Rumex pulcher (*Polygonaceae*) in the Bulgarian flora: distribution, morphology, and karyology

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Abstract. Of the four subspecies of *Rumex pulcher* distributed in Europe, three occur in Bulgaria. The type subspecies *pulcher* is widely distributed in the country, while *R. pulcher* subspp. *woodsii* and *raulinii* are registered in the more southern parts of the country with Mediterranean climatic conditions. We have applied a set of classical (morphological and karyological) and modern (genome size measurements and scanning microscopy) methods to identify the taxonomically most reliable features of the three taxa. An identification key for the three subspecies is presented. The chromosome number of the three subspecies is $2n = 20$. Their genome size (1C-values) varies between 0.76 and 0.88 pg.

Key words: Bulgaria, genome size, karyology, morphology, *Rumex pulcher*, scanning microscopy

Introduction

Rumex pulcher L. is a polymorphic species represented in Europe by four subspecies (Akeroyd 1993). According to this author, *R. pulcher* L. subsp. *pulcher* and *R. pulcher* subsp. *woodsii* (De Not) Archang. are distributed in Bulgaria. Prior to Vulev (1966), no subspecific taxa have been recognized in *R. pulcher* for the Bulgarian flora (Stojanov & Stefanov 1924, 1933, 1948; Stojanov & al. 1966). In the latest Bulgarian floristic literature (Delipavlov 2003), *R. pulcher* subsp. *raulinii* (Boiss.) Rech. f. is also reported for the national flora. Our field studies confirm the three subspecies for the territory of Bulgaria.

Rumex pulcher subsp. *pulcher* is widely distributed across the country and often behaves as a weed. The remaining two subspecies are of more restricted distribution in areas with clearly expressed Mediterranean climatic conditions.

Different authors have treated the morphological variation of *R. pulcher* at different taxonomic ranks (Hayek 1924; Rechinger 1932; Cullen 1967). We present here the results of a comparative morphological study of the three subspecies distributed in Bulgaria, including scanning microscopy of the valves and fruits.

The karyology of *R. pulcher* s.l. has been studied by different authors from various geographic regions (Table 1). Most of the counts, including earlier ones from Bulgaria (Stoeva 1987; Raycheva 2005), show a diploid chromosome number $2n = 20$. Only Dahlgren & al. (1971) and Garcia & al. (1989) have reported a tetraploid number for *R. pulcher* subsp. *woodsii* from Spain.

Table 1. References on the chromosome counts for the subspecies of *R. pulcher* distributed in Bulgaria.

Taxon	2n	Origin	Reference
R. pulcher subsp. *pulcher*	20	Turkey	Degraeve (1975)
	20	Bulgaria	Stoeva (1987)
	20	Central Europe	Löve & Löve (1961)
	20	Spain	Garcia & al. (1989)
	20	Sweden	Löve (1967)
	20	Denmark	Ichikawa & al. (1971)
	20	California	Löve (1986)
	20	Japan	Himi & al. (1999)
	20	Greece	Baltisberger (1991)
	20	Bulgaria	Raycheva (2005)
R. pulcher subsp. *woodsii*	20	Albania	Strid (1971)
	40	Spain	Dahlgren & al. (1971)
	40	Balearic Islands	Garcia & al. (1989)
	20	Bulgaria	Raycheva (2005)
	20	Italy	Löve (1967)
R. pulcher subsp. *raulinii*	20	Greece	Löve (1967)
	20	Bulgaria	Raycheva (2005)

In the last decades, estimation of the amount of DNA contained in an organism's chromosome complement (C-value) and also, in polyploids, in the constituent chromosome sets (Cx-value), has proven to be a reliable taxonomic tool, as the closely related species or subspecific entities can measurably differ in their genome size (Bennett & al. 2000; Gregory 2005). Genome size is measured with flow cytometry and Feulgen densitometry. Some plant species pose significant technical problems caused by protein- and DNA-binding endogenous metabolites (Greilhuber 1986). The representatives of subgenus *Rumex* are known to have such compounds in their cells. So far the DNA content of *R. pulcher* has not been measured (Bennett & Leitch 2004).

To our knowledge, no data on the microscopic characteristics (SEM electron microscopy) of the valves of *R. pulcher* have been published so far. Taking into consideration that valves provide a number of taxonomically reliable morphological features, we present data about the surface of the valves and the tuberculae.

The present study of *R. pulcher* s.l. provides data for a clear delimitation of subspp. *pulcher*, *woodsii* and *raulinii* in the Bulgarian flora.

Material and methods

The herbarium collections of *R. pulcher* s.l. in the three national herbaria (SOM, SOA and SO) have been studied. For the karyological studies, genome size estimation and scanning microscopy, plant material has been collected from 15 natural accessions in Bulgaria, in the period 2003–2005 (Table 2). Voucher specimens were deposited in the herbarium SOA (Agrarian University, Plovdiv). The chorological information has been processed according to Kozhuharov & al. (1983), mapped and included in a data base with the help of dSOA software (Stoyanov 2003). The floristic regions follow the standard accepted in *Flora R Bulgaria*.

