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Specific habitat preferences of *Psilotum nudum* (L.) P. Beauv on tree fern (Cyatheaceae): insight from Bali botanic gardens

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Abstract

Psilotum nudum is an epiphytic fern belonging to the Family Psilotaceae. In Bali Botanic Garden, this plant grows on the stem of tree fern, but not all stands of tree fern become phorophytes for *P. nudum*. Studying *P. nudum* ecology in the Bali Botanic Garden is crucial in conservation efforts. The research was conducted in the Bali Botanic Garden area, using field observation methods on tree ferns located on the right and left of the main route of the garden. The data collected include tree fern species as hosts, the number of *P. nudum* individuals (clumps), *P. nudum* growing height and associated plants. The results showed that *P. nudum* was only found growing on one species of tree fern (phorophyte), *Alsophila orientalis*. *Psilotum nudum* is also associated with 43 species of plants. The most common plant species are *Davalia denticulata* (10.6 %), *Asplenium nidus* (8.13 %) and *Dischidia* sp. (6.91 %). Host tree fern height has a weak but insignificant correlation with the number of *P. nudum* individuals (r = - 0.14, p = 0.5). The higher the *Alsophila* tree, the fewer *P. nudum* individuals were found. Host tree fern height was weakly correlated with the number of associated plant species (r= 0.24, p= 0.22). This data helps complete ecological data on collection plants that can be a reference in their development and cultivation efforts.

Keywords

Alsophilla orientalis; autecology; plant association; porophyte

Introduction

Psilotum is one of two genera in the fern Family Psilotaceae. Members of this family are known as the most primitive vascular plants (1) and are often called living fossils. Previously, Psilotaceae was grouped within the fern allies based on their morphology. Still, molecular studies suggested that this family is one of the earliest-diverging groups of ferns, with Ophioglossaceae as the most closely related family (2, 3). This genus has two species members, *Psilotum nudum* (L.) P. Beauv. and *Psilotum complanatum* Sw., and also one hybrid between them, *Psilotum* × *intermedium* W. H. Wagner (4, 5)

Psilotum nudum is the most widespread species among *Psilotum*. It was reported that this species has medicinal potency to arrest diarrhoea (6). This rootless fern has a very different morphology and is easily recognized by highly reduced and scale-like leaves, the upright dichotomously branched stem and large synangia. *Psilotum nudum* came from the Greek psilo, which refers to the naked or exposed synangia and Latin nudum, which refers to the lack of clear big leaves (7, 8). Globally, this species is recorded in the IUCN Red List of Threatened Species with Least Concern category (9). Regionally, Japan listed this species in

the Near Threatened category (9), while Korea putted this species in the Endangered category (10) and China in the Vulnerable category (11). *Psilotum nudum* can be found in the tropics and sub-tropics region (12). It grows on forest floors, rocky or sandy soil, or epiphytes on the trunk of other plants, such as tree ferns (7, 8).

In Cibodas Botanic Garden, *P. nudum* can be found epiphyte on *Cyathea junghuhniana*'s trunk (13). In Bali Botanic Garden, *P. nudum* grows naturally on tree fern trunks from Family Cyatheaceae and other plants. Cyatheaceae can be found easily in this conservation area, growing natively or planted along the main road or as a collection. Still, not all of Cyatheaceae become phorophytes for *P. nudum*. To enhance the ecological information on *P. nudum*, this research aims to investigate the microhabitat characteristics of *P. nudum* in the Bali Botanic Garden conservation area, including identifying the host species of *P. nudum*, examining the vertical distribution of *P. nudum* on its phorophytes and also identifying the plant species that coexist with *P. nudum* on its phorophytes. This data helps complete ecological data on collection plants that can be a reference in their development and cultivation efforts.

Materials and Methods

Research location

The research was conducted in "Eka Karya" Bali Botanic Garden – BRIN (later called Bali Botanic Garden), Candikuning, Tabanan, Bali, in June-July 2022. Bali Botanic Garden has an area of about 157.5 hectares and is located at an altitude of 1.250 - 1.450 m asl (14). Data from Collection Registration Data of Bali Botanic Garden, Bali Botanic Garden weather station recorded data from 2008-2022 show the air temperature ranges from 18.7-20.9 °C with an average air humidity of 89 % and an average rainfall of 1005.76 mm per year.

