

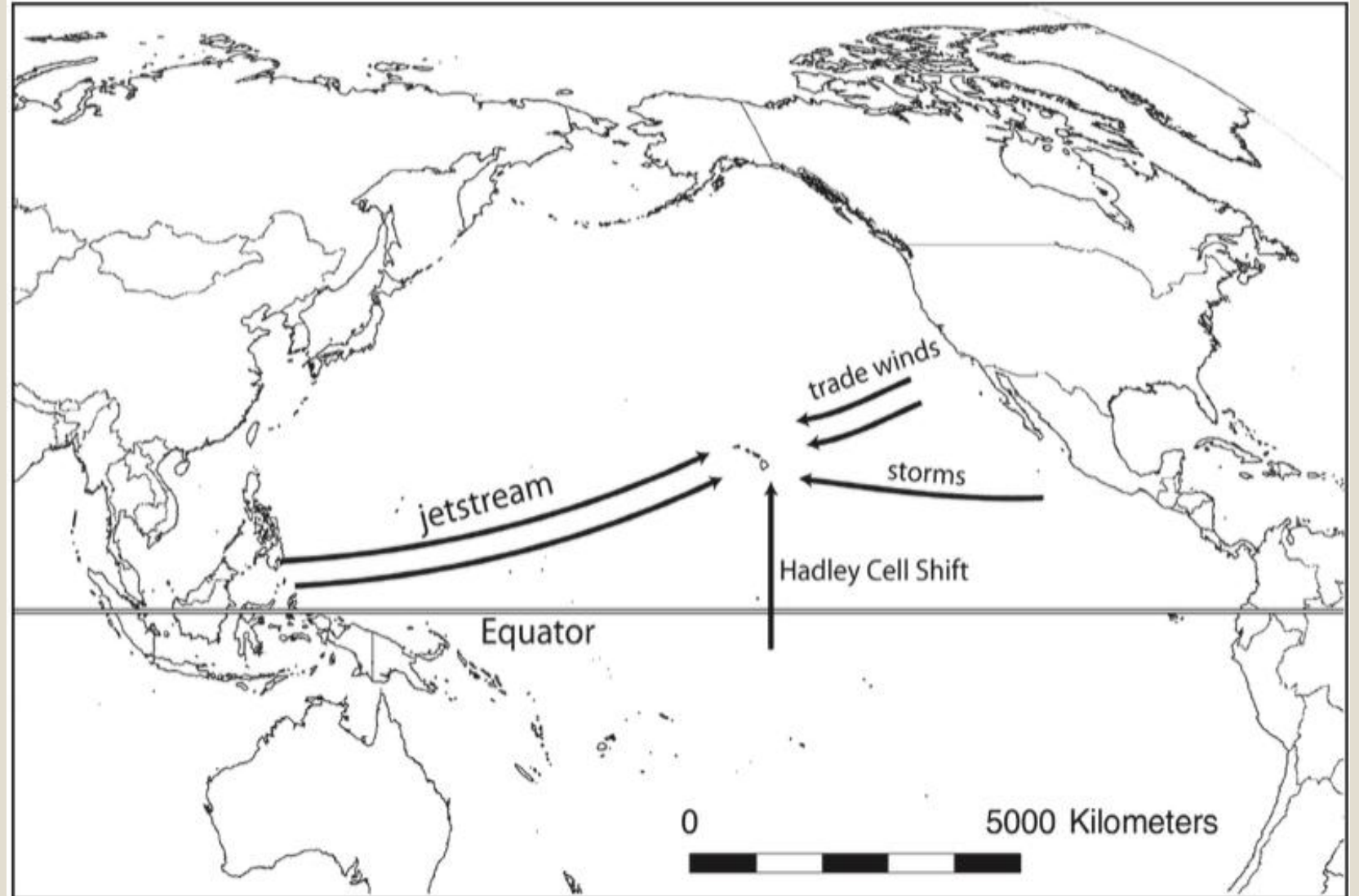


MOLECULAR BIOGEOGRAPHY AND ORIGINS OF THE HAWAIIAN FERN FLORA

Molly Flagstad - Biogeography

Possible Climate-Based or Weather-Based spore dispersal hypotheses

- 1) The Subtropical Jetstream
- 2) The Trade Winds
- 3) Storms from Southern Mexico and/or Central America
- 4) Combined influence of seasonal southern shift of the ITCZ, Hadley Cell air movement, and trade winds



Species Studied and Hypotheses

- 1) *Dryopteris* - 3 of the 5 subtropical jetstream
- 2) *Polystichum* - both subtropical jetstream
- 3) *Hymenophyllum* - at least 1 subtropical jetstream
- 4) *Adenophorus* - trade winds or storm system
- 5) *Grammitis* - possibly 1 subtropical jetstream
- 6) *Lellingeria* - any but the subtropical jetstream

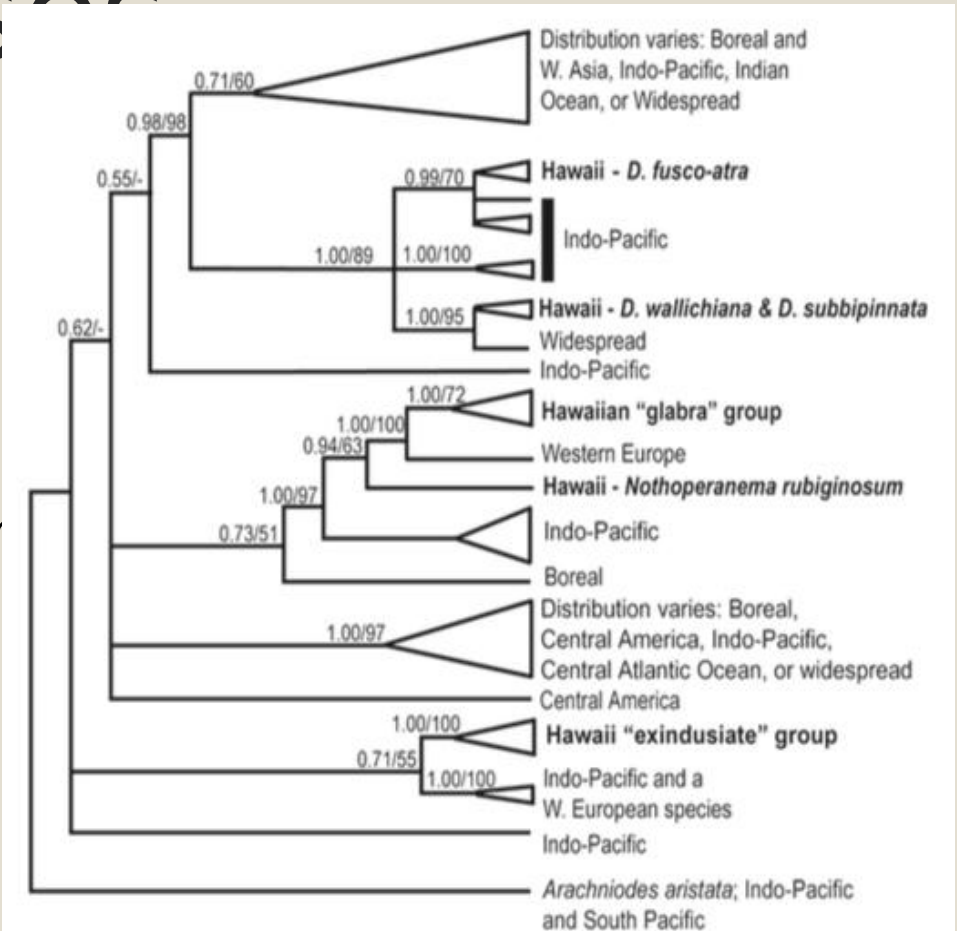


FIG. 2. Summary phylogeny of a combined Bayesian analysis of *rbcL* and the *trnL-F* IGS for *Dryopteris* (Dryopteridaceae) modified from Geiger and Ranker (2005). Hawaiian clades are named and geographic distributions are indicated for each non-Hawaiian clade or taxon. Posterior probabilities are the first number above each branch and were obtained from running 1,000,000 generations of which one tree was sampled per 100 trees generated. The second number above each branch (when present) represents maximum parsimony bootstrap values from 1303 repetitions and 10 random stepwise addition replicates each.