Plant material collected by the authors has been used for scanning microscopy (Table 2). The objects have been observed directly, without any physical or chemical treatment. The valves have been prepared for observation prior to scanning, following the methodology of Terziisky & Atanasov (1977) and Terziisky (1983). The observations have been conducted with a JEOL electron scanning microscope, with scanning adaptor JSM-5500.

A classical squash technique has been applied for the karyological studies. Root tips were pretreated with 0.05 % colchicine for 2 hours, fixed in ethanol:glacial acetic acid (3:1) for at least 2 h at room temperature or for 24 h in the refrigerator, and stored in 96 % ethanol until required. Hydrolysis was conducted in 1N HCl at 60 °C for 20–40 min. Then the root tips were transferred into HCl:di-ethyl ether (1:1) for 9 min at 60 °C, washed thoroughly in distilled water and stained with haematoxylin after Gomori (Melander & Wingstrand 1953) for 20–25 min at 60 °C. Finally, the root tips were squashed in 45 % acetic acid and mounted in Canadian balsam.

Nuclear C-values of *R. pulcher* were investigated by the two available methods: flow cytometry and Feulgen densitometry.

The nuclei isolation procedure for flow cytometry was based on Galbraith & al. (1983). Young leaves of *Hordeum vulgare* 'Ditta' (1C = 5.02 pg DNA, Doležel & al. 1998) served as internal standard. After preparation of the nuclei suspension and its incubation with propidium iodide (PI) as described in Baranyi & Greilhuber (1996), the measurement was conducted on a Ploidy Analyser II (PAII, Partec, Münster, Germany) equipped with a mercury lamp and the appropriate filter combination (TK560, EM520). Depending on the availability of the plant material, one to three individuals were measured from every accession. For each individual and isolate, three runs of 5000 counts each were measured. Coefficients of variation of G_1 peaks of *R. pulcher* and *H. vulgare* mostly did not exceed 3 %.

Table 2. List of accessions studied and methods applied to *R. pulcher* s.l. in Bulgaria.

No	Taxon, locality, UTM, voucher number	Methods applied
R. pulcher* subsp. *pulcher		
1	Black Sea Coast (*Southern*): grassy places around Sozopol, 20 m, 03.07.2004; NG-59 (*Raycheva*)	K, Map, M
2	Black Sea Coast (*Southern*): Kraimorie village, 20 m, 23.06.2003, NG-67 (*Raycheva*)	Fl, Fe, Map, M
3	Danubian Plain: Somovit village, Nikopol district, 50 m, 25.06.2003, LJ-24 (*Raycheva*) SOA 56363	K, Map, M
4	Forebalkan (*West*ern): hay meadows along the left bank of Bela Reka River, near Dolna Bela Rechka village, Montana district, 400 m, 27.6.2002, FN-98 43,1218 N, 23,1931 E, (*Dimitrova*)	Fl, Fe, Map, M
5	Rhodopi Mts (*Eastern*): near Ivailovgrad – Doupkata Protected Area, 104 m, 14.07.2005, MF-29 (*Raycheva*)	SEM, Map, M
6	Rhodopi Mts (*Eastern*): village Odrintsi, 194 m, 15.07.2005, MF-28 (*Raycheva*)	Fe, Map, M
7	Thracian Lowland: near Pyasuchnik dam, 300 m, 22.06.2003, KG-99 (*Raycheva*) SOA 56407	Fl, Fe, K, Map, M
R. pulcher* subsp. *woodsii		
8.	Black Sea Coast (*Southern*): light oak forests near Sinemorets village, 40 m, 03.07.2004, NG-85 (*Raycheva*) SOA 57068	Fl, Fe, K, Map, M
9*	Rhodopi Mts (*Eastern*): betwen Siv Kladenets and Odrintsi villages, 194 m, 15.07.2005, MF-28 (*Raycheva*) SOA 56931	Fl, Fe, Map, M
10*	Rhodopi Mts (*Eastern*): near Belopolyane village, in sunflower fields, 170 m, 15.07.2005; MF-38 (*Raycheva*) SOA 56929	SEM, Fe, Map, M
11*	Rhodopi Mts (*Eastern*): abandoned lands and pastures near Ivailovgrad, 160 m, 15.07.2005, MF-29 (*Raycheva*) SOA 56930	Fe, Map, M
R. pulcher* subsp. *raulinii		
12*	Black Sea Coast (*Southern*): rare oak forests near Sinemorets village, 40 m, 03.07.2004, NG-85 (*Raycheva*) SOA 57067	Fl, Fe, K, Map, M
13*	The Valley of Strouma River: Kresna town, 180 m, 18.06.2005, FM-72 (*Raycheva*) SOA 56926	K, Map, M
14*	Belasitsa Mts: between Skrut and Klyuch villages, 300 m, 18.06.2005, F-L68 (*Raycheva*) SOA 56927	SEM, Fl, Fe, Map, M
15*	Rila Mts: Dupnitsa town, above the road tunnels, 510 m, 19.06.2005, FM-78 (*Raycheva*) SOA 56928	K, Map, M

Fl – flow cytometry; **Fe** – Feulgen densitometry; **SEM** – scanning electron microscopy; **K** – karyological studies with classical haematoxylin method; **Map** – mapped locality; **M** – gross-morphological studies; * – plants from a new floristic region.

