REVIEW ARTICLE



# Studies on the Bulgarian members of the family Chenopodiaceae s. stricto: a review

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Academic editor: Josef Settele | Received 27 November 2021 | Accepted 7 March 2022 | Published 27 April 2022

**Citation:** Boneva VS, Petkova NT (2022) Studies on the Bulgarian members of the family Chenopodiaceae *s. stricto*: a review. BioRisk 18: 17–34. https://doi.org/10.3897/biorisk.18.78548

#### Abstract

The Bulgarian members of Chenopodiaceae are mainly ruderal and weed species; another part are halophytes. Over the last two decades, phylogenetic molecular studies have led to a number of taxonomic changes in the above mentioned family. Changes have also occurred in one of the largest genera – *Chenopodium*. The aim of the present study is to review the research on Bulgarian members of the Chenopodiaceae family. The data available in the literature on the taxonomic composition, chorology, morphological features and karyological variability of the species from their Bulgarian populations has been studied. A review of the phytochemical studies of Chenopodiaceae plants from their Bulgarian populations has been made. The systematized data is presented in chronological order, which allows for tracing the current level of study on the family in Bulgaria and opportunities for new research.

#### **Keywords**

Bulgaria, Chenopodiaceae, chorology, karyology, morphology, phytochemical investigations

## Introduction

The family Chenopodiaceae is relatively large and worldwide it numbers about 1600 species belonging to more than 100 genera, spread more widely in the moderate and subtropical regions (Kühn et al. 1993; Kadereit et al. 2003).

The greater part of the Bulgarian members of the family belongs to the group of the highly movable ruderal plants and weeds with habitats in the central and

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southern part of the continent but having successfully spread in Bulgaria's thermal zone and in the western parts of its territory (Stefanov 1943). Their wide distribution is related to the ability of most species to grow under extreme conditions such as high air and soil temperature (Ilyin 1936). Some of the species are North American, North African or Australian and have spread over the territory of the country due to human activity. The last part is the number of species belonging to the Mediterranean and Boreal floral element, as well as those distributed on most of the continents. Another part of the Bulgarian members of the family are halophytes inhabiting the periodically flooded, muddy and sandy terrains on the periphery of the hyperhaline and, less frequently, the brackish Black Sea lakes. They form the communities of the annual halophytes in the Black Sea salt lakes (Tzonev and Gussev 2015). The globalization we have witnessed over the last two decades has led to the penetration of many foreign species, and molecular research has led to new taxonomic changes and decisions in the family.

The objective of the present study is to review the chorological, morphological, karyological, and phytochemical studies regarding the Bulgarian members of the family Chenopodiaceae.

# Data about the taxonomic composition and distribution of the bulgarian members of the family Chenopodiaceae

## Available literature from the last decades of the 19th century

The first data about the distribution of the Bulgarian members of the family Chenopodiaceae was published in the 19<sup>th</sup> century by: Bornmüller (1888), Georgiev (1889), Yavashev (1890), Toshev (1895), and Urumov (1897). The information there occurring has been summarized by Velenovský (1891, 1898) in the two editions of his Flora bulgarica.

The 1st edn. of Flora bulgarica (Velenovský 1891) listed 28 Chenopodiaceae species belonging to 9 genera. Concerning *Chenopodium*, the following 12 species were reported: *C. bonus-henricus* L., *C. rubrum* L., *C. glaucum* L., *C. polyspermum* L. *C. hybridum* L., *C. murale* L., *C. urbicum* L., *C. album* L., *C. ficifolium* Sm., *C. opulifolium* Schrad., *C. vulvaria* L., *C. botrys* L. For *Atriplex* 4 representatives were reported, i.e. *A. rosea* L., *A. laciniata* L., *A. patula* L., and *A. hastata* L. The genus *Kochia* is represented by 4 species in Velenovský's work: *K. scoparia* Schrad., *K. prostrata* Schrad., *K. arenaria* Roth., and *K. sedoides* Schrad. Two species of *Camphorosma* (*C. monspeliaca* L. and *C. ovata* WK.) and two of *Suaeda* (*S. maritima* Dumort. and *S. altissima* Pall.) were recognized. Finally, just one species was given for four genera: *Beta trygina* B. K, *Blitum virgatum* L., *Salicornia herbacea* L, *Salsola kali* L.

In the  $2^{nd}$  edn. of Flora bulgarica Supplementum (Velenovský 1898) new information concerning the distribution has been included regarding: 6 species from genus *Chenopodium – C. bonus-henricus, C. hybridum, C. urbicum, C. botrys, C. vulvaria, C. album*; 3 species belonging to genus *Atriplex – A. rosea, A. patula, A. tatarica*; 2 species from genus *Beta – B. trygina, B. maritima* and 1 species from each of genus *Blitum (B. virgatum), Kochia (K. scoparia), Suaeda (S. heterophylla), Salsola (S. kali).* 

Therefore, a total of 32 Chenopodiaceae species were reported for Bulgaria in the 19<sup>th</sup> century, including data about the distribution in the country.

## Available literature from the 20<sup>th</sup> century

In the first two decades of the 20<sup>th</sup> century the main source of information about the Bulgarian Chenopodiaceae species was works by Urumov (1901, 1904, 1905, 1906, 1908a, 1908b, 1908c, 1909, 1913, 1917, 1923), Kovachev (1903), Davidov (1904, 1905), Neichev (1905), Petkov (1907), Stranski (1921), and Yordanov (1923–1924).

