PHYLOGENETIC COMPARATIVE ECOLOGY OF THE FERNS OF MOOREA, FRENCH POLYNESIA

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Abstract. Ferns (pteridophytes or monilophytes) are abundant in tropical forests on oceanic islands, coexisting in similar habitats. Closely related fern species exhibit variation in functional traits, traits that are responsible for allocation of plant resources. Possible functional trait correlation, with environmental factors, was tested in ferns on Moorea, French Polynesia. Overall, this study examined whether the correlations observed among fern species of Moorea reflect the historical patterns of correlated evolutionary change. Three functional traits, specific leaf area (SLA), frond size, and stomata density (SD), and three environmental factors, canopy cover, elevation, and substrate preference, were measured in ten fern species on Moorea. Fern species surveyed were selected to represent the major fern clades. Linear regression statistical analysis was used to test for possible correlation between traits, and trait correlation was mapped on the fern phylogeny to test for similar evolutionary patterns. No significant linear correlation was observed between the SLA, frond size, or SD and canopy cover or elevation, but some correlation was observed between all functional traits and substrate preference. All measured correlation patterns reflected the evolutionary correlation patterns. Future studies should include extensive sampling of fern lineages, functional traits, and environmental factors and include other vascular plant species. Understanding how plant functional traits respond to changing environmental conditions will help us predict how future environmental changes, such as climate change and other disturbances, will affect the plant and larger ecological system.

Key words: ferns; pteridophytes; monilophytes; tropical rainforest; French Polynesia; comparative ecology; phylogenetics; functional traits

INTRODUCTION

Understanding the processes generating variations in co-occurring species is a central goal of ecology (Kraft *et al*. 2007). A common approach in ecological studies is examining how organisms interact with their environment (Webb *et al.* 2002, Ackerly 2009). Comparative ecological studies examine how well adapted organisms are to their surrounding environments (Harvey and Purvis 1991, Freckleton *et al*. 2002, Poulin *et al*. 2011). Since different organisms respond to changing environmental conditions in different ways the differences, and similarities, in their response to their environment can reveal insights about a species' ecology (Ackerly 2009). Comparative ecological studies use evolutionary or taxonomic relationships to compare variations in different species and, include ecological and phylogenetic components (Harvey and Purvis 1991, Freckleton *et al*. 2002). The ecological approach examines how functional traits vary with changing environmental conditions (Ackerly 2003), while the phylogenetic

approach examines whether character states are ancestral or derived, and what mechanisms drove divergent evolutionary events (Webb 2000).

Phylogenetic studies in ecology tell us how species are related one to another, and how character states (or traits) vary between different groups or clades (Ackerly 2009). Species are also separated into groups based on similarities and differences of morphological and molecular data (Pryer *et al*. 2004). This grouping of species allows us to construct a phylogenetic tree with extant or extinct groups to model relationships between species (Smith *et al.* 2006). By studying the evolution of character states on the phylogeny we can infer ancestral states, the direction of evolutionary change in a trait, and the possible occurrence of convergent evolution (Felsenstein 2004).

In addition to phylogenetic analysis comparative ecology also has an ecological component that specifically focuses on organismal functional traits (Harvey and Purvis 1991). Plant functional traits are those that are directly responsible for attaining

resources, such as light, water, nutrients, and CO_2 that the plant requires for growth and survival (Ackerly 2003, Cornelissen *et al.* 2003). Plant functional traits, however, are not isolated characters; they interact with one another, and with different environmental conditions to influence plant survival (Ackerly and Donoghue 1998). By examining the correlation of functional traits, instead of separate individual traits, through time you can learn what types of historical evolutionary changes have occurred (Marquez and Knowles 2007, Shipley 2007). The environmental and ecological variation of functional traits can be a result of phenotypic plasticity (Ackerly *et al.* 2000, Ackerly 2003), ecological sorting (Ackerly 2003), or adaptation by natural selection (Ackerly 2003, Marquez and Knowles 2007). Heritable functional traits arise through natural selection, while other functional traits arise during development or in response to ecological changes (Ackerly 2003). Natural selection can be rapid, leading to changes in phenotypes and sometimes leading to adaptive radiation events and divergence between close relatives (Ackerly 2003).

Despite functional trait variation closely related species or lineages often do exhibit a high degree of phenotypic and ecological similarity, which usually means that little evolutionary change has occurred since the original species divergence from a common ancestor (Ackerly 2003). Phenotypic similarities though also arise from parallel or convergent evolutionary events that have occurred independently since divergence (Ackerly and Donoghue 1998, Ackerly 2003). Absence of evolutionary change can be a result of time, lack of genetic variation, and from stabilizing selection which favors intermediate forms and maintains the ancestral state (Harvey and Purvis 1991, Ackerly 2003). The role of stabilizing selection can be strong, resulting from species favoring their habitat where they have adapted instead of expanding into new ones, leading to niche conservation over time (Harvey and Purvis 1991, Ackerly 2003, 2009). According to the competitive exclusion principle, or Gause's Law, closely related species should not be able to live close together if they utilize the same resources, as one will eventually out compete the other (Hardin 1960). A consequence of competitive exclusion, resulting when closely related species live together, is that the competing species will evolve distinguishing functional traits that allow them to be more

competitive than other species (Hardin 1960). These functional traits become adaptive traits if performance is enhanced, resulting in improved resource acquisition, growth, survival, and reproduction relative to other forms (Ackerly 2003). How closely related species respond to environmental change is a question comparative ecological studies attempt to answer (Shipley 2007).

In tropical rainforests significant clustering of closely related species occurs, probably due to similar habitat preferences over different clades (Webb 2000). Tropical rainforests are characterized by richness of species, which are maintained by disturbance events that keep the community in a nonequilibrium state (Connell 1978). Tropical forests on oceanic islands are isolated systems, making them excellent models for evolutionary and adaptive radiation studies (Connell 1978, Nitta 2006). One group of plants abundant on tropical islands is ferns, also known as monilophytes, a large diverse group of vascular seed-free plants (Smith *et al.* 2006). There is significant fern diversity in the tropics, but the habitat preference is narrow as many ferns coexist in similar niches (Hietz and Briones 1998). Ferns are the most ancient vascular plant lineage, representing a distinct path in vascular plant evolution, which we can study to determine functional trait evolutionary patterns (Karst and Lechowicz 2007).

Certain relationships among important functional traits may have been conserved throughout the evolution of vascular plants (Shipley *et al.* 2006), but these relationships have not been extensively studied in ferns. Cornelissen *et al.* (2003), published a handbook of protocols, standardizing and measuring plant functional traits, resulting in many vascular plant species being measured (Hietz and Briones 1998, Cornelissen *et al.* 2003, Wright *et al.* 2004, Karst and Lechowicz 2007, Shipley *et al.* 2006, Shipley 2007), but interestingly very few fern species. Karst and Lechowicz (2007) and Saldana *et al.* (2006) did collect functional trait information on fern species in temperate rainforests, but few studies have been conducted on fern functional traits in tropical forests on oceanic islands. Most plant functional trait studies focus on understanding how traits respond to changing environmental conditions, their effects on competitive strengths and defenses, or their effects on biogeochemical cycles (Cornelissen *et al.* 2003). These studies have usually focused on a subset of plant functional

traits, as some traits are more important than others in regards to a plants response to their environment. Many fern functional trait studies focus on leaf traits (specific leaf area, leaf size, leafy dry matter content, leaf N and P concentration, physical strength of leaves, leaf lifespan, leaf phenology, photosynthetic pathway, gas exchange, leaf frost resistance), or whole-plant traits (growth form, life form, plant height, clonality, spinescence, or flammability) (Cornelissen *et al.* 2003, Karst and Lechowicz 2007). One reason leaf traits are studied in ferns in particular is they provide a great deal of information about hydraulic characteristics and carbon balance, important components of fern fitness (Saldana *et al.* 2006).

The island of Moorea in French Polynesia is a rich environment to study ferns, as they disproportionally represent the tropical habitat of land plants, creating a disharmonic assemblage of plant species (Nitta 2006). Over 100 species of fern are found on Moorea, coexisting in similar habitats on the island (Murdock and Smith 2003, Murdock and Nitta 2011, Nitta *et al.* 2011). The goal of this study was to examine whether phenotypic correlations, observed among fern species of Moorea, reflect the historical patterns of correlated evolutionary change. There were three primary objectives: (1) Examining whether a suite of ecologically important functional traits correlated with environmental conditions, (2) Examining whether the interspecific correlation patterns observed reflect similar evolutionary divergence patterns, and (3) Examining the
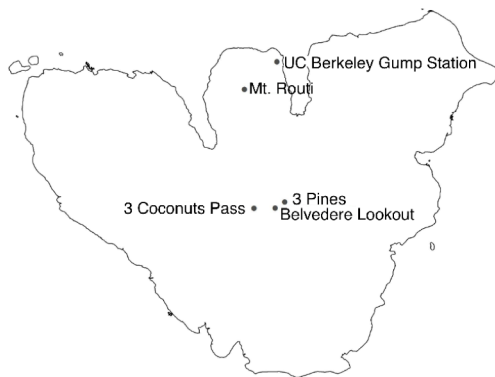
specialization of substrate preference of fern species through history to determine whether specialization of substrate is a derived character state. For these studies I compiled data for certain fern leaf functional traits related to resource allocation and use, specifically water assimilation and conservation, specific leaf area (SLA), stomata density, and frond size, and also conducted a phylogenetic comparative study of the correlated evolution among the fern functional traits and environmental conditions, canopy cover, substrate preference, and elevation (Appendix A outlines the specific hypotheses tested in this study).

METHODS

Study site

Moorea, French Polynesia is a high volcanic island (134 square km) surrounded by a fringing and barrier coral reef. The island is located in the South Pacific Ocean and is the second largest island in the Society Islands Archipelago. The majority of the interior of the island is composed of a tropical rainforest dominated by *Inocarpus fagiferus*, *Hibiscus tiliaceus*, and *Angiopteris evecta* at low to mid elevations (100m-400m) and *Metrosideros collina*, *Weinmannia parviflora*, and a large variety of epiphytes at higher elevations (400m and above) (Nitta 2006). Hiking trails across the interior of Moorea range in elevation from sea level (0m) to the top of the highest peak, Mount Toihea (1207m). Plants surveyed were adjacent to the following hiking trails: Three Coconuts Pass (260m-420m), Mount Rotui (50m-400m), Three Pines
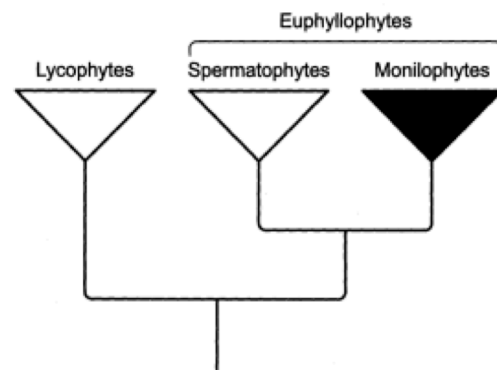

FIG. 1. Moorea, French Polynesia map depicting study sites: UC Berkeley Gump Station, Mt. Routi, 3 Pines, 3 Coconuts Pass, and the Belvedrere Lookout. Courtesy of the Geospatial Innovation Facility, University of California, Berkeley.

FIG. 2. Phylogeny depicting the relationships of major vascular plant lineages. All nodes have received bootstrap support ≥ 70 (adapted from Smith *et al.* 2006).

(260m-320m), and Belvedere Lookout to Marae Trail (120m-260m) (Fig. 1). Ferns were also sampled from the UC Berkeley Gump Station (0m-50m), located near the mouth of Cook's Bay. Sampling in the field involved physical collection of specimens and gathering of ecological data, including canopy cover, substrate preference, elevation, and GPS coordinates for each specimen.

