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Chromosome and Pollen Morphology of Amaranthus hybridus L. and Amaranthus retroflexus L. in Bulgaria

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Abstract. The chromosome and pollen morphology in the Bulgarian populations of *Amaranthus hybridus* L. and *Amaranthus retroflexus* L. was studied. The diploid chromosome number $2n = 34$ was found. The metacentric type of chromosomes was predominant in all studied populations. In the karyotype of *A. hybridus*, the length of the metaphase chromosome ranged from 0.38 µm to 1.60 µm, and the total haploid length of the chromosome set - from 11.44 µm to 13.50 µm. The length of the chromosomes in *A. retroflexus* ranged from 0.18 µm to 2.27 µm, and the total haploid length of the chromosome set - from 13.01 µm to 23.32 µm. Pollen morphology was examined using a scanning electron microscope (SEM). Pollen was defined as a spherical, pantoporate type. The pollen diameter in *A. hybridus* varied from 18.93 µm to 22.11 µm, and in *A. retroflexus* - from 16.75 µm to 22.21 µm. Differences in the number and diameter of pores have been found between the two species.

Key words: *Amaranthus hybridus* L.; *Amaranthus retroflexus* L.; chromosome number; karyology; pollen morphology; idiograms; Bulgaria.

Introduction

Genus *Amaranthus* L. (Amaranthaceae Juss.) comprises about 65-80 species, spread mainly in tropical, subtropical and warm-moderate areas. Worldwide, some of the species are spread as introduced and naturalized weeds (Mosyakin & Robertson, 1996, 2003; Bojian et al., 2003; Mujica & Jacobsen, 2003; Iamonico, 2016). The representatives of the genus are predominantly edible food plants and a significant part thereof are categorized as agricultural weeds or pseudocereals all over the world (Costea & Halmajan, 1996; Mosyakin & Robertson, 2003). Some species are grown due to their ornamental qualities (Brenner et al., 2000; Jäger et al., 2008; Shukla et al., 2004, 2006, 2010).

In the Bulgarian flora the genus is represented by 13 species (Kovachev, 1966; Assyov & Petrova, 2012; Petrova, 2018). These are spread mainly ruderally, along roads, gardens and agricultural areas, partially in parks and gardens. The present study includes two species - *Amaranthus hybridus* L. and *Amaranthus retroflexus* L.

Up to that moment the Bulgarian populations of both species have not been an object of a detailed study. Chorological data point to their wide spreading all over the country (Assyov & Petrova, 2012). Their habitats are mainly ruderal terrains, orchards and vegetable gardens. They can be found up to 1000 m a.s.l. *A. hybridus* is considered one of the most dangerous weeds in the words. *A.*

retroflexus is a widely spread weed for the Bulgarian flora (Petrova et al., 2013).

The karyological data are scarce and incomplete, partially due to difficult taxonomic differentiation of the species. Worldwide, *Amaranthus* L. comprise a genus exhibiting variation in the chromosome numbers and ploidy level, but interspecies variation is minimum (Pal, 1982). The latest report about the chromosome number of the genus is from the end of the XXth century.

Therefore, the aim of the present study is to investigate the chromosomal and pollen analysis of the species *Amaranthus hybridus* L. and *Amaranthus retroflexus* L. in Bulgaria.

Materials and Methods

The present study includes three populations of each species. The data comprise three floristic regions in the country (Table 1). To establish the species chromosome number and karyotype durable preparation were prepared from the metaphase plates of root tips. All root tips were prepared from seeds collected natural habitats of *A. hybridus* and *A. retroflexus* and germinated under laboratory conditions. The root tips were treated and squashed following the methodology by Grozeva (2007).

The chromosome type was determined in relation to the centromere index I = s / s + l, according to the classification recommended by Grif & Agapova (1986). The karyograms and ideograms were been processed by the Adobe Photoshop 2020 and Karyo Type Win 2018 software. The data were obtained on the basis of three metaphase plates from each population. The interchromosome asymmetry was calculated by means of index A_2 (Zarco, 1986). To determine intrachromosome asymmetry the following indexes were used: general shape stated as percentage - TF% (Huziwara, 1962); percentage of karyotype asymmetry - Ask% (Arano, 1963); symmetry index - Syi (Greilhuber & Speta, 1976); intrachromosome asymmetry A_1 (Zarco, 1986); asymmetry level A (Watanabe et al., 1999); the four categories of Stebbins (1971) - SKS: from A to D according to the arm ratio and the centromere location in the chromosome. Each of the four categories has three subtypes determined according to the largest/smallest chromosomes ration (Table 2).