Working methodology

The number of *Psilotum nudum* growing epiphytes on tree ferns was calculated by observing each tree fern from the family Cyatheaceae clan that grew within the maximum distance of two meters on the right-left side of the main ring road of Bali Botanic Garden as shown in Fig. 1. All tree ferns from the family Cyatheaceae clan found along the road were counted and observed with 160 individual tree ferns. The observation did not include tree ferns growing other than along the main road.

Tree ferns with *P. nudum* growing epiphytes on the trunk were then recorded, including the species name of the tree fern, the number of *P. nudum* individuals found and the height of growth on the trunk of the tree ferns. Other plants that were found growing together with *P. nudum* are also recorded. The coordinates of where the tree fern grows are determined using Garmin GPS and then plotted on Google Earth software. The data collected include the species of Cyatheaceae overgrown by *P. nudum*, the types of epiphytic plants associated with *P. nudum*, the individual number of *P. nudum* and other epiphytic plants, the height of *P. nudum* on the tree fern trunk and the total height of the tree fern. The microclimate, including light intensity, temperature and air humidity, is also measured using Lutron. Meanwhile, the humidity of the trunk of tree fern was measured using a moisture meter TK100.

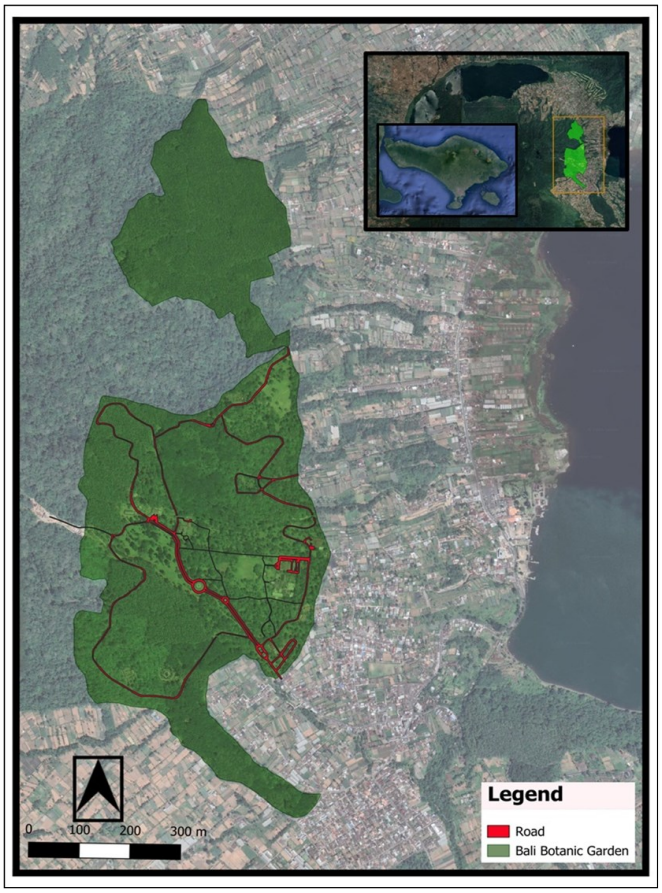

Fig. 1. Map of research location.

Data analysis

The data obtained are processed descriptively and statistically. The Spearman correlation coefficient is used to determine the correlation between tree height, the number of individuals (clumps) of *P. nudum* and other plant associations. The correlation is done using R Studio software. The suitability of *P. nudum* with other epiphytic types was obtained using relative frequency (FR) (15). Relative frequency is used to determine the distribution of plants associated with *P. nudum,* as given in Eqn. 1.

$$\text{Relative frequency (FR)} = \times \frac{\text{Frequency of one species}}{\text{Frequency of all species}} \quad \text{Eqn. 1}$$

