

1 **Article – Discoveries section**

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3 **Genome-wide sequence information reveals recurrent hybridization among diploid**  
4 **wheat wild relatives**

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19

20 **Abstract:** Many conflicting hypotheses regarding the relationships among crops and wild species  
21 closely related to wheat (the genera *Aegilops*, *Amblyopyrum*, and *Triticum*) have been postulated. The  
22 contribution of hybridization to the evolution of these taxa is intensely discussed. To determine  
23 possible causes for this, and provide a phylogeny of the diploid taxa based on genome-wide sequence  
24 information, independent data was obtained from genotyping-by-sequencing and a target-enrichment  
25 experiment that returned 244 low-copy nuclear loci. The data were analyzed with Bayesian, likelihood  
26 and coalescent-based methods. *D* statistics were used to test if incomplete lineage sorting alone or  
27 together with hybridization is the source for incongruent gene trees. Here we present the phylogeny of  
28 all diploid species of the wheat wild relatives. We hypothesize that most of the wheat-group species  
29 were shaped by a primordial homoploid hybrid speciation event involving the ancestral *Triticum* and  
30 *Am. muticum* lineages to form all other species but *Ae. speltoides*. This hybridization event was  
31 followed by multiple introgressions affecting all taxa but *Triticum*. Mostly progenitors of the extant  
32 species were involved in these processes, while recent interspecific gene flow seems insignificant.  
33 The composite nature of many genomes of wheat group taxa results in complicated patterns of diploid  
34 contributions when these lineages are involved in polyploid formation, which is, for example, the case  
35 in the tetra- and hexaploid wheats. Our analysis provides phylogenetic relationships and a testable  
36 hypothesis for the genome compositions in the basic evolutionary units within the wheat group of  
37 Triticeae.

38

39 **Keywords:** *Aegilops*, *Amblyopyrum*, crop wild relatives, evolution, genotyping-by-sequencing,  
40 hybridization, nuclear single-copy genes, target-enrichment, phylogeny, *Triticum*, wheat

## 41 Introduction

42 Different molecular marker types resulted in widely incongruent hypotheses of relationships for the  
43 species belonging to the wheat wild relatives (WWR) of the grass tribe Triticeae (Mason-Gamer and  
44 Kellogg 1996; Escobar et al. 2011; Bernhardt 2015; Glémin et al. 2019), i.e. the genera *Aegilops*,  
45 *Amblyopyrum*, and *Triticum* (van Slageren 1994; Kilian et al. 2011). Thus, despite their economic  
46 importance both as crops and as wild species contributing to the continued improvement of wheat, no  
47 comprehensive and generally agreed phylogeny for these species is currently available. This hampers  
48 the understanding of the evolution of morphological, physiological, and genetic traits, the  
49 biogeography of the species and their environmental adaptation, polyploid formation, speciation, and  
50 ultimately the search for useful alleles for plant breeding.

51 Hybridization is an important evolutionary process (Mallet et al. 2016). It describes the crossing of  
52 individuals belonging to different species. On the homoploid level, i.e. if no whole-genome  
53 duplication is involved, hybridization results in first generation ( $F_1$ ) offspring that possesses half of  
54 the genome of each of its parents. If this  $F_1$  generation becomes reproductively isolated from its  
55 parents and evolves into a new species the process is termed homoploid hybrid speciation. If over  
56 time repeated backcrossing with one parent dilutes the contribution of the second parent this process  
57 is called introgression and means that genomic material (nuclear, chloroplast or mitochondrial DNA)  
58 can cross species borders. In contrast, incomplete lineage sorting (ILS) describes the process where  
59 during speciation DNA polymorphisms occurring in an ancestral taxon, are stochastically passed on to  
60 daughter taxa. Depending on the allele composition in individuals at certain genomic loci,  
61 phylogenetic analyses can arrive at different species relationships when different individuals and/or  
62 loci are analyzed (Maddison 1997). As ILS mostly depends on population sizes together with  
63 mutation rates, the process of lineage sorting can be modeled in a coalescent framework (Kingman  
64 1982). Although it is not always possible to discern hybridization from ILS, multi-locus coalescent  
65 analyses including multiple individuals per species can in part overcome this problem (Green et al.  
66 2010; Durand et al. 2011; Pease and Hahn 2015; Yu and Nakhleh 2015; Solís-Lemus and Ané 2016;  
67 Wen and Nakhleh 2018; Chao Zhang et al. 2018).

68 The recent advent of genomic data for *T. aestivum* (International Wheat Genome Sequencing  
69 Consortium 2014, 2018), an allohexaploid with three subgenomes (termed **A**, **B**, and **D**), and the  
70 related diploid species *Ae. tauschii* (Jia et al. 2013; Luo et al. 2013, 2017) and *T. urartu* (Ling et al.  
71 2013), allows for the comparative analyses of genome structure and gene content. Marcussen et al.  
72 (2014), when analyzing relationships among the three subgenomes of wheat, postulated that the **D**-  
73 genome lineage, occurring in *Ae. tauschii*, is of homoploid hybrid origin involving the ancestors of  
74 the **A** (occurring in *T. urartu*) and **B** genomes (similar to *Ae. speltooides*). This finding spurred a  
75 discussion regarding a hybrid origin of *Ae. tauschii* (Li et al. 2015a, b; Sandve et al. 2015). El  
76 Baidouri et al. (2017) analyzed sequences of homeologous genes and transposable elements derived

77 from *T. aestivum* (**ABD**), tetraploid *T. durum* (**AB**), *T. urartu* (**A**), *Ae. speltooides* (**B**), and *Ae. tauschii*  
78 (**D**). They deduced that about six million years ago (Mya) an ancestral **D** genome introgressed into a  
79 homoploid hybrid of the ancestral **A** and **B** genomes. The ancestral **D** genome went extinct sometime  
80 later. Today's **D** genome, occurring in diploid *Ae. tauschii* and as one subgenome in *T. aestivum* and  
81 other polyploid species of *Aegilops*, is, therefore, a hybrid genome combining three genomes (El  
82 Baidouri et al. 2017). As the **B** genome of polyploid wheat is different from its closest extant relative  
83 *Ae. speltooides*, they assumed that the **B** genome itself might also have been introgressed by species of  
84 the **S** genome group of *Aegilops* sect. *Sitopsis*. Recently, Glémin et al. (2019) developed a new  
85 framework to investigate hybridizations. Based on transcriptome data for all species, they proposed a  
86 complex scenario of hybridizations identifying *Am. muticum* (**T**), instead of *Ae. speltooides* (**B**), as an  
87 ancestor of the **D**-genome lineage and at least two more hybridization events.

88 In Triticeae it is generally agreed that the diploid taxa and cytotypes form the basic units of evolution  
89 and are involved in different combinations in the formation of polyploid taxa (Kellogg 2015).

90 Polyploids occur mostly as allopolyploid taxa combining the genomes of different parental species  
91 after hybridization and whole-genome duplication (WGD). Except for Glémin et al. (2019), the recent  
92 studies of the evolution of wheat included only a few species and mostly single individuals (although  
93 with huge amount of genome data) of wheat wild relatives. Here we describe the analyses of two  
94 genome-wide datasets obtained for all diploid species of *Aegilops*, *Amblyopyrum*, and *Triticum* and  
95 always multiple individuals per taxon to improve the understanding of evolutionary relationships in  
96 the wheat group. This work employs DNA sequences of 244 nuclear low-copy genes uniformly  
97 distributed among all seven chromosomes of the taxa. These were obtained through a set of gene-  
98 specific hybridization probes used to enrich the target loci prior to next-generation sequencing (Hyb-  
99 seq; Weitemier et al. 2014). Based on this set of genes, species relationships were calculated using  
100 diverse phylogenetic algorithms. In addition, genome-wide single-nucleotide polymorphism (SNP)  
101 data was obtained through genotyping-by-sequencing (GBS; Elshire et al. 2011). Both datasets were  
102 compared for signals of directed introgression and hybridization. Our results provide species  
103 relationships within the wheat group taxa, and lead to new hypotheses on far-reaching hybridization  
104 and introgression influencing the evolutionary origins and composition of all extant basic diploid  
105 genomes in this species group.

