Morphological vs molecular differentiation of *Juniperus seravschanica* Kom. in Kyrgyzstan

Master thesis submitted by

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I.Dedication

Dedicated to my beloved parents

Nusupova Roza

and

Sultangaziev Esenbek

II.Declaration

I declare that the Master thesis is an original work and no material in this thesis has previously been submitted at this or other universities.

III.Acknowledgment

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VII. Summary

Zeravschan juniper (*Juniperus seravschanica* Kom.) is an evergreen, long living dioecious conifer tree species. Its naturally grows in Central Asian countries (Kyrgyzstan, Uzbekistan, Kazakhstan, Tajikistan) in Afghanistan, Pakistan as well as in Iran and Oman. Biogeography of the species in its range distribution is studied. On the species level Zeravschan juniper considered as least concerned, but locally in all above mentioned countries the species is under heavy anthropogenic pressure. Up until now, there were no attempts to investigate population differentiation of the species in Kyrgyzstan. Here we try to shed light on gap of knowledge with aid of morphological and molecular techniques.

Intra-and among-population variations of *Juniperus seravschanica* were morphologically examined in eight populations originating from elevations of 1300–2200ma.s.l.in Kyrgyzstan. Eleven traits of needles and cones were studied on 70 vouchers. In addition, height, diameter, stem form, and sex of 172 trees were recorded in the field in order to test whether male trees invest more in vegetative growth than females and if sex ratio is shaped by (limited)environmental resources. Morphological differences among populations were small, but needle length, width and thickness were statistically different. However, differences based on needle traits were independent from geographical, altitudinal and environmental distances. In sharp contrast to studies in other *Juniperus* species, sex ratio in *J. seravschanica* was strongly female biased (3.5females:1male). Moreover, no correlation between the habitat conditions and the sex ratio was detected, suggesting that within the altitudinal range of this species , females occur more frequently . This has implications for sustainable use and the conservation of *J. seravschanica* populations. It is likely, that due to the higher investment of male individuals in vegetative growth males are more exploited than females. An average effective population size of 70% of the respective census suggests that conservation measures and non-selective logging regimes are required to allow reproduction and a natural regeneration of this species.

Conservation of these plant's genetic resources is to be based on an understanding of factors that have shaped species' genetic variation. Novel plastid DNA markers (two minisatellites, one transversion, one indel) were identified and applied to investigate haplotype diversity and population structure in Kyrgyzstan. In total 540 individuals from 15 populations were analyzed and 11 haplotypes were detected. Strong divergence between populations from northern and southern Kyrgyzstan was evident from haplotype

distribution. Gene diversity ranged from 0.083 to 0.765, and was higher in southern (0.687) than in northern populations (0.540). A similar pattern was detected in allelic richness. AMOVA revealed 11.9 % of variation due to differences among regions, 1.5 % among populations, and 86.6 % within populations. N_{ST} was not significantly different from G_{ST} (0.125) giving no evidence for a phylogeographic pattern. These results indicate that the north of Kyrgyzstan was relatively recently colonized by migrants from southern populations probably associated with more favorable conditions during the early Holocene. The Fergana Valley and humid Fergana Range were identified as significant ecological barriers to gene flow between northern and southern populations.

Key words Central Asia - *Juniperus seravschanica* Kom - Phenotypic variation - Sex ratio- conservation – cpDNA- minisatellite - phylogeography - Kyrgyzstan.

VIII. Zusammenfassung

Der serafschanische Wacholder (*Juniperus seravschanica* Kom.) ist eine immergrüne, langlebige, heterözische Nadelbaumspezies. Sie kommt natürlich in Zentralasien (Kirgisistan, Usbekistan, Kasachstan, Tadschikistan), in Afghanistan, Pakistan sowie im Iran und Oman vor. Die Biogeographie der Spezies in ihrer Verbreitung ist erforscht. Als Spezies ist der serafschanische Wacholder als nicht bedroht eingestuft, unterliegt allerdings in allen oben genannten Ländern starkem anthropogenem Druck. Bis zu diesem Zeitpunkt gab es keine Versuche, die Abgrenzung der Populationen der Spezies in Kirgisistan zu untersuchen. Wir versuchen hier, diese Wissenslücke mit Hilfe von morphologischen und molekularen Techniken zu beleuchten.

Variationen innerhalb und zwischen den Populationen des *Juniperus seravschanica* wurden innerhalb von acht Populationen morphologisch untersucht, die in Höhen zwischen 1300 Metern und 2200 Metern Seehöhe in Kirgisistan angesiedelt waren. Elf Charakteristiken der Nadeln und Zapfen wurden anhand von 70 Mustern untersucht. Zusätzlich wurden Höhe, Durchmesser, die Form des Stammes und Geschlecht von 172 Exemplaren im Feld gemessen, um festzustellen, ob männliche Bäume intensiver in vegetatives Wachstum investieren als weibliche, und ob das Geschlechterverhältnis durch die (begrenzte) Verfügbarkeit von Umweltressourcen beeinflusst wird. Morphologische Unterschiede zwischen den Populationen waren klein, aber Breite, Dicke und Länge der Nadeln waren statistisch unterschiedlich. Allerdings waren die Unterschiede bezüglich der Nadelmerkmale unabhängig von geographischer und umweltbezogener Distanz und Höhe. In scharfem Kontrast zu Studien anderer *Juniperus*-Spezies war das Geschlechterverhältnis des *J. seravschanica* stark weiblich dominiert (3,5 weiblich:1 männlich). Weiters konnte keine Korrelation zwischen den Bedingungen im Habitat und dem Geschlechterverhältnis ausgemacht werden, was nahelegt, dass weibliche Pflanzen innerhalb der Höhenzone der Spezies öfter vorkommen. Dies hat Implikationen für nachhaltige Nutzung und Erhaltung der Populationen der *J. seravschanica*. Es ist wahrscheinlich, dass männliche Exemplare aufgrund des höheren Investments in vegetatives Wachstum stärker ausgebeutet werden als weibliche. Eine durchschnittliche effektive Populationsrate von 70% des respektiven Zensus suggeriert, dass Schutzmaßnahmen und aselektive Abholzung die Reproduktion und natürliche Regeneration der Spezies ermöglichen müssen.

Die Erhaltung der genetischen Ressourcen der Pflanze muss sich in einem Verständnis der Faktoren, die die genetische Variation der Pflanze beeinflusst haben, gründen. Neue

Marker der plastidären DNS (zwei Minisatelliten, eine Transversion, eine Indel) wurden identifiziert und angewendet, um haplotype Diversität und Populationsstruktur in Kirgisistan zu untersuchen. Insgesamt wurden 540 Individuen aus 15 Populationen analysiert und 11 Haplotypen gefunden. Starke Divergenz zwischen Populationen aus den nördlichen und südlichen Regionen Kirgisistans war aus der haplotypen Verteilung ersichtlich. Die Gendiversität lag zwischen 0,083 und 0,765, und war höher in südlichen (0,687) als in nördlichen Populationen (0,540). Ein ähnliches Bild bot sich in der allelen Reichhaltigkeit. AMOVA fand 11,9% an Variation durch regionale Unterschiede, 1,5% innerhalb von Populationen und 86,6% innerhalb von Populationen auf. N_{ST} unterschied sich nicht nennenswert von G_{ST} (0.125), was keinen Hinweis auf ein phylogeographisches Muster zulässt. Diese Resultate deuten an, dass der Norden Kirgisistans erst vor relativ kurzer Zeit von Migranten aus den südlichen Populationen kolonialisiert wurde, möglicherweise im Zusammenhang mit günstigeren Bedingungen während des frühen Holozän. Das Ferghanatal und das feuchte Ferghanagebirge wurden als signifikante ökologische Barrieren für den Genfluss zwischen nördlichen und südlichen Populationen ausgemacht.

Schlagwörter:: Zentral Asien- *Juniperus seravschanica* Kom.- Phenotypic variation, cpDNA, minisattellites, phylogeography, Kirgisistan

IX. Introduction

Zeravschan juniper (*Juniperus seravschanica* Kom.) is an evergreen, long living dioecious conifer tree species. Its naturally grows in Central Asian countries (Kyrgyzstan, Uzbekistan, Kazakhstan, Tajikistan) in Afghanistan and Pakistan as well as in Iran and Oman (Adams, 2004, 2014). This study comprises the first morphological and molecular investigation covering an entire part of the geographical range of *Juniperus seravschanica* Kom, in Kyrgyzstan. At the whole range of species distribution on the basis of a literature review attempt to draw a biogeography of the species was performed. The results of our studies were published in 2 scientific papers.

X. Research objectives

The overall aim of this study was to investigate the biological diversity of Zeravshan juniper (*Juniperus seravschanica* Kom.) in Kyrgyzstan. The assessment was based on the analysis of morphological traits, current ecological condition as well as molecular analysis of population genetic structure, which leads to development an appropriate conservation measures on a species level.

The main objectives of the research were:

1. To understand biogeographical pattern of the species throughout its whole range
2. To measure and assess morphological variation using needle and cone traits
3. To compare growth patterns of male and female individuals
4. To compare sex ration among the populations in order to check whether sex ration changes with changing of environmental conditions
5. To investigate spread of the species after the Last Glacial Maximum and to elucidate patterns of genetic diversity, population differentiation and gene flow. Fifteen natural populations of *J. seravschanica* from Kyrgyzstan were analyzed, employing novel plastid minisatellites together with haplotype specific PCR and PCR-RFLP markers.

VIII. Biogeographical history of *Juniperus seravschanica* Kom. in Central Asia

Biogeography is a very broad science, including such scientific disciplines like e.g.: biology, geography, geology, ecology, palaeontology, climatology, systematic and evolutionary biology, as well as phylo- and/or population genetics. It helps us to understand past and present distribution of plant and animal species and other organisms in our planet (McDonald, 2003). A central aim of biogeography is to understand when and how modern patterns of species diversity and distribution developed and how individual taxa reached their current locations (Milne & Abbott, 2002)

The genus *Juniperus* L. consists of approximately 70 species and 28 varieties (Adams, 2014). It is the second most diverse genus of the conifers. Genus divided into the 3 sections: *Caryocedrus* (with only one specie *Juniperus drupacea*); *Juniperus*, with 12 species, and *Sabina* with 55 species. There are three primary centers of *Juniperus* L. diversity: deserts of Mexico and the south-western USA, the Mediterranean region and western China-**Central Asia** (Adams R. 2004). *Juniperus* L. probably originated in Eurasia, and was a part of the south Eurasian Tethyan vegetation of the Eocene to Oligocene. It reached America once at this time, once in the Miocene and once more recently (Mao et al., 2010).

The species of interest is *J. seravschanica* belongs to the section *Sabina.* It mainly occupies foothills of Central Asian mountains reaching Arabian peninsula (Oman) on the western range of its distribution. Globally, Zeravshan juniper is considered as least concerned tree species, only having vulnerable status in Oman (Patzelt, 2008). The Central Asian name of juniper is “kara-archa” meaning in the Persian language “kara”-black and “arsa” - juniper. It was described by a Russian botanist V.L Komarov in 1932 on the Zeravshan river basin in the Pamir–Alai mountain system. On the basis of morphological traits Zeravshan juniper was considered as a synonym of *J. exelsa* Bieb. (Riedl, 1968); *J. polycarpos* (Farjon, 2005) or *J. polycarpos* var. *seravschanica* (Adams, 2004a). But a recent study by Adams (2016) redefines *J. seravschanica* as *J. exelsa* var. *seravschanica* based on analysis of cpDNA data. However, due taxonomic uncertainty of Zeravshan juniper, in this paper the name *J. seravshanica* is used.

Distribution and ecology of Zeravschan juniper.

A core distributional range of Zeravshan juniper is in Tien-Shan and Pamir-Alai mountain system. Scattered, small populations of it were recently found in the Southern Iran, Northern Oman (Adams et al., 2014) and more recently scattered individuals in western Turkey. Within Central Asian part of the western range are reached Nuratau and Kugitang mountain ridges; the northern boundary runs along the slopes of the Talas and Karatau range; the southern through the mountains of Afghanistan, to Ziarat Forests of Pakistan (Komarov, 1932, Zakirov, 1969) (Fig.1).

In the dry areas of **Afghanistan** 10 m high *J. seravschanica- J. semiglobosa* woodlands are found. The upper part of the forest belt on the northern slopes of the Hindu Kush is formed by open mixed juniper-pistachio woodland. However, most areas of juniper woodlands have been cut for fuel wood consumption and mature stands are rare (Breckle, 2007). Living juniper woodland are very degraded and intensively used for animal grazing. No information is available regarding the conservational status of the tree due to continuous military conflicts in Afghanistan in last two decades.

Juniper woodlands (*J. excelsa, J. polycarpos, J. polycarpos* var. *turcomanica*) are typical for the Irano-Turanian area and occur in the southern slopes of Alborz and Khorassan-Kopedagh mountains in the north-east of **Iran** and neighboring Turkmenistan**.** Recently, presence of Zeravschan juniper in the territory of Iran was confirmed by Adams et al. (2014a). It mainly grows in the southern part of Iran in Kuhbanan, Khabr and Rabor mountain ranges. These populations are found near the border to Pakistan and Oman. There is no detailed information about the area covered by this species and an admixture of other juniper species within Iran makes it difficult to delineate ecological niches of each juniper species growing in Iran. There is evidence of admixture of *J. polycarpos* var. *turcomanica* with Zeravshan juniper within eastern and southern distribution in Iran. According to findings of Adams et al. (2014a), presence of Zagros mountain range serve as natural barrier for distribution of juniper species into northern population of Iran.

In **Kazakhstan**, Zeravshan juniper stands mainly grow in the Aksu-Dzhabagly Reserve and occupy south-western slopes of Bala-Baldarbek and Baldarbek mountain ranges at altitude 1300-2000m.a.s.l. Total area covered by this juniper species is about 3300 ha (Karamysheva 1973). It is the far northern boarder of distribution of the species.

Populations were severely exploited during the second World War by wood cuttings as well as grazing.

Within **Kyrgyzstan** range, it grows form 1000 to 2300 m a.s.l. in Turkestan, Alai, Fergana, Chatkal, Talas, Inner Tien Shan and Kavak mountain ranges (Muhamedshin, 1967). Habitat of the specie is forming sparse juniper woods in lower and middle parts of the timber zone, in pure stands or mixed with *J. semiglobosa* Rgl. Pure Zeravshan juniper woodlands occupy an area about 8,400 hectares with an average wood stocking of 30.4 m^3/ha (Musuraliev, 2004). Zeravshan juniper in the upper limit of its distribution (2300 – 2400 m) is represented by old trees and the absence of natural regeneration (Dzhanaeva, 1958). Gan et al. (1959) observed juniper trees under the age of 1000 years in the Tien-Shan and Gursky (1949) found a 2000 years old Zeravshan juniper in the Western Pamir Mountains. The growth of young trees depends entirely on the environmental conditions. At an altitude of 1,000 – 1,300 m, they are usually depressed, resulting in short stature, strong tapering and curvature of its trunk. At higher altitudes from 1,300 to 2,300 m, trees grow bigger. At higher altitudes the growth is slowed down. Zeravshan juniper grows better when in mixed with *Acer turkestanicum* (Zapyagaeva, 1976).

Regarding the good correlation between pollen percentages and tree cover in Juniper woodlands (Beer et al., 2007), high values of *Juniperus* pollen (>40%) indicates that 1.8 Ma (Pleistocene) ago, the area had been located within the *Juniperus* woodlands.

So far Zeravschan juniper was mistakenly treated as *J. excelsa var. polycarpos* in **Oman** (Fischer and Gardner, 1995). Analyzing nDNA and cpDNA region of juniper species (Adams et al. 2014b) confirmed the presence of Zeravschan juniper in Oman. It grows in Al-Hajar Mountains south-west of Muskat at altitudes 1,450 – 3,000m. Juniper grows with admixture of *Olea europaeae*, *Daphne mucronata* Royle, *Lonicera aucheri* Jaub & Spach, *Monotheca buxifolia* Falc or *Berberis* spec. Indigenous people consider juniper as holy tree (Matwani, 2011) , protecting it from illegal wood cuttings and collections. Locally people use it for medical purposes: treatments from stomach ache; diabetes; admixture with lemon juice to threat malaria. Women wear dried cone berries as decorative necklaces. According to Fischer and Gardner (1995), Matwani, (2011) and other researchers the main threat for juniper populations are: climate change, anthropogenic pressure via tourism, pastoralism and removal of highly nutritional soil cover and exporting it to neighboring United Arab Emirates.

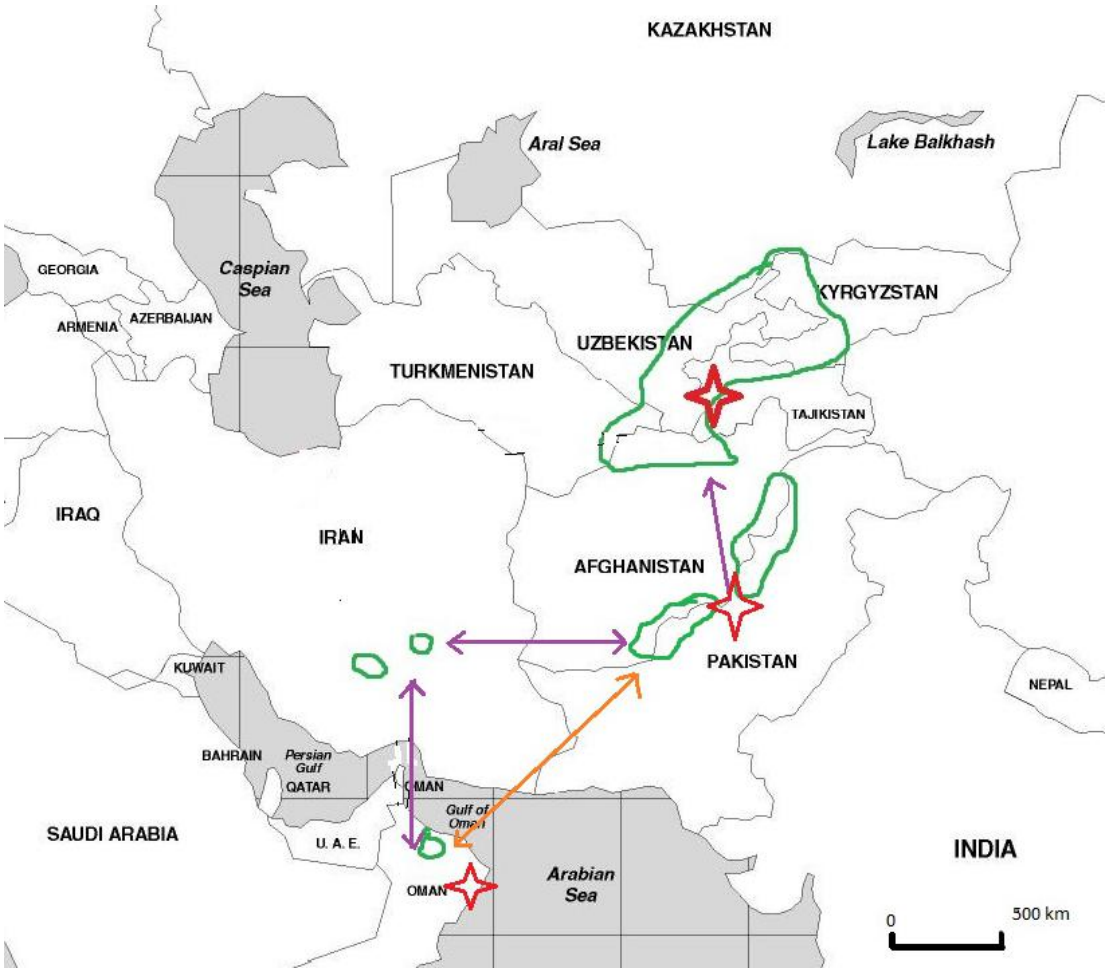

Fig.1. Distribution map of Zeravshan juniper (*Juniperus seravshanica* Kom). Red stars indicate possible refugias (arrows show possible migration routes, different colours indicate alternative migration routes).

