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GLOBAL CHLOROPLAST PHYLOGENY AND BIOGEOGRAPHY OF BRACKEN (*PTERIDIUM*; DENNSTAEDTIACEAE)[1]

JOSHUA P. DER,[2,4] JOHN A. THOMSON,[3] JERAN K. STRATFORD,[2,5] AND PAUL G. WOLF[2]

[2]Department of Biology, Utah State University, 5305 Old Main Hill, Logan, Utah 84322 USA; and
[3]National Herbarium of New South Wales, Botanic Gardens Trust, Mrs Macquaries Road, Sydney, NSW 2000, Australia

Bracken ferns (genus *Pteridium*) represent an ancient species complex with a natural worldwide distribution. *Pteridium* has historically been treated as comprising a single species, but recent treatments have recognized several related species. Phenotypic plasticity, geographically structured morphological variation, and geographically biased sampling have all contributed to taxonomic confusion in the genus. We sampled bracken specimens worldwide and used variable regions of the chloroplast genome to investigate phylogeography and reticulate evolution within the genus. Our results distinguish two major clades within *Pteridium*, a primarily northern hemisphere Laurasian/African clade, which includes all taxa currently assigned to *P. aquilinum*, and a primarily southern hemisphere Austral/South American clade, which includes *P. esculentum* and *P. arachnoideum*. All European accessions of *P. aquilinum* subsp. *aquilinum* appear in a monophyletic group and are nested within a clade containing the African *P. aquilinum* taxa (*P. aquilinum* subsp. *capense* and *P. aquilinum* subsp. *centrali-africanum*). Our results allow us to hypothesize the maternal progenitors of two allotetraploid bracken species, *P. caudatum* and *P. semihastatum*. We also discuss the biogeography of bracken in the context of the chloroplast phylogeny. Our study is one of the first to take a worldwide perspective in addressing variation in a broadly distributed species complex.

Key words: allopolyploidy; Dennstaedtiaceae; fern; hybridization; phylogeography; population; *Pteridium*; worldwide.

Describing any biological characteristic of organisms requires adequate sampling so that statements are universally valid. Thus, one should consider the range of variation within the taxon (be it taxonomic, morphological, physiological, ecological, genetic, or geographic) to accurately describe diversity within the group (Hillis, 1998). To capture such variation, one must sample sufficiently to understand both the limits and typical ranges of variation. Such sampling means collecting and examining representatives across the distribution of the taxon. In general, sampling across a taxon's range is easier to do for lower taxonomic levels because successively smaller clades encompass increasingly narrow ranges. However, sampling across geographic ranges becomes logistically challenging for species or species complexes with worldwide (or nearly so) distributions.

Examples of widespread species for which a global perspective has been taken include highly mobile birds (Burg and Croxall, 2004), invertebrates (Lee, 2000; Boyer et al., 2007), and fungi (Hibbett, 2001; Banke and McDonald, 2005). Within green plants, worldwide distributions are common among "invasive" species, i.e., those with recent (less than 500 years), human-mediated, widespread distributions. Examples include aquatic weeds (Barrett, 1989) among others (Holm et al., 1997). At least one study has examined evolutionary history in a widespread invasive species, *Cardamine flexuosa* (Lihová et al., 2006), but few examples, if any, have examined the phylogenetic structure of a species complex with an ancient (preagricultural; 10 000 years) worldwide distribution (but see Bakker et al., 1995). For a species to occupy such a broad range, it must have a wide ecological amplitude (i.e., ecological valence) or be able to adapt quickly to a wide range of local environmental conditions without speciation. Additionally, to achieve a wide distribution, a species must have a high dispersal and colonization ability, or be an old taxon with a broad ancestral distribution, or both. The genus *Pteridium* Gled. ex Scop. (bracken fern, Dennstaedtiaceae) represents one such taxon, having achieved a natural worldwide distribution and occupying diverse habitats (Page, 1976, 1986; Holm et al., 1997). Fossil evidence indicates that bracken had achieved a worldwide distribution by the Oligocene, ~23.8 mya (reviewed by Page, 1976), and several lines of evidence indicate that bracken can disperse and establish following long distance dispersal by spores (Punetha, 1991; Rumsey et al., 1991).

Pteridium is often treated as a monotypic genus after Tryon (1941), but most contemporary systematists recognize the genus as a species complex in need of taxonomic revision (Page, 1976; Brownsey, 1989; Page and Mill, 1995; Thomson, 2000b). Bracken ferns are easily recognized and well differentiated from other genera in Dennstaedtiaceae, and many infrageneric taxa within *Pteridium* have high levels of geographically based morphological structure. However, great confusion in defining infrageneric taxa has resulted from the fact that bracken has high levels of phenotypic plasticity, few diagnostic morphological characters, and the presence of intermediate phenotypes where different morphological forms come into contact, demonstrating that reproductive barriers are incomplete (Page, 1976). These factors, coupled with local taxonomic judgments

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[4] Author for correspondence (e-mail: jpder@biology.usu.edu)

[5] Present address: Lineberger Comprehensive Cancer Center, University of North Carolina-Chapel Hill, 450 West Drive, CB# 7295, Chapel Hill, NC 27599-7295 USA

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based on geographically biased sampling, has led to a large number of local forms being described as new species, subspecies, or varieties, resulting in a multiplicity of names (Tryon, 1941; Page, 1976; Thomson, 2000a). The genus is distributed worldwide and is notorious as a weed because of its exceptional ability to grow rhizomatously in dense patches, overgrowing open fields and pasture (Tryon, 1941; Holm et al., 1997).

Bracken ferns have a long and complex taxonomic history. The first bracken species were described by Linnaeus in the genus *Pteris* L. (Linnaeus, 1753). Later authors followed this generic circumscription, but Agardh (1839) was the first to examine specimens worldwide and set the brackens apart as section *Ornithopteris* J. Agardh (Tryon, 1941; Brownsey, 1989). Later, Hooker (1858), in a comprehensive treatement of *Pteris*, subsumed all brackens as varieties of *Pteris aquilina* L. Various authors segregated the brackens from *Pteris*, but it was not until Kuhn (1879) defined *Pteridium aquilinum* (L.) Kuhn that the brackens were widely accepted as a distinct genus (Tryon, 1941). The last global revision of the genus was Tryon's (1941) monograph, in which he reduced more than 135 previously named variants into a single species, with two subspecies containing 12 varieties. Subsequent authors have continued to modify the taxonomy of bracken, but most works reflect a geographically limited perspective (Brownsey, 1989; Page and Mill, 1995; Wolf et al., 1995; Speer, 2000; Thomson and Alonso-Amelot, 2002; Gureyeva and Page, 2005; Thomson et al., 2005, 2008). Recent evidence suggests that two *Pteridium* taxa have allopolyploid hybrid origins, and these two tetraploids are currently recognized as segregate species (Tan and Thomson, 1990b; Thomson, 2000a, b; Thomson and Alonso-Amelot, 2002).

Recent work has reexamined the systematic utility of morphological characters (Thomson and Martin, 1996), characterized the structure of the chloroplast genome (Tan and Thomson, 1990a; Tan, 1991), and used genetic fingerprinting to examine evolutionary history in *Pteridium* (Thomson, 2000a, b). Much of this work contributes toward a taxonomic revision of the genus. The typification of *Pteridium aquilinum* (L.) Kuhn has been revisited, and nomenclature within 'latiusculum' morphotypes (N. America, Europe and northeast Asia) has been clarified (Thomson, 2004; Thomson et al., 2008).

Global perspectives on phylogeography can be inferred using chloroplast DNA sequence variation to detect and reconstruct historical evolutionary events, including population demographics, migration, colonization, and both ancient and contemporary hybridization events (Rieseberg and Soltis, 1991; McCauley, 1995; Rieseberg et al., 1996; Ennos et al., 1999). Variation in chloroplast sequence data has been used to reconstruct phylogenetic relationships among plants as diverse and ancient as land plants to variation among recently diverged populations within a single species. *Pteridium* is a well-defined and evolutionarily isolated genus comprising several closely related taxa. Examining patterns of chloroplast variation presents an excellent opportunity to infer patterns of widespread dispersal, colonization, and divergent evolutionary history on a global scale. This study represents one of the only studies of worldwide variation in a terrestrial plant.

