

Article

Two New Species of *Hymenogaster* (Hymenogastraceae, Agaricales) from China Based on Morphological and Molecular Markers

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Abstract: Two new species from China, *Hymenogaster pseudoniveus* and *H. zunhuaensis*, are described and illustrated based on morphological and molecular evidence. *Hymenogaster pseudoniveus* is distinguished from other species of the genus based on the color of peridium, which is snow white to white when unexcavated but then turns yellowish to earth yellow, and the basidiospores, which are ornamented with nearly longitudinally arranged ridges up to 2 µm high. *H. zunhuaensis* is diagnosed by its dirty white to pale yellow peridium, yellow brown to brown gleba, and the smaller ($L_m \times W_m = 11.7 \mu\text{m} \times 9.8 \mu\text{m}$) broadly ellipsoidal to subglobose basidiospores ($Q = 1.1\text{--}1.3$). ITS/LSU-based phylogenetic analysis supports the erection of the two new species, each placed in distinct clades with strong statistical support, suggesting that they represent two distinct species novel to science. Based on the morphological and molecular evidence, we have published two new species of *Hymenogaster*. A key for *Hymenogaster* species from China is provided.

Keywords: Basidiomycota; hypogaeus; phylogeny; taxonomy



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1. Introduction

As a globally widespread genus, the *Hymenogaster* are found in Europe [1–4], Asia [5,6], North America [7] of the Northern Hemisphere, Australia [8], Oceania [9,10], South America [11], and Central Africa [12] of the Southern Hemisphere. In China, *Hymenogaster* has been recorded across 21 provinces or cities, notably in areas such as Hebei, Shaanxi, Shanxi, and Yunnan [13,14], which are recognized for their high species richness.

As hypogaeous fungi, the *Hymenogaster* species symbiotically form ectomycorrhizae with various tree species, helping plants uptake nutrients and rebuild the ecosystems [6,15–22]. They also serve as food sources for small mammals in the forest, such as *Clethrionomys gapperi*, *Napaeozapus insignis*, *Sus scrofa*, *Tamias striatus*, *Tamiasciurus hudsonicus*, and *Zapus hudsonius*, as well as some birds and invertebrates, and in this way, these small animals can also help spread *Hymenogaster* spores [23–26].

To date, about 170 species of this group have been reported globally [27], but only 32 species and variants of *Hymenogaster* have been reported in China, and most of them lack molecular data. Recently, seven species have been identified based on morphological and molecular evidence [14]. In this study, two new species were recognized from our collections based on morphological and molecular evidence, which have been described and illustrated.

2. Materials and Methods

2.1. Morphological Studies

Fresh specimens were collected from Hebei, Shanxi, Shaanxi, and Yunnan provinces over a period of several years in China and were subsequently dried and deposited at BJTC

(Herbarium Biology Department, Capital Normal University). Several older specimens were also examined from HMAS (Herbarium Mycologicum Academiae Sinicae, Institute of Microbiology, Chinese Academy of Sciences). Macroscopic characters were recorded from fresh specimens whenever possible. Microscopic characters were examined in both fresh and dried materials by mounting free-hand sections of basidiomata in 5% KOH and Melzer’s reagent [28]. Basidiospores dimensions, excluding ornamentation, were based on 30 spores for each basidiome, with the measurements presented as the diameter (mean diam ± SD, n = 30) for each species. For scanning electron microscopy (SEM), spores were scraped from the dried gleba, placed onto double-sided adhesive tape that was mounted directly on the SEM stub, coated with an 8 nm thick platinum–palladium film using an ion-sputter coater (HITACHI E-1010), and examined with a HITACHI S-4800 SEM.

2.2. DNA Extraction, PCR Amplification, Sequencing and Nucleotide Alignment

Dried gleba was ground by shaking for 45 s at 30 Hz 2–4 times (Mixer Mill MM 301, Retsch, Haan, Germany) in a 1.5 mL tube along with a 3 mm diameter tungsten carbide ball, and total genomic DNA was extracted using the modified CTAB method [29]. The internal transcribed spacer (ITS) region of nuclear ribosomal DNA (nrDNA) was amplified using primers ITS1f/ITS4 [29,30]. The 28S large subunit (nrLSU) nrDNA region was amplified using primers LR0R/LR5 [31]. Polymerase chain reactions (PCRs) were performed in 50 µL reactions containing 2 µL of each DNA primer (10 µM), 25 µL of 2 × Master Mix (Tiangen Biotech (Beijing) Co., Beijing, China), 17 µL ddH₂O, and 4 µL of dNTPs. Amplification reactions were implemented as follows: for the ITS gene—an initial denaturation at 95 °C for 4 min, followed by 35 cycles at 95 °C for 30 s, 55 °C for 45 s, 72 °C for 1 min, and a final extension at 72 °C for 10 min; for the nrLSU gene—an initial denaturation at 95 °C for 4 min, followed by 35 cycles at 95 °C for 30 s, 55 °C for 1 min, 72 °C for 1 min, and a final extension at 72 °C for 10 min. The PCR products were sent to Beijing Zhongkexilin Biotechnology Co., Ltd. (Beijing, China) for purification and sequencing. Validated sequences are stored in the NCBI database (<http://www.ncbi.nlm.nih.gov/>, accessed on 10 April 2024) under the accession numbers provided; other sequences used in this study were downloaded from GenBank (Table 1).

Table 1. Sources of specimens and GenBank accession numbers for sequences used in this study. Chinese sequences from basidiomates are in bold, and newly generated sequences are in red.

Taxon Name in Analysis	Taxon Name	Collection	Country	GenBank Accession Number	
				ITS	nrLSU
<i>Anamika lactariolens</i> AY818352	<i>Anamika lactariolens</i>	taxon:301353		AY818352	-
<i>Anamika lactariolens</i> NR_119524	<i>Anamika lactariolens</i>	HC 88/95		NR_119524	-
<i>Hymenogaster arenarius</i> BJTC FAN786 China	<i>Hymenogaster arenarius</i>	BJTC FAN786	China	PP467413	PP467449
<i>Hymenogaster arenarius</i> BJTC FAN856 China	<i>Hymenogaster arenarius</i>	BJTC FAN856	China	PP467414	PP467450
<i>Hymenogaster arenarius</i> GU479233 Germany	<i>Hymenogaster arenarius</i>	it10_26_2	Germany	GU479233	-
<i>Hymenogaster arenarius</i> GU479272 Germany	<i>Hymenogaster arenarius</i>	it5_2	Germany	GU479272	-
<i>Hymenogaster arenarius</i> GU479278 Germany	<i>Hymenogaster arenarius</i>	it6_3	Germany	GU479278	-
<i>Hymenogaster bulliardii</i> GU479261 Germany	<i>Hymenogaster bulliardii</i>	it20_4	Germany	GU479261	-
<i>Hymenogaster bulliardii</i> GU479262 Germany	<i>Hymenogaster thwaitesii</i>	it20_4_1	Germany	GU479262	-

Table 1. Cont.

