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Abstract

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Chromosome numbers are presented for 32 taxa of angiosperms from Romania, mainly from mountainous regions in the Carpathians. Counts on 12 species are reported for the first time for Romanian and 14 for Carpathian plants. For *Silene dubia* a new ploidy level is reported. Karyotypes are presented for several species, mainly of *Achillea* and *Ranunculus*. Taxonomic and phytogeographical aspects are discussed.

Additional key words: chromosome numbers, karyotypes, Carpathians

Introduction

Karyological data are essential information for any organism and many karyological investigations have been performed, providing important characters for plant systematics and evolutionary analysis (Stace 2000). Nevertheless, the chromosome numbers of only about 25 % of all angiosperms are known (Bennett 1998). Besides variation in chromosome number (including ploidy level and aneuploidy), karyological data can show variation in absolute and relative chromosome size, in chromosome morphology and in staining properties of the chromosomes (Sharma & Sen 2002). The description of chromosome morphology and the presentation in a karyogram is a powerful method to characterise genomes.

Many reported chromosome numbers are more or less uncertain because their basis is poor: plants from a single population or even one single individual were investigated, or reported chromosome count are not well documented, e.g., lacking indications of origin or voucher specimens. This reduces the usefulness of the results, especially within taxonomically difficult groups where chromosome numbers are often variable. Stace (2000) defines three main conditions for chromosome numbers to be useful: the investigated plants should originate from known wild localities, voucher specimens must be deposited in a designated and accessible herbarium, and counts have to be performed on several plants in each population of a given taxon. Furthermore, individuals of several populations from different geographic regions should be investigated.

In 1997 we undertook a field trip to the southern part of the Carpathians in Romania to collect plant material for ongoing biosystematic projects (e.g., *Ranunculus alpestris* group). Later in the same year, the first author travelled to the same region together with Marianne Ring for a very short visit to sample *R. crenatus*. For our investigations living plants were collected and transferred to the greenhouse at ETH Zurich. As not many karyological data from Romanian plants are available, living plants (and in a few cases seeds) of interesting plant species of different families were additionally sampled and karyological investigations were carried out on the plants cultivated in our greenhouse. A few of these data have been published earlier (Frey & al. 2003: *Erigeron annuus* (L.) Pers. with $2n = 3x = 27$ [voucher *13359*]; Baltisberger &

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Widmer 2009: *Ranunculus alpestris* L. with $2n = 2x = 16$ [voucher *13354*] and *R. crenatus* Waldst. & Kit. [voucher *13401*]), the main portion, however, is presented here with remarks on taxonomy and phytogeography.

Material and methods

The karyological investigations were carried out on root tips. These were pretreated with colchicine (0.05 %) for 0.5 to 2 hours, then fixed in ethanol/acetic acid (3:1) and stained and squashed in lacto-propionic orcein (Dyer 1963). To determine chromosome numbers, 5–10 metaphases were counted for each individual investigated, and, where possible, several individuals were investigated per site. The numbers of investigated individuals per site are indicated in Table 1. Karyotypes are described using the terminology by Levan & al. (1964). The chromosomes are named according to the position of the centromers, which is expressed with the ratio long arm to short arm (R=LA/SA): metacentric (arm ratio 1–1.7), submetacentric (ratio 1.7–3), subtelocentric (ratio 3–7), acrocentric (ratio more than 7) and telocentric (chromosome only with one arm).

The taxa are arranged in alphabetical order by families, genera and species. The nomenclature follows Euro+Med (2006+) for *Asteraceae, Geraniaceae, Poaceae* and *Rosaceae* (already treated in Euro+Med). For the other families the Romanian flora (Săvulescu 1952–76) and "Flora Europaea" (Tutin & al. 1968–80, 1993) were used. The identification numbers represents the first author's collecting number of the herbarium specimens (in parenthesis herbarium specimens of plants cultivated from the wild source), which all are deposited in Z/ZT.

Literature concerning chromosome numbers was checked using the indexes by Federov (1969), Moore (1973–77), Goldblatt (1981–88), Van Loon (1987) and Goldblatt & Johnson (1990–2006).

Results and discussion

Asteraceae

Achillea distans Willd. – $2n = 6x = 54$ (Fig. 1A)
SW of Brasov, SW of Zarnesti, Piatra Craiului, NE to NW exposed calcarous rocks, 1800–1850 m, 23.7.1997, *13330 (14025).*

Achillea distans is a rather tall yarrow (up to 1.2 m) growing in the Alps, the Carpathians as well as in mountains of the Balkan peninsula. It belongs to the polymorphic and difficult group of *A. millefolium* L., which comprises plants from the diploid to the octoploid level. Most indications for *A. distans* give the hexaploid level with $2n = 6x = 54$ chromosomes as is the case with the plants investigated here, representing the first count on native Carpathian plants. The karyotype consists of 42 metacentric and 12 submeta- to subtelocentric chromosomes (Fig. 1A). The same karyotype is indicated by Baltisberger (1992). The 12 submeta- to subtelocentric chromosomes probably all have satellites but not always all satellites are visible, a phenomenon that has been observed earlier in other *Achillea* species (e.g., Baltisberger & Baltisberger 1995; Baltisberger 2002).

