

# Phylogeny of Graphostromatacea with Three Species Isolated in China

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## Research Article

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# Abstract

Three species possessing characteristics of *Graphostromataceae* were observed in China. Morphology of the described species with illustrations and their phylogeny based on regions of internal transcribed spacers (ITS), the second largest subunit of the RNA polymerase II (*RPB2*),  $\beta$ -tubulin (*TUB2*) and  $\alpha$ -actin (*ACT*) are provided. Two new species and one new record from China were identified. Morphologically, *Biscogniauxia glaucae* sp. nov. differs from *B. atropunctata* var. *maritima*, *B. citriformis* var. *macrospora*, *B. fuscilla* and *B. mediterranea* by its stromata with raised margins, with clear outlines, with punctate ostioles openings and ascospores which are equilateral with broadly rounded ends, with a straight germ slit on the more concave side, nearly full spore length, lacking appendages and sheathes. ITS sequence difference between *Graphostroma guizhouensis* sp. nov. and type strain of *G. platystomum* is 7%, which support identifying it as a new species. *Camillea broomeana* with scanning electron microscope description of ascospores was illustrated as a new record from China. *Cryptostroma* was proposed in *Graphostromataceae* based on molecular data. *Vivantia* was accepted in *Graphostromataceae* based on its morphological characteristics and *Nodulisporium* anamorphs which is similar with that of *Biscogniauxia*.

# Introduction

Graphostromataceae M.E. Barr, J.D. Rogers & Y.M. Ju was erected to accommodate *Graphostroma platystomum* (Schwein.) Piroz. which was first belonged to Xylariaceae (Barr et al. 1993, Pirozynski 1974). *Graphostroma platystomum* was separated from Diatrypaceae, and placed in Xylariaceae (Pirozynski 1974). Up to now, five genera, *Biscogniauxia* Kuntze, *Camillea* Fr., *Graphostroma* Piroz., *Obolarina* Pouzar, and *Vivantia* J.D. Rogers, Y.M. Ju & Cand. were listed in the family of Graphostromataceae (Wijayawardene et al. 2020).

*Graphostroma* was introduced to accommodate some fungi resembling *Diatrype stigma* (Hoffm. ex Fr.) Fr. as a monotypic genus of *Xylariaceae* (Pirozynski 1974). *Graphostroma platystomum* (Schw.) Piroz. was designated as type species. Since the features of two-layered stroma, diatrypoid configuration of short-broken ascumata, holoblastic conidiogenesis of the *Nodulisporium* anamorph, faintly amyloid apical ring of asci, *G. platystomum* was proposed to under a new family of Graphostromataceae (Barr et al. 1993). Currently, only one species was accepted in the genus of *Graphostroma*.

*Nummularia* Tul. & C. Tul. was introduced in 1863 (Tulasne and Tulasne 1863). The name, *Biscogniauxia* Kuntze was used for the sexual morph of genus (Pouzar, 1979). *Biscogniauxia* was reviewed by Ju et al. (1998), and forty-nine taxa were accepted. In their study, morphological differences between similar genera were discussed, and a key to the species of *Biscogniauxia* was provided. The morphological characteristics of *Biscogniauxia* are as follows: stromata widely effuse with separate ostioles at the surface; perithecia mostly in a layer, but sometimes polystichous; asci 8-spored, cylindrical, with or without an amyloid apical ring; ascospores uniseriate, rarely biseriate, ellipsoid, and brown, with or without germ slits (Ju et al. 1998; Vasilyeva et al. 2007).

*Camillea* was erected to include taxa with applanate, carbonaceous, cylindrical, or broadly conic-truncate, bipartite stromata with light coloured ascospores (Fries 1849). *Camillea leprieurii* (Mont.) Mont. was designated as the lectotype by Montagne (1855). Læaessøe et al. (1989) reviewed the *Camillea* and made morphological descriptions of most species. Based on phylogenetic analysis, Wendt et al. (2018) accepted it as a genus of Graphostromataceae, which was followed by Daranagama (2018).

During investigation of Xylariales from Chian, three taxa possessing features of Graphostromataceae were collected. Based on morphological and molecular data, we identified them as *Biscogniauxia glaucae*, *Graphostroma guizhouensis*, *Camillea broomeana*. Detail descriptions were given in this paper. Meanwhile, we discuss the systematic classification of Grapholomataceae and suggest that *Vivantia* and *Cryptostroma corticale* (Ellis & Everh.) P.H. Greg. & S. Waller should be included in this family.

## Materials And Methods

### Collection, isolation and morphology

Specimens with black spots on their surfer were collected from Guizhou province, China. Materials were placed into paper bags with desiccant silica gel and taken to laboratory for examinations. Specimen collection information including location and collection time was recorded. Pure cultures were isolated using single-spore isolation (Chomnunti et al. 2014). Cultures were stored in screw cap centrifuge tubes containing potato-dextrose agar (PDA) medium at 4°C and were preserved in 2 mL screw cap centrifuge tubes with 10% glycerol at - 20°C. Macroscopic characteristics were observed using Olympus SZ61 stereomicroscope and photographed with a fitted Canon 700D digital camera (Liu et al. 2018). The contents of the stomata were picked out and mounted in water and Melzer's reagent for anatomical examination. Characteristics of asci, ascospores, and ascus apical apparatus were photographed using a Nikon digital camera (700D) fitted to a microscope (Nikon Ni). The dimensions of 30 ascospores, 20 asci, and 20 ascus apical apparatus were measured with Tarosoft® image framework (v. 0.9.0.7).

