

1 **Contribution to Neotropical data of *Geastrum* section *Corollina* (Basidiomycota):**  
2 **Two new earth–stars from Caatinga vegetation, Brazil**

3 Julieth de Oliveira Sousa\*<sup>1</sup>, George Sidney Baracho <sup>2</sup>, María Paz-P. Martín <sup>3</sup>, Iuri  
4 Goulart Baseia <sup>4</sup>

5  
6 \*<sup>1</sup>julieth.oliveira.sousa@gmail.com

7 Programa de Pós-Graduação em Sistemática e Evolução  
8 Universidade Federal do Rio Grande do Norte  
9 Campus Universitário Natal 59072–970, Brazil

10  
11 <sup>2</sup> Universidade de Pernambuco, Instituto de Ciências Biológicas  
12 Laboratório de Biologia Vegetal, 50.100–130, Recife, Brazil.

13  
14 <sup>3</sup> Departamento de Micología  
15 Real Jardín Botánico–CSIC  
16 Plaza de Murillo 2, Madrid, Spain

17  
18 <sup>4</sup> Departamento de Botânica e Zoologia  
19 Universidade Federal do Rio Grande do Norte  
20 Campus Universitário Natal 59072–970, Brazil  
21

22 **Abstract**

23 The Caatinga is a unique phytogeographical domain of semi-arid vegetation in  
24 northeastern Brazil. Although, it includes rare and endemic birds and mammals, it is  
25 poorly represented in the Brazilian Conservation Area Network. In this paper we  
26 describe two new species of the genus *Geastrum* section *Corollina*: *G. caatingense* and  
27 *G. parvistellum*, based on morphological data and molecular analyses of ITS/LSU  
28 nrDNA. Species description, images, and taxonomic discussion are provided.

29

30 **Keywords** gasteroid fungi · Geastraceae · phylogeny · systematic · taxonomy ·

31

## 32 Introduction

33 Caatinga is a unique phytogeographical domain in Brazil, comprising about 10% of  
34 the territory of this “continental” country. The vegetation grows under semi-arid  
35 conditions, a climate unusual in a tropical region. It is mainly characterized by scarcer  
36 and concentrated rains (mean of less than 1 mm per year), mean temperature of 26–28  
37 °C and a high level of evapotranspiration (Prado 2003, Leal et al. 2005, Moro et al.  
38 2016). The Caatinga includes rare and endemic taxa, but just 2% of its area is protected  
39 in conservation units (Castelletti et al. 2003).

40 Recently, the genus *Geastrum* has been a focus of systematic and taxonomic studies  
41 (Kasuya et al. 2012, Jeppson et al. 2013, Zamora et al. 2014). Historically the genus is  
42 considered as of subcosmopolitan distribution, and has been investigated more  
43 intensively in temperate areas (Palearctic and Nearctic regions) than in tropical areas.  
44 However, this scenario has changed in the last decade, during which more than ten new  
45 species of the genus were discovered from Brazil (Fazolino et al. 2008, Silva et al.  
46 2013, Cabral et al. 2014a, Cabral et al. 2014b, Sousa et al. 2015, Crous et al. 2016,  
47 2017, 2018, Cabral et al. 2017).

48 One of the 14 sections recently proposed for the genus *Geastrum* is the section  
49 *Corollina* J.C. Zamora. It groups species with very heterogeneous morphology, mainly  
50 recognized by peristome fibrillose to irregularly plicate, basidioma frequently saccate,  
51 but rarely arched, exoperidium hygroscopic or not, mycelial layer normally not  
52 encrusting debris, rarely encrusted, endoperidial body sessile (-Zamora et al. 2014). The  
53 sect. *Corollina* is divided into three subsections: subsect. *Lageniformia* J.C. Zamora,  
54 mainly characterized by strongly delimited, fibrillose peristome, presence of horn-like  
55 crystal on rhizomorphs and mycelial layer composed of generative hyphae; *Marginata*  
56 P. Ponce de León, mainly characterized by strongly delimited fibrillose peristome,  
57 presence of acicular crystals on rhizomorphs and two sub-layers in the mycelial layer,  
58 outer layer with skeletal hyphae and inner layer with generative hyphae; and subsect.  
59 *Plicostomata* V.J. Staněk, mainly characterized by non-delimited or weakly delimited  
60 peristome, irregularly plicate, presence of acicular crystals on rhizomorphs and two  
61 sub-layers in the mycelial layer, outer layer with skeletal hyphae and inner layer with  
62 generative hyphae.

63 The sect. *Corollina* has some undetermined species (*Geastrum* sp.) (Zamora et al.  
64 2014). Furthermore, there are few studies with *Corollina*' specimens from Neotropical  
65 Region, where exists high potential to found undescribed species for science  
66 (Hawksworth, 2001). Thus, the aimed of this study is to enhance the knowledge of the  
67 sect. *Corollina*, describing two news species of from Neotropical region, Brazil  
68 (Caatinga vegetation), with analyses based on morphological and molecular data.

69

## 70 Materials and Methods

71

### 72 Colletion Site and Morphological Analysis

73 The specimens were collected during the rainy season of 2015 in an area of  
74 Caatinga *sensu stricto* vegetation (6°36'18" S, 38°32'44" W, 273 meters above sea  
75 level), localized in the ecoregion of “Depressão Sertaneja Setentrional” (Velloso et al.  
76 2002). Morphological studies were performed according to Sousa et al. (2014a, 2014b)  
77 with dried basidiomata at variable stages of development. Color definition was based on  
78 Korerup & Wanscher (1978). For light microscopy (LM), slides containing  
79 basidiospores, eucapillitial, and exoperidial hyphae mounted on 5% (w/v) KOH were  
80 examined under a Nikon Eclipse Ni light microscope coupled with a Nikon DS-Ri

81 camera. Thirty basidiospores were measured at 1000 × magnification including  
82 ornamentation. Scanning Electron Microscopy (SEM) was used to observe ultrastructure  
83 of basidiospores ornamentation, eucapillitium and endoperidial surfaces. Statistical  
84 measurements of basidiospores given in the description followed Sousa et al. (2017). All  
85 analyzed samples have been deposited in the collection of the Federal University of Rio  
86 Grande do Norte, Natal, Brazil (UFRN–Fungos).

