Shape analysis of moss (Bryophyta) sporophytes: Insights into land plant evolution[1]

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PREMISE OF THE STUDY: The alternation of generations life cycle represents a key feature of land-plant evolution and has resulted in a diverse array of sporophyte forms and modifications in all groups of land plants. We test the hypothesis that evolution of sporangium (capsule) shape of the mosses—the second most diverse land-plant lineage—has been driven by differing physiological demands of life in diverse habitats. This study provides an important conceptual framework for analyzing the evolution of a single, homologous character in a continuous framework across a deep expanse of time, across all branches of the tree of life.

METHODS: We reconstruct ancestral sporangium shape and ancestral habitat on the largest phylogeny of mosses to date, and use phylogenetic generalized least squares regression to test the association between habitat and sporangium shape. In addition, we examine the association between shifts in sporangium shape and species diversification.

RESULTS: We demonstrate that sporangium shape is convergent, under natural selection, and associated with habitat type, and that many shifts in speciation rate are associated with shifts in sporangium shape.

CONCLUSIONS: Our results suggest that natural selection in different microhabitats results in the diversity of sporangium shape found in mosses, and that many increasing shifts in speciation rate result in changes in sporangium shape across their 480 million year history. Our framework provides a way to examine if diversification shifts in other land plants are also associated with massive changes in sporophyte form, among other morphological traits.

KEY WORDS BAMM; Bryophyta; diversification; morphometrics; morphospace; Ornstein-Uhlenbeck shifts; phylogeny; sporangium; sporophyte

The origin of land plants (embryophytes) in the Ordovician or earlier was the pivotal evolutionary event in Earth's history, setting in motion a complex series of geochemical processes in the geosphere, affecting carbon cycling in both the geosphere and atmosphere, initiating the start of land-based ecosystems, and significantly impacting the evolutionary trajectories of fungi and metazoa (Berner, 1997; Bateman et al., 1998; Gensel and Edwards, 2001; Labandeira, 2005; Graham et al., 2014). A key feature in the success of land plants is their life cycle involving an alternation of generations in which two distinct plant bodies are produced—a diploid sporophyte and a haploid gametophyte (Graham and Wilcox, 2000). Overcoming significant issues of life on land involving water loss, transport of water and carbon, protection from ultraviolet light, and dispersal of spores has dramatically shaped the evolution of the

gametophyte and sporophyte (Bateman et al., 1998; Niklas and Kutschera, 2010; Graham et al., 2014; Proctor, 2014; Raven and Edwards, 2014; Haig, 2015). The evolution of a multicellular diploid phase in land plants facilitated long-distance dispersal of mitotic spores across a more arid landscape, whereas male gametes have limited dispersibility on land. The trend in the evolution of these life phases has been from a gametophyte-dominant (liverworts, mosses, hornworts) to a sporophyte-dominant life phase (angiosperms, gymnosperms, lycophytes, and monilophytes).

The success of land plants is apparent in the diversification of the nonvascular and gametophyte dominant mosses (Bryophyta) with over 12,700 species (Crosby et al., 1999; Laenen et al., 2014), second after angiosperms (Magnoliophyta) with about 270,000 species (Soltis and Soltis, 2004), and likely exceeding even the ferns (Monilophyta) with about 10,000 species (Schneider et al., 2004; Smith et al., 2006). The moss life cycle consists of an alternation between a long-lived, photosynthetic gametophyte stage with water-dependent fertilization, and a relatively short-lived sporophyte stage that remains attached to the gametophyte and disperses spores

into the wind. In the time since their divergence from the rest of land plants at least 460 Ma (Clarke et al., 2011; Magallón et al., 2013), mosses have come to occupy an astonishing diversity of ecological habitats including water, rocks, desert soils, tree bark, carrion, and even fecal matter. A persistent problem in understanding land-plant colonization is how the transition from a gametophyte-dominant to sporophyte-dominant life history occurred (Bennici, 2008), especially with regard to sporophyte elaboration. Emerging genomic evidence suggests that sporophyte elaborations in vascular plants are the result of changes in genes currently serving a regulatory function in nonvascular plants (Tomescu et al., 2014). However, it is still unclear what exactly the branched vegetative axis of vascular plants is homologous to, compared to the sporophyte of nonvascular plants (Kato and Akiyama, 2005; Qiu et al., 2012), although it is likely not the sporangium of the latter. Nevertheless, the unbranched, elaborated sporophytes of nonvascular plant lineages may provide useful analogies for elucidating trends in

sporophyte evolution in vascular plants, especially if we can employ approaches that can quantify an entire organism.

We conducted such an analysis by examining the evolution of sporophyte shape (here restricted to the sporangium) across the expansive phylogenetic and ecological space occupied by mosses using elliptic Fourier analysis (EFA) (Kuhl and Giardina, 1982) in a phylogenetic context (Klingenberg and Gidaszewski, 2010). We test the hypothesis of Vitt (1981) that sporophyte features, including sporangium shape, are evolutionary adaptations to specific habitats (Fig. 1; Table 1), an understanding of which "will greatly clarify ideas of evolutionarily parallelisms and adaptive specialization [in mosses]" (Vitt, 1981, p. 166).

As the moss sporophyte functions to produce and disperse spores, selection should favor tall sporophytes that are elevated above the gametophyte and above the boundary layer to be transported long distances on air currents (Vitt, 1981; Johansson et al., 2014). However, a highly elevated sporophyte increases water loss,

FIGURE 1 Representative diversity of moss sporophytes. (A) *Aphanorrhegma serratum* (Funariaceae), an ephemeral species; (B) *Amblystegium varium* (Amblystegiaceae), a mesic species; (C) *Bryum lisae* var. *cuspidatum* (Bryaceae), an ephemeral species; (D) *Fissidens subbasilaris* Hedw. (Fissidentaceae), a mesic species; (E) *Trematodon longicollis* (Bruchiaceae) a dry terrestrial species; (F) *Schistidium rivulare* (Grimmiaceae) a xerophytic species. (All photographs courtesy of Robert Klips.)

