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Taxonomy & Inventories

Geastrum suae sp. nov. (Geastraceae, Basidiomycota) a new species from Yunnan Province, China

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Abstract

Background

Geastrum is the largest genus of Geastraceae and is widely distributed all over the world. Four specimens which belong to *Geastrum* were collected during our scientific expedition to Cangshan Mountain, Yunnan, China. Based on morphological characteristics and phylogenetic analysis, a new species was introduced.

New information

Geastrum suae is characterised by its large basidiomata (height 35–70 mm, diameter 18–37 mm) with long stipe (height 10–45 mm), smooth pink exoperidium and sessile globose endoperidial body. Phylogenetic analysis has been carried out, based on the internal

transcribed spacer (ITS) and large subunit ribosomal ribonucleic acid (nrLSU) sequence data. The illustration and description for the new taxa are provided.

Keywords

Geastraceae, ITS, nrLSU, taxonomy, phylogeny

Introduction

Geastrum Pers. is the largest genus of Geastraceae and was established by Persoon (1794). *Geastrum* is commonly known as the earthstars with worldwide distribution and the most species-diverse in the family Geastraceae. Up to now, there are 140 valid species in this genus (Wijayawardene et al. 2022, Zhou et al. 2022, Cabral et al. 2022, Wang and Bau 2023). *Geastrum* clearly differs from *Myriostoma* by a single endodermal stoma (Sousa et al. 2014). Due to the non-splitting ectoderm and the poorly-developed endoperidium being different from *Geastrum*, researchers thought that *Radiigera* is one of the genera closely related to *Geastrum* (Sunhede 1989, de Toledo and Castellano 1996). Later, some studies have found that specimens in *Radiigera* are nested in *Geastrum* (Hosaka et al. 2006, Hosaka and Castellano 2008, da Silva et al. 2013), but the relationship between these two genera has not been studied in depth until Jeppson et al. (2013) classified the species of *Radiigera* into the genus *Geastrum*. Species of this genus are distributed globally, especially in temperate and tropical regions, such as Brazil-Amazon and Europe (de León 1968, da Silva et al. 2013, Jeppson et al. 2013, Zamora et al. 2014, Cabral et al. 2014a, Cabral et al. 2014b, Sousa et al. 2015, Crous et al. 2016, Cabral et al. 2017, Crous et al. 2017, Sousa et al. 2017, Crous et al. 2018a, Crous et al. 2018b, Assis et al. 2019, Finy et al. 2021, Rodrigues et al. 2021). However, the taxonomic relationship under the genus was chaotic (Zamora et al. 2013) until Zamora et al. (2014) divided it into 14 Sections using polygenic analysis, viz. Sect. Campestria, *Corollina*, *Elegantly*, *Exareolata*, *Fimbriata*, *Fornicata*, *Geastrum*, *Hariotia*, *Hieronymia*, *Myceliostroma*, *Papillata*, *Pseudoilmbata*, *Schmidelia* and *Trichaster*.

In China, the early systematic report of *Geastrum* can be found in "Fungi in China" (Deng 1963) and "The Confluence of Chinese Fungi" (Dai 1979). Zhou et al. (2007) detailed descriptions of 16 species of *Geastrum* in China in "Flora Fungorum Sinicorum-Geastraceae and Nidulariaceae". Later, three new records and nine new species were reported (Han and Bau 2016, Zhou et al. 2022, Wang and Bau 2023).

Four specimens which belong to *Geastrum* were collected during our scientific expedition to Cangshan Mountain, Yunnan, China. Morphological and phylogenetic analysis revealed that these specimens are the same species and are different from other species in *Geastrum*. Therefore, we introduced it as a new species and provided the detailed description and illustration.

Materials and methods

Morphological description

Macro-morphological descriptions were based on fresh specimens, which were photographed in the field with notes and laboratory supplemental measurements. The colour is compared with the standard colours in the colorhexa website (https://www.colorhexa.com). Micro-morphological data were obtained from the fresh specimens and observed by using a light microscope, following Accioly et al. (2019). Sections were studied at magnification of up to 1000× using a NiKon eclipse Ni microscope and phase contrast illumination and scanning electron microscope (SEM) analysis was done under a Shimadzu SSX–550. Preparation of the material examined under SEM followed da Silva et al. (2011). Microscopic features and measurements were made from slide preparations stained with 5% potassium hydroxide (KOH). Basidiospore features, hyphal system, colour, sizes and shapes were recorded and photographed. Measurements were made using the Image Framework v.0.9.7 to represent variation in the size of basidiospores, 5% of measurements were excluded from each end of the range and extreme values are given in parentheses.

The abbreviation for spore measurements (n/m/p) denote “n” spores measured from “m” basidiocarps of “p” specimens. Basidiospore dimensions (and “Q” values) are given as (a) b–av–c (d), where “a” represents the *minimum*, “d’ the largest, “av” the average “b” and “c” covers a *minimum* of 90% of the values. “Q” is the length/width ratio of a spore inside view and “Qm” for the average of all spores ± standard deviation. Voucher specimens are deposited in the Herbarium of Cryptogams, Kunming Institute of Botany Academia Sinica (KUN-HKAS).

