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Geastrum chamelense (Geastraceae, Agaricomycetes), a new species with setose endoperidium from the tropical dry forest in Jalisco, Mexico

Geastrum chamelense (Geastraceae, Agaricomycetes), una nueva especie con endoperidio setoso del bosque tropical caducifolio en Jalisco, México

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Abstract:

Background and Aims: *Geastrum* is characterized by stelliform basidiomata, exoperidium with three layers, sessile or stalked endoperidium, and sulcate, plicate, folded or fibrillose peristome, distinctly or indistinctly delimited, sometimes with mycosclereids. The objective of this study is to describe and illustrate *Geastrum chamelense* with morphological, ecological and molecular data as a new species from the Chamela Biological Station, Jalisco, Mexico.

Methods: Basidiomata collections with different degrees of maturity gathered in 2010 and 2011 from tropical dry forest at the Chamela Biological Station in Jalisco state are described macro- and microscopically. The type material is deposited in the fungal collections of the herbaria ENCB and MEXU. The extraction of DNA, as well as the phylogenetic analyses of ITS, LSU, *atp6* and *rpb1* sequences, are based on the holotype.

Key results: *Geastrum chamelense* is distinguished by its greyish brown basidiomata, pseudofoeniculate, fleshy exoperidium, not hygroscopic, sessile endoperidium, subglobose to depressed with peristome plicate, not delimited, and setae present. The latter character is shared with *Geastrum setiferum* from Brazil, but that species has shorter and wider setae (95-215 × 20-47 μm) than *G. chamelense* (102-330 × 10.2-15.3 μm). From a phylogenetic perspective, *G. chamelense* is sister to *G. hieronymi* and *G. cf. calceum*, while *G. setiferum* is not related, as it appears in a separate clade.

Conclusions: *Geastrum chamelense* is recognized as a new species based on morphological, ecological and molecular data.

Key words: earthstars fungi, Geastrales, phylogeny, taxonomy.

Resumen:

Antecedentes y Objetivos: *Geastrum* se caracteriza por presentar basidiomas esteliformes, exoperidio con tres capas, endoperidio sésil o estipitado, peristoma sulcado, plegado, doblado o fibriloso, distintiva o indistintivamente delimitado, algunas veces con micoescleroides. El objetivo de este estudio es describir e ilustrar a *Geastrum chamelense* con datos morfológicos, ecológicos y moleculares como una especie nueva de la Estación de Biología Chamela, Jalisco, México.

Métodos: Las colecciones de basidiomas con diferentes grados de madurez recolectados en 2010 y 2011 del bosque tropical caducifolio de la Estación de Biología Chamela en el estado de Jalisco se describen macro y micromorfológicamente. El material tipo está depositado en las colecciones micológicas de los herbarios ENCB y MEXU. La extracción de ADN y el análisis filogenético se basó en secuencias de ITS, LSU, *atp6* y *rpb1* del holotipo.

Resultados clave: *Geastrum chamelense* se distingue por sus basidiomas pardo grisáceos, exoperidio pseudofoenicado, carnoso, no higroscópico y endoperidio sésil, subgloboso a deprimido con peristoma plegado, no delimitado, y presencia de setas. Este último carácter se comparte con *Geastrum setiferum* de Brasil, pero esta tiene setas más cortas y anchas (95-215 × 20-47 μm) que *G. chamelense* (102-330 × 10.2-15.3 μm). Desde una perspectiva filogenética, *G. chamelense* tiene como grupo hermano a *G. hieronymi* y *G. cf. calceum*, mientras que *G. setiferum* no está relacionado porque aparece en un clado separado.

Conclusiones: *Geastrum chamelense* es reconocida como una especie nueva basada en datos morfológicos, ecológicos y moleculares.

Palabra clave: estrellas de tierra, filogenia, Geastrales, taxonomía.

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Introduction

The Chamela Biological Station of the Universidad Nacional Autónoma de México is located within the Chamela-Cuixmala Biosphere Reserve in the municipality La Huerta, Jalisco state, Mexico (Fig. 1). The station has a predominance of tropical dry forest, which safeguards one of the best conserved tropical communities, and this has great biological and ecological importance (Noguera et al., 2002). Although Mexico has extensive areas with tropical dry forest, 160 000 km² approximately (Challenger, 1998), knowledge of mycobiota in this kind of vegetation and particularly in the Chamela Biological Station is scarce, compared to the great diversity of fungal species in the tropical dry forest (Hawksworth, 1993) and the station. There are several studies that include species from the tropical dry forest such as those from Esqueda et al. (1999), Raymundo et al. (2009, 2014, 2017), Salinas-Salgado et al. (2012), Valenzuela et al. (2012), Álvarez et al. (2016), Contreras-Pacheco et al. (2018), and Reyes et al. (2020). However, few studies are known from the Chamela Biological Station: Ramírez-López

et al. (2012), Bautista-Hernández et al. (2015), and Raymundo (2021).

Seventeen species of *Geastrum* Pers. have been registered in this type of ecosystem (Esqueda et al., 1996, 1999, 2000, 2003, 2009; Pérez-Silva et al., 1999; Guzmán, 2003; Calonge and Mata, 2004; Calonge et al., 2004; Herrera et al., 2005; Bautista-Hernández et al., 2015). This genus belongs to the family Geastraceae, order Geastrales, subclass Phallomycetidae, class Agaricomycetes, subphylum Agaricomycotina of the phylum Basidiomycota (Index Fungorum, 2021). Three species, *Geastrum fimbriatum* Fr., *G. saccatum* Fr. and *G. violaceum* Rick, have been cited from the Chamela Biological Station (Pérez-Silva et al., 1999; Bautista-Hernández et al., 2015). This genus is characterized by stelliform basidiomata, exoperidium with three layers, sessile or stalked endoperidium, sulcate, plicate, folded or fibrillose peristome, distinctly or indistinctly delimited. Microscopically, it can present ornamented basidiospores and mycosclereids or protruding hyphae (Sunhede, 1989), and setae (Baseia and Milanez, 2002). During explorations

