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蕨上之蕨：探索台灣蕨類附生配子體棲身在台灣杪欏之上

Ferns on ferns: an exploration of epiphytic fern gametophytes growing on *Alsophila spinulosa* in Taiwan

Alexandria Quinlan

指導教授: 胡哲明, 郭立園

Advisors: Jer–Ming Hu, Ph.D.; Li–Yaung Kuo, Ph.D.

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本論文係Alexandria Quinlan君（學號: R09B44022）在國立臺灣大學生態學系、所完成之碩（博）士學位論文，於民國2022年7月7日承下列考試委員審查通過及口試及格，特此證明

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國立臺灣大學生態學與演化生物學研究所 澤大衛 博士 _____
Dr. David Zelený, National Taiwan University (Committee Chair)

國立臺灣大學生態學與演化生物學研究所 胡哲明 博士 _____
Dr. Jer-Ming Hu, National Taiwan University (Co-advisor)

國立清華大學生命科學院分子與細胞生物研究所 郭立圜 博士 _____
Dr. Li-Yaung Kuo, National Tsing Hua University (Co-advisor)

所長(director) _____（簽名 Signature）

中華民國111年7月21日（日期）

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ABSTRACT

Tree ferns have been documented as significant hosts for epiphytes and are often composed of distinct communities that are considerably different from other hosts. Particularly, exclusive growth of epiphytic fern species has been exhibited on tree ferns, however, this fern–on–tree–fern epiphytism has yet to be substantially explored for the gametophyte generation. This study focused on epiphytic fern gametophytes growing on low trunks of a tree fern species, *Alsophila spinulosa* (Cyatheaceae), and nearby angiosperm hosts. Fern gametophyte surveys were conducted in two seasons, during the months of June and October, in a subtropical forest in Taiwan. These surveys sought to understand what factors make tree ferns optimal habitats for gametophytes, and whether there are notable differences between the composition of fern gametophyte communities on tree ferns compared with other hosts. Gametophytes were identified using an Illumina Miseq approach (Illumina inc., San Diego, CA, USA) which involved sequencing multiplexed *trnL–F* amplicons derived from tissue–direct PCR. Environmental surveys were conducted for each season, in which we recorded relative humidity (RH) and measured canopy openness and light availability. Overall, October had a higher abundance of individuals in the epiphytic gametophyte community which could be a phenological association. Tree ferns harbored a significantly higher abundance and species richness of gametophytes than angiosperm hosts, and hosted more cordiform gametophytes that were mostly from accidental and facultative fern epiphytes. In comparison, angiosperms had a higher abundance of non–cordiform individuals. In addition, two independent gametophytes were found on the surveyed hosts, *Callistopteris apiifolia* and a *Haplopteris yakushimensis* species. Generally, plots with a higher species richness of sporophytes also had a higher richness of gametophytes. Results from the environmental survey showed that the RH for all surveyed

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hosts was most frequently over 95%, however the angiosperm host spent considerably more time below 95% and 85% RH than tree ferns. The stable RH revealed on the tree fern trunks, which presumably results from the moist root mantle, allowed for a high diversity of fern gametophytes. There was overall no statistical significance for the relationship between canopy openness and gametophyte diversity. Nevertheless, in each season, the plot with the highest total light also had the high species richness and abundance for fern gametophytes. Importantly, this study is among the first insights of epiphytic gametophyte communities on tree ferns.

Keywords: Cyatheaceae, epiphytes, ferns, gametophytes, multiplexed amplicon sequencing, relative humidity, tissue–direct PCR, tree ferns, *trnL–F*

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INTRODUCTION

Fern–on–tree fern epiphytism

Epiphytes, plants that grow on other plants without parasitism, has long been a topic of interest for ecologists and botanists; with a physical dependence on their host plants, an interspecies relationship is realized. Among these epiphyte–host relationships, ecologists have highlighted distinct epiphytic communities growing on tree ferns (Oliver, 1930). Tree ferns have a long history of being host to epiphytes; evidence of epiphytic growth on the Paleozoic Marattialean tree fern *Psaronius* emphasizes that this ecological phenomenon has ancient origins in the early Permian (Rößler, 1999). Similarly, research on a fossilized rhizome of a Jurassic Osmundaceous fern revealed that it likely hosted a complex community of epiphytes (McLoughlin & Bomfleur, 2016). Evolution of epiphytic assemblages on angiosperm hosts is comparatively recent, where the angiosperm–dominated forests of the late Cretaceous and early Cenozoic provided novel ecological niches for plants to radiate into the canopy (Nitta et al., 2020). The development of this heterogeneous canopy presents an important influence for the diversification of many of the present–day epiphytic plants growing on tree ferns and other hosts.

Recognized as an "ecosystem unto itself" (Rößler, 1999), further studies have illustrated the hyper diversity of epiphytic growth on tree ferns (Medeiros, 1993; Roberts et al., 2005, Schmitt & Windisch, 2010). Host comparisons have been conducted where it was found that tree ferns host a higher abundance and species richness of epiphytes than angiosperm hosts (Moran et al., 2003; Mehltreter et al., 2005). Particularly, certain epiphytic fern species were observed to exclusively grow on tree ferns (Moran et al., 2003, Mehltreter, 2008; Lehnert, 2019), presenting an extreme case of host specificity that is predominantly found in fern epiphytes (Wagner et al., 2015). Fern–on–tree fern epiphytism was further highlighted by Lin (2019) who explored the

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evolution and ecology of fern species which are termed “Tree Fern Specialists”. A recent study on one of these tree fern specialists, namely *Vaginularia junghuhnii*, revealed that, in addition to the sporophyte, the gametophyte of this species presumably has restricted growth on tree fern trunks (Wu et al., 2022). Nevertheless, for specialists and generalists alike, there has been a primary focus on the sporophyte generation of fern epiphytes. Thus, a question still remains: Are tree ferns also a more significant host for the gametophyte generation of epiphytic ferns?

Significance of the gametophyte generation

First, we will discuss the significance of the gametophyte generation of ferns. Ferns are unique in that they have two independently existing generations, the haploid gametophyte and the diploid sporophyte, where the gametophyte has many responsibilities including sexual reproduction, habitat selection, and migration (Farrar et al., 2008). However, the gametophyte generation is often overlooked. They are an essential part of the fern life cycle, yet, only a few studies have investigated their ecology (Dassler & Farrar, 2001; Ebihara et al., 2013, 2019; Watkins et al., 2007a, b; Quinlan et al., 2022; Wu et al., 2022). Gametophytes can be difficult to study, due to their obscure morphology and small size, and are thus hard to identify to species level without DNA–identification. Regardless, this generation may tell a different ecological story than the sporophyte generation.

Unlike sporophytes, gametophytes lack vascular tissue and stomata, have poorly developed to absent cuticles, and produce rhizoids instead of roots (Raghavan, 1989). With vastly different morphologies and physiologies, these generations can often have diverging habitat requirements and preferences (Nitta et al., 2021). Furthermore, gametophytes often have more widespread distributions than their conspecific sporophytes (Dassler & Farrar, 1997;

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Watkins et al., 2007a; Pinson et al., 2017; Nitta et al., 2021; Pinson et al., 2022). This has been confirmed by field studies such as Ebihara et al. (2019) where the sporophyte of *Pleurosoriopsis makinoi* growing at a lower elevation preferred open canopy sites while its gametophytes grew at a site on shady rocks along a river. There have been other cases of this "separation of generations" (Pinson et al. 2017) where fern gametophyte populations persist without a conspecific sporophyte nearby, and often do so with their self–proliferating gemmae. Since their distributions may not overlap, and to unveil the potential of independent gametophytes, it's crucial to study both generations.

Gametophyte ecomorphology

Fern gametophyte life forms are further diversified by their ecomorphology, where a cordiform, short–lived morphotype is generally associated with terrestrial growth and non–cordiform, long–lived morphotypes are associated with epiphytism (Farrar et al., 2008). The long–lived morphotype is key to adapting to an epiphytic habitat, where abiotic and biotic factors are much different than those on the ground. Growing above the competition of the terrestrial environment, non–cordiform gametophytes can slowly develop into the next generation. However, compared with terrestrial habitats, water and proximity for outcrossing is not as guaranteed in the canopy. Since water is required for the motile sperm of fern gametophytes, proximity is crucial for sexual reproduction. The ribbon, strap and filamentous morphotypes have adapted a branching of the gametophyte thallus, increasing the opportunities for outcrossing (Dassler & Farrar, 2001). Other non–cordiform species opt for asexual reproduction, where they develop the ability to produce gemmae (Farrar et al., 2008). These are small, vegetative propagules that disperse to give rise to a new, yet clonal, population. Even so, beyond sexual

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reproduction, gametophytes still require moisture for survival; their single–cell layer thick tissue deems them generally dry–intolerant and their lack of stomata and cuticle inhibit their ability to control water loss. While some gametophytes have evolved to moderately tolerate desiccation (Watkins et al., 2007b), the majority of species require a moist environment for stability.

Tree fern structure and its significance for fern gametophytes

Root mantle characteristics

The root mantle of tree ferns can provide a moisture–stable habitat for gametophytes throughout their development. Composed of adventitious roots, the mantle favors epiphyte establishment (Johansson, 1974), and has a high water retention rate (Mehltreter et al., 2005). Additionally, as one of the few fern families with arborescent trunks (Large & Braggins, 2004), tree ferns are remarkably sturdy. This is largely due to their sclerenchyma; unlike wood which is produced by a vascular cambium, sclerenchyma is hardened tissue that runs along the stem of the tree fern's "trunk", surrounding the conducting tissues of xylem and phloem. Additional support is provided by the external layer of dense, interlocking roots (i.e. root mantle), which is usually two to five times wider than the stem's diameter (Moran, 2004). Recently, studies of the tree fern genome (Huang et al., 2022) found the presence of high lignin content in the xylem, which further supports the rigid, wood–like quality of a tree fern's root mantle.

Suitability for fern gametophytes

These characteristics of the root mantle come together to create a strong substrate with a sponge–like quality that is especially suitable for fern gametophytes. The moist substrate is also frequented by bryophyte mats, which, in addition to the intricate weaving of the tree fern mantle, provide spaces where non–cordiform gametophytes can persist until favorable conditions occur

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for gemmae production or sporophyte establishment (Farrar et al., 2008). As highlighted by Watkins and Cardelús (2009), the gametophyte generation is a time for "habitat exploration". This is confirmed by the observation of not only epiphytic, but also hemiepiphytic and terrestrial species using tree ferns as a substrate for establishment (Schneider & Schmitt, 2011; Lin, 2019). In many ways, the moist mesh of roots can imitate a terrestrial environment, creating microhabitats that would otherwise not be available in canopy environments. This supports the likelihood that there will be a complex community of fern gametophytes growing on tree ferns. We seek to explore this community and to determine factors that could influence the composition of fern gametophytes.

Rationale

Epiphytes and the protection of tree ferns

Over 30% of the world's ferns are epiphytic (Kress, 1986), which accounts for 10% of the world's total vascular epiphytes (Zotz et al., 2021). Being that an epiphytic habitat is quite representative in ferns, researchers are determined to understand the factors influencing habitat and host preferences. With their fibrous root mantle and moist substrate, tree ferns are a substantial host for fern epiphytes (Moran et al., 2003), and as highlighted in the introduction, there are some species with a preference for or restriction to growing on tree ferns (Moran et al., 2003; Schmitt & Windisch, 2010; Lin, 2019.) The abundance of epiphytic bryophyte (Beever, 1984; Roberts et al., 2005) and angiosperm species (Gaxiola et al., 2008; Dawes & Burns, 2020) growing along the mantle further illustrate the diversity and importance of a tree fern habitat. However, tree ferns are known to be overharvested for their gardening potential (Windisch, 2002), prompting the Convention of International Trade in Endangered Species of Wild Fauna

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and Flora to incorporate Cyatheaceae and *Dicksonia* species to protect them from overexploitation (IUCN, 2008). Importantly, we can learn from local communities on how to sustainably harvest tree ferns (e.g. only harvesting when there is an immediate need) so that they may continue to hold ecological and cultural significance (see Appendix for interview with Yaya Huwat from the Skadang tribe). Additionally, tree fern species in Taiwan are experiencing a fungal infestation causing wilt disease, leading to death within two months (Fu et al., 2013). Therefore, we should continue to highlight tree ferns as optimal microhabitats to encourage further measures to be taken for their protection and conservation.

Ecology of spore–producing organisms and progress of fern gametophyte research

In general, spore–producing plants are poorly understood, where Roberts (2005) has highlighted the importance of developing ecological knowledge for the biodiversity–protection measures of these organisms. Tree ferns have long been pioneers in forest ecosystems, influencing community assembly both in the canopy and on the ground (Brock, 2018). In addition, with a rich fossil record, the study of tree ferns is an opportunity to perceive historical ecosystems (Rößler, 1999; Bippus et al., 2019). Specifically, this study will be among the first insights of epiphytic fern gametophyte communities on tree ferns, illustrating the importance of this habitat for fern gametophyte growth. Any study which explores gametophyte distributions can promote further understanding of the ecology, phenology, and evolution of gametophytes. This study in particular seeks to understand what factors make tree ferns optimal habitats for gametophytes, and if there are notable assemblages of gametophytes on tree ferns compared with other hosts.

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Aims of this study

The purpose of this study is to further demonstrate the significance of tree ferns as a substrate for epiphytic fern growth by highlighting the gametophyte stage of ferns growing on Cyatheaceae in Taiwan. This study aims to (1) measure and compare the abundance and species composition of epiphytic fern gametophytes growing on *Alsophila spinulosa* and nearby angiosperm hosts, (2) record presence/absence of epiphytic fern sporophyte species growing on hosts, and (3) measure environmental variables of tree fern and angiosperm hosts to evaluate factors which likely influence the gametophyte community. Specifically, we will be measuring relative humidity (RH) and canopy coverage.

With reference to previous studies that recorded the diversity of epiphytic fern sporophytes growing on tree ferns, it is expected that (1) tree ferns will also host a higher abundance and diversity of fern gametophytes than angiosperm hosts. As highlighted, the substrate of the tree fern trunk is quite different from that of an angiosperm; composed of many overlapping roots where both space and moisture are provided for gametophyte establishment. These characteristics are expected to be suitable for not only a larger diversity of gametophyte species, but also a diversity of gametophyte types including both cordiform and non–cordiform morphotypes, and epiphytic and accidentally epiphytic lifestyles.

Additionally, it is hypothesized that the (2) species composition of fern gametophytes and sporophytes will vary; the gametophyte generation will likely be more abundant and diverse than the sporophyte generation. There are many processes that must occur in order for sporophyte production to take place; not all species that establish as gametophytes on hosts will develop into a viable sporophyte. Further, non–cordiform fern gametophytes, the morphotype associated with epiphytism, are at an advantage due to their dispersal ability and phenology as long–lived

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perennials (Farrar et al., 2008). Their conspecific sporophytes may not be nearby and could be more affected by seasonal shifts (Ebihara et al., 2013).

A final expectation is that (3) tree ferns will maintain stable, high levels of relative humidity (RH). In 2019, Lin recorded the RH of a tree fern and angiosperm host in Taiwan for a 24–hour period and found that the RH was much higher and more stable for tree ferns than angiosperms. With additional support from Mehltreter's (2005) study highlighting the high water retention rate of the tree fern root mantle, it is expected that the stable RH and moist habitat of tree ferns will be influential factors for epiphytic fern gametophyte communities.

MATERIALS AND METHODS

Field surveys and study site

Community survey

Community surveys of fern gametophytes growing on tree fern and angiosperm hosts were conducted in Wulai, Taiwan at an elevation of 700 m in a small valley located near Neidong Forest Road (內洞林道). The vegetation of Neidong forest is best characterized as a *Pyrenaria–Machilus* winter monsoon forest type (Li et al., 2013). Neidong forest has a year long rainy season with over 3,600 mm of annual precipitation and a mean annual temperature of 20°C (桶後 weather station: https://e–service.cwb.gov.tw/HistoryDataQuery/ [accessed 23 May 2022]). Surveys were conducted during the months of June and October 2021, as gametophyte populations have been recorded to experience peaks in abundance during these two months (Quinlan et al., 2022). For each survey, 3 circular plots were determined along either side of a stream, resulting in 6 plots total, each 5m in diameter with a tree fern at the center (see Fig.1).

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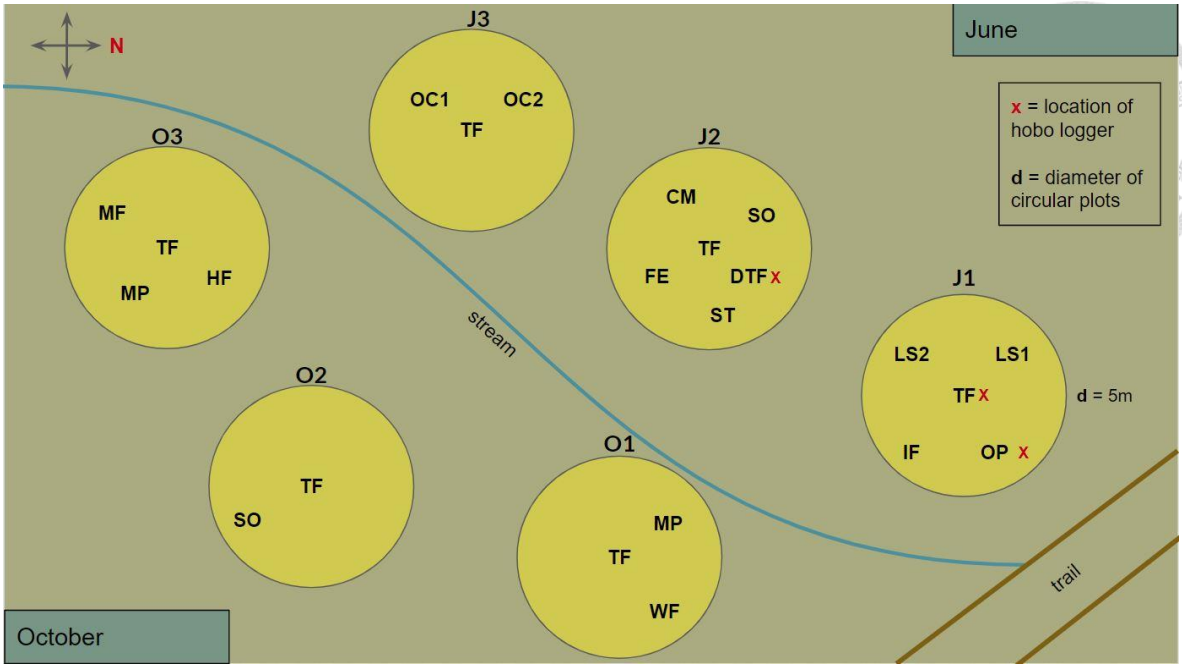

Figure 1. Site diagram.

