**Geastrum dolomiticum**, a new earthstar species from Central Europe

Péter Finy1 · Viktor Papp2 · Dániel G. Knapp3 · Károly Bóka3 · Gábor M. Kovács3 · Bálint Dima3

Received: 14 December 2020 / Accepted: 11 May 2021 / Published online: 7 June 2021
© The Author(s) 2021

**Abstract**
The recently revised *Geastrum minimum* species complex in sect. *Geastrum* subsect. *Quadrifida* revealed that the name *G. minimum* is a nomen ambiguum and dubium and was collectively used for at least two European species (viz. *G. granulosum* and *G. marginatum*). During the morphological revision of the Hungarian materials labelled as *G. minimum*, different crystal structures were found on the endoperidial body of some specimens than those of characteristic for *G. granulosum* and *G. marginatum*. These collections were exclusively found on open rocky grassy vegetation on dolomite bedrock in Hungary. Multigene phylogenetic analyses involving nrITS, nrLSU, *rpb1*, *atp6* and *tef1-α* sequences of the collections with unique crystal morphology and ecology revealed that these form a distinct clade in close relationship with *G. granulosum* s.l. (i.e. specimens from Europe and North America). Based on molecular evidence, macro- and micromorphology as well as X-ray Powder Diffraction (XRD) characterisation of the mesoperidial crystals, here we propose the new species *Geastrum dolomiticum*.

**Keywords** Geastrales · Phallomycetidae · Protein coding genes · SEM-microscopy · Taxonomy

**Introduction**
The worldwide distributed genus *Geastrum* Pers. is one of the largest genera of gasteroid fungi, encompassing ca. 130 species (He et al. 2019). The taxonomy of *Geastrum* was intensively studied in the recent years and several new species were described from many parts of the world (e.g. Hemmes and Desjardin 2011; Zamora et al. 2015; Accioly et al. 2019; Crous et al. 2019), although most of the novel species have been found in South America, viz. Brazil’s semi-arid region or in the Amazonas region (Silva et al. 2013; Cabral et al. 2014a, b, 2017; Sousa et al. 2015, 2019; Crous et al. 2016, 2017, 2018a, b, Assis et al. 2019). In contrast to the tropical regions, the genus *Geastrum* is considered as well-studied in Europe (e.g. Sunhede 1989; Calonge and Zamora 2003; Zamora and Calonge 2007). Taxonomy and systematics of European earthstars (*Geastrum* and *Myriostoma* Desv.) have been reviewed by Jeppson et al. (2013), who accepted 30 *Geastrum* s. str. species for the old continent based on morphological observations and multigene analyses. Zamora et al. (2014) revised the section *Schmidelia*, and proposed *Geastrum senoretiae* J.C. Zamora as a new species from Spain. In a later study on phylogeny and classification of *Geastrum* sect. *Geastrum*, Zamora et al. (2015) described three additional European species, two from Spain (G. benitoi J.C. Zamora and G. meridionale J.C. Zamora) and one from the United Kingdom (G. britannicum J.C. Zamora). Therefore, *Geastrum* s. str. currently encompasses 34 species in Europe.

Among the European earthstars, 25 *Geastrum* species were known from Hungary (Central Europe), which have indicated an exceptional species richness of the genus (e.g. Jeppson 2013; Finy and Jeppson 2021). Taxonomic studies of *Geastrum* have a long tradition in Hungary. At the beginning of the twentieth century in his monographic book, Hollós (1903) already reported 21 *Geastrum* species from Hungary. Besides the valuable chorological data, Hollós (1901) also described new earthstar species from Hungary, such as *G. hungaricum* Hollós, which grows in steppe and dry grassland habitats, and produces an extremely small, hygroscopic basidiome. The species has gained legal protection...
in Hungary since 2006 (Siller et al. 2006). Lately, intensive fieldwork has been taken place devoted to Hungarian earth-stars from the sandy forest steppe region of the Carpathian Basin (Rimóczi et al. 2011). In 2015, a peculiar Geastrum species was found in Hungary growing exclusively on dolomite bedrock. Macroscopically it resembled to G. granulosum Fuckel, but based on microscopic and molecular phylogenetic data it differs from all known European species.

In this study we aimed to clarify the taxonomy of those Hungarian collections found on dolomite, based on macro- and micromorphological features of the basidiome, X-ray Powder Diffraction (XRD) characterisation of the mesoperidial crystals and multigene phylogenetic analyses.

Materials and methods

Morphological study

In this study, altogether nine specimens collected from autumn to spring (Table 1) were examined. Type specimens including holotype and paratypes were deposited in the herbarium of the Hungarian Natural History Museum, Budapest (BP) under the accession numbers BP111140–BP111144. All other examined specimens were deposited in the Department of Plant Anatomy, Eötvös Loránd University (abbreviated further as ELTE). Dried mature fruiting bodies were used for macro- and microscopic examination. For light microscopy, samples were mounted in water or in Lactophenol-cotton blue and heated to boiling temperature. The samples were examined with Reichert Polyvar and Olympus BH-2 microscopes. Spore dimensions were inclusive of spore wall ornamentation. Terminology mostly followed Sunhede (1989) and Zamora et al. (2015). Small pieces of peridium and gleba from dried basidiomes were prepared, fixed to stubs, coated with gold and examined under a Hitachi S2460N (Hitachi Ltd., Tokyo, Japan) scanning electron microscope at 22 kV accelerating voltage.

