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# Journey to the west: Migration patterns of the riparian montane genus *Myricaria*

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#### ABSTRACT

*Myricaria* Desv. (Tamaricaceae) is a genus of riparian plants found in montane regions throughout Eurasia. We present molecular phylogenies covering the entire Eurasian range of the genus using the chloroplast interspacer region *psbA-trn*H, the chloroplast region *trnL-trn*F, and the nuclear ribosomal Internal Transcribed Spacer (ITS). Biogeographical analyses of the DNA sequencing data based on Takhtajan's Floristic Provinces using a Bayesian Binary Markov Chain Monte Carlo reconstruction show that the origin of *Myricaria* is the Tibetan Floristic Province, specifically in the Qinghai-Tibetan Plateau. A molecular clock analysis was also performed. We estimate that the crown age of *Myricaria* is around 22.8 Ma and that the common ancestor of all European *Myricaria* occurred around 8.8 Ma ago. By covering the entire native range of the genus, our analyses confirm previous hypotheses on the origin and migration patterns of *Myricaria*, namely a central Asian centre of origin, followed by a westward migration to Europe via the central Asian mountain ranges, the Caucasus, and Eastern Europe. The divergence times and migration routes of *Myricaria* are remarkably similar to that of the genus *Hippophae* L.

# 1. Introduction

The biogeography of the European Alps and their links to the other Eurasian mountains (from the Middle East to central and eastern Asia) have been studied from various angles for over 150 years. For example, Christ (1867) explored the possibility that taxa in the European Alps originate in Asia, while Ball (1879) discussed the idea that Arctic plants could have migrated from mountainous regions as global temperatures increased during the tertiary period. More recently, a study by Kadereit et al. (2008) looks at the occurrences of various taxa present in both Asia and the European Alps, and how these taxa are connected to each other. Their survey, based on the available literature at the time, illustrates how genus-level phylogenetic studies in particular can help elucidate general patterns of distribution and migration between the European Alps and the Asian mountain ranges. They count 653 vascular plant genera in the European Alps (excluding recent neophytes), of which 429 genera are also found in the Himalayas; this means that roughly 66% of European Alpine genera can also be found in Asia. Focussing on taxa at the genus level is useful as genera are convenient monophyletic units which often cover both Europe and Asia and can contribute to the broader understanding of alpine migration patterns summarised in Kadereit et al. (2008). Since 2008, researchers have been building on this body of work. Examples of such studies include Surina et al. (2014),

which discusses the disjunct distribution of *Wulfenia* Jacq. (Plantaginaceae); the ancestor of *Wulfenia* comes from the European-Mediterranean region. More recently, Kadereit *et al.* (2019) found that the genus *Callianthemum* C.A.Mey. (Ranunculaceae) migrated twice independently from Asia to Europe; the authors suggest that one of these migrations is through southern Siberia.

Kadereit et al. (2008) note that there are two general patterns of migration of European Alpine taxa; either via the "northern" route, or the "southern" route. The northern route connects Europe to a central Asian centre of origin via the Arctic, while in the southern route, taxa have a Mediterranean centre of origin, from whence they spread to the European Alps and also to Asia via the Caucasus, Hindukush, Pamir, Karakorum and Tien Shan mountain ranges.

In this context we focus on *Myricaria* Desv. (Tamaricaceae), a genus of montane shrubs with a wide Eurasian distribution. *Myricaria* is one of the 429 genera present in the European Alps which are also found in the Himalayas. Populations can also be found in Scandinavia, the Balkans, the Caucasus, the central Asian mountain ranges, Siberia, central and northern China, and the Qinghai-Tibetan Plateau (GBIF Secretariat, 2023). The present distribution of *Myricaria* matches Kadereit's southern intervening areas, but not the northern one. The trends described by Kadereit *et al.* (2008) would therefore predict that European *Myricaria* has a Mediterranean centre of origin, rather than a central Asian one.

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This would contradict the predictions made by other authors, for example Bobrov (1967), who suggests a central Asian centre of origin. Since the migration pathways of *Myricaria* from Asia to Europe have not been investigated using molecular data, this study is the first to test which migration pattern *Myricaria* follows.

*Myricaria* is not only an alpine genus, but also a riparian one. According to the Interpretative manual of European riparian forests and shrublands (Milanović and Stupar, 2021), *Myricaria* is a dominant plant in the scrub vegetation of subalpine to submontane river gravel bars of the temperate and boreal European mountains and the Caucasus, forming a key part of the alliance Salicion eleagno-daphnoidis. The species composition of this alliance consists of 27 species belonging to 22 genera, of which *Myricaria* is one (Milanović and Stupar, 2021).