For quantitative Feulgen staining (Feulgen & Rossenbeck 1924), seedlings of *R. pulcher* were co-fixed in 4 % neutral formaldehyde solution, together with primary root tips of the standard organism *Pisum sativum* 'Kleine Rheinländerin' (1C = 4.42 pg DNA, Greilhuber & Ebert 1994). These samples were post-fixed with methanol acetic acid (3:1) and afterwards stored in 96 % ethanol in the freezer until use. Following the preparation method of Greilhuber & Ebert (1994), rehydrated material was hydrolyzed in 5N HCl for 75 min at 20.0 °C (Greilhuber & Baranyi 1999; Greilhuber & Temsch 2001) and stained in Schiff´s reagent for 1.5 hours at room temperature. After washing out in SO_2-water, the plant tissue was squashed in 45 % acetic acid, briefly rinsed in 96 % ethanol, and finally air-dried. Three slide pairs of *R. pulcher* and the standard tissue were produced in parallel per accession. For each slide, 20 $G_{1/0}$ or 10 telophase plus 10 prophase nuclei were measured. We have used the Cell Image Retrieval and Evaluation System (CIRES, Kontron, Munich, Germany) for measurement.

Results and discussion

Chorology and ecology

Rumex pulcher subsp. *pulcher* occurs in the Mediterranean region, Balkan Peninsula, Asia Minor, Hungarian lowlands, Atlantic West Europe. It has spread into Asia and got naturalized in America (Akeroyd 1993). In Bulgaria, the subspecies grows in open grassy habitats, along roads, and as a weed in agricultural phytocoenoses across the country, from 0 up to 1500 m a.s.l. (Fig. 1, Table 3).

Rumex pulcher subsp. *woodsii* is abundant and often occurs as a ruderal plant in the Aegean region, SW Asia and the central part of East Mediterranean. The subspecies has been introduced in Central and West Europe and different parts of America and South Africa. In Bulgaria, the taxon has been reported in literature from the Black Sea Coast (*Southern*), Balkan Range (*Eastern*) and Toundzha Hilly Country. In the course of the current study, the subspecies was confirmed for the Black Sea Coast (*Southern*), and registered for the first time

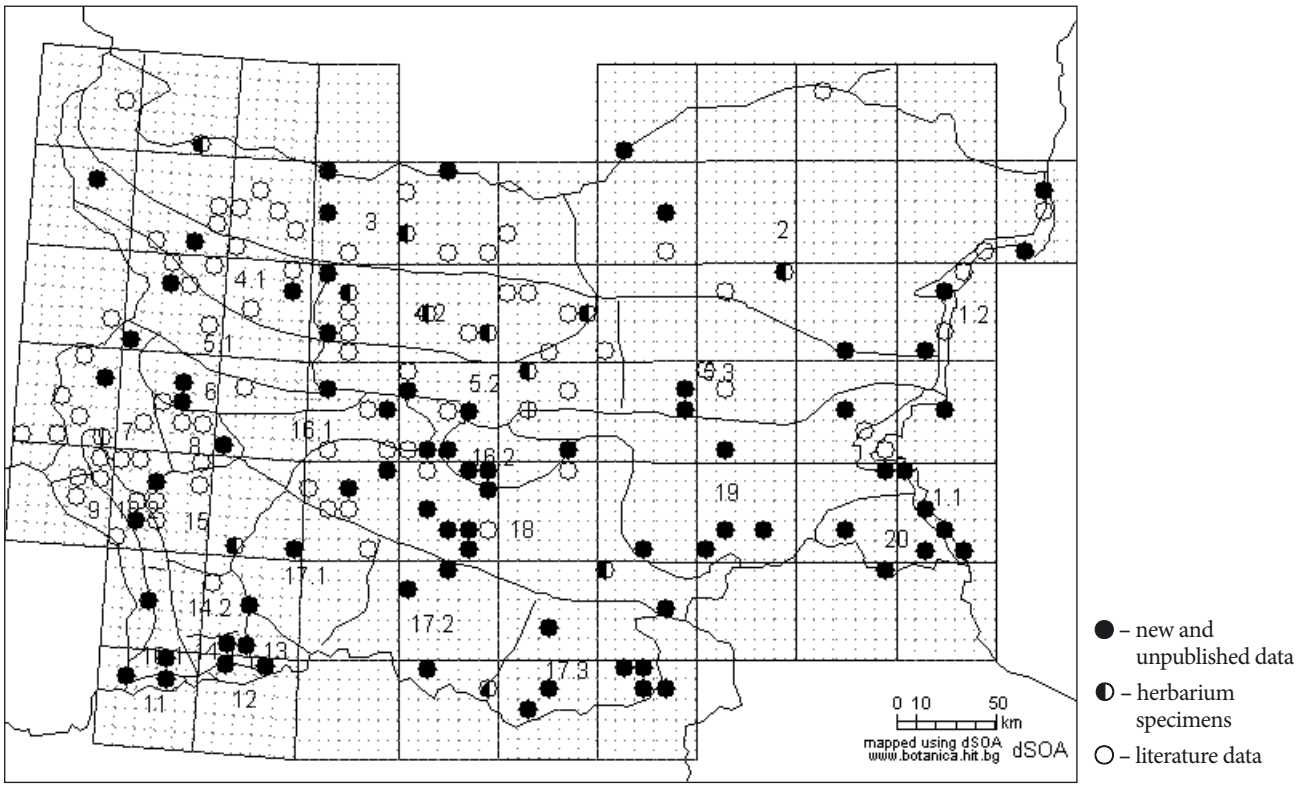


Fig. 1. Distribution of *R. pulcher* subsp. *pulcher* in Bulgaria.

