Taxonomy and new records of Graphidaceae lichens in Western Pangasinan, Northern Philippines

Weenalei T. Fajardo¹,²* and Paulina A. Bawingan¹

Abstract

There are limited studies on the diversity of Philippine lichenized fungi. This study collected and determined corticolous Graphidaceae from 38 collection sites in 10 municipalities of western Pangasinan province. The study found 35 Graphidaceae species belonging to 11 genera. Graphis is the dominant genus with 19 species. Other species belong to the genera Allographa (3 species), Fissurina (3), Phaeographis (3), while Austrotrema, Chapsa, Diorygma, Dypiolabia, Glyphis, Ocellularia, and Thelotrema had one species each. This taxonomic survey added 14 new records of Graphidaceae to the flora of western Pangasinan.

Keywords: Lichenized fungi, corticolous, crustose lichens, Ostropales

Introduction

Graphidaceae is the second largest family of lichenized fungi (Ascomycota) (Rivas-Plata et al. 2012; Lücking et al. 2017) and is the most speciose of tropical crustose lichens (Staiger 2002; Lücking 2009). The inclusion of the initially separate family Thelotremataceae (Mangold et al. 2008; Rivas-Plata et al. 2012) in the family Graphidaceae made the latter the dominant element of lichen communities with 2,161 accepted species belonging to 79 genera (Lücking et al. 2017). However, the currently circumscribed family is projected to have about 1,850 species due to the rapid rate of new lichen discoveries (Rivas-Plata & Lücking 2013; Lücking et al. 2014). In fact, in 2014, an additional 175 new species of Graphidaceae were reported in one publication alone (Sohrabi et al. 2014).

There are many studies on Graphidaceae lichens all over the world, with most of them done in South America. Significant taxonomical contribution in the Philippines was done by Edvard August Vainio, the father of Philippine lichenology, which led to the description of 118 Graphidaceae in 1921 (Tabaquero et al. 2013). There are currently 459 described Graphidaceae in the country (Parmmen et al. 2012). Most recent surveys resulted in the characterization of six new species (Lumbsch et al. 2011; Tabaquero et al. 2013; Rivas-Plata et al. 2014). In the northwestern part of Luzon in the Philippines (Region 1), an account on the Graphidaceae lichens was conducted only from the Hundred Islands National Park (HINP), Alaminos City, Pangasinan (Bawingan et al. 2014). The study reported 32 identified lichens, including 17 Graphidaceae belonging to the genera Diorygma, Fissurina, Graphis, Thecaria and Thelotrema. Except for this one study in the HINP, there are no other records of lichens in Pangasinan, in particular, the Graphidaceae microlichens, which are commonly found in semi-exposed areas in tropical forests (Lücking et al. 2013).

Hence, this present study continued the inventory of Graphidaceae lichens in Pangasinan, specifically the corticolous Graphidaceae lichens or those that grow on the barks of trees. With the current pace of land-use change and conversion in Pangasinan, there is a need to study these organisms before they become endangered or extinct. Moreover, lichens act as biological indicators (Nimis et al. 1991, Rivas-Plata et al. 2008b) and are known for their medicinal uses (Singh 2011). Hence, there is a need to conserve and preserve them.

Materials and Methods

Study Area and Sampling Sites

The study area included 38 collection sites in 10 municipalities of western Pangasinan namely Agno, Alaminos, Anda, Bani, Bolinao, Burgos, Dasol, Infanta, Mabini and Sual.
Sampling sites represent different ecological areas selected based on their accessibility, safety and security during the time of the visit. Local Department of Environment and Natural Resources (DENR) personnel guided the researchers in the selection of sampling sites. Field surveys were done subsequently after the approval of the municipal mayors.

The sampling sites included coastal tourism areas (beach and islands), riparian zones (rivers and falls), forest reserves, caves, and parks. Fig. 1 shows the various collection sites per locality. The quantitative transect sampling method was employed. Rare, conspicuous and cryptic groups are more likely collected by this type of sampling (Cáceres et al. 2007).

**Sampling Procedures**

*Sampling in forested area.* The collection was done from 10-15 lichen-rich trees along a 100 m distance parallel, but 5 m away from the main trail to a distance of 30 m inward; trees were 5 m apart (Tabaquero et al. 2013).

*Sampling in coastal areas.* At each location, two transects were laid out not less than 1 km apart. A 50 m transect trail was deployed perpendicular to the high tide line. Transect began at the upper limit of the rockweed zone and moved landward to the area at which terrestrial vascular plants or lichens were seen (Brodo & Sloan 2004).

*Sampling in the riparian zones of wetlands.* Riverbanks and wetland areas were sampled similarly to coasts. However, the transect line started from the edge of the bank near the river moving landward (Brodo & Sloan, 2004).

*Sampling near the roads.* A 15 m transect was placed parallel to the road. The collection was done on trees 5 m apart (Klos et al., 2009).

