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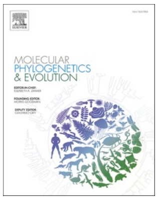

Spatio-temporal evolution of shrub willows (*Salix* subgenus *Vetrix* clade) on a global scale

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ABSTRACT

The spatio-temporal evolution of woody plant lineages in the Holarctic is still understudied, limiting our understanding of evolutionary processes that promote higher diversity at higher latitudes in some lineages, which contradicts the latitudinal gradient hypothesis which predicts higher species richness in the tropics. Shrub willows (*Salix* subgenus *Vetrix*) comprise about 350 species of which many are adapted to arctic-alpine ecosystems and thus form an important element across the Holarctic region. In this study, we utilized RAD sequencing to estimate the spatio-temporal evolution of the *Vetrix* clade based on about 140 species covering most of its morphological and geographical diversity. The resolved phylogeny revealed four lineages: the Pan-Himalayan clade, the North American clade and two clades containing species from Eurasia. Widespread circumpolar species form a hybridogenetic grade between the Eurasian and North American clades. Our results confirm that shrub willows originated in the Tertiary, probably in Asia, and that diversification coincided with the climatic cooling in the Pleistocene. At least two radiations were observed, in the Pan Himalayas and in North America. Speciation was further shaped by migration and dispersal in Eurasia, likely accelerated by the uplift of mountain chains, the closure of the Turgai Strait and expansion of suitable habitats. The dated phylogeny revealed that speciation of arctic lineages predates adaptation to high latitudes, as also evident from the fossil record. Small wind-dispersed seeds enabled shrub willows to colonize the expanding arctic tundra regions in the Miocene and their recolonization after the LGM. Given the high observed number of polyploid species equally distributed in all clades and indications of genetic admixture, we assume that species diversity in shrub willows was additionally increased by hybridization and polyploidization.

1. Introduction

The Holarctic is the largest described floristic biome encompassing temperate to arctic regions of the Northern Hemisphere. It is characterized by tree and shrub species of the Pinaceae, Fagaceae, Betulaceae, and Salicaceae families (Liu et al., 2023). Paleobotanical reconstructions indicate that global cooling in the Tertiary offered ecological opportunities for preadapted plant groups to diversify within the expanding seasonal biomes (Tiffney and Manchester, 2001; Donoghue, 2008). The origin and historical biogeography of the temperate forest biome have received extensive attention (Wen, 1999; Donoghue and Smith, 2004; Wen et al., 2010) and several woody plants were analyzed on a worldwide scale, e.g., oaks, beeches, elms and *Rhododendron* (Manos and Stanford, 2001; Shrestha et al., 2018; Hipp et al., 2020; Zhang et al., 2022; Zhou et al., 2022). Likewise, the history of the arctic flora was focus of certain studies (Alsos et al., 2007; Zhang et al., 2023; Markley

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et al., 2025). However, the phylogenetic and biogeographical history of woody plant lineages that span the temperate-arctic boundary and reach into arctic tundra ecosystems remains understudied, despite their ecological importance. Studying those taxa will provide insights into how plants adapt to colder climate conditions.

The theory of the latitudinal gradient of species diversity predicts highest species richness in the tropics that gradually declines towards higher latitudes (Pianka 2002). This pattern is evident in most Holarctic plant groups (Liu et al., 2025). One notable exception is genus *Salix* L. (Salicaceae) which has its highest diversity in the northern hemisphere and a lack of diversity in the tropical regions (Kouki, 1999; Skvortsov, 1999). Species diversity patterns result from the net effects of speciation, extinction and dispersal acting over evolutionary time-scales (Wiens and Donoghue, 2004). The species richness of willows in the Holarctic is not yet fully understood. It has been proposed that willow diversification was affected by ecological niche availability, polyploidization and hybridization (Skvortsov, 1999; Wagner et al., 2020), which fits to the global trend of a higher frequency of polyploid plants in northern Holarctic regions (Rice et al. 2019). Intercontinental dispersals, local radiations and climatic oscillations in the geological past may have further spurred willow diversification, but the impact of these factors has not yet been examined (Skvortsov, 1999; Percy et al., 2014; Wu et al., 2015).

The genus *Salix* comprises about 450–500 species of woody trees, shrubs and dwarf shrubs. Over half of them are found in China, more than 160 in Eurasia, and about 140 in North America (Fang et al., 1999; Skvortsov, 1999; Argus, 2010). *Salix* is taxonomically complex as a result of dioecy, reduced floral characteristics and a tendency for frequent hybridization. Additionally, many willow species are polyploids (Suda and Argus, 1968), and exhibit high phenotypic plasticity that further challenges taxonomic classification. Following Skvortsov (1999), *Salix* has been traditionally divided into three subgeneric groups: *Salix* subgen. *Salix* s.l. (“tree willows”) and the two subgenera *Salix* subgen. *Vetrix* (“shrub willows”) and *Salix* subgen. *Chamaetia* (“dwarf willows”). Recent research showed that the distinction of the latter two subgenera is not supported by molecular data, but that they rather form a single monophyletic lineage where species of both subgenera are intermingled (Wu et al., 2015; Wagner et al., 2018, 2020, 2021a, 2023). Since a formal taxonomic revision is still lacking, we will hereafter refer to them as the “*Vetrix* clade” or shrub willows. About two thirds of the willow species belong to this clade. They show a remarkable scope of life forms, ranging from creeping arctic-alpine dwarf shrubs to medium-sized trees (Argus, 1997; Skvortsov, 1999; Wagner et al., 2018). Most species of the clade inhabit the Holarctic floristic region (sensu Takhtajan, 1986), where they form an ecologically central element in riparian wetland and arctic-alpine habitats (Lautenschlager-Fleury and Lautenschlager-Fleury, 1994). The *Vetrix* clade therefore provides an excellent model system for studying the spatio-temporal evolution of cold-adapted woody plants in the Northern Hemisphere.

The age and geographic origin of the *Vetrix* clade remains uncertain. Skvortsov (1999) suggested that the clade originated in the early Tertiary and that the diversification of *Salix* subgenus *Salix* predates the diversification of *Salix* subg. *Vetrix* and *Salix* subgen. *Chamaetia*. The fossil record for *Salix* subg. *Salix* s.l., genus *Populus* and their common ancestor places the origin of the genus *Salix* in North America (Boucher et al., 2003), with the oldest known fossils identified as *Salix* subg. *Salix*, dating back to the Early Eocene (Collinson, 1992). For shrub willows, the fossil record is sparse. A 23 Ma old specimen found in Alaska with recognizable leaf and catkin was reliably assigned to *Salix* subgenus *Vetrix* (Wolfe, 1987). In Europe, *Salix* fossils date back to the middle Oligocene and were assigned to subtropical tree willows (subg. *Salix*), while no fossil record of shrub willows exists (Collinson, 1992). The origin of the shrub willow clade in the Northern Hemisphere thus remains unclear. Because it is difficult to assign leaf fossils to certain taxonomic groups, and because the fossil record is unequally distributed across the Northern Hemisphere, a biogeographical reconstruction based solely on the fossil record is challenging (Wu et al., 2015). Molecular phylogenetics are therefore necessary to gain reliable insights into the evolutionary history and biogeography of willows.

Studies using traditional Sanger sequencing were able to distinguish between subtropical and temperate tree willows (*Salix* subg. *Salix* s.l.) and found some evidence for a split into a “New World” and an “Old World” clade, but the *Vetrix* clade remained unresolved (Chen et al., 2010; Wu et al., 2015). Wu et al. (2015) hypothesized an origin in the Oligocene/Miocene for the *Vetrix* clade. They assumed that cold-tolerant species could continuously migrate via the Bering Land Bridge (BLB) and the North Atlantic Land Bridge (NALB), resulting in an intercontinental gene exchange and a wide Holarctic distribution, that prevented the formation of geographically distinct clades (Wu et al., 2015). More recently, Chen et al. (2025) showed an increased speciation rate for shrub willows in the late Oligocene, suggesting that climate cooling and tectonic events affected their diversification. Previous studies suggested convergent evolution of adaptations to harsh climates as another factor driving speciation at high elevations (Wagner et al., 2018; Marinček et al., 2024), however, the origin of arctic species and lineages is less understood and the impact of niche availability on the diversification of the *Vetrix* clade was addressed only partially. Arctic willows might be derived from temperate-boreal species that have originated well before the Pleistocene glaciations and were preadapted to arctic climates as suggested by Skvortsov (1999). Alternatively, their speciation might be of a more recent origin, driven by glaciations during the Pleistocene.

It also remains unclear whether the distribution patterns of willows in the Northern Hemisphere are a result of an intercontinental dispersal, vicariance or regional radiations, as was assumed in the Hengduan Mountains (He et al., 2021) and in North America (Marinček et al., 2024). The wide distribution of some Holarctic species (e.g., *S. hastata*) might as well be a result of multiple dispersal events.

