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INVENTORY, ASSOCIATION, AND HABITAT CHARACTERISTICS OF HUPERZINE A NATURAL RESOURCES IN THE CIBODAS BOTANICAL GARDENS, WEST JAVA, INDONESIA

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Abstract – Huperzine A (HupA) is a lycopodium alkaloid that is important in the treatment of alzheimer's symptoms. Until now, the raw material for this compound comes from the Lycopodiceae family, which is harvested directly from nature. However, not too much ecological information exists for this plant family, while responsible utilization would require a sufficient baseline. This research aims to carry out an inventory of HupA natural resources and study their association and habitat characteristics in the Cibodas botanical gardens. A survey was conducted on 29 host trees using a purposive sampling method. Three species of HupA natural resources were identified, including *Phlegmariurus pinifolius* (Trevis.) Kiew, *Phlegmariurus squarrosus* (G. Forst.) Á. Löve & D. Löve, and *Phlegmariurus phlegmaria* (L.) Holub. The most abundant and evenly distributed species was *P. pinifolius*, while the least abundant and restricted distribution was *P. phlegmaria*. *Phlegmariurus pinifolius* showed no association with other vascular epiphytes, while *P. squarrosus* and *P. phlegmaria* showed associations. The species diversity of vascular epiphytes is significantly correlated with elevation and relative air humidity. Based on ecological aspects, we suggest *P. pinifolius* and *P. squarrosus* be developed in further bioprospecting studies.

Keywords: huperzine a, lycopodiaceae, phlegmariurus, association, vascular epiphytes, alzheimer

Introduction

The genus *Phlegmariurus* (Herter) Holub, commonly known as tassel-ferns, is a member of the Lycopodiaceae family, a group of delicate pendant ferns with branching strobili (Field, 2011). In the history of its taxonomy, tassel-ferns have been placed in several genera, even once placed in a separate family, Huperziaceae (Rothmaler, 1944; Ching, 1978). Initially, the tassel ferns were classified by Linnaeus into the genus *Lycopodium* using *Lycopodium phlegmaria* L. from the Paleotropics (Linnaeus, 1753). This broader definition of *Lycopodium* was generally adopted in the taxonomic literature until the 1950s, including the taxonomic literature of Lycopodiaceae in the Indonesian region (van Alderwerelt van Rosenbergh, 1915, 1917; Backer & Posthumus, 1939). Furthermore, the tassel-ferns have been placed in some genera, such as *Urostachys* Herter (Herter, 1908, 1949a, 1949b, 1950; Nessel, 1939) and *Huperzia* Bernhardi

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(Bernhardi, 1801; Trevisan De Saint-Leon, 1874; Holub, 1985; Ollgaard, 1987). In one of the famous revisions of the global Lycopodiaceae, Ollgaard (1987) assigned the epiphytic tassel-ferns to the genus *Huperzia* Bernh based on their morphological similarity. As a result, *Huperzia* s.l. (broad definition) is still often adopted today in various flora literature and some applied research, such as biochemical and biopharmaceutical. However, based on the results of various phylogenetic studies using both molecular and non-molecular character analysis, the epiphytic tassel-ferns clearly separate from the genus *Phlegmariurus* (Herter) Holub, which is part of the subfamily Huperzioideae, together with *Phylloglosum* Kunze and *Huperzia* s.s. (narrow definition) plants (Ollgaard, 2015; Field et al., 2016; PPG, 2016). At this moment, *Phlegmariurus* (Herter) Holub consists of epiphytic tassel-fern species from the tropics and the Southern Hemisphere as well as unique secondary terrestrial species from the Andes (Field et al., 2016; Testo et al., 2018).

Phlegmariurus contains roughly 250 species and is the most numerous in the Lycopodiaceae (PPG, 2016; Testo et al., 2018). *Phlegmariurus* species are widely distributed in tropical and subtropical regions, occupy various altitudes ranging from sea level to 5000 m ASL, and have different habits, such as epiphytes, terrestrial, and rupicolous (Testo et al., 2018). *Phlegmariurus* grows in moist habitat and is commonly found in mountain forests and alpine grasslands. *Phlegmariurus* also grows very slow, taking 15 to 20 years from spore germination to maturity (Ma et al., 2006; Luo et al., 2010). These plants, like other members of Lycopodiaceae, have a mycoheterotrophic gametophyte that gets a carbon source from a symbiotic relationship with fungi by forming mycorrhizal associations (Merckx et al., 2013).

Several species of *Phlegmariurus* have been used for medicinal purposes and food supplements, including for the treatment of fever, rheumatism, pain of the joints and waist, injuries from falls, swelling due to poisoning, bone fractures, and cancer (Ma et al., 2006; Silalahi et al., 2015; Xu et al., 2019). There are studies to investigate medicinal chemical compounds from *Phlegmariurus*. One of the important findings was the Huperzine A (HupA), an alkaloid compound that can act as a strong acetylcholinesterase inhibitor and is promising as a treatment for Alzheimer's symptoms (Ma et al., 2006; Yang et al., 2016). The compound was originally found in *Huperzia serrata* but in lower content compared to some of *Phlegmariurus* species, i.e. *P. squarrosus*, *P. pinifolius*, and *P. phlegmaria* (Ma et al., 2005; Ishiuchi et al., 2013). However, it is important to note that *Phlegmariurus* populations in some areas are disrupted due to slow growth, habitat loss, and their high potency, resulting in overharvesting for medicinal purposes (Ma et al., 2006).

