



Genetic and ecological differentiation of *Caragana jubata* (Pall.) Poir.

Elena V. Hantemirova^{1*}, Maria G. Horeva² & Varvara A. Bessonova¹

Elena V. Hantemirova^{1*}
e-mail: hantemirova@ipae.uran.ru

Maria G. Horeva²
e-mail: mkhoreva@ibpn.ru

Varvara A. Bessonova¹
e-mail: bessonova1varechka@gmail.com

¹ Institute of Plant and Animal Ecology,
Ural Division RAS, Ekaterinburg, Russia

² Institute of Biological Problems of the
North FEB RAS, Magadan, Russia

* corresponding author

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ABSTRACT

Caragana jubata (Pall.) Poir. is a cold-tolerant mountain shrub and important medicinal species of Fabaceae family with a disjunctive pan-Asian range. Sequences of the *atpH-atpI* and *trnL-trnF* intergenic spacers of chloroplast DNA and of the internal transcribed spacer region, ITS-1, of the nuclear ribosomal DNA were used to investigate the intraspecific evolution and phylogeography of this species. Eleven cpDNA haplotypes were identified. The population subdivision is very high ($G_{ST} = 0.743$, $N_{ST} = 0.943$), suggesting a distinct phylogeographic structure ($N_{ST} > G_{ST}$, $P < 0.001$). Phylogenetic analyses of the eleven haplotypes were clustered into four clades, consistent with their respective distributions in four separate regions: Tibet, Tien Shan, Buryatia and Magadan Region. This pronounced phylogeographic gap is also confirmed by nrITS data, however, there is some mixture of genotypes in the Magadan and Buryat populations. Genetic evidence suggests that the center of origin of *C. jubata* was Tibet and adjacent mountain systems, from where the species spread westwards along continuous mountain chains towards the Tien Shan and northeastwards to the coast of the Sea of Okhotsk and further northwards to Arctic. Divergence of Tien Shan populations of *C. jubata* may have been caused by climate change in the Pleistocene associated with increased aridity and expansion of deserts, while subsequent climate-induced cycles of range contraction/expansion increased geographic isolation and fragmentation of more northern populations. Marginal northern populations now occupy sites with the most favourable environmental conditions. Special attention is paid to the Okhotsk populations of this species preserved in Magadan Region.

Keywords: *Caragana jubata*, disjunctions, nrITS, cpDNA, Magadan, Tibet, Tien Shan

РЕЗЮМЕ

Хантемирова Е.В., Хорева М.Г., Бессонова В.А. Эколого-генетическая дифференциация *Caragana jubata* (Pall.) Poir. *Caragana jubata* (Pall.) Poir. – холодостойкий горный кустарник и важный лекарственный вид семейства Fabaceae с дизъюнктивным паназиатским ареалом. Последовательности межгенных спейсеров *atpH-atpI* и *trnL-trnF* хлоропластной ДНК и внутренней транскрибируемой спейсерной области ITS-1 ядерной рибосомной ДНК были использованы для изучения внутривидовой эволюции и филогеографии этого вида. У *C. jubata* выявлено 11 гаплотипов хпДНК. Популяционная дифференциация высокая ($G_{ST} = 0.743$, $N_{ST} = 0.943$), что свидетельствует об отчетливой филогеографической структуре ($N_{ST} > G_{ST}$, $P < 0.001$). В результате филогенетического анализа 11 гаплотипов были сгруппированы в четыре клады, что соответствует их распространению в четырех отдельных регионах: Тибете, Тянь-Шане, Бурятии и Магаданской области. На этот выраженный филогеографический разрыв указывают и данные nrITS, однако в магаданской и бурятской популяциях наблюдается некоторое смешение генотипов. Генетические данные свидетельствуют о том, что центром происхождения *C. jubata* были Тибет и прилегающие горные системы, откуда вид распространился на запад вдоль непрерывных горных цепей в сторону Тянь-Шаня и на северо-восток к побережью Охотского моря и далее на север в Арктику. Дивергенция тянь-шаньских популяций *C. jubata* могла быть вызвана изменением климата в плейстоцене, связанным с увеличением засушливости и расширением пустынь, в то время как последующие климатические циклы сокращения/расширения ареала усилили географическую изоляцию и фрагментацию более северных популяций. В настоящее время маргинальные северные популяции занимают места с наиболее благоприятными экологическими условиями. Особое внимание уделяется охотским популяциям этого вида, сохранившимся в Магаданской области.

