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## Morphological and phylogenetic features of the Crimean population of *Juniperus deltoides* R.P. Adams

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**Abstract.** *Juniperus deltoides* is a relict species from the Tertiary Period. It is a typical representative of the Mediterranean group of the section *Juniperus*. It is included in the Red Books of the Republic of Crimea and the city of Sevastopol. Until recently, it was believed that a population of *J. oxycedrus* grew in Crimea. Currently, *J. deltoides* is described as a cryptic species, morphologically difficult to distinguish from *J. oxycedrus*. As a result, it became necessary to conduct a series of detailed studies to determine the morphological and phylogenetic features of the Crimean cryptic population in order to identify it as being one of the species of the cryptic pair. The studies were carried out in two stages: at the first stage, the morphological features of the vegetative and generative organs and their difference from *J. oxycedrus* were determined; the second stage included genetic research. The length of the needles of the Crimean population is  $12.94 \pm 0.19$  mm, which corresponds to the Eastern Italian population of *J. deltoides*. At the same time, the width of the needles is  $1.39 \pm 0.02$  mm, which is typical of the Portuguese population of *J. oxycedrus*. The dimensions of the cones are  $d_1$  (conditional height) =  $7.54 \pm 0.14$  mm, and  $d_2$  (conditional width) =  $9.11 \pm 0.09$  mm, which is more in line with *J. deltoides*. The shapes of the cones are very diverse. Some individuals have cones, the covering scales of which are visually indistinguishable, and their tops are completely fused. A similar phenomenon is characteristic of the Western Mediterranean populations of *J. oxycedrus*. Morphological analysis of the vegetative and generative organs of *J. deltoides* showed that when these two traits are combined, it is not possible to reliably distinguish between *J. deltoides* and *J. oxycedrus* individuals. Nuclear (ITS internal transcribed spacer) and chloroplast (petN-psbM, trnS-trnG) non-coding regions of the genome were used for genetic analysis. Studies have shown that the nuclear regions of genes have greater variability than chloroplast regions. The sequences obtained in this work formed a clade with *J. deltoides* samples 9430 and 9431 (BAYLU) growing in Turkey, which makes it possible to assign the samples studied to *J. deltoides*.

**Key words:** *Juniperus deltoides*; population; needles; cone berries; cryptic view; phylogenetic analysis; nuclear genes; chloroplast genes.

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## Морфологические и филогенетические особенности крымской популяции *Juniperus deltoides* R.P. Adams

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**Аннотация.** *Juniperus deltoides* – это реликтовый вид третичного периода. Является типичным представителем средиземноморской группы секции *Juniperus*. Включен в Красные книги Республики Крым и города Севастополя. До недавнего времени считалось, что на территории Крыма произрастает популяция *J. oxycedrus*. Сейчас *J. deltoides* описывают как криптический вид, морфологически сложно отличимый от *J. oxycedrus*. В результате возникла необходимость проведения ряда детальных исследований по определению морфологических и филогенетических особенностей криптической популяции Крыма с целью установления ее принадлежности к одному из видов криптической пары. Исследования проходили в два этапа: на первом этапе определяли морфологические особенности вегетативных и генеративных органов и их отличие от *J. oxycedrus*, на вто-

ром – выполняли генетические исследования. Длина хвои крымской популяции составляет  $12.94 \pm 0.19$  мм, что соответствует восточноитальянской популяции *J. deltooides*. При этом ширина хвои равна  $1.39 \pm 0.02$  мм, что соответственно португальской популяции *J. oxycedrus*. Размеры шишкоягод составляют:  $d_1$  (условно высота) –  $7.54 \pm 0.14$  мм,  $d_2$  (условно ширина) –  $9.11 \pm 0.09$  мм, что больше соответствует *J. deltooides*. Форма шишкоягод варьирует весьма сильно. Встречаются особи с шишкоягодами, кроющие чешуи которых визуально не отличимы, их верхушки полностью срстаются. Подобное явление характерно для западносредиземноморских популяций *J. oxycedrus*. Морфологический анализ вегетативных и генеративных органов *J. deltooides* показал, что при сочетании двух этих признаков достоверно различить особи *J. deltooides* и *J. oxycedrus* не представляется возможным. Для генетического анализа использовали ядерные (внутренний транскрибируемый спейсер ITS) и хлоропластные (petN-psbM, trnS-trnG) некодирующие участки генома. Исследования показали, что ядерные участки генов обладают большей вариабельностью, чем хлоропластные. Последовательности, полученные в данной работе, образовали кладу с образцами *J. deltooides* 9430 и 9431 (BAYLU), произрастающими в Турции, что позволяет отнести изученные образцы к виду *J. deltooides*.

Ключевые слова: *Juniperus deltooides*; популяция; хвоя; шишкоягоды; криптический вид; филогенетический анализ; ядерные гены; хлоропластные гены.

