# Diversity, composition and distribution of lichens along elevational gradients in the tropical mountain forest of Gunung Nuang, Selangor, Malaysia.

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Keywords: biodiversity, epiphytic lichen, Malaysia, montane forest, tropical lichen

#### Abstract

This study aims to explore how lichen diversity, composition and distribution vary with altitude, and environmental factors (temperature and relative humidity). The study was conducted in the forest of Gunung Nuang, Selangor at five sites (at different altitudes) which were chosen as sampling stations. Forty-four lichen species were identified. Their diversity, composition and distribution correlated significantly with the forest's altitude and environmental factors, increasing at higher altitudes. While Graphidaceae and Physciaceae species were present at all altitudes, the dominant species changed according to altitude: some species of Parmeliaceae were found only at higher altitudes (601–1493 m), while C. *xanthina* and a few species from Physciaceae were present only at lower altitudes (0–600m). These findings will provide additional information about the lichens of the tropical montane forest of Malaysia, enhancing knowledge on how to manage and sustain lichens in this type of forest.

## Profile Protected area Gunung Nuang Mountain range Titiwangsa Main Range of Peninsular Malaysia

#### Introduction

Lichen diversity and species composition are varied, depending on environmental conditions and type of ecosystem (Nimis et al. 2002). Lichens can be found growing from low-tide level to mountain summits, and from arctic and desert to tropical climate areas (Nash 2008; Abas et al. 2018). Lichens are unique dual-organisms (comprising fungus as mycobiont, and bluegreen algae or cyanobacteria as photobiont) which can show immediate responses to changes in environmental conditions (Abas et al. 2020). Some species are able to grow under harsh environmental conditions, while even slight changes in their environment cause others to perish. Lichens have three different growth forms: crustose, which grow attached to the substratum; foliose, which are leaf-like and loosely attached to the substratum; and fruticose, which are bush-like, and either hang, or grow upright on the substratum (Gaurav & Upreti 2016). As well as their ecological roles, lichens with their metabolites have numerous biological properties, including antimicrobial, antiprotozoal, antiviral, anti-proliferative, anti-inflammatory, analgesic, antipyretic, anti-termite, antioxidant, cytotoxic, enzyme inhibitory, insecticidal, wound healing, and antitumor properties (Yilmaz et al. 2004; Kosanić et al. 2013; Rajan et al. 2016).

Malaysia, a tropical country, is known for its diverse and unique species of flora and fauna, but the study of lichen in Malaysia is far from complete (Abas & Wang 2017). Most tropical lichens grow abundantly only at higher altitudes, such as in the montane forest, on hill summits, and in mountain ranges (Chongbang et al. 2018). The first lichen samples in Malaysia were recorded by Beccari (1904), who carried out a study

on lichen in Sarawak. Galloway et al. (1994) prepared a bibliography of Malaysian lichenology comprfFising 90 entries, and an additional list comprising a further 192 entries (Galloway et al. 1997). Seven genera of lichens collected from Bario, Sarawak were identified by Din et al. (1998). Zakaria et al. (2010) reported on the morphology and chemical constituents of Cladia aggregata collected from Gunung Jerai, Kedah. The chemical components of a few specific samples of Heterodermia flabellata and Heterodermia leucomela from Gunung Jerai, Kedah and the Cameron Highlands, Pahang were described by Din et al. (2010). Lichens of North Eastern Langkawi and Gunung Machincang, Kedah were investigated by Zulkifly and Merican (2005), and lichen diversity and species composition (36 species were found) at Gunung Machincang were studied by Zulkifly et al. (2011). Urban lichen diversity and distribution in Kuala Lumpur have been analysed by Abas & Awang (2017), and in Kota Kinabalu by Abas et al. (2020). Abas et al. (2019a) have reported 12 lichen species in Teluk Nipah, Pulau Pangkor, Perak as well.

Studies carried out in tropical forests have shown that environmental factors (e.g. temperature and humidity) and the forests' structure will vary significantly with altitude (Boonpeng et al. 2017). Other research has shown that lichen diversity, composition and distribution correlate significantly with, and are influenced by, environmental factors and the forest structure (Sulaiman et al. 2018; Abas et al. 2019b). Altitude has a strong connection with, and significant effects on, lichen diversity, composition and distribution. The results of the present study are consistent with the findings of Zulkifly et al. (2011), Upadhyay et al. (2018), and Abas et al. (2019a, b), who all concluded that lichen diversity, composition and distri-



Figure 1 – Map of Gunung Nuang, Selangor

bution increase significantly, the higher the forest's altitude.

