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Karyology of the *Chenopodiastrum* s. Fuentes et al. (Amaranthaceae) from Bulgaria

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Abstract

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The karyotypes of *Chenopodiastrum murale* and *Chenopodiastrum hybridum* were examined for the first time in their Bulgarian populations. Diploid chromosome number $2n = 18$ was found. The karyotype of 8 pairs of metacentric and 1 pair of submetacentric chromosomes was established for the *C. murale* populations. The total length of the chromosomes varied from 1.4 to 2.55 μm. For the populations of *C. hybridum* the submetacentric pairs of chromosomes were a total of 3 pairs and the metacentric, respectively, were 6 pairs. The total length of the chromosomes varied from 1.42 to 5.7 μm. Clustering of the species based on karyotype features grouped them into separate clusters. Higher values for mean centromeric asymmetry (Mca) is registered for *C. hybridum.* Idiograms of the studied populations were presented.

Keywords: Chenopodiastrum; chromosome number; karyotype; Bulgaria

Introduction

The genus *Chenopodium* L. (Amaranthaceae) is a very diverse group of species, numbering about 150 species (Kühn, 1993). According to recent molecular phylogenetic analyses the remaining major part of the genus has been divided into seven different genera *Chenopodiastrum* S. Fuentes et al., *Chenopodium* s.str., *Lipandra* Moq., *Oxybasis* Kar. & Kir, *Blitum* L., Dysphania R. Br. and Teloxys L. (Fuentes-Bazan et al., 2012 a, b).

Genus *Chenopodiastrum* included 5 species: *Chenopodiastrum murale* (L.) S. Fuentes, Uotila & Borsch; *Chenopodiastrum hybridum (*L.) S. Fuentes, Uotila & Borsch; *Chenopodiastrum coronopus* (Moq.) S. Fuentes, Uotila & Borsch; *Chenopodiastrum badachschanicum* (Tzvelev) S. Fuentes, Uotila & Borsch; *Chenopodiastrum simplex* (Torrey). They are characterized by vesicular trichomes stems and leaves; not dimorphic flowers; 5 perianth segments with prominent midvein visible inside; exclusively horizontal seeds distinctly pitted to sometimes rugulose or almost smooths (Fuentes-Bazan et al., 2012 a, b).

For the Bulgarian flora up to this moment 2 species were reported: *Chenopodiastrum hybridum* (L.) S. Fuentes, Uotila & Borsch (syn.: *Chenopodium hybridum***)** and *Chenopodiastrum murale* (L.) S. Fuentes, Uotila & Borsch (syn.: *Chenopodium murale*) (Yordanov et al., 1966; Assyov & Petrova, 2012).

C. hybridum inhabits all over the country in ruderal or anthropophytic communities up to 1000 m a.s.l. It is frequently a weed in the row crops. It forms not numerous populations on moderately moist soils and prefers shadier terrains.

C. murale occurs on ruderalized habitats in settlements on open or shadier terrains from sea level up to 1000 m a.s.l. It forms populations small in number, of limited area, preferring moderately moist soils.

The present study is part of a multi-year complex research of genus *Chenopodium* s.l. Here we presented karyotype of *Chenopodiastrum* species from their Bulgarian populations.

Materials and Methods

The karyological analysis includes 2 populations of *Chenopodiastrum hybridum* and 3 populations of *C. murale* (Table 1).

The chromosome number has been determined on durable squash preparations on metaphase plates from root tips of seeds germinated in laboratory conditions, collected from the natural habitats of the species. The collected roots have been processed according to the method of Grozeva (2007b). Metaphase plates have been observed on an Olympus BX51 light microscope. The chromosome type is determined by the centromeric index , according to the methodology of Grif & Agapova (1986). The mean length of chromosomes; sum of the length of the haploid chromosome number (hcl) are determined and the karyotype formula is indicated. Karyograms and idiograms were obtained using Adobe Photoshop CS6. At least five metaphase plates have been measured from each population. The herbarium specimens of the karyologically studied plants have been deposited at the Institute of Biodiversity and Ecosystem Research at the Bulgarian Academy of Sciences.