106

## 107 **Results and Discussion**

### 108 **Sequence assembly of the target-enriched loci**

109 Loci for target-enrichment were selected via the comparison of available genome information from  
110 different Poaceae like *Brachypodium distachyon*, rice and sorghum, barley and wheat (Vogel et al.  
111 2010; Matsumoto et al. 2011; Mayer et al. 2011), aiming for orthologous loci with an even

112 distribution on the genome (SI Materials and Methods). Our design of capture probes was finally  
113 based on 451 loci evenly distributed over the **A**, **B**, and **D** genomes of *T. aestivum* (Table S1, Figure  
114 S1).

115 Target-enrichment and Illumina sequencing resulted in 140 million raw reads and 116 million reads  
116 after quality filtering. On average 6% of the reads mapped to the chloroplast genome. Of the 451 loci,  
117 25 (5%) were not sufficiently captured (i.e. not captured in most taxa) and were excluded from further  
118 analyses. The capture efficiency was usually taxon/accession independent, indicating no (strong)  
119 influence of probe design on the capture efficiency (Table S1, Table S2). The sequences retrieved for  
120 the 426 well captured nuclear loci were combined into multiple sequence alignments. Visual  
121 inspection of these alignments often showed genus- or species-specific patterns of ambiguous  
122 positions. Allelic diversity is assumed to be much lower than 1%. This threshold was set based on a  
123 comparison with Jakob et al. (2014) that reported an allelic diversity clearly lower than 1% for the  
124 analysis of six single-copy loci of large populations of *Hordeum vulgare* subsp. *spontaneum*. Thus,  
125 single-copy loci of heterozygous individuals can be expected to show noticeably less than 1% of  
126 ambiguous positions in assembled sequences. Since sequenced accessions within a species mainly  
127 share the same combinations of polymorphic positions, this points to the existence of paralogous gene  
128 copies for a locus, either functional or as pseudogenes, rather than to heterozygous loci. The  
129 proportion of ambiguous positions per accession and locus was estimated (Table S3). An average of  
130 more than 1% of ambiguous sites in more than five species was detected for 62 (~15%) captured loci.  
131 These loci were considered as mainly multi-copy and excluded from further analyses. Moreover, very  
132 short or not variable loci were excluded. The median of the mean coverage for the 244 remaining loci  
133 was 25X. Large deviations in the mean coverage result from the actually achieved sequencing depth  
134 (Table S4a). The loci used for phylogenetic inference had on average a length of 2,278 bp, 43% of  
135 non-variable sites and a pairwise-identity of 88% (Table S4b). Concatenation of the 244 nuclear loci  
136 in a supermatrix resulted in an alignment with a total length of 555,543 bp.

137

### 138 **Phylogenies based on target-enrichment data**

139 *Supermatrix approach* - The first step of our analysis procedure was to use DNA sequences of nuclear  
140 genes enriched through hybridization probes for Illumina sequencing to infer phylogenetic  
141 relationships from quality filtered alignments. In addition to the wheat group taxa, we included four  
142 diploid species as outgroups representing the barley genus *Hordeum* (Table S5). Maximum likelihood  
143 (ML) and Bayesian phylogenetic inference (BI) of the concatenated DNA sequences of all loci (i.e.  
144 creating a supermatrix with 555,543 alignment positions) resulted in the phylogenetic relationships  
145 provided in Figure S2. In this tree *Ae. speltoides* and *Am. muticum* form a clade that is sister to all  
146 other taxa analyzed. Within the latter, *Triticum* is sister group of the remainder of *Aegilops* species.

147 When analyzing the same dataset with maximum parsimony (MP), *Triticum* and *Ae. speltoides/Am.*  
148 *muticum* exchange their respective positions in the phylogenetic tree (Fig. S3).

149

150 *Coalescent-based phylogenetic inference* - As data concatenation could potentially result in strong  
151 support for wrong species relationships (Xi et al. 2015), gene trees were used to infer a coalescent-  
152 based species tree. Individual ML gene trees were used as input for ASTRAL (Mirarab et al. 2014;  
153 Chao Zhang et al. 2018), which models ILS under the multispecies coalescent (MSC) model (Degnan  
154 and Rosenberg 2009) to deduce species relationships. The resulting phylogeny places *Triticum* as  
155 sister to *Amblyopyrum* and all *Aegilops* species (Fig. 1A and S4), a topology similar to the one found  
156 by MP analysis of the supermatrix (Fig. S3). *Aegilops markgrafii/Ae. umbellulata* form a clade with  
157 *Ae. comosa/Ae. uniaristata* (clade **CUMN**), although with very low statistical support (Fig. 1A).

158 While all 244 individual ML gene trees were in conflict to each other and accessions of the same  
159 species may be widely scattered in single topologies (data not shown), all supermatrix phylogenetic  
160 approaches (Fig. S2, S3), the ASTRAL analysis (Fig. S4), and the unrooted network obtained via  
161 SPLITSTREE (Fig. S5) revealed species to be monophyletic. We, therefore, conclude that ongoing gene  
162 flow between species is not significantly impacting the data and extant species can be considered as  
163 units.

164 Low support values in the ASTRAL tree (Fig. 1A and S4) correspond to branches with topological  
165 differences when comparing to the supermatrix phylogenies indicating conflicting phylogenetic  
166 signal. The degree of gene tree/species tree conflict was investigated in detail with PHYPARTS (Smith  
167 et al. 2015), as it could also stem from hybridization/introgression instead of ILS. For most clades  
168 comprising several species, no major alternative to the ASTRAL topology could be identified (Fig. S6).  
169 However, the clades of **CUMN** and **DS** present in the ASTRAL tree were supported by only seven and  
170 20 out of 244 gene trees, respectively. For the former clade, there were five alternative topologies  
171 found to be more frequent involving members of the **CUMN** clade together with either *Ae. tauschii*  
172 (**D**) or the *Triticum* species (**A**): **UD** with 14 supporting topologies, **CD** 12, **MND** 10, **AU** 9, and **ND**  
173 8. In the case of **DS**, there were 20 alternative topologies that grouped *Ae. speltoides* (**B**) instead of  
174 *Ae. tauschii* (**D**) together with sect. *Sitopsis* (**S**).

175 In multi-locus analyses, *Ae. speltoides* always forms a moderately supported clade with *Am. muticum*  
176 (**T**), and, as in previous studies (e.g. Petersen et al. 2006; Li et al. 2015a; Bernhardt et al. 2017), it is  
177 always clearly separated from the other species of *Aegilops* sect. *Sitopsis* (**S**), as well as from the  
178 remaining *Aegilops* species. In the following we will use sect. *Sitopsis*\* to indicate that we refer to the  
179 **S**-genome group of sect. *Sitopsis* excluding *Ae. speltoides* (**B**) that was earlier placed within this group  
180 (van Slageren 1994). *Aegilops tauschii* (**D**), although assumed to be either a homoploid hybrid  
181 between the **A**- and **B**-genome lineages (Marcussen et al. 2014; Sandve et al. 2015) or the **A**-, **B**-, and

182 **D**-genome ancestors (El Baidouri et al. 2017), results in all our analyses as sister of sect. *Sitopsis*\*.  
183 This indicates that an **S**-genome progenitor may have played a role in its formation. This close  
184 relationship was not previously postulated, although Marcussen et al. (2014) used sequences of the **S**-  
185 genome species *Ae. sharonensis* (International Wheat Genome Sequencing Consortium 2014).  
186 However, they excluded them from additional analyses, as they assumed *Ae. sharonensis* itself to be a  
187 hybrid involving the **B**-genome lineage. Our data show that not only *Ae. sharonensis* is closely related  
188 to *Ae. tauschii* but that shared genome parts most probably involve the entire sect. *Sitopsis*\*. Although  
189 the relationship to the **B** genome was not found in this initial analysis, it clearly indicates a more  
190 complex evolutionary history of the *Ae. tauschii* genome and perhaps also that of sect. *Sitopsis*\* in  
191 comparison to what was heretofore hypothesized.

192 Although the discordant topologies revealed by PHYPARTS are potentially better resolved by  
193 modeling ILS, they may also result from past hybridizations or gene flow among species. Both  
194 processes would violate the assumption of the coalescent analysis that only ILS contributes to  
195 deviating gene-tree topologies. Therefore, our sequence data were further analyzed to uncover past  
196 hybridization and introgression events.

