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The Paraphyly of *Osmunda* is Confirmed by Phylogenetic Analyses of Seven Plastid Loci

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Abstract—To resolve phylogenetic relationships among all genera and subgenera in Osmundaceae, we analyzed over 8,500 characters of DNA sequence data from seven plastid loci (*atpA*, *rbcL*, *rbcL–accD*, *rbcL–atpB*, *rps4–trnS*, *trnG–trnR*, and *trnL–trnF*). Our results confirm those from earlier anatomical and single-gene (*rbcL*) studies that suggested *Osmunda* s.l. is paraphyletic. *Osmunda cinnamomea* is sister to the remainder of Osmundaceae (*Leptopteris*, *Todea*, and *Osmunda* s.s.). We support the recognition of a monotypic fourth genus, *Osmundastrum*, to reflect these results. We also resolve subgeneric relationships within *Osmunda* s.s. and find that subg. *Claytosmunda* is strongly supported as sister to the rest of *Osmunda*. A stable, well-supported classification for extant Osmundaceae is proposed, along with a key to all genera and subgenera.

Keywords—ferns, *Osmunda*, Osmundaceae, *Osmundastrum*, paraphyly, plastid DNA.

Osmundales is the smallest but most ancient order of leptosporangiate ferns and occupies an important phylogenetic position as sister to all other extant leptosporangiates (Hasebe et al. 1995; Pryer et al. 2004; Schuettpelz and Pryer 2007). Numerous fossil representatives are known from the Permian onwards (Tidwell and Ash 1994) and fossil representatives of *Osmunda* L. are known since the Triassic (Phipps et al. 1998; Vavrek et al. 2006). The single extant family Osmundaceae is characterized by rhizomes with a highly distinctive anatomy in transverse section that is consistent across the family and unique for ferns (Hewitson 1962; see Fig. 13-20 on pg. 267 in Gifford and Foster 1989), sporangia that are not organized into sori, green spores, and a unique suite of reproductive characters that appears intermediate between eusporangiate and leptosporangiate ferns (Kramer 1990). Osmundaceae sporangia develop from multiple initial cells and produce hundreds of spores, both traits associated with eusporangiate fern lineages (Bierhorst 1971; Ogura 1972). The sporangia also have a rudimentary patch-like annulus that causes longitudinal dehiscence, distinct from all other annulus morphologies present in leptosporangiate ferns (Bierhorst 1971).

Osmundaceae is commonly thought to comprise three extant genera: *Osmunda*, *Leptopteris* C. Presl, and *Todea* Willd. ex Bernh. (Kramer 1990; Smith et al. 2006). *Leptopteris* and *Todea* share many characters, including monomorphic leaves and sporangia that follow veins on uncontracted fertile pinnae (Kramer 1990), but the two genera are readily distinguished. *Leptopteris*, with about six species, has filmy leaves that lack stomata and sporangia sparsely arranged on the abaxial surface; *Todea*, with two species, has coriaceous leaves with stomata and sporangia densely covering the abaxial surface (Hennipman 1968; Brownsey 1981). *Osmunda* has been distinguished from these two genera by its contracted fertile pinnae and contains eight to nine species that have been recognized in three subgenera (Kramer 1990): subg. *Osmunda* L. with three species, subg. *Osmundastrum* (C. Presl) C. Presl with two species, and subg. *Plenasium* (C. Presl) J. Smith with three to four species. Although most authors define *Osmunda* in this manner, there have been indications that the genus may not be monophyletic. Anatomical studies of extant and fossil species of subg. *Osmundastrum* by Miller (1967, 1971) led him to conclude that *O. cinnamomea* was not closely related to the rest of *Osmunda* and that *O. claytoniana* should be transferred to subg. *Osmunda*. Miller (1967, 1971) also recommended that all three subgenera be elevated to generic level, as previously suggested by other authors (Tagawa 1941; Bobrov 1967). Using this taxonomic approach, the genus *Osmundastrum* C. Presl would include one extant and one fossil species (Miller 1967). Miller's suggestions were not widely accepted and most subsequent studies did not adopt *Osmundastrum* sensu Miller (e.g. Stein and Thompson 1975; Sobel and Whalen 1983; Li and Haufler 1994).

