

*Biogeographical Origins and Phylogeny of the Endemic
Hawaiian Species of Cibotium (Dicksoniaceae): a Molecular
Perspective Based on atp β Sequences*


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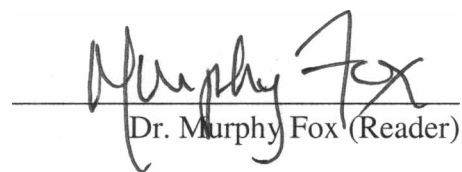
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Table of Contents

Acknowledgements.....	iii
Abstract.....	v
List of tables.....	vi
List of figures.....	vii
Introduction.....	1
Materials and Methods.....	9
Results.....	14
Discussion.....	16
Literature Cited.....	18

Abstract

A great deal of speculation exists as to the biogeographical origins and phylogeny of endemic Hawaiian ferns; yet only a handful of studies has actually examined the evolution of these endemics and their relationships to continental species. The fern genus *Cibotium* (Dicksoniaceae), consisting of four Hawaiian endemics and five non-Hawaiian species, is the focus of this study. The goals of my research were to resolve whether Hawaiian *Cibotium* is monophyletic and to test the hypothesis that the common ancestor of Hawaiian *Cibotium* originated from the Indo-Pacific region. Although the long-distance dispersal mechanism of fern spores to Hawaii was not directly investigated, a pathway for the colonizing ancestor can be inferred based on the geographical location of the closest non-Hawaiian relative. The chloroplast DNA fragment *atpβ* was sequenced for eighteen taxa and phylogenetic analyses were performed using both maximum parsimony and maximum likelihood. These analyses strongly indicate that the genus *Cibotium* is monophyletic and suggest that the colonizing ancestor of the Hawaiian species is of South American origin. From this we may infer that the ancestral spores arrived on Hawaii via the combined effects of Hadley cells, trade winds, and seasonal shifts in the inter-tropical convergence zone (ITCZ).

List of Tables

Table 1: Species, collection localities, collectors, and native distribution of taxa.....	10
Table 2: Primer pairs, reaction reagents, and cycling conditions used for PCR amplification and sequencing of <i>atpβ</i>	12

List of Figures

Figure 1: Maximum parsimony tree for all sampled taxa based on the analysis of <i>atpβ</i> sequences.....	15
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Introduction

The Hawaiian Islands have long been regarded as a natural laboratory for studying the speciation, evolution, and biogeographical origins of vascular floras that are found nowhere else in the world. All Hawaiian endemic species evolved in extreme isolation, as geological evidence suggests that the Hawaiian Archipelago has never been connected to a substantial landmass (Simon, 1987). The Hawaiian Islands are considered the world's most remote island group because the high islands presently lie 4000 km from the nearest continent, North America, and 1600 km from Polynesia, the nearest archipelago. The extant islands vary in age, since they were born one by one from volcanic eruptions that began 85 million years ago and that still fire today on the Big Island of Hawaii, the youngest island of the archipelago (est. 500,000 yr) (Carson and Clague, 1995). The islands were formed in a conveyor-belt-like style as the relentlessly moving Pacific plate carried them from the southeast to the northwest, passing over a stationary hotspot, or area of high thermal energy. On their northwest voyage, the islands are reshaped and leveled by the pressures of time and sea. The older islands eventually sink into the sea, but new islands continue to form and increase the length of the Hawaiian Island chain. For the past 85 million years, the continued formation of islands has offered an extended availability of new habitats for colonizing species, which may explain the dramatic species diversification and high endemism displayed by Hawaiian flora (Wagner and Funk, 1995); 89% of Hawaiian angiosperms and 71% of Hawaiian pteridophytes are endemic (Palmer, 2003).

