DIVERSITY AND MOLECULAR PHYLOGENY OF THE LICHEN GENUS *GRAPHIS* (GRAPHIDACEAE) IN THAILAND

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A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy in Environmental Biology

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ความหลากหลายและสายสัมพันธ์ทางวิวัฒนาการเชิงโมเลกุลของไลเคน สกุล *Graphis* (Graphidaceae) ในประเทศไทย

นางสาวอรัณย์ภัค พิทักษ์พงษ์

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรดุษฎีบัณฑิต

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DIVERSITY AND MOLECULAR PHYLOGENY OF THE LICHEN GENUS *GRAPHIS* (GRAPHIDACEAE) IN THAILAND

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อรัณย์ภัค พิทักษ์พงษ์ : ความหลากหลายและสายสัมพันธ์ทางวิวัฒนาการเชิงโมเลกุลของไลเคน สกุล *GRAPHIS* (GRAPHIDACEAE) ในประเทศไทย (DIVERSITY AND MOLECULAR PHYLOGENY OF THE LICHEN GENUS *GRAPHIS* (GRAPHIDACEAE) IN THAILAND) อาจารย์ที่ปรึกษา : รองศาสตราจารย์ ดร.หนูเดือน เมืองแสน, 285 หน้า.

ไลเคนสกุล *Graphis* เป็นไลเคนสกุลหนึ่งของวงศ์ Graphidaceae เป็นไลเคนในกลุ่ม crustose ซึ่งไลเคนวงศ์ Graphidaceae ส่วนใหญ่พบในเขตร้อน ซึ่งประเทศไทยเป็นประเทศที่มีอากาศร้อนชื้นมีระบบนิเวศหลากหลายเป็นผลให้มีไลเคนชนิด *Graphis* ได้หลากหลาย แต่ยังไม่มีการศึกษาของสกุลนี้อย่างเป็นระบบ วัตถุประสงค์ของการศึกษาเป็นการสำรวจความหลากหลายของไลเคนสกุล *Graphis* ในสภาพป่าที่แตกต่างกันและเป็นการหาความสัมพันธ์ของปัจจัยสิ่งแวดล้อม สำหรับการศึกษาด้านอนุกรมวิธานของไลเคนสกุล *Graphis* ในประเทศไทย โดยทำการสุ่มเก็บตัวอย่างใน 12 พื้นที่ (11 อุทยาน และ 1 สถานีวิจัย) นำไปจัดจำแนกชนิดไลเคนในห้องปฏิบัติโดยการศึกษาทางสัณฐานวิทยา ทางกายวิภาค ทางเคมี และทางโมเลกุล ได้ทำการเพิ่มจำนวนและหาลำดับนิวคลีโอไทด์ในยีน mitochondrial small subunit ribosomal (mtSSU) และ large subunit of nuclear ribosomal (nuLSU) และนำข้อมูลไปวิเคราะห์หาสายสัมพันธ์เชิงวิวัฒนาการด้วยวิธี Maximum likelihood และ Bayesian analyses จากการระบุชนิดพบไลเคนชนิด *Graphis* 32 ชนิด โดยพบไลเคนชนิดใหม่ 1 ชนิดและ 6 ชนิดเป็นไลเคนที่มีรายงานเป็นครั้งแรก การวิเคราะห์สายสัมพันธ์ของไลเคนสกุล *Graphis* ร่วมกับหลักฐานทางสัณฐานวิทยาและเคมี สามารถยืนยันไลเคนชนิดใหม่ของโลก 1 ชนิด คือ *Graphis koratensis* Pitakpong Kraichak Lücking ในการศึกษานี้ได้จัดทำรูปวิธานระดับชนิด ให้คำบรรยายลักษณะทางพฤกษศาสตร์ ตัวอย่างที่ทำการศึกษา เอกสารอ้างอิงตามหลักภาพถ่าย ระบุตัวอย่างต้นแบบ และภาพถ่าย ในการศึกษาพบไลเคนสกุล *Graphis* พบโดยทั่วไปในป่าหลายแบบที่มีช่วงความสูงเหนือระดับน้ำทะเลระหว่าง 150-1,000 เมตร บางชนิดมีการกระจายหลายแหล่งที่อยู่และบางชนิดพบเฉพาะพื้นที่

การศึกษาปัจจัยแวดล้อมที่มีผลต่อความหลากชนิดของไลเคนสกุล Graphidaceae ได้ดำเนินการในสถานีวิจัยสิ่งแวดล้อมสะแกราช โดยทำการศึกษาไลเคนบนต้นไม้ 85 ต้น ด้วยวิธี cluster sampling ทำการศึกษาในป่าดิบแล้งและป่าเต็งรังขนาดแปลง 20 × 20 เมตร จำนวน 10 แปลง พร้อมบันทึกชนิดของต้นไม้ เส้นรอบวงต้นไม้ ความเป็นกรดด่าง ร่องลึกของเปลือกไม้ และเปอร์เซ็นต์ความชื้นเปลือกไม้ จากตัวอย่างต้นให้อาศัย 85 ต้น พบ 12 สกุล 25 ชนิด โดยพบสกุลดังนี้ *Carbacanthographis* (2 ชนิด) *Diorygma* (1 ชนิด) *Dyplolabia* (1 ชนิด) *Fissurina* (2 ชนิด) *Graphina* (1 ชนิด) *Graphis* (9 ชนิด) *Hemithecium* (2 ชนิด) *Pallidogramme* (1 ชนิด)

Phaeographis (3 ชนิด) *Platygramme* (1 ชนิด) *Platythecium* (1 ชนิด) และ *Sarcographa* (1 ชนิด) โดยพบความหลากชนิดของไลเคนมากที่สุดอยู่บนต้นเต็ง และต้นมะกอกเกลื้อน จำนวน 12 ชนิด จากการวิเคราะห์ Canonical Correspondence Analysis (CCA) พบว่าปัจจัยที่ผลต่อความหลากชนิดไลเคนสกุล Graphidaceae มากที่สุดคือ ความเป็นกรดด่างของเปลือกไม้

สาขาวิชาชีววิทยา | ลายมือชื่อนักศึกษา____________
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ARUNPAK PITAKPONG : DIVERSITY AND MOLECULAR PHYLOGENY OF THE LICHEN GENUS *GRAPHIS* (GRAPHIDACEAE) IN THAILAND. THESIS ADVISOR : ASSOC. PROF. NOODUAN MUANGSAN, Ph.D. 285 PP.

GRAPHIS/ LICHEN/ DIVERSITY/ DISTRIBUTION/ THAILAND

The crustose lichenized genus *Graphis* is one of the largest genera of tropical family Graphidaceae. Since Thailand is located in a hot and humid climatic zone, a variety of tropical ecosystems are maintained and hence are able to support a number of *Graphis* species, but no systematic report of the genus has been completed. The objectives of the present study were to investigate the diversity of genus *Graphis* in different forest types in Thailand and to examine its relationship with environmental factors. For the systematic study of genus *Graphis* in Thailand, lichen specimens were randomly sampled in twelve localities (eleven national parks and one research station). The collected lichens were then taken to the laboratories for identification on the basis of morphological, anatomical, chemical, and molecular studies. Two molecular markers, the mitochondrial small subunit ribosomal (mtSSU) and nuclear large subunit ribosomal (nuLSU) genes, were amplified, sequenced, and then used for phylogenetic analyses with Maximum likelihood and Bayesian approaches. Thirty-two species, six new records, and one new species of the genus *Graphis* were found in this study. Molecular data, along with morphological and chemical evidence, supported the identity of the new species, *Graphis koratensis* Pitakpong, Kraichak, Lücking. Keys to species for the examined specimens were constructed, including

type, bibliographies, and photographs. Ecologically, *Graphis* species distributed in many forest types in the altitude range of 150-1,000 meters. Some species were widespread in many habitats, while some are more restricted.

The study of the effect of environmental factors on species richness of lichens family Graphidaceae was conducted at Sakaerat Environmental Research Station. Lichen sampling on tree trunk was conducted on 85 trees by the cluster sampling technique. Ten plots in the dry dipterocarp and dry evergreen forests with size of 20 × 20 square meters were randomly chosen. For the study of host plants, various substrate factors were measured, including circumference of tree, pH of bark, bark crevice depth, and percent moisture. The results found 12 genera and 25 species of Graphidaceous lichens including the genera *Carbacanthographis* (2 spp.), *Diorygma* (1 sp.), *Dyplolabia* (1 sp.), *Fissurina* (2 spp.), *Graphina* (1 sp.), *Graphis* (9 spp.), *Hemithecium* (2 spp.), *Pallidogramme* (1 sp.), *Phaeographis* (3 spp.), *Platygramme* (1 sp.), *Platythecium* (1 sp.), and *Sarcographa* (1 sp.). The highest species richness of lichen species was found on *Shorea obtusa* and *Canarium subulatum* about 12 lichen species. Canonical Correspondence Analysis (CCA) showed the most pronounced factor affecting the species richness of Graphidaceous lichens was bark pH.

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CHAPTER I

INTRODUCTION

1.1 Background

Graphidaceae family is the largest family of crustose lichens, most dominant and distributed mainly in tropical crustose lichen communities (Hale, 1981; Sipman and Harris, 1989; Archer, 2000; Staiger, 2002; Lücking *et al*., 2009; Riva Plata *et al*., 2010; Riva Plata and Lücking, 2012). The family Graphidaceae was divided into four genera (Staiger, 2002) according to the ascospores are transversely septate or muriform, colourless or greyish brown; *Graphis* (hyaline, transverse), *Graphina* (hyaline muriform), *Phaeographis* (grey-brown, transverse) and *Phaeographina* (grey-brown, muriform) (Lücking, 2009; Lücking *et al*., 2009).

Graphis is a genus of lichenized fungi in the family Graphidaceae, order Ostropales, class Lecanoromycetes and division Ascomycota. It is more than 400 species, *Graphis* is the largest genus of tropical crustose microlichens (Lücking *et al*., 2008; 2009; Seavey and Seavey, 2011). Its apothecia (spore-bearing structures) are called lirellae because of their unusual linear ridge like form whereas most lichen apothecia are shaped like round balls, disks, or cups. Depending upon the species, the lirellae may form dots, straight or wavy lines, irregular glyph-like shapes, radiating star-shaped clusters or complex labyrinth form patterns, lirellate ascomata and hyaline, transversely septate ascospores (Müller, 1880; 1882; Lücking, 2009; Lücking *et al*., 2009).

Graphis genus is characteristic elements of tropical forest ecosystems, being most diverse in lowland rain forest, dry forest and savanna vegetation (Lücking *et al*., 2009). *Graphis* is lirellate ascomata with well-developed, convergent labia and mostly closed disc, partly to fully carbonized excipulum, mostly non-inspersed hymenim, hyaline, transversely septate to muriforom amyloid ascopores, iodine reaction, chemistry, was proposed to genus concept (Staiger, 2002; Kalb *et al*., 2004; Lücking *et al*., 2008), including another genera such as *Acanthothecis*, *Anomomorpha*, *Carbacanthographis*, *Dioryma*, *Dyplolabia*, *Fissurina*, *Platythecium* and *Thalloloma* (Wirth and Hale, 1963; 1978; Archer, 1999; 2000). So molecular phylogenetic support identification of *Graphis* genus, molecular data are necessary to confirm to species of lichens (Kalb *et al*., 2004; Staiger and Grube, 2004; Staiger *et al*., 2006; Mangold *et al*., 2008; Aptroot and Sipman, 2007)

In Thailand, the genus *Graphis* has approximately 45 species (Lichen Research Unite, 1994). They are most commonly found on bark, in shaded to exposed micro sites with each genus having its particular niche preferences. Most *Graphis* genera are excellent indicators of ecosystem health and can be used in standardized protocols to assess the conservation status of forest fragments in the tropics. Due to Thailand is situated in a hot and humid climatic zone, a variety of tropical ecosystems are maintained. There are various types of forest ranging from tropical rain forest to mangrove forest in Thailand, and hence are able to support a larger variety of *Graphis* species but little information has been reported. Therefore, the aims of this study were to investigate the diversity of *Graphis* in different forest types in Thailand and to examine its relationship to environmental factors.

1.2 Research Objectives

The objectives of the study were;

1) To identify and classify lichens in the genus *Graphis* in Thailand.

2) To investigate diversity and distribution of genus *Graphis* in Thailand.

3) To investigate the diversity of the genus *Graphis* and correlation with tree factors at Sakaerat Environmental Research Station.

1.3 Research Hypotheses

The research hypotheses of the study were;

1) High diversity of lichens in the genus Graphis are supported by tropical climate of Thailand, and molecular data could confirm to species identification of lichens.

2) Diversity of genus *Graphis* is influenced by environmental factors such as bark pH and tree speices in Sakaerat Environmental Research Station.

1.4 Scope and Limitations

The scope and limitations of the study were;

1) The work was carried out between January 2013 and December 2014 using materials obtained from various localities and herbarium specimens from Thailand and abroad.

2) The work included the morphology, anatomy, chemical, ecology and molecular studies of the genus *Graphis* in Thailand.

3) Lichen specimens in Thailand were collected and preserved to be identified, described and used for construction of a key to species.

1.5 Expected Results

This study was aimed to provide information about;

1) The systematics and relationships among the taxa in the genus *Graphis* in Thailand.

2) The ecology and diversity of the genus *Graphis* in each forest type of Thailand.

3) The providing important information may be useful for regulation and management purposes of the National Park, Wildlife and Plant Conservation Department and Lichen Herbarium.

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CHAPTER II

LITERATURE REVIEW

2.1 Lichens

Lichens consist of two quite different organisms growing together in intimate association a fungus and an alga resulting in a stable thallus (Alvin and Kershaw, 1966; Hawksworth and Hill, 1984).

- Lichen fungi, about 20% of all fungi are lichenized (Purvis, 2000) and over 95% of the fungi that form lichens are members of the Ascomycota (Baron, 1999).

- Lichen algae, there are about 25 genera of green algae, a few golden algae, one brown alga and 12 genera of cyanobacteria (or blue-green algae) that become associated in lichens as photobiont (Brodo *et al*., 2001)

There are an estimated 13,500 to 18,000 species of lichens, extending from the tropics to the polar regions. Some of them grow on the bark of temperate trees or as epiphytes on the leaves of trees in tropical rain forests. Others occupy some of the most inhospitable environments on earth, growing on cooled lava flows and bare rock surfaces, where they help in the process of soil formation, and on desert sands (Hale, 1983; Nash III, 1996; Sipman and Aptroot, 2001). This is particularly true for crustose epiphytic microlichens which, for many groups, have their highest diversity in the tropics (Aptroot and Sipman, 1997; Sipman and Aptroot, 2001; Lücking *et al*., 2009a). Lücking *et al*. (2009a) estimated that about half of the predicted global

26,000–28,000 lichen species occur in the tropics, even considering that the tropics cover less than 25% of the Earth's land surface.

2.2 Occurrence of Lichens

Lichens are found growing in wide variety of situations from the Arctic to Antarctic and all regions in between. Some lichens are able to live where there is no other vegetation and thus prove important colonizers of bare rocks. They may grow on leaves, bark of tree, soil, bare rock and many other similar situations. Based on their place of occurrence, lichens may fall in following groups (Sharma, 1989):

- Corticolous: Lichens developing on bark of trees, e.g. species of *Paralia*, *Alectoria*, *Usnea*, *Graphis*, etc.

- Lignicolous: Lichens developing directly on wood, e.g. *Calicicum*, *Chaenotheca*, *Cyphelium*, etc.

- Saxicolous: Lichens developing on rocky substrata, e.g. *Verrucaria*, *Porina*, *Dermatocarpon*, *Xanthora*, etc.

- Terricolous: Lichen growing on the ground, e.g. *Cladonia*, *Lecidea granulose*, *Collematenax*, etc.

- Marine: Lichens developing on siliceous rocky shores of sea, e.g. *Verrucaria mucosa*, *Caloplacen tummarinae*, *Caloplaca marina*, etc.

- Freshwater: Lichens developing on hard siliceous rocks in freshwater, e.g. *Hymene lialacustris*, *Ephebalanata*, etc.

Many man-made substrata, such as leather, silk, wool, hairs, bone, glass fibre, timber, walls, paints, sculptures, asbestos-cement, worked-iron, glass etc. may also be colonized by lichens (Hawksworth and Hill, 1984).

There are three main kinds of lichens, distinguishable on general habit of growth and the manner of attachment to the object on which they grow (Alvin and Kershaw, 1966):

- Crustose lichens: the thallus is like a crust and usually lacks any distinct lobes. It is closely attached to the substratum by the whole of its lower surface and normally is impossible to separate without breaking it.

- Foliose lichens: the thallus in this type usually creeps horizontally and is rather like a leaf or scale or more usually a system of leaves or scales. It is normally attached to the substratum by means of root-like threads called rhizine.

- Fruticose lichens: the thallus is erect and bush-like or pendent and tassel like. It is attached only at the base.

2.3 Reproduction and Dispersal of Lichens

As occurs in most fungi, the vast majority of lichenized ascomycetes have a sexual and an asexual life cycle. Within lichens, usually only the mycobiont expresses the full sexual and asexual reproduction. The typical sexual and asexual fruiting structures of the individual symbionts, lichenized as ascomycetes have evolved a number of vegetative propagules, by which both partners are distributed. There is considerable variation among ascomata and according to their morphology and anatomy, several types of fruit bodies exist. There are two very common vegetative structures, isidia and soridia (Nash *et al.*, 2002). Virtually all lichens produce their spores in sac-like structure termed “asci”. The fruiting bodies containing the asci are known either as apothecia, where they are more or less open, or as perithecia, when they are enclosed in a flask-like body (Baron, 1999).

2.4 Benefits of Lichens

The benefits of lichens could be classified as follows (Brodo *et al*., 2001; Lichen Research Unit and Lichen Herbarium, 1994; Nash III, 1996):

- Food: Lichens consist of no actual carbohydrate or even cellulose. However, they have lichenin at hyphae cell walls of fungal which could be used as food. For example, in the Scandinavian, *Cetraria islandica* (Iceland lichen) has been taken as food and as a medicine to better food digestion in a body. Besides, it has been pound to be mixed with flour or potatoesin times of famine to stretch their meager supplies.

- Medicine: The ancient Egyptians used lichens as ingredients in medicines and herbs. In the 15th Century, people used lichens for treatment such as *Usnea barbata*, *Lobaria pulmonaria*, *Xanthoria parietina* and *Peltigera canina*, etc. A recent study on the *Umbilicaria esculenta* species shows that it produces substances that can inhibit the growth of HIV virus.

- Dyes: Lichens have been used, since the ancient Egypt age, as dyes. The one well-known is *Rocella tinctoria*, giving color in purple tone. France and Holland have produced lichens in term of industry. With their property of being sensitive to the pH, they are therefore used as colors of the litmus. In medical study and research nowadays, lichens have been used for chromosome dying.

- Perfume: In France, *Evenia prunestri* and *Lobaria pulmonaria* are ingredients in perfume. Apart from making good smell, they keep the smell stay longer.

- Lichenometry: Because lichens grow so slowly, a relatively small circular patch can actually be very old. In the arctic, crustose species such as the map lichens add only a fraction of millimeter of radial growth each year.

- Poison: Although lichens produce various kinds of organic acids causing a bit irritation after taken, most lichens contain no poison. Two types of lichens found to have poison are *Letharia vulpine* and *Cetraria pinastri* that were used as poison to foxes by the European.

- Indicators of environmental conditions: Lichens are sensitive to changes in atmosphere and micro-climate conditions and have been used as environmental bioindicators. The sensitivity of many lichens to atmospheric pollutions such as oxides of sulfur, nitrogen and heavy metals, has been used to assess air quality.

2.5 Influence of Environmental Parameters to Lichen Diversity

Lichens are able to colonize environments that have extremes of humidity, temperature and light and they often occur in places where few other living things are able to survive (Purvis, 2000; Wolseley and Aguirre-Hudson, 1997a).

- Water: Lichens cannot control their water content, the level of humidity in the atmosphere and in their substrate that is the determining factor since, just like sponges or blotting paper.

- Temperature: Lichens can withstand wide ranges of temperature and hence adjust to seasonal changes to a much greater extent than the higher plants. Their slow growth rate and their adaptability are in large measure due to their being able to dry out, yet maintain a low level of respiration.

- Light: lichens which rely on algae or cyanobacteria must have light for the process of photosynthesis by their photobiont.

- Substances: Lichen substances have been described from lichens and new compounds are being discovered all the time. Many of these substances, which belong to chemically diverse classes of compounds including aromatic compounds depsides, depsidones and carotenoids are unique to lichen fungi.

- Metals: A feature of lichens is their capacity to absorb and tolerate heavy metals inside the thallus.

- Environmental changes in seasonal tropical forests: Although foliose and fruticose lichens may grow up to 1 cm a year or more in the wet season, crustose lichens grow very slowly 1-2 mm a year. Growth is radial, the youngest part being on the outside edge, so that lichens measuring 20 cm may be between 100-200 years old. These species are only found in old growth forests where conditions have been stable over long periods of time, allowing reproduction and establishment of slow growing individuals.

2.6 Family Graphidaceae

Graphidaceae are the dominant family of tropical crustose lichens and in its current circumscription, the largest of abundance and diversity with nearly 2,000 names described and more than 600 species currently accepted in about 20 genera (Staiger, 2002; Lücking, 2009b). The emended family Graphidaceae contains about 45 genera and possibly some 1,200 species (Neuwirth and Lücking, 2009) and in 2013, 2000 species by molecular study (Rivas Plata *et al*., 2013).

Classification genera of Graphidaceae

Watson (1929) reported 13 genera in Graphidaceae i.e. *Acanthographina*, *Anomalographis*, *Enterodictyon*, *Glyphis*, *Graphina*, *Graphis*, *Helmithocarpon*, *Medusulina*, *Phaeographina*, *Phaeographis*, *Sarcographa*, *Sarcographina*, and *Xyloshistes*

Krik *et al*. (2001) reported 15 genera in Graphidaceae i.e. *Acanthothecis*, *Anomalographis*, *Cyclographina*, *Diplogramma*, *Glyphis*, *Graphina*, *Graphis*, *Gymnographosis*, *Gyrostomum*, *Helminthocarpon*, *Medusilina*, *Phaeographina*, *Phaeographis*, *Sarcographa* and *Sarcographina*.

Staiger (2002) classified Graphidaceae into 22 genera by using morphology and chemical characteristics of lichens i.e. *Acanthothecis*, *Anomalographis*, *Anomomopha*, *Carbacathographina*, *Diorygma*, *Fissurina*, *Glaucinaria*, *Glyphis*, *Graphis*, *Gymnographa*, *Gymnographosis*, *Helmithecium*, *Leiorreuma*, *Phaeographina*, *Phaeographis*, *Platygramme*, *Platythecium*, *Sarcographa*, *Solenographa*, *Thalloloma* and *Thecaria*.

The current classification of Graphidaceae is very different from previous versions, distinguishing over 50 genera in the core group alone, whereas previous treatments recognized 12-14 genera. Growing evidence from molecular data continuously increases the number of clades recognized at the genus level and the actual number of genera is predicted to be possibly close to 90. Some genera are now well-known and well-supported but others such as *Chapsa*, *Fissurina* and *Phaeographis* require more studies to resolve their classification with confidence. A few genera have not yet been sequenced at all (Lucking *et al*., 2011).

The Graphidaceae, generally called “graphid”, forms a large but very poorly known family of corticolous, crustose lichens widely distributed in tropical regions (Wirth and Hale, 1963). It is a widely distributed family, mainly tropical to subtropical (McCarthy, 2009). The fruiting bodies are linear ascomata with two parallel lines of white or black, sometimes branching, called “lirelli form”. There are generally found on barks and occasionally on leaves and rock (Sutjaritturakan, 2002).

A phylogenetic study of the lichen family Graphidaceae is presented. Most genera of the family, as well as selected representatives of the closely related Thelotremataceae, are included. The results of the Bayesian analysis of combined mtSSU and nuLSU rDNA sequence data were compared with recently introduced concepts of genera. The evolutionary pattern of morphological characters of the ascomata such as exciple carbonization, paraphyses types, and ascospore characters (colour, septation, and Lugols reaction) are critically discussed (Staiger *et al.*, 2006). Lücking *et al.* (2011) reported 23 genera in molecular phylogeny of Graphideceae i.e. *Allographa*, *Anomomopha*, *Creographa*, *Diorygma*, *Discographis*, *Ectographis*, *Fiegographa*, *Glyphis*, *Graphis*, *Halegrapha*, *Hemithecium*, *Leiorreuma*, *Malmographa*, *Pallidogramme*, *Phaeographis*, *Platygramme*, *Platythecium*, *Pliariona*, *Sarcographa*, *Schistophoron*, *Thalloloma*, *Thecaria* and *Thecographa*, as shown in the Figure 2.1.

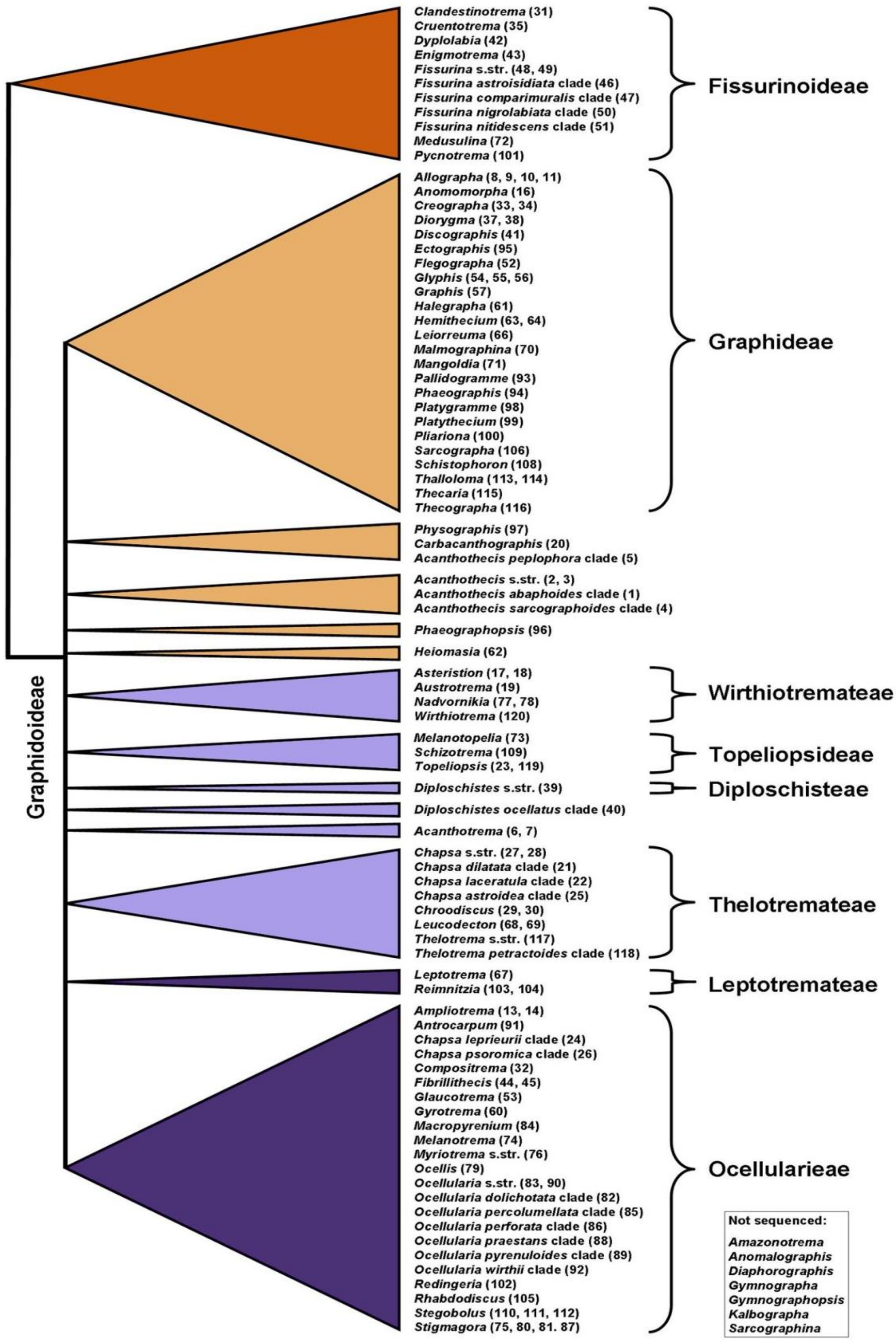

Figure 2.1 Graphidaceae lichens in the world (Lücking *et al*., 2011).

The Graphidaceae has *Trentepohlia* as photobiont. The mycobiont produces perithecium shaped ascomata embed in the thallus. Their ascomata are linear in outline called “lirellate” or lirelli form. They are mostly semiemergent or emergent, less immersed with thalline margins. Its ascomata is composed of colorless to black disc, plane to concurved. The lip-like ascomata may be absolutely closed or widely opened exposing the disc surface. The ascospores in Graphidaceae have different characteristic such as Opegrapha is an ascospore with cylindrical locule characteristic (Lücking *et al.*, 2009b).

Table 2.1 Members of genera of the Graphidaceae proposed by previous reports.

Watson (1929)	Krik *et al.* (2001)	Staiger (2002)	Lücking *et al.* (2011)
Acanthographina	*Acanthothecis*	*Acanthothecis*	*Allographa*
Anomalographi	*Anomalographis*	*Anomalographis*	*Anomomopha*
Enterodictyon	*Cyclographina*	*Anomomopha*	*Creographa*
Glyphis	*Diplogramma*	*Carbacathographina*	*Diorygma*
Graphina	*Glyphis*	*Diorygma*	*Discographis*
Graphis	*Graphina*	*Fissurina*	*Ectographis*
Helmithocarpon	*Graphis*	*Glaucinaria*	*Fiegographa*
Medusulina	*Gymnographosis*	*Glyphis*	*Glyphis*
Phaeographina	*Gyrostomum*	*Graphis*	*Graphis*
Phaeographis	*Helminthocarpon*	*Gymnographa*	*Halegrapha*
Sarcographa	*Medusilina*	*Gymnographosis*	*Hemithecium*
Sarcographina	*Phaeographina*	*Helmithecium*	*Leiorreuma*
Xyloshistes	*Phaeographis*	*Leiorreuma*	*Malmographa*
	Sarcographa	*Phaeographina*	*Pallidogramme*
	Sarcographina	*Phaeographis*	*Phaeographis*
		Platygramme	*Platygramme*
		Platythecium	*Platythecium*
		Sarcographa	*Pliariona*
		Solenographa	*Sarcographa*
		Thalloloma	*Schistophoron*
		Thecaria	*Thalloloma*
			Thecaria
			Thecographa

Distribution of the Graphidaceae

Investigations of the Graphidaceae were carried out extensively in various part of the world. In Solomon Islands, 16 genera and 75 species were found (Archer, 2006). There were 19 genera and 163 species from Australia (Archer, 2009), 42 species from Galapagos Islands (Bungartz *et al*., 2010), 13 genera and 77 species from northeastern Brazil (Cáceres, 2007), 12 genera and 57 species from Parama (Forono, 2009), 3 genera and 22 species from New Zealand (Hayward, 1977), 4 genera and 65 species from America (Wirth and Hale, 1978), and 15 genera and 57 species from Mexico (Wirth and Hale, 1963)

Studies of the Graphidaceae in Thailand

The first report of the Graphidaceae in Thailand was done by Vainio (1909) in which lichens in Koh Chang Island, Trat Province were collected. Four genera and 40 species of Graphidaceae of Graphidaceae were found. Two species from Koh Tao, Suratthanee Province was reported by Paulson (1930). Wolseley *et al.* (1997) studied the diversity and distribution of the lichen flora of Thailand and found 15 genera and 74 species of lichens in the family Graphidaceae. Sutjaritturakan (2002) reported 9 genera and 127 species the lichens Graphidaceae at Khao Yai National Park, Nakhon Ratchasima province. Boonpragob *et al.* (2009a) showed that lichens on Samaesarn Island and Kram Island were dominated by the Graphidaceae. Poengsungnoen *et al.* (2010) reported the identification of 96 species and 14 genera from Phu Luang Wildlife Sanctuary, Loei province.

2.7 Genus *Graphis*

Characteristics of genus *Graphis* (Lücking, 2009)

Thallus colour: Most species have a white-grey thallus due to the presence of large clusters of calcium oxalate crystals in and above the photobiont layer. A few taxa have an olive-green color, similar to *Phaeographis* and related genera, because the crystal clusters are located below the photobiont layer.

Vegetative propagules: Only four species have isidia and one (*Graphis sorediosa*) has soralia. Isidia are usually numerous in *Graphis isidiata*, *Graphis isidiza*, and *Graphis patwardhanii*, but scattered and easily overlooked in *Graphis stellata*, so careful inspection of the thallus surface is required.

Lirella emergence: Lirella emergence can be categorized into four states 1) immersed (upper part of hymenium more or less level with thallus surface, labia flush or slightly emergent; 2) erumpent (upper part of hymenium above, but lower part below thallus level; lirellae with gently sloping margins; 3) prominent (hymenium fully above thallus level; lirellae with steeply sloping margins; 4) sessile (lirellae basally constricted. Variation of lirella emergence on a single thallus depends mostly on age, with the lirellae in most cases starting out as fissures in the thallus. Lirella emergence is the best observed in transverse sections of mature lirellae towards the thallus centre under the dissecting microscope or, with some experience, from surface view, using a light source at an angle of 45 degrees (light sources at higher angles will make the lirellae appear flatter than they actually are).

Thalline margin of lirellae: Because of the carbonized excipulum, the labia in *Graphis* species are black. However, in many species they are covered by a thalline margin, leaving only the upper part of the labia exposed or covering them completely.

A thalline margin is absent when the black labia are erumpent to sessile and the black color reaches down to the thallus level; by default, immersed lirellae must have at least a lateral thalline margin. A basal thalline margin is developed when it covers less than half of the overall height of the lirellae, whereas a lateral thalline margin usually is at level with the height of the lirellae and only leaves the upper, horizontal part of the labia exposed and black. Many species have an apically thin complete thalline margin: the lateral part of the thalline margin is thick and contains a photobiont layer, whereas the upper part consists of a thin, hyaline cortex only; in this case the upper part of the labium appears dark grey. In species with an apically thick complete thalline margin, the margin is uniformly composed of cortex and photobiont layer up to the top and the black excipulum surface is more or less invisible. The nature of the thalline margin varies with age: even in species with eventually prominent lirellae lacking a thalline margin, young lirellae (or the tips of the lirellae) first appear as immersed fissures or narrow black lines with a thalline margin. Thus, the nature of the thalline margin should be observed in mature lirellae towards the centre of the thallus.

Labia and disc pruinosity: Many species exhibit pruinose labia or discs when exposed. Variation of this character is little understood, but most of the material available indicates that this feature is species specific.

Labium striation: Labium striation has been used as an important taxonomic feature in the past (Wirth and Hale, 1978) and was also taken up by Staiger (2002). However, since labium striation seems to be connected to the formation of new hymenia (Staiger, 2002), it appears that entire *versus* striate labia represent developmental stages of the same species rather than a taxonomically important character.

Excipulum carbonization: *Graphis* is partially characterized to complete carbonized excipulum. Wirth and Hale (1963, 1978), and Staiger (2002) distinguished several groups characterized by their degree of excipulum carbonization shown in Figure 2.2 (Rivas Plasta *et al.*, 2011). It is not yet well-understood whether the thick carbonized basal excipulum in certain species of *Graphis* is homologous with the excipulum in other genera or, at least in part, represents a carbonized hypothecium, as in *Leiorreuma*, *Sarcographa*, and *Thecaria* (Staiger, 2002).

Hymenium inspersion: Hymenium inspersion is another character that was rarely used in the taxonomy of Graphis, but turned out to be relevant at the species level (Staiger, 2002; Lücking *et al.*, 2008; Lücking, 2009). The importance of this character is underlined by the fact that two different types of inspersion occur within the genus and that these types are largely restricted to three species groups.

Ascospores: Species of Graphis typically have hyaline, I+ violet-blue, distoseptate ascospores with lens-shaped lumina. The iodine reaction is concentrated in the endospore and usually strong, giving a violetblue to violet-brown colour (Staiger, 2002), except at the tips where the lumina tend to be smaller with less endospore. In a few species (e.g., Graphis chrysocarpa, Graphis mucronata, Graphis pittieri), the ascospores become grey-brown. Some variation is found in the degree of the endospore thickenings, especially in taxa with muriform ascospores. The number of ascospores per ascus should be carefully assessed observing several asci from different lirellae. Ascopores are often discharged prematurely or their number is difficult to assess within the ascus. If there are more than four ascospores per ascus, the actual number is most probably eight.

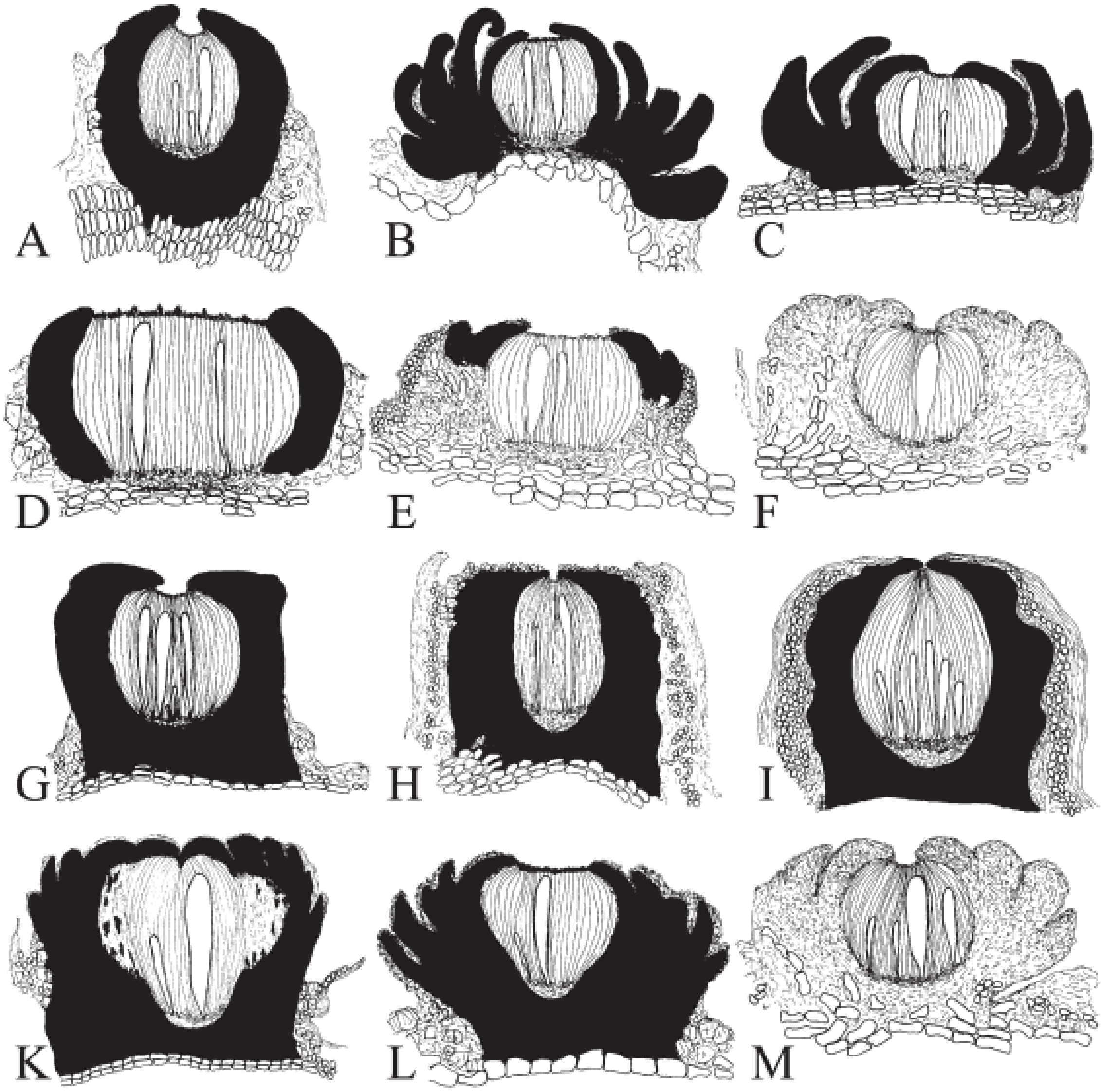

Figure 2.2 Variation of excipulum carbonization in genus *Graphis* (Rivas Plasta *et al*., 2011). A = complete; B = nearly complete, striate; C = lateral, striate; D = lateral, entire; E = apical, striate; F = uncarbonized; G = complete, entire; H = complete, entire; I = complete, entire; K = complete, striate; L = complete, striate; M = uncarbonized.

Secondary chemistry: Most species of *Graphis* lack secondary substances. Species with a simple chemistry with norstictic or stictic acids and accessory substances are particularly common. Salazinic and protocetraric acids are comparatively rare.

The lichen genus *Graphis* (Lecanoromycetes: Ostropales: Graphidaceae) is the largest genus of tropical lichens, commonly found in (semi-)exposed situations in tropical lowland to montane and dry forests (Cáceres, 2007; Lücking *et al.*, 2008; 2009b; Plata *et al.*, 2011). It is characterized by a crustose thallus, rounded to lirellate with carbonized exciples; nonamyloid, functionally unitunicate asci with apical wall thickenings, hyaline, amyloid ascospores with lens shaped lumina, and a trentepohlioid photobiont (Wirth and Hale, 1963; 1978; Archer, 2001; Staiger, 2002; Lücking *et al*., 2009b). It is the largest tropical lichen genus, with more than 300 accepted species worldwide (Kirk *et al*., 2008; Lücking *et al*., 2009b). Recent molecular study has confirmed the placement of the genus *Graphis* within family Graphidaceae (Mangold *et al*., 2008; Joshi *et al*., 2010). The first three types were discovered in Thailand whereas the last one in Costa Rica. Moreover, Rivas Plasta and Lücking (2012) found two new types of lichens: *Graphis apertoinspersa* Rivas Plasta & Lücking and *Graphis pitmanii* Rivas Plasta & Lücking in Amazonian Peru. Next, Joshi *et al*. (2013) found a new lichen named *Graphis koreana* S. Joshi & Hur in South Korea. Another discovery of two new lichens by Sipman (2014) included *Graphis murali-elegans* Sipman. (found in Costa Rica) and *Graphis nigroglobosa* Sipman. (found in Venezuela). Van den Broeck *et al*. (2014), in addition, found two new species of Graphidaceae from tropical Africa, namely, *Graphis aptrootiana* Van den Broek, Lücking & Ertz. and *Graphis vandenboomiana* Ertz, Lücking & Van den Broeck. In addition to the aforementioned discoveries, existence of other new species of lichens in Thailand was also reported. Poengsungnoen *et al*. (2014) discovered two new species: *Graphis subdussii* Poengs. & Kalb. and *Graphis subinsulana* Poengs. & Kalb. in Loei, while Sutjaritturakan *et al*. (2014) found *Graphis australosiamensis* Sutjaritt. & Kalb. in Chumpon.

Distribution of the genus *Graphis*

Graphis is generally corticolous but some saxicolous taxa are known. Species can be found throughout all forests types. It is a common genus that is widespread in tropical and sub-tropical regions, with some taxa in temperate regions (Lücking, 2009a). In Australia, 43 species of *Graphis* were found (Filson, 1996), 19 new species of *Graphis* (Müller, 1887; 1895). A world-wide key to the genus *Graphis* is presented, and 330 species are accepted (Lucking *et al.*, 2009). Lucking *et al.* (2010) described the new species *Graphis collinsiae* Lucking & Lumbsch from the Fiji Islands, *Graphis insulana* aggregate and is intermediate between *Graphis insulana* (Mull. Arg.) Lucking & Sipman and *Graphis subhiascens* (Mull. Arg.) Lucking. Jia (2011) a new species in the lichen genera *Graphis* Adans., *Graphis paradussii* Z. F., was reported from southern China. Seavey and Seavey (2011) reported a total of 31 species of the genus *Graphis* from Everglades National Park. In addition, the following eleven species were newly reported from North America: *Graphis analoga* Nyl., *Graphis cincta* (Pers.) Aptroot, *Graphis chlorotica* Massal., *Graphis crebra* Vain., *Graphis dendrogramma* Nyl., *Graphis filiformis* Adaw. & Makhija, *Graphis furcata* Fee, *Graphis modesta* Ahlbr., *Graphis neoelongata* Zenker, *Graphis* *renschiana* (Mull.Arg.) Stizenb. and *Graphis supracola* A. W. Archer. Weerakoon *et al.* (2012) described four new species of *Graphis* from Sri Lanka. Lucking and McCune (2012) identified *Graphis* genus from the Paleotropics. There are few studies on *Graphis* genus in Thailand, because of small sample and difficult for identification.

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CHAPTER III

IDENTIFICATION OF LICHEN GENUS *GRAPHIS* IN THAILAND

3.1 Abstract

Genus *Graphis* were wide in Tropical region, about 400 species in the world and largest of family Graphidaceae. The aim of this study was to classify the lichen genus *Graphis* in 12 localities in Thailand. The identification of species levels on the basis of morphological, anatomical, chemical, and molecular studies. From the study of 12 localities, 32 species of genus *Graphis* were found in Thailand. In this case, One of 32 was a new species and six of them were new of the crustose lichen genus Graphis recorded in Thailand. The new species was named *Graphis koratensis* Pitakpong, Kraichak, Lücking. It can be characterized by lirelline ascocarps with whitish grey or grey-green pruina along the slit, transversely septate ascospores, and the presence of norstictic acid. The six new records in Thailand were reported includind *Graphis cincta* (Pers.) Aptroot, *Graphis jejuensis* K. H. Moon, M. Nakan. & Kashiw., *Graphis nigrocarpa* Adaw. & Makhija, *Graphis renschiana* (Müll. Arg.) Stizenb., *Graphis seminuda* Müll. Arg. and *Graphis subserpentina* Nyl. In this chapter, picture keys for explaining those 32 species of genus *Graphis* that found in Thailand.

3.2 Introduction

Of more than 400 species, the lichens genus *Graphis* stands as one of the largest genus of microlichens (Lücking *et al*., 2009; 2014; Rivas Plata *et al*., 2011; 2013). The genus traditionally includes taxa with lirellate ascomata, and hyaline, traversely septate ascospore, commonly found in dry and semi-exposed tropical forests. According to the comprehensive work by Staiger (2002), the number of described species with the genus has grown steadily due to the publication of the world-wide key (Lücking *et al*., 2009) and concerted efforts in taxonomic revisions and collection of the now-expanded family Graphidaceae (Sohrabi *et al*., 2014). The diversity and monophyly of the genus is the area under active research, as the genus appears to be heterogeneous in their morphological (Staiger, 2002) and genetic characters (Rivas Plata *et al*., 2011; 2013).

As widely perceived, there is a wide diversity of lichenized genus *Graphis*. Since early studies were solely based on morphological data, only a small number of this family of lichen was discovered. Nevertheless, with the supplement of anatomical, chemical, and molecular data, classification of lichens was reconstructed. This consequentially led to discoveries of new types of lichens. Lücking *et al*. (2012), for example, found four new types of lichens, namely, *Graphis norvestitoides* Sutjaritturakan, *Graphis arbusculaeformis* (Vain.) Lücking, *Graphis rongklaensis* Sutjaritturakan, and *Graphis pseudoaquilonia* Lücking. The first three types were discovered in Thailand whereas the last one in Costa Rica. Moreover, Rivas Plasta and Lücking (2012) found two new types of lichens: *Graphis apertoinspersa* Rivas Plasta & Lücking. and *Graphis pitmanii* Rivas Plasta & Lücking in Amazonian Peru. Next, Joshi *et al*. (2013) found a new lichen named *Graphis koreana* S. Joshi & Hur in South Korea. Another discovery of two new lichens by Sipman (2014) include *Graphis murali-elegans* Sipman. (found in Costa Rica) and *Graphis nigroglobosa* Sipman. (found in Venezuela). Van Den Broeck *et al*. (2014), in addition, found two new species of Graphidaceae from tropical Africa, namely, *Graphis aptrootiana* Van Den Broek, Lücking & Ertz. and *Graphis vandenboomiana* Ertz, Lücking & Van Den Broeck. In addition to the aforementioned discoveries, existence of other new species of lichens in Thailand was also reported. Poengsungnoen *et al*. (2014) discovered two new species: *Graphis subdussii* Poengs. & Kalb. and *Graphis subinsulana* Poengs. & Kalb. in Loei, while Sutjaritturakan *et al*. (2014) found *Graphis australosiamensis* Sutjaritt. & Kalb. in Chumpon.

Inspite of a large extent of dry and semi-exposed tropical forests– a suitable habitat for the genus-all over the country, the study of *Graphis* in Thailand has been moderately sporadic (Rundel and Boonpragob, 2009). Since the earliest report by Vainio in Koh Chang Flora (Vainio, 1909), 59 named species of the genus have been reported so far from Thailand, mostly as part of larger area-based studies of the family Graphidaceae or the tribe Graphidoideae (Vainio, 1909; 1921; Nakashi *et al*., 2001; Aptroot *et al*., 2007; Papong *et al*., 2007; Mongkolsuk *et al*., 2011; Poengsungnoen *et al*., 2010). In many cases, the genus represents the largest portion of lichen diversity. For example, in the study of Graphidoid lichens in Phu Luang Wildlife Sanctuary, *Graphis* constitutes the largest genus in the study with 38.5 percent of the species richness (35 out of 91 species) of the family (Poengsungnoen *et al*., 2010). Similarly, in the study of Khao Yai National Park, 34 *Graphis* species were reported, making it the largest genus for both macro- and microlichens in the study (Lichen Research Unit and Lichen Herbrarium, 1994). Despite this level of diversity, a country-wide study on the genus has yet to be completed.

Therefore, the purpose of this study was to classify the lichen genus *Graphis* on the basis of morphological, anatomical, chemical, and molecular studies to optimize the accuracy of lichen classification.

3.3 Materials and Methods

Study areas:

The lichen speciemens were randomly collected in twelve localities (eleven national parks and one research station) as shown in Table 3.1, Figure 3.1, and reseach plane in Figure 3.2.

Laboratories:

The experiments of the presented study were conducted at two laboratories: the laboratory at the Center for Scientific and Technological Equipment, Suranaree University of Technology, Nakhon Ratchasima, Thailand and Pritzker Laboratory for Molecular Sytematics and Evolution, The Field Museum, Chicago, Illinois, United States of America (USA).

Table 3.1 Field trip sites in Thailand.