An *rbcL* study of Osmundaceae found *O. cinnamomea* to be sister to the rest of Osmundaceae, including *Leptopteris* and *Todea* (Yatabe et al. 1999). This single gene study also found *O. claytoniana* sister to a clade containing subg. *Plenasium* and subg. *Osmunda*. Although this relationship was not well supported, Yatabe et al. (2005) proposed a new subgenus, *Claytosmunda* Yatabe, Murakami & Iwatsuki, to accommodate the putative phylogenetic position of *O. claytoniana*. The most recent classification for Osmundaceae recognizes four genera (Yatabe et al. 2005): *Osmundastrum*, *Todea*, *Leptopteris,* and *Osmunda* with its three subgenera (*Claytosmunda*, *Plenasium,* and *Osmunda*).

In the current study, we reconstruct the first multilocus phylogeny for Osmundaceae, assess relationships within and among all genera and subgenera, and seek to settle the taxonomic and nomenclatural uncertainty that surrounds *Osmunda*. Our highly resolved phylogeny allows us to evaluate previous classifications and recommend which, if any, should be followed.

Materials and Methods

Taxon Sampling—We sampled 24 accessions representing 13 ingroup and four outgroup species (Appendix 1). Ingroup sampling included all described extant genera and subgenera of Osmundaceae, with multiple accessions for four species to assess intraspecific and geographic variation. We selected outgroup taxa from the gleichenioid lineage based on its phylogenetic proximity to Osmundaceae (Pryer et al. 2004).

DNA Isolation, Amplification, and Sequencing—DNA extraction, amplification, sequencing and assembly for seven plastid loci (*atpA*, *atpB–rbcL*, *rbcL–accD*, *rbcL*, *rps4–trnS*, *trnG–trnR*, and *trnL–trnF*) followed es-

tablished protocols (Schuettpelz and Pryer 2007; Korall et al. 2007; Metzgar et al. 2007). A total of 155 new sequences were generated specifically for this project and are available in GenBank (Appendix 1).

***Sequence Alignments*—**Sequence alignments were performed manually using MacClade version 4.06 (Maddison and Maddison 2003). There were no insertions or deletions (indels) in the protein-coding *rbcL* alignment and the *atpA* data set was easily aligned with only a single, unambiguous four-codon indel that was clearly delimited. The *rbcL–accD*, *rbcL–atpB*, *rps4–trnS*, *trnG–trnR*, and *trnL–trnF* alignments all included some indels. No gap coding method was employed; however, some regions were excluded from these data sets due to ambiguities in the alignment (29 bp in *rbcL–accD*, 129 bp in *rbcL–atpB*, 51 bp in *rps4–trnS*, 39 bp in *trnG–trnR*, and 146 bp in *trnL–trnF*). For each of these five data sets, some noncoding portions with very divergent sequences between ingroup and outgroup taxa resulted in highly ambiguous alignments. To preserve maximum phylogenetic resolution within the ingroup (where alignment was unproblematic), these portions of the ambiguously aligned outgroup sequences were deleted and treated as missing data in the analyses (529 bp in *rbcL–accD*, 537 bp in *rbcL–atpB*, 422 bp in *rps4–trnS*, 655 bp in *trnG–trnR*, and 667 bp in *trnL–trnF*).

***Data Set Combinability*—**Using MrBayes version 3.1.1 (Huelsenbeck and Ronquist 2001; Ronquist and Huelsenbeck 2003), Bayesian Markov chain Monte Carlo (B/MCMC) analyses were run for each single locus data set using the same settings as for the combined data matrix analysis (see below). The seven majority-rule consensus topologies were inspected for topological conflicts using a threshold of 0.95 posterior probability or higher for the B/MCMC analyses. We observed no topological conflict among data sets and hence all seven were combined into a single data set. The seven-locus combined data matrix contained 8,628 bp (17.5% of cells were missing data) and is available in TreeBASE (study number S1897).