Taxon Name in Analysis	Taxon Name	Collection	Country	GenBank Accession Number	
				ITS	nrLSU
<i>Hymenogaster</i> cf. <i>niveus</i> MT005942 Germany	<i>Hymenogaster</i> xxx	KR-M-0044217	Germany	MT005942	-
<i>Hymenogaster</i> cf. <i>niveus</i> MT005967 Germany	<i>Hymenogaster niveus</i> c 'ryptic species 3'	KR-M-0044314	Germany	MT005967	-
<i>Hymenogaster</i> cf. <i>rehsteineri</i> MT005990 Germany	<i>Hymenogaster rehsteineri</i>	KR-M-0044423	Germany	MT005990	-
<i>Hymenogaster citrinus</i> BJTC FAN1079 China	<i>Hymenogaster citrinus</i>	BJTC FAN1079	China	PP467412	PP467448
<i>Hymenogaster citrinus</i> BJTC FAN883 China	<i>Hymenogaster citrinus</i>	BJTC FAN883	China	PP467410	PP467446
<i>Hymenogaster citrinus</i> BJTC FAN915 China	<i>Hymenogaster citrinus</i>	BJTC FAN915	China	PP467411	PP467447
<i>Hymenogaster glacialis</i> AF325634	<i>Hymenogaster</i> sp.	GP 5302	-	AF325634	-
<i>Hymenogaster griseus</i> AF325636 USA	<i>Hymenogaster griseus</i> 2	Trappe 12841	USA	AF325636	-
<i>Hymenogaster knappii</i> GU479287 Germany	<i>Hymenogaster thwaitesii</i>	it9_2	Germany	GU479287	-
<i>Hymenogaster latisporus</i> BJTC FAN1134 China	<i>Hymenogaster latisporus</i>	BJTC FAN1134, holotype	China	PP467404	PP467440
<i>Hymenogaster luteus</i> cf. <i>trigonospora</i> GU479306 Hungary	<i>Hymenogaster luteus</i>	zb1457	Hungary	GU479306	-
<i>Hymenogaster luteus</i> GU479341 Hungary	<i>Hymenogaster luteus</i>	zb2603	Hungary	GU479341	-
<i>Hymenogaster luteus</i> var. <i>berkeleyanus</i> GU479273 Germany	<i>Hymenogaster bulliardii</i>	it5_21	Germany	GU479273	-
<i>Hymenogaster luteus</i> var. <i>subfuscus</i> GU479358 Hungary	<i>Hymenogaster griseus</i> 2	zb37	Hungary	GU479358	-
<i>Hymenogaster luteus</i> var. <i>trigonospora</i> GU479334 Hungary	<i>Hymenogaster luteus</i>	zb235	Hungary	GU479334	-
<i>Hymenogaster lycoperdineus</i> GU479353 Hungary	<i>Hymenogaster griseus</i> 2	zb3533	Hungary	GU479353	-
<i>Hymenogaster megasporus</i> GU479239 Germany	<i>Hymenogaster megasporus</i>	it12_1	Germany	GU479239	-
<i>Hymenogaster megasporus</i> GU479286 Germany	<i>Hymenogaster megasporus</i>	it8_5_1	Germany	GU479286	-
<i>Hymenogaster minisporus</i> BJTC FAN1244 China	<i>Hymenogaster minisporus</i>	BJTC FAN1244, holotype	China	PP467407	PP467443
<i>Hymenogaster niveus</i> GU479255 Germany	<i>Hymenogaster niveus</i> c 'ryptic species 1'	it17_3	Germany	GU479255	-
<i>Hymenogaster niveus</i> GU479307 Hungary	<i>Hymenogaster</i> xxx	zb1461	Hungary	GU479307	-
<i>Hymenogaster niveus</i> GU479344 Hungary	<i>Hymenogaster niveus</i> c 'ryptic species 3'	zb28	Hungary	GU479344	-
<i>Hymenogaster niveus</i> KU878613 USA	<i>Hymenogaster</i> xxx	SC14_3	USA	KU878613	-
<i>Hymenogaster olivaceus</i> GU479292 Belgium	<i>Hymenogaster citrinus</i>	dt8293	Belgium	GU479292	-
<i>Hymenogaster olivaceus</i> GU479313 Hungary	<i>Hymenogaster citrinus</i>	zb1645	Hungary	GU479313	-

Table 1. Cont.

Taxon Name in Analysis	Taxon Name	Collection	Country	GenBank Accession Number	
				ITS	nrLSU
<i>Hymenogaster olivaceus</i> GU479317 Hungary	<i>Hymenogaster citrinus</i>	zb1817	Hungary	GU479317	-
<i>Hymenogaster olivaceus</i> GU479332 Hungary	<i>Hymenogaster citrinus</i>	zb2300	Hungary	GU479332	-
<i>Hymenogaster olivaceus</i> GU479345 Hungary	<i>Hymenogaster thwaitesii</i>	zb2804	Hungary	GU479345	-
<i>Hymenogaster olivaceus</i> GU479359 Hungary	<i>Hymenogaster luteus</i>	zb3721	Hungary	GU479359	-
<i>Hymenogaster papilliformis</i> BJTC FAN1002 China	<i>Hymenogaster papilliformis</i>	BJTC FAN1002	China	PP467396	PP467432
<i>Hymenogaster papilliformis</i> BJTC FAN1070 China	<i>Hymenogaster papilliformis</i>	BJTC FAN1070	China	PP467399	PP467435
<i>Hymenogaster papilliformis</i> BJTC FAN1074 China	<i>Hymenogaster papilliformis</i>	BJTC FAN1074, holotype	China	PP467400	PP467436
<i>Hymenogaster papilliformis</i> BJTC FAN1109 China	<i>Hymenogaster papilliformis</i>	BJTC FAN1109	China	PP467402	PP467438
<i>Hymenogaster papilliformis</i> BJTC FAN1156 China	<i>Hymenogaster papilliformis</i>	BJTC FAN1156	China	PP467406	PP467442
<i>Hymenogaster papilliformis</i> BJTC FAN1266 China	<i>Hymenogaster papilliformis</i>	BJTC FAN1266	China	PP467408	PP467444
<i>Hymenogaster papilliformis</i> BJTC FAN1267 China	<i>Hymenogaster papilliformis</i>	BJTC FAN1267	China	PP467409	PP467445
<i>Hymenogaster papilliformis</i> BJTC FAN655 China	<i>Hymenogaster papilliformis</i>	BJTC FAN655	China	PP467381	PP467417
<i>Hymenogaster papilliformis</i> BJTC FAN807 China	<i>Hymenogaster papilliformis</i>	BJTC FAN807	China	PP467384	PP467420
<i>Hymenogaster papilliformis</i> BJTC FAN820 China	<i>Hymenogaster papilliformis</i>	BJTC FAN820	China	PP467385	PP467421
<i>Hymenogaster papilliformis</i> BJTC FAN891 China	<i>Hymenogaster papilliformis</i>	BJTC FAN891	China	PP467388	PP467424
<i>Hymenogaster papilliformis</i> BJTC FAN944 China	<i>Hymenogaster papilliformis</i>	BJTC FAN944	China	PP467389	PP467425
<i>Hymenogaster papilliformis</i> BJTC FAN958 China	<i>Hymenogaster papilliformis</i>	BJTC FAN958	China	PP467391	PP467427
<i>Hymenogaster papilliformis</i> BJTC FAN960 China	<i>Hymenogaster papilliformis</i>	BJTC FAN960	China	PP467392	PP467428
<i>Hymenogaster papilliformis</i> BJTC FAN980 China	<i>Hymenogaster papilliformis</i>	BJTC FAN980	China	PP467393	PP467429
<i>Hymenogaster papilliformis</i> BJTC FAN983 China	<i>Hymenogaster papilliformis</i>	BJTC FAN983	China	PP467394	PP467430
<i>Hymenogaster papilliformis</i> BJTC FAN992 China	<i>Hymenogaster papilliformis</i>	BJTC FAN992	China	PP467395	PP467431
<i>Hymenogaster parksii</i> AF325638 USA	<i>Hymenogaster gardneri</i>	Trappe 13296	USA	AF325638	-
<i>Hymenogaster parksii</i> JN022510 USA	<i>Hymenogaster gardneri</i>	SOC1643	USA	JN022510	-
<i>Hymenogaster perisporius</i> BJTC FAN1038 China	<i>Hymenogaster perisporius</i>	BJTC FAN1038	China	PP467397	PP467433
<i>Hymenogaster perisporius</i> BJTC FAN1049 China	<i>Hymenogaster perisporius</i>	BJTC FAN1049	China	PP467398	PP467434
<i>Hymenogaster perisporius</i> BJTC FAN1076 China	<i>Hymenogaster perisporius</i>	BJTC FAN1076	China	PP467401	PP467437
<i>Hymenogaster perisporius</i> BJTC FAN1126 China	<i>Hymenogaster perisporius</i>	BJTC FAN1126	China	PP467403	PP467439

