# Amino acid transporter inventory of the *Selaginella* genome

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Amino acids play fundamental roles in a multitude of functions including protein synthesis, hormone metabolism, nerve transmission, cell growth, production of metabolic energy, nucleobase synthesis, nitrogen metabolism, and urea biosynthesis. *Selaginella* as a member of the lycophytes is part of an ancient lineage of vascular plants that had arisen ∼400 million years ago. In angiosperms, which have attracted most of the attention for nutrient transport so far, we have been able to identify many of the key transporters for nitrogen. Their role is not always fully clear, thus an analysis of *Selaginella* as a representative of an ancient vascular plant may help shed light on the evolution and function of these diverse transporters. Here we annotated and analyzed the genes encoding putative transporters involved in cellular uptake of amino acids present in the *Selaginella* genome.

**Keywords: *Selaginella*, amino acid, transporter**

## INTRODUCTION

Amino acids play fundamental roles in a multitude of functions including protein synthesis, hormone metabolism, nerve transmission, cell growth, production of metabolic energy, nucleobase synthesis, nitrogen metabolism, and urea biosynthesis. In multicellular organisms, many of the nitrogenous compounds are transported between cells (Lalonde et al., 2004). Long distance transport of organic nitrogen in the plant is probably more complex as compared to sugar transport given the large number of amino acids. The transported substrates are highly diverse, including 20 proteogenic amino acids, GABA, and a variety of amino acid analogs and even oligopeptides, but also many other N-containing compounds. Furthermore, amino acids are not only transported in the phloem but also in xylem, and they exchange between phloem and xylem in a complex manner (Atkins, 2000). If we take into account that nitrogen metabolism is highly compartmentalized; it appears reasonable to expect a large number of transporter genes. Amino acid transporters for cellular import were initially identified by suppression cloning in yeast, and they comprise primarily two major families: the amino acid transporter superfamily 1 (ATF1) and the amino acid–polyamine–choline (APC) transporter superfamily (Frommer et al., 1993, 1995; Lalonde et al., 2004). Transporters for the efflux of amino acids and for transport across the vacuolar membrane may be encoded by uncharacterized members of this family or may be encoded by yet uncharacterized membrane protein genes.

### THE AMINO ACID TRANSPORTER SUPERFAMILY 1

In contrast to the other amino acid transporter families, ATF1 members were first described in plants (Frommer et al., 1993). ATF1 members contain 9–11 putative membrane spanning domains with cytosolic N- and extracellular C-termini (Chang and Bush, 1997). Within the ATF1 family, six subfamilies were found: amino acid permeases (AAPs), "lysine/histidine" transporters (LHTs), ProTs, γ-aminobutyric acid transporters (GATs), ANTs, and AUXs. (i) AAPs have eight members in *Arabidopsis*. The *Arabidopsis* AAPs, which mediate $Na^+$-independent, $H^+$-coupled uptake of a wide spectrum of amino acids, represent the best-characterized members of the superfamily (Fischer et al., 2002). Slight differences in substrate specificity exist among the different members complicating an exact assessment of their *in vivo* function. (ii) LHTs were named according to the substrate selectivity originally determined for AtLHT1. However, more detailed studies on the biochemical properties of AtLHT1 and AtLHT2 in *Saccharomyces cerevisiae* revealed that compared to basic amino acids their affinities for neutral and acidic amino acids are much higher (Hirner et al., 2006). Consequently, the LHTs are now classified as high affinity transporters for these groups of amino acids. LHT1 has been shown to play an important role in the acquisition of amino acids by *Arabidopsis* roots (Hirner et al., 2006). (iii) ProTs transport the amino acid proline, glycine betaine, and related quaternary ammonium compounds as well as the amino acid γ-aminobutyric acid (GABA) with moderate or low affinity (Grallath et al., 2005). (iv) GATs transport GABA and GABA-related compounds. (v) Aromatic and neutral amino acid transporter 1 (ANT1)-like proteins, of which only AtANT1 has been characterized so far, functions as a moderate affinity transporter for aromatic and neutral amino acids (Chen et al., 2001). (vi) The AUX (auxin-resistant) subgroup. AtAUX1 was initially reported to be a putative auxin-influx carrier on the basis of genetic data and the analysis of mutants in *Arabidopsis*. The expression of *AtAUX1* in *Xenopus* oocytes provided unambiguous evidence that AUX1 is capable of importing auxin into cells (Yang et al., 2006).

