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Stasis and convergence characterize morphological evolution in eupolypod II ferns

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• *Background and Aims* Patterns of morphological evolution at levels above family rank remain underexplored in the ferns. The present study seeks to address this gap through analysis of 79 morphological characters for 81 taxa, including representatives of all ten families of eupolypod II ferns. Recent molecular phylogenetic studies demonstrate that the evolution of the large eupolypod II clade (which includes nearly one-third of extant fern species) features unexpected patterns. The traditional 'athyrioid' ferns are scattered across the phylogeny despite their apparent morphological cohesiveness, and mixed among these seemingly conservative taxa are morphologically dissimilar groups that lack any obvious features uniting them with their relatives. Maximum-likelihood and maximum-parsimony character optimizations are used to determine characters that unite the seemingly disparate groups, and to test whether the polyphyly of the traditional athyrioid ferns is due to evolutionary stasis (symplesiomorphy) or convergent evolution. The major events in eupolypod II character evolution are reviewed, and character and character state concepts are reappraised, as a basis for further inquiries into fern morphology.
• *Methods* Characters were scored from the literature, live plants and herbarium specimens, and optimized using maximum-parsimony and maximum-likelihood, onto a highly supported topology derived from maximum-likelihood and Bayesian analysis of molecular data. Phylogenetic signal of characters were tested for using randomization methods and *fitdiscrete*.
• *Key Results* The majority of character state changes within the eupolypod II phylogeny occur at the family level or above. Relative branch lengths for the morphological data resemble those from molecular data and fit an ancient rapid radiation model (long branches subtended by very short backbone internodes), with few characters uniting the morphologically disparate clades. The traditional athyrioid ferns were circumscribed based upon a combination of symplesiomorphic and homoplastic characters. Petiole vasculature consisting of two bundles is ancestral for eupolypods II and a synapomorphy for eupolypods II under deltran optimization. Sori restricted to one side of the vein defines the recently recognized clade comprising Rhachidosoraceae through Aspleniaceae, and sori present on both sides of the vein is a synapomorphy for the Athyriaceae *sensu stricto*. The results indicate that a chromosome base number of $x = 41$ is synapomorphic for all eupolypods, a clade that includes over two-thirds of extant fern species.
• *Conclusions* The integrated approach synthesizes morphological studies with current phylogenetic hypotheses and provides explicit statements of character evolution in the eupolypod II fern families. Strong character support is found for previously recognized clades, whereas few characters support previously unrecognized clades. Sorus position appears to be less complicated than previously hypothesized, and linear sori restricted to one side of the vein support the clade comprising Aspleniaceae, Diplaziopsidaceae, Hemidictyaceae and Rachidosoraceae – a lineage only recently identified. Despite $x = 41$ being a frequent number among extant species, to our knowledge it has not previously been demonstrated as the ancestral state. This is the first synapomorphy proposed for the eupolypod clade, a lineage comprising 67 % of extant fern species. This study provides some of the first hypotheses of character evolution at the family level and above in light of recent phylogenetic results, and promotes further study in an area that remains open for original observation.

Key words: Athyriaceae, character state reconstruction, convergent evolution, Diplaziopsidaceae, eupolypods II, morphological evolution, Polypodiales, rate of evolution, Rhachidosoraceae, symplesiomorphy, Woodsiaceae.

INTRODUCTION

Despite a rich history of inquiry (e.g. Goebel, 1900; Bower, 1928; Christensen, 1938; Campbell, 1940; Ching, 1940; Holttum, 1947; Ogura, 1972; Tryon and Tryon, 1982; Kramer and Green, 1990) patterns of fern evolution have long confounded systematists, with each new investigation seemingly producing a new hypothesis (and a corresponding new classification of relationship; reviewed by Smith, 1995; Rothfels *et al.*, 2012*b*). More recently, authors of cladistic studies focusing on the evolution of vascular plants have performed the herculean task of parsing the large body of morphological, anatomical, cytogenetic and phytochemical data into character state matrices (Pryer *et al.*, 1995; Stevenson and Loconte, 1996; Kenrick and Crane, 1997; Schneider *et al.*, 2002, 2009; Schneider, 2007). Given their emphasis on broad

relationships, these studies focused heavily on the deepest branches within the fern phylogeny, with generally sparse sampling of the most species-rich order of ferns, the Polypodiales. Recent advances establishing phylogenetic relationships within the Polypodiales (Sano *et al.*, 2000*a*; Pryer *et al.*, 2001, 2004; Wang *et al.*, 2003; Schneider *et al.*, 2004*b*; Wikström and Pryer, 2005; Korall *et al.*, 2006; Schuettpelz *et al.*, 2006; Schuettpelz and Pryer, 2007; Kuo *et al.*, 2011; Rothfels *et al.*, 2012*a*) have left us in an awkward position; whereas the polypod phylogeny is increasingly well understood, patterns of morphological evolution at levels above families are largely unknown.

The need for study is particularly apparent among the eupolypods (= crown Polypodiales), which comprise approximately 67 % of extant fern species (Schneider *et al.*, 2004*b*). Although the families of the two major eupolypod clades – eupolypods I and eupolypods II – have been provided with detailed descriptions (Smith *et al.*, 2006; Rothfels *et al.*, 2012*b*), synapomorphies for eupolypod clades at or above the level of family have not been identified. Within families, many studies have made explicit statements about character evolution and contributed to concepts of character delimitation (Blasdell, 1963; Brown, 1964; Liew, 1971; Hennipman and Roos, 1982; Hovenkamp, 1986, 1996; Moran, 1987; Rödl-Linder, 1990; Bosman, 1991; Moran, 1991; Pacheco and Moran, 1999; Cranfill and Kato, 2003; Ranker *et al.*, 2004; Janssen and Schneider, 2005; Kreier and Schneider, 2006; Labiak *et al.*, 2010*a*, *b*; Moran *et al.*, 2010*b*; Moran and Prado, 2010; Sundue, 2010*b*; Sundue *et al.*, 2010). However, these studies have narrow taxonomic breadth, focusing primarily on characters among species and closely related genera. Between these two foci – deep branches of the fern phylogeny and branch tips within families – a large gap remains.

We aim to narrow this gap by investigating the morphological evolution of eupolypod ferns. We focus on the eupolypods II, a clade for which the recent study of Rothfels *et al.* (2012*a*) provides a robust phylogenetic foundation. As currently circumscribed, eupolypods II is a large clade, comprising over 2600 species in ten families (Rothfels *et al.*, 2012*b*). These families include some of the largest genera of ferns: *Asplenium* (Aspleniaceae, approx. 700 spp.), *Cyclosorus* (Thelypteridaceae, approx. 650 spp.), *Diplazium* (Athyriaceae, approx. 400 spp.), *Athyrium* (Athyriaceae, approx. 200 spp.) and *Blechnum* (Blechnaceae, approx. 150 spp.; richness estimates from Kramer *et al.*, 1990*a*, *b*; Kramer and Viane, 1990; Smith, 1990; Liu *et al.*, 2009). Recognition of the eupolypod II clade is recent, the name itself having been coined by Schneider *et al.* (2004*b*), and its composition only established as a result of subsequent densely sampled studies (Schuettpelz and Pryer, 2007; Kuo *et al.*, 2011; Rothfels *et al.*, 2012*a*).

Rothfels *et al.* (2012*a*) assembled a molecular dataset (five plastid loci) for 67 eupolypod II species and 14 outgroup taxa. Two results from that study stand out as demanding further investigation and motivated our investigation of this clade. First, included in eupolypods II are several apparently synapomorphy-rich lineages with long histories of taxonomic recognition – i.e. Aspleniaceae, Thelypteridaceae, Onocleaceae and Blechnaceae – whose relationships to each other are obscure as inferred from morphological data. What characters unite these seemingly disparate groups? Second, Woodsiaceae *sensu* Smith *et al.* (2006) (the 'athyrioids') was revealed to be polyphyletic. Some if its

components – i.e. Cystopteridaceae, Hemidictyaceae and Woodsiaceae *sensu* Rothfels *et al.* (2012*b*) – have historically been of uncertain placement. Their position as evolutionarily distant from the bulk of Woodsiaceae *sensu* Smith *et al.* (2006) is thus not too surprising. However, two other lineages – Rhachidosoraceae and Diplaziopsidaceae – have always been considered closely allied with the athyrioids, prior to molecular phylogenies. Not only have these three clades invariably been considered confamilial, but the former two have nearly always been considered congeneric with each of the two large athyrioid genera (Rhachidosoraceae in *Athyrium* and Diplaziopsidaceae in *Diplazium*, respectively; Kato, 1975; Kato and Darnaedi, 1988). Is this scattering of the 'athyrioid morphology' across the tree due to evolutionary stasis (symplesiomorphy) or convergent evolution? Here, we develop a large morphological matrix to address these questions. Simultaneously, we review the major events in eupolypod II character evolution, and critically reappraise character and character state concepts, with the goal of providing a basis for further inquiries into fern morphology.
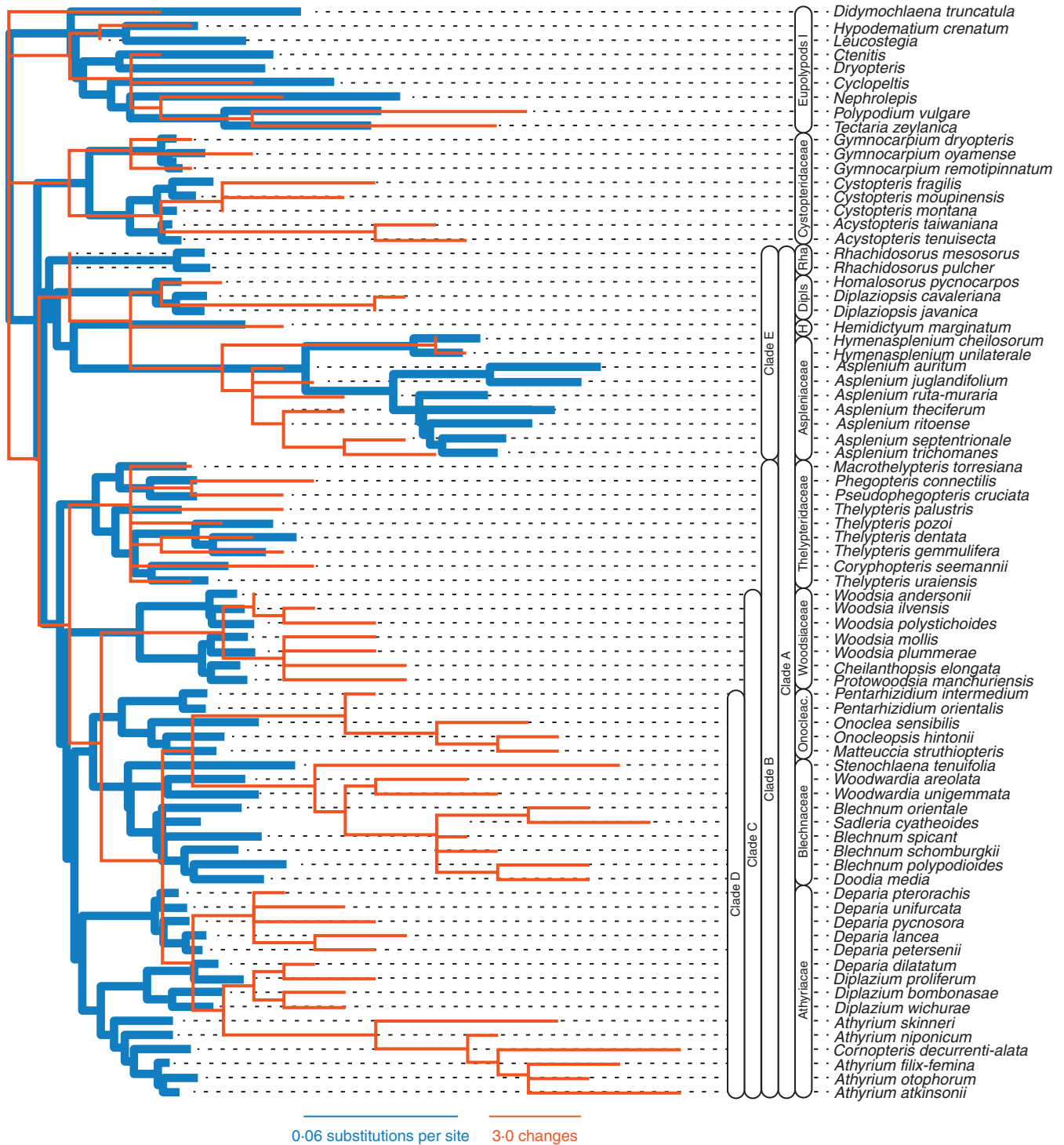
MATERIALS AND METHODS

Taxon selection

We based our study on the topology from Rothfels *et al.* (2012*a*), hereafter referred to as the '2012 topology' (Fig. 1). The 81 taxa sampled included 67 from eupolypods II and 14 outgroup accessions comprising members of eupolypods I, Pteridaceae and Dennstaedtiaceae. This sampling included the deepest divergences as suggested by previous molecular (particularly Sano *et al.*, 2000*a*; Tzeng, 2002; Schuettpelz and Pryer, 2007; Kuo *et al.*, 2011) and morphological (chiefly Kato and Darnaedi, 1988; Wang *et al.*, 2004) studies. The focus on deep divergences required sparse sampling within the large families (Athyriaceae, Blechnaceae and Thelypteridaceae); conclusions about finer-scaled patterns in these families must await more densely sampled studies.

