

Diversity of the genus *Chondrilla* L. (Asteraceae) in Eastern Europe

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Manuscript received: 24 October 2023. Revision accepted: 3 April 2024.

Abstract. Kashin AS, Parkhomenko AS, Kondratieva AO, Kritskaya TA, Bogoslov AV, Shilova IV. 2024. Diversity of the genus *Chondrilla* L. (Asteraceae) in Eastern Europe. *Biodiversitas* 25: 1901-1910. The genus *Chondrilla* (Asteraceae) has a complex taxonomic structure due to widespread apomictic reproduction. This study aimed to identify the variability and clarify the status of *Chondrilla* species growing in Eastern Europe (European Russia and Western Kazakhstan). The study of morphological variability in *Chondrilla* species was assessed using 28 morphometric parameters. Moreover, 840 specimens from 28 populations of 8 *Chondrilla* species from Eastern Europe were analyzed. Principal Component Analysis (PCA), Nonmetric Multi-Dimensional Scaling (NMDS), recursive decomposition, and random forest algorithm were used for analysis. The ordination methods divided the studied samples into two groups: the first group included samples of *C. ambigua* and *C. pauciflora*, and the second group included all remaining samples. Following the resulting binary trees, obtained by recursive decomposition method and the random forest algorithm, a determination key was compiled for the *Chondrilla* species of Eastern Europe. The high level of morphological variation in *Chondrilla* and its distribution characteristics revealed in this study by numerical taxonomy and recursive decomposition methods, along with our previous studies results, show that only *C. ambigua* is a morphologically and genetically well-separated species, including *C. pauciflora* as a triploid cytotype; *C. canescens*, *C. graminea*, *C. latifolia* and *C. juncea* are synonyms with the priority name *C. juncea* and should be considered as subspecies; and *C. brevirostris* and *C. laticoronata* are hybrids with *C. ambigua* as maternal parent.

Keywords: *Chondrilla*, diversity, Eastern Europe, numeric taxonomy, recursive decomposition method

INTRODUCTION

The genus *Chondrilla* L. includes two subgenus, namely *Chondrilla* and *Brachyrinchus* (Leonova 1989) and about 30 species (WFO 2023). It is widespread in the steppe and desert regions of Eurasia and North Africa. Most of the species have extensive areas, including secondary ones (Tremetsberger et al. 2013). *Chondrilla juncea*, which has a natural range in Eurasia and North Africa was accidentally introduced into Australia, Argentina, Canada, and the USA in the mid-20th century, where nowadays recognized as an invasive weed in wheat-growing regions. Moreover, invasions in North America, Australia, and Argentina were independent events, probably with different geographical origins of donors (Gaskin et al. 2013). *Chondrilla laticoronata* also expands its habitat to the southern part of Russia. This was approved by the existing of herbarium specimens from Volgograd, Astrakhan Provinces, and Stavropol Territory of Russia (MW; SARBG). Conversely, endemic species of the genus with restricted areas are known, such as *C. chondrilloides* from Eastern Alps (Woellner et al. 2019, 2022; Info Flora 2020) - a diploid, obligately sexual species as is *C. ambigua* (Orsenigo et al. 2019). On the contrary, the range of *C. ambigua* is extensive and covers the south of Western Siberia, Turkmenistan, Kazakhstan, Uzbekistan, and China (Leonova 1989).

At least 8 species of *Chondrilla* occur naturally in Eastern Europe (Eastern Ukraine, European Russia, and Western Kazakhstan). Six of them belong to subgenus *Chondrilla*,

namely *C. acantholepis* Boiss., *C. brevirostris* Fisch. & C.A.Mey., *C. canescens* Kar. & Kir., *C. graminea* M.Bieb., *C. juncea* L., *C. latifolia* M.Bieb.; and two species belong to subgenus *Brachyrinchus*, namely *C. ambigua* Fisch. ex Kar. & Kir. and *C. pauciflora* Ledeb.. In addition, populations of *C. laticoronata* (subgenus *Chondrilla*) have been increasingly discovered. *C. juncea* and *C. graminea* are found in Ukraine and Russia (Black Sea and Volga-Don regions, Crimea, and Caucasus). *C. latifolia* is widespread in the Middle Dnieper and Lower Don regions, Crimea and Caucasus. *C. acantholepis* is found in the Crimea and Caucasus. *C. brevirostris* and *C. canescens* are common in the Trans-Volga and Lower Volga regions of Russia and Western Kazakhstan. *C. laticoronata* has not previously been recorded in European Russia. *C. ambigua* are found in the Lower Volga region of Russia and Western Kazakhstan. *C. pauciflora* is probably found only in Western Kazakhstan.

No extensive and targeted comparative molecular genetic studies of the genus have previously been carried out. The polymorphism study using molecular genetic methods of DNA analysis was carried out in populations of *C. juncea*, but only to assess the invasive potential of the species and the geographic origin of the invasive genotypes (Gaskin et al. 2013). In addition, several species of *Chondrilla* have been used as outgroups in studies of other systematic groups from Asteraceae (Fernández-Mazuecos et al. 2016). According to Kashin et al. (2017), it was revealed that *C. acantholepis*, *C. canescens*, *C. graminea*, *C. juncea*, *C. latifolia*, *C. brevirostris*, and *C. laticoronata*

are facultative apomicts. An exception was *C. ambigua*, obligately sexual species.

There is still no clear understanding of the taxonomic structure of *Chondrilla*. Apomictic reproduction and widespread distribution (van Dijk 2003) make it difficult to distinguish among species. For example, *C. juncea* and *C. graminea* are considered by some authors as independent species (Leonova 1989), while others combine them into one species *C. juncea* (Global Compositae Checklist 2023). Several authors also include *C. canescens*, *C. brevirostris* (Tutin et al. 1976), and *C. latifolia*, *C. acantholepis* (Tutin et al. 1976; Nasseh 2010) under the priority name of *C. juncea*. This study aimed to identify the diversity and clarify the status of *Chondrilla* species from European Russia and Western Kazakhstan, based on the results of previously conducted studies, supplemented by numeric taxonomy and recursive partitioning methods.

