LEAF MORPHOGENESIS OF THE GIANT FERN (*ANGIOPTERIS EVECTA*) IN FRENCH POLYNESIA

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Abstract. Understanding the effects that environmental conditions have on the development of organisms is critical, given the rapid environmental changes currently occurring across the globe. The giant fern, *Angiopteris evecta*, was used as a model system for studying the interactions between ecology and development. To understand the factors affecting *A. evecta* leaf compoundness, the heteroblastic and heterophyllic influences on its leaf development were investigated. The heteroblastic series of *A. evecta* was diagrammed based off of field observations. Heterophyllic influences were examined by recording the number and type of variant pinnules, and comparing the environmental conditions at the sites at which they were found. The heteroblastic series revealed the transition from simple leaf forms to increasingly compound forms as ferns aged. The tripinnate variation, in which pinnules were divided into pinnulets, was found to significantly increase under conditions of high canopy cover, high elevation, and low *A. evecta* density. These findings imply compound leaves are more beneficial in sun-dappled environments, but that these benefits decrease when ferns are crowded at low elevations. A comparison of field specimens to herbaria vouchers revealed that variations are present throughout most of *A. evecta*'s range, and that this is not a localized phenomenon. This study found no instances in which leaves became less compound, implying that it is either highly unbeneficial or genetically impossible. Future research should expand upon this study by including a phylogenetic comparison of leaf variations across fern species.

Key words: Angiopteris evecta; *heteroblasty*; *heterophylly; compoundness; morphogenesis; Moorea, French Polynesia*

INTRODUCTION

The field of morphogenesis examines the developmental processes responsible for generating the form of an organism (Hall 1999). Leaves are of particular interest when studying morphogenesis because a variety of forms may be produced by the same shoot (Goliber *et al.*, 1999). Morphogenetic changes in leaf form can be classified as heteroblastic, if different forms are produced as the shoot develops without regard to the environment, or as heterophyllic, if switches between possible morphologies are environmentally induced (Zotz, Wilhelm, and Becker 2011). These concepts are not inherently isolated from each other: the environment can influence the progression of the heteroblastic series and shoot age can influence heterophyllic variations (Goliber 1999). However, distinguishing heterophyllic changes from heteroblastic changes is important for determining a plant's phenotypic plasticity (Zotz, Wilhelm, and Becker 2011). An improved understanding of the extent of phenotypic plasticity has significant implications for conservation planning.

Examining the degree to which leaves are compound is a useful vehicle for studying developmental variation because it is affected by both heteroblastic and heterophyllic factors. Some species exhibit heteroblastic changes in compoundness, such as *Acacia koa*, which has pinnately compound leaves as a juvenile and simple leaves as an adult (Poethig 2010). Other species display heterophyllic changes, such as *Neobeckia aquatica*, which varies in compoundness depending on the amount of light and water

present (Goliber 1999). Other studies have described the ecological conditions in which a compound leaf is advantageous over a simple leaf. The morphology of compound leaves purportedly facilitates gas exchange (Gurevitch and Schuepp, 1990), reduces herbivore damage (Brown and Lawton, 1991), promotes heat dissipation (Balding and Cunningham, 1976), enables rapid growth (Malhado *et al*. 2010) and supports foliar growth in environments with low light and high evaporation (Niinemets 1998).

A handful of studies have quantified the occurrence of pinnation in ferns. Klugel and Kessler (2007) suggested that high laminar thickness and low dissection are defensive strategies for ferns against extreme elevations and climates. Pryer and Hearn (2009) found that the final leaf form that Marsileaceous ferns achieve is dependent on the levels of nutrients present.

Angiopteris evecta, a Marattiaceae fern common in Moorea, French Polynesia (Murdock and Smith 2003), is a good model system for studying the morphogenesis of leaf development. The first leaves that emerge from its sporophytes are simple leaves, but as the shoot ages bipinnate leaves are formed (Bloomquist 1922; Farmer 1892). Heterophyllic influences on *A. evecta* have not been extensively studied, but the Eriophyidae mite *Trioza fletcheri* has been found to cause galls on *A. evecta* leaves in India (Balick, Furth and Cooper-Driver 1978; Bera, Patra and Ghorai 2003). Variations in *A. evecta* pinnules are present in populations in Moorea in varying quantity and degree, and have not been previously studied. One distinct type of variation, in which a pinnule splits into pinnulets and becomes tripinnate, increases the order of compoundness.