The association and ecology of *Phlegmariurus* highlight the importance of the relationships between plants, fungi, and other organisms in maintaining healthy ecosystems (Gilliam, 2006; Higgins et al., 2007; Debbab et al., 2012; Bunce et al., 2013; Jia et al., 2016). Furthermore, Cibodas Botanic Gardens (CBG), located in the lower montane zone (1300–1425 meters above sea level) on the slopes of Mount Gede Pangrango, is a conservation area that has wild and introduced huperzioid plant collections, including *Phlegmariurus* and *Huperzia* species. CBG has a total land area of 84.9 hectares, comprising garden and collection areas as well as 8.43 hectares of remnant forest (Nurdiana & Buot JR, 2021). Nasution (2014) reported that there are five species of *Phlegmariurus* (recorded with the *Huperzia* name) in CBG, i.e., *Huperzia carinata, H. gnidioides, H. phlegmaria, H. serrata,* and *H. squarrosa.* However, there is no updated information related to the ecological aspects of *Phlegmariurus* species in the CBG area. This research focuses on the inventory and habitat characteristics of potential natural resources of HupA in the Cibodas Botanical Gardens area. Location selection was based on previous research in the Cibodas Botanical Garden (Nasution 2014). He recorded that several *Phlegmariurus* species inhabit this garden. However, previous studies did not explain the abundance and habitat characteristics. These data are important as a consideration in the bioprospecting of these species. We assume that botanical gardens can be used as a model to study the trends in the abundance and habitat preferences of *Phlegmariurus* to support bioprospecting. This initial information is important to support bioprospecting research on this group of plants. This data is also needed to support sustainable use and avoid extinction in nature due to overharvesting.

Material and methods

Study area

This research was carried out from January to April 2023 at the Cibodas Botanical Gardens (CBG), West Java, Indonesia (Fig. 1). Study sites included a collection garden and remnant forests inside the CBG area. The CBG region is located on the slopes of Mount Gede Pangrango at 1300–1425 meters above sea level (S 06^{0}44.515', E 107^{0}00.290'). According to Mutaqien & Zuhri (2011), the average annual rainfall for CBG is 2,950 mm, the average air temperature is 20 ^{0}C, and the average relative air humidity is 80%. This garden covers an area of 85 hectares, and around 10% is a remnant forest with a tropical mountain rainforest ecosystem.

Sampling method

We conducted a comprehensive survey of the Cibodas Botanical Garden area, including the collection garden area and remnant forest. The purposive sampling method was used to determine the plot location. The first tree recorded as a *Phlegmariurus* habitat was made into a sampling plot. The sampling plot is rectangular with dimensions of 0.5 x 2 m (1 m^2). The plot was placed on the trunk of the host tree where *Phlegmariurus* was found. We limited the sampling location to tree trunks because some of the host trees were tree ferns that did not have branches and twigs. Then, we inventoried all *Phlegmariurus* species and other vascular epiphyte species in the plot to determine their associations. We continued our inventory of the four nearest trees and also recorded plant species in the 1 m^2 plot. Subsequently, we shifted our focus to another *Phlegmariurus* host tree and repeated the same process by sampling its four closest neighbours.

Herbarium specimens were collected for identification. We referred to Varenflora voor Java (Backer & Posthumus, 1939) and van Alderwerelt van Rosenbergh (1915; 1916) as references for the identification of *Phlegmariurus* and ferns. Meanwhile, for identification of other plant species, we referred to the Flora Malesiana Series and van Steenis (1972). We also measured habitat characteristics. The parameters that were measured included host tree diameter, tree bark thickness, air temperature, relative air humidity, elevation, and light intensity.

Data analysis

We used species-area curves to analyze whether the number of plots sampled represented all species diversity at the study site (Krebs 1999). In order to analyze vegetation data, we calculated the important value index (IVI) proposed by Ellenberg & Mueller-Dombois (1974). The Shannon-Wienner diversity index (Odum et al., 1971) was also utilized to analyze the vegetation data. To assess association, we used a contingency table analysis (Ellenberg & Mueller-Dombois, 1974). We analyzed the Spearman rank correlation between measured parameters and species abundance and species diversity of vascular epiphytes using R software. The strength of the correlation referred to Kuckartz et al. (2013). There were five categories based on the value of r: no correlation (0.0 < 0.1), low correlation (0.1<0.3), medium correlation (0.3<0.5), high correlation (0.5<0.7), and very high correlation (0.7<1).

Results

Vegetation Data

A comprehensive survey was carried out in the Cibodas Botanical Gardens area. This initial survey was conducted to

Figure 1. Location of sampling plots in Cibodas Botanical Gardens, West Java, Indonesia.

determine the first host trees to be sampled. We established the first sampling plot on the host tree of *Phlegmariurus* that we first recorded. Then, we continued until 29 host trees were obtained. The location of these plots is in the southern part of the Cibodas Botanical Garden area (Fig. 1).

We did not find any *Phlegmariurus* species in the northern part of the Cibodas Botanical Gardens. A total of 64 species of vascular epiphytes were recorded, and three species of *Phlegmariurus* were identified (Tab. 1).

