

A Revisited Study on Phylogeography and Phylogenetic Diversity of *Myricaria* (Tamaricaceae)

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Abstract

As the first part of the present study, the ancestral distribution origin of *Myricaria* at a Eurasian perspective, a genus of Tamaricaceae, was re-evaluated by comparing three different methods, including Statistical Dispersal-Vicariance Analysis (S-DIVA), Dispersal-Extinction-Cladogenesis Analysis (DEC) and Bayesian Binary MCMC Analysis (BBM). In addition, Parsimony Analysis of Endemicity (PAE) was adopted to identify areas of endemism and test whether the endemic areas could be congruent to the origin of distribution. My results showed that two of the three ancestral distribution methods consistently identified East Asia as the historical origin of distribution, which was in line with a previous study using the distributional information of *Myricaria* in China only based on classical dispersal-vicariance method. PAE further supported such an observation, indicating that PAE could be an option for identifying origin of distribution. Further, it was found that the emigration of *Myricaria* from East Asia to the rest of Eurasian regions only occurred when the drastic uplifting of Qinghai-Tibet Plateau emerged. As another part of the study, I quantified the phylogenetic diversity patterns of *Myricaria* species using six phylogenetic diversity metrics, for the purpose of identifying the highest-priority species for conservation. For those species endemic to the origin of distribution, it was found that two species were assigned the highest conservation values under the phylogenetic framework, *M. laxiflora* and *M. elegans* var. *tsetangensis*.

Keywords: Historical biogeography; Species extinction; Biodiversity conservation; Areas of endemicity

Introduction

The small genus *Myricaria* is a part of the family Tamaricaceae, composed of around 11 species over the world [1]. Most *Myricaria* species have distributional ranges in China, especially in the Himalayan region [2]. Its ancestral origin is generally believed to be derived from Himalayan region. Due to increasing habitat fragmentation and industrialization of the native habitats, many species are believed to undergo extinction threats in China [1].

In previous studies [1,2], Himalayan region (Qinghai-Tibet Plateau) was believed to be the historical origin *Myricaria* by utilizing statistical dispersal-vicariance analysis. However, dispersal-vicariance analysis might be misleading due to its incapability to identify extinction events [3]. Here, by utilizing different methods to reconstruct ancestral distributional ranges [4,5], the purpose of the present study is to re-evaluate the validity of the Himalayan-origin hypothesis. I am in particular focusing on the discrepancy caused by different ancestral range reconstruction methods. I also introduce the Parsimony Analysis of Endemicity (PAE) [6,7] as a new way to identify the origin of distribution of *Myricaria* taxa. I regard that both origin of distribution and areas of endemism are two identical quantities, although they should have different biological foundations. If the areas of endemism identified by PAE are concordant with the origin of distribution reconstructed by ancestral range inference, PAE might be a potentially promising option for identifying the origin of distribution. Otherwise, PAE would not be recommended. In a summary, the central objective of my study is to offer alternative statistical diagnoses on the distributional origin of the genus.

Besides, though conservation strategies have been advocated for the genus [1], no quantitative assessment of conservation priorities for each species has been proposed. As such, it should be of some help to utilize phylogenetic diversity methods to quantify the extinction threats of the species and evaluate the conservation values of each species [8-11]. Therefore, in the present study, another objective is to quantify

conservation priorities of *Myricaria* species on the basis of phylogenetic perspectives.

To avoid possible repetitions of the previous studies [1,2], I am not discussing in detail the phylogenetic patterns of the *Myricaria* species. Instead, my study will focus on the comparative methods for identifying the ancestral distribution, the historical biogeographic scenarios and the conservation priority of *Myricaria* species.

Materials and Methods

Sequence data

DNA sequences for *Myricaria* taxa were collected for the nuclear ITS region (partial sequences of ITS1 and ITS2, complete sequence of 5.8S) and *psbA-trnH* intergenic spacer, which have been widely used in the molecular phylogenetic analysis. These sequences, available from GenBank database were directly obtained from previous works [1,2]. The list of species and associated GenBank sequence accession numbers are presented in Table 1.

