

Short Communication:

New records of *Graphis* (Graphidaceae, Ascomycota) in Bali Island, Indonesia

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Abstract. Hardini J, Kasiamdari RS, Santosa, Purnomo. 2018. Short Communication: New records of *Graphis* (Graphidaceae, Ascomycota) in Bali Island, Indonesia. *Biodiversitas* 19: 112-118. *Graphis* is a genus of the lichen family Graphidaceae, which is found living attached to *Plumeria* spp. trees as its substrate. The purpose of this study was to determine the species diversity of *Graphis* in Bali Island, Indonesia. This study was conducted between July 2014 and December 2014, at three locations, namely Denpasar City, Klungkung district, and Tabanan district, at the altitudes of 35 m, 69 m and 1269 m asl. respectively. Seven species were found as new records namely *Graphis modesta*, *Graphis immersicans*, *Graphis palmicola*, *Graphis nana*, *Graphis dupaxana*, *Graphis maritima* and *Graphis subradiata*.

Keywords: Bali Island, Graphidaceae, lichen, new record, taxonomy

INTRODUCTION

Lichen is an important pioneer on soil and rock surface, such as in the newly opened forest, burning forests and traces of volcanic lava flows (Campbell et al. 2003), and does not require high living conditions (Tjitrosoepomo 1986). Lichen is one of the epiphytic organisms generally regarded as an indicator of air quality (Shukla and Upreti 2011) which can be found in abundance in the bark of a tree, a rock, or a variety of other substrates (Hale 1974; Nash 2008). There is a cosmopolitan and adaptable lichen that may be found in various ecosystem types of the world. However, the general properties of lichen vegetation in an area is determined mainly by climatic variations and altitude (Zulkifly et al. 2011) and lichen can be found from the tropics to the polar region (Ahmadjian 1993; Nash 2008).

Graphidaceae lichen is the most dominant crustose lichen communities in tropical ecosystems (Hale 1974; Lucking et al. 2009), and is the largest family of tropical crustose lichen that comprises more than 1,800 species (Lucking et al. 2014). *Graphis* is the largest element in this family (Lucking 2009) and is one of the largest genera in Graphidaceae, consisting of more than 300 taxa (Lucking et al. 2009; Rivas Plata et al. 2011). Several data about Graphidaceae lichen in Indonesia have been known, namely data of eight genera (*Graphina*, *Graphis*, *Phaeographina*, *Phaeographis*, *Sarcographa*, *Sarcographina*, *Glyphis* and *Fissurina*) from Bogor, Cibodas (Indonesia) and Singapore (Sipman, 2003); 23 species of *Graphis* from Java Island, Krakatau, Sulawesi,

Kalimantan (Lucking et al. 2009), and 82 species (Lucking et al. 2014).

In Bali Island, Indonesia, there are a lot of *Plumeria* spp. trees planted. *Plumeria* is a tropical plant that flourishes in lowlands to an altitude of 700 m asl., and is one of the ornamental plants at homes, offices, and public parks (Wrasiasi et al. 2011). The flowers are mainly used for religious ceremonies in Bali (Adiputra, 2011). *Plumeria* trees have thin and smooth bark on which the lichen family Graphidaceae live. The taxonomic study of Graphidaceae Indonesia is very limited, so the aim of this research was to collect data about *Graphis* in Bali Island, Indonesia.

MATERIALS AND METHODS

Study area

Lichen was collected from Denpasar City (69 m asl., 8°38'9"S 115°12'44"E, temperature 31°C, humidity 56%); Klungkung district (35 m asl., 8°33'17"S 115°27'35"E, temperature 38°C, humidity 54%); Tabanan district (1269 m asl., 8°16'10"S 115°9'48"E, temperature of 25°C, humidity of 58% (Figure 1).