MATERIALS AND METHODS

Plant materials

Plant materials were carried out in 2022 in 28 populations of 8 *Chondrilla* species (Figure 1) from Astrakhan, Volgograd, Rostov, Saratov regions, R. Kalmykia, and Western Kazakhstan (Table 1, Figure 2). The sample size was 30 specimens per population and the total sample size was 840 specimens.

Procedures

The morphometric parameters of leaves and stems were measured in field conditions. The anthodiums and achenes collected in the field were dried and then microscopied and photographed in the laboratory for more accurate measurement. From 1 to 3 plants per population were taken as herbarium specimens and were repositied in the Herbarium of the Saratov State University Botanical Garden (SARBG, collector Shilova I. V.). The study did not involve *C. acantholepis* specimens because they had not been separated from *C. juncea* by any previously used methods. The morphological differences between these two species were insignificant (Leonova 1989).

In most of the studied territory, plants of most species from the subgenus *Chondrilla*, namely *C. canescens*, *C. graminea*, *C. juncea*, and *C. latifolia* coexist in sympatric populations and demonstrate a continuous spectrum of transitions in various combinations according to taxonomically significant traits from one extreme form to another. Therefore, samples selected for analysis were as close as possible to a particular species in terms of morphological characteristics complex (Parkhomenko et al. 2023).

Twenty eight parameters (14 quantitative and 14 qualitative) of vegetative organs, inflorescences, and fruits were observed (Table 2). Qualitative characteristics were measured using a point system. The majority of characteristics are used in global and regional determination keys and floras as diagnostic traits for identifying *Chondrilla* species (Tutin et al. 1976; Leonova 1989; Nasseh 2010).

Data analysis

Ordination methods were used to identify the main patterns of variation in morphological parameters, namely Principal Component Analysis (PCA) for quantitative traits only and Nonmetric Multi-Dimensional Scaling (NMDS) for the entire dataset. Data processing and statistical analysis were performed in R ver. 4.1.2 (R core team 2021). Spearman correlation coefficients were calculated before the analysis. If the correlation coefficients for pairs of variables were >0.90 , one variable from the pair was excluded from the analysis (Nobis et al. 2016; Maia and Goldenberg 2019; Cruz-Lustre et al. 2020). All variables were standardized before the analysis (Biye et al. 2016; Finot et al. 2018). The missing data were replaced using the iterative imputation procedure.

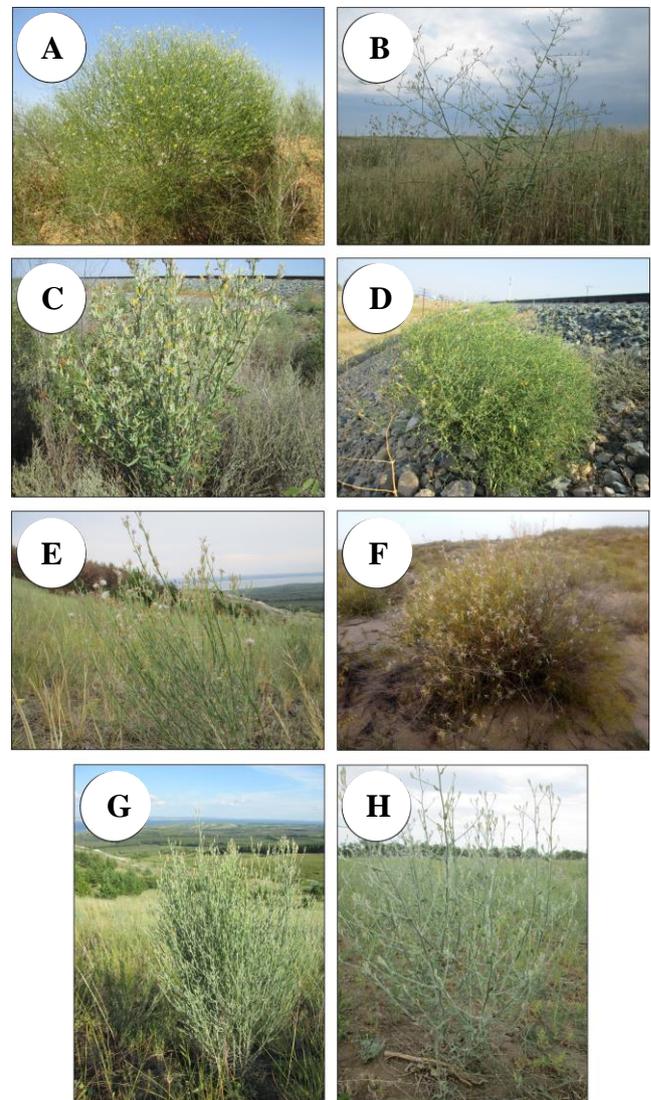


Figure 1. *Chondrilla* species used in the study. A. *C. ambigua*, B. *C. brevirostris*, C. *C. canescens*, D. *C. laticoronata*, E. *C. graminea*, F. *C. pauciflora*, G. *C. juncea*, H. *C. latifolia*