Microphotographs were modified appropriately without changing features, and were arranged as a plate using Adobe Photoshop CS6. The specimens were deposited in herbarium of Guizhou medical university (GMB), herbarium of Guizhou agricultural college (GACP) and herbarium of Kunming Institute of Botany (KUN), and living cultures were deposited in Guizhou medical university culture collection (GMBC).

### DNA extraction, PCR amplification and sequencing

Pure cultures were grown on PDA at 25°C for 2 weeks. The fungal mycelia were scratched off with sterilized scalpel. Genomic DNA was extracted from fresh fungal mycelia using the BIOMIGA fungus genomic DNA extraction kit (GD2416, Biomiga, USA), following the manufacturer's instructions. DNA extractions were stored at - 20°C. Region of internal transcribed spacers (ITS) was amplified with primers ITS4 and ITS1 (White et al. 1990; Gardes and Bruns 1993). Partial  $\beta$ -tubulin (*TUB2*) was amplified with primers Bt2a and Bt2b (Glass and Donaldson 1995). The second largest subunit of the RNA polymerase II

(*RPB2*) was amplified using primers (*RPB2*-5F, -7cF, and -7cR) introduced by Liu et al. (1999). The PCR primer pair ACT-512F/ACT-783R (Carbone and Kohn 1999) was used to amplify portions of the  $\alpha$ -actin gene (*ACT*). The components of a 25  $\mu$ L volume PCR mixture were as follows: double distilled water 9.5  $\mu$ L, PCR Master Mix 12.5  $\mu$ L, 1  $\mu$ L of each primer, 1  $\mu$ L template DNA. Qualified PCR products were checked with 1.5% agarose gel electrophoresis stained with GoldenView and sent to Sangon Co., China, for sequencing. The PCR conditions were: initial denaturation step at 95°C for 2 min, 35 cycles of 95°C for 30 seconds, 52°C (*ITS*), 61°C (*TUB2*), 54°C (*RPB2*), 58°C (*ACT*) for 45 s, 72°C for 1 min, and a final extension at 72°C for 5 min.

## Sequence alignment and phylogenetic analyses

All strains for phylogenetic analyses were chosen based on Wendt et al. (2018) and top hits of ITS blasted in the GenBank database. Sequences downloaded from GenBank database for construction of phylogenetic tree are listed in supplement table. ITS, *TUB2*, *RPB2* and *ACT* sequence data were assembled respectively using the MAFFT v.7.110 online programme (<http://mafft.cbrc.jp/alignment/server/>) with the default settings. Multiple sequence alignments of ITS, *TUB2*, *RPB2* and *ACT* were combined and manually adjusted to reduced redundant gaps using BioEdit v.7.0.5.3 (Hall 1999). The maximum likelihood analyses were carried out in RAxML 7.4.2 Black Box (<https://www.phylo.org/>, Stamatakis et al. 2008). The final ML search was conducted using the GTR GAMMA + I model. All free model parameters were estimated by RAxML with ML estimates of 25 per site rate categories. The phylogenetic analyses were performed for Bayesian inference in MrBayes 3.2.7 online with default parameters (<https://www.phylo.org/>, Stamatakis et al. 2008). Markov chain Monte Carlo (MCMC) sampling in MrBayes v.3.2.2 (Ronquist et al. 2012) was used to determine the posterior probabilities (PP) (Rannala and Yang 1996). Six simultaneous Markov chains were run for 1000000 generations and trees were sampled every 1000th generation. Phylogenetic trees were viewed and arranged using FigTree v1.4.0. and edited with Microsoft PowerPoint and Adobe Photoshop CS6. The alignment was uploaded in TreeBASE ([www.treebase.org/treebase-web/home.html](http://www.treebase.org/treebase-web/home.html)) under ID 27791 for ITS-*RPB2*-*TUB2*-*ACT* alignment and tree.

## Results

### Phylogenetic analyses

Graphostromataceae taxa and closely related families were included in the phylogenetic analyses with two strains of *Hypoxylon rickii* (MUCL 53309) and *H. pulicicidum* (MUCL49879) as outgroup (Fig. 1). Datasets of ITS, *RPB2*, *ACT* and *TUB2* were aligned respectively, and were combined to form a dataset of ITS-*RPB2*-*TUB2*-*ACT*. The dataset of ITS-*RPB2*-*TUB2*-*ACT* consisted 67 taxa, including the two sequences from two outgroup taxa, and contained 2785 alignable characters, 1347 constant characters, 1248 phylogenetically informative characters, and 190 parsimony-uninformative variable characters. Final ML optimization likelihood is -43391.566516. Topology of the complete RAxML and MrBayes trees were

consistent. The tree showed that *Camillea*, *Obolarina*, *Graphostroma* and *Cryptostroma* were nested within *Biscogniauxia* with a well-supported (100/1). Sequences of *Biscogniauxia glaucae* showed a close relationship with *B. formosana* with bootstrap (100/1), and showed as a distinct branch. GMBC0218 was nested in group of *Camillea*, which showed a very close kinship with *Camillea obularia* (Fr.) Læssøe, J.D. Rogers & Lodge. *Graphostroma guizhouensis* formed a distinctly branch within the group of *Graphostroma*.

## SEM

About 5 mm sample of stromata for SEM investigations was processed by gold coating under critical point dryer (K850). Scanning electron microscopy (SEM Inspect) was carried out to examine the preparations. The images were saved and processed using Adobe Photoshop CS6.

