Revealing Phylogenetic Relationship Among *Secale* Species: Preliminary Results

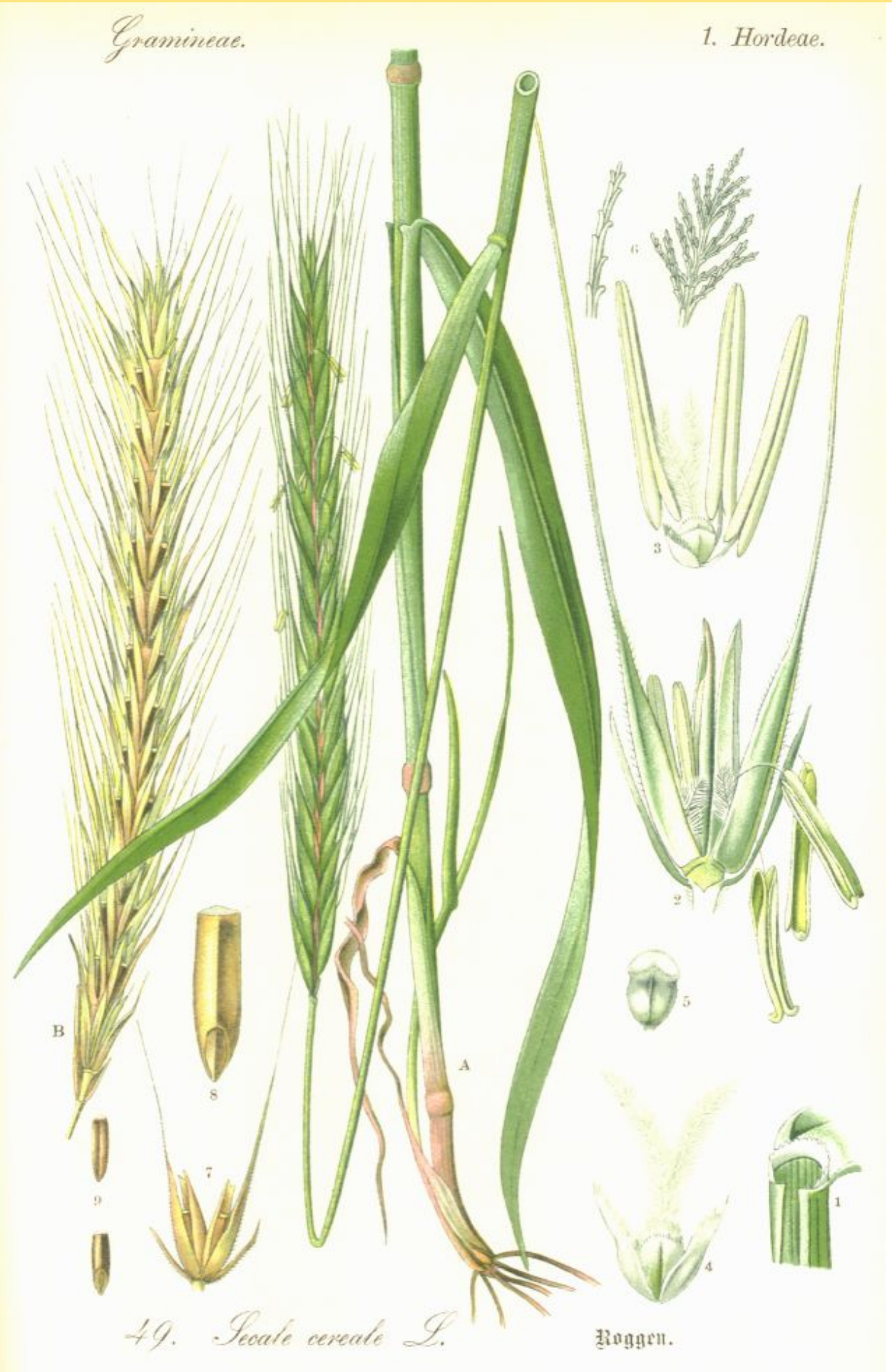
Öncü Maracı*, Raşit Bilgin, Hakan Özkan
*Boğazici University, Institute of Environmental Sciences, Hisar Kampüs, 34342, Bebek, İstanbul, Turkey.
Phone: +90 212 359 4601, E-mail: oncu.maraci@boun.edu.tr