**Lichen Phorophytes and Collection**

Lichen phorophytes were mostly native trees such as *Antidesma bunius* (Linn.) Sprengel, *Calophyllum inophyllum* Linn., *Ficus glomerata* Roxb., *Shorea polysperma* Merr., *Terminalia catappa* Linn. and *Toona calantas* Merill and Rolfe. Other species of trees found in the area were *Acacia auriculiformis* A. Cunn. ex Benth., *Cocos nucifera* L., *Mangifera indica* L., *Syzygium cumini* (L.) Skeels, *Vitex parviflora* Juss. and *Tamarindus indica* L. Older trees were selected for lichen collection because their trunks tend to have more diverse lichen flora compared to younger representatives (Lücking et al. 1996; Fritz et al. 2009).

The selected phorophytes had the following characteristics: freestanding well-lit trees with inclination of the trunk not exceeding 10°; no evidence of disturbance or pathologies and damaged decorticated parts of the trunk (Nimis et al. 2000; Asta et al. 2002; Aprile et al. 2011); and the trunk did not have more than 25% cover of bryophytes (Castello & Skert 2005).

A sampling grid (microplot) was laid onto the phorophyte consisting of four vertical ladders of 10 cm x 50 cm, each divided into five 10 cm x 10 cm unit areas. Each of the four ladders was placed to one of the four cardinal directions (north, south, east, and west) with the base at 1.5 m high from the ground (Castello & Skert 2005; Cáceres et al. 2007; Aprile et al. 2011). Collection sites and phorophytes were photographed using Nikon D3200 DSLR and Garmin GPSMAP 64sc, which were automatically geotagged.

The standard protocol for field collection was followed to avoid or cause minimal damage to the tree trunk (Nayaka 2014). Colorless nail polish was applied to scraped areas to prevent the entry of pathogenic organisms. At most three thalli per species per collection site were collected for morpho-anatomical examination and vouchering. Examination and segregation of specimens resulted in 1,232 lichen collections. The herbarium vouchers are deposited at the Pangasinan State University (PSU) - Lingayen Biology Laboratory.

**Lichen Characterization and Identification**

The features essential for species identification of Graphidaceae lichens are the following: color and texture of the thallus; characteristics of ascomata such as their morph, form, emergence, branching, color, disc color, presence of pruin, margin, striation, and color of the rim; carbonization of excipulum and hypothecium; inspersion of its hymenium; number of ascospores in every ascus; the shape, locule number, length and width, septation, color and color reaction to Iodine solution of ascospores; and presence of lichenic acid (Staiger 2002; Lücking et al. 2009; Tabaquero et al. 2013).

Thin free-hand sections of ascomata and other structures were made using a sharp razor blade (Joshi et al. 2018). The sections were placed in water on a plain slide and covered with a glass slip and examined under a compound microscope using a Motic binocular compound microscope (Speed Fair Co., Ltd, Hong Kong) with 400x magnification. All morphometric measurements (e.g. spore size) were done in water mounts under the compound microscope using a calibrated eyepiece with a total magnification of 400x. Photographs of images viewed under the oil immersion objective were taken using Huawei Nova5T smartphone.

Spot color tests were performed for the initial determination of the lichen acids present using 10% aqueous KOH solution (K test), an aqueous solution of Ca(ClO)₂ (C test), an ethanolic solution of paraphenylenediamine (P test), and Iodine solution (I test). The stability of the KOH was tested on *Graphis persicina* ascocarp; a color change to green in the
**Figure 1.** Map of the collection sites per locality in western Pangasinan.
hymenium indicated a stable solution. A drop of each of these solutions was placed in separate areas on the thallus surface or the medulla then observed as to the color reactions (Hale 1967; Elix 1992). A color change to yellow using K test indicated the presence of stictic acid; under the microscope, this color change was observed as a yellow effusion along the thalline margin. A color change in the medulla from yellow to red in the K test indicated the presence of norstictic acid or salazinic acid. C test or KC+ test showing a rose pink color change indicated the presence of gypogrocytic acid. On the other hand, change in the color to blue-violet, red-violet upon addition of iodine solution indicated an amyloid property of ascospore or hymenium.

An extremely sensitive test done was observing crystal formation under the compound microscope (May et al. 2001). Thin sections of the thallus were placed on a glass slide and a drop of a reagent (K or P) was added to observe color reactions and the formation of crystals (Lücking 2009; Benatti 2012). Under the microscope, a K+ yellow effusion without crystallization indicated the presence of stictic acid; the formation of long reddish needle-shaped crystals with a stellate formation indicated the presence of salazinic acid; norstictic acid crystals were shorter with no star-like formation. A P+ test that indicated the presence of protocetraric acid shows formation of red to deep red granules (not needle-shaped crystals).