Two survey seasons (June and October, respectively) consisting of 3 plots each, on either side of a stream. Plots are labeled by first letter of the month, and number of the plot. Abbreviations inside are for the hosts, and the red x denotes the location of hobo loggers. Names of host abbreviations are provided in Table 2.

Initial criteria for plot selection included finding tree fern hosts with an epiphytic community established, which also had angiosperm hosts growing nearby for comparison. Each plot consisted of one tree fern, with the exception of Plot 2 from June, where, in addition to the sampled live tree fern, one dead tree fern within the 5 m diameter was surveyed to see how lack of frond growth affects the fern gametophyte community. All tree ferns within the plots were *Alsophila spinulosa*. Any angiosperm hosts within the plot that had a diameter less than 1 π cm were not surveyed. Notably, this study required destructive sampling (i.e. removal of gametophytes for identification), thus the trunks from each survey period were only sampled once.

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For the gametophyte survey, abundance and species composition were recorded beneath 1m on tree fern and angiosperm hosts. The surveyed area of the trunks was further broken into 2 zones, below 50 cm and above 50 cm respectively. For a standardized sampling method, a garden net with 2.5 × 2.5 cm squares was wrapped around the trunks to develop subplots for collection. The number of gametophyte populations within each square determined the collection of individuals. While cordiform individuals are distinguishable, determining individuals of non–cordiform morphotypes can be difficult due to their branching patterns. Consequently, all cordiform individuals within a given square were collected, whereas if there was only one non–cordiform population within the 2.5 × 2.5 cm square, this was considered as one individual. Previous studies of sampling gametophyte populations assisted in establishing this method (Nitta et al., 2017). Gametophytes from each host were placed in respective collection boxes and brought back to the lab for processing, including cleaning, photographing for voucher images, and tissue–direct PCR identification (method described below).

To compare the species composition of sporophytes and gametophytes and note the presence of conspecific sporophytes, all sporophyte species observed along the trunks were recorded. Sporophytes were morphologically identified in the field except for when there were cryptic juveniles, which were brought back to the lab for further analysis. Sporophytes were recorded as presence/absence of species (see Table A1). For species whose gametophytes were observed without a conspecific sporophyte, additional databases (TAIF: https://taif.tfri.gov.tw/tw/index.php; Taiwan Biodiversity Network: https://www.tbn.org.tw/) were referenced to determine if records of their sporophytes exist nearby. Species nomenclature follows the TPG (2019, 2021)'s classification system.

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While not a focus of the study, the same sampling protocol was utilized to collect fern gametophytes from *Alsophila podophylla* hosts outside of the plotting sites. In June, three *A. podophylla* hosts were surveyed in a different locality, approximately 500 m away from the study site. In October, three *A. podophylla* hosts were surveyed within 5 to 10 m of the three plots. These hosts were not included in the environmental survey or statistical analyses. All gametophytes collected from these hosts followed the same DNA–based identification protocol below.

Environmental survey

To test the microclimate of hosts, HOBO temperature/relative humidity (RH) sensors (S–THB–M002, Onset, Bourne MA, USA) were used and recorded with HOBOware (ONSET Computer Corporation, Bourne, MA, USA). The loggers were programmed to record RH and temperature every 10 minutes and were placed in the field from May 15th to October 16th, 2021. Loggers were installed at 1.5 m on the trunks of one living tree fern, one dead tree fern, and one angiosperm (see Fig. 1 for placement). Only the data from May 15th to August 23rd was analyzed due to faulty loggers and inconsistent recording.

Additionally, canopy coverage and light availability was measured using a hemispherical lens (D5500, NIKON, Japan; 4.5mm F2.8 EX DC HSM Circular Fisheye, SIGMA, Japan). The camera was situated 1 m out from the tree fern at the center of the plot, and a photo was taken in each quadrant (N, S, E, W). The four photos from each plot were processed in Gap Light Analyzer (Simon Fraser University, Cary Institute of Ecosystem Studies, Millbrook, NY, USA). After the photos were analyzed, the data values from each set of four photos were averaged to obtain a final total of canopy openness and light availability for each plot.

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DNA–based gametophyte identification

Tissue–direct PCR

Fern gametophyte samples were identified using an Illumina Miseq Approach (Fig. 2) which involved sequencing multiplexed *trnL–F* amplicons that were derived from tissue–direct PCR, an extraction–free method for gametophyte identification (Li et al., 2010). Due to its small size, DNA extraction of a gametophyte could compromise the entire specimen, preventing further morphological studies. For pretreatment, after cleaning the samples with soft brushes in water, a healthy, green piece of gametophyte tissue (within 1 mm^2 size) was detached from each sample using tweezers and placed in a respective PCR tube with 20 µL ddH_2O. The tweezers were cleaned with 75% alcohol between slicing of different gametophyte samples. The tissues were then fragmented by cycles of freezing with liquid nitrogen, dissolving using an ultrasonic cleaner, and spinning down the liquified samples in a mini–centrifuge. These steps were repeated until gametophyte tissue was no longer visible (see "Optimized protocol of TD–PCR" in Wu et al., 2022). These raw extractions were used as templates for subsequent PCR experiments. For the PCR reagents, 1 µL of the template was added to 5 µL ddH_2O, 7.5 µL super–red PCR master mix (Biotools, New Taipei City, Taiwan) and 0.5 µL of each primer for a total reaction volume of 15 µL. The following barcoded primer set was used for PCR amplification and sequencing: FernF4121_br01 to br20 and FernL5675_br01 to br20 (Kuo et al., unpublished) (See Table A2 in appendix). Additionally, a primer set targeting a longer *trnL–F* region (i.e. including *trnL* intron), FernL0725 paired with FernF4121 (Wu et al., 2022), was used for all filamentous gametophytes as well as for any samples that failed with the barcoded primer set.

The amplification program was performed on a SimpliAmp Thermal Cycler (Thermo Fisher Scientific, Waltham, Massachusetts, USA) beginning with a one initial denaturation step

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for 5 min at 94°C, followed by 35 cycles of 1 minute at 94°C, 30 seconds at 55°C, and 30 seconds (for the multiplexed *trnL–F* amplicons) or 60 seconds (for amplicons of a longer *trnL–F* region) at 72°C, ending with an extension period of 10 minutes at 72°C. If amplification was completed with the barcoded primer set, the samples were sequenced using the llumina Miseq PE 300 platform (method below) and Next Generation Sequencing (NGS) was performed by Health GeneTech Corp. (Taoyuan, Taiwan). For the samples sequenced with the longer, non–barcoded primers (F4121, L0725), an ABI 3730XL DNA Analyzer (Thermo Fisher Scientific) was used for Sanger sequencing, which was performed by Genomics BioSci & Tech (New Taipei City, Taiwan).

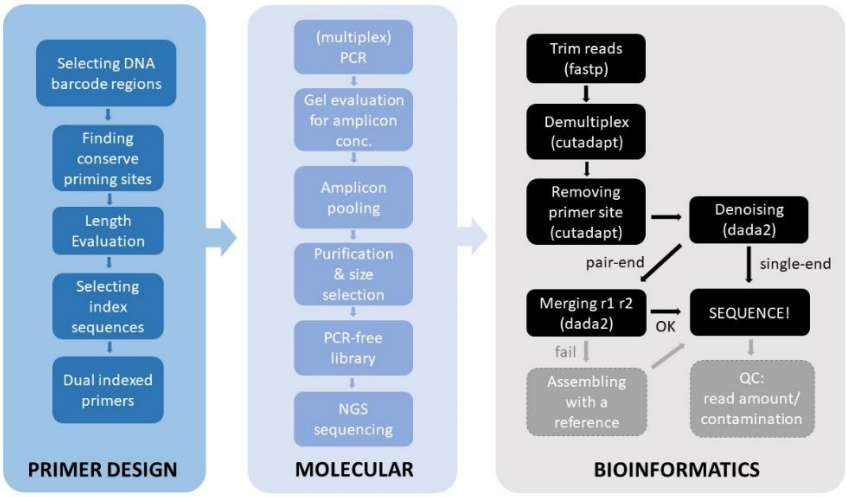

Figure 2. DNA–based identification protocol.

"Power Barcoder" created by Li–Yaung Kuo (unpublished). Includes initial primer design, molecular protocol, and follow up bioinformatics with sequence processing.

Illumina Miseq Platform and Library preparation for Next Generation Sequencing

For the Illumina Miseq platform (Illumina inc., San Diego, CA, USA), samples needed to be pooled together for sequencing. Amplicon pooling consisted of measuring the concentration and length of the amplification in order to determine the amount of each sample that will be

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added to the pool. The length for all samples was between 400–500 bp. Amplicons were separated into 4 concentration levels depending on the strength of the band in the electrophoresis (Table 1). Samples with a low concentration (e.g. 9 ng/1.5 µL) had a larger volume in the pool (e.g. 5 µL) than a sample with a high concentration (1.76 µL added for a concentration of 25.5 ng/1.5 µL). Once all samples were pipetted into a 2.5 mL tube, 20–40 µL of a sugary blue dye was added to the solution to enhance visibility for the DNA fragment extraction protocol (described below). The solution of pooled amplicons was then gently mixed using a pipette and pulsing with a mini–centrifuge.

Table 1. Amplicon pooling for samples sent to Next Generation Sequencing.

Samples were labeled with different levels depending on their concentration, which determined what volume to add to the amplicon pool. The number of samples in each level for each season are provided.

level	Concentration	Amount added to pool	June samples	October samples
1	9 ng/ 1.5 µL	5 µL	37	70
2	9–12 ng/ 1.5 µL	3.75 µL	32	58
3	12–18 ng/ 1.5 µL	2.5 µL	52	44
4	25.5 ng/ 1.5 µL	1.76 µL	36	69
Total:			157	241

To remove nonspecific DNA products, the amplicon pool was loaded in a TAE 0.8% agarose gel with electrophoresis at 50 volts for~ 1 hr. After the electrophoresis, relevant DNA fragments (300~500 bp) were cut out of the gel and ~250 mg of the gel slice was added to a 1.5 mL tube. To recover the DNA from the slice, a detailed protocol was followed using the Large DNA Fragments Extraction Kit (Geneaid Biotech Ltd., New Taipei City), which included cycles of dissolving the agarose gel with a concentrated sodium buffer, binding the DNA fragments with silica beads, and removing contaminants with a wash buffer. Finally, an elution buffer was

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used to obtain a high concentration of purified DNA. The purified DNA was then cleaned with KAPA pure beads (Roche KAPA Biosystems, Basel, Switzerland), as it is recommended to perform a genomic DNA cleanup prior to library construction for NGS workflows. The final DNA concentration and purity were checked with NanoDrop (Thermo Fisher Scientific, Waltham, Massachusetts, USA) with an additional concentration check using Quantus™ Fluorometer (Promega, Madison, Wisconsin, USA).

The clean, purified DNA was used for NGS library preparation following the KAPA HyperPrep Kit protocol (Roche KAPA Biosystems, Basel, Switzerland). Since the samples were dually–barcoded, a PCR–free library was implemented to avoid the disruption of the barcodes. The total volume of the library was 15 µL; this volume was determined using the size selection protocol in the HyperPrep kit. This step was followed by end repair ligation, adapter ligation, post–ligation cleanup, library amplification, and post–amplification clean–up protocols detailed in the kit. The library was sent to Next Generation Sequencing where the Miseq Reagant Kit v3 (600–cycle; MS–102–3003) was used for sequencing.

Demultiplexing protocol and BLAST methods

After the sequences were received from NGS, a demultiplex protocol was performed (Fig. 2) where the reads were separated by their barcoded primers. After sequence trimming and removal of the primer site with Cutadapt (Martin, 2011), reads were then uploaded to R 4.0.03 (R Core Team 2020) for denoising and merging using "dada2" (https://github.com/benjjneb/dada2) and "devtools" packages. The quality of final sequences was assessed by checking purity and read amount. The sequences were then aligned and BLASTed (Camacho et al., 2009) against a local *trnL–F* sequence database of Taiwanese ferns (Kuo et al., unpublished). In the case where the blast result did not match the morphotype of the

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sample (i.e. a cordiform gametophyte being matched with a *Haplopteris* species), additional outputs from Dada2 were investigated, including the fully merged file, and r1 and r2 sequences. If the blast results from r1 and r2 sequences matched, and had a purity over 95, this was considered the correct taxa for the sample. If local blast results of sequence identity were under 99% (e.g. *Antrophyum* and *Haplopteris* sequences), blast results from NCBI GenBank were additionally referenced to determine the correct taxa. For samples which were Sangers sequenced, quality of the sequence was assessed by analyzing the AB1 files, followed by the same BLASTN identification protocol used for NGS samples.

Data analysis

Microclimate investigation

To process the microclimate data, relative humidity (RH) records from the HOBO loggers were downloaded from HOBOware (Onset, Bourne MA, USA). Daily minimum RH values were obtained using transform functions from the "tidyverse" package in R, and graphed using "ggplot2". The data was further explored using two drought thresholds of 95% and 85% RH which were categorized to determine how long the hosts (live tee fern, dead tree fern, angiosperm) spent under a given threshold (https://github.com/alex–quinlan/thesis_data/tree/main/RH%20data). Length of time was further broken down into a range of durations from 0.5 to 7 hours for 85% RH, and 0.5 to 19 hours for 95% RH. The frequency per day that a host spent at these given durations were then graphed using the "ggplot2" package in R. To analyze the significance of the difference in RH threshold frequencies, ANOVA was performed using "ggpubr", "car", and "ggplot2" packages in R. The analysis of variance function

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(aov) was used, followed by a Tukey post–hoc test using the TukeyHSD function which explains the variance.

Canopy cover photos were uploaded to Gap Light Analyzer (GLA) along with topographical data including slope, aspect, elevation, and GPS coordinates of the plot. This data assists the GLA program with estimates of canopy coverage values. The outputs of GLA were used to determine canopy openness, direct light, diffuse light, and total light of each plot.

Community survey data

Non–metric multidimensional scaling (NMDS) using Bray–Curtis distance was performed to generate the following comparisons: June versus October gametophyte species compositions, gametophyte species compositions versus sporophyte species compositions per host, and gametophyte species composition versus mature sporophyte species composition. These comparisons were generated in R using "vegan" and "tidyverse" packages. For the June versus October gametophyte species composition, the ordihull function was used to generate polygons surrounding each season's hosts.

A one–way ANOVA was performed using the "ggpubr", "car", and "ggplot2" packages in R to determine whether there was a significant difference in species richness and abundance between tree fern and angiosperm hosts. This was followed by using the TukeyHSD function to generate a Tukey post–hoc test which explains the variance. Additionally, a Decorana was performed using the DCA function, which analyzes the heterogeneity of the species composition dataset.

Finally, the "ggscatter" function from the "tidyverse" package in R was used to explore area–abundance and area–species richness relationships. Pearson correlation coefficient was employed due to the normal distribution of the variables and their linear relationships. To further

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normalize the data, values were log–transformed. Correlations between gametophyte species richness and host surface area as well as gametophyte abundance and host surface area were explored. Surface area of the hosts was estimated using the area of a cylinder, where the height was one meter and the radius was determined by halving the diameter of the host. For exploring area correlations with sporophyte species richness, diameter at breast height (DBH) was used as sporophyte species presence was recorded along the entire trunk, and the SA of the entire trunk was not measured.

RESULTS

Community survey

Fern gametophyte abundance and area–abundance relationship

In total, 180 fern gametophytes were collected from hosts in June, and 287 were collected in October, 2021. The one–way ANOVA and Tukey post–hoc test revealed that on average, tree ferns had a significantly higher abundance ($F = 14.42$, $p < 0.001$) of fern gametophytes than angiosperm hosts. Generally, a larger proportion of individuals occupied the zone beneath 50 cm on the trunks (Fig. 3). Further, all hosts from the third plot of each season experienced low gametophyte abundance. In June, the tree fern in the second plot (TF_J2) had the highest abundance with 55 individuals, followed by the dead tree fern (DTF_J2) which hosted 28 individuals. For angiosperms, an *Oreocnide pedunculata* (OP2_J3) host had the most individuals with 10 gametophytes collected. Additionally, there were three angiosperm hosts in June, *Lagerstroemia subcostata, Ficus erecta* var. *beecheyana,* and *Saurauia tristyla* var. *oldhamii,* which had no gametophyte growth and were therefore not included in species composition data. In October, the tree fern in plot 1 (TF_O1) had the highest gametophyte abundance with 88

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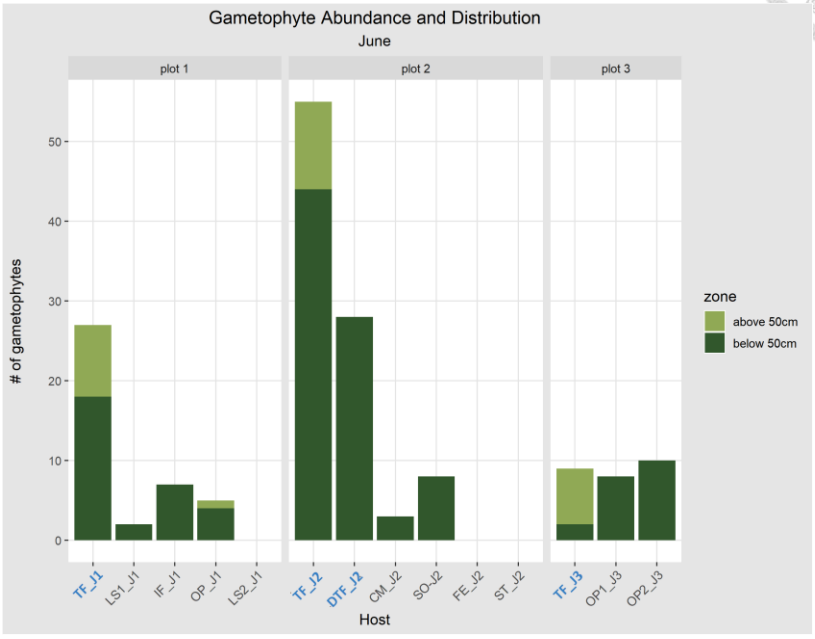
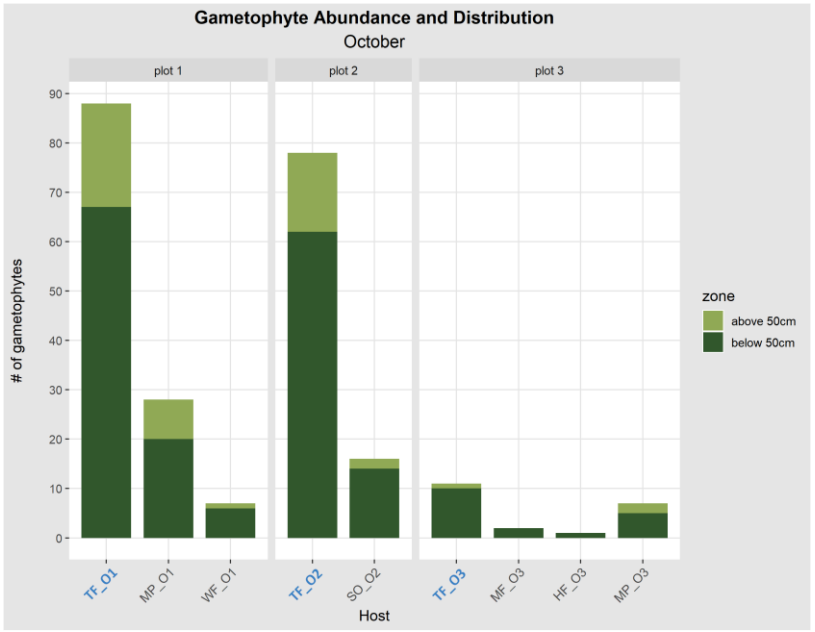
individuals, followed by the second tree fern (TF_O2) which hosted 78 individuals. A *Mallotus paniculatus* host (MP_O1) from the first plot with 28 gametophyte individuals had the highest abundance for angiosperms.



