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Morphological characteristics and phylogenetic analyses revealed *Geastrum yunnanense sp. nov.* (Geastrales, Basidiomycota) from Southwest China

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Abstract

A new species (*Geastrum yunnanense*) from Yunnan Province in Southwest China is described and illustrated based on morphological and molecular evidence. This species is characterized by the globular or ovate endoperidial body, shallowly saccate exoperidium, and spherical basidiospores (2.0–2.5 × 1.9–2.5 µm in diameter). Sequences of the internal transcribed spacers (ITS) and large subunit (nrLSU) of the nuclear ribosomal DNA (rDNA) markers of the studied samples were generated, and the phylogenetic analyses were performed with maximum likelihood, maximum parsimony, and Bayesian inference methods. The phylogenetic analyses inferred from ITS+nrLSU dataset indicated that *G. yunnanense* nested within the genus *Geastrum*, forming a monophyletic lineage sister to *G. velutinum*, and *G. javanicum*. A full description, illustrations, and phylogenetic analysis results for the new species are provided.

Key words: 1 new species, Earthstar fungus, Geastraceae, Phylogeny, Taxonomy, Yunnan Province

Introduction

Fungi represent one of the most diverse groups of organisms on the earth, with an indispensable role in the processes and functioning of ecosystems (Hyde 2022). Most taxa of the phylum Basidiomycota drive the carbon cycling in forest soils, mediate mineral nutrition of plants, and alleviate carbon limitations of other soil organisms as the decomposers and mutualists of plants and animals being the fundamental ecological roles (Tedersoo *et al.* 2014). The genus *Geastrum* Pers. (1794: 85) is widely known as the earthstars, belonging to the family Geastraceae (Basidiomycota) (Wang & Bau 2023). This group of taxa is recorded on all continents, except Antarctica, mostly in the forest humus layer, occasionally seen on rotten wood or sand and grassland, which is characterized by exoperidium splitting into rays at maturity (Wang & Bau 2023, Wu *et al.* 2024).

The genus *Geastrum* was first proposed by Persoon (1794) and typified by *G. coronatum* Pers. (1801: 132). The Gasteromycete means "stomach fungus" and these fungi produce their spores inside the fruiting body that are enclosed inside an outer covering called peridium, and the genus *Geastrum* is considered one of the most genera-diverse of gasteroid fungi (Chouhan & Panwar 2021, Zhang *et al.* 2023). *Geastrum* is characterized by the stelliform basidiomata, exoperidium with three layers, sessile or stalked endoperidium, and sulcate, plicate, folded or fibrillose peristome, distinctly or indistinctly delimited, sometimes with mycosclereids. Both MycoBank database (http://www.MycoBank.org; 12 September 2024) and Index Fungorum (http://www.indexfungorum.org; 12 September 2024) have registered 387 specific and infraspecific names in the genus *Geastrum*, but the actual number of species has been estimated to be around 130 (He *et al.* 2019, Finy *et al.* 2021, Wijayawardene *et al.* 2022), of which 35 species have been recorded from China (Wu *et al.* 2024).

The systematic reports of *Geastrum* in China are based on the morphology (Deng 1963, Dai 1979, Zhou *et al.* 2007). Phylogenetic relationships among European earthstars were inferred using sequence data from the nuclear ribosomal DNA internal transcribed region (ITS1, 5.8S, and ITS2), nuclear large subunit (nrLSU), and translation elongation factor

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1-alpha (*tef*1-α) showed that the phylogenetic analyses recovered eleven clades including 31 morphological species, and proposed the close relationship between *Myriostoma coliforme* (Dicks.) Corda (1842) and *Geastrum* supported by the molecular data (Jeppson *et al*. 2013). Based on phylogenetic analyses of four molecular markers as 5.8S nrDNA, nrLSU, the largest subunit of ribosomal polymerase II (*rpb*1), and subunit 6 of ATP synthase (*atp*6), indicated that a new subdivision of the genus *Geastrum* was presented, and the phylogenetic and morphological boundaries among three genera *Geastrum*, *Myriostoma* Desv. (1809: 103), and *Radiigera* Zeller (1944: 628) (Geastraceae) were evaluated and *Myriostoma* represented a different phylogenetic lineage within *Geastraceae* which was confirmed as distinct from *Geastrum* (Zamora *et al*. 2014). Multigene phylogenetic analyses involving ITS, nrLSU, *rpb1*, *atp6,* and *tef1-α* sequences revealed that the genus *Geastrum* formed a distinct clade and had a close relationship with *G. granulosum* s.l. (Finy *et al*. 2021). Based on morphological observation combined with phylogenetic analysis of ITS-nrLSU-*rpb1*-*atp6*, *G. yanshanense* C.L. Hou, Hao Zhou & Ji Qi Li (2022: 9) and *G. beijingense* C.L. Hou, Hao Zhou & Ji Qi Li (2022: 11), were introduced from Yanshan Mountains (Zhou *et al.* 2022). Based on morphological observation combined with phylogenetic analysis through ITS+nrLSU, seven new *Geastrum* species were introduced from China (Wang & Bau 2023). Phylogenetic analyses based on sequences of ITS, nrLSU, and *atp6* regions showed that *G. sanglinense* Y.Q. Wu & Shu R. Wang (2024: 13) is sister to *G. yanshanense* and *G. rubellum* P.-A. Moreau & C. Lécuru (2019: 17) (Wu *et al.* 2024).

During investigations on Earthstars fungi in China, we found a *Geastrum* specimen that could not be assigned to any described species. Based on morphological characteristics and molecular phylogenetic analyses, the specimen is here described as *G. yunnanense sp. nov.*

Material and Methods

Sample collection and herbarium specimen preparation

The fresh basidiomata of fungi growing on the ground were collected from Dali in Yunnan Province, P.R. China. The samples were photographed using a Jianeng 80D camera (Tokyo, Japan) *in situ* and fresh macroscopic details were recorded. All the photographs were focus-stacked and merged using Helicon Focus Pro 7.7.5 software. Specimens were dried in an electric food dehydrator at 40 °C (Hu *et al.* 2022), and then sealed and stored in an envelope bag and deposited in the herbarium of the Southwest Forestry University (SWFC), Kunming, Yunnan Province, P.R. China.