### THE AMINO ACID–POLYAMINE–CHOLINE TRANSPORTER SUPERFAMILY

In the absence of structural data, computer-aided predictions of secondary structures were used to categorize plant APC

**Table 1 | Number of gene from the different amino acid transporters subfamilies found in *Arabidopsis*, Rice, *Selaginella*, *Physcomitrell a*, and *Chlamydomonas* genomes (nd, not determined).**

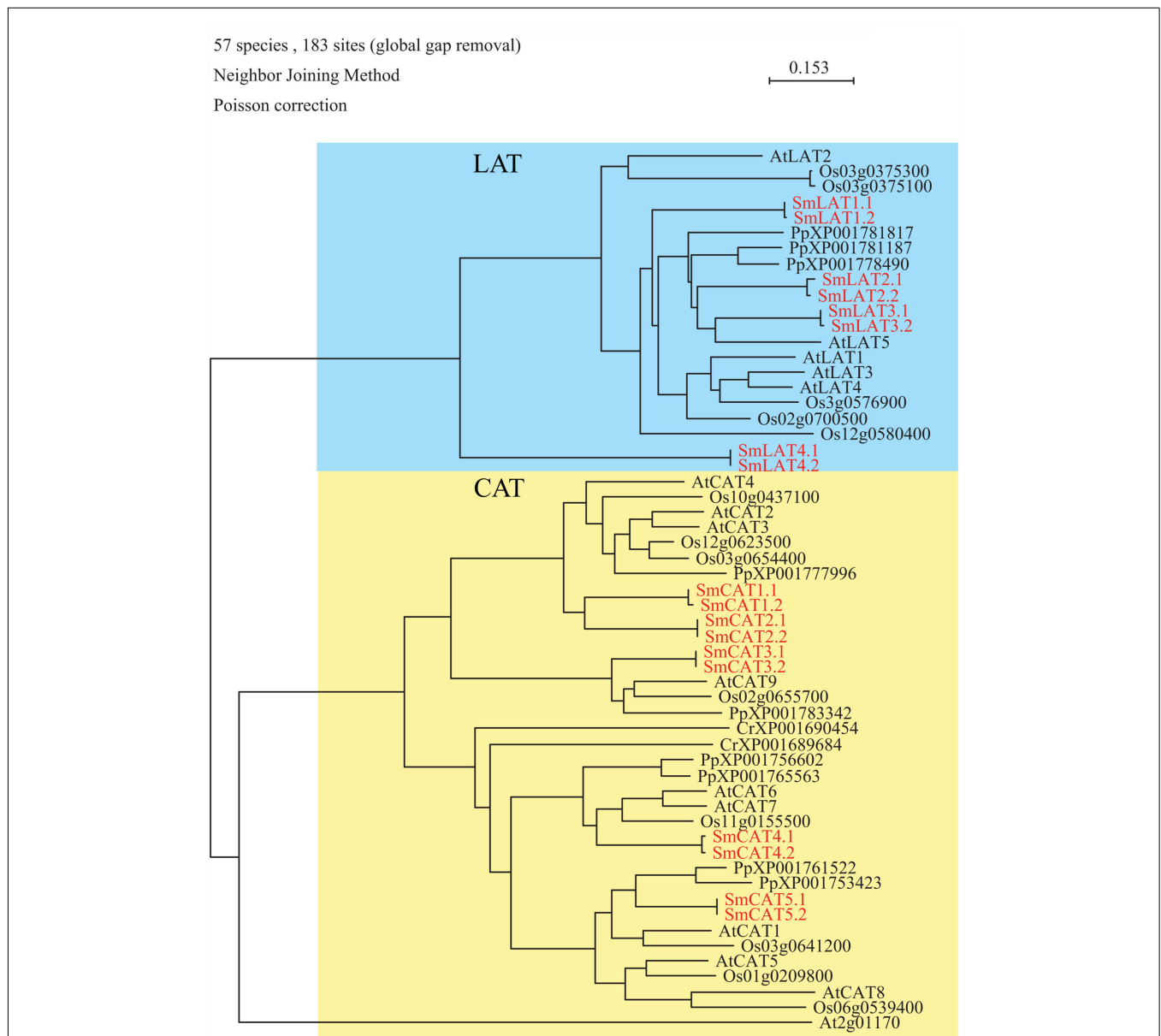| Gene function | Gene name | Gene used for query | Number of putative orthologs | | | | |
|---|---|---|---|---|---|---|---|
| | | | *Arabidopsis* | Rice | *Selaginella* | *Physcomitrella* | *Chlamydomonas* |
| Amino acid transport | AAP, LHT, ProT, Aux, GAT, ANT1 | | 48 | 57 | nd | nd | Nd |
| | LAT | AtLAT1 | 5 | 4 | 4 | 3 | 0 |
| | CAT | AtCAT1 | 10 | 8 | 5 | 6 | 3 |
| | mBAC | AtmBAC1 | 2 | 4 | 10 | 8 | 6 |
| | OEP16 | AtOEP16.1 | 4 | 2 | 1 | 1 | 0 |
| | DIT2 | AtDIT2 | 2 | 2 | 3 | 4 | 2 |