Introduction

Genus *Secale* belongs to the grass tribe Triticeae of Poacea family and includes annual and perennial species. In addition to cultivated rye, weedy and wild species also belong to the genus. All of the taxa, except for a cultivated tetraploid form, are diploids with 14 chromosomes. Although taxonomy of the genus is still a matter of debate, according to most widely accepted classification, the taxon contains four species. *Secale cereale*, the annual outbreeder species, includes weedy and wild forms. Although the species has a broad distribution from Scandinavia to Southern Chile, it is a typical representative of Mediterranean flora. *Secale strictum* is a complex group containing both outbreding and inbreeding subspecies. *Secale vavilovii* is an annual and interbreeding species characterized by being shorter than the other species. *Secale sylvestre*, the annual, wild and self-pollinating species, is morphologically most distinct.

Although central Asia is accepted to be the center of genetic origin, exact timing and localities of beginning of rye cultivation and domestication is still a controversial issue. Similarly there is not a consensus about the evolutionary history of *Secale* genus. Studies employing genetic markers indicated that *Secale sylvestre* was the first species to separate from others in the course of evolution (Petersen, 1993; Chikmawati et al., 2005; Skuza et al., 2007) and it is the only species well separated.

Secale cereale and *S. vavilovii* are considered to have a common origin and diverged relatively recently (Cuadrado and Jouve, 2002). *Secale strictum* was shown to be the most propable ancestor of *S. cereale* (Rilley, 1955; Khush and Stebbins, 1961). *Secale vavilovii* is the intermediate form between perennial wild ryes and cultivated rye (Zohary, 1971). Based on mithocondrial RFLP analysis, Skuza et al. (2007) grouped genus *Secale* into two sections: first group includes two species with the highest genetic similarity *S. sylvestre* and *S. cereale segetale*. The second group includes the subspecies of *S. strictum*, *S. vavilovii*, and *S. cereale*. In this study, in order to elucidate the phylogenetic relationships among *Secale* species and to gain new insights about taxonomy and the degree of genetic diversity of the genus 142 different accessions were analyzed by nuclear SSR and chloroplastic SNP markers.



Otto Thome. 1903. Flora von Deutschland. Plate 49.

Material and Methods

Sample Collection

In this study, a total of 142 different accessions of *Secale* genus were investigated including landraces and improved varieties of cultivated rye, wild, and weedy forms of *S. cereale*, *S. vavilovii*, *S. strictum,* and *S. sylvestre*) from different eco-geographical areas with a concentrated focus on Turkey and Fertile Crescent (Fig. 1).