In his analyses of Hawaiian flora, Fosberg (1948) notes that “Especially little has been written specifically on the relationships of Hawaiian genera and species to their relatives elsewhere.” Few phylogenetic studies have been performed on endemic Hawaiian pteridophytes even though the plants constitute 16% of native Hawaiian vascular flora (Fosberg, 1948; Wagner, 1988). Since the first comprehensive phylogenetic analysis of any lineage of Hawaiian ferns was conducted by Ranker et al. (2003); only five additional studies have been completed (Hennequin et al., 2003; Ranker et al., 2004; Geiger and Ranker, 2005; Scheider et al., 2004; Schneider et al., 2005). Obviously more phylogenetic studies are needed in order to expand our knowledge of the evolutionary history of endemic Hawaiian ferns. In particular, it would be enlightening to determine the continental origins of Hawaiian pteridophytes should augmentations be needed due to habitat destruction and the consequent reduction of the native floras. This study examined the biogeography and phylogenetic relationships of the tree fern genus *Cibotium*, with the main focus upon the four endemic Hawaiian species.

The genus *Cibotium* (Dicksoniaceae) consists of nine species: three in Asia, two in Mexico and Central America, and four in Hawaii (Kramer, 1990). All recognized Hawaiian species: *C. chamissoi*, *C. menziesii*, *C. nealiae*, and *C. glaucum* are endemic to Hawaii, although their ancestry and biogeographical origins have never been ascertained. *Cibotium chamissoi* was the first species to receive a taxonomic description in 1824, when Kaulfuss published *Cibotium* (Palmer, 1994). By the early 1900’s, botanists recognized six species of Hawaiian *Cibotium* (Palmer, 1994), but an extensive taxonomic survey by Palmer (1994) recognized only four.

Instead of scales, *Cibotium* are noted for possessing long, silk-like hairs, varying in color and texture (Palmer, 1994). Among Hawaiian species, *C. chamissoi* is noted for dark coarse hair, distinguishing it from *C. glaucum*, a fern with golden hair located exclusively at the base of the petiole (Wagner, 1988). Classifications of *C. menziesii* and *C. nealiae* are much more difficult, as the two species are morphologically similar.

Differing views on classification, distribution, and nomenclature have seriously hampered our current understanding of Hawaiian *Cibotium*. Until recently there was no clear definition of species which resulted in incorrect identifications of specimens, as well as confusion in assigning nomenclature (Ripperton, 1924; Wick and Hashimoto, 1971). Morphologically, few phylogenetic studies have been done on Hawaiian *Cibotium*, aside from an examination of spore morphology by Gastony (1981), from which he surmised the Hawaiian species to be closely related to other genera of the Dicksoniaceae family, such as *Thyrsopteris* and *Lophosoria* (Gastony, 1981). As for morphological differences within the *Cibotium* genus, the spores of the Hawaiian and Central American species show a similar degree of distal ridge development, which differs from the extensive distal and equatorial ridge development of the Asiatic species (Gastony, 1981). Palmer (1994) considered *Cibotium* to be a taxonomically difficult genus because species share many similar characteristics, such as leaf size, spore development, and hair and trunk variations (Becker, 1984).

Within the past twenty years, several taxonomic treatments of *Cibotium* have been published (Wagner, 1988; Palmer, 1994). Still the genus remains poorly

understood due to a lack of usable herbarium specimens needed for research and to the absence of phylogenetic studies. The sparse scientific data elucidating Hawaiian *Cibotium*'s evolutionary origins are solely from inconclusive studies of spore morphology and from conventional taxonomic treatments (Gastony, 1981; Becker, 1984; Palmer, 1994). Molecular data are changing the methods scientists use to trace phylogeny, as morphological data alone can be misleading or insufficient in forming well-supported hypotheses. Molecular data from the chloroplast genome have been successfully used to elucidate phylogenetic relationships in pteridophytes (Wolf, 1997) and in particular the gene *atpβ* has shown to be useful for phylogenetic analyses.