Morphology

The macromorphological descriptions were based on field notes and photos captured in the field and laboratory and followed the color terminology of Petersen (1996). Micromorphological data were obtained from the dried specimens following observation under a light microscope (Zhao & Wu 2017). Drawings were made with the aid of a fungus plotter (Zhao *et al.* 2023). The measurements and drawings were made from slide preparations stained with Cotton Blue (0.1 mg aniline blue dissolved in 60 g pure lactic acid), Melzer's reagent (3 g potassium iodide, 1 g crystalline iodine, 44 g chloral hydrate, and 40 ml distilled water) and 5% potassium hydroxide. Spore size data, excluded 5% of the measurements from each end of the range, showing them in parentheses. The following abbreviations were used: KOH = 5% potassium hydroxide water solution, CB+ = cyanophilous, CB = cotton clue, CB– = acyanophilous, IKI = Melzer's reagent, IKI– = both inamyloid and indextrinoid. Q = variation in the L/W ratios between the specimens studied and n = a/b (number of spores (a) measured from a given number (b) of specimens). Q_m represented the average Q of basidiospores measured ± standard deviation.

DNA extraction, PCR amplification, sequencing, and phylogenetic analyses

The CTAB rapid plant genome extraction kit-DN14 (Aidlab Biotechnologies Co., Ltd, Beijing, China) was used to obtain genomic DNA from the dried fungal specimens according to the manufacturer's instructions (Zhao & Wu 2017). The nuclear ribosomal ITS region was amplified with ITS5 and ITS4 primer pair (White *et al.* 1990). The nuclear nrLSU region was amplified with the LR0R and LR7 primer pair (Vilgalys & Hester 1990, Rehner & Samuels 1994). The PCR procedure for ITS was as follows: initial denaturation at 95 °C for 3 min, followed by 35 cycles at 94 °C for 40 s, 58 °C for 45 s, and 72 °C for 1 min, and a final extension of 72 °C for 10 min. The PCR procedure for nrLSU was as follows: initial denaturation at 94 °C for 1 min, followed by 35 cycles at 94 °C for 30 s, 48 °C for 1 min, and 72 °C for 1.5 min, and a final extension of 72 °C for 10 min. The PCR products were purified and sequenced at Kunming Tsingke Biological Technology Limited Company (Yunnan Province, China). All of the newly generated sequences were deposited in NCBI GenBank (https://www.ncbi.nlm.nih.gov/genbank/) (Table 1).

TABLE 1. Names, localities, sample numbers and corresponding GenBank accession numbers of the taxa used in this study. The new species are in bold.

Species	Locality	Sample No.	GenBank Accession No.		References
			ITS	nrLSU	
Geastrum albonigrum	Panama	MA-Fungi 36140-2	KF988349	KF988468	Zamora *et al.* 2014
G. albonigrum	Brazil	UFRN:Fungos 1989	KJ127026	KJ127019	Cabral *et al.* 2017
G. argentinum	Argentina	MA-Fungi 82605	KF988353	KF988473	Zamora *et al.* 2014
G. argentinum	Argentina	LPS 48446	KF988352	KF988472	Zamora *et al.* 2014
G. austrominimum	Australia	MEL:2292062	KP687490	KP687451	Zamora *et al.* 2015
G. austrominimum	Australia	MEL:2276089	KP687491	KP687452	Zamora *et al.* 2015
G. beijingense	China	BJTC 153	MZ508875	MZ509379	Zhou *et al.* 2022
G. benitoi	Spain	Herb. Zamora 76	KF988338	KF988470	Zamora *et al.* 2014
G. benitoi	Spain	MA-Fungi 68191	KF988350	KF988469	Zamora *et al.* 2014
G. berkeleyi	Spain	MA-Fungi 74668	KF988354	KF988474	Zamora *et al.* 2014
G. britannicum	England	K(M) 60288	EU784242	–	Zamora *et al.* 2015
G. britannicum	England	K(M) 79617	EU784243	–	Zamora *et al.* 2015
G. brunneocapillatum	Brazil	UFRN:Fungos:2286	MH634996	MH635029	Wu *et al.* 2024
G. campestre	USA	MICH 28566	KF988358	KF988480	Zamora *et al.* 2014
G. corollinum	Spain	MA-Fungi 5746	KF988359	KF988481	Zamora *et al.* 2014
G. corollinum	Sweden	Herb. Sunhede 7744	KF988360	KF988482	Zamora *et al.* 2014
G. coronatum	Spain,	Herb. Zamora 522	KF988362	KF988484	Zamora *et al.* 2014
G. coronatum	USA	MICH 28567	KF988363	KF988485	Zamora *et al.* 2014
G. elegans	Spain	Herb. Zamora 189	KF988366	KF988488	Zamora *et al.* 2014
G. elegans	Sweden	UPS F-560810	KF988367	KF988489	Zamora *et al.* 2014
G. entomophilum	Brazil	MA-Fungi 70785	KF988368	KF988490	Zamora *et al.* 2014
G. fimbriatum	Spain	Herb. Zamora 234	KF988369	KF988491	Zamora *et al.* 2014
G. fimbriatum	Sweden	Herb. Sunhede 7739	KF988370	KF988492	Zamora *et al.* 2014
G. flexuosum	Sweden	UPS F-119844	KF988371	KF988493	Zamora *et al.* 2014
G. floriforme	Spain	Herb.Zamora 453	KF988373	KF988495	Zamora *et al.* 2014
G. floriforme	Spain	MA-Fungi 69173	KF988372	KF988494	Zamora *et al.* 2014
G. fornicatum	Spain	Herb.Zamora 255	KF988374	KF988496	Zamora *et al.* 2014
G. fornicatum	Spain	MA-Fungi 30749	KF988375	KF988497	Zamora *et al.* 2014
G. fuscogleba	USA	NY Trappe 9500	KF988377	KF988499	Zamora *et al.* 2014
G. fuscogleba	USA	NY Trappe 1071	KF988376	KF988498	Zamora *et al.* 2014
G. glaucescens	Argentina	MA-Fungi 83762	KF988378	KF988500	Zamora *et al.* 2014
G. glaucescens	Argentina	MA-Fungi 83763	KF988379	KF988501	Zamora *et al.* 2014
G. gorgonicum	Cape Verde	MA:Fungi:92118	MN754045	MN754083	Wang & Bau 2023
G. gorgonicum	Cape Verde	MA:Fungi:92116	MN754046	MN754084	Wang & Bau 2023
G. hansagiense	Hungary	GBL1	MN582753	–	Wang & Bau 2023
G. hariotii	Argentina	MA-Fungi 83765	KF988381	KF988504	Zamora *et al.* 2014
G. hieronymi	Argentina	MA-Fungi 83767	KF988344	KF988509	Zamora *et al.* 2014
G. hieronymi	Argentina	MA-Fungi 83766	KF988384	KF988508	Zamora *et al.* 2014
G. hungaricum	Czech	Sunhede 5993	KP687500	KP687461	Zamora *et al.* 2015
G. hungaricum	Spain	Zamora 611	KP687501	KP687462	Zamora *et al.* 2015
G. javanicum	Brazil	UFRN-Fungos 1215	KJ127031	–	Cabral *et al.* 2017
G. kotlabae	Spain	MA-Fungi 35963	KF988385	KF988510	Zamora *et al.* 2014
G. kotlabae	Spain	Herb.Zamora 440	KF988386	KF988511	Zamora *et al.* 2014

