

Diversification and vicariance of desert plants: Evidence inferred from chloroplast DNA sequence variation of *Lagochilus ilicifolius* (Lamiaceae)



Xiao-Yang Gao^{a,d}, Hong-Hu Meng^{a,b,d}, Ming-Li Zhang^{a,c,*}

^a Key Laboratory of Biogeography and Bioresource in Arid Land, Xinjiang Institute of Ecology and Geography, Chinese Academy of Sciences, Urumqi 830011, China

^b Key Laboratory of Tropical Forest Ecology, Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences, Mengla 666303, China

^c Institute of Botany, Chinese Academy of Sciences, Beijing 100093, China

^d University of Chinese Academy of Sciences, Beijing 100049, China

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ABSTRACT

To document arid Northern China as a diversification center for desert plants, and to better understand the mechanisms of desert taxa diversification, we used five cpDNA spacers (*trnL-trnF*, *rps16*, *psbA-trnH*, *psbK-psbI* and *trnS-trnG*) to investigate *Lagochilus ilicifolius* in all distribution areas, including Northern China, Mongolia and Russia. Phylogenetic analyses showed that *L. ilicifolius* comprises two distinctive lineages, one distributed in China, and another in Mongolia–Russia. Our data confirmed that arid Northern China, is a distinctive area with many endemic genera. Biogeographic inferences, based on a Bayesian uncorrelated lognormal model together with molecular dating, suggested that the main diversification within the species occurred in the Pleistocene (*ca.* 1.38–0.3 Ma), resulting from the transition of the climate of Eurasia to a dry-cold pattern as well as the desertification caused the vicariance of desert plants.

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1. Introduction

To understand factors that influence organismal diversity is a fundamental goal in biology (Darwin, 1872; Simpson, 1961); and to appreciate the drivers and mechanisms of species diversification and persistence is of central interest to biogeography, evolutionary biology and conservation genetics (Lexer et al., 2013). The global distribution of plant diversity and endemism is uneven, with concentrations of species in the Andes, the Atlantic coastal forests of Brazil, the southern tip of Africa, the East Indies and the eastern Himalayas (Mutke and Barthlott, 2005). Thus, in China, many plants studies have focused on the Sino-Japanese Floristic Region of East Asia where harbors species more abundantly and ranks prominently among diversity centers of the world's temperate regions, which was referred to as the core of the Himalayan hotspot, is one of the greatest concentrations of biodiversity in the world due to its high level of species diversity and generic richness (Myers et al., 2000). These regions of high endemism were the most important glacial refugia for their Tertiary representatives ('relics')

* Corresponding author. Key Laboratory of Biogeography and Bioresource in Arid Land, Xinjiang Institute of Ecology and Geography, Chinese Academy of Sciences, 818# South Beijing Road, Urumqi 830011, Xinjiang, China. Tel.: +86 991 7823152; fax: +86 991 7823151.

E-mail address: zhangml@ibcas.ac.cn (M.-L. Zhang).

throughout the Quaternary ice–age cycles (Qiu et al., 2011). But arid regions in China, especially Alxa–Helan Mountain Range, are considered one of eight diversification centers within China, which contain a number of endemic genera (Wu et al., 2010; Zhao and Zhu, 2003). This region, designation is supported by Maxent and Domain species distribution model simulations of the ranges of 13 genera, indicated the Alxa of Inner Mongolia to be the most noticeable endemic area (Ma et al., 2012). However, this enrichment of diversification and endemism in arid zones has not drawn much attention; and only a few studies have called for conservation for its desert taxa, such as for *Ammopiptanthus* (Ge et al., 2005), *Tetraena mongolica* (Ge et al., 2011), *Helianthemum songaricum* (Su et al., 2011) and *Gymnocarpus przewalskii* (Ma and Zhang, 2012).