Summary Phylogeny for
Dryopteris

Summary phylogenies of *rbcL* sequence data (3) and *rbcL* and *trnL-F* for *Polystichum* (4)

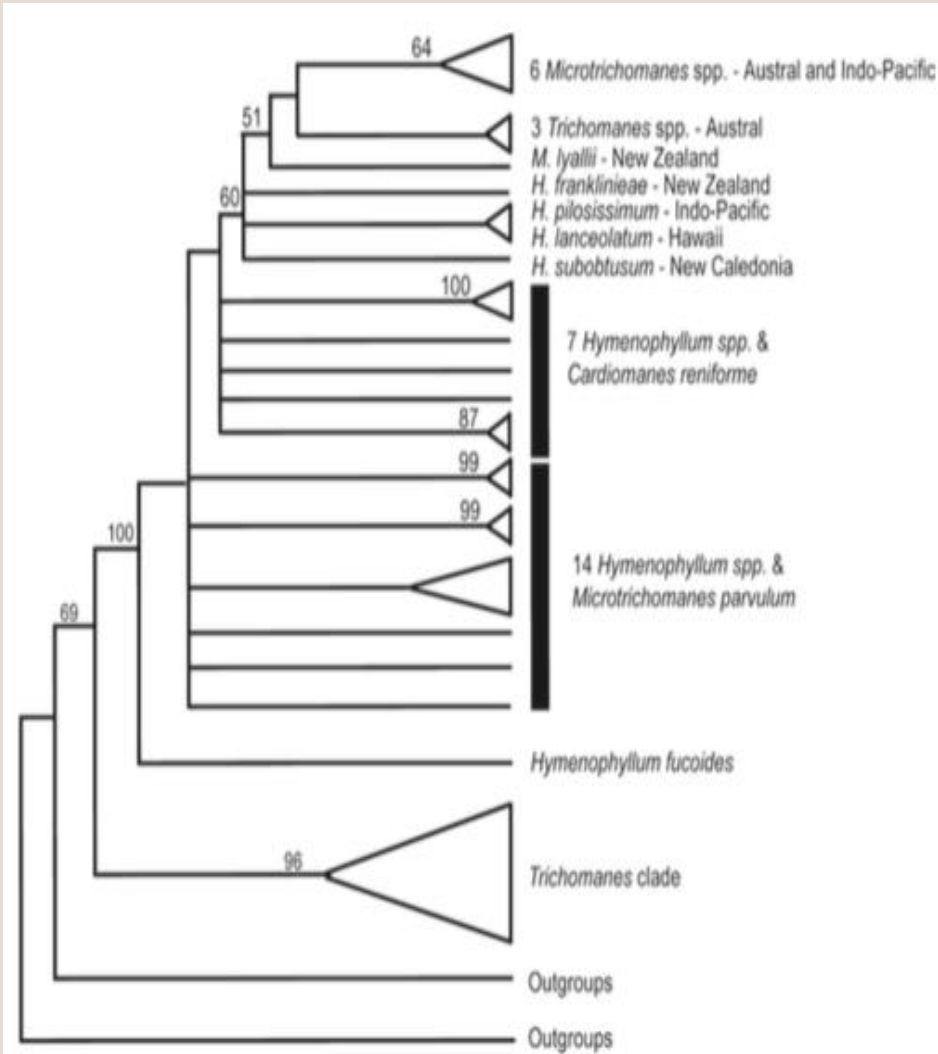


FIG. 3. Summary phylogeny of the strict consensus tree of 4806 most parsimonious trees based on *rbcL* sequence data modified from Fig. 1 of Ebihara et al. (2004). Numbers above each branch represent maximum parsimony bootstrap values.

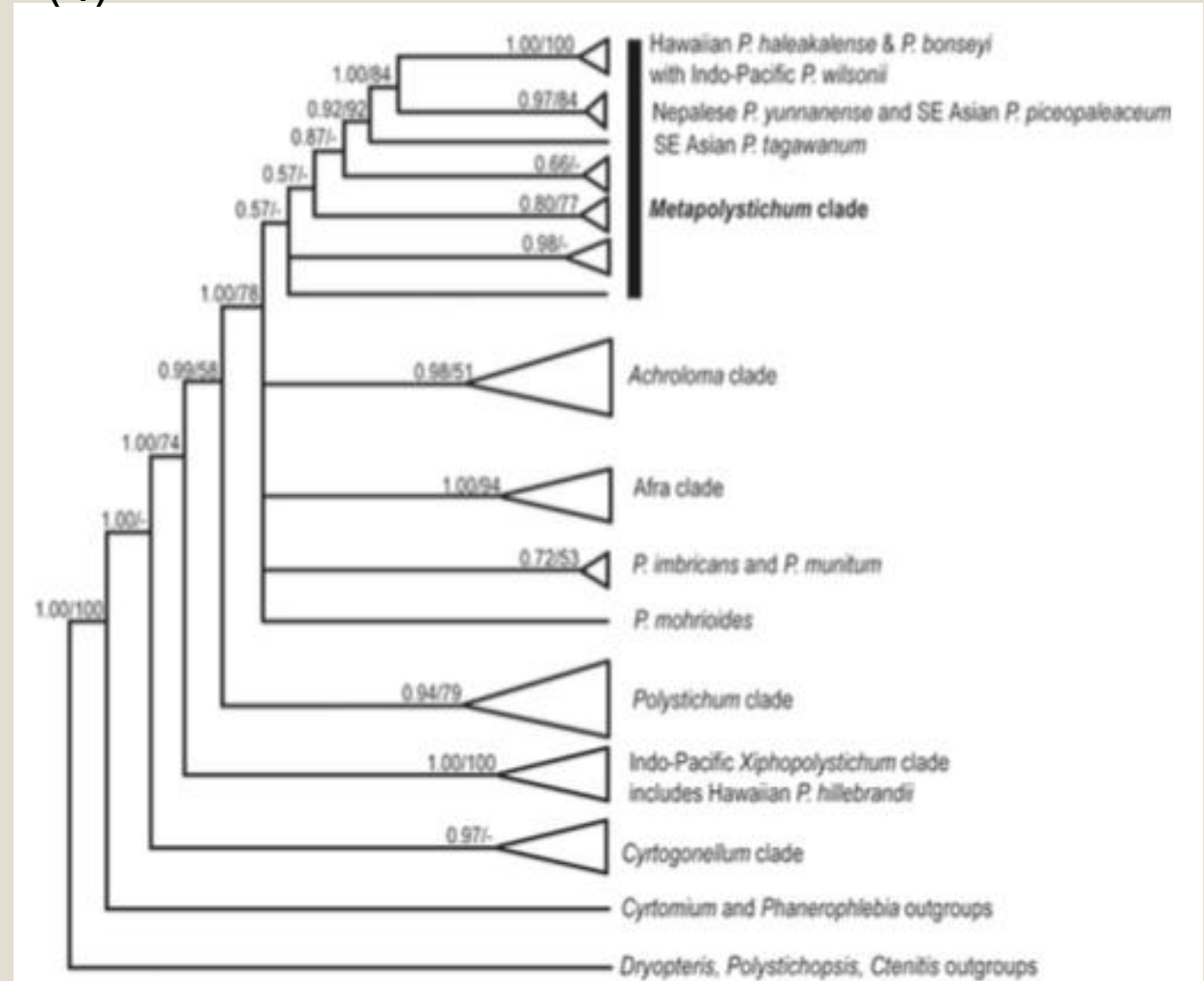


FIG. 4. Summary phylogeny of a combined Bayesian analysis of *rbcL* and the *trnL-F* IGS for *Polystichum* (Dryopteridaceae) modified from Driscoll and Barrington (in press). Posterior probabilities are the first number above each branch and were obtained from running 1,000,000 generations of which one tree was sampled per 100 trees generated. The second number (when present) represents maximum parsimony bootstrap values from 100 repetitions.