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TABLE 1. (Continued)

Species	Locality	Sample No.	GenBank Accession No.		References
			ITS	nrLSU	
G. kuharii	Argentina	MA-Fungi 83795	KF988463	KF988598	Zamora *et al.* 2014
G. kuharii	Argentina	MA:Fungi:86913	KP687502	KP687463	Zamora *et al.* 2015
G. lageniforme	Spain	Herb.Zamora 316	KF988339	KF988514	Zamora *et al.* 2014
G. lageniforme	Spain	Herb.Zamora 207	KF988388	KF988513	Zamora *et al.* 2014
G. laneum	China	HMJAU65711	OP964640	OP964638	Wang & Bau 2023
G. laneum	China	HMJAU65704 *	OP964641	OP964639	Wang & Bau 2023
G. litchi	China	HMJAU65716 *	OQ360756	OP964619	Wang & Bau 2023
G. marginatum	Spain	ERRO 2012112609	KP687504	KP687465	Zamora *et al.* 2015
G. marginatum	Czech	PRM:842884 *	KP687507	KP687468	Zamora *et al.* 2015
G. melanocephalum	Spain	Herb.Zamora 34	KF988395	KF988522	Zamora *et al.* 2014
G. melanocephalum	Sweden	Herb.Sunhede 7737	KF988396	KF988523	Zamora *et al.* 2014
G. meridionale	Spain	Herb. Zamora 252 *	KF988412	KF988540	Zamora *et al.* 2014
G. michelianum	Spain	Herb.Zamora 227	KF988398	KF988525	Zamora *et al.* 2014
G. microphole	China	HMJAU65721	OP964637	OP964644	Wang & Bau 2023
G. microphole	China	HMJAU65720 *	OP964636	OP964643	Wang & Bau 2023
G. minimum	Spain	Herb.Zamora 191	KF988400	KF988528	Zamora *et al.* 2014
G. minimum	Sweden	Herb.Sunhede 7746	KF988401	KF988529	Zamora *et al.* 2014
G. minutisporum	Argentina	CORD15	KM260665	–	Wu *et al.* 2024
G. mirabile	Japan	TNS:KH-JPN10-714	JN845109	JN845227	Kasuya *et al.* 2012
G. mongolicum	China	HMJAU65762	OP964647	OP964645	Wang *et al.* 2023
G. mongolicum	China	HMJAU65763 *	OP964648	OP964646	Wang *et al.* 2023
G. morganii	Canada	Herb.Lebeuf HRL0177 *	KF988406	KF988534	Zamora *et al.* 2014
G. neoamericanum	Brazil	UFRN:Fungos:2302 *	MH635001	MH635040	Zhou *et al.* 2022
G. neoamericanum	French	LIP:JLC 12030103	MH635014	MH635038	Zhou *et al.* 2022
G. ovalisporum	Bolivia	MA-Fungi 47184	KF988411	KF988539	Zamora *et al.* 2014
G. oxysepalum	China	HMJAU65727 *	OP964632	OP964622	Wang & Bau 2023
G. oxysepalum	China	HMJAU65728	OP964633	OP964623	Wang & Bau 2023
G. parvisporum	Argentina	BAFC:51926	MG196037	MG196035	Zamora *et al.* 2019
G. parvisporum	Argentina	MA-Fungi 83793	KF988461	KF988596	Zamora *et al.* 2014
G. parvistriatum	Spain	Zamora 272	JN943162	JN939572	Zhou *et al.* 2022
G. pectinatum	Belgium	MA-Fungi:43295	KP687517	KP687479	Zamora *et al.* 2015
G. pleosporum	Cameroon	MA-Fungi 56971	KF988416	KF988544	Zamora *et al.* 2014
G. pouzarii		MA-Fungi 2944	KF988417	KF988545	Zamora *et al.* 2014
G. pseudolimbatum	Spain	Herb.Zamora 231	KF988419	KF988547	Zamora *et al.* 2014
G. pseudolimbatum	Sweden	UPS F-560804	KF988420	KF988548	Zamora *et al.* 2014
G. pseudosaccatum	China	HMJAU65769 *	OP964628	OP964634	Wang & Bau 2023
G. pseudosaccatum	China	HMJAU65781	OP964625	OP964635	Wang & Bau 2023
G. pusillipilosum	Beazil	UFRN:Fungos 2315	KX761175	KX761176	Crous *et al.* 2016
G. quadrifidum	USA	MICH 72512	KF988423	KF988551	Zamora *et al.* 2014
G. rubellum	Brazil	UFRN:Fungos:2844	MH634999	MH635031	Zhou *et al.* 2022
G. rubropusillum	Brazil	UFRN:Fungos:2308	MH634994	MH635027	Zhou *et al.* 2022
G. rufescens	Spain	Herb.Zamora 274	KF988425	KF988553	Zamora *et al.* 2014
G. saccatum	Japan	UPS F-530056	KF988428	KF988558	Zamora *et al.* 2014

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TABLE 1. (Continued)