Determination of the level of association between P. nudum and host trees using the Jaccard Index (JI) and Dice Index (DI) (16). The calculation uses 1 for presence (presence) and 0 if not (absence), as given in Eqns 2 and 3.

$$DI = \frac{2a}{2a+b+c} \quad \text{Eqn. 2}$$

$$JI = \frac{a}{a+b+c} \quad \text{Eqn. 3}$$

a = The frequency of *P. nudum* growing on j-type host trees

b = The frequency of *P. nudum* growing on non-j-type host trees

c = The frequency of epiphytic spikes other than *P. nudum* growing on host trees of the -j type

If the values of DI and JI are equal to 0, then there is no association between *P. nudum* and the host tree type -j. If the values of DI and JI are equal to 1, then the association is maximum. Three classes were used to determine the association level: low, medium and high. The type of plant whose name is unknown is photographed and specimens are taken to be identified at the Herbarium of the Tabanan Hortus Botanicus Baliense.

Results

Population of *Psilotum nudum*

Member of Cyatheaceae found at the research site consists of two species, namely *Alsophila orientalis* (Kunze) R. M. Tryon (156 individuals) and *Sphaeropteris glauca* (Blume) R. M. Tryon (four individuals). However, *Psilotum nudum* was attached only to the *A. orientalis* trunk (Table 1). These populations were found at 29 trunks of *A. orientalis* from a total sample of 160 tree fern trunks (18.1 %).

Table 1. The presence of *P. nudum* on the trunk of a tree fern at Bali Botanic Garden

Psilotum nudum	*Alsophila orientalis*	*Sphaeropteris glauca*
Presence	29	0
Absence	127	4
Total	156	4

Psilotum nudum in the Bali Botanical Gardens was only found on *A. orientalis* trunks and none was found on *S. glauca* trunks (Fig. 2). Fig. 3 presented the visual trunk differences between *A. orientalis* and *S. glauca* in the Bali Botanical Gardens. The differences in morphological characters are presented in Table 2. The microclimatic trunk humidity of tree ferns was measured using a TK 100 moisture meter. The results showed that the water content in the trunk of *A. orientalis* ranged between 20.6 - 27.3 %, more significant than that in the trunk of *S. galuca* (10.6 - 18.3 %). *Psilotum nudum* epiphyte on tree fern is most commonly found along the main road between areas 2 and 3 of the Bali Botanic Garden (area marked red in Fig. 2). On the approximately 338 m long road, as many as 91 *A. orientalis* trees were found and 25 were covered with *P. nudum. Psilotum nudum* is found at a height of 30 - 450 cm above ground level, while the distribution of *P. nudum* is mainly found at the trunk of *Alsophila orientalis* at 160-200 cm above ground level (Table 2). The height of the *Alsophila orientalis* trunk in the Bali Botanic Garden was 5.89 ± 2.03 m. The results of the Spearman Correlation Test showed a weak but not significant correlation between tree height and the number of individuals ($r = -0.14$, $p = 0.5$) (Fig. 3).