Molecular phylogenetic study

For molecular identification, ITS (internal transcribed spacer) region of the nrDNA, the universal fungal barcode region (Schoch et al. 2012) was amplified using the Phire® Plant Direct PCR Kit (Thermo Scientific, USA) and the primer pairs ITS1F/ITS4 (White et al. 1990; Gardes and Bruns 1993) as described in Papp and Dima (2018). For amplifying further four loci a prior total DNA extraction with the E.Z.N.A. SP Fungal DNA Mini Kit was applied. The primers LR0R (Rehner and Samuels 1994) and LR5 (Vilgalys and Hester 1990) were used to amplify the partial 28S nrRNA gene (LSU) of the nrDNA operon region. The partial RNA polymerase II largest subunit (rpb1) was amplified with RPB1-Af and RPB1-Cr (Matheny et al. 2002) and part of the mitochondrial ATPase subunit 6 (atp6) using the primers atp6-2 and atp6-3 (Kretzler and Bruns 1999). The primers EF1-983F and EF1-2218R (Rehner and Buckley 2005) were used to amplify part of the translation elongation factor 1α (tef1-α). Sequencing of the amplicons was carried out with the primers used for amplification by LGC Genomics (Berlin, Germany). The sequences were compiled from electropherograms using the Staden software package (Staden et al. 2000). Sequences of each locus were aligned separately with sequences of respective species from GenBank (Table 1) using E-INS-i method of the online MAFFT version 7 (Katoh and Standley 2013). The alignments were checked and edited in MEGA7 (Kumar et al. 2016).

Two datasets were used in the phylogenetic analyses. The ‘subsection-level’ dataset was used to gain information about the phylogenetic position of our sequences among those of Geastrum sequences representing subsect. Quadrifida and subsect. Hungarica sensu Zamora et al. (2015). The second dataset represented sequences of only G. granulosum related specimens. For the two datasets, multi-locus Bayesian analysis (BI) were performed with MrBayes 3.1.2 (Ronquist and Huelsenbeck 2003) using the GTR + G nucleotide substitution model. The GTR + G nucleotide substitution model was used with ML estimation of base frequencies and a ML bootstrap analysis with 1,000 replicates was used to test the support of the branches. The phylogenetic trees were visualized and edited using MEGA7 and deposited in TreeBASE (www.treebase.org) as submission 27948.

Following Zamora et al. (2015), the ITS, LSU, rpb1 and atp6 sequences were used for ‘subsection-level’ phylogenetic analyses and considered as separate partitions. These four regions of a selected subset of species were supplemented with additional two partitions (tef1-α, and indels from ITS region) to determine the phylogenetic relationships of G. granulosum related species and to improve phylogenetic resolution. The indels in the ITS region were coded (Nagy et al. 2012) using the simple indel coding algorithm (Simmons et al. 2001; Young and Healy 2003) with the program FastGap (Borchesgenius 2009). In the BI analysis, the two-parameter Markov (Mk2 Lewis) model was used for the indel partition of the dataset and the GTR + G model for the nucleotide partitions. In the ML analyses, in addition to the nucleotide partitions (GTR + G), the indel data were treated as binary data (BIN).
Table 1 Details of *Geastrum* specimens comprised in this study. Species, country and state/province, herbarium voucher numbers, and GenBank accession numbers of each loci (ITS, LSU, rpb1, atp6, tef1-α) are presented. Specimens and the new sequences generated in this study are shown in bold