Regions	Study sites	Provinces
Northern	DoiInthanon National Park	Chiang Mai
	Khun Khan National Park	Chiang Mai
North Eastern	Sakaerat Environmental Research Station (SERS)	Nakhon Ratchasima
	Phujong-Nayoi National Park	Ubon Ratchathni
	Phu Pha Terb National Park	Mukdahan
Central	Phu Hin Rong Kla National Park	Phitsanulok
	Phu Toei National Park	Suphanburi
Eastern	Pang Sida National Park	Sakaew
	Khao Sip Ha Chan National Park	Jantaburi
Western	Thong Pha Phum National Park	Kanjanaburi
	Kaeng Krachan National Park	Phetchaburi
Southern	Hat Chao Mai National Park	Trang

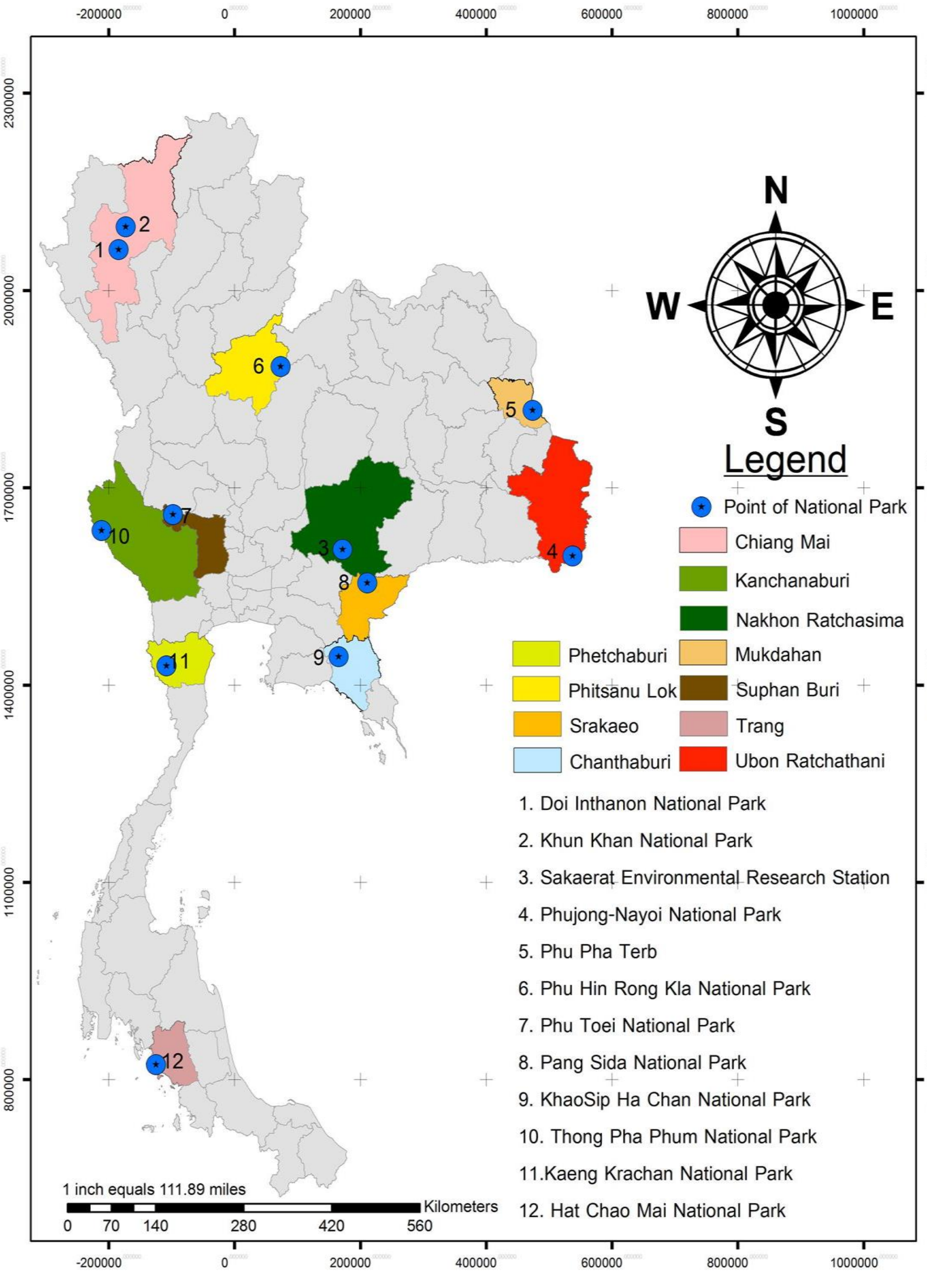

Figure 3.1 Map of collecting localities in Thailand.

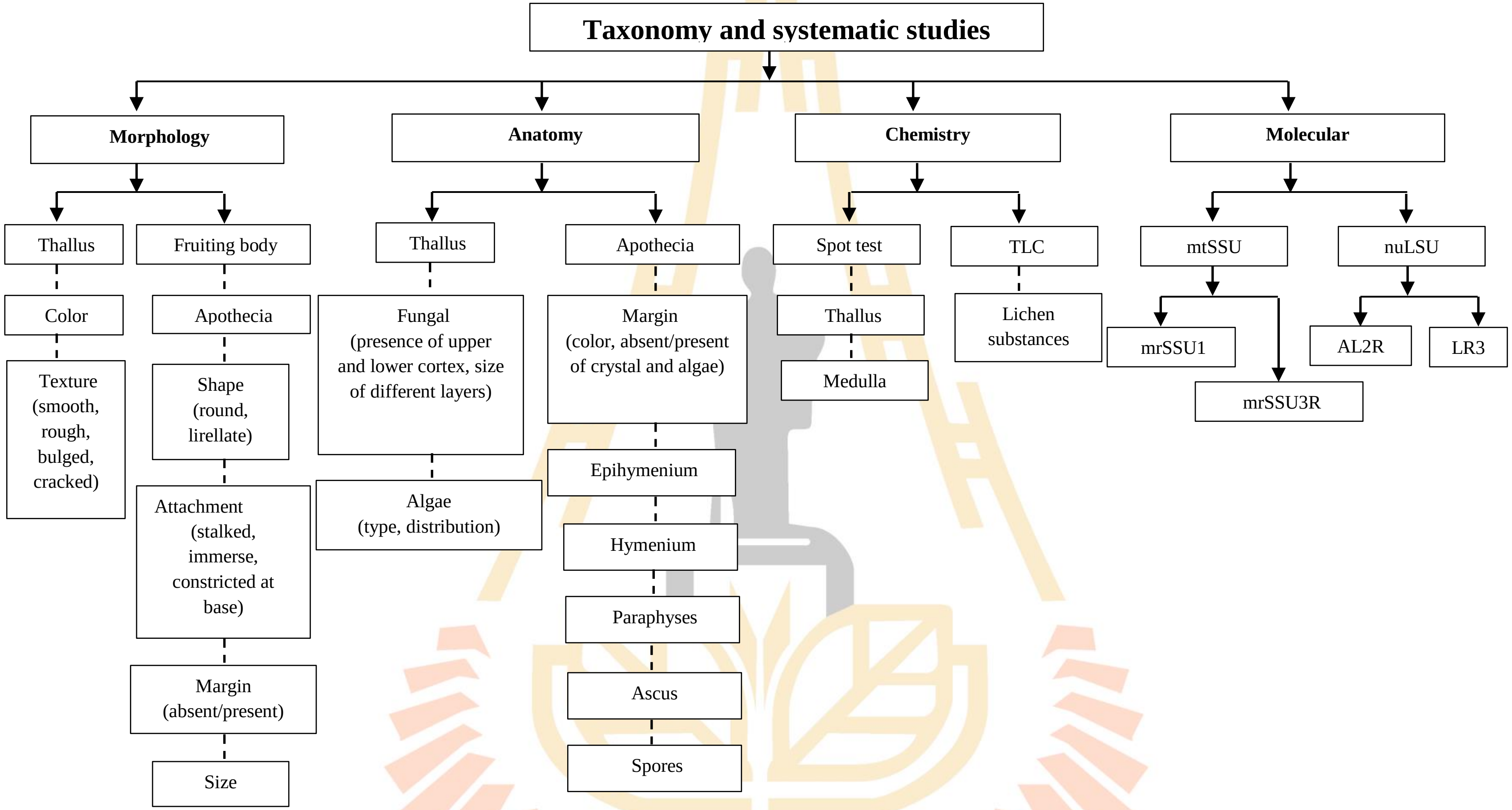

Figure 3.2 Characters used for lichen taxonomic study.

Identification of lichens

Once to the collection of samples from the localities mentioned in Table 3.1 was completed, the lichens were taken to the laboratories for identification on the basis of morphological, anatomical, chemical and molecular studies (Figure 3.2) using keys provided by Archer (2001a, b), Staiger (2002), Lücking *et al*. (2009), and Joshi *et al*. (2013).

Morphological study

Identification of lichens based on morphology was conducted using a low magnification stereomicroscope (Olympus- SZX12, Tokyo, Japan) at magnifications of X7 to X90 to investigate their thallus color, texture, shape, and size. The morphological study regarding fruiting body of the sample lichen genus *Graphis* revealed that the samples belonged to the apothecia. Moreover, the morphological study also investigated whether the mouth of apothecia was open or closed, they were scattered, isolated, or in dense cluster. The specimens were later exposed to ultraviolet (UV) light of wavelengths 254 to 365 nm to examine the light reflected from the lichens' thallus.

Anatomy study

Once the morphological study was carried out, an anatomical analysis was performed. The anatomical identification was conducted with a light coumpound microscope (Olympus-BH2, Tokyo, Japan) at magnifications of x40 to x1000 in combination with specimen section for the examinations of their thallus and fruiting body.

The thallus examination was done on the basis of the studies of cortex (upper cortex, algal layer, lower cortex) and types of algae and fungal, distribution of algae (heteromerous or homeomerous) and fungi hyphae (vertical or horizontal).

The investigation of fruting body is essential to the study of lichen genus *Graphis*. This part of the research placed its foci on the studies of spore (simple, septate, muliform), color (hyaline, brown), shape, size, number of spores in an ascus, color of ascocarp wall (exiple), presence or absence of crytals, heights of hyphae (hymenium, suhymenium, hypothecium), separation and layering of paraphyses.

Chemistry study

Color spot test

Four types of chemicals implemented in the color spot test included 10% aqueous potassium hydroxide solution (K), aqueous solution of calcium hypochlorite (C), aqueous solution of paraphyenldiamine (Pd), and Iodine solution (I). First, the chemicals were dropped on the thallus surface and the upper layer of medulla to test whether there was any color reaction. Then the reaction was observed; a change in color indicated the test to be positive (+) whereas the nonpresence of the color showed the test to be negative (-) (Brodo *et al*., 2001; Orange *et al*., 2001).

Thin layer chromatography (TLC)

On the basis of the methods of White and James (1985); Elix and Ernst-Russell (1993) and Orange *et al*. (2001), thin layer chromatography (TLC) is a study that identifies lichen substances in thallus by means of applying spots on the TLC plate. In the present study, first, the specimens were digested into small sizes. Then, acetone was added to extract the substances of lichens. The extract was later dropped on the 20 × 20 cm TLC plate with each spot was placed at 2 cm distance. Then, a solvent was applied on each spot through a capillary. Next, the TLC plate was placed into the TLC tank, in which Solvent C (Toluence-acetic acid (170:30)) was applied. The plate was kept in the tank for 40-50 min. Subsequently, the plate was taken out and dried. Then, the plate was exposed to UV light, and the spots with chemical reaction were marked. To highlight the chemical reactions on the spots, the plate was exposed to a 10% sulphuric acid solution and heated at 110 °C for 3-5 min.

Molecular study

A molecular study is the study that identifies lichens through DNA extraction. This study was performed when morphological, anatomical, and chemical data have shown unclear results or failed to clearly identify lichens. In other words, a molecular study was employed to confirm species of lichens. Phylogenetic tree was developed to separate the taxa from each other and analyze character evolution with appropriate data analysis (Swofford, 2002).

3.4 Results and Discussion

From our recent collection of 1,704 specimens, a total of 536 were identified as *Graphis* spp. Thirty-two species of *Graphis* were found in Thailand, *Graphis assamensis* Nagarkar & Patw., *Graphis assimilis* Nyl., *Graphis cincta* (Pers.) Aptroot, *Graphis descissa* Müll. Arg., *Graphis duplicata* Ach., *Graphis elongata* Zenker., *Graphis emersa* Mull.Arg., *Graphis falvovirens* Makhija & Adaw., *Graphis furcata* Fée, *Graphis glaucescens* Fée, *Graphis handelii* Zahlbr., *Graphis hossei* Vain., *Graphis intricata* Fée., *Graphis jejuensis* K. H. Moon, M. Nakan. & Kashiw., *Graphis koratensis* Pitakpong, Kaichak & Lücking, *Graphis librata* C. Knight, *Graphis lineola* Ach., *Graphis longiramea* Müll. Arg., *Graphis longula* Kremp., *Graphis nanodes* Vain., *Graphis nigrocarpa* Adaw. & Makhija., *Graphis pinicola* Zahlbr., *Graphis renschiana* (Müll. Arg.) Stizenb., *Graphis rhizocola* (Fée) Lücking & Chaves., *Graphis rimulosa* (Mont.) Trevis., *Graphis seminuda* Müll. Arg., *Graphis streblocarpa* (Bel.) Nyl., *Graphis subserpentina* Nyl., *Graphis subvelata* Stirt., *Graphis tenella* Ach., *Graphis verminosa* Müll. Arg., *Graphis vittata* Müll. Arg.

Twenty-eight species (for key species) of *Graphis* from herbarium in Ramkhamhang University were included, *Graphis intermedians* Vain., *Graphis streimannii* A. W. Archer., *Graphis subregularis* A. W. Archer., *Graphis pavoniana* Fée., *Graphis dussii* Vain., *Graphis longispora* D. D. Awasthi & S. R. Singh., *Graphis pyrrhocheiloides* Zahlbr., *Graphis caesiella* Vain., *Graphis dendrogramma* Zahlbr., *Graphis supracola* A. W. Archer., *Graphis ochrocheila* Vain., *Graphis rongklaensis* Sutjaritturakan., *Graphis analoga* Nyl., *Graphis consimilis* Vain., *Graphis sauroidea* Leight., *Graphis rustica* Kremp., *Graphis marginata* Raddi., *Graphis subdisserpens* Nyl., *Graphis subvittata* Adaw. & Makhija., *Graphis trichospora* Vain., *Graphis stenotera* Vain., *Graphis proserpens* Fée., *Graphis glaucocinerea* Vain., *Graphis striatula* (Ach.) Spreng., *Graphis leptospora* Vain., *Graphis lumbricina* Vain., *Graphis norvestitoides* Sutjaritturakan., and *Graphis myrtacea* (Müll. Arg.) Lücking.

Therefore, sixty species of *Graphis* key to species in Thailand and thirty-two species are listed and described in alphabetical order (Figure 3.3-3.34).

KEY TO THE SPECIES

1 Labia entire 1

Labia striate...... 45

2 Excipulum apically 3

Excipulum completely carbonized.. .... .30

3 Expiculum laterally carbonized 4

Expiculum apically (peripherally) carbonized .... *G. streblocarpa*

4 Hymenium inspersed with oil droplets; ascospores transversely septate 5

Hymenium clear.. 11

5 Norstictic acid...6

No substances.. 8

6 Ascospores 15-45 µm; lirellae variable 7

Ascospores 40-70 µm long; lirellae erumpent............................... *G. streimannii*

7 Disc erumpent; Excipulum laterally carbonized ....*G. handelii*

Disc concealed; Lateral thalline margin .... *G. cincta*

8 Ascospores 40-80 µm, 11-17-septate 9

Ascospores 20-40 µm long, 5-11-septate 10

9 Lirellae erumpent, with basal thalline margin ....*G. intermedians*

10 Disc concealed...*G. lineola*

Lirellae distinctly elongate, with thick lateral thalline margin............*G. jejuensis*

11 Ascospores transversely septate 12

Ascospores (terminally to regularly) muriform.. 25

12 Ascospores 45-135 µm 13

Ascospores 15-45 µm 17

13 Norstictic or stictic acid... 14

No substances.. .... *G. pavoniana*

14 Lirellae variable; labia thick 15

Lirellae prominent to sessile, with thick lateral thalline margin .... *G. dussii*

15 Lirellae emergent to prominent, with apically thin thalline margin, strait to

curved, sparsely branched .... *G. koratensis*

Lirellae immersed to erumpent, with (thick) lateral thalline margin..................16

16 Lirellae elongate and irregularly branched; thallus smooth to uneven;

ascospores 45-135 µm.. *G. longispora*

Lirellae radiately branched; ascospores 60-100 µm.. .... *G. longiramea*

17 Norstictic, salazinic, stictic, or protocetraric acid 18

No substances 22

18 Salazinic and norstictic acid 19

Salazinic acid only..21

19 Lirellae variable, short and sparsely branched .... *G. librata*

Lirellae elongate, irregularly branched...20

20 Disc exposed, white-pruinose... *G. pyrrhocheiloides*

Disc concealed; white-pruinose.. *G. caesiella*

21 Lirellae irregularly branched with basal to lateral thalline margin or

labia pruinose .... *G. dendrogramma*

Lirellae erumpent, short to elongate and irregularly branch .... *G.supracola*

22 Lirellae prominent, with basal thalline margin, elongate and irregularly branched, excipulum laterally carbonized .... *G. librata*

Lirellae erumpent, short to elongate, thin, flexuose, with gently sloping thalline margins 23

23 Lirellae irregularly branched 24

Lirellae thin branched .... *G. furcata*

24 Lirellae irregularly branched; excipulum laterally carbonized; ascospores 25-35 × 10-12 µm .... *G. pinicola*

Lirellae irregularly branched; ascospore 9-15 µm .... *G. elongata*

25 Norstictic, salazinic, or stictic acid 26

No substances 29

26 Ascospores 1(-2) per ascus, (40-)50-150 µm 27

Ascospores (2-)4-8 per ascus, 20-50 µm 28

27 Lirellae erumpent, with (thick) lateral thalline margin, elongate and irregularly branched .... *G.subserpentina*

Lirellae erumpent to prominent, with apically thick complete thalline margin, short and unbranched .... *G. rongklaensis*

28 Lirellae prominent, with basal thalline margin .... *G. analoga*

Lirellae erumpent, with lateral thalline margin .... *G. renschiana*

29 Lirellae with basal thalline margin; ascospores 30-40 µm .... *G. consimilis*

Lirellae with lateral thalline margin; ascospores 30-50 µm .... *G. nanodes*

30 Hymenium clear 31

Hymenium inspersed 44

31 Ascospores transversely septate 32

Ascospores (terminally to regularly) muriform.................................. *G. acharii*

32 Ascospores 50-120 µm 33

Ascospores 15-50 µm 39

33 Lichexanthone (UV+ yellow); lirellae prominent .... *G. sauroidea*

Lichexanthone absent (UV−); lirellae and ascospores variable 34

34 Norstictic, stictic, and/or salazinic acids 35

No substances 38

35 Stictic acid, norstictic and salazinic acids absent (K+ yellow),

lirellae prominent, with thick lateral thalline margin,

elongate and irregularly branched.. *G. rustica*

Norstictic and/or salazinic acid present, sometimes additionally stictic acid ..36

36 Lirellae prominent; ascospores 60-120 µm .... *G. marginata*

Lirellae prominent 37

37 Lirellae with thick lateral thalline margin, norstictic acid............... *G. nigrocapa*

Lirellae with complete thalline margin; salazinic and stictic acid .. *G. assamensis*

38 Ascospores 70-130 µm .... *G. rhizocola*

Ascospores 40-70 µm 42

39 Lirellae immersed to erumpent, with lateral thalline margin ......*G. subdisserpens*

Lirellae prominent, with (thick) lateral to complete thalline margin 40

40 Labia white pruinose ....*G. seminuda*

Labia non-pruinose 41

41 Lirellae very long and radiately branched, lateral thalline margin thin

.... *G. falvovirens*

Lirellae elongate and irregularly branched, lateral thalline margin thick .... *G. descissa*

42 Norstictic acid... 43

No substances .... *G. intricata*

43 Lirellae erumpent, with lateral thalline margin .... *G. assimilis*

Lirellae prominent, lacking thalline margin .... *G. emersa*

44 Ascospores transversely septate .... *G. arbusculaeformis*

Ascospores (terminally to regularly) muriform.............................. *G. subvelata*

45 Excipulum apically (to peripherally) carbonized only, inner and basal parts noncarbonized, hymenium clear 46

Excipulum laterally to completely carbonized; hymenium clear or rarely inspersed... 53

46 Ascospores transversely 47

Ascospores (terminally to regularly) muriform.............................. *G. semirigida*

47 Norstictic and/or stictic acid 48

No substances 51

48 Norstictic acid, sometimes additionally stictic acid 49

Stictic acid only 50

49 Norstictic, stictic, and constictic acids; ascospores 20-60 µm long; lirellae prominent, with basal thalline margin, short and sparsely branched .... *G. subvittata*

Norstictic acid only; ascospores 75–100 µm long; lirellae prominent, with apically thin complete thalline margin, elongate and irregularly branched .... *G. trichospora*

50 Lirellae erumpent, with lateral thalline margin, ascospores 30-50 µm
.... *G. vittata*

Lirellae erumpent to prominent, lacking thalline margin, short and
sparsely branched; ascospores 20-40 µm ....*G. stenotera*

51 Labia white-pruinose 52

Labia non-pruinose ....*G. proserpens*

52 Ascospores 15-17-septate, 30-48 × 6-8 µm.............................. .*G. glaucocinerea*

Ascospores 7-9-septate, 25-35 × 5-7 µm......................................*G. glaucescens*

53 Excipulum laterally carbonized, basally absent or thin and non-carbonized ...54

Excipulum completely carbonized, basal carbonized part thin to thick 57

54 Norstictic acid...*G. verminosa*

No substances 55

55 Ascospores small 56

Ascospores small to medium-sized (30–65 µm long); lirellae prominent, lacking
or with basal thalline margin, elongate and irregularly branched
....*G. striatula*

56 Lirellae erumpent to prominent, elongate and irregularly brancehed,
ascospore 7-11-septate ....*G. duplicata*

Lirellae erumpent, with lateral thalline margin, short and sparsely,
branched, ascospore 5-9-septate ....*G. tenella*

57 Hymenium inspersed 58

Hymenium clear 59

58 Ascospores terminally muriform, lirellae prominent ....*G. phaeospora*

Ascospore regularly muriform, lirellae variable ....*G. subflexibilis*

59 Ascospores transversely septate 60

Ascospores (terminally to regularly) muriform.. 63

60 Norstictic acid... 61

No substances 62

61 Lirellae erumpent to prominent, with persistent, apically thin thalline margin, elongate and irregularly branched, with fine striation; ascospores 6-10 µm .... *G. leptospora*

Lirellae prominent, with thin thalline margin that often flakes off to expose black labia, short and sparsely branched, with very distinct and coarse striation; ascospores 15-21 µm .... *G. lumbricina*

62 Lirellae erumpent to sessile, lacking thalline margin or with basal thalline margin; short and sparsely branched; ascospores 30-50 × 7-13 µm ....*G. rimulosa*

Lirellae erumpent, with lateral thalline margin, elongate and irregularly branched, short and sparsely branched; ascospores 50-70 × 10-13 µm ....*G. longula*

63 Ascospores terminally muriform, with longitudinal septa in terminal segments; lirellae prominent, with apically thin complete margin, elongate and irregularly branched; norstictic acid............................*G. norvestitoides*

Ascospores regularly muriform or submuriform with at least a few longitudinal septa in middle segments, 1-2 per ascus; lirellae erumpent, with lateral thalline margin; no substances .... *G. myrtacea*

Description of species *Graphis*

Thirty-two species of *Graphis* are listed and described in alphabetical order.

1. *Graphis assamensis* Nagarkar & Patw.

Description: Thallus corticolous, white, whitish grey; surface smooth. Apothecia lirelliform, black, lips closed, labia non-pruinose, lirellae erumpent to prominent, 1.0-6.0 mm long and 0.1-0.3 mm wide. Exciple laterally carbonized; lirellae with complete thalline margin. Hymenium clear, 115-150 µm high, paraphyses simple. Ascospores 8 per ascus, hyaline, transversely septate, 4-8 septate, 50-95 × 25-35 µm, I+ blue.

Chemistry: Stictic acid (Thallus; PD + orange, K + yellow, C -, KC -).

Habitat: On bark of trees in dry dipterocarp forest, dry evergreen forest, mixed forest and montane forest at 250-1,500 m elevations.

Distribution: India and Eastern Palaeotropics**.**

Specimens examined: **THAILAND.** Misiones: Doi Inthanon National Park, Chiang Mai Province, Pitakpong-611 (SUT); Sakaerat Environmental Research Station, Nakhon Ratchasima Province, Pitakpong-194 (SUT); Phu Hin Rong Kla National Park, Phitsanulok Province, Pitakpong-448 (SUT); Thong Pha Phum National Park, Kanjanaburi Province, Pitakpong-1015 (SUT).

Discussion: The species resembles *Graphis cervina* Müll. Arg. immersed-erumpent ascomata, short, sparsely branched, lateral thalline margin, labia entire, exciple completely carbonized, hymenium clear, ascospores transversely septate and similar geographical distribution. *Graphis cervina* Müll. Arg. differs in ascospores.

A.

B.

Figure 3.3 Thallus (A) and spore (B) of *Graphis assamensis* Nagarkar & Patw.

2. *Graphis assimilis* Nyl.

Description: Thallus corticolous, cream, white, surface smooth. Apothecia lirelliform, black, lips closed or becoming slightly open, semi-immersed to sessile, thin, sinuous, simple or branched, 0.5-4 mm long and 0.1-0.5 mm wide. Exciple completely or almost completely carbonized. Hymenium clear, 95-120 μm high, paraphyses simple. Ascospores 8 per ascus, hyaline, transversely septate, 8-11 septate, 28.5-45 × 8-10 μm, I+ blue.

Chemistry: Norstictic acid (Thallus; PD + orange, K + yellow, C -, KC -).

Habitat: On bark of trees in dry dipterocarp forest, dry evergreen forest, mixed forest and montane forest at 200-800 m elevations.

Distribution: Central and South America, Indonesia, Singapore, Eastern Palaeotropics.

Specimens examined: **THAILAND.** Misiones: Sakaerat Environmental Research Station, Nakhon Ratchasima Province, Pitakpong-135 (SUT); Phujong-Nayoi National Park, Ubon Ratchathni Province, Pitakpong-78 (SUT); Phu Pha Terb National Park, Mukdahan Province, Pitakpong-374 (SUT); Phu Toei National Park, Suphanburi Province, Pitakpong-741 (SUT); Pang Sida National Park, Sakaew Province, Pitakpong-841 (SUT); Kaeng Krachan National Park, Phetchaburi Province, Pitakpong-1321 (SUT).

Discussion: *Graphis assimilis* Nyl. closely resembles with *Graphis koreana* S. Jpshi & Hur. *Graphis cervina* Mull. Arg. similar species with short and sparsely branched, different ascospore size 15-25 μm. *Graphis* *assimilis* Nyl. is also comparable to *Graphis koreana* S. Jpshi & Hur. in having entire labia, short, sparsely branched lirellae and a thallus containing norstictic acid, but differs in ascomata with a lateral thalline margin, white thallys, while *Graphis koreana* with a green thallus.

A.

B.

Figure 3.4 Thallus (A) and spore (B) of *Graphis assimilis* Nyl.

3. *Graphis cincta* (Pers.) Aptroot.

Description: Thallus corticolous, white, white to pale fawn, surface smooth. Apothecia lirelliform, black, lips closed, scattered, simple, straight, curved or sinuous, sessile, 0.5-2.0 mm long and 0.1-0.3 mm wide. Exciple laterally carbonised, conspicuously open at the base. Hymenium clear, 110-140 µm high, paraphyses simple. Ascospores 8 per ascus, hyaline, transversely septate, 5-8 septate, 26.0-40.0 × 7-10 µm, I+ blue.

Chemistry: Norstictic acid (Thallus; PD + orange, K + yellow, C -, KC -).

Habitat: On bark of trees in dry dipterocarp forest, dry evergreen forest, mixed forest and beach forest at 50-500 m elevations.

Distribution: West Indies, Costa Rica, India, Japan, the Philippines, Vanuatu and Pantropical.

Specimens examined: **THAILAND.** Misiones: Sakaerat Environmental Research Station, Nakhon Ratchasima Province, Pitakpong-162 (SUT); Phujong-Nayoi National Park, Ubon Ratchathni Province, Pitakpong-35 (SUT); Pang Sida National Park, Sakaew Province, Pitakpong-847 (SUT); Khao Sip Ha Chan National Park, Jantaburi Province, Pitakpong-905 (SUT); Hat Chao Mai National Park, Trang Province, Pitakpong-1446 (SUT).

Discussion: *Graphis cincta* (Pers.) Aptroot. is a distinct species with ascospores small. The similar *Graphis inspersa* Zahlbr. differs in lirellae elongate and slender.

A.

B.

Figure 3.5 Thallus (A) and spore (B) of *Graphis cincta* (Pers.) Aptroot.

4. *Graphis descissa* Müll. Arg.

Description: Thallus corticolous, white, whitish grey; surface smooth. Apothecia lirelliform, black, lips closed, labia non-pruinose; Lirellae elongate and irregularly branched, 1.0-3.0 mm long and 0.1-0.3 mm wide. Exciple laterally carbonized, with a lateral thalline margin; labia striate, convergent. Hymenium clear, 105-120 µm high, paraphyses simple. Ascospores 8 per ascus, hyaline, transversely septate, 5-8 septate, 25-40 × 7.5-10 µm, I+ blue.

Chemistry: Stictic acid (Thallus; PD + orange, K + yellow, C -, KC -).

Habitat: On bark of trees in dry dipterocarp forest, dry evergreen forest, pine forest, mixed forest and montane forest at 200-2,000 m elevations.

Distribution: Australia, Neotropics and Eastern Palaeotropics.

Specimens examined: **THAILAND.** Misiones: Doi Inthanon National Park, Chiang Mai Province, Pitakpong-618 (SUT); Khun Khan National Park, Chiang Mai Province, Pitakpong-542 (SUT); Sakaerat Environmental Research Station, Nakhon Ratchasima Province, Pitakpong-108 (SUT); Phu Hin Rong Kla National Park, Phitsanulok Province, Pitakpong-412 (SUT); Phu Toei National Park, Suphanburi Province, Pitakpong-708 (SUT); Thong Pha Phum National Park, Kanjanaburi Province, Pitakpong-1085 (SUT).

Discussion: This species is characterized by elongate and irregularly branched, lateral thalline margin thick, transversely septate ascospores and stictic acid. It is close to *Graphis flavovirens* Makhija & Adaw., but the former species has ascospores broader.

A.

B.

Figure 3.6 Thallus (A) and spore (B) of *Graphis descissa* Müll. Arg.

5. *Graphis duplicata* Ach.

Description: Thallus corticolous, white, cream; surface smooth and dull. Apothecia lirelliform, black, lips closed, disc concealed, prominent to sessile, conspicuous, numerous, dispersed, unbranched, straight, 1.0-3.0 mm long and 0.2-0.4 mm wide. Exciple laterally carbonized, lacking thalline margin; labia striate, convergent, large cluster of crystals below the exciple. Hymenium clear, 85-100 μm high, paraphyses simple. Ascospores 8 per ascus, hyaline, transversely septate, 8-11 septate, 35-45 × 6.5-8 μm, I+ blue.

Chemistry: No substances detected (Thallus; PD -, K -, C -, KC -).

Habitat: On bark of trees in dry dipterocarp forest, dry evergreen forest and mixed forest at 200-800 m elevations.

Distribution: South America, China, Indonesia, Australia and Pantropical.

Specimens examined: **THAILAND.** Misiones: Phujong-Nayoi National Park, Ubon Ratchathni Province, Pitakpong-42 (SUT); Phu Pha Terb National Park, Mukdahan Province, Pitakpong-355 (SUT); Phu Hin Rong Kla National Park, Phitsanulok Province, Pitakpong-483 (SUT); Pang Sida National Park, Sakaew Province, Pitakpong-821 (SUT); Hat Chao Mai National Park, Trang Province, Pitakpong-1421 (SUT).

Discussion: The morphologically, *Graphis duplicata* Ach. is similar to *Graphis schizograpta* Müll. Arg., which is distinguished by a laterally carbonized exciple.

A.

B.

Figure 3.7 Thallus (A) and spore (B) of *Graphis duplicata* Ach.

6. *Graphis elongata* Zenker.

Description: Thallus corticolous, white, pale fawn, off-white; surface subtuberculate to tuberculate and dull. Apothecia lirelliform, black, lips closed, conspicuous, erumpent, numerous, irregularly to radially branched, slightly curved, 1.0-3.0 mm long and 0.2-0.4 mm wide. Exciple laterally carbonized, with thick lateral thalline margin; labia entire, convergent. Hymenium clear, 85-110 μm high, paraphyses simple. Ascospores 8 per ascus, hyaline, transversely septate, 10-16 septate, 55-75 × 10-13.5 μm, I+ blue.

Chemistry: No substances detected (Thallus; PD -, K -, C -, KC -).

Habitat: On bark of trees in dry dipterocarp forest, dry evergreen forest, mixed forest and montane forest at 250-1,900 m elevations.

Distribution: Brazil and Australia.

Specimens examined: **THAILAND.** Misiones: Doi Inthanon National Park, Chiang Mai Province, Pitakpong-645 (SUT); Phujong-Nayoi National Park, Ubon Ratchathni Province, Pitakpong-69 (SUT); Phu Hin Rong Kla National Park, Phitsanulok Province, Pitakpong-491 (SUT); Phu Toei National Park, Suphanburi Province, Pitakpong-745 (SUT); Thong Pha Phum National Park, Kanjanaburi Province, Pitakpong-1198 (SUT).

Discussion: *Graphis uruguayensis* Lücking. also produces erumpent and irregulary branched ascomata, transversely septate ascospores and no lichen compounds but differs from *Graphis elongata* Zenker. in ascospore broader.

A.

B.

Figure 3.8 Thallus (A) and spore (B) of *Graphis elongata* Zenker.

7. *Graphis emersa* Mull.Arg.

Description: Thallus corticolous, white, whitish grey; surface smooth to subtuberculate and dull. Apothecia lirelliform, black, lips closed, sessile, numerous, conspicuous, dispersed, unbranched and straight, 1.0-3.0 mm long and 0.1-0.3 mm wide; disc concealed. Exciple completely carbonized, lacking a thalline margin; labia entire, convergent. Hymenium clear, 105-120 µm high, paraphyses simple. Ascospores 8 per ascus, hyaline, transversely septate, 7-11 septate, 32-45× 7.5-8.5 µm, I+ blue.

Chemistry: Norstictic acid (Thallus; PD + orange, K + yellow, turning red, C -, KC -).

Habitat: On bark of trees in dry dipterocarp forest, dry evergreen forest, pine forest, mixed forest and montane forest at 200-1,900 m elevations.

Distribution: Thailand, Japan, Australia and Pantropical.

Specimens examined: **THAILAND.** Misiones: Doi Inthanon National Park, Chiang Mai Province, Pitakpong-627 (SUT); Khun Khan National Park, Chiang Mai Province, Pitakpong-518 (SUT); Phujong-Nayoi National Park, Ubon Ratchathni Province, Pitakpong-51 (SUT); Phu Hin Rong Kla National Park, Phitsanulok Province, Pitakpong-474 (SUT); Phu Toei National Park, Suphanburi Province, Pitakpong-723 (SUT); Thong Pha Phum National Park, Kanjanaburi Province, Pitakpong-1143 (SUT).

Discussion: *Graphis emersa* Mull.Arg. is a distinct species with entire labia, completely carbonized exciple without a thalline margin, clear hymenium, transversely septate ascospores and norstictic acid. The similar *Graphis meridionalis* M. Nakan. differ in 1-2 spored asci.

A.

B.

Figure 3.9 Thallus (A) and spore (B) of *Graphis emersa* Mull.Arg.

8. *Graphis falvovirens* Makhija & Adaw.

Description: Thallus corticolous, white, whitish grey; surface smooth. Apothecia lirelliform, black, lips closed, labia non-pruinose; Lirellae elongate and irregularly branched, 1.0-6.0 mm long and 0.1-0.3 mm wide. Exciple laterally carbonized, labia non-pruinose; lirellae very long and radiately branched, lateral thalline margin thin. Hymenium clear, 85-100 µm high, paraphyses simple. Ascospores 8 per ascus, hyaline, transversely septate, 4-8 septate, 18-40 × 5.5-8 µm, I+ blue.

Chemistry: Stictic acid (Thallus; PD + orange, K + yellow, C -, KC -).

Habitat: On bark of trees in dry dipterocarp forest, dry evergreen forest, mixed forest and montane forest at 250-800 m elevations.

Distribution: India, Neotropics and Eastern Palaeotropics.

Specimens examined: **THAILAND.** Misiones: Phujong-Nayoi National Park, Ubon Ratchathni Province, Pitakpong-76 (SUT); Phu Pha Terb National Park, Mukdahan Province, Pitakpong-263 (SUT); Phu Toei National Park, Suphanburi Province, Pitakpong-783 (SUT); Pang Sida National Park, Sakaew Province, Pitakpong-823 (SUT); Kaeng Krachan National Park, Phetchaburi Province, Pitakpong-1342 (SUT).

Discussion: *Graphis flavovirens* Makhija & Adaw. is chemically similar *Graphis descissa* Müll. Arg. but differs in longer irregularly branched and inspersed hymenium.

A.

B.

Figure 3.10 Thallus (A) and spore (B) of *Graphis falvovirens* Makhija & Adaw.

9. *Graphis furcata* Fée.

Description: Thallus corticolous, greyish white to pale fawn, surface smooth, slightly glossy. Apothecia lirelliform, black, lips closed, sessile, simple, 0.5-3.0 mm long and 0.1-0.3 mm wide, with an inconspicuous basal thalline margin. Exciple laterally carbonised, conspicuously open at the base. Hymenium clear, 60-95 µm high, paraphyses simple. Ascospores 8 per ascus, hyaline, transversely septate, 8-10 septate, 20.0-32.0 × 6-8 µm, I+ blue.

Chemistry: No substances detected (Thallus; PD -, K -, C -, KC -).

Habitat: On bark of trees in dry dipterocarp forest, dry evergreen forest, mixed forest and mangroves forest at 100-850 m elevations.

Distribution: Central and South America, Angola and the Philippines.

Specimens examined: **THAILAND.** Misiones: Sakaerat Environmental Research Station, Nakhon Ratchasima Province, Pitakpong-146 (SUT); Phu Pha Terb National Park, Mukdahan Province, Pitakpong-241 (SUT); Pang Sida National Park, Sakaew Province, Pitakpong-808 (SUT); Khao Sip Ha Chan National Park, Jantaburi Province, Pitakpong-976 (SUT); Kaeng Krachan National Park, Phetchaburi Province, Pitakpong-1263 (SUT); Hat Chao Mai National Park, Trang Province, Pitakpong-1463 (SUT).

Discussion: *Graphis furcata* Fée is most similar *Graphis scripta* (L.) Ach in ascospore long, with differs in labia non-pruinose or rarely thinly white-pruinose. The other similar species, *Graphis foliicola* Vain. differ from *Graphis furcata* Fée. in thallus not well-developed.

A.

B.

Figure 3.11 Thallus (A) and spore (B) of *Graphis furcata* Fée.

10. ***Graphis glaucescens*** **Fée**

Description: Thallus corticolous, white, cream; surface smooth, farinose-sorediate. Apothecia lirelliform, black, lips closed, sessile, numerous, lirellae immersed to erumpent, with lateral thalline margin and pruinose labia, elongate and irregularly branched, 1.0-3.0 mm long and 0.1-0.3 mm wide; disc concealed. Exciple completely carbonized, lacking a thalline margin; labia entire, convergent. Hymeniumclear, 100-120 µm high, paraphyses simple. Ascospores 8 per ascus, hyaline, transversely septate, 6-10 septate, 28-45 × 7-8.5 µm, I+ blue.

Chemistry: No substances detected (Thallus; PD -, K -, C -, KC -).

Habitat: On bark of trees in dry dipterocarp forest, dry evergreen forest, mixed forest, pine forest and montane forest at 300-2,000 m elevations.

Distribution: Australia and Pantropical.

Specimens examined: **THAILAND.** Misiones: Doi Inthanon National Park, Chiang Mai Province, Pitakpong-678 (SUT); Khun Khan National Park, Chiang Mai Province, Pitakpong-593 (SUT); Phu Hin Rong Kla National Park, Phitsanulok Province, Pitakpong-415 (SUT); Phu Toei National Park, Suphanburi Province, Pitakpong-771 (SUT); Thong Pha Phum National Park, Kanjanaburi Province, Pitakpong-1230 (SUT).

Discussion: *Graphis glaucescens* Fée. is similar in lirellae morphology and no thallus compound to *Graphis bulacana* Vain. and *Graphis bulacana* Vain., which differs in smaller ascospores.

A.

B.

Figure 3.12 Thallus (A) and spore (B) of *Graphis glaucescens* Fée.

11. *Graphis handelii* Zahlbr.

Description: Thallus corticolous, cream, cream to yellowish grey; surface smooth to dull. Apothecia lirelliform, black, epruina, lips closed, erumpent, inconspicuous, sparse, dispersed, unbranched, straight, 0.5-2.0 mm long and 0.1-0.3 mm wide; disc concealed. Exciple laterally carbonized or sometimes slightly thin completely carbonized, lateral thalline margin; labia entire, convergent; usually including small crystals. Hymenium inspersed, 85-120 μm high, paraphyses simple. Ascospores 8 per ascus, hyaline, transversely septate, 6-10 septate, 25-40 × 6-8μm, I + blue.

Chemistry: Norstictic acid (Thallus; PD + orange, K + yellow, turning red, C -, KC -).

Habitat: On bark of trees in dry dipterocarp forest, dry evergreen forest and mixed forest at 50-800 m elevations.

Distribution: Paraguay, India, China and Pantropical.

Specimens examined: **THAILAND.** Misiones: Sakaerat Environmental Research Station, Nakhon Ratchasima Province, Pitakpong-149 (SUT); Phujong-Nayoi National Park, Ubon Ratchathni Province, Pitakpong-53 (SUT); Phu Hin Rong Kla National Park, Phitsanulok Province, Pitakpong-403 (SUT); Thong Pha Phum National Park, Kanjanaburi Province, Pitakpong-1211 (SUT); Hat Chao Mai National Park, Trang Province, Pitakpong-1463 (SUT).

Discussion: *Graphis handelii* Zahlbr. is most similar in entire labia, laterally carbonized exciple, inspersed hymenium, transversely septate ascospore to *Graphis crebra* Vain. which differs in disc white-pruinose.

A.

B.

Figure 3.13 Thallus (A) and spore (B) of *Graphis handelii* Zahlbr.

12. *Graphis hossei* Vain.

Description: Thallus corticolous, white, white grey; surface subtuberculate and dull, weakly developed with scattered crystals. Apothecia lirelliform, black, epruina; lips black; disc concealed, conspicuous, erumpent to prominent, numerous, sparsely branched, almost straight, 1.0-3.0 mm long and 0.1-0.3 mm wide; disc concealed. Exciple completely carbonized, with a basal to lateral thalline margin; labia entire, convergent. Hymenium clear, 115-150 μm high, paraphyses simple. Ascospores 4-8 per ascus, hyaline, transversely septate, 12-17 septate, 58-85 × 9-13 μm, I+ blue.

Chemistry: No substances detected (Thallus; PD -, K -, C -, KC -).

Habitat: On bark of trees in dry dipterocarp forest, dry evergreen forest, mixed forest and montane forest at 350-2,000 m elevations.

Distribution: Costa Rica, China, Thailand, Indonesia, the Philippines and Pantropical.

Specimens examined: **THAILAND.** Misiones: Doi Inthanon National Park, Chiang Mai Province, Pitakpong-656 (SUT); Phujong-Nayoi National Park, Ubon Ratchathni Province, Pitakpong-53 (SUT); Phu Pha Terb National Park, Mukdahan Province, Pitakpong-368 (SUT); Pang Sida National Park, Sakaew Province, Pitakpong-817 (SUT); Thong Pha Phum National Park, Kanjanaburi Province, Pitakpong-1093 (SUT); Kaeng Krachan National Park, Phetchaburi Province, Pitakpong-1315 (SUT).

Discussion: The sympatric *Graphis hossei* Vain. also has entire labia, a laterally carbonized exciple, a clear hymenium, transverselly septate ascospores and produces no lichen compounds. It is similar to *Graphis tenuis* Vain. and *Graphis nematodiza* Vain., but it has smaller ascospores.

A.

B.

Figure 3.14 Thallus (A) and spore (B) of *Graphis hossei* Vain.

13. ***Graphis intricata*** **Fée.**

Description: Thallus corticolous, white, cream, pale grey; surface smooth or sometimes subtuberculate and dull. Apothecia lirelliform, black, lips closed, labia non-pruinose; lirellae elongate to very long and irregularly to radiately branched, 1.0-3.0 mm long and 0.2-0.5 mm wide, epruina; disc concealed. Exciple laterally carbonized, with a lateral thalline margin; labia striate, convergent. Hymenium clear, 85-100 μm high, paraphyses simple. Ascospores 8 per ascus, hyaline, transversely septate, 5-7 septate, 15-28 × 6.5-8 μm, I+ blue.

Chemistry: Norstictic acid (Thallus; PD + orange, K + yellow, turning red, C -, KC -).

Habitat: On bark of trees in dry dipterocarp forest, dry evergreen forest, mixed forest and beach forest at 50-800 m elevations.

Distribution: South America, India and Pantropical.

Specimens examined: **THAILAND.** Misiones: Phujong-Nayoi National Park, Ubon Ratchathni Province, Pitakpong-47 (SUT); Phu Toei National Park, Suphanburi Province, Pitakpong-770 (SUT); Pang Sida National Park, Sakaew Province, Pitakpong-819 (SUT); Kaeng Krachan National Park, Phetchaburi Province, Pitakpong-1326 (SUT); Hat Chao Mai National Park, Trang Province, Pitakpong-1576 (SUT).

Discussion: The sympatric *Graphis confinis* C. Knight & Mitt., *Graphis tapetica* Zahlbr. and *Graphis hunana* Zahlbr. entire labia, completely carbonized exciple without a thalline margin, clear hymenium, transversely septate ascospores and norstictic acid but differs from *Graphis intricata* Fée. in comparatively smaller ascopores.

A.

B.

Figure 3.15 Thallus (A) and spore (B) of *Graphis intricata* Fée.

14. *Graphis jejuensis* K. H. Moon, M. Nakan. & Kashiw.

Description: Thallus corticolous, greyish white to pale fawn, surface smooth, slightly glossy. Apothecia lirelliform, black, lips closed, sessile, simple, 0.5-3.0 mm long and 0.1-0.3 mm wide. Exciple laterally carbonised, conspicuously open at the base. Hymenium clear, 70-100 µm high, paraphyses simple. Ascospores 8 per ascus, hyaline, transversely septate, 7-9 septate, 18.5-25.0 × 5-8 µm, I+ blue.

Chemistry: No substances detected (Thallus; PD -, K -, C -, KC -).

Habitat: On bark of trees in dry dipterocarp forest, dry evergreen forest, mixed forest and montane forest at 250-1,800 m elevations.

Distribution: South Korea.

Specimens examined: **THAILAND.** Misiones: Doi Inthanon National Park, Chiang Mai Province, Pitakpong-695 (SUT); Khun Khan National Park, Chiang Mai Province, Pitakpong-579 (SUT); Sakaerat Environmental Research Station, Nakhon Ratchasima Province, Pitakpong-157 (SUT); Phujong-Nayoi National Park, Ubon Ratchathni Province, Pitakpong-33 (SUT); Phu Hin Rong Kla National Park, Phitsanulok Province, Pitakpong-492 (SUT); Phu Toei National Park, Suphanburi Province, Pitakpong-785 (SUT); Thong Pha Phum National Park, Kanjanaburi Province, Pitakpong-1076 (SUT).

Discussion: *Graphis jejuensis* K. H. Moon, M. Nakan. & Kashiw. is given as carbonized apically or in the upper half and similar with *Graphis pertricosa* (Kremp.) A. W. Archer. which is known with such a character combination, differing in the muriform ascospores and norstictic acid.

A.

B.

Figure 3.16 Thallus (A) and spore (B) of *Graphis jejuensis* K. H. Moon, M. Nakan. & Kashiw.

15. *Graphis librata* C. Knight.

Description: Thallus corticolous, white-grey to yellowish; surface smooth and dull, slightly cracked; cortex whitish grey, loose. Apothecia lirelliform, black, erumpent, spares, dispersed, unbranched, straight, 0.5-2.0 mm long and 0.1-0.3 mm wide, with a thin pruina; disc concealed. Exciple laterally carbonized or sometimes slightly thin completely carbonized, lateral thalline margin; labia entire, convergent; usually including small crystals. Hymenium inspersed, 85-120 μm high, paraphyses simple. Ascospores 8 per ascus, hyaline, transversely septate, 6-8 septate, 25-32 × 6-8μm, I+ blue.

Chemistry: Norstictic acid (Thallus; PD + orange, K + yellow, turning red, C -, KC -).

Habitat: On bark of trees in dry dipterocarp forest, dry evergreen forest, mixed forest, pine forest and montane forest at 350-2,000 m elevations.

Distribution: U.S.A., South Africa, India, New Zealand and Pantropical.

Specimens examined: **THAILAND.** Misiones: Doi Inthanon National Park, Chiang Mai Province, Pitakpong-672 (SUT); Khun Khan National Park, Chiang Mai Province, Pitakpong-525 (SUT); Phu Hin Rong Kla National Park, Phitsanulok Province, Pitakpong-478 (SUT); Thong Pha Phum National Park, Kanjanaburi Province, Pitakpong-1062 (SUT).

Discussion: *Graphis librata* C. Knight. is a short and sparsely branched, ascomata with concealed disc, entire labia and with slightly pruina, laterally carbonized exciple, a clear hymenium, transversely septate ascospores and norstictic acid. It is similar to *Graphis filiformis* Adaw. & Makhija. but *Graphis librata* has the shorter and erumpent lirella.

A.

B.

Figure 3.17 Thallus (A) and spore (B) of *Graphis librata* C. Knight.

16. *Graphis lineola* Ach.

Description: Thallus corticolous, white, white grey; surface subtuberculate and dull, weakly developed with scattered crystals. Apothecia lirelliform, black, lips black; disc concealed, conspicuous, numerous, sparsely branched, 1.0-4.0 mm long and 0.1-0.3 mm wide; disc concealed. Exciple completely carbonized, labia non-pruinose; lirellae erumpent, with lateral thalline margin, convergent. Hymenium clear, 85-100 µm high, paraphyses simple. Ascospores 8 per ascus, hyaline, transversely septate, 5-10 septate, 25-40 × 6-8 µm, I+ blue.

Chemistry: No substances detected (Thallus; PD -, K -, C -, KC -).

Habitat: On bark of trees in dry dipterocarp forest, dry evergreen forest and mixed forest at 300-800 m elevations.

Distribution: Costa Rica and Pantropical.

Specimens examined: **THAILAND.** Misiones: Phujong-Nayoi National Park, Ubon Ratchathni Province, Pitakpong-86 (SUT); Phu Pha Terb National Park, Mukdahan Province, Pitakpong-322 (SUT); Pang Sida National Park, Sakaew Province, Pitakpong-822 (SUT); Kaeng Krachan National Park, Phetchaburi Province, Pitakpong-1285 (SUT).

Discussion: *Graphis lineola* Ach. is a short and sparsely branched labia entire, excipulum laterally carbonized, hymenium inspersed, ascospores transversely septate and norstictic acid. It is similar to *Graphis submarginata* Lücking., but *Graphis lineola* Ach. has disc concealed, labia non-pruinose.

A.

B.

Figure 3.18 Thallus (A) and spore (B) of *Graphis lineola* Ach.

17. *Graphis longiramea* Müll. Arg.

Description: Thallus corticolous, greenish yellow; surface smooth or sometimes subtuberculate and dull. Apothecia lirelliform, black, lips closed, erumpent, conspicuous, numerous, dispersed, sparsely branched, straight or curved, 1.0-6.0 mm long and 0.1-0.3 mm wide, epruina; disc slightly open. Exciple laterally carbonized, thick laterally with a thaline margin; labia entire, convergent. Hymenium clear, 185-210 µm high, paraphyses simple. Ascospores 8 per ascus, hyaline, transversely septate, 9-18 septate, 80-95 × 7-13 µm, I+ blue.

Chemistry: Stictic acid (Thallus; PD + orange, K + yellow, C -, KC -).

Habitat: On bark of trees in dry dipterocarp forest, dry evergreen forest and mixed forest at 100-700 m elevations.

Distribution: India and Pantropical.

Specimens examined: **THAILAND.** Misiones: Phu Pha Terb National Park, Mukdahan Province, Pitakpong-308 (SUT); Pang Sida National Park, Sakaew Province, Pitakpong-834 (SUT); Khao Sip Ha Chan National Park, Jantaburi Province, Pitakpong-995 (SUT); Hat Chao Mai National Park, Trang Province, Pitakpong-1530 (SUT).

Discussion: *Graphis longiramea* Müll. Arg. is distinguished simple to branched ascomata with open disc and entire labia, a laterally carbonized exciple, a thick lateral thalline margin, a clear hymenium, transversely septate ascospores more than 70 µm long and stictic. It closes to *G. sitapurensis* Makhija & Adaw. but differs by smaller ascospores and a thin lateral thalline margin.

A.

B.

Figure 3.19 Thallus (A) and spore (B) of *Graphis longiramea* Müll. Arg.

18. *Graphis longula* Kremp.