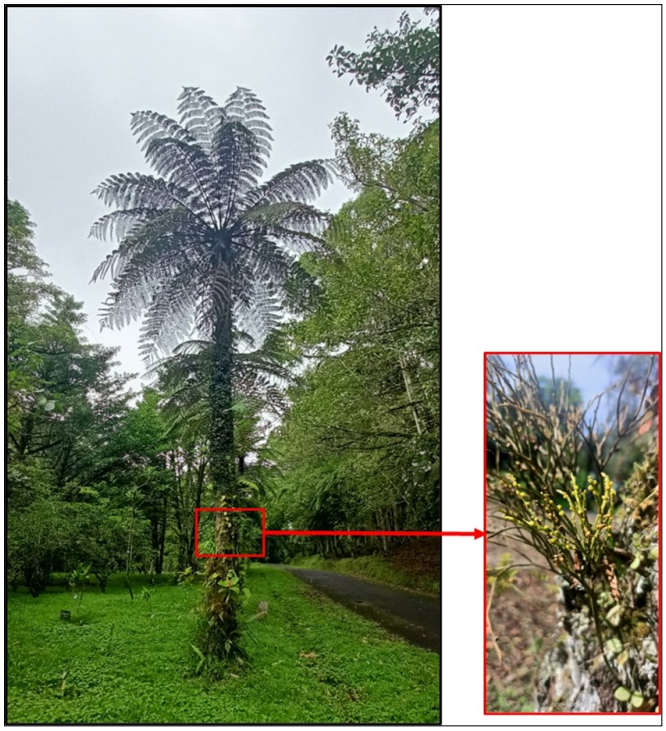

Fig. 2. *Psilotum nudum* epiphyte on the trunk of *Alsophila orientalis.*

Fig. 3. Trunk morphology A: *A. orientalis* B: *S. glauca*.

Plant association with *Psilotum nudum*

The association between *P. nudum* and *A. orientalis* compared with *P. nudum* with *S. glauca* falls into the high category according to the Jaccard index and Dice index (Table 2). *Psilotum nudum* is found on the trunk of *Alsophila orientalis,* along with 42 other plant species (Table 5). The most common plant species are *Davalia denticulata* (10.6 %), *Asplenium nidus* (8.13 %) and *Dischidia* sp. (6.91 %). The Spearman correlation test results showed a weak relationship between tree height and the number of plant species associated with *Psilotum nudum* ($r=0.24$, $p=0.22$). Meanwhile, the number of associated plant species with the number of *Psilotum nudum* individuals found epiphytes on *Alsophila orientalis* trunks showed no correlation ($r=0.14$, $p=0.05$), as presented in Fig. 6.

Habitat preference of *P. nudum*

Psilotum nudum in Bali Botanic Garden is an epiphyte on *Alsophilla orientalis* trunk distributed at an elevation between 1348 to 1389 m asl. The habitat of *P. nudum* has a temperature range of 22.6- 28.5 °C, a relative humidity range of 62.4 - 86.6 % and a light intensity range of 677- 16520 lux (Table 6).

Table 2. Morphological characters of *Alsophila orientalis* dan *Sphaeropteris glauca*

Organs	*Alsophila orientalis*	*Sphaeropteris glauca*
Trunk	Treelike growth habit, with an erect trunk that rarely branches, dead frond usually still connected with the trunk	Often very tall and much thickened by adventitious roots at the base, only when old showing leaf-scars in the upper part
Stipe	Stipe dark, to at least 50 cm; scales abundant, dark; pneumatophores 4-7 mm long, in a single row.	Stipe to 100 cm long, glaucous, purplish towards the base, usually strongly spiny
Sori	Sori near costules; indusium a relatively thin brown cup	Sori near costules, lacking indusia
Spore	Trilete, brown	Trilete, brown