The gathered chorological information was summarized in the 1<sup>st</sup> edn. of Flora of Bulgaria (Stoyanov and Stefanov 1924), where family Chenopodiaceae is presented by 44 species and 13 genera. Cyclolobae C. A. Mey. comprises members of the following genera: *Atriplex* (7), *Bassia* (2), *Beta* (2), *Camphorosma* (3), *Chenopodium* (12), *Corispermum* (3), *Kochia* (3), *Polycnemum* (3 species), *Salicornia* (1), *Spinacia* (1). Spirolobae comprises 3 genera – *Petrosimonia* (1), *Salsola* (3), *Suaeda* (3).

In the period prior to the publication of the 2<sup>nd</sup> edn. of Flora of Bulgaria (Stoyanov and Stefanov 1933), data about the Chenopodiaceae plants was published mainly by Urumov (1926, 1928, 1929a, 1929b, 1930).

The 2<sup>nd</sup> edn. of the Flora of Bulgaria (Stoyanov and Stefanov 1933) reports 39 Chenopodiaceae species (13 genera): *Atriplex* (7), *Bassia* (2), *Beta* (2), *Camphorosma* (2), *Chenopodium* (12), *Corispermum* (3), *Kochia* (3), *Petrosimonia* (1), *Polycnemum* (3 species), *Salicornia* (1), *Salsola* (2), *Spinacia* (1), *Suaeda* (2).

From the members of the family included in the 1<sup>st</sup> edn. of Flora of Bulgaria 5 of the species were removed: *Camphorosma ruthenicum* M.B., *Corispermum canescens* Kit., *Corispermum orientale* Lam., *Salsola toseffi* Urumov, and *Suaeda heterophylla* Bunge.

Later chorological data about the species in the family was published by Rechinger (1933), Urumov (1935), and Baev (1947) which were included in the 3<sup>rd</sup> edn. of the Flora of Bulgaria (Stoyanov and Stefanov 1948). Forty one species of Chenopodiaceae are mentioned, belonging to 13 genera: *Atriplex* (7), *Bassia* (2), *Beta* (2), *Camphorosma* (2), *Chenopodium* (13), *Corispermum* (2), *Kochia* (3), *Petrosimonia* (1), *Polycnemum* (3), *Salicornia* (1), *Salsola* (2), *Spinacia* (1), *Suaeda* (2). In the same edition 2 new species of Bulgarian flora are included in family Chenopodiaceae – *Chenopodium opulifolium* Schrad. and *Corispermum hyssopifolium* L. *C. opulifolium* was mentioned as a ruderal plant widely spread in the country, while the second one was discovered in an area on the sands of the Danubian islands from the Belene group (isle of Milka and isle of Persina).

In subsequent years new data about the distribution and the ecology of Chenopodiaceae was found in works by Stoyanov et al. (1954), Kiryakov et al. (1951), and Kolev (1956, 1959).

The next edition (4<sup>th</sup>) of Flora of Bulgaria (Stoyanov et al. 1966) the author reported one new genus and species for Bulgaria, i.e. *Ceratocarpus arenarius*.

The most exhaustive information about the family Chenopodiaceae in our country was given by Yordanov et al. (1966) in Flora of the People's Republic of Bulgaria. A total of 48 Chenopodiaceae species were reported, belonging to 15 genera: *Atriplex* (7), *Bassia* (2), *Beta* (2), *Camphorosma* (2), *Ceratocarpus* (1), *Chenopodium* (15), *Corispermum* (2), *Halimione* (2), *Kochia* (3), *Petrosimonia* (1), *Polycnemum* (3), *Salicornia* (2), *Salsola* (2), *Spinacia* (1), *Suaeda* (3). Members of the family are 4 cultivated species as well as *Atriplex hortensis*, *Spinacia oleracea*, *Beta vulgaris*, *Kochia scoparia*. *Chenopodium ambrosioides* was published as new species for the country and it was reported for the first time for the sands near Varna and the experimental field in Ovcha kupel.

Following the publication of Flora of the People's Republic of Bulgaria floristic reports have been presented supplementing the data about the family Chenopodiaceae in Bulgarian flora (Delipavlov and Dimitrov 1973; Panov 1975, 1987; Delipavlov and Cheshmedzhiev 1984; Cheshmedzhiev 1988; Meshinev et al. 1994).

Summarized studies about the family during that period are presented in the Field Guide to the vascular plants in Bulgaria (Andreev 1992), where Chenopodiaceae is represented by 48 species (14), as follows: *Atriplex* (8), *Bassia* (2), *Beta* (2), *Camphorosma* (2), *Ceratocarpus* (1), *Chenopodium* (16), *Corispermum* (2), *Halimione* (2), *Kochia* (2), *Petrosimonia* (1), *Polycnemum* (3), *Salicornia* (2), *Salsola* (2), *Suaeda* (3).

It is noted that *Bassia sedoides* distribution in Bulgaria has not been confirmed by Andreev (1992). The publication does not mention any cultivated species. Concerning the genus *Chenopodium*, a new record from the Northern Black Sea coast has been reported, i.e. *Chenopodium chenopodioides* (L.) Aellen. (= *C. botryoides* Sm.; see e.g., Iamonico 2014). Among the representatives of genus *Atriplex* two new species have been added to the national flora, i.e. *A. micrantha* (= *A. heterosperma*) and *A. halimus*. The first species is indicated as in doubt, whereas *A. halimus* has been reported for the Northern Black Sea coast. Philipova-Marinova et al. (1997) indicated *C. botryoides* as new for the country from the territory of Shabla Lake.