***Phylogenetic Analyses of the Combined Data Matrix*—**Models of sequence evolution for maximum likelihood (ML) and B/MCMC analyses were selected using Modeltest 3.6 (Posada and Crandall 1998). For the ML analysis of the combined data set, the TIM + I + G model (transitional model, incorporating invariable sites and rate variation among sites) was selected. For the B/MCMC analyses, the TIM + G model was selected for *atpA*, *rbcL–accD*, and *rbcL–atpB*, the HKY + G model (Hasegawa et al. 1985) was selected for *trnG–trnR*, the TrN + G model (Tamura and Nei 1993) was selected for *rbcL*, and the K81uf + G model (Kimura 1981) was selected for *rps4–trnS* and *trnL–trnF*.

Maximum parsimony (MP) and ML analyses of the full combined data matrix were run using PAUP* 4.0b10 (Swofford 2002) and B/MCMC analyses were performed using MrBayes version 3.1.1 (Huelsenbeck and Ronquist 2001; Ronquist and Huelsenbeck 2003). The MP heuristic search was run with 1,000 random addition sequence (RAS) replicates with tree-bisection-reconnection (TBR) branch swapping, and the MP bootstrap analysis was performed using 500 replicates, each with five RAS and TBR branch swapping. The ML heuristic search was run with 500 RAS replicates with TBR branch swapping, and the ML bootstrap analysis included 500 replicates, each with 10 RAS and TBR branch swapping. The B/MCMC analysis was performed using four separate tree searches, each composed of four chains, running for 10 million generations each. The B/MCMC analyses were performed with default priors, trees being sampled every 1,000 generations, and data partitioned by locus with separate sequence evolution models for each locus (see above; if the selected model could not be implemented, the next more complex model was used). Stationarity was determined to have occurred after 2,500,000 generations in each analysis by plotting likelihood scores, and the first 2,500 trees were excluded as the burnin. Post-burnin trees from all four runs were pooled, and a majority-rule consensus tree with average branch lengths and posterior probabilities computed from the resulting 30,000 trees, using the "sumt" command in MrBayes.

Results

The MP analysis of the full seven-locus combined data set resulted in two equally most parsimonious trees (3148 steps, CI = 0.803, RI = 0.877), which yielded a well-resolved strict consensus tree (tree not shown). The ML analysis found a single optimal tree (−lnL = 27497.97284; tree not shown). The Bayesian analysis resulted in a majority-rule consensus tree with a well-resolved topology (Fig. 1). The MP strict consensus tree, ML optimal tree and Bayesian majority-rule consensus tree were identical. All intergeneric and interspecific relationships were resolved and well supported (Fig. 1). We considered any node to be well supported if it had a posterior probability (PP) $\geq$ 0.99, a ML bootstrap value (MLBS) $\geq$ 90%, *and* a MP bootstrap (MPBS) $\geq$ 90%.

Our results show that *Osmunda* s.l. is paraphyletic, with the taxon traditionally treated as *O. cinnamomea* sister to the rest of the family, including *Leptopteris* and *Todea* (Fig. 1); this supports the recognition of *Osmundastrum* as a separate genus with a single extant species, *O. cinnamomeum* (L.) C. Presl. Within-species variation for *O. cinnamomeum* resolved the two New World collections (Jamaica and U.S.A.) together as sister to a Japanese collection (Fig. 1). The small genus *Todea* forms a monophyletic group and is sister to a monophyletic *Leptopteris* (Fig. 1). Our molecular data were able to distinguish all species sampled, including the two *Todea* species, even though *T. papuana* was only sequenced at two loci (part of *rps4–trnS* and *trnL–trnF*; Appendix 1). Two collections of *L. hymenophylloides*, one from New Zealand and one from cultivation (unknown wild origin), exhibited very little molecular divergence. We reveal a robustly supported placement of *O. claytoniana* as sister to the remainder of *Osmunda* (Fig. 1); strong evidence for three clades within *Osmunda* supports the recognition of three subgenera: subg. *Osmunda*, subg. *Plenasium*, and the recently described subg. *Claytosmunda*.