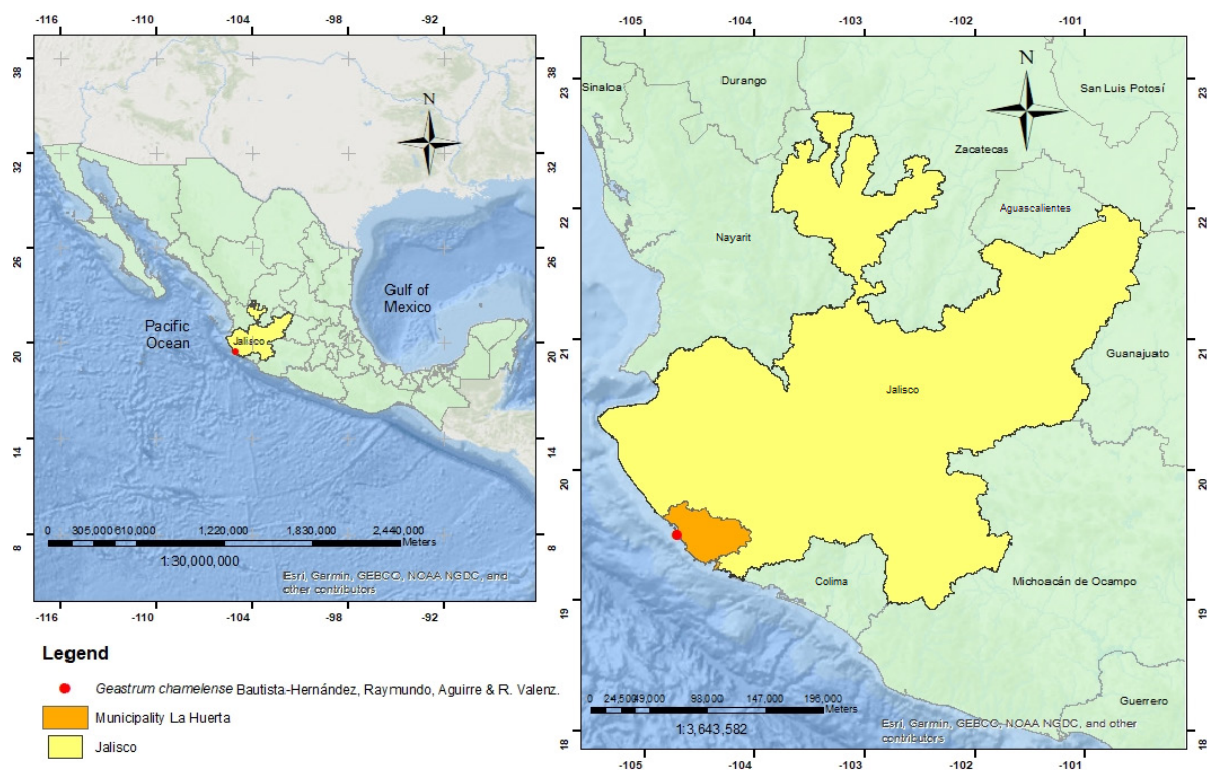


Figure 1: Type locality of *Geastrum chamelense* Bautista-Hernández, Raymundo, Aguirre & R. Valenz. in the Chamela Biological Station, Chamela, Jalisco, Mexico.



carried out in the station about a decade ago, a great diversity of *Geastrum* species was observed, and several specimens with particular macro- and micromorphological characteristics uncommon in the genus called our attention. The objective of this study is to describe and illustrate *Geastrum chamelense* as a new species for the Chamela Biological Station, based on morphological, ecological and molecular data.

Materials and Methods

Field work

Material of the undescribed species was collected in September 2010 and 2011 in the Chamela Biological Station, Jalisco, Mexico, ca. 19°27'2.1"N, 105°01'33"W, 250 m a.s.l. (Fig. 1, coordinates and elevation were obtained with a Garmin Etrex 10 GPS, Kansas City, USA). The local ecosystem belongs to the semideciduous tropical forest, according to Rzedowski (2006), with *Brosimum alicastrum* Sw. and *Celtis monoica* Hemsl. as the dominant tree species. The holotype was deposited in the fungal collection in the Herbarium of the Escuela Nacional de Ciencias Biológicas of the Instituto Politécnico Nacional (ENCB) and the isotype in the fungal collection of the National Herbarium of Mexico (MEXU) of the Instituto de Biología of the Universidad Nacional Autónoma de México (UNAM).

Morphological analyses

This taxonomic study was based on collections of basidiomata with different degrees of maturity. Morphological examinations were conducted using protocols outlined by Sunhede (1989). The colour of the sporomata was coded according to Kornerup and Wanscher (1978), which is indicated in parentheses in the description. For the microscopic study, temporary preparations were made in 70% alcohol and 5% potassium hydroxide (KOH) to elaborate descriptions of colour, size, shape of basidiospores, setae, and hyphae. The length and width of thirty basidiospores and setae were measured with a micrometric scale. Using scanning microscopy, gleba and endoperidium preparations were made, observing the detail of the spore and capillitium ornamentation, as well as the surface of the endoperidium. For the morphometric study, an optical microscope (MO; Primo Star, Carl Zeiss, Göttingen, Germany)

and a scanning electron microscope (SEM; Hitachi Su 1510, Hitachi, Japan) were used. To prepare the taxonomic key, the abovementioned species known to occur in the study area were incorporated.