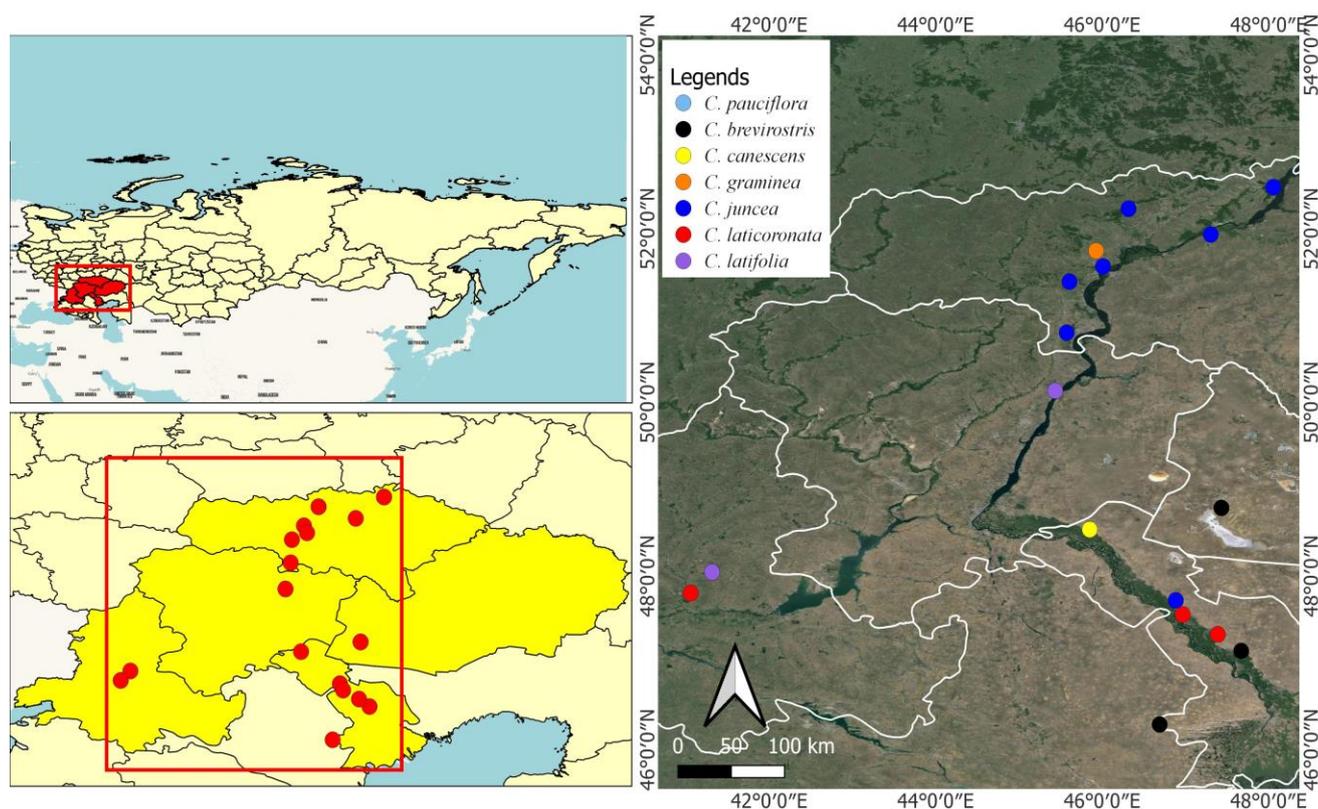


Figure 2. Location of the studied populations of *Chondrilla* species

Table 1. Source of plant materials used in the study

Species	Population name	Coordinates		Altitude (m asl)	Location
		Lat. (N)	Lon. (E)		
<i>C. ambigua</i> Fisch. ex Kar. & Kir.	DOS_A	46°54'33"N	47°55'22"E	-18	Russia, Astrakhanskaya Prov., Krasnoyarskiy distr., near Dosang
<i>C. brevisrostris</i> Fisch. & C.A.Mey.	BOL	47°59'23"N	46°33'3"E	2	Russia, Astrakhanskaya Prov., Akhtubinskiy distr., near Bolkhunuy
	WER	47°43'8"N	46°53'11"E	-4	Russia, Astrakhanskaya Prov., Akhtubinskiy distr., near Verblyuzhiy
	VLN	47°8'13"N	47°40'21"E	-17	Russia, Astrakhanskaya Prov., Kharabalinskiy distr., near Vol'noe
	DOS_B	46°17'23"N	46°41'23"E	-25	Russia, Kalmykia Republic, Yashkul'skiy distr., near Khulkhuta
<i>C. canescens</i> Kat. et Kir.	KAZB	48°47'4"N	47°26'5"E	-2	Kazakhstan, Bokeyordinskiy distr., near Urda
	HVL_CAN	52°28'44"N	48°3'33"E	227	Russia, Saratovskaya Prov., Khvalynskiy distr., near Khvalynsk
	AL_CAN	52°13'49"N	46°19'6"E	184	Russia, Saratovskaya Prov., B.-Karabulakskiy distr., near Alekseevka
<i>C. graminea</i> M.Bieb.	KAP	48°32'10"N	45°51'7"E	3	Russia, Astrakhanskaya Prov., Akhtubinskiy distr., near Kapustin Yar
	HVL_GR	52°28'44"N	48°3'33"E	227	Russia, Saratovskaya Prov., Khvalynskiy distr., near Khvalynsk
<i>C. juncea</i> L.	AL_GR	52°13'49"N	46°19'6"E	184	Russia, Saratovskaya Prov., B.-Karabulakskiy distr., near Alekseevka
	PRI	51°44'47"N	45°55'45"E	112	Russia, Saratovskaya Prov., Atkarskiy distr., near Prirechnoe
<i>C. laticoronata</i> Leonova	BS	51°33'57"N	46°0'42"E	101	Russia, Saratovskaya Prov., Saratov, Botanical Garden
	VOL	51°56'3"N	47°18'26"E	19	Russia, Saratovskaya Prov., Marksovskiy distr., near Volkovo
	HVL_JUN	52°28'44"N	48°3'33"E	227	Russia, Saratovskaya Prov., Khvalynskiy distr., near Khvalynsk
	AL	52°13'49"N	46°19'6"E	184	Russia, Saratovskaya Prov., B.-Karabulakskiy distr., near Alekseevka
	BAZJ	47°48'13"N	41°3'27"E	14	Russia, Rostovskaya Prov., Konstantinovskiy distr., near Bazki
	KMHJ	50°8'5"N	45°26'15"E	103	Russia, Volgogradskaya Prov., Kamyshinskiy distr., near Kamyshin
	MELJ	50°48'11"N	45°34'32"E	289	Russia, Saratovskaya Prov., Krasnoarmeyskiy distr., near Melovoye
<i>C. latifolia</i> M.Bieb.	PPVJ	51°23'30"N	45°36'32"E	255	Russia, Saratovskaya Prov., Saratovskiy distr., near Popovka
	WER_L	47°43'8"N	46°53'11"E	-4	Russia, Astrakhanskaya Prov., Akhtubinskiy distr., near Verblyuzhiy
	SAS	47°33'21"N	46°58'12"E	-18	Russia, Astrakhanskaya Prov., Kharabalinskiy distr., near Sasykoli
	TAM	47°19'44"N	47°23'34"E	-16	Russia, Astrakhanskaya Prov., Kharabalinskiy distr., near Tambovka
<i>C. pauciflora</i> Ledeb.	KAZL	48°47'4"N	47°26'5"E	-2	Kazakhstan, Bokeyordinskiy distr., near Urda
	BAZL	47°48'13"N	41°3'27"E	14	Russia, Rostovskaya Prov., Konstantinovskiy distr., near Bazki
	ROST	48°2'43"N	41°18'54"E	100	Russia, Rostovskaya Prov., Tatsinskiy distr., near Verkhnekol'tsov
<i>C. juncea</i> L.	KMH	50°8'5"N	45°26'15"E	103	Russia, Volgogradskaya Prov., Kamyshinskiy distr., near Kamyshin
<i>C. pauciflora</i> Ledeb.	KAZP	48°47'4"N	47°26'5"E	-2	Kazakhstan, Bokeyordinskiy distr., near Urda