Discussion

Numerous taxonomic treatments have been recommended for Osmundaceae (Hewitson 1962; Miller 1971; Kramer 1990; Yatabe et al. 2005), leaving the proper classification of the family shrouded in mystery. Our seven-locus data set resulted in a highly resolved, well-supported phylogeny that allows us to clearly delimit genera and subgenera. Echoing anatomical studies (Miller 1967, 1971) and an *rbcL* study (Yatabe et al. 1999), we support the recognition of *Osmundastrum* (sensu Miller 1971) as a separate genus with a single extant species, *O. cinnamomeum*. We recognize both *Todea* and *Leptopteris*, and find that *Osmunda* s.s. consists of three subgenera: subg. *Osmunda*, subg. *Plenasium*, and the recently described subg. *Claytosmunda*. Our results show convincing support for the classification proposed by Yatabe et al. (2005) and also remove Hewitson's (1962) and Miller's (1967) observations and conclusions regarding *O. cinnamomeum* from obscurity. This intergeneric and infrageneric classification should prove stable and long-lasting.

***Osmundastrum*—**Our results clearly show that *Osmunda* s.l. is paraphyletic, with the taxon traditionally treated as *O. cinnamomea* sister to the rest of the family, including *Leptopteris* and *Todea* (Fig. 1). Therefore, we encourage the use of *Osmundastrum* at the genus level and recognize *Osmundastrum cinnamomeum* (referred to only as *O. cinnamomeum* from here on) as its sole extant species. This concurs with the findings of an earlier *rbcL* study (Yatabe et al. 1999) and the taxonomic conclusions drawn from it (Yatabe et al. 2005). Our results also support the anatomical work by Hewitson (1962) and Miller (1967, 1971) that separated *O. cinnamomeum* from the rest of Osmundaceae based on two anatomical traits. The first of these is the presence of a second endodermis in the stele, which appears in cross-sections of the stem and is located between the xylem cylinder and the pith (all Osmundaceae possess an endodermis in the stem between the pericycle and the inner cortex; see Figs. 8–9 on pgs. 74–75