Of these 22 genera, all but one are included in the survey by Kadereit et al. (2008). Of the remaining 21 genera, only 5 (Daucus L., Eupatorium L., Hippophae L., Medicago L., and Myricaria) had a "southern" distribution. So far, it appears that there are no biographical studies available on the centre of origin of Daucus. However, literature suggests that Medicago and Eupatorium spread to Asia via Europe or the Mediterranean. In the case of Medicago, the Mediterranean region has been found to be an important centre from which migration to the east occurred (Liu et al., 2023). Meanwhile, Eupatorium migrated from the New World (Schmidt and Schilling, 2000) to Europe via the Atlantic, from whence it was able to spread to Asia via Europe. In both these cases, patterns of migration follow the trends shown by Kadereit et al. (2008), that alpine genera with a southern distribution migrate to Asia from Europe. Hippophae (Eleagnaceae) appears to be the only genus in this vegetation alliance with a southern distribution that bucks the trend; instead, this genus has a central Asia centre of origin, spreading to Europe via central Asian mountain ranges (Jia et al., 2012; Jia and Bartish, 2018).

#### 1.1. Adaptations and origins of Myricaria

Adaptations of *Myricaria* to alpine river habitats include its root structures which enable these plants to anchor themselves to gravelly riverbanks, and to access groundwater (Sitzia *et al.*, 2021). As a result, other plants (such as *Salix* L. species) are able to establish themselves in the vicinity as the substrate is made more hospitable by *Myricaria*. Therefore, this genus is considered ecologically important due to its species' status as both indicator and pioneer species (Harzer *et al.*, 2018).

Although *Myricaria* belongs to the family Tamaricaceae, which is made up of mainly halophytic, xerophytic and rheophytic genera, *Myricaria* is adapted to non-saline habitats. The study by Dörken *et al.* (2017) on the leaves and salt glands of *Myricaria germanica* (L.) Desv. showed that these salt glands excrete high concentrations of calcium and magnesium rather than sodium. The authors hypothesise that these glands were originally adaptations for saline environments but are now co-opted for calcium-rich soils.

A detailed biogeographic hypothesis has been put forward for the genus *Myricaria* by Bobrov in 1967: *Myricaria* originated in central Asia and migrated to Europe via the central Asian mountain ranges, the Caucasus, and Eastern Europe. Bobrov (1967) also hypothesised that the divergence of *Myricaria* and *Tamarix* L. was caused by diverging adaptations to differing salinities; *Myricaria* to riparian freshwater habitats and *Tamarix* to saline valley plains, consistent with the findings of Dörken *et al.* (2017). Further, Bobrov suggested that the divergence and radiation of *Myricaria* is associated with geological changes in central Asia during the Neogene and the Pleistocene.

Bobrov (1967) was the last review of *Myricaria* as a whole genus covering its entire geographic range. More recently, the phylogeography of *Myricaria* has been explored using molecular methods in the context of *Myricaria* found within China (Liu *et al.*, 2009; Wang *et al.*, 2009; Zhang *et al.*, 2014). These studies conclude that the Himalayan region is the centre of origin of Chinese *Myricaria* populations. Although the phylogeography of *Myricaria* has been studied for Chinese populations,

the history of other populations and of the genus as a whole has not been examined, including the circumstances under which *Myricaria* diverged from its sister genus *Tamarix*.

# 1.2. Aims of the study

In order to fill the knowledge gap of the phylogeny of *Myricaria* outside of China, we present molecular phylogenies which include individuals sampled from herbarium specimens collected from across the Eurasian distribution of the genus. Our sample size is strengthened by incorporating sequence data from previous authors, in particular data from Liu *et al.* (2009), Wang *et al.* (2009) and Zhang *et al.* (2014).

We aim to test Bobrov's (1967) hypotheses about the biogeography of the genus against the general patterns of alpine flora described by Kadereit *et al.* (2008) using Bayesian Binary Markov Chain Monte Carlo (BBM) reconstruction. The framework of Takhtajan's (1986) Floristic Provinces will be used in this context, as it is a well-established hierarchical floristic system. By extending the geographical range of sampling for molecular phylogenetics to cover the entire Eurasian distribution of *Myricaria*, we aim to obtain a clearer understanding of its pathways of migration west to Europe. We also aim to estimate divergence times of European and Siberian clades of *Myricaria*.

The species boundaries in *Myricaria* have proven to be problematic for nearly 200 years (Ehrenberg 1827). To give a rough estimate of species numbers, there are 26 species names listed on the International Plant Names Index (IPNI) (POWO, 2024), of which 13 are listed as "accepted" by the Plants of the World Online (POWO) database. The Flora of China (Yang and Gaskin, 2007) also estimates 13 species, of which four are endemic to China. Based on phylogenetic data using whole chloroplast genomes and the ITS region, Hu *et al.* (2023) show that many of the putative species found in China are in fact a species complex. Moreover, in Siberia, the described *Myricaria* species include numerous synonyms (Lyakh 2013). Therefore, one could argue that *Myricaria* can be classified into considerably fewer than 13 species. The difficulties in elucidating species boundaries in *Myricaria* reflect that of its sister genus, *Tamarix*, which is "one of the taxonomically most complex genera among the angiosperms" (Villar *et al.*, 2019).