Central parts of the Qinghai–Tibet Plateau (QTP) might have been uplifted to present heights in early Miocene, which compelled Tethys Ocean retreated from Central Asia, establishing the beginning of an arid climate (Guo et al., 2002; Sun et al., 2010). That aggravated climatic aridification and cooling (Clark et al., 2005; Fang et al., 2005) in the Pleistocene, which sharply increased aridity in the Chinese deserts (Ding et al., 2005; Fang et al., 2002). Also, it changed the hydrology and climate of these regions, played a significant role in determining the local geographic distribution and evolutionary history of plant species (Meng and Zhang, 2011, 2013; Su and Zhang, 2013). As a dynamic ecosystem, the deserts interaction among vicariance, dispersal and habitat shift, often promotes diversification of desert plants (Meng et al., 2014). Plants, as primary producers, also play a key role in maintaining these ecosystems. Desert ecosystems currently cover large Earth's land surface and are continuously expanding. Desertification and ongoing deterioration in arid, semi-arid regions have recently focused attention on the urgent need to protect desert ecosystems. Greater comprehension of the diversification and vicariance of desert plants may provide important insights into the processes of aridification and desertification of these regions, because evolutionary history might have preserved the genetic imprint in these processes.

Evolutionary history of *L. ilicifolius* has suggested that diversification was consistent with aridification and desert expansion during the middle Pleistocene; and Helan Mountains might be suggested as the diversification center of the species (Meng and Zhang, 2011). However, previous study of *L. ilicifolius* was limited to populations in China and lacked distribution areas from Mongolia and Russia, and conclusions were inferred from only two chloroplast DNA (cpDNA) intergenic spacers. In addition to Northern China, *L. ilicifolius* occurs in southwestern Siberia (Tuvinskaya), southeast Mongolia and Leningrad (presently, St. Petersburg) (Komarov, 1954) (Fig. 1). So, the evolutionary history and biodiversity of *L. ilicifolius* will be better understood if we give full consideration to plants in all distribution areas, including China, Mongolia and Russia.

Here, we used an integration of phylogenetic, biogeographic, and molecular dating methods to reconstruct the evolutionary history of *L. ilicifolius*. The distribution of this species spans desert steppe and grassland, sandy areas as well as semidesert thickets on gentle slopes, which offers a unique opportunity to disentangle the evolutionary processes underlying botanical evolution in the arid regions. So, we investigate sequence variation of five chloroplast cpDNA spacers in samples of *L.*

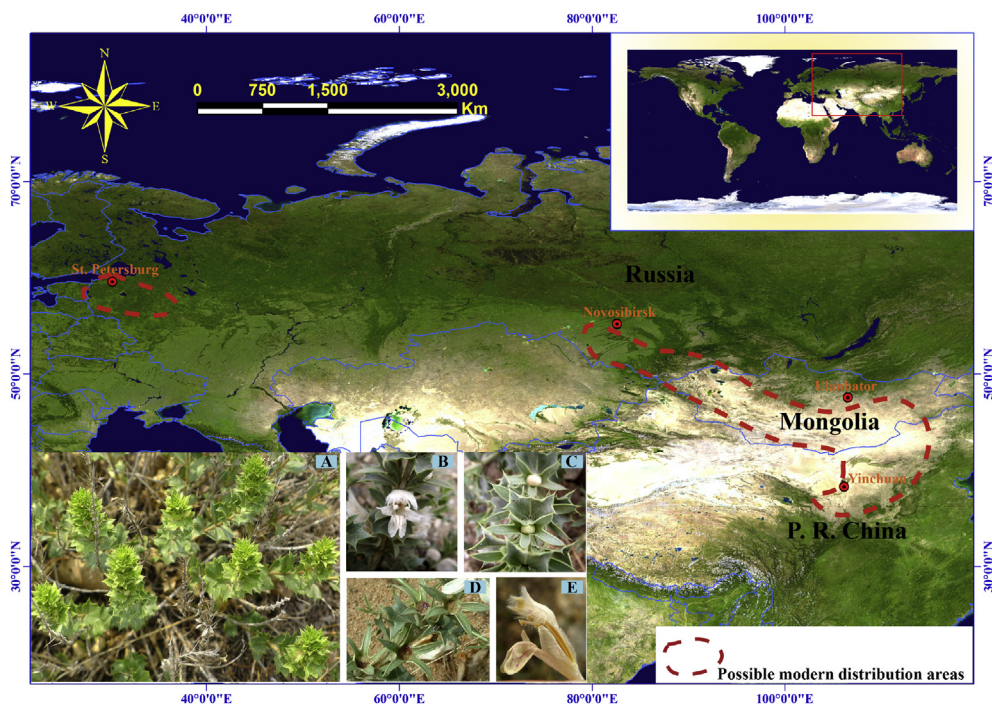


Fig. 1. Distribution map of *Lagochilus ilicifolius* showing the habitat areas in Northern China, Mongolia and Russia. (A) Adult nature plant of *L. ilicifolius*, (B) flower, (C) flower bud, (D) seeds and (E) special feature of flower of a different individual morphology.