Juniper forest is the most extensive vegetation type dominated by *Juniperus excelsa* subspecies *polycarpos* (synonym to *Juniperus seravschanica* Kom.) in the northern mountain ranges of **Balochistan (Pakistan).** This extensive open woodland is spread between 2,100-3,000 m but at higher elevation the trees become stunted and dwarf or form large prostrate patches on rocks especially on wind exposed slopes in Ziarat and Zarghun ranges. Generally above 2,800 m juniper trees are gradually replaced by curious cushion-shaped dwarf shrubs. Several species found in juniper tract are endemic to Balochistan or extend their distribution to neighboring areas of Afghanistan and Iran. These species with restricted range of distribution increase the importance of these mountains. These mountains are centre of endemism in the region. Some of these endemic species associated with juniper forests include *Berchemia pakistanica, Amygdalus brahuica,Cotoneaster afghanica, Cotoneaster rechingeri, Cerasus rechingeri, Spirea brahuica, Aitchisonia rosea, Gaillonia afghanica, G. macrantha.* Juniper forests include some of the oldest trees of the

country but this national heritage is facing threat from a plant parasite -the dwarf mistletoe (*Arceuthobium oxycederii*). (Akhter &.Mirza, 2006).

In **Tajikistan**, juniper grows in Zeravshan, Darvaz, Vachsh, Chozretish, Peter I and Gissar mountain ranges at altitude 1,600 – 2,600 (2800) m asl. (Zapryagaeva, 1954). It was originally described in the Zeravschan mountain ranges of Tajikistan by Komarov (1932). Juniper woodlands do not form closed canopy stands with pure juniper trees. In mountain ridges it mixes with *Acer turkestanicum, Cercis Griffithii* and *Amygdalus bucharica* and in lower elevations and river banks with *Populus alba.* On Zeravshan mountain range juniper grows at altitudes from 1,600 to 2,600m a.s.l., forming at rocky slopes very sparse, thickets bordering in the bottom with thickets of *Cerasus verrucosa*, *Ephedra equisetina*, *Atraphaxis spinosa,* and *A. pyrifolia*. Zeravshan juniper is highly threatened by anthropogenic pressure. Former clear cuttings of the most valuable individuals together with uncontrolled animal grazing played a major role in degradation of this forests (Zapryagaeva, 1954).

Juniper forest is a major tree species in **Uzbekistan**. They constitute a special vegetation belt with a clear timber setting on the slopes of Turkestan, Kurama, Alai, Zeravshan, Fergana, Chatkal, Hissar and Babatag ranges. The total juniper forested area cover about 192,000 hectares with a total volume of 4,135.5 thousand m^3 (Kayimov, 2011). These stands are typically composed of three juniper species, namely, *Juniperus seravschanica*, *J. semiglobosa* and *J. turkestanica*. As a result of the study of the juniper stands occurring in different forest regions of Uzbekistan the best performing stands were selected (the total area is 550 ha) and a reserve with Zeravshan juniper (1,260 ha) was designated (Alexandrovsky, 1998).

Glacial refugia

There is a little evidence of glacial refugia. However, the species must have survived in some parts of its current distribution in Arabian Peninsula, Himalaya Mountains or in Central Asia, from where its expansion started. According to Kamelin (1973), populations of Zeravshan juniper might have been preserved in Turkestan Mountain Range (on the boarder of three central Asian countries (Kyrgyzstan, Uzbekistan and Tajikistan)) (Fig.1). During the Palaeogene, almost entire range of species distribution (Mediterranean area and Central Asia) was dominated by a shallow sea (Tethys) and today's mountains were archipelago of small islands. The Tethyan-Tertiary region was characterized by subtropical forests composed of evergreen trees and shrubs (Takhtajan, 1969). To the north, generally reaching the latitude of modern Kazakhstan subtropical forests were graded into Arco-Tertiary (Turgai) forests composed of temperate deciduous hardwood conifers and some broad-leaved evergreens (Makulbekov, 1972). Since the late Oligocene the Mediterranean flora started to invade eastwards and finally reached Central Asia (Kamelin, 1979). According to Kostenko (1955) in the mid-Pliocene in Kuhistan (central part of species' distribution), including Turkestan ridge, was a mid mountainous country. While the territory was occupied at the beginning with a Tropical-Tertiary evergreen (Poltavian) flora, already in Miocene it was replaced with temperate Arco-Tertiary mixed coniferous and deciduous (Turgai) flora (Fig.2).

It is assumed that, along with the extinction and partial preservation of Poltavian flora there has been an overlapping and mixture of preserved formations Poltavian flora and distributed deciduous Turgai flora (Ovchinnikov, 1955). This was facilitated by the emerging of mountain ranges. The upper part of the mountains were occupied by Turgai mesophyllic forest flora and represented by such species as hornbeam, linden, birch, alder, oak, maple, ash, walnut with an admixture of various conifers, cedar, spruce, pine, etc. However, according to Ovchinnikov (1955), "forest vegetation in places then the relief was combined with more or less xerophyllic woody and herbaceous formations that reflected both the earlier Palaeocene relations and local xeromorphic evasion, for example, on the rocky slopes of the same Turgai species formations." Junipers have been a part of these forests had nature of admixture with more common deciduous mesophyllic species (Ovchinnikov, 1958). Since the late Pliocene, the restructuring of Turgai vegetation due to the general climate cooling and powerful mountain formation processes begun.

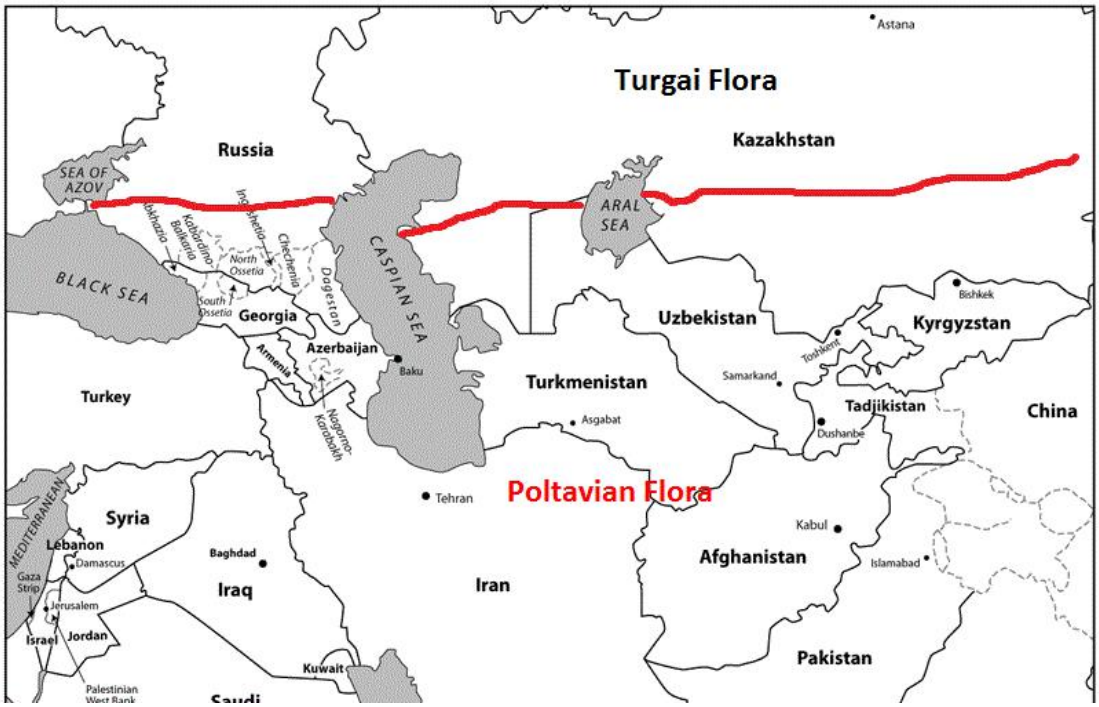

Fig.2. Distribution of Turgai and Poltavian flora

Continuing uplift at the end of the Pliocene and the beginning of the Pleistocene created conditions for the development of glaciations. Especially powerful glaciations experienced central part of Kuhistan - Zeravshan range, Zeravshan and Yagnob valleys. On the outskirts of Kuhistan (the southern slope of the Hissar mountain range and probably the northern slope of the Turkestan mountain range) glaciations was less significant (Konnov 1966) which probably could serve as a refugia sites for plant species. It is possible that the current forest and meadow flora of northern regions derived from southern refugia that could have persisted during the cold and dry climate of the Pleistocene. Some of these refugia are believed to have been in the nearby Fergana Valley, the Alai or the Gissar-Darvaz regions (Kamelin 1973), as exemplified by the current presence there of *Platanus orientalis, Juglans regia, Acer turkestanicum, Punica granatum* and *Diospyros lotus* (Kamelin, 1973).

Periods of climate cooling in the Pleistocene enhancing glaciation processes alternated with drier and warmer interglacial periods. However, throughout the Pleistocene aridization of the climate occurred. Last (Würm) cooling phase had smaller glaciation effects (Grosset, 1962). The vegetation was subjected to destruction and transformation during the Pleistocene. The main core of Pliocene thermophyllic flora was destroyed. Extant part gave rise to a new, previously absent types of vegetation. On the top of the mountains evolved formations of cryophilic mountain-steppe vegetation and on the basis of so-called Pleistocene floristic complex (Krashenninikov 1937, 1939 ; Ovchinnikov 1946,

1955, 1957), mountain taiga spruce, juniper forests (Ovchinnikov, 1958) of junipers close to modern *Juniperus turkestanica* and *J. semiglobosa*. Down side hills were dominated with mixed juniper - maple forest with juniper close to modern Zeravshan juniper *Juniperus seravschanica*.

Relatively few details are known on the vegetation history of that area, but accumulated evidence indicates that during the last glacial maximum (LGM; ca. 18,000 yrs BP) and early Holocene very cold (permafrost) and arid conditions prevailed in the region (Aubekerov & Gorbunov, 1999). After these desert conditions a warmer and more humid period (8,000 – 5,000 yrs BP) with more extensive vegetation cover followed. Later the climate became drier but stable in the region (Adams & Faure, 1997). The first half of the Holocene was obviously warmer and relatively moist compared to the current climate (Ranov & Sidorov, 1960). In the second half of the Holocene a further cooling continued the process of climate aridization. During that time, juniper - broadleaved forests changed to pure juniper forests.

Centuries of human influence enhanced the thinning of juniper forests, steppe communities, and erodible slopes creating conditions for dthe evelopment of tragacanth and petrophytic vegetation types. Modern flora of the area shows the transformed relationship with the older Pleistocene, Pliocene flora and distribution of individual species and the mountain profile phytocenoses reflects about going now shifts (Kamelin, 1973).

It has been suggested that Zeravshan juniper survived the Pleistocene in Hajar mountain range of Oman being part of continuous population during the last Pleistocene ice advance (ending ~15,000 years BP) which forced juniper population to lower and cooler areas. (Adams, 2014b) According to Miller and Nyberg (1991) continuous populations of juniper from Pakistan to south Iranian populations may have been source populations for long distance migration (common for *Juniperus* on islands, Adams, 2014) from across the Arabian Golf. Kurschner (1998) listed Omani juniper among some other taxa of Irano-Turanian origin and described them as invaders of Arabian Peninsula.

Table. 1. General overview of Zeravshan juniper populations distribution.

Country	Area (ha)	Min. Temp. (° C)	Max. Temp. (°C)	Precipitation, (mm)	Natural regeneration	Seed dispersal	Diseases	Anthropogenic /ecological pressure	Citation
Afghanistan	?	-5	34	327	vp	?	?	fuel wood	World Bank Report 2014
Iran	3500000*	5	30	228	vp	birds	dwarf mistletoe, witches broom	logging, grazing, habitat loss	Pirani et al., 2011
Kazakhstan	3300	-5,4	20	250	vp	birds	juniper seed mite	grazing, logging	Karamysheva, 1973
Kyrgyzstan	8400	-4,6	32,4	593	p	birds	juniper seed mite, *Trisetacus kirghisorum Megastigmus Dalm*.	grazing, logging	Musuraliev, 2004, Ciesla, 2002
Oman	?	-3,6**°C**	36,6	322	vp (lowland) p (highland)	birds	forest decline, dieback	climate change, forest fire, firewood for shepherds, tourism	Fischer and Gardner, 1995
Pakistan	86000*	-0,8	29	250-323	vp	birds	dwarf mistletoe,	fuel wood, grazing (goat), deficient in rain fall	Sarangazai et,al., 2013
Tajikistan	150000*	-3,4	30,1	691	vp	birds	juniper seed mite	fogging, grazing, habitat loss	Djanaeva, 1969,
Uzbekistan	192000*	-0,4	35,2	206-300	P	birds	juniper seed mite	fuel wood, grazing	Aleksandrovsky , 1996

*Including other juniper species; vp-very poor; p-poor; ?-unknown

Pollination, seed dissemination and regeneration of Zeravschan juniper

Zeravschan juniper is a 10-15 (up to 20m) evergreen dioecious tree with separate male and female trees. Presence of bisexual individuals (female and male generative organs are present in the same tree) were also recorded (Fischer and Gardner, 1995, Sultangaziev et al. 2010, Matwani, 2011). Understanding the system of pollination and dissemination of the pollen and seed material allows us to understand the patterns of species mating system as well as gene flow.

Male strobili (cones) are terminal on branchlets, globose or subglobose, 3-4x 2-3 mm, green maturing yellowish-brown; have 8-10 microsporophylls, peltate, with rounded entire margins, bearing 3-4 abaxial microsporangia near the lower margin containing spherical pollen (Fig. 3). Male individuals start pollination at an early age (25-30 years). Pollen shed is during April-May. There are no detailed studies on pollen dissemination, pollen count per tree or other biological peculiarities of the pollen. Generally, junipers are effective and large amount pollen producers. For example a single individual of north American juniper *Juniperus ashei* produced approximately up to 523 billion pollen grains (Bunderson et al., 2012). In addition, the pollen grains are small and lightweight and can be transported over great distances. Pollen weight ranges from $2{,}5x10^{-6}$ mg, 4,0mg $x10^{-6}$ and 4,8mg x 10^{-6} for *J. ashei, J. pinchotii*, and *J. monosperma* respectively (Bunderson & Levitin, 2015). The distance that pollen travel after release depends on the pollen type, and meteorological conditions of the region. Increased air humidity can cause additional weightto the pollen grains and hence increasing its settling rates. Markgraf (1980) showed that in mountainous areas daytime upslope wind transport juniper pollen from lower elevations to the upland. Weather conditions with rainy days during pollen shed cause pollen deficiency and formation of seed cones with no embryo (Djanaeva, 1976).

The pollen dispersal of a single isolated tree of *Juniperus excelsa* was studied by collecting the pollen in a distance of 3, 6, 12, 24, 48, 96 m from the source. The results showed that the pollen frequency at a distance of 96 meters was 0.5% of the source frequency (Khalique & Perveen, 1997).

Fig. 3. Male cones of *Juniperus seravshanica* Kom in Kyrgyzstan.

The majority of the pollen released is deposited near the source trees. Settling range of the juniper pollen grains is commonly estimated by Stoke's law (Gregory, 1973). By using Stoke's law for United States juniper species (*.s ashei, J. monosperma* and *J. pinchotii*) Bunderson and Levitin (2015) have calculated settling rate of the pollen. The settling rate was 0.73 – 0.88 cm/sec, 1.09 – 1.29 cm/sec and 1.0 - 1.24 cm/ sec, respectively. If we assume, that settling rate of Zeravschan juniper is roughly the average settling rate of above mentioned species and assume a certain release height, one can calculate the time the pollen grain will remain in the air. We take settling rate of 1.24 cm/s (as an example) with absence of vertical wind it will still take approximately 20 - 25 minutes for a pollen relased 15 m above the ground to reach the ground. Beer (2007) roughly estimated the flying distances of juniper pollen ranging from 20 m up to 2 km, which is close to Zhang et al. (2005) estimates of *J. przewalski*.

Investigation of pollen in other juniper species from mountain areas Markgraf (1980) found an elevational abundance of pollen grains. At elevation 740 m abundance of pollen is 400 grains/day/cm^2/tree, at elevation of 2000 m 1285 grains, at elevation of 1500 m 2920 grains and at upper elevations only up to 100 grains.

Female strobili have 4 - 6 pointed scales each containing 0-2 ovules. Formation of female generative organs (ovules) starts in the previous autumn in inner parts of small branchlets (Fig.4) before the pollination. It is hard to differentiate them from vegetative branches. During the pollination period, drops of fluid come out from the strobili which catches

juniper pollen and help to transport it into ovule. Pollination mechanism was studied in *J. excelsa* and family *Cupressaceae*. In all species, the contact of pollen grains with ovule nucellus is carried out with the aid of a pollination drop. The capacity of the ovule of perceiving pollen grains does not change during 4-14 days depending on the species (Ahani et al., 2013). Pollination through wind and starts at 1,000 – 1,200 m at the beginning of March, and at an altitude of 2,400 m in late April or early May. During development the fleshy scales of female cones fuse, forming indehiscent strobili often referred as "berries" (Fig.4).