Species	Locality	Sample No.	GenBank Accession No.		References
			ITS	nrLSU	
G. sanglinense	China	HMSAU 15023	OP050118	OP050163	Wu *et al.* 2024
G. sanglinense	China	HMSAU 15024	OP050119	OP050164	Wu *et al.* 2024
G. schmidelii	Sweden	UPSF-560805	KF988435	KF988565	Zamora *et al.* 2014
G. schweinitzii	Argentina	MA-Fungi 83779	KF988437	KF988567	Zamora *et al.* 2014
G. smardae	Spain	Herb.Zamora 527	KF988441	KF988574	Zamora *et al.* 2014
G. smardae	Canada	Herb.Lebeuf HRL 0160	KF988440	KF988573	Zamora *et al.* 2014
G. smithii	Argentina	MA-Fungi 83783	KF988442	KF988575	Zamora *et al.* 2014
G. striatum	Sweden	MA-Fungi 86672	KF988443	KF988577	Zamora *et al.* 2014
G. tenuipes	Australia	CANB:775658	KP687527	KP687489	Zamora *et al.* 2015
G. tenuipes	Australia	CANB:738350	KP687526	KP687488	Zamora *et al.* 2015
G. thanatophilum	USA	MICH 72012	KF988364	KF988486	Zamora *et al.* 2014
G. triplex	Madagascar	UPS F-014630(213863)	KF988444	KF988578	Zamora *et al.* 2014
G. triplex	Argentina	MA-Fungi 83784	KF988445	KF988579	Zamora *et al.* 2014
G. velutinum	Argentina	MA-Fungi 83785	KF988446	KF988581	Zamora *et al.* 2014
G. velutinum	Argentina	MA-Fungi 83786	KF988447	KF988582	Zamora *et al.* 2014
G. violaceum	Agentina	MA-Fungi 82487	KF988451	KF988586	Zamora *et al.* 2014
G. violaceum	Agentina	BAFC 51671	KF988450	KF988585	Zamora *et al.* 2014
G. yanshanense	China	BJTC 381 *	MZ508878	MZ509383	Zhou *et al.* 2022
G. yanshanense	China	BJTC 057	MZ508879	MZ509384	Zhou *et al.* 2022
G. yunnanense	**China**	**CL Zhao 24800 ***	**PP511307**	–	**Present study**
G. yunnanense	**China**	**CL Zhao 24893**	**PP511308**	**PP511310**	**Present study**
G. yunnanense	**China**	**CL Zhao 24922**	**PP511309**	**PP511311**	**Present study**
Schenella pityophila	Spain	Herb.Zamora 531	KF988347	KF988465	Zamora *et al.* 2014

*Indicates type material (holotype).

Sequencher 4.6 (GeneCodes, Ann Arbor, MI, USA) was used to assemble and edit the generated sequence reads. Sequences were aligned in MAFFT 7 (https://mafft.cbrc.jp/alignment/server/) using the "G-INS-I" strategy. The alignment was adjusted manually using AliView version 1.27 (Larsson 2014). The dataset was aligned first, and then the sequences of ITS+nrLSU were combined with Mesquite version 3.51. The combined ITS+nrLSU sequences were used to infer the position of the new species in the genus *Geastrum* and related species. Sequences of *Schenella pityophila* (Malençon & Riousset) Estrada & Lado (2005: 147) retrieved from GenBank were used as an outgroup in the combined ITS+nrLSU analysis (Wang & Bau 2023).

Maximum parsimony (MP), Maximum Likelihood (ML), and Bayesian Inference (BI) analyses were applied to the combined three datasets. Approaches to phylogenetic analyses followed (Zhao & Wu 2017). MP analysis was performed in PAUP* version 4.0b10 (Swofford 2002). All of the characters were equally weighted and gaps were treated as missing data. Trees were inferred using the heuristic search option with Tree Bisection and Reconnection (TBR) branch swapping and 1000 random sequence additions. Max trees were set to 5000, branches of zero length were collapsed and all most parsimonious trees were saved. Clade robustness was assessed using bootstrap (BT) analysis with 1000 replicates (Felsenstein 1985). Descriptive tree statistics tree length (TL), the consistency index (CI), the retention index (RI), the rescaled consistency index (RC), and the homoplasy index (HI) were calculated for each most-parsimonious tree generated. ML was inferred using RAxML-HPC2 through the Cipres Science Gateway (www.phylo.org) (Miller *et al.* 2012). Branch support (BS) for ML analysis was determined by 1,000 bootstrap replicates and evaluated under the gamma model.

The best-evolutionary model of each alignment was estimated using jModelTest (Guindon & Gascuel 2003, Posada 2008) under the Akaike information criterion. MrModeltest 2.3 (Nylander 2004) was used to determine the best-fit evolution model for the dataset for Bayesian Inference (BI). Bayesian Inference was performed with MrBayes 3.1.2 with a general time reversible (GTR+I+G) model of DNA substitution and a gamma distribution rate variation

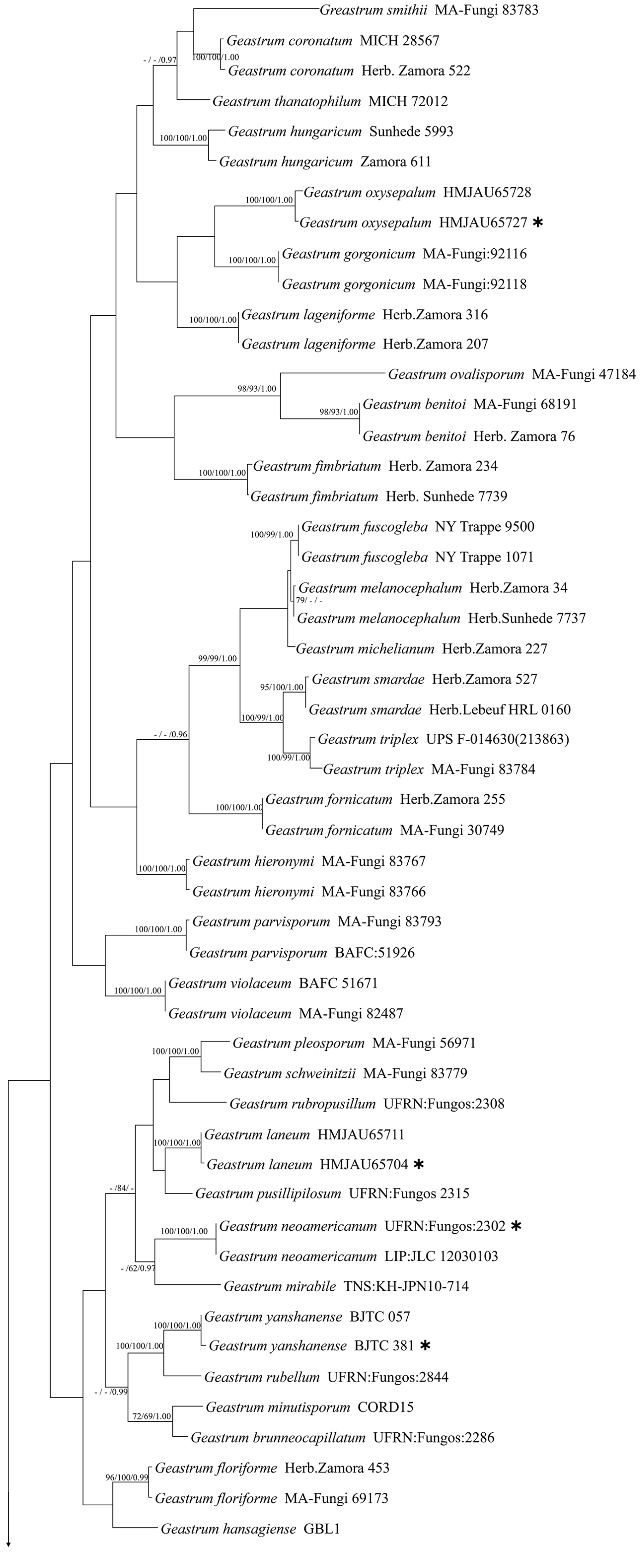

FIGURE 1. Maximum parsimony strict consensus tree illustrating the phylogeny of one new species and related species in *Geastrum* based on ITS + nrLSU sequences. Branches are labeled with maximum likelihood bootstrap values ≥ 70%, parsimony bootstrap values ≥ 50%, and Bayesian posterior probabilities ≥ 0.95, respectively. New species accessions are in bold, *Indicates type material (holotype).

