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CHROMOSOME COUNTS IN SOME BULGARIAN TAXA OF SUBGENUS *RUMEX* (*POLYGONACEAE*)

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ABSTRACT. The chromrsome number of 30 Bulgarian populations referring to 12 species and 3 subspecies of the subgenus *Rumex* has been estabilished. Some taxa investigated – *R. alpinus, R. conglomeratus, R. sanguineus, R. rulcher* spp. *pulcher, divaricatus* and *raulinii* are diploid with 2n=20; *R. obtusifolius*, *R. palustris*, *R. maritimus* and *R. stenophyllus* are tetraploid with 2n=40; *R. crispus* and *R. patientia* are hexsaploid with 2n=60; *R. cristatus* are octoploid with 2n=80 and *R. hydrolapathum* are 20-ploid whit 2n=200.

Unlikely most of the existing data in which *R. stenophyllus* Ledeb. is reported as hexaploid the Bulgarian accessions of this species are tetraploid. *Rumex conglomeratus* has been considered as tetraploid but Bulgarian populations are diploid. The results of the current study for *R. palustris* differ from the counts from other origin which show hexaploid chromosome number.

The lower ploidy levels (up to 2n=6x) correlate with a potential for wider distribution and adaptation to different ecological conditions. The octoploid and the 20-ploid taxa are of more restricted distribution and narrower ecological range.

The different ploidy levels, the wide range of ecological plasticity, the high reproductive and hybridizing potential explain the wide distribution of this group far beyond its natural areals.

KEW WORDS: caryology, *Rumex*, *Polygonaceae*, chromosome counts, distribution.

INTRODUCTION

The representatives of the polymorph and large subgenus *Rumex* are unevenly studied karyologically. The biggest number of polyploids in the whole genus *Rumex* are found in this group - 2n=20,40, 60, 80, ca. 200; x= 10 (Ichikawa et al. 1971, Degraeve 1975). The high morphological variability makes difficult the clear

discretion of the morphological syndromes. The difference in the ploidy level relates to the specificities of the taxa. Therefore the ploidy level and geographic distribution are very much significant for the identification of taxa. The subgenus comprises taxa with different phytogeography. Some of them have very high distribution potential and are distributed all over the world (*R. crispus* L. and *R. conglomeratus* Murray) and sometimes is difficult to make difference between the primary and secondary areas of distribution. Others are of restricted distribution and grow in very specific ecological conditions (Rechinger, 1949). In Europe 4 species and 2 subspecies are endemic to the continent. Very often more than one species can be found in one and the same locality which is a prerequisite for very active natural interspecific hybridization.

According to the most recent chorological literature (Delipavlov et al., 2003) 16 species of *Rumex* subgenus *Rumex* are distributed in the Bulgarian flora, of which 13 are perennials and three are annuals.

The chromosomes of subgenus *Rumex* are very small with unclear morphology. Therefore the karyological studies are restricted mainly to chromosome counts and the information about the karyotype features is very scanty. The karyological studies from different parts of the worlds shoe considerable variation in chromosome numbers. The investigations are mainly on the species with wide distribution, while species of local distribution are much less studied. There are single data about the locally distributed R. *kerneri* Borbas. (Degraeve, 1975) and R. *cristatus* DC. (Ichikawa et al., 1971).

In Bulgaria the docks have not been a subject to systematic karyological studies. The only chromosome reports are by (Stoeva 1985, 1987) who gives information about the chromosome number of four species from single localities.

MATERIAL AND METHODS

Thirty Bulgarian accessions of 12 species have been investigated (Table 1). The studies are based on plant collections and for one of the species - on seeds from a herbarium specimen in SO. Herbarium specimens of all studied accessions are deposited in SOA. The accessions are mapped using UTM-Grid coordinates and following the floristic regions accepted in Flora R Bulgaria (Kozhuharov at al. 1983).

Root tips were pre-treated with 0,05% colchicine for 2 hours at room temperature (18-20ºC) and subsequently fixed in ethanol:acetic acid (3:1). Then the root tips were hydrolized in 1n HCl at 60ºC for 9 minutes. The staining is with hamaetoxylin after Gomori (Melander & Wingstrand, 1953) for 20 minutes. When the staining was not satisfactory some of the root tips were additionally stained with 4% orcein. Permanent slides are prepared after dehydrating in butyl alcohol and xylol and mounted in Canadian balsam.

