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A new species and a new record of the lichen family Graphidaceae from China

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Abstract

Based on morphological, chemical and molecular data, a new species, *Platygramme guizhouensis* is described to science, and *Phaeographis decipiens* is reported as new record to China. *Platygramme guizhouensis* features lirelliform apothecia with discs open, black, epruinose, proper exciple apically carbonized, wedge-shaped. Asci cylindrical to clavate, 8-spored. Ascospores brown, elongate-ellipsoid, 9–12 transverse septa, I+ violet-purple. Detailed descriptions and illustrations of the new species and the new record are provided.

Key words: Lichenized fungi, Graphidales, Morphology, Phylogeny, Taxonomy

Introduction

Graphidaceae (Graphidales) is one of the largest groups of crustose lichens and has a wide distribution in tropical regions (Kirk *et al.* 2008; Lumbsch *et al.* 2007; Mangold *et al.* 2008; Wijayawardene *et al*. 2021). Staiger (2002) published a monograph on the family Graphidaceae, revising the spore-based generic system originally established by Müller Arg. (1880a, 1880b, 1882). Several genera were reintroduced with this revised concept, including *Platygramme* Fée (1874: 29) and *Phaeographis* Fée (1882: 36). *Platygramme,* originally described by Fée (1874), is a genus predominantly distributed in tropical and subtropical regions. It is characterized by lirelliform ascomata, proper exciple that is carbonized either apically or laterally and often wedge-shaped, inspersed hymenium, and greyish to pale brown ascospores that are transversely septate or muriform. Currently, 31 species of the genus *Platygramme* are recognized worldwide (Archer & Elix 2007; Cáceres *et al*. 2014; Jia & Lücking 2017a, b; Kalb & Kalb 2017; Lüecking *et a*l. 2011; Mtro *et al*. 2021; Poengsungnoen *et al*. 2014; Seavey *et al*. 2014; Seavey *et al*. 2017; Sutjaritturakan *et al*. 2014; Tripp *et al*. 2010; Jia *et al*. 2013). *Phaeographis* was established by Müller Arg (1882). It is characterized by brown, I+ wine-red ascospores, generally inspersed hymenia; poorly developed and uncarbonized excipula; and lirellae with exposed discs (Archer 2006; Cáceres 2007; Lücking *et al.* 2008; Staiger 2002). This genus comprises approximately 230 species worldwide, 27 of which have been recorded in China (Jia & Lücking 2017a, b; Jia *et al.* 2016; Li *et al.* 2014; Wang *et al.* 2013; Chen *et al*. 2023).

Materials & Methods

Sample collection & Morphological observations

The specimens were collected from Kuankuoshui Nature Reserve and Congjiang Yueliangshan Nature Reserve in Guizhou Province, China, and are preserved in the Lichen Herbarium Kunming Institute of Botany (KUN-L), Chinese

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Academy of Sciences, Yunnan, China. The micromorphology was examined using a stereomicroscope (OLYMPUS SZX16) and a compound microscope (OLYMPUS DP72). Photographs were taken after measurement of asci and ascospores using ImageJ software v. 1.50d. The sizes are given as (min–) ($\bar{x}$ – SD) – ($\bar{x}$ + SD) (–max), where 'min' and 'max' are the extreme values observed, $\bar{x}$ is the arithmetic mean and SD is the corresponding standard deviation (Zhurbenko *et al.* 2024).

Chemical component analyses

The color reactions on thallus and medulla were complished using 10% potassium hydroxide (KOH, K), saturated sodium hypochlorite solution (NaClO, C), and 95% ethyl alcohol (P). Sections were stained with Lugol's solution (I). Lichen substances were identified by thin-layer chromatography (TLC) employing solvent system C (toluene/acetic acid = 17/3) (Orange *et al.* 2001).