ilicifolius, which enables us to contemplate: 1) is there a diversification center of desert plants in the arid zone of China? 2) did the progressive aridification in arid zones determine the diversification and vicariance of desert species, such as *L. ilicifolius*?

2. Materials and methods

2.1. Taxon sampling

Our sampling within the species *L. ilicifolius* attempted to include as wide a geographic diversity as possible, covering its distributional range including China, Mongolia and Russia. From the viewpoint of morphology, *Lagochilus* and *Panzeria* are derived from the genus *Leonurus* (Wu et al., 2003). Thus, *Leonurus turkestanicus*, *Leonurus glaucescens*, and *Panzeria alashanica* were used as outgroups in the analyses. Natural individuals from 14 populations (localities in Inner Mongolia, Ningxia, Shaanxi and Gansu) and outgroups were collected in China during field investigation and collections in 2010. Some plant materials of Mongolia and Russia were obtained from herbarium specimens (Table 1 and Table 2). In the field work, young and healthy leaves were sampled randomly, and quickly dried with silica gel in the field and stored frozen until DNA extraction. Voucher specimens were deposited in the Herbarium of Xinjiang Institute of Ecology and Geography, Chinese Academy of Science (XJBI). All the materials and GenBank accession numbers are presented in Tables 1 and 2.

2.2. Methods

2.2.1. DNA extraction, amplification and sequencing

Total genomic DNA was extracted from silica gel-dried leaf tissue using modified cetyltrimethylammonium bromide (CTAB) protocol (Cullings, 1992). Isolated DNA was amplified using five cpDNA regions; *trnL-trnF* (Taberlet et al., 1991), *rps16* (Shaw et al., 2005), *psbA-trnH* [*psbA-trnH-f* (Sang et al., 1997), *psbA-trnH-r* (Tate and Simpson, 2003)], *psbK-psbI* (Lahaye et al., 2008) and *trnS-trnG* (Shaw et al., 2007) were performed using the published primers of the cited authors (Table 3). Polymerase chain amplification (PCR) and cycle sequencing followed the methods described in the previous study (Meng and Zhang, 2013). PCR products were examined by gel electrophoresis using a 0.8% agarose gel in a 0.5 × TAE (pH 8.3) buffer, and stained with EB (ethidium bromide) to confirm single products. PCR products were purified using QIAquick Gel Extraction Kits. The same primers were used for sequencing, at Sangon Biotech (Shanghai) Co., Ltd., China.

DNA sequences were edited using SeqMan (Lasergene, DNASTAR Inc., Madison, Wisconsin, USA), and consensus sequences were obtained from each individual. Multiple sequence alignment was carried out in Clustal X 1.81 (Thompson et al., 1997), and refined and adjusted manually. Regions of ambiguous alignment were excluded from the analyses.

2.2.2. Phylogenetic analyses

Five cpDNA spacers were concatenated and analyzed as one sequence in phylogenetic analyses. The combined data set was analyzed under maximum parsimony (MP) in PAUP* 4.0 b10 (Swofford, 2002) and Bayesian inference (BI) relying on MrBayes 3.1.2 (Ronquist and Huelsenbeck, 2003). For MP analysis, we used heuristic searching, starting trees obtained via stepwise addition, tree bisection reconnection branch swapping, steepest descent, and the MulTrees and Collapse options in effect,

Table 1

List of sampled *Lagochilus ilicifolius*, their sources, and Genbank accession numbers. The abbreviations, "XJBI" and "Herb." denote the wild collected and herbarium materials, respectively.

