Botanica SERBICA 43 (1): (2019) 13-21

Original Scientific Paper

Karyological and morphological variation within *Petrosimonia brachiata* Bunge in Bulgaria

Neli Grozeva[1*], Mima Todorova[2] and Dimitar Pavlov[2]

1 Department of Biology and Aquaculture, Faculty of Agriculture, Trakia University, Stara Zagora, Bulgaria
2 Department of Plant Crops, Faculty of Agriculture, Trakia University, Stara Zagora, Bulgaria

ABSTRACT: We studied karyological and morphological variability of the only representative of the genus *Petrosimonia* (Amaranthaceae) in Bulgaria and evaluated the current state of its populations in the country. The results indicated that Bulgarian populations of *Petrosimonia brachiata* have a diploid chromosome number of $2n = 16$. In the population from the Pomorie Lake, the karyotype consists of six pairs of metacentric and two pairs of sub-metacentric chromosomes. In the population from the Atanasovsko Lake, satellites were observed on one pair of sub-metacentric chromosomes. Statistical analysis demonstrated that the main source of phenotype variation in the species is within the populations. Vegetative traits are more variable than generative ones. No significant variability was found in the studied quantitative characteristics or pollen morphology. Given the biological type of the species (annual) and its limited distribution in Bulgaria, the status of the two populations of *P. brachiata* cannot be regarded as stable, and the danger of a potential threat to them in the future is a possibility not to be excluded.

Keywords: Amaranthaceae, karyotype, chromosome number, morphology, chorology

Received: 02 November 2018 Revision accepted: 19 February 2019

UDC: 582.643.21:581.4:576.316(497.2)
DOI: https://doi.org/10.2298/BOTSERB1901013G

INTRODUCTION

Halophytes exhibit special morphological and physiological adaptations to survive in saline ecosystems (Wucherer *et al.* 2005; Flowers & Colmer 2008). Interest in halophytic plants has grown during recent years. Many publications can be found in the literature that deal with using halophytes as helpful tools for rehabilitation of degraded lands and soil protection (Qadir & Oster 2004; Wucherer *et al.* 2005), as a potential resource for developing bio-energy (Abideen *et al.* 2011), as a viable alternative to transgenic technologies for development of tolerance (Sardo & Hamdy 2005), and as agents for capturing and long-term sequestering of atmospheric CO_2 (Glenn *et al.* 1993).

The typical annual halophytes that occur in Bulgaria dwell in periodically flooded muddy and sandy places on the periphery of the hyperhaline and more rarely brackish marshes along the coast of the Black Sea (Tzonev *et al.* 2008; Tzonev & Gussev 2011). They have limited distribution and the latest expert appraisals define them as endangered. There are some very rare and protected plants in the communities of annual halophytes living in coastal salt marshes by the Black Sea, among which are representatives of the genera *Petrosimonia*, *Hamilione*, etc.

The genus *Petrosimonia* Bunge [Amaranthaceae sensu APG IV (2016)] includes between 11 and 15 species, distributed in South-East Europe, as well as in Central and South-West Asia (Zhu *et al.* 2003; Berberoglu *et al.* 2004; Atamov *et al.* 2006; Toderich *et al.* 2009; Uotila 2011; Krasnova *et al.* 2013; Ibrahim 2014). In the Bulgarian flora, this genus is represented by a single species – *Petrosimonia brachiata* Bunge (Yordanov & Kuzmanov 1966; Assyov & Petrova 2012). It is protected by the Biological Diversity Act (2002), and is included in the Red Book of the Republic of Bulgaria (Stoeva 2011) in the category "critically endangered". This species belongs to the group of leaf-succulent eu-

*correspondence: grozeva@uni-sz.bg

halophytes (Wucherer *et al.* 2005) and forms populations on saline sands and clay, on dikes of salt-pans and on the seashore and in open halophytic herbaceous communities (Stoeva 2011). The species has very limited distribution in Bulgaria: so far, only two populations have been located, in the southern part of Bulgaria's Black Sea coast, viz., the – Atanasovsko Lake and the Pomorie Lake (Grozeva 2004; Stoeva 2011). Chorological data on *P. brachiata* can be found in some publications about halophytic plants in Bulgaria and about the flora and plants of the Burgas wetlands (Kochev *et al.* 1971; Grozeva 2004, 2005; Grozeva *et al.* 2004; Stoyanov 2010). The relationship between soil salinity and the distribution of *P. brachiata* on territory of the Pomorie and Atanasovsko Lakes was assessed in a previous study (Todorova *et al.* 2014). Currently, both populations are within protected territories, but they have never been the subject of a comprehensive study.

The purpose of the present study was to investigate the intrapopulation and interpopulation karyological and morphological variability of *P. brachiata* in Bulgaria, evaluate the area occupied by its populations and estimate the number of specimens in them.

