

## Article

# *Diorygma tiantaiense* sp. nov. and a Checklist and Key to *Diorygma* Species from China

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**Abstract:** A new species *Diorygma tiantaiense* Z.F. Jia, sp. nov. was found, which is characterized by a corticolous thallus with norstictic acid, oval or oblong apothecia, immersed to  $\pm$  raised discs with white pruina, and large muriform ascospores ( $120\text{--}210 \times 35\text{--}60 \mu\text{m}$ ). Detailed morphological descriptions, photographs, and a comparison and discussion of similar species are provided. A checklist and key to the species of *Diorygma* known from China is presented.

**Keywords:** lichenized fungi; lecanoromycetes; graphidales; graphidaceae; taxonomy

## 1. Introduction

The lichenized fungi genus *Diorygma* was established by Eschweiler (1824) [1], and *D. hieroglyphicum* (Pers.) Staiger & Kalb was chosen as the lectotype for the genus by Staiger (2002) [2]. Kalb et al. (2004) described the monograph of 24 species known in the world of the genus [3]. Feuerstein et al. (2014) described three new species and a revised world key which contained 52 species [4]. Subsequently, another species was described from tropical to subtropical distribution [5–9]. Wimalasena et al. (2022) described the genus to include 77 species [10]. The total has now increased to 85 [11–13]. The genus *Diorygma* belongs to Graphidaceae, Graphidales, Ostropomycetidae, and Lecanoromycetes, usually on bark [3,10,14,15]. *Diorygma* is characterized by a crustose, off-white to pale olive-green thallus; lirelliform to irregularly rounded ascomata with a pruinose disc; exciple uncarbonized or sometimes carbonized; hymenium hyaline, not interspersed, branched or anastomosing paraphyses with a thick gelatinous wall; asci clavate, 1–8-spored; ascospores hyaline (rarely brownish), transversely septate to mostly muriform; substances including norstictic or stictic acid or the protocetraric acid complex [3,6,11].

Ten species of the genus *Diorygma* from China were reported: *Diorygma erythrellum* [ $\equiv$  *Graphina erythrellum*], *D. fuscum*, *D. hieroglyphicum*, *D. hololeucum*, *D. isabellinum* [ $\equiv$  *Graphina isabellina*], *D. junghuhnii* [= *Graphina mendax*], *D. macgregorii*, *D. megasporum*, *D. pruinatum*, and *D. soozanum* [ $\equiv$  *Graphina soozana*] [3,6,7,16–23]. Here, we describe a new species to science collected in Zhejiang Province, China, for which we propose the name *Diorygma tiantaiense*. We provide a working key and the descriptions and known distribution of the Chinese species.

## 2. Materials and Methods

### 2.1. Specimens and Morphology

The specimens were collected from Zhejiang, Hunan, Sichuan, Guizhou, Guangdong, Guangxi, Yunnan, Fujian and Hainan provinces, China, and deposited in the Fungarium of College of Life Sciences, Liaocheng University (LCUF), the Herbarium Mycologicum Academiae Sinicae-Lichenes (HMAS-L), and the Botanical Herbarium, Shandong Normal University (SDNU). The collected time and location of the specimens are described for each species in the part of taxonomy. A dissecting microscope (Olympus SZX16) was used to observe the structure of apothecia, and compound microscope (Olympus BX53)



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for microscopic characters. Measurements of apothecia, exciples, paraphysis, asci, and ascospores were obtained from mature vertical sections of fruit bodies mounted in water.

## 2.2. Chemistry

Ascospores were tested by using Lugol's solution (1% iodine solution). Spot tests were performed on the thallus surface (10% KOH, saturated aqueous NaOCl, and saturated p-phenylenediamine in ethanol). The lichen substances were detected and identified by thin-layer chromatography (TLC), using solvent C [24–26].

## 2.3. DNA Extraction and PCR Sequencing

Genomic DNA was extracted from ascomata of the specimens by using the Hi-DNA-secure Plant Kit (Tiangen, Beijing, China) according to the manufacturer's protocol. The nuLSU region was amplified using the primer pair AL2R/LR6 [27]. Reactions were carried out in a 50 µL reaction system containing 2 µL of primer solution, 2 µL of genomic DNA, 19 µL of ddH<sub>2</sub>O, and 25 µL of 2×Taq PCR Master Mix (Tiangen, Beijing, China). PCR conditions were as follows: initial denaturation for 5 min at 94 °C and 35 cycles of 94 °C for 30 s, 52 °C for 30 s, followed by an extension at 72 °C for 90 s, and a final extension at 72 °C for 10 min. The target products of PCR were purified and sequenced at Biosune Biotechnology Company (Jinan, China).

## 2.4. Phylogenetic Analysis

The newly generated sequence of the new species, *Diorygma tiantaiense*, was submitted to GenBank. Nineteen related sequences for phylogenetic tree construction were downloaded from GenBank and *Trapelia placodioides* Spribille Bjoerk 09 was selected as the outgroup (Table 1). The alignment was undertaken by applying Geneious 9.0.2 with the option of MUSCLE Phylogenetic relationships to be inferred using maximum likelihood (ML) and Bayesian inference (BI) on the CIPRES Scientific gateway portal (<http://www.phylo.org/portal2/> (accessed on 3 February 2024) [28]. ML was performed using RAxML-HPC BlackBox v. 8.2.12 [29], with a GTRGAMMA model and bootstrap statistics calculated from 1000 bootstrap replicates. For BI analysis, jModelTest 2.1.6 [30] was used to determine the best-fit model. Based on the result, we used the GTR+I+G model for nuLSU. BI analysis was performed using MrBayes on XSEDE (3.2.7a) [31] on CIPRES with 2 independent runs, searching for 10,000,000 generations. Each run included four independent chains and sampling every 1000 generations. After discarding the burn-in, the remaining 75% were used to calculate the consensus tree [29,31–34]. Bootstrap support above 70% and posterior probabilities above 0.95 were considered significant support values. The generated phylogenetic tree was visualized under FigTree v.1.4.3.