Table 1. Cont.

Taxon Name in Analysis	Taxon Name	Collection	Country	GenBank Accession Number	
				ITS	nrLSU
<i>Hymenogaster perisporius</i> BJTC FAN606 China	<i>Hymenogaster perisporius</i>	BJTC FAN606	China	PP467379	PP467415
<i>Hymenogaster perisporius</i> BJTC FAN651 China	<i>Hymenogaster perisporius</i>	BJTC FAN651, holotype	China	PP467380	PP467416
<i>Hymenogaster perisporius</i> BJTC FAN768 China	<i>Hymenogaster perisporius</i>	BJTC FAN768	China	PP467383	PP467419
<i>Hymenogaster perisporius</i> BJTC FAN846 China	<i>Hymenogaster perisporius</i>	BJTC FAN846	China	PP467386	PP467422
<i>Hymenogaster perisporius</i> BJTC FAN850 China	<i>Hymenogaster perisporius</i>	BJTC FAN850	China	PP467387	PP467423
<i>Hymenogaster perisporius</i> BJTC FAN952 China	<i>Hymenogaster perisporius</i>	BJTC FAN952	China	PP467390	PP467426
<i>Hymenogaster populetorum</i> GU479252 Germany	<i>Hymenogaster griseus</i> 1	it16_1_1	Germany	GU479252	-
<i>Hymenogaster populetorum</i> GU479289 Hungary	<i>Hymenogaster griseus</i> 1	aszodvt_1991	Hungary	GU479289	-
<i>Hymenogaster populetorum</i> GU479304 Hungary	<i>Hymenogaster griseus</i> 1	zb1436	Hungary	GU479304	-
<i>Hymenogaster populetorum</i> GU479327 Hungary	<i>Hymenogaster griseus</i> 2	zb2097	Hungary	GU479327	-
<i>Hymenogaster populetorum</i> GU479328 Hungary	<i>Hymenogaster griseus</i> 2	zb2105	Hungary	GU479328	-
<i>Hymenogaster populetorum</i> GU479340 Hungary	<i>Hymenogaster griseus</i> 1	zb2576	Hungary	GU479340	-
<i>Hymenogaster populetorum</i> GU479356 Hungary	<i>Hymenogaster griseus</i> 2	zb3594	Hungary	GU479356	-
<i>Hymenogaster populetorum</i> JF908082 Italy	<i>Hymenogaster griseus</i> 2	17022	Italy	JF908082	-
<i>Hymenogaster pruinatus</i> GU479242 Germany	<i>Hymenogaster huthii</i>	it12_3_1	Germany	GU479242	-
<i>Hymenogaster pruinatus</i> GU479308 Hungary	<i>Hymenogaster bulliardii</i>	zb1485	Hungary	GU479308	-
<i>Hymenogaster pruinatus</i> GU479366 Hungary	<i>Hymenogaster huthii</i>	zb95	Hungary	GU479366	-
<i>Hymenogaster pseudoniveus</i> BJTC FAN874 China	<i>Hymenogaster pseudoniveus</i>	BJTC FAN874	China	PP622380	PP622367
<i>Hymenogaster pseudoniveus</i> BJTC FAN916 China	<i>Hymenogaster pseudoniveus</i>	BJTC FAN916	China	PP622383	PP622370
<i>Hymenogaster pseudoniveus</i> BJTC FAN967 China	<i>Hymenogaster pseudoniveus</i>	BJTC FAN967	China	PP622382	PP622369
<i>Hymenogaster pseudoniveus</i> BJTC FAN1069 China	<i>Hymenogaster pseudoniveus</i>	BJTC FAN1069	China	PP622379	PP622366
<i>Hymenogaster pseudoniveus</i> BJTC FAN1075 China	<i>Hymenogaster pseudoniveus</i>	BJTC FAN1075, holotype	China	PP622378	PP622365
<i>Hymenogaster pseudoniveus</i> BJTC FAN1238 China	<i>Hymenogaster pseudoniveus</i>	BJTC FAN1238	China	PP622384	-
<i>Hymenogaster pseudoniveus</i> BJTC FAN1251 China	<i>Hymenogaster pseudoniveus</i>	BJTC FAN1251	China	PP622381	PP622368
<i>Hymenogaster rehsteineri</i> GU479259 Germany	<i>Hymenogaster rehsteineri</i>	it2_4_1	Germany	GU479259	-

Table 1. Cont.

