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Ferns richness along environmental gradients in a tropical forest ecosystem

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Abstract
The fern species richness assessment was done along environmental gradients in Ayer Hitam forest reserve, Johor, Peninsular Malaysia. This was done to ascertain the distributional pattern of the ferns as influenced by the environmental gradients in this forest. The collection of the fern species was done using a preferential non-random sampling technique. Temperature, humidity, light intensity, and elevation data were obtained at each point of collection of the ferns. The influence of these environmental gradients on the distribution of the species was determined using detrended correspondence analysis (DCA) and general linear model (GLM). A total of 67 ferns were identified at all the study sites. Two ferns namely *Asplenium antiquum* and *Pteris longipinnula* were first recorded in this study. In all the sites, light intensity ranges from 337.9 – 8098 lux, temperature ranges from 28.5 - 33°C, humidity ranges from 66.24 - 88.80%, and elevation ranges from 14–33m. *Tectaria singaporeana* and *Nephrolepis biserrata* are the only species that are widely distributed across all the study sites. The DCA revealed that the light intensity influences the distribution of the fern species in this forest. However, the GLM showed that temperature had a positive influence on the distribution of the ferns while light intensity had a negative influence on them.

Keywords: Ferns diversity; Microclimate; Muar; Peninsular Malaysia; *Tectaria singaporeana*

Introduction

Ferns and lycophytes are known to be the second largest among the different groups of vascular plants due to having about 11000 species (PPG, 2016). They are important ecological components of tropical forests by constituting more than 20% of the total plant cover (Linares-Palomino et al., 2008; Salazar et al., 2015). The diversity of ferns and lycophytes in biotic communities has been described as closely related to the environmental gradients of such communities and can be used as bioindicators of environmental stability (Zuquim, 2015). The availability of water (high humidity), light intensity, and topography are mostly strongly correlated with the richness and diversity of ferns in many tropical and sub-tropical regions of the world (Krömer et al., 2013; Salazar et al., 2015). Although these ferns-environmental gradients relationships are still very complex and lots need to be done to fully understand the mechanisms involved (Kessler et al., 2014).

Studies on the distribution of plants along elevation and climatic gradients have been more focused on angiosperms, only a few have addressed the influence of these environmental gradients on fern species distribution (Kessler 2000, 2001; Hemp, 2002). The elevational gradient has been used to assess the relationship between species ecology and their responses to climatic changes in the environments (Korner, 2000). In other words, microclimatic factors such as temperature, light intensity, and humidity explain the distribution of fern species along an elevation gradient (Othman *et al.*, 2015). The distribution of ferns along elevation gradients has revealed that fern species are richer at mid-elevation gradients comprising more epiphytes than terrestrials (Kessler et al., 2011; Krömer et al., 2013).

All these environmental factors are products of global climate change which has been reported to be one of the major factors responsible for the habitat change of many plants across the world (Sharpe, 2019). Most importantly, an increase in global earth temperature has been predicted to lead to a greater loss of plants' distributional ranges (Warren et al., 2018). Therefore, studies on the fern species distribution along environmental gradients can be used to indicate the quality of forests (Othman et al., 2015). This study aimed to assess the diversity and richness of ferns along environmental gradients in Ayer Hitam forest, Johor, Malaysia. Their distribution patterns as influenced by the environmental conditions and their conservation statuses were elucidated in this study.

Material and methods

Study area

This scientific expedition was carried out in Ayer Hitam forest, which is a lowland reserved forest located in Muar, Johor, Malaysia (Fig. 1). Muar (Bandar Maharani) is known to be one of the most tourist-visited cities in Johor, Malaysia. This town has an approximate land mass of 234,612 ha out of which 83% of it was reported to be covered by natural forests in the year 2010. In the year 2019, almost 138ha of its natural forests were lost due to several anthropogenic factors (https://www.globalforestwatch.org). Muar can be described as a city with a year-round tropical climate. The mean monthly temperature ranges between 21.4 and 32.7ºC. The city's mean monthly precipitation ranges from 112 to 238 mm. The people of Muar engage in the cultivation of some major cash and food crops including rubber, oil palm, coconut, cocoa, fruits, and vegetables. Besides these, they are also involved in fish, poultry, and livestock farming.