Sample collection and identification

The specimens were collected and identified in December 2014 to July 2015 and deposited in the herbarium of Laboratory of Plant Systematics (LabPS), Faculty of Biology Universitas Gadjah Mada, Yogyakarta, Indonesia. Stereomicroscope (Olympus CX22) with 7x to 45x at magnifications were used to observe the

morphological features. Anatomical features were studied under light microscope XSZ-107BN with 40x to 1000x magnifications using Optilab microscope camera. Thin hand-cut sections of thalli and ascomata were observed in water and lactophenol aniline blue 1%. Lichen substances were identified using thin layer chromatography (TLC) following standard methods in solvent C (Orange et al. 2010). The chemical color tests used potassium hydroxide (K), calcium or sodium hypochlorite (C), parphenylenediamine (P), and iodine solution (J) (Wirth 1995). Microcrystals test (Hale 1974; Huneck and Yoshimura 1996) was performed by crystallizing reagents: GE (glycerine-acetic acid), GAW (glycerine alcohol-water), GAOt (glycerine alcohol o-toluidin), GAAa (glycerine alcohol aniline). Photographs were taken using Nikon Coolpix S220 digital camera.

Description was made based on the data obtained from the results of morphological, anatomical, and chemical characterization, followed by identification. Identification was performed using the identification key of *Graphis* lichen (Lucking et al. 2009); and journals related to lichen family Graphidaceae, as follows: Hale (1961), Wirth and Hale (1978), Dobson (1992), Wirth (1995), Huneck and

Yoshimura (1996), Archer (1999; 2000; 2007), Sipman (2003), Nash (2008), Lucking et al. (2009), Lucking (2009), Rivas Plata et al. (2010), Seavey and Seavey (2011), Rivas Plata et al. (2012), Jia and Kalb (2013), and Lucking R (2014, pers. comm.).

RESULTS AND DISCUSSION

Taxonomic description of these newly recorded species
Graphis modesta Zahlbr. (1911); The Lichenologist 41 (4): 408 (2009); The Bryologist 114 (4): 779 (2011) (Figures 2A-a)

Thallus corticolous, crustose, whitish-gray, uneven thallus surface, slightly white pruina, many oxalate crystals on the thallus surface and between layers of algae; ascomata lirellae, erumpent, short, 1-2 mm, radiate branched; labia entire; disc exposed; lateral thalline margin; excipulum completely carbonized; yellowish to brownish hymenium, 82-122 μm high, clear, paraphyses simple; ascospores brownish-gray, no halonate, ascospores 8 per ascus, transversely 7-9 septate, 8-10 locular, 18-34 x 5-7 μm , I+ blue.