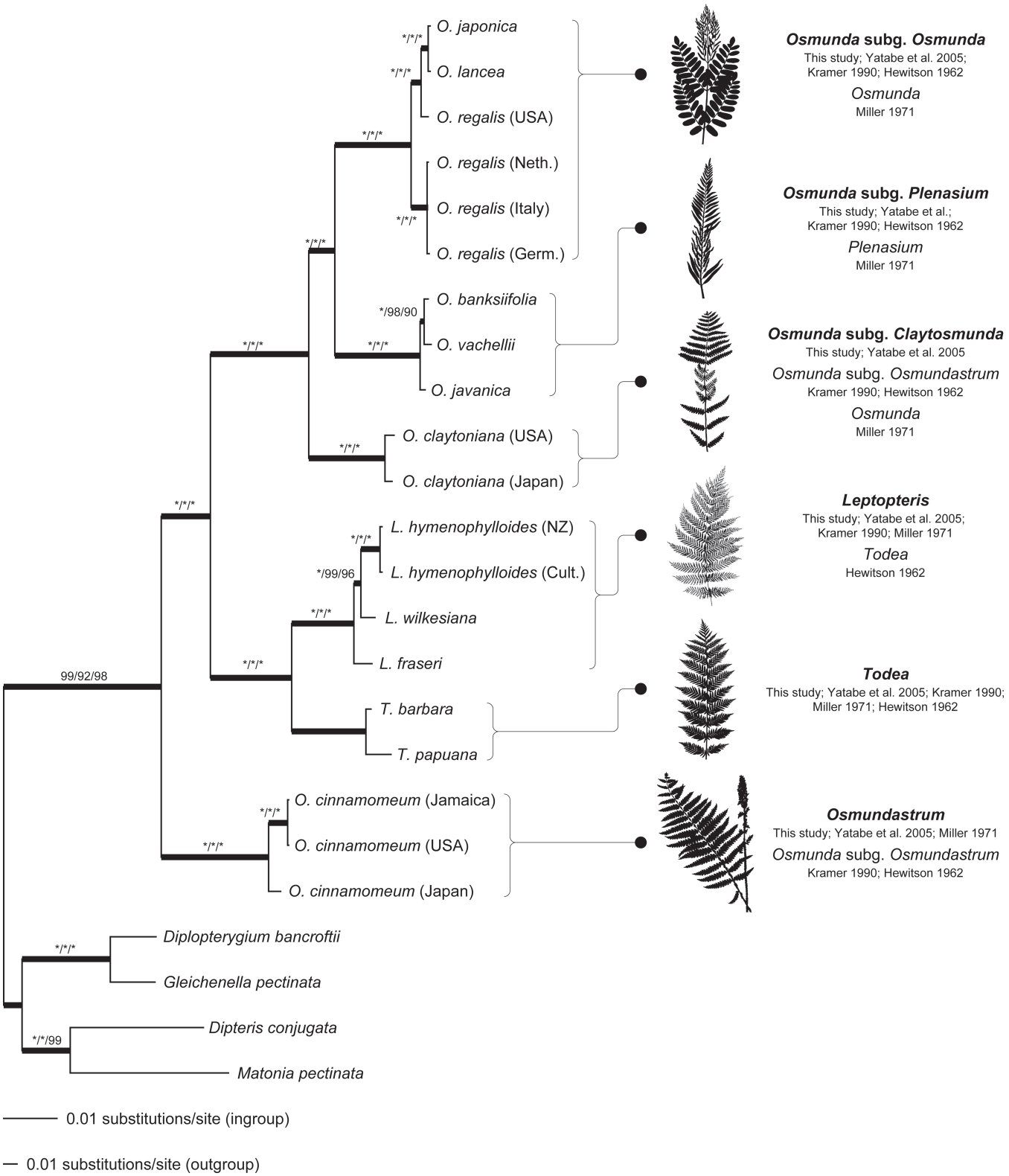

FIG. 1. 50% majority-rule consensus tree resulting from Bayesian (B/MCMC) analyses of the combined seven-locus data set, depicting the topology and average branch lengths in Osmundaceae. *Diplopterygium*, *Dipteris*, *Gleichenella,* and *Matonia* are outgroups. To increase clarity of ingroup relationships, branch lengths outside Osmundaceae (including branch leading to Osmundaceae) are shown at 0.25 scale. All divergences were well supported by all three measures (PP $\geq$ 0.99, MLBS $\geq$ 90, MPBS $\geq$ 90) and are shown as thickened branches with support values above each branch (PP/MLBS/MPBS; 1.00 PP and 100% BS values indicated by asterisks). Multiple accessions of the same taxon are distinguished by their geographical origin in parentheses. Silhouettes identifying a representative of each clade are modified from Hewitson (1962; *O. cinnamomeum*, *O. claytoniana*, and *O. javanica*), Hoshizaki and Moran (2001; *T. barbara* and *L. hymenophylloides*), and Berry et al. (1995; *O. regalis*). Our taxonomic recommendations are in bold type alongside the silhouettes, above those favored by previous authors.

of Hewitson 1962 for a clear comparison of Osmundaceae rhizome cross-sections). The second character pertains to the number of clusters of thick-walled cells forming the sclerenchyma ring surrounding the vascular strand in the petiole base. *Osmundastrum cinnamomeum* possesses three of these clusters that can be observed in cross-section through the stipular region of the petiole (see Fig. 7 on pg. 71 of Hewitson 1962 for a comparison of the disposition of sclerenchyma in Osmundaceae petiole base cross-sections). *Osmundastrum* can be distinguished by pinnate-pinnatifid leaf dissection, dimorphic leaves, contracted fertile pinnae, a tuft of hairs present abaxially on photosynthetic pinnae near the rachis and reddish brown hairs on petioles (see key).

Palynological (Hanks and Fairbrothers 1981) and serological (Petersen and Fairbrothers 1971) studies were generally inconclusive on the paraphyly of *Osmunda*, concluding that *O. cinnamomeum* and *O. claytoniana* were more closely related to each other than to *O. regalis*; a flavonoid study (Sobel and Whalen 1983) reaffirmed an Osmundaceae with the traditional three genera. DNA hybridization studies first suggested that *O. cinnamomeum* and *O. claytoniana* were more closely related to each other than to *O. regalis* (Stein and Thompson 1975), but this was later refuted (Stein et al. 1979). An isozyme study of *O. cinnamomeum*, *O. claytoniana*, and *O. regalis* concluded that *O. cinnamomeum* was sister to the other two species (Li and Haufler 1994). Although these studies produced valuable insights regarding the biology and genetics of Osmundaceae, their results are inconclusive and mostly taxonomically uninformative due to limited taxon sampling.