**FIGURE 1 | Phylogenetic tree of amino acid polyamine choline (APC) superfamily transporters.** The alignment was obtained from ClustalW and tree was build using PAUP software. At, *Arabidopsis thaliana*; Cr, *Chlamydomonas reinhardtii*; Os, *Oryza sativa*; Pp, *Physcomitrella patens*; Sm, *Selaginella moellendorffii*. Red letters indicate a *Selaginella moellendorffii* gene.

16 species , 239 sites (global gap removal)
Neighbor Joining Method
Poisson correction

**FIGURE 2 | Phylogenetic tree of DiT2 (dicarboxylate) transporter family.**
The alignment was obtained from ClustalW and tree was build using PAUP
software. At, *Arabidopsis thaliana*; Cr, *Chlamydomonas reinhardtii*; Os,
*Oryza sativa*; Pp, *Physcomitrella patens*; Sm, *Selaginella moellendorffii*. Red
letters indicate a *Selaginella moellendorffii* gene.
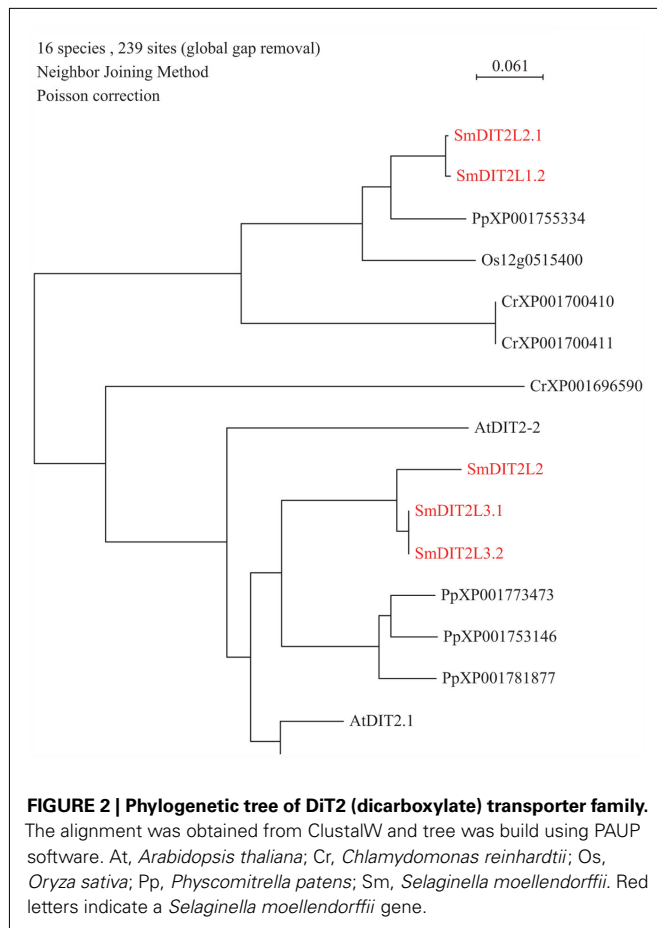
transporters into two subgroups (http://aramemnon.botanik.
uni-koeln.de/). Members of the cationic amino acid transporters
(CATs) are probably built from 14 putative transmembrane
domains, with homologs found in animals (Wipf et al., 2002). The
second subfamily comprises proteins with 12 putative transmem-
brane domains (Wipf et al., 2002). In short, amino acid transport
mediated by members of the APC family from different organ-
isms is diverse, uses $Na^+$- or $H^+$-coupling by co- or antiport and
serve functions in uptake and nutrition (Su et al., 2004; Yang et al.,
2010).