DNA extraction, amplification, and sequencing

The DNA was obtained from herbarium specimens (Table 1). The CTAB protocol of Martínez-González et al. (2017) was used to extract genomic DNA. The DNA was quantified with a Nanodrop 2000c (Thermo Scientific™, Wilmington, USA). We prepared dilutions from each sample at 20 ng/μl to amplify the next four regions (Table 2): mitochondrial ATPase subunit 6 (*atp6*), nuclear large subunit ribosomal DNA (LSU), Internal Transcribed Spacer (ITS) and the largest subunit of RNA polymerase II gene (*rpb1*). The reaction mixture for PCRs was performed on a final volume of 15 μl containing 1× buffer, 0.8 mM dNTPs mix, 20 pmol of each primer, 2 units of GoTaq DNA (Promega, USA) and 100 ng of template DNA. The PCR products were verified by agarose gel electrophoresis. The gels were run for 1 h at 95 V cm⁻³ in 1.5% agarose and 1× TAE buffer (Tris Acetate-EDTA). The gel was stained with GelRed (Biotium, USA) and the bands were visualized in an Infinity 3000 transilluminator (Vilber Lourmat, Eberhardzell, Germany). The amplified products were purified with the ExoSAP Purification kit (Affymetrix, USA), following the manufacturer's instructions. They were quantified and prepared for the sequence reaction using a BigDye Terminator v. 3.1 (Applied Biosystems, USA). These products were sequenced in both directions with an Applied Biosystem model 3730XL (Applied BioSystems, Foster City, USA), at the Instituto de Biología, UNAM. The sequences obtained were compared with the original chromatograms to detect and correct possible reading errors. The sequences of both strands of each of the genes were analyzed, edited and assembled using the BioEdit v. 7.0.5 (Hall, 1999) to generate a consensus sequence which were compared with those deposited in GenBank (2020), using the tool BLASTN v. 2.2.19 (Zhang et al., 2000).

Phylogenetic analyses

To explore the phylogenetic relationships of the new species, an alignment was made based on the taxonomic sampling employed by Zamora et al. (2014). Each gene region



Table 1: GenBank accession numbers corresponding to the sequences used in the phylogenetic analyses. In bold the accession of the new species.

Species name	Isolate/Voucher/strain	GenBank Accessions			
		ITS	nrLSU	<i>rpb1</i>	<i>atp6</i>
<i>Schenella pityophila</i> (Malençon & Rioussel) Estrada & Lado	Zamora 530	KF988346	KF988464	KF988599	KF988734
<i>Myriostoma coliforme</i> Desv.	Zamora 496	KF988337	KF988466	KF988601	KF988736
<i>Geastrum albonigrum</i> Calonge & M. Mata	MA-Fungi 36140-2	KF988349	KF988468	KF988603	KF988738
<i>Geastrum</i> aff. <i>arenarium</i>	MA-Fungi 68191	KF988350	KF988469	KF988604	KF988739
<i>Geastrum</i> aff. <i>arenarium</i> 2	Zamora 76	KF988338	KF988470	KF988605	KF988740
<i>Geastrum argentinum</i> Speg.	LPS 48446	KF988352	KF988472	KF988607	KF988742
<i>Geastrum argentinum</i> 2	MA-Fungi 82605	KF988353	KF988473	KF988608	KF988743
<i>Geastrum berkeleyi</i> Massee	MA-Fungi 74668	KF988354	KF988474	KF988609	KF988744
<i>Geastrum</i> cf. <i>calceum</i>	UFRN-Fungos 723	KF988340	KF988477	KF988612	KF988747
<i>Geastrum campestre</i> Morgan	Zamora 283	JN943167	JN939575	JN991286	KF988748
<i>Geastrum chamelense</i> Bautista-Hernández, Raymundo, Aguirre & R. Valenz.	T. Raymundo 3504 (ENCB)	OL653145	OL653165	OL67687	OL676806
<i>Geastrum corollinum</i> (Batsch) Hollós	MA-Fungi 5746	KF988359	KF988481	KF988616	KF988751
<i>Geastrum corollinum</i> 2	Sunhede 7744	KF988360	KF988482	KF988617	KF988752
<i>Geastrum coronatum</i> Pers.	Zamora 266	KF988361	KF988483	KF988618	KF988753
<i>Geastrum elegans</i> Vittad.	Zamora 189	KF988366	KF988488	KF988623	KF988758
<i>Geastrum elegans</i> 2	UPS F-560810	KF988367	KF988489	KF988624	KF988759
<i>Geastrum entomophilum</i> Fazolino, Calonge & Baseia	MA-Fungi 70785	KF988368	KF988490	KF988625	KF988760
<i>Geastrum fimbriatum</i> Fr.	Zamora 234	KF988369	KF988491	KF988626	KF988761
<i>Geastrum fimbriatum</i> 2	Sunhede 7739	KF988370	KF988492	KF988627	KF988762
<i>Geastrum flexuosum</i> (L.S. Domínguez & Castellano) Jeppson & E. Larss.	UPS F-119844	KF988371	KF988493	KF988628	KF988763
<i>Geastrum floriforme</i> Vittad.	MA-Fungi 69173	KF988372	KF988494	KF988629	KF988764
<i>Geastrum floriforme</i> 2	Zamora 453	KF988373	KF988495	KF988630	KF988765
<i>Geastrum fornicatum</i> (Huds.) Hook.	Zamora 255	KF988374	KF988496	KF988631	KF988766
<i>Geastrum fornicatum</i> 2	MA-Fungi 30749	KF988375	KF988497	KF988632	KF988767
<i>Geastrum fuscoglebum</i> (Zeller) Jeppson & E. Larss.	Trappe 1071	KF988376	KF988498	KF988633	KF988768
<i>Geastrum fuscoglebum</i> 2	Trappe 9500	KF988377	KF988499	KF988634	KF988769
<i>Geastrum glaucescens</i> Speg.	MA-Fungi 83762	KF988378	KF988500	KF988635	KF988770
<i>Geastrum glaucescens</i> 2	MA-Fungi 83763	KF988379	KF988501	KF988636	KF988771
<i>Geastrum hariatii</i> Lloyd	MA-Fungi 80070	-----	KF988503	KF988638	KF988773
<i>Geastrum</i> aff. <i>hariatii</i>	MA-Fungi 78296	KF988382	KF988505	KF988640	KF988775
<i>Geastrum hieronymi</i> Henn.	MA-Fungi 83766	KF988384	KF988508	KF988643	KF988776
<i>Geastrum hieronymi</i> 2	MA-Fungi 83767	KF988344	KF988509	KF988644	KF988777
<i>Geastrum kotlabae</i> V.J. Staněk	MA-Fungi 39563	KF988385	KF988510	KF988645	KF988778
<i>Geastrum kotlabae</i> 2	Zamora 440	KF988386	KF988511	KF988646	KF988779
<i>Geastrum</i> aff. <i>kotlabae</i>	MA-Fungi 33300	KF988387	KF988512	KF988647	-----
<i>Geastrum lageniforme</i> Vittad.	Zamora 207	KF988388	KF988513	KF988648	KF988780
<i>Geastrum</i> aff. <i>lageniforme</i>	MA-Fungi 79056	-----	KF988515	KF988650	KF988782
<i>Geastrum michelianum</i> (Sacc.) W.G. Sm.	Sunhede 7738	KF988397	KF988524	KF988659	KF988791



Table 1: Continuación.

