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# CYTOTAXONOMY OF *CENTAUREA* SECTION *MESOCENTRON* (ASTERACEAE) IN TÜRKIYE

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## Abstract

This study aims to examine chromosome morphologies of the genus *Centaurea* from Türkiye. Chromosome counts were performed during somatic metaphase using squash technique. The clearest metaphase stages were identified, and the chromosomes were analyzed using an imaging system. The chromosome number for *C. solstitialis* subsp. *carneola* was found to be 2n=18, while the other taxa were determined to have 2n=16. *C. verutum* is differentiated from other taxa with the same chromosome number based on its karyotype formula. In conclusion, it can be inferred that the data obtained from karyomorphological studies contribute to the differentiation of taxa.

## Introduction

*Centaurea*, a significant genus within the tribe Cardueae of the Asteraceae family, encompasses a variety of herbaceous forms as well as some shrubs. These plants are generally characterized by their unarmed leaves (Susanna and Garcia-Jacas, 2007). A notable characteristic of *Centaurea* is its lateral hilum (Dittrich, 1968) and its distinctive floral arrangement, which features prominent sterile peripheral florets that lack staminodes (Wagenitz and Hellwig, 1996). The shape of the scarious bract appendages is a crucial morphological trait for species classification within this genus. *Centaurea* primarily thrives in the Mediterranean and Irano-Turanian regions, although some species, especially those in section Jacea, can be found in temperate areas of Europe (Hilpold *et al.*, 2014).

This genus includes around 650 species globally, making it the largest within the subtribe Centaureinae (Mabberley, 2008). Türkiye serves as a key center of diversity for *Centaurea* (Wagenitz, 1986), and recent studies have identified 247 taxa (İlçim and Demir, 2023; Uysal *et al.*, 2024). Among these, 145 taxa are endemic to Türkiye, yielding an endemism rate of 58.7%.

In Türkiye, the section *Mesocentron* is represented by three taxa (Wagenitz, 1975), and with the inclusion of *Centaurea verutum* L., this number has risen to four (Duran *et al.*, 2014). Of these, two taxa are endemic, resulting in an endemism rate of 50%. The identification key in the Flora of Turkey indicates that taxa within this section are differentiated solely by flower color, spine color, and spine length (Wagenitz, 1975).

Cytotaxonomy is a specialized area within cytogenetics that systematically examines karyological features for evolutionary insights (Siljak-Yakovlev and Peruzzi, 2012). Chromosomes, particularly those of plants, serve as valuable resources for various types of cytogenetic research (Guerra, 2012). Since an organism's genetic information is carried by its chromosomes, any alterations in chromosome number (such as polyploidy or diploidy) and structure (including inversions, deletions, or translocations) play a crucial role in plant evolution and speciation. However, simply knowing the number of chromosomes is insufficient for fully understanding the evolutionary history of a group (Weiss-Schneeweiss and Schneeweiss, 2013);

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*Corresponding author. Email: emrahsirin@selcuk.edu.tr karyomorphology can provide additional insights. In some instances, ecological and morphological data alone may not adequately clarify the evolutionary relationships among species. In these cases, combining cytotaxonomy with molecular data can enhance analysis (Venora *et al*., 2008). The karyotype describes the phenotypic characteristics of chromosomes, including their number, size, arm ratio, centromere position, and other fundamental features (Levin, 2002).

This study aims to uncover the karyomorphological characteristics of species within the section *Mesocentron*, examine the chromosomal relationships among closely related species, and explore the extent of chromosomal variation both within and between the studied taxa.

## Material and Methods

The specimens from the section *Mesocentron* were gathered from different locations, as detailed in Table 1, and preserved in the herbarium of the Biology Department at Selçuk University (KNYA). Only mature achenes were chosen for periodic germination to facilitate chromosomal analysis. Chromosome counts were performed during somatic metaphase using squash technique. Metaphase plates were obtained from primary root meristems. The samples underwent pretreatment with 0.002 M 8-hydroxyquinoline for 8 hours at 4°C, followed by fixation in Karnoy for 24 hrs at the same temperature. Hydrolysis was conducted using 5 N HCl for 30 minutes at room temperature, and the material was then stained with 1% aceto-orcein.