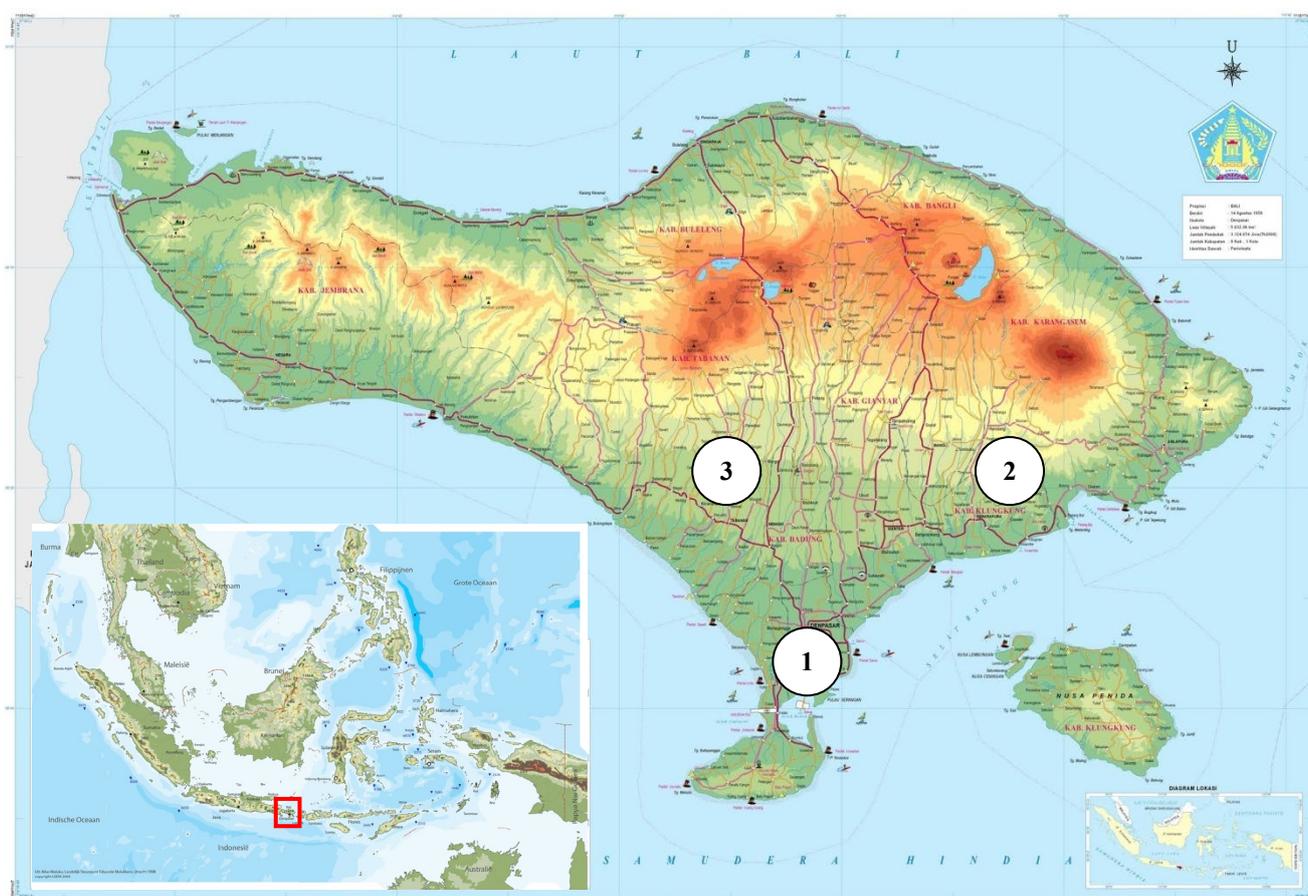


Figure 1. Study area of *Graphidaceae* lichen diversity: 1. Denpasar City, 2. Klungkung District, 3. Tabanan District, Bali Island, Indonesia

Chemistry: Thallus K+, P-, C-, KC-; stictic acid; microcrystalline test identified as *nephroarctin* acid, *baeomycesic* acid.

Specimen examined: Indonesia: Bali Island: Lumintang Village-Denpasar City, 69 m asl., on bark of *Plumeria* spp., December 2014, Jun-LD3 (LabPS).

Distribution: Solomon Island, Everglades National Park (Florida), South Western Ghats of India.

Note: *Graphis discarpa* resembles *Graphis modesta* in having stictic acid, but distinguished from *G. modesta* by concealed disc and lirellae with thick complete thalline margin (Lucking et al. 2009). *G. modesta* also resembles *G. handelii* characterized by shorter lirellae, branched and non-pruinose, hymenium interspersed and norstictic acid presence. *Graphis crebra* is also similar to *G. modesta*, but differs in its lateral carbonized exciple, and norstictic acid presence (Seavey & Seavey 2011).

Graphis immersicans A.W. Archer (Figures 2B-b)

Holotype: *Graphis leptocarpa* var. *invita* Vain., *Annales Academiae Scientiarum Fennicae* 15 (6): 205 (1921)

Holotype: *Graphis immersicans* A.W. Archer *Aust. Syst. Bot.* 14 (2): 262 (2001) (Lucking et al. 2009)

Thallus corticolous, crustose, brown, uneven surface thallus, many oxalate crystals on the of thallus surface and between layers of algae; ascumata lirellae, erumpent, black, short, 1-3 mm, partially branched; labium striate; disc exposed with white pruina; lateral thalline margin; excipulum completely carbonized; yellowish to brownish hymenium, 73-108 µm high, clear, paraphyses simple; ascospores gray-brown, halonate, ascospores 6-8 per ascus, transversely 6-7 septate, 7-8 locular, 15-22 x 5-7 µm, I + blue.

Chemistry: thallus K-, C-, P-, KC-; microcrystalline test identified as *acetylprotentol* acid, *thiophanic* acid, *pseudoplacodiolic* acid, *barbatine* acid, *sekikaic* acid, *confluentia* acid, *psoromic* acid.

Specimen examined: Indonesia: Bali Island: Lumintang Village-Denpasar City, 69 m asl., on bark of *Plumeria* spp., December 2014, Jun-LD3 (LabPS).

Distribution: Philippines, Australia.

Note: *Graphis immersicans* resembles *G. oxyclada*, but differs in its erumpent lirellae, with lateral thalline margin, short to elongated and sparsely to irregularly branched.