The objectives of this present study are threefold. First, we examine chloroplast DNA variation within *Pteridium* in a global phylogenetic context to ascertain evolutionary patterns of divergence, dispersal and colonization. Second, we examine maternal ancestry in the two tetraploid taxa hypothesized to be of hybrid origin. Third, we establish a clear framework for developing hypotheses of evolutionary history that can be tested with comparative nuclear sequence data. These results also provide important information necessary for taxonomic revision of infrageneric taxa in *Pteridium*.

MATERIALS AND METHODS

Taxonomic sampling—Sampling was designed to cover the range of morphological and geographical diversity within *Pteridium*, representing nearly all currently recognized species, and most infraspecific taxa, with the exception of *P. aquilinum* subsp. *feei* (W. Schaffn. ex Fée) J. A. Thomson, Mickel & Mehltreter, endemic to central Mexico. Determination of specimens used in this study follow Thomson and Alonso-Amelot (2002), Thomson (2004), and Thomson et al. (2005, 2008). In addition to the materials used here, a large series of specimens from major herbaria has been examined in the course of the taxonomic revisions listed. We sampled 77 bracken specimens, most of which were included previously in the taxonomic studies described. Tissue samples were collected from either wild sources or from sporophytes grown in a common garden derived from known wild sources and propagated from rhizome segments or mass spore sowings (Thomson, 2000a). Complete voucher information with geographic sources and GenBank accession numbers is provided in Appendix 1.

DNA extraction, PCR amplification, and sequencing—Total genomic DNA was extracted from tissue that had been silica-dried, freeze-dried, or pickled in CTAB/NaCl/ascorbate (Thomson, 2002). The chloroplast markers *trnS*^{GGA}–*rpS4* spacer+gene and *rpL16* intron were amplified in 25-μL polymerase chain reactions (PCR) using the fern-specific primers published in Small et al. (2005). Each PCR reaction contained 25–50 ng of template DNA, 1× Promega PCR buffer (Promega, Madison, Wisconsin, USA), 1.5 mM $MgCl_2$, 0.20 mM of each dNTP, 0.25 μM each of the forward and reverse primers, and ~12.5 U *Taq* polymerase. Most PCR reactions were completed under a standard temperature cycle procedure beginning with a 2 min denaturation step at 94°C; followed by 30 cycles of 94°C, 48°C and 72°C each for 1 min; finishing with a 7 min elongation step at 72°C. Problematic samples were amplified using a slow ramp thermal cycle protocol that began with a 2 min denaturation step at 95°C, followed by 30 cycles in which the sample was denatured at 95°C for 1 min, dropped to 45°C for 1 min, then slowly raised to 68°C at a rate of 0.2°C/sec and held at 68°C for 4 min. After cycling, reactions were held at 68°C for 10 min to complete elongation of PCR products. PCR products were directly cycle-sequenced in both directions using the PCR primers and ABI BigDye Terminator version 3.1 chemistry (Applied Biosystems, Foster City, California, USA).

Sequence alignment and phylogenetic analyses—Sequences were checked against electropherograms and manually edited, if necessary, using the program 4Peaks version 1.7 (Griekspoor and Groothuis, 2006). Sequences were manually aligned using the program Se-Al version 2.0a11 (Rambaut, 2002). The aligned concatenated data matrix is available in TreeBase (http://www.treebase.org; study number S2235). Maximum parsimony (MP) analyses were performed on the two-marker concatenated data set in the program PAUP* version 4.0b10 (Swofford, 2002). All nucleotide site characters were unordered and equally weighted, treating gaps as "missing" data. Heuristic MP searches used tree-bisection-reconnection (TBR) branch swapping on starting trees generated from 10 000 random stepwise addition sequence replicates, holding 10 trees at each addition step. All the most parsimonious trees were saved, and the strict consensus of these was calculated. MP bootstrap support was assessed from 10 000 bootstrap replicates using heuristic searches with TBR branch swapping on starting trees generated from 10 random stepwise addition sequence replicates and saving all the most parsimonious trees.

Models of DNA sequence evolution used in Bayesian phylogenetic inference (BI) were selected for each chloroplast marker using the second order Akaike information criterion (AICc) implemented in the program MrModelTest version 2.2 (Nylander, 2004), using likelihood scores estimated in PAUP* for the neighbor-joining tree under alternative models of evolution. The total number of alignment sites in each data partition was used as the sample size for AICc calculations (Posada and Buckley, 2004). Akaike weights and evidence ratios were examined to help guide selection of the best-fit model of molecular evolution for each partition (Burnham and Anderson, 2002).

Bayesian analysis was performed in the parallel version of the program MrBayes version 3.1.2 (Ronquist and Huelsenbeck, 2003; Altekar et al., 2004). Data were partitioned for the two chloroplast markers, allowing model parameters for each partition to vary independently while linking topology and branch

lengths across partitions. Two independent runs, with six chains each, were conducted simultaneously for 10 000 000 generations. Parameter estimates were sampled every 1000 generations. The average standard deviation of split frequencies and the potential scale reduction factor (PSRF) were calculated after discarding the first 25% of the generations (2.5 million generations) as burn-in to assess topological and parameter convergence (respectively) between the two runs. Bayesian inference clade credibility values (i.e., posterior probabilities for clades) and tree posterior probabilities were also calculated after the first 2.5 million generations were discarded as burn-in.

Alignment of two outgroup taxa [*Dennstaedtia davalloides* (R. Br.) T. Moore and *Hypolepis muelleri* N. A. Wakef.] with the *Pteridium* sequences was not possible due to a high level of divergence (both sequence substitution saturation and ambiguous indels). Inclusion of these taxa in preliminary phylogenetic analyses contributed to the destabilization of clades within *Pteridium*. Because of this, the midpoint rooting method implemented in PAUP* was used to root the *Pteridium* phylogeny in both MP and BI analyses. While this approach is not ideal, it has yielded acceptable results in other taxa when appropriate data are not available (Schuettpelz and Hoot, 2006). The monophyly of *Pteridium* and the midpoint root was confirmed by outgroup phylogenetic analysis of *rbcL* sequences from a subset of our sampled *Pteridium* taxa aligned with additional outgroup taxa from Dennstaedtiaceae (data not shown).

Biogeographic analysis—Broad geographic regions were mapped onto the phylogeny using the parsimony criterion to elucidate major historical biogeographic events. Geographic areas were coded as six unordered character states (Hawaii, North/South America, Europe, Asia/India, Africa, Austral) and traced onto the tree using the program Mesquite version 2.5 (Maddison and Maddison, 2008). Specimens in cultivation were coded from the geographic area of their source.

RESULTS

Sequence characteristics—The aligned *trnS–rpS4* spacer+gene and *rpL16* intron data matrices included 1018 and 753 nucleotide sites with 29 and 25 variable sites, respectively. Of those variable sites, 22 were parsimony-informative in the *trnS–rpS4* spacer+gene, and 21 sites were parsimony-informative in the *rpL16* intron. There were two 5-bp repeat sequence indels located 32 nucleotides apart in the *trnS–rpS4* spacer. Individuals either lacked one or both of the repeat sequences, resulting in three observed indel haplotypes. These haplotypes have been reported previously (Thomson et al., 2008), and our study is consistent with those findings, so we adopt their nomenclature here (haplotypes A, B, and C). This study newly establishes the haplotypes for *Pteridium aquilinum* subsp. *decompositum* (Gaudich.) Lamoureux ex J. A. Thomson, *P. caudatum* (L.) Maxon, and *P. semihastatum* (Wall. ex J. Agardh) S. B. Andrews, and extends coverage of *P. aquilinum* subsp. *pinetorum* (C. N. Page & R. R. Mill) J. A. Thomson to eastern Europe. Haplotype B (the presence of the first repeat sequence, GTTTT) was observed in *P. aquilinum* subsp. *aquilinum* from Europe and both *P. aquilinum* subsp. *centrali-africanum* Hieron. and *P. aquilinum* subsp. *capense* (Thunb.) C. Chr. in Africa, while haplotype C (the presence of the second repeat sequence, AGTCT) was observed in *P. arachnoideum* (Kaulf.) Maxon in Central and South America, *P. esculentum* (G. Forst.) Cockayne in Australia, New Zealand, and New Caledonia, *P. semihastatum* in Australia and Malaysia, and a single accession of *P. caudatum* from Costa Rica. Haplotype A (the absence of both repeat sequences) was observed in the remaining taxa (i.e., *P. aquilinum* from Asia and North America and the remaining *P. caudatum* accessions). Comparison of bracken haplotypes with those found in *Dennstaedtia davalliodes* and *Hypolepis muelleri* reveals that haplotype C is likely to be plesiomorphic. Parsimony character reconstruction of these indels on our phylogeny indicates that the second repeat was lost in the common ancestor of *P. aquilinum*, and the first repeat was gained in the common ancestor of the African and European *P. aquilinum* subspecies. The evolution of these indels is mapped on the Bayesian phylogeny (Fig. 1).