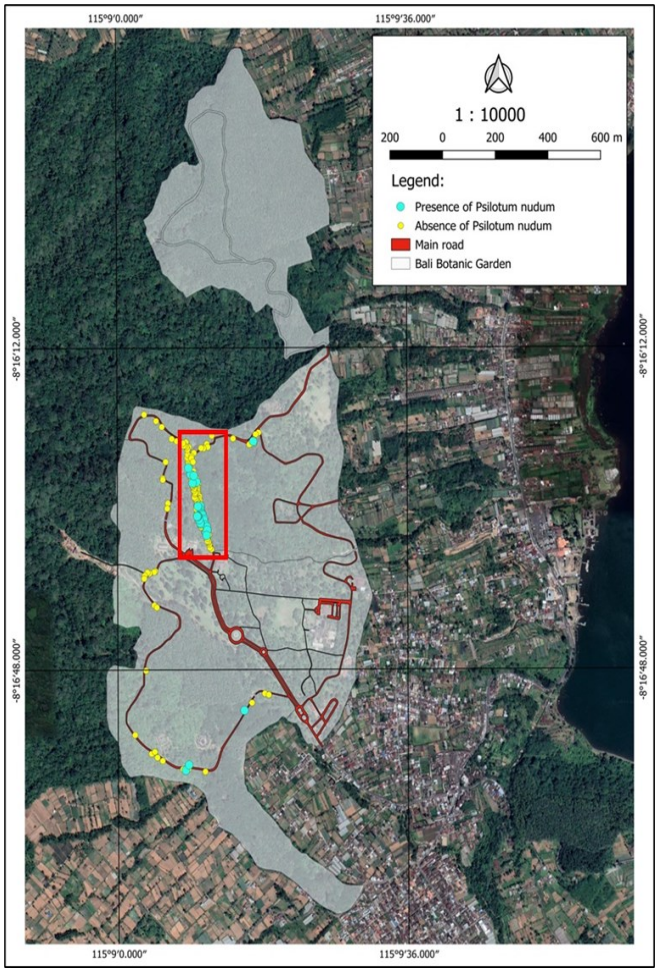

Fig. 4. Map of the presence of *Psilotum nudum* on tree fern along the main road in the Bali Botanic Garden.

Table 3. Altitude, number of individuals and percentage of *P. nudum*

Height (cm)	Number of individuals (clumps)	Percentage (%)
0 – 50	6	7.2
60 – 100	8	9.6
110 – 150	15	18.1
160 – 200	29	35
210 – 250	8	9.6
260 – 300	13	15.7
310 – 350	3	3.6
360 – 400	0	0
410 – 450	1	1.2

Table 4. The level association of *P. nudum* with phoprophyte

No	Species pairs	Level Association	
		Jaccard Index (JI)	Dice Index (DI)
1.	*Psilotum nudum* × *Alsophila orientalis*	0.39 High	0.57 High
2.	*Psilotum nudum* × *Sphaeropteris glauca*	0 Low	0 Low

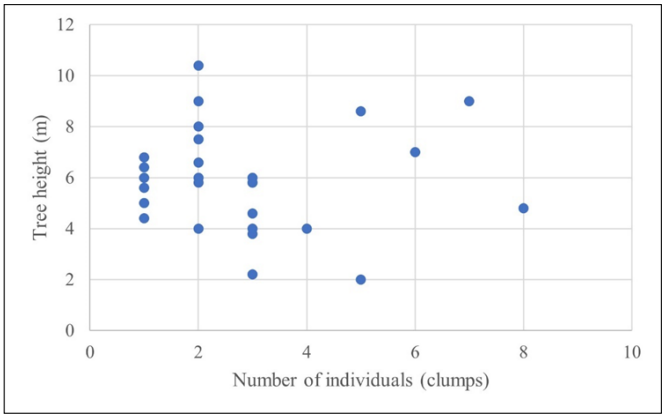

Fig. 5. The relationship between the height of *A. orientalis* trunk and the number of individuals of *P. nudum.*