## Introduction

The genus *Juniperus* L. is the largest in the cypress family (Cupressaceae Bartl.), assigned to the juniper subfamily (*Juniperoideae* Endl.), and includes 76 species (Pisarev, 2007; Adams, 2014b).

Junipers are distinguished by significant polymorphism, on the basis of which a number of subgenera, sections and series are distinguished in the genus. The taxonomy of the genus is based on two distinctive morphological features: the structure of generative organs (cones) and the structure of vegetative organs (needles) (Kolesnikov, 1974).

The genus was first described in 1700; since that time, the taxonomy of the genus has undergone significant changes (Novikov et al., 2014). Currently, the genus is divided into three sections, among which: the *Caryocedrus* section counting one species – *J. drupacea* Labill., the *Juniperus* section (synonymous with *Oxycedrus*), which includes 14 species, and the *Sabina* section, which consists of the remaining 61 species (Pisarev, 2007; Abaimov, 2009; Adams, 2014b). At the same time, the *Caryocedrus* section was often considered as a separate genus, but PCR studies conducted by R.P. Adams proved its common origin with the *Juniperus* section (Adams, 2014b).

Five species of junipers grow in the Crimea (*J. communis* L., *J. deltooides* R.P. Adams, *J. excelsa* M.-Bieb., *J. foetidissima* Willd., *J. sabina* L.), which belong to two sections – *Juniperus* and *Sabina*. All of them are included in the Red Books of the Republic of Crimea and the city of Sevastopol (Yena, Fateryga, 2015; Red Book..., 2018).

*Juniperus deltooides* is a relict species from the Tertiary period. It is a typical representative of the Mediterranean group of the *Juniperus* section. *J. deltooides* is common in the Mediterranean and the Middle East. To a large extent, its range is limited to the Mediterranean climate, but in the Balkans it occurs in more continental conditions. The northern border of its range passes in the Crimea. The area of the cryptic population of the Crimea, according to 2006 data, is 4843 hectares (Adams, 2014b; Plugatar, 2015; Farjon, 2017; Rajčević et al., 2020; Sadykova, Neshataeva, 2020; Yousefi et al., 2021).

Until recently, it was believed that a population of *J. oxycedrus* grows on the territory of Crimea. This species was included in the Guide to Higher Plants of Crimea (Rubtsov, 1972). However, the scientist R.P. Adams, on the basis of DNA sequencing, found that in most of the Mediterranean –

from Italy to the east through Turkey to the mountains of the Caucasus and Iran (including the Crimea), a juniper other than *J. oxycedrus* is common, which he described as a new species – *J. deltooides*. At the same time, no direct analysis of the genetic material from the territory of Crimea was carried out. Adams made a similar conclusion based on the geographic localization of populations. In his works, he described that junipers growing west of Italy belong to the species *J. oxycedrus*, and junipers found to the east are *J. deltooides* (Adams et al., 2005; Adams, 2014a; Roma-Marzio et al., 2017).

Currently, *J. deltooides* is described as a cryptic species that is morphologically difficult to distinguish from *J. oxycedrus* (Adams et al., 2005; Adams, 2014a; Roma-Marzio et al., 2017). As a result, it became necessary to conduct a series of detailed studies to determine the morphological and phylogenetic features of the Crimean population of *J. deltooides*, in order to establish its belonging to one of the species of the cryptic pair.

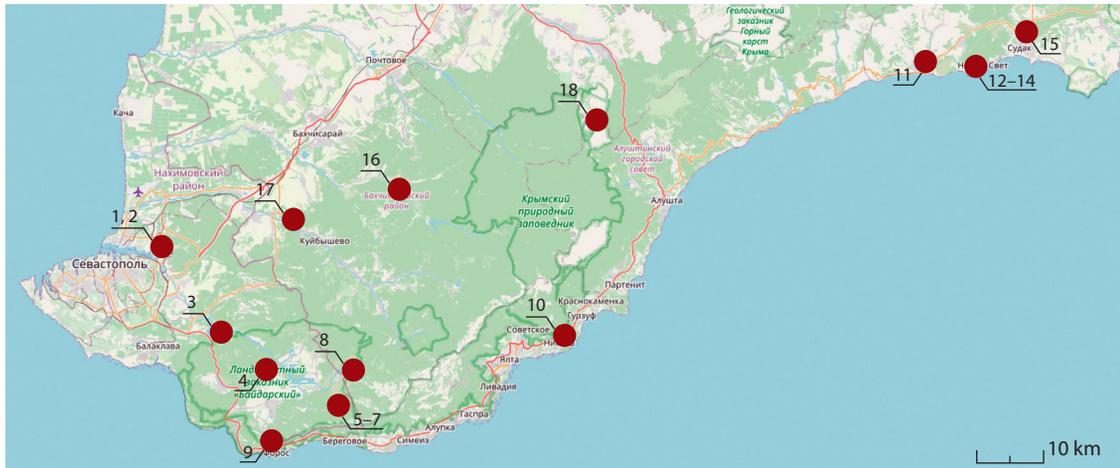
The study includes two main tasks: determining the correspondence between the morphological features of the vegetative and generative organs of the cryptic population of the Crimea to the species *J. deltooides*; conducting genetic studies using nuclear and chloroplast regions of marker sequences.