Ключевые слова: *Caragana jubata*, дизъюнкции, nrITS, cpDNA, Магадан, Тибет, Тянь-Шань

Quaternary climate fluctuations led to range shifts, range expansion, range contraction and/or range fragmentation of many plant and animal species and has profoundly shaped present species distributions and spatial genetic structure. Species with disjunctive ranges are of particular interest in a phylogeographic studies. Disjunctions can occur as a result of some catastrophic climate change, ice sheet formation,

sea transgressions, land uplift and subsidence, desertification, volcanic activity. Altogether a disjunct distribution of plants could either be the result of long-distance dispersal from a source area into a suitable new area or the consequence of disruption of the previously continuous distribution range.

The object of our study is a species of the Fabaceae family with an extensive but disjunctive pan-Asian range. It

is *Caragana jubata* (Pall.) Poir., a cold-tolerant shrub capable of surviving in extreme conditions of cold deserts in high mountains. These properties are associated with the predominance of genes encoding proteins involved in plant growth and development at low temperature (Bhardwaj et al. 2013). *Caragana jubata* has long been used in traditional Tibetan medicine. The center of origin of this species is considered to be the eastern part of the Tibetan Plateau, where there is still a high density of finds of this mountain species. In addition, the species is widespread in the north-eastern Himalayas, central and southern Tien Shan. In Siberia, the range of the species extends from Altai to the Aldan Plateau and in Khangai in the south, where it occupies mountain taiga and mountain tundra habitats (Fig. 1A). Farther north, the species appears near the Arctic, at the mouth of the Lena River near the Kharaulakh Range. The easternmost locations of *C. jubata* are in the area of Magadan, on the coast of the Sea of Okhotsk, where *C. jubata* is considered as a relict species (Churiulina et al. 2020).

The striking diversification within the genus *Caragana*, giving rise to some 100 species of arid and cold habitats, is thought to have been resulted from the Qinghai-Tibetan Plateau uplift (QTP) and the drying out of interior Asia. As a result of the QTP uplift, the climate of this area became more arid and colder, which probably led to the origin and divergence of groups of organisms adapted to cold and drought. The clade of the genus *Caragana* comprising sections *Bracteolatae* and *Jubatae* pro parte, inferred to have originated at 7.63 Ma in the QTP, likely in response to cooling climate (Zhang et al. 2009, Zhang & Fritsch 2010).

The population and genetic structure of species on the Tibetan Plateau, one of the major centers of biodiversity in the Northern Hemisphere (Wen et al. 2014) and one of the most topographically complex regions on Earth (Zhang et al. 2002) is currently actively explored. It turned out that different plant species of this region show contrasting patterns of responses to Quaternary climatic fluctuations. High diversity and strong differentiation of populations from different parts of the plateau were revealed. Many widespread temperate plant groups of the Northern Hemisphere, which have the highest taxonomic diversity in mountainous areas of Asia, originated in the QTP and adjacent highlands before migrating to other regions of the Northern Hemisphere and undergoing divergence, e.g., *Hippophaë rhamnoides* L. (Jia et al. 2012) and *Rhodiola rosea* L. (Zhang et al. 2014).

Phylogeographic studies have provided evidence for several routes of such migrations. From the highlands of the QTP and adjacent areas along the so-called Central Asian corridor, some species migrated towards the European Alpine System (EAS, a biogeographic region covering the Pyrenees, Alps, Carpathians and Northern Balkans). From there, the plants could spread to the Arctic regions (Kadereit et al. 2008). For some species, such as *Saxifraga oppositifolia* L., expansion from the QTP towards northern Siberia could also have occurred, followed by migration from here to both the east and west (Abbott et al. 2000). Northern glacial survival and extensive post-glacial migration in the Arctic region have also been reported in *Vaccinium uliginosum* L. (Alsos et al. 2005) and *Juniperus communis* L. (Hantemirova et al. 2017). Suggest that the mountain species *Rhodiola rosea* had at least