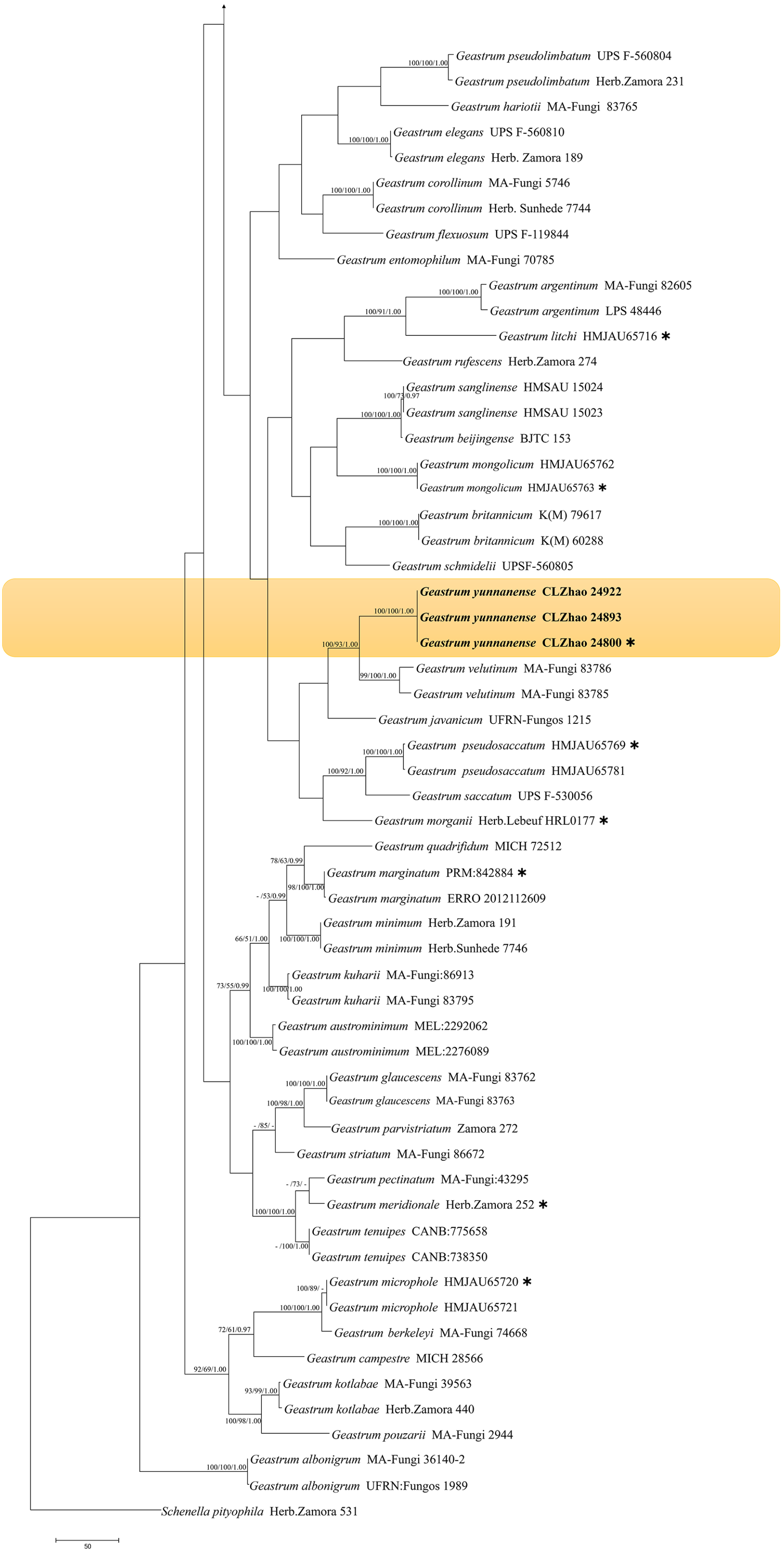

FIGURE 1. *Cont.*

across sites (Ronquist & Huelsenbeck 2003). Four Markov chains were run for two runs from random starting trees for 80 million generations combined ITS+nrLSU sequences (Fig. 1), with trees and parameters sampled every 100 generations. The first quarter of all the generations were discarded as burn-ins. A majority rule consensus tree of all remaining trees and posterior probabilities were calculated. Branches were considered significantly supported if they received a maximum likelihood bootstrap value (BS) of ≥70%, a maximum parsimony bootstrap value (BT) of ≥70%, or Bayesian posterior probabilities (BPP) of ≥0.95.

Results

Phylogenetic analyses

The combined ITS+nrLSU dataset (Fig. 1) included sequences from 109 fungal specimens representing 70 species. The dataset had an aligned length of 1664 characters, of which 963 characters are constant, 127 are variable and parsimony-uninformative, and 574 are parsimony-informative. Maximum parsimony analysis yielded 5000 equally parsimonious trees (TL = 4770, CI = 0.2520, HI = 0.7480, RI = 0.5503, RC = 0.1387). The best model for the ITS dataset estimated and applied in the Bayesian analysis was GTR+I+G (lset nst = 6, rates = invgamma; prset statefreqpr = dirichlet (1,1,1,1). The bayesian analysis and ML analyses resulted in a similar topology to that of the MP analysis with an average standard deviation of split frequencies = 0.009493 (BI), and the effective sample size (ESS) across the two runs is double the average ESS (avg. ESS) = 1499. The phylogenetic tree (Fig. 1) inferred from ITS+nrLSU sequences revealed that *Geastrum yunnanense* is sister to *G. velutinum* Morgan (1895: 38) and *G. javanicum* Lév. (1846: 161) with 100% BS, 93% BT, and 1.00% BPP support.