Character and character state coding

We scored a total of 79 characters (Appendix 1), including those of habit, roots, rhizomes (stems), leaves (including development), phyllotaxis, petioles, laminae and axes, sori and sporangia, spores, gametophytes, and chromosome base numbers. Characters that required microscopy (sporangial stalk width, and number of annular cells) were visualized and photographed on a light microscope. States were scored as polymorphic when variation existed. Our matrix was completed with data from literature resources including general texts on fern systematics and morphology (Bower, 1923, 1928; Campbell, 1940; Ogura, 1972; Tryon and Tryon, 1982; Tryon & Lugardon, 1991; Kramer and Green, 1990) and more specific monographic, floristic and morphological studies (Slosson, 1906; Pickett and Thayer, 1927; Holttum, 1940, 1949, 1954, 1958, 1959, 1960; Copeland, 1947; Stokey and Atkinson, 1952; Wagner, 1952; Momose, 1958, 1959, 1960*a*, *b*, 1964, 1965*a*, *b*; Wilson, 1959; Blasdell, 1963; Atkinson and Stokey, 1964; Brown, 1964; Ching, 1964, 1978; Nayar *et al.*, 1966; Bir, 1969*b*, 1971; Bir and Trikha, 1970; Iwatsuki, 1970; Liew, 1971; Nayar and Kaur, 1971; Kato, 1972, 1973, 1975, 1977, 1979*a*, *b*, 1984;

Fig. 1. Maximum-likelihood phylogram of the molecular dataset from Rothfels *et al.* (2012*a*, blue) compared with maximum-parsimony branch lengths of the morphological data set (orange). Letters A–G refer to clades cited in the text. Family names follow Rothfels *et al.* (2012*b*) (Onocleac. = Onocleaceae; H = Hemidictyaceae; Dipls = Diplaziopsidaceae; Rha = Rhachidosoraceae).

Atkinson, 1973; Sledge, 1973; Masuyama, 1975*a*, *b*; Huang, 1981; Holttum, 1982; von Aderkas, 1983; von Aderkas and Green, 1986; Kramer, 1987; Nauman, 1989; Kato and Kramer, 1990; Kramer and Green, 1990; Kramer *et al.*, 1990*b*; Price, 1990; Murakami and Moran, 1993; Liu *et al.*, 2009;

Mendoza-Ruiz and Pérez-García, 2009). Characters 29, 31, 75, 78 and 79 were scored entirely from the literature. Many of our character and character state concepts were taken from previous studies, particularly Pryer and Smith (1995), Stevenson and Loconte (1996), Moran *et al.* (2007, 2010*a*) and Sundue *et al.*

(2010). The final data matrix is available on TreeBASE [14610]. Representative voucher specimens used to score the characters are listed in Supplementary Data Appendix S1.

Character state reconstruction

Character matrices were assembled and optimizations performed using *Winclada v1·7* (Nixon, 1999–2002), and *Mesquite v2·75* (Maddison and Maddison, 2006). Ancestral character-state reconstructions were performed under both maximum-parsimony (MP) and maximum-likelihood (ML), on the 2012 topology. Parsimony reconstructions were performed using *Winclada*, except for characters 74 (mean number of annular cells), and 75 (chromosome base number), where the number of states exceeded that supported by *Winclada*; these were optimized using *Mesquite*. *Winclada* MP optimizations were explored using acctran, deltran and unambiguous optimizations. ML optimizations were performed using *Mesquite*, under the Mk1 model (Lewis, 2001). All characters were treated as unordered and qualitative, including those (e.g. chromosome base number and number of vascular bundles in petiole vasculature) that have numerical character states.

Tests for phylogenetic signal

We tested for phylogenetic signal in each character using two methods. First, we compared the length of a character (number of changes under MP) when optimized on the 2012 topology with the average length of the same character when optimized onto 1000 81-taxa Yule trees simulated in *Mesquite*. Secondly, we used *fitDiscrete* in the R package *Geiger* (Harmon *et al.*, 2007; R Development Core Team, 2011) by optimizing the value of λ (Pagel, 1999; Freckleton *et al.*, 2002) for each character on the phylogeny using ML. We compared the likelihood of the free model to that of a model where λ was constrained to zero (no phylogenetic signal) using a likelihood ratio test. This was performed for 49 of the 79 characters (we did not test characters that had a length of 0 or were autapomorphic, or where there were large amounts of missing data). For characters with small amounts of missing data, we performed the test by dropping the taxa that lacked data.

A topology derived from an analysis of morphological characters was not the focus of our investigation. We did, however, perform an MP analysis of the matrix to compare the resulting character statistics [length, consistency index (CI), retention index (RI)] on the most parsimonious trees with those from the same characters when optimized on the 2012 topology. This MP analysis was performed using *TNT* (Goloboff *et al.*, 2008), with 2000 iterations of the parsimony ratchet (Nixon, 1999), 4 % up- and down-weighting, 60 iterations of tree drift and 15 rounds of tree fusion, followed by branch swapping to completion.

RESULTS

Character values

The final matrix (TreeBase accession 14610) consisted of 6399 cells (79 characters scored for 81 taxa). Missing data ('?')

constituted 2 % of the matrix and inapplicable ('–') cells constituted 8 %. Three characters were invariant (21: young leaves covered in mucilage; 44: central ridge of rachis; 56: hamate hairs), and four were autapomorphic (4: proliferous roots; 49: submarginal collecting vein; 57: hairs forked stellate or stalked-stellate; 72: paraphyses), leaving 72 characters that were informative under our equally weighted unordered model. MP analysis of these characters alone found 12 800 most-parsimonious trees (not shown) with length = 528, CI = 0·24 and RI = 0·60. Optimized onto the 2012 topology these characters had a length of 592 steps, a CI of 0·21 and an RI of 0·54. The length, CI and RI of each character optimized onto the morphological tree compared with the same metrics when optimized onto the 2012 topology are presented in Supplementary Data Table S1.

Molecular versus morphological rates of evolution

Morphological and molecular branch lengths of the 2012 topology followed a similar general pattern: long branches among families and genera and short branches along the backbone (Fig. 1). The ratio of molecular to morphological branch lengths is particularly high for the Aspleniaceae and Rhachidosoraceae (these taxa have relatively faster rates of molecular than morphological evolution), whereas the opposite pattern characterizes *Athyrium* and the clade comprising Blechnaceae and Onocleaceae (Fig. 1).

Phylogenetic signal and character state reconstruction

We were able to reject the null hypothesis that the character lengths did not differ from random for 64 of the 79 characters. The eight characters for which the null hypothesis could not be rejected (10, 17, 27, 35, 45, 46, 52, 58) had high levels of homoplasy as indicated by their low CI and RI values (Supplementary Data Table S1). The seven remaining characters either had lengths of 0 or were autapomorphic, and therefore were not tested. We were unable to reject the null hypothesis in 13 out of the 49 tests. These characters included all of those for which the null hypothesis could not be rejected using randomization, as well as nine others (10, 12, 13, 14, 25, 26, 45, 46, 52). These characters also had relatively low CI and RI values.

Excluding autapomorphies, 14 state changes occurred without homoplasy among ingroup taxa (Table 1); the remaining state changes were homoplastic. Under acctran reconstruction, the number of character state changes without homoplasy increased to 17, and under deltran there were 14 (Supplementary Data Fig. S1).

DISCUSSION

Character values and phylogenetic signal

Tests for phylogenetic signal using randomization and Pagel's λ demonstrate that most of the morphological characters are non-randomly distributed on the 2012 topology, lending further credence to the assertion that both data types reflect a common evolutionary history. Not surprisingly, characters whose optimization does not differ significantly from random are highly homoplastic, characterized by low CI and RI (Supplementary Data Tables S1, S2). We also find evidence for general similarity

Table 1. *Unambiguous synapomorphies for eupolypod II ferns*

Character	Character state	Clade supported
3	Roots fleshy and pale	Diplaziopsidaceae
20	Fertile lamina reflexed and enclosing sori	Onocleaceae
26	Petioles with a proximal articulation above the base	*Woodsia ilvensis* + *W. polystichoides*
29	Xylem bundles in petiole distally united forming an x-shape	Aspleniaceae
40	Leaf margin decurrent and forming lateral ridges along rachis	Aspleniaceae
48	Smooth leaf margin	Athyriaceae
51	Mature leaf with abundant anthocyanins	Athyriaceae
69	Indusium composed of strap-shaped lobes	Woodsiaceae
69	Indusium deltate	Onocleaceae
73	Sporangia stalk uniseriate in the middle	Aspleniaceae
75	Chromosome base number of 34	Blechnaceae
75	Chromosome base number of 42	*Cystopteris* + *Acystopteris*
75	Chromosome base number of 39	*Hymenasplenium*
77	Chlorophyllous spores	Onocleaceae

between the optimizations of the morphological and molecular data sets; nearly half of our characters had the same number of steps when optimized on the most parsimonious topologies from the morphological data as they did on the 2012 molecular topology (Supplementary Data Table S1).

Morphological evolution early in the eupolypod II radiation

Very few characters bear witness to the early eupolypod II divergences. The distribution of branch lengths for the optimized morphological data resembles that for the molecular phylogeny of Rothfels *et al.* (2012a; Fig. 1) and fits an ancient rapid radiation model (long ingroup branches distributed among short backbone internodes; Whitfield and Lockhart, 2007; Jian *et al.*, 2008; Rothfels *et al.*, 2012a). The relatively few character changes mapping to these deep branches is not surprising (if there were many such characters, we would expect these clades to have been recognized long ago). Morphological and molecular characters appear to have evolved in comparable ways during the early diversification of eupolypods II (these early divergences were not, for example, associated with bursts of morphological evolution).

No character changes unambiguously map to the base of the eupolypods II under MP reconstruction (Supplementary Data Fig. S1). However, confluence of the rachis sulcus wall with the pinna costa supports eupolypods II under acctran reconstruction, and the presence of two vascular bundles at the base of the petiole (Fig. 2B) supports eupolypods II using deltran; this result is also supported by ML character reconstruction (0·986 proportional likelihood). Petiole vasculature has long attracted the attention of systematists (Presl, 1848 [1847]; Ching, 1936; Keating, 1968; Lin and DeVol, 1977, 1978) and the presence of two vascular bundles has been used to unite some of the eupolypod II taxa (Kato and Kramer, 1990), yet no previous system has proposed a classification uniting what amounts to nearly all of the eupolypods with two vascular bundles in their petioles (this character occurs infrequently among eupolypods I and the rest of the Polypodiales). That the eupolypods II were not recognized and circumscribed earlier suggests that systematists did not allow for a classification that weighted this character so heavily, and indeed fern classification of the 19th century favoured reproductive characters of the sporangium, sorus and indusium to define taxa at higher ranks (Paris and Barrington, 1990).

