

## **A computational analysis of Salt Overly Sensitive 1 homologs in halophytes and glycophytes**

Cherin E. Kim<sup>1</sup> and Ray A. Bressan<sup>2</sup>

<sup>1</sup> West Lafayette Jr./Sr. High School, West Lafayette, IN, USA

<sup>2</sup> Department of Horticulture and Landscape Architecture, Purdue University, West Lafayette, IN, USA

Corresponding Author:

Ray A. Bressan

Department of Horticulture and Landscape Architecture, Purdue University, 625 Agriculture Mall, West Lafayette, IN, 47907, USA

bressan@purdue.edu

**Abstract**

Soil salinity is one of the most serious impediments to global agricultural productivity. Although most terrestrial plants are glycophytes which cannot tolerate high salt concentrations, a small fraction of species are halophytes. Exactly what allows these extremophile plants to survive in saline conditions is not yet well understood. Several studies have established the Salt Overly Sensitive (SOS) pathway as the canonical model for the mechanism responsible for salt tolerance. The SOS pathway involves interplay among  $\text{Na}^+$ - $\text{H}^+$  antiporters for transporting sodium, and the activation of the kinase that phosphorylates the transporter. Among them, SOS1, a plasma membrane  $\text{Na}^+$ - $\text{H}^+$  antiporter, has been shown to be a critical component for maintaining salt homeostasis by pumping sodium out of cells upon activation. Therefore, it is of great interest to evaluate any differences of SOS1 in halophytes as compared to glycophytes. Here we report a computational analysis of the primary and secondary structures of eight halophytes and seven glycophytes. ClustalW alignment of the protein sequences as a whole reveals no regions conserved specifically in only halophytes or in only glycophytes. In addition, the key regulatory residues at the C-terminus of SOS1, S1136 and S1138, which were shown to be the phosphorylation sites by the kinase SOS2, were completely conserved in all 15 halophytes and glycophytes. The four amino acids, G136, R365, G777, and G784, in which alterations affect the function of SOS1, are mostly conserved in the 15 species. The 14-3-3 binding site in the C-terminus which is important in the phosphorylation step of SOS1 in the SOS signal transduction cascade is also well conserved. Furthermore, the number of transmembrane helices for each species is between 9 and 12 and there is no significant difference between halophytes and glycophytes. If halophytes present any special feature of SOS1, it likely involves the presence (halophytes) or absence (glycophytes) of a SOS1-interacting component.

## Introduction

Salt stress is a major abiotic stress factor that significantly impacts agricultural output. Approximately 20% of the world's cultivated land and nearly half of all irrigated lands are affected by salinity (Zhu, 2001). High salinity can adversely affect plants by inducing physiological responses at the cellular level such as ion imbalance and hyperosmotic stress as well as by altering outward physical characteristics through wilting, drying, and death (Zhu, 2001). However, plants differ in their ability to tolerate high salt concentrations. Halophytes, which make up around 1% of the world's flora, are an extremophile group of plants that are able to survive in highly saline soils (Flowers and Colmer, 2008; Flowers and Colmer, 2015). In contrast, the majority of plants, including most commercial crops, are glycophytes, which are not necessarily salt-intolerant but cannot survive in environments where the salt concentration is too high (Garg et al., 2013; Ji et al., 2013). Even so, the line separating halophytes from glycophytes is not discrete. There is actually a continuum of salt tolerance degrees with very salt-sensitive glycophytes at the low end and highly salt-tolerant halophytes at the high end (Rozema and Schat, 2012). Furthermore, within halophytes, many dicotyledonous halophytes grow optimally in 50-250 mM NaCl while monocotyledonous halophytes prefer less than 50 mM NaCl (Flowers and Colmer, 2008). We limit our species to dicotyledonous halophytes only in order to minimize any conflicting effects due to phylogenetics on salt tolerance ability.

The factors that make halophytes different from glycophytes are not yet well understood. However, the mechanism(s) by which plants respond to increased salt concentrations has been extensively studied. The protein kinase cascade involving Salt Overly Sensitive (SOS) 1, 2, and 3 has emerged as a predominant model for salt stress responses, as shown in Fig. 1. The SOS signal transduction pathway is activated when SOS3, a calcium-binding protein, binds with  $\text{Ca}^{2+}$ , which is well-known as a "warning" signal for  $\text{Na}^+$  toxicity (Ji et al., 2013; Zhu, 2001). SOS3 then interacts with and activates SOS2, a serine/threonine kinase, and SOS2 phosphorylates SOS1, a plasma membrane  $\text{Na}^+\text{-H}^+$  antiporter, resulting in  $\text{Na}^+$  being transported out from the cell.

SOS1 in particular has been identified as playing a key role in the reactions of plants to salinity stress. Mutants of SOS1 display stunted growth and significant leaf discoloration in environments with high  $\text{Na}^+$ , whereas transgenic *sos1-1* mutants containing the wild-type *SOS1* gene controlled by the cauliflower mosaic virus 35s promoter show no or little difference in

growth in salt compared with the wild type, demonstrating that the *SOS1* locus is necessary, but not necessarily sufficient, for salt tolerance (Shi et al., 2000). In addition, *SOS1* expression is up-regulated in plants in response to NaCl stress (Shi et al., 2000). The C-terminal region, where phosphorylation sites and 14-3-3- binding sites are located, is also proposed to be important for SOS1 function in plant salt tolerance (Shi et al., 2000).