Recently, studies leveraging high throughput, next-generation sequencing approaches have elucidated the phylogenetic relationships among shrub willows (Wagner et al., 2018, 2020, 2023; Sanderson et al., 2023; Marinček et al., 2024; Ogutcen et al., 2024). In particular, RAD sequencing (Baird et al., 2008) proved to be suitable for analyzing the species rich *Vetrix* clade (Wagner et al., 2018, 2020). The origin and the divergence of shrub willows leading to the high species numbers in the Northern Hemisphere can be tested solely with a global biogeographical analysis of spatio-temporal diversification based on a worldwide sampling of the *Vetrix* clade.

Here, we will utilize genome-wide RAD sequencing data to reconstruct a resolved phylogenetic tree based on a representative worldwide sampling of over 30 % of the *Vetrix* clade covering a wide spectrum of the cladeś morphological and geographical diversity. We aim to reconstruct the spatio-temporal evolution of shrub willows to test, (1) whether the proposed origin and diversification in the Tertiary could be confirmed, (2) whether diversification is due to radiations in certain areas or is constant over time, (3) whether the adaptation to cold climates observed in arctic species predates the glacial periods of the Pleistocene, and (4) whether vicariance, migration via land bridges and/or dispersals were responsible for the observed distribution of shrub willows in the Northern Hemisphere.

2. Materials & Methods

2.1. Sampling

For this study, we sampled 303 individuals representing 136 species sampled in North America, Eurasia and China and covered more than a third of the extant species of the *Vetrix* clade. Six species of *Salix* subgenus *Salix* s.l., as well as one previously published European *S. triandra* L. sample (Wagner et al., 2018) were included as outgroups (Marinček et al., 2024) (Appendix Table S1, see Supplementary Data with this article). Data from 68 samples were newly generated for this study, while the remaining samples were previously published (Wagner et al., 2018, 2020, 2023; Marinček et al., 2024; Pittet et al., 2025b, 2025a).

Plant identification to the species level was done by *Salix* experts using existing regional and global literature (Fang et al., 1999; Skvortsov, 1999; Argus, 2010; Hörandl et al., 2012), regional floras (Aeschimann and Lauber, 2004; Jonsell, 2004; Castroviejo, 2012) and voucher specimens in GOET. We aimed to sample representatives of all sections. To have a comparatively balanced sampling, we used one to four accessions per species. For widespread species or species that appeared polyphyletic in previous studies (e.g., *S. glauca*), more than four accessions were used.

2.2. DNA extraction and RAD sequencing

The DNA of all samples was extracted using the DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) following the manufacturers instructions with slight modifications as described in Marinček et al. (2024). After quality check with Nanodrop 2000 (Thermo Fischer Scientific, Waltham, USA) and Qubit 3.0 Fluorometer (Thermo Fisher Scientific, Waltham, Massachusetts, USA), the DNA extracts were sent to Floragenex, Inc. (Portland, Oregon, USA). Sequencing libraries were prepared following Baird et al. (2008) using the restriction enzyme *Pst*I, size selection to 300–500 base pairs (bp) with Pippin Prep (Sage Science, Beverly, Massachusetts, USA) and barcoding by individual sample. Multiplexed libraries were sequenced on an Illumina sequencing machine. The quality of the resulting single-end sequence reads was checked using FastQC v.0.10.1 (Andrews, 2010). We demultiplexed the reads with ipyrad v.0.9.52 (Eaton and Overcast, 2020) and used it to run the de-novo assembly for 303 individuals using the high-performance cluster of the Gesellschaft für Wissenschaftliche Datenverarbeitung mbH Göttingen (GWDG HPC, Göttingen, Germany). The ipyrad pipeline processes raw reads to concatenated loci alignments. It includes assembly of loci within samples as well as clustering across samples (Eaton and Overcast, 2020). After initial investigation of optimal assembly parameters, the clustering threshold was kept at 85 % as has been done in previous studies of the *Salix* subg. *Vetrix* clade (e.g., Wagner et al., 2018, 2020). We applied default settings except the following parameters: A maximum of four alleles per site were allowed in the final consensus sequence and consensus sequences were then clustered across samples. For initial tests, we used different settings for the parameter "minimum number of samples sharing a locus (m)" on the full dataset, namely m7, m10, m13, m16. These datasets were selected as thresholds for 50 %, 33 %, 25 % and 20 % of retained loci and then compared by calculating phylogenies. To summarize the congruence and discrepancies in topology when using datasets resulting from different thresholds of shared loci (m), we utilized the PhyloFusion option in SplitsTree 6 to create a hybrid network (Huson and Bryant, 2024). For the final phylogenetic analyses, we used the filtering of m10, which retained after all filtering steps approximately one third of all RAD loci.

2.3. Phylogenetic relationships and genetic structure analysis

The reconstruction of phylogenetic relationships for the final dataset was done in IQ-TREE v2.2.6 (Minh et al., 2020) based on the concatenated RAD loci of the m10 dataset. The software identified the TVM + F + I + G4 as the best fitting substitution model for our dataset. Statistical support of clades was estimated by an ultrafast bootstrap with 1000 replicates (Chernomor et al., 2016). The resulting tree with the corresponding statistical scores was visualized in FigTree (Rambaut, 2014).

To observe the genetic structure, we performed the sNMF analysis included in the R package LEA v.3.0.0 (Frichot and François, 2015). This approach is appropriate for mixed-ploidy datasets, as it does not rely on the assumptions of the Hardy–Weinberg equilibrium, and is therefore most suitable for analyzing polyploid species (Frichot and François, 2015). We used the.ugeno file which contains only the information of a single SNP per locus and that is automatically generated by ipyrad, following a set of criteria described in the ipyrad manual. We investigated genetic clusters (K) from 1 to 14, set the ploidy level to 4 to account for samples with higher ploidies, and performed three repetitions per K. The sNMF analysis also generates a cross-entropy plot that can be used as a criterion for the selection of the most likely number of ancestral K-value. Though we were not interested in finding the number of ancestral clades, we used the K-value with the lowest cross-entropy as the best dataset to investigate the genetic structure. Then the calculated probabilities for each of the K clades were visualized as bar plots in MS Excel.

2.4. Dated phylogeny and ancestral area reconstruction

For estimating the divergence times, we reduced the dataset to one sample per species. The exceptions were non-monophyletic *S. glauca* (3) and *S. myrtilloides* (2), here we added one accession per lineage, and for *S. disperma* and *S. bebbiana* for which we included one accession per subspecies. The selection was based on the reconstructed phylogeny, and we retained 143 accessions. We used the ipyrad's .snp file, a dataset of 764,003 aligned SNPs. To retain the tree topology, as calculated for the complete m10 dataset, we created a constraint tree that was used to fix the topology with the –g option in IQ-TREE. We explored two approaches, the penalized likelihood and MCMC, to estimate the divergence times.

The penalized likelihood and maximum likelihood method for molecular dating is implemented in the *chronos* function of the R package ape v.5.7.1 (Paradis and Schliep, 2019). This method is computationally efficient and well-suited for large datasets, making it appropriate for the scale of our study. We imported the single-tips phylogenetic tree that was calculated with constrained topology of all-tips. We set the calibration bounds on the root node between the minimum of 23 and the maximum of 69 Ma, based on the results of previous molecular dating studies (De Mestier et al., 2022; Liu et al., 2022), as is thoroughly explained in Marinček et al. (2024). We tested different options of the smoothing parameter lambda and determined the best value to be set at the default 0. Finally, we ran the *chronos* analysis 100 times, calculated average node ages of all iterations, and used them to reconstruct a tree with estimated divergence times.

We performed a second dating analysis using the MCMC in BEAST 2 v.2.7.5 (Bouckaert et al., 2014) following the settings in Marinček et al. (2024) and applying an optimized relaxed clock approach with a GTR + Γ substitution model with four rate categories and a Yule model for speciation. We defined the maximum and minimum age on the root of our phylogeny, in the form of a uniform prior between 23 and 69 Ma, corresponding with the settings used in the penalized likelihood estimation.. To keep a fixed topology that was reconstructed for the complete m10 dataset, we used the results of the penalized likelihood analysis as a starting tree and a constraint tree for BEAST. This ensured that the set calibration prior fits the tree and the analysis can initialize. Two independent MCMC analyses were run with 50 million generations each, and the output log files were investigated with Tracer v.1.7.2 (Rambaut et al., 2018) to assess convergence. The log files and the trees files of the two separate runs were combined with LogCombiner from the BEAST package, discarding the first 25 % of the logs as burn-in. With TreeAnnotator (Heled and Bouckaert, 2013), the combined trees file was used to generate a maximum clade credibility tree with common ancestor heights.