After pollination, there is a long period of "berries" maturation. Complete maturation occurs after the second year of pollination (Djanaeva, 1965). One can always observe unripe (green) and ripe (bluish- blackish) cones on the trees. The time of cone maturation is October- November. At the maturity cones does not open and seeds are not released.

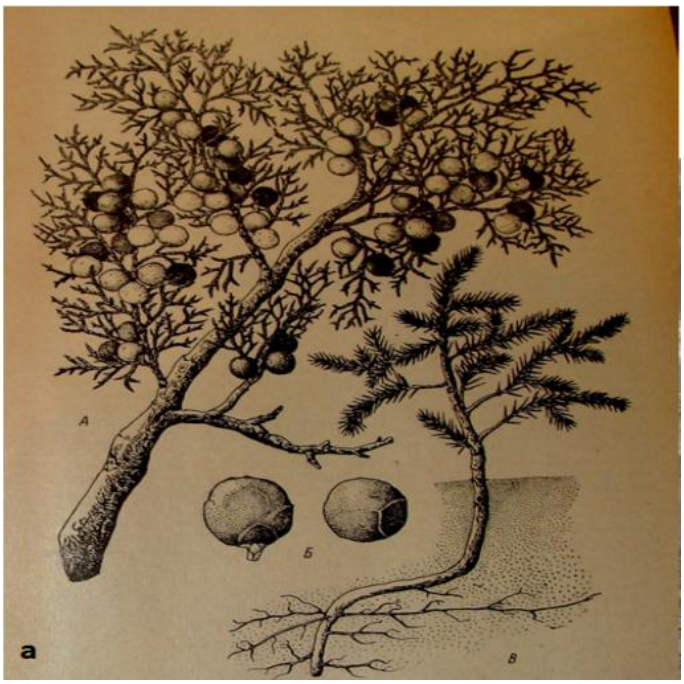

Fig. 4. Female cones (berry-like) of *Juniperus seravshanica* (Djanaeva, 1965)

There is an extensive loss of potential seed production between pollination and seed maturation with many fruits failing to fill or being eaten by insects or other predators. Junipers may develop fully develop seed coats but without an embryo or endosperm (Djanaeva, 1969; Chambers et al., 1999 and reference therein), The proportion of filled seeds vary from 6.1 % to 85.2 % (Aleksandrovsky & Abdurazakova, 1996) in other juniper *J. osteosperma* ranged from 0 % to 17.3 % (Fuentes & Schupp, 1998), for *J. excelsa* from

Lebanon 5 to 42 % (Douaihy et al., 2013). The proportion of filled seed for bi-sexual (ambisexual) tree of *J. arizonica* from USA was 0 to 100 % (Adams, 2014c)

There are several other reasons for formations of empty seeds:

- unproductive (sterile) pollen,
- unproductive (sterile) ovule,
- asynchronous male and female maturation,
- unfavorable climatic condition for fertilization.

One of the explanations of empty seed formation is rainy weather causing a lack of pollen, i.e. female trees will not be fertilized by pollen, but still develop seed without an embryo (Lanner, 1964 and reference therein). This aspect of fertilization of Zervshan juniper need to be studied to evaluate positive and negative influence of rainy or foggy conditions. Another reason for empty seediness might be that female ovules catch a pollen from other juniper tree species (*J. semiglobosa, J. turkomanica, J. polycarpos*) growing close by in admixture with Zeravshan juniper trees. Pollen captured by pollination drop will not be able to pollinate female ovule, therefore seeds with empty embryos will be formed. The pollination drop that captured pollen will not be secreted again, so fertilization does not take place (Jagel & Doerken, 2015).
Under natural conditions, male and female strobili can mature at different times of the year, i.e. male strobili mature too late to fertilize the female strobili which are receptive for only 10-12 days before the cone scales start to close (Djavanshir, 1974).

Seed cones are large, 8-12mm in diameter, subglobose, strongly pruinose, blackish-blue, containing high amount of resin (comparing with other juniper species in Kyrgyzstan), very hard, with a woody pericarp layer. Through time, cone slowly dry out, shrivel and fall in winter or stay on the trees until spring. Per cone 2-8, often 4 seeds are found. Seeds are 5 -8 mm in length, 5 – 6 mm in width, reddish-brown, claw like, and of triangular shape. The weight of 1000 cones are 350 - 750 g (on average 500 g) and 1000 seeds weigh 42 – 67 g (on average 54 g). There are approximately 1500 - 2700 seed cones per 1 kg (on average 2000 (Djanaeva, 1965)). In a detailed study of the species in Oman, Fischer and Gardner (1995) counted 48,815 seeds obtained from a single tree. Annual Fructification is more abundant than in other juniper speciessuch as *J. ashei* in central Texas (Chambers et al., 1999). Fruiting of juniper *in vivo* starts at 25 - 30 years, sustainable fruiting around 50 - 70 years when Zeravshan juniper individuals are more tolerant to summer droughts. Generally,

Zeravshan juniper starts frutification earlier than other junipers species in the region. Fruitification of species last until old age. Djanaeva, 1965 observed female individuals with fruit at age of 800-1200, but the size and amount of the fruits were less than middle aged individuals. Stable and good quality seeds are collected from pre-mature and mature juniper trees. Generally mast years happen every 4-8 years (Daneshvar, 2015). Purpose of the masting in juniper trees were studied in detail for Thurifer juniper species in Spain (Montesinos et al., 2012). On the one hand masting allows to satiate seed predating animals, and on the other side increases the efficiency of pollination. Interestingly is to mention that female individuals invest only 1 % of their resources, whereas male individuals invest 2 % by producing large amount of pollen (Montesinos et al., 2012). Female individuals are able to distribute limited resources between growth and reproduction which is consisted with resource switch hypothesis (Monks & Kelly, 2006). They do not invest current years resources into reproduction as do male individuals on cost of their growth. Reproduction resources will be allocated from the end of last masting until the next masting event. Concentration of nitrogen, potassium in cones are 2-3 times higher than in twigs and branches (Ganquelin et al., 2002). Artificial resource availability may increase a mast-flowering, but not necessary mast seeding will occur. Mast seeding events with initially small flower set is common in junipers.

From an evolutionary point of view, the acquisition of “berry-like” seeds, increasing dispersibility, could have promoted allopatric speciation, as well as permitting rapid change of ranges in response to climate change (Farjon, 2005). Depending on the climatic condition of the region juniper cones after ripening fall down to ground and either go to dormancy stage (seed bank) or will be eaten by birds or seed dispersing animals. The fleshy pericarp of the cones is attractive to seed dispersal animals from one side, but from another side it can be a disadvantageous for seed germination. Dried pericarp does not allow humidity to enter into the seeds, which inhibits the effect of seed dormancy. Durimg this stage seeds can stay up to 5 years (Djanaeva, 1965). Seed dormancy is an evolutionary adaptation to harsh, unpredictable environment conditions, escape competition for optimizing reproductive success in randomly varying environmental conditions by dispersing germination by space and time.

Causes of delayed germination:

- impermeable seed coats,
- immature embryos,

- embryo dormancy.

Normally, seeds sown in spring after winter cold-moist stratification for 6 weeks can break seed dormancy (Daneshvar, 2015). Seeds sowed right after cone ripens by removing of flesh pericarp (it is avoid of seed in going to dormancy) on well prepared soil facilitate seed germination (Ahani et al.,2013). Seedlings will germinate the next spring (Djanaeva, 1965).

Further losses to invertebrate and vertebrate predation on developing seeds have a significant impact on seed production. Mostly seeds are destroyed by seed eating mite-*Megastigmus spec.*, and *Trisetacus kirghisorum* which destroys up to 30 – 50 % of the seeds in mast years and 80 % in normal years, respectively (Aleksandrovsky & Abdurazakova, 1996). Unaffected trees produce two times more seed that infected ones (Chambers et al., 1999). Juniper seeds are endo-zoochorously disseminated (Santos et al., 1999). Bird species *Turdus* spec., common chaffinch (*Fringilla coelebs coelebs)* (Fig.5), white-winged grosbeak (*Mycerobas carnipes*), Alpine chough (*Pyrrhocorax graculus*) as well as rodents (rabbits, mice, stone marten scats) have the ability to disperse seeds to short-distance (few hundred meters) by eating cone berries, digest the fleshy pericarp and disseminate them through droppings (Konnov, 1966), whereas carnivores disseminate up to 4.9 – 6.7 km (Santos et al., 1999). Interestingly to mention is that domestic animals, i.e. sheep are able to disseminate juniper seeds more effectively than carnivore (Santos et al., 1999). Dispersal season vary from species to species. In Zeravshan juniper cone (seed) dispersal season occurs from end of spring until end of winter (pers. observation), for congeneric *J. thurifera* occurs from middle of autumn until end of winter (Santos et al. 1999).

Fig. 5. Common chaffinch (*Fringilla coelebs coelebs)* is a generalist juniper seed distributor

The fleshy pericarp can contain high energy. For example in *J. monosperma* the cone has an energy content of 1,32 kJ (Salomonson, 1978; Balda, 1987). Bird species of the *Turdus* family and other smaller birds pass the thick, hard seed through digestive system undamaged, increasing the chances for germination and dissemination them throughout the space while others like the white-winged grosbeak (*Mycerobas carnipes*) may damage and feed on seeds (Konnov, 1966). Greater gut passage time increase the distance of dispersal, and seed scarification by gut acids (Travest es al., 2001). Gut passage time for birds is frequently less than an hour; whereas for other animals it can last from one day up to a week (Chambers et al., 1999).

The distribution of the common rock thrush (*Monticola saxatilis*) coincides with the Zeravschan juniper distribution. Within this geographic region several juniper species are distributed (Farjon, 1992; Adams, 2004; Sehhatisabet et al., 2006). Fruits of juniper serve as a nutrition base for the birds. The nearest populations of Zeravshan juniper are situated in southern Iran (Geno Mountains) 300 km far from the African wintering area of the common thrushes (Sehhatisabet et al., 2006). The next adjacent juniper populations are located as far as 500 km at the Pakistani and Afghanistan territory from Africa (Fig.1). Habitat fragments of Zeravshan juniper potentially act as stepping-stones for frugivorous birds. A common rock thrush (*Monticola saxatilis*) is a long distance migratory bird flying distance up to 1,000 km (Cramp, 1988). Due to long distance flight birds need to stop for rest and feed to continue their travel to the wintering places. On the way back to Africa birds need to overfly through Kyzyl-Kum, Karakum Deserts with relative little amount of food diversity. According to Bulyuk and Shamuradov (2001) birds make stopover right at the edge of Karakum desert in Kopetdag mountain range in Turkmenistan. Estimated an average stopover time for migrating thrushes was estimated up to 3 days (Young & Moore, 1994). There is lack of detailed information on migrating thrushes from breeding areas to wintering places. According to above mentioned migrating pattern of the common rock thrushes one can assume that birds need to make 2-3 stopovers during the migrating period. By doing so birds disseminate juniper seeds over large distances, hence homogenizing effect of gene flow are taking places. Adams et al. (2014b) could not differentiate Zeravshan juniper individuals from Pakistan with Kazakh individuals. Most probably, closely situated populations of juniper in Pakistan and Kyrgyzstan, Kazakhstan, Uzbekistan and Tajikistan are well connected by visiting breeding and migrating thrushes, rather than Pakistani and Iranian populations. Iranian populations of the species are relatively small of (Fig.1) and might be colonized not long time ago and subject to

population leading to isolation by distance or hybridization with other juniper species growing in the region (Adams et al., 2014b). More detailed research on that region is needed to understand the population differentiation in this region.

Natural regeneration of the Zeravshan juniper is an important aspect of its reproduction. It is recognized as not sufficient in the whole area of its distribution. There are several reasons of insufficient natural regeneration:

- low percentage of seed viability,
- seed dormancy,
- lack of seeds,
- climatic instability,
- disturbance by domestic animals (also positive by creating micro-sites as well as seed distribution).

Natural regeneration in Zeravschan juniper is occurring in areas higher 1,500 m. In this altitudes bush under story under the forest stand is well presented. Bush layer serve as a protection against domestic animals as well as providing valuable moisture conditions in dry semi-arid climate of Central Asia and Arabian Peninsula.

Anthropogenic pressure on Zeravshan juniper populations.

Growing in the lower elevation part of the mountains, Zeravshan juniper is suffering from anthropogenic pressure. In the past, Zeravshan juniper forest occupied a much larger area within its natural boundaries. This is still evidenced by the names of localities where juniper forests vanished and only solitary or old specimens are still found at the foothills and treeless slopes. For many centuries, juniper forests have been subject to heavy use. Wood has been used as a building material for the manufacture of household utensils, for heating and cooking. A huge quantity of wood had been used to make charcoal. Zapryagaeva (1958) notes: “Charcoal burning was common practice of the local population, which annually for 6 months goes to the forest for charcoal burning”. Along Zeravshan river basin alone annually 34,000 trees were cut for charcoal burning. In addition, 30,000 upright tree trunks were shipped off to Samarkand for construction purposes (Fedchenko, 1950). In the thirties of the last century juniper wood was widely used in pencil manufacturing, construction purposes as well as for fuel wood. During the Second World War juniper woodlands were the main bases of fuel wood supply for the

Kyrgyzstan. Such excessive use of juniper woodlands has led to depletion of its resources. Juniper forests extended to 479.300 hectares in 1930, while by 1956 the area decreased to 355.300 hectares, and nowadays only 280,000 hectares of juniper forests are found (Musuraliev, 2004).

The area between the rivers Irsu and Mashat (Kazakhstan) were covered by *Juniperus-Malus-Crataegus* forests about 1,000 years ago. But these forests were easily accessible at a time when people already lost their local forests. Deforestation of juniper forests was also facilitated by nomadic herders, for centuries they grazed their large herds on pastures at altitudes of 600 – 2,000 meters a.s.l. (Karamysheva 1973).

In the past, Zeravschan juniper in the Pamir-Alai and Tien Shan mountains were exterminated. Coal burning, slash and burn, creating on-site forest pastures and hay fields contributed to the reduction of its areas. In medieval times, Shakhristan area was part of the ancient land-owning region of Central Asia called Usrushany (Negmatov, 1957). In the cities of residence flourished various crafts - weaving, pottery and forgings. Mining production was developed in the eastern parts around Minka and Marsmandy (on the territory of the modern city Suluktu, (Kyrgyzstan). It is interesting that for melting ore and forgings the best fuel considered was juniper charcoal (Andreev, 1926). Therefore it is plausible that the greatest damage in juniper forests was near the mining centers such as in Southern Kyrgyzstan and Zeravshan river basins.

Researchers have noted numerous traces of forest fires in juniper woodlands (Konnov, 1966). Resistant to degradation, juniper wood helps to ensure that traces of fires persist for very long time. The main causes were from unattended nomad fires places or from uncontrolled pasture fires to clear the land from weeds (Konnov, 1966).

In the Central Kopetdag only during recent 40 - 50 years juniper forests have been reduced by 30 – 40 % (Alibekov & Alibekova, 2007).

Nowadays Zeravshan juniper in southern Kyrgyzstan rarely forms close stands. Typically, the number of trees per hectare do not exceed 180 - 200 (Dzhanaeva , 1965) reducing significantly the ecological role of juniper.

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XII. Part A. Morphometric traits and sexual dimorphisms do not differentiate populations of Zeravshan juniper (*Juniperus seravschanica* Kom.) in Kyrgyzstan

Introduction

Zeravshan juniper (*Juniperus seravschanica*) is a wind-pollinated shrub or small tree. It is a slowly growing species that attains heights up to 20 m and a diameter at breast height of 50 -100 cm after approximately 300 years (Djanaeva, 1965). Although most trees are dioecious, there are cases were bisexual individuals are found (Fischer and Gardner, 1995; Djanaeva, 1965 and this study).

It is widely naturally occurring in south-eastern Uzbekistan, southern Kazakhstan, western Kyrgyzstan, Tajikistan, northern and eastern Afghanistan, north-eastern Pakistan and northern India. It is generally highly drought resistant.

In Kyrgyzstan, three types of juniper forests are found at altitudes between 900 and 3,700 m asl. Usually the lower altitudinal belt (900-2300 m asl) is formed by *J. seravschanica, J. semiglobosa* is found at elevations between 1,400 and 3,100 m asl and the upper belt is occupied by *J. pseudosabina* at altitudes beyond 2,500 m asl. Although the ecological importance if these juniper forests have been well recognized, the area is constantly decreasing. Since 1980, approximately 20 % has been lost due to intensive cuttings, forest fires, and excessive cattle grazing, bringing the natural regeneration virtually to a halt. This is also due to the irregular seed production and small quantity of viable seeds of Zeravshan juniper (Aleksandrovsky and Abdurazakova, 1996) in the wake of insufficient pollination that usually starts at the end of March or beginning of April. Supplemental artificial pollination resulted into up to 2.5 times higher yields than under natural conditions (Chub, 2003). Juniper seeds are shed 2 years after pollination.

On a species level, Zeravshan juniper is not threatened, however, locally or regionally juniper forests are under increasing pressure from human activities, often heavily fragmented and thus its genetic resources are endangered. Ideally, the conservation and sustainable use of Zervshan juniper should be based on findings of the species' geographic differentiation of adaptively relevant traits such as drought resistance, seed productivity and survival or - in short - is based on Darwinian fitness. In addition, estimates of the effective population size would be helpful. However, for the majority of plant species these data are not available and conservationists are forced to draw conclusion on much more

limited data sets, sometimes on purely descriptive information only. Biochemical and molecular variation may be used as a supplemental source and do not substitute each other (e.g., Perrie and Brownsey, 2005).

J. servaschanica was firstly described by Komarov in 1932 belonging to the section *Sabina*. However, it is sometimes also treated as a subspecies of *J. excelsa* (cf. Farjon, 2005; p. 291) or to be a variety of *J. polycarpos* (Adams, 2001). Disregarding of its still unclear taxonomic position, its intraspecific variability has not been studied yet.

Intraspecific variability of different *Juniperus* species has been mainly studied by means of chemical components such as terpenoids (e.g. Flake et al., 1964), essential oils (e.g. Adams, 2001) or molecular markers (e.g. Zhang et al., 2008). Morphometric traits (e.g. Marcysiak et al. 2007) have been seldom employed. For the first time to the best of the authors' knowledge, this study tackles morphometric variation of needles and cones of *J. seravschanica* by comparing eight natural Kyrgyz populations. In addition, descriptive data on growth (diameter and height) and stem form will be provided. Another aim of the study was to compare the sex ratio among the populations in order to address the question whether sex ratio changes with ecological conditions. In dioecious species, the reproductive effort of females is usually higher than of males and consequently in habitats with low availability of resources male-biased sex ratios are to be expected (Ortiz et al., 2002).