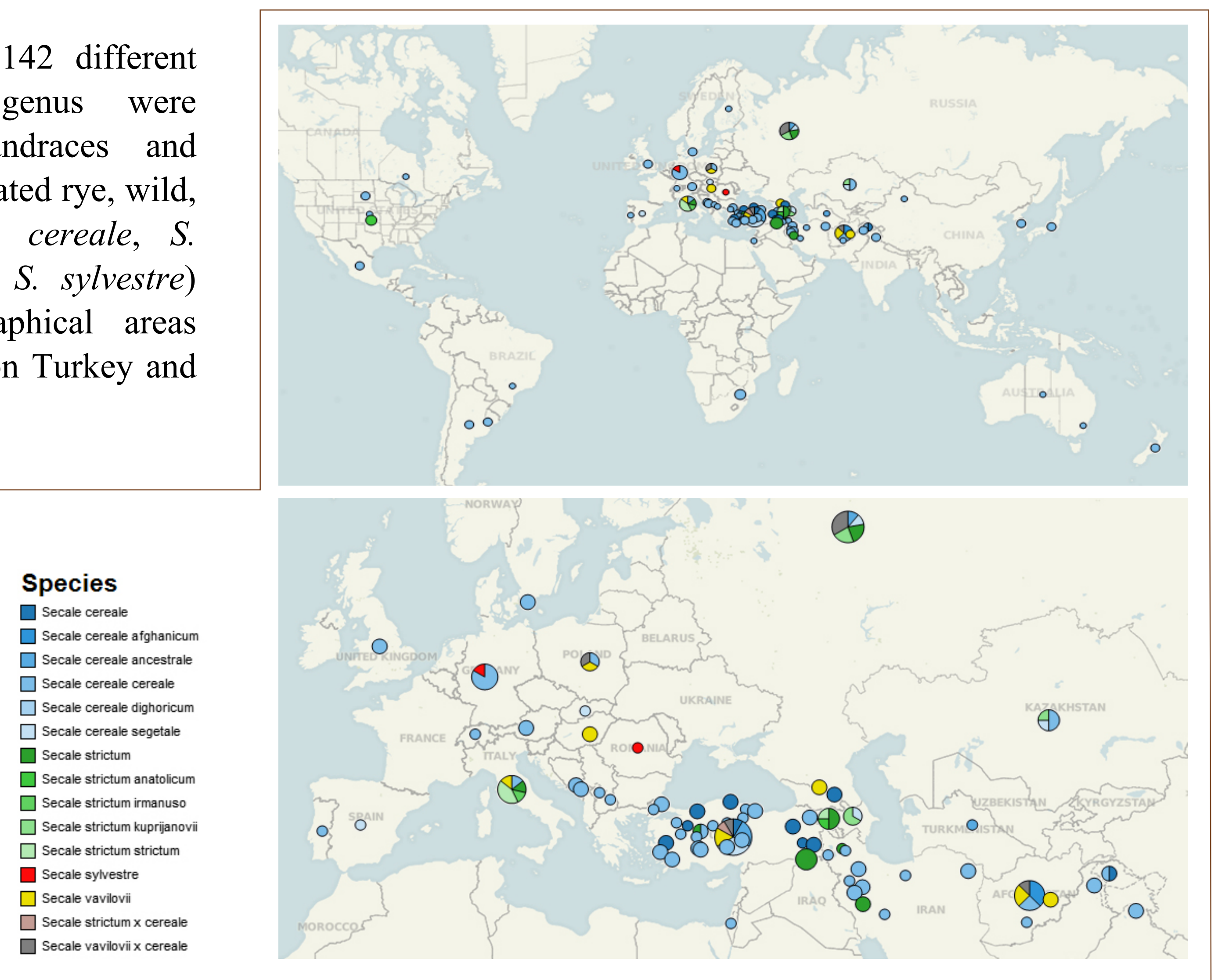


Figure 1
Localities of *Secale* samples analysed in the study. Color codes shows the taxon identifications of at suspecific level.weedy forms of *S. cereale*, *S. vavilovii*, *S. strictum,* and *S. sylvestre*) from different eco-geographical areas with a concentrated focus on Turkey and Fertile Crescent (Fig. 1).

Genetic Analyses

The DNA was extracted from each individual plant by CTAB protocol. A total of 729 samples were included in the SSR study, in which 10 nuclear SSR loci were amplified according to the protocol described in Khlestkina et al. (2004). This was followed by genotyping and determination of allele sizes using the software program Genemarker V2.2.0 (Softgenetics). To eliminate genotyping errors in the data, the results were checked using MICRO-CHECKER (Oosterhout et al. 2004).

Chloroplastic intergenic region ndhF-rpl32 were amplified using IGR and 643R primers following the protocol described by Yamane and Kawahara (2005) for 86 samples. The amplified fragments were sequenced using the forward primer. Sequencing data was edited with Sequencher v.3.1 (Gene Codes Corp.) and a Bayesian phylogenetic tree was constructed by using BEAST v1.6.1 (Drummond and Rambaut 2007). The MCMC analysis was run for 10 × 10^6 generations and sampled every 1000th; the first 10% were discarded as burn-in. Yule Process was used for the tree prior with A UPGMA starting tree.

For microsatellite analysis, 10 SSR markers were used. The microsatellite scores were analyzed for distinct clusters with the software Structure (Pritchard et al., 2000), whose results were interpreted with the help of STRUCTURE HARVESTER (Earl & Von Holdt, 2012). We used 2, 3, and 4 clusters, and the cluster memberships that were below 0.5 were left as unassigned.

Results

In **ndhF-rpl32 chloroplastic intergenic region**, a total of 28 haplotypes were identified, eleven of which were shared in more than one individual. Except *S. sylvestre*, the remaing *Secale* species shared most of the haplotypes, without exhibiting a species level structuring (Fig. 2).

Bayesian construction of haplotypes recovered two shallowly seperated clades (Fig. 3). In the first clade haplotypes shared by *S. cereale*, *S. vavilovii*, and *S. strictum* samples clustered together and the second clade formed by *S. sylvestre* haplotypes. The divergence between these clades were approximately 1.3%.