This study aimed to identify heteroblastic and heterophyllic influences on leaf development in *A. evecta*. I hypothesized that pinnule variations increasing the order of compoundness are heterophyllic and are triggered by abiotic conditions. Data were also collected in regards to a hypothesis that the pinnule variations are heterophyllic but are due to biotic factors, such as herbivory or parasitism. This study also expanded upon Farmer's (1892) work on the first four leaves of the sporophyte by detailing additional leaves in the heteroblastic series.

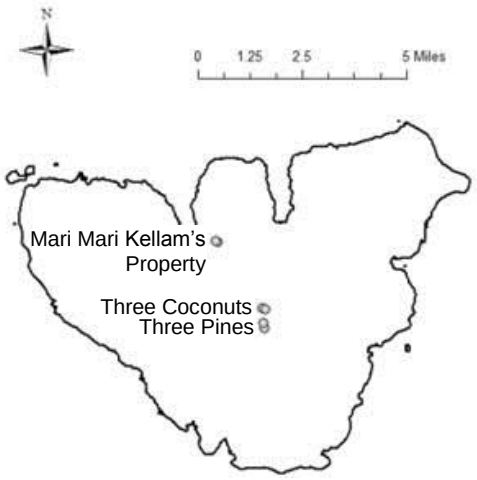

FIG. 1. Map of sites sampled in this study. Base map courtesy of the Geospatial Innovation Facility, UC Berkeley

METHODS

This study was conducted on the island of Moorea in French Polynesia (17.53° S, 149.83° W) during October and November of 2014.

Study organism

Angiopteris evecta is commonly known by the English names of giant fern, king's fern, mule's-foot fern, and oriental vessel fern, and by the Tahitian name of nahe. It is native to Polynesia, Melanesia, Micronesia, Australia, and New Guinea, but has invaded other tropical areas (NBII and ISSG 2010). It inhabits tropical areas with annual temperatures of 19-27 °C, annual precipitations of 1,054-5,447 mm, and elevations of 0-1,492 m, and is tolerant of sun, shade, slope, and mild disturbance (NBII and ISSG 2010).

TABLE 1. Environmental characteristics of each site sampled in the *Heterophylly study*.

Site	Area	Coordinates	Elevation (m)	Proximity to stream (m)	Percent cover	*A. evecta* density (per meter)	Soil N	Soil P	Soil K
1	Three Pines	-17°32.198, -149°49.714	127	122.0	74.0	0.067	very low	trace	very low
2	Three Pines	-17°32.217, -149°49.646	179	10.0	83.1	0.154	trace	trace	very low
3	Three Coconuts	-17°32.614 , -149°49.681	274	23.3	76.6	0.291	trace	very low	medium low
4	Three Coconuts	-17°32.489, -149°49.702	310	253.3	78.9	0.024	trace	trace	very low
5	Mari Mari's	-17°30.801, -149°50.691	66	24.1	88.8	0.003	low	very low	medium low
6	Mari Mari's	-17°30.781, -149°50.706	100	3.5	84.1	0.034	very low	medium low	low

Like other pteridophytes, *A. evecta* undergoes alternation of generations from haploid gametophyte to diploid sporophyte (Haupt 1940). *Angiopteris evecta*'s exact lifespan is unknown but considered to be well over 100 years (NBII and ISSG 2010). *A. evecta* can reproduce vegetatively via auricles located at the base of its fronds or sexually via spores (Haupt 1940; Matthes *et al.* 2011).

Study area

Three sampling areas were selected along an elevational gradient (Fig. 1). The Three Coconuts trail was chosen as the high elevation area, the Three Pines trail as the mid-elevation area, and Mari Mari Kellam's private property as the low elevation area. Both Three Pines sites were typified by high amounts of *Inocarpus fagifer* and moderate *Miconia calvensis*. At Three Coconuts, high amounts of *Hibiscus tiliaceus*, *Miconia calvensis,* and small ferns were present. Both sites at Mari Mari Kellam's property were home to tall woody plants such as *Neonauclea forsteri*, *Hibiscus tiliaceus,* and *Cocos nuciferas*.