Material and Methods

Eight populations of *J. seravschanica* were sampled in Kyrgyzstan in 2007 and 2008 (Table 2, Figs. 6,7). Ecological data of the samples sites are compiled in Tab. 3. Seventy vouchers (approx. 30 cm) were taken randomly from 4 -15 adult trees per population. In order to avoid sampling from closely related individuals, a minimum distance of 10 m was chosen between trees; on average sampled trees were 46 m apart. Vouchers including cones (if present) were gathered separately from every individual. The samples of cones and specimens were collected mostly from the north-west or western parts of the crowns, at a height of 2-2.5 m above ground. Currently the vouchers are maintained in the Institute of Botany, University of Life Sciences, Vienna, Austria. Diameter at breast height (dbh), tree height, stem form, and sex were recorded for 172 trees.

The voucher measurements were performed on dry material following closely the method described for *J. phoenicea* (Mazur et al., 2003). Three parts of each branchlet were chosen,

i.e. top, left and right, and short fragments of the one-year old growth were analyzed. In total, 2952 measurements of scales and 400 of cones were taken under a stereoscope microscope of 8x magnification with a scaled ocular.

The populations were compared on the basis of the following morphological traits:

(1) length of apical needle,

(2) length of lateral needle,

(3) width of apical needle,

(4) width of lateral needles,

(5) thickness of ultimate apical branchlet,

(6) thickness of ultimate lateral branchlets,

(7) number of apical scales,

(8) number of lateral scales,

(9) cone length,

(10) cone diameter (average of 2 independent measures in an angle of 90°),

(11) seed number per cone.

The main statistics, as arithmetic mean, standard deviations and variation coefficient were calculated for each individual separately and for each population. The interactions between the morphological traits were checked with Pearson's correlation coefficient. Due to different samples sizes cone related traits were excluded.

Table 3. Sampled populations of *Juniperus seravschanica* in Kyrgyzstan

Population	Longitude	Latitude	No. of vouchers	No.of cone bearing trees
Andarak (1)	69°28’	39°44’	8	70
Arka (2)	69°58’	39°58’	8	30
Batken (3)	70°54’	39°51’	15	120
Uch-korgon (4)	71°44’	39°56’	9	70
Nookat (5)	72°21’	40°12’	14	90
Suttuu bulak (6)	72°27’	41°29’	6	-
Kayindy (7)	72°46’	41°34’	6	-
Talas (8)	71°47’	42°46’	4	20
Total			**70**	**400**

Fig. 6. A typical pure Zeravshan juniper forest stand in the Province of Batken (Kyrgyzstan), [2000 m asl]

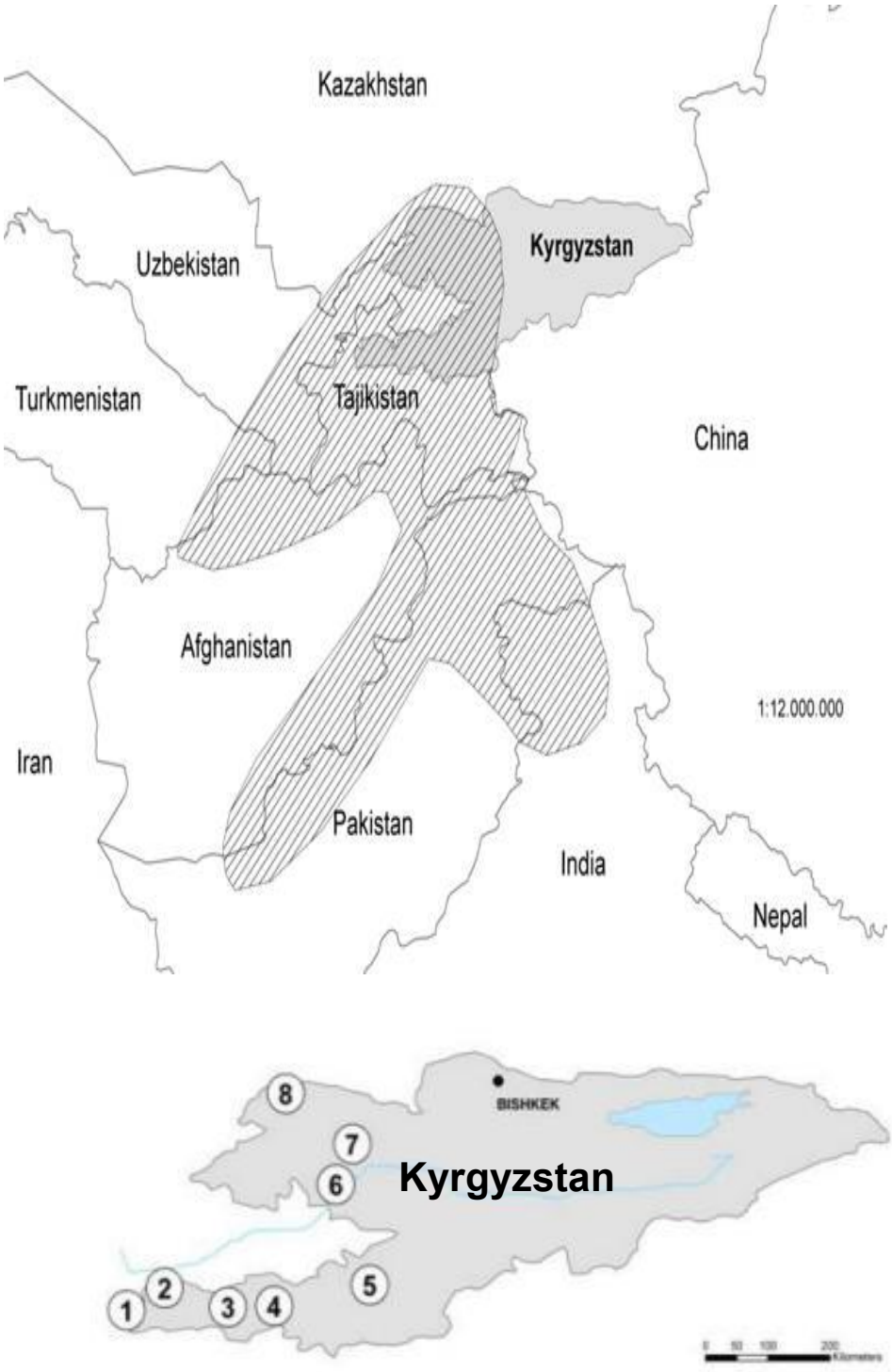

Fig. 7. Natural distribution of *J. seravshanica* in Central Asia and sampled populations in Kyrgyzstan

Table 3. Ecological conditions, sex ratio, and growth traits of sampled populations.

Pops	Alt., m	MAT, (°C)	APS, (mm)	MI	MTV, (°C)	PV, (mm)	MIV	Sex			DBH, (cm)	H, (m)	Stem form		
													Single	Double	Triple
1	1850	9.3	590	32.83	14.79	33.25	124.3	14	5	1	19.8	8.9	14	3	3
2	1550	11.3	412	51.69	18.33	31.69	149.0	18	5	-	16.1	6.95	11	7	5
3	2200	8.5	648	28.61	17.59	29.8	154.3	16	4	2	18.6	6.1	16	5	1
4	1750	8.3	648	28.37	16.78	31.25	142.8	16	5	1	23.1	7.5	14	5	3
5	1600	10.3	435	46.68	18.1	29.81	157.1	16	7	-	13.2	6.5	10	9	4
6	1300	8.8	626	30.04	12.27	34.5	107.6	19	3	-	38.5	8.6	21	1	-
7	1450	9.6	471	41.63	13.22	34.3	112.8	17	5	1	37.7	7.2	20	2	2
8	1300	10.6	394	52.41	16.96	29.71	151.2	14	3	-	17.0	7.0	12	5	-
Total / *Mean*	*1625*	*9.58*	*528*	*39.03*	*16.00*	*31.78*	*137.3*	**130**	**37**	**5**	*23*	*7.34*	**118**	**37**	**18**

MAT-mean annual temperature; APS-annual precipitation sum; MI-moisture index; MTV-mean temp. Vegetation time; PV- precipitation veg. time; MIV-moisture index veg.time;

Morphometric data were analyzed by ANOVA followed by Student-Newman-Keuls test for a multiple comparison of means. Homogeneity of variances was previously checked with the Bartlett test. Traits that were statistically different among the populations were further used to calculate pairwise Euclidean distances between the populations. These distances were used for the UPGMA-dendrogram and for correlations with geographical and altitudinal distances employing Mantel tests. Moreover, ecological data based on precipitation and temperature data, i.e. mean annual temperature (MAT), mean temperature during vegetation time (April-September) (MTV), annual precipitation sum (APS), precipitation sum in vegetation time (PV) annual moisture index (MI) and moisture index during vegetation time (MIV) were correlated with the Euclidian distances of the morphometric measures. As vegetation time the period between April and September was chosen. The moisture indices were calculated according to formulas of Wang et al. (2006): MI=(MAT+10)/(APS/1000) and MIV=(MTV+10)/(PV/1000). All statistical calculations for correlations, ANOVA, Bartlett test, Student-Newmann-Keul's test were performed with STATISTICA8.0. Mantel tests were drawn with GenAlEx 6.0 (Peakall and Smouse, 2006).

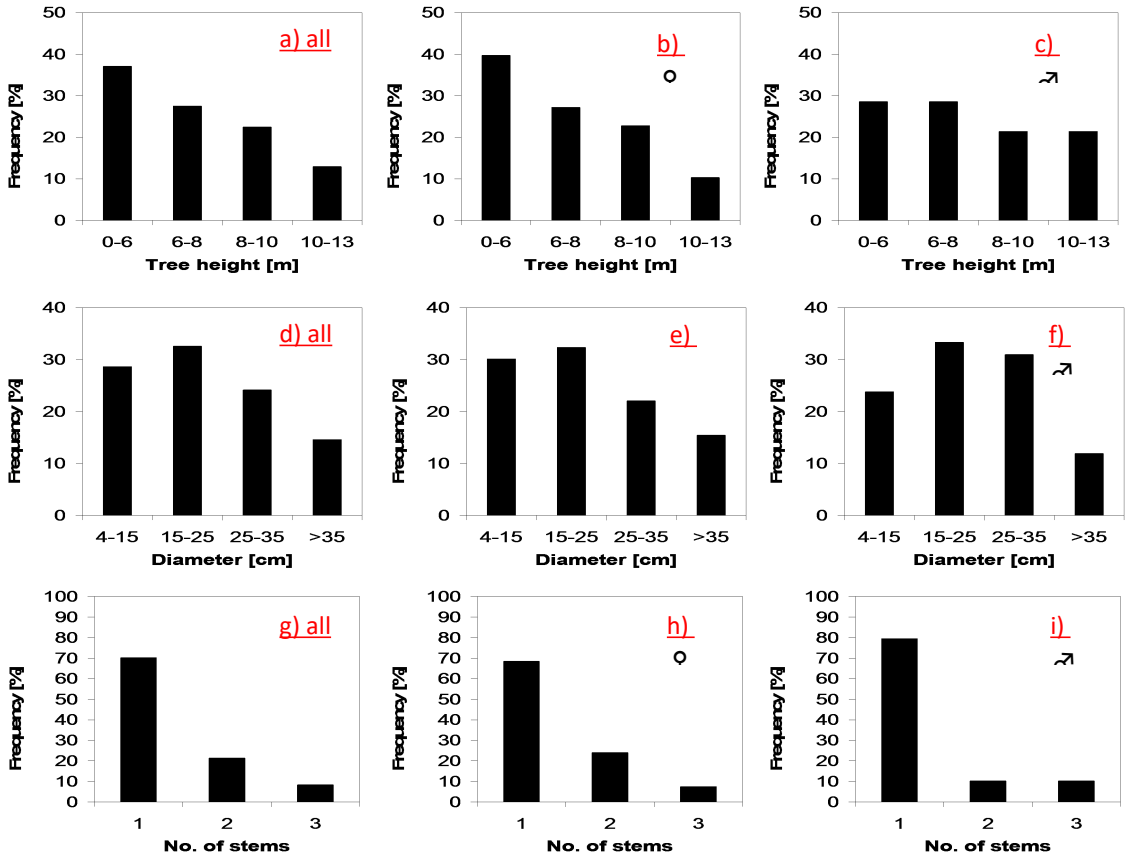

Fig. 8. Height and diameter at breast height distribution for all individuals (a, d, g), the female population (b, e, h), and the male population (c, f, i).

Table 4. Correlation coefficient of needle based traits

	Length of apical needle	Width apical needle	Width lateral needle	Thickness apical branchlet	Thickness lateral branchlet	Number of apical scale	Number of lateral scale
Length lateral needle	0.32*						
Width apical needle	0.48*	0.39*					
Width lateral needle	0.15	0.46*	0.48*				
Thickness apical branchlet	0.56*	0.29*	0.72*	0.47*			
Thickness lateral branchlet	0.19	0.36*	0.41*	0.69*	0.54		
Number of apical scale	-0.52*	-0.28*	-0.29*	-0.39*	-0.18	-0.25*	
Number of lateral scale	-0.28*	-0.77	-0.14*	-0.40*	-0.14	-0.24*	0.19

* Significance level P< 0.05

To analyse differences in vegetative growth between female and male individuals, the field measurements of dbh and height were assigned to distinct classes, where each class contained at least 5 individuals. A G-test for goodness of fit was used to test for differences in the dbh and height class distribution.

Based on the census of female and male trees (N_f and N_m) in all populations, the effective population size (N_e) was estimated as

$$N_e \approx \frac{4 N_f N_m}{N_f + N_m}$$

Bisexual trees were not considered.

Results

In Table 3 sex ratio and growth traits are recorded. All populations showed a female biased sex ratio. In total, 130 females, 37 males and 5 trioecious specimen were observed. In 50 % of the populations bisexual trees were found.

The average size of individuals within populations ranged from 6.1 m in Pop. 3 to 8.9 m in pop. 1, while the average dbh varied more considerably from 16.1 cm up to 38.5 cm, probably reflecting differences in age structure and population history. Since the sex ratio within the populations was similar (Table 3), all trees were pooled and the frequency distribution of dbh and height presented in Fig.8. Although a comparison of the sub figures 8b and 8c as well as the sub figures 8e and 8f indicates a trend for higher and thicker trees among the male individuals, the G-test could did not detect significant differences among the frequency distributions of the single sexes compared to all trees.

The analysis of the morphometric traits revealed an unimodal frequency distribution with homogeneous variances for all traits. Correlations between the traits are given in Table 4, showing significant correlation between the most needle traits.

An ANOVA for morphometric differences between the populations showed highly significant differences for the most needle traits (Tables 5 and 6), but not for the cone characteristics. The values of variation coefficient of the characters ranged from 7.8 % (width of lateral needle) to 20.1 % (number of seeds per cone) (Table 6)

Table 5. Results of ANOVA for 11 morphometric traits based on eight populations

Traits	SQ	FG	MQ	F	P
Apical needle length	2.169	7	0.310	5.369	.000
Lateral needle length	0.87	7	0.124	3.373	.004
Width of apical needle	0.43	7	0.061	5.576	.000
Width of lateral needle	0.227	7	0.032	6.965	.000
Thickness of ultimate apical branchlet	0.872	7	0.125	7.567	.000
Thickness of ultimate lateral branchlet	0.214	7	0.031	2.873	.012
Number of apical scales	61.61	7	8.802	1.416	.215
Number of lateral scales	93.48	7	13.350	1.752	.113
Cone length	8.13	5	1.626	1.214	.324
Cone diameter	6.262	5	1.252	.916	.482
Seed number per cone	4.256	5	0.851	1.458	.229

Table 6. Significant (P< 0.05) pairwise liner contrasts between populations based on different morphometric traits(Newman Keuls's post hoc test)

Populations	Andarak(1)	Arka (2)	Batken (3)	Uch-korgon (4)	Nookat (5)	Suttuu bulak (6)
Arka (2)	3, 5					
Batken (3)		1, 3, 4, 5				
Uch-korgon (4)		1, 4, 5				
Nookat (5)		2, 3, 4				
Suttuu bulak (6)	5		1, 3, 4,5	1, 4, 5	5	
Kayindy (7)		4, 5, 6				4, 5, 6
Talas (8)	4	1, 4, 5			1	1, 4, 5

Relevant traits were: (1) length of apical needle, (2) length of lateral needle, (3) width of apical needle, (4) width of lateral needle, (5) thickness of ultimate apical branchlet, (6) thickness of ultimate lateral branchlets.

A multiple comparison of means, as calculated by the Student-Newman-Keul test revealed the populations no. 2 (Arka) and 6 (Suttuu bulak) to be the strongest outliers. This is reflected also by the UPGMA-dendrogram (Fig. 4): here, pop. 2 and 6 form a single group with a large distance to the others. Within the remaining 6 populations, Andarak (pop. 1) forms a single group, while the pop. 5 and 7 on the one side and the pop. 3, 4, and 8 on the other side group together. In order to test if the observed variation in needle traits is driven by geographical, altitudinal or environmental variation, a series of Mantel test was employed (Tab. 8). However, none of the tested correlations was significant.

The average effective population size was estimated to be approximately 70 % of the census size.

Discussion

A morphometric analysis of nine Zeravshan juniper populations from Kyrgyzstan showed significant variation in length, wide and thickness of needles. However, this variation could not be related to geographical, altitudinal or any of the environmental variables tested. But what are the reasons for this variation? In general, morphometric differences in needle characteristics could be caused either by inherited genetic variation or by short-term adaptation to the environmental conditions during needle growth and elongation.

Patterns of inherited genetic variation of morphometric traits in tree species reflect evolutionary responses to environmental conditions often corresponding to distinct geographic ranges of races or varieties. For example, shoots and needles of eleven *Abies alba* populations were thoroughly analyzed morphologically in a common garden experiment (*ex situ*); populations originating from Southeastern Europe differed clearly from those of Western and Central Europe (Aas et al., 1993). While a morphometric differentiation among different populations in a common environment is most suitable, such plant material is not available in most cases and, hence, conclusions based on morphological comparisons *in situ* are much more limited. *In situ* studies were exemplarily done in *Acacia victoriae* confirming three subspecies within Australia (Ariati et al., 2007) or *Pinus ponderosa*, where morphometric traits can easily differenciate two varieties in North America (e.g. Wright et al., 1969). Also within the genus *Juniperus*, morphological differences among populations of a single species have been found for characteristics of cones, seeds and leaflets, but not for needle traits. For example, tree populations originating from two subspecies of *Juniperus phoenicea*, i.e. subspecies *turbinata* and

phoenicea (Mazur et al., 2003) or ten populations of *J. oxycedrus* sampled throughout its Mediterranean range (Klimko, 2007) could be readily differentiated. Inherited genetic variations should at least correlate with geographic or environmental distance, because patterns of differentiation are most readily interpreted as the result of drift, limited gene flow and selection triggered by different environmental conditions. However, it was somewhat unexpected, that all climatic variables tested were not significantly Mantel-correlated with the traits. In particular, we expected the water-supply related measures to correlate with needle characteristics, because for selection often water availability is one of the major differentiating environmental elements (e.g., Grant et al., 1989) also resulting into contrasting physiological performances or needle length differences (Peguero-Pina et al., 2007). It is prompting to speculate, that ecological and morphometric pair-wise distances were too small based on the present sampling. On a restricted geographic scale, morphometric differentiation among *Juniperus* populations seems to be generally weak (Mazur et al., 2004).

