DIVERSITY, ECOLOGY AND SECONDARY METABOLITE OF LICHEN FAMILY GRAPHIDACEAE AT PHU LUANG WILDLIFE SANCTUARY, LOEI PROVINCE

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Abstract: Five hundred and thirty-one graphidacean specimens were collected at Phu Luang Wildlife Sanctuary from barks and rocks in seven forest types; coniferous forest (CF), dry dipterocarp forest (DDF), dry evergreen forest (DEF), lower montane scrub (LMS), lower montane rain forest (LMRF), mixed deciduous forest (MDF) and tropical rainforest (TRF). Ninety-one taxa of an unclear generic affiliation and 16 genera were identified; *Acanthothecis*, *Carbacanthographis*, *Diorygma*, *Dyplolabia*, *Glyphis*, *Graphis*, *Fissurina*, *Hemithecium*, *Leiorreuma*, *Pallidogramme*, *Phaeographis*, *Platygramme*, *Platythecium*, *Sarcographa*, *Thalloloma* and *Thecaria*. Thirty-five taxa were first records for Thailand and 17 taxa were expected to be new species to science. The lichen chemical compounds were analyzed by thin layer chromatography, 16 compounds were recognized and one of them was an unknown substance. The greatest diversity of taxa were 63 species found in LMS and the second greatest taxa densities were 36 species in LMRF. The lowest diversity was found in DEF and CF, which represented 9 and 8 species respectively.

Introduction: The crustose lichen family Graphidaceae is formed from fungi in Ascomycota and green algae (Chlorophyta) of the genus *Trentepohlia* as a mutualistic association.[1, 2, 3, 4, 5] The characteristics of the graphidioid members of lichen family Graphidaceae are; ± lirellate apothecia with usually simple paraphyses (or with only a few ramifications), functionally unitunicate asci and ascospores with lens-shaped lumina.[2] Normally, almost all of the Graphidaceae are predominantly corticolous, some are saxicolous and a few are foliicolous. These lichens are mainly distributed in tropical countries such as Mexico, Costa Rica, Brazil, India, parts of Australia, Thailand and the Philippines, and subtropical regions.[6, 7] Some taxa are also found in temperate zones[8] such as New Zealand.[9] The purposes of this study are to investigate the diversity, ecology and their metabolites of graphidioid lichens at Phu Luang Wildlife Sanctuary.

Methodology: The diversity, ecology and secondary metabolite of lichen in family Graphidaceae at Phu Lung Wildlife Sanctuary were explored in seven forest types which were coniferous forest (CF), dry dipterocarp forest (DDF), dry evergreen forest (DEF), lower montane scrub (LMS), lower montane rain forest (LMRF), mixed deciduous forest (MDF) and tropical rainforest (TRF). Five hundred and thirty-one graphidacean specimens on barks and rocks in the seven forest types were examined with stereo compound microscrope (Olympus model SZ30) at 10-40 magnification and light microscope (OLYMPUS-CH) at 100-1000× magnification. Anatomical features studied on hand-cut sections of thallus and ascomata mounted in water, 10% KOH (K). The iodine reaction of the hymenium was studied in Lugol's iodine solution after pretreatment with K (KI). The secondary products of the specimens were characterized by spot test and thin layer chromatography (TLC)

according to White & Jame (1985).[10] Taxa were determined according to Lücking et al. (2009)[2], Archer (2006)[11], Kalb et al. (2004)[12], Staiger (2002)[5] and Awasthi (1991).[13]

Results, Discussion and Conclusion: Ninety one taxa of lichen in family Graphidaceae at Phu Lung Wildlife Sanctuary belong to an unclear generic affiliation and 16 genera; *Acanthothecis, Carbacanthographis, Diorygma, Dyplolabia, Glyphis, Graphis, Fissurina, Hemithecium, Leiorreuma, Pallidogramme, Phaeographis, Platygramme, Platythecium, Sarcographa, Thalloloma* and *Thecaria*. The majority of the taxa were encountered in genera *Graphis* which compose of 32 species and 3 unidentified species and *Phaeographis* represented by 9 species and 8 unidentified species. Thirty-five taxa are first records for Thailand and 17 taxa were expected to be new species to science (Table 1).