Adenophorous and Grammitis

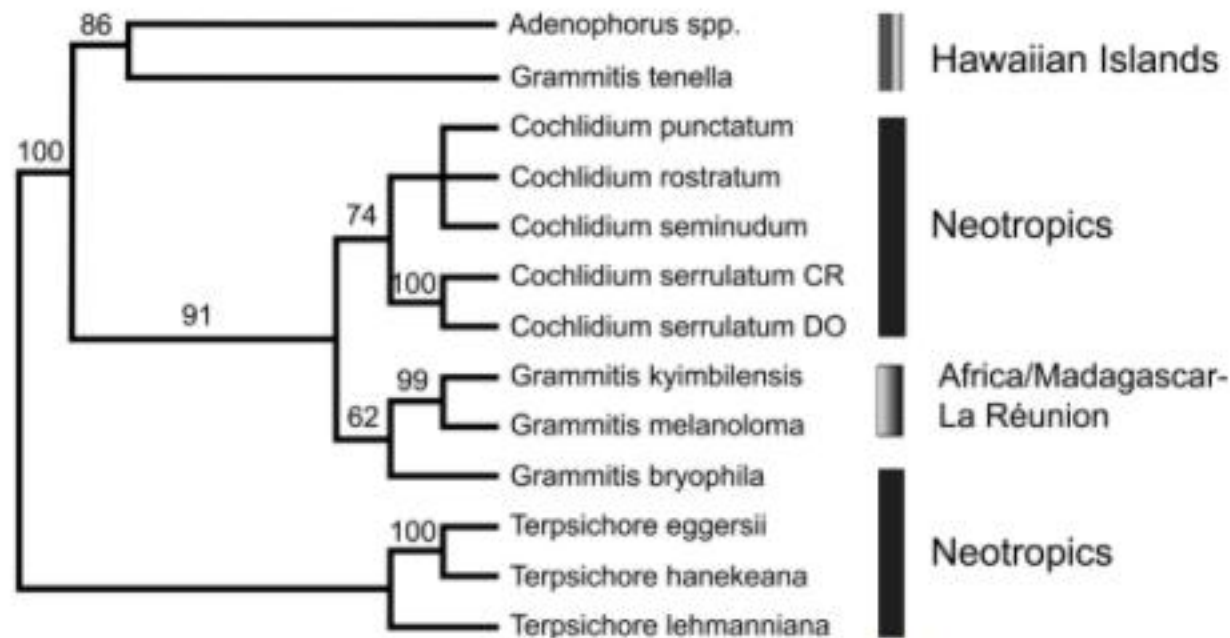


FIG. 5. Strict consensus tree of the three most parsimonious trees from branch-and-bound parsimony analysis of *rbcl* and *atp8*, with bootstrap values reflecting 100 branch-and-bound bootstrap replicates for Hawaiian *Adenophorus* + *Grammitis tenella* and their non-Hawaiian relatives.

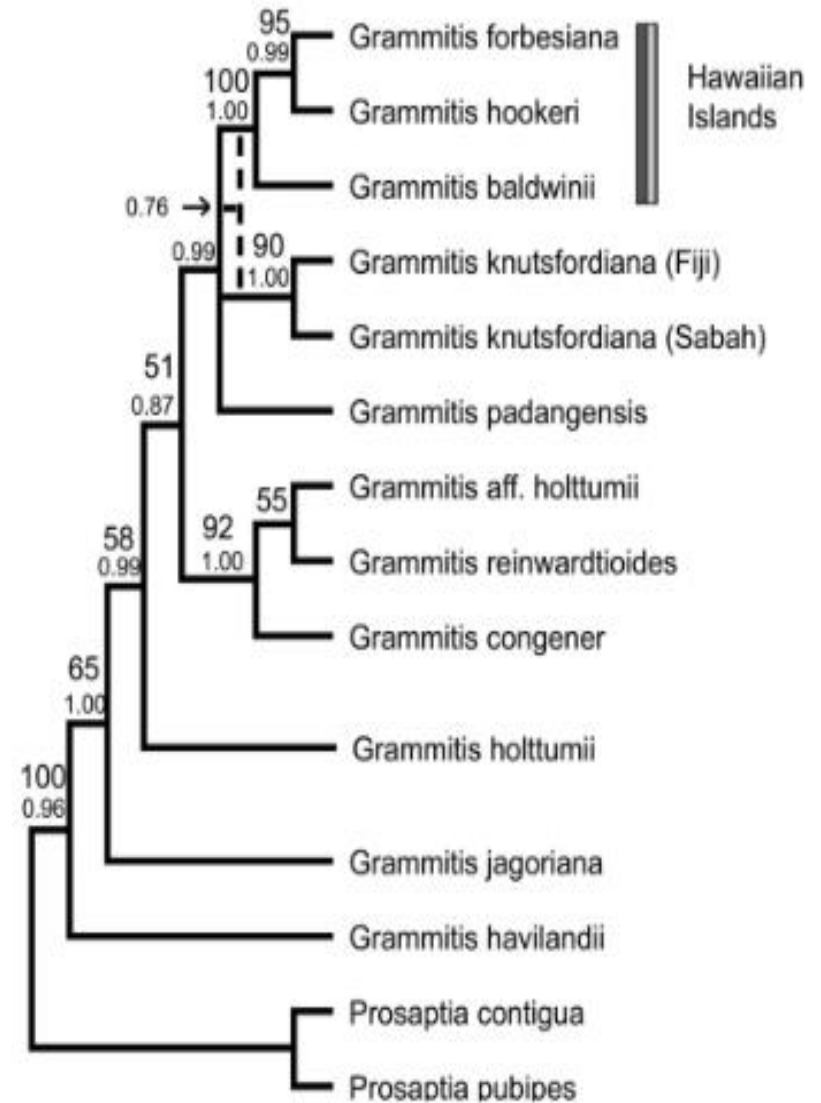
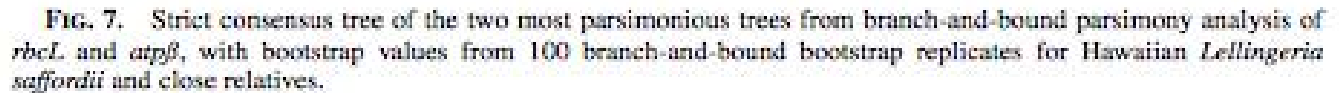


FIG. 6. Strict consensus tree of the two most likely trees resulting from a GTR maximum likelihood analysis based on sequences of *rbcl*, *atp8*, and *trnL-F* for the *Grammitis hookeri* clade and relatives. The top numbers above branches are the parsimony bootstrap values, and the bottom numbers are posterior probabilities from Bayesian analysis. Dashed lines indicate the support for a sister relationship between the Hawaiian clade and *G. knutsfordiana* in the Bayesian analysis with a posterior probability of 0.76.

