ABSTRACT

Brachypodium sylvaticum has been selected as a model for perennial grasses, and considerable genomic resources have been generated and a reference genome and several resequenced pan genome accessions are available for this species. Despite these genomic advances, the evolution and systematics of diploid B. sylvaticum s.l. is almost unknown. The B. sylvaticum complex is formed by up to seven taxonomically close micro-taxa which differentiate from typical B. sylvaticum s.s. based on a few morphological features. Moreover, some of them show some largely disjunct geographic distributions on both sides of their native Palearctic region. In this study, we used a phylogenomic approach including representative populations from the oriental and occidental distribution range of B. sylvaticum micro-taxa to elucidate their evolutionary relationships and assess the systematic value of the morphological features that separate them. A combined plastome and nuclear phylogenetic tree supports an early split and high divergence of the oriental lineage, showing the close relationship of the Himalayan B. sylvaticum var. breviglume lineages to the Pacific B. miserum / B. kurielense clade, and the contrasting large homogeneity and low divergence of the occidental European, N African and SW and C Asian lineages, with several B. sylvaticum s.s., B. spryginii, and B. glaucovirens samples showing identical or similar sequences. Divergence time estimate analysis suggests that the oriental lineage diverged from the common ancestor in the early Pleistocene (2.0 Ma), followed by subsequent colonization and isolations in the Himalayas (2.0 – 1.7 Ma) and the Far East (0.36 Ma) in more recent times, while the occidental lineage split in the Mid-Late Pleistocene (0.97 Ma), followed by rapid radiation and postglacial spread in the western Palearctic during the last thousand years.

Keywords: Brachypodium sylvaticum complex, diploid populations, discriminant morphological traits, eastern and western Eurasian micro-taxon, phylogenetics, plastomes, rDNA 35S gene, systematics
Since its proposal as a model perennial grass system (Steinwand et al. 2013, Gordon et al. 2016), Brachypodium sylvaticum has attracted the attention of the scientific community as a suitable model plant for understanding the molecular mechanisms that caused the transitions between perennial-annual plants (Hu et al. 2011, Scholthof et al. 2018, Friedman 2020), and to design strategies for improving perennial energy crops (Carroll & Somerville 2009, Dohleman & Long 2009) and develop perennial grain crops (Glover et al. 2010, Gordon et al. 2016). This conceptual framework has conveyed the generation of large genomic and biological resources (e.g., transformation and inbred lines, Steinwand et al. 2013; transcriptome atlases, Fox et al. 2013; salt tolerance responses, Sade et al. 2018) for B. sylvaticum, paralleling those of its well-known annual congener B. distachyon (Scholthof et al. 2018). A reference genome has been assembled for one Brachypodium sylvaticum accession (B. sylvaticum-Ain1; Phytozome https://phytozome-next.jgi.doe.gov/info/BsylvaticumAin_1_v2_1) and current research on the genomic resequencing of other B. sylvaticum lines aims to generate a pangenome for this model perennial grass (Joint Genome Institute, Community Science Program; https://jgi.doe.gov/brachypodium-model-grass-genus-bioenergy/).

