Bangladesh J. Plant Taxon. **25**(2): 159-166, 2018 (December)

A NEW SPECIES OF *GLYPHIS* ACH. AND THREE NEW RECORDS OF *GRAPHIS* ADANS. (GRAPHIDACEAE) FROM BALI ISLAND, INDONESIA

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Keywords: *Glyphis batuana* sp. nov.; Graphidaceae, New records; Taxonomy; Bali Island.

Abstract

Glyphis batuana Hardini, Kasiamdari & *Purnomo* sp. nov. is a new species of lichenized fungus found on the bark of the Frangipani tree (*Plumeria* sp.). The new species from Batuan village (Gianyar districts), Bali Island, Indonesia is described and illustrated. It is characterized by its lirelliform, unbranched ascomata, entire labia, black, open disc with brown pruina, completely carbonized excipulum, 8-spored asci with 8-10 locular ascospores, and lack of secondary substances. A key to species of *Glyphis* Ach. in Indonesia is provided. Three new records of *Graphis* Adans., namely *G. conferta* Zenker, *G. immersella* Mull.Arg. and *G. nilgiriensis* Adaw. & Makhija are also reported.

Introduction

The Island of Bali is located in the central part of Indonesia, corresponding to the province of the same name. The lowland area of Bali has many Frangipani trees (*Plumeria* spp., mostly *P. rubra* L.), native in the Neotropics but cultivated world-wide and in Indonesia these are planted in open areas for religious ceremonies. Frangipani trees have a thin and smooth skin and are often overgrown with crustose lichens, particularly of the family Graphidaceae. Graphidaceae is the largest family of tropical crustose lichens, and consists of 79 genera, including *Glyphis* Ach. and *Graphis* Adans. (Lücking *et al.*, 2014, 2017). During a survey in the lowland areas of Bali (around Batuan Village in Gianyar District), a new species of *Glyphis* was discovered. This genus is characterized by brown-pruinose ascomata and carbonized excipula, together with hyaline, distoseptate, amyloid ascospores. The genus currently includes seven species, namely *G. atrofusca* (Mull. Arg) Lücking, *G. cicatricosa* Ach., *G. dictyospora* Staiger, *G. duriuscula* Stirt., *G. latissima* (Vain.) Zahlbr., *G. substriatula* (Nyl.) Staiger, and *G. scyphulifera* (Ach.) Staiger (Archer, 2009; Lücking *et al*. 2014, 2017).

Glyphis cicatricosa Arc. is the most common and widespread species, with a pantropical distribution. It was reported from Singapore (Sipman, 2003), the Philippines (Tabaquero, 2013), and also from Hong Kong, China (Aptroot, 1999), Australia (Archer, 2004), India (Singh and Sinha, 2010), Bolivia (Flakus *et al*., 2013; Kukwa *et al*., 2013), and Venezuela (Fuenmayor, 2013). In Indonesia, *Glyphis cicatricosa* is reported from Bogor and Cibodas (Sipman, 2003) and from Java (Groenhart, 1936). Other species of *Glyphis* found in Indonesia were also reported by Groenhart (1936), which are *G. verrucosa* Mont. *et* Bosch (Java), *G. javanica* Mull.Arg. (Java), *G. labyrinthica* Ach. (Bogor, Gunung Gede, Java), G. *lactea* Mull.Arg (Bogor), *G. heterostycha* Hepp. (Bogor, Cibodas, Gunung Salak, Java), *G. heteroclyta* Mont. (Bogor, Cibodas, Gunung

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Salak, Java), G. *Leprieurii* Mont (Bogor, Cibodas, Gunung Salak, Java), and *G. tricosa* Ach. (Java).