The majority of character changes within the eupolypod II phylogeny occur at the family level or above (Fig.1) regardless of optimization (Supplementary Data Fig. S1). For example, a total of 32 character changes occur along the stem branches of the ten eupolypod II families; under acctran and deltran optimization these clades are supported by 61 and 51 character changes, respectively. By comparison, the deeper clades are supported by a total of only four unambiguous characters (12 and eight changes under acctran and deltran, respectively). These data are consistent with our initial premise, that eupolypod II ferns comprise a number of apomorphy-rich families whose morphological relationships to each other are obscure; the rapid radiation of the eupolypods II was not accompanied, at least originally, by extensive morphological evolution.

Clades that were not widely recognized prior to molecular evidence are supported by few characters in our results. Character support for these groups includes conspicuously pale and fleshy roots supporting the Diplaziopsidaceae (Fig. 2A), dimorphic leaves supporting Onocleaceae + Blechnaceae, veins reaching the leaf margin supporting Cystopteridaceae, and single-sided sori supporting the clade Rhachidosoraceae + Diplaziopsidaceae + Hemidictyaceae + Aspleniaceae (Fig. 3B–E).

Polyphyly of the Woodsiaceae

Characters used by Smith *et al.* (2006) in their description of the Woodsiaceae are predominantly plesiomorphic when optimized onto the 2012 topology, including: terrestrial habit, with non-clathrate and glabrous scales, petioles with two vascular bundles distally fused into a single U- or V-shaped bundle, usually holomorphic leaves with predominantly free veins, and elongate sori that are abaxial with a linear indusium. Other character states used in their description to account for variation among the taxa are synapomorphic albeit homoplastic, and do not unite large groups; at most they unite individual families. These include: erect rhizomes, glandular scales, anastomosing veins, and round sori that are exindusiate or with reniform or J-shaped indusia.

Kato and Kramer's (1990) Physematieae comprises similar taxa to the Woodsiaceae *sensu* Smith *et al.* (2006), but was defined using a different suite of characters, also revealed here to be symplesiomorphic, apomorphic or homoplastic. Homoplastic characters include petiole bases swollen or forming trophopods
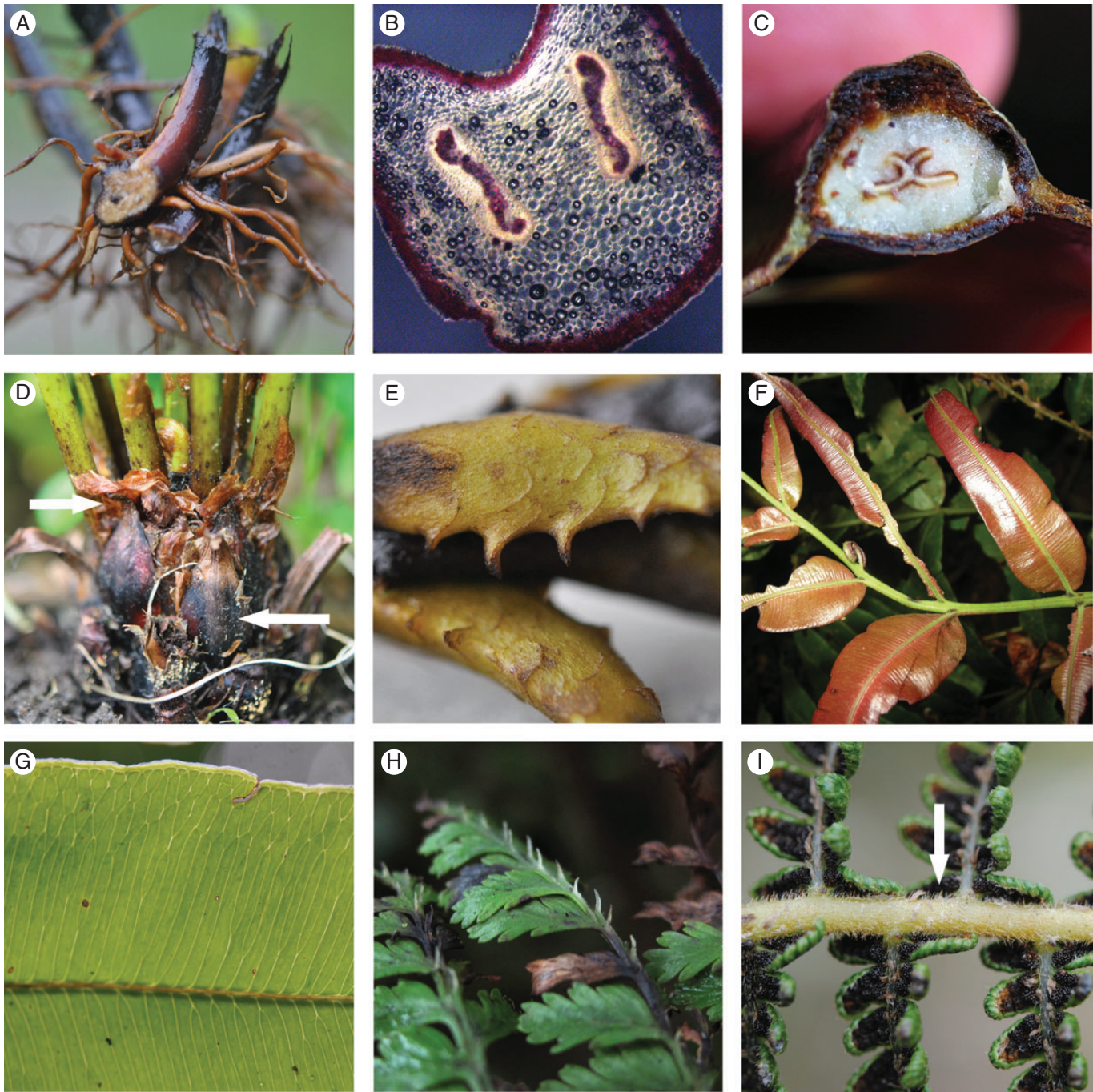
FIG. 2. Morphological features in eupolypod II ferns. (A) Pale fleshy roots, *Homalosorus pycnocarpos*; (B) petiole base with two vascular bundles, *Homalosorus pycnocarpos*; (C) medial portion of petiole with two vascular bundles fused into an x-shape, *Asplenium nidus* (photo: Tom Ranker, used with permission); (D) trophopods, left arrow points to aborted leaf apex, right arrow points to thickened persistent starch-filled petiole base, *Matteuccia struthiopteris*; (E) pneumatophores, *Deparia acrostichoides*; (F) reddish young leaves, *Salpichlaena volubilis*; (G) anastomosing veins and membranaceous leaf margin, *Hemidictyum marginatum*; (H) pinna costae with adaxial projections, *Athyrium microphyllum*; (I) arrow points to whitish acicular hairs, *Thelypteris supina*.

(Fig. 2D), petioles with pneumatophores (Fig. 2E) and sulcus grooves continuous between axes (Fig. 4D). Plesiomorphic characters include elongate sori (Fig. 3B–I), adaxially sulcate axes (Fig. 4B–G), veins that are free rather than anastomosing, sori that are along veins rather than at vein tips (Fig. 3C), and petioles with two vascular bundles at the base (Fig. 2B).

Thus, our results indicate that the scattering of the 'athyrioid' morphology across the tree is due both to morphological stasis (symplesiomorphy) and to the parallel evolution of a suite of derived characters; however, any adaptive significance of these characters is unclear. This result is reminiscent of the polyphyly in other fern groups studied recently, which were also defined by
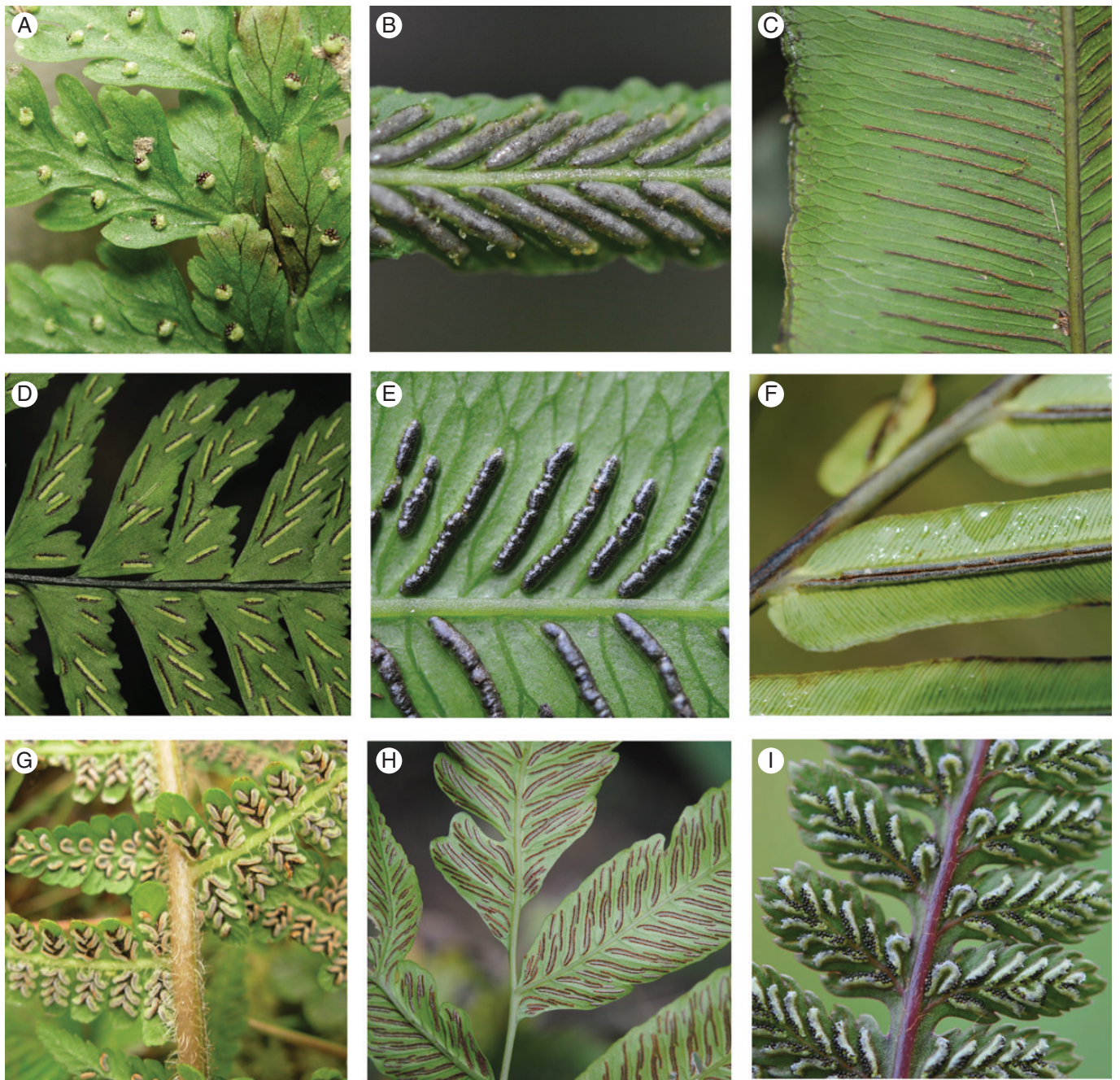
FIG. 3. Soral and indusial characters of eupolypod II ferns. (A) Round sori, *Cystopteris fragilis*; (B–F) elongate sori restricted to one side of the vein: (B) *Homalosorus pycnocarpos*; (C) *Hemidictyum marginatum*; (D) *Hymenasplenium laetum*; (E) *Diplaziopsis javanica*; (F) *Blechnum orientale*; (G–I) elongate sori along both sides of the vein: (G) *Athyrium dolosum* (=*Deparia* sect. *Lunathyrium*); (H) *Diplazium riparium*; (I) *Athyrium niponicum*.

homoplastic or plesiomorphic characters: *Bolbitis* (Moran *et al.*, 2010*a*), *Lellingeria* (Labiak *et al.*, 2010*b*), *Terpsichore* (Ranker *et al.*, 2004; Sundue, 2010*a*, *b*), and *Cheilanthes*, *Pellaea* and *Notholaena* (Kirkpatrick, 2007; Prado *et al.*, 2007; Rothfels *et al.*, 2008; Johnson *et al.*, 2012). In several of these cases new characters were identified in support of the novel phylogenetic relations. In *Terpsichore*, for example, the re-circumscription of the genus and its allies based on molecular data resulted in a classification that was more congruent with morphological data than was the original pre-molecular

classification (Sundue, 2010*b*). In general, however, this is not the case in eupolypods II – the clades that were not widely recognized prior to the results of Rothfels *et al.* (2012*a*) have weak character support. Furthermore, the characters that unite them are mostly homoplastic, appearing elsewhere in the tree.