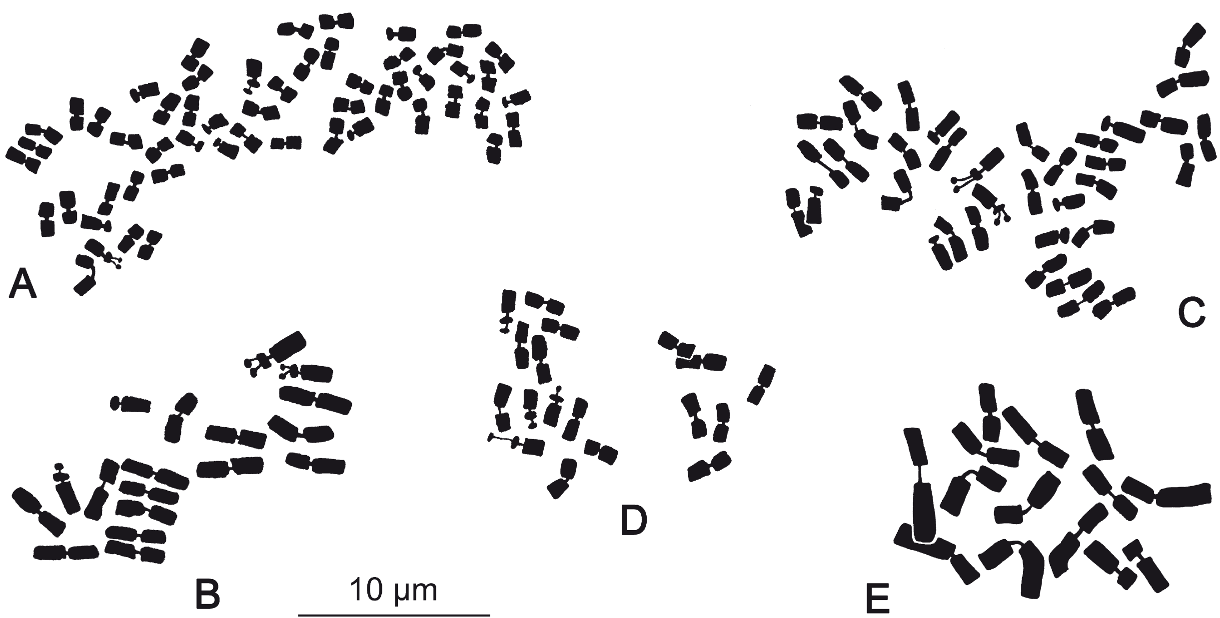

Fig. 1. Somatic metaphases – A: *Achillea distans* ($2n = 54$); B: *Achillea lingulata* ($2n = 18$); C: *Achillea millefolium* s.l. ($2n = 36$); D: *Achillea oxyloba* subsp. *schurii* ($2n = 18$); E: *Podospermum roseum* ($2n = 14$).

Table 1. Alphabetical list of the investigated species, with the identification numbers referring to the herbarium specimens deposited in Z/ZT, the number of investigated individuals (N), the chromosome numbers (2*n*) and the sources. – L = living plants, S = seeds; + = first count on plants from the Carpathians, * = first count on plants from Romania, ° = new ploidy level.

Species	Voucher	Source	N	2*n*
Asteraceae: *Achillea distans*	*13330*	L	6	54+
A. lingulata	*13346*	L	6	18+
A. millefolium s.l.	*13335*	L	6	36
A. oxyloba subsp. *schurii*	*13331*	L	6	18
Antennaria dioica	*13303*	L	4	28+
Centaurea nervosa	*13307*	L	1	22+
Gnaphalium sylvaticum	*13313*	L	3	56*
Pilosella aurantiaca	*13301*	L	5	36
Podospermum roseum	*13304*	L	5	14*
Campanulaceae: *Campanula carpatica*	*13319*	L	4	34*
Caryophyllaceae: *Dianthus carthusianorum*	*13343*	L	7	30
Dianthus superbus	*13328*	L	6	60
Silene dubia	*14014*	L	5	24*
S. dubia	*13308*	L	4	48°
S. pusilla	*13311*	L	3	24+
Geraniaceae: *Geranium caeruleatum*	*13347*	L	13	28+
Geranium phaeum	*13326*	L	3	28+
G. pratense	*13317*	L	2	28*
Lamiaceae: *Nepeta nuda*	*13332*	L	2	18+
Stachys alpina	*13333*	L	3	30*
S. annua	*13315*	S	11	34*
Teucrium chamaedrys	*13334*	L	5	62+
Orchidaceae: *Nigritella rhellicani*	*13353*	L	3	80+
Poaceae: *Setaria pumila*	*13406*	S	6	72*
Ranunculaceae: *Aconitum vulparia*	*13345*	L	8	16
Consolida regalis	*13318*	S	6	16*
Ranunculus acris	*13310*	L	8	14+
R. oreophilus	*13329*	L	4	16*
R. repens	*13309*	L	4	32+
R. sardous	*13314*	S	4	16*
Rosaceae: *Geum montanum*	*13306*	L	5	42*
Potentilla crantzii	*14036*	L	2	28+
Scophulariaceae: *Veronica officinalis*	*13312*	L	4	36+

Achillea lingulata Waldst. & Kit. – $2n = 2x = 18$ (Fig. 1B)
SW of Brasov, SW of Zarnesti, S slope of Piatra Craiului, meadow, 1600–1650 m, 24.7.1997, *13346 (14034).*

Achillea lingulata has simple, densely leafy stems up to 50 cm, each with one corymb of up to 30 capitula. Only a few species of *Achillea* have undivided leaves, this species is one of them, its leaves being spathulate to lanceolate. The capitula are middle sized, the involucre is 5–8 mm in diameter and the white, suborbicular ligules are c. 3 mm long. It grows in alpine meadows and rocky slopes in the E and S Carpathians and scattered in mountains of the Balkan peninsula southwards to N Greece.

Two ploidy levels are indicated in the literature, diploid with $2n = 18$ and tetraploid with $2n = 36$, based on plants from botanical gardens, from Bosnia-Hercegovina and from Bulgaria. Our investigated plants showed 18 chromosomes and thus are diploid, being the first count on native material from the Carpathians. The karyotype consists of 14 metacentric and 4 submeta- to subtelocentric chromosomes, the latter with satellites (Fig. 1B). Karyotypes of diploid as well as tetraploid plants from Bosnia-Hercegovina are presented in Međedović & Šiljak (1978, repeated in Međedović 1984). The karyotypes are similar to the one found by us (including the satellites on the 4 smaller chromosomes).