The *atpβ* gene codes the β chain of ATP synthase, the transmembrane complex responsible for coupling ATP synthesis with proton transport across the chloroplast membrane. The *atpβ* region of the chloroplast genome is 1497 bp long and located downstream from *rbcL*, another gene which is often sequenced for use in phylogenetic studies (Wolf, 1997). Wolf (1997) determined *atpβ* to be more informative than *rbcL* at resolving older divergence events, possibly because of its slower rate of divergence.

atpβ sequences are valuable in resolving relationships within and among families of angiosperms (Hoot and Crane, 1995). Wolf (1997) concluded that *atpβ* sequencing also has phylogenetic utility for ferns and that a combined analysis of *atpβ* and *rbcL* sequences produces a better supported phylogenetic hypothesis than does the use of one set alone. Kleist (2006; in prep) is generating *rbcL* and *trnL-F* sequences for *Cibotium* that will later be combined with the *atpβ* sequences from this

study to possibly produce a more informed description of *Cibotium*'s evolutionary origins. If the region of origin is known, we can then infer the long-distance dispersal mechanism responsible for carrying the ancestral spores to Hawaii.

All regional floras have arrived on Hawaii via long distance dispersal, either by air or by sea. Scientists have proposed a variety of sources for Hawaiian floras, ranging from North America to Asia. Although the Hawaiian Islands lie closer to North America, it is believed that most Hawaiian flora originated in the South Pacific region (Mueller-Dombois and Fosberg, 1998). Alternatively, Lindqvist and Albert (2002) propose that the endemic Hawaiian mint *Stachys* (Lamiaceae) was derived from North America. A recent study of the Hawaiian members of the pteridophyte genus *Dryopteris* (Dryopteridaceae) suggests that the majority of Hawaiian *Dryopteris* clades have SE Asiatic origins (Geiger and Ranker, 2005). Other studies of Hawaiian biogeography suggest that the Hawaiian endemic *Gunnera panke* is a sister species to the South American subspecies *Misandra* (Wanntorp and Wanntorp, 2003).

With the exception of the Hawaiian endemic *Adenophorus* and the above mentioned Hawaiian dryopterids, most likely being of neotropical origin (Ranker et al., 2003), the origins of other Hawaiian pteridophytes are virtually unknown. Fosberg (1948) proposed that "the majority of plants arrived in Hawaii from islands to the southwest (Indo-Pacific)." He (1948) was unable to rule out the possibility of an American ancestor(s). His studies, based on comparative morphology, suggest the following regions of origin for endemic Hawaiian pteridophytes: Indo-Pacific

(Indonesia, Southeast Asia, and the neighboring Pacific islands); 48%, Pantropical 20.8%; American 11.9%, Boreal 4.4%; and Austral 3.7% (Fosberg, 1948).

There is general consensus among scientists that wind dispersal of spores is the most likely method of dispersal and subsequent colonization (Carlquist, 1980). Studies also show that fern spores can be exposed to high amounts of UV radiation at altitudes up to 12,000 m and still be capable of germinating under favorable conditions (Gradstein and van Zanten, 1987). Three climate-based dispersal pathways of spores to the Hawaiian Islands have been proposed: the jet stream (Ratner, 1955), trade winds, and a combination of the effects of Hadley cells and shifts in the inter-tropical-convergence zone (ITCZ) (Wright et al., 2001).

If ancestral spores originated from the Indo-Pacific, they may have been transported to Hawaii via the Pacific jet stream. The jet stream occurs in the upper troposphere as a fast-flowing band of air, fluctuating between speeds of 195 kph and 115 kph (Carlquist, 1980) as it flows from west to east (Ratner, 1955). During storms, spores can be lofted into the jetstream and carried from SE Asia to the Hawaiian Islands in a relatively short amount of time (2-4 days) (Ratner, 1955).

Alternatively, spores originating in America could have migrated to Hawaii via the northern trade winds, which is a large mass of low elevation winds supplied by the North Pacific anticyclone. Traveling southwest, the trade winds pass over most of the Hawaiian Islands, which could explain how spores were dispersed from America (Wright et al., 2001).