**Table 1.** Specimen and sequences used in the phylogenetic analysis. The newly generated sequence is shown in bold.

Species	Specimen	nuLSU
<i>Diorygma antillarum</i>	MPN322	JX046465
<i>Diorygma hieroglyphicum</i>	Wirth 26647	AY640015
<i>Diorygma junghuhnii</i>	Lumbsch 205391	JX421474
<i>Diorygma junghuhnii</i>	Kalb 33937	AY640018
<i>Diorygma karnatakense</i>	CRG668RATO14	OP235520
<i>Diorygma karnatakense</i>	CRG668RATO12	OP235518
<i>Diorygma karnatakense</i>	CRG668RATO10	OP235517
<i>Diorygma karnatakense</i>	CRG668RATM05	OP235516
<i>Diorygma karnatakense</i>	CRG668RATO13	OP235519
<i>Diorygma poitaei</i>	DNA3210	HQ639627

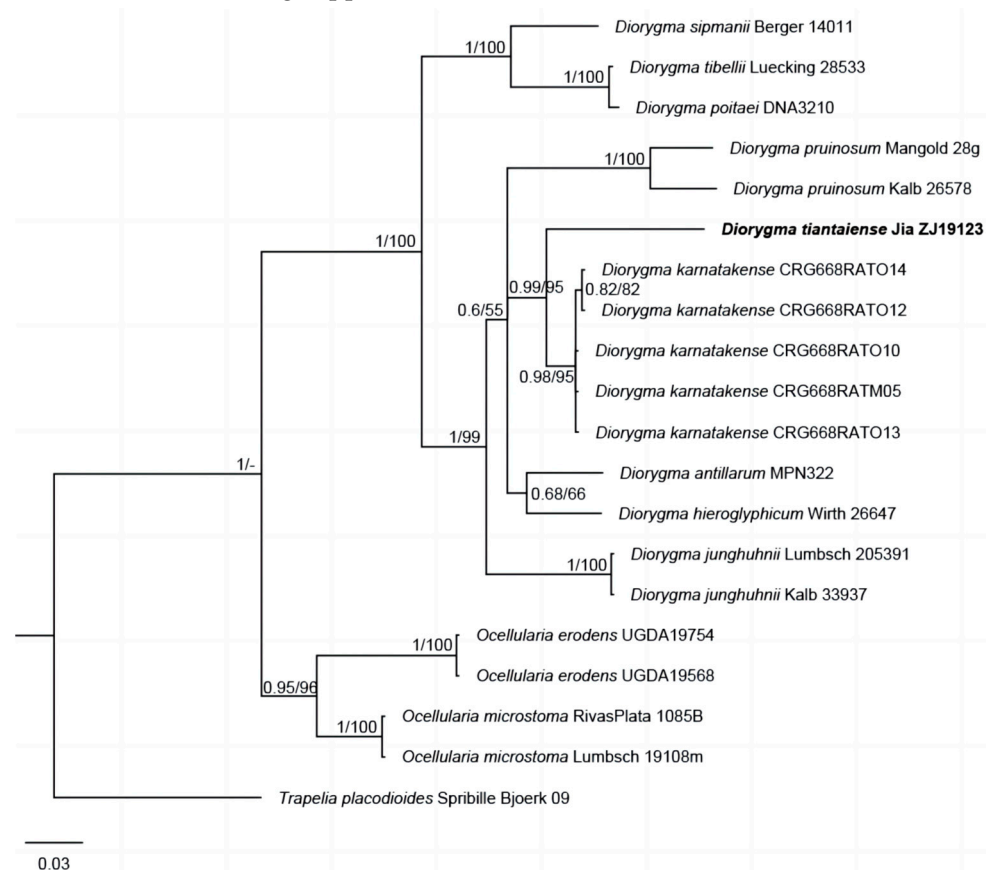
Table 1. Cont.

Species	Specimen	nuLSU
<i>Diorygma pruinorum</i>	Kalb 26578	AY640014
<i>Diorygma pruinorum</i>	Mangold 28g	JX421476
<i>Diorygma sipmanii</i>	Berger 14011	AY640020
<i>Diorygma tiantaiense</i>	Jia ZJ19123	<b>MW750692</b>
<i>Diorygma tibellii</i>	Luecking 28533	JX421475
<i>Ocellularia erodens</i>	UGDA19568	MK542902
<i>Ocellularia erodens</i>	UGDA19754	MK542900
<i>Ocellularia microstoma</i>	Lumbsch 19108m	JX421575
<i>Ocellularia microstoma</i>	RivasPlata 1085B	JX421577
<i>Trapelia placodioides</i>	Spribille Bjoerk 09	MH627046

### 3. Results and Discussion

#### 3.1. Phylogenetic Results

The phylogenetic trees obtained from maximum likelihood (ML) and Bayesian Inference (BI) exhibited similar topologies; we therefore present only the BI tree (Figure 1). The phylogenetic analyses strongly supported the two clades of *Diorygma* and *Ocellularia* (PP = 1). The nuLSU sequences supports that our specimen belongs to the *Diorygma* genus, and *D. tiantaiense* is shown as sister to the clade consisting of *D. karnatakense* B. O. Sharma & Khadilkar with strong support (PP = 0.99; ML = 95%).