## Taxonomy

*Biscogniauxia glaucae* Q.R. Li, J.C. Kang & K.D. Hyde, sp. nov. Figure 2

MycoBank number: MB 835896;

Etymology: In reference to the host, *Quercus glauca* Thunb..

Holotype: GMB0007

*Saprobic* on dead bark of *Q. glauca*. **Sexual morph:** *Stromata* 3–5 cm long, 1–2 cm wide and 0.3–0.4 cm high, widely effused, flat, black, bipartite, confluent, with raised margins, perithecium immersed, with black ostioles. Stromatal tissue between perithecia composed entirely of carbonaceous tissue. *Ostioles* openings punctate, discretely encased by carbonaceous stromatal tissue, equal or slightly lower than stromatal surface, black. *Perithecia* 250–350  $\mu\text{m}$  high, 130–260  $\mu\text{m}$  wide (av. = 285  $\times$  183  $\mu\text{m}$ ,  $n = 10$ ), solitary, black, carbonaceous, immersed, in vertical section, obovoid, globose to subglobose, ostiolate. *Peridium* 45–70  $\mu\text{m}$  thick, black. *Paraphyses* not observed. *Asci* 131–180  $\times$  10.5–12.5  $\mu\text{m}$  (av. = 152.5  $\times$  11.5  $\mu\text{m}$ ,  $n = 20$ ), 8-spored, unitunicate, cylindrical, short-pedicellate, apically round, with a J+, wedge apical apparatus in Melzer's reagent, 3–4  $\mu\text{m}$  high, 7.5–9.5  $\mu\text{m}$  wide. *Ascospores* 13.5–16  $\times$  7.5–9.5  $\mu\text{m}$  (av. = 15  $\times$  8.5  $\mu\text{m}$ ,  $n = 30$ ), uniseriate, unicellular, dark brown to black, more or less equilateral with broadly rounded ends, smooth-walled, with a straight germ slit on the more concave side, nearly full spore length, lacking appendages and sheathes. **Asexual morph:** undetermined.

Culture characteristics: Colonies growing on PDA, reached 5 cm diam. after one week at 25 °C, white, cottony, flat, low, dense, fructifications were not observed in culture.

Material examined: CHINA, Guizhou province, Guiyang city, Forest Park of Guiyang, on dead bark of *Q. glauca*, March, 2014, Q.R. Li, GZ72, (GMB0007, holotype, exatype living culture GMBC0007; GACP2014QR58, isotype),.

Other material examined: CHINA, Guizhou province, Guiyang city, Huaxi Park, on dead bark of unknown plant, June, 2014, Q.R. Li, GZH96 (GMB0029, living culture GMBC0029; GACP2014QR79)..

## Note

There were four species collected from China in the examined specimens by Ju et al. (1998). Most of them were obtained and published in Taiwan (Ju and Rogers 2001; Kuo et al. 2018). Currently, nine species were collected and introduced from China (Ju and Rogers 2001; Vasilyeva et al. 2009; Ariyawansa et al. 2015; Ma et al. 2020). Here we introduce a new species of *Biscogniauxia* found from China mainland. Molecular phylogenetic studies based on *ITS*,  $\beta$ -tubulin, *RPB2* and *ACT* sequences indicated that *B. glaucae* form a distinct branch from other species and supported *B. glaucae* as a new species of *Biscogniauxia*. In morphology, this species has a similar ascospores size range to *B. atropunctata* var. *maritima* (Lar. N. Vassiljeva) Y.M. Ju & J.D. Rogers, *B. citriformis* var. *macrospora* Van der Gucht & Whalley, *B. fuscella* (Rehm) F. San Martín & J.D. Rogers and *B. mediterranea* (De Not.) Kuntze. However, stromata of *B. glaucae* have clear raised margins, with clear outlines which differ from those of them (Ju et al. 1998; Ju and Rogers 2001). Moreover, *B. fuscella* has white outer dehiscing layer and nearly equilateral ascospores with a cellular appendage on immature ascospores and some mature ascospores (Ju et al. 1998). *Biscogniauxia atropunctata* var. *maritime* shows different characteristics in having ostioles of slightly higher than the stromatal surface, with openings papillate and ascospores with narrowly rounded ends (Ju et al. 1998; Ju and Rogers 2001). Ascospores of *B. citriformis* var. *macrospora* are almost C-shaped with pinched ends, with spore-length on more convex side (Ju et al. 1998). *Biscogniauxia mediterranea* can be readily separated from the *B. glaucae* in having ostioles higher than stromatal surface, with openings coarsely papillate (Ju et al. 1998). The phylogenetic tree (Fig. 2) shows that *B. glaucae*, *B. formosana* and *B. cylindrispora* have a close relationship. However, *B. formosana* ( $15\text{--}22.5 \times 8\text{--}11 \mu\text{m}$ ) and *B. cylindrispora* ( $20\text{--}26 \times 10\text{--}14 \mu\text{m}$ ) has larger ascospores than those of *B. glaucae*.