87

## 88 **Molecular Analyses**

89 Genomic DNA was extracted from approximately 10 mg of gleba from dried  
90 basidiomata as described in Sousa et al. (2017). Amplifications were carried out using  
91 illustra™ PureTaq™ Ready–To–Go™ PCR Beads (Healthcare, Buckinghamshire,  
92 UK) with a final volume of 25 µl. Two regions were amplified by Polymerase Chain  
93 Reaction (PCR): Internal Transcribed Spacer (ITS nrDNA), including the 5.8S of the  
94 ribosomal RNA, and Large SubUnit (LSU nrDNA), with the primer pairs ITS1F/ITS4B  
95 (White et al. 1990, Gardes & Bruns 1993) and LR0R/LR7 (Vilgalys & Herster 1990,  
96 Rehner & Samuels 1994), respectively. When amplifications were weak (less than 10  
97 ng/ µl), nested–PCR to ITS and seminested–PCR to LSU was done using 1 µl of  
98 amplification product from the first PCR. For the nested–PCR to ITS, the primers  
99 ITS5/ITS4 were used (White et al. 1990), and for seminested–PCR to LSU, the primers  
100 LR0R/LR5 were used (Vilgalys & Herster 1990, Rehner & Samuels 1994). Before  
101 sequencing, 20 µl of the amplification products were purified using Speedtools PCR  
102 Clean–up Kit (Biotools, B & M Labs, S.A). Purified PCR products were then sequenced  
103 at Macrogen (Seoul, Korea), with the primer pairs used in the amplification.

104 Editing and consensus assembly of DNA sequences were performed using  
105 Sequencher v.4.1.4 (Gene Codes, Ann Arbor, Michigan, USA). Sequences were  
106 submitted to GenBank under the accession numbers indicated in Table 1. Both ITS and  
107 LSU sequences were compared with homologous *Geastrum* sequences retrieved from  
108 GenBank. The multiple alignments were optimized visually in MEGA v. 5.2 (Kumar et  
109 al. 2016). Sequences of two specimens of *Geastrum hungaricum* (MJ9317 and MJ8915)  
110 were included as outgroup. The concatenated alignment was analyzed by Maximum  
111 Parsimony (MP), using PAUP\* v.4.0b10 during phylogenetic analysis. A heuristic  
112 search was performed, with branch swapping and using the TBR algorithm, with initial  
113 trees obtained by stepwise addition of random additional sequences repeated 10 times,  
114 and bootstrap (MPbs) of ten thousand replicates. A Maximum Likelihood Analysis  
115 (ML) was also carried out using RAxML in the CIPRES portal (CIPRES Science  
116 Gateway v.3.3), with GTRGAMMA as the model of evolution (Stamatakis 2014), with  
117 bootstrap (MLbs) of one thousand replicates.

118

## 119 **Results**

120

### 121 **Molecular analyses**

122 The concatenated dataset (ITS/LSU) included 38 specimens of *Geastrum* (36  
123 ingroup sequences of Sect. *Corollina*, and two of *G. hungaricum* as outgroup).  
124 Seventy–two sequences were retrieved from GenBank and four were newly generated in  
125 this study (Table 1, the new sequences are shown in bold.). In MP analysis of 1643  
126 positions, 1262 positions were constant, 140 parsimony–uninformative, and 241 were  
127 parsimony–informative. Gaps were treated as "missing data". Parsimony tree scores  
128 were identical for the eight most parsimonious trees obtained: Consistency Index (CI) =  
129 0.5457, Retention Index (RI) = 0.7033 and Homoplasy Index (HI) = 0.4543. The strict

130 consensus of these trees is shown in Fig. 1. The three ML consensus trees have similar  
131 topology (not shown). Bootstrap percentages (MPbs and MLbs) are indicated in Fig.1.

132 Morphological and phylogenetic analyses of the specimens represent two species  
133 new to science, i.e. *Geastrum caatingense* J.O. Sousa, M.P. Martín & Baseia, and  
134 *Geastrum parvistellum* J.O. Sousa, M.P. Martín & Baseia. Both are included in sect.  
135 *Corollina*. *Geastrum caatingense* grouped in subsect. *Marginata*, as a sister species of  
136 two specimens from Spain (*Geastrum* sp. 1) in Fig. 1; however this relationship is not  
137 well supported (less than 50 bootstrapsupport). *Geastrum parvistellum* grouped in  
138 subsect. *Plicostomata*, with a sequence of undetermined *Geastrum* (*Geastrum* sp. 2) in  
139 Fig. 1 from Australia (MEL 2382911) and this relationship is strongly supported by  
140 good bootstrap values (MPbs = 98, MLbs = 100).

141

## 142 Taxonomy

143

144 *Geastrum caatingense* J.O. Sousa, M.P. Martín & Baseia (Fig. 2)

145 MYCOBANK NO.: MB825153

146 Diagnosis: This species is mainly recognized by hygrosopic rays, distinctly delimited,  
147 fibrillose peristome, ephemeral mycelial layer which expose the coriaceous fibrous  
148 layer with longitudinal cracks. *Geastrum caatingense* is close to *Geastrum corollinum*,  
149 however *G. caatingense* differs by non-pruinose endoperidium, non-ephemeral  
150 mycelial layer with presence of encrustations, and larger basidiospores ( $5.7\text{--}6.5 \times 5.2\text{--}$   
151  $6.2 \mu\text{m}$ ). *Geastrum saccatum* is also similar to *G. caatingense*, but *G. caatingense* has  
152 hygrosopic rays, as well as an encrusted mycelial layer and a fibrous layer with  
153 longitudinal cracks.

154 Types: BRAZIL. Paraíba State: Triunfo, 28.II.2015, G.S. Baracho 12.180 (holotype  
155 UFRN-Fungos 2843, isotype UFRN-Fungos 2960). GenBank accession ITS =  
156 MH253884, LSU = MH253886.

157 Etymology: “*caatingense*” refers to the vegetation of the type locality.

158 Expanded basidiomata saccate, 7–14 mm high (including peristome)  $\times$  11–29 mm  
159 wide. Exoperidium splitting into 6–10 rays, revolute when fresh, involute, rolling up  
160 above the endoperidial body or planar when dry, hygrosopic; Mycelial layer pallid  
161 orange (5A3), papery, strongly encrusted with debris, peeling away in irregular patches  
162 with age; Fibrous layer white to orange white (5A2), coriaceous, with longitudinal  
163 cracks; Pseudoparenchymatous layer dark brown (6E4; 7F4), persistent when young or  
164 peeling away in irregular patches with age. Endoperidial body orange gray (6B2),  
165 brownish gray (6C2) to yellowish white (5A2), depressed globose to subglobose, 5–12  
166  $\times$  9–16 mm, sessile, surface glabrous, without pruinose material. Apophysis absent  
167 or reduced, lighter color than endoperidium. Pedicel inconspicuous ( $< 0.5$  mm high),  
168 lighter than endoperidium. Peristome finely fibrillose, distinctly delimited by orange  
169 white (5A2) line, strongly conic (up to 3.2 mm high, up to 5.2 mm diam.) lighter than  
170 endoperidium. Gleba grayish brown (6F3). Mycelial layer composed of hyaline to  
171 yellowish hyphae, 2.5–3.2  $\mu\text{m}$  diam., unbranched, thick-walled (0.5–1.0  $\mu\text{m}$ ), surface  
172 encrusted, lumen evident. Fibrous layer composed of hyaline hyphae, 4.2–7.9  $\mu\text{m}$  diam.,  
173 thin-walled (0.7–1.2  $\mu\text{m}$ ), surface not encrusted, lumen evident. Pseudoparenchymatous  
174 layer composed of hyaline subglobose to elongated hyphal cells, 17.0–46.0  $\times$  17.8–34.1  
175  $\mu\text{m}$ , thick-walled (0.8–1.5  $\mu\text{m}$ ). Endoperidium composed of interlaced hyphae.  
176 Eucapillitial hyphae yellowish, 3.8–6.7  $\mu\text{m}$  wide, thin walled (0.5–0.8  $\mu\text{m}$ ), surface