It is also important to note that some authors consider the name *Myricaria elegans* Royle to be a synonym of *Myrtama elegans* (Royle) Ovcz. & Kinzik – and that *Myrtama elegans* is a monospecific genus in its own right (Ovchinnikov, 1981; Zhang et al., 2006). In this analysis we include specimens identified as *Myricaria elegans*, and we assume for now that these individuals are part of the genus *Myricaria*, as recent phylogenetic studies show consistently that *Myricaria* (including *Myricaria elegans*) is monophyletic.

Due to these issues regarding species boundaries in *Myricaria*, we focus on molecular and geographical data from herbarium specimens rather than assigned species names to plot the patterns of migration. This study focusses on the biogeography and migration patterns of *Myricaria* species and does not attempt to taxonomically revise the genus, but we hope that our study can provide some insights into potential for further taxonomy work.

# 2. Materials and methods

# 2.1. Taxon sampling

Takhtajan's (1986) Floristic Provinces were used to divide the native range of *Myricaria* into ecologically and floristically representative areas. We generated 164 new sequences from 66 individuals, then supplemented with data from previous studies (Gaskin and Schaal, 2003; Zhang *et al.*, 2006; Wang *et al.*, 2009; Zhang *et al.*, 2014b; Yin *et al.*, 2015; Villar *et al.*, 2019; Han *et al.*, 2021; Chi *et al.*, 2019; Yao *et al.*, 2019; Wang *et al.*, 2020; Liang *et al.*, 2019; Werth and Scheidegger, 2011; Liu *et al.*, 2009) and other sequences uploaded onto GenBank (Sayers *et al.*, 2021), reaching a total of 178 individuals representing all

floristic regions in the full geographic range of *Myricaria*. This also includes specimens of *Reaumuria* L. and *Tamarix* as outgroups. Specimens were sampled from herbaria M, BRNU, BEOU, and the living collection of the Botanical Garden München-Nymphenburg, Germany. An overview of all the specimens (including authors and GenBank accession numbers) used can be found in Table S1 (supplementary material).

#### 2.2. Molecular methods

The CTAB protocol (Doyle and Doyle, 1987) was followed for DNA extraction. Two cpDNA regions, the *psbA-trn*H chloroplast intergenic spacer region and the *trnL-trn*F chloroplast region, and the nrDNA region ITS (Internal Transcribed Spacer) were amplified for this study. These regions were selected so that our dataset could be compatible with data from GenBank and previous studies. The plastid region *rpl16* and nuclear region *ycf16* were also trialled, but sequencing quality from the herbarium material was generally poor and they were therefore not included in this study.

All regions were amplified in a 25  $\mu$ L total reaction volume, consisting of 0.25  $\mu$ L Phusion DNA Polymerase, 5  $\mu$ L 5 x Phusion HF buffer, 2  $\mu$ L of dNTPs at 2.5 mM (200  $\mu$ M), and 1.25  $\mu$ L of both forward and reverse primers at 10  $\mu$ M each (0.5  $\mu$ M). Between 0.5 pg and 5 ng of template DNA was used per 25  $\mu$ L reaction to amplify *psbA-trn*H and *trnL-trn*F, while 25 ng to 125 ng DNA was used per reaction for ITS. Primers used in all the PCR processes (Taberlet *et al.*, 1991; Sang *et al.*, 1997; Blattner, 2000) can be found in Table S2 (supplementary material).

The *psbA-trn*H region was amplified using a protocol of 98 °C for 30 s, followed by 35 cycles of 98 °C denaturation for 30 s, 48 °C annealing for 30 s, and 72 °C elongation for 45 s, then final elongation 72 °C for 5 min. The *trnL-trn*F region was amplified in two parts using the primer pairs My-trnLF-c2 (which was designed especially for this study) with A49855, and B49873 with A50272; Taberlet *et al.* (1991) was consulted to help design My-trnLF-c2 and inform primer pairing. The cycling protocol used was 98 °C for 30 s, then 35 cycles of 98 °C for 30 s, 53 °C for 30 s, and 72 °C for 45 s, and elongation at 72 °C for 5 min. The ITS region was also amplified in two parts using the primer pairs ITS-A with ITS-C, and ITS-D with ITS-B (Blattner, 2000). The PCR cycle was 98 °C for 30 s, finishing with 72 °C final elongation for 5 min.