Study organisms

Euphyllophytes is an informal subdivision of vascular plants that makes up over 99% of all vascular plants (Smith *et al.* 2006). Euphyllophytes are broken into two groups, the spermatophytes (seed plants) and the monilophytes (ferns and fern allies) (Fig. 2). This study focuses solely on the monilophytes (also called the pteridophytes), and within the division, specifically the ferns. Ferns are vascular and spore-bearing plants without seeds and are generally split into three groups: whisk ferns, eusporangiate ferns, and leptosporangiate ferns (Smith *et al.* 2006). Ferns are a diverse group (Vasco *et al.* 2013) and can have simple leaves or compound leaves, once or twice pinnate (Pryer *et al.* 2004). The entire fern leaf is called a frond, and the individual leaflets in a compound leaf are called pinna (Fig. 3). Species examined in this
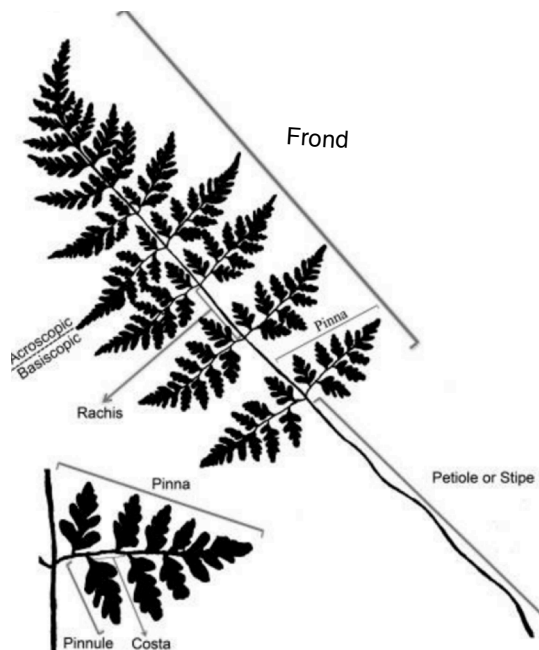

FIG. 3. General fern descriptions and terminology (adapted from Vasco *et al.* 2013).

study belong to different families within the division monilophytes, and have different phenotypic morphologies as well as different phylogenetic relationships. For this study, the following nine fern families were sampled: Davalliaceae, Dryopteridaceae, Hymenophyllaceae, Lomariopsidaceae, Marattiaceae, Polypodiaceae, Psilotaceae, Pteridaceae, and Thelypteridaceae (Fig. 4).

Taxonomic selection

Ten terminal taxa were selected to represent major lineages of ferns for the comparison of correlated functional trait evolution. All selected ferns were growing in the tropical rainforest on Moorea. Fern species chosen for this study were widespread on the

Class Marattiopsida
    Order Marattiales
        Family Marattiaceae
            Genus *Angiopteris*
                Angiopteris evecta
Class Polypodiopsida
    Order Polypodiales
        Family Davalliaceae
            Genus *Davallia*
                Davallia solida
        Family Dryopteridaceae
            Genus *Bolbitis*
                Bolbitis lonchophora
            Genus *Teratophyllum*
                Teratophyllum wilkesianum
        Family Lomariopsidaceae
            Genus *Nephrolepis*
                Nephrolepis hirsutula
        Family Polypodiaceae
            Genus *Microsorum*
                Microsorum grossum
        Family Pteridaceae
            Genus *Adiantum*
                Adiantum trapeziforme
        Family Thelypteridaceae
            Genus *Amphineuron*
                Amphineuron opulentum
    Order Hymenophyllales
        Family Hymenophyllaceae
            Genus *Crepidomanes*
                Crepidomanes bipunctatum
Class Psilotopsida
    Order Psilotales
        Family Psilotaceae
            Genus *Psilotum*
                Psilotum nudum

FIG. 4. Taxonomic relationships of the ten sampled monilophyte or fern species (Pryer *et al.* 2004, Nitta *et al.* 2011)

island, and represented terminal taxa from a wide diversity of fern clades. All ferns included in this study have life-history variation, but they do all coexist in similar habitats on Moorea. The following ten fern species were sampled: *Adiantum trapeziforme, Amphineuron opulentum, Angiopteris evecta, Bolbitis lonchophora, Crepidomanes bipunctatum, Davallia solida, Microsorum grossum, Nephrolepis hirsutula, Psilotum nudum,* and *Teratophyllum wilkesianum* (Fig. 4)*.* In order to best approximate and cover the entire fern phylogeny of Moorea, the subset of selected species were chosen from a broad array of fern groups, representing the major lineages within a clade. Including all species of a clade is neither feasible nor necessary for a group as large as monilophytes in order to obtain a hypothesis about phylogenetic relationships and functional trait evolution (Ackerly and Donoghue 1998). Table 1 below shows general information (frond morphology, common elevation, and substrate preference) of the ferns surveyed in this study.

Sampling methods

TABLE 1. Selected general fern characteristics. Table key: F=Frond Morphology: 1=1 Pinnate, 2=2 Pinnate; E=Elevation: Low (L)=0m-100m, Mid (M)=100m-300m, High (H)=300m-500m; S=Substrate: T=Terrestrial, E=Epiphytic, L=Lithophyte (adapted from Murdock and Smith 2003, Murdock and Nita 2011, Nitta *et al.* 2011, University and Jepson Herbaria at University of California, Berkeley (Moorea Digital Flora)).

Fern Species	F	E	S
A. trapeziforme	2*	M/H	T
A. opulentum	1	M	T
A. evecta	1	M/H	T
B. lonchophora	1	M	T/E/L
C. bipunctatum	2	M	E/L
D. solida	2	All	T/E/L
M. grossum	1**	All	T/E/L
N. hirsutula	1	All	T/E/L
P. nudum	2*	All	T/E
T. wilkesianum	2	M	T/E/L

Notes: *Species frond morphology is compound, but classified as forked rather than twice pinnate. ***M. grossum* is commonly found with a compound frond, once pinnate, but it is also sometimes found as a simple frond with no pinnate.

Fern specimens were collected from five sites with differing environmental conditions; therefore not all ten ferns were sampled at each site. Table 2 below states at which sites each fern species were found. For each fern species 16 individuals were sampled. Fern sampling occurred on clearly marked and traveled trails or roads; and all ferns collected were visible from the trails or road. Sampling was conducted by hiking along the paths and visually scanning for the selected ferns. When a fern was observed, it was sampled only if it was fully mature, contained sterile and vegetative fronds, and contained no visible signs of disease. When a fern met all of the specified requirements ecological data was recorded and the individual fern physically sampled. Three mature, sterile, and healthy fern fronds were collected from each individual fern, with the exception of *A. evecta* in which only three portions of the frond were sampled. Only sampling the tip or the apex of *A. evecta* standardized the sampling of the frond, which is too large to sample in entirety. For the remainder of the ferns the three selected fronds were on the outer portion of the plant receiving the most sunlight (Cornelissen *et al.* 2003). Frond stalks were cut directly under the basal pinna so only the upper fleshy 'leafy' portion of the fern remained. Canopy cover was estimated using a spherical densitometer (Model C, Robert E. Lemmon, Forest Densitometers) and the

TABLE 2. Study sites in which selected fern species were sampled. "X" means the fern was sampled from that study site, and empty cell means the fern was not sampled from that site Table key: G=UC Berkeley Gump Station (0m-50m); B=Belvedere to Marae Trail (126m-260m); P=3 Pines (260m-320m); C=3 Coconuts Pass (260m-420m); R=Mount Rotui (50m-400m).

Fern Species	G	B	P	C	R
A. trapeziforme		X	X	X	
A. Opulentum		X	X		
A. evecta		X	X		
B. lonchophora		X	X		
C. bipunctatum		X	X		
D. solida	X	X	X	X	
M. grossum	X	X	X	X	X
N. hirsutula	X	X	X	X	
P. nudum		X	X	X	
T. wilkesianum		X	X		

elevation and the GPS location were recorded with a handheld GPS device (etrex 20, Garmin 2011). The substrate in which the fern was growing was also recorded.

Fern identification was done using the "Diagnostic Key to Pteridophytes of Moorea" from the University and Jepson Herbaria at the University of California at Berkeley (Murdock and Smith 2003, Murdock and Nitta 2011, Nitta *et al.* 2011; http://ucjeps.berkeley.edu/moorea/fern_key .html). This key included all of the pteridophyte (fern) species known to grow on Moorea with photographs, field and herbarium, of all of the species. Most fern identification was done in the field, but some samples and photographs were taken to examine in the lab as necessary. Voucher specimens were deposited in the University Herbarium, UC Berkeley.

Comparative ecological study

Three functional traits were studied: specific leaf area (SLA), frond size, and stomata density (SD). All measured functional trait data was combined and averaged for all ten fern species. Functional trait correlation with environmental factors was then compared across ferns, without specifying a species, to determine if the ferns in general showed any correlation trends.

Specific leaf area. Specific leaf area (SLA) is a functional trait in plants that is related to water availability, leaf size, and temperature (Wright *et al.* 2007). The SLA is a function of leaf dry matter content and leaf thickness as both contribute to the SLA depending on the habitat and the plant in question (Wilson *et al.* 1999, Wright *et al.* 2007). For the purpose of this study 'leaves' will be referred to as 'fronds,' which is proper nomenclature for ferns (Fig. 2). As described above, selected fronds contained no visible signs of disease, were fully mature, were sterile and vegetative, and were sampled from the outer canopy fronds with the most sunlight. For this study, the rachis (stalk-like midrib of a fern (Fig. 2)) was included as part of the frond leaf. All frond types, simple or once or twice pinnate were measured similarly. All frond measurements were calculated within 48 hours of collection in order to minimize shrinkage due to dehydration (Wright *et al.* 2007). All samples were washed with water and patted dry prior to SLA measurements.

SLA is calculated by dividing the leaf area by the oven-dry mass. The frond area was calculated from a photograph analyzed with ImageJ software (Rasband 1997, US National Institutes of Health; http://www.nih.gov/). All photographs were taken with a tripod at a set distance and angle. A ruler was included in all photographs and used for software measurement calibration. After area measurements, frond samples were placed in a drying oven at 75 C for 72 hours. Dry mass was measured directly after removal from drying oven.

Frond size. The size of a frond is generally related to the availability of water and other resources a plant needs to grow (Wright *et al.* 2007). For this study, fern fronds were measured with a vernier caliper (0.05mm). For simple fronds the entire frond was measured, while for compound fronds, only the first pinna was measured. Unless specified, all samples listed are compound fronds. For compound fronds (once or twice pinnate), the fourth pinna down from the frond apex of each sample of the first pinnate was selected. Frond measurements included: pinna width (mm), length (mm), and thickness (mm).

Stomata density. Stomata are pores found on the epidermis of leaves and stems that control gas exchange and water loss in plants (Hultine and Marshall 2000; Atala 2012). Stomata have been linked to plant water conversation mechanisms because the guard cells on either side of the stoma can regulate the size of the opening of the pore depending on availability of water and gases in the plant (Hultine and Marshall 2000; Atala 2012). The stomata density is the number of pores per leaf surface area. Shifts in the stomata density have been attributed to changing levels of atmospheric CO_2, and water conservation necessity (Hultine and Marshall 2000). For this portion of the study three pinna were selected, one from frond base, middle, and apex. For simple fronds, the base, middle, and apex sections of the frond were treated similarly to the different pinna of the compound frond. All trichomes, or hairs on the surface of the frond, were removed and a layer of clear nail polish was applied to the pinna surface (Hultine and Marshall 2000). An epidermis peel and impression was captured with the nail polish and was examined under a compound microscope (40X power). Stomata were counted in three separate fields of views (Diameter=4250 micrometers) for each of the three pinna epidermis impressions. Average stomata density (number of stomata per square micrometer) was calculated for each of the three sections as well. Stomata densities

were measured on all surveyed ferns except *P. nudum* and *C. bipunctatum* due to procedural difficulties in obtaining an epidermis peel and the fern not having any stomata respectively.