MATERIALS AND METHODS

Morphological and karyological analyses were carried out on both populations known to exist in Bulgaria – near the Atanasovsko Lake and the Pomorie Lake (Table 1).

The Atanasovsko and Pomorie Lakes are a part of the most significant complex of wetlands along the Black Sea coast of Bulgaria – the Burgas wetlands. A hyper-saline lake near the coastline, the Atanasovsko Lake is divided into two parts by the Burgas – Varna road. Both parts of it have been salt-pans since 1906. The Pomorie Lake is a natural hyper-saline lagoon separated from the Black Sea by a sand bar. Since 2001 it has been declared a Protected Site. The climate in the studied area is continental-Mediterranean. The absolute maximum temperature exceeds 40°C, while the absolute minimum is -20.3°C (Tsenkova - Bratoeva *et al.* 2010). The annual average air temperature is 12.3°C, while in January it is 2°C and in August 22°C. The average precipitation is between 520 and 580 mm. The highest values are reached in June and November, the lowest in August and September.

A karyological analysis (determination of the number and morphology of chromosomes) was done on metaphase plates obtained from root tips of seeds germinating under laboratory conditions in Petri dishes. Seeds were collected in the natural habitat of the species. Roots were treated and squashed according to Grozeva (2007). The chromosome type was determined using the centromere index I = s/s+l according to the classification proposed by Grif & Agapova (1986). Five metaphase plates were measured for each population. Intrachromosomal and interchromosomal karyotype asymmetries were estimated with the coefficient of variation of chromosome length (CV_{cl}) according to Paszko (2006) and the mean centromeric asymmetry (M_{ca}) proposed by Peruzzi & Eroğlu (2013). The voucher specimens are deposited in the herbarium of the Institute for Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences (SOM).

The performed morphological analysis took into account 15 quantitative characters reported in 30 specimens of each population: 1. stem height (mm); 2. basal leaf length (mm); 3. basal leaf width (mm); 4. basal leaf length/width ratio; 5. upper leaf lamina length (mm); 6. upper leaf lamina width (mm); 7. upper leaf lamina length/width ratio; 8. bracteole length (mm); 9. bracteole width (mm); 10. bracteole length/width ratio; 11. flower diameter (mm); 12. flower petiole length (mm); 13. seed length (mm); 14. seed width (mm); and 15. seed length/width ratio.

Plant height was recorded on site, the other traits on collected herbarium parts.

The morphological data were processed using the Statistica 7 program. The population means and coefficients of variation (CV%) were estimated for each character of each population. Their values were used in comparing various characters within and between the populations. The relative contribution of intra- and interpopulation variation to the overall variation of each studied quantitative character of the two studied population was evaluated by one-way ANOVA.

The following qualitative traits were also recorded: shape, colour and indumentum of stem, bracteoles and perianth segments; colour and indumentum of leaf lamina; degree of perianth connation; anther shape; pericarp colour; and seed shape and colour.

Scanning electron microscopy was used for a more detailed study of pollen morphology and the qualitative traits. Studies were conducted on dry plant parts. Observations were performed and documentation obtained with a JEOL JSM-5500 scanning electron microscope in a laboratory of the Faculty of Chemistry and Pharmacy of St. Kliment Ohridski University in Sofia (the University of Sofia). At least 15 pollen grains from each population were studied. Specimens were prepared according to the technique for observation of air-dried pollen. Anthers were isolated, put into petri dishes and kept at room temperature for 48 h. The pollen was sprinkled on microscope slides and dried at room temperature. Materials prepared for observation were coated with gold for 90 seconds in an argon-ionising sphere. The fastening of the contact holder was made with a silver paste. The pollen terminology used is according to Erdtman (1986) and Perveen & Qaiser (2012). The data of scanning electron microscopic measurements are presented in generalised form for the species due to the absence of significant variability within and between populations.

Table 1. Studied populations of *Petrosimonia brachiata*.
*– data published by Grozeva (2015)

Locality	$2n$	Ecological conditions	Area and population size
Black Sea Coast (*southern*), Pomorie Lake protected area, west of the salt museum, population´s central point: N 42°34.243', E 27°37.397'.	16*	Saline areas around the lake, sandy terrains, light to high salinity and neutral to alkaline reaction, at elevation of 1-2 m. Halophyte community dominated by *Petrosimonia brachiata*. Accompanying species: *Suaeda maritima* (L.) Dumort., *Bassia hirsuta* (L.) Asch., *Salicornia europaea* L., *Atriplex tatarica* L., *Suaeda altissima* Pall., *Artemisia santonicum* L., *Aster tripolium* L.	Diffuse spatial structure, number during the studied period: between 1700 and 1800 specimens; area about 2460 m^2.
Black Sea Coast (*southern*), Atanasovsko Lake, Burgas – Pomorie road, population´s central point: N 42°33.103', E 29°29.008',	16	Saline areas around the lake, Southern Black Sea Coast, Atanasovsko Lake, at the northern end of the lake near the Burgas – Pomorie road, high level of salinity and alkaline to strong alkaline reaction, at elevation of –1.5 m. Halophyte community dominated by *Salicornia europaea.* Accompanying species: *Bassia hirsuta* and *Petrosimonia brachiata.*	Diffuse spatial structure, number during the studied period: between 240 and 270 specimens; area 95 m^2.

