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The mitochondrial genomes of bryophytes

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Abstract

In contrast to the highly variable mitogenomes of vascular plants, the composition and architecture of mitogenomes within the three bryophyte lineages appear stable and invariant. Currently, complete mitogenomes are available from 113 bryophyte accessions of 71 genera and 28 orders. Liverworts and mosses hold a rich mitochondrial (mt) gene repertoire among land plants with 40–42 protein-coding genes, whereas hornworts maintain the smallest functional gene set among land plants, of only around two dozen protein-coding genes, with the majority of ribosomal genes pseudogenized and all cytochrome c maturase genes lost. The rRNA and tRNA genes are also conserved and rich in mosses and liverworts, whereas subject to patchy losses in hornworts. In contrast to the conserved gene set, intron content varies significantly with only one intron shared among the three bryophyte lineages. Bryophytes hold relatively compact mitogenomes with narrow size fluctuations. Among the three bryophyte lineages, intergenic spacers and repeat content are smallest in mosses, largest in hornworts, and intermediate in liverworts, mirroring their size differences and levels of structural dynamics among the three lineages. Mosses, with the least repeated sequences, show the most static genome structure; whereas hornworts, with a relatively large set of repeated sequences, experience 1–4 rearrangements; liverworts, with intermediate repeat levels, see only one structural variant that requires two inversions to gain collinearity with the mitogenome of other liverworts. Repeat sequences were evoked to explain the mt gene order rearrangements in hornwort and liverwort mitogenomes; with the latter also supported by sequencing read evidence, which suggests that the conserved mitogenome structure observed in bryophyte lineages might be shaped by low repeat recombination level, and/or along with the intensified nucleus' surveillance. Mitochondrial RNA editing is abundant in hornworts, with medium frequency and high variation in liverwort species, and generally limited in mosses, reflecting the diversity of nuclear encoded PPR proteins that are functionally related to RNA editing processes.

Keywords: Bryophytes, mitochondrial genomes, gene content, introns, recombination

Introduction

Vascular plant mitogenomes are well-known for their big size, dynamic genome structure, conserved gene content, and RNA editing (Palmer & Herbon 1988; Palmer *et al.* 2000). A typical vascular plant mitogenome holds an average size of ~470 Kb, encodes 20~42 protein coding genes, 10~20 tRNAs and three rRNAs, which altogether take about 25–30 Kb of the total mitogenome length, leaving ~80% of the mitogenome as non-coding regions (Dong *et al.* 2019a). These non-coding regions harbor abundant intracellular transferred sequences from either nuclear or plastid genomes (Alverson *et al.* 2010), horizontal transferred sequences from mitochondrial (mt) DNAs of other organisms (Rice *et al.* 2013), anonymous sequences of unknown origin (Mower *et al.* 2012), and numerous repeated sequences (Dong *et al.* 2018b). Many studies have shown that repeated sequences in intergenic spacers could mediate intragenomic and intermolecular recombinations in plant mitochondria, which could have contributed to the structural dynamics of vascular plant mitogenomes (Lilly & Havey 2001; Darracq *et al.* 2010; Sloan 2013; Skippington *et al.* 2015).

Relationships among the three bryophyte lineages (mosses, liverworts, and hornworts) and vascular plants has long been controversial, with up to nine competing topologies being identified (reviewed by Puttick *et al.* 2018). Although nuclear (Morris *et al.* 2018; Puttick *et al.*, 2018; One Thousand Plant Transcriptomes Initiative 2019) and plastid (Gitzendanner *et al.* 2017; Sousa *et al.* 2020) evidences tend to support bryophytes as a monophyletic group, mitochondrial data seem preferring a paraphyletic bryophyte, with the three lineages, liverworts, mosses, and hornworts form a grade, and give rise to the vascular plant clade (Liu *et al.* 2014a; this study). Comparing to vascular

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plants, bryophyte lineages show distinctively stable mitogenome size and gene content (Liu *et al.* 2012). Bryophyte mitogenomes demonstrate much smaller sizes (100–242 Kb) with narrow size fluctuations compared with that of the large and variable mitogenomes of vascular plants. Each bryophyte lineage holds a constant protein-coding gene (PCG) set with very small variations (Liu *et al.* 2014b; Dong *et al.* 2018a; Dong *et al.* 2019b). Liverworts and mosses hold the most complete mt gene repertoire of 40–42 PCGs among land plants, whereas hornwort mitogenomes show distinctly reduced numbers of functional genes, with most ribosomal protein genes lost or pseudogenized (Dong *et al.* 2018a). The intron content is also conserved within each bryophyte lineage, whereas highly variable among them (Mower *et al.* 2012). These genome features might suggest that each bryophyte lineage follows its own evolutionary path.

One of the most prominent features of bryophyte mitogenomes is that they show astoundingly conserved structural evolution in each lineage (Liu *et al.* 2014b). The hornwort mitogenomes show limited structural variations among the sequenced species representing four orders, since they can be reconciled with each other by just one to four inversions and translocations (Li *et al.* 2009; Xue *et al.* 2009; Dong *et al.* 2018a; Villarreal *et al.* 2018). The moss mitogenomes (as of Sep. 2020), spanning the moss tree of life, kept the same gene order. The liverwort mitogenomes also show exactly the same genome structure (Wang *et al.* 2009; Ślipiko *et al.* 2017; Dong *et al.* 2019a), with only one exception: the mitogenome of *Gymnomitrion concinnatum* (Gymnomitriaceae, Jungermanniales), which requires two inversions to align with other liverwort mitogenomes (Myszczyǹski *et al.* 2018).