## Materials and methods

In order to conduct morphological and phylogenetic studies within the population, test areas of 0.2 hectares were laid at an altitude of 40 to 620 m above sea level, in various edapho- orographic conditions from Inkerman to Sudak (Fig. 1).

According to generally accepted methods, 10 model trees were identified within the test areas (Yarmishko, Lyanguzova, 2002). For each model tree, 30 cones were measured in two mutually perpendicular planes (conventional width and height). In addition, according to the determinant key developed by Adams for *J. deltooides* (Adams, 2014a), the degree of accretion of cone scales was visually determined.

To determine the parameters of the vegetative organs, the length and width of the needles were measured and the average error was determined (30 needles for each model tree). Then, a cross section of the needles was carried out in order to establish the presence or absence of curvature of the adaxial surface of the needles. The shape of the base of the needles was determined (Adams, 2014a, b).



**Fig. 1.** Scheme of the location of sample plots in the cryptic populations of the Crimean Mountains.

1, 2 – the vicinity of the city of Inkerman; 3 – mt. Chirka-Kayasy; 4 – mt. Samnalykh; 5–7 – mt. Kara-Dag; 8 – mt. Tolaka- Bair; 9 – mt. Dragon; 10 – Cape Martyan; 11 – mt. Papaya-Kaya; 12, 13 – mt. Koba-Kaya; 14 – mt. Sokol; 15 – mt. Karshiters; 16 – Kullu-Kaya rocks; 17 – the vicinity of the village of Kudrino; 18 – mt. Chatyr-Dag.

**Table 1.** Nucleotide sequences of primers and PCR protocol used in this work (Hojjati et al., 2018)

Sequence name	Size, b.p.	Primer sequences	PCR Protocol
ITS	1100	F: GGAAGGAGAAGTCGTAACAAGG	1. 94 °C – 4 min
		R: CTTTCTCCGCTTATTGATATG	2. 34 cycles: 94 °C – 40 s
petN-psbM	764	F: AACGAAGCGAAAATCAATCA	50 °C – 40 s
		R: AAAGAGAGGGATTCTGATGGA	3. 72 °C – 7 min
trnS-trnG	700	F: GCCGCTTTAGTCCACTCAGC	
		R: GAACGAATCACACTTTACCAC	

18 samples of *J. deltooides* from different geographical locations of the Crimean peninsula were selected for genetic studies (see Fig. 1). DNA isolation from needles was carried out using the DNeasy Plant Mini Kit (Qiagen, Germany). The quantity and quality of the isolated DNA were analyzed using an Inplen nanophotometer (Germany). For PCR analysis, nuclear (internal transcribed spacer ITS) and chloroplast (petN-psbM, trnS-trnG) non-coding regions of the genome were used. Marker gene amplifications were performed using the universal primers and protocols described earlier (Table 1, Hojjati et al., 2018), using the ScreenMix reagent kit (Eurogen, Russia).

Sequencing of the obtained fragments was carried out on the genetic analyzer NANOPHOR-05 (Syntol, Russia) in the Resource Centre “Molecular Structure of Matter”. Electrophoregrams unsuitable for analysis were obtained for two samples during sequencing of the ITS nuclear fragment, and the nucleotide sequences of 16 samples of the Crimean population were further studied (Table 2). The obtained sequences of ITS, petN-psbM and trnS-trnG were compared with those available in the database of the National Center for Biotechnology Information (<https://www.ncbi.nlm.nih.gov/>). The samples for comparison were taken from (Hojjati

et al., 2018) and are indicated in Table 2. The alignment of nucleotide sequences for each marker site and their integration into the combined matrix was carried out in the MegaX program (Kumar et al., 2018). Phylogenetic reconstruction was performed using the Bayesian method implemented in MrBayes version 3.2.6 (Ronquist et al., 2012).

Haplotype diversity (HD), nucleotide diversity ( $\pi$ ), and the number of nucleotide substitutions (M) were calculated for each species using DnaSP 6.0 (Rozas et al., 2017).

The relationships between haplotypes of sequences from three marker sites were reconstructed by the TCS method implemented in the PopArt program (Bandelt et al., 1999).

