

# Gametophyte and sporophyte morphology are phylogenetically correlated in mosses, indicating coadaptation and parent–offspring conflict

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## Introduction

The relationship between parents and their offspring presents a striking paradox wherein parents must balance the investment of limited resources between provisioning their offspring to increase their chances for survival and maturation, and reserving resources for their own survival and future reproduction (Trivers, 1974). This is a complex and dynamic interaction that is common in both animals (Godfray, 1995a; Crespi & Semeniuk, 2004) and plants (Mazer, 1987; Haig & Westoby, 1988). Cooperation and conflict between parents and their offspring, particularly in relation to resources, have immediate implications for survival and far-reaching effects on fitness and evolution (Parker & Macnair, 1979; Godfray, 1995b). In the majority of plant lineages, parent–offspring interactions are limited to early developmental stages (Ellner, 1986; Haig & Wilczek, 2006), whereas in bryophytes (mosses, liverworts, hornworts), offspring sporophytes are physically attached to their parental gametophytes throughout their entire lifespan (During, 1979; Haig, 2012). Thus, bryophytes present a unique system to broaden our understanding of the parent–offspring relationship.

The sporophytes of bryophytes are physically attached to and dependent on the parental gametophyte for both water

## Summary

- Parent–offspring relationships present a paradox wherein parents must balance limited resources between provisioning their offspring to increase their chances of survival and maturation, and reserving resources for their own survival and future reproduction. Bryophytes provide a unique system to explore this relationship due to the dependency of sporophytes on parental gametophytes throughout their lifespan.
- We investigate the morphological evolution of gametophyte and sporophyte characters to test for evidence of coadaptation in the Dicranaceae Schimp. and Grimmiaceae Arn. We also examine these morphological features in Grimmiaceae species with different sexual systems to test for higher levels of parent–offspring conflict in species that are exclusively outcrossing. Our study is the first to test this prediction with empirical data.
- Our study reveals significant correlations between parental gametophyte and offspring sporophyte morphology, which provides evidence of coadaptation. We found that species with unisexual gametophytes have larger calyptrae, which may decrease offspring resource acquisition, as well as larger capsules and larger setae, which may increase resource acquisition, than species with bisexual gametophytes.
- These findings suggest that the sexual system influences the relationship between gametophyte and sporophyte morphology, indicating higher levels of parent–offspring conflict in outcrossing species.

and photosynthates throughout their life (Kubásek *et al.*, 2021). This translocation of resources occurs at the base of the sporophyte through the foot (placenta region), which is embedded in and intermingled with tissues of the gametophyte (Ligrone & Gambardella, 1988). In mosses, there is a second area of physical contact between the haploid and diploid phases. The moss calyptra is a small cap of gametophyte tissue that covers and protects the apical region and seta meristem of the sporophyte from dehydration during early development (Budke *et al.*, 2013). The calyptra also plays a role in resource translocation by decreasing evaporation from the sporophyte apex, which decreases the transport through the sporophyte (Bopp & Stehle, 1957; Whitaker & Budke, 2021) and can potentially decrease the transpirational pull of water and nutrients from the gametophyte. This ability to decrease water loss from the sporophyte apex is due to a waxy cuticle that covers the exterior of the calyptra (Budke *et al.*, 2011, 2012).

Two frameworks that help us to understand the relationship between parents and their offspring are the coadaptation hypothesis and the parental conflict hypothesis. The coadaptation hypothesis predicts that offspring and their parents have shared interests that result in developmental coordination, evolutionary adaptations, and ultimately resource allocation that positively

affects offspring survival, development, and fitness (Kölliker *et al.*, 2005; Wolf & Hager, 2006). Concurrently, the parental conflict hypothesis predicts that resource limitation drives evolutionary adaptations in offspring to maximize resource acquisition (Shaanker *et al.*, 1995) and in parents to limit offspring resource acquisition (Parker & Macnair, 1979). Over 13 000 moss species (Patel *et al.*, 2021) exhibit morphological variation in the structures involved in the parent–offspring relationship of both the gametophyte (placenta, Ligrone *et al.*, 1993; cuticle, Budke & Goffinet, 2016; calyptra, Budke, 2024) and the sporophyte (water-conducting cells, Héban, 1977; foot, Ligrone *et al.*, 1993; stomata, Renzaglia *et al.*, 2020). In particular, both calyptrae and sporophytes range in size from extremely small in *Physcomitrium patens* (Hedw.) Mitt. (calyptra length = 0.2 mm; sporophyte height = 1 mm; Goffinet, 2007) to relatively large in *Dawsonia polytrichoides* R.Br. (calyptra length up to 20 mm; sporophyte height up to 36 mm; Hyvönen, 2012).

Under the coadaptation hypothesis, larger parental calyptrae are predicted to provide better protection for the apical region and seta meristem of the sporophyte offspring during early developmental stages (Budke *et al.*, 2013). Providing this protection for longer developmental periods could enable the production of a larger capsule containing more spores and the growth of a longer seta, resulting in positive impacts on spore dispersal and offspring fitness. Concurrently, under the parental conflict hypothesis, large parental calyptrae are predicted to limit resource uptake by decreasing the transpirational pull of the sporophyte (Bopp & Stehle, 1957; Whitaker & Budke, 2021) enabling the parental gametophyte to retain more resources for itself. By contrast, sporophyte offspring with larger capsules and longer setae (stalks) have larger surface areas and are elevated above the still air of the laminar boundary layer, potentially increasing evaporation from the sporophyte and thus increasing the pull of resources from the attached gametophyte parent, which would also be predicted by the parental conflict hypothesis.

Mosses also vary in terms of their sexual system, with 57% of species having unisexual (dioicous) and 43% bisexual (monoicous) gametophytes (Villarreal & Renner, 2013). The sexual system of a species can also affect the parent–offspring relationship by influencing the relatedness between parents and their offspring. Unisexual parents, which can be at most 50% related to their offspring, are predicted to exhibit higher levels of genetic conflict with their offspring due to the genomic contribution of the other parent (Haig, 2012). By contrast, bisexual parents, which can be up to 100% genetically related to their offspring, are predicted to exhibit lower levels of conflict (Haig & Wilczek, 2006) and potentially higher levels of developmental coordination (Haig, 2014). The influence of morphological and sexual system variation on parent–offspring relationships in mosses is ripe for study using comparative phylogenetic methods, which account for relatedness between species and can reveal novel patterns of adaptation that are not apparent when evolutionary history is ignored (Felsenstein, 1985; Harvey & Pagel, 1991).