TABLE 1. Moss sporophyte characters associated with different habitats, based on Vitt (1981). Xerophytic taxa may be either epipetric or epiphytic. Blank cells indicate no distinctive sporophytic features.

	mesic	submerged aquatic	emergent aquatic	xerophytic	ephemeral	dry terrestrial
peristome	well-developed	reduced/absent	well- developed	reduced/ absent	absent	—
sporangium shape	curved-cylindrical	ovate to oblong	curved-cylindrical	erect, oblong- ovate	ovate	erect
setae	elongate	—	elongate	short, straight	—	curved, twisted

resulting in an evolutionary tension between maintaining water balance, source of nutrition, and increasing dispersal capability (Vitt, 1981; Haig, 2013; Johansson et al., 2014). Mosses may achieve tall sporophytes via the elongation of the seta, sporangium (changes in shape), or both. Based solely on observational correlations of mosses with particular habitats, Vitt provided adaptive reasons for the occurrence of particular sporangium shapes in different micro-habitats. Although his reasoning was not fully developed, it hinged around the water balance issue raised above. In mesic environments (with a closed or open canopy), where maintaining water balance is relatively easy, the development of elongate, curved sporangia that facilitate maximal spore dispersal in more turbulent air currents as a result of vibration of the elongate sporangium is favored (Johansson et al., 2014). In contrast, species in exposed, consistently dry habitats tend to have elongate erect sporangia, although Vitt provided no evolutionary rationale for this phenomenon. Vitt also noted convergence of sporangium shape in xerophytic (epiphytic, epipetric) mosses, seasonally submerged aquatic mosses, and mosses of seasonally moist bare soil (ephemerals). Species in these three micro-habitats tend to converge toward more erect and stout sporangia. Vitt attributed this phenomenon to adaptations to account for water balance demands in habitats that fluctuate widely in water availability (e.g., tree bark, seasonally inundated rocks in streams, outcrops) and that also tend to receive high intensity photons, which results in increased chloroplast damage from ultraviolet light. Additionally, because gametophytes in these more exposed conditions are frequently desiccated, photosynthetic output, and therefore, nutrient allocation to sporophyte tissue is limited, favoring shapes that maximize the ratio of sporangial volume to surface area. Vitt regarded mesophytic mosses with well-developed peristomes and curved, cylindrical sporangia as plesiomorphic in these characteristics, while mosses adapted to xerophytic conditions (epiphytic or epipetric species) have sporophytes apomorphic in these characteristics, with reduced peristomes and ovate-to-oblong sporangia.

Tests for association between sporangium shape and habitat type have received mixed results in the literature (Buck et al., 2000; Hedenäs, 2000, 2001; Huttunen et al., 2012). Here we reconstruct sporangium shape as a quantitative measure on a well-sampled moss phylogeny, and correlate changes in sporangium shape to ecological diversification over the last 480 Ma. To do this, we combine a large set of tools in the growing field of comparative methods, which have been underemployed in plant biology. Specifically, we employ a methodology that reduces the potential for bias in character coding and that allows for the quantification of mathematically complex traits in a comparative context.

MATERIALS AND METHODS

Phylogenetic methods—*Sequence Data*—Our taxon sampling constitutes the most thoroughly sampled phylogeny of mosses to date in terms of tips. Prior to this, the best taxonomic sampling has been

that of Cox et al. (2010), who used three genomic segments and 660 species as generic exemplars (but see Chang and Graham (2014) for a more nucleotide-rich plastid phylogeny). We used a supermatrix approach of increasing taxa and gene sampling, which has been shown to generate reliable phylogenetic insights at all levels within the Tree of Life, even with over 90% missing data (Wiens and Tiu, 2012; Hinchliff and Roalson, 2013; Roure et al., 2013; Soltis et al., 2013). We compiled sequence data from 21 loci including 14 plastid markers (*atpB-rbcL*, *atpF-atpH*, *ndhF*, *psbA-trnH*, *psbB-psbN-psbH*, *psbE-psbF-psbL-psbJ*, *rbcL*, *rpoC1*, *rps4-trnS*, *rps4-trnT-trnL*, *rpl2*, *rpl16*, *trnG, trnL-trnL-trnF*), five mitochondrial markers (*atp1*, *atp6-rps7*, *nad2*, *nad4-nad5*, *nad5*), and two ribosomal markers (internal transcribed spacer regions, ITS, and 26S rDNA) from GenBank from a total of 3121 mosses and 102 outgroups (82% missing sequences) (Appendix S1, see Supplemental Data with the online version of this article for individual locus and concatenated alignments, see the Dryad database, http://dx.doi:10.5061/dryad.0643c). Three genes (ITS, *rps4-trnS*, and *trnL-trnL-trnF*) had at least 1 800 taxa represented. If multiple sequences for a species were present in GenBank, only the longest was used. To minimize missing data, we checked the synonymy of species using Tropicos (http://www.tropicos.org/).

Taxa sampling—The nonmonophyly of bryophytes has been contentious in the past few years (Cox et al., 2014; Ruhfel et al., 2014; Wickett et al., 2014). However, we used the prevailing idea that mosses are more closely related to hornworts (Anthocerotophyta) than liverworts (Marchantiophyta). Hornwort and liverwort taxa were removed from resulting trees for sporangium shape analyses, reconstruction of ancestral habitats, and species diversification analyses. Therefore, the relationships of mosses to these phyla do not affect downstream analyses.

Alignment, phylogenetic analysis, and molecular dating—We aligned sequences using MAFFT version 7 (Katoh and Standley, 2013). Because of the expected deep divergence in the data set, the direction of sequences were adjusted according to the first sequence to account for reversed orthologous sequences in GenBank, and the FFT-NS-I slow, iterative refinement strategy was employed. While ITS is generally fast-evolving in angiosperms, molecular evolution in mosses is two-to-three times lower than in vascular plants (Stenøien, 2008), allowing for more straightforward alignment. All alignments were concatenated and a phylogeny was reconstructed using maximum likelihood (ML) as implemented in RAxML (Stamatakis, 2014) under the GTR+G model. We did not conduct model testing, but justify the use of this model given that Cox et al. (2010) found GTR+I+G to be the best fitting model for *rps4-trnS* and *nad5* in their moss-wide study. Branch support was assessed through 500 bootstrap replicates. As the large size of the ML tree precluded doing a dating analysis in BEAST (Drummond et al., 2012), we used penalized likelihood, as implemented in the R (R Core Team, 2014) package *ape* (Paradis et al., 2004). To calibrate the tree, lambda was set to 0.1 and the ranges of 12 secondary dates

were used based on recent literature and fossils (Appendix S2). The maximum and minimum root age were set using 815 Ma as the oldest estimate for the origin of land plants (Clarke et al., 2011) and 450 Ma as a 10 Ma offset from the 460 Ma minimum age estimate for land plants estimated by Magallón et al. (2013).