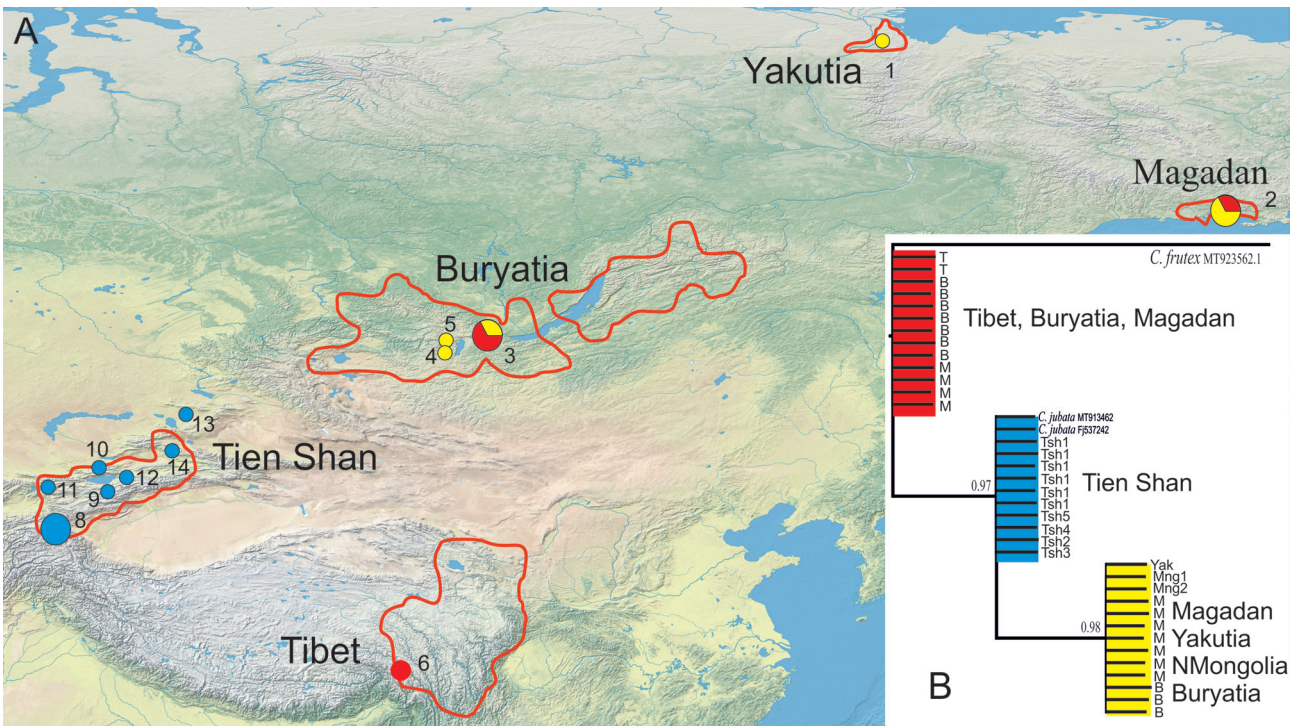


Figure 1 (A). Localization of the populations and individual samples of *Caragana jubata*, the ranges are given according to Churyulina et al. (2020). Distribution of genotypes (ITS-1). The size of the circles is related to the number of samples. Small circles correspond to single samples. The numbers of populations and samples correspond to those given in Table 1. The color of the sectors corresponds to the color of the genotypes in Fig. 1B. (B) Phylogenetic tree constructed in MrBayes v. 3.2.3 for all *C. jubata* samples. The numbers above the horizontal line represent the posterior probability (PP) of the value. The color indicates the three different genotypes. Abbreviations correspond to the population names in Table 1.

two different migration paths that caused the origin of two evolutionary lineages. The species migrated from QTP westwards along the Ural Mountains and eastwards through the mountain ranges of Eastern Siberia (Kozyrenko et al. 2011).

It should be noted that, in contrast to *C. jubata*, all of the above species and many other arcto-alpine species, such as *Dryas octopetala*, have extensive modern ranges throughout Eurasia and often in North America as well. It is known that Quaternary climate fluctuations have repeatedly led to the spreading of extensive periglacial landscapes with a high proportion of xerophytic and arctoalpine vegetation (Zhang & Xiang 2016). It is suggested that the ranges of these species may have expanded in the late Pliocene–early Pleistocene, when the modern tundra ecosystem was formed in response to climate cooling (Hultén 1937).

What did not allow the *C. jubata* to spread as widely and what historical reasons determined the boundaries of its modern range? Did the species' weakly expand ability or a low adaptability to new habitats in response to climatic fluctuations? And why, having extended so far northwards, does this species now occupy only local habitats in the Lena River delta and the Magadan area? Whether there was a decrease of diversity during migration to the north or did the species evolve at high latitudes and form new haplotypes? The decrease of diversity in the direction from the QTP to the Altai Mountains has been recorded, for example, in *Pedicularis longiflora* Rudolph. (Yang et al. 2008), a mountain species with an Asian range similar to *C. jubata*, but limited to the north by Buryatia and Altai. Investigation the genetic structure of *C. jubata* populations in the current range using different types of markers may elucidate of these problems.