Phylogenetic analysis and reconstruction

Sequence alignment was done using Cluster X program [12]. The region with ambiguous alignment was manually excluded using BioEdit program [13]. Phylogenetic analysis was performed using maximum parsimony method (MP) on PAUP version 4b10

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No.	GenBank ID	Species	ED	TD	ES	UPD	PE	BED
1	EU240605	<i>M wardii</i> *	7.766	0.033	6.276	5.898	9.775	6.711
2	AY572413	<i>Mgermanicassp germanica</i>	6.721	0.027	4.907	4.679	5.681	2.44
3	EU240606	<i>M platyphylla</i>	5.11	0.02	2.894	2.398	5.955	2.339
4	EU240604	<i>M squamosa</i>	5.11	0.02	2.894	2.398	5.555	1.939
5	EU240600	<i>M paniculata</i> *	4.392	0.02	2.139	1.513	6.426	2.683
6	AF484746	<i>M germanica ssp alopecuroides</i>	3.848	0.019	1.283	0.427	5.291	1.412
7	EU240596	<i>M bracteata</i>	3.848	0.019	1.283	0.427	5.291	1.412
8	EU240602	<i>M pulcherrima</i>	6.273	0.023	4.269	4.142	8.397	5.058
9	EU240607	<i>M prostrata</i>	8.534	0.042	7.144	5.366	5.937	3.114
10	EU240608	<i>M rosea</i>	8.534	0.042	7.144	5.366	6.831	4.008
11	EU240609	<i>M laxiflora</i> *	11.332	0.083	11.332	9.937	13.006	10.551
12	EU240594	<i>M elegans</i>	15.384	0.333	15.384	5.484	8.428	6.778
13	EU240595	<i>M elegans var tsetangensis</i> *	15.384	0.333	15.384	5.484	12.084	10.434

Table 1: Phylogenetic diversity measurements of *Myricaria* species based on the Bayesian MCMC maximum clade credibility tree presented in Figure 2. Endemic species only found in East Asia (China) were marked in asterisks. GenBank accession numbers for the sequences utilized for the present study are provided as well.

package [14]. Partition homogeneity test was performed to see the heterogeneity of the combined data for maximum parsimony analysis [1]. Most-parsimonious trees were obtained by 1000 replicates of random sequence addition using Tree Bisection-Reconnection (TBR) branch swapping under the Fitch criterion. The Bayesian tree was constructed using the program MrBayes [15]. The evolutionary model GTR+Gamma was selected using MrModeltest [16] under the Akaike information criterion. The Markov chain Monte Carlo chains were run simultaneously for 1000000 generations and trees were sampled for each 1000 generation. Two independent chains were run simultaneously. Average standard deviation of split frequencies was used as the diagnosis of stationary status of the MCMC chains. The first 25% trees were regarded as the unstable burn-in ones and discarded, the remaining trees were used to construct 50% majority rule consensus tree [4].

Molecular dating

Because of the lack of fossil records [2], I used the relaxed molecular clock Bayesian model [17] to date the tree with BEAST software by inputting it as the starting tree [18]. The general substitution rate ($u = 1.0 \times 10^{-9} \text{ss}^{-1} \text{year}^{-1}$) of the plastid sequence was used [4,19]. 1000000 generations and trees were sampled for each 1000 generation. Stationary status of MCMC chains were checked using Tracer [20]. The maximum clade credibility tree was constructed using TreeAnnotator [18] after pruning the first 400 sampled trees. The tree was viewed and edited using FigTree software [21].

Ancestral range reconstruction

Prior to the reconstruction of historical ranges, I defined the following five bigeographic regions by using Qinghai-Tibet Plateau as the reference center: East Asia (A), covering the ranges of south, western and eastern part of China, but excluding the northern part of China; South Asia (S), covering the countries including India, Nepal, Pakistan and others; Central Asia (C), including the countries in that region, like Afghanistan and others; North Asia (D), including northern part of China (Xinjiang, Inner Mongolia), Mongolia and Eastern part of Russia; and Europe (E).

Reconstruction of ancestral ranges were implemented using the software RASP [5,22]. Three methods available were all carried out, including S-DIVA method [22,23], Bayesian Binary MCMC method (BBM) [4,5] and dispersal-extinction-cladogenesis method (DEC) [3].