DNA extraction, PCR amplification and sequencing

The DNA extractions were performed from a small piece of the dried basidioma by using Trelief ™ Plant Genomic DNA Kit from Tsingke Biotechnology Co., Ltd (Beijing, China). Two DNA regions were amplified: the internal transcribed spacer nuclear ribosomal DNA (ITS), nuclear ribosomal large subunit (nrLSU) with the primer pairs ITS1F/ITS4 and LR0R/LR5, respectively (Table 1).

Table 1.

Amplification primers information used in this study.

Gene	Primer	Primer sequence (5′-3′)	References
ITS	ITS1F	CTTGGTCATTTAGAGGAAGTAA	Gardes and Bruns (1993)
	ITS4	TCCTCCGCTTATTGATATGC	White et al. (1990)
nrLSU	LR0R	ACCCGCTGAACTTAAGC	Vilgalys and Hester (1990)
	LR5	ATCCTGAGGGAAACTTC	Vilgalys and Hester (1990)

PCR reactions (25 µl) contained mixture: 12.5 µl 2X SanTaq PCR Master Mix (including $MgCl_2$, dNTP, Taq DNA Polymerase, PCR buffer, loading etc.), 1 µl each of primer, 2 µl DNA solution and 9.5 µl sterilised distilled H_2O. The PCR cycling for ITS and nrLSU was as follows: initial denaturation at 94°C for 5 min, followed by 35 cycles at 94°C for 30 sec, 53°C for 30 sec and 72°C for 50 sec and a final extension of 72°C for 10 min. The PCR products were visualised via UV light after electrophoresis on 1% agarose gels stained with ethidium bromide. Successful PCR products were sent to Sangon Biotech Limited Company (Shanghai, China), using forward PCR primers. When sequences have heterozygous INDELS or ambiguous sites, samples were sequenced bidirectionally to make contigs of the amplified regions or verify the ambiguous sites. Raw DNA sequences were assembled and edited in Sequencher 4.1.4 and the assembled DNA sequences were deposited in GenBank (Table 2).

Table 2.

Species, specimens, Collection locality and GenBank accession numbers of sequences used in this study (newly-generated sequences are indicated in bold).

Species	**Strain/Voucher**	**Collection locality**	**GenBank Accession No.**	
			ITS	**nrLSU**
Geastrum mirabile	strain: 228-394	Japan	AB509736	-
Geastrum javanicum	TNS:TKG-GE-90902	Japan	JN845100	JN845218
Geastrum mirabile	TNS:KH-JPN10-714	Japan	JN845109	JN845227
Geastrum parvistriatum	MA-Fungi 69583	Spain	JN943160	JN939560
Geastrum parvistriatum	Herb. Zamora 272	Spain	JN943162	JN939572
Geastrum striatum	Herb. Zamora 257	Spain	JN943164	JN939557
Geastrum campestre	Herb. Zamora 283	Spain	JN943167	JN939575
Geastrum aff. *arenarium*	Herb. Zamora 76	Spain	KF988338	KF988470
Geastrum lageniforme	Herb. Zamora 316	Spain	KF988339	KF988514
Geastrum cf. *calceum*	UFRN-Fungos 723	Brazil	KF988340	KF988477
Geastrum cf. *calceum*	MA-Fungi 83761	Argentina	KF988341	KF988478
Geastrum aff. *hariotii*	Börge Petterson 2070	Mozambique	KF988342	KF988507
Geastrum cf. *saccatum*	Herb. Sunhede 7749	Australia	KF988343	KF988556
Geastrum hieronymi	MA-Fungi 83767	Argentina	KF988344	KF988509
Geastrum cf. *stipitatum*	Herb. Zamora 528	Brazil	KF988345	KF988576
Geastrum albonigrum	MA-Fungi 36140-2	Panama	KF988349	KF988468
Geastrum aff. *arenarium*	MA-Fungi 68191	Spain	KF988350	KF988469
Geastrum cf. *arenarium*	MA-Fungi 83760	Argentina	KF988351	KF988471
Geastrum argentinum	LPS 48446	Argentina	KF988352	KF988472
Geastrum argentinum	MA-Fungi 82605	Argentina	KF988353	KF988473
Geastrum berkeleyi	MA-Fungi 74668	Spain	KF988354	KF988474