Description: Thallus corticolous, white, white grey; surface smooth and dull. Apothecia lirelliform, black, lips black; erumpent to prominent, spare, irregulary branched, straight to partly curved, 1.0-4.0 mm long and 0.1-0.3 mm wide; epruina; disc concealed. Exciple completely carbonized, with a basal to thin lateral thalline margin, with crystals; labia striate, convergent. Hymenium clear, 105-150 µm high, paraphyses simple. Ascospores 8 per ascus, hyaline, transversely septate, 5-8 septate, 45-70 × 6-10 µm, I+ blue.

Chemistry: No substances detected (Thallus; PD -, K -, C -, KC -).

Habitat: On bark of trees in dry dipterocarp forest, dry evergreen forest, mixed forest and montane forest at 250-1,300 m elevations.

Distribution: Brazil and Pantropical.

Specimens examined: **THAILAND.** Misiones: Khun Khan National Park, Chiang Mai Province, Pitakpong-547 (SUT); Phujong-Nayoi National Park, Ubon Ratchathni Province, Pitakpong-54 (SUT); Khao Sip Ha Chan National Park, Jantaburi Province, Pitakpong-948 (SUT); Thong Pha Phum National Park, Kanjanaburi Province, Pitakpong-1181 (SUT).

Discussion: *Graphis longula* Kremp. is a labia striate, excipulum completely carbonized, hymenium clear, ascospores transversely septate. It differs from *Graphis asterizans* Nyl. by lirellae short to elongate, stellately branched and thallus verrucose.

A.

B.

Figure 3.20 Thallus (A) and spore (B) of *Graphis longula* Kremp.

19. *Graphis nanodes* Vain.

Description: Thallus corticolous, whitish-grey to dark grey; surface smooth and dull. Apothecia lirelliform, black, lips black; erumpent, inconspicuous, numerous, dispersed, unbranched, straight, 1.0-4.0 mm long and 0.1-0.3 mm wide, epruina; disc concealed. Exciple laterally carbonized, with a lateral thalline margin; labia entire, convergent. Hymenium clear, 85-100 μm high, paraphyses simple. Ascospores 8 per ascus, hyaline, ellipsoidal, muriform, 7-12 transverse septa × 3-7 longitudinal septa, 25-40 × 8-12 μm, I+ blue.

Chemistry: No substances detected (Thallus; PD -, K -, C -, KC -).

Habitat: On bark of trees in dry dipterocarp forest, dry evergreen forest, mixed forest and beach forest at 50-1,500 m elevations.

Distribution: The Philippines, South Korea and Pantropical.

Specimens examined: **THAILAND.** Misiones: Phu Pha Terb National Park, Mukdahan Province, Pitakpong-352 (SUT); Pang Sida National Park, Sakaew Province, Pitakpong-856 (SUT); Khao Sip Ha Chan National Park, Jantaburi Province, Pitakpong-913 (SUT); Hat Chao Mai National Park, Trang Province, Pitakpong-1457 (SUT).

Discussion: *Graphis nanodes* Vain. is a labia entire, excipulum laterally carbonized, hymenium clear, ascospores muriform and no compound substances. It is similar to *Graphis consimilis* Vain. but differs in lirellae with basal thalline margin.

A.

B.

Figure 3.21 Thallus (A) and spore (B) of *Graphis nanodes* Vain.

20. *Graphis nigrocarpa* Adaw. & Makhija.

Description: Thallus corticolous, white, whitish grey, surface smooth, dense with a few crystals. Apothecia lirelliform, black, lips closed, erumpent to prominent, numerous, conspicuous, dispersed, sparsely branched, straight or curved, 1.5-6.0 mm long and 0.1-0.3 mm wide. Exciple completely carbonized, with a thick lateral thalline margin; labia entire, convergent. Hymenium clear, 95-120 µm high, paraphyses simple. Ascospores 8 per ascus, hyaline, transversely septate, 10-15 septate, 45-55 × 7.5-8 µm, I+ blue.

Chemistry: Norstictic acid (Thallus; PD+ orange, K + yellow, turning red, C -, KC -).

Habitat: On bark of trees in dry dipterocarp forest, dry evergreen forest, mixed forest and beach forest at 100-800 m elevations.

Distribution: India and Eastern Palaeotropics.

Specimens examined: **THAILAND.** Misiones: Phujong-Nayoi National Park, Ubon Ratchathni Province, Pitakpong-72 (SUT); Phu Pha Terb National Park, Mukdahan Province, Pitakpong-301 (SUT); Pang Sida National Park, Sakaew Province, Pitakpong-884 (SUT); Khao Sip Ha Chan National Park, Jantaburi Province, Pitakpong-1005 (SUT); Kaeng Krachan National Park, Phetchaburi Province, Pitakpong-1350 (SUT); Hat Chao Mai National Park, Trang Province, Pitakpong-1680 (SUT).

Discussion: The species resembles *Graphis nigrocarpa* Adaw. & Makhija. in having branched ascomata with entire labia, a clear hymenium, transversely septate ascospores and a similar geographical distribution.

But *Graphis nigrocarpa* Adaw. & Makhija differ in chemical characters.

A.

B.

Figure 3.22 Thallus (A) and spore (B) of *Graphis nigrocarpa* Adaw. & Makhija.

21. *Graphis pinicola* Zahlbr.

Description: Thallus corticolous, white, whitish grey, surface smooth. Apothecia lirelliform, black, lips closed, erumpent to prominent, numerous, conspicuous, dispersed, sparsely branched, straight or curved, 1.0-4.0 mm long and 0.1-0.3 mm wide. Exciple completely carbonized, with a thick lateral thalline margin; labia entire, convergent. Hymenium clear, 90-120 µm high, paraphyses simple. Ascospores 8 per ascus, hyaline, transversely septate, 8-10 septate, 25-40 × 6.5-8 µm, I+ blue.

Chemistry: No substances detected (Thallus; PD -, K -, C -, KC -).

Habitat: On bark of trees in dry dipterocarp forest, dry evergreen forest and mixed forest at 80-800 m elevations.

Distribution: Florida and Pantropical.

Specimens examined: **THAILAND.** Misiones: Phujong-Nayoi National Park, Ubon Ratchathni Province, Pitakpong-64 (SUT); Phu Hin Rong Kla National Park, Phitsanulok Province, Pitakpong-476 (SUT); Pang Sida National Park, Sakaew Province, Pitakpong-855 (SUT); Khao Sip Ha Chan National Park, Jantaburi Province, Pitakpong-941 (SUT); Kaeng Krachan National Park, Phetchaburi Province, Pitakpong-1291 (SUT); Hat Chao Mai National Park, Trang Province, Pitakpong-1597 (SUT).

Discussion: *Graphis pinicola* Zahlbr. and *Graphis castanopsidis* Zahlbr. are similar in lirellae with lateral thalline margin, transversely septate ascospores, and no chemical character in thallus. *Graphis pinicola* Zahlbr. differs in larger ascopores.

A.

B.

Figure 3.23 Thallus (A) and spore (B) of *Graphis pinicola* Zahlbr.

22. *Graphis renschiana* (Müll. Arg.) Stizenb.

Description: Thallus corticolous, white, whitish grey, surface smooth. Apothecia lirelliform, black, lips closed, erumpent, numerous, elongate and irregularly branched, 1.0-4.0 mm long and 0.1-0.3 mm wide. Exciple completely carbonized, labia entire, convergent. Hymenium clear, 80-100 µm high, paraphyses simple. Ascospores 8 per ascus, hyaline, transversely septate, 8-10 septate, 20-40 × 7.5-15 µm, I+ blue.

Chemistry: Norstictic acid (Thallus; PD+ orange, K + yellow, turning red, C -, KC -).

Habitat: On bark of trees in dry dipterocarp forest, dry evergreen forest and mixed forest at 100-1,500 m elevations.

Distribution: Florida and Pantropical.

Specimens examined: **THAILAND.** Misiones: Khun Khan National Park, Chiang Mai Province, Pitakpong-501 (SUT); Phu Hin Rong Kla National Park, Phitsanulok Province, Pitakpong-450 (SUT); Pang Sida National Park, Sakaew Province, Pitakpong-833 (SUT); Kaeng Krachan National Park, Phetchaburi Province, Pitakpong-1327 (SUT); Hat Chao Mai National Park, Trang Province, Pitakpong-1423 (SUT).

Discussion: *Graphis renschiana* Müll. Arg. also produces entire labia, lirellae with lateral thalline margin, excipulum laterally carbonizded, ascoprotes 8 per ascus but differs from *Opegrapha gracilis* Fr. and *Graphis antillarum* var. *manilensis* Vain. in ascospores size.

A.

B.

Figure 3.24 Thallus (A) and spore (B) of *Graphis renschiana* (Müll. Arg.) Stizenb.

23. ***Graphis rhizocola*** **(Fée) Lücking & Chaves.**

Description: Thallus corticolous, whitish-grey to dark grey; surface smooth and dull. Apothecia lirelliform, black, lips blacka slightly covered by white pruina; disc concealed; prominent, conspicuous, sparse, dispersed, unbranched, straight to curved, 1.0-4.0 mm long and 0.1-0.3 mm wide, epruina; disc concealed. Exciple completely carbonized, complete thalline margin; labia entire, convergent. Hymenium clear, 85-100 μm high, paraphyses simple. Ascospores 8 per ascus, hyaline, transversely septate, 15-20 setate, 75-100 × 12-16 μm, I+ blue.

Chemistry: No substances detected (Thallus; PD -, K -, C -, KC -).

Habitat: On bark of trees in dry dipterocarp forest, dry evergreen forest, mixed forest and montane forest at 300-1,800 m elevations.

Distribution: South America, United Kingdom, Brazil, Costa Rica and Neotropics.

Specimens examined: **THAILAND.** Misiones: Doi Inthanon National Park, Chiang Mai Province, Pitakpong-632 (SUT); Phu Hin Rong Kla National Park, Phitsanulok Province, Pitakpong-481 (SUT); Phu Toei National Park, Suphanburi Province, Pitakpong-723 (SUT); Thong Pha Phum National Park, Kanjanaburi Province, Pitakpong-1053 (SUT); Kaeng Krachan National Park, Phetchaburi Province, Pitakpong-1309 (SUT).

Discussion: *Graphis rhizocola* (Fée) Lücking & Chaves. also produces entire, excipulum completely carbonized, hymenium clear and ascospores transversely septate but differs from *Graphis conglomerata* Spreng. in lirellae short and stellately branched.

A.

B.

Figure 3.25 Thallus (A) and spore (B) of *Graphis rhizocola* (Fée) Lücking & Chaves.

24. *Graphis rimulosa* (Mont.) Trevis.

Description: Thallus corticolous, white, whitish grey, surface smooth and dull. Apothecia lirelliform, black, lips closed, conspicuous, sulcate, scattered, sessile, rarely subimmersed, straight, curved or sinuous, simple or rarely branched, 1.0-3.0 mm long and 0.1-0.4 mm wide. Exciple completely carbonized, labia entire. Hymenium clear, 78-120 µm high, paraphyses simple. Ascospores 8 per ascus, hyaline, transversely septate, 8-10 septate, 30-48 × 7.5-12 µm, I+ blue.

Chemistry: No substances detected (Thallus; PD -, K -, C -, KC -).

Habitat: On bark of trees in dry dipterocarp forest, dry evergreen forest, mixed forest and beach forest at 100-800 m elevations.

Distribution: Australia.

Specimens examined: **THAILAND.** Misiones: Phujong-Nayoi National Park, Ubon Ratchathni Province, Pitakpong-24 (SUT); Phu Toei National Park, Suphanburi Province, Pitakpong-706 (SUT); Pang Sida National Park, Sakaew Province, Pitakpong-802 (SUT); Khao Sip Ha Chan National Park, Jantaburi Province, Pitakpong-957 (SUT); Hat Chao Mai National Park, Trang Province, Pitakpong-1519 (SUT).

Discussion: *Graphis rimulosa* (Mont.) Trevis. is characterised by the completely carbonised proper exciple and the absence of lichen compounds. The species is distinguished from the chemically similar *Graphis subtenella* Müll.Arg. by the completely carbonised proper exciple and the larger ascospores.

A.

B.

Figure 3.26 Thallus (A) and spore (B) of *Graphis rimulosa* (Mont.) Trevis.

25. ***Graphis seminuda*** **Müll. Arg.**

Description: Thallus corticolous, whitish grey; surface smooth to subtuberculate and dull. Apothecia lirelliform, black, lips closed, conspicuous, sulcate, scattered, sessile, rarely subimmersed, straight, curved or sinuous, simple or rarely branched, 1.0-5.0 mm long and 0.2-0.4 mm wide. Exciple completely carbonized, labia entire. Hymenium clear, 95-140 μm high, paraphyses simple. Ascospores 8 per ascus, hyaline, transversely septate, 12-18 septate, 45-65 × 7.5-12 μm, I+ blue.

Chemistry: No substances detected (Thallus; PD -, K -, C -, KC -).

Habitat: On bark of trees in dry dipterocarp forest, dry evergreen forest, mixed forest and montane forest at 200-1,800 m elevations.

Distribution: India and Pantropical.

Specimens examined: **THAILAND.** Misiones: Doi Inthanon National Park, Chiang Mai Province, Pitakpong-683 (SUT); Khun Khan National Park, Chiang Mai Province, Pitakpong-515 (SUT); Phu Hin Rong Kla National Park, Phitsanulok Province, Pitakpong-498 (SUT); Thong Pha Phum National Park, Kanjanaburi Province, Pitakpong-1064 (SUT); Kaeng Krachan National Park, Phetchaburi Province, Pitakpong-1252 (SUT).

Discussion: *Graphis seminuda* var. *sublaevis* Müll. Arg. and *Graphis catherinae* A. W. Archer., which are most similar to *Graphis seminuda* Müll. Arg., have distinctly white pruinose ascomatal discs.

A.

B.

Figure 3.27 Thallus (A) and spore (B) of *Graphis seminuda* Müll. Arg.

26. ***Graphis streblocarpa*** **(Bel.)Nyl.**

Description: Thallus corticolous, whitish grey, pale greyish green; surface smooth to tuberculate and dull. Apothecia lirelliform, black, lips closed, erumpent, conspicuous, numerous, dispersed, sparsely branched, straight or curved, 1.0-6.0 mm long and 0.1-0.3 mm wide,epruina; disc slightly open. Exciple laterally carbonized or sometimes apically carbonized, usually with a lateral thalline margin; labia entire, convergent. Hymenium clear, 155-180 µm high, paraphyses simple. Ascospores 1-4 per ascus, hyaline, muriform, 8-15 transverse septa × 3-5 longitudinal septa, 78-90 × 25-30 µm, I+ blue.

Chemistry: Stictic acid (Thallus; PD + orange, K + yellow, C -, KC -).

Habitat: On bark of trees in dry dipterocarp forest, dry evergreen forest and mixed forest at 80-1,200 m elevations.

Distribution: India, Thailand, the Philippines, Australia, Neotropics and Eastern Palaeotropics.

Specimens examined: **THAILAND.** Misiones: Phu Hin Rong Kla National Park, Phitsanulok Province, Pitakpong-407 (SUT); Phu Toei National Park, Suphanburi Province, Pitakpong-735 (SUT); Hat Chao Mai National Park, Trang Province, Pitakpong-1482 (SUT).

Discussion: *Graphis streblocarpa* (Bel.) Nyl. also produces entire labia, excipulum apically carbonized, hymenium clear, ascospores muriform and stictic acid but differs from *Graphis japonica* (Müll. Arg.) A. W. Archer & Lücking. in smaller ascopores.

A.

B.

Figure 3.28 Thallus (A) and spore (B) of *Graphis streblocarpa* (Bel.) Nyl.

27. ***Graphis subserpentina*** **Nyl.**

Description: Thallus corticolous, white; surface smooth and dull. Apothecia lirelliform, black, lips closed, epumpent to prominent, conspicuous, numerous, dispersed, sparsely to irregulary branched, straight, curved, 1.0-3.0 mm long and 0.2-0.4 mm wide, thinly pruina; disc concealed. Exciple laterally carbonized, sometimes the base almost closed, covered by a thin complete thalline margin with scattered small crystals; labia entire, convergent. Hymenium clear, 140-185 µm high, paraphyses simple and thicken at tips. Ascospores 1-2 per ascus hyaline, muiform, 85-120 × 28.5-35 µm, I+ blue.

Chemistry: Norstictic acid (Thallus; PD + orange, K + yellow, turning red, C -, KC -).

Habitat: On bark of trees in dry dipterocarp forest, dry evergreen forest and mixed forest at 200-800 m elevations.

Distribution: India, Sri Lanka, Malaysia, Singapore, the Philippines, Australia and Pantropical.

Specimens examined: **THAILAND.** Misiones: Sakaerat Environmental Research Station, Nakhon Ratchasima Province, Pitakpong-184 (SUT); Phujong-Nayoi National Park, Ubon Ratchathni Province, Pitakpong-97 (SUT); Phu Pha Terb National Park, Mukdahan Province, Pitakpong-329 (SUT); Phu Toei National Park, Suphanburi Province, Pitakpong-729 (SUT); Khao Sip Ha Chan National Park, Jantaburi Province, Pitakpong-968 (SUT); Thong Pha Phum National Park, Kanjanaburi

Province, Pitakpong-1125 (SUT); Kaeng Krachan National Park, Phetchaburi Province, Pitakpong-1301 (SUT).

Discussion: *Graphis subserpentina* Nyl. is characterized by erumpent to remarkable elongate and irregularly branched ascomata, whith a thick lateral to complete thalline margin and norstictic acid in thallus. It is similar *Graphis insulana* (Müll. Arg.) Lücking & Sipman., *Graphis saxicola* (Müll. Arg.) A. W. Archer. and *Graphis streblocarpa* (Bél.) Nyl., *Graphis insulana* but differs by an inspersed hymenium *Graphis saxicola* has smaller ascospores, while *Graphis streblocarpa* produces stictic acid in the thallus.

A.

B.

Figure 3.29 Thallus (A) and spore (B) of *Graphis subserpentina* Nyl.

28. *Graphis subvelata* Stirt.

Description: Thallus corticolous, whitish-grey, yellowish white; surface smooth and dull. Apothecia lirelliform, black, erumpent, conspicuous, sparse, dispersed, sparsely branched, straight to rarely curved, 1.0-4.0 mm long and 0.1-0.3 mm wide, epruina; disc concealed. Exciple completely carbonized with basal thalline margin; labia entire, convergent. Hymenium inspersed, 80-95 µm high, paraphyses simple. Ascospores 8 per ascus, hyaline, submuriform, 6-10 transverse septa × 1-2 longitudinal septa, 28-45 × 8-12 µm, I+ blue.

Chemistry: No substances detected (Thallus; PD -, K -, C -, KC -).

Habitat: On bark of trees in dry dipterocarp forest, dry evergreen forest and mixed forest at 250-700 m elevations.

Distribution: Thailand, Australia and Pantropical.

Specimens examined: **THAILAND.** Misiones: Khun Khan National Park, Chiang Mai Province, Pitakpong-509 (SUT); Phujong-Nayoi National Park, Ubon Ratchathni Province, Pitakpong-81 (SUT); Phu Hin Rong Kla National Park, Phitsanulok Province, Pitakpong-416 (SUT); Phu Toei National Park, Suphanburi Province, Pitakpong-742 (SUT).

Discussion: *Graphis subvelata* Stirt. is characterized by entire labia, a laterally carbonized excipulum, an inspersed hymenium, muriform ascospores and no lichens compounds, *Graphis pilarensis* Cáceres & Lücking. is similar, but differs by very large ascospores.

A.

B.

Figure 3.30 Thallus (A) and spore (B) of *Graphis subvelata* Stirt.

29. *Graphis tenella* Ach.

Description: Thallus corticolous, white, greyish-white to pale fawn, surface smooth and slightly shiny. Apothecia lirelliform, black, lips closed, erumpent to prominent, irregulary branched, straight, curved, sinuous, 0.5-3.5 mm long and 0.15-0.3 mm wide. Exciple apically carbonized and pale brown below with a lateral thalline margin. Hymenium clear, 68-90 µm high, paraphyses simple. Ascospores 8 per ascus, hyaline, transversely septate, 4-8 septate, 17.4 -20.8 × 6.8-9.2 µm, I + blue.

Chemistry: No substances detected (Thallus; PD -, K -, C -, KC -).

Habitat: On bark of trees in dry dipterocarp forest, dry evergreen forest and mixed forest at 250-800 m elevations.

Distribution: Brazil, Australia, Argentina, Costa Rica, Socotra, Zanzibar, Japan, Hawaii, Indonesia, Papua New Guinea, New Caledonia, New Zealand and Pantropical.

Specimens examined: **THAILAND.** Misiones: Phujong-Nayoi National Park, Ubon Ratchathni Province, Pitakpong-73 (SUT); Phu Hin Rong Kla National Park, Phitsanulok Province, Pitakpong-422 (SUT); Pang Sida National Park, Sakaew Province, Pitakpong-899 (SUT); Khao Sip Ha Chan National Park, Jantaburi Province, Pitakpong-990 (SUT); Thong Pha Phum National Park, Kanjanaburi Province, Pitakpong-1188 (SUT).

Discussion: The species resembles *Graphis tenella* Ach. in having laterally carbonised proper exciple, the small ascospores and the absence of lichen compounds but differs from *Graphis duplicata* Ach.in lacking thalline margin or with basal thalline margin, elongate and irregularly branched.

A.

B.

Figure 3.31 Thallus (A) and spore (B) of *Graphis tenella* Ach.

30. *Graphis verminosa* Müll. Arg.

Description: Thallus corticolous, white, cream, pale grey; surface smooth or sometimes subtuberculate and dull. Apothecia lirelliform, black, lips closed, erumpent to prominent, conspicuous, numerous, dispersed, simple to rarely branched, straight or curved, 1.0-3.0 mm long and 0.2-0.5 mm wide, epruina; disc concealed. Exciple laterally carbonized, with a lateral thalline margin; labia striate, convergent. Hymenium clear, 105-140 µm high, paraphyses simple to sparsely branched at the epithecial region. Ascospores 8 per ascus, hyaline, transversely septate, 18-30 septate, 55-85 × 6.5-8 µm, I+ blue.

Chemistry: Norstictic acid (Thallus; PD + orange, K + yellow, turning red, C -, KC -).

Habitat: On bark of trees in dry dipterocarp forest, dry evergreen forest, mixed forest and montane forest at 200-1,500 m elevations.

Distribution: India and Eastern Palaeotropics.

Specimens examined: **THAILAND.** Misiones: Doi Inthanon National Park, Chiang Mai Province, Pitakpong-667(SUT); Khun Khan National Park, Chiang Mai Province, Pitakpong-543 (SUT); Sakaerat Environmental Research Station, Nakhon Ratchasima Province, Pitakpong-172 (SUT); Phujong-Nayoi National Park, Ubon Ratchathni Province, Pitakpong-15 (SUT); Phu Hin Rong Kla National Park, Phitsanulok Province, Pitakpong-456 (SUT); Phu Toei National Park, Suphanburi Province, Pitakpong-764 (SUT).

Discussion: *Graphis verminosa* Müll. Arg. is most similar in thallus compound and lirellae morphology to *Graphis semirigida* with difference in muriform ascospores.

A.

B.

Figure 3.32 Thallus (A) and spore (B) of *Graphis verminosa* Müll. Arg.

31. *Graphis vittata* Müll. Arg.

Description: Thallus corticolous, cream, greyish green, surface smooth and slightly shiny. Apothecia lirelliform, black, lips closed, erumpent, irregulary branched, straight, curved, sinuous, 0.5-4.5 mm long and 0.1-0.3 mm wide. Exciple apically carbonized and pale brown below with a lateral thalline margin, labia striate. Hymenium clear, 80-110 µm high, paraphyses simple. Ascospores 8 per ascus, labia non-pruinose, hyaline, transversely septate, 7-11 septate, 35.5 -55.5 × 6.8-8.0 µm, I + blue.

Chemistry: Stictic acid (Thallus; PD + orange, K + yellow, C -, KC -).

Habitat: On bark of trees in dry dipterocarp forest, dry evergreen forest and mixed forest at 250-800 m elevations.

Distribution: Indonesia, China and Taiwan

Specimens examined: **THAILAND.** Misiones: Khun Khan National Park, Chiang Mai Province, Pitakpong-536 (SUT); Phu Toei National Park, Suphanburi Province, Pitakpong-755 (SUT); Pang Sida National Park, Sakaew Province, Pitakpong-945 (SUT); Kaeng Krachan National Park, Phetchaburi Province, Pitakpong-1374 (SUT).

Discussion: *Graphis vittata* Müll. Arg. also produces erumpent to prominent ascomata, striate labia, an apically carbonized exciple, a clear hymenium, transversely septate ascospores and stictic acid but differs from *Graphis stenotera* Vain. in thalline margin and has smaller ascospores.

A.

B.

Figure 3.33 Thallus (A) and spore (B) of *Graphis vittata* Müll. Arg.

New species

32. *Graphis koratensis* Pitakpong, Kraichak, Lücking.

Type: Thailand, Nakhon Ratchasima Province, Sakaerat Environmental Research Station, dry dipterocarp forest, located on a height of 380 meters above sea level, tree trunks, 2014, Pitakpong D205 (SUT-holotypus, F-isotypus).

Description: Thallus corticolous, 3-6 cm diam., 150-250 µm thick in cross section, continuous; surface smooth to uneven, pale whitish- grey or grey-green, cortex distinct 10-15 µm, algal layer continuous 50-75 µm, medulla 100-150 µm thick, with clusters of calcium oxalate crystals. Ascomata lireliform, emergent to prominent, straight to curved, sparsely branched, with apically thin thalline margin, 2-7 mm long, 0.3-0.5 mm wide, 0.1-0.15 mm high, labia thick, appearing greyish black but with pruina along the slit, Proper exciple completely carbonized, 100-140 µm wide, hymenium clear, 100-150 µm high, asci, 20-25 µm long, 110-140 µm wide. Ascospore 8 per ascus, oblong to narrowly fusiform, transversely, 11-19 septate, 70-115 µm long, 9-15 µm wide, colourless.

Chemistry: Norstictic acid (thallus in section with K+ yellow efflux forming red, needle–shaped crystals, C–, P+ orange, UV–).

Etymology: The epithet was derived from the Thai word *Korat*, referring to the traditional name of the type locality (Nakhon Ratchasima).

Remarks: This new species is closely related to *G. caesiella* Vain. in having labia white-pruinose, lirellae elongate and irregularly branched, but also differs in its colour of thallus, ascospore and the colour of K reaction test. While the new species has whitish-grey or grey-green thallus and large spores, *G. caesiella* Vain. has white thallus with smaller ascospore (20-40 × 6-8 μm). The new species also is tested K+ yellow, while *G. caesiella* Vain. tested K+ orange-red.

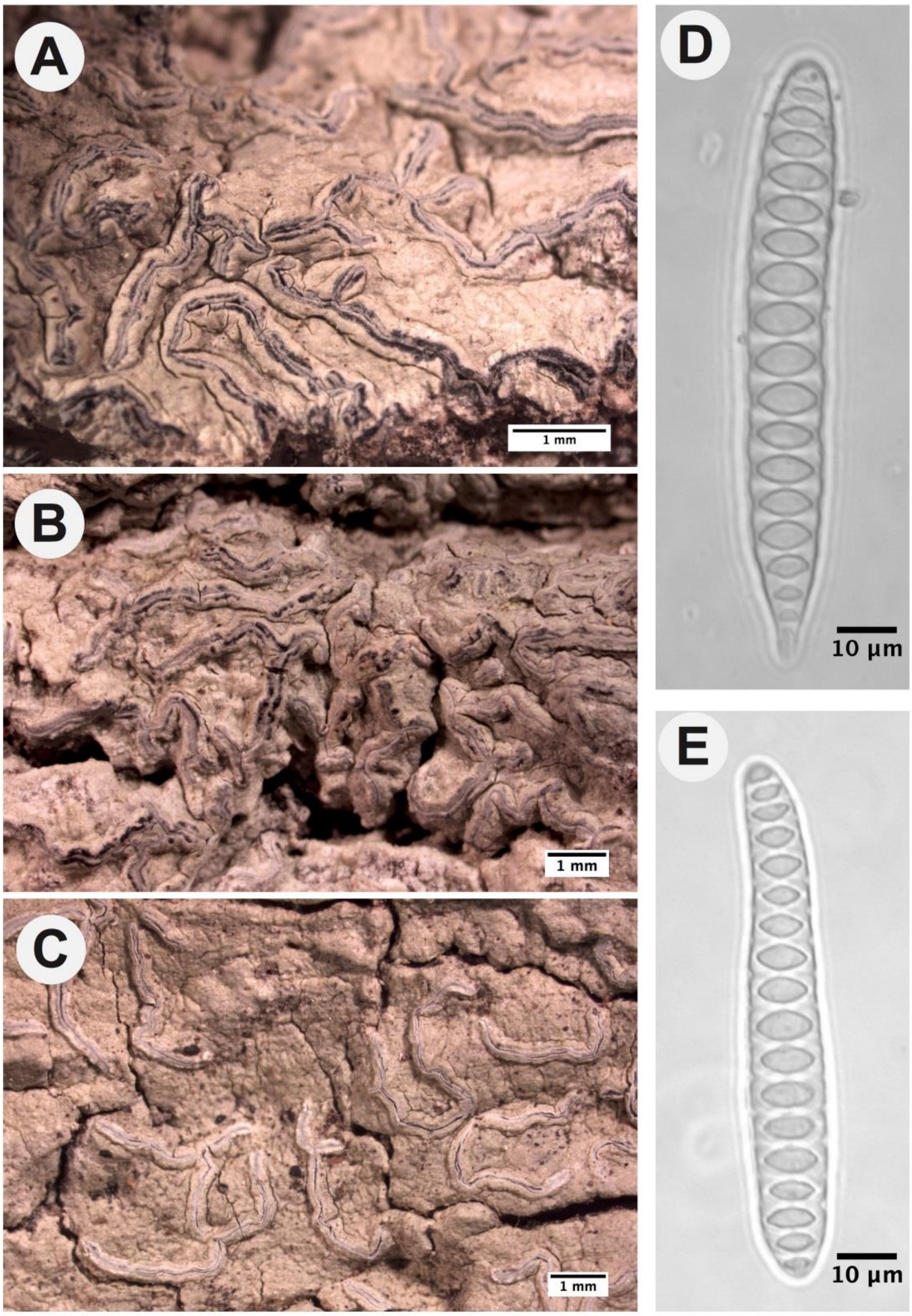

Figure 3.34 *Graphis koratensis* Pitakpong, Kraichak, Lücking. **A-B**: Thallus and ascomata showing lirellae from Pitakpong D205, **C**: from Pitakpong A04, and **D-E:** ascospores from Pitakpong D205.

3.5 Conclusion

For the identification of lichens morphology, anatomy, chemistry and molelular data were used. The study found that 6 of 32 species of *Graphis* identified were reported for the first time (*Graphis cincta* (Pers.) Aptroot, *Graphis jejuensis* KH Moon, M. Nakan. & Kashiw., *Graphis nigrocarpa* Adaw. & Makhija., *Graphis renschiana* (Müll. Arg.) Stizenb., *Graphis seminuda* Müll. Arg., and *Graphis subserpentina* Nyl.) and one species of the *Graphis* lichens was proposed as the new world record (*Graphis koratensis* Pitakpong, Kraichak, Lücking).

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CHAPTER IV

PHYLOGENY OF LICHEN GENUS *GRAPHIS* IN THAILAND

4.1 Abstract

Phylogeny is the evolutions development of organism as they change through time. Evidences from morphological, anatomical, and chemical data show that all organisms on earth are genetically related and genealogical relationships of living things can be represented by evolutionary tree. The aim of this studied is to confirm new species by molecular studies. The phylogeny of the genus *Graphis* based on two loci of mitochondrial small subunit ribosomal DNA (mtSSU) and large subunit of nuclear ribosomal DNA (nuLSU) were carried out to elucidate the position of new species. Maximum likelihood and Bayesian analyses were used to estimate the phylogenetic trees for a set of *Graphis* species. It was found that phylogeny study supported the synonymy of genus *Graphis* and confirmed new species (*Graphis koratensis* Pitakpong, Kraichak, & Lücking). Its morphologically similar to *Graphis caesiella*, the new species differs in a number of apothecia characters and chemistry. The new species is also phylogenetically distinct from *Graphis caesiella* with strong supports from both the Maximum likelihood and Bayesian analysis, 98 and 0.91, respectively. From these morphological, anatomical, chemical, and molecular studies,

the new *Graphis* species named *Graphis koratensis* Pitakpong, Kraichak, Lücking were found with the clear phylogeny data.

4.2 Introduction

All previous evolutionary studies of lichens used only morphological and chemical data. The major complications in using these data are difficulties in defining homologies especially in structurally simple organisms like fungi and the lack of suitable characteristics. Investigations based on molecular data have been shown to be a valuable tool in current taxonomy of lichenized fungi. Previously, molecular data used in lichenized fungal phylogenetics revealed that single genes may fail to resolve a phylogeny. Phylogeny is the evolutionary development and history of organismal lineages as they change through time. It implies that different species arise from previous forms via descent. Evidence from morphological, biochemical, and gene sequence data suggest that all organisms on earth are genetically related and genealogical relationships of living things can be represented by a vast evolutionary tree, the tree of life. Organisms that are alive today are the leaves of this giant tree, and if we could trace their history back down the branches of the tree of life, we would encounter their ancestor, which lived thousands or millions or hundreds of millions of years ago. Organisms have evolved through the ages from ancestral forms into more derived forms. New lineages generally retain many of their ancestral features, which are then gradually modified and supplemented with novel traits that help them to better adjust to the environment they live in. Studying the phylogeny of organisms can help us explain similarity and differences among plants, animals, and microorganisms. The tree of life thus provides a rigorous framework to guide research in all biological sub disciplines, and it is therefore an ideal model for the organization of biological knowledge (Soltis *et al*., 2005; Simpson, 2010).

Although secondary metabolites have been used in taxonomy at different levels, in many cases molecular data do not correspond with the chemical variation and the correlation between them has to be evaluated for each case de novo. As recent molecular studies show, subtle morphological and chemical characters can support the distinction of phylogenetic lineages as species (Divakar *et al*., 2005; 2006), which suggests that the real number of species is higher than previously known.

In the absence of a phylogeny estimate based on morphology, morphological data could be discussed in narrative form relative to an accepted phylogeny based on molecular data (McDade *et al*., 2000). Such an approach suffers from the lack of a corroborated accurate phylogeny even in the presence of high bootstrap values and reduces morphology to a subsidiary role in classification. The incongruent morphological characters are interpreted as a simple model of character evolution-plesiomorphy, synapomorphy and convergence – and a molecular phylogeny still need more analysis to discover a procedure. Other approaches such as combined analysis of morphological and molecular data remain controversial (Klung and Wolfe, 1993; Nixon and Carpenter, 1996) and are cautioned against because of a lack of critical detail on anatomy. Poorly circumscribed morphological characters combined with molecular data are unlikely to provide a clearer understanding of phylogeny. Recently, there have been several critical studies of particular morphological structures that have increased our understanding of phylogenetic issues (Schönenberger and Endress, 1998; Manktelow, 2000).

There have been molecular systematic studies addressing the higher level systematics of Graphidaceae (Fernández-Brime *et al*., 2013). Graphidaceae is currently accepted to include the previously independent families Thelotremataceae (Rivas Plata *et al*., 2013), Gomphillaceae, Asterothyriaceae, and Solorinellaceae (Baloch *et al*., 2010; Rivas Plata *et al*., 2012a; b), Futhermore, it is the second largest family of lichenized fungi and the dominant element of lichen communities in tropical regions, with over 1800 accepted species (Lücking *et al*., 2009; Rivas Plata *et al*., 2012a; 2013). Quantitative extrapolations and molecular data available for species complexes, as well as continuous new discoveries, suggest that the family may actually contain well over 2000 species (Lücking *et al*., 2011; Rivas Plata *et al*. 2012b; 2013; Sipman *et al*., 2012).

The general aim of the present work was to use large subunit of nuclear ribosomal DNA (nuLSU) and small subunit of mitochondrial ribosomal DNA (mtSSU) sequences in order to clarify the phylogenetic relationships in genus *Graphis* and related taxa as well as species delimitation in *Graphis koratensis*.

4.3 Materials and Methods

A major step to resolve the phylogeny of many species belonging to the former Graphidaceae was made by Rivas Plata *et al*. (2013) and Lumbsh *et al*. (2014). Their analysis of the mitochondrial small subunit ribosomal DNA (mtSSU rDNA) and large subunit of nuclear ribosomal DNA (nuLSU) showed that the infrageneric aggregations are probably more different than previously thought.

Mitochondria are eukaryotic intracellular organelles which are present in animals, plants and fungi. These organelles contain DNA that codes for specific proteins and is self-replicated. The sizes of mtDNA molecules are in fungi range from about 30 to 176 kb (Griffiths, 1996). Due to a rapid rate of sequence divergence in mitochondrial genome, mtDNA is widely used in many studies of eukaryotic populations or evolutionary biology (Taylor, 1986). The sequence data obtained from mitochondrial rDNA are mostly used to study phylogenetic relationships in various groups of fungi and lichenized fungi for several reasons. There are eight universal conserved regions which form the minimal core secondary structure of mtSSU rRNA, important for protein biosynthesis (Gray *et al*., 1984). In this study, one forward primer (mrSSU1) and three reverse primers (mrSSU2R, mrSSU3R and MSU7) were employed, solid boxes (U1-U8) indicate universal conserved regions and unshaded boxes indicate variable and semi-conserved regions. The sequence data, especially those of mtSSU rDNA were used to detect genetic variability and taxonomic position at different taxonomic levels of lichenized fungi such as in cyanobacterial lichens in the suborder Lecanoromycetes (Lumbsch *et al*., 2004), Parmeliaceae (Crespo *et al*., 2007) and Graphidaceae (Lücking *et al*., 2012; 2014; Rivas Plata *et al*., 2013).

The large subunit of nuclear ribosomal DNA (nuLSU) comprises the 5.8S and the 25-28S genes which contains relatively conserved and divergent domains (Hopple and Vilgalys, 1999). The conserved regions show great similarity in secondary structure even between prokaryotes and eukaryotes hence are considered essential for ribosome function, while the divergent domains (D domains) are variable areas which contain a valuable source of phylogenetic information (Chilton, 2003). In fungal systematics, D domain sequence data, especially D1-D3 data are extensively used for higher and lower level phylogenies of basidiomycetes and yeasts but rarely in the lichenized fungi. Later in 2000, Döring and colleagues designed PCR specific primers for nuLSU of lichenized ascomycetes and used in combination with other previously designed primers. The result revealed successful amplifications of all primers. The nuLSU sequence data have been used to re-examine the systematic position of various lichen groups such as baeomycetoid lichens Porinaceae (Grube *et al*., 2004), Trypetheliaceae (Del Prado *et al*., 2006), Ostropales (Lumbsch *et al*., 2007) Roccellaceae (Tehler and Irestedt, 2007), Parmeliaceae (Crespo *et al*., 2007; Divakar *et al*., 2010), and Graphidaceae (Staiger *et al*., 2006; Lücking *et al*., 2012; 2014; Rivas Plata *et al*., 2013).

This study is performed when morphological, anatomical, and chemical data have shown unclear results or failed to clearly identify lichens. Phylogenetic tree was developed to separate the taxa from each other and analyze character evolution with appropriate data analysis (Swofford, 2002).

DNA extraction

To perform a DNA extraction, the lichen specimen was first disrupted. Then, a 15 µl of extraction solution (DNA extraction using Red-Extract Sigma Kit (Sigma Aldrich, USA) was added. After that, the specimen was heated at 95°C for 15 minutes. Finally, a 15 µl of dilution solution (dilutions of 9:1 of genomic DNA extraction) was added.

PCR amplification

Two DNA regions: mitochondrial small subunit of the ribosomal DNA (mtSSU) and large subunit of the nuclear ribosomal DNA (nuLSU) were used in the present study. The primers for the PCR amplifications were nuLSU: AL2R3

(Mangold *et al*., 2008) and LR3 (Vilgalys and Hester, 1990) and mtSSU: mrSSU1 and mrSSU3R (Zoller *et al*., 1999) (Table 4.1).

PCR reactions contained 2.5 µl Sigma RED-Extract-N-Amp™ PCR, 0.5 µl of each primer (10 µM), 2 µl genomic DNA extract and 4 µl distilled water for a total of 10 µl. Thermal cycling conditions were as follows:

i) for nuLSU: initial denaturation for 5 min at 94°C, followed by 35 cycles of 30 s at 95°C, 30 s at 58°C, 1 min at 72°C, and a final elongation for 10 min at 72°C

ii) for mtSSU: initial denaturation for 5 min at 95°C, followed by 35 cycles of 45s at 94°C, 1 min at 50°C, 1 min 30 s at 72°C, and a final elongation for 10 min at 72°C.

Table 4.1 Primers for PCR amplifications of the nuLSU and mtSSU gene.

Primer	F/R	Sequence	References
AL2R	F	GCGAGTGAAGCGGCAACAGCTC	Mangold *et al*. (2008)
LR3	R	GGTCCGTGTTTCAAGAC	Vilgalys and Hester (1990)
mrSSU1	F	AGCAGTGAGGAATATTGGTC	Zoller *et al*. (1999)
mrSSU3R	R	ATGTGGCACGTCTATAGCCC	Zoller *et al*. (1999)

Amplification products were visualized on 1% agarose gels strained with ethidium bromide. The product was purified by the cutting of the target bands and digesting of the gel materials at 70°C for 10 min or until it turned liquid. Then it was cooled to 45°C-heat block for 5 min, treated with 1 µl of gelase (Epicentre

Biotechnologies, Madison, WI, USA) and incubated at 45°C for at least 3 hour or left overnight.

DNA sequencing

The procedure of cycle sequencing was performed according to the instruction manual provided by BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems). For each reaction of 10 µl, 4.6 µl of nuclease-free water, 1.25X sequencing buffer, each of the primers at 0.4 mM concentration, 1.0 µl of purified DNA template and 1.5 µl of BigDye Terminator was added to the PCR beads. The solution was mixed well by inversion and then spun down. It was a liquoted into a 96-well PCR plate. After sealing the plate with PCR film the solution was mixed by vortexing for 3 sec. The plate was then placed in the thermal cycler (Eppendorf). Sequencing profile used included an initial heating step at 94°C for 3 min, followed by 25 cycles of 96°C for 10 sec, 50°C for 5 sec and 60°C for 4 min. Once the cycle sequencing reactions were completed, the products were precipitated with 25 µl of 100% ethanol, mixed with 1 µl of 3M NaOAC, and 1 µl of EDTA, before they were loaded on an ABI PRISM^{TM} 3730 DNA Analyzer (Foster City, California, USA). Sequence fragments obtained were assembled with Geneious 8.0.3 (Drummond *et al*., 2014) manually inspected and adjusted.

Phylogenetic analysis

The purposes of phylogenetic studies are to reconstruct evolutionary relationships of organisms and to estimate the time of divergence between those organisms since they last shared a common ancestor (Hall, 2001). The present study investigated the evolution of lichen genus *Graphis* and its evolutionary relationship. A new species of lichen was discovered in the present study. In this study, the molecular data including DNA sequences of partial mtSSU and nuLSU gene were used for phylogenetic analyses. Two methods of phylogenetic tree reconstruction, maximum likelihood (ML) and Bayesian approach were employed.

Maximum likelihood (ML) method seeks the tree with the largest log-likelihood. The term ln L_{tree} was the log-likelihood of observing the alignment under the chosen evolutionary model, given that particular tree with its branching order and branch lengths (Hall, 2004). ML analyses were performed using the program GARLI (Genetic Algorithm for Rapid Likelihood Inference) v0.96 (Zwickl, 2006). The program was able to perform maximum likelihood tree searches on large data sets in a number of hours. These analyses were carried out assuming the general time-reversible model of nucleotide substitution (Rodriguez *et al*., 1990), including estimation of invariant sites and assuming a discrete gamma distribution with six rate categories (GTR+I+G). Bootstrap analysis was performed with 2000 pseudoreplicates.

Bayesian approach uses an evolutionary model to derive the probability of data matrix given a tree topology and model parameter. In this study, Bayesian inference (BI) of phylogeny was performed using the software MrBayes 3.1.2 (Huelsenbeck and Ronquist, 2001) and the online tool at http://www.phylo.org/sub_sections/portal (Miller *et al*., 2010). Mr.Bayes uses a simulation technique called Markov Chain Monte Carlo (MCMC) to approximate the posterior probabilities of trees. The posterior probability of each branch was calculated by counting the frequency of trees that were visited during the course of the MCMC analysis. The analysis was performed using the general time-reversible (GTR) model (Rodriguez *et al*., 1990) including estimation of invariant sites and assuming a discrete gamma distribution with six rate categories (GTR+I+G) for a single gene as well as a combined analysis of different nucleotide regions. Data sets were partitioned. Each partition was allowed to have its own parameter values (Nylander *et al*., 2004). No molecular clock was assumed. Heating of chains was set to 0.2. Posterior probabilities were approximated by sampling trees using a variant of MCMC method. Number of generations was 10 millions. To avoid autocorrelation, only every 1000th tree was sampled. The first 4000 generations were discarded as burn in. The log-likelihood scores of sample points against generation time were plotted using TRACER v1.4.1 (http://tree.bio.ed.ac.uk/software/tracer/) to ensure that stationarity was achieved after the first 4000 generations (the log-likelihood values of the sample points reached a stable equilibrium value) (Huelsenbeck and Ronquist, 2001). In addition, AWTY (Nylander *et al*., 2004; 2007) was used to compare split frequencies in different runs and then cumulative split frequencies were plotted to ensure that stationarity was reached. A majority rule consensus tree with average branch lengths was calculated using the “sumt” option of MrBayes.

Posterior probabilities were obtained for each clade. Only clades with bootstrap support > 70% under the ML analyses and posterior probabilities > 0.95 in the Bayesian analysis were considered as strongly supported. Phylogenetic trees were visualized using the program. Both ML and Bayesian analyses were performed on the CIPRES super computer cluster (Miller *et al*., 2010). The topology from the ML analysis was illustrated using the R-package ape (Paradis *et al*., 2004).

4.4 Result and Discussion

Data of 48 representative samples were assembled using mtSSU and nuLSU sequences. Specimens and the sequences used for molecular analyses are listed in Table 4.2 These species plus *Diorygma antillarum* and *Diorygma minisporum* available from GenBank were selected as out group based on previous phylogenetic studies in genus *Graphis* (Nelsen *et al*., 2012; Rivas Plata *et al*., 2013). Four new sequences were generated in this study, including 2 mtSSU and 4 nuLSU sequence. A combined data matrix of 42 unambiguously aligned characters which was used for phylogenetic analyses of 21 characters of mtSSU and 34 characters of nuLSU. Phylogenetic trees were obtained from the combined and separated data sets based on Maximum likelihood and Bayesian analyses.

In our analysis, the core Graphidaceae is divided into two strongly supported clades representing genera *Allographa* and *Graphis* (Figures 4.1). The genus *Allographa* includes species of the *Graphis cinerea* group with large ascospores but genus *Graphis* with small ascospore, moreover thallus morphology also appears to be a good predictor of clade relationships, with white, strongly crystalline thalli characteristic of *Graphis* and *Allographa* (Rivas Plata *et al*., 2013).

Phylogenetic analysis of genus *Graphis* was conducted based on the mtSSU and nuLSU sequences. Forty-eight taxa were used in this analysis. There were so many problems in the analysis of mtSSU and nuLSU sequence data; because of ambiguity, the sequences were difficult to align and the DNA could not be amplified. Unfortunately, their DNA bands did not show or their sequences were still messy. So, more than a half of the samples were excluded from this analysis and only four samples were analyzed. However, the limited analysis from this region showed the tree support quite well, and it further was necessary to include this region in a combined analysis. In this analysis, 4 samples of *Graphis* sp. A (Pitakpong A04 (SUT); Pitakpong 311 (SUT); Pitakpong D205 (SUT); Pitakpong E108 (SUT)) were clearly, by strong support of high bootstrap support value from the maximum likelihood analysis and the posterior probability from the Bayesian Analysis (ML = 98; PP = 0.98 respectively), which is used to confirm giving a new species. *Graphis* sp. A (new species), is named as *Graphis koratensis* Pitakpong, Kraichak, Lücking. (Chapter III).

According to Grube and Kroken (2000), recognition of a new species is possible when single-gene phylogeny shows the strongly supported monophyly of the corresponding lineage and is also supported by clear phenotypic character. In our case, however, at the moment it seems to be better to regard specimens of *Graphis koratensis*. As the morphological characters, distributional and ecological data do not clearly define *Graphis koratensis*, no taxonomic innovation is proposed until more molecular multilocus data are available.

Graphis koratensis forms a strongly supported monophyletic clade to true *Graphis caesiella* (Figure 4.1). The morphological character of *Graphis koratensis* is closely related to *Graphis caesiella*, but from phylogenetic position of *Graphis koratensis* was mentioned because they were clearly distinguished from *Graphis caesiella*, beside the difference in some morphological characters, differs in its colour of thallus, ascospore and the colour of K reaction test. The *Graphis koratensis* has whitish-grey or grey-green thallus and large spores, *Graphis caesiella* has white thallus with smaller ascospore (20-40 × 6-8 μm). The *Graphis koratensis* also is tested K+ yellow, while *Graphis caesiella* tested K+ orange-red (Lücking *et al*., 2009).

The phylogenetic position of *Graphis koratensis* close to the *Graphis handelii* is rather surprising, but from morphological study, *Graphis handelii* has disc non-pruinose, excipulum laterally carbonized, ascospores 5-11-septate (20-40 × 6-9 µm), but *Graphis koratensis* has disc pruinose, ascospore 11-19-septate (70-115 × 9-15 µm) (Lücking *et al*., 2009).

Table 4.2 Specimens used for studying the generic status of the genus *Graphis*, with localities, reference collection details and GenBank accession numbers. The missing sequences are indicated with a dash.

Species	Voucher information	GenBank Acc. No.	
		mtSSU	**nuLSU**
Diorygma antillarum	*Lücking* 33019 (F)	JX046454	JX046467
D. minisporum	*Lumbsch* 19543v (F)	HQ639598	HQ639626
Graphis albissima	*Rivas Plata* 1004D (F)	HQ639604	–
G. anfractuosa	*Hernandez* 1340 (F)	HQ639618	–
G. angustata	*Lücking* 28102 (F)	HQ639612	HQ639632
G. caesiella 1	*Berger* 17247 (Hb. Berger)	DQ431975	AY640028
G. caesiella 2	*Lumbsch* 20540i (F)	JX421065	–
G. caesiella 3	*Lumbsch* 20530a (F)	JX421066	–
G. cinerea	*Kalb* 26950 (Hb. Kalb)	DQ431988	DQ431947
G. gracilescens	*Lücking* 33942B (Hb. Kalb)	DQ431976	DQ431936
G. chrysocarpa	*Lücking* No. 00-35 (Hb. Kalb)	DQ431987	–
G. dichotoma	*Rivas Plata* 2088 (F)	–	HQ639633
G. furcata	*Rivas Plata* 1172Q (F)	HQ639607	–
G. handelii	*Green* GR4BH	KC592281	–
G. illinata	*Lumbsch* 19639 (F)	HQ639614	HQ639634
G. amplicate 1	*Lücking* 28527 (F)	–	HQ639653
G. amplicate 2	*Rivas Plata* 0103A (F)	HQ639602	–
G. amplicate 3	*Lücking* 28104 (F)	–	HQ639655
G. amplicate 4	*Lücking* 28039 (F)	–	HQ639654
G. amplicate 5	*Lücking* 16103a (F)	KJ440975	KJ440928
G. amplicate 6	*Lücking* 16103b (Hb. Kalb)	DQ431978	DQ431939
G. leptoclada	*Lumbsch* 20535b (F)	JX421068	JX421509
G. librata 1	*Lücking* 28007 (F)	–	HQ639637
G. librata 2	*Lücking* 28001 (F)	KJ440976	KJ440929
G. librata 3	*Lücking* 28001b (F)	HQ639621	HQ639636
G. pavoniana	*Lücking* 16100c (Hb. Kalb)	DQ431986	DQ431946
G. proserpens	*Rivas Plata* 2065 (F)	HQ639619	–
G. pseudocinerea 1	*Lücking* 26537 (F)	HQ639620	HQ639639
G. pseudocinerea 2	*Lücking* 26531 (F)	–	HQ639638
G. pseudocinerea 3	*Lücking* 26532a (F)	–	HQ639640
G. pseudoserpens 1	*Lücking* 28048 (F)	–	HQ639642
G. pseudoserpens 2	*Lücking* 28003 (F)	–	HQ639641

Table 4.2 Specimens used for studying the generic status of the genus *Graphis*, with localities, reference collection details and GenBank accession numbers. The missing sequences are indicated with a dash (Continued).