Achillea millefolium L. s.l. – $2n = 4x = 36$ (Fig. 1C)
SW of Brasov, SW of Zarnesti, on the road from Zarnesti to Piatra Craiului, stony meadow, 1000 m, 23.7.1997, *13335 (14030).*

Achillea millefolium s.l. is a difficult polyploid complex occurring in Europe, W Asia and North America. The central European taxa are rather well understood but the knowledge of taxa outside Central Europe is incomplete. Introgression and hybridization seem to be frequent and identification is often difficult, uncertain or even impossible. Concerning *A. millefolium* s.l. Romania belongs to these poorly investigated areas. That is why we cannot give a proper determination of the investigated plants, which all are tetraploid with $2n = 36$ chromosomes. This is a common and widespread ploidy level within *A. millefolium* s.l. The karyotype consists of 28 metacentric and 8 submeta- to subtelocentric chromosomes, the latter with satellites (Fig. 1C).

Achillea oxyloba subsp. ***schurii*** (Sch. Bip.) Heimerl – $2n = 2x = 18$ (Fig. 1D)
SW of Brasov, SW of Zarnesti, Piatra Craiului, NE to NW exposed calcarous rocks, 1800–1850 m, 23.7.1997, *13331 (14026).*

Achillea oxyloba is characterized by pinnatifid leaves and stems up to 20 cm with only one rather big capitulum (involucre c. 10 mm in diameter, ligules white and 6–10 mm long). Two subspecies are recognized that are both growing in mountain rocks, pastures and screes. They have distinct geographic distribution areas, with subspecies *oxyloba* occurring in the SE Alps, and subspecies *schurii* being restricted to the E and the S Carpathians.

The investigated plants proved to be diploid with 2*n* = 18 chromosomes. This confirms the counts by Speta (1971) and Tăcină (1979) on Romanian plants as well as by Stephanik on plants from the Ukrainian Carpathians in 1977 (cited in Goldblatt 1984). The karyotype consists of 14 metacentric and 4 submeta- to subtelocentric chromosomes, the latter with satellites (Fig. 1D). This corresponds with the karyotype indicated by Stephanik (cited in Goldblatt 1984) and mostly with that by Tăcină (1979).

Antennaria dioica (L.) Gaertn. – 2*n* = 4*x* = 28
ESE of Timisoara, ESE of Caransebes, Muntii Tarcului, N of Mount Tarcu, Muntele Mic, meadow with rocks, 1550–1600 m, 21.7.1997, *13303 (14012)*.

Antennaria dioica is a widespread species in Eurasia and North America. In southern Europe it is restricted to mountains (e.g., Pyrenees, Appennini). This is the first count on Carpathian plants, with 2*n* = 28 chromosomes confirming the numerous indications in the literature.

Centaurea nervosa Willd. – 2*n* = 2*x* = 22
ESE of Timisoara, ESE of Caransebes, Muntii Tarcului, N of Mount Tarcu, Muntele Mic, meadow with rocks, 1550–1600 m, 21.7.1997, *13307*.

Centaurea nervosa belongs to the group of *C. uniflora* Turra, which grows in montane and alpine regions of the Alps, N Appennini, S Carpathians and in northern and central parts of the Balkan peninsula. The six taxa of the group have partly overlapping distribution areas. *C. nervosa* has the widest distribution area, occurring nearly throughout the range of the group and being the only representative in the Carpathians. It is rather variable and three subspecies are recognized, subsp. *davidovii* (Urum.) Hayek in Bulgaria, subsp. *promota* E. Gamal-Eldin & Wagenitz in Greece and subsp. *nervosa* throughout the range of the species and therefore the only taxon of the *C. uniflora* group in the Carpathians. Ours are the first Carpathian plants investigated and showed 22 chromosomes, thus confirming the reports in the literature.

Gnaphalium sylvaticum L. – 2*n* = 56
ESE of Timisoara, ESE = of Caransebes, Muntii Tarcului, N of Mount Tarcu, on the road from Caransebes to Muntele Mic, forest, 900 m, 21.7.1997, *13313 (14018)*.

Gnaphalium sylvaticum grows in woods, heaths and grassland throughout Europe. The investigated plants showed 2*n* = 56 chromosomes. This is the first count on plants from Romania, confirming the count of Pogan & al. (1990) on plants from the Polish Carpathians as well as many further reports in the literature.

Pilosella aurantiaca (L.) F. W. Schultz & Sch. Bip. – 2*n* = 4*x* = 36
ESE of Timisoara, ESE of Caransebes, Muntii Tarcului, N of Mount Tarcu, Muntele Mic, meadow with rocks, 1550–1600 m, 21.7.1997, *13301 (14011)*.

Pilosella aurantiaca (= *Hieracium aurantiacum* L.) grows mainly in the mountains of Europe. As this species shows beautiful orange to red ligules, it is widely cultivated for ornaments. It rather often naturalises and therefore grows also in non-mountainous areas. The basic chromosome number of the genera *Pilosella* and *Hieracium* is *x* = 9. Many species show several ploidy levels, which is also the case for *P. aurantiaca* with diploid (2*n* = 18) to octoploid (2*n* = 72) plants. All investigated plants from the Carpathians were tetraploid with 2*n* = 36 chromosomes.

Podospermum roseum (Waldst. & Kit.) Gemeinholzer & Greuter – 2*n* = 2*x* = 14 (Fig. 1E)
ESE of Timisoara, ESE of Caransebes, Muntii Tarcului, N of Mount Tarcu, Muntele Mic, meadow with rocks, 1550–1600 m, 21.7.1997, *13304 (14013)*.