*Camillea broomeana* (Berk. & M.A. Curtis) Læssøe, J.D. Rogers & Whalley, Mycol. Res. 93(2): 149 (1989) Fig. 3

Mycobank number: MB 135957;

Saprobic on the surface of stem of deciduous plant, *Cerasus* sp.. **Sexual morph:** Stromata widely effused, irregular in outline, black, erumpent through bark, edges raised, up to 4 cm length, up to 3 mm thick, first covered with an ectostromatal and host tissue layer which is readily lost. *Ectostroma* brown to black, crust, easily separated without clear ostioles. *Entostroma* carbonaceous, black, with a layer of ostioles on the surface, perithecia embedded. *Ostioles* indistinct papillate, central. *Perithecia*  $295\text{--}380 \times 195\text{--}264 \mu\text{m}$  (av. =  $320 \times 245 \mu\text{m}$ ), ovoid to cylindrical. *Paraphyses* abundant, persistent, smooth,  $3 \mu\text{m}$  wide, slightly longer than asci. *Asci*  $144.5\text{--}190.5 \times 7.5\text{--}15.5 \mu\text{m}$  (av. =  $167 \times 10 \mu\text{m}$ , n = 30), 8-spored, unitunicate, cylindrical, rounded at apex, with a J+, jar-shape apical apparatus in Melzer's reagent,  $2\text{--}4 \mu\text{m}$  high,  $3\text{--}5 \mu\text{m}$  wide. *Ascospores*  $10\text{--}15 \times 4.5\text{--}6.5 \mu\text{m}$  (av. =  $13 \times 5.5 \mu\text{m}$ , n = 30), one-celled, uniseriate,

dilute brown, ellipsoid, mostly 1-guttulate, rounded ends, with rough warty ornamentation, without a germ slit. Asexual morph: Not observed on the surface of stromata and PDA medium.

Material examined: CHINA, Hainan province, Wuzhishan city, Emerald Park, (18°48′46.17″N, 109°30′45.54″E), on stem of *Cerasus* sp., November, 2020, Q.R. Li, 2020FCGY11, altitude: 475 m (GMB0218, exatype living culture GMBC0218; HKAS 112698).

## Note

There are two species of *Camillea* with echinulate ascospores, *Camillea broomeiana* and *C. signata* (Jong & Benjamin) Læaessøe, J. D. Rogers & Whalley. *Camillea signata* differs from *C. broomeiana* by its annulate ostioles (Læaessøe et al. 1989). Most species of *Camillea* have been discovered from in the Neotropics and the largest concentration of species occurs in the Amazon region (Læaessøe et al. 1989). No *Camillea* species by far was reported from China mainland. This is the first discovery of *Camillea* species in China mainland.

*Graphostroma guizhouensis* **sp. nov.** Q.R. Li Fig. 4

Mycobank number: MB XXX;

Etymology: In reference to the collection location.

Holotype: GMB0219

Saprobic on the surface of small branches of unknown deciduous plant. **Sexual morph:** *Stromata* black, widely effused, irregular in outline, black, up to 3 cm length, containing two layers. *Ectostroma* brown to black, crust, carbonaceous, easily separated, with cracks, without clear ostioles. *Entostroma* cracked, with a layer of ostioles on the surface, perithecia embedded. *Ostioles* crater-like, central. *Perithecia* 285–322 × 175–234  $\mu\text{m}$  (av. = 302 × 198  $\mu\text{m}$ ), bottle-shaped, pyriform with a neck, with hyaline wall. *Paraphyses* not observed. *Asci* 25–39 × 3.5–6  $\mu\text{m}$  (av. = 31.5 × 5  $\mu\text{m}$ ), 8-spored, unitunicate, clavate, short-stalked, rounded at apex, with a non-amyloid ring in the apical apparatus. *Ascospores* 6–7.5 × 1–1.5  $\mu\text{m}$  (av. = 6.8 × 1.4  $\mu\text{m}$ ), one-celled, colorless, reniform, equilateral, rounded ends, without germ slit. **Asexual morph:** Not observed on the surface of stromata and PDA medium.

Examined specimen: CHINA, Guizhou Province; Guiyang city, Aha lake (26°36′50.21″N, 106°40′15.78″E), on dead wood, August, 2020, collector: Sihan Long, 2020AH23, altitude: 1108 m (GMB0219, holotype, exatype living culture GMBC0219; HKAS 112684, isotype).

Additional examined specimen: CHINA, Guizhou Province; Guiyang, Aha lake (26°32′50.37″N, 106°40′16.65″E), on dead wood, August, 2020, collector: Yinhui Pi, 2020AH28, Altitude: 1090 m (GMB0008, living culture GMBC0008).

## Note

Here we would like to propose a new species of *Graphostroma*, although *Graphostroma guizhouensis* shows similar features with *G. platystomum*. A comparison of the ITS region DNA sequence data between *G. guizhouensis* and *G. platystomum* (CBS 270.87, type material of *G. platystomum*) revealed base pair differences of 7% which supports establishment of *G. guizhouensis* as a new taxon. We speculate that *Graphostroma platystomum* should be a group containing some cryptic species. Their morphological characteristics are very similar, but their DNA sequences are quite different. And DNA sequences should be a main diagnostic feature for the genus of *Graphostroma*.

## Discussion

*Nummularia* was introduced in 1863 to accommodate the fungi which have flattened disc-shaped stromata reminiscent of coins. *Obolarina*, *Camillea* and *Biscogniauxia* all possess *Nummularia*-like anamorphs. *Biscogniauxia* was adopted for the genus (Pouzar 1979). *Obolarina* was separated from *Biscogniauxia* with *Obolarina dryophila* (Tul. & C. Tul.) Pouzar (= *Nummularia dryophila* Tul. & C. Tul.) as the type species (Pouzar 1986). *Obolarina dryophila* possesses spiral germination slits of rather large spores which differ from those of *Biscogniauxia*. *Obolarina* and *Biscogniauxia* have almost the same morphological characteristics, such as the morphology of the stromata, asci, ascospores. Ju et al. (1998) reviewed the *Biscogniauxia* and presented a key that contained genera with close affinities. They figured out that the main difference between *Obolarina* and *Biscogniauxia* is that *Obolarina* lacks an ascapical ring. *Camillea* is characterized by dark brown or black and carbonaceous bipartite stromata, asci with blue apical rings in Melzer's iodine reagent and hyaline or light brown ornamented ascospores lacking a germ slit (Læaessøe et al. 1989). These characteristics of *Camillea* can clearly distinguish it from *Obolarina* and *Biscogniauxia*.