Statistical analysis

Linear regression statistical analysis tests were used to determine trait correlation within a fern species (R Core Team 2013, RStudio Team 2015). For each fern species (and for combined and averaged fern data overall), simple linear regressions were conducted between the following functional traits and environmental factors to test for a linear relationship between the variables: (1) SLA vs. canopy cover and elevation; (2) frond size vs. canopy cover and elevation; and (3) SD vs. canopy cover and elevation. In each linear regression, the null hypothesis tested was that the functional trait had no linear correlation with the environmental variable (slope (m)=0). Regression data and best-fit lines were visualized with ggplot2 (Wickham 2009, R Core Team 2013, RStudio Team 2015).

For species that established in multiple substrates (epiphytic, lithophytic, or terrestrial), functional traits (SLA, frond size, and stomata density) were compared against substrate preference using an ANOVA test (R Core Team 2013, RStudio Team 2015). All data was log-transformed prior to analysis to meet the requirements of the Shapiro-Wilk normality test (Royston 1982, R Core Team 2013, RStudio Team 2015).

A power analysis (Cohen 1992, R Core Team 2013, RStudio Team 2015) was used to determine the appropriate sample size needed for this study. The expected effect size was calculated using a linear regression model (R Core Team 2013, RStudio Team 2015) with alpha=0.05.

Phylogenetic analysis

Phylogenetic analysis was used to determine if the correlations measured in the comparative ecological portion of this study match the evolution patterns of traits, and whether any evolutionary correlations among trait-divergences existed. Characters were mapped on a molecular phylogenetic tree (adapted and pruned from Rothfels *et al.* 2015 (Appendix E shows the phylogenetic tree used with branch lengths)) in the phylogeny software, Mesquite version 3.04 (Maddison and Maddison 2015, version 3.04). The phylogeny was used to hypothesize the evolutionary relationships between the ten fern species used in this study. The PDAP (Phenotypic Diversity Analysis Programs) package in Mesquite (Midford *et al.* 2005) was used to test for a correlation between two continuous-valued traits (Felsenstein 1985, Garland *et al.* 1992), and the character trace feature on Mesquite was used to map and substrate preference on the phylogeny (Maddison and Maddison 2015, version 3.04). The test results were then compared against the correlation results obtained from the ecological trait correlation measurements.

RESULTS

Comparative ecological study

Below are the results of functional trait, SLA, frond size, and stomata density correlation, with environmental factors, canopy cover, elevation, and substrate. Table 3 summarizes the averaged functional trait and environmental factor correlation data for all ten fern species. Table 4 summarizes linear regression statistical analysis results (residual standard error, R^2, F-value, P-value, and slope and Y—intercept of the best fit regression line) from combined and averaged fern data (Appendix C lists the statistical analysis results for all ten fern species).

Environmental Factors. Variation in measured canopy cover for all ferns ranged over 18.40% cover. *M. grossum* was able to establish in the most open canopy, 72.28% cover, while *A. trapeziforme* was able to establish in the most covered canopy, 90.67% cover. The average canopy cover measured was 85.96% cover.

Elevation measurements ranged over 72.88 m for all ferns. *M. grossum* was able to establish at the lowest average elevation, 143.13 m, while *P. nudum* was able to establish at the highest average elevation, 216.00 m. The average elevation overall was 180.88 m.

Ferns establish on one, two, or three substrate types, terrestrial, epiphyte or lithophtye. On average, ferns surveyed established on 2.1 substrates. The following ferns established on only one substrate type: *A. trapeziforme* (terrestrial), *A. Opulentum* (terrestrial), *A. evecta* (terrestrial), and *P. nudum* (epiphyte). *C. bipunctatum* was the only fern to establish on two substrate types (epiphyte and lithophyte), and the remaining five ferns (*B. lonchophora, D. solida, M. grossum, N. hirsutula,* and *T. wilkesianum,* established

TABLE 3. Averaged functional trait (SLA, FS, SD) and environmental factor (CC and E) data for each fern. Overall average, minimum, maximum, and range value for each trait are included. Table Key: E=elevation; CC=Canopy cover; SLA=specific Leaf Area; FS=Frond size; SD=Stomata Density; S=Substrate number occupied; A=Average fern data overall; m=Minimum; M=Maximum; R=Range.

	SD (μm^2)	SLA (cm^2/g)	FS (mm^2)	E (m)	CC (%)	S
Ad	12.10	2.63	2.48	196.81	90.67	1
Am	12.39	2.88	2.92	185.19	86.03	1
An	9.11	2.28	3.84	191.50	90.38	1
Bo	5.23	2.59	3.36	184.44	90.32	3
Cr	n/a	2.19	1.88	180.38	86.76	2
Da	26.07	2.40	3.35	164.00	82.04	3
Mi	7.55	2.41	3.10	143.13	72.28	3
Ne	12.18	2.56	2.44	163.94	88.04	3
Ps	n/a	2.33	1.50	216.00	84.37	1
Te	7.93	2.69	3.06	183.38	88.67	3
A	11.57	2.50	2.79	180.88	85.96	2.1
m	Bo 5.23	Cr 2.19	Ps 1.50	Mi 143.13	Mi 72.28	1
M	Da 26.07	Am 2.88	An 3.84	Ps 216.00	Ad 90.67	3
R	20.84	0.69	2.34	72.88	18.40	2

Notes: *Ad=*A. trapeziforme;* Am=*A. Opulentum;* An=*A. evecta;* Bo=*B. lonchophora;* Cr=*C. bipunctatum;* Da=*D. solida;* Mi=*M. grossum;* Ne=*N. hirsutula;* Ps=*P. nudum;* Te=*T. wilkesianum.*

on all three substrate types (terrestrial, epiphyte, and lithophyte).

Specific leaf area. As listed in Table 3, SLA differed between each fern species over a range of 20.84 cm²/g. *C. bipunctatum* had the smallest average SLA value, 2.19 cm²/g, and *A. Opulentum* had the largest average SLA, 2.88 cm²/g. The average SLA value overall was 2.50 cm²/g.

SLA was not significantly linearly correlated with elevation or canopy cover in any of the ten selected ferns. Table 1 in Appendix C lists the linear regression statistical analysis results (residual standard error, R², F-value, P-value, and slope and Y—intercept of the best fit regression line) between the correlation of SLA and environmental factors (elevation and canopy cover). Seven ferns (*A. opulentum, N. hirsutula, T. wilkesianum, A. trapeziforme, A. evecta, P. nudum,* and *M. grossum)* showed a positive,

TABLE 4. Combined and averaged fern regression values and best-fit line slope and intercept of all functional traits (SLA, FS, SD) and environmental variables (CC and E). Table Key: E=elevation; CC=Canopy cover; SLA=specific Leaf Area; FS=Frond size; SD=Stomata Density; RSE=Residual Standard Error on 8 degrees of freedom (6 degrees of freedom for SD); R²=Multiple R-Squared; b=Y-Intercept; m=Slope; F=F-statistic on 1 and 8 degrees of freedom (1 and 6 degrees of freedom for SD); p=P-Value. All values computed in R statistic software (R Core Team 2013, RStudio Team 2015).

Trait	RSE	R^2	b	m	F	p
SLA vs E	0.224	0.00	2.488	0.000	0.000	0.990
SLA vs CC	0.219	0.05	1.773	0.008	0.413	0.539
FS vs E	0.699	0.15	5.319	-0.014	1.449	0.263
FS vs CC	0.760	0.00	2.616	0.002	0.002	0.965
SD vs E	6.800	0.03	23.266	-0.066	0.214	0.660
SD vs CC	6.825	0.03	26.087	-0.169	0.168	0.696

but insignificant, correlation between SLA and elevation. Three ferns (*D. solida, C. bipunctatum,* and *B. lonchophora*) showed a negative, but insignificant correlation (Fig. 1; Appendix D). All fern species, except *A. evecta,* showed a positive, but insignificant, correlation between SLA and canopy cover (Fig 2; Appendix D). *A. evecta* showed a negative insignificant correlation between SLA and canopy cover.

Combined and averaged SLA and environmental factor (canopy cover and elevation) correlation data also showed no correlation between the traits (Fig. 5). The regression line between SLA and both canopy cover and elevation was approximately zero and horizontal, showing no correlation at all between the traits. Table 4 lists the statistical analysis results (residual standard error, R², F-value, P-value, and slope and Y—intercept of the best fit regression line) between the combined and averaged correlation data of SLA and environmental factors (elevation and canopy cover).

Average SLA for species establishing on multiple substrates did vary across substrate

FIG. 6 Combined and averaged functional trait and Substrate correlation. Stomata density (top), frond size (middle), and SLA (bottom). Key: *=Significant; SD=Stomata Density; SLA=Specific Leaf Area. All graphs made in R statistic software (R Core Team 2013, RStudio Team 2015).
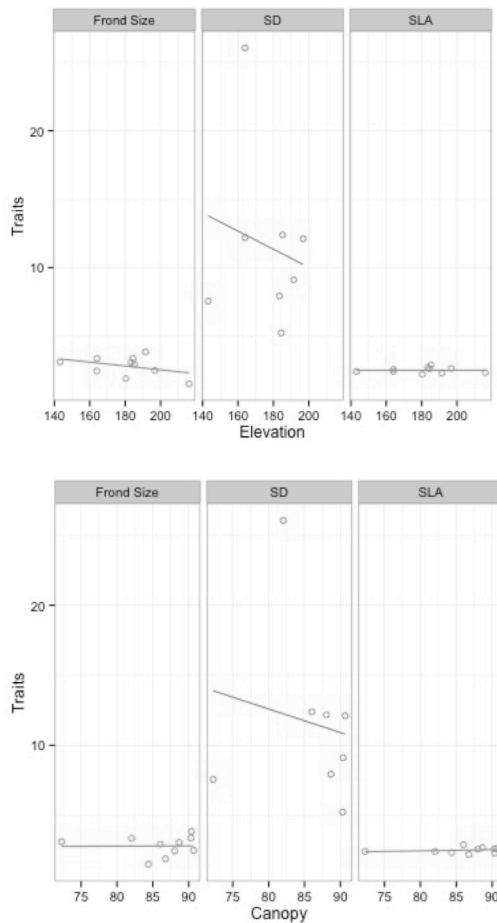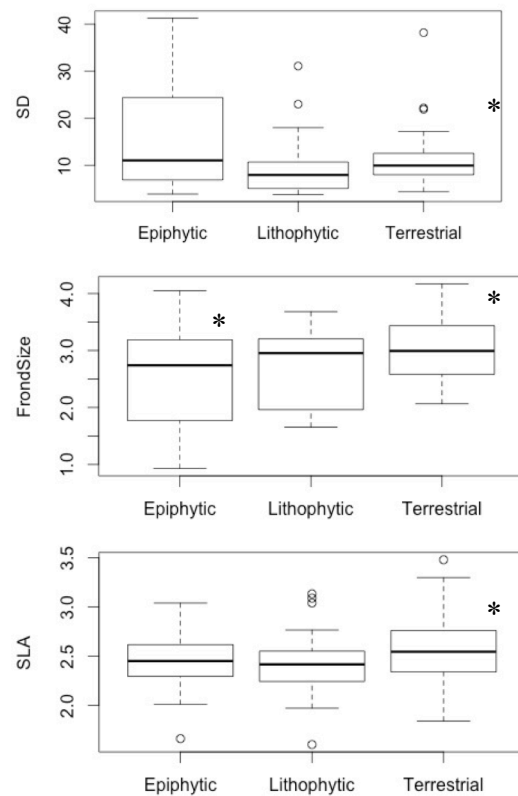
FIG. 5 Combined and averaged functional trait data and Elevation (top) and Canopy Cover (bottom) correlation. Key: SD=Stomata Density; SLA=Specific Leaf Area; Elevation=meters; Canopy Cover=% cover. All graphs made with ggplot in RStudio (Wickham 2009, R Core Team 2013, RStudio Team 2015).

types (Fig. 6). Terrestrial fern SLA was larger on average than SLA from epiphytes or lithophytes. Terrestrial SLA was significantly larger than epiphyte SLA ($p < 0.05$), but insignificantly larger than lithophytes ($p > 0.05$). Averaged ANOVA statistical analysis results from substrate and functional trait correlations are summarized in Table 5. Appendix C (Table 4) summarizes all ten fern species' ANOVA statistical analysis results.