197

198 *Network approach based on gene tree topologies from target-enrichment data* - Even though methods  
199 to infer phylogenetic networks are under constant development (e.g. (Yu et al. 2011; Yu and Nakhleh  
200 2015; Solís-Lemus and Ané 2016; Wen et al. 2016; Wen and Nakhleh 2018; Chi Zhang et al. 2018),  
201 the analysis of multiple loci, individuals, and species while modeling ILS and reticulations remains  
202 computationally expensive (Hejase and Liu 2016; Wen et al. 2018). Thus, resource demanding  
203 methods such as full maximum-likelihood or Bayesian inference (Yu et al. 2014; Wen and Nakhleh  
204 2018) failed to infer networks from our entire sequence data. We, therefore, used different strategies  
205 of data partitioning by reducing the number of individuals or loci. However, these approaches gave  
206 incoherent results across replicates (not shown).

207 Nevertheless, we were able to obtain phylogenetic networks from the 244 gene tree topologies under  
208 the multispecies network coalescent (MSNC) using maximum pseudo-likelihood as implemented in  
209 PHYLONET (Yu and Nakhleh 2015). We allowed for zero to five reticulations (Fig. S7a-f). If no  
210 hybridization was assumed, the tree with the best log pseudo-likelihood (-7,617,218) had a topology  
211 similar to the one obtained via ASTRAL (Fig. 1A, S4). However, poorly supported clades were  
212 dissolved resulting in a grade with *Triticum* as sister to the rest of the species, *Am. muticum* and *Ae.*  
213 *speltoides* not being monophyletic, and *Ae. comosa*/*Ae. uniaristata* and *Ae. markgrafii*/*Ae.*  
214 *umbellulata* not clustering together. PHYLONET also retrieved the ASTRAL topology among the top  
215 five trees with a slightly lower log pseudo-likelihood (-7,617,519). The network with four  
216 hybridization nodes (Fig. 2, S7e) was selected with the Akaike information criterion as best-fit. In this

217 network, hybridizations are nested within each other. This suggests a sequence of hybridization  
218 events, the first one involves the ancestors of *Am. muticum* and the *Triticum* clade each contributing  
219 approximately equal proportions (0.54 and 0.46, respectively) to the common ancestor of all other  
220 species except *Ae. speltoides*. This confirms the scenario inferred by Glémin et al. (2019) identifying  
221 *Am. muticum* instead of *Ae. speltoides* as one of the genome donors (Marcussen et al. 2014). Sect.  
222 *Sitopsis*\* appears as sister to both *Ae. tauschii* and *Ae. markgrafii* and to be introgressed by *Ae.*  
223 *speltoides* (0.31). Finally, the *Ae. comosa/Ae. uniaristata* clade is sister to *Ae. markgrafii* with an  
224 additional introgression of the *Triticum* clade (0.29). However, phylogenetic networks inferred from  
225 gene tree topologies under maximum pseudo-likelihood are not necessarily uniquely encoded by their  
226 system of rooted triples and this analysis may return an equivalent network to the true network (Yu  
227 and Nakhleh 2015). In this case, the authors suggest investigating the obtained network with other  
228 methods and/or data. Here we used GBS to generate genome-wide SNP data from all taxa to evaluate  
229 this scenario.

230

### 231 **DNA polymorphisms obtained through genotyping-by-sequencing (GBS)**

232 *Sequence assembly of the GBS data* - To obtain genome-wide SNP data, a two-enzyme GBS analysis  
233 (Poland et al. 2012) was performed by cutting the genome with a frequent and a rare-cutting  
234 restriction enzyme then sequencing 100 bp of the DNA fragments directly adjacent to the rare  
235 restriction sites following Wendler et al. (2014). This method was shown to target the coding parts of  
236 the genome (Schreiber et al. 2019). Thus, it can be used to compare SNP patterns between species,  
237 which might, in their non-coding genome regions, already be too diverse for meaningful comparisons.  
238 As *Hordeum* and the wheat group lineage were already separated 15 Mya (Marcussen et al. 2014),  
239 their genomes have diverged substantially. Therefore, we included *Dasypyrum villosum* and  
240 *Taeniatherum caput-medusae* as outgroups. These taxa are outside of the wheat group genera  
241 (Bernhardt et al. 2017) but still close enough to share multiple GBS loci.

242 On average 1.65 million reads per sample were obtained from Illumina sequencing. After filtering and  
243 clustering on average 222,185 clusters remained per sample. After consensus calling per cluster the  
244 number of loci per individual in the assembly was on average 21,000 (with a minimum of 8,472 loci  
245 for accession AE 739 of *Ae. speltoides* and maximum of 28,469 loci for accession PI 560122 of *Am.*  
246 *muticum*). In total 140,072 loci having 444,618 phylogenetic informative sites were kept for  
247 downstream analysis when specified that at least four individuals had to share a locus (Table S6).

248

249 *GBS-based phylogenetic relationships* - To analyze phylogenetic relationships based on the GBS data  
250 we conducted an analysis in TETRAD within the IPYRAD package (Eaton 2014;  
251 <https://github.com/dereneaton/ipyrad>). TETRAD uses a single SNP per GBS locus and conducts quartet



252 analyses to infer a species tree that is consistent under the multispecies coalescent. The phylogenetic  
253 tree (Fig. 1B, S8) supports the topology of the supermatrix tree of the target-enrichment data (Fig. S2)  
254 with respect to the relative positions of *Triticum* and *Ae. speltoides*/*Am. muticum* and of the ASTRAL  
255 tree regarding the MN and UC taxa forming together a weakly supported clade (Fig. 1A). The  
256 unrooted phylogenetic network computed by SPLITSTREE (Fig. S9) is concordant with the one for  
257 target-enrichment data (Fig. S5) showing that species are monophyletic and can be considered as units  
258 for the detection of hybridization.

259 Even though Zhu and Nakhleh (2018) developed a method (i.e. MLE\_BiMarkers) able to deal with  
260 more than 50 taxa and four hybridizations using bi-allelic markers under the maximum pseudo-  
261 likelihood, we could not process our dataset in a reasonable timeframe (i.e. analyses did not finish  
262 within 30 days). We assume that the complexity of the relationships, including putative nested  
263 hybridization and introgression events (Fig. 2) complicate the inference of a network from the GBS  
264 data. Nonetheless, we assessed hybrid relationships with Four- and Five-taxon *D* statistics. Those  
265 methods, based on the frequency of shared polymorphisms between taxa, are less computing  
266 intensive.

267

268 *GBS-based D statistics for the detection of hybridization and direction of introgression* - Under a  
269 neutral model of sequence evolution, and if speciation events occur in rapid succession, ILS should  
270 result in similar amounts of shared polymorphisms among species derived from a common ancestor.  
271 However, if hybridization is involved, the amount of shared alleles shifts towards the species  
272 connected through gene flow in comparison to the background signal contributed by ILS. *D* statistics,  
273 also known as ABBA–BABA test (Green et al. 2010a; Durand et al. 2011), is able to discern  
274 hybridization from ILS by analyzing allele distribution in three taxa in comparison to an outgroup.

275 All Four-taxon *D* statistic tests were performed species-wise on unlinked SNPs with the routine Dtrios  
276 of DSUITE (Malinsky 2019). First, *D. villosum* was set as outgroup to test if *Ta. caput-medusae* was  
277 involved in hybridizations with any members of the WWR (Fig. S10). *Taeniatherum caput-medusae*  
278 then was used as outgroup for all following tests as no hybridization signal was found. A total of 220  
279 tests were performed of which 64 were significant (*p* value < 0.05 after Benjamini-Yekutieli  
280 correction) with *D* statistics ranging between 0.10 and 0.33 (Fig. 3, Table S7). All species were  
281 involved in potential hybridizations. The strongest signal revealed a relationship between both  
282 *Triticum* species and *Ae. markgrafii*/*Ae. umbellulata*, and to a lesser extent *Ae. comosa*/*Ae. uniaristata*  
283 and *Ae. tauschii*. A similar, though weaker, pattern was also found for *Am. muticum*. *Aegilops*  
284 *markgrafii* also showed a strong tie with the members of sect. *Sitopsis*\* (**S**). This analysis also  
285 confirmed the strong and exclusive relationships between *Ae. speltoides* and the latter.