Looking at *L. saffordii* and *L. subcoriacea*



Summary and Conclusions

- Molecular phylogenies from 6 fern genera reviewed
- Evidence found for each of the four dispersal mechanisms
- Northern subtropical jetstream spore dispersal method for several taxa
- Indo-Pacific Origin: two endemic *Polystichum* lineages, three of the five endemic *Dryopteris* lineages, and possibly the *Grammitis hookeri* clade
- *Adenophorous* and *Grammitis* are sister to a mostly neotropical clade – trade winds or storm
- Two species of *Lellingeria* share a common ancestor of neotropical origin dispersed to the Pacific Islands from trade winds or a storm

TABLE I
SPECIES, COLLECTION AND VOUCHER INFORMATION, AND GENBANK ACCESSION NUMBERS. DASHES INDICATE THAT GENBANK ACCESSION NUMBERS ARE CITED IN PUBLICATIONS CITED IN THE TEXT. ND=NO DATA

Species	Locality, collector & number (herbarium)	GenBank numbers new in this study		
		<i>rbcL</i>	<i>atpB</i>	<i>trnL-F</i> IGS
<i>Cochlidium serrulatum</i> (Sw.) L. E. Bishop	Costa Rica, A. Rojas 3004 (UC)	EF178614	ND	ND
<i>Cochlidium serrulatum</i> <i>Grammitis baldwinii</i> (Baker) Copel.	Dominica, S. R. Hill 29102 (UC) Hawaiian Islands, USA, K. Wood 10575 (PTBG)	EF178615	EF178632	ND
<i>Grammitis congener</i> Bl.	Sabah, Malaysia, Ranker 2126 (BORH, SAN, SNP, COLO)	EF178616	EF178633	EF178649
<i>Grammitis dolichosora</i> (Copel.) Copel.	Sabah, Malaysia, Ranker 2183 (BORH, SNP)	EF178617	EF178634	EF178650
<i>Grammitis forbesiana</i> W. H. Wagner	Hawaiian Islands, USA, Ranker 1321 (COLO)	EF178618	EF178635	ND
<i>Grammitis havilandii</i> (Baker) Copel.	Sabah, Malaysia, Ranker 2179 (BORH, SAN, SNP, COLO)	–	–	EF178651
<i>Grammitis holttumii</i> Copel.	Sabah, Malaysia, Ranker 2180 (BORH, SAN, SNP, COLO)	EF178619	EF178636	EF178652
<i>Grammitis</i> aff. <i>holttumii</i>	Sabah, Malaysia, Ranker 2175 (BORH, SAN, SNP, COLO)	EF178620	EF178637	EF178653
<i>Grammitis hookeri</i> (Brack.) Copel.	Sabah, Malaysia, Ranker 2176 (BORH, SAN, SNP, COLO)	EF178621	EF178638	EF178654
<i>Grammitis jagoriana</i> (Mett. ex. Kuhn) Tagawa	Hawaiian Islands, USA, Ranker 1116 (COLO)	–	–	EF178655
<i>Grammitis knutsfordiana</i> (Baker) Copel.	Sabah, Malaysia, Ranker 2152 (SNP)	EF178622	EF178639	EF178656
<i>Grammitis knutsfordiana</i> (Baker) Copel.	Fiji, J. Game 95-81 (UC)	–	–	EF178657
<i>Grammitis kyimbilensis</i> (Brause ex. Brause & Hieron.) Copel.	Sabah, Malaysia, Ranker 2176 (BORH, SAN, SNP, COLO)	EF178623	EF178640	EF178658
<i>Grammitis padangensis</i> (Baker) Copel.	Madagascar, Kessler 12773 (UC)	EF178624	EF178641	EF178659
<i>Grammitis reinwardtioides</i> Copel.	Sabah, Malaysia, Ranker 2159a (BORH, SAN, SNP, COLO)	EF178625	EF178642	EF178660
<i>Lellingeria major</i> (Copel.) A. R. Sm. & R. C. Moran	Sabah, Malaysia, Ranker 2160 (BORH, SAN, SNP, COLO)	EF178626	EF178643	EF178661
<i>Lellingeria saffordii</i> (Maxon) A. R. Sm. & R. C. Moran	Ecuador, M. Lehnert 895 (GOET)	EF178627	EF178644	ND
<i>Lellingeria subcoriacea</i> (Copel.) A. R. Sm. & R. C. Moran	Hawaiian Islands, USA, Ranker 1892 (BISH, COLO)	EF178628	EF178645	EF178662
<i>Lellingeria subcoriacea</i> (Copel.) A. R. Sm. & R. C. Moran	Marquesas Islands, D. Lorence 8944 (PTBG)	EF178629	EF178646	ND
<i>Lellingeria</i> sp. <i>Lellingeria</i> sp.	Marquesas Islands, D. Lorence 8944 (PTBG)	EF178629	EF178646	ND
<i>Prosaptia configua</i> C. Presl	Ecuador, M. Lehnert 1232 (GOET)	EF178631	EF178647	ND
<i>Prosaptia pubipes</i> Copel.	Bolivia, M. Lehnert 718 (GOET)	EF178630	EF178648	ND
	Taiwan, Chiou 97-09-12-05 (COLO, TAIF, UC)	–	–	EF178663
	Fiji, J. Game 95-65 (UC)	–	–	EF178664

Molecular Phylogeny and Adaptive Radiation of the Endemic Hawaiian *Plantago* Species

Molly Flagstad – Biogeography

Adaptive Radiation is a process in which organisms diversify rapidly from an ancestral species into a multitude of new forms, particularly when a change in the environment makes new resources available, creates new challenges, or opens new environmental niches.

- **Why *Plantago*?**

- Large genus with worldwide distribution
- Variety of habitats
- Differences in growth form, leaf morphology, and reproductive features
- Understudied and little is known

Morphological and ecological characteristics provide support - subdividing into 13 distinct morphotypes

TABLE 1. Morphological and ecological variation in Hawaiian *Plantago*. H, Hawai'i; K, Kaua'i; eM, East Maui; wM, West Maui; Mo, Moloka'i; eO, eastern O'ahu; wO, western O'ahu.

Taxon/Morphotype	Island(s)	Habitat	Growth form ^b	Leaf pubescence	Leaf arrangement ^c	Leaf attachment	Floral position to spike
<i>P. hawaiensis</i> ^a	H	Subalpine shrubland	Herb	Glabrous	Basal	Petiolate	Ascending
<i>P. hawaiensis</i> ^a and <i>P. pachyphylla</i>	H,eM, wM,Mo	Bog	Herb, robust	Glabrous or sparse abaxial tomentum	Equitant	Sessile	Ascending
<i>P. pachyphylla</i>	wM	Bog	Herb, minute	Glabrous	Rosette	Sessile	Ascending
<i>P. pachyphylla</i>	wM	Rainforest	Herb, robust	Dense abaxial tomentum	Basal	Petiolate	Ascending
<i>P. pachyphylla</i>	Mo	Wet ridge	Herb, robust	Dense adaxial and abaxial tomentum	Basal	Sessile	Ascending
<i>P. pachyphylla</i>	K, eO	Bog/Wet ridge	Herb	Glabrous	Rosette	Sessile	Ascending
<i>P. pachyphylla</i>	K	Bog/Wet ridge	Herb, minute	Glabrous	Rosette	Sessile	Ascending
<i>P. pachyphylla</i>	K	Bog/Wet ridge	Herb	Dense adaxial and abaxial tomentum	Rosette	Sessile	Ascending
<i>P. pachyphylla</i>	K	Bog	Herb, minute	Glabrous or dense adaxial tomentum	Rosette	Sessile	Ascending
<i>P. princeps</i> var. <i>anomala</i> ^a	K	Rainforest	Shrub, erect	Glabrous	Whorled	Sessile	Perpendicular
<i>P. princeps</i> var. <i>laxifolia</i> ^a	eM, wM	Rainforest	Shrub, erect	Glabrous	Whorled	Petiolate	Perpendicular
<i>P. princeps</i> var. <i>longibracteata</i> ^a	K	Waterfall	Subshrub, prostrate	Glabrous	Whorled	Petiolate	Ascending
<i>P. princeps</i> var. <i>princeps</i> ^a	eO, wO	Mesic forest	Shrub, erect	Glabrous	Whorled	Petiolate	Perpendicular

^aFederally listed endangered species

^bRobust = plants greater than 8 inches in height; minute = plants less than one inch in height; erect = vertical; prostrate = lying on the ground.

^cBasal = leaves that arise from the base of the stem and are spirally arranged; equitant = leaves overlapping in two ranks; rosette = dense radiating cluster of leaves at ground level, the stem very short or lacking; whorled = three or more leaves arising from a node at the tips of stems.