177 encrusted, densely verrucose, lumen evident. Basidiospores brownish, subglobose, 5.7–  
178 6.5 × 5.2–6.2 μm [ $x = 6.0 \pm 0.2 \times 5.8 \pm 0.2$ ,  $Q_m = 1.04$ ,  $n = 30$ ], with ornamentation less  
179 conspicuous under LM; densely verrucose under SEM, with columnar to triangular  
180 warts (0.9–1.7 μm high), slight pointed or rounded apex, with some confluent tips;  
181 apiculus inconspicuous.

182 Habitat and distribution: Found in Caatinga vegetation, growing on sandy soil covered  
183 by leaf–litter.

184

185 *Geastrum parvistellum* J.O. Sousa, M.P. Martín & Baseia

Fig. 3

186 MycoBank: MB825154

187

188 Diagnosis: This species is mainly recognized by small basidiomata (6–12 mm wide),  
189 sub–hygroscopic rays, encrusted mycelial layer, irregularly plicate and weakly delimited  
190 peristome. *Geastrum parvistellum* is close to *G. morganii*, however *G. parvistellum* has  
191 smaller basidiomata (6–12 mm wide), involute rays, ephemeral and encrusted mycelial  
192 layer. *Geastrum arenarium* is also a similar species to *G. parvistellum*, but *G.*  
193 *parvistellum* has smaller basidiomata (6–12 mm wide), non–pruinose endoperidial body,  
194 weakly delimited peristome, and larger basidiospores (4.8–6.5 × 4.8–6.3 μm).

195

196 Type: BRAZIL. Paraíba State: Triunfo, 28.II.2015, G.S. Baracho 12.181 (holotype  
197 UFRN–Fungos 2841, isotype UFRN–Fungos 2961). GenBank accession ITS=  
198 MH253885, LSU = MH253887).

199 Etymology: “*parvistellum*” (derived from Latin: *parvo* = small; *stella* = star) refers to  
200 the small size of the basidiomata.

201 Expanded basidiomata saccate to arched, 6–8 mm high (including peristome) × 6–  
202 12 mm wide. Exoperidium splitting into 5–7 involute rays, rolling up under the  
203 endoperidial body, sub–hygroscopic; Mycelial layer grayish orange (5B3), wooly,  
204 strongly encrusted with debris, persistent or peeling away in some basidiomata; Fibrous  
205 layer white (5A1), coriaceous; Pseudoparenchymatous layer brown (6E5) to dark brown  
206 (7F5), persistent, rimose. Endoperidial body orange gray (5B2), brownish orange (5C3)  
207 to dark brown (7F4), subglobose, 3–7 × 5–8 mm, sub–sessile, surface furfuraceous.  
208 Apophysis absent. Pedicel absent or inconspicuous (< 1 mm high), lighter than  
209 endoperidium. Peristome irregularly plicate, fibrillose with age, weakly delimited,  
210 slightly depressed on the endoperidium or mammiform, concolorous or lighter than  
211 endoperidium (up to 1 mm high, up to 5.2 mm diam.) Gleba grayish brown (5F3).  
212 Mycelial layer composed of hyaline hyphae, 1.2–3.2 μm diam., thin-walled (< 0.5 μm)  
213 surface encrusted, lumen evident, some clamped hyphae present. Fibrous layer  
214 composed of hyaline hyphae, 3.3–4.9 μm diam., thin-walled (0.5–0.9 μm), surface non-  
215 encrusted, lumen evident. Pseudoparenchymatous layer composed of subglobose, oval  
216 to elongated hyphae, 17.3–36.7 × 14.4–23.9 μm, thick-walled (0.4–1.2 μm).  
217 Eucapillitial hyphae brownish, sinuous, unbranched, 3.2–5.3 μm diam., thin-walled  
218 (0.5–0.9 μm), surface slight verrucose, encrusted, lumen evident. Basidiospores  
219 brownish, globose to subglobose, 4.8–6.5 × 4.8–6.3 μm [ $x = 5.5 \pm 0.5 \times 5.4 \pm 0.4$ ,  $Q_m =$   
220 1.02,  $n = 30$ ], ornamentation less conspicuous under LM; verrucose under SEM, formed  
221 by triangular to cylindrical warts, slightly truncated, with planar to slightly pointed  
222 apex, 0.9–1.2 μm high; apiculus inconspicuous.

223 Habitat and distribution: Found in Caatinga vegetation, growing on sandy soil covered  
224 by leaf-litter.  
225

## 226 Discussion

227

228 *Geastrum caatingense* is morphologically very similar to species of subsect.  
229 *Marginata*, and our phylogenetic analyses confirm the placement of the species in this  
230 subsection. *G. caatingense* is related to *G. corollinum* (Batsch) Hollós and *G. diosiae*  
231 J.C. Zamora, both have saccate basidiomata, very well delimited peristome, and  
232 hygroscopic rays; however, these two species have smaller basidiospores (4.5–5 µm  
233 diam. in *G. corollinum* and 4–5 µm diam. in *G. diosiae*) (Sunhede 1989, Crous et al.  
234 2015). Moreover, *G. corollinum* has a pruinose endoperial body, and ephemeral  
235 mycelial layer without encrustations in contrast to *G. caatingense*; and *G. diosiae* has  
236 sessile and pruinose endoperial body, and absence of longitudinal cracks on the fibrous  
237 layer (Sunhede 1989, Crous et al. 2015).