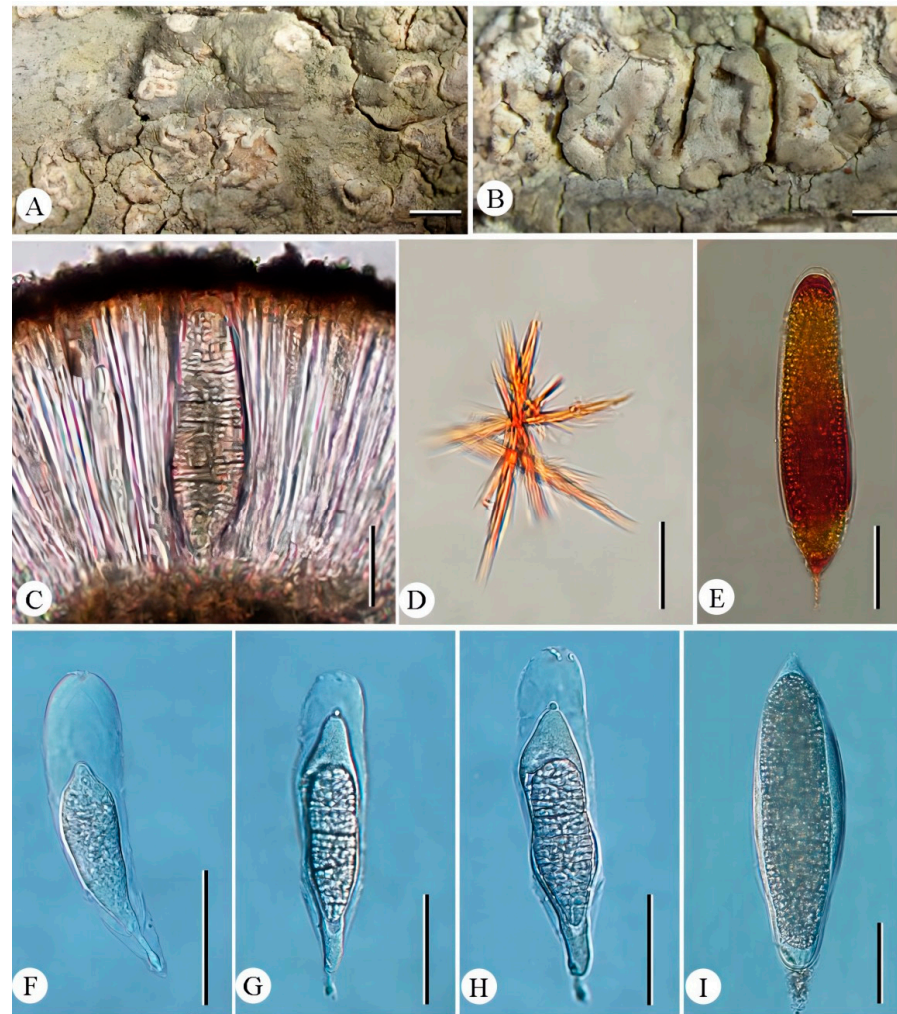
**Figure 1.** The ML phylogeny inferred from nuLSU sequences of *Diorygma tiantaiense* and closely related species (with *Trapelia placodioides* Spribille Bjoerk 09 as outgroup). The evolutionary tree was inferred by using the Bayesian method based on the GTR+I+G model. Bootstrap support values are given on the side of nodes. BI-PP > 0.95 (left) and ML-BS > 70% (right) are considered to be strongly supported. The newly generated sequence is in bold.

### 3.2. Taxonomy

Eleven species of *Diorygma* from China are reported in this paper, and one of them is new to science. *D. pachygraphum* (Nyl.) Kalb, Staiger & Elix [ $\equiv$  *Graphis pachygrapha* Nyl., Acta Soc. Sci. Fenn. 7: 472, 1863.], reported from China [21–23], is excluded in the present paper because of the synonym, the current name is *Allographa pachygrapha* (Nyl.) Lücking & Kalb in Mycobank. *D. poitaei* (Fée) Kalb et al. was reported in China as *Graphina* cf. *virginea* (Eschw.) Müll. Arg. [35]. However, after consulting the literature and examining photos, we found that its appearance is not like *D. poitaei*, which is different from the previously reported of Kalb et al. in 2004. In addition, the number and size of its ascospores are also different from the latter, which have (4–6) 6–8/ascus, ascospores 40–65  $\times$  10–18  $\mu$ m [3]. Therefore, it is not found in China now, and we correct it here. A working key to the Chinese species is provided. The descriptions, microphotographs, and discussions for the species new to China are also given.