To trace the symmetry of the established karyotypes the following has been used: coefficient of variation of chromosome length (Paszko, 2006), where A_2 is interchromosomal asymmetry index A_2 of Romero Zarko (1986); mean centromeric asymmetry (Peruzzi & Eroğlu, 2013), where A is degree of asymmetry of karyotype proposed by Watanabe et al. (1999).

The parameters describing the karyotype characteristics were statistically processed with Statistica 10 for Windows (for Cluster analysis) and Pirouette 4.5 (for Principal Component Analysis) software. Principal component analysis (PCA) was performed to establish the contribution of each karyotypic parameter to the ordination of species. To group the studied populations by karyotype similarity Cluster analysis (CA) was used.

Results and Discussion

As a result of the conducted karyological study in genus *Chenopodiastrum* diploid chromosome number $2n = 2x = 18$ has been established (Table 1). The data obtained correlate to the available ones about genus *Chenopodium* s.l. (Snogerup 1995; Runemark, 1996; Goldblatt & Johnson, 2000). Karyomorphological data about the studied populations are presented in Table 2. The data show that the karyotype of the Bulgarian populations of the species from the genus *Chenopodiastrum* is of 2 types of chromosomes: metacentric and submetacentric (Table 2). In all studied populations of the species belonging to the genus, the metacentric chromosomes are dominant. Satellites have not been registered.

The registered diploid chromosome number for the studied Bulgarian populations of *Chenopodiastrum murale* conforms to the data by Winge (1917), Rohweder (1937), Heiser & Whitaker (1948), Kawatani & Ohno (1956), Mehra & Malik (1963), Uotila (1973), Crompton & Bassett (1975, 1976), Queiros (1975), Skalinska (1978), Sidhu (1979), Bir & Sid-

Table 1. Studied populations from genus *Chenopodiastrum*

Species	Population`s location	2*n*	GPS coordinates, altitude (m)
C. murale, mur1	Balkan Range (Eastern), town of Sliven, grassed areas in the streets.	18*	42° 40′ N, 26º 19′ E, 243
C. murale, mur2	Balkan Range (Central), town of Kotel, ruderalized terrains.	18*	42° 53′ N, 26º 27′ E, 643
C. hybridum, hyb1	Black Sea Coast (Southern), town of Burgas, ruderalized terrains.	118*	42° 30′ N, 27º 28′ E, 30
C. hybridum, hyb2	Danube plain, town of Belene, ruderalized terrains.	18*	43° 39′ N, 25º 07′ E, 80
C. hybridum, hyb3	Rhodope Mts (Eastern), town of Kardzhali, ruderalized terrains.	18*	41° 39′ N, 25º 38′ E, 275

* – data published by Grozeva (2007a)

Table 2. Karyomorphometric data for the studied *Chenopodiastrum* populations, chromosome size variation (µm) - short (S) and long (L), total haploid chromosome length (hcl, µm), interchromosomal asymmetry indices (A_2); coefficient variation of chromosome length (CV_{cl}), degree of asymmetry of karyotype (A), mean centromeric asymmetry (M_{ca})