Species name	Isolate/Voucher/strain	GenBank Accessions			
		ITS	nrLSU	rpb1	atp6
<i>Geastrum michelianum</i> 2	Zamora 227	KF988398	KF988525	KF988660	KF988792
<i>Geastrum minimum</i> Schwein.	Zamora 191	KF988400	KF988528	KF988663	KF988795
<i>Geastrum morganii</i> Lloyd	Lebeuf HRL0177	KF988406	KF988534	KF988669	-----
<i>Geastrum</i> aff. <i>morganii</i>	Zamora 367	KF988407	KF988535	KF988670	KF988801
<i>Geastrum ovalisporum</i> Calonge & Mor.-Arr.	MA-Fungi 47184	KF988411	KF988539	KF988674	KF988805
<i>Geastrum parvistriatum</i> J.C. Zamora & Calonge	MA-Fungi 69583	JN943160	JN939560	JN991291	KF988806
<i>Geastrum parvistriatum</i> 2	Zamora 272	JN943162	JN939572	JN991283	KF988807
<i>Geastrum pectinatum</i> Pers.	Zamora 252	KF988412	KF988540	KF988675	KF988808
<i>Geastrum pleosporum</i> Duoanla-Meli	MA-Fungi 56971	KF988416	KF988544	KF988679	KF988811
<i>Geastrum pouzarii</i> V.J. Staněk	MA-Fungi 2944	KF988417	KF988545	KF988680	KF988812
<i>Geastrum pouzarii</i> 2	Sunhede 7494	KF988418	KF988546	KF988681	KF988813
<i>Geastrum pseudolimbatum</i> Hollós	Zamora 231	KF988419	KF988547	KF988682	KF988814
<i>Geastrum quadridum</i> DC. ex Pers.	Zamora 170	KF988421	KF988549	KF988684	KF988816
<i>Geastrum rufescens</i> Pers.	Zamora 253	KF988424	KF988552	KF988687	KF988819
<i>Geastrum rufescens</i> 2	Zamora 274	KF988425	KF988553	KF988688	KF988820
<i>Geastrum schmidelii</i> Vittad.	Zamora 279	KF988434	KF988564	KF988699	KF988831
<i>Geastrum schmidelii</i> 2	UPS F-560805	KF988435	KF988565	KF988700	KF988832
<i>Geastrum setiferum</i> Baseia	MA-Fungi 83781	-----	KF988571	KF988706	KF988837
<i>Geastrum smardae</i> V.J. Staněk	Lebeuf HRL 0160	KF988440	KF988573	KF988708	KF988839
<i>Geastrum smithii</i> Lloyd	MA-Fungi 83783	KF988442	KF988575	KF988710	KF988841
<i>Geastrum</i> cf. <i>stipitatum</i>	Zamora 528	KF988345	KF988576	KF988711	-----
<i>Geastrum striatum</i> DC.	Zamora 257	JN943164	JN939557	JN991288	KF988842
<i>Geastrum striatum</i> 2	MA-Fungi 86672	KF988443	KF988577	KF988712	KF988843
<i>Geastrum violaceum</i> Rick	BAFC 51671	KF988450	KF988585	KF988720	KF988851
<i>Geastrum violaceum</i> 2	MA-Fungi 82487	KF988451	KF988586	KF988721	KF988852

Table 2: Primers used in the amplification and sequencing of the DNA fragments.

Loci/Segment	Primer	Sequence 5'- 3'	T(°C)	Reference
ITS	ITS5	GGAAGTAAAAGTCGTAACAAGG	57	White et al., 1990
	ITS4	TCCTCCGCTTATTGATATGC	57	White et al., 1990
LSU	LROR	ACCCGCTGAACTTAAGC	54	White et al., 1990
	LR3	GGTCCGTGTTTCAAGAC	60	White et al., 1990
rpb1	Afrpb1	GARTGYCCDGGDCAYTTYGG	52	Wu et al., 2014
	Acrpb1	CCNGCDATNTCRTTRTCCATRTA	52	Wu et al., 2014
ATP6	atp6-5	ATYGCTTTAGAAAGTTTMTTGC	56	Giachini, 2004
	atp6-6	GGDATRAARWAWGARAARAARTG	55	Giachini, 2004



was independently aligned using the online version of MAFFT v. 7 (Katoh et al., 2002, 2017; Katoh and Standley, 2013). Alignments were reviewed in PhyDE v. 10.0 (Müller et al., 2005), followed by minor manual adjustments to ensure character homology between taxa. The matrices were formed for ITS by 64 taxa (667 characters), for LSU by 63 taxa (875 characters), *atp6* by 64 taxa (451 characters), while that of *rpb1* consisted of 64 taxa (684 characters). The aligned matrices were concatenated into a single matrix (64 taxa, 2677 characters). Eight partitioning schemes were established: one for the ITS, one for the LSU, three to represent the codon positions of the gene region *atp6* and three for the *rpb1* gene region, which were established using the option to minimize the stop codon with Mesquite v. 3.70 (Maddison and Maddison, 2021). The best evolutionary model for alignment was sought using PartitionFinder v. 2 (Lanfear et al., 2014, 2016; Frandsen et al., 2015). Phylogeny was performed with Bayesian inference using MrBayes v. 3.2.6 x64 (Huelsenbeck and Ronquist, 2001). The information block for the matrix included two simultaneous runs, four Montecarlo chains, temperature set to 0.2 and sampling 10 million generations (standard de-

viation ≤ 0.1) with trees sampled every 1000 generations. The convergence of the chains was displayed in Tracer v. 1 (Suchard et al., 2018). The highest credibility phylogram of the clades recovered with TreeAnnotator v. 1.8 (Bouckaert et al., 2014) was chosen with a 25% burn-in. Trees were visualized and optimized in FigTree v. 1.4.4 (Suchard et al., 2018).