In this study we present the comparison of SOS1 protein sequences of eight halophytes and seven glycophytes. We looked for distinctly conserved regions as a whole, at key mutation positions, and near the C-terminus, and we determined the number of transmembrane helices in each species to analyze secondary structure.

## Materials & Methods

SOS1 and SOS1 homologs were identified using the BLASTp search of the NCBI protein database, using previously identified SOS1 from *Arabidopsis* from The Arabidopsis Information Resource (TAIR) gene database (arabidopsis.org). The top BLASTp hit was used as the homolog for each species. Protein sequences were aligned using ClustalW alignment in the sequence alignment editor BioEdit. The number of transmembrane helices were determined using the TMHMM Server version 2.0 (<http://www.cbs.dtu.dk/services/TMHMM/>), which predicts transmembrane helices in proteins.

## Results & Discussion

To identify any regions conserved uniquely in either halophytes or glycophytes in SOS1, we first collected the SOS1 protein sequences of eight halophytes and seven glycophytes, using NCBI Blast with *Arabidopsis thaliana* SOS1 as a query. The halophyte species analyzed were *Eutrema parvulum*, *Eutrema halophila*, *Salicornia dolichostachya*, *Aeluropus littoralis*, *Suaeda salsa*, *Salicornia brachiata*, *Mesembryanthemum crystallinum*, and *Distichlis spicata* (Table 1). The glycophyte species analyzed were *Arabidopsis thaliana*, *Solanum lycopersicum*, *Oryza sativa* Indica group, *Sorghum bicolor*, *Gossypium hirsutum*, *Selaginella moellendorffii*, and *Physcomitrella patens* (Table 1). ClustalW alignment was used to compare the sequences at the primary level. Although there is variation among species, no differentiating regions between halophyte and glycophyte protein sequences were found at the primary level. That is, there were

no regions where all of the halophyte sequences had the same (or similar) amino acid and all of the glycophyte sequences did not have that amino acid, or vice versa (Fig. 2).

Several *sos1* mutant alleles in *Arabidopsis thaliana* were initially isolated from a mutant screen looking for mutants having altered salt response. When treated with 100mM NaCl, *sos1* mutants showed sensitive responses such as arrested growth, chlorosis in older leaves, and dark color in young leaves. The genetic mapping study revealed that four of these alleles (*sos1-3*, *sos1-8*, *sos1-9*, and *sos1-12*) had missense mutations in SOS1 which result in amino acid substitutions such as Gly 136 to Glu, Arg 365 to Cys, Gly 777 to Glu, and Gly 784 to Asp in *sos1-3*, *sos1-8*, *sos1-9*, and *sos1-12*, respectively. This indicates that the amino acids at these particular positions are crucial (Shi et al., 2000). Sequence alignment showed that amino acids are mostly conserved at these crucial missense mutation areas (Fig. 3A). At region A, only *S. brachiata* showed a deviation from the amino acids as its sequence had arginine instead of glycine at this position. Mutation regions B and D were completely conserved in all species and at region C, there was another substitution of arginine for glycine, this time with *S. lycopersicum*.

It has been proposed that there are key regulatory residues at the C-terminus of SOS1. Previously, 1136 Serine and 1138 Serine in the C-terminus of Arabidopsis SOS1 were shown to be the phosphorylation sites by SOS2 (Quintero et al., 2010). These two amino acid positions were completely conserved in all 15 halophytes and glycophytes (Fig. 3B). Arabidopsis SOS2 phosphorylates these serine residues and facilitates binding by a 14-3-3 protein. An alignment of the SOS1 protein sequences of *A. thaliana* and *P. patens* shows strong conservation, suggesting a common mechanism of sodium tolerance shared by plants (Kleist et al., 2014). The sequences were also strongly conserved at this 14-3-3 binding site in the C-terminus (Fig. 3B). These data show that since both the sequences as a whole, key mutation areas, and important binding sites are all mostly totally conserved, halophyte-glycophyte differences are most likely not evident at the primary sequence level.

The number of transmembrane helices was determined for each species. All species had between 9 and 12 transmembrane helices but no distinct pattern in either glycophytes or halophytes was found.

In conclusion, there appears to be no major differences in SOS1 between halophytes and glycophytes at the primary and secondary levels. Considering that SOS1 is required but not sufficient for improved salt tolerance in other species as well as SOS1's pivotal role in the

signaling cascade, the regulation interplay of *SOS1* and related genes, rather than their sequences or structures, should be further investigated to differentiate halophytes and glycophytes from a molecular perspective. Alternatively, a comparison of *SOS1*-interacting proteins obtained from halophytic and glycophytic species may be a more promising strategy to find factors that affect the differential role of *SOS1* in halophytes and glycophytes.

With the explosion of the size of the human population in recent years, it has become increasingly relevant to explore alternatives to traditional food sources. Considering that a large percentage of land cannot be used to cultivate agricultural crops due to high soil salinity, halophytes may be used as models to transgenetically improve conventional crops or be domesticated for mass consumption in their own right (Flowers and Colmer, 2015). Therefore, further research involving halophytes is necessary in order to understand the underlying mechanisms of plant salt tolerance.