| Species            | Collection site     | Herbarium voucher | GenBank accession numbers |
|--------------------|---------------------|-------------------|---------------------------|
|                    |                     |                   |                           |
| *G. austrominimum* | Australia, New South Wales | CANB 748741 | – | KP687529 KP687531 KP687572 – |
|                    | Australia, New South Wales | MEL2276089 | | KP687490 KP687451 KP687532 KP687573 – |
|                    | Australia, Victoria | MEL 2292062 | | KP687491 KP687452 KP687533 KP687574 – |
|                    | Australia, Victoria | MEL 2358014 | | KP687492 KP687453 KP687534 KP687575 – |
| *G. cf. calceum 1* | Argentina, Tucumán | MA-Fungi 83761 | | KP687493 KP687454 KP687535 KP687576 – |
| *G. cf. calceum 2* | Brazil, Rio Grande do Norte | UFRN-Fungos 723 | | KP688340 KP688477 KP688612 KP688747 – |
| *G. dolomiticum*   | Hungary, Fejér | FP20150909/FP111140 (FP05) (holotype) | MT569463 MT569455 MT572903 MT572900 MT593358 |
|                    | Hungary, Veszprém | FP20151015/FP111142 (FP07) | MT569464 MT569456 MT572904 MT572901 MT593359 |
|                    | Hungary, Veszprém | FP20151227/FP111144 (FP09) | MT569465 – – – – |
|                    | Hungary, Veszprém | FP20151027/FP111143 (FP17) | MT569467 MT569458 MT572905 – MT593360 |
|                    | Hungary, Fejér | FP20140223/FP111141 (FP38) | MT569469 MT569460 MT572906 MT572902 MT593361 |
| *G. hungaricum*    | Hungary | M98915 (GB) | KC581964 KC581964 – – KC758603 |
|                    | Slovakia | MJ9317 (GB) | KC581963 KC581963 – – – |
|                    | Czech Republic, Reporyje | Sunhede 5993 | KP687500 KP687461 KP687542 KP687582 – |
|                    | Spain, Toledo | Zamora 611 | KP687501 KP687462 KP687543 KP687583 – |
| *G. granulosum 1*  | Russia, Rostov | K(M) 154623 | JN845105 JN845223 – JN845347 – |
|                    | Spain, Madrid | MA-Fungi 69175 | KP687497 KP687458 KP687539 KP687579 – |
|                    | Sweden, Öland | Sunhede 7746 | KP988401 KP988529 KP988664 KP988796 – |
|                    | Spain, Madrid | Zamora 191 | KP988400 KP988528 KP988663 KP988795 – |
| *G. granulosum 2*  | Hungary, Fejér | FP20160221 (FP01) | MT569461 MT569453 – – – |
|                    | Hungary, Fejér | FP20150325 (FP03) | MT569462 MT569454 – – MT572898 |
|                    | Hungary, Pest | FP20141214 (FP11) | MT569466 MT569457 – – MT572899 |
|                    | Hungary, Fejér | FP20141213 (FP37) | MT569468 MT569459 – – – |
|                    | Sweden | MJ9529 | KC581957 KC581957 – – KC758598 |
| *G. kuharii*       | Argentina, Buenos Aires | MA-Fungi 83795 | KP988463 KP988598 KP988733 KP988864 – |
|                    | Argentina, Entre Ríos | MA-Fungi 86913 | KP687502 KP687463 KP687544 KP687584 – |
|                    | Argentina, Buenos Aires | MA-Fungi 86914 | KP687503 KP687464 KP687545 KP687585 – |
| *G. marginatum*    | Spain, Canary Islands | ERRO 2012112069 | KP687504 KP687465 KP687546 KP687586 – |
|                    | Spain, Madrid | MA-Fungi 31530 | KP988404 KP988532 KP988667 KP988799 – |
|                    | Spain, Jaén | MA-Fungi 32395 | KP687505 KP687466 KP687547 KP687587 – |
|                    | Spain, Madrid | MA-Fungi 48129 | KP687506 KP687467 KP687548 KP687588 – |
|                    | Sweden, Gotland | MA-Fungi 86669 | KP988405 KP988533 KP988668 KP988800 – |
|                    | USA, Arizona | MICH 28119b | KP988403 KP988531 KP988666 KP988798 – |
|                    | Czech Republic, Bohemia | PRM 842884 (holotype of *G. minimum* var. *fumosicollum*) | KP687507 KP687468 KP687549 – – |
| *G. quadrifidum*   | Sweden, Uppland | MA-Fungi 86671 | KP988422 KP988550 KP988685 KP988817 – |
Table 1 (continued)

| Species            | Collection site | Herbarium voucher | GenBank accession numbers |
|--------------------|-----------------|-------------------|---------------------------|
|                    |                 |                   | ITS | LSU | rpb1 | atp6 | tefl-α          |
| USA, Colorado      | MICH 72512      | KF988823          | KF988851 | KF988868 | KF988818 | – |
| Sweden, Södermanland | SF-45993   | JN845119          | JN845237 | – | – | – |
| Spain, Orense      | Zamora 139     | KP687523          | KP687485 | KP687566 | KP687603 | – |
| Spain, Huesca      | Zamora 170     | KP988421          | KP988549 | KP988684 | KP988816 | – |
| Spain, Cuenca      | Zamora 300     | KP687524          | KP687486 | KP687567 | KP687604 | – |
| Sweden             | MJ7151          | KC581958          | KC581958 | – | – | KC758599 |
| Sweden             | MJ7249          | KC581959          | KC581959 | – | – | KC758600 |
| Geastrum sp.       | Japan, Aomori  | TNS TKG-GE-91002  | JN845118 | JN845236 | – | JN845360 |

Fig. 1 Maximum Likelihood (RAxML) tree of ITS, LSU, rpb1 and atp6 sequences of *G. dolomiticum* and other *G. marginatum* representing subsect. *Hungarica* and subsect. *Quadrifida* sensu Zamora et al. (2015). RAxML bootstrap support values (≥ 70) are shown above branches and before slashes, Bayesian posterior probabilities (≥ 0.90) are shown below branches and after slashes. Materials collected by P. Finy (FP) in Hungary are bold. Holotypes are marked with T. Specimens from the subsect. *Hungarica* were served as multiple outgroup. The scale bar indicates 0.01 expected changes per site per branch.
X-Ray powder diffraction (XRD) measurement of calcium-oxalate

The measurements of the calcium-oxalate samples were carried out with Bruker D8 Advance instrument. The following parameters were configured: (i) Sample holder: Si low background sample holder (PMMA), (ii) Rotation: 30/min, (iii) Range: 5–80° (two theta), (iv) Mode: continuous scan, (v) Detector type: LYNXEYE XE (energy dispersive), (vi) X-Ray source: Cu-anode (Kα: 1.54184 Å), (vii) X-Ray optics: Bragg–Brentano, (viii) Generator power: 1600 W (40 mA, 40 kV). Samples were prepared using 1–5 mg sample that was gently homogenized in an achate mortar with a pestle to make fine powder. The grinded powder was mounted in a round sample holder (Si low background PMMA) and smooth surface was prepared by pressing it with a glass plate.