Graphis palmicola Makhija and Adaw. *Mycotaxon* 91: 378 (2005) (Figures 2C-c)

Thallus corticolous, crustose, brownish-yellow, uneven surface thallus, many oxalate crystals on the thallus surface and between layers of algae; ascumata lirellae, erumpent, black, elongate with tapered tip, 2-4 mm, unbranched; labia entire; disc exposed; lateral thalline margin; excipulum completely carbonized; yellowish to brownish hymenium, 86-108 µm high, clear, paraphyses simple; ascospores hyalin and halonate, ascospores 8 per ascus, transversely 5-7 septate, 6-8 locular, 14-23 x 5-7 µm, I+ blue.

Chemistry: Thallus K+, P-, C-, KC-; microcrystalline test identified as *lichexanthone* acid, *divaricatic* acid, *placodiolic* acid, *fumarprotocetraric* acid.

Specimen examined: Indonesia: Bali Island: Lumintang Village-Denpasar City, 69 m asl., on bark of *Plumeria* spp., December 2014, Jun-LD3 (LabPS).

Distribution: India

Note: *Graphis palmicola* resembles *G. deserpens* Vain. which has irregularly branched lirellae and muriform ascospores.

***Graphis nana* (Fee) Spreng.** (1927) *The Lichenologist* 41 (4): 396 (2009) (Figures 2D-d)

Synonym: *Opegrapha nana* Fee, *Essai Cryptog. Écorc. Officin.*, tab. XV.p. 26 (1824)

Thallus corticolous, crustose, brownish-white, uneven thallus surface, many oxalate crystals between layers of algae; ascumata lirellae, sessile, black, short, 0.-1.5 mm, unbranched; labia entire; disc concealed; lacking thalline margin; excipulum laterally carbonized; I +ve, yellowish to brownish hymenium, 41-64 µm high, clear, paraphyses simple; ascospores hyaline, ascospores 8 per ascus, transversely 6-8 septate, 7-9 locular, 17-28 x 5-7 µm, I+ blue.

Chemistry: Thallus K-, P-, C-, KC-; microcrystalline test identified as *leucotylin*, *placodiolic* acid, *fumarprotocetraric* acid.

Specimen examined: Indonesia: Bali Island: Gunaksa Village, Klungkung Regency, 35 m asl., on bark of *Plumeria* spp., January 2015, Jun-LD3 (LabPS).

Distribution: South America.

Note: *Graphis nana* resembles *G. urendrae* which has prominent, basal thalline margin, elongated and partly branched lirellae.

Graphis dupaxana Vain. (Figures 3E-e)

Lectotype: *Graphis dupaxana* Vain., *Annales Academiae Scientiarum Fennicae* 15 (6): 241 (1921), Philippines.

Holotype: *Graphis moultonii* Vain., *Annales Academiae Scientiarum Fennicae* 15 (6): 243 (1921), Indonesia (Borneo).

Holotype: *Graphis leioplaca* Müll. Arg., *Flora (Regensburg)* 63 (2): 20 (1880), Brazil.

Thallus corticolous, crustose, grayish-white, uneven thallus surface, soralia present, many oxalate crystals on the thallus surface and between layers of algae; ascumata lirellae, erumpent, black, short, 0.5-3mm, rare and irregularly branched; labia striate; disc closed; lacking thalline margin; excipulum completely carbonized; yellowish to brownish hymenium, 58-79 µm high, clear, paraphyses simple; ascospores hyaline, ascospores 8 per ascus, transversely 8-12 septate, 9-13 locular, 23-34 x 5-7 µm, I + blue.

Chemistry: Thallus K-, P-, C-, KC-; microcrystalline test identified as *acetoxyhopan* acid, *sordidone*, *dissectic* acid, *placodiolic* acid, *barbatic* acid.

Specimen examined: Indonesia: Bali Island: Baturiti village, Tabanan Regency, 1269 m asl., on bark of *Plumeria* spp., February 2015, Jun-LD3 (LabPS).

Distribution: Borneo (Indonesia), Philippines, Brazil.

Note: This species resembles *G. rimulosa*, but differs in ascospores size *G. rimulosa* has 30-50 x 7-13 µm

ascospores, whereas *G. dupaxana* has 20-40 x 5-9 µm ascospores. According to Lucking et al. (2009) *Graphis moultonii* Vain. was the holotype from Borneo (Indonesia), which has prominent lirellae character, basal thalline margin; transversely 5-11 septa, ascospores 20-35 x 5-8 µm.