## References

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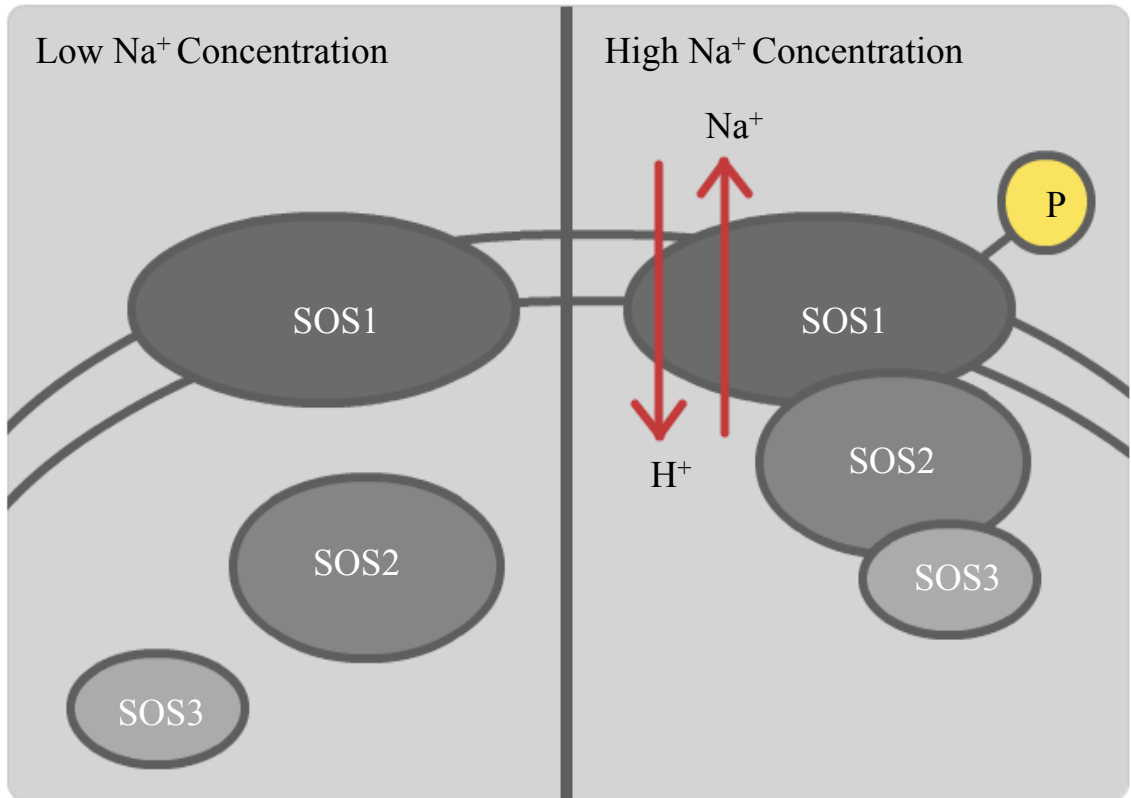
Zhu JK. 2001. Plant salt tolerance. *Trends in Plant Science* 6:66-71.

