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 rp1b1 sequences, are based on the holotype.

Key results: Geastrum chamelense is distinguished by its greyish brown basidiomata, pseudofornicate, 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. hieronymyi 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 mycosclereidos. 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, así como las análisis filogenéticos de ITS, LSU, atp6 y rp1b1 se basaron en el holotipo.

Resultados clave: Geastrum chamelense se distingue por sus basidiomas pardo grisáceos, exoperidio pseudofornicado, carnoso, no higroscópico, endoperidio sésil, subgloboso a depresado con peristoma plegado, no delimitado, y presencia de setas. Este último caracter 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. hieronymyi 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.
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 (Noguerá 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, sub-class 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...
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 1x 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 1x 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             |
|                                        |                        | rpb1              |
|                                        |                        | atp6              |
| Schenella pityophila (Malençon & Riousset) Estrada & Lado | Zamora 530 | KF988346 | KF988464 | KF988599 | KF988734 |
| Myriostoma coliforme Desv.             | Zamora 496             | KF988337 | KF988466 | KF988601 | KF988736 |
| Geastrum albogranum Calonge & M. Mata  | MA-Fungi 36140-2       | KF988349 | KF988468 | KF988603 | KF988738 |
| Geastrum aff. arenarium                 | MA-Fungi 68191         | KF988350 | KF988469 | KF988604 | KF988739 |
| Geastrum aff. arenarium 2               | Zamora 76              | KF988338 | KF988470 | KF988605 | KF988740 |
| Geastrum argentinum Spec.              | LPS 48446              | KF988352 | KF988472 | KF988607 | KF988742 |
| Geastrum argentinum 2                  | MA-Fungi 82605         | KF988353 | KF988473 | KF988608 | KF988743 |
| Geastrum berkeleyi Massee              | MA-Fungi 74668         | KF988354 | KF988474 | KF988609 | KF988744 |
| Geastrum cf. calceum                   | UFRN-Fungos 723        | KF988340 | KF988477 | KF988612 | KF988747 |
| Geastrum campestre Morgan              | Zamora 283             | JN943167 | JN993575 | JN991286 | KF988748 |
| **Geastrum chamelense** Bautista-Hernández, Raymundo, Aguirre & R. Valenz. | T. Raymundo 3504 (ENCB) | OL653145 | OL653165 | OL67687 | OL676806 |
| Geastrum corollinum (Batsch) Hollós    | MA-Fungi 5746          | KF988359 | KF988481 | KF988616 | KF988751 |
| Geastrum corollinum 2                  | Sunhede 7744           | KF988360 | KF988482 | KF988617 | KF988752 |
| Geastrum coronatum Pers.               | Zamora 266             | KF988361 | KF988483 | KF988618 | KF988753 |
| Geastrum elegans Vittad.               | Zamora 189             | KF988366 | KF988488 | KF988623 | KF988758 |
| Geastrum elegans 2                     | UPS F-560810           | KF988367 | KF988489 | KF988624 | KF988759 |
| Geastrum entomophilum Fazolino, Calonge & Baseia | MA-Fungi 70785 | KF988368 | KF988490 | KF988625 | KF988760 |
| Geastrum fimbriatum Fr.                | Zamora 234             | KF988369 | KF988491 | KF988626 | KF988761 |
| Geastrum fimbriatum 2                  | Sunhede 7739           | KF988370 | KF988492 | KF988627 | KF988762 |
| Geastrum flexuosum (L.S. Dominguez & Castellano) Jeppson & E. Larss. | UPS F-119844 | KF988371 | KF988493 | KF988628 | KF988763 |
| Geastrum floriforme Vittad.            | MA-Fungi 69173         | KF988372 | KF988494 | KF988629 | KF988764 |
| Geastrum floriforme 2                  | Zamora 453             | KF988373 | KF988495 | KF988630 | KF988765 |
| Geastrum fornicatum (Huds.) Hook.      | Zamora 255             | KF988374 | KF988496 | KF988631 | KF988766 |
| Geastrum fornicatum 2                  | MA-Fungi 30749         | KF988375 | KF988497 | KF988632 | KF988767 |
| Geastrum fuscochlebomin (Zeller) Jeppson & E. Larss. | Trappe 1071 | KF988376 | KF988498 | KF988633 | KF988768 |
| Geastrum fuscochlamidum                | Trappe 9500            | KF988377 | KF988499 | KF988634 | KF988769 |
| Geastrum glaucescens Spec.             | MA-Fungi 83762         | KF988378 | KF988500 | KF988635 | KF988770 |
| Geastrum glaucescens 2                 | MA-Fungi 83763         | KF988379 | KF988501 | KF988636 | KF988771 |
| Geastrum hariotii Lloyd                | MA-Fungi 80070         | ------- | KF988503 | KF988638 | KF988773 |
| Geastrum aff. hariotii                 | MA-Fungi 78296         | KF988382 | KF988505 | KF988640 | KF988775 |
| Geastrum hieronymi Henn.               | MA-Fungi 83766         | KF988384 | KF988508 | KF988643 | KF988776 |
| Geastrum hieronymi 2                   | MA-Fungi 83767         | KF988344 | KF988509 | KF988644 | KF988777 |
| Geastrum kotlabae V.J. Staněk          | MA-Fungi 39563         | KF988385 | KF988510 | KF988645 | KF988778 |
| Geastrum kotlabae 2                    | Zamora 440             | KF988386 | KF988511 | KF988646 | KF988779 |
| Geastrum aff. kotlabae                 | MA-Fungi 33000         | KF988387 | KF988512 | ------- | ------- |
| Geastrum lageniforme Vittad.           | Zamora 207             | KF988388 | KF988513 | KF988648 | KF988780 |
| Geastrum aff. lageniforme              | MA-Fungi 79056         | ------- | KF988515 | KF988650 | KF988782 |
| Geastrum michelianum (Sacc.) W.G. Sm.  | Sunhede 7738           | KF988397 | KF988524 | KF988659 | KF988791 |
### Table 1: Continuación.