Frond size. As described in Table 3, frond size differed between each fern species over a range of 2.34 mm^2. *P. nudum* had the smallest average frond size, 1.50 mm^2, and *A. evecta* had the largest average frond size, 3.84 mm^2. The average frond size overall was 2.79 mm^2.

Frond size was not significantly correlated with elevation or canopy cover in any of the ten selected ferns. Table 2 in Appendix C lists the statistical analysis results (residual standard error, R^2, F-value, P-value, and slope and Y—intercept of the best fit regression line) between the correlation of frond size and environmental factors (elevation and canopy cover). Although not significant, in five of the fern species (*A. evecta, T. wilkesianum, A. trapeziforme, C. bipunctatum,* and *B. lonchophora*), a positive linear correlation was observed between frond size and elevation (Fig. 3; Appendix D). In the other five fern species (*A. opulentum, D. solida, P. nudum, N. hirsutula,* and *M. grossum*), a negative, but insignificant, linear correlation was observed between frond size and elevation (Fig. 3; Appendix D). In six ferns (*A. opulentum, D. solida, N. hirsutula, T. wilkesianum, A. trapeziforme,* and *C. bipunctatum)* an insignificant positive correlation was observed between frond size and canopy cover (Fig. 4; Appendix D). Three ferns (*A. evecta, B.*

lonchophora, and *P. nudum*) had a negative insignificant correlation, and one fern (*M. grossum)* had no correlation (b=0) between frond size and canopy cover (Fig. 4; Appendix D).

Combined and averaged frond size and environmental factor (canopy cover and elevation) correlation data also showed no correlation between the traits (Fig. 5). The regression line between frond size and both canopy cover and elevation was approximately zero and horizontal, showing no correlation at all between the traits. Table 4 lists the statistical analysis results (residual standard error, R^2, F-value, P-value, and slope and Y—intercept of the best fit regression line) between the combined and averaged correlation data of frond size and environmental factors (elevation and canopy cover).

Frond size for species establishing on multiple substrates did vary across substrate types (Fig. 6). Terrestrial frond size was larger on average than frond size of epiphytes or lithophytes. Terrestrial fern frond sizes were significantly larger than both epiphyte and lithophyte fern frond sizes (p<0.05). Averaged ANOVA statistical analysis results from substrate and functional trait correlations are summarized in Table 5. Appendix C (Table 4) summarizes all ten fern species' ANOVA statistical analysis results.

Stomata density. As described in Table 3, stomata density differed between each fern species over a range of 20.84 μm^2. *B.*

TABLE 5. Averaged fern ANOVA values for all functional traits (SLA, frond size, Stomata density) and Substrate. Table Key: S=Substrate; SLA=specific Leaf Area; FS=Frond size; SD=Stomata Density; RSE=Residual Standard Error on 156 degrees of freedom (124 degrees of freedom for SD); SS=Sum of Squares; MS=Mean square; F=F-statistic on 3 and 156 degrees of freedom (3 and 124 degrees of freedom for SD); p=P-value. All values computed in R statistic software (R Core Team 2013, RStudio Team 2015).

Trait	RSE	SS	MS	F	p
SLA vs S	0.29	0.92	0.31	3.71	0.01
SD vs S	6.59	551	186.8	4.30	0.006
FS vs S	0.68	9.54	3.18	6.89	0.0002

lonchophora had the smallest average stomata density, 5.23 μm^2, and *D. solida* had the largest average stomata density, 26.07 μm^2. The average stomata density overall was 11.57 μm^2.

Stomata density was not significantly correlated with elevation or canopy cover in any of the ten selected ferns. Table 3 in Appendix C lists the statistical analysis results (residual standard error, R^2, F-value, P-value, and slope and Y—intercept of the best fit regression line) between the correlation of stomata density and environmental factors (elevation and canopy cover). Although not significant, in five of the fern species (*A. Opulentum, B. lonchophora, D. solida, M. grossum,* and *T. wilkesianum*), a positive linear correlation was observed between stomata density and elevation (Fig. 5; Appendix D). In the other three fern species (*A. trapeziforme, A. evecta,* and *N. hirsutula*), a negative, but insignificant, linear correlation was observed between stomata density and elevation (Fig. 5; Appendix D). In five ferns (*D. solida, T. wilkesianum, B. lonchophora, A. trapeziforme,* and *A. evecta)* an insignificant positive correlation was observed between stomata density and canopy cover (Fig. 6; Appendix D). Three ferns (*A. Opulentum, M. grossum,* and *N. hirsutula*) had an insignificant, negative correlation between stomata density and canopy cover (Fig. 6; Appendix D).

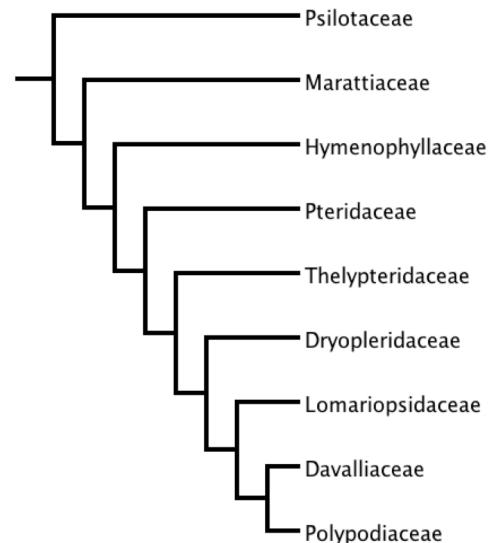Combined and averaged stomata density

FIG. 7 Fern phylogeny used for study. Terminal taxa listed as families. Pruned and adapted from Rothfels *et al.* 2015. Tree adapted in Mesquite (W. Maddison and D. Maddison 1997-2015).

and environmental factor (canopy cover and elevation) correlation data also showed no correlation between the traits (Fig. 5). The regression line between stomata density and both canopy cover and elevation was negative, but insignificant, showing no correlation at all between the traits. Table 4 lists the statistical analysis results (residual standard error, R^2, F-value, P-value, and slope and Y—intercept of the best fit regression line) between the combined and averaged correlation data of stomata density and environmental factors (elevation and canopy cover).

Stomata density for species establishing on multiple substrates did vary across substrate types (Fig. 6). Terrestrial fern stomata densities were larger on average than stomata density from epiphytes or lithophytes. Terrestrial stomata density was significantly larger than epiphyte stomata density ($p<0.05$), but insignificantly larger than stomata density of lithophytes ($p>0.05$). Epiphyte stomata densities were also significantly larger than lithophyte stomata density ($P<0.05$). Averaged ANOVA statistical analysis results from substrate and functional trait correlations are summarized in Table 5. Appendix C (Table 4) summarizes all ten fern species' ANOVA statistical analysis results.

Statistical analysis

TABLE 6.   Averaged fern phylogenetic correlation analysis. All regression lines run through the origin. Table Key: SLA=Specific Leaf Area; FS=Frond Size; SD=Stomata Density; m=Slope; PPM= PPM=Pearson Product-Moment Correlation Coefficient; F=F-Value; R^2=Regression Coefficient; p=P-Value; df=Degrees of Freedom.

Traits	m	PPM	F	R^2	p	df
SLA vs E	0.005	0.527	2.70	0.28	0.07	7
SLA vs CC	0.008	0.285	0.62	0.08	0.23	7
FS vs E	-0.005	-0.141	0.14	0.02	0.36	7
FS vs CC	-0.005	-0.049	0.02	0.00	0.45	7
SD vs E	0.143	0.308	0.52	0.10	0.25	5
SD vs CC	0.441	0.372	0.80	0.14	0.21	5

All data was log-transformed in order to meet the requirements of the Shapiro-Wik normality test ($p>0.05$). All fern species data, as well as combined and averaged fern data, fail to reject the null hypothesis that functional traits (SLA, frond size, and stomata density) are linearly correlated with environmental factors (elevation and canopy cover) (slope (m)=0). Functional trait correlation with substrate preference is significant, and does reject null the hypothesis that all functional trait data is the same across all substrate types.

Power analysis conducted for linear regression indicates that if more samples were used a significant correlation would be found between functional traits (SLA, frond size, and stomata density) and environmental factors (canopy cover and elevation). In order to determine a significant linear correlation between fern functional traits and environmental factors, an average of 390 samples would need to be tested for each fern species in the ecological study, and an average of 550 samples would need to be tested overall for combined fern data.
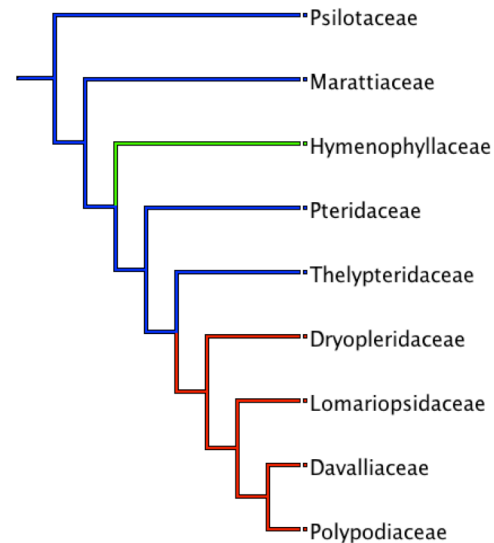
Phylogenetic analysis

FIG. 8 Substrate preference character state mapped on the fern phylogeny used for study. Terminal taxa listed as families. Key: Blue=1 substrate; Green=2 substrates; Red=3 substrates. Tree was pruned and adapted from Rothfels et al. 2015. Character state mapped in Mesquite (W. Maddison and D. Maddison 1997-2015).

The fern phylogeny (adopted from Rothfels *et al.* 2015) was cut down (pruned) to include only the ten selected species for this study (Fig. 7). Appendix E includes the fern phylogeny used in this study with branch lengths.

Substrate preference was mapped on a tree in the phylogeny software, Mesquite version 3.04 (W. Maddison and D. Maddison 1997-2015). Later diverging species show a generalist type of behavior in which they prefer to live on many substrates (terrestrial, epiphyte, and lithophyte) (Fig. 8). All later diverging ferns, after the split between *A. opulentum* (Thelypteridaceae family) and the rest of the later ferns (Dryopleridaceae, Lomariopsidaceae, Davalliaceae, and Polypodiaceae families), do not show a preference for substrate (e.g. they live as terrestrial, epiphytic, and lithophytic plants).

Most early fern species are specialists, meaning they prefer one specific substrate, however, the filmy fern (*C. bipunctatum*) preferred to live on two substrates (epiphyte and lithophyte) (Fig. 8). Early fern families, specializing on one substrate include, Psilotaceae (epiphyte), Marattiaceae (terrestrial), Pteridaceae (terrestrial), and Thelypteridaceae (terrestrial) families.

