Gene transfer from the mitochondrion into the nucleus is a corollary of the endosymbiont hypothesis. The frequent and independent transfer of genes for mitochondrial ribosomal proteins is well documented with many examples in angiosperms, whereas transfer of genes for components of the respiratory chain is a rarity. A notable exception is the nad7 gene, encoding subunit 7 of complex I, in the liverwort Marchantia polymorpha, which resides as a full-length, intron-carrying and transcribed, but conserved pseudogene in the chondriome, whereas its functional counterpart is nuclear encoded. To elucidate the patterns of pseudogene degeneration, we have investigated the mitochondrial nad7 locus in 12 other liverworts of broad phylogenetic distribution. We find that the mitochondrial nad7 gene is nonfunctional in 11 of them. However, the modes of pseudogene degeneration vary: whereas point mutations, accompanied by single-nucleotide indels, predominantly introduce stop codons into the reading frame in marchantiid liverworts, larger indels introduce frameshifts in the simple thalloid and leafy jungermanniid taxa. Most notably, however, the mitochondrial nad7 reading frame appears to be intact in the isolated liverwort genus Haplomitrium. Its functional expression is shown by cDNA analysis identifying typical RNA-editing events to reconstitute conserved codon identities and also confirming functional splicing of the 2 liverwort-specific group II introns. We interpret our results 1) to indicate the presence of a functional mitochondrial nad7 gene in the earliest land plants and strongly supporting a basal placement of Haplomitrium among the liverworts, 2) to indicate different modes of pseudogene degeneration and chondriome evolution in the later branching liverwort clades, 3) to suggest a surprisingly long maintenance of a nonfunctional gene in the presumed oldest group of land plants, and 4) to support the model of a secondary loss of RNA-editing activity in marchantiid liverworts.

Introduction

In contrast to animals, the mitochondrial DNA of plants is characterized by larger genomic complexity and significant evolutionary plasticity. Additional genes, the presence of introns, the incorporation of foreign DNA from the nucleus and the chloroplast, frequent genomic recombination, and an ongoing functional gene transfer from the mitochondrion to the nucleus are typical features of plant mitochondrial genomes (Knoop 2004). Functional transfer of individual genes from the mitochondrion to the nuclear genome is known to occur frequently and independently in flowering plants (Adams et al. 2001, 2002; Adams and Palmer 2003). In most instances, genes for proteins of the small (rps genes) or large (rpl genes) subunits of mitochondrial ribosomes or tRNA genes are subject to gene transfer, sometimes also subunits of complex II of the respiratory chain (sdh genes). The core set of typical mitochondrial genes encoding subunits of the respiratory chain protein complexes I (nad genes), III (cox genes), and IV (cob) and of the ATPase (atp) is generally found to be universally conserved in the mitochondrial genomes of land plants (embryophytes) and in green algae. A noteworthy exception is the cob2 gene, for which the establishment of a functional gene copy in the nucleus and the following inactivation and disintegration of the original mitochondrial copy can be traced in leguminous plants (Nugent and Palmer 1991; Covello and Gray 1992; Adams et al. 1999). The quick degeneration of a formerly functional mitochondrial gene into a pseudogene after establishment of a functional gene copy in the nucleus is typical of angiosperms. Indeed, a complete loss of the nonfunctional mitochondrial gene barely leaving traces are often the first conclusive hint for a functional gene transfer in the history of a respective taxon (Adams et al. 2000).

A second interesting example of a core respiratory subunit gene transfer event is the case of nad7 in the liverwort Marchantia polymorpha, in fact also the only clearly documented example of functional gene transfer in a non-angiosperm land plant (Kobayashi et al. 1997). The functional nuclear copy of nad7 in Marchantia has a typical 5’ reading frame extension encoding the appropriate target sequence for organellar import. Somewhat in contrast to what is generally observed in angiosperms, the mitochondrial nad7 copy in Marchantia is turned into a nonfunctional pseudogene through the introduction of 6 stop codons but otherwise remains intact and complete over the full extension of the reading frame from start to stop (Oda et al. 1992). This may indicate an evolutionary recent event of gene transfer and could suggest the presence of functional nad7 genes in mitochondria of related liverworts.