For each specimen, a template-primer mix was prepared, consisting of 20 ng to 50 ng (for sequences between 500 and 1000 bp) of PCR product, 3.2  $\mu$ L of the relevant primer, and purified water to a total volume of 7  $\mu$ L. The samples were then cycle sequenced, cleaned, and sequenced using the BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, 2002) using an ABI 3730 capillary sequencer (Thermo Scientific) by personnel of the Genomics Service Unit of the Institute of Genetics of Faculty of Biology, LMU Munich. Using MEGA-X v.10.2.4 (Kumar *et al.*, 2018), electropherograms were manually checked and mistakes corrected, DNA sequences were combined and aligned using the MUSCLE algorithm.

#### 2.3. Phylogenetic analyses

*Myricaria* ITS, *psbA-trn*H and *trnL-trn*F sequences from previous studies were downloaded from GenBank and added into our own dataset, as shown in Table S1. The *psbA-trn*H and *trnL-trn*F sequences in this combined dataset were concatenated using the Concatenate tool on MEGA-X v.10.2.4. ModelTest-NG (Darriba *et al.*, 2020) was used to calculate the best substitution models for the alignments, which were GTR+ $\Gamma$  for both the ITS alignment and the concatenated *psbA-trn*H and *trnL-trn*F alignment. For both alignments, RAxML v.8. (Edler *et al.*, 2021; Stamatakis, 2014) was used to calculate Maximum Likelihood (ML) trees, while MrBayes v.3.2.7 (Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003) was used to calculate Bayesian Inference (BI) trees (5 million generations). For the ML trees, autoMRE (a

bootstrap-based criterion) was used. In MrBayes, the parameters of the MCMC analysis were set to 50,000 samples; the frequency of samples set to 100; 25% burn-in; 10 chains; and temperature of 0.1. These parameters were determined experimentally to ensure good mixing and convergence. The GTR+ $\Gamma$  model was selected with equal among-site rate variation, following the results of the aforementioned ModelTest-NG before making the phylogenetic analyses. Since the *psbA-trn*H and *trnL-trn*F alignment contains individuals that lack one gene or the other, the analysis was also run using only individuals where both gene sequences were available.

#### 2.4. Biogeographical reconstruction

We use Takhtajan's Floristic Provinces (1986) as a framework for *Myricaria* distribution across Eurasia. The floristic regions form a hierarchical system that divides all landmasses in the world according to the flora found in these areas. The Eurasian range of *Myricaria* covers many floristic provinces forming three regions and two subkingdoms. Table 1 shows the breakdown of the distribution of *Myricaria* and the letter assigned to each floristic region. Each specimen in the study is assigned a letter according to its collection provenance.

RASP 4 (Yu et al., 2020b) was used to estimate geographical ancestral states. A Bayesian binary Markov chain Monte Carlo (MCMC) (BBM) analysis was performed on each gene tree. BBM analyses use the ancestral state reconstruction algorithm of MrBayes to infer ancestral distributions (Ronquist and Huelsenbeck, 2003). This analysis was selected because it tolerates polytomies and because it supported data points with missing geographical provenance data which were sometimes not available or specific enough on GenBank. Maximum number of areas was set to four; this means that the maximum number of floristic provinces where a single node can be distributed is assumed not to exceed four. The MCMC analysis parameters were set to the same as the aforementioned MrBayes analysis. The BBM analysis estimates probabilities for the possible ancestral ranges (the floristic province) at every node. These probabilities are shown as node pies, where the most likely floristic province is represented by a letter in the middle of the pie (Table 1), and areas with less than 5% probability are shown in black.

# 2.5. Molecular clock dating

BEAST v.2.7 (Bouckaert *et al.*, 2019) was used on the CIPRES Gateway (Miller *et al.*, 2010) to estimate divergence times on the concatenated *psbA-trn*H and *trnL-trn*F alignment. BEAUti (Drummond *et al.*, 2012) was used to input settings. For site model, estimate substitution rate was set to gamma, category count 4, and estimate shape 1.0. Default settings were used for substitution model rates, while

Table 1

Overview of Takhtajan's (1986) Floristic Provinces covered in this study. Each province is assigned a letter code for biogeographical reconstructions.

Subkingdom	Region	Province	Code
Boreal	Circumboreal	Central Europe and Northern Europe	А
		Illyrian or Balkan	С
		Euxine	Μ
		Caucasian	В
		Altai-Sayan, Central Siberian and	0
		Transbaikalian	
	Eastern Asian	Northern Chinese	К
		Central Chinese	J
		Sikang-Yunnan	Ι
		Eastern Himalayan	Н
Tethyan	Irano-	Central Anatolian and Armeno-Iranian	Ν
	Turanian	Turkestanian	L
		Western Himalayan	D
		Central Tien Shan	G
		Mongolian	F
		Tibetan	E

frequencies were set to estimated. Prior settings were: tree set to Yule Model (a simple model appropriate for sequences from different species). The clock model was set to random local clock with rate changes Poisson, which are the setting recommended by Drummond and Suchard (2010). The mean clock rate was set to an exponential distribution with mean 1. The birth rate was set to log normal, with mean set to 0.1 and standard deviation of log transform set to 1. This was derived from stem age estimated by Yao *et al.* (2019) according to the relationship set down by Steel and Mooers (2010). In accordance with the divergence estimates of Terrones and Juan (2023), a calibration prior with a mean of 26.5 Ma was set for the (monophyletic) *Myricaria* stem age, normally distributed with a standard deviation of 1.2.