3a.



3b.

Figure 3a, b. Gametophyte abundance per season with zones.

Gametophyte abundance on hosts and their distribution in the given zones for June (3a) and October (3b). Names for host abbreviations can be seen in Table 2. Blue text denotes tree fern hosts while black text are angiosperm hosts.

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Table 2. Lists of hosts.

List of hosts by season with abbreviation, scientific, and Chinese name provided, as well as # of gametophyte individuals, and # of gametophyte and sporophyte species growing on the trunks. Diameter at breast height (DBH) ranged from 2 to >30 π cm. Location of hosts in the plot can be seen in Figure 1. The bottom of the table includes data of the *A. podophylla* hosts outside of the plotting regions.

Host abbrev.	Scientific name	Chinese name	DBH range	# of gametophyte individuals	# of gametophyte species	# of sporophyte species
June						
TF_J1	*Alsophila spinulosa*	臺灣桫欏	20–30	27	6	8
LS1_J1	*Lagerstroemia subcostata*	九芎	2–10	2	1	0
IF_J1	*Ilex formosana*	糊樗	2–10	7	3	0
OP_J1	*Oreocnide pedunculata*	長梗紫麻	10–20	5	3	6
LS2_J1	*Lagerstroemia subcostata*	九芎	10–20	0	0	0
TF_J2	*Alsophila spinulosa*	臺灣桫欏	> 30	55	10	15
DTF_J2	dead TF (*Alsophila spinulosa*)	臺灣桫欏	10–20	28	7	14
CM_J2	*Cinnamomum micranthum*	土樟	2–10	3	1	0
SO_J2	*Schefflera octophylla*	江某	10–20	8	6	3
FE_J2	*Ficus erecta* var. *beecheyana*	牛奶榕	2–10	0	0	0
ST_J2	*Saurauia tristyla* var. *oldhami*	水冬瓜	2–10	0	0	0
TF_J3	*Alsophila spinulosa*	臺灣桫欏	10–20	9	2	6
OP1_J3	*Oreocnide pedunculata*	長梗紫麻	2–10	8	3	1
OP2_J3	*Oreocnide pedunculata*	長梗紫麻	10–20	10	5	2
October						
TF_O1	*Alsophila spinulosa*	臺灣桫欏	10–20	88	14	8
MP_O1	*Mallotus paniculatus*	白匏子	> 30	28	9	11
WF_O1	*Wendlandia formosana*	水金京	10–20	7	1	3
TF_O2	*Alsophila spinulosa*	臺灣桫欏	20–30	78	11	6
SO_O2	*Schefflera octophylla*	江某	10–20	16	7	2
TF_O3	*Alsophila spinulosa*	臺灣桫欏	> 30	11	5	9
MF_O3	*Maesa formosana*	臺灣山桂花	2–10	2	2	0
HF_O3	*Helicia formosana*	山龍眼	2–10	1	1	0
MP_O3	*Mallotus paniculatus*	白匏子	> 30	7	5	3
AP_J1	*Alsophila podophylla*	鬼桫欏	10–20	4	2	1
AP_J2	*Alsophila podophylla*	鬼桫欏	10–20	2	2	1
AP_J3	*Alsophila podophylla*	鬼桫欏	10–20	2	2	0
AP_O1	*Alsophila podophylla*	鬼桫欏	10–20	15	3	0
AP_O2	*Alsophila podophylla*	鬼桫欏	20–30	19	7	1
AP_O3	*Alsophila podophylla*	鬼桫欏	10–20	16	6	2

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June had a positive and significant (r = 0.87, p < 0.0001) correlation with diameter at breast height (DBH) and abundance, where, as host diameter increased, so did the number of gametophyte individuals on their trunk (Fig. 4). October hosts did not experience such a significant correlation with DBH increase and abundance. However, area–abundance and area–species richness relationships were still revealed when all hosts from both seasons are considered. Host surface area overall had a significant (r = 0.72, p = 0.0003) and positive correlation with fern gametophyte abundance (Fig. 5) as well as with gametophyte species richness (r = 0.72, p = 0.0003). Additionally, DBH had a positive correlation with sporophyte species richness (r = 0.88, p < 0.0001) (Fig. 6).

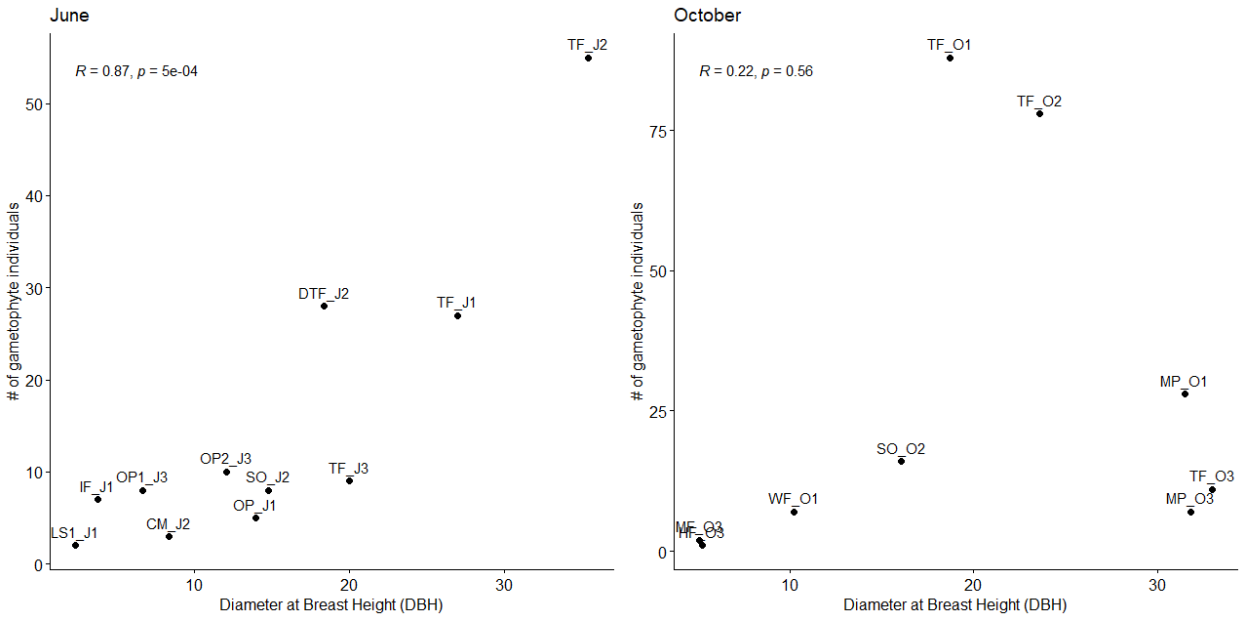

Figure 4. Pearson correlation for DBH–abundance relationship in June and October.

The DBH of June hosts (on left) were positively and significantly correlated with gametophyte abundance with a Pearson r value of 0.87 and a p–value of 5e–04. This correlation was not evident for the October hosts (on right), which had both a low Pearson r value and an insignificant p–value. Points are labeled with host abbreviations.

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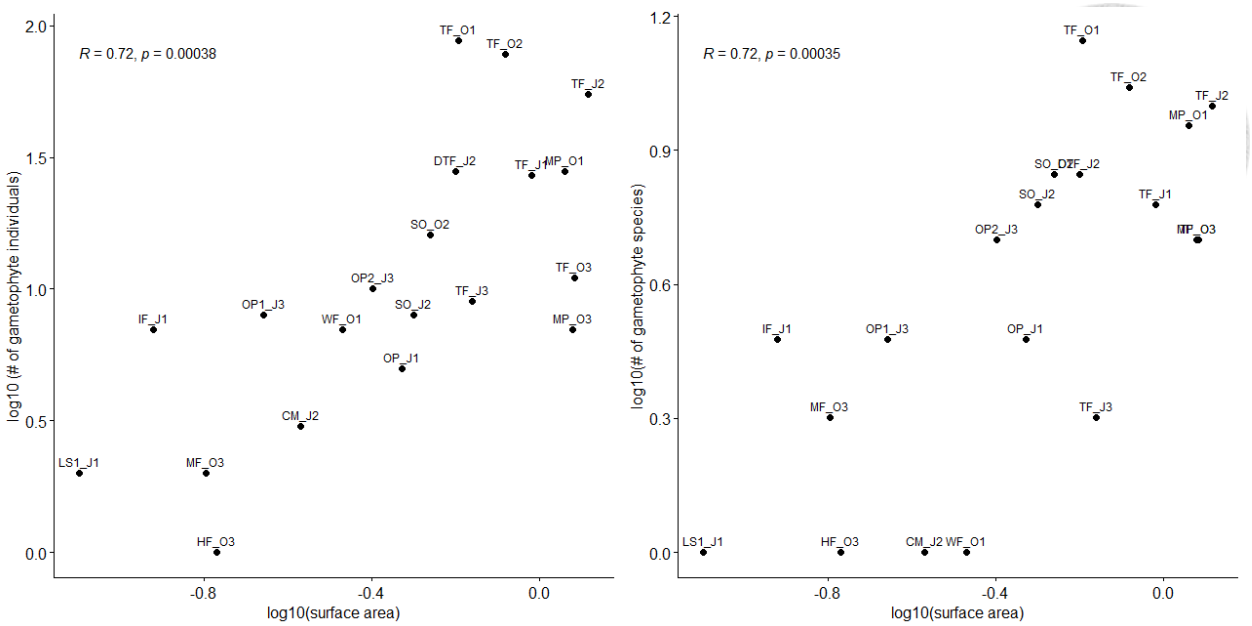

Figure 5. Pearson correlation for surface area with gametophyte richness.

Using the Pearson correlation coefficient, a significant and positive relationship was revealed for surface area–gametophyte abundance relationship (left) and surface area–gametophyte species richness (right). Values were log–transformed. Points are labeled with host abbreviations.

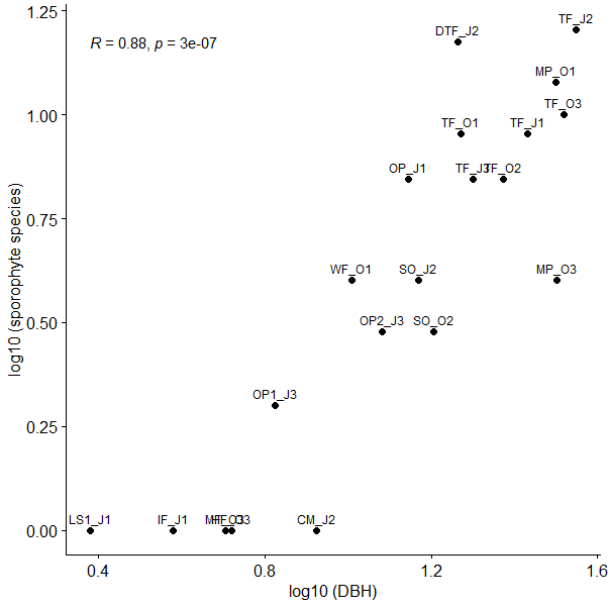

Figure 6. Pearson correlation for DBH and sporophyte species richness.

Using Pearson correlation coefficient, a significant and positive relationship was revealed for DBH with sporophyte species richness. Values were log–transformed. Points are labeled with host abbreviations.

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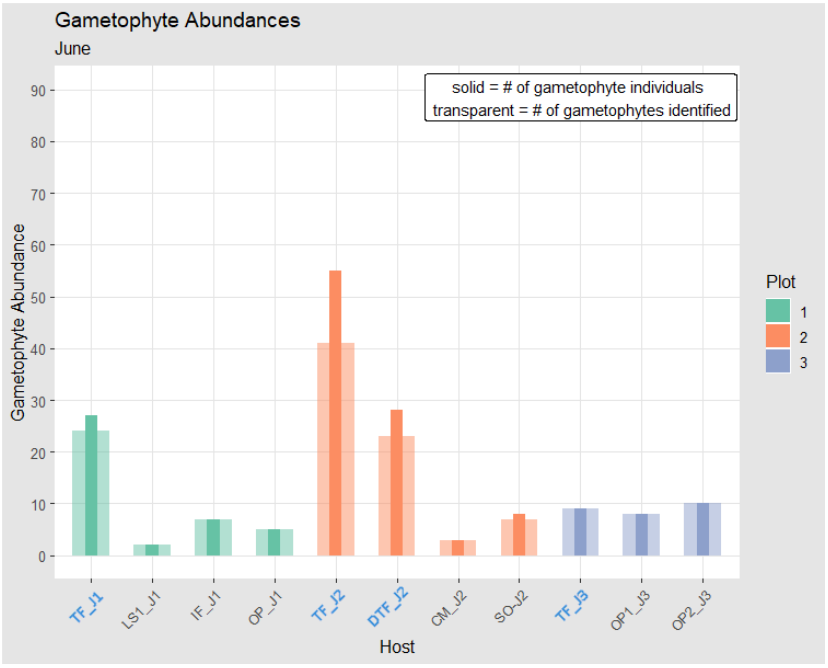
Gametophyte species composition

In June, 167/180 gametophyte samples had visible amplifications in the gel electrophoresis, yielding a 93% success rate. For October, there was a 94% success rate with 271/287 samples successfully amplified. Samples that did not succeed either had poor tissue, insufficient tissue fragmentation, or contamination. Out of these samples, 148 (137 NGS, 11 Sangers) from June were successfully identified to species level, as well as 263 (239 NGS, 24 Sangers) samples from October (included in the successful samples are 8 from June as well as 44 samples from October collected from *Alsophila podophylla* hosts outside of the plotting regions). Unidentified species either had low purity levels, low read amount, low % identity match in BLAST results, or a combination of these characteristics. Figure 7 shows another illustration of fern gametophyte abundances, with a representation of number of individuals vs. number of individuals successfully identified per host. In total, between the two seasons, 32 gametophyte species were identified. Figure 8 shows a breakdown of the gametophyte species per season, with the respective host that they are growing on.



7a.

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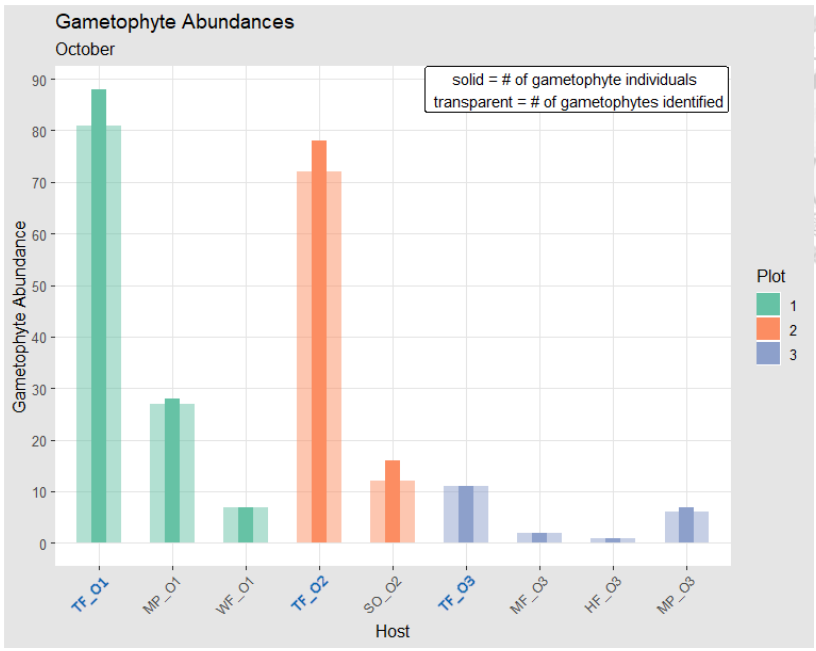

7b.

Figure 7a, b. Gametophyte abundances per season with number of individuals identified.

Each season is broken into respective plots; Plot 1 green, Plot 2 orange, and Plot 3 blue. The blue text in the x–axis denotes tree fern hosts and the black text denotes angiosperm hosts. The thinner, solid bar represents the # of gametophytes collected from the trunk. The wider, transparent bar represents the number successfully identified.