We assessed within-species variation for *O. cinnamomeum* and found the two New World collections (Jamaica and U.S.A.; Fig. 1) together are sister to a Japanese collection. Because they are clearly distinct based on nucleotide data, it is possible that New World and Asian individuals of *O. cinnamomeum* represent distinct species, as suggested by Yatabe et al. (1999). *Osmundastrum cinnamomeum* is remarkable for its age; with fossils known from the Late Cretaceous (Serbet and Rothwell 1999), it has existed for at least 70 million years.

Todea and Leptopteris—The small genus *Todea* forms a monophyletic group and is sister to a monophyletic *Leptopteris* (Fig. 1). This result confirms long-standing hypotheses that the two genera are closely related (Hewitson 1962; Yatabe et al. 1999). Given how readily the two genera can be separated using morphological characters (see key) and the strong bootstrap and posterior probability support values for each genus, we see no basis to sink *Leptopteris* into *Todea*, as suggested by Hewitson (1962). Our molecular data were able to distinguish all species sampled, including the two *Todea* species, even though *T. papuana* was only sequenced at two loci (part of *rps4–trnS* and *trnL–trnF*; Appendix 1). Two collections of *L. hymenophylloides*, one from New Zealand and one from cultivation (unknown wild origin), exhibited very little molecular divergence.

Osmunda—We confirm the monophyly of *Osmunda* s.s., consisting of all traditionally accepted *Osmunda* species except *Osmundastrum cinnamomeum*. Within *Osmunda* s.s. our analyses identify three well-supported clades corresponding to the easily distinguished subgenera *Claytosmunda*, *Plenasium*, and *Osmunda* (described below), supporting the classification proposed by Yatabe et al. (2005). Although Miller (1967, 1971) suggested elevating subgenus *Plenasium* to the genus level, we see no reason to follow this suggestion and instead support the recognition of three subgenera within *Osmunda* s.s. The existence of a known hybrid between two of these subgenera (Wagner et al. 1978) recommends against recognizing them as separate genera. *Osmunda* is characterized by hemidimorphic or dimorphic leaves that are pinnate to pinnate-pinnatifid or bipinnate in dissection and herbaceous to subcoriaceous in texture.

Osmunda subg. Claytosmunda—We reveal a robustly supported placement of *O. claytoniana* as sister to the remainder of *Osmunda*, validating the naming of subg. *Claytosmunda* based on an unsupported relationship in an *rbcL* phylogeny (Yatabe et al. 2005). *Osmunda claytoniana* was originally included in subg. *Osmundastrum* together with *O. cinnamomeum* (Hewitson 1962; Kramer 1990), although Miller (1967) placed it in subg. *Osmunda* based on anatomical similarities. The only known North American *Osmunda* hybrid is derived from *O. claytoniana* and *O. regalis* (Wagner et al. 1978), suggestive of a close relationship between *O. claytoniana* and the rest of *Osmunda* s.s. Furthermore, the fossil *O. wehrii* Miller (Miller 1982) is morphologically intermediate between *O. claytoniana* and subg. *Osmunda*.

Accessions of *O. claytoniana* from Japan and U.S.A. were highly similar, but sequence data could only be obtained for three loci (*rbcL*, part of *rps4–trnS*, and *trnL–trnF*; Appendix 1) for the U.S.A. collection. A fossil species, *O. claytoniites* Phipps et al. is known from the Triassic with gross leaf morphology that is remarkably similar to *O. claytoniana*, suggesting that *O. claytoniana* has perhaps been in morphological stasis for at least 200 million years and also that the genus *Osmunda* is at least this old. This subgenus is characterized by herbaceous, hemidimorphic, pinnate-pinnatifid leaves (see key).

Osmunda subg. Plenasium—This monophyletic group of Asian species is easily distinguished morphologically from other species of *Osmunda*. The extremely short branch lengths observed in the topology for this subgenus (Fig. 1), coupled with an earlier contention that *O. vachellii* and *O. javanica* are conspecific based on morphology (Hewitson 1962), suggest that subg. *Plenasium* is in need of critical reevaluation with increased geographic sampling and examination of herbarium specimens. This subgenus possesses evergreen, hemidimorphic, pinnate leaves, with fertile pinnae positioned medially (see key).