Table 1. List of species, families and Important Value Index of vascular epiphytes on sampling sites in Cibodas Botanical Garden

No.	Species	Families	RD (%)	RF (%)	IVI (%)
1	*Aeschynanthus radicans* Jack	Gesneriaceae	7.41	8.17	15.58
2	*Phlegmariurus pinifolius* (Trevis.) Kiew	Lycopodiaceae	6.83	5.77	12.60
3	*Haplopteris elongata* (Sw.) E.H.Crane	Pteridaceae	6.26	5.29	11.54
4	*Phlegmariurus squarrosus* (G.Forst.) Á.Löve & D.Löve	Lycopodiaceae	5.97	4.33	10.29
5	*Davallia hymenophylloides* (Blume) Kuhn	Davalliaceae	5.00	4.81	9.81
6	*Liparis cespitosa* (Lam.) Lindl.	Orchidaceae	7.31	2.40	9.72
7	*Peperomia tetraphylla* (G.Forst.) Hook. & Arn.	Piperaceae	5.58	3.85	9.43
8	*Davallia denticulata* (Burm.f.) Mett.	Davalliaceae	3.46	5.29	8.75
9	*Ageratina riparia* (Regel) R.M.King & H.Rob.	Asteraceae	5.10	1.44	6.54
10	*Dendrobium crumenatum* Sw.	Orchidaceae	3.37	2.88	6.25
11	*Goniophlebium percussum* (Cav.) W.H.Wagner & Grether	Polypodiaceae	2.60	2.88	5.48
12	*Selaginella involvens* (Sw.) Spring	Selaginellaceae	4.33	0.96	5.29
13	*Ceratostylis* sp.	Orchidaceae	3.27	1.92	5.20
14	*Heptapleurum scandens* (Blume) Seem.	Araliaceae	1.06	3.37	4.42
15	*Loxogramme scolopendrioides* (Gaudich.) C.V.Morton	Polypodiaceae	2.79	1.44	4.23
16	*Eria* sp.	Orchidaceae	1.92	1.92	3.85
17	*Nephrolepis davallioides* (Sw.) Kunze	Nephrolepidaceae	2.12	1.44	3.56
18	*Asplenium nidus* L.	Aspleniaceae	1.15	2.40	3.56
19	*Antrophyum reticulatum* (G.Forst.) Kaulf.	Pteridaceae	2.79	0.48	3.27
20	*Phlegmariurus phlegmaria* (L.) Holub	Lycopodiaceae	1.83	1.44	3.27
21	*Asplenium salignum* Blume	Aspleniaceae	0.87	2.40	3.27
22	*Octarrhena parvula* Thwaites	Orchidaceae	2.50	0.48	2.98
23	*Medinilla laurifolia* Blume	Melastomataceae	1.44	1.44	2.89
24	*Medinilla alpestris* (Jack) Blume	Melastomataceae	0.96	1.92	2.89
25	*Ficus villosa* Blume	Moraceae	0.67	1.92	2.60
26	*Goniophlebium subauriculatum* (Blume) C.Presl	Polypodiaceae	0.67	1.92	2.60
27	*Microsorum membranifolium* (R.Br.) Ching	Polypodiaceae	0.67	1.92	2.60
28	*Ficus ampelos* Burm.f.	Moraceae	0.58	1.92	2.50
29	*Pyrrosia albicans* (Blume) Ching	Polypodiaceae	1.06	0.96	2.02
30	*Belvisia mucronata* (Fée) Copel.	Polypodiaceae	0.58	1.44	2.02
31	*Ficus cuspidata* Reinw. ex Blume	Moraceae	0.58	1.44	2.02
32	*Davallia pentaphylla* Blume	Davalliaceae	0.96	0.96	1.92
33	*Davallia repens* (L.f.) Kuhn	Davalliaceae	0.87	0.96	1.83
34	*Ficus punctata* Thunb.	Moraceae	0.87	0.96	1.83
35	*Vaginularia trichoidea* Fée	Pteridaceae	0.29	1.44	1.73
36	*Asplenium laserpitiifolium* Lam.	Aspleniaceae	0.58	0.96	1.54
37	*Goniophlebium persicifolium* (Desv.) Bedd.	Polypodiaceae	0.77	0.48	1.25
38	*Grammitis* sp.	Grammitidaceae	0.29	0.96	1.25

39	*Coelogyne ventricosa* (Blume) Rchb.f.	Orchidaceae	0.67	0.48	1.15
40	*Agalmyla parasitica* (Lam.) Kuntze	Gesneriaceae	0.19	0.96	1.15
41	*Belvisia spicata* (L.f.) Mirb. ex Saff.	Polypodiaceae	0.48	0.48	0.96
42	*Haplopteris ensiformis* (Sw.) E.H.Crane	Pteridaceae	0.38	0.48	0.87
43	*Bulbophyllum* sp.	Orchidaceae	0.29	0.48	0.77
44	*Poikilospermum suaveolens* (Blume) Merr.	Urticaceae	0.29	0.48	0.77
45	*Davallia sessilifolia* Blume	Davalliaceae	0.19	0.48	0.67
46	*Hoya* sp.	Apocynaceae	0.19	0.48	0.67
47	*Medinilla eximia* (Jack) Blume	Melastomataceae	0.19	0.48	0.67
48	*Viola javanica* W.Becker	Violaceae	0.19	0.48	0.67
49	*Begonia cucullata* Willd.	Begoniaceae	0.10	0.48	0.58
50	*Blumea balsamifera* (L.) DC.	Asteraceae	0.10	0.48	0.58
51	*Coelogyne miniata* (Blume) Lindl.	Orchidaceae	0.10	0.48	0.58
52	*Elaphoglossum* sp.	Lomariopsidaceae	0.10	0.48	0.58
53	*Ficus benjamina* L.	Moraceae	0.10	0.48	0.58
54	*Ficus variegata* Blume	Moraceae	0.10	0.48	0.58
55	*Neonauclea excelsa* (Blume) Merr.	Rubiaceae	0.10	0.48	0.58
56	*Oxalis corniculata* L.	Oxalidaceae	0.10	0.48	0.58
57	*Pothos scandens* L.	Araceae	0.10	0.48	0.58
58	*Paspalum* sp.	Poaceae	0.10	0.48	0.58
59	*Psilotum nudum* (L.) P.Beauv.	Psilotaceae	0.10	0.48	0.58
60	*Selliguea enervis* (Cav.) Ching	Polypodiaceae	0.10	0.48	0.58
61	*Smilax zeylanica* L.	Smilacaceae	0.10	0.48	0.58
62	*Tetrastigma* sp.	Vitaceae	0.10	0.48	0.58
63	*Vanda tricolor* Lindl.	Orchidaceae	0.10	0.48	0.58
64	*Youngia japonica* (L.) DC.	Asteraceae	0.10	0.48	0.58