The anamorphic fungi *Cryptostroma corticale* introduced as type species of *Cryptostroma* can cause sooty bark disease of *Acer pseudoplatanus* L. (Gregory et al. 1951). Stroma was described as two layers of "floor stroma" and "roof stroma" by Gregory et al. (1951). Pirozynski (1974) established a new genus, *Graphostroma* to accommodate *G. platystomum*, and placed it among Xylariaceae. Molecular systematic studies indicated the close affinity of *Graphostroma* and *Biscogniauxia* (Smith et al. 2003). Based on ITS rDNA sequences, Koukol et al. (2015) treated *C. corticale* as a Hypoxyloideae clade of Xylariaceae which closed to *G. platystomum* and *Biscogniauxia*. In this study, we get the similar result. *Cryptostroma* was listed in Xylariales genera incertae sedis (Wijayawardene et al. 2020). Here, we propose to place *Cryptostroma* in Graphostromataceae.

*Vivantia* was accepted in the Amphisphaeriaceae to include species with features of bipartite, carbonaceous, applano-pulvinate stromata, cylindrical asci with apical ring bluing in Melzer's reagent and two-celled, subhyaline ascospores without germ slit (Rogers et al. 1996). Two-layers, carbonaceous stromata and *Nodulisporium* anamorphs indicate its affinity with *Biscogniauxia*. *Vivantia* was listed in the family of Graphostromataceae without any evidences (Wijayawardene et al. 2020). In this paper we provides evidences and would like accept *Cryptostroma* and *Vivantia* in Graphostromataceae, based on



their common features, bipartite stromata, asci with apical ring bluing in Melzer's reagent, light-colored ascospores, *Nodulisporium* or *Xylocladium* like anamorphs.

Molecular phylogenetic analysis indicated that species of Graphostromataceae form monotypic family with high value (100/1), and *Biscogniauxia* is not a monotypic genus. *Biscogniauxia* was divided into 8 clades. *Obolarina*, *Camillea* and *Graphostroma* clustered in the branch of *Biscogniauxia*, have close relationship with *Biscogniauxia*. *Obolarina*, *Camillea* and *Graphostroma* form a monophyletic group respectively. *Biscogniauxia mediterranea* and *B. rosacearum* (clade 1) indicate a close relationship with *Obolarina*. Nevertheless, *Biscogniauxia mediterranea* have an apical ring of ascus bluing in Melzer's iodine reagent and dark brown ascospores (Ju et al. 1998). *Biscogniauxia rosacearum* was published as an endophytic fungus without description of sexual morph. *Biscogniauxia granmoi* and *B. marginata* (clade 6) have close genetic relationship with *Cryptostroma* and *Graphostroma*. However, the ascospores of *B. granmoi* and *B. marginata* are brown which distinct from those of *Cryptostroma* and *Graphostroma* (Ju et al. 1998, Læssøe et al. 1999). In terms of the morphological characteristics of ascospores, the differences between *Camillea*, *Graphostroma*, *Cryptostroma* and *Biscogniauxia* are very obvious. However, phylogeny showed that *Camillea*, *Graphostroma*, and *Cryptostroma* were in the branch of *Biscogniauxia*. We speculate that the morphological diagnostic features currently used are insufficient for the systematic classification of *Biscogniauxia* and its related genera. *Biscogniauxia* may be divided into some genera to maintain the uniformity of genus characteristics. Compared with the published morphological species, there are relatively few species with available DNA sequences of *Biscogniauxia* and *Camillea*, which leads to the confusion of kinship of *Camillea*, *Obolarina* and *Cryptostroma*. We will not make arbitrary divisions of this genus for the time being. We look forward to more available DNA sequences or new diagnostic features to solve the systematic classification problem of *Biscogniauxia*.