Results

Multigene phylogenetic analyses were carried out using two datasets comprising 47 strains and 3513 characters, and 18 strains and 4557 characters including gaps. According to the results, the studied specimens from Hungary belong to Geastrum sect. Geastrum subsect. Quadrifida representing different lineages (Fig. 1). The nine specimens from Hungary comprised in this study (marked with an asterisk in the Taxonomy part) were grouped together with G. granulosum specimens collected in Europe and in the USA. Four of the Hungarian specimens (FP01, FP03, FP11 and FP37) belong to the clade consist of various G. granulosum collection from Europe (Fig. 1). Five of our specimens (FP05, FP07, FP09, FP17 and FP38) represent a well-supported, relatively heterogeneous clade beside the G. granulosum lineages (Fig. 2).
the five samples, FP07 is grouped with FP17, and FP09 is with FP38, which two form together a sister clade with FP05 (Fig. 2). The difference in the sequences of the novel clade compared with the two groups comprising *G. granulosum* specimens from Europe, and *G. granulosum* specimens from the USA were 1.9 and 1.0% for ITS, 0.3% and 0.5% for *rpb1*, and 1.0% and 0.9% for *atp6*. Although, *tef1-α* sequences of *G. granulosum* specimens from the USA were not available, this locus showed remarkable distance between the European *G. granulosum* clade and the novel clade (3.5%) with relatively low intragroup heterogeneity (0.1% and 0.6%, respectively).

The XRD analysis of the samples prepared from the studied specimens (viz. *G. granulosum* and other Hungarian specimens growing on dolomite) showed characteristic peaks that can verify the presence of calcium-oxalate crystals. However, on *G. granulosum* we observed calcium oxalate dihydrate (COD) crystals, but on the specimens found on dolomite, calcium oxalate monohydrate (COM) crystals were found (Fig. 3).

Results of the molecular phylogenetic analyses and the calcium-oxalate investigation reinforce our hypothesis that the lineage comprising five of the specimens with unique morphological characteristics (for comparison with *G. granulosum*, *G. marginatum* and *G. austrominimum*, see Table 2) collected in Hungary, represent a novel species within *Geastrum* subsection *Quadrifida*.

**Discussion**

The new species, *Geastrum dolomiticum*, is mainly characterized by the small fruiting body, the lack of big COD crystals, the crested spore ornamentation and the habitat. *Geastrum granulosum* may also occur in calcareous rocky grasslands, but it has a wider ecological range, most common in open sandy steppe areas.

The recent integrative taxonomic study including morphological, molecular, ecological, and chorological data by Zamora et al. (2015) proposed that the collectively used *Geastrum minimum* Schwein. name is better to treat as *nomen ambiguum* and *dubium* since it includes at least four cryptic species from which two of them occur in Europe (i.e. *G. granulosum* Fückel and *G. marginatum* Vittad.), furthermore the protologue of *G. minimum* is not enough detailed to know which species was described by Schweinitz (1822). During the taxonomic revision of all material labelled in the Hungarian National History Museum (BP) as well as in private herbaria under the name *Geastrum minimum*, we found that the collections deposited in BP represent *G. granulosum* (= *G. queletii* Hazsl., see Zamora et al. 2015) due to the presence of large and regular COD crystals on the basidiomes. Among our private collections we discovered specimens with different, irregular or when regular than smaller, predominantly COM crystals. These samples originated from calcareous open rocky grasslands on dolomite bedrock. Phylogenetic analyses of the nrITS, nrLSU, *rpb1*, *atp6*, and *tef1-α* sequences revealed that these specimens with the unique crystal morphology and habitat belong to a separate lineage in *Geastrum* sect. *Geastrum* subsect. *Quadrifida* which we suggest as novel species and described here as *G. dolomiticum*. On the other hand, based on solely the crystal morphology, there is another species in Europe (*G. marginatum*) having small crystals on the endoperidial body (<70(–95) µm), however, *G. dolomiticum* when having regular crystals they are even smaller (<50 µm) than those of *G. marginatum*. Furthermore, the stalk of *G. marginatum* is dark compared to *G. dolomiticum* which has white stalk. The former species was only verified by two collections in Hungary and grows on grassy habitats on more or less acidic
and only rarely on slightly calcareous bedrocks, while the latter species prefers calcareous dolomite bedrocks.

*Geastrum dolomiticum*, the most similar species to *G. dolomiticum*, based on our analyses, seems to be a widespread species in Hungary occurring in dry steppe-like sandy grasslands as well as rocky habitats in the Hungarian mountain ranges on limestone and dolomite. There are examples that sandy grassland species occurs also on calcareous rocky habitats (e.g. *Infundibulicybe glareosa* (Röllin & Monthoux) Harmaja, *Gastrosporium simplex* Mattir., and *Tulostoma calcareum* Jeppson, Altés, G. Moreno & E. Larss). In contrast, *G. dolomiticum* has so far been found exclusively on dry rocky grassland on dolomite. Based on our phylogenetic analyses (Figs. 1, 2), a closely related North American species seems to belong in the *G. granulosum–G. dolomiticum* lineage too. This North American clade was collectively treated under *G. granulosum* in Zamora et al. (2015), according to the available phylogenetic data, it might belong to another species, viz. *G. minimum* s. str. However, this name is currently not in use as discussed above and in Zamora et al. (2015). The morphological delimitation of the North American lineage of *G. granulosum* and the clarification of the name *G. minimum* needs further investigations.