#### Key to the species of *Diorygma* known from China

- |  |                          |
|--|--------------------------|
| 1a. Asci 2–8-spored.....   | 2                        |
| 1b. Asci 1-spored .....  | 5                        |
| 2a. Ascospores < 70 $\mu$ m long.....  | 3                        |
| 2b. Ascospores $\geq$ 70 $\mu$ m long.....   | 4                        |
| 3a. Mainly norstictic acid present, connorstictic acid present, stictic acid absent or seldom; lirellae $\pm$ raised, disc with a thin white pruina; ascospores hyaline, 10–12/2–4 locular, 40–53 $\times$ 12–15 $\mu$ m .....                               | <i>D. erythrellum</i>    |
| 3b. Mainly stictic acid present, constictic, hypostictic, and hypoconstictic acids absent or seldom; lirellae immersed to $\pm$ raised, disc with a thick white pruina; ascospores hyaline to brownish, 10–14/3–4 locular, 40–60 $\times$ 12–18 $\mu$ m..... | <i>D. fuscum</i>         |
| 4a. Protocetraric acid present; lirellae raised or adnate, disc open, with a thick white pruina; spores 2–4(–6 or –8)/ascus, 25–40/5–8 locular, 120–200 $\times$ 30–40 $\mu$ m.....  | <i>D. hololeucum</i>     |
| 4b. Protocetraric acid absent, stictic, $\alpha$ -acetylconstictic and constictic acids present; lirellae immersed, disc narrow to slightly open, with a thick greyish pruina; spores 2–6/ascus, 30–60/7–12 locular, 80–200 $\times$ 20–50 $\mu$ m.....      | <i>D. megasporum</i>     |
| 5a. Stictic and norstictic acids absent, protocetraric acid present; disc open, with a thick white pruina; ascospores 25–40/6–10 locular, 90–170 $\times$ 20–50 $\mu$ m .....  | <i>D. pruinusum</i>      |
| 5b. Stictic and (or) norstictic acids present .....  | 6                        |
| 6a. Stictic, norstictic, constictic, and cryptostictic acids present; lirellae immersed, disc narrow to open, with a whitish or yellowish pruina; ascospores 20–30/6–10-locular, 80–150 $\times$ 20–40 $\mu$ m .....   | <i>D. hieroglyphicum</i> |
| 6b. Mainly norstictic acid present, connorstictic and stictic acids absent or seldom .....   | 7                        |
| 7a. Lirellae immersed when older, disc open, with a greyish to brownish pruina; ascospores 25–30/6–10 locular, 70–125 $\times$ 20–40 $\mu$ m .....   | <i>D. junghuhnii</i>     |
| 7b. Lirellae raised or $\pm$ raised when older.....  | 8                        |
| 8a. Ascospores 110–120 $\times$ 35–48 $\mu$ m; labia obvious, disc narrow when mature; hymenium I–.....  | <i>D. isabellinum</i>    |
| 8b. Ascospores $\geq$ 120 $\mu$ m long.....  | 9                        |
| 9a. Norstictic, connorstictic, cryptostictic and stictic acids present; disc distinctly open, with a white or greyish pruina; ascospores 135–180 $\times$ 35–50 $\mu$ m.....   | <i>D. macgregorii</i>    |
| 9b. Cryptostictic and stictic acids absent, norstictic and (or) connorstictic acids present... ..  | 10                       |
| 10a. Lirellae $\pm$ raised when mature, disc wide; ascospores 120–210 $\times$ 35–60 $\mu$ m, I– .....   | <i>D. tiantaiense</i>    |
| 10b. Lirellae prominently raised when mature, disc narrow to open; ascospores 110–140 $\times$ 35–45 $\mu$ m, I+ violet blue .....   | <i>D. soozanum</i>       |

*Diorygma tiantaiense* Z.F. Jia, sp. nov. (Figure 2).

**Figure 2.** *Diorygma tiantaiense* (holotype, ZJ19123): (A,B) thallus with ascocarps; (C) apothecium section; (D) norstictic acid crystals by using 10% potassium hydroxide; (E) ascus in Lugol's solution; (F–I) asci in various developmental stages. Scale bars: (A) = 2 mm; (B) = 0.5 mm; (C) = 50  $\mu$ m; (D) = 20  $\mu$ m; (E–I) = 50  $\mu$ m.

**Mycobank:** MB 852800

**Diagnosis:** Differs from *Diorygma karnatakense* by its ascocarps  $\pm$  raised when mature, whitish to greyish disc, divergent exciple, ascospores 1/ascus, I–, and the absence of salazinic acid.

**Type:** China, Zhejiang Province, Tiantai County, Huading National Forest Park, on bark of *Rhododendron simsii* Planch., 29°15'16" N, 121°05'30" E, alt. 950 m, 27/IV/2019, Z.F. Jia ZJ19123 (Holotype, LCUF; GenBank MW750692 for LSU).

**Etymology:** The species epithet refers to the locality of holotype.

**Description:** Thallus corticolous, crustose, pale grey to greenishgrey, 100–130  $\mu$ m thick, surface uneven to slightly rugose, without soralia or isidia; algal layer 40–60  $\mu$ m thick; medulla poorly developed. Ascocarps numerous, oval or oblong, flexuous and branched, immersed in the thallus when young, becoming open and  $\pm$  raised when mature, whitish to greyish, rounded at the ends, 0.5–3  $\times$  0.4–2 mm; disc surrounded by entire raised thalline margins, open, rarely convex, with thick and white pruina, sometimes sparse; exciple divergent, laterally uncarbonized, basally and laterally brownish, consisting of a weakly and irregularly or brownish hyphal tissue intermingled with parts of the substrate; hymenium 150–220  $\mu$ m high, not inspersed, I–; epithecium usually distinctly developed, consisting

of intermingled anastomosing, hyaline or brownish paraphysis tips with short  $\pm$  globular cells, hyaline granules, and dead hyphae; paraphyses 1–2  $\mu\text{m}$  diam., with a gelatinous wall, often anastomosing, especially in the upper part of the hymenium and near the asci, sometimes branched at the tip. Ascospores 1/ascus, hyaline, muriform, dense spore locules of equal size, 120–210  $\times$  35–60  $\mu\text{m}$ , I–, with thin halo.

**Chemistry:** K+ red, P+ yellow to red; norstictic acid.

**Ecology and distribution:** This species is distributed in a subtropical forest in the southeast of China, growing on bark. The type location is in China.

**Additional specimen examined:** China. Zhejiang Province, Tiantai County, Huading National Forest Park, on bark of *Rhododendron simsii*, 29°15'16" N, 121°05'30" E, alt. 950 m, 27/IV/2019, Z.F. Jia ZJ19124, ZJ19125 (LCUF).

