Mycoscience VOL.65 (2024) 12–18

Mycoscience

An open access journal for general mycology

The Mycological Society of Japan

Short communication

Geastrum sanglinense, a new species from the Manghe Rhesus Monkey National Nature Reserve, China

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ABSTRACT

A novel species of earthstar from China, *Geastrum sanglinense* is described. Phylogenetic analyses based on sequences of the nuclear ribosomal DNA internal transcribed spacer (ITS), large subunit nuclear ribosomal RNA (nrLSU), and subunit 6 of ATP synthase (*atp6*) regions showed that the species belongs to subsect. *Epigaea* in sect. *Myceliostroma*. The sequences of the new taxon formed a sister group to *G. yanshanense* and *G. rubellum*. This species was mainly characterized by scattered or clustered basidiomata (1.9–2.2 cm in width × 2.3–2.5 cm in height), small to medium-sized saccate exoperidium (1.9–4.3 cm diam. when expanded), smooth endoperidial bodies (1.2–2.7 cm diam.), and globose to subglobose basidiospores (3.7–4.1 µm diam.), surface with short columnar warts. The species can also be distinguished by ITS, nrLSU, and *atp6* sequences. The new species was described in detail and can provide a reference for the investigation of macrofungi resources in Shanxi Province, China.

Keywords: *Basidiomycota*, *Geastraceae*, morphology, multi-gene phylogeny, taxonomy

Article history: Received 5 October 2023, Revised 7 November 2023, Accepted 7 November 2023, Available online 31 January 2024.

The genus *Geastrum* Pers. belongs to *Geastraceae*, *Geastrales*, *Phallomycetidae*, *Agaricomycetes*, and *Basidiomycota* (Kirk, Cannon, Minter, & Stalper, 2008). It is characterized by exoperidium splitting into rays at maturity and is widely known as the earthstars, which have a worldwide distribution and have been recorded from all continents except Antarctica (Kasuya et al., 2012a). The areas with more in-depth research on *Geastrum* are mainly concentrated in Europe, North America, South America, and East Asia (Japan) (Lloyd, 1902, 1907; Sunhede, 1989; Douanla, Langer, & Calonge, 2005; Kirk et al., 2008; Leite, Assis, Silva, Sotão, & Baseia, 2011; Kasuya, Hosaka, Uno, & Kakishima, 2012b; Jeppson, Nilsson, & Larsson, 2013; Zamora, Calonge, & Martín, 2013; Zamora, Kuhar, Castiglia, & Papinutti, 2014a; Zamora, Calonge, Hosaka, & Martín, 2014b; Zamora, Calonge, & Martín, 2015; Accioly et al., 2019). The species richness of genus is extremely high in China, so a growing number of scholars are paying attention to *Geastrum* species. To date, 34 species of *Geastrum* have been reported in China (Zhou & Yang, 2002; Han & Bau, 2016; Wang & Bau, 2023). In the past 10 years, an increasing number of molecular sequences have been used in the taxonomy of *Geastrum*, including the internal transcribed spacer (ITS) and large subunit nuclear ribosomal RNA (nrLSU) (Cabral et al., 2014). Zamora et al. (2014b) used ITS, nrLSU, the largest subunit of RNA polymerase II (*rpb1),* and subunit 6 of ATP synthase (*atp6*) polygenic sequences to explore *Geastrum* species worldwide and divided *Geastrum* into fourteen groups. This indicates that the species diversity of *Geastrum* worldwide is underestimated.

Sprawled across mountains and deep valleys, the Manghe Rhesus Monkey National Nature Reserve is located in the southern part of Shanxi Province (35°02′55″ to 35°17′20″ N, 112°22′10″ to 112°31′55″ E) (Liu, Zhao, Guo, & Wu, 1998). The Reserve covers *c.* 5573 ha, reaching the south boundary of Shanxi Province. The climate is a continental monsoon type, and annual precipitation is between 750 and 800 mm. 874 species of seed plants belong to 390 genera and 103 families in the Reserve (Zhang, 2019). The vegetation preserves the oak-like deciduous broad-leaved forest community, mainly *Quercus variabilis* Blume and *Q. baronii* Skan. Fungal diversity, including macrofungi in this area has not been well investigated because of its large scale. To contribute to our knowledge of the fungal diversity in the area, field surveys of macrofungi were conducted in recent years. We found one undescribed species of *Geastrum* in this area and here formally described it as a new taxon with a detailed description and the results of phylogenetic analyses.