Shape analysis—We used geometric morphometric analyses of moss sporangia in the framework of an explicit phylogenetic hypothesis (Lavin et al., 2008; Klingenberg and Gidaszewski, 2010; Klingenberg et al., 2012; Smith and Hendricks, 2013). Scanned line drawings of moss sporangia gathered from the literature representing all major lineages and including representatives from all continents (Appendices S3–S5) were imported into GIMP 2.8 (http://www.gimp.org) and outlined. Because sporangia vary in their shape when dry, and species may or may not have dehiscent sporangia, care was taken to ensure that the images were of moist sporangia with an operculum still attached (if operculate). For indehiscent species, we viewed the tissue that was distal-most from the foot of the sporophyte as homologous with the operculum tissue in operculate species. Setae were not included because (1) they are rarely completely depicted in illustrations, (2) length has been shown to be highly variable within a species (Shaw and Beer, 1999; Ligrone et al., 2012), and (3) the seta-like gametophytic pseudopodia of some moss clades (*Andreaea*, Sphagnales, *Takakia*) are not homologous with the seta. If the species possessed a sporophytic seta, a small portion of it was retained in the outline.

Images of the sporangial shapes were converted into 8-bit images using Fiji (Schindelin et al., 2012) and then imported into R. To reduce twisting of the outlines, we set two landmarks on either side of the proximal end of the sporangium (point on the seta or near the pseudopodium), and calculated harmonic amplitudes for the outlines using the default of 32 harmonics using EFA as implemented in the *Momocs* package (Bonhomme et al., 2014). This method attempts to fit trigonometric functions (harmonics) to the shape outlines, each composed of four coefficients (amplitudes). We then subjected the 128 amplitudes (4 coefficients × 32 harmonics) to a principal components analysis (PCA) to determine which harmonics were most important in describing the variation in sporangium shape. This approach also eliminated noise that may be caused by over-fitting harmonics. We used the principal component (PC) that described most of the shape variation of the sporangium as a shape variable to reconstruct the ancestral state of that component on the chronogram of the most likely ML tree using the "contMap" function in the *phytools* package (Revell, 2012).

To test phylogenetic niche (trait) conservatism (i.e., sporangium shape similarity in closely related species), we also examined phylogenetic signal in sporangium shape using Blomberg's K statistic (Blomberg et al., 2003) in the R package *picante* (Kembel et al., 2010). The K statistic compares the observed signal in a trait on a phylogeny to the signal under a Brownian motion model of trait evolution. A value of $K = 1$ indicates that the trait values of the tips are correlated as expected under the Brownian model, whereas a $K = 0$ indicates no correlation under the Brownian expectation. The statistical significance of the K statistic was evaluated using a null model of shuffling taxa across the tips of the phylogeny.

Because of the size of the inferred moss phylogeny and the likelihood of phylogenetic signal varying across subclades within mosses, we also examined phylogenetic signal in reduced trees that represent individual orders or other subclades using the "extract.clade" function in *ape*. We also employed disparity-through-time in *geiger*

to compare levels of phenotypic (sporangium shape) disparity within and among clades through the history of the tree, relative to that expected under a null model of Brownian motion (the disparity index) (Harmon et al., 2003).

Habitat reconstruction—To explicitly test the hypothesis of Vitt (1981), we assigned outlined species one of five possible habitat types from habitat descriptions from the literature (Appendix S4). These habitats include aquatic (submerged or emergent in water), mesic (woodlands, hummocks in wetlands), ephemeral (arable fields, roadside ditches), dry terrestrial (gravel roadsides, dunes, deserts), and xerophytic (epiphytic or epipetric) (Fig. 1). While habitat is a more complex variable than that portrayed here (Hedenäs, 2001), we used this simple coding to test the hypothesis of Vitt (1981). The wide ecological amplitude inhabited by mosses, and the difficulty in incorporating climatic data as a single quantitative variable necessitated combining many habitat types in this manner. However, these five habitat types effectively span this breadth of ecological variation seen across mosses. Species whose habitat preferences were insufficiently described in the literature were not scored and removed from the analysis. For species known to be polymorphic for habitat, the most common habitat based on descriptions in the literature was selected. Ancestral habitat was reconstructed on the same chronogram as sporangium shape using the rerooting method of Yang et al. (1995) as implemented in the *phytools* package (Revell, 2012) under a symmetric model of rates. A likelihood ratio test selected the all rates different model as the best-fitting model of trait evolution, but use of this model with this function is discouraged in the package documentation. To test for phylogenetic niche conservatism in the discrete, multistate habitat character, we examined phylogenetic signal using Pagel's λ as implemented by the fitDiscrete function in *geiger* (Harmon et al., 2008), and used the likelihood ratio test, approximated by a χ^2 distribution to compare the negative log likelihood with no signal (i.e., using the tree transformed λ =0) to that when λ was estimated using the original tree topology. Similarly, as with sporangium shape, we also examined phylogenetic signal in habitat for individual orders or other subclades.

Testing for correlation of habitat and shape—We assessed the correlation of shape with habitat type using phylogenetic generalized least-squares regression (PGLS) by fitting a generalized least squares model using the R package *nlme* (Pinheiro et al., 2015) with the correlation matrix derived from the method described by Blomberg et al. (2003) and implemented in *ape* (Paradis et al., 2004) with a starting value of $g = 0.7$.