Results

Molecular analysis

The ITS, LSU, *atp6* and *rpb1* sequences obtained from *Geastrum chamelense* were deposited in GenBank (Table 2). In the Bayesian analysis, the standard deviation between the chains stabilized at 0.002 after 10 million generations, indicating MC3 reached a stationary phase. To confirm that the sample size was sufficient, the parameter file was examined in Tracer v. 1.6 (Suchard et al., 2018): all parameters had an estimated sample size of over 1500. The posterior probabilities (PP) obtained were estimated by generating a strict consensus tree in MrBayes. Bayesian inference analysis recovered a well-supported clade (PP=1) of the new species (Fig. 2).

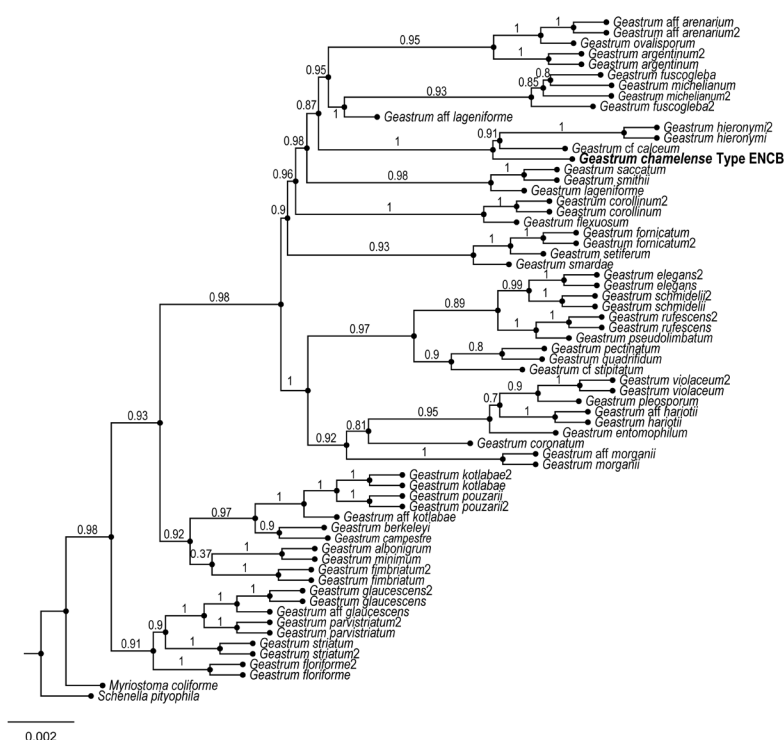


Figure 2: Bayesian inference phylogram of ITS, LSU, *rpb1*, *atp6* sequences. The new species *Geastrum chamelense* Bautista-Hernández, Raymundo, Aguirre & R. Valenz. is shown in bold.



The topology of the phylogenetic tree is similar to that reported by Zamora et al. (2014). The new species is phylogenetically distant from *G. setiferum* Baseia, the taxon with which it bears the greatest morphological similarity, sharing the presence of setae in the endoperidium. It forms a well-supported clade with *G. hieronymi* Henn. and *G. cf. calceum*.

Key to species of *Geastrum* found in the Chamela Biological Station, Jalisco, Mexico

- 1a. Basidiomata with setae on the endoperidial surface
..... *Geastrum chamelense*
Bautista-Hernández, Raymundo, Aguirre & R. Valenz.
- 1b. Basidiomata without setae on the endoperidial surface 2
- 2a. Exoperidium pink to purplish
..... *Geastrum violaceum* Rick
- 2b. Exoperidium brown grey, brown orange, beige 3
- 3a. Basidiomata growing on rotten wood or dead leaves, with white subiculum
..... *G. schweinitzii* (Berk. & M.A. Curtis) Zeller.
- 3b. Basidiomata growing on soil, without white subiculum 4
- 4a. Basidiomata separating the mycelial layer from the exoperidium easily at maturity *G. javanicum* Lév.
- 4b. Basidiomata with arachnoid aspect, exoperidium with longitudinal ridges, the mycelial layer not easily separating from the exoperidium *G. lageniforme* Vittad.

Taxonomy

Phylum Basidiomycota

Subphylum Agaricomycotina

Class Agaricomycetes

Subclass Phallomycetidae

Order Geastrales

Family Geastraceae

Geastrum chamelense Bautista-Hernández, Raymundo, Aguirre & R. Valenz., **sp. nov.** Figs. 3, 4, 5.

TYPE: MEXICO. Jalisco, municipality La Huerta, Reserva de la Biosfera Chamela-Cuixmala, Chamela Biology Station, km 60 Barra de Navidad - Puerto Vallarta highway, Eje Central, 250 m, 19°27'2.1"N, 105°01'33"W, 28.IX.2010. *T. Raymundo* 3504 (holotype: ENCB!, isotype: MEXU!); Mycobank: MB839090.

Geastrum chamelense is distinguished from other species of the genus *Geastrum* by its depressed, globose, semiformicate basidiomata, exoperidium 60 mm diameter, splitting into 4-7 rays, setose endoperidium, setae 102-330 × 10.2-15.3 µm, plicate peristome, not delimited; basidiospores 4.2-5 µm, globose, densely warty, dark brown.

Basidioma unexpanded, semihypogaeous, depressed globose, light brown (5D6), 30 mm diameter; exoperidium 60 mm diameter, splitting into 4-7 rays, with the tips recurved, semiformicate horizontally, brown grayish (10E3), consistency carnosus, in dry specimens not hygroscopic; mycelial layer attached to the litter, dimitic, 2.1-2.8 µm wide, with skeletal hyphae, thick-walled, aseptate, not branched, greenish yellow, light brown (5D6); fibrous layer 3.2-4 µm wide, thick-walled, light yellow; pseudoparenchymatous layer 14.4 -34.8 × 12-24 µm, globose to subglobose, brown yellow; without mycelial cords; endoperidium setose, sessile, subglobose to depressed, 15 × 20-30 mm, concolorous with exoperidium, constituted by interwoven hyphae, thick-walled, greenish yellow, 5.1-6.8 µm wide; setae 102-330 × 10.2-15.3 µm, thick-walled, lumen narrow; peristome plicate, not delimited; mycosclereids absent; gleba chocolate brown to blackish; basidiospores 4.2-5 µm diameter, globose, densely warty, dark brown in KOH 5%; basidia not observed; capillitial hyphae 5.6-7.2 µm diameter, aseptate, brown in KOH 5%, thick-walled, lumen narrow, not branched, surface with small warts and litter.