Graphis is one of the largest genera in Graphidaceae. Groenhart (1936) reported 30 species of *Graphis* found in Indonesia, of which four species were recorded from Bogor (*G. bataviana* A. Zahlbr., *G. bogoriensis* A. Zahlbr., *G. karsteni* A. Zahlbr., *G. schiffneri* A. Zahlbr.), four species from Cibodas (*G. curtiuscula* A. Zahlbr., *G. inamoena* A. Zahlbr., *G. overimii* A. Zahlbr., *G. treubii* A. Zahlbr.) and 22 species from Java (*G. afzelii* Ach., *G. angustata* Eschw., *G. aphanes* Mont. *et* Bosch., *G. cinerea* Fee, *G. duplicata* Ach., *G. elegans* Ach., *G. flavens* Müll.Arg., *G. grammitis* Fee, *G. intricata* Fee, *G. javanica* A. Zahlbr., *G. lineola* Ach., *G. ovata* Mass., *G. radiata* Nyl., *G. regularis* Müll.Arg., *G. rimulosa* Trev., *G. schizograpta* Müll.Arg., *G. scripta* Ach., *G. stenospora* Müll.Arg, *G. subassimilis* Müll.Arg, *G. tenella* var. *flavicans* Müll.Arg, *G. vittata* Müll.Arg, *G. zollingeri* A. Zahlbr.). Whereas, Lücking *et al.*, (2009) reported 20 species of *Graphis*, which were three species from Borneo (*G. dupaxana* Vain., *G. marginata* Raddi, *G. sarawakensis* Hale *ex* Lücking), one species from Celebes (*G. rustica* Kremp.), 12 species from Java (*G. assimilis* Nyl., *G. chlorotica* A. Massal, *G. crassilabra* Müll.Arg., *G. curtiuscula* Zahlbr., *G. duplicata* Ach., *G. flavens* Müll.Arg., *G. hossei* Vain., *G. leptoclada* Müll.Arg., *G. regularis* Müll.Arg., *G. subassimilis* Müll.Arg., *G. submarginata* Lücking, *G. vittata* Müll.Arg.), one species from Krakatau (*G. filiformis* Adaw. & Makhija), one species from Malang Java (*G. leucaenae* Aptroot), and also reported were *G. schiffneri* Zahlbr., and *G. japonica* (Mull.Arg.) A.W. Archer & Lücking. Lücking *et al.* (2014) also states that there were still many lichen family of Graphidaceae that not yet known and predicted to be mostly found in Southeast Asia one of which is Indonesia.

This study aimed to explore lichen Graphidaceae in Batuan village (Gianyar district) and Bunutbolong village (Jembrana district) and reported lichen taxa which were a new species and a new record for Indonesia. Further detail observations of morphological characters, anatomy, chemistry in samples of lichen Graphidaceae, resulted in a conclusion for new species, *Glyphis batuana* Hardini, Kasiamdari & Purnomo. and newly recorded *Graphis conferta* Zenker, *Graphis immersella* Mull. Arg., and *Graphis nilgiriensis* Adaw. & Makhija, which were described in this paper.

Material and Methods

Study area

This research was conducted on the Bali Island with two locations: Gianyar district and Jembrana district (Fig. 1). Gianyar district is located at an altitude of 113 m a.s.l., temperature of 40□C, humidity of 40%, and Jembrana district is located at an altitude of 491 m a.s.l., temperature 25□C, humidity of 70%.

Sample collection and identification

The specimens were collected in July through December 2014 from the Gianyar District and Jembrana District, Bali Island, and deposited in the herbarium of Biology Museum, Faculty of Biology Universitas Gadjah Mada, Yogyakarta, Indonesia. The stereomicroscope (Olympus CX22) at magnifications of 7x to 45x were used for observation of morphological features. The anatomical features were studied under a light microscope XSZ-107BN at magnifications of 40x to 1000X with Optilab microscope digital camera. Thin hand-cut sections of thalli and ascomata were observed in water, 10% KOH and Iodine solutions. Lichen substances were identified by thin layer chromatography (TLC) following standard methods in solvent C and color spot tests as described by Orange *et al.* (2010). 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). The photographs were taken using a Nikon Coolpix S220 digital camera.