Morphological characteristics of major eupolypod II clades

Our analysis of morphological evolution across the eupolypod II phylogeny yielded many further insights beyond those of the
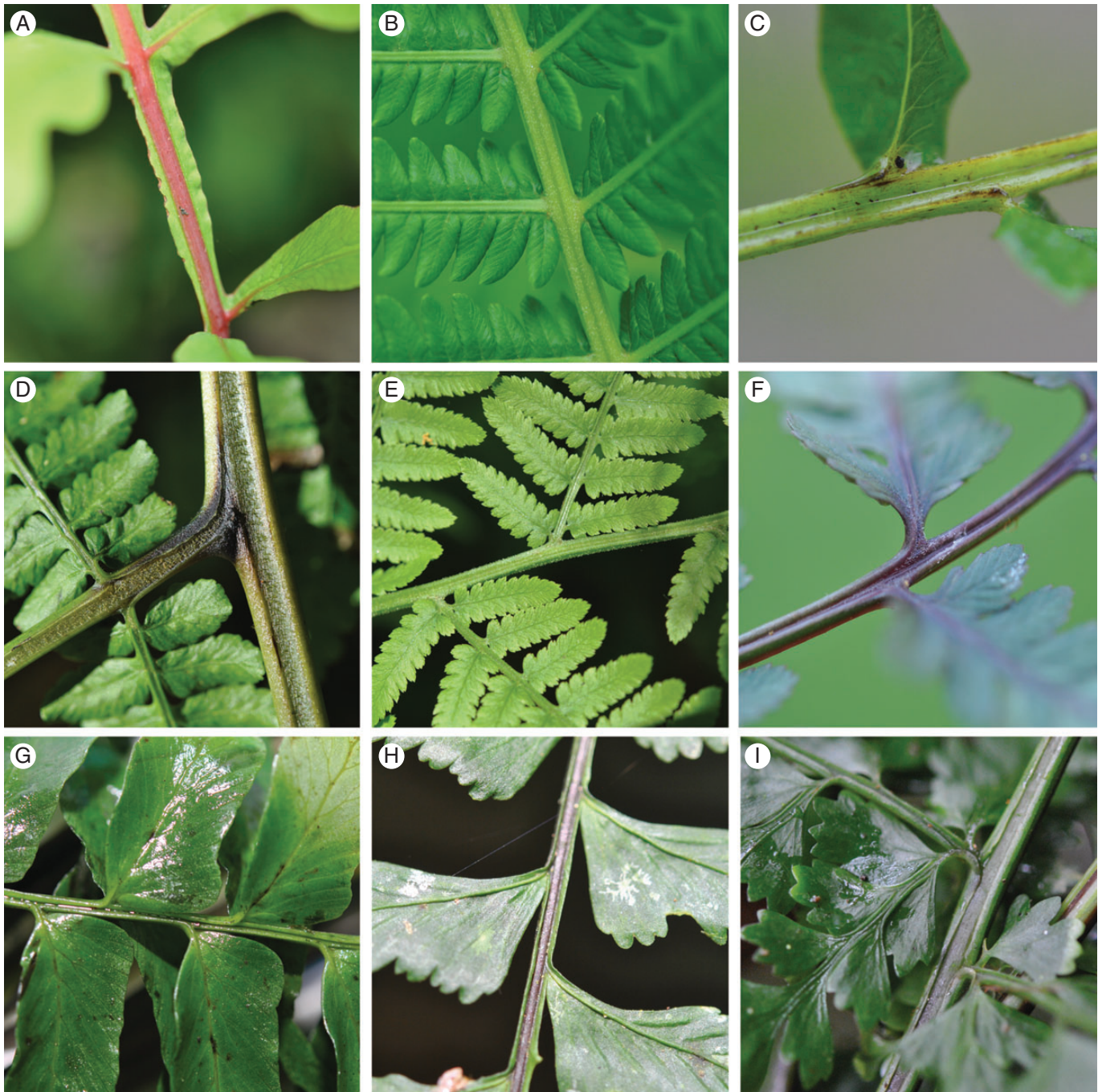
Fɪɢ. 4. Rachis–costa architecture in eupolypod II ferns. (A) *Onoclea sensibilis*; (B) *Matteuccia struthiopteris*; (C) *Homalosorus pycnocarpos*; (D) *Diplazium assimile*; (E) *Deparia acrostichoides*; (F) *Athyrium japonicum*; (G) *Hymenasplenium riparium*; (H) *Asplenium harpeodes*; (I) *Asplenium uniseriale*.

two main patterns discussed above. In the following section we discuss the main findings in select clades, in an approximately top-to-bottom order as presented in Fig. 1.

Eupolypods II. The value of petiole vasculature has been highlighted before (Ching, 1936; Keating, 1968; Lin and DeVol, 1977, 1978), but the specific character state of two vascular bundles at the base of the petiole has only recently been forwarded as a uniting character for the eupolypods II (Schuettpelz and Pryer, 2007; Rothfels *et al.*, 2012*b*).

Cystopteridaceae. The placement of *Cystopteris sensu lato* (s.l.) and *Gymnocarpium* was not agreed upon by systematists prior to early molecular phylogenetic studies (Wolf *et al.*, 1994; Hasebe *et al.*, 1995), which found support for their sister relationship, and more recent studies (Schuettpelz and Pryer, 2007; Kuo *et al.*, 2011; Rothfels *et al.*, 2012*a*), which found support for their position as sister to the remainder of eupolypods II. Their close relationship was anticipated by Diels (1899), who placed them in the Woodsiinae; however, this group also included unrelated taxa with basal indusia. Tryon and Tryon (1982) commented

that characters exhibited by *Cystopteris* affiliated it to '*Athyrium* and perhaps *Gymnocarpium*', but they did not list specific characters. Not surprisingly, we find few morphological characters that these taxa have in common. Unambiguous character changes include long creeping rhizomes, and veins reaching the leaf margins. Both of these characters are homoplastic; however, the character of veins reaching the leaf margins is a useful character for distinguishing *Cystopteris* from *Woodsia*, with which it can easily be confused when sterile.

Gymnocarpium. As pointed out by Kato and Kramer (1990), the petiole vasculature and leaf architecture of *Gymnocarpium* is similar to that of the athyrioid ferns. The distinctive swollen pinna bases of *Gymnocarpium* are often used as diagnostic characters because they are rare among ferns; not surprisingly, they act as a synapomorphy here. Although commonly described as round in floristic treatments, the sori of *Gymnocarpium* are slightly elongate, with the sporangia spread along the vein. The chromosome base number $x = 40$ also supports the genus and distinguishes it from the remaining members of the Cystopteridaceae, but with homoplasy; this number also occurs in clade D (Onocleaceae – Athyriaceae) and contributed to Kato and Kramer's (1990) argument that *Gymnocarpium* was related to those ferns.

Clade E, Rhachidosoraceae – Aspleniaceae. The grouping of Rhachidosoraceae, Diplaziopsidaceae, Hemidictyaceae and Aspleniaceae was not suggested prior to molecular phylogenetic studies. Whereas the latter three families form a well-supported clade, the position of Rhachidosoraceae has not yet been well supported (Rothfels *et al.*, 2012*a*). Genera that constitute the first three families were long considered to be allied with *Diplazium* and *Athyrium* (e.g. Kato and Kramer, 1990) because of their morphological similarity. This similarity extends into Aspleniaceae; some *Hymenasplenium* are remarkably similar in gross morphology to members of *Diplazium* (Smith, 1976). Distinguishing members of clade E from those of the Athyriaceae is not always easy. However, sori restricted to one side of the vein – sometimes referred to as 'asplenioid' sori (Fig. 3B–E) – supports this clade, and sori occurring on two sides of a vein – often called 'diplazioid' or 'back-to-back' sori (Fig. 3G–I) – are predominant in Athyriaceae. Thus, this relatively simple character is an important aid in diagnosing these two clades. There are, however, exceptions: diplazioid sori, for example, occur in some Aspleniaceae (Fig. 3D) and also rarely in *Diplaziopsis* and *Homalosorus pycnocarpos* (Slosson, 1906). In Aspleniaceae they tend to be restricted to small portions of the lamina where vein groups meet. Holttum (1947) suggested that these aberrations are probably the result of lamina fusion or reduction. More problematic is that many species of Athyriaceae, particularly in *Deparia* and *Athyrium*, commonly have one-sided sori either sporadically or throughout their laminae. These exceptions weaken the diagnostic power of these characters; nonetheless, the overall pattern of character optimization on the 2012 topology is simple compared with that required by previous classifications.

Rhachidosoraceae. These present an array of characters that are emblematic of the problems with the previous classifications of 'athyrioid' ferns. Many of the characters that define the Rhachidosoraceae also occur among Athyriaceae, including the rachis–costa architecture, the minute corniculae and in some species, scales that are present at the base of the adaxial pinnae costae. At the same time, the single-sided 'asplenioid' sori and clathrate scales of the Rhachidosoraceae are characters that they share with the Aspleniaceae. These two character states are uncommon in Athyriaceae species and so are useful characters for distinguishing these families.

Diplaziopsidaceae. The conspicuously soft, pale, fleshy roots of *Homalosorus* (Fig. 2A) and *Diplaziopsis* have been noted previously (Bir, 1969*a*; Price, 1990); however, a novel finding of this study is that they serve as a synapomorphy for the Diplaziopsidaceae. Anatomical study is needed to further characterize how they differ from the typical black wiry roots of other eupolypod II ferns.

Hemidictyum. This has long been viewed as a morphologically isolated taxon whose relationships are unclear (Stolze *et al.*, 1994); our character optimizations confirm its unique morphology. Seven character changes support *Hemidictyum*, including two autapomorphic characters – proliferous roots and a submarginal collecting vein (Fig. 2G) – yet characters uniting *Hemidictyum* to its sister taxon, Aspleniaceae, are nearly absent. A large number of characters are shared with *Diplaziopsis*, including anastomosing veins, isodromous venation, expanded vein endings and a terminal pinna conform to the lateral pinnae, but these are interpreted as homoplastic in our results.

Aspleniaceae. These have strong character support. They also lie along one of longest branches in the 2012 topology (Rothfels *et al.*, 2012*a*; Fig. 1). Two synapomorphies, vascular bundles united distally in the petiole forming an x-shape (Fig. 2C) and sporangial stalks that are uniseriate in the middle, occur without homoplasy in eupolypods II. Clathrate rhizome scales and an epiphytic habit support the family under deltran and acctran reconstruction, respectively. Additional sampling of this large family, however, is necessary to accurately determine ancestral states of some key characters such as habit, which varies widely in the family (Schneider *et al.*, 2004*a*).

Hymenasplenium. This was described by Hayata (1927) in his article on the systematic importance of stem vasculature. Hayata's illustration depicts the dorsal distichous leaf arrangement, ventral root insertion and long-creeping rhizome that distinguish *Hymenasplenium* from *Asplenium*, characters that are also recovered here. Murakami and Moran (1993) additionally pointed out that *Hymenasplenium* differs from *Asplenium* in its rachis–costa architecture. Our results demonstrate that the rachis–costa architecture of *Hymenasplenium* (Fig. 4G) is similar to that of many former 'athyrioids', including Athyriaceae (excluding *Deparia*), Cystopteridaceae, Diplaziopsidaeae and Rhachidosoraceae. *Asplenium*, by contrast, has rachis–costa architecture otherwise not seen among eupolypod II ferns (Fig. 4H–I), one that is characterized by a terete and alate rachis with wings that are confluent with the basiscopic pinna margin.