## Results and discussion

In 2014, R.P. Adams (2014a) developed and published a determinant key for *J. deltooides*, which makes it possible to distinguish individuals of this species from *J. oxycedrus*. According to the determinant, the maximum needle length of *J. deltooides* is less than that of *J. oxycedrus* and equals 13.0 mm (for *J. oxycedrus* it is 15.0 mm). The length of the needles of the Crimean population of *J. deltooides* is  $12.94 \pm 0.19$  mm, which corresponds to the dimensions declared by Adams. At the same time, a significant number of

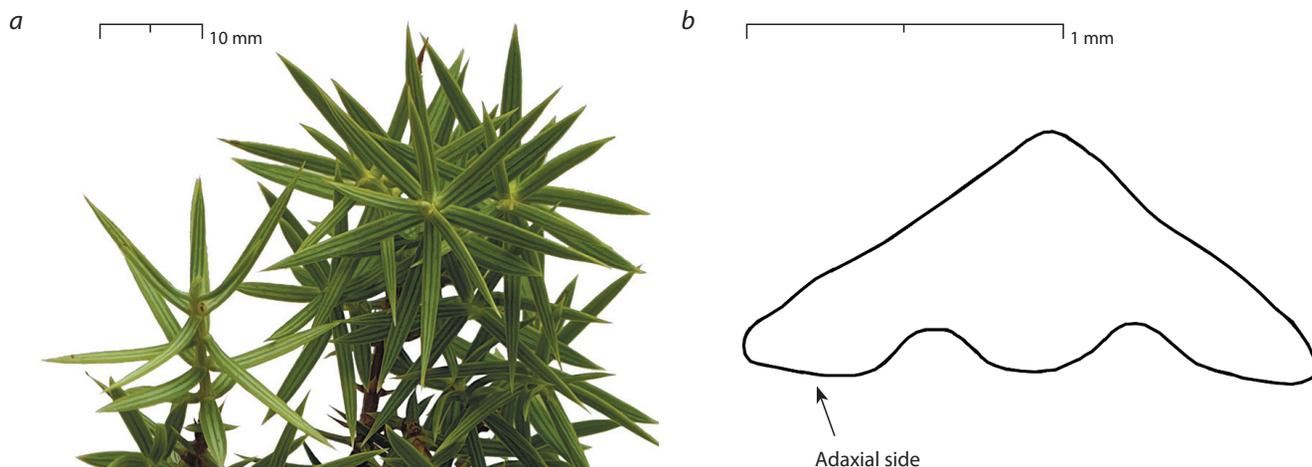
**Table 2.** Codes, geographical location and Genbank codes of the analyzed samples

Sample code / species*	Location	Sequences	Genbank code
CRMD1	mt. Chirka-Kayasy	ITS	OP303319
		petN-psbM	OP321223
		trnS-trnG	OP321239
CRMD4	mt. Samnalykh	ITS	OP303320
		petN-psbM	OP321224
		trnS-trnG	OP321240
CRMD7	mt. Kara-Dag	ITS	OP303321
		petN-psbM	OP321225
		trnS-trnG	OP321241
CRMD10		ITS	OP303322
		petN-psbM	OP321226
		trnS-trnG	OP321242
CRMD14		ITS	OP303323
		petN-psbM	OP321227
		trnS-trnG	OP321243
CRMD16	mt. Tolaka-Bair	ITS	OP303324
		petN-psbM	OP321228
		trnS-trnG	OP321244
CRMD19	mt. Papaya-Kaya	ITS	OP303325
		petN-psbM	OP321229
		trnS-trnG	OP321245
CRMD22	mt. Sokol	ITS	OP303326
		petN-psbM	OP321230
		trnS-trnG	OP321246
CRMD25	mt. Koba-Kaya	ITS	OP303327
		petN-psbM	OP321231
		trnS-trnG	OP321247
CRMD27		ITS	OP303328
		petN-psbM	OP321232
		trnS-trnG	OP321248
CRMD28		ITS	OP303329
		petN-psbM	OP321233
		trnS-trnG	OP321249
CRMD29		ITS	OP303330
		petN-psbM	OP321234
		trnS-trnG	OP321250
CRMD33	mt. Karshiters	ITS	OP303331
		petN-psbM	OP321235
		trnS-trnG	OP321251
CRMD35	The vicinity of the city of Inkerman	ITS	OP303332
		petN-psbM	OP321236
		trnS-trnG	OP321252
CRMD38		ITS	OP303333
		petN-psbM	OP321237
		trnS-trnG	OP321253
CRMD40	mt. Chatyr-Dag	ITS	OP303334
		petN-psbM	OP321238
		trnS-trnG	OP321254
BAYLU 13730* <i>J. communis</i>	Bulgaria	ITS	LC420860
		petN-psbM	LC420856
		trnS-trnG	LC420859