238 *Geastrum flexuosum* (L.S. Domínguez & Castellano) Jeppson & E. Larss. and  
239 *Geastrum saccatum* Fr. are phylogenetic close to *G. caatingense*. Nevertheless, the  
240 morphology could clearly *G. flexuosum*, because it has hypogeous sequestrate  
241 basidiomata (Jeppson et al. 2013); while *G. saccatum* is distinct to *G. caatingense* by  
242 non-hygroscopic rays, absence of longitudinal cracks on the fibrous layer and non-  
243 encrusted mycelial layer (Sunhede 1989) (Table 2).

244 Another species with morphology similar to *G. caatingense* is *G. hungaricum*  
245 Hollós. Although these species are phylogenetic distant (*G. hungaricum* is grouped in  
246 *Geastrum* section), both have involute rays, distinct delimited peristome, mycelial  
247 encrusted and palling away with age. However, *G. hungaricum* has strongly  
248 hygroscopic rays, a pseudoparenchymatous layer with thicker walled hyphal cells (> 1.5  
249 µm vs upto 1.5 µm), and basidiospores with smaller ornamentation (up to 0.7 µm vs  
250 up to 1.7 µm) (Sunhede 1989, Zamora et al. 2015).

251 The phylogenetic analyses showed *Geastrum parvistellum* grouped in subsect.  
252 *Plicostomata* with *Geastrum morganii* Lloyd and *Geastrum violaceum* Rick. These  
253 three species have irregularly plicate peristome with delimitation weak or absent. On  
254 the other hand, *G. violaceum* is easily distinct to *G. parvistellum* by its endoperidium  
255 and exoperidium color, purple to violet, and smaller basidiospores (2.7–3.1 µm diam.)  
256 (Sousa et al. 2014a); while *G. morganii* is distinct to *G. parvistellum* by mycelial layer  
257 persistent, non-encrusted, revolute rays and larger basidiomata (9–28 mm wide) (Sousa  
258 et al. 2014b) (Table 2).

259 In the section *Corollina*, another species are similar to *G. parvistellum*, as *G.*  
260 *corollinum*. But, *G. corollinum* has a pruinose endoperidium, distinctly delimited  
261 peristome, ephemeral mycelial layer without encrustations and smaller basidiospores  
262 (4–5 µm) (Sunhede 1989, Kuhar et al. 2012). *Geastrum lageniforme* and *G. saccatum*  
263 could also recall *G. parvistellum*, however, these two species differ from *G.*  
264 *parvistellum* due to absence of encrustations on the mycelial layer and distinctly  
265 delimited peristome (Sunhede 1989). Additionally, *Geastrum saccatum* var. *parvulum*  
266 Speg. is similar to *G. parvistellum*, mainly due to the small size of the basidiomata (15–  
267 20 mm wide). However, *G. saccatum* var. *parvulum* is distinct by having non-  
268 hygroscopic rays, sessile endoperidial body and smaller basidiospores (3–5 µm diam.)  
269 (Dios et al. 2017).

270 Although the phylogenetic analyses showed that *G. parvistellum* belongs to subsect.  
271 *Plicostomata*, morphologically it is very similar to *Geastrum arenarium* Loyd. (sect.

272 *Geastrum*); both have an encrusted mycelial layer, semi-hygroscopic rays and  
273 sessile endoperidial body. Nevertheless, *G. arenarium* has larger basidiomata (15–30  
274 mm wide), a pruinose endoperidial body, distinctly delimited peristome and smaller  
275 basidiospores (up to 4 µm diam.) (Bates 2004, Kuhar et al. 2012).

276

### 277 **Acknowledgements**

278 The first author would like to thank Coordenação de Aperfeiçoamento de Pessoal de  
279 Nível Superior (CAPES, Brazilian agency) for four months of doctorate international  
280 scholarship in Madrid–Spain; the second author thanks the Universidade de  
281 Pernambuco (Programa de Fortalecimento Acadêmico/PFA), Maria Batista de Freitas  
282 (D. Carminha) and family (sítio Jenipapeiro, Triunfo, PB), for the logistical support and  
283 assistance during fieldwork. Authors are grateful to the Conselho Nacional de  
284 Desenvolvimento Científico e Tecnológico (CNPq—Brazil, Projeto Pesquisador  
285 Visitante Especial PVE/407474/2013–7) for providing the financial support; and also to  
286 Prof. Marian Glenn for English revision.

287

### 288 **References**

289

- 290 Baseia, I.G. & Galvão, T.C. (2002): Some interesting Gasteromycetes (basidiomycota)  
291 in dry areas from Northeastern Brazil. – *Acta botanica brasílica*. 16: 1–8.
- 292 Bates, S.T. (2004): Arizona members of the Geastraceae and Lycoperdaceae  
293 (Basidiomycota, Fungi). – Master’s Thesis, Arizona State University,  
294 <http://www.azfungi.org/stbates/STBThesis1.pdf>.
- 295 Cabral, T.S., Silva, B.D.B., Marinho, P., & Baseia, I.G. (2014a): *Geastrum rusticum*  
296 (Geastraceae, Basidiomycota), a new earthstar fungus in the Brazilian Atlantic  
297 rainforest – A molecular analysis. – *Nova Hedwigia*. 98: 265–272.
- 298 Cabral, T.S., Silva, B.D.B., Ishikawa, N.K., Alfredo, D.S., Braga-Neto, R. et al.  
299 (2014b): A new species and new records of gasteroid fungi (Basidiomycota) from  
300 Central Amazonia, Brazil. – *Phytotaxa*. 183: 239–253.
- 301 Cabral, T., Sousa, J.O., Silva, B.D.B., Martín, M.P., Clement, C.R. et al. (2017): A  
302 remarkable new species of *Geastrum* with an elongated branched stipe. –  
303 *Mycoscience*. 58: 344–350.
- 304 Castelletti, C.H.M., Santos, A.M.M., Tabarelli, M. & Da Silva, J.M.C. (2003): Quanto  
305 ainda resta da Caatinga? Uma estimativa preliminar. – In: Leal, I.R., Tabarelli, M.  
306 & Da Silva, J.M.C. (Eds.): *Ecologia e conservação da Caatinga*. pp. 719–734.  
307 Pernambuco, Brazil.
- 308 Crous, P.W., Wingfield, M.J., Burgess, T.I., Carnegie, A.J., Hardy, G.E.St.J. et al.  
309 (2017): Fungal Planet description sheets: 625–715. – *Persoonia*. 39: 270–467.
- 310 Crous, P.W., Wingfield, M.J., Burgess, T.I., Hardy, G.E.St.J., Crane C. et al. (2016):  
311 Fungal Planet description sheets: 469–557. – *Persoonia* 37: 218–403.
- 312 Crous, P.W., Wingfield, M.J., Burgess, T.I., Hardy, G.E.St.J., Gené J. et al. (2018):  
313 Fungal Planet Description Sheets: 716–784. – *Persoonia*. 40: 240–393.
- 314 Crous, P.W., Wingfield, M.J., Le Roux, J.J., Richardson, D.M., Strasberg, D. et al.  
315 (2015): Fungal Planet Description Sheets: 371–399. – *Persoonia*. 35: 264–327.
- 316 Dios, M.M., Moreno, G., Zamora, J.C. & Altés, A. (2017): Algunos hongos gasteroides  
317 epigeos interesantes de Catamarca (Argentina) – *Lilloa*. 54: 154–169.
- 318 Fazolino, E.P., Calonge, F.D. & Baseia, I.G. (2008): *Geastrum entomophilum*, a new  
319 earthstar with an unusual spore dispersal strategy. – *Mycotaxon*. 104: 449–453.