In order to infer variation in diversification rates between clades and through time, we used BAMM v.2.5.0 (Rabosky, 2014; Rabosky et al., 2014) on the time-calibrated ultrametric tree implementing a speciation-extinction model and 50 million generations to ensure that effective sample sizes were above 200. To account for the incomplete sampling, we defined the sampling fractions for the *Vetrix* clade (33 % of the 337 species worldwide), the Pan-Himalayan clade (22 % of 95 species in this region) and subg. *Salix* s.l. (8 % of the species worldwide) based on Argus (2010). The outputs were examined to find the best estimate of rate shifts for the dated tree, and the results were visualized with R using the packages BAMMtools v.2.1.10 (Rabosky et al., 2014), ape and coda v.0.19.4 (Plummer et al., 2006).

To test the influence of vicariance, migration and/or dispersal on the spatio-temporal evolution, we correlated the dated phylogeny with the geographic history of lineages. By comparison of ancestral areas with major geological events we gain indirect insights into the mechanisms shaping the current distribution. We reconstructed the ancestral areas for internal nodes of the ultrametric tree using the R package 'BioGeoBEARS' v.1.1.2 (Matzke, 2014). For the analysis we defined six major areas in the Northern Hemisphere, roughly following (Takhtajan, 1986). We aimed at implementing a good setting of areas based on the natural distribution ranges of extant species while avoiding artificial separations, such as country borders. Therefore, the distribution ranges of the extant willow species were taken from literature and online databases (Skvortsov, 1999; Argus, 2010; GBIF.org, 2024) and were implemented in the area definition. The six areas were A: Europe eastwards until the Ural mountains referred to as "Europe" in the following, B: Northern Asia including Siberia North of the central Asian steppe referred to as "Siberia" in the following for readability, C: "Asia", comprising southern Asia including the Pan Himalaya region and regions south of the Caucasus mountain range and southern Turkey, D:

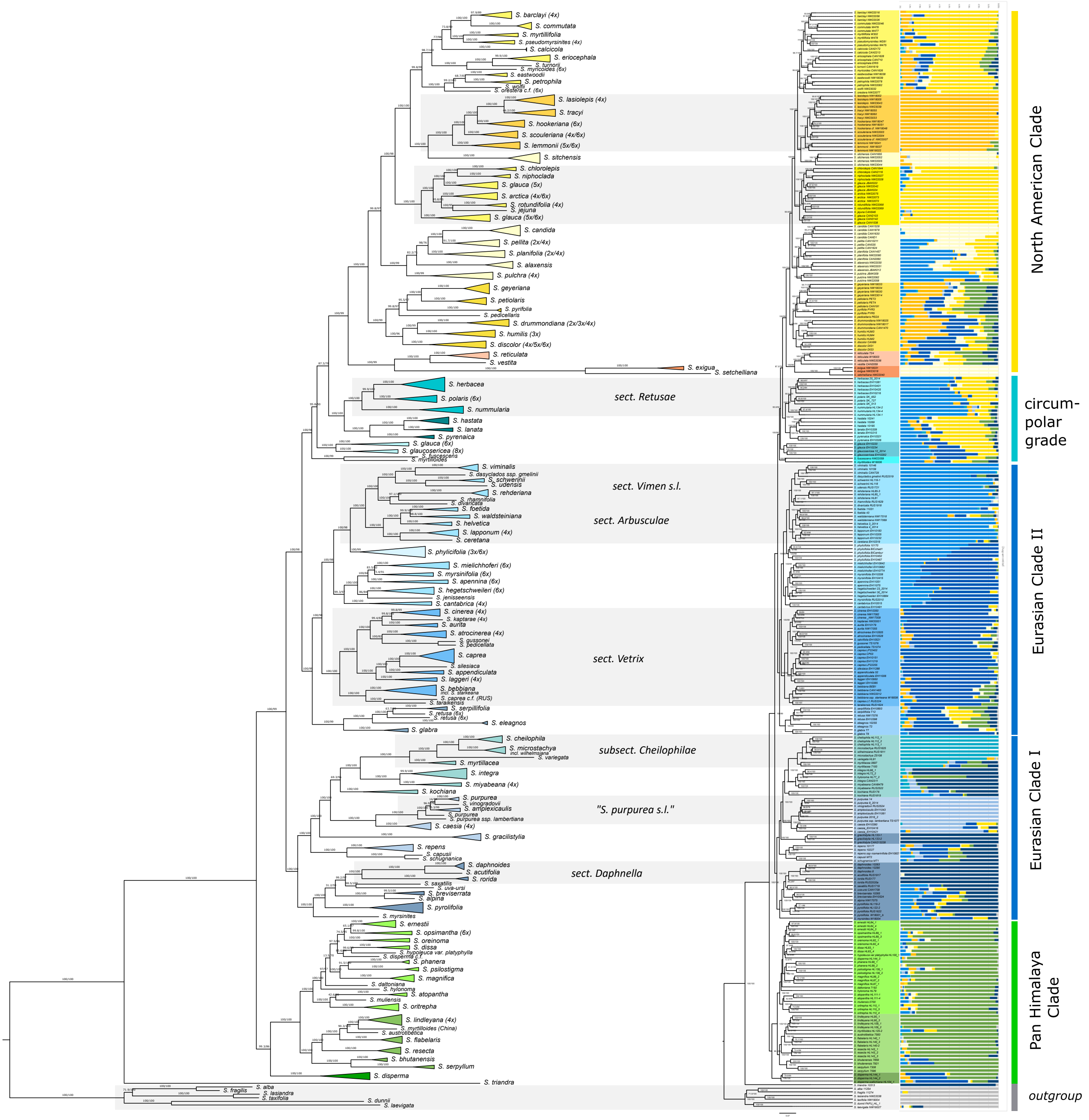

Fig. 1. Phylogenetic relationships and genetic structure results (k = 10) of 303 taxa representing 136 species of subgeneric *Vetrix* clade based on 67,654 RADloci. The main clades and taxa discussed in the text are highlighted in the maximum likelihood tree on the left. Statistical support values are shown above branches and monophyletic species groups are summarized in triangles for better illustration, while relationships of individual samples were displayed in the right tree. The genetic structure results of the sNMF analysis are presented as bar blots for each individual, in which the color-coding denotes clade relationships. Six species of subg. *Salix* s. l. were used as outgroup. Ploidies higher than diploid for species within the *Vetrix* clade are indicated next to the species name.

Western North America, E: Eastern North America, F: South Western North America and Northern Mexico. We considered including a seventh area defined as “arctic”, however, since several species occur only in a part of the circumpolar arctic region (e.g., only in Alaska, Beringia, or Northern Europe) we decided against this approach in order to have a more precise depiction of the distribution pattern. Three models and their respective + J versions were tested: DEC, DEC + J, DIVALIKE, DIVALIKE + J, BAYAREALIKE, and BAYAREALIKE + J. The results of the DEC + J model were finally selected for illustration.

2.5. *Biogeographical stochastic mapping (BSM)*

Following ancestral range reconstruction, we performed biogeographical stochastic mapping (BSM) in BioGeoBEARS using the best-fitting model to estimate the relative numbers of dispersal for the six biogeographic regions we specified (Ree, 2005; Ree et al., 2005; Dupin et al., 2017). Using 100 pseudoreplicated BSM biogeographical histories, we calculated relative dispersal events for the six regions throughout the shrub willow’s entire evolutionary history following (Matos-Maraví et al., 2021). The results were visualized with the R package *qgraph* (Epskamp et al., 2012). We set the minimum dispersal value and cutoff values in *qgraph* to visualize dispersal networks for overall dispersal, to 1 and 4, respectively.

3. Results

3.1. *RAD sequencing dataset*

The m10 ipyrad dataset with 33 % of all loci, which we identified as the most appropriate for our study, contained a total of 67,654 RAD loci and 764,003 SNPs after all filtering steps. The resulting alignment showed 87.83 % missing data. On average, 6928 RAD loci per sample were retained. Three samples, however, yielded lower numbers of raw reads and consequently a lower number of observed RAD loci. Setting the number of samples sharing loci to ten in the ipyrad pipeline was also beneficial to account for differences in initial read number and allowed us to keep these samples in our analysis.