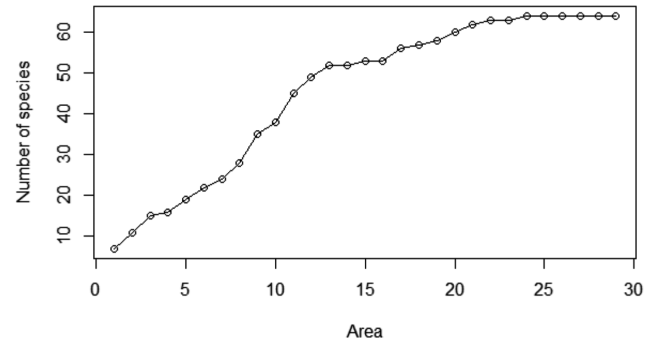

Figure 2. The species-area curve showed flat curve when sampling area reached 29 plot (29 m^2).

The three species belong to the Lycopodiaceae family, including *Phlegmariurus pinifolius* (Trevis.) Kiew, *Phlegmariurus squarrosus* (G. Forst.) Á. Löve & D. Löve, and *Phlegmariurus phlegmaria* (L.) Holub. We analyzed the number of sampling plots made with species-area curves. The species-area curve shows a horizontal line when the number of samplings has reached 29 plots (Fig. 2).

We analyzed the role of the inventoried vascular epiphytes by calculating the importance value index (IVI). In Table 1, *Aeschynanthus radicans* had the highest IVI and dominated compared to other species, followed by *P. pinifolius* and *H. elongata*. *Phlegmariurus pinifolius* was the most abundant and had the most even distribution among the three *Phlegmariurus* species, followed by *P. squarrosa* in the second place. Meanwhile, *P. phlegmaria* had the lowest IVI value compared to the two previous *Phlegmariurus* species. This species also had a lower value of relative density and relative frequency among the three *Phlegmariurus* species.

Association of ***Phlegmariurus***

We used a contingency table to calculate the X^2 value in the association analysis between *Phlegmariurus* species and other vascular epiphytic plants in the plot. There is a significant association if the X^2 value is greater than the X^2 table. Even though *P. piniflius* was the most abundant and had the highest IVI compared with two other *Phlegmariurus*, we didn't find any association of this species with other vascular epiphytes in our plot (Tab. 2).

There was also no association between *P. pinifoliu*s, *P. squarrosus* and *P. phlegmaria*.

We have discovered an association between *P. squarrosus* and other vascular epiphytes within the observation plots (Tab. 3). Eleven species of vascular epiphytes are significantly associated with *P. squarrosus*. The most frequent family associated with *P. squarrosus* is Moraceae, which encompasses three distinct species: *Ficus cuspidata*, *F. villosa*, and *F. ampelas*.

Table 2. Association analysis of *P. pinifolius* with other vascular epiphytes in the sampling plot (X^2 table = 3.84)

No.	Species	Family	X^2	Association*
1	*Coelogyne miniata*	Orchidaceae	2.779502315	Not Associated
2	*Davallia denticulata*	Davalliaceae	2.541771886	Not Associated
3	*Goniophlebium subauriculatum*	Polypodiaceae	1.937217262	Not Associated
4	*Ficus variegata*	Moraceae	1.937217262	Not Associated
5	*Ceratostylus* sp.	Orchidaceae	1.937217262	Not Associated
6	*Peperomia tetraphylla*	Piperaceae	1.712165179	Not Associated
7	*Haplopteris ensiformis*	Pteridaceae	1.701736111	Not Associated
8	*Pyrrosia albicans*	Polypodiaceae	1.701736111	Not Associated
9	*Blumea balsamifera*	Asteraceae	1.701736111	Not Associated
10	*Liparis caespitosa*	Orchidaceae	1.701736111	Not Associated

*Associated if $X^2 > X^2$ table

Table 3. Association analysis of *P. squarrosus* with other vascular epiphytes in the sampling plot (X^2 table = 3.84)

No	Species	Family	X^2	Association*
1	*Schefflera scandens*	Araliaceae	15.8887987	Associated
2	*Ficus cuspidata*	Moraceae	14.25385802	Associated
3	*Ageratina riparia*	Asteraceae	14.25385802	Associated
4	*Medinilla laurifolia*	Medinillaceae	14.25385802	Associated
5	*Aeschynanthus radicans*	Gesneriaceae	9.298744658	Associated
6	*Ficus villosa*	Moraceae	6.132579365	Associated
7	*Ficus ampelos*	Moraceae	6.132579365	Associated
8	*Davallia hymenophylloides*	Davalliaceae	5.686523908	Associated
9	*Pyrrosia albicans*	Polypodiacae	5.542989418	Associated
10	*Selaginella caulescens*	Selaginellaceae	5.542989418	Associated
11	*Agalmyla parasitica*	Gesneriaceae	5.542989418	Associated
12	*Grammitis* sp.	Grammitidaceae	5.542989418	Associated

*Associated if $X^2 > X^2$ table

The association of *P. phlegmaria* with other vascular epiphytes was also observed in all plots. Table 4 presents the associations based on X^2 values, wherein six species belonging to six distinct families were found to associate with *P. phlegmaria*. Notably, among these associated species were two fern species: *Goniophlebium percissifolium* and *Haplopteris elongata*.