**Table 1.** SOS1 homologs of 9 halophytic species and 8 glycophytic species.

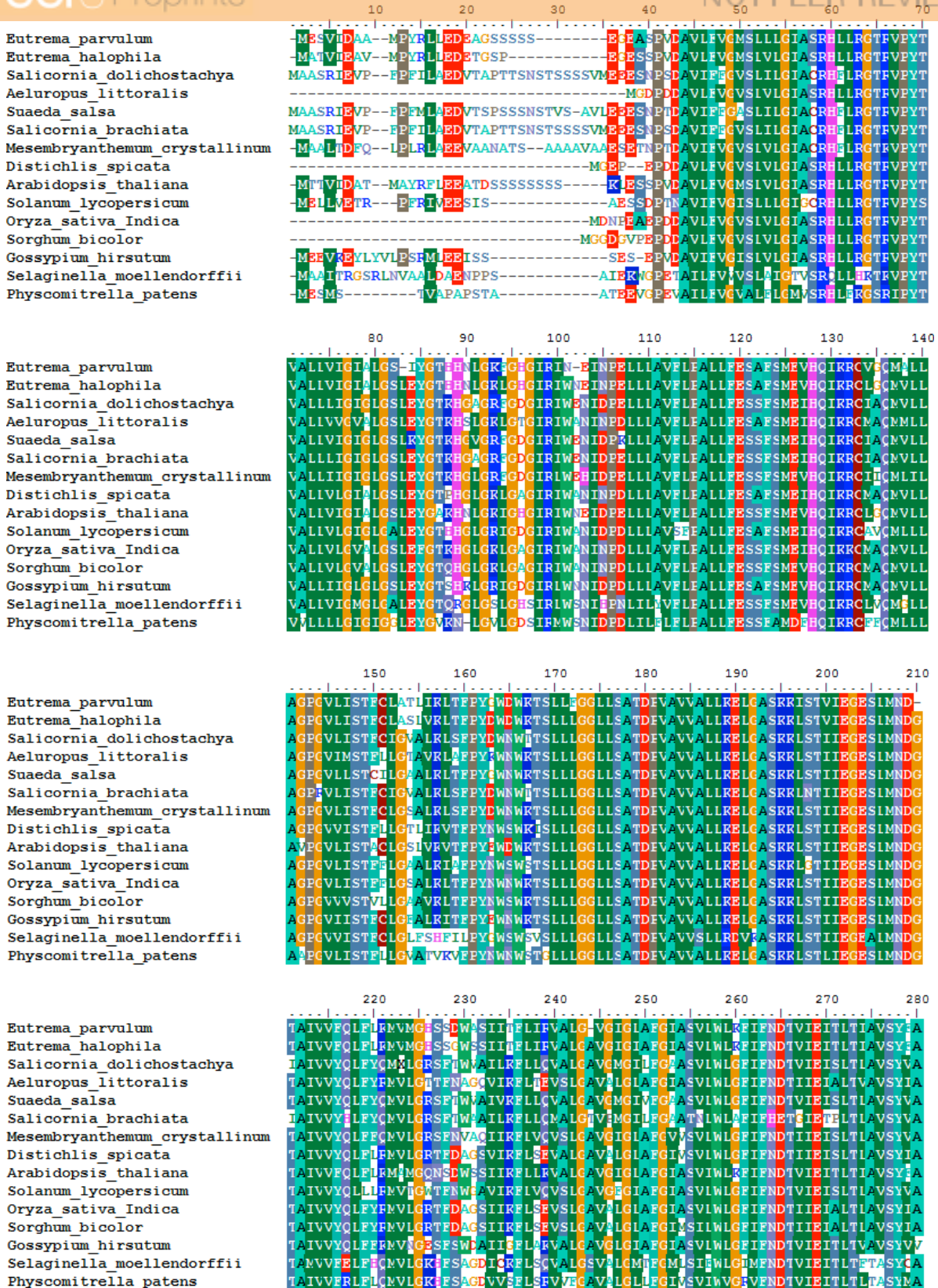
Species Name	Reference	Number of transmembrane helices	Gene Name	Resources
<b>Halophytes</b>				
<i>Eutrema parvulum</i>	Oh et al. (2010)	12	>gi 312190386 gb ADQ43186.1  salt overly sensitive 1 [Schrenkiella parvula]	http://thellungiella.org, NCBI
<i>Eutrema halophila</i>	Wang et al. (2006)	12	>gi 229914867 gb ACQ90592.1  salt overly sensitive 1-like protein [Eutrema halophilum]	http://thellungiella.org, NCBI
<i>Salicornia dolichostachya</i>	Rozmea and Schat (2013)	11	>gi 781786798 emb CDL70804.1  plasma membrane Na <sup>+</sup> /H <sup>+</sup> antiporter [Salicornia dolichostachya]	NCBI
<i>Aeluropus littoralis</i>	Rozmea and Schat (2013)	13	>gi 359843922 gb AEV89922.1  plasma membrane Na <sup>+</sup> /H <sup>+</sup> antiporter [Aeluropus littoralis]	NCBI
<i>Suaeda salsa</i>	Rozmea and Schat (2013)	12	>gi 584609172 gb AHJ14584.1  Na <sup>+</sup> /H <sup>+</sup> antiporter [Suaeda salsa]	NCBI
<i>Salicornia brachiata</i>	Rozmea and Schat (2013)	10	>gi 214028396 gb ACJ63441.1  salt overly sensitive 1 [Salicornia brachiata]	NCBI
<i>Mesembryanthemum crystallinum</i>		10	>gi 124301347 gb ABN04858.1  salt-overly-sensitive 1 [Mesembryanthemum crystallinum]	NCBI
<i>Distichlis spicata</i>		12	>gi 301087449 gb ADK60916.1  plasma membrane Na <sup>+</sup> /H <sup>+</sup> transporter [Distichlis spicata]	NCBI
<b>Glyphytes</b>				
<i>Arabidopsis thaliana</i>	Essah et al. (2003)	9	>gi 22325422 ref NP_178307.2  sodium/hydrogen exchanger 7 [Arabidopsis thaliana]	https://www.arabidopsis.org
<i>Solanum lycopersicum</i>		12	>gi 350535282 ref NP_001234698.1  plasmalemma Na <sup>+</sup> /H <sup>+</sup> antiporter [Solanum lycopersicum]	NCBI
<i>Oryza sativa Indica group</i>	Rozmea and Schat (2013)	12	>gi 218187326 gb EEC69753.1  hypothetical protein OsI_39290 [Oryza sativa Indica Group]	NCBI
<i>Sorghum bicolor</i>	Wang et al. (2014)	12	>gi 242086498 ref XP_002443674.1  hypothetical protein SORBIDRAFT_08g023290 [Sorghum bicolor]	NCBI
<i>Gossypium hirsutum</i>	Rozmea and Schat (2013)	12	>gi 846328855 gb AKN19929.1  salt overly sensitive 1 [Gossypium hirsutum]	NCBI
<i>Selaginella moellendorffii</i>		11	>gi 302753756 ref XP_002960302.1  hypothetical protein SELMODRAFT_75049, partial [Selaginella moellendorffii]	NCBI
<i>Physcomitrella patens</i>		12	>gi 33352144 emb CAD91921.1  putative Na/H antiporter [Physcomitrella patens]	NCBI





**Figure 1. Model for salt response in plants.**

Under highly saline conditions, the Salt Overly Sensitive (SOS) pathway is triggered when SOS3, a calcium-binding protein, activates SOS2, a serine/threonine kinase, which in turn phosphorylates SOS1, a Na<sup>+</sup>/H<sup>+</sup> antiporter.