DNA extraction, PCR amplification & sequencing

Genomic DNA was extracted directly from apothecia using a fungal genomic DNA extraction kit (Solarbio, China). The primer pairs mrSSU1/mrSSU3R and LR0R/LR5 were used to amplify sequences of mitochondrial small subunit rRNA (mtSSU) and the nuclear large subunit of rDNA (nrLSU) (Kraichak *et al.* 2015, Vilgalys *et al.* 1990). Polymerase Chain Reaction (PCR) was performed using a Mastercycler (Bio-RAD T-100, Singapore) for 25-µL reactions with 12.5 µL of 2 × dNTPs Mix (Solarbio, China), 8 µL of double-distilled water (ddH_2O), 1.0 µL for each 10 mM primer, and 2.5 µL of DNA template. The PCR conditions were as follows: (1) for mtSSU, initial denaturation at 95 ℃ for 5 min, denaturation at 94 ℃ for 45 s, annealing at 50 ℃ for 60 s, extension at 72 ℃ for 90 s, followed by 38 cycles, and a final extension at 72 ℃ for 10 min, then hold at 4 ℃/+∞. (2) for nrLSU, the other steps are the same as (1) mtSSU, except for the annealing step, which is at 55 ℃ for 60 s (Kraichak *et al.* 2015). PCR products were detected by 1% agarose gel electrophoresis and sequenced by Tsingke Biotech Co., Ltd. (Chongqing, China).

Phylogenetic analyses

The quality of sequence was checked by chromatograms using BioEdit 7.0.9.0. Forward and reverse sequences were assembled using ContigExpress 6.0.620.0. The sequences were subjected to BLASTn searches (https://blast.ncbi.nlm.nih.gov/Blast.cgi) to confirm their affiliation and identify potential sample contamination. Newly generated sequences were submitted to GenBank (Table 1). Additional sequences used for the inner group were downloaded from the NCBI website (https://www.ncbi.nlm.nih.gov), while taxon *Diorygma poitaei* (Fée) Kalb, Staiger & Elix (2004: 164), was selected as the outgroup (Medeiros *et al.* 2022). Phylogenetic analyses were conducted using the OFPT program (Zeng *et al.* 2023) following its protocol: Each gene region's dataset was initially aligned using the 'auto' strategy (based on data size) in MAFFT (Katoh *et al.* 2013) and subsequently trimmed using the 'gappyout' method (based on gap distribution) in TrimAl (Capella-Gutiérrez *et al.* 2009). Single gene phylogenetic trees were constructed using IQTREE Web Server (http://iqtree.cibiv.univie.ac.at/) to confirm well-supported branches. The best-fit nucleotide substitution models for each dataset were selected based on the Bayesian Information Criterion (BIC) from twenty-two common DNA substitution models with rate heterogeneity by ModelFinder (Kalyaanamoorthy *et al.* 2017). All datasets were concatenated with partition information for subsequent phylogenetic analyses. Maximum likelihood analysis with 1000 replicates was performed using ultrafast bootstrap approximation (Hoang *et al.* 2018) combined with the SH-like approximate likelihood ratio test (SH-aLRT) (Guindon *et al.* 2010) in IQ-TREE (Nguyen *et al.* 2015). The consensus tree was summarized based on the extended majority rule. Maximum likelihood analysis was also conducted with the RAxML-HPC2 on ACCESS (8.2.12) employing the GTRGAMMA approximation with a rapid bootstrap analysis of 1000 replicates (Miller *et al.* 2010, Stamatakis 2014). Bayesian inference was performed using MrBayes (Ronquist *et al.* 2012), with two parallel Metropolis-coupled Markov Chain Monte Carlo runs (one 'cold' chain and three heated chains), sampling trees every 1000 generations. The run was automatically stopped when the average standard deviation of split frequencies fell below 0.01, and the resulting tree was summarized after discarding the first 25% samples. The resulting trees were visualized in FigTree v1.4.4. The new taxon was registered in Index Fungorum (2025) and Faces of Fungi database (Jayasiri *et al.* 2015).