Using the morpho-anatomical features and chemistry of the lichens, the collected specimens were identified using various taxonomic keys (Staiger 2002; Archer 2006, 2009; Sipman 2005, 2008a, 2008b; Rivas-Plata et al. 2008; Lücking et al. 2009, Rivas-Plata et al. 2010; Joshi et al. 2013, Lücking et al. 2014; Lücking et al. 2016). A key for the identification of the lichens in the Graphidaceae from western Pangasinan was prepared.

Results

Field collections in the 38 sampling sites in western Pangasinan led to the identification of 35 Graphidaceae species distributed in 11 genera (Table 1). There were four thelotremoid genera comprised of Austrotrema, Chapsa, Ocellularia and Thelotrema. Most of the identified Graphidaceae were graphiphid lichens with 31 species belonging to the genera Allographa, Diorygma, Dyplolabia, Fissurina, Glyphis, Graphis and Phaeographis. Graphis was the dominant genus with 17 identified species and 2 unidentified. This total number of species indicated low species richness considering the large number of sampling areas. Evaluation of existing publications on Graphidaceae lichens showed that 14 new records (indicated with an asterisk) are included in this study.

Thelotremoid Graphidaceae

Genus Austrotrema

It is a new genus described by Medeiros et al. (2017). The genus name refers to the occurrence of the type species in the Australian–Southeast Asian region. The genus is circumscribed by small, pore-like apothecia with double margin, non-amyloid to faintly amyloid (I+) ascospores, and presence of stictic acid (Medeiros et al. 2017).

*Austrotrema bicinctulum* (Nyl.) I. Medeiros, Lücking & Lumbsch (2017)

It was formerly placed under the genus Thelotrema but was separated due to molecular phylogenetic evidence (Medeiros et al. 2017). *A. bicinctulum* was previously collected in Thailand (Schumm & Aptroot 2012; Buaruang et al. 2017). It has lepadinoid ascocarp, which is immersed to erumpent with free excipulum and double margin (Rivas-Plata et al., 2010). Its ascospores are transversely septate, hyaline and non-amyloid (I-), 18-23 x 5-8 μm in size with 8-10 locule. Stictic acid (K+Y, P+, C-) present. (Fig. 2a)

Genus Chapsa

The genus Chapsa was described in 1860 (Joshi et al. 2018); however, the group was brought up lately by Frisch et al. (2006) to include species earlier classified in Thelotrema, Chroodiscus, Ocellularia, and Myriotrema. The genus Chapsa accommodates a group of theletremoid lichens with a trentepohlioid photobiont, chroodiscoid ascomata, an exciple with lateral paraphyses, and almost thin- to thick-walled ascospores (Messuti et al. 2010). It comprises 62 species with pantropical or subtropical distribution (Lücking et al. 2017).

*Chapsa indica* A. Massal. (1860)

The species has records in Australia, Brazil, Guyana, Malaysia, Singapore and Thailand (Lumbsch et al. 2014; The Catalogue of Life Partnership 2018). *C. indica* is recognized by its lepocarpedid ascomata with brown disc covered by thick white-felt pruina. This type of ascomata is immersed to erumpent with irregular almost erect marginal lobes that usually break apart (Rivas-Plata et al. 2010). Its hymenium is non-amyloid. Ascospores are transversely septate, hyaline and non-amyloid (I-), 39.91 x 5.8 μm in size with 25-27 locules. No lichenic acid (K-, P-, C-) detected. (Fig. 2b)

Genus Ocellularia

Ocellularia is the largest genus in the Graphidaceae after
**Table 1.** Graphidaceae microlichens in Western Pangasinan

<table>
<thead>
<tr>
<th>Genera</th>
<th>Graphidaceae species</th>
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| **Allographa** Chevall. (1824) | *Allographa fujianensis* (Z.F.Jia & J.C.Wei)  
Lücking & Kalb (2018)  
*Allographa laubertiana* (Fée) Lücking & Kalb (2018)  
*Allographa pilarensis* (Cáceres & Lücking)  
Lücking & Kalb (2018) |
| **Austrotrema** I. Medeiros, Lucking & Lumbsch (2017) | *Austrotrema bicinctulum* (Nyl.)  
I. Medeiros, Lucking & Lumbsch (2017) |
| **Chapsa** A.Massal. (1860) | *Chapsa indica* A. Massal. (1860)  
*Chapsa* sp. |
| **Diorygma** Eschweiler (1824) | *Diorygma hieroglyphicum* (Pers.)  
Staiger & Kalb (2004) |
| **Dyplolabia** A.Massal. (1854) | *Dyplolabia afzelii* (Ach.) A. Massal. (1854)  
*Fissurina* Fée (1825)  
*Fissurina comparilis* (Nyl.) Nyl. (1888)  
*F. “chroodiscoides”* Sipman (2008)  
*Fissurina* sp.  
| **Graphis** Adans. (1763) | *Graphis analoga* (Nyl.) Zahlbr. (1909)  
*G. cincta* (Pers.) Aptroot (2009)  
*G. consimilis* Vain (1907)  
*G. dendrogramma* Nyl. (1875)  
*G. furcata* Fée (1825)  
*G. glaucescens* Fée (1824)  
*G. immersicans* Archer (2001)  
*G. imshaugii* M. Wirth & Hale (1978)  
*G. leptocarpa* Fée (1824)  
*G. lineola* Ach. (1810)  
*G. modesta* Zahlbr. (1911)  
*G. nanodes* Vain. (1921)  
*G. persicina* G. Mey. & Flot. (1843)  
*G. pinicola* Zahlbr. (1930)  
*Graphis* sp.1  
*Graphis* sp. 2  
*Ocellularia* G. Mey (1825)  
*Ocellularia lumbschii* S. Joshi & Hur (2015) |
*P. intricans* (Nyl). Staiger (2002)  
*P. nylanderi* (Vain.) Zahlbr. (1923) |
| **Thelotrema** Ach. (1803) | *Thelotrema adjectum* Nyl. (1886) |
Graphis, with 400 currently recognized species (Lücking et al. 2017). The genus is characterized by having a brown to carbonized excipulum lacking periphysoid and a carbonized columella (da Silva Cáceres 2006).