Primary Objectives of the Study

- 1) Present the first phylogeny of the Hawaiian *Plantago* radiation
- 2) Assess the monophyly of the group **Monophyly** – group of organisms that are classified in the same taxon and share a common ancestor
- 3) Explain relationships among Hawaiian *Plantago* taxa and morphotypes, including an assessment of possible hybridization between species
- 4) Determine modes of speciation
- 5) Investigate the historical biogeography of the group

Materials and Methods

◦ **Taxon Sampling and DNA Extraction** – total of 21 Hawaiian *Plantago* individuals were collected from every island and included for analysis

◦ **Molecular Markers** – The internal transcribed spacer (ITS) and external spacer (ETS) regions of the nrDNA have both been shown to provide species-level phylogenetic signal

◦ **Amplification and DNA Sequencing** - the four gene regions were amplified and sequenced using standard primers

◦ **Sequence Alignment and Phylogenetic Analysis** – maximum parsimony, maximum likelihood, and Bayesian methods were used to estimate phylogenies for all three data sets (nuclear, chloroplast and combined)

Results

Coded Indels Partition

Chloroplast

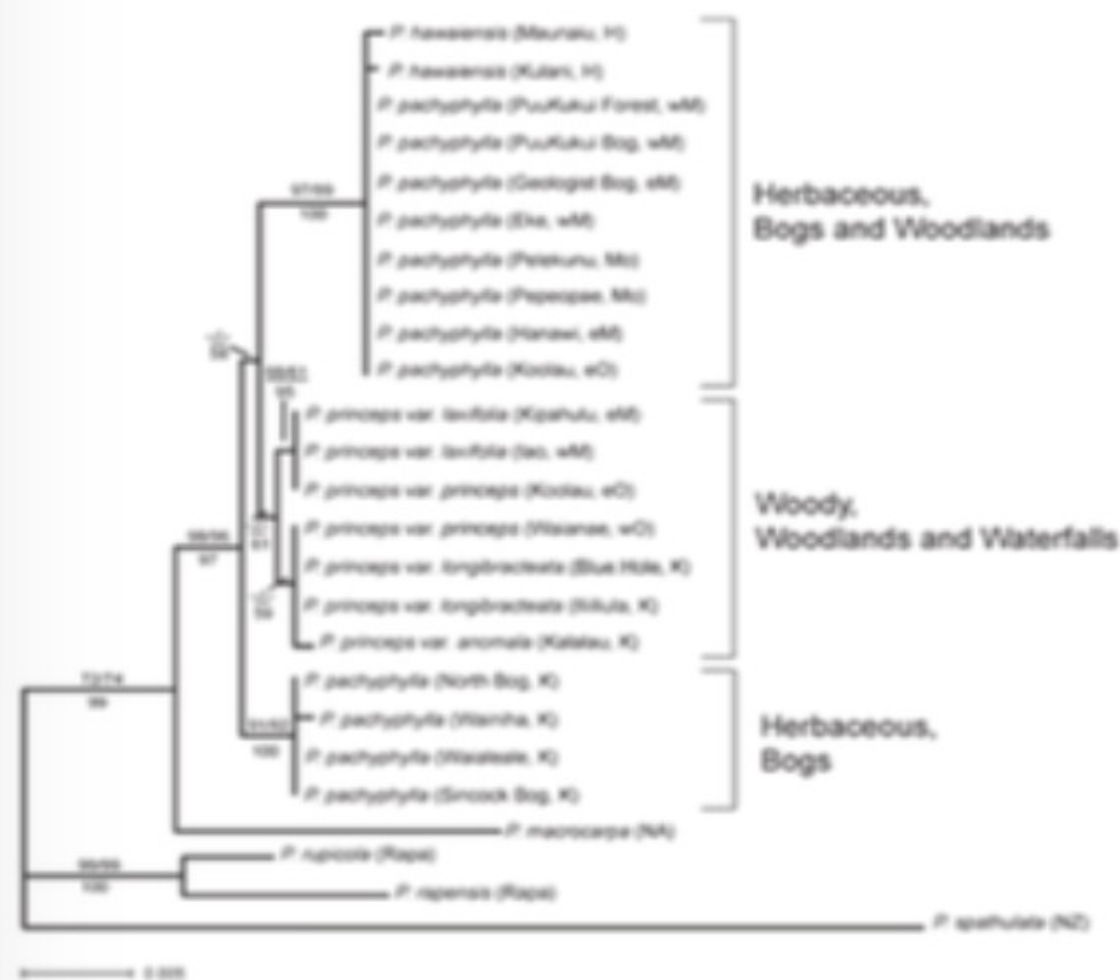
Separate Partition Analysis Analysis

Combined

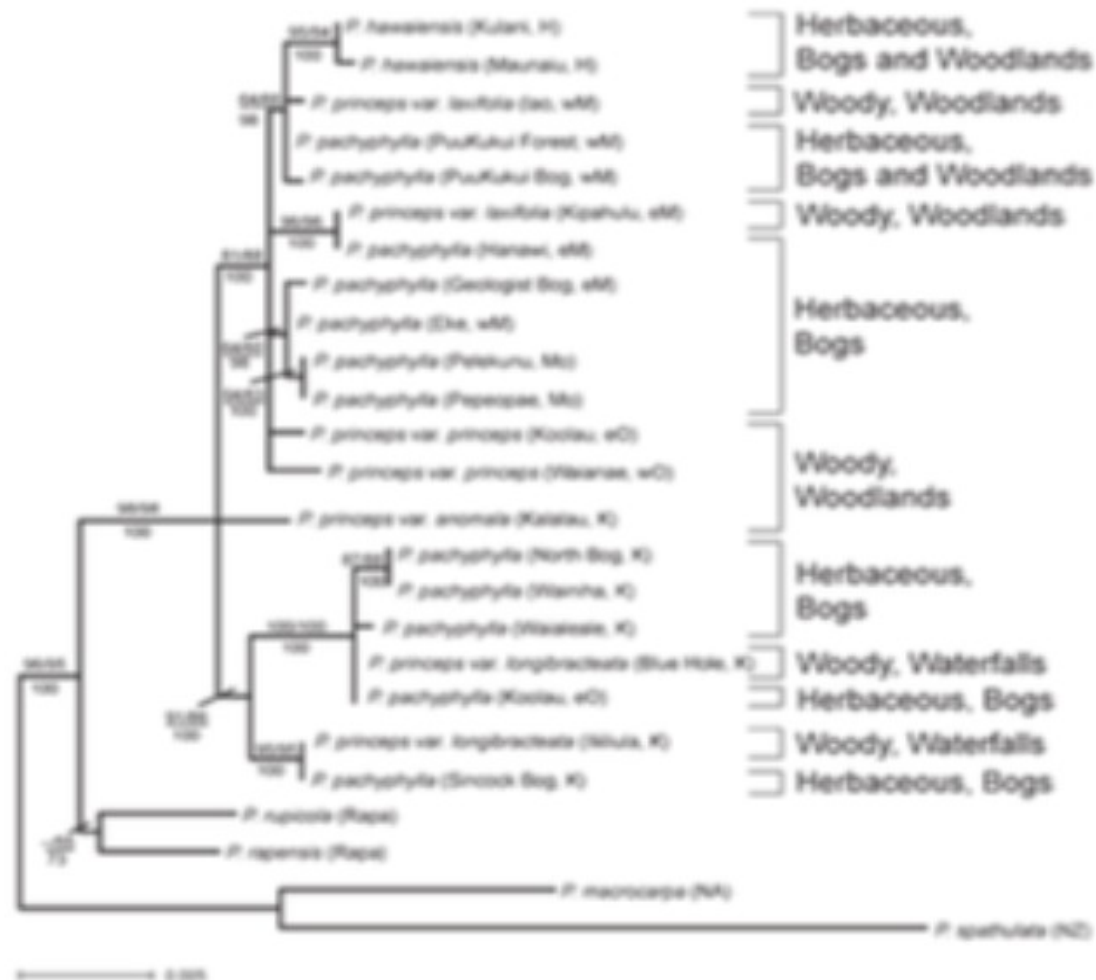
TABLE 2. Sequence characteristics for each gene partition.