Podospermum roseum is the now preliminarily accepted name for *Scorzonera rosea* Waldst. & Kit. (see Euro+Med 2006+). This species is one of the three taxa of the group of *P. purpureum* (L.) W. D. J. Koch & Ziz. The group has a wide European distribution area from central Germany and Italy eastwards to SE Russia and the taxa are treated as species or subspecies (Euro+Med 2006+). *P. roseum* subsp. *peristericum* (Formánek) Gemeinholzer & Greuter occurs only in Greece and Macedonia, the other two are widespread and grow more or less throughout the range of the group. Both *P. purpureum* as well as *P. roseum* occur in the Carpathians. Ours are the first plants from Romania investigated and showed 2*n* = 14 chromosomes, confirming the counts on plants from other parts of the Carpathians (Frey & al. 1977 and Skalinska & al. 1978 from Poland, Pashuk 1987 from Ukraine) as well as most indications in the literature from other areas. The karyotype consists of 12 meta- and 2 submetacentric chromosomes, the latter being the smallest chromosomes (Fig. 1E).

Campanulaceae

Campanula carpatica Jacq. – 2*n* = 2*x* = 34
SW of Brasov, SW of Zarnesti, S slope of Piatra Craiului, NE to NW exposed calcarous rocks, 1800–1850 m, 23.7.1997, *13319 (14022)*.

Campanula carpatica has beautiful big flowers and grows on calcarous rocks exclusively in the Carpathians. The investigated plants showed 2*n* = 34 chromosomes. This first record for Romania confirms the counts on Slovakian plants (Kovanda 1983; Murin 1993).

Caryophyllaceae

Dianthus carthusianorum L. s.l. – 2*n* = 2*x* = 30
SW of Brasov, SW of Zarnesti, on the road from Zarnesti to Piatra Craiului, rocks and screes, 1050–1100 m, 24.7.1997, *13343 (14031), 13344 (14032)*.

Dianthus carthusianorum grows in the southern parts of Europe, from W Europe to Ukraine. It is a very variable species, which has been subdivided by various authors but the delimitations of the infraspecific taxa are not clear. Nevertheless, in the Carpathians several subspecies are indicated (Jalas & Suominen 1986). All indications in the literature (including plants from the Carpathians) give the same chromosome number of $2n = 30$ for all subspecies as we found.

Dianthus superbus L. – $2n = 4x = 60$
SW of Brasov, SW of Zarnesti, Piatra Craiului, NE to NW exposed calcarous rocks, 1800–1850 m, 23.7.1997, *13328 (14024)*.

Dianthus superbus is an Eurosiberian species and occurs in most of Europe except in the West (e.g., Britain, Portugal and Spain). It is rather variable and infraspecific taxa at various taxonomic levels have been described. Often three subspecies are recognized (Jalas & Suominen 1986). *D. superbus* subsp. *stenocalyx* (Trautv. ex Juz.) Kleopow is restricted to SC Russia and N Ukraine. The other two subspecies grow more or less throughout the range of the species, subsp. *superbus* mostly in lowlands and subsp. *alpestris* Kablík ex Čelak in higher mountains. The plants investigated here were morphologically intermediate between the two widespread subspecies. Most indications in the literature for all taxa within *D. superbus* as well as from many geographical areas (including the Carpathians) give $2n = 2x = 30$ chromosomes. We found $2n = 4x = 60$ chromosomes confirming a single count published by Krogulevich in 1978 on plants from Siberia (see Goldblatt 1988).

Silene dubia Herbich – $2n = 2x = 24$, $2n = 4x = 48$
ESE of Timisoara, ESE of Caransebes, Muntii Tarcului, N of Mount Tarcu, Muntele Mic, meadow with rocks, 1550–1600 m, 21.7.1997 *(14014)* ($2n = 24$); ibid., *13308 (14016)* ($2n = 48$).

Silene dubia belongs to the extremely variable *S. nutans* L. Several taxa are described within this complex and sometimes recognized at different taxonomic levels (e.g., *S. brachypoda* Rouy from S France and N Spain or *S. livida* Willd. in southern Europe). *S. dubia* is a Carpathian endemic. *S. nutans* seems to be karyologically uniform, all indications in the literature give $2n = 2x = 24$. The only indication in the literature for *S. dubia* shows the same chromosome number based on plants from the Ukrainian Carpathians (Tasenkevitch & al. 1989). Ours are the first Romanian plants investigated karyologically and showed two different ploidy levels. The tetraploid number $2n = 48$ is new for *S. dubia*.

Silene pusilla Waldst. & Kit. – $2n = 2x = 24$
ESE of Timisoara, ESE of Caransebes, Muntii Tarcului, N of Mount Tarcu, Muntele Mic, wet place, 1500 m, 21.7.1997, *13311*.

The *Silene pusilla* group consists of about ten taxa endemic to mountains of S Europe and is characterized by its winged seeds with characteristic dorsal structures. As this character is unique within *Silene,* the group has sometimes been treated as a separate genus *Heliosperma*. The only species in the Carpathians is the widespread and rather variable *S. pusilla,* occurring from the Pyrenees to the W Carpathians. This is the first count on Carpathians plants, confirming the counts from other areas.

Geraniaceae

Geranium caeruleatum Schur – $2n = 2x = 28$
SW of Brasov, SW of Zarnesti, S slope of Piatra Craiului, meadow, 1900–2100 m, 24.7.1997, *13347 (14035).*

Geranium caeruleatum occurs in the Carpathians and in the mountains of the Balkan peninsula. It is related to and sometimes treated as subspecies of *G. sylvaticum* L., which has a much wider distribution area all over Europe and also grows in the Carpathians. This is the first count on plants from the Carpathians, confirming the only other count on plants from Bulgaria.