In flowering plants (angiosperms), the functional mitochondrial nad7 gene carries 3 or 4 group II introns (Bonen et al. 1994), and the 2 upstream introns are also conserved in mosses (Pruchner et al. 2001), but the liverwort gene contains none of the angiosperm-like introns. Two unrelated group II introns are present in different positions in the nad7 pseudogene of Marchantia. The presence of unrelated introns in mitochondrial genes of liverworts in comparison with other land plants is a general observation (Pruchner et al. 2001; Knoop 2004). Although the mitochondrial nad7 pseudogene was shown to be transcribed in Marchantia, no splicing of the 2 introns was detectable (Takemura et al. 1995), and this raises the possibility that the lack of splicing functionality was involved in pseudogene degeneration. The secondary structures of the 2 Marchantia introns mostly conform with the group II intron consensus, but in both cases, intron and exon binding sites are not...
Evolution of a Pseudogene 1069

Table 1
List of Liverworts Investigated in This Study

<table>
<thead>
<tr>
<th>Taxonomy</th>
<th>Species</th>
<th>Voucher</th>
<th>Size/accession number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haplomitriopsida</td>
<td>Haplomitrium mnioides (Lindb.) Schust.</td>
<td>M. Shimamura s.n.</td>
<td>DNA: 3570 bp/EF010864</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>cDNA: 916 bp/EF010865</td>
</tr>
<tr>
<td>Marchantiopsida Blasiopsida</td>
<td>Blasia pusilla L.</td>
<td>J. Heinrichs 2291</td>
<td>1032 bp/EF010866</td>
</tr>
<tr>
<td>Marchantiopsida, sensu stricto</td>
<td>Marchantia polymorpha L.</td>
<td>J. Heinrichs 2291</td>
<td>5668 bp/NC 001660</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1942 bp, Kobayashi et al. (1997)</td>
</tr>
<tr>
<td></td>
<td>Marchantia polymorpha L., nuclear nad7</td>
<td>J. Heinrichs 2291</td>
<td>1276 bp/EF010867</td>
</tr>
<tr>
<td></td>
<td>Bueciera romaniaca Radian</td>
<td>Ulm collection s.n.</td>
<td>1267 bp/EF010868</td>
</tr>
<tr>
<td></td>
<td>Conocephalum conicum (L.) Underw.</td>
<td>Groth and Schwertfeger s.n.</td>
<td>1276 bp/EF010869</td>
</tr>
<tr>
<td></td>
<td>Monosolenium tenerum Griff./Sunita Kapila and SS Kumar</td>
<td>Groth and Schwertfeger s.n.</td>
<td>1283 bp/EF010870</td>
</tr>
<tr>
<td></td>
<td>Lunularia cruciata (L.) Dum. ex Lindb.</td>
<td>Groth and Schwertfeger s.n.</td>
<td>1142 bp/EF010871</td>
</tr>
<tr>
<td>Jungernanniopsida simple thalloids</td>
<td>Aneura pinguis (L.) Dumort.</td>
<td>MGM031218-01SC</td>
<td>1198 bp/EF010872</td>
</tr>
<tr>
<td>Jungernanniopsida leafy liverworts</td>
<td>Lepidogyra hodgsoniae Grolle</td>
<td>MGM031218-02SC</td>
<td>1232 bp/EF010873</td>
</tr>
<tr>
<td></td>
<td>Calypogeia muelleriana (Schiffner) K. Müller</td>
<td>J. Heinrichs 4375</td>
<td>1233 bp/EF010874</td>
</tr>
<tr>
<td></td>
<td>Frullania tunarisci (L.) Dumort.</td>
<td>J. Heinrichs 4382</td>
<td>1230 bp/EF010875</td>
</tr>
<tr>
<td></td>
<td>Harpantus flotovianus (Nees) Nees</td>
<td>J. Heinrichs 4390</td>
<td>nucleus: 680 bp/EF010876</td>
</tr>
<tr>
<td></td>
<td>Scapania nemorea (L.) Grolle</td>
<td>J. Heinrichs 4372</td>
<td>1233 bp/EF010877</td>
</tr>
</tbody>
</table>