Partition	<i>N</i> ^a	Length (bp)	Aligned length (bp)	Parsimony informative characters	Variable, parsimony uninformative characters	Coded indels
ITS	25	664–675	548	22	32	5
ETS	25	763–775	693	19	29	9
<i>ndhF-rpl32</i>	25	776–844	801	25	30	22
<i>rpl32-trnL</i>	24	790–842	806	17	38	13
Combined nuclear regions	25	n/a	1241	41	61	14
Combined plastid regions	25	n/a	1607	42	68	35
Combined all regions	20	n/a	2848	77	134	49

^aThe number of individuals in each data set after removing those with identical sequences within a population. Five discordant individuals were also removed from the combined analysis of all regions.



Nuclear



Chloroplast

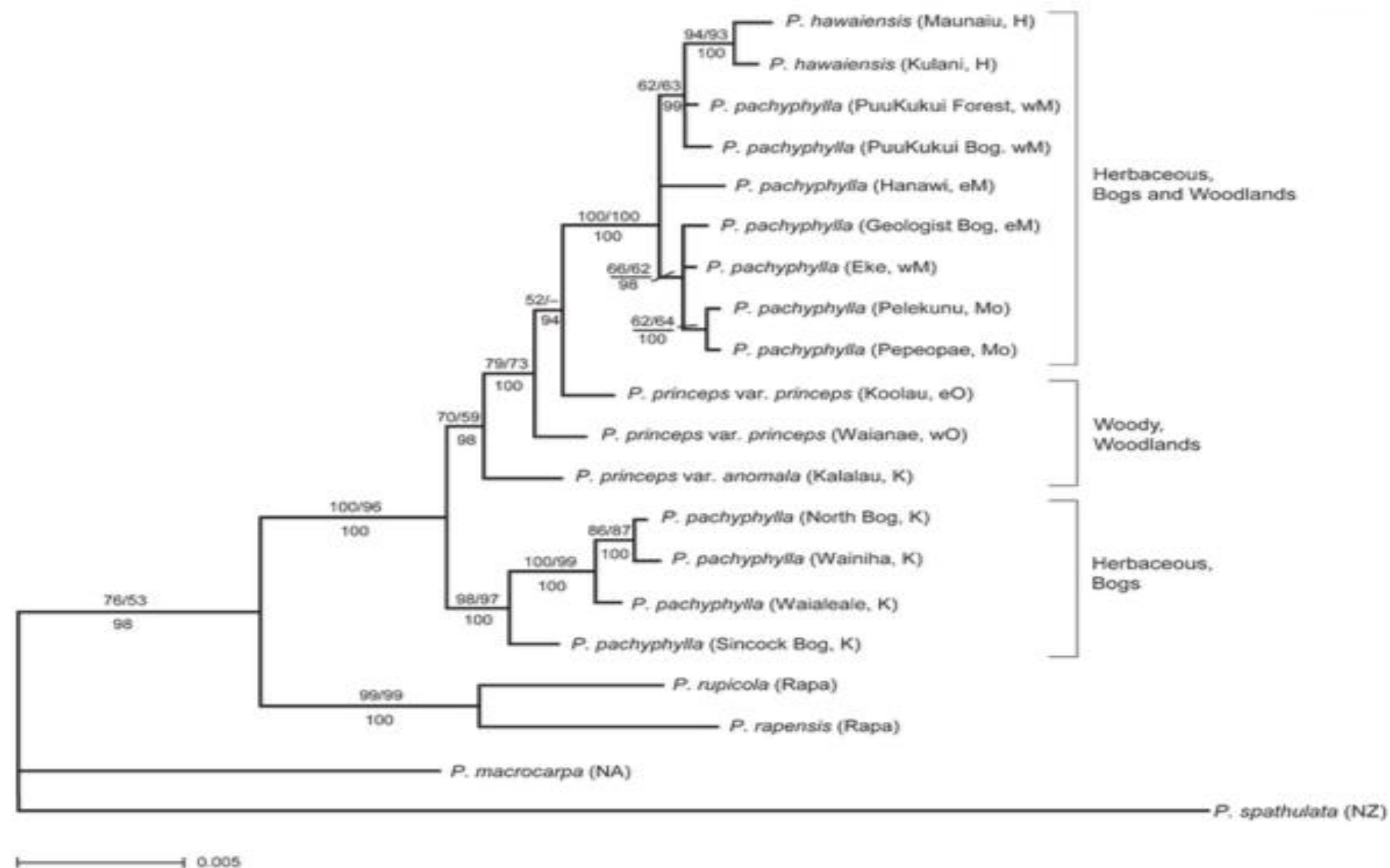


Fig. 2. Bayesian consensus tree for Hawaiian *Plantago* of the combined data set (ITS, ETS, *rpl32-trnL*, and *ndhF-rpl32*) run for 1 million generations with model TIM+I+G and score $-\ln L = 5450.71$. Above branches are bootstrap values (MP/ML) and below branches are Bayesian posterior probability scores. Refer to Table 1 and Fig. 1 for location abbreviations. Note: Woodlands refers to forests and shrublands, and bogs include wet ridge tops.

Hybridization and Historical Biogeography

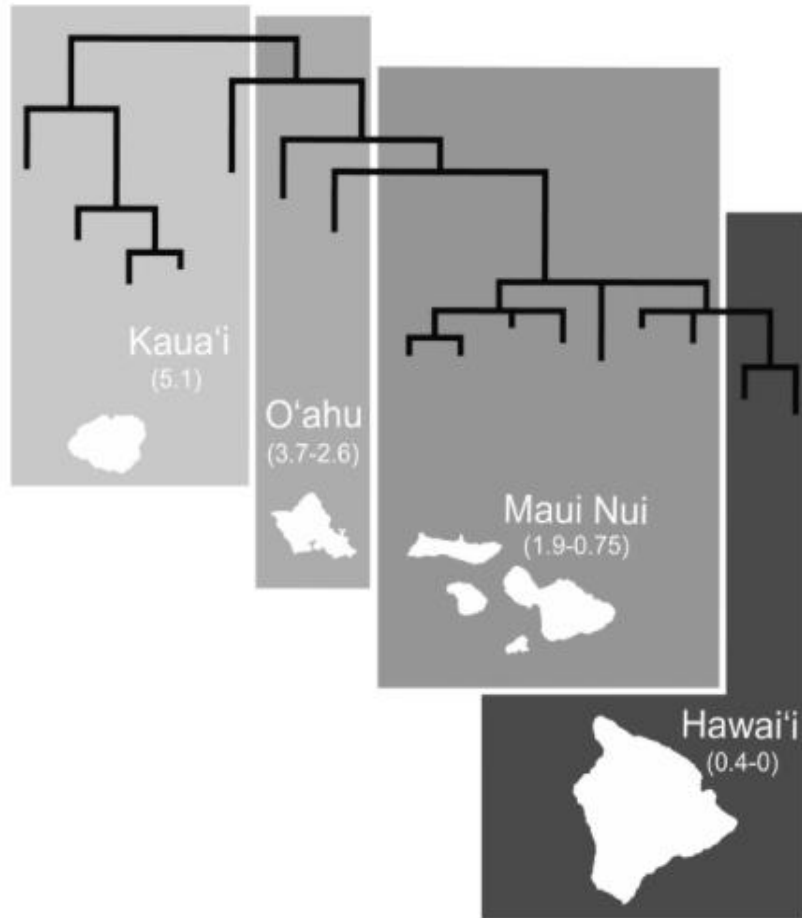


Fig. 3. Biogeographic distribution of Hawaiian *Plantago* morphotypes included in the combined analysis. In parentheses is island age in millions of years.