Source	Code	<i>trnL-trnF</i>	<i>rps16</i>	<i>psbA-trnH</i>	<i>psbK-psbI</i>	<i>trnS-trnG</i>
Inner Mongolia, China, (XJBI)	CN01	KC890946	KC890898	KC890970	KC890922	KC890989
Inner Mongolia, China, (XJBI)	CN02	KC890947	KC890899	KC890971	KC890923	KC890990
Ningxia, China, (XJBI)	CN03	KC890948	KC890900	KC890972	KC890924	KC890991
Ningxia, China, (XJBI)	CN04	KC890949	KC890901	KC890973	KC890925	KC890992
Ningxia, China, (XJBI)	CN05	KC890950	KC890902	KC890974	KC890926	KC890993
Ningxia, China, (XJBI)	CN06	KC890951	KC890903	KC890975	KC890927	KC890994
Ningxia, China, (XJBI)	CN07	KC890952	KC890904	KC890976	KC890928	KC890995
Ningxia, China, (XJBI)	CN08	KC890953	KC890905	KC890977	KC890929	KC890996
Ningxia, China, (XJBI)	CN09	KC890954	KC890906	KC890978	KC890930	KC890997
Shaanxi, China, (XJBI)	CN10	KC890955	KC890907	KC890979	KC890931	KC890998
Shaanxi, China, (XJBI)	CN11	KC890956	KC890908	KC890980	KC890932	KC890999
Gansu, China, (XJBI)	CN12	KC890957	KC890909	KC890981	KC890933	KC891000
Gansu, China, (XJBI)	CN13	KC890958	KC890910	KC890982	KC890934	KC891001
Gansu, China, (XJBI)	CN14	KC890959	KC890911	KC890983	KC890935	KC891002
West Siberia, Russia, (Herb.)	RU01	KC890939	KC890891	KC890963	KC890915	
Tuvinskaya, Russia, (Herb.)	RU02	KC890940	KC890892	KC890964	KC890916	
Leningrad, Russia, (Herb.)	RU03	KC890944	KC890896	KC890963	KC890920	
Leningrad, Russia, (Herb.)	RU04	KC890945	KC890897	KC890968	KC890921	KC890988
Gobi Altai, Mongolia, (Herb.)	MN01	KC890941	KC890893	KC890969	KC890917	KC890987
Mandalgobi, Mongolia, (Herb.)	MN02	KC890942	KC890894	KC890966	KC890918	
East-Gobi, Mongolia, (Herb.)	MN03	KC890943	KC890895	KC890967	KC890919	

Table 2List of outgroups (*Leonurus turkestanicus*, *L. glaucescens* and *Panzeria alashanica*) and Genbank accession numbers.

Species	<i>trnL-trnF</i>	<i>rps16</i>	<i>psbA-trnH</i>	<i>psbK-psbI</i>	<i>trnS-trnG</i>
<i>Leonurus turkestanicus</i> (XJBI)	KC890936	KC890888	KC890960	KC890912	KC890984
<i>Leonurus glaucescens</i> (XJBI)	KC890938	KC890890	KC890962	KC890914	KC890986
<i>Panzeria alashanica</i> (XJBI)	KC890937	KC890889	KC890961	KC890913	KC890985

Table 3Prime sequences of cpDNA *trnL-trnF*, *rps16*, *psbA-trnH*, *psbK-psbI* and *trnS-trnG*.

Prime	Sequence	Resource
<i>trnL-trnF</i>	F-(5'-3')	CGA AAT CGG TAG ACG CTA CG
	R-(5'-3')	ATT TGA ACT GGT GAC ACG AG
<i>rps16</i>	F-(5'-3')	AAA CGA TGT GGT ARA AAG CAA C
	R-(5'-3')	AAC ATC WAT TGC AAS GAT TCG ATA
<i>psbK-psbI</i>	F-(5'-3')	TTA GCC TTT GIT TGG CAA G
	R-(5'-3')	AGA GTT TGA GAG TAA GCA T
<i>psbA-trnH</i>	F-(5'-3')	GTT ATG CAT GAA CGT AAT GCT C
	R-(5'-3')	CGC GCA TGG TGG ATT CAC AAT CC
<i>trnS-trnG</i>	F-(5'-3')	AAC TCG TAC AAC GGA TTA GCA ATC
	R-(5'-3')	GAA TCG AAC CCG CAT CGT TAG