**Table 2 (end)**

Sample code / species*	Location	Sequences	Genbank code
BAYLU 8765* <i>J. communis</i>	Armenia	ITS	LC420870
		petN-psbM	LC420866
		trnS-trnG	LC420869
BAYLU 9431* <i>J. deltoides</i>	Turkey	ITS	LC420895
		petN-psbM	LC420891
		trnS-trnG	LC420894
BAYLU 8795* <i>J. drupacea</i>	Greece	ITS	LC420930
		petN-psbM	LC420926
		trnS-trnG	LC420929
BAYLU 8796* <i>J. drupacea</i>		ITS	LC420935
		petN-psbM	LC420931
		trnS-trnG	LC420934
BAYLU 8785* <i>J. excelsa</i>		ITS	LC420800
		petN-psbM	LC420796
		trnS-trnG	LC420799
BAYLU 9433* <i>J. excelsa</i>	Turkey	ITS	LC420850
		petN-psbM	LC420846
		trnS-trnG	LC420849
BAYLU 5645* <i>J. foetidissima</i>	Greece	ITS	LC420900
		petN-psbM	LC420896
		trnS-trnG	LC420899
BAYLU 9039* <i>J. oxycedrus</i>	France	ITS	LC420880
		petN-psbM	LC420876
		trnS-trnG	LC420879
BAYLU 9040* <i>J. oxycedrus</i>		ITS	LC420885
		petN-psbM	LC420881
		trnS-trnG	LC420884
TARI IRN30492* <i>J. oxycedrus</i>	Iran	ITS	LC420974
		petN-psbM	LC420970
		trnS-trnG	LC420973
BAYLU 14171* <i>J. polycarpus</i> var. <i>polycarpus</i>	Azerbaijan	ITS	LC420790
		petN-psbM	LC420786
		trnS-trnG	LC420789
BAYLU 8757* <i>J. polycarpus</i> var. <i>turcomanica</i>	Turkmenistan	ITS	LC420690
		petN-psbM	LC420686
		trnS-trnG	LC420689
BAYLU 14316* <i>J. sabina</i>	Azerbaijan	ITS	LC420910
		petN-psbM	LC420906
		trnS-trnG	LC420909
BAYLU 9430* <i>J. deltoides</i>	Turkey	ITS	LC420890
		petN-psbM	LC420886
		trnS-trnG	LC420889
BAYLU 14317* <i>J. sabina</i>	Azerbaijan	ITS	LC420920
		petN-psbM	LC420916
		trnS-trnG	LC420919
TARI 17035* <i>J. foetidissima</i>	Azerbaijan, Iran	ITS	LC420989
		petN-psbM	LC420985
		trnS-trnG	LC420988

\*The samples taken for comparison from (Hojjati et al., 2018), their species are indicated.



**Fig. 2.** Needles of the Crimean cryptic population (localization – mt. Tolaka-Bair):  
 a, general view; b, a schematic representation of the curvature of the adaxial surface of the needles.



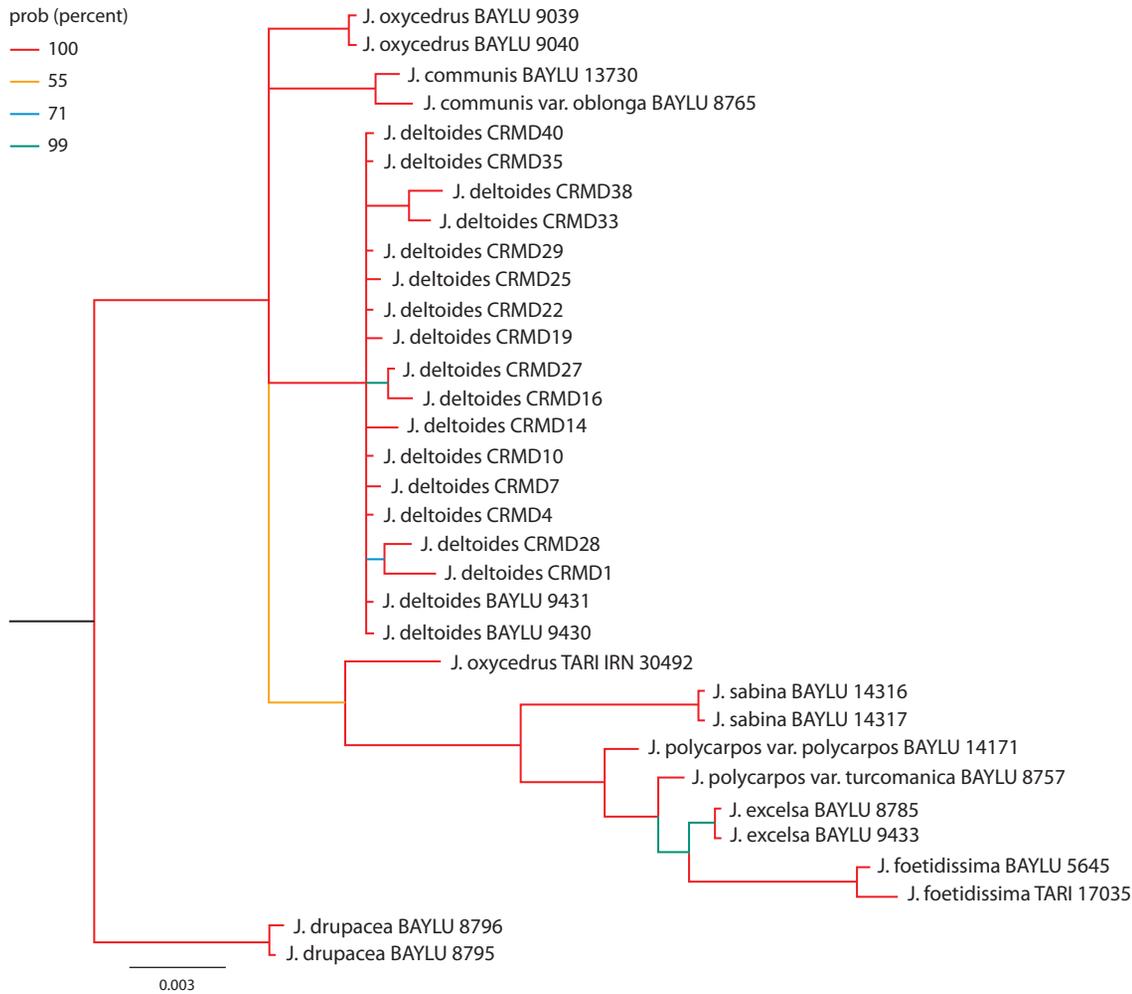
**Fig. 3.** Morphological heterogeneity of cones of the cryptic population of Crimea.