Heteroblasty study

The heteroblastic series of *A. evecta* was examined from gametophyte to bipinnate sporophyte. Gametophytes and sporophytes were identified in the field at Three Pines and Three Coconuts until multiple had been found showing each of the early leaf forms, up to the point where a frond contained multiple pinna. Based on these field observations, a generalized diagram of *Angiopteris evecta*'s progression throughout the heteroblastic series was created.

Heterophylly study

Angiopteris evecta was examined at two randomly selected areas within each of the three sites, for a total of six sampling areas. At each sampling area, thirty individuals located closest to the randomly-selected center were sampled. This resulted in a total sample size of 180 individuals. On each individual, every pinnule was examined for variations in form. Variations were recorded under specific categories that were later generalized based upon similar characteristics.

A suite of ecological factors were reco rded on a site-wide scale at each sampling area (Table 1). The elevation and coordinate location were measured from the center. Percent cover was also estimated from the center using a densiometer. Proximity to stream was determined by averaging the distance of the closest and farthest *A. evecta* individuals sampled from the nearest stream. Density was estimated by measuring the distance from the farthest *A. evecta* sampled to the center. A soil sample was taken at the center point of each site and measured for Nitrogen, Phosphorous, and Potassium content using a soil test kit.

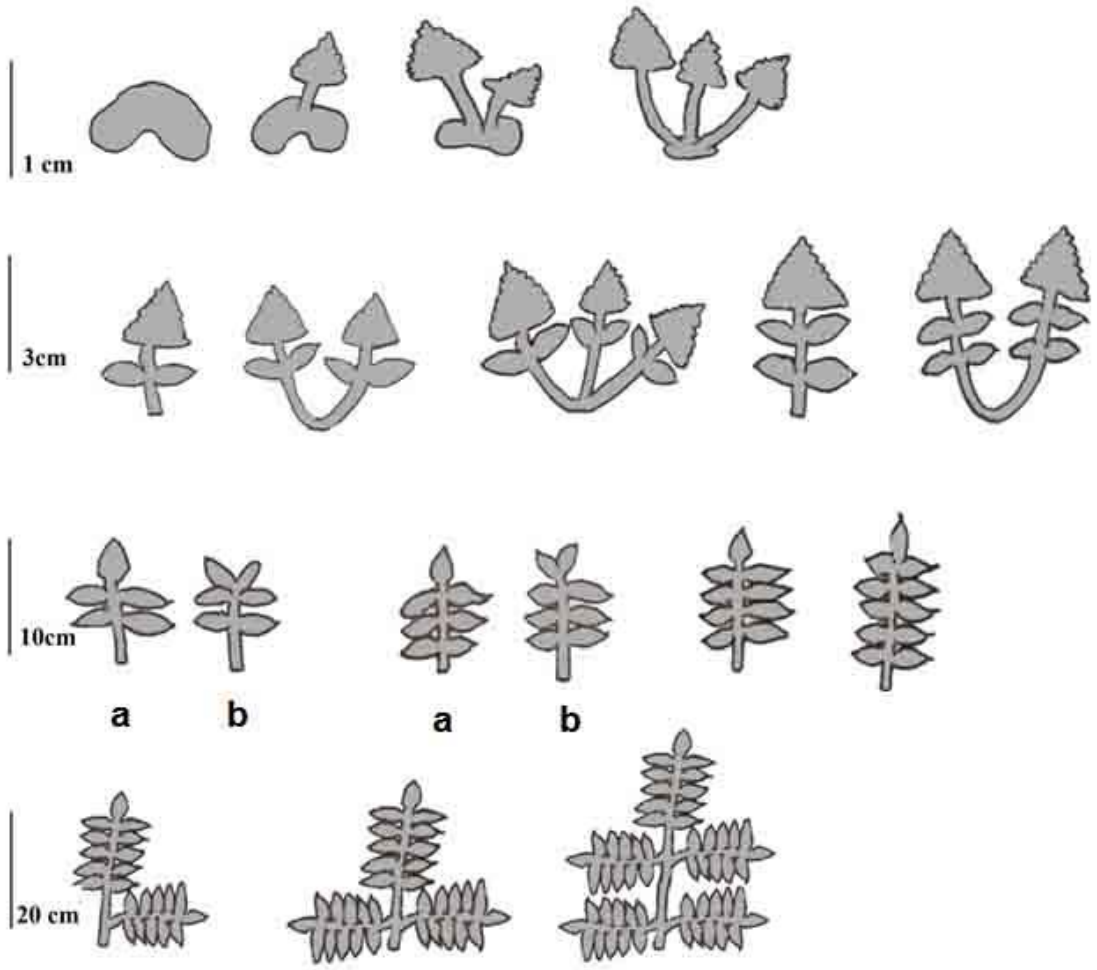

FIG. 2. Diagram of the heteroblastic series of *A. evecta*. "A" and "B" sketches indicate alternate forms where either an odd or even number of pinnules may be present, and are possible for each subsequent leaf in the series.

Herbaria Study

Ninety voucher specimens for *A. evecta* were examined at the University and Jepson Herbaria at UC Berkeley on December 4, 2014. Vouchers were examined for pinnule variations following the same procedure as the *Heterophylly study*. The location, year, and elevation the vouchers were collected at were recorded when available.

Statistical analyses

Analysis of data was done using the R statistical software package (R Development Core Team 2014). A Poisson regression was run in R for each variation category, with the number of occurrences of that variation as the dependent variable and the environmental conditions (elevation, percent cover, proximity to nearest stream, soil nutrient content, and *A. evecta* density) as the independent variables. A mixed-effects model was used with individual ferns as the fixed effect and frond length as an offset. A model selection approach was used to determine the best fit model, using ANOVA to test for significant differences between models.