So far, the amino acid transporters that have been identified fall
into at least five gene families (Rentsch et al., 2007) and display dif-
ferent substrate selectivity and affinity as well as distinct subcellular
localization (Wipf et al., 2002). Recently at bidirectional amino
acid transporter 1 (*AtBAT1*) a new AA transporter, which is not a
member of any previously defined amino acid transporter family,
was identified (Dundar and Bush, 2009). Direct measurement of
amino acid transport and yeast growth experiments demonstrated
that AtBAT1 exhibits transport activity for alanine, arginine, gluta-
mate, and lysine, but not for GABA or proline. Significantly, unlike
other amino acid transporters described in plants to date, *AtBAT1*
displayed both export and import activity. BAT1 is a single copy
gene in the *Arabidopsis* genome, and its mRNA is ubiquitously
expressed in all organs. BAT1 may thus be key for cellular efflux,
a step needed for transport between cells and organs, similar as

the sucrose efflux transporters of the SWEET family (Chen et al.,
2011).

*Selaginella* is part of an ancient lineage of vascular plants that
had arisen ∼400 million years ago. An analysis of *Selaginella* as a
representative of an ancient vascular plant may help shed light on
the evolution and function of the diverse nutrient transporters.
Here we analyzed the genes for transporters involved in cellular
uptake of amino acids present in the *Selaginella* genome. We car-
ried out a similar analysis for the *Selaginella* complement of genes
for transporters involved in cellular uptake of ammonium, urea,
and cellular uptake of sugars (DeMichele et al., FiPS, in revision;
Lalonde et al., FiPS, in revision).