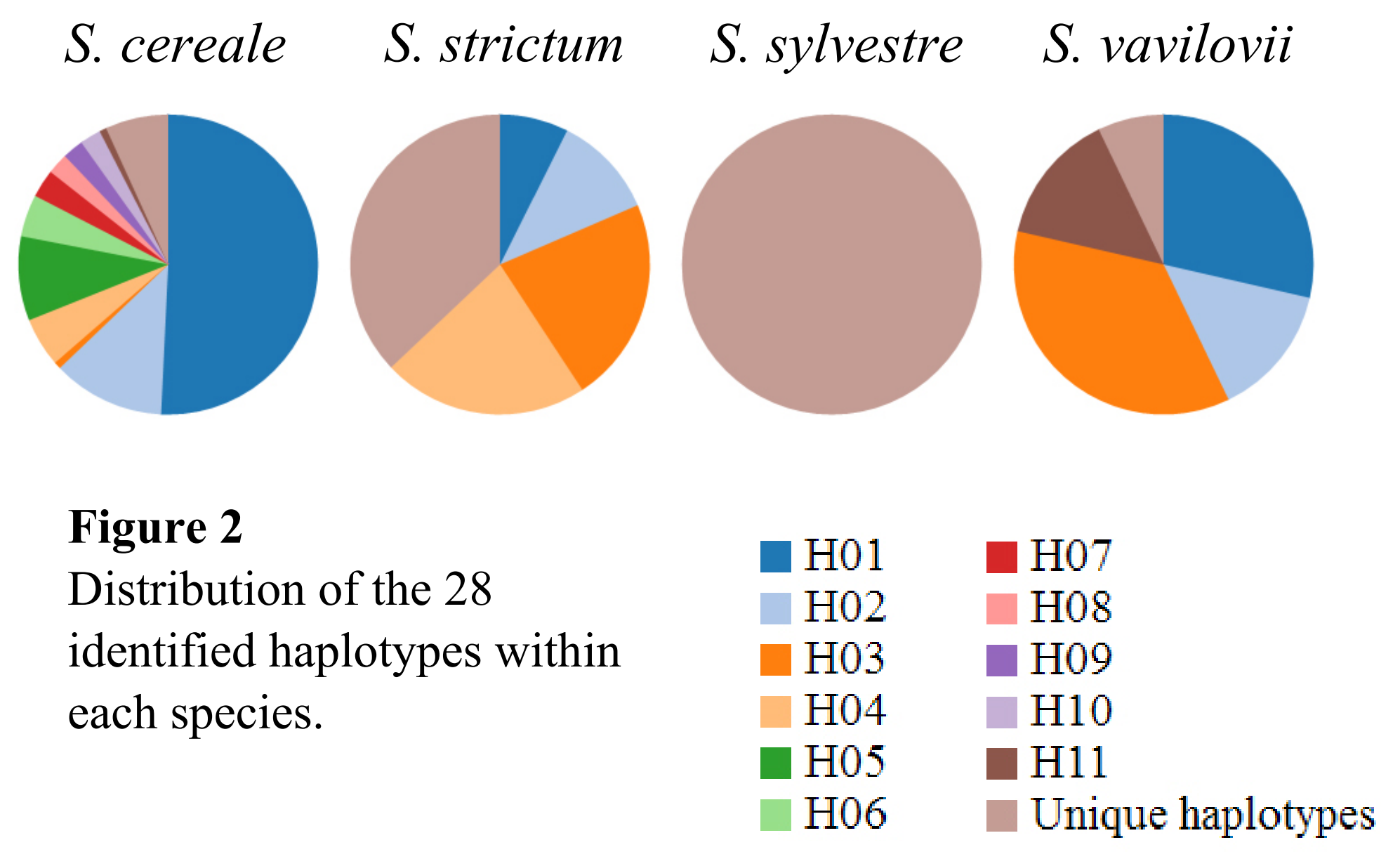


Figure 2
Distribution of the 28 identified haplotypes within each species.