Diversification analysis—A recent study by Laenen et al. (2014) examined diversification in nonvascular plants using MEDUSA (Alfaro et al., 2009). However, their diversification analysis incorrectly assumed monophyly of genera (for a summary of problems related to generic paraphyly in mosses see Grundmann et al., 2006; Harris, 2008) and only accounted for three quarters of moss species. Correct application of diversification analyses requires that (1) all tips serving as placeholders represent clades based on the current best hypothesis, and (2) all species in a clade be accounted for. We conducted a diversification analysis using Bayesian Analysis of Macroevolutionary Mixtures (BAMM) version 1.0 (Rabosky, 2014) on our moss-wide data set (including all tips, whether or not they had sporangia represented) to detect shifts in speciation. Priors for

BAMM were generated using the R package BAMMtools v.2.0.2 (Rabosky, 2014), which selected a Poisson prior of 1.0. Two independent MCMC chains of 150,000,000 generations were run in BAMM and convergence was assessed by computing the effective sample sizes of log likelihoods, as well as the number of shift events present in each sample using the R package *coda* v. 0.16-1 (Plummer et al., 2006). After removing 10% of sampled generations as burn-in, we analyzed the BAMM output using BAMMtools and computed the 95% credible rate shift configurations using the Bayes factors criterion for including core shifts. This procedure computes Bayes factors evidence associated with a rate shift for every branch in the phylogeny and excludes all unimportant nodes using the Bayes factor criterion. Large Bayes factors (BF > 5) values are taken as strong evidence for a shift on a branch in the tree. We also estimated the rate-shift configuration with the highest maximum a posteriori (MAP) probability ("the best shift configuration") after excluding all noncore shifts (BF < 5).

Because of rampant paraphyly at the family level and below (Cox et al., 2010), tips were assigned to order as a grouping variable using the checklist of Crosby et al. (1999) and the classification of Goffinet et al. (2009), although in the pleurocarps and Dicraniidae we used (informal) modified order definitions to maintain monophyly. The fraction of missing species was calculated based on a total count of 12,737 species (Appendix S5).

Shifts in sporangium shape—To examine the location of sporangium shifts on the phylogenetic tree, we fitted PC 1 of sporangium shape using an Ornstein-Uhlenbeck (OU) model as implemented in the "OUshifts" function in the R package *phylolm* (Ho and Ané, 2014). We employed the modified Bayesian Information Criterion (mBIC) to detect the optimal number of sporangium shifts and set the maximum number of shifts allowed to 200.

RESULTS

Phylogeny—Our ML analysis of the data matrix of 3226 taxa and 54 985 aligned bp is largely consistent with previously published studies (Cox et al., 2010; Chang and Graham, 2014) (see Appendices S6–S8). A clade comprising Sphagnopsida plus Takakiopsida is sister to the rest of mosses, followed by a grade of Andraeaeosida plus Andraeaobryopsida, and then Oedipodiopsida. Polytrichopsida plus Tetraphidopsida form a clade sister to Bryopsida. Buxbaumiidae is sister to the rest of Bryopsida, followed by a grade of Diphysciidae, Gigaspermales, and then Funariales plus Diphysciales and Encalyptales. Timmiidae is sister to Dicraniidae and Bryidae, both of which are monophyletic with the exception that Catoscopiaceae falls sister to Dicraniidae instead of within Bryidae. Lower taxonomic levels within Dicraniidae are largely paraphyletic and poorly supported. Within Bryidae, Splachnales are sister to the rest of the clade. Bartramiales are sister to Hedwigiales, and Orthotrichales are sister to a paraphyletic assemblage of pluerocarpous and pseudo-pleurocarpous mosses (Rhizogoniales and Hypnanae sensu Goffinet et al., 2009). These results differ from previous studies mainly in the placement of the Tetraphidopsida as sister to Polytrichopsida as opposed to part of a grade (but see Volkmar and Knoop, 2010), as well as in the placement of Gigaspermales. However, the placement of the latter clade has also been controversial and it possesses multiple unique genomic features (Goffinet et al., 2007) suggesting that further research is needed to confidently place the order.

Shape analysis—We obtained sporangium outlines for 1090 taxa representing the full range of phylogenetic, geographical, habitat, and morphological diversity in the mosses (Appendices S3–S5). The first two principal components (PCs) of the EFA harmonics describe a total of 84.2% (68.7, 15.5%) of the total variation in sporangium shape. They largely capture, respectively, sporangium shape (round vs. linear) and curvature (erect vs. concave) (Fig. 2). A scatter plot of PC 1 vs. PC 2 (Fig. 2) reveals a large amount of convergence and divergence with respect to sporangium shape. Ancestral-state reconstruction of sporangium shape based on PC 1 for 886 tips that matched tips in the chronogram confirms recurrent convergence in sporangium shape in mosses (Fig. 3). The ancestral state at many deeper nodes is reconstructed as "average" sporangium shape, but there is considerable elongation of the sporangium in the more nested lineages. Most rounded sporangium shapes are inferred to have evolved relatively recently, with the exception of Bartramiales and Sphagnales. Dicranales exhibits a large amount of diversity in sporangium shape with many exceptionally elongate and rounded lineages.

The Blomberg's *K* statistic showed significant phylogenetic signal in sporangium shape across the mosses (p = 0.012) despite a low *K* value (K = 0.0028)—low values are expected in trees with large numbers of taxa (Harmon-Threatt and Ackerly, 2013). However, sporangium shape is not inferred to have evolved in a phylogenetically conservative fashion (i.e., closely related species are quite different in shape) within a number of orders or subclades (Table 2). These clades (Hedwigiales + Bartramiales, Bryales, Orthotrichales, and pleurocarps) all exhibited significant shifts in sporangium shape and/or species diversification (see below). Only one clade (Catoscopiales + Dicranales) exhibited significant shifts in sporangium shape and shows significant phylogenetic signal, although it is large in size (250 tips). Mirroring the results of phylogenetic conservatism, the Disparity-through-time (dtt) plot demonstrates significant departure from the simulated 95% confidence intervals starting 250–280 Ma (Fig. 4). This is coincident with the diversification of the orders whose descendants show significant shifts in sporangium shape (see below).

Correlation between shape and habitat—We were able to confidently score 785 species for habitat type. Ancestral habitat reconstruction (Appendix S6) at the older nodes was ambiguous. Clearer ancestral state reconstructions were found at younger nodes. Although phylogenetic signal exists for habitat overall across the mosses, Pagel's λ values were often well below 1.0 (strong phylogenetic signal), often with values of λ less than 0.75, although λ was significantly different from 0 (some phylogenetic signal). The distribution of habitat in Bryales lacked phylogenetic signal (i.e., was not significantly different from λ = 0 or no signal) (Table 2).