## MATERIALS AND METHODS
The *Selaginella moellendorffii* genome was annotated using the
JGI Annotation pipeline, which combines several gene prediction,
annotation, and analysis tools. Gene predictors used for annota-
tion include Fgenesh, Fgensh+, and Genewis (Banks et al., 2011).
Over 110,000 *Selaginella* ESTs were clustered, converted into
putative full-length (FL) genes and directly mapped to genomic
sequences or used to extend predicted gene models into FL genes
by adding 5′ and/or 3′ UTRs to the models. Since multiple gene
models were generated for each locus, a single representative model
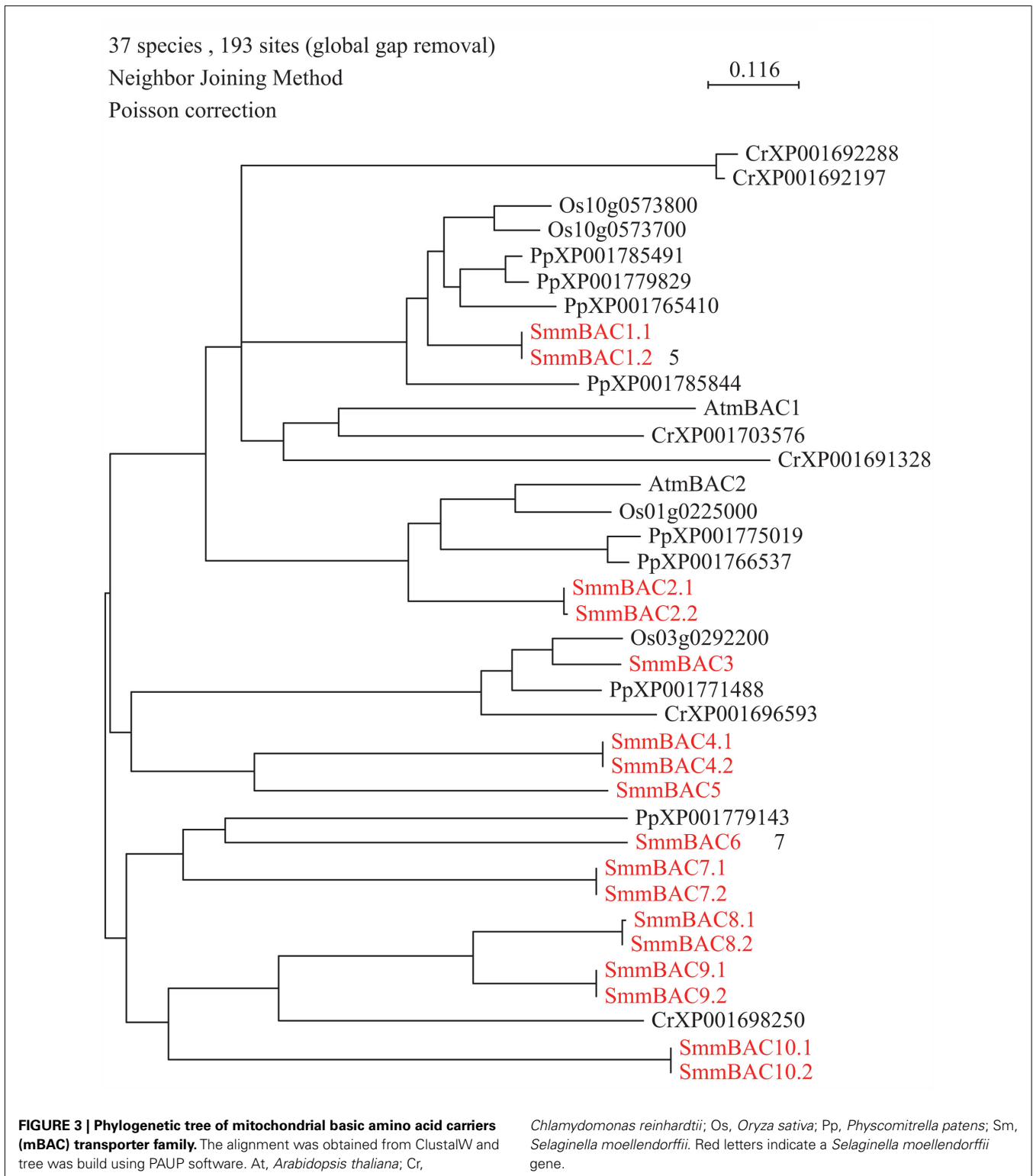was chosen based on homology and EST support and used for
further analysis.

All predicted gene models were functionally annotated by
homology to annotated genes in the NCBI non-redundant set.
Each gene model was analyzed for domain content and structure
using InterProScan and classified according to Gene Ontology,
eukaryotic orthologous groups (KOGs) and KEGG metabolic
pathways. In total, 34,292 gene models were predicted for the
*Selaginella* assembly (Banks et al., 2011).