Phylogenetic analyses—Maximum parsimony (MP) analysis of the concatenated data set resulted in 12 most parsimonious trees with a length of 59 (consistency index, CI = 0.9153; retention index, RI = 0.9892). The best fit model used in Bayesian inference (BI) was GTR+Γ and HKY+Γ for *trnS–rpS4* spacer+gene and *rpL16* intron, respectively. The AICc weight of the best fit model was 1.4 times greater than the next-best model for *trnS–rpS4* spacer+gene and 1.3 times greater for *rpL16* intron. The topology of the MP strict consensus tree (Fig. 2A) is congruent with the BI phylogeny (Fig. 1). MP bootstrap support (BS) and BI posterior probabilities of clades (PP, i.e., clade credibility values) are reported on the tree for supported nodes (Fig. 1). Two fully supported clades are resolved at the base of the phylogeny, separating *P. arachnoideum*, *P. esculentum*, and *P. semihastatum* from *P. aquilinum.* There is a basal polytomy within the *P. aquilinum* clade. A single accession of the tetraploid species *P. caudatum* is grouped with *P. semihastatum* in the former clade, while the remaining *P. caudatum* accessions are grouped with subspecies *pseudocaudatum* (Clute) Hultén in the *P. aquilinum* clade.

Biogeographic analysis—Mapping geographic areas onto the phylogeny revealed a number of biogeographic patterns (Fig. 2A). The Austral taxon *P. esculentum* is found in a clade with *P. arachnoideum* from South and Central America and *P. semihastatum* from Southeast Asia and Australia, forming a group with a post-Gondwanan (after Africa split away) southern continental distribution referred to here as the Austral/South American clade. Within the *P. aquilinum* clade, basal biogeographic patterns are unresolved, but the European *P. aquilinum* subsp. *aquilinum* accessions emerge from within the African brackens and *P. aquilinum* subsp. *decompositum* in Hawaii is in a clade with all of the sampled accessions of *P. aquilinum* subsp. *pubescens* (Underw.) J. A. Thomson, Mickel & Mehltreter from western North America. The locations of specimens included in this study are indicated on a global map (Fig. 2B).

DISCUSSION

Phylogenetic relationships among bracken taxa—*Pteridium* species form two distinct and fully supported basal clades (100% maximum parsimony bootstrap—BS, 1.0 Bayesian posterior probability—PP). The first clade contains *P. esculentum* from Australia, New Caledonia, and New Zealand and *P. arachnoideum* from the neotropics, but neither of these species form distinct monophyletic groups. The tetraploid species *P. semihastatum* and a single accession of the tetraploid species *P. caudatum* from Costa Rica are included in this first main clade and together are monophyletic (85% BS, 1.0 PP). This tetraploid group is derived from a clade containing an accession of *P. esculentum* from New Caledonia (55% BS, 0.99 PP). The second main clade in *Pteridium* includes all of the *P. aquilinum* accessions we sampled and three of the four *P. caudatum* accessions we included in our analysis (Costa Rica and northern South America). This second main *Pteridium* clade (the *P. aquilinum* clade) is fully supported as monophyletic (100% BS, 1.0 PP), but phylogenetic relationships of lineages within this

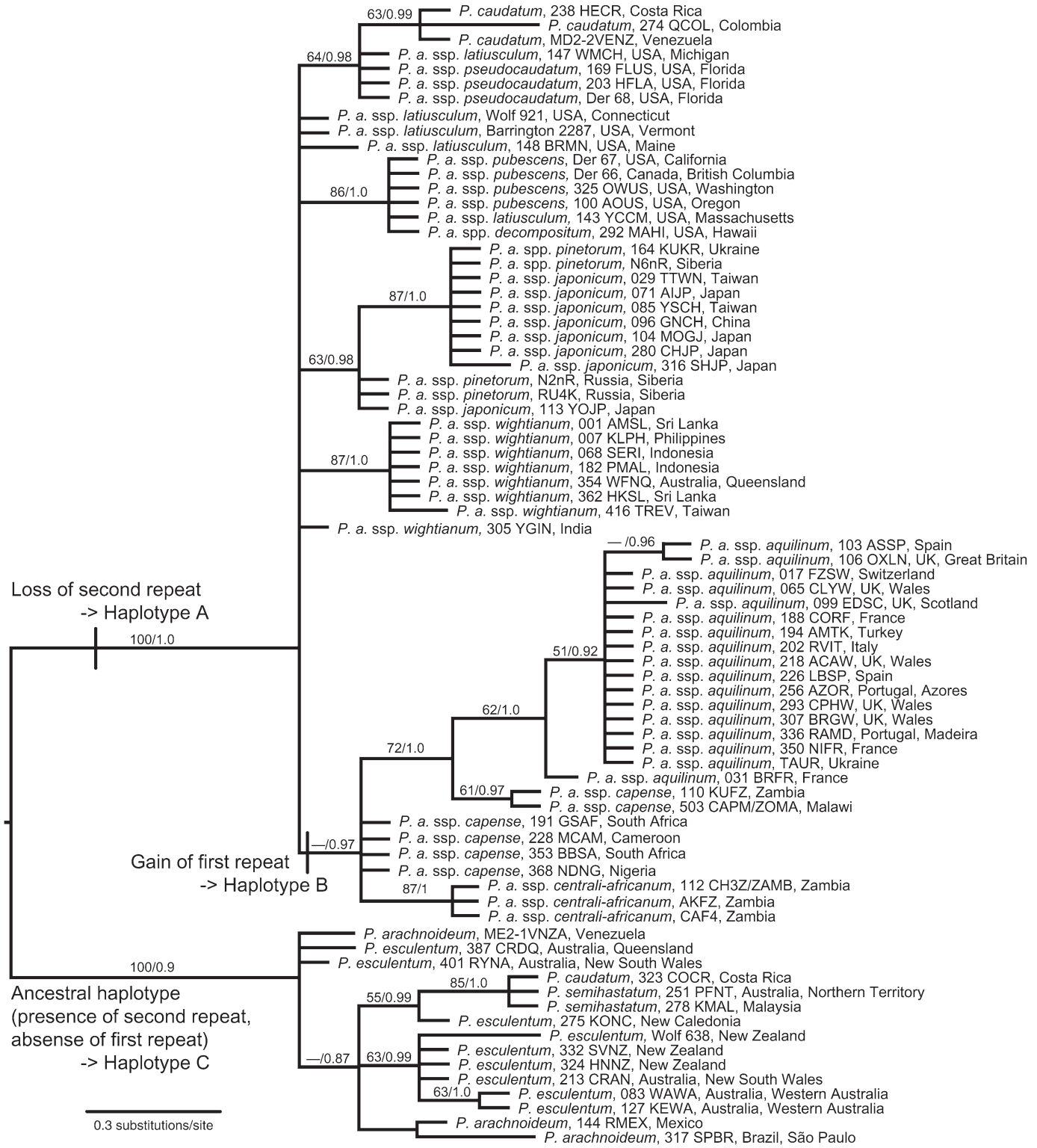


Fig. 1. Bayesian phylogram inferred from the concatenated *trnS-rpS4* spacer+gene and *rpL16* intron alignment. Branch lengths are proportional to the number of nucleotide substitutions/site. Support values from maximum parsimony bootstrap analysis (BS) and Bayesian posterior probabilities for clades (PP) are given above branches (BS/PP). Clades receiving less than 50% BS or 0.5 PP are indicated with a dash (—).