Table 2. Morphometric parameters of *Chondrilla* specimens

Parameter	Units/points
1. Length of the anthodium pedicel	mm
2. Character of pubescence on the third cauline leaf from the bottom	1 - no pubescence 2 - cobwebby 3 - bristly
3. Location of bristly pubescence on the third cauline leaf from the bottom	1 - at the top along the edge 2 - along the edge and below on middle vein 3 - at the top along the edge and below on middle vein
4. Length of the third cauline leaf from the bottom	cm
5. Width of the third cauline leaf from the bottom	mm
6. Presence of bristly cover on stem	1 - absent 2 - only near the base of the stem 3 - all over the stem
7. Direction of the bristles on stem	1 - perpendicular to stem 2 - down bent 3 - up bent
8. Density of bristly stem cover on the vertical segment of 6 mm	pcs
9. Length of bristles	mm
10. Thickness of bristles	1 - capillaceous 2 - coarse
11. Bristly pubescence of the anthodium	1 - absent 2 - present
12. Number of flowers in the anthodium	pcs
13. Number of inner leaflets of spathe	pcs
14. Character of bristly cover on inner leaflets of spathe	1 - absent 2 - small solitary 3 - short and sparse 4 - long and crowded, coarse
15. The density of the bristles (number of bristles on the middle vein of the spathe inner leaflet)	pcs
16. Size of the bristles on the middle vein of the spathe inner leaflet	mm
17. Length of the extended part of the achene	mm
18. Width of the extended part of the achene	mm
19. Presence of the tubercles and squamules on the achene	1 - absent 2 - tubercles are present 3 - squamules are present
20. Location of the squamules on the extended part of the achene	1 - near the top 2 - on 1/4-1/3 length from top
21. Shape of the squamules on the extended part of the achene	1 - wide and entire 2 - long entire, overlapping each other 3 - small and sharp tubercles and squamules (top ones are quite long) 4 - tridentate 5 - trilobate 6 - with the deep and wide hollow in the middle
22. Length of rostellum	mm
23. Width of rostellum	mm
24. Presence of the articulation of rostellum	1 - absent (rostellum does not break off) 2 - weakly outlined (rostellum breaks off incorrectly) 3 - present
25. Thickening of the rostellum	1 - absent 2 - slight thickening 3 - pronounced clavate
26. Presence of the coronet	1 - the coronet is absent or outlined in the form of rare tubercles and small squamules 2 - the coronet is well developed
27. Size of the coronet squamules	mm
28. Shape of the coronet squamules	1 - wide blunt 2 - obtuse tridentate 3 - trilobate, with a more developed middle lobe 4 - wide and short deep-trilobate, with lobes of almost the same length (for some achenes, the squamules are reduced to small tubercles) 5 - entire acuminate 6 - entire ovate or oblong 7 - wide, truncated, and gnawed at the top 8 - rounded lati-spathulate

Note: The quantitative parameters are measured in units; the qualitative parameters are measured in points

The NMDS analysis was carried out using the matrix of Gower distances, calculated by Gower.dist() function from the 'shipunov' package (Shipunov 2022). The NMDS was performed using the metaMDS function from the 'vegan' package (Oksanen et al. 2020). To identify the relationships between the observed pattern of variation and the most biologically meaningful environmental factors, spatial data with 0.5 arc minutes (1 km) resolution were obtained for each *Chondrilla* population from the WorldClim ver. 2.0 database (Fick and Hijmans 2017). This dataset included 19 bioclimatic variables and altitude. The latitude and longitude were also taken into account in the analysis. The environmental parameters were projected onto the ordination plot as vectors using the envfit() function from the 'vegan' package. The direction of the vector indicated the fastest change in the values of the corresponding variable (direction of gradient); the vector's length was proportional to the correlation between the environmental variable and the ordination axes (strength of gradient). The correlations between the environmental variables and ordination axes were tested using a Monte Carlo permutation test.

The random forest algorithm was applied to all subsets following the already determined species identity. We used the supervised classification approach described by Shipunov and Efimov (2015). This method takes an existing classification and creates binary trees with structures similar to dichotomous keys (Therneau et al. 2014). The results of the recursive decomposition were used to construct a dichotomous key.

RESULTS AND DISCUSSION

Principal Component Analysis (PCA)

The first two principal components explain 52% of the total variation in morphometric parameters of *Chondrilla* populations (Figure 3). The scatter plot shows a clear separation of specimens by species along the first principal component. On the left side of the ordination plot is a separate cloud formed by points corresponding to *C. ambigua* and *C. pauciflora* specimens. The points corresponding to *C. ambigua* specimens occupy a more peripheral position. The specimens of *C. ambigua* and *C. pauciflora* have the longest anthodium pedicels, the longest extended part of the achene, and maximally wide and short rostellum.