The summarized data from the studies about the Bulgarian members of the family Chenopodiaceae in the 20<sup>th</sup> century showed that the species diversity of the family comprised 48 species belonging to 14 genera.

## Available literature from the new millennium

Chorological studies of the members of Chenopodiaceae from the end of the  $20^{\text{th}}$  century and the beginning of the  $21^{\text{st}}$  century have been published by Delipavlov and Cheshmedzhiev (2011). Fifty species have been listed for the family (15), as follows: *Atriplex* (8), *Bassia* (1), *Beta* (2), *Camphorosma* (2), *Ceratocarpus* (1), *Chenopodium* (17), *Corispermum* (2), *Halimione* (2), *Kochia* (3), *Petrosimonia* (1), *Polycnemum* (3), *Salicornia* (2), *Salsola* (2), *Spinacia* (1), *Suaeda* (3). The composition of the genus *Chenopodium* comprises the 16 species indicated by Andreev (1992) and 1 of the species published by Panov (1987) – *C. acuminatum* – confirmed for the Northern Black Sea coast. *Bassia sedoides* has been excluded from the genus *Bassia*. Four cultivated species were reported - *Atriplex hortensis*, *Spinacia oleracea, Beta vulgaris, Kochia scoparia*.

Conspectus of the Bulgarian vascular flora by Assyov and Petrova (2006) registered 50 species of Chenopodiaceae belonging to 13 genera: *Atriplex* (9), *Bassia* (1), *Beta* (2), *Camphorosma* (2), *Chenopodium* (18 species), *Corispermum* (2), *Halimione* (2), *Kochia* (3), *Petrosimonia* (1), *Polycnemum* (3), *Salicornia* (2), *Salsola* (2), *Suaeda* (3). The composition of genus *Chenopodium* included 1 new species for the country, *C. schraderianum* Schult.

Five Chenopodiaceae species are protected by the Biological Diversity Act (2007): Bassia hirsuta, Halimione pedunculata, H. portulacoides, Petrosimonia brachiata, and Suaeda heterophylla. The Red Data Book of the Republic of Bulgaria (Peev et al.2015) includes 4 species, i.e. B. hirsuta, Corispermum marschalii, P. brachiata, and S. heterophylla. B. hirsuta and C. marschalii have been referred to the IUCN category EN (endangered), whereas *P. brachiata* and *S. heterophylla* are assessed as CE (critically endangered). B. hirsuta, P. brachiata and S. heterophylla are part of the communities of annual halophytes in the salty lakes along the Black Sea coast. The annual halophyte communities are among the protected natural habitats pursuant to Directive 92/43/ EEC (1992). The habitat is included in Annex 1 of Biological Diversity Act (2007) and in the Red Data Book of the Republic of Bulgaria by Tzonev and Gussev (2015) in the category "endangered" habitat. The data about B. hirsuta, P. brachiata, and S. heterophylla populations are found in some works concerning the Black Sea coast vegetation and in the protected area management plans of Burgas wetlands (Michev et al. 2003; Grozeva et al. 2004; Grozeva 2004, 2005; Tzonev et al. 2008; Stoyanov 2009; Todorova et al. 2014).

In the last decade the genus *Chenopodium* has been subject to a number of studies resulting in changes in the species composition of both the genus and the family Chenopodiaceae. Grozeva (2007a) reported a new species for the country *Chenopodium pumilio*. Based on a taxonomic revision Grozeva (2009) suggested *Chenopodium acuminatum* to be deleted from the members of the Bulgarian flora as incorrectly identified. This change was later recorded by Assyov et al. (2012).

Six new species and one subspecies from the genus *Chenopodium* (*C. strictum* Roth, *C. striatiforme* Murr, *C. album* subsp. *pedunculare* (Bertol.) Arcang., *C. pratericola* Rydb., *C. probstii* Aellen, and *C. missouriense* Aellen) have been reported for the Bulgarian flora by Grozeva (2009, 2010a, 2010b, 2012a, 2012b).

The changes that had occurred in the taxonomic composition of Chenopodiaceae have been recorded in the 4<sup>th</sup> edn. of Conspectus of Bulgarian vascular flora (Assyov et al. 2012), where 56 species (14 genera) are listed as follows: *Atriplex* (9), *Bassia* (1), *Beta* (2), *Camphorosma* (2), *Ceratocarpus* (1), *Chenopodium* (23), *Corispermum* (2), *Kochia* (3), *Petrosimonia* (1), *Polycnemum* (3 species), *Salicornia* (2), *Salsola* (3), *Spinacia* (1), *Suaeda* (3).

The Euro+Med Plant Base (Uotila 2011) reported 53 species and 19 genera for Bulgaria: Atriplex (8), Bassia (4), Beta (2), Blitum (2), Camphorosma (2), Ceratocarpus (1), Chenopodiastrum (2), Chenopodium (6), Corispermum (2), Dysphania (4), Halimione (2), Lipandra (1), Oxybasis (4), Petrosimonia (1), Polycnemum (3 species), Salicornia (2), Salsola (2), Spinacia (1), Suaeda (3). The differences in the composition of genus *Chenopodium* between Conspectus of Bulgarian vascular flora (Assyov et al. 2012) and The Euro+Med Plant Base (Uotila 2011) are because Euro+Med Plant Base (Uotila 2011) gives the composition of *Chenopodium s. str.*, including the genera *Blitum*, *Chenopodiastrum*, *Dysphania*, *Lipandra*, *Oxybasis*, while Conspectus of Bulgarian vascular flora (Assyov et al. 2012) presents *Chenopodium s. lat*.