individuals were found (in the vicinity of the city of Inkerman and the village of Kudrino; on the mountains of Kara-Dag, Koba-Kaya, Dragon, on the rocks of Kullu-Kaya and on Cape Martyan), the length of the needles of which is from 18 to 20 mm. These individuals aroused the greatest interest for further research. Needle width, according to Adams (2014a), is also a defining feature. Under the conditions of the Crimea, this indicator is  $1.39 \pm 0.02$  mm, which corresponds to the western group of junipers, namely *J. oxycedrus*.

The needles had no differences in color and shape of the base. All needles are light green with a deltoid base, which is typical for *J. deltoides* (Fig. 2, a). At the same time, the cross section of the needles showed that a significant part of the individuals (34 %) are characterized by the curvature of the adaxial surface of the needles (see Fig. 2, b). According to Adams (2014a), this is a distinctive feature of *J. oxycedrus*. The length of the needles with this type of stomatal bands is  $11.87 \pm 0.24$  mm. Thus, it was found that the morphological features of the needles of the Crimean population of *J. deltoides* simultaneously exhibit signs of both *J. deltoides* and *J. oxycedrus*. On the basis of the identified features of the vegetative organs, it is not possible to attribute individuals to one of the species.

The second distinctive morphological feature of junipers are cones. In the case of the cryptic pair, *J. deltoides*/*J. oxycedrus*, the main role in determining the species is played by the inoculation of cone scale tips, and to a lesser extent, by the size and color of the cone of cones, the presence or absence of plaque on them.

The cones of the Crimean cryptic population have a significant number of morphological variants (Fig. 3). At the same time, the coloration is almost the same for all of them, and the smoke-blue coating appears to varying degrees, regardless of the shape of the cones. The shape, in turn, differs very much (from spherical to triangular). There are individuals with cones, the covering cone scale tips of which are visually indistinguishable and their tops are completely fused. A similar phenomenon is characteristic of *J. oxycedrus*. At the same time, the needles of these individuals are defined as the needles of *J. deltoides*. The second type of cones is almost triangular due to clearly visible three covering cone scale tips (characteristic of *J. deltoides*). The needles of these individuals show signs of both species. To a greater extent, there are intermediate variants, in which the bases of the cone scale tips grow together, and their tops move away from each other to varying degrees.



**Fig. 4.** A phylogenetic tree constructed by the Bayes method based on a combined sequence including nuclear (ITS) and chloroplast (petN-psbM, trnS-trnG) non-coding regions of the genome.

Node support values are shown in color.

The size of the cones turned out to be the most stable trait and differed within the error on all test areas. The average sizes of cones are:  $d_1$  (conditional height) is  $7.54 \pm 0.14$  mm, and  $d_2$  (conditional width) is  $9.11 \pm 0.09$  mm, which is more consistent with the Turkish population of *J. deltooides*.

Thus, the morphological analysis of the vegetative and generative organs of *J. deltooides* showed that when these two characters are combined; it is not possible to reliably distinguish between individuals of *J. deltooides* and individuals of *J. oxycedrus*. *J. oxycedrus* is a basal species, while *J. deltooides* is a cryptic one. As a result, the issue of conducting phylogenetic studies is especially acute for determining the systematic belonging of the Crimean population to one of the species.