The cloud of points formed by specimens of the remaining 6 species of *Chondrilla* is situated in the central and right parts of the ordination plot. This cloud has its distribution features. The points corresponding to specimens of *C. brevirostris* and *C. laticoronata* are placed mostly on the left side. Their location along the first principal component is also associated with the length of the anthodium pedicel, the length of the extended part of the achene, and the length of rostellum. The specimens of *C. canescens* and *C. latifolia* occupy the right part of the described cloud. In this case, specimens of *C. latifolia* occupy the most peripheral position. The incomplete separation of these species is associated with the width of the leaf, the density of bristly stem cover, number of inner leaflets of the spathe, length of the rostellum, and size of

the coronet squamules.

The points corresponding to specimens of *C. graminea* and *C. juncea* are placed predominantly in the right and central parts of the single cloud and significantly mixed. At the same time, they are mixed with points on the left (*C. brevirostris* and *C. laticoronata*) and points on the right (*C. canescens* and *C. latifolia*). The latter circumstance indicates significant polymorphism of *C. graminea* and *C. juncea*.

The strength of association between morphometric parameters and the main pattern of variation is represented in Table 3. The greatest contribution to the observed separation of species is made by the characteristics of anthodium and achene. The maximum correlation (>0.70) with the first Principal Component (PC1) was noted for the following parameters: number of flowers in the anthodium, number of inner leaflets of spathe, length of the expanded part of the achene, length, and width of rostellum. The maximum correlation (>0.70) with the second Principal Component (PC2) was noted for the size of the bristles on the middle vein of the spathe inner leaflet (Table 3).

Nonmetric Multi-Dimensional Scaling (NMDS)

The scatterplot shows a separation of *C. ambigua* and *C. pauciflora* specimens from specimens of other species in the same way as for PCA. However, the separation between these two species is clearer in this analysis (Figure 4). Their separation from specimens of other species and each other occurs mainly along the first axis. Specimens from *C. latifolia* populations are compact but mixed with specimens of *C. canescens*, *C. juncea*, and *C. graminea*. The specimens of *C. canescens* are mixed with specimens of *C. graminea* and *C. juncea*. The clouds of *C. brevirostris* and *C. laticoronata* are the most overlapped. At the same time, both of them are not completely separated from *C. juncea*.

Table 3. Correlation of quantitative morphometric parameters with ordination axes

Morphometric parameter	Correlation	
	PC1	PC2
Length of the anthodium pedicel	-0.55	0.15
Length of the third cauline leaf from the bottom	0.44	0.20
Width of the third cauline leaf from the bottom	0.53	0.52
Density of bristly stem cover on the vertical segment of 6 mm	0.62	0.14
Length of bristles	0.43	0.21
Number of flowers in the anthodium	0.88	-0.25
Number of inner leaflets of spathe	0.83	-0.22
The density of the bristles (number of bristles on the middle vein of the spathe inner leaflet)	0.39	0.67
Size of the bristles on the middle vein of the spathe inner leaflet	-0.42	0.73
Length of the extended part of the achene	-0.78	-0.01
Width of the extended part of the achene	-0.31	-0.05
Length of rostellum	0.87	0.04
Width of rostellum	-0.84	0.16
Size of the coronet squamules	0.54	0.25

Note: Correlation >0.70 highlighted in bold, PC1: The first Principal Component, PC2: The second Principal Component

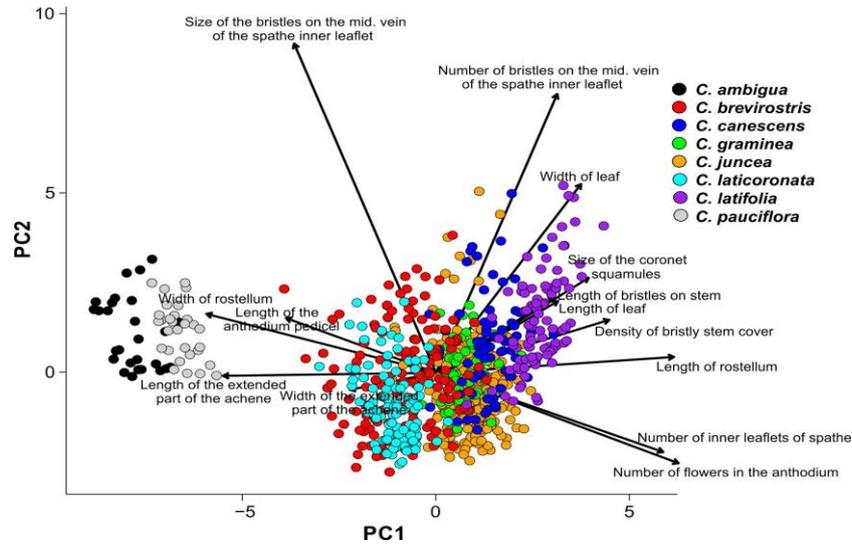


Figure 3. Principal component analysis of 840 specimens from 28 populations of 8 *Chondrilla* species. PC1: The first Principal Component, PC2: The second Principal Component