**Table 1. Localities of the studied *Mesocentron* taxa.**

| Taxa | Endemic (E) | Locality | Collector number |
|---|---|---|---|
| *C. solstitialis* L. subsp. *carneola*(Boiss.) Wagenitz | E | İçel: Mut, roadside,187 m, 19 vii 2023 | *E. Şirin* 805 |
| *C. solstitialis* L. subsp. *pyracantha*(Boiss.) Wagenitz | E | İçel: Anamur, roadside 356 m, 19 vii 2023 | *E. Şirin* 806 |
| *C. solstitialis* L. subsp. *solstitialis* | | Konya: Şelçuklu, roadside, 1212 m, 21 vii 2023 | *E. Şirin* 807 |
| *C. verutum*L. | | Gaziantep: Gaziantep–Kilis road, interior of red lentil field, 703 m, 07 vi 2023 | *E. Şirin* 795 |

Preparations were made permanent following Bowen's method (1956). For each taxon, a minimum of 10 metaphases were analyzed, and the clearest metaphase image was captured at 100X magnification with the Olympus DP-72 digital camera connected to the Olympus BX53 microscope.

The chromosome nomenclature proposed by Levan *et al*., (1964) was utilized, designating m and sm for metacentric and submetacentric chromosomes, respectively. Karyotype asymmetry was assessed using the average centromere index (CI), the ratio of the shortest to longest chromosomes, and the A1 and A2 indices. Additionally, total form percentage (TF%, Huziwara 1962), Arano index of karyotype asymmetry (AsK%, Arano, 1963), interchromosomal asymmetry index–interchromosomal asymmetry index (A1–A2, Romero-Zarco, 1986), relative variation in chromosome length ($CV_{cl}$, Paszko, 2006) and Stebbins classification (Stebbins, 1971) values have been determined. Idiograms for the taxa were generated with the Karyo Measure analysis system (Mahmoudi and Mirzaghaderi, 2023).

The karyomorphological traits of four taxa were evaluated, and their correlation coefficients were computed. These taxa were subsequently classified using a clustering analysis technique, specifically the unweighted pair group method with arithmetic mean (UPGMA), which incorporated similarity and standardized variables. To distinguish the *Mesocentron* taxa, 16 quantitative karyomorphological traits were chosen, and their averages were determined. The cluster analysis was performed utilizing Euclidean distances and the UPGMA method via PAST 4.03 software (Fig. 3) (Hammer *et al*., 2001).

Principal component analysis (PCA) was used to identify the most important traits for data interpretation and summarization. Eigenvalues were represented in a two-dimensional scatter plot along the first and second principal components (PC1 and PC2), reflecting the greatest variation (Fig. 4). Box-plot graphs were created based on data regarding A1, A2, $CV_{CL}$, and $CV_{CI}$ (Fig. 5). Both PCA and box plot analyses were carried out using Origin 2018 software (Moberly *et al*., 2018).

## Results and Discussion

*C. solstitialis* subsp. *carneola:* This investigation is the first chromosome count and morphology report of the endemic taxon. Karyological data and asymmetry values are as follows: 2n=18, x=9, PL=2x, HCL=12.96, TF%=42.01, AsK%=57.98, S%=24.96, KF=8m+1sm, AI=3.20, A1=0.28, A2=0.39, $X_{ca}$=16.62, $X_{ci}$=0.41, $CV_{cl}$=39.93, $CV_{ci}$=8.03, Stebbins=4C (Tables 2–3, Figs. 1–2).