Note.—Sequences of the amplicons are given with their respective lengths and have been deposited in the database under the novel accession numbers indicated. Sequences from Chara vulgaris (NC_005255), Marchantia polymorpha, and Physcomitrella patens (1713035479) included for comparison were taken from the database.

s.n., sine numero

completely compatible. As no other plant group with a functional mitochondrial nad7 gene shares these particular introns, it is as yet unclear whether they were correctly spliced at any time in evolution.

Liverworts represent an evolutionary old land plant clade and are in fact possibly the phylogenetic sister group to a clade comprising all other land plants including mosses, hornworts, lycophytes, monilophytes (ferns, horsetails, and whisk ferns), and seed plants (angiosperms and gymnosperms) (Qi et al. 2006). Mitochondrial gene sequences including the positionally stable group I and group II introns have contributed to current models of land plants phylogeny (Qi et al. 1998; Groth-Malonek and Knoop 2005; Groth-Malonek et al. 2005). Algae closely related to the land plants such as Chara vulgaris (Turnel et al. 2003) and Chaetosphaeridium globosum (Turnel et al. 2002) carry functional nad7 genes in their mitochondria, but these genes do not contain any introns. Mosses have functional mitochondrial nad7 genes and carry 2 of the angiosperm-type group II introns (Hashimoto and Sato 2001; Pruchner et al. 2001). Hence, the data indicate presence of a functional nad7 gene in the ancestor of all embryophytes and a subsequent degeneration into a pseudogene in Marchantia and possibly in related liverwort taxa.

An improved understanding of liverwort phylogeny is currently emerging from multigene studies (Davis 2004; Forrest and Crandall-Stotler 2004, 2005; He-Nygren et al. 2004; Frey and Stech 2005; Heinrichs et al. 2005; Knoop V, unpublished data). The classical morphological distinction of complex thalloid taxa (marchantiid) versus simple thalloid and leafy (metzgeriid/jungermanniid) taxa is well corroborated by the molecular analyses, but several novel insights have emerged. We wished to trace the evolutionary history of the nad7 pseudogene in liverworts more and less closely related to Marchantia. The objective of our study was 2-fold: 1) to address the modes of mitochondrial gene disintegration in a plant group so far not in the focus of gene transfer studies and 2) to obtain additional molecular data useful for phylogeny reconstruction in this ancient land plant group.

Materials and Methods

Plant taxa under study are listed in table 1. Total nucleic acids were extracted from green plant material in the presence of cetyl-trimethyl-ammonium-bromide. DNA and RNA were differentially precipitated in the presence of 3 M lithium acetate. OmniScript Reverse Transcriptase (Qiagen, Hiden, Germany) was used for cDNA synthesis. Polymerase chain reaction (PCR) amplification assays contained 1 μl template DNA or cDNA (approximately 10 ng–0.5 μg), 1 unit Taq DNA polymerase (Genaxxon, Biberach, Germany) or Silverstar Taq (Eurogentec, Seraing, Belgium), 5 μl corresponding 10× PCR buffer, 2–3 mM MgCl2, 200 μM dNTPs each, 0.2 mM of each primer, 2–4% DMSO (Dimethyli sulfonide), and double-distilled water added up to 50 μl. A typical amplification assay included initial denaturation at 92 °C for 1 min, followed by 10 cycles of 92 °C for 1 min, 57–50 °C for 1 min, 72 °C for 2 min, followed by 30 cycles of 92 °C for 1 min, 50 °C for 1 min, 72 °C for 2–2.5 min, and a final step of synthesis for 15 min at 72 °C. Primers used for the DNA assays were n7i336up (5′-ggt agg act ctc gta att gga tgg c-3′) and n7i1113do (5′-gtt gtc acc cag aca ata acc-3′), for the nuclear gene assay 7E1+ (5′-cag cata cct gta ggt g-3′) and 7E3− (5′-cc aac aca ata tct cga gta cc-3′), and for the cDNA assays were nad7up2 (5′-tac gct gcn car gaa cay gc-3′) and nad7down2 (5′-ttc atc tct ctc tcc aac cgt aat-3′). Primers to amplify the 5′ and 3′ termini of the mitochondrial nad7 open reading frame (ORF) in Haplomitrium were nad7up2v3 (5′-ccg tag ata ttt atc gta tgt ttg tta gaa tgg g-3′) and nad7is366do (5′-ggt ttc cga agt tyc cga tgg agg aac g-3′) and nad7is656up (5′-tac mgc agt aga agc rce taa agg-3′) and nad7downstream (5′-gtc cta cct gtt cca caa tga gtc-3′), respectively. PCR
fragments were sequenced directly on an ABI 3100 capillary sequencer using the BigDye Terminator Cycle Sequencing v2.0 kit (PE Biosystems, Foster City, CA), or cloned into the pGEM-T Easy vector (Promega, Manheim, Germany) and sequenced on an ALF Express II (Amersham Biosciences) using the Sequenase Cy5 Dye Terminator kit (GE Healthcare, Munich, Germany), or were commercially sequenced (Macrogen Inc., Korea). Sequences were aligned with MEGA3 (Kumar et al. 2004) using the implemented Clustal algorithm and manually adjusted.