A Mann-Whitney U test was used to test if the frequencies of pinnule variations recorded in the *Heterophylly study* for field specimens and the frequencies recorded in the *Herbaria study* for voucher specimens were the same across all types of variation categories.

RESULTS

Heteroblastic Study

The developmental stages of gametophytes and young sporophytes were identified and diagrammed to create the heteroblastic series of *A. evecta* leaf development (Fig. 2). The earliest leaves are simple leaves. As more leaves are produced, the gametophyte shrinks until it is no longer present. The next leaves produced on the sporophyte are compound leaves. As the sporophyte ages, more leaflets are produced on each new leaf. Eventually, the terminal leaflet shrinks and becomes similar in form to the rest of the leaflets. Once this stage has been reached, an odd or even number of leaflets may be present on the leaf. After this stage, bipinnate leaves begin to be produced as more pinna develop and the sporophyte ages.

Heterophyllic Study

After examining 180 *A. evecta* across six sites, twelve specific categories of variations were identified. These were then grouped based on similar characteristics into the four general categories of indented pinnules, warped pinnules, split pinnules, and pinnules with pinnulets (Appendix A).

TABLE 2. Frequency of pinnule variations broken down by site and category.

Site	Indent	Warp	Split	Pinnulet	Total
1 (TP)	601	72	10	104	787
2 (TP)	648	99	16	92	855
3 (TC)	1062	49	12	153	1276
4 (TC)	3545	776	44	475	4840
5 (M)	820	95	43	148	1106
6 (M)	1260	197	64	132	1653
Total	7936	1288	189	1104	10517

Note: TP = Three Pines, TC = Three Coconuts, M= Mari Mari's Property

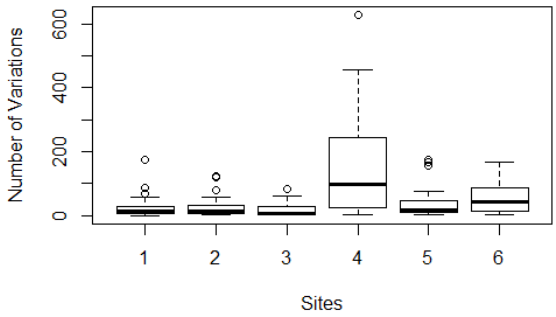

FIG. 3. Boxplots of the total number of variant pinnules at each site.

The frequency of pinnule variations differed across variation types and sites sampled at. Indented pinnules were the most common of the variant morphologies found, while split pinnules were the least common (Table 2). An average of 68.1±102.7 variant pinnules per fern was found. Site 4, located at Three Coconuts, had the greatest number of total pinnule variations present, as well as the greatest variance (Fig. 3).