Table 1. Data about the studied *A. hybridus* and *A. retroflexus* populations in Bulgaria.

Species	Population locality	Coordinates	Altitude (m)	Floristic regions	2n
A. hybridus L.	Elin Pelin	N42^{o}67.047″ E023^{o}59.701″	544	Sofia Region	34
	Pavel banya	N42^{o}35.344″ E025^{o}12.515″	406	East Sredna Gora	34
	Plovdiv	N42^{o}08.086″ E024^{o}47.862″	157	Thracian Plane	34
A. retroflexus L.	Asenovgrad	N42^{o}00.745″ E024^{o}52.317″	238	Thracian Plane	34
	Zvanichevo	N42^{o}11.380″ E024^{o}15.000″	221	Thracian Plane	34
	Plovdiv	N42^{o}08.017″ E024^{o}48.049″	155	Thracian Plane	34

Table 2. Intrachromosomal asymmetry indexes (Stebbins, 1971).

Ratio: largest/smallest chromosomes	Proportion of chromosomes with arm ratio > 2:1			
	0.0	0.01 – 0.5	0.51 – 0.99	1.0
< 2:1	1 A	1 B	1 C	1 D
2:1 – 4:1	2 A	2 B	2 C	2 D
> 4:1	3 A	3 B	3 C	3 D

The morphological characterization of pollen was done by scanning electron microscope (SEM). The study was carried out at the laboratory of the Faculty of Chemistry and Pharmacy at „St. Kliment Ohridski" University of Sofia. The data were obtained from a minimum of ten pollen grains for each studied population. Herbarized plant parts were used. The herbarized materials are mounted on a metal tripod, covered with gold particles in an ionizing chamber and observed under scanning electron microscope (JEOL 5510). The pollen terminology used conforms to Erdthman (1952), Kremp (1965), Walker & Doyle (1975). The following morphological characteristics were determined: 1) Pollen diameter (maximum diameter, μm) - D_1; 2) Polar axis (diameter perpendicular to D_1, μm) - D_2; 3) Distance among three adjacent pores forming a triangle with sides as close as possible to the highest grain focus (μm) - C; 4) C/D_1 ratio; 5) Total number of pores - TNP; 6) Pore diameter (μm) - PD; 7) Pore area (μm^2) - PA; 8) Number of spinules per 100 μm^2 - NS/100 μm^2; 9) Number of spinules in the pores – NSP; 10) Polar shape; 11) Equatorial shape. The statistical analysis was carried out by Microsoft Excel 2010.

Results

Karyology. As a result of the karyological study of the species *A. hybridus* and *A. retroflexus* in Bulgaria diploid chromosome number $2n = 34$ was established in the six studied populations (Table 1). The karyomorphological data are presented in Table 3. The ideograms of both species are given on Fig. 1.

Two types of chromosomes were reported: meta- and submetacentric. Predominant in both species are the metacentric chromosomes. In the population from Elin Pelin a karyotype was established formed only by metacentric chromosomes – $2n = 34m$. In the other two *A. hybridus* populations, diploid chromosome number $2n = 32m + 2sm$ was reported for the population from Pavel banya, and $2n = 27m + 7sm$ for the population from Plovdiv. In two of the three studied *A. retroflexus* populations – Asenovgrad and Plovdiv, identical $2n = 31m + 3sm$ karyotype was recorded. In the third population, the one from Zvanichevo, similar data were registered - $2n = 33m + 1sm$. The chromosome size varies from 0,74 μm for the representatives from Elin Pelin to 1,68 μm for the ones from Zvanichevo. From all studied karyological data the shortest arm is 0,11 μm long and the longest one - 1,38 μm. Both arm values were registered in the same *A. retroflexus* population from Asenovgrad. The total sum of the haploid chromosome length is within small limits - from 11,44 μm to 13,50 μm for the *A. hybridus* populations and from 13,01 μm to 23,32 μm for *A. retrofexus* population. The data are supplemented by the results obtained for the asymmetry index (A_2) given in Table 3.