Habit and habitat: growing gregarious on soil in tropical dry forest.

Distribution: only known from the type locality.



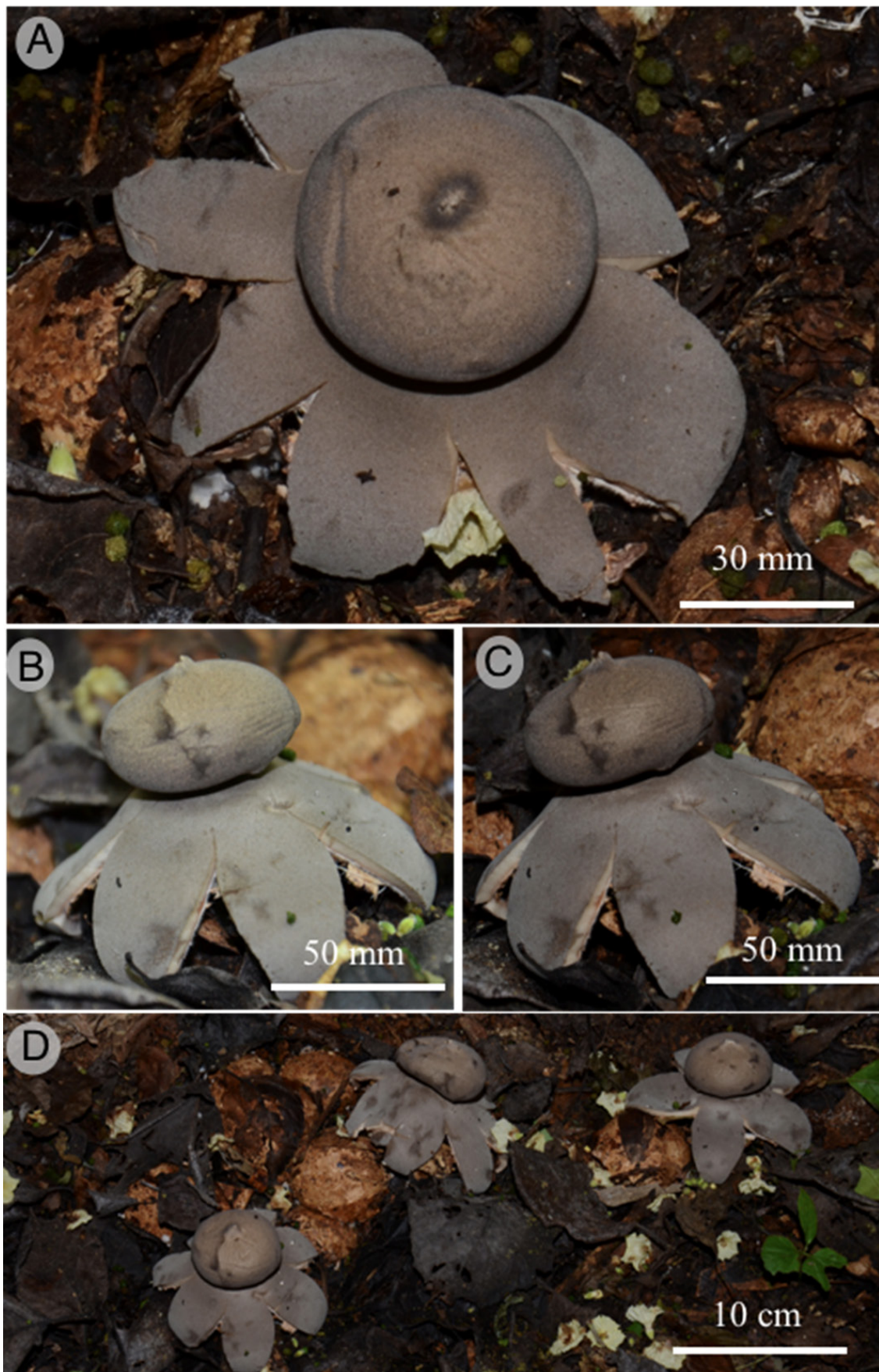


Figure 3: *Geastrum chamelense* Bautista-Hernández, Raymundo, Aguirre & R. Valenz. A. endoperidium showing the peristome; B-C. basidiomata showing the expanded exoperidium; D. basidiomata in the field.