Table 2. Karyomorphometric data for the studied populations of *Petrosimonia brachiata*. Chromosome size variation (μm) – short arm (S) and long arm (L); total sum of haploid chromosome length (Hcl, μm).

Population	**Karyotype formula**	**S**	**L**	**Hcl**	**CV$_{cl}$**	**M$_{ca}$**
1. Pomorie Lake	$2n$ = 12m + 4sm	1.64	2.28	15.92	5.43	2.38
2. Atanasovsko lLake	$2n$ = 12m + 2sm + 2sm^{sat}	1.75	2.69	16.49	4.23	1.42

RESULTS

Karyology. A chromosome number of $2n$ = 16 showing eight pairs of homologous chromosomes was found in the two studied populations of *P. brachiata* (Fig. 1). The average length of chromosomes in the studied populations ranged from 1.99 μm in the population of the Pomorie Lake to 2.06 μm for that of the Atanasovsko Lake (Table 2). The longest of chromosome pairs is 2.69 μm long, the shortest 1.64 μm long.

The population of the Pomorie Lake shows a karyotype with six pairs of metacentric chromosomes and two pairs of sub-metacentric chromosomes, i.e., $2n = 2x$ = 12m + 4sm. Values of the interchromosomal and intrachromosomal karyotype asymmetry indices were 4.23 and 1.42 for CV$_{cl}$ and M$_{ca}$, respectively.

The karyotype of the Atanasovsko Lake´s population differs from that of the Pomorie Lake´s population in the presence of satellites on one of the pairs of sub-metacentric chromosomes: $2n = 2x$ = 12m + 2sm + 2sm^{sat}. Analysis of the interchromosomal and intrachromosomal karyotype asymmetry indices showed values of 5.43 for CV$_{cl}$ and 2.38 for M$_{ca}$.

Morphology. Data on the studied quantitative traits are presented in Table 3. Intrapopulation variability in both of the studied populations was established on the basis of the coefficient of variation. Values of the coefficient of variation were different in regard to individual traits of each population, vegetative traits being always more variable than generative ones. Statistically significant differences between the two studied populations were found for 10 parameters: plant height; basal leaf length and length/width ratio; upper leaf length and width; bracteole length and length/width ratio; flower petiole length; and seed width and length/width ratio. No statistically significant difference was found for the following parameters: basal leaf width; upper leaf length/width ratio; bracteole width; flower diameter; and seed length.

The results obtained from ANOVA showed that intrapopulation variability is dominant (Table 3).

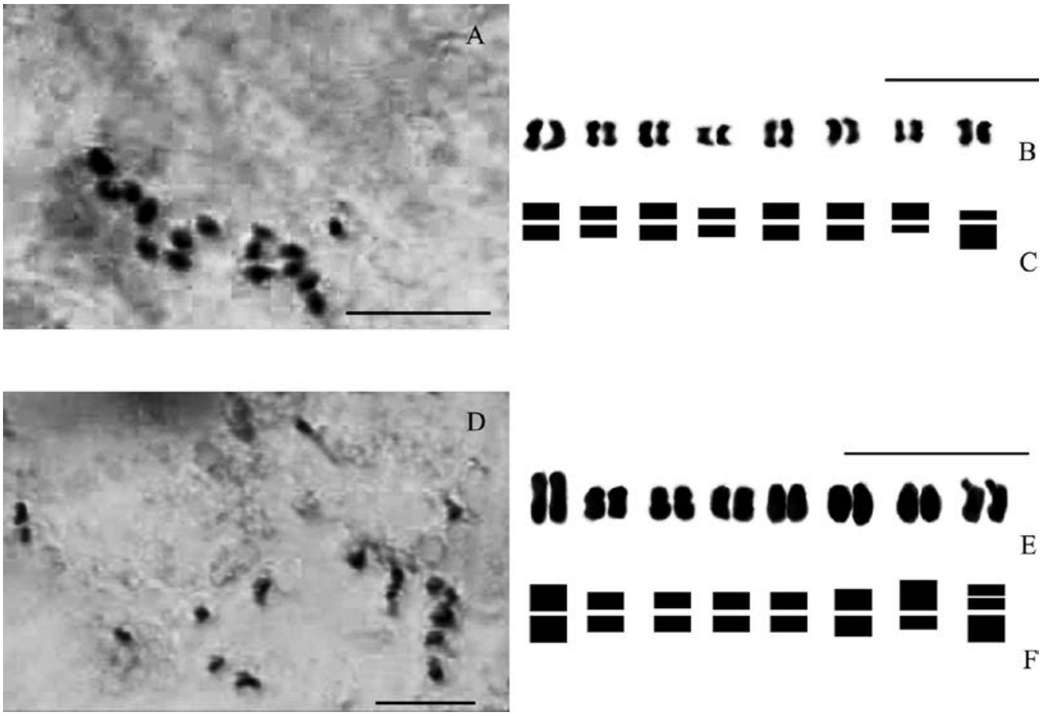

Fig. 1. *Petrosimonia brachiata* Bunge.: A, D – microphotographs of root tip mitosis; B, E – karyograms; C, F – idiograms; scale bar 10 µm.