TABLE 1. Species name, voucher/culture and their GenBank accession numbers used in this study. The newly generated sequences are shown in black bold. The absence of GenBank accession numbers is shown by 'NA'.

Species name	Voucher/Culture	Location	GenBank accession numbers	
			nrLSU	mtSSU
Phaeographis asteroides	Common 9113J	USA	NA	JX421245
P. alcicornis	Lücking 24118	Thailand	NA	JX421242
P. alcicornis	Common 9117	USA	NA	JX421243
P. alcicornis	Common 9128	USA	NA	JX421244
P. brasiliensis	Kalb 33154	Dominican Republic	AY640022	DQ431958
P. caesioradians	Kalb 33917	Australia	AY640021	DQ431968
P. decipiens	Lücking 29118	Bolivia	NA	JX421246
P. decipiens	**KUN-L 0093703**	**China**	**PQ565565**	**PO512826**
P. elliptica	CBS:130777	USA	MH878104	KC592285
P. flavescens	Common 9095	USA	NA	JX421250
P. flavescens	Common 9076	USA	NA	JX421252
P. intricans	Kalb 38864	Thailand	JX421602	JX421254
P. intricans	Kalb 38883	Thailand	JX421601	JX421253
P. leiogrammodes	Common 9111C	USA	NA	JX421258
P. leiogrammodes	Common 9084C	USA	NA	JX421257
P. lobata	Berger 19598	Bermuda	DQ431944	DQ431984
P. lobata	Mangold 30a	Australia	NA	JX421259
P. lobata	Lumbsch 19636	Mexico	JX421603	JX421260
P. platycarpa	Lücking 31094a	Brazil	NA	JX421266
P. platycarpa	Mangold 30za	Australia	JX421604	JX421263
P.subfulgurata	Common 9118A	USA	NA	JX421281
P. schizoloma	Common 9118D	USA	NA	JX421272
P. schizoloma	Common 9118B	USA	NA	JX421271
P. spondaica	Lumbsch 19633	Mexico	JX421609	JX421280
P. spondaica	Lumbsch 19634	Mexico	JX421608	JX421279
P. spondaica	Lücking RLD 071	Mexico	DQ431943	DQ431983
Platygramme australiensis	Kalb 33930	Australia	AY640024	DQ431970
Pl.caesiopruinosa	Lumbsch 19636b	Mexico	JX421611	HQ639599
Pl.caesiopruinosa	Common 9118I	USA	NA	JX421287
Pl.caesiopruinosa	Common 9084F	USA	NA	JX421286
Pl.caesiopruinosa	Common 9067	USA	NA	JX421284
Pl. guizhouensis	**KUN-L 0093702**	**China**	**PQ565564**	**PQ535887**
Pl. impudica	Rivas Plata 1022D	Philippines	JX421613	JX421289
Pl. impudica	Rivas Plata 1021C	Philippines	JX421612	JX421288
Pl. impudica	Kalb 38818	Thailand	NA	JX421290
Pl. praestans	Common 9129	USA	NA	JX421291
Pl. pachnodes	LaGreca 2659	USA	ON507246	ON507270
Pl. pachyspora	Jia YN222493	China	OQ676636	OQ676625
Pl. pachyspora	Jia YN222445	China	OQ676635	NA
Diorygma poitaei	Lücking 28538	Nicaragua	HQ639627	HQ639596