Species/ population	Karyotype formula	S	L	hcl	A2	CV_{CL}	A	M_{CA}
1. *C. murale*, Kotel	2*n* = 16m + 2sm	1.42	2.53	17.01	0.04	4.43	0.01	1.27
2. *C. murale*, Sliven	2*n* = 16m + 2sm	1.40	2.55	17.03	0.04	4.21	0.01	1.42
3. *C. hybridum*, Burgas	2*n* = 12m + 6sm	2.40	5.59	28.52	0.02	2.34	0.02	2.41
4. *C. hybridum*, Belene	2*n* = 12m + 6sm	2.45	5.70	28.68	0.02	2.28	0.02	2.17
5. *C. hybridum*, Kardzhali	2*n* = 12m + 6sm	1.42	4.33	23.44	0.03	3.35	0.02	2.38

hu (1980), Dvorak et al. (1980), Schwarzova (1980), Dvorak & Dadakova (1984), D'Ovidio (1986; 1987), Granado *et* al. (1988), Runemark (1996), Al-Turki et al. (1999; 2000), Rahiminejad & Gornall (2004), Rahiminejad (2006). Haploid chromosome number n = 9 has also been reported about the species by Dvorak et al. (1980). The sizes of the chromosomes in the two populations studied are very similar, with mean length of 1.89 µm, the longest recorded chromosome has length of 2.55 µm and the shortest one has length of 1.40 µm. The ratio between the longest and the shortest chromosome in the population from the town of Kotel is 1.78:1, with a difference in their size of 1.11 µm. In the population from the town of Sliven the ratio between the longest and the shortest chromosome is 1.82:1, the difference in their size being 1.15 µm. Higher CV_{CL} values have been recorded for the population from Kotel. The karyotype established for the Bulgarian populations of 8 pairs of metacentric and 1 pair of submetacentric chromosomes (Fig. 1, A-B) differs from the one reported by Dvořák & Dadakova (1984) for the population of *C. murale* from the village of Sedlec in the South Moravian Region of the Czech Republic, in which dominant were the submetacentric chromosome pairs.

The diploid chromosome number established for the studied 3 populations of *C. hybridum* from the town of Burgas, the town of Belene and the town of Kardzhali confirms the information from literature data from various parts of the species area (Winge, 1917; Kjellmark, 1934; Cooper, 1935; Löve, 1954; Kawatani & Ohno, 1956; Gadella & Kliphuis, 1963; Ferakova, 1974; Magic & Majovsky, 1974; Skalinska, 1978; Schwarzowa, 1978; Murin et al., 1980; Dvorák, 1989; Stepanov, 1994; Lövkvist & Hultgård, 1999; Rahiminejad & Gornall, 2004; Rahiminejad, 2006, Chepinoga & Gnutikov, 2014, Lomonosova et al., 2014). The mean length of chromosomes in all three studied populations is 3.77 µm for the ones from the town of Burgas and the town of Belene, 2.60 µm for the population from the town of Kardzhali. The longest chromosome has length of 5.70 µm and the shortest one has length of 1.42 µm. The ratio between the longest and the shortest chromosome in the populations from the town of Burgas and the town of Belene is 2.33:1, with a difference in their size from 3.19 µm for the first and 3.25 µm for second population. The ratio between the longest and the shortest chromosome in the third studied population from the town of Kardzhali is 3.05:1, with a difference of 2.91 µm in the size. A karyotype of 6 pairs of metacentric and 3 pairs of submetacentric chromosomes has been established (Fig.1 C-E). The highest CV_{CL} and M_{CL} values have been registered for the *C. hybridum* populations from Kardzhali and from Burgas. The population from Belene has CV_{CL} and M_{CL} values in the middle of the two.

When comparing the studied populations of *C. hybridum* and *C. murale* by similarity of karyotipic parameters it has been established that the populations are grouped and form two clusters (Fig. 2). The first cluster included the populations of *C. murale*, while the second one – those of *C. hybridum.* The greatest degree of similarity has been registered among the populations of *C. murale.* Great similarity has been found between the two populations of *C. hybridum* from Burgas and Belene as well, forming a subcluster, while