A third climate-based method of dispersal via the ITCZ, was proposed (Wright et al., 2001) in order to explain an unusual case of trans-equatorial dispersal

of *Metrosideros polymorpha* (Myrtaceae) seeds from the Marquesas Islands to the Hawaiian Islands. Phylogenetic analyses strongly suggested a relationship between Hawaiian *Metrosideros* and those of the Marquesas Islands, 3000 km south southeast of the Hawaiian Archipelago. In order to reach the Hawaiian Islands, the seeds must cross the equatorial zone, an area marked by climatic discontinuity, then pass through the Northeastern trade winds. The ITCZ lies between the northern and southern hemispheres and denotes the atmospheric discontinuity which usually restricts low altitude air masses (possibly carrying spores) from crossing the equator (Wright et al., 2001). However, the ITCZ drifts south in the late summer/early fall period, and occasionally incorporates the Marquesas Islands into a northern hemisphere circulation. If equatorial Hadley cells move air (carrying spores from the southern hemisphere) northward away from the equator, eventually the air will descend just northeast of the Hawaiian Archipelago (Wright et al., 2001). The northeasterly trade winds could then transport the spores carried in the Hadley cells to their final destination, the Hawaiian Archipelago (Wright et al., 2001). This scenario is the third proposed mechanism, accounting for trans-equatorial dispersal.

The goals of this thesis were two-fold. First, the phylogenetic relationships among Hawaiian *Cibotium* species and their non-Hawaiian relatives were examined. Fosberg (1948) hypothesized that Hawaiian *Cibotium* are monophyletic, having a single colonizing ancestor. This hypothesis remains largely untested and since plants could have been carried to Hawaii from several directions, it is possible that *Cibotium* has multiple colonizing ancestors. Geiger and Ranker (2005) observed that the endemic species of Hawaiian *Dryopteris* were not monophyletic and that there were

at least five different successful dispersal events of dryopteroid ferns to Hawaii.

Using this phylogenetic framework, *Cibotium*'s biogeographical origins and method of long distance dispersal to Hawaii were also investigated in this study.

Accordingly, the second objective of my research was to clarify *Cibotium*'s origins, by testing Fosberg's general hypotheses of origin through comparative analyses of sequences of the *atp β* gene. As a polymorphic species, *Cibotium* was an excellent candidate for molecular analysis. I hypothesized that *Cibotium* is monophyletic and that the colonizing ancestor(s) which gave rise to the four Hawaiian species, originated in the Indo-Pacific region.

Materials and Methods

Taxon sampling and DNA extraction

Samples from seven species of *Cibotium* (Dicksoniaceae), including three currently recognized Hawaiian species, one of which is represented by two individuals (*C. scheidei*), along with two species from North and Central America, and two species from Southeast Asia, were analyzed in this study (Table 1). Past morphological data (Gastony, 1981) suggest that a number of species in the Dicksoniaceae and Cyatheaceae families (both Order Cyatheaales) are closely related; and from these families, eleven outgroup species were sampled. A total of eighteen pteridophyte species were analyzed (Table 1).

The chief source of DNA was extracted from samples collected by colleagues in the field and preserved in silica gel. In some cases, DNA was extracted from the leaf material of herbarium specimens donated by various collectors. Several new *atpβ* sequences were generated in this study and will eventually be submitted to GENBANK. Genomic DNA was extracted from 0.03 g of these silica gel dried or herbarium specimens using the FastDNA® kit protocol with the FastPrep® Instrument supplied by Q-Biogene. Samples were homogenized in the FastPrep® Instrument at speed 4.0 for 30 seconds.