The highest diversities of lichens found in LMS, 63 taxa from 14 genera, due to this forest consists of bush trees, such as *Lithocarpus recurvatus* Barnett, *Rhododendron lyi* H. Lev, high elevation 1000-1400 meters above sea level, strong high light intensities and good aeration circumstances which preference for graphidoid lichens setting in colonization.[14] The lower taxa diversities were in DDF, DEF, LMRF, MDF, TRF. CF had the lowest taxa which only present 8 species (Figure 1) because it is very different from other forest types due to its wet and cool climate as well as strong and high light intensities, and finally pine as dominant trees. It is traditionally known that at mature, the pine stem barks are peeled out by its self before the lichens setting in colony.

Lichen substances were analyzed by TLC; 29% lacking lichen substances and 71% contains lichen substances which compose of 11 depsidones and 1 depside, 2 quinone, 1 xanthone; 21% of norstictic acid, 19% stictic acid and constictictic acid 9%. Other lichen substances which are present in less frequently including connorstictic acid, cryptostictic acid, echinocarpic acid, hyposalazinic acid, hypostictic acid, isohypocrellin, lecanoric acid, lichenxanthone, menegazziaic acid, peristictic acid, protocetraric acid, salazinic acid and unknown substances (Figure 2).

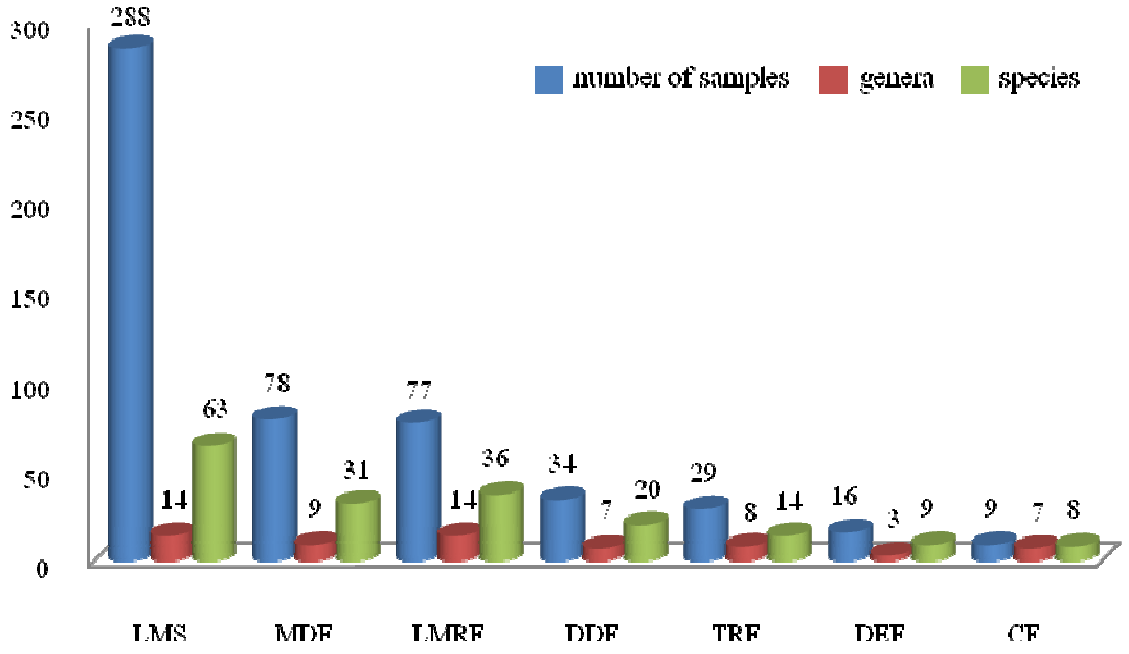

Figure 1. The number of specimens with genera and species in the seven forest types.

Table 1 Recorded, New records and New Species of the Lichen Family Graphidaceae at Phu Luang Wildlife Sanctuary