Parsimony Areas of Endemicity (PAE)

Areas of endemism [6,7,24] were defined as the areas where two or more species that have sympatric distribution. PAE is a cladistic method for identifying areas of endemism, in which the clades were identified as endemic areas when the lineages leading to these clades were supported by two or more synapomorphies [25]. In the empirical studies of historical biogeography, PAE has been widely employed [25,26]. In the present study, I employ it to find the areas of endemism for *Myricaria* and compare whether the identified endemic areas could be fully congruent with the reconstructed origin of distribution from the abovementioned ancestral range reconstruction methods. The abovementioned five biogeographic regions were used as the operating areal units for the PAE analysis.

Phylogenetic diversity analysis of *Myricaria* species

The following species-based phylogenetic diversity metrics were calculated, including evolutionary distinctiveness (ED) [8,27], taxonomic distinctiveness (derived from ED index, but the branch lengths are ignored and all treated equally during the calculation) (TD) [28], equal splits (ES) [8,29], unshared phylogenetic diversity (i.e., the terminal branches linking to the external species directly) (UPD) [27], Phylogenetic Endemism (PE) [30,31] and Biogeographically weighted phylogenetic Distictiveness (BED) [32] indices. The definition and calculation of these indices were not present here for simplicity, and readers could refer to the original papers for further details.

Results

Phylogenetic history of *Myricaria* species

The constructed 50% major-rule consensus MP tree is highly identical to the previous studies [1,2]. Thus, it is not presented here for simplicity. The dated maximum clade credibility Bayesian tree (Figure 1) based on was highly similar to the MP tree and previous studies [1,2], using GTR+G as the evolutionary model which was selected by MrModelTest. The ancestor of all *Myricaria* species has a clade age around 25 million years ago (Ma) (95% HPD: 14.9~35.9 Ma). For all the subsequent analyses, this dated Bayesian tree was used to infer the origin of distribution for *Myricaria* species and identify conservation values of species since it contained branch length information and fully dichotomous.

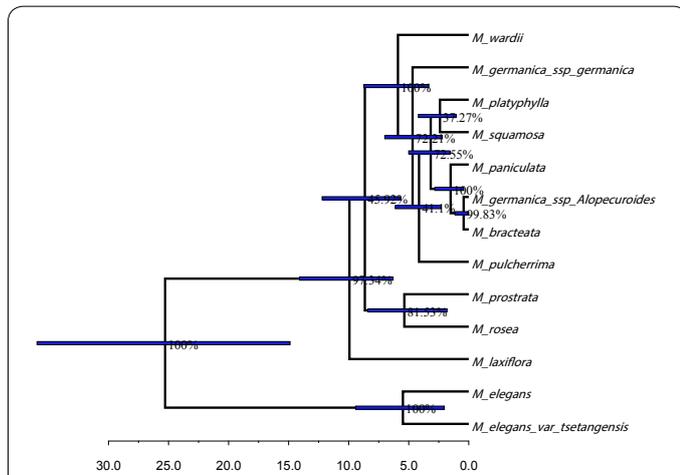


Figure 1: Dated maximum clade credibility tree with posterior probability supports showed aside the nodes. Bars on the nodes indicated the highest probability density interval of node ages. Values aside the nodes indicated that posterior support of the clades.

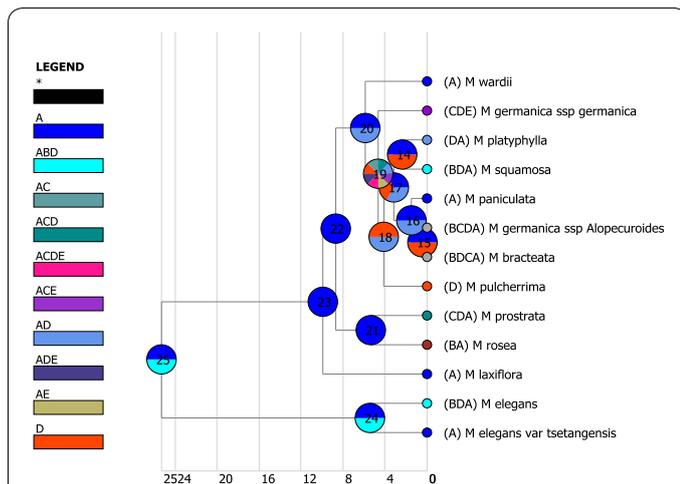


Figure 2: S-DIVA analysis on the ancestral ranges of *Myricaria* species. Area codes: A-East Asia; B-South Asia; C-Central Asia; D-North Asia; E-Europe.

