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 (Wrasiaiet 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°09’48"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), paraphenylenediamine (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), GAAn (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, baeomycetic acid.

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


Distribution: 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 inspersed 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)


Thallus corticolous, crustose, brown, uneven surface thallus, many oxalate crystals on the of thallus surface and between layers of algae; ascomata 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; ascosporae gray-brown, halonate, ascospores 6-8 per ascus, transversely 6-7 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., December 2014, Jun-LD3 (LabPS).

Distribution: South America.

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


Thallus corticolous, crustose, brownish-white, uneven thallus surface, many oxalate crystals between layers of algae; ascomata lirellae, sessile, black, short, 0.1-1.5 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; ascosporae hyaline, ascosporae 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 acetyprotentol acid, thiophanic acid, pseudoplacidiolic acid, barbatine acid, sekikaic acid, confluentic acid, psoromic acid.

Specimen examined: Indonesia: Bali Island: Lumintang Village-Denpasar City, 69 m asl., on bark of Plumeria spp., January 2015, Jun-LD3 (LabPS).

Distribution: South America.

Note: Graphis nana resembles G. modesta, but differs in its erumpent lirellae, branched and irregularly branched lirellae.

Graphis palmicolia 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; ascomata 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; ascosporae hyaline and halonate, ascosporae 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: Indonesia.

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


Thallus corticolous, crustose, grayish-white, uneven thallus surface, soralia present, many oxalate crystals on the thallus surface and between layers of algae; ascomata 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; ascosporae hyaline, ascosporae 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 acetoxhyopan acid, sordidone, dissectic acid, placodiolic acid, barbatic acid.

Specimen examined: Indonesia: Bali Island: Baturiti Village-Denpasar City, 69 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 moultouii* 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.


**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; yelllowish to brownish hymenium, 67-92 μm high, clear, paraphyses simple; ascospores hyaline, ascospores 6-8 per ascus, muriform, halonate, 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:** 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 lirelliform 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 lirela, 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).

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).


**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 thalline margin ........... Graphis maritima

3a. Excinipulum laterally carbonized, lacking thalline margin, disc closed, lirellae short, sessile ................. G. nana
3b. Excinipulum completely carbonized ..................... 4

4a. K positive (yellow), stictic acid, lirellae short, erumpent, ascospores 18-34 x 5-7 μm, ascospores 8 per ascus, 8-10 locular, 7-9 septate ............. G. modesta
4b. K negative, no substance, lirellae elongate, prominent, ascospores 14-23 x 5-7 μm, 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 thalline margin, erumpent, ascospores 15-22 x 5-7 μm, ascospores 6-8 per ascus, 7-8 locular, 6-7 septate ........... G. immersicans
6b. Lirellae lacking thalline margin, prominent, ascospores 23-34 x 5-7 μm, 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 Makhiha & 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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