Brachypodium sylvaticum has also been the subject of large taxonomic, cytogenetic, evolutionary and ecological research. This perennial grass presents the broadest native distribution of all worldwide Brachypodium species, covering the entire Paleartic region, from Macaronesia (West) to New Guinea (East) and from Scandinavia and Siberia (North) to northern Africa and Asia (South) (Catalán et al. 2016). It is also the only known invasive perennial Brachypodium taxon in the New World (Rosenthal et al. 2008, POWO https://powo.science.kew.org/taxon/urn:lsid:ipni.org:names:393196-1, accessed 16 April 2023). Morphologically, B. sylvaticum separates from other Brachypodium congeners based on the possession of short and slender rhiizomes, nodding panicle, densely hairy habit and long-awned lemma (Schippmann 1991). It is a self-compatible perennial species (Khan & Stace 1999, Steinwand et al. 2013), adapted to mesic and nemoral habitats of humid forest (Schippmann 1991). Some of its features are also shared by the tropical and South African B. flexum and the Malagasy B. madagascariensis, though they differ from the former in their shorter panicles, spikelets and awns (Catalán et al. 2016). The B. sylvaticum complex includes up to seven micro-taxes; B. sylvaticum s. s., described from England, is the most widespread species in Europe, N Africa, and SW and C Asia (Schippmann 1991). The remaining six cryptic micro-taxes were described from different regions of eastern Europe, Asia, and Malesia. They were formerly synonymized with B. sylvaticum, although some of them were later disgregated from it (B. sylvaticum var. breviglumis, B. sylvaticum var. pseudodorstelbyon, B. kurilense, B. miserum, B. pu­becens, B. spryginii; Keng 1982, Tzvelev 1983, 2015, Probatova & Skolovskaya 1985, Tzvelev & Probatova 2019) based on features related to the length of glumes, plant pubescence and height of the plant. All the B. sylvaticum complex taxa are further characterized by a constant diploid chromosome number of $2n = 2x = 18$ and a chromosome base number of $x = 9$ (Wolny & Hasterok 2009, Catalán et al. 2016, Tzvelev & Probatova 2019, Decena et al. 2023). A close taxon to the B. sylvaticum complex is the eastern Mediterranean – SW Asian endemic B. glaucoviridis, which is also a diploid species but showing a smaller chromosome number and chromosome base number ($2n = 2x = 16; x = 8$) than B. sylvaticum s. s. (Wolny & Hasterok 2009, Catalán et al. 2016).

Phylogenetic studies of Brachypodium based on plastid and nuclear loci have consistently reconstructed B. sylvaticum as a main lineage of the recently evolved core-perennial Brachypodium clade (Catalán & Olmstead 2000, Catalán et al. 2016, Díaz-Pérez et al. 2018). Molecular dating analysis inferred a recent Mid-Late Pleistocene origin for the B. syl­vaticum s. l. lineages (1.2–0.2 Ma; Díaz-Pérez et al. 2018). A phylogenomic survey based on transcriptome data also supported a recent split of B. sylvaticum s. s. from its close B. pisum sister lineage in the Pleistocene (1.4 Ma; Sancho et al. 2022). Fluorescent In Situ Hybridization (FISH)-based comparative chromosome barcoding (CCB) analysis characterized the karyotypic profiles of B. sylvaticum s. s. ($x = 9$) and of B. glaucoviridis ($x = 8$) and hypothesized their derived origins from either ancestral $x = 10$ or intermediate-ancestral $x = 9$ Brachypodium karyotypes through descendant disjunct disjunct (Lusinska et al. 2019, Sancho et al. 2022); however, the B. sylvaticum s. s. barcoded karyotype could not be differentiated from those of other close diploid core perennial $x = 9$ species (B. arbuscula, B. pisum) probably due to their recent divergences from the common ancestor (Sancho et al. 2022).

Despite the enormous advances attained in the genomic study of the model perennial grass B. sylvaticum s. s., the systemsatics of the B. sylvaticum complex micro-taxa is almost unknown. Taxonomically, the morphological characters used to separate several of these micro-taxes are extremely plastic and variable, and have been considered of doubtful taxonomic relevance in some cases (Tzvelev 2015, Catalán et al. 2016). Nonetheless, some of these taxa show large disjunct geographic distributions in both extremes of Eurasia, which could have led to genetic isolation and evolutionary divergence. Moreover, no evolutionary study has included representative samples of oriental and occidental populations of B. sylvaticum s. l. yet. Therefore, the objectives of our study were to conduct a phylogenomic study of the B. sylvaticum complex micro-taxa using whole plastid genome (plastome) and nuclear ribosomal DNA (rDNA) 35S gene sequences to elucidate their evolutionary relationships and to assess the systematic value of the morphological features that separate them. Our study also aimed to infer the divergence ages of these lineages and to estimate their respective ancestries.

**MATERIAL AND METHODS**

**Sampling and taxonomic identification**

A total of 18 Brachypodium sylvaticum s. s. samples collected from different European, N African and Asian populations, plus six samples of close B. sylvaticum complex taxa [B. sylvaticum var. breviglumis (2), B. kurilense (1), B. miserum (1), B. glaucoviridis (1), B. spryginii (1)], five samples from other congeners [B. pisum (2), B. arbuscula (1), B. distachyon (1), B. stacei (1)] and three outgroup grass samples (Triticum
Phylogenetics of *Brachypodium sylvaticum* uncovers two divergent oriental and occidental micro-taxa lineages

asticum, *Oryza sativa*, *Sorghum bicolor*) were included in the evolutionary and systematic study (Fig. 1, Table 1). The taxonomic identification of the studied materials was based on previous taxonomic treatments of *Brachypodium* (Keng 1982, Schippmann 1991, Catalán et al. 2012, Tzvelev & Probatova 2019). Chromosome and ploidy level information was retrieved from these studies and from a broad evo-phylogenomic study of *Brachypodium* perennial species performed by Decena et al. (2023, and unpub. data).