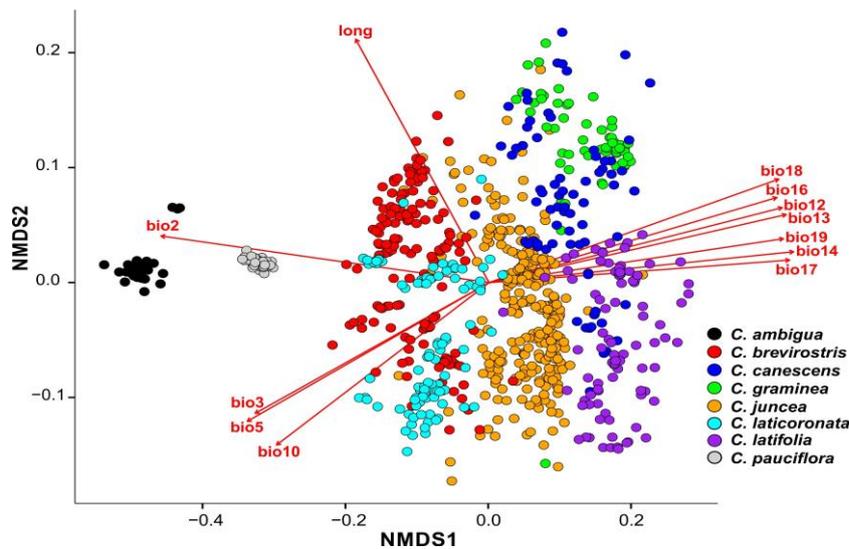


Figure 4. Nonmetric multidimensional scaling of 840 specimens from 28 populations of 8 *Chondrilla* species

The observed pattern of variation is most closely associated with qualitative characteristics (Table 4). The parameters with the greatest contribution are the location and shape of the squamules on the extended part of the achene ($R^2 = 0.32$; $R^2 = 0.27$), the presence of a bristly cover of the stem ($R^2 = 0.21$), the location of bristly pubescence on the third cauline leaf from the bottom ($R^2 = 0.19$), and the presence of the articulation of rostellum ($R^2 = 0.19$) (Table 4). The most significant quantitative characteristics were the leaf width ($R^2 = 0.23$), the coronet squamules' size ($R^2 = 0.19$), and the width of the extended part of the achene ($R^2 = 0.15$) (Table 4).

The biplot shows only significant bioclimatic factors that make the greatest contribution to explaining the observed variation (with R^2 values >0.30) (Figure 4). The factors most correlated with the first axis are bio2, bio18,

bio14, bio12, bio17, bio13, bio16, and bio19. All of them, except for bio2 is precipitation factors (Table 5).

Recursive decomposition method and random forest algorithm

Several binary trees considering all the analyzed morphological characteristics (quantitative and qualitative) were created. The first tree included all 8 *Chondrilla* species under study on the presumption that all of them are considered as well-distinct (Figure 5A). The tree has a rather intricate structure. It can be seen that the main confusion comes from *C. brevisrostris* specimens. Regarding the width of the leaf and the length of rostellum, the species is very polymorphic and contrasts with itself. Therefore, the use of this tree to formulate dichotomous keys was unacceptable.

Table 4. Relationship of *Chondrilla* morphometric parameters with NMDS axes

Morphometric parameters	R ²	Pr (>r)
Quantitative		
Length of the anthodium pedicel	0.05	0.14
Length of the third cauline leaf from the bottom	0.02	0.36
Width of the third cauline leaf from the bottom	0.23	0.00
Density of bristly stem cover on the vertical segment of 6 mm	0.07	0.07
Length of bristles	0.06	0.08
Number of flowers in the anthodium	0.01	0.54
Number of inner leaflets of spathe	0.00	1.00
The density of the bristles (number of bristles on the middle vein of the spathe inner leaflet)	0.06	0.06
Size of the bristles on the middle vein of the spathe inner leaflet	0.05	0.11
Length of the extended part of the achene	0.04	0.23
Width of the extended part of the achene	0.15	0.01
Length of rostellum	0.01	0.79
Width of rostellum	0.06	0.09
Size of the coronet squamules	0.19	0.00
Qualitative		
Character of pubescence on the third cauline leaf from the bottom	0.00	1.00
Location of bristly pubescence on the third cauline leaf from the bottom	0.19	0.00
Presence of a bristly cover on stem	0.21	0.00
Direction of the bristles on stem	0.09	0.00
Thickness of bristles	0.14	0.00
Bristly pubescence of the anthodium	0.06	0.02
Character of bristly cover on inner leaflets of spathe	0.09	0.01
Presence of the tubercles and squamules on the achene	0.00	1.00
Location of the squamules on the extended part of the achene	0.32	0.00
Shape of the squamules on the extended part of the achene	0.27	0.00
Presence of the articulation of rostellum	0.19	0.00
Thickening of the rostellum	0.10	0.01
Presence of the coronet	0.00	1.00
Shape of the coronet squamules	0.10	0.01

Note: R²: The coefficient of determination, Pr (>r): The significance level. The higher values of R² are in bold

Specimens of *C. brevisrostris* were excluded from the analysis to construct a new binary tree. Additionally, to avoid confusion, specimens of *C. laticoronata* with the shape of coronet squamules atypical for this species (the smooth-edged ovoid shape instead of a three-lobed one) were also excluded. The proportion of such individuals was 3% of the total number of studied individuals of this species. However, the new tree also contained several contradictions (Figure 5B). *Chondrilla canescens* contrasted with itself due to the wide range of variation in the leaf's width. On the contrary, *C. ambigua*, *C. pauciflora*, and *C. laticoronata* were separated quite well based on the rostellum's length and width and the squamules' presence on the extended part of the achene. For a clearer separation of species on dendrogram, the specimens of *C. juncea*, *C. graminea*, *C. canescens* and *C. latifolia* were combined into one group with the priority name *C. juncea* (Figure 5C).