Species	Voucher information	GenBank Acc. No.	
		mtSSU	nuLSU
G. rhizocola 1	*Lücking* 28512 (F)	–	HQ639644
G. rhizocola 2	*Lücking* 28548 (F)	–	HQ639645
G. rhizocola 3	*Lücking* 28502 (F)	–	HQ639643
G. rimulosa	*Rivas Plata* 1021H (F)	JX421069	–
G. scripta 1	*Tønsberg* 42518 (BG)	KJ440969	KJ440922
G. scripta 2	*Nelsen* MN499 (F)	KJ461720	KJ440935
G. scripta 3	*Neuwirth* 11834 (ABL)	KJ440977	KJ440932
G. sp.A	*Pitakpong* A04 (SUT)	KP862882	KP862884
G. sp.A	*Pitakpong* 311 (SUT)	KP862883	KP862887
G. sp.A	*Pitakpong* D205 (SUT)	–	KP862885
G. sp.A	*Pitakpong* E108 (SUT)	–	KP862886
G. streblocarpa	*Rivas Plata* 1015E (F)	–	HQ639646
G. tenella	*Rivas Plata* 1007G (F)	–	HQ639647
G. tsunodae	*Lücking* 26096 (F)	–	JX421511
G. vestitoides	*Rivas Plata* 2078 (F)	–	HQ639648
G. xanthospora	*Lücking* 26535 (F)	–	HQ639649

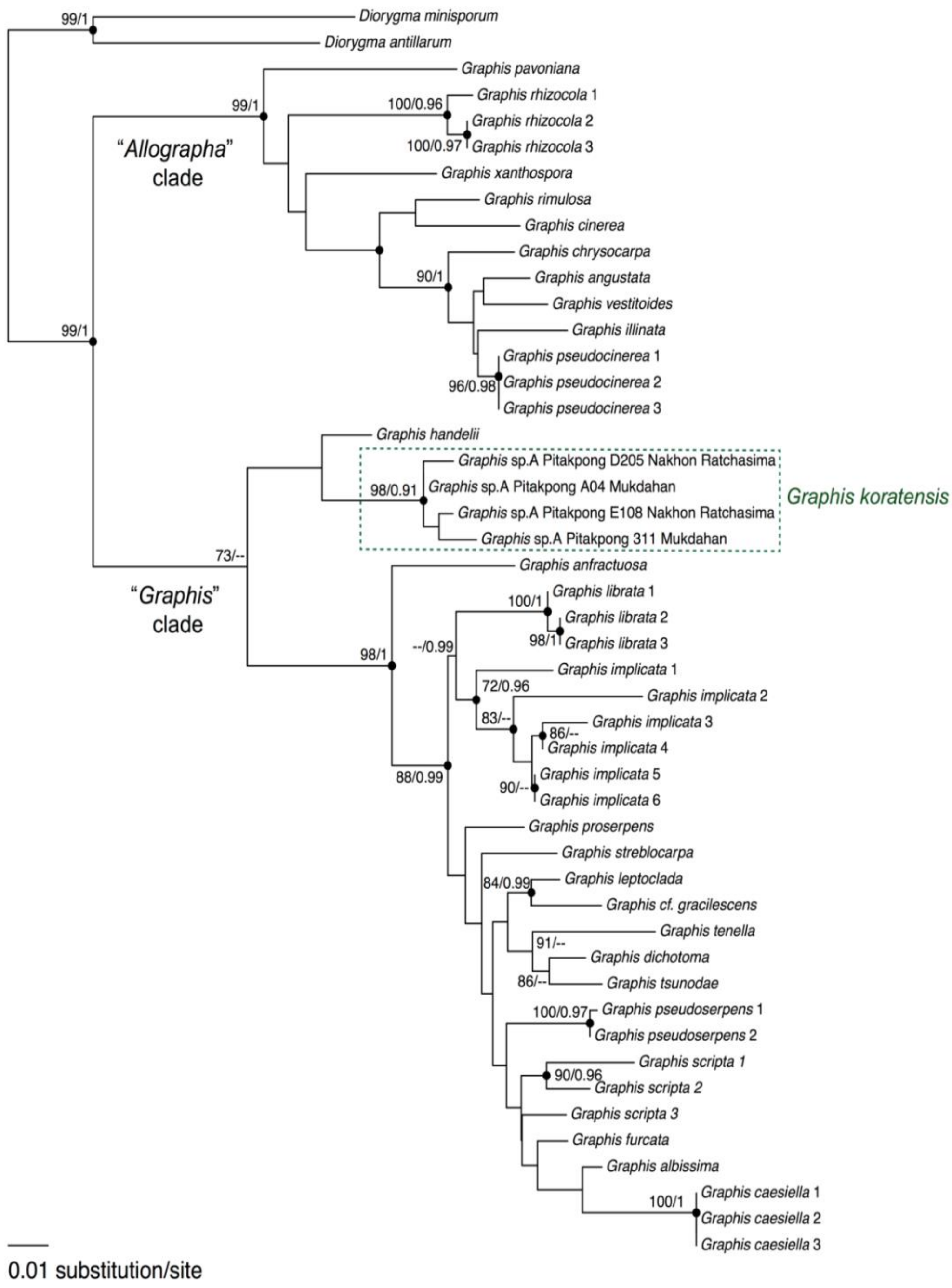

Figure 4.1 Phenogram illustrating phylogenetic relationships among lichens genus *Graphis*. The ML support > 70 and PP > 0.95 are considered strong supports and reported here. The circles indicate the node with strong supports from both analyses.

4.5 Conclusions

The phylogenetic analyses of combined sequence data provided the best phylogenetic reconstruction of studied taxa. Nevertheless, the result from this analysis suggested that some morphological and anatomical characters are variable in some taxa of the studied group. Therefore, using additional characters, such as more gene regions, more taxa from various geographic regions, distributional and ecological data, and even more detail of further morphological (thallus size, fruiting body) and anatomical (ascospore, spore size) studies may support more accurate or correct identification.

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CHAPTER V

THE DISTRIBUTION OF GENUS *GRAPHIS* (GRAPHIDACEAE) IN THAILAND

5.1 Abstract

Distributions of each species of lichens are very different in each forest area and the altitude. One important factor of the lichens distribution is altitude. This chapter therefore study distributions of each species of genus *Graphis* in 12 localities of Thailand. The study were carried out in dry evergreen forest, montane forest, rain forest, moist evergreen, mixed forest, evergreen forests, dry dipterocarp forest, pine forest, mangroves forest, and beach forest. Coordinates of found lichen species in each area will be represented by a Global Positioning System (GPS). The study of distribution of 32 species of genus *Graphis* (Graphidaceae) in Thailand based on field collection show that thirty-two species were found in dry dipterocarp, dry evergreen and mixed forests at altitude in the range of 150-1000 m. Fourteen species were found in montane forest i.e. *Graphis assamensis*, *G. assimilis*, *G. descissa*, *G. elongate*, *G. emersa*, *G. falvovirens*, *G. glaucescens*, *G. hossei*, *G. jejuensis*, *G. librata*, *G. longula*, *G. rhizocola*, *G. seminude*, and *G. verminosa*. Five species were found in beach forest i.e. *G. cincta*, *G. intricate*, *G. nanodes*, *G. nigrocarpa*, and *G. rimulosa*. Four species were found in pine forest i.e. *G. descissa*, *G. emersa*, *G. glaucescens* and *G. librata* at Phu Hin Rong Kla National Park, Phu Toei National Park and Khun Khan

National Park. And only one species (*G. furcata*) was found in mangrove forest at Hat Chao Mai National Park.

5.2 Introduction

The importance of distribution of a variety of creatures, both small and large, caused by the internal components of the ecosystem which influences on environmental change and consequently impacts on the distribution of a variety of organisms (Song *et al*., 2015) which lichens can be used as indicators of forest conditions. So the distribution and the different of lichens species can indicate internal elements within the environment. The factors affect the distribution of organisms such as altitudes, climate, temperature, humidity, and rainfall etc. (Ingerpuu *et al*., 2005; Ah-Peng *et al*., 2007; Belinchón *et al*., 2015).

Thailand is a country located in the tropical zone with having temperature around 21-36°C and 50-380 mm of rainfall. The forest spreads over mountain altitude areas. Thailand has a height ranging from 0-2,600 meters above the sea level north to south and 5-1,250 km east to west, with a coastline of approximately 1,840 km on the Gulf of Thailand and 865 along the Andaman sea (LePoer, 1989). It has a prevalence of forest and biodiversity that is different in each area. Forest type at different altitude areas affected the distribution of a variety of organisms (Hosseini *et al*., 2013).

The Graphidaceae is distributed mainly in tropical and subtropical regions. *Graphis* is by far the largest genus of tropical crustose microlichens, a group that has been neglected but accounts for most of the diversity in tropical lichen biota (Rivas Plata *et al*., 2012a; b; 2013). In Thailand, genus *Graphis* commonly grows in the forest. The genus *Graphis* was selected to investigate the distribution of lichens in

Thailand because it could be found commonly in the tropical zone. The objective of this study was to explore the distribution of lichens in the genus *Graphis* in Thailand by using GIS (geographical information system).

5.3 Materials and Methods

Study areas:

The study was carried out between July 2013 and July 2514. A total of 12 study areas were spread across the country (Table 5.1 and Figure 5.1). Thailand has altitude between 0-2,500 meters above a sea level, temperatures between 21-36°C, and rainfall levels between 50-380 mm. Each area has altitude, with different forest types, including dry evergreen forest, montane forest, rain forest, moist evergreen, mixed forest, evergreen forests, dry dipterocarp forest, pine forest, mangroves forest, and beach forest (The Forest Herbarium, 2011; Santisuk, 2012).

A location map was created based on the natural geographical distribution of species using a global positioning system with ArcGIS 10.1 (Environmental Systems Research Institute [ESRI], 2012). An overview of the distribution of each species in these taxa within the genus was made from labels on ecological observations during field collecting in natural habitats.

Table 5.1 List of localities for collecting sites.

Site studies	Provinces	Forest types
Doi Inthanon National Park	Chiang Mai	1.Dry evergreen forest 2.Montane forest 3.Rain forest 4.Moist evergreen 5.Mixed forest
Khun Khan National Park	Chiang Mai	1.Evergreen forests 2.Dry dipterocarp forest 3.Pine forest 4.Mixed forest
Sakaerat Environmental Research Station	Nakhon Ratchasima	1.Dry evergreen forest 2.Dry dipterocarp forest
Phujong-Nayoi National Park	Ubon Ratchathni	1.Mixed evergreen forest 2.Dry evergreen forest
Phu Hin Rong Kla National Park	Phitsanulok	1.Dry evergreen forest 2.Montane forest 3.Pine forest 4.Rain forest
Phu Toei National Park	Suphanburi	1.Mixed evergreen forest 2.Pine forest 3.Dry evergreen forest
Pang Sida National Park	Sakaew	1.Dry evergreen forest 2.Rain forests 3.Mixed forest 4.Dry dipterocarp forest
Khao Sip Ha Chan National Park	Jantaburi	1.Rain forest 2.Dry evergreen forest 3.Dry dipterocarp forest
Thong Pha Phum National Park	Kanjanaburi	1.Evergreen forest 2.Dry evergreen forest 3.Mountain forest 4.Mixed forest
KaengKrachan National Park	Phetchaburi	1.Dry evergreen forest 2.Dry dipterocarp forest
Hat Chao Mai National Park	Trang	1.Evergreen forest 2.Beach forest 3.Mangrove forest

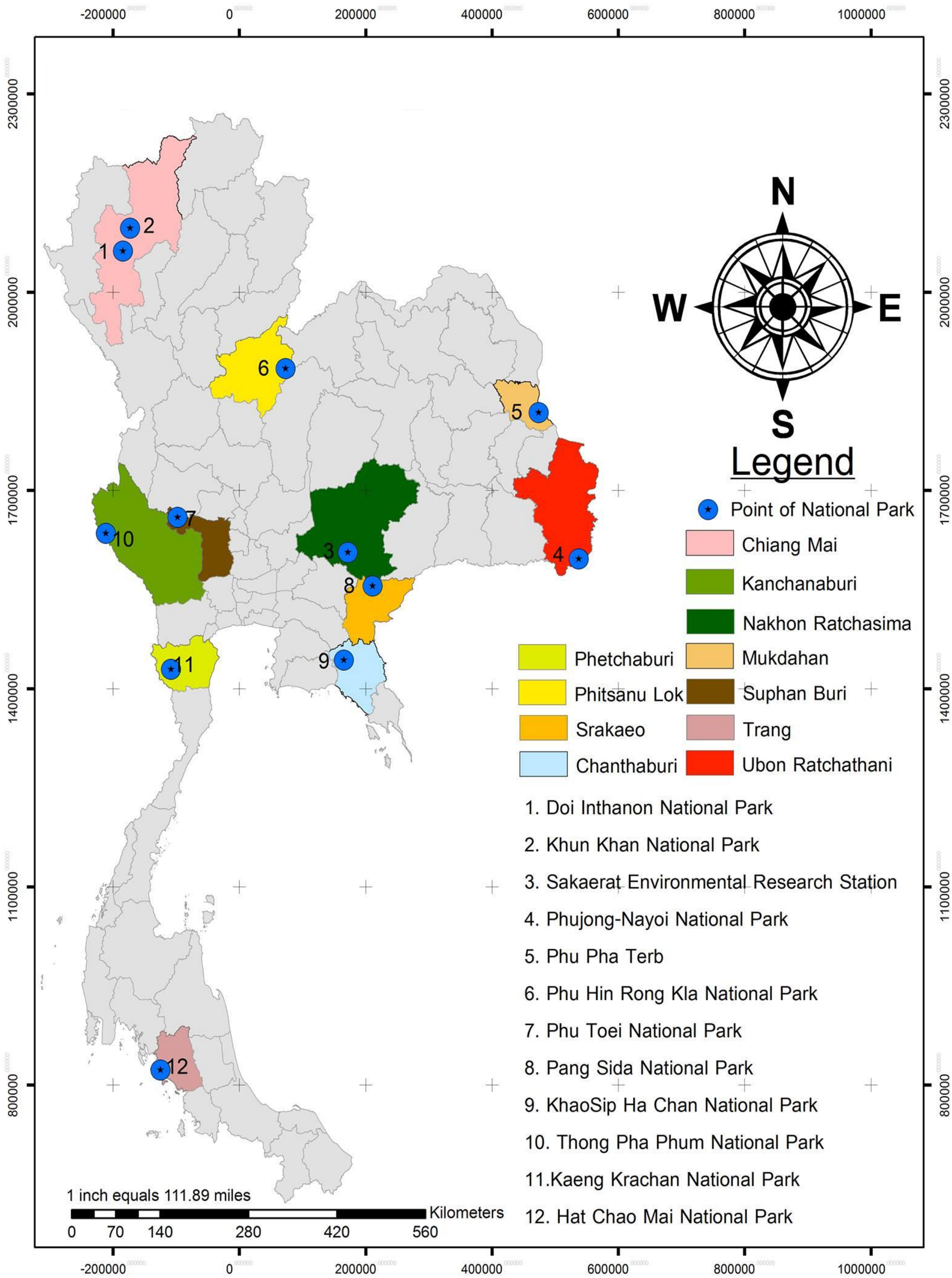


Figure 5.1 Map of collecting localities in Thailand.

Species identification (as described in CHAPTER III)

Morphological study: To identify the lichen specimens in this study, characteristics of thallus, lichen coloring, spore-producing bodies, each specimen was identified to the genus and species in laboratory, using a stereo and compound microscopes.

Chemical study: Lichen substances were identified by spot test (Orang *et al*., 2001) and thin-layer chromatography (TLC) according to standard methods of Culberson and Kristinsson (1969) and White and James (1985).

Molecular study: DNA sequences of the mitochondrial ribosomal small subunit (mtSSU) and nuclear large subunit (nuLSU), DNA extraction, PCR amplification, PCR amplification, PCR purification, cycle-sequencing and phylogenetic analyses were carried out.

Data analysis

We used the Pearson's correspondence analysis to test the significance of lichen species and environmental package in R 3.2.1 (Available from http://www.R-project.org).

5.4 Results and Discussions

We found 536 *Graphis* specimens of 12 studied areas. A total of 32 species were identified (Table 5.2). In each area, it was found that species richness was different. Phujong-Nayoi National Park found the most diversity of lichens (20 species), followed by Phu Hin Rong Kla National Park (17 species), Pang Sida

National Park (16 species), Phu Toei National Park (15 species), and Thong Pha Phum National Park (14 species) etc. (Figure 5.2)

Table 5.2 The distribution of lichen species at sites in the Thailand.

Lichen species	State distribution											
	Doi	Khun	Sak	Phuj	PhuP	PhuH	PhuT	PangS	KhaoS	Thong	Kaeng	HatCh
Graphis assamensis Nagarkar & Patw.	✓		✓			✓				✓		
Graphis assimilis Nyl.			✓	✓	✓		✓	✓			✓	
Graphis cincta (Pers.) Aptroot			✓	✓				✓	✓			✓
Graphis descissa Müll. Arg.	✓	✓	✓			✓	✓			✓		
Graphis duplicata Ach.				✓	✓	✓		✓				✓
Graphis elongata Zenker.	✓			✓		✓	✓			✓		
Graphis emersa Mull.Arg.	✓	✓		✓		✓	✓			✓		
Graphis falvovirens Makhija & Adaw.				✓	✓		✓	✓			✓	
Graphis furcata Fée			✓		✓			✓	✓		✓	✓
Graphis glaucescens Fée	✓	✓				✓	✓			✓		
Graphis handelii Zahlbr.			✓	✓		✓				✓		✓
Graphis hossei Vain.	✓			✓	✓			✓		✓	✓	
Graphis intricata Fée				✓			✓	✓			✓	✓
Graphis jejuensis K. H. Moon, M. Nakan. & Kashiw.	✓	✓	✓	✓		✓	✓			✓		
Graphis koratensis Pitakpong, Kaichak & Lücking			✓	✓	✓							
Graphis librata C. Knight	✓	✓				✓				✓		
Graphis lineola Ach.				✓	✓			✓			✓	
Graphis longiramea Müll. Arg.					✓			✓	✓			✓
Graphis longula Kremp.		✓		✓					✓	✓		
Graphis nanodes Vain.					✓			✓	✓			✓
Graphis nigrocarpa Adaw. & Makhija				✓	✓			✓	✓		✓	✓
Graphis pinicola Zahlbr				✓		✓		✓	✓		✓	✓
Graphis renschiana (Müll. Arg.) Stizenb		✓				✓		✓			✓	✓
Graphis rhizocola (Fée) Lücking & Chaves	✓					✓	✓			✓	✓	
Graphis rimulosa (Mont.) Trevis.				✓			✓	✓	✓			✓
Graphis seminuda Müll. Arg.	✓	✓				✓				✓	✓	
Graphis streblocarpa (Bel.) Nyl.						✓	✓					✓
Graphis subserpentina Nyl.			✓	✓	✓		✓		✓	✓	✓	
Graphis subvelata Stirt.		✓		✓		✓	✓					
Graphis tenella Ach.				✓		✓		✓	✓	✓		
Graphis verminosa Müll. Arg.	✓	✓	✓	✓		✓	✓					
Graphis vittata Müll. Arg.		✓					✓	✓			✓	

Abbreviation: Doi=Doi Inthanon National Park, Khun=Khun Khan National Park, Sak=Sakaerat Environmental Research Station, Phuj=Phujong-Nayoi National Park, PhuP=Phu Pha Terb National Park, PhuH=Phu Hin Rong Kla National Park, PhuT=Phu Toei National Park, PangS=Pang Sida National Park, KhaoS=Khao Sip Ha Chan National Park, Thong=Thong Pha Phum National Park, Kaeng=Kaeng Krachan National Park, and HatCh=Hat Chao Mai National Park.

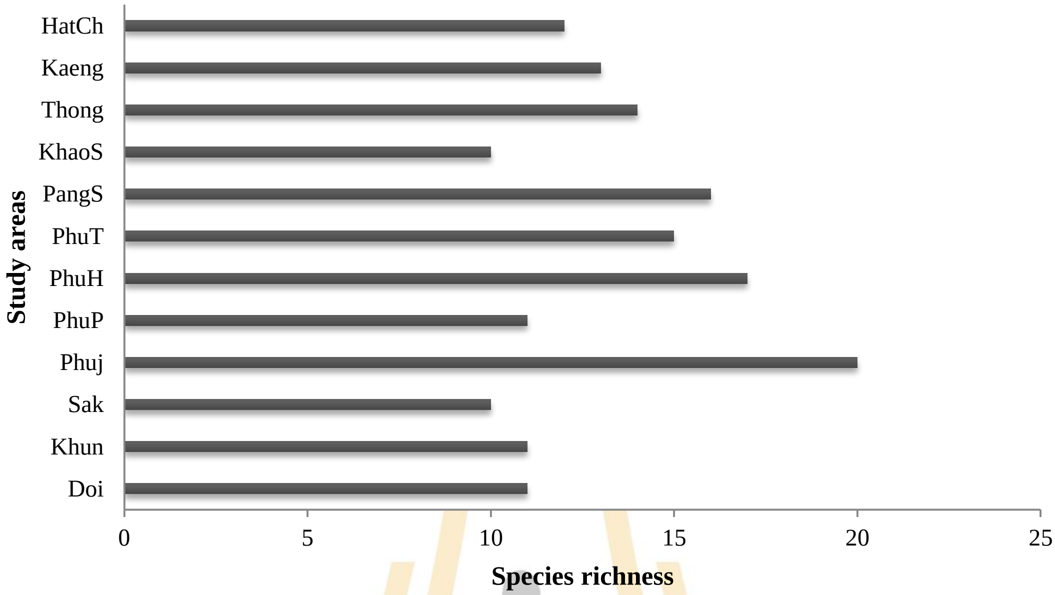


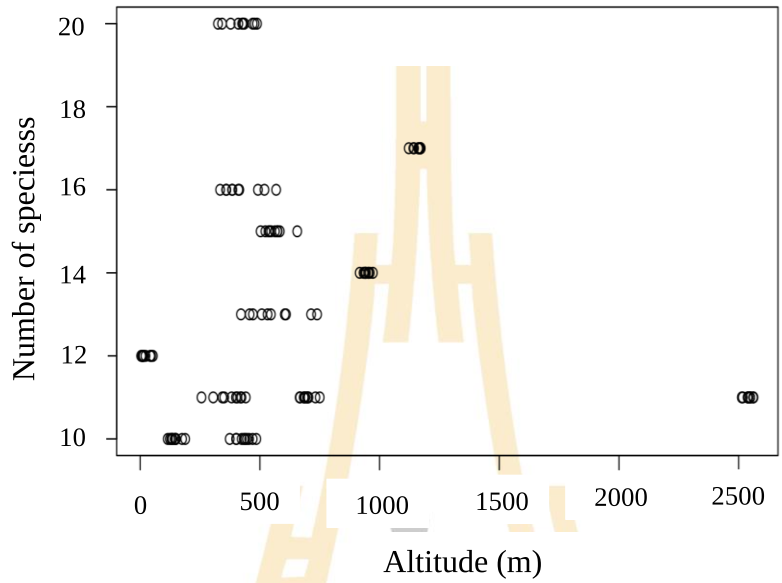
Figure 5.2 The species richness at each area in Thailand. Abbreviation: Doi=Doi Inthanon National Park, Khun=Khun Khan National Park, Sak=Sakaerat Environmental Research Station, Phuj=Phujong-Nayoi National Park, PhuP=Phu Pha Terb National Park, PhuH=Phu Hin Rong Kla National Park, PhuT=Phu Toei National Park, PangS=Pang Sida National Park, KhaoS=Khao Sip Ha Chan National Park, Thong=Thong Pha Phum National Park, Kaeng=Kaeng Krachan National Park, and HatCh=Hat Chao Mai National Park.

From the study of lichens at different altitude in a range of 4-2,562 m above sea level, Hat Chao Mai National Park, had the lowest altitude (4 m) whereas the highest (2,562 m) was Doi Inthanon National Park. Average altitudes of 12 study areas were in the range of about 300-800 m above sea level (Figure 5.3A). In addition, the relationship between the species diversity of lichens and altitudes was higher when the height decreases as calculated by the equation $y = -0.0002x + 13.454$, $r2 = 0.0014$ (Figure 5.3B).

From the study of lichens at the average levels of rainfall during the period July 2556 to July 2557, average values of rainfall were 0.7 to 512.6 mm. The park with the lowest rainfall level (0.7 mm) was Phujong-Nayoi National Park whereas Khao Sip Ha Chan National Park had the highest average level of rainfall (512.6 mm). Lichens were most found at the rainfall levels approximately 50-250 mm (Figure 5.4A). The relationship of the various types of lichens with the rainfall was calculated by this equation $y = -0.0127x + 15.159$, $r2 = 0.0487$ (Figure 5.4B).

The study of lichens in the average temperature during the period July 2556 to July 2557 found temperature average of 21.3 to 31.65°C. The lowest temperature was found (21.3°C) at Doi Inthanon National Park whereas Thong Pha Phum National Park was a park that had the highest average temperature (31.65°C). Lichens was mostly found in the average temperature of 28-29°C (Figure 5.5A). In addition, the relationship of the species diversity of lichens and the average temperature was calculated by the equation $y = 1.587x - 31.068$, $r2 = 0.1821$ (Figure 5.5B).

A.



B.

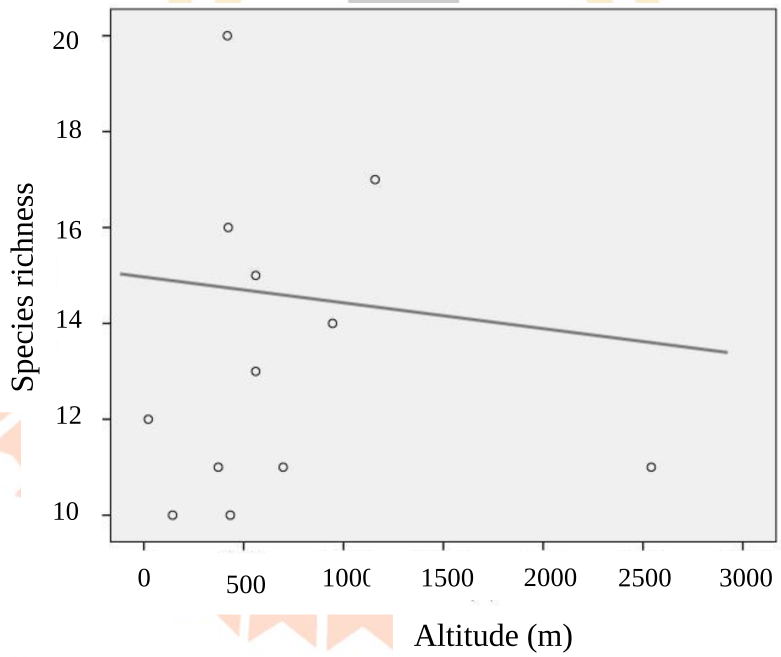
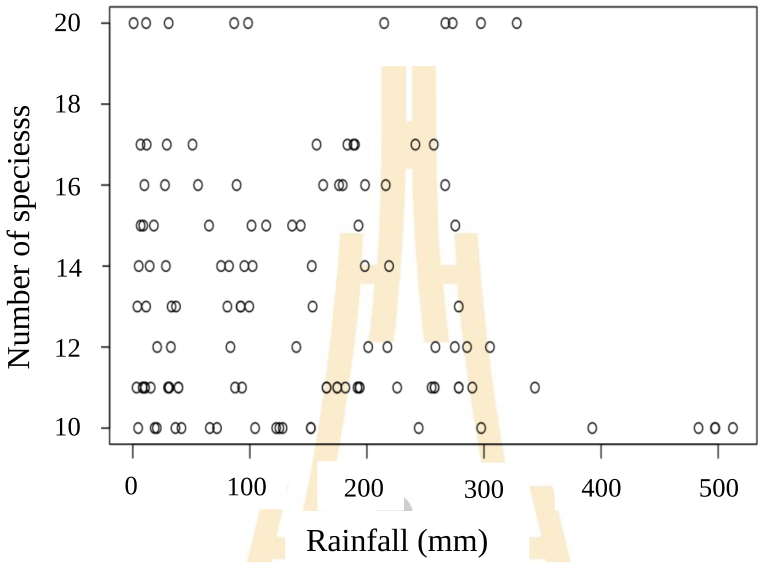


Figure 5.3 The relationship between altitude and number of species (A), and species richness (B) of lichens in Thailand. The polynomial equation is $y = -0.0002x + 13.454$, $r^2 = 0.0014$.

A.



B.

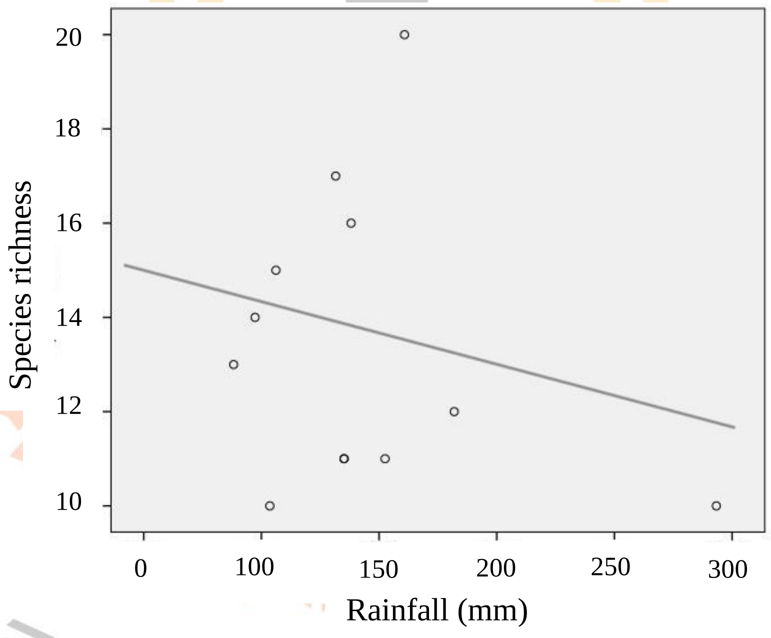
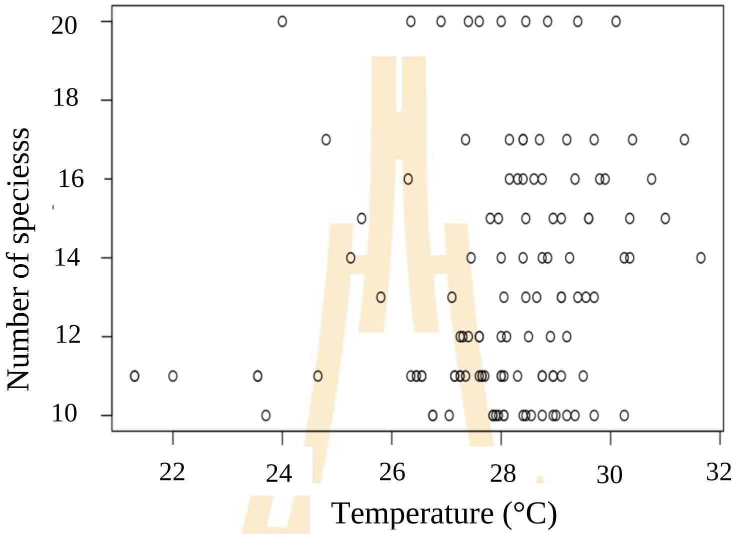


Figure 5.4 The relationship between rainfall and number of species (A), and species richness (B) of lichens in Thailand. The polynomial equation is $y = -0.0127x + 15.159$, $r^2 = 0.0487$.

A.



B.

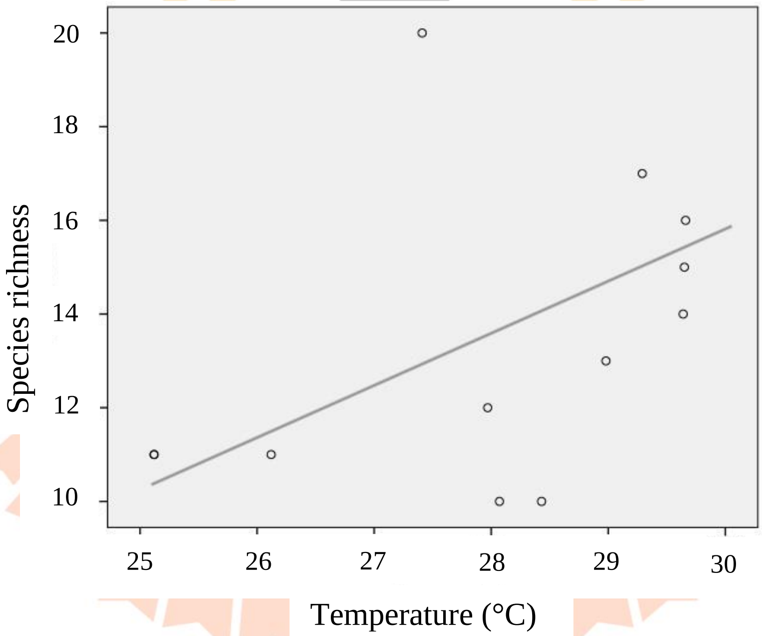


Figure 5.5 The relationship between temperature and number of species (A), and species richness (B) of lichens in Thailand. The polynomial equation is $y = 1.587x – 31.068$, $r^2 = 0.1821$.

Pearson's correlations showed the relationships of altitude, the average rainfall and average temperature with lichen species richness of 32 species including *Graphis assamensis* Nagarkar & Patw. (A1), *G. assimilis* Nyl. (A2), *G. cincta* (Pers.) Aptroot. (A3), *G. descissa* Müll. Arg. (A4), *G. duplicata* Ach. (A5), *G. elongata* Zenker. (A6), *G. emersa* Mull.Arg. (A7), *G. falvovirens* Makhija & Adaw. (A8), *G. furcata* Fée. (A9), *G. glaucescens* Fée. (A10), *G. handelii* Zahlbr. (A11), *G. hossei* Vain. (A12), *G. intricata* Fée. (A13), *G. jejuensis* K. H. Moon, M. Nakan. & Kashiw. (A14), *G. koratensis* Pitakpong, Kraichak, Lücking. (A15), *G. librata* C. Knight. (A16), *G. lineola* Ach. (A17), *Graphis longiramea* Müll. Arg. (A18), *Graphis longula* Kremp. (A19), *Graphis nanodes* Vain. (A20), *Graphis nigrocarpa* Adaw. & Makhija. (A21), *Graphis pinicola* Zahlbr. (A22), *G. renschiana* (Müll. Arg.) Stizenb. (A23), *G. rhizocola* (Fée) Lücking & Chaves. (A24), *G. rimulosa* (Mont.) Trevis. (A25), *G. seminuda* Müll. Arg. (A26), *G. streblocarpa* (Bel.) Nyl. (A27), *G. subserpentina* Nyl. (A28), *G. subvelata* Stirt. (A29), *G. tenella* Ach. (A30), *G. verminosa* Müll. Arg. (A31) and *G. vittata* Müll. Arg. (A32) as shown in the Table 5.3

The positive correlation was found between altitude and 12 species of lichens including *Graphis assamensis* Nagarkar & Patw. (A1), *G. descissa* Müll. Arg. (A4), *G. elongata* Zenker. (A6), *G. emersa* Mull.Arg. (A7), *G. glaucescens* Fée. (A10), *G. hossei* Vain. (A12), *G. jejuensis* K. H. Moon, M. Nakan. & Kashiw. (A14), *G. librata* C. Knight. (A16), *G. rhizocola* (Fée) Lücking & Chaves. (A24), *G. seminuda* Müll. Arg. (A26), *G. subvelata* Stirt. (A29), and *G. verminosa* Müll. Arg. (A31). Whereas, the negative correlation was obtained between altitude and 20 species of lichens including *Graphis assimilis* Nyl. (A2), *G. cincta* (Pers.) Aptroot. (A3), *G. duplicata* Ach. (A5), *G. falvovirens* Makhija & Adaw. (A8), *G. furcata* Fée. (A9), *G. handelii*

Zahlbr. (A11), *G. intricata* Fée. (A13), *G. koratensis* Pitakpong, Kraichak, Lücking. (A15), *G. lineola* Ach. (A17), *G. longiramea* Müll. Arg. (A18), *G. longula* Kremp. (A19), *G. nanodes* Vain. (A20), *G. nigrocarpa* Adaw. & Makhija. (A21), *G. pinicola* Zahlbr. (A22), *G. renschiana* (Müll. Arg.) Stizenb. (A23), *G. rimulosa* (Mont.) Trevis. (A25), *G. streblocarpa* (Bel.) Nyl. (A27), *G. subserpentina* Nyl. (A28), *G. tenella* Ach. (A30), and *G. vittata* Müll. Arg. (A32). These results indicated that high altitude decreased at high altitude.

Average rainfall levels were positively correlated with 22 lichen species including *Graphis assamensis* Nagarkar & Patw. (A1), *G. assimilis* Nyl. (A2), *G. cincta* (Pers.) Aptroot. (A3), *G. duplicata* Ach. (A5), *G. elongata* Zenker. (A6), *G. falvovirens* Makhija & Adaw. (A8), *G. furcata* Fée. (A9), *G. handelii* Zahlbr. (A11), *G. intricata* Fée. (A13), *G. lineola* Ach. (A17), *G. longiramea* Müll. Arg. (A18), *G. nanodes* Vain. (A20), *G. nigrocarpa* Adaw. & Makhija. (A21), *G. pinicola* Zahlbr. (A22), *G. renschiana* (Müll. Arg.) Stizenb. (A23), *G. rhizocola* (Fée) Lücking & Chaves. (A24), *G. rimulosa* (Mont.) Trevis. (A25), *G. streblocarpa* (Bel.) Nyl. (A27), *G. subserpentina* Nyl. (A28), *G. tenella* Ach. (A30), and *G. vittata* Müll. Arg. (A32). On the other hand, Average rainfall levels were negatively correlated with 10 lichen species including *Graphis descissa* Müll. Arg. (A4), *G. emersa* Mull. Arg. (A7), *G. glaucescens* Fée. (A10), *G. hossei* Vain. (A12), *G. jejuensis* K. H. Moon, M. Nakan. & Kashiw. (A14), *G. koratensis* Pitakpong, Kraichak, Lücking. (A15), *G. librata* C. Knight. (A16), *G. longula* Kremp. (A19), *G. seminuda* Müll. Arg. (A26), *G. subvelata* Stirt. (A29), and *G. verminosa* Müll. Arg. (A31). These results indicated that lichen richness increased in high rainfall levels.

Additionally, Temperature showed a positive correlation with 10 lichen species including *Graphis cincta* (Pers.) Aptroot. (A3), *G. duplicata* Ach. (A5), *G. furcata* Fée. (A9), *G. longiramea* Müll. Arg. (A18), *G. longula* Kremp. (A19), *G. nanodes* Vain. (A20), *G. nigrocarpa* Adaw. & Makhija. (A21), *G. pinicola* Zahlbr. (A22), *G. rimulosa* (Mont.) Trevis. (A25), and *G. tenella* Ach. (A30). In contrast, temperature showed a negative correlation with 22 lichen species including *Graphis assamensis* Nagarkar & Patw. (A1), *G. assimilis* Nyl. (A2), *G. descissa* Müll. Arg. (A4), *G. elongata* Zenker. (A6), *G. emersa* Mull. Arg. (A7), *G. falvovirens* Makhija & Adaw. (A8), *G. glaucescens* Fée. (A10), *G. handelii* Zahlbr. (A11), *G. hossei* Vain. (A12), *G. intricata* Fée. (A13), *G. jejuensis* K. H. Moon, M. Nakan. & Kashiw. (A14), *G. koratensis* Pitakpong, Kraichak, Lücking. (A15), *G. librata* C. Knight. (A16), *G. lineola* Ach. (A17), *G. renschiana* (Müll. Arg.) Stizenb. (A23), *G. rhizocola* (Fée) Lücking & Chaves. (A24), *G. seminuda* Müll. Arg. (A26), *G. streblocarpa* (Bel.) Nyl. (A27), *G. subserpentina* Nyl. (A28), *G. subvelata* Stirt. (A29), *G. verminosa* Müll. Arg. (A31), and *G. vittata* Müll. Arg. (A32). These results exhibited that lichen richness declined in high temperature.

Table 5.3 Pearson's correlation between *Graphis* species and environmental factors in the study areas.

	Site	Alt	Temp	Rain	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	A11	A12	A13	A14	A15	A16	A17	A18	A19	A20	A21	A22	A23	A24	A25	A26	A27	A28	A29	A30	A31
Alt	-.515**																																		
Temp	.327**	-.170																																	
Rain	.124	-.131	.194*																																
A1	-.306**	.646**	.015	-.160																															
A2	-.048	-.360**	.099	-.158	-.354**																														
A3	.171	-.535**	.082	.228*	-.239**	.169																													
A4	-.481**	.577**	-.038	-.216*	.707**	-.333**	-.507**																												
A5	.122	-.281**	.031	.067	-.239**	.169	.314**	-.507**																											
A6	-.220*	.580**	.062	-.125	.478**	-.169	-.371**	.507**	-.029																										
A7	-.433**	.574**	-.069	-.135	.354**	-.333**	-.507**	.667**	-.169	.845**																									
A8	.122	-.297**	.095	-.103	-.598**	.845**	-.029	-.507**	.314**	-.029	-.169																								
A9	.433**	-.574**	.069	.135	-.354**	.333**	.507**	-.667**	.169	-.845**	-1.000**	.169																							
A10	-.317**	.654**	-.044	-.161	.478**	-.507**	-.714**	.845**	-.371**	.657**	.845**	-.371**	-.845**																						
A11	.122	-.126	.121	-.062	.478**	-.169	.314**	.169	.314**	.314**	.169	-.371**	-.169	-.029																					
A12	0.000	.295**	-.037	-.127	.000	.333**	-.169	-.333**	.169	.169	0.000	.507**	0.000	-.169	-.169																				
A13	.464**	-.390**	.180*	-.061	-.598**	.507**	.314**	-.507**	.314**	-.029	-.169	.657**	.169	-.371**	-.029	.169																			
A14	-.610**	.513**	-.064	-.194*	.598**	-.169	-.314**	.845**	-.314**	.714**	.845**	-.314**	-.845**	.714**	.371**	-.169	-.314**																		
A15	-.417**	-.256**	-.119	-.023	.000	.577**	.293**	-.192*	.293**	-.098	-.192*	.293**	.192*	-.488**	.293**	.192*	-.098	.098																	
A16	-.357**	.720**	-.128	-.113	.625**	-.707**	-.598**	.707**	-.239**	.478**	.707**	-.598**	-.707**	.837**	.120	.000	-.598**	.598**	-.408**																
A17	.102	-.274**	.017	-.052	-.500**	.707**	.120	-.707**	.478**	-.239**	-.354**	.837**	.354**	-.598**	-.239**	.707**	.478**	-.478**	.408**	-.500**															
A18	.408**	-.501**	.017	.286**	-.500**	.000	.478**	-.707**	.478**	-.598**	-.707**	.120	.707**	-.598**	-.239**	.000	.120	-.837**	.000	-.500**	.250**														
A19	-.051	-.154	-.059	.167	-.125	-.354**	.120	.000	-.239**	.120	.354**	-.239**	-.354**	.120	.120	.000	-.239**	.239**	.000	.250**	-.125	-.125													
A20	.408**	-.501**	.017	.286**	-.500**	.000	.478**	-.707**	.478**	-.598**	-.707**	.120	.707**	-.598**	-.239**	.000	.120	-.837**	.000	-.500**	.250**	1.000**	-.125												
A21	.481**	-.577**	.038	.216*	-.707**	.333**	.507**	-1.000**	.507**	-.507**	-.667**	.507**	.667**	-.845**	-.169	.333**	.507**	-.845**	.192*	-.707**	.707**	.707**	.000	.707**											
A22	.529**	-.371**	.183*	.186*	-.354**	0.000	.507**	-.667**	.507**	-.169	-.333**	.169	.333**	-.507**	.169	0.000	.507**	-.507**	-.192*	-.354**	.354**	.354**	.000	.354**	.667**										
A23	.317**	-.157	.058	-.062	-.239**	-.169	-.029	-.169	.314**	-.371**	-.169	-.029	.169	-.029	-.029	-.169	.314**	-.314**	-.488**	.120	.120	.120	-.239**	.120	.169	.507**									
A24	.122	.617**	.135	-.229*	.478**	-.169	-.714**	.507**	-.371**	.657**	.507**	-.029	-.507**	.657**	-.029	.169	-.029	.371**	-.488**	.478**	-.239**	-.598**	-.239**	-.598**	-.507**	-.169	-.029								
A25	.366**	-.501**	.155	.232*	-.598**	.169	.657**	-.507**	.314**	-.029	-.169	.314**	.169	-.371**	-.029	-.169	.657**	-.314**	-.098	-.598**	.120	.478**	.120	.478**	.507**	.507**	-.029	-.371**							
A26	-.122	.654**	-.075	-.187*	.478**	-.507**	-.714**	.507**	-.371**	.314**	.507**	-.371**	-.507**	.657**	-.029	.169	-.371**	.371**	-.488**	.837**	-.239**	-.598**	.120	-.598**	-.507**	-.169	.314**	.657**	-.714**						
A27	.306**	-.100	.161	-.018	.000	-.192*	-.098	.192*	.293**	.293**	.192*	-.098	-.192*	.293**	.293**	-.577**	.293**	.098	-.333**	.000	-.408**	.000	-.408**	.000	-.192*	.192*	.293**	.293**	.293**	-.098					
A28	.171	-.371**	.121	-.005	-.120	.507**	.029	-.169	-.314**	.029	-.169	.371**	.169	-.314**	.029	.169	.029	-.029	.488**	-.478**	.239**	-.120	.239**	-.120	.169	-.169	-.657**	.029	.029	-.314**	-.293**				
A29	-.357**	.021	-.017	-.061	-.125	.000	-.239**	.354**	.120	.478**	.707**	.120	-.707**	.478**	.120	-.354**	.120	.598**	.000	.250**	-.125	-.500**	.250**	-.500**	-.354**	.000	.120	.120	.120	.120	.408**	-.120			
A30	.220*	-.096	.216*	.147	.120	-.169	.314**	-.169	.314**	.314**	.169	-.029	-.169	-.029	.314**	.169	-.029	.029	-.098	.120	.120	.120	.478**	.120	.169	.507**	-.029	-.029	.314**	-.029	-.098	.029	.120		
A31	-.770**	.439**	-.140	-.126	.354**	0.000	-.169	.667**	-.169	.507**	.667**	-.169	-.667**	.507**	.169	-.333**	-.169	.845**	.192*	.354**	-.354**	-.707**	.000	-.707**	-.667**	-.333**	-.169	.169	-.169	.169	.192*	-.169	.707**	-.169	
A32	.102	-.144	.077	-.160	-.500**	.354**	-.239**	.000	-.239**	-.239**	.000	.478**	.000	.120	-.598**	.000	.478**	-.120	-.408**	-.125	.250**	-.125	-.125	-.125	.000	.000	.478**	.120	.120	.120	.000	-.120	.250**	-.239**	.000

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

From 32 taxa of the genus *Graphis* in Thailand, the distributions of lichens were analyzed and mapping of the distribution of each species is shown in Figures 5.6 to 5.39.

G. assamensis in dry dipterocarp forest, dry evergreen forest, mixed forest, and montane forest at 250-1500 m elevations at Doi Inthanon National Park, Sakaerat Environmental Research Station, Phu Hin Rong Kla National Park, and Thong Pha Phum National Park (Figure 5.6).

G. assimilis in dry dipterocarp forest, dry evergreen forest, mixed forest, and montane forest at 200-800 m elevations at Sakaerat Environmental Research Station, Phujong-Nayoi National Park, Phu Pha Terb National Park, Phu Toei National Park**,** Pang Sida National Park, and Kaeng Krachan National Park (Figure 5.7).

G. cincta in dry dipterocarp forest, dry evergreen forest, mixed forest, and beach forest at 50-500 m elevations at Sakaerat Environmental Research Station, Phujong-Nayoi National Park, Pang Sida National Park, Khao Sip Ha Chan National Park, and Hat Chao Mai National Park (Figure 5.8).

G. descissa in dry dipterocarp forest, dry evergreen forest, pine forest, mixed forest, and montane forest at 200-2000 m elevations at Doi Inthanon National Park, Khun Khan National Park, Sakaerat Environmental Research Station, Phu Hin Rong Kla National Park, Phu Toei National Park, and Thong Pha Phum National Park (Figure 5.9).

G. duplicate in dry dipterocarp forest, dry evergreen forest, and mixed forest at 200-800 m elevations at Phujong-Nayoi National Park, Phu Pha Terb National Park, Phu Hin Rong Kla National Park, Pang Sida National Park, and Hat Chao Mai National Park (Figure 5.10).

G. elongate in dry dipterocarp forest, dry evergreen forest, mixed forest, and montane forest at 250-1900 m elevations at Doi Inthanon National Park, Phujong-Nayoi National Park, Phu Hin Rong Kla National Park, Phu Toei National Park, and Thong Pha Phum National Park (Figure 5.11).

G. emersa in dry dipterocarp forest, dry evergreen forest, pine forest, mixed forest, and montane forest at 200-1900 m elevations at Doi Inthanon National Park, Khun Khan National Park, Phujong-Nayoi National Park, Phu Hin Rong Kla National Park, Phu Toei National Park, and Thong Pha Phum National Park (Figure 5.12).

G. falvovirens in dry dipterocarp forest, dry evergreen forest, mixed forest, and montane forest at 250-800 m elevations at Phujong-Nayoi National Park, Phu Pha Terb National Park, Phu Toei National Park, Pang Sida National Park, and Kaeng Krachan National Park (Figure 5.13).

G. furcata in dry dipterocarp forest, dry evergreen forest, mixed forest, and mangroves forest at 100-850 m elevations at Sakaerat Environmental Research Station, Phu Pha Terb National Park, Pang Sida National Park, Khao Sip Ha Chan National Park, Kaeng Krachan National Park, and Hat Chao Mai National Park (Figure 5.14).

G. glaucescens in dry dipterocarp forest, dry evergreen forest, mixed forest, pine forest, and montane forest at 300-2000 m elevations at Doi Inthanon National Park, Khun Khan National Park, Phu Hin Rong Kla National Park, Phu Toei National Park, and Thong Pha Phum National Park (Figure 5.15).

G. handelii in dry dipterocarp forest, dry evergreen forest, and mixed forest at 50-800 m elevations at Sakaerat Environmental Research Station, Phujong-Nayoi

National Park, Phu Hin Rong Kla National Park, Thong Pha Phum National Park, and Hat Chao Mai National Park (Figure 5.16).

G. hossei in dry dipterocarp forest, dry evergreen forest, mixed forest, and montane forest at 350-2000 m elevations at Doi Inthanon National Park, Phujong-Nayoi National Park, Phu Pha Terb National Park, Pang Sida National Park, Thong Pha Phum National Park, and Kaeng Krachan National Park (Figure 5.17).

G. intricata in dry dipterocarp forest, dry evergreen forest, mixed forest, and beach forest at 50-800 m elevations at Phujong-Nayoi National Park, Phu Toei National Park, Pang Sida National Park, Kaeng Krachan National Park, and Hat Chao Mai National Park (Figure 5.18).

G. jejuensis in dry dipterocarp forest, dry evergreen forest, mixed forest, and montane forest at 250-1800 m elevations at Doi Inthanon National Park, Khun Khan National Park, Sakaerat Environmental Research Station, Phujong-Nayoi National Park, Phu Hin Rong Kla National Park, Phu Toei National Park, and Thong Pha Phum National Park (Figure 5.19).

G. koratensis in dry dipterocarp forest, and dry evergreen forest at Sakaerat Environmental Research Station, Phujong-Nayoi National Park, and Phu Pha Terb National Park (Figure 5.20).