**Genomic data and phylogenetic analysis**

Total genomic DNA isolation and genome scaffolding sequencing procedures are detailed in Moreno-Aguilar et al. (2020) and Decena et al. (2023). Genomic data for plastome reconstruction and nuclear rDNA 35S genes were retrieved from the respective reference genomes of *B. sylvaticum* An1, *B. distachyon* Bd21 and *B. stacei* ABR114 accessions (Sancho et al. 2018, 2022) and downloaded from Phytozome (https://phytozome-next.jgi.doe.gov//). Assembly of plastomes and rDNA 35S sequences of the remaining *Brachypodium* samples was performed using NovoPlasty v. 2.7.1 (Dierckxsens et al. 2017) and read-mapping strategies to the reference 35S sequences with Geneious Prime 2023, respectively. Multiple sequence alignments (MSA) were performed separately for entire plastomes and nuclear 35S sequences using MAFFT.v7.450 (Katoh & Standley 2013), and removal of low quality regions from each of the MSAs was performed with TrimAl v. 1.2rev59 (Capella-Gutierrez et al. 2009) followed by manual curation.

Maximum likelihood (ML) phylogenetic trees were reconstructed for each separated data set with IQ-TREE 1.6, imposing the best-fit nucleotide substitution model, ac-

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### Table 1. List of the *Brachypodium sylvaticum* complex samples