In ITS BLAST results, the top ten taxa were *Geastrum velutinum* (Maximum record descriptions: Max score 640; Total score 640; Query cover 88%; E value 2e-178; Ident 89.12%). In nrLSU BLAST results, the top ten taxa were *Geastrum floriforme*, *G. velutinum*, *G. xerophilum*, *G. schmidelii*, *G. gorgonicum* and *G. gorgonicum* (Maximum record descriptions: Max score 2398; Total score 2398 Query cover 100%; E value 0.0; Ident 98.60%).

Taxonomy

Geastrum yunnanense X. Yang & C.L. Zhao, *sp. nov.* Figs. 2–4

MycoBank no.: MB 852965

***Diagnosis*:**—It differs in its unexpanded basidiomata with a few white mycoderma, not easily dislodged mycelial layer, a monomitic hyphal structure with tawny to yellow to pale brown generative hyphae, and spherical basidiospores.

***Etymology*:**—*yunnanense* (Lat.) refers to the locality "Yunnan Province" of the holotype.

***Holotype*:**—CHINA. Yunnan Province, Dali, Weishan County, Qinghua Town, Qinghua Nature Reserve, 25°0′57″N, 100°13′17″E, elev. 2071.6 m, on the ground, 18 October 2022, CLZhao 24800 (SWFC).

Fruiting body:—Unexpanded basidiomata, 7–25 mm in size, with a few white mycoderma. Expanded basidiomata medium-sized, 2–4 cm in size. Exoperidium: shallowly saccate, 0.3–0.5 mm thick, dehiscence often less than halfway down, splits into 7–9 lobes at maturity, lobes 5–12 mm wide, tapered at the front end, rolled outwards to underneath exoperidial disc, turn to brittle and thin upon drying. Pseudoparenchymatous layer: smooth surface, camel, not deciduous, aseptic collar, thinner and fragile when dry. Fibrous layer: yellowish-white, tightly attached to the mycelial layer. Mycelial layer: henna, visible villus in a felted form, without debris, not easily dislodged.

***Endoperidial body*:**—Globular, 10–20 mm in diameter, projecting apically, 0.5–2 mm in length, sessile. Endoperidium: brownish grey, with a smooth surface and greyish villus visible under the dissecting microscope. Peristome: broadly conical, silkily fibrillose, darker in color than the endoperidium, distinctly delimited.

***Hyphal structure*:**—Monomitic; generative hyphae tawny to yellow to pale brown, thick-walled, rarely branched; IKI+, CB+, tissues unchanged in KOH; capillitial hyphae: up to 4–6 µm in diameter, thick-walled, tawny, rarely branched. Exoperidium pseudoparenchymatous layer inside, formed of the angular cell structured, 16.0–52.5 × 8.0–19.5 µm; fibrous layer in the middle, formed of the interlacing filament tissue, fibrous hyphae thick-walled to solid, (2.6)3.1–4.1(4.4) µm in diameter; the mycelium layer outside, mycelium hyphae thick-walled to solid, (4.2–)4.3–6.4(–7.2) µm in diameter.

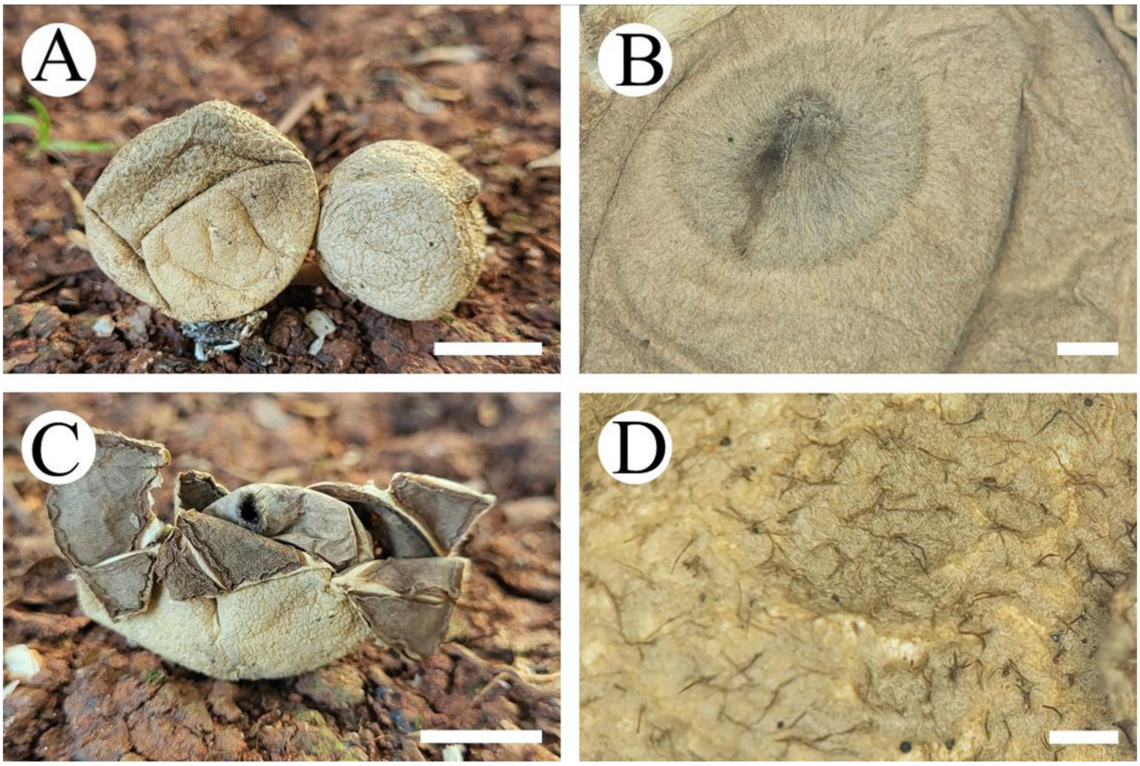

FIGURE 2. *Geastrum yunnanense* (A from CLZhao 24893, B–D from CLZhao 24800), (A) Unexpanded basidiomata; (B) Peristome; (C) Basidiomata; (D) Mycelial layers; Bars: (A, C) = 1cm, (B, D) = 1 mm. Photoplate by: Xin Yang.