Table 1. Species, collection localities, collectors, and native distribution of taxa

Species	Family	Collection Locality	Collector and number	Native Distribution
<i>Alsophila spinulosa</i> Wall. ex Hook	Cyatheaceae	Taiwan	Ranker 2056	India, Taiwan, China, Thailand, Philippines
<i>Alsophila tahitensis</i> (Brackenr.) Copel.; Bull. Bernice P.	Cyatheaceae	Tahiti	Ranker 1910	Tahiti
<i>Calochlaena villosa</i> (C. Chr.) M. D. Turner & R. A. White	Dicksoniaceae	Unknown	Unknown	Australia, Papua New Guinea, Indonesia
<i>Cibotium barometz</i> (L.) J. Sm.	Dicksoniaceae	Taiwan	Ranker 2094	Assam, Taiwan, SE Asia, S. Pacific Islands
<i>Cibotium chamissoi</i> Kaulf	Dicksoniaceae	USA	Ranker 1873	Hawaii
<i>Cibotium cummingii</i> Kunze	Dicksoniaceae	Taiwan	Yi-Hsiu Lai 105	Taiwan, Borneo, Philippines
<i>Cibotium glaucum</i> (Sm.) Hook. & Arn.	Dicksoniaceae	USA	Ranker 1895	Hawaii
<i>Cibotium menziesii</i> Hook.	Dicksoniaceae	USA	Ranker 1996	Hawaii
<i>Cibotium nealea</i> Degen.	Dicksoniaceae	USA	Ranker 1994	Hawaii
<i>Cibotium regale</i> Verschaff & Lem.	Dicksoniaceae	Unknown	RBGE-19991856	Central America, Mexico, Honduras
<i>Cibotium schiedei</i> Schlecht. & Cham.	Dicksoniaceae	Unknown	RBGE - 19696415	Mexico
<i>Cyathea metteniana</i> (Hance) C. Chr. & Tardieu	Cyatheaceae	Taiwan	Ranker 2066	China, Indochina, Taiwan, Japan
<i>Cyathea speciosa</i> Humb. & Bonpl. ex Willd.	Cyatheaceae	Unknown	Lorence 9017	Colombia, Venezuela
<i>Dicksonia antarctica</i> * Labill	Dicksoniaceae	Australia	W.A. Weber 13898	SE Australia, Tasmania
<i>Dicksonia arborescens</i> L'Her.	Dicksoniaceae	Unknown	RBGE-20000259	St. Helena
<i>Dicksonia blumei</i> Moore	Dicksoniaceae	Unknown	RBGE - 19530207	Sumatra, Philippines
<i>Dicksonia squarrosa</i> Swartz.	Dicksoniaceae	New Zealand	Pukieti Rhododendron Trust; RBGE - 20030795	New Zealand
<i>Lophosoria quadripinnata</i> (J.F. Gmel.) C. Chr.	Lophosoriaceae	Chile	RBGE - 20041842	Mexico, Honduras, C. America, S. America
<i>Thyrsopteris elegans</i> Kunze	Dicksoniaceae	Unknown	RBGE - 20031267	Unknown

* Asterik denotes that this species has a GenBank accession number: U93829

PCR amplification and sequencing

The *atpβ* segment, a 1497 bp region of the cpDNA genome was PCR-amplified and sequenced. PCR amplification was accomplished by using a combination of primers designed by Wolf (1997) in 50 μL reactions, following the conditions described in Table 2. I amplified *atpβ* as four overlapping fragments, using four primer pairs: 470F + 609R, 672F2 +1334R, 1163F2 +1592R, and 1419F + 1365R; sequencing primers were 470F, 965F, 1163F2, and 1419F, respectively. Repeated sequencing attempts using 672F2+1334R failed in most cases and modifications were made by pairing 1334R with 965F. The PCR conditions of Ranker et al. (2003) were used with modifications shown in Table 2.