From data by Grozeva (2018b) the family Chenopodiaceae is represented in Bulgarian flora by 51 species and 2 subspecies [*Chenopodium strictum* subsp. *striatiforme* (Murr) Uotila and *C. album* subsp. *pedunculare* (Bertol.) Arcang.] and the author does not include the cultivated species *Beta vulgaris* and *Spinacia oleracea* in the species composition.

The summarized data about the taxonomic composition of family Chenopodiaceae is presented in Table 1.

## Morphological studies on members of Chenopodiaceae in Bulgar-ia

Detailed studies on the morphology of Chenopodiaceae in Bulgaria were conducted, mainly on *Chenopodium s. lat.* In particular, the following species were investigated: *Chenopodium album* and *Dysphania botrys* from 18 populations (Grozeva and Cvetanova 2008), *Blitum bonus-henricus* from 10 populations with ecological notes (Grozeva and Cvetanova 2011) and distribution in the country (Grozeva 2011), *C. pratericola* (Grozeva 2012a), the genus *Dysphania* (Grozeva and Cvetanova 2013), *C. probstii* and *C. missouriense* (Grozeva 2014), *Blitum virgatum* (Grozeva and Cvetanova 2016). Further taxa studies were *Bassia hirsuta* (Grozeva and Todorova 2014) and *Petrosimonia brachiata* (Grozeva et al. 2019a).

## Karyological studies on Chenopodiaceae in Bulgaria

The first karyological data about Chenopodiaceae in Bulgaria was published by Markova (1968) on *Dysphania multifida* (population from the Danubian plain) resulting in a tetraploid chromosome number 2n = 36. These data was later confirmed by Grozeva and Stoeva (2006) on populations of the species from Sredna Gora Mountain, the Eastern Rhodopes and the Thracian lowland and by Grozeva and Cvetanova (2013) on populations from the Central Stara planina, the Danubian plain and the Thracian lowland.

Kožuharov and Kuzmanov (1969) specified for *Suaeda altissima* diploid chromosome number 2n = 18 for populations from the Southern Black Sea coast and these data were confirmed by Grozeva (2010a, 2015a, 2015b).

Cheshmedzhiev (1976) published for *Beta trigyna* populations from Eastern Stara planina hexaploid chromosome number 2n = 54.

Popova and Ceschmedjiev (1978) reported for *Kochia scoparia* population from the experimental field of Agricultural University of Plovdiv diploid chromosome number 2n = 18. These data were confirmed by Grozeva (2015a, 2015b) for a population of the species from the Thracian lowland.