286 An extension of *D* statistics is the  $D_{\text{FOIL}}$  test (Pease and Hahn 2015) that allows not only the detection  
287 of hybridization in the presence of ILS but also infers the direction of introgression in a five-taxon  
288 phylogeny. This analysis only accepts an alignment of five sequences, therefore we created consensus  
289 sequences for each species.  $D_{\text{FOIL}}$  tests were performed with *Ta. caput-medusae* used as the outgroup,  
290 to polarize the comparisons of all species. Altogether 216 unique combinations of five taxa were  
291 tested but only 143 tests were considered after removing tests that did not fulfill the requirements of  
292 estimated divergence times (see Material and Methods; Pease and Hahn 2015). On average 292,602  
293 alignment positions (233,791–379,867) were used resulting in 6,738 (952–10,354) SNP patterns that  
294 could be compared (Table S7; Fig. 4). Overall, the relationships inferred are similar to the ones  
295 identified by the ABBA–BABA test (Fig. 3; Table S6), however, directions of gene flow could be  
296 inferred for nine relationships (11 tests). A large proportion of tests (42) revealed undirected patterns  
297 involving three taxa indicative of complex or ancient introgressions, or reciprocal gene flow.  
298 Evidence of introgression/hybridization was found for all species (Fig. 4a-k), with a low number of  
299 significant tests involving *Ae. uniaristata* and *Ae. umbellulata* (Fig. 4e-f) and a high number involving  
300 *Ae. markgrafii* and *Ae. longissima* (Fig. 4g and 4k). This analysis confirms the close relationships  
301 between the members of sect. *Sitopsis*\* (**S**) and *Ae. speltoides* (**B**), but, in contrast to the network  
302 inferred with PHYLONET (Fig. 2),  $D_{\text{FOIL}}$  identifies gene flow from **S** to **B** (Fig. 4b). Among the  
303 members of sect. *Sitopsis*\*, *Ae. longissima* (**Sl**) appeared as a major introgressor of **B** but also of *Ae.*  
304 *comosa* (**M**), *Ae. markgrafii* (**C**), and *Ae. tauschii* (**D**) (Fig. 4k). This may explain the high number of  
305 tests returning undirected signal involving those four species. The close relationship between *Triticum*  
306 species and the **CUMND** clade was confirmed although no direction could be inferred (Fig. 4c). This  
307 analysis also suggests that *Am. muticum* was affected by gene flow from *Ae. comosa* and *Ae. tauschii*  
308 (Fig. 4a).

309

### 310 **Homoploid hybrid speciation and major introgressions**

311 In the following, we describe our hypothesis for the evolution of WWR (Fig. 5). Overall, the scenario  
312 inferred is similar to the one identified by Glémin et al. (2019). Nonetheless, as we did not focus on  
313 identifying the progenitors of the “**D**-genome lineage” we are able to propose a more complete  
314 picture. However, as the relationships we identified are highly reticulate, there are partly alternative  
315 scenarios possible. We limit our interpretation to the most strongly supported relationships to avoid  
316 false positives (Eaton et al. 2015).

317 As our phylogenetic analyses revealed the monophyly of all species we are certain that hybridizations  
318 and introgressions involved mainly ancestral taxa and not the extant species. Our results suggest that  
319 there are different groups of taxa, i.e. lineages that introgressed others, lineages that are recipients of

320 introgressions from one or several taxa and/or lineages that originated via homoploid hybrid  
321 speciation.

322 We hypothesize that most of the wheat-group species were shaped by a primordial homoploid hybrid  
323 speciation event, i.e. that the *Triticum* lineage merged with the ancestor of *Am. muticum* to form all  
324 other species but *Ae. speltoides*. This hybridization event was followed by multiple introgressions  
325 affecting all taxa but *Triticum*. In contrast to Glémin et al. (2019), we do not find an introgression of  
326 *Triticum* into *Am. muticum*, instead our results indicate that *Am. muticum* may have been introgressed  
327 by *Ae. umbellulata* or the common ancestor of the **CUMND** clade (Fig. 4a, S7d). Previously  
328 published chloroplast phylogenies (Bordbar et al. 2011; Bernhardt et al. 2017) support this event of  
329 introgression into **T**, as the chloroplast of *Am. muticum* does not group with *Ae. speltoides* in the  
330 chloroplast phylogeny, although both are sister taxa in nuclear phylogenies. These results highlight  
331 the pivotal role of *Am. muticum*, instead of *Ae. speltoides* in the formation of the WWR.

332 For *Ae. speltoides* (**B**) conflicting results were obtained with either sect. *Sitopsis*\* (**S**) being  
333 introgressed by **B** (Fig. 2) or the other way around (Fig. 4b). This suggests that either reciprocal gene  
334 flow occurred between those species or that at least one of the applied methods revealed false  
335 positives. Both methods have drawbacks: phylogenetic networks obtained under maximum pseudo-  
336 likelihood may not be true but rather equivalent to the true network (Yu and Nakhleh 2015), and *D*  
337 statistics are only analyzing three or four taxa simultaneously. Nevertheless, sect. *Sitopsis*\*, and  
338 especially *Ae. longissima* that has been described as an outcrossing taxon (Escobar et al. 2010), was  
339 repeatedly identified as an introgressor as it exhibits relationships with all taxa except the *Triticum*  
340 lineage (Fig. 4k).

341 Signals for the involvement of the sect. *Sitopsis*\* genomes can be found in *Ae. comosa* (**M**) and *Ae.*  
342 *markgrafii* (**C**), for which a hybrid origin has been recently proposed (Danilova et al. 2017). Both taxa  
343 presented patterns of introgressions different from their respective sister species *Ae. umbellulata* and  
344 *Ae. uniaristata*. These two species were involved in the least number of hybridizations. This seems to  
345 indicate that **C** and **M** lineages diverged from their sister species due to minor introgressions from *Ae.*  
346 *longissima* or other species of the sect. *Sitopsis*\*. It is further suspected that *Ae. longissima* or sect.  
347 *Sitopsis*\* strongly introgressed another, possibly extinct (El Baidouri et al. 2017), member or the  
348 progenitor of the **CUMN** clade to form *Ae. tauschii*, as the observed pattern does not resemble a  
349 simple sister-species relationship (Fig. 3, 4h, S6). *Aegilops tauschii*, therefore, displays similarities  
350 with *Triticum* (**A**), *Ae. comosa* (**M**) and, to a lower extent, *Am. muticum* due to the primordial  
351 homoploid hybrid speciation, and is, through its sect. *Sitopsis*\* parent, connected to *Ae. speltoides*.

352 In addition to the major evolutionary scenario developed in this work, past or present gene flow  
353 among the different lineages of WWR cannot be ruled out entirely, whenever species come into  
354 contact with each other (Arrigo et al. 2011; Bernhardt et al. 2017). The existence of extinct ancestral

355 lineages (Brassac and Blattner 2015) that could not be sampled may, in general, mislead the results of  
356 *D* statistics (Beerli 2004; Slatkin 2005). However, in that case *D* statistics are expected to return  
357 mostly false-negative test results (Pease and Hahn 2015) instead of arriving at wrong species  
358 connections. On the other hand, although we took a conservative approach, ancestral population  
359 structure, non-random mating, and small effective population sizes, characteristic of inbreeding  
360 species like most wheat wild relative species, could lead to high *D* statistic values (Eriksson and  
361 Manica 2012; Martin et al. 2015). New methods accounting for demographic processes at the scale of  
362 a genus are necessary to overcome this limitation.

363

## 364 **Conclusions**

365 We obtained DNA sequences of 244 nuclear low-copy genes evenly distributed among the Triticeae  
366 chromosomes and genome-wide single-nucleotide polymorphism for all diploid species of the WWR.  
367 A combination of different phylogenetic and network approaches together with *D* statistics revealed  
368 ancient complex reticulated processes partly involving multiple rounds of introgression as well as at  
369 least one homoploid hybrid speciation during the formation of the extant taxa.