- 320 Gardes, M. & Bruns, T.D. (1993): ITS primers with enhanced specificity for  
321 Basidiomycetes applications to the identification of mycorrhizae and rusts. –  
322 Molecular Ecology. 1: 113–118.
- 323 Hawksworth, D. L. (2001): The magnitude of fungal diversity: the 1.5 million  
324 species estimate revised. – Mycological Research 105 (12): 1422–1432.
- 325 Jeppson, M., Nilsson, H.R. & Larsson, E. (2013): European earthstars in Geastraceae  
326 (Geastrales, Phallomycetidae) – a systematic approach using morphology and  
327 molecular sequence data. – Systematics and Biodiversity. 11 (4): 437–465.
- 328 Kornerup, A. & Wanscher, J.H. (1978): Methuen Handbook of Colours. – 3rd edn. Eyre  
329 Methuen, London.
- 330 Kasuya, T., Hosaka, K., Uno, K. & Kakishima, M. (2012): Phylogenetic placement of  
331 *Geastrum melanocephalum* and polyphyly of *Geastrum triplex*. – Mycoscience.  
332 53(6): 411–426.
- 333 Kuhar, F., Castiglia, V. & Papinutii, L. (2012): *Geastrum* species of the Riojia province,  
334 Argentina. – Mycotaxon. 122, 145–156.
- 335 Kumar, S., Stecher, G. & Tamura, K. (2016) MEGA7: Molecular Evolutionary  
336 Genetics Analysis Version 7.0 for Bigger Datasets. 2016. – Mol. Biol. Evol 33(7):  
337 1870–1874.
- 338 Leal, I.R., da Silva, J.M.C., Tabarelli, M. & Lacher, J.R. T.E. (2005): Mudando o curso  
339 da conservação da biodiversidade na Caatinga do Nordeste do Brasil. –  
340 Megadiversidade. 1: 139–146.
- 341 Moro, M.F., Lughadha, E.M., Araújo, F.S. & Martins, F.R. (2016): A Phytogeographical  
342 Meta analysis of the Semiarid Caatinga Domain in Brazil. – The Botanical Review.  
343 82: 91–148.
- 344 Prado, D. (2003): As Caatingas da América do Sul. – In: Leal, I., Tabarelli, M. & Silva,  
345 J.M.C. (eds). Ecologia e Conservação da Caatinga. Recife. Ed. Universitária UFPE.
- 346 Rehner, S.A. & Samuels, G.J. (1994): Taxonomy and phylogeny of *Gliocladium*  
347 analyzed from nuclear large subunit ribosomal DNA sequences. – Mycological  
348 Research. 98: 625–634.
- 349 Silva, B.D.B., Cabral, T.S., Marinho, P., Ishikawa, N.K. & Baseia, I.G. (2013): Two  
350 new species of *Geastrum* (Geastraceae, Basidiomycota) from Brazil. – Nova  
351 Hedwigia. 96: 445–456.
- 352 Silva, B.D.B., Sousa, J.O. & Baseia, I.G. (2011): Discovery of *Geastrum xerophilum*  
353 from the Neotropics. – Mycotaxon. 118: 355–359.
- 354 Sousa, J.O., Baracho, G.S. & Baseia, I.G. (2015): *Geastrum laevisporum*: a new  
355 earthstar fungus with uncommon smooth spores. – Mycosphere 6: 501–507.
- 356 Sousa, J.O., Morais, L.A., Nascimento, Y.M. & Baseia, I.G. (201a) – Updates on the  
357 geographic distribution of three *Geastrum* species from Brazilian semi-arid region.  
358 – Mycosphere 3: 467– 474.
- 359 Sousa, J.O., Silva, B.D.B. & Baseia, I.G. (2014b): *Geastrum* from the Atlantic Forest in  
360 northeast Brazil –new records for Brazil. –Mycotaxon 129: 169–179.
- 361 Sousa, J.O., Suz, L.M., García, M.A., Alfredo, D.S., Conrado L.M. et al. (2017): More  
362 than one fungus in the pepper pot: integrative taxonomy unmasks hidden species  
363 within *Myriostoma coliforme* (Geastraceae, Basidiomycota). – PloS ONE 12(6):  
364 e0177873.
- 365 Stamatakis, A. (2014): RAxML Version 8: A tool for phylogenetic analysis and post-  
366 analysis of large phylogenies. – Bioinformatics. 30: 1312–1313.
- 367 Sunhede, S. (1989): Geastraceae (Basidiomycotina): morphology, ecology, and  
368 systematics with special emphasis on the North European species. – Synopsis  
369 Fungorum 1. Fungiflora, Oslo, Norway.

370 Velloso, A.L., Sampaio, E.V.S.B. & Pareyn, F.G.C. (2002): Ecorregiões propostas para  
371 o bioma Caatinga. Associação Plantas do Nordeste, Instituto de Conservação  
372 Ambiental. – The Nature Conservancy do Brasil, Recife. Available from  
373 [http://www.mma.gov.br/estruturas/203/\\_arquivos/ecorregioes\\_site\\_203.pdf](http://www.mma.gov.br/estruturas/203/_arquivos/ecorregioes_site_203.pdf)  
374 Vilgalys, R. & Hester, M. (1990): Rapid genetic identification and mapping of  
375 enzymatically amplified ribosomal DNA from several *Cryptococcus* species. –  
376 *Journal of Bacteriology*. 172: 4238–4246. PMID: 2376561  
377 White, T.J., Bruns, T. & Taylor, J. (1990): Amplification and direct sequencing of  
378 fungal ribosomal RNA genes for phylogenetics. – In: Innes, M.A., Gelfand, D.H.,  
379 Sninsky, J.J. & White, T.J. (eds), *PCR protocols: A guide to methods and*  
380 *applications*. Academic Press, Inc.; pp. 315–322. California, San Diego.  
381 Zamora, J.C., Calonge, F.D., Hosaka, K. & Martín, M.P. (2014): Systematics of the  
382 genus *Geastrum* (Fungi: Basidiomycota) revisited. – *Systematics and Phylogeny*.  
383 *Taxon*. 63: 447–497.  
384 Zamora, J.C., Calonge, F.D. & Martín, M.P. (2015): Integrative taxonomy reveals an  
385 unexpected diversity in *Geastrum* section *Geastrum* (Geastrales, Basidiomycota). –  
386 *Persoonia*. 34: 130–165.  
387  
388  
389  
390  
391  
392  
393  
394  
395  
396  
397  
398  
399  
400  
401  
402  
403  
404  
405  
406  
407  
408  
409  
410  
411  
412  
413  
414  
415  
416  
417