Note: This figure includes also data on *R. pulcher* s.l. from the Bulgarian floristic literature prior to Vulev (1966), where the subspecific variation has not been considered.

Table 3. Chorological data on *Rumex pulcher* s.l. in Bulgaria.

Literature sources		Herbarium specimens	
Floristic regions	**Altitude**	**Floristic regions**	**Altitude**
R. pulcher* L. subsp. *pulcher			
1.1 (Urumov 1908b); **1.2** (Urumov 1901; Davidov 1905; Rechinger 1932, 1933); **2** (Urumov 1901; 1905a; Kovachev 1903; Rechinger 1932; Stoeva 1987); **3** (Urumov 1898, 1901, 1902, 1910, 1917, 1925, 1926, 1928, 1935a); **4.1** (Velenovský 1898; Urumov 1898, 1901, 1905a, 1917, 1925, 1926, 1928, 1935a; Rechinger 1932, 1933); **4.2** (Urumov 1897, 1926, 1928); **5.1** (Urumov 1902, 1905b, 1935a); **5.2** (Urumov 1897, 1898, 1917, 1929b; Neichev 1908; Baev 1947); **5.3** (Velenovský 1891; Urumov 1909); **6** (Velenovský 1891; Urumov 1905b, 1906, 1910, 1929a, 1930, 1935b); **7** (Urumov 1905b, 1913, 1935b); **8** (Urumov 1929a, 1930); **9** (Urumov 1904, 1913, 1935b); **11** (Urumov 1905a); **14.1** (Urumov 1923); **15** (Urumov 1904, 1906); **16.1** (Urumov 1902, 1905a, 1908a, 1935b); **16.2** (Urumov 1904; 1910, 1929b); **17.1** (Urumov 1906, Rechinger 1932); **18** (Velenovský 1891; Urumov 1906, 1908b, 1917, 1929b; Rechinger 1932); **19** (Urumov 1909, 1910). Vulev (1966), Andreev (1992), and Delipavlov (2003) report the taxon for whole territory of the country	up to 2000 m	1.1; 1.2; 2; 3; 4; 4.2; 5.2; 5.3; 6; 7; 8; 9; **10***; 11; **12***; **13***; 14.1; 15; 16.1; 16.2; 17.1; **17.2***; **17.3***; 18; 19; **20***	up to 1100 m
R. pulcher* subsp. *woodsii			
1.1 (Panov 1987); **5.3** (Panov 1987); **19** (Panov 1987). Andreev (1992) and Delipavlov (2003) report the taxon for the Black Sea Coast, Balkan Range (*Eastern*) and Toundzha Hilly Country.	–	1.1; **17.3***	up to 200 m
R. pulcher* subsp. *raulinii			
Vulev (1966), Andreev (1992), and Delipavlov (2003) report the taxon for Black Sea Coast, Forebalkan (*Eastern*) and Thracian Llowland.	–	1.1; **10***; **11***; **15***	up to 500 m

1 – Black Sea Coast (**1.1** – *Southern*, **1.2** – *Northern*); **2** – NE Bulgaria; **3** – Danubian Plain; **4** – Forebalkan (**4.1** – *Western*, **4.2** – *Eastern*); **5** – Balkan Range (**5.1** – *Western*, **5.2** – *Central*, **5.3** – *Eastern*); **6** – Sofia region; **7** – Znepole region; **8** – Vitosha region; **9** – West Frontier Mts; **10** – The Valley of Strouma River (**10.1** – *Southern*, **10.2** – *Northern*); **11** – Mt Belasitsa; **12** – Mt Slavyanka; **13** – The Valley of Mesta River; **14** – Pirin Mts (**14.1** – *Southern*, **14.2** – *Northern*); **15** – Rila Mts; **16** – Mt Sredna Gora (**16.1** – *Western*, **16.2** – *Eastern*); **17** – Rhodopi Mts (**17.1** – *Western*, **17.2** – *Central*, **17.3** – *Eastern*); **18** – Thracian Lowland; **19** – Toundzha Hilly Country; **20** – Mt Strandzha; * – new data.

in the Rhodopi Mts (*Eastern*) (Fig. 2, Table 3). The subspecies occurs in secondary herbaceous plant communities, with *Lolium perenne* L., *Dactylis glomerata* L., *Aegilops triuncialis* L., *Alopecurus myosuroides* Huds., *Cirsium* sp., *Filago vulgaris* Lam., *Plantago lanceolata* L., *Eryngium campestre* L., *Hypericum perforatum* L., *Daucus guttatus* Sm., and *Potentilla pedata* Willd.