Taxon Name in Analysis	Taxon Name	Collection	Country	GenBank Accession Number	
				ITS	nrLSU
<i>Hymenogaster rehsteineri</i> GU479293 Luxembourg	<i>Hymenogaster rehsteineri</i>	dt8455	Luxembourg	GU479293	-
<i>Hymenogaster rehsteineri</i> MT005953 Germany	<i>Hymenogaster rehsteineri</i>	KR-M-0044018	Germany	MT005953	-
<i>Hymenogaster rubyensis</i> AY945303 USA	<i>Hymenogaster</i> sp.	Fogel 2698	USA	AY945303	-
<i>Hymenogaster</i> sp. MK027200 Slovenia	<i>Hymenogaster niveus</i> c 'ryptic species 1'	FV4_04	Slovenia	MK027200	-
<i>Hymenogaster subalpinus</i> AF325640 USA	<i>Hymenogaster gardneri</i>	Trappe 22752	USA	AF325640	-
<i>Hymenogaster tener</i> EU784363 UK	<i>Hymenogaster tener</i>	RBG Kew K(M)102406	UK	EU784363	-
<i>Hymenogaster tener</i> GU479250 Germany	<i>Hymenogaster tener</i>	it15_3	Germany	GU479250	-
<i>Hymenogaster tener</i> GU479253 Germany	<i>Hymenogaster intermedius</i>	it16_2, holotype	Germany	GU479253	-
<i>Hymenogaster thwaitesii</i> GU479258 Germany	<i>Hymenogaster thwaitesii</i>	it2_2	Germany	GU479258	-
<i>Hymenogaster thwaitesii</i> GU479264 Germany	<i>Hymenogaster thwaitesii</i>	it3_2	Germany	GU479264	-
<i>Hymenogaster variabilis</i> BJTC FAN1141 China	<i>Hymenogaster variabilis</i>	BJTC FAN1141	China	PP467405	PP467441
<i>Hymenogaster variabilis</i> BJTC FAN656 China	<i>Hymenogaster variabilis</i>	BJTC FAN656, holotype	China	PP467382	PP467418
<i>Hymenogaster vulgaris</i> EU784365	<i>Hymenogaster rehsteineri</i> c 'ryptic species 3'	RBG Kew K(M)27363	-	EU784365	-
<i>Hymenogaster vulgaris</i> JQ724028 Sweden	<i>Hymenogaster rehsteineri</i> c 'ryptic species 3'	GN_4d_I	Sweden	JQ724028	-
<i>Hymenogaster</i> zunhuaensis BJTC FAN1061 China	<i>Hymenogaster</i> zunhuaensis	BJTC FAN1061	China	PP622373	PP622361
<i>Hymenogaster</i> zunhuaensis BJTC FAN1062 China	<i>Hymenogaster</i> zunhuaensis	BJTC FAN1062, holotype	China	PP622376	PP622363
<i>Hymenogaster</i> zunhuaensis BJTC FAN1083 China	<i>Hymenogaster</i> zunhuaensis	BJTC FAN1083	China	PP622374	PP622362
<i>Hymenogaster</i> zunhuaensis BJTC FAN1105 China	<i>Hymenogaster</i> zunhuaensis	BJTC FAN1105	China	PP622372	PP622360
<i>Hymenogaster</i> zunhuaensis BJTC FAN1162 China	<i>Hymenogaster</i> zunhuaensis	BJTC FAN1162	China	PP622375	-
<i>Hymenogaster</i> zunhuaensis BJTC FAN1249 China	<i>Hymenogaster</i> zunhuaensis	BJTC FAN1249	China	PP622377	PP622364
<i>Hymenogaster</i> zunhuaensis BJTC FAN1259 China	<i>Hymenogaster</i> zunhuaensis	BJTC FAN1259	China	PP622371	PP622359
Uncultured Agaricales HM105539 China	<i>Hymenogaster minisporus</i>	QL054	China	HM105539	-
Uncultured fungus EU554705 Canada	<i>Hymenogaster</i> sp.	A2N_88	Canada	EU554705	-
Uncultured fungus EU554717 Canada	<i>Hymenogaster</i> sp.	A3E_60	Canada	EU554717	-
Uncultured <i>Hymenogaster</i> LT980461 China	<i>Hymenogaster minisporus</i>	taxon:522720	China	LT980461	-

2.3. Phylogenetic Analysis

The ITS-LSU combined dataset was assembled and aligned utilizing the MAFFT algorithm [32], adhering to default parameters, and manually adjusted to allow maximum sequence similarity in Se-AL v.2.03a [33]. Alignments of all datasets used in this study were submitted to TreeBASE (No. 31314). Maximum likelihood (ML) and Bayesian inference (BI) analysis were used to construct a phylogenetic tree separately using two methods. ML analysis was performed with RAxML v.8.0.14 [34–36] employing the GTRGAMMAI substitution model with the parameters unlinked, which was determined by MrModeltest v.2.3 [37]. ML bootstrap replicates (1000) were computed in RAxML using a rapid bootstrap analysis and a search for the best-scoring ML tree was conducted. The ML trees were visualized using TreeView v.32 [38]. Clades with bootstrap support (BS) $\geq 70\%$ were considered significant [39]. BI was conducted using MrBayes v.3.1.2 [40] as an additional method of evaluating branch support. In the BI analysis, after selecting the best substitution models (GTRGAMMAI) determined by MrModeltest [37], two independent runs of four chains were conducted for 1,440,000 Markov chain Monte Carlo (MCMC) generations using the default settings. Average standard deviations of split frequency values were far less than 0.01 at the end of the generations. Trees were sampled every 100 generations after burn-in (well after convergence), and a 50% majority-rule consensus tree was constructed and visualized using TreeView [38]. Clades with Bayesian posterior probability (PP) ≥ 0.95 were considered significantly supported [41].

3. Results

3.1. Molecular Phylogenetics

The ITS and LSU datasets were concatenated to find the phylogenetic positions of the new species described in this study. The final alignment of this comprehensive dataset comprises 111 sequences from 25 different species, inclusive of 26 newly generated sequences derived from our collections. The length of the aligned dataset was 1360 bp after the exclusion of poorly aligned sites, with 618 bp for ITS and 742 bp for nrLSU. Sequences were analyzed by ML and BI, which yielded identical tree topologies, and only the tree inferred from the ML analysis is shown (Figure 1). The sequences isolated from our collections were grouped into two independent clades with strong statistical support (Figure 1), suggesting that they represent two distinct species novel to science. We described them as *Hymenogaster zunhuaensis* sp. nov. and *H. pseudoniveus* sp. nov. in this paper. *Hymenogaster zunhuaensis* further clustered together with *H. minisporus* with relatively strong statistical support, suggesting that both species are related. *Hymenogaster pseudoniveus* is resolved in an independent clade with strong statistical support, and it is basal to a large clade formed by *H. minisporus*, *H. latissporus*, *H. variabilis*, *H. zunhuaensis*, and three undescribed species.

3.2. Taxonomy

Hymenogaster pseudoniveus L. Fan & T. Li, sp. nov. (Figure 2)

MycoBank: MB853450

Etymology: *pseudoniveus*, referring to the snow white basidiome when freshly excavated, which is similar to that of *Hymenogaster niveus*.

Holotype: China. Shanxi Province, Linfen City, Pu County, Wulu Mountain, Gelaozhang Village, alt. 1700 m, 26 October 2017, in soil under *Pinus tabulaeformis* Blume, LT054 (BJTC FAN1075).