Graphis maritima (A.W. Archer) A.W. Archer, Syst. Biodiv. 5 (1): 16 (2007) (Figures 3F-f)

Synonym: *Graphina maritima* A.W. Archer, Mycotaxon 89 (2): 322 (2004)

Thallus corticolous, crustose, grayish-white, uneven and wrinkled thallus surface, many oxalate crystals on thallus surface and between layers of algae; ascomata lirellae, prominent, black, variable (short, straight, curve, or notched), 0.5-2 mm, unbranched; labia entire, disc closed; lateral thalline margin; excipulum completely carbonized; yellowish to brownish hymenium, 67-92 µm high, clear, paraphyses simple; ascospores hyaline, ascospores 6-8 per ascus, muriform, halonate, 9-10 x 2-4 locular, 23-45 x 10-15 µm.

Chemistry: Thallus K⁻, P⁻, C⁻, KC⁻; I⁺; Microcrystalline test identified as *bellidiflorin* acid, *placodiolic* acid, *ergosterol*, *dissectic* acid, *acetylportentol* acid, *barbatic* acid.

Specimen examined: Indonesia: Bali Island: Baturiti Village, Tabanan Regency, 1269 m asl., on bark of *Plumeria* spp., February 2015, Jun-LD3 (LabPS).

Distribution: Solomon Island.

Note: This species resembles *G. comma* (Ach.) Spreng. but differs in the size of ascospores, basal thalline margin. According to Archer and Elix (2007), this species is previously only known in the Solomon Islands but now is reported that it is also present in North Queensland. This species resembles *G. platycarpa* Eschw., but differs in the apical exciple character and the larger ascospores size which is between 50-70 x 14-20 µm, and similar to *G. geraensis* is at a glance in having septate ascospores.

Graphis subradiata (Nyl.) Lucking (Figures 3G-g)

Graphis subradiata (Nyl.) Lücking, The Lichenologist 41 (4): 441 (2009)

Synonym: *Graphis analoga* var. *subradiata* Nyl., Acta Societatis Scientiarum Fennicae 7: 465 (1863), *Graphina analoga* var. *subradiata* (Nyl.) Zahlbr., Catalogus Lichenum Universalis 2: 395 (1923)

Thallus corticolous, crustose, grayish-white, uneven and wrinkled thallus surface, many oxalate crystals on the thallus surface and between layers of algae; ascomata lirellae, erumpent, black, short, curved irregularly, 0.5-5 mm, rare and irregularly branched; labia striate; disc closed; lateral thalline margin; excipulum completely carbonized; yellowish to brownish hymenium, 98-127 µm, clear, paraphyses simple; ascospores hyaline, ascospores 4-8 per ascus, muriform, 25-45: 9-15 µm.

Chemistry: Thallus K⁻, P⁻, C⁻, KC⁻; microcrystalline test identified as *placodiolic* acid, *leucotylin* acid, *sordidone*, *dissertic* acid, *thiophanic* acid.

Specimen examined: Indonesia: Bali Island: Baturiti Village, Tabanan Regency, 1269 m asl., on bark of *Plumeria* spp., February 2015, Jun-LD3 (LabPS).

Distribution: Colombia

Note: *G. subradiata* resembles *G. multisulcata* (Mull. Arg.) Lucking and Chaves but differs in very short and unbranched lirellae.

Discussion

In this study, Graphidaceae lichens were found in all three locations, but they were rarely found in a place with an altitude of 1269 m (Baturiti village), that are dominated by foliose lichen and moss. Graphidaceae lichen is an important element of crustose lichen community in the tropics, easily found at lower altitudes. The main character is the ascomata lireliform which a line or elongated in shape.

The morphological, anatomical, chemical characteristics of thallus and ascomata lirellae varied. Generally thallus lichen is white-gray in color. Lichen samples collected had brownish-white, brown, green, yellow, and brownish yellow in color. The emergence of the lirellae was categorized into four types, immersed, erumpent, prominent and sessile, and all four types were clearly distinguished by the cross-sectional lirellae (Lucking et al. 2009). Thalline margin was absent, lateral or complete. The relative length of the lirellae was found very short (1-2 mm), short (2-3 mm), elongated (> 3-10 mm). The branching patterns of lirellae were unbranched, sparse branched, and radiate branched. Disc lirellae was observed in open and close positions.