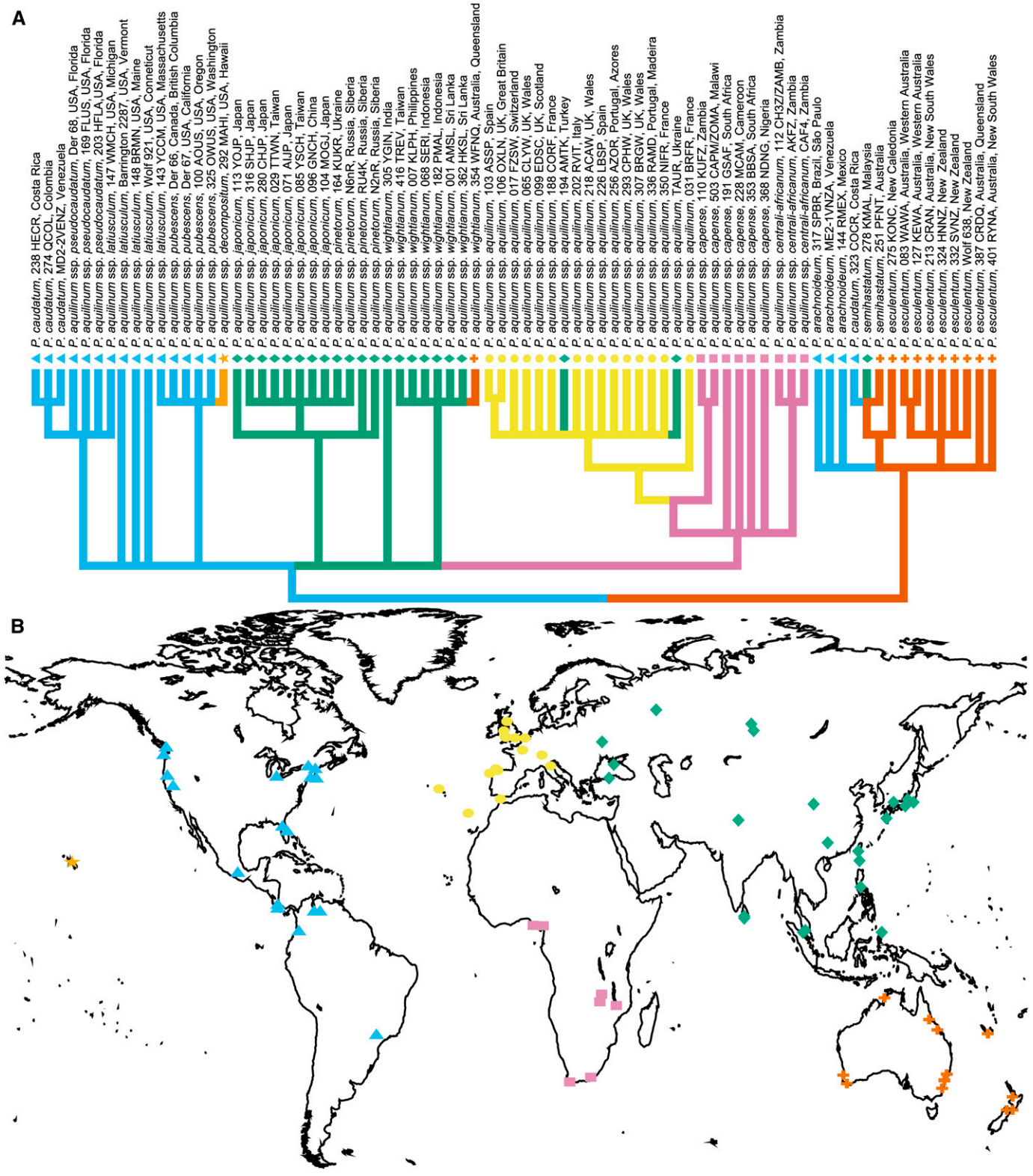


Fig. 2. Biogeography of bracken. (A) Broad geographic distribution of bracken specimens traced onto the maximum parsimony phylogeny using parsimony-based character reconstruction. Geographic areas were coded as six unordered character states: Hawaii, North/South America, Europe, Asia/India, Africa, Austral. (B) Global map of sampled bracken specimens, color-coded consistently with the geographic area character states in (A).

group are not resolved. The three *P. caudatum* accessions in the *P. aquilinum* clade are supported as monophyletic with 63% BS and 0.99 PP and are included in a polytomy with all of the *P. aquilinum* subsp. *pseudocaudatum* accessions we sampled (Florida, USA) and a *P. aquilinum* subsp. *latiusculum* (Desv.) Hultén accession from Michigan, USA (64% BS, 0.98 PP). *Pteridium aquilinum* subsp. *latiusculum* accessions are scattered throughout the *P. aquilinum* clade and therefore is not monophyletic in our analyses. This phylogenetic pattern in *P. aquilinum* subsp. *latiusculum* may be explained if this taxon is primarily defined by plesiomorphies (morphologically) and/or if it is susceptible to widespread hybridization (Speer et al., 1998). Together, *P. aquilinum* subsp. *japonicum* (Nakai) Á. Löve & D. Löve from coastal Asia and *P. aquilinum* subsp. *pinetorum* from the boreal Asian interior form a clade supported by 63% BS and 0.98 PP, but neither subspecies forms a monophyletic group. *Pteridium aquilinum* subsp. *decompositum* (Gaudich.) Lamoureux ex J. A. Thomson from Hawaii and *P. aquilinum* subsp. *pubescens* from western North America and a single *P. aquilinum* subsp. *latiusculum* accession from Massachusetts, USA, form a polytomy, supported by 86% BS and 1.0 PP. This pattern suggests a trade wind dispersal route from North America to Hawaii consistent with other wind-dispersed fern taxa (Geiger et al., 2007). *Pteridium aquilinum* subsp. *wightianum* (J. Agardh) W. C. Shieh from the Himalayas of northern India (isolate YGIN) emerges from the *P. aquilinum* clade polytomy, while the remaining *P. aquilinum* subsp. *wightianum* accessions from Sri Lanka and Southeast Asia form a monophyletic group supported by 87% BS and 1.0 PP. Isolate YGIN was also distinguished from other *P. aquilinum* subsp. *wightianum* by both a distinctive nuclear genome marker and morphology in an earlier study (Thomson, 2000a). All the *P. aquilinum* subsp. *aquilinum* accessions we sampled (Europe) are monophyletic (62% BS, 1.0 PP), but emerge from a paraphyletic grade of African *P. aquilinum* subsp. *capense*. Also emerging from *P. aquilinum* subsp. *capense* is *P. aquilinum* subsp. *centrali-africanum*, which is supported as monophyletic with 87% BS and 1.0 PP.

We emphasize here that apparent paraphyly at the level of species or below in our data set should not be used to recircumscribe taxa for two reasons. First, we have examined only variation in chloroplast DNA. Because of lineage sorting and recombination, we have no reason to expect exactly the same patterns for nuclear genes (Soltis et al., 1992). Second, some models of speciation predict a high frequency of paraphyly among recently diverged species (Rieseberg and Brouillet, 1994; Funk and Omland, 2003).

Hybridization and the origin of the tetraploid species—Although the majority of *Pteridium* taxa are at the same ploidy level ($2N = 104$; Page, 1976), recent evidence suggests that *P. semihastatum* and *P. caudatum* are tetraploid taxa ($4N = 208$; Tan and Thomson, 1990b; Thomson, 2000a, b; Thomson and Alonso-Amelot, 2002). Allopolyploidy appears to be a common speciation process in plants, and it often involves a triploid intermediate (Ramsey and Schemske, 1998). Two main types of evidence for allopolyploid speciation can be gathered. Nuclear markers, preferably fixed for different alleles in the different parents, can show additive effects in allotetraploids, and simultaneously reveal both parents, whereas uniparentally inherited markers will reveal one parent and whether there have been multiple origins (Soltis and Soltis, 1993). Although we have no evidence for the mechanism of inheritance of chloroplast DNA in *Pteridium*, several studies have demonstrated maternal inheritance in other species of ferns (Gastony and Yatskievych, 1992; Vogel et al., 1998).