For MCMC, default settings were used, except chain length was set to 30 million with a pre-burn in of 3 million, to ensure adequate sampling across the parameters. The resulting tree was formatted using FigTree v.1.4.4 (Rambaut, 2018).

# 3. Results

# 3.1. Phylogenies

Two gene trees were calculated using both the BI and ML algorithms using the data summarised in Table S1 (in supplementary materials). Fig. 1 shows the phylogeny generated from the concatenated chloroplast *psbA-trnH* and *trnL-trnF* regions. Since the BI and ML algorithms resulted in the same tree topology, Fig. 1 is annotated with both BI posterior probabilities and ML bootstrap values in brackets. To test that missing

data did not skew our analysis, we calculated a phylogeny from a subset of the concatenated chloroplast data including only individuals where both the *psbA-trn*H and *trnL-trn*F gene regions were available. The result of this phylogenetic analysis of the reduced chloroplast data is very similar to that of the analysis of the full concatenated chloroplast dataset shown in Fig. 1. The resulting smaller tree can be seen in Figure S1 (in supplementary materials).

Fig. 2 shows the phylogeny calculated from the nuclear ITS sequences. Again, Fig. 2 shows the BI posterior probabilities and ML bootstrap values on the same tree, as the algorithms agreed on the tree topology.

Since species delineation in the genus is uncertain and there is strong evidence (Liu *et al.*, 2009; Wang *et al.*, 2009; Lyakh, 2013; Zhang *et al.*, 2014; Hu *et al.*, 2023) that currently accepted species do not reflect clear phylogenetic or biological entities, this study adopts a purely cladistic approach. Clades in the concatenated *psbA-trn*H and *trnL-trn*F tree are colour-coded (Fig. 1).

The ITS and the concatenated *psbA-trn*H and *trnL-trn*F trees are incongruent. For example, in the concatenated *psbA-trn*H and *trnL-trn*F tree all specimens coloured pink on Figs. 1 and 2 (deposited in the herbaria under the names *Myricaria wardii* C.Marquand and *Myricaria rosea* W.W.Sm.) form a clade with a posterior probability of 0.92, but in the ITS tree (Fig. 2) only some specimens form a clade with posterior probability 0.92, while the remaining specimens are part of a polytomy. The orange clade (deposited under the name "*Myricaria prostrata* Hook.f. & Thomson") is also incongruent; in the ITS tree, the two individuals form a clade with strong support values (posterior probability 1;



Fig. 1. Molecular phylogeny reconstruction using concatenated chloroplast *psbA-trn*H intergenic spacer and *trnL-trn*F regions in the plant genus *Myricaria*. Fig. 1a gives an overview of the general tree topology. Fig. 1b and 1c shows posterior probabilities above 0.9 from Bayesian inference analysis (using MrBayes) and bootstrap values above 70 (in brackets) from maximum likelihood (using RAxML).



Fig. 2. Molecular phylogeny reconstruction using the nuclear ITS region in the plant genus *Myricaria*. Fig. 2a gives an overview of the general tree topology. Fig. 2b and 2c shows posterior probabilities above 0.9 from Bayesian inference analysis (using MrBayes) and bootstrap values above 70 (in brackets) from maximum likelihood (using RAxML). Specimen colours match those of Fig. 1 for ease of reference.

bootstrap value 89), but they do not group together on the concatenated *psbA-trn*H and *trnL-trn*F tree. The concatenated *psbA-trn*H and *trnL-trn*F tree shows that individuals collected in Europe (clade coloured green, posterior probability 1) and individuals collected in Asia (clade coloured brown, posterior probability also 1) form clear clades. The ITS tree is less clear in this regard, but the European clade is largely consistent (with three exceptions).

# 3.2. Biogeographical analyses

Takhtajan's (1986) Floristic Provinces were used as a framework to assign individuals of Myricaria into floristic regions. BBM analyses show the most likely ancestral states at each node on each tree (Figs. 3 and 4). Both BBM analyses show that the most likely ancestral location of the genus is the Tibetan Province, represented by the letter E. Differences between the topologies of the two gene trees mean that there are also some differences between the most likely distributions at each node. The analysis using the chloroplast data (Fig. 3) suggests that diversification of the main lineages took place within the Tibetan Province with subsequent spread to the Caucasian Province (B) and the Mongolian Province (F), which were gateways to western Europe and China, respectively. Meanwhile, Fig. 4 suggests that the ancestors of all lineages sister to the dark blue clade (Fig. 2; herbarium specimens labelled as Myricaria elegans Royle) originated in the Eastern Himalayan Province, whence they moved back to the Tibetan Province, before spreading to other areas.