<table>
<thead>
<tr>
<th>Taxon</th>
<th>Code</th>
<th>Location, herbarium acronym</th>
<th>2n</th>
<th>Ploidy</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>B. sylvaticum</em> (Huds.) P. Beauv. var. <em>sylvaticum</em></td>
<td>Bsylvaticum_A11</td>
<td>Tunisia: Ain Draham, JGI</td>
<td>18</td>
<td>2x</td>
<td>Sancho et al. 2022</td>
</tr>
<tr>
<td></td>
<td>Bsylvaticum_S11</td>
<td>Turkey: Sinop, JGI</td>
<td>18</td>
<td>2x</td>
<td>Decena et al. 2023</td>
</tr>
<tr>
<td></td>
<td>Bsylvaticum29</td>
<td>Greece: E Vertania, B100281411</td>
<td>18</td>
<td>2x</td>
<td>Decena et al. 2023</td>
</tr>
<tr>
<td></td>
<td>Bsylvaticum30</td>
<td>Denmark: Zealand, B100560906</td>
<td>18</td>
<td>2x</td>
<td>Decena et al. 2023</td>
</tr>
<tr>
<td></td>
<td>Bsylvaticum31</td>
<td>Germany: Ober Bayern, M0177011</td>
<td>18</td>
<td>2x</td>
<td>Decena et al. 2023</td>
</tr>
<tr>
<td></td>
<td>Bsylvaticum32</td>
<td>Russia: Kaluga, M01769847</td>
<td>18</td>
<td>2x</td>
<td>Decena et al. 2023</td>
</tr>
<tr>
<td></td>
<td>Bsylvaticum54</td>
<td>Morocco: Rif Mountains, UZ</td>
<td>18</td>
<td>2x</td>
<td>Decena et al. 2023</td>
</tr>
<tr>
<td></td>
<td>Bsylvaticum63</td>
<td>Morocco: Mulay-Idriss, UZ</td>
<td>18</td>
<td>2x</td>
<td>Decena et al. 2023</td>
</tr>
<tr>
<td></td>
<td>Bsylvaticum434</td>
<td>Ukraine: Krym: USDA PI639821</td>
<td>18</td>
<td>2x</td>
<td>Decena et al. 2023</td>
</tr>
<tr>
<td></td>
<td>Bsylvaticum446</td>
<td>Iran: Ardehils, USDA PI380758</td>
<td>18</td>
<td>2x</td>
<td>Decena et al. 2023</td>
</tr>
<tr>
<td></td>
<td>Bsylvaticum467</td>
<td>Spain: Huesca, Bespen, UZ</td>
<td>18</td>
<td>2x</td>
<td>Decena et al. 2023</td>
</tr>
<tr>
<td></td>
<td>Bsylvaticum470</td>
<td>Spain: Guipuzcoa: San Sebastian, UZ</td>
<td>18</td>
<td>2x</td>
<td>Decena et al. 2023</td>
</tr>
<tr>
<td></td>
<td>Bsylvaticum476</td>
<td>France: Hautes Pyrenees, Barbazan, UZ</td>
<td>18</td>
<td>2x</td>
<td>Decena et al. 2023</td>
</tr>
<tr>
<td></td>
<td>Bsylvaticum501</td>
<td>France: Alpes Maritime. Roquetfort les Pins, UZ</td>
<td>18</td>
<td>2x</td>
<td>Decena et al. 2023</td>
</tr>
<tr>
<td></td>
<td>Bsylvaticum506</td>
<td>France: Herault, Saint Jean de Fos, UZ</td>
<td>18</td>
<td>2x</td>
<td>Decena et al. 2023</td>
</tr>
<tr>
<td></td>
<td>Bsylvaticum508</td>
<td>France: Aude, Villeseque des Corbieres, UZ</td>
<td>18</td>
<td>2x</td>
<td>Decena et al. 2023</td>
</tr>
<tr>
<td></td>
<td>Bsylvaticum552</td>
<td>Spain: Cadiz, Tarifa, UZ</td>
<td>18</td>
<td>2x</td>
<td>Decena et al. 2023</td>
</tr>
<tr>
<td></td>
<td>Bsylvaticum554</td>
<td>Spain: Malaga, Canillas, UZ</td>
<td>18</td>
<td>2x</td>
<td>Decena et al. 2023</td>
</tr>
<tr>
<td><em>B. sylvaticum</em> var. <em>breviglume</em> Keng</td>
<td>Bbreviglumec35</td>
<td>Tibet: Gorgongyamda, LD135398</td>
<td>18</td>
<td>2x</td>
<td>Mo et al. 2023</td>
</tr>
<tr>
<td></td>
<td>Bbreviglumec34</td>
<td>Pakistan: Hazara, Paras-Shogran, M0175636</td>
<td>18</td>
<td>2x</td>
<td>Decena et al. 2023</td>
</tr>
<tr>
<td><em>B. glaucovirens</em> (Marks) T. Durand &amp; BD Jacks</td>
<td>Bglaucovirens</td>
<td>Greece, Crete, B3151</td>
<td>16</td>
<td>2x</td>
<td>Decena et al. 2023</td>
</tr>
<tr>
<td><em>B. kurilense</em> (Proch.) Koidz.</td>
<td>Bkurilense9</td>
<td>Russia: Kuril Islands, Iturup, VLA 1625</td>
<td>18</td>
<td>2x</td>
<td>Tzvelev &amp; Probatova 2019, Decena et al. 2023</td>
</tr>
<tr>
<td><em>B. miserum</em> (Thuils.) Koidz.</td>
<td>Bmiserum67</td>
<td>Japan: Honshu, Aramakioaob, RIKEN</td>
<td>18</td>
<td>2x</td>
<td>Tzvelev &amp; Probatova 2019</td>
</tr>
<tr>
<td></td>
<td>B sprigini Tzvelev</td>
<td>B sprigini29</td>
<td>Russia: Krasnodarski Krai, VLA 11652</td>
<td>18</td>
<td>2x</td>
</tr>
<tr>
<td><em>B. pinnaatum</em> (L.) P. Beauv.</td>
<td>Bpinnatum505</td>
<td>Norway: USDA PI345964</td>
<td>18</td>
<td>2x</td>
<td>Decena et al. 2023</td>
</tr>
<tr>
<td></td>
<td>Bpinnatum515</td>
<td>Kazakhstan: USDA PI440176</td>
<td>18</td>
<td>2x</td>
<td>Decena et al. 2023</td>
</tr>
<tr>
<td><em>B. arbuscula</em> Gay ex Knoche</td>
<td>Barbuscula302</td>
<td>Spain: Canary Islands, La Gomera, INIA</td>
<td>18</td>
<td>2x</td>
<td>Sancho et al. 2022, Decena et al. 2023</td>
</tr>
<tr>
<td>Triticum aestivum L.</td>
<td>cultivar</td>
<td>42</td>
<td>6x</td>
<td>Genbank</td>
<td></td>
</tr>
<tr>
<td><em>Oryza sativa</em> L.</td>
<td>cultivar</td>
<td>24</td>
<td>2x</td>
<td>Genbank</td>
<td></td>
</tr>
<tr>
<td><em>Sorghum bicolor</em> (L.) Moench</td>
<td>cultivar</td>
<td>20</td>
<td>2x</td>
<td>Genbank</td>
<td></td>
</tr>
</tbody>
</table>