## Declarations

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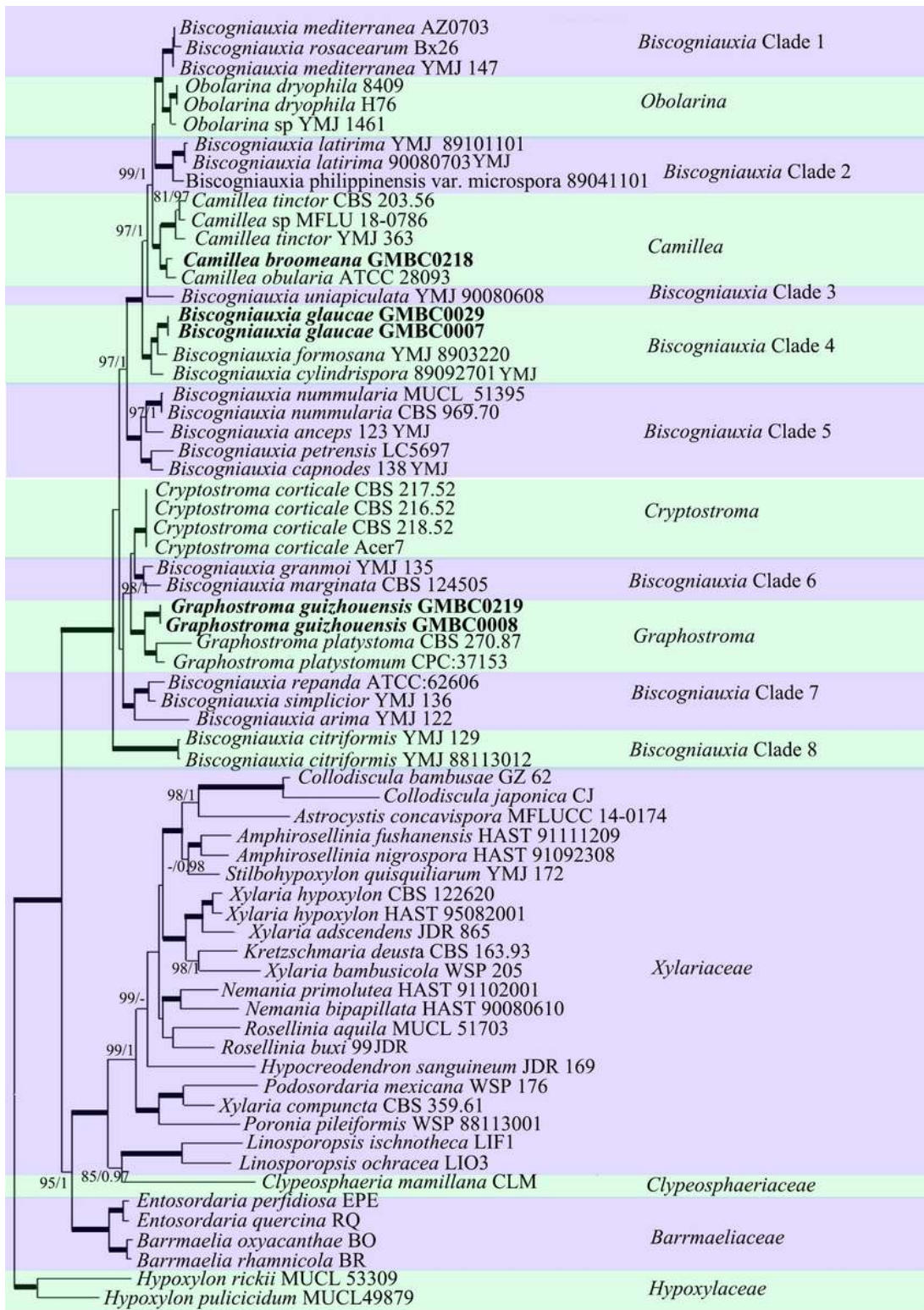
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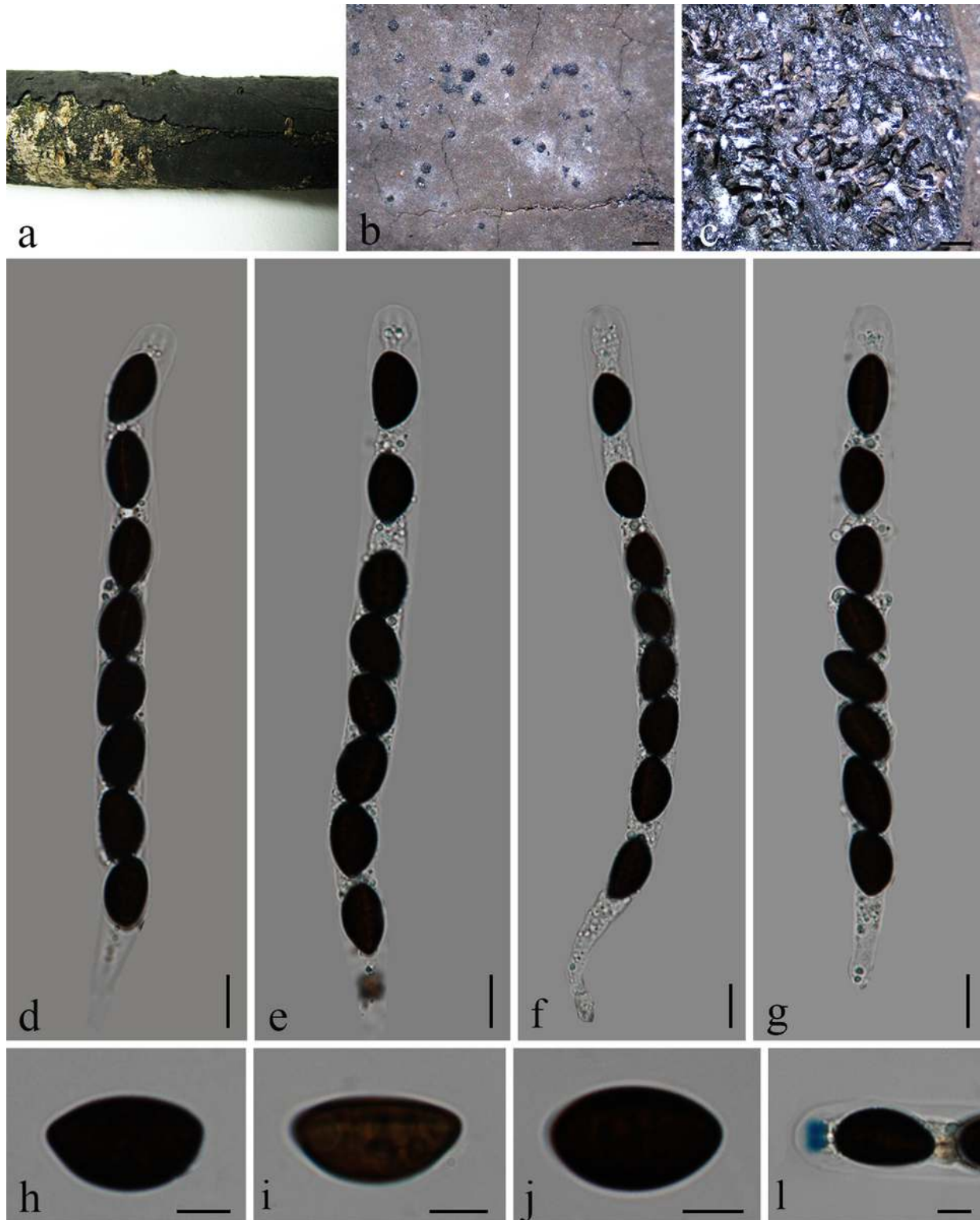
## Figures