Table 3. Mean, coefficient of variation (CV%) and percentage of intrapopulation variation in the overall morphological variation (SSw) of *Petrosimonia brachiata* populations for each of the 15 studied quantitative characters.
Differences of specific parameters between the two populations are statistically significant at *P<0.05; **P<0.01 and ***P<0.001 if they do not have the same letters.

Para meter	Populations and population variation				
	Atanasovsko Lake	**CV**	**Pomorie Lake**	**CV**	**SSw%**
1.	131.77***	36.65	265.73***	40.17	59.49
2.	9.8***	25.75	11.87***	26.99	87.09
3.	**1.42a**	23.02	**1.38a**	24.11	99.72
4.	7.10**	27.83	8.89**	29.08	84.83
5.	5.37**	15.62	15.62	22.38	15.62
6.	1.08**	14.13	14.13	13.11	14.13
7.	**5.04a**	18.65	18.65	19.26	18.65
8.	3.03**	31.32	3.61**	26.37	88.95
9.	**0.81a**	24.31	**0.87a**	23.77	99.49
10.	3.83**	25.45	4.83**	22.17	88.84
11.	**1.53a**	20.32	**1.70a**	20.65	96.02
12.	0.16***	5.12	0***	3.15	39.62
13.	**1.55a**	12.54	**1.56a**	11.3	99.88
14.	0.98***	13.36	1.07***	9.98	80.44
15.	1.59***	12.52	1.46***	12.83	79.49