3.2. *Phylogenetic relationships and genetic structure analysis*

Almost all species appeared monophyletic, the few exceptions probably might be due to misidentifications and/or allopolyploid origin

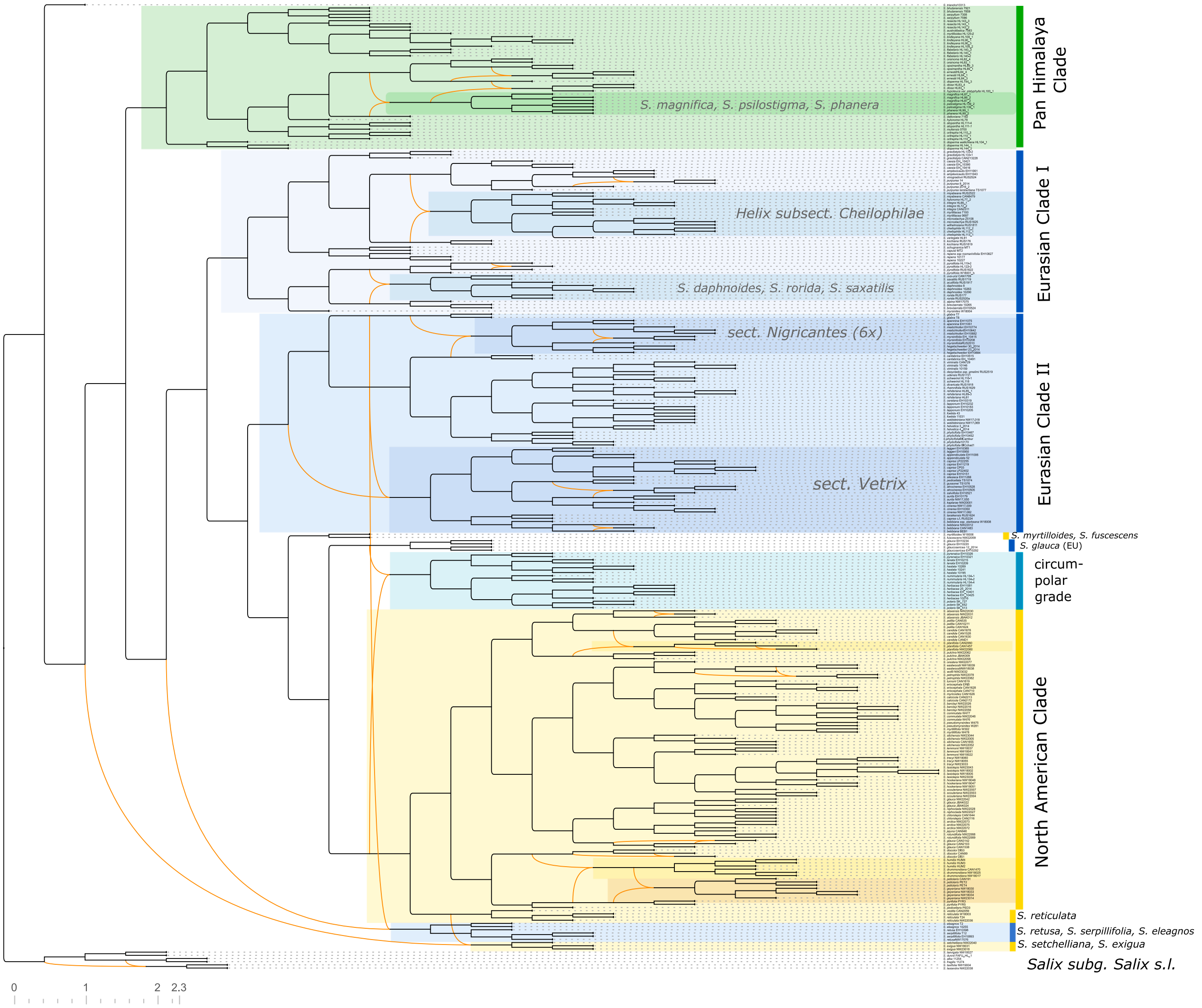

Fig. 2. PhyloFusion hybrid network combining the trees resulting from three different underlying SNP datasets coming from the applied thresholds of minimum shared loci (m10, m13 and m16) of the ipyrad pipeline. Incongruences in the topology are displayed and highlighted in orange. The four main clades are highlighted using the same color coding as in Fig. 1. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

(e.g., *S. glauca, S. disperma,* and *S. myrtilloides c.f.*). The first diverging clade was well supported (BS 100) and consisted of 21 species from the Pan-Himalayan region (PH clade, Fig. 1). The Eurasian clade I (BS 100) included species from Eurasia, several of which are exclusively distributed in Europe, but also some widespread taxa as well as *S. uva-ursi* distributed in Eastern North America. Species of sections *Daphnella*, *Helix* and *Helix* subsect. *Cheilophilae* fell into this clade. The likewise strongly supported Eurasian clade II contained species of sections *Arbusculae*, *Nigricantes*, *Vetrix,* and *Vimen* which are mainly distributed in Europe. This clade included a subclade of European hexaploids (BS 99) (*S. myrsinifolia, S. apennina, S. hegetschweileri,* and *S. mielichhoferi*). The North American (NA) clade (BS 100) was situated in a derived position of the phylogeny and containing mainly species that are restricted in their distribution range to the American continent. The species that formed a paraphyletic group between Eurasian clade II and the North American clade included many widespread, Holarctic species and will thus be referred to as "circumpolar grade". It consisted of widespread dwarf shrubs such as *S. herbacea* but also high polyploid shrub species, such as *S. glauca*. Within this grade, the three species *S. herbacea, S. nummularia,* and *S. polaris* form a clade (BS 100) in a sister position to a well-supported clade containing *S. hastata, S. lanata,* and *S. pyrenaica* (BS 100). To compare phylogenies generated from different loci outputs, we used a PhyloFusion hybrid network to visualize a summary of contradicting topologies (Fig. 2). This showed that *S. reticulata* and its relatives are of hybrid origin, resulting from a hybridization between the "circumpolar clade" and an ancestral lineage. *Salix setchelliana* and *S. exigua* were shown to be genetically related to an ancient shrub willow lineage and the North American clade. Further, the PhyloFusion indicated a hybrid origin for the *Helix* subsection *Cheilophilae* clade, the clade containing S. *daphnoides, S. rorida,* and *S. saxatilis*, the clade containing members of section *Vetrix*, the "hexaploid clade" of sect. *Nigricantes*, the "circumpolar clade" and some smaller clades within the Pan Himalayan and the North American clade (Fig. 2).

Genetic cluster analysis using sNMF and an optimal number of clusters of k = 10 showed several (sub)clades as distinct genetic clusters with little or no admixture (Fig. 1). For example, most individuals of species within the Pan-Himalayan clade, with the exception of *S. disperma*, displayed a high proportion of shared genetic ancestry (green) and exhibited similar admixture patterns, thereby forming a distinct cluster (Fig. 1). In Eurasian clade I, the *S. purpurea* s.l. subclade and members of Section *Helix* subsect. *Cheilophilae* were each fit as homogeneous clusters. *Salix gracilistyla* and *S. daphnoides*, along with their sister taxa (sect. *Daphnella*), also grouped into a single cluster, despite not being monophyletic. Other species in this clade, such as the dwarf shrubs of section *Myrtosalix* (*S. alpina*, *S. breviserrata*, *S. uva-ursi*) and boreal *S. pyrolifolia*, showed a mixture of clusters. A similar pattern was observed in Eurasian clade II. Species of sections *Vetrix* and *Vimen* s.l. each formed uniform clusters. In contrast, higher polyploid species, *S. mielichhoferi*, *S. myrsinifolia*, *S. apennina*, *S. hegetschweileri*, *S. phylicifolia* s.l., and *S. cantabrica*, displayed varyingly complex mixtures of other clusters. The widespread species in the "circumpolar grade" also exhibited admixture, sharing genetic components from the *Vimen* cluster (light blue) and the NA clade (yellow). Procumbent dwarf shrubs (*S. herbacea*, *S. polaris*, *S. nummularia*; section *Retusae*) had a greater proportion of *Vimen* ancestry, while medium-sized shrubs (*S. lanata*, *S. hastata*, *S. pyrenaica*) showed more affinity to the North American cluster. The profiles of *S. reticulata* and *S. vestita* showed contributions from multiple clusters. In the NA clade, *S. lasiolepis*, *S. tracyi*, *S. hookeriana*, and *S. scouleriana* formed a uniform cluster. A subclade containing arctic species (*S. arctica*, *S. chlorolepis*, *S. rotundifolia, and S. glauca*) also shared a distinct genetic cluster (bright yellow). *Salix sitchensis*, *S. candida*, and *S. exigua*/*S. setchelliana* formed a separate cluster without signs of admixture (light yellow). The remaining clades and species displayed high levels of admixture, incorporating genetic components from both Eurasian (blue colours) and Pan-Himalayan (green) clades (Fig. 1).