According to the Bayesian Information Criterion (BIC), and estimating 1000 ultrafast bootstrap replicates (BS) and SH-rt test with 1000 replicates for the branch support of the best tree (Nguyen et al. 2015). Due to the overall congruence of the plastome and the rDNA topologies, both data sets were concatenated into a combined data matrix and used to compute a combined ML plastome+35S tree. Ancestral divergence times of the Brachypodium lineages were estimated with BEAST 2 (Bouckaert et al. 2014) imposing independent site substitution models for each partition, log-normal relaxed clock and Yule tree linked models, a broad uniform distribution prior for the uncorrelated lognormal distribution (ucld) mean (lower = 1.0E-6; upper = 0.1) and an exponential prior for ucld standard deviation (SD).

Five nodes of the tree were calibrated using secondary age constrains for the crown node of grasses (BOP + PACMAD clade; normal prior mean = 55.0 Ma, SD = 0.5 Ma), the BOP clade (Brachypodium + Oryza; normal prior mean = 51.7 Ma, SD = 1.9 Ma), the Pooidae clade (Brachypodium + Triticeae, normal prior mean = 33.2 Ma, SD = 9.52 Ma), the Brachypodium clade (normal prior mean = 32.2 Ma, SD = 9.5 Ma) and the Brachypodium core-perennial clade (normal prior mean = 2.95 Ma, SD = 1.49 Ma), following the grass-wide dating analysis of Sancho et al. (2018). We ran 600 million Markov chain Monte Carlo (MCMC) generations in BEAST2 with a sampling frequency of 1,000 generations. The adequacy of parameters was checked using TRACER v. 1.66 with all the parameters showing Effective Sample Size (ESS) > 200. A Maximum clade credibility (MCC) tree was computed after discarding 10 % of the respective saved trees as burn-in.

**R E S U L T S**

**Plastome and nuclear rDNA 35S phylogenies**

The full Brachypodium plastome data set included 136,380 filtered positions and the 35S data set 6,237 positions. Whole plastome sequences of the Brachypodium sylvaticum complex species were highly conserved in terms of synteny and gene number: they contained a total of 133 genes (76 protein coding genes, 20 non-redundant rRNAs, four rRNAs in both inverted repeats, four pseudogenes, and two hypothetical open reading frames) and a structure and length similar to that of the B. stacei plastome (Sancho et al. 2018). The 35S showed a conserved structure and similar average lengths along its aligned transcriptional unit in all the samples studied (5’-external transcribed spacer (ETS) (~500 bp), 18S gene (~1,818 bp), internal transcribed spacers and 5.8S gene (ITS1-5.8S-ITS2) (~590 bp), 25S gene (~3,350 bp).

The best ML plastome tree retrieved a strong to well-supported topology for most Brachypodium lineages (100–79 % bootstrap support (BS), Fig. 2A). The earliest splits were those of the annual B. stacei and B. distachyon lineages, followed by the divergence of the Brachypodium core-perennial clade lineages. Within it, the Canarian B. arbuscula lineage branched off first, and the next divergences corresponded to the B. sylvaticum-complex oriental clade and then that of the sister B. sylvaticum-complex occidental clade and B. pinna-tum clade lineages. The oriental clade showed in turn the successive splits of the strongly supported Himalayan B. sylvaticum var. breviglume lineages and Pacific B. miserum / B. kurilense lineages, while the B. sylvaticum occidental clade reconstructed the nesting of B. glaucovirens and B. spryginii within this broadly homogeneous and non-geographically structured lineage, with several tips collapsing in polytomies (Fig. 2A).