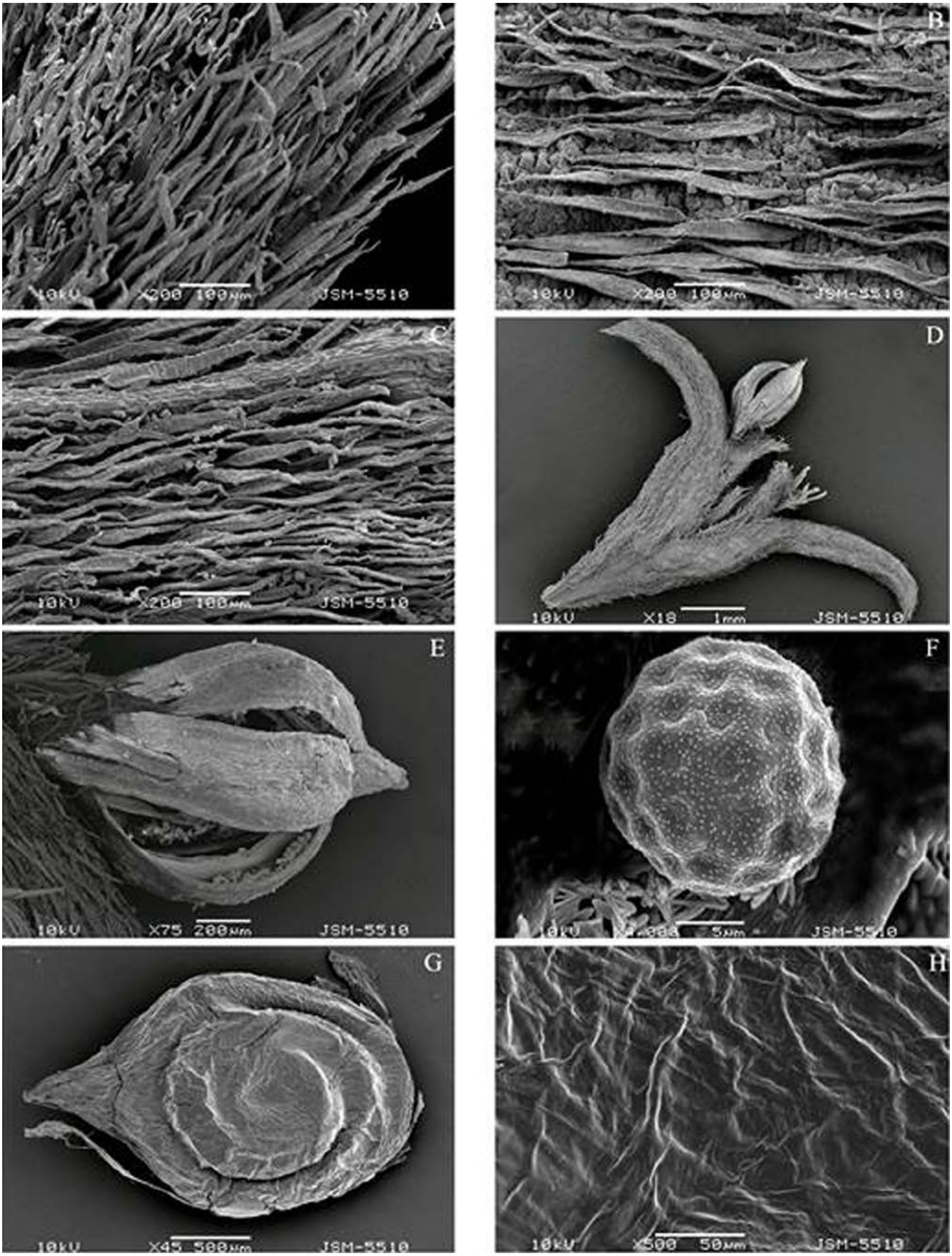

Fig. 2. Scanning electron micrographs of *Petrosimonia brachiata* Bunge.: A – indumentum of stem; B – upper leaf surface; C – lower leaf surface; D – flower: E – anthers; F – pollen grain; G – fruit; H – seed surface.

The stems of all studied specimens were slightly ribbed, light-green, grey-green to reddish at the beginning of vegetation and red during flowering and fruiting, densely pubescent with long, semiappressed hairs (Fig. 2A). The leaf lamina of the lower and upper stem leaves were linear, rarely linear-elliptical, with a sharply pointed tip, entire edge and visible middle vein, grey-green, sessile, hairy with densely pubescent with long, semiappressed hairs (Fig. 2B-C).

In all studied specimens, bracteoles were narrowly lanceolate, boat-shaped and narrow at the top, pointed and tightly engaging the perianth segments and always longer than them, grey-green, juicy, densely pubescent with long, semiappressed hairs (Figure 2D). Perianth segments were five , narrowly lanceolate, pointed, fused at the base, grey-green, densely pubescent with long, semiappressed hairs (Fig. 2D). During flowering, perianth segments were more or less juicy, in early fruiting they acquire a cartilaginous texture and at the end of the phenophase (probably under pressure of the growing fruit) they split into thin lengthwise strips and are separated. The pistil had a prolonged egg-shaped ovary, short style and two thin stigmas. Stamens were five, fused on an obscure disc, almost equal in length with the bracteoles. Anthers had a short appendage on the apex (Fig. 2E). Length of the appendage was 0.2-0.3 mm and it was attached by lateral margins, making the joined anthers umbrella-shaped.

Among members of the two studied populations, no significant variability of pollen morphology was established. The pollen of *P. brachiata* was spheroidal, pantoporate, 17.7-20.8 µm in diameter (Fig. 2F). Exine ornamentation was densely scabrate. Pores were circular, 0.8-1.2 µm in diameter, the pore plate sunken, with scabrae.

The pericarp was yellowish, translucent, non-hairy, with a layered structure and highly folded surface, not fused with the seed coat (Fig. 2G). Seeds were vertical, oval, flattened. The seed coat (testa) was dark grey to black, clearly streaked in the form of highly elongated stitches along the edge and peripheral parts on both sides of the seed and less so in the central ones (Fig. 2H).

Status of the studied populations. In three-year field studies of the two *P. brachiata* populations, reductions in their area or in the number of their specimens were not registered (Table 1). Factors with an adverse effect were not been found during the present study. Invasive and competing plant species were not observed. Anthropogenic impact was limited.

DISCUSSION

The nature of pollen grains and karyotype morphology are important bases in the task of clarifying relationships and they are considered to be of significant value in observing the effects of environmental and climatic factors on plants (Malih 2017). The chromosome number of $2n = 16$ counted in the Bulgarian populations of *P. brachiata* corresponds to the data reported by Lomonosova (2005) for the population from Russia (Republic of Kalmytia, Manych Valley, Great Almaty Lake). Our results indicating a basic chromosome number of $x = 8$ correspond with those of Doulatyari *et al.* (2009) and Ghaffari *et al.* (2015) for the genus *Petrosimonia*. Karyotype asymmetry is a well-known parameter in karyological studies (Eroğlu 2015). According to the M_{ca} and CV_{cl}. values, the karyotype of the population from the Pomorie Lake is slightly more asymmetrical than that from the Atanasovsko Lake. General characteristics of the pollen (pollen and pore diameter, exine ornamentation) of the two *P. brachiata* populations exhibited the features specified by Perveen & Qaiser (2012) as the *Atriplex stocksii*-type. The data pertaining to exine ornamentation were in line with those provided by Mosyakin & Tsymbalyuk (2006) for the genus *Petrosimonia*. The morphological results obtained for the analysed *P. brachiata* populations agree with data for other species of Amaranthaceae in the Bulgarian flora (Grozeva & Cvetanova 2008, 2011, 2013), for which it was also established that vegetative traits are more variable than generative ones and that seed dimensions (i.e., length, width and the ratio between them) are among the more conservative traits. Stem height was one of the most variable traits in both populations and values for this trait were twice as high for plants from the Pomorie Lake, where *P. brachiata* is dominant in the community. Fairly great intrapopulation variability was also recorded for leaf lamina length and bracteole length, specimens from the Pomorie Lake compared with those from the Atanasovsko Lake having longer leaf laminas and bracteoles.