370 Based on our comprehensive taxon sampling we are able to propose a detailed scheme of events that  
371 shaped the close relatives of wheat, and is much more complex than previously suggested (Marcussen  
372 et al. 2014; Li et al. 2015a, b; Sandve et al. 2015; El Baidouri et al. 2017). With two independent  
373 datasets, we were not only able to confirm the scenario developed by Glémin et al. (2019) and that  
374 seems to best reflect the evolution of wheat wild relatives but also to uncover more complex pattern of  
375 inter-specific gene flow. Our hypothesis is congruent with the proposed formation of the **D**-genome  
376 lineage through homoploid hybrid speciation (Marcussen et al. 2014) but proposes, in agreement with  
377 Glémin et al. (2019), *Am. muticum* together with the *Triticum* lineage as progenitors. Furthermore, we  
378 suggest that *Ae. longissima* or members of sect. *Sitopsis*\* played an important role in the formation of  
379 *Ae. comosa* (**M**), *Ae. markgrafii* (**C**), and *Ae. tauschii* (**D**). We propose that *Ae. tauschii* belongs to the  
380 **CUMN** clade but was introgressed by *Ae. longissima* or sect. *Sitopsis*\* thus appearing as its sister  
381 species. Moreover, our data provide evidence of gene flow between sect. *Sitopsis*\* and the **B**-genome  
382 lineage, a hypothesis raised by El Baidouri et al. (2017) and Glémin et al. (2019). We also show that  
383 *Am. muticum* cannot be separated from *Aegilops*, as it is sister-taxon to *Ae. speltoides* for nuclear data  
384 and is both a progenitor of and introgressed by other *Aegilops* species as shown from *D* statistics and  
385 plastid phylogenies (Bordbar et al. 2011; Bernhardt et al. 2017). As the here proposed scenario is  
386 highly reticulate, it is necessary to obtain extensive genome information for all diploid species of this  
387 group to test predictions regarding composite genomes. Hybrid speciation and introgression should  
388 influence genome organization, the presence of syntenic blocks, and the occurrence of different  
389 transposable elements within the basic and hybrid lineages of the wheat group taxa. In more general

390 terms the question remains if the important role of hybrid speciation and introgression we found in the  
391 wheat group is a peculiarity of these taxa or if it plays an important role in most grasses or generally  
392 in plant evolution but was not yet detected, as studies using an approach similar to ours are still  
393 mainly in their infancy.

394

## 395 **Materials and Methods**

### 396 **Plant materials**

397 We analyzed 97 individuals representing all diploid species of the WWR with multiple individuals  
398 plus three outgroup taxa (i.e. *Dasypyrum*, *Hordeum*, *Taeniatherum*) of the grass tribe Triticeae (Table  
399 S5). All materials were grown from seed and identified based on morphological characters if an  
400 inflorescence was produced. Vouchers of the morphologically identified materials were deposited in  
401 the herbarium of IPK (GAT). Genome size and ploidy level of 83 individuals were initially verified  
402 by flow cytometry and genomic DNA was extracted as in Bernhardt et al. (2017).

403

### 404 **Design of capture probes and library preparation for target-enrichment**

405 We used the assembly of *H. vulgare* cv. ‘Morex’ (Mayer et al. 2012), the only Triticeae draft genome  
406 that was available at the time of bait design, to select loci for which orthology could be confirmed  
407 when comparing them to the fully sequenced grass genomes of *Brachypodium distachyon*, rice, and  
408 sorghum (Vogel et al. 2010; Matsumoto et al. 2011; Mayer et al. 2011). Subsequently, one locus was  
409 selected every 0.5 cM on all *H. vulgare* chromosomes. These loci were used for BLAST comparisons  
410 (Altschul et al. 1990) against available data of *Brachypodium*, rice, sorghum, barley, and wheat.  
411 Multiple sequence alignments were built including full-length cDNA (fl-cDNA) and genomic DNA  
412 sequences. Finally, 451 loci were chosen for the design of hybridization probes, if they showed (i) a  
413 conserved exon-intron structure, (ii) a total length of exonic region larger than 1000 bp with (iii) a  
414 minimum size of single exons being 120 bp, and (iv) introns separating adjacent short exons being  
415 smaller than 400 bp. The design of capture probes for the selected loci was finally based only on fl-  
416 cDNAs from *H. vulgare* and *T. aestivum*, two distantly related Triticeae taxa, and *Brachypodium*  
417 *distachyon*, which was used to broaden the taxonomic spectrum. Capture probes for each of the loci  
418 were designed on exon sequences of all three species. The loci used for bait-design are evenly  
419 distributed over the **A**, **B**, and **D** genomes of *T. aestivum* (Table S1, Figure S1). The total exonic  
420 sequence information considered in bait design amounts to 690 kb. Custom PERL scripts were used to  
421 design bait sequences that were submitted to the web-based application eARRAY (Agilent  
422 Technologies). A detailed description of the bait design can be found in the Supplementary  
423 Information (SI) Material and Methods.

424 For each of the selected 69 samples (Table S5) 3 µg genomic DNA were sheared into fragments  
425 having an average length of 400 bp. The sheared DNA was used in a sequence-capture approach  
426 (SureSelect<sup>XT</sup> Target Enrichment for Illumina Paired-End Sequencing, Agilent Technologies). All  
427 samples were barcoded, pooled, and sequenced on the Illumina HiSeq 2000 or MiSeq. For further  
428 details see SI Material and Methods.

429

### 430 **Library construction and sequencing for genotyping-by-sequencing (GBS)**

431 GBS and Illumina sequencing were performed for 57 individuals (Table S5) following Wendler et al.  
432 (2014). *Dasypyrum villosum* and *Taeniatherum caput-medusae* were included as outgroup taxa. For  
433 each individual, 200 ng genomic DNA were digested by two restriction enzymes *Pst*I-HF (CTGCAG,  
434 NEB Inc.) and *Msp*I (CCGG, NEB Inc.). Sequencing was done on an Illumina HiSeq 2500 obtaining  
435 100 bp single-end reads.

436

### 437 **Target-enrichment data assembly and analyses**

438 *Assembly* - The loci were assembled in a two steps procedure. First, all 451 loci were assembled in a  
439 fast and non-stringent approach to evaluate if the capture worked sufficiently and if the loci are truly  
440 single-copy in most of the taxa. For each sample, the sequence reads were mapped to the barley  
441 genome assembly (Mayer et al. 2012) using the Burrows-Wheeler Alignment (BWA) Tool v. 0.7.8  
442 (Li and Durbin 2009). Consensus sequences were called using SAMTOOLS version 1.1. (Li et al.  
443 2009; Li 2011) and converted into FASTA sequences using VCFUTILS and SEQTK version 1.0 (Heng  
444 Li, <https://github.com/lh3/seqtk>). The percentage of ambiguous sites was determined for each  
445 sequence in locus-wise multiple sequence alignments. Allelic diversity is assumed to be much lower  
446 than 1% for single- and low-copy-number loci (for comparison see Jakob et al. 2014). Thus, a high  
447 percentage of ambiguous positions for sequences of the same species are assumed to reflect the  
448 presence of paralogous gene copies. Finally, loci with an average number of ambiguous sites >1% in  
449 six or more species of *Aegilops* and *Triticum* were considered as multi-copy (Table S3). Then, the loci  
450 found to be mainly low-copy-number loci were kept and selected for a refined assembly procedure if  
451 they had a length of at least 1000 bp, contained less than 25% of missing data and at least 15% of  
452 parsimony-informative positions, as identified with PAUP\*4.0a146 (Swofford 2002). The refined  
453 assembly was performed in GENEIOUS v. 10.0.5 (Kearse et al. 2012) as it can reliably assemble short  
454 insertions and deletions (Smith 2015). For further details see SI Materials and Methods.

455 *Phylogenetic analyses* - To infer the phylogeny of the wheat relatives we adopted an analysis  
456 approach consisting of the following steps. After aligning the sequences for all loci separately, (i)  
457 models of sequence evolution were determined for each locus. (ii) Gene trees were inferred for each

458 locus by maximum likelihood (ML). (iii) The degree of gene tree/species tree conflict was  
459 investigated in detail with PHYPARTS. (iv) Concatenated sequences from all loci (supermatrix) were  
460 used for Bayesian phylogenetic inference (BI), maximum likelihood (ML), maximum parsimony  
461 (MP) and NEIGHBORNET analyses. (v) Multispecies coalescent-based analyses were conducted to  
462 infer species trees from the ML gene trees. (vi) Phylogenetic networks were calculated based on the  
463 ML gene tree topologies. These analysis steps are detailed below.

464 *Gene tree inference* - Individual gene trees were inferred using RAXML v. 8.1 (Stamatakis 2014)  
465 under the GTRCAT model, rapid bootstrapping of 100 replicates and search for the best-scoring ML  
466 tree. To reduce noise from the data, the ML trees were further processed by contracting low support  
467 branches (bootstrap values < 10) as suggested by (Chao Zhang et al. 2018) with the Newick utilities  
468 function `nw_ed` and rerooted using the MRCA of *Hordeum* as outgroup with the function `nw_reroot`  
469 (Junier and Zdobnov 2010).