Genus	Species	References for nomen- clature
1. Beta	Beta trigyna Waldst. & Kit., Beta vulgaris L. subsp. maritima (L.) Arcang.	Iamonico (2019)
2. Bassia	Bassia hirsuta (L.) Asch., Bassia laniflora (S.G.Gmel) A. J. Scott. (= Salsola	Uotila (2011)
	laniflora S.G.Gmel), Bassia prostrata (L.) Beck (= Kochia prostrata (L.) Schrad.),	
	Bassia scoparia (L.) A. J. Scott (= Kochia scoparia (L.) Schrad.)	
3. Camphorosma	Camphorosma annua Pall., Camphorosma monspeliaca L. subsp. monspeliaca	Uotila (2011)
4. Atriplex	Atriplex prostrata DC. (= A. hastata L.), A. micrantha Lebed. (= A. heterosperma	Kadereit et al. (2010),
	Bunge) subsp. micrantha, A. hortensis L., A. sagittata Borkh. (= A. nitens Sch-	Uotila (2011)
	kuhr), A. oblongifolia Walds. & Kit., A. patula L., A. rosea L., A. tatarica L.	
5. Blitum	Blitum bonus-henricus (L.) Rchb. (= Chenopodium bonus-henricus), Blitum	Fuentes-Bazan et al.
	virgatum L. (= Chenopodium foliosum (Moench) Asch.)	(2012)
6. Ceratocarpus	Ceratocarpus arenarius L.	Uotila (2011)
7. Chenopodiastrum	Chenopodiastrum murale (L.) S.Fuentes, Uotila & Borsch (= Chenopodium	Fuentes-Bazan et al.
	murale), Chenopodiastrum hybridum (L.) S. Fuentes, Uotila & Borsch. (= Che-	(2012)
	nopodium hybridum L.)	
8. Chenopodium	Chenopodium vulvaria L., Chenopodium ficifolium Sm.,	Fuentes-Bazan et al.
	Chenopodium pratericola Rydb., Chenopodium betaceum Andrz. (= C. strictum	(2012) Mosyakin (2018
	Roth s.lat.), Chenopodium opulifolium Schrad. ex W.D.J.Koch & Ziz, Chenopo-	for C. Betaceum) Iamo-
	dium missouriense Aellen, Chenopodium probstii Aellen, Chenopodium album	nico & Mosyakin (2018
	L., <i>Chenopodium album subsp. pedunculare</i> (Bertol.) Arcang. (= C. pedunculare)	for C. album subsp.
		pedunculare)
9. Corispermum	Corispermum nitidum Kit. Ex Schult.	
10. Dysphania	Dysphania ambrosioides (L.) Mosyakin et Clements L. (= Chenopodium ambro-	Fuentes-Bazan et al.
	sioides L.), <b>Dysphania botrys</b> (L.) Mosyakin et Clements (= C. botrys L.), <b>Dyspha</b> -	(2012)
	nia multifida (L.) Mosyakin et Clements (= C. multifidum L.), Dysphania pumi-	
	<i>lio</i> (R. Br.) Mosyakin et Clements (= <i>C. pumilio</i> R. Br.), <i>Dysphania schraderiana</i>	
11. Halimione	(Schult.) Mosyakin et Clements (= <i>C. schraderianum</i> Schult.)	K 1 1 (2010)
	Halimione pedunculata (L.) Aellen (basionym?), Halimione portulacoides (L.) Aellen (basionym?)	Kadereit et al. (2010)
12. Lipandra	<i>Lipandra polysperma</i> (L.) S. Fuentes, Uotila & Borsch (=Chenopodium polysper-	
	mum L.)	
13. Oxybasis	Oxybasis chenopodioides ( L. ) S. Fuentes, Uotila & Borsch	Fuentes-Bazan et al.
15. 0xjousis	(= <i>Chenopodium chenopodioides</i> L.), <b>Oxybasis glauca</b> (L.) S.Fuentes, Uotila &	(2012)
	Borsch (= <i>C. glaucum</i> L.), <b>Oxybasis rubra</b> (L.) S.Fuentes, Uotila & Borsch (= <i>C.</i>	(2012)
	rubrum L.), Oxybasis urbica	
	( L.) S.Fuentes , Uotila & Borsch (= <i>C, urbicum</i> L.)	
14. Salicornia	Salicornia europaea L. subsp. europaea, Salicornia perennans Willd. subsp.	Kadereit et al. (2012)
	perennans	
15. Petrosimonia	Petrosimonia brachiata Bunge	Uotila (2011)
16. Salsola	Salsola tragus L. (= S. ruthenica Iljin), Salsola soda L.	Akhani et al. (2007)
17. Suaeda	Suaeda altissima (L.) Pall., Suaeda maritima (L.) Dumort.,	Uotila (2011)
	Suaeda heterophylla Bunge	
18. Polycnemum	Polycnemum arvense L., Polycnemum heuffelii Lang., Polycnemum majus	Masson & Kadereit
	A.Braun	(2013)
19. Spinacia	Spinacia oleracea L.	

Table 1. List of members belonging to the family Chenopodiaceae, according to literature dates.

Van Loon and Van Setten (1982) reported for *Blitum bonus-henricus* population from Rila mountain tetraploid chromosome number 2n = 36. These data were confirmed by Grozeva and Stoeva (2006) for populations from the Western Rhodopes, Pirin and Central Stara planina and by Grozeva and Cvetanova (2011) for populations from the Central Rhodopes, Western Stara planina, Rila, Slavyanka, and Vitosha mountains.

Van Loon and Van Setten (1982) reported for *Blitum virgatum* populations from Shipka, Central Stara planina diploid chromosome number 2n = 18. These data were

confirmed by Grozeva and Stoeva (2006) for other populations of the species from Central Stara planina, Western Rhodopes and Tundzha hilly country by Grozeva and Cvetanova (2016) as well as for populations from Rila, Western and Central Rhodopes, Belasitsa, Tundzha hilly country.

Grozeva and Stoeva (2006) published for the first time chromosome numbers for 4 species from the family Chenopodiaceae, as follows: diploid chromosome number 2n = 18 for *Bassia hirsuta* and *Corispermum nitidum* for populations from the Southern Black Sea coast and for *Dysphania botrys* from the Danubian plain, North-Eastern Bulgaria and Central Stara planina; tetraploid chromosome number 2n = 32 for *Dysphania ambrosioides* populations from the Danubian plain and the Thracian lowland.

Grozeva (2007b) reported for the first time diploid chromosome number 2n = 18from the Bulgarian populations of 8 species: Oxybasis chenopodioides from the Northern Black Sea coast; Chenopodiastrum hybridum from the Southern Black Sea coast; Danubian plain and the Eastern Rhodopes; O. glauca from the Northern Black Sea coast and the Danubian plain; C. murale from Central and Eastern Stara planina; O. rubra from North-Eastern Bulgaria; Lipandra polysperma from Eastern Sredna gora, Strandzha and Thracian lowland; C. ficifolium from the Northern Black Sea coast, North-Eastern Bulgaria and Thracian lowland; Atriplex tatarica from the Southern Black Sea coast (Grozeva 2007b). For the newly established species Dysphania pumilio diploid chromosome number 2n = 18 was published (Grozeva 2007b) and for the 2 new for the country North American species Chenopodium missouriense and C. probstii – hexaploid chromosome number 2n = 54 (Grozeva 2010a). The chromosome number for 3 species was reported for the first time: A.hastata 2n = 18 from the Danubian plain and the Thracian lowland, A. patula 2n = 36 from the Eastern Rhodopes and Eastern Sredna Gora, Suaeda maritima 2n = 36 from the Southern Black Sea coast (Grozeva 2010a).