Five fresh materials of *Geastrum* were collected in the Manghe Rhesus Monkey National Nature Reserve, Shanxi Province, China, in summer and autumn between 2016 and 2017. All collections were dried in a hot air oven at 45 °C, and then stored in specimen boxes. All specimens were deposited in the Herbarium of Mycology of Shanxi Agricultural University (HMSAU), China. Macroscopic

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characteristics were recorded from fresh materials during the sampling and collection expedition. Descriptions include the number of rays, diameter, and color of the exoperidium, structure and color of the mycelial layer, fibrous layer, and pseudoparenchymatous layer, the diameter and color of the endoperidial region, presence or absence of a stalk, peristome type, and whether there is peristome ring. Microstructures were studied using light microscopy (Eclipse Ni-U, with a Ri2 camera, Nikon, Tokyo, Japan). Small pieces of different parts of the basidiomata were mounted in 5% KOH (w/v), observe the microstructure of the basidiospores, capillitial threads, fibrous layer, and mycelial layer under the 100× oil immersion objective lens, with 10× oculars, and the pseudoparenchymatous layer under the 40× oil immersion objective lens, with 10× oculars. The pseudoparenchymatous layer was mounted in 5% KOH (w/v) and 1% Congo Red (w/v) for good contrast. At least 50 randomly selected basidiospores were measured at 1000× magnification. Meanwhile, the spores, the height of the ornamentation and capillitial threads were observed by scanning electron microscopy. For scanning electron microscopy, air-dried samples were mounted on a sample holder covered with double-sided adhesive tape, coated with pure gold in a sputter coater, and observed with a JSM-7500F SEM (JEOL, Tokyo, Japan). The following abbreviations were used in the descriptions: *avg* for the average length of the measured basidiospores, and *n* for the number of randomly measured basidiospores (Bas, 1969). Color codes (*e.g.*, 8B5) were described following Kornerup and Wanscher (1978). Morphological terms were applied of Sunhede (1989) and Zhou et al. (2007).

The genomic DNA of five *Geastrum* specimens was extracted from dried materials using the E.Z.N.A.® Forensic DNA extraction kit (Omega Bio-Tek, Norcross, Georgia, USA). PCR amplification was performed with the primer pairs ITS8-F/LR3 (Dentinger, Margaritescu, & Moncalvo, 2010) or ITS8-F/5.8S and 5.8SR/LS1R (Vilgalys & Hester, 1990; Hausner, Reid, & Klassen, 1993) for the ITS region, the primer pairs LR0R/LR5 (Cubeta, Echandi, Abernethy, & Vilgalys, 1991; Vilgalys & Hester, 1990) for the nrLSU partial region, and the primer pairs *atp6*-1/*atp6*-2 or *atp6*-3/*atp6*-2 (Kretzer & Bruns, 1999) for the *atp6* partial region. Successfully amplified products were cleaned using an E.Z.N.A.® Cycle-Pure Kit (Omega Bio-Tek) and sequenced at Tsingke Biotechnology Co., Ltd. (Xi'an, China). Following DNA sequencing, chromatograms of partial sequences were initially edited and assembled using the SeqMan of the DNASTAR 5.01 software package (DNASTAR, Inc., Madison, USA). Newly acquired sequences have been deposited in GenBank as ITS (OP050116–OP050120), nrLSU (OP050161–OP050165), and *atp6* (OP056323–OP056324).