The main source of phenotype variation is intrapopulation variation. The probable cause of strong intrapopulation variability is the observed uneven distribution of soil moisture during vegetation, a factor which affects the development of specimens.

A possible cause of the obtained interpopulation differences with respect to 10 of the total of 15 quantitative characters included in the morphological analysis could lie in differences of soil salinisation at the growing sites, considering that *P. brachiata* is a halophyte. The level of soil salinity on territory of the Atanasovsko Lake was greater than soil salinisation on territory of the Pomorie Lake. The mean values of electrical conductivity for soil samples taken near the Atanasovsko Lake were twice as great as those for samples from the Pomorie Lake. The maximum value of EC = 42.6 mS/cm for samples from the Atanasovsko Lake area was much higher than that of the Pomorie Lake samples (with EC = 12.61 mS/cm). Soil from the Atanasovsko Lake was characterised by an alkaline reaction, with values of pH (H_2O) between 8 and 9.2, as compared to samples from the Pomorie Lake, with values of pH (H_2O) between 7.1 and 8.9, which represent a neutral to alkaline reaction of the samples (Todorova *et al.* 2014). In support of this hypothesis is the fact that the number of specimens and size of the population of the Pomorie Lake are much greater compared to that of the Atanasovsko Lake (Table 1). In the population of the Pomorie Lake, *P. brachiata* is dominant and soil conditions in the area of occurrence of this population probably are more favourable for it and have contributed to acceleration of vegetative growth. Another reason for the variability found could also lie in the identified karyotype differences (Fig. 1). Despite the statistically proven differences in the reported values of 10 studied parameters, the recorded variation is within the range of species variation.

The populations of *P. brachiata* were within protected areas for which management plans have been implemented. They were included in the NATURA 2000 European ecological network. Despite the relatively constant population size and area over the three-year study, their condition cannot be considered as stable. For example, having in mind that the average number of seeds per plant was between 15 and 35, the size of each population was far from the theoretically possible value. According to our observations, the main reason for this is the fact that some of the seeds fail to reach the soil, being washed away by lake water and/or blown away by air currents to an environment inappropriate for their development. In addition, some seeds do not reach full maturity due to late development of the plants or prolonged drought in parts of the populations.

CONCLUSION

The diploid chromosome number established for the Bulgarian population of *P. brachiata* was $2n = 2x = 16$. The karyotypes consist of metacentric and sub-metacentric chromosomes, the metacentric ones being prevalent. Pollen morphology showed a uniform type of characteristics – pantoporate, spheroidal, with a spinulose tectum. The karyotype and morphology of the pollen of *Petrosimonia brachiata* are herein reported for the first time for its Bulgarian populations. Relationships are established between the degree of intrapopulation morphological variability and the distribution of soil moisture, as well as between the degree of interpopulation variability and differences in karyotypes and environmental conditions, especially the soil reaction and soil salinity. Despite the conservation measures that have been taken to protect them, the status of the two populations of *P. brachiata* cannot be regarded as stable, and it is necessary to monitor the populations and react promptly in the event of an adverse effect.

Acknowledgements – This work was financially supported with funds approved for projects E2/13 and 5P/15 of Trakia University´s Faculty of Agriculture. The authors express sincere thanks to the anonymous reviewers for their valuable advice.

REFERENCES

Abideen Z, Ansari Z & Khan M. 2011. Halophytes: potential source of ligno-cellulosic biomass for ethanol production. *Biomass and Bioenergy* **35**(5): 1818–1822.

APG VI. 2016. An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG IV. *Botanical Journal of the Linnean Society* **181**: 1–20.

Assyov B & Petrova A. 2012. *Conspectus of the Bulgarian Vascular Flora. Distribution Maps and Floristic Elements*. Fourth revised and enlarged edition. Bulgarian Biodiversity Foundation, Sofia.

Atamov V, Aktoklu E, Cetin E, Aslan M & Yavuz M. 2006. Halophytication in Harran (Şanlıurfa) and Amik Plain (Hatay) in Turkey. *Phytologia Balcanica* **12**(3): 401–412.

Berberoglu S, Yimaz K & Özkan C. 2004. Mapping and monitoring of coastal wetlands of the Çukurova Delta in the Eastern Mediterranean region. *Biodiversity and Conservation* **13**(3): 615–633.

Biological Diversity Act. 2002. Decree No 283 approved by the 39th National Assembly on 2nd August 2002. *Darzhaven Vestnik No 77/09.08.2002*.