Result

Phylogenetic analyses

The final dataset comprised 41 taxa with 1,192 aligned characters, including gaps (nrLSU: 1–809bp; mtSSU: 810–1192bp). The RAxML tree was constructed with a final ML optimization likelihood value of -4805.595667. The parameters for the GTR+I+G model of combined nrLSU and mtSSU were as follows: estimated base frequencies: A = 0.30, C = 0.17, G = 0.27, T = 0.26, substitution rate AC = 0.42, AG = 4.10, AT = 2.39, CG = 0.87, CT = 10.78 and GT=1.00. Bayesian posterior probabilities from MCMC analysis showed a final average standard deviation of split frequencies = 0.009999. The topologies from both ML and Bayesian analyses were verified manually and largely concurred (Fig. 1). The phylogenetic tree indicates that *Phaeographis* is polyphyletic, with the new collection Liu & Yang Y323 closely related to *P. decipiens* Müll. Arg. (1887: 26), supported by a high value (97/1.00). The new species, *Platygramme guizhouensis* (Fu & Zheng KKS7), is sister to *Pl. impudica* (A.W. Archer) A.W. Archer (2005: 77) and *Pl. pachnodes* Fée (1874: 30), with strong support.

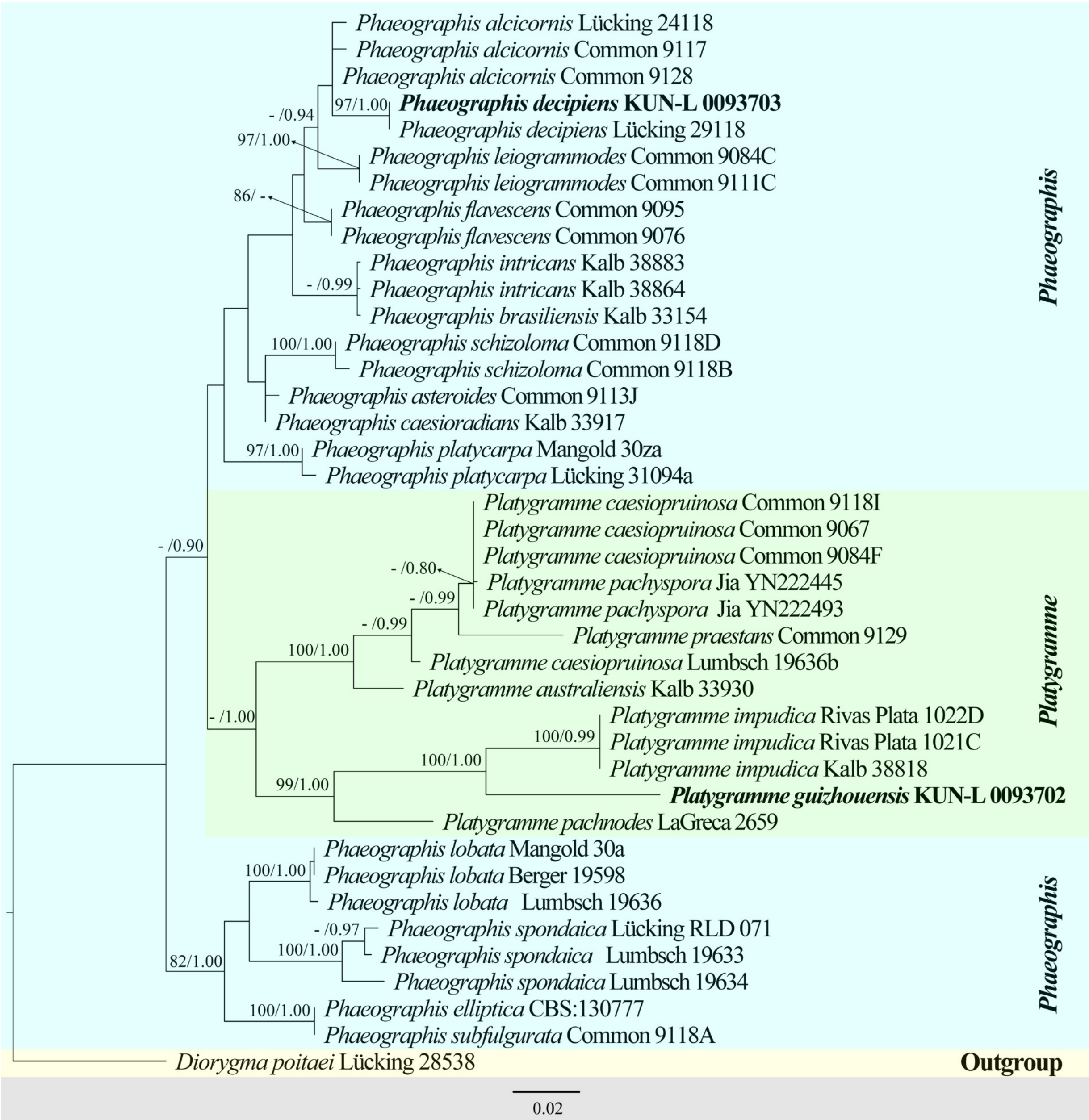


FIGURE 1. RAxML analysis based on combined nrLSU and mtSSU sequence data. Bootstrap support values for maximum likelihood (MLBS ≥ 75%), and the Bayesian Posterior Probabilities (BIPP ≥ 0.90) are shown near the nodes as BS/PP. *Diorygma poitaei* (Lücking 28538) was used as outgroup taxa. The newly generated sequence is shown in bold.

Taxonomy

Platygramme guizhouensis Wei Wu, Shu H. Jiang & S.B. Fu, *sp. nov.*

Differing from *Platygramme coccinea* in the absence of stictic acid and its chemical composition.