**Discussions:** *Diorygma tiantaiense* is characterized by the oval or oblong apothecia, the large muriform ascospores (20–210  $\times$  35–60  $\mu\text{m}$ ) and the presence of norstictic acid only. The species is shown as sister to the clade consisting of *D. karnatakense*, but different in the latter having concealed and brownish black disc, convergent exciple, KI+ in hymenium, ascospores 1–8/ascus, longer ascospores (75–220  $\times$  18.5–51.5  $\mu\text{m}$ ), I+ blue violet, and the presence of salazinic acids [36]. Morphologically, it resembles *D. africanum* Kalb, Staiger & Elix, *D. reniforme* (Fée) Kalb, Staiger & Elix, *D. salvadoriense* Kalb, Staiger & Elix, and *D. soozanum* (Zahlbr.) M. Nakan. & Kashiw. in ascocarps, but is distinguished from those species by *D. africanum* having protocetraric acid and the absence of norstictic acid, *D. reniforme* having larger ascospores (110–230  $\times$  35–80  $\mu\text{m}$ ) and the presence of norstictic, protocetraric, and salazinic acids, *D. salvadoriense* having wider ascospores (150–200  $\times$  50–75  $\mu\text{m}$ ) and the presence of norstictic and salazinic acids, *D. soozanum* having thick white pruinose discs and the presence of norstictic and connorstictic acids [3].

*Diorygma erythrellum* (Mont. & Bosch) Kalb, Staiger & Elix, Symb. Bot. Upsal. 34(1): 150, 2004.  $\equiv$  *Ustalia erythrella* Mont. & Bosch, in Junghuhn, Pl. Jungh. 4: 478, 1856.  $\equiv$  *Graphina erythrella* (Mont. & Bosch) Zahlbr. Cat. Lich. Univers. 2: 405 1923. Thallus crustose, greenish grey; surface smooth; pseudocortex well developed. Ascocarps lirellae, 0.5–5  $\times$  0.3–0.5 mm,  $\pm$  raised, sometimes branched, isabelline. Disc narrow to open, with a thin white pruina; Exciple divergent, uncarbonized, brown. Hymenium clear, 120–150  $\mu\text{m}$  high. Ascospores 8/ascus, hyaline, muriform, spore locules of equal size, 10–12/2–4 locular, 40–53  $\times$  12–15  $\mu\text{m}$ .

**Chemistry:** Norstictic acid, connorstictic acid, stictic acid (TLC).

**Ecology and distribution:** This species is a pantropical species, which is distributed in tropical to subtropical forests, growing on bark. Known from China, Java, the Philippines, Australia, New Caledonia, Thailand, and Sumatra [3].

**Specimens:** China. Fujian Province: Wuyishan City, Mt. Wuyishan, alt. 540 m, 27/V/2007, Z.F. Jia FJ457 (LCUF). Hainan Province: Qiongzong County, Mt. Wuzhishan, alt. 700 m, 21/VII/2009, J. Li HN09077 (LCUF). **Discussions:** This species is similar to *Diorygma fuscum* Jian Li bis & Z.F. Jia, but differs in the latter having opened discs with a thick and white pruina, and stictic acid as major chemistry, with norstictic acid absent [6]. The size of ascospores in specimens from China is smaller than the previously reported of Kalb et al. in 2004 [3]. Position in key of Feuerstein et al. (2014): couplet 22(21) Ascospores 30–65  $\times$  12–20  $\mu\text{m}$ ; thallus with smooth cortex [4].

*Diorygma fuscum* Jian Li bis & Z.F. Jia, Mycotaxon 131(3): 718, 2016. Thallus crustose, pale grey to olivegrey, surface uneven to slightly rugose or warty; pseudocortex indistinctly developed, partly lacking. Ascocarps lirellate, 1–4  $\times$  0.3–2 mm, sometimes branched, immersed to  $\pm$  raised, whitish. Disc with thick and white pruina. Exciple divergent, laterally uncarbonized, carbonization sometimes at the basal position. Hymenium clear, 100–180  $\mu\text{m}$  high, I+ weakly bluish violet. Ascospores 8/ascus, hyaline to brownish, muriform, spore locules of equal size, 10–14/3–4-locular, 40–60  $\times$  12–18  $\mu\text{m}$ , I+ violet, with thin halo.

**Chemistry:** Stictic acid, constictic, hypostictic and hypoconstictic acids (TLC).

**Ecology and distribution:** This species is distributed in subtropical forests, growing on bark. Known from China [6].

**Specimens examined:** Type: China. Fujian Province, Jianou City, Fangdao Town, Wanmulin, alt. 310 m, on bark, 3/VI/2007, Q.F. Meng FJ1280 (HMAS-L 137193); alt. 540 m, 2/VI/2007, J. Li FJ1066 (HMAS-L 137199).

**Discussions:** This species is similar to *D. pruinosum* (Eschw.) Kalb et al., which differs in the latter having 1-spored asci, larger ascospores, and the presence of protocetraric acids [3]. This species is similar to *D. poitaei*, but differs in having opened discs, a slightly carbonized proper exciple at the base, and the presence of stictic acid (major), while *D. poitaei* contains hypostictic and hypoconstictic acids (major),  $\alpha$ -acetylhypoconstictic, constictic, and stictic acids (minor, trace or absent) [3].

*Diorygma hieroglyphicum* (Pers.) Staiger & Kalb, in Kalb et al., Symb. Bot. Upsal. 34(1): 151, 2004.  $\equiv$  *Opegrapha hieroglyphica* Pers., Ann. Wetter. Gesellsch. Ges. Naturk. 2(1): 16, 1810. Thallus crustose, whitish grey or greenish, surface rough and matt, sometimes verrucose; pseudocortex indistinctly developed, with crystallization. Ascocarps lirellate, 0.5–3  $\times$  0.4–0.8 mm, oblong, numerous or branched, immersed, whitish. Discs narrow to open and covered with whitish or yellowish pruina. Exciple divergent, uncarbonized, and indistinctly developed. Hymenium clear, 100–150  $\mu$ m high, I+ weakly blue to violet blue. Ascospores 1/ascus, hyaline, muriform, oblong, with thick wall, spore locules of equal size, 20–30/6–10-locular, 80–150  $\times$  20–40  $\mu$ m, I+ violet blue.