Fern functional traits, SLA, frond size, and stomata density, were not significantly correlated with environmental factors, canopy cover and elevation. Fig. 9 and Fig. 10 show all functional trait, SLA, frond size, and stomata density, correlation with canopy cover and elevation respectively. SLA and stomata density correlation, with both canopy cover and elevation, yielded a positive but insignificant slope, while, frond size correlation, with both canopy cover and
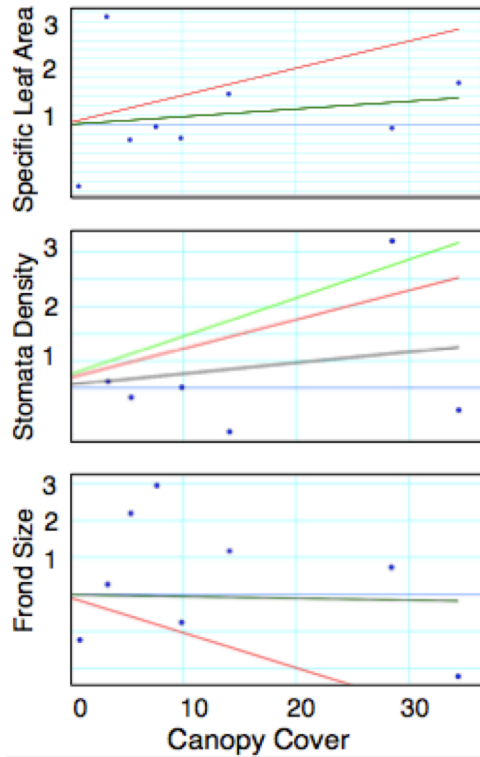

FIG. 9 Fern functional traits, Specific Leaf Area (top), Stomata Density (middle), Frond Size (bottom) phylogenetic correlation with Canopy Cover (% cover). Key: Regression Lines: Black is ordinary least squares; Green is major axis; Red is reduced major axis. Trait correlation (Felsenstein 1985, Garland et al. 1992) analysis done in Mesquite (W. Maddison and D. Maddison 1997-2015) with PDAP package (Midford et al. 2005).
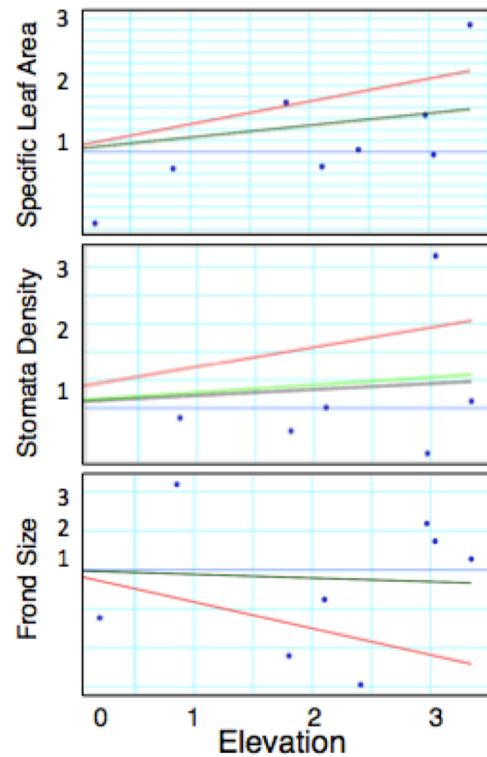

FIG. 10 Fern functional traits, Specific Leaf Area (top), Stomata Density (middle), Frond Size (bottom) phylogenetic correlation with Elevation (meters). Key: Regression Lines: Black is ordinary least squares; Green is major axis; Red is reduced major axis. Trait correlation (Felsenstein 1985, Garland et al. 1992) analysis done in Mesquite (W. Maddison and D. Maddison 1997-2015) with PDAP package (Midford et al. 2005).

elevation, yielded an insignificant but negative slope. Table 6 lists the statistical analysis results from the phylogenetic analysis correlation (Slope of regression line, Pearson Product-Moment Correlation Coefficient, F-Value, Regression Coefficient, P-Value, Degrees of Freedom).

DISCUSSION

Although this study sampled ferns from the major groups of fern lineages on Moorea, sampling is still inadequate with respect to the rich diversity of fern species (Pryer *et al.* 2004). Of the ferns selected for this study some fern families have received more extensive research then others, especially the Hymenophyllaceae or filmy ferns (Hennequin *et al.* 2003, Nitta 2006). Other families, including the Psilotaceae, Marattiaceae, Pteridaceae, Thelypteridaceae, Dryopleridaceae, Lomariopsidaceae, Davalliaceae, and the Polypodiaceae fern families need further research. The fern group, Polypods (Appendix B), encompassing the most diverse fern lineage, is in particular need of additional research (Pryer *et al*. 2004).

Monilophytes are an ecologically and morphologically diverse group. The ferns of Moorea represent this diversity as a large portion of the vascular plant species found in the interior forests of the island are ferns (Nitta 2006). Moorean ferns occur from low (0m) to high (above 500m) elevations as epiphytes, lithophytes, and terrestrial plants and exhibit a wide variety of morphological characteristics. The characteristics of the different ferns have evolved to match the niche occupied by each species, which explains why closely related ferns may differ in frond size, SLA, stomata density, and other functional traits (Karst and Lechowicz 2007). Patterns of functional trait diversity within closely related species arise as a result of environmental variations, such as elevation and canopy cover preference. By mapping functional traits onto a phylogenetic tree, larger evolutionary patterns of divergence events become evident.

In this study although it was hypothesized that a correlation would exist, I found no significant linear correlations between fern functional traits, SLA, frond size, and SD, and environmental factors, elevation and canopy cover, in the ecological or phylogenetic components. As previous studies (Ackerly and Conoghue 1998, Karst and Lechowicz 2007, Wright *et al.* 2007) suggest that a correlation between the functional traits and environmental characteristics exist, the lack of linear correlation in my study may have resulted from limitations with the experimental procedure.

This study was limited by factors, including time, labor, and accessibility. The time and labor constraints limited the number of fern lineages selected and the individual sample size for each species. As demonstrated by the statistical power analysis run on the data, a larger sample size may have resulted in a significant correlation observed in all species. The inaccessibility of many fern habitats on the island also severely limited this study. The island of Moorea is a high volcanic island with extremely steep mountain slopes, which makes hiking to high elevation forests (over 500m) difficult and impossible in many cases. Due to inaccessibility to higher elevations ferns sample were mainly collected in low to mid elevations (100m-300m). Further studies should include a larger variation in sample site selection and larger fern lineage and individual collections.

Although insignificant some trait correlations, positive and negative, were observed in each sample. It was hypothesized that ecologically important fern functional traits are positively correlated with elevation, and negatively correlated with canopy cover, due to water availability and conservation mechanisms in the plant. I found, however, that some ferns exhibited positive, and some ferns exhibited negative, correlation trends for both elevation and canopy cover. As this result is different than hypothesized, some possible explanations arise; first closely related fern species have different functional traits related to water conservation, responding differently to environmental factors. This phenotypic functional trait variation may be due to variation in evolutionary divergent events (Ackerly 2003) or life history characteristics of the fern species (Ackerly *et al*. 2000). Second, unpredicted results may have arisen due to some of the limitations explained above. The results of the study may also be skewed due to inadequate sampling. R^2 values measured from the linear regression were extremely low, meaning the best-fit line did not actually fit the data. With a larger sample group, originally observed negative correlations may be positive correlations. As described above future studies should include a larger sample group over a wider range of environmental variation to determine most accurate results.

As hypothesized fern functional traits, SLA, frond size, and stomata density, did vary with substrate preference. Larger functional traits were found on ferns growing as terrestrial plants. This result was expected as terrestrial plants grow in more nutrient rich soil than epiphytes or lithophytes (Vasco *et al.* 2013). This excess of nutrients allows the plant to allocate more resources to growing and niche expansion, without having to focus on pure survival. With more plant resources functional traits have the opportunity to grow larger and quicker, allowing SLA, frond size, and stomata density to increase. Future studies could examine the primary nutrient content of soil found under terrestrial plants, epiphytes, and lithophytes to determine factors that may contribute to the larger functional traits in terrestrial plants trend observed.

Using the best estimate of fern phylogeny (Rothfels *et al.* 2015), I was able to provide several insights on morphological evolution within the ferns of Moorea. The sampling and measurement of important phenotypic functional traits allowed me to reconstruct the evolution of critical character states and attempt to determine and understand their implications for the biology and systematics of the ferns. It was predicted that early diverging ferns would be generalists, occupying many substrates, while later diverging ferns would be specialists, occupying only one substrate. In this study, however, I observed the opposite. A possible explanation is that as fern species coexist in similar habitats the ability to occupy multiple substrates may be advantageous for survival, as generalist behavior allows for a wider range of niches to be utilized (Ackerly 2003). Future studies could explore whether substrate preference evolution in ferns evolves once or multiple times in a species.

This study was intended to provide a family and species level comparative ecological evaluation about the ferns of Moorea. Intending to better understand the evolutionary history of the lineages of ferns on the Island. By studying fern systematics we can answer questions about divergent events that may have led to the vast diversity of fern species on Moorea.

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APPENDIX A

    Specifically I tested the following hypotheses: (1) Ecologically important fern leaf function traits are positively correlated with elevation and negatively correlated with canopy cover. As the fern has more availability to acquire water at higher elevations due to increased rainfall SLA, stomata density, and leaf size will increase. Conversely, as the forest canopy cover increases and the fern has less available sunlight and water the plant will allocate more resources to simply surviving and SLA, stomata density, and leaf size will all decrease. (2) In ferns that exhibit generalist substrate preference, functional traits will be correlated with habitat type. SLA, stomata density, and leaf size will be highest on ferns found in terrestrial soils, intermediate on epiphytic soils on trees, and lowest as lithophytes on rocks. (3) All interspecific correlation patterns observed will reflect the trait-pair evolutionary divergence patterns of historical phylogenetic data.
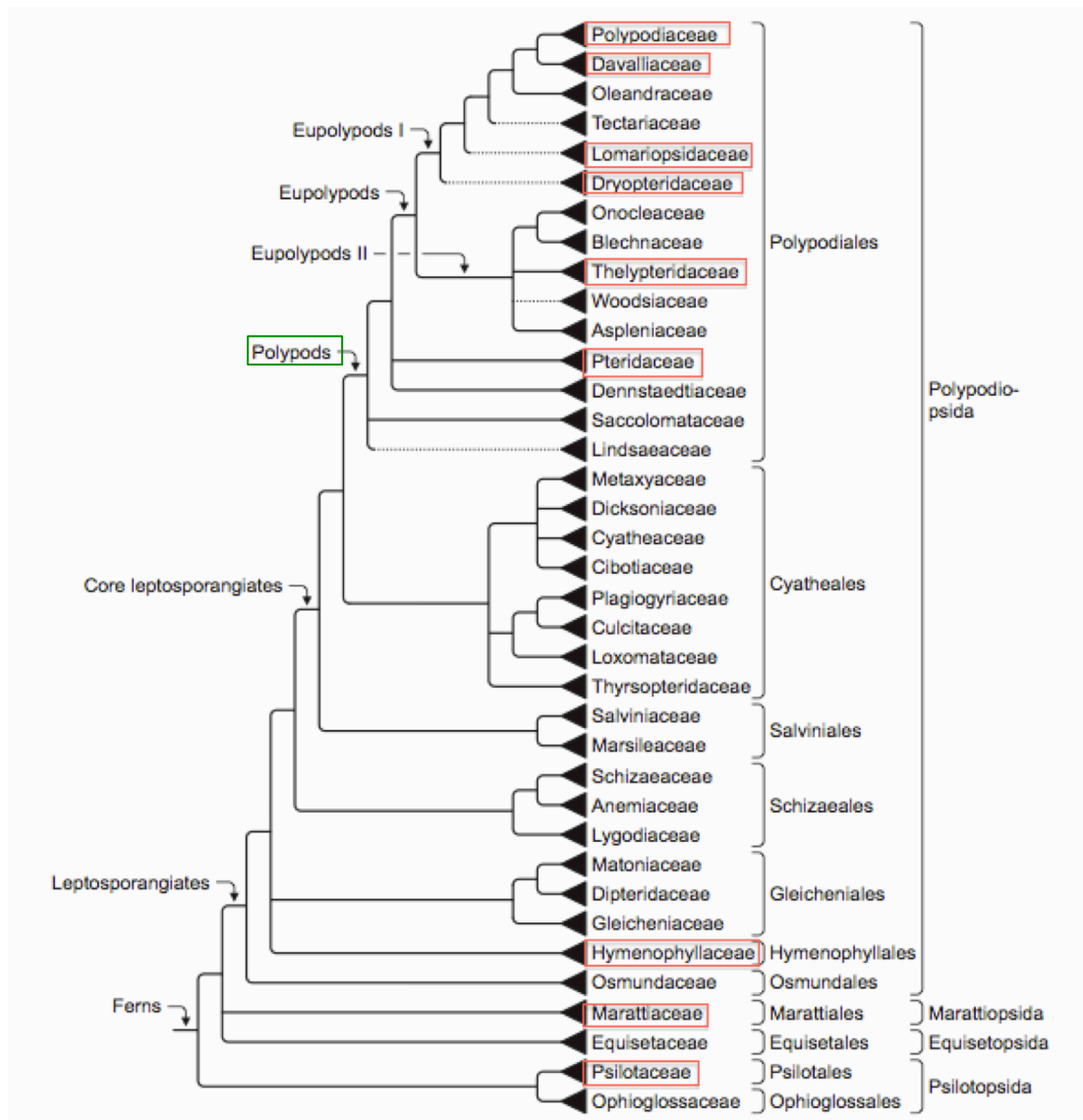
FIG 1. Accepted phylogeny depicting fern relationships upon which my study tree is based. Terminal taxa are family names; nine surveyed fern families are boxed in red. Solid lines have received bootstrap support of over 70, while dotted lines received less support. Family, order, and class names are listed on the right, and common names are listed on the left. The Polypods group, the largest of all fern groups, is boxed in green on the left. (Adapted from Smith et al. 2006).