Results

Five of the 6 stop codons in the mitochondrial nad7 gene of liverworts Marchantia polymorpha are located between the 2 large group II introns nad7i336 and nad7i1113 in the liverwort M. polymorpha (fig. 1, intron designations follow Dombrovska and Qiu 2004). Hence, the large central exon between them was an attractive region to address pseudogene evolution assuming that the 2 introns would be conserved in other liverwort taxa. Design of primers targeting the mitochondrial introns could ensure amplification of the mitochondrial copy in liverworts rather than a potential nuclear version. Moreover, this approach circumvented the risk of PCR failure using primers targeting terminal exon regions given the large group II intron sizes or after potential disintegration of gene termini through recombination events.

The amplification and sequencing of the nad7 region with primers anchoring in the 2 liverwort-specific introns nad7i336 and nad7i1113 (fig. 1) was indeed successful for a taxonomically diverse spectrum of liverworts. PCR products of expected sizes around 1300 bp were obtained with the exception of Blasia where a product of about 1000 bp was retrieved (table 1). All PCR products were sequenced to clarify the status of the mitochondrial nad7 sequences. We first checked other taxa of the marchantiid group of complex thalloid liverworts closely related to Marchantia. As in Marchantia, the nad7 reading frames were also found to carry stop codons in Bucegia, Conocephalum, Lunularia, and Monosolenium (fig. 2). Surprisingly, one of the Marchantia stop codons (s3765) is shared among all taxa (except Blasia), and 2 other ones (s3483 and s4064) are shared with all taxa except Lunularia (and Blasia). The 2 remaining stops are unique to Marchantia, and one novel stop codon each is identified in Lunularia and Bucegia. As in Marchantia, there are no reading frameshifts in the closely related taxon Bucegia and only 1 or 2 single-nucleotide frameshifts, respectively, in the other taxa. These very minor differences and the otherwise high degree of sequence similarities even in a pseudogene correlate well with the extreme degree of sequence conservation of functional mitochondrial genes in the marchantiids that had been observed before (Beckert et al. 1999).

The genus Blasia is of particular interest to the phylogeny of liverworts because recent molecular data have strongly suggested its inclusion among the marchantiids in a basal position instead of its classic assignment to the simple leafy, juncagnemid taxa. Unusually, in Blasia, we observed an internal deletion of 238 bp removing a large portion of the nad7 reading frame (fig. 2). Interestingly, this large deletion is immediately upstream of a codon insertion (in3878 + 3) universally present in the marchantiid taxa. Neither frameshifts nor stop codons were observed in the regions flanking the large deletion in Blasia, possibly suggesting that those observed in the derived marchantiids were gained after separation from the Blasia lineage and so possibly support its basal placement.