This study provides a first attempt to disentangle the biogeographic history of *C. jubata* using nuclear and chloroplast markers in order to determinate patterns of current disjunctive distribution of this species and compare marginal northern populations with populations from the species' center of origin.

MATERIALS AND METHODS

Samples of *C. jubata* were carried out in the three geographical locations in Asian Russia: in the Tien Shan Mountains (Kyrgyzstan, Osh Region), Tunka District of Burya-

tia and Magadan Region (Cape Kharbiz). Leaf samples of this species were collected from 12–24 plants in each population and dried in silica gel (Table 1). Material from herbarium collections of *C. jubata* was also used: 5 specimens from the herbarium of Moscow State University (Seregin 2024), numbers are given in Table 1, and two specimens from southeast Tibet collected by A.N. Berkutenko. Total DNA was isolated from all samples using the CTAB method (Devey et al. 1996). DNA from these samples was used as a matrix for PCR amplification of the internal transcribed spacer ITS-1, a non-coding region of the gene that separates the 18S rRNA gene from the 5.8S rRNA gene (White et al. 1990). These sequences were compared with *C. jubata* samples (MT913462 and FJ537242.1.) from the NCBI. *Caragana frutex* (L.) C. Koch (MT923562.1) was used as an external group. A total of 36 accessions from 10 geographical locations were studied using ITS-1 marker.

The *trnL-trnF* region (internal primers *trn e* and *trn f*) (Taberlet et al. 1991) and *atpH-atpI* (Grivet et al. 2001) were used to study the variability cpDNA of *C. jubata*. The *atpH-atpI* region was not successfully amplified in all herbarium samples (in Table 1, these samples are marked with an asterisk). This is, for example, for two Tibetan samples collected by A.N. Berkutenko. Therefore, as Tibetan material in this instance the complete genome of *C. jubata* from Qinghai (GWHBJYN00000000) from the NGDC database was used (Yuan et al. 2022). Sixty-five samples were analyzed for the first cpDNA region and 30 samples for the second region. *Caragana tangutica* Maxim ex Kom. (OR49170) was used as outgroup. The primer sequences were synthesized in Syntol Company (Moscow, Russia). Sequencing of PCR products was performed on a genetic analyzer NANOFOR-05 (Syntol, Russia).

The obtained nucleotide sequences were aligned using the ClustalW algorithm in the BioEdit (Hall 1999), followed by manual evaluation of the read quality. Indels were counted as missing data for all samples. All nucleotide sequences were deposited in the NCBI GenBank database (numbers PP319204 to PP319214 and PP355546 to PP355548).

Several approaches were used to study the population structure and phylogenetic analyses of *C. jubata*. Using the software package Arlequin v. 3.5.1.2 (Excoffier & Lischer 2011), we determined the level of differentiation and the

Table 1. Populations of *Caragana jubata* and their genetic diversity measures (cpDNA)

No.	Locality		Lat., N	Lon., E	Alt. (m)	<i>N</i>	Haplotype	<i>He</i>	π
1*	Yakutia, MSU Herbarium MW0102975, Lena River	Yak	70°25'	125°43'	64	1			
2	Magadan, Cape Harbiz	M	59°30'	151°31'	348	16	h5(2), h6(1), h7(1), h8(12)	0.228	0.0004
3	Buryatia Tunkinsky district, Moygoty	B	51°38'	101°23'	1011	24	h1(1), h2(5), h3(19), h4(1)	0.256	0.0016
4*	Northern Mongolia, MSU herbarium MW0182778	Mng1	50°47'	99°39'	2000	1			
5*	Northern Mongolia, MSU herbarium MW0182780	Mng2	50°36'	99°55'	2000	1			
6*	China, southeast Tibet	T	30°44'	95°12'	4100	2			
7	China, Tibet, Qinghai Province GWHBJYN00000000		32°37'	97°11'	4372	1	h11	0	0
8	Tien Shan, Kyrgyzstan, Osh	Tsh1	39°38'	73°49'	3070	12	h9(12)	0	0
9	Tien Shan, Kyrgyzstan, Atbashi ridge	Tsh2	41°48'	77°42'	3866	1	h9	0	0
10	Tien Shan, Kyrgyzstan, Djetimbel ridge	Tsh3	42°48'	76°47'	3561	1	h10	0	0
11	Tien Shan, Kyrgyzstan, MSU herbarium MW0843866	Tsh4	41°60'	75°20'	2600	1	h9	0	0
12	Tien Shan, Kyrgyzstan, MSU herbarium MW0843860	Tsh5	42°33'	78°55'	3117	1	h10	0	0
13*	China, Xinjiang, Tacheng MT913462		46°44'	82°59'	555	1			
14*	China, Xinjiang, Zhaosu, FJ537242.1		43°10'	81°07'	1927	1			