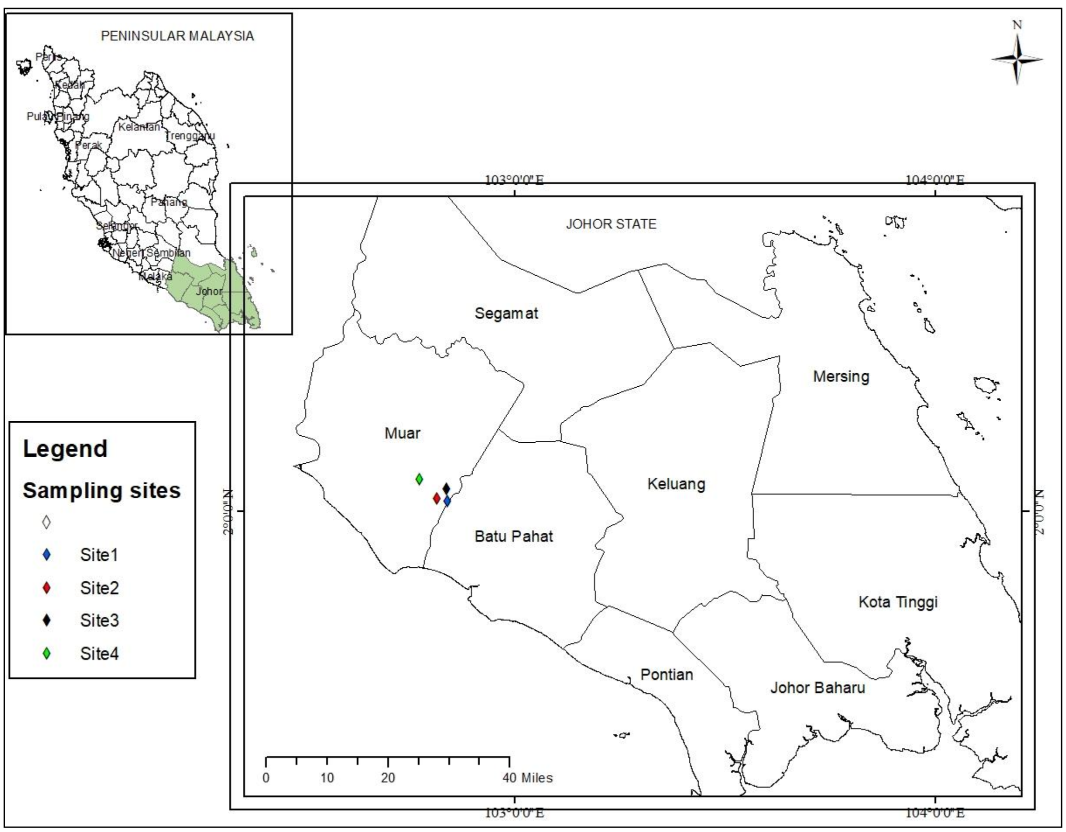


Figure 1. Study area map of the forest sites in Johor, Malaysia

Plant Collection and Identification Techniques

The collection of the ferns was done using the preferential non-random sampling technique as described by Akomolafe and Rahmad (2019). Four different sampling sites were chosen for study within the forest based on ease of accessibility and based on the presence of any fern species there. The complete and healthy fern fronds were collected and identified. At each point of plant collection, the light intensity and temperature were measured with an Urceri MT-912 light meter. Relative humidity was measured with a HT-86 humidity meter. Elevation and geographical coordinates of the sampling sites were measured using a hand-held Global Positioning System (GPS) device. The taxonomic identities, naturalization, and conservation status of the fern species were established using the flora of Peninsular Malaysia (Parris et al., 2010, 2013, 2020), fern flora of Malaysia (Piggott, 1988), the International Plant Names Index (IPNI, 2020), Malaysia Biodiversity Information System (MyBIS), and International Union for Conservation of Nature and Natural resources (IUCN). The voucher specimens of the collected and identified ferns were deposited at the herbarium of the Universiti Sains Malaysia.

Statistical analyses

The fern species richness of the forests was estimated using an incidence-based rarefaction and extrapolation analysis. This was accomplished with 500 bootstrap replicates using iNEXT software (Chao et al., 2016). The 95% confidence intervals of the species accumulation curves were used to determine the significant difference in the species richness. An overlap in the confidence intervals shows there is no significant difference in the species richness between the sites. This analysis is important because it quantifies the actual number of species in the sampled sites which might not have been adequately observed on the field. The similarity of the sites in terms of the fern species found there was analyzed using a Bray-Curtis index with a single linkage algorithm in BioDiversity Pro software. Principal component analysis (PCA) was done to see the pattern of variations in the distribution of the ferns at the different sites. Detrended correspondence analysis (DCA) which is a multivariate analysis used by ecologists for arranging multiple species along environmental gradients was employed in this study. This analysis was able to isolate the important environmental factors closely related to the distribution of the fern species. In order to have further robust explanations of the factors that drive the fern species distribution along the environmental gradients, we did a simple general linear model (GLM) with the species richness as a response variable and the environmental factors as an explanatory variable. The PCA, DCA, and GLM were achieved using PAST 3 software.