The optimal ML 35S tree was less resolved than the plastome tree (Fig. 2B). This phylogeny also recovered the successive divergences of the early splitting B. stacei, B. distachyon and B. arbuscula lineages, followed by those of the more recently evolved core-perennials. Within the most recently in-core group, all members of the B. sylvaticum-complex oriental lineages plus a few members of the occidental lineage (B. spry-ginii, and B. sylvaticum s. s. samples from eastern, central, and SW Europe) formed a moderately supported clade (76 % BS) with low internal resolution except for the strongly supported Pacific B. miserum / B. kurilense clade (96 % BS). The remaining members of the occidental clade collapsed...
Phylogenetics of *Brachypodium sylvaticum* uncovers two divergent oriental and occidental micro-taxa lineages

Figure 2: Maximum Likelihood phylogenomic trees of the *Brachypodium sylvaticum* complex taxa studied and other representative species of the genus. A – plastome tree cladogram. B – nuclear rDNA 35S gene tree cladogram. Ultrafast bootstrap / SH-aalrt support values are indicated above the branches. *Sorghum bicolor*, *Oryza sativa* and *Triticum aestivum* outgroups were used to root the trees. *Brachypodium sylvaticum* complex oriental and occidental lineages are shown in blue and green colors, respectively.
in a series of nested polytomies in a moderately supported clade that also included the B. pinnatum samples (78 % BS); part of these lineages formed another moderately supported B. sylvaticum s. s. pro partim subclade (79 %) without clear geographic structure (but with most samples from the western Mediterranean region) (Fig. 2B).

**Bayesian dated tree**

We applied a total-evidence approach to retrieve a consensus phylogeny for this diploid *Brachypodium* tree and to infer the nodal ages of the *B. sylvaticum*-complex lineages (Fig. 3). The resolution of the combined plastome+35S tree reflected the matrilineal plastome topology, and the Bayesian dating analysis of its maximum clade credibility tree inferred a Mid-Miocene (9.04 Ma) origin for the MRCA of *Brachypodium* and a Late-Pliocene (2.71 Ma) origin for that of the core-perennial clade. The splits of the crown ancestors of the *B. sylvaticum*-oriental (2.0 Ma) and *B. sylvaticum*-occidental (0.97 Ma) ancestors were estimated to have occurred during the early Pleistocene, while the subsequent splits spanned the Late-Quaternary (Fig. 3). The dating analysis indicated that the *B. sylvaticum* var. *breviglume* ancestors were comparatively older (2.00–1.72 Ma) than that of the young Pacific *B. miserum* / *B. kurilense* group (0.36 Ma), while the western taxa showed recent but different ancestries (*B. spriginnii* and close lineages 0.52 Ma; *B. glaucovirens* and close lineages 0.29 Ma) (Fig. 3).

**DISCUSSION**

Our study has unveiled the existence of two main divergent lineages within the hitherto considered single monophyletic species *B. sylvaticum* or unclearly resolved *B. sylvaticum* complex taxa (Figs 1, 2, 3; Catalán et al. 2016, Díaz-Pérez et al. 2018). Although *B. sylvaticum* s. l. has been taxonomically split into several minor satellite taxa in its eastern and western distribution ranges (Tzvelev 1983, Scholz 2007, Tzvelev & Probatova 2019), the evolutionary relationships of both the oriental and occidental taxa and populations of *B. sylvaticum* s. l. were mostly unknown. Our analysis has demonstrated that this cytologically homogeneous diploid species complex is composed of two main lineages which are distributed in two largely disjunct Himalayan–Pacific and Euro-Mediterranean–Siberian regions (Figs 1, 2). Our plastome-based phylogeny clearly separated the two main *B. sylvaticum* s. l. diploid lineages, placing the oriental taxa within a first diverging clade, and the occidental taxa within a subsequently split *B. sylvaticum* s. s. clade (Fig. 2A), sister to its close *B. pinnatum* relative, a resolution mostly congruent with that of the rDNA 35S tree (Fig. 2B). The oriental clade showed the successive divergences of Himalayan *B. sylvaticum* subsp. *breviglume* lineages from their respective ancestors, assumed to have occurred in the Early Pleistocene (Gelasian, 2.0–1.7 Ma), while the two Pacific close species *B. miserum* and *B. kurilense* were inferred to have diverged from their common ancestor in the Middle Pleistocene (Chibabian, ~0.3 Ma; Fig. 3). The purported colonization ages of the Tibet and pan-Himalayan Pakistan mountains agreed with previous findings about the migrations of other cool grass lineages in South-Eastern Asia as well as components of the Himalayan flora (Shen et al. 2017) in the Late Pleistocene–Early Pleistocene. Population genetic analyses detected high rates of genetic diversity for Tibetan population of *B. sylvaticum* var. *breviglume* (Mo et al. 2013). The close relationship uncovered for the Far East *B. kurilense* and *B. miserum* lineages concurred with the main contribution of southern Japanese floristic elements to the northern Kurils’ flora (Pietsch et al. 2003). The survival and adaptation of these diploid lineages to the cold climate conditions of NE Asia could have been favored by the existence of glacial refugia in western Beringia (Lozhkin et al. 2018).