N = number of specimen, *He* = haplotype diversity, π = nucleotide diversity. * – samples used for ITS analysis only.

distribution of genetic variation among haplotype groups (analysis of molecular variation, AMOVA). The F_{ST} value was obtained to measure the molecular divergence between populations and population groups. Molecular diversity indices, haplotype diversity (H_e) and nucleotide diversity (π), were calculated using the same program. Phylogenetic relationships between ITS genotypes and cpDNA haplotypes with *C. frutex* and *C. tangutica* as outgroups were reconstructed using NETWORK ver. 4.6.1.2 (Median Join method, MJ) (Bandelt et al. 1999) and MrBayers ver. 3.2.3. (Ronquist & Huelsenbeck 2003). Coding of mutations was 0 and 1. Each insertion or deletion was coded, regardless of size, as a single mutational event. Phylogeographic structure test was performed with the PERMUT v1.0 program (Pons & Petit 1996). Using this program, two measures of population differentiation (G_{ST} , N_{ST}) were obtained at 1000 permutations and then compared with each other.

RESULTS

The ITS-1 sequences nrDNA variation

The consensus ITS1–5.8S sequence of the 36 *C. jubata* samples had a length of 248 bp. ITS-1 exhibited two parsimony informative nucleotide substitutions – one nucleotide substitution at position 65 (replacing T with C) and a nucleotide substitution at position 221 (replacing A with T), corresponding to three haplotypes. ITS tree generated exhibited largely resolved topology with high bootstrap support (Fig. 1B). Three clades (I, II, and III) were identified. Clade I included samples *C. jubata* distributed in Buryatia and Tibet and few from Magadan, clade II comprised samples from Magadan, but it also contains a small number of Buryat samples. In addition, this cluster included a sample from Yakutia (Lena River delta) and two samples Mng1 and Mng2 from Northern Mongolia. The third clade consists totally from the Tien Shan samples (Fig. 1A, 1B). These are samples from different regions of Kyrgyzstan, as well as two samples from the gene bank (China, Xinjiang Uyghur AR).

Chloroplast variation and haplotype distribution

The aligned sequences of the trn e and trn f site of the *trnL-trnF* and *atpH-atpI* region were 402 bp and 1321 bp long, respectively, for a total length of the combined alignments of 1723 bp. More variable sites were found in the *atpH-atpI* region, where seven indels and five single nucleotide polymorphisms (SNPs) occurred. In the *trnL-trnF* region, only one indel and one single nucleotide substitution mutations were found. Tibetan sample № 7 from Qinghai Province has a large deletion of 500 bp in the *atpH-atpI* region. Two SNPs in the *atpH-atpI* chloroplast region were detected in all Magadan samples, which are not found in any other population, including samples from the GenBank. Same mutations are also found only in *Caragana kozlovii* Kom. (KX349219.1), an endemic of the southeastern part of the Tibetan Plateau. In combination, these polymorphisms defined 11 haplotypes (Fig. 2 A, B; Table 1). Haplotype diversity (h) ranged from 0.000 to 0.25 and nucleotide diversity (π) ranged from 0.000 to 0.0016 with a maximum in the populations from Buryatia. The level of N_{ST}

(0.943) was significantly higher than G_{ST} (0.743; $P < 0.001$), indicating significant phylogeographic structure across the species' range. Hierarchical analysis of molecular variance (AMOVA) showed that $F_{ST} = 86\%$.

We used MrBayers ver. 3.2.3 to establish the maximum parsimonious tree of haplotypes with one sample of *C. tangutica* as an outgroup. A single tree was obtained (Fig. 2A, B) and four clades were identified. Clade I included haplotype 11 fixed in a single sample from Tibet, clade II comprised four haplotypes (h1, h2, h3, h4) distributed in Buryatia, clade III consisted of a four haplotypes (h5, h6, h7, h8) from Magadan region and clade IV consisted of a two haplotypes (h9 and h10) in Tien-Shan. The minimum spanning network analyses also clustered all haplotypes into four groups with the same topological relationships (Fig. 2C). H3 haplotype is most common in the Buryatia populations, h8 in the Magadan populations, h9 in the Tien-Shan populations. No haplotype was shared between these regional distributions.