In Malaysia, there has been limited study of the specific lichens of tropical montane forest, and few studies are similar to our own. In this respect, Zulkifly et al. (2011) on Gunung Machincang, Langkawi, and Shahpuan et al. (2019) on foliicolous lichen in the Borneo Rainforest, Sabah, stand out. However, Shahpuan et al.'s study of composition and distribution is only very basic. Abas et al. (2019a, b) and Abas & Awang. (2017) focused solely on lichen diversity in the lowland area, and more specifically on how lichen diversity was affected by air pollution and urbanization.

Information on lichen diversity, composition and distribution is still lacking for montane forests, especially in Malaysia. In addition, the uncontrolled development of land and recreational activity at Gunung Nuang have affected the diversity of the fauna. Hence, this study aims to identify and analyse lichen diversity, composition and distribution in Gunung Nuang, Selangor. It also aims to investigate the spatial distribution of lichens with regard to different elevations of Gunung Nuang. It could also raise awareness of how environmental changes at Gunung Nuang have affected the diversity of the mountain's flora and fauna.

## Study area

Gunung Nuang is located in Hulu Langat District, Selangor. Rising to 1493 m (4898 feet), Gunung Nuang is the highest mountain in the state of Selangor, while part of its peak lies in the Titiwangsa Main Range of Peninsular Malaysia. The peak also borders on Pahang state and is close to the Pahang-Selangor-Negeri-Sembilan border tripoint. Gunung Nuang is covered by an extensive montane forest, making it an attractive tourist destination for hiking and mountain climbing. There are three hiking routes to the peak, all of them built by the Malaysian Department of Wildlife and National Parks. Two of them start in Selangor - one at Kuala Pangsun in Hulu Langat, the other at Kampung Kemensah in Gombak. The third route originates in Bukit Tinggi. The Genting Highlands in Pahang are visible (especially at night) from the peak of Gunung Nuang, which is accessible from the Kuala Pangsun route. The climb involves a two-hour hike on a very steep track, then an optional stop at Camp Lolo, followed by a four-hour trek to the start of the climb proper and a six-hour push to the summit. Gunung Nuang is vulnerable to global climate change. In 1990, Gunung Nuang's lowest recorded temperature was 14°C, but in 2015 it had increased to 18°C. This temperature increase is due also to the development, since 1995, of the nearby Semenyih Dam, and the numerous recreational activities conducted in the region since 1997, with the opening of its famous trail (Forest Department Peninsular Malaysia 2019).

The study was conducted at five sites along the hiking trail of Gunung Nuang, Selangor, between the Kuala Pangsun entrance and Gunung Nuang's peak (Figure 1). According to Zulkifly et al. (2011), the ecological stands of tropical forests vary every 200–400 m. In order to observe the change in lichen diversity, distribution and composition along elevational

Table 1 – Environmental factors for each sampling site. Source: Malaysia Meteorological Department (2019).

Site	Coordinates	Altitude [m]	Relative Humidity [%]	Temperature [°C]
1	3.216894 N, 101.883468 E	0–299	80.7	32.5
2	3.235284 N, 101.901734 E	300–599	81.1	31.8
3	3.246242 N, 101.905081 E	600–899	82.5	27.1
4	3.256777 N, 101.905365 E	900–1199	87.9	23.2
5	3.263782 N, 101.903943 E	1 200–1 493	90.3	21.3

Research



Figure 2 – The Shannon-Weiner Index.

gradients, therefore, this study used mostly 300 m intervals in selecting the study sites: Site 1 (0-300 m a.s.l.), Site 2 (300–599 m a.s.l.), Site 3 (600–999 m a.s.l.), Site 4 (1000-1299 m a.s.l), and Site 5 (1300-1493 m a.s.l.). The study area has a warm tropical climate, with annual average minimum and maximum temperatures of 19°C and 33°C respectively; monthly rainfall can be as low as zero in January and as high as 342 mm in December, when there is heavy monsoon rain, which starts in November (Malaysian Meteorological Service 2018). The study sites included protected tropical rainforest reserves; diverse species and a variety of forest structures are distributed throughout the region. In addition, Gunung Nuang is also a protected area (Category VI: Protected Area with sustainable use of natural resources) under the management of Selangor State Foresty Department.

## Method

## Sampling procedures and data analysis

The sampling of lichens was carried out at five sites along the trail that leads to the summit of Gunung Nuang. The environmental conditions of the study sites, such as temperature, humidity and altitude, were recorded (see Table 1). The sampling plot at each site was determined using a stratified random sampling method for an area of 20 m x 20 m, or 400 m<sup>2</sup>, the size being based on a preliminary site investigation, as recommended by Khaien et al. (2018). Only epiphytic lichens were collected; the coordinates of the lichens were noted.