Table 5. Relationship of environmental factors with NMDS axes

Environmental factor	Name	R ²	Pr (>r)
Annual mean temperature	bio1	0.26	0.02
Mean diurnal range	bio2	0.63	0.02
Isothermality	bio3	0.38	0.02
Temperature seasonality (coefficient of variation)	bio4	0.13	0.02
Maximum temperature of the warmest month	bio5	0.41	0.02
Minimum temperature of the coldest month	bio6	0.15	0.02
Temperature annual range	bio7	0.23	0.02
Mean temperature of the wettest quarter	bio8	0.03	0.02
Mean temperature of the driest quarter	bio9	0.00	0.68
Mean temperature of the warmest quarter	bio10	0.36	0.02
Mean temperature of the coldest quarter	bio11	0.19	0.02
Annual precipitation	bio12	0.52	0.02
Precipitation of the wettest month	bio13	0.53	0.02
Precipitation of the driest month	bio14	0.54	0.02
Precipitation seasonality (coefficient of variation)	bio15	0.03	0.02
Precipitation of the wettest quarter	bio16	0.50	0.02
Precipitation of the driest quarter	bio17	0.52	0.02
Precipitation of the warmest quarter	bio18	0.52	0.02
Precipitation of the coldest quarter	bio19	0.51	0.02
Elevation	alt	0.30	0.02
Latitude	lat	0.30	0.02
Longitude	long	0.38	0.02

Note: R²: The coefficient of determination, Pr (>r): The significance level. The higher values of R² are in bold

Based on the latest version of binary tree (Figure 5C), the determination key for *Chondrilla* species native to European Russia and Western Kazakhstan was created as follows:

- 1a. Length of rostellum more than or equal to 2.9 mm 2
- 1b. Length of rostellum less than 2.9 mm 6
- 2a. Width of leaf more than 9.8 mm 3
- 2b. Width of leaf less than 9.8 mm 4
- 3a. Length of the anthodium pedicel more than or equal to 1.8 mm *C. juncea* ssp. *canescens*
- 3b. Length of the anthodium pedicel less than 1.8 mm *C. juncea* ssp. *latifolia*
- 4a. The anthodium has bristly pubescence *C. juncea* ssp. *graminea*
- 4b. The bristly pubescence of anthodium is absent 5
- 5a. The stem is bristly pubescent along its entire length *C. juncea* ssp. *canescens*
- 5b. The stem is bristly pubescent only at the basal part or completely bare *C. juncea* ssp. *juncea*
- 6a. Width of rostellum less than or equal to 0.29 mm 7
- 6b. Width of rostellum more than 0.29 mm 8
- 7a. Width of the extended part of the achene less than 0.7 mm, length of rostellum-less than 1.87 mm *C. x brevisrostris*
- 7b. Width of the extended part of the achene more than 0.7 mm, length of rostellum-more than 1.87 mm *C. x laticoronata*
- 8a. The extended part of the achene is smooth *C. ambigua*
- 8b. The extended part of the achene covered by tubercles and squamules *C. ambigua* var. *pauciflora*

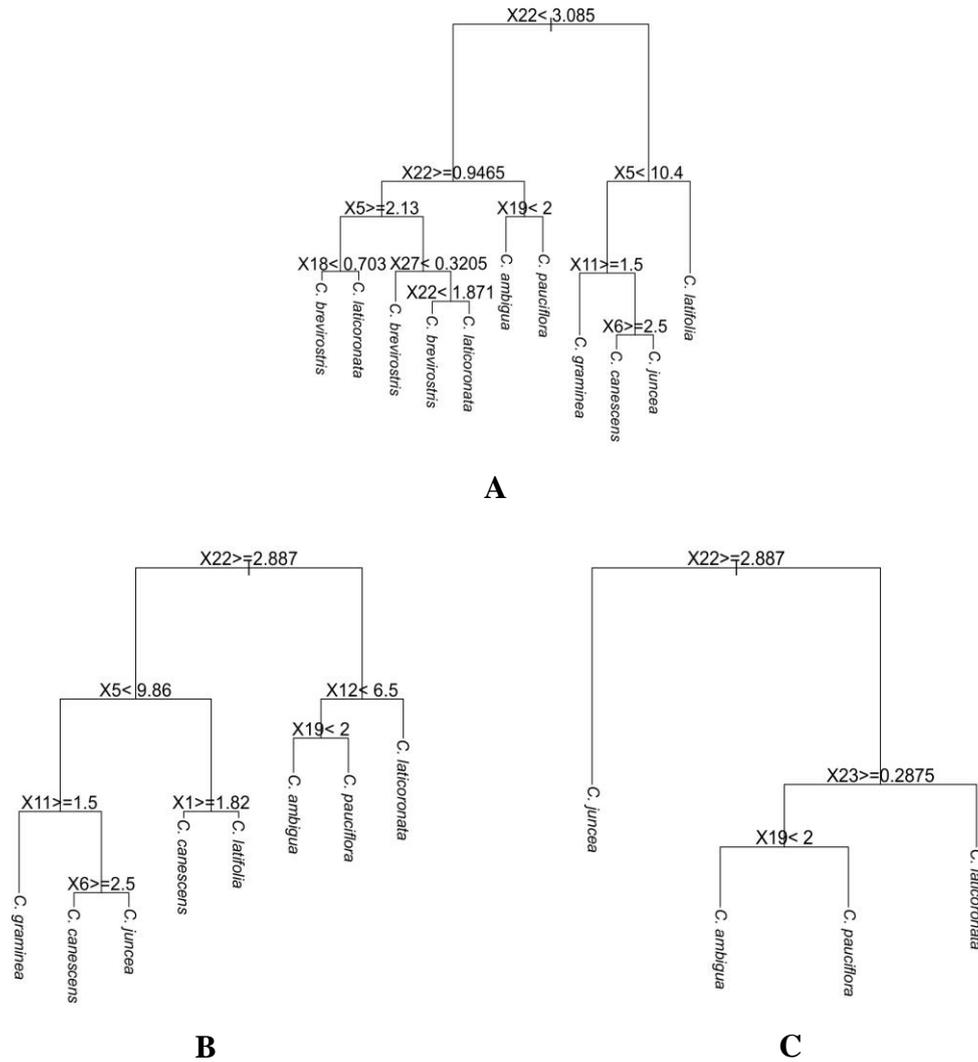


Figure 5. Binary trees obtained as a result of recursive decomposition of all morphological parameters based on a given species identity. A. Species identity is given following the classification of Leonova (1964, 1989), B. The same, but except for *C. brevirostris*, *C. Chondrilla juncea*, *C. graminea*, *C. canescens*, and *C. latifolia* are combined into one group *C. juncea*. X1: Length of the anthodium pedicel, X5: Width of the third cauline leaf from the bottom, X6: Presence of bristly cover on stem, X11: Bristly pubescence of the anthodium, X12: Number of flowers in the anthodium, X18: Width of the extended part of the achene, X19: Presence of the tubercles and squamules on the achene, X22: Length of rostellum, X23: Width of rostellum, X27: Size of the coronet squamules