The disc was opened when it had full hydration, but was closed in dry conditions. However, some species had opened discs even in dry conditions. The samples were found to have entire labia and striate labia. According to Lucking (2009), there was no correlation between the combination of the emergence of lirella, thalline margin, relative lirellae length, and branching with labia striation. Excipulum on *Graphis* is generally carbonized. Almost half of all *Graphis* species have complete carbonized excipulum, about one-third have lateral carbonized excipulum and other species have apical carbonized excipulum (Lucking 2009).

Graphis species were characterized by hyaline ascospores, positive reaction to iodine, ascospores septa with lens-shaped lumina. Ascospores changed during development. Young ascospores had thin endospores and weak iodine reactions. Matured ascospores, post-matured ascospores, and endospores were destroyed when the iodine reactions disappeared, and often ascospores shriveled. The number of ascospores per ascus should be observed from several asci of different lirellae. Ascospores were often lost prematurely or their numbers were difficult to quantify in the ascus. If there are more than four ascospores per ascus, the most likely number is eight (Lucking et al. 2009).

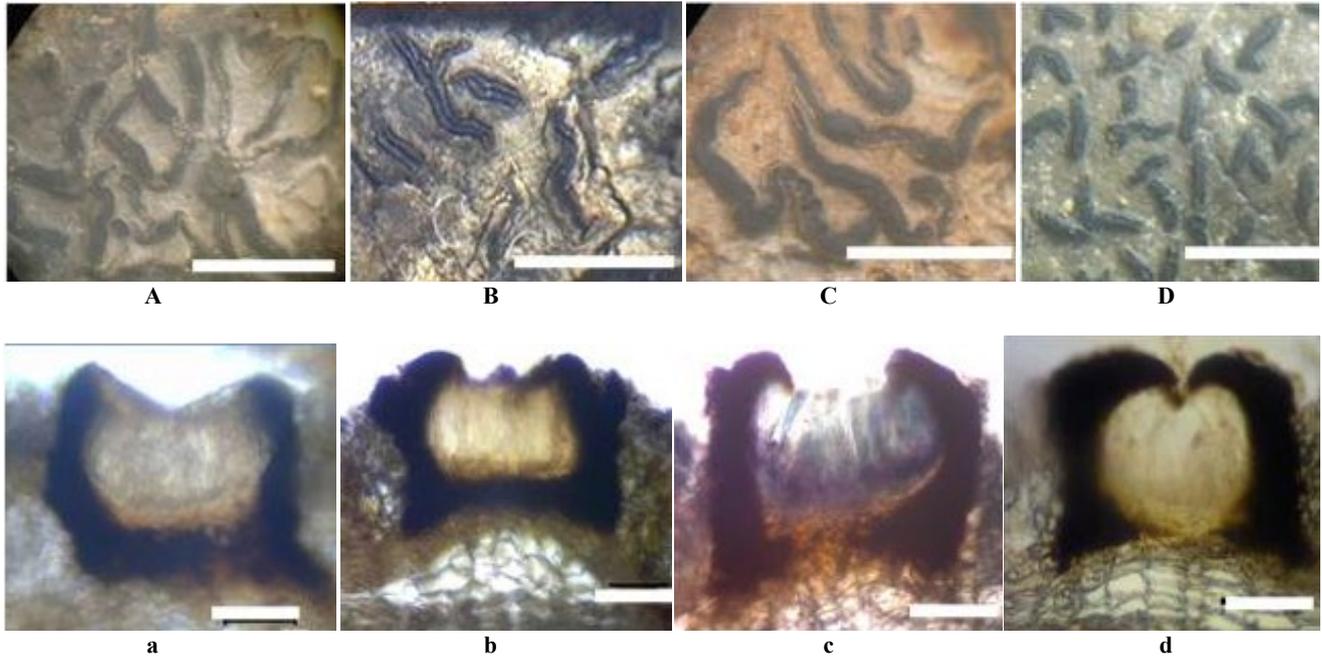
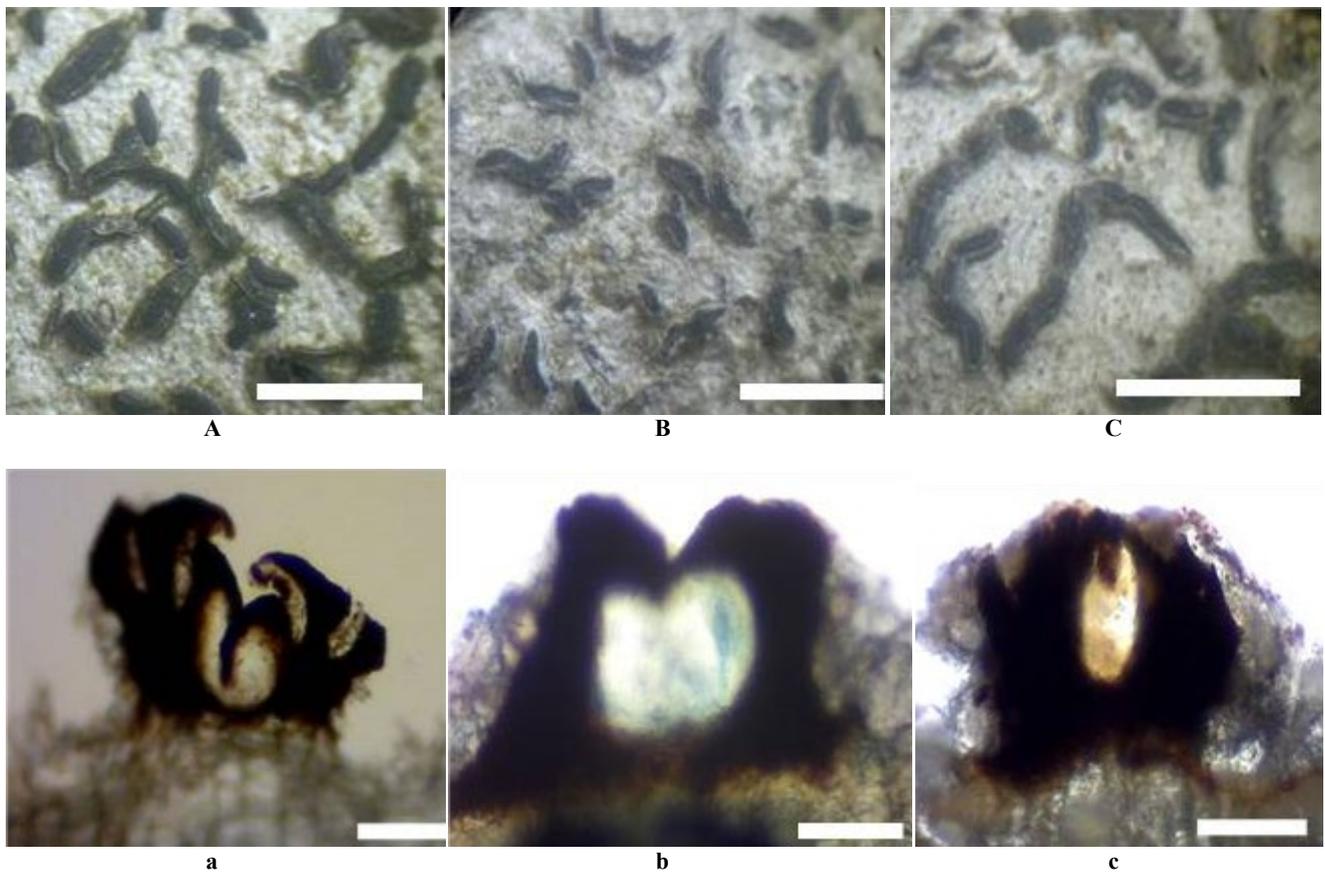


Figure 2. A-D. Lirellae morph ; a-d. cross section of ascomata. A,a. *Graphis modesta*, B,b. *Graphis immerscans*, C,c. *Graphis palmicola*, D,d. *Graphis nana*. Scale bars: A-D = 2 mm; a-d = 100 μ m



Figures 3. E-F. Lirellae morph ; e-f. cross section of ascomata. E-e. *Graphis dupaxana*, F-f. *Graphis maritima*, G-g. *Graphis subradiata*. Scale bars: E-F = 2 mm; e-f = 100 μ m

Ascospore sizes in a limited species varied, the biggest one being twice as big as the smallest one (for example, 25-50 µm, 50-100 µm, 100-200 µm). The results of the study were followed by a class of ascospore sizes (Lucking 2009), range of size: very small (15-25 µm), small (25-45 µm, mostly 30-40 µm ascospores), medium (45-80 µm, mostly ascospores 50-70 µm), large (80 to 150 µm, mostly ascospores 100-130 µm) and very large (150-300 µm, mostly ascospores 180-250 µm). The size of ascospores should be measured in adult ascospores only, i.e. fully developed endospores and various sizes should be assessed, measuring at least ten sample ascospores, with different ascus and from various lirellae (Lucking et al. 2009).