Osmunda subg. Osmunda—This monophyletic subgenus consists of *O. regalis* and two closely related Asian species. Our results indicate an interesting biogeographic divergence within the clade: European accessions (Netherlands, Italy, and Germany) of *O. regalis* are sister to a clade composed of a North American *O. regalis* accession, *O. japonica,* and *O. lancea*. The paraphyly of *O. regalis* and the limited divergence among species in this clade could be indicative of ongoing speciation, the presence of cryptic species within *O. regalis*, or that *O. lancea* and/or *O. japonica* are conspecific with *O. regalis* (Hewitson 1962). More extensive geographic sampling and detailed study of herbarium specimens is needed to fully answer this question. This disjunct biogeographic pattern of sister taxa occurring in eastern North America and eastern Asia (Wen 2001) is also present in many other fern genera (e.g. *Diplazium* and *Deparia*; Kato and Iwatsuki 1983; Kato 1993). Subg. *Osmunda* has hemidimorphic, bipinnate leaves with fertile pinnae positioned apically, or dimorphic, bipinnate leaves (see key).

Key to the Genera and Subgenera of Osmundaceae

1. Leaves monomorphic; sporangia following veins on abaxial surface of uncontracted, photosynthetic pinnae 2
   2. Leaves filmy (only a few cells thick), stomata absent; sporangia sparsely arranged ..... *Leptopteris*
   2. Leaves coriaceous, stomata present; sporangia densely arranged, appearing confluent ..... *Todea*
1. Leaves hemidimorphic or dimorphic; sporangia on contracted, nonphotosynthetic pinnae 3
   3. Leaves subcoriaceous, pinnate-pinnatifid, dimorphic; photosynthetic pinnae with tufts of hairs on abaxial surface near rachises ..... *Osmundastrum*
   3. Leaves herbaceous or subcoriaceous, pinnate to pinnate-pinnatifid or bipinnate, hemidimorphic or dimorphic; photosynthetic pinnae without tufts of hairs on abaxial surface near rachises 4
      4. Leaves subcoriaceous, evergreen, pinnate ..... *Osmunda* subg. *Plenasium*
      4. Leaves herbaceous, deciduous, pinnate-pinnatifid or bipinnate 5
         5. Leaves pinnate-pinnatifid, hemidimorphic with fertile pinnae positioned medially ..... *Osmunda* subg. *Claytosmunda*
         5. Leaves bipinnate, hemidimorphic with fertile pinnae positioned apically, or leaves fully dimorphic ..... *Osmunda* subg. *Osmunda*

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APPENDIX 1. List of accessions used in phylogenetic analyses. Format: taxon, Fern DNA Database (http://www.pryerlab.net/DNA_database.shtml) accession number, voucher, collection locality, GenBank accession number for *atpA*, *rbcL*, *rbcL–accD*, *rbcL–atpB*, *rps4–trnS*, *trnG–trnR*, *trnL–trnF* (in that order; dashes indicate missing sequence data). GenBank accession numbers in parantheses indicate sequences in GenBank prior to this study. Geographical origins for multiple accessions of the same species are given in parentheses, following the species name.

Outgroup—*Diplopterygium bancroftii* (Hook.) A. R. Sm., 172, *Smith 2569* (UC), Mexico: Edo. Veracruz, EF588669, EF588691, EF588735, EF588713, (AY612657), EF588778, EF588800. *Dipteris conjugata* Reinw., 140, Fiji: Viti Levu, *Game 98/106* (UC), EF588670, EF588692, EF588736, EF588714, (AY612658), EF588779, EF588801. *Gleichenella pectinata* (Willd.) Ching, 3425, *Christenhusz 4240* (TUR, UPR), Puerto Rico: Roncador, EF588671, EF588693, EF588737, EF588715, EF588757, EF588780, EF588802. *Matonia pectinata* R. Br., 475, *Hasebe 27620* (TI), Malaysia: Selangor, EF588672, (U05634), EF588738, EF588716, (AY621666), EF588781, EF588803.