Table.7 Measurements of 11 morphometric traits : (1) length of apical needle (mm) , (2) length of lateral needle (mm),(3) width of apical needle(mm), (4) width of lateral needles (mm), (5) thickness of ultimate apical branchlet (mm), (6)thickness of ultimate lateral branchlets(mm), (7)number of apical scales, (8)number of lateral scales,(9)cone length (mm),(10)cone diameter (average of 2 independent measures in an angle of 90°) (mm),(11)seed number per cone.

Characters Statistic	Populations	1	2	3	4	5	6	7	8	9	10	11
Mean	1	1.75	1.16	0.86	0.75	1.21	0.923	13.50	15.62	11.51	10.91	2.66
	2	1.377	1.15	0.72	0.67	0.90	0.860	13.75	17.62	10.41	9.98	2.72
	3	1.84	1.34	0.98	0.83	1.24	0.980	12.06	15.13	10.32	10.21	3.03
	4	1.81	1.45	0.90	0.83	1.16	1.010	12.33	14.55	11.09	10.74	3.36
	5	1.60	1.44	0.90	0.78	1.14	0.960	13.28	13.92	10.49	10.21	3.24
	6	1.43	1.31	0.77	0.73	0.93	0.870	15.00	15.66	-	-	-
	7	1.64	1.44	0.87	0.84	1.14	1.040	13.33	13.50	-	-	-
	8	1.97	1.30	0.86	0.87	1.18	0.990	11.20	14.50	10.46	10.31	4.05
	All samples	1.67	1.32	0.85	0.78	1.11	0.95	13.05	15.06	10.71	10.35	3.17
Minimum	1	1.57	1.02	0.75	0.70	1.04	0.83	11.00	12.00	10.65	9.74	2.00
	2	1.17	0.97	0.59	0.62	0.73	0.73	9.00	16.00	9.60	9.70	2.30
	3	1.26	0.76	0.81	0.70	1.08	0.87	8.00	12.00	9.20	9.22	2.30
	4	1.70	1.04	0.80	0.65	1.03	0.93	10.00	10.00	9.41	9.50	2.10
	5	1.34	1.21	0.76	0.68	0.94	0.79	10.00	12.00	8.87	9.58	2.20
	6	1.17	1.20	0.63	0.65	0.76	0.81	12.00	12.00	-	-	-
	7	1.46	1.21	0.82	0.80	1.04	0.82	12.00	12.00	-	-	-
	8	1.87	1.13	0.73	0.79	0.97	0.92	8.00	12.00	9.40	9.60	3.90
	All	1.4	1.06	0.73	0.69	0.94	0.83	8.5	12.2	9.52	9.55	2.46

	samples	4							5			
Maximum	1	2.25	1.32	1.00	0.82	1.29	1.07	17.00	19.00	14.01	13.91	3.50
	2	1.49	1.51	0.84	0.73	1.03	0.99	18.00	22.00	11.10	11.07	3.10
	3	2.41	1.65	1.29	0.97	1.40	1.13	18.00	25.00	12.60	11.43	4.40
	4	2.23	1.93	1.15	0.97	1.30	1.18	15.00	20.00	13.44	12.93	5.66
	5	2.01	1.74	1.06	0.93	1.44	1.34	18.00	18.00	12.45	12.67	4.50
	6	1.58	1.52	0.99	0.83	1.12	0.92	18.00	18.00	-	-	-
	7	1.88	1.90	0.93	0.87	1.27	1.18	18.00	15.00	-	-	-
	8	2.25	1.54	1.00	0.92	1.31	1.10	15.00	18.00	11.50	11.40	4.20
Variation coefficient	All samples	2.01	1.63	0.80	0.88	1.27	1.11	17.12	19.37	12.51	12.23	4.22
	1	12.10	9.40	11.90	6.10	9.40	8.60	15.30	17.40	10.00	14.00	20.30
	2	9.70	14.80	11.00	6.00	10.70	10.60	19.30	13.90	7.00	7.00	14.80
	3	18.70	16.60	11.80	7.70	8.20	7.60	23.70	22.90	7.70	7.30	18.10
	4	15.20	18.00	13.00	12.90	9.60	9.40	14.60	23.60	15.10	12.60	35.60
	5	12.30	10.70	9.30	9.40	15.40	14.80	18.50	15.50	10.90	10.80	26.40
	6	11.20	8.50	20.70	10.50	16.80	4.90	16.30	13.20	-	-	-
	7	10.30	16.50	5.50	3.40	6.90	14.30	18.20	9.10	-	-	-
	8	9.30	12.80	12.70	6.40	15.20	6.00	26.40	20.70	14.30	12.10	5.20
	All samples	12.35	13.41	11.98	7.80	11.40	9.52	19.03	17.01	10.83	10.63	20.06

Table 8. Mantel test correlation of values using needle-based characters (MAT-mean annual temperature; APS-annual precipitation sum; MI-moisture index; MTV- mean temperature at vegetation time; PV-precipitation at vegetation time; MIV- moisture index at vegetation time.

Value	Geographical distance	Altitudinal distance	MAT	APS	MI	MTV	PV	MIV
P	.570	.411	.363	.334	.308	.221	.528	.505

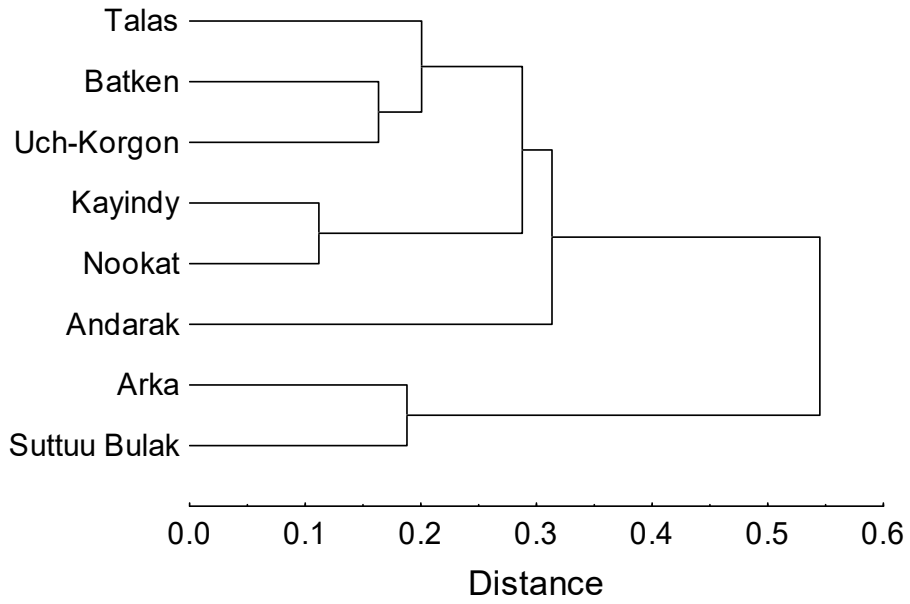

Fig 9. UPGMA-Dendrogram of Euclidian distances between populations of *J .seravschanica* Kom

A short-term morphological/physiological adaptation of the needle characters to the water supply in the past and recent growing seasons could be another explanation for the observed pattern. Such drought effects have been observed for a range of other species as well, for example in *Picea abies* (Palátová, 2004), *Pinus canariensis* (Grill et al., 2004), or *Pinus cembroides* (Poulos and Berlyn, 2007). Larcher (1994) explained this adaptation by lesser photosynthetic production and a reduced cell elongation evoked by smaller turgor pressure. Due to the remote location of the studied populations and difficulties to get

precise weather data from this region we cannot test if drought periods within the last few years are responsible for the observed needle variation.

For reliable conclusions on the causes and meaning of needle variation, common garden experiments or at least heritability estimates of the quantified traits would be helpful. However, these data are not available for *J. seravschanica* and hence only scientific guesses are possible. Even in widely distributed conifers of high economic value, heritability estimates of the traits used in this study are very limited. For Ponderosa pine, Grant et al. (1989) reported a high heritability for the primary needle length, but a low heritability for the secondary needle length, which was first of all shaped by the environment. Moreover, in Douglas-fir it is known, that needle anatomy including needle length is not independent on age (Apple et al., 2002). Ontogenetic effect may further interfere with needle morphs. In conclusion, it is unknown whether the measured needle traits simply reflect imprints of environmental factors on the phenotypic expression, the traits are exclusively genetically controlled or are – to a varying extent – controlled by genes and shaped by environmental factors.

It is well known that in plant species reproductive success may be compensated by less vegetative (somatic) growth, i.e. differences in vegetative growth rate may be associated with differences in reproductive investment of males and females (e.g. Obeso, 2002). In dioecious species, the reproductive effort in females is usually higher than in males and in consequence in habitats with low availability of resources male-biased sex ratios are to be expected. While the sex ratio in six populations of *J. oxycedrus* scarcely deviated from unity (Ortiz et al., 1998), in *J. communis* both male and female biased sex ratio were found in different populations (Marion and Houle, 1996) and more recently Ortiz et al. (2002) showed that sex ratio in *J. communis* subsp. *alpina* shifted towards male-biased ratios along an altitudinal gradient. The findings of the present study were somehow unexpected, because in contrast to our expectations we could not detect different sex ratios in different ecological environments. Moreover, differences in vegetative growth between males and females were also anticipated; but besides a slight trend towards higher and thicker trees among the male populations differences were statistically weak.

Estimates of effective population size are important for the assessment of vulnerability. As a rough estimate approximately 70 % of the census can be considered. Although this estimate might be slightly conservative since bisexual trees were not considered, it may be helpful for plant conservation.

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XIII. Part B. North-south population subdivision of *Juniperus seravschanica* in Kyrgyzstan revealed through novel plastid DNA markers

Introduction

Population structure and phylogeography of forest tree species have been the aim of a multitude of studies. At present the population history of many tree species in Europe and North America is known (e.g., Petit et al., 2003; Soltis et al. 2006). Diverse modes of species movements after glaciations or drastic changes in population structure like bottlenecks have been detected. This knowledge is important to understand processes that have shaped current genetic patterns, as well as to develop management strategies to conserve species effectively (Frankham et al., 2002). However, very little is known on the population history of forest trees from other regions of the temperate north, e.g. Central Asia. Relatively few details are known on the vegetation history of that area, but accumulated evidence indicates that during the last glacial maximum (LGM; ca. 18,000 yrs BP) and early Holocene very cold (permafrost) and arid conditions prevailed in the region (Aubekerov & Gorbunov, 1999) that drove the forest to refugia further south. After these desert conditions a warmer and more humid period (8000-5000 yrs BP) with more extensive vegetation cover followed. Later the climate became drier but stable in the region (Adams & Faure, 1997). Here we analyze the genetic diversity and population differentiation of a forest tree species in Kyrgyzstan to fill knowledge gaps on the vegetation history of the region.

Kyrgyzstan is a mountainous country that is located in the western (outer) part of the Tien-Shan range. The climate is mainly semi-arid and only 4.2% of the land surface is covered by continuous forests (Musuraliev, 2004). These are predominately juniper (*Juniperus* L., *Cupressaceae*) forests (locally called "archa") which are found typically on dry foothills or at mid to high altitudes between 1000 and 3500 m asl, where trees take on prostrate growth. Natural juniper forests in Kyrgyzstan occur in two parts of the country: the south-west and the north-west, with the Fergana Valley and the humid Fergana Range separating the distribution. The most important contribution to the understanding of the vegetation history of western Kyrgyzstan was provided by Beer et al. (2007; 2008a, b). Their findings indicate that open juniper forests were present in the south-west (Alay Range) and north-west (Chatkal Range) at least since 6000 years BP and since that time juniper pollen records have remained relatively stable. In contrast, pollen of this genus was far less

common close to the Fergana Range in the north-west of the country (Beer et al., 2008b), indicating that *Juniperus* has markedly increased its abundance there since ca. 1000 years BP. Before 10,000 BP large parts of the region were probably affected by permafrost (Aubekerov & Gorbunov, 1999). In consequence it can be hypothesized that tree populations currently occurring in the area are of relatively recent origin, and that colonization originated from refugia further south, since the extremely cold and dry conditions during the LGM and early Holocene very likely did not sustain woody vegetation.

Juniperus seravschanica Kom. (= *J. polycarpos* var. *seravschanica* [Kom.] Kitam.) is a member of the section *Sabina* (Adams, 2004; Farjon, 2005) within the genus. It is a wind-pollinated, slow growing shrub or small tree occurring mainly in open stands. The species is dioecious and female trees produce fleshy cones that are eaten and dispersed by birds. Zeravshan juniper is a typical species of mixed juniper forests and grows on mountain slopes and foothills between 1000 and 2500 m asl of western Kyrgyzstan (Djanaeva, 1965); other common species with similar ecology and distribution include *J. semiglobosa* Regel and *J. pseudosabina* Fisch. & Mey. (Muhamedshin, 1967; Chub, 2003). The Kyrgyz range of Zeravshan juniper forms the northern part of the species' distribution. It is also found in parts of Tajikistan, Pakistan, India, and Afghanistan (Fig. 10C). The species plays an important role in conserving and regulating water regime and protecting soils from erosion and settlements from mudslides (Kosmynin et al., 2008). At present illegal logging and over-grazing by livestock (Chorfi et al., 2004) cause decline and fragmentation of natural populations. In addition, global warming with an already observed increase of warmer and drier periods in the area (Bolch, 2007) threatens these forests. Despite the ecological importance of *J. seravschanica* very little is known on its genetic diversity.

In the present study, we focus on plastid DNA which is putatively paternally inherited in junipers (Provan et al., 2008). Plastid DNA has already been used to characterize genetic diversity and population structure in other *Juniperus* species in Asia (Zhang et al., 2005; Opgenoorth et al., 2010; Guo et al. 2010) and Europe (Provan et al., 2008; Juan et al. 2011). Here we investigate the population history of *J. seravschanica* as a model for the juniper forest community in the region to test hypotheses regarding the spread of the species after the LGM and to elucidate patterns of genetic diversity, population differentiation and gene flow. Fifteen natural populations of *J. seravschanica* from Kyrgyzstan were analyzed, employing novel plastid minisatellites together with haplotype specific PCR and PCR-RFLP markers.

Materials and methods

Population sampling

Samples were collected from 15 populations covering the entire distribution of the species in Kyrgyzstan (Table 9, Fig. 10A). Twig samples were collected from 16 to 78 individuals per population, with a distance of at least 50 m between trees. The latitude, longitude and altitude of each tree were recorded. In total 540 samples of *J. seravschanica* were collected. All samples were immediately dried in silica gel for transport, and then stored at -20°C until DNA was extracted.

DNA extraction, amplification, and sequencing

Total DNA was extracted from ca. 50 mg of plant material using the DNeasy Plant Mini Kit (Qiagen) according to the manufacturer's protocol. DNA concentration was measured on a Nanodrop Spectrophotometer ND-1000 (Peqlab). A preliminary screening of the plastid genome using a set of universal primers to identify phylogenetically useful polymorphisms was conducted on 16 individuals (two from each of eight different populations, evenly dispersed over the covered range). The following regions were amplified using the PCR primers from the respective papers: *atp*H-*atp*I, *atp*I-*rpo*C2, *petB*-*petD* (Grivet et al., 2001), *trnS*-*trnG,* (Hamilton, 1999), *trn*V intron (Wang et al., 2003), *psb*C-*trn*S, *trn*D-*trn*T (Demesure et al., 1995), and *trn*T-*trn*F (Taberlet et al., 1991). PCR was performed in 20 µL volumes, containing ca. 80 ng plant DNA, 1x PCR buffer, 2.5 mM $MgCl_2$, 0.5 mM each dNTPs, 0.2 µM of each primer, and one unit of Platinum *Taq* polymerase (Invitrogen). PCR conditions were 94° C for 3 min, followed by 35 cycles of 94° C for 1 min, 55° C (for *atp*H-*atp*I, *atp*I-*rpo*C2, *trn*S-*trn*G, *trn*T-*trn*F, *trn*D-*trn*T, *trn*V intron, *pet*B-*pet*D); 50° C (for *psb*C-*trn*S) 1 min, and 72° C for 2 min plus a final extension of 72° C for 7 min. PCRs were carried out on a PTC200 Gradient thermal cycler (MJ). Double-stranded PCR products were purified using the NucleoSpin Extract II PCR Purification Kit (Macherey-Nagel) and commercially sequenced from both directions using the same primers as in the PCR. The sequences of the examined regions were aligned using ClustalX2 (Larkin et al., 2007). Minor adjustments of the alignment were done manually.