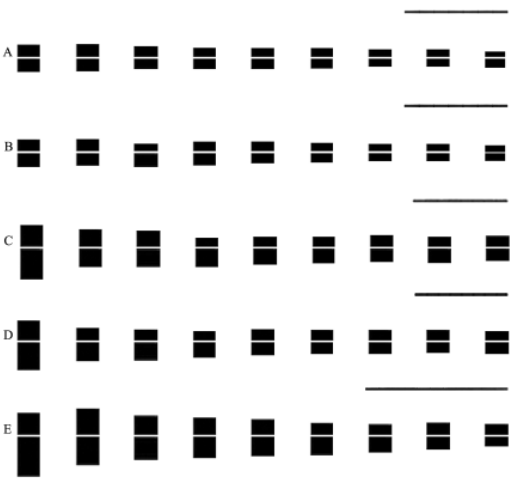

Fig. 1. Idiograms of *Chenopodiastrum* populations, 2*n* = 18: A) *C. murale*, Kotel; B) *C. murale*, Sliven; C) *C. hybridum*, Burgas; D) *C. hybridum*, Belene; E) *C. hybridum*, Kardzhali, scale bar 10 µm

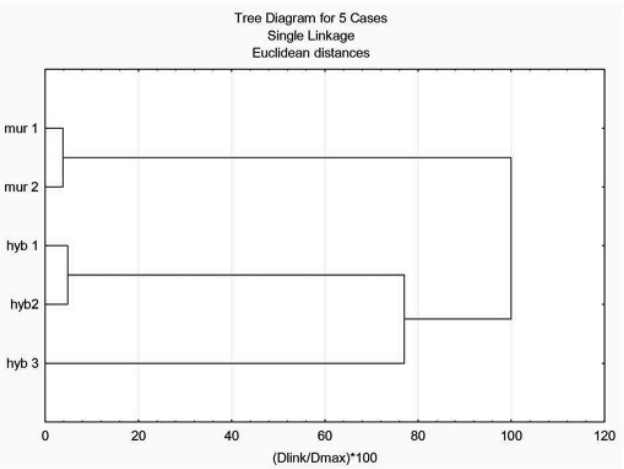

Fig. 2. Dendrogram of CA of *Chenopodiastrum* populations based on karyotype data
*numbering of populations follows the indications in Table 1

the third population of the species from Kardzhali had greater values of metric distance.

PCA of the karyotipic parameters for the 2 species from genus *Chenopodiastrum* reveals that factor 1 contributed 99.47% of the total variance and had the greatest positive correlation with the total sum of haploid chromosome length (0.95) and long arm of chromosomes (0.18) (Table 3). Factor 2 contributed 0.47% of the total variation and has the greatest positive correlation with the coefficient of variation of chromosome length (0.92) and the lowest relations with the long arm of chromosomes (-0.27) and centromeric index (-0.27).

Table 3. Principal component analysis based on karyotype parameters of studied *Chenopodiastrum* populations

Parameter	Factor 1 (99.47%)	Factor 2 (0.47%)
Short arm	0.075923	-0.024765
Long arm	0.176306	-0.270591
Total length	0.091905	0.144432
Arm ratio	0.106058	-0.006225
Centromeric index	0.100382	-0.245826
hcl	0.954526	-0.056027
A2	0.001110	0.008924
CVCL	0.125739	0.917393
A	0.000674	-0.000566
MCA	0.080308	-0.002890

Conclusion

The chromosome numbers registered for Bulgarian populations of *C. murale* and *C. hybridum* were diploid based on basic chromosome number x = 9. The karyotype consisted of metacentric and submetacentric chromosomes with the metacentric ones being dominant. Satellites have not been found. The mean dimension of chromosomes varies from 1.89 μm in the *C. murale* population from the town of Kotel to 3.18 μm in that of *C. hybridum* from the town of Belene. Higher values of the coefficient of variation of chromosome length (CVcl) have been registered for the populations of *C. murale.* The total sum of haploid chromosome length (hcl) was within the range from 17.01 to 28.68 μm, with the *C. hybridum* populations showing higher values for mean centromeric asymmetry (Mca). The karyotype morphology of these species has been counted for the first time from Bulgarian populations.

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