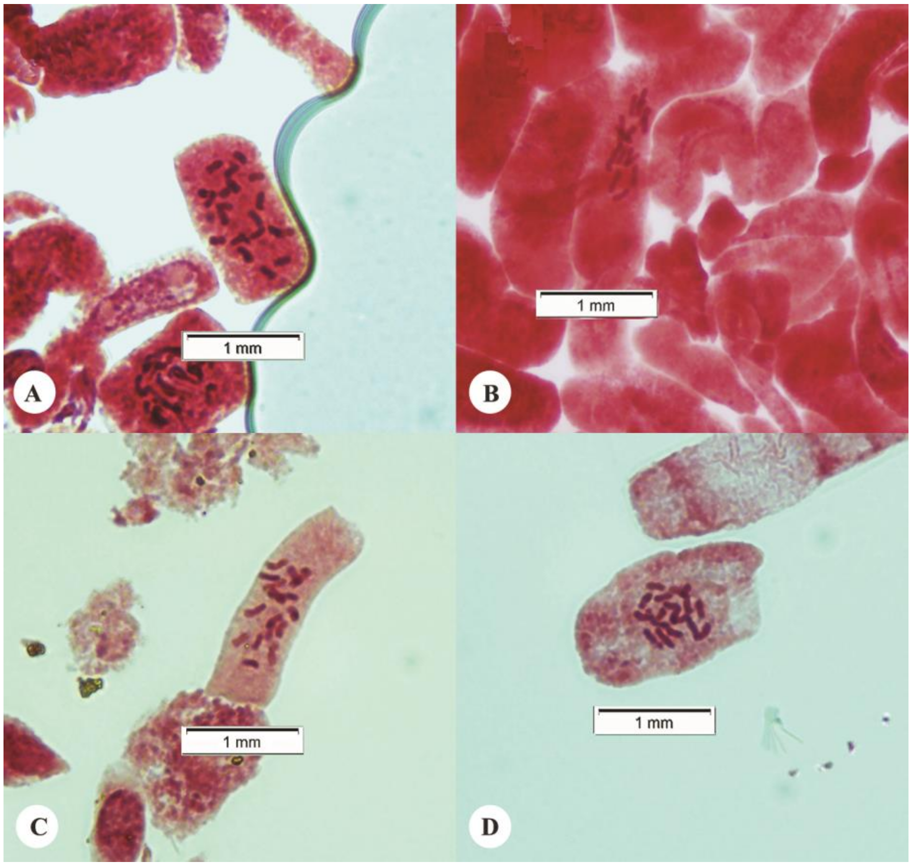

Fig.1. Mitotic metaphase chromosomes of taxa belonging to section *Mesocentron*. A. *C. solstitialis* subsp. *carneola*, B. *C. solstitialis* subsp. *pyracantha*, C. *C.solstitialis* subsp. *solstitialis*, and D. *C. verutum.*

*C. solstitialis* subsp. *pyracantha:* This research is the first chromosome count and morphology report of the endemic taxon. The following presents the karyological data and asymmetry values: 2n=16, x=8, PL=2x, HCL=12.47, TF%=39.20, AsK%=60.79, S%=62.45, KF=5m+3sm, AI=2.67, A1=0.32, A2=0.15, $X_{ca}$=20.49, $X_{ci}$=0.39, $CV_{cl}$=15.65, $CV_{ci}$=17.07, Stebbins=4A (Tables 2–3, Figs. 1–2).

**Table 2. Karyological characteristic parameters of the studied *Mesocentron* taxa.**

| Taxa | 2n | x | PL | HCL | TF%* | AsK%** | S% | KF |
|---|---|---|---|---|---|---|---|---|
| *C.solstitialis* subsp. *carneola* | 18 | 9 | 2x | 12.96 | 42.01 | 57.98 | 24.65 | 8m+1sm |
| *C. solstitialis* subsp. *pyracantha* | 16 | 8 | 2x | 12.47 | 39.20 | 60.79 | 62.45 | 5m+3sm |
| *C. solstitialis* subsp. *solstitialis* | 16 | 8 | 2x | 13.94 | 38.71 | 61.28 | 64.03 | 5m+3sm |
| *C. verutum* | 16 | 8 | 2x | 14.86 | 41.54 | 58.45 | 47.50 | 6m+2sm |