According to the classification of Stebbins (1971) there are four different types of symmetry. For the *A. hybridus* representative these are: 1B – Elin Pelin, 1C – Pavel banya and 2C – Plovdiv. In *A. retroflexus* the index is of the following type: 1B – Zvanichevo, 1C – Asenovgrad and 2C – Plovdiv. The intrapopulation symmetry shows similar results for the TF% and Ask% indexes. The lowest and the highest Syi value is found in the *A. hybridus* populations and varies: from 72,71 – Plovdiv to 82,72 – Pavel banya. The lowest A_1 and A values are found in the population from Zvanichevo (*A. retroflexus*), and high ones are registered in the population from Plovdiv (*A. hybridus*). Correlation data are given in Table 4.

Pollen morphology

The palynological analysis was made on the basis of ten qualitative and quantitative traits (Table 5, Fig. 2). The data show that all studied populations are characterized by spherical pollen. The pollen is pantoporate, elliptically jagged and covered by numerous spinules.

Morphometric studies showed similar results for diameter D_1 in *A. hybridus* populations: from 20,93 μm – Plovdiv to 21,78 μm – Elin Pelin. Greater range of the values was registered in *A. retroflexus*: from 18,57 μm – Asenovgrad to 22,43 μm – Zvanichevo. The last two populations contain both the smallest and the largest D_2 value in the six studied habitats. The polar axis varies from 14,93 μm to 22 μm. The measured distance between the

pores is with similar results, but the highest values were reported in the two populations from Plovdiv. The C/D1 ratio is within the limits from 0,152 to 0,200. The smallest pore diameter was measured in the Plovdiv *A. hybridus* population. The highest value of the diameter – 1,84 µm was measured in both species. The pollen area varies from 2,06 µm to 2,66 µm. The smallest total number of spinules - 279 and number of spinules in the pores – 7,25 were reported in the population from Asenovgrad.