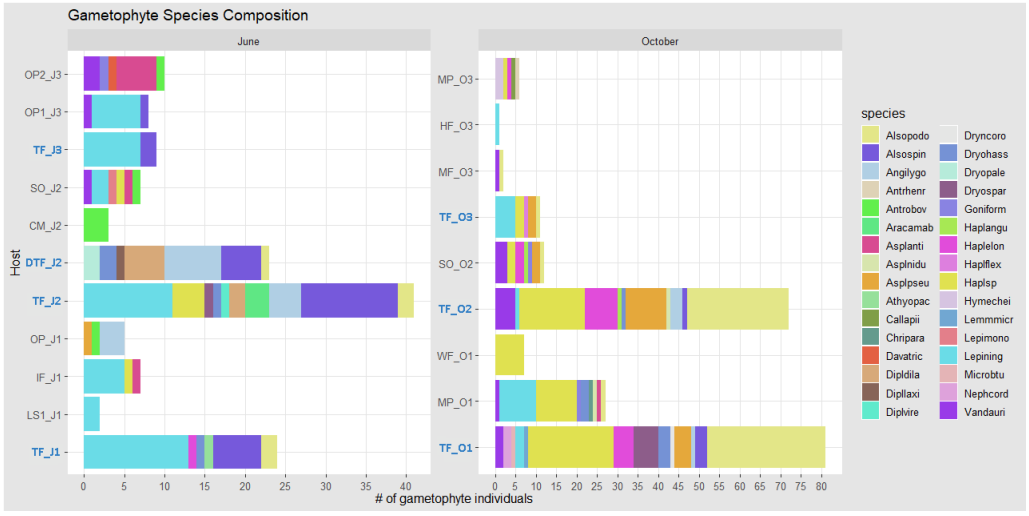

Figure 8. Gametophyte species composition per season.

Species composition of gametophytes growing on hosts with tree ferns in blue text, and angiosperms in black. Abbreviations of gametophyte species are provided in the legend, next to their respective color. Species names of gametophytes can be referenced in Table 3.

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Table 3. Gametophyte species list.

List of gametophytes by scientific name, Chinese name, and abbreviation. For sporophyte presence, 'x' indicates presence of conspecific sporophyte nearby (within plotting regions). * indicates species whose sporophyte were not directly observed, however there are records of this species in this area and/or is a common species in this ecosystem. Life form is 'E' for epiphytic, 'A' for accidental, and 'F' for facultative (note: only 3 species are facultative – *Nephrolepis cordifolia*, *Dryopteris hasseltii*, and *Hymenasplenium cheilosorum*).

Gametophyte species	Chinese name	Species abbreviation	Life form	Morphotype	Present on tree fern	Present on angiosperm	Sporophyte present
Alsophila podophylla	鬼桫欏	Alsopodo	A	cordiform	x	x	x
Alsophila spinulosa	台灣桫欏	Alsospin	A	cordiform	x	x	x
Angiopteris lygodiifolia	觀音座蓮	Angilygo	A	cordiform	x	x	x
Antrophyum henryi	車前蕨屬	Antrhenr	E	non-cordiform		x	
Antrophyum obovatum	車前蕨	Antrobov	E	non-cordiform		x	x
Arachniodes amabilis	屋久複葉耳蕨	Aracamab	A	cordiform	x		*
Asplenium antiquum	山蘇花	Asplanti	E	cordiform		x	x
Asplenium nidus	臺灣山蘇花	Asplnidu	E	cordiform	x	x	x
Asplenium pseudolaserpitiifolium	大黑柄鐵角蕨	Asplpseu	E	cordiform	x	x	x
Athyrium opacum	黑葉貞蕨	Athyopac	A	cordiform	x		x
Callistopteris apiifolia	毛桫蕨	Callapii	E	non-cordiform		x	
Christella parasitica	密毛小毛蕨	Chripara	A	cordiform		x	*
Davallia trichomanoides	海洲骨碎補	Davatric	E	cordiform		x	x
Diplazium dilatatum	廣葉鋸齒雙蓋蕨	Dipldila	A	cordiform	x		x
Diplazium laxifrons	冲绳双盖蕨	Dipllaxi	A	cordiform	x		*
Diplazium virescens	疏葉雙蓋蕨	Diplvire	A	cordiform	x		*
Drynaria coronans	崖薑蕨	Dryncoro	E	cordiform	x		x
Dryopteris hasseltii	哈氏假複葉耳蕨	Dryohass	F	cordiform	x	x	x
Dryopteris paleolata	魚鱗蕨	Dryopale	A	cordiform	x		*
Dryopteris sparsa	長葉鱗毛蕨	Dryospar	A	cordiform	x		x
Goniophlebium formosanum	臺灣水龍骨	Goniform	E	cordiform		x	x
Haplopteris anguste-elongata	姬書帶蕨	Haplangu	E	non-cordiform	x	x	x
Haplopteris elongata	垂葉書帶蕨	Haplelon	E	non-cordiform	x	x	x
Haplopteris flexuosa	書帶蕨	Haplflex	E	non-cordiform	x		
Haplopteris yakushimensis	屋久書帶蕨	Haplsp	E	non-cordiform	x	x	
Hymenasplenium cheilosorum	薄葉孔雀鐵角蕨	Hymechei	F	non-cordiform		x	*
Lemmaphyllum microphyllum	伏石蕨	Lemmmicr	E	cordiform	x		x
Lepidomicrosorum ningpoense	攀援星蕨	Lepimono	E	non-cordiform	x	x	x
Lepisorus monilisorus	擬芨瓦葦	Lepining	E	non-cordiform		x	x
Microlepia obtusiloba	團羽鱗蓋蕨	Microbtu	A	cordiform	x		*
Nephrolepis cordifolia	腎蕨	Nephcord	F	cordiform	x		x
Vandenboschia auriculata	瓶蕨	Vandauri	E	non-cordiform	x	x	x

A one–way ANOVA followed by a Tukey post–hoc test revealed that tree ferns had a significantly higher species richness ($F = 6.43$, $p < 0.05$) of fern gametophytes than angiosperm hosts. June and October had distinct species compositions, which is explained further with the NMDS below. The most abundant fern gametophyte species in June were *Lepidomicrosorium*

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ningpoense and *Alsophila spinulosa* while a *Haplopteris yakushimensis* species and *Alsophila podophylla* were most abundant in October. The gametophyte appendix (Table 3) provides names for the abbreviations used in figures, as well as information on whether the species grew on tree ferns, angiosperms, or both, and if their conspecific sporophyte was recorded in the plotting region. With the exception of three species (see Presence of Independent Gametophytes), conspecific sporophytes for all gametophyte species were observed either directly in the plots, nearby, or had GBIF records in the area.

Sporophyte species composition

In Table 2 the number of sporophyte species growing on a given host is provided. In June, plot 2 had the highest richness of sporophyte species, with 15 species growing on TF_J2 and 14 species growing on the dead tree fern (DTF_J2). In October, the first plot had the highest richness for sporophytes, with an angiosperm (MP_01) hosting 11 fern sporophyte species, and TF_O1 hosting 8 species. Generally, for both seasons, as sporophyte species richness increased in a plot, so did gametophyte species richness. In addition, the sporophyte appendix (Table A1) provides a look at the sporophytes growing in each plot, whether they were mature or immature, what host type they were growing on, and if their conspecific gametophyte was collected in the study. Mature sporophytes were those which were adult and producing spores; immature sporophytes were either juvenile or sterile.

Species composition comparisons with non–metric multidimensional scaling (NMDS)

The NMDS which compared the gametophyte species composition of June and October (Fig. 9) revealed that the seasons have varied compositions, with the hosts from either season forming distinct clusters in the ordination. Additionally, a Decorana test revealed that June had a higher eigenvalue, identifying this season as having a more heterogeneous gametophyte species

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composition than October. The species most responsible for the diverging compositions amongst seasons were *Antrophyum obovatum* (Pteridaceae), *Diplazium dilatatum* (Diplaziaceae), and *Dryopteris paleolata* (Dryopteridaceae) in June, and *Hymenasplenium cheilosorum* (Aspleniaceae), *Haplopteris anguste–elongata* (Pteridaceae), and *Microlepia obtusiloba* (Dennstaedtiaceae) in October. Some of the commonly shared species between each season were *Lepidomicrosorum ningpoense* (Polypodiaceae), *Dryopteris hasseltii* (Dryopteridaceae), and *Alsophila podophylla* (Cyatheaceae).

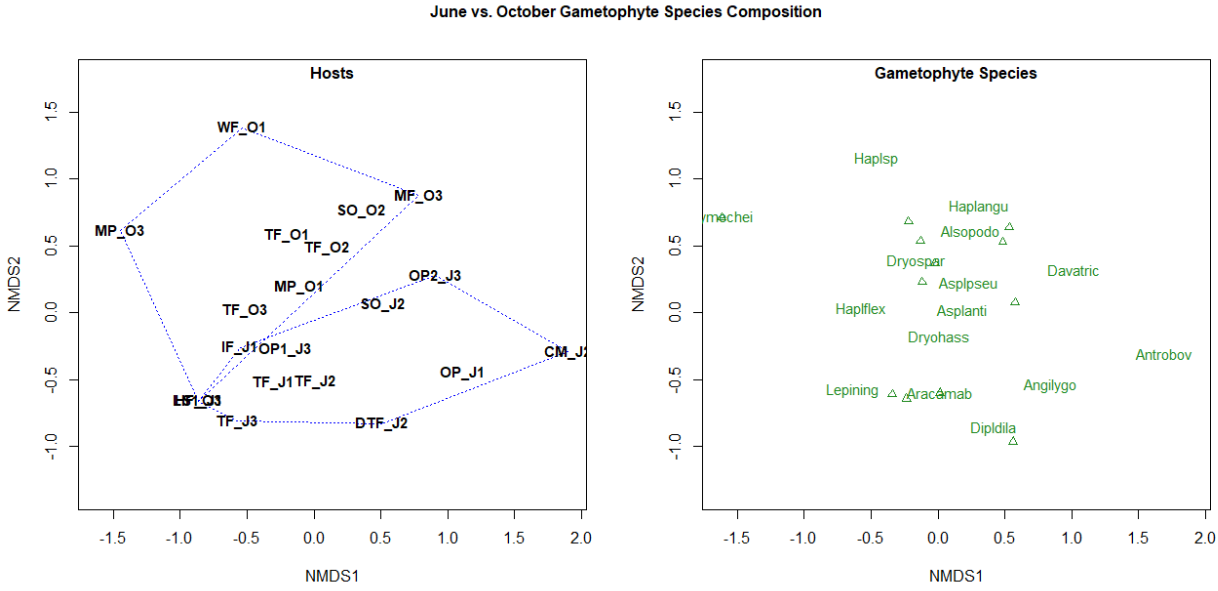

Figure 9. NMDS comparing June and October gametophyte species composition.

Non–Metric Multidimensional Scaling created with Bray–Curtis distance. The left plot illustrates similarity between hosts, and the right plot shows gametophyte species responsible for those distances. June ('_J') and October ('_O') hosts formed clusters highlighted by the polygons with dashed blue lines. "Ordihull" was used to determine which gametophyte species had the most importance, and created triangles in place where multiple species were present.

The separate ordinations which illustrate each season (Fig. 10) highlight that the tree ferns have more similar gametophyte species compositions while the angiosperm hosts are more scattered throughout the plot, concluding that their compositions were more dissimilar. While

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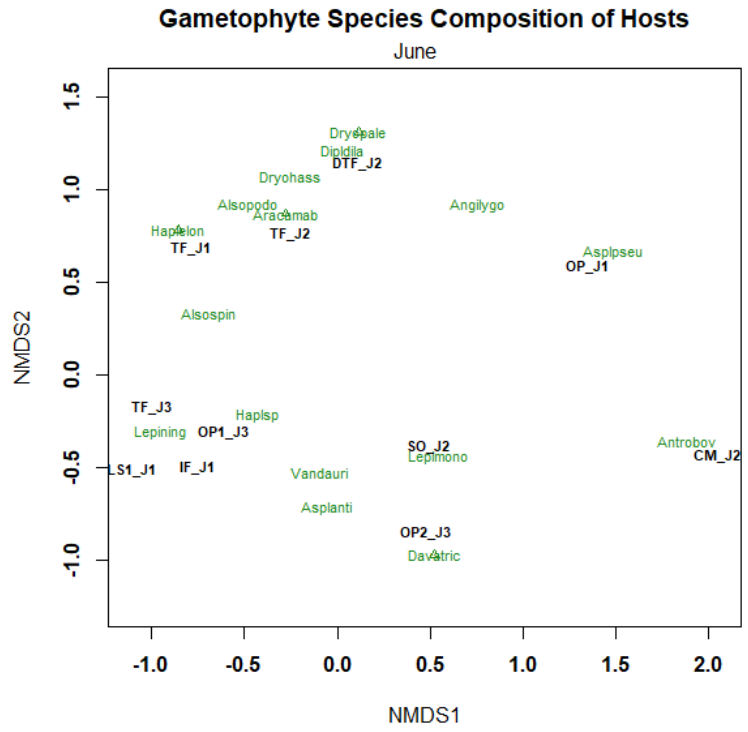
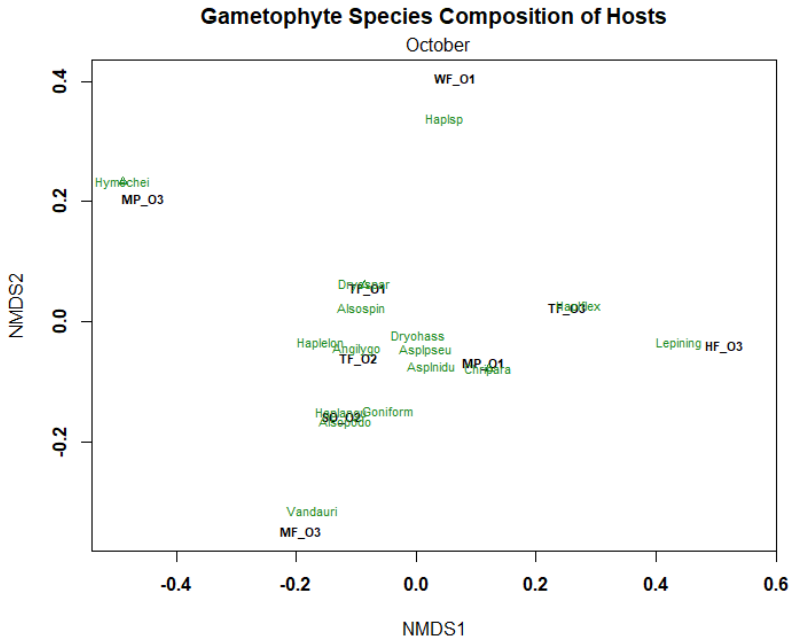


10a.



10b.

Figure 10a, b. Individual NMDS for June and October gametophyte species composition.

Non–metric multidimensional scaling comparing species compositions on hosts in June (10a) and October (10b). Black text are the host abbreviations. Green text are the gametophyte species present on the hosts.

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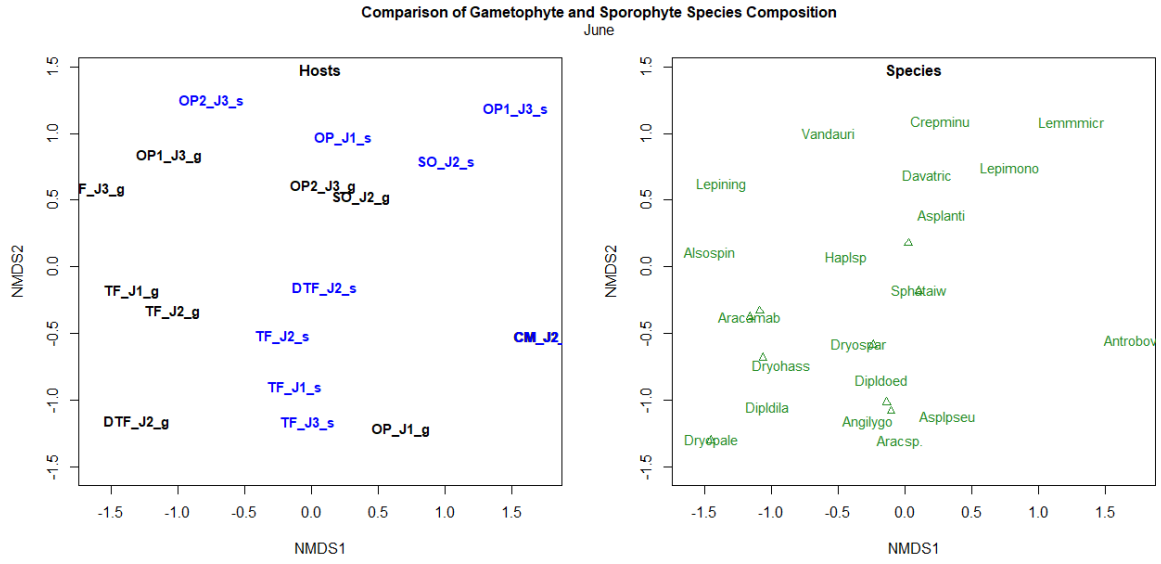
there was no significant preference of gametophyte species on a given host type, *Alsophila podophylla* (Cyatheaceae), *Alsophila spinulosa* (Cyatheaceae), and *Haplopteris elongata* (Pteridaceae) individuals grew more often on tree ferns, while *Vandenboschia auriculata* (Hymenophyllaceae) gametophytes grew more often on angiosperms (see species counts in Table A3 in appendix). There were 12 gametophyte species only found on tree ferns while 6 species were only found on angiosperms (Table 3), however the abundances of these species were quite low and did not yield significance preference of host type.

Figure 11 represents NMDS ordinations which compare gametophyte and sporophyte species compositions for each season. In June, an example of similarity is illustrated by the second tree fern which had a more cohesive species composition when comparing gametophyte (TF_J2_g) and sporophyte (TF_J2_s) species. On the other hand, the first tree fern had more diverging compositions (TF_J1_g and TF_J1_s) with these assemblages being further apart in the ordination. In October, the tree fern hosts generally had more cohesive compositions while the angiosperm hosts were more divergent when comparing gametophyte and sporophyte compositions.



11a.