*Ocellularia lumbschii* S. Joshi & Hur (2015)

It was first collected in Vietnam (Joshi et al. 2015). This species has ocellularioid ascocarp, clear hymenium, apically carbonized exciple, and apically carbonized columella. Ascospores are transversely septate, hyaline and amyloid (I+), 15-29 x 7-8 μm in size with 4-10 locules. Stictic acid (K+, P-, C-) present. (Fig. 2c)

**Genus Thelotrema**

There are 106 acknowledged species of *Thelotrema* worldwide (Lücking et al. 2017). The genus *Thelotrema* is circumscribed by the lepadinoid or rarely urceolarioid or ocellularioid apothecia, with more or less free excipulum and a distinct split between excipulum and thalline margin, thalline margin usually entire but free excipulum often undulate to fissured; ascospores often with thick wall (Rivas-Plata et al. 2010)

*Thelotrema adjectum* Nyl. (1886)

It has records in Cuba, India, and the USA (The Catalogue of Life Partnership 2018). It is recognized by round and immersed ascocarps with flesh-colored inner excipulum, small and roundish to irregular pores. It has clear hymenium, hyaline and amyloid (I+ red-violet) muriform ascospores with 31-47 x 5-8 μm size and 10-12 x 2-4 locules. No lichen acid (K-, P-, C-) detected. (Fig. 2d)

**Graphidoid Graphidaceae**

### Genus Allographa

The formal placement of *Allographa* into the Graphidaceae led to the transfer of 182 Graphis and Hemithecium species in this genus (Lücking & Kalb, 2018). Species under the genus have usually simple, prominent, broader, completely carbonized and often with striate lirellae. They have large ascospores with numerous septa and exhibit type B (asci and ascospores hardly discernible because of large and irregular oil droplets) hymenium inspersion (Lücking et al. 2017). However, no
Figure 3. Morphoanatomical features of graphidoid Graphidaceae under stereomicroscope (20x) and compound light microscope (400x). (a) muriform ascospore of *A. fujianensis* (b) uncarbonized lirella of *A. laubertiana* (c) muriform ascospore of *A. pilarensis* (d) radially branched lirellae of *G. imshaugii* (e) completely carbonized excipulum of *G. imshaugii* (f) scripta morph of lirellae of *G. modesta* (g) striated lirellae of *G. nilgiriensis* (g) scripta morph of lirellae of *G. sundarbanensis* (h) brown ascospore in inspersed hymenium of *P. nylanderi* (i) uncarbonized lirellae with pruinose disc of *P. caesioradians*. 
particular character or certain combination of characters would easily demarcate these genera. We have three species with new records, namely *A. fujianensis* with a record in China, *A. laubertiana* documented in Australia, Dominica and Peru (Staiger 2002; Archer 2009) and *A. pilarensis* in Brazil (The Catalogue of Life Partnership 2018).


The lirella of this species has *hossei*-morph characterized by erumpent to prominent and sparsely branched entire labia with basal thalline margin (Rivas-Plata *et al.* 2010). It has also clear hymenium, completely carbonized exciple, hyaline and amyloid (I+) muriform ascospores with 23-39 x10-13 μm size and 8-10 x 2-4 locules. Stictic acid (K+Y, P-, C-) present. (Fig. 3a)

*Allographa laubertiana* (Fée) Lücking & Kalb (2018)

It is characterized by lirellate ascomata with raised thalline margins concolorous with the thallus. It has clear hymenium and non-carbonized exciple. Ascospores are hyaline, amyloid (I+) transversely septate with 26-42 x 7-8 μm size and 8-14 locules. No lichenic acid (K-, P- & C-) detected. (Fig. 3b)