This study explores coadaptation and conflict between parents and offspring. Parental investment in a larger calyptra is predicted to both provide protection for the developing moss

sporophyte offspring and decrease the ability of the offspring to acquire resources by reducing the amount of exposed surface area, while offspring investment in a larger capsule and longer seta increases the ability of the offspring to acquire resources via larger surface areas. We also predict that there will be higher levels of conflict for unisexual vs bisexual species and we predict that this will result in a morphological arms race where unisexual species have larger calyptrae, larger capsules, and longer setae than bisexual species. Following from these predictions, we first examined the relationship between the size of the calyptra, capsule, and seta across two morphologically diverse moss families (Dicranaceae Schimp. and Grimmiaceae Arn.) to determine whether there is evidence of coadaptation. Secondly, we examined the relationship between these morphological features in Grimmiaceae alone, since it contains both unisexual and bisexual species, to determine whether there is evidence of higher levels of parent–offspring conflict in these features for species that are exclusively outcrossing.

## Materials and Methods

### Morphological data collection

We employed phylogenetic comparative analyses to explore the morphological evolution of characters involved in parent–offspring relationships across mosses in Dicranaceae and Grimmiaceae. These are relatively large families with world-wide distributions that exhibit morphological variation in both the gametophyte and sporophyte phases (Hedenäs & Bisang, 2004; Erzberger *et al.*, 2016). In Dicranaceae, the calyptra ranges in size from extremely small in *Pseudephemerum nitidum* (Hedw.) Loeske (calyptra length = 0.3 mm; Britton, 1913) to large in *Dicranum japonicum* Mitt. (calyptra length = 6 mm; Chien *et al.*, 1999). In this family, the sporophyte length ranges from short in *Cladophascum gymnomitrioides* (Dixon) Dixon (sporophyte height = 0.6 mm; Magill, 1982) to long in *D. japonicum* (sporophyte height = 43 mm; Chien *et al.*, 1999). In Grimmiaceae, calyptrae range in size from small in *Schistidium marginale* H.H. Blom, Bedn.-Ochyra & Ochyra (calyptra length = 0.1 mm; Blom *et al.*, 2016), covering only the operculum, to large in *Coscinodon cribrosus* (Hedw.) Spruce (calyptra length = 2.3 mm; Loeske, 1913), covering half of the capsule or more. In this family, the sporophyte length ranges from short in *Schistidium cryptocarpum* Mogensen & H.H. Blom (sporophyte length = 0.5 mm; Allen, 2005) to long in *Niphotrichum canescens* (Hedw.) Bednarek-Ochyra & Ochyra (sporophyte length = 17 mm; Ochyra *et al.*, 2003). In addition, these families have different sexual systems. While Dicranaceae (*sensu stricto*; La Farge *et al.*, 2002) contains only unisexual species, Grimmiaceae has both unisexual and bisexual species (Ireland, 2007), which enables us to test the following predictions in these two moss families.

Morphological data were collected from the literature for as many Dicranaceae and Grimmiaceae species as possible for sexual system, capsule length, seta length, and calyptra length. Length was often the only quantitative metric reported in the literature

for these structures and thus, these metrics were used as proxies for the surface area of the sporophyte and the surface area of the sporophyte covered by the calyptra. This approach is frequently employed in plant ecology and physiology research, where surface area is either difficult to measure directly or not reported (Schraeder *et al.*, 2021). The sporophyte morphology described in the literature focuses exclusively on mature forms, when seta elongation is complete. When morphological data could not be gathered from the literature, herbarium specimens at the University of Tennessee (TENN) were examined and calyptrae and/or sporophytes were measured. When measuring capsules and setae, we ensured that only mature sporophytes with fully expanded capsules were selected, which occurs only after seta elongation is complete. Calyptra length was also measured from herbarium specimen images archived on the Consortium of Bryophyte Herbaria portal ([bryophyteportal.org](http://bryophyteportal.org)). Species names were standardized for all taxa using the online taxonomic name resolution service (Boyle *et al.*, 2013; accessed 17 December 2023).

### Phylogeny construction

A clustering analysis in PyPHLAWD (v.1.0; Smith & Walker, 2019) was used to retrieve sequences for members of Dicranaceae, Grimmiaceae, and Scouleriaceae S.P. Churchill. The latter family is known to be sister to the other two (Bechteler *et al.*, 2023) and was used as the outgroup. Clusters containing < 25 taxa were discarded to reduce the proportion of missing data. Species present in the morphological dataset that were not recovered by PyPHLAWD in any of the clusters were manually downloaded from GenBank, and the sequence data for these species were added to the appropriate cluster. We filtered these sequence data by (1) excluding sequences from hybrids or taxa that were only identified to the genus level and (2) selecting sequences from published peer-reviewed papers over those not referenced in the literature.

Each cluster of sequences was aligned using MAFFT (7.505-GCC-11.3.0; Katoh & Standley, 2013) with default parameters (FFT-NS-1 strategy). The aligned clusters were concatenated using 2MATRIX (v.1.0; Salinas & Little, 2014) for subsequent phylogenetic analyses. Using IQ-TREE (v.2.2.2.6; Minh *et al.*, 2020), we constructed a maximum likelihood tree based on a concatenated matrix of all the clusters. We used IQ-TREE to estimate the best partitioning scheme for these data and the substitution model for each partition. Additionally, we estimated support at each node using 1000 ultra-fast bootstrap replicates. All analyses were performed on the HPC cluster (Rocky) at the University of Tennessee ([https://wiki.nimbios.org/Rocky\\_User\\_Guide](https://wiki.nimbios.org/Rocky_User_Guide)). To convert the resulting maximum likelihood tree to an ultrametric tree with branch lengths in millions of years, we used TREEPL (v.1.0; Smith & O'Meara, 2012). Fossils of *Tricarinnella crassiphylla* Savoretti, Bippus, Stockey, G.W. Rothwell & Tomescu (Savoretti *et al.*, 2018) and *Campylopodium allonense* Konopka, Herend. & P.R. Crane (Konopka *et al.*, 1998) were used for dating and were applied to the stem nodes of Dicranaceae and Grimmiaceae, respectively. The configuration file for running TREEPL is provided in Supporting Information Table S1.

### Phylogenetic comparative analyses

Ancestral character states were inferred using a maximum likelihood approach. Reconstructions of continuous characters (calyptra length, capsule length, seta length) were performed using the *fastAnc* function and visualized with *contMap* using the R package PHYTOOLS (Revell, 2012). The sexual system was analyzed as a discrete character. We used *fitMK* in PHYTOOLS to identify the most suitable transition rate model by evaluating its predictive performance using the Akaike information criterion (AIC) weights. We tested three Markov models featuring distinct patterns of evolution: 'ER', equal rates; 'SYM', symmetric; and 'ARD', all rates different. We estimated phylogenetic signal for the continuous characters for Dicranaceae and Grimmiaceae separately using Pagel's  $\lambda$  (Pagel, 1999). We calculated these measures using the *phylosig* function in the R package PHYTOOLS. We tested for correlated evolution between all pairs of continuous characters using phylogenetic generalized least squares (PGLS) implemented using *ppls* in the R package CAPER (Orme, 2013). Then, to test whether there was a significant difference in the length of the calyptra, capsule, and seta between unisexual and bisexual species, we conducted a phylogenetic paired *t*-test using the *phyl.pairedt-test* function in the R package PHYTOOLS. In addition, to determine the impact of the sexual system on the correlations between both the calyptra length and capsule length, as well as calyptra length and seta length, we performed a phylogenetic generalized analysis of covariance (ANCOVA) using the R package NLM (Pinheiro *et al.*, 2019).