Geranium phaeum L. – $2n = 2x = 28$
SW of Brasov, SW of Zarnesti, Piatra Craiului, NE to NW exposed calcarous rocks, 1800–1850 m, 23.7.1997, *13326 (14023).*

Geranium phaeum is a Central European species extending eastwards to Ukraine. It is rather frequently cultivated as an ornamental and sometimes has escaped from gardens. Ours are the first Carpathian plants investigated and showed the same chromosome number as given in the literature.

Geranium pratense L. – $2n = 2x = 28$
ESE of Timisoara, on the road from Simeria to Sebes, ruderal place; 22.7.1997, *13317 (14020).*

Geranium pratense is an Eurasian species rather widespread in Europe. It is frequently cultivated for ornament and naturalised widely. Ours are the first Romanian plants investigated and showed the same chromosome number as given in the literature.

Lamiaceae

Nepeta nuda L. – $2n = 18$
SW of Brasov, SW of Zarnesti, on the road from Zarnesti to Piatra Craiului, stony meadow, 1000 m, 23.7.1997, *13332 (14027).*

Nepeta nuda is an Eurasian species growing in Europe in the southern and eastern parts. The chromosome number has been established for the first time on Carpathian plants. With $2n = 18$ it corresponds with the indications in literature.

Stachys alpina L. – $2n = 30$
SW of Brasov, SW of Zarnesti, on the road from Zarnesti to Piatra Craiului, stony meadow, 1000 m, 23.7.1997, *13333 (14028).*

The group of *Stachys germanica* L., to which *S. alpina* belongs to, occurs in Europe and eastwards to the Caucasian region and comprises about ten species, which all but one (namely *S. alpina*) are taxonomically very dif-

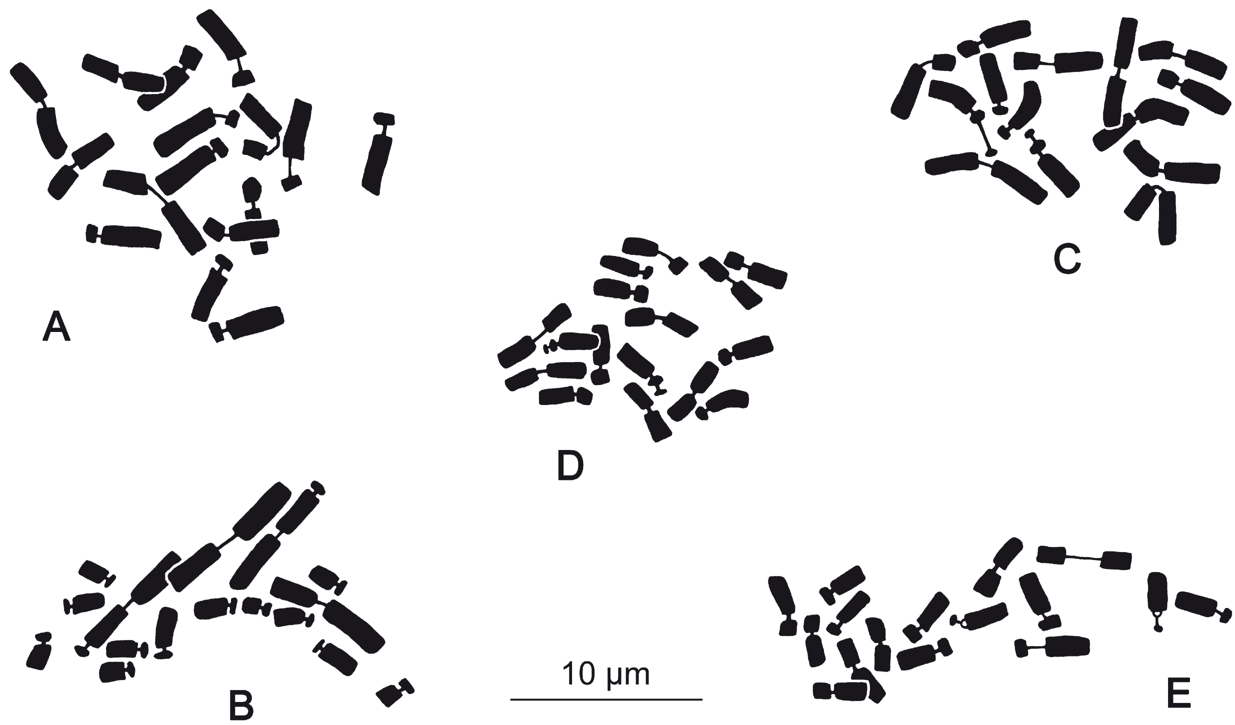

Fig. 2. Somatic metaphases – A: *Aconitum vulparia* (2*n* = 16); B: *Consolida regalis* (2*n* = 16); C: *Ranunculus acris* (2*n* = 14); D: *R. oreophilus* (2*n* = 16); E: *R. sardous* (2*n* = 16).

ficult and treated at various taxonomic levels. *S. alpina* is morphologically rather homogenous and well defined by glandular hairs on the stems. No Romanian plants have been investigated so far. With 2n = 30 they showed the same chromosome number as given for plants from the Carpathians in Poland (Pogan & al. 1980) as well as numerous counts from elsewhere.

Stachys annua L. – 2*n* = 34
ESE of Timisoara, on the road from Simeria to Sebes, ruderal place; 22.7.1997, *13315 (14019)*.

Stachys annua is a mostly annual weed growing in cultivated fields, ruderal and other open habitats. It originates in the E Mediterranean region but has been naturalised in Europe, SW Asia, N Africa and occasionally even in North America. The investigated plants showed 2*n* = 34 chromosomes. This is the first count on Romanian plants, confirming previous indications in the literature.