with no upper limit for the number of trees held in memory; support values for all nodes (on a 50% majority rule bootstrap tree) were calculated with the same settings as above for 1000 replicates; 10 searches with random taxon additions were conducted for each replicate, and the strict consensus tree of all shortest trees was saved. For BI analysis, the best-fitting model for cpDNA was chosen by Modeltest 3.7 (Posada and Crandall, 1998). We used the default of one cold and three heated Markov chain Monte Carlo chains (MCMC), starting from random initial trees, and chains were run for 6,000,000 generations while sampling every 200th. The default options in MrBayes were used for chain heating and mixing. We discarded a burn-in of the first 2,000,000 generations and used 20,000 trees from the posterior distribution to obtain a majority rule consensus tree.

2.2.3. Divergence time estimation

Bayesian relaxed method was implemented in BEAST 1.5.4 (Drummond and Rambaut, 2007) to explore divergence time. BEAUti interface was used to create an input file for BEAST, in which a general time reversible (GTR) nucleotide substitution model with gamma + invariant sites was applied using uncorrelated lognormal model. Ten million generations of the MCMC chains were run, sampling every 1000th. After discarding the first 1000 trees as burn in, the samples were summarized in the maximum clade credibility tree using TreeAnnotator 1.4.8 (Drummond and Rambaut, 2007), with the posterior probability limit setting to 0.5, and summarizing mean node heights. Final trees were evaluated and edited in FigTree 1.3.1 (Rambaut, 2007). Statistical support for the clades was determined by assessing the Bayesian posterior probabilities. In the estimation of divergence time, we used published nucleotide substitution rates of 0.28% M yr⁻¹, in accordance with the substitution rate of cpDNA spacers in previous study (Meng and Zhang, 2011, 2013). Because there are neither *Lagochilus* fossil records nor specific substitution rates available by which to calibrate a molecular clock, these estimates are provisional and should be interpreted with caution, which provide approximations that allow us to hypothesize possible scenarios under the lineages have diverged.

2.2.4. Biogeographic analyses

Although many biogeographic analyses have used dispersal-vicariance analysis (DIVA) (Ronquist, 1997) to infer ancestral distributions (Baird et al., 2010; Harris et al., 2013), DIVA requires fully bifurcated trees. Because our Bayesian trees were not fully resolved, we used RASP 1.1 that implements the S-DIVA (statistical dispersal-vicariance analysis) method but allows uncertainties in phylogenetic trees (Yu et al., 2010).

All *L. ilicifolius* species were coded as the current distribution according to the phylogenetic tree. Based on the extant distributions of the species in the Bayesian tree, we defined three areas to assess the historical biogeography of the *L. ilicifolius* clade, Mongolia to Novosibirsk (A), St. Petersburg (previously Leningrad) (B), and North China (C). We used Bayesian trees from the phylogenetic analyses (10,000 trees, excluding the remote outgroup of *Leonurus*) as input for S-DIVA. The condensed tree was computed using these 9000 trees (excluding the burn-in 1000 trees); the 'maxarea' was set to three and state frequencies were estimated.

3. Results

3.1. Phylogenetic relationships

Five cpDNA region matrix exploited two different analyses, the maximum parsimonious (MP) tree and Bayesian inference (BI) tree yielded essentially identical topologies, but only the Bayesian analysis tree with parsimony bootstrap (BP) values in

the MP analysis and Bayesian posterior probability (PP) values were presented here for higher resolution (Fig. 2). *L. ilicifolius* was strongly supported as monophyletic (PP = 1.00, BP = 100%) with two well-supported groups on the phylogenetic tree: Clade A, including taxa in Mongolia and Russia (PP = 1.00, BP = 94%); Clade B with the taxa in Chinese arid lands (PP = 1.00, BP = 98%) (Fig. 2). In the analysis, Clade A supported two subclades: A-1 (PP = 1.00, BP = 93%) and A-2 (PP = 1.00, BP = 85%), which represented taxa from Mongolia to Novosibirsk, and St. Petersburg, respectively.