Gene predictions were checked by aligning *Selaginella* amino
acid transporter sequences with known plant amino acid trans-
porters in the ClustalW software (Thompson et al., 1994). Phylo-
genetic trees were build using PAUP software (Swofford, 1998).
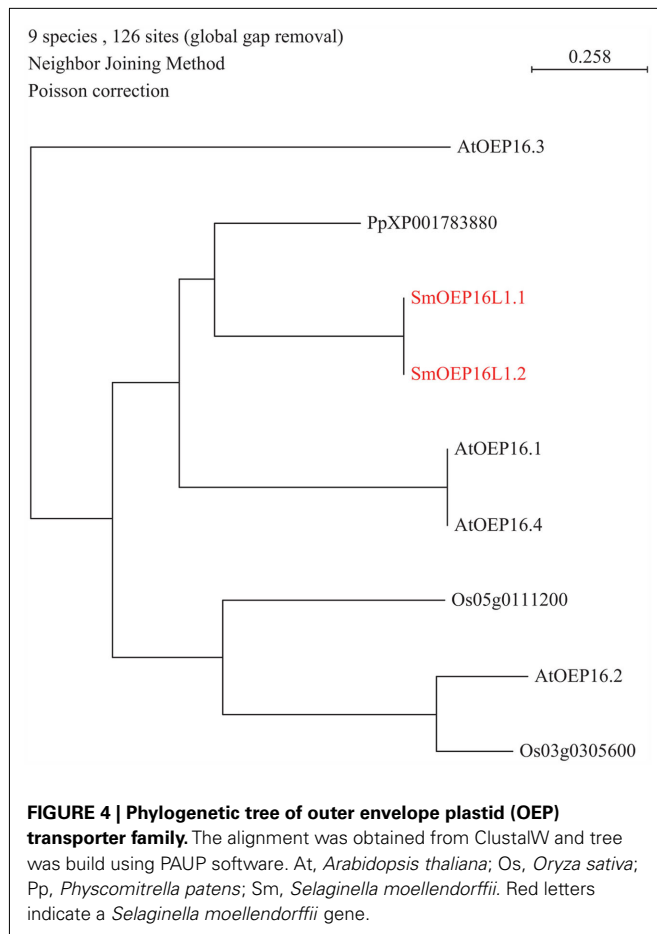
## RESULTS AND DISCUSSION
### AMINO ACID TRANSPORT IN *SELAGINELLA*
Analysis of the *S. moellendorffii* genome indicated the presence
of nine amino acid transporters belonging to the APC trans-
porter superfamily (**Table 1**; **Figure 1**), four belonging to the
ʟ-type Amino acid Transporter clade (respectively annotated as
LAT1.1 (and a putative allele LAT1.2), LAT2.1 (and a putative
allele LAT2.2), LAT3.1 (and a putative allele LAT3.2), and LAT4.1
(and a putative allele LAT4.2), and five belonging to the CAT
clade (respectively annotated as CAT1.1 (and a putative allele
CAT1.2), CAT2.1 (and a putative allele CAT2.2), CAT3.1 (and a
putative allele CAT3.2), CAT 4.1 (and a putative allele CAT4.2),
and CAT5.1 (and a putative allele CAT5.2). Thus *Selaginella* pos-
sesses the same number of LATs as rice (4), which is similar to
the number found in *Arabidopsis* (5) (**Table 1**). In *Physcomitrella
patens* only three LAT homologs were found whereas none were
found in the *Chlamydomonas reinhardtii* genome. According to
our analyses, and to be able to perform a direct comparison, the
annotation in *Physcomitrella* needs to be improved, since in many
cases the start codon does not appear to be assigned correctly,

**FIGURE 3 | Phylogenetic tree of mitochondrial basic amino acid carriers (mBAC) transporter family.** The alignment was obtained from ClustalW and tree was build using PAUP software. At, *Arabidopsis thaliana*; Cr, *Chlamydomonas reinhardtii*; Os, *Oryza sativa*; Pp, *Physcomitrella patens*; Sm, *Selaginella moellendorffii*. Red letters indicate a *Selaginella moellendorffii* gene.

putative sequencing errors may have led to apparent frame shifts, and in several cases intron locations are possibly not assigned correctly. The *Selaginella* annotation carried out here and the gene models may prove useful for improving the *Physcomitrella* genome annotation.

Concerning the second clade of amino acid transporters from the APC superfamily the situation is different (**Figure 1**). Less CAT type transporters were found in mosses and green algae (*Selaginella*: 5, *Physcomitrella*: 6, *Chlamydomonas*: 3) compared to 8 in rice, and 10 in *Arabidopsis* (**Table 1**).

**FIGURE 4 | Phylogenetic tree of outer envelope plastid (OEP) transporter family.** The alignment was obtained from ClustalW and tree was build using PAUP software. At, *Arabidopsis thaliana*; Os, *Oryza sativa*; Pp, *Physcomitrella patens*; Sm, *Selaginella moellendorffii*. Red letters indicate a *Selaginella moellendorffii* gene.