Rumex pulcher subsp. *raulinii* is distributed in the Aegean region. The subspecies has been reported in the Bulgarian literature from the Black Sea Coast, Forebalkan, Balkan Range (*Central*), and Thracian Lowland. Our revision of the Bulgarian herbaria has revealed one specimen belonging to *R. pulcher* subsp. *raulinii* (SV: coll. *B. Davidov*, SOM 17832, in humidis Deli Orman, July 1903) that had been first determined as *R. palustris*, and subsequently revised by B. Achtarov as *R. pulcher*. We have confirmed the taxon for the Black Sea Coast (*Southern*), and discovered it for the first time in the Valley of River Strouma, Mt Belasitsa, and Rila Mts (Fig. 3, Table 3). It occurs seldom in thin oak forests (*Quercus cerris* L., *Q. frainetto* Ten.), accompanied by *Briza maxima* L., *Poa bulbosa* L., *P. sylvicola* Guss., *Bromus tectorum* L., *Lolium perenne* L., *Festuca pratensis* Huds., *Cistus incanus* L., and *Cynosurus cristatus* L.

Morphological characteristics

Rumex pulcher s.l. shows a typical tendency to xerophytization, e.g, formation of fleshy tubers on the main root, reduction in the leaf size, distinctly fiddle-shaped leaves, and indumentum on stems and leaves. These features appear in all three subspecies but are most clearly expressed in the typical one.

Rumex pulcher is a perennial plant. In *R. pulcher* subsp. *woodsii*, tubers can be frequently found on the main root. Typical for this taxon is the fiddle-like shape of the basal leaves, but our observations have shown that this cannot be used as a diagnostic feature on suspecific level, since it is regularly found in the three subspecies distributed in Bulgaria. This is contrary to Rechinger (1932), according to whom the fiddle-shaped leaves distinguish *R. pulcher* subsp. *woodsii* from *R. pulcher* subspp. *pulcher* and *raulinii*. The leaf and stem indumentum varies considerably in the three taxa. It is mostly papillose, and seldom (in subsp. *woodsii*) consists of unicellular trichomes. The stem height also varies between the subspecies but in general the typical subspecies has shorter, flexuose, often intricate stems, while *R. pulcher* subsp. *woodsii* has high stems with long lateral divaricate branches.

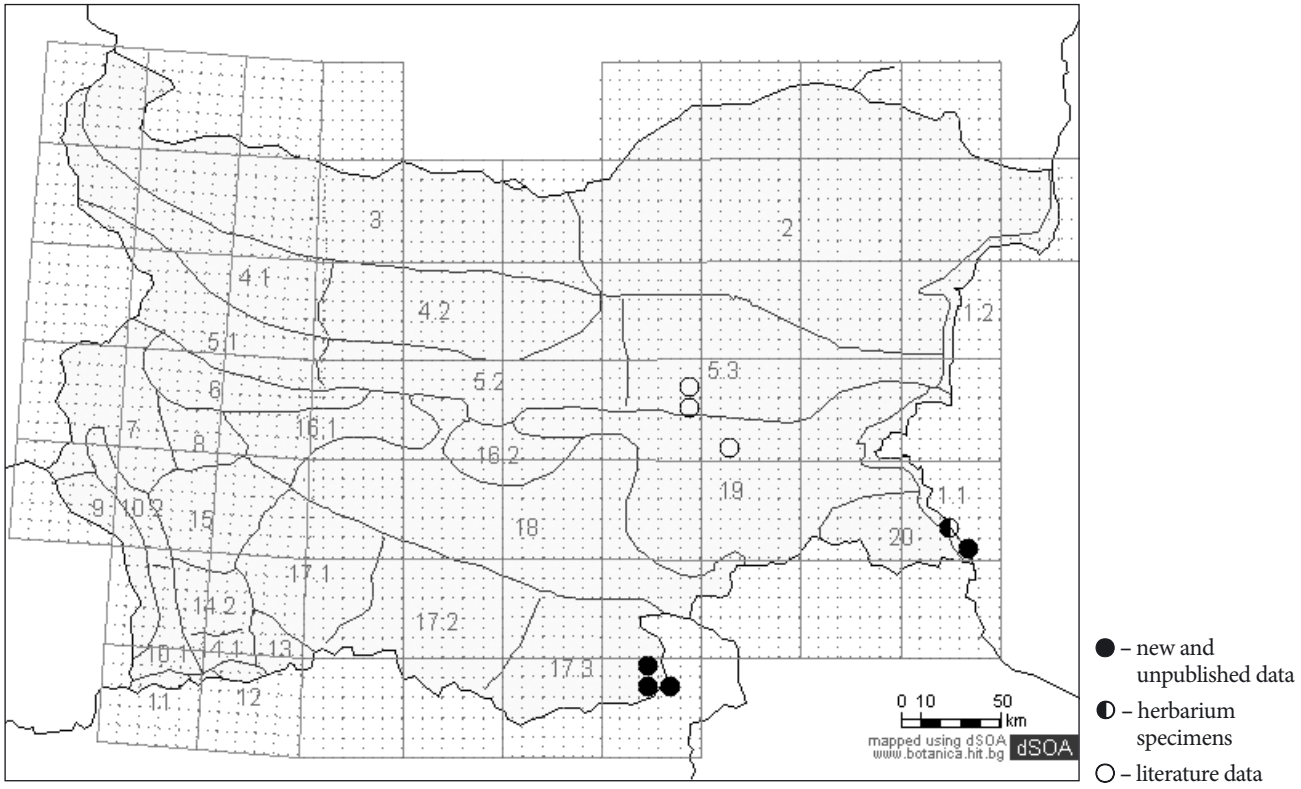


Fig. 2. Distribution of *R. pulcher* subsp. *woodsii* in Bulgaria.