A total of 34 sets of sequences were selected for molecular phylogenetic analyses based on the Basic Local Alignment Search Tool (BLAST) results from a GenBank search and a previous study of *Geastrum,* sect. *Myceliostroma* Henn. (Zhou, Li, & Hou, 2022; Zamora et al., 2014b; Accioly et al., 2019) (Table 1). *Geastrum striatum* DC. and *G. glaucescens* Speg. were used as outgroups for phylogenetic analyses since these species are placed in the sect. *Geastrum* based on the study of Zamora et al. (2014b). Sequences of ITS, nrLSU and *atp6* were separately aligned using Muscle algorithms in MEGA X (Tamura, 1992; Kumar, Stecher, Knyaz, & Tamura, 2018). For all partitions of the data (ITS, nrLSU, and *atp6*), tests of evolutionary models in MEGA X found that T92+G was the best model based on ln(*L*) scores, so this model was used to construct the maximum likelihood (ML) phylogenetic tree using MEGA X. The branch support value-maximum likelihood bootstrap (MLbs) were obtained by 1000 repeats. The data partition homogeneity test (Farris, Källersjö, Kluge, & Bult, 2005) performed in PAUP* v.4.0a169 (Swofford, 2003) allowed combination of three regions (ITS, nrLSU, and *atp6*) ($P = 0.24$). The maximum parsimony (MP) phylogenetic tree was constructed using PAUP* v.4.0a169 software, invoking the bootstrap method with a heuristic search set up to 1000 trees (MaxTrees = 1000), and the branch-swapping algorithm with tree-bisection-reconnection. Branches collapsed (creating polytomies) if the maximum branch was zero, using nonparametric bootstrap searches (Felsenstein, 1985), full-heuristic conditions could be set, 1000 replicates were made, the branch support value-maximum parsimony bootstrap (MPbs) was calculated, and the same parametric setting was conducted for heuristic exploration. If more than 1 MP tree was obtained, the optimal tree was determined using a similarity-based Shimodaira-Hasegawa topology test (Shimodaira & Hasegawa, 1999). A Bayesian Inference (BI) molecular phylogenetic tree was constructed using the Markov chain Monte Carlo method in MrBayes 3.2.6 (Ronquist et al., 2012). MrBayes settings for the best-fit model were selected by AIC in MrModeltest 2.3 (Nylander, 2004) for determining the best model for each gene. The best-fit model for ITS was HKY+I+G, and for nrLSU and *atp6*, both were GTR+I+G. Four chains were run simultaneously, and each chain independently randomly generated the tree using a total of 2,000,000 generations and then obtained the posterior probability (pp).

The phylogenetic tree constructed based on ITS, nrLSU, and *atp6* contains 1453 base pairs with ITS 505 base pairs, nrLSU 583 base pairs, and *atp6* 363 base pairs. The MP analysis results showed that TL (tree length) = 1019, CI (consistency index) = 0.6192, and RI (retention index) = 0.7849. The MP, ML, and BI phylogenetic trees showed that the topological structure was similar. The ML tree constructed based on the three genes ITS, nrLSU, and *atp6* are shown in Fig. 1. Number above branches are MLbs values, MPbs values, and pp values.

The phylogenetic tree constructed is similar to the branching structure reported by Accioly et al. (2019). The present new species in sect. *Myceliostroma* formed an independent lineage with strong statistical support (MLbs = 100, MPbs = 100, pp = 1) and was a sister to *G. yanshanense* C.L Hou, Hao Zhou & Jiqi Li and *G. rubellum* P.-A. Moreau & C. Lécuru. The new species can also be well distinguished from other species of the phylogenetic tree within sect. *Myceliostroma*.

Taxonomy

Geastrum sanglinense Y.Q. Wu & Shu R. Wang, sp. nov.

Figs. 2, 3, 4.

MycoBank no.: MB 845733

Diagnosis: Differs from *Geastrum rubellum* P.-A. Moreau & C. Lécuru by the light grayish orange pseudoparenchymatous layer (pinkish gray in *G. rubellum*), larger endoperidial body without apophysis, and smaller basidiospores (3.7–4.1 μm). (Accioly et al., 2019). Differs from *G. fimbriatum* Fr. by smaller basidiospores, fibrillose peristome and rhizomorphs covered with narrow prismatic crystals (Leite et al., 2011). Also differs from *G. yanshanense* by the light grayish orange pseudoparenchymatous layer (yellowish brown in *G. yanshanense*), and the larger basidiospores (2.8–3.3 μm in *G. yanshanense*) (Zhou et al., 2022). This species is also distinguishable from these taxa by their ITS, nrLSU, and *atp6* sequences.