Doulatyari A, Ghaffariq SM & Akhani H. 2009. A cytological study of fourteen halophytic species of tribes Caroxyloneae and Salsoleae (Chenopodiaceae) from Iran. *Cytologia* **74**(1): 79–87.

Erdtman G. 1986. *Pollen morphology and plant taxonomy: Angiosperms* (Vol. **1**). Brill Archive.

Eroğlu HE. 2015. Which chromosomes are subtelocentric or acrocentric? A new karyotype symmetry/asymmetry index. *Caryologia* **68**: 239 – 245.

Flowers TJ & Colmer TD. 2008. Salinity tolerance in halophytes. *New Phytologist* **179**: 945–963.

Ghaffari S M, Targol Z B & Akhani CH. 2015. Cytology of SW Asian Chenopodiaceae: new data from Iran and a review of previous records and correlations with life forms and C_4 photosynthesis. *Plant Systematics and Evolution* **301**(2): 501–521.

Glenn E, Squires V, Olsen M & Frye R. 1993. Potential for carbon sequestration in the drylands. *Water, Air and Soil Pollution* **70**: 341–355.

Grif VG & Agapova N D. 1986. Methods of description of plant karyotypes. *Botanicheskii Zhurnal* **71**: 550–553.

Grozeva N. 2004. Family Chenopodiaceae plants with conservation value. *Journal of Balkan Ecology* **7**: 125–134.

Grozeva N. 2005. The flora of the Atanassovsko lake Natural Reserve. In: Gruev M, Nikolova M & Donev A (eds.), *Proceedings of the Balkan Scientific Conference of Biology in Plovdiv (Bulgaria)*, pp. 381–396, Plovdiv, Bulgaria.

Grozeva N. 2007. *C. pumilio* (Chenopodiaceae): a species new to the Bulgarian flora. *Phytologia Balcanica* **13**: 331–334.

Grozeva N. 2015. Reports. In: Marhold K (ed.), IAPT/IOPB chromosome data 19. *Taxon* **64**(5): 1071, E1-E21.

Grozeva N & Cvetanova Y. 2008. Variability among the populations of two *Chenopodium* genus species. *Proceedings 7 Scientific Conference of Ekology and Health*, Academic Publishing House of Agrarian University, Plovdiv, pp. 135–140.

Grozeva N & Cvetanova Y. 2011. *Chenopodium bonus-henricus* (Perennial goosefoot) in Bulgaria. I. Population variability. *Trakia Journal of Sciences* **9**: 1–7.

Grozeva N & Cvetanova Y. 2013. Karyological and morphological variations within the genus *Dysphania* (Chenopodiaceae) in Bulgaria. *Acta Botanica Croatica* **72**(1): 49–69.

Grozeva N, Miteva Tsh, IVanov P & Videv V. 2004. The Flora on the Atanasovsko Lake, presented through. A Web-based Information. *Journal of Balkan Ecology* **7**: 362–373.

Ibrahim T. 2014. Important fodder crops growıng in the watered haylands of northern terrıtorıes of the Lower Caucasus. *European Academic Research* **2**: 2137–2143.

Kochev H, Ganchev S & Yordanov D. 1971. The halophylic vegetation in Bulgaria. *Proceedings of the Institute of Botany, BAS* **21**: 5–50.

Krasnova A, Kuzmichev A & Dzhalalova M. 2013. Features of littoral ecotone of middle Caspian Sea (within the Terek-Kuma Lowland). *Arid Ecosystems* **3**: 150–155.

Lomonosova M. 2005. Chromosome numbers of Chenopodiaceae species from Russia and Kazakhstan. *Botanicheskii Zhurnal* **90**(7): 1132–1134.

Malih HR. 2017. Pollen grain morphology characters and the chromosomal number of some Chenopodiaceae plants. *Journal of Thi-Qar Science* **6**(2): 39–44.

Mosyakin SL & Tsymbalyuk MG. 2006. Phylogenetic significance of palynomorphological peculiarities of representatives of the family Chenopodiaceae Vent. *Ukrayins'kyi Botanicheskii Zhurnal* **63**(4): 502–514.

Paszko A. 2006. A critical review and a new proposal of karyotype asymmetry indices. *Plant Systematics and Evolution* **258**: 39–48.

Peruzzi L & Eroğlu HE. 2013. Karyotype asymmetry: again, how to measure and what to measure? *Comparative Cytogenetics* **7**(1): 1–9.

Perveen A & Qaiser M. 2012. Pollen flora of Pakistan – LXX: Chenopodiaceae. *Pakistan Journal of Botany* **44**(4): 1323–1333.

Qadir M & Oster JD. 2004. Crop and irrigation management strategies for saline-sodic soils and waters aimed at environmentally sustainable agriculture. *Science of the Total Environment* **323**: 1–19.