Clade B, Thelypteridaceae – Athyriaceae. Similar to other backbone nodes, the clade of Thelypteridaceae – Athyriaceae is supported by only a single character under unambiguous optimization: laminae that are 1-pinnate-pinnatifid. However, acctran and deltran optimizations find four characters each in support of this node.

Thelypteridaceae. Supporting characters in our results closely match those used in general family descriptions (e.g.

Christensen, 1913; Ching, 1936; Morton, 1963; Smith, 1990), particularly the presence of acicular hairs (Fig. 2I), and hairs present on rhizome scales (on the surface and/or margins). As anticipated by Kramer (1987), catadromous venation supports the Thelypteridaceae as well.

Woodsiaceae. These are supported by the basal indusium that is divided into narrow strap-shaped processes, the same characters that have been used to distinguish the genus since its inception (Brown, 1810). Here, we also find that the epipetric habit, suberect rhizomes and a raised receptacle support this clade under all optimizations. Dromy in leaf venation may also support this clade, but our results are ambiguous. All of the species examined were found to be polymorphic, exhibiting both anadromy and isodromy in different leaves.

Clade F, Onocleaceae + Blechnaceae. Bower (1914, 1928) championed a close relationship between Onocleaceae and Blechnaceae based on shared leaf dimorphism and what he interpreted as similarities in the sori. Lloyd (1971) presented careful arguments demonstrating that the soral similarities were superficial; the indusium of the Blechnaceae and the similar-looking recurved leaf margin of the Onocleaceae are not homologous. Furthermore, both the ontogeny and the relationship of the sori to venation differ in the two families. Only the condition of having morphologically different sterile and fertile leaves is potentially homologous. Thus, the strong molecular support for the sister-relationship of the families evokes a sense of irony; Bower appears to be right, but for the wrong reasons. As interpreted here, fertile leaf dimorphism is the only character uniting the two families unambiguously. Monomorphy is also present in Blechnaceae, and denser taxon sampling is required to determine which state is ancestral. Several other characters support the clade using acctran or deltran optimization (Supplementary Data Fig. S1B, C), but are highly homoplastic.

Blechnaceae. These are supported here by three characters regardless of the optimization used: petioles with more than two vascular bundles at the base, veins with costular anastomoses and leaves that are reddish when young. With its climbing rhizome and acrostichoid sori, *Stenochlaena* departs strongly from the remainder of the family. The effect is that several of the characters usually considered diagnostic of Blechnaceae do not optimize onto the branch leading to the most recent common ancestor of the family, but rather on the next clade. This is the case for sori borne upon a costal commissure (Fig. 3F), considered the hallmark of the family, and catadromous leaf venation, which is widespread in the family (Kramer, 1987). Taxonomic sampling in the 2012 topology, however, is limited. In Cranfill and Kato's (2003) topology, *Stenochlaena* is nested deeper among other Blechnaceae.

Athyriaceae. These are unambiguously supported by the presence of a soral arrangement where one or two indusia open to face away from a single vein, commonly called a 'back-to-back' arrangement (Fig. 3G–I). It is important to note that soral arrangement is scored separately from indusium shape. Sori back-to-back includes both the 'athyrioid' J-shaped indusia where the sorus wraps around the vein (Fig. 3I), as well as 'diplazioid' where the sorus is divided into two parts (Fig. 3H). Subsequent character transformations away from this state do

occur, however, in some species of *Deparia*, *Cornopteris* and *Pseudocystopteris* (=*Athyrium*).

Deparia. This is supported here by petiole bases enlarged and forming trophopods, and by catenate hairs that are reduced from scales. Under acctran reconstruction, it is also supported by petiole bases with pneumatophores (Fig. 2E) and spores with a tuberculate perispore. The transition from scales to catenate hairs is particularly distinctive, and was used by Kato (1973, 1984) and Sano *et al.* (2000*b*) to circumscribe the genus. This character state is, however, homoplastic. Similar hairs occur in *Diplazium* (species treated in *Callipteris* by Pacheco and Moran, 1999), *Acystopteris* and some species of *Woodsia* (e.g. *W. mollis*) – other species of *Woodsia* have septate hairs, but these are not part of a reduction series beginning with broad scales. Kato also championed the use of rachis grooves that are not continuous with those of the pinna costae (Fig. 4E). Although present in *Deparia*, non-continuous rachis grooves are symplesiomorphic in this clade. The transition to continuous grooves supports the clade of *Diplazium* and *Athyrium s.l.* under acctran.

Evolution of critical characters

Scoring the morphological character states of a diverse sample of eupolypods II, and then reconstructing those characters onto a phylogenetic hypothesis, yielded many insights into the nature and evolution of specific characters. Many of these insights are relevant beyond the eupolypods II, and we discuss them here.

Petiole vasculature. When composed of two bundles this is demonstrated here to be ancestral for eupolypods II. With the exception of most members of Blechnaceae and a few species of Thelypteridaceae (Holttum, 1982), all eupolypod II ferns have two crescent- or strap-shaped vascular bundles at the base of their petioles (Fig. 2B), making this a powerful diagnostic character for the clade. Most members of the sister lineage – eupolypods I – have two large adaxial bundles as well as a semicircular arc of smaller bundles, a state that we code as 'more than two bundles'. The majority of the remaining Polypodiales (Lindsaeaceae, Dennstaedtiaceae, Saccolomataceae, Pteridaceae) have a single bundle. Although two or more bundles occur sporadically in Pteridaceae (Tryon *et al.*, 1990) and Dennstaedtiaceae (*Lonchitis*, *Monachosorum*), a single bundle appears to be the ancestral state for Polypodiales given the topologies presented in Schuettpelz and Pryer (2008) and Schuettpelz *et al.* (2007).

The presence of two vascular bundles was previously reported as a synapomorphy for eupolypods II (Schuettpelz *et al.*, 2006); however, this result depends on the manner in which the character is coded. The presence of two vascular bundles acts as a synapomorphy only if the character is interpreted as having two states (two bundles versus any number of bundles other than two). If more states are recognized, then the optimization is ambiguous as to the origin of the two bundles.

Three states were employed in our analysis, and therefore the character acts as a synapomorphy for eupolypods II under deltran optimization alone. Under acctran, the transition to two bundles occurs in the common ancestor of all eupolypods. ML reconstruction finds the likelihood of being ancestral/derived for each of our three states to be nearly equal (data not shown).

Using character states comparable to ours, Hernández-Hernández *et al.* (2012) reported a transition from a single bundle to two, and then to multiple bundles. However, those results are derived from an incorrectly drawn phylogeny that features a non-monophyletic eupolypods II [they cite Schuettpelz and Pryer (2008) as the source of their phylogeny, but that reference does feature a monophyletic eupolypods II]. Using the 2012 topology a transition series between these states is only recovered under acctran optimization.

An alternative coding would be to assume that the large adaxial bundles in the petiole of eupolypods I and II are homologous and code the states as one bundle vs. two, and consider the presence of the abaxial ring of small bundles as a separate character. The similar xylem pattern of the large bundles supports their homology; however, this homology should be assessed anatomically. Using this coding, the transition from one to two bundles supports the eupolypods and the presence of the abaxial ring of smaller bundles is a synapomorphy for eupolypods I.

Chromosome base number. Investigation of chromosome base numbers has had a tremendous impact on fern systematics (Manton, 1950; Manton and Sledge, 1954; Holttum, 1982; Tryon and Tryon, 1982) and has figured prominently in the circumscription of eupolypod II genera (Manton and Sledge, 1954; Kato, 1977). Results presented here corroborate their utility in that base numbers are consistent within most genera (Fig. 5B). We also find that chromosome base numbers are useful at higher taxonomic levels. Results presented here indicate that $x = 41$ is synapomorphic (proportional likelihood = 0·933) for all eupolypods, a clade that includes over two-thirds of extant fern species (Fig. 5). Despite $x = 41$ being a frequent number among extant species in both lineages, to our knowledge it has not previously been demonstrated as the likely ancestral character state. Additional sampling is not expected to change this result; early diverging members of eupolypods I have $x = 41$, and the sister groups to the eupolypods have different numbers. Among eupolypods II, $x = 41$ is also the ancestral condition, with at least seven transitions to other numbers. Six of these transitions are reductions; the transition to a higher number ($x = 42$) occurs only in the clade of *Cystopteris* + *Acystopteris*. Nakazato *et al.* (2008) demonstrate a strong correlation between chromosome number and genome size in ferns, and therefore we can interpret reductions in chromosome number as chromosome losses rather than fusions and thus that the overall pattern of genome evolution in eupolypod II ferns is marked by a series of independent reductions in genome size.

Sori and indusia. The use of soral and indusial shape to delineate fern genera dates back to Linnaeus (1753) and J. E. Smith (1793), and has since been relied upon heavily as a diagnostic character in eupolypod ferns (Tryon, 1952). Determining homology between types of sori, however, is a challenge because there are few criteria by which to judge them. Here we delineate sori as round, elongate or acrostichoid, and evaluate their position relative to the leaf margin, and to veins, their position upon the veins and whether the receptacle is raised or flat. We also examine the presence, shape and attachment of the indusium. Using this character state scheme, ancestral state reconstructions suggest that the ancestor of the eupolypods II was an indusiate plant with an elongate and laterally attached sorus, and a flat receptacle that was abaxial on the leaf, and positioned dorsally along the veins, with a linear indusium.

Elongate sori occur in approximately two-thirds of eupolypod II species, and can be roughly divided into three forms: asplenioid (sporangia confined to a single side of each vein), diplazioid (sporangia 'back-to-back' on both sides of veins, but not crossing over the vein itself) and athyrioid (sorus hooking over the vein tip in a J or horseshoe shape). Bower (1928) and Holttum (1947) hypothesized that diplazioid sori evolved from either a J- or horseshoe-shaped sorus that was interrupted distally, and asplenioid sori evolved by abortion of the small arm in J-shaped sori. Our results do not support these hypotheses. We find that ancestors of *Diplazium*, *Athyrium* and *Asplenium* had elongate and one-sided sori – diplazioid and J-shaped sori then evolved from that ancestral condition, rather than via the reductions hypothesized by Bower and Holttum.

Conversely, in an ironic vindication of the historical emphasis on reproductive characters, our results suggest that sorus position requires a simpler explanation under the 2012 topology than with the most recent classifications [i.e. Woodsiaceae *sensu* Smith *et al.* (2006) or Physematieae *sensu* Kato and Kramer (1990)]. This is because the Rhachidosoraceae, Diplaziopsidaceae and Hemidictyaceae form a clade with the Aspleniaceae rather than with the Athyriaceae. Thus, in our results, sori restricted to one side of the vein defines the clade comprising Rhachidosoraceae through Aspleniaceae, and sori present on both sides of the vein is a synapomorphy (with the exceptions detailed) for the Athyriaceae (*sensu* Rothfels *et al.*, 2012b).