Species	Strain/Voucher	Collection locality	GenBank Accession No.	
			ITS	nrLSU
Geastrum berkeleyi	Herb. Sunhede 7724	Sweden	KF988355	KF988475
Geastrum berkeleyi	Herb. Zamora 504	Sweden	KF988356	KF988476
Geastrum campestre	Herb. Sunhede 7575	Sweden	KF988357	KF988479
Geastrum campestre	MICH 28566	USA	KF988358	KF988480
Geastrum corollinum	MA-Fungi 5746	Spain	KF988359	KF988481
Geastrum corollinum	Herb. Sunhede 7744	Sweden	KF988360	KF988482
Geastrum coronatum	Herb. Zamora 266	Spain	KF988361	KF988483
Geastrum coronatum	Herb. Zamora 522	Sweden	KF988362	KF988484
Geastrum coronatum	MICH 28567	USA	KF988363	KF988485
Geastrum aff. *coronatum*	MICH 72012	USA	KF988364	KF988486
Geastrum aff. *coronatum*	MICH 72014	USA	KF988365	KF988487
Geastrum elegans	Herb. Zamora 189	Spain	KF988366	KF988488
Geastrum elegans	UPS F-560810	Sweden	KF988367	KF988489
Geastrum entomophilum	MA-Fungi 70785	Brazil	KF988368	KF988490
Geastrum fimbriatum	Herb. Zamora 234	Spain	KF988369	KF988491
Geastrum fimbriatum	Herb. Sunhede 7739	Sweden	KF988370	KF988492
Geastrum flexuosum	UPS F-119844	Sweden	KF988371	KF988493
Geastrum floriforme	MA-Fungi 69173	Spain	KF988372	KF988494
Geastrum floriforme	Herb. Zamora 453	Spain	KF988373	KF988495
Geastrum fornicatum	Herb. Zamora 255	Spain	KF988374	KF988496
Geastrum fornicatum	MA-Fungi 30749	Spain	KF988375	KF988497
Geastrum fuscogleba	NY Trappe 1071	USA	KF988376	KF988498
Geastrum fuscogleba	NY Trappe 9500	USA	KF988377	KF988499
Geastrum glaucescens	MA-Fungi 83762	Argentina	KF988378	KF988500
Geastrumglaucescens	MA-Fungi 83763	Argentina	KF988379	KF988501
Geastrum aff. *glaucescens*	MA-Fungi 83764	Argentina	KF988380	KF988502
Geastrum hariotii	MA-Fungi 83765	Argentina	KF988381	KF988504
Geastrum aff. *hariotii*	MA-Fungi 78296	Brazil	KF988382	KF988505
Geastrum aff. *hariotii*	MA-Fungi 78289	Brazil	KF988383	KF988506
Geastrum hieronymi	MA-Fungi 83766	Argentina	KF988384	KF988508
Geastrum kotlabae	MA-Fungi 39563	Spain	KF988385	KF988510
Geastrum kotlabae	Herb. Zamora 440	Spain	KF988386	KF988511
Geastrum aff. *kotlabae*	MA-Fungi 33300	Tanzania	KF988387	KF988512
Geastrum lageniforme	Herb. Zamora 207	Spain	KF988388	KF988513
Geastrum aff. *lageniforme*	MA-Fungi 83768	Argentina	KF988389	KF988516

Species	Strain/Voucher	Collection locality	GenBank Accession No.	
			ITS	nrLSU
Geastrum aff. *lageniforme*	COFC Hama 327	Niger	KF988390	KF988517
Geastrum aff. *lageniforme*	MA-Fungi 83770	Argentina	KF988391	KF988518
Geastrum aff. *lageniforme*	MA-Fungi 83769)	Argentina	KF988392	KF988519
Geastrum aff. *lageniforme*	MA-Fungi 78398	Portugal	KF988393	KF988520
Geastrum aff. *lageniforme*	Herb. Ribes 221210-01	Spain	KF988394	KF988521
Geastrum melanocephalum	Herb. Zamora 34	Spain	KF988395	KF988522
Geastrum melanocephalum	Herb. Sunhede 7737	Sweden	KF988396	KF988523
Geastrum michelianum	Herb. Sunhede 7738	Sweden	KF988397	KF988524
Geastrum michelianum	Herb. Zamora 227	Spain	KF988398	KF988525
Geastrum aff. *michelianum*	MA-Fungi 83771	Argentina	KF988399	KF988527
Geastrum minimum	Herb. Zamora 191	Spain	KF988400	KF988528
Geastrum minimum	Herb. Sunhede 7746	Sweden	KF988401	KF988529
Geastrum minimum	MICH 72010	USA	KF988402	KF988530
Geastrum minimum	MICH 28119	Spain	KF988403	KF988531
Geastrum minimum	MA-Fungi 31530	USA	KF988404	KF988532
Geastrum minimum	MA-Fungi 86669	Sweden	KF988405	KF988533
Geastrum morganii	Herb. Lebeuf HRL0177	Canada	KF988406	KF988534
Geastrum aff. *morganii*	Herb. Zamora 367	Spain	KF988407	KF988535
Geastrum aff. *morganii*	Herb. Zamora 525	Spain	KF988408	KF988536
Geastrum aff. *morganii*	MA-Fungi 83772	Argentina	KF988409	KF988537
Geastrum aff. *morganii*	MA-Fungi 83773	Argentina	KF988410	KF988538
Geastrum ovalisporum	MA-Fungi 47184	Bolivia	KF988411	KF988539
Geastrum pectinatum	Herb. Zamora 252	Spain	KF988412	KF988540
Geastrum pectinatum	UPS F-560803	Sweden	KF988413	KF988541
Geastrum pectinatum	UPS F-09935 (161483)	Tanzania	KF988414	KF988542
Geastrum pectinatum	MA-Fungi 83774	Argentina	KF988415	KF988543
Geastrum pleosporum	MA-Fungi 56971	Cameroon	KF988416	KF988544
Geastrum pouzarii	MA-Fungi 2944	Czechoslovakia	KF988417	KF988545
Geastrum pouzarii	Herb. Sunhede 7494	Czechoslovakia	KF988418	KF988546
Geastrum pseudolimbatum	Herb. Zamora 231	Spain	KF988419	KF988547
Geastrum pseudolimbatum	UPS F-560804	Sweden	KF988420	KF988548
Geastrum quadrifidum	Herb. Zamora 170	Spain	KF988421	KF988549
Geastrum quadrifidum	MA-Fungi 86671	Sweden	KF988422	KF988550
Geastrum quadrifidum	MICH 72512	USA	KF988423	KF988551
Geastrum rufescens	Herb. Zamora 253	Spain	KF988424	KF988552