Discussion

This study showed that in the analysis, which took into account quantitative traits, the *Chondrilla* specimens were separated into two groups. The first group included specimens of *C. ambigua* and *C. pauciflora*, and the second included all other specimens. Analysis using both quantitative and qualitative traits separated species in similar ways, but *C. ambigua* and *C. pauciflora* distinguished from each other more clearly. Primarily it was due to qualitative characteristics, such as form and location of squamules on the extended part of achene having a great contribution to the first axis. Specimens of *C. ambigua* have no squamules and tubercles on the extended part of achene, while specimens of *C. pauciflora* have small but well distinguishable squamules and tubercles, these characteristics are considered as separation determining. However, these characters are not enough to consider these two taxa as independent

species, given their genetic similarity, which was shown both by molecular markers (Kashin et al. 2019), and chromosome morphology (Parkhomenko et al. 2019). *Chondrilla ambigua* and *C. pauciflora* share the common plastid haplotype, and the ribotypes are separated from each other by only two mutational steps. The genealogical networks of chloroplast (*trnT-trnF*) and nuclear (ITS1-5.8S-ITS2) DNA sequences clearly unite them into one cluster (Kashin et al. 2019). Within groups of homologous chromosomes, the chromosomes of *C. pauciflora* ($3x=15$) are completely identical to *C. ambigua* ($2x=10$) and differ only in number. In contrast, the chromosome morphology of *C. juncea* or *C. brevirostris* in III and IV pairs varies a lot (Parkhomenko et al. 2019). Based on the whole set of results, we conclude that *C. pauciflora* is a variety (triploid cytotype) of the diploid *C. ambigua*.

Based on morphological analysis, there is also no clear separation between *C. brevirostris* and *C. laticoronata*, *C. juncea*, *C. graminea*, *C. canescens*, and *C. latifolia*. According to morphological characters, *C. juncea* is located between the "*C. brevirostris* + *C. laticoronata*" and "*C. graminea* + *C. canescens* + *C. latifolia*" groups. "*C. brevirostris* + *C. laticoronata*" group is closest to the "*C. ambigua* + *C. pauciflora*" group in terms of morphological characters (Figures 3 and 4). A similar pattern was observed in the combined network of haplo- and ribotypes constructed using the statistical parsimony method (Kashin et al. 2019). It was shown that *C. brevirostris* and *C. laticoronata* have the same chloroplast haplotypes as *C. ambigua* and *C. pauciflora*. The ribotypes of these species in the ITS1–5.8S–ITS2 region were excluded from the analysis due to heterozygosity, indicating their hybrid nature. The same work presents a dendrogram constructed by the NeighborNet method, which more clearly divided the sequences into two separate clusters: the first cluster included specimens of *C. ambigua* and *C. pauciflora*; and the second cluster included *C. juncea*, *C. graminea*, *C. latifolia*, and *C. canescens*, grouped into subclusters without any taxonomic correspondence. *C. brevirostris* and *C. laticoronata* were located in this network between the two main clusters. However, ISSR analysis and modeling in the NewHybrids program showed that all three species, the amphimictic *C. ambigua*, and the facultative apomictic *C. brevirostris* and *C. laticoronata*, are equally well separated from each other. All three species were classified as independent parent classes. It follows that both *C. brevirostris* and *C. laticoronata* can originate from *C. ambigua* by hybridizing the latter with some species from the subgenus *Chondrilla* that were not included in the analysis, with the formation of stable, predominantly apomictic clones. They could have arisen as a result of two independent hybridization events in the past. The agreement of plastid haplotypes among three species points to *C. ambigua* as a maternal parent in both cases. The male parents in these hybridizations were most likely two different species from the subgenus *Chondrilla*. The position of *C. brevirostris* and *C. laticoronata* between *C. ambigua* and species from subgenus *Chondrilla* in the NeighborNet network supports this hypothesis (Kashin et al. 2019).

The presented analysis of relationship between the observed pattern of morphological variation and bioclimatic factors show that the greatest contribution to the species separation is made by precipitation parameters (all except bio15) and mean diurnal range of temperature (bio2) (Figure 4). Moreover, the analysis showed that these parameters may be associated with *Chondrilla* variation in taxonomically significant traits used to identify species, such as location and shape of squamules on the extended part of achene, width of the leaf, or the presence of bristly cover on stem. These results prove the presence of high intraspecific morphological variation in *C. canescens*, *C. graminea*, *C. latifolia*, *C. juncea*, *C. brevirostris*, and *C. laticoronata*, which makes it difficult to identify them correctly during the field study. To simplify this problem, we presented an updated determination key for *Chondrilla* species from European Russia and Western Kazakhstan

based on the last version of binary tree (Figure 5C) taking into account new interpretation of *Chondrilla* taxonomy based on the results of recent morphometric (Parkhomenko et al. 2023), molecular (Kashin et al. 2017, 2019), and cytogenetic (Parkhomenko et al. 2019) studies.

In conclusion, the high level of intraspecific morphological diversity of Eastern European *Chondrilla* species along with previously obtained data on genetic polymorphism, confirms the necessity for taxonomical revision of the genus. In this regard, the new interpretation proposes integration of *C. canescens*, *C. graminea*, *C. latifolia*, and *C. juncea* into one species with the priority name *C. juncea*, inclusion of *C. pauciflora* in *C. ambigua* as a triploid cytotype and also supports assumptions about the hybrid nature of *C. brevirostris* and *C. laticoronata*.

ACKNOWLEDGEMENTS

We thank The Russian Science Foundation (project No. 22-24-00340, fiscal year 2023) for financially supporting this study through <https://rscf.ru/project/22-24-00340/>.

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