G. librata in dry dipterocarp forest, dry evergreen forest, mixed forest, pine forest, and montane forest at 350-2000 m elevations at Doi Inthanon National Park, Khun Khan National Park, Phu Hin Rong Kla National Park, and Thong Pha Phum National Park (Figure 5.21).

G. lineola in dry dipterocarp forest, dry evergreen forest, and mixed forest at 300-800 m elevations at Phujong-Nayoi National Park, Phu Pha Terb National Park, Pang Sida National Park, and Kaeng Krachan National Park (Figure 5.22).

G. longiramea in dry dipterocarp forest, dry evergreen forest, and mixed forest at 100-700 m elevations at Phu Pha Terb National Park, Pang Sida National Park, Khao Sip Ha Chan National Park, and Hat Chao Mai National Park (Figure 5.23).

G. longula in dry dipterocarp forest, dry evergreen forest, mixed forest, and montane forest at 250-1300 m elevations at Khun Khan National Park, Phujong-Nayoi National Park, Khao Sip Ha Chan National Park, and Thong Pha Phum National Park (Figure 5.24).

G. nanodes in dry dipterocarp forest, dry evergreen forest, mixed forest, and beach forest at 50-1500 m elevations at Phu Pha Terb National Park, Pang Sida National Park, Khao Sip Ha Chan National Park, and Hat Chao Mai National Park (Figure 5.25).

G. nigrocarpa in dry dipterocarp forest, dry evergreen forest, mixed forest, and beach forest at 100-800 m elevations at Phujong-Nayoi National Park, Phu Pha Terb National Park, Pang Sida National Park, Khao Sip Ha Chan National Park, Kaeng Krachan National Park, and Hat Chao Mai National Park (Figure 5.26).

G. pinicola in dry dipterocarp forest, dry evergreen forest, and mixed forest at 80-800 m elevations at Phujong-Nayoi National Park, Phu Hin Rong Kla National Park, Pang Sida National Park, Khao Sip Ha Chan National Park, Kaeng Krachan National Park, and Hat Chao Mai National Park (Figure 5.27).

G. renschiana in dry dipterocarp forest, dry evergreen forest, and mixed forest at 100-1500 m elevations at Khun Khan National Park, Phu Hin Rong Kla National

Park, Pang Sida National Park, Kaeng Krachan National Park, and Hat Chao Mai National Park (Figure 5.28).

G. rhizocola in dry dipterocarp forest, dry evergreen forest, mixed forest, and montane forest at 300-1800 m elevations at Doi Inthanon National Park, Chiang Mai Province, Phu Hin Rong Kla National Park, Phu Toei National Park, Thong Pha Phum National Park, and Kaeng Krachan National Park (Figure 5.29).

G. rimulosa in dry dipterocarp forest, dry evergreen forest, mixed forest, and beach forest at 100-800 m elevations at Phujong-Nayoi National Park, Phu Toei National Park, Pang Sida National Park, Khao Sip Ha Chan National Park, and Hat Chao Mai National Park (Figure 5.30).

G. seminuda in dry dipterocarp forest, dry evergreen forest, mixed forest, and montane forest at 200-1800 m elevations at Doi Inthanon National Park, Khun Khan National Park, Phu Hin Rong Kla National Park, Thong Pha Phum National Park, and Kaeng Krachan National Park (Figure 5.31).

G. streblocarpa in dry dipterocarp forest, dry evergreen forest, and mixed forest at 80-1200 m elevations at Phu Hin Rong Kla National Park, Phu Toei National Park, and Hat Chao Mai National Park (Figure 5.32).

G. subserpentina in dry dipterocarp forest, dry evergreen forest, and mixed forest at 200-800 m elevations at Sakaerat Environmental Research Station, Phujong-Nayoi National Park, Phu Pha Terb National Park, Phu Toei National Park, Khao Sip Ha Chan National Park, Thong Pha Phum National Park, and Kaeng Krachan National Park (Figure 5.33).

G. subvelata in dry dipterocarp forest, dry evergreen forest, and mixed forest at 250-700 m elevations at Khun Khan National Park, Phujong-Nayoi National Park, Phu Hin Rong Kla National Park, and Phu Toei National Park (Figure 5.34).

G. tenella in dry dipterocarp forest, dry evergreen forest, and mixed forest at 250-800 m elevations at Phujong-Nayoi National Park, Phu Hin Rong Kla National Park, Pang Sida National Park, Khao Sip Ha Chan National Park, and Thong Pha Phum National Park (Figure 5.35).

G. verminosa in dry dipterocarp forest, dry evergreen forest, mixed forest, and montane forest at 200-1500 m elevations at Doi Inthanon National Park, Khun Khan National Park, Sakaerat Environmental Research Station, Phujong-Nayoi National Park, Phu Hin Rong Kla National Park, and Phu Toei National Park (Figure 5.36)

G. vittata in dry dipterocarp forest, dry evergreen forest, and mixed forest at 250-800 m elevations at Khun Khan National Park, Phu Toei National Park, Pang Sida National Park, and Kaeng Krachan National Park (Figure 5.37).

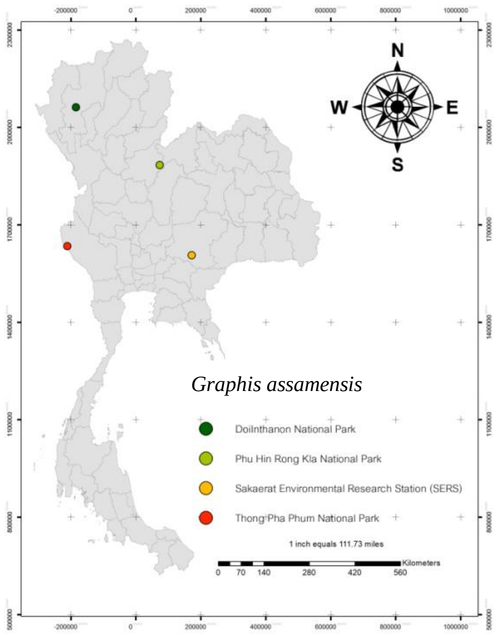


Figure 5.6 Distribution of *Graphis assamensis* Nagarkar & Patw. in Thailand.

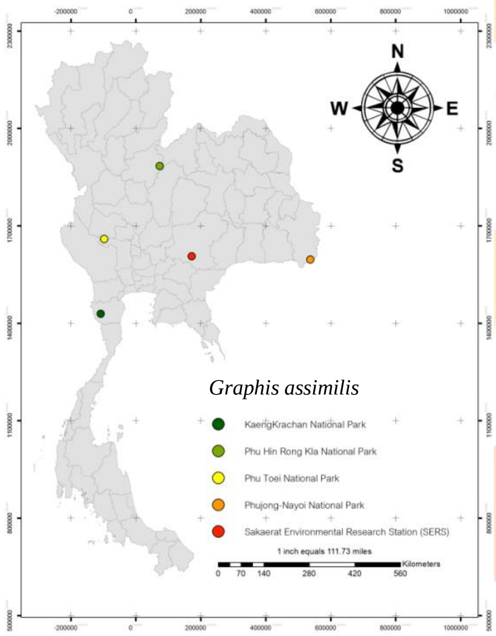


Figure 5.7 Distribution of *Graphis assimilis* Nyl. in Thailand.

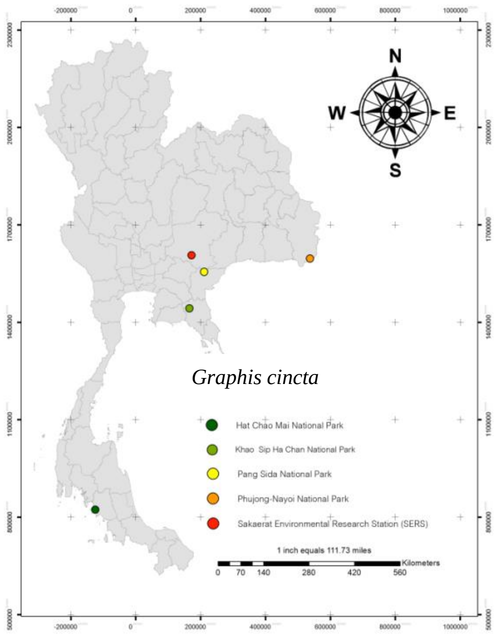


Figure 5.8 Distribution of *Graphis cincta* (Pers.) Aptroot. in Thailand.

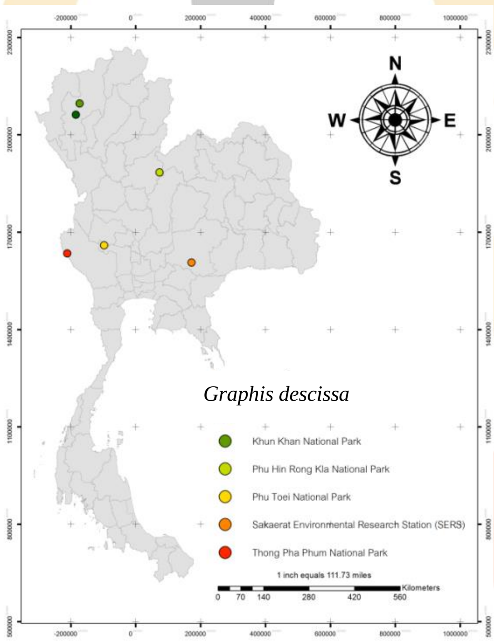


Figure 5.9 Distribution of *Graphis descissa* Müll. Arg. in Thailand.

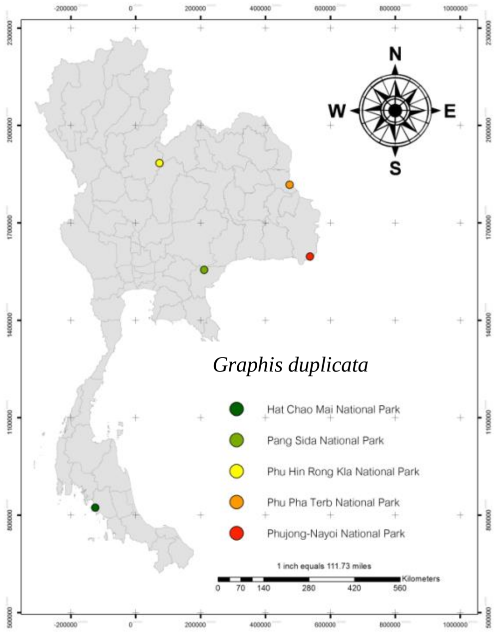


Figure 5.10 Distribution of *Graphis duplicata* Ach. in Thailand.

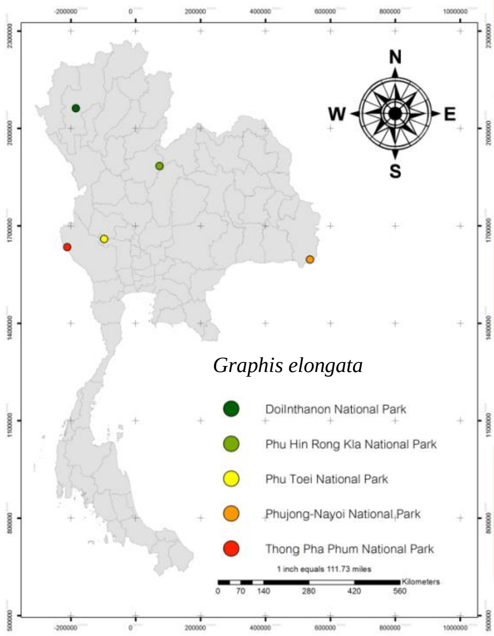


Figure 5.11 Distribution of *Graphis elongata* Zenker. in Thailand.

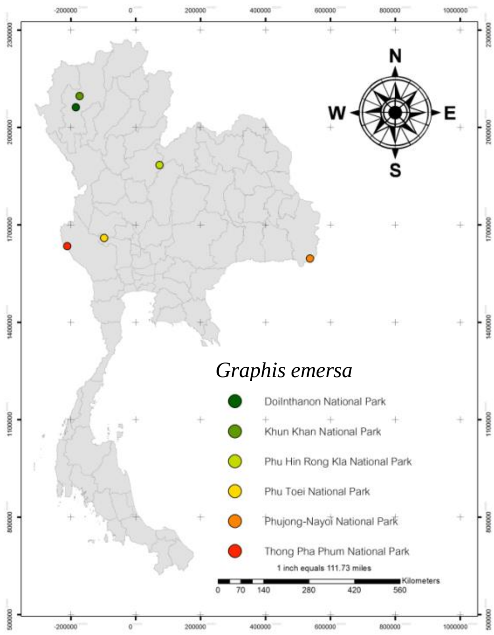


Figure 5.12 Distribution of *Graphis emersa* Mull. Arg. in Thailand.

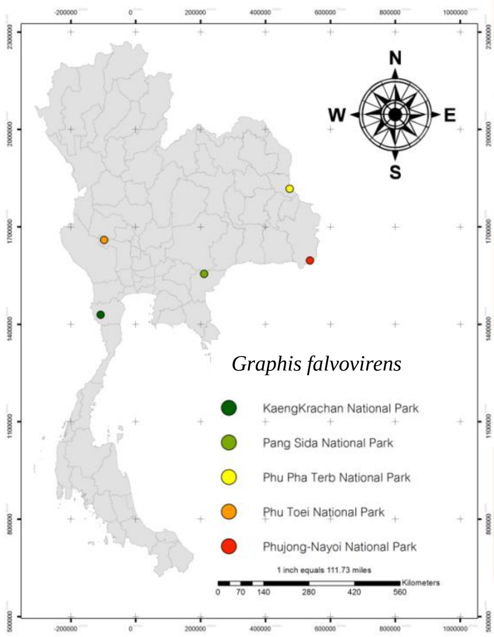


Figure 5.13 Distribution of *Graphis falvovirens* Makhija & Adaw in Thailand.

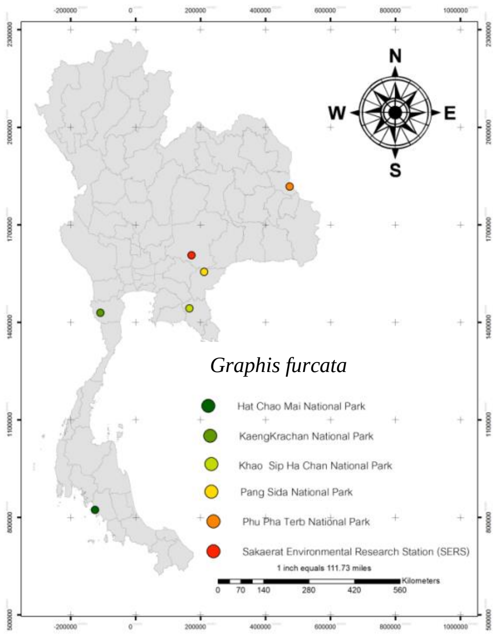


Figure 5.14 Distribution of *Graphis furcata* Fée. in Thailand.

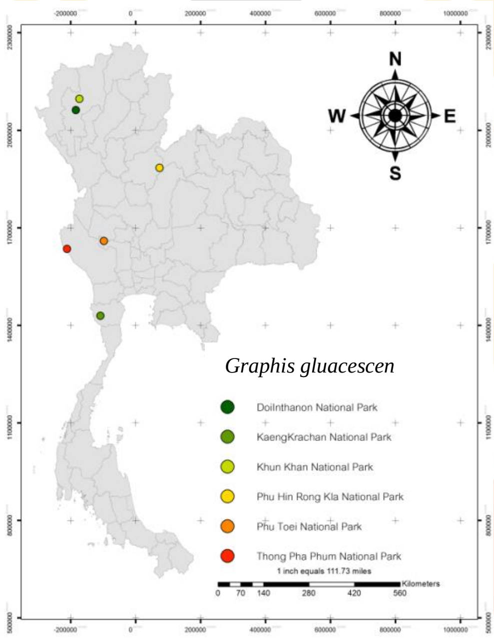


Figure 5.15 Distribution of *Graphis glaucescens* Fée. in Thailand.

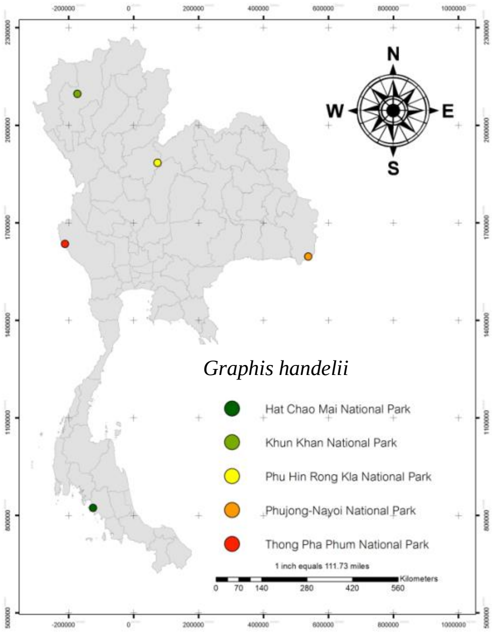


Figure 5.16 Distribution of *Graphis handelii* Zahlbr. in Thailand.

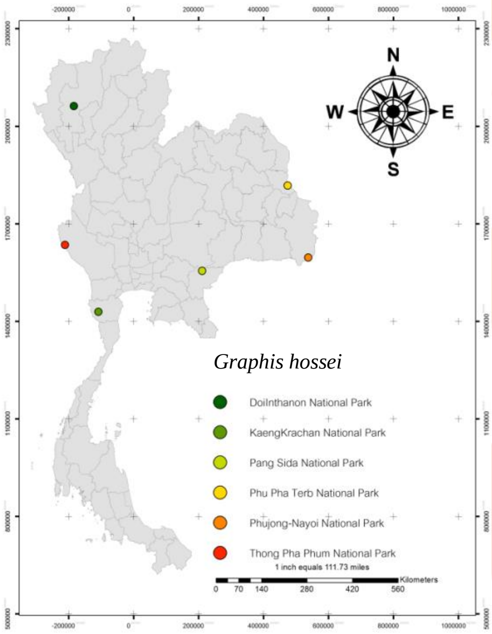


Figure 5.17 Distribution of *Graphis hossei* Vain. in Thailand

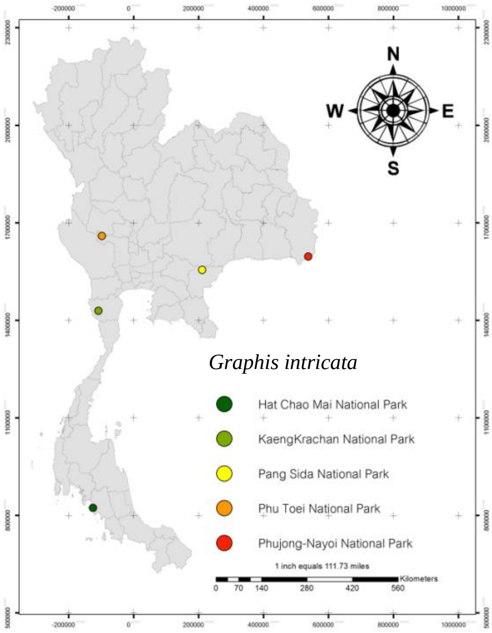


Figure 5.18 Distribution of *Graphis intricata* Fée. in Thailand.

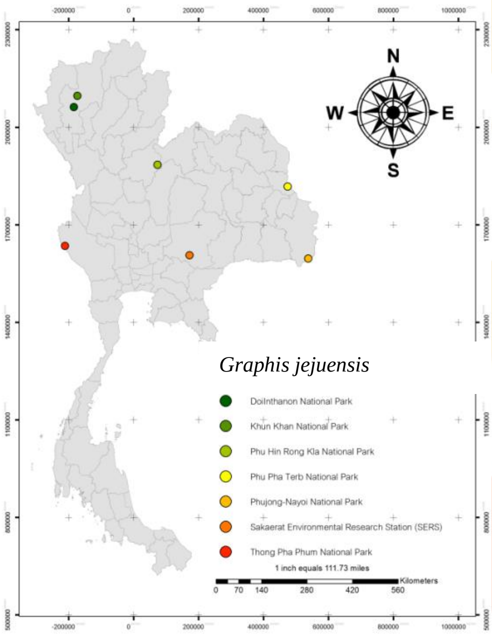


Figure 5.19 Distribution of *Graphis jejuensis* K.H. Moon, M. Nakan. & Kashiw. in Thailand.

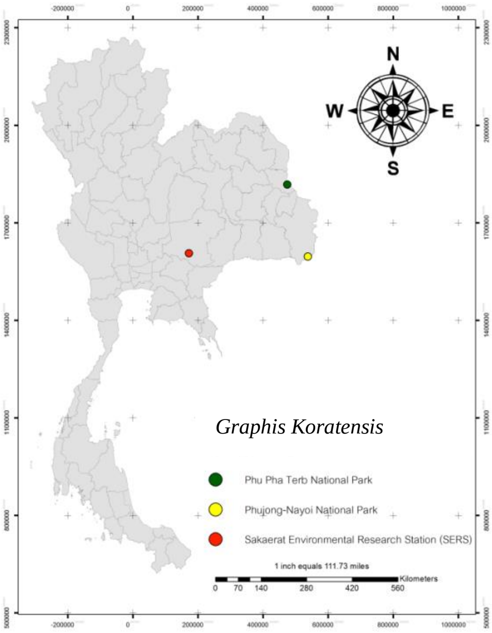


Figure 5.20 Distribution of *Graphis koratensis* Pitakpong, Kraichak, Lücking. in Thailand.

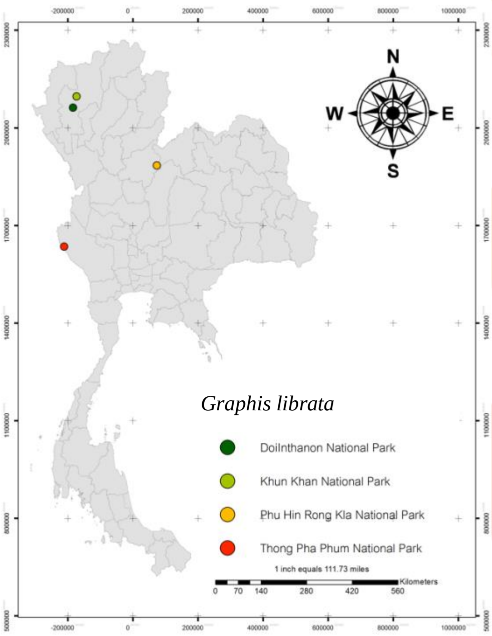


Figure 5.21 Distribution of *Graphis librata* C. Knight. in Thailand.

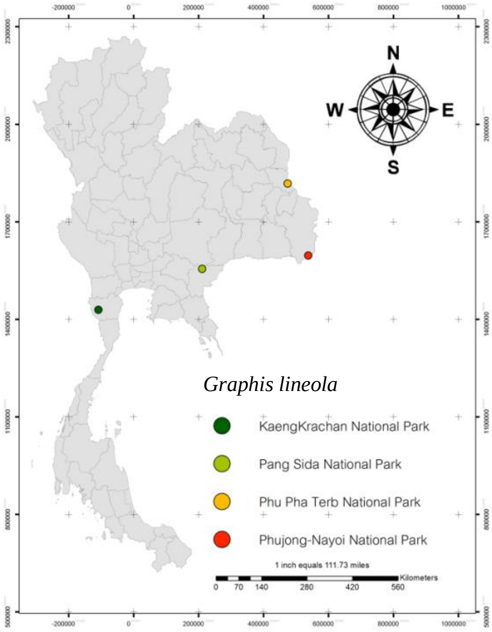


Figure 5.22 Distribution of *Graphis lineola* Ach. in Thailand.

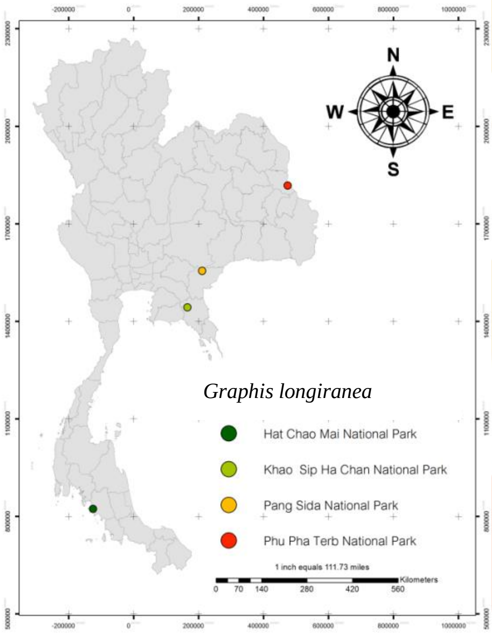


Figure 5.23 Distribution of *Graphis longiramea* Müll. Arg. in Thailand.

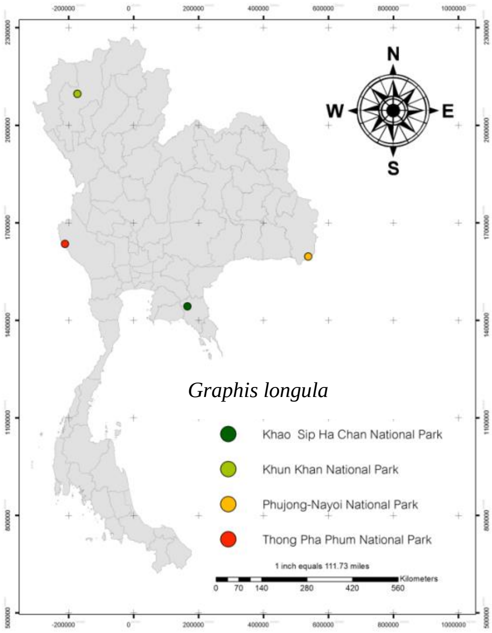


Figure 5.24 Distribution of *Graphis longula* Kremp. in Thailand.

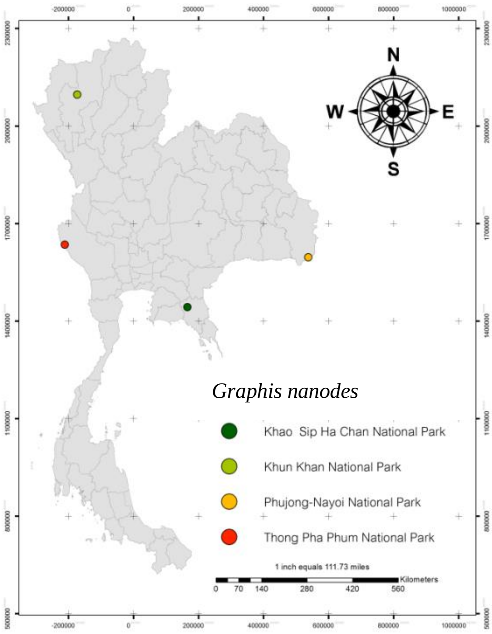


Figure 5.25 Distribution of *Graphis nanodes* Vain. in Thailand.

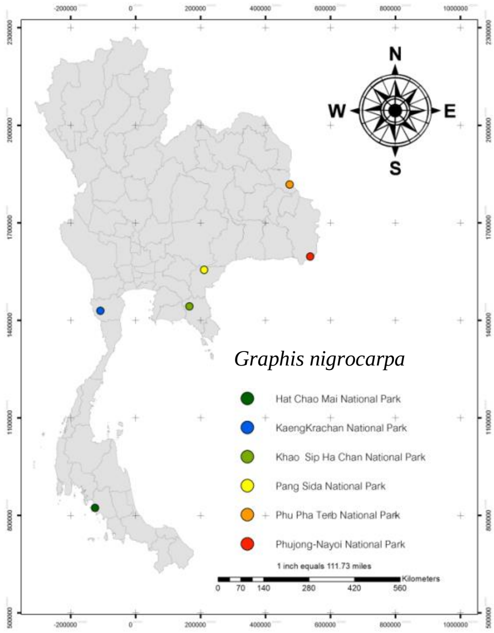


Figure 5.26 Distribution of *Graphis nigrocarpa* Adaw. & Makhija. in Thailand.

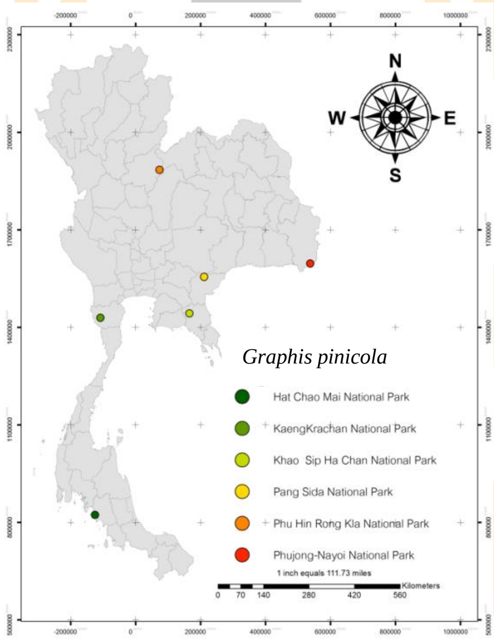


Figure 5.27 Distribution of *Graphis pinicola* Zahlbr. in Thailand.

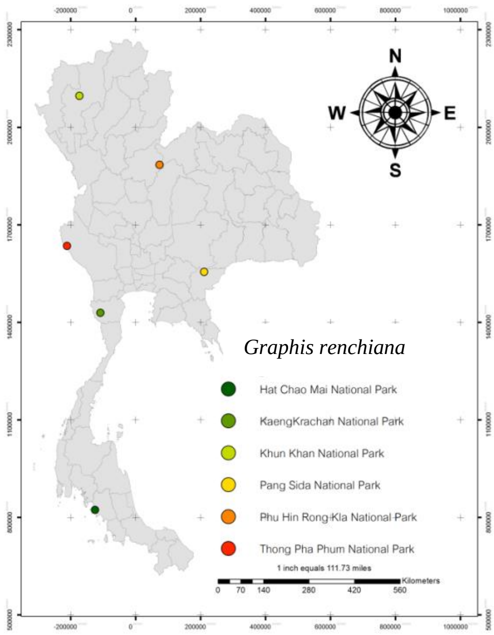


Figure 5.28 Distribution of *Graphis renschiana* (Müll. Arg.) Stizenb. in Thailand.

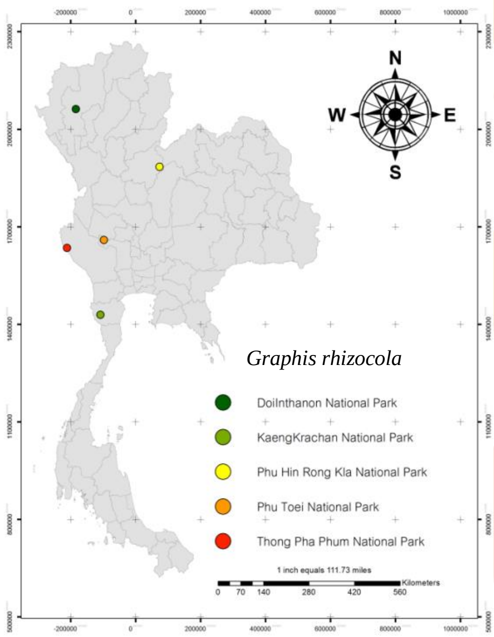


Figure 5.29 Distribution of *Graphis rhizocola* (Fée) Lücking & Chaves. in Thailand.

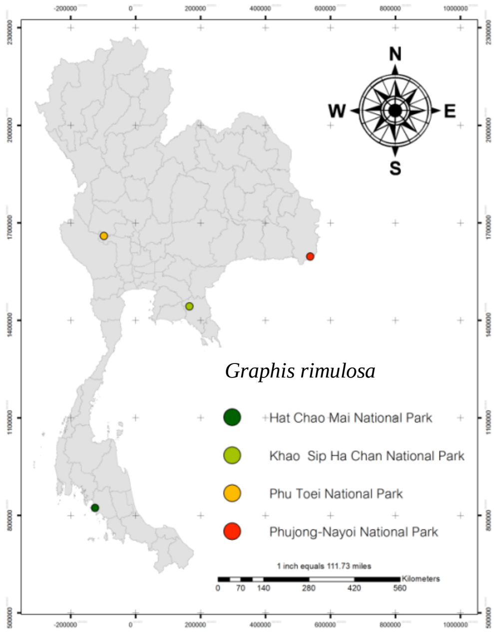


Figure 5.30 Distribution of *Graphis rimulosa* (Mont.) Trevis. in Thailand.

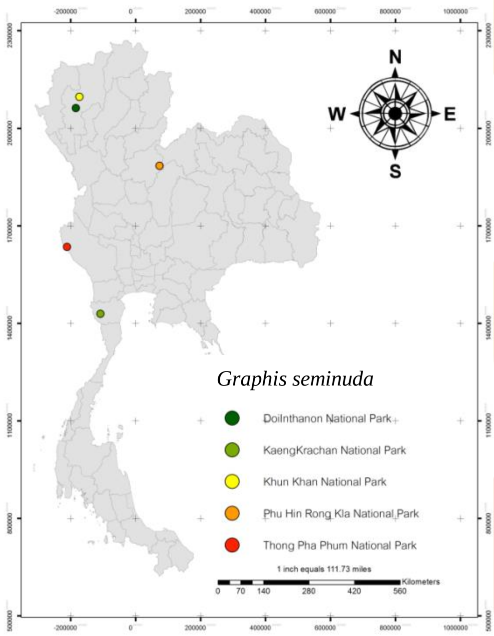


Figure 5.31 Distribution of *Graphis seminuda* Müll. Arg. in Thailand.

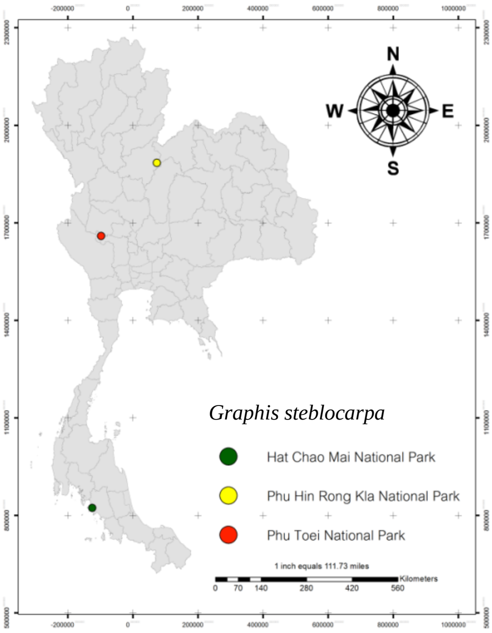


Figure 5.32 Distribution of *Graphis streblocarpa* (Bel.) Nyl. in Thailand.

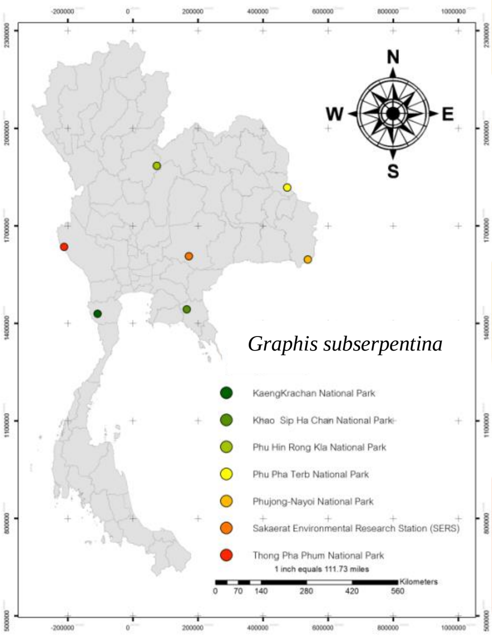


Figure 5.33 Distribution of *Graphis subserpentina* Nyl. in Thailand.

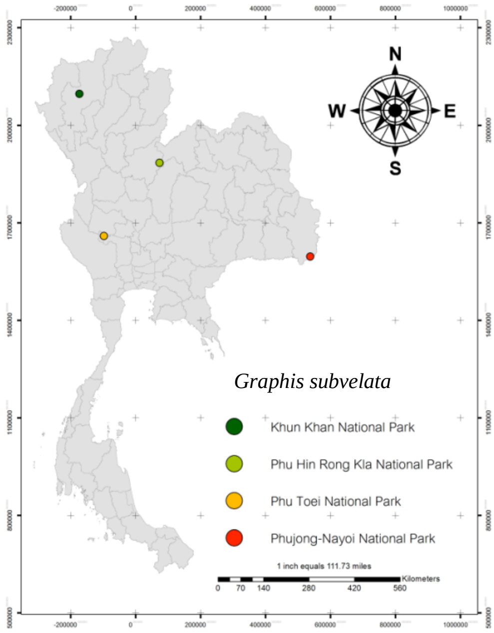


Figure 5.34 Distribution of *Graphis subvelata* Stirt. in Thailand

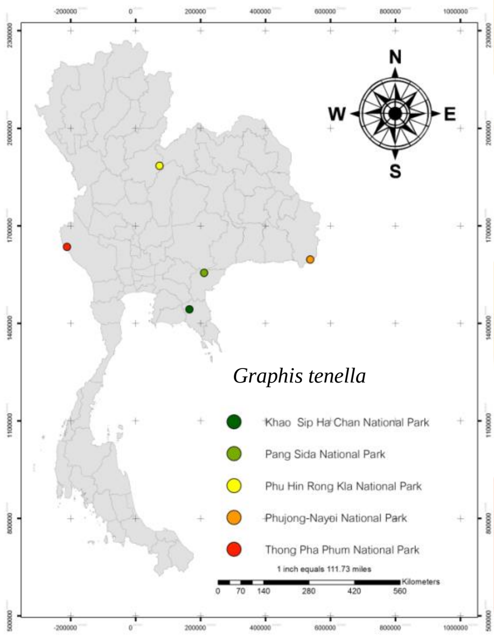


Figure 5.35 Distribution of *Graphis tenella* Ach. in Thailand.

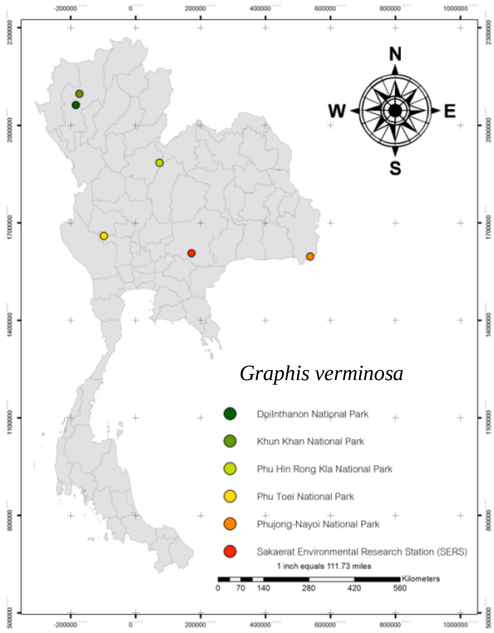


Figure 5.36 Distribution of *Graphis verminosa* Müll. Arg. in Thailand.

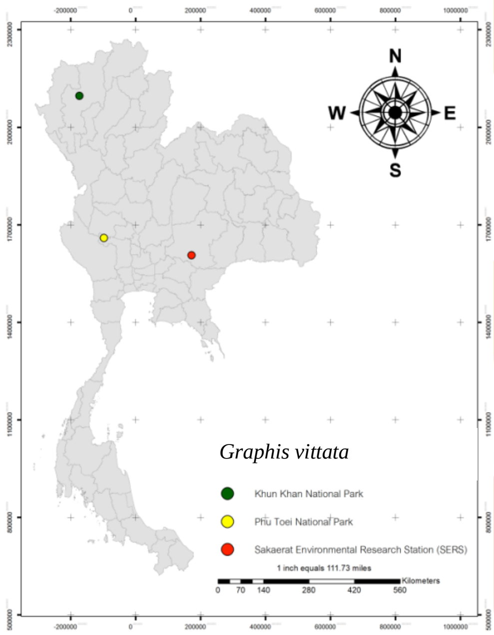


Figure 5.37 Distribution of *Graphis vittata* Müll. Arg. in Thailand.

The lichens distribution depended on a complex set of environmental and substrate-related explanatory variables, acting from the tree to the landscape level (Nimis *et al*., 2002), several authors pointed out the role of climatic factors (Hauck and Spribille, 2005; Giordani, 2007). Our study showed lichen richness decreased at high altitude with the positive correlation. Kessler *et al.* (2011) reported that species richness-elevation relationships have received considerable attention during the last two decades, as a response to the major challenge of documentation and explanation of global and regional gradients of species richness, while some studies suggested that species richness decreases monotonically with elevation (Bachman *et al*., 2004). The study of the distribution of lichens in the genus *Graphis* at altitude in the range of 4-2,562 m. found distinct species of lichens but there was no different in diversity. Doi Inthanon National Park, the highest altitude park of this study area (2,562 m) exhibited 11 lichen species including *G. assamensis* Nagarkar & Patw., *G. descissa* Müll. Arg., *G. elongata* Zenker., *G. emersa* Mull.Arg., *G. glaucescens* Fée., *G. hossei* Vain., *G. jejuensis* K. H. Moon, M. Nakan. & Kashiw., *G. librata* C. Knight., *G. rhizocola* (Fée) Lücking & Chaves., *G. seminuda* Müll. Arg., and *G. verminosa* Müll. Arg. On the other hand, Hat Chao Mai National Park Park, the lowest altitude area (4 m) found 12 species including *G. cincta* (Pers.) Aptroot, *G. duplicata* Ach., *G. furcata* Fée., *G. handelii* Zahlbr., *G. intricata* Fée., *G. longiramea* Müll. Arg., *G. nanodes* Vain., *G. nigrocarpa* Adaw. & Makhija., *G. pinicola* Zahlbr., *G. renschiana* (Müll. Arg.) Stizenb., *G. rimulosa* (Mont.) Trevis., and *G. streblocarpa* (Bel.) Nyl. From the correlation of both areas, different species were found at $p > 0.05$. The total species richness of lichens varied strongly with mid elevations in accordance with Grytnes *et al.* (2006) who studied the lichens from similar habitats in Himalayan. The lichen species richness tends to peak at intermediate elevations, which is in accordance with other similar studies worldwide (Wolseley and Aguirre-Hudson, 1997; Negi and Upreti 2000; Wolf and Alejandro, 2003; Pinokiyo *et al.*, 2008; Baniya *et al*., 2010; Rai *et al.*, 2012). Moreover, Dastych (1985) reported a negative relationship between tardigrade species richness and altitude in samples collected in Spitsbergen. As indicated by Hortal *et al*. (2009), tardigrade species richness showed a unimodal distribution along an altitudinal gradient. Although some authors (Dastych, 1987) reported that the number of species increased with altitude, a closer examination of their data in fact indicated a unimodal distribution of tardigrade richness. These limits could be overcome by referring bioclimatic observations to a higher geographic scale (2,000 m) such as at regional or local level. We agreed with Walther *et al*. (2002) that regional changes, being spatially heterogeneous, are relevant in the context of ecological response to climatic change. We found that both temperature and rainfall had less influence on lichen distribution, with the range of 21.3-31.65ºC and 0.7-512.6 mm respectively.

Shrestha and St. Clair (2011) applied correlation analysis and found that comparative increase in proportion of crustose growth forms in Mana can be attributed to increasing altitude, low relative humidity and lower atmospheric temperature in mountainous habitats of Mana. Still, the overall meso-climatic regimes of the environment in which the lichen grows (such as rainfall, humidity, exposure, insulation, and temperature) seem to be chiefly responsible for the development and maintenance of tardigrade populations (Schuster and Greven, 2007; Hortal *et al.*, 2009; Kaczmarek *et al.*, 2011). Therefore, it seems reasonable to conclude that neither the type of vegetation nor differences in lichen species significantly influenced lichen distribution along the altitudinal gradients investigated in the present study. The specific habitat which is appropriate to plant growth depends on many factors. Vidal (1979), a bioclimatic area results from a combination of temperature and humidity. The appropriate environment is necessary to support their life cycle.

5.5 Conclusions

In this study, 32 species of genus *Graphis* were investigated throughout Thailand. *Graphis* had higher distribution probabilities in the mid altitude than in the high and low regions of Thailand. The differences in environmental conditions may affect their growth, so if their habitats are lost or changed, they may die out. These taxa have a risk to disappear easily. These findings are essential for the effective conservation of lichens in Thailand, not only with respect to estimating the species distribution ranges of *Graphis*, but also for identifying the environmental factors limiting lichen distribution. At present, the climate change may be cause of the disappear from the forest.

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CHAPTER VI

FACTORS INFLUENCING SPECIES RICHNESS OF LICHENS IN THE FAMILY GRAPHIDACEAE AT SAKAERAT ENVIRONMENTAL RESEARCH STATION, NAKHON RATCHASIMA

6.1 Abstract

Research concerning ecological relationships between lichens and substrate factors in Sakaerat Environmental Research Station is limited. This study aimed to determine the variation of species richness of family Graphidaceae in Sakaerat Environmental Research Station to substrate factors, including circumference of tree, pH of bark, bark crevice depth, and percent moisture on tree. In addition, lichen specificity of the tree species *Shorea obtusa*, *Shorea siamensis*, *Dipterocarpus obtusifolius*, *Lagerstroemia floribunda*, *Canarium subulatum*, *Hopea ferrea*, *Pterocymbium javanicum*, *Shorea sericeifolia*, and *Hopea odorata* was determined On 85 sampled trees, 25 lichen species were found. Canonical correspondence analysis (CCA) showed a segregation pattern in the species composition due to circumference of tree, pH of bark, bark crevice depth, and percent moisture. Based on our study, the most pronounced factor affecting species richness of lichens was bark pH.

6.2 Introduction

Lichen is a small living organism living on the surfaces of natural things such as barks, leaves, soil, rocks, insects, and construction materials such as concrete, metal, signs, and sheet, etc. It can grow on any surface (Lücking, 1998; Purvis, 2000). The lichens growing on tree bark or trunk, rock, moss, and soil are called corticolous, saxicolous, gauscicolous, and tericolous, respectively (Dobson, 1981; Hale, 1979). In deciduous forest, richness of lichens has been reported (Nordén *et al*., 2007). Different species of lichens prefer, or only grow on different tree species (Mezaka *et al*., 2008; Meier and Paal, 2009). Growth substrates are ecologically important impact on the diversity and distribution of lichens (Brodo, 1973; Wolseley and Aguirre-Hudson, 1997; Brodo *et al*., 2001; Cáceres *et al*., 2007; Rosabal *et al*., 2010). Properties of the substrates are important features to the distribution of lichens in particular texture, water holding capacity, and pH of bark (Barkman, 1958; van Herk *et al*., 2001; Sutton *et al*., 2004; Frati *et al*., 2006; Sparrius, 2006; Wolseley *et al*., 2006; Cáceres *et al*., 2007; Jüriado *et al*., 2009; Spier 2010; Rosabal *et al*., 2013). Tree species, tree size and bark crevice depth are also factors for epiphytic diversity (Fritz *et al*., 2008; Ranius *et al*., 2008; Lie *et al*., 2009). Moreover, the position of lichen cover also depends on the size of the tree (Belinchón *et al*., 2007; Johansson *et al*., 2007; Ranius *et al*., 2008). Diameter of the trunk is also considered in studies of substrate ecology (Cáceres *et al*., 2007; Amo de Paz and Burgaze, 2009). Bark moisture is also a factor that influences lichen diversity and distribution on the same tree (Brodo, 1973). Therefore, trees species are very important on variation of the cover of lichens (Gauslaa and Solhaug, 1995; Marmor *et al*., 2013).

The purpose of this study was to study the species richness of lichens in the family Graphidaceae associated with circumference of tree, pH of bark, bark crevice depth, percent moisture, and directions to find lichens on tree at Sakaerat Environmental Research Station (SERS). The trees selected for the study were *Shorea obtusa* Wall. Ex Blume, *Shorea siamensis* Miq., *Dipterocarpus Obtusifolius* Teijsm.ex Miq., *Lagerstroemia floribunda* Jack., *Canarium subulatum* Guillaumin., *Hopea ferrea* Laness., *Pterocymbium javanicum* R. Br., *Shorea sericeifolia* C. E. C. Fisch. and Hutch., and *Hopea odorata* Roxb. because these trees are common in Sakaerat Environmental Research Station.

6.3 Materials and Methods

Study areas

Field studies were carried out from October 2013 to March 2014 in dry evergreen and dry dipterocarp forests of Sakaerat Environmental Research Station (SERS), classified as a UNESCO Biosphere Reserve since 1967. The reserve, covering 78.09 km^2, is located in Nakhon Ratchasima province at north-eastern Thailand (Figure 6.1). The climate is typically tropical, with annual rainfall ranges from 244 to 950 mm, annual temperature ranges from 20.5 to 35°C, and relative humidity ranges from 60 to 86%. SERS has two major natural forest types: dry evergreen forest (DEF) and dry dipterocarp forest (DDF). In dry evergreen forest, dominant tree species are *Hopea ferrea* Laness., *Pterocymbium javanicum* R. Br., *Shorea sericeifolia* C. E. C. Fisch and Hutch., and *Hopea odorata* Roxb. Dry dipterocarp forest has been dominated by common trees such as *Shorea obtusa* Wall.

ex Blume, *Shorea siamensis* Miq., *Dipterocarpus obtusifolius* Teijsm. ex Miq., *Lagerstroemia floribunda* Jack., and *Canarium subulatum* Guillaumin.

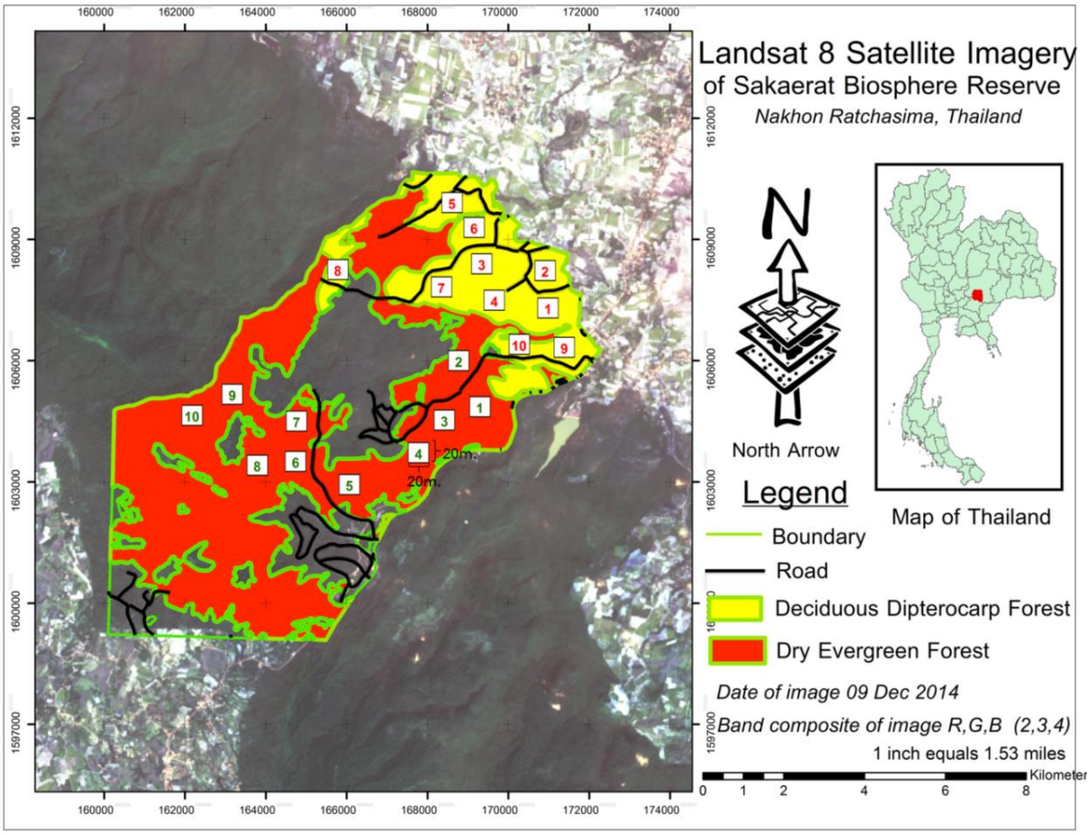

Figure 6.1 Map of Sakaerat Environmental Research Station (SERS).