Holotype: CHINA, Shanxi Province, Yangcheng County, the Manghe Rhesus Monkey National Nature Reserve, located near Huiquan Village, approx. 35°17′21″ N, 112°24′23″ E, 1090.08 m a.s.l, on rotting leaves in a mixed forest dominated by *Populus* L. and *Quercus* L., 5 Aug 2016, leg. S.R. Wang and F.K. Guo, HMSAU 15020 (The Herbarium of Mycology of Shanxi Agricultural Univer-

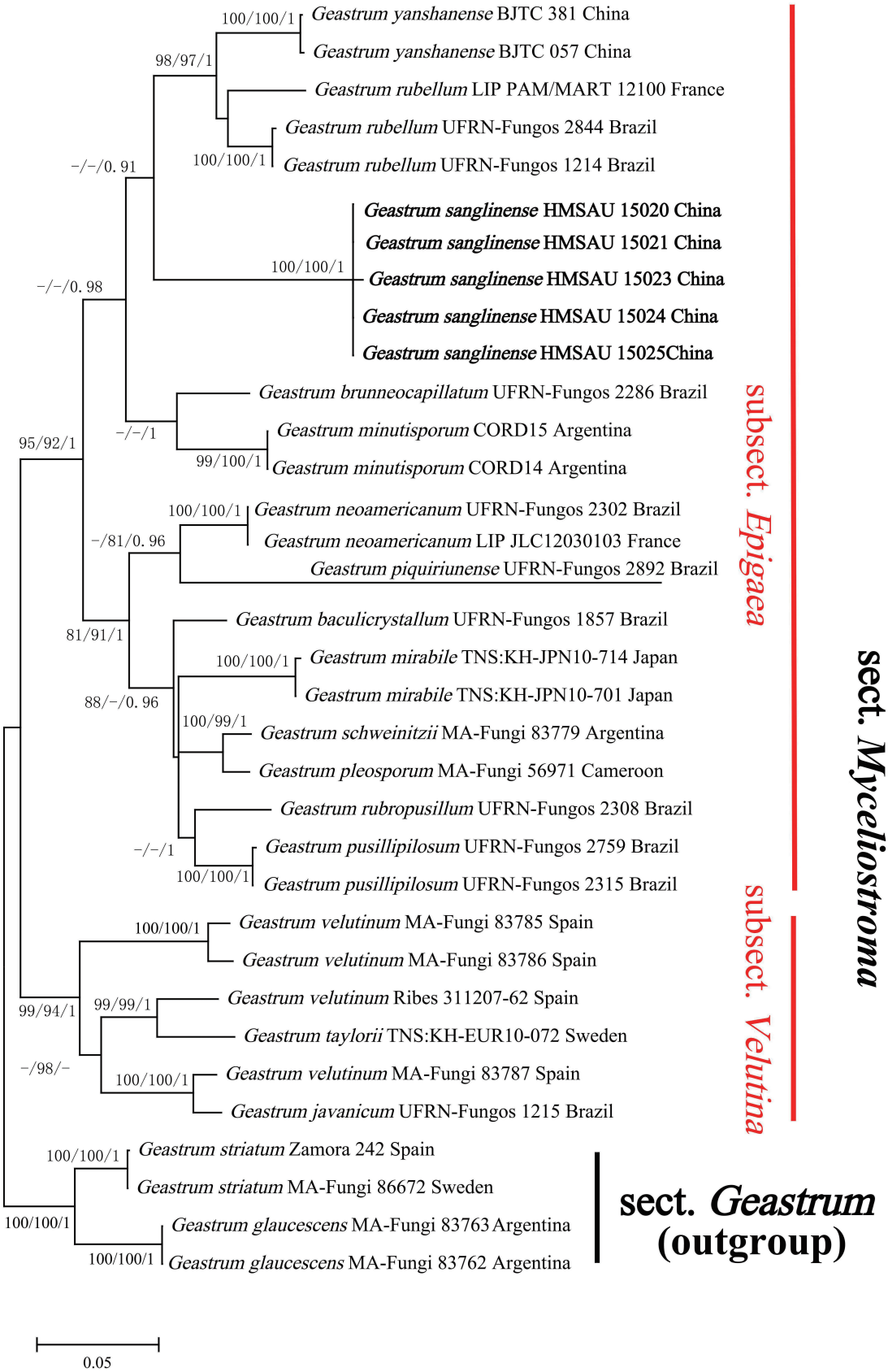

Fig. 1 – Multigene phylogenetic tree of *Geastrum* sect. *Myceliostoma* obtained from the maximum likelihood analysis of the ITS, nrLSU, and *atp6* gene regions. The numbers above branches are maximum likelihood bootstrap (MLbs), maximum parsimony bootstrap (MPbs) values and Bayesian posterior probability (pp) values. The support values (MLbs ≥ 75%, MPbs ≥ 75% or pp ≥ 0.9) are given at the branches. The scale bar indicates 0.05 expected changes per site per branch. All terminals are labelled by their collection numbers and names by which they were identified; accession numbers are listed in Table 1.