## 3.3. Divergence times

The BEAST analysis (Fig. 5) gives us some estimates of divergence times for European, Chinese, and Siberian clades. As the stem age of *Myricaria* was calibrated with a prior with mean 26.5 million years, the analysis shows that a common ancestor of all European *Myricaria* occurred around 8.8 Ma ago, with a 95% confidence interval between 5.7 and 12.2 Ma ago. Meanwhile, the common ancestor of specimens from the Central Chinese Province date to 5.2 Ma ago (95% confidence interval between 1.9 and 8.7 Ma ago). The common ancestor of *Myricaria* in the Mongolian, Altai-Sayan, Central Siberia and Transbaikalian Provinces occurred 4.7 Ma ago (95% confidence interval between 1.9 and 7.8 Ma ago).

# 4. Discussion

This study confirms the biogeographic hypotheses of Bobrov (1967), with regards to the centre of origin of *Myricaria*, and its patterns of migration from Central Asia to Siberia and Europe. Specifically, our biogeographical analyses show that the centre of origin of *Myricaria* is Takhtajan's Tibetan Province. This province includes the Pamirs and the Tibetan Plateau, bounded by the Trans-Alay, Hindukush and Karakorum mountain ranges (Takhtajan, 1986). By sampling from the entire Eurasian distribution of *Myricaria*, our result updates and extends previous studies which estimate the origin of the genus to be within the Himalayan Mountain region (Zhang *et al.*, 2014).

The phylogenetic reconstruction and BBM analyses yielded different



**Fig. 3.** BBM analysis using RASP 4 of the concatenated chloroplast *psbA-trn*H intergenic spacer and *trnL-trn*F regions. Each node pie shows the probabilities of each node of belonging to the different floristic provinces, and the most likely floristic province is represented by a letter in the middle of the pie. Areas with less than 5% probability is shown in black. The colours and letters representing the floristic regions correspond to the insert map.



Fig. 4. BBM analysis using RASP 4 of the nuclear ITS region. Each node pie shows the probabilities of each node of belonging to the different floristic provinces, and the most likely floristic province is represented by a letter in the middle of the pie. Areas with less than 5% probability is shown in black. The colours and letters representing the floristic regions correspond to the insert map.

tree topologies between the combined *psbA-trn*H and *trnL-trn*F tree and the ITS tree. The analyses from the two datasets agree on the broader aspects of *Myricaria* biogeography; for example the Tibetan Province centre of origin, the general direction of migration, and that the European specimens largely clade together. However, the ITS tree differs on the details of migration between the nodes.

The maternally inherited cpDNA is less susceptible to phenomena like recombination and incomplete lineage sorting than the nuclear ITS

region. Therefore, we use the results from this dataset to summarise our findings in Fig. 6. For consistency, each floristic province is coloured with the same colour code as in Figs. 3 and 4 and shows the same letter identifier as Table 1. Every black arrow represents a migration event from one node to another as seen in Fig. 3. The black arrows with white numbers represent the timeframes of key migrations taken from the BEAST analysis shown in Fig. 5. These numbers show the estimated time interval during which a migration event occurred.



Fig. 5. Tree showing results of BEAST analysis of the concatenated chloroplast *psbA-trn*H intergenic spacer and *trnL-trn*F regions. Nodes marked with an asterisk denotes posterior probability of at least 0.99 (excluding outgroups). Divergence times (Ma) with 95% confidence intervals of major clades are shown on the tree and specimens in each clade are shown in the boxes on the right. Colour and letter keys show the floristic province and corresponds to the colours used in Figs. 3 and 4.

# 4.1. Significance of the Qinghai-Tibetan Plateau

The term "Qinghai-Tibetan Plateau" (QTP) is used inconsistently by different authors. In a narrow sense, it is restricted to the plateau interior, and is roughly equivalent to Takhtajan's Tibetan Province. In a wider sense, it also includes the Hengduan Mountains and the eastern part of the Himalayas (Yu *et al.*, 2020a). This distinction is important floristically because the plateau interior is relatively species poor (Takhtajan, 1986; Yu *et al.*, 2020a). There are only around 1000 plant species in the Tibetan Province, with the central plateau region consisting mainly of halophytes (Takhtajan, 1986). In contrast, the Hengduan mountains and the Himalayas (part of Takhtajan's Eastern Himalaya and Sikang-Yunnan Provinces) are the most species diverse mountain regions in the northern hemisphere, with high levels of endemism and high levels of hybridisation (Sun *et al.*, 2017; Wu *et al.*, 2020a).