**Figure 2. Amino acid sequence alignment of 8 halophytic and 7 glycophytic species.**

Figure 2 (continued)

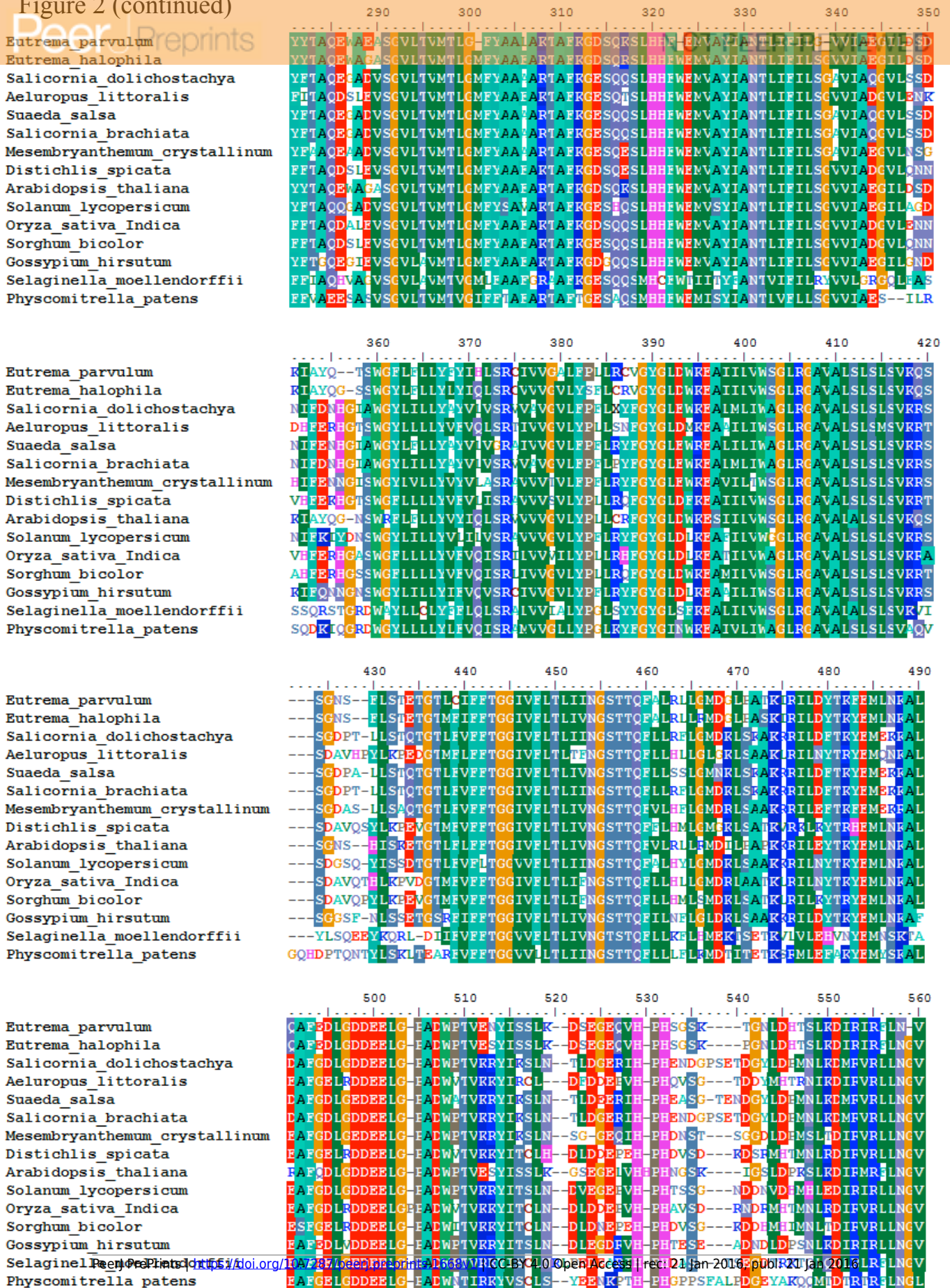
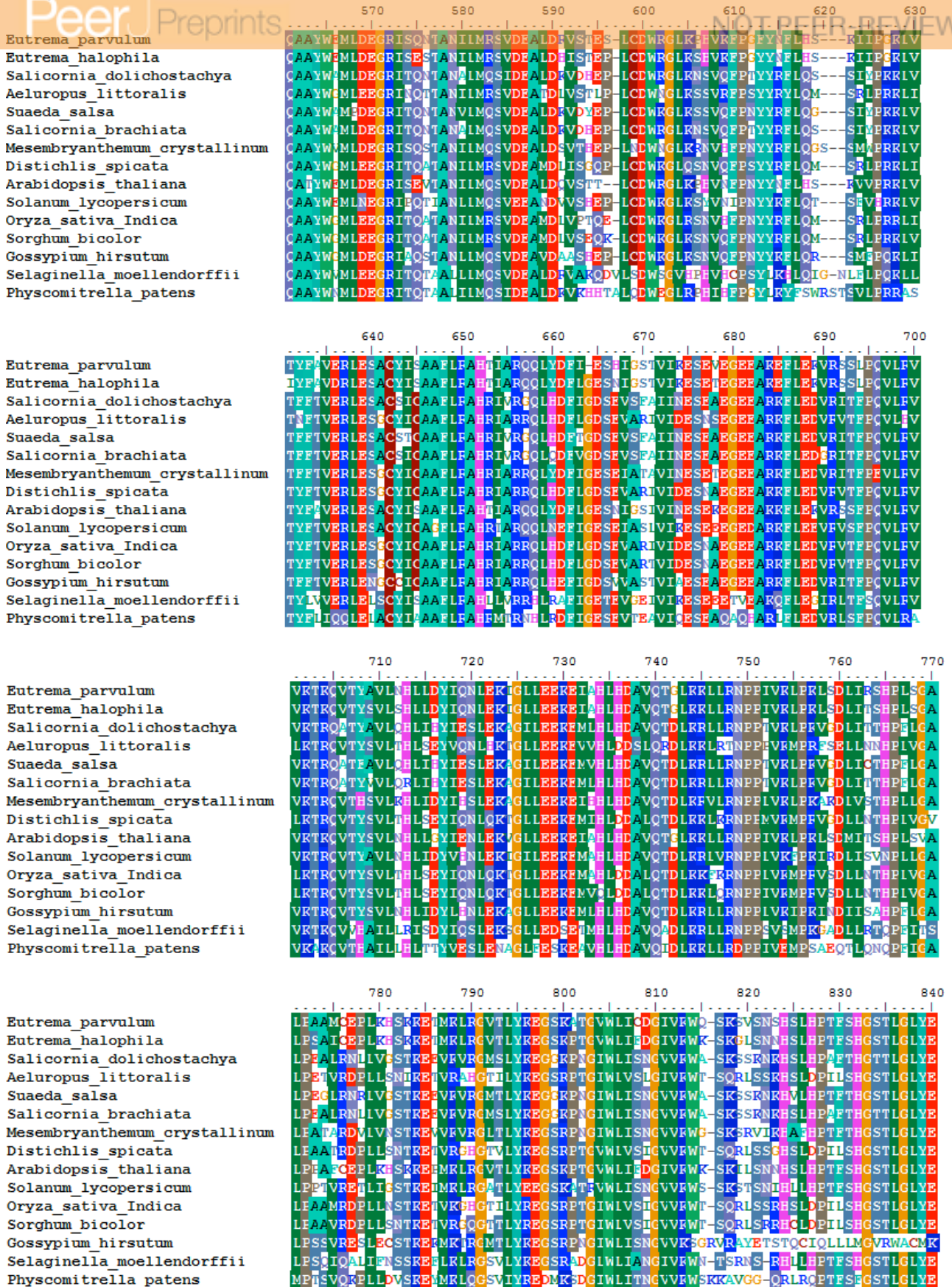