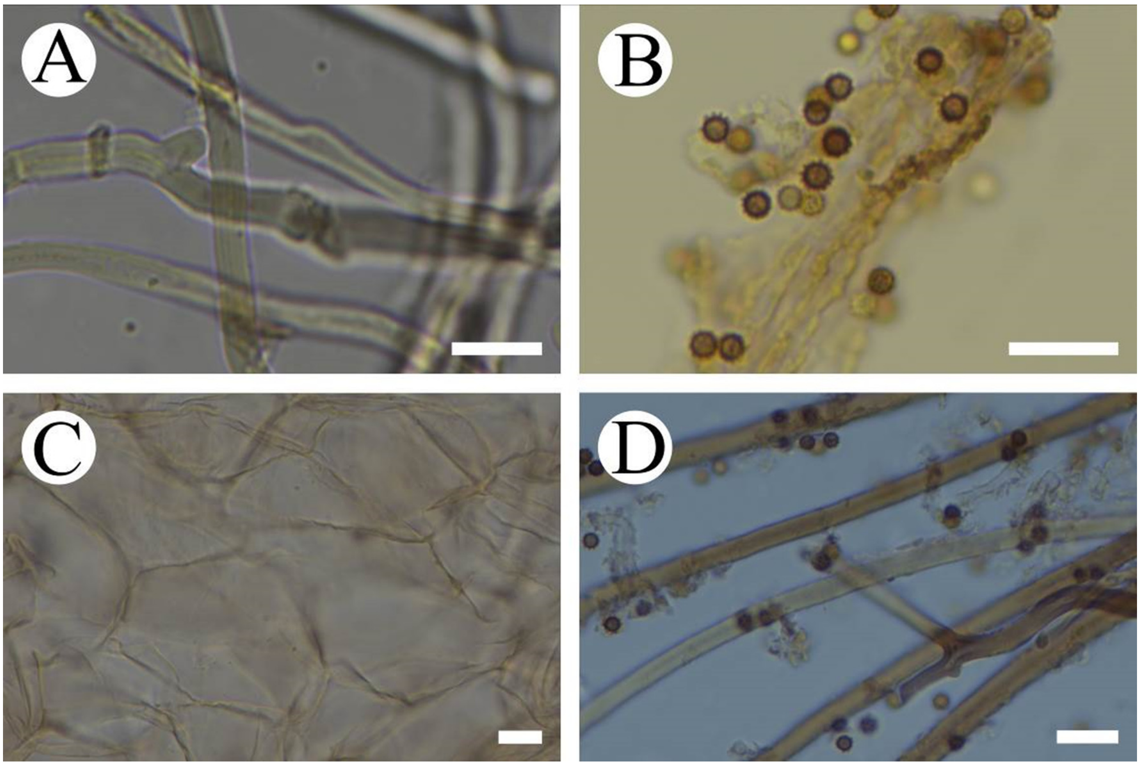

FIGURE 3. *Geastrum yunnanense* (A–D from CLZhao 24800), (A) Mycelium hyphae; (B) Basidiospores; (C) Pseudoparenchymatous layer; (D) Capillital hyphae; Bars: (A–D) =10 µm. Photoplate by Xin Yang.

***Basidiospores*:**—Spherical, (1.7–) 2.0–2.5 (–2.9) × (1.7–) 1.9–2.5 (–2.9) µm in diameter, yellowish brown to dark brown in contact with 5% KOH solution, IKI–, CB–, spores surface with delicately echinulate, 0.4–0.8(–1.0) µm in length, Q= 1.01, Qm=1.01±0.03.

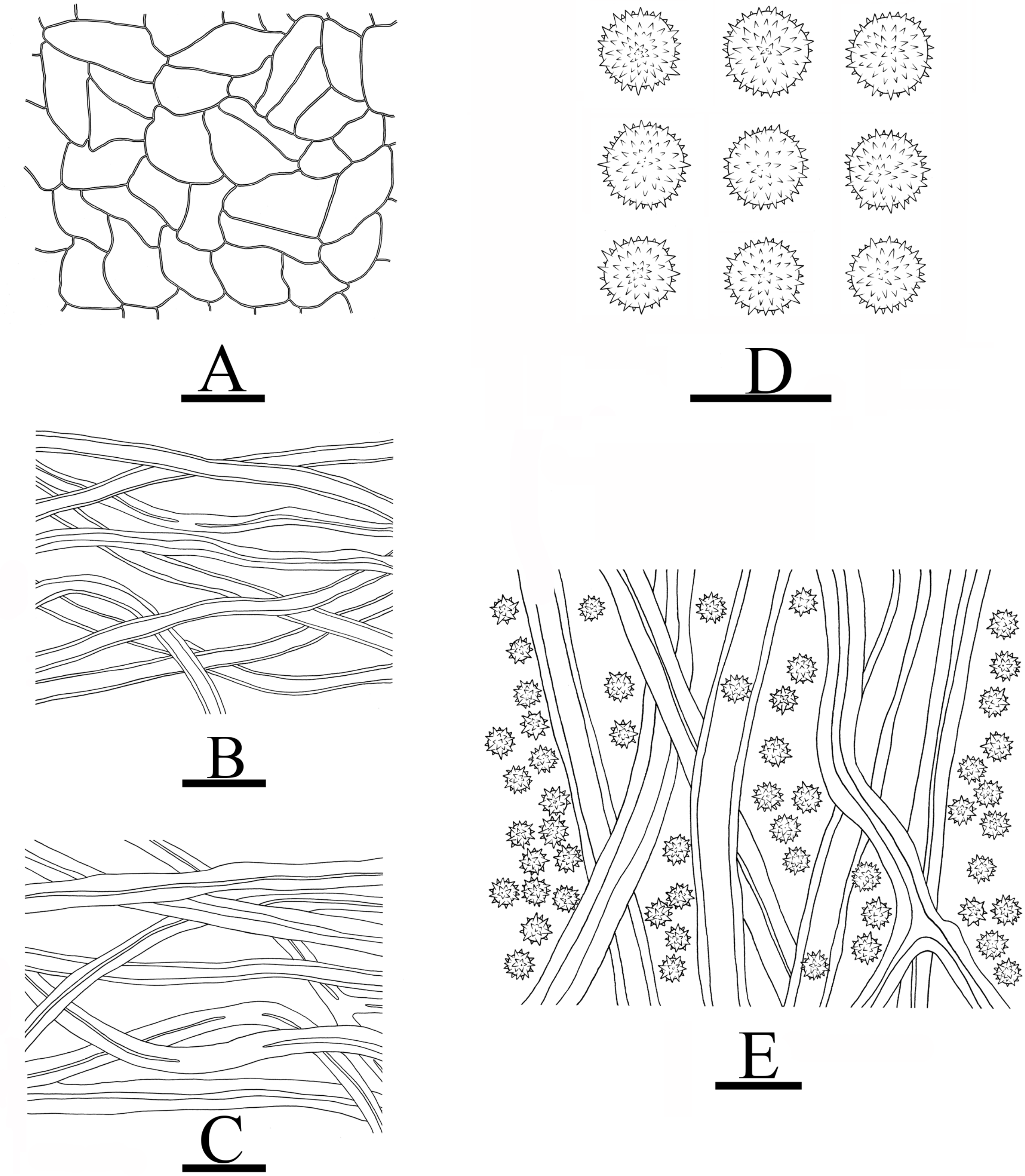

FIGURE 4. *Geastrum yunnanense* (A–E from CLZhao 24800), (A) Pseudoparenchymatous layer; (B) fibrous hyphae; (C) Mycelium hyphae; (D) Basidiospores; (E) Capillital hyphae; Bars: (A–C, E) = 10 µm, D = 5 µm. Drawings by Xin Yang.