Habitat Characteristics

We measured and recorded several parameters of habitat characteristics in each sampling plot. A summary of the measurement results is presented in Tab. 5.

We found that *P. phlegmaria* had a limited distribution and was only found in three plots. Meanwhile, the other two species of *Phlegmariurus* had a more even distribution. *Phlegmariurus pinifolius* was present in 12 plots, and *P. squarrosus* was found in nine plots. These plots were spread across collection gardens and remnant forest areas. *Phlegmariurus pinifolius* was mostly found in collection garden plots. A total of nine plots of this species, or around 75% of the sampling area, were found in the garden collection. On the other hand, *P. phlegmaria* is more commonly found in remnant forest plots. *Phlegmariurus squarrosa* was almost equal between plots in the garden (five plots) and in the remnant forest (four plots). About half of the hosts for *P.*

Table 4. Association analysis of *P. phlegmaria* with vascular epiphytes in sampling plot (X^2 table = 3.84)

No.	Species	Family	X^2	Association*
1	*Goniophlebium percissifolium*	Polypodiaceae	22.82886905	Associated
2	*Smilax zeylanica*	Smilacaceae	22.82886905	Associated
3	*Tetrastigma* sp.	Vittaceae	22.82886905	Associated
4	*Bulbophyllum* sp.	Orchidaceae	22.82886905	Associated
5	*Haplopteris elongata*	Pteridaceae	13.12813283	Associated
6	*Schefflera scandens*	Araliacae	5.296266234	Associated
7	*Aeschynanthus radicans*	Gesneriaceae	2.145864152	Not associated
8	*Davallia hymenophylloides*	Davalliaceae	1.111204147	Not associated
9	*Nephrolepis davallioides*	Nephrolepidaceae	0.937232906	Not associated
10	*Davallia denticulata*	Davalliaceae	0.538257576	Not associated

*Associated if $X^2 > X^2$ table

pinifolius and *P. squarrosus* were tree ferns (Cyatheaceae). However, we did not find any *P. phlegmaria* on the tree fern. We conducted a Spearman correlation analysis to investigate the relationship between habitat characteristics with the species abundance and diversity of vascular epiphytes at our research site. Six parameters were analyzed, including tree diameter, tree bark thickness, air temperature, air relative humidity, elevation, and light intensity. Correlation analysis between species abundance of vascular epiphytes and measured parameters is displayed in Fig. 3.

In our sampling plot, we only found a significant correlation between vascular epiphytes abundance with elevation. The correlations of the other five parameters were weak and insignificant. We found that vascular epiphytes diversity had a significant correlation with elevation and humidity (Fig. 4). The correlation strengths were medium. There was no significant correlation between species diversity and the other four measured parameters.

Table 5. The summary of *Phlegmariurus* spp. habitat characteristics in Cibodas Botanical Garden

No	Parameters	All species of Vascular epiphytes	*P. pinifolius*	*P. squarrosus*	*P. phlegmaria*
1	Tree host diameter (cm)	62.51±35.41	49.03±30.20	47.49±25.90	100.17±17.18
2	Bark tree thickness (mm)	5.33±1.91	5.73±1.58	5.62±1.30	4.78±1.71
3	Air Temperature (^{0}C)	24.04±1.32	23.44±0.91	24.18±1.44	23.50±0.52
4	Air Relative Humidity (%)	73.90±6.35	76.83±6.93	77.00±5.94	77.33±6.35
5	Elevation (m ASL)	1387.28±20.72	1397.83±18.38	1393.33±22.62	1377±21.93
6	Light intensity (Lux)	3654.24±2072.64	4023.25±1993.05	4137.44±2206.04	2727±2234.09
7	Plot location	Garden (14), remnant forest (15)	Garden (9), remnant forest (3)	Garden (5), remnant forest (4)	Garden (1), remnant forest (2)
8	Tree host species		*Helicia formosana* , *Echinocarpus australis*, *Spathodea campanulata*, *Ficus variegata*, *Cyathea contaminans* (2), *Cyathea junghuhniana* (3), *Cyathea raciborskii* , *Magnolia grandiflora*, *Magnolia sumatrana*	*Ficus variegata*, *Ostodes paniculata*, *Cyathea contaminans*, *Cyathea junghuhniana* (3) *Magnolia grandiflora*, *Macropanax dispermus*, *Magnolia sumatrana*	*Melaleuca alternifolia*, *Magnolia sumatrana*, *Sloanea sigun*