*Allographa pilarensis* Cáceres & Lücking (2007)

It has ascocarps that are *negrosina*-morph characterized by prominent complete thin thalline margin and sparsely branched lirellae. The hymenium is inspersed and with laterally carbonized exciple. Ascospores are hyaline and amyloid (I+) muriform with 78-96 x 21-24 μm size and >8x2-4 locules. No lichenic acid detected. (Fig. 3c)

**Genus Dyplolabia**

This genus, initially placed under *Graphis* is distinguished by the erumpent to prominent ascomata forming labia, with thick carbonization and thick white cover containing lecanoric acid (Kalb *et al.* 2016). Ascospores are hyaline and non-amylloid (I-) with 3-septate lenticular lumina (Sipman 2008a). There are only four known species of *Dyplolabia* (Lücking *et al.* 2017). Of these four, the Philippines has contributed one new species, *Dyplolabia dalvaiwiana* described in 2016 with the type specimen coming from Mt. Palali, Nueva Vizcaya (Kalb *et al.* 2016).

**Dyplolabia afzelii** (Ach.) A. Massal. (1854)

It has lateral carbonization with labia thickly pruinose. Ascospores are transversely septate with 13-26 x 7-8 μm size and 4-locule or 3-septate. Lecanoric acid is present in the pruina of the ascocarp (K-, P-, C+ red).

**Genus Fissurina**

*Fissurina* comprises 160 species worldwide (Lücking *et al.* 2017). Many species have recently been added to the genus as a consequence of a molecular and phylogenetic revision of the Graphidaceae (Rivas-Plata *et al.* 2012a). The genus accommodates species with mostly slit-like lirellae (Archer 2009; Staiger 2002).

**Fissurina comparilis** (Nyl.) Nyl. (1888)

This is recognized by elongate ascocarp seemingly labiate, however, fissurinoid with thin, dark-brownish margins and closed above the disc. Ascospores are hyaline to faintly brown, transversely septate with 10-16 x 7-8 μm size and 4-locules. Stictic acid (K+Y, P-, C-) present.

**Fissurina “chroodiscoides”** Sipman (2008)

The ascocarp of this species is *dumastii*-type, which is concolorous with thallus outside and conspicuously white inside. Ascospores are hyaline, non-amylloid (I-) muriform with 20-26 x 10-11μm size and 6-8 x 2-3 locule. No lichen acids detected.

**Fissurina sp. 1**

This species is distinguished by aggregate, radiate lirellae, forming a pseudostroma (ascocarp with several discs) with inconspicuous and gaping labia. Ascospores are hyaline to faintly brown, amyloid (I+ blue-violet) transversely septate with 15-17 x 7-8 μm size and 4-locules. No lichen acid detected.
Genus *Glyphis*

The genus contains seven known species (Lücking et al. 2017). The distinguishing features under the genus include exposed disc, with brown powdery cover (brown pruina) and anastomosing paraphyses. Hymenium is clear with brown granular tips and thick jelly-like walls (Staiger 2005; Sipman 2008a). Ascospores are continuously hyaline.


This species is recognized by rounded sessile ascocarps with dark brown disc; hyaline, I+ blue-violet muriform ascospores, 26-29 x 10-13 μm size, 8-10x 2-4 locules. No lichen acid detected.

Genus *Graphis*

Recent molecular and data mining placed 271 species of *Graphis* in the core Graphidaceae (Lücking et al. 2018). The thallus of *Graphis* is white-gray caused by abundant calcium oxalate crystals in or above the photobiont layer, a more or less prosoplectenchymatous cortex, extremely rarely sorediate or insidiate, but having distinctly lirellate apothecia (Lücking 2009). Various species of the genus are distinguished by the lirella morph combinations, spore characteristics, and chemistry. We have seven (7) new records out of the 19 species for *Graphis*, namely: *G. distincta* with previous records only in India (Lücking et al. 2009); *G. imshaugii* previously known only in New Mexico; *G. modesta* in Solomon Islands and India; *G. nilgiriensis* in India and *G. sundarbanensis* documented from Brazil, Fiji, Papua, Seychelles and Vanuatu (The Catalogue of Life Partnership 2018).

*Graphis* (Nyl.) Zahlbr. (1909)

It is characterized by erumpent to prominent, sparsely to irregularly branched lirellae (*hossei*-morph), clear hymenium, laterally carbonized exciple, hyaline and amyloid (I+) muriform ascospores, 21-26 x 7-8 μm size and 4-6 x 2-4 locules. Norstictic acid present (K+Y then forming red crystals with no star-like formation, P+, C-).

*Graphis cincta* (Pers.) Aptoort (2009)

It has *lineola*-morph lirellae characterized by short and sparsely branched ascocarp with lateral thalline margin, concealed disc and entire non-pruinose labia (Lücking et al. 2009). It has inspersed hymenium, laterally carbonized exciple, hyaline and amyloid (I+) transversely septate ascospores, 26-39 x 13-16 μm size, 8-10x 2-4 locules. Norstictic acid present.