Recorded		New records		New species
Carbacanthographis candidata	*Leiorreuma hypomelaenum*	*Acanthothecis clavulifera*	*Graphis rustica*	*Acanthothecis* sp. 1
Carbacanthographis marcescens	*Leiorreuma melanostalazans*	*Acanthothecis dialeuca*	*Graphis stenotera*	*Acanthothecis* sp. 2
Diorygma hieroglyphicum	*Pallidogramme chlorocarpoides*	*Carbacanthographis coccospora*	*Graphis subregularis*	*Fissurina* sp. 1
Diorygma stiticum	*Pallidogramme chrysenteron*	*Diorygma erythrellum*	*Graphis subvelata*	*Graphis* sp. 1
Dyplolabia afzelii	*Phaeographis brasiliensis*	*Diorygma macgregorii*	*Graphis subvittata*	*Graphis* sp. 2
Fissurina incrustans	*Phaeographis caesioradians*	*Diorygma magaspermum*	*Graphis urandrae*	*Graphis* sp. 3
Glyphis cicatricosa	*Phaeographis colligata*	*Fissurina cingalina*	*Graphis verminosa*	*Leiorreuma* sp. 1
Graphis assimilis	*Phaeographis dendroides*	*Graphis elongata*	*Graphis vittata*	*Phaeographis* sp. 1
Graphis duplicata	*Phaeographis intricans*	*Graphis furcata*	*Hemithecium implicatum*	*Phaeographis* sp. 2
Graphis emersa	*Phaeographis schizoloma*	*Graphis handelii*	*Hemithecium oryzaeforme*	*Phaeographis* sp. 3
Graphis hossei	*Phaeographis subdividens*	*Graphis longispora*	*Hemithecium rufopallidum*	*Phaeographis* sp. 4
Graphis librata	*Platygramme caesioprunosa*	*Graphis longula*	*Phaeographis decipiens*	*Phaeographis* sp. 5
Graphis longiramea	*Platygramme jambosae*	*Graphis marginata*	*Phaeographis quadrifera*	*Phaeographis* sp. 6
Graphis nanodes	*Platygramme pudica*	*Graphis nigrocarpa*	*Platygramme discurrens*	*Phaeographis* sp. 7
Graphis pyrrhocheiloides	*Platythecium dimorphodes*	*Graphis norstictica*	*Platythecium pertenellum*	*Phaeographis* sp. 8
Graphis streblocarpa	*Platythecium serpentinellum*	*Graphis novopalmicola*	*Sarcographa verrucosa*	*Platygramme* sp. 1
Graphis subdisserpens	*Sarcographa labyrinthica*	*Graphis rhizocola*	*Thalloloma anguinum*	unknown genus
Graphis subserpentina	*Thecaria montagnei*	*Graphis semirigida*		
Graphis supracola	*Thecaria quassiicola*			
Graphis tenella				

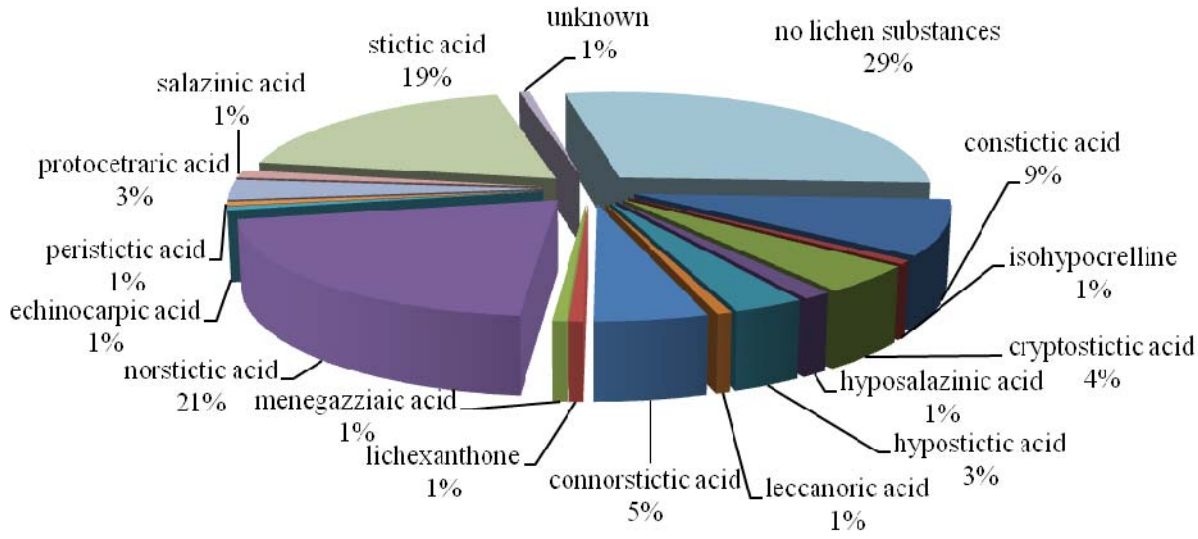

Figure 2. The proportion of lichen substances of Graphidaceae in Phu Luang Wildlife Sanctuary.

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Keywords: lichen, Graphidaceae, diversity, ecology, secondary metabolite