**Figure 1**

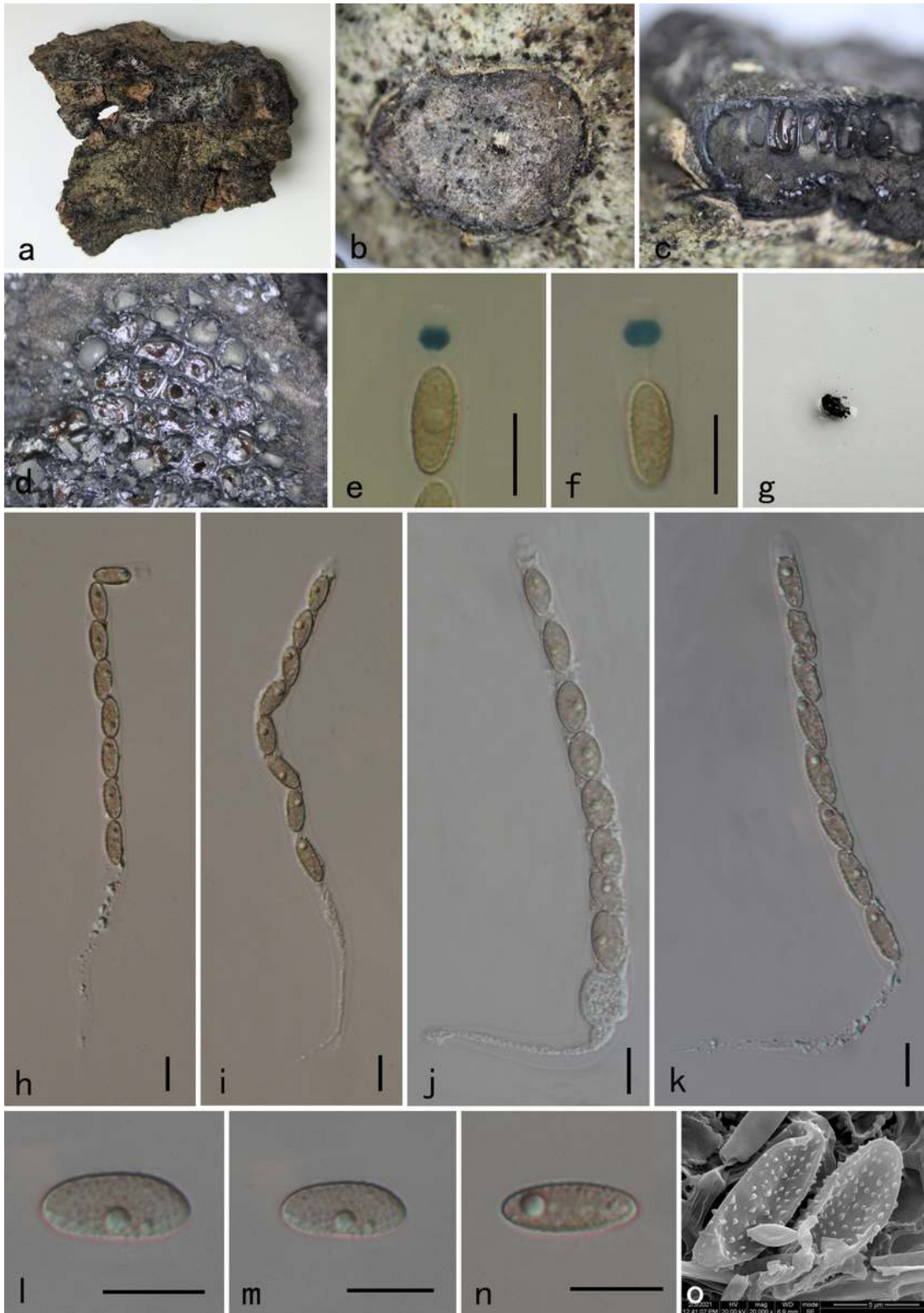
Phylogeny of Graphostromataceae and its allies obtained from a Maximum Likelihood analysis of the combines of ITS, RPB2, TUB2 and ACT using RAxML-HPC BlackBox software online. *Hypoxylon rickii* (MUCL 53309) and *H. pulvicidum* (MUCL49879) were taken as outgroup. Strains numbers were followed by their names. Bayesian posterior probabilities >0.95 and bootstrap support values for maximum

likelihood (ML) higher than >75% are marked above the nodes; an en-dash ("-") indicates a value <0.95 (PP) or <75% (BS). The bold branches indicate the support values are 100/1.