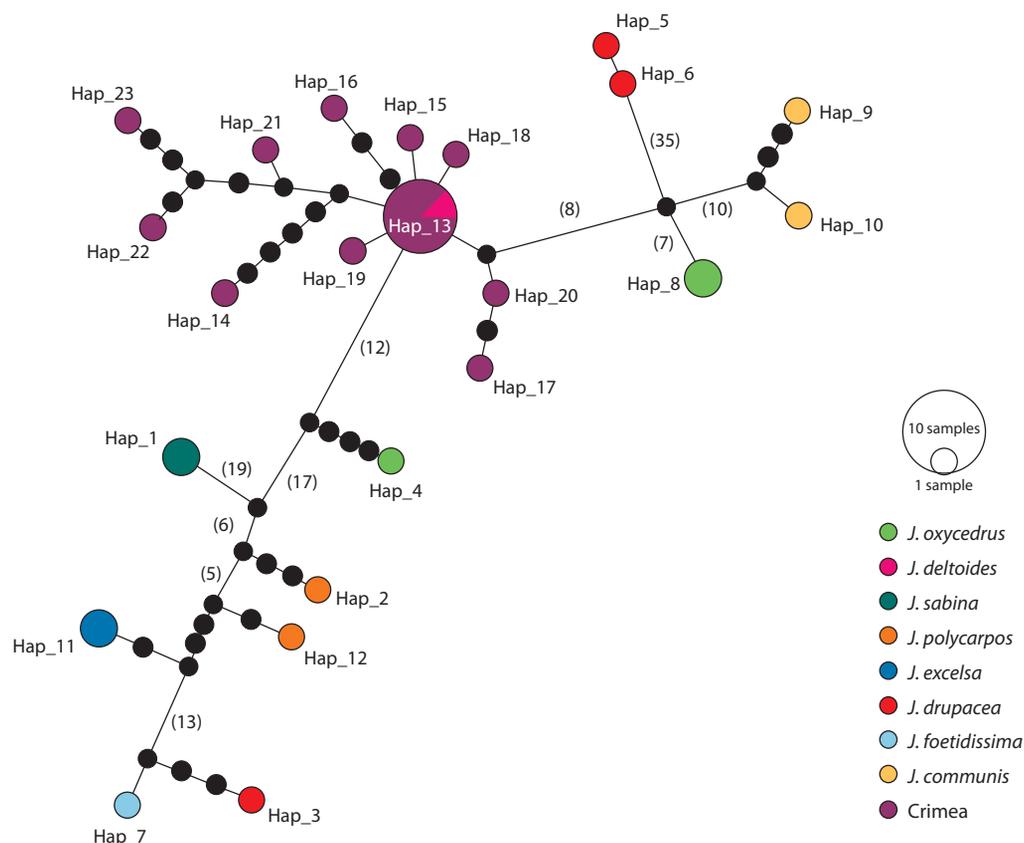
For phylogenetic analysis, the nucleotide sequences of three marker sites (ITS, petN-psbM and trnS-trnG) of 16 Crimean samples and 17 samples from the work (Hojjati et al., 2018) were used (see Table 2).

Phylogenetic trees constructed from individual marker sequences are presented in Supplementary Materials 1–3<sup>1</sup>. The use of ITS and petN-psbM marker sequences allowed

us to obtain topologies where each species forms a separate clade and their phylogenetic definition is unambiguous. The topology obtained by analyzing the trnS-trnG sequences does not allow separating the species of *J. communis* and *J. deltooides*.

A phylogenetic tree was also constructed taking into account all the nucleotide fragments studied in this work (Fig. 4). It can be seen that the samples of each species form clades with high (more than 75 %) support. The exception is the specimen *J. oxycedrus* TARI IRN 30492, for which the phylogenetic definition is not unambiguous: according to the sequences trnS-trnG and petN-psbM, it forms a clade with samples of the species *J. deltooides*, and according to the fragment of ITS and the analysis of the combined sequences, it forms a clade with species of the section *Sabina*. All the samples from the Crimea studied in this work formed a clade with sequences of the species *J. deltooides* 9430 and 9431 (BAYLU) growing in Turkey. In this clade, three pairs of samples (CRMD1 and CRMD28, CRMD16 and CRMD27, CRMD33 and CRMD38) form separate branches with high support, but there is no correlation between them by phenotypes and geographical location.

<sup>1</sup> Supplementary Materials 1–3 are available in the online version of the paper: [http://vavilov.elpub.ru/jour/manager/files/Suppl\\_Lantushenko\\_Engl\\_27\\_4.pdf](http://vavilov.elpub.ru/jour/manager/files/Suppl_Lantushenko_Engl_27_4.pdf)



**Fig. 5.** Haplotypic network constructed by the TCS method.

The color indicates belonging to the species, the size of the circle is the number of samples in the haplotype, small distances are represented as dots (1 point – 1 replacement), for large ones the number of substitutions is given in brackets.

**Table 3.** Characteristics of the nucleotide sequences studied in this work

Parameter	Length	H	Hp	$\pi$	M
ITS	1295	10	0.867	0.00419	14
petN-psbM	896	4	0.442	0.00140	7
trnS-trnG	785	3	0.242	0.0005	3
Combined sequence	2976	11	0.875	0.002	24

Note. H is the number of haplotypes, Hp is the haplotypic diversity,  $\pi$  is the nucleotide diversity, M is the number of mutations.