**Taxonomic treatment**

*Geastrum dolomiticum* Finy, Dima & V. Papp, sp. nov.—

**Type:** Hungary, Fejér County, near Csór, in open grassland on dolomite, among mosses and grasses in *Seseli leucospermi-Festucetum pallentis*, 8 Sep 2015 *P. Finy* FP20150908 (holotype: BP 111140, FP05*; isotype: ELTE). [Mycobank # MB 835789]. GenBank ITS (MT569463), LSU (MT569455), *rpb*1 (MT572903), *atp*6 (MT572900), *tef*1–α (MT593358) (Figs. 4, 5).

**Etymology:** The epithet refers to the habitat requirement of the species, in open rocky grasslands on dolomite bedrock.

**Description:** Exoperidium 8–22 mm in diam, arched, splitting to the middle in 6–12 non-hygroscopic rays, sometimes they roll towards the endoperidial body. Fibrous layer thin, papyraceous, whitish coloured when denuded. Pseudo-parenchymatous layer pale cream, ochraceous to brownish.

### Table 2 Comparison of morphological and ecological characters among the specimens examined of *Geastrum dolomiticum*, the European *G. granulosum* and *G. marginatum*. The features of *G. austrominimum* is taken from Zamora et al. (2015)

|                    | *G. dolomiticum* | *G. granulosum* 1 | *G. marginatum*     | *G. austrominimum* |
|--------------------|------------------|-------------------|--------------------|---------------------|
| **Peristome**      | Fibrillose, distinctly delimited | Fibrillose, mostly distinctly delimited | Fibrillose, distinctly delimited | Fibrillose, mostly distinctly delimited |
| **Diameter of the endoperidial body** | 3–9 mm | 4–12 mm | 6–9 mm | 5–10 mm |
| **Apophysis**      | Distinct apophysis | Distinct apophysis | More or less distinct apophysis | Present or absent |
| **Stalk**          | Whitish to cream | Whitish to cream | Brownish, cream at the base | Brownish |
| **Diameter of the exoperidium** | 8–22 mm | 9–35 mm | 12–25 mm | 17–35 mm |
| **Exoperidial rays** | 6–12 | 6–11 | 6–9 | 6–11 (–13) |
| **Mesoperidial crystals** | Aggregates of irregular shaped or flaky COM 5–50 μm in diam, sometimes mixed with bipyramidal COD 10–50 μm in diam | Bipyramidal COD 60–160 μm in diam | Bipyramidal COD 25–45 μm in diam | Rounded COM scales 30–105 μm in diam, less abundant bipyramidal COD 20–130 (–200) μm in diam |
| **Diameter of spores with ornamentation** | 4.7–5.1 × 4.5–4.9 μm | 4.3–5 × 4.1–4.8 μm | 4.6–5.5 × 4.3–5.3 μm | 4.5–6.5 μm |
| **Spore ornamentation** | Isolated or coalescing crest-like warts | Verrucose to irregularly pilate warts | Verrucose to irregularly pilate warts | Verrucose to irregularly pilate warts |
| **Capillitial hyphae** | 3–5 μm wide | 4–7 μm wide | 2–7 μm wide | 5–8.5 μm wide |
| **Cell wall of pseudo-parenchymatous layer** | 1–2 μm thick | thin, up to 1 μm | thin, up to 1 μm | thin, up to 1 μm |
| **Habitat**        | Rocky dolomitic grasslands | Various types of xeric grasslands | Acidic sandy grasslands | Grasslands, savannas, shrublands, forests in Australasia |
1–2 mm thick, covered by a dense mesoperidial crystalline matter, not persisting in old basidiomes. Mycelial layer persisting, intermixed with debris from the substrate. Endoperidial body 3–9 mm diam, greyish, greyish white, cream, brownish, more colourful on younger specimens, covered by a whitish mesoperidial crystalline matter. Peristome fibrillose, flat to broadly conical, up to 1 mm high, white, mostly well-delimited, with a whitish delimitation line. Lighter than the endoperidial body, except for the older, discoloured basidiomes. Stalk more or less stout, round, sometimes ellipsoid in cross section, 0.2–1.0 mm high, whitish to cream. Apophysis present, 0.5–1 mm high, concolorous with the endoperidial body, darker in the lower part, remains coloured. Columella cylindrical, intruding to the half or more into the gleba. Gleba chocolate brown. Basidiospores globose, (4.34)4.66–5.07(5.54) × (4.10)4.46–4.87(5.29) µm, L = 4.83, W = 4.7, Q = (1.00)1.01–1.08(1.12), Qav = 1.04, n = (100/2), with 0.2–0.5 µm high warts, ornamentation isolated or coalescing crest-like warts. Basidia not observed. Capillitial hyphae max. 3.0–5.0 µm wide, light brown, thick-walled with sparse surface debris, no lumen or very narrow. Endoperidial body composed of thick-walled up to 4 µm wide hyaline hyphae with narrow lumen. Peristomal hyphae up to 4 µm wide, light brown. Mesoperidial crystalline aggregates of COM 5–50 µm in diam, irregular shaped or flaky, sometimes mixed with bipyramidal COD which 10–50 µm in diam, covering the endoperidial surface and the pseudoparenchymatous layer (Figs. 5a, c). Pseudoparenchymatous layer thick-walled (1–2 µm thick) composed of 25–50×10–25 µm variously shaped elongated cells. Hyphae