PCR products were purified, using ExoSAP-IT (USD Corp.), then diluted before being sent to Macrogen Sequencing in South Korea. ChromasPro Version 1.32 (Technelysium Pty Ltd.) was used to edit all sequences by means of visual inspection of electropherograms. All *atpβ* fragments were edited manually and the complete *atpβ* sequence of *Dicksonia antarctica* was used to assist in manual adjustments which allowed more accurate alignments of the base pairs. For all DNA sequences studied, only the *Dicksonia antarctica* sequence was registered in GenBank (Table 1).

Table 2. Primer pairs, reaction reagents, and cycling conditions used for PCR amplification and sequencing of *atpβ*.

Primers	PCR Reaction Reagents (per rxn)	Cycling Conditions
470F + 609R	PCR buffer: 5.0μL, MgCl ₂ : 5.0μL, dNTP: 4.0μL, Primer 470F: 1.5μL, Primer 609R: 1.5μL, Taq: 0.15μL, BSA: 2.5μL, dH ₂ O: 28.9μL, Sample DNA: 1.5μL	1.)94°C(180s) 2.)94°C(60s) 3.)42°C(60s) 4.)72°C(180s) 5.)repeat 2-4, 35X 6.)72°C(600s)
672F2+1334R	PCR buffer: 5.0μL, MgCl ₂ : 3.0μL, dNTP: 4.0μL, Primer 672F2: 2.0μL, Primer 1334R: 2.0μL, Taq: 0.30μL, BSA: 2.5μL, dH ₂ O: 27.2μL, Sample DNA: 3.0μL	1.)94°C(45s) 2.)94°C(60s) 3.)42°C(60s) 4.)72°C(180s) 5.)repeat 2-4, 35X 6.)72°C(600s)
965F+1334R	PCR buffer: 5.0μL, MgCl ₂ : 5.5μL, dNTP: 4.0μL, Primer 965F: 1.75μL, Primer 1334R: 1.75μL, Taq: 0.23μL, BSA: 2.5μL, dH ₂ O: 28.9μL, Sample DNA: 3.0μL	1.)94°C(45s) 2.)94°C(60s) 3.)40°C(60) 4.)72°C(180s) 5.)repeat 2-4, 35X 6.)72°C(600s)
1163F2+1592R 1419F+1365R	PCR buffer: 5.0μL, MgCl ₂ : 3.0μL, dNTP: 4.0μL, Primer 1163F2: 2.0μL, Primer 1592R: 2.0μL, Taq: 0.30μL, BSA: 2.5μL, dH ₂ O: 27.2μL, Sample DNA: 3.0μL	1.)94°C(180s) 2.)94°C(60s) 3.)42°C(90s) 4.)72°C(120s) 5.)repeat 2-4, 30X 6.)72°C(700s)

Phylogenetic analyses

Two phylogenetic analyses, maximum parsimony (MP) and maximum likelihood (ML), were conducted on the *atpβ* data set. First, MP analysis was performed as implemented in PAUP* 4.0 (Swofford, 2002). All characters were unordered and equally weighted. I employed the heuristic search algorithm with 1000 random addition sequence replicates with MulTrees activated, and with TBR branch swapping. Also, using the same settings as above, bootstrap analyses were conducted with 1000 repetitions and ten random stepwise addition replicates.

Second, ModelTest 3.7 (Posada and Crandall, 1998) was used to determine the model of evolution that best fit the data for maximum likelihood (ML) analysis. The GTR + I + G (Tavare, 1986) was the best fit model for ML. A ML bootstrap analysis was performed with 100 repetitions and 10 random stepwise addition replicates each.

Results

For the eighteen *atpβ* sequences obtained, 1300 bp of the gene were sequenced. Across these sequences, 1218 bp were invariant, 112 bp were variable, and 73 bp were parsimony-informative.