In most species of Graphidaceae, mature spores react with almost all iodine concentrations and rapidly change to the blue-violet color. The reaction seems to be fairly constant, so it is very useful in species identification. Most *Graphis* species are lack of secondary substances (Archer 2006; Lucking 2009). Norstictic or stictic acid is very common in *Graphis*, whereas salazinic and protocetraric acids are relatively rare.

The secondary substance in *Graphis* is identified by the spot test. Most lichen researchers also use spot test techniques to routinely identify lichen substances, and the use of TLC is needed in dubious cases. Microchemical reactions with thallus and lirellae slices use 10-20% KOH solution (to test the presence of norstictic, salazinic, or stictic acid). Continuous pale color to bright yellow efflux without crystal formation indicates stictic acid, the characteristic of stictic-yellow acids is easily recognizable (Lucking et al. 2009).

Seven lichen samples identified based on the morphological, anatomical and chemical characters were *Graphis modesta* Zahlbr. (Lucking et al. 2009; Seavey and Seavey 2011), *G. immersicans* A.W. Archer (Lucking et al. 2009), *G. palmicola* Makhija and Adaw. (Lucking et al. 2009), *G. nana* (Fee) Spreng. (Lucking et al. 2009), *G. dupaxana* Vain. (Lucking et al. 2009), *G. maritima* (A.W.Archer) A.W.Archer (Lucking et al. 2009), *G. subradiata* (Nyl.) Lucking (Lucking et al. 2009).

Key to the species *Graphis* in Bali Island, Indonesia

- 1a. Labia entire 2
- 1b. Labia striate 5
- 2a. Ascospores transversely septa 3
- 2b. Ascospores muriform, ascospores 6-8 per ascus, 23-45 x 10-15 µm, lirellae prominent, lateral thallin margin *Graphis maritima*
- 3a. Excipulum laterally carbonized, lacking thallin margin, disc closed, lirellae short, sessile *G. nana*
- 3b. Excipulum completely carbonized 4
- 4a. K positive (yellow), stictic acid, lirellae short, erumpent, ascospores 18-34 x 5-7µm, ascospores 8 per ascus, 8-10 locular, 7-9 septate *G. modesta*
- 4b. K negative, no substance, lirellae elongate, prominent, ascospores 14-23 x 5-7µm, ascospores 8 per ascus, 6-8 locular, 5-7 septate *G. palmicola*

- 5a. Ascospores transversely septa 6
- 5b. Ascospores muriform, lateral thalline margin, prominent, ascospores 24-55 x 9-15 µm, ascospores 4-8 per ascus ... *G. subradiata*
- 6a. Lirellae with lateral thallin margin, erumpent, ascospores 15-22 x 5-7µm, ascospores 6-8 per ascus, 7-8 locular, 6-7 septate *G. immersicans*
- 6b. Lirellae lacking thallin margin, prominent, ascospores 23-34 x 5-7µm, ascospores 8 per ascus, 9-13 locular, 8-12 septate *G.dupaxana*

In conclusion, new record of *Graphis* (Graphidaceae) was collected for the first time in Bali Island, which was discussed in this study. The results of this study are expected to be the basis for further lichen research and studies of the diversity of Graphidaceae in other areas. Seven species of *Graphis* have been found and newly reported, namely: *Graphis modesta* Zahlbr., *Graphis immersicans* A.W. Archer, *Graphis palmicola* Makhija & Adaw., *Graphis nana* (Fee) Spreng., *Graphis dupaxana* Vain., *Graphis maritima* (A.W. Archer) A.W.Archer, *Graphis subradiata* (Nyl.) Lucking. All species were identified based on morphological, anatomical and chemical characteristics.

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