Fig. 4 Habitat and macromorphology of Geastrum dolomiticum: a habitat in Öskü (dolomitic grassland); b typical habitat of G. dolomiticum in Csákberény; c basidiomes of G. dolomiticum (FP20151015, paratype – BP111142); d basidiomes of G. dolomiticum (FP20150908, holotype – BP111140). Photos: P. Finy
Geastrum dolomiticum, a new earthstar species

Fig. 5 Morphological characteristics of *Geastrum dolomiticum* (a–c) and *G. granulosum* (d–f). a Basidioma of *G. dolomiticum* (FP20150908, holotype); b basidiospores (FP20150908, holotype—BP111140); c crystalline aggregates of calcium oxalate monohydrate (COM) (FP20151227, paratype—BP111144); d basidioma of *G. granulosum* (FP20150214); e basidiospores (FP20141213); f crystalline aggregates of calcium oxalate dihydrate (COD) (FP20141213). Scale bars: 5 mm (a, d). Photos: K. Bőka (b, c, e, f), P. Fíny and V. Papp (a, d)
of the fibrous layer hyaline, thick-walled up to 5 µm wide, lumen visible. Columella hyphae thick-walled up to 5 µm wide.

Diagnosis: Exoperidium 8–22 mm in diam, endoperidial body 3–9 mm in diam, Mesoperidial crystalline aggregates of COM irregularly shaped or flaky, 5–50 µm in diam, sometimes mixed with bipyramidal COD. 10–50 µm in diam. Basidiospores globose, 4.5–5.1×4.5–4.9 µm, with isolated or coalescing crest-like warts. Pseudoparenchymatous layer with thick-walled (1–2 µm thick) cells.

Ecology and distribution: Geastrum dolomiticum grows in small groups in calcareous open rocky grassland on dolomite bedrock. The habitat is characterized by heliophilous vegetation e.g. grasses such as Festuca pallens Host and Stipa eriocaulis Borbás as well as cryptogams like mosses and lichens (Bölöni et al. 2011). On approximately horizontal places among the rocks, the undeveloped dolomite soils are mixed up with rubbles and powdered stones. The strong edaphical stress prevents the vegetation from closure and succession (Kun et al. 2005), the soil surface is covered mainly by mosses and lichens, where mature fruiting bodies occur. Unexpanded fruiting bodies develop deep in the moss layer. Accompanying macrofungi on the sites are Lycoperdon lividum Pers., Tulostoma brumale Pers., T. calcareum, and T. kotlabae Pouzar. These habitats are host of many endemic T. kotlabae lividum, and Tulostoma brumale T. calcareum Pers., Pers., Lycoperdon.

Host and distribution: Geastrum dolomiticum grows in small groups in calcareous open rocky grassland on dolomite bedrock. The habitat is characterized by heliophilous vegetation e.g. grasses such as Festuca pallens Host and Stipa eriocaulis Borbás as well as cryptogams like mosses and lichens (Bölöni et al. 2011). On approximately horizontal places among the rocks, the undeveloped dolomite soils are mixed up with rubbles and powdered stones. The strong edaphical stress prevents the vegetation from closure and successsion (Kun et al. 2005), the soil surface is covered mainly by mosses and lichens, where mature fruiting bodies occur. Unexpanded fruiting bodies develop deep in the moss layer. Accompanying macrofungi on the sites are Lycoperdon lividum Pers., Tulostoma brumale Pers., T. calcareum, and T. kotlabae Pouzar. These habitats are host of many endemic T. kotlabae lividum, and Tulostoma brumale T. calcareum Pers., Pers., Lycoperdon.

Additional specimens examined: HUNGARY. Fejér County, near Csákóvár, Lóllás-tető, in locis graminosis, 21 Oct 1955, L. Baksay, Szűjkőné (BP23235); near Csákóvár, in declivo graminoso, 20 Jul 1961, J. Ujhelyi (BP31425); near Csákóvár, Haraszt-hegy, in Seseli leucospermi-Festucetum pallentis, 12 Aug 2016, P. Finy FP20160812 near Csákóberény, in Seseli leucospermi-Festucetum pallentis, 17 Dec 2016, P. Finy FP20161217, ibid., 18 Nov 2017, P. Finy FP20171118, ibid., 25 Nov 2017, P. Finy FP20171125; Iszkaszentgyörgy, in Seseli leucospermi-Festucetum pallentis, 16 Dec 2017, P. Finy FP20171216; Lovasberény, in calcareous pasture, 23 Feb 2014, P. Finy FP20140223 (BP111141, FP38*, paratype); Veszprém County, near Öskü, in Seseli leucospermi-Festucetum pallentis, 15 Oct 2015, P. Finy FP20151015 (BP111142, FP07*, paratype), ibid., 27 Oct 2015, P. Finy FP20151027 (BP111143, FP17*, paratype), ibid., 19 Sep 2017, P. Finy FP20170919; near Várpalota, Baglyas, in Seseli leucospermi-Festucetum pallentis, 11 Feb 2017, P. Finy FP20170211; near Várpalota, Tési-fennsík, in Seseli leucospermi-Festucetum pallentis, 27 Dec 2015, P. Finy FP20151227 (BP111144, FP09*, paratype).
Geastrum dolomiticum, a new earthstar species