The lichen samples were taken to the Algae Lab at the National University of Malaysia (UKM) for identification by observing their morphological traits under stereoscopic and optical microscopes. The external characteristics of the lichens, such as the lobes, thallus, pycnidia, rhizines, ciliates and apothecia, were analysed thoroughly (Nimis et al. 2002). A spot test was used to determine the presence of acid (the lichen's secondary metabolites) in the medulla and cortex (Sipman 2009). All the data acquired from both techniques (morphological observation and spot test) were recorded and incorporated into the Lichen Herbarium of UKM, Malaysia.

Lichen species richness and composition were calculated and presented as percentages of the total



Figure 3 – Simpson's Diversity Index.

number of samples, using a cluster bar chart. Next, an independent t-test was used to examine the difference in species composition between lower- and higher-frequency species (Sevgi et al. 2019). Species diversity was analysed using the two most common diversity indices - the Shannon-Weiner Index and Simpson's Diversity Index. The Shannon-Weiner Index is more useful in identifying rare species; Simpson's Diversity Index is more useful and effective for more abundant species (Simpson 1949; Barnes et al. 1998). The Similarity Index was used to investigate which lichens were endemic to certain sites (according to altitudinal difference). Correspondence Analysis (CA) was used to illustrate the variation in the species distribution and abundance along the elevational gradient in Gunung Nuang, Selangor (Benzecri 1973). All the statistical analyses were performed using the R program version 4.0, and SPSS statistical software version 23.0.

### Results

#### Lichen species diversity

A total of 44 lichen species belonging to 11 families were collected from Gunung Nuang, Selangor. The highest number of species collected was at site 5 (35 species), followed by Site 4 (32), Site 3 (31), Site 2 (25), and lastly Site 1 (21).

The diversity of lichen species was determined using the Shannon-Weiner Index (Figure 2) and Simpson's Diversity Index (Figure 3). The diversity varied significantly among sampling sites (ANOVA, p < 0.05), with Site 5 having the greatest diversity, showing that lichen species diversity tends to increase with increased altitude.

#### Lichen species composition and distribution

The lichen species composition and distribution varied within and among sampling sites. Bar charts were produced to analyse lichen distribution and composition for each of the sampling sites (Figure 4: Site 1; Figure 5: Site 2; Figure 6: Site 3; Figure 7: Site 4; Figure 8: Site 5). Lichen species from Graphidaceae, Physiaceae, Pyrenulaceae and Chryptotaceae were found at all sampling sites, but the particular species found varied from site to site. Some species were found at only one site – for example, *Chrysothrix xanthina* at Site 1, and *Parmotrema mellissi* and *Parmelia sulcata* at Site 5.







Figure 5 – Distribution of lichen species for Site 2 (301–600m).



Figure 6 – Distribution of lichen species for Site 3 (601–900m).



Figure 7 – Distribution of lichen species for Site 4 (901–1200m).

As shown in Figure 4, there are two dominant species at Site 1, namely *Graphis librata* and *Pyrenula ochraceoflava* (both 12.7% of samples). These are followed by *Dirinaria applanata* (10.5%), *Dirinaria picta* (9.0%), *Chrysothrix xanthina* (7.7%), *Graphis casiella* (6.6%), *Gra-* phis hiascens (6.6%), Dirinaria aegialita (5.0%), and Pyxine berteriana (5.0%). Other species – Phaegraphis caesioradians (1.1%), Rinodina oxydata (1.1%), Ocellularia papilillata (0.5%) – had percentages significantly below 5%. The majority of the samples from Site 1 were from the



Figure 8 – Distribution of lichen species for Site 5 (1201–1493 m).

Table 2 – The Similarity Index data for each sampling site

		2	5	1	0
	Site 1	Site 2	Site 3	Site 4	Site 5
Site 1		0.570681	0.433249	0.331155	0.28833
Site 2	0.570681		0.623501	0.480167	0.424508
Site 3	0.433249	0.623501		0.663968	0.631356
Site 4	0.331155	0.480167	0.663968		0.816479
Site 5	0.28833	0.424508	0.631356	0.816479	

crustose group, and only *Chrysothrix xanthina* was from the leprose group. Graphidaceae and Physciacea are the dominant lichen families for Site 1, both families being represented there by at least five species.