Origin of distribution of *Myricaria* species

Both BBM and S-DIVA methods congruently identified East Asia is the origin of distribution of *Myricaria* species at the node of 25 (Figures 2, 3). In contrast, DEC method (Figure 4) returned a very confusing diagnosis on the origin of distribution of *Myricaria* because either single region or combined regions were supported with very low probabilities in the root. PAE method further verified that East Asia is the areas of endemism, as supported by the four endemic species (Figure 5). Thus, in my study, without specific references, the inferred origin of distribution is identical to the identified area of endemism for *Myricaria* species, both meaning East Asia (Qinghai-Tibet Plateau).

Phylogenetic diversity patterns of *Myricaria* species

As showed in Table 1, the six phylogenetic diversity indices quantified the relative importance of priority species for conservation under different phylogenetic perspectives. In specific, *M. elegans* and *M. elegans var. tsetangensis* have equally highest ED, TD, and ES indices, while *M. laxiflora* has highest UPD, PD, and BED indices.

Discussion

Evolutionary history of *Myricaria* species

Based on my fossil-free analysis, I estimated that the most recent ancestor for all the *Myricaria* species could be dated back to 25 Ma. My estimation is based on the substitution rate of plastid sequence. In the previous study [2], the likely earliest occurrence time of Tamarix was used as the reference dating time of the occurrence of *Myricaria* ancestor at the time of 57 Ma.

The previous work [2] suggested the main divergence events occurred at the time during the Late Pliocene and Early Pleistocene (approximately 2.3~1.46 Ma). My re-analysis was a bit different from such an observation with some discrepancies on the time interval. As evidenced by Figure 1, most cladogenesis events happened around the time frame during 5~2.5 Ma, which is a bit earlier than the previous observation [2]. My estimation of the late burst of species divergence during 5~2.5 Ma is fully consistent with beginning time of the principal glaciation events happened in the Qinghai-Tibet Plateau (around 4.43~1.21 Ma) [2,33,34].

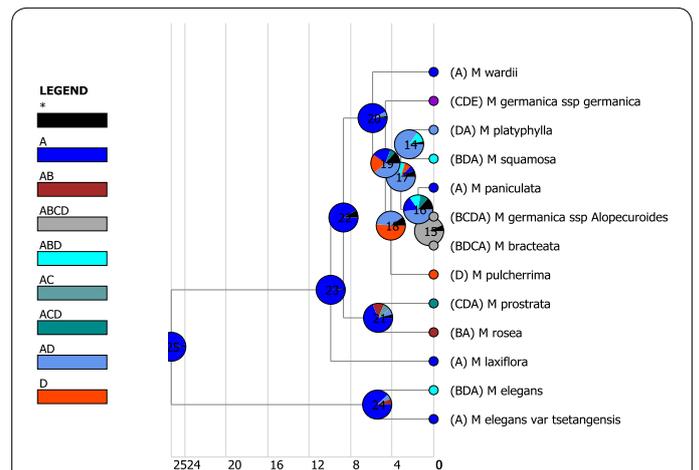


Figure 3: BBM analysis on the ancestral ranges of *Myricaria* species. Area codes: A-East Asia; B-South Asia; C-Central Asia; D-North Asia; E-Europe.

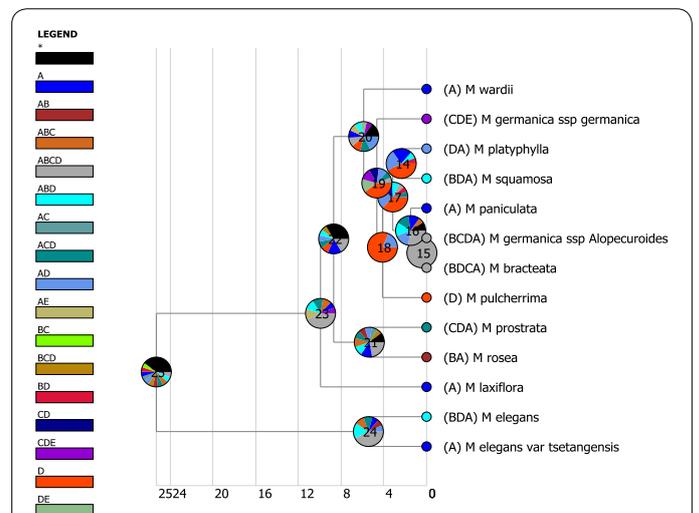


Figure 4: DEC analysis on the ancestral ranges of *Myricaria* species. Area codes: A-East Asia; B-South Asia; C-Central Asia; D-North Asia; E-Europe.