Pteridium caudatum and *P. semihastatum* have been shown to be tetraploid bracken species based on DNA *c*-values, spore size, guard cell length, and morphology of the cells in the false indusium (Thomson, 2000a, b; Thomson and Alonso-Amelot, 2002). Hypothesized allopolyploid origins have been inferred from intermediate morphology and additive AP-PCR DNA genotypes of these two species (Thomson, 2000a; Thomson and Alonso-Amelot, 2002). These studies have suggested putative progenitors of *P. caudatum* to be *P. aquilinum* subsp. *pubescens* in the north and *P. arachnoideum* in the south (Thomson and Alonso-Amelot, 2002). These data have similarly been used to infer the putative progenitors of *P. semihastatum* in Southeast Asia and northern Australasia as *P. aquilinum* subsp. *wightianum* (=*P. revolutum* sensu Brownsey, 1989) and *P. esculentum* (Thomson, 2000a; Thomson and Alonso-Amelot, 2002). Our data based on the phylogeny of chloroplast sequences support the maternal parentage of *P. semihastatum* to be *P. esculentum*, while *P. caudatum* emerges from two parts of the phylogeny (with North American *P. aquilinum* subsp. *semicaudatum/latiusculum* and embedded in the Austral/South American clade, which includes *P. arachnoideum*, *P. esculentum*, and *P. semihastatum*). Two possible explanations for the phylogenetic placement of our *P. caudatum* accessions seem plausible: (1) reciprocal and multiple hybrid origins of this species or (2) a single hybrid origin of *P. caudatum* with the maternal parent as *P. aquilinum* subsp. *pseudocaudatum/latiusculum* with subsequent introgression of the tetraploid *P. semihastatum* chloroplast genomes. Additionally, the Austral/South American parent of *P. caudatum* may be an unsampled haplotype of the co-occurring *P. arachnoideum* from South America or long-distance hybridization from an Austral member of that clade. Long-distance hybridization in bracken has been documented between eastern North America and Scotland (Rumsey et al., 1991). To evaluate these hypotheses, we need nuclear genetic data to determine parentage and additivity of genomic elements in the allotetraploid species. Nuclear sequence data promise to be especially useful in elucidating specific phylogenetic relationships among the various bracken taxa (Schuettpelz et al., 2008).

Phylogeography of bracken—*Pteridium* occurs throughout the world, except in hot and cold desert regions. Range-wide sampling is necessary to adequately assess variation within the genus and delineate taxa. Only one such study has been accomplished in *Pteridium*: that of Tryon (1941). While the varieties in Tryon's treatment are often geographically based, he only briefly discussed the geographic ranges of each taxon and instead focused on the morphological differences between the varieties. Page (1976) expanded and updated the ecological and phytogeographic information known for each of Tryon's varieties, but emphasized that the adoption of Tryon's nomenclature was done for practical reasons rather than validation of these taxa and that an updated worldwide revision of the genus was needed. While our sampling strategy attempts to cover the range of variation in *Pteridium* worldwide, our study would benefit from additional sampling from Madagascar, South and Central America, the Caribbean, and parts of North America.

Recent phylogenetic analyses of ferns, which have included extensive taxonomic sampling and have used fossils for molecular date calibrations, have estimated divergence dates between *Pteridium* and *Dennstaedtia* to be about 114 mya (Schneider

et al., 2004) and 92 mya (Pryer et al., 2004). These estimates represent upper ages for the evolution of *Pteridium* and correspond to the Cretaceous period. Fragments of fossils attributable to *Pteridium* are known from the Tertiary period as far back as the Oligocene (33.7–23.8 mya) from Europe to Australia, suggesting that even by this time, bracken may have achieved a widespread distribution (Page, 1976). During the time when bracken likely originated and the earliest divergences may have occurred (from 100 to 30 mya), Africa and India separated from a southern landmass that included Australia, Antarctica, and South America (Scotese, 2001). This separation may correspond to the basal divergence among brackens, splitting the Austral/South American clade from the *P. aquilinum* clade of African and Laurasian affinity. The polytomy in the *P. aquilinum* clade may represent a rapid radiation of African and Indian bracken as they moved north, colliding with a warm temperate and paratropical Laurasia by the end of the Paleocene (about 50 mya). Alternatively, the polytomy in the *P. aquilinum* clade may result from insufficient data to distinguish phylogenetic relationships among lineages in the clade.

As continents have come to their modern global locations, and with the onset of the current ice age, the Quaternary glaciation beginning 2.5 mya, there have been periods of extensive ice sheets and cold, dry steppe coming into Europe and North America and likely driving bracken into southern refugia. These repeated glacial periods may be invoked to explain the phylogeographic pattern whereby the European brackens are derived from within the African taxa if Africa served as a source for colonization of Europe during recent geological times. The strong geographic structure observed at large (continental) scales among bracken taxa is consistent with patterns expected under a vicariance model, with range expansion across land bridges and only occasional long-distance dispersal resulting in hybrid forms. This evidence contradicts the common assumption in ferns that repeated long-distance dispersal of spores will erode biogeographic patterns resulting from vicariance in widespread species via constant gene flow (Tryon, 1985; Wolf et al., 2001). Recent empirical evidence in *Ceratopteris* Brongn. shows that genetic reproductive barriers can accumulate quickly in allopatry, thereby restricting gene flow and potentially maintaining the signature of vicariance (Nakazato et al., 2007).

Conclusions—This study highlights the importance of range-wide perspectives when undertaking evolutionary and monographic studies. Any taxonomic revision of *Pteridium* must reflect a global perspective and should include information from diverse areas of research, including morphology, cytology, genetics, and reproductive biology. To evaluate the biogeographic and hybrid origin hypotheses presented here, we will need a better-resolved species phylogeny with increased population-level sampling that incorporates nuclear data and fossils for date calibration. These data might also allow us to examine additional reticulation and lineage sorting in the evolutionary history of *Pteridium* as well as evaluate contemporary and historical gene flow.

LITERATURE CITED

AGARDH, J. G. 1839. Recensio specierum generis pteridis. Lundae typis Berlingianis, Berlin, Germany.

ALTEKAR, G., S. DWARKADAS, J. HUELSENBECK, AND F. RONQUIST. 2004. Parallel Metropolis coupled Markov chain Monte Carlo for Bayesian phylogenetic inference. *Bioinformatics* 20: 407–415.

BAKKER, F. T., J. L. OLSEN, AND W. T. STAM. 1995. Global phylogeography in the cosmopolitan species *Cladophora vagabunda* (Chlorophyta) based on nuclear rDNA internal transcribed spacer sequences. *European Journal of Phycology* 30: 197–208.

BANKE, S., AND B. A. MCDONALD. 2005. Migration patterns among global populations of the pathogenic fungus *Mycosphaerella graminicola*. *Molecular Ecology* 14: 1881–1896.

BARRETT, S. C. H. 1989. Waterweed invasions. *Scientific American* 261: 90–97.

BOYER, S. L., R. M. CLOUSE, L. R. BENAVIDES, P. SHARMA, P. J. SCHWENDINGER, I. KARUNARATHNA, AND G. GIRIBET. 2007. Biogeography of the world: A case study from cyphophthalmid Opiliones, a globally distributed group of arachnids. *Journal of Biogeography* 34: 2070–2085.

BROWNSEY, P. J. 1989. The taxonomy of bracken (*Pteridium*: Dennstaedtiaceae) in Australia. *Australian Systematic Botany* 2: 113–128.

BURG, T. M., AND J. P. CROXALL. 2004. Global population structure and taxonomy of the wandering albatross species complex. *Molecular Ecology* 13: 2345–2355.

BURNHAM, K. P., AND D. R. ANDERSON. 2002. Model selection and multimodel inference: A practical information-theoretic approach. Springer-Verlag, New York, New York, USA.

ENNOS, R. A., W. T. SINCLAIR, X.-S. HU, AND A. LANGDON. 1999. Using organelle markers to elucidate the history, ecology and evolution of plant populations. *In* P. M. Hollingsworth, R. M. Bateman, and R. J. Gornall [eds.], Molecular systematics and plant evolution, 1–19. Taylor & Francis, London, UK.

FUNK, D. J., AND K. E. OMLAND. 2003. Species-level paraphyly and polyphyly: Frequency, causes, and consequences, with insights from animal mitochondrial DNA. *Annual Review of Ecology Evolution and Systematics* 34: 397–423.

GASTONY, G. J., AND G. YATSKIEVYCH. 1992. Maternal inheritance of the chloroplast and mitochondrial genomes in cheilanthoid ferns. *American Journal of Botany* 79: 716–722.

GEIGER, J. M. O., T. A. RANKER, J. M. R. NEALE, AND S. T. KLIMAS. 2007. Molecular biogeography and origins of the Hawaiian fern flora. *Brittonia* 59: 142–158.

GRIEKSPOOR, A., AND T. GROOTHUIS. 2006. 4Peaks, version 1.7. Computer program distributed by the authors, website http://mekentosj.com/4peaks [accessed 28 February 2006].