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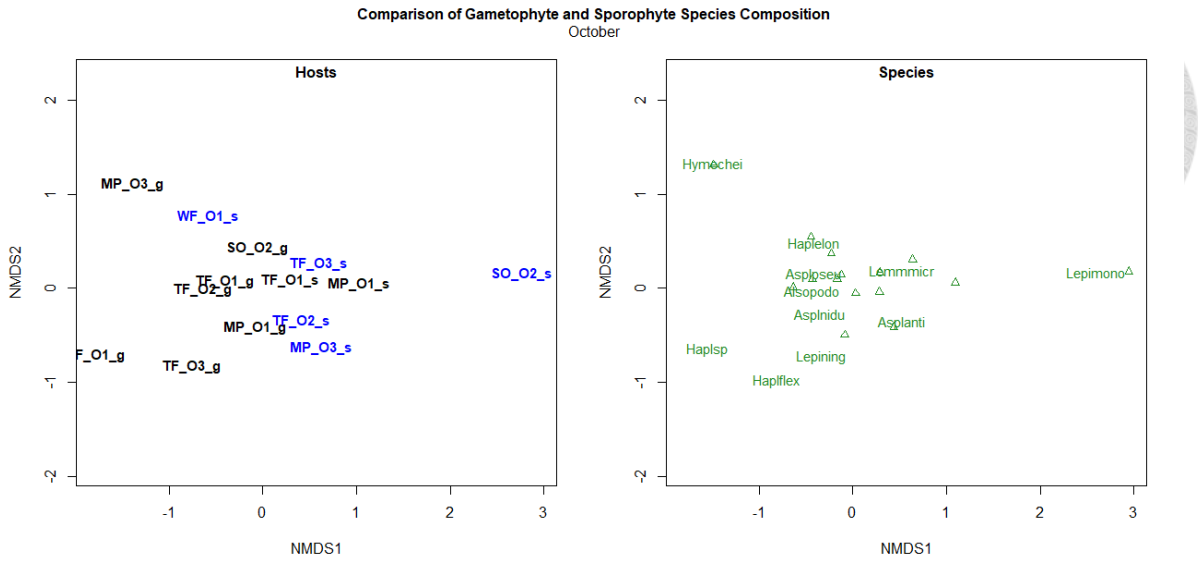

11b.

Figure 11a, b. NMDS comparing sporophyte and gametophyte species composition.

An NMDS comparing sporophyte and gametophyte species composition for each season with hosts in the left plot, where black text represents the gametophyte species composition on the host and the blue text represents sporophyte composition. The fern species influencing these distances are in green on the right.

For tree ferns, species which often existed both as a sporophyte and gametophyte on hosts were *Dryopteris hasseltii* (Dryopteridaceae), *Dryopteris sparsa* (Dryopteridaceae), and *Alsophila spinulosa* (Cyatheaceae). For angiosperms, *Vandenboschia auriculata* (Hymenophyllaceae) and *Lepidomicrosorum ningpoense* (Polypodiaceae) often grew with a conspecific sporophyte on hosts (Table A1, Table A3). The comparison of mature sporophyte species composition with gametophyte species composition is illustrated in Figure 12. For June, only the first and second tree fern (TF_J1, TF_J2), the dead tree fern (DTF_J2), and an *Oreonicide pedunculata* host (OP1_J1) from the first plot had presence of mature fern sporophytes. In October, two tree ferns (TF_O1, TF_O3) and two angiosperm hosts had mature fern sporophytes (MP_O1, SO_O2).

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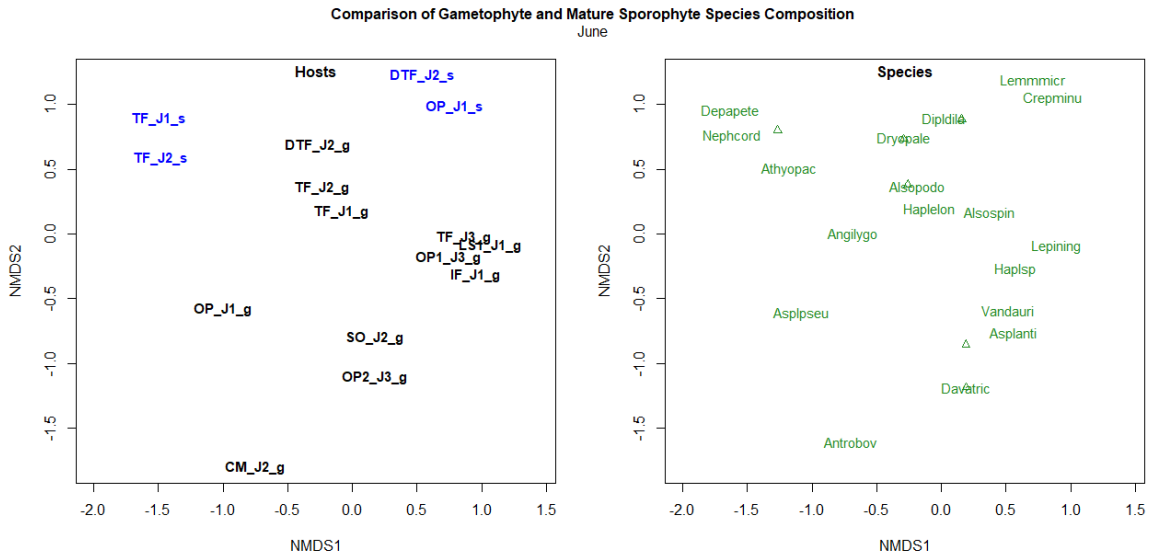
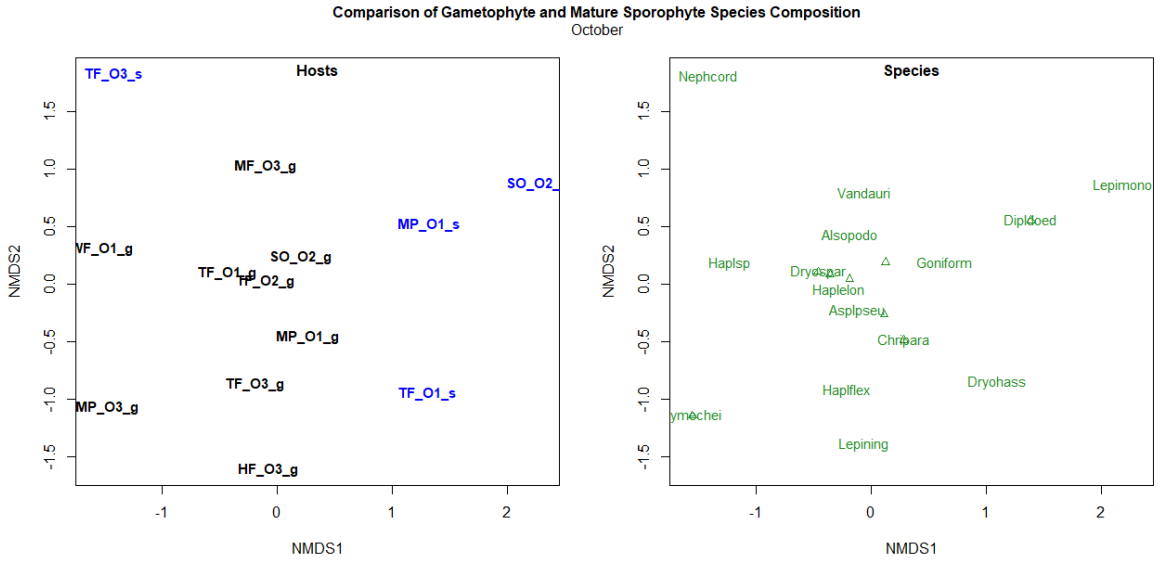


12a.



12b.

Figure 12a, b. NMDS comparing mature sporophyte and gametophyte species composition.

An NMDS similar to Figure 11, where only hosts which had mature sporophyte species composition are included. In each season, only 4 hosts had mature epiphytic fern sporophytes present.

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Presence of independent gametophytes

A final result from the community survey is the presence of independent gametophytes which had no sporophytes growing nearby. One vittaroid species and one filmy fern species fit this criterion in our study; *Haplopteris yakushimensis* and *Callistopteris apiifolia* were found in the plotting regions without presence of their conspecific sporophytes, nor were there any observations of these sporophyte species in nearby regions. *Haplopteris yakushimensis* was found in both June and October while *C. apiifolia* was only found growing in the October plots. *H. yakushimensis* (represented by the yellow color in Fig. 8) had a high abundance and was found on a variety of hosts, with 6 individuals collected in June and 59 collected in October (Table A3). Only 2 *C. apiifolia* were found, one growing on an angiosperm host in plot 3 (MP_O3), and one growing on an *Alsophila podophylla* host within five meters of plot 3 (Fig. 17).

Two additional vittaroid species, *Antrophyum henryi* and *Haplopteris flexuosa*, had no observed sporophyte in the region, however, databases showed records of these sporophytes in nearby areas). *Antrophyum henryi* was found growing on MP_03 (Fig. 8), as well as two *A. podophylla* hosts (Fig. 17). The *H. flexuosa* gametophyte was found growing on the tree fern in the third plot in October (TF_O3). Initially, it was thought that the *H. flexuosa* individual was also *Haplopteris yakushimensis*, however, upon further analysis of the DNA sequence and BLAST results, it was determined that this sample had a 99% match with *H. flexuosa*. Photos of all four species revealed the presence of self–proliferating gemmae (Fig 13).

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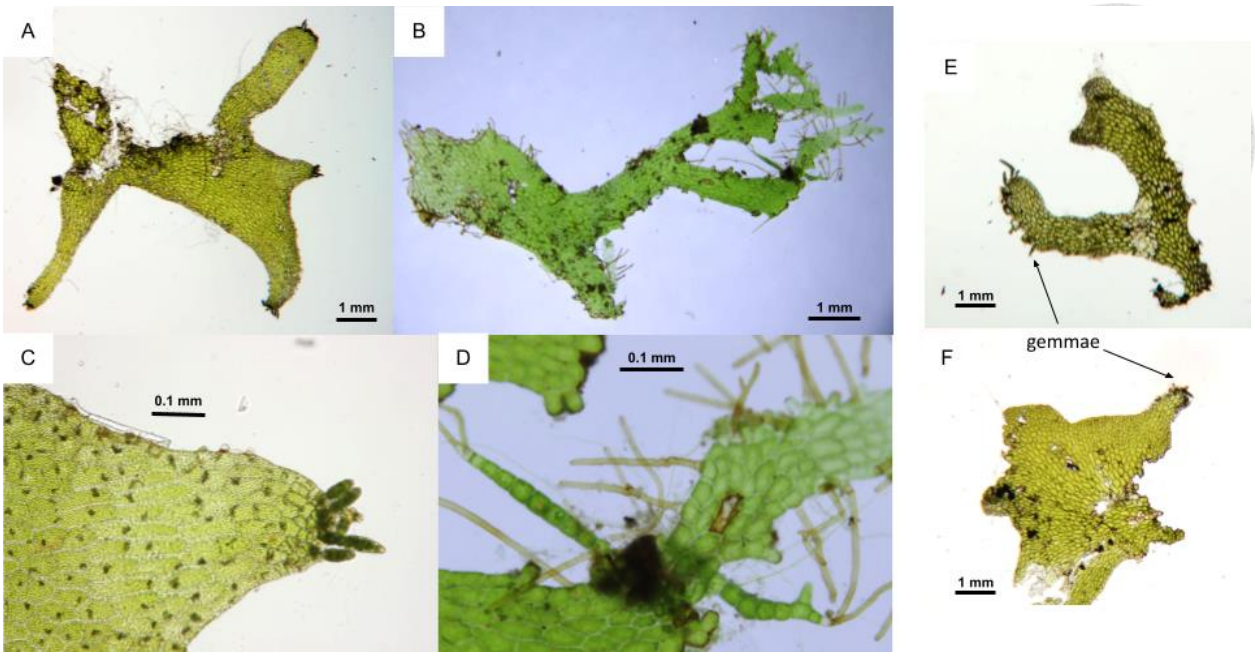

Figure 13. Images of independent gametophytes and other gemmae–producing species.

A–D: Independent gametophytes, with a close up of their gemmae. (A) (B) *Haplopteris yakushimensis*. (C)(D) *Callistopteris apiifolia*. E, F: Other gemmae producing species. (E) *Haplopteris flexuosa*. (F) *Antrophyum henryi*.

Table 4. Gametophytes by type.

The top table represents the gametophyte types as # of individuals on hosts, while the bottom table represents the types as # of species on hosts. *Angiopteris lygodiifolia* was not included in morphotype counts due to its irregular shape (Fig. 14).

Group	Morphotype		Life Form		Propagation	
	Cordiform	Non–cordiform	Epiphytic	Accidental	Gemmae producing	Non–gemmae producing
By host type						
Tree Fern	141	105	137	124	66	195
Angiosperm	22	72	85	12	36	61
By season						
June	63	65	74	65	11	128
October	103	112	135	84	91	128

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Group	Host Type		Season	
	Tree Fern	Angiosperm	June	October
Morphotype				
Cordiform (n = 20)	16	9	13	13
Non–cordiform (n = 11)	6	10	7	9
Life Form				
Epiphytic (n = 17)	13	14	10	14
Accidental/Facultative (n = 15)	13	6	11	9

Gametophyte types and morphologies

In the gametophyte survey, there were 20 cordiform species and 11 non–cordiform species. Table 4 shows the number of cordiform and non–cordiform individuals on either host type, as well as the number of cordiform and non–cordiform species on host types. Figure 14 shows photos of these morphotypes in the study. Overall, tree ferns hosted more cordiform individuals than angiosperm hosts, and angiosperms hosted considerably more non–cordiform individuals. Tree ferns also hosted more cordiform species with 16 out of the 20 species growing on their trunks, while angiosperms only hosted 9 of the 20 species. Angiosperm hosts had 10 out of the 11 non–cordiform species growing on their trunks, and tree ferns hosted 6 out of the 11 species. There were no considerable differences between morphotype presence in either season.

Tree ferns hosted considerably more gametophyte species which were recorded as accidental epiphytes with 124 individuals collected from their trunks; in comparison, only 12 individuals that were accidental species were collected from angiosperm trunks. Tree ferns hosted 13 out of these 15 species, while angiosperms hosted only 6. Species which have an

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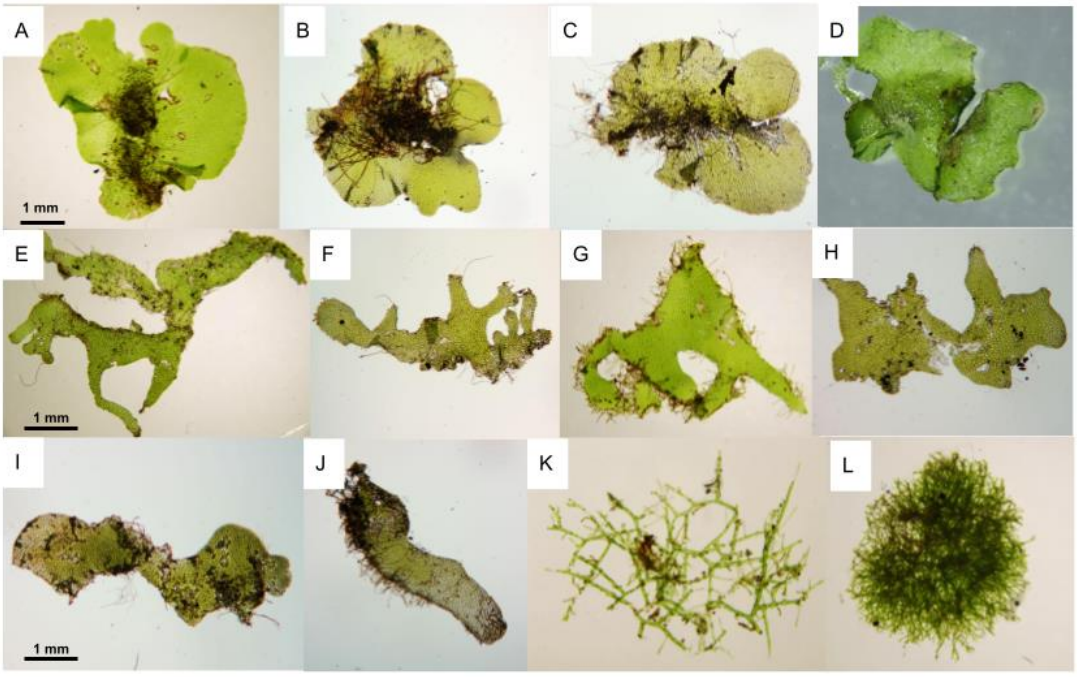
epiphytic lifestyle had a balanced distribution on both host types with 13 out of 17 species growing on tree ferns and 14 out of 17 species growing on angiosperms.

An additional type are gametophyte species which have the ability to self–proliferate, those being gemmae–producing gametophytes. There were only 6 gemmae producing species in the study, *Haplopteris anguste–elongata*, *Haplopteris elongata*, *Haplopteris flexuosa*, *Haplopteris yakushimensis*, *Callistopteris apiifolia*, and *Vandenboschia auriculata*. Most species were found growing on both tree fern and angiosperm hosts, with the exception of *H. flexuosa*, which was found growing on one tree fern host in October. For individual count, 66 gemmae–producing individuals grew on tree ferns and 36 grew on angiosperm hosts. Yet, to put numbers into perspective, gemmae–producing individuals made up 59% of the fern gametophytes growing on angiosperms, while they only made up 34% of the gametophyte abundance on tree ferns.



14a.

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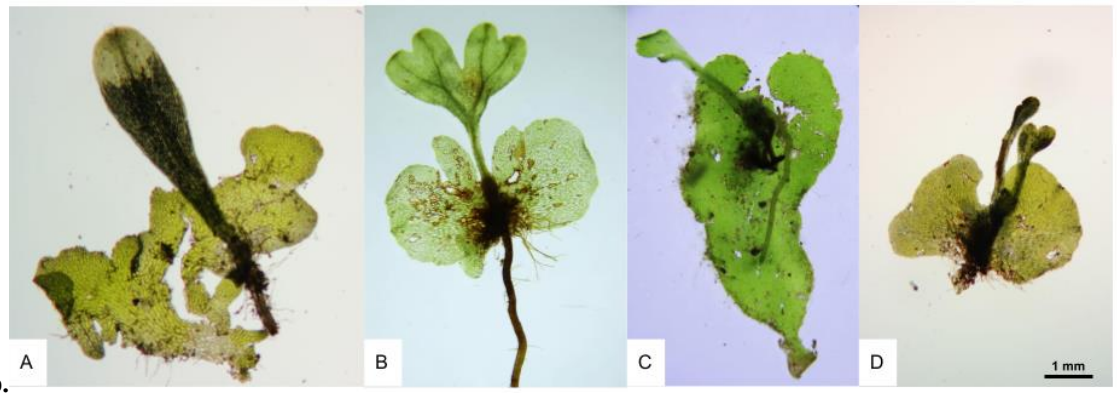

14b.