The somatic chromosome number is counted in at least 10 well spread metaphase plates for each accession.

The metaphase plates are photographed with digital camera Samsung V5 (3,2 M pixels). This photos are used as basis for the drawings.

RESULTS AND DISCUSSION

Of all 16 species distributed in Bulgaria 30 accessions of 12 species (ten perennials and two annuals) have been karyologically studied. It has been established diploids (2n=2x=20), tetraploids (2n=4x=40), two hexaploids (2n= 6x=60), one octoploid (2n=8x=80) and 20-ploid (2n=20x=200) (Table 2, figs. 1 and 2).

The diploid accessions belong to the perennial *R. alpinus* L., *R. conglomeratus* Murray, *R. sanguineus* L., *R. pulcher spp. pulcher, divaricatus* (L.) Murb. and *raulinii* (Boiss.) Rech. f. The diploid species are widely distributed. Some of them are have live in more specific ecological conditions while are others are more tolerant in respect to altitude and habitat. For instance *Rumex alpinus* is a synanthrop, nytrophille species distributed in the mountains of Europe, North Iran, and in Caucasus. It is also naturalized in North America. *Rumex sanguineus* inhabits light forest coenoses and grows in whole Europe (except for its most northern parts), North Africa, Central and South Russia, North Iran, North America. Most aggressive of the diploids is *R. conglomeratus*, who has large vertical tolerance (0 - 1800 m alt.), is widely distributed (Europe, North Africa, Asia, North America) and can be found in various habitats - ruderal habitats, along rivers, in meadows. For this species Menshikova (1964) reported a tetraploid chromosome number from Russian accessions. The chromosome number for the Bulgarian accessions (2n=2x=20, fig. 2e) coincides with the data in Degraeve (1975) and Löve (1967).

Rumex pulcher is a diploid species that grows in South and East Europe, Caucasus, South-West Asia, and is naturalized in North America and Australia. The species is highly polymorph and has four subspecies, three of which can be found in Bulgaria as well. The areal of ssp. *pulcher* is within the range of the species, ssp. *divaricatus* grows in East and South Mediterranean region and South-West Asia and ssp. *raulinii* is with more restricted distribution that includes Bulgaria, Greece and the Aegean region. The populations of the three subspecies can be found in sandy and grassy places, along rivers and the Black Sea coast, in anthropogenic and ruderal habitats.

The polyploids comprise a bigger group, eight species, and their distribution is more divers.

The annual species distributed in Bulgaria are tetraploid: *R. maritimus* L. (fig. 1b) and *R. palustris* Sm. *Rumex maritimus* was found in Bulgaria only (Dimitrov, 1997). Bulgaria is the southernmost range of its areal that Central Europe, Scandinavia, Japaneese – Chineese area, Mongolia, and Siberia. The species grows also in the Mediterranean region, including Africa and Morroco but is more scarce there. According to the data in the karyological literature the species is diploid with the exception of the Russian populations (Menshikova 1964).

Rumex palustris grows on the periphery of natural and artificial water basins in Europe, West Asia, the Mediterranean region, rarely in Africa. The Bulgarian populations are tetraploid (fig. 2f). These results coincides with the data in Löve (1942) and Degraeve (1975). Hexaploid chromosome number was reported from UK (Ichikawa et al., 1971).

Tetraploids are also the perennial species *R. obtusifolius* L. and *R. stenophyllus* Ledeb. The highly polymorph *R. obtusifolius* is with high morphological variation arranged in four subspecies. The species is distributed in Central Europe, the Balkans, Siria, Iran and is naturalized in East Asia, Africa and America. It has high reproductive potential and ruderal and subruderal characteristics. The four subspecies can be found in Bulgaria spp. *obtusifolius, subalpinus* (Schur) Čelac., *silvestris* (Wallr.) Rech. and *transiens* (Simonkai) Rech. f. The chromosome count from the Bulgarian accessions coincides with the data from the numerous karyological studies (fig. 1a) on accessions from different phytogeograpic regions (Mulligan 1959, Löve 1961, Ichikawa et al. 1971).