Morphological work was carried out on the unknown collected specimen and were done by comparison and assessment of voucher specimen of the new taxa with that of the holotypes, isotypes and specimens the herbaria of images the lichen with The Field Museum (Robert Lücking, 2008, www.discoverlife.org), images the lichens with the Tropical Plant Guides and Discover Life. Specimens were also identified with the relevant literature, *viz.* Hayward (1977), Archer (1999, 2000, 2004, 2005, 2007), Sipman (2003) and Lücking *et al.* (2009, 2012, 2014), and then were consulted for identification and confirmation of specimens diagnosed as new taxa with curator for lichens.

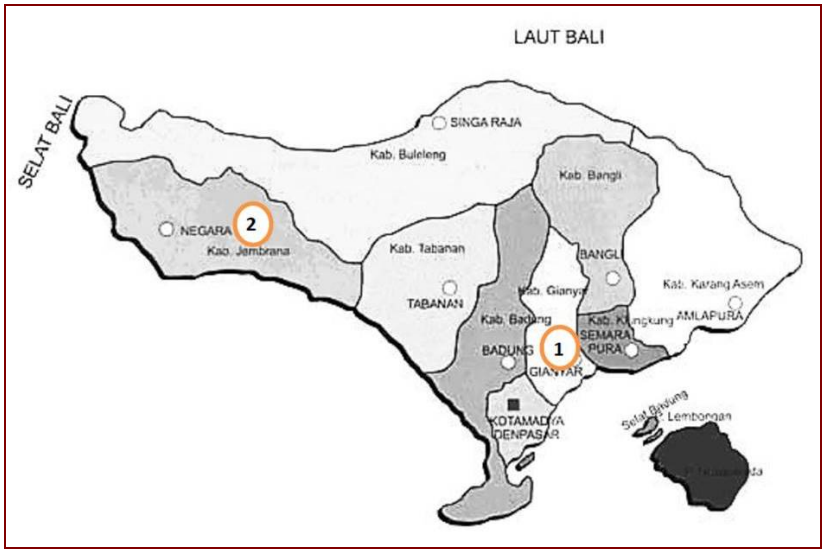

Fig. 1. Study area at the Bali Island. 1. Batuan village (Gianyar districts); 2. Bunutbolong village (Jembrana district).

Results and Discussion

Glyphis batuana Hardini, Kasiamdari & Purnomo, **sp. nov**. (**Fig. 2**).

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Diagnosis: The new species, *Glyphis batuana* differing from *G. atrofusca* (Mull.Arg.) Lücking in the transversely septate ascospores and completely carbonized excipulum and from *G. cicatricosa* in the non-pseudostromatic lirellae and smaller ascospores.

Type: INDONESIA, Bali Island, Gianyar district, Batuan village, on the tree bark of Frangipani (*Plumeria* sp.), house plants and plants by the road, altitude 113 m a.s.l., 8°34’59”S - 115°16’2”E, July 2014, Jun-BG5 (Holotype, MBY).

Thallus corticolous, crustose, 3-5 cm, continuous, whitish green, surface smooth to uneven, rough; crystalline, 100-150 thick in cross-section. Cortex indistinct. Photobiont layer densely interspersed with calcium oxalate crystals, 50-70 um thick. Crystalline layer with cluster of calcium oxalate crystals scattered on the thallus surface and the bottom of the algae layers. Medulla indistinct.

Ascomata numerous, lirelliform, erumpent. Lirellae lacking thalline margin, short to elongate, 1-4 mm long and 0.15 – 0.30 mm broad, sinuous, unbranched to sparsely branched. Labia entire, black. *Disc* exposed, reddish brown with chocolate-brown pruina. Excipulum completely carbonized, in upper part 15-20 µm thick, laterally 15-30 µm thick. Hymenium hyaline, clear, 85-95µm high. Paraphyses unbranched, filiform, hyaline, sometimes pale brown at the tips. Asci clavate, 8-spored, 75-100 x 7-20 µm, I- blue. Ascospores hyaline, fusiform, distoseptate, transversely 7-9 septate, about 19-26 x 5-7 µm, halo not seen, I- blue.