Figure 2 (continued)



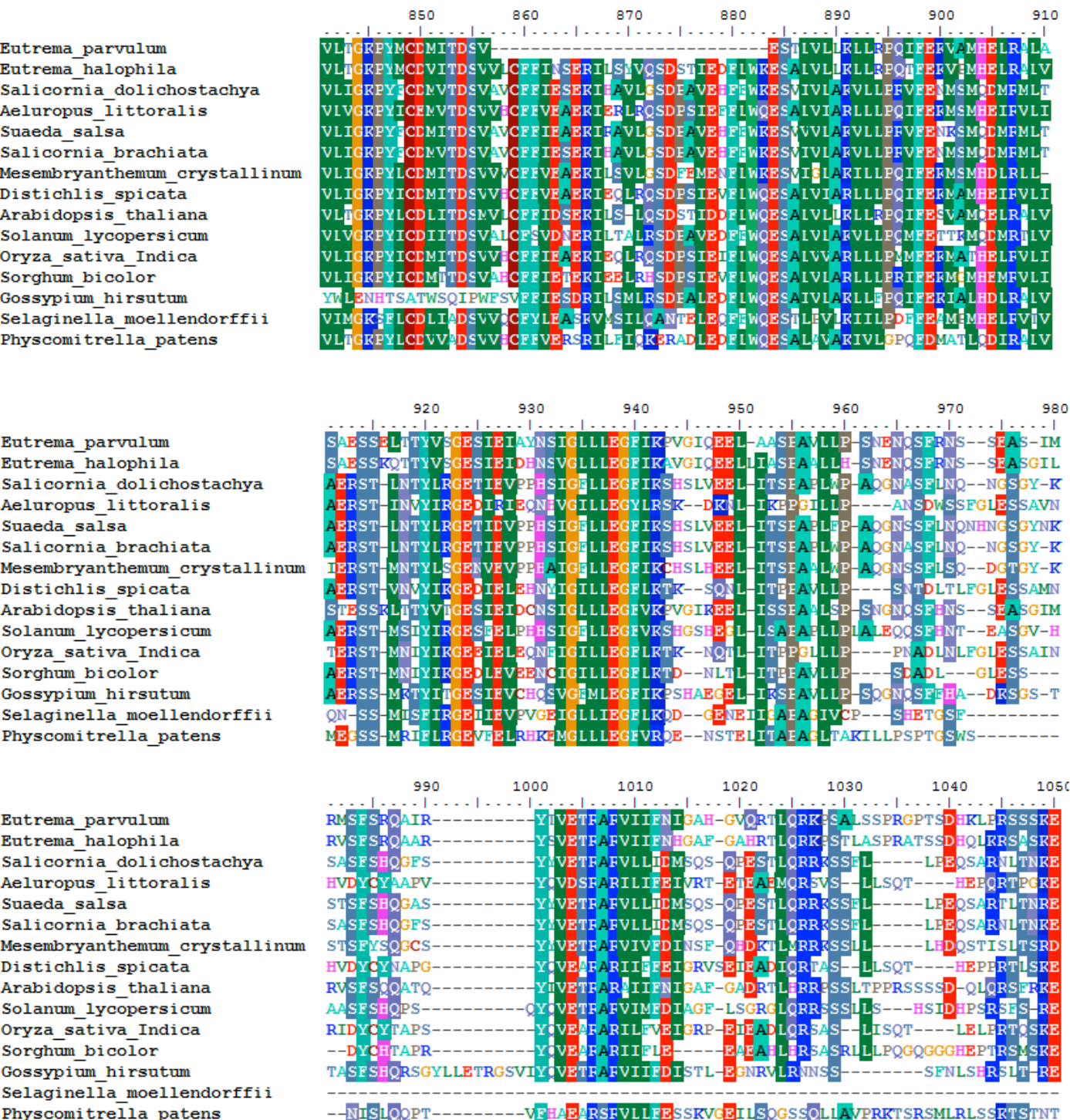
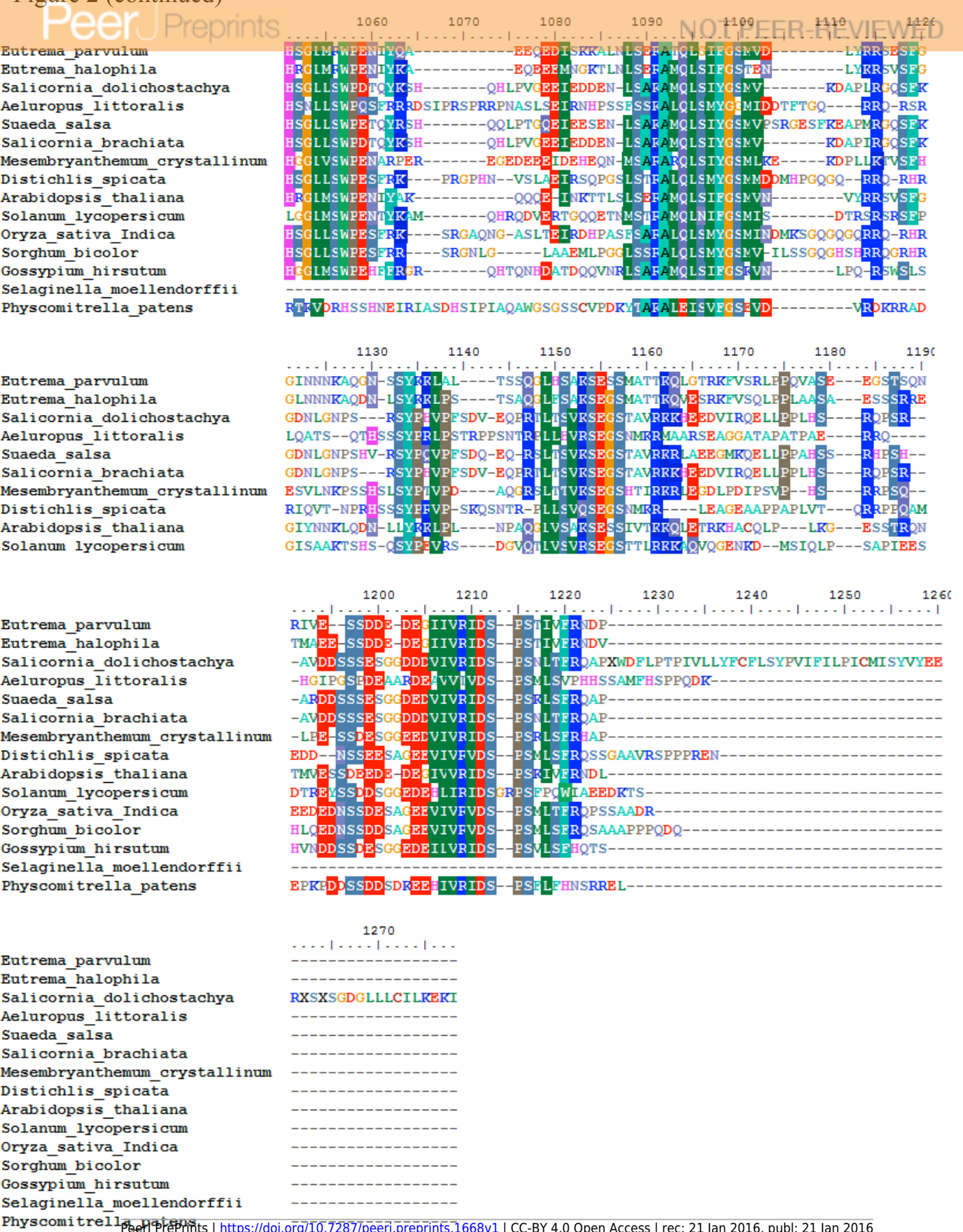
