

Figure S1. CENH3 nucleotide sequences from barley species virtually translated into proteins and aligned. Accession numbers are given in Table S1. The sequence of canonical histone H3.3 in *H. vulgare* was retrieved from GenBank (accession number 769350).

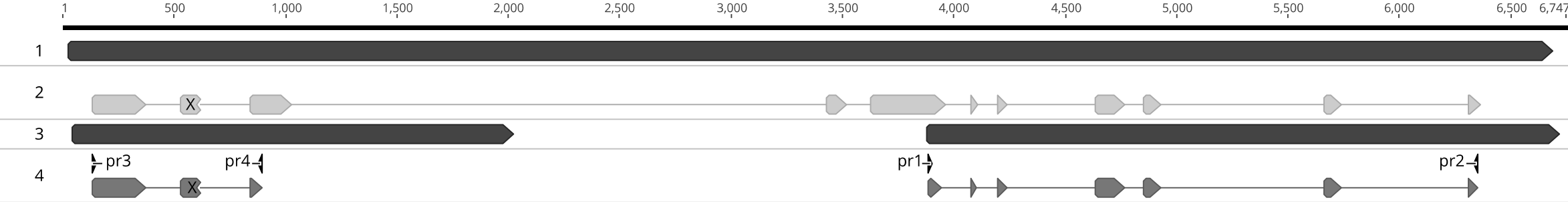
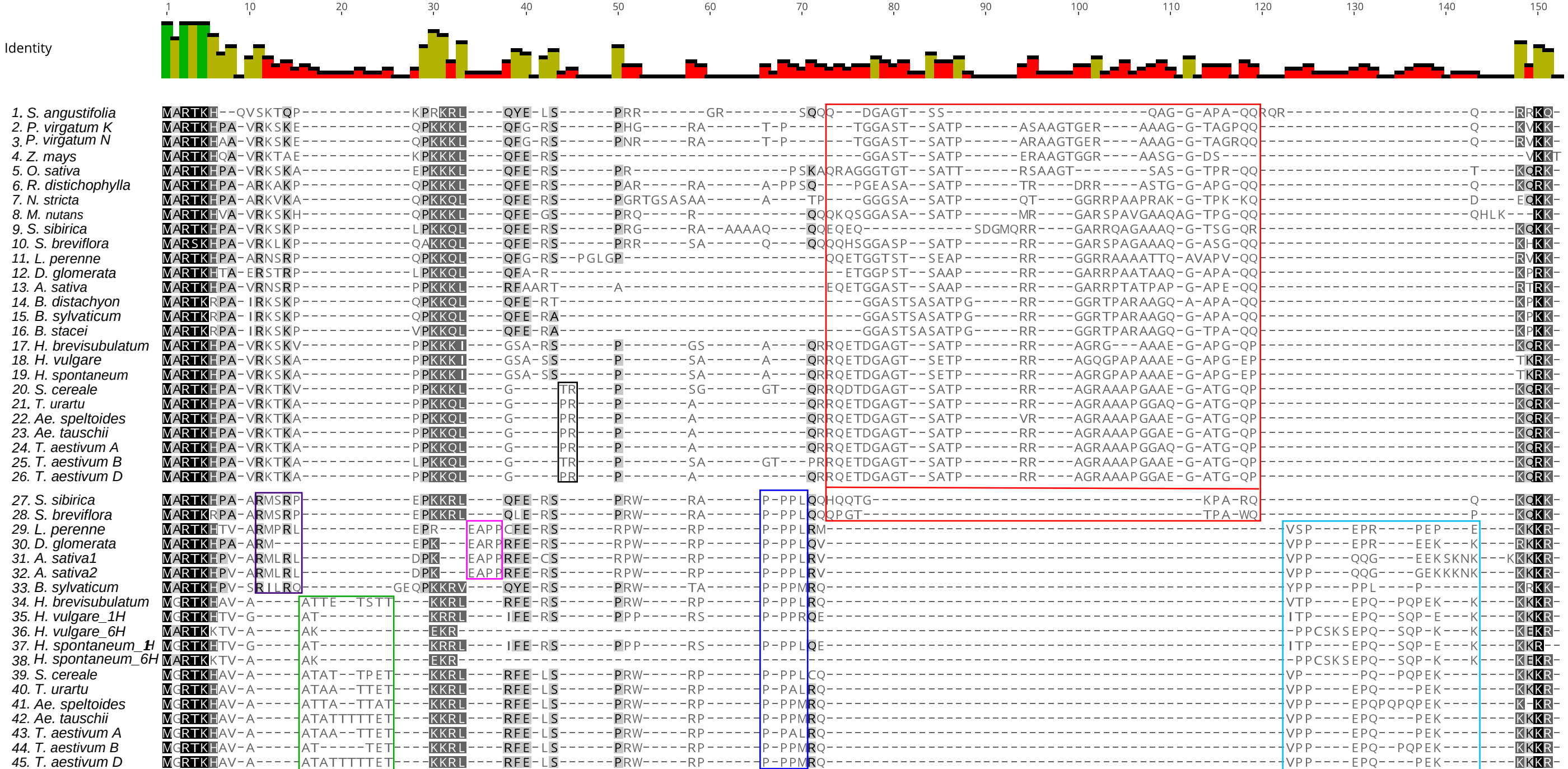


Figure S3. Experimental confirmation of the presence of a stop codon in exon 2 of β CENH3 in *B. distachyon*.