Results

Influence of the Environmental Gradients on the Ferns richness and Diversity

Thirty-nine (39) fern species were identified at site 1 with the following ranges of environmental factors; light (337.9 – 8098 lux), temperature (30.1 – 32.5^{o}C), humidity (76.40 – 84.27 %) and elevation (14 – 28m). At site 2, fifteen (15) fern species were identified along the following ranges of environmental factors; light (414.9 lux), temperature (33^{o}C), humidity (66.24%), and elevation (15m). Also, at site 3, fourteen (14) fern species were observed along the following environmental factors; light (588.8 – 607.2 lux), temperature (28.5 – 29.4^{o}C), humidity (84.16 – 88.80%) and elevation (18 – 27m). At site 4, ten (10) fern species were observed having the following environmental ranges; light (1423 lux), temperature (29.0^{o}C), humidity (81.08%), and elevation (33m). In all the sites, a total of 67 fern species belonging to 14 families were enumerated (Table 1). Aspleniaceae and Lindsaeaceae had the highest number of species (12 and 11 respectively) while Hypodematiaceae and Marattiaceae are represented by only one species each (Fig. 2). *Tectaria singaporeana* is the only species found common to all the study sites. *Nephrolepis biserrata* is also the next species which was found common in only three out of the four study sites. The incidence-based rarefaction and extrapolation species curve shows that site 1 had the highest estimated species richness (90) which is significantly different from all the other sites (Fig. 3). Sites 2, 3, and 4 had the species richness of 30, 35 and 20 respectively. As for the variations in the distribution of the species, the principal components (PCs) 1 and 2 accounted for a total variation of 47.15% and 29.47% respectively (Fig. 4). Site 1 and Site 3 significantly contributed to PC 1 while site 2 significantly contributed to PC 2. All the species encountered are within the 95% eclipse. The similarity among the sites in terms of the fern species is shown in the dendrogram (Fig. 5). This revealed a close relationship between site 2 and site 4 with the highest similarity index of 30%. The DCA analysis indicated that all the fern species in this forest were influenced more along the light intensity gradient than along other environmental factors measured (Fig. 6). The GLM revealed that the light intensity (Figure 7A, r^2 = 0.009, P = 0.428, slope = -249.3, intercept = 1598), humidity (Figure 7B, r^2 = 0.003, P = 0.645, slope = -0.557, intercept = 80.61) and elevation (Figure 7C, r^2 = 0.000, P = 0.835, slope = -0.17, intercept = 17.62) had inverse effects on the distribution of the ferns. However, the temperature exhibited a direct relationship with the distribution of the ferns (Figure 7D, $r^2 = 0.007$, $P = 0.491$, slope = 0.161, intercept = 30.86).

Table 1. The presence/absence of fern species at the study sites
√ represents presence, X represents absence

S/N	NAMES	FAMILY	SITE 1	SITE 2	SITE 3	SITE 4
1	*Adiantum aethiopicum* L.	Adiantaceae	√	X	X	X
2	*Adiantum capillus-veneris* L.	Adiantaceae	X	X	√	X
3	*Adiantum trapeziforme* L.	Adiantaceae	√	X	X	X
4	*Angiopteris evecta* (Forst.) Hoffm.	Marattiaceae	√	√	X	X
5	*Arachniodes haniffii* (Holtt.) Ching	Dryopteridaceae	√	X	X	X
6	*Asplenium antiquum* Makino	Aspleniaceae	√	X	X	X
7	*Asplenium australasicum* Hook.	Aspleniaceae	√	X	X	X
8	*Asplenium batuense* Alderw.	Aspleniaceae	X	X	X	√
9	*Asplenium caudatum* G. Forst.	Aspleniaceae	√	X	X	X
10	*Asplenium nitidum* Sw.	Aspleniaceae	X	√	X	X
11	*Asplenium longissimum* Blume	Aspleniaceae	X	X	√	X
12	*Asplenium nidus* L.	Aspleniaceae	√	√	X	√
13	*Asplenium normale* Don	Aspleniaceae	X	X	√	X
14	*Asplenium polyodont* G. Forst.	Aspleniaceae	X	√	X	X
15	*Asplenium robustum* Blume	Aspleniaceae	X	√	X	X
16	*Asplenium scortechinii* Bedd.	Aspleniaceae	X	√	X	X
17	*Asplenium belangeri* Kunze	Aspleniaceae	X	X	X	√
18	*Blechnum orientale* L.	Blechnaceae	√	X	X	X
19	*Blechnum patersonii* (R.Br.) Mett.	Blechnaceae	X	X	√	X
20	*Bolbitis heteroclita* (C. Presl) Ching	Dryopteridaceae	√	X	X	X
21	*Bolbitis lonchophora* (Kunze) C.Chr.	Dryopteridaceae	X	√	X	X
22	*Bolbitis quoyana* (Gaudich.) Ching	Dryopteridaceae	√	X	X	X
23	*Bolbitis sinuata* (C. Presl.) Hennipman	Dryopteridaceae	√	X	X	X
24	*Bolbitis virens* (Hook. & Grev.) Hennipman	Dryopteridaceae	√	X	X	X
25	*Coryphopteris viscosa* (Baker) Holttum	Thelypteridaceae	X	X	√	X
26	*Davallia divaricata* Blume	Davalliaceae	X	√	X	X
27	*Davallia denticulata* (Burm. f.) Mett.	Davalliaceae	√	X	X	X
28	*Davallia solida* (G. Forst.) Sw.	Davalliaceae	√	X	X	X
29	*Dicranopteris linearis* var. *linearis* (Burm.f.) Underwood	Gleichenaceae	√	X	X	X
30	*Drynaria rigidula* (Sw.) Bedd.	Polypodiaceae	√	X	X	X
31	*Drynaria sparsisora* (Desv.) T. Moore	Polypodiaceae	X	√	X	X
32	*Dryopteris sparsa* (Don) Kuntze	Dryopteridaceae	√	X	X	√
33	*Heterogonium pinnatum* (Copel.) Holttum	Tectariaceae	√	X	X	X
34	*Leucostegia pallida* (Mett.) Copel.	Hypodematiaceae	X	√	X	X
35	*Lindsaea bouillodii* Christ	Lindsaeaceae	√	X	X	X
36	*Lindsaea ensifolia* Sw.	Lindsaeaceae	√	X	X	X
37	*Lindsaea lancea* (L.) Bedd.	Lindsaeaceae	√	X	X	X
38	*Lindsaea lucida* Bl.	Lindsaeaceae	X	X	X	√
39	*Lindsaea oblanceolata* Alderw.	Lindsaeaceae	√	X	X	X
40	*Lindsaea orbiculata* (Lam.) Mett.	Lindsaeaceae	X	X	X	√
41	*Lindsaea rigida* J.Sm.	Lindsaeaceae	√	X	X	X
42	*Lindsaea scandens* Hook.	Lindsaeaceae	√	X	X	X
43	*Microlepia speluncae* (L.) Moore	Lindsaeaceae	X	√	X	X
44	*Nephrolepis biserrata* (Sw.) Schott	Nephrolepidaceae	√	√	X	√
45	*Nephrolepis cordifolia* (L.) Presl	Nephrolepidaceae	X	√	X	√
46	*Nephrolepis exaltata* (L.) Schott	Nephrolepidaceae	√	X	X	X
47	*Nephrolepis undulata* (Sw.) J.Sm.	Nephrolepidaceae	√	X	X	X
48	*Odontosoria chinensis* (L.) J.Sm.	Lindsaeaceae	√	X	X	X
49	*Osmolindsaea odorata* (Roxb.) Lehtonen & Christenh.	Lindsaeaceae	√	X	X	X