Etymology: The specific epithet of *Glyphis batuana* refers to the location where the new taxon was discovered, Batuan village.

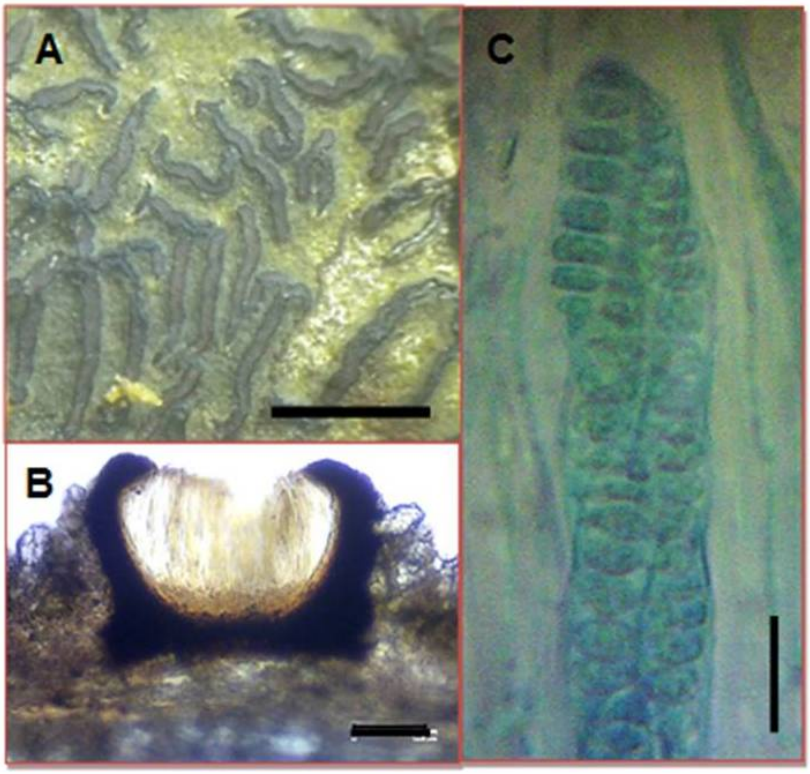

Fig. 2. A-C. *Glyphis batuana* Hardini, Kasiamdari & Purnomo **sp. nov.**. A. Habit; B. Cross section of ascomata; C. Ascospores. Scale bars: A = 2 mm, B = 100 µm; C = 10 µm.

Chemistry: Thallus and medulla K–, P–, C–, KC–, no substances detected in thin-layer chromatography.

Distribution and habitat: The type locality is situated in the lowland area of the Bali Island (113 m a.s.l.). The region has many Frangipani trees for religious ceremonies purposes, are planted in open ground with temperature of 38°C, humidity 40%. The new species is found in the young Frangipani tree that has smooth skin and thin. The new species is dispersed in small patches on thin tree bark with other species of *Graphis* and collected at a height of 1-1.5 m from tree base in exposed condition. The region is considered as eastern paleotropics, similar to other regions in Singapore, The Phillipines, India, Vietnam and Cambodia.

Notes: The new species *Glyphis batuana* is characterized by brownish green thallus, lirellae erumpent, elongate, sinuous and sparsely branched, thalline margin absent or lacking, labium entire and black, disc pruinose brown and exposed, completely carbonized exciple, transversely septate ascospores of about 19-26 x 5-7 µm, 7-8 septate, 8 spored per ascus, hymenium clear and 85-95 µm thick, no lichen substances found (Fig. 2). The organism resembles *G. atrofusca* (Mull.Arg.) Lücking in having ascomata lirelliform, open, scattered, sometimes branched. All species of *Glyphis* have this pruina and generally a carbonized excipulum and mostly lack substances. *G. atrofusca* basically differs in the muriform ascospores and laterally carbonized excipulum. Another similar species of the group is *G. cicatricosa* Ach., which has disc dark reddish brown, completely carbonized, 8 spores asci, that are comparable to *G. batuana*, but is distinct in having an ascomata immersed in conspicuous raised white pseudostromata, stromata rounded, richly branched, crowded and covering the surface of the stromata, thicker hymenium of about 120–160 µm, larger ascospores of 32–55 × 8–12 µm (Staiger, 2002; Archer, 2004). *G. scyphulifera* (Ach.) Staiger, has ascomata reddish brown, exciple completely carbonized, 8 per ascus, but differs by its rounded ascomata and muriform ascospores (Staiger, 2002). *G. substriatula* has elongate lirellae with distinct brown pruina along slit and lacking thalline margin (Straiger, 2005; Lücking *et al.*, 2014).