DISCUSSION

We propose that *C. jubata* populations from the current disjunctive ranges are remnants of a past (Tertiary) widespread species. The center of origin of the species was most likely the Tibetan plateau and neighbouring mountain systems, which is confirmed by the results of cpDNA variability analysis, where the Tibetan haplotype is most strongly differentiated and ancestral (Fig. 2). The strong differentiation of *C. jubata* revealed in this study, can be explained using two hypotheses: 1) closely related haplotypes were formed locally already after dispersal from QTP, 2) dispersal to the mountains of Central Asia (Tien Shan) and North-East Eurasia occurred from different parts of QTP along the mountain chains connecting these regions.

The second hypothesis is supported by the fact that both types of markers confirm the isolation of Tien-Shan populations of *C. jubata*. Like to some other species, *C. jubata* apparently had a western migration route from the QTR, which allowed it to reach the Tien Shan Mountains and spread further northwards along the Altai-Sayan mountainous country. Isolation of *C. jubata* populations in the Tien Shan mountains could have occurred due to the formation of a wide arid belt in northern China. Deserts located deep in Eurasia and controlled by continental dry air masses expanded significantly during the Pleistocene cooling (Meng et al. 2015). Phylogenetic studies of many desert species in northwest China revealed the barrier effect of desert on species distribution and differentiation (Gao et al. 2014). For example, cpDNA analysis revealed three highly divergent geographic clades in *Juniperus sabina* L. in China. The authors suggest that this species probably occurred widely across China before the Pliocene, but was gradually divided into three separate regions by the developing deserts (Guo et al. 2010).

The second migratory pathway of *C. jubata* from QTP may have been in a northeastern direction. The species may have dispersed from the south-eastern part of the QTP, which is a refugium for many species, through the Great Khingan to the Stanovoi Range and the coast of the Sea