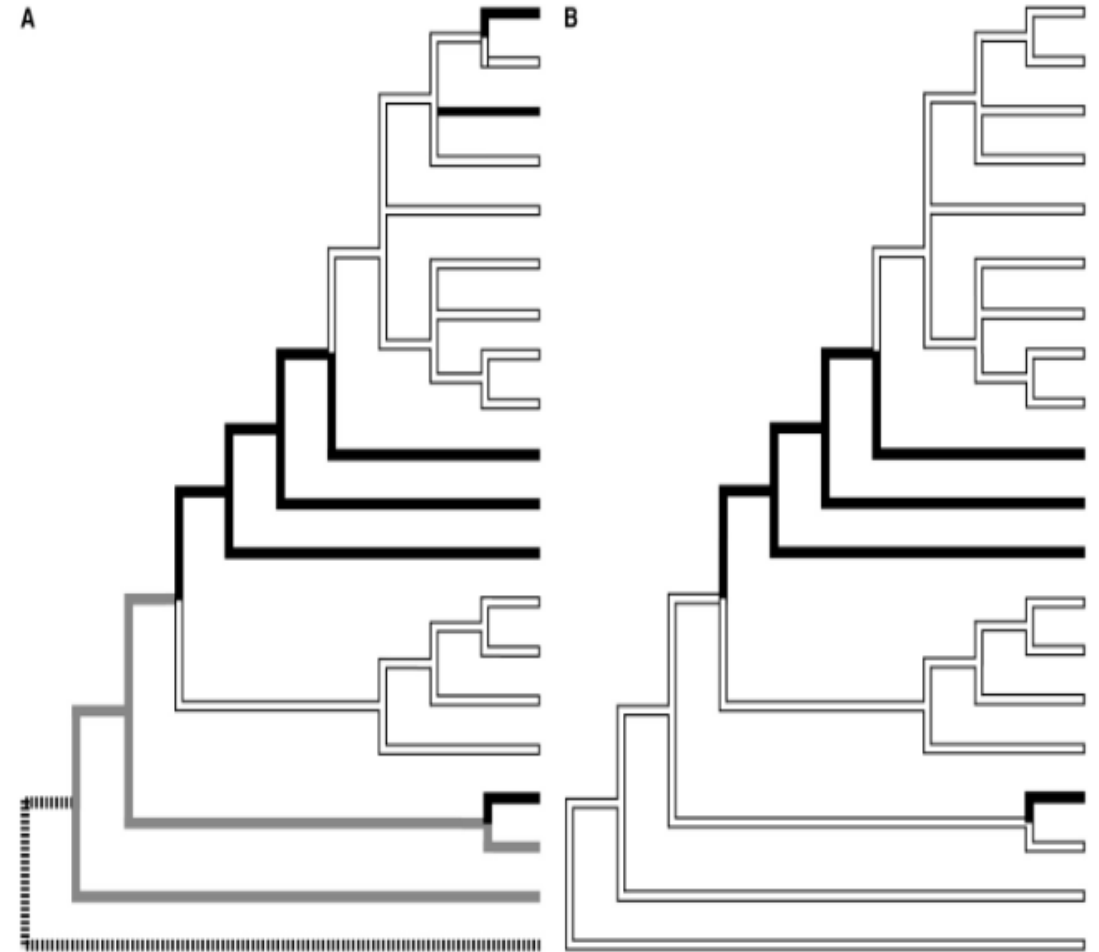


Fig. 4. Reconstruction of ancestral states mapped onto Bayesian consensus tree of the combined data set for Hawaiian *Plantago*. (A) Habitat (white = open bogs; black = woodlands; gray = coastal; dashed = unknown). (B) Growth form (white = herbaceous; black = woody).

Conclusions

- - Apparent monophyly of Hawaiian *Plantago* along with its morphological and ecological diversity suggest that it is an example of adaptive radiation in the Hawaiian flora
- - Hybridization has played a role in the evolutionary history of some lineages in the Hawaiian Islands
- - Analyses presented have confirmed the morphological complexity and reinforced need for taxonomic revision of *Plantago* along with many other plant groups

Molecular Biogeography and Origins of the Hawaiian Fern Flora

The Hawaiian islands are located around 4000 kilometers from the nearest continent. Because of the isolation of the islands, a unique flora arose that includes around 200 native ferns and lycophytes that are similar to ferns but have unique leaves called microphylls. $3/4^{\text{th}}$ of these are endemic to the islands. Because the Hawaiian islands originated from a volcano, abiotically dispersed organisms had to have arrived by water or wind. Fern spores are most likely to have been dispersed by the wind, and because of this, the patterns of air movement are important when looking into the geographic origins of the ancestors of the ferns in Hawaii. The goal of this paper is to evaluate molecular phylogenetic and resulting biogeographical analyses in a comparative context to assess whether long-distance dispersal to the archipelago has been essentially random across groups or if there are common patterns across groups due to the influence of shared historical processes.

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The researchers of this paper have identified four possible climate-based or weather-based spore dispersal hypotheses that could have resulted in the movement of ancestral spores to the Hawaiian Islands. (1) the northern subtropical jetstream, moving spores from Indo-Pacific regions; (2) the trade winds, dispersing spores from Central and North America; (3) storms carrying spores from southern Mexico and/or Central America; and (4) a dispersal mechanism carrying spores from the South Pacific across the equator resulting from the combined influence of a seasonal southern shift of the Intertropical Convergence Zone (ITCZ), Hadley Cell air movement, and the trade winds.

The first method, the subtropical Jetstream, flows from west to east, and accelerates as it moves eastward from SE Asia and decelerates as it moves over the Hawaiian Islands.

Researchers propose that spores could be moved up into the Jetstream by storms and then moved from SE Asia and/or Malaysia to the Islands in just 2-4 days.

The second method of dispersal is the trade winds. The clockwise-rotating North Pacific anticyclone feeds into the trade wind belt, drawing northerly air masses towards the equator, making the trade winds a possible dispersal method of spores from America.

The third possible spore dispersal method is from regular, tropical storm and hurricane tracks from southern Mexico and C. America. Storms that make it to the Hawaiian Islands from these areas can do so in 7-10 days.

The fourth and final spore dispersal pathway is through the trade winds in combination with the effects of Hadley Cells and a seasonal shift southward of the inter-tropical convergence zone.

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Researchers looked into 3 fern genera including *Dryopteris*, *Polystichum*, and *Hymenophyllum*. They studied three additional genera with new analyses including *Adenophorus*, *Grammitis*, and *Lellingeria*. All six of these are represented in the Hawaiian Islands by at least one endemic lineage.

The researchers hypothesized that three of the five endemic *Dryopteris* lineages, both of the endemic *Polystichum* lineages, at least one endemic *Hymenophyllum* lineage in the Hawaiian Islands, and, perhaps, one endemic *Grammitis* lineage resulted from ancestral spores of each lineage dispersing to the Hawaiian Islands via the northern subtropical jetstream. *Adenophorus* is sister to a mostly neotropical clade, therefore, it is likely that the ancestor of the Hawaiian clade dispersed to the Hawaiian Islands via the trade winds or a storm system.

Although the researchers only sampled about 25% of the genus of *Dryopteris*, their results suggested ancestral dispersal of *Dryopteris* to the Hawaiian islands via the northern subtropical Jetstream. Figure 2 shows us the summary phylogeny of a combined analysis of genes for

Dryopteris. In this figure the Hawaiian clades are named and geographic distributions are indicated for each non-Hawaiian clade or taxon.

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Both of these next two phylogenies take a look at two evolutionary genes. Figure 3 displays the data found from research analyzed from 60 rbcL sequences from members of *Hymenophyllaceae*. They found weak evidence for a sister relationship between two different species in Borneo and New guinea. This was just a piece of evidence that could prove that the Hawaiian colonizing ancestor was Indo-Pacific in distribution and the dispersal of spores was done via the northern subtropical Jetstream.

Polystichum is up next with 3 endemic species in the Hawaiian Islands. Researchers conducted a molecular phylogenetic study of 50 polystichum species including these 3 species. They obtained the cpDNA regions rbcL and trnL-F IGS to determine whether the three endemic Hawaiian species comprise a monophyletic group and to infer their geographic region of origin by determining their closest non-hawaiian relatives. Figure 4 displays the relationships they found, specifically two numerous species found in SE Asia and the Himalayas. This supported the hypothesis that the northern subtropical Jetstream was the mode of spore dispersal of the lineages of *polystichum* to the Hawaiian Islands.

