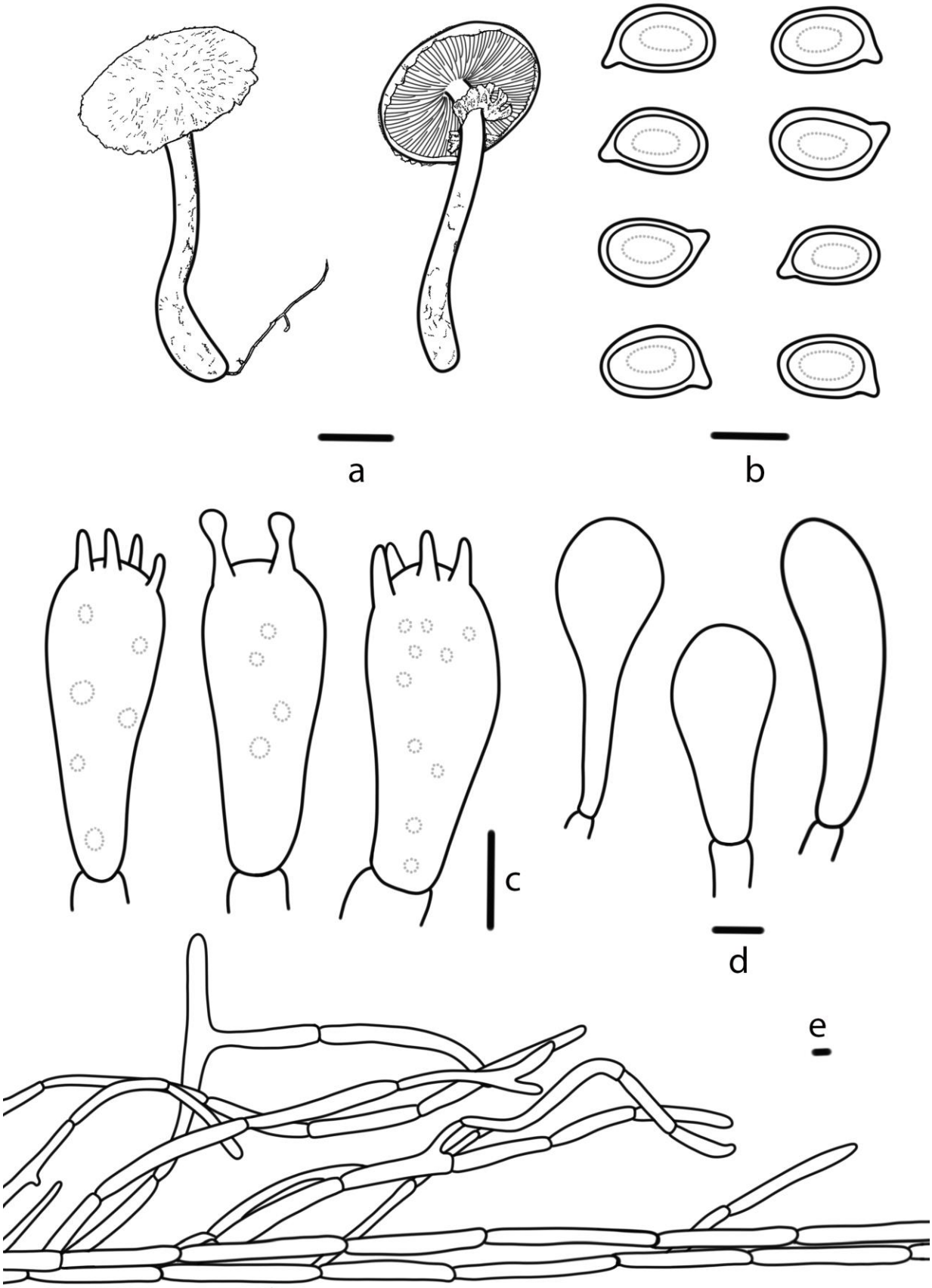

Figure 7. *Agaricus velutinosus* (HMJAU 67764, HMJAU 67768) (**a**) Basidiomata, (**b**) Basidiospores, (**c**) Basidia, (**d**) Cheilocystidia, (**e**) Pileipellis. Bars: (**a**) =1 cm, (**b–e**) =5 μm.

Basidiospores (4.7) 4.9–5.8 (5.9) × (2.9) 3.0–3.7 (3.8) µm, [Xav = 5.2 × 3.4], Q = 1.35–1.76, Qav = 1.52, ellipsoid to elongate-ellipsoid, smooth, brown, thick-walled, guttulate. **Basidia** 14–20 × 5–7 µm, clavate, 4(2) spored, sterigmata 1–3 µm long. **Cheilocystidia** abundant, clavate, broadly clavate, or globose with a long peduncle, 19–32 × 7–13 µm. **Pleurocystidia** absent. **Pileipellis** a cutis of cylindrical hyphae, 5–11 µm wide, slightly constricted at the septa.