GUREYEVA, I. I., AND C. N. PAGE. 2005. Towards the problem of bracken taxonomy in Siberia. *Taxonomy Notes of the Krylov Herbarium of Tomsk State University* 95: 18–26.

HIBBETT, D. S. 2001. Shiitake mushrooms and molecular clocks: Historical biogeography of *Lentinula*. *Journal of Biogeography* 28: 231–241.

HILLIS, D. 1998. Taxonomic sampling, phylogenetic accuracy, and investigator bias. *Systematic Biology* 47: 3–8.

HOLM, L., J. DOLL, E. HOLM, J. PANCHO, AND J. HERBERGER. 1997. World weeds: Natural histories and distribution. John Wiley and Sons, New York, New York, USA.

HOOKER, W. J. 1858. Species filicum, vol. II, *Adiantum–Ceratopteris*. William Pamplin, London, UK.

KUHN, M. 1879. Cryptogamae vasculares. *In* Carl C. von der Decken [ed.], Reisen in Ost-Afrika in den Jahren 1859–1865, Band III, Abtheilung III, 11. C. F. Winter, Leipzig, Germany.

LEE, C. E. 2000. Global phylogeography of a cryptic copepod species complex and reproductive isolation between genetically proximate "populations". *Evolution* 54: 2014–2027.

LIHOVÁ, J., K. MARHOLD, H. KUDOH, AND M. A. KOCH. 2006. Worldwide phylogeny and biogeography of *Cardamine flexuosa* (Brassicaceae) and its relatives. *American Journal of Botany* 93: 1206–1221.

LINNAEUS, C. VON. 1753. Species plantarum, vol. II. Laurentii Salvii, Stockholm, Sweden.

MADDISON, W. P., AND D. R. MADDISON. 2008. Mesquite: A modular system for evolutionary analysis, version 2.5. Computer program and documentation distributed by the authors, website: http://mesquiteproject.org [accessed 13 July 2008].

MCCAULEY, D. E. 1995. The use of chloroplast DNA polymorphismin studies of gene flow in plants. *Trends in Ecology & Evolution* 10: 198–202.

NAKAZATO, T., M. K. JUNG, E. A. HOUSWORTH, L. H. RIESEBERG, AND G. J. GASTONY. 2007. A genomewide study of reproductive barriers between allopatric populations of a homosporous fern, *Ceratopteris richardii*. *Genetics* 177: 1141–1150.

NYLANDER, J. A. A. 2004. MrModeltest 2.2. Program distributed by the author, Evolutionary Biology Centre, Uppsala University, website: http://www.abc.se/~nylander/ [accessed 17 April 2007].

PAGE, C. N. 1976. The taxonomy and phytogeography of bracken—A review. *Botanical Journal of the Linnean Society* 73: 1–34.

PAGE, C. N. 1986. The strategies of bracken as a permanent ecological opportunist. *In* R. Smith and J. Taylor [eds.], Ecology, land use and control technology. Parthenon Publishing, Carnforth, UK.

PAGE, C. N., AND R. R. MILL. 1995. The taxa of Scottish bracken in a European perspective. *Botanical Journal of Scotland* 47: 229–247.

POSADA, D., AND T. R. BUCKLEY. 2004. Model selection and model averaging in phylogenetics: Advantages of Akaike information criterion and Bayesian approaches over likelihood ratio tests. *Systematic Biology* 53: 793–808.

PRYER, K. M., E. SCHUETTPELZ, P. G. WOLF, H. SCHNEIDER, A. R. SMITH, AND R. CRANFILL. 2004. Phylogeny and evolution of ferns (monilophytes) with a focus on the early leptosporangiate divergences. *American Journal of Botany* 91: 1582–1598.

PUNETHA, N. 1991. Studies on atmospheric fern spores at Pithorgarh (northwest Himalaya) with particular reference to distribution of ferns in the Himalayas. *In* T. N. Bhardwaja and O. B. Gena [eds.], Aspects of plant sciences, vol. 13, Perspectives in pteridology present and future, part I, 145–161. Today and Tomorrow's Printers & Publishers, New Delhi, India.

RAMBAUT, A. 2002. Se-Al: Sequence Alignment Editor, version 2.0a11. Department of Zoology, University of Oxford, website: http://evolve.zoo.ox.ac.uk [accessed 15 March 2006].

RAMSEY, J., AND D. W. SCHEMSKE. 1998. Pathways, mechanisms, and rates of polyploid formation in flowering plants. *Annual Review of Ecology Evolution and Systematics* 29: 467–501.

RIESEBERG, L. H., AND L. BROUILLET. 1994. Are many plant species paraphyletic? *Taxon* 43: 21–32.

RIESEBERG, L. H., AND D. E. SOLTIS. 1991. Phylogenetic consequences of cytoplasmic gene flow in plants. *Evolutionary Trends in Plants* 5: 65–84.

RIESEBERG, L. H., J. WHITTON, AND C. R. LINDER. 1996. Molecular marker incongruence in plant hybrid zones and phylogenetic trees. *Acta Botanica Neerlandica* 45: 243–262.

RONQUIST, F., AND J. HUELSENBECK. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19: 1572–1574.

RUMSEY, F. J., E. SHEFFIELD, AND C. H. HAUFLER. 1991. A re-assessment of *Pteridium aquilinum* (L.) Kuhn in Britain. *Watsonia* 18: 297–301.

SCHNEIDER, H., E. SCHUETTPELZ, K. M. PRYER, R. CRANFILL, S. MAGALLÓN, AND R. LUPIA. 2004. Ferns diversified in the shadow of angiosperms. *Nature* 428: 553–557.

SCHUETTPELZ, E., A. L. GRUSZ, M. D. WINDHAM, AND K. M. PRYER. 2008. The utility of nuclear gapCp in resolving polyploid fern origins. *Systematic Botany* 33: 621–629.

SCHUETTPELZ, E., AND S. B. HOOT. 2006. Inferring the root of *Isoëtes*: Exploring alternatives in the absence of an acceptable outgroup. *Systematic Botany* 31: 258–270.

SCOTESE, C. R. 2001. Atlas of Earth history. PALEOMAP Project, Arlington, Texas USA.

SMALL, R. L., E. B. LICKEY, J. SHAW, AND W. D. HAUK. 2005. Amplification of noncoding chloroplast DNA for phylogenetic studies in lycophytes and monilophytes with a comparative example of relative phylogenetic utility from Ophioglossaceae. *Molecular Phylogenetics and Evolution* 36: 509–522.

SOLTIS, D. E., AND P. S. SOLTIS. 1993. Molecular data and the dynamic nature of polyploidy. *Critical Reviews in Plant Sciences* 12: 243–273.

SOLTIS, D. E., P. S. SOLTIS, AND B. G. MILLIGAN. 1992. Intraspecific cpDNA variation: Systematic and phylogenetic implications. *In* D. E. Soltis, P. S. Soltis, and J. J. Doyle [eds.], Molecular systematics of plants, 117–150. Chapman and Hall, New York, New York, USA.

SPEER, W. D. 2000. A systematic assessment of British and North American *Pteridium* using cpDNA gene sequences. *In* J. A. Taylor and R. T. Smith [eds.], Bracken fern: Toxicity, biology and control, 37–42. International Bracken Group, Aberystwyth, Wales.

SPEER, W. D., C. R. WERTH, AND K. W. HILU. 1998. Relationships between two infraspecific taxa of *Pteridium aquilinum* (Dennstaedtiaceae). II. Isozyme evidence. *Systematic Botany* 23: 313–325.

SWOFFORD, D. L. 2002. PAUP*: Phylogenetic analysis using parsimony (*and other methods), version 4.0b10. Sinauer, Sunderland, Massachusetts, USA.

TAN, M. K. 1991. Evolutionary studies of the chloroplast genome in *Pteridium. Plant Science* 77: 81–91.

TAN, M. K., AND J. A. THOMSON. 1990a. Evolutionary studies of the chloroplast genome in *Pteridium. In* J. A. Thomson and R. T. Smith [eds.], Bracken 89: Bracken biology and management, 95–104. Australian Institute of Agricultural Science, Sydney, Australia.

TAN, M. K., AND J. A. THOMSON. 1990b. Variation of genome size in *Pteridium. In* J. A. Thomson, and R. T. Smith [eds.], Bracken 89: Bracken biology and management, 87-93. Australian Institute of Agricultural Science, Sydney, Australia.