TABLE 1. SLA and environmental variables (canopy cover and elevation) regression values and best-fit line slope and intercept from all ten surveyed ferns. Table Key: SLA=Specific Leaf Area; RSE=Residual Standard Error on 14 degrees of freedom; R^2=Multiple R-Squared; b=Y-Intercept; m=Slope; F=F-statistic on 1 and 14 degrees of freedom; p=P-Value. All values computed in R statistic software (R Core Team 2013, RStudio Team 2015).

Correlation	Fern Species	RSE	R^2	b	m	F	p
SLA vs Canopy cover	A. trapeziforme	0.1694	0.2082	0.70057	0.02124	3.681	0.07567
SLA vs Canopy cover	A. Opulentum	0.2372	0.3845	1.52015	0.01586	3.745	0.0104
SLA vs Canopy cover	A. evecta	0.1309	0.1628	3.42088	-0.01263	2.722	0.1212
SLA vs Canopy cover	B. lonchophora	0.2235	0.03462	1.883999	0.007832	0.5021	0.4902
SLA vs Canopy cover	C. bipunctatum	0.2364	0.01773	1.66859	0.00604	0.2528	0.623
SLA vs Canopy cover	D. solida	0.1423	0.1155	1.948356	0.005563	1.828	0.1979
SLA vs Canopy cover	M. grossum	0.1362	0.2584	1.883999	0.007832	4.379	0.04437
SLA vs Canopy cover	N. hirsutula	0.304	0.1075	0.57813	0.02254	1.686	0.2151
SLA vs Canopy cover	P. nudum	0.2614	0.1402	1.34308	0.01165	2.282	0.1531
SLA vs Canopy cover	T. wilkesianum	0.1601	0.2813	0.62314	0.02328	4.481	0.03454
SLA vs Elevation	A. trapeziforme	0.1903	0.000557	2.5967068	0.0001516	0.007802	0.9309
SLA vs Elevation	A. Opulentum	0.2993	0.01987	2.681911	0.001093	0.2839	0.6025
SLA vs Elevation	A. evecta	0.142	0.01472	2.1806812	0.0005169	0.2092	0.6544
SLA vs Elevation	B. lonchophora	0.2274	3.698e-05	2.601e+00	-5.299e-05	0.0005177	0.9822
SLA vs Elevation	C. bipunctatum	0.238	0.004071	2.2999351	-0.0005952	0.05723	0.8144
SLA vs Elevation	D. solida	0.1472	0.05327	2.4708022	-0.0004029	0.7878	0.3898
SLA vs Elevation	M. grossum	0.1563	0.1563	1.883999	0.007832	0.3439	0.5669
SLA vs Elevation	N. hirsutula	0.2545	0.3745	2.253699	0.001885	4.382	0.01176
SLA vs Elevation	P. nudum	0.2303	0.3329	1.574006	0.003483	3.986	0.01929
SLA vs Elevation	T. wilkesianum	0.1692	0.1965	2.152251	0.002919	3.424	0.08546

TABLE 2: Frond size and environmental variables (canopy cover and elevation) regression values and best-fit line slope and intercept from all ten surveyed ferns. Table Key: FS=Frond Size; RSE=Residual Standard Error on 14 degrees of freedom; R^2=Multiple R-Squared; b=Y-Intercept; m=Slope; F=F-statistic on 1 and 14 degrees of freedom; p=P-Value. All values computed in R statistic software (R Core Team 2013, RStudio Team 2015).

Functional Trait	Fern Species	RSE	R^2	b	m	F	p
FS vs Canopy cover	A. trapeziforme	0.1545	0.03333	1.841332	0.007013	0.4826	0.4986
FS vs Canopy cover	A. Opulentum	0.2341	0.0264	2.63810	0.00326	0.3796	0.5477
FS vs Canopy cover	A. evecta	0.1712	0.1163	5.0646	-0.0136	1.843	0.1961
FS vs Canopy cover	B. lonchophora	0.21	0.003009	3.556582	-0.002135	0.04225	0.8401
FS vs Canopy cover	C. bipunctatum	0.1417	0.01109	1.636372	0.002854	0.157	0.6979
FS vs Canopy cover	D. solida	0.3547	0.006651	3.09124	0.00314	0.09373	0.764
FS vs Canopy cover	M. grossum	0.1218	0.001936	3.0819119	0.0002419	0.02716	0.8715
FS vs Canopy cover	N. hirsutula	0.1871	0.02826	1.844550	0.006817	0.4072	0.5337
FS vs Canopy cover	P. nudum	0.3892	0.01427	1.935254	-0.005171	0.2027	0.6594
FS vs Canopy cover	T. wilkesianum	0.1592	0.1588	1.63017	0.01608	2.643	0.1263
FS vs Elevation	A. trapeziforme	0.1456	0.1407	2.085762	0.001989	2.293	0.1522
FS vs Elevation	A. Opulentum	0.2363	0.00797	3.0191541	-0.0005432	0.1125	0.7423
FS vs Elevation	A. evecta	0.1786	0.03843	3.631995	0.001064	0.5596	0.4668
FS vs Elevation	B. lonchophora	0.2087	0.01564	3.177919	0.001008	0.2224	0.6445
FS vs Elevation	C. bipunctatum	0.1391	0.04678	1.666529	0.001205	0.687	0.4211
FS vs Elevation	D. solida	0.3307	0.1363	3.597496	-0.001516	2.209	0.1594
FS vs Elevation	M. grossum	0.1	0.3263	3.174971	-0.000528	6.78	0.02082
FS vs Elevation	N. hirsutula	0.1735	0.1645	2.5655572	-0.0007369	2.756	0.1191
FS vs Elevation	P. nudum	0.387	0.02532	1.787508	-0.001336	0.3637	0.5561
FS vs Elevation	T. wilkesianum	0.1734	0.002292	3.0028139	0.0002898	0.03216	0.8602

TABLE 3. SD and environmental variables (canopy cover and elevation) regression values and best-fit line slope and intercept from all eight surveyed ferns (*P.nudum* and *C. bipunctatum* were excluded from the SD study). Table Key: SD=Stomata Density; RSE=Residual Standard Error on 12 degrees of freedom; R^2=Multiple R-Squared; b=Y-Intercept; m=Slope; F=F-statistic on 1 and 12 degrees of freedom; p=P-Value. All values computed in R statistic software (R Core Team 2013, RStudio Team 2015).

Functional Trait	Fern Species	RSE	R^2	b	m	F	p
SD vs Canopy cover	*A. trapeziforme*	3.239	0.1069	-12.7485	0.2740	1.676	0.2164
SD vs Canopy cover	*A. Opulentum*	2.515	0.04264	16.24941	-0.04489	0.6236	0.4429
SD vs Canopy cover	*A. evecta*	1.805	0.05316	0.64900	0.09357	0.786	0.3903
SD vs Canopy cover	*B. lonchophora*	1.059	0.04164	1.53708	0.04083	0.6082	0.4484
SD vs Canopy cover	*D. solida*	6.648	0.1167	4.6179	0.2615	1.849	0.1954
SD vs Canopy cover	*M. grossum*	2.55	0.008458	8.31768	-0.01062	0.1194	0.7348
SD vs Canopy cover	*N. hirsutula*	3.629	0.01021	19.11005	-0.07877	0.1445	0.7096
SD vs Canopy cover	*T. wilkesianum*	1.83	0.002407	6.0715	0.0209	0.03379	0.8568
SD vs Elevation	*A. trapeziforme*	3.091	0.1868	21.93867	-0.04999	3.216	0.09455
SD vs Elevation	*A. Opulentum*	2.529	0.03143	10.22333	0.01169	0.4543	0.5113
SD vs Elevation	*A. evecta*	1.814	0.0433	11.30760	-0.01150	0.6336	0.4393
SD vs Elevation	*B. lonchophora*	1.077	0.008453	4.522427	0.003809	0.1193	0.7349
SD vs Elevation	*D. solida*	6.912	0.04532	23.21912	0.01738	0.6646	0.4286
SD vs Elevation	*M. grossum*	2.049	0.3598	5.882873	0.011648	7.868	0.01404
SD vs Elevation	*N. hirsutula*	2.903	0.3668	15.642439	-0.021151	8.109	0.01291
SD vs Elevation	*T. wilkesianum*	1.707	0.1318	3.67032	0.02320	2.125	0.1669

TABLE 4. Averaged fern ANOVA values for all functional traits (SLA, FS, SD) and Substrate. Table Key: SLA=specific Leaf Area; FS=Frond size; SD=Stomata Density; RSE=Residual Standard Error on 13 degrees of freedom; SS=Sum of Squares; MS=Mean square; F=F-statistic on 2 and 13 degrees of freedom; p=pr (>F). All values computed in R statistic software (R Core Team 2013, RStudio Team 2015).