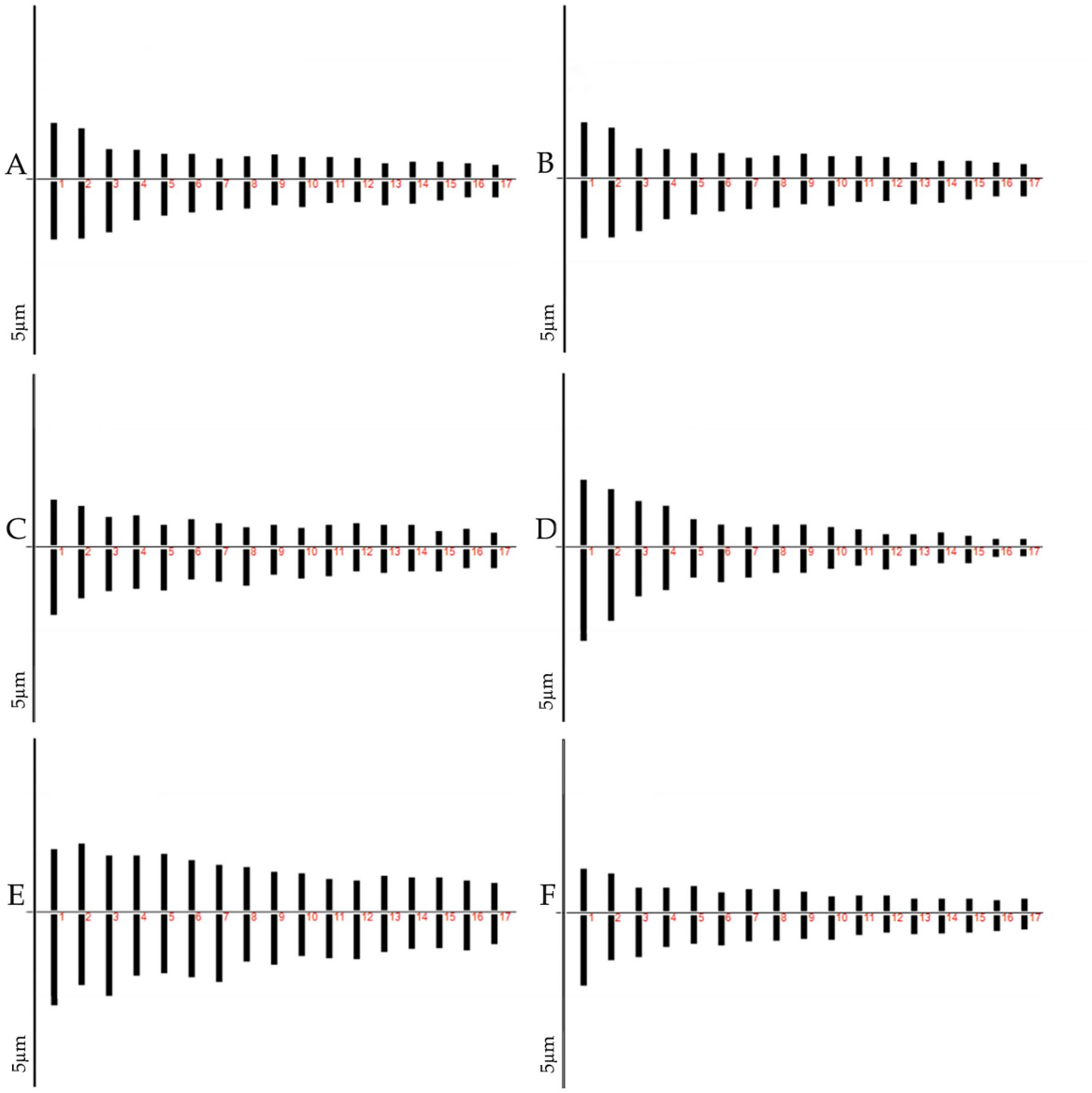

Fig. 1. Idiograms of *Amaranthus hybridus* and *Amranthus retroflexus*, 2*n*=34.
A. hybridus: A) Elin Pelin, B) Pavel banya, C) Plovdiv;
A. retroflexus: D) Asenovgrad, E) Zvanichevo, F) Plovdiv; scale bar 5 µm.

Table 3. Karyomorphometric data for the representatives of *A. hybridus* and *A. retroflexus* in Bulgaria. *Legend:* Chromosome size variation (µm) – short (S) and long (L); total sum of the haploid chromosome length (hcl, µm).

Population	Karyotype formula	S	L	hcl	Inter index	Intrachromosomal index					
					A_2	SKS	TF%	Ask%	Syi	A_1	A
A. hybridus L.											
Elin Pelin	2*n* = 34m	0.17	0.57	11.44	0.21	1B	44.78	55.22	81.11	0.19	0.11
Pavel banya	2*n* = 32m + 2sm	0.17	0.84	13.00	0.44	1C	45.27	54.73	82.72	0.18	0.10
Plovdiv	2*n* = 27m + 7sm	0.13	1.00	13.50	0.37	2C	42.10	57.90	72.71	0.26	0.15
A. retroflexus L.											
Asenovgrad	2*n* = 31m + 3sm	0.11	1.38	13.01	0.74	1C	44.34	55.66	79.66	0.19	0.11
Zvanichevo	2*n* = 33m + 1sm	0.39	1.29	23.32	0.31	1B	44.88	55.12	81.43	0.17	0.10
Plovdiv	2*n* = 31m + 3sm	0.16	1.33	13.58	0.47	2C	42.53	57.47	74.01	0.24	0.14

Table 4. Correlations for asymmetry indexes of the studied *Amaranthus* species from Bulgaria. *Legend:* *Correlation is significant at p<0.05.

Indexes	A_2	TF%	Ask%	Syi	A_1	A
A_2	1,000					
TF%	-0,087*	1,000				
Ask%	0,087	-1,000*	1,000			
Syi	-0,093*	1,000	-1,000*	1,000		
A_1	0,021*	-0,976*	0,976	-0,974*	1,000	
A	0,012*	-0,989*	0,989	-0,988*	0,996	1,000

Table 5. Some important palynological characteristics of the studied *Amaranthus* taxa (all measured values are in µm).