Habit, habitat, and distribution: Solitary or gregarious in broad-leaved forest in summer. Currently, only known from Jilin Province, China.

Species-specific ITS markers in *A.* sect. *Minores*: ttagaTttcat@77, gcaatCtgctg@158, gatgtAgggac@169, ggtttAtatgc@299, gaaccGgtttg@592, gataaCtatct@621, and ctaatAgtctc@673.

Additional specimens examined: China, Jilin Province: Qianjin Experimental Forestry Farm, Jiaohe City, 25 August 2023, Tolgor Bau & Xia Wang, W23082521 (HMJAU 67769); Red Rock National Forest Park, Huadian City, 28 August 2023, Tolgor Bau & Xianyan Zhou, Y2382816 (HMJAU 67762), Y2382817 (HMJAU 67763), Y2382818 (HMJAU 67764), Y2382822 (HMJAU 67765), Y2382823 (HMJAU 67766), Y2382824 (HMJAU 67767).

Notes: *A. velutinosus* belongs to *A.* sect. *Minores*. *Agaricus velutinosus* is similar to *A. pseudopallens* M.Q. He & R.L. Zhao, but *A. pseudopallens* has larger basidiomata (pileus diameter 2.3-3.8 cm), annulus single, smooth, and cheilocystidia absent [45].

In the phylogenetic tree (Figure 1), *A. velutinosus* and *A. huijsmanii* Courtec. formed a sister clade with a high support value. However, the pileus of *A. huijsmanii* is larger (1.4–4.0 cm), and cheilocystidia spherical, sometimes catenulate [17].

Agaricus daqinggouensis T. Bau & S.E. Wang, **sp. nov.** (Figures 2 k–l, 8)

MycoBank: MB 851073

Etymology: '*daqinggouensis*' refers to the type locality, Daqinggou National Nature Reserve, of this species.

Holotype: China, Inner Mongolia Autonomous Region: Daqinggou National Nature Reserve, Tongliao City, 122° 10′ 23.1″ E, 42° 47′ 35.6″ N, alt. 208 m, 19 August 2022, Tolgor Bau & Shi-En Wang, E2208287 (HMJAU 67756).

Diagnosis: Pileus white (2A1) to greyish white (2B1), lamellae pale red (9A3), then reddish brown (8E8) and finally brownish black (8F8), stipe with bulbous base, becoming yellow (3B8) at the base on cutting, and cheilocystidia nearly globose or pyriform, sometimes catenulate.

Pileus 2.5–12.9 cm in diameter, hemispherical, truncate conical to plane, slightly depressed at the center, surface dry, white (2A1), greyish white (2B1) or pastel grey (2C1), darker at disc, nearly smooth, or with greyish white (2B1) fine fibrils, margin appendiculate by annulus remnants. **Lamellae** 0.4–0.9 cm broad, pale red (9A3), then reddish brown (8E8), finally brownish black (8F8), free, crowded, intercalated with numerous lamellulae. **Stipe** 4.0–12.8 cm long, 0.6–2.4 cm thick, nearly cylindrical with bulbous base, white (2A1) to honey yellow (4D6), hollow, sometimes with white (2A1) rhizoids, nearly smooth, or fibrillose below the annulus (especially at the base). **Annulus** superior, double, with irregular floccose squamules on the lower surface, white (2A1), then reddish brown (8E8), membranous, pendant and persistent. **Context** of the pileus up to 0.8–1.4 cm thick, white (2A1), greyish white (2B1), becoming yellow (3B8) at stipe base on cutting, with no special odor.

Basidiospores (4.8) 5.0–6.2 (6.3) × (3.2) 3.3–3.4 (3.5) µm, [Xav = 5.5 × 3.4], Q = 1.38–1.82, Qav = 1.63, ellipsoid to elongate-ellipsoid, smooth, brown, thick-walled, guttulate. **Basidia** 13–19 × 5–7 µm, clavate, 4(2)-spored, sterigmata 1–3 µm long. **Cheilocystidia** 7–20 × 7–14 µm, abundant, nearly globose, or pyriform, sometimes catenulate. **Pleurocystidia** absent. **Pileipellis** a cutis of cylindrical hyphae, 5–12 µm wide, hyaline, slightly constricted at the septa.