3.2. Temporal and spatial diversification

The chronogram divergence times from the dating analyses at the intraspecific level (Fig. 3) showed that initial separation of *L. ilicifolius* clades was in late Pliocene. Within Clade A, species of Mongolia–Russia, the divergence time of the Mongolian–Novosibirsk species (A-1) was in the Pleistocene at ca. 1.38 Ma; that of the St. Petersburg species (A-2) was at ca. 1.72 Ma; diversification of Clade B occurred at ca. 1.80 Ma, respectively. The most probable ancestral area and highest posterior probability values of ancestral area reconstructions were inferred from statistical dispersal–vicariance analysis (S-DIVA), indicating that the most recent common ancestor had likely already spread in China and the western regions of Mongolia–Russia (Fig. 3) at the time of divergence of the main clades.

4. Discussion

4.1. Diversification of *L. ilicifolius*

Our results, based on the combined analyses of five plastid regions, support the monophyly of intraspecific phylogeny in *L. ilicifolius* (Fig. 2), which comprises two clades: Clade A from the Mongolian taxa extending to Novosibirsk and St. Petersburg; Clade B from Northern China. The previous study was hampered by a lack of taxa from Russia and Mongolia, although the results suggested that the diversification among *L. ilicifolius* lineages in Northern China occurred during the middle Pleistocene (Meng and Zhang, 2011). When we re-examined taxa representing the additional distribution areas, there still seemed to have been rapid diversification in the Pleistocene (Fig. 3).

During Pleistocene and Holocene, environments in the Mongolian Altai and western Mongolia varied between arid and semi-arid conditions due to climatic fluctuations (Grunert and Lehmkuhl, 2004). The extent of Pleistocene ice in the Russian

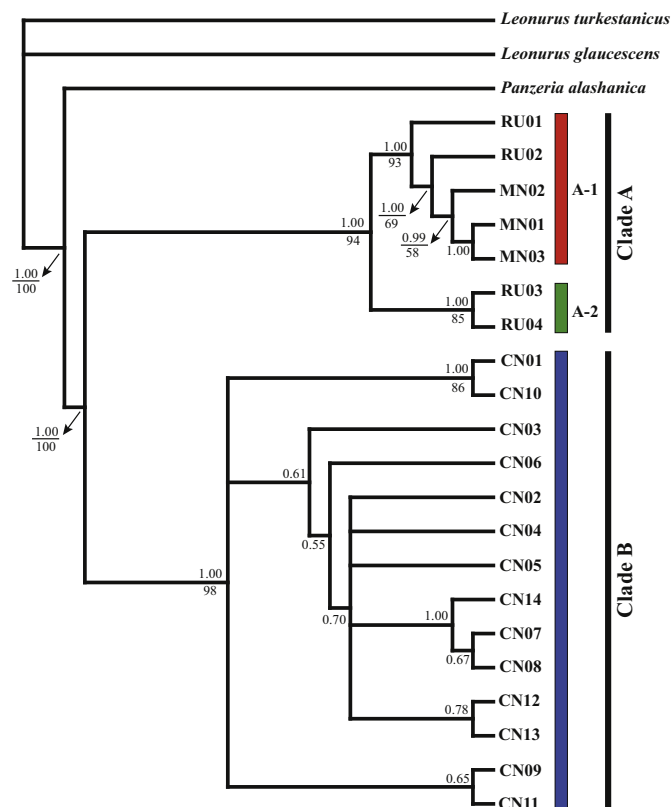


Fig. 2. The phylogenetic relationships among extant *Lagochilus ilicifolius* species based on five cpDNA spacer sequences. Numbers above branches are support values from Bayesian inference and bootstrap resampling.