**Chemistry:** K+ red, P+ yellow to red; stictic acid, norstictic acid, constictic acid, and cryptostictic acid (TLC).

**Ecology and distribution:** This species is a pantropical species, which is distributed in tropical to subtropical coastal rainforests, growing on bark. Known from China, Africa, Singapore, Papua New Guinea, Philippines, Australia, and Southwest Pacific Island Countries [3,37].

**Specimens examined:** China. Fujian Province: Wuyishan City, Mt. Wuyishan, alt. 500 m, 27/V/2007, Z.F. Jia FJ450 (LCUF). Hainan Province: Mangrove near the Qiongzhan city, alt. 0m, 24/V/2007, Q.F. Meng M402 (HMAS-L 128726), M405 (HMAS-L 128727); Baoting contry, Mt. Qixianling, alt. 110 m, 25/VII/2009, M. Liu HN09386 (HMAS-L 115519); Qiongzhan County, Limushan National Forest Park, alt. 630 m, 24/IX/2008, Z.F. Jia HN003 (LCUF). Yunnan Province: Mengla County, Rainforest Valley Xishuangbanna National Park of Tropical Rainforests, alt. 570 m, 21/VIII/2011, Q. Ren YN-R-08 (LCUF); Pingbian County, Dawei Mountain Sand Pearl Bottom, alt. 900 m, 23/VIII/2011, Z.F. Jia 11-419, 11-421, 11-452 (LCUF).

**Discussions:** This species is similar to *Diorygma pruinosum*, but differs in the latter having obviously opened discs, and stictic acid absent [3]. It is morphologically similar to *D. megasporum* Kalb, Staiger & Elix, but differs in the latter having ascospores 2–6/ascus [3]. Position in key of Feuerstein et al. (2014): couplet 36 (34) Stictic acid present in addition to norstictic acid; ascospores 95–150(–170)  $\times$  30–45  $\mu$ m [4].

*Diorygma hololeucum* (Mont. & Bosch) Kalb, Staiger & Elix, Symb. Bot. Upsal. 34(1): 155, 2004.  $\equiv$  *Graphis hololeuca* Mont. & Bosch, Pl. Jungh. 4: 473, 1856. Thallus crustose, white, creamy white to grey, surface rough and matt; pseudocortex indistinctly developed or thin, with crystallization. Ascocarps lirellate, 1–7  $\times$  0.5–1.5 mm, scattered, elongated to oblong, numerous or branched, raised or adnate, whitish. Discs open, covered with a thick and white pruina. Exciple divergent, uncarbonized, poorly developed. Hymenium clear, 180–250  $\mu$ m high, I+ weakly violet blue. Ascospores 2–4(–6 or –8)/ascus, muriform, hyaline, oblong, with thin gelatinous wall at ends, spore locules of equal size, 25–40/5–8-locular, 120–200  $\times$  30–40  $\mu$ m, I+ violet blue.

**Chemistry:** K–, P–; protocetraric acid (TLC).

**Ecology and distribution:** This species is a pantropical species, which is distributed in tropical to subtropical rainforests, growing on bark. Known from China, Philippines, Papua New Guinea, Malaysia, Indonesia [3]; and Australia [38].

**Specimens examined:** China. Hainan Province: Ledong County, Mt. Jianfengling Core Area, alt. 950 m, 2/X/2008, J. Li HN081449 (HMAS-L 117001).

**Discussions:** This species is similar to *Diorygma megasporum*, but differs in the latter having immersed ascocarps, with narrow to slightly opened discs, and containing stictic acid,  $\alpha$ -acetylconstictic acid, and constictic acid [3]. Position in key of Feuerstein et al. (2014): couplet 12(11) Ascospores  $125(-250) \times 30-40(-50) \mu\text{m}$  [4].

*Diorygma isabellinum* (Zahlbr.) Z.F. Jia & Lücking, MycoKeys 25: 24, 2017. = *Graphina isabellina* Zahlbr., in Handel-Mazzetti, Symb. Sinic. 3: 58, 1930. Thallus crustose, creamy white, somewhat yellowish, surface rough and warty. Ascocarps lirellate,  $2-4.5 \times 0.2-0.35$  mm, elongate, single and rarely branched. Labia obvious. Discs closed to slightly open and proper margin conspicuous; Exciple uncarbonized. Hymenium clear,  $160-180 \mu\text{m}$  high, I-. Ascospores 1/ascus, muriform, hyaline, ellipsoid,  $110-120 \times 35-48 \mu\text{m}$ , I+ violet.

**Chemistry:** Norstictic acid, connorstictic acid (TLC).

**Ecology and distribution:** This species is a subtropical species, growing on bark. Known from China [7,39]. **Specimens examined:** China. Hunan Province: Changsha City, Mt. Yuelushan, alt. 250 m, 27/I/1918, Handel-Mazzetti 11437 (W).

**Discussions:** *Diorygma isabellinum* was reported in China as *Graphina isabellina* Zahlbr. [39], and then was recombined as *D. isabellinum* [7]. This species is similar to *D. junghuhnii* (Mont. & Bosch) Kalb, Staiger & Elix, but the latter differs in having I+ blue-violet hymenium and smaller ascospores sized  $(60-80-125) \times 21-42 \mu\text{m}$  [3].

*Diorygma junghuhnii* (Mont. & Bosch) Kalb, Staiger & Elix, Sym. Bot. Upsal. 34(1): 157, 2004.  $\equiv$  *Graphis junghuhnii* Mont. & Bosch, Pl. Jungh. 4: 471, 1856. = *Graphis mendax* Nyl., Anns Sci. Nat., Bot., sér. 4 11: 244 1859. Thallus crustose, whitish, greyish or greenish, surface rough and matt, verrucose; pseudocortex absent or thin, with crystallization. Ascocarps lirellate,  $1-6 \times 0.5$  mm, scattered, elongated to nearly round, single or irregular branched, immersed, whitish. Discs open, covered with greyish to brownish pruina. Exciple slightly divergent, indistinctly developed, uncarbonized. Hymenium clear,  $100-130 \mu\text{m}$  high, I+ blue. Ascospores 1/ascus, muriform, hyaline, oblong, with thin gelatinous wall at ends, spore locules of equal size,  $25-30/6-10$ -locular,  $70-125 \times 20-40 \mu\text{m}$ , I+ violet blue.