Sporangia

Sporangial characters map to some of the deepest nodes in the fern phylogeny. Notable among these are the transition from the eusporangium to the leptosporangium [defining the Polypodiidae *sensu* Pryer *et al.* (2008) or the Polypodiopsida *sensu* Smith *et al.* (2006)], and the various orientations of the annulus, which diagnose many of the leptosporangiate orders (Bower, 1928; Pryer *et al.*, 1995, 2008). Within eupolypods II, our results corroborate a previous finding: a sporangial stalk that is uniseriate in the middle is a synapomorphy for the Aspleniaceae; other eupolypod II taxa have stalks two or three cells wide in the middle (Bower, 1928; Wagner, 1952; Wilson, 1959; Smith, 1976). We also examined mean cell number in the annulus (Fig. 5A). This character tracked some of the major patterns of divergence among eupolypod II taxa. In our MP results, backbone nodes beginning with clade A are resolved with a mean number of 15, and the Aspleniaceae is supported by a mean number of 19. These conclusions, however, do not have support under ML. Another pattern that we detected was the increase in annular cell number in the clade comprising Onocleaceae and Blechnaceae. In these ferns, the mean number of cells in the annulus ranges from 14 to 35. The Onocleaceae contain the highest numbers, ranging from 24 to 35 cells, whereas the other eupolypod II taxa have 11–19 cells. Whether these differences relate to spore size or some other feature warrants further investigation. Smith (1976) found the shape of the annulus post dehiscence to be useful for distinguishing *Diplazium* from *Hymenasplenium*. We agree that the character appears to be useful, but we were unable to define discrete character states in this study.
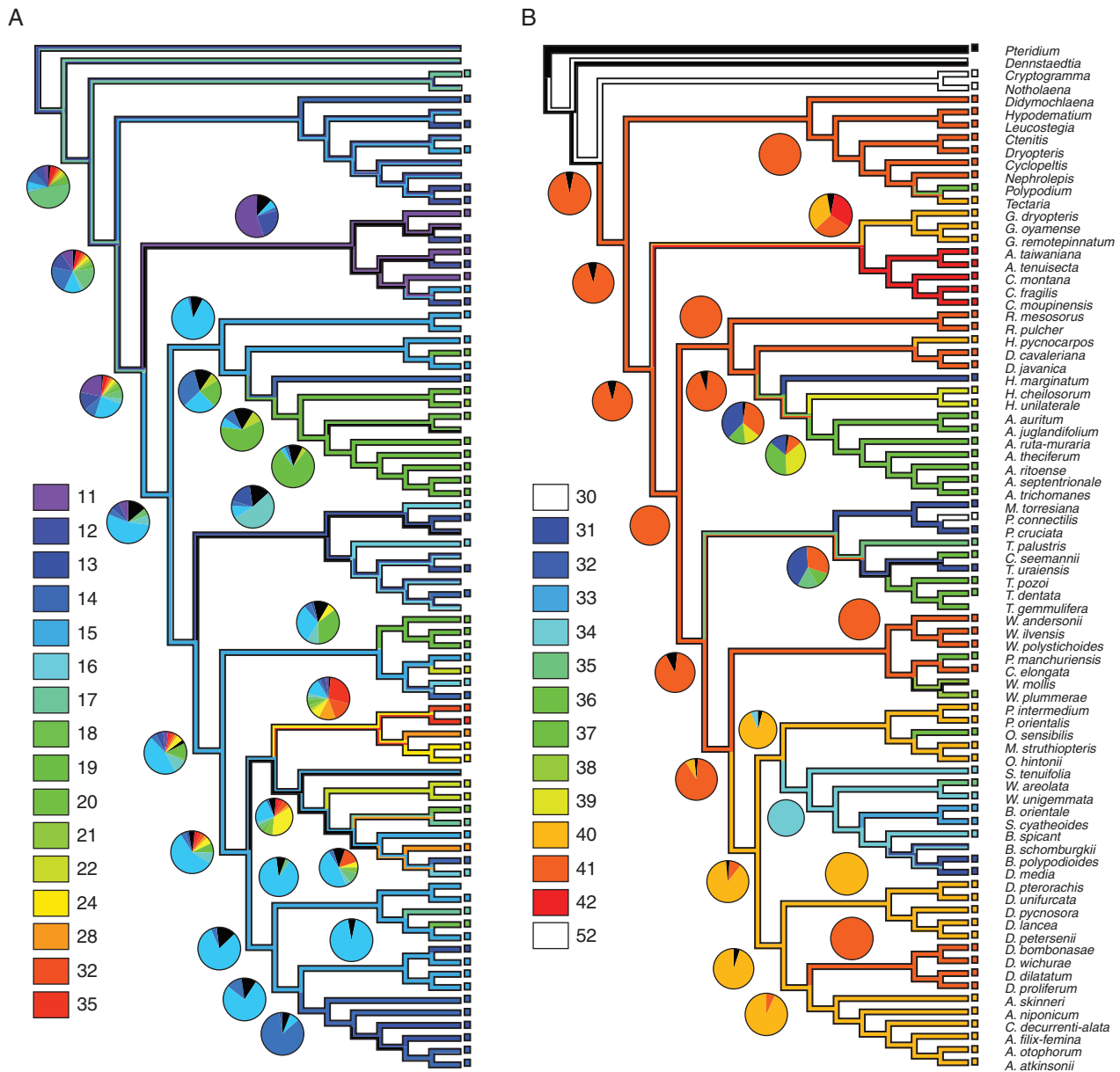
Fɪɢ. 5.　Ancestral state analysis for eupolypod II ferns. Branch colours depict maximum-parsimony optimization. Pie charts depict proportional likelihoods. (A) Character 74, mean number of annulus cells in the sporangia. (B) Character 75, chromosome base numbers.

Rachis–costa architecture. In many ferns, the adaxial side of the rachis and pinna rachis exhibit a series of sulci or ridges that have been referred to as the rachis–costa architecture (Murakami and Moran, 1993). This suite of characters can be observed at the juncture where a pinna departs from the rachis (Fig. 4A–I). Its use as a diagnostic character was pioneered by Holttum (1954, 1958, 1959, 1960), and it has also featured prominently in the circumscription of eupolypod II genera. For example, Kato (1977, 1984) segregated *Deparia* from *Athyrium* and *Diplazium* based on whether the sulcus of the rachis is continuous with that of the second-order axis, Murakami and Moran (1993) used the presence of a ridge that bridges the pinna–rachis juncture to distinguish *Hymenasplenium* (as *Asplenium* sect. *Hymenasplenium*) from *Asplenium*, and Holttum (1960)

used the presence of sulcate axes to distinguish *Metathelypteris* and the phegopteroid genera from the other thelypteroid ferns which lack them.

We interpret rachis–costa architecture as three characters: whether axes are sulcate adaxially (Fig. 4B–G) or not (Fig. 4A, H–I), whether the channels of the sulci are continuous with that of the next order (Fig. 4D), and whether the walls of the rachis sulci are confluent with those of the pinna costae (Fig. 4C–D, F–G), which Murakami and Moran (1993) referred to as a ridge. We also considered whether the leaf margin is decurrent and thickened, forming wings or lateral ridges on either side of the rachis (Fig. 4H, I). Kato (1975, 1977) paid close attention to the shape of the adaxial channel itself, distinguishing between flat-bottomed, and u- and v-shaped grooves; however,

we found the distinction between those character states difficult to interpret and did not include this character in our analysis.

In our results, an adaxially sulcate rachis is the plesiomorphic condition and occurs in nearly all eupolypod II species, with losses in some Thelypteridaceae, *Woodwardia unigemmata* and *Asplenium*. Sulci that are continuous with that of the next order occur among most eupolypod II taxa that were traditionally treated as athyrioids: *Athyrium*, *Cornopteris*, *Diplazium*, *Diplaziopsis*, *Homalosorus* and *Rhachidosorus*. As championed by Kato (1977), *Deparia* always has sulci that are discontinuous between orders (Fig. 4E); however, discontinuous or weakly continuous sulci also occur in some *Athyrium* (e.g. *A. nipponicum*; Fig. 4F) and *Diplazium*, somewhat diminishing the diagnostic power of the character. A more powerful character for distinguishing *Deparia* from other Athyriaceae is whether the sulci walls of the rachis are confluent with the pinna costae. This condition is never present in the species of *Deparia* studied here, whereas it is present in all other Athyriaceae species examined. The character state is homoplastic, however, because it also occurs throughout the Cystopteridaceae, and at the base of the clade including *Rhachidosorus*–Aspleniaceae, with a loss in *Hemidictyum*. *Hymenasplenium* also has rachis sulci walls that are confluent with the pinna costa walls (Fig. 4G), but this has not been observed in the remaining Aspleniaceae. In *Asplenium*, the rachis is not sulcate: it is bordered on each side by alae that are confluent with the basiscopic edge of the lamina (Fig. 4H, I), rather than the pinna costa. The resulting rachis–costa architecture is similar looking, but not homologous. Our observations on the difference between *Asplenium* and *Hymenasplenium* are consistent with the rachis cross-sections presented by Regalado and Prada (2011). It should be stressed, however, that rachis–costa characters should be further investigated with a larger sampling of *Asplenium*. Nonetheless, in our results the rachis–costa architecture exhibited by *Asplenium* does not appear elsewhere among eupolypod II ferns. Similar rachis–costa architecture does, however, appear in some eupolypod I ferns, in particular *Lastreopsis*, *Pteridrys*, *Rumohra* and some Davalliaceae; it was referred to as the *Davallia*-type by Holttum (1960).

Chlorophyllous spores. Fern spores differ in whether their chloroplasts have differentiated prior to germination (Campbell, 1905; Marengo, 1949; Gantt and Arnott, 1965; Sheffield and Bell, 1987), and this condition is known to be associated with certain intrinsic biological traits. Chlorophyllous spores have evolved multiple times in unrelated groups of ferns (Lloyd and Klekowski, 1970; Sundue *et al.*, 2011). Among the eupolypod II species sampled here, chlorophyllous spores evolved once, acting as an unambiguous character supporting the Onocleaceae. Two other records of green spores for eupolypod II species not included in our study include *Blechnum nudum* (Stone, 1961) and *Blechnum discolor* (M. Sundue, unpubl. data). These species, and Onocleaceae, are most abundant along stream banks, floodplains and other periodically flooded habitats, suggesting that adaptation to similar habitat conditions may have driven the evolution of this character multiple times in eupolypods II.

Trophopods. Wagner and Johnson (1983) used the term trophopod to describe a swollen and enlarged, starch-filled petiole base that persists on rhizomes of some fern species (Fig. 2D, right arrow). As noted by Johnson (1986), trophopods are left on the rhizome by two different processes. In the first case, the trophopod is left following senescence of the distal portion of a fully developed leaf. These trophopods can be diagnosed when frayed remains of the lower petiole persist upon the apex of the trophopod. In the second case, the leaf apex aborts shortly after development of the petiole base. Consequently, the distal petiole and lamina never develop. This second type can be identified by the necrotic crosier that is often persistent on the apex of the trophopod (Fig. 2D, left arrow). Goebel (1900, p. 350) applied the term cataphyll to these leaves in *Matteuccia struthiopteris*, a term that Johnson (1986) later adopted to distinguish this type of trophopod from the first. Trophopods of both types were independently described from *Pteris wallichiana* J. Agardh. by Chandra and Nayar (1970), and can also be found scattered throughout the leptosporangiate ferns (e.g. Osmundales, *Plagiogyria* and Dryopteridaceae; Wagner and Johnson, 1983; Johnson, 1986; Sundue, 2011). Eupolypods II, however, are particularly rich with trophopods and home to some of the best-developed examples. We studied trophopods by scoring the presence of enlarged leaf bases that were persistent upon the rhizome. We did not score the two types of trophopods separately, because we were often unable to distinguish them in herbarium specimens. Trophopods are gained seven times in eupolypods II. Four of these gains are autapomorphic for single species (given our sample), and two of the gains define clades (Onocleaceae and *Deparia*). Enlarged petiole bases also appear within *Athyrium*, with a single gain followed by a single loss. Lellinger (2002) stated that trophopods were a feature of temperate species. Among eupolypod II ferns, we concur that trophopods are perhaps best developed among predominantly temperate groups (Onocleaceae, Athyriaceae, *Cystopteris*). However, trophopods are also present among sub-tropical and tropical species (*Acystopteris tenuisecta*, *Hypodematium crenatum*, *Macrothelypteris torresiana*, *Rhachidosorus mesosorus*) and previous studies indicate that they occur in tropical and sub-tropical species outside of the eupolypods II as well (e.g. *Pteris wallichiana*). Whether trophopods are in fact more prevalent among temperate plants should be examined more thoroughly.

We also scored the presence of pneumatophores (Fig. 2E), wing-like protuberances found on some petiole bases (Iwatsuki, 1970; Kato, 1984). These are an elaboration of the aerophore that is present in all Polypodiales (Davies, 1991). We did not anticipate pneumatophores as being associated with trophopods, but to our surprise we found that, in eupolypods II, pneumatophores only appear in species that also have enlarged leaf bases.