Figure 14a, b. Voucher Specimen – photos of gametophytes and sporelings

Top set of photos (16a) includes different gametophyte species to illustrate morphotypes. A–C: Cordiform. (A)(B) *Alsophila podophylla*, (C) *Dryopteris sparsa*, D: Irregular; *Angiopteris lygodiifolia*. E–H: Non–cordiform, Ribbon. (E) *Haplopteris yakushimensis*, (F) *Haplopteris elongata*, (G) *Callistopteris apiifolia*, (H) *Antrophyum henryi*. I–J: Non–cordiform, irregular; *Lepidomicrosorum ningpoense*. K–L: Non–cordiform, filamentous; *Vandenboschia auriculata*. Scale bar provided at the start of each row.

Bottom set of photos (16b) includes development of sporelings from gametophyte thallus. (A) *Haplopteris elongata*, (B)(D) *Alsophila spinulosa*, (C) *Hymenasplenium cheilosorum.* Scale bar provided in photo D.

Environmental survey

Relative humidity data

All three of the recorded hosts spent most of their time at values above 95% relative humidity from mid–May to the end of August, 2021. Figure 15 shows the daily minimum value for relative humidity for each host during this time period, where a high percentage of their max values (not represented) were at 100% RH. Generally, the live tree fern and the dead tree fern spent longer periods of time above 85% RH than the angiosperm host. Figure 16 illustrates the frequency per day that a host remained under a given threshold, as well as the duration of time that the host remained there (0.5 to 7 hours). The longest time that the angiosperm host remained under 85% RH was ~6 hours, while the dead tree fern was 5 hours, and the live tree

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fern was 2 hours. For 95% RH, the longest period below the threshold was ~7 days for the angiosperm host, 12 hours for the dead tree fern, and 10 hours for the live tree fern. These longer periods of drought occurred in July, with the angiosperm experiencing 7 consecutive days under 95% between July 14th and July 21st, 2021.

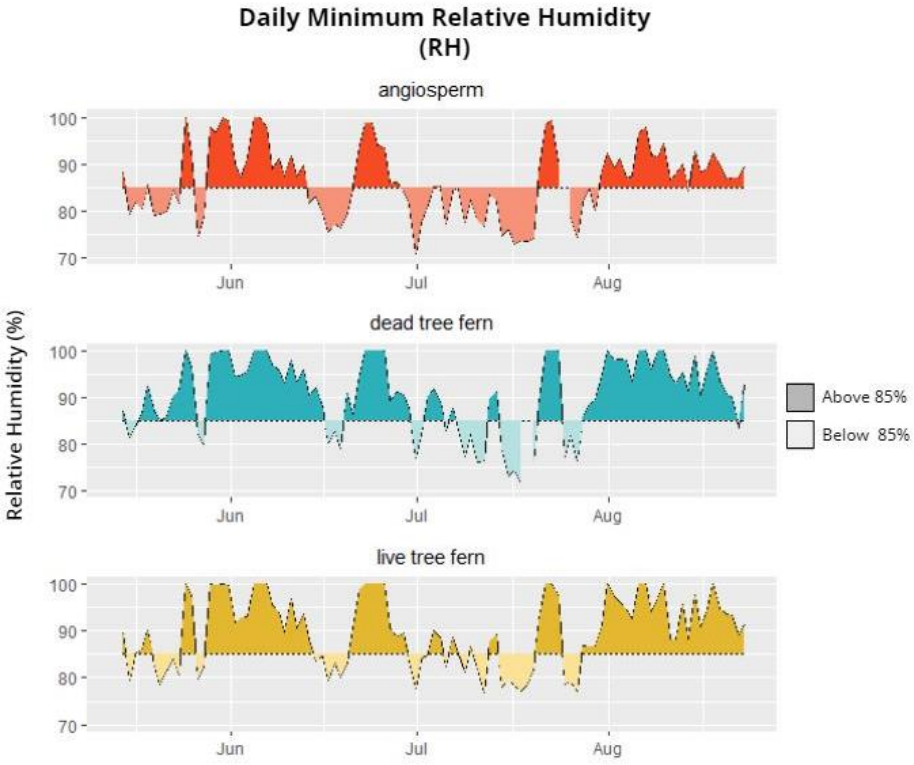

Figure 15. Daily minimum relative humidity of hosts.

This figure represents the daily minimum RH value that a host experienced from Mid–May to the end of August. Red represents the angiosperm host, blue is the dead tree fern, and yellow is the live tree fern. The darker value of the color represents when the daily minimum RH was above 85%, and the lighter colored value is below 85%.

The ANOVA and Tukey post hoc test, which analyzed the variance in the frequency of durations that a host spent under a given threshold, revealed that the angiosperm host spent significantly more time below 85% ($p < 2e–16$) and 95% RH ($p < 0.00001$, $p < 0.00003$) than both the live tree fern and dead tree fern, respectively. A comparison of the live tree fern and dead tree fern at either threshold was not significant.

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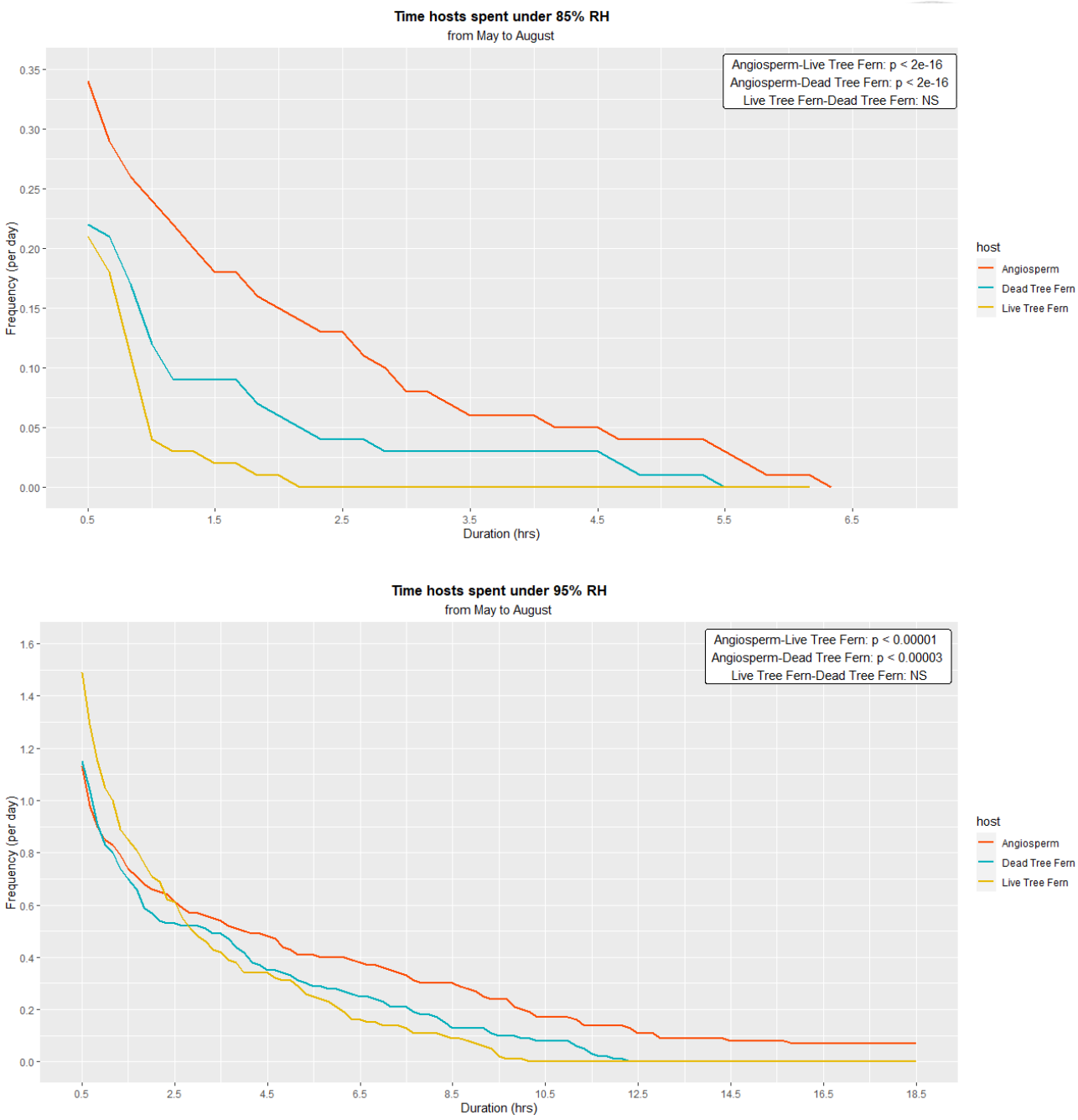

Figure 16. Frequency of durations that hosts spent under thresholds.

These figures show the frequency of durations that a host spent under the given threshold with angiosperm in red, dead tree fern in blue, and live tree fern in yellow. Results from the ANOVA test showing the angiosperm spending significantly more time below either threshold is provided in the upper right hand corner of the graphs. The data used to produce this graph can be viewed at https://github.com/alex–quinlan/thesis_data/tree/main/RH%20data

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Canopy coverage and total light

While there was no significant influence of canopy coverage revealed for fern gametophyte abundance/species richness, Table 5 shows that, in each season, the plot with the highest total light also had the high species richness and abundance for fern gametophytes, as well as the highest species richness for fern sporophytes. The third plot in each season had the lowest total light estimates, as well as the lowest abundance and species richness for fern gametophytes.

Table 5. Canopy openness and light values by plot.

This table shows data per plot. The # of gametophyte individuals, gametophyte species, and sporophyte species per m^2 were calculated to reveal potential relationships with light. This table reveals that the plots with the highest total light (highlighted in yellow) in each season also had a high # gametophyte individuals, gametophyte species richness, and sporophyte species richness. Light and canopy openness was calculated in Gap Light Analyzer (GLA). Light levels were compared within seasons, instead of between, as light availability and sun path changes from season to season.

Plot	# of gameto–phytes/ m^2	# of gameto–phyte species/ m^2	# of sporo–phytes species / m^2	Total surface area of hosts (m^2)	Direct light (mol/m^2/d)	Diffuse light (mol/m^2 /d)	Total light (mol/m^2 /d)	% Canopy openness
June Plot 1	21	5	6	1.98	16.32	16.78	33.10	16.6
June plot 2	32	6	8	2.87	16.47	18.11	34.57	17.09
June Plot 3	19	5	6	1.31	15.18	16.99	32.17	13.93
Oct Plot 1	58	8	8	2.13	11.99	17.28	29.27	8.86
Oct Plot 2	68	8	6	1.38	9.53	16.22	25.75	13.96
Oct Plot 3	7	3	4	2.74	8.6	15.73	24.57	8.81

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Alsophila podophylla hosts

The species composition and abundance of gametophytes growing on *Alsophila podophylla* hosts is represented in Figure 17. The *A. podophylla* hosts in June had considerably less gametophyte growth than those in October. The host near plot 2 in October (AP2_O) had one of the highest abundances with 15 individuals. It also had the highest species richness with 7 gametophyte species and had the highest DBH value of 26. Some of the more abundant gametophyte species found growing on *A. podophylla* hosts were *Vandenboschia auriculata* (Hymenophyllaceae), *Alsophila podophylla* (Cyatheaceae), *Haplopteris yakushimensis* (Pteridaceae), and *Asplenium pseudolaserpitiifolium* (Aspleniaceae).

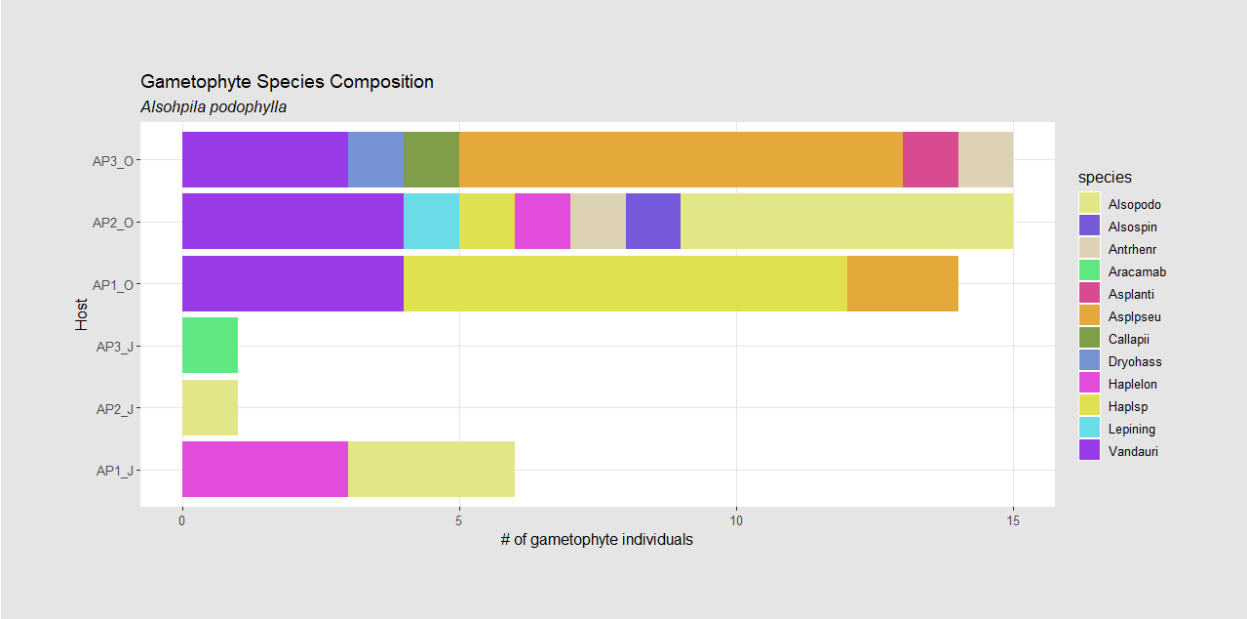

Figure 17. Gametophyte species composition of *Alsophila podophylla* hosts.

A figure showing the gametophyte species growing on *A. podophylla* hosts. Location of the hosts in each season can be found in table 2. AP1–3_J are from the June survey and AP1–3_O are from October. The names of the gametophyte species can be found with their corresponding abbreviation in table 3.

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DISCUSSION

Studying epiphytic community compositions is a complex process which involves considering many factors, especially when working with organisms as small and cryptic as fern gametophytes. For this study, we aimed to survey the community of epiphytic fern gametophytes growing on *Alsophila spinulosa* and nearby angiosperms in Taiwan, and to evaluate factors which likely influence the community.

With respect to our expectations (see "aims of this study"), we found that (1) tree ferns had both a higher abundance and species richness of fern gametophytes than angiosperm hosts. This corroborated our hypothesis, however, the latter half of our expectation assumed that tree ferns would also be host to a higher variety of gametophyte types, those being cordiform vs. non–cordiform, and epiphytic vs. accidental. While tree ferns hosted a higher abundance of cordiform and accidental gametophytes, angiosperms hosted a higher abundance of non–cordiform and epiphytic individuals. As hypothesized, we also found that (2) fern gametophyte and sporophyte species compositions varied, and confirmed that the gametophyte generation was more diverse and abundant than the sporophyte generation in our study. Finally, (3) our findings for relative humidity corroborated our hypothesis where we found that the tree fern hosts maintained stable, high levels of RH. Specifically, they remained above the 85% and 95% thresholds more frequently than the angiosperm host. While we did not set a hypothesis for canopy coverage and light, we found that in each season, the plot with the highest total light also had the highest species richness and abundance for fern gametophytes, as well as the highest species richness for fern sporophytes. We will now address important discussion points which seek to explain these findings.

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Significance of tree fern as host for epiphytic fern gametophytes

Relative humidity characteristics

In 2005, Mehltreter conducted a study establishing that tree ferns were able to support a higher abundance and species richness of epiphytes than angiosperm hosts. This was highlighted by his experiment which found that tree ferns have a higher water retention capacity than angiosperms. The relative humidity surveys we conducted can serve as an addition to the argument that epiphyte diversity is correlated with moisture availability. As mentioned, all measured hosts spent most of their time at RH values above 95; this was not surprising given the year–long rainy season in Neidong. Regardless of the almost consistent presence of moisture in this locality, the angiosperm host still spent significantly more time below 85% and 95% RH, dropping below the thresholds more frequently, and remaining there longer than the other hosts (Fig. 16). In comparison, both the dead tree fern and live tree fern maintained relatively stable RH values, with less frequency and shorter durations under either threshold. This result can be projected to other tree fern and angiosperm hosts within the plotting regions, concluding that the higher abundance and species richness of fern gametophytes on tree ferns is in part due to their relative humidity. Many ecologists who have studied epiphyte diversity cite humidity retention in the bark as the single most important factor in epiphyte surveys, which often explains the richness and viability of epiphytes (Castro Hernández et al., 1999; Callaway et al., 2002; Mehltreter et al., 2005).

Humidity and moisture are especially important for the gametophyte generation of ferns, given the necessity of water for protection of dehydration due to their single–layer tissue, and for their mating via flagellate sperms. Correspondingly, the establishment of the 85% threshold was informed by Lin's (2019) study, where he established that RH values below 85 are not suitable

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for desiccation intolerant fern gametophytes. He exposed gametophytes to drought intensities for 24 hours, and then rehydrated them to find that the photochemical efficiency values for most of the species did not recover (preferred range is .79 to .84 Fv/Fm). Notably, all species in his experiment were vittarioids. Watkins (2007b) did a similar study where he found that desiccation tolerance is exhibited in fern gametophytes, and the degree of tolerance is linked to habitat preference. For example, a fern gametophyte species growing in an exposed canopy was very tolerant to desiccation and was able to recover, while a species on the forest floor was not. Given that all collected gametophytes in our study were under 100 cm, it is likely that the gametophytes growing in this region have a drought response that is more similar to the "mid–canopy" or "understory" species in Watkin's study. However, our study did not include sufficient zoning to explore desiccation intensities. Nevertheless, water often collects at the base of trees, improving the moisture and humidity of low trunks (Yao–Moan Haung; personal observation). This is further supported by a study on epiphytic moss and lichen, which cited that tree bases are more sheltered by higher humidity (Sales et al., 2016). The low trunks of hosts in this study likely provide a habitat that would be suitable for fern gametophyte species which are drought intolerant.