Grozeva (2010b) published for the first time data about the chromosome number of 7 species from the genera: Atriplex (A. nitens, A. rosea, A. hortensis, A. oblongifolia), Chenopodium (C. opulifolium, C. urbicum, C. vulvaria) and Oxybaisis (O. urbica). Diploid chromosome number 2n = 18 was established for populations of: A. nitens from the Eastern Rhodopes; A. rosea from the Southern Black Sea coast and the Thracian lowland; A. hortensis from the Danubian plain and the Thracian lowland; O. urbica from North-East Bulgaria; C. vulvaria from the Eastern Rhodopes. Tetraploid chromosome number 2n = 36 was registered for populations of A. oblongifolia from the Thracian lowland and hexaploid chromosome number 2n = 54 for C. opulifolium populations from North-Eastern Bulgaria and the Thracian lowland.

For the newly established for the Bulgarian flora species *Chenopodium pratericola* diploid chromosome number 2n = 18 was reported (Grozeva 2012a).

Grozeva (2012b) reported for the first time data about the chromosome number of *Chenopodium album subsp. album*, *C. album* subsp. *pedunculare*, *C. betaceum* (sub. *C. striatiforme* and *C. strictum*), and *Suaeda heterophylla*. For *C. album* populations from the Northern Black Sea coast, Eastern Rhodopes and the Thracian lowland and for *C. album* subsp. *pedunculare* from the Northern Black Sea coast hexaploid chromosome number 2n = 54 was reported. For *C. betauceum* populations from the Southern Black Sea coast, Danubian plain and the Eastern Rhodopes tetraploid chromosome number 2n = 36 was established. For *S. heterophylla* population from the Southern Black Sea coast diploid chromosome number 2n = 18 was reported.

Grozeva and Cvetanova (2013) registered for the first time diploid chromosome number 2n = 18 for *Dysphania schraderiana* population from the Eastern Sredna gora mountain. Data about the karyotype of 27 populations of the species from genus *Dysphania* (*D. ambrosioides*, *D. botrys*, *D. multifida*, *D. pumilio* and *D. schraderiana*) was published. Polyploidy and disploidy are cited as basic evolutionary mechanisms in the genus.

Grozeva (2013) reported karyological data about 14 Chenopodiaceae species from their Bulgarian populations. For the first time the chromosome number of the species *Bassia laniflora, Salsola tragus* and *Salicornia europaea* subsp. *europaea* was reported for their populations from the Southern Black Sea coast. For *S. tragus* tetraploid chromosome number 2n = 36 was registered and for *B. laniflora* and *S. europaea* diploid chromosome number 2n = 18. The chromosome numbers known from literature data about 11 species (*Atriplex oblongifolia, A. tatarica, Bassia hirsuta, Chenopodium album subsp. album, C. betaceum* (*sub. C. Strictum*), *Corispermum nitidum, Dysphania pumilio, Lipandra polysperma, Oxybasis chenopodioides, O. rubra, O. urbica*) were confirmed (Grozeva 2013).

Grozeva (2015a) reported for the first time data about the chromosome number from the Bulgarian populations of 4 species (*Atriplex heterosperma*, *Bassia prostrata*, *Salicornia perennans*, *and Salsola soda*). For *A. heterosperma* population from the Southern Black Sea coast chromosome number 2n = 36 was registered. For *B. prostrata* population from the Thracian lowland and for the *S. perennans* and *S. soda* populations from the Southern Black Sea coast diploid chromosome number 2n = 18 was reported. The chromosome numbers known from literature data from the Bulgarian populations of two species (*Suaeda heterophylla* and *S. maritima*) were confirmed.

Grozeva (2015b) reported for the first time diploid chromosome number 2n = 16 for a *Petrosimonia brachiata* population from the Southern Black Sea coast and confirmed the chromosome number known from Bulgarian populations of *Blitum virgatum, Chenopodium album subsp. pedunculare, C. probstii, C. pratericola, Salicornia europaea* subsp. *europaea, Suaeda altissima, S. maritima.* Data about the karyotype of the species from genus *Bassia (B. hirsuta, B. laniflora, B. prostrata, and B. scoparia)* was published (Grozeva and Gospodinova 2016).

The karyological variability was traced and the karyotype of *Blitum virgatum* (Grozeva and Cvetanova 2016), of the species from genus *Salsola* (Grozeva et al. 2018) and genus *Atriplex* (Grozeva 2018a) was established.

Grozeva and Atanassova (2019) studied the karyotype of *Chenopodiastrum murale* and *Ch. hybridum* from their Bulgarian populations. Grozeva et al. (2019b) established diploid chromosome number 2n = 16 and described the karyotype of *Petrosimonia brachiata* from populations of the species from the Southern Black Sea coast. Diploid chromosome number 2n = 18 and data about the karyotype of *Oxybasis chenopodioides*, *O. glauca* and *O. urbica* were reported (Grozeva et al. 2019b).

For the members of family Chenopodiaceae from their Bulgarian populations diploid (2n = 16, 18), tetraploid (2n = 32, 36) and hexaploid (2n = 54) chromosome numbers and two types of chromosomes – metacentric and submetacentric – were found (Grozeva 2018b). Basic chromosome numbers x = 9 and x = 8 were registered with the diploid ones being the dominant, followed by tetraploid and hexaploid species (Grozeva 2018b). According to data by Petrova and Vladimirov (2020), 80.4% of the Chenopodiaceae species have been studied karyologically. The data about the chromosome number of the studied species has been systematized in Chromosome atlas of the Bulgarian vascular plants (Petrova and Vladimirov 2020).