3.3. Dated phylogeny and ancestral area reconstruction

We used two approaches to reconstruct the temporal diversification of the shrub willows. The dated phylogeny based on the unlinked SNP data of the reduced dataset (one sample per species) using pseudolikelihood revealed a crown age of 35.7 Ma for the *Vetrix* clade (Fig. 3, Sup. Fig. S1). The PH clade separated from the other clades and diversified about 32 Ma. The diversification of the Eurasian clades I and II started 30.4 Ma and 27.7 Ma, respectively. The North American clade (incl. widespread species *S. reticulata*) showed an age of about 24 Ma. The clade of exclusively North American species started its diversification about 22 Ma. The analysis using BEAST based on the same data set revealed generally younger ages of diversification (Sup. Fig. S1). It estimated the diversification of the *Vetrix* clade to start 24.9 Ma, while the stem age was estimated to be about 35.2 Ma. The Pan-Himalayan clade showed a crown age of 19.7 Ma. The Eurasian clades I and II diversified about 18.7 Ma and 17.7 Ma, respectively. The North American clade was 15.79 Ma old. The BAMM analysis showed a shift in speciation rate between the outgroup and the *Vetrix* clade and an increase in speciation rate at about 30 – 20 Ma which flattened throughout the past 10 Ma (Sup. Fig. S1, S2).

The results of the biogeographic reconstruction based on six major regions are shown in Figs. 3, 4. The Pan Himalayan clade originated and diversified in Asia (C). The Eurasian clade I is composed of two subclades which showed a potential origin in Europe (A) and Asia (C) with several dispersal events or migration from Europe (A) to Siberia (B) and Asia (C) (Fig. 3). Remarkably, also one North American endemic, *S. uva-ursi*, is situated within this clade, while its sister species occurs in Northern Asia (B). Like Eurasian clade I, Eurasian clade II originated in Europe (A), with the majority of species occurring in Europe (A). However, one derived subclade indicated migration and dispersal from Europe (A) to Siberia (B) and Asia (C), respectively, at about 10 Ma and includes wide-spread species occurring in the Palearctic region (ABC or BC), e.g., *S. viminalis* and *S. schwerinii*. The observed circumpolar distribution of the "circumpolar grade" started in Europe (A). Our BSM analysis recovered high dispersal among Europe, North and South Asia (A, B, C, respectively) throughout its evolutionary history. The majority of transfers in the Old World from region A towards region B (13.28) as well as from region C towards region B (10.87; Fig. 4). Overall, the direction of estimated dispersal events was unbalanced in all cases, although the number of estimated events from area C towards region A and vice versa were quite similar (4.07 and 6.51, respectively). Based on our analysis, the species migrated about 18 Ma ago to the West to areas D (Western North America) and E (Eastern North America) and to the East into the area B (Siberia), meaning via the Beringian land bridge (BLB) and the North Atlantic land bridge (NALB), to achieve their current broad distribution range. Finally, the ancestors of the North American clade started their diversification in Western North America (D), followed by migration east and south to areas E and F, respectively.

Interestingly, we observe both: either an East-West disjunction of sister species (e.g., *S. humilis, S. drummondiana*) or occurrence in both areas (D + E) (e.g., *S. pedicellaris*, *S. petiolaris,* and *S. pyrifolia*). This was further supported by BSM as we recovered higher relative dispersal rates (12.25) from Western North America (D) to Eastern North America (E) and Southwestern America and Northern Mexico (F). A minor number of transfers from the Old World to the New World and vice versa were observed. However, we recovered the highest relative dispersal rates from Western North America (D) to Siberia (B) throughout its evolutionary history (5.29). We note that five species are attributed to the recovered trends throughout mid to late Miocene (Fig. 3). *Salix arctica* is widespread in the Holarctic and *S. rotundifolia* occurs in Alaska and Siberia, both are small arctic willows originating in North America, while closely related *S. niphoclada* is a medium sized shrub showing similar distribution pattern as *S. rotundifolia. Salix pulchra* and *S. alaxensis* are medium sized shrubs to trees with distribution ranges reaching even further into Siberia towards Europe.

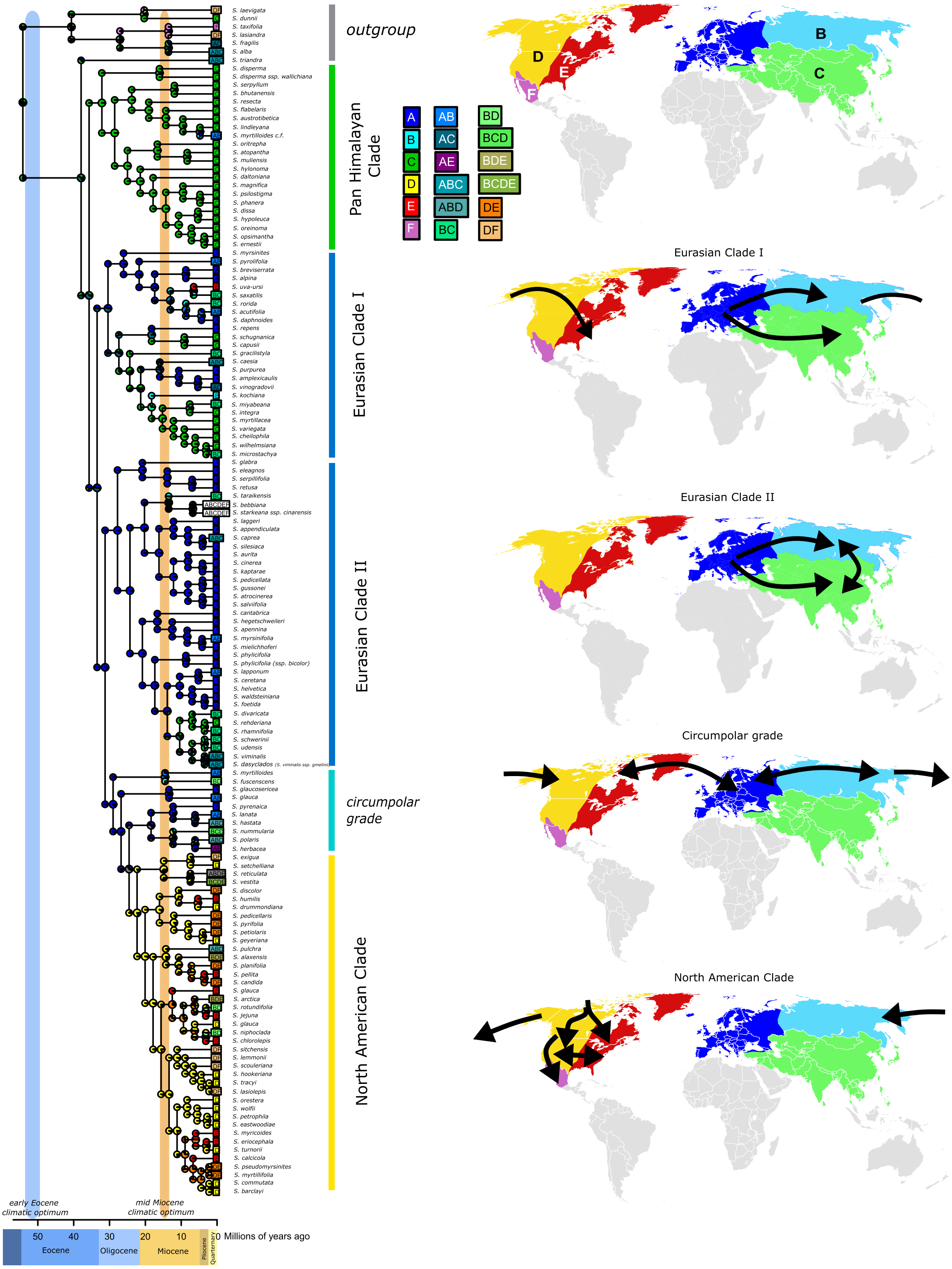

Fig. 3. Ancestral area reconstruction of shrub willows using the DEC + J model in BioGeoBEARS based on one sample per species and the time-calibrated tree estimated using the unlinked SNP dataset. The area coding included six areas defined after Takhjatan (1986) (see Material and Methods). For color coding see legend next to uppermost map indicating the six areas A-F. The maps with arrows on the right-hand side illustrate potential migration and dispersal routes for each major clade/grade except the Pan-Himalayan clade. Two important climatic periods are highlighted across the tree: The early Eocene climatic optimum (blue bar) and the mid Miocene climatic optimum (orange bar). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