1. A schematic of locus LOC100830307 retrieved from GenBank. 2. A schematic of a 1776-bp transcript from the locus. Exons appear as white finger-post arrows. 3. A schematic of sequences in locus LOC100830307 homologous to β CENH3 and α CENH3 in the rye genome [25]. 4. Products of RT-PCR with primers of 1 and 2 for α CENH3 and primers 3 and 4 for β CENH3. Arrows point to the primer positions, stop codons are shown by cross. The upper scale shows the dimensions in bp.



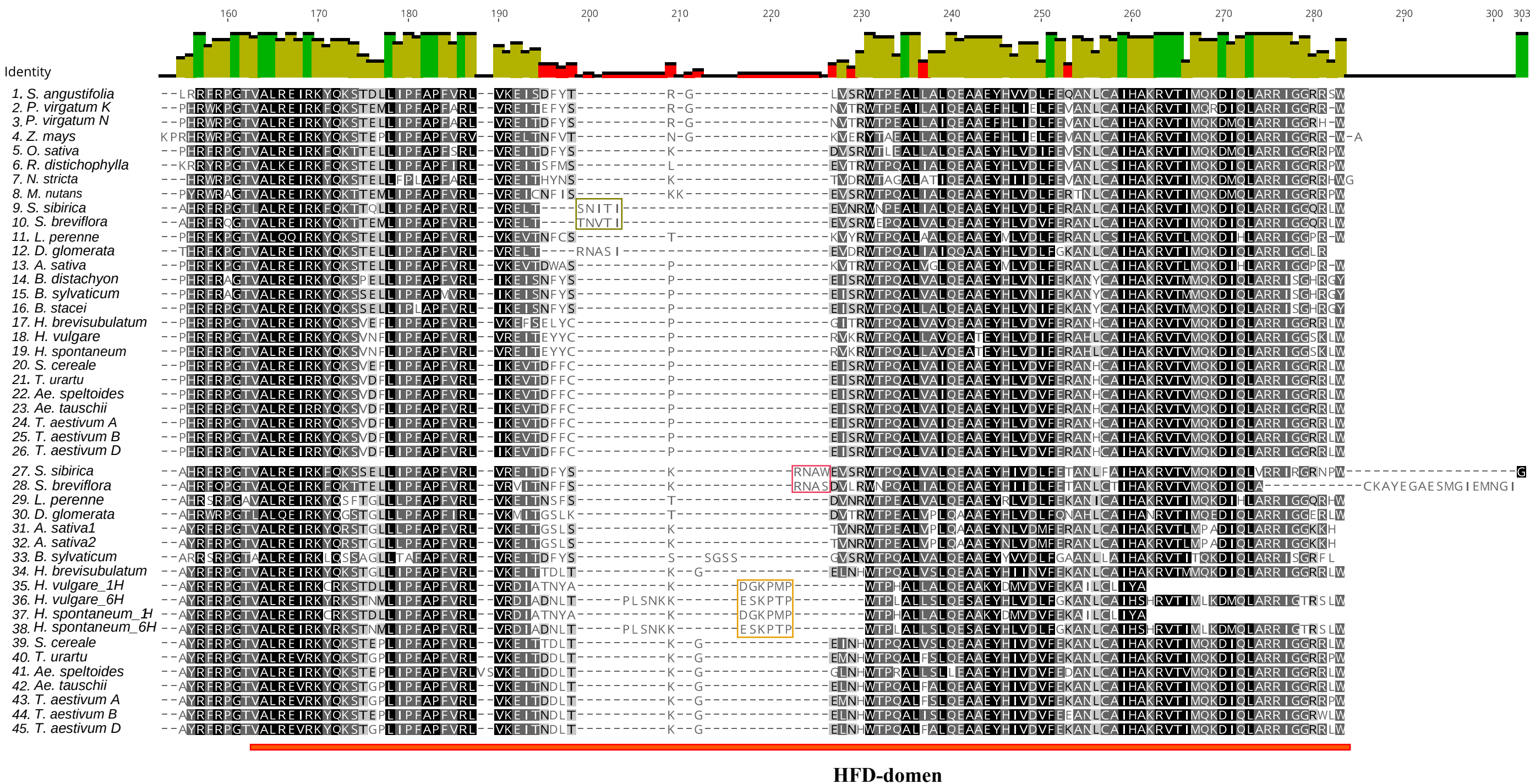


Figure S4. A multiple alignment of the amino acid sequences of the CENH3 proteins using the Bali-Phy program [35]. BALi-Phy is software that estimates multiple sequence alignments and evolutionary trees from DNA, amino acid, or codon sequences. It uses likelihood-based evolutionary models of substitutions and insertions and deletions to place gaps. Redelings showed that BALi-Phy had 3.5 times fewer alignment errors than MUSCLE and MAFFT on simulated data [35]. For convenience, the alpha and beta forms are grouped into two separate blocks: 1 – 26 (α CENH3) and 27 – 45 (β CENH3). Amino acid residues identical in all species are shown as white letters on the black background. Amino acid motifs specific for certain tribes and genera are contained within rectangular boxes with different border colors.