The karyological review showed that until 2021 data about the chromosome number and the karyotype of *Beta vulgaris* subsp. *maritima*, *Camphorosma annua*, *C. monspeliaca*, *Ceratocarpus arenarius*, *Halimione pedunculata*, *H. portucaloides*, *Polycnemum arvense*, *P. heuffelii*, *P. majus*, and *Spinacia oleracea* from their Bulgarian populations was lacking.

## Phytochemical studies of Chenopodiaceae in Bulgaria

According to the Medicinal Plants Act (2000), 11 species of Chenopodiaceae are considered as medicinal plants: *Atriplex rosea*, *Blitum bonus-henricus*, *B. virgatum*, *Camphorosma monspeliaca*, *Chenopodium album* subsp. *album*, *C. vulvaria*, *Dysphania botrys*, *Lipandra polysperma*, *Oxybasis rubra*, *Salicornia europaea*, *Salsola tragus*.

Data on the phytochemical composition of medicinal plants of the family Chenopodiaceae so far are known from the Bulgarian populations of two species, i.e. *B. bonus-henricus* and *B. virgatum*.

The phytochemical investigations of genus *Chenopodium* revealed many compounds with a vast variety of structural patterns. The chenopods contained minerals, primary metabolites- carbohydrates, amino acids, nonpolar constituents, proteins, aromatic cytokinins, hormones and secondary metabolites – flavonoids, saponins, terpenes, sterols, alkaloids and vitamins (Nedialkov and Kokanova-Nedialkova 2020).

The aerial parts of *Blitum. bonus-henricus* are a rich source of bioactive compounds. Among them thirty-six compounds were distinguished including twenty-two saponins of eight sapogenins (phytolaccagenin, bayogenin, medicagenic acid,  $2\beta$ -hydroxygypsogenin,  $2\beta$ -hydroxyoleanoic acid, 2-hydroxy-30-nor-gypsogenin, 2-hydroxyakebonic acid and akebonic acid), twelve flavonoid glycosides of 6-methoxykaempferol, isorhamnetin, patuletin, spinacetin and two ecdysteroids (20-hydroxyecdysone and polypodine B). It was reported that glycosides of spinacetin and patulenin were the predominant compounds in the aerial parts of *C. bonus-henricus* (Kokanova-Nedialkova et al. 2020; Kokanova-Nedialkova and Nedialkov 2021). The occurrence of sapogenins 2-hydroxy-30-nor-gypsogenin, 2-hydroxyakebonic acid, and akebonic acids was also detected. The flavonoid and saponin-rich fractions showed in vitro hepatoprotective and antioxidant activity comparable to those of flavonoid complex silymarin (60 µg/mL) in a model of metabolic bioactivation, induced by CCl4. (Kokanova-Nedialkova et al. 2020). Nine

flavonol glycosides of patuletin, 6-methoxykaempferol and spinacetin were reported to reduce lipid damage, showed antioxidant activity, neuroprotective, anti-α-glucosidase, and lipase activities and possessed no toxic on the HepG2 cell line (Kokanova-Nedialkova and Nedialkov 2021). B. bonus-henricus possessed emollient, laxative, anthelmintic, antianemic and vermifuge properties. It is used also as expectorant and for the treatment of inflammated wounds (Kokanova-Nedialkova et al. 2016; Kokanova-Nedialkova et al. 2009). The MeOH extract (60 µg/ml) of the aerial parts of C. bonus-henricus showed hepatoprotective and antioxidant activities comparable to those of flavonoid complex silymarin in an in vitro model of metabolic bioactivation, induced by tetrachloromethane. Along with the decreased MDA quantity and increased level of GSH, seven days pre-treatment of rats with the MeOH extract (100 mg/kg/daily) also prevented the tetrachloromethane-caused oxidative damage by increasing antioxidant enzyme activities (CAT, SOD, GPx, GR and GST) (Kokanova-Nedialkova et al. 2016). Extracts from roots of C. bonus-henricus L. radix showed radical scavenging activity evaluated by DPPH assay as IC50 was above 200 µg/ml (Nikolova et al. 2011).Moreover, ecdysteroids (especially 20-hydroxyecdysone, derivatives of makisterone A and polypodine B), triterpene saponins (3-O-β-**D**-glucopyranosyl-phytolaccagenin-28-α-**L**-arabinopyranosyl ester, 3-O-Bglucuronopyranosyl-bayogenin-28-O-B-glucopyranosyl ester, 3-O-B-glucopyranosylbayogenin-28-α-L-arabinopyranosyl ester, 3-O-β-glucuronopyranosyl-medicagenic acid-28- $\beta$ -xylopyranosyl(1 $\rightarrow$ 4)- $\alpha$ -rhamnopyranosyl(1 $\rightarrow$ 2)- $\alpha$ -arabinopyranosyl ester), 6-methoxyflavonol glycosides(6-methoxykaempferol 3-O-[ $\beta$ -apiofuranosyl(1 $\rightarrow$ 2)]- $\beta$ glucopyranosyl( $1\rightarrow 6$ )- $\beta$ -glucopyranoside), flavonoid glycosides (spinacetin 3-O-[ $\beta$ apiofuranosyl $(1\rightarrow 2)$ ]- $\beta$ -glucopyranosyl $(1\rightarrow 6)$ - $\beta$ -glucopyranoside and spinacetin 3-O-gentiobioside) were found in the roots of B. bonus-henricus (Kokanova-Nedialkova et al. 2009; Kokanova-Nedialkova et al. 2013; Kokanova-Nedialkova et al. 2015). Spinacetin 3-O-gentiobioside possessed stronger DPPH and ABTS radical scavenging activity (IC\_{50} 0.44 \pm 0.008 mM and 0.089 \pm 0.002 mM) (Kokanova-Nedialkova et al. 2015). Fifteen saponins of six sapogenins were identified in roots of this plant, as saponins bonushenricoside A, 3-O-B-D-glucuronopyranosylbayogenin-28-O-B-Dglucopyranosyl ester, 3-O-\beta-Dglucuronopyranosyl-medicagenic acid-28-O-β-Dxylopyranosyl  $(1 \rightarrow 4)$ - $\alpha$ -L-rhamnopyranosyl $(1 \rightarrow 2)$ - $\alpha$ -Larabinopyranosyl ester, 3-O-β-D-glucuronopyranosyl2β-hydroxygypsogenin-28-O-β-D-glucopyranosyl ester (4), 3-O-α-L-rabinopyranosyl-bayogenin-28-O-β-Dglucopyranosyl ester and bonushenricoside B deminstrateds possessed antioxidant and hepatoprotective effect (Kokanova-Nedialkova et al. 2019). Due to their root bioactive compounds infusions from them were used for the treatment of bronchitis, laryngitis, rheumatism, gout, constipation, dermatitis, eczema (Kokanova-Nedialkova et al. 2013). Moreover, the hepatoprotective activity of MeOH extracts from the roots of C. bonus-henricus as reported (Kokanova-Nedialkova et al. 2013). The glycosides of phytolaccagenin, bayogenin, medicagenic acid, 2\beta-hydroxygypsogenin, 2\beta-hydroxyoleanoic acid and oleanoic acid are a promising and safe class of hepatoprotective agents (Kokanova-Nedialkova et al. 2019). In plant material of *B. bonus-henricus* also sterols and vitamins were detected, especially vitamin C (Kokanova-Nedialkova et al. 2009).