Leaf coloration. Reddish young leaves occur in scattered groups of ferns, most notably the adiantoids (*Adiantum* + the vittarioids; Schuettpelz *et al.*, 2007; Sundue, 2011), and the Blechnaceae (Tryon and Tryon, 1982). Crowden and Jarman (1974) extracted anthocyanins from the leaves of *Blechnum procerum* Sw., and these compounds are thought to be responsible for the colour of young leaves. As leaves mature, the red colour is masked by chlorophyll. Although homoplastic across ferns (and vascular plants more broadly), here reddish young leaves are an unambiguous character supporting the Blechnaceae (Fig. 2F), an anticipated result based on previous reports (Tryon and Tryon, 1982). To our surprise, however, we also observed reddish leaves in all species of *Athyrium s.l.* that were sampled, visible in the petiole, rachis and

sometimes the lamina. However, unlike members of the Blechnaceae, *Athryium s.l.* retain a reddish colour in maturity (Figs 3I, 4F). These colour characteristics have been noted occasionally in taxonomic (Mickel and Smith, 2004, p. 138; Liu *et al.*, 2009) and horticultural literature (Mickel, 2003, p. 106), or presented in photographs (Knapp, 2011), but have not previously been used to define this group. The character is an unambiguous synapomorphy in our analysis. However, more detailed investigation into its nature and distribution is warranted. *Athyrium* comprises approx. 200 species (Liu *et al.*, 2009); sampling additional taxa may yield a different result.

Indument. Scales are a common type of leaf indument in ferns and scaly leaves are the ancestral condition in eupolypods II. Their loss is homoplastic in our analysis, with losses having occurred at least seven times. One large clade supported by the absence of scales from the mature leaves is that which includes Diplaziopsidaceae, Hemidictyaceae and Aspleniaceae. This unanticipated result is interesting because it offers character support to this previously unrecognized clade; however, denser sampling in *Asplenium* is certainly warranted – many *Asplenium* species not sampled in the 2012 topology have scaly leaves (Tryon and Tryon, 1982). Like leaf scales, leaf hairs are homoplastic in eupolypods II. Unlike scales, however, leaf hairs are reconstructed as absent from the ancestor of the eupolypods II, and are gained at least six times in the clade. They are present without subsequent loss in both Thelypteridaceae and Woodsiaceae. Thelypteridaceae are further supported by having acicular hairs (Fig. 2I), which do not occur elsewhere in the eupolypods II. Another clade supported by an indument character is *Deparia*, which bears catenate hairs. These hairs comprise one end of a morphological reduction series that begins with broad scales. This was one of the principal characters used by Kato (1977) and later Sano *et al.* (2000*b*) to circumscribe *Deparia*. Unanticipated by us, this character is homoplastic in eupolypods II; catenate hairs derived from reduced scales can also be found in *Acystopteris* and *Woodsia mollis*. Similar hairs occur in species of *Diplazium*, namely *D. immensum*, and the species treated as *Callipteris* by Pacheco and Moran (1999); these species should be prioritized in future phylogenetic studies. Most other characters describing types of hairs and scales did not support any clade; namely: leaf hairs gland-tipped, hairs hamate, hairs forked, stellate or stalked-stellate, and hairs catenate and not reduced from a scale.

We also scored the presence of hairs similar to root hairs, but which are present on the lower portions of the petioles. These were present in *Cystopteris moupinensis*, *Homalosorus pycnocarpos* and *Athyrium skinneri*. Among outgroup taxa, they also occurred in *Pteridium aquilinum*. These hairs are easily overlooked and often lost in specimens and have received little previous attention in the literature. Their function is unknown. Rhizomes of *P. aquilinum* are clearly subterranean, and the root-hair-like indument appears on the portion of the petiole just below ground level. Whether this is the case in the eupolypod II ferns studied here requires further investigation.

Growth habit. Eupolypods II are predominantly terrestrial ferns. A change from this state to either an epipetric or epiphytic growth habit occurs as few as three times (acctran reconstruction), or as many as five times (deltran reconstruction) on the 2012 topology (Supplementary Data Fig. S1). An epipetric growth habit appears most frequently in the Aspleniaceae and Woodsiaceae.

Aspleniaceae comprise approx. 700 spp., an estimated 50 % of which are epiphytes (Schneider *et al.*, 2004*a*); only three of these were included in our study. Schneider *et al.* (2004*a*), with a taxon sample of 71 Aspleniaceae species, found multiple character state changes between epiphytism and non-epiphytism in the family. Interestingly, the epiphytic habit is otherwise rare in eupolypods II, appearing only in a few species of *Blechnum* not included in the 2012 topology. Blechnaceae also exhibit other growth habits that are rare among eupolypods II, including plants that climb by rhizomes (*Stenochlaena*, some *Blechnum*), plants that climb by twining indeterminate leaves (*Salpichlaena*), and plants with arborescent and sometimes massive rhizomes. The arborescent habit appears twice independently in our Blechnaceae sample, in *Sadleria cyatheoides* and *Blechnum schomburgkii*. *Sadleria* is a Hawaiian endemic genus of six species, four of which produce arborescent rhizomes up to 3·5 m tall (Palmer, 2002). *Blechnum schomburgkii* is a member of *Blechnum* sect. *Lomariocycas*, a group of about seven species (Morton, 1959) that are known to attain heights of about 3 m. The 2012 topology, however, does not include other arborescent and sub-arborescent taxa in the family; they should be included in subsequent studies.

CONCLUSIONS

Our investigation of morphological evolution of the eupolypods II produced one of the largest morphological matrices for the ferns to be analysed with modern phylogenetic methods. Our integrated approach synthesizes morphological studies with current phylogenetic hypotheses and provides explicit statements of character evolution in the eupolypod II fern families. We find strong character support for previously recognized clades, whereas few characters support previously unrecognized clades. Sorus position appears to be less complicated than previously hypothesized, and linear sori restricted to one side of the vein support the clade comprising Aspleniaceae, Diplaziopsidaceae, Hemidictyaceae and Rhachidosoraceae – a lineage only recently identified. Despite $x = 41$ being a frequent number among extant species, to our knowledge it has not previously been demonstrated as the ancestral state. This is the first synapomorphy proposed for the eupolypod clade, a lineage comprising 67 % of extant fern species. This study provides some of the first hypotheses of character evolution at the family level and above in light of recent phylogenetic results, and promotes further study in an area that remains open for original observation.

SUPPLEMENTARY DATA

Supplementary data are available online at www.aob.oxfordjournals.org and consist of the following. Table S1: character statistics when optimized onto the 2012 topology, from an analysis of the morphological data alone, and results from the randomization test. Table S2: results from FitDiscrete. Appendix S1: representative vouchers used for character state determinations. Figure S1: maximum parsimony optimization of 77 characters (1–73, 76–79).

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APPENDIX 1. CHARACTERS AND CHARACTER STATES

Habit – 1. Plant habit: terrestrial (0); epipetric (1); epiphytic (2).

Roots – 2. Root insertion: radial (0); ventral (1). **3.** Root texture: wiry and dark (0); fleshy and pale (1). **4.** Proliferous roots: absent (0); present (1).

Rhizomes – 5. Rhizome length: short-creeping (0); long-creeping (1). **6.** Rhizome habit: prostrate (0); erect (1); sub-erect (decumbent) (2). **7.** Rhizome commonly branched: no (0); yes (1). **8.** Erect rhizomes massive and arborescent: no (0); yes (1). **9.** Erect rhizome with stilt roots: no (0); yes (1). **10.** Rhizome forming stolons: no (0); yes (1). **11.** Rhizome scales: absent (0); present (1). **12.** Rhizome pubescent: no (0); yes (1). **13.** Rhizome scales: non-clathrate (0); clathrate (1); weakly clathrate (2). **14.** Rhizome scale margin: eglandular (0); glandular (1). **15.** Rhizome scales bearing distinct hairs different from marginal cilia: no (0); yes (1). **16.** Rhizome scales with teeth formed by two adjacent cells: no (0); yes (1).

Leaves – 17. Leaf arrangement: distichous (0); more than 2 ranks (1). **18.** Leaf positioning: radial (0); dorsal only (1). **19.** Fertile leaves dimorphic: holomorphic (0); dimorphic (1); partially dimorphic (2). **20.** Fertile lamina reflexed and enclosing the sori: no (0); yes (1). **21.** Young leaves covered in mucilage: no (0); yes (1). **22.** Leaves cleanly abscising from the rhizome: no (0); yes (1).

Petioles – 23. Petiole bases enlarged (forming trophopods): no (0); yes (1). **24.** Old petiole bases persistent: no (0); yes (1). **25.** Pneumatophores upon petiole base (*sensu* Iwatsuki, 1970; Kato, 1984): absent (0); present (1). **26.** Petioles with a proximal articulation above the base: no (0); yes (1). **27.** Petiole base bearing rhizoids: no (0); yes (1). **28.** Number of vascular bundles in petiole: one (0); two (1); more than two (2). **29.** Shape of vascular bundles when united distally in leaf: U or V shaped (0); X shaped (1). **30.** Petiole colour: stramineous, grey, brown, or greenish, pink or reddish (0); castaneous or blackish (1). (Many ferns have petioles that are blackish at their base near where they are below the level of the soil, but otherwise stramineous or greenish. These plants are scored here as state 0. State 1 is reserved for petioles that are dark-coloured throughout.) **31.** Xylem shape in main vascular bundles of the petiole: C-shaped (0); hippocampiform (1).

Laminae and axes – 32. Blade dissection: simple (0); 1-pinnate (1); 1-pinnate-pinnatifid (2); 2-pinnate (3); 2-pinnate-pinnatifid (4); 3-pinnate (5); 3-pinnate-pinnatifid (6); 4-pinnate (7); deeply 1-pinnatifid (8). **33.** Dromy at leaf base:

catadromous (0); anadromous (1); isodromous (2). **34.** Lamina base with series of reduced pinnae: no (0); yes (1). **35.** Leaf apex: conform or nearly so (0); pinnatifid or non-conform (1). **36.** Pinnae base: not articulate or swollen (0); swollen (1); articulate. **37.** Veins reaching leaf margin: no (0); yes (1). **38.** Vein fusion: non-anastomosing (0); anastomosing (1); with costular areoles only (2). **39.** Vein endings: undifferentiated (0); expanded or with hydathodes (1). **40.** Leaf margin decurrent and forming lateral ridges along rachis: no (0); yes (1). **41.** Axes sulcate adaxially: no (0); yes (1). **42.** Sulcae of axes continuous with next order: not continuous (0); continuous (1). **43.** Pinna costa sulcus wall confluent with rachis sulcus wall: no (0); yes (1). **44.** Central ridge present or absent: absent (0); present (1). **45.** Pinna costae with an adaxial projection: absent (0); present (1). **46.** Scales or corniculae in sulcae at base of pinnae adaxially: absent (0); present (1). **47.** Leaf margin: not differentiated (0); transparent (1); translucent (2); thickened and opaque (3); membranaceous (4). **48.** Translucent margin type: smooth (0); nodulose (1); denticulate (2). **49.** Submarginal collecting vein: absent (0); present (1). **50.** Leaf reddish when young: no (0); yes (1). **51.** Leaf reddish when mature: no (0); yes (1). **52.** Nectiferous glands: absent (0); present (1).

Laminar indument – 53. Leaf hairs: absent (0); present (1). **54.** Leaf hairs gland-tipped: absent (0); present (1). **55.** Hairs acicular: no (0); yes (1). **56.** Hairs hamate: no (0); yes (1). **57.** Hairs forked-stellate or stalked-stellate: no (0); yes (1). **58.** Leaf hairs catenate (not including hairs reduced from a scales as part of a reduction series): no (0); yes (1). **59.** Catenate hairs reduced from scales as part of a reduction series: no (0); yes (1). **60.** Leaf scales of mature leaves: absent (0); present (1). **61.** Leaf indument glandular other than capitate hairs: absent (0); present (1).