References

Accioly T, Sousa JO, Moreau P-A, Lécureu C, Silva BDB, Roy M, Gardes M, Baseia IG, Martín MP (2019) Hidden fungal diversity from Neotropics: Geastrum hirsutum, G. schweinitzii (Basidiomycota, Geastrales) and their allies Hidden fungal diversity. PLoS ONE 14:e0211388. https://doi.org/10.1371/journal.pone.0211388

Assis NM, Freitas-Neto JF, Sousa JO, Barbosa FR, Baseia IG (2019) Geastrum hyalinum (Basidiomycota, Geastraceae), a new species from Brazilian Southern Amazon. Stud Fungi 4:83–89. https://doi.org/10.5943/sif/4/1/11

 Bölöni J, Molnár Zs, Kun A (eds) (2011) Magyarország élőhelyei. A hazai vegetációtípusok leírása és határozása. ÁNÉR 2011. MTA ÖBKı, Vác

Borchsenius F (2009) FastGap 1.2. Department of Bio-sciences, Aarhus University, Aarhus. Available at: https://www.aubot.dk/FastGap_home.htm

Cabral TS, Silva BDB, Marinho P, Baseia IG (2014a) Geastrum rusticum (Geastraceae, Basidiomycota), a new earthstar fungus in the Brazilian Atlantic rainforest—a molecular analysis. Nova Hedwigia 98:265–272. https://doi.org/10.1127/0029-5035/2013/0158

Cabral TS, Silva BDB, Ishikawa NK, Alfredo DA, Braga-Neto R, Clement CR, Baseia IG (2014b) A new species and new records of gastroid fungi (Basidiomycota) from Central Amazonia Brazil. Phytotaxa 183:239–253. https://doi.org/10.11646/phytotaxa.183.4.3

Cabral T, Sousa JO, Silva BDB, Martín MP, Clement CR, Baseia IG (2017) A remarkable new species of Geastrum with an elongated branched stipe. Mycoscience 58:344–350. https://doi.org/10.1016/myc.2017.03.004

Calonge FD, Zamora JC (2003) Geastrum arenarium, encontrado en España y nuevo para Europa. Bol Soc Micol Madrid 27:59–61

Crous PW, Wingfield MJ, Burgess TI, Hardy GEStJ, Crane C, et al (2016) Fungal Planet description sheets: 469–557. Persoonia 37:218–403. https://doi.org/10.3767/003158516X694499

Crous PW, Wingfield MJ, Burgess TI, Carnegie AJ, Hardy GEStJ, et al (2017) Fungal Planet description sheets: 625–715. Persoonia 39:270–467. https://doi.org/10.3767/persoonia.2017.39.11

Crous PW, Wingfield MJ, Burgess TI, Hardy GEStJ, Gené J, et al (2018a) Fungal Planet description sheets: 716–784. Persoonia 40:239–392. https://doi.org/10.3767/persoonia.2018.40.10

Crous PW, Luangsa-ard JJ, Wingfield MJ, Carnegie AJ, Hernández-Restrepo M et al (2018b) Fungal Planet description sheets: 785–867. Persoonia 41:238–417. https://doi.org/10.3767/persoonia.2018.41.12

Crous PW, Wingfield MJ, Lombard L, Roets F, Swart WJ et al (2019) Fungal Planet description sheets: 951–1041. Persoonia 43:223–425

Finé P, Jeppson M (2021) Noteworthy European records of the xerothermic earthstar Geastrum xerophilum from the Hungarian natural inland sand dunes. Mikol Közlem, Clusiana (in press)

Gardes M, Bruns TD (1993) ITS primers with enhanced specificity for basidiomycetes—application to the identification of mycorrhizae and ruts. Molec Ecol 2:113–118. https://doi.org/10.1111/j.1365-294X.1993.tb00005.x

He MQ, Zhao R-L, Hyde KD, Begerow D, Kemler M et al (2019) Notes, outline and divergence times of Basidiomycota. Fungal Divers 99:105–367

Hemmes DE, Desjardin DE (2011) Earthstars (Geastrum, Myriostoma) of the Hawaiian Islands including two new species, Geastrum litchiforme and Geastrum reticulatum. Pacific Sci 65:477–496

Hollos L (1901) Új Gasteromyceta-Fajok Magyarországból. Math Term Értes 19:504–512

Hollos L (1903) Magyarország Gasteromycetái. Franklin társulat, Budapest

Jeppson M (2013) Jordstjärnor – en bestämningsguide. – Mykologiska Publikationer 6. Sveriges Mykologiska Förening, Göteborg

Jeppson M, Nilsson RH, Larsson E (2013) European earthstars in Geastraceae (Geastrales, Phallomycetidae)—a systematic approach using morphology and molecular sequence data. Syst Biodivers 11:437–465. https://doi.org/10.1080/14772000.2013.857367

Katoh K, Standley DM (2013) MAFFT: multiple sequence alignment software version 7: improvements in performance and usability. Mol Biol Evol 30:772–780. https://doi.org/10.1093/molbev/ms5014

Kretzer A, Bruns TD (1999) Use of atp6 in fungal phylogenetics: an example from the Boletales. Mol Phylogen Evol 13:483–492. https://doi.org/10.1006/mpev.1999.0680