50	*Pityrogramma calomelanos* (L.) Link	Pteridaceae	X	√	X	X
51	*Pteridrys australis* Ching	Tectariaceae	X	X	√	X
52	*Pteris cretica* L.	Pteridaceae	X	X	√	X
53	*Pteris longipinnula* Wall.	Pteridaceae	X	X	√	X
54	*Pteris semipinnata* L.	Pteridaceae	X	X	√	X
55	*Pteris vittata* L.	Pteridaceae	√	X	X	X
56	*Pyrrosia lanceolata* (L.) Farw.	Polypodiaceae	√	X	X	√
57	*Pyrrosia longifolia* (Burm. f.) Morton	Polypodiaceae	X	X	√	X
58	*Pyrrosia piloselloides* (L.) M.G. Price	Polypodiaceae	X	X	√	X
59	*Sticherus truncatus* (Willd.) Nakai	Gleicheniaceae	√	X	X	X
60	*Tectaria coadunata* (J.Sm) C. Chr.	Tectariaceae	√	X	X	X
61	*Tectaria crenata* Cav.	Tectariaceae	√	X	X	X
62	*Tectaria decurrens* (Pr.) Copel	Tectariaceae	√	X	X	X
63	*Tectaria grandidentata* (Ces.) Holttum	Tectariaceae	√	X	X	X
64	*Tectaria impressa* (Fee) Holttum	Tectariaceae	X	X	√	X
65	*Tectaria oligophylla* (Rosenst.) C. Chr.	Tectariaceae	√	X	X	X
66	*Tectaria singaporeana* (Wall. ex Hook. & Grev.) Copel.	Tectariaceae	√	√	√	√
67	*Thelypteris paleata* (Copel.) Holttum	Thelypteridaceae	X	X	√	X