Notes: PL, ploidy level; HCL, Total chromosome length of the haploid complement; TF%, Total form percentage; AsK%, Arano index of karyotype asymmetry; S%, Symmetry index; MCA, Mean Centromeric Asymmetry; KF, Karyotype Formula.
*(Huziwara, 1962); ** (Arano, 1963)

**Table 3. Karyotypes of *Mesocentron* taxa using different methods of evaluating karyotype asymmetry.**
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| Taxa | AI | A1* | A2* | $X_{ca}$ | $X_{ci}$ | $CV_{cl}$** | $Cv_{ci}$** | Stebbins*** |
|---|---|---|---|---|---|---|---|---|
| *C.solstitialis* subsp. *carneola* | 3.20 | 0.28 | 0.39 | 16.62 | 0.41 | 39.93 | 8.03 | 4C |
| *C. solstitialis* subsp. *pyracantha* | 2.67 | 0.32 | 0.15 | 20.49 | 0.39 | 15.65 | 17.07 | 4A |
| *C. solstitialis* subsp. *solstitialis* | 1.27 | 0.36 | 0.15 | 22.34 | 0.38 | 15.59 | 8.19 | 4A |
| *C. verutum* | 3.30 | 0.28 | 0.27 | 17.11 | 0.41 | 27.45 | 12.05 | 4B |

Notes: AI, karyotype asymmetry index; A1, interchromosomal asymmetry index; A2, interchromosomal asymmetry index; $X_{ca}$, mean centromeric asymmetry; $X_{ci}$, Mean centromeric index; $CV_{cl}$, relative variation in chromosome length; $CV_{ci}$, Coefficient of variation of centromeric index; Stebbins: types, classification of karyotypes in relation to their degree of asymmetry according to Stebbins (1971). *(Romero-Zarco, 1986); **(Paszko, 2006); *** (Stebbins, 1971).

*C. solstitialis* subsp. *solstitialis*: Karyological data and asymmetry values are as follows: 2n=16, x=8, PL=2x, HCL=13.94, TF%=38.71, AsK%=61.28, S%=64.03, KF=5m+3sm, AI=1.27, A1=0.36, A2=0.15, Xca=22.34, Xci=0.38, CVcl=15.59, CVci=8.19, Stebbins=4A (Tables 2–3, Figs. 1–2).

*C. verutum*: The following presents the karyological data and asymmetry values: 2n=16, x=8, PL=2x, HCL=14.86, TF%=41.54, AsK%=58.45, S%=47.50, KF=6m+2sm, AI=3.30, A1=0.28, A2=0.27, Xca=17.11, Xci=0.41, CVcl=27.45, CVci=12.05, Stebbins=4B (Tables 2–3, Figs. 1–2).

According to UPGMA dendrogam, *C. solstitialis* subsp. *pyracantha* and *C. solstitialis* subsp. *solstitialis* are the closest taxa, while *C. solstitialis* subsp. *carneola* is the most distant taxon. It can be suggested that the different chromosome count of *C. solstitialis* subsp. *carneola* may have contributed to this situation (Fig. 3).