Index Fungorum number: IF903029; Facesoffungi number: FoF 16965; Fig. 2

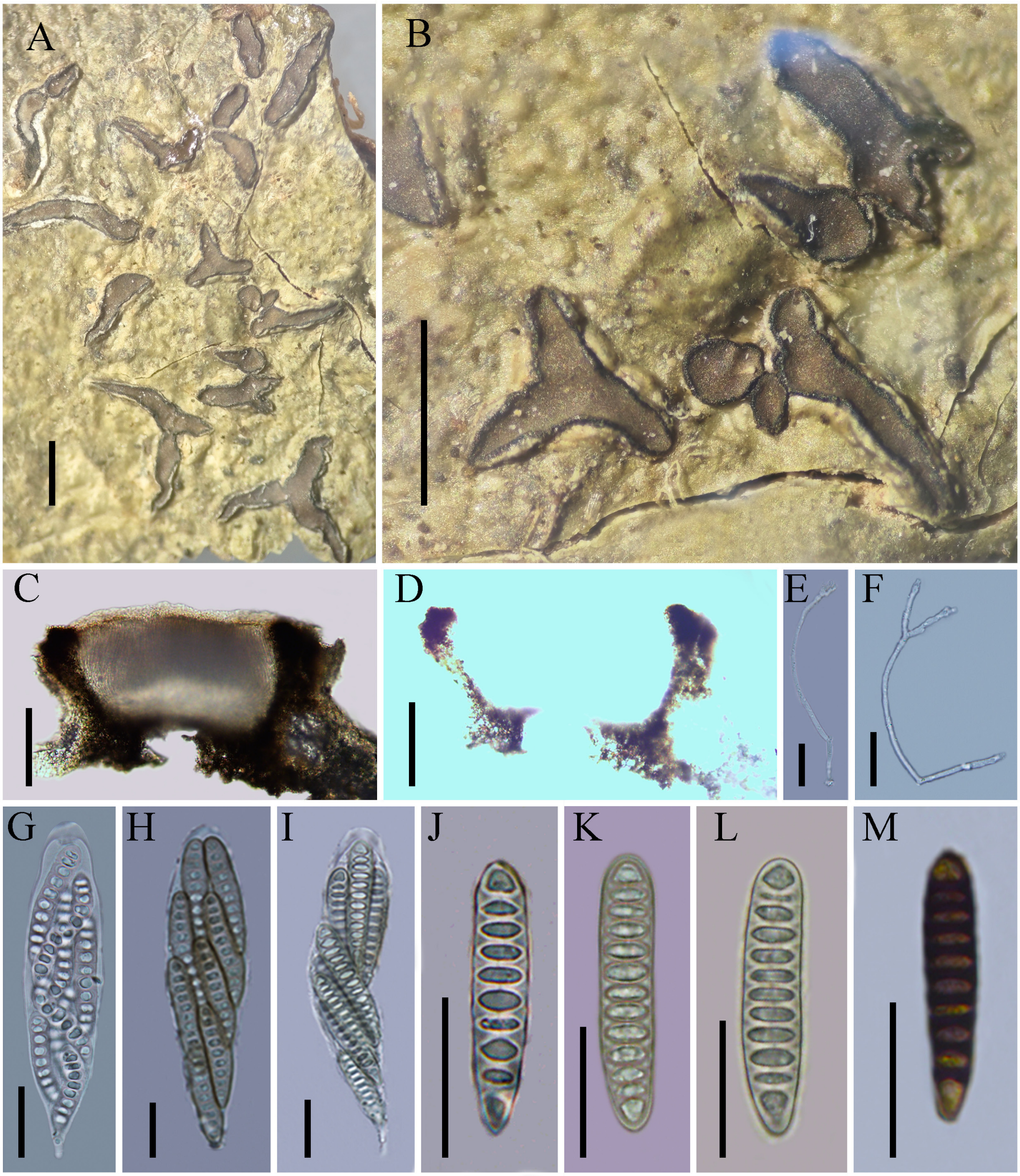


FIGURE 2. *Platygramme guizhouensis* (KUN-L 0093702: holotype). A–B. Thallus with ascomata. C. Cross section of apothecium. D. Cross section of apothecium (Under strong light). E–F. Paraphyses. G–I. Ascus. J–L. Ascospores (in water). M. Ascospore (in IKI). Scale bars: A–B = 1 mm, C–D = 100 µm, E–M = 20 µm

Etymology: The specific epithet "*guizhouensis*" refers to the locality where the holotype was collected.

Description: Thallus corticolous, crustose, thin, tightly attached to the substratum, weakly greyish-green to yellow-green, rough, dull, lacking isidia and soredia, prothallus absent. Apothecia lirelliform, scattered or aggregated, erumpent, simple or irregularly branched, curved, and either terminally rounded or acute, measuring 0.6–5 mm long, and 0.3–0.6 mm wide. Disc plane or slightly concave, epruinose. Proper exciple apically carbonized, wedge-shaped. Epihymenium pale yellow-orange, 16–25 µm high. Hymenium pale orange, inspersed, 170–210 µm high, I–. Paraphyses simple or branched, aseptate, 1.5–2.5 µm wide. Hypothecium weakly brown, 10–20 µm high. Asci cylindrical to clavate, 8-spored, 95–128 × 20–30 µm. Ascospores brown, elongate ellipsoid, transversely septate, 9–12-septate, (18–)27–46(–48) × (5.5–)6.5–9.5(–10) µm ($\bar{x}$= 36.5 × 7.9 µm, n = 20), I+ violet-purple. Pycnidia not observed.

Chemistry: Thallus K+ yellow, C–, KC+ yellow, P–, TLC: stictic acid.

Material examined: China, Guizhou Province, Zunyi City, Suiyang County, Kuankuoshui Nature Reserve, 28°14'0.60″N, 107°09'45.66″E, alt. 1561 m, 16 November 2023, *Shao-Bin Fu & Wei-Wei Zheng* KKS7 (KUN-L 0093702, holotype).

Distribution and habitat: Known only from the type locality, Kuankuoshui Nature Reserve Guizhou, China. The new species grew on the bark at alt. 1561 m.