## Results

### Morphology

Morphological characteristics for 78 species of Dicranaceae and 116 species of Grimmiaceae were obtained from a combination of the literature and TENN herbarium specimens. The calyptrae of five species were measured from bryophyte portal photos of herbarium specimens housed at Duke University (DUKE), University of Michigan (MICH), and The New York Botanical Garden (NY).

Calyptra length of Dicranaceae ( $n = 45$ ) ranged from 0.35 to 8.00 mm, while those of Grimmiaceae species ( $n = 49$ ) ranged from 0.20 to 2.37 mm. In Dicranaceae, the seta length ( $n = 76$ ) and capsule length ( $n = 69$ ) ranged from 1.00 to 45.00 mm and 0.55 to 7.00 mm, respectively. For Grimmiaceae, the seta length ( $n = 104$ ) and capsule length ( $n = 83$ ) ranged from 0.10 to 15.00 mm and 0.50 to 3.00 mm, respectively. The sexual system for 58 species of Dicranaceae and 98 species of Grimmiaceae was determined based on the literature. All 58 Dicranaceae (*sensu stricto*) species were reported in the literature as unisexual. Grimmiaceae contains both types of sexual systems, and 56 species were reported as unisexual and 42 as bisexual in this family (Table S2).

### Phylogenetic analyses

The PyPHLAWD searches yielded 17 clusters for species in Dicranaceae, Grimmiaceae, and Scouleriaceae. From these, seven

clusters comprising 25 or more taxa were retained for the following analyses. The selected clusters include four chloroplast regions (*matK*, *rbcL*, *rps4*, *rps4-trnT*), one mitochondrial region (*nad5*), and two nuclear regions (5.8S ribosomal RNA, 26S ribosomal RNA). The four chloroplast regions – *rps4*, *matK*, *rbcL*, and *rps4-trnT* – consisted of a total of 743, 2612, 1428, and 2103 aligned nucleotides, respectively. Among these, 192, 290, 129, and 327 were identified as parsimony-informative for each region, respectively. The mitochondrial gene *nad5* contained 1951 aligned nucleotides, with 105 that were parsimony-informative. For the nuclear ribosomal DNA genes, 5.8S and 26S, there were 1414 and 1876 aligned nucleotides, respectively, with 495 and 684 that were parsimony-informative. In total, the concatenated matrix included 12 127 aligned nucleotides and contained 243 taxa, with 101 taxa for Dicranaceae, 135 taxa for Grimmiaceae, and seven species of Scouleriaceae. There were 3652 variable sites, of which 2222 were parsimony informative (Table S3). The list of GenBank accession numbers for taxa used in this study is provided in Table S4.

Seven partitions were determined using IQ-TREE, one for each gene region, with the following models identified: 5.8S (TPM2u + F + I + G4), *matK* and *rps4* (TVM + F + I + G4), *rps4-trnT* (GTR + F + I + G4), *nad5* and *rbcL* (TIM + F + I + G4), and 26S (GTR + F + I + G4). The maximum likelihood (ML) tree from the concatenated analysis is shown in Fig. S1, with bootstrap support (BS) displayed on the branches. Dicranaceae and Grimmiaceae were identified as sister groups with 100% BS. Our tree supports a monophyletic Dicranaceae *sensu stricto*, consistent with La Farge *et al.* (2002). Within Dicranaceae *sensu stricto*, four well-supported subclades are recognized. (1) *Dicranoloma* (Renauld) Renauld clade plus *Wardia* Harv. & Hook. (99% BS): This clade supports the paraphyly of *Dicranoloma* with *Braunfelsia* Paris, aligning with La Farge *et al.* (2002) hypothesis of a close relationship between these taxa. (2) Dicranoideae Broth. (100% BS): This clade consists of two strongly supported sister taxa: The *Dicranum* Hedw. clade (97% BS), comprising *Dicranum*, *Chorisodontium* (Mitt.) Broth., *Orthodicranum* (Bruch & Schimp.) Loeske, and *Paraleucobryum* (Lindb. ex Limpr.) Péterfi. The *Holomitrium* Brid. clade (99% BS), including *Holomitrium* and *Eucamptodontopsis* Broth., forms a well-supported group within Dicranoideae. (3) *Dicnemon* Schwägr. plus *Mesotus* Mitt. (86% BS): *Dicnemon* is resolved as monophyletic (100% BS) and sister to *Mesotus*. Together, they form a clade sister to the *Dicranum* and *Dicranoloma* clades. And (4) *Leucoloma* Brid. (100% BS): This genus is resolved as monophyletic and sister to the remaining Dicranaceae, consistent with Gama *et al.* (2015). These results corroborate previous studies (La Farge *et al.*, 2000, 2002).

The ML tree supports the monophyly of Grimmiaceae (100% BS; Fig. S1). Within Grimmiaceae, two primary clades are resolved: (1) a clade comprising *Racomitrium* Brid. *sensu lato* and *Grimmia* Hedw. (69% BS) and (2) a clade including *Schistidium* Brid., *Coscinodon* Spreng., and the remaining *Grimmia* species (67% BS). Recently, *Racomitrium* has been segregated into four genera – *Racomitrium*, *Niphotrichum* (Bedn.-Ochyra) Bedn.-Ochyra & Ochyra, *Codriophorus* P. Beauv., and

*Bucklandiella* Roiv. (Ochyra *et al.*, 2003). This classification is also corroborated by our data. Our results support the monophyly of *Racomitrium sensu stricto*, which includes *R. lanuginosum* (Hedw.) Brid. and *R. pruinosum* (Wilson) Müll. Hal. (100% BS), and the monophyly of *Niphotrichum* (100% BS). Members of *Bucklandiella* and *Codriophorus* are polyphyletic, distributed across several different clades, which is consistent with Larraín *et al.* (2013). Our results corroborate the paraphyletic nature of *Grimmia* as currently defined (Hedderson *et al.*, 2004) and support the monophyly of *Schistidium* (90% BS), which is resolved as sister to *Grimmia*, consistent with Hernández-Maqueda *et al.* (2008) based on chloroplast sequence data. We find that several *Coscinodon* species are scattered within *Grimmia*, which supports Hernández-Maqueda *et al.* (2008) and reinforces their suggestion that *Coscinodon* should be merged with *Grimmia*.