Table 1. Sequences used in the phylogenetic analyses. New sequences derived for this study are in bold font.

Species	Voucher	Collection locality	GenBank accession No.		
			ITS	nr LSU	atp6
Geastrum baculicrystallum	UFRN-Fungos 1857	Brazil	MH635018	MH635035	-
G. brunneocapillatum	UFRN-Fungos 2286	Brazil	MH634996	MH635029	-
G. glaucescens	MA-Fungi 83763	Argentina	KF988379	KF988501	KF988771
G. glaucescens	MA-Fungi 83762	Argentina	KF988378	KF988500	KF988770
G. hirsutum	UFRN-Fungos 1214	Brazil	KJ127029	JQ683662	JQ683670
G. javanicum	UFRN-Fungos 1215	Brazil	KJ127031	JQ683663	JQ683669
G. minutisporum	CORD14	Argentina	KM260664	-	-
G. minutisporum	CORD15	Argentina	KM260665	-	-
G. mirabile	TNS:KH-JPN10-701	Japan	JN845107	JN845225	JN845349
G. mirabile	TNS:KH-JPN10-714	Japan	JN845109	JN845227	JN845351
G. neoamericanum	UFRN-Fungos 2302	Brazil	MH635001	MH635040	-
G. neoamericanum	LIP JLC12030103	France	MH635014	MH635038	-
G. pleosporum	MA-Fungi 56971	Cameroon	KF988416	KF988544	KF988811
G. piquiriunense	UFRN-Fungos 2892	Brazil	MH260269	MH260270	-
G. pusillipilosum	UFRN-Fungos 2315	Brazil	KX761175	KX761176	-
G. pusillipilosum	UFRN-Fungos 2759	Brazil	KX761177	KX761178	-
G. rubellum	UFRN-Fungos 2844	Brazil	MH634999	MH635031	-
G. rubellum	LIP PAM/MART 12100	France	MH635010	MH635037	-
G. rubropusillum	UFRN-Fungos 2308	Brazil	MH634994	MH635027	-
G. sanglinense	**HMSAU 15020**	**China**	**OP050116**	**OP050161**	**-**
G. sanglinense	**HMSAU 15021**	**China**	**OP050117**	**OP050162**	**-**
G. sanglinense	**HMSAU 15023**	**China**	**OP050118**	**OP050163**	**OP056323**
G. sanglinense	**HMSAU 15024**	**China**	**OP050119**	**OP050164**	**OP056324**
G. sanglinense	**HMSAU 15025**	**China**	**OP050120**	**OP050165**	**-**
G. schweinitzii	MA-Fungi 83779	Argentina	KF988437	KF988567	KF988834
G. striatum	Zamora 242	Spain	JN943163	JN939559	KP687606
G. striatum	MA-Fungi 86672	Sweden	KF988443	KF988577	KF988843
G. taylorii	TNS:KH-EUR10-072	Sweden	JN845204	JN845329	JN845435
G. velutinum	Ribes 311207-62	Spain	KF988448	KF988583	KF988849
G. velutinum	MA-Fungi 83787	Spain	KF988449	KF988584	KF988850
G. velutinum	MA-Fungi 83786	Spain	KF988447	KF988582	KF988848
G. velutinum	MA-Fungi 83785	Spain	KF988446	KF988581	KF988847
G. yanshanensis	BJTC 381	China	MZ508878	MZ509383	MZ571184
G. yanshanensis	BJTC 057	China	MZ508879	MZ509384	MZ571185

Note: "-" means no relevant genetic information.

