

## Background

The genus *Centaurea* s.l. is one of the richest and most taxonomically complex of the Asteraceae family. It includes between 400 and 700 species, of which more than 70 are found in Bulgaria – a territory considered as one of the secondary centers of speciation of the genus. There is a large number of endemics (Bulgarian and Balkan) with endemism reaching up to 50% in some groups, such as *Cyanus* gr. Due to the ongoing active speciation in the Balkans, the boundaries between closely related taxa cannot be easily established based entirely on morphological features. Thus, the taxonomy of the genus in the Bulgarian flora is not well resolved yet. The application of molecular marker approaches would significantly improve and accelerate the discovery of new plant species and studying population dynamics.

## Aim of the study

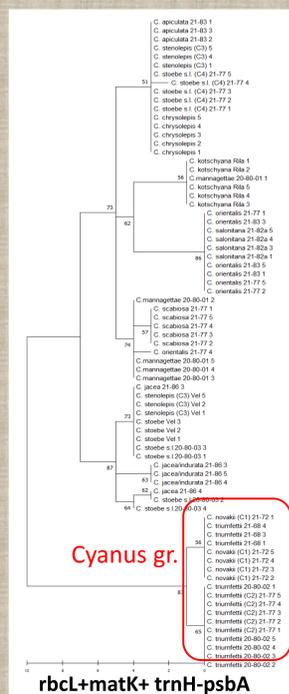
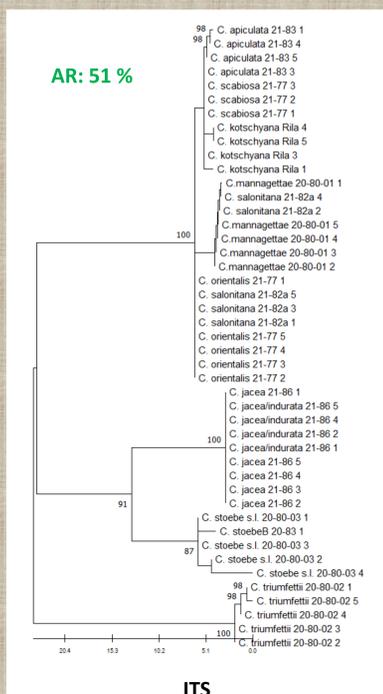
The aim of the study is to assess the genetic diversity and phylogenetic relationship of representatives of the genus *Centaurea* from different floristic regions in Bulgaria by using DNA barcoding method.

## Results

1. The level of genetic discrimination of *Centaurea* specimens and the success of PCR amplification (AR) differed between DNA barcode regions.
2. The rbcL region showed the lowest level of genetic differentiation (2 haplotypes), with the specimens of *C. triumfettii* and *C. novakii* ('*Cyanus*' group) forming a distinct cluster.
3. trnH-psbA and ITS barcode regions showed a genetically variable genus (appr. 6 haplotypes) and individualized each species, while showing traces of possible hybridization between populations of different species.



Fig. 1 Phylogenetic trees of *Centaurea* taxa based on barcode regions rbcL, trnH-psbA, ITS and a consensus tree of plastid markers.



## Material and Methods

### Plant material

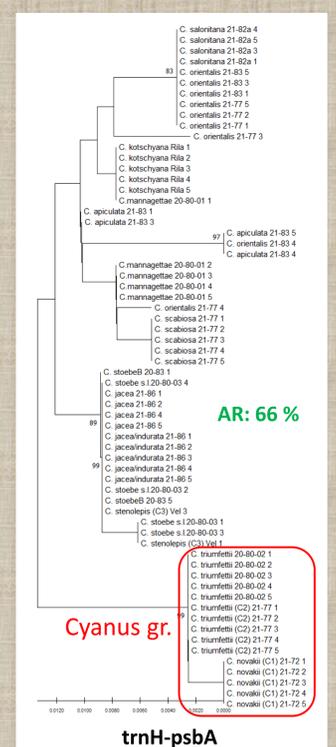
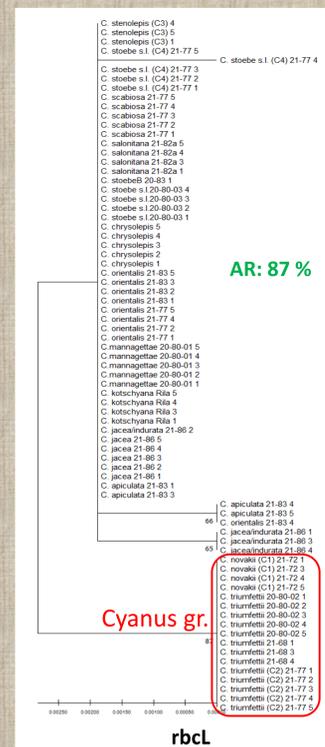
Plant material comprised silica gel-dried leaves of specimens from 15 populations (5 individuals per population) of the genus *Centaurea* collected from different floristic regions in Bulgaria.

Taxon	Floristic region
<i>C. apiculata</i>	Northeast Bulgaria (21-83)
<i>C. jacea</i>	Balkan Range (Western) (21-86)
<i>C. kotschyana</i>	Rila Mts
<i>C. mannagettae</i>	Pirin Mts (20-80)
<i>C. orientalis</i>	Znepole Region (21-77)
<i>C. salonitana</i>	Black Sea Coast (Northern) 21-82
<i>C. scabiosa</i>	Znepole Region (21-77)
<i>C. stenolepis</i>	Rhodopi Mts (Western)
<i>C. stoebe</i>	Znepole Region (21-77), Pirin Mts (20-80, 20-83)
<i>C. novakii</i>	Rhodopi Mts (Central) (21-72)
<i>C. triumfettii</i>	Znepole Region (21-77), Pirin Mts (20-80)



### Methodology

We applied DNA barcoding method based on sequences of barcodes for plants psbA-trnH, matK, rbcL and ITS. Candidate DNA barcode sequences for each barcode region were edited and aligned in MEGA-X software. The same software was used for the construction of phylogenetic trees using a bootstrap method with 500 replicates.



## Conclusions

1. We have obtained preliminary pioneer data about the genetic diversity within the genus *Centaurea* and taxonomic relationship between different species and populations of the genus in Bulgaria.
2. Different barcode regions have different discrimination power.
3. The preliminary results suggest that the '*Cyanus*' group is well separated from the '*Centaurea* s.str.' group which corresponds well to their treatment as different subgenera or genera. Within '*Cyanus*', grouping of taxa corresponds well to the morphology of the species. Within '*Centaurea* s.str.', although a relatively low number of species has been included, grouping of taxa in most cases is congruent with the morphological characters.
4. Presence of incongruent tree topologies for particular species which implies the need for further analyses – repetition of analyses, addition of more presumably closely related species.