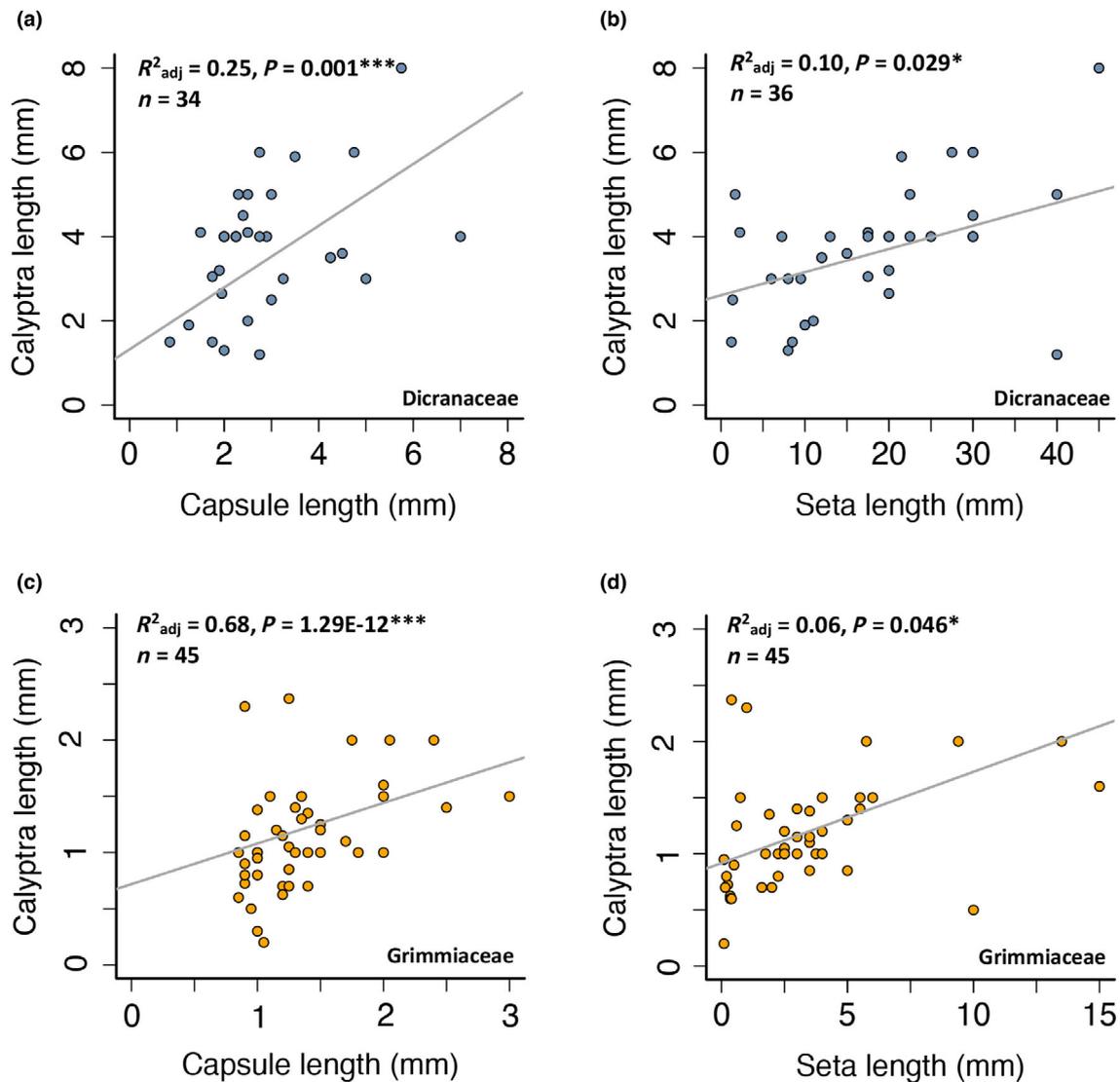
### Phylogenetic signal and ancestral state reconstruction

We conducted tests to determine the phylogenetic signal for the continuous characters for both families separately using Pagel's  $\lambda$  (Pagel, 1999). When  $\lambda = 0$ , it is interpreted as an absence of phylogenetic signal. Conversely,  $\lambda = 1$  indicates that there is a strong phylogenetic signal in the examined characters. In Dicranaceae, seta length had the strongest and significant phylogenetic signal ( $\lambda = 0.52$ ,  $P = 0.001$ ), while capsule and calyptra length showed lower levels of phylogenetic signal that were both not significantly different from the null hypothesis of lambda (Table S5), indicating less morphological similarity among closely related species (Figs S2–S5). In Grimmiaceae, seta length showed the strongest significant phylogenetic signal among the characters tested ( $\lambda = 0.99$ ,  $P = 7.65E-19$ ), indicating a significant resemblance among closely related species. Capsule length exhibited a moderate ( $\lambda = 0.67$ ) and significant phylogenetic signal ( $P = 6.43E-12$ ), similar to the pattern observed for calyptra length, which showed a moderate ( $\lambda = 0.57$ ) and significant phylogenetic signal ( $P = 0.0005$ ; Table S5).

To visualize the morphological variation in these characters across the phylogeny, we performed ancestral state reconstructions for three continuous characters, calyptra length, capsule length, seta length, and one binary character, sexual system. Due to limitations in available data from the literature and herbarium specimens without sporophytes, some species lacked all morphological characters. Consequently, for each reconstruction, the tree was pruned to eliminate the species that were not present in the morphological dataset. As a result of this pruning, each of these reconstructions had a different sample size that included the following number of species (both families and outgroup): calyptra length ( $n = 84$ ), capsule length ( $n = 135$ ), seta length ( $n = 164$ ), and sexual system ( $n = 146$ ; refer to Figs S2–S5; Table S6).

### Phylogenetic comparative analyses

We tested for correlations between continuous characters for Dicranaceae and Grimmiaceae separately using PGLS, as detailed in Table S7, Figs 1, and S6. In both families, we observed significant positive correlations between capsule and seta length, as well



**Fig. 1** Correlation between calyptra length and both capsule length and seta length in (a, b) Dicranaceae (blue-gray) and (c, d) Grimmiaceae (yellow-orange) species. Analyses were performed using phylogenetic generalized least squares (PGLS), with adjusted  $R^2$  values,  $P$ -values ( $P$ ), and sample sizes ( $n$ ) reported. The solid dark-gray line represents the PGLS linear regression fitted to the data. \*,  $P < 0.05$ ; \*\*\*,  $P < 0.001$ .

as between calyptra length and both capsule and seta length. Based on PGLS analyses, the strongest correlations were observed between calyptra and capsule length in both Dicranaceae (Adj  $R^2 = 0.25$ ,  $P = 0.001$ ) and Grimmiaceae (Adj  $R^2 = 0.68$ ,  $P = 1.29\text{E-}12$ ). In Dicranaceae, the correlations between capsule and seta (Adj  $R^2 = 0.09$ ,  $P = 0.016$ ) and also between calyptra and seta (Adj  $R^2 = 0.10$ ,  $P = 0.029$ ) were weak but statistically significant. In Grimmiaceae, the correlation between capsule and seta (Adj  $R^2 = 0.14$ ,  $P = 0.0004$ ) was slightly stronger and more significant than the correlation between calyptra and seta (Adj  $R^2 = 0.06$ ,  $P = 0.046$ ). We also tested for a correlation between the percentage of the capsule covered by the calyptra (calyptra : capsule ratio) and seta length in both families. A calyptra : capsule ratio greater than one indicates that the calyptra is longer than the capsule, whereas a ratio less than one indicates that the