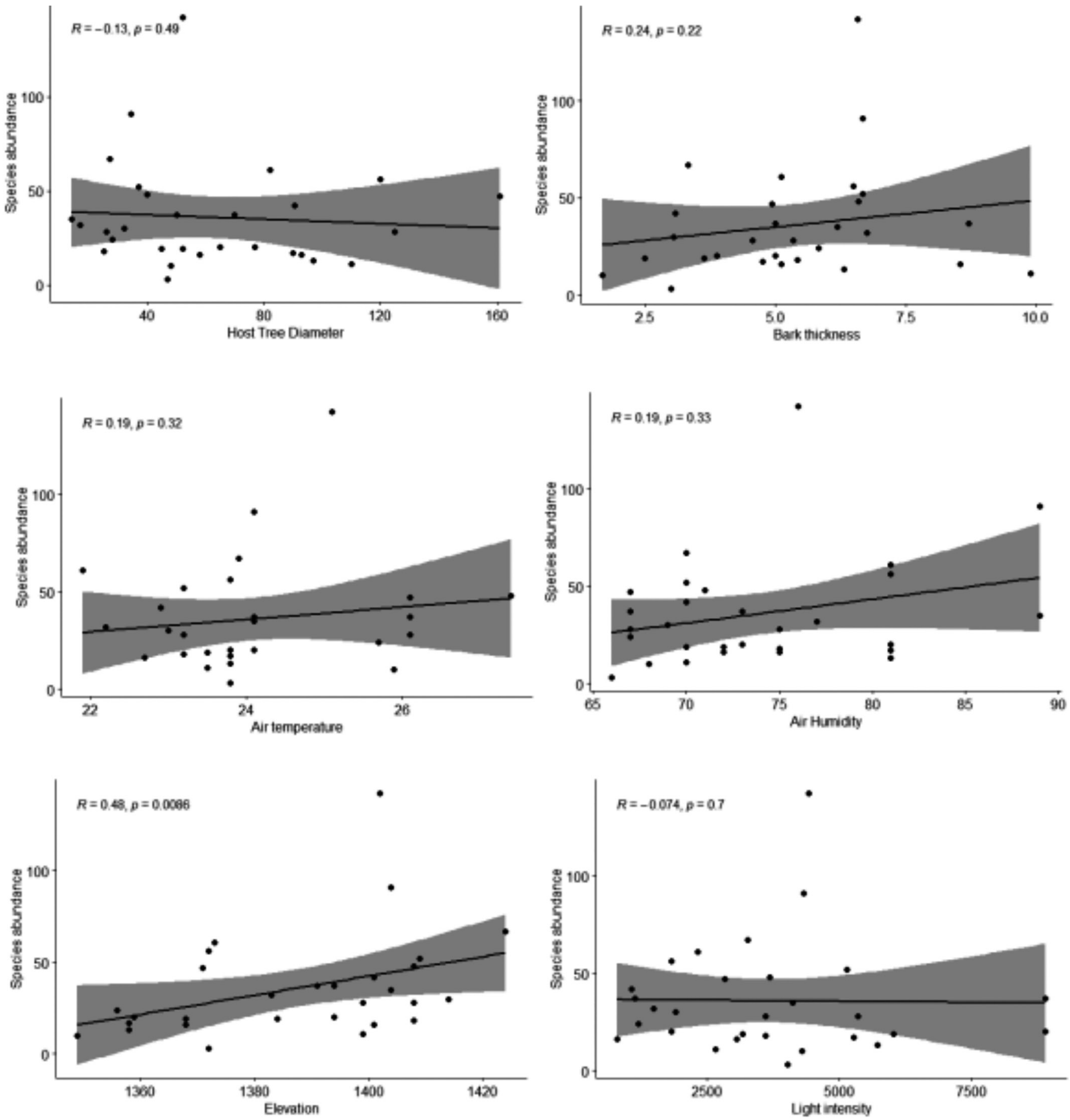

Figure 3. Correlation of vascular epiphytes abundance with habitat characteristics in the Cibodas Botanical Gardens.

Discussion

We found 64 species of vascular epiphytes and identified three species of *Phlegmariurus* that potentially become Huperzine A natural sources. The three species were *Phlegmariurus pinifolius* (Trevis.) Kiew, *Phlegmariurus squarrosus* (G. Forst.) Á. Löve & D. Löve, and *Phlegmariurus phlegmaria* (L.) Holub. All three species are epiphytic plants, classified into the *Phlegmariurus* genus and the Lycopodiceae family. The identification of these three species was in line with previous studies that stated that these three species were distributed in the mountains of Java (van Alderwerelt