Rumex stenophyllus Ledeb. grows in open sunny places along water basins and in ruderal habitats in Central Asia, East and West Siberia, West Europe, rarely in Scandinavia, UK, the Netherlands. It is naturalized in America. Three ploidy levels have been established for this species. The most frequent one is the hexaploid chromosome number, 2n=60, but also diploid and tetraploid reported by Menshikova (1964). Bulgarian accessions are tetraploid, 2n=4x=40 (fig. 2a).

Hexaploids (2n=6x=60) are *R. crispus* L. and *R. patientia* L.

Rumex crispus is cosmopolitantly distributed in Europe, Asia and America in grassy places, along roads, often in ruderal communities and forms dense populations. The chromosome number from the Bulgarian localities coincide with the data from different phytogeographic regions (fig.1d).

The haxaploid *R. patientia* L. (fig.1f) is a polymorph species with three subspecies in its area of distribution. The species grows in South-East Europe, South-West Asia, the southern parts of Russia-in-Europe. The species has been cultivated as well, often behaves as ruderal and is naturalized in many parts of the world. The three subspecies can be found in Bulgaria: ssp. *patientia*, ssp. *recurvatus* (Rech.) Rech. f. and ssp. *orientalis* (Bernh.) Danser. The results from the Bulgarian localities coincide with the data from different phytogeographic regions.

The octoploid *R. cristatus* DC. (fig. 1e), 2n=8x=80 is a perennial species of restricted distribution: Balkan peninsula, Italy, Turkey, is reported from Hungary and is naturalized in Ukraine (Eremko, 1997). The chromosome number from Bulgaria corresponds with the results from UK reported by Ichikawa et al. (1971). Data from other phytogeographic regions are not available.

The highest chromosome number is this of *R. hydrolapathum* Huds. (fig. 2c) – a perennial species, distributed in most of Europe (secondary distribution in the Balkan peninsula), Caucasus, South-West Asia. The Bulgarian accessions have 2n=20x=200, which is one of the highest chromosome numbers registered for the genus as a whole. This number coincides with the data in Löve (1942) based on plants from Scandinavia (Table 2).

Following the areas of occurrence, the habitats and ploid levels of the species, belonging to the subgenus *Rumex* in Bulgaria, the impression is that the most aggressive ones are the perennial tetraploid *R. obtusifolius*, the hexaploid *R. crispus* and the diploid *R. conglomeratus*. The octaploids are local endemites with limited occurrence.

CONCLUSIONS

Fourteen taxa of subgenus *Rumex* native to Bulgaria have been studied karyologically. The chromosome numbers of eight species and three subspecies are reported for the first time from Bulgarian accessions. Eight of the studied taxa are polyploids and four are diploids. The tetraploid chromosome number of *R. maritimus* and *R. palustris* correlates with the annual biological type.

Different ploidy levels are registered among perennials - di-, tetra- and hexaploid levels.

Unlikely most of the existing data in which *R. stenophyllus* is reported as hexaploid the Bulgarian accessions of this species are tetraploid. *Rumex conglomeratus* has been considered as tetraploid but Bulgarian populations are diploid. The results of the current study for *R. palustris* (2n=4x=40) differ from the counts from other origin which show hexaploid chromosome number.

The lower ploidy levels (up to 2n=6x) correlate with a potential for wider distribution and adaptation to different ecological conditions. The octoploid and the 20-ploid taxa are of more restricted distribution and narrower ecological range.

The different ploidy levels, the wide range of ecological plasticity, the high reproductive and hybridizing potential explain the wide distribution of this group far beyond its natural areals.