470 *Supermatrix phylogeny* - Multiple sequence alignments of all 244 loci were concatenated. Bayesian  
471 inference was performed in MRBAYES v. 3.2.6 (Ronquist et al. 2012) on CIPRES, Cyberinfrastructure  
472 for Phylogenetic Research Science Gateway 3.3 (Miller et al. 2010). The best-fitting models of  
473 sequence evolution were estimated by making the MCMC sampling across all substitution models as  
474 described in Bernhardt et al. (2017). *Hordeum vulgare* was set as outgroup. An alternative approach  
475 to visualize the variation in the data was conducted by computing an unrooted phylogenetic network  
476 via SPLITSTREE v. 4.14.8 (Huson and Bryant 2006). The tool was run using the algorithms  
477 Uncorrected P, NeighborNet and EqualeAngle for the matrix of the 244 concatenated target-  
478 enrichment loci.

479 An MP analysis of the supermatrix was conducted in PAUP\* v. 4.0a146 (Swofford 2002) to see if the  
480 phylogeny obtained by BI is sufficiently robust with regards to different analysis algorithms. The MP  
481 analysis was run using a heuristic search with 100 random-addition sequences and tree bisection and  
482 reconnection (TBR) branch swapping, saving all shortest trees. Node support was evaluated by 500  
483 bootstrap re-samples with the same settings but without random-addition sequences.

484 *Coalescent-based species tree estimation* - The effect of gene tree conflicts due to ILS was addressed  
485 using the short-cut coalescence method ASTRAL (Mirarab et al. 2014; Chao Zhang et al. 2018), which  
486 is able to estimate the true species tree with high probability, given a sufficiently large number of  
487 correct gene trees under the multispecies coalescent model. ASTRAL-III v. 5.6.3 was run using 244 the  
488 ML edited and rerooted gene trees pre-estimated in RAXML.

489 *Differences among gene trees* - PHYPARTS (Smith et al. 2015) was used to summarize the amount of  
490 concordant and conflicting phylogenetic signal from the 244 ML gene trees with the ASTRAL  
491 topology as species tree. Visualization of the output was done as in Kates et al. (2018) and Villaverde

492 et al. (2018), and using the `phypartspiecharts.py` script of M. Johnson available at  
493 [www.github.com/mossmatters/phyloscripts](http://www.github.com/mossmatters/phyloscripts).

494 *Maximum pseudo-likelihood gene tree-based phylogenetic networks estimation* - Throughout all  
495 analyses *Ae. sharonensis* groups within *Ae. longissima* and *T. monococcum* within *T. boeoticum*. This  
496 is in accord with what is already known about these species, i.e. *Ae. sharonensis* and *Ae. longissima*  
497 are closely related taxa, and the unified or separate treatment of the two *Triticum* taxa is debated (van  
498 Slageren 1994; Bernhardt 2015). Here we use *Ae. sharonensis* and *T. boeoticum* if accessions were  
499 assigned to this taxon in the donor seed bank. However, due to their strong genetic similarity we treat  
500 *Ae. sharonensis* and *Ae. longissima* as well as *T. boeoticum* and *T. monococcum* con-specific.

501 The effect of gene tree conflicts due to hybridizations was investigated with the maximum pseudo-  
502 likelihood method `InferNetwork_MPL` (Yu and Nakhleh 2015) included in the package `PHYLONET`  
503 (Than et al. 2008; Wen et al. 2018). The set of ML gene trees analyzed with `ASTRAL` was used as  
504 input for `PHYLONET` allowing for zero to five hybridizations, other options were left to default. For  
505 each analysis, the best network was recorded and they were compared using the Akaike information  
506 criterion (AIC; Akaike 1974). As suggested by Yu et al. (2012) and Morales-Briones et al. (2018), the  
507 number of parameters was set to the number of branches plus the number of hybridization  
508 probabilities being estimated. The network with the lowest AIC score was selected as the best-fit  
509 multi-species network. The network was visualized with `DENDROSCOPE` (Huson and Scornavacca  
510 2012).

511

#### 512 **Assembly and analysis of GBS data.**

513 The assembly of the GBS data was performed *de novo* using `IPYRAD v. 0.7.17` (Eaton 2014;  
514 <https://github.com/dereneaton/ipyrad>), with strict filtering for adapters and restricting the maximum  
515 number of heterozygous sites per locus to 25%. Default settings were used for the remaining  
516 parameters.

517 A species tree based on `SVDQUARTETS` (Chifman and Kubatko 2014) under multispecies coalescence  
518 was estimated using `TETRAD`, as implemented in `IPYRAD v. 0.7.17` with 100 bootstrap replicates. For  
519 comparison with the target-enrichment data `SPLITSTREE v. 4.14.8` (Huson and Bryant 2006) was run  
520 using the methods `Uncorrected P`, `NeighborNet` and `EqualeAngle` to compute unrooted phylogenetic  
521 networks for 807,909 SNPs of the GBS analysis.

522

#### 523 **Identification of hybrid taxa.**

524 We used Four-taxon *D* statistics (Green et al. 2010a; Durand et al. 2011; Eaton and Ree 2013) for the  
525 GBS data to identify candidate lineages involved in the introgressive hybridization within a fixed



526 phylogeny (((P1, P2) P3), O). Under ILS alone, the number of shared single-nucleotide  
527 polymorphisms (SNPs) resulting in an incongruent topology (i.e. ABBA and BABA) are expected to  
528 be equivalent. If P3 was involved in an introgressive event with P1, it will share more SNPs with P1  
529 (i.e. BABA patterns), than with P2 (i.e. ABBA patterns).

530 The VCF file generated by IPYRAD was first filtered with SAMTOOLS/BCFTOOLS (Li 2011) retaining  
531 only unlinked SNPs. Four-Taxon *D* statistic tests were performed using the routine Dtrios of DSUITE  
532 (Malinsky 2019; <https://github.com/millanek/Dsuite>). We first tested if *Taeniatherum caput-medusae*  
533 was involved in any introgressions. As no hybridization signal was found (Fig. S10) and because it is  
534 sharing more loci with the WWR than *D. villosum*, *Ta. caput-medusae* was used as outgroup taxon for  
535 all following tests. The VCF file was further processed to exclude all *D. villosum* individuals and  
536 DTRIOS was used to perform 220 tests. The ASTRAL topology (Fig. 1A) was used to specify species  
537 relationships. *D* statistics significance was assessed using jackknife (Green et al. 2010) on blocks of  
538 100 SNPs. The function *p.adjust* in R 3.5.3 (R Core Team 2019) was used to apply a Benjamini-  
539 Yekutieli correction (Benjamini and Yekutieli 2001). All 220 tests are summarized in Table S7. The  
540 results were visualized with the Ruby script “plot\_d.rb” available from M. Matschiner  
541 (<https://github.com/mratschiner>).

542 The  $D_{\text{FOIL}}$  test (Pease and Hahn 2015; <https://github.com/jbpease/dfoil/>) was used on the GBS data. It  
543 relies on a symmetric five-taxon phylogeny (((P1, P2), (P3, P4)), O) to identify the direction of  
544 introgressions among the candidate taxa identified using the Four-Taxon *D* statistic. All tests were  
545 performed on species-specific consensus sequences. For each species, the alignment of all loci was  
546 used to call a consensus sequence that represented all diversity within the species. Therefore, we used  
547 the “0% identical” threshold in GENEIOUS that minimizes the number of ambiguities. A custom  
548 workflow in GENEIOUS was used to create datasets of five species including *Ta. caput-medusae* as  
549 outgroup. For all tests, we made sure that the estimated divergence times fit the assumptions of the  
550 program, i.e. that P1 and P2 diverged after P3 and P4 in forward time, by excluding all tests that  
551 raised the warning “b” (Table S8). We also used a feature of  $D_{\text{FOIL}}$ , i.e.  $D_{\text{FOIL,alt}}$ , that excludes single  
552 derived-allele count for tests with an error warning “c” (Table S8) following Leduc-Robert and  
553 Maddison (2018). As a total of 216 tests were conducted, a Benjamini-Yekutieli correction  
554 (Benjamini and Yekutieli 2001) was applied to all four statistics for each test with the function  
555 *p.adjust* in R 3.5.3 (R Core Team 2019). A significance level of 0.01 was then used on the adjusted *p*  
556 values to identify patterns of introgression.

557

558 **Contribution of Authors**

559 Designed study: FRB, NB, BK. Coordinated study: NB. Provided data or materials: EMW, BK.  
560 Performed experiments: NB. Analyzed data: NB, JB, XD, FRB, and CHP. NB and FRB wrote the  
561 initial manuscript. All authors contributed to and approved the final version.

