

# Phylogenetic relationships between *Chondrilla* L. species in European Russia based on plastid DNA sequencing

Alexandr Kashin<sup>1,\*</sup>, Ivan Schanzer<sup>2</sup>, Tatyana Kritskaya<sup>1</sup>, and Alena Parkhomenko<sup>1</sup>

<sup>1</sup>Saratov State University, Botanic Garden, Saratov, 410012, Russia

<sup>2</sup>Main Botanical Garden, Herbarium (MHA), Moscow, 127276, Russia

**Abstract.** The article examines the genetic diversity in 54 populations of 9 *Chondrilla* species (*C. acantholepis*, *C. ambigua*, *C. brevirostris*, *C. canescens*, *C. graminea*, *C. juncea*, *C. laticoronata*, *C. latifolia*, and *C. pauciflora*) in European Russia. Plastid DNA segments trnT-trnF are selected as markers. Reconstruction of evolutionary networks based on the principle of maximum parsimony reveals that the sample is divided into four groups, where group 1 is *C. ambigua*, 2 – *C. brevirostris*, 3 – *C. laticoronata*, 4 – *C. acantholepis*, *C. canescens*, *C. graminea*, *C. juncea*, and *C. latifolia*. The findings show that *C. acantholepis*, *C. canescens*, *C. graminea*, *C. juncea* and *C. latifolia* are to be treated as synonyms under the name of *C. juncea*.

## 1 Introduction

*Chondrilla* genus is comprised by approximately 30 species that form two subgeni and four sections [1]. The genus is wide spread in the steppes and deserts of Eurasia and North Africa. There are no less than 7 in vivo *Chondrilla* species in European Russia. Six of them – *C. acantholepis* Boiss., *C. brevirostris* Fisch. et Mey, *C. canescens* Kat. et Kir., *C. graminea* Bieb., *C. juncea* L., and *C. latifolia* Bieb.- belong to the section *Chondrilla* of the subgenus *Chondrilla*. The seventh species – *C. ambigua* Fisch. – belongs to the subgenus *Brachyrynchus* [1]. Lately, more *C. laticoronata* Leonova populations are being spotted [2, 3].

Currently, there is no consistency in representing the taxonomic structure of the *Chondrilla* genus. Apomictic reproduction that is highly characteristic of the *Chondrilla* species [4, 5] makes it problematic to single out the species and distinguish between them as well as causes contradiction in evaluating their rank and taxa's relationships. The embryological analysis and the analysis of seed productivity show that, out of all the studied species in European Russia, *C. ambigua* is the only obligate amphimictic species while all the remaining taxa (*C. acantholepis*, *C. canescens*, *C. graminea*, *C. juncea*, *C. latifolia*, *C. brevirostris*, and *C. laticoronata*) are facultative apomicts [3, 5].

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\* Corresponding author: [kashinas2@yandex.ru](mailto:kashinas2@yandex.ru)

The present paper attempts to identify the relationships between the *Chondrilla* species in Southern Russia, based on the plastid DNA sequencing.

## 2 Materials and Methods

### 2.1 Sampling

The specimens were collected from the natural populations of 9 *Chondrilla* species (*C. acantholepis*, *C. ambigua*, *C. brevirostris*, *C. canescens*, *C. graminea*, *C. juncea*, *C. laticoronata*, *C. latifolia*, and *C. pauciflora*) in the Astrakhan, Volgograd, Penza, Samara, Saratov, and Ulyunovsk oblasts as well as the Republic of Kalmykia and the Republic of Crimea. Three plants were selected from every population.

### 2.2 DNA Extraction and Sequencing

DNA was extracted from the ligules (petal-like corollas) after they had been dried in silica gel, using NucleoSpin® Plant II (MACHEREY-NAGEL, Germany) according to the manufacturer's protocol.

Primers *a* and *b*, *c* and *d*, *e* and *f* were used to perform the amplification of the plastid DNA segments *trnT-trnL* and *trnL-trnF*, including the intron *trnL* [6].

Sequencing was carried out in the ABI PRISM 3130 XL sequenator using the reagent kit BIG DYE TERMINATOR kit ver. 3.1 produced by the Sintol company (Moscow, Russia).

### 2.3 Statistical Analysis

The forward and reverse sequences were manually edited and aligned in BioEdit 7.0.5.3. program [7]. The haplotype extraction and building of rootless network employed the method of statistical parsimony in TCS v. 1.21 program [8]. Only the nucleotide recombinations were taken into account; the indels were considered missing data. In order to root the obtained network, the data were further subject to the Neighbor Joining analysis in the program SplitsTree4 ver. 4.11.3[9]. An outgroup was comprised of the DNA sequences of *Helenium arizonicum* S.F.Blake (DQ395174.1) and *Giillardia comosa* A.Gray (DQ395152.1) obtained from GenBank.

## 3 Results and Discussion

The associated alignment of the spacers *trnT-trnL* and *trnL-trnF* and the intron *trnL* included 1292 positions, 13 of which were variable (contained recombinations). Furthermore, the research identified the duplication of the TATTT fragment in all specimens excluding *C. ambigua*, *C. pauciflora*, and *C. brevirostris*; the deletion of GAAA fragment in all specimens except for *C. ambigua*, *C. pauciflora*, *C. brevirostris*, and *C. laticoronata*; and the duplication of ATTACAAAT fragment in all *C. ambigua*, *C. brevirostris*, *C. pauciflora*, and *C. laticoronata* specimens. The analysis in the TCS program allowed for uniting *Chondrilla* sequences in 15 haplotypes. To reconstruct haplotype genealogy, all haplotypes were mapped in one network (probability of parsimonious decision >95%) that included 26 haplotypes, 11 of which were absent in the sample and were hypothetical transitional haplotypes (Fig. 1 on the left). Topologically, 4 clades, or groups were singled out within the network; each group was constituted of the internal haplotype and the closest terminal haplotypes designated with letters and numbers.



(A). The latter two species are characterized by high heterozygosity of the ITS sequences [10]. The second evolutionary route is represented by the species with the haplotype groups B-F. Based on ITS ribotypes, *C. laticoronata* and *C. breviostris*, though possessing the same chloroplast haplotypes as *C. ambigua*, are drastically different from *C. ambigua* and completely heterozygous. Both facts provide strong evidence that *C. laticoronata* and *C. breviostris* derived from the hybridization of *C. ambigua* with some other species that resulted in the formation of new stable apomictic clones.

In conclusion, there is no doubt that *C. ambigua* is an independent species. *C. breviostris* and *C. laticoronata* are hybrid groups, the parental forms of which are missing in the sample. The obtained data prove that *C. acantholepis*, *C. canescens*, *C. graminea*, *C. juncea*, and *C. latifolia* are the specimens of one and the same species under the priority name of *C. juncea*.

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