Repeated sequences play essential roles in the structural dynamics of plant mitogenomes (Palmer *et al.* 2000) and number of repeats appears to be positively correlated with number of structural rearrangements (Liu *et al.* 2014b). The static mitogenome structure of mosses could possibly be explained by the absence of repeated sequences within intergenic spacers (Liu *et al.* 2014b). Hornwort mitogenomes contain the most repeat sequences among bryophytes, i.e., ~46 repeats (>50 bp, >85% BLAST identity) of small and medium size, which might explain their relatively liable mitogenome structure. Liverwort mitogenomes have an average amount of 20 pairs of repeated sequences in their intergenic spacer regions, ranging from nine pairs in *Treubia lacunosa* to 59 pairs in *Marchantia paleacea*, apparently holding the potential for structural rearrangements. The only known structural mitogenome variant, found in *Gymnomitrion concinnatum* (Myszczyǹski *et al.* 2018), holds 45 repeats. Repeat abundance and structural dynamics seem to be positively correlated among bryophyte lineages on the large. Here we reviewed the mt gene content and the structure of all the available 113 bryophyte mitogenomes (as of Sep. 2020) spanning 71 genera, and 28 of the total 54 orders of bryophytes (Goffinet & Shaw 2009) (**Supplementary Table S1)**, to summarize the peculiar features of bryophyte mitogenomes, including gene content, genome structure, and RNA editing.

Bryophyte mitochondrial genome size

Mitogenomes of vascular plants are highly variable in size—i.e., from ~66 Kb in *Viscum scurruloideum* (Skippington *et al.* 2015) to 11.3 Mb in *Silene conica* (Sloan *et al.* 2012). By contrast, mitogenomes within each of the three bryophyte lineages exhibit rather narrow size variation (Dong *et al.* 2019a). The 49 mitogenomes from 34 moss genera (e.g., Liu *et al.* 2014b; Vigalondo *et al.* 2016) are typically smaller (101–141 Kb, median ~107 Kb) than those of other land plants (Mower 2020). The six mitogenomes of hornworts, sampled from four genera (Li *et al.* 2009; Xue *et al.* 2009; Dong *et al.* 2018a; Villarreal *et al.* 2018; Gerke *et al.* 2019; Frangedakis *et al.* 2020), are larger in size (185–242 Kb, average, ~208 Kb). The 56 mitogenomes, from 33 liverwort genera (Oda *et al.* 1992; Wang *et al.* 2009; Ślipiko *et al.* 2017; Myszczyǹski *et al.* 2018; Dong *et al.* 2019a), are intermediate in size between those of mosses and hornworts (142–187 Kb, median ~164 Kb). The mitogenome size variation within the three bryophyte lineages is much narrower compared to that in angiosperms (i.e., ~200-fold range); however, the evolutionary history of any of these three major lineages spans much longer than that of angiosperms (Morris *et al.* 2018). The rather stable mt exome is consistently the smallest component of the bryophyte mitogenomes (~34 Kb). Consequently, changes in total mitogenome size are shaped by the variation of size in intergenic spacers, which is consistent with what has been seen in vascular plants (**Fig. 1, Supplementary Table S2**).