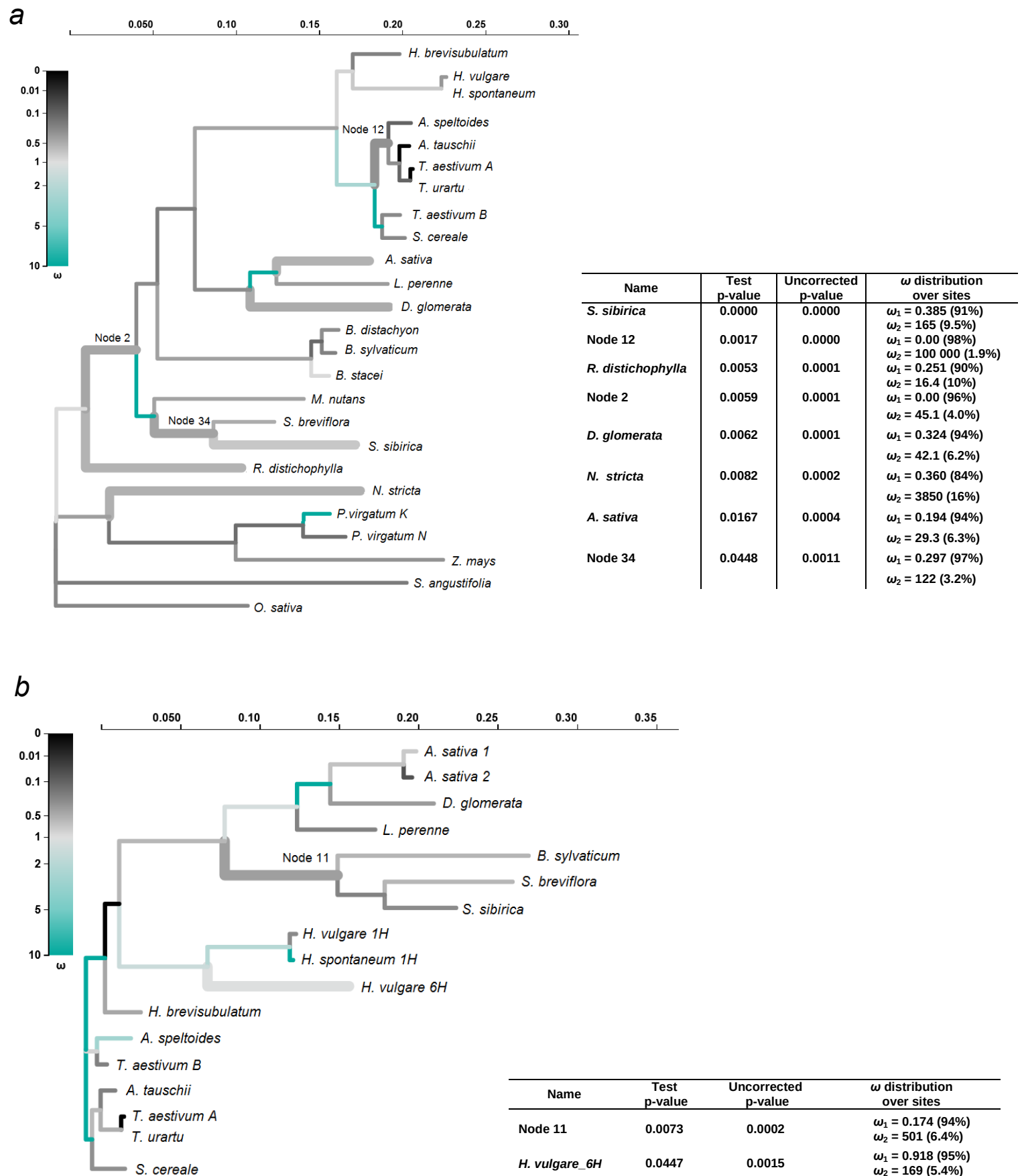


Figure S5. Branch-site (BSREL) analysis of positive selection for the α CENH3 (a) and β CENH3 (b) [40]. The upper scale indicates the length of branches. Branch lengths are scaled to the expected number of substitutions per amino acid and branch colors indicate the strength of selection (dN/dS or ω). Green, positive selection ($\omega > 5$); black, purifying selection ($\omega = 0$); gray, neutral evolution ($\omega = 1$). The proportion of each color represents the fraction of the sequence undergoing the corresponding class of selection. Branch thickness reflects the amount of statistical support for evolution under episodic diversifying positive selection as determined by BSREL. The tables show the branches and nodes in which BSREL found evidence of episodic diversifying selection at $P < 0.05$.