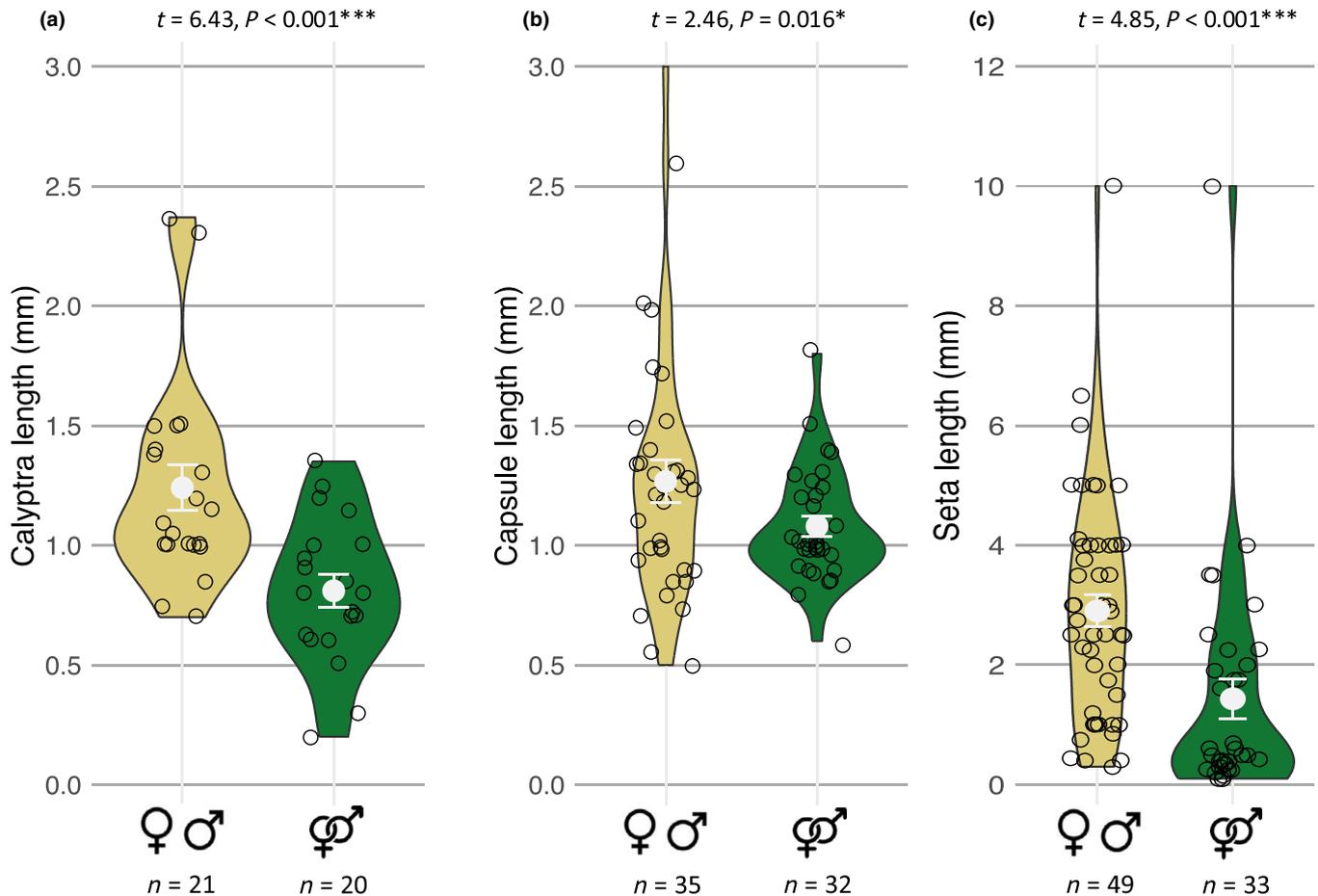
calyptra is shorter than the capsule. Our results show no significant correlation between capsule coverage and seta length in either family (Fig. S6).

We tested for differences between unisexual and bisexual species of Grimmiaceae for continuous morphological characters using phylogenetic paired  $t$ -tests. All three tests demonstrated statistically significant differences between unisexual and bisexual species (Table 1). Notably, seta length exhibited the highest estimated phylogenetic mean difference (phylogenetic mean difference = 2.39,  $P < 0.001$ ), indicating a significantly larger difference in seta size between unisexual and bisexual species. Capsule size (phylogenetic mean difference = 0.60,  $P = 0.016$ ) and calyptra size (phylogenetic mean difference = 0.50,  $P < 0.001$ ) also displayed significant differences between the sexual systems, albeit with smaller mean differences compared to

**Table 1** Phylogenetic paired *t*-tests: comparison of calyptra, seta, and capsule sizes between unisexual and bisexual species in Grimmiaceae.

Morphological characters	Unisexual species	Bisexual species	<i>t</i>	df	<i>P</i>	Phylogenetic mean difference	Log-likelihood
Calyptra	<i>n</i> = 21	<i>n</i> = 20	6.43	38	< 0.001***	0.50	-26.6384
Capsule	<i>n</i> = 35	<i>n</i> = 32	2.46	64	0.016*	0.60	-51.54
Seta	<i>n</i> = 49	<i>n</i> = 33	4.85	79	< 0.001***	2.39	-163.312

Sample sizes (*n*), *t*-values (*t*), degrees of freedom (df), *P*-values (*P*), and phylogenetic mean differences are reported for each comparison. \*, *P* < 0.05; \*\*\*, *P* < 0.001.



**Fig. 2** Violin plots of the distribution of (a) calyptra length, (b) capsule length, and (c) seta length, all in mm, for Grimmiaceae species. Density estimations for unisexual species are on the left of each plot in sand and bisexual species are on the right in green. Open circles display the data from each species. The white solid points represent the mean, and whiskers (error bars) represent the standard error of the mean  $\pm 1$  (SEM). Additionally, the results of Phylogenetic paired *t*-test, with *t*-test (*t*), *P*-value (*P*), and sample sizes (*n*) are reported. All characters exhibited statistically significant differences between unisexual and bisexual species. Additional details can be found in Table 1. \*, *P* < 0.05; \*\*\*, *P* < 0.001.

seta length (Fig. 2). There were also higher levels of variance (SEM) among the unisexual species than among the bisexual species for these two characters (capsule: unisexual = 0.08, bisexual = 0.04; calyptra: unisexual = 0.09, bisexual = 0.07; Fig. 2).