*Graphis consimilis* Vain. (1907)

It is distinguished by erumpent to prominent, sparsely to irregularly branched lirellae (*hossei*-morph), clear hymenium, laterally carbonized exciple, hyaline and amyloid (I+) muriform ascospores with 20-42 x 13-16 μm size and 8-10x 2-3 locules. No lichen acid detected.

*Graphis dendrogramma* Nyl. (1875)

It has *caesiella* to *dendrogramma*-morph ascocarp characterized by radiately branched lirellae, concealed disc and non-pruinose entire labia. It has also clear hymenium, laterally carbonized exciple, hyaline and amyloid (I+) transversely septate ascospores with 15-31 x 7-8 μm size and 6-10 locules. Stictic acid present (K+Y, P+, C-).


It has *scripta*-morph ascocarps characterized by sparsely or radiately lirellae, exposed disc and pruinose entire labia. It has clear hymenium, laterally carbonized exciple, hyaline and amyloid (I+) transversely septate ascospores with 18-21 μm size and 6-8 locules. Stictic and protocetraric acids detected (K+Y, P+, C-).

*Graphis furcata* Fée (1825)

It is recognized by corticated thallus, sparsely to irregularly branched lirellae, immersed to erumpent, with lateral thalline margin, pruinose entire labia, concealed disc (*caesiella*-morph ascocarp), clear hymenium, lateral carbonization of exciple, hyaline and amyloid (I+) transversely septate ascospores with 18-26 x 7-8 μm size and 8-10 locules. No lichenic acid detected.

*Graphis glaucescens* Fée (1824)

It has *glaucescens*-morph ascocarps characterized by erumpent to prominent, sparsely to irregularly branched lirellae, concealed disc and pruinose entire labia. It is recognized also by its clear hymenium, apically carbonized exciple, hyaline and amyloid (I+) transversely septate ascospore with 31-45 x 7-8 μm size and 8-10 locules. No lichenic acid detected.

*Graphis immersicans* Archer (2001)

Ascocarps are *lineola* to *deserpens*-morph, disc not exposed, clear hymenium, completely carbonized exciple, hyaline and amyloid (I+) transversely septate ascospores with 18-29 x 7-8 μm size and 6-8 locules. No lichenic acid detected.

*Graphis imshaugii* M. Wirth & Hale (1978)

It has very long and radiately branching lirellae (*dendrogramma*-morph), labia pruinose, disc concealed, clear hymenium, completely carbonized exciple, hyaline and amyloid (I+) transversely septate ascospores with 13-31 x 7-8 μm size.
and 6-8 locules. Stictic acid present. (Figs. 3d, 3e)

* Graphis leptocarpa Fée (1824)  
  Ascocarps are lineola-morph, disc not exposed, inspersed hymenium, laterally carbonized exciple, hyaline and amyloid (I+) transversely septate ascospores with 23-26 x 7-8 μm size, 6-8 locules. Stictic acid present.

* Graphis lineola Ach. (1810)  
  Ascocarps are lineola-morph, disc not exposed, inspersed hymenium, laterally carbonized exciple, hyaline and amyloid (I+) transversely septate ascospores with 23-31 x 7-8 μm size, 8-10 locules. No lichen acid detected.

* Graphis modesta Zahlbr. (1911)  
  It has ascocarps that are either dendrogramma- or lineola-morph, clear hymenium, completely carbonized exciple, hyaline and amyloid (I+) transversely septate ascospore with 15-21 x 7-10 μm size, 8-10 locules. Stictic acid (K+Y, P+, C-) present. (Fig. 3f)

* Graphis nanodes Vain. (1921)  
  Ascocarps are lineola-morph, clear hymenium, laterally carbonized exciple, hyaline and amyloid (I+) muriform ascospores with 26-39 x 10-13 μm size, 8-10 x 2-4 locules. No lichen acid detected.

* Graphis nilgiriensis Adaw. & Makhija (2006)  
  It has striatula-morph ascocarp characterized by prominent, sparsely branched lirellae lacking thalline margin, concealed disc and non-pruinose striated labia. It has also clear hymenium, completely carbonized exciple, hyaline and amyloid (I+) transversely septate ascospores with 18-26 x 5-6 μm size, 8-10 locules. Stictic acid present. (Fig. 3g)

* Graphis persicina G. Mey. & Flot. (1843)  
  It is recognized by its negrosina-morph lirellae, hymenium and epithecium inspersed with red pigments, laterally carbonized exciple, hyaline and amyloid (I+) transversely septate ascospores with 26-44 x 7-10 μm size, 9-15 locules. Isohypocrelline present (K+ Green, P+, C-).