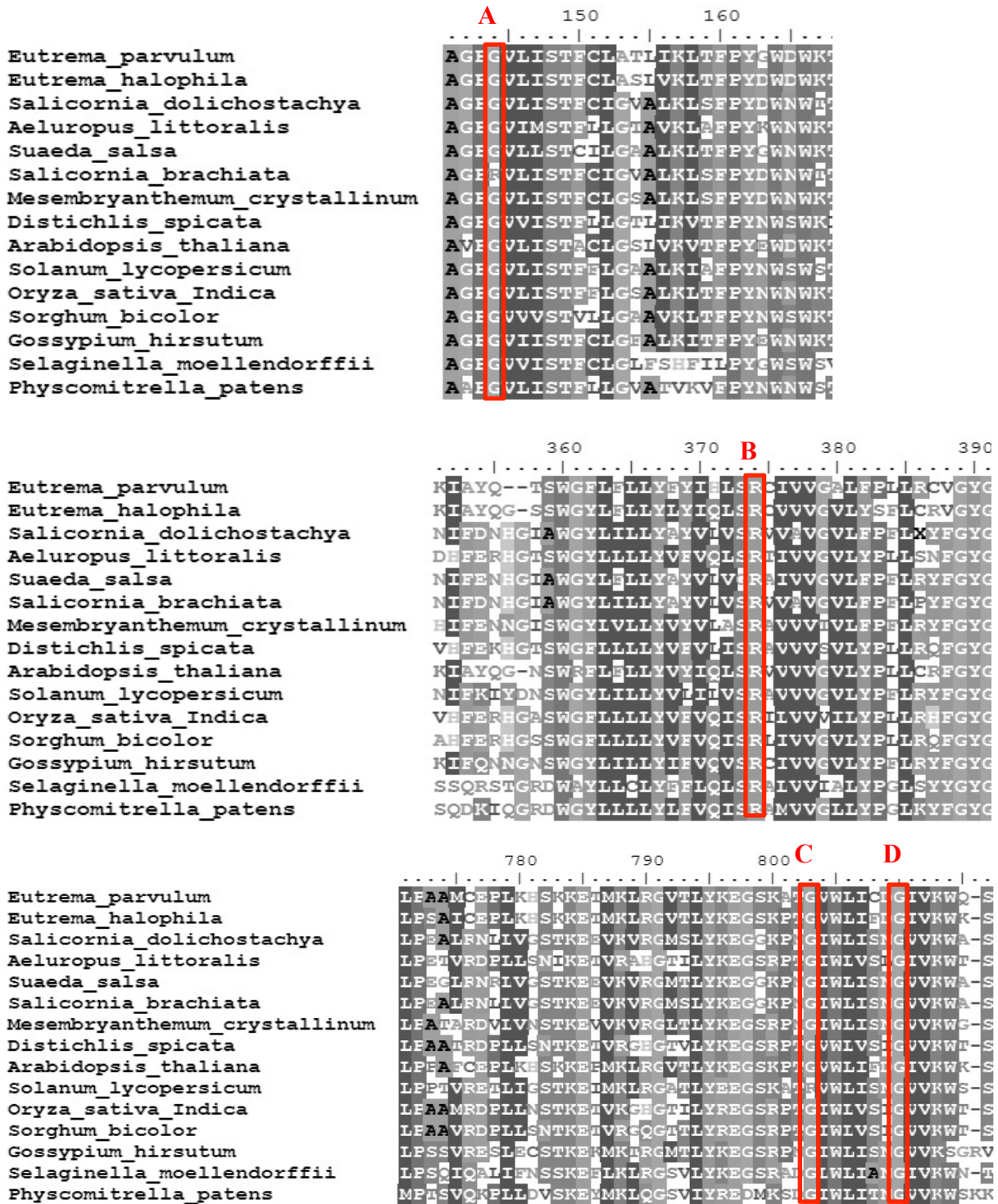
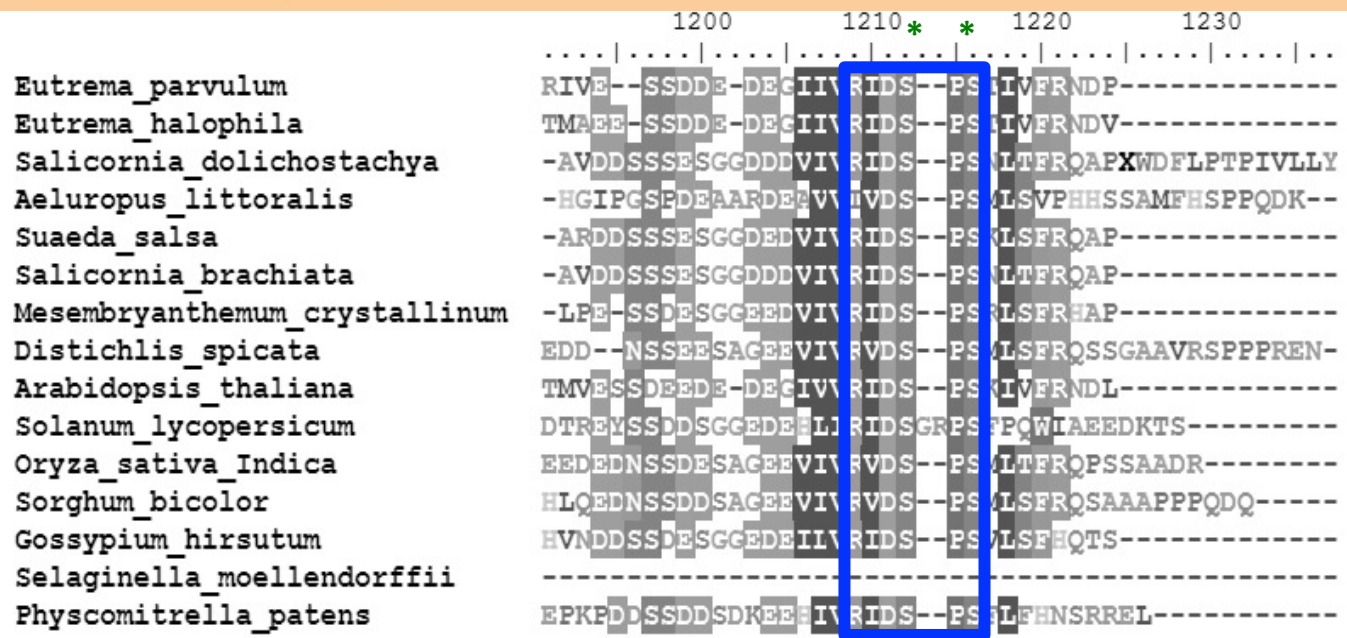


Figure 2 (continued)









**Figure 3. Crucial amino acid positions.**

3A. A-D. *sos1* missense mutation positions.

3B. SOS2 phosphorylation sites.