Lichen sampling

Lichen sampling on tree trunk was conducted by cluster sampling technique in order to reduce the time of data collection in each sample plot. The layout of the squares along the line (transect) was located far from the road. Ten plots in the dry dipterocarp and dry evergreen forests with size of 20 × 20 m^2 were positioned. Lichens in the family Graphidaceae on 85 trees were collected. In the dry dipterocarp forest, 46 trees were studied, which belong to *Shorea obtusa* Wall. ex Blume., *Shorea* *siamensis* Miq., *Dipterocarpus obtusifolius* Teijsm. ex Miq., *Lagerstroemia floribunda* Jack., and *Canarium subulatum* Guillaumin. In dry evergreen forest, there were also 39 trees belonging to *Hopea ferrea* Laness., *Pterocymbium javanicum* R.Br., *Shorea sericeifolia* C. E. C. Fisch. and Hutch., and *Hopea odorata* Roxb. (Table 6.1) and trunk characteristics of the trees were shown in Figure 6.2.

Table 6.1 List of the tree species and number of the sampled trees for each species (abbreviations used for this thesis).

Family	Species	Number of tree	Abbrev.
Dipterocarpaceae	*Shorea obtusa* Wall. ex Blume.	17	Sho-obtu
Dipterocarpaceae	*Shorea siamensis* Miq.	13	Sho-siam
Dipterocarpaceae	*Dipterocarpus obtusifolius* Teijsm. ex Miq.	5	Dip-obtu
Lythraceae	*Lagerstroemia floribunda* Jack.	7	Lag-flor
Burseraceae	*Canarium subulatum* Guillaumin.	4	Can-subu
Dipterocarpaceae	*Hopea ferrea* Laness.	15	Hop-ferr
Sterculiaceae	*Pterocymbium javanicum* R. Br.	7	Pte-java
Dipterocarpaceae	*Shorea sericeifolia* C. E. C. Fisch. and Hutch.	7	Sho-seri
Dipterocarpaceae	*Hopea odorata* Roxb.	10	Hop-odor

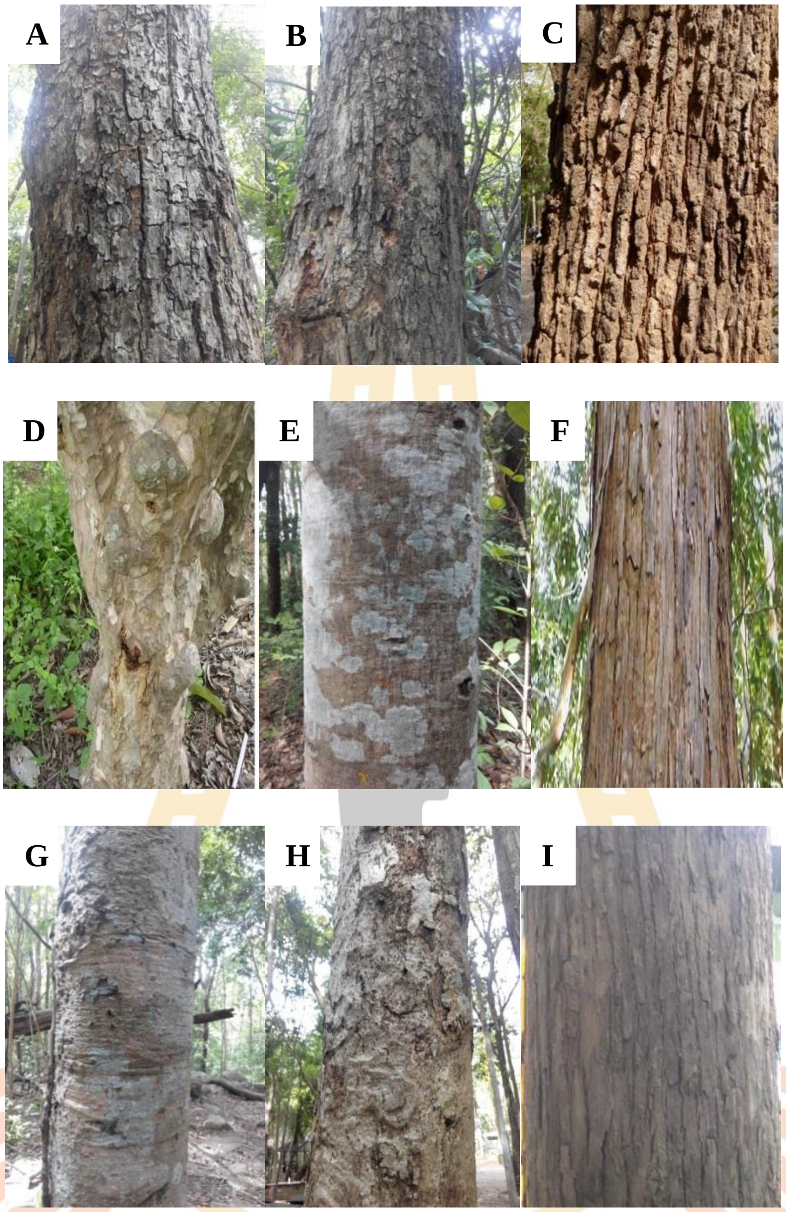

Figure 6.2 Trunk characteristics of each tree species (A: *Shorea obtusa* Wall. ex Blume., B: *Shorea siamensis* Miq., C: *Dipterocarpus obtusifolius* Teijsm. ex Miq., D: *Lagerstroemia floribunda* Jack., E: *Canarium subulatum* Guillaumin., F: *Hopea ferrea* Laness., G: *Pterocymbium javanicum* R. Br., H: *Shorea sericeifolia* C. E. C. Fisch. and Hutch., and I: *Hopea odorata* Roxb.)

The frequency of lichen species was obtained by counting a number of lichens that grow on a tree using a quadrat (surveying grid frame) of 20 × 50 cm for a height of 1.30 m of the trees (Pérez- Pérez *et al*., 2011). The direction the grid frame and types of trees were recorded. Lichen samples were collected and identified in the laboratory by stereo microscope, light microscope, spot test, UV light and Thin-layer chromatography (TLC) and using key described by Aptroot *et al*. (2008; 2009); Staiger (2002); Lücking *et al*. (2008; 2009a; 2009b).

Measurement of diameter of the trunk and bark crevice depth, moisture percentage, and pH of bark

At 1.3 m height, measurement of diameter of the tree trunk by cables and record. The bark crevice depth was measured with a vernier on the north side, which was suggested by Barkman (1958).

For determination of the moisture percentage, the barks were weighed before drying. Then, the weighed barks were soaked into 20 ml of water for 24 hr and then weighed to get the weight after soaking into water. Moisture percentage of bark was calculated as following formula; dry weight/sample weight × 100 (Manwiller, 1975; Rosabal *et al*., 2013)

For measurement of bark pH, bark samples were collected using a knife to cutting the bark with a depth of 3.0 mm from the north side of a tree at 1.3 m height. Tree bark samples were cut in small pieces (medium size 0.001 g). An amount of 0.5 g of bark pieces was shaken in 20 ml 1M KCl (pH 5.5) solution for one hour in 100 ml flasks and pH measured with a pH meter (Mežaka *et al*., 2008; Mežaka *et al.*, 2012).

Data analysis

The lichen species and tree species with factors were determined using Canonical Correspondence Analysis (CCA), general linear mixed model (GLMM), akaike's information criterion (AIC), and the correlations between lichen species and factors were analyzed using Spearman's correlations for continuous predictor variables and analysis of variance (ANOVA) for categorical variables in R version 3.1.2 statistical package R (Venables and Smith, 2010; Mathiopoulos, 2011).

6.4 Results and Discussions

Lichen species

From the study of lichens in the family Graphidaceae on 85 trees at the height of 1.3 m, 11 genera and 25 species of lichens were found (Table 6.2) including the genera *Carbacanthographis* (2 spp.), *Diorygma* (1 sp.), *Dyplolabia* (1 sp.), *Fissurina* (2 spp.), *Graphina* (1 sp.), *Graphis* (9 spp.), *Hemithecium* (2 spp.), *Pallidogramme* (1 sp.), *Phaeographis* (3 spp.), *Platygramme* (1 sp.), *Platythecium* (1 sp.), and *Sarcographa* (1 sp.).

Platygramme jambosae Zahlbr. was the most common found, followed by *Graphis pinicola* Zahlbr., *Graphis subserpentina* Nyl (17), *Diorygma reniforme* (Fée) Kalb, Staiger & Elix, and *Dyplolabia afzelii* (Ach.) A. Massal. Lichen species that showed the most cover area was *Graphis pinicola* Zahlbr. (321 cm^2), followed by *Platygramme jambosae* Zahlbr. (285 cm^2), *Pallidogramme chlorocarpoides* (Nyl.). Staiger, Kalb & Lücking (210 cm^2), *Diorygma reniforme* (Fée) Kalb, Staiger & Elix (199 cm^2), and *Sarcographa labyrinthica* (Ach.) Müll. Arg. (192 cm^2), respectively.

In addition, one new species found in Thailand was *Graphis koratensis* Pitakpong, Kraichak, Lücking sp. nov., and the new record species in Thailand were *Graphis cincta* (Pers.) Aptroot and *Graphis jejuensis* KH Moon, M. Nakan. & Kashiw (APPENDIX A).

Table 6.2 The frequency of lichens in SERS.

Species	Frequency									
	Sho-obtu	Sho-siam	Dip-obtu	Lag-flor	Can-subu	Hop-ferr	Pte-java	Sho-seri	Hop-odor	Total
Carbacanthographis crassa	3							1	1	5
Carbacanthographis marcescens		1		6	3	2				12
Diorygma reniforme	1		6		3		3		3	16
Dyplolabia afzelii		7		1	5	1			2	16
Fissurina rubiginosa		1		4			2			7
Fissurina dumastioides	2			3	2			3		10
Graphina incrustans			3		3	1	3			10
Graphis assimilis	6	1						1		8
Graphis cincta				1	6		2			9
Graphis descissa	1		3			2		2		8
Graphis furcata		1			2		1		3	7
Graphis jejuensis	3	2							2	7
Graphis koratensis	5		3		3					11
Graphis pinicola			8		6		4			18
Graphis subserpentina	5	3		2		2		5		17
Graphis verminosa		2			7					9
Hemithecium aphaneomicrosporum		1					2			3
Hemithecium laubertianum			3				2			5
Pallidogramme chlorocarpoides			4		6	1	3	1		15
Phaeographis crispata	5			4					2	11
Phaeographis phurueaensis	1			1				2	1	5
Phaeographis subdividens			7	2						9
Platygramme jambosae	3	12			2		2		2	21
Platythecium serpentinellum		2								2
Sarcographa labyrinthica	2		8	2						12

Abbreviations: *Shorea obtuse*: Sho-obtu, *Shorea siamensis*: Sho-siam, *Dipterocarpus obtusifolius*: Dip-obtu, *Lagerstroemia floribunda*: Lag-flor, *Canarium subulatum*: Can-subu, *Hopea ferrea*: Hop-ferr, *Pterocymbium javanicum*: Pte-java, *Shorea sericeifolia*: Sho-seri, and *Hopea odorata*: Hop-odor.

The study of lichens on nine trees, which are *Shorea obtusa*, *Shorea siamensis*, *Dipterocarpus obtusifolius*, *Lagerstroemia floribunda*, *Canarium subulatum*, *Hopea ferrea*, *Pterocymbium javanicum*, *Shorea sericeifolia*, and *Hopea odorata*, showed the most species richness of lichen species on *Shorea obtusa* and *Canarium subulatum* about 12 lichen species. Frequency of lichen species was found the most common on *Canarium subulatum* (48 lichen species). However, the lowest species richness, frequency was determined on *Hopea ferrea* in Table 6.3, and Figures 6.3-6.4.

Table 6.3 Species richness, frequency and cover area values for each lichen species.

	Sho-obtu	Sho-siam	Dip-obtu	Lag-flor	Can-subu	Hop-ferr	Pte-java	Sho-seri	Hop-odor
Speiceis richness	12	11	9	10	12	6	10	7	8
Frequency	37	33	45	26	48	9	24	15	16

Abbreviations: *Shorea obtuse*: Sho-obtu, *Shorea siamensis*: Sho-siam, *Dipterocarpus obtusifolius*: Dip-obtu, *Lagerstroemia floribunda*: Lag-flor, *Canarium subulatum*: Can-subu, *Hopea ferrea*: Hop-ferr, *Pterocymbium javanicum*: Pte-java, *Shorea sericeifolia*: Sho-seri, and *Hopea odorata*: Hop-odor.

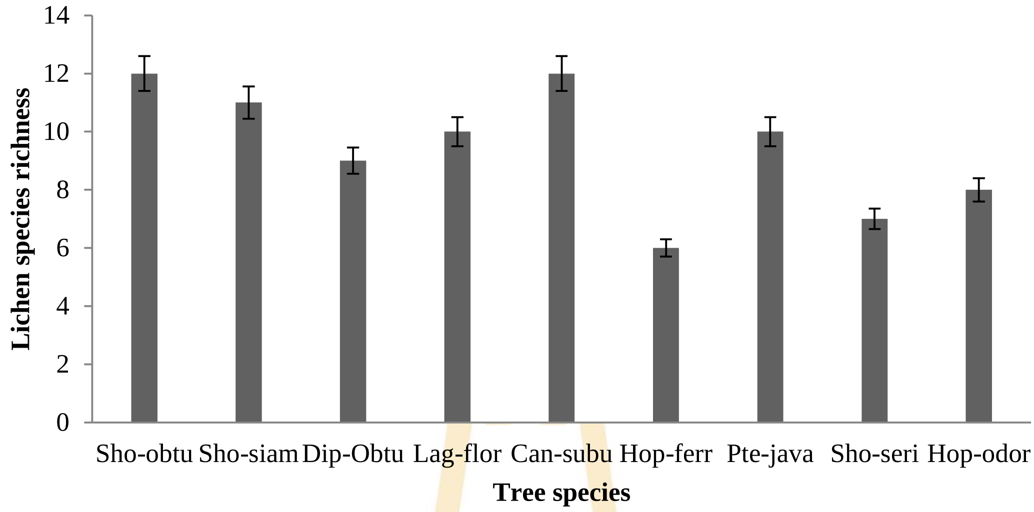

Figure 6.3 Species richness values for each lichen species on different tree.

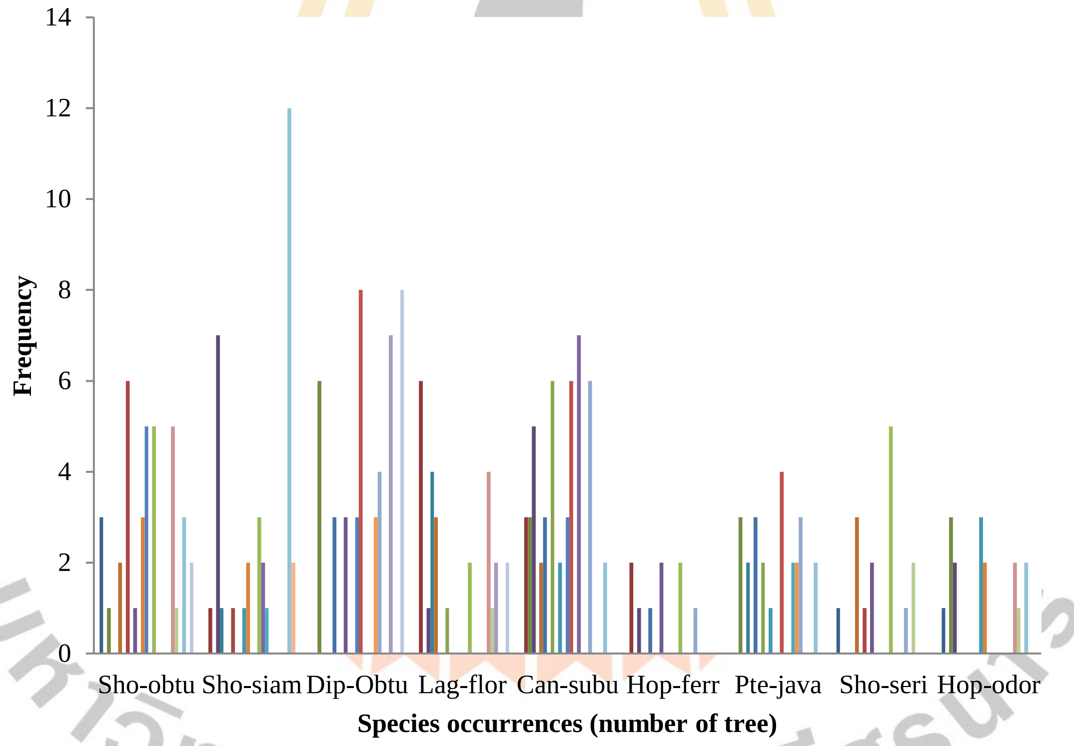

Figure 6.4 Frequency values for each lichen species found on trees.

Tree characteristics

1) Circumference of tree

The study of lichens on 85 trees found nine tree species with a circumference between 50-288 cm and most of the trees are in the range of 50-100 cm circumference. *Hopea odorata* (288 cm) had the widest circumference, followed by *Hopea ferrea* (283 cm) whereas *Shorea obtusa* had least circumference of 50 cm Mean and standard deviation (± SD) values of circumference of the trunk were calculated for each tree specices (Table 6.3). The study of the correlations between the circumference of the tree species and the richness of lichens found the correlation being statistically significant (Figure 6.5).

2) pH of bark

The pH values of the barks were between 3.6 and 6.1 (average 4.73). The pH of the most trees was acidic. pH of the bark of each species was most found in the range of 4 to 5.5. Each tree had the mean and standard deviation (± SD) values of pH of barks. *Hopea odorata* had the highest pH at 6.1 and *Hopea ferrea* had the lowest value at 3.6 (Table 6.3). The study of the correlations between pH of the tree bark and the richness of lichens found the correlation being statistically significant (Figure 6.6).

Table 6.3 Mean and standard deviation (±SD) values of circumference of the trunk and pH of bark for each tree species.

Species	Circumference (cm)		pH	
	Mean	±SD	Mean	±SD
Shorea obtusa	65.41	12.55	4.63	0.58
Shorea siamensis	70.46	11.39	4.89	0.52
Dipterocarpus obtusifolius	74.00	11.87	4.94	0.72
Lagerstroemia floribunda	101.00	22.97	4.63	0.36
Canarium subulatum	83.75	17.37	5.08	0.33
Hopea ferrea	144.13	64.87	4.71	0.66
Pterocymbium javanicum	96.43	27.67	4.85	0.69
Shorea sericeifolia	101.43	43.59	4.42	0.32
Hopea odorata	171.60	80.84	4.60	0.70

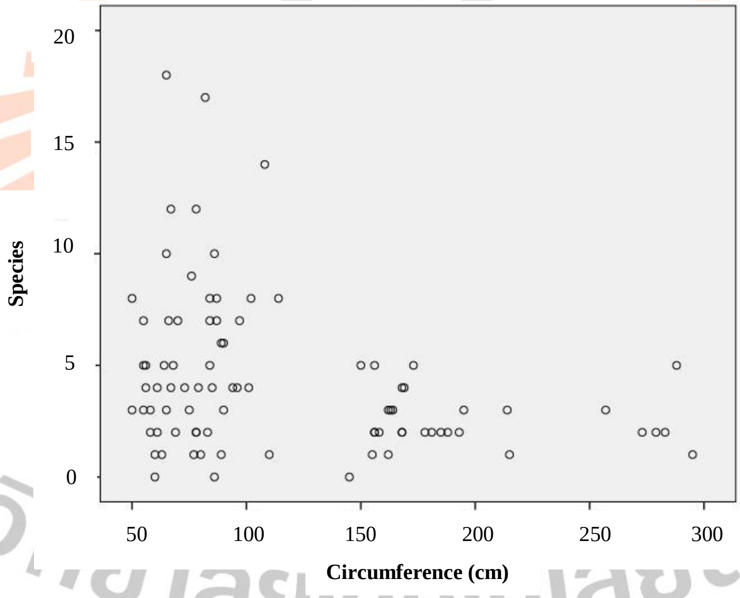

Figure 6.5 Correlations between lichen species and tree circumference (cm) (significant Rs = -0.266; N = 85; p = 0.14 at the 0.05 level).

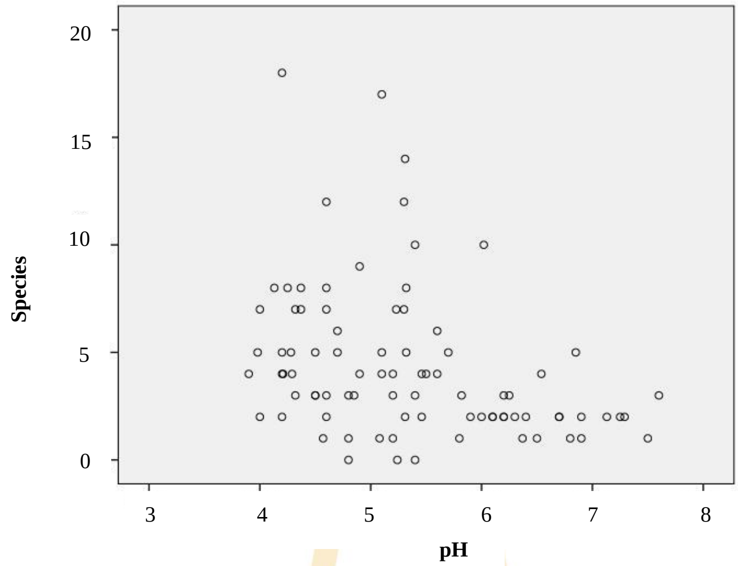

Figure 6.6 Correlation between lichen species and tree pH (significant Rs = -0.424; N = 85; p = 0.0001 at the 0.01 level).

3) Moisture percentage

The mean and standard deviation (± SD) values of bark moisture and bark crevice depth of bark for each tree species were shown in Table 6.4. It was found that the percentage of moisture ranged from about 8 to 56.3. *Pterocymbium javanicum* had the highest moisture percent (56.3%) whereas *Shorea siamensis* had lowest percent. The percentage of moisture most found in the bark was 11-25%, as shown in (Table 6.4). The study of the correlations between the percentage of bark moisture and the species richness of lichens found the correlation being statistically significant (Figure 6.7).

4) Bark crevice depth

The survey found that tree species having the hightest value of bark crevice depth was *Shorea siamensis* (5.1 cm) and the lowest depth value was the bark of

Pterocymbium javanicum (0.3 cm). The depth of bark found the most in the range of 0.3-1.8 cm, as shown in (Table 6.4). The study of the correlations between the bark crevice depth and the species richness of lichens found the correlation being statistically significant (Figure 6.8).

Table 6.4 Mean and standard deviation (±SD) of percent moisture and bark crevice depth of bark for each tree species.

Species	Percent moisture		Bark crevice depth (cm)	
	Mean	±SD	Mean	±SD
Shorea obtusa	15.35	2.78	3.41	0.71
Shorea siamensis	16.58	5.80	3.00	0.87
Dipterocarpus obtusifolius	42.72	2.55	1.04	0.76
Lagerstroemia floribunda	33.93	9.80	0.87	0.70
Canarium subulatum	37.55	6.47	0.43	0.10
Hopea ferrea	17.35	4.39	1.31	0.37
Pterocymbium javanicum	45.17	7.40	0.49	0.11
Shorea sericeifolia	36.9	5.17	1.19	0.55
Hopea odorata	19.3	4.11	1.24	0.48

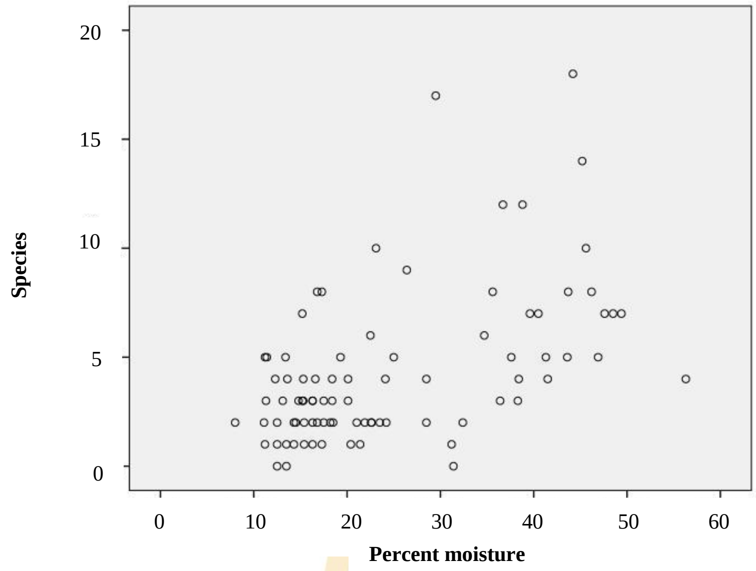

Figure 6.7 Correlations between lichen species and percent moisture of bark (significant Rs = -0.510; N = 85; p = 0.0001 at the 0.01 level).

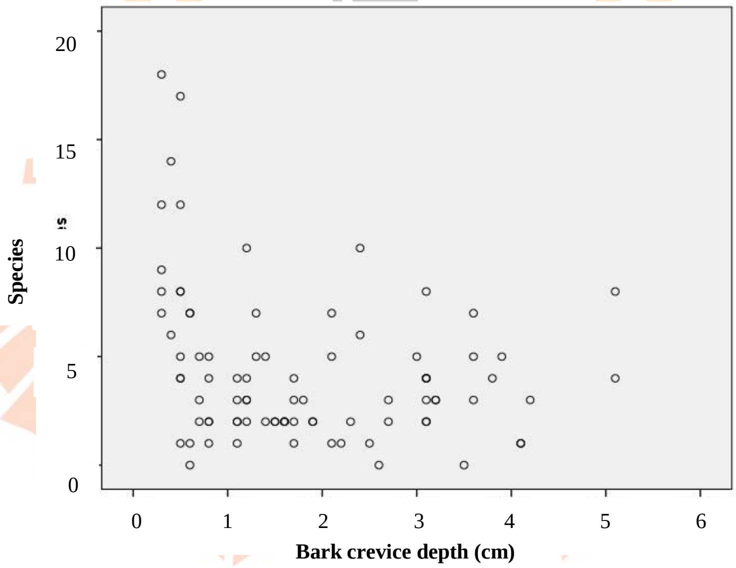

Figure 6.8 Correlations between lichen species and bark crevice depth (cm) (significant Rs = -0. 272; N = 85; p = 0.012 at the 0.05 level).

Directions to find lichens on tree were mainly found in the north (31specimens), followed by east (24 specimens), south (15 specimens) and west (14 specimens) as shown in Figure 6.9. *Shorea obtusa*, *Dipterocarpus Obtusifolius*, *Lagerstroemia floribunda* and *Pterocymbium javanicum* were found in the north more than other directions. *Shorea siamensis*, *Canarium subulatum* and *Hopea ferrea* were found in the south whereas *Shorea sericeifolia* was in the east. Lichens were found on *Shorea sericeifolia* in the south while *Hopea odorata* found in the north and east as well.

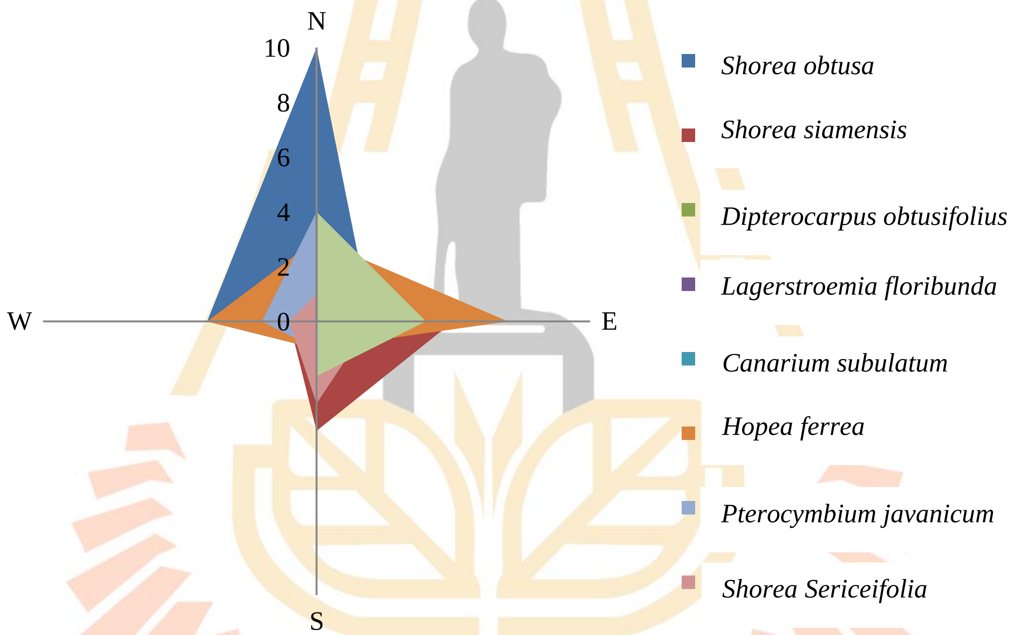

Figure 6.9 Directions of lichen collection on each tree species.

The correlations between species and factors such as circumference, bark crevice depth, percent moisture and pH were analyzed using CCA (Figure 6.10, Table 5.6), and it was found that pH has affected with lichen species more than other factor. *Fissurina rubiginosa*, *Carbacanthographis marcescens*, *Hemithecium aphaneomicrosporum*, and *Fissurina dumastioides* were correlated with the bark crevice depth. *Platygramme jambosae*, *Graphis subserpentina*, and *Carbacanthographis crassa* were correlated with the circumference of trees. *Graphis verminosa* and *Graphina incrustans* were associated with percent moisture. From CCA analysis, pH value was high which was related to *Lagerstroemia floribunda*, *Graphis verminosa*, and *Graphina incrustans.*

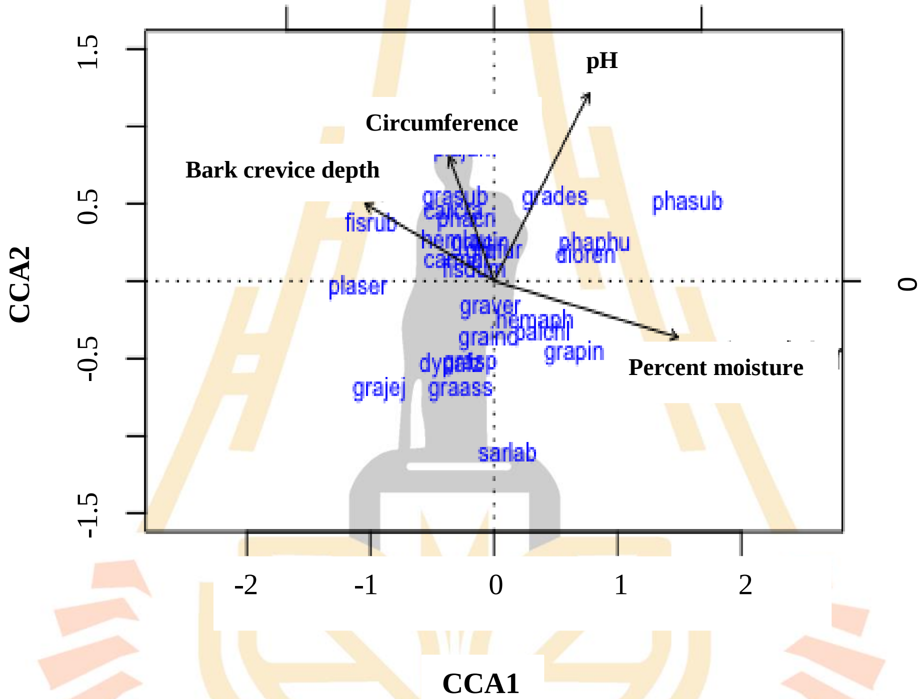

Figure 6.10 Canonical correspondence analysis (CCA) ordination of 25 lichen species with circumference, bark crevice depth, and percent moisture.

Table 6.5 CCA ordination of 25 lichen species with factors of 85 trees.

Lichen Species	CCA1	CCA2
Carbacanthographis crassa (Müll. Arg.) Staiger & Kalb	-0.33	0.45
Carbacanthographis marcescens (Fée) Staiger & Kalb	-0.30	0.14
Diorygma reniforme (Fée) Kalb, Staiger & Elix	0.74	0.18
Dyplolabia afzelii (Ach.) A. Massal.	-0.35	-0.53
Fissurina rubiginosa (Fée) Staiger.	-0.99	0.38
Fissurina dumastioides (Fink) Staiger.	-0.16	0.10
Graphina incrustans (Fée) Müll. Arg.	-0.05	-0.36
Graphis assimilis Nyl.	-0.27	-0.70
Graphis cincta (Pers.) Aptroot	-0.11	0.24
Graphis descissa Müll. Arg.	0.49	0.55
Graphis furcata Fée	0.00	0.20
Graphis jejuensis K. H. Moon, M. Nakan. & Kashiw.	-0.93	-0.69
Graphis koratensis Pitakpong, Kraichak, Lücking sp. nov.	-0.19	-0.53
Graphis pinicola Zahlbr.	0.65	-0.46
Graphis subserpentina Nyl.	-0.31	0.54
Graphis verminosa Müll. Arg.	-0.03	-0.17
Hemithecium aphaneomicrosporum Makhija & Adaw.	0.33	-0.26
Hemithecium laubertianum (Fée) Staiger	-0.30	0.28
Pallidogramme chlorocarpoides (Nyl.) Staiger, Kalb & Lücking	0.39	-0.33
Phaeographis crispata Kalb & Mathes-Leicht.	-0.22	0.40
Phaeographis phurueaensis Poengs. & Kalb.	0.82	0.24
Phaeographis subdividens (Leight.) Müll. Arg.	1.57	0.51
Platygramme jambosae Zahlbr.	-0.23	0.85
Platythecium serpentinellum (Nyl.) Stainger.	-1.10	-0.04
Sarcographa labyrinthica (Ach.) Müll. Arg.	0.11	-1.10

5) Candidate models predicting lichen occurrence

For *Carbacanthographis crassa*, *Carbacanthographis marcescen*s, *Dyplolabia afzelii*, *Fissurina rubiginosa*, *Graphina incrustans*, *Graphis cincta*, *Graphis descissa*, *Graphis furcate*, *Graphis jejuensis*, *Graphis koratensis*, *Graphis subserpentina*, *Graphis verminosa*, *Hemithecium aphaneomicrosporum*, *Hemithecium laubertianum*, *Pallidogramme chlorocarpoides*, *Phaeographis crispate*, and *Platygramme jambosae*, there was the most support from the model that combined lichen species with circumference of trunk, pH of bark, percent moisture, and bark crevice depth more than for any of the components alone (Table 6.6). Of the factors in the full model, the most support was given to bark crevice depth for *Carbacanthographis crassa*, *Fissurina rubiginosa*, *Graphina incrustans*, *Hemithecium aphaneomicrosporum*, and *Platygramme jambosae*. pH of bark was the best support of full model for *Carbacanthographis marcescen*s, *Graphis jejuensis*, *Graphis koratensis*, and *Graphis subserpentina*. The circumference of trunk supported of the model for *Dyplolabia afzelii*, and *Pallidogramme chlorocarpoides*. In addition, percentage moisture supported of factor models for *Graphis cincta*, *Graphis descissa*, *Graphis furcate*, *Graphis verminosa*, *Hemithecium aphaneomicrosporum*, and *Phaeographis crispate*. These four factors affected the occurrence of lichens on the trees by a positive relationship between species of lichens and each factor. In addition, this model alone received the most support for predicting *Diorygma reniforme*, *Fissurina dumastioides*, *Graphis pinicola*, *Phaeographis phurueaensis*, *Phaeographis subdividens*, and *Sarcographa labyrinthica*. This is likely a reflection of the high variability and low number of occurrences of these species. On the other hand, the model did not support the occurence of *Diorygma reniforme*, was the percentage moisture of bark. The *Fissurina dumastioides* and *Sarcographa labyrinthica* were not supported by the pH of bark as well as *Graphis pinicola* and *Phaeographis phurueaensis*. The species which was not based on any factor was *Platythecium serpentinellum* (Appendix B).

For predicting the occurrence of lichens, candidate models were developed by focusing on the relationship of factors that could affect the occurrence of lichens (Table 6.7). The full model showed the support for lichens including *Carbacanthographis crassa*, *Carbacanthographis marcescens*, *Dyplolabia afzelii*, *Fissurina rubiginosa*, *Graphina incrustans*, *Graphis cincta*, *Graphis descissa*, *Graphis furcate*, *Graphis jejuensis*, *Graphis koratensis*, *Graphis subserpentina*, *Graphis verminosa*, *Hemithecium aphaneomicrosporum*, *Hemithecium laubertianum*, *Pallidogramme chlorocarpoides*, *Phaeographis crispate*, and *Platygramme jambosae*. Some factors also influenced the occurrence of lichens. In addition to these factors, which could affect the occurrence of lichens, the bark characteristics were also other factors that affected the incidence of lichens. Direction had no effect the occurrence of lichens. Tree species with most other variables but most significantly with factor (APPENDIX C, D).

Table 6.6 Comparison of candidate model predicting the occurrence of lichen species on trees with environmental factors in SERS. Full model: lichen species diversity are influenced by all the measured variables (circumference, pH, percent moisture and bark crevice depth); Null: lichen species diversity are random with regard to the measured variables (circumference, pH, percent moisture and bark crevice depth); The best model has a value of 0. All models with value ≤ 2 have substantial support (Burnham and Anderson, 2002) shown in bold. AIC = Akaile's information criterion, N = 85 for all species.

Factors	CarCra	CarMar	DiRen	DyAfz	FisRub	FisDum	Grana	GraAss
Full model	**0.00**	**0.00**	2.50	**0.00**	**0.00**	2.03	**0.00**	4.97
Circumference (cm)	2.09	2.14	3.53	0.80	1.71	**0.00**	1.02	6.09
pH	2.14	1.35	4.44	1.64	1.41	1.60	1.02	**0.00**
Percent moisture	1.92	2.06	0.76	2.06	2.02	3.18	0.66	6.14
Bark crevice depth (cm)	0.48	1.81	**0.00**	2.08	0.87	4.16	0.91	6.82
Null model	10.18	4.76	6.89	2.48	8.19	7.15	9.44	10.19

Factors	GraCin	GraDes	GraFur	GraJej	GraKor	GraPin	GraSub	GraVer
Full model	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	2.81	**0.00**	**0.00**
Circumference (cm)	2.15	2.14	1.38	2.15	2.30	2.04	2.13	1.66
pH	2.14	2.14	1.39	1.77	0.71	4.03	0.85	0.88
Percent moisture	1.68	0.27	0.06	1.84	4.83	**0.00**	1.55	0.01
Bark crevice depth (cm)	2.15	1.30	0.87	1.96	4.42	0.77	2.15	0.80
Null model	10.49	10.39	5.42	11.57	2.83	1.89	10.13	7.96

Table 6.6 Comparison of candidate model predicting the occurrence of lichen species on trees with environmental factors in SERS. Full model: lichen species diversity are influenced by all the measured variables (circumference, pH, percent moisture and bark crevice depth); Null: lichen species diversity are random with regard to the measured variables (circumference, pH, percent moisture and bark crevice depth); The best model has a value of 0. All models with value ≤ 2 have substantial support (Burnham and Anderson, 2002) shown in bold. AIC = Akaile's information criterion, N = 85 for all species (Continuous).

Factors	HemAph	HemLau	PalChl	PhaCri	PhaPhu	PhaSub
Full model	**0**	**0**	**0**	**0**	1.99	3.67
Circumference (cm)	2.14	2.11	0.06	1.9	3.96	5.9
pH	2.08	1.78	1.76	1.97	3.85	6.62
Percent moisture	0.04	2.15	0.57	0.86	**0**	**0**
Bark crevice depth (cm)	0.72	0.8	1.23	2.14	0.46	5.17
Null model	6.16	5.81	2.97	8.19	10.12	4.77

Factors	PlaJam	PlaSer	SarLab
Full model	**0**	0.01	5.5
Circumference (cm)	1.17	0.15	1.51
pH	2.13	0.16	**0**
Percent moisture	1.8	1.07	4.53
Bark crevice depth (cm)	0.61	1.18	4.39
Null model	11.15	**0**	2.41

Table 6.7 Spearman's correlations between lichen species and predictor variables in SERS.

Species	Circumference	pH	Moisture	Bark crevice depth	Tree species	Bark characteristics	Direction
Carbacanthographis crassa	0.041	0.008	-0.052	0.143	-0.003	-0.109	**-0.54**
Carbacanthographis marcescens	0.006	-0.09	-0.029	-0.029	-0.06	0.156	0.026
Diorygma reniforme	0.022	-0.03	0.154	-0.231	0.122	0.158	0.078
Dyplolabia afzelii	-0.113	-0.08	0.105	-0.012	-0.075	-0.126	-0.078
Fissurina rubiginosa	0.194	0.167	-0.07	0.065	0.103	0.019	0.122
Fissurina dumastioides	-0.028	0.081	0.104	-0.116	0.032	-0.061	0.074
Graphina incrustans	0.022	-0.112	0.142	-0.128	0.024	-0.038	0.025
Graphis assimilis	-0.118	**-0.284**	0.043	0.012	-0.106	0.07	-0.116
Graphis cincta	0.059	0.003	-0.043	0.017	-0.035	-0.004	0.071
Graphis descissa	0.009	0.036	0.113	-0.085	0.076	-0.076	0.025
Graphis furcata	0.042	0.096	0.164	-0.134	0.105	0.105	0.029
Graphis jejuensis	-0.027	0.045	-0.06	0.065	0.031	-0.109	-0.054
Graphis koratensis	-0.105	-0.228	0.092	-0.121	-0.166	-0.115	-0.103
Graphis pinicola	-0.07	-0.034	**0.327**	**-0.311**	0.061	0.003	0.128
Graphis subserpentina	0.027	-0.108	0.04	0.006	0.023	0.122	-0.035

Table 6.7 Spearman's correlations between lichen species and predictor variables in SERS (Countinuous).

Species	Circumference	pH	Moisture	Bark crevice depth	Tree species	Bark characteristics	Direction
Graphis verminosa	-0.08	-0.188	0.115	-0.146	-0.052	0.007	-0.066
Hemithecium aphaneomicrosporum	0.019	0.048	0.188	-0.114	0.14	0.184	-0.048
Hemithecium laubertianum	0	-0.07	-0.006	-0.122	-0.002	0.038	0.025
Pallidogramme chlorocarpoides	-0.119	-0.068	0.189	-0.096	0.027	-0.085	0.028
Phaeographis crispata	-0.024	-0.041	0.109	-0.068	-0.01	-0.064	0.018
Phaeographis phurueaensis	0.113	-0.052	0.12	-0.19	0.141	-0.016	0.078
Phaeographis subdividens	-0.084	-0.038	**0.278**	-0.182	-0.063	0	0.021
Platygramme jambosae	-0.035	0.042	0.015	0.022	-0.018	0.047	-0.093
Platythecium serpentinellum	-0.076	-0.085	-0.107	0.116	-0.085	0.063	-0.13
Sarcographa labyrinthica	-0.16	-0.213	-0.001	0.027	-0.126	-0.052	-0.062

N = 85, degrees of freedom = 83, critical r for 0.05 level of significance = 0.250. All of the correlations in bold are significant.

Based on our findings the species richness of lichens in Sakaerat Environmental Research Station, depended on several factors such as, trees species, circumference, pH, percent moisture, bark crevice depth, bark surface characteristics, and directions to find lichens on tree. The results of this study found that the circumference, pH, and bark crevice depth of the bark showed significantly negative correlation with the diversity of lichens growing on trees. The percent moisture and bark surface characteristics were positively correlated at $P < 0.0001$, which was a very strong relationship. From the CCA analysis, the most influencing factors on species diversity of lichens were bark pH, followed by the percent of bark moisture while the circumference and bark crevice depth showed less influence on the diversity of lichens and in accordance with the relationship between trees species and the diversity of lichens. This correlation was consistent with the analysis by the CCA. Results found that the most important factors that affected the lichens were pH of the bark, circumference of the tree, and surface characteristics. The percent of moisture and bark crevice depth were less important factors for affecting the diversity of lichens. When lichen species were analyzed with the types of each tree, it indicated that each tree was unspecific to each species of lichens.

Candidate models were developed for predicting the influence of environmental factors on lichen occurrence; these focused on the roles of trees species, circumference, pH, percent moisture, bark crevice depth, bark surface characteristics, and directions to find lichens on tree. Full model showed the highest support with significant delta value for lichen occurrence as well as those of pH and percent moisture content of the bark. However, only the direction to finding lichen was non-significant support for lichen occurrence.

The results of this study were consistent with studies of Amo de Paz and Burgaz (2009), and Rosabal *et al*. (2013) who found pH and the diameter of the trunk were factors for prediction of lichen composition by a negative relationship. The study of Kantvilas and Jarman (2004) found that if the pH of lichens were more than 5 time, the density of lichens was found less. The studies of Cáceres *et al*. (2007); Wolsely and Aguirre Hudson (1997) indicated that the range of the pH of the bark in the tropical rainforest area was 4 to 5.5, and Worlseley and Pryor (1999) also showed that lichens were not found with low pH or acidic conditions. Mistry and Berardi (2005) found that the bark with high pH was not suitable for the growth of lichens. The findings of Hauck and Jürgens (2008), and Hauck *et al*. (2008) showed that lichens were uncorrelated with pH. Larsen *et al.* (2007) found that bark pH seemed to affect the types of lichens. Carlsson (2004) reported that the relationship between tree grith (diameter) of oak and the species diversity of lichens was significantly correlated. However, Jüriado *et al.* (2009) found the diameter was not correlated with increase of lichen species was consistent with our study. The study of Carlsson (2004) also found that bark crevice depth showed significant correlation with diameter of trees. The studies of Ellis and Coppins (2006), Johansson, (2008), and Jüriado *et al*. (2009) show negative relationship between the circumferences of the tree with a variety of lichens. This was consistent with our studies. In addition, it was found that the surface of the bark affected species richness of lichens because if the bark surface was smooth, lichens were found more than those growing on rough bark. A study by Cáceres *et al.* (2007) found that the relationship betwen bark characteristic with lichen diversity was negatively correlated. Berg *et al.* (2013) found that young tree with smooth bark and shallow bark crevice was a support for occurrence of lichens more than old tree. It was also found that percent of moisture of bark had no relation to the diversity of lichens and frequency of lichen, which was consistent with our study using CCA. Wolsely and Aguirre Hudson (1997) found that the tree with low moisture content of the bark was a less support for lichens than those with high moisture. This was consistent with the analysis by using our statistical models. The study of Spier *et al*. (2010), by creating a model of lichen species between pH and tree species found that the pH value had more significant value than tree species which was accordance with our finding. However, the study of Spier *et al*. (2010) reaffirmed that not only pH was a factor for predicition of the emergence of lichen but also there are several factors that affected the occurrence of lichens, especially pollution climate which is an important factor for the encounter with lichens.

6.5 Conclusions

In conclusion, The most species richness of lichen species was found on *Shorea obtusa* and *Canarium subulatum*, about 12 lichen species. Lichen species cooccurred less in *Hopea ferrea* and *Shorea sericeifolia* than *Shorea siamensis, Dipterocarpus obtusifolius*, *Lagerstroemia floribunda*, *Pterocymbium javanicum*, and *Hopea odorata*. The finding found 11 genera and 25 species of lichens on trees. On the basis of the study, it can be concluded that only pH is an important factor for lichen richness.

6.6 References

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CHAPTER VII

CONCLUSION AND RECOMMENDATION

7.1 Conclusion

In this study, lichens in the genus *Graphis* (Graphidaceae) found in Thailand were investigated. *Graphis*, a crustose lichen, was commonly found in the tropical zone, but a minority of the genus has been not clearly identified due to its smaller and more complex morphology of the lichens in comparison with others. The results of this study revealed species diversity, species distribution, and environmental factors affecting the diversity of the lichens in the genus *Graphis* found in Thailand, and environmental factors affecting species richness of lichens which are belonging to the family Graphidaceae in Sakaerat Environmental Research Station, Nakhon Ratchasima province, Thailand. Also, obtained data of these lichens provides informative basis for the study of classification and distribution of other lichen species existing in the country.

From this study, thirty two lichen species were identified in the genus *Graphis*, which were collected from 12 localities. Morphological, anatomical, chemical, and molecular techniques were used for lichen identification. The study of morphology was determined using a low magnification stereomicroscope and then common characteristics of external structures such as color, surface, shape, size, and the color and dust of the fruiting body, were recorded. The anatomical study was conducted to determining the cross section parts of thallus and apotheciam under the light microscope and stereomicroscope, which were included distribution of algae, fungi hyphae, the thickness of cortex, apotheciam (exciple color, size, spore arrangement, ascus, spore type, and spore color). Each lichen species is able to produce unique chemicals which indicate the type of lichens. To study the chemical, spot test and Thin layer chromartography (TLC) were carried out. Moreover, molecular technique was applied to confirm the lichen species. The technique should be modified depending on lichen species. The phylogenetic and evolutionary studies are very useful for identifying unknown taxa and confirming the determination of new taxa, since some species were only found as vegetative parts in the field collection. This study found one new species and six new records of the crustose lichens in the genus *Graphis* discovered in Thailand.

Graphis had higher distribution probabilities in the mid altitude than in the high and low regions of Thailand. The differences in environmental conditions may affect their growth, so if their habitats are lost or changed, they may die out. These taxa have a risk to disappear easily. These findings are essential for the effective conservation of lichens in Thailand, not only with respect to estimating the species distribution ranges of *Graphis*, but also for identifying the environmental factors limiting lichen distribution. At present, the climate change may be cause of the disappear from the forest.

Lichens in the family Graphidaceae in Sakaerat Environmental Research Station, Nakhon Ratchasima was determined the species richness in the relationship circumference of tree, pH of bark, bark crevice depth, and percent moisture on tree. The most species richness of lichen species on *Shorea obtusa* and *Canarium* *subulatum*, about 12 lichen species. Lichen species occurred less in *Hopea ferrea* and *Shorea sericeifolia* than *Shorea siamensis*, *Dipterocarpus obtusifolius*, *Lagerstroemia floribunda*, *Pterocymbium javanicum*, and *Hopea odorata*. The finding found 11 genera and 25 species of lichens on trees. On the basis of the study, it can be concluded that only pH is important for lichen richness.

7.2 Recommendation

From this study, there were many lichens found but the minority of identified lichen species in the genus *Graphis* was obtained. Therefore, the investigation of the morphology of lichen species in the genus *Graphis* is needed in laboratory scale before actually conducted in the fields area. However, due to the high complexity of the lichen species in the study, field errors commonly happen. Therefore, laboratory studies to identify the species of lichens should use every aspects including the morphological, anatomical, chemical studies coupled with the molecular technique in order to confirm the results and correct identification of the obvious examples.

APPENDICES

APPENDIX A

DESCRIPTION OF THE FAMILY GRAPHIDACEAE IN SAKAERAT ENVIRONMENTAL RESEARCH STATION

***Carbacanthographis crassa* (Müll. Arg.) Staiger & Kalb.**

Synonymy: *Graphina crassa* Müll. Arg. (1895)

Phaeographina crassa (Müll. Arg.) Redinger (1934)

Description: Thallus corticolous, cream, white, surface smooth and slightly shiny; cortex grey, Ascomata lirelliform, numerous, conspicuous, dispersed, sparsely branched, straight, slightly curved, sessile, 2.0-10.0 mm long and 0.7-1.0 mm wide; lips white; disc concealed. Exciple uncarbonized, yellowish brown, lacking thalline margin; labia striate, well developed, convergent. Hypothecium indistinct. Hymenium strongly inspersed, 100-135 µm high, I -. Epihymenium black, paraphyses simple to sparsely branched. Ascospores hyaline, muriform, I + violet-red.

Chemistry: Stictic acid

Figure 1 *Carbacanthographis crassa* (Müll. Arg.) Staiger & Kalb.

***Carbacanthographis marcescens* (Fée) Staiger & Kalb.**

Synonymy: *Graphis marcescens* Fée, *Essai Crypt. Exot.* (Paris), 1, 38 (1825).

Graphina marcescens (Fée) Müll. Arg., *J. Mem. Soc. Phys. Hist Nat.Geneve*, *29*(8), 42 (1887).

