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

Jordan S. Metzgar,1 Judith E. Skog,2,3 Elizabeth A. Zimmer,4 and Kathleen M. Pryer1,5

1Department of Biology, Duke University, Durham, North Carolina 27708 U.S.A.
2Department of Environmental Science and Policy, George Mason University, Fairfax, Virginia 22030 U.S.A.
3National Science Foundation, Division of Biological Infrastructure, 4201 Wilson Blvd., Arlington, Virginia 22203 U.S.A.
4Department of Botany and Laboratory of Analytical Biology, Smithsonian Museum Support Center, 4210 Silver Hill Road, Suitland, Maryland 20746 U.S.A.
5Author for correspondence (pryer@duke.edu)

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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; Schuettelz and Pryer 2007). Numerous fossil representatives are known from the Permian onwards (Tidwell and Ash 1994) and fossil representatives of Osmunda L. are known from 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).

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 data were easily aligned using 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 ambiguous 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 (Huelserbeck 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 atpB, rbcL–accD, and rbcL–atpB, the HKY + G model (Hasegawa et al. 1985) was selected for trnG–trnR, the TN + G model (Tamura and Nei 1993) was selected for rbcL, and the K8uf + 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 (Huelserbeck and Ronquist 2003; Ronquist and Huelserbeck 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) ≥ 0.99, a ML bootstrap value (MLBS) ≥ 90%, and a MP bootstrap (MPBS) ≥ 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. Clavotomuda.

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. Clavotomuda. 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 ≥ 0.99, MLBS ≥ 90, MPBS ≥ 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.
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 clear 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 paralogy 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 Hauffner 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. papuanu 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 Claytoniastrum, 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. Claytoniastrum—We reveal a robustly supported placement of O. claytoniana as sister to the remainder of Osmunda, validating the naming of subg. Claytoniastrum 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. vachelli 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. vachelli and O. javonica 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 paralogy 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).
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LITERATURE CITED


Vavrek, M. J., R. A. Stockey, and G. W. Rothwell. 2006. Osmunda vancouverensis sp. nov. (Osmundaceae), permineralized fertile frond seg-


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.


Osmunda vachellii Hook., 793, Mickel & Beitel s.n. (UC), China: Hong Kong, EF588686, EF588708, EF588729, EF588772, EF588795, EF588818.


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