**Chemistry:** K+ red, P+ yellow to red; norstictic acid and connorstictic acid (TLC).

**Ecology and distribution:** This species is a pantropical species, which is distributed in tropical to subtropical rainforests, growing on bark. Known from China, Togo (Central and Southern West Africa), Tanzania (East Africa), Philippines, Australia, Fiji, Costa Rica, Guatemala, Guyana, Brazil, etc. [3]; Thailand, Australia, and South Pacific island countries [38].

**Specimens examined:** China. Fujian Province: Wuyishan City, Mt. Wuyishan, alt. 500 m, 27/V/2007, Z.F. Jia FJ445, FJ446 (LCUF). Guangdong Province: Xinyi City, Mt. Datianding, alt. 1700 m, 5/XI/2010, H.Y. Wang 20107250, 20107750, 20107958 (SDNU). Guangxi Province: Longsheng County, Huaping, alt. 900 m, 6/VI/2001, J.B. Chen 20032-1-3 (HMAS-L 030808-10). Hainan Province: Ledong County, The tropical arboretum in the Mt. Jianfengling, alt. 650 m, 30/IX/2008, Z.F. Jia HN080692 (HMAS-L 127471); Wuzhishan City, Mt. Wuzhishan, alt. 680 m–880 m, 28/IX/2008, J. Li, HN081254 (HMAS-L 127474), HN081259 (HMAS-L 117008).

**Discussion:** *Diorygma junghuhnii* is similar to *D. soozanum*, but differs in the latter having narrow to open discs covered with white pruina, and I+ weakly blue in hymenium [3]. It is similar to *D. macgregorii* (Vain.) Kalb, Staiger & Elix in appearance and the chemical substance, but differs in the latter having larger spores  $(135-185) \times 40-63 \mu\text{m}$  [3]. Position in key of Feuerstein et al. (2014): couplet 41(40) Hymenium completely I+ blue-violet; ascospores  $(60-80-125) \times 21-42 \mu\text{m}$  [4].

*Diorygma macgregorii* (Vain.) Kalb, Staiger & Elix, Sym. Bot. Upsal. 34(1): 159, 2004.  $\equiv$  *Helminthocarpon pervarians* var. *macgregorii* Vain., Ann. Acad. Sci. Fenn., Ser. A 15(6): 266, 1921. Thallus crustose, creamy white, grey or greenish grey, surface rough and matt with small granula; pseudocortex absent or thin, with crystallization. Ascocarps lirellate,



1–5 × 0.5–2 mm, scattered, oval to elongated, branched, distinctly raised. Discs distinctly open, covered with white or greyish pruina. Exciple divergent, indistinctly developed, uncarbonized. Hymenium clear, 140–200 µm high, I+ weakly violet blue near exciple. Ascospores 1/ascus, muriform, hyaline, oblong, with the central cells larger than the peripheral ones, 135–180 × 35–50 µm, I+ violet blue.

**Chemistry:** K+ red, P+ yellow to red; norstictic acid, connorstictic acid, cryptostictic acid, stictic acid (TLC).

**Ecology and distribution:** This species is a pantropical species, which is distributed in tropical to subtropical rainforests, growing on bark. Known from China, Southeast Asia, and Papua New Guinea [3].

**Specimens examined:** China. Hunan Province: Sangzhi County, Mt. Badagongshan, alt. 1400 m, 19/VIII/1997, J.B. Chen, D.P. Wang, S.L. Wang 9633 (HMAS-L 121023). Hainan Province: Changjiang County, Mt. Bawangling, alt. 980 m, 18/V/2007, Q.F. Meng M488 (HMAS-L 128742); Ledong County, Mt. Jianfengling, alt. 180 m, 1/X/2008, J. Li, HN081360 (HMAS-L 117074). Guizhou province: Tongren City, Mt. Fanjingshan, alt. 1570 m, 1/IX/1963, J.C. Wei 621 (HMAS-L 047731).

**Discussion:** This species is similar to *Diorygma hieroglyphicum*, but differs in the latter having immersed lirellae with narrow to open discs and smaller spores sized 95–150(–170) × 30–45 µm [3]. It is similar to *D. junghuhnii*, but differs in the latter having smaller spores sized (60–)80–125 × 21–42 µm [3].

*Diorygma megasporum* Kalb, Staiger & Elix, Symb. Bot. Upsal. 34(1): 160, 2004. Thallus crustose, whitish, creamy white, yellowish, or weakly grayish green, surface rough and matt, with small granula; pseudocortex indistinctly developed or thin, with crystallization. Ascocarps lirellate, 0.5–5 × 0.1–0.7 mm, scattered, sub round to elongated, branched, immersed. Discs narrow to slightly open, and covered with a thick greyish pruina. Exciple divergent, uncarbonized, poorly developed. Hymenium clear, 170–200 µm high, I+ violet blue on upper parts and exciple. Ascospores 2–6/ascus, muriform, hyaline, irregular round, with thick wall, spore locules of equal size, 30–60/7–12-locular, 80–200 × 20–50 µm, I– or I+ violet blue.