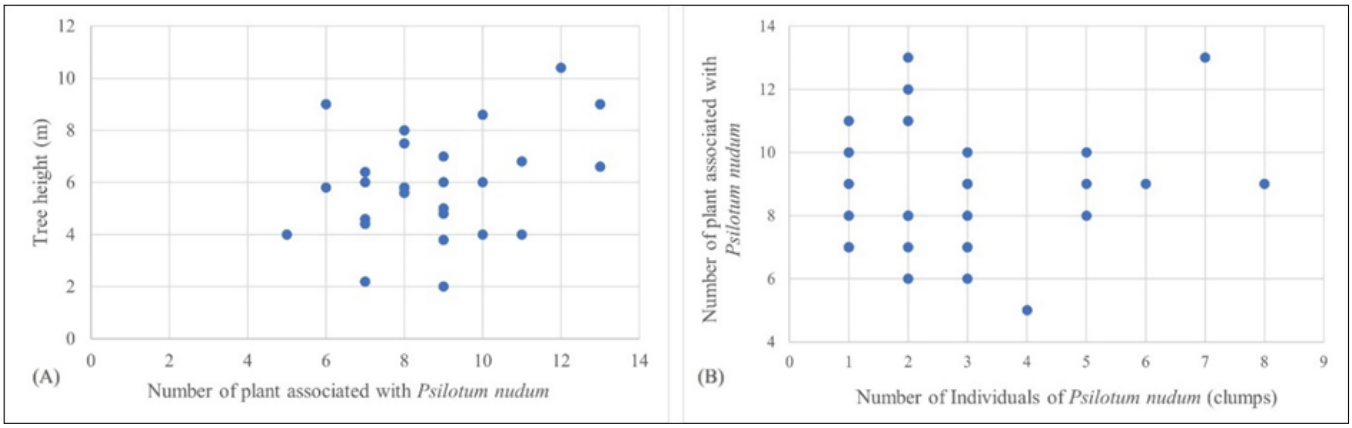

Fig. 6. The relationship between the height of *A. orientalis* and the number of plant species associated with *P. nudum* (A) The relationship between the number of individuals of *P. nudum* and the number of plants associated with *P. nudum* (B).

Table 5. Plant species association with *Psilotum nudum*

No	Species name	Family	Frequency	Relative Frequency (%)
1	*Ageratina riparia* (Regel) R. M. King & H. Rob	Asteraceae	11	4.4
2	*Ageratina* sp.	Asteraceae	2	0.8
3	*Agrostophyllum* sp.	Orchidaceae	2	0.8
4	*Asplenium nidus* L.	Aspleniaceae	20	8
5	*Lepisorus spicatus* (L. f.) Li Wang	Polypodiaceae	5	2
6	*Bulbophyllum absconditum*	Orchidaceae	1	0.4
7	*Bulbophyllum* sp.	Orchidaceae	1	0.4
8	*Coelogyne carnea*	Orchidaceae	1	0.4
9	*Davallia denticulate* (Burm. f.) Mett	Polypodiaceae	26	10.6
10	*Davallia pentaphylla* Blume	Polypodiaceae	14	5.69
11	*Davallia repens* (L. f.) Kuhn	Polypodiaceae	8	3.25
12	*Dendrobium crumenatum*	Orchidaceae	1	0.4
13	*Dischidia* sp.	Apocynaceae	17	6.8
14	*Epipremnum pinnatum* (L.) Engl.	Aracaceae	8	3.2
15	*Eria* sp.	Orchidaceae	1	0.4
16	*Ficus fistulosa* Reinw. ex Blume	Moraceae	2	0.8
17	*Ficus punctata* Thunb	Moraceae	12	4.8
18	*Ficus repens* Rottler	Moraceae	1	0.4
19	*Ficus* sp	Moraceae	6	2.4
20	*Goniophlebium subauriculatum* (Blume) C. Presl	Polypodiaceae	12	4.8
21	*Huperzia squarrosa* (G. Forst.) Trevis	Lycopodiaceae	2	0.8
22	*Hoya* sp.	Apocynaceae	4	1.6
23	*Lindsaea* sp.	Lindsaeaceae	2	0.8
24	*Loxogramme avenia* (Blume) C. Presl	Polypodiaceae	2	0.8
25	*Mycetia cauliflora* Reinw.	Rubiaceae	1	0.4
26	*Nauclea* sp.	Rubiaceae	1	0.4
27	*Nephrolepis cordifolia* (L.) C. Presl	Polypodiaceae	14	5.6
28	*Nephrolephis biserrata* (Sw.) Schott	Polypodiaceae	8	3.2
29	*Piper* sp.	Piperaceae	9	3.6
30	*Procris* sp.	Urticaceae	2	0.8
31	*Pyrrosia glabra* (Desv.) Fraser-Jenk	Polypodiaceae	12	4.8
32	*Saurauia* sp.	Actinidiaceae	15	6
33	*Schleffera* sp.	Araliaceae	4	1.6
34	*Selliguea enervis* (Cav) Ching	Polypodiaceae	3	1.2
35	*Sphaerostephanos* sp.	Aspleniaceae	2	0.8
36	*Thrixspermum* sp.	Orchidaceae	2	0.8
37	*Vaginularia trichoidea* Fee	Pteridaceae	1	0.4
38	*Vaginularia paradoxa* (Fee) Miq.	Pteridaceae	5	2
39	*Haplopteris elongate* (Sw.) E.H. Crane	Pteridaceae	1	0.4
40	*Haplopteris ensiformis* (Sw.) E.H. Crane	Pteridaceae	2	0.8
41	*Vittaria* sp.	Pteridaceae	6	2.4
42	*Wendladia* sp.	Rubiaceaea	1	0.4
	Total		**250**	**100**