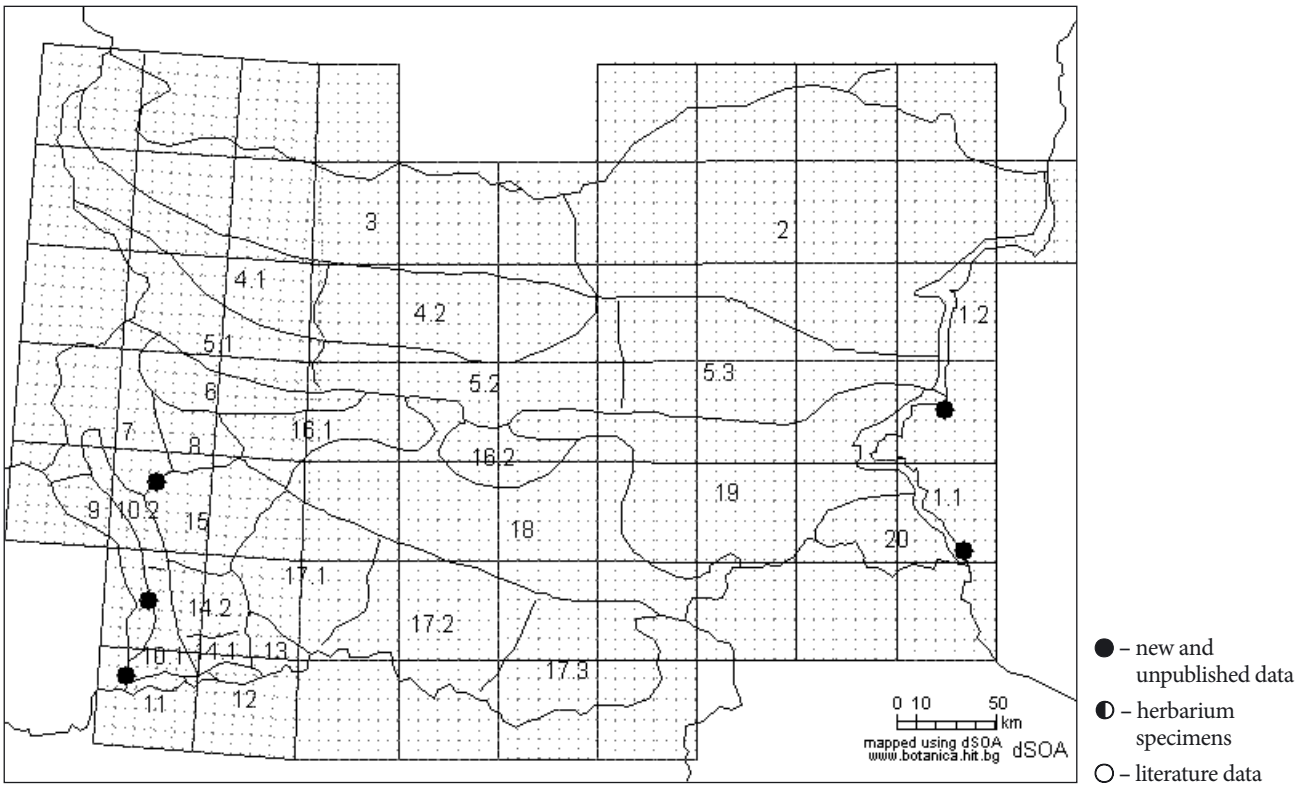


Fig. 3. Distribution of *R. pulcher* subsp. *raulinii* in Bulgaria.

Our observations have shown that most of the taxonomically reliable morphological features for subgenus *Rumex* can be found in the valves of the mature plants (length/width of valves, tuberculae and ochreae, size and number of spines) (Table 4).

Table 4. Morphological parameters of the valves of *R. pulcher* s.l.

Character	Taxon		
	subsp. *pulcher*	subsp. *woodsii*	subsp. *raulinii*
	x±Sx (mm)	x±Sx (mm)	x±Sx (mm)
Valve length	4.73±0.02	5.46±0.03	5.34±0.03
Valve width	3.00±0.03	4.65±0.03	2.96±0.02
Tubercule length	2.38±0.03	2.81±0.02	2.48±0.03
Tubercule width	1.18±0.01	1.55±0.01	1.13±0.02
Ochrea length	2.33±0.03	2.84±0.02	2.00±0.02
Ochrea width	1.46±0.02	1.84±0.02	1.50±0.02

The identification key given below is based on morphological features that have proven to be most discrete and reliable for identification of the three subspecies. The data related to plant habitus, morphology of basal leaves and indumentum type are considered reliable for the purpose and are not used, in contrast to Akeroyd (1993).