Our PGLS results indicate that habitat is a significant predictor of sporangium shape for dry terrestrial, ephemeral, mesic, and xerophytic mosses (Table 3). Importantly, this demonstrates that unrelated species living in similar habitats possess similar sporangium shapes (Fig. 3; Appendix S6).

Diversification analysis—Diversification analyses across mosses using BAMM detected 19 shifts in speciation rate (18 increases and 1 decrease) (Table 4; Appendix S8) across mosses (Fig. 3). All but two shifts in speciation rate were younger than 200 Ma (Table 4). The most ancient increase in speciation rate dates to the Mid/Late Devonian (382 Ma).
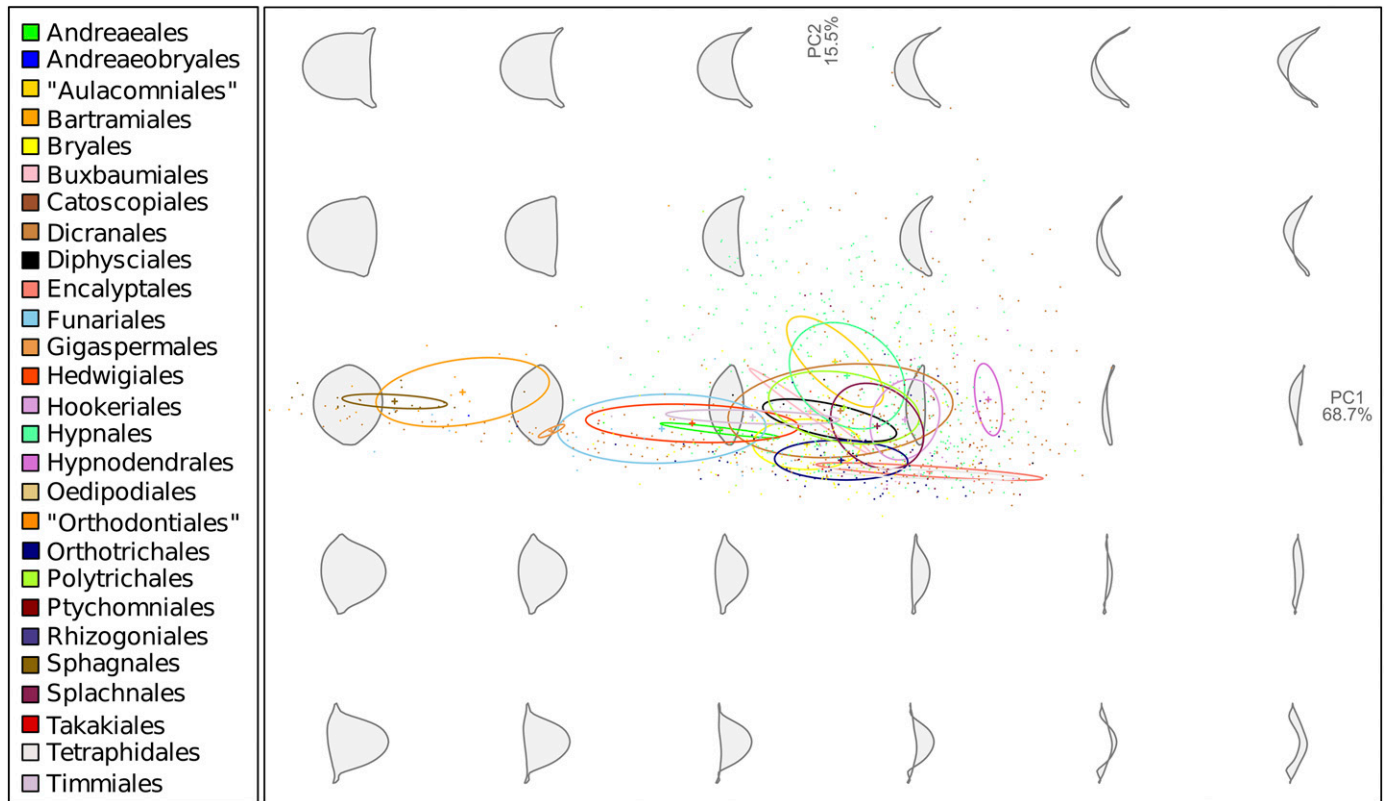
FIGURE 2 PC 1 vs. PC 2 for sporangium shape of 1090 species of mosses. Circles represent the 50% confidence ellipses around the centroid for each color-coded order. The background represents the sporangium shape based on PC 1 vs. PC 2 for a particular portion of the morphological space. Unoccupied areas represent hypothetical sporangium shapes. For taxonomic assignment see Appendix S4.

Sporangium shape shifts—Of the maximum of 200 allowable trait shifts in the model, "OUshifts" selected a model containing 50 shifts in sporangium shape (Table 4; Appendices S7 and S9). Our model estimated the strength of selection, $\alpha = 0.1$. The α value was used to compute the time in which the trait reaches half the distance to the optimum trait value (the phylogenetic half-life or $\log(2)/\alpha$), which was 6.65 Ma. This phylogenetic half-life represents 1.3% of the total depth of the phylogeny. Most shifts are concentrated toward the tips of the tree, but five shifts appear to have occurred earlier than 200 Ma. Most shifts in sporangium shape occurred in Bryales, Pottiaceae (Dicranales), Hookeriales, and Hypnales (Fig. 3). Of the 50 detected shifts in sporangium shape, 30 follow 10 of the detected increases in speciation, often by a few nodes. The speciation shift in *Sphagnum* and a shift in sporangium shape occur on the same branch.

DISCUSSION

We present here the largest phylogenetic analysis of mosses (Bryophyta) to date, and use this to examine the evolution of sporangium shape and its correlation with habitat. We draw three main conclusions: (1) sporangium shape has evolved in a convergent fashion throughout the diversification of mosses, (2) changes in sporangium shape are the result of natural selection in differing microhabitats, and (3) shifts in speciation rate are associated with, and appear to result in, shifts in sporangium shape. Based on a

disparity-through-time analysis, much of this evolution (as reflected in high variance within subclades vs. between subclades) began around 250–280 Ma (Fig. 4).