Species	Strain/Voucher	Collection locality	GenBank Accession No.	
			ITS	nrLSU
Geastrum rufescens	Herb. Zamora 274	Spain	KF988425	KF988553
Geastrum cf. *saccatum*	MA-Fungi 47185-2	Bolivia	KF988426	KF988554
Geastrum cf. *saccatum*	MA-Fungi 83775	Argentina	KF988427	KF988555
Geastrum cf. *saccatum*	UPS F-530056	Japan	KF988428	KF988558
Geastrum cf. *saccatum*	MA-Fungi 83777	Argentina	KF988429	KF988559
Geastrum cf. *saccatum*	Herb. Zamora 260	Spain	KF988430	KF988560
Geastrum cf. *saccatum*	Herb. Zamora 461	Spain	KF988431	KF988561
Geastrum cf. *saccatum*	COFC Hama 343	Niger	KF988432	KF988562
Geastrum cf. *saccatum*	MA-Fungi 83778	Argentina	KF988433	KF988563
Geastrum schmidelii	Herb. Zamora 279	Spain	KF988434	KF988564
Geastrum schmidelii	UPS F-560805	Sweden	KF988435	KF988565
Geastrum cf. *schweinitzii*	S Henrik Kylin 1983 30.X	Papua New Guinea	KF988436	KF988566
Geastrum cf. *schweinitzii*	MA-Fungi 83779	Argentina	KF988437	KF988567
Geastrum cf. *schweinitzii*	MA-Fungi 36141	Panama	KF988438	KF988568
Geastrum cf. *schweinitzii*	MA-Fungi 83780	Argentina	KF988439	KF988569
Geastrum smardae	Herb. Lebeuf HRL 0160	Canada	KF988440	KF988573
Geastrum smardae	Herb. Zamora 527	Spain	KF988441	KF988574
Geastrum smithii	MA-Fungi 83783	Argentina	KF988442	KF988575
Geastrum striatum	MA-Fungi 86672	Sweden	KF988443	KF988577
Geastrum "*triplex*"	UPS F-014630 (213863)	Madagascar	KF988444	KF988578
Geastrum "*triplex*"	MA-Fungi 83784	Argentina	KF988445	KF988579
Geastrum cf. *velutinum*	MA-Fungi 83785	Argentina	KF988446	KF988581
Geastrum cf. *velutinum*	MA-Fungi 83786	Argentina	KF988447	KF988582
Geastrum cf. *velutinum*	Herb. Ribes 311207-62	Spain	KF988448	KF988583
Geastrum cf. *velutinum*	MA-Fungi 83787	Peru	KF988449	KF988584
Geastrum violaceum	BAFC 51671	Argentina	KF988450	KF988585
Geastrum violaceum	MA-Fungi 82487	Argentina	KF988451	KF988586
Geastrum sp.1	MA-Fungi 83788	Argentina	KF988452	KF988587
Geastrum sp.1	MA-Fungi 83789	Argentina	KF988453	KF988588
Geastrum sp.2	MA-Fungi 31143	Spain	KF988454	KF988589
Geastrum sp.2	MA-Fungi 37546	Spain	KF988455	KF988590
Geastrum sp.3	MA-Fungi 83790	Argentina	KF988456	KF988591
Geastrum sp.4	MA-Fungi 83791	Peru	KF988457	KF988592
Geastrum sp.5	Herb. Zamora 145	Spain	KF988458	KF988593
Geastrum sp.5	Herb. Zamora 450	Spain	KF988459	KF988594