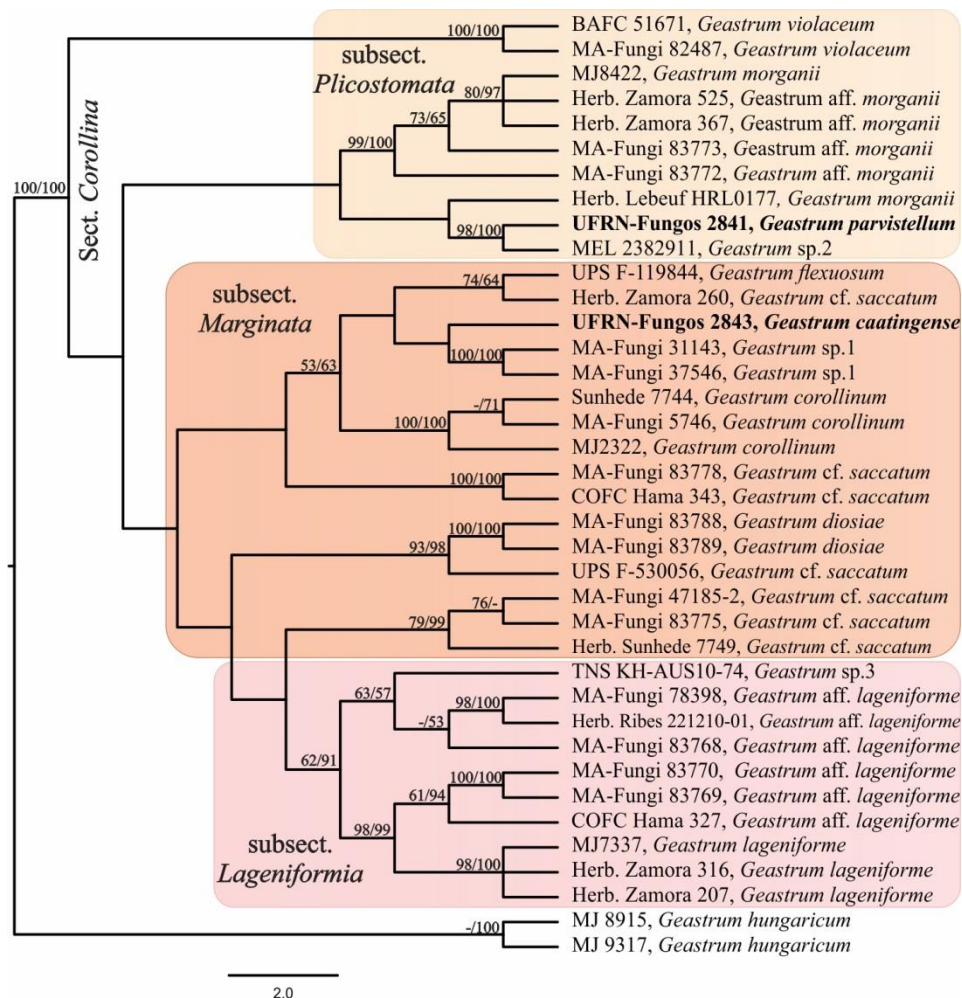
418 Table 1. *Geastrum* species included in the molecular analyses with their country,  
 419 collection number and GenBank accession numbers of internal transcribed spacer (ITS)  
 420 and large subunit (LSU) of nuclear ribosomal DNA. The new sequences in bold.

| Species                                 | Locality  | Collection number     | GenBank accession number |                 |
|---|-----------|-----------------------|--------------------------|-----------------|
|   |           |                       | ITS                      | LSU             |
| <i>Geastrum caatingense</i> sp. nov.    | Brazil    | UFRN-Fungos 2843      | <b>MH253884</b>          | <b>MH253886</b> |
| <i>Geastrum corollinum</i>              | Sweden    | MJ2322                | KC581972                 | KC581972        |
|   | Spain     | MA-Fungi 5746         | KF988359                 | KF988481        |
|   | Sweden    | Herb. Sunhede 7744    | KF988360                 | KF988482        |
| <i>Geastrum diosiae</i>                 | Argentina | MA-Fungi 83788        | KF988452                 | KF988587        |
|   | Argentina | Ma-Fungi 83789        | KF988453                 | KF988588        |
| <i>Geastrum flexuosum</i>               | Sweden    | UPS F-119844          | KF988371                 | KF988493        |
| <i>Geastrum lageniforme</i>             | Spain     | Herb. Zamora 207      | KF988388                 | KF988513        |
|   | Spain     | Herb. Zamora 316      | KF988339                 | KF988514        |
|   | Slovakia  | MJ7337                | KC581966                 | KC581966        |
| <i>Geastrum</i> aff. <i>lageniforme</i> | Argentina | MA-Fungi 83768        | KF988389                 | KF988516        |
|   | Niger     | COFC Hama 327         | KF988390                 | KF988517        |
|   | Argentina | MA-Fungi 83770        | KF988391                 | KF988518        |
|   | Argentina | MA-Fungi 83769        | KF988392                 | KF988519        |
|   | Portugal  | MA-Fungi 78398        | KF988393                 | KF988520        |
|   | Spain     | Herb. Ribes 221210-01 | KF988394                 | KF988521        |
|   | Canada    | Herb. Lebeuf HRL0177  | KF988406                 | KF988534        |
| <i>Geastrum morgani</i>                 | France    | MJ8422                | KC581971                 | KC581971        |
|   | Spain     | Herb. Zamora 525      | KF988408                 | KF988536        |
| <i>Geastrum</i> aff. <i>morgani</i>     | Spain     | Herb. Zamora 367      | KF988407                 | KF988535        |
|   | Argentina | MA-Fungi 83772        | KF988409                 | KF988537        |
|   | Argentina | MA-Fungi 83773        | KF988410                 | KF988538        |
|   | Brasil    | UFRN-Fungos 2341      | <b>MH253885</b>          | <b>MH253887</b> |
| <i>Geastrum</i> cf. <i>saccatum</i>     | Argentina | MA-Fungi 83775        | KF988427                 | KF988555        |
|   | Bolivia   | MA-Fungi 47185-2      | KF988426                 | KF988554        |
|   | Australia | Herb. Sunhede 7749    | KF988343                 | KF988556        |
|   | Japan     | UPS F-530056          | KF988428                 | KF988558        |
|   | Argentina | MA-Fungi 83778        | KF988433                 | KF988563        |
|   | Niger     | COFC Hama 343         | KF988432                 | KF988562        |
|   | Spain     | Herb. Zamora 260      | KF988430                 | KF988560        |
|   | Spain     | MA-Fungi 31143        | KF988454                 | KF988589        |
| <i>Geastrum</i> sp.1                    | Spain     | MA-Fungi 37546        | KF988455                 | KF988590        |
|   | Australia | MEL 2382911           | KP012780                 | KP012780        |
| <i>Geastrum</i> sp.2                    | Australia | TNS KH-AUS10-74       | JN845177                 | JN845301        |
| <i>Geastrum violaceum</i>               | Argentina | BAFC 51671            | KF988450                 | KF988585        |
|   | Argentina | MA-Fungi 82487        | KF988451                 | KF988586        |
| Outgroup                                |           |                       |                          |                 |
| <i>Geastrum hungaricum</i>              | Hungary   | MJ8915                | KC581963                 | KC581963        |
|   | Slovakia  | MJ9317                | KC581964                 | KC581964        |