Figure 1

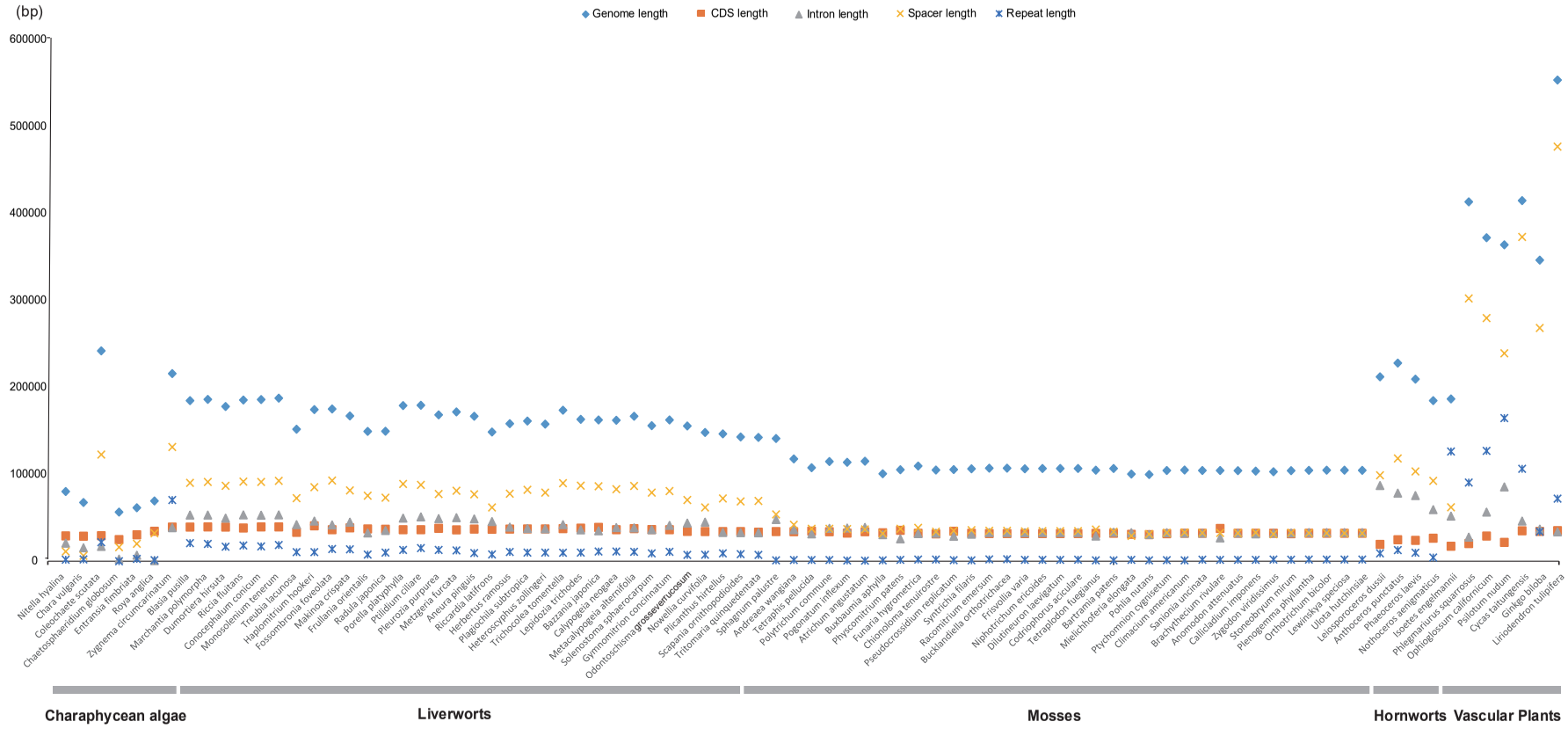

FIGURE 1. Comparison of mt genome size, intergenic, intronic, exonic, and repeat contents among 86 selected land plant genera. The order of the taxa is sorted by the phylogeny as shown in Figure 2.

Phylogenetic reconstructions based on mitogenome sampling at accession-(**Supplementary Figs. S1a and S1b**), species-(**Supplementary Figs. S2a and S2b**), and genus-(**Supplementary Figs. S3a and S3b**) level generally yielded consistent topology in deep relationships. We thus performed ancestral state reconstruction of genome size using the genus-level phylogeny. The obvious expansions of bryophyte mt intergenic spacers and introns, compared to that of the green algae, might posit such event in the common ancestor of embryophytes (**Supplementary Fig. S4**). Considering that recent phylogenomic reconstructions propose Zygnematales (Gitzendanner *et al.* 2018; Cheng *et al.* 2019; One Thousand Plant Transcriptomes 2019) as the sister group of extant embryophytes, and that some mitogenomes from *Zygnema* have already demonstrated distinct genome size expansions (**Fig. 1, Supplementary Table S2**), the first mitogenome expansion in land plants might have predated the split of embryophytes from its streptophyte algal ancestors, the second and the most significant expansion started in the ancestor of vascular plants (**Supplementary Fig. S4**). These genome size expansions are mainly caused by the accumulation of repeat sequences and AT-rich intergenic spacers, mobile elements and introns (Burger *et al.* 2003). Although there may be some trade-offs between the genome size expansion and energy allocations of increased DNA synthesis pressure, plant mitogenome expansion might possibly hold potential for complex regulatory roles, as has been indicated for nuclear non-coding DNAs (Bennetzen 2000; Burger *et al.* 2003; Kumar *et al.* 2010), to cope with the stress of energy demands and repair the damage of the reactive oxygen species (ROS) released in respiration and/or oxidative phosphorylation during plant terrestrialization.

Bryophyte mitochondrial gene content

Mitogenomes of vascular plants encoded a variable set of genes—i.e., 13–64 (Petersen *et al.* 2015), excluding duplicated genes and open reading frames (ORFs), whereas the mitogenomes of the three bryophyte lineages are conserved in gene content (Liu *et al.* 2012). The 49 mitogenomes from 34 moss genera (e.g., Liu *et al.* 2014b; Vigalondo *et al.* 2016) constantly encode 40 protein-coding mt genes (PCGs), 24 tRNAs, and three rRNAs. The 56 mitogenomes from 33 liverwort genera (Oda *et al.* 1992; Wang *et al.* 2009; Ślipiko *et al.* 2017; Myszczyǹski *et al.* 2018; Dong *et al.* 2019a) hold a relatively larger gene set, including 39–43 PCGs, 25–27 tRNAs, and three rRNAs. The six mitogenomes of hornworts sampled from four genera (Li *et al.* 2009; Xue *et al.* 2009; Dong *et al.* 2018a; Villarreal *et al.* 2018; Gerke *et al.* 2019; Frangedakis *et al.* 2020) encoded a reduced gene set with 21–23 PCGs, 18–23 tRNAs, and 3 rRNAs. Around ten protein-coding genes are pseudogenized in each of the hornwort mitogenomes, most of them being ribosomal protein genes (**Figs. 2 and 3**).