Habit, habitat, and distribution: Gregarious or scattered in broad-leaved forest, coniferous forest or in grassland in summer. Currently, only known from Jilin Province and Inner Mongolia Autonomous Region, China.

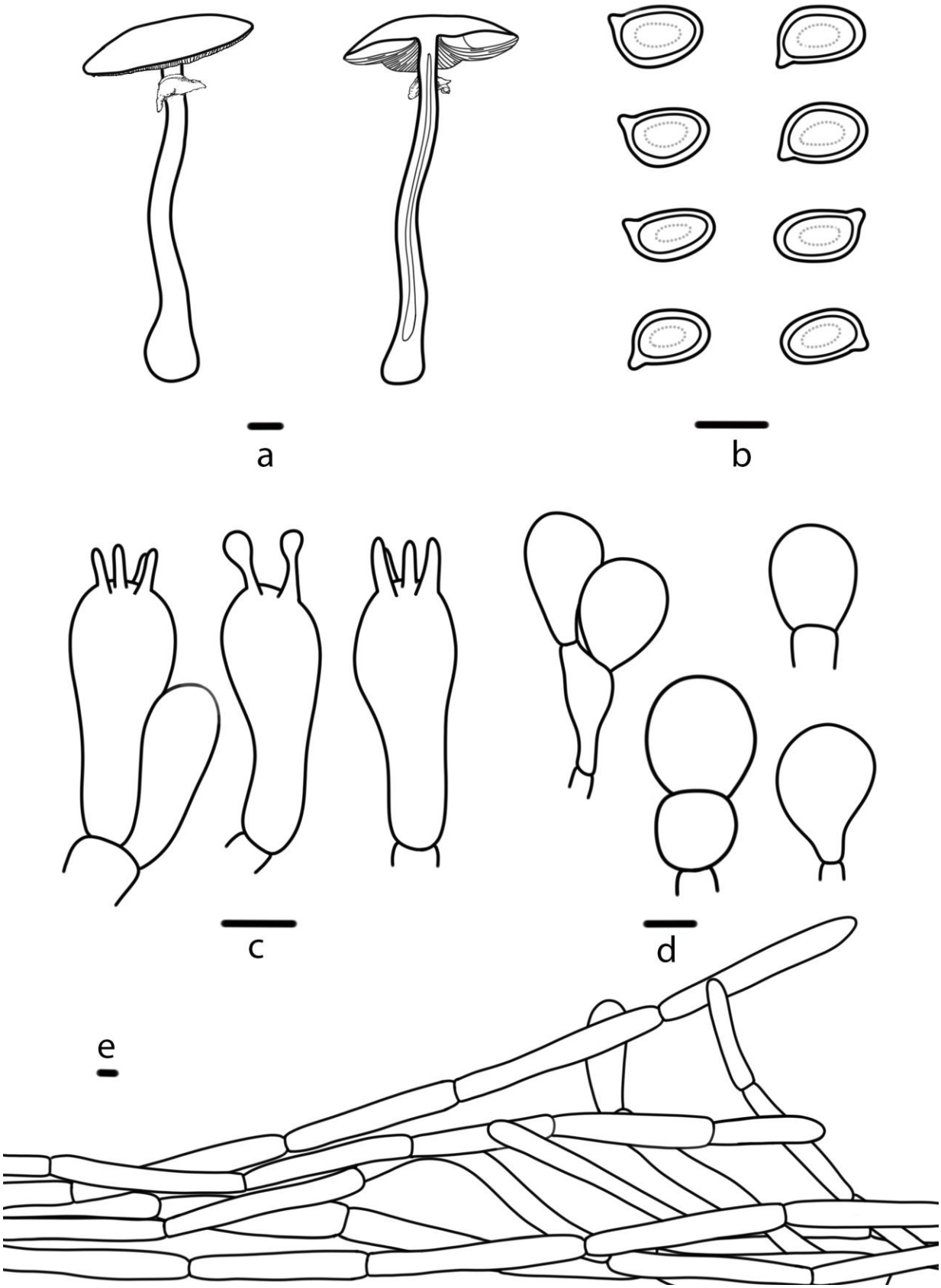

Figure 8. *Agaricus daqinggouensis* (HMJAU 67756, HMJAU 67759) (**a**) Basidiomata, (**b**) Basidiospores, (**c**) Basidia, (**d**) Cheilocystidia, (**e**) Pileipellis. Bars: (**a**) =1 cm, (**b**–**e**) =5 μm.

Species-specific ITS markers in *A.* sect. *Xanthodermatei*: tggatTtgagg@167, accctTtaaac@264, and gttttCacatg@288.