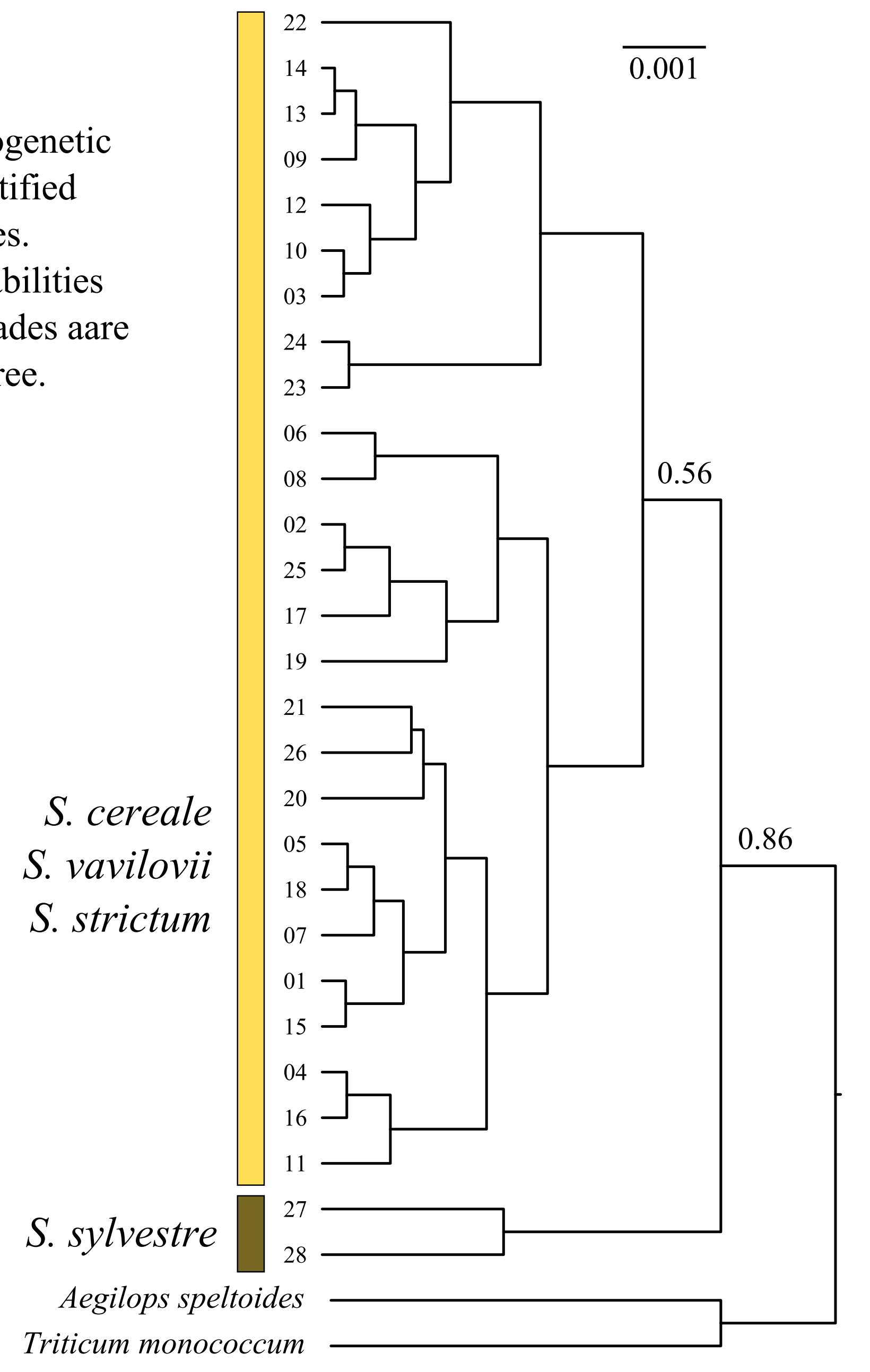


Figure 3
Bayesian phylogenetic tree of the identified ndhF haplotypes. Posterior probabilities of the major clades aare shown on the tree.

The STRUCTURE analysis were run for **six polimorphic microsatellite** regions (REMS-1187, REMS-1254, REMS-1323, REMS-1264, REMS-1238, and REMS-1303), which amplified for most of the individuals. The runs for both, independent and correlated allele frequencies, indicated different numbers of clusters, and did not reveal any clear separation neither with regards to the species identifications of the samples (**Fig. 4**) nor their geographical distributions. The only observed pattern was the cluster compostions within *S. cereale* and *S. vavilovii*; in all runs these species had similar ratios of the assigned cluster memberships.