* Graphis pinicola Zahlbr. (1930)  
  It is recognized by its lineola-morph lirellae, clear hymenium, laterally carbonized exciple, hyaline and amyloid (I+) transversely septate ascospores with 15-34 x 7-8 μm size, 6-10 locules. No lichenic acid detected.

  It is identified by its scripta-morph lirellae, exposed disc, clear hymenium, laterally carbonized exciple, hyaline and amyloid (I+) transversely septate ascospores with 18-23 x 7-8 μm size, 8-14 locules. Stictic acid detected. (Fig. 3h)

* Graphis sp 1.  
  It has negrosina-morph ascocarps with discs widely exposed. The hymenium and epithecium are inspersed with red pigments. Ascospores are hyaline, amyloid (I+) transversely septate with 28-56 x 7-8 μm size, 8-15 locules. Isohypocrelline (K+ Green, P+, C-) present. 

* Graphis sp 2.  
  It has lineola-morph, immersed with simple to sparsely branched lirellae sometimes separated by thallus by slits. It is also characterized by clear hymenium, laterally carbonized exciple, and hyaline amyloid (I+) transversely septate ascospores with 26-31 x 5-8 μm size, 8-10 locules. Stictic and protocetraric acid detected (K+Y, P+, C-).

**Genus Phaeographis**

The genus Phaeographis with 180 species of lichens (Lücking *et al.* 2017) is characterized by simple to irregularly branched, rarely discoid, open lirelliform ascomata, disc pruinose or epruinose. We have two new records: *Phaeographis caesioradians* (Leight.) A.W. Archer (2005) previously known only in the Dominican Republic, Indonesia, Malaysia, Papua New Guinea, and Singapore and, *Phaeographis nylanderi* (Vain.) Zahlbr. (1923) collected only in Singapore and Taiwan (Aptroot & Sparrius 2011; The Catalogue of Life Partnership 2018).

* Phaeographis caesioradians (Leight.) A.W. Archer (2005)  
  It is recognized by its stellate branched ascocarps with margins forming a whitish rim around the exposed, grey, flat disc. It has also clear hymenium, light brown, muriform ascospores, 28-34 x 10-16 μm, 4-6 x 1-2 locale. No lichenic acid detected. (Fig. 3i)

* Phaeographis intricans* (Nyl.) Staiger (2002)  
  Ascocarps are densely stellate-branched, raised, stromatoid, widely exposed, grey flat disc. It has clear hymenium, non-carbonized exciple, hyaline to light brown, transversely septate ascospores, 15-18 x 5-8 μm, 4 locules. Norstictic acid present.

* Phaeographis nylanderi* (Vain.) Zahlbr. (1923)  
  Ascocarps are round to slightly elongate, branched, with raised margins forming a dark rim around the widely exposed,
Key to the species of Graphidaceae lichens in Western Pangasinan

1a Ascomata elongate, sometimes rounded, excipulum hyaline to carbonized, usually prosplectenchymatous, periphysoids often absent 2 (Graphidoid-type)
1b Ascomata rounded to rarely elongate, excipulum hyaline, rarely carbonized, usually paraplectenchymatous, periphysoids often present 31 (Thelotremaoid-type)

2a Apothecia rounded to elongate, opening by irregular cracks in thallus, resulting in irregularly-shaped ascoecarp when mature (fissurinoid) 3
2b Apothecia not fissurinoid 5

3a Ascospores transversely-septate 4
3b Ascocarp immersed, spores muriform, no lichen acids detected 44, “chroodiscoides”

4a Ascocarp pseudostromatous, labia inconspicuous and gaping, spores 15-17 x 7-8 μm 96-21 μm, Phaeographis caesioradians
4b Ascocarp not pseudostromatous, labia partly carbonized, spores 10-16 x 7-8 μm 8 µm, Phaeographis intricans

5a Lirellae sessile, rounded, disc pruinose brown, spores muriform, no lichen acids detected 6
5b Lirellae lirellate, ascospores transversely septate to muriform, may contain lichenic acids 7

6a Lirellae with open disc, hymenium I+ blue, spores muriform and large, 44-130 x 15-26 μm 17 x 7 μm, Diorygma hieroglyphicum
6b Lirellae open to exposed disc, hymenium I-, spores transversely septate, not as large 7

7a Lirellae non-carbonized 8
7b Lirellae carbonized 10

8a Lirellae elongate, simple and prominent, spores transversely septate, 26-42 x 7-8 μm, 8-14 locules 9
8b Lirellae rounded to stellate-branched, spores transversely septate to muriform 10

9a Lirellae stellate-branched 10
9b Lirellae rounded to slightly elongate, not stellate-branched, spores muriform 22 µm, Phaeographis nylanderi

10a Spores muriform, brown, 28-34 x 10-16 μm 22-34 µm, Phaeographis caesioradians
10b Spores transversely septate, hyaline to faintly brown, 15-18 x 5-8 μm 15-20 µm, Phaeographis leptocarpa