At Site 2, as Figure 5 shows, the most dominant species was again *Graphis librata*, with 12.4%. This was followed by *Dirinari picta* (11.44%), *Graphis scripta* (10.44%), *Graphis furcata* (7.0%), *Graphis hiascens* (6.97%), *Pyrenula ochraceoflava* (6.97%), *Dirinaria applanata* (6.47%), *Graphis caesiella* (5.0%), and *Graphis glaucescens* (5.0%). The lowest figures for Site 2 were for *Phaeographis leioplacella* (0.5%) and *Pyrenula petivica* (0.99%). The majority of the species collected at the site were from the crustose lichen group. Graphidaceae and Physciaceae are again the most dominant families here, represented by at least 5 species each.

Figure 6 shows the composition distribution of lichen species for Site 3. The most dominant species was *Graphis caesiella*, with 10.19%, followed by *Dirinaria picta* (8.8%), *Graphis furcata* (7.41%), *Trypethelium tropicum* (6.02%), *Cryptothesia granularis* (6.02%), and *Graphis librata* and *Pramotrema praesorediosum* (both 5.56%). *Coccocarpia erythroxyli* and *Cryptothesia striata* were among the lichen species that scored less than 5%. The majority of the lichens found here belong to the crustose group, but foliose lichens such as *Coccocarpia erythroxyli* and *Pramotrema praesorediosum* were also collected at this site. The most dominant family was Graphidaceae (12 species found); 2 species of Parmeliaceae and 1 of Coccocarpiaceae were also found at Site 3.

Site 4 recorded 32 lichen species (see Figure 7). The most dominant species here was *Graphis caesiella* (7.81%), followed by *Graphis librata* (7.58%), *Trypethelium epileucodes* (7.19%), *Lepraria usnica* (6.47%), *Graphis scripta* (6.12%), and *Trypethelium tropicum* (5.4%). *Coc*-

*cocarpia erythroxyli* and *Parmotrema tinctorum* both accounted for less than 5% of samples. Crustose lichens dominated, but the foliose group was also represented by several species. Graphidaceae was the dominant lichen family at Site 4; other species belonged to the Collemataceae family.

Figure 8 shows the lichen species composition and distribution for Site 5. The most dominant species was *Parmotrema praesorediosum* (8.59%), followed by *Trypethelium epileucodes* (7.42%), *Trypethelium eluteriae* (6.64%), *Graphis caesiella* (6.25%), *Coccocarpia pellita* and *Lepraria usnica* (5.08%). *Bulbothrix isidiza* and *Parmotrema mellissii* each accounted for less than 5% of samples. Both crustose and foliose lichen groups were found abundantly. Graphidaceae was the dominant lichen family, but Parmeliaceae were also well represented.

### The Similarity Index for the sampling sites

The Similarity Index (Table 2) showed how the sites differed according to the presence or absence and abundance of species. The similarity between Sites 4 and 5 was higher than for Sites 3 and 4, or for Sites 3 and 5. Sites 1 and 5 have the lowest similarity (less than 30%).

The sampling sites can be classified into two main groups, based on their ecological distances. A dendrogram (Figure 9) shows that the ecological distances between Sites 3 and 5, and between Sites 1 and 2 were the closest. Thus altitudes of 601–1 493 m can be considered as one group, with altitudes of 0–600 m falling into a separate group.

## Effects of altitude on lichen species diversity, composition and distribution

Lichen diversity, composition and distribution varied according to altitude, and environmental conditions such as humidity and temperature. The differences between lichen diversity and abundance correlated significantly with the location's altitude, temperature and humidity. Altitude and environmental factors had a stronger effect on species distribution from 0 to 1493 m. Correspondence analysis (CA) showed that there was a very clear correlation between species diversity and distribution on the one hand, and sam-



Figure 9 – Dendrogram showing the similarity of species abundance for all sites.

pling site altitude on the other (Figure 10). The first and second axes of CA show more than 60% of the total fitted variation. The weighted average scores for lichen diversity at different altitudes vary greatly, with the abundance and distribution of lichens spreading into different quarters of the ordination graph. Where a sampling point in the graph is located close to a particular species, that species is expected to be highly abundant and frequent at the sampling point. For example, Chrysothrix xanthina, Pyxine cocoes, Pyxine berteriana, Hyperphyscia adglutinata and Dirinaria aegialita are located close to Site 1, which means that these species are abundant only at Site 1; a higher altitude than that of Site 1 was not an appropriate condition and habitat for this species. It should be noted that a small vector angle between species corresponds to a strongly positive association, and a 180° vector angle between species corresponds to a negative correlation.