Diagnosis: *Hymenogaster pseudoniveus* is characterized by the color of the peridium that is snow white to white when freshly excavated, turning yellowish to earth yellow, with a slight reddish tinge, brown to dark brown gleba, and the basidiospores ornamented with ridges scattered, sometimes longitudinally arranged, occasionally verrucose, up to 2 μm high.

Basidiome subglobose to irregular globose, 0.8–2.5 cm diam, soft and elastic, snow white to white when freshly excavated, which turns yellowish to earth yellow or light yellow brown, with a distinct depression at the white sterile base. Surface smooth, glabrous.

Peridium 110–265 µm thick, composed of elliptic cells of $4.1\text{--}9.3 \times 4.3\text{--}9.6$ µm, light yellow brown to pale yellow, and more or less parallel interwoven hyphae of $2.1\text{--}3.9$ µm broad, light yellow brown. Gleba has a slight reddish tinge, brown to dark brown at maturity, loculate, locules irregular oblong to subglobose, empty, filled with spores at maturity. Hymenium $18\text{--}30$ µm thick. Hymenial cystidia $29\text{--}44$ µm long, only present when young, collapses and disappears at maturity. Basidia clavate, 2–3-spored, mostly 2-spored, sterigmata short, $1\text{--}3(4)$ µm, basidia collapses and disappears at maturity. Basidiospores ellipsoidal to broadly ellipsoidal, yellow brown to dark brown at maturity, ornamented with ridges up to 2 µm high, ridges scattered, sometimes longitudinally arranged, occasionally verrucose, $(10.2\text{--})13\text{--}17(20) \times (8\text{--})10\text{--}13(14)$ µm ($L_m \times W_m = 15.3 \pm 1.1 \times 11.4 \pm 0.8$, $n = 30$), $Q = 1.2\text{--}1.5$ ($Q_{av} = 1.3$), excluding ornamentations, with gelatinous perisporium when young, with a pronounced apex, obtuse, nearly hyaline, $2\text{--}3$ µm high, with appendix, truncate, nearly hyaline, $1\text{--}2$ µm long.

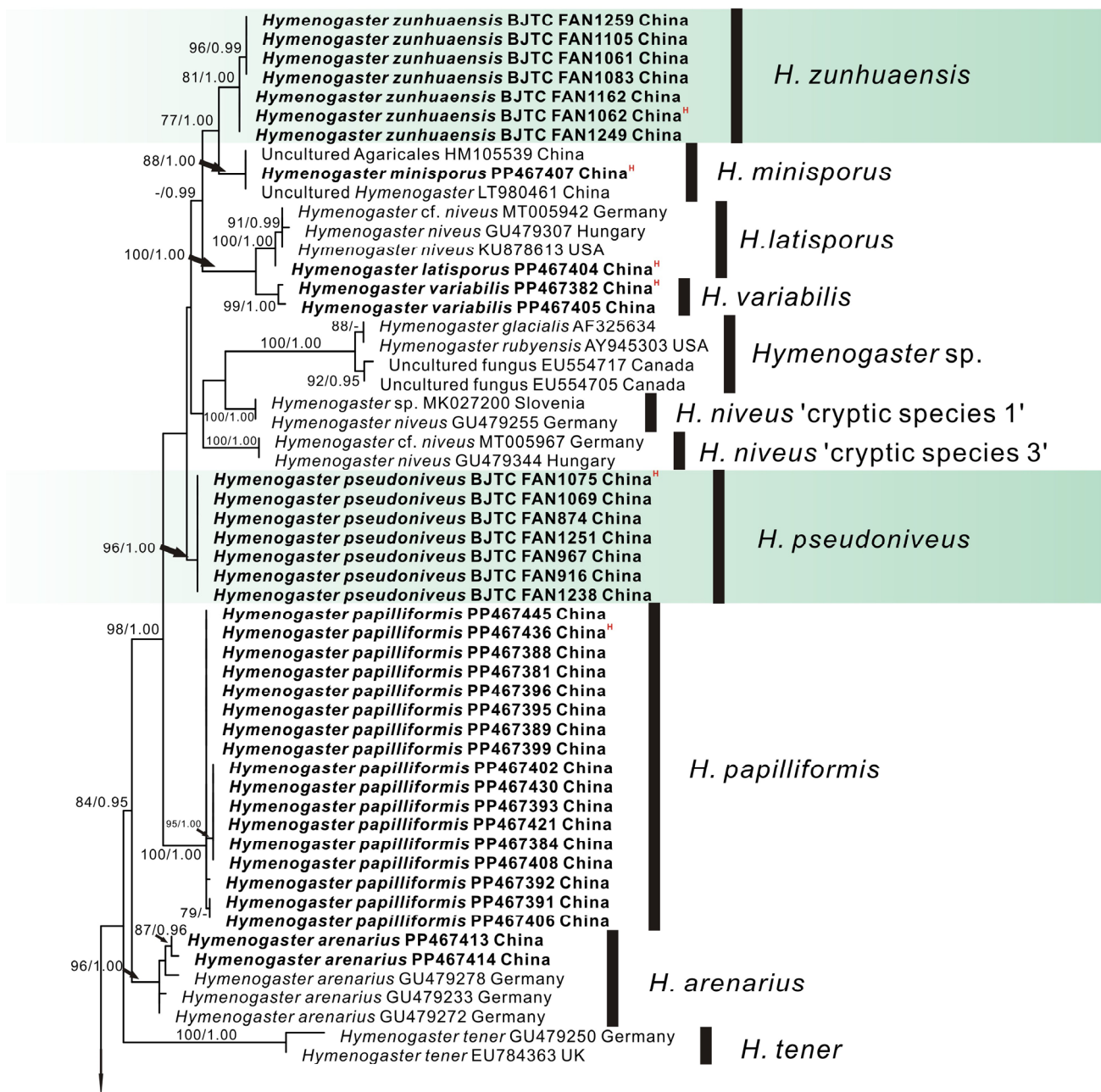


Figure 1. Cont.