Certain environmental conditions were found to have significant effects on pinnule variations (Fig. 4). As sites became located closer to streams, the occurrence of indented pinnules (p=3.35e-7) and warped pinnules (p=1.31e-7) increased. As elevation increased, the number of pinnules with pinnulets increased (p=5.01e-6) but the number of warped pinnules decreased (p=2.19e-3). As *A. evecta* became more dense, the number of pinnules with pinnulets decreased (p=9.35e-4). As the canopy cover increased, the amount of pinnules with pinnulets increased as well (p=2.58e-4). Finally, as the level of nutrients in the soil increased, the number of split pinnules increased (p=0.0159). The level of soil nutrients was included in the best fit model for pinnules with pinnulets, but was not found to have a significant effect (p=0.0553).

Herbaria study

All types of variation categories found in the field were found in the herbaria specimens as well. Field specimens examined during the *Heterophylly study* were found to have a higher frequency of all types of variation categories – indented pinnules (p=5.8e-11), warped

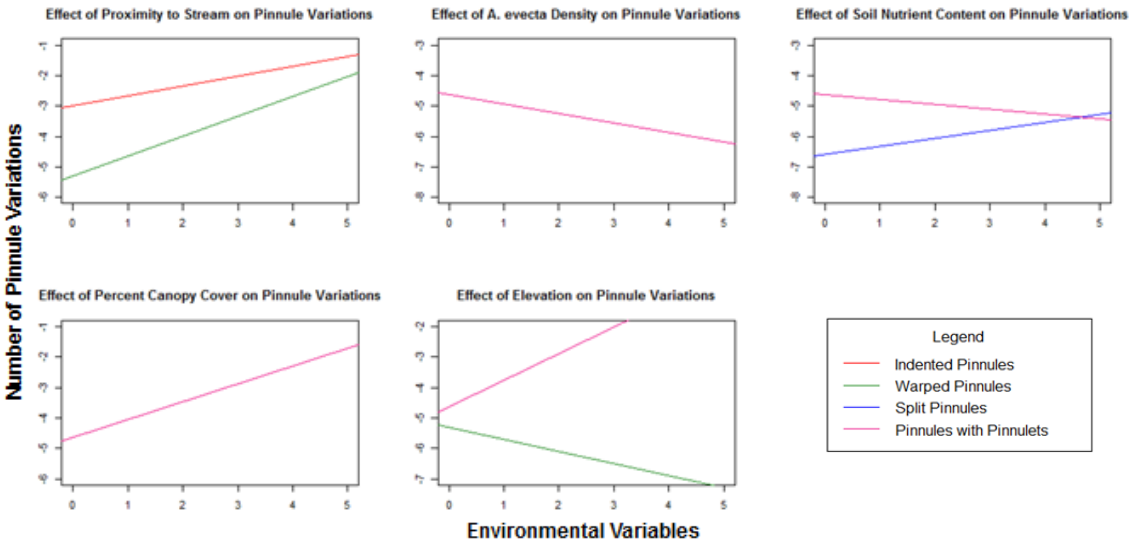

FIG 4. Plots of best fit regression slopes sorted by environmental factors.

pinnules (p=1.7e-8, split pinnules (p=3.7e-6) and pinnules with pinnulets (p=2.2e-16) – than the herbaria specimens.

DISCUSSION

Heteroblasty Study

The first four leaves observed of the sporophyte are highly similar to those described in Farmer's 1892 study, implying that the beginning of *A. evecta*'s heteroblastic series has not altered noticeably over the past 120 years.

Angiopteris evecta leaves only become more compound as the heteroblastic series progresses. At no stage in the series do leaves return to a more simple leaf form. It may only be genetically possible for them to increase in compoundness as they age.

Because these results are inferences based on field observations, there may be incorrectly ordered or missing stages. A laboratory study growing a gametophyte into a mature sporophyte under controlled conditions would provide a more definitive result, but would require many years due to the incredible lifespan of both the gametophyte and sporophyte stages. This approach would also permit the identification of heterophyllic influences on the heteroblastic series, which was beyond the scope of this study.