Species	Strain/Voucher	Collection locality	GenBank Accession No.	
			ITS	nrLSU
Geastrum sp.6	MA-Fungi 83792	Argentina	KF988460	KF988595
Geastrum sp.7	MA-Fungi 83793	Argentina	KF988461	KF988596
Geastrum sp.7	MA-Fungi 83794	Argentina	KF988462	KF988597
Geastrum sp.8	MA-Fungi 83795	Argentina	KF988463	KF988598
Geastrum hirsutum	UFRN-Fungos 1214	Brazil	KJ127029	-
Geastrum javanicum	UFRN-Fungos 1215	Brazil	KJ127031	-
Geastrum minutisporum	CORD14	Argentina	KM260664	-
Geastrum minutisporum	CORD15	Argentina	KM260665	-
Geastrum pusillipilosum	UFRN:Fungos 2315	Brazil	KX761175	KX761176
Geastrum pusillipilosum	UFRN:Fungos 2759	Brazil	KX761177	KX761178
Geastrum piquiriunense	UFRN:Fungos:2892	Brazil	MH260269	MH260270
Geastrum hirsutum	INPA:259950	Brazil	MH634993	MH635026
Geastrum rubropusillum	UFRN:Fungos:2308	Brazil	MH634994	MH635027
Geastrum baculicrystallum	UFRN:Fungos:2835	Brazil	MH634995	MH635028
Geastrum brunneocapillatum	UFRN:Fungos:2286	Brazil	MH634996	MH635029
Geastrum rubellum	UFRN:Fungos:2844	Brazil	MH634999	MH635031
Geastrum neoamericanum	UFRN:Fungos:2302	Brazil	MH635001	MH635040
Geastrum courtecuissei	LIP:FH 2004090503	Guadeloupe	MH635003	MH635033
Geastrum rubellum	LIP:CL/MART 8067B	Martinique	MH635009	-
Geastrum rubellum	LIP:PAM/MART 12.100	Martinique	MH635010	MH635037
Geastrum neoamericanum	LIP:JLC 12030103	French	MH635014	MH635038
Geastrum suae	**HKAS 123795 (Holotype)**	**China**	**ON529511**	**ON529515**
Geastrum suae	**HKAS 123794**	**China**	**ON529512**	**ON529516**
Geastrum suae	**HKAS 123793**	**China**	**ON529513**	**ON529517**
Geastrum suae	**HKAS 123796 (Paratype)**	**China**	**ON529514**	**ON529518**
Geastrum hariotii	MA-Fungi 80070	Dominican Republic	-	KF988503
Geastrum aff. *lageniforme*	MA-Fungi 79056	Brazil	-	KF988515
Geastrum cf. *saccatum*	MA-Fungi 83776	Argentina	-	KF988557
Geastrum cf. *schweinitzii*	S Henrik Kylin 842	Fiji	-	KF988570
Geastrum cf. *velutinum*	MA-Fungi 73247	India	-	KF988580
Geastrum michelianum	Herb. Ribes 231208-31	Spain	-	KF988526
Geastrum setiferum	MA-Fungi 83781	Argentina	-	KF988571
Geastrum setiferum	MA-Fungi 83782	Argentina	-	KF988572
Geastrum velutinum	BJTC 221	China	-	MZ509382
Geastrum velutinum	BJTC 598	China	MZ508877	-

Species	Strain/Voucher	Collection locality	GenBank Accession No.	
			ITS	nrLSU
Geastrum yanshanense	BJTC 381	China	MZ508878	MZ509383
Geastrum yanshanense	BJTC 057	China	MZ508879	MZ509384
Geastrum yanshanense	BJTC 255	China	MZ508880	-
Schenella pityophila	Herb. Zamora 530	Spain	KF988346	KF988464
Schenella pityophila	Herb. Zamora 531	Spain	KF988347	KF988465
Myriostoma coliforme	MA-Fungi 83759	Argentina	KF988348	KF988467

Sequence alignment

Sequence data of two partial loci, internal transcribed spacer region (ITS) and the large subunit ribosomal RNA gene (nrLSU) were analysed. All the sequences, except those which were obtained from this study, were selected from GenBank for phylogenetic analyses (Table 2). Sequences were aligned using the online version of MAFFT v.7 (http://mafft.cbrc.jp/alignment/server/) (Katoh and Standley 2013) and adjusted using BioEdit v. 7.0.9 (Hall 1999) by hand to allow maximum alignment and minimise gaps. Ambiguous regions were excluded from the analyses and gaps were treated as missing data. AliView 1.19-beta was used to convert the alignment fasta file to Phylip and Nexus format for phylogenetic analysis. Phylogenetic analyses were obtained from Maximum Likelihood (ML) and Bayesian Inference (BI).

Molecular phylogenetic analyses

The Maximum Likelihood (ML) and Bayesian Inference (BI) methods were used to analyse the combined dataset of ITS and nrLSU sequences. ML analysis was conducted with RAxML-HPC2 on the CIPRES Science Gateway (Miller et al. 2010), involving 100 ML searches; all model parameters were estimated by the programme. The ML bootstrap values (ML-BS) were obtained with 1000 rapid bootstrapping replicates.

Bayesian analysis was performed with MrBayes v.3.2 (Ronquist et al. 2012), with the best-fit model of sequence evolution estimated with MrModelTest 2.3 (Nylander et al. 2008) to evaluate posterior probabilities (PP) (Rannala and Yang 1996, Zhaxybayeva and Gogarten 2002) by Markov Chain Monte Carlo (MCMC) sampling. Six simultaneous Markov chains were run for 100,000,000 generations, trees were sampled every 500th generation and 200,000 trees were obtained. The first 50,000 trees, representing the burn-in phase of the analyses, were discarded, while the remaining 150,000 trees were used for calculating posterior probabilities in the majority rule consensus tree (the critical value for the topological convergence diagnostic is 0.01).

The phylogenetic tree was visualised with FigTree version 1.4.0 (Rambaut 2012) and made in Adobe Illustrator CS5 (Adobe Systems Inc., USA). Sequences derived in this study were deposited in GenBank (http://www.ncbi.nlm.nih.gov).

Taxon treatment

Geastrum suae Z.Q. Zhang C.H. Li & Z.L. Luo, sp. nov.