The haplotypic network constructed from the nuclear and chloroplast regions of the genome for the samples listed in Table 2 is shown in Figure 5. It can be seen that the Crimean population of *J. deltoides* is characterized by a quite large number of haplotypes: 11.

In the previously studied populations of juniper trees of other species (*J. excelsa*, *J. polycarpus*, and *J. foetidissima*) on the northern border of the distribution area, in the Crimea, the Caucasus and Dagestan (Sadykova et al., 2021), much smaller haplotypic diversity was found: sequences of 17 *J. excelsa* samples form two haplotypes, 16 *J. foetidissima* samples form four haplotypes, 15 samples of *J. polycarpus* – one haplotype.

The genetic variability of nuclear and chloroplast regions of genes was analyzed. The analysis of the parameters presented

in Table 3 allows us to conclude that the greatest variability is characteristic of the ITS nuclear fragment, and the least variability is characteristic of the trnS-trnG fragment. The greatest variability of the nuclear sites of marker nucleotide sequences is characteristic of other juniper species (Mao et al., 2010; Hojjati et al., 2018).

It follows from Figure 5 that the nucleotide sequences of CRMD4, CRMD10, CRMD22, CRMD29, CRMD35, CRMD40 samples formed a common haplotype with the sequences BAYLU:9430 and BAYLU:9431.

The sequences obtained in this work formed a clade with *J. deltoides* 9430 and 9431 (BAYLY) specimens growing in Turkey (Table 4). Thus, the analysis carried out in this work allows us to attribute the studied samples to the species *J. deltoides*.

**Table 4.** List of haplotypes of nucleotide sequences of samples of the Crimean population and samples taken for comparison from the work (Hojjati et al., 2018)

Haplotype	Species
Hap_1	<i>Juniperus sabina</i> BAYLU:14317, BAYLU:14316
Hap_2	<i>Juniperus polycarpos</i> var. <i>polycarpos</i> BAYLU:14168
Hap_3	<i>Juniperus foetidissima</i> TARI 17035
Hap_4	<i>Juniperus oxycedrus</i> TARI IRN: 30492
Hap_5	<i>Juniperus drupacea</i> BAYLU:8796
Hap_6	<i>Juniperus drupacea</i> BAYLU:8795
Hap_7	<i>Juniperus foetidissima</i> BAYLU:5645
Hap_8	<i>Juniperus oxycedrus</i> BAYLU:9040, BAYLU:9039
Hap_9	<i>Juniperus communis</i> var. <i>oblonga</i> BAYLU:8765
Hap_10	<i>Juniperus communis</i> BAYLU:13730
Hap_11	<i>Juniperus excelsa</i> BAYLU:9433, BAYLU:8785
Hap_12	<i>Juniperus polycarpos</i> var. <i>turcomanica</i> BAYLU:8757
Hap_13	<i>Juniperus deltooides</i> BAYLU:9430, BAYLU:9431, CRMD4, CRMD10, CRMD22, CRMD29, CRMD35, CRMD40
Hap_14	<i>Juniperus deltooides</i> CRMD1
Hap_15	<i>Juniperus deltooides</i> CRMD7
Hap_16	<i>Juniperus deltooides</i> CRMD14
Hap_17	<i>Juniperus deltooides</i> CRMD16
Hap_18	<i>Juniperus deltooides</i> CRMD19
Hap_19	<i>Juniperus deltooides</i> CRMD25
Hap_20	<i>Juniperus deltooides</i> CRMD27
Hap_21	<i>Juniperus deltooides</i> CRMD28
Hap_22	<i>Juniperus deltooides</i> CRMD33
Hap_23	<i>Juniperus deltooides</i> CRMD38

## Conclusion

Based on the studies of vegetative organs, it was found that the length of the needles of individuals of the Crimean cryptic population is  $12.94 \pm 0.19$  mm, which is typical for this species. At the same time, there are individuals with needles of much greater length (18–20 mm). The cross section of the needles, regardless of its length, in 34 % of cases shows signs of *J. oxycedrus*, expressed in the curvature of its adaxial surface.

Cones manifest significant morphological heterogeneity. Their shape varies from spherical to triangular, depending on the degree of fusion of the covering cone scale tips. At the same time, it was found that the same individuals can simultaneously show signs of *J. deltooides* (by vegetative organs) and signs of *J. oxycedrus* (by generative organs).

Thus, we can conclude that these morphological features are not reliable for determining the systematic affiliation of individuals. Thus, the only possible way to determine it is to conduct genetic research.

Phylogenetic analysis has shown that nuclear regions of genes have greater variability than chloroplast ones. The sequences obtained in this work for the Crimean population formed a clade with samples of *J. deltooides* 9430 and 9431 (BAYLU) growing in Turkey, which makes it possible to attribute the studied samples to the species *J. deltooides*.

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