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The study distributions of lichen genus *Graphis* (Graphidaceae) in Thailand

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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. Therefore, study distributions of each species of genus *Graphis* in 12 localities of Thailand. The study was 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-twospecies 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* and only one species (*G. furcata*) was found in mangrove forest.
Keywords : *Graphis*, lichen, distribution, Thailand

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).

The Graphidaceae is distributed mainly in tropical and subtropical regions. *Graphis* is by far the largest genus of tropical crustosemicrolichens, 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 can 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).

Methodology

1. Study areas

The study was carried out between July 2013 and July 2514. A total of 12 study areas were spread across the country (Figure 1). Thailand has altitude between 0–2,500 meters above a sea level, temperatures between 21-36℃, and rainfall levels between 50-380mm. 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.

2. Species identification

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); Culberson and Kristinsson (1969); Orang *et al*. (2001); Staiger (2006); Lücking and Rivas Plata (2008); Lücking (2009); Lücking *et al*. (2009) 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.

3. 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).

Results and Discussion

We found 536 *Graphis* specimens of 12 studied areas. A total of 32 species were identified (Table 1) and only new species in Sakaerat Environmental Research Station (Figure 2). In each area, it was found that species richness was different. Phujong-Nayoi National Park possesses the highest 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 2)

From the study of lichens at different altitude in a range of 4-2,562 m above the mean sea level, Hat Chao Mai National Park, was 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 the mean sea level (Figure 3). In addition, the relationship between the species diversity of lichens and altitudes revealed the higher attitude, the lower diversity as calculated by the equation y = -0.0002x + 13.454, r2 = 0.0014 (Figure 4).

Twelve species of lichens showing the positive correlation with altitude includes *Graphis assamensis* Nagarkar & Patw. (A1), *G. descissa* Müll. Arg. (A4), *G. elongate* Zenker. (A6), *G. emersa* Mull.Arg. (A7), *G. glaucescens* Fée. (A10), *G. hossei* Vain. (A12), *G. jejuensis* K. H. Moon (A13), M. Nakan. & Kashiw. (A14), *G. librata* C. Knight. (A16), *G. rhizocola* (Fée) Lücking & Chaves. (A24), *G. seminude* Müll. Arg. (A26), *G. subvelata* Stirt. (A29), and *G. verminosa* Müll. Arg. (A31), whereas 20 species with the negative correlation includes *Graphis assimilis* Nyl. (A2), *G. cincta* (Pers.) Aptroot. (A3), *G. duplicata* Ach. (A5), *G. falvovirens* Makhija & Adaw. (A8), *G. furcate* Fée. (A9), *G. handelii* Zahlbr. (A11), *G. intricate* 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).

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 the higher 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 thegenus *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. elongate* 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, the lowest altitude area (4 m) found 12 species including *G. cincta* (Pers.) Aptroot, *G. duplicata* Ach., *G. furcate* 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 atp > 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; Negiand and Upreti 2000; Wolf and Alejandro, 2003; Pinokiyo *et al.*, 2008; Baniya *et al*.,2010; Rai *et al.*, 2012).

Conclusion

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 the respect to estimate the species distribution ranges of *Graphis*, but also the identification of the environmental factors limiting lichen distribution. At the present, the climate change may be a cause of their disappearance from the forest.

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Table 1. 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 = KhaoSip Ha Chan National Park, Thong = Thong Pha Phum National Park, Kaeng = Kaeng Krachan National Park, and HatCh = Hat Chao Mai National Park.