- MycoBank MB845193

Materials

Holotype:

a. scientificName: ***Geastrum suae***; kingdom: Fungi; phylum: Basidiomycota; class: Agaricomycetes; order: Geastrales; family: Geastraceae; genus: ***Geastrum***; verbatimElevation: 2160 m; locationRemarks: China, Yunnan Province, Dali City, Cangshan Mountain; verbatimLatitude: 25°43′36.97″N; verbatimLongitude: 100°07′16.46″E; year: 2020; month: September; day: 4; habitat: Terrestrial; fieldNotes: grows in groups on the ground in mixed coniferous and broad-leaved forests, with thick humus; recordNumber: SJ582; recordedBy: Zheng-Quan Zhang; type: KUN-HKAS 123795; occurrenceID: 6D676216-9572-5A8D-A7C3-4C445C671395

Paratype:

a. scientificName: ***Geastrum suae***; kingdom: Fungi; phylum: Basidiomycota; class: Agaricomycetes; order: Geastrales; family: Geastraceae; genus: ***Geastrum***; verbatimElevation: 2221 m; locationRemarks: China, Yunnan Province, Dali City, Cangshan Mountain; verbatimLatitude: 25°40′16.38″N; verbatimLongitude: 100°09′08.42″E; year: 2020; month: October; day: 14; habitat: Terrestrial; fieldNotes: grows in groups on the ground in mixed coniferous and broad-leaved forests, with thick humus; recordNumber: MB015; recordedBy: Chao-Hai Li; type: KUN-HKAS 123796; occurrenceID: F58D4D0C-33BB-541B-A92B-7AF436F12F49

Other materials:

a. scientificName: ***Geastrum suae***; kingdom: Fungi; phylum: Basidiomycota; class: Agaricomycetes; order: Geastrales; family: Geastraceae; genus: ***Geastrum***; verbatimElevation: 2208 m; locationRemarks: China, Yunnan Province, Dali City, Cangshan Mountain; verbatimLatitude: 25°40′28″N; verbatimLongitude: 100°08′59″E; year: 2021; month: September; day: 3; habitat: Terrestrial; fieldNotes: grows in groups on the ground in mixed coniferous and broad-leaved forests, with thick humus; recordNumber: SJ2501; recordedBy: K. Wang; type: KUN-HKAS 123793; occurrenceID: 9213A508-19C3-5A9C-8E34-8DCDD9D4C170

b. scientificName: ***Geastrum suae***; kingdom: Fungi; phylum: Basidiomycota; class: Agaricomycetes; order: Geastrales; family: Geastraceae; genus: ***Geastrum***; verbatimElevation: 2350 m; locationRemarks: China, Yunnan Province, Yangbi County, Cangshan Mountain; verbatimLatitude: 25°41′59″N; verbatimLongitude: 100°02′00″; year: 2021; month: October; day: 1; habitat: Terrestrial; fieldNotes: grows in groups on the ground in mixed coniferous and broad-leaved forests, with thick humus; recordNumber: SJ2500; recordedBy: G. H. Yang; type: KUN-HKAS 123794; occurrenceID: CE65C858-1CD7-53B3-B545-F1750391FB8E

Description

Unexpanded basidiomata 13–28 mm, cylindrical to ellipsoidal, very light grey (#fdfdfd) to very pale red (#ffe6e6) with a slight protrusion, rough. Expanded basidiomata height

35–70 mm, diameter 18–37 mm, deep saccate, **Exoperidium** splitting into 6, arched, not hygrometric, prosthecae length 23–35 mm, diameter 5–13 mm, exoperidium attached to the rhizomorphs. Rhizomorphs with 0.1–5.4 µm hyphae, fibrous and transparent, white (#ffffff). **Mycelial layer** 49.5–59.0 µm, consisting of transparent hyphae (1.0–3.5 µm) with thin walls and no septum, curved. **Fibrous layer** 6.5–16.5 µm, transparent, curved, thick-walled hyphae (1.1–5.0 µm) smooth, transparent to cream (#fffdd0), pure red (#e60000) to dark red (#9a0000) when stained with Congo red. **Pseudoparenchymatous layer** 2.5–19.3 × 2.7–30.4 µm, irregular shape, mycelium is transparent when fresh, pure orange (#ffa500) to moderate pink (#cc6691) when stained with Congo red, the thickness of the pseudoparenchyma layer is about 1.0–1.3 mm, very soft pink (#d98ca0). **Endoperidial body** 11–23 mm, globose, sessile, very light grey (#dfdfdf) to dark grey (#a0a0a0), with lighter reticulation. Endoperidial surface with some protruding hyphae, endoperidium is interwoven by transparent hyphae, fibrous. **Peristome** fibrillose, unpleated, wide conical, with obvious oral margin ring. Columella obvious very light grey (#f4f4f4 to #e0e0e0). **Eucapillitium hyphae** 1.0–5.5 µm, thick-walled, with distinct cavities, smooth, the ends tapering and are bluntly rounded (Fig. 1).