We conducted phylogenetic ANCOVA to test for correlations between calyptra length and capsule length, calyptra length and seta length, and capsule coverage and seta length, while considering the effect of sexual system for Grimmiaceae (Table 2; Fig. 3). For these analyses, the data were log-transformed to account for differences in scale and to improve the model's assumptions. The

results showed a significant correlation between calyptra length and capsule length ( $F = 130.14$ ,  $P < 0.0001$ ; Table 2). However, neither the main effect of the sexual system nor the interaction term between capsule length and sexual system was significant (Fig. 3a). This suggests that while calyptra length and capsule length are significantly correlated; this correlation does not differ between unisexual and bisexual species.

A weak correlation was observed between calyptra length and seta length ( $F = 8.33$ ,  $P = 0.006$ ; Table 2). In contrast to the relationship between calyptra length and capsule length,

**Table 2** Results of phylogenetic analysis of covariance on the correlations between calyptra length and capsule length, calyptra length and seta length, and percentage of capsule covered by calyptra (calyptra : capsule ratio) and seta length in Grimmiaceae, accounting for the sexual system.

Model	Term	numDF	F	P
Calyptra ~ Capsule × Sexual system	Intercept	1	0.12	0.72
	log(Capsule length)	1	130.14	< 0.0001***
	Sexual system	1	2.40	0.13
	log(Capsule length) : Sexual system	1	1.71	0.19
Calyptra ~ Seta × Sexual system	Intercept	1	0.04	0.84
	log(Seta length)	1	8.33	0.006**
	Sexual system	1	0.59	0.44
	log(Seta length) : Sexual system	1	13.25	0.0009***
Percentage of capsule covered ~ Seta × Sexual system	Intercept	1	0.29	0.59
	log(Seta length)	1	6.63	0.01*
	Sexual system	1	2.67	0.11
	log(Seta length) : Sexual system	1	21.41	0.0001***

Degrees of freedom of term (numDF), *F*-value (*F*), and *P*-value (*P*) are reported. \*, *P* < 0.05; \*\*, *P* < 0.01; \*\*\*, *P* < 0.001.

described previously, the interaction term between seta length and sexual system was significant ( $F = 13.25$ ,  $P = 0.0009$ ; Fig. 3b), indicating that the correlation between calyptra length and seta length differs between unisexual and bisexual species (Table 2). In addition, we observed a weak correlation between the percentage of capsule covered by the calyptra and seta length ( $F = 6.63$ ,  $P = 0.01$ ; Table 2). The phylogenetic ANCOVA revealed a significant interaction between seta length and sexual system ( $F = 21.41$ ,  $P = 0.0001$ ; Fig. 3c). This indicates that the relationship between seta length and capsule coverage differs significantly between the two sexual systems.

## Discussion

We present a comprehensive analysis focused on the morphological evolution of structures involved in parent–offspring coadaptation and conflict that takes into account phylogenetic relatedness using Dicranaceae and Grimmiaceae as study systems. We have two main findings. (1) We observed significant positive correlations in both families between calyptra length and capsule length, as well as calyptra length and seta length, which is evidence supporting coadaptation between these parental gametophyte and offspring sporophyte structures. (2) Our study revealed significant differences between the morphology of unisexual and bisexual Grimmiaceae species. Unisexual species have longer calyptrae, longer capsules, and taller setae than bisexual species. These morphological differences may reflect higher levels of parent–offspring conflict for species that are exclusively outcrossing. Additionally, we found significant differences in the relationships between calyptra length and seta length, as well as the percentage of the capsule covered by the calyptra between species with different sexual systems.

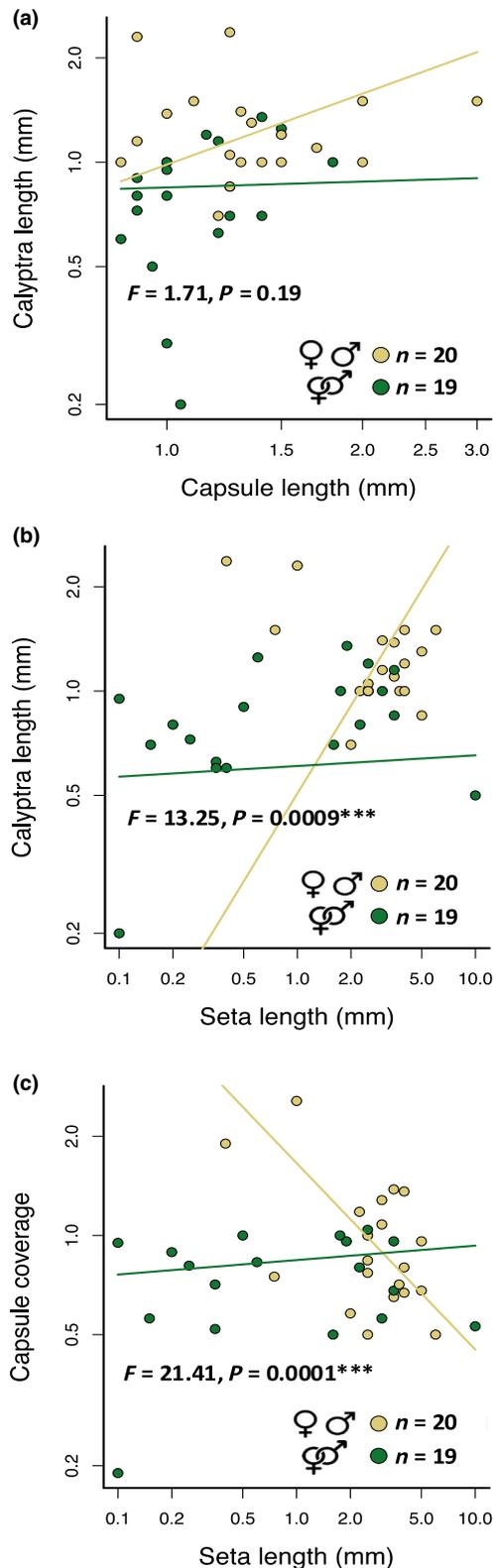
### Coadaptation and morphological coordination

The coadaptation hypothesis predicts that parents and their offspring have shared interests, which result in coadapted strategies that positively impact offspring fitness (Wolf & Hager, 2006). In flowering plants, this has been studied most often during seed

development, focusing on the diploid parent and triploid endosperm (reviewed in Xu & Zhang, 2023; Boccaccini *et al.*, 2024). The endosperm acts as a nourishing, placenta-like tissue for the developing embryo and directly impacts offspring development and fitness. Garcia *et al.* (2005) found evidence for coadaptation between the maternal integuments and the offspring endosperm, wherein integument cell elongation and endosperm growth coordinate to influence seed size and thus the size of the developing embryo.