Shape is associated with habitat—Morphometric analysis of sporangium shape indicates that roundness and curvature, elements hypothesized to be important ecological adaptations of mosses (Vitt, 1981), are important in describing the variability in shape throughout mosses (Fig. 2). Similar sporangium shapes have clearly evolved repeatedly with surprisingly similar shapes in completely unrelated groups. There is an overall trend toward increasing elaboration of the sporangium (here realized as curvo-linear in shape). In this case, linearity and curvature allows for an increased range of spore dispersal via a decrease in the amount of air turbulence that is necessary to cause spore release from a vibrating sporophyte (Johansson et al., 2014).

Even in xerophytic clades, sporangia that are more rounded than their mesophytic relatives are still markedly elongate (cf. Hypnales in Fig. 3). Clades with more rounded sporangia are on the whole more recent in origin than clades with elongate sporangia. Many of the rounded-sporangium clades, especially those that lack an operculum, appear to be short-lived compared with the dominance in space and time of clades with elongate and operculate sporangia. The recent appearance and general rarity of these clades suggests that the long-term success of rounded, especially inoperculate sporangia, is limited, although we find no evidence for reduced speciation or increased extinction in this study. In the short term, round inoperculate sporangia
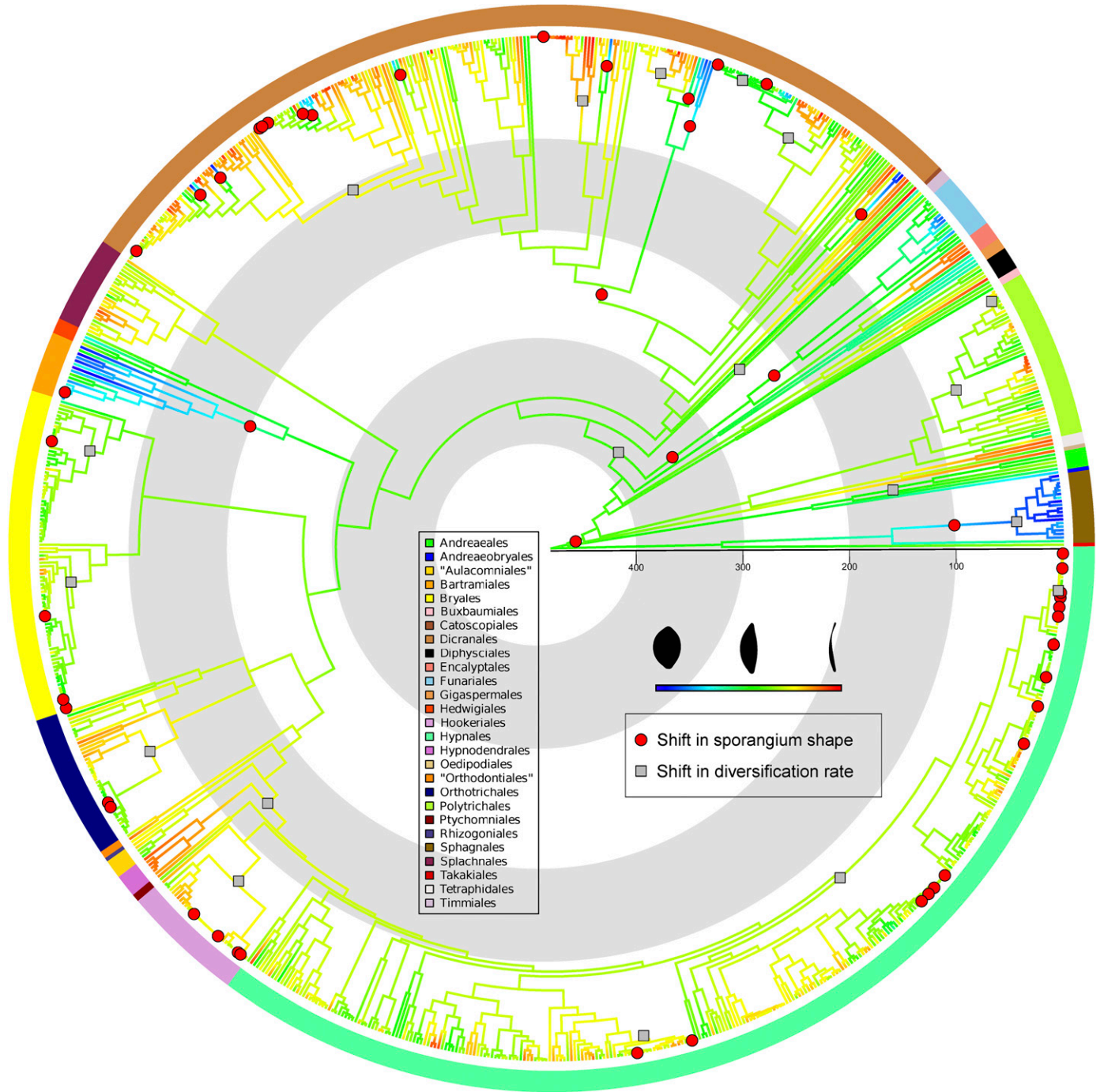
FIGURE 3 Chronogram of mosses. Branch color indicates the ancestral sporangium shape based on PC 1. Tip labels correspond to order (or nearest approximate) as in Appendix S4. Shifts in sporangium shape are indicated by red circles; shifts in speciation rate are noted with gray squares.

allow for mass dispersal of a large number of spores in highly temporary and extremely patchy disturbed habitats. However, in the long-term, low dispersibility and high rates of self-fertilization may lead to increased homozygosity and eventual extinction (Klips, 2015). Vitt (1981) hypothesized that elongate, curved sporangia are the plesiomorphic character state in mosses. However, we find little support for this. Our results suggest that the ancestor of mosses had an elongate, straight sporangium. However, we do show that rounded-to-oblong sporangia are apomorpic in sporangium shape, as he hypothesized.