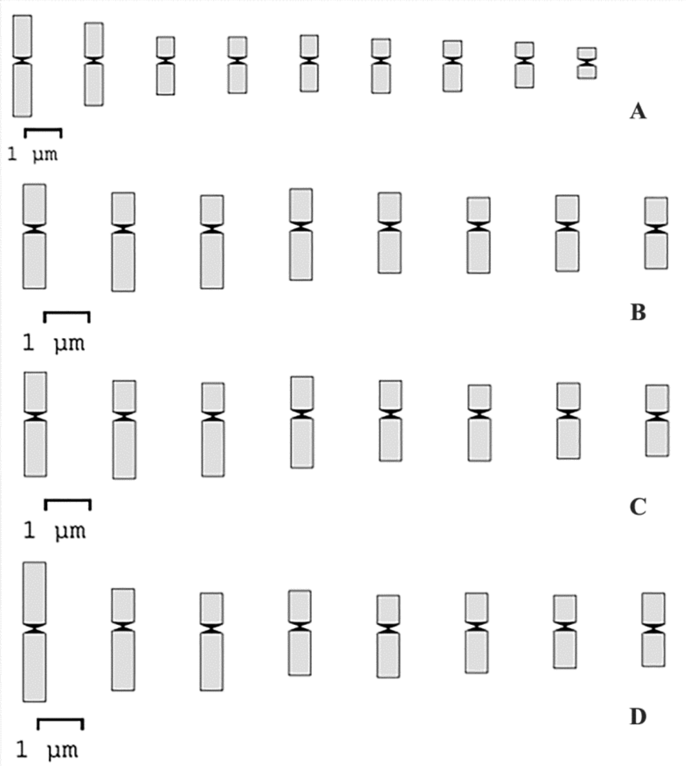

Fig. 2. Idiograms of taxa belonging to section *Mesocentron*. A. *C. solstitialis* subsp. *carneola*, B. *C. solstitialis* subsp. *pyracantha*, C. *C.solstitialis* subsp. *solstitialis*, and D. *C. verutum.*

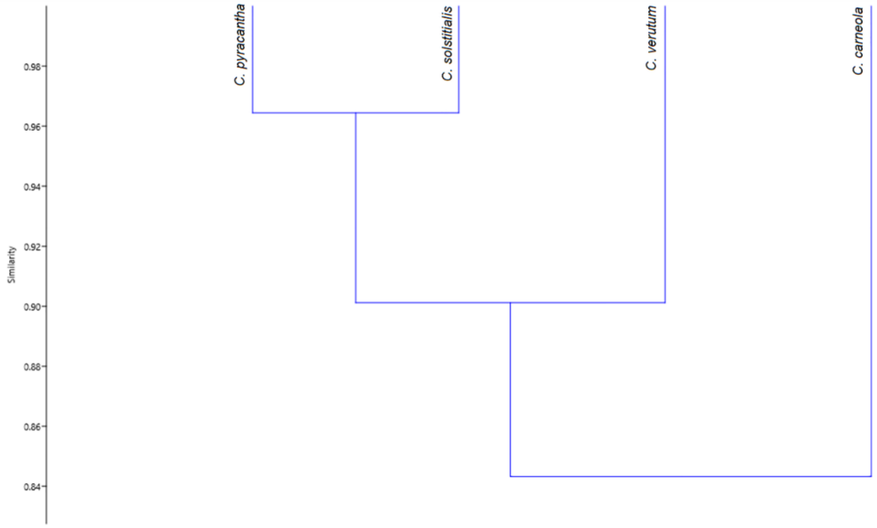

Fig. 3. The UPGMA dendrogram shows similarity distance of the studied *Mesocentron* taxa according to investigated characters.

In regard to PCA analysis, while creating the graph, data on A1, A2, $CV_{cl}$, and $Cv_{ci}$ indexes. As a result, *C. solstitialis* subsp. *solstitialis* and *C. verutum* were positioned closely, while *C. solstitialis* subsp. *carneola* and *C. solstitialis* subsp. *pyracantha* took separate positions (Fig. 4).

Considering the box-plot graphics, just like in the PCA analysis, data on A1, A2, $CV_{cl}$, and $Cv_{ci}$ indexes were used. The box plot displayed a thin or thick appearance according to the reference range of the relevant character. For example, the values for the A1 and A2 indices are close to each other, resulting in a narrow box plot. In contrast, the values for the $CV_{cl}$ and $CV_{ci}$ indices are not close to each other, leading to a wider box plot (Fig. 5).