Preliminary phytochemical screening of the aerial parts of *Blitum virgatum* shows the presence of carbohydrates, flavonoids, phytosterols, saponins and alkaloids/amins. Moisture content (6.05%) and total ash (12.19%) of aerial parts were also determined (Kokanova-Nedialkova et al. 2014a). The main secondary metabolites found in B.virgatum were terpenes (Kokanova-Nedialkova et al. 2009). This group of monocyclic hydrocarbon monoterpenoids includes limonene,  $\alpha$ -terpinene and its  $\gamma$ -isomer,  $\alpha$ -terpinolen, β-phellandrene and three related derivatives that were found in this plant. It contains also aromatic monoterpenoid p-cymene, carvacrol, tymol,  $\alpha$ -pinene and  $\beta$ -pinene, camphor and camphene, monocyclic sesquiterpenoids as β-caryophyllene (Kokanova-Nedialkova et al. 2009) and the oleanane triterpene 30-normedicagenic acid (Kokanova-Nedialkova et al. 2014b). The aerial parts of this plant also contain phenolic compounds. New flavonol glycosides were detected as 6-methoxy kaempferol-3-O-β-gentiobioside, gomphrenol-3-O- $\beta$ -gentiobioside and gomphrenol-3-O-[6-O-( $\beta$ -Dglucopyranosyl)- $\beta$ -D-glucopyranoside], as well as the already known compounds patuletin-3-O-\beta-gentiobioside and spinacetin-3-O-β-gentiobioside. The decoction prepared from C. foliosum was evaluated as a potential source of flavonoids with a radical-scavenging activity. The highest radical-scavenging activity evaluated by DPPH and ABTS methods were patuletin-3-O- $\beta$ -gentiobioside and 6-methoxykaempferol-3-O-ßgentiobioside, which were comparable with those of classic antioxidant ascorbic acid (Kokanova-Nedialkova et al. 2014a). The new acylated flavonol triglycoside namely gomphrenol-3-O-(5"-O-E-feruloyl)- $\beta$ -D-apiofuranosyl-(1 $\rightarrow$ 2)[ $\beta$ -Dglucopyranosyl- $(1\rightarrow 6)$ ]- $\beta$ -D-glucopyranoside was also identified in the aerial parts of the plant collected from Beglika, Western Rhodopes, Bulgaria. This compound demonstrated low activity radical scavenging activity evaluated by DPPH and ABTS radicals and lack of antioxidant activity evaluated FRAP assay and inhibition of lipid peroxidation (LP) in the linoleic acid system by the ferric thiocyanate method. However, in combination with CCl4, gomphrenol-3-O-(5<sup> $\circ\circ\circ$ </sup>-O-E-feruloyl)- $\beta$ -D-apiofuranosyl-(1 $\rightarrow$ 2)[ $\beta$ -D-glucopyranosyl- $(1\rightarrow 6)$ ]- $\beta$ -D-glucopyranoside reduced the damage caused by the hepatotoxic agent and preserved cell viability and GSH level, decreased LDH leakage and reduced lipid damage at the highest concentration  $(100 \ \mu\text{g/mL})$  (Kokanova-Nedialkova et al. 2014b).

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