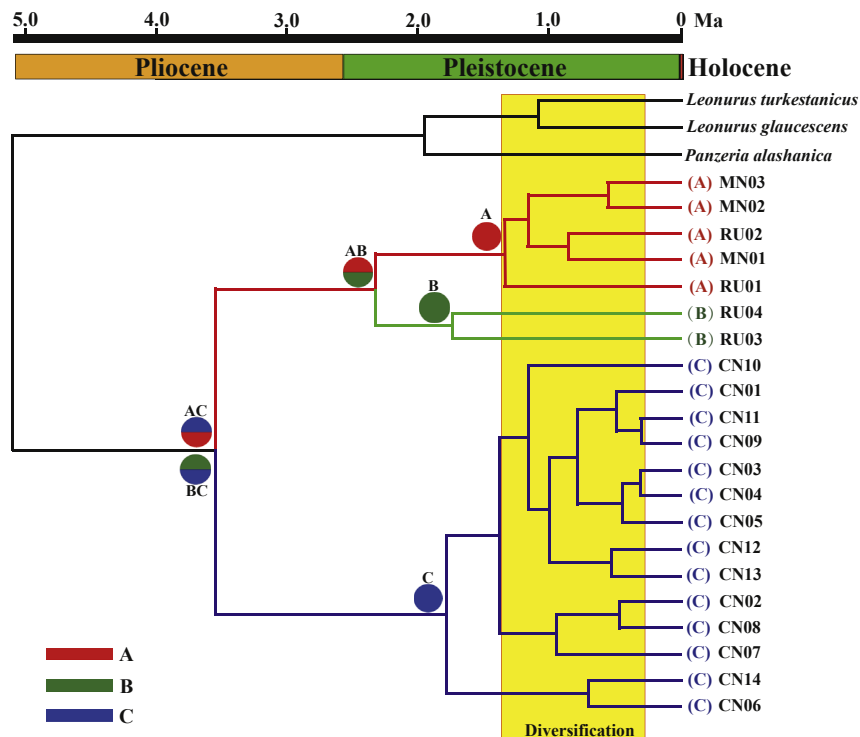


Fig. 3. Time tree of *Lagochilus ilicifolius*. The tree topology, derived from BEAST analyses of all samples. Branch colors reflect biogeographic designations (for species at tips) and ancestral area reconstruction. The upper circles indicate the results of S-DIVA. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Altai is still under debate, however, Pleistocene glaciers in the eastern Altai and especially in the northern part of the Mongolian Altai has been proven. Glaciers were restricted to several isolated mountain systems (Lehmkuhl et al., 2004). The more limited extent of present and Pleistocene glaciers in the eastern part of the Russian and Mongolian Altai was the result of decreasing precipitation from west to east. Moreover, Pleistocene climatic in Siberia during cold-arid stages was recorded by loess deposition in a uniform palaeoclimatic pattern (Chlachula, 2003). Quaternary aridification, expansion of the Arctic ice-sheet driven by ongoing global cooling; successive uplifts in the Himalayan–Tibetan complex enhanced the summer monsoon and brought wetter climates to India and Southeast Asia. But the moisture could not reach the Asian interior, because the uplifted Himalayan–Tibetan topography blocked airflow from the south (Guo et al., 2002). Meanwhile, uplifts in the QTP strengthened the winter monsoon, causing additional mid-latitude to high-latitude Asian interior cooling (An et al., 2001; Rea et al., 1998). As a result, the interior of Asia, including Northern China, Mongolia and Siberia, became increasingly dry and cold during the Pleistocene, which strongly affecting the biota within these regions. As a drought tolerant plant, *L. ilicifolius* might have been driven to rapid diversification in the mountains or at the edges of deserts as an adaptation to the environment.

4.2. Vicariance

Molecular dating and ancestral area reconstruction indicate that *L. ilicifolius* originated from the interior of Eurasia (Fig. 3), which might appear to be in line with the hypothesis that *Lagochilus* originated and differentiated along the coasts of Tethys Ocean (Wu et al., 2003). In addition, aridification in the Pleistocene did promote the diversification and vicariance of *L. ilicifolius* (Meng and Zhang, 2011, 2013). The common ancestors of the *L. ilicifolius* clades had likely spread from Mongolia–Russia into adjacent western China by the start of the Mid-Pliocene. The lineages rapidly diversified through the Pleistocene (Fig. 3) as the climate of Eurasia transitioned to a dry-cold pattern.