Table 6. Habitat characteristics of *P. nudum* in the research sites

Tree Fern Number	Number of *P. nudum* epiphytes at tree fern	Relative Humidity (%)	Lux (Klux)	Temperature (°C)	Elevation (m asl)
8	5	78	815	24.2	1365
10	5	72	903	24.6	1367
17	1	82.6	5020	24.8	1371
18	1	86.5	2750	23.9	1371
19	2	79.1	2000	22.8	1371
22	5	77.3	1528	23.8	1371
23	1	77.3	1528	23.8	1371
25	2	80.4	2940	24	1371
33	3	76.2	1770	25	1379
47	2	81.6	1345	24.6	1389
51	8	81.8	16520	25.9	1390
54	6	72.5	15710	28.5	1390
76	7	67.2	2570	28.2	1366
77	2	67.7	3870	28.3	1351
82	3	77.7	2030	23.6	1388
97	1	77.4	3420	23.3	1393
102	1	78.4	1104	23.1	1393
117	2	77.6	2460	22.6	1389
118	3	78	1782	22.85	1389
120	2	75.75	3720	24.3	1389
121	2	77.7	3160	23.4	1389
122	6	73.8	3180	23.9	1389
123	3	73.8	4280	25.2	1389
125	3	73.2	5860	25.9	1389
129	1	73.5	1520	25.5	1389
131	3	68.8	3740	25.4	1389
132	3	77.3	677	23.3	1389
155	1	62.4	842	24.6	1348
Average		75.84± 5.32	3520.85± 3943.59	24.7±1.61	

Discussion

We found two species of tree fern from Family Cyatheaceae that grew within the maximum distance of two meters on the right and left sides of the main ring road of Bali Botanic Garden. Those are *Alsophila orientalis* (156 individuals) and *Sphaeropteris glauca* (four individuals). Some were grown natively, and some others were planted as border plants. The trunk of those tree ferns become phorophytes for other plants, including the whisk fern *Psilotum nudum*.

Psilotum nudum can be found in living epiphytes on other plants or terrestrials. The epiphytic plants are adapted to their unusual and limited habitat (17). As epiphytes, *P. nudum* are attached to different plants and get nutrients from non-terrestrial sources. In Iran, *P. nudum* grows as an epiphyte on the trunk of *Parrotia persica* C. A. May in the Ramsar riparian forest on a river bank (586 m. asl) and as lithophyte in crevices among rocks near streams in the Ata-Kuh forest (207 m. asl) (18). *P. nudum* also found epiphytes on trees or between branches on humus, limestone and rocky soil around the coast in Oelmuke Hamlet, Tasinifu Village, Mutis District, North Central Timor, NTT (less than 500 m. asl) (19)

From those 160 individual samples of tree fern, *P. nudum was* only found at 29 trunks of *A. orientalis*. This species was among the top five host plants (phorophytes) found at Bukit Pengelengan near Bali Botanic Garden and was the most suitable in the surrounding area (15). Previously, the species was misidentified as *Cyathea latebrosa*, but it is now known as *A. orientalis*. He also stated that host trees favoured by epiphytic ferns usually have thick, grooved or filamentous skin texture and hard bark.

Bali Botanic Garden, *P. nudum* was not found in *Sphaeropteris glauca* trunk samples. In Cibodas Botanic Garden, *P. nudum* were found only at *Cyathea junghuhniana* (synonym of *Alsophila junghuhniana*) and were not found attached to *Cyathea contaminans* (synonym of *Sphaeropteris glauca*) either (13). The trunk structure is thought to affect the absence of *P. nudum* in *C. contaminans* because the trunk contains a structure formed from the former frond shedding. So, *P. nudum* found in Bali Botanic Garden attaches to *A. orientalis* while *P. nudum* in Cibodas Botanic Garden attaches to *C. junghuhniana* (= *A. junghuhniana*). This species-specific relationship is suggested because the holding capacity of *A. orientalis* is higher compared with *S. glauca* trunk found the epiphytes *Tillandsia usneoides* and *Polypodium polypoides* were highly associated with the water-holding capacity of the host tree's bark (20).