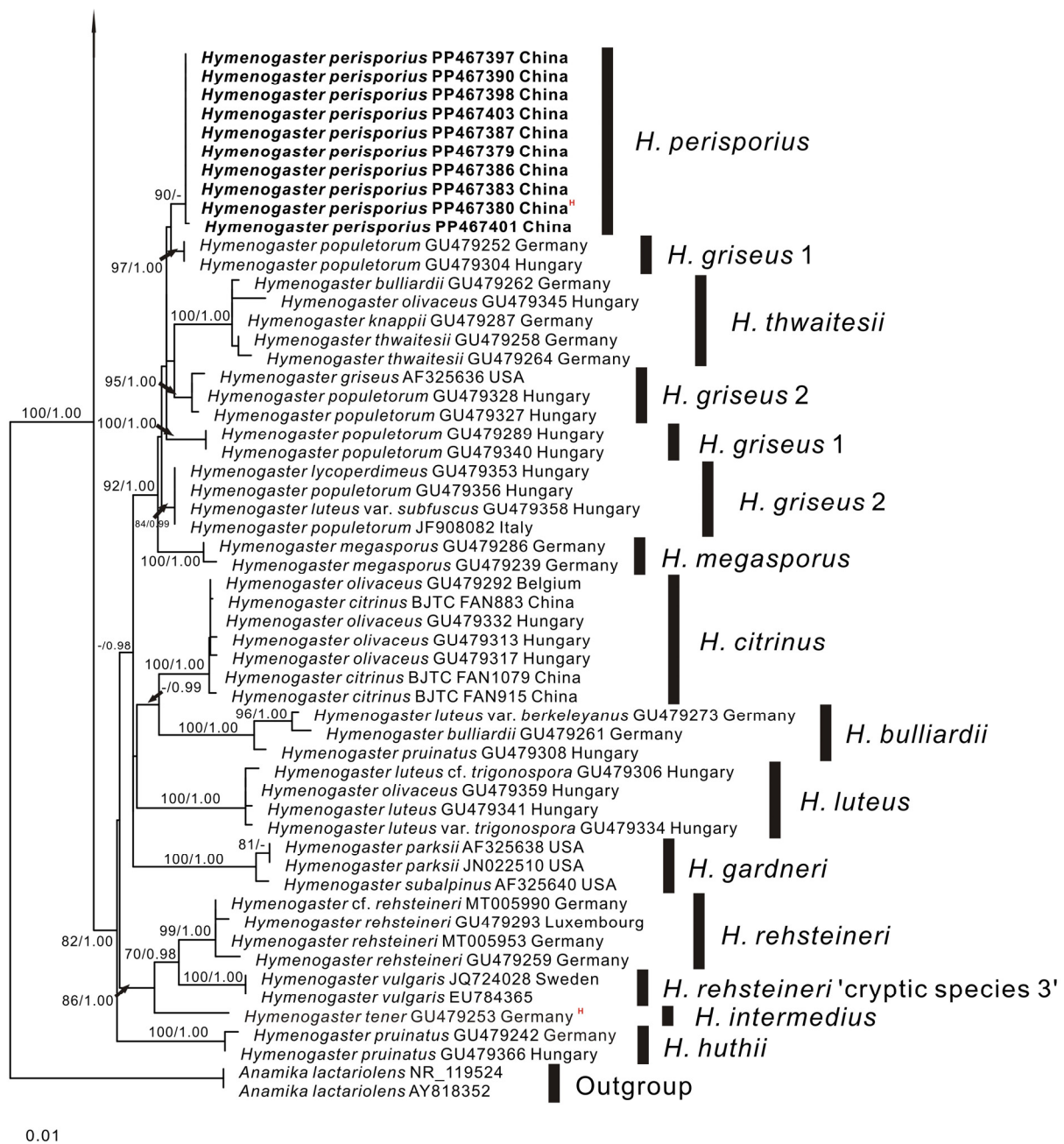


Figure 1. Phylogeny derived from maximum likelihood analyses of the ITS/LSU sequences from *Hymenogaster*. Two sequences of *Anamika lactariolens* were selected as the outgroup. Values on the left represent the likelihood of bootstrap support values ($\geq 70\%$). Values on the right represent significant Bayesian posterior probability values (≥ 0.95). Chinese sequences from basidiomates are in bold; two new species in this study are in green background. Super index “H” in red means “Holotype”.

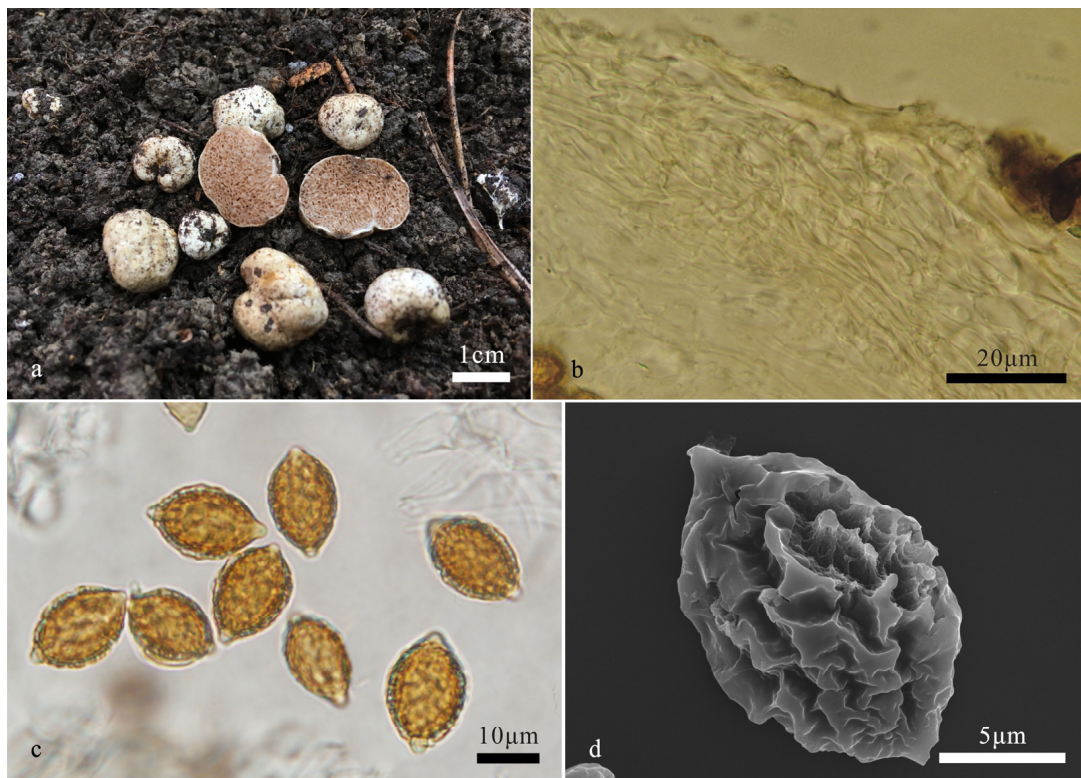


Figure 2. *Hymenogaster pseudoniveus* (BJTC FAN1075, holotype). (a) Basidiomes. (b) Peridium under LM. (c) Basidiospores under LM. (d) Basidiospore under SEM.

Habit, habitat, and distribution: hypogeous, gregarious, in the soil under *Betula platyphylla* Sukaczew, *Castanea mollissima* Blume, *Larix gmelinii* (Rupr.) Rupr., *Pinus armandii* Franch., *P. bungeana* Zucc. ex Endl., *P. tabuliformis* Carr., *Quercus mongolica* Fisch. ex Ledeb., Hebei, Shanxi and Shaanxi Provinces, Northern China.