THOMSON, J. A. 2000a. Morphological and genomic diversity in the genus *Pteridium* (Dennstaedtiaceae). *Annals of Botany* 85: 77–99.

THOMSON, J. A. 2000b. New perspectives on taxonomic relationships in *Pteridium. International Bracken Group Special Publications* 4: 15–34.

THOMSON, J. A. 2002. An improved non-cryogenic transport and storage preservative facilitating DNA extraction from 'difficult' plants collected at remote sites. *Telopea* 9: 755–760.

THOMSON, J. A. 2004. Towards a taxonomic revision of *Pteridium* (Dennstaedtiaceae). *Telopea* 10: 793–803.

THOMSON, J. A., AND M. E. ALONSO-AMELOT. 2002. Clarification of the taxonomic status and relationships of *Pteridium caudatum* (Dennstaedtiaceae) in Central and South America. *Botanical Journal of the Linnean Society* 140: 237–248.

THOMSON, J. A., A. C. CHIKUNI, AND C. S. MCMASTER. 2005. The taxonomic status and relationships of bracken ferns (*Pteridium*: Dennstaedtiaceae) from sub-Saharan Africa. *Botanical Journal of the Linnean Society* 148: 311–321.

THOMSON, J. A., AND A. B. MARTIN. 1996. Gnarled trichomes: An understudied character in *Pteridium. American Fern Journal* 86: 36–51.

THOMSON, J. A., J. T. MICKEL, AND K. MEHLTRETER. 2008. Taxonomic status and relationships of bracken ferns (*Pteridium*: Dennstaedtiaceae) of Laurasian affinity in Central and North America. *Botanical Journal of the Linnean Society* 157: 1–17.

TRYON, R. M. 1941. A revision of the genus *Pteridium. Rhodora* 43: 1–67.

TRYON, R. 1985. Fern speciation and biogeography. *Proceedings of the Royal Society, Edinburgh* 86B: 353–360.

VOGEL, J. C., S. J. RUSSELL, F. J. RUMSEY, J. A. BARRETT, AND M. GIBBY. 1998. Evidence for maternal transmission of chloroplast DNA in the genus *Asplenium* (Aspleniaceae, Pteridophyta). *Botanica Acta* 111: 247–249.

WOLF, P. G., H. SCHNEIDER, AND T. A. RANKER. 2001. Geographic distributions of homosporous ferns: Does dispersal obscure evidence of vicariance? *Journal of Biogeography* 28: 263–270.

WOLF, P. G., E. SHEFFIELD, J. A. THOMSON, AND R. B. SINCLAIR. 1995. Bracken taxa in Britain: A molecular analysis. *In* R. T. Smith and J. A. Taylor [eds.], Bracken: An environmental issue, 16–20. University of Leeds, Leeds, UK.

APPENDIX 1. Taxa, vouchers, localities, and GenBank accessions for bracken specimens included in this study. Voucher specimens are deposited in the following herbaria: NSW = National Herbarium of New South Wales, Australia; UTC = Intermountain Herbarium, Utah State University, USA.

Taxon — Voucher: *Collector* (Herbarium accession) Sample isolate ID; Locality; Latitude; Longitude; GenBank accesions: *trnS–rpS4* spacer+gene; *rpL16* intron.