Suitability for accidental epiphytes

Tree ferns have been noted as important pioneer species in forest ecosystems where their trunks can provide structural support to plants without strictly epiphytic lifestyles (Mehltreter et al., 2005; Schneider & Schmitt, 2011; Dawes & Burns, 2020; Machado et al., 2021). For example, seed plants normally growing in terrestrial environments have been observed using the complex weaving of the root mantle as a place to germinate (Dawes & Burns, 2020). Further, humus accumulation has been observed on tree fern microsites, a substrate

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characteristic that is required for most accidental epiphytes (Machado et al., 2021). This, along with the moisture held in by the sponge–like root mantle allows tree ferns to simulate a terrestrial–like environment, providing habitats for species which would usually spend their life on the ground. *Angiopteris lygodiifolia* (Marattiaceae) is an example of an accidental epiphyte, with records of both the gametophyte and sporophyte generation growing on tree fern hosts in this study (Tables A1 and A3). Both generations prefer moist sites; the gametophyte of *A. lygodiifolia* is often found growing on moist rocks (Ogura–Tsujita et al., 2013), and the sporophyte can be found throughout the moist understory of subtropical forests (personal observation).

Additionally, hemiepiphytism has been exhibited in fern gametophytes where they begin their life epiphytically; as they develop into a sporophyte, their roots help them crawl down to the ground or climb further up the tree. In 2012, Lagomarsino et al. studied this habit in *Elaphoglossum amygdalifolium* (Dryopteridaceae) where the species was observed growing epiphytically as a gametophyte; as the species develops into a sporeling, a long root extends to the ground and the sporophytes begin to crawl down with assistance of their creeping rhizomes. This has similarly been illustrated by Nitta & Epps (2009) with *Vandenboschia collariata* (Hymenophyllaceae) growth patterns in Costa Rica. Our study was not long enough to observe this behavior, but is a notable insight for the accidental epiphytic gametophytes in the site. It would be beneficial to see a long–term study which follows the life cycle of hemiepiphytic ferns in Taiwan, and how their habitat preferences may be associated with host substrate.

Importance of Alsophila spinulosa *in subtropical forests*

As seen by the survey of the *Alsophila podophylla* trunks, *Alsophila spinulosa* hosts had a higher abundance and species richness of fern gametophytes. The two tree ferns species often

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coexist in the shaded habitats of forest environments while a third species, *Sphaeropteris lepifera*, is often found nearby in open habitats (Chiu et al., 2015). While all three species belong to Cyatheaceae, their substrates vary in thickness, texture, and moisture availability. Compared with the *A. spinulosa* hosts, *A. podophylla* hosts had a thinner root mantle, usually more exposure of tree fern leaf scars, and a larger presence of tree fern "skirts" (personal observation). Many of the studies that describe tree fern substrate as an important habitat for epiphytes highlight the complex webbing of the trunk as a significant influence. Additionally, while bare leaf scars are still a suitable space for epiphyte establishment, this may not be the case for trunks with skirts. Tree fern skirts are described as the retention of fronds which create a layer of fringe around the trunk; this has been observed to inhibit epiphyte establishment (Page & Brownsey, 1986). Importantly, the findings from our survey indicate that *A. spinulosa* trunks could be providing a unique tree fern habitat for epiphytic fern gametophytes that cannot sufficiently be provided by other tree fern species.

Relationship between morphotype, life form, and host preference

Cordiform vs. non–cordiform

The results from Table 4 illustrate that tree ferns were able to host more cordiform fern gametophytes – a morphotype often associated with terrestrial habitats where moisture is more guaranteed. Importantly, not all cordiform gametophytes in this study were accidental epiphytes; *Asplenium* species and other cordiform gametophytes with an epiphytic lifestyle were found growing on both host types (Table 3). Angiosperm hosts more frequently hosted strictly epiphytic species of a non–cordiform morphotype. Tree ferns were also able to host non–cordiform gametophytes with an epiphytic lifestyle, however, a large proportion of the tree fern

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hosts' trunks were dominated by cordiform gametophyte growth.

It is important to note that some of the cordiform individuals on tree ferns were likely coming from the tree fern itself; In June, *Alsophila spinulosa* gametophytes were abundant on the tree fern trunks. As the *Alsophila spinulosa* fronds from above release their spores, they disperse to find their preferred, often terrestrial site; with a large surface area (average DBH for tree fern hosts: ~25) and suitable moisture requirements, many spores are bound to land on their own trunk. Spores from accidental species may land on angiosperm hosts as well, but the likelihood of successful germination is lower than the spores landing on terrestrial sites or tree fern hosts. Importantly, accidental epiphytic species prefer moist environments (e.g. tree fern hosts), while strictly epiphytic species may tolerate habitats that experience more frequent periods of drought (e.g. angiosperm hosts).

With respect to the higher abundance of non–cordiform gametophytes on angiosperm trunks, they were also host to a higher proportion of self–proliferating gametophytes (Table 4). As mentioned, non–cordiform gametophytes are generally long–lived and able to persist both with branching of the gametophyte thallus and production of gemmae. With the higher relative humidity recorded on tree ferns, it was assumed that these long–lived gametophytes would be better supported by the moist root mantle than the bark of the angiosperm hosts. Still, drought tolerance is more often associated with epiphytism, and non–cordiform gametophytes in particular have the ability to retain more water with their complex, three–dimensional morphologies (Farrar et al., 2008). Nevertheless, self–proliferating gametophytes were relatively abundant on both host types and there were likely other factors influencing their distributions such as disturbance, competition for space, or dispersal ability from their conspecific sporophyte (or lack thereof – see below).

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Independent gametophytes

Both of the independent gametophyte species —*Callistopteris apiifolia,* and *Haplopteris yakushimensis*— found in this study are known to exhibit an independent lifestyle (Pinson et al., 2017). Independent fern gametophytes are often non–cordiform, and occupy epiphytic habitats where the branching of their gametophyte thallus and gemmae production allow them to persist as a colony. Most independent gametophytes use gemmae for vegetative propagation (Fig. 13), and production of these propagules is dominant in vittarioids (Pteridaceae), filmy ferns (Hymenophyllaceae), and grammitids (Polypodiaceae) (Farrar, 1967). For tropical localities, theories as to why gametophytes develop an independent lifestyle are being formed, as the current theory of extinction of their sporophyte counterpart due to glaciations and climate change does not hold in these warmer, more stable climates (Kuo et al., 2017). It is presumed that *Callistopteris apiifolia* engaged in long–distance dispersal from a locality of conspecific sporophytes, and has since persisted as an independent colony with self–proliferating gemmae. This could also be the case for *Haplopteris flexuosa* and *Antrophyum henryi,* the other two non–cordiform gametophytes which have records of sporophytes nearby, but none were observed.

On the other hand, the independent gametophyte of *Haplopteris yakushimensis*, which was recently discovered by Kuo et al. (2017), has no highly genetically identical sporophyte species in East Asia and has so far been found growing in a few localities in Japan and Taiwan; in Japan, the species grew terrestrially, while in Taiwan, it was found terrestrially and epiphytically on *Cryptomeria japonia.* With our study, another Taiwan locality of *H. yakushimensis* has been established with distribution on *Alsophila spinulosa* hosts as well as angiosperm hosts (Fig. 8). While certainly more numerous on the tree fern host type (Table A3), it seems this species can adapt to various niches and grow on multiple host types.

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Sporophyte production and potential for phenological associations

Sporophyte production

The sporophytes present in this study were recorded along the entire trunk of the hosts, where most of the sporophytes were labeled as immature, meaning that they lacked spores and were often juveniles (Table A1). Table A1 also highlights how, for plot 3 in each season, with the exception of one species in October, there were no mature sporophytes recorded. While the mature sporophyte species composition did not seem to be an influential factor for any of the plots (Fig. 12), the lack of mature growth in Plot 3 of each season could be an explanation for the low gametophyte abundance on these hosts. In addition, the abundance of immature sporophytes could be indicative of sporophyte viability; it is possible that the hosts in our study are not providing a suitable habitat for the production of mature sporophytes. While many sporelings were observed in the study (Fig. 14b), we did not sufficiently record the different developmental stages of sporophytes. It is also possible that some of the sporophyte species in this study develop their spores in months besides June or October, and that we were observing sporophytes before they had reached their window of fertility.

Phenological associations

The high abundance of gametophytes in October could be a phenological association. Our recently published study (Quinlan et al., 2022) which analyzed the results of a serial survey of *Alsophila podophylla* gametophytes, concluded that gametophyte abundance is associated with the phenology of spore release; 2 to 4 months after the highest peak in spore release, the highest peak of gametophyte abundance followed. While this study focused on one gametophyte species (namely *Alsophila podophylla*), there was additional data collected during the one–year

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survey period, including recording all gametophyte individuals in established plots; these records found that overall, the highest abundance of gametophytes was in October (Kuo et al., unpublished data). This thesis corroborates these findings, both with fern gametophytes having a higher abundance in October than June, and with *A. podophylla* being one of the most abundant species for gametophyte individuals in October (Fig. 8). However, phenological associations cannot be confirmed; as highlighted above, few mature sporophytes were recorded on our surveyed trunks, and measurements of spore release which is an important insight for fern phenology, were not conducted. Though some fern species have relatively short dispersal distances (~3 m) (Rose & Dassler, 2017), the high diversity of species in island ecosystems attest to ferns' capacity for long–distance dispersal (Tryon, 1970; Kessler et al., 2010). It is possible that there were species in our study with a high dispersal ability which had parents growing in areas outside of the plotting region.

Fern gametophyte ecophysiology: relationship with light

While there was no significant correlation with canopy openness and gametophyte richness, in each season, the plot with the highest total light also had high abundance and species richness, plot 2 in June, and plot 1 in October, respectively (Table 5). Although we did not measure the specific light levels of each trunk, the dead tree fern in June (DTF_J2) likely had the highest exposure to light with no frond growth present to shade the trunk. Notably, the dead tree fern had comparable gametophyte richness and abundance to the nearby living tree fern. Regarding light factors, ecophysiological studies of gametophytes are relatively few, however, in 2006, Watkins et al. observed that gametophytes growing epiphytically are better adapted to light than terrestrial gametophytes. Additionally, in 2007, Watkins performed a series of disturbance

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treatments on gametophytes and found that as light increased with disturbance, so too did gametophyte density. While fern gametophytes have also been recorded to grow in moist, shady nooks of rocks (Pinson et al., 2017) and can better adapt to low–light conditions than their conspecific sporophyte (Ebihara et al., 2019), it is apparent that response to light varies from species to species, and that gametophytes can adapt to a wide–range of light conditions.

Another important factor to consider with light is spore germination. If a fern gametophyte is to successfully establish, the site needs to first be suitable for spores. In Suo et al.'s (2015) comprehensive study on fern spores' response to light, it was concluded that optimal light intensity and illumination varies from species to species. However, it was generally found that low light intensity is conducive to spore germination, and that some fern spores required long periods of light irradiation for successful germination. With the high level of gametophyte abundance in the high light plots of our study, and the comparatively low gametophyte abundance in the low light plots (Plot 3 from each season), it can be assumed that light was a factor influencing the successful germination of spores on these trunks. Further, light availability is quite varied along the trunk of tree ferns, especially if they have retained some of their "skirt" mentioned above (Page & Brownsey, 1986). This variation could be sustaining fern gametophytes with a diverse range of light demands, allowing a richness of species to colonize the various light regimes of a tree fern trunk.

This could also be support for the high abundance of accidental gametophyte species on tree ferns. In addition to the moisture availability of tree ferns, the trunk could be providing microhabitats that accidental species cannot easily find in a terrestrial habitat, such as light quantity and quality. Interestingly, Dawes & Burns (2020) found that shade–intolerant small–seeded, woody species that can fit into the webbing of tree ferns are using these hosts as an

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elevated microsite to escape light competition at ground level. With respect to the long periods of irradiation that the spores of some fern species require for successful germination, and the varied light regimes that tree ferns provide, it is possible that some of the accidental gametophyte species in this study were similarly using tree ferns as an elevated habitat to gain more access to light. However, further *in situ* studies of the physiological characteristics of fern gametophyte species should be done to draw such conclusions.

The case of the dead tree fern

While not a focus of my study, I had an interest in exploring the ecological importance of tree fern trunks even when dead (a trunk with a rotted pith and a crown lacking frond growth). With only one dead tree fern surveyed in the study, significant conclusions cannot be made about the suitability of dead tree fern hosts compared with others. However, it is worthwhile to note that the dead tree fern (DTF_J2) had similar relative humidity levels with the live tree fern host and a comparable richness of fern gametophyte individuals, gametophyte species, and sporophyte species (Table 2). Additionally, without survey of the dead tree fern, the presence of *Dryopteris paleolata* gametophytes would not have been realized, and the abundance of 6 other species would have decreased (Fig. 8). Accordingly, Ogle (2000) has recorded a similar variety of epiphytes on live and dead tree ferns, labeling dead tree ferns as an integral part of forest ecosystems. Likewise, snags (standing dead trees) have long been highlighted as an essential part of forest structure and functioning, often providing a home for lichen, fungi, insects, birds, and other creatures (Guby & Dobbertin, 1996). Importantly, Johansson (1974) documented that epiphytes can persist on hosts long after their death. Thus, dead trunks should be considered in epiphyte surveys to sufficiently understand the ecological role of a host species, and to provide protection to hosts when necessary.

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Important insights and potential caveats

Habitat complexity of the host & species–area relationships

There is a general consensus in biological diversity that a positive relationship exists between area and species richness; as area increases, the number of species inhabiting that area increases. This consensus holds true for epiphytic habitats where, as the hosts' trunk size increases, so does the species richness of epiphytes growing on the host. While this relationship was observed in our study (Figs. 4–6), other factors such as age and environmental diversity are considered by some ecologists to be more influential in explaining species richness (Flores–Palasios & García-Franco, 2006). For the former factor, ecologists argue that over time, more diaspores have the potential to land on the host, allowing for richness to be accumulated with age. For the latter, it is argued that as the habitat becomes more complex (e.g. branching, humus accumulation from biomass), heterogeneous environments are created which encourage a diversity of species (Po–Ju Ke; personal communication). Age was not recorded in this study, as age of tree ferns are difficult to determine (Blair et al., 2017), however the other two factors (i.e. area and habitat complexity) will be discussed more here as potential caveats of the study.

The diameter at breast height of all species was recorded along with the surface area of the surveyed region on the hosts. It is important to note that the tree ferns in our site were generally larger than the angiosperms; while the substrate of tree ferns has been recorded as being more favorable for epiphyte establishment, the smaller size of the angiosperm trunks is an important factor to consider. With a comparatively larger area than the angiosperms, it is reasonable that the tree fern trunks hosted a higher abundance and species richness of fern gametophytes. Further, due to limitations in sampling methods (i.e. steep slopes inhibiting us to climb the hosts), the entire trunks of the hosts were not surveyed. For angiosperms, zones higher

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up in the canopy often have a higher diversity of epiphytes than the lower trunks. Particularly, higher zones are where complex branching occurs, along with biomass accumulation which can provide pockets of moisture and nutrients (Steege & Cornilessen, 1989). Whereas tree ferns have also been observed to have an accumulation of organic matter (Machado et al., 2021), it could be argued that, with their unbranching root mantle, tree ferns offer less habitat heterogeneity than angiosperm hosts. By omitting the more diverse zones of the angiosperm hosts from our survey, our estimates of diversity on their trunks could be deficient. Nevertheless, with the generally high diversity of epiphytes in the upper zones of tree fern trunks (Schneider & Schmitt, 2011), I expect that the entire trunk of a tree fern would still host significantly more fern gametophytes than the entire trunk of an angiosperm. However, a follow–up survey which includes sufficient zoning would be beneficial to confirm comparisons of these host types.

Observation of other epiphytic organisms

A diversity of other epiphytic organisms was observed in this study which possibly affected fern gametophyte growth. Specifically, an abundance of lichen and bryophyte growth was observed on the hosts (Fig. 18). The lichen in particular caused a controversy in June, where many collected samples (~50) assumed to be filamentous gametophytes were actually a species of lichen known as *Coenogonium linkii* (Coenogoniaceae) (Table A4). After placing the supposed "filamentous gametophytes" under a microscope, apothecia (fungal fruiting body with spores) were observed, which are characters unique to lichens (Fig. 18, C). Most of the samples were collected from tree ferns; it is possible that this species fulfills a similar niche as *Vandenboschia auriculata* (Hymenophyllaceae), the primary filamentous gametophyte in this study. This potential presence of biotic competition could be an explanation for the higher abundance of filamentous gametophytes on angiosperms.

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While no specific species of bryophytes seemed to compete for space with fern gametophytes, a variety of species were observed on tree fern trunks (Fig. 18). Bryophyte mats have been noted as beneficial for long–lived gametophytes, where they can weave their way through the moist habitat and persist until outcrossing or self–proliferation can occur (Farrar et al., 2008). Additional aspects of fern gametophytes' relationship with bryophytes have been brought to light such as epiphytic gametophytes' ability to tolerate allelopathic compounds that bryophytes release (McCarthy, 2007) and the facilitative role that moss may have in fern fertilization due their water storage capacity (Harrington & Watts, 2021). If this thesis topic were to be further investigated, it would be advantageous to explore biotic factors and how interaction with other organisms may encourage or inhibit fern gametophyte growth.

Figure 18. Bryophytes and lichen on tree fern trunk

A–C are photos of the lichen species *Coenogonium linkii*. (A) *C. linkii* growing in the web of the root mantle. (B) A colony of *C. linkii* within the 2.5x2.5cm garden net. (C) A microscopic photo of the apothecia of *C. linkii*. D–F are photos of unidentified bryophyte species growing on the tree fern root mantle.