Identification key

1 Valves clearly dentate, oblong to narrowly ovate, 4.5–5.5 mm long, 3(–3.4) mm wide, with 4–6 irregular spines longer than 1 mm; branches short, flexuose, often intricate……**2**

1* Valves slightly dentate, rounded in shape, 5.2–6 mm long, 4.2–5.2 mm wide, with 6–8 almost regular 0.5–0.8 mm long spines; branches long, divaricate……**subsp. *woodsii***

2 Some spines 3–3.5 mm long ……**subsp. *raulinii***

2* Some spines 1.5–2 mm long ……**subsp. *pulcher***

Scanning microscopy supports the significance of the valves morphology for identification of the three subspecies. Furthermore, the surface of the tuberculae also shows some differences that correspond to the subspecific taxonomic concept. In *R. pulcher* subsp. *raulinii* their structure is more robust and papillose as compared to the other two taxa.

The surface of the valves is similar in all three subspecies, but when compared to other species in subgenus *Rumex,* their thick and robust venation outlines

a clear tendency towards xerophytization. In *R. pulcher* subsp. *woodsii* the surface of the valves is larger and spines are shorter (0.4–0.8 mm). In the remaining two subspecies the valves are narrower and elongated, while the spines are longer and more robust, reaching 2.8–3.2 mm and exceeding the valve width.

The tuberculae are usually three in number, only in *R. pulcher* subsp. *woodsii* two of them are reduced to nodes. The surface of the valve tuberculae is irregularly concave-undulate, but distinctly differs in the three taxa. Most clearly differentiated are the concave structures in *R. pulcher* subsp. *raulinii,* where the pits are strongly undulated (Plate I, 3b), while in the remaining two subspecies the pit surface is smooth. The difference in valve architecture correlates with the gross-morphology of the three taxa and their ecological characteristics. *Rumex pulcher* subsp. *pulcher*, which shows the strongest tendency to xerophytization, has the smallest concave structures with irregular shape as compared to the remaining two subspecies.

Karyology and genome size variation

Further studies (in addition to Raycheva 2005) on the karyology of *R. pulcher* in Bulgaria have shown stable chromosome numbers $2n = 2x = 20$ for the three subspecies (Fig. 4).

Apart from classical karyology, we have applied flow cytometry and Feulgen densitometry to test for nuclear C-value variation among the Bulgarian subspecies of *R. pulcher*. An earlier comparison with the Feulgen stained samples fixed in formaldehyde or methanol acetic acid (3:1) revealed the presence of secondary metabolites in the present material (Fig. 5). Such substances are known as inhibitors of DNA staining (Greilhuber 1986, 1988), but susceptibility of the two approaches (flow cytometry and Feulgen densitometry) to that problem is different. A strong influence of inhibitors can often be demonstrated, when results with flow cytometry and Feulgen densitometry diverge widely. However, in our investigation we found good data agreement, when formaldehyde fixed material was used for Feulgen densitometry.

Fig. 4. Metaphase plate of *R. pulcher* subsp. *woodsii*. Scale bar = 5 μm.

The mean 1C-values were 0.76 pg DNA in *R. pulcher* subsp. *woodsii*, 0.83 pg DNA in *R. pulcher* subsp. *raulinii*, and 0.82 pg DNA in *R. pulcher* subsp. *pulcher*. The C-value of *R. pulcher* subsp. *woodsii* was approx. 7 % lower than the values of the other two subspecies (Table 5). This difference underlines the more specific morphological syndrome of *R. pulcher* subsp. *woodsii* and its more divergent position within *R. pulcher* s.l.