562

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572 enriched nuclear loci (Dataset S1), the demultiplexed fasta-file of the barcoded reads for each  
573 accession used for GBS and the matrix for the filtered loci (Dataset S2) are published via e!DAL  
574 (Arend et al. 2014) at <http://dx.doi.org/XXX>.

575

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791 **Figure Legends**

792 **Figure 1. Comparison of coalescent-based phylogenetic trees for the diploid wheat wild**  
793 **relatives.** Triticeae-specific genome designations are provided for the respective clades. Fully  
794 supported nodes are indicated by asterisks. **A** Schematic representation of the multi-species coalescent  
795 tree calculated from separate maximum likelihood gene trees of 244 target-enriched low-copy loci  
796 using ASTRAL. Numbers at nodes depict local posterior probabilities. **B** Consensus cladogram derived  
797 from a TETRAD analysis of GBS data. Numbers along branches are bootstrap support values (%).

798 **Figure 2. Phylogenetic network inferred under the multispecies network coalescent (MSNC)**  
799 **from the 244 gene tree topologies using maximum pseudo-likelihood.** The network with four  
800 reticulations was selected as best-fit among zero to five hybridizations calculated with the routine  
801 InferNetwork\_MPL of PHYLONET under the Akaike information criterion (Fig. S7). Reticulations are  
802 indicated by blue arcs with major contribution of species to hybrid lineages indicated by bold lines.  
803 Numbers represent estimated inheritance probabilities.

804 **Figure 3. Heatmap summarizing Four-taxon  $D$  statistic tests using *Taeniatherum caput-medusae***  
805 **as outgroup.** The plot is based on 220 tests. It shows the  $D$  statistic results and their significance for  
806 each pair of species. Red and blue indicate high and low  $D$  statistic values, respectively. The intensity  
807 of the color corresponds to the  $p$  value (in log-scale) assessed using the block jackknife procedure and  
808 corrected with Benjamini-Yekutieli for multiple testing. All  $D$  statistic results are summarized in  
809 Table S7.

810 **Figure 4. Representation of  $D_{\text{FOIL}}$  results for genotyping-by-sequencing data.** All significant  
811 relationships after Benjamini-Yekutieli correction are shown on a modified version of the TETRAD  
812 species tree. Each tree shows all significant relationships for a focal taxon. An arrow tip indicates the  
813 direction of hybridization/introgressions between two taxa. Undirected relationships involving three  
814 taxa are shown using a branched line. Taxa not contributing to hybridization signal for the focal taxon  
815 are shown in grey for easier visualization. All  $D_{\text{FOIL}}$  results are summarized in Table S8.

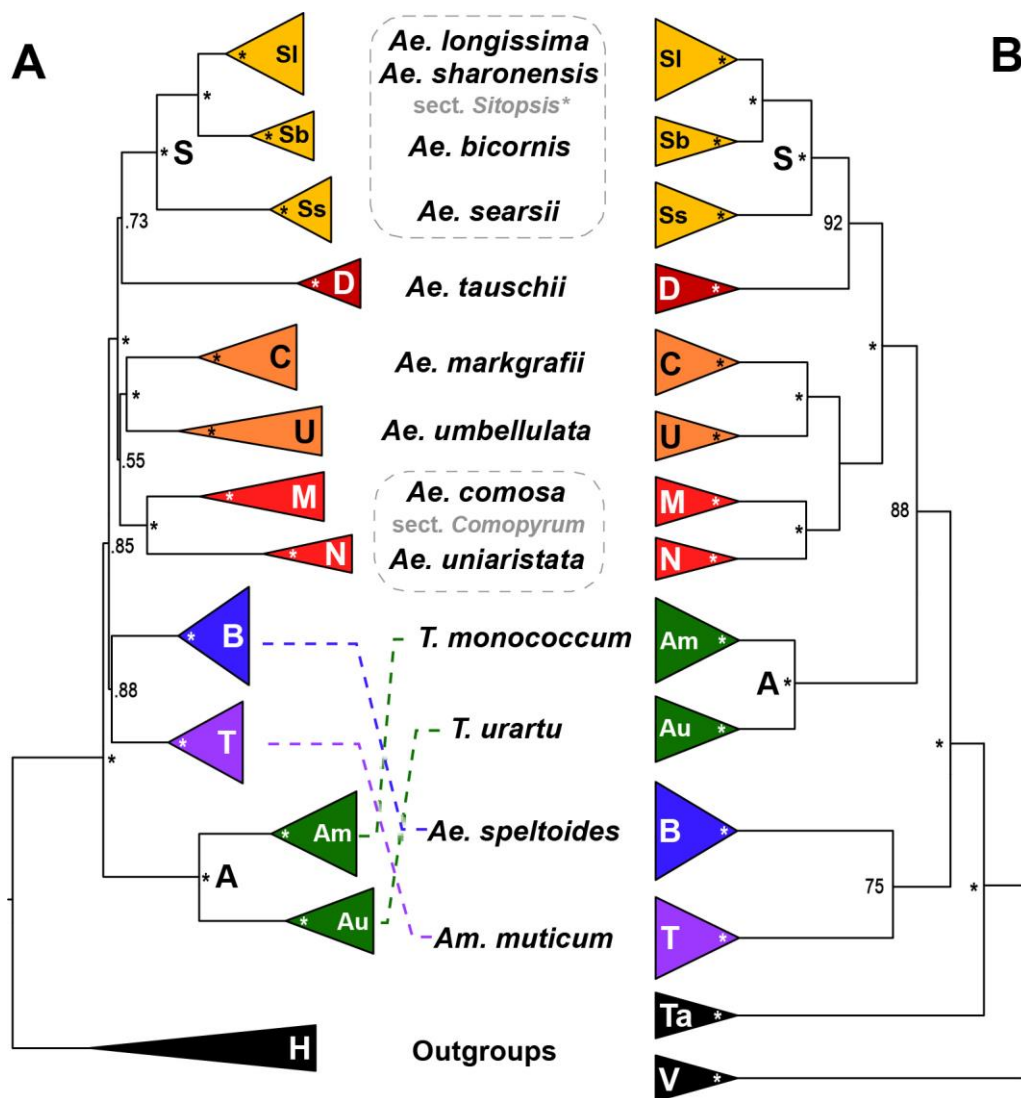
816 **Figure 5. Total evidence evolutionary scenario for the wheat wild relatives.** All diploid *Aegilops*  
817 species except *Ae. speltoides* are derived from an initial homoploid hybridization event involving the  
818 ancient **A** (*Triticum*) and **T** (*Am. muticum*) lineages (1). Strong signals of introgression were found for  
819 *Am. muticum* (from the U/C group; 2) and between *Ae. speltoides* and sect. *Sitopsis* (3). For the latter,  
820 introgression seems to have happened in both directions. Weaker signals of introgression (dashed  
821 arrows) were found by GBS-based  $D$  statistics from the *Triticum* (**A**) into the **M/N** lineage (4), and (5)  
822 from sect. *Sitopsis* into *Ae. tauschii* (**D**), *Ae. markgrafii* (**C**) and *Ae. comosa* (**M**).

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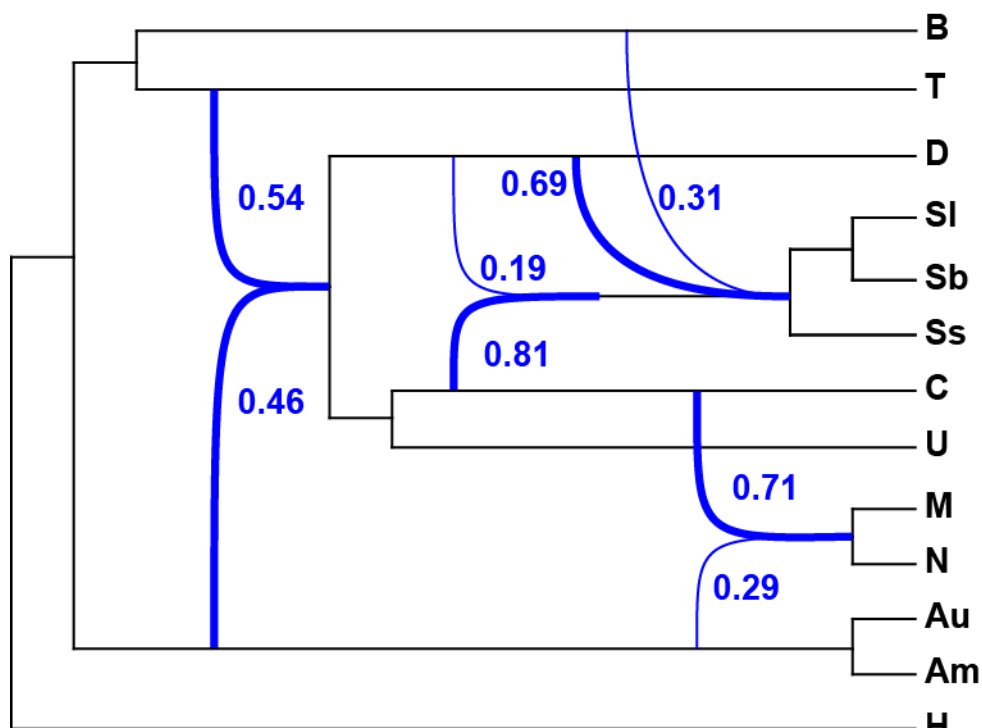
826 **Figure 1**