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Moving on to *Adenophorous* and *Grammitis*, figure 5 shows a branch and bound analysis of a combined rbcL and atpBeta dataset from all of the taxa shown. The researchers found that because two Old World species were supported as derived within the Neotropical clade that is sister to the Adenophorous and Gammitis clade, they hypothesized that the ancestor of the Hawaiian clade had a Neotropical distribution to the Hawaiian Islands either through the trade winds or a storm.

In figure 6, several analyses were conducted of *rbcl*, *atpBeta*, and *trnL-F* from the taxa shown. Among the three endemic species, *Grammitis hookeri* and *Grammitis forbesiana* were strongly supported as sister taxa, and *G. baldwinii* as their sister. However, this tree does not support the Hawaiian clade as a sister group of four Indo-Pacific species due to the bootstrap relationships being so low.

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Finally, *Lellingeria saffordii* is the third grammitid lineage endemic to the Hawaiian Islands. A branch and bound analysis and a branch and bound bootstrap analysis. *Lellingeria saffordii* was strongly supported as a sister to another species which is present in the Society Islands. Because of these findings the researchers hypothesized a common ancestor of these two species is of Neotropical origin and dispersed from the trade winds or a storm.

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Lellingeria

CONCLUSIONS

Phylogenies were studied from six fern genera including the *Dryopteris*, *Hymenophyllum*, *Polystichum*, *Adenophorus*, *Grammitis*, and *Lellingeria*. This six were chosen as they all are represented in the Hawaiian Islands by at least one endemic species.

From this, evidence was found for each of the four described dispersal method.

The northern subtropical Jetstream is supported to be the spore dispersal mechanism for several taxa.

The endemic lineages supported by molecular phylogenetic analysis to be of Indo-Pacific origin include the two endemic *Polystichum* lineages, 3 of the 5 endemic *Dryopteris* lineages, and possibly the endemic *grammitis hookeri* clade. However, the Hadlet cell is a possible mechanism as well.

Because Adenophorous and Grammitis are sister to a mostly Neotropical clade, the likely ancestor of this clade traveled to the islands from the trade winds or a storm.

And finally, from the last figure, because two species of *Lellingeria* are hypothesized to share a common ancestor of Neotropical origin, the mode of dispersal for this clade was also from the trade winds or a storm.

MOLECULAR phylogeny and ADAPTIVE RADIATION of the ENDEMIC Hawaiian *Plantago* species (Plantaginaceae)

Ocean islands offer isolation from source populations and a close association of a diversity of habitats. The evolutionary consequences are rapid speciation events often coupled with remarkable examples of adaptive radiation.

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In evolutionary biology, **adaptive radiation** is a process in which organisms diversify rapidly from an ancestral species into a multitude of new forms, particularly when a change in the environment makes new resources available, creates new challenges, or opens new environmental niches. The Hawaiian Islands are an ideal environment to study these topics due to the isolation and different environments.

In order for further understanding of adaptive radiation and island evolution in a Hawaiian plant group, researchers focused on the endemic Hawaiian *Plantago* L. species. *Plantago* is a large genus with worldwide distribution and the species occurs in a variety of habitats such as bogs,

wet ridge tops, subalpine shrubland, mesic forest, rainforest, and waterfalls and have diverged in growth form (including woodiness), leaf morphology, and reproductive features. This species is also understudied and relatively poorly known. The researchers of this paper were interested in the Hawaiian *Plantago* radiation comprised of six endemic taxa showing inter- and intraspecific morphological and ecological diversity. The rDNA internal (ITS) and external (ETS) transcribed spacers and two recently described chloroplast spacers, *ndhF--rpl32* and *rpl32-trnL*, were sequenced to study phylogenetic relationships within this morphologically complex group. Their analysis provided strong evidence for the monophyly of Hawaiian *Plantago*, which suggests that the lineage arose from a single long-distance dispersal event. The most recent treatment of the Hawaiian flora recognizes only three endemic species and four varieties of *Plantago*. However, these species' considerable inter- and intraspecific morphological and ecological diversity has led to broad disagreement regarding the number of taxa that should be recognized and their relationships to one another.

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The researchers conducted a molecular phylogenetic analysis to delimit species, establish relationships among them, and examine evolutionary patterns in this poorly known group using DNA internal (ITS) and external (ETS) transcribed spacers in combination with two rapidly evolving spacer regions from the chloroplast genome, *ndhF-rpl32* and *rpl32-trnL*. In Table 1, we see the morphological and ecological variation in Hawaiian *Plantago*. Characteristics like habitat, leaf arrangement and attachment provide support for loosely subdividing Hawaiian *Plantago* into 13 distinct morphotypes.

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OBJECTIVES

From this research, there were 5 main objectives of this study. The first was to present the first phylogeny of the Hawaiian *Plantago* radiation, 2nd to assess the monophyly of the group, 3rd to

explain the relationships among these taxa and possible hybridization, 4th determine modes of speciation, and 5th to investigate the historical biogeography of the group.

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MATERIALS AND METHODS

In order to study these objectives, the researchers used different methods and materials. The first was of course taxon sampling and DNA extraction. With the amount of variability among the *Plantago*, the researchers collected leaf material from all known extant morphologically and geographically distinct populations. And within the islands, samples were collected from different volcanoes to give age structure within each island. A total of 21 Hawaiian *Plantago* individuals were collected from every island and included for analysis.

For Molecular markers, nuclear and chloroplast DNA gene regions were obtained for phylogenetic analysis. By including gene regions from the nuclear DNA which is biparentally inherited and chloroplast DNA which is uniparentally inherited, these genomes allowed the researchers to assess the differences among the phylogenies. This gave insight to hybridization.

They amplified the four gene regions and sequenced with standard primers, aligned the sequences and used Maximum parsimony, maximum likelihood, and Bayesian methods to estimate phylogenies for nuclear, chloroplast and combined data sets.

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On to the results..

Coded indels did not contribute a significant amount of phylogenetic information to any of the data sets and therefore were omitted, although the number of coded indels are in table 2.

For Separate Partition analysis, the different gene regions did not vary much in the amount of phylogenetic information they contained. Also, the nuclear and chloroplast gene partitions did not differ in total number of parsimony informative characters.

Nuclear Partition – The nuclear data set strongly supports the monophyly of Hawaiian *Plantago* and moderately supports a sister relationship with the western north American species, *Plantago macrocarpa*. In general, the three major nrDNA lineages most closely correspond with morphology and habitat type.

Monophyly of Hawaiian *Plantago* is also strongly supported by the chloroplast data set with a couple differences included a different sister relationship.

In the combined analysis, there was also strong support for the monophyly of Hawaiian *Plantago*.

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This figure shows the phylogenetic trees generated for both the nuclear analysis and chloroplast analysis. There are many differences between the two, making groupings that are different suggesting difference inheritance among species.

(CHANGE SLIDE)

This next figure comes from the combined analysis and shows a primary division that separates the herbaceous, bog forms on Kauai from the woody and herbaceous forms occurring in the woodlands and bogs on all of the islands. In Hawaiian *Plantago*, the nuclear and chloroplast phylogenies suggest that woodiness was likely secondarily derived from the herbaceous growth form.

(CHANGE SLIDE)

As far as hybridization goes, there were a few species that exhibited differences among the nuclear and chloroplast gene trees. Researchers suggest that from this evidence, high interfertility, wind pollination, and breeding system of the *Plantago* taxa, hybridization is strongly possible among these species.

CONCLUSIONS

In conclusion, the apparent monophyly of Hawaiian *Plantago* along with its morphological and ecological diversity suggest that it is yet another example of adaptive radiation in the Hawaiian flora.

The work done for this paper further suggests that hybridization has played a role in the evolutionary history of this lineage along with others in the islands.

And finally analyses presented have reinforced the need for revisions of *Plantago* and other Hawaiian plant groups.