Pteridium aquilinum* subsp. *aquilinum — *T. Reichstein* (NSW 420392) 017 FZSW; Switzerland, Filzbach; 47.12; 9.10; FJ177240; FJ177159; *R. Prelli* (NSW 420391) 031 BRFR; France, Britagne, Erquy; 48.38; 2.27; FJ177241; FJ177160; *M. E. Gillham* (NSW 420404) 065 CLYW; United Kingdom, Wales, Neath Valley; 51.65; -3.80; FJ177242; FJ177161; *A. F. Dyer* (NSW 420393) 099 EDSC; United Kingdom, Scotland, Pentland Hills; 55.5; -3.25; FJ177243; FJ177162; *R. Viane* (NSW 420405) 103ASSP; Spain, Asturias Province, Villanueva; 43.37; -5.83; FJ177244; FJ177163; *M. E. Gillham* (NSW 420396) 106 OXLN; United Kingdom, England, London, Oxshott Common; 51.38; -0.42; FJ177245; FJ177164; *F. Piccoli* (NSW 420390) 188 CORF; France, Corsica, Haute Corse; 42.57; 9.30; FJ177246; FJ177165; *E. Sheffield* (NSW 420403) 194 AMTK; Turkey, Amasra, Black Sea Coast; 41.73; 32.40; FJ177247; FJ177166; *F. Piccoli* (NSW 420394) 202 RVIT; Italy, Ravenna; 44.42; 12.2; FJ177248; FJ177167; *C. N. Page* (NSW 420408) 218 ACAW; United Kingdom, Wales, Caernarfon; 53.13; -4.27; FJ177249; FJ177168; *B. Capezedo & A. E. Salvo* (NSW 420374) 226 LBSP; Spain, Cadiz, Los Barrios; 36.18; -5.50; FJ177250; FJ177169; *A. C. Jermy* (NSW 420397) 256 AZOR; Portugal, Azores, Terceira, Agra; 38.70 -27.20; FJ177251; FJ177170; *M. E. Gillham* (NSW 420380) 293 CPHW; United Kingdom, Wales, Caerphilly Mt.; 51.35; 3.12; FJ177252; FJ177171; *M.E. Gillham* (NSW 420399) 307 BRGW; United Kingdom, Wales, Bridgend; 51.52; -3.58; FJ177253; FJ177172; *R. Viane* (NSW 420388) 336 RAMD; Portugal, Madeira, Rocha Alta; 32.65; -16.88; FJ177254; FJ177173; *R. Prelli* (NSW 420376) 350 NIFR; France, Nice, Cagnes-sur-Mer; 43.67; 7.15; FJ177255; FJ177174; *V. Korzhenevsky* (NSW 627463) TAUR; Ukraine, Caucasus Mountains, Crimea; 44.98; 34.62; FJ177288; FJ177207. ***Pteridium aquilinum* subsp. *capense*** (Thunb.) C. Chr. — *C. S. McMaster* (NSW 617745) 110 KUFZ; Zambia, Kundalila Falls; -12.45; 30.13; FJ177256; FJ177175; *V. Rashbrook* (NSW 420332) 191 GSAF; Republic of South Africa, Grahamstown; -33.19; 26.32; FJ177257; FJ177176; *Watling* (NSW 420334) 228 MCAM; Cameroon, Mundamba; 4.42; 8.87; FJ177258; FJ177177; *P. J. Myerscough* (NSW 420333) 353 BBSA; Republic of South Africa, Betty's Bay, Silver Sands; -34.35; 18.9; FJ177260; FJ177179; *B. E. Okoli* (NSW 420328) 368 NDNG; Nigeria, Niger Delta; 4.83; 6.25; FJ177262; FJ177181; *A. C. Chikuni* (NSW 617743) 503CAPM/ZOMA; Malawi, Zomba Plateau; -15.3; 35.32; FJ177263; FJ177182. ***Pteridium aquilinum* subsp. *centrali-africanum*** Hieron. — *C. S. McMaster* (NSW 617746) 112 ZAMB/CH3Z; Zambia, Mkushi, Chilongoma Hills; -14.38; 29.53; FJ177264; FJ177183; *C. S. McMaster* (NSW 617747) AKFZ; Zambia, Kundalila Falls; -12.45; 30.13; FJ177265; FJ177184; *C. S. McMaster* (NSW 617757) CAF4; Zambia, Mkushi, Chilongoma Hills; -14.38; 29.53; FJ177266; FJ177185. ***Pteridium aquilinum* subsp. *decompositum*** (Gaudich.) Lamoureux ex J. A. Thomson — *C. W. Smith* (NSW 420357) 292 MAHI; USA, HI, Maui, Haleakala National Park; 20.75; -156.17 FJ177268; FJ177187. ***Pteridium aquilinum* subsp. *japonicum*** (Nakai) Á. Löve & D. Löve — *S.-M. Chew* (NSW 420347) 029 TTWN; Taiwan, Nankang, Taipei; 25.00; 121.02; FJ177269; FJ177188; *R. Hirose* (NSW 420335) 071 AIJP; Japan, Honshu, Aichi Prefecture; 34.49; 137.12; FJ177270; FJ177189; *K. U. Kramer* (NSW 420340) 085 YSCH; China, Kwansi, Guilin, Yao Shan; 25.35; 110.18; FJ177271; FJ177190; *Y.-J. Zhang* (NSW 420336) 096 GNCH; China, Gansu Province; 35.00; 105.00; FJ177272; FJ177191; *R. Hirose* (NSW 420351) 104 MOGJ; Japan, Honshu, Nagano Prefecture, Mt. Ogura; 36.17; 138.25; FJ177273; FJ177192; *T. Kumashiro* (NSW 420356) 113 YOJP; Japan, Kyushu, Kagoshima Prefecture; 31.58; 130.55; FJ177274; FJ177193; *R. Hirose* (NSW 420348) 280 CHJP; Japan, Honshu, Chiba Prefecture; 35.60; 140.10; FJ177275; FJ177194; *T. Miyata* (NSW 420349) 316 SHJP; Japan, Honshu, Shimane Prefecture; 35.47; 133.03; FJ177276; FJ177195. ***Pteridium aquilinum* subsp. *latiusculum*** (Desv.) Hultén — *E. Klekowski* (NSW 420315) 143 YCCM; USA, MA, Yarmouth; 41.70; -70.23; FJ177277; FJ177196; *W. H. Wagner Jr* (NSW 420310) 147 WMCH; USA, MI, Waterloo; 42.15; -84.24; FJ177278; FJ177197; *D. Barrington* (NSW 420311) 148 BRMN; USA, ME, Bridgton; 44.04; -70.42; FJ177279; FJ177198; *D. Barrington* (UTC 247719) DSB 2287; USA, VT, Colchester, Niquette Bay State Park; 44.58; -73.20; FJ177280; FJ177199; *P. Wolf* (UTC 249671) PGW 921; USA, CT, SE of Storrs, Spring Hill; 41.79; -72.22; FJ177281; FJ177200. ***Pteridium aquilinum* subsp. *pinetorum*** (C. N. Page & R. R. Mill) J. A. Thomson — *V. Bogatyr* (NSW 420402) 164 KUKR; Ukraine, Kiev; 50.43; 30.5; FJ177235; FJ177154; *E. A. Ershova & A. I. Shmakov* (NSW 807731) N2nR; Russia, Siberia, Altai Plain; 53.35; 83.73; FJ177236; FJ177155; *E. A. Ershova* (NSW 807726) N6nR; Russia, Siberia, Novosibirsk; 55.03; 82.92; FJ177237; FJ177156; *O. N. Perestoronina* (NSW 807736) RU4K; Russia, Kirov Region; 58.60; 49.65; FJ177238; FJ177157. ***Pteridium aquilinum* subsp. *pseudocaudatum*** (Clute) Hultén — *E. Sheffield* (NSW 420317) 169 FLUS; USA, FL, Cape Kennedy; 28.47; -80.47; FJ177282; FJ177201; *P. G. Wolf & M. D. Windham* (NSW 420316) 203 HFLA; USA, FL, Hawthorne; 29.55; -82.08; FJ177283; FJ177202; *P. Soltis* (UTC 249632) Der 68; USA, FL, Paynes Prairie State Preserve; 29.52; -82.30; FJ177299; FJ177218. ***Pteridium aquilinum* subsp. *pubescens*** (Underw.) J. A. Thomson, Mickel & Mehltreter — *D. Barrington* (NSW 419173) 100 AOUS; USA, OR, Ashland; 42.23; -122.73; FJ177284; FJ177203; *J. Schneller* (NSW 420312) 325 OWUS; USA, WA, Olympic Peninsula; 47.55; -124.24; FJ177285; FJ177204; *J. Der* (UTC 249629) JPD 66; Canada, British Columbia, Vancouver; 49.26; -123.26; FJ177297; FJ177216; *J. Der* (UTC 247718) JPD 67; USA, CA, Feather River Canyon; 39.74; -120.71; FJ177298; FJ177217. ***Pteridium aquilinum* subsp. *wightianum*** (J. Agardh) W. C. Shieh — *R. D. E Jayesekara* (NSW 419562) 001 AMSL; Sri Lanka, Ambewela; 7.03; 80.59; FJ177289; FJ177208; *J. V. Pancho* (NSW 420371) 007 KLPH; Philippines, Luzon, Kinabuhayan; 14.25; 121.5; FJ177290; FJ177209; *T. Partomihardjo* (NSW 419534) 068 SERI; Indonesia, Molucca Island, Seram; 3.10; 129.05; FJ177291; FJ177210; *R. Kiew* (NSW 420251) 182 PMAL; Malaysia, Pahang; 4.00; 102.5; FJ177292; FJ177211; *S. P. Khullar* (NSW 419570) 305 YGIN; India, Garhwal, Yamunotri Hills ; 30.92; 78.47; FJ177293; FJ177212; *J. A. Thomson* (NSW 420257) 354 WFNQ; Australia, N. Queensland, Wallaman Falls; -18.55; 145.80; FJ177294; FJ177213; *M. D. Dassayanake* (NSW 419571) 362 HKSL; Sri Lanka, Hakgala; 6.55; 80.48; FJ177295; FJ177214; *S. J. Moore* (NSW 705077) 416 TREV; Taiwan, Taitung Hsieh; 23.27; 120.96; FJ177296; FJ177215. ***Pteridium arachnoideum*** (Kaulf.) Maxon — *B. Perez-Garcia* (NSW 420308) 144 RMEX; Mexico, Molango, Río Malila; 20.48; -98.44; FJ177221; FJ177140; *M. G. E. Noronha & P. G. Windisch* (NSW 420303) 317 SPBR; Brazil, Sao Paulo; -22.42; -49.00; FJ177219; FJ177138; *M. E. Alonso-Amelot* (NSW 505771) ME2-1VNZA; Venezuela, Mérida, Cerro La Bandera; 8.42; -69.03; FJ177220; FJ177139. ***Pteridium caudatum*** (L.) Maxon — *J. Villalobos-Salazar & M. Firenczi* (NSW 420247) 238 HECR; Costa Rica, Heredia; 10.00; -84.08; FJ177222; FJ177141; *J. Lenne* (NSW 420295) 274 QCOL; Colombia, Valle, Quisquina; 3.60; -76.48; FJ177223; FJ177142; *A. C. Jermy & T. G. Walker* (NSW 420305) 323 COCR; Costa Rica, Cordillera; 9.15; -83.8; FJ177224; FJ177143; *M. E. Alonso-Amelot* (NSW 505770) MD2-2VENZ; Venezuela, Merida, La Hechicera; 8.42; -71.03; FJ177225; FJ177144. ***Pteridium esculentum*** (G. Forst.) Cockayne — *J. A. Thomson* (NSW 420273) 083 WAWA; Australia, WA, Waroona; -32.51; 115.59; FJ177226; FJ177145; *J. A. Thomson* (NSW 420274) 127 KEWA; Australia, WA, Kenton; -34.57; 117.01; FJ177227; FJ177146; *J. A. Thomson* (NSW 420848) 213 CRAN; Australia, NSW, Craven; -32.15; 151.95; FJ177228; FJ177147; *J. A. Thomson* (NSW 420264) 275 KONC; New Caledonia, Mt. Koghi; -22.17; 166.50; FJ177229; FJ177148; *C. Surman* (NSW 420267) 324 HNNZ; New Zealand, South Island, Nelson, Hira Forest; -41.18; 173.17; FJ177230; FJ177149; *C. Surman* (NSW 420289) 332 SVNZ; New Zealand, North Island, Wellington, Stokes Valley; -41.07; 175.08; FJ177231; FJ177150; *J. A. Thomson* (NSW 420879) 387 CRDQ; Australia, N. Qld, Credition; -21.20; 148.53; FJ177232; FJ177151; *J. A. Thomson* (NSW 420269) 401 RYNA; Australia, NSW, Sydney, Ryde; -33.47; 151.05; FJ177233; FJ177152; *P. Wolf* (UTC 250545) PGW 638; New Zealand, North Island, Ruakura; -37.78; 175.31; FJ177234; FJ177153. ***Pteridium semihastatum*** (Wall. ex J. Agardh) S. B. Andrews — *P. Brocklehurst & G. Wightman* (NSW 420865) 251 PFNT; Australia, NT, Petherick's Forest Park; -13.06; 130.24; FJ177286; FJ177205; *Mujamil & T. J. Ho* (NSW 419554) 278 KMAL; Malaysia, Selangor, Kapar; 3.07; 101.24; FJ177287; FJ177206.