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CONCLUSION

This study further demonstrates the significance of tree ferns as an optimal habitat for epiphytic fern growth by highlighting the gametophyte stage of ferns growing on Cyatheaceae in Taiwan. Though specific host type preferences by gametophyte species were not observed in this study, Wagner et al. (2015) cite that, even if epiphytes have a host preference, they are still capable of using a broad range of hosts that occupy a similar niche. This theory could be especially true for fern gametophytes which often have wide distributions throughout diverse habitats. Nevertheless, tree ferns were host to a larger abundance and species richness of fern gametophytes than other hosts, likely due to their stable relative humidity and the characteristics of their substrate. Chiefly, this study is among the first insights of epiphytic fern gametophyte communities on tree ferns.

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APPENDIX

Cultural significance of tree ferns in Taiwan – Interview with Yaya Huwat

The Truku tribe includes indigenous groups located in and around what is now Taroko National Park on the East coast of Taiwan. In particular, The Skadang (Datong/大同) tribe lives in a village at ~1,000m above sea level that is only accessible by foot. While many Truku inhabitants relocated to Tmbarah village outside of the national park's visitor center, about 10 years ago, six or seven households returned to the mountains to reclaim their ancestral lands and reestablish a permanent living here (Cheung, 2020). A visit to the village includes a 4 to 5 hour steep trek; inhabitants of the tribe frequently take this trek to obtain resources from the towns below, but most food and living materials are produced in the village through practices such as subsistence farming.

Tree ferns are among many plants the Skadang tribe work with in their village. I had the opportunity to speak with tribal elder and natural farming practitioner Yaya Huwat about her tribe's relationship with tree ferns. Tree ferns have long been a cultural representation of the Skadang tribe with both historical and current uses. Their primary use is structural; Yaya shared, "We may have wood and steel nowadays, but the strength of tree ferns in our home is commemorative of our historical use of these plants." Yaya used tree fern trunks as the main support for a guest house on the historical site of her childhood home (Fig. S1). The trunks here are likely *Alsophila spinulosa*, known in Truku as grul galux. Galux means "black" in Truku, which distinguishes this tree fern from grul mbanan (*Sphaeropteris lepifera*), mbanan meaning "red". Generally, grul galux is preferred for beams due to their thick, strong root mantle (Fig. S2). Yaya shared that a larger piece of grul mbanan is required to be as strong as grul galux.

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Figure A1. Yaya's building project with tree fern beams on the historical location of her childhood home.

Figure A2. Comparison of tree fern types for beam support.

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In addition to tree ferns being commemorative, Yaya shared the practicality of using them instead of wood. She has observed that wood decays quicker than both tree ferns and bamboo (also used for building), and that termites do not seem to eat the trunks of the tree fern. Also, tree ferns are quite abundant around the Skadang village. Consistent with ecological studies on tree fern distribution (Chiu et al., 2015), Yaya has observed colonies of grul mbanan (*Sphaeropteris lepifera*) growing in open, sunny areas, while grul galux (*Alsophila spinulosa*) grows in the shady understory of the forest (Fig. S3).

Figure A3. A Grove of Tree Ferns on the trek to Skadang Village.

Yaya communicated the fundamental importance of tree ferns in her village by describing their harvesting practices. Importantly, they only cut down the older tree ferns, never harvest the young ones, and save harvesting for when there is an immediate need. Yaya put it simply by stating, “If we don’t need, we don’t cut. We leave them standing.” Additionally, a single tree fern trunk (5–7m tall) can serve many purposes; along with being used as beams, the thick trunks are often used as stair steps, pots for plants, and a bed in which the soft, large leaves of grul

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mbanan can be laid out for napping. (We had a nice laugh about not using grul galux for naps due to the spikes that run down the stems of the leaves.) When I asked about cutting down the trunks, Yaya also shared how she talks to plants in her garden when she harvests them, often telling them, “I’m going to cut you, I’m going to use you now.” She believes other members of the Skadang tribe share this personal connection to plants in the Datong region. Having always lived in the high mountains, they have built relationships and developed practices with surrounding plants which have become an essential part of their culture.

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I would like to thank Candice Jee and Han Cheung for making the interview with Yaya Huwat possible. Candice is an artist in residence in the Skadang village and has taken inspiration from tree ferns and their cultural significance for her artwork. Han Cheung is a journalist with Taipei Times who writes compassionate stories about Taiwan’s history and current events. A special thank you to Yaya for hosting me in her home village and sharing her plant wisdom with me. To all of the members of the Skadang tribe that I met while on my trek for sharing stories and making me feel welcome, Mhuway su' balay.

Figure A4. Interview with Yaya Huwat.

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Table A1. Sporophyte composition recorded in this study.

Appendix of sporophyte species recorded in study, organized by plot.

Species	Chinese name	Immature or Mature (I/M)	On tree fern	On angiosperm	Gametophyte present in surveyed month
JUNE – PLOT 1 (13 species)					
Angiopteris lygodiifolia Rosenst.	觀音座蓮	I	+		+
Asplenium antiquum Makino	山蘇花	I	+	+	+
Asplenium pseudolaserpitiifolium Ching	大黑柄鐵角蕨	I	+		+
Crepidomanes minutum (Blume) K.Iwats. subsp. minutum	團扇蕨	M		+	
Davallia trichomanoides Blume	海洲骨碎補	I		+	+
Deparia petersenii (Kunze) M.Kato var. petersenii	假蹄蓋蕨	M	+		
Diplazium doederleinii (Luerss.) Makino	德氏雙蓋蕨	I	+		
Dryopteris hasseltii (Blume) C.Chr.	哈氏假複葉耳蕨	I	+		+
Dryopteris sparsa (D.Don) Kuntze	長葉鱗毛蕨	M	+		+
Lemmaphyllum microphyllum C.Presl	伏石蕨	M		+	
Lepidomicrosorum ningpoense (Baker) L.Y.Kuo	攀援星蕨	M		+	+
Nephrolepis cordifolia (L.) C.Presl	腎蕨	M	+		
Vandenboschia auriculata (Blume) Copel.	瓶蕨	I		+	+
JUNE – PLOT 2 (22 species)					
Alsophila spinulosa (Wall. ex Hook.) R.M.Tryon	台灣桫欏	I	+		+
Angiopteris lygodiifolia Rosenst.	觀音座蓮	I	+		+
Antrophyum obovatum Baker.	車前蕨	I		+	+

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Asplenium antiquum Makino	山蘇花	I	+	+	+
Asplenium pseudolaserpitiifolium Ching	大黑柄鐵角蕨	I	+		+
Athyrium opacum (D.Don) Copel.	黑葉貞蕨	M	+		+
Davallia trichomanoides Blume	海洲骨碎補	I	+		+
Diplazium dilatatum Blume	廣葉鋸齒雙蓋蕨	M	+		+
Diplazium doederleinii (Luerss.) Makino	德氏雙蓋蕨	I	+		
Drynaria coronans (Wall. ex Mett.) J.Sm. ex T.Moore	崖薑蕨	I	+		
Dryopteris hasseltii (Blume) C.Chr.	哈氏假複葉耳蕨	I/M	+		+
Dryopteris sparsa (D.Don) Kuntze	長葉鱗毛蕨	I	+		+
Goniophlebium formosanum (Baker) Rödl–Linder	水龍骨屬	I	+		+
Haplopteris elongata (Sw.) E.H.Crane	垂葉書帶蕨	I	+		+
Lemmaphyllum microphyllum C.Presl	伏石蕨	I/M	+ (M)	+ (I)	
Lepidomicrosorum ningpoense (Baker) L.Y.Kuo	攀援星蕨	I	+		+
Lepisorus monilisorus (Hayata) Tagawa	擬芨瓦葦	I		+	+
Nephrolepis cordifolia (L.) C.Presl	腎蕨	M	+		
Psilotum nudum (L.) P.Beauv.	松葉蕨	I	+		
Pteris wallichiana J.Agardh	瓦氏鳳尾蕨	I	+		
Sphaerostephanos taiwanensis (C.Chr.) Holttum ex C.M.Kuo	臺灣圓腺蕨	I	+		
Vandenboschia auriculata (Blume) Copel.	瓶蕨	I	+		+
JUNE – PLOT 3 (8 species)					
Alsophila podophylla Hook.	鬼柳櫂	I	+		+
Arachniodes sp.	複葉耳蕨屬	I	+		

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Asplenium antiquum Makino	山蘇花	I	+		+
Asplenium pseudolaserpitiifolium Ching	大黑柄鐵角蕨	I	+		+
Diplazium dilatatum Blume	廣葉鋸齒雙蓋蕨	I	+		+
Lemmaphyllum microphyllum C.Presl	伏石蕨	I		+	
Lepidomicrosorum ningpoense (Baker) L.Y.Kuo	攀援星蕨	I		+	+
Vandenboschia auriculata (Blume) Copel.	瓶蕨	I		+	+
OCT – PLOT 1 (16 species)					
Alsophila podophylla Hook.	鬼柳櫧	I	+		+
Asplenium antiquum Makino	山蘇花	I	+	+	+
Asplenium pseudolaserpitiifolium Ching	大黑柄鐵角蕨	I	+	+	+
Crepidomanes minutum (Blume) K.Iwats. subsp. minutum	團扇蕨	I/M		+	
Davallia griffithiana Hook.	杯狀蓋骨碎補	I		+	
Davallia trichomanoides Blume	海洲骨碎補	I		+	
Diplazium doederleinii (Luerss.) Makino	德氏雙蓋蕨	M		+	
Dryopteris hasseltii (Blume) C.Chr.	哈氏假複葉耳蕨	I/M	+ (I/M)	+ (I)	+
Dryopteris sparsa (D.Don) Kuntze	長葉鱗毛蕨	I	+		+
Goniophlebium formosanum (Baker) Rödl–Linder	臺灣水龍骨	I/M	+ (I)	+ (I/M)	+
Haplopteris elongata (Sw.) E.H.Crane	垂葉書帶蕨	I	+	+	+
Lemmaphyllum microphyllum C.Presl	伏石蕨	I		+	+
Lepidomicrosorum ningpoense (Baker) L.Y.Kuo	攀援星蕨	I		+	+

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Lepisorus monilisorus (Hayata) Tagawa	擬芨瓦葦	I		+	
Nephrolepis cordifolia (L.) C.Presl	腎蕨	I	+		+
Vandenboschia auriculata (Blume) Copel.	瓶蕨	I		+	+
OCT – PLOT 2 (8 species)					
Alsophila podophylla Hook.	鬼柳櫳	I	+		+
Asplenium antiquum Makino	山蘇花	I	+		+
Asplenium pseudolaserpitiifolium Ching	大黑柄鐵角蕨	I	+		+
Haplopteris elongata (Sw.) E.H.Crane	垂葉書帶蕨	I	+		+
Histiopteris incisa (Thunb.) J.Sm	栗蕨	I	+		
Lepisorus monilisorus (Hayata) Tagawa	擬芨瓦葦	M		+	
Nephrolepis cordifolia (L.) C.Presl	腎蕨	I	+		
Pyrrosia lingua (Thunb.) Farw.	石葦	I		+	
OCT – PLOT 3 (11 species)					
Alsophila podophylla Hook.	鬼柳櫳	I	+		+
Alsophila spinulosa (Wall. ex Hook.) R.M.Tryon	台灣柳櫳	I	+		+
Asplenium antiquum Makino	山蘇花	I	+	+	+
Asplenium pseudolaserpitiifolium Ching	大黑柄鐵角蕨	I	+		+
Goniophlebium formosanum (Baker) Rödl–Linder	臺灣水龍骨	I	+		+
Haplopteris elongata (Sw.) E.H.Crane	垂葉書帶蕨	I	+		+
Lemmaphyllum microphyllum C.Presl	伏石蕨	I	+		+
Lepidomicrosorum ningpoense (Baker) L.Y.Kuo	攀援星蕨	I		+	+
Nephrolepis biserrata (Sw.) Schott	長葉腎蕨	I	+		

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Nephrolepis cordifolia (L.) C.Presl	腎蕨	I/M	+		
Vandenboschia auriculata (Blume) Copel.	瓶蕨	I		+	+

Table A2. Primer Appendix.

Primer Name	Target Region	Direction	Sequence 5'–3'	Reference
FernF4121 (br01 – br20)	trnF	F	GGnnnnnnnnGGATTTTCAGTCCYCTGCTCT	Kuo, unpublished
FernF4121 (non–barcoded)	trnF	F	GGATTTTCAGTCCYCTGCTCT	Wu et al. 2022
FernL5675	trnL3'exon	R	TTnnnnnnnnTGAGGGTTCGANTCCCTCTA	Kuo, unpublished
FernL0725	trnL	R	ATGGCGRAATGGTAGACGC	Wu et al. 2022

Table A3. Gametophyte Appendix.

Note: gametophytes growing on *Alsophila podophylla* hosts are not included here.

Fern Gametophytes		June			October		
Species name	Family	On tree fern	On angiosperm	Total	On tree fern	On angiosperm	Total
Alsophila podophylla Hook.	Cyatheaceae	5		5	54	3	57
Alsophila spinulosa (Wall. ex Hook.) R.M.Tryon	Cyatheaceae	25	1	26	4		4
Angiopteris lygodiifolia Rosenst.	Marattiaceae	11	3	14	3		3
Antrophyum henryi Heiron.	Pteridaceae					1	1
Antrophyum obovatum Baker.	Pteridaceae		6	6			
Arachniodes amabilis (Blume) Tindale var. amabilis	Dryopteridaceae	3		3			
Asplenium antiquum Makino	Aspleniaceae		7	7		1	1
Asplenium nidus L.	Aspleniaceae				1	1	1

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Asplenium pseudolaserpitiifolium Ching	Aspleniaceae		1	1	16	2	18
Athyrium opacum (D.Don) Copel.	Athyriaceae	1		1			
Callistopteris apiifolia (C.Presl) Copel.	Hymenophyllaceae					1	1
Christella parasitica (L.) H.Lév. ex Y.H.Chang	Thelypteridaceae					1	1
Davallia trichomanoides Blume	Davalliaceae		1	1			
Diplazium dilatatum Blume	Diplaziaceae	7		7			
Diplazium laxifrons Rosenst.	Diplaziaceae	1		1			
Diplazium virescens Kunze var. virescens	Diplaziaceae	1		1			
Drynaria coronans (Wall. ex Mett.) J.Sm. ex T.Moore	Polypodiaceae				1		1
Dryopteris hasseltii (Blume) C.Chr.	Dryopteridaceae	4		4	4	2	6
Dryopteris paleolata (Pic.Serm.) Li Bing Zhang	Dryopteridaceae	2		2			
Dryopteris sparsa (D.Don) Kuntze	Dryopteridaceae	1		1	6		6
Goniophlebium formosanum (Baker) Rödl–Linder	Polypodiaceae		1	1		2	2
Haplopteris anguste–elongata (Hayata) E.H.Crane	Pteridaceae				1	1	1
Haplopteris elongata (Sw.) E.H.Crane	Pteridaceae	1		1	13	3	16
Haplopteris flexuosa (Fée) E.H.Crane	Pteridaceae				1		1
Haplopteris yakushimensis C.W.Chen & Ebihara	Pteridaceae	4	2	6	39	20	59
Hymenasplenium cheilosorum (Kunze ex Mett.) Tagawa	Aspleniaceae					2	2

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Lemmaphyllum microphyllum C.Presl	Polypodiaceae				1		1
Lepidomicrosorum ningpoense (Baker) L.Y.Kuo	Polypodiaceae	31	15	46	8	10	18
Lepisorus monilisorus (Hayata) Tagawa	Polypodiaceae		1	1			
Microlepia obtusiloba Hayata	Dennstaedtiaceae				1		1
Nephrolepis cordifolia (L.) C.Presl	Oleandraceae				2		2
Vandenboschia auriculata (Blume) Copel.	Hymenophyllaceae		4	4	2	5	7

Table A4. *Coenogonium linkii* reference sequence.

A reference sequence for the lichen samples was obtained from Dr. Ko–Hsuan Chen at Academia Sinica.

Coenogonium linkii Ehrenb.	KHC 154_ITS1F–LR3 CCCGCCGAAAGCCCTTTCGAAAATCcTTTTCGAGAAACCCCCTGAAGACAGAACGATCGCGAAA TCACACGAACCAAAAAACTTTCAACAACGGATCTCTTGGTTCCGGCAACGATGAAGAACGCAG CGAAATGCGATAAGTAATGTGAATTGCAGAATTTTGTGAATCATCGAATCTTTGAACGCACATT GCGCCCTCCGGCATTCCGGGGGGCACGCCTGTTCGAGCGTCATTTGGTCACTCAAGCCCGGCTT GGTGTTGGATGCCTCCGGGGTGGAGCGTCCGAAATGCAGCGGCTCGGTCGTCCGGGGAAGGA ACGCACTGGTGAAAGTGGTCGAGTGTCGGCCACCGTTTCCCCCCCCCCCGGGCGGTCGTCGCC GTAAgATCGCGGCCGGGCAGCCGGCCCCGACCCTCGCAGCGATATTGACCTCGGATCAGGCGG GAGTACCCGCTGAACTTAAGCATATCAATAAGCGGAGGAAAAGAAACCAACAGGGATTGCCCC AGTAACGGCGAGTGAAGCGGCAAGAGCTCAAATTTGAAATCTGGCTCCCCCGGGGGTCCGAG TTGTAATTTGCAGAAGGTGCCTCGGGGACGGACCTCGGCCCAAGTCCTCTGGAACGGGGCGTC GCAGAGGGTGAGAATCCCGTACGGCCGGGGCCTACCCCCGCACGAGGCCCTTTCGACGAGTC GAGTTGTTTGGGAATGCAGCTCAAAACGGGTGGTAAATTTCATCCAAAGCTAAATACCGGCCG GAGACCGATAGCGCACAAGTAGAGTGATCGAAAGATGAAAAGCACTTTGGAAAGAGAGTTAA AAAGCACGTGAAATTGTTGAAAGGGAAGCGCTTGCGGCCAGACTCGCCCGCGGGTGCTCAGC CGTCCCCCCGGGGCCGGTGCACTCACC

Additional supplementary data and R scripts can be seen here:

https://github.com/alex–quinlan/thesis_data

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