We found support for the hypothesis that the moss sporangium exhibits adaptations characteristic of specific habitats, and these adaptations often appear in a convergent manner. Although the association may perhaps be weak in some parts of moss phylogeny, and some of the variability in sporangium shape may be due to phylogenetic relatedness based on our tests for phylogenetic signal, it is clear that sporangium shape has evolved at least partially in conjunction with habitat type in many of the clades for which Blomberg's *K* suggests no phylogenetic signal (Table 2). Our habitat coding is

TABLE 2. Phylogenetic signal in sporangium shape and habitat across mosses and selected subclades. Blomberg's *K* statistic or testing phylogenetic signal in the continuous trait of sporangium shape, and Pagel's λ for testing phylogenetic signal in discrete trait of habitat. Significant P-values (*) indicate phylogenetic signal, a nonsignificant value indicates closely related species in the clade are more dissimilar in shape or habitat than expected; NA indicates that there is no phylogenetically informative habitat variability in that clade. The two rightmost columns indicate how many sporangium shape or speciation rate (diversification) shifts occurred in that clade.

clade	clade size	sporangium shape		habitat		shape shifts	diversification shifts
		K	P	λ	P		
Bryophyta	886	0.0028	0.012*	0.88	0*	50	19
Takiales + Sphagnales	20	0.645	0.004*	NA	NA	1	1
Tetraphidiales + Polytrichales	47	0.082	0.002*	0.61	0.0059*	0	2
Encalyptales + Funariales	20	0.27	0.001*	1.0	0.00055*	2	0
Catoscopiales + Dicranales	250	0.014	0.002*	0.91	0*	17	5
Splachnales	23	0.35	0.04*	0.99	0.014*	0	0
Hedwigiales + Bartramiales	20	0.090	0.15	0.66	0.014*	2	0
Bryales	87	0.021	0.25	0.36	0.13	4	2
Orthotrichales	38	0.0080	0.31	NA	NA	2	1
pleurocarps	360	0.0012	0.83	0.80	0*	20	5

inherently coarse and may not be capturing shape variation because of differences in sporangium exposure (i.e., in aquatic species with immersed vs. emergent sporangia; see Table 1). Furthermore, our analysis of trait shifts suggests that several sporangium shifts are accompanied by shifts in habitat, although some nodes are ambiguously reconstructed as a result of a large amount of habitat shifting (Table 3; Appendix S6). A shift in both habitat and sporangium shape is seen in many clades, most notably in *Bryum*, *Sphagnum*, *Scouleria*, and *Weissia*. Several shifts in habitat appear to be associated with more gradual shifts in sporangium shape based on the ancestral-state reconstruction of shape (i.e., *Leucobryum*, Fig. 3). Coarse habitat coding may also limit our results in this regard. For example, in Orthotrichales there are multiple sporangium shape shifts in *Orthotrichum*, but no concurrent habitat shifts. Nevertheless, *Orthotrichum* is the most xerophytic genus of the family, and sporangium shape has likely changed to cope with slightly drier and exposed microhabitats in our "xerophytic" category (Vitt, 1973). This microhabitat shift is not captured in our analysis.

The adaptive value of changes in sporangium shape is further emphasized in our mapping of OU shifts onto the moss tree. Our model estimate of α = 0.1, and a low phylogenetic half-life of 1.3% of the total depth of the phylogeny, both suggest strong selective pressure on this trait.

Sporangial evolution is associated with speciation—Our diversification analysis indicates that a total of 10 of 18 shifts that led to increasing moss species diversification are associated with shifts in sporangium shape, although few of these shifts appear to be associated with habitat shifts (Table 4). Our diversification results closely match those of two recent analyses of diversification in land plants using other approaches and taxa sampling (Fiz-Palacios et al., 2011; Laenen et al., 2014). Our use of a Poisson prior of 1.0 (which rejects most moves that increase the number of shifts) suggests that this may be a conservative estimate of the number of shifts. There is one case of an increase in speciation rate and shift in sporangium shape in *Sphagnum*. Our single detected decrease in speciation rate in Timmiales is not surprising because the order is old (crown age of ca. 250 Ma) and small (only eight extant species), suggesting that much extinction has occurred in it.

Some recent clades show a shift in shape with no (or an ambiguous) shift in habitat, suggesting a time lag in sporophytic adaptation to differing habitats (Donoghue and Sanderson, 2015). The

distribution of these shifts is nonrandom in mosses, with a highly disproportionate number of shifts in sporangium evolution and speciation rate occurring in clades with low values of K that differ significantly from 1 (Table 2), with the exception of the large and polymorphic Dicranales (sensu this study).

These observations support two general conclusions. First, given the association of speciation rate with sporangium shape, diversification in mosses is associated with morphological changes that resulted in substantial evolutionary novelty. Many of these morphological changes involved sporangium shape, although changes
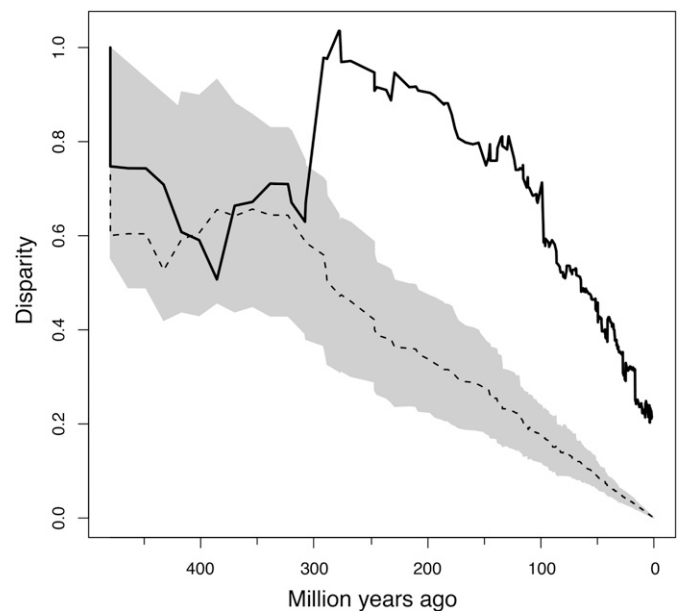

FIGURE 4 Disparity-through-time (dtt) plots of sporangium shape across the phylogenetic history of mosses. The disparity index is the average distance between measured and mean simulated disparity values. High disparity values indicate high variance within subclades; low values indicate conservation within subclades, but high variance among subclades. Solid black lines are measured values of disparity, dotted lines are the median disparity of 100 data sets simulated under Brownian motion, and gray areas are 95% confidence intervals of simulations.