Additional specimens examined: China, Inner Mongolia Autonomous Region: Daqinggou National Nature Reserve, Tongliao City, 19 August 2022, Tolgor Bau & Shi-En Wang, E2208285 (HMJAU 67755), E2208295 (HMJAU 67757), same location, 16 July 2023, Tolgor Bau & Qianru Liu, R23796 (HMJAU 67760). China, Jilin Province: Hongye Valley, Jiaohe City, 24 July 2022, Tolgor Bau & Shi-En Wang, E220723 (HMJAU 67758); Longtan Mountain, Jilin City, 20 June 2023, Shi-En Wang, E2307182 (HMJAU 67759).

Notes: *A. daqinggouensis* belongs to *A.* sect. *Xanthodermatei*. The superficial morphological characteristics of *A. daqinggouensis* and *A. arvensis* Schaeff. are similar. However, *A. arvensis* differs in having basidiospores usually broadly ellipsoid to ellipsoid, rarely subglobose, smaller Qav (Qav = 1.34) and larger Xav (Xav = 6.9 × 5.18), and odour of anise or bitter almonds when the pileus margin is bruised [17].

In the phylogenetic tree (Figure 1), *A. berryessae* Kerrigan, *A. deardorffensis* Kerrigan, *A. moelleroides* Guinb. & L.A. Parra, and *A. tibetensis* J.L. Zhou & R.L. Zhao, are the closely related species to *A. daqinggouensis*. While *A. daqinggouensis* and *A. moelleroides* formed a sister clade. However, *A. moelleroides* has a pileus with dense grain-brown squamules and white to brown lamellae [17]. *Agaricus berryessae* has a cylindrical stipe often tapered toward base, odor mildly phenolic (stronger when dried), and larger basidia (19–26 × 5.5–7.5) [3]. *Agaricus deardorffensis* has dark brown squamules at the center of the pileus and narrower cheilocystidia (11–18.5 × 4.5–8 μm) [3]. *Agaricus tibetensis* has dark brown squamules at the center of the pileus, cheilocystidia absent [47].

4. Discussion

The macroscopic and microscopic characteristics of many *Agaricus* species overlap, therefore, molecular data and phylogenetic analysis are necessary to identify *Agaricus* species having similar morphological features [3, 7, 17]. *Agaricus griseopileatus*, a novel species examined in this study, serves as an exemplary illustration. The morphological distinction between *A. griseopileatus* and *A. arvensis* Schaeff. poses challenges, whereas their differentiation in phylogenetic analysis is straightforward. The exclusive base on ITS sequences for phylogenetic analysis of *Agaricus* is not methodologically rigorous [25]. The more DNA regions, nrLSU + *tef1-a*, should be added to ensure the accuracy of species identification and the stability of phylogenetic relationships.

Agaricus daqinggouensis belongs to *A.* sect. *Xanthodermatei.* However, certain species within *A.* sect. *Xanthodermatei* and *A.* sect. *Hondenses* exhibit toxicity, such as *A. xanthodermus* Genev., which can induce gastrointestinal symptoms [49–51]. Despite in lack of research, *A. daqinggouensis* is unsuitable for consumption due to potential toxicity. Because some *Agaricus* species are so similar in morphological characteristics, it is difficult to identify *Agaricus* species in the wild. Refraining from consuming wild *Agaricus* species is advised as a precautionary measure against mushroom poisoning. Furthermore, there is a need for further investigation into the toxic components, mechanisms of poisoning, detoxification processes, and other aspects related to the toxicity of *Agaricus* species.

Agaricus floccularis, *A. aurantipileatus* and *A. griseopileatus* are members of *A.* sect. *Arvenses*. Currently, *A.* sect. *Arvenses* has not been reported to contain any toxic species, with several exhibiting nutritional or medicinal potential, such as cultivated species including *A. arvensis* Schaeff., *A. augustus* Fr., and *A. subrufescens* Peck. [3, 17, 25]. We postulate that *A. floccularis*, *A. aurantipileatus* and *A. griseopileatus* are also edible species that may have potential medicinal and edible values as new cultivatable species. Particularly noteworthy, *A. griseopileatus* was reported to be edible by some locals, which is precious fungal resources worthy of further excavation and development.

This study encompassed six new species in China, thereby enriching the species diversity of *Agaricus* in Northeast China. In the phylogenetic tree (Figure 1), some species could not be determined. For instance, *Agaricus* sp. HMJAU 67828, collected from Jilin Province, consisted of immature basidiomata, with only one specimen obtained; thus, it

has ~~been not published~~. The findings of this study indicate the potential existence of undiscovered species in Northeast China needs to be studied further.

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