Vicariance had a significant role in the diversification of *L. ilicifolius* either. Dramatic crustal deformation induced by the Indo-Asian collision contributed to the intensifying drought in the interior of Eurasia. Alongside climatic shift, massive mountains and deeply carved valleys appeared and the deserts expanded, which acted as barriers to distribution and resulted in differentiation that subsequently gave rise to three subclades. The effects of climate-driven vicariance are evident in other arid zone taxa, such as the cryptic vicariance in the historical assembly of a Baja California Peninsular Desert biota (Garrick et al., 2009; Riddle et al., 2000). But few studies have been carried out on the vicariance of species adapted to open areas (arid zones) interspersed in the interior of Eurasia. For *L. ilicifolius* in Northern China, the physiographical heterogeneity of the Helan Mountains area may have given rise to geographical and probably ecological isolation (Meng and Zhang, 2011).

Phylogenetic relationships indicate that Clade A and Clade B are two distinctive lineages (Fig. 2). It is interesting that the clades have a genetic break between the geographic regions of China and Mongolia–Russia. DIVA analysis demonstrates that *L. ilicifolius* was likely to have originated from an arid adapted ancestor and then has been restricted to a more narrow ecosystem niche in response to drastic environment changes (extremely dry and cold) in the interior of Eurasia during the Pliocene (Fig. 3). In China, the distribution areas of *L. ilicifolius* were under pressure by mountains and deserts: the north-south Helan Mts. and the west-east Yinshan Mts.; at the north of the distribution areas are the Ulanbuhe and Kubuqi Deserts, and on the west and east are the Tengger Desert and Mu Us Sandy Land, respectively. These deserts can be considered as geographic barriers, but the Loess Plateau appears to have provided an ecological corridor for northward migration (Meng and Zhang, 2011). When all distribution areas of *L. ilicifolius* are taken into account, our results propose a “line” along the edges of the deserts, linking the Helan and Yinshan Mts., as a biogeographic barrier across this region. That is to say, there is a genetic break between China and the rest of arid Eurasia. In particular, the Yinshan Mts. might be the geographic barrier that resulted in the development of genetic isolation between China lineage and the Russia–Mongolia lineage. As a biogeographic boundary, Wallace’s line was proposed on the basis of numerous taxa, even some that are marine (Barber et al., 2000). Here, although we recommend the “line”, limitations in our ability to estimate geographic barriers more accurately confining the robustness of our inferences; information from additional taxa should reveal whether these findings apply to other desert plants.

4.3. Implications of conservation in Northern China

L. ilicifolius is mainly restricted to arid environments, and these regions could be defined as the exclusive biodiversity of a region. The area inhabited by *L. ilicifolius* in Northern China is considered as a diversification center contains many endemic genera (Wu et al., 2010; Zhao and Zhu, 2003). An area of endemism contains taxa found nowhere else and could be catalogued as irreplaceable and of high priority for conservation. However, there is still no evidence to prove that the area is a distinctive region. To the best of our knowledge, the spatial partitioning of genetic diversity in *L. ilicifolius* and integrated previous view points which offered information for conservation practices. Our results reveal that China clade is a distinctive evolutionary lineage (Figs. 2 and 3). Moreover, the area was simulated using distribution models, suggesting that the Alxa of Inner Mongolia is the most noticeable endemic area (Ma et al., 2012).

Arid areas of Northern China are vast, especially since the sand deserts are developing and expanding further by current anthropogenic processes (Zhong and Qu, 2003). Conservation of the genetic resources of desert plants is crucial to combat desertification, to prevent further degradation of the fragile ecosystems in arid and semi-arid regions, and to sustain biodiversity in the deserts (Meng et al., 2014). According to the results, *L. ilicifolius* in China constitutes a separate evolutionary clade, and this area is confirmed as a diversification center (Meng and Zhang, 2011). So, strategies applicable to this area should be adopted for these populations for in situ and ex situ conservation of genetic diversity. Finally, the researched taxa are rare in this region, which limits conservational strategies and management decisions. Therefore, samples from different lineages in various habitats, such as from different mountain slopes and sandy deserts, should be taken into account for conservation, because desertification in these regions is increasing and the genetic diversity of desert plants is constantly diminishing.

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