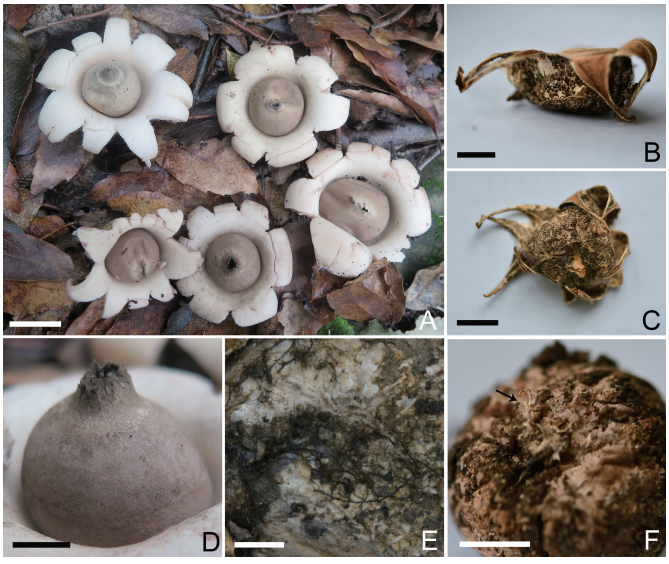

Fig. 2 – Macroscopic characteristics of *Geastrum sanglinense* (HMSAU15020). A: Expanded basidiomata. B, C: Dried basidiomata covered with debris. D: Brownish gray and fibrillose peristome. E: Blackish gray rhizomorphs attached to the base of the expanded basidiomata. F: Grayish yellow rhizomorphs attached to the base of the unexpanded basidiomata. *Bars*: A, B, C 1 cm; D, F 5 mm; E 1 mm.

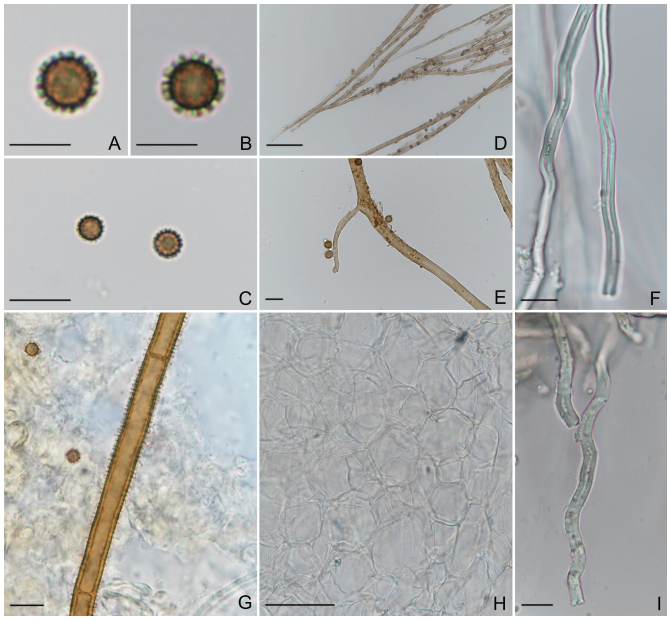

Fig. 3 – Microscopic characteristics of *Geastrum sanglinense* (HMSAU15020). A–C: Basidiospores under a light microscope. D, E: Capillitial threads with short branches. F: Skeletal hyphae of the mycelial layer. G: Brownish orange, and thick-walled generative hyphae of the mycelial layer. H: Pseudoparenchymatous layer. I: Hyaline and sinuous hyphae of the fibrous layer. *Bars*: A, B 5 μm; C, E–G, I 10 μm; D, H 50 μm.