| Functional Trait	Fern Species	RSE	SS	MS	F	p
SLA vs Substrate	*A. trapeziforme*	n/a	n/a	n/a	n/a	n/a
SLA vs Substrate	*A. Opulentum*	n/a	n/a	n/a	n/a	n/a
SLA vs Substrate	*A. evecta*	n/a	n/a	n/a	n/a	n/a
SLA vs Substrate	*B. lonchophora*	0.2090175	0.1562	0.07812	1.788	0.206
SLA vs Substrate	*C. bipunctatum*	0.2356927	0.0186	0.01865	0.336	0.572
SLA vs Substrate	*D. solida*	0.1208935	0.1304	0.06521	4.462	0.0335
SLA vs Substrate	*M. grossum*	0.1310394	0.1270	0.06351	3.699	0.0535
SLA vs Substrate	*N. hirsutula*	0.2992409	0.2855	0.14274	1.594	0.24
SLA vs Substrate	*P. nudum*	n/a	n/a	n/a	n/a	n/a
SLA vs Substrate	*T. wilkesianum*	0.1953983	0.0027	0.00134	0.035	0.966
FS vs Substrate	*A. trapeziforme*	n/a	n/a	n/a	n/a	n/a
FS vs Substrate	*A. Opulentum*	n/a	n/a	n/a	n/a	n/a
FS vs Substrate	*A. evecta*	n/a	n/a	n/a	n/a	n/a
FS vs Substrate	*B. lonchophora*	0.2147216	0.0199	0.00993	0.215	0.809
FS vs Substrate	*C. bipunctatum*	0.1402324	0.00893	0.008929	0.454	0.511
FS vs Substrate	*D. solida*	0.3590533	0.0971	0.04855	0.377	0.693
FS vs Substrate	*M. grossum*	0.1146032	0.03725	0.01862	1.418	0.277
FS vs Substrate	*N. hirsutula*	0.1684239	0.1355	0.06773	2.388	0.131
FS vs Substrate	*P. nudum*	n/a	n/a	n/a	n/a	n/a
FS vs Substrate	*T. wilkesianum*	0.1701157	0.0455	0.02276	0.786	0.476
SD vs Substrate	*A. trapeziforme*	n/a	n/a	n/a	n/a	n/a
SD vs Substrate	*A. Opulentum*	n/a	n/a	n/a	n/a	n/a
SD vs Substrate	*A. evecta*	n/a	n/a	n/a	n/a	n/a
SD vs Substrate	*B. lonchophora*	0.8220936	7.584	3.792	5.611	0.0175
SD vs Substrate	*C. bipunctatum*	n/a	n/a	n/a	n/a	n/a
SD vs Substrate	*D. solida*	6.432569	162.6	81.31	1.965	0.18
SD vs Substrate	*M. grossum*	2.041542	37.60	18.799	4.51	0.0325
SD vs Substrate	*N. hirsutula*	3.465154	30.2	15.10	1.257	0.317
SD vs Substrate	*P. nudum*	n/a	n/a	n/a	n/a	n/a
SD vs Substrate	*T. wilkesianum*	1.83236	3.36	1.681	0.501	0.617
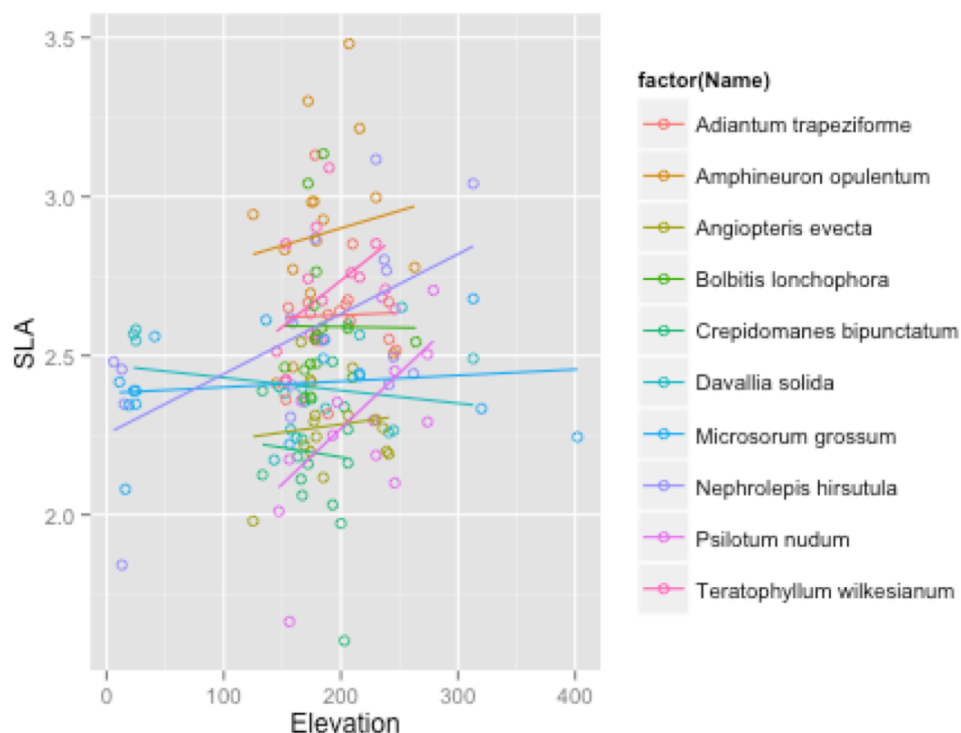
FIG. 1 SLA and Elevation. Figure Key: y-axis=SLA (cm^2/g); x-axis=Elevation (meters) Each color represents a different fern species; colored lines are the linear regression line for each species. All graphs made with ggplot in RStudio (Wickham 2009, R Core Team 2013, RStudio Team 2015).
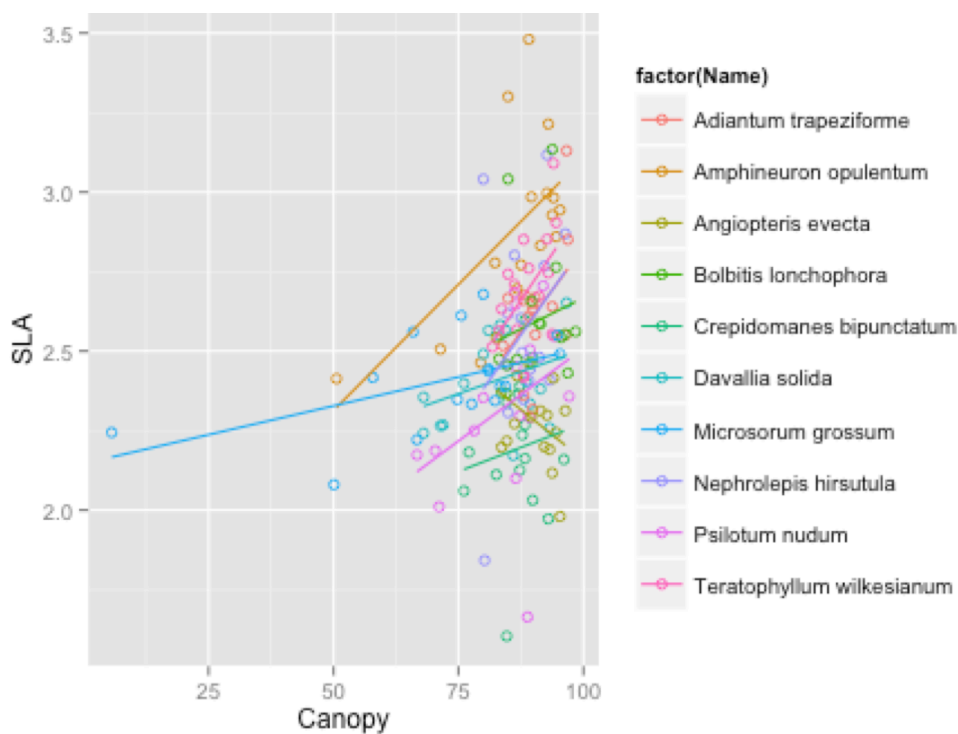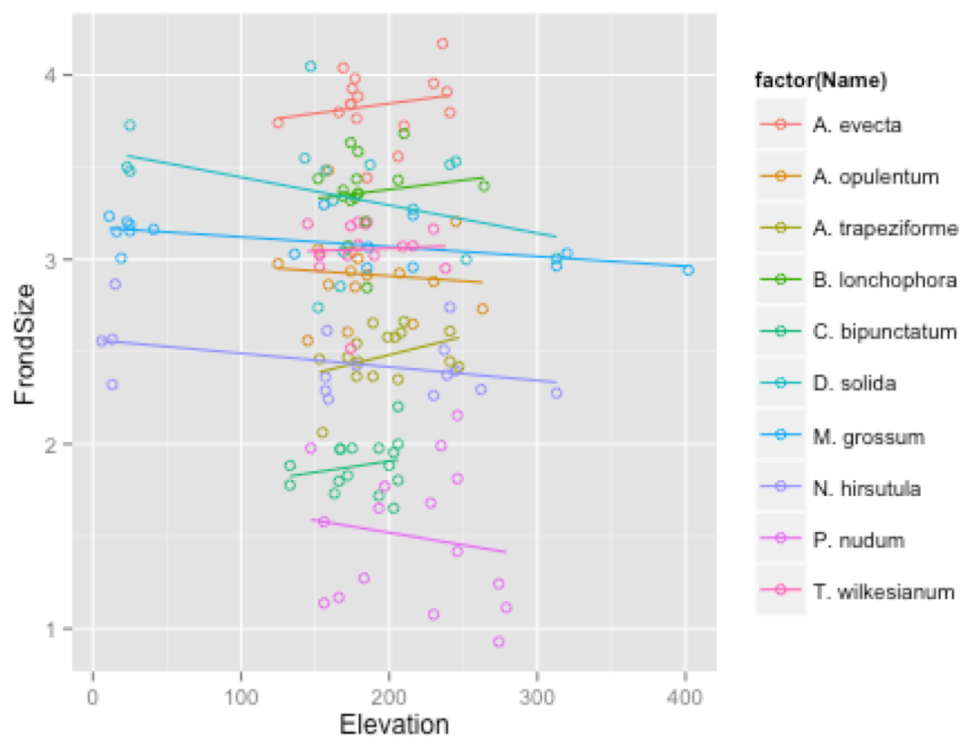