Mt gene content represents the most conserved component across embryophytes, even streptophytes, despite independent gene losses in some lineages (**Figs. 2 and 3**). Mitogenomes of Liverworts and mosses hold a highly similar gene repertoire, with only a few exceptions. The *nad7* gene is pseudogenized in all sequenced hornworts and all liverwort genera, but for *Haplomitrium*; whereas, this gene appears to be independently pseudogenized in four moss lineages: *Tetraphis*, *Buxbaumia*, *Pohlia*, and *Mielichhoferia*. Two ribosomal protein genes (*rps8* and *rps10*) and two tRNA genes (trnN*guu* and trnS*gcu*), that are universally present in liverwort mitogenomes, are absent in all of mosses. One tRNA gene (trnT*guu*) that occurred in the mosses and the *Phaeoceros* and *Leiosporoceros* hornworts, is absent from all liverworts, but for *Haplomitrium*. Among liverworts, *Treubia* possesses the smallest set of mt PCGs (i.e., 39) and lacks all Cytochrome c genes (*ccmB*, *ccmC*, *ccmFN* and *ccmFC*) (Liu *et al.* 2012). A complete set of Cytochrome c genes is also lacking from all hornwort mitogenomes (Dong *et al.* 2018a), lycophytes (Grewe *et al.* 2011), and algae (Mower *et al.* 2012). The parallel loss of Cytochrome c genes in various plant lineages might suggest functional cooption by an alternative heme attachment pathway (Guo *et al.* 2017), rather than their transfer to the nucleus, based on transcriptome evidence and increasing genome resources available (Mower 2020). In contrast to liverworts and mosses, hornwort mitogenomes are featured by heavy gene losses and pseudogenizations (Dong *et al.* 2018a). This high number of retained and shared pseudogenes suggests that their deletion rate is low, and hence that gene relicts may be maintained for long period in hornwort mitogenomes. As suggested by Xue *et al.* (2009), the long retention period of these pseudogenes may not be simply explained by conservative evolution of hornwort mitogenomes but may rather indicate that these pseudogenes possibly play some functional role, such as on gene regulations, as demonstrated in rice (Guo *et al.* 2009).

Figure 2

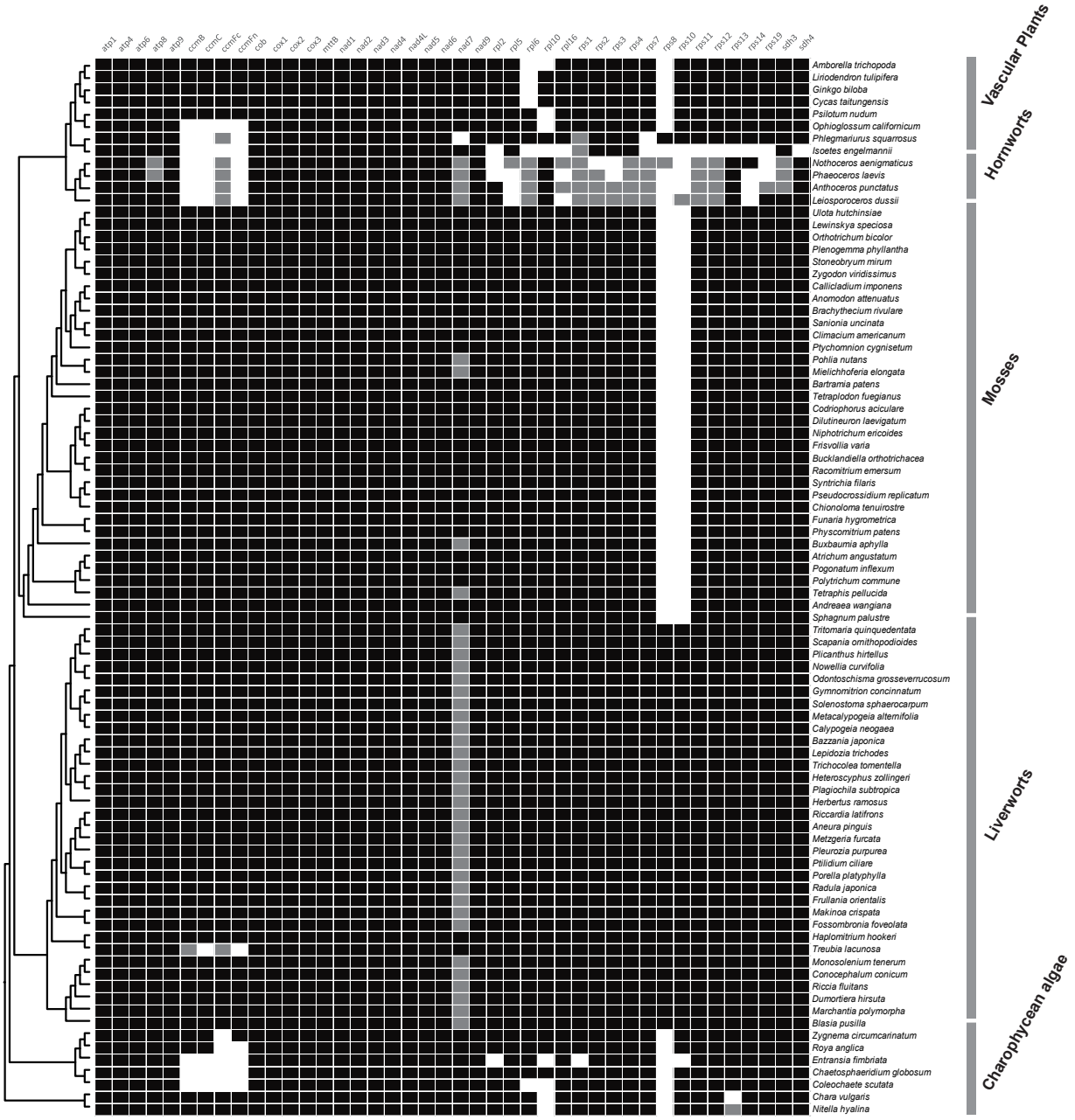

FIGURE 2. Protein gene content among land plants. Presence, absence, and pseudogenization of genes were indicated in black, white, and gray, respectively. The phylogeny is inferred with IQ-TREE2 based on the concatenated amino acid sequences of 41 conserved mitochondrial protein coding genes (see **Supplementary Figs. S3a and S3b**).