The current range of *Myricaria* within China corresponds to areas of high soil salinity, including northern China, Inner Mongolia and the Tibetan Plateau (Thomas *et al.*, 2014; Liu and Wang, 2021). The salinification of the Qaidam Basin in the north-eastern region of QTP is estimated to have occurred 29 to 42 Ma ago, which coincided with patterns of global cooling and aridification (Ye *et al.*, 2020).

There is no current geological consensus on timing of the uplift of the QTP. In fact, there has been some controversy on the reliability of evidence regarding conclusions of orogeny in this area and its connection to the development of the local flora (Favre *et al.*, 2015; Renner, 2016; Muellner-Riehl, 2019; Mao *et al.*, 2021; Spicer *et al.*, 2020). However,

most recent estimates indicate a series of uplift events between 20 and 38 Ma ago, by which time the basin had reached an elevation of more than 4000 m according to Ding *et al.* (2022). This is consistent with estimates of the timing of the split between *Myricaria* and its sister genus *Tamarix*, dated between 24.5 and 28.5 Ma ago (Yao *et al.*, 2019; Terrones and Juan, 2023). We suggest that the salinisation of the QTP allowed for colonisation of the area by the common ancestor of *Tamarix* and *Myricaria*, with the complex history of its uplift potentially playing a role in their split into separate genera and the subsequent adaptation of *Myricaria* to its alpine riparian habitat. The uplift of the other mountainous regions in central Asia, believed to have occurred later than that of the QTP (Caves *et al.*, 2017), may well have played a key role as it facilitated the spread and diversification of *Myricaria* beyond the plateau.

#### 4.2. Adaptation to montane riparian freshwater habitats

The family Tamaricaceae consists of three accepted genera, *Reaumuria, Tamarix* and *Myricaria. Reaumuria* is sister to the other two genera (Yao et al., 2019). *Reaumuria* occurs in desert environments, with some species known to withstand extreme aridity (Shi et al., 2013). *Tamarix* is particularly associated with saline river valleys of lowland deserts (Ohrtman and Lair, 2013), while *Myricaria* is found in valleys of freshwater mountain rivers, on pebble and sandy deposits, which are often subject to flooding (Bobrov, 1967; Kudrnovsky and Stöhr, 2013). *Tamarix* is highly adapted to regions of elevated soil and water salinity, regulating its mineral uptake through ion exclusion at root uptake and,



**Fig. 6.** Summary and integration of all cpDNA results, including BBM analysis results in the context of Takhtajan's (1986) floristic provinces (colours and letters corresponding to the key in Fig. 3), all migration routes from the BBM (black arrows). The divergence time estimates (Ma before present) for key migrations, taken from the BEAST analysis, are shown by white numbers on the black arrows; upper and lower bounds are taken from the date estimates of the most recent ancestor in the origin and the earliest ancestor in the destination province. The boundaries of the northern migration patterns described by Kadereit *et al.* (2008) are shown in red and blue.

importantly, recretion through specialised glands (Ohrtman and Lair, 2013). The findings of Dörken *et al.* (2017) and Karpova *et al.* (2022) highlight that species of *Myricaria*, despite being specialised on freshwater habitats, still possess functional salt glands. The presence of those glands in both *Myricaria* and *Tamarix* suggests that the common ancestor of *Tamarix* and *Myricaria* occurred in saline environments. While *Tamarix* remained in saline environments, *Myricaria* species may have moved into freshwater river valleys during the uplift of the mountains. The co-option of these salt glands to the needs of riparian mountain habitats is not yet fully understood, but the results of Dörken *et al.* (2017) suggest that it could be crucial for the success of (at least) *M. germanica* in highly calciferous environments.

# 4.3. Migration to Europe

The BBM analysis also supports Bobrov's (1967) conclusion that western and northern Europe (Central European and Northern European Provinces) was the last to be colonised by *Myricaria* lineages as the genus spread west from its central Asian centre of origin. Kadereit *et al.* (2008) observes two patterns of migration of European Alpine taxa; either migration occurs from an Asian centre of origin via a "northern" route (via the Arctic), or a Mediterranean centre of origin for taxa in the European Alps that are connected to Asia via the Caucasus, Hindukush, Pamir, Karakorum and Tien Shan mountain ranges (the "southern" connection). In this latter model, these southern mountain ranges do not play a significant role in migrations to Europe.