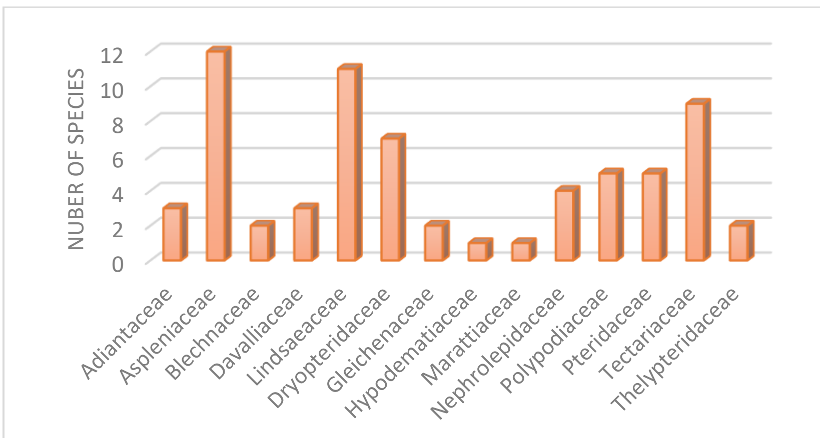


Figure 2. The number of species in each of the ferns families

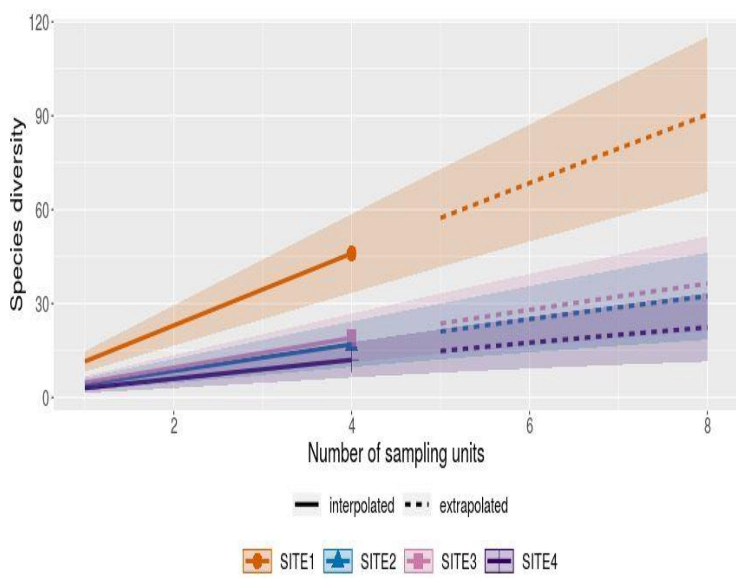


Figure 3. Incidence-based rarefaction and extrapolation curve for the fern species richness of the sampling sites

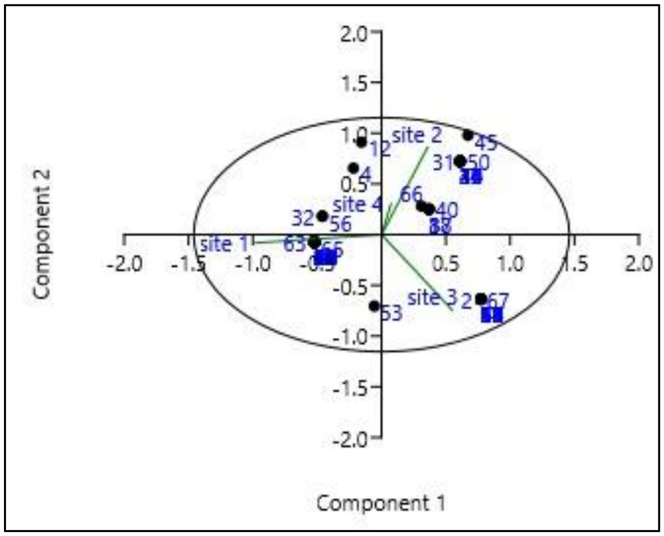


Figure 4. PCA showing the variations in the distribution of the ferns at different sites. The species numbers (1-67) represent each fern species as presented in Table 1

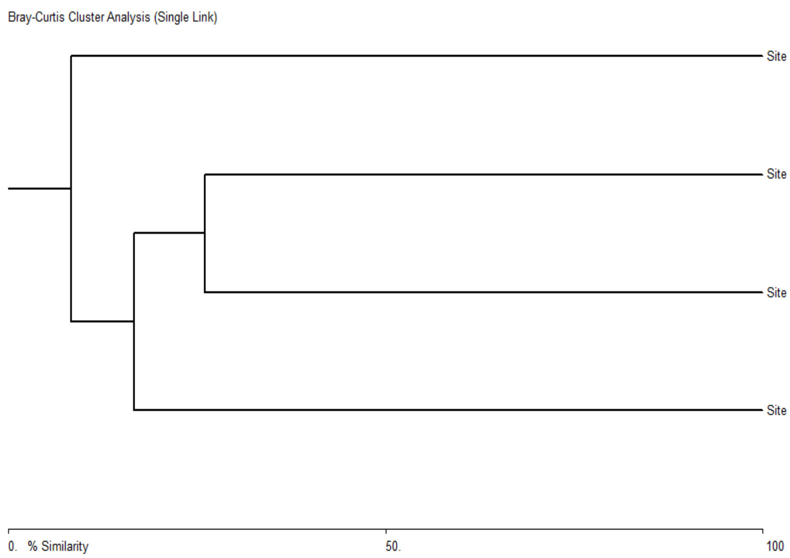


Figure 5. The similarity index of the study sites

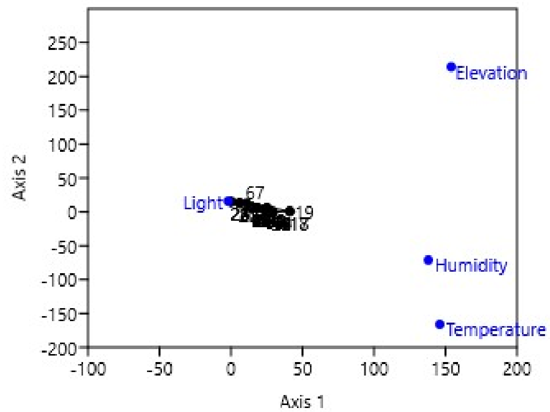
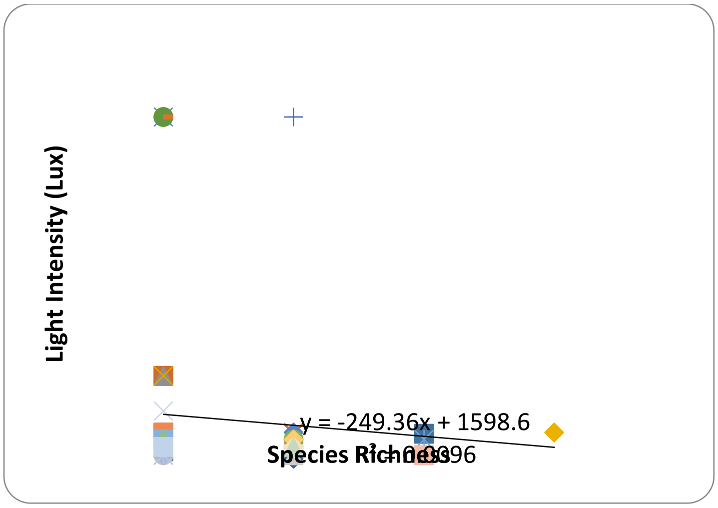