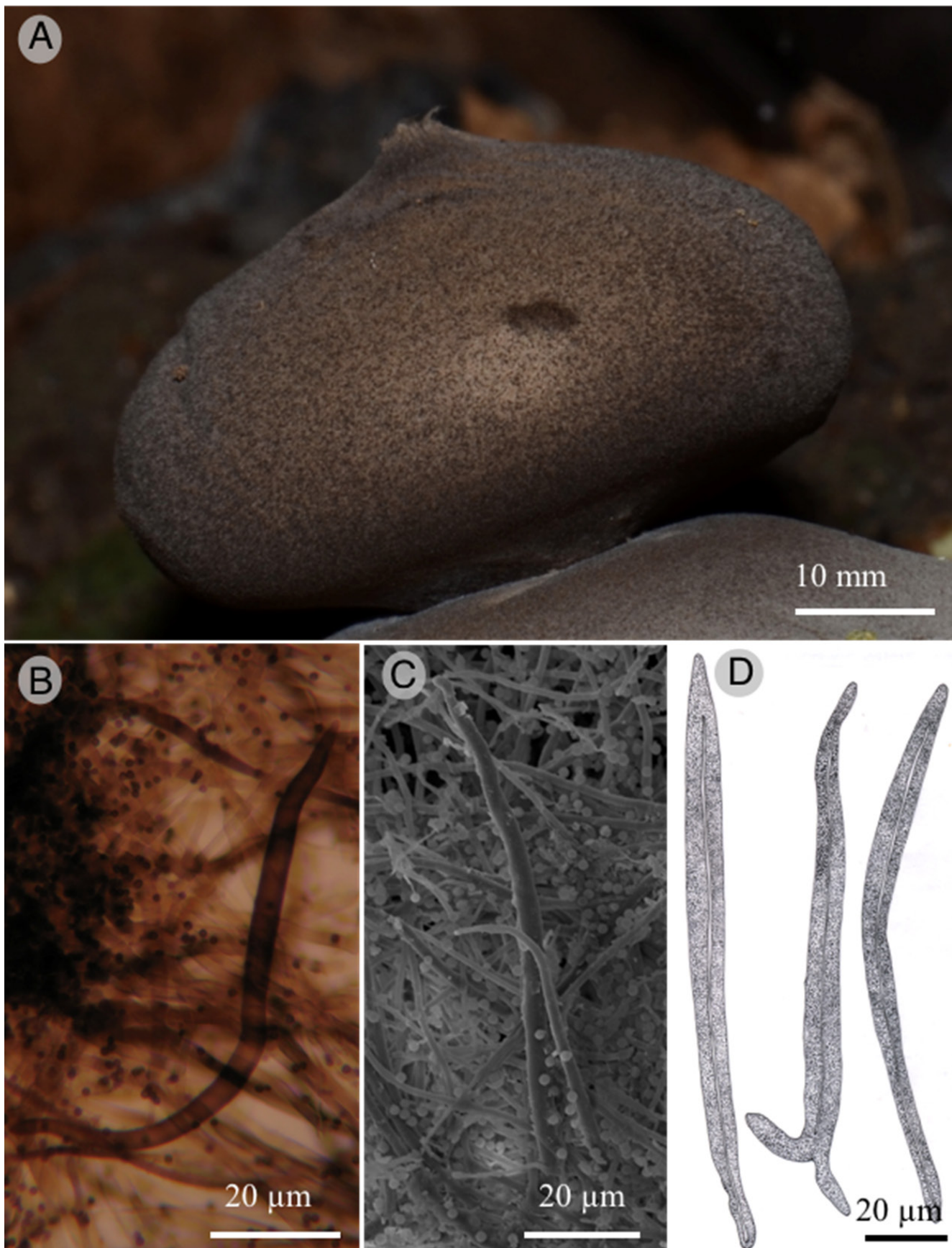


Figure 4: *Geastrum chamelense* Bautista-Hernández, Raymundo, Aguirre & R. Valenz. A. endoperidial body, the blackish appearance is due to the presence of setae; B. setae (MO); C. setae (SEM); D. setae (drawing).

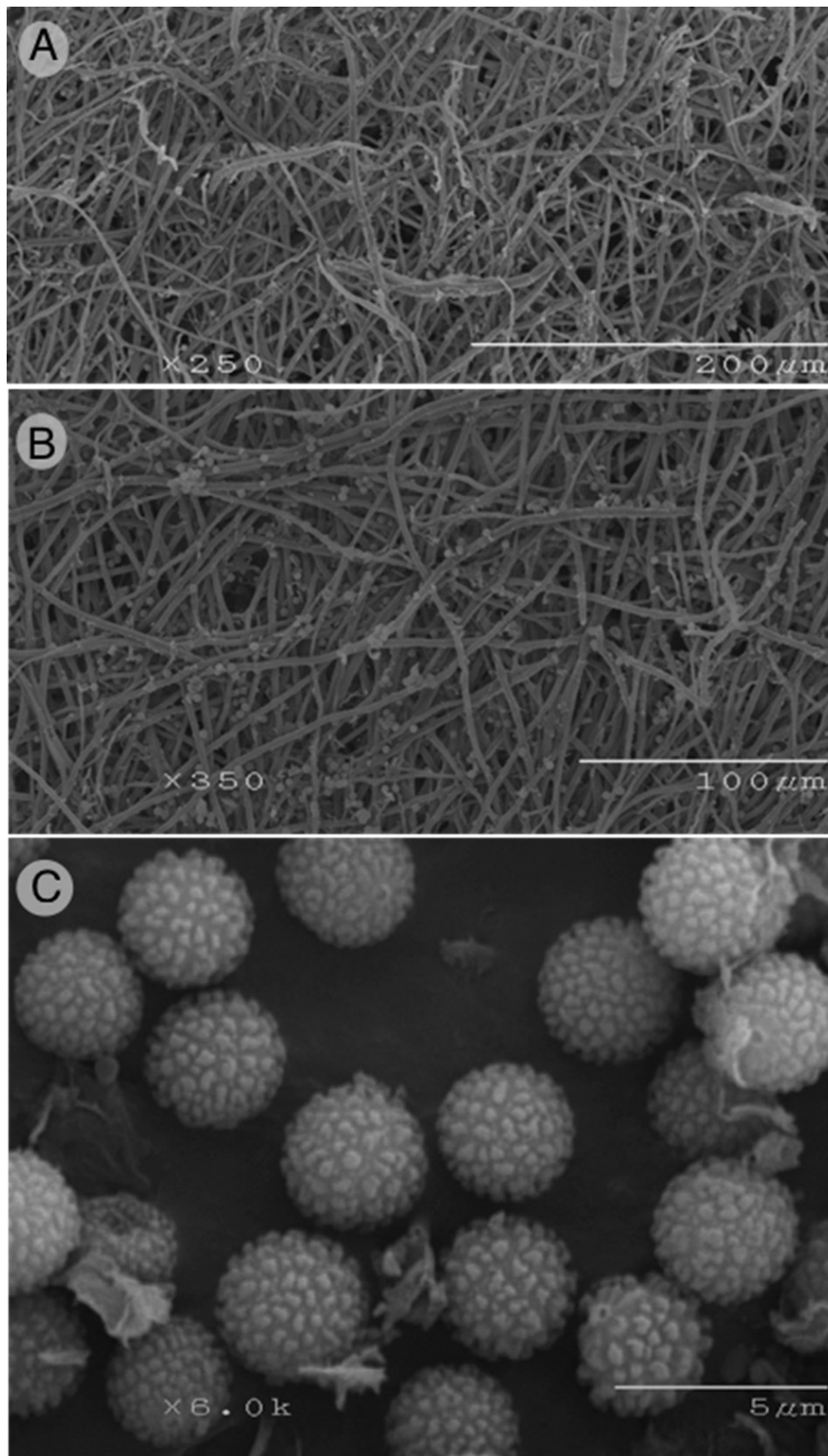


Figure 5: *Geastrum chamelense* Bautista-Hernández, Raymundo, Aguirre & R. Valenz. A. endoperidium surface showing the setae (SEM); B. capillitium (SEM); C. basidiospores (SEM).