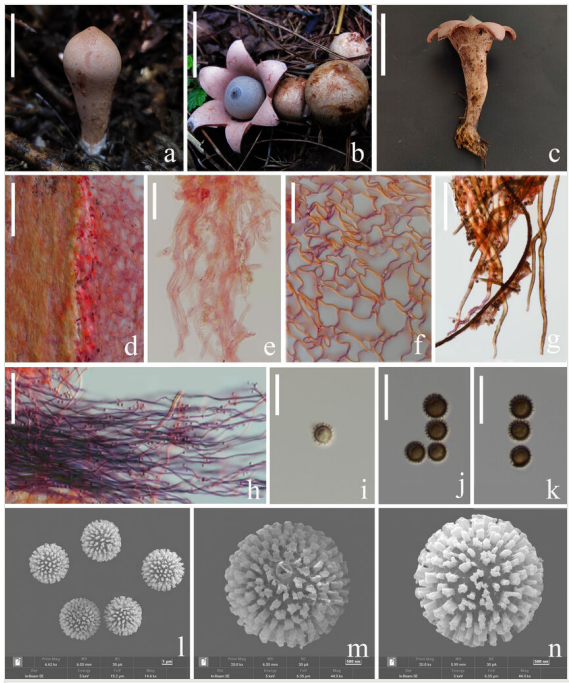

Figure 1. doi

Geastrum suae (KUN-HKAS 123795, holotype). **a** fresh unexpanded fruiting bodies; **b, c** fresh mature fruiting bodies; **d** mycelial layer, fibrous layer and pseudoparenchymatous layer; **e** hyphae of mycelial layer; **f** pseudoparenchymatous layer (cells in the stack); **g, h** eucapillitium hyphae; **i-k** basidiospores (LM); **l-n** basidiospores (SEM). Scale bars: a = 10 mm; b, c, e = 20 mm; d = 80 µm; f, g, i-k = 10 µm; h = 70 µm; l = 1 µm; m, n = 500 nm.

Basidiospores globose: Holotype (40/2/1) 4.5–5.3–6.0 × (4.5)5.0–5.4–6.0 µm, Q = (0.80)0.83–1.12(1.14), Qm= 0.98 ± 0.08, n = 40, including spines truncated at the apex ornamentation, with 0.2–0.5 µm high warts, ornamentation isolated or coalescing crest-like warts. Basidia not observed.

Diagnosis

Geastrum suae is characterised by long stipes and larger basidiomata; Pseudoparenchymatous layer is pink, smooth; globose endoperidial body, grey; the ends of eucapillitium hyphae taper and are bluntly rounded; and they live in groups.

Etymology

The species is named *suae* (Lat.), in memory of the Chinese mycologist Prof. Hong-Yan Su, who kindly helped the authors in many ways and sadly passed away on 3 May 2022 during the preparation of the current paper.

Habit

It grows in groups on the ground in mixed coniferous and broad-leaved forests where there are *Alnus nepalensis* and *Pinus yunnanensis*, with thick humus. Currently, it is known only from Cangshan Mountain.

Analysis

Phylogenetic analysis

Firstly, we constructed the ML tree of *Geastrum* genus, based on ITS (1–540 bp) and nrLSU (541–1498 bp) genes and found that *G. suae* is in Sect. Mycelioatroma. The Maximum Likelihood bootstrap values (ML) equal to or greater than 70% are given above each node (Fig. 2), with the Final ML Optimisation Likelihood: -24127.230142. The aligned matrix had 856 distinct alignment patterns, with 6.78% completely undetermined characters or gaps. The base frequency and rate are as follows: A = 0.274187, C = 0.208839, G = 0.265219, T = 0.251755; rate AC = 1.202699, AG = 3.054698, AT = 1.472914, CG = 0.671195, CT = 5.726232, GT = 1.000000; gamma distribution shape: α = 0.269052. Therefore, we constructed the ML tree and Bayesian tree of Sect. Mycelioatroma, based on ITS and nrLSU genes and clarified the position of *G. suae* in this Section. The dataset is composed of ITS and nrLSU genes, comprising a total of 1478 characters including gaps, ITS (1–591 bp) and nrLSU (592–1478 bp), including 35 taxa with *Myriostoma coliforme* (MA-Fungi 83759) as the outgroup taxon (Fig. 3). The best fit model for the combined 2-gene 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 phylogenetic analysis of ML and BI produces similar topology. The combined dataset analysis of RAxML generates a best-scoring tree (Fig. 3), with the Final ML Optimisation Likelihood value of -7513.207751. The aligned matrix had 584 distinct alignment patterns,

with 21.33% completely undetermined characters or gaps. The base frequency and rate are as follows: A = 0.272494, C = 0.207593, G = 0.257821, T = 0.262093; rate AC = 1.093594, AG = 2.765430, AT = 1.755140, CG = 0.441983, CT = 5.721217, GT = 1.000000; gamma distribution shape: α = 0.243957. Bootstrap support values with ML greater than 70% and Bayesian posterior probabilities (PP) greater than 0.95 are given above the nodes (Fig. 3).

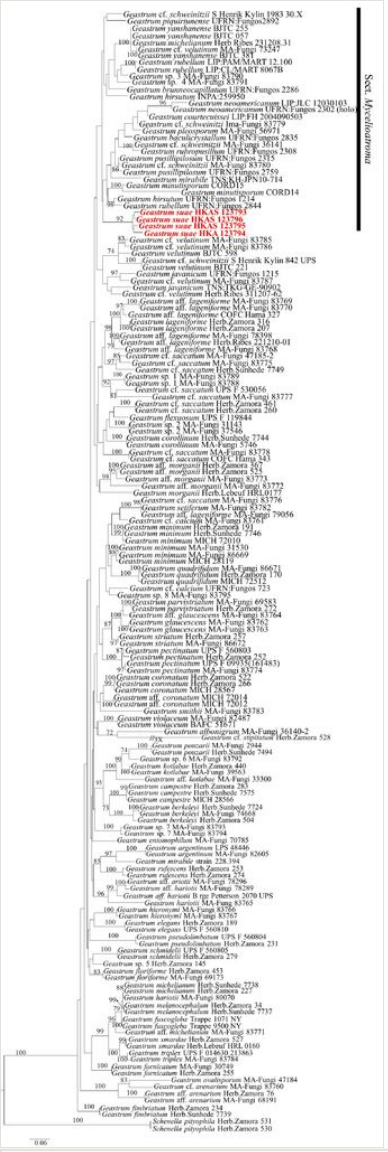

Figure 2. doi

Phylogenetic tree of *Geastrum* species and related taxa, based on ITS and nrLSU sequence data.