In the next step, we included phylogenetically more distant taxa of the Jungermanniopsida (simple thalloid and leafy taxa) into our survey. Again, our taxonomic spectrum was wide enough to include representatives from the well-established clades as based on recent insights on liverwort phylogeny. As in the marchantiids, the nad7 reading frame was found destroyed in all cases. However, whereas single-nucleotide frameshifts or stop codons render nad7 a pseudogene in the marchantiids, oligonucleotide insertions and deletions of up to 25 bases are characteristic of nad7 pseudogenes in the Jungermanniopsida (fig. 2). Three frameshifting indels are shared by all Jungermanniopsidi taxa: in3551+2, in3764+5, and in3902–25. Other indels occur independently and at particularly high frequency in Aneuira, a simple thalloid liverwort and member of supposedly early diverging groups of the Jungermanniopsida, which shows 16 indels in total.
in the central nad7 exon. All other taxa show fewer indels, up to 9 in Lepidogyna.

The clear distinction of jungermanniid and marchantiid taxa is also clearly supported by 8 codon changes, which appear as synapomorphies of the Jungermanniopsida and one as synapomorphy for the clade of marchantiids including Blasia (fig. 2). A further 13 such changes confirm a clade of 4 derived jungermanniid taxa.

Although these data so far suggested that nad7 is generally a pseudogene in liverwort mitochondria, we finally included the genus Haplomitrium in our taxon sampling. As in the case of Blasia, molecular data had recently suggested a new taxonomic placement, possibly at the base of liverworts as a whole (Crandall-Stotler et al. 2005). The complete nad7 coding region between the 2 liverwort-type introns was found intact in Haplomitrium. Neither frameshifts nor stop codons or frame-conserving codon indels were found. Moreover, no significant mutations affecting conserved amino acids were observed other than some codon exchanges that could potentially be corrected by the C-to-U type of RNA editing typical for plant organellar genes to reconstitute codon identities, which were previously found in abundance in this genus in a study of the nad5 gene (Groth-Malonek et al. 2005). To address whether the Haplomitrium sequence actually represents a functional sequence, we used RT–PCR. Because functional splicing of the liverwort intron sequences would be a fundamental prerequisite for expression, we extended the amplicon with primers anchoring in the upstream and downstream flanking exons (fig. 1). An RT (reverse transcription)–PCR product of a size expected for correct splicing was obtained and sequenced. Comparison of cDNA and DNA sequences indeed confirmed correct and precise splicing at the expected sites and showed complete sequence identity between cDNA and DNA except for all 15 expected RNA-editing positions to reconstitute conserved codon identities as now confirmed by the cDNA (fig. 3). A remote possibility would be that the cDNA could have been derived from a transcript of a nad7 gene copy very recently transposed into the nucleus in Haplomitrium that has not accumulated any nucleotide exchanges. To address this possibility, we wished to clone the 5' and 3' terminal sequences of the nad7 ORF in Haplomitrium. This proved difficult given that flanking intergenic sequences are generally only rarely conserved in plant mitochondrial DNA and required several attempts with different primers. However, in the end, we succeeded to amplify and clone the 5' end of the Haplomitrium nad7 gene with a downstream primer anchoring in nad7i336 and a 5' primer anchoring 39 bp upstream of the nad7 start codon. The 5' end of the Haplomitrium nad7 gene is colinear with the mitochondrial homologues in other taxa and identical with the cDNA sequence overlapping in the exon upstream of i336 except for 3 further codons found to be edited as could be expected (fig. 3). The methionine start codon in the Haplomitrium nad7 ORF is located at the same position as the start codon in other mitochondrial nad7 genes. In addition, a stop—only 12 codons upstream