Heterophylly Study

Out of the four general categories of variations found, the only one to truly increase compoundness is the pinnules with pinnulets variation. Indented pinnules, warped pinnules, and split pinnules are still simple leaves, albeit with altered margins. This may also be the only true heterophyllic category, as it is possible that the indented, warped, and split pinnules are derived from a pinnule that was produced by the shoot in the standard form and later altered in form as it was growing.

Although they may not be of a heterophyllic nature, the correlations found between the indented, warped, and split pinnules and certain environmental variables shed light on factors influencing *A. evecta* leaf morphology. Since the number of indented and warped pinnules increase near streams, and the number of warped pinnules increases at low elevations, the herbivores and parasites which are likely responsible for these variations are probably found in low, riparian environments. Split pinnules may be more common in areas with high levels of soil nutrients as this supports greater laminar expansion (Witkowski and Lamont 1991).

The correlation between high percent cover environments and high numbers of

pinnules with pinnulets is supported by Niinemets's (1998) study showing compound leaves to be advantageous in low light environments. When the canopy cover increases, environments become sun-dappled, and highly dissected leaves can compensate for this by minimizing the resources used to cover more area (Xu *et al.* 2009). Although a study by Klugel and Kessler (2007) shows that simpler leaves are advantageous in extreme elevations, the results of this study imply that it is more common in higher elevations. This could be because the elevations in Moorea are not high enough for the result found in the Klugel and Kessler's study to occur. In regards to the effect of *A. evecta* density on pinnule variation, it is possible that any benefit conferred by the expanded area covered by the leaf diminishes when the plants are crowded, so leaves are less likely to be tripinnate.

Herbaria Study

The higher frequency of all types of variant pinnules found in the *Heterophylly study* than in the *Herbaria study* could be because botanists seek out "normal specimens" to use for their vouchers, or could be related to the location or year they were collected from. Future studies should examine this possibility in further detail.

The herbaria vouchers reveal that variant pinnules are not just a phenomenon unique to a couple populations in Moorea. The herbaria specimens show that these variations occur in locations as widespread as China, India and Jamaica. This implies that the ability to produce pinnules with variant forms must be inherently fixed within the genetic code and not easily changed.

Broader Implications and Future Directions

The heteroblasty and heterophylly studies completed on *A. evecta* further our understanding of the processes that are responsible for generating the form of an organism within its own lifetime. This fits into the larger field of "eco-devo": the study of the interactions between ecology and development. To gain an even more holistic view, future studies should incorporate a cross-species comparison. The pinnules with pinnulets variation has been observed in other species in the *Angiopteris* family, and the split pinnule variation was observed in Moorea on the mangrove fern, *Acrostichum aureum*. Assessing variation on a phylogenetic basis would give the occurrence of variations an evolutionary context as well, and expand the scope to changes beyond an individual's own lifetime.

An improved understanding of this subject area is crucial in the context of the current rapid, widespread changes occurring to the global environment. Being able to predict if organisms will be able to successfully respond to changing conditions and what this response will be provides invaluable information for conservation efforts.

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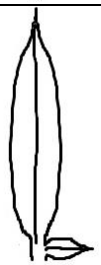
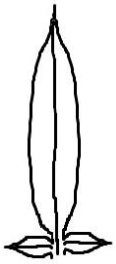
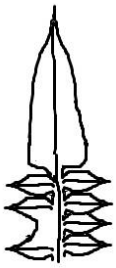
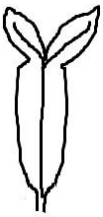
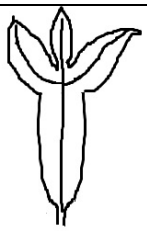
APPENDIX A

Categorization and description of variant pinnule morphologies.