**Figure 2**

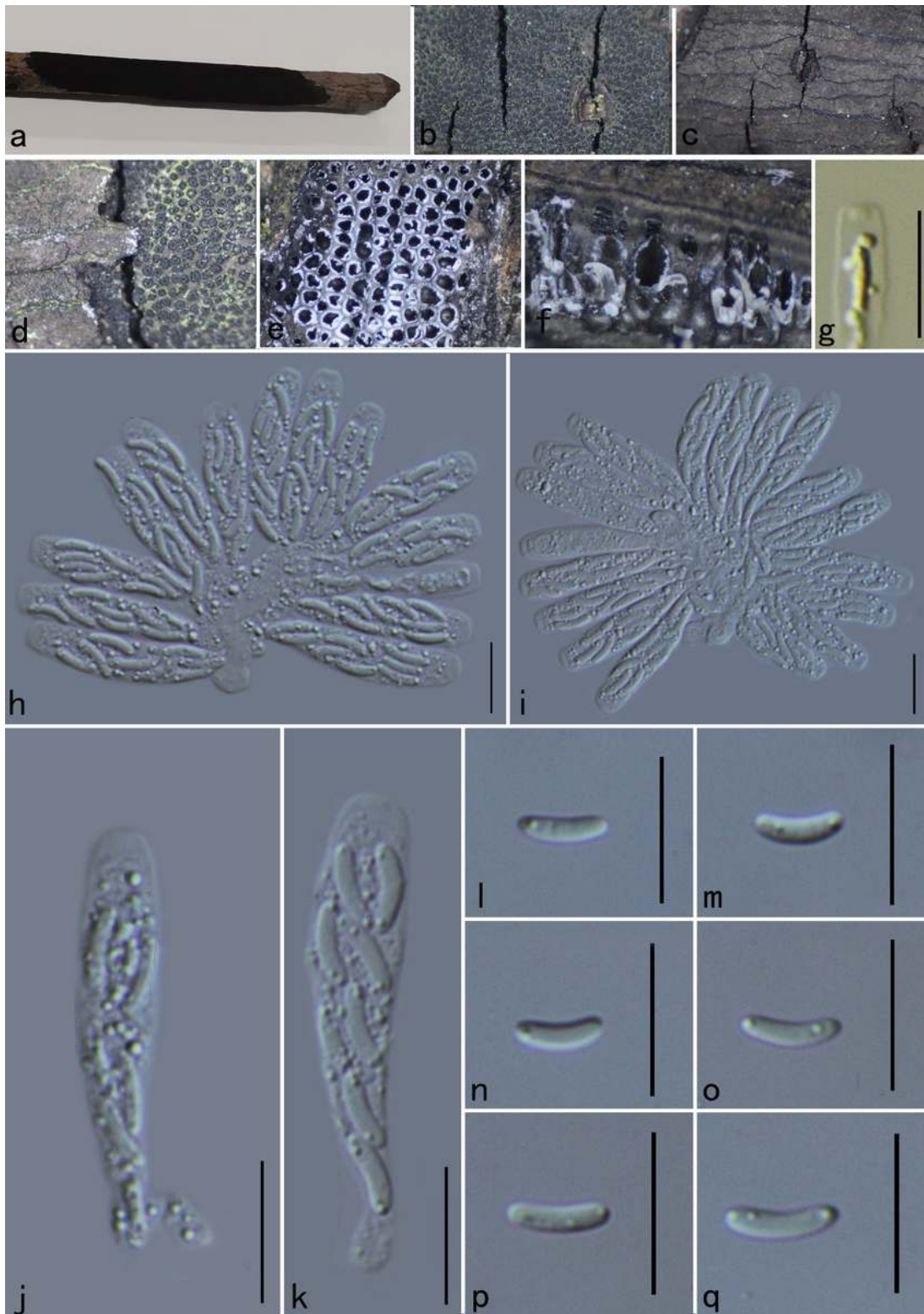
*Biscogniauxia glaucae* (GMB0007, holotype) a Material. b Ascomata on the surface of host. c Section of ascomata. d–j Asci with ascospores (stained in Melzer's reagent). h–j Ascospores. l Ascus apex with a J+ apical apparatus. Scale bars: b, c=200  $\mu$ m, d–g=10  $\mu$ m, h–l=5  $\mu$ m



**Figure 3**

*Camillea broomeiana* (GMB0218). a Material. b the stromata on stem surface of *Cerasus yedoensis*. c longitudinal section of ascomata. d Cross-section of ascomata. e,f Ascus apex with a J+ apical apparatus (stained in Melzer's reagent). g Fragments of stromata in KOH without pigments. h–k Asci with ascospores l–o Ascospores with one guttulate and rough warty ornamentation e. Scale bars: e,f =10  $\mu$ m, h–n=10  $\mu$ m.





**Figure 4**

*Graphostroma guizhouensis*. (GMBC0219, holotype) a Material. b the inner layer of stromata c the outer layer of stromata. d the stromata on the surface of unknown plant. e Cross-section of asci (inner layer of stromata). f Longitudinal section of asci (inner layer of stromata) g Ascus apex with a non-amyloid ring in Melzer's reagent. h–k Asci with ascospores. l–q Ascospores. l Scale bars: g–k = 10  $\mu$ m

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