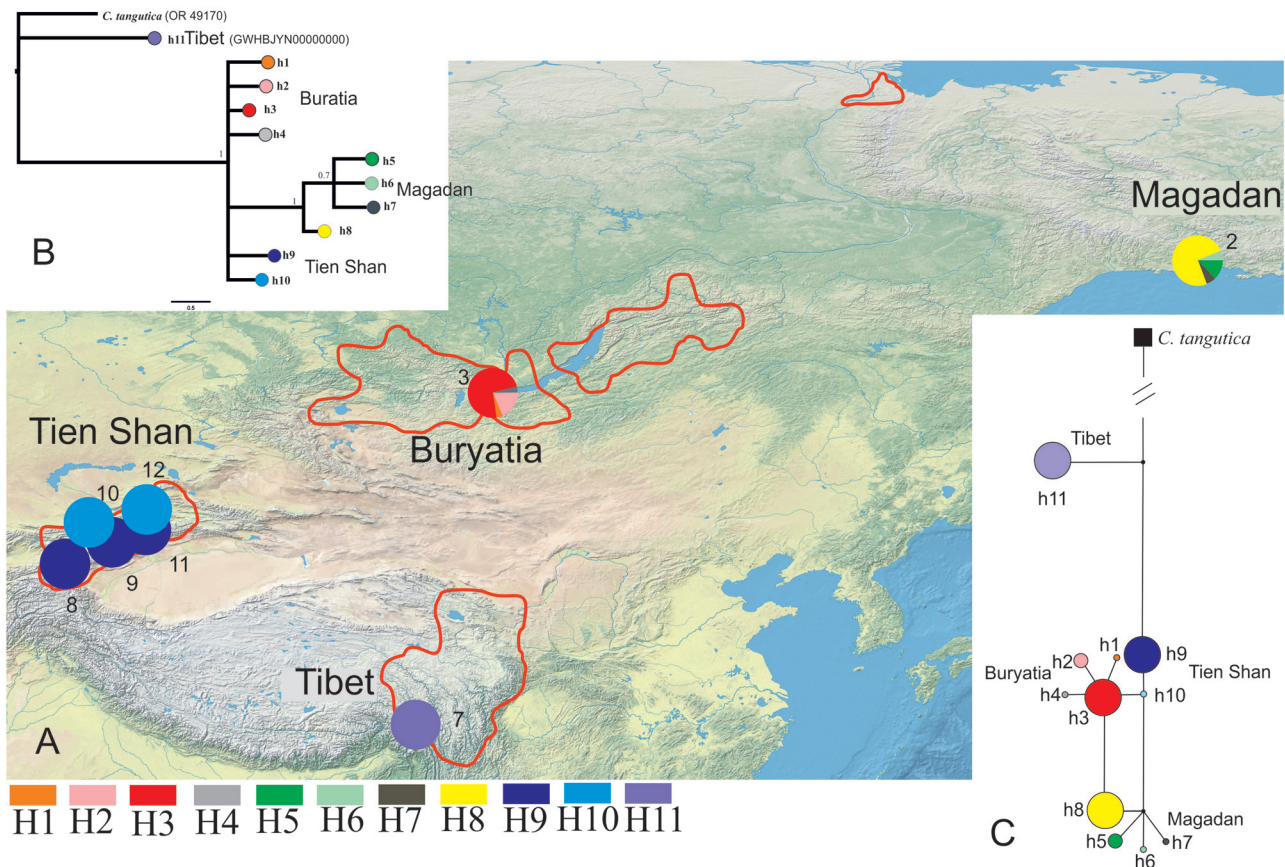


Figure 2 (A) Localization of the populations of *Caragana jubata* and distribution of cpDNA haplotypes (h1-h11). The numbers of the populations correspond to those given in Table 1. (B) Phylogenetic tree constructed in MrBayes v. 3.2.3 based on the results of cpDNA variability. The numbers above the horizontal line represent the posterior probability (PP) of the value. (C) cpDNA haplotype network constructed using the MJ method. The numbers indicate haplotypes. The size of the circle depends on the frequency of occurrence of the haplotype

of Okhotsk and further north to the Arctic latitudes and the west coast of North America (Alaska, Yukon). The repeatedly noted affinity of the Tibetan-Himalayan flora with the floras of Siberia and the Far East is explained by the migration of plant species along continuous mountain ranges connecting these geographical areas. A striking example of such floristic connections is *C. jubata* itself, as well as some species and groups of closely related species, in the distribution of which significant disjunctions are observed. For example, *Potentilla biflora* D.F.K. Schltdl., which has an extensive Central Asian, passing into the Siberian part of the range, appears in Okhotia on the Dzhugdzhur ridge and further to the east with a large gap it is found in Beringia (Chukotka, Alaska) (Berkutenko 2013). Three species have been described in the ancient calcephalous genus *Megadenia*: *M. pygmaea* Maxim. (northeast Tibet), *M. bardumovii* Popov (Tunkinskaya valley and Eastern Sayan), *M. speluncorum* Vorob., Vorosch. et Gorovoj (Primorye). The identity of these species by morphological features was proved by A.N. Berkutenko (1998), but then the differences in the anatomical structure of their petioles were still revealed (Gorovoy et al. 2011). Differentiation of *Megadenia* into three vicariant species was confirmed using chloroplast markers, but the nuclear and mitochondrial fragments were found to be similar in all samples (Artyukova et al. 2014). Species of the genus *Hedinia* are equally debatable, although

there is no doubt that their distribution also marks the trans-Asian migration route linking the flora of Tibet and Beringia. *Hedinia tibetica* (Thorns.) Ostenf. was described from Tibet, *H. mongolica* (Kom.) Velichkin from Mongolia, then *Hedinia* is found in Altai and described as *H. altaica* Pobed., and from western Chukotka – *H. chukotica* (Botsch. et Petrovsky) Balandin, Korobkov et Jurtzev. However, according to A.N. Berkutenko (1996), they all belong to the same species – *Hedinia tibetica* (Thomson) Ostenf. (synonym of *Smelowskia tibetica* (Thomson) Lipsky).

Thus, we can see that *Megadenia* and *Hedinia*, as well as *C. jubata*, have a very discontinuous and fragmented Asian range. Suffice it to say that *M. speluncorum* was found in a single habitat on limestone outcrops at the entrance to a cave in the south of Sikhote-Alin. It is difficult to ascertain to what extent the geographical isolation of these species has affected their taxonomic differentiation.

Isolated populations of *C. jubata* in Magadan Region are not as sparse as populations of *Megadenia*, but nevertheless, this species is rare here and is known from 12 localities (Konratiev et al. 2019, Mochalova 2023, GBIF 2023). Scanned herbarium specimens (49 from Magadan Region and 4 from Khabarovsk Territory) are available at <https://herbarium.ibpn.ru/>, as well as in GBIF (Mochalova et al. 2024).