Sardo V & Hamdy A. 2005. Halophytes: a precious resource**.** *Options Méditerranéennes: Série B. Etudes et Recherches* **53**: 119-128

Stoeva M. 2011. *Petrosimonia brachiata*. In: Peev D (ed), *Red data book of the Republic of Bulgaria. Vol.1. Plants and Fungi*. http://e-ecodb.bas.bg/rdb/bg/vol1/04A2.html

Stoyanov D. 2010. The higher flora of Pomorie Lake. In: Radev R, Heebaum G, Michev T & Profirov L (eds.), *Proceedings of the Integrated management plan of Protected area „Pomorie lake" BG 0000152 and Protected area „Pomorie" BG 0000620,* BSPB, pp. 66–71, Green Balkans.

Toderich K, Shuyskaya E, Ismail S, Gismatullina L, Radjabov TB, Bekchanov B & Aralova D**.** 2009. Phytogenic resources of halophytes of Central Asia and their role for rehabilitation of sandy desert degraded rangelands. *Land Degradation and Development* **20**(4): 386–396.

Todorova M, Grozeva N, Pleskuza L, Yaneva Z, Gerdgikova M. 2014. Relationship between soil salinity and *Bassia hirsuta*, *Salicornia europaea* agg. and *Petrosimonia brachyata* distribution on the territory of Pomorie lake and Atanasovsko lake. *Agricultural Science and Technology* **4**: 465–470.

Tsenkova-Bratoeva A, Koleva W & Ivancheva Y. 2010. Climatic conditions in the area of Pomorie Lake and its surrounding lands. In: Radev R, Heebaum G, Michev T & Profirov L (eds.), *Proceedings of the Integrated management plan of Protected area „Pomorie lake" BG 0000152 and Protected area „Pomorie" BG 0000620,* BSPB, pp. 9-18, Green Balkans.

Tzonev R & Gussev Ch. 2011. Communities of annual halophytes in coastal salt marshes at the Black Sea. In: Biserkov V (ed.) *Red data book of the Republic of Bulgaria, vol.* ***3****. Natural habitats*. http://e-ecodb.bas.bg/rdb/en/vol3/04A2.html

Tzonev R, Lysenko T, Gusev G & Zhelev P. 2008. The halophytic vegetation in South-East Bulgaria and along the Black Sea Coast. *Hacquetia* **2**: 95–121.

Uotila P. 2011.Chenopodiaceae (pro parte majore). In: *Euro+Med PlantBase - the information resource for Euro-Mediterranean plant diversity*. Published on the Internet http://ww2.bgbm.org/EuroPlusMed/

Wucherer W, Veste M, Herrera Bonilla O & Breckle S. 2005. Halophytes as useful tools for rehabilitation of degraded lands and soil protection. *Proceedings of the first international forum on Ecological construction of the Western Beijing, Beijing*: 87–94.

Yordanov D & Kuzmanov B. 1966. *Petrosimonia* Bunge. In: Yordanov D (ed.), *Flora of the People's Republic of Bulgaria* **3**, p. 565, Aedibus Academiae Scientiarum Bulgaricae, Serdicae.

Zhu G, Mosyakin SL & Clements SE. 2003. Chenopodiaceae: *Petrosimonia* Bunge In: Wu ZY, Raven PH & Hong DY (eds.), *Flora of China*, **5**. *Ulmaceae through Basellaceae*, pp. 376–378, Science Press, Beijing and Missouri Botanical Garden Press, St. Louis. http://www.efloras.org/florataxon.aspx?flora_id=2&taxon_id=124744

Botanica SERBICA

REZIME

Kariološka i morfološka variranja unutar vrste *Petrosimonia brachiata* Bunge u Bugarskoj

Neli Grozeva, Mima Todorova i Dimitar Pavlov

Istraživana je kariološka i morfološka varijabilnost jedinog predstavnika roda *Petrosimonia* (Amaranthaceae) u Bugarskoj i ocenjeno je trenutno stanje populacija. Rezultati pokazuju da bugarske populacije *Petrosimonia brachiata* imaju diploidan broj hromozoma $2n = 16$. U populaciji sa jezera Pomorje, kariotip se sastoji od 6 parova metacentričnih i 2 para submetacentričnih hromozoma, a u populaciji sa Atanasovskog jezera sateliti su uočeni na jednom paru submetacentričnih hromozoma. Statističke analize su ukazale na glavni izvor fenotipske varijabilnosti unutar populacija. Vegetativne osobine su varijabilnije od generativnih. Nije pronađena značajna varijabilnost istraživanih kvantitativnih karakteristika i morfologije polena. Status dve populacije *P. brachiata*, sudeći prema jednogodišnjosti i ograničenom rasprostranjenju u Bugarskoj, ne može se oceniti kao stabilan i potencijalna pretnja po njih u budućnosti nije isključena.

Ključne reči: Amaranthaceae, kariotip, broj hromozoma, morfologija, horologija