Kumar S, Stecher G, Tamura K (2016) MEGA7: Molecular evolutionary genetics analysis Version 7.0 for bigger datasets. Mol Biol Evol 33:1807–1874. https://doi.org/10.1093/molbev/msw054

Kun A, Tóth T, Szabó B, Koncz J (2005) A dolomitjelenség: közettani, talajtani és növényzeti összefüggések (kőzet-, talaj- és növényelemzések magyarországi mészők- és dolomit szigkepekre). Bot Közlem 97:1–25

Matheny PB, Liu YJ, Ammirati JF, Hall BD (2002) Using RPB1 sequences to improve phylogenetic inference among mushrooms (Inocybe, Agaricales). Amer J Bot 89:688–698. https://doi.org/10.1073/ajb.89.4.688

Nagy LG, Kocsabé S, Csana Z, Kovács GM, Petkovits T, Vágvölgyi C, Papp T (2012) Remind the gap! Insertion–deletion data reveal neglected phylogenetic potential of the nuclear ribosomal internal transcribed spacer (ITS) of fungi. PLoS ONE 7:e49794. https://doi.org/10.1371/journal.pone.0049794

Rehner SA, Buckley E (2005) A Beauveria phylogeny inferred from nuclear ITS and EFl-a sequences: evidence for cryptic diversification and links to Cordyceps telemorphs. Mycologia 97:84–98. https://doi.org/10.3852/mycologia.97.1.84

Rehner SA, Samuels GJ (1994) Taxonomy and phylogeny of Glomus analysed from nuclear large subunit ribosomal DNA sequences. Mycol Res 98:625–634. https://doi.org/10.1016/s0953-7562(98)00409-7

Rimóci I, Jeppson M, Benedek L (2011) Characteristic and rare species of Gasteromycetes in Eupannomorphic. Fungi non delineati 56–57. Edizioni Candusso, Alessio

Ronquist F, Huelsenbeck JP (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19:1572–1574. https://doi.org/10.1093/bioinformatics/btg180

Schoch CL, Seifert KA, Huhndorf S, Robert V, Spouge JL, Levesque CA, Chen W, and Fungal Barcoding Consortium (2012) Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi. Proc Natl Acad Sci USA 109:6241–6245. https://doi.org/10.1073/pnas.1117018109

Schweinitz LD (1822) Synopsis fungorum Carolinae superioris secundum observationes. Schriften Naturf Ges Leipzig 1:20–130

Silva BDB, Cabral TS, Marinho P, Ishikawa NK, Baseia IG (2013) Two new species of Geastrum (Geastraceae, Basidiomycota) found in Brazil. Nova Hedwigia 96:445–456. https://doi.org/10.1127/0029-5035/2013/0089

Silvestro D, Michalak I (2012) raxmlGUI: a graphical front-end for RAxML. Organisms Diversity Evol 12:335–337. https://doi.org/10.1007/s13127-011-0056-0

Simmons MP, Ochoterena H, Carr TG (2001) Incorporation, relative homoplasy, and effect of gap characters in sequence-based phylogenetic analysis. Syst Biol 50:454–462. https://doi.org/10.1080/10635150120427
Sousa JO, Baracho GS, Baseia IG (2015) Geastrum laevisporum: a new earthstar fungus with uncommon spores. Mycosphere 6:501–507. https://doi.org/10.5943/mycosphere/6/4/12

Sousa JO, Baracho GS, Martín MP, Baseia IG (2019) Contribution to Neotropical data of Geastrum section Corollina (Basidiomycota): Two new earth-stars from Caatinga vegetation, Brazil. Nova Hedwigia 109:161–175. https://doi.org/10.1127/nova_hedwigia/2019/0524

Staden R, Beal KF, Bonfield JK (2000) The Staden package, 1998. Meth Molec Biol 132:115–130. https://doi.org/10.1385/1-59259-192-2:115

Stamatakis A (2014) RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics 30:1312–1313. https://doi.org/10.1093/bioinformatics/btu033

Sunhede S (1989) Geastraceae (Basidiomycotina): Morphology, ecology and systematics with special emphasis on the North European species. Syn Fungorum 1:1–534

Vilgalys R, Hester M (1990) Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several Cryptococcus species. J Bacteriol 172:4238–4246. https://doi.org/10.1128/jb.172.8.4238-4246.1990

White TJ, Bruns T, Lee S, Taylor JW (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (eds) PCR protocols: a guide to methods and applications. Academic Press, New York, pp 315–322

Young ND, Healy J (2003) GapCoder automates the use of indel characters in phylogenetic analysis. BMC Bioinformatics 4:6. https://doi.org/10.1186/1471-2105-4-6

Zamora JC, Calonge FD (2007) Geastrum parvistriatum, una nueva especie encontrada en España. Bol Soc Micol Madrid 31:139–149

Zamora JC, Calonge FD, Martín MP (2014) Combining morphological and phylogenetic analyses to unravel systematics in Geastrum sect. Schmidelia. Mycologia 106:1199–1211. https://doi.org/10.3852/14-072

Zamora JC, Calonge FD, Martin MP (2015) Integrative taxonomy reveals an unexpected diversity in Geastrum section Geastrum (Geastrales, Basidiomycota). Persoonia 34:130–165. https://doi.org/10.3767/003158515X687443

Publisher’s Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.