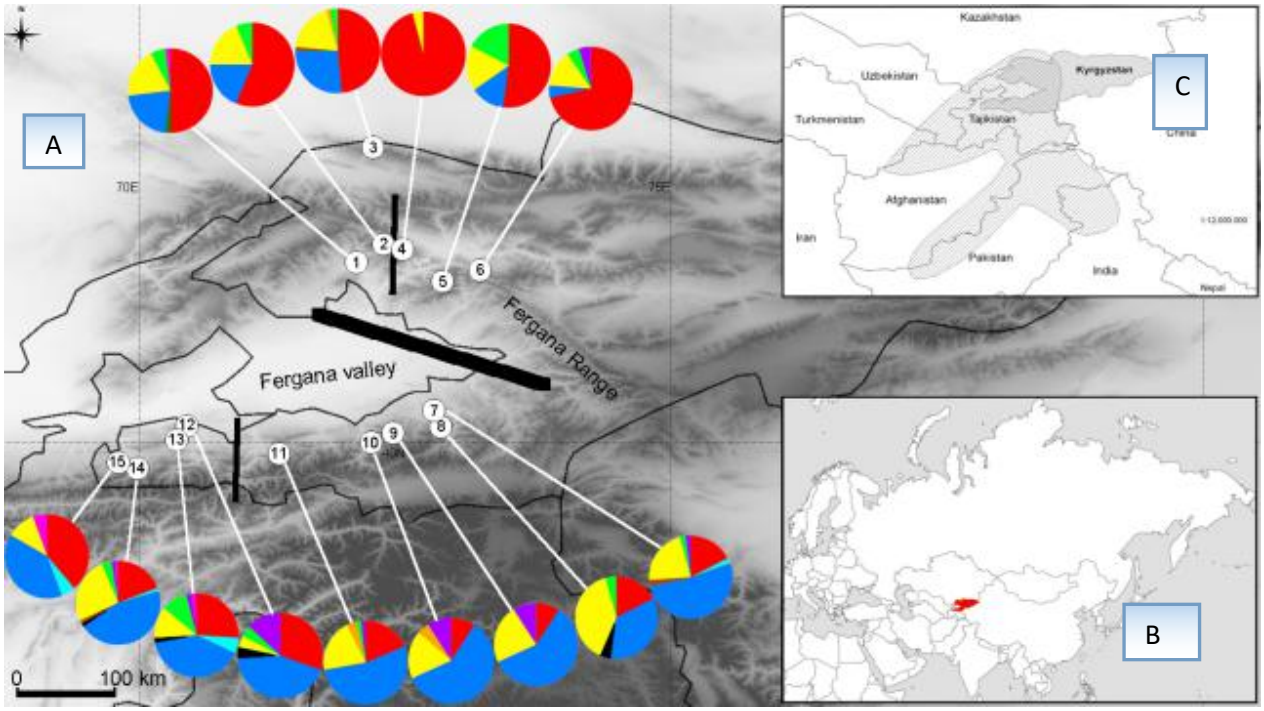

Fig.10. (A) Geographical position and distribution of plastid DNA haplotypes in 15 populations of *J.seravschanica* Kom from Kyrgyzstan. Colors correspond to the haplotypes in the legend. Genetic boundaries computed with BARRIER are indicated by black bars with the first barrier in bold, number indicates percent support. (B) Locality map of the study region. (C) Distribution range of *J.seravschanica* Kom. According to Adams (2004).

Subsequently polymorphisms detected in the *trn*V, *trn*D-*trn*T, and *atp*H-*atp*I regions were used to characterize genetic diversity in the species. Allele specific PCR was used to genotype a single nucleotide polymorphism (SNP) in the *trn*V intron revealed by sequencing. Nested selective forward primers were designed for this polymorphism following the approach of Provan et al. (2008). The PCR protocol was as follows: initial template denaturation was programmed at 94ºC for 3 min, followed by 11 touchdown cycles of denaturation at 94º C for 1 min, annealing at 65ºC for 1 min (-0.7º C per cycle), extension at 72º C for 1 min, followed by 24 cycles of denaturation at 94º C for 1 min, annealing at 58º C for 1 min, extension at 72º C for 1 min, and final extension 72º C for 5 min. PCR was carried out in a total volume of 10 µL containing 60 ng of genomic DNA, 10 pmol of forward primer (GCTATACGGGCTCGAAC), 10 pmol of reverse primer (TACCTACTATTGGATTTGAACC) and 10 pmol of nested SNP-selective primer (TCACAGTTTAGTCTTAAAAATAG or TCACAGTTTAGTCTTAAAAATAT, respectively), 1x PCR buffer, 200 µM of each dNTP's, 2.5 mM $MgCl_2$ and 0.25 U of Platinum *Taq* Polymerase (Invitrogen). PCR products were separated on 2% agarose gels

and visualized with ethidium bromide staining. A PCR-RFLP approach (Lowe *et al*., 2004) was implemented for the screening of all individuals for length variation in the *trn*D-*trn*T region. *Pfe*I was used to digest the 673bp PCR product from the *trn*D-*trn*T region according to the manufacturer‘s instructions (Fermentas).

Fragments in the *atp*H-*atp*I region containing a plastid minisatellite were amplified with a primer pair designed using FastPCR (Kalendar et al., 2009): RTTTTATAAATGAGCAAATGATT, ATAGGTGAATCCATGGAGGG. The forward primer was labelled with a fluorescent dye (D4; Sigma-Aldrich). Amplification was performed in a PCR mixture containing a 1x buffer S (containing 2 mM $MgCl_2$), 0.2 µM of forward and reverse primers, 50 µM of dNTPs and 0.25 units of *Taq* polymerase (Peqlab). PCR cycling program was following: initial denaturation at 94ºC for 5 min, then 30 cycles of 1min at 94ºC, 1 min annealing at 58ºC, 1 min at 72ºC and final elongation at 72ºC for 10 minutes. In total 540 individuals were genotyped using a CEQ8000 Genetic Analysis System (Beckman Coulter) with an internal size standard for exact determination of minisatellite length polymorphisms.

Data analyses

Gene diversity was estimated for each population as ^{2} where p_i is the frequency of the i^{th} haplotype (Nei, 1973). Total haplotype diversity (H_T) and gene diversity due to variation within populations (H_S) were estimated in order to assess the proportion of total haplotype diversity due to frequency differences among populations (G_{ST}). The presence of a phylogeographic structure was tested by comparing G_{ST} with N_{ST}. The latter measure takes size similarities between haplotypes into account, thus the relationship between both measures can reveal whether phylogeographic structure is present. These calculations were done with PERMUT (Pons & Petit, 1996). A UPGMA dendrogram based on a distance matric using Nei’s pairwise distance corrected for uneven sample size was constructed in PHYLIP v3.68 (Felsenstein 2005) from data of the 15 populations. In order to account for different sample sizes, haplotype richness with rarefaction (R) was calculated using CONTRIB (Petit et al., 1998), setting the minimum number of samples per population to 15.

A Mantel test was applied in GenAlex v6.2 (Peakall & Smouse, 2006) to test for an isolation-by-distance (IBD) pattern. Euclidian distances between sampling locations and Nei’s genetic distance were employed. This analysis was performed for the whole data set and separately for northern and southern population groups. In order to use ARLEQUIN v3.5 (Excoffier & Lischer, 2010), haploid data were binary coded: the SNP polymorphism

was coded as RFLP data and the number of minisatellite repeats were coded with "1" and "0" according to presence or absence of the minisatellite repeats (Navascues et al., 2006). Indels, SNPs, and minisatellites probably have different mutation rates (Cozzolino et al., 2003); however, the indels and SNP were located only on few peripheral haplotypes (Fig. 6) and occurred in low frequency, therefore all loci were treated the same way. Inter-population differentiation and differentiation between regions were estimated from haplotype frequencies calculated within the analysis of molecular variance (AMOVA) framework (Excoffier et al., 1992). A minimum spanning network based on relationship between plastid DNA haplotypes was constructed using ARLEQUIN 3.5.

Boundaries of sharp changes in haplotype frequencies were evaluated using BARRIER v2.2 (Manni et al., 2004). This analytical method makes use of Monmonier's maximum difference algorithm (Monmonier, 1973) to find edges associated with the highest rates of change in a distance matrix. The algorithm is applied to a network of geographic distances among populations using Delaunay triangulation. Barriers are placed perpendicular to edges that correspond to the largest genetic distances (Nei's corrected pairwise distance) and are continued across adjacent edges in order to maximize genetic distance until the barrier reaches the limit of the network space, or a previously determined barrier. One hundred bootstrapped distance matrices were used to evaluate consistencies of barriers.

Results

Identification and variation of minisatellite sequences

Except the polymorphisms detected in the *trn*V, *trn*D-*trn*T, and *atp*H-*atp*I regions all other sequenced regions were monomorphic in *J. seravschanica*. Sequencing of the *atp*H-*atp*I PCR products (GenBank HQ341798-HQ341803) revealed that the observed length polymorphism was due to variation in the number of copies of a 32 bp minisatellite and a 5 bp deletion. This minisatellite corresponds to the 5' end of the intergenic spacer region adjacent to the stop codon of the *atp*I gene in *Cryptomeria japonica* (GenBank AP010967). Capillary gel electrophoresis analysis at this locus allowed the identification of six length variants ranging from 123 bp to 283 bp, corresponding to one to six perfect repeats of the minisatellite motif (including the flanking region). A PCR product 5 bp shorter than observed in all other individuals for the *atp*H-*atp*I minisatellite region was obtained in seven individuals, and these were sequenced to confirm the cause of this polymorphism. In all of these samples a 5 bp deletion within the primary minisatellite motif was revealed.

The occurrence of this deletion was centred in the southern range of the species as three samples originated from Kempir Oi (Pop 13), one from Karhana (Pop 15), one from Andarak (Pop 14), and one from Abshir ata (Pop 7). These six individuals carried two repeats of the minisatellite (GenBank HQ341802; haplotype 2). Only a single sample from a northern population (Padysha ata, Pop 1) carried the same 5 bp deletion, but only one repeat of the minisatellite (GenBank HQ341803; haplotype 3).

PCR-RFLP and sequencing of the *trn*D-*trn*T intergenic spacer region identified two different size variants (639 and 673 bp, respectively). The cause for this size polymorphism was another 34 bp repetitive minisatellite (GenBank HQ341806-HQ341807). Variation was low for this locus, as only 7 individuals (Pop 3: 2, Pop 7: 1; Pop 11: 2, Pop 10: 1, Pop 14: 1) were affected. No further haplotype variants were detected when all sampled individuals were screened using PCR-RFLP. Further within the *trn*V intron region (734bp) a single transversion (T to G) at site 586 was detected (GenBank HQ341804-HQ341805). Allele specific PCR was utilized to screen all individuals for the latter polymorphism. In total four individuals differed in this point mutation, these originated from four different populations from the southern Kyrgyz range (Table 9).

Genetic diversity and population structure

Screening all 540 individual trees for polymorphisms in the *atp*H-*atp*I, *trn*D-*trn*T and *trn*V intron regions resulted in eleven different plastid haplotypes (H1 – H11; Table 10). Haplotype frequencies and diversity estimates of each population are presented in Table 8 and their geographical distribution are depicted in Fig. 10A; the minimum spanning network showing relationships between haplotypes is given in Fig. 11.

Table 10. Variable sites of the aligned sequences of four plastid DNA fragments in 11 haplotypes of *J. seravschanica* (*-*atp*H-*atp*I minisatellite motif: ATTAAAAGACTAAGTCCAATTAATTAAATTAA; § *trn*D-*trn*T minisatellite motif: AAAAATATTCAAATCCCTAGGAATTTGTCAATAT). The first six numbers of the binary code indicate the number of repeats in the atpH-atpI minisatellite locus; the following numbers indicate presence or absence of *atp*H-*atp*I 5 bp deletion, *trn*D-*trn*T minisatellite repeat, and *trn*V intron SNP, respectively.

Haplotype no.	Binary Code	Locus			
		***atp*H-*atp*I MS* no. of repeats**	***atp*H-*atp*I 5 bp del**	***trn*D-*trn*T MS§ no. of repeats**	***trn*V intron SNP**
1	100000000	1	0	0	T
2	110000100	2	1	0	T
3	100000100	1	1	0	T
4	110000000	2	0	0	T
5	110000001	2	0	0	G
6	110000010	2	0	1	T
7	111000000	3	0	0	T
8	111000010	3	0	1	T
9	111100000	4	0	0	T
10	111110000	5	0	0	T
11	111111000	6	0	0	T

The three most common haplotypes (H1, H4, and H7) strongly dominated in both northern and southern populations with a compound frequency of 91.9 % and 87.3 %, respectively (89.3% total average). However, different haplotypes were predominant in the north and south: in the northern populations (Pop 1 to Pop 6) the dominant haplotype was H1, with Bakai (Pop 4) almost fixed for this haplotype. Haplotype (H3) was private to Padysha ata (Pop 1) in northern Kyrgyzstan. In the southern populations (Pop 7 to Pop 15) the

dominant haplotype was H4 and haplotype diversity within populations was higher (Fig. 10A; Tab. 9). Haplotypes H2 and H5 were private to populations in the south. The UPGMA distance tree supported a northern and a southern group of populations (Fig. 12).

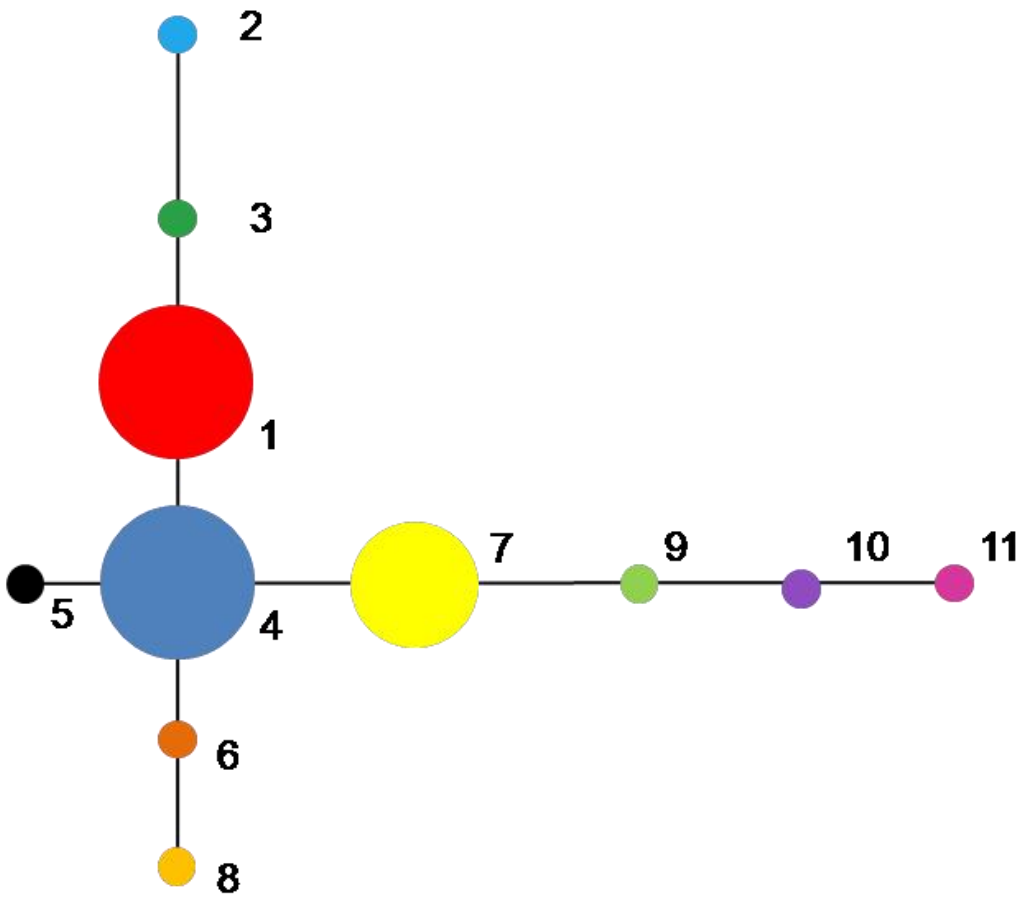

FIG. 11. Minimum spanning network of relationships between plastid DNA haplotypes detected in 15 populations of *Juniperus seravschanica* in Kyrgyzstan. Numbers indicate haplotypes. The three most common haplotypes are indicated by larger circles; circle size not to scale.

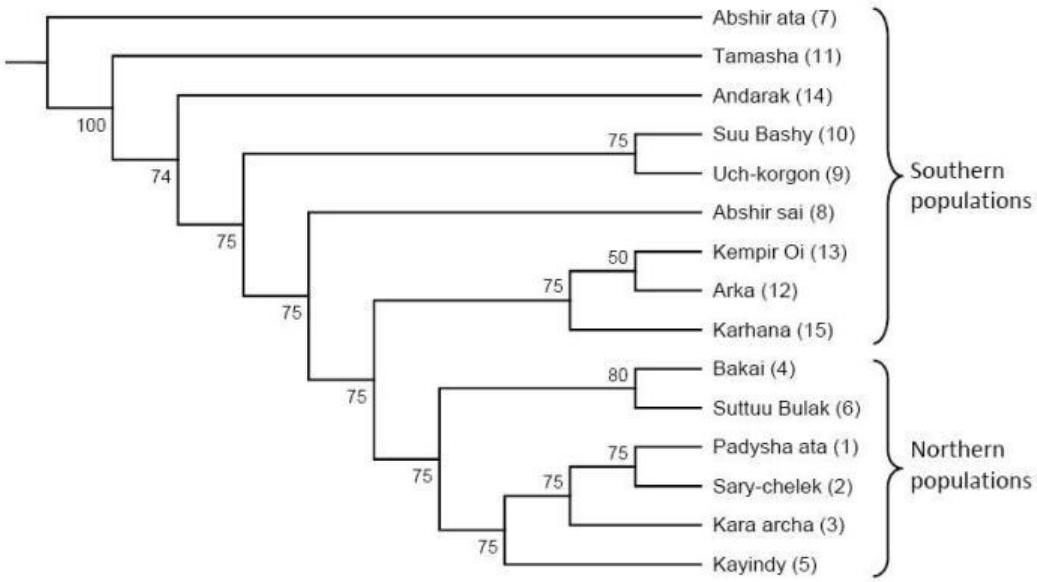

FIG. 12. UPGMA tree based on Nei's corrected pairwise genetic distances between 15 natural populations of *Juniperus seravschanica* in Kyrgyzstan (population numbers according to Table 9).

Allelic richness was significantly higher in the southern populations than in the northern ones (3.47 vs. 2.61; $U = 48$, df = 1, $P = 0.012$), even when omitting Bakai (Pop 4; U=39, df = 1, $P = 0.028$) which showed very low levels of haplotype diversity as mentioned above. Total haplotype diversity was estimated as H_T=0.713 (SE=0.014) and within population diversity as H_S=0.609 (SE=0.042). Population differentiation G_{ST} equalled to 0.125 (SE=0.052). N_{ST} (0.124 [SE=0.050]) was similar to G_{ST} indicating no phylogeographic structure for the species in Kyrgyzstan. A significant correlation between genetic and geographic distance was detected with a Mantel test for the whole set of populations (R^2=0.102; P=0.01). When the analysis was done separately for northern and southern population groups the Mantel test was significant only in the southern populations (R^2=0.129; P=0.02). Northern populations (G_{ST}=0.058) were stronger differentiated than southern ones (G_{ST}=0.013); N_{ST} again was not significantly different from G_{ST}.

The analysis of molecular variance (AMOVA) revealed that 86.6 % of the variation was found within populations, whereas 11.9 % of the variation was found between regions (northern vs. southern populations) and only 1.5 % among populations within regions (Table 10). The BARRIER analysis detected several significant barriers to gene flow; the

first and most important one was the Fergana Valley and Fergana Range separating northern from southern populations (99 % bootstrapped support) (Fig. 10A).