![Graphical overview of the nad7 exon region between the introns nad7i336 and nad7i1113 for Marchantia polymorpha and the 12 liverworts investigated in this study. Asterisks represent stop codons (s), rhombs represent single-nucleotide frameshifts, up and down arrows indicate insertions or deletions that result in frameshifts (in), the open squares indicate codon indels that do not disrupt the reading frame. The respective positions are indicated with the M. polymorpha nucleotide position of the nad7 gene (including introns) followed by the number of inserted (+) or deleted (−) nucleotides, respectively. The hatched box represents the deletion of a major part of the central nad7 exon upstream of i3878 in Blasia. Presumed phylogenetic relationships of the taxa are shown to the left, summarizing findings from several recent studies (Davis 2004; Forrest and Crandall-Stotler 2004; He-Nygré et al. 2004). Cladistic support for some of the nodes (node identifier above branch) also comes from several synapomorphic codon changes (number below branch) in the nad7 pseudogene amplicon (for amino acid positions of the Chara sequence refer to fig. 3. Node 1: D123G, L176P, Y183H, G206S, S219G, G227E, L244S, I262T, Q272R, S304D, H/R310Q, L314P, L340S; node 2: M122T, R151G, S183Y, M191V, M275T, F340L, N344D, G364E; node 3: E135G, G161E; node 4: A163G; node 5: R196C; and node 6: E336S). Loss of RNA editing has presumably occurred at node 6 (arrow).]
(coincidentally conserved at this position in the Marchantia mitochondrial sequence)—clearly documents absence of a potential targeting sequence extension that could be indicative of a nuclear gene copy. Efforts to amplify the 3' end of the Haplomitrium nad7 gene in the end were successful with a 5' primer anchoring in the coding region upstream of i1113 and a 3' primer anchoring immediately downstream of the stop codon in Haplomitrium to determine the conserved 3' end of nad7. Again, the sequence is perfectly colinear with its mitochondrial homologues in other taxa (fig. 3), including the highly conserved carboxy terminal amino acid motif GEVDR and the stop codon position and is identical with the cDNA sequence except for the confirmation of an expectedly edited proline codon (fig. 3).

Finally, we wished to verify that a functional nad7 gene copy resides in the nucleus of jungermanniid species. A PCR amplification with the exon-based primers initially employed to retrieve the functional nuclear counterpart in Marchantia (Kobayashi et al. 1997) was successful in amplifying the corresponding sequence (from amino acid positions 24 through 253) in Harpanthus. We were unable to amplify this region from other taxa in our sampling, a result likely due to a combination of lower sequence conservation in the more divergent nuclear gene copies and genome complexities. In the case of Haplomitrium, however, the failure to detect any nad7 copy other than the obviously mitochondrial one with this and any of the other primer combinations used could certainly also reflect a phylogenetic status before nuclear
gene transfer, which may have occurred only in the stem lineage of the remaining liverworts after split from *Haplotrichium* (fig. 2). The (presumably nuclear) *Harpanthus* sequence is, as expected, free of group II (and other) introns and frameshifts and shares most sequence similarity with the *Marchantia* nuclear *nad7* gene (fig. 3). Several codon positions in the *Harpanthus* *nad7* sequence are shared with the *Marchantia* nuclear *nad7* sequence and are not shared with any of the mitochondrial sequences included in these analyses (C89L, C91I/V, I110L, L141M, S155A, T207V, Q212E, V228I, C229K, A236S). This suggests an ancient gene transfer establishing the functional nuclear *nad7* gene before the split of jungermanniid and marchantiid taxa.

**Discussion**

Numerous examples of frequent and independent gene transfer from the mitochondrion to the nucleus have been reported, however, mainly for genes encoding ribosomal proteins among angiosperms. No example for a functional gene transfer of any one of the *nad* genes among flowering plants had been reported (Adams et al. 2002). Hence, the case of *nad7* gene transfer in a liverwort (Kobayashi et al. 1997) is noteworthy in 2 respects: it is the only known functional gene transfer event from the mitochondrion in non-angiosperm land plants and the only one that involves one of the 9 mitochondrially encoded *nad* genes.