Teucrium chamaedrys L. – 2*n* = 62
SW of Brasov, SW of Zarnesti, on the road from Zarnesti to Piatra Craiului, stony meadow, 1000 m, 23.7.1997, *13334 (14029)*.

The distribution area of *Teucrium chamaedrys* covers the Mediterranean area, reaches northwards the Netherlands and S Poland and eastwards SW Russia. It is a very variable species and many infraspecific taxa have been described but are mostly not recognized. Many different chromosome numbers have been reported, ranging from 2*n* = 32 to 2*n* = 96. It seems that no correlation exists between chromosome number and geographical distribution (Baltisberger 1993). Ours are the first Carpathian plants investigated and showed one of the widespread numbers also reported from Spain, France, Italy, Serbia, Bulgaria and Greece (Baltisberger 1993).

Orchidaceae

Nigritella rhellicani Teppner & Klein – 2*n* = 4*x* = 80
SW of Brasov, SW of Zarnesti, S slope of Piatra Craiului, meadow, 1900–2100 m, 24.7.1997, *13353*.

Nigritella rhellicani (= *N. nigra* (L.) Rchb. f. p.p.) grows in meadows of Europe. In N Europe it grows in the lowlands but in the central and southern parts of Europe it is restricted to the mountains. In the Carpathians three taxa of *Nigritella* are known, *N. rhellicani*, *N. rubra* (Wettst.) K. Richter and the recently discovered E Carpathian narrow endemic *N. carpatica* (Zapat.) Teppner & al. (1994). The basic chromosome number within *Nigritella* is *x* = 20 and diploid to pentaploid plants are known. The first plants investigated from the Carpathians showed 80 chromosomes and thus were tetraploid.

Poaceae

Setaria pumila (Poir.) Roem. & Schult. – 2*n* = 8*x* = 72
E of Timisoara, Lugoj, near Hotel Tirol, ruderal place; 24.8.1997, *13406 (14037)*.

Setaria pumila is probably native to S and Central Europe but introduced nearly all over the world. This annual weed grows in open habitats (mainly cultivated fields and ruderal sites). Two ploidy levels are known, $2n = 4x = 36$ and $2n = 8x = 72$. The first Romania plants investigated proved to be octoploid.

Ranunculaceae

Aconitum vulparia Rchb. – $2n = 2x = 16$ (Fig. 2A)
SW of Brasov, SW of Zarnesti, S slope of Piatra Craiului, rocky meadow, 1600–1650 m, 24.7.1997, *13345 (14033)*.

The species complex *Aconitum vulparia* (= *A. lycoctonum* L.) consists of perennial plants with rhizomes, with violet, blue or yellow flowers, in which the helmet is c. 3 times as high as wide and the nectary-spurs are spirally curved. This group has long been considered as difficult caused by high morphological variability and assumed hybridization. Morphological variability within and among populations is high, morphological characters have little or no systematic value and sequence variation (using ITS and *psbA-trnH*) is very low (Utelli & al. 2000). These authors conclude that the group should be treated as one morphologically highly variable species in which colour morphs could probably be treated as subspecies.

In the Carpathians several colour morphs occur, violet (*Aconitum septentrionale* Koelle), blue (*A. moldavicum* Hacq.), yellow (*A. vulparia* Rchb., including *A. lasianthum* (Rchb.) Simk.) and yellowish (*A. lasiostomum* Rchb. ex Besser). The investigated plants showed yellow flowers and proved to be diploid with $2n = 16$ chromosomes. The number corresponds to indications on Romanian plants (Tăcină 1980, as *A. lasianthum*) as well as on plants from the Slowakian Carpathians (Micieta 1981) and confirms all indications in the literature. The karyotype consists of 4 metacentric, 4 submetacentric and 8 subtelocentric chromosomes (Fig. 2A).

Consolida regalis S. F. Gray – $2n = 2x = 16$ (Fig. 2B)
ESE of Timisoara, on the road from Simeria to Sebes, ruderal place; 22.7.1997, *13318 (14021)*.

Consolida regalis is an annual weed growing in cultivated fields, ruderal and other open habitats. It originates in S Europe (probably the Mediterranean region) but has been introduced and naturalised in Europe and Asia. This first count on Romanian plants confirms previous indications in the literature. The karyotype consists of 4 big metacentric chromosomes, 2 of them with satellites, and 12 much smaller subtelocentric chromosomes (Fig. 2B).

Ranunculus acris L. – $2n = 2x = 14$ (Fig. 2C)
ESE of Timisoara, ESE of Caransebes, Muntii Tarcului, N of Mount Tarcu, Muntele Mic, wet place, 1500 m, 21.7.1997, *13310 (14008)*.

The *Ranunculus acris* group is widespread in Europe, with several species being recognized (sometimes partly treated at subspecific level). All taxa have a basic chromosome number of $x = 7$, which is uncommon in *Ranunculus* with mostly $x = 8$. Diploid and tetraploid taxa are known. *R. acris* is always diploid with $2n = 14$ chromosomes, which is confirmed here for the first time on Carpathian plants. The karyotype consists of 6 metacentric and 8 submeta- to subtelocentric chromosomes (Fig. 2C). Two chromosomes with rather small short arms bear satellites. Similar karyotypes for *R. acris* (sometimes without any indications about satellites) are given by Gregson (1965), Goepfert (1974), Marchi & al. (1975), Baltisberger & Baltisberger (1995), and Baltisberger (2006). The same karyotype is given for other taxa of the group, e.g., for *R. borealis* Trautv. ($2n = 2x$; Goepfert 1974, Baltisberger unpubl.), *R. friesianus* Jord. ($2n = 2x$; Baltisberger unpubl.), *R. granatensis* Boiss. ($2n = 4x$; Goepfert 1974; Diosdado & Pastor 1992), *R. lanuginosus* L. ($2n = 4x$; Goepfert 1974; Marchi & al. 1975; Sopova & Sekovski 1982; Baltisberger & al. 1996), *R. serbicus* Vis. ($2n = 4x$; Goepfert 1974; Marchi & al. 1975; Sopova & Sekovski 1982; Baltisberger & Baltisberger 1995; Baltisberger 2002, 2006) and *R. strigulosus* Schur ($2n = 4x$; Goepfert 1974).