| Species name                              | Isolate/Voucher/strain | GenBank Accessions |
|-------------------------------------------|------------------------|--------------------|
| *Geastrum michelianum* 2                 | Zamora 227             | KF988398 KF988525  |
| *Geastrum minimum* Schwein.              | Zamora 191             | KF988400 KF988528  |
| *Geastrum morganii* Lloyd                | Lebeuf HRL0177         | KF988406 KF988534  |
| *Geastrum aff. morganii*                 | Zamora 367             | KF988407 KF988535  |
| *Geastrum ovalisporum* Calonge & Mor.-Arr. | MA-Fungi 47184        | KF988411 KF988539  |
| *Geastrum parvistriatum* J.C. Zamora & Calonge | MA-Fungi 69583       | JN943160 JN939560  |
| *Geastrum parvistriatum* 2               | Zamora 272             | JN943162 JN939572  |
| *Geastrum pectinatum* Pers.              | Zamora 252             | KF988412 KF988540  |
| *Geastrum pleosporum* Duolanla-Meli      | MA-Fungi 56971         | KF988416 KF988544  |
| *Geastrum pouzarii* V.J. Staněk          | MA-Fungi 2944          | KF988417 KF988545  |
| *Geastrum pouzarii* 2                    | Sunhede 7494           | KF988418 KF988546  |
| *Geastrum pseudolimbatum* Hollós         | Zamora 231             | KF988419 KF988547  |
| *Geastrum quadrifidum* DC. ex Pers.      | Zamora 170             | KF988421 KF988549  |
| *Geastrum rufescens* Pers.               | Zamora 253             | KF988424 KF988552  |
| *Geastrum rufescens* 2                   | Zamora 274             | KF988425 KF988553  |
| *Geastrum schmidelii* Vittad.            | Zamora 279             | KF988434 KF988564  |
| *Geastrum schmidelii* 2                  | UPS F-560805           | KF988435 KF988565  |
| *Geastrum setiferum* Baseia              | MA-Fungi 83781         | KF988441 KF988571  |
| *Geastrum smardae* V.J. Staněk           | Lebeuf HRL 0160        | KF988440 KF988573  |
| *Geastrum smithii* Lloyd                 | MA-Fungi 83783         | KF988442 KF988575  |
| *Geastrum cf. stipitatum*                | Zamora 528             | KF988435 KF988576  |
| *Geastrum striatum* DC.                  | Zamora 257             | KF988443 KF988577  |
| *Geastrum striatum* 2                    | MA-Fungi 86672         | KF988450 KF988585  |
| *Geastrum violaceum* Rick                | BAFC 51671             | KF988451 KF988586  |
| *Geastrum violaceum* 2                   | MA-Fungi 82487         | KF988451 KF988587  |

### 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   | TCCTCCGCTTATATATGC  | 57    | White et al., 1990 |
| LSU          | LROR   | ACCCGCTGAACATTAAC  | 54    | White et al., 1990 |
|              | LR3    | GGTCCGTGGTTCAAGAC  | 60    | White et al., 1990 |
| rpb1         | Afrpb1 | GARTGYCCDGGDCAYTTYGG| 52    | Wu et al., 2014  |
|              | Acrpbl | CCNGCDATNTCRTTRCTCATRTA| 52    | Wu et al., 2014  |
| ATP6         | atp6-5 | ATYGCTTTAGAAGTTYTMTTG | 56    | Giachini, 2004 |
|              | atp6-6 | GGDATRAARWAWGARARAARTG| 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 ma-
trix (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 Mesqui-
te v. 3.70 (Maddison and Maddison, 2021). The best evolu-
tionary model for alignment was sought using PartitionFin-
der 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 simul-
taneous 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 exa-
mined 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 analy-
sis recovered a well-supported clade (PP=1) of the new spe-
cies (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 .................................................. Geastrum chamelense Bautista-Hernández, Raymundo, Aguirre & R. Valenz.

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 ............................................................................. G. javanicum Lév.

4a. Basidiomata separating the mycelial layer from the exoperidium easily at maturity ........ G. lageniforme Vittad.

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 do 3504 (holotype: ENCB!, isotype: MEXU!); Mycobank: MB839090.

Geastrum chamelense is distinguished from other species of the genus Geastrum by its depressed, globose, semifornicate 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, semihypogeous, depressed globose, light brown (5D6), 30 mm diameter; exoperidium 60 mm diameter, splitting into 4-7 rays, with the tips recurved, semifornicate horizontally, brown grayish (10E3), consistency carnosine, 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).

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

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

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

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

Discussion

*Geastrum 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.

*Geastrum setiferum* has setae of 95-215 × 20-47 µm (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 × 12-16 µm. 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 2.5-4 µm diameter (Baseia and Milanez, 2002; Trierveiler-Pereira et al., 2011), while in *G. chamelense*, they are 4.2-4.9 µm 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 µm in diameter and warty (Ponce de León, 1968). Therefore, the differences are notable between both species.

Conclusions

*Geastrum 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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