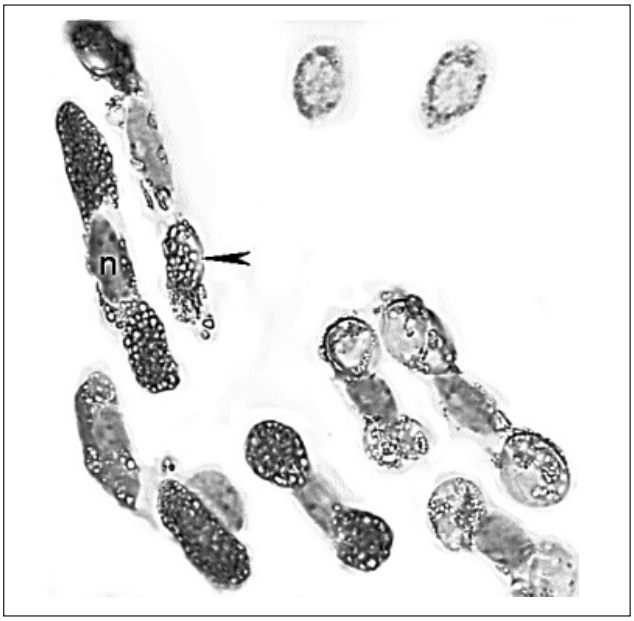
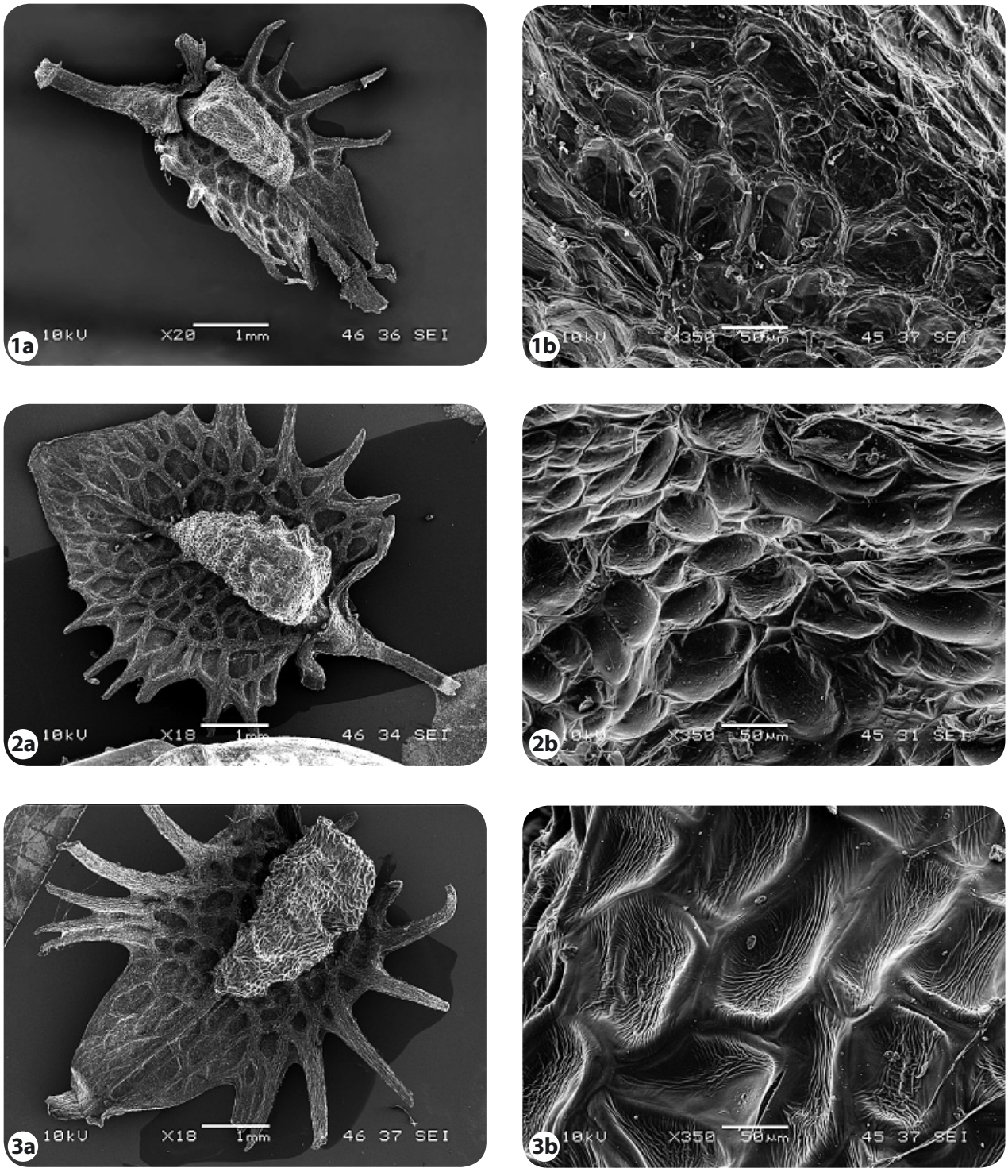


Fig. 5. Root-tip cells of *R. pulcher*; fixation in 4 % formaldehyde, Feulgen staining. Arrowhead shows polymerized secondary compounds (probably condensed tannins) in many cells and (n) shows regular staining of nuclei.

Table 5. Comparative DNA data obtained by flow cytometry and Feulgen densitometry. Locality numbers follow the sequence in Table 2.

Locality No	Flow cytometry pg/1C±S.D	Feulgen densitometry pg/1C±S.D
R. pulcher L. subsp. pulcher		
2	0.80±0.02	0.83±0.05
4	0.79±0.02	0.81±0.04
6	–	0.81±0.03
7	0.79±0.02	0.88±0.08
R. pulcher subsp. wodsii		
8	0.76±0.01	0.76±0.06
9	–	0.70±0.06
10	–	0.81±0.04
11	–	0.81±0.09
R. pulcher subsp. raulinii		
12	0.80±0.02	0.82±0.03
14	–	0.85±0.004

Plate I



SEM of valves and ochreae surface of the three subspecies of *R. pulcher*: 1a, 1b, subsp. *pulcher*; 2a, 2b, subsp. *woodsii*; 3a, 3b, subsp. *raulinii*.

Conclusions

The biosystematic study of *R. pulcher* in Bulgaria has confirmed the existence of three subspecies in the national flora: *R. pulcher* subspp. *pulcher*, *woodsii* and *raulinii*. Morphological studies and genome size estimations have confirmed the more divergent position of *R. pulcher* subsp. *woodsii* in the plant group.

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