In mosses, evidence of coadaptation has been observed between the parental gametophytes and offspring sporophytes. In an analysis of over 350 species from the Funariales M. Fleisch, Polytrichales Cavers, and Pottiales M. Fleisch, Hedderson & Longton (1995) found that taller gametophytes produced sporophytes with well-developed dispersal mechanisms (opercula and peristome teeth) as well as many small spores. They also analyzed seta length, but did not find any significant relationships or evidence of coadaptation for this feature. Studying 27 diverse species, He *et al.* (2023) found a significant positive correlation between gametophyte and sporophyte biomass. However, these analyses were not carried out using phylogenetic comparative methods (Harvey & Pagel, 1991) and thus these findings could potentially be driven by species relatedness rather than evolutionary independent coadaptation. Taking phylogeny into account, Crawford *et al.* (2009) found a correlation between species with longer setae also having small spores, which could reflect coadaptation for increased dispersibility.

Our results provide the first evidence supporting coadaptation between the parental calyptra and offspring capsule in mosses. The development of the capsule occurs from the apical region of the sporophyte, which is initially surrounded by the gametophytic calyptra (Crum, 2001; Budke, 2019). The physical presence of the calyptra protects the apical region from environmental stresses, specifically dehydration (Lorch, 1920; Budke *et al.*, 2013). The calyptra also plays a crucial role in capsule maturation (Zielinski, 1909). Removal of the calyptra during early development results in undeveloped or malformed capsules (Herzfelder, 1923). The strong positive correlation between calyptra length and capsule length we observed in both families



(Fig. 1a,c) supports the hypothesized morphological coadaptation between these structures. This correlation does not significantly differ between unisexual and bisexual species in the Grimmiaceae (Fig. 3a); thus, these data do not support the

**Fig. 3** Scatterplots and regression lines illustrating the relationships between (a) calyptra length and capsule length, (b) calyptra length and seta length, and (c) percentage of capsule covered by the calyptra (calyptra : capsule ratio) and seta length in Grimmiaceae species. Each point represents an individual species, untransformed and displayed on a log scale, with unisexual species in sand and bisexual species in green. The regression lines represent model predictions from the phylogeny-corrected analysis of covariance (PANCOVA) for each sexual system. The PANCOVA models tested: (a) the interaction between log capsule length and sexual system on log calyptra length; (b) the interaction between log seta length and sexual system on log calyptra length; and (c) the interaction between log seta length and sexual system on log percentage of coverage.  $F$ -values ( $F$ ) and  $P$ -values ( $P$ ) are reported for each model. Sexual system did not significantly affect the relationship between calyptra length and capsule length, but it significantly influenced the relationships between calyptra length and seta length, and between capsule coverage and seta length. Further details are provided in Table 2.  $^{***}, P < 0.001$ .

parental conflict hypothesis influencing the relationship between these features. The functional integration between the calyptra and capsule may be crucial for sporophyte fitness, and thus any advantages a sporophyte might gain by having a longer capsule, such as a larger surface area enabling it to extract more resources from the gametophyte, would be limited by the size of the calyptra. Thus, increases in capsule size would only be successful and thus confer fitness advantages, such as increases in spore production, if concurrently linked with increases in calyptra size. Consequently, these two structures may be unable to evolve independently.

We do not find strong evidence of coadaptation between the parental calyptra and offspring seta. These features are positively correlated in both families, but the relationship is weak (Fig. 1b, d), especially when compared to the relationship between the calyptra and the capsule (Fig. 1a,c). The seta meristem is located below the apical region and during early development it is also covered by the calyptra. Similarly, the calyptra also protects the seta meristem from dehydration (Lorch, 1920; Budke *et al.*, 2013) and is required for normal seta development (Herzfelder, 1923). When the calyptra is removed, the seta meristem produces an indeterminate, obconic-shaped stalk that is wider and shorter than when the calyptra is present (French & Paolillo, 1976). Seta length has an indirect impact on dispersal and fitness by influencing the distance the capsule is elevated from the substrate. Thus, the functional integration between the calyptra and the seta may be less critical compared to the calyptra and capsule. Consequently, increases in seta length resulting in a larger sporophyte surface area, which enables the acquisition of more resources from the gametophyte, may not necessitate concurrent increases in calyptra length.

#### Parent–offspring conflict and a morphological arms race

The parental conflict hypothesis posits that under conditions of resource scarcity, there will be selective pressure favoring morphological adaptations that increase the parent's ability to preserve resources, as well as morphologies that enhance the offspring's capacity to obtain resources (Shaanker *et al.*, 1995). In bryophytes,

the sporophyte remains physically connected to the parental gametophyte throughout its life and relies on nutrients from the parent (Ligrone & Gambardella, 1988). Parental gametophytes have limited resources to allocate between their own survival and the survival of their offspring sporophytes; thus, a lifelong conflict over resources arises (Haig & Wilczek, 2006; Haig, 2012). Sporophyte transpiration may influence the pull of resources from the gametophyte into the sporophyte and ultimately play a role in parent–offspring conflict. Bopp & Stehle (1957) found that water moved 1.3× faster through the sporophytes of *Funaria hygrometrica* Hedw. when the calyptra was removed, exposing the surface area of the sporophyte beneath, compared to when it was present. Whitaker & Budke (2021) replicated these experiments under controlled laboratory conditions for both *F. hygrometrica* and *Physcomitrium pyriforme* (Hedw.) Hampe. They found that calyptra removal increased sporophyte transpiration rates for both species over 2× compared to individuals where the calyptra was not removed, confirming the findings of Bopp & Stehle (1957). The ability of the calyptra to slow water movement is likely due to the waxy cuticle that covers its exterior (Budke *et al.*, 2011). Additionally, species with taller sporophytes tend to have thicker calyptra cuticles (Budke & Goffinet, 2016). This adaptation is likely to reduce sporophyte transpiration, which could decrease the resources extracted by the offspring sporophyte from the parental gametophyte.

On the other side of this tug-of-war, the offspring could extract more resources from the parent by increasing capsule and/or seta size. This results in surface area increases and also elevates the capsule above the still air of the boundary layer, thus increasing the transpirational pull of the sporophyte. However, our knowledge about the influence of capsule and/or seta size on resource acquisition is limited due to a lack of experimental research.

The ability to self-fertilize is a key difference between unisexual and bisexual species (Eppley *et al.*, 2007). In bisexual gametophytes, when self-fertilization occurs, the resulting sporophyte inherits all of its genetic material from a single parent, making it 100% related to the attached gametophyte (Solbrig, 1976). By contrast, in species with unisexual gametophytes, the sporophyte can be at most 50% related to the attached parental gametophyte (Haig & Wilczek, 2006). This variation in genetic relatedness is predicted to result in different levels of parent–offspring conflict. Haig (2012) posited that bisexual mosses should exhibit lower levels of conflict than unisexual species, with sporophytes of bisexual species having smaller capsules and shorter setae than those of unisexual species. Our study is the first to test this prediction with empirical data.