The largest population of *C. jubata* is located on the Mt. Skif (Okhotsk-Kolyma watershed, Ola basalt plateau),

two smaller ones are on the shore of the Ola Lagoon between the Atargan Spit and Cape Kharbiz, as well as on Cape Grey (the coast opposite the island of Misunderstandings). The remaining populations are fragmented and small. Preferring slopes of southern exposure, the North Okhotsk caragana grows on dry rocky, gravelly and meadow coastal slopes at an altitude of several tens to 300 m above sea level, as well as in the upper belt of mountains in forbs meadows and in mountain tundra, on rocks and gravelly plateau at an altitude of more than 1000 m above sea level. Meadow communities with *C. jubata* on coastal slopes often intersperse with stone birch forests (*Betula lanata* (Regel) V. Vassil.) On the Ola Plateau, caragana is found as part of both meadow and dryad communities, sometimes extending under the sparse canopy of larch (*Larix cajanderi* Mayr).

Unlike the Buryat caragana, which grows in the form of a shrub with tall, erect branches, the North Okhotsk caragana has the life form of a semi-spread or creeping shrub with arched branches not exceeding 50 cm in height, and forms rather dense clumps. Settling in the cracks of almost vertical rocks, caragana acquires an unusual life form: plants hang from the rocks in an arched manner or extend along vertical walls (Andriyanova et al. 2004).

As it turns out the northern habitats of *C. jubata* not only in the Magadan Region, but also at the mouth of the Lena River are confined to areas with high air humidity. Perhaps the dryness of the substrate on which this species grow, in the presence of dense pubescence of shoots and leaves, is compensated by a large amount of precipitation. Apparently, similar conditions did not preserved inside the continent, which caused extinction *C. jubata* there and its survival in areas with a coastal climate, not necessarily with a lot of precipitation, but certainly with high relative humidity.

Association with carbonate soils also may be account for the formation of the modern range of *C. jubata*. On the example of disjunctive range of *Artemisia rupestris* L., it was inferred that the very absence of continuous distribution of carbonate soils leads to the inevitability of rupture of the range of this species, as well as of other calcophilous plants (Vasiliev 1963). However, it turns out that *C. jubata* in Magadan Region, unlike the northernmost points in the Republic of Sakha Yakutia, on the Lena River is not found on outcrops of carbonate rocks, and its localities are confined mainly to volcanogenic rocks of basic composition (Ola basalt plateau, Harbiz – Atargan), although there are also on acidic rocks-granitoids (the Cape Grey, Zavyalova and Misunderstandings islands, Staritsky Peninsula).

The disjunction of the Okhotsk part of the *C. jubata* range from the main Central Asian part suggests that this species existed in this territory as early as in the Pliocene, survived in few refugia in the Pleistocene, and expanded its range during warming at the beginning of the Holocene. A mountain species, *C. jubata* was already adapted to harsh ecological conditions, which allowed it to exist and evolve for a long time in high latitudes under conditions of continentalisation, xerisation and climate cooling. The specific ITS mutation presumably originated here and then spread southwards to the Sayan Mountains. This mutation is not neither in the Tien Shan nor in Tibet. Now East Sayan

Mountains are where this species is most abundant and, according to our data, has the highest genetic diversity (of the three populations studied). Although the Magadan and Buryat populations have different chloroplast haplotypes, the structure of nuclear DNA indicates possible secondary contacts between them (Fig. 1). Buryat group has a small mix of Magadan genotypes, and Magadan group has a small mix of Buryat genotypes.

The range of caragana, as well as some tree species such as Siberian spruce, then declined and fragmented in the north. This range reduction is related to climatic cooling as well as to sea level rise and the formation of the modern coastline of the Okhotsk Sea.

Thus, we would expected that whether the *C. jubata* dispersed from one or different locations in Tibet, its Tien Shan populations were isolated from the rest as a result of climate change in the Pleistocene associated with increasing aridity and expanding deserts. At the same time, the species was widely distributed in the north and formed new closely related haplotypes there. Subsequent climate-induced cycles of range contraction/expansion increased geographic isolation and fragmentation of northern populations. At present, marginal northern populations occupy places with the most favourable ecological conditions.

Not changeable enough genetic markers and the absence of population samples of this species in Tibet and the Lena delta do not allow us to reliably reconstruct the phylogeographic history of *C. jubata* and to determine the time of divergence of the identified genetic lineages. It remains to be established involving a larger number of specimens and different genetic methods in order to elucidate on the evolutionary history of this unique species of the genus *Caragana*.

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