The highly diversifying history of the oriental *B. sylvaticum* complex group contrast with the large genomic homogeneity of the occidental lineage. The latter group was

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**Figure 3** Bayesian maximum clade credibility dated chronogram of *Brachypodium sylvaticum* complex taxa and other congeners constructed with BEAST2 using plastome and rDNA 35S data showing estimated nodal divergence times (medians, in Ma) above branches. Stars indicate secondary nodal calibration priors (means ± SD, in Ma) for the crown nodes of the Poaceae, BOP, *Brachypodium* + core pooids, *Brachypodium*, and *Brachypodium* core-perennial clades. *Brachypodium sylvaticum* complex oriental and occidental lineages are shown in blue and green colors, respectively.
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composed of European, Asian, and North African B. sylvaticum s. s. plus B. glaucovirens and B. spryginii lineages that did not reveal a strong geographic pattern in any of the phylogenetic trees reconstructed (Fig. 2). Several samples shared the same plastome sequence and collapsed in large polytomies in the plastome-based phylogenetic tree (Fig. 2A), while they split into independent but mostly invariable and unresolved lineages, sometimes mixed with oriental Himalayan and B. pinnatum lineages, in the 35S-based phylogenetic trees (Fig. 2B). Their origins from the crown ancestor were dated to more recent times than those of the oriental lineages, having presumably occurred in the last five hundred thousand years (Fig. 3). These results point towards a rapid spread of genomically homogeneous occidental B. sylvaticum s. s. lineages across the western Palearctic in late glacial and postglacial times with occasional crosses with oriental lineages (Figs. 2, 3). Moreover, some of the characterized B. sylvaticum s. s. invasive genotypes in northwestern North America were apparently introduced from occidental European ancestors (Rosenthal et al. 2008).

The taxonomic implications of the phylogenetic results obtained in this study support the oriental micro-taxon B. sylvaticum var. breviglume, B. kurilense, and B. misurnum as distinct to typical B. sylvaticum s. s. based on their short lower glume (more than 1.5 times shorter than the upper glume), which is shorter in B. sylvaticum var. breviglume (3–5 mm) than in the Pacific taxa (4.5–6 mm) (Keng 1982, Tzvelev 2015). Brachypodium kurilense, also subordinated to B. misurnum in some taxonomic treatments, departs from the latter taxon based on the short prickles and hairiness of its spikelet pedicel and axis (Probatova & Skolovskaya 1985, Tzvelev 2015). These characters, although potentially variable, are in general fixed in the populations and constitute therefore phylogenetic signal traits of systematic value. The studied occidental micro-taxon (B. glaucovirens, B. spryginii) have distribution ranges that overlap with that of B. sylvaticum s. s. in the eastern Mediterranean and SW Asia, and in Ciscaucasia, respectively (Fig 1, Tzvelev 2015). Brachypodium glaucovirens, formerly synonymized to B. sylvaticum, was later recognized as a separate species (Scholz 2007). Morphologically it shows intermediate features, resembling B. sylvaticum in its short rhizome and long awn, and B. pinnatum in its bright green leaf color, broad leaf ribs, and erect panicle, and thus being a taxon of purported hybrid origin between the two species (Schippmann 1991). Our phylogenies corroborate this hypothesis and support a B. sylvaticum s. s. type maternal progenitor species for B. glaucovirens (Fig. 2). Brachypodium spryginii differentiates from B. sylvaticum s. s. based on its more abundant plant pubescence and longer hairs (Tzvelev 2015). However, these variable characters are probably plastic and of low systematic value.

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