The same karyotype is also shown by *Ranunculus* taxa with $x = 7$ that do not belong to the *R. acris* group, e.g., the Mediterranean *R. constantopolitanus* (DC.) d'Urv. (Kurita 1957; Goepfert 1974), *R. fibrillosus* C. Koch (Baltisberger 1991) and *R. velutinus* Ten. (Goepfert 1974; Marchi & al 1975; Sopova & Sekovski 1982; D'Ovidio & al 1986; Baltisberger unpubl.), as well as the western American *R. californicus* Benth., *R. occidentalis* Nutt. and *R. uncinatus* D. Don (Goepfert 1974). This karyotype might probably be typical for all taxa of *Ranunculus* with $x = 7$, which form clearly separated clades in molecular phylogenies (Hörandl & al. 2005; Paun & al. 2005). Karyotypes may therefore indicate phylogenetic relationships.

Ranunculus oreophilus M. Bieb. – $2n = 2x = 16$ (Fig. 2D)
SW of Brasov, SW of Zarnesti, Piatra Craiului, NE to NW exposed calcarous rocks, 1800–1850 m, 23.7.1997, *13329 (14010)*.

This taxon was for a long time known as *Ranunculus oreophilus* and its identity was clear. The name *R. breyninus* Crantz, reestablished by Aeschimann & Heitz (1996) for this species, was used for plants of various groups and had therefore been considered as doubtful (Landolt & Hess 1954). The uncertainty of its identity remains.

Ranunculus oreophilus was included in the group of *R. montanus* Willd. but recognized as distinct by its special characters (Landolt 1954, 1956). The core of the group occurs in mountains of Europe but does not exist in the Caucasus, while *R. oreophilus* does not only grow in European mountains but also in the Caucasus. As shown in molecular phylogenies, *R. oreophilus* is related to the Caucasian *R. cappadocicus* Willd. Both are clearly distinct from the *R. montanus* group but nevertheless part of the same broader clades (Hörandl & al. 2005: clade XII; Paun & al. 2005: clade VIIId-g), a result that is also supported by morphological and biogeographical characters.

The investigated plants proved to be diploid with $2n = 16$ chromosomes. This is the first count on Romanian plants and it confirms the indication on plants from the Ukrainian Carpathians (N Carpathians; Pashuk 1987) as well as most indications from further areas. Only Sopova & Sekovski (1982) reported tetraploid plants. The karyotype consists of 6 metacentric, 6 submetacentric, 2 subtelocentric and 2 subtelo- to acrocentric chromosomes, the latter with satellites (Fig. 2D). A similar karyotype is given by Goepfert (1974) and Agapova (1983) for *R. oreophilus* as well as for the related *R. cappadocicus* and by Sopova & Sekovski (1982) and Masci & al. (1987) for *R. oreophilus*.

Although *R. oreophilus* does not belong to the *R. montanus* group, it shows a similar karyotype as all investigated species of this group: *R. aduncus* Gren & Godr. (Masci & al. 1987; Baltisberger unpubl.), *R. apenninus* Chiov. (Masci & al. 1987), *R. carinthiacus* Hoppe (Sopova & Sekovski 1981; Dickenmann 1982; Diosdado & Pastor 1992; Baltisberger unpubl.), *R. gouanii* Willd. (Goepfert 1974; Diosdado & Pastor 1992), *R. grenierianus* Jord. (Goepfert 1974; Dickenmann 1982), *R. montanus* (Goepfert 1974; Sopova & Sekovski 1982; Masci & al. 1987), *R. montanus* s.l. (Baltisberger 1984, 2002, 2006), *R. pollinensis* [Terr.] Chiov. (Masci & al. 1987), *R. ruscinonensis* Landolt (Diosdado & Pastor 1992) and *R. venetus* Huter (Masci & al. 1987).

Several species thought not to be closely related with *Ranunculus oreophilus,* with *R. cappadocicus* or with the *R. montanus* group, are situated in molecular phylogenies in the same clades (clade XII in Hörandl & al. 2005, clade VIIId-g in Paun & al. 2005) and also show rather similar karyotypes. These are *R. cortusifolius* Willd. (Goepfert 1974; Baltisberger & al. 1990; Baltisberger & Widmer 2006), *R. creticus* L. (Goepfert 1974; Baltisberger & Widmer 2005), *R. cupreus* Boiss. & Heldr. (Baltisberger & Widmer 2005), *R. millefoliatus* Vahl (Goepfert 1974; Sopova & Sekovski 1982; Baltisberger unpubl.), *R. psilostachys* Griseb. (Sopova & Sekovski 1982; Baltisberger, unpubl.) and *R. paludosus* Poir. (Baltisberger unpubl.). It seems that also in this case (similar to the case discussed under *R. acris,* see above) the karyotypes support molecular phylogenies.

Ranunculus repens L. – $2n = 4x = 32$
ESE of Timisoara, ESE of Caransebes, Muntii Tarcului, N of Mount Tarcu, Muntele Mic, wet place, 1500 m, 21.7.1997, *13309 (14007)*.

Ranunculus repens is widespread in Eurasia and N Africa and has been introduced and naturalised in North America. It is a very variable species but no satisfactory taxonomic treatment is available so far. The first plants from the Carpathians investigated so far proved to be tetraploid, corresponding with most of the numerous indications in the literature.