Our study found significant differences between the morphology of unisexual and bisexual Grimmiaceae that could indicate a morphological arms race driven by higher levels of conflict in unisexual species (Haig, 2012). We found significantly longer calyptrae, longer capsules, and longer setae (Fig. 2), as well as higher levels of variation in the calyptra and capsule morphology in unisexual species. A longer calyptra results in more sporophyte surface area covered, thus decreasing the transpirational pull of resources from the gametophyte (Bopp & Stehle, 1957; Whitaker & Budke, 2021). On the opposite side, longer capsules and taller

setae result in sporophytes with larger surface area potentially increasing the transpirational pull of resources from the gametophyte. The higher levels of morphological variation in the unisexual species could indicate ongoing selection driven by the parent–offspring conflict.

We also found significant differences in the relationships between calyptra length and seta length, as well as the percentage of the capsule covered by the calyptra between species with different sexual systems (Fig. 3b,c). Unisexual species with longer calyptrae also had longer setae, which indicates that both sides of the parent–offspring conflict are enhancing their abilities to impact the transpirational pull of resources from the parental gametophyte. Unisexual species with longer setae also had a lower proportion of their capsule length covered by the calyptra. This could indicate that the two sides of this morphological arms race are not balanced, with the offspring increasing the transpirational pull via larger seta surface area outcompeting the capacity of the calyptra to decrease the transpirational pull of the capsule by covering a larger percentage of its length. Calyptra development is completed and its mature morphology is achieved before detaching from the leafy gametophyte (Budke *et al.*, 2012). In many species, this separation is driven by the activity of the dividing seta meristem and elongating seta (Budke, 2019). This may enable the offspring sporophyte to shorten the time available for calyptra development, thus limiting calyptra size. Undertaking experimental studies across an array of morphologically diverse taxa will enable us to expand our understanding of the factors that influence sporophyte transpiration in mosses.

### Spore dispersal and sexual system

The main function of the sporophyte is to produce and disperse spores (Keddy, 1981). Positive correlations between plant height and seed dispersal have been demonstrated in over 500 species of flowering plants (Soons *et al.*, 2004; Travis *et al.*, 2010; Thomson *et al.*, 2011). In bryophytes, Johansson *et al.* (2014) demonstrated that long setae enable spores to catch the wind more effectively, potentially enhancing long-distance dispersal. Selection should favor tall sporophytes that are elevated above the gametophyte and the still air of the boundary layer, facilitating spore dispersal over long distances (Vitt, 1981). Mosses can achieve taller sporophytes via longer setae and/or longer capsules (Rose *et al.*, 2016). The significant difference in seta length between unisexual and bisexual species suggests that selective pressures may favor longer setae in unisexual species, potentially enhancing spore dispersal and increasing outcrossing opportunities.

Capsule morphology also plays a critical role in spore dispersal. In many mosses, spore release is coordinated by hygroscopic movements of the peristome teeth (Patterson, 1953; Gallenmüller *et al.*, 2018). Elongated, curved capsules also facilitate spore dispersal by turbulent air currents causing vibrations (Johansson *et al.*, 2014). However, our understanding of the role of capsule size in dispersal is limited. Larger capsules could be advantageous for unisexual species by increasing sporophyte height, thereby enhancing opportunities for outcrossing.

Plants exhibit a wide variety of sexual systems ranging from unisexual to bisexual (Barrett, 2002), which have been found to be correlated with life-history traits, such as flower (Vamosi *et al.*, 2003) and fruit (Muenchow, 1987) morphology. In mosses, unisexuality has been found to be phylogenetically correlated with small spores (Crawford *et al.*, 2009). This suggests that evolutionary shifts to separate sexes may be enhanced by small spores, which may play a role in dispersal (Zanatta *et al.*, 2016), thus increasing the likelihood of male and female gametophytes being located in close proximity. Studies have also found that unisexual mosses have taller gametophytes (During, 2007; Crawford *et al.*, 2009). These authors hypothesized that taller gametophytes enhance spore dispersal, especially in acrocarpous species, where the sex organs and later the sporophyte are located at the tip of the main stem. Our study extends this understanding by demonstrating that unisexual moss species also have larger calyptrae, capsules, and setae, which are also likely to enhance reproductive success via increased spore dispersal, particularly in acrocarpous species.

## Conclusion

Due to their unique lifecycle, bryophytes experience a lifelong relationship between the parental gametophyte and offspring sporophyte that influences the coordination and conflict over resources. This study investigated the morphological evolution of structures involved in this relationship using two moss families, Dicranaceae and Grimmiaceae. Our findings provide evidence of morphological coadaptation between the parental gametophyte and offspring sporophyte, including significant positive correlations between parental gametophyte and offspring sporophyte morphology. Our study also provides evidence of parent–offspring conflict, suggesting the evolution of both parental adaptations to minimize and offspring adaptations to maximize resource acquisition. Additionally, the significant differences in the relationships between morphological features for species with different sexual systems align with predictions of higher parent–offspring conflict in outcrossing species. This study highlights the complex interplay between morphological evolution, sexual system, and parent–offspring relationships in bryophytes. Further investigation into the evolution of the developmental mechanisms underlying these morphological patterns will provide deeper insights into the parent–offspring relationships in plants.

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## Competing interests

None declared.

## Author contributions

MN conducted the data curation, formal analysis, investigation, wrote the original draft of the manuscript, and contributed to visualization. JMB contributed to conceptualization, funding acquisition, and supervision. MN and JMB reviewed and edited the manuscript.

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## Data availability

The data that support the findings of this study are available within the paper (see the [Results](#) section; Tables 1, 2; Figs 1–3) and its Supporting Information (Tables S1–S7; Figs S1–S6).

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## Supporting Information

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

**Fig. S1** Maximum likelihood tree using seven genes, obtained from IQ-TREE.

**Fig. S2** Ancestral character state reconstruction of calyptra length.

**Fig. S3** Ancestral character state reconstruction of capsule length.

**Fig. S4** Ancestral character state reconstruction of seta length.

**Fig. S5** Ancestral character state reconstruction of sexual system.

**Fig. S6** Correlations between seta length and both capsule length and percentage of capsule covered by calyptra in Dicranaceae and Grimmiaceae.

**Table S1** The TREEPL configuration file for constructing the time-calibrated ultrametric tree.

**Table S2** List of morphological characters and sexual systems of species in Dicranaceae, Grimmiaceae, and Scouleriaceae.

**Table S3** Number of taxa, alignment length, number of variable sites, parsimony-informative sites, and best models for each gene used to generate the maximum likelihood phylogeny.

**Table S4** GenBank accession numbers and voucher specimen for taxa used in this study.

**Table S5** Results of tests for phylogenetic signal of the morphological characters used in the study using Pagel's lambda ( $\lambda$ ) values.

**Table S6** Three models of character evolution (ARD, all rates different; ER, equal rates; SYM, symmetric) were tested for fit using the AIC.

**Table S7** Phylogenetic generalized least-squares (PGLS) models testing the correlation between each possible pair of morphological characters.

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