**Chemistry:** K+ yellow, P+ yellow to orange; stictic acid, α-acetylconstictic acid and constictic acid (TLC).

**Ecology and distribution:** This species is a pantropical species, growing on bark. Known from China and India [3].

**Specimens examined:** China. Guizhou Province: Tongren City, Mt. Fanjing Mountain, Scissor Gorge, alt. 1570 m, 6/IX/1963, J.C. Wei 0675 (HMAS-L 047752) **Discussions:** This species is similar to *Diorygma hololeucum*, but differs in the latter having protocetraric acid, and adnate ascocarps with open discs [3]. This species is recorded containing chemical components such as norstictic and hypostictic acids by Kalb et al. (2004); however, we did not detect these components in Chinese specimens. Position in key of Feuerstein et al. (2014): couplet 49(48) Ascospores 231–244 × 59–76 µm [4].

*Diorygma pruinatum* (Eschw.) Kalb, Staiger & Elix, Sym. Bot. Upsal. 34(1): 166, 2004. ≡ *Leiogramma pruinatum* Eschw., in von Martius, Icon. Plant. Cryptog. 2: 12, 1828. ≡ *Cyclographina pruinosa* (Eschw.) D. D. Awasthi, Norw. J. Bot. 26: 175, 1979. Thallus crustose, creamy white, greenish or pale grey, surface rough and matt, partially cracked; pseudocortex distinctly developed and partially indistinctly developed, with crystallization. Ascocarps lirellate, 1–3 × 0.2–1 mm, scattered, elongated to round, single or branched, immersed to ± raised. Discs wide open and covered with a thick and white pruina. Exciple slightly divergent, indistinctly developed, uncarbonized to slightly carbonized. Hymenium clear, 110–190 µm high, I+ weakly violet blue. Ascospores 1/ascus, muriform, hyaline, oblong, spore locules of equal size, 25–40/6–10-locular, 90–170 × 20–50 µm, I+ violet blue. **Chemistry:** K–, P–; protocetraric acid (TLC).

**Ecology and distribution:** This species is a pantropical species, which distributed in tropical to subtropical forests, growing on bark. Known from China, Cameroon, Nigeria (West Coast African country), Kenya (Equatorial East African country), and Tanzania Nigeria

(East African countries), Indonesia, Singapore, Papua New Guinea (Western Pacific countries), Australia, Scotland, Brazil, etc. [3]; Australia, Philippines, and Solomon Islands [37]. **Specimens examined:** China. Fujian Province: Jian'ou City, Fangdao Town, Wanmulin, alt. 600 m, 2/VI/2007, Q.F. Meng FJ645 (LCUF). Hainan Province: Ledong Country, Mt. Jianfengling, Rainforest Valley, alt. 670 m, 16/V/2007, Q.F. Meng M231 (HMAS-LHMAS-L128760), M333 (128737), M362 (HMAS-L128761).

**Discussion:** This species is similar to *Diorygma hololeucum*, but differs in the latter having 2 or more spores with larger spores sized  $125\text{--}230\text{--}(250) \times 30\text{--}45\text{--}(50) \mu\text{m}$  [3]. It is similar to *D. macgregorii* in disc and the size of spores, but differs in the latter having norstictic acid [3]. Position in key of Feuerstein et al. (2014): couplet 15(13) Ascospores up to  $150 \mu\text{m}$  long (or very rarely to  $170 \mu\text{m}$  long),  $95\text{--}150\text{--}(170) \times 19\text{--}50 \mu\text{m}$ ; peripheral and central ascospore locules of more or less equal size [4].

*Diorygma soozanum* (Zahlbr.) M. Nakan. & Kashiw. [as 'soozana'], in Nakanishi, Kashiwadani & Moon, Bull. Natn. Sci. Mus., Tokyo, B 29(2): 86, 2003.  $\equiv$  *Graphina soozana* Zahlbr., Feddes Repert. Spec. Nov. Regni veg. 31: 215, 1933. Thallus crustose, creamy white to pale grey, surface slightly rough and matt, cracked partialy; pseudocortex distinctly developed, with small crystallization. Ascocarps lirellate,  $1\text{--}5 \times 0.4\text{--}0.6 \text{ mm}$ , scattered, oblong to enlarged, single or branched, prominently raised. Discs narrow to open, covered with white pruina. Exciple slightly divergent, indistinctly developed, uncarbonized. Hymenium clear,  $130\text{--}160 \mu\text{m}$  high, I+ weakly violet blue. Ascospores 1/ascus, muriform, hyaline, oblong, spore locules of equal size,  $20\text{--}30/7\text{--}8\text{-locular}$ ,  $110\text{--}140 \times 35\text{--}45 \mu\text{m}$ , I+ violet blue. **Chemistry:** K+ red, P+ yellow to red; norstictic acid and connorstictic acid (TLC).

**Ecology and distribution:** This species is a pantropical species, which is distributed in tropical to subtropical rainforests, growing on bark. Known from China and Japan [3,20].

**Specimens examined:** China. Fujian Province: Wuyishan City, Mt. Wuyishan, alt. 500 m, 27/V/2007, Z.F. Jia FJ470, FJ453, FJ454 (LCUF); Jian'ou City, Fangdao Town, Wanmulin, alt. 320 m, 3/VI/2007, Q.F. Meng FJ942, FJ943 (LCUF). Sichuan Province: Dujiangyan City, Xiangshui Cave, alt. 1750 m, 13/VIII/1997, J.C. Wei 97141 (HMAS-L 055260). Yunnan Province: Luxi City, Mt. Santai, alt. 1340 m, 28/XI/1980, Y.M. Jiang 552-2 (HMAS-L 047681).

**Discussion:** This species is similar to *Diorygma tuberculosum* (Stirt.) Kalb, Staiger & Elix, but differs in the latter having small and unequal spores, I- [3]. It is similar to *D. junghuhnii*, but differs in the latter having immersed ascocarps, with thickly pruinose discs, and I+ distinctly bluish violet in hymenium [3]. Position in key of Feuerstein et al. (2014): couplet 41(40) Hymenium weakly I+ blue-violet (mostly laterally); ascospores  $110\text{--}145 \times 36\text{--}45 \mu\text{m}$  [4].

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