## Discussion

Lichen species that belong to the Graphidaceae and Physciaceae families were present at all forest altitudes (Sites 1-5), but the specific species that emerged were different. For example, among the Graphidaceae Phaeographis intricans and Graphis japonica were present only at 601-1 493 m (Sites 3-5), while Heterodermia japonica, of the Physciaceae family, was found only at 901-1493 m (Sites 4 and 5). On the other hand, Dirinaria aegialita, Pyxine cocoes, Pyxine berteriana and Hyperphyscia adglutinata (all Physciaceae) were present at 0-300 m (Site 1) only. Similarly, Chrysothrix xanthina (a leprose lichen) was found only at 0-300 m. These differences can be explained by the differing environmental factors between altitudes. For example, altitudes of 0-300 m are lowland areas, which are vulnerable to air pollution and human activities. Anthropogenic activities lead to increased nitrogen and sulphur in the atmosphere, and only acidophile and nitrophile types of lichens can survive in high concentrations of these elements. Parmeliaceae, Coccocarpiaceae and Collemataceae species were present only at high altitudes (601–1493 m; Sites 3–5). These three families are foliose-type lichens, which only grow at low temperatures in areas of high humidity. A study by Sipman (2009)



Figure 10 - CA showing the relationship between species distribution and elevation for each sampling site.

concluded that foliose-type lichens can grow abundantly at higher altitudes in regions with tropical climates. In addition, most of the Parmeliaceae, such as *Parmotrema tinctorum*, *Parmotrema mellissii* and *Coccocarpia pellita*, are highly sensitive to air pollution, which is why these species are found commonly only in pristine and remote forests.

Species distribution correlated strongly with climatic conditions, especially temperature and relative humidity. Furthermore, the abundance of species has been proved to vary according to altitudinal zone. Our study found that Chrysothrix xanthina, Pyxine cocoes, Pyxine berteriana, Dirinaria aegialita, Rinodina oxydata and Hyperphyscia adglutinata grew exclusively at altitudes of 0-300 m, with average coverage of 33%. Dirinaria picta, Dirinaria applanata and Physcia atrostriata grew in abundance at 301-600 m, where they were 55% more abundant than at lower altitudes. Graphis japonica and Cryptothesia striata were found exclusively at 601-900 m, with average coverage of 44% for both species. Several Parmeliaceae, Coccocarpiacea and Collemataceae species, such as Parmotrema tinctorum, Parmotrema praesorediosum, Parmotrema mellissii, Bulbothrix isidiza, Parmelia sulcata, Coccocarpia pellita, Coccocarpia erythroxyli and Collema poeltii, were found thriving at 901 m and above, with average coverage of 53%.

Our study found that some lichen species, such as *Cryptothesia granularis*, *Ocellularia crocea, Graphis librata* and *Graphis caesiella*, were present at almost all altitudes. This is in agreement with the findings of Zulkifly et al. (2011), who found that some Graphidaceae and Cryptothesiacea species were common in montane forest at all altitudes. This may be because of the ability of this lichen family to adapt, making it less vulnerable to its surroundings even if there are changes in the quality of the environment (Abas et al. 2018).

Geographically, tropical forests are located in equatorial regions (Khamis & Nizam 2013). This gives the forests a unique structure which makes them suitable habitats for thousands of endemic bryophyte and lichen species (Sevgi et al. 2019). The number of lichen species found in Gunung Nuang, Selangor (44 species) was significantly higher than that found for the montane forest at Gunung Machincang, Langkawi (33 species) (Zulkifly et al. 2011). Somewhat fewer lichen species again were found in Araucaria forest of Southern Brazil (27 species) (Kaffer et al. 2009). The varying numbers of lichen species present in different areas may be due to physical geographical boundaries and climatic variation.

## Conclusion

In this study, we found that lichen species diversity, composition and distribution in Gunung Nuang, Selangor increase with the montane forest's altitude. The dominant lichen species also varied according to altitude. Although the diversity does not change significantly between 0 and 600 m (Sites 1 and 2), at altitudes of 601-1493 m (Sites 3-5) it did do so, with the presence here of a few endangered lichen species. Given the link between altitudinal gradient and environmental factors (lower temperature and higher humidity at higher altitudes) which are essential for lichen growth and forest productivity, our study's findings are consistent with earlier studies that show a positive correlation between lichen species and forest altitude. The study also verified that some lichen species present in Gunung Nuang, Selangor, such as Parmotrema mellissii, Collema poeltti and Coccocarpia pellita, are threatened by the loss of forest and by human activities. These species need to be protected and managed to ensure their survival in this tropical montane forest.

Our findings add to knowledge of Malaysia's lichens, and have implications for phytogeography and ecology, especially montane forest ecology, giving insight into how elevation gradient and variation of environmental factors can determine the diversity, composition and distribution of lichens. The results of our study will thus be of use in determining appropriate management techniques in tropical regions more widely.

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