Figure 6. Detrended correspondence analysis of the fern species along environmental gradients in the study sites



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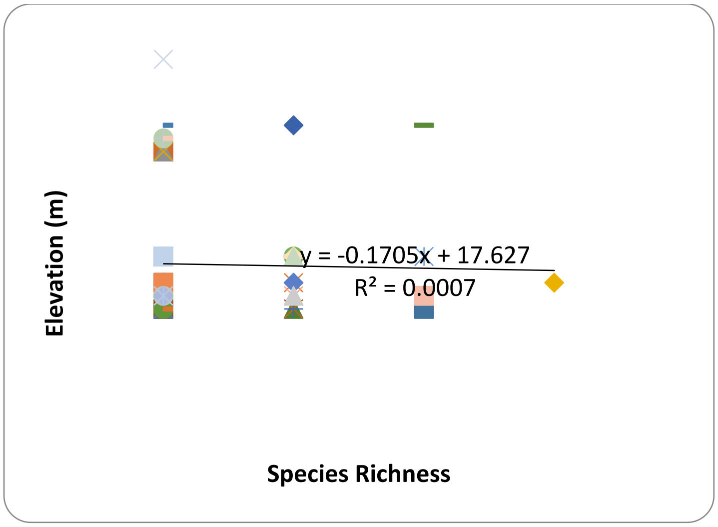
7B



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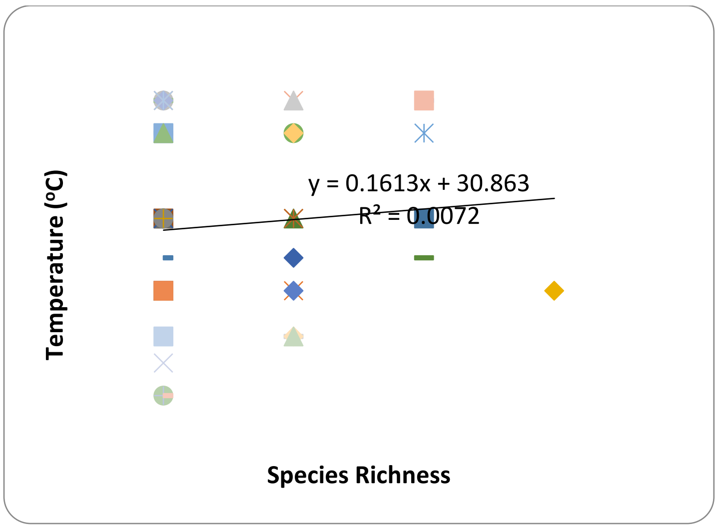


7D

Figure 7. General linear models showing the effects of the environmental gradients: (A) Light Intensity on the fern species richness (B) Humidity on the fern species richness (C) Elevation on the fern species richness (D) Temperature on the fern species richness.

Conservation Statuses of the Ferns

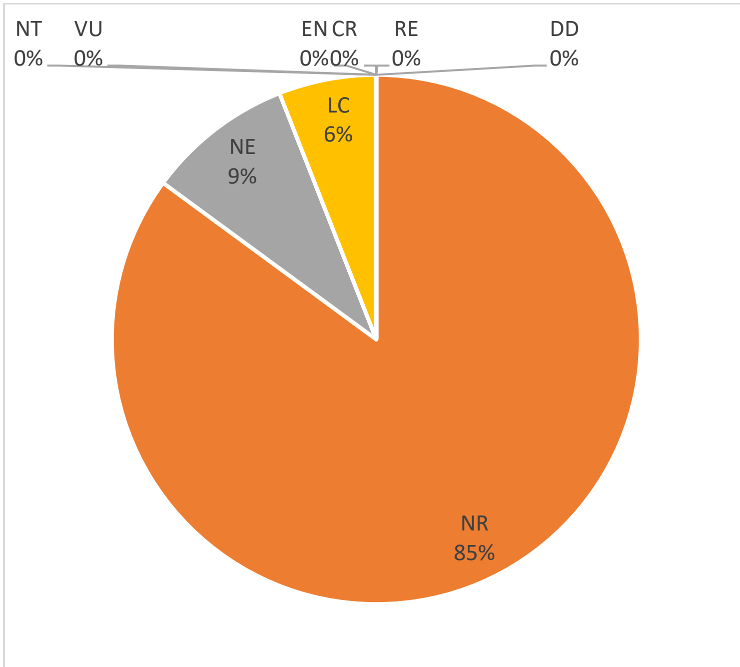
The IUCN red list status of all the observed ferns in this study revealed only four ferns namely *Adiantum capillus-veneris*, *Nephrolepis undulata*, *Pteris vittata*, and *Dicranopteris linearis* as least concern (Table 2). Others were categorized as either not recorded or not evaluated and this amounts to 85% and 9% respectively (Fig. 8A). On the contrary, the Malaysia biodiversity red list assessment status showed that 66% of the ferns are not evaluated, 4% have no records, 27% are least concern, 2% is data deficient and 1% is near threatened (Fig. 8B). Out of all these, two ferns namely *Asplenium antiquum* and *Pteris longipinnula* were first mentioned in this study.