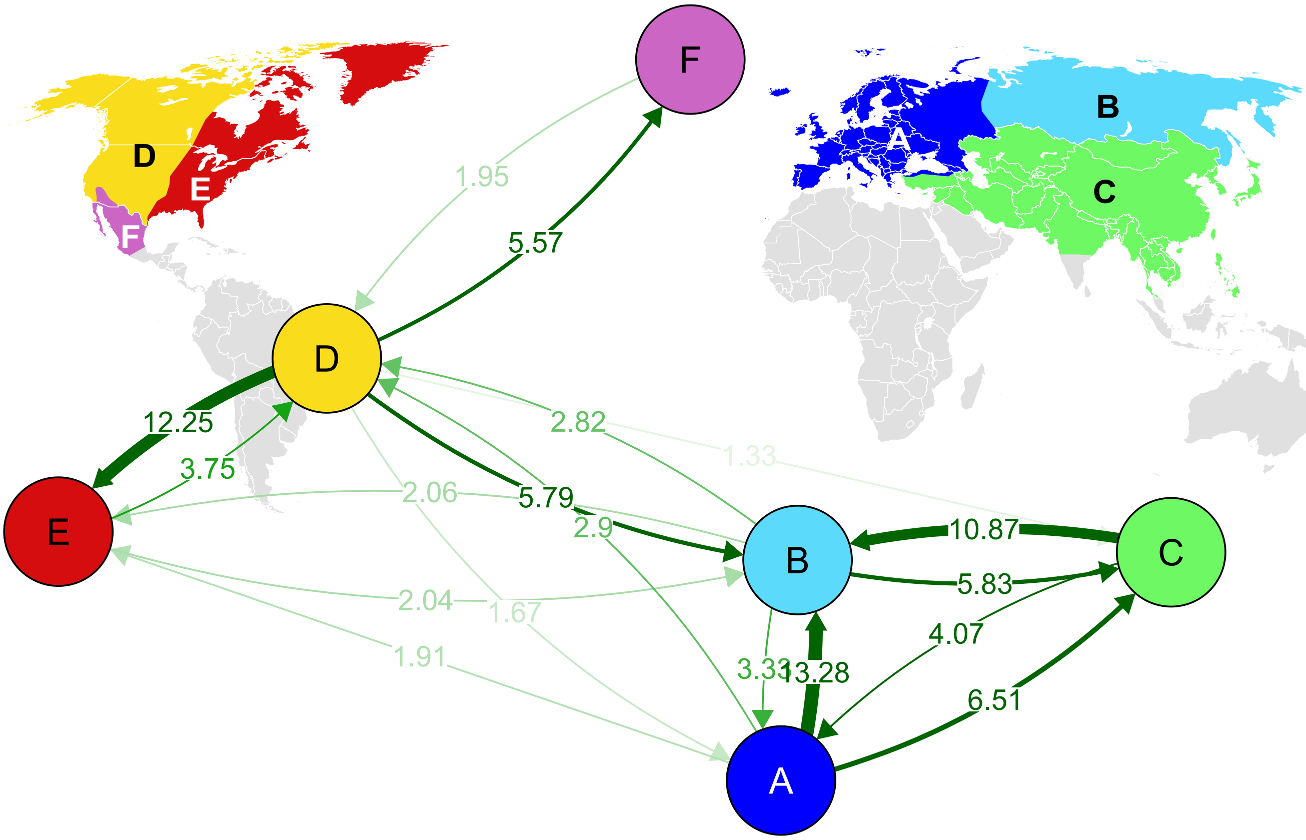

Fig. 4. Biogeographical stochastic mapping (BSM) results based on the unconstrained DEC + J Model of the biogeographical analyses. Color coding refers to the defined six regions shown on the map in the background.

4. Discussion

4.1. Methodological considerations

Resolving the backbone of the shrub willow phylogeny remains a challenge, as evident from contemporary studies (Marinček et al. 2024, Sanderson et al. 2023, Chen et al. 2025). Ancient and recent hybridization, introgression, and polyploidy contribute to uncertainty in key nodes. The processing of RADseq data in the ipyrad pipeline reduces allelic complexity and allows to integrate diploid and polyploid taxa (Wagner et al. 2020). Concatenation of SNP datasets for inferring robust phylogenies of taxa with different ploidy levels and on different taxonomic levels was used in several studies (Eaton et al., 2017, He et al., 2021, Hipp et al., 2020, Wagner et al., 2020, 2023). This simplification, however, is not suited to investigate detailed mechanisms of reticulation pattern. Using different thresholds in the parameter settings, that is, different SNP datasets, can produce different topologies for the main clades, as demonstrated in Marinček et al. (2024). We addressed these complications by visualizing conflicting clades in a PhyloFusion hybrid network (Fig. 2). Putatively admixed groups identified in the genetic structure analysis (Fig. 1), such as the "circumpolar grade," often shift their positions in the tree topology depending on the underlying dataset. The circumpolar grade appears to be "of hybrid origin", with ancestors of the North American clade and of the European *S. glauca* lineage as putative parents (Fig. 2). The hybrid nature of these taxa is also apparent in the mixed genetic clusters (Fig. 1). Similarly, the results for the clade of higher polyploids in section *Nigricantes* (Fig. 1) supports an allopolyploid origin, consistent with previous findings in tetraploid willows (Wagner et al., 2020). Interestingly, even genetically uniform clades, such as members of section *Helix* subsection *Cheilophilae* and section *Vetrix*, are inferred as of hybrid origin in the PhyloFusion network. This reticulate evolutionary history complicates biogeographical reconstructions, as hybrid origins can distort tree topologies and obscure the placement of allopolyploid lineages (McDade, 1992). Therefore, the phylogenetic positions shown represent one of several plausible hypotheses for these admixed subclades (Hipp et al., 2019). Currently, no network-based tools are available to analyze the spatio-temporal evolution of reticulate groups like shrub willows. While comparing plastid and nuclear data is a common strategy in hybrid studies, plastome data have repeatedly proven uninformative for shrub willows (Percy et al., 2014; Wagner et al., 2021b; Gambhir et al., 2025) and could thus not be used for biogeographical analyses.

Despite these challenges, we based our study on the well-supported tree topology shown in Fig. 1, which includes a robust set of genetic markers. The four major clades appear stable and provide a solid framework for the reconstruction of key biogeographical events. In the effort to place the generated phylogeny on a time scale, we employed two independent molecular dating strategies based on two very different computational approaches while using the same calibration settings that were set in consideration to the latest studies and fossil evidence, as explained in Marinček et al., 2024. The similar outputs strengthen our discussion on potential paleo geological and paleo climatic drivers of speciation. Finally, our sampling covered the geographical and taxonomic diversity of the *Vetrix* clade, however, only few samples from the Far North of Russia and the Caucasus region were included. We circumvented the putative effects of sampling gaps by including samples of the same section and wide-spread species occurring in these regions.

4.2. Phylogenetic relationships within the observed four main clades

In this study we used a comprehensive sampling representing the worldwide distribution and morphological diversity of the *Vetrix* clade. The species appeared mainly monophyletic and the observed four main clades (PH clade, Eurasian clades I and II, NA clade) were congruent with previous findings (Wagner et al., 2020, 2023; He et al., 2021; Marinček et al., 2024). In agreement with He et al. (2021), the Pan Himalayan clade, that in our study included seven additional species, showed a genetically uniform lineage in sister position to the remaining clades (Fig. 1). This supports the hypothesis of a species radiation in this area (He et al., 2021). The only exception is *S. disperma*, a species taxonomically placed in section *Vetrix* (He, 2018), whose placement could not be confirmed by our data and which showed admixed genetic structure, indicating a reticulate evolutionary history.

The relationships within Eurasian Clade I and II were in accordance with previous studies on shrub willows (Wagner et al., 2020, 2021a, 2023; Marinček et al., 2024). In Eurasian clade I, species of section *Helix* subsection *Cheilophilae* (treated as a section by Skvortsov (1999)) formed a well-supported clade with a uniform genetic structure, confirming their close relationships. Additional samples from Russia and Asia also grouped within this clade, supporting Skvortsov's (1999) sectional classification. In contrast, the subclade containing *S. repens*, *S. repens* subsp. *rosmarinifolia*, *S. schugnanica* (also sometimes regarded as subspecies of *S. repens,* Skvortsov (1999)), and *S. capusii* showed signs of admixture. The species of this clade occupy a rather large distribution area ranging from Western Europe to Eastern Asia. Skvortsov (1999) reported morphological diversity as well as putative hybrid zones for *S. repens* and *S. rosmarinifolia*. Notably, *S. capusii* and *S. schugnanica* displayed genetic contributions from the Pan-Himalayan clade in the sNMF analysis (Fig. 1), likely reflecting their Central Asian distribution and potential introgression from Pan-Himalayan species.

In Eurasian clade II, two uniform genetic clusters were observed, referring to the section *Vetrix* (dark blue) and sections *Vimen* s.l. and *Arbusculae* (light blue), respectively (Fig. 1), hence supporting the existing taxonomic classification based on morphological data (Skvortsov, 1999). We found indications of genetic admixture in the polyploid taxa that were assumed to be allopolyploids (Fig. 1). These findings are in accordance with previous studies on polyploid willow species (Wagner et al., 2020, 2023).

The "circumpolar grade" consisted of widely distributed species with a high amount of genetic admixture: the arctic dwarf willows *S. herbacea*, *S. polaris* and *S. nummularia,* and the arctic shrubs *S. lanata, S. hastata* (and *S. pyrenaica*) (Fig. 1.). They showed genetic structure of both, the North American clade and *S. glauca*. The admixture patterns observed in the sNMF analysis and PhyloFusion network (Figs. 1, 2) likely reflect accumulated introgression from multiple lineages and geographical regions facilitated by the wide circumpolar distribution of these species (Skvortsov, 1999; Argus, 2010). Similar pattern of local introgression was also observed in *Betula* (Thomson et al., 2015).