Etymology: the specific epithet *chamelense* refers to the Chamela Biological Station, where this species has been collected.

Additional material examined: MEXICO. Jalisco, municipality La Huerta, Chamela-Cuixmala Biosphere Reserve, Chamela Biology Station, km 60 Barra de Navidad - Puerto Vallarta highway, Eje Central, 50 m, 19°27'2.1"N, 105°01'33"W, 18.IX.2011, *T. Raymundo 4064* (ENCB); loc. cit., *R. Valenzuela 14534* (ENCB); loc. cit., 28.IX.2010, *E. Aguirre y S. Bautista-Hernández* (MEXU 27044).

-*Gastrum javanicum*, Búho, 23.IX.2012, *E. Aguirre y S. Bautista-Hernández* (MEXU 28929).

-*Gastrum lageniforme*, Eje Central, 27.IX.2013, *E. Aguirre y S. Bautista-Hernández* (MEXU 28996).

-*Gastrum schweinitzii*, Eje Central, 18.IX.2011, *E. Aguirre-Acosta* (MEXU 28883).

-*Gastrum violaceum*, Tejón, 21.X.2009, *E. Aguirre y S. Bautista-Hernández* (MEXU 25836).

Discussion

Gastrum chamelense was macroscopically characterized because the basidiomata, when mature, have a grey to greyish brown colour, a globose depressed endoperidium, folded and non-delimited peristoma, and a non-hygroscopic and arcuate exoperidium. Microscopically, it presented densely ornamented spores and setiform hyphae on the surface of the endoperidium, which, under a stereomicroscope, were observed as small erect blackish brown spines.

A similar species is *G. setiferum*, described from Brazil (Baseia and Milanez, 2002; Trierveiler-Pereira et al., 2011) and Argentina (Castiglia et al., 2013), which presents setae in the endoperidium. However, macro- and micromorphological differences delimit both taxa, such as the peristome, colour of the basidiome, and size of the setae and spores, indicating that this is a new species. Baseia and Milanez (2002) described *G. setiferum* with a fibrillose to almost sulcate and defined peristoma, while Trierveiler-Pereira et al. (2011) mentioned that it was fibrillose to slightly plicated. However, the specimen from Argentina was reported as conical to mammiform, with the apex truncated, finely plicated, and not delimited (Castiglia et al., 2013), showing a heterogeneity with this

characteristic in both Brazilian and Argentinian specimens, in addition to presenting a pseudo-stipe and apophysis (Trierveiler-Pereira et al., 2011; Castiglia et al., 2013). In the Mexican specimens, few folds were observed, and it was sessile, not delimited, and without apophysis. Furthermore, the colour of the endoperidium differed because in the specimens from Brazil and Argentina, the tones ranged from greyish orange to light brown, while in the Mexican ones, it was greyish brown. Although macroscopically they are similar, we considered that the differences between the microscopic characteristics were preponderant for the separation of both species.

Gastrum setiferum has setae of $95\text{--}215 \times 20\text{--}47 \mu\text{m}$ (Baseia and Milanez, 2002; Trierveiler-Pereira et al., 2011; Castiglia et al., 2013), differing notably in the proposed taxon which has setae that are longer and slender, measuring up to $330 \times 12\text{--}16 \mu\text{m}$. Additionally, the setae were observed with dichotomous terminations, a character that was not addressed by the aforementioned authors. Regarding the size of the spores, those of *G. setiferum* measure $2.5\text{--}4 \mu\text{m}$ diameter (Baseia and Milanez, 2002; Trierveiler-Pereira et al., 2011), while in *G. chamelense*, they are $4.2\text{--}4.9 \mu\text{m}$ diameter, including ornamentation in both species. Therefore, our new species has larger spores.

Molecular analysis showed that *G. hieronymi* is found in the same clade as *G. chamelense*, sharing an arched exoperidium, fibrillose peristome, endoperidial surface with spines, which are formed by bundles of hyphae (Zamora et al., 2014) or strongly asperate with acute or subpyramidal spicules (Ponce de León, 1968). Although these authors refer to *G. hieronymi* as having a stalked endoperidial body and prominent apophysis, this character was not observed in the studied specimens. Regarding the microscopic characteristics, the spores of *G. hieronymi* are much larger, up to $6 \mu\text{m}$ in diameter and warty (Ponce de León, 1968). Therefore, the differences are notable between both species.

Conclusions

Gastrum chamelense is recognized as a new species based on morphological, ecological and molecular data. Although this species is close to *G. setiferum* because they share the setiferous elements in the endoperidium, the macro-



and micromorphological characters and its position in the phylogenetic hypothesis based on ITS, LSU, *rpb1* and *atp6* markers were decisive to separate them as different species. Worldwide, *Geastrum* has 109 valid species (Index Fungorum, 2021); of those, 29 species (26.6%) have been reported in Mexico, including *G. chamelense*. It is important to continue with taxonomic studies of this genus to contribute with new records and new species for the Mexican mycobiota.

Author contributions

SBH, TR, EAA and RV carried out the collections of the studied material. SBH and EAA carried out the revision of the herbarium material, the elaboration of preparations, the measurements, as well as the identification and descriptions of the specimens. SBH, TR, EAA and RV corroborated the taxonomic identification. SBH, TR and RV took the photographs of the specimens in the field. CRMG extracted the DNA and realized amplification and phylogenetic analysis. SBH wrote the manuscript with the support of TR, EAA CRMG and RV. All authors contributed to the discussion, revision and approval of the final manuscript.

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