Ingroup—*Leptopteris fraseri* (Hook. f. & Grev.) C. Presl, 739, *Cranfill s.n.* (UC), Royal Botanic Garden Kew, EF588673, EF588694, EF588739, EF588717, EF588758, EF588782, EF588804. *Leptopteris hymenophylloides* (A. Rich.) C. Presl (NZ), 941, *Smith 2603* (UC), New Zealand: Waipoua Kauri Forest, EF588674, EF588695, EF588740, EF588718, EF588759, EF588783, EF588805. *Leptopteris hymenophylloides* (Cult.), 3729, *Cubey 56* (E), Royal Botanic Garden Edinburgh, EF588675, EF588696, EF588741, EF588719, EF588760, EF588784, EF588784. *Leptopteris wilkesiana* (Brack.) H. Christ, 912, *van der Werff 16025* (UC, MO), New Caledonia: Prov. du Nord, EF588676, EF588697, EF588742, EF588720, EF588761, EF588785, EF588807. *Osmunda banksiifolia* (C. Presl) Kuhn, 3726, *Cubey 55* (E), Royal Botanic Garden Edinburgh, EF588677, EF588698, EF588743, EF588721, EF588762, EF588786, EF588808. *Osmunda claytoniana* L. (USA), 3918, *Bradley 7411* (GMUF), USA: Virginia,—, EF588699,—,—, EF588763,—, EF588809. *Osmunda claytoniana* (Japan), 3749, *Nemoto 99-307* (KYO), Japan: University of Tokyo Nikko Botanical Gardens, EF588678, EF588700, EF588744, EF588722, EF588764, EF588787, EF588810. *Osmunda japonica* Thunb., 3752, *Yatabe 99-0303* (KYO), Japan: Shizuoka Prefecture, EF588679, EF588701, EF588745, EF588723, EF588765, EF588788, EF588811. *Osmunda javanica* Blume, 3727, *Cubey 49* (E), Royal Botanic Garden Edinburgh (original source: Sri Lanka: Central Province), EF588680, EF588702, EF588746, EF588724, EF588766, EF588789, EF588812. *Osmunda lancea* Thunb., 3751, *Hirai 99-0305* (KYO), Japan: University of Tokyo Koishikawa Botanical Gardens, EF588681, EF588703, EF588747, EF588725, EF588767, EF588790, EF588813. *Osmunda regalis* L. (USA), 3434, *Christenhusz 4245* (DUKE, TUR), USA: North Carolina, EF588682, EF588704, EF588748, EF588726, EF588768, EF588791, EF588814. *Osmunda regalis* (Neth.), 3528, *Christenhusz 4271* (DUKE, TUR), Netherlands: Hengelo, EF588683, EF588705, EF588749, EF588727, EF588769, EF588792, EF588815. *Osmunda regalis* (Italy), 3730, *Schwertfeger s.n.* (GOET), Italy: Piemonte, EF588684, EF588706, EF588750, EF588728, EF588770, EF588793, EF588816. *Osmunda regalis* (Germ.), 3731, *Schwertfeger s.n.* (GOET), Germany: Sachsen, EF588685, EF588707, EF588751, EF588729, EF588771, EF588794, EF588817. *Osmunda vachellii* Hook., 793, *Mickel & Beitel s.n.* (UC), China: Hong Kong, EF588686, EF588708, EF588752, EF588730, EF588772, EF588795, EF588818. *Osmundastrum cinnamomeum* (L.) C. Presl (Jamaica), 2596, *Christenhusz 3380* (IJ, TUR), Jamaica: Clarendon, EF588687, EF588709, EF588753, EF588731, EF588773, EF588796, EF588819. *Osmundastrum cinnamomeum* (USA), 3433, *Christenhusz 4244* (DUKE, TUR), USA: North Carolina, EF588688, EF588710, EF588754, EF588732, EF588774, EF588797, EF588820. *Osmundastrum cinnamomeum* (Japan), 3750, *Hasebe 27624* (TI, KYO), Japan: University of Tokyo Nikko Botanical Gardens, EF588689, EF588711, EF588755, EF588733, EF588775, EF588798, EF588821. *Todea barbara* (L.) Moore, 3602, *Schuettpelz 547* (GOET), Germany: Alter Botanischer Garten Göttingen, EF588690, EF588712, EF588756, EF588734, EF588776, EF588799, EF588822. *Todea papuana* Hennipm., 3919, *Hauk 20072* (US), Royal Botanic Garden Kew (original source: Papua New Guinea),—,—,—,—, EF588777,—, EF588823.