We report that the mitochondrial *nad7* pseudogene sequences are generally present in liverworts across a wide taxonomic range. These sequences carry both liverwort-type group II introns known from *M. polymorpha*, nad7i336 and nad7i1113. Although the mitochondrial pseudogene of *Marchantia* is transcribed, both introns are not spliced. In contrast, mosses apparently have functional *nad7* genes in their mitochondria, which carry 2 upstream angiosperm-type group II introns nad7i140 and nad7i209 (Hashimoto and Sato 2001; Pruchner et al. 2001). This finding in itself is not surprising given that plant mitochondrial introns are generally stable in position within a particular phylogenetic clade (Beckett et al. 1999; Dombrovska and Qiu 2004; e.g., Vangerow et al. 1999; Pruchner et al. 2002; Qiu et al. 2006). Notably, the very different occurrence of mitochondrial introns in liverworts and other land plants is strong evidence for the deepest dichotomy of embryophyte phylogeny (Qiu et al. 1998, 2006).

The persistence of the mitochondrial *nad7* pseudogene in liverworts is a puzzling observation. If liverworts indeed are the sister group to all other land plants and in so far a phylogenetically very old clade, presumably exceeding an age of 400 Myr, why is a nonfunctional pseudogene conserved for so long in their mitochondrial genomes with only minor changes—notably very minor ones in the marchantiid subgroup?

*Nad7* sequence analysis indicates that the mode of pseudogene degeneration differs in the 2 liverwort subclades. Whereas the *nad7* gene is rendered nonfunctional through introduction of stop codons and single-base indels in the marchantiids, indels of several bases dominate in the jungermanniid taxa. The fact that all surveyed members within each taxon share similar classes of mutations suggests that different modes of pseudogenization arose early in the 2 extant clades. One of the stop codons (s3765) is conserved in all surveyed complex thalloid liverworts and also occurs in *Aneura*, the earliest diverging branch of the Jungermanniopsida in our sampling. The large gap that was found in *Blasia* is, when aligned with other liverwort sequences, placed in a region that includes the position of this highly conserved stop codon. Hence, this could possibly be the one stop codon that initiated the loss of functionality of the mitochondrial *nad7* gene at least in marchantiids, if not the entire clade of non-*Haplotrichium* liverworts when assuming a later reversal in the jungermanniids. Currently, the most parsimonious explanation for the observations is the establishment of a functional *nad7* copy in the nucleus at least before the split of jungermanniid and marchantiid taxa. This is well supported by our finding of a functional copy of *nad7* in the jungermanniid taxon *Harpanthus*, which exhibits a high degree of similarity to the nuclear *nad7* sequence of *Marchantia*.

The presence of a functionally spliced and edited mitochondrial *nad7* copy in *Haplotrichium* is particularly noteworthy in other respects. First, it serves as an independent genomic character that supports the basal phylogenetic placement of this genus among liverworts as a whole. Furthermore, it clearly supports the idea that both liverwort-type group II introns, nad7i336 and nad7i1113, were present in a functional mitochondrial *nad7* gene of the prymordial liverwort ancestor and were correctly spliced at that time. Finally, the finding also lends strong support to a phylogenetically plausible scenario of secondary loss of RNA-editing activity (fig. 2), which is absent in the marchantiid liverworts (Steinhauser et al. 1999), instead of independent gain of editing activity in *Haplotrichium*, the Jungermanniopsida, and the clade of all other land plants.

Disintegration of the mitochondrial gene copy after functional transfer to the nucleus is frequently so fast in angiosperms that absence of a mitochondrial gene copy had been taken as an indicator of gene transfer in the first place (Adams and Palmer 2003). A notable exception has only very recently been reported for the survival of *rps14* pseudogenes among the grasses for maybe some 80 Myr (Ong and Palmer 2006). The dominating point mutations and small-scale indels observed in liverworts stand in contrast to the otherwise (quick) recombinational disruption of pseudogenes known in angiosperms. The high recombinational activity typical for the mitochondrial DNAs of angiosperms may be a later evolutionary gain in the tracheophyte lineage, not present to that extent in liverworts. The new observations are in line with the absence of disrupted (trans-splicing) introns in bryophytes, which instead are conventionally cis-arranged there (Malek and Knoop 1998; Dombrovksa and Qiu 2004; Groth-Malonek et al. 2005).

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