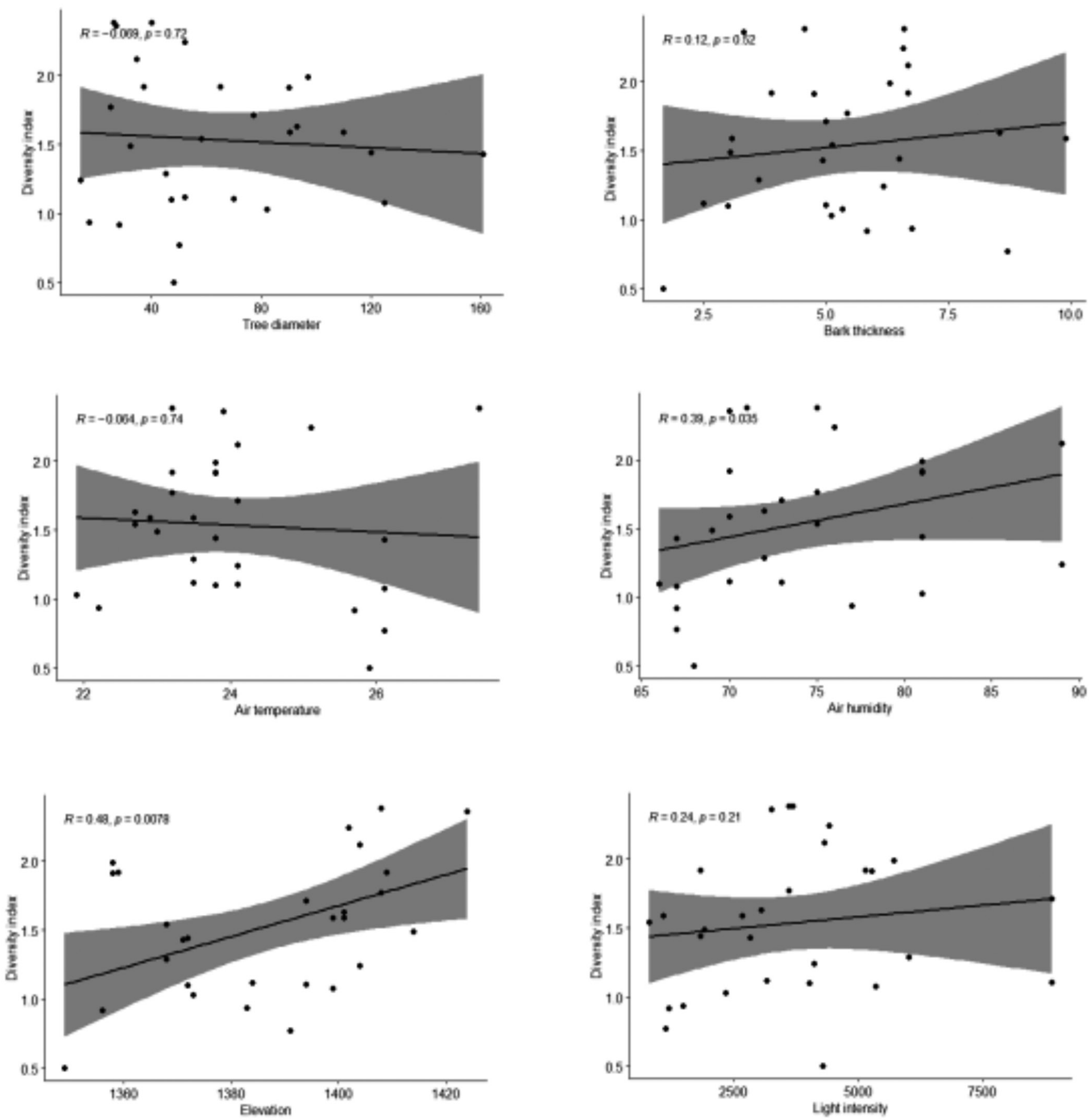

Figure 4. Correlation of vascular epiphytes diversity with habitat characteristics in the Cibodas Botanical Gardens.

van Rosenbergh, 1915, 1917; Backer & Posthumus, 1939; Nasution, 2015; Hartini, 2015). Meanwhile, HupA producers from other genera, such as *Huperzia*, were not found in our sampling plot.

Differences in habitat factors at least influence the distribution of the three species of *Phlegmariurus* in the garden and remnant forest. All *P. phlegmaria* were recorded on the bigger tree, which provided a lower air temperature, higher relative air humidity and lower light intensity (Tab. 5). The garden area is more open than the remnant forest area, thus influencing its microclimatic factors. It can be concluded that *P. phlegmaria* prefers higher humidity habitat. According to

Hoshizaki and Moran (2001), *P. phlegmaria* prefers moist and wet habitats, while *P. squarrosus* and *P. pinifolius* prefer habitats with better drainage.

We sampled 29 host trees and took an inventory of vascular epiphytes in a 1 m^2 plot on the tree trunk. The species-area curve shows the relationship between the size of the sampling area and the number of vascular epiphytes. In this study, the curve has reached an asymptote (Fig. 2), so it is predicted that the number of sampling plots is sufficient to inventory species diversity in the research area. In another study by Nurahma et al. (2005), at the same location, the curve reached an asymptote before reaching 30 sampling plots.

We calculated the IVI of 64 vascular epiphytes, which is the sum of relative frequency and relative density. The highest IVI value is occupied by *A. radicans*, followed by *P. pinifolius*, *H. elongata*, *P. squarrosus*, and *D. hymenophylloides*. These five species had higher IVI because they had a high relative density and relative frequency compared to other vascular epiphytes. A higher relative density indicates that the species has a high abundance, while a high relative frequency indicates that the species is more evenly distributed than other species in the study area.

Phlegmariurus pinifolius exhibited a higher relative density and relative frequency, followed by *P. squarrosus* and *P. phlegmaria*, respectively. *Phlegmariurus phlegmaria* showed the lowest IVI value compared to other *Phlegmariurus*. The lowest IVI value of *P. phlegmaria* is influenced by its lower abundance and limited distribution in the research location. This species only occurred in three plots, with a total of 19 individuals. It also has the lowest relative frequency, which indicates that the distribution of *P. phlegmaria* is more limited than two other species, most likely due to its preference of the shadier and more humid habitat (Hoshizaki and Moran 2001). This is supported by the microhabitat data (Tab. 5), which shows a lower average air temperature, higher air humidity, and lower light intensity for this species. *Phlegmariurus phlegmaria* also has a lower abundance than the other two species of *Phlegmariurus*. This low abundance is probably due to differences in microhabitat characteristics.