Bryophyte mitochondrial intron content

Vascular plant mitogenomes contain a large and variable set of introns, ranging in numbers from three (*Viscum album,* Petersen *et al.* 2015) to 37 (*Selaginella moellendorffii*, Hecht *et al.* 2011) (average, ~21). Generally, the intron content is much more conserved within, rather than among, major lineages of land plants (Mower *et al.* 2012). Bryophyte mitogenomes tend to hold more introns than vascular plants (generally 20–25) do. There are 28–38 (average, ~33) introns in hornworts (Dong *et al.* 2018a), 26–29 (average, ~27) in mosses (Liu *et al.* 2014b), and 23–32 (average, ~28) in liverworts, if the two vestigial introns of the pseudogenized *nad7* are included. The intron set is conserved within each bryophyte lineage and varies significantly among the three lineages (**Fig. 4**). Mosses hold a stable intron set, with intron variation only observed in the *rrnS* gene—i.e., group I intron *rrnSi839g1* is only present in a few lineages

(*Atrichum*, *Pogonatum*, *Tetraphis*, *Andreaea*, and *Sphagnum*). Liverworts hold a relatively variable intron set, with intron variations observed in six genes—i.e., *atp1*, *cob*, *cox1*, *nad4L*, *rrn18*, and *rrn26*. Complex thalloid liverworts harbor on average more introns (~32) than simple thalloid (~25) and leafy liverworts (~23). Hornworts also hold a large and dynamic intron set, with intron variation observed in 10 genes—i.e., *atp1*, *atp9*, *cob*, *cox1*, *cox2*, *cox3*, *nad4*, *nad5*, *rps3*, and *rps13*. Liverwort mitogenomes share four and six introns with mosses and hornworts, respectively. Hornwort mitogenomes have 13 introns in common with mosses. However, only one group II intron (*atp9i95g2*) is shared among all three bryophyte lineages. This low among-lineage intron sharing level might lend additional support to the purported ancient divergence time of the three lineages, as would do the independent intron gains and losses during the evolutionary history of the three bryophyte lineages.