## AMINO ACID TRANSPORTERS IN OTHER GENE FAMILIES

Several other protein families appear to be responsible for transport across the membranes of organelles. Transporters have been identified that facilitate amino acid transport from the cytosol into plastids. In the outer envelope, OEP16 from pea forms a cation-selective high-conductance channel with a strong bias for amino acids and amines (Pohlmeyer et al., 1997). So far, the only amino acid transporter identified to operate at the inner plastid envelope is DiT2.1 (dicarboxylate transport), belonging to the divalent anion:Na$^+$ symporter (DASS) family. DiT2.1 functions in glutamate/malate exchange essential for the photorespiration (Renné et al., 2003).

Mitochondrial amino acid transporters have been found in plants that, like the yeast and animal mitochondrial transport systems, are members of the mitochondrial carrier family (MCF). In *Arabidopsis*, two AtmBACs (mitochondrial basic amino acid carriers) have been functionally characterized, and their substrate selectivity resembles that of the corresponding human proteins (Catoni et al., 2003).

### AtDIT2 homologs

So far, the only amino acid transporter identified to operate at the inner plastid envelope is AtDiT2.1 (dicarboxylate transport), belonging to the DASS family. DiT2.1 functions in glutamate/malate exchange essential in the photorespiratory pathway;

transport activity for its closest homolog, AtDiT2.2, has not been demonstrated yet. Analysis of *S. moellendorffii* genome indicates the presence of three putative DIT2 amino acid transporters (DIT2L2.1, DIT2L2.2, and DIT2L3.1 (putative paralog DIT2L3.2; **Table 1**; **Figure 2**). DIT2L2 is most closely related to DIT2L3.1 and 3.2. It is interesting to note that a similar numbers of DIT2s is found in mosses and algae (*Selaginella*: 3, *Physcomitrella*: 4, *Chlamydomonas*: 2) as in both *Arabidopsis* (2) and rice (3) (**Table 1**).

### AtmBAC1 homologs

Mitochondrial amino acid transporters have been found in plants that, as in yeast and animals, are members of the MCF. In *Arabidopsis*, two AtmBACs (mitochondrial basic amino acid carriers) have been functionally characterized. Their substrate selectivity resembles that of the corresponding human proteins. Up to 10 genes homologous to AtmBAC1 were found in *S. moellendorffii* (**Table 1**; **Figure 3**). At present, it is not possible to determine which of these 10 members of the MCF may transport amino acids, because MCFs transport a wide spectrum of compounds.

### AtOEP16.1 homologs

A major part of amino acid assimilation and biosynthesis of plants occurs in plastids. Amino acids pass the outer envelope via OEP proteins forming cation-selective high-conductance channels permeable to amines and amino acids. A single OEP16 homolog was found in both the *S. moellendorffii* and *P. patens* genomes (**Table 1**; **Figure 4**), whereas no homolog was found in the *C. reinhardtii* genome. Notably, part of the nitrogen assimilation pathway is cytosolic in algae (Fischer and Klein, 1988). It will be interesting to study amino acid transport across the plastid membrane in green algae to determine which proteins may be responsible for transport of amino acids across the outer envelope. Mosses and higher plants may have shifted nitrogen assimilation to the plastid and may thus have required high capacity transporters such as OEP16 to sustain high flux rates.

## CONCLUSION

Taken together, the haploid genome of the spikemoss *Selaginella* contains at least 23 amino acid transporter homologs. In addition, *Selaginella* also contains a single homolog of the plastid amino acid transporters (AtOEP16.1 homologs) whereas *Chlamydomonas* contains none, which could reflect evolutionary differences in the physiology of amino acid metabolism and transport from green algae to mosses to higher plants. Unfortunately, at present it is not possible to assess the physiological function of these transporters in *Selaginella* due to the lack of tools for gene replacement and transformation. However the genome of the moss *Physcomitrella* appears to contain a similar complement of amino acid transporters, and all necessary tools for analyzing the physiological function of these genes are available. The identification of the amino acid transporters from the "ancient" *Selaginella* and the understanding of their functions may contribute to our understanding of the evolution of nitrogen compartmentation and translocation.

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