A total of 64 species were identified and belonged to 27 families. The families with the highest number of species are Polypodiaceae (9 species), Orchidaceae (9 species), Moraceae (6 species), Davalliaceae (5 species), and Pteridaceae (4 species). Ferns dominated the family with the largest number of species, including Polypodiaceae, Davalliaceae, and Pteridaceae. Research on vascular epiphytes in mountain forests in Guatemala was also dominated by orchid and fern species (Catling & Lefkovitch, 1989). The high abundance of ferns is supported by suitable microclimatic conditions in the study area. Ferns need moist conditions to grow well, especially in the gametophyte phase. The measurement results at the research location presented in Table 5 have shown relatively low temperatures and high humidity at the research location. Large trees are suitable hosts for vascular epiphytes, especially ferns. According to Zhao et al. (2015), the size of the host tree had a significant effect on the presence of vascular epiphytes. The study site is located at an elevation 1349 – 1424 m above sea level, which is classified as a submontane or lower montane forest ecosystem van Steenis, 1972. The submontane forest zone has relatively low average air temperature, high air humidity, and low light intensity that are suitable for *Phlegmariurus*. *Phlegmariurus phlegmaria* prefers shadier and more moist habitat compared to *P. squarrosus* (Hoshizaki and Moran, 2001; Hartini, 2015).

An association study was carried out to determine whether the *Phlegmariurus* species are associated with each other. Association analysis showed that there was no association between the three species of *Phlegmariurus*. This indicates that the three species of *Phlegmariurus* are independent of each other and are thought not to use the same resources in their habitat. However, an association was found between *Phlegmariurus* and several vascular epiphyte species in the observation plot. *Phlegmariurus pinifolius* does not show associations, while both *P. squarrosus* and *P. phlegmaria* associate with other epiphytic plants on host trees. The highest X^2 value for *P. pinifolius* is less than the X^2 table value (3.84). Table 2 displays the ten species with the highest X^2 value, with the results being less than the X^2 table value. As a result, it is possible to conclude that *P. pinifolius* has no association with other vascular epiphytes. Meanwhile, based on the X^2 values shown in Table 3 and Table 4, we discovered an association for both *P. squarrosus* and *P. phlegmaria* with other vascular epiphytes in the plots. *Phlegmariurus squarrosus* was found to be associated with 11 vascular epiphytes. It is more numerous than *P. phlegmaria* (six species). This is conceivable since *P. phlegmaria* has a restricted distribution, appearing in only three plots. Two fern species and four spermatophytes are associated with *P. phlegmaria*. Meanwhile, seven of the 11 vascular epiphytes associated with *P. squarrosa* were spermatophytes, two species of ferns, and two species of Lycopods. In comparison to the other two *Phlegmariurus* species, *P. squarrosus* had more extensive associations with vascular epiphytes at the research site. The association between two species shows whether the two species grow in the same environment, have the same distribution, and depend on each other (Kusmana, 1995). *Phlegmariurus pinifolius* does not show associations with other species, so it can be concluded that this species is more adaptive and does not depend on other species. This is also supported by the higher IVI and more abundant compared to other *Phlegmariurus* species.

We calculated the Shannon-Wiener diversity index on each plot and analyzed the correlations of the measured factors. Only the elevation parameter shows a significant correlation with the diversity and abundance of vascular epiphytic species. Meanwhile, the correlation between elevation and abundance was medium-strong and positive. A positive relationship indicated that abundance would increase with increasing elevation. Meanwhile, humidity only significantly correlates with species diversity. The correlation strength was medium. We suspect that the lack of significant correlation between the other parameters measured was due to the location of the sampling plots close to each other. A significant correlation between diversity and elevation was also proposed by Zhao et al. (2015). They also found that the diameter of the tree host also showed a significant correlation with the vascular epiphyte diversity. This study did not show the same result because the sampling was limited to a 1 m^2 sampling plot and located on tree trunks. We did not inventory branches and twigs because some of the host trees at the research location were tree ferns that did not have branches and twigs. The correlation between elevation and humidity on diversity and abundance is in accordance with the statements of Hartini (2015) also Hoshizaki and Moran (2001), which state that the habitat of *Phlegmariurus* is in the submontane zone with shady and humid conditions. In line with increasing elevation, the temperature will decrease, and the humidity will increase. This is a suitable habitat condition for the growth of *Phlegmariurus*. The implication for bioprospecting is that it is necessary to consider that the harvesting location chosen is a suitable habitat so that it continues to grow optimally even when harvesting activities are carried out.

The *Phlegmariurus* species exhibiting optimal abundance and even distribution were proposed for further bioprospecting studies. Drawing from the ecological data and HupA content from previous studies, we strongly recommend *P. pinifolius* and *P. squarrosus* in further bioprospecting studies. Meanwhile, we do not suggest *P. phlegmaria* due to its low abundance and restricted distribution in Cibodas Botanical Garden.

Conclusions

We identified three species of *Phlegmariurus* that are potential natural resources of Huperzine A, including *P. pinifolius*, *P. squarrosus*, and *P. phlegmaria,* in the Cibodas Botanical Gardens. *Phlegmariurus pinifolius* had the highest abundance and was evenly distributed, while *P. phlegmaria* showed low abundance and limited distribution. *Phlegmariurus squarrosus* and *P. phlegmaria* showed associations with other vascular epiphytes, while *P. pinifolius* did not show significant associations. In our sampling plot, elevation showed a significant correlation with species diversity and species abundance. Meanwhile, relative air humidity only showed a significant correlation with species diversity. We suggested further bioprospecting research on *P. pinifolius* and *P. squarrosus* and do not recommend *P. phlegmaria* due to its low abundance and limited distribution in the study area.

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