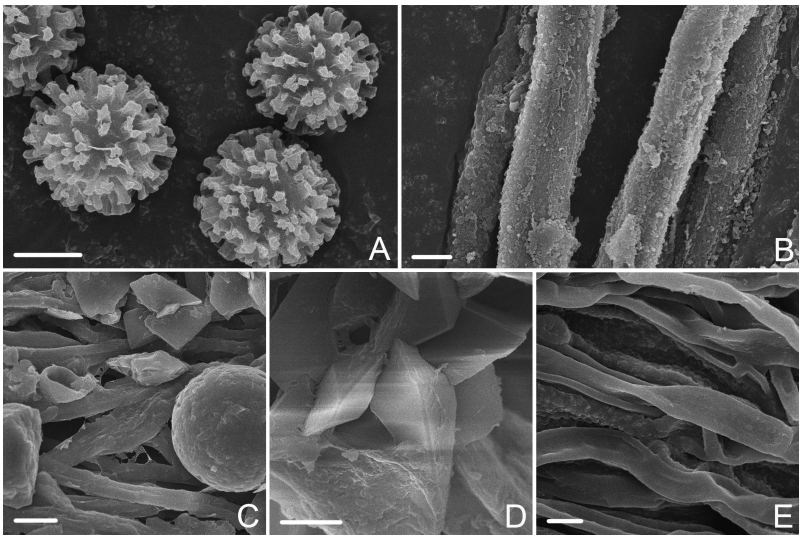

Fig. 4 – Microscopic characteristics of *Geastrum sanglinense* (HMSAU 15020) under SEM. A: Basidiospores with short columnar warts. B: Capillitial threads covered with dense warty structures. C, D: The hyphae surface of grayish yellow rhizomorphs covered by irregular prism-shaped crystals. E: the hyphae surface of blackish gray rhizomorphs was smooth or rough. *Bars*: A–C 2 μm; D 1 μm; E 2 μm.

sity, collection number W10387).

Gene sequences ex-holotype: OP050116 (ITS), OP050161 (nrLSU).

Etymology: *sanglinense* (Latin), referring to Sanglin Village, the Manghe Rhesus Monkey National Nature Reserve, from where the holotype was collected.

Basidiomata clustered or scattered. Unexpanded basidiomata 1.9–2.2 cm in width and 2.3–2.5 cm in height, epigeous, subglobose to obovate, grayish yellow (4C3 to 4B3), and as well as having coarse surface with encrusting debris. Expanded basidiomata small to medium-sized, 1.9–4.3 cm diam., saccate. Exoperidium split into 5–8 rays, which were triangular rays, each ray was different in width, approximately 1.1–1.9 cm, not hygrometric. Mycelial layer brownish gray (5D2–3), covered with small amounts of debris, persistent, and had a surface free of incrustations. Fibrous layer dark grayish orange (5A2 to 5C2), coriaceous. Pseudoparenchymatous layer thick, with shallow cracks, light grayish orange (6B2) when fresh, dark brown (7C2–4) when dried, persistent or cracking on the base of rays. Endoperidial body 1.2–2.7 cm diam., smooth, subglobose or oblate spherical, grayish brown (6D3), sessile, apophysis absent. Mesoperidium absent or very poorly developed. Peristome fibrillose 2–3 mm wide, 2–5 mm high, brownish gray (6E4), darker than endoperidium, some with peristome ring, up to 1 cm diam.. Columella well-developed. Mature gleba powdery or cottony, dark brown (7F7). The base attached with a very small number of rhizomorphs, blackish gray (G1) rhizomorphs attached to the base of the expanded basidiomata, growing on the ground surface; grayish yellow (1B2–3) rhizomorphs attached to the base of the unexpanded basidiomata, growing underground.

Basidiospores (3.3–)3.7–4.1(–5.0) μm diam. (avg = 3.9 μm, n = 50, not including columnar warts), globose, light brown, with columnar warts on the surface. Under the scanning electron microscope (SEM), the short columnar warts with flat tops could be observed, partly columnar warts were connected at the apex, which were 0.4–0.7 μm high. Capillitial threads abundant, 3.6–5.7 μm diam., thick-walled (0.6–1.0 μm), light brown, lumen narrowing or even absent, strongly attenuating towards the ends, mostly unbranched or rarely with short branches, the surface covered with dense warty protrusions under SEM. Mycelial layer double-layered, of skeletal hyphae 4.3–6.6 μm diam., sinuous, lumen conspicuous. Generative hyphae 4.7–6.7 μm diam., thick-walled (0.9–1.2 μm), brownish orange, lumen evident. Pseudoparenchymatous layer composed of thick-walled (0.7–1.9 μm) hyphal cells, 56–71 × 40–43 μm, subglobose to irregular oval, brownish gray, reddish orange stained with Congo Red. Fibrous layer composed of hyaline hyphae, 3.4–4.6 μm diam., sinuous, thick-walled (1.2–2.1 μm), lumen evident. Under SEM, the hyphal surface of grayish yellow rhizomorphs covered by irregular prism-shaped crystals; the hyphae surface of blackish gray rhizomorphs smooth or rough.