The heuristic MP analysis of the *atpβ* dataset produced one most parsimonious tree, characterized by a length (L) of 158 steps, a consistency index (CI) of 0.75 (including uninformative characters), and a retention index (RI) of 0.84 (Fig. 1). The model of data that best explained the entire *atpβ* dataset used in maximum likelihood analysis was the GTR + I + G (Tavare, 1986). The most parsimonious tree was identical to the ML bootstrap topology (Fig. 1). However, the tree was not well resolved, as indicated by the number of polytomies. There was also low branch support for the placement of the *Cibotium* genus (52% MP bootstrap) (Fig. 1.).

The monophyly of *Cibotium* is 100% supported by both the MP and ML bootstrap values. Within the *Cibotium* clade, one sequence of *C. schiedei* is the first-branching species and all seven *Cibotium* species form a well-supported clade (100% MP bootstrap). The Hawaiian species weren't supported as monophyletic, as *C. menziesii* was weakly supported (52% MP bootstrap) in the MP analysis as sister to the remaining *Cibotium* species. *C. chamissoi* and *C. glaucum* fell out among the rest of the species in the unresolved clade. These analyses grouped the sequences for the two *C. schiedei* samples separately, suggesting that the sequences were different for the same species.

Relationships among outgroup taxa that were enforced by both ML and MP analyses (100% bootstrap support) include the sister-taxon relationship between *Calochlaena villosa* and *Cyathea speciosa*, and the sister-taxon relationship between *D. squarrosa* and *D. antarctica*. From these analyses, all outgroup genera for which there was more than one species represented were supported as either paraphyletic or polyphyletic (Fig. 1).