In the North American clade, phylogenetic relationships reflect the recent findings of Marinček et al. (2024). The genetic structure is uniform in several clades, but the early branching clades showed a high amount of admixture (Fig. 1). This corresponds with the findings of Sanderson et al. (2023) and can be explained by the comparatively young age (17 Ma) of this clade allowing a high amount of recent, local introgression combined with ancient hybridization events. Analogously, local introgression rather than patterns of shared biogeographical history was observed in North American birches based on plastid haplotypes (Thomson et al., 2015). This hypothesis is supported further by a series of chloroplast capture events observed in North American willows (Gambhir et al., 2025). Next, the number of polyploid taxa in this clade is high. Our data supports that most of them could be allopolyploids as they show high amounts of genetic admixture. The high ploidy levels complicate the phylogenetic placement of taxa within the North American clade (Sanderson et al., 2023). The PhyloFusion network (Fig. 2) showed some clades being of hybrid origin, e.g., the *humilis-drummondiana-petiolaris-geyeriana* clade, as these also showed high amounts of admixture in the sNMF analysis (Fig. 3). Nevertheless, the observed subclades themselves were stable and most species appeared monophyletic.

4.3. *Spatio-temporal evolution of major clades*

We will focus on the spatio-temporal patterns of the whole group and the major well-supported clades in the Holarctic. According to our data, shrub willows likely originated and started to diversify in the Asian region C in the late Eocene, although with some degree of uncertainty, which might result from the small number of outgroup taxa that influenced the composition of the basal nodes. The crown age of the *Vetrix* clade in our study is in accordance with previous findings (Wu et al., 2015; He et al., 2021; Marinček et al., 2024; Ogutcen et al., 2024). In contrast, Kikuchi et al. (2023), Percy et al. (2014) and Zhang et al. (2018) suggested a younger divergence time for the *Vetrix* clade at about 14 Ma. However, as pointed out by Marinček et al. (2024) and should be noted here, one of the main reasons for contrasting results might be the use of different calibration strategies.

At the estimated time of divergence, region C in Asia was a unified landmass with a warm-temperate climate, prior to the uplift of the Himalayan-Tibetan plateau. Eastern Asia is a known centre of origin for many woody lineages (Donoghue and Smith, 2004). Several studies (Skvortsov, 1999; Wu et al., 2015; Zhang et al., 2018; Ogutcen et al., 2024) suggested a later diversification of shrub willows than of tree willows (subgenus *Salix* s.l.), a pattern also supported by fossil evidence (Collinson, 1992). This diversification coincided with global cooling at the Eocene–Oligocene boundary (White et al., 1997), aligning with shrub willows' adaptations to temperate and cool climates. Our results also indicate a rise in diversification rates during the Oligocene (Suppl. Fig. S2). Compared to other woody lineages, shrub willows are thus relatively young. For instance, diversification in Fagaceae began in the Paleocene (Zhou et al., 2022), in Betulaceae during the Eocene–Oligocene (Yang et al., 2022), and in Ulmaceae (elms) around 45–50 Ma (Zhang et al., 2022). This difference may stem from ecological specialization: unlike forest trees, willows, especially shrubs, were preadapted to open tundra habitats that appeared later in geological time scale. One key preadaptation (exaptation) could involve their small, airborne seeds lacking endosperm, making seedling survival dependent on immediate photosynthesis and requiring light and moist soils (Karrenberg et al., 2002; Gramlich and Hörandl, 2016). Arctic and alpine tundra provided ideal conditions for their germination. Similarly, *Betula*, which shares these habitat preferences (Perala and Alm, 1990), also arose around 20 Ma (Yang et al., 2022). Skvortsov (1999) suggested that the development of boreal groups in subgenus *Vetrix* took place in several regions in the Holarctic, including Europe, Asia, and North America predating the Quaternary. This fits our observations of several geographically distinct clades and was further supported by our BSM results (Fig. 4), which revealed a higher number of transfer occurrences within the Old World (A, B, C) and the New World (D, E, F), respectively, than between both. Later, with ongoing global cooling in the Pleiocene and Pleistocene, reticulate evolution and polyploidization probably contributed to diversification. Polyploid plants are in general more frequent at higher latitudes, and cool temperature is the strongest driver for this distribution pattern (Rice et al. 2019). Given the data type used here and the challenges of dating hybrid origins, we could only indirectly infer the extent and exact timing of these reticulate processes by observing the number and distributions of polyploid species, and their genetic admixture in the diverse clades.

4.3.1. *Pan Himalayas*

The diversification of the Pan-Himalayan clade occurred in the early Miocene, probably driven by the rapid uplift of the Himalayas and the origin of many new habitats along elevation gradients (Xing and Ree, 2017; Muellner-Riehl et al., 2019). The PH clade is distributed exclusively in Asia (C) (Fig. 3) and thus our data based on a broader sampling confirmed the findings of a radiation by He et al. (2021). This was further supported by a slightly higher diversification rate for this clade compared to the remaining shrub willow clades. Similar radiations in this area have been observed in other widespread plant genera such as

Rhododendron L. (Mo et al., 2022), *Ranunculus* L. (Emadzade et al., 2015), and *Saussurea* DC. (Zhang et al., 2021).

4.3.2. Eurasia

The ancestor of the Eurasian clades I and II originated either in Asia (C) or Europe (A). Skvortsov (1999) stated that boreal *Vetrix* lineages are missing in southeast Asia and thus suggested a relatively young age and diversification of those lineages in Europe. However, based on our results the ancestors of shrub willows already existed in Europe 23 Ma. This is similar to the findings of Zhang et al. (2022) who showed that elms (*Ulmus*) dispersed from East Asia to Europe in the early to middle Paleocene and middle Miocene, diverging in Europe about 20 Ma. In the Tertiary, Europe was split by the Paratethys and the Tethys remnants system with several smaller island systems until the late Oligocene. The closure of the Turgai Strait at its northern end in the Paleocene, and its retreat to the south in the late Eocene, facilitated floral exchanges between Asia and Europe (Tiffney and Manchester, 2001). The Eurasian clades I and II each showed ancestry in Europe and migration and dispersal events from and to Siberia (B) and Asia (C) followed by speciation in the respective regions (Fig. 3). The slow climate cooling (Zachos et al., 2001) probably aided the speciation in the *Vetrix* clade. In the Miocene, the uplift of many mountain chains to high elevations (Himalayas, European Alps, Zagros Mountains, and Rocky Mountains) provided ample opportunities for habitat differentiation in altitudinal zones and speciation in mountain systems (Nagy and Grabherr, 2009; Potter and Szatmari, 2009). In the lowlands, global aridification could have forced willows to restrict their habitats to riversides and wetlands, which could have further fostered diversification due to geographical and ecological isolation.

The Eurasian clade I originated and diversified in area A (Europe) in the Miocene. Most species of this clade occur in Europe, while the species of section *Helix* speciated after migration to Asia (area C) about 20 Ma (Fig. 3). Species of *Helix* subsection *Cheilophilae* are nowadays endemic to this region. According to Skvortsov (1999), the group of morphologically related sections *Helix*—*Flavidae*—*Cheilophilae* has an exclusively Eurasian distribution with the center of diversity in Asia. He assumed that the group probably emerged there in very remote areas from primitive ancestors of the subgenus *Vetrix*. This is confirmed by our results, which showed the likely origin of this group in Asia (C). Migration back to Europe (A) is assumed for the species *S. purpurea* s.l., which diverged into several (sub-) species within the last 3 Ma.

Eurasian clade II shows a pattern similar to Eurasian clade I. The origin was in Europe (A) in the Miocene and most lineages diversified in this region (Fig. 3). However, eastward migration to Siberia (B) and Asia (C) was observed for ancestors of the clade that contains sections *Arbuscella* (*S. rhamnifolia, S. rehderiana,* and *S. divaricata*) and *Vimen* (*S. udensis, S. schwerinii)*. According to our dating, the migration within this clade to areas B and C took place in the middle to late Miocene (10 Ma). At that time, the Asian Mountain ranges already existed, but major geological activity in the Himalayan region, such as the uplift of the Tibetan Plateau, was still ongoing, (Xing and Ree, 2017; Muellner-Riehl et al., 2019). This uplift and the Himalayas, divided Asia into the central arid regions and the subtropical, wet southeastern regions, which could have enhanced further diversification. The two wide-spread species of section *Vimen* (*S. viminalis, S. dasyclados*), sometimes referred to as one species, are situated in a derived position and probably spread after diversification in B/C to their current distribution range.