Table 2. Conservation and native status of the ferns at the Johor Forest

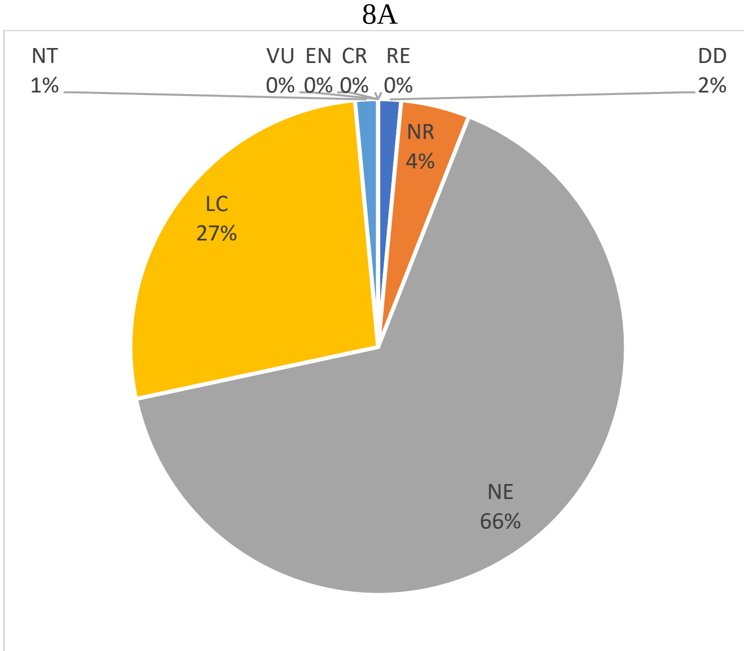
S/N	Species	IUCN Redlist Status 2020	Peninsular Malaysia Redlist status 2020	Native to Malaysia?
1	*Adiantum aethiopicum*	NE	NE	X
2	*Adiantum capillus-veneris*	LC	LC	√
3	*Adiantum trapeziforme*	NE	NE	X
4	*Angiopteris evecta*	NE	LC	√
5	*Arachniodes haniffii*	NE	NE	√
6	*Asplenium antiquum*	NE	NR (1st mention)	X
7	*Asplenium australasicum*	NE	LC	√
8	*Asplenium batuense*	NR	NE	√
9	*Asplenium caudatum*	NR	LC	√
10	*Asplenium nitidum*	NR	LC	√
11	*Asplenium longissimum*	NR	LC	√
12	*Asplenium nidus*	NR	LC	√
13	*Asplenium normale*	NR	NE	√
14	*Asplenium polyodont*	NR	LC	√
15	*Asplenium robustum*	NR	LC	√
16	*Asplenium scortechinii*	NR	LC	√
17	*Asplenium belangeri*	NR	NE	√
18	*Blechnum orientale*	NR	NE	X
19	*Blechnum patersonii*	NR	NE	√
20	*Bolbitis heteroclita*	NR	NR	√
21	*Bolbitis lonchophora*	NR	NE	X
22	*Bolbitis quoyana*	NR	NE	√
23	*Bolbitis sinuata*	NR	NE	√
24	*Bolbitis virens*	NR	LC	√
25	*Coryphopteris viscosa*	NR	LC	√
26	*Davallia divaricata*	NR	NE	√
27	*Davallia denticulata*	LC	NE	√
28	*Davallia solida*	NR	NT	X
29	*Dicranopteris linearis* var. *linearis*	NR	LC	√
30	*Drynaria rigidula*	NR	LC	√
31	*Drynaria sparsisora*	NR	NE	√
32	*Dryopteris sparsa*	NR	NE	√
33	*Heterogonium pinnatum*	NR	NE	√
34	*Leucostegia pallida*	NR	DD	√
35	*Lindsaea bouillodii*	NR	NE	X
36	*Lindsaea ensifolia*	NR	NE	√
37	*Lindsaea lancea*	NR	NE	X
38	*Lindsaea lucida*	NR	NE	√
39	*Lindsaea oblanceolata*	NR	NE	√
40	*Lindsaea orbiculata*	NR	NE	√
41	*Lindsaea rigida*	NR	NE	√
42	*Lindsaea scandens*	NR	NE	√
43	*Microlepia speluncae*	NR	NE	X
44	*Nephrolepis biserrata*	NR	LC	√
45	*Nephrolepis cordifolia*	NR	NE	√
46	*Nephrolepis exaltata*	NR	NE	X
47	*Nephrolepis undulata*	LC	NE	X
48	*Odontosoria chinensis*	NR	NE	√
49	*Osmolindsaea odorata*	NR	NE	X
50	*Pityrogramma calomelanos*	NR	NE	√
51	*Pteridrys australis*	NR	NE	√
52	*Pteris cretica*	NR	NE	√
53	*Pteris longipinnula*	NR	NR (1st mention)	X
54	*Pteris semipinnata*	NR	NE	√
55	*Pteris vittata*	NR	NE	√
56	*Pyrrosia lanceolata*	LC	LC	√
57	*Pyrossia longifolia*	NR	LC	√
58	*Pyrrosia piloselloides*	NR	LC	√
59	*Sticherus truncates*	NR	NE	X
60	*Tectaria coadunata*	NR	NE	√