Description: Thallus corticolous, yellowish green; surface smooth and matt; cortex yellowish green, loose. Ascomata lirelliform, unbranched to sparsely branched, straight or curved, prominent to sessile, 1.0-5.0 mm long and 0.2-0.4 mm wide, covered by a white pruina; lips black; disc concealed. Exciple laterally carbonized, laterally covered by a thalline margin with small crystals; labia entire, convergent. Hymenium clear, 62-82 µm high, I -. Epihymenium indistinct; paraphyses simple; periphysoids short and unbranched. Asci clavate, 58-81 × 13-18 µm, with 4-8 ascospores. Ascospores hyaline, muriform, I + pale violet.

Chemistry: Salazinic acid, protocetaric acid, and norstictic acid.

Figure 2 *Carbacanthographis marcescens* (Fée) Staiger & Kalb.

***Diorygma reniforme* (Fée) Kalb, Staiger & Elix.**

Description: Thallus corticolous, yellowish green; surface smooth and matt; cortex yellowish green, loose. Ascomata lirelliform, unbranched to sparsely branched, straight or curved, Ascocarps variously shaped, with exposed disc, immersed, surrounded by swollen thallus border, with more or less constricted base; hymenium 150-200 μm, clear, I+ weakly blue; Ascospores 1/ascus, muriform, 110-230 × 35-80 18 μm

Chemistry: Norstictic, salazinic, protocetraric acids.

Figure 3 *Diorygma reniforme* (Fée) Kalb, Staiger & Elix.

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

Synonymy: *Graphis afzelii* Ach., *Cat. Lich. Univers. 2*: 291 (1814)

Description: Thallus corticolous, pale yellow to grayish green; surface smooth and dull; cortex yellow to grayish green.Ascomata lirelliform, numerous, conspicuous, dispersed, unbranched and straight, sessile, 1.0-4.0 mm long and 0.5-0.8 mm wide, lips whit, covered by a thick white pruina. Exciple thick laterally carbonized, lacking a thalline margin; labia entire, convergent. Hymenium clear, 103-142 µm high, I -. Epihymenium whitish gray, 4-7 µm high; paraphyses simple. Asci ellipsoid, 86-123 × 19-27 µm with 8 ascospores. Ascospores hyaline, transversely septate, 3 septate, 15-20.5 × 6-8.5 µm, I + violet.

Chemistry: Lecanoric acid.

Figure 4 *Dyplolabia afzelii* (Ach.) A. Massal.

Fissurina rubiginosa **(Fée) Staiger, Biblioth.**

Description: Thallus corticolous, *smooth, green, olive green to pale green, lirelliform, inconspicuous, immersed, indicated by a thin line, sometimes slightly open, slightly raised and paler than* the thallus, straight, curved or sinuous, disc slit-like, narrow, scarcely open in mature apothecia. Epihymenium hyaline, indistinct, with greyish or brownish granules, 10-15 µm high. Hymenium hyaline, clear, Paraphyses straight to bent, unbranched, with slightly thickened tips, moderately to distinctly conglutinated, 1-2 µm thick. Ascospores hyaline, muriform, thick walled, 18-25 × 5-9 µm, with 6-8 × 2-3 locules, I –.

Chemistry: No lichen compound detected.

Figure 5 *Fissurina rubiginosa* (Fée) Staiger, Biblioth.

***Fissurina dumastioides* (Fink) Staiger.**

Description: Thallus corticolous, grey-green, stramineous, rough, rugose, thick, cracked. long, simple to branched, visible as fissures in the thallus, immersed, flexuose, ends obtuse to acute. Disc very narrow, sunken. Exciple non-striate, non-carbonized, present at the base, converging at the apical portion, covered by the thalline margin up to the top. Hymenium hyaline. Hypothecium indistinct. Paraphyses simple. Ascospores oval, hyaline, transversely septate, 3 septate, 10-12 × 3-6 µm, I + violet.

Chemistry: Stictic acid.

Figure 6 *Fissurina dumastioides* (Fink) Staiger.

Graphina incrustans **(Fée) Müll. Arg.**

Synonymy: *Graphis incrustans* (Fée) Nyl., *Mem. Soc. Sci. Nat Cherourg 5*: 130 (1857).

Description: Thallus corticolous, pale greyish green; surface smooth and very shiny. Ascomata fissurine, numerous, inconspicuous, dispersed, sparsely branched, straight, erumpent. Exciple uncarbonized, laterally with a thalline margin; labia entire, convergent. Hypothecium hyaline, 10-16 µm high. Hymenium clear, 74-95 µm high, I -. Epihymenium hyaline; paraphyses simple to sparsely branched and slightly warty at epithecial region, periphysoids short and branched. Ascospores hyaline, 3 transverse septa, 14-25 × 8-13 µm, I -.

Chemistry: No lichen compounds detected.

Figure 7 *Graphina incrustans* (Fée) Müll. Arg.

***Graphis assimilis* Nyl.**

Description: Thallus corticolous, cream, white, surface smooth. Apothecia lirelliform, black, lips closed or becoming slightly open, semi-immersed to sessile, thin, sinuous, simple or branched, 0.5-4 mm long and 0.1-0.5 mm wide. Exciple completely or almost completely carbonized. Hymenium clear, 95-120 µm high, paraphyses simple. Ascospores 8 per ascus, hyaline, transversely septate, 8-11 septate, 28.5-45 × 8-10 µm, I+ blue.

Chemistry: Norstictic acid.

Figure 8 *Graphis assimilis* Nyl.

Graphis cincta **(Pers.) Aptroot.**

Description: Thallus corticolous, white, white to pale fawn, surface smooth. Apothecia lirelliform, black, lips closed, scattered, simple, straight, curved or sinuous, sessile, 0.5-2.0 mm long and 0.1-0.3 mm wide. Exciple laterally carbonised, conspicuously open at the base. Hymenium clear, 110-140 μm high, paraphyses simple. Ascospores 8 per ascus, hyaline, transversely septate, 5-8 septate, 26.0-40.0 × 7-10 μm, I + blue.

Chemistry: Norstictic acid.

Figure 9 *Graphis cincta* (Pers.) Aptroot.

***Graphis descissa* Müll. Arg.**

Description: Thallus corticolous, white, whitish grey; surface smooth. Apothecia lirelliform, black, lips closed, labia non-pruinose; Lirellae elongate and irregularly branched, 1.0-3.0 mm long and 0.1-0.3 mm wide. Exciple laterally carbonized, with a lateral thalline margin; labia striate, convergent. Hymenium clear, 105-120 μm high, paraphyses simple. Ascospores 8 per ascus, hyaline, transversely septate, 5-8 septate, 25-40 × 7.5-10 μm, I+ blue.

Chemistry: Stictic acid.

Figure 10 *Graphis descissa* Müll. Arg.

***Graphis furcata* Fée.**

Description: Thallus corticolous, greyish white to pale fawn, surface smooth, slightly glossy. Apothecia lirelliform, black, lips closed, sessile, simple, 0.5-3.0 mm long and 0.1-0.3 mm wide, with an inconspicuous basal thalline margin. Exciple laterally carbonised, conspicuously open at the base. Hymenium clear, 60-95 μm high, paraphyses simple. Ascospores 8 per ascus, hyaline, transversely septate, 8-10 septate, 20.0-32.0 × 6-8 μm, I+ blue.

Chemistry: No substances detected.

Figure 11 *Graphis furcata* Fée.

***Graphis jejuensis* K. H. Moon, M. Nakan. & Kashiw.**

Description: Thallus corticolous, greyish white to pale fawn, surface smooth, slightly glossy. Apothecia lirelliform, black, lips closed, sessile, simple, 0.5-3.0 mm long and 0.1-0.3 mm wide. Exciple laterally carbonised, conspicuously open at the base. Hymenium clear, 70-100 µm high, paraphyses simple. Ascospores 8 per ascus, hyaline, transversely septate, 7-9 septate, 18.5-25.0 × 5-8 µm, I+ blue.

Chemistry: No substances detected.

Figure 12 *Graphis jejuensis* K. H. Moon, M. Nakan. & Kashiw.

Graphis koratensis **Pitakpong, Kraichak & Lücking sp. nov.**

Description: Thallus corticolous, 3–6 cm diam., 150–250 µm, with clusters of calcium oxalate crystals. Proper exciple completely carbonized, 100–140 µm wide, hymenium clear, 100–150 µm high, asci, 20–25 µm long, 110–140 µm wide. Ascospores 8 per ascus, oblong to narrowly fusiform, transversely, 11–19 septate, 70–115 µm long, 9–15 µm wide, colourless.

Chemistry: Norstictic acid.

Figure 13 *Graphis koratensis* Pitakpong, Kraichak, Lücking.

***Graphis pinicola* Zahlbr.**

Description: Thallus corticolous, white, whitish grey, surface smooth. Apothecia lirelliform, black, lips closed, erumpent to prominent, numerous, conspicuous, dispersed, sparsely branched, straight or curved, 1.0-4.0 mm long and 0.1-0.3 mm wide. Exciple completely carbonized, with a thick lateral thalline margin; labia entire, convergent. Hymenium clear, 90-120 µm high, paraphyses simple. Ascospores 8 per ascus, hyaline, transversely septate, 8-10 septate, 25-40 × 6.5-8 µm, I+ blue.

Chemistry: No substances detected

Figure 14 *Graphis pinicola* Zahlbr.

***Graphis subserpentina* Nyl.**

Description: Thallus corticolous, white; surface smooth and dull. Apothecia lirelliform, black, lips closed, epumpent to prominent, conspicuous, numerous, dispersed. Exciple laterally carbonized, sometimes the base almost closed, covered by a thin complete thalline margin with scattered small crystals; labia entire, convergent. Hymenium clear, 140-185 µm high, paraphyses simple and thickened at tips. Ascospores 1-2 per ascus hyaline, muiform, 85-120 × 28.5-35 µm, I+ blue.

Chemistry: Norstictic acid.

Figure 15 *Graphis subserpentina* Nyl.

Graphis verminosa **Müll. Arg.**

Description: Thallus corticolous, white, cream, pale grey; surface smooth or sometimes subtuberculate and dull. Apothecia lirelliform, black, lips closed, erumpent to prominent, conspicuous, numerous, dispersed, simple to rarely branched, straight or curved, 1.0-3.0 mm long and 0.2-0.5 mm wide, epruina; disc concealed. Exciple laterally carbonized, with a lateral thalline margin; labia striate, convergent. Hymenium clear, 105-140 µm high, paraphyses simple to sparsely branched at the epithecial region. Ascospores 8 per ascus, hyaline, transversely septate, 18-30 septate, 55-85× 6.5-8 µm, I+ blue.

Chemistry: Norstictic acid.

Figure 16 *Graphis verminosa* Müll. Arg.

Hemithecium aphaneomicrosporum **Makhija & Adaw.**

Description: Thallus corticolous, greenish grey; surface smooth and dull; cortex grey. Ascomata lirelliform, numerous, inconspicuous, dispersed, sparsely branched, straight or slightly curved to curved, prominent, epruina; lips bright yellow; disc concealed. Exciple uncarbonized and brown to dark brown, complete below hymenium, slightly brown striate at lateral labia. Paraphyses simple. Ascospores hyaline, transversely septate, 8-13 septae, 32-42 × 6-9.6 µm, I + blue.

Chemistry: Stictic acid.

Figure 17 *Hemithecium aphaneomicrosporum* Makhija & Adaw.

***Hemithecium laubertianum* (Fée) Staiger.**

Synonymy: *Graphis laubertiana* Fée. Essai Crypt. Écorc. 41 (1825).

Graphis argopholis C.Knight ex Müll.Arg., Flora 70: 401 (1887)

Hemithecium argophole (C.Knight ex Müll.Arg.) A.W.Archer, Telopea 11: 74(2005).

Description: Thallus off-white, thick, smooth, dull. Ascomata white, inconspicuous, immersed, numerous, crowded, curved or sinuous, often branched, visible as a slit with pale marginal lines on the surface of the thallus, 1–2 mm long, 0.2–0.3 mm wide. Proper exciple pale yellow-brown, complete. Hymenium 125–150 µm thick, I–. Ascospores 8 per ascus, transversely septate, 6–10-locular, 28–32 × 7–8 µm, I+ blue.

Chemistry: No lichen compounds detected.

Figure 18 *Hemithecium laubertianum* (Fée) Staiger.

***Pallidogramme chlorocarpoides* (Nyl.) Staiger, Kalb & Lücking**.

Synonymy: *Graphis chlorocarpoides* Nyl., *Flora* 49: 133 (1866).

Phaeographina chlorocarpoides (Nyl.) Zahlbr., *Cat. Lich. Univers. 2*:435 (1923).

Description: Thallus corticolous, grey to yellowish green; surface smooth and slightly shiny. Ascomata lirelliform, numerous, conspicuous, dispersed, sparsely branched, straight, slightly curved, sessile. Exciple uncarbonized, yellowish brown, lacking thalline margin; labia striate, well developed, convergent. Hymenium strongly inspersed, 137-185 µm high, paraphyses simple to sparsely branched. Ascospores pale brown, muriform, 31-40 transverse septate, 93-140 × 17- 28 µm, I + violet-red.

Chemistry: Stictic acid and constictic acid.

Figure 19 *Pallidogramme chlorocarpoides* (Nyl.) Staiger, Kalb & Lücking.

***Phaeographis crispate* Kalb & Mathes-Leicht.**

Description: Thallus corticolous, off-white to white; surface smooth and dull. Ascomata lirelliform, numerous, inconspicuous, clustered. Exciple uncarbonized to apically weakly darkened, lacking a thalline margin; labia entire, divergent. Hypothecium hyaline, paraphyses simple to sparsely branched at the tips. Ascospores dark brown, transversely septates, 5-7 septate, 15-28 × 7.5-10 µm, I + reddish-brown.

Chemistry: Stictic acid, norstictic acid.

Figure 20 *Phaeographis crispate* Kalb & Mathes-Leicht.

***Phaeographis phurueaensis* Poengs. & Kalb.**

Description: Thallus corticolous, greyish to green; smooth and dull; cortex grey. Ascomata lirelliform, immersed, numerous, inconspicuous, clustered, irregularly to radiately branched, straight or curved, 1.5-3.0 mm long and 0.1-0.2 mm wide, white pruinose, lips white; disc white, slightly open. Exciple uncarbonized, laterally with a thalline margin; labia entire. Hymenium clear, paraphyses simple, branched at the tips. Ascospores brown, transversely septates, 3-5 septate, 15-25 × 7.5-9 µm, I + reddish-brown.

Chemistry: Stictic acid.

Figure 21 *Phaeographis phurueaensis* Poengs. & Kalb.

***Phaeographis subdividens* (Leight.) Müll. Arg.**

Synonymy: *Graphis subdividens* Leight., *Trans. Linn. Soc. London 27*: 177 (1869). (Lit.: Archer, 2001, 2006).

Description: Thallus corticolous, creamy, pale greyish green; smooth to subtuberculate and matt; cortex grayish, weakly organized, with clusters of large crystals. Ascomata lirelliform, numerous, conspicuous, dispersed, irregularly branched, straight or slightly curved, prominent, terminally acute, 2.0-5.0 mm long and 0.2-0.3 mm wide, with a white pruina; lips whitish; disc concealed to slightly open. Exciple dark brown to pale carbonized below, lacking a thalline margin; labia entire to slightly striate, convergent. Hypothecium pale brown, 7-13 μm high. Hymenium inspersed with numerous oil globules, paraphyses simple. Ascospores brown, transversely septate, 5 septate, elongate, thick-walled, 19-32 × 7.9-8.5 μm, I + reddish brown.

Chemistry: No lichen compounds detected.

Figure 22 *Phaeographis subdividens* (Leight.) Müll. Arg.

***Platygramme jambosae* Zahlbr.**

Synonymy: *Phaeographina jambosae* Zahlbr., *Ann. Cryptog. Exot.*, 1: 143 (1928). (Lit.: Redinger, 1936).

Description: Thallus corticolous, pale olive green, yellowish green; subtuberculate and dull, cortex pale olive. Ascomata lirelliform, numerous, conspicuous, dispersed, sparsely branched, straight or curved, erumpent, 2.0-8.0 mm long and 0.2-0.4 mm wide, epruina; lips black; disc black, open. Exciple laterally carbonized, with a lateral thalline margin; labia entire, divergent. Hypothecium hyaline, paraphyses simple. Ascospores pale brown, muriform, 65-83 × 15.5-19.4 µm, I + reddish brown.

Chemistry: No lichen compounds detected.

Figure 23 *Platygramme jambosae* Zahlbr.

***Platythecium serpentinellum* (Nyl.) Stainger.**

Synonymy: *Graphis serpentinella* Nyl., Acta Soc. Sci. Fenn. 7: 469 (1863).

Phaeographis serpentinella (Nyl.) Zahlbr., Cat. Lich. Univers. 2: 386 (1923).

Description: Thallus surface smooth and dull; cortex grey. Ascomata lirelliform, sparse, inconspicuous, dispersed, irregularly branched, slightly curved to radiate, immersed, 2.0-4.0 mm long and 0.2-0.3 mm wide, epruina; lips indistinct; disc grey-black, open. Exciple uncarbonized to apically weakly carbonized, dark grey below, with a lateral to thin complete thalline margin with abundant crystals at lateral labia; labia entire. Hymenium clear, 75-117 µm high, paraphyses simple. Ascospores pale brown, muriform, 12-15.8 × 5.7-8 µm, I + reddish violet.

Chemistry: No lichen compounds detected.

Figure 24 *Platythecium serpentinellum* (Nyl.) Stainger.

***Sarcographa labyrinthica* (Ach.) Müll. Arg.**

Synonymy: *Glyphis labyrinthica* Ach., *Syn. Lich*: 107 (1814).

Graphis labyrinthica (Ach.) Vain. *Acad. Sci. Fenn. 15*: 230 (1921). (Lit.: Brodo, Sharnoff, & Sharnoff, 2001).

Description: Thallus corticolous, pale olive green, greyish green; smooth or subtuberculate and shiny; cortex grey. Ascomata lirelliform, very numerous, conspicuous, always clustered in a white stroma, straight or curved, thin white pruina; irregularly branched, lips grey; disc slightly open, grey, stroma round, oval, more or less elongate. Exciple completely carbonized, lacking a thalline margin; labia entire, divergent. Hypothecium hyaline to pale brown. Hymenium inspersed, paraphyses simple, sparsely branched at the tips. Ascospores brown or dark brown, transversely septate, 3 septate, 13.2-19.5 × 5.5-8.3 μm, I + reddish brown.

Chemistry: Stictic acid and cryptostictic acid

Figure 25 *Sarcographa labyrinthica* (Ach.) Müll. Arg.

APPENDIX B

THE PROBABILITY OF LICHEN SPECIES OCCURRENCE IN RELATION TO ENVIRONMENTAL FACTORS

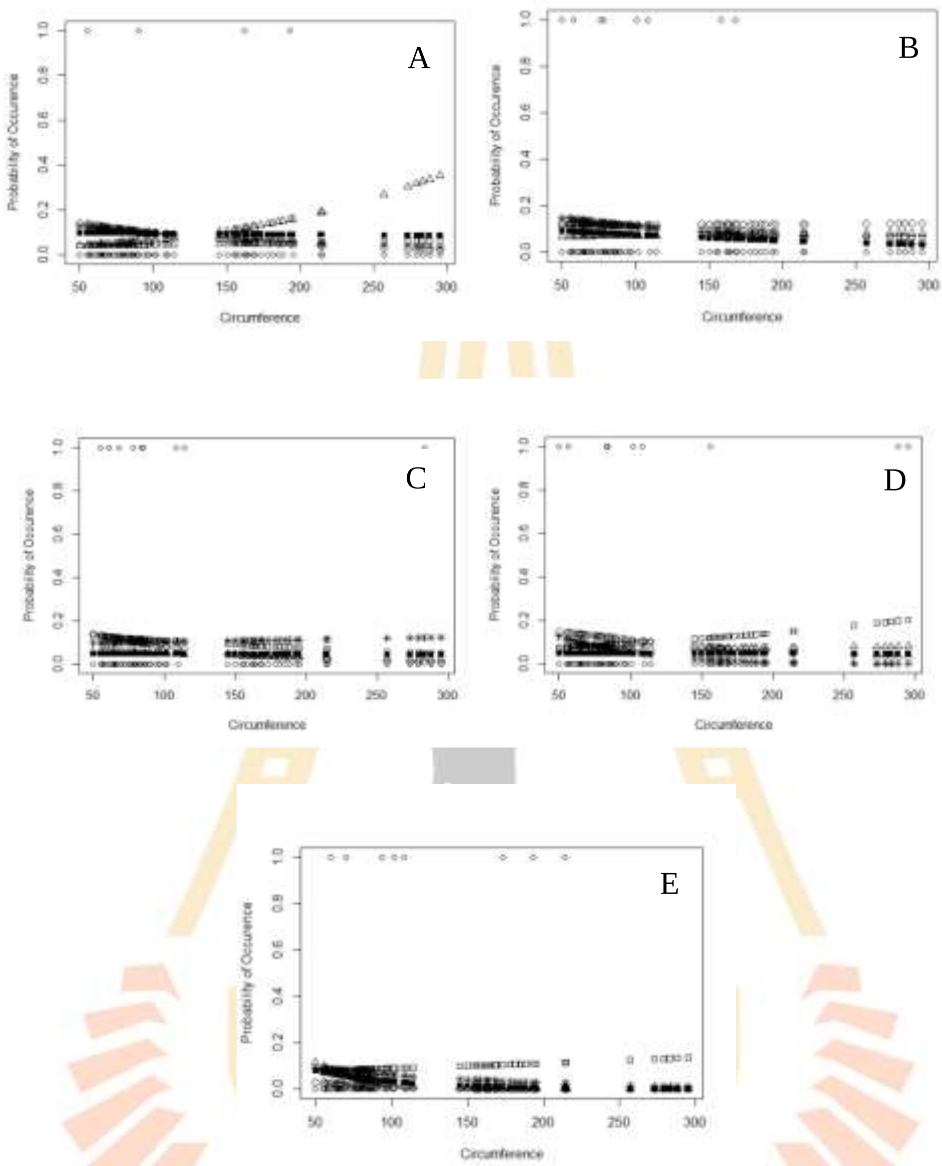

Figure 1 The probability of lichen species occurrence in relation to circumference in the full model. Curves wee fitted using binomial regression. (A): (○) CarCra; (✳) CarMar; (□) DiRen; (■) DyAfz; (△) FisRub, (B): (○) FisDum; (✳) Graphina; (□) GraphisAss; (■) GraCin; (△) GraDes, (C): (○)GraFur; (✳) GraJej; (□) GraKor; (■) GraPin; (△) GraSub, (D): (○) GraVer; (✳) HemAph; (□) HemLau; (■) PalChl; (△) PhaCri, and (E): (○) PhaPhu; (✳) PhaSub; (□) PlaJam; (■) PlaSer; (△) SarLab.

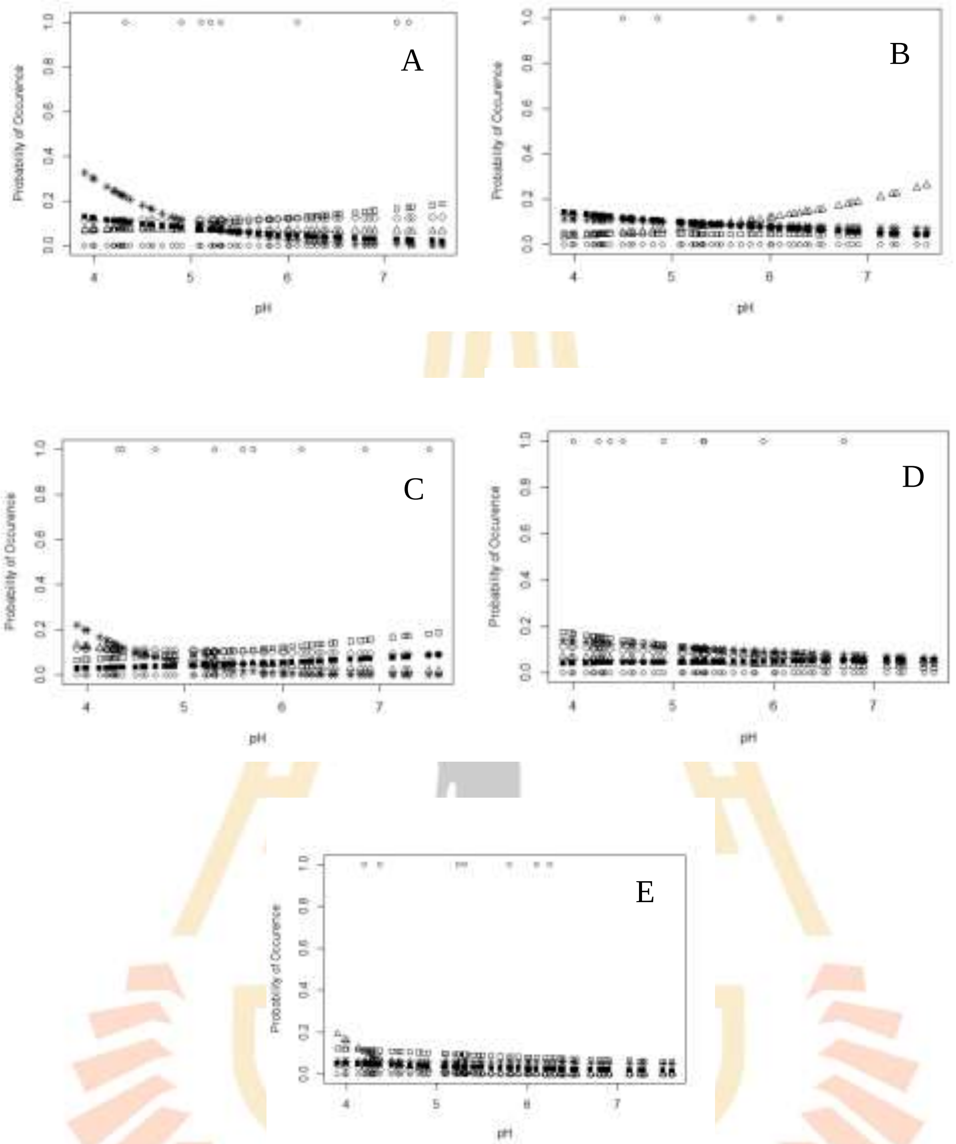

Figure 2 The probability of lichen species occurrence in relation to pH in the full model. Curves wee fitted using binomial regression. (A): (○) CarCra; (✳) CarMar; (□) DiRen; (■) DyAfz; (Δ) FisRub, (B): (○) FisDum; (✳) Graphina; (□) GraphisAss; (■) GraCin; (Δ) GraDes, (C): (○)GraFur; (✳) GraJej; (□) GraKor; (■) GraPin; (Δ) GraSub, (D): (○) GraVer; (✳) HemAph; (□) HemLau; (■) PalChl; (Δ) PhaCri, and (E): (○) PhaPhu; (✳) PhaSub; (□) PlaJam; (■) PlaSer; (Δ) SarLab.

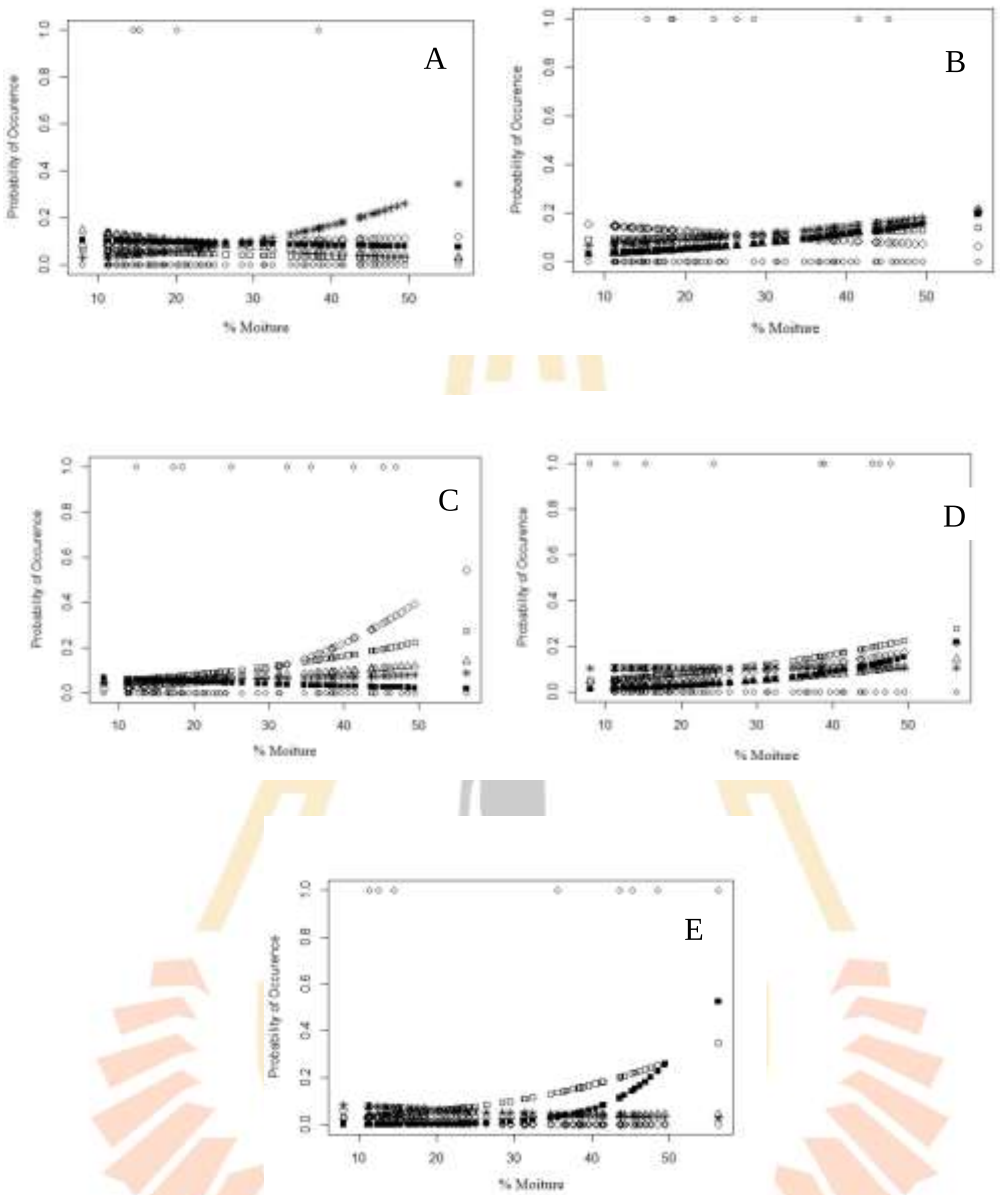

Figure 3 The probability of lichen species occurrence in relation to % moisture in the full model. Curves wee fitted using binomial regression. (A): (○) CarCra; (✳) CarMar; (□) DiRen; (■) DyAfz; (△) FisRub, (B): (○) FisDum; (✳) Graphina; (□) GraphisAss; (■) GraCin; (△) GraDes, (C): (○) GraFur; (✳) GraJej; (□)GraKor; (■) GraPin; (△) GraSub, (D): (○) GraVer; (✳) HemAph; (□) HemLau; (■) PalChl; (△) PhaCri, and (E): (○) PhaPhu; (✳) PhaSub; (□) PlaJam; (■) PlaSer; (△) SarLab.

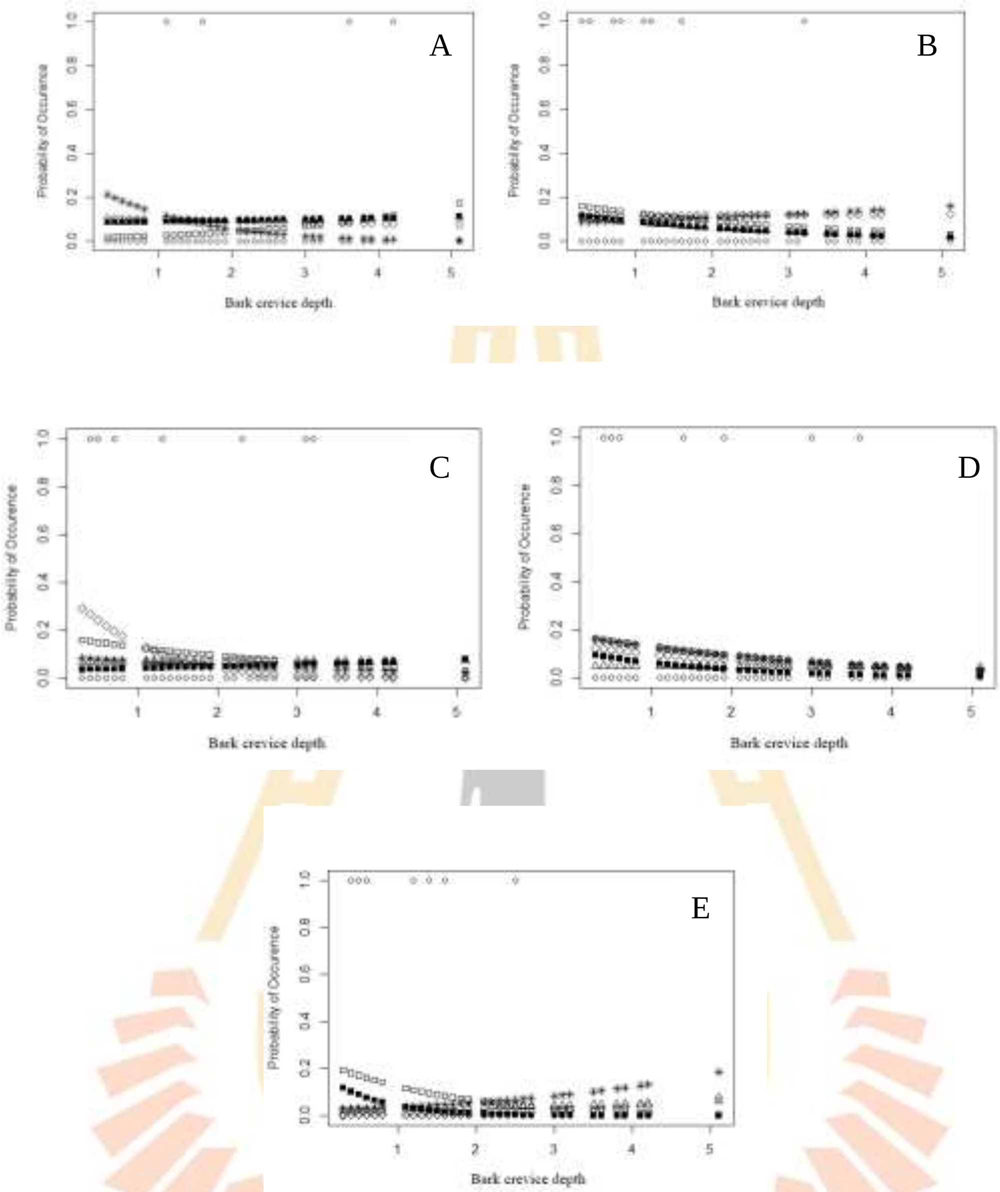

Figure 4 The probability of lichen species occurrence in relation to bark crevice depth in the full model. Curves wee fitted using binomial regression. (A): (○) CarCra; (✳) CarMar; (□) DiRen; (■) DyAfz; (△) FisRub, (B): (○) FisDum; (✳) Graphina; (□) GraphisAss; (■) GraCin; (△) GraDes, (C): (○)GraFur; (✳) GraJej; (□) GraKor; (■) GraPin; (△) GraSub, (D): (○) GraVer; (✳) HemAph; (□) HemLau; (■) PalChl; (△) PhaCri, and (E): (○) PhaPhu; (✳) PhaSub; (□) PlaJam; (■) PlaSer; (△) SarLab.

APPENDIX C

ACTUAL OCCURRENCE AND PROBABILITY CURVES OF LICHEN SPECIES WITH ENVIRONMENTAL FACTORS

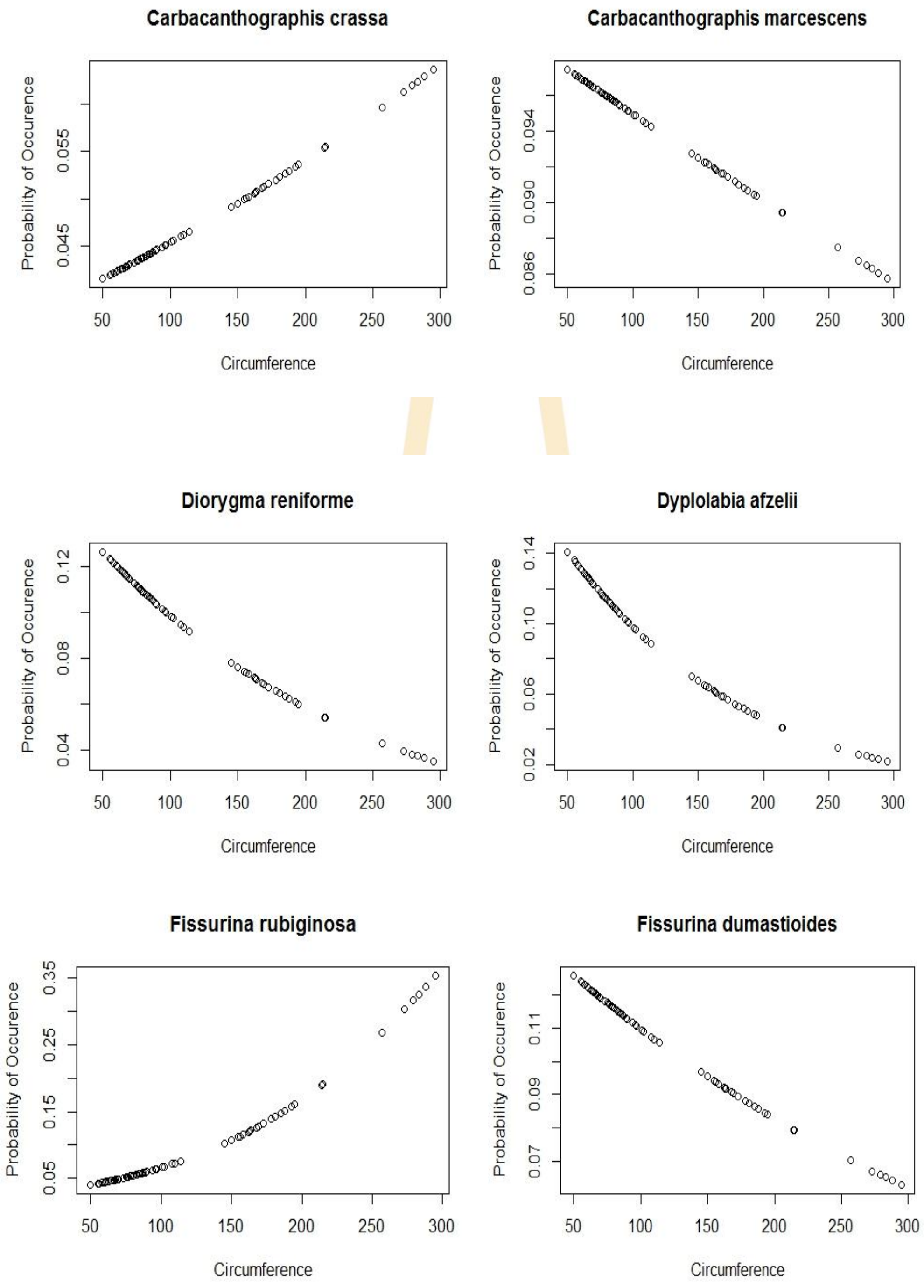

Figure 1 Actual occurrence (marked with dots) and probability curves of lichen species by different values of circumference on tree.

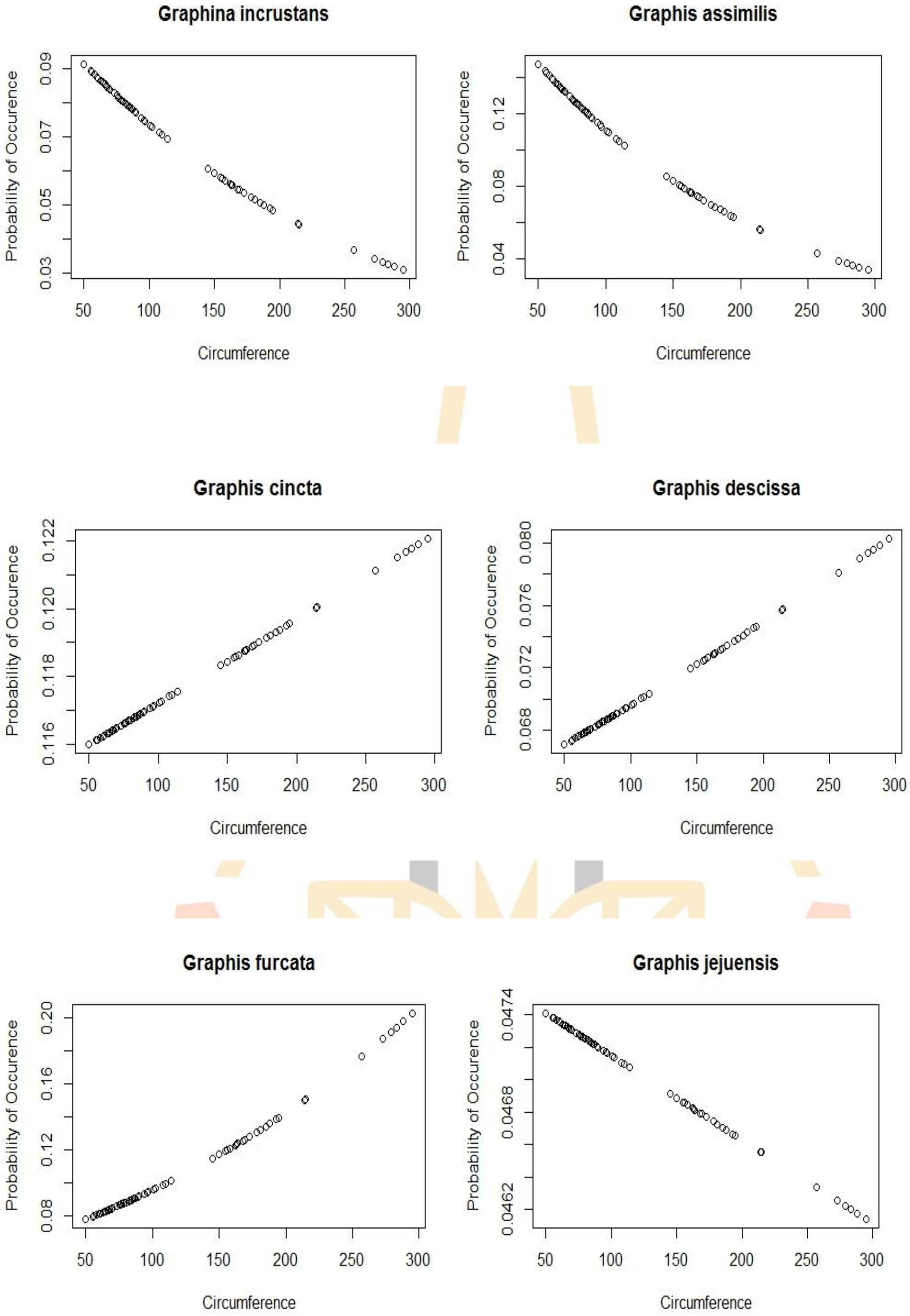

Figure 1 Actual occurrence (marked with dots) and probability curves of lichen species by different values of circumference on tree (Continuous).

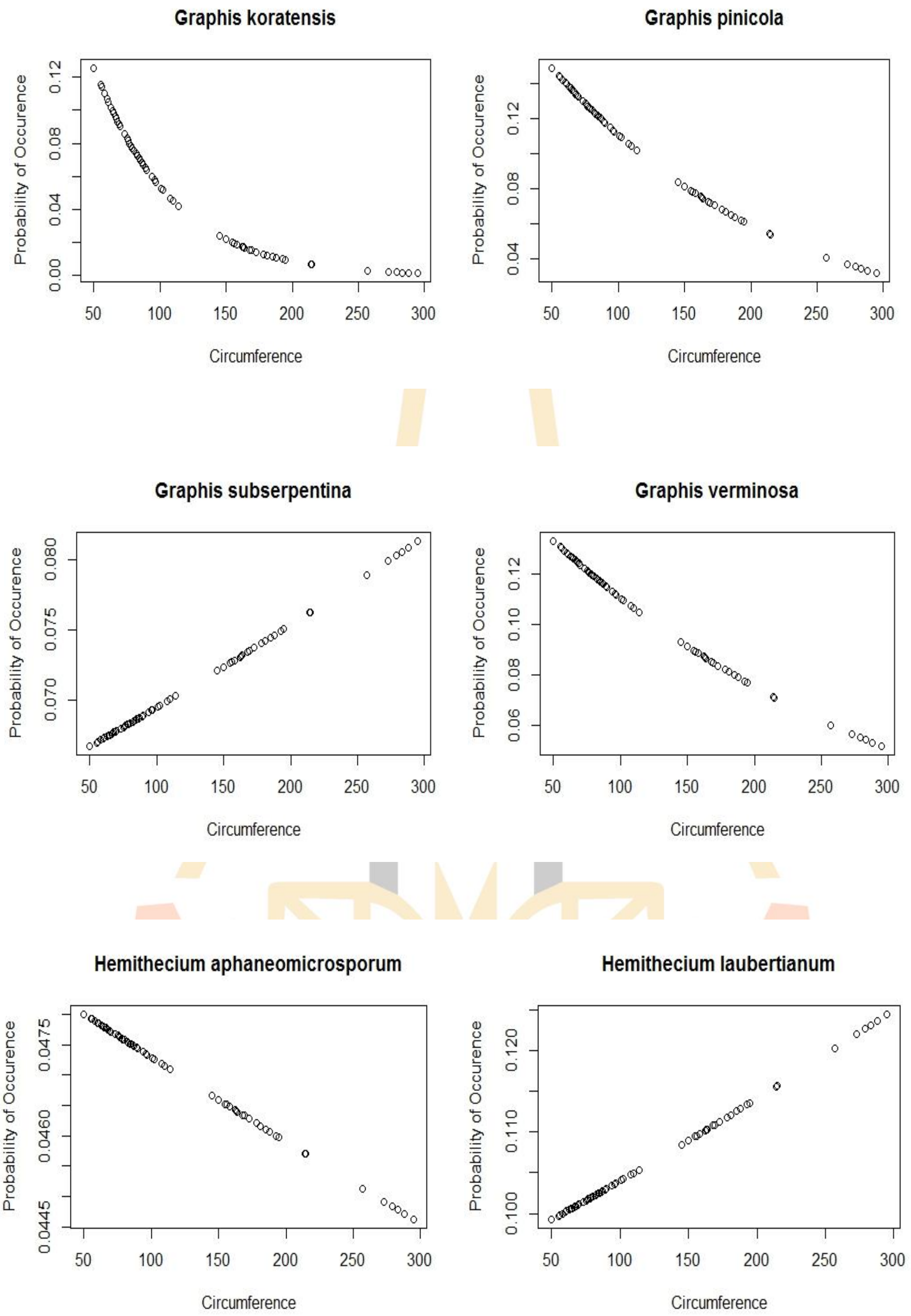

Figure 1 Actual occurrence (marked with dots) and probability curves of lichen species by different values of circumference on tree (Continuous).

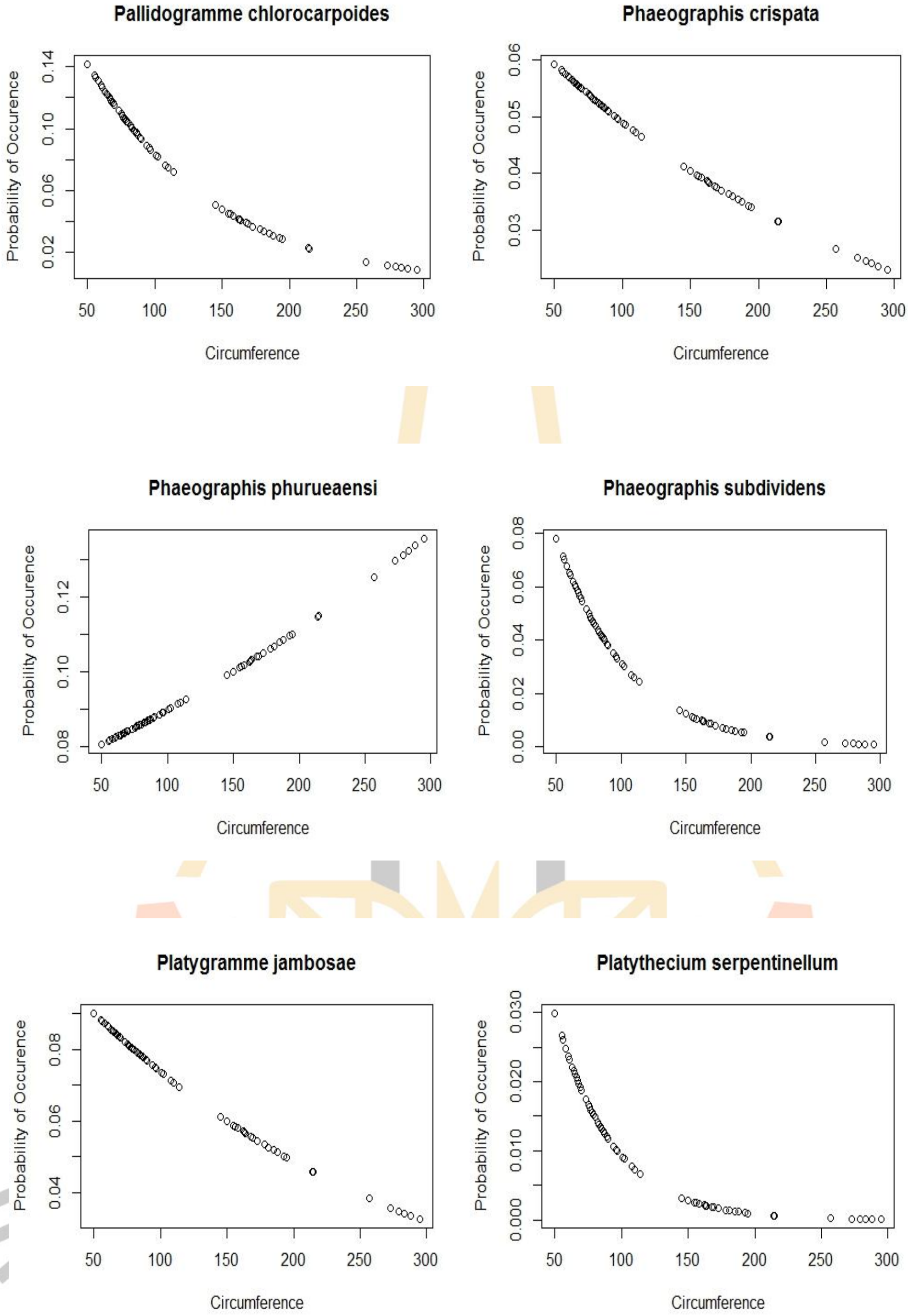

Figure 1 Actual occurrence (marked with dots) and probability curves of lichen species by different values of circumference on tree (Continuous).

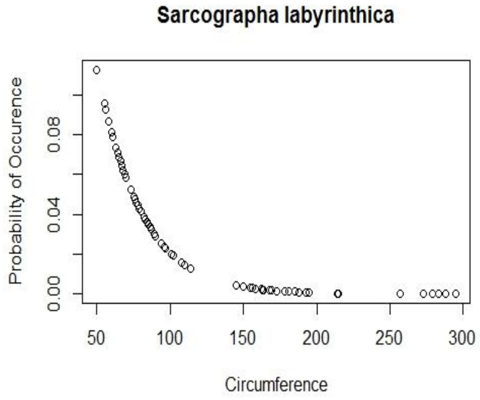

Figure 1 Actual occurrence (marked with dots) and probability curves of lichen species by different values of circumference on tree (Continuous).