Key to the genus *Glyphis* in Indonesia

1.	Ascospores transversely septate	2
-	Ascospores muriform	3
2.	Ascomata pseudostromatic; ascospores 32-55 x 8-12 µm	*G. cicatricosa*
-	Ascomata lirellate, not in pseudostromata; ascospores 19-26 x 5-7 µm	*G. batuana* **sp. nov.**
3.	Ascomata rounded, sessile; ascospores 12-15 µm broad	*G. scyphulifera*
-	Ascomata lirellate	4
4.	Excipulum laterally carbonized	*G. atrofusca*
-	Excipulum completely carbonized	*G. substriatula*

New records of *Graphis* Adans.

Three species of *Graphis* (*G. conferta* Zenker, *G. immersella* Mull.Arg. and *G. nilgiriensis* Adaw. & Makhija) have been reported for the first time from Bali, which are new records for Indonesia. There are no previous reports about the collection of these three species of *Graphis* from Bali or any other part of Indonesia.

Graphis conferta Zenker, Pharmaceutische Waarenkunde (Eisenach) 1(3): 166 (1829). (**Fig. 3A**).

Thallus corticolous, crustose, brown, smooth surface shiny; ascomata lirellate, black, short, 0.2-3.0 mm, unbranched, sessile, flat labium, disc covered; basal thalline margin, excipulum completely carbonized, yellow hymenium, 100-110 µm high, clear; ascospores hyaline, 8 spored ascus, 20-28 x 5-7 µm, 6-10 septate, I + blue; No subtances.

Specimen examined: Indonesia: Bali Island: Jembrana district: Bunutbolong village, 491 m a.s.l., on bark of Frangipani (*Plumeria* sp.), July 2014, MBY.

Graphis immersella Mull.Arg. Bull. Herb. Boissier 3: 319 (1895). T: Cairns, Qld, 1893, J. F. Shirley 1793*;* lecto: *G. fide* A.W. Archer, Telopea 8: 281 (1999). (**Fig. 3B**).

Synonym: *Graphis leptalocarpa* A.W. Archer, (*Holotype:* Solomon Islands) Mycotaxon 83: 364 (2002); *G. manhaviensis* Zahlbr. (*Holotype:* China, Handel-Mazzetti, H. 1930. Symbolae Sinica 3:1-254).

Thallus corticolous, crustose, whitish-grey, surface of thallus uneven; ascomata lirellate, immersed, black, short to elongate, 2-5 mm, sparsely branched, lirellae variable; labia entire, non pruinose; disc exposed; lateral thalline margin; excipulum laterally carbonized; I –ve, yellowish to brownish hymenium, 90-115 µm high, clear; ascospores hyaline, 8 spored ascus, 5-7 septate, I + blue, 19-25 x 5-7 µm; K + yellow, thallus containing stictic acid.

The materials examined similar to *G. immersella* which has been described by Archer (1999, 2005) and Lücking *et al.* (2009), but this species has longer lirellae (2-5 mm), some branched dichotom at the end or middle. It has been previously reported in Australia (Archer 1999), and Solomon Islands (Archer, 2007).

Specimen examined: Indonesia: Bali Island: Jembrana district, Bunutbolong village, 491 m a.s.l., on bark of Frangipani (*Plumeria* sp.), July 2014, Jun-BJ12, MBY.