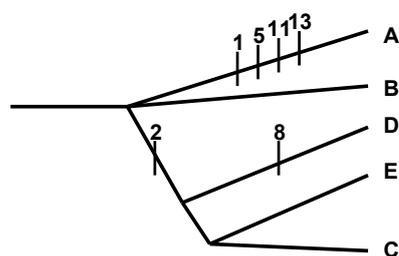


Figure 5: Identification of areas of endemism for *Myricaria* species using PAE. Bars on the branch lengths are supporting synapomorphies. This is a 50% major-rule MP tree constructed using PAUP. Area codes: A-East Asia; B-South Asia; C-Central Asia; D-North Asia; E-Europe. The corresponding species names for the numeric codes please refer to Table 1.

During the time, another paramount geological event is the significant uplift of Qinghai-Tibet Plateau, which was happening during the time of Late Pliocene and Early Pleistocene [2,35,36]. These series of events, the glaciation and uplifting of plateau, are fully concordant with the rapid branching of *Myricaria* species. Thus, historical geological events have profoundly driven the dispersal, extinction and speciation of *Myricaria*, causing the accelerating divergence of the genus during that time window as discussed below.

Historical biogeographic analysis of distributional ranges of *Myricaria*

Because DEC method returned uninformative results on the ancestral distribution of *Myricaria* species (Figure 4), I excluded the discussion referring to the method. Other two methods, S-DIVA and BBM methods were the focused ones.

As showed in (Figure 2, Figure 3), it was found that the “out-of-East Asia” historic episodes only happened after the drastic uplifting of Qinghai-Tibet Plateau at 5 Ma. During the orogenic movement of the plateau, many lineages of *Myricaria* processed a series of dispersal events from East Asia to other Eurasian regions. Interestingly, no matter what methods were used, dispersal was always identified as the principal driver of contemporary distribution of *Myricaria* species. In contrast, the contribution of vicariance or extinction events is quite rare. 22 dispersal events were congruently found by S-DIVA and BBM methods. In contrast, only 2 vicariance events and 1 extinction event were found by S-DIVA method; while only 1 extinction event and no vicariance events were identified by BMM method.

Contribution of endemic species in the origin of distribution to the diversity of *Myricaria* species

Based on PAE (Figure 5), there are four endemic species characterizing the origin of distribution of *Myricaria* in East Asia, which are *M. laxiflora*, *M. elegans var. tsetangensis*, *M. paniculata*, and *M. wardii*. Based on the phylogenetic diversity scenario, it is predicted that the loss of these four species would lead to the loss of ED by 38%, TD by 46%, ES by 43%, UPD by 43%, PE by 42% and EBD by 52%. Not surprisingly, the loss of EBD would be the highest since it is strongly correlated to the endemism status of species.

One *Myricaria* species, *M. laxiflora*, is subjected to immediate extinction due to Three Gorges Dam Project [2]. This species is endemic to Three Gorges reservoir only (Chongqing City and Hubei Province), listed as one of endangered plant species in China. It has gained much public and scientific awareness [2,37,38]. Conservation strategies on

the species have been carried out in recent years. As mentioned above, it has the highest UPD, PE and BED values; second highest ED, TD and ES values (Table 1).

Another endemic species *M. elegans var. tsetangensis* might be overlooked up to date because it is a subspecies and not subjected to immediate extinction due to dam construction. However, from the perspective of phylogenetic diversity indices, it has highest ED, TD and ES values, second highest PE and BED values. Finally, the contribution of another two endemic species, *M. paniculata* and *M. wardii*, to the global phylogenetic diversity pattern is not so remarkable. But still, *M. wardii* has second highest UPD value (Table 1).

In conclusion, conservation priority should be given to *M. laxiflora* and *M. elegans var. tsetangensis* on the basis of their unique contribution to global diversity of *Myricaria* genus and the diagnosis of areas of endemism.

Conflict of Interests

The author declares that there is no conflict of interests regarding the materials of the paper.

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