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Table 1. Kariologically studied accessions of species from subgenus *Rumex*

Taxon	Floristic region*, UTM position, locality and voucher
R. alpinus L.	(8) UTM: 34TFN81, 1810 m alt., Vitosha mt., near Aleko chalet, Raycheva, 19.08.2003.
	(15) UTM:34TGM27, 1550 m alt., grassy places near Belmeken dam, Raycheva, 14.07.2003.
R. conglomeratus Murray	
	(17.2) UTM: 35TLG 25, 600 m alt., grassy places above Ruen village, Raycheva, 03.07.2003.
R. crispus L.	(3)UTM: 35TLJ01, 100 m alt., near Plevem, Raicheva, 25.06.2003
	(1.1)UTM: 35TNG68, 20 m alt., after Arkutino, along Ropotamo river, Raycheva, 03.07.2004.
R. cristatus DC.	(5.2) UTM: 35TLG23, after Karnare village towards Beklemeto pass, Raycheva, 25.06.2003.
	(1.1)UTM:35TNG59, 40 m alt., grassy places near Sozopol, Raycheva, 03.07.2004.
R. hydrolapathum Huds.	(18) UTM: 35TKG99, 300m alt., west part of Pjasachnik dam, Raycheva, 22.06.2003.
R. maritimus L.	(1.1) UTM: 35TNG76, 20 m alt., the quay of Tzarevo, Raycheva, 03.07.2004.
	(1.2) UTM: 35TNH89, 40m alt., between Varna and Golden Sands resort, Raycheva, 17.06.2004.
R. obtusifolius L	
	(1.2) UTM: 35TNH76, 20 m alt., Kamchia reserve, D. Stojanov SO-98411, 14.08.1996.
R. palustris Sm.	(2) UTM 35TMJ15, 40 m alt., on the bank of Danube river by Russe, Raycheva, 04.08.2004.
	(2) UTM: 35TNJ28, 25 m alt., along the Danube river near Silistra, Raycheva, 4.08.2004.
R. patientia L.	
	(17.2) UTM: 35TLC13, 660 m alt., grassy places near Luky, Raycheva, 13.07.2003
R. pulcher L.	(4)UTM: 34TGN08, 650 m alt., Vratza Balkan, Vratzata, along Leva river, Raycheva,
ssp. ***pulcher***	05.09.2004.
	(5.2)UTM: 35TLH32, 750 m alt., along Bjala reka river near Kalofer, Raycheva, 24.07.2004.
*ssp.**divaricatus***(L.) Murb.	(17.2) UTM: TLJ25, 435 m alt., on the bank of Ruensko lake, Raycheva, 03.07.2003.
	(5.3) UTM:35TNH65, 20 m alt., along Kamchia river after Staro Oryahovo , Raycheva,
ssp.***raulinii*** (Boiss.)	17.06.2004.
Rech. f.	
R. sanguineus L.	(5.1) UTM: 34TFN78, 980 m alt., Petrohan pass, near Gintzi village, Raycheva,

R.stenophyllus Ledeb.	07.09.2004. (1.1) UTM: 35TNH41, 5 m alt., the old salt deposites near Pomorie, Raycheva, 17.06.2004. (16.1) UTM:35TKH90, 300 m alt., near Pyasachnik dam, Raycheva, 22.06.2003. (5.2) UTM: 35TLH03, 540 m alt., Karnare village, Raycheva, 25-06-2003. (1.1) UTM:35TNG85, 40 m alt., rare oak forests near Sinemoretz village, Raycheva, 03.07.2004. (1.1)UTM:35TNH72, 50 m alt., Emine cape, near the military base, Raycheva, 17.06.2004 (1.1) UTM:35TNG85, 40 m alt., rare oak forests near Sinemoretz village, Raycheva, 03.07.2004. (18) UTM: 35TLG17, 140 m alt., Ostrova locality near Plovdiv, Raycheva, 26.05.2003. (4) UTM:34TGN1, 850 m alt., near Ledenika cave, Vratza Balkan, Raycheva, 05.09.2004 (2) UTM:35TMJ15, 40 m alt., on the bank of the Danube river by Russe, Raycheva, 04.08.2004 (3) UTM: 34TFP55,50 m alt., on the bank of the Danube river near Archar, Raycheva, 06.09.2004. (2)UTM:35TNJ28, 20 m alt., on the bank of the Danube river near Silistra, Raycheva, 04.08.2004.