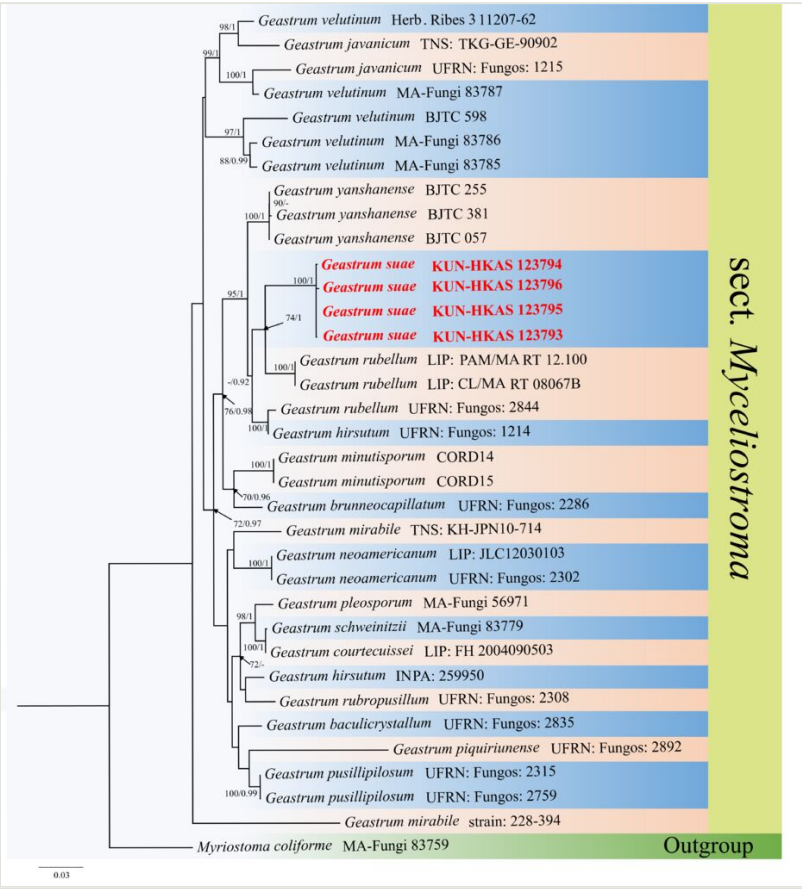

Figure 3. doi

Phylogenetic tree of the new *Geastrum* species and related taxa which belong to sect. Myceliostroma, based on ITS and nrLSU sequence data. Branches are labelled with bootstrap values (ML) higher than 70% and posterior probabilities (PP) higher than 0.95. The new species are shown in red bold.

Phylogenetic analysis showed that four new collections of *G. suae* clustered together with high bootstrap support and are sister to *G. rubellum* with good bootstrap support (74% ML/ 1 PP Fig. 3).

Discussion

Geastrum suae can be easily recognised by the basidiomata with pink neat, smooth 6-lobed ectoderm, globose sessile endoperidium and longer prosthecae.

In the phylogenetic inferences, *Geastrum suae* is sister to *G. rubellum*, which is known from the biome Tropical and Subtropical Moist Broadleaf Forests in Brazil (Accioly et al. 2019) (Fig. 3). Morphologically, both species share similar characteristics of the mesopodal

basidiomata, but *G. rubellum* has reddish to brownish exoperidium with longer exoperidium hairs. *G. suae* hardly has such hairs and the reddish pseudoparenchymatous layer in *G. rubellum* also clearly differentiates *G. suae*. Not only that, but *G. rubellum* also has reddish to brownish exoperidium with a verrucose to hairy mycelial layer, while the exoperidium of *G. suae* is almost smooth. Their size is different, the expanded basidiomata saccate of *G. rubellum* being 10 mm high × 8.5–30 mm wide, while *G. suae* is 35-70 mm high × 18–37 mm wide. The warts on the basisiospore of *G. suae* are shorter than those of *G. rubellum*. The pseudoparenchymatous layer of *G. rubellum* is pure (or mostly pure) pink (#fa007d) when fresh, brownish-grey when dried, but is very pale red (#ffccd5) for *G. suae*. The ITS comparison between our specimen (KUN-HKAS 123795) and *G. rubellum* (LIP: PAM/MART 12.100) revealed a 53 bp difference in a total of 542 bp. The nrLSU comparison between *G. suae* (KUN-HKAS 123795) and *G. rubellum* (LIP: PAM/MART 12.100) revealed 11 bp difference in a total of 809 bp (Accioly et al. 2019). It is worth noting that *G. rubellum* is distributed in the Neotropics (Accioly et al. 2019). Combined with the above analysis, we introduce *Geastrum suae* as a new species.

Acknowledgements

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