Table 11. AMOVA results for 15 populations of *Juniperus seravschanica* from Kyrgyzstan. Groups were defined by northern and southern populations.

Source of variation	Degrees of freedom	Sum of squares	Variance components	Percentage of variation	P value
Among groups	1	18.98	0.07114	11.89	0.0000
Among populations within groups	13	10.67	0.00878	1.47	0.0255
Within populations	525	272.126	0.51833	86.64	0.0001
Total	539	301.776	0.59825		

The second barrier (75 % bootstrap support) separated the northern populations Sary-chelek (Pop 2) and Bakai (Pop 4), the latter population being small and having the least haplotype diversity of all investigated populations. Further barriers were subdividing the remaining populations but were not as well supported (not shown).

Discussion

Population differentiation and gene flow

The 15 populations of *J. seravschanica* investigated in this study showed moderate population differentiation (Table 10). The G_{ST} value (0.125) was relatively high compared to 29 species with paternally inherited markers (G_{ST}~0.10; Petit et al., 2005), but lower than found in the congeneric *J. przewalskii* Komarov (G_{ST}=0.772; Zhang et al., 2005), and *J. sabina* (G_{ST}=0.926; Guo et al. 2010) sampled from a wider geographic range.

Comparison between G_{ST} and N_{ST} revealed no significant differences, indicating a short period of population divergence, not reflected in haplotype size differences. However, G_{ST} was higher in northern vs. southern populations (0.058 and 0.013, respectively).The observed comparatively low level of genetic divergence is in agreement with the low level of phenotypic variation in the species (Sultangaziev et al., 2010). The Mantel test indicated a weak but significant IBD pattern for the total data set as well as for populations in the south. Isolation-by-distance was not found in the northern populations. This together with the differences in G_{ST} and allelic richness clearly indicates disruption of gene flow among northern populations. Genetic drift appears to be an important evolutionary mechanism in the latter region. The two groups were also clearly identified in the UPGMA distance tree (Fig. 12): the southern group with relatively high diversity and the northern group with less variation. The BARRIER analysis corroborated these findings. Haplotype diversity was strongly partitioned due to regional drift, with more than 10 % of the variation found between regions and only 1.5 % of the variation between populations within regions. This geographic pattern supports the scenario of post-glacial spread from the south to the north (Petit et al., 2003). On the regional level *Juniperus* species with similar ecology often exhibit low levels of population differentiation following postglacial range expansion (e.g., Juan, et al., 2011; Guo et al., 2010). With the applied highly polymorphic minisatellite markers we were able to identify landscape effects on the regional level.

The low differentiation observed within regions suggests that pollen flow is efficient particularly among southern populations, whereas in latitudinal direction pollination appears inhibited due to the predominant westerly winds (Weischet & Endlicher, 2000). Pollen of *Juniperus* is among the smallest tree pollen with very low sedimentation velocity and therefore has high capacity for long distance dispersal (Durham, 1946). However, for wind pollination also pollen production and the density of the trees (Schueler et al., 2005; Schueler & Schlünzen, 2006) play an important role and may limit successful long-range pollen dispersal of *J. seravschanica*. For example, Zhang et al. (2005) report observations that in *J. przewalskii* effective pollen dispersal by wind may not exceed more than 2 km. This is probably a main factor for the higher differentiation of northern populations, which are usually more fragmented than southern ones.

Seed dispersal by birds is the other main gene flow agent in the region. In Kyrgyzstan, seeds of *Juniperus* are mainly dispersed by thrushes and finches (e.g., *Turdus viscivorus* L., *Mycerobas carnipes merzbacheri* Schalow; Kovshar, 1983), which are bound to the juniper forests. Flocks of thrushes can fly easily between distant populations, and search efficiently

for sites of higher cone availability, even across extensive geographical regions (Jordano, 1993). However, they probably forage regionally and therefore very likely do not cross the ecologically aberrant Fergana Valley and Fergana Range, which adds to explain the observed levels of inter-population gene flow. Bakai (Pop 4) was almost fixed for one haplotype despite the fact that another population (Sary-chelek, Pop 2) was situated close-by. Bakai (Pop 4) consisted of very old trees (approximately 300-400 year old), which were not coning at all (and attracting no seed dispersers). Strong drift effects due to harsh environment at that site may explain the pattern observed there.

Population history

The minimum spanning network between the 11 haplotypes (Fig. 11) shows, that the central and thus probably ancestral haplotype is H4. This haplotype is the most common in the southern range. Range expansion and population growth in connection with drift effects may have caused dominance of the derived haplotype H1 in the north. However, the split between north and south must be a relatively recent event, since only one (very rare) private haplotype was detected in the north. Based on our results (pattern of allelic richness, haplotype network) we propose that warmer and more humid conditions after the LGM allowed *J. seravschanica* to colonize the south of Kyrgyzstan from either local or close-by refugia. Then it crossed the Fergana Valley and spread to the north of the country. The densely forested Fergana Range very likely impeded the northward spread of the species locally, as indicated by pollen records (Beer et al., 2008b). The humid conditions caused by mid-latitude cyclones from the Mediterranean region (Syed et al., 2006) have even allowed the establishment of dominant hardwood forests in the Fergana Range. It is likely that after the spread of the species to the north the increasingly drier climate caused disruption of further gene flow from the south to the north. The patterns of haplotype diversity, with the highest diversity in the south, as well as the larger population sizes in the south support this hypothesis. Northern populations, in contrast, consist mostly of smaller stands with fewer individuals probably due to the harsher climatic conditions (O. Sultangaziev, personal observation). Therefore genetic drift appears to have profoundly affected populations in the north. The complete phylogeography of the species cannot be determined at present. More sampling from a larger area is needed to clarify this matter.

The pattern of north-south subdivision of *J. seravschanica* in Kyrgyzstan detected in this study has probably also been influenced by anthropogenic factors. The densely populated

and agriculturally intensively exploited lowland in the Fergana Valley (clearance of forests for agriculture, livestock grazing) cause another barrier to the dispersal of *J. seravschanica*. The human settlement in the valley started approximately 3000-2000 years BP and is strongly associated with the Silk Road that ran through it (Harmatta, 1994). Today human interference also extends to the mountain slopes that border the valley, where *J. seravschanica* would occur naturally. Remnants of juniper trunks indicate that the forest line was 400 – 600 m lower than at present (Buttoud & Junusova, 2000). Therefore, it is possible that human settlement has helped to the maintain or even intensify gene flow disruption between north and south.

Conservation implications

In this study the genetic diversity of *J. seravschanica* in Kyrgyzstan was investigated for the first time using novel plastid DNA markers, providing one of the first insights into population dynamics of forest tree species in Central Asia. Populations in the south harbour higher genetic diversity and are strongly differentiated from northern populations. Together with pollen data obtained in the study region (Beer et al. 2007, 2008a, b) this pattern indicates a relatively recent spread of the species from the south to the north during a more humid period. At present intense exploitation and deforestation in the region have led to the degradation of natural juniper forests. This development in connection with the increasingly drier conditions caused by climate change increases aridification (Buttoud & Junusova, 2000; Chorfi et al., 2004). The observed population structure shows the predominant effect of climate and landscape on gene flow. Management needs to take this into account, e.g. in planting schemes aiming to better connect northern populations. Populations from the north of the range could either suffer from lack of genetic variation, therefore be more vulnerable to climatic stress, or on the other hand could represent genetic resources that are adapted to harsh climate. Common garden experiments will be necessary to evaluate performance of populations from the north and south in terms of resilience to climate change and performance under drought conditions. Based on the results of this study we suggest that conservation efforts should consider northern and southern populations as separate entities.

Novel minisatellite markers in the plastid genome of *J. seravschanica*

Two novel minisatellites in the plastid genome have been identified in this study. These are the first minisatellites in the plastid genome of *Juniperus spp*. Although the plastid genome contains similar hypervariable regions as the nuclear genome (Powell et al., 1995), minisatellites have been less frequently found than single nucleotide microsatellites (Weising & Gardner, 1999). Conifer examples come from *Pseudotsuga* Carrière (Hipkins et al., 1995) and *Pinus* L. (Tian et al., 2008). The repeat units of the minisatellites identified in this study are large (32 bp and 34 bp, respectively), even allowing the main haplotypes to be scored on agarose gels. Certainly these markers will be valuable for future investigations in other parts of the species' range. Size homoplasy has been shown to affect the analysis of data derived from repetitive markers in the plastid genome (e.g., Vendramin et al., 1999). However, we presume low significance of this factor on the patterns observed with the novel minisatellite markers studied.

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Table 9. Origin of samples, genetic diversity estimates and plastid DNA haplotypes frequencies in 15 natural populations of *Juniperus seravschanica* from Kyrgyzstan.

Pop. no.	**Population name**	**Latitude (N)**	**Longitude (E)**	**Altitude (m)**	**N**	**nh**	**H**	**SEH**	**R [15]-1**	**Haplotype frequencies**										
										H1	H2	H3	H4	H5	H6	H7	H8	H9	H10	H11
Northern populations																				
1	Padysha ata	41° 39'	71° 38'	1700	52	6	0.677	0.049	3.189	0.500	0.000	0.019	0.212	0.000	0.000	0.192	0.000	0.058	0.000	0.019
2	Sary-chelek	41° 47'	71° 55'	1275	16	4	0.650	0.105	2.938	0.563	0.000	0.000	0.188	0.000	0.000	0.188	0.000	0.063	0.000	0.000
3	Kara archa	42° 46'	71° 47'	1300	68	6	0.666	0.037	2.804	0.485	0.000	0.000	0.265	0.000	0.015	0.191	0.015	0.029	0.000	0.000
4	Bakai	41° 46'	71° 58'	1300	24	2	0.083	0.075	0.625	0.958	0.000	0.000	0.000	0.000	0.000	0.042	0.000	0.000	0.000	0.000
5	Kayindy	41° 34'	72° 46'	1450	23	4	0.680	0.079	2.953	0.522	0.000	0.000	0.130	0.000	0.000	0.174	0.000	0.174	0.000	0.000
6	Suttuu Bulak	41° 29'	72° 27'	1200	21	5	0.486	0.124	3.128	0.714	0.000	0.000	0.048	0.000	0.000	0.143	0.000	0.048	0.048	0.000
	Sub-total				204	9														
	Mean				34.0	4.500	0.540	0.078	2.606	0.624	0.000	0.003	0.141	0.000	0.003	0.155	0.003	0.062	0.008	0.003

Southern populations

7	Abshirata	40° 12'	72° 21'	1900	46	7	0.663	0.056	3.263	0.174	0.022	0.000	0.522	0.000	0.022	0.217	0.000	0.022	0.020
8	Abshir sai	40° 06'	72° 22'	1600	23	5	0.723	0.054	3.296	0.174	0.000	0.000	0.348	0.043	0.000	0.391	0.000	0.043	0.000
9	Uch-korgon	39° 56'	71° 44'	1750	22	4	0.610	0.094	2.817	0.091	0.000	0.000	0.591	0.000	0.000	0.227	0.000	0.000	0.091
10	Suu Bashy	39° 51'	70° 54'	2200	22	5	0.628	0.101	3.495	0.091	0.000	0.000	0.591	0.000	0.000	0.182	0.045	0.000	0.091
11	Tamasha	40° 01'	71° 54'	2000	54	6	0.646	0.052	2.994	0.185	0.000	0.000	0.537	0.000	0.000	0.204	0.037	0.019	0.019
12	Arka	39° 59'	69° 59'	1500	23	6	0.727	0.065	3.925	0.304	0.000	0.000	0.435	0.043	0.000	0.043	0.000	0.043	0.130
13	Kempir Oi	39° 58'	69° 58'	1650	50	8	0.765	0.039	4.373	0.260	0.060	0.000	0.400	0.020	0.000	0.120	0.000	0.100	0.020
14	Andarak	39° 44'	69° 28'	1850	78	9	0.703	0.033	3.405	0.192	0.013	0.000	0.449	0.013	0.013	0.256	0.000	0.038	0.013
15	Karhana	39° 43'	69° 31'	2050	18	5	0.719	0.072	3.647	0.389	0.056	0.000	0.389	0.000	0.000	0.111	0.000	0.000	0.000
	Sub-total				336	10													
	Mean				37.3	6.11	0.686	0.063	3.469	0.203	0.013	0.000	0.474	0.013	0.003	0.193	0.003	0.023	0.043

	3	1	7	3	8	7	7	0	4	3	4	5	9	9	3	0
	54															
Total	0	11														
Total		5.46	0.62	0.06	3.12	0.37	0.01	0.00	0.34	0.00	0.00	0.17	0.00	0.04	0.02	0.00
mean	36	7	8	9	3	3	0	1	0	8	3	9	6	2	9	7

N = number of trees analyzed; nh = observed number of haplotypes; H = Nei's (1973) gene diversity; SE H = standard error H; R [15]-1 = allelic richness after rarefaction to 15 samples computed using Petit et al. (1998)

XIV. Conclusions and recommendations

Our study showed that differentiation of populations based on morphological traits were weak as comparing to the molecular markers. But on the other hand morphometric data measured in the field showed essential results in sex ratio, female/male growth pattern and effective population size. Interestingly, in contrast to other studies in *Juniperus* species strong 3,5-1 female domination in all populations were observed. Analysis of growth patterns in male and female individuals showed slightly higher and thicker trees among the male individuals. Estimation of effective population size based on female and male trees showed 70%, which means every third tree does not participate in sexual reproduction.

Using newly developed plastid DNA markers in Zeravschan juniper we were able to differentiate northern and southern population subdivision. Populations in the south harbour higher genetic diversity and are strongly differentiated from northern populations. Together with pollen data obtained from the other studies this pattern indicates a relatively recent spread of the species from the south to the north during a more humid period of LGM. The observed population structure shows the predominant effect of climate and landscape on gene flow. Populations from the north of the range could either suffer from lack of genetic variation, therefore be more vulnerable to climatic stress, or on the other hand could represent genetic resources that are adapted to harsh climate.

Based on the results of this thesis we suggest that conservation efforts should be based on molecular markers data, while morphological traits do not strongly differentiate populations of Zeravschan juniper. When there is need in conservation of Zeravschan juniper we suggest consider northern and southern populations as separate entities.

XV. Appendix

Haplotype 1 (H1)

- *MS1*

>AZTAL3

TGGTATCTCCTCTTAGATTAATAAATGGTGGATAATATGATAGTTTTATCTATT
GATTTGATTGTTCACGTTCAACTAGAGAACAATTTCTTTTCTTTGAAAACAACT
CAATTTTGATTCGACAAAATGGTATTAAAAAATTATATAATACAAAAAAGGTA
AATCCTCTACCCTTCCTTATATTATATTCTTTTTTAGATGAAATATCCTATCTCT
AATTCTTTAGAGATAGAATATAGAAACAAACTATGTACATAAAACGTATGTAT
CCCTTCGAGTCATTGCTCGAAACCGGGATACACACATAGTTTACTAAACTAATT
CCCATTAGTAAACCTATTTATTAATGTAGATATGAATCTACAAATATGATCTAT
TCTATCTATTGATTTTATCGACGGGTGAGGTGATCCTTAATCTTTCGACTAAGA
TCTTAGAGATTCAGTATTTTCATTTATGACTATAATTAAGGGAGAATCAAAATG
GAAAGAACATTTAACGTTTTATAAATGAGCAAATGATTATTATTAATTTGAATT
AAAAGACTAAGTCCAATTAATTAAATTAAATAAAAGACTAAGTCCAA

- *MS2*
- *trnV*
- *trnD*

Haplotype 2 (H2)

- *MS1*
- *MS2*

>AZKEAM20

TGGTATCTCCTCTTAGATTAATAAATGGTGGATAATATGATAGTTTTATCTATT
GATTTGATTGTTCACGTTCAACTAGAGAACAATTTCTTTTCTTTGAAAACAACT
CAATTTTGATTCGACAAAATGGTATTAAAAAATTATATAATACAAAAAAGGTA
AATCCTCTACCCTTCCTTATATTATATTCTTTTTTAGATGAAATATCCTATCTCT
AATTCTTTAGAGATAGAATATAGAAACAAACTATGTACATAAAACGTATGTAT
CCCTTCGAGTCATTGCTCGAAACCGGGATACACACATAGTTTACTAAACTAATT
CCCATTAGTAAACCTATTTATTAATGTAGATATGAATCTACAAATATGATCTAT
TCTATCTATTGATTTTATCGACGGGTGAGGTGATCCTTAATCTTTCGACTAAGA
TCTTAGAGATTCAGTATTTTCATTTATGACTATAATTAAGGGAGAATCAAAATG
GAAAGAACATTTAACGTTTTATAAATGAGCAAATGATTATTATTAATTTGAATT
AAAAGACTAAGTCCAATTAATTAAATTAAATTAAAAGACTAAGTCCAATTAAT
TAAATTAAATTAAAAGACTAAGTCCAATTAATTAAATTAATGATGACCCTCCA
TGTTT

- *trnV*
- *trnD*

Haplotype 3 (H3)

- *MS1*
- *MS2*

>AZPAM49

TGGTATCTCCTCTTAGATTAATAAATGGTGGATAATATGATAGTTTTATCTATT
GATTTGATTGTTCACGTTCAACTAGAGAACAATTTCTTTTCTTTGAAAACAACT
CAATTTTGATTCGACAAAATGGTATTAAAAAATTATATAATACAAAAAAGGTA
AATCCTCTACCCTTCCTTATATTATATTCTTTTTTAGATGAAATATCCTATCTCT
AATTCTTTAGAGATAGAATATAGAAACAAACTATGTACATAAAACGTATGTAT
CCCTTCGAGTCATTGCTCGAAACCGGGATACACACATAGTTTACTAAACTAATT
CCCATTAGTAAACCTATTTATTAATGTAGATATGAATCTACAAATATGATCTAT
TCTATCTATTGATTTTATCGACGGGTGAGGTGATCCTTAATCTTTCGACTAAGA
TCTTAGAGATTCAGTATTTTCATTTATGACTATAATTAAGGGAGAATCAAAATG
GAAAGAACATTTAACGTTTTATAAATGAGCAAATGATTATTATTAATTTGAATT
AAAAGACTAAGTCCAATTAATTAAATTAAATTAAAAGACTAAGTCCAATTAAT
TAAATTAATGATGACCCTCCATGGTTTTCACCTA

- *trnV*
- *trnD*

Haplotype 4 (H4)