Figure 3

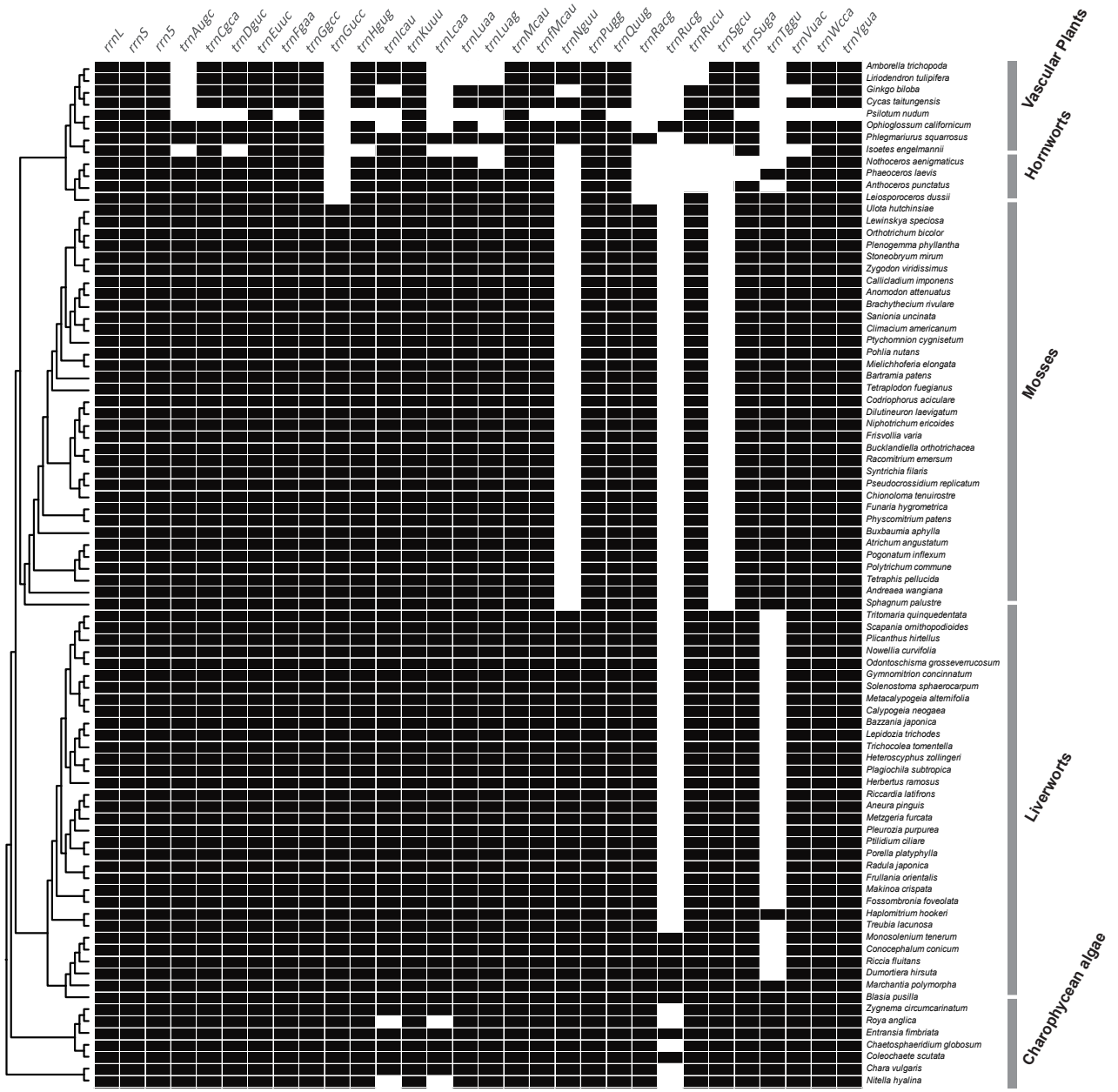

FIGURE 3. RNA gene content among land plants. Presence, and absence of RNA genes were indicated in black, and white, respectively.

Figure 4

FIGURE 4. Intron content among land plants. Presence, absence, pseudogenization, and trans-splicing of introns were indicated in black, white, gray, and cross, respectively.

Based on our phylogeny reconstructed from a concatenated amino acid dataset of 41 mt protein-coding genes (**Fig. 4, Supplementary Figs. S3a and S3b**), introns appear to flourish in the ancestor of the embryophytes, given that the streptophyte algae sister lineages contain only a few introns (**Supplementary Fig. S5**). Lineages of mosses and liverworts sister to species-rich clades tend to hold a more complete set of genes. The grade composed of liverwort lineages Haplomitriopsida and Marchantiopsida contains more introns than the species-rich clades this grade leads to (especially clade B of Jungermanniales), reflecting conserved intron content as well as paralleled reduction in intron content in liverworts (**Supplementary Fig. S5**). Mosses such as *Sphagnum* also contain more (i.e., 29) introns than their sister moss clades with only 26/27. However, this might not hold the truth for hornworts, a much less diversified bryophyte group, *Leiosporoceros* (sister to all other hornworts) contains less introns in its mitogenome than *Anthoceros* (which is sister to all hornworts, but for *Leiosporoceros*), suggesting either independent intron gains in *Anthoceros* or parallel losses in the rest of hornworts. Zumkeller *et al.* (2020) suggested parallel gains of *cox2i98* in the *Anthoceros* mitogenome by duplicative invasion, while loses of introns have usually been explained by gene conversions/retrotransposition or even horizontal gene transfers (Hepburn *et al.* 2012).

Bryophyte mitochondrial intergenic content

Intergenic spacers compose the bulk of the mitogenome of land plants, for example, accounting for about 80% in vascular plant mitogenomes (Mower *et al.* 2012). Spacer regions also comprise the bulk in bryophyte mitogenomes, with mosses having the smallest spacer size, hornworts the largest, and liverworts somewhere in between. Moss mt spacers range from 29 to 53 Kb (average, ~35 Kb), accounting for 29 to 38% (average, ~32%) of the mitogenome, comparable to that of the coding regions (average, ~32 Kb, ~30%). Liverwort mt spacers constitute the largest portion of their mitogenomes, ranging from 62 to 92 Kb with an average of ~81 Kb, and accounting for 42% to 53% (average, ~49%) of the mitogenome length. Hornwort mt spacers range from 93 to 118 Kb (average, ~103 Kb), accounting for 47 to 52% (average, ~49%) of the mitogenome (**Fig. 1, Supplementary Table S2**).

The spacer region of bryophyte mitogenomes is composed of ORFs, pseudogene fragments, nuclear homologous sequences, dispersed repeated sequences, SSRs, and other non-coding sequences of unknown origin. Nuclear transferred sequences accumulated to an average of ~15 Kb (42%) and ~16 Kb (20%) for mosses (Liu *et al.* 2014b) and liverworts (Dong *et al.* 2019a), respectively, and ~23 Kb (20%) for hornworts (i.e., *Anthoceros* mitogenomes). ORFs usually make up the second largest part of the bryophyte mt spacers, with an average ratio of 16%, 5%, and 20% for liverworts, mosses, and hornworts, respectively (Liu *et al.* 2012; Liu *et al.* 2014b). Moss mitogenomes lack repeated sequences in their intergenic spacers, liverworts hold on average 4% of repeated sequences, whereas hornworts hold 9% of repeated sequences in their intergenic spacer regions. The relatively higher repeated sequence content might imply higher potential for mitogenome recombination, as a positive correlation between the number of repeated sequences and the gene rearrangements has been suggested (Darracq *et al.* 2010; Liu *et al.* 2014b).