***Additional specimens examined (paratypes)*:**—China, Yunnan Province, Dali, Weishan Country, Qinghua Town, Qinghua Nature Reserve, E 100°13′17″, N 25°0′57″, elev. 2071.6 m, on the angiosperm ground, 18 October 2022, CLZhao 24893, CLZhao 24922 (SWFC!).

***Ecology and distribution*:**—The climate of the collection site is north subtropical plateau monsoon, in which the forest type is evergreen angiosperm forest, and samples were collected on the ground. So far, it has only been found in Yunnan Province, China.

Discussion

The present study describes *Geastrum yunnanense* as a new species based on phylogenetic analyses and morphological characteristics. Phylogenetic analysis of ITS+nrLSU dataset (Fig. 1) shows, *G. yunnanense* is sister to *G. velutinum* and *G. javanicum*. However, morphologically *G. velutinum* differs from *G. yunnanense* by having the easily dislodged mycelial layer, and larger basidiospores (3–4 µm in diameter) (Zhou *et al.* 2007); *G. javanicum* differs from *G. yunnanense* in its deeply saccate exoperidium, capillitial hyphae without branches and larger basidiospores 3.0–4.0 µm in diameter (Zhou *et al.* 2007).

Morphologically, *G. morganii* Lloyd (1902: 80), *G. saccatum* Fr. (1829: 16), *G. pseudosaccatum* T. Bau & X. Wang (2023:15) *Geastrum yanshanense* C.L Hou, Hao Zhou & Jiqi Li (2022:9), *Geastrum suae* Z.Q. Zhang, C.H. Li & Z.L. Luo (2023: 10), *G. melanorhynchum* T. Bau & X. Wang (2023: 17), *G. oxysepalum* T. Bau & X. Wang (2023: 19), are similar to *G. yunnanense* by the sessile endoperidial body and mycelial layer without debris. However, *G. morganii* differs by its larger basidiospores (4–5.5) µm in diameter (Zhou *et al.* 2007); *G. saccatum* differs by its peristome with obvious oral margin ring and easily dislodged mycelial layer (Zhou *et al.* 2007); *G. pseudosaccatum* differs by its peristoma having ringed with a distinctly bulge and unbranched capillitial hyphae (Wang & Bau 2023); *G. yanshanense* differs in its deep saccate and expanded basidiomata, peristome with obvious oral margin ring and obvious columella (Zhou *et al.* 2022); *G. suae* differs by its long stipes, larger basidiomata (height 35–70 mm, diameter 18–37 mm), peristome with obvious oral margin ring and obvious columella (Zhang *et al.* 2022); *G. melanorhynchum* differs by its peristome with obvious oral margin ring and exoperidium dehiscence often greater than halfway down (Wang & Bau 2023); and *G. oxysepalum* differs by its peristome with obvious oral margin ring and unbranched capillitial hyphae (Wang & Bau 2023).

Geastrum laneum T. Bau & X. Wang (2023: 8), *G. microphole* T. Bau & X. Wang (2023: 22) and *G. mongolicum* T. Bau & X. Wang (2023: 12) are similar to *G. yunnanense* by its fibrous layer attached to the mycelial layer. However, *G. laneum* differs by its soft and thin exoperidium, and unbranched capillitial hyphae and encrusted with debris mycelial layer (Wang & Bau 2023); *G. microphole* differs by its easily dislodged and encrusted with debris mycelial layer, stipitate endoperidial body and with an apophysis (Wang & Bau 2023); and *G. mongolicum* differs by its easily dislodged and encrusted with debris mycelial layer, and arched or deep saccate exoperidium (Wang & Bau 2023). Morphologically, *G. yunnanense* resembles two closely related species in this genus: *G. javanicum,* and *G. velutinum* (Table 2).

In ecology and biogeography, macrofungi are an extensively studied group of Basidiomycota, which are an important group in the forest ecosystem (Deng & Zhao 2023, Duan *et al.* 2023, Yang *et al.* 2023, Yuan *et al*. 2023, Zhou *et al.* 2023, Zhang *et al.* 2024, Lu *et al.* 2024). The family Geastraceae is an extensively studied group of Basidiomycota (Zhou *et al*. 2007, Finy *et al.* 2021, Wang & Bau 2023). However, the *Geastrum* species diversity in China is still not well known, especially in the subtropical and tropical areas. In the present study, a new species is introduced from Yunnan Province, which will further enrich our knowledge of the macrofungal diversity in Southwest China.

TABLE 2. A morphological comparison of *Geastrum yunnanense* and two closely related species in the genus *Geastrum*.

Species name	Unexpanded basidiomata	Basidiospores	Expanded basidiomata	Exoperidium	Stalk	Capillitial hyphae	References
Geastrum javanicum	13–21 mm in high	Globose; 4.0–5.0 µm in diam	17–29 mm high	5–7 rays; Mycelial layer not persistent;	sessile	straight to more or less sinuous; 2.0–7.0 µm in diam	Pereira *et al.* (2011)
Geastrum velutinum	19–22 mm in high	Globose; (2.5–) 4.0–4.0 (–5.5) µm in diam	19–50 mm high	5–7 (-8) rays; Mycelial layer not persistent;	sessile	unbranched; 4.0–6.0 µm in diam	Zhou *et al.* (2007)
Geastrum yunnanense	7–25 mm in high	Spherical; (1.7–) 2.0–2.5 (–2.9) × (1.7–) 1.9–2.5 (–2.9) µm in diam	20–40 mm high	7–9 rays; Mycelial layer not easily dislodged	sessile	rarely branched; 2.0–6.0(–8.0) µm in diam	**Present study**

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