Additional specimens examined: China. Hebei Province, Tangshan City, Zunhua County, Zhangzhuangzi Village, alt. 100 m, 16 November 2019, in soil under *Castanea mollissima*, LT124 (BJTC FAN1238); Shanxi Province, Yuncheng City, Yuanqu County, Lishan Town, Shunwangping scenic spot, alt. 2276 m, 17 October 2016, in soil under *Pinus armandii*, YXY026 (BJTC FAN662), CM015 (BJTC FAN667), SXY011 (BJTC FAN672), CM011 (BJTC FAN675), CM016 (BJTC FAN679), HKB030 (BJTC FAN705), WYW027 (BJTC FAN709); Lvliang City, Jiaocheng County, Pangquangou, alt. 1897 m, 8 September 2017, in soil under *Larix gmelinii*, HKB099 (BJTC FAN874); Linfen City, Xi County, Shenjiagou, alt. 1321 m, 10 September 2017, YXY 080 (BJTC FAN894), HKB114 (BJTC FAN940); Linfen City, Pu County, Wulu Mountain, Gelaozhang Village, alt. 1321 m, 10 September 2017, in soil under *Pinus bungeana*, LT024 (BJTC FAN916), LT027 (BJTC FAN919), LT028 (BJTC FAN920), LT029 (BJTC FAN921), HKB107 (BJTC FAN933), HKB108 (BJTC FAN934), 11 September 2017, HKB109 (BJTC FAN943), HKB118 (BJTC FAN945), XYY037 (BJTC FAN963), Chaoyanggou, alt. 1645 m, 11 September 2017, in soil under *Quercus mongolica*, XYY041 (BJTC FAN967); Gelaozhang Village, alt. 1321 m, 12 September 2017, in soil under *Pinus bungeana*, HKB128 (BJTC FAN997); Gelaozhang Village, alt. 1730 m, 26 October 2017, in soil under *Quercus* sp., XYY068 (BJTC FAN1068), XYY069 (BJTC FAN1069), alt. 1700 m, in soil under *Pinus tabuliformis*, LT054 (BJTC FAN1075); Yuncheng City, Xia County, Sijiao Town, Xigou Village, Taikuanhe, alt. 900 m, 26 October 2017, in soil under *Pinus tabuliformis*, HKB155 (BJTC FAN1117); Shaanxi Province, Yanan City, Huangling County, Diantou Town, Huangling National Forest Park, alt. 1100 m, 15 September 2020, in soil under *Larix gmelinii*, LT135 (BJTC FAN1251).

Hymenogaster zunhuaensis L. Fan & T. Li, sp. nov. (Figure 3)

Mycobank: MB853448

Etymology: *zunhuaensis*, referring to the locality where the type specimen was collected.

Holotype: China. Hebei Province, Tangshan City, Zunhua County, alt. 107 m, 17 September 2017, in soil under *Castanea mollissima*, XYY062 (BJTC FAN1062).

Diagnosis: *Hymenogaster zunhuaensis* is characterized by the dirty white to pale yellow peridium, yellow brown to brown gleba, and small, broadly ellipsoidal to subglobose basidiospores.

Basidiomes subglobose to irregular globose, gibbous, 1.0–2.5 cm diam, soft and elastic, dirty white to pale yellow, partial with pale brown when fresh, earth yellow to yellow brown when dry, with a distinct depression at the sterile base. Surface smooth, glabrous.

Peridium 70–280 μm thick, pseudoparenchymatous, composed of ellipsoid cells of $7\text{--}12 \times 12\text{--}17 \mu\text{m}$ in diam, with some interwoven hyphae of $2.8\text{--}3.9 \mu\text{m}$ broad, pale yellow to nearly hyaline. Gleba has reddish to rusty tinge, yellow brown to brown when maturity, deep brown when dry, loculate, locules irregular, oblong or irregular globose, empty, filled with spores at maturity. Hymenium $27.8\text{--}55 \mu\text{m}$ thick. Hymenial cystidia hypha-like, $42\text{--}60 \mu\text{m}$ long, only present when young, collapses and disappears at maturity. Basidia narrow clavate, not inflate on the apex, 1–3-spored, mostly 2-spored, $42.5\text{--}65 \mu\text{m}$ long, sterigmata $2\text{--}4 \mu\text{m}$ long, basidia collapses and disappears at maturity. Basidiospores broadly ellipsoidal to subglobose, yellow brown to dark brown at maturity, ornamented with short ridges of about $1 \mu\text{m}$ high, some ridges anastomosed, forming an irregular reticulum, $10\text{--}13.4\text{--}(15.6) \times (7.2\text{--})8.2\text{--}11.6\text{--}(13.3) \mu\text{m}$ ($L_m \times W_m = 11.7 \pm 0.9 \times 9.8 \pm 0.8$, $n = 30$), $Q = 1.1\text{--}1.3$ ($Q_{av} = 1.2$), excluding ornamentations, without gelatinous perisporium, with a pronounced apex, obtuse, nearly hyaline, $2\text{--}3 \mu\text{m}$ high, with appendix, truncate, occasionally tenuous, hyaline, $1\text{--}3 \mu\text{m}$ long.

Habit, habitat, and distribution: hypogeous, gregarious, in the soil under *Castanea mollissima*, *Quercus acutissima* Carruth., *Q. mongolica*, *Q. palustris* Münchh., and *Q. variabilis* Blume, Hebei, Shaanxi, Shanxi, and Yunnan provinces, China.