Mitochondrial repeats, recombinations, and genome structure of bryophytes

Repeated sequences play an important role in plant mitogenome structure stability since they can mediate intragenomic homologous recombinations leading to inversions and translocations of genomic regions (André *et al.* 1992; Mower *et al.* 2012; Skippington *et al.* 2015). Mitogenomes of vascular plants hold abundant repeated sequences, including several large repeats (>1000 bp), many medium-sized repeats (100–1000 bp), and numerous small repeats (50–100 bp). Increased repeat length and identity facilitate intragenomic recombinations (Mower *et al.* 2012) and may, hence, account for the fluid genome structure of vascular plant mitogenomes. In contrast, the mitogenomes of the three bryophyte lineages usually have fewer repeated sequences and are structurally more stable. The mitogenome of mosses contains only few and, moreover, small repeated sequences and is, hence, structurally nearly static (Liu *et al.* 2014b). Hornworts contain relatively more repeated sequences (~46 pairs of small and medium-sized repeats), which may explain the few described rearrangements distinguishing the four assembled mitogenomes (Dong *et al.* 2018a). The mitogenome of liverworts contains repeated sequences of intermediate abundance and size (14 pairs of small repeated sequences of 50–100 bp, and 14 pairs of medium-sized repeats of 100–900 bp) between those of mosses and hornworts (Dong *et al.* 2019a).

Although empirical studies suggest that repeats longer than 50 bp and with an identity above 85% may mediate

recombination (André *et al.* 1992; Marechal & Brisson 2010), the recombination activity of repeats is actually positively correlated with the length of repeated sequences, with small repeats (<100 bp) rarely inducing recombinations (Dong *et al.* 2019a; Wynn & Christensen 2019). Our previous study (Dong *et al.* 2019a) has examined the recombination rates by investigating the paired-end sequence reads related to all repeats within the 50–250 bp range for all the 29 liverwort samples and found recombination evidence for 26 repeats in 16 species, 40% of the liverwort species show no evidence of recombination. The mitogenomes of nine leafy liverworts appear to recombine more frequently than those of complex thalloids (three species) and simple thalloids (two species). The detection of repeat recombinations for two thirds of liverwort species with an average of two active repeats per species might indicate repeat recombinations occurred, but in low frequencies (Dong *et al.* 2019a). Considering five repeats longer than 250 bp exist on average per species in liverworts, which have not been investigated for recombination, it is very likely that some of these larger repeats (250–900 bp) would also allow for recombination to take place. As recombination and structure fluidity is supposed to be positively correlated, liverwort mitogenomes may represent an intermediate structural stage between those of static mosses and relatively dynamic hornworts. Similar studies are needed to be conducted for mosses and hornworts to quantify the recombination level and evaluate the structural dynamics of all bryophyte mt genomes. The stable mitogenome structure of each bryophyte lineage that spans 300~400 million years (Morris *et al.* 2018) is surprising given that liverwort and hornwort mitogenomes may potentially recombine, and alternative genome conformations could have coexisted. The apparent paradox of structural stasis of the mitogenome during the evolution of bryophytes despite evidence of ongoing recombination may be partially explained by the following caveats. First, bryophyte mitogenomes generally contain small and medium-sized repeats that recombine less frequently than long repeats do (Dong *et al.* 2019a). Second, bryophytes might have a strengthened nuclear surveillance system to keep the stability of mitogenomes, as has been suggested by Dong *et al.* (2019a). It is also possible that gene organization is essential to the transcription of polycistronic operons in liverworts and mosses (Liu *et al.* 2014b); hence, an identical gene order would be maintained across all liverwort lineages despite the existence of alternative genome conformations.

Bryophyte mitochondrial RNA editing

RNA editing is a co-or post-transcriptional process that changes the content of the transcripts, resulting in the translated protein to deviate from that encoded by the genomic sequence (Covello & Gray 1993; He *et al.* 2016). Nuclear and organellar RNA editing systems have opposite effects on protein diversity (Sloan 2017), since nuclear RNA editing in animals and fungi increases the protein diversity and creates novel protein types (Sloan 2017), whereas organellar RNA editing in plants restores conserved amino acid sequences (Knoop 2011; Bentolila *et al.* 2012; Sloan 2017). RNA editing occurs in organellar genomes in almost all land plant lineages but for the marchantiid liverworts (Rüdinger *et al.* 2008). Angiosperms contain about 200–500 RNA editing sites in their mitogenomes (Oldenkott *et al.* 2014). Lycophytes seem to possess an extreme amount of editing sites, e.g., the mitogenome of *Sellaginella moellendorffii* has 2,139 such sites in only 18 protein coding genes (Hecht *et al.* 2011). Hornwort *Anthoceros agretis* holds 496 C-to-U and 403 U-to-C editing in its mt transcripts (Gerke *et al.* 2019; Frangedakis *et al.* 2020). Mosses seem to hold the least amount of RNA editing sites, e.g., *Physcomitrium* holds 11 C-to-U RNA editing events in its mt transcripts (Rüdinger *et al.* 2009).

Using genomic and transcriptomic data, our previous study (Dong *et al.* 2019b) has identified 4,694 RNA editing sites in the mitogenomes of 33 liverwort species, 91% of these occurring as nonsynonymous editing. The abundance of RNA editing site varies greatly among different liverwort lineages, species, and genes. Liverwort order Haplomitriales (with *Haplomitrium* sampled here) demonstrates the highest editing level among liverwort species, whereas its sister order Treubiales has the fewest editing sites, in addition to the complex thalloids clade, which have no editing sites detected. Simple thalloid groups, such as *Aneura* and *Fossombronia*, show the second highest RNA editing frequency, followed by the Jungermanniales; with the exception of two *Herbertus* species, with distinctly reduced editing level. Porellales is the least edited leafy liverwort order. The pattern of species poor lineages showing higher editing levels in liverworts resembles that of angiosperms but opposes to that of the hornworts (Duff & Moore 2005; Gerke *et al.* 2019; Frangedakis *et al.* 2020) and ferns (Knie *et al.* 2016). In angiosperms, mt RNA editing is frequently reported from 300 to 500 editing sites, whereas some non-eudicot angiosperm lineages, such as *Amborella* (Rice *et al.* 2013) and *Liriodendron*, hold up to 800 editing sites (Richardson *et al.* 2013), and some eudicot lineages, such as *Silene noctiflora*, hold only 189 editing sites (Sloan *et al.* 2010). Ferns linages, such as Equisetales, and hornworts, such as *Leiosporoceros*, hold relatively less RNA editing sites than their more species-rich counterparts' (Knie *et al.* 2016).