Habitat and distribution: most likely, saprophytic; growing in groups on the ground in a mixed forest dominated by *Populus* and *Quercus*. Collected from July to September, in Shanxi Province, China.

Additional specimens examined: CHINA, Shanxi Province, Yangcheng County, the Manghe Rhesus Monkey National Nature Reserve, Sanglin Village, located near Qianzhuang Country, approx. 35°15′7″ N, 112°27′23″ E, 630.3 m a.s.l, on rotten leaves in a forest dominated by *Quercus*, 27 Aug 2017, leg. S.R. Wang and F.K. Guo, HMSAU 15021 (collection number: G10455); CHINA, Shanxi Province, Yangcheng County, the Manghe Rhesus Monkey National Nature Reserve, Sanglin Village, located near Huiquan Country, approx. 35°17′21″ N, 112°24′23″ E, 1090.08 m a.s.l, on rotting leaves in a forest dominated by *Populus* and *Quercus*, 2 Aug 2016, leg. S.R. Wang and F.K. Guo, HMSAU 15023, HMSAU 15024, HMSAU 15025 (collection numbers: W10280, W10252, and W10264, respectively).

Coments

The phylogenetic tree (Fig. 1) based on ITS, nrLSU, and *atp6* showed that *Geastrum* is a multilineage genus, which is consistent with previous studies (Lloyd, 1902, 1907; Sunhede, 1989; Douanla et al., 2005; Kirk et al., 2008; Leite et al., 2011; Kasuya et al., 2012b; Jeppson et al., 2013; Zamora et al., 2013, 2014a, 2014b, 2015; Accioly et al., 2019). Elsewhere, sect. *Myceliostroma* is divided into subsect. *Epigaea* Dissing & M. Lange and subsect. *Velutina* J.C. Zamora (Zamora et al., 2014b). Our multigene phylogenetic analyses indicated that the new species *G. sanglinense* belongs to subsect. *Epi-*

gaea. This subsection was characterized by its well-developed rhizomorph system; basidiomata not caespitose, were medium-sized (endoperidial body 10–25 mm diam.); rhizomorph cystidioid elements largely vesiculose, were covered with narrow prismatic crystals. Phylogenetically, *G. sanglinense* has an independent position in the phylogenetic tree and forms a clade with *G. yanshanense* and *G. rubellum* which is weekly supported (less than 75% of MLbs and MPbs, 0.91 of pp). Morphologically, *G. yanshanense* can be distinguished from *G. sanglinense* by a pinkish gray exoperidium, a smaller size of endoperidial body (8–13 mm) and basidiospores, (2.7–3.2 × 2.8–3.3 μm, including columnar warts) (Zhou et al., 2022). *Geastrum rubellum* also differs from *G. sanglinense* by way of its smaller endoperidial body (6 × 3–10 mm) than that of *G. sanglinense* besides the pinkish-gray exoperidium (Accioly et al., 2019). Furthermore, the new species *G. sanglinense* resembles *G. fimbriatum* in the size and color of the endoperidial body and rays, but *G. fimbriatum* has a smaller basidiospore (3–3.5 μm diam.) and its rhizomorphs are not covered with narrow prismatic crystals (Leite et al., 2011), which is inconsistent with the distinguishing characteristic of the subsect. *Epigaea*.

Whether it is phylogenetic analysis or morphological observation, the new species can be distinguished from their similar species. Therefore, we identified it as a new species of *Geastrum*. Further studies will focus on the collection of specimens in Shanxi Province to enrich the species diversity of the genus *Geastrum*.

Disclosure

The authors declare no conflicts of interest. All the experiments undertaken in this study complied with the current laws of the countries in which they were performed.

Acknowledgments

This study was supported in part by the Scientific Start-up Program of Shanxi Agricultural University (2014YJ18), the Special Scientific Research Project of Agricultural Region Construction in Shanxi Province (SXNGJSKYZX201903), and the Engineering Technology Research Center for Edible Fungi (Shanxi Province). We thank Dr. Taiga Kasuya (Department of Biology, Keio University, Japan) and Dr Tiezheng Wei (Mycological Herbarium, Institute of Microbiology, Academia Sinica, China) for their suggestions and guidance.

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