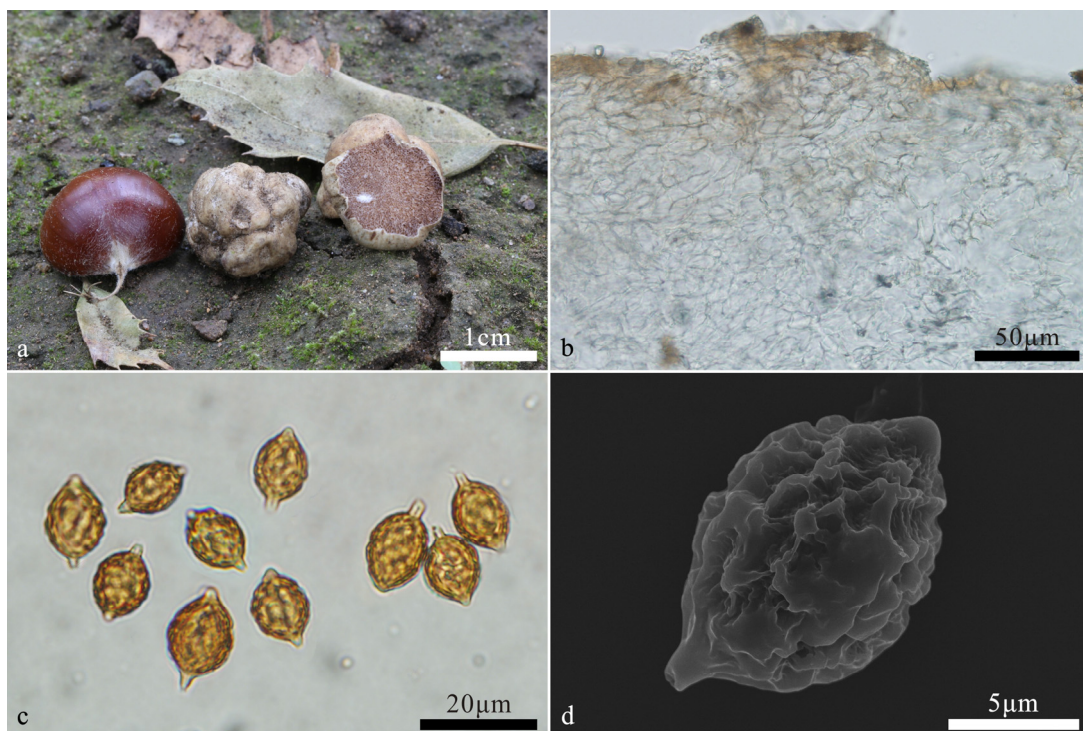


Figure 3. *Hymenogaster zunhuaensis* (BJTC FAN1062, holotype). (a) Basidiomes. (b) Peridium under LM. (c) Basidiospores under LM. (d) Basidiospore under SEM.

Additional specimens examined: China. Shanxi Province, Yuncheng City, Yuanqu County, Lishan Town, Houwentang Village, alt. 1250 m, 4 June 1988, in soil under mixed forest, J.L. Wang 414 (HMAS83127 ex MHSU 2031), 7 July 1990, Y. Ma, B. Qiao & X.K. Bai

386 (HMAS96762 ex MHSU 2032), 1 August 1990, Y. Ma & B. Qiao 377 (HMAS81693, HMAS 83128 ex MHSU 2033); Yunnan Province, Kunming City, Heilongtan Park, 10 August 1990, in soil under *Quercus acutissima*, M.C. Chang & L. Wang 408 (HMAS83129 ex MHSU 2034); Hebei Province, Chengde City, Pingquan County, Liaoheyuan National Forest Park. alt. 1193 m, 1 October 2018, in soil under *Quercus mongolica*, GLJ010 (BJTC FAN1162); Tangshan City, Zunhua County, alt. 107 m, 17 September 2017, in soil under *Castanea mollissima*, XYY061 (BJTC FAN1061), XYY063 (BJTC FAN1063), XYY064 (BJTC FAN1064), XYY065 (BJTC FAN1065), LT080 (BJTC FAN1161); Shanxi Province, Yuncheng City, Xia County, Sijiao Town, Yujialing Village, alt. 970 m, 27 October 2017, in soil under *Quercus variabilis*, LT056 (BJTC FAN1082), LT057 (BJTC FAN1083), LT058 (BJTC FAN1084), LT060 (BJTC FAN1086), YXY125 (BJTC FAN1093), XYY073 (BJTC FAN1103), XYY075 (BJTC FAN1105), XYY077 (BJTC FAN1107), Xigou Village, alt. 900 m, 27 October 2017, in soil under *Quercus* sp., YXY132 (BJTC FAN1100), HKB148 (BJTC FAN1110), HKB149 (BJTC FAN1111), HKB150 (BJTC FAN1112), HKB157 (BJTC FAN1119), alt. 1057 m, 29 October 2017, in soil under *Castanea mollissima*, HKB163 (BJTC FAN1127); Jincheng City, Yangcheng County, Manghe Natural Reserve, alt. 580 m, 31 October 2017, YXY145 (BJTC FAN1145), YXY146 (BJTC FAN1146), Sijiao Town, alt. 1270 m, 5 October 2020, in soil under *Quercus palustris*, LT 141 (BJTC FAN1256), alt. 970 m, in soil under *Quercus* sp., LT143 (BJTC FAN1258), LT144 (BJTC FAN1259), alt. 1370 m, LT145 (BJTC FAN1260); Shaanxi Province, Hanzhong City, Fuoping country, Liangfengya Natural Reserve, alt. 890 m, 12 September 2020, in soil under *Castanea mollissima*, LT133 (BJTC FAN1249).

4. Discussion

Hymenogaster pseudoniveus is differentiated from other species of *Hymenogaster* based on the color of peridium changes, a slight reddish tinge, brown to dark brown gleba, and spore ornamentations up to 2 μm high. *Hymenogaster pseudoniveus* is similar to *H. arenarius* [42] in spore shape, but *H. pseudoniveus* has larger spores and longer apex than *H. arenarius* ($L_m \times W_m = 13.3 \mu\text{m} \times 10.2 \mu\text{m}$, 1–1.5 μm).

Hymenogaster zunhuaensis is similar to *H. gilkeyae* [43] and *H. minisporus* [14] in spore size. However, the peridium is earth yellow to yellowish and relatively uniform in thickness (112.5–185 μm thick) in *H. gilkeyae*, while it is of a different color with very variable thickness in *H. zunhuaensis* (see description). *Hymenogaster minisporus* clustered together with new species in the phylogenetic tree (Figure 1), while the former has light brown gleba, with a small apex (1–1.3 μm high), which is different from those of *H. zunhuaensis*.

Currently, nine *Hymenogaster* species have been supported with morphological and molecular data in China. A key for them is provided below.

Key to the species of *Hymenogaster* from China:

- | | |
|-----------------------------------------------------------------------------------------------------|-------------------------|
| 1. Basidiome pale yellow, white to dirty white when fresh | 2 |
| 1. Basidiome earth yellow to yellow brown when fresh | 6 |
| 2. Gleba reddish brown to brown when fresh | 3 |
| 2. Gleba light brown when fresh | <i>H. minisporus</i> |
| 3. Basidiospores length >17 μm | 4 |
| 3. Basidiospores length $\leq 17 \mu\text{m}$ | 5 |
| 4. Basidiospores 21–25.5 \times 14–18.5 μm | <i>H. citrinus</i> |
| 4. Basidiospores 17–22 \times 12–15 μm | <i>H. perisporius</i> |
| 5. Basidiospores 10–13.5 \times 8–11.5 μm | <i>H. zunhuaensis</i> |
| 5. Basidiospores 13–17 \times 10–13 μm | <i>H. pseudoniveus</i> |
| 6. Peridium exhibits substantial variation in thickness, differing by at least 120 μm | 7 |
| 6. Peridium exhibits substantial variation in thickness, differing by at the most 120 μm | 8 |
| 7. Basidiospores broad ellipsoidal to subglobose, $Q = 1.1$ –1.3 | <i>H. variabilis</i> |
| 7. Basidiospores broad fusiform to broad citriform, $Q = 1.3$ –1.4 | <i>H. papilliformis</i> |
| 8. Basidiospores fusiform, $Q = 1.2$ –1.4 | <i>H. arenarius</i> |
| 8. Basidiospores broadly ellipsoidal to subglobose, $Q = 1.1$ –1.3 | <i>H. latisporus</i> |

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