General Categories	Description	Sub-categories	Description	Diagram
Normal	Pinnule is oblong-elliptical in shape with a beaked tip. The costule extends straight from the base to the apex of the pinnule.	N/A	N/A	
Indentation	The margin deviates from the standard outline and extends inwards at least once.	Single Indent	The margin deviates inwards for a portion of the pinnule. The margin may or may not reach the costule, but does not extend along it.	
		Gap	The margin deviates inwards and disappears, such that the costule forms the new leaf edge for a portion of the pinnule.	
		Multiple Indents	The pinnule displays more than one indent.	
Pinnule with Pinnulet	The pinnule has at least one pinnulet, in which the costule forks and extends into a smaller leaflet attached to the	One Pinnulet	The pinnule has a basal pinnulet on one side of the costule.	

	pinnule.	Two Pinnulets	The pinnule has a basal pinnulet on both sides of the costule.	
		Many Bulges	The pinnule has three or more pinnulets. These begin at the base and terminate at some point before the apex. The pinnulets may or may not be paired. The lamina may return to the standard outline or may be warped.	
Warp	The pinnule form is greatly variant from the standard state. The costule may end abruptly or be curved, but does not split or give rise to pinnulets. The lamina is not indented, buy may be misshapen in other ways.	M-shape	The pinnule is emarginate: the costule terminates early, but the lamina continues growing upwards on either side of it.	
		Curved	The costule departs dramatically from the standard straight condition.	
		Curled	The margins of the pinnule are curled under the lamina.	
		Circinate	The pinnule does not develop a beaked tip, and the lamina has a circular form.	
Split	The costule of the pinnule splits.	Twice Split	The costule splits in two directions, and continues growth laterally. Growth may be even or uneven between the two divisions.	

		Thrice Split	The costule splits into three directions, and continues growth both terminally and laterally. Growth may be even or uneven among the three divisions.	

APPENDIX B

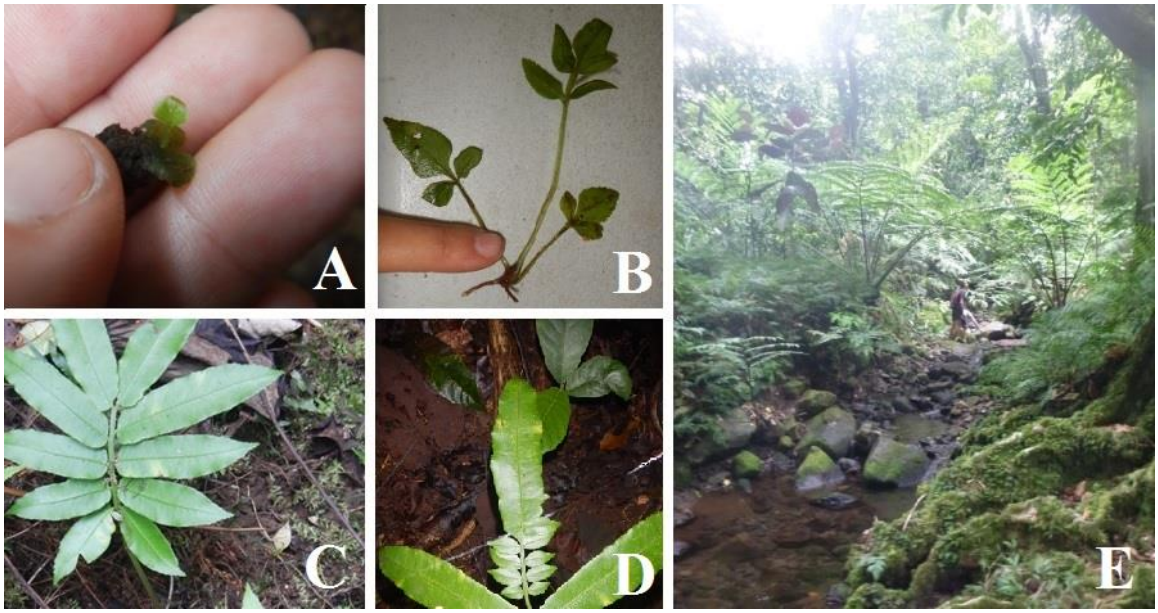



Plate of photographs of *A. evecta*. A: *Angiopteris evecta* gametophyte and sporophyte with the first simple leaf. B: Some of the first compound leaves to be produced by the young sporophyte. C: Young *A. evecta* sporophyte in the pinnate form, before it becomes bipinnate. D: *Angiopteris evecta* pinnule with pinnulets, in the tripinnate stage. E: Mature, bipinnate *A. evecta* towers over hikers and forms a dominant part of the forest understory.