Populations	D_1	D_2	C	C/D_1	TNT	PD	PA	NS/100 µm²	NSP	Polar shape	Equatorial shape
Amaranthus hybridus L.											
Elin Pelin	21.78	20.25	3.37	0.152	34.33	1.74	2.38	336	8.45	circular	elliptic-trucate
Pavel banya	21.57	19.89	3.64	0.167	33.20	1.84	2.66	414	10.00	circular	elliptic-trucate
Plovdiv	20.93	20.30	3.97	0.176	40.00	1.62	2.06	486	9.50	circular	elliptic-trucate
Amaranthus retroflexus L.											
Asenovgrad	18.57	14.93	3.57	0.190	32.00	1.65	2.14	279	7.25	circular	elliptic-trucate
Zvanichevo	22.43	22.00	3.37	0.160	42.00	1.84	2.65	331	6.00	circular	elliptic-trucate
Plovdiv	21.43	19.00	3.81	0.200	34.66	1.69	2.24	369	8.00	circular	elliptic-trucate

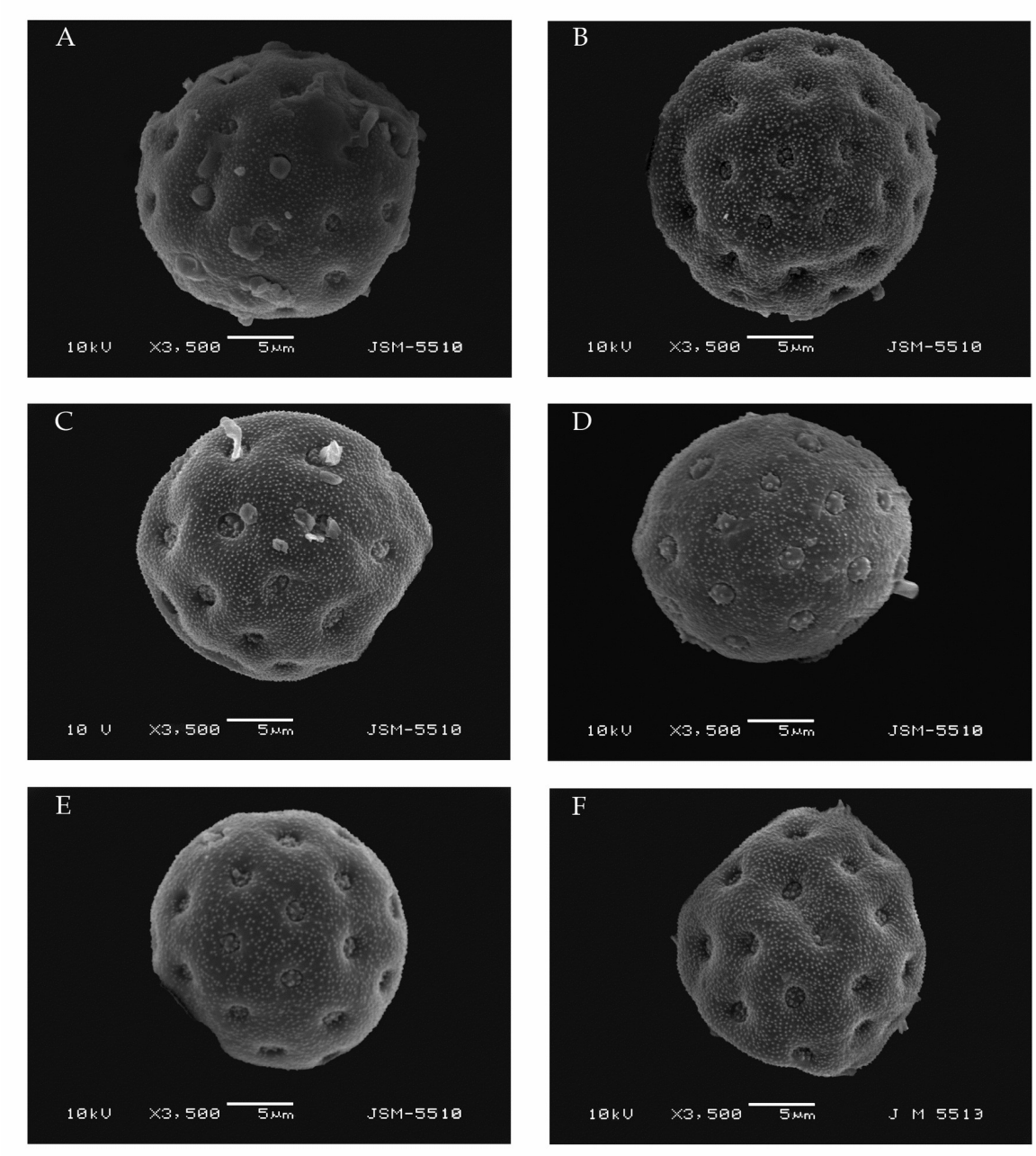

Fig. 2. Pollen microphotography.
Amaranthus hybridus: A) Elin Pelin, B) Pavel banya, C) Plovdiv;
Amaranthus retroflexus: D) Asenovgrad, E) Zvanichevo, F) Plovdiv.

Discussion

Karyology

Amaranthus L. are a genus with great variability in the chromosome number and ploidy level. The genus is tribasic (Prajitha & Thoppil, 2018). Three gametic numbers are reported about it: n = 14, 16, 17 (Greizerstein & Poggio, 1992, 1995, 1997; Carretero, 1985, 1991). Two chromosome numbers have been reported about *A. hybridus*: 2n = 32, 34 (Queirós, 1989; Carretero, 1991). The karyotype contains meta- and submetacentric chromosomes. More frequent is the metacentric type, sometimes with satellites present (Radwan et al., 2014;

Prajitha & Thoppil, 2018). *A. retroflexus*, similarly to *A. hybridus*, is known for the diploid chromosome number 2*n* = 32, 34 (Javurkova, 1980; Dmitrieva et al., 1986; Song et al., 2002; Marhold, 2007; Zykova et al., 2018; Lomonosova, 2018). Metacentric chromosomes predominate in the karyotype, satellites have not been registered (Radwan et al., 2014).