421 Table 2

422 Comparative table with morphologic characteristics from species of section *Corollina*.

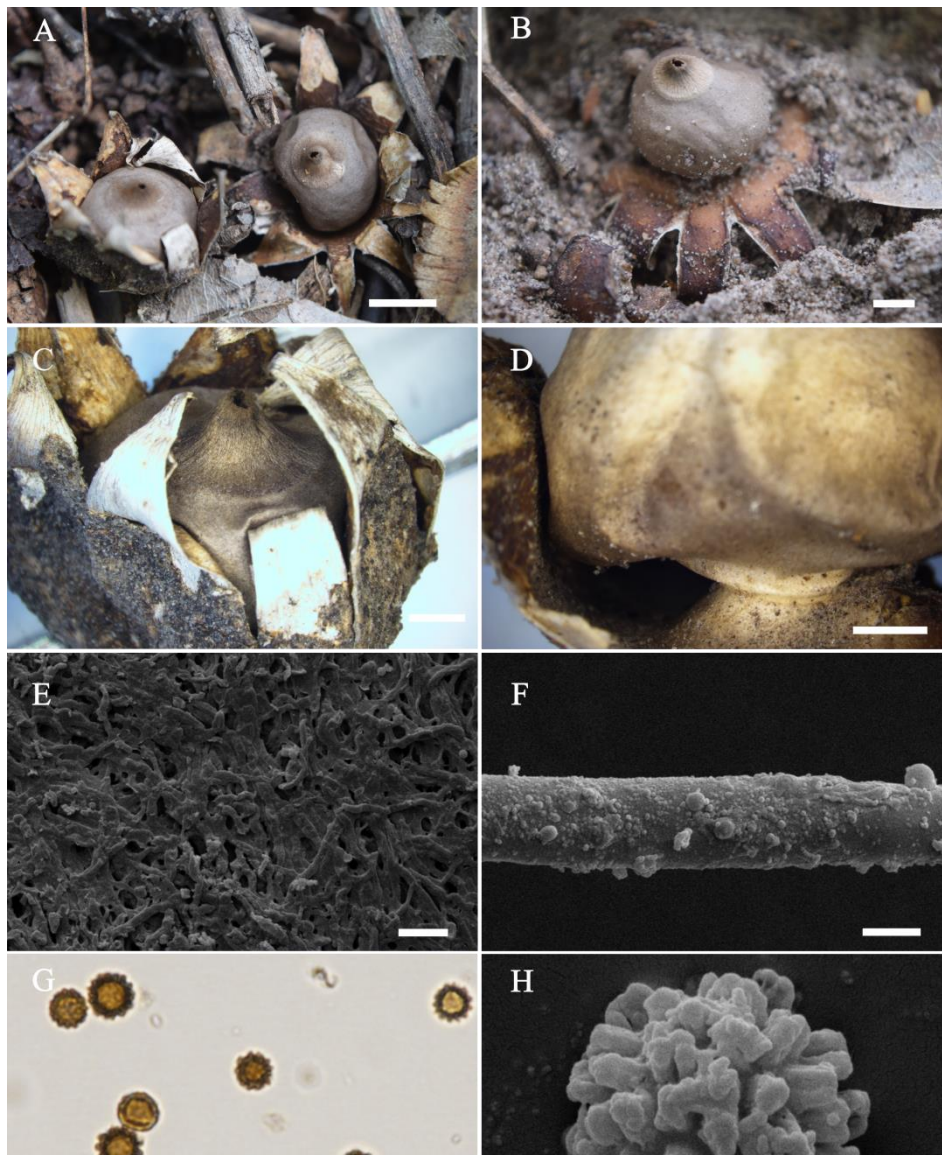
|                               | <i>Section Corollina</i>    |                                  |                           |                                       |                          |                                    |  |                       |   |
|-------------------------------|-----------------------------|----------------------------------|---------------------------|---------------------------------------|--------------------------|------------------------------------|--|-----------------------|---|
|                               | <i>Subsec. Plicostomata</i> |                                  |                           | <i>Subsec. Marginata</i>              |                          |                                    |  |                       | <i>Subsec. Lageniformia</i>                         |
|                               | <i>G. morganii</i>          | <i>G. parvistellum</i>           | <i>G. violaceum</i>       | <i>G. caatingense</i>                 | <i>G. corollinum</i>     | <i>G. diosiae</i>                  | <i>G. saccatum</i>   | <i>G. flexuosum</i>   | <i>G. lageniforme</i>                               |
| Basidiomata wide (mm)         | 9–28                        | 6–12                             | 9–26                      | 11–29                                 | 11–30                    | 5–14                               | 8–44   | 20–40                 | 15–44   |
| Exoperidium                   | non-hygroscopic             | sub-hygroscopic                  | non-hygroscopic           | hygroscopic                           | strongly hygroscopic     | strongly hygroscopic               | non-hygroscopic  | non-hygroscopic       | non-hygroscopic                                     |
| Mycelial layer                | non-encrusted, persistent   | encrusted, rarely non-persistent | non-encrusted, persistent | encrusted, rarely non-persistent      | non-encrusted, ephemeral | encrusted, normally non-persistent | non-encrusted, persistent, rarely with longitudinal cracks | encrusted, persistent | non-encrusted, persistent, with longitudinal cracks |
| Endoperidium surface          | with protruding hyphae      | furfuraceous                     | glabrous                  | glabrous                              | pruinose                 | glabrous or inconspicuous pruinose | glabrous   | reduced or lacking    | glabrous  |
| Pedicel                       | absent                      | inconspicuous (< 0.5 mm high)    | absent                    | absent or inconspicuous (< 1 mm high) | absent or inconspicuous  | absent                             | absent   | absent                | absent  |
| Peristome                     | irregularly plicate         | irregularly plicate              | irregularly plicate       | fibrillose                            | fibrillose               | fibrillose                         | fibrillose   | absent                | fibrillose  |
| Peristome delimitation        | absent                      | absent or weak                   | absent                    | distinct                              | distinct                 | distinct                           | distinct   | absent                | distinct  |
| Basidiopores size (-µm diam.) | 4.5–6                       | 4.8–6.5                          | 2.7–3.1                   | 5.2–6.5                               | 4.5–5                    | 4–5                                | 4.5–6  | 4–5                   | 4.5–5   |