11a Lirellae apically carbonized, spores transversely septate, 31-45 x 7-8 μm no lichenic acids detected 12
11b Lirellae laterally to completely carbonized 12

12a Lirellae laterally carbonized 13
12b Lirellae completely carbonized 22

13a Hymenium inspersed 14
13b Hymenium clear 18

14a Hymenium without pigment 15
14b Hymenium with pigment 17

15a Lirellae negrosina-morph, large ascospores, 78-96 x 21-24 μm 8-10 x 2-5 μm, Allographa laubertiana
15b Lirellae different types, spores not as large 16

16a K+ Y, stictic acid present 18 x 7-8 μm, Graphis lineola
16b K-, stictic acid absent 18 x 7-8 μm, Graphis leptocarpa

17a Lirellae negrosina-morph, disc not widely opened, K+ green (Isohypocrelline present) 20 x 7-8 μm, Graphis persicina
17b Lirellae irregularly branched, widely opened disc, K+ green (Isohypocrelline present) 19 x 7-8 μm, Graphis sp. 1

18a Ascospores muriform 21
18b Ascospores transversely septate 21
19a Lirellae *hossei*-morph with basal thalline margin ______________________________________________________________ 20
19b Lirellae *lineola*-morph with lateral thalline margin __________________________________________________________ 20
Graphis nanodes
20a Norstictic acid present, K+ red ____________________________________________________________ 20
20b Norstictic acid absent, K- _______________________________________________________________ 22
Graphis analoga
21a P-, protocetraric acid absent _______________________________________________________________ 22
21b P+, protocetraric acid present ____________________________________________________________ 26
Graphis consimilis
22a Stictic acid present, K+ ____________________________ 20
Graphis dendrogramma
22b Stictic acid absent ____________________________________________________________ 23
Graphis nanodes
23a Labia pruinose ___________________________________________________________ 24
23b Labia non-pruinose or rarely thinly white-pruinose ____________________________________________ 25
Graphis sundarbanensis
24a Disc exposed, *scripta*-morph ____________________________________________________________ 26
24b Disc concealed, *caesiella*-morph __________________________________________________________ 27
Graphis cincta
25a K+ red, norstictic acid present ____________________________________________________________ 27
Graphis pinicola
25b K- norstictic acid absent _________________________________________________________________ 28
Graphis sp. 2
26a Disc concealed ____________________________________________________________ 29
26b Disc open ____________________________________________________________ 30
Graphis distincta
27a Labia striae ____________________________________________________________ 30
27b Labia entire ____________________________________________________________ 31
Graphis nilgiriensis
28a K+Y, stictic acid present _________________________________________________________________ 32
28b K-Y, stictic acid absent _________________________________________________________________ 33
Graphis modesta
29a Disc exposed ____________________________________________________________ 33
29b Disc concealed ____________________________________________________________ 34
Graphis imshaugii
30a Lirellae *caesiella*-morph ____________________________________________________________ 34
30b Lirellae *hossei*-morph ____________________________________________________________ 35
Allographa fujianensis
31a Ascocarp erumpent to prominent with thick thalline margin and narrow pore (ocellularioid), with apically carbonized columella, K+ yellow, stictic acid ____________________________________________________________ 31
Ocellularia lumbschii
31b Ascocarp not showing same features, with or without lichenic acids ____________________________________________ 32
Chapsa indica
32a Ascocarp immersed-erumpent and open by irregular marginal lobes that remain erect or break apart (leprocarpoid), disc pruinose, ascospores large, no lichenic acids detected ____________________________________________ 33
Chapsa indica
32b Ascocarp immersed-erumpent to prominent, with free excipulum and distinct double margin, thalline margin bulging and entire (lepadinoid) ____________________________________________________________ 34
Austrotrema bicinctulum
33a Inner excipulum thin, entire to slightly split, concolorous with the thallus, K+ yellow, with stictic acid, spores medium, transversely septate ____________________________________________________________ 35
Austrotrema bicinctulum
33b Inner excipulum thick, apothecial disc flesh-colored, spores larger, muriform, no lichenic acids detected __ Thelotrema adjectum
dark brown, flat disc, hymenium inspersed. Ascospores are hyaline to light brown, oblong muriform, 24-29 x 7-8 μm, 6-18 x 2-4 locules. No lichen acid detected. (Fig. 3)

**Conclusion and Recommendations**

Over-all, there is a low number of Graphidaceae species collected in western Pangasinan. The presence of new records signifies that there are still undiscovered species in the area. Further survey of the Graphidaceae microlichens in the whole Pangasinan province should be undertaken.

**Acknowledgments**

The researchers would like to express their gratitude to Department of Environment and Natural Resources-Region 1 for the granting of gratuitous permits, Commission on Higher Education for the dissertation grant given to the first author, local government units of western Pangasinan for their assistance during the field surveys and the Pangasinan State University-Lingayen Campus for the laboratory assistance.

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