*Floristic regions: 1- Black Sea coast (1.1-South, 1.2-North), 2-North-East Bulgaria, 3-Danube plane, 4-Balkan Foothills (4.1-West, 4.2-East), 5-Stara Planina mt. (5.1-West, 5.2-Central, 5.3-East), 15- Rila mt., 16-Sreda Gora mt. (16.1-West, 16.2-East), 17-Rhodopi mt. (17.1-West, 17.2-Central, 17.3-East), 18-Thracian plane.

Table 2. Chromosome counts for the studied Bulgarian representatives of subgenus *Rumex*

Taxon	2n	Previous studies (origin of accessions)	Author	Data from the current study (2n)
R. alpinus L.	20	Romania	Löve (1967)	**20**
	20	Danmark	Ichikawa et al. (1971)	
	20	Belgium	Degraeve (1975)	
	20	Bulgaria	Stoeva (1987)	
R. conglomeratus Murray	40	Russia	Menshikova (1964)	**20**
	20	Belgium	Degraeve (1975)	
	20	Sweden	Löve (1967)	
R. crispus L.	60	Canada	Mulligan (1957)	**60**
	60	Canada	Ichikawa et al. (1971)	
	60	Bulgaria	Stoeva (1987)	
R. cristatus DC.	80	UK	Ichikawa et al. (1971)	**80**
	80	Germany	Ichikawa et al. (1971)	
R. hydrolapathum Huds.	200	Scandinavia	Löve (1942)	**200**
	c.100	Russia	Menshikova (1964)	
	c.200	Germany	Ichikawa et al. (1971)	
	c.200	Danmark	Ichikawa et al. (1971)	
	c.200	Belgium	Ichikawa et al. (1971)	
	c.200	Poland	Ichikawa et al. (1971)	
	c.200	Russia	Ichikawa et al. (1971)	
	c.200	Netherlands	Ichikawa et al. (1971)	
R. maritimus L.	40	India	Datta (1952)	**40**
	40	Canada	Ichikawa et al. (1971)	
	40	USA	Ichikawa et al. (1971)	
	20, 40	Russia	Menshikova (1964)	
R. obtusifolius L	40	Canada	Mulligan (1959)	**40**
	40	C Europe	Löve (1961)	
	40	Danmark	Ichikawa et al. (1971)	
R. palustris Sm.	60	UK	Ichikawa et al. (1971)	**40**
	60	Danmark	Ichikawa et al. (1971)	
	40	Scandinavia	Löve (1942)	
	40	Belgium	Degraeve (1975)	
R. patientia L.	60	C Europe	Löve (1961)	**60**
	60	Georgia	Löve (1967)	
	60	Danmark	Ichikawa et al. (1971)	
R. pulcher L.	20	Russia	Menshikova (1964)	
	20	Turkey	Degraeve (1975)	
	20	Bulgaria	Stoeva (1987)	
ssp. pulcher	20	C Europe	Löve (1961)	**20**
	20	Sweden	Löve (1967)	

ssp. *divaricatus* (L.) Murb.	20	Italy	Löve (1967)	**20**
ssp. *raulinii* (Boiss.) Rech. f.	20	Greece	Löve (1967)	**20**
R. sanguineus L.	20	Sweden	Löve (1967)	**20**
	20	Danmark	Ichikawa (1971)	
	20	Danmark	Degraeve (1975)	
	20	Bulgaria	Stoeva (1985)	
R. stenophyllus Ledeb.	20,40	Russia	Menshikova (1964)	**40**
	60	Hungary	Pólya (1950)	
		Canada	Mulligan (1957)	
		Canada	Löve (1967)	
		Belgium	Degraeve (1975)	
	62-67	Romania	Tarnavschi (1948)	

Figure. 1. Drawings of mitotic root-tip cells of
a-*R. obtusifolius*, 2n=40; b-*R. maritimus*, 2n=40; c-*R. sanguineus*, 2n=20; d-*R. crispus*, 2n=60; e-*R. cristatus*, 2n=80; f- *R. patientia*, 2n=60. Scale bars = 5 μm.