4.3.3. Arctic regions

Willow species represent a major component of the arctic flora that has a complex history. Two recent reviews on arctic species (Zhang et al., 2023; Markley et al., 2025) highlighted the different distribution patterns and historical implications in the Holarctic. Markley et al. (2025) proposed that in most lineages cold tolerance evolved about 3–5 Ma, corresponding to the relatively young origin of the arctic tundra and shrublands. This hypothesis fits Zhang et al. (2023), who stated that the evolution of cold-adapted plant species in the arctic accelerated around 2.6 Ma. Wolfe and Upchurch (1987) reported that a truly arctic tundra ecosystem in North America was unlikely to exist as a clearly defined biome until the Pliocene-Pleistocene boundary. Our results suggested an origin of arctic willows at about 12 Ma (Fig. 3, Suppl. Fig. S1). This indicates that most arctic *Salix* lineages are considerably older than the arctic ecoregions they inhabit, while the adaptations to this habitat (e.g., cold tolerance, or dwarf shrub growth) might have evolved later. Skvortsov (1999) assumed that preadaptations to arctic habitats existed in willow species originating from high mountain regions and that these species could rapidly and successfully colonize the emerging arctic regions leading to subsequent diversification. Our results showed that arctic-alpine adaptations (e.g., dwarf growth) evolved several times independently, further contributing to regional radiations and thus supporting Skvortsosv's hypothesis. Besides the "circumpolar grade", we observed species adapted to arctic regions in other clades, such as *S. myrsinites* and *S. saxatilis* in Eurasian clade I. As discussed before, the mode of seedling development could have been another exaptation of willows to the arctic tundra. The rapid movement to and between arctic regions could be attributed to the high dispersal ability of most plant species in the arctic flora including the dwarf willows (Birks, 2008). Alsos et al. (2007) found evidence for frequent long-distance dispersals into the arctic after the last glacial maximum that contributed to the recolonization of certain regions like Svalbard. This was further supported by comparison of 32 angiosperm lineages revealing that a long-distance dispersal corridor existed between the Arctic and Western North America (Zhang et al., 2023). This aligns with our findings of a close relationship of the species of the circumpolar grade and the North American clade. The circumpolar distribution of several arctic species (*S. reticulata, S. herbacea, S. polaris,* and *S. glauca*) might have also been possible via migration over ice-free land bridges such as Beringia that existed before and during the glaciation periods of the Pleistocene due to lower sea levels (Tiffney and Manchester, 2001; Wen et al., 2010). Although the origin of species predates these glaciation periods, the current distribution might have been affected by them. Establishment in the postglacial re-colonization phase might have been accelerated by the establishment of more suitable habitats. Rapid (re-) colonization of northern regions after the LGM was also observed in dwarf birch *Betula nana* (Thórsson et al., 2010) and other plant lineages (summarized in Alsos et al., 2007).

4.3.4. North America

Wolfe and Upchurch (1987) stated that next to diversification on mountains, the observed diversity of cold-adapted willows of the *Vetrix* clade is the result of several migratory episodes to North America in the Neogene. However, our results clearly suggest a single origin of most North American lineages followed by subsequent diversification within the North American clade (Figs. 1, 3) (Marinček et al., 2024). The migration to North America gave rise to a radiation which was already inferred by Percy et al. (2014) and Marinček et al. (2024). Based on our data, the divergence took place about 20–15 Ma (Fig. 3, Suppl. Fig. S1), while Percy et al. (2014) assumed a younger radiation starting about 10 Ma (termed "haplotype group 1", which contains most shrub willow species of subgenus *Vetrix*). The oldest known *Vetrix* fossil, dated to approximately 23 Ma and attributed to section *Glaucae*, was discovered in Alaska. This finding suggests that *Vetrix* first appeared in Beringia during the early Oligocene following a migration from Asia (Wolfe and Upchurch, 1987). While it slightly predates our molecular divergence estimates, it still falls within a reasonable time range. It should be noted that both molecular dating and fossil age determination are associated with inherent uncertainties based on the data and methodology used. Besides that, several migrations or range expansion events during the late Eocene–early Oligocene transition may have allowed widespread Northeast Asian species to reach Alaska via the Bering Land Bridge before the diversification of North American willows began. In contrast to genera such as *Quercus* and *Ulmus*, which dispersed from North

America to Europe (Hipp et al., 2020; Zhang et al., 2022), our results point to a circumpolar or European origin for the North American shrub willow species (Fig. 3). Nevertheless, in both scenarios, the North Atlantic Land Bridge (NALB) and Bering Land Bridge (BLB) facilitated species migration across the Holarctic and into and out of North America, although the BSM results (Fig. 4) revealed only few transitions from the Old World to the New World. Notably, much older *Salix*-related fossils from North America were dated to the Middle Eocene (46–50 Ma) but have been identified as tree willows (*Salix* subgenus *Salix s.l.*) (Collinson, 1992; Boucher et al., 2003). These differences in age and evolutionary history suggest that the biogeographic histories of the two major subclades within *Salix*, tree and shrub willows, should be treated separately, as was suggested by Skvortsov (1999) and has been demonstrated for other temperate tree genera such as white and red oaks (Hipp et al., 2018; Manos and Hipp, 2021).

Several phylogenetic sister species pairs within the North American clade exhibit an East–West distribution pattern (e.g., *Salix drummondiana* – *S. humilis*, *S. rotundifolia* – *S. jejuna*). Argus (2010) previously noted a similar pattern for *S. humilis* and *S. pellita*, two species that were clearly separated in our phylogeny. The observed disjunct distributions likely result from allopatric speciation. While Argus highlighted morphological distinctions among such species, he offered no explanation for the biogeographic pattern. Comparable East–West patterns have been documented in European alpine willows, such as *S. foetida* – *S. waldsteiniana* and *S. alpina* – *S. breviserrata*, which are attributed to survival in Quaternary glacial refugia (Marinček et al., 2023; Pittet et al., 2025b). In North America, the Rocky Mountains may have acted as a barrier, or alternatively, glaciation and subsequent deglaciation could have created separate eastern and western refugia, promoting allopatric divergence. An East–West split has also been observed in North American oaks, where both red and white oak clades show parallel vicariant divergence (Hipp et al., 2019; Manos and Hipp, 2021). However, the divergence of these two oak clades occurred much earlier (ca. 35–45 Ma) than the divergence times we estimated for North American shrub willows. In addition to this East–West pattern, our data support a general North–South migration trend for shrub willows in North America, originating in cooler northern regions and diversifying southward into areas like California and Arizona. Wolfe and Upchurch (1987) similarly inferred from fossil records that *Vetrix* lineages migrated south during the Middle Miocene. The availability of suitable habitats along mountain ranges and river corridors may have facilitated this movement. Although not directly tested in this study, ongoing hybridization and the high frequency of polyploidy in North American shrub willows (Sanderson et al., 2023; Marinček et al., 2024) suggest that reticulate evolution has played a significant role in shaping species richness. Future studies with broader sampling across subclades could further illuminate the complex, network-like evolutionary patterns underlying species diversification in this region.

5. Conclusion

Lineage-specific investigations are essential to understand the complex history of Holarctic species. In our study, we focused on shrub willows, a group that contradicts the latitudinal gradient in species richness and tried to circumscribe the mechanisms leading to the high species richness in the Northern Hemisphere. Using a genome-wide SNP marker approach and a comprehensive worldwide sampling, we estimated the diversification history of shrub willows on a global scale. Our results indicate that lineage divergence occurred before the establishment of arctic tundra habitats and the climatic oscillations of the Pleistocene. Cold-adapted shrub willows subsequently colonized the newly emerging northern habitats, facilitated by their small, easily dispersed seeds, which enabled them to expand across circumpolar regions. We further show that diversification within the *Vetrix* clade arose from more than one center of origin and was promoted by the expansion of arctic–alpine habitats following global cooling in the Miocene. In addition to geological processes such as mountain uplift, we assume that hybridization and polyploidization further contributed to increasing species diversity.

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CRediT authorship contribution statement

Pia Marinček: Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis. **Jing Vir Leong:** Writing – review & editing, Validation, Methodology, Investigation. **Loic Pittet:** Writing – review & editing, Methodology, Data curation. **Ètienne Léveillé-Bourret:** Investigation, Methodology, Resources, Writing – review & editing. **Tommi Nyman:** Writing – review & editing, Resources. **Maria Tomoshevich:** Writing – review & editing, Resources. **Evgeny Banaev:** Writing – review & editing, Resources. **Li He:** Writing – review & editing, Resources. **Elvira Hörandl:** Writing – review & editing, Resources. **Martin Volf:** Writing – review & editing, Supervision, Resources, Funding acquisition. **Natascha D. Wagner:** .

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ympev.2025.108480.

Data availability

Raw sequence data is available from National Center for Biotechnology Information under the BioProject number PRJNA433286 (detailed accession numbers are listed in Table S1 in supporting information).

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