423

424 Fig. 1. Strict consensus tree of the eight most parsimonious trees of concatenated ITS  
 425 and LSU nrDNA sequences of *Geastrum* indicated in Table 1. Numbers over branches  
 426 are parsimony bootstrap (MPbs) and maximum likelihood bootstrap (MLbs) values.  
 427 Holotypes of the new species here described are indicated in bold. Locality, collection  
 428 numbers, and GenBank codes are indicated in Table 1.  
 429





430

431

432 Fig. 2. *Geastrum caatingense* sp. nov. A. Fresh basidiomata *in situ* (UFRN–Fungos

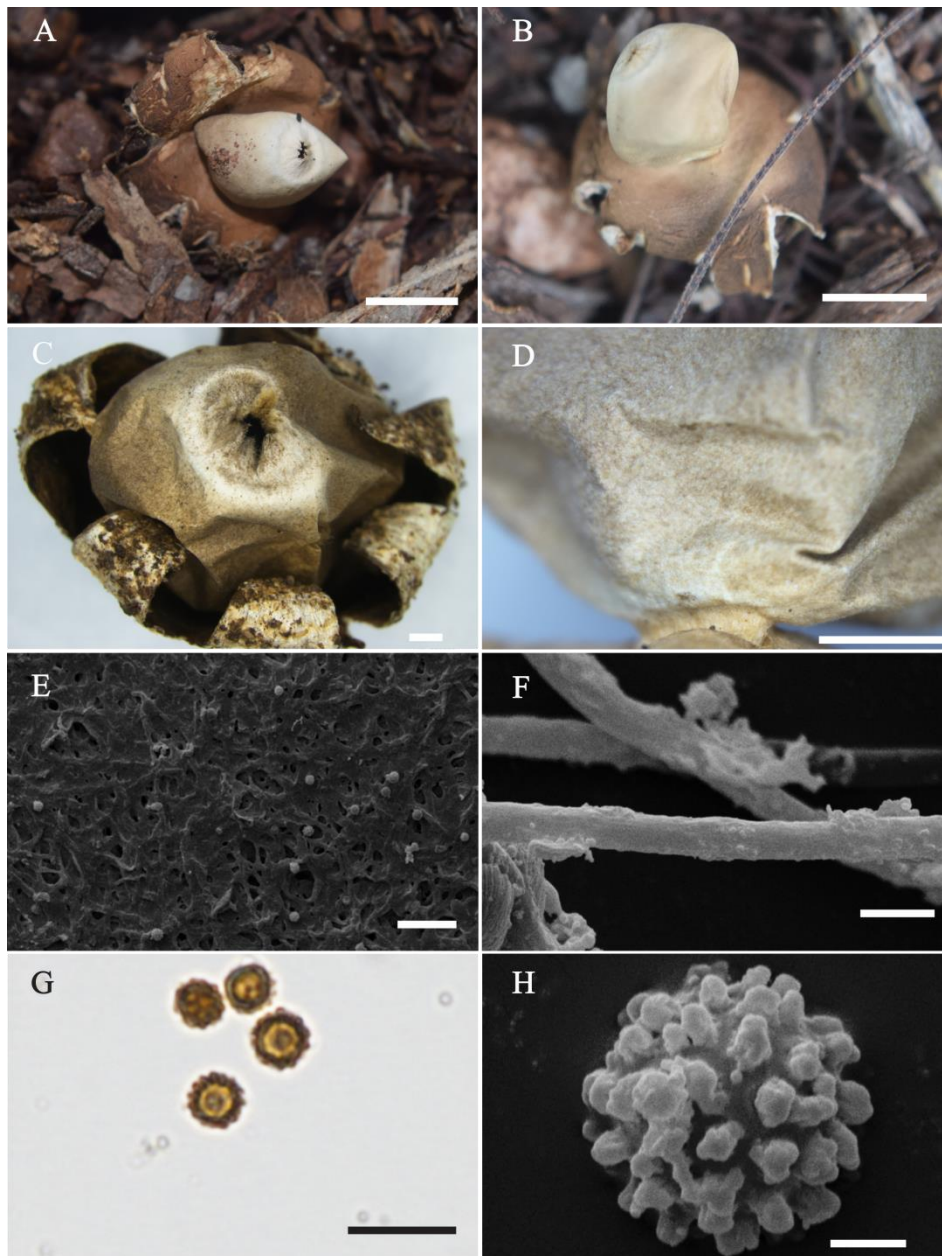
433 2843, holotype). B. Fresh basidioma *in situ*. C (UFRN–Fungos 2960, isotype).

434 Peristome detail. D. Endoperidial body detail. E. Endoperidium surface under SEM. F.

435 Eucapillitialy hypha under SEM. G. Basidiospores under LM. H. basidiospore under

436 SEM. Scale bars: A–B = 5 mm, C–D = 2 mm, E = 20  $\mu$ m, F = 2  $\mu$ m, G = 10  $\mu$ m, H = 1

437  $\mu$ m.



438

439

440 Fig. 3. *Geastrum parvistellum* sp. nov. A. Fresh basidioma *in situ* (UFRN–Fungos 2841,  
 441 holotype). B. Fresh basidioma *in situ* (UFRN–Fungos 2961, isotype). C. Peristome  
 442 detail. D. Pedicel detail. E. Endoperidium surface under SEM. F. Eucapillitial hyphae  
 443 under SEM. G. Basidiospores under LM. H. Basidiospore under SEM. Scale bars: A–B  
 444 = 5 mm, C–D = 1 mm, E = 20  $\mu$ m, F = 2  $\mu$ m, G = 10  $\mu$ m, H = 1  $\mu$ m.