Notes: The new species is characterized by lirelliform apothecia with a plane or slightly concave, epruinose disc, proper exciple apically carbonized, wedge-shaped. Asci cylindrical to clavate, 8-spored, brown ascospores with transversely septa (9–12-septate), and sized 27–46 × 6.5–9.5 µm. Morphologically, the new species resembles *Platygramme coccinea* F. Seavey & J. Seavey (2014: 399), but it differs in chemical composition. The new species containing stictic acid while *P. coccinea* lacks lichen substance (Frederick *et al.* 2014). It is also similar to *Pl. australiensis* Staiger & Matthes (2002: 355) and *Pl. muelleri* (A.W. Archer) Staiger (2002: 364), however, the ascospores of the latter two similar species are muriform while the ascospores are transversely septate in the new species. Moreover, the similar species have no lichen substances while the new species contains stictic acid (Kalb & Kalb 2017; Jia *et al*. 2013).

Phylogenetic analysis of combined mtSSU and nrLSU sequence data indicates that the species is most closely related to *Platygramme impudica* A.W. Archer. Morphologically, *Pl. guizhouensis* possesses an open disc but *Pl. impudica* has closed one. The asci of new species contain eight spores but one spore in asci for *Pl. impudic*a. Besides, the ascospores in *Pl. guizhouensis* are exclusively transversely septate nevertheless they are muriform in *Pl. impudica*. According to the TLC result, the new species contains stictic acid, whereas *Pl. impudica* lacks lichen substances (Archer 2005).

Phaeographis decipiens Müll. Arg., Mémoires de la Société de Physique et d'Histoire Naturelle de Genève 29 (8): 26 (1887) Fig. 3

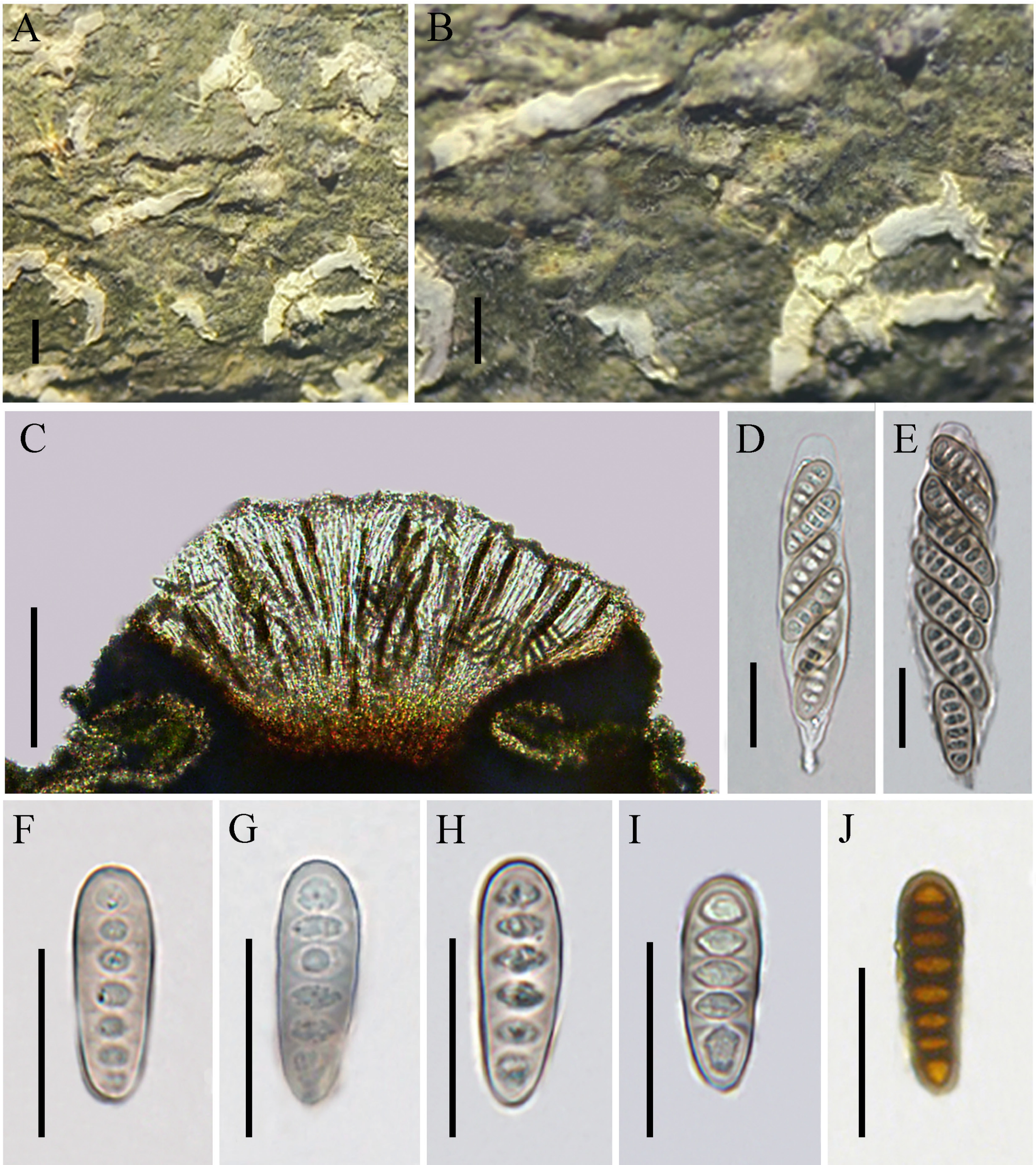
Description: Thallus corticolous, crustose, surface light olive green, smooth, waxy or slightly shiny. Apothecia lirelliform, prominent, slightly curved, irregularly branched, 1–6 mm long, 0.2–0.6 mm wide. Disc flat, with white pruina. Exciple thin, weakly and irregularly carbonized. Epihymenium brown, 13–20 µm high. Hymenium hyaline, inspersed, 180–300 µm high, I–. Paraphyses hyaline, with an inflated tip. Hypothecium brown, 30–47 µm high. Asci cylindrical to clavate, 8-spored, 43–74 × 9–18 µm, with a slight thickening of the ascal wall at the apex. Ascospores brown, with transverse septa, 5–8 locular, (12–)18–27(–32) × (5–)7–9.5(–11) µm ($\bar{x}$= 22.4 × 8.2 µm, n = 20), I+ reddish-brown. Pycnidia not observed.