The RNA editing frequency is also variable among different gene groups in bryophytes as evidenced by the high levels of editing in respiration-complex and photosynthesis-related protein genes, in contrast to the low levels of editing in ribosomal protein genes (Dong *et al*. 2019b), which is consistent with the findings in angiosperms (Edera *et al.* 2018). The abundance of liverwort RNA editing sites shows strong positive correlation with GC content, and the diversity of each of the five types of PPR PLS proteins that are functionally related to RNA editing process (Takenaka *et al.* 2008; Takenaka *et al.* 2013).

Future prospects

Overall, each of the three bryophyte lineages has kept a stable mt gene content and mitogenome structure. The conserved evolution of liverwort mitogenomes might be explained by low recombination levels, selective pressures, and intensified nucleus' surveillance. Hornwort mitogenomes are relatively more dynamic than those of liverworts and mosses. These three lineages differ in content of repeat sequences and frequency of rearrangements, suggesting that the mechanisms maintaining mitogenome structure might be different among these lineages. Given the low frequency of repeat recombinations observed in liverwort mitogenomes, possibly also in hornworts (Dong *et al.* 2018a), it would be interesting to understand how the mitogenome structure of liverworts and hornworts could be conserved over such a long evolutionary history. High-throughput sequencing technologies and accumulation of genetic resources have opened up new venues for tackling this question. Moreover, despite the conserved mt gene sets maintained across embryophytes, the intron set varied significantly among different plant lineages, and the patterns and mechanisms of intron losses and gains still await future investigation. Last but not least, as RNA editing genetically restores conserved amino acid sequences in plant organellar genes, and shows strong correlations with GC content and substitution rates, RNA editing could impact phylogenetic reconstruction, as has been demonstrated in organellar phylogenetic reconstruction of bryophytes (Bell *et al.* 2020). Although many pioneering studies have addressed the topic, this issue is still not settled, and should be evaluated in future studies, perhaps with dense sampling for mosses and hornworts.

Methods

Complete mitochondrial genomes of bryophytes were downloaded from GenBank organellar genome database (https://www.ncbi.nlm.nih.gov/genome/browse/?report=5#!/overview/). After removing those unverified and uncircularized ones, 113 accessions, representing 98 species, 71 genera and 28 orders, were retained. The genome features and statistics were summarized and curated in Geneious v10.0.2 (Biomatters, New Zealand). For phylogenetic reconstruction, we assembled three data matrices, representing the accession-(128 accessions), species-(113 species), and genus-(86 genera) level sampling of mitochondrial genomes, respectively. For each data set, eight vascular plants (two angiosperms, two gymnosperms, two ferns and two lycophytes) and seven charaphycean algae outgroups were included. Forty-one conserved mitochondrial protein-coding genes (PCGs) were extracted in Geneious v10.0.2 (Biomatters, New Zealand) for all taxa, including *atp1*, *atp4*, *atp6*, *atp8*, *atp9*, *ccmB*, *ccmC*, *ccmFC*, *ccmFN*, *cob*, *cox1*, *cox2*, *cox3*, *nad1*, *nad2*, *nad3*, *nad 4*, *nad4L*, *nad5*, *nad6*, *nad7*, *nad9*, *rpl10*, *rpl16*, *rpl2*, *rpl5*, *rpl6*, *rps1*, *rps10*, *rps11*, *rps12*, *rps13*, *rps14*, *rps19*, *rps2*, *rps3*, *rps4*, *rps7*, *sdh3*, *sdh4*, and *tatC*. For each dataset, each mitochondrial gene was translated to amino acid sequences and aligned with MAFFT (Katoh *et al.* 2005) in Geneious v10.0.2 (Biomatters, New Zealand) to create an amino acid alignment. The alignment was further trimmed for ambiguous portions by GBLOCKS (Talavera & Castresana 2007) with the least stringent settings and concatenated to generate concatenated amino acid alignment. PartitionFinder (Lanfear *et al.* 2012) was used for selection of the optimal data partition scheme and the associated amino acid substitution models, with an initial partitioning strategy by genes, resulting in 14, 13, and 13 partitions for the extensive 128-accession, 113-species, and 86-genus dataset, respectively. IQ-TREE2 (Quang *et al.* 2020) was used for maximum likelihood tree reconstruction with 1000 ultra-fast bootstrap replicates with the partitions and models generated by PartitionFinder (**Supplementary Figs. S1a**–**S3b**). Ancestral state reconstruction of genome size, spacer length, intron length is performed based on the genus-level topology in Mesquite v3.6.1 (Maddison & Maddison 2019) (**Supplementary Fig. S4**). Intron gains and losses along the genus-level phylogeny were inferred with software Count (Csuos 2010) (**Supplementary Fig. S5**).

Supplementary Materials

Supplementary figures S1a-S5, and tables S1-S2 are available online at https://doi.org/10.11646/bde.43.1.9.

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Competing Interests

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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