TABLE 3. Phylogenetically controlled generalized least-squares regression of moss sporangium shape based on PC 1 vs. microhabitat on 770 residual *df* and with a residual standard error of 1.92. Significant P-values (*) indicate a slope for that variable is significantly different from zero.

variable	coefficient	SE	T	P
intercept	−0.25	0.56	−0.44	0.6578
dry terrestrial	0.27	0.01	20.34	0.0000*
ephemeral	0.13	0.03	3.75	0.0002*
mesic	0.20	0.005	40.38	0.0000*
xerophyte	0.18	0.009	19.75	0.0000*

in shape need not have been the only morphological feature explored following species diversification events. It is difficult to say whether morphological novelty preceded or followed the shifts in speciation rate given the differing sizes of the trees on which we conducted our trait and speciation diversification analyses and some uncertainty in the placement of shifts on particular nodes of the trees that is inherent with methods of modeling shifts. However, our results suggest that diversification of sporangia followed increases in speciation in mosses rather than preceding them, ruling them out as causative agents in these cases.

Second, while associated to sporangium shape, there is a mismatch between shifts in habitat and shifts in sporangium shape. This is especially evident in recent clades, where there are large shifts in sporangium morphology but little ecological change, at least as characterized by our habitat coding. Mismatch between the evolution of sporophytic characters and the colonization of new habitats in mosses is not a novel finding. For example, Huttunen et al. (2012) found a lack of a one-to-one correspondence between several sporophytic traits and colonization of epiphytic habitats in Neckeraceae and Lembophyllaceae. For three of four correlated traits in their study, shifts in morphology occurred after colonization of novel habitats.

Taking our results as a whole, we propose that species diversification leads to rapid colonization of novel niche space. Natural selection acting on sporangium shape to optimize dispersal output in terms of both volume of spores produced and dispersal distance, coupled with the need to avoid desiccation in variably moist environments, led to elongated sporangia in moist and reliably stable environments. Elongation of sporangia maximized dispersal distance via a decrease in the amount of air turbulence that is necessary to cause spore release from a vibrating sporophyte. In contrast, natural selection also favored rounded sporangia in hydrologically variable sites to maximize sporangial volume and reduce desiccation via an increased surface-area-to-volume ratio.

Vitt (1981) suggested that peristome morphology and seta length are likely also associated with habitat (we would also add sporangium length). Phylogenetically controlled analyses of the evolution of these and perhaps other morphological features of the sporophyte should be examined in the future. The interplay of these sporophyte-associated features should be examined in the framework of phylogenetically controlled multivariate regression and shift detection, once such analytical methods have been developed.

We do not specifically infer any drivers of species diversification within mosses and have limited our discussion to the phenotypic results of diversification events. However, increases in speciation rate are undoubtedly associated with additional traits. In the sporophyte, these may include the peristome type or calyptra morphology. The shifts in species diversification uncovered here may not consistently be correlated with selection on particular sporangium shapes. For example, shifts in speciation rates seen within the deeper node of Polytrichaceae and within *Pogonatum* may be associated with the acquisition, respectively, of "polytrichopsid" peristome teeth with an associated epiphram and angled sporangia (Smith, 1971; Bell and Hyvönen, 2008), and of pitted peristome teeth (Smith, 1971). We also detected a striking shift in speciation rate following the evolution of the gametophytic pleurocarpous habit (in which sporangia are produced laterally instead of terminally on branches).

TABLE 4. Bayesian Analysis of Macroevolutionary Mixtures (BAMM) speciation rate shifts detected across the moss phylogeny and the number of sporangium shape shifts in the descendant of each branch. All diversification shifts led to an increase in speciation rate, except for Timmiales. Associated shifts in habitat or sporangium shape are indicated; "ASR" indicates that a shift is indicated in the ancestral-state reconstruction of sporangium shape under a Brownian motion process, but this shift was not detected as significant under an Ornstein-Uhlenbeck process.

clade	increase/ decrease	timing (Ma)	habitat shift	number of associated sporangium shifts
arthrodontus mosses	increase	382.41	no	48
Timmiales	decrease	333.45	no	—
Andreaea	increase	179.26	yes	—
pleurocarps	increase	125	no	20
Polytrichaceae	increase	103.47	ambiguous	—
Pottiaceae	increase	98.03	no	9
core Hypnales	increase	72.23	no	14
Dicranaceae	increase	69.05	no	1
Hookeriales	increase	55.57	no	4
Sphagnum	increase	46.1	yes	1
Grimmiaceae	increase	44.45	no	1
Mniaceae	increase	42.24	no	1
Bryaceae	increase	31.54	no	3
Leucobryaceae	increase	26.56	yes	ASR
Orthotrichum	increase	10.64	no	2
Neckeraceae	increase	9.74	no	2
Pogonatum	increase	9.68	ambiguous	ASR
Grimmia	increase	6.35	no	2
Sematophyllaceae	increase	3.27	ambiguous	6

Implications for land-plant evolution—This study represents the first quantitative examination of moss sporophyte evolution (here the sporangium), but it is also one of a few studies looking at the evolution of a morphological character on a broad phylogenetic scale (but see Mahler et al., 2013; Stournaras et al., 2013) or covering such a deep expanse of time. The convergent evolution of the moss sporangium in specific habitats and the success of these shifts in morphology (as measured by species diversification differences) provide perhaps one of the better examples of a general pattern: land-plant body plans are capable of extensive morphological homoplasy due to adaptive convergence on similar structural solutions that reflects nearly identical requirements for plant growth and survival, regardless of phylogenetic relatedness (Niklas, 2000, 2014). Given the generality of the association between major body plan changes, diversification, and habitat in mosses, it seems logical to hypothesize that such drastic changes of the sporophyte body plan (or other morphological traits) may also be responsible for (or result from) major diversification events in vascular plants, including angiosperms, although the selective pressure that results in major changes in other groups are likely different. While the intricate and more complex sporophyte shapes of vascular plants make their analysis more difficult, the presence of relatively simple body plans in some lineages (e.g., in ferns, Ophioglossaceae; in angiosperms Aizoaceae, Bromeliaceae, Cactaceae, Euphorbiaceae) may provide examples for applying similar approaches. Our study provides a framework for quantifying morphological changes across these other organisms. It demonstrates in particular that sporangium shape in mosses has been molded by natural selection in specific microhabitats, and that the diverse morphospace exhibited by moss sporangia is likely the result of habitat shifts following speciation events that led to major clades.

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