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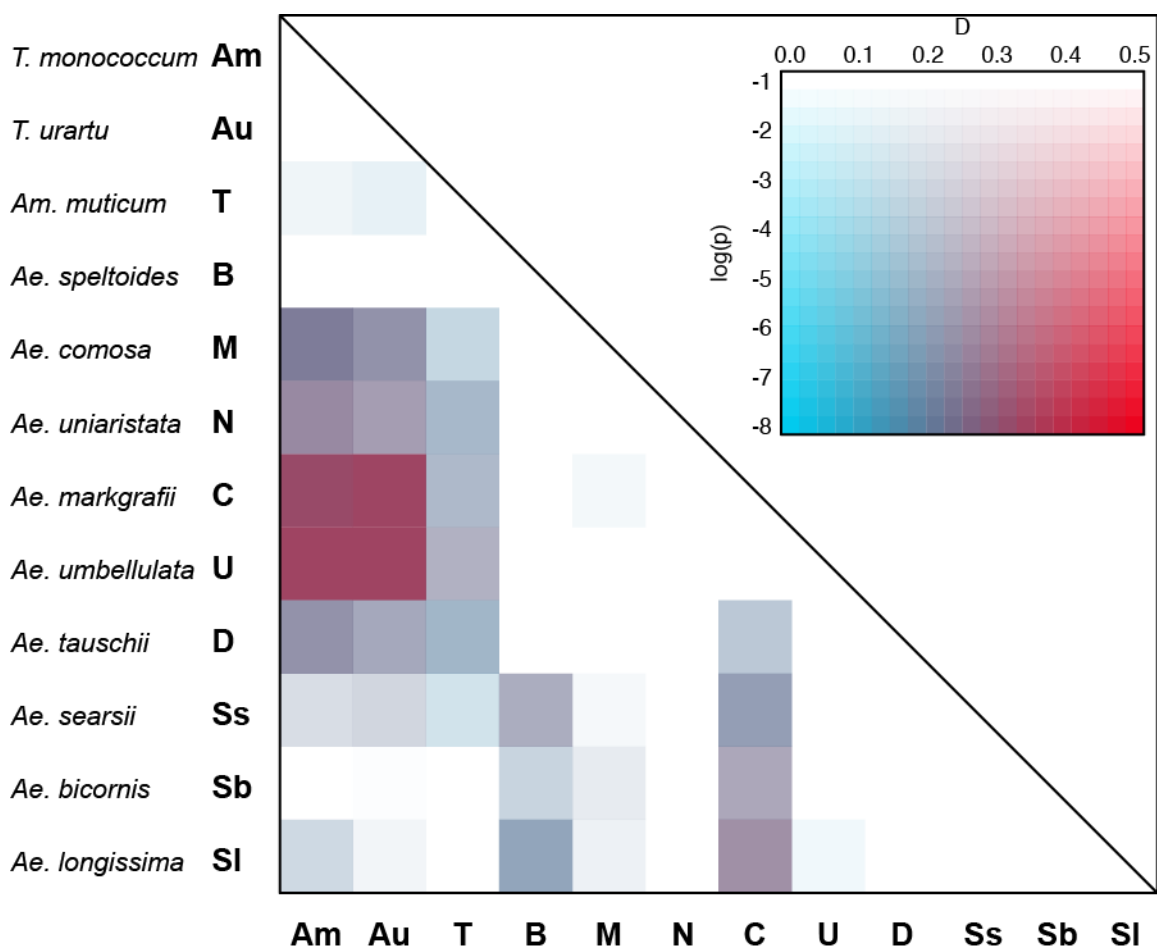
828

829 **Figure 2**



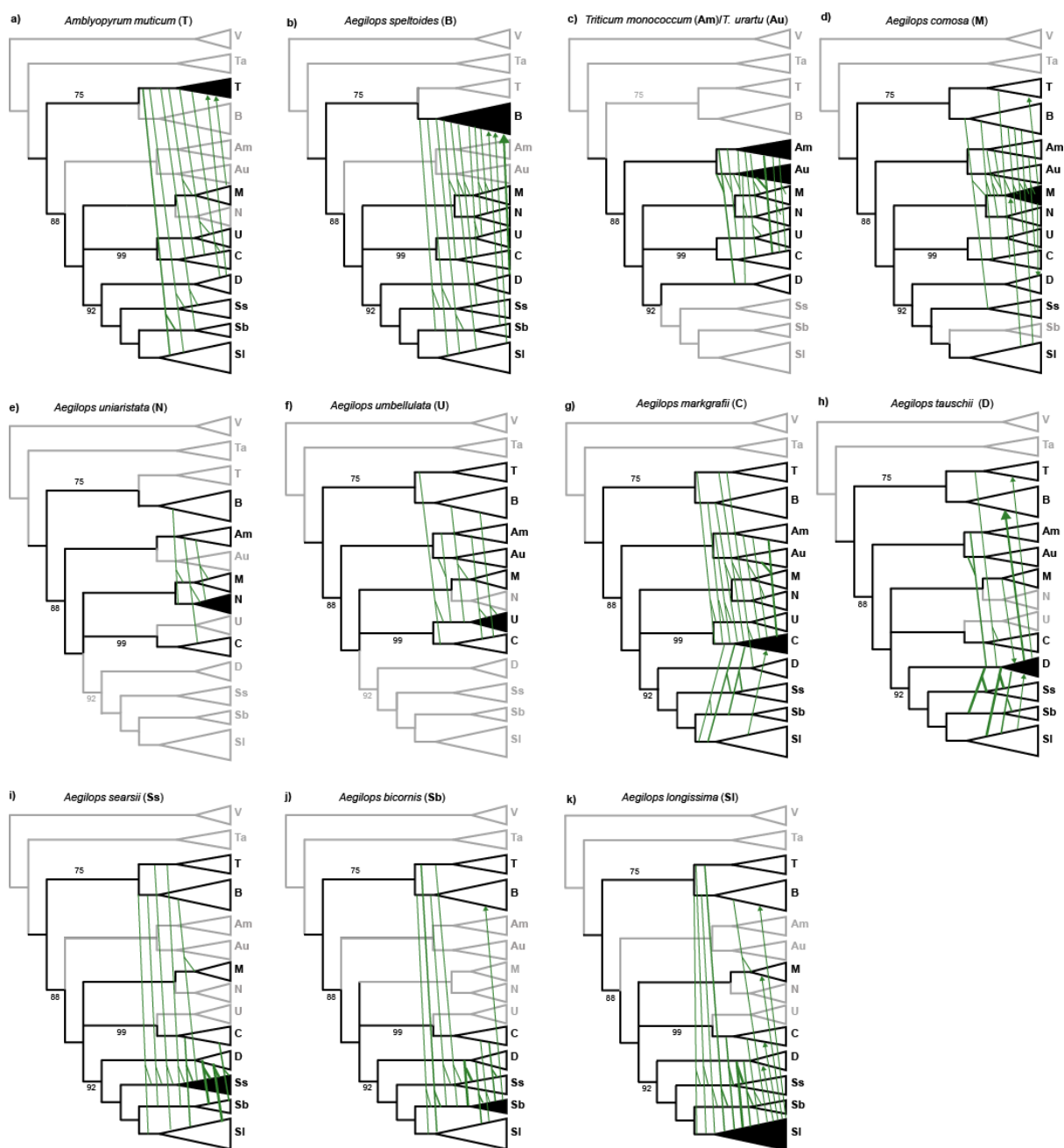
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831 **Figure 3**



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833 **Figure 4**



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836 **Figure 5**