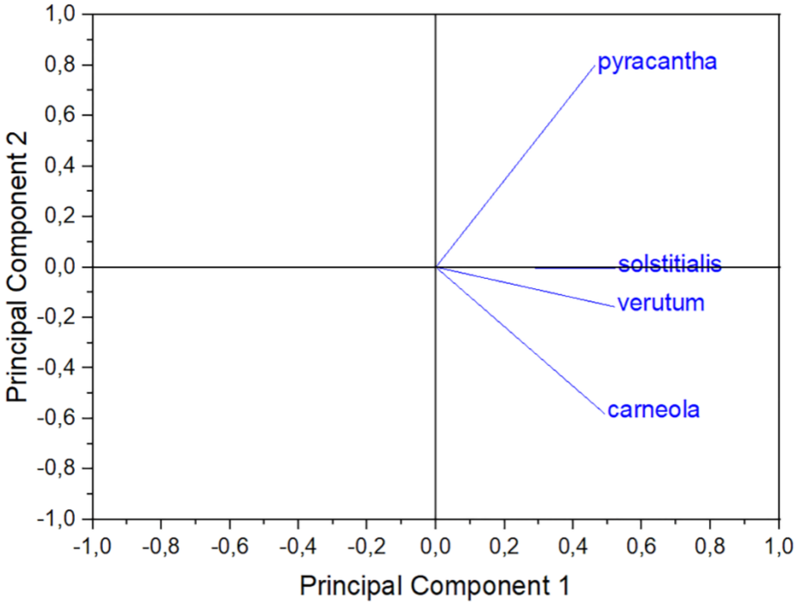

Fig. 4. PCA analysis of achene morphological characteristics.

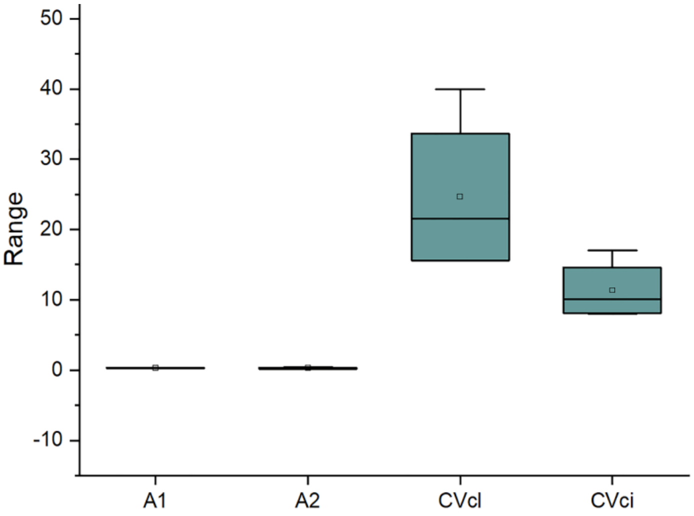

Fig. 5. Box-plot graphics of karyological indexes.

The quantity, dimensions, and asymmetrical characteristics of chromosomes are significant attributes that aid in understanding the evolutionary relationships among species (Eroglu*et al*., 2013). The role of karyology in the systematic classification of different genera within the subtribe Centaureinae has been validated through connections among karyological, morphological, and molecular data (Wagenitz and Hellwig, 1996; Hellwig, 2004).

Karyotype asymmetry serves as a valuable indicator of the overall morphology of plant karyotypes. Alterations in genome characteristics are frequently linked to the evolution of more advanced plant species. An alternative approach to assessing karyotype asymmetry has been suggested, which takes into account both innerchromosomal asymmetry (A1) and interchromosomal asymmetry (A2) indices (Romero-Zarco, 1986).

A1 and A2 values were found to be lower than those reported in previous studies on *Rhaponticoides* and *Centaurea* taxa (Uysal *et al*., 2015; Uysal *et al*., 2017; Şirin *et al*., 2019). This suggests that the section *Mesocentron* comprises more symmetric karyotypes and less evolved species.