The latest karyological data about genus *Amaranthus* L. in Bulgaria were by Cheshmedziev (1994). Three species were published, one of which is *A. hybridus* with diploid chromosome number 2*n* = 32. The information is for the floristic region of the Thracian plane, more specifically Plovdiv region. There were no data about the chromosome number of *A. retroflexus*.

The base chromosome number for the three studied *A. hybridus* populations is n = 17. The karyotype formulas of the following populations in the country have been established: Elin Pelin - 2*n* = 34m, Pavel banya - 2*n* = 32m + 2sm and Plovdiv - 2*n* = 27m + 7sm. They do not confirm the diploid chromosome number of 2n = 32 established so far, but conform to the taxonomic analyses worldwide. The base chromosome number for the three studied *A. retroflexus* populations is *n* = 17. The karyotype formulas of the following populations in the country have been established: Asenovgrad - 2*n* = 31m + 3sm, Zvanichevo - 2*n* = 33m + 1sm and Plovdiv - 2*n* = 31m + 3sm. The results are new for Bulgaria.

Pollen morphology

In modern systematics pollen morphology is extremely useful for clarifying the systemic relations on inter- and intraspecies level (Iwanami et al., 1988). The currently existing morphological characteristics of pollen give grounds to classify it to *Amaranthus* type with pores type II typical of that type of pollen (Borsch, 1998). Our data largely confirm the already published ones.

The pollen in all six populations has typical spherical shape covered by numerous perforations. It is characterized by small size and a big number of pores. The difference between the species is found in the pollen size, which is bigger in *A. retroflexus* (18,57 – 22,43 µm). The C index is high (3,81 µm, 3,97 µm) in both species. The values have been reported for the same region. That could be due to the same conditions of the environment and could be used as a chorological trait in current studies of the species (Arora & Modi, 2008).

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