In contrast to the southern migration patterns out of the Mediterranean or the northern migration across the Arctic described by Kadereit et al. (2008), in the case of *Myricaria*, the southern mountains were key to the migration from its Central Asian centre of origin to the European Alps. In fact, the Caucasus acts as a secondary centre of origin, as the common ancestor of *Myricaria* growing in central and northern European and Mediterranean came from the Caucasian Province. Therefore, in the case of *Myricaria*, neither is there any evidence of a "northern" pattern of migration, nor is the centre of origin is the Mediterranean as put forward by the "southern migration" hypothesis (Kadereit et al., 2008). This is consistent with geological estimates that during the Pleistocene Glacial Maximum, there was only a distance of around 200 km between the Scandinavian and Alpine glaciers, meaning that the Scandinavian and central European mountain habitats were essentially united (Ehlers and Gibbard, 2007). *Myricaria* lineages would have been easily able to spread through central Europe to Scandinavia.

# 4.4. Phylogeny and taxonomy of Myricaria

Although the aim of this study is not primarily taxonomic, our results are consistent with the findings of previous authors. Building on the findings of previous authors (Zhang *et al.*, 2014; Wang *et al.*, 2009; Hu *et al.*, 2023), our phylogeny also shows that the relationships between species within the genus remain unresolved.

In particular, the incongruence between our nuclear ITS tree and chloroplast regions is in accordance with the results of Hu *et al.* (2023) and aligned with known phenomena in *Tamarix* (Terrones and Juan, 2023). This adds to the overwhelming evidence that current species delineations within *Myricaria* are polyphyletic, and further work is required to elucidate these relationships. Reasons for this incongruence could be incomplete lineage sorting, hybridisation, or recombination. Future work on this topic could include Next Generation Sequencing methods to explore these possible reasons in more detail.

#### 4.5. Association with Hippophae

As was already noted by Bobrov (1967), *Myricaria* species have a remarkable association with the sea buckthorn genus, *Hippophae*. This genus contains seven species, only one of which, *H. rhamnoides* L., is found in Europe (Swenson and Bartish, 2002). This genus co-occurs in characteristic vegetation groups across the range of *Myricaria*, including Tibet (Li and Wu, 1991), Ladakh (Joshi *et al.*, 2005), the Caucasus region

# (Pietsch, 1967) and Switzerland (Volk, 1939).

Another similarity of the genus *Hippophae* to *Myricaria* is that both genera have incongruent gene trees (Bartish *et al.*, 2002; Hu *et al.*, 2023). In both cases, this points to intrageneric hybridisation events in the history of each genus.

A phylogeographical study of *H. rhamnoides* has revealed a very similar history of origin in the QTP with subsequent dispersal across Eurasia via the Caucasian mountains (Jia *et al.*, 2012), making it plausible that such associations among plant species could have been maintained across millions of years. In fact, fossil-calibrated dating of the genus *Hippophae* has revealed divergence times that are remarkably similar to our *Myricaria* results; estimates by Jia and Bartish (2018) based on cpDNA indicate that all species within *Hippophae* had diversified between 17.6 and 21.2 Ma ago. The similarities between *Hippophae* and *Myricaria* may well be due to their shared riparian habitat, though we are not aware of any general migration patterns of riparian taxa compared to non-riparian taxa. It remains to be determined by future studies if the biogeographic and migration patterns found in *Myricaria* and *Hippophae* are more generally shared by other riparian plant genera.

#### 5. Conclusions

By combining newly sequenced samples from outside of China with sequences from previous studies, we generated two molecular phylogenies, the first using the chloroplast intergenic spacer regions psbA-trnH and the trnL-trnF chloroplast regions, and the second using the nuclear ribosomal ITS region. The phylogeny and biogeographical analysis presented elucidate the biogeography of the genus Myricaria. Its centre of origin is situated in Takhtajan's Tibetan Province. The migration of Myricaria to Europe is confirmed to have progressed through the Caucasus and the remaining southern mountain ranges, including the Hindukush, Pamir, Karakorum and Tien Shan mountain ranges. This pattern of diversification and migration mirrors that of the genus Hippophae, which has a very similar distribution and biogeographical history. These results confirm and substantiate the biogeographical conclusions of Bobrov (1967): the genus Myricaria has a centre of origin in central Asia (specifically in the QTP). The increase in soil salinity of the QTP may have played a key role in the establishment of Tamaricaceae in the region, and its uplift may well have contributed to the subsequent divergence of the genus Myricaria from the genus Tamarix and to the specialisation of Myricaria to montane riparian habitats. Finally, we also conclude that species delineations within Myricaria across its entire Eurasian range remain unresolved.

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# CRediT authorship contribution statement

Kailin Sun: Writing – review & editing, Writing – original draft, Visualization, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. Silke Werth: Writing – review & editing, Validation, Supervision, Resources, Methodology, Funding acquisition, Conceptualization.

# 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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# Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.flora.2024.152639.

# Data availability

Sequence data has been uploaded onto GenBank and accession numbers are listed in the supplementary material.

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