- *MS1*

>KJS45

TGGGTATCTCCTCTTAGATTAATAAATGGTGGATAATATGATAGTTTTATCTAT
TGATTTGATTGTTCACGTTCAACTAGAGAACAATTTCTTTTCTTTGAAAACAAC
TCAATTTTGATTCGACAAAATGGTATTAAAAAATTATATAATACAAAAAAGGT
AAATCCTCTACCCTTCCTTATATTATATTCTTTTTTAGATGAAATATCCTATCTC
TAATTCTTTAGAGATAGAATATAGAAACAAACTATGTACATAAAACGTATGTA
TCCCTTCGAGTCATTGCTCGAAACCGGGATACACACATAGTTTACTAAACTAAT
TCCCATTAGTAAACCTATTTATTAATGTAGATATGAATCTACAAATATGATCTA
TTCTATCTATTGATTTTATCGACGGGTGAGGTGATCCTTAATCTTTCGACTAAG
ATCTTAGAGATTCAGTATTTTCATTTATGACTATAATTAAGGGAGAATCAAAAT
GGAAAGAACATTTAACGTTTTATAAATGAGCAAATGATTATTATTAATTTGAAT
TAAAAGACTAAGTCCAATTAATTAAATTAAATTAAAAGACTAAGTCCAATTAA
TTAAATTAA

- *MS2*
- *trnV*
- *trnD*

Haplotype 5 (H5)

- *MS1*

>KJS3

TGGGTATCTCCTCTTAGATTAATAAATGGTGGATAATATGATAGTTTTATCTAT
TGATTTGATTGTTCACGTTCAACTAGAGAACAATTTCTTTTCTTTGAAAACAAC
TCAATTTTGATTCGACAAAATGGTATTAAAAAATTATATAATACAAAAAAGGT
AAATCCTCTACCCTTCCTTATATTATATTCTTTTTTAGATGAAATATCCTATCTC
TAATTCTTTAGAGATAGAATATAGAAACAAACTATGTACATAAAACGTATGTA
TCCCTTCGAGTCATTGCTCGAAACCGGGATACACACATAGTTTACTAAACTAAT
TCCCATTAGTAAACCTATTTATTAATGTAGATATGAATCTACAAATATGATCTA
TTCTATCTATTGATTTTATCGACGGGTGAGGTGATCCTTAATCTTTCGACTAAG
ATCTTAGAGATTCAGTATTTTCATTTATGACTATAATTAAGGGAGAATCAAAAT
GGAAAGAACATTTAACGTTTTATAAATGAGCAAATGATTATTATTAATTTGAAT
TAAAAGACTAAGTCCAATTAATTAAATTAAAATAAAAGACTAAGTCCAATTAA
TTAAATTAA

- *MS2*

- *trnV*

>AZArM40

CAAATTTCTTATTACCAAAATGATTTGAACTGTTCCAAGAACCCAACATGCATT
TTTATTGCATTGGGCTCTTTCATTAACTGATGGAAAAATAAGTTAGTCTGCCAT
TCTTTTCCGGAACAGAACAGATAATAAGATGGCTCCGTTTGCTTGAAACGAAT
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GTCATGCTTAGACATAGATTCTCCAAATGGAGTCTCTCTCACCCCAAAATAAG
AATATGAGACTTCATACACCTCAAAGTTCATAGAGCGAAAAGAAATGTTTTTT
GAGATCCTCGTACGTATTATACTCATTATGTCTGACATTGAATGCCCCCGAGAT
TGACCATATCAACTAGATCTATAGTTCGAATCATAGAACGAACTTCATCTTTGT
CATGCTATTGTTCTCCTAATTACATAGAGTAAGACTAAAATCTATTTTATGAAC
CGAATGAACTAATAAACTCTTCTGAAAACCATTGGCGCACGTGTAAACGAGGT
GCTCTACCTAGCTGAGCTATAGCCCTTCACAGTTTAGTCTTAAAAATAGACTAA
TAAAATAGATCACTTTCTGTCAAGATGATATTTGATTTAATCATTCTTAAGGGC

ATATTGTTTATATGTCTAATTATGCCTAGAATTTAGGTATGACTCAATAAAGAA
CCTACTTAACTCAGTGGTTAGAGTATCGTTCAACG

- *trnD*

>AZAnL4

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CTTTTATCTTTCAAATTTATATTCTATATCAAAAGTATCTGTTCGTTCCGATTCT
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TTTCTTATCTGATAGATAAATATCTATCTCAATCTATCAAGTTGGTATTGGGCC
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TTTTTTCATATTTCATTATTTAATATTCTTCTTGTGTTGTCAGGATCGATAATAG
AACTATATTCAATTAATTTAGTTCTATTATTGACCCCATTATTTGGCATCTATCG
AATATGTAATAGACTTCTCCAT

Haplotype 6 (H6)

- *MS1*

>AZAAL13

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GATTTGATTGTTCACGTTCAACTAGAGAACAATTTCTTTTCTTTGAAAACAACT
CAATTTTGATTCGACAAAATGGTATTAAAAAATTATATAATACAAAAAAGGTA
AATCCTCTACCCTTCCTTATATTATATTCTTTTTTAGATGAAATATCCTATCTCT
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GAAAGAACATTTAACGTTTTATAAATGAGCAAATGATTATTATTAATTTGAATT
AAAAGACTAAGTCCAATTAATTAAATTAAATTAAAAGACTAAGTCCAATTAAT
TAAATTAAATAAAAGACT

- *MS2*

- *TrnV*

TTTCTTATTACCAAAATGATTTGAACTGTTCCAAGAACCCAACATGCATTTTTA
TTGCATTGGGCTCTTTCATTAACTGATGGAAAAATAAGTTAGTCTGCCATTCTT
TTCCGGAACAGAACAGATAATAAGATGGCTCCGTTTGCTTGAAACGAATCATT
TTTCGGAAGCAATCCCAAAGTGCTTCAAAAGATTCTCTTGACGTAGGTCTGTCA
TGCTTAGACATAGATTCTCCAAATGGAGTCTCTCTCACCCCAAAATAAGAATAT
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CCTCGTACGTATTATACTCATTATGTCTGACATTGAATGCCCCCGAGATTGACC
ATATCAACTAGATCTATAGTTCGAATCATAGAACGAACTTCATCTTTGTCATGC
TATTGTTCTCCTAATTACATAGAGTAAGACTAAAATCTATTTTATGAACCGAAT
GAACTAATAAACTCTTCTGAAAACCATTGGCGCACGTGTAAACGAGGTGCTCT
ACCTAGCTGAGCTATAGCCCTTCACAGTTTAGTCTTAAAAATATACTAATAAAA
TAGATCACTTTCTGTCAAGATGATATTTGATTTAATCATTCTTAAGGGCATATT
GTTTATATGTCTAATTATGCCTAGAATTTAGGTATGACTCAATAAAGAACCTAC
TTAACTCAGTGGTTAGAGTATCGTTTCA

- *trnD*

>Andarak7(trnD)

GACTAACTGTCTAAAAAAAACTGTGCCGTGTGCCGGGTCGTATTTTTGAAAAC
TTTTATCTTTCAAATTTATATTCTATATCAAAAGTATCTGTTCGTTCCGATTCTT
TACTCTATTACAGTATAGGATTAGAGGGTAGGGGATTCAAAAACCAATTACCT
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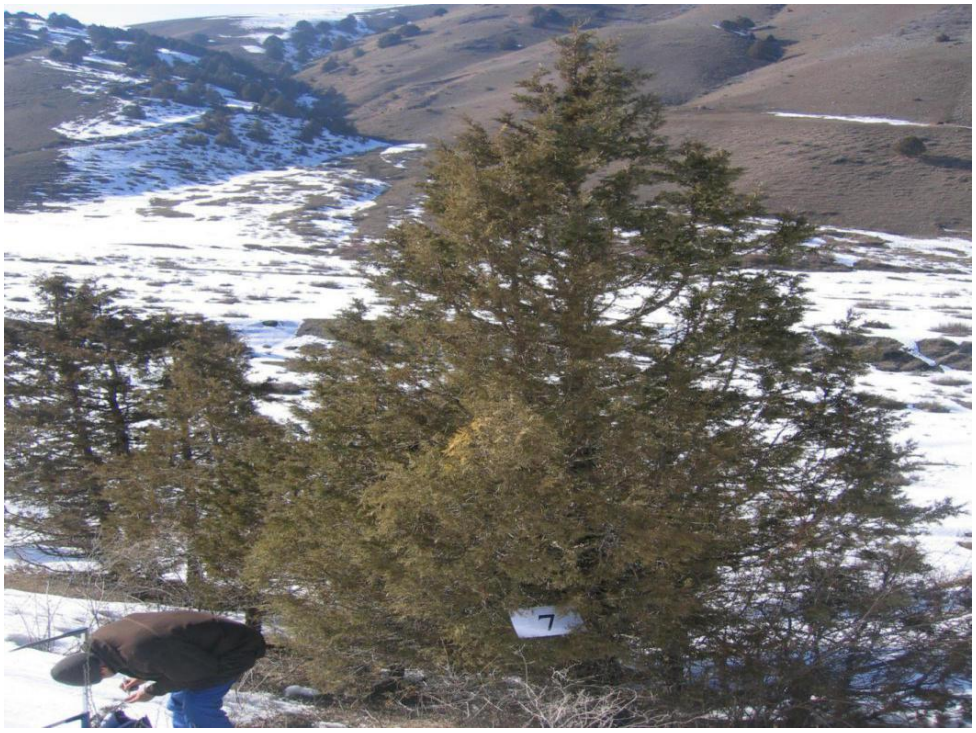

Haplotype 7 (H7)

- *MS1*

>AZAAM19

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TCAATTTTGATTCGACAAAATGGTATTAAAAAATTATATAATACAAAAAAGGT
AAATCCTCTACCCTTCCTTATATTATATTCTTTTTTAGATGAAATATCCTATCTC
TAATTCTTTAGAGATAGAATATAGAAACAAACTATGTACATAAAACGTATGTA
TCCCTTCGAGTCATTGCTCGAAACCGGGATACACACATAGTTTACTAAACTAAT
TCCCATTAGTAAACCTATTTATTAATGTAGATATGAATCTACAAATATGATCTA
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ATCTTAGAGATTCAGTATTTTCATTTATGACTATAATTAAGGGAGAATCAAAAT
GGAAAGAACATTTAACGTTTTATAAATGAGCAAATGATTATTATTAATTTGAAT
TAAAAGACTAAGTCCAATTAATTAAATTAAATTAAAAGACTAAGTCCAATTAA
TTAAATTAAATTAAAAGACTAAGTCCAATTAATTAAATTAAAAATAAAAGACT
AAGTCCCA

Haplotype 8 (H8)

- *MS1*

>AZAAM19

CATGGTATCTCCTCTTAGATTAATAAATGGTGGATAATATGATAGTTTTATCTA
TTGATTTGATTGTTCACGTTCAACTAGAGAACAATTTCTTTTCTTTGAAAACAA
CTCAATTTTGATTCGACAAAATGGTATTAAAAAATTATATAATACAAAAAAGG
TAAATCCTCTACCCTTCCTTATATTATATTCTTTTTTAGATGAAATATCCTATCT
CTAATTCTTTAGAGATAGAATATAGAAACAAACTATGTACATAAAACGTATGT
ATCCCTTCGAGTCATTGCTCGAAACCGGGATACACACATAGTTTACTAAACTA
ATTCCCATTAGTAAACCTATTTATTAATGTAGATATGAATCTACAAATATGATC
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AGATCTTAGAGATTCAGTATTTTCATTTATGACTATAATTAAGGGAGAATCAAA
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ATTAAAAGACTAAGTCCAATTAATTAAATTAAATTAAAAGACTAAGTCCAATT
AATTAAATTAAATTAAAGACTAAGTCCAATTAATTAA

- *MS2*
- *TrnV*
- *trnD*

>Andarak7

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TACTCTATTACAGTATAGGATTAGAGGGTAGGGGATTCAAAAACCAATTACCT
TTCTTATCTGATAGATAAATATCTATCTCAATCTATCAAGTTGGTATTGGGCCG
AGCTGGATTTGAACCAGCGTAGGCATATTGCCAACGAATTTACAGTCCGTCCC
CATTAGCCACTCGGGCATCGACCCAGGAAAAAATGGGAAGTTGATTATAGTAC
CTAATTAGGTACTATAATGACTTTCCTAGCCTACCTAGTACCCCTAGGGGAAAT
CGAATCCCCGTTGCCTCCTTGAAAGAGAGATGTCCTGGGCCACTAGACGATAG
GGGCTATTGTTATAATATTCAAATCCCTAGGAATTTGTCAATATAAAAATATTC
AAATCCCTAGGAATTTGTCAATATAAAAGAATCTTTTTTCATATTTCATTATTT
AATATTCTTCTTGTGTTGTCAGGATCGATAATAGAACTATATTCAATTAATTTA

GTTCTATTATTGACCCCATTATTTGGCATCTATCGAATATGTAATAGACTTCTCC
AT

Haplotype 9 (H9)

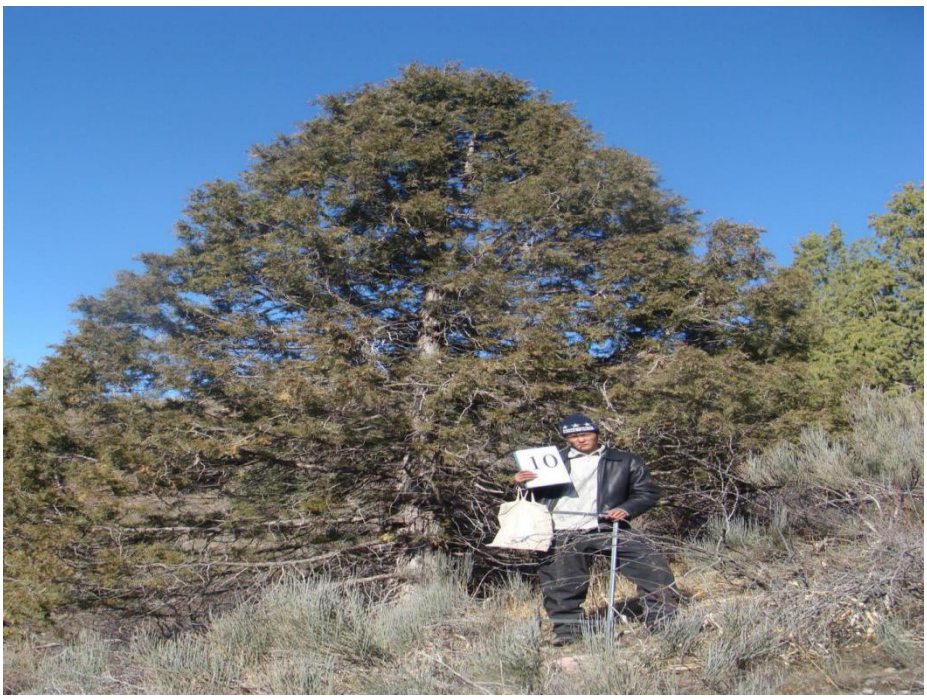
- *MS1*

>AZPAM10

TGGTAATCTCCTCTTAGATTAATAAATGGTGGATAATATGATAGTTTTATCTAT
TGATTTGATTGTTCACGTTCAACTAGAGAACAATTTCTTTTCTTTGAAAACAAC
TCAATTTTGATTCGACAAAATGGTATTAAAAAATTATATAATACAAAAAAGGT
AAATCCTCTACCCTTCCTTATATTATATTCTTTTTTAGATGAAATATCCTATCTC
TAATTCTTTAGAGATAGAATATAGAAACAAACTATGTACATAAAACGTATGTA
TCCCTTCGAGTCATTGCTCGAAACCGGGATACACACATAGTTTACTAAACTAAT
TCCCATTAGTAAACCTATTTATTAATGTAGATATGAATCTACAAATATGATCTA
TTCTATCTATTGATTTTATCGACGGGTGAGGTGATCCTTAATCTTTCGACTAAG
ATCTTAGAGATTCAGTATTTTCATTTATGACTATAATTAAGGGAGAATCAAAAT
GGAAAGAACATTTAACGTTTTATAAATGAGCAAATGATTATTATTAATTTGAAT

TAAAAGACTAAGTCCAATTAATTAAATTAAATTAAAAGACTAAGTCCAATTAA
TTAAATTAAATTAAAAGACTAAGTCCAATTAATTAAATTAAATTAAAAGACTA
AGTCCAATTAATTAAATTAA

- *MS2*
- *TrnV*
- *trnD*



Haplotype 10 (H10)

- *MS1*

>Arka 15

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TCAATTTTGATTCGACAAAATGGTATTAAAAAATTATATAATACAAAAAAGGT
AAATCCTCTACCCTTCCTTATATTATATTCTTTTTTAGATGAAATATCCTATCTC
TAATTCTTTAGAGATAGAATATAGAAACAAACTATGTACATAAAACGTATGTA
TCCCTTCGAGTCATTGCTCGAAACCGGGATACACACATAGTTTACTAAACTAAT
TCCCATTAGTAAACCTATTTATTAATGTAGATATGAATCTACAAATATGATCTA
TTCTATCTATTGATTTTATCGACGGGTGAGGTGATCCTTAATCTTTCGACTAAG
ATCTTAGAGATTCAGTATTTTCATTTATGACTATAATTAAGGGAGAATCAAAAT

GGAAAGAACATTTAACGTTTTATAAATGAGCAAATGATTATTATTAATTTGAAT
TAAAAGACTAAGTCCAATTAATTAAATTAAATTAAAAGACTAAGTCCAATTAA
TTAAATTAAATTAAAAGACTAAGTCCAATTAATTAAATTAAATTAAAAGACTA
AGTCCAATTAATTAAATTAAATTAAAAGACTAAGTCCAATTAATTAAATTAA

- *MS2*
- *TrnV*
- *trnD*

Haplotype 11 (H11)

- *MS1*

>AZArM19

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AAATCCTCTACCCTTCCTTATATTATATTCTTTTTTAGATGAAATATCCTATCTC
TAATTCTTTAGAGATAGAATATAGAAACAAACTATGTACATAAAACGTATGTA
TCCCTTCGAGTCATTGCTCGAAACCGGGATACACACATAGTTTACTAAACTAAT

TCCCATTAGTAAACCTATTTATTAATGTAGATATGAATCTACAAATATGATCTA
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GGAAAGAACATTTAACGTTTTATAAATGAGCAAATGATTATTATTAATTTGAAT
TAAAAGACTAAGTCCAATTAATTAAATTAAATTAAAAGACTAAGTCCAATTAA
TTAAATTAAATTAAAAGACTAAGTCCAATTAATTAAATTAAATTAAAAGACTA
AGTCCAATTAATTAAATTAAATTAAAAGACTAAGTCCAATTAATTAAATTAAA
TTAAAAGACTAAGTCCAATTAATTAAATTAA

- *MS2*
- *TrnV*
- *trnD*