Based on the A2 index, the values ranged from 0.15 to 0.39 (Table 3). Specifically, the lowest value was found in *C. solstitialis* subsp. *pyracantha*and *C. solstitialis* subsp. *solstitialis*, while the highest was observed in *C. solstitialis* subsp. *carneola*. Furthermore, mostly taxa exhibited symmetrical karyotypes. These results indicate that chromosomal exchanges (crossovers) within the section *Mesocentron* are restricted.

$X_{CA}$ (as $M_{CA}$) and $CV_{CL}$ are the most suitable parameters for measuring intra- and inter-species asymmetry (Peruzzi and Altınordu, 2014). *C.solstitialis*subsp. *carneola*has the highest $CV_{CL}$ value at 39.93, which distinguishes it from other species. *C. solstitialis*subsp. *solstitialis*has the lowest $CV_{CL}$ value at 15.59. The $X_{CA}$ values of the section *Mesocentron* range from 16.62 to 22.34. The highest value is observed in *C. solstitialis*subsp. *solstitialis*, while the lowest value is observed in *C. solstitialis*subsp. *carneola*.

Uysal *et al*.(2015) reported the AI values for certain *Centaurea* species to range from 1.71 to 3.64. Our findings are somewhat aligned with these values, with AI observed between 1.27 and 3.30. The taxa exhibit symmetrical karyotypes, predominantly in *C. verutum*, while *C. solstitialis* subsp. *solstitialis* displays the least. Overall, the studied taxa seem to be characterized by symmetrical karyotypes and a predominance of m chromosomes. Among the various indices utilized to characterize chromosomes, our results suggest that AI may be preferred, as it shows a stronger correlation compared to other indices. We propose that AI plays a significant role in differentiating closely related species.

According to Garcia-Jacas *et al*. (1996), the distinction between primitive and derived groups within the Centaureinae subtribe is established at x = 12. The fundamental chromosome numbers of x = 12 and lower (such as 8 and 9) are observed in the more advanced groups. Therefore, it can be inferred that section *Mesocentron* is among the most advanced groups in this subtribe.

Numerous chromosome counts have been conducted for the species *C. solstitialis*, all reported as 2n=16 (Strid, 2015; Baeza *et al*., 2016;Carev *et al*., 2016;Carev *et al*., 2017; Semple and Watanabe, 2023). The chromosome count of *C. solstitialis* subsp. *carneola* (2n=18) has been found to be different from that of *C. solstitialis* subsp. *solstitialis* and *C. solstitialis* subsp. *carneola*. It was intriguing to observe that the subspecies exhibited different chromosome counts. The possibility of classifying the variety as a separate species was considered, though the morphological differences were minimal. A similar situation has been noted in the varieties of *Draba helleriana* Greene (Ward, 1983; Ward and Spellenberg, 1988; Warwick and Al-Shehbaz, 2006).

*C. solstitialis* subsp. *carneola* is distinguished from other taxa by its different chromosome count (2n=18). Additionally, it differentiates based on values of TF% (highest), AsK% (lowest), A2 (highest), $X_{ca}$ (lowest), $CV_{cl}$ (highest), and $CV_{ci}$ (lowest). *C. solstitialis* subsp. *pyracantha* is distinguished from other taxa by having the lowest HCL and the highest $CV_{ci}$. *C. solstitialis* subsp. *solstitialis* is distinguished from other taxa by having the lowest TF%, the highest AsK%, the highest S%, the lowest AI, the highest A1, the highest $X_{ca}$, the lowest $X_{ci}$, and the lowest $CV_{cl}$. *C. verutum* is distinguished from other taxa by its karyotype formula (6m+2sm). It is also characterized by having the highest HCL and the highest AI. Based on all these results, we can conclude that karyomorphological characteristics contribute to the differentiation of taxa in the *Mesocentron* section.

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