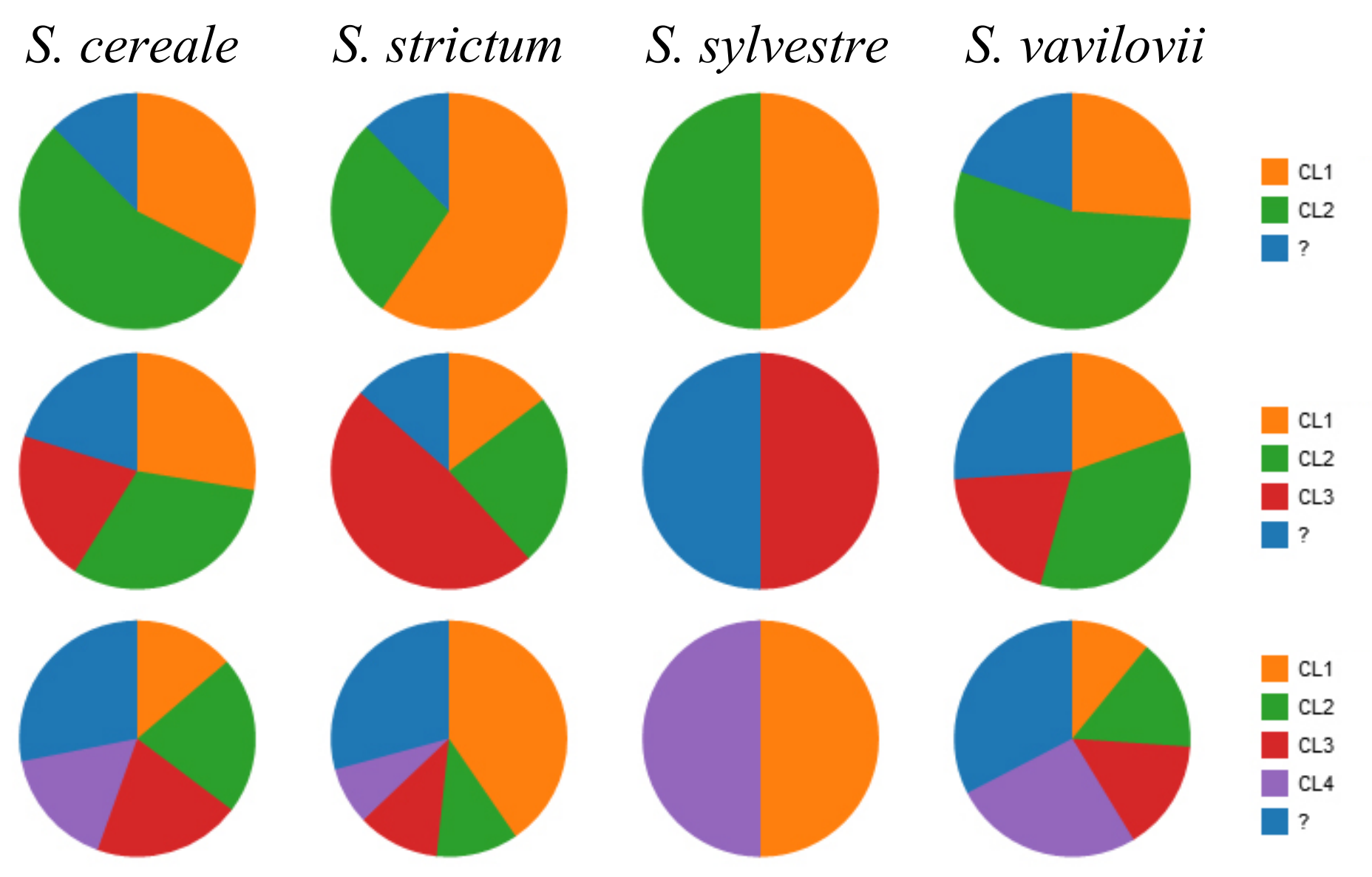


Figure 4
Distribution of clusters assigments for each each species: K=2, K=3, and K=4 from up to down. Individuals, which cannot be assigned to any clusters are shown as "?."

Discussion

Phylogenetic reconstruction of chloroplastic intergenic region ndhF-rpl32 recovered only two shallowly seperated clades, indicating a low level of chloroplastic polymorphism among the *Secale* genus. Except *S. sylvestre* samples, rest of the species shared most of their haplotypes, and therefore, their phylogenetic relations could not be resolved. Similarly, the analyses of SSR markers did not recovered any clusters at the species level. Although they showed high allelic diversity within the genus, the members of all the species had overlapping cluster assignments, which might indicate their shared ancestry or past introgression between populations.

The analyses carried out with chloroplastic SNP and nuclear SSRs revealed that these markers are not sufficient to resolve phylogenetic relationships among all accessions included in the study. Evolutionary history of genus Secale has to be studied in more detail using other markers like nuclear SNPs and iPBS markers. The lack of any structure according to the nuclear microsatellite data is probably due to the permanence of ancestral genotypes and/or intensive introgression between species.

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