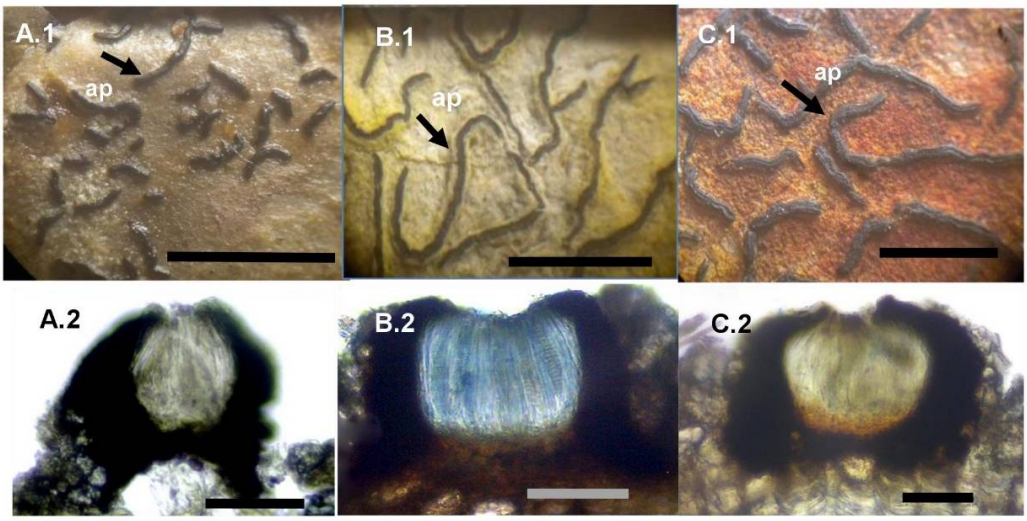

Fig. 3. A. *Graphis conferta* Zenker: A1. Thallus with apothecia (scale=2 mm), A2. Section of apothecium with hymenium, ascus and ascospores (scale=50 µm); B. *Graphis immersella* Mull.Arg.: B1. Thallus with apothecia (scale=2 mm), B2. Section of apothecium with hymenium, ascus and ascospores (scale = 50 µm); C. *Graphis nilgiriensis* Adaw. & Makhija: C1. Thallus with apothecia (scale = 2 mm), C2. Section of apothecium with hymenium, ascus and ascospores (scale = 50 µm); ap = apothecium.

Graphis nilgiriensis Adaw. & Makhija, Mycotaxon 96: 59 (2006). (**Fig. 3C**).

Thallus corticolous, crustose, yellowish-orange, the surface uneven; ascomata lirellate, sessile, black, elongated, irregularly curved, striate, 1- 5 mm long, unbranched; labium entire; disc closed; basal thalline margin; excipulum completely carbonized; yellowish to brownish hymenium, of 74-99 µm high, clear; ascospores hyaline, 8 spored ascus, 5-9 septate, I + blue, 20-50 x 5-8 µm; K + yellow, containing stictic acid.

This species has been reported previously from India (Adawadkar and Makhija, 2006). Thallus colour of this species is yellowish orange, may be experiencing discoloration due to decomposition of chlorophyll (Lücking *et al.*, 2009) and ascomata length 1-5 mm.

Specimen examined: Indonesia: Bali Island: Jembrana district: Bunutbolong village, 491 m a.s.l., on bark of Frangipani (*Plumeria* sp.), July 2014, Jun-BJ12, MBY.

Acknowledgments

We express our thanks to Dr. Robert Lücking (Curator for cryptogams: lichens, fungi, bryophytes), Botany Department, The Field Museum, Chicago, Illinois, USA, for many discussions about the taxa of *Glyphis* and *Graphis* (Graphidaceae).This study is part of a Doctoral dissertation at the Biological Science Program, Faculty of Biology UGM, and funded by the Directorate General of Higher Education (DGHE), Ministry of Education and Culture, Republic of Indonesia for the Scholarship of National Graduate Study Program.

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(Manuscript received on 16 October 2017; revised on 1 November 2018)