Sori – 62. Sorus shape: round (0); elongate (1); acrostichoid (2). **63.** Sorus position: sub-marginal (0); abaxial (1). **64.** Sorus relation to vein: at vein ending (0); along vein (1); sub-terminal at vein ending (2); spreading from veins (3); on a costal commissure (4); on a sub-marginal commissure (5). **65.** Sorus position or sporangial position relative to vein or commisure: one side (0); two sides (1); on top (2). **66.** Receptacle: flat (0); raised (1). **67.** Indusia: absent (0); present (1). **68.** Indusium attachment relative to sporangia: lateral (0); basal (1); central (2). **69.** Indusium shape: linear (0); reniform (1); round (2); deltate (3); strap shaped (4); theciform (5); j-shaped (6); triangular and cup-like (7). **70.** Indusium glandular: no (0); yes (1). **71.** Indusium pubescent: no (0); yes (1). **72.** Paraphyses (sterile structures, e.g. hairs or scales, present among sporangia and emerging from the receptacle): absent (0); present (1). **73.** Sporangial stalk at middle: uniseriate (0); two or three cells wide (1). **74.** Mean number of cells in annulus: 11 (0); 12 (1); 13 (2); 14 (3); 15 (4); 16 (5); 17 (6); 18 (7); 19 (8); 20 (9); 21 (10); 22 (11); 24 (12); 28 (13); 32 (14); 35 (15).

Chromosomes base number – 75. Chromosome base number: 30 (0); 31 (1); 32 (2); 33 (3); 34 (4); 35 (5); 36 (6); 37 (7); 38 (8); 39 (9); 40 (10); 41 (11); 42 (12); 52 (13).

Spores and gameotphytes – 76. Spore colour: tan (0); brown (1); black (2); yellow (3). **77.** Spores chlorophyllous: no (0); yes (1). **78.** Perispore: sharp ridges (0); broad folds (1); spines (2); tuberculate (3); foliose (4); granulate (5); shallowly reticulate (6); essentially smooth (7); rugose (8); papillate (9).

79. Gametophyte thallus margin indument: glabrous (0); unicellular marginal papillae (1).

APPENDIX 2. CHARACTER STATES CHARACTERIZING MAJOR CLADES

Character state changes that map to the stem branches of the major clades under the three optimization methods (unambiguous, acctran, deltran).

Eupolypods II: Unambiguous: none. Acctran: 43(1) rachis sulcus wall confluent with pinna costae. Deltran: 28(0) petioles with two vascular bundles at the base (Fig. 3B).

Cystopteridaceae: Unambiguous: 5(1) rhizome long-creeping, 37(1) veins reaching leaf margin. Acctran: 5(1), 7(1) rhizome commonly branched, 39(0) vein endings undifferentiated, 60(0) leaf scales absent from mature leaves, 69(3) indusium round (Fig. 2A), 75(10) base number 40. Deltran: 5(1), 37(1), 43(1) rachis sulcus wall confluent with pinna costa.

Gymnocarpium: Unambiguous: 36(1) pinnae swollen at the base, 67(0) indusium absent. Acctran: 36(1), 54(1) gland-tipped leaf hairs present, 67(0). Deltran: 7(1) rhizome commonly branched, 36(1), 60(0) leaf scales absent from mature leaves, 67(0), 75(10) $x = 40$.

Cystopteris + *Acystopteris*: Unambiguous: 47(1) leaf margin translucent, 62(0) sori round, 66(1) receptacle raised, 75(12) $x = 42$. Acctran: 32(6) laminae 3-pinnate, 47(1), 62(0), 66(1), 74(0) mean number of annulus cells 11. Deltran: 47(1), 62(0), 66(1), 69(3) indusium deltate, 74(0), 75(12), 79(1) margin of gametophyte thallus papillate.

Acystopteris: Unambiguous: 6(2) rhizome sub-erect, 14(1) rhizome scale margin glandular, 33(2) venation isodromous, 53(1) leaf hairs present, 59(1) catenate hairs in a reduction series beginning in broad scales present, 61(1) leaf glands present, 76(0) spores tan. Acctran: 6(2), 7(0) rhizome usually unbranched, 14(1), 33(2), 53(1), 59(1), 61(1), 76(0). Deltran: 6(2), 14(1), 32(6) laminae 3-pinnate-pinnatifid, 33(2), 53(1), 59(1), 60(0) leaf scales absent from mature leaves, 61(1), 76(0).

Cystopteris: Unambiguous: 13(2) rhizome scales weakly clathrate, 68(1) indusium basally attached. Acctran: 13(2), 24(0) petiole bases not persistent, 60(1) leaf scales present, 68(1). Deltran: 13(2), 68(1).

Clade A: Unambiguous: 74(4) mean number of annulus cells 15. Acctran: 39(1) vein ending expanded or with hydathodes; 78(0) perispore with sharp ridges. Deltran: none.

Clade E: Unambiguous: 65(0) sori on one side of the vein. Acctran: 13(1) scales clathrate, 47(1) leaf margin translucent, 65(0), 79(0) margin of gametophyte thallus glabrous. Deltran: 43(1) pinna costa sulcus wall continuous with rachis sulcus wall, 65(0).

Rhachidosoraceae: Unambiguous: 42(1) sulcae of axes continuous with those of the next order. Acctran: 23(1) petiole bases swollen, 39(0) vein endings undifferentiated, 42(1), 78(3) perispore tuberculate. Deltran: 13(1) scales clathrate, 42(1), 47(1) leaf margin translucent.

Diplaziopsidaceae: Unambiguous: 3(1) roots soft and fleshy. Acctran: 3(1), 13(0) rhizome scales non-clathrate, 34(1) lamina base with a series of reduced pinnae, 79(1) margin of gametophyte thallus papillate. Deltran: 3(1), 39(1) vein endings expanded, 47(1) leaf margin translucent.

Homalosorus Unambiguous: 27(1) petiole bases provided with indument similar to root hairs, 75(10) $x = 40$. Acctran: 27(1), 75(10). Deltran: 27(1), 34(1) lamina base with series of reduced pinnae, 75(10).

Diplaziopsis Unambiguous: 6(1) rhizome erect, 14(1) rhizome scale margin glandular, 24(0) petiole bases persistent upon the rhizome, 33(2) dromy at leaf base isodromous, 35(0) terminal pinna conform to lateral pinnae, 38(1) veins anastomosing, 70(1) indusium glandular. Acctran: 6(1), 14(1), 24(0), 33(2), 35(0), 38(1), 70(1). Deltran: 6(1), 14(1), 24(0), 33(2), 35(0), 38(1), 70(1), 79(1) margin of gametophyte thallus papillate.

Hemidictyum: Unambiguous: 4(1) roots proliferous, 33(2) venation at leaf base isodromous, 35(0) terminal pinna conform to lateral pinnae, 38(1) veins anastomosing, 43(0) pinna costa sulcus wall not confluent with rachis sulcus wall, 49(1) submarginal collecting vein present, 74(3) mean number of cells in annulus 14, 78(1) chromosome base number $x = 31$. Acctran: 4(1), 13(2) rhizome scales weakly clathrate, 33(2), 35(0), 38(1), 43(0), 47(3) leaf margin membranaceous, 49(1), 74(3), 78(1). Deltran: 4(1), 6(2) rhizome sub-erect, 13(2) rhizome scales weakly clathrate, 33(2), 35(0), 38(1), 39(1), vein endings expanded, 43(0), 47(3), 49(1), 74(3), 75(1), 78(1).

Aspleniaceae: Unambiguous: 29(1) petiole vascular bundles uniting distally to form an x-shape, 73(0) sporangial stalk uniseriate in the middle, 74(8) mean number of annular cells 19. Acctran: 1(1) plants epiphytic, 24(0) old petiole bases not persistent, 29(1), 73(0). Deltran: 13(1) rhizome scales clathrate, 29(1), 31(0), 73(0), 75(12).

Hymenasplenium: Unambiguous: 2(1) root insertion ventral, 5(1) rhizome long-creeping, 17(0) leaves distichous, 18(1) leaves dorsal only, 22(1) leaves cleanly abscising from rhizome, 30(1) petiole castaneous, 37(1) veins reaching the leaf margin, 75(9) $x = 39$. Acctran: 2(1), 5(1), 6(0) rhizome decumbent, 17(0), 18(1), 22(1), 30(1), 37(1). Deltran: 2(1), 5(1), 17(0), 18(1), 22(1), 24(0) petiole bases not persistent, 30(1), 37(1).

Asplenium: Unambiguous: 40(1) leaf margin decurrent and forming lateral ridges along rachis, 41(0) axes terete adaxially, not sulcate, 75(6) $x = 36$. Acctran: 40(1), 41(0). Deltran: 40(1), 41(0).

Clade B: Unambiguous: 32(2) laminae 1-pinnate-pinnatifid. Acctran: 32(2), 43(0) pinna costa sulcus wall not confluent with rachis sulcus wall, 53(1) leaf hairs present, 62(0) sori round. Deltran: 32(2), 39(1) vein endings either expanded or with hydathodes, 78(0) perispore with sharp ridges, 79(1) margin of gametophyte thallus papillate.

Thelypteridaceae: Unambiguous: 33(0) venation catadromous, 55(1) hairs acicular (Fig. 3I), 69(1) indusium reniform. Acctran: 15(1) rhizome scales bearing distinct hairs, 33(0), 55(1), 69(1), 71(1) indusium pubescent. Deltran: 33(0), 53(1) leaf hairs present, 55(1), 62(1) sorus round, 69(1).

Woodsiaceae: Unambiguous: 1(1) plants epipetric, 6(2) rhizomes sub-erect, 66(1) receptacle raised, 68(1) indusium basal, 69(4) indusium splitting into strap-shaped segments. Acctran: 1(1), 6(2), 58(1) leaf hairs catenate, but not reduced from scales, 64(2) sori sub-terminal on veins, 66(1), 68(1), 69(4). Deltran: 1(1), 6(2), 53(1) leaf hairs present, 62(0) sori round, 66(1), 68(1), 69(4).

Clade F: Unambiguous: 19(1) fertile leaves dimorphic. Acctran: 7(1) rhizomes commonly branched, 19(1), 37(1)

veins reaching leaf margin, 64(0) sorus terminal on vein, 78(1) perispore with sharp ridges. Deltran: 19(1), 78(1).

Onocleaceae Unambiguous: 20(1) fertile lamina reflexed and enclosing sori, 23(1) petiole bases enlarged and forming trophopods (Fig. 3D), 66(1) receptacle raised, 69(7) indusium deltate and bullate, 77(1) spores chlorophyllous. Acctran: 6(1) rhizome erect, 20(1), 23(1), 62(0) sori round, 64(0) sori terminal on vein, 66(1), 69(7), 77(1). Deltran: 20(1), 23(1), 62(0), 64(0), 66(1), 69(7), 77(1).

Blechnaceae: Unambiguous: 28(1) petioles with more than two vascular bundles, 38(2) veins with costular anastomoses only, 50(1) leaves reddish when young (Fig. 3F), 75(4) $x = 34$. Acctran: 28(1), 32(1) leaves 1-pinnate, 35(0) leaf apex conform or nearly so, 38(2), 50(1), 64(1) sori along vein, 75(4), 76(0) spores tan. Deltran: 28(1), 38(2), 50(1), 75(4).

Athyriaceae: Unambiguous: 65(1) sori back-to-back, 74(8) mean number of annular cells 14. Acctran: 65(1), 79(0) perispore with sharp ridges. Deltran: 65(1).

Deparia: Unambiguous: 23(1) petiole bases enlarged forming trophopods, 59(1) catenate hairs reduced from scales. Acctran: 23(1), 25(1) pneumatophores present on petiole base (Fig. 3E), 59(1), 78(3) spores with tuberculate perispore. Deltran: 23(1), 59(1).

Diplazium Unambiguous: 75(11) $x = 41$. Acctran: 6(2) rhizome sub-erect, 32(1) lamina 1-pinnate, 47(0) leaf margin not differentiated, 60(0) leaf scales absent from mature leaves, 75(11). Deltran: 9(1) erect rhizome supported by stilt roots, 75(11). The absence of scales from mature leaves should be re-examined with denser taxon sampling. Many species of *Diplazium* do have scales present on the mature leaves and our result is likely biased by our sampling.

Athyrium incl. *Cornopteris* and *Pseudocystopteris*: Unambiguous: 48(0) leaf margin smooth and translucent, 50(1) leaves reddish when young, 51(1) mature leaf reddish, 69(6) indusium j-shaped (Fig. 2I). Acctran: 48(0), 50(1), 51(1), 69(6). Deltran: 48(0), 50(1), 51(1), 69(6).