61	*Tectaria crenata*	NR	NE	√
62	*Tectaria decurrens*	NR	NE	√
63	*Tectaria grandidentata*	NR	NE	√
64	*Tectaria impressa*	NR	NE	√
65	*Tectaria oligophylla*	NR	NE	√
66	*Tectaria singaporeana*	NR	NE	X
67	*Thelypteris paleata*	NR	NE	√

KEY: RE – Regionally Extinct; CR - Critically endangered; EN – Endangered; VU – Vulnerable; NT – Near Threatened; LC – Least Concern; DD – Data deficient,; NE – Not Evaluated; NR – No Record.



8A



8 B

Figure 8. Percentage distribution of the conservation status of the ferns (A) IUCN status (B) Malaysia status

Discussion

The distribution of *Tectaria singaporeana* and *Nephrolepis biserrata* at most of the study sites shows that they are species that could adapt to varying degrees of microclimates. The wide difference between the observed and estimated species richness of the fern species in these sites revealed the true state of what is available in the field and what could have been observed if the sampling was adequate (Zuquim, 2015). The slight influence of humidity on the ferns observed in this study supported the hypothesis that fern species richness and diversity in tropical forests are promoted by water availability (Linares-Palomino et al., 2008; Krömer et al., 2013). In the same vein, the influence of temperature on the fern species richness is described to be such that lower temperature decreases evapotranspiration rates thereby increasing the humidity of the forests, hence increasing the diversity and richness of the ferns (Krömer et al., 2013). The influence of elevation on the richness of ferns in Peninsular Malaysia has been reported earlier (Kessler et al., 2011). It was revealed that ferns richness increased with increasing elevation and this had a corresponding positive correlation with the environmental humidity. However, in our study, elevation is not the main environmental gradient influencing the ferns in the study sites. All these ferns were found at lower elevations.

The importance of light intensity and temperature on the growth and distribution of ferns has also been reported by a study on fern diversity along environmental gradients in Thailand (Sathapattayanon & Boonkerd, 2006). They reported that low light intensity influenced the richness of ferns but negatively affected the diversity. This was also observed in this study whereby light intensity had an inverse relationship with the fern's richness. It is very significant to note that the elevation range of all the forests studied is between 14–33m. A similar study of ferns distribution in some urban areas of Pahang and Kedah, Malaysia has reported ferns to be mostly found along high elevations of between 200-500m (Othman *et al.*, 2015). However, some of the ferns they reported as found at high elevations such as *Dicranopteris linearis, Angiopteris evecta*, and *Pityrogramma calomelanos* were also found at lower elevations in this study. This revealed that those ferns are not restricted only to high elevations.

The assessment of the IUCN red list status of ferns in this study further indicated that less attention has been given to the conservation status of ferns in Malaysia, and perhaps globally. This is because over 90% of the ferns were categorized under NR and NE in the IUCN red list. A similar observation was also reported concerning the ferns of Kedah, Malaysia whereby the IUCN status of the ferns was also lacking (Akomolafe & Rahmad, 2019). The Malaysian biodiversity assessment report was also lacking in fern species data due to over 60% of the ferns categorized as NE. This calls for an immediate comprehensive conservation assessment of ferns in Malaysia by both the local biodiversity agencies and the IUCN.

Also, *Davallia solida* which is classified as near threatened needs to be properly conserved in order to avoid it being vulnerable or endangered. *Angiopteris helferiana* which is popular in some Southern Asian countries (Lamichhane et al., 2019), was first reported in this study as a fern in Malaysia. All the newly mentioned ferns were not formally reported to be found in Malaysia by Parris et al. (2010) and Piggott (1988). They are also not mentioned in the Peninsular Malaysia biodiversity assessment portal. However, further taxonomic descriptions particularly using molecular techniques are needed to substantiate these newly mentioned species.

Conclusion

This study revealed that the fern species identified in these studied forests were positively influenced mostly by low light intensity and increasing temperature. The influence of these environmental gradients on the distribution of the fern species at the various study sites of this forest revealed that any corresponding change in the fern species richness and distribution could detect the change in the microclimatic conditions of the forests. Considering the paucity of information on the conservation statuses of the ferns in Malaysia, relevant government agencies and ecologists must pay close attention to the ecology and conservation of this group of plants.

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