Ranunculus sardous Crantz – $2n = 2x = 16$ (Fig. 2E)
ESE of Timisoara, on the road from Simeria to Sebes, ruderal place; 22.7.1997, *13314 (14009)*.

Ranunculus sardous is a Mediterranean, morphologically very variable annual species of disturbed, often wet places. It looks similar to and is often hardly to distinguish from other annual species such as *R. angulatus* C. Presl (from Sicily), *R. cordiger* Vis. (from Corsica and Sardegna), *R. marginatus* d'Urv. (SE Europe) and *R. parvulus* L., which grow at similar sites and probably should be united to a single, variable taxon under the name *R. sardous*. *R. trilobus* Desf., which is also a rather similar annual, differs in leaves, flowers and mericarps and is therefore kept as a separate species (Strid 2002).

Diploid, tetraploid and hexaploid plants are known from *Ranunculus sardous*. Ours are the first plants from Romania investigated karyologically so far and were diploid with $2n = 16$ chromosomes. The karyotype consists of 4 metacentric, 2 submetacentric, 8 subtelo- to acrocentric and 2 telocentric chromosomes, the latter with satellites (Fig. 2E). Similar karyotypes are given by Goepfert ($2n = 2x$; 1974), Sopova & Sekovski ($2n = 2x$; 1982), Fujishima ($2n = 6x$; 1990) and Diosdado & Pastor ($2n = 2x$; 1993).

In the molecular phylogenies by Hörandl & al. (2005) and Paun & al. (2005) *Ranunculus sardous* is found in clades with *R. bulbosus* L., *R. neapolitanus* Ten. and *R. polyanthemos* L. These species show rather similar karyotypes as *R. sardous* (*R. bulbosus:* Goepfert 1974; Diosdado & Pastor 1992; *R. neapolitanus:* Goepfert 1974; *R. polyanthemos:* Goepfert 1974; Baltisberger 1980).

Ranunculus marginatus and *R. trilobus* are morphologically and ecologically similar to *R. sardous* and show similar karyotypes, too (*R. marginatus:* Goepfert 1974; Lentini & al. 1988; Baltisberger & Baltisberger 1995; *R. trilobus*: Goepfert 1974; Diosdado & Pastor 1993; Baltisberger unpubl.). These two species are situated in the same clade as *R. sardous* in Paun & al. (2005) but not in Hörandl & al. (2005) where they are clearly separated. Several other species (not resembling *R. sardous*) also show different positions in the two phylogenies but rather similar karyotypes, e.g., *R. muricatus* L. (Goepfert 1974; Diosdado & Pastor 1993), *R. pensylvanicus* L.fil. and *R. repens* L. (Goepfert 1974). The correlation between karyotype and position in molecular phylogenies seems here not to be as strong as in the two cases of *R. acris* and *R. oreophilus*.

Rosaceae

Geum montanum L. – $2n = 6x = 42$
ESE of Timisoara, ESE of Caransebes, Muntii Tarcului, N of Mount Tarcu, Muntele Mic, meadow with rocks, 1550–1600 m, 21.7.1997, *13306 (14015)*.

Geum montanum is a rather variable species of Central and S European mountains. Several infraspecific taxa have been described but usually are not recognized. Two ploidy levels are known, $2n = 4x = 28$ and $2n = 6x = 42$.

Plants investigated from different regions of the Carpathians are all hexaploid (Krahulkova 1994). Ours are the first plants from Romania investigated and also proved to be hexaploid.

Potentilla crantzii (Crantz) Beck – $2n = 4x = 28$
SW of Brasov, SW of Zarnesti, S slope of Piatra Craiului, meadow, 1900–2100 m, 24.7.1997 *(14036)*.

Potentilla crantzii occurs in meadows and on rocky grounds in Eurasia and North America. In Central and S Europe it is confined to mountainous regions. Many ploidy levels are indicated in the literature, ranging from $2n = 4x = 28$ to $2n = 12x = 84$, as well as aneuploid chromosome numbers, mainly between 42 and 49. Ours are the first plants investigated from the Carpathians and proved to be tetraploid with $2n = 4x = 28$ chromosomes.

Scrophulariaceae

Veronica officinalis L. – $2n = 4x = 36$
ESE of Timisoara, ESE of Caransebes, Muntii Tarcului, N of Mount Tarcu, on the road from Caransebes to Muntele Mic, forest, 900 m, 21.7.1997, *13312 (14017)*.

Veronica officinalis is an Eurasian and North American species growing in woods and heaths. Two ploidy levels are known, $2n = 2x = 18$ and $2n = 4x = 36$. Ours are the first plants from the Carpathians investigated and proved to be tetraploid.

General comments

Among the 32 species investigated from the Carpathian region in Romania five species were sampled in ruderal places in the lowlands and all were investigated karyologically for the first time on plants from Romania *(Consolida regalis, Geranium pratense, Ranunculus sardous, Setaria pumila, Stachys annua)*. 27 species were collected in mountainous areas, of which 14 have been investigated for the first time for the whole Carpathian region, seven for the first time on Romanian plants. In *Silene dubia* we found for the first time tetraploid plants with $2n = 4x = 48$ chromosomes in addition to the already known diploid plants.

The genus *Ranunculus* shows a broad variation concerning morphology, life cycle, ecology, geographic distribution as well as karyotypes. It seems that karyology (especially karyotypes) are a useful tool to support the phylogeny of the genus as (at least some) clades in molecular phylogenies show homogenous karyotypes but differ from each other. Further investigations in molecular but also in karyotype analysis are required to test the correspondence of molecular and karyotype data for the entire genus and to elucidate the evolution within *Ranunculus*.

Although the genus *Achillea* has also a broad variation concerning morphology, life cycle, ecology, and geographic distribution as *Ranunculus,* the karyotypes seem to be the same in the whole genus. Karyotypes are therefore not useful to get insights into the evolution of *Achillea*.

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