Chemistry: Thallus K+ pale yellow, C–, KC–, P–, TLC: stictic acid.

Material examined: China, Guizhou Province, Congjiang County, Yueliangshan Nature Reserve, 25°24'33.87″N, 108°34'46.59″E, alt. 973 m, 18 October 2023, *Bo Liu & Ze Yang* Y323 (KUN-L 0093703).

Notes: The species is characterized by lirelliform apothecia, a plane disc covered with white pruina, a thin exciple and weakly to irregularly carbonized exciple, cylindrical to clavate asci that are 8-spored. Ascospores, brown, transversely septa (5–8 locules), sized 18–27 × 7–9.5 µm.

Phylogenetic analysis indicates this species forming a well-supported singleton clade (BS = 100, PP = 0.97) with *P. decipiens* (Lücking 29118) clearly distinguishes from other members of the genus *Phaeographis*. Morphologically, it is similar to *P. platycarpa* Müll. Arg. (1894: 284), but differs in color reaction of ascospores; *P. platycarpa* shows an I+ blue-purple reaction (vs. I+ reddish-brown here). In terms of chemical composition, *P. platycarpa* contains norstictic acid, while this collection has stictic acid (Müller Arg 1894). It is also similar to *P. heterochroides* Zahlbr. (1930: 53), although the latter has a disc with gray-brown pruina and larger ascospores (25–30 × 7.5–10 µm vs. 18–27 × 7–9.5 µm) (Zahlbruckner 1930).



FIGTURE 3. *Phaeographis decipiens* (KUN-L 0093703). A–B. Thallus with ascomata. C. Cross section of apothecium. D–E. Ascus. F–I. Ascospores (in water). J. Ascospore (in IKI). Scale bars: A–B = 1 mm, C = 100 µm, D–J = 20 µm.

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