Microclimate, including air temperature, air humidity and light intensity in Bali Botanic Garden (24.7 °C, 75.84 % and 3,520.85 Lux) is higher compared to Cibodas Botanic Garden (21.97 °C, 65.23 % and 2,629.12 Lux) (13).These microclimate conditions are suitable for the growth of *P. nudum* and never been reported before. However, Cibodas botanic garden is located at 1300-1450 m above mean sea level, *P. nudum* was only found as an epiphyte on *Cyathea jughuhniana* (13). Plant distribution, richness and abundance are affected by macro and microclimate, which results from a combination of environmental factors and habitat altitude (21). *Psilotum nudum* was not found alone in its phorophytes. It is found at the trunk of *A. orientalis* along with 42 other plant species and

the most common plant species are *Davalia denticulata* (10.6 %), *Asplenium nidus* (8.13 %) and *Dischidia* sp. (6.91 %). Epiphytic plant communities provide an ideal system for evaluating the specific interactions of each species. Vascular epiphytic plants are constituents of up to 10 % of plants in nature (20).

Some species of tree fern can provide a favourable habitat for many epiphytes. It is possible to find large numbers of epiphytes in part of tree fern trunks containing adventive roots (22). The trunk of *A. orientalis is* covered with adventitious roots, which is ideal for some other epiphytic plants to grow on it. It's fibrous, airy and has water retention. Tree ferns, especially *Cyathea* trunks, are often used as a growing medium for epiphyte plants, flower pots and other crafts. This is what causes its population in nature to decrease. In addition, *Cyathea spp* is also included in threatened plants in the CITES index. Tree fern trunks are specific hosts and not for all epiphytes. The morphology of the trunk and the microclimate around it will influence the settlement of the epiphytes (22). Apart from the microclimate, the type of epiphyte is also influenced by the dispersal of spores and shoot development, which affects growth (23). *Davalia denticulata* and *Asplenium nidus* are ferns. In Cibodas Botanic Garden, the highest number of epiphytic plants found in *C. contaminans* and *C. junghuhniana* were also fern group (13).

The higher the Cyatheaceae trunk, the fewer *P. nudum* individuals were found. It assumed that at the top level of the trunk, the microclimate, such as light and temperature, is higher than the lower part and did not support the life of *P. nudum.* However, our results differ from those found in Adams, Ilocos Norte, northern Luzon, Philippines, where *P. nudum* was observed at the top of the tree trunk (24). There was no influence/ correlation (R^2 = 0.058) between host tree height and vertical distribution of *Asplenium nidus* (25).

Contrary to our findings, the more plant species are associated, the fewer individuals are found due to competition for nutrients and growing space. The number of *Psilotum nudum* individuals found at the trunk of *Alsophila orientalis* was not influenced by the number of associated plants. It is thought to be more influenced by the growth of *Psilotum nudum* shoots.

Conclusion

In Bali Botanic Garden's main ring road, tree ferns, *Psilotum nudum* grew only on the host tree (phorophyte) *Alsophila orientalis*. This plant is associated with 42 species of other plants. The most common associated plant species are *Davalia denticulata* (10.6 %), *Asplenium nidus* (8.13 %) and *Dischidia* sp (6.91 %). Host tree height has a weak but insignificant correlation with the number of *P. nudum* individuals (r = - 0.14, p = 0.5). The higher the Cyatheaceae trunk, the fewer *P. nudum* individuals were found. Host tree height was weakly correlated with the number of associated plant species (r= 0.24, p= 0.22). This data helps complete ecological data on plant collection that can be used as a reference in their development and cultivation efforts.

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Authors' contributions

IDPD conceptualized and contributed by giving essential suggestions. IDPD, SFH, WSL, AR and MBA conducted field surveys and collected data. WSL identified the specimens. SFH and AR carried out the field data analysis. All authors performed the experiments, authored and reviewed the draft of the article and approved the final draft.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare

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