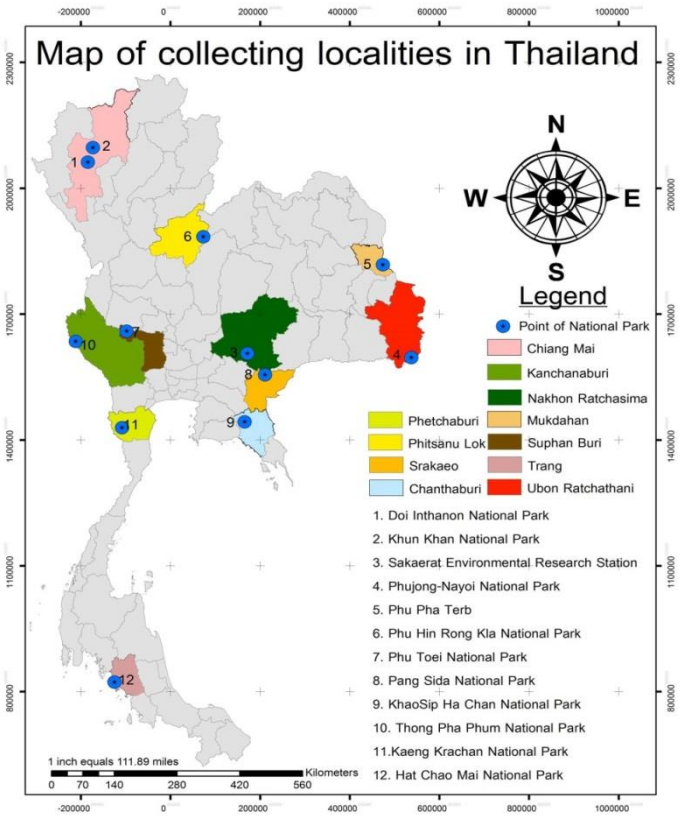

Figure 1. Map of collecting localities in Thailand.

Figure 2. *Graphis koratensis* Pitakpong, Kraichak & Lücking sp. nov.

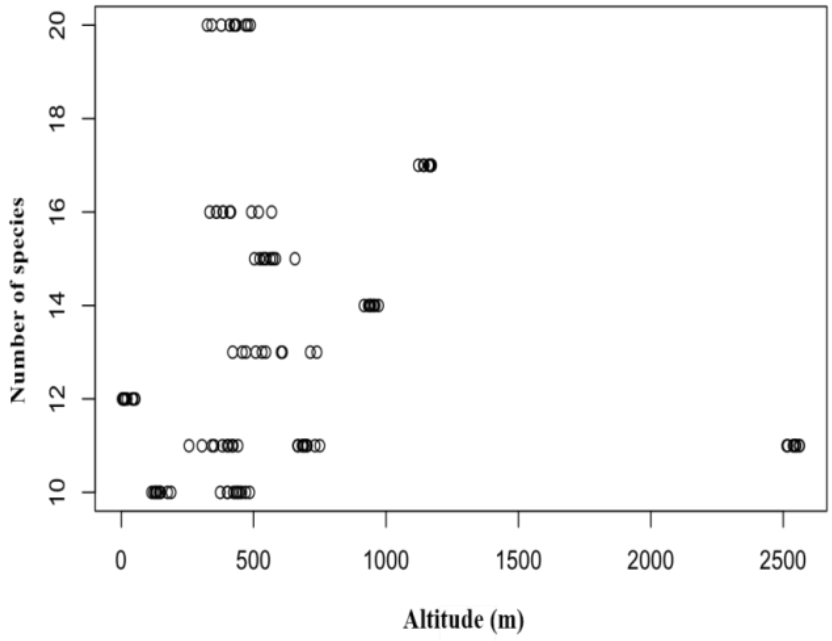

Figure 3. The relationship between altitude and number of species.

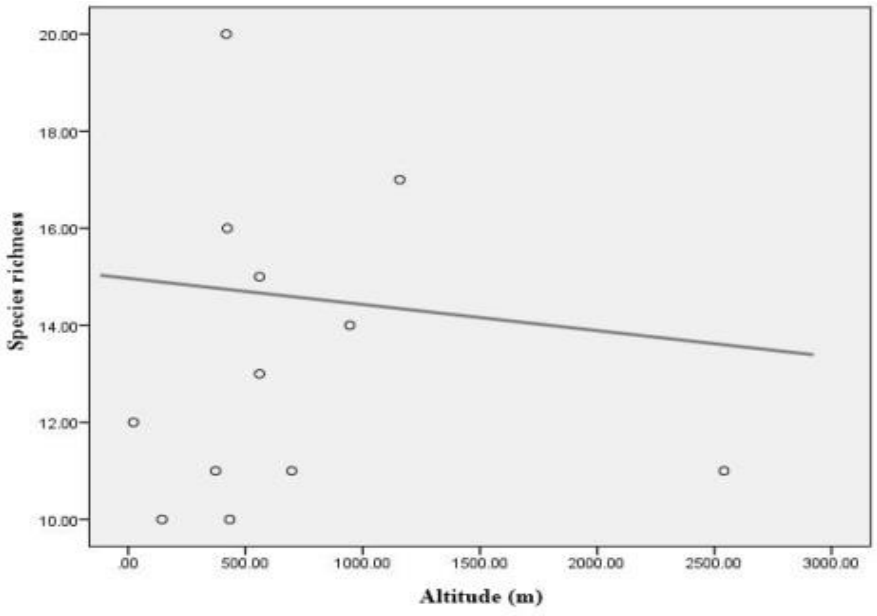

Figure 4. The relationship between altitude and species richness. The polynomial equation is y = -0.0002x + 13.454, r2 = 0.0014