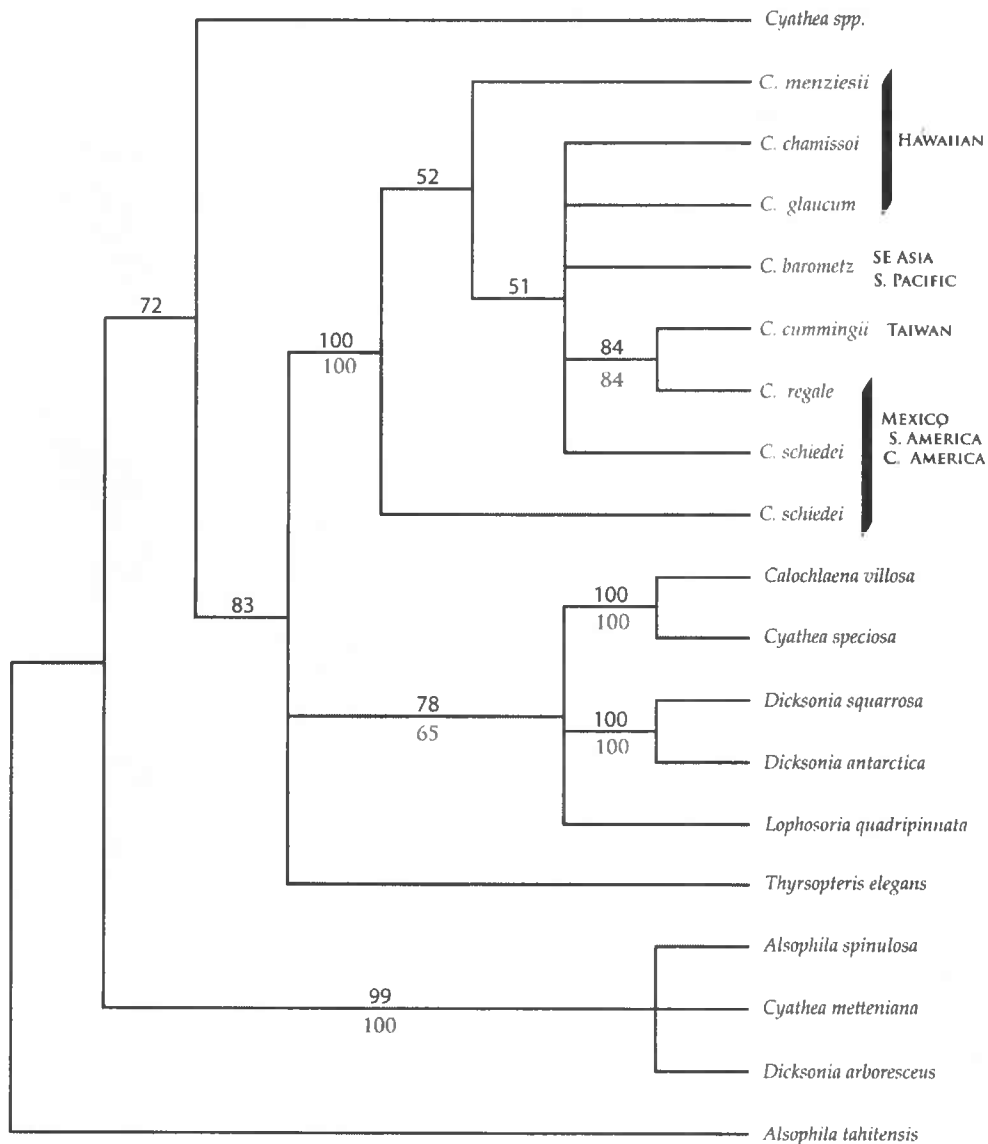


Fig. 1. Maximum parsimony bootstrap consensus tree for all sampled taxa based on the analysis of *atpβ* sequences. Continental locations are shown to the right of taxon names. Maximum parsimony bootstrap values are above branches; maximum likelihood bootstrap values are below branches where maximum likelihood analysis resolved the same relationships.

Discussion

Both MP and ML analyses of the *atpβ* data set strongly support my hypothesis that *Cibotium* is monophyletic, thus suggesting that the Hawaiian species share a common ancestor with continental *Cibotium*. However, the analyses provide a fairly uncertain relationship between the Hawaiian *Cibotium* and their potential ancestor. Only parsimony analyses weakly suggests that *Cibotium* has a South American origin, maximum likelihood does not (Fig. 1); and the analyses are contrary to Fosberg's (1948) Indo-Pacific hypothesis. The South American region is identified as the potential biogeographical origin because of the relationship between *C. schiedeii* and all other *Cibotium*. A South American origin is also supported by phylogenetic analyses using *Cibotium rbcL* and chloroplast intergenic spacers *trnL-F* (Kleist; 2006 in prep) and *trnG-R* sequences.

In order to arrive in Hawaii, spores from South America may have been dispersed via the coordinated movement of the ITCZ band, trade winds, and Hadley cells (Wright et al., 2001). This phenomenon occurred in the unique dispersal event of *Metrosideros* spores of the Marquesas Islands to Hawaii, but is rare because of the obstacle posed by crossing the equator (Wright et al., 2001). Further conclusions regarding the biogeographical relationships among the species within the *Cibotium* genus can not be drawn due to the lack of resolution in the phylogenetic tree.

The lack of resolution may have occurred because the entire *atpβ* sequence could not be obtained from every sample. Problems with primer pairs prevented the collection of all 1300 bp used in sequence analyses, creating a margin of error. If variable segments were missing, it is very likely that erroneous relationships would

be indicated by MP and/or ML trees. Also, the entire *Cibotium* genus was not represented in the study, including one of the four Hawaiian species. Consequently the phylogenetic relationships were not resolved and could not be estimated among the entire genus.

Although useful in resolving relationships within families, *atpβ* sequences may not be capable of resolving relationships among species within a genus. The next step in this study would be to combine the *atpβ* dataset with *rbcL* and the chloroplast intergenic spacers *trnL-F* and *trnG-R* to test the evolutionary relationships among the studied species in more detail (Wolf, 1997). The geographical spread of the sampled species should also be broadened to include more species from South America as well as all nine *Cibotium* species. The biogeographical origins of *Cibotium* could not be obtained from these analyses; however evidence does support my hypothesis that Hawaiian *Cibotium* is monophyletic.

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