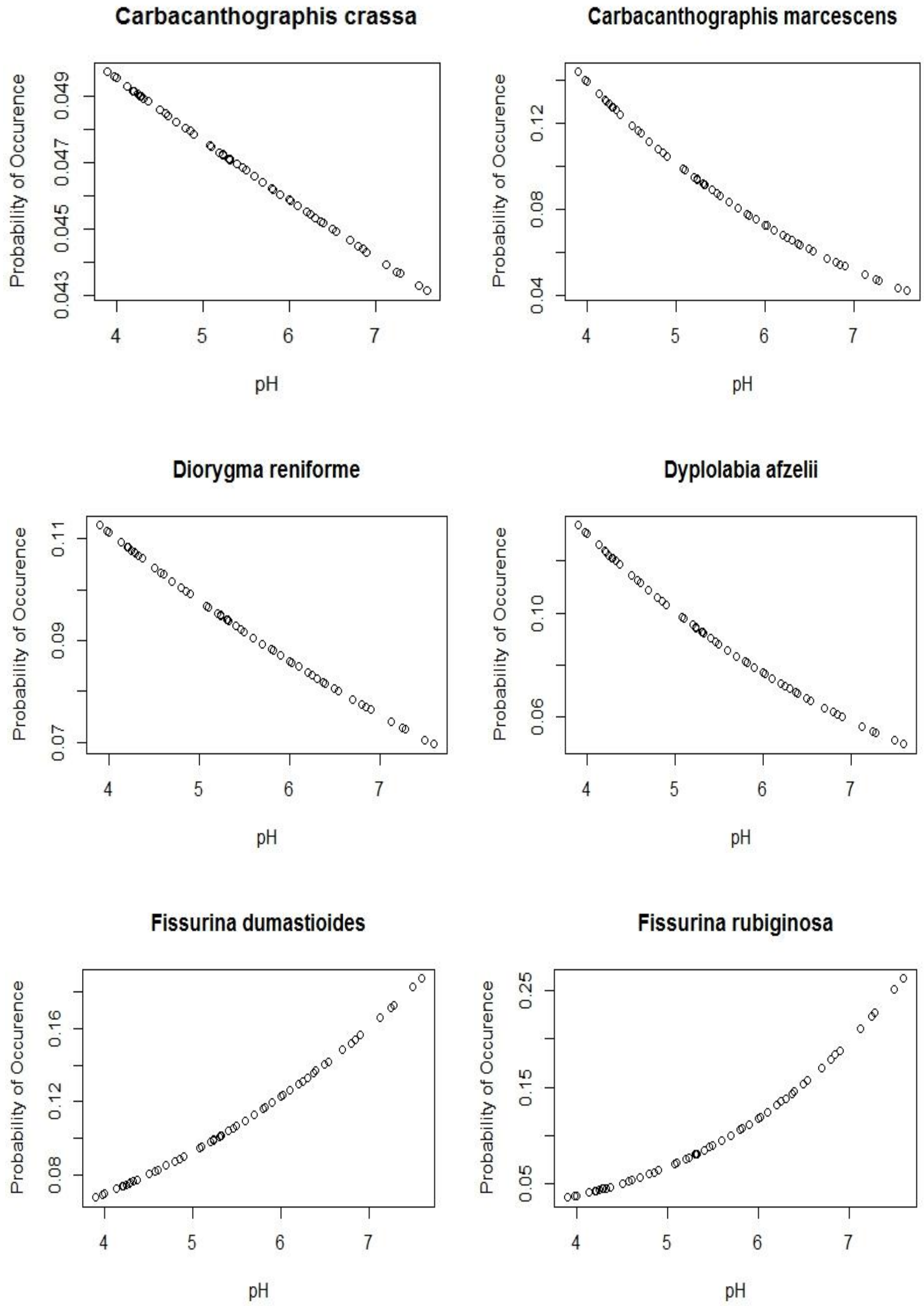

Figure 2 Actual occurrence (marked with dots) and probability curves of lichen species by different values of pH on tree.

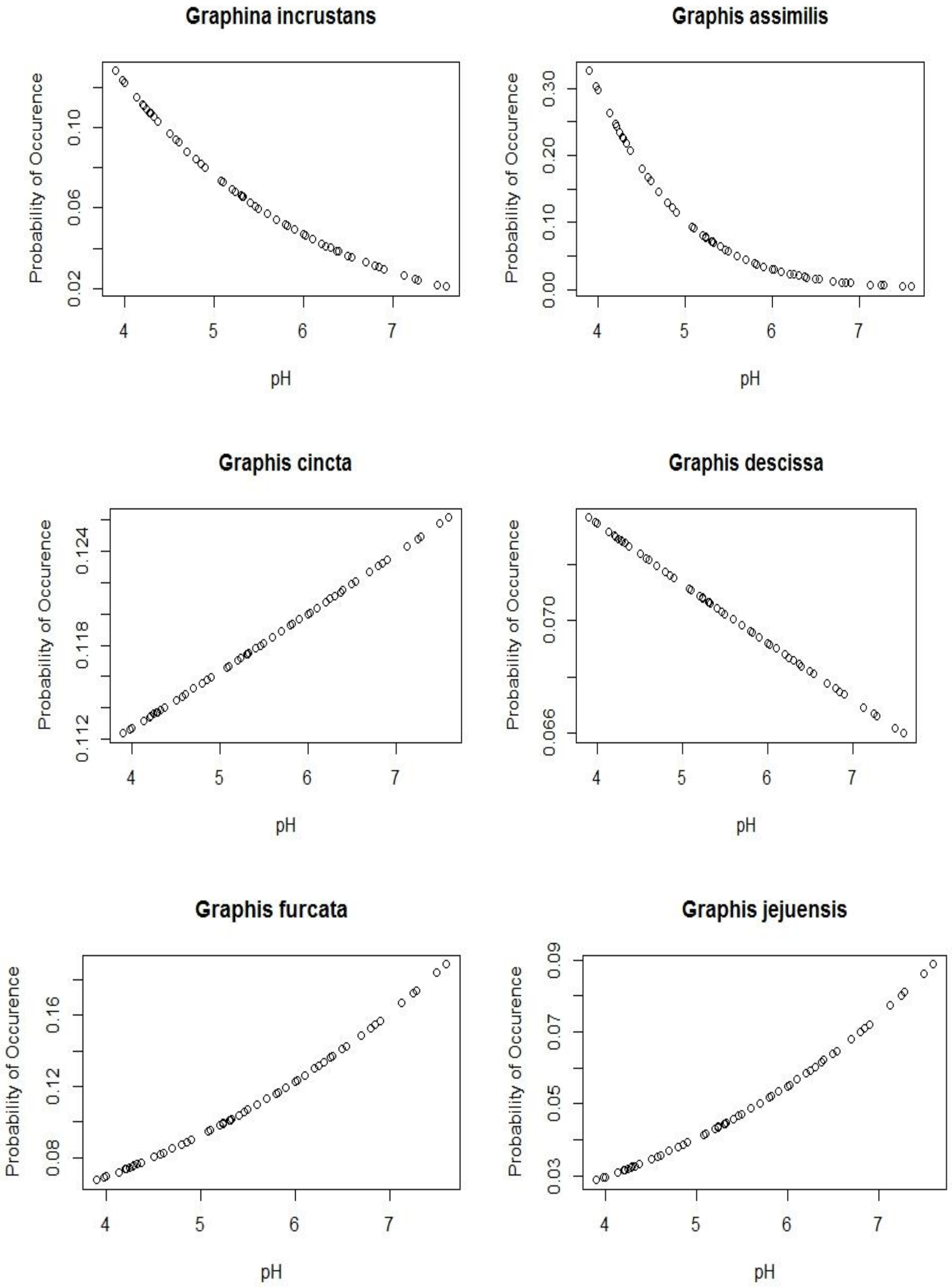

Figure 2 Actual occurrence (marked with dots) and probability curves of lichen species by different values of pH on tree (Continuous).

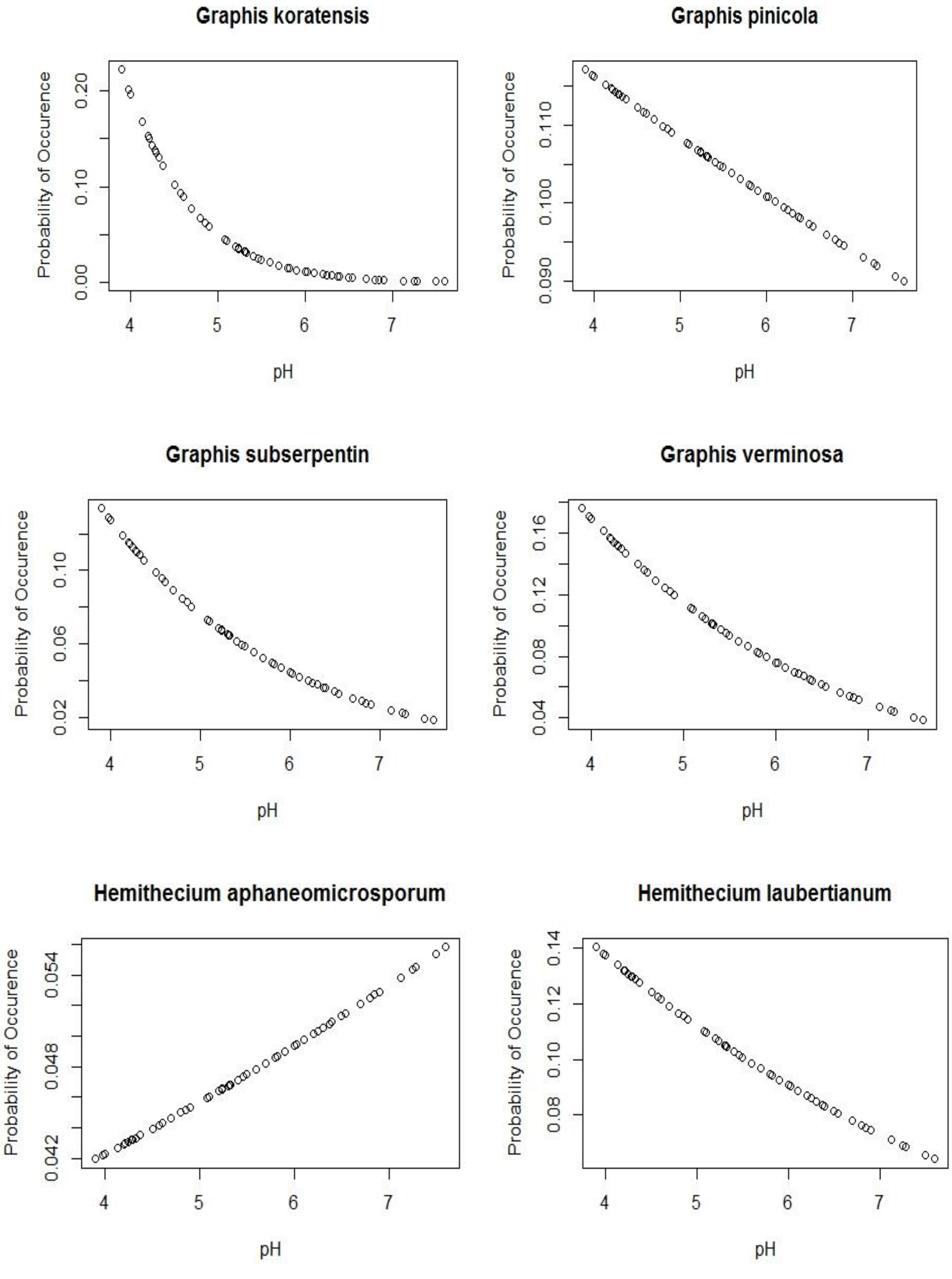

Figure 2 Actual occurrence (marked with dots) and probability curves of lichen species by different values of pH on tree (Continuous).

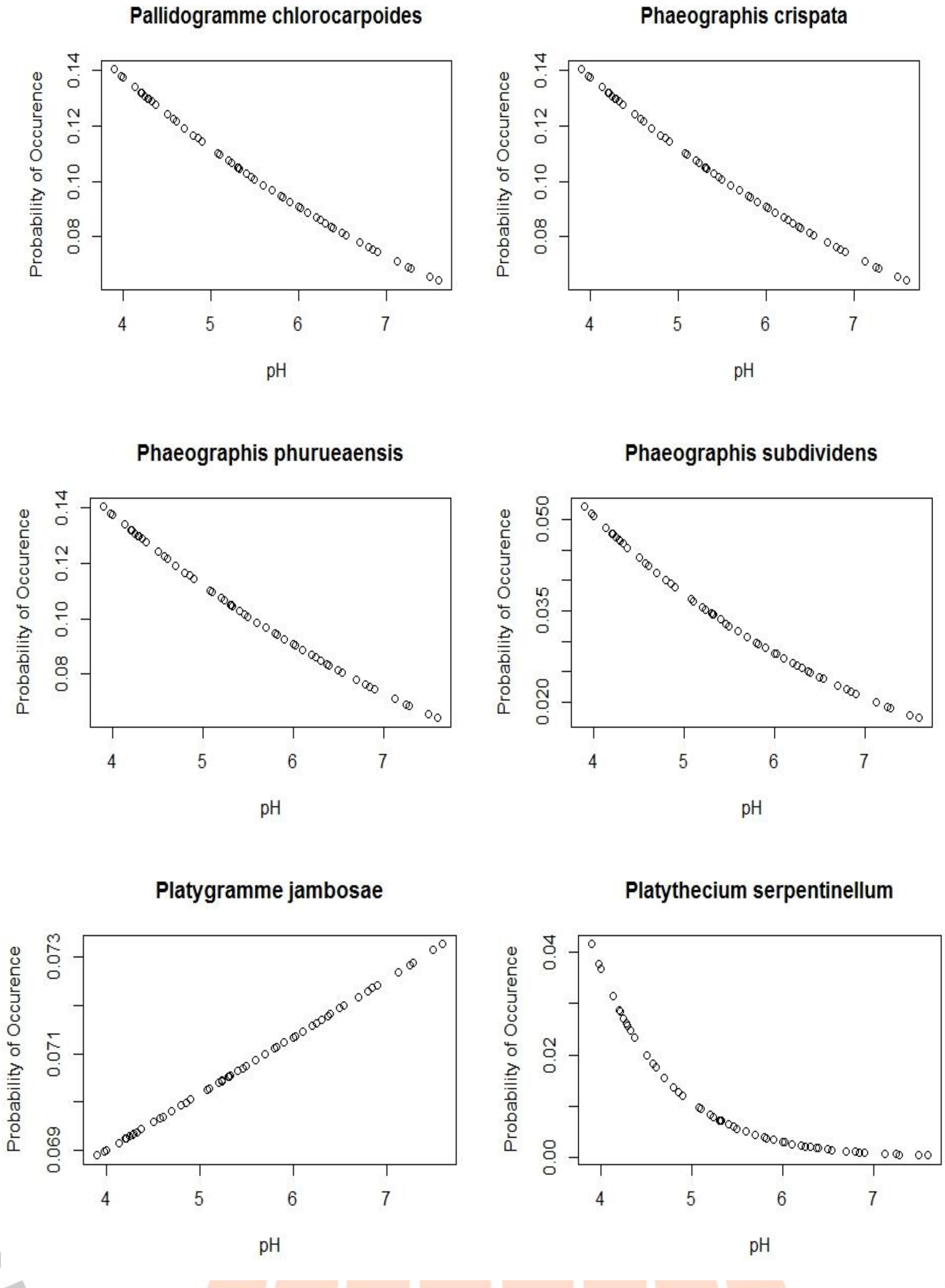

Figure 2 Actual occurrence (marked with dots) and probability curves of lichen species by different values of pH on tree (Continuous).

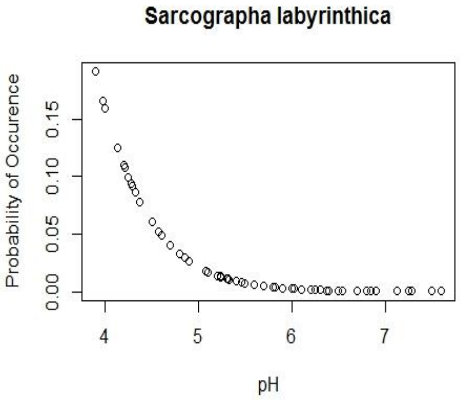

Figure 2 Actual occurrence (marked with dots) and probability curves of lichen species by different values of pH on tree (Continuous).

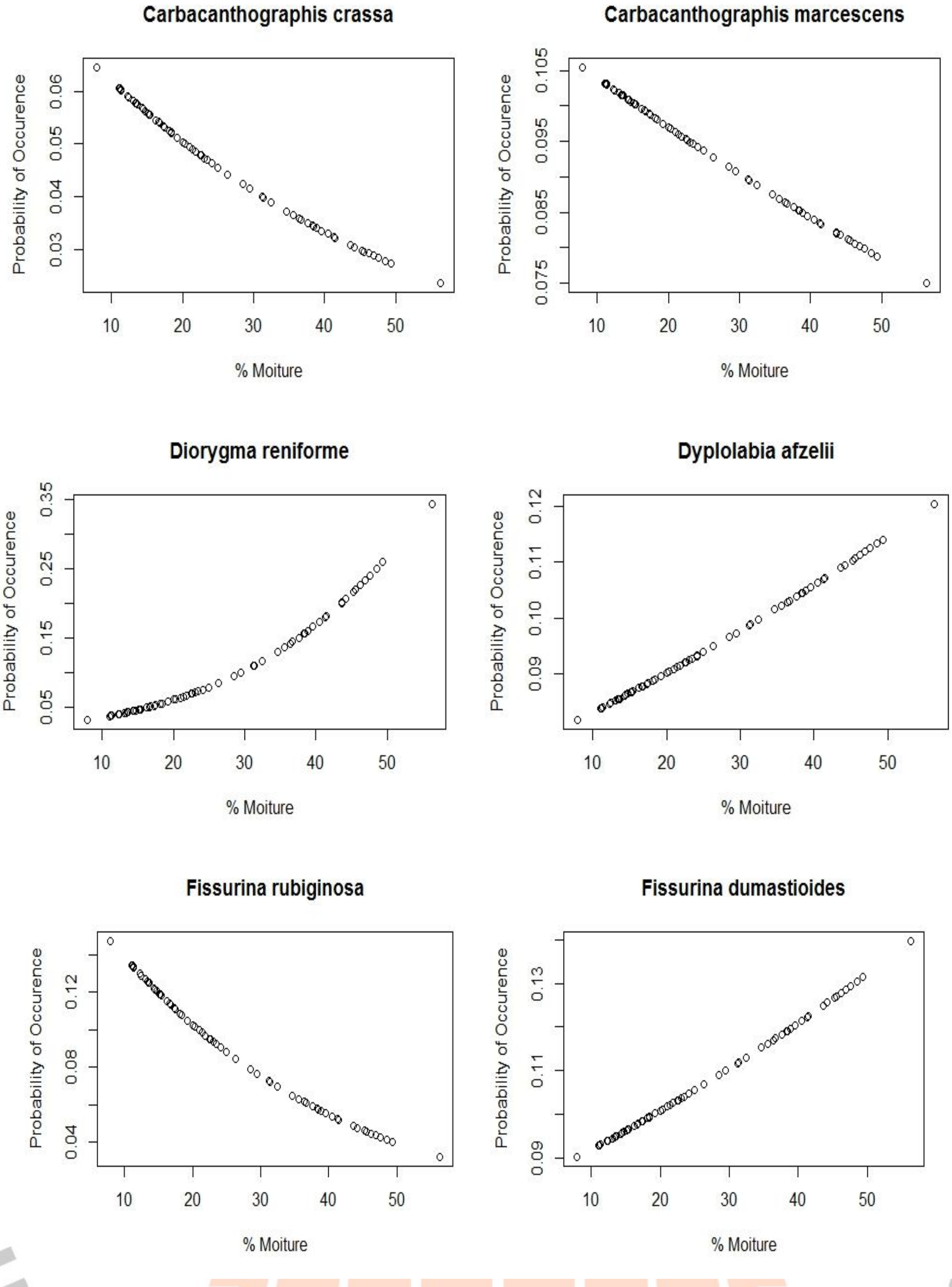

Figure 3 Actual occurrence (marked with dots) and probability curves of lichen species by different values of percent moisture on tree.

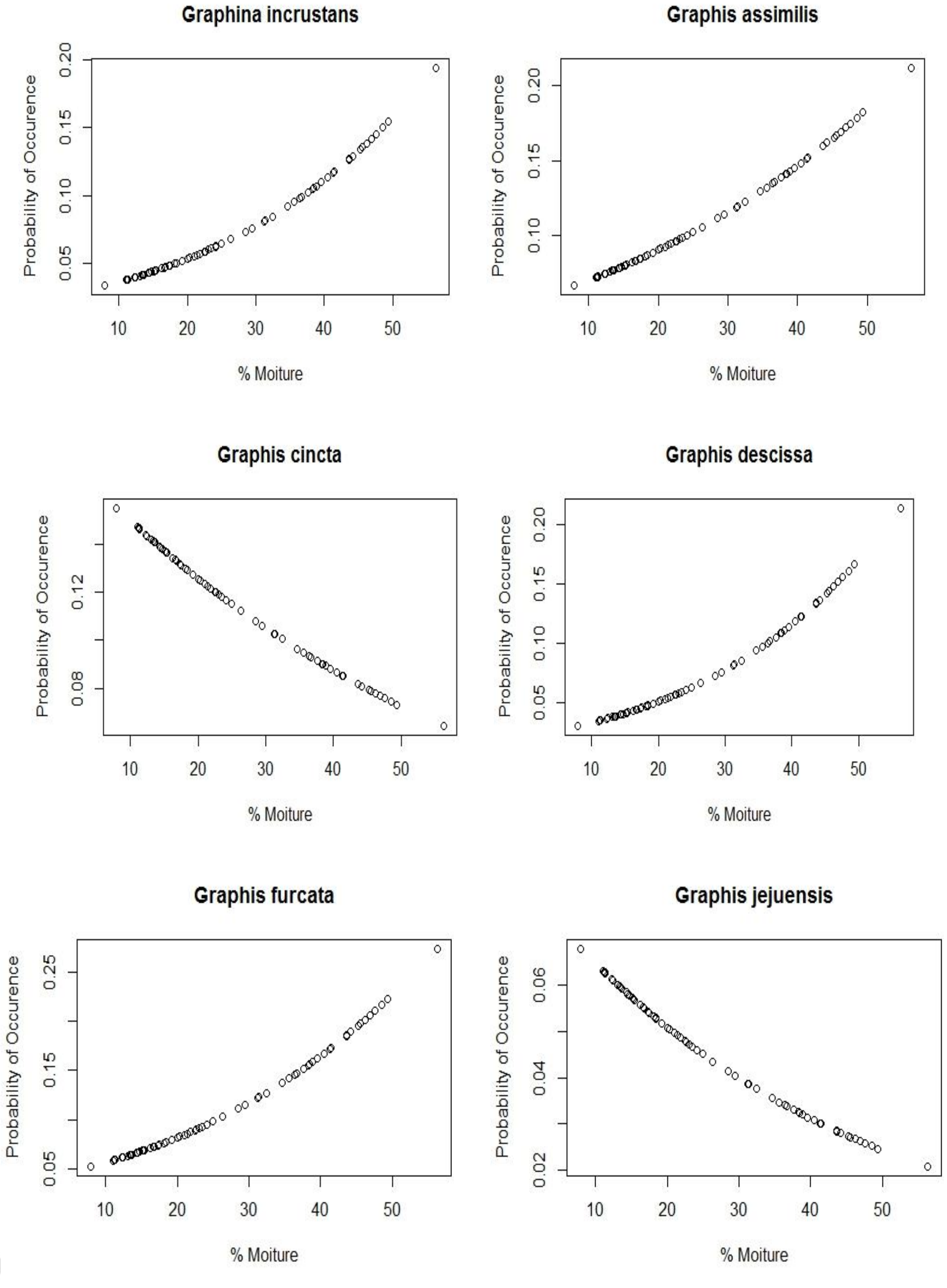

Figure 3 Actual occurrence (marked with dots) and probability curves of lichen species by different values of percent moisture on tree (Continuous).

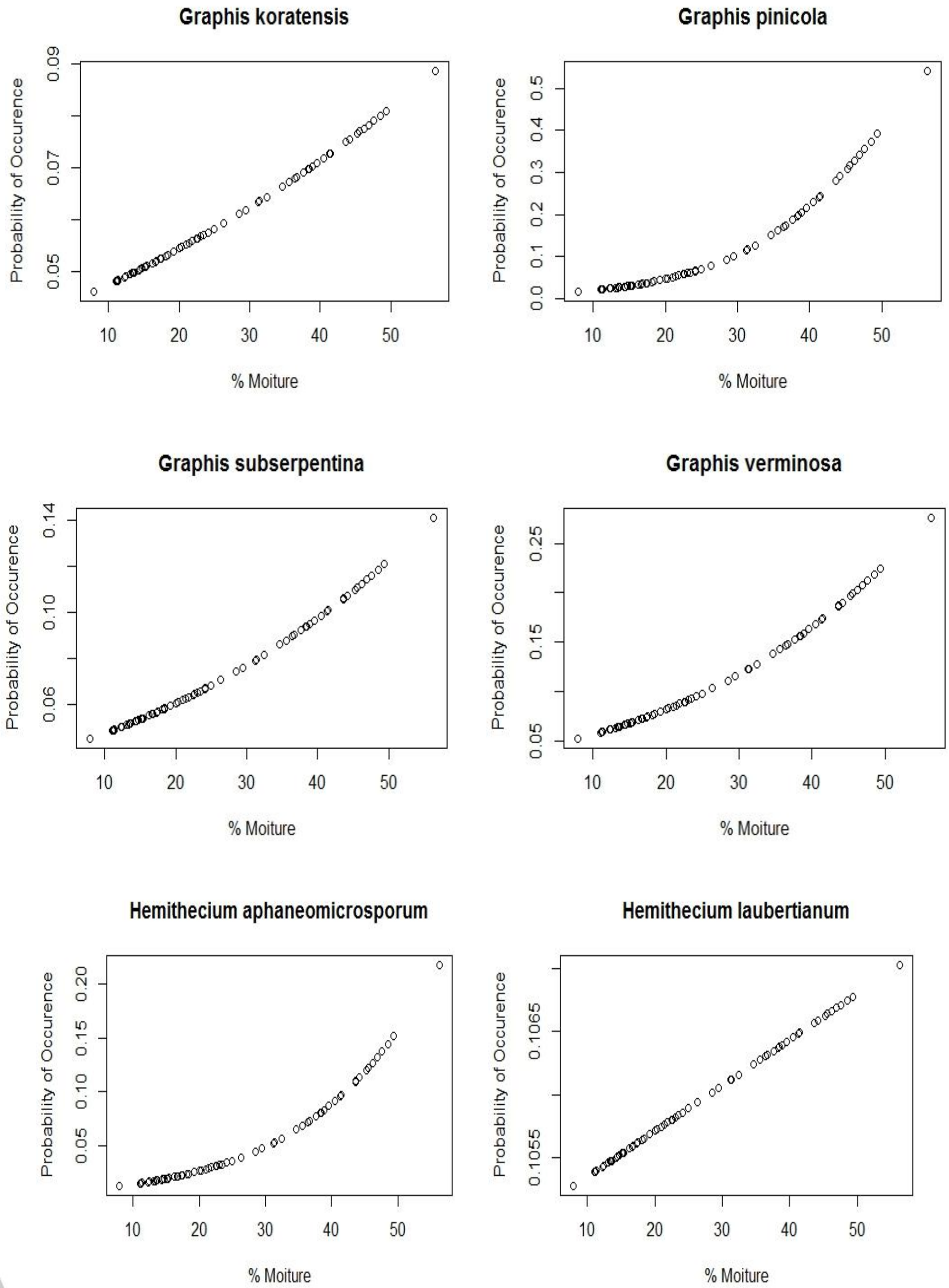

Figure 3 Actual occurrence (marked with dots) and probability curves of lichen species by different values of percent moisture on tree (Continuous).

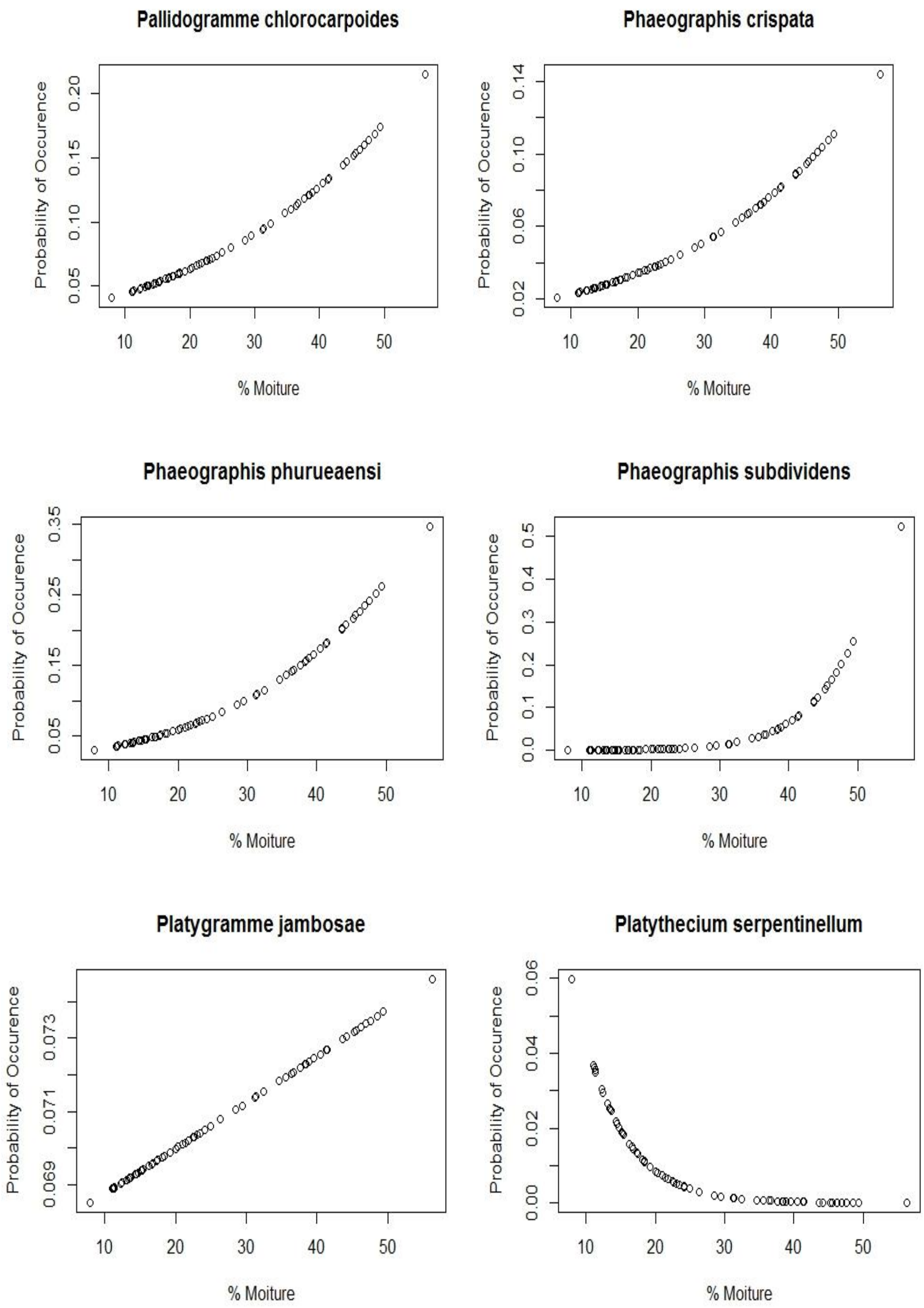

Figure 3 Actual occurrence (marked with dots) and probability curves of lichen species by different values of percent moisture on tree (Continuous).

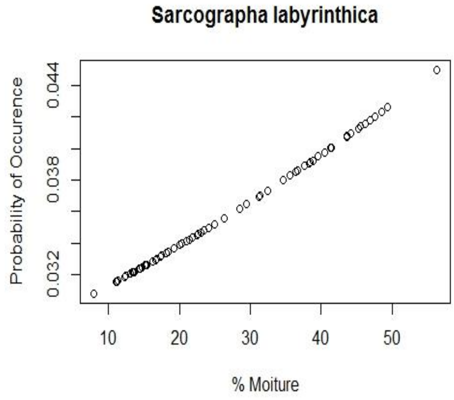

Figure 3 Actual occurrence (marked with dots) and probability curves of lichen species by different values of percent moisture on tree (Continuous).

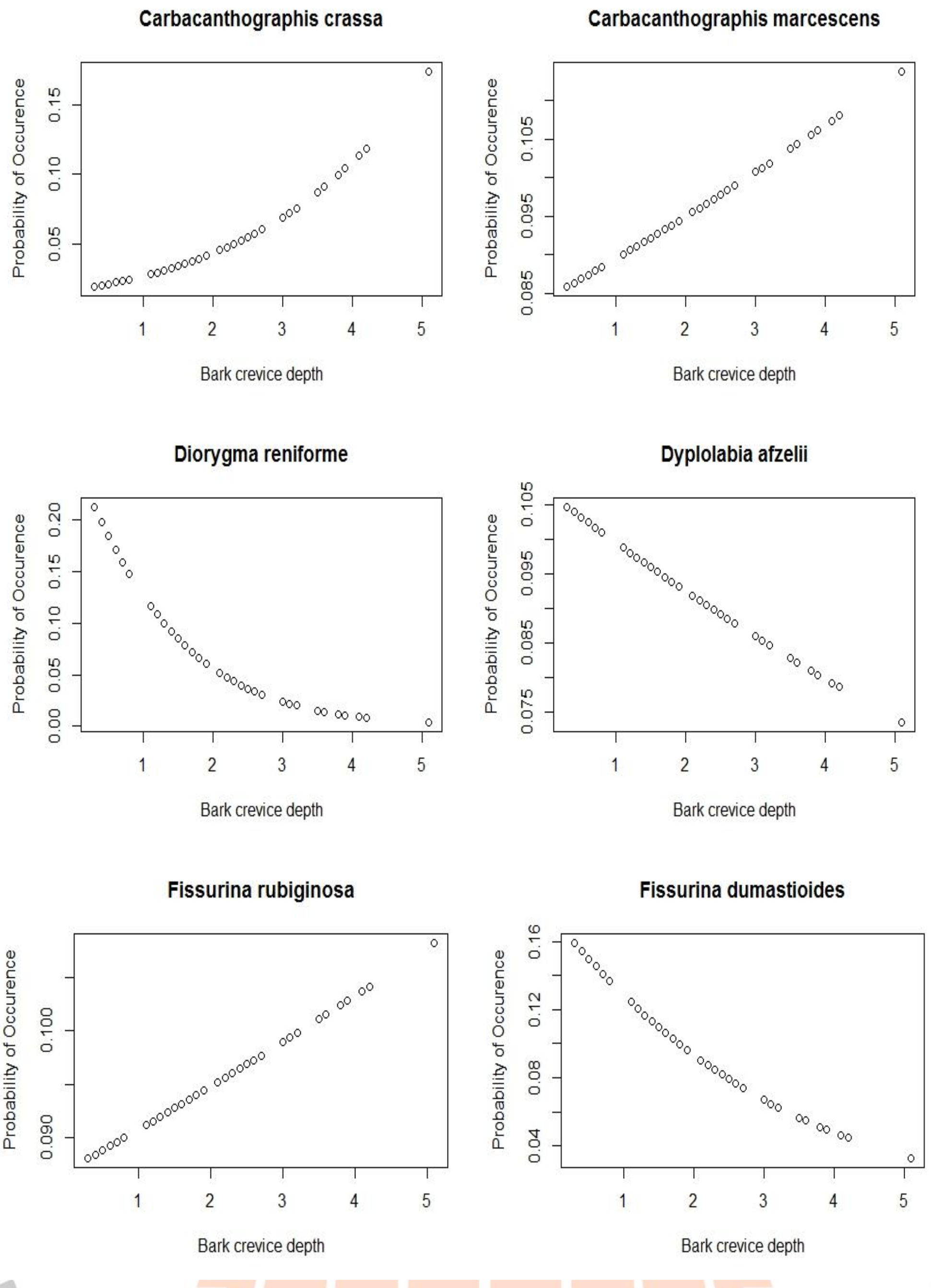

Figure 4 Actual occurrence (marked with dots) and probability curves of lichen species by different values of bark crevice depth (cm) on tree.

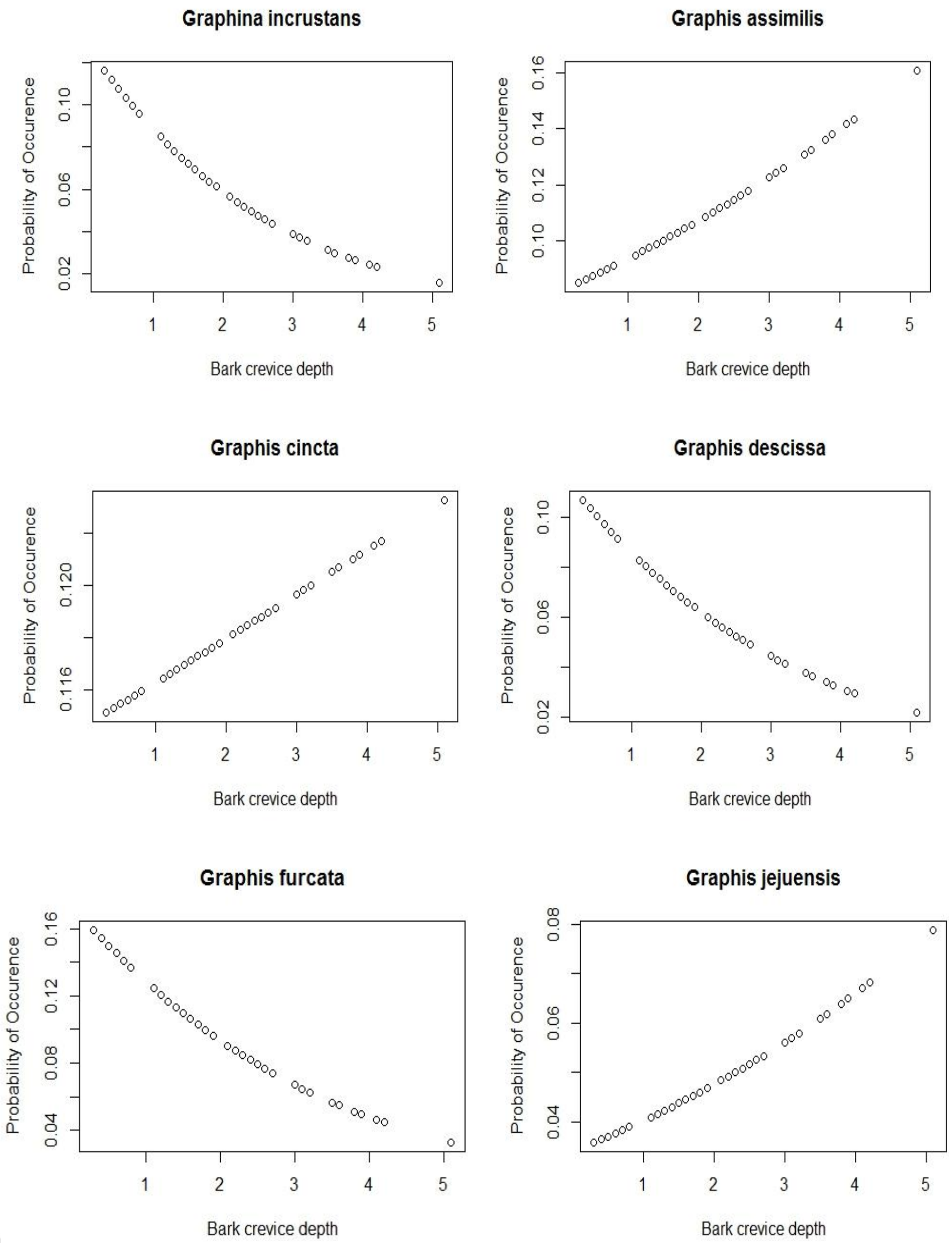

Figure 4 Actual occurrence (marked with dots) and probability curves of lichen species by different values of bark crevice depth (cm) on tree (Continuous).

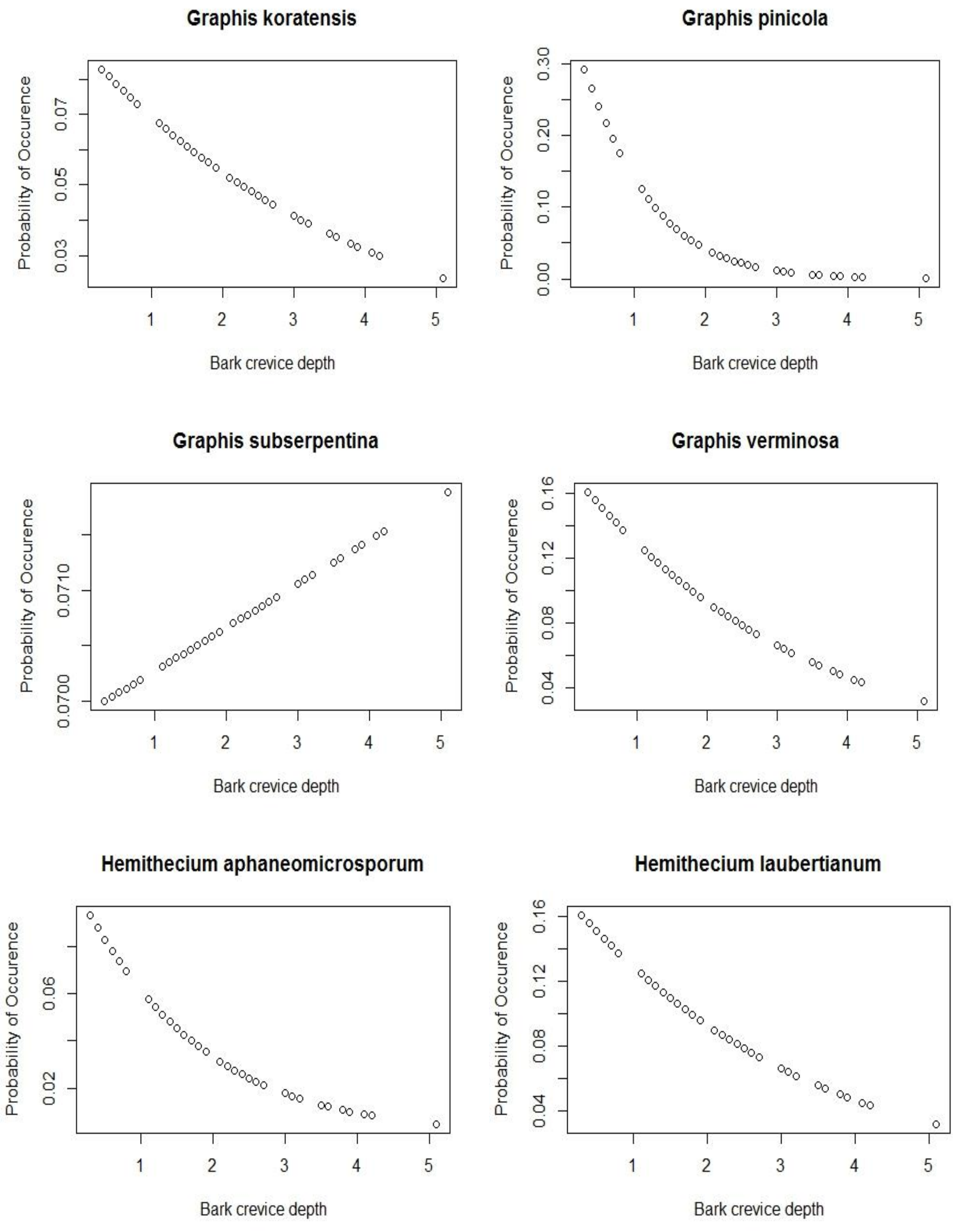

Figure 4 Actual occurrence (marked with dots) and probability curves of lichen species by different values of bark crevice depth (cm) on tree (Continuous).

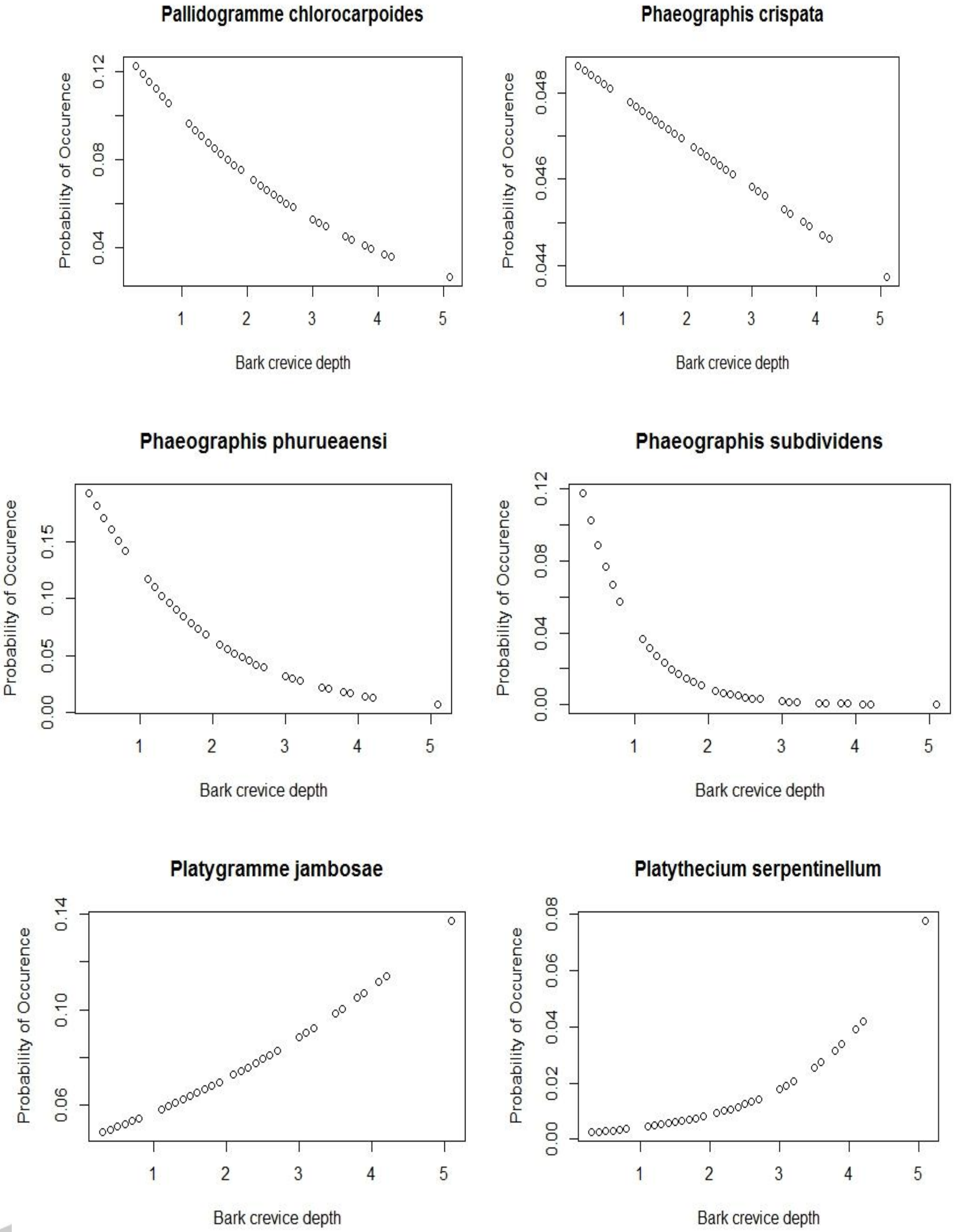

Figure 4 Actual occurrence (marked with dots) and probability curves of lichen species by different values of bark crevice depth (cm) on tree (Continuous).

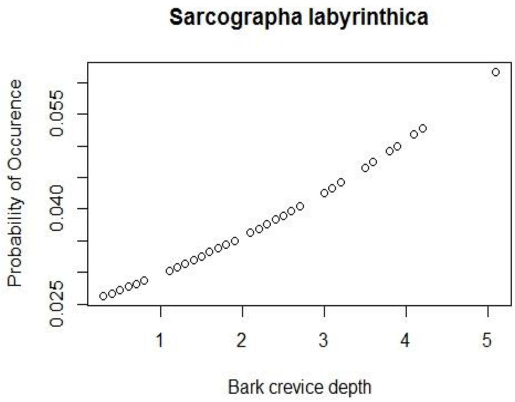

Figure 4 Actual occurrence (marked with dots) and probability curves of lichen species by different values of bark crevice depth (cm) on tree (Continuous).

APPENDIX D

ANALYSIS OF VARIANCE BETWEEN CLASSES OF ENVIRONMENTAL FACTORS AND CONTINUOUS PREDICTOR VARIABLES

Table 1 Analysis of variance between classes of bark characteristics and continuous predictor variables.

Factors	Rough		Smooth		Crack		F	P
	STDV	**V**	**STDV**	**V**	**STDV**	**V**		
Circumference (cm)	12.13	147.08	32.26	1040.58	51.09	2610.39	104.426	**<0.0001**
pH	0.57	0.33	0.66	0.44	0.77	0.59	44.989	**<0.0001**
Percent moisture	4.31	18.58	7.74	59.97	4.31	18.54	146.220	**<0.0001**
Bark crevice depth (cm)	0.80	0.64	0.59	0.34	0.41	0.17	122.371	**<0.0001**

P-values for variables that varied significantly (*P* < 0.05) with lichen species are shown in bold. N = 85.

Table 2 Analysis of variance between classes of direction and continuous predictor variables.

Factors	North		East		West		South		F	P
	STDV	**V**	**STDV**	**V**	**STDV**	**V**	**STDV**	**V**		
Circumference (cm)	64.3	4134.8	51.83	2686.36	75.69	5729.07	46.96	2205.21	0.635	0.594
pH	0.91	0.83	1.05	1.11	1	1.01	0.84	0.71	1.229	0.305
Percent moisture	13.03	169.8	8.86	78.54	11.75	138.09	13.4	179.57	0.696	0.557
Bark crevice depth (cm)	1.24	1.54	1.05	1.11	1.45	2.09	0.91	0.82	0.812	0.491

P-values for variables that varied significantly (*P* < 0.05) with lichen species. N = 85.

Table 3 Analysis of variance between tress species and continuous predictor variables.

Factors	Sho-obtu		Sho-siam		Dip-Obtu	
	STDV	**V**	**STDV**	**V**	**STDV**	**V**
Circumference (cm)	12.550	157.507	11.392	129.769	11.874	141.000
pH	0.57728	0.333	0.49392	0.244	0.72019	0.519
Percent moisture	2.77852	7.720	5.80228	33.666	2.5548	6.527
Bark crevice depth (cm)	0.70929	0.503	0.87464	0.765	0.76026	0.578

Table 3 Analysis of variance between tress species and continuous predictor variables (Continuous).

Factors	Lag-flor		Can-subu		Hop-ferr	
	STDV	**V**	**STDV**	**V**	**STDV**	**V**
Circumference (cm)	22.971	527.667	17.366	301.583	38.193	1458.695
pH	0.36106	0.130	0.3327	0.111	0.85477	0.731
Percent moisture	9.52885	90.799	6.4707	41.870	4.39218	19.291
Bark crevice depth (cm)	0.71813	0.516	0.09574	0.009	0.37007	0.137

Table 3 Analysis of variance between tress species and continuous predictor variables (Continuous).

Factors	Pte-java		Sho-seri		Hop-odor		F	P
	STDV	V	STDV	V	STDV	V		
Circumference (cm)	27.67	765.619	41.061	1686	66.071	4365.378	29.855	<0.0001
pH	0.69014	0.476	0.7413	0.55	0.60843	0.37	13.789	<0.0001
Percent moisture	7.40714	54.866	5.15687	26.593	4.111	16.9	45.83	<0.0001
Bark crevice depth (cm)	0.1069	0.011	0.55205	0.305	0.47656	0.227	33.462	<0.0001

P-values for variables that varied significantly ($P < 0.05$) with lichen species. N = 85.

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Publications

Pitakpong, A., Ekaphan, K., Khwanruan, P., Nooduan, M., Pongthep, S., H. Thorsten, L. and Robert, L. (2015). New species and records of the lichen genus Graphis (Graphidaceae, Ascomycota) from Thailand. **The Lichenologist**. 47(5): 335-342.

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