FIG. 2 SLA and Canopy Cover. Figure Key: y-axis=SLA (cm^2/g); x-axis=Canopy Cover (% cover). Each color represents a different fern species; colored lines are the linear regression line for each species. All graphs made with ggplot in RStudio (Wickham 2009, R Core Team 2013, RStudio Team 2015).
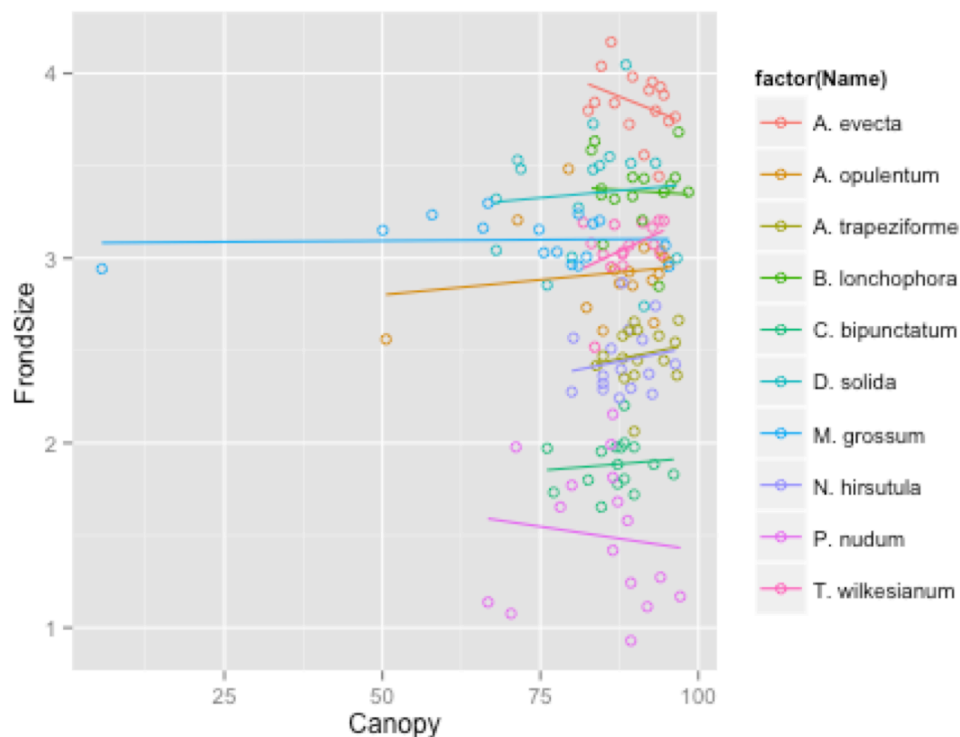
FIG. 3 Frond Size and Elevation. Figure Key: y-axis=Frond Size (mm^2); x-axis=Elevation (meters). Each color represents a different fern species; colored lines are the linear regression line for each species. All graphs made with ggplot in RStudio (Wickham 2009, R Core Team 2013, RStudio Team 2015).
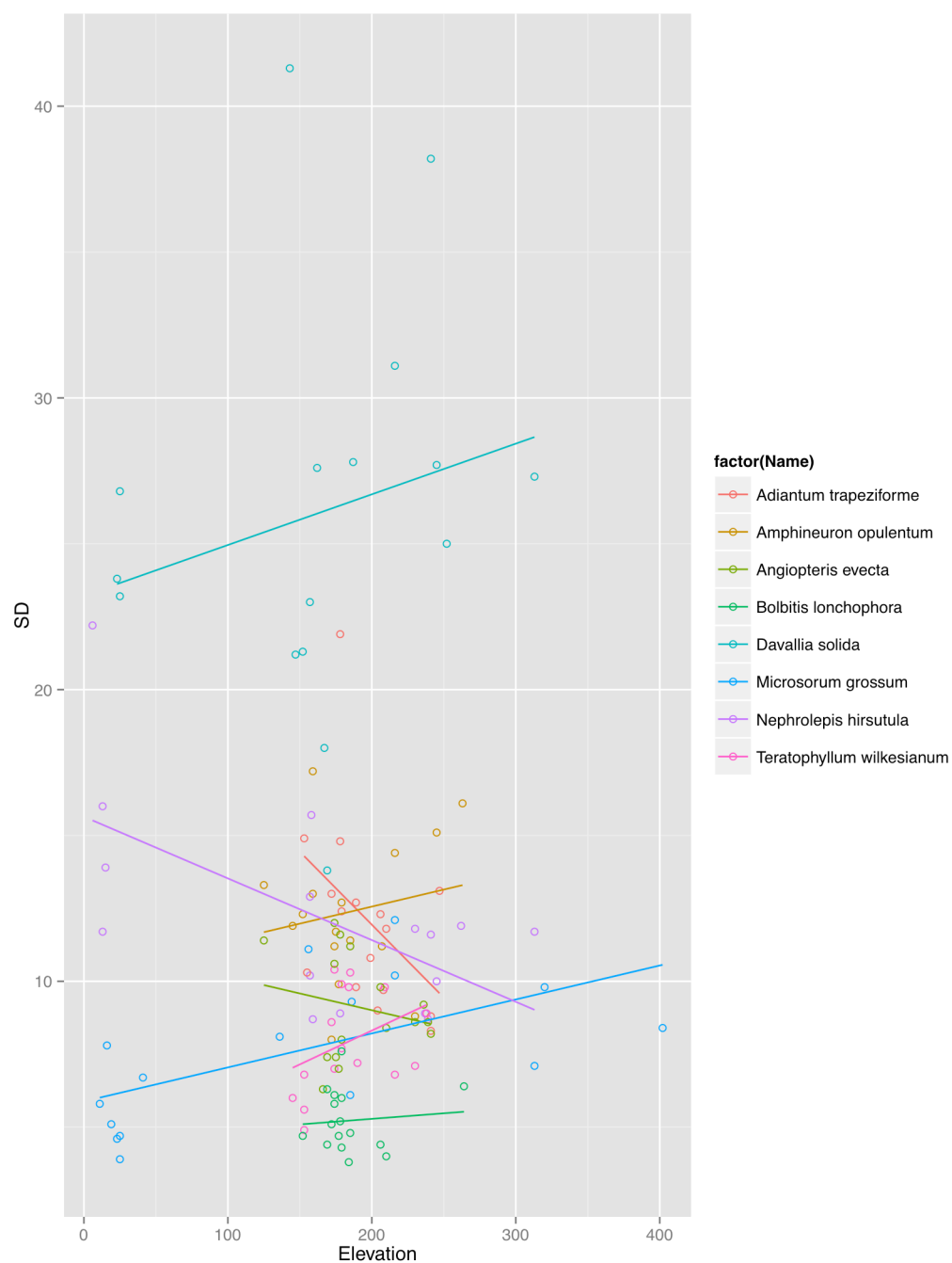

FIG. 4 Frond Size and Canopy Cover. Figure Key: y-axis=Frond Size (mm^2); x-axis=Canopy Cover (% cover). Each color represents a different fern species; colored lines are the linear regression line for each species. All graphs made with ggplot in RStudio (Wickham 2009, R Core Team 2013, RStudio Team 2015).

F IG . 5 Stomata Density and Elevation. Figure Key: y-axis=Stomata Density (μm^2); x-axis=Elevation (meters). Each color represents a different fern species; colored lines are the linear regression line for each species. All graphs made with ggplot in RStudio (Wickham 2009, R Core Team 2013, RStudio Team 2015).
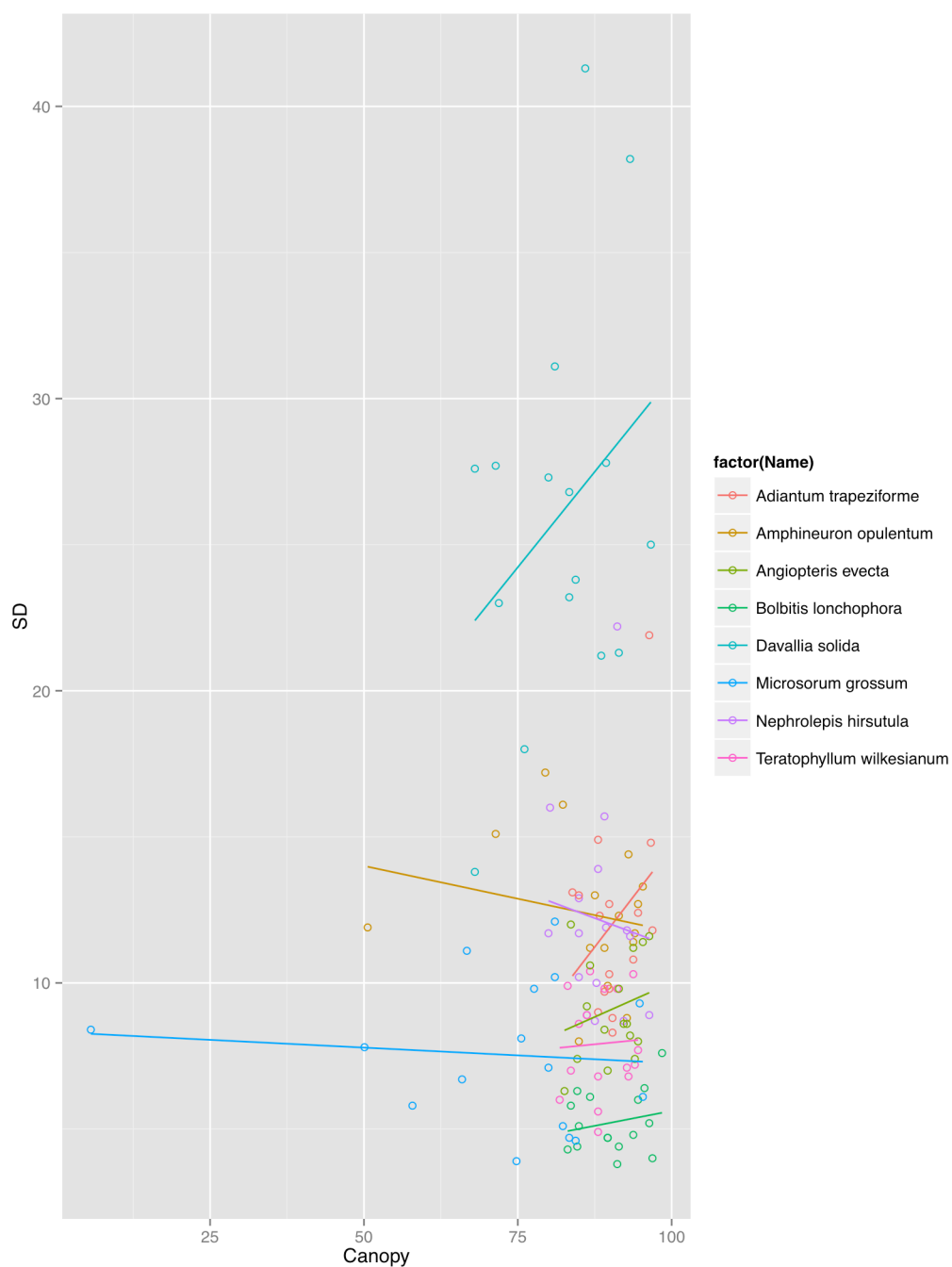
FIG. 6 Stomata Density and Canopy Cover. Figure Key: y-axis=Stomata Density (μm^2); x-axis=Canopy Cover (% cover). Each color represents a different fern species; colored lines are the linear regression line for each species. All graphs made with ggplot in RStudio (Wickham 2009, R Core Team 2013, RStudio Team 2015).
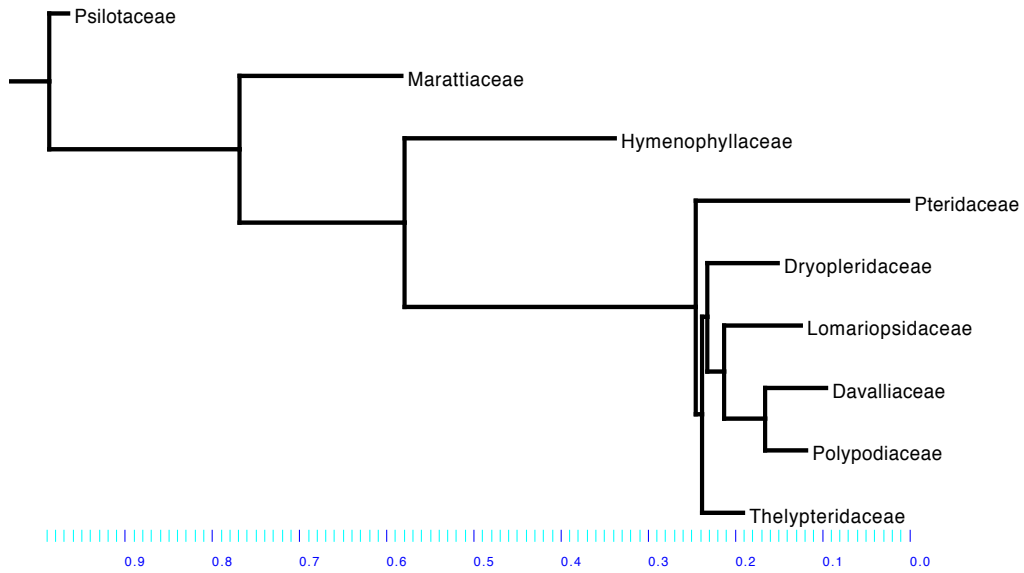
FIG. 1 Fern phylogeny used for study. Branch length scale included on bottom. Terminal taxa listed as families. Pruned and adapted from Rothfels et al. 2015. Tree adapted in Mesquite (W. Maddison and D. Maddison 1997-2015).