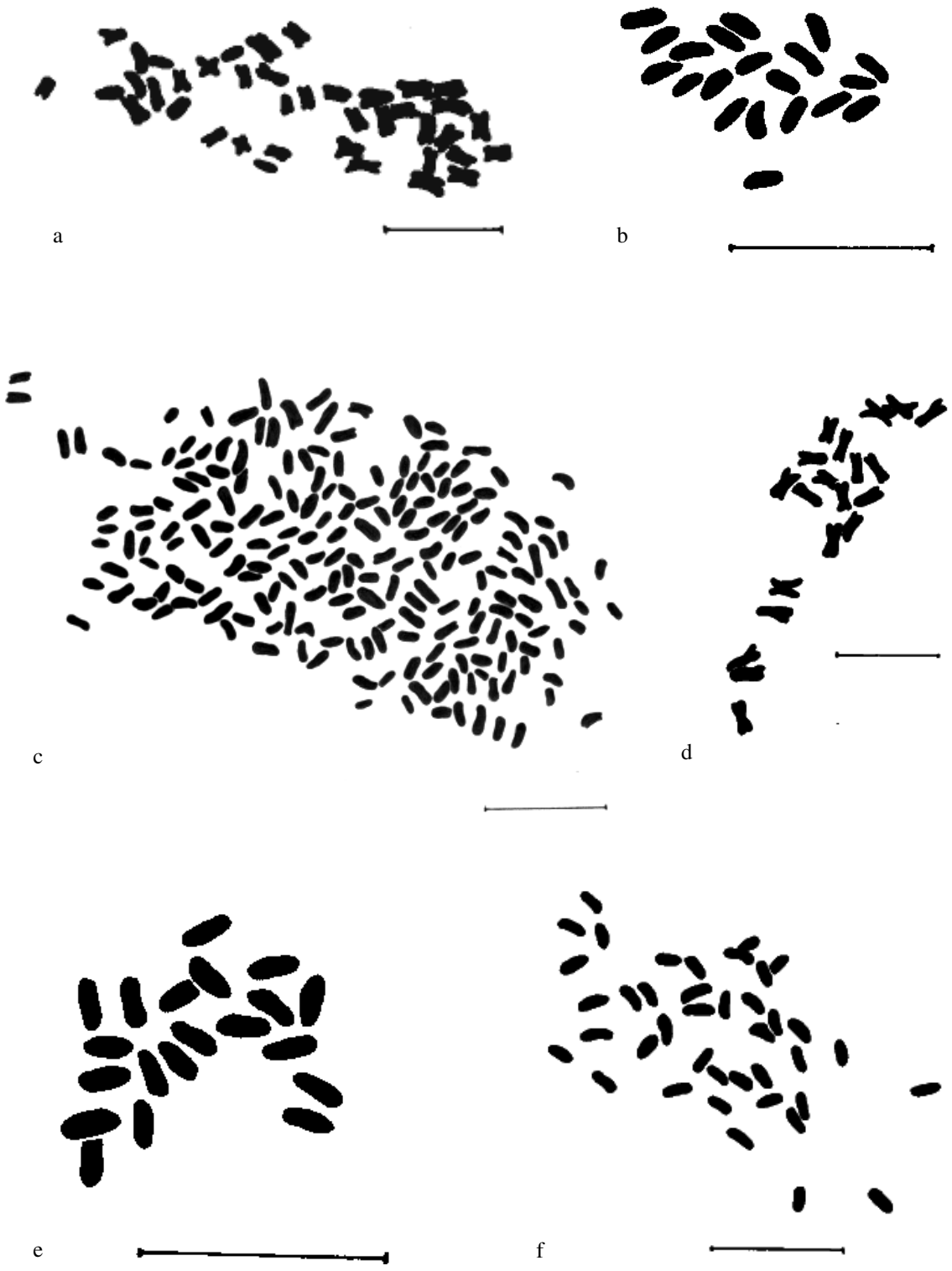

Figure. 2. Drawings of mitotic root-tip cells of
a-*R. stenophyllus*, 2n=40; b-*R. alpinus*, 2n=20; c-*R.hydrolapathum*, 2n=200; d-*R. pulcher*, 2n=20; e-*R.conglomeratus*, 2n=20; f-*R. palustris*, 2n=40.
Scale bars =5 μm.