

MORPHOLOGICAL AND KARYOLOGICAL STUDY OF THE SPECIES OF THE FAMILY GERANIACEAE JUSS. OF ARMENIAN FLORA

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In connection with taxonomic study of representatives of the *Geraniaceae* family of Armenian flora. Usually during the species identification the structure of the root system, life form, leaf morphology, flower structure, inflorescence, carpels structure are taken into account. The karyological research of the genus *Geranium* growing in Armenian flora has revealed the following: for 8 species we discovered diploid and tetraploid cytoraces. The basic chromosome numbers $x = 9, 10, 13, 14$ shows up in the genus *Geranium*.

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Introduction. The *Geraniaceae* Juss. family is rather complicated from taxonomic point of view and includes about 10 genera and 700 species [1]. Majority of species are growing in temperate zone of the Earth and in high mountains of tropical zone. The species are characterized by polymorphism, which makes it difficult to identify them. The *Geraniaceae* family is represented by two genera and 26 species (*Geranium* – 20 species, *Erodium* – 6) in Armenia. These species are distributed in 8 and 2 sections in *Geranium* and *Erodium* genera correspondingly. One additional species from each genera can be found in the territory of the republic, as they grow in adjacent territories, and their ecological features allow them to find favorable conditions in Armenia. In connection with the taxonomic processing of representatives of the family *Geraniaceae* in Armenia an analysis of the diagnostic features species of the family was carried out. The main features that play a role in the construction of dichotomous keys for determining species and sections are identified. Their taxonomic significance has been assessed [2].

Determination of chromosome numbers is the first step in any karyological study. As a result of the accumulation of factual material, this level opens up wide possibilities for using karyological data in systematics. The study of chromosome numbers, in some cases, makes it possible to clarify the issues of speciation and

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phylogenetic relationships. The accumulation of data on chromosome numbers makes it possible to carry out a more differentiated approach to understanding a taxon, species, or even family.

Information about the chromosome numbers of species, growing on the territory of Armenia, is presented in the atlas "Chromosomal numbers of flowering plants of the Armenian flora" [3], which summarizes data on 900 species from more than 2000 populations, 51 families belonging to 320 genera. Until now, representatives of the family *Geraniaceae* of the Armenian flora have not been studied karyologically.

Materials and Methods. The main method for study of the taxonomy of the family is the comparative morphological one, supplemented by karyological and botanical-geographical methods. The morphological characteristics of the species were studied on herbarium specimens and on fresh material. The herbarium collections kept at the Institute of Botany after A. Takhtajyan National Academy of Sciences of the Republic of Armenia (ERE), the Botanical Institute of the Russian Academy of Sciences (LE), the Museum of Natural History of Vienna (W) and the Chair of Botany and Mycology of YSU (ERCB) were studied, as well as own and previously unprocessed collections of species of the family *Geraniaceae*. Regional "Flora" and databases of Plants for a future were studied.

Database search.: <http://www.ibiblio.org>., the Linnaen Plant Name Typification Project.: <http://www.nhm.ac.uk/research-curation/research/projects/linnaean-typification/database/>; the Linnean Collections.: <http://www.linnean-online.org/>.

The determination of the number of chromosomes and the description of the species karyotype were carried out by light microscopy. The seeds were germinated on wet filter paper in Petri dishes in the laboratory (21°C). The karyological investigations were made on the mitotic metaphases of the meristematic cells from root tips. The root tips were pretreated in 0.4% colchicines solution for 2 h; fixed in fluid 3:1 alcohol and glacial acetic acid for at least 2 h at room temperature. After hydrolysis in HCl 1 N for 10–15 min at 60°C the root tips were stained in Schiff reagent at 1.5 h. Then the root tips were squashed on a glass slide with 45% acetic acid. For all chromosome counts, a minimum of 10 plates were examined for each taxon. The stained roots were washed in warm water and placed on a glass slide. The colored tip of the rootlet (0.5–1.0 mm) was crushed in 45% acetic acid under a coverslip. After a preliminary examination of the smears under a microscope, the preparations were placed in butyl alcohol for 5 min, then in xylene for 5 min, and were placed in Canadian balsam. The karyological investigation was based on the samples of species of the genus *Geranium* (*Geraniaceae*) collected by R. Adamyan, K. Tamanyan, G. Fayvush during expeditions in 2003–2010 to the different regions of Armenia. Herbarium material of the Institute of Botany of National Academy of Sciences (ERE) and Yerevan State University (EREU) was studied.

Results and Discussion. The genus *Geranium* L. is represented mainly by annuals and perennials herbs. In general, the genus is characterized by simple, palmately dissected, petiolate or sessile leaves, usually with stipules. All species of geranium have stipules. They can be membranous or leathery in texture, brown or green in color, pubescent or glabrescent. Stipules are lanceolate in shape, oblong-

ovate, ovate and always with a pointed apex. In some cases, they can serve as a diagnostic species feature.

The inflorescence of geranium is of the sympodial type and is characterized as a cymoid or closed thyrus, where directly under the flower at the top of the main axis, one or more lateral axes develop, repeating the structure of the main axis [4].

Pedicels can be two-flowered or one-flowered, erect or deflexed during bearing. The flower of representatives of the genus is actinomorphic, bisexual or less often unisexual. The perianth consists of 5 sepals and 5 petals. Sepals are usually with 3 or 5 (7) veins, membranous margin and awn at apex. Petals are integral or emarginate at apex, with or without short clawed, pubescent or without pubescence at the base. There are 10 stamens in two circles, all with anthers. The stamens of the outer circle are shorter and opposite of the petals, while the stamens of the inner circle are longer and alternate with the petals.

Fruits in geranium syncarpic consists of 5 mericarps, the cavities of the nests of which gradually narrow upwards, forming a column ("beak"), specific to *Geraniaceae* family. There are five nectars alternating with petals. The sterigma of the genus *Geranium* develops from the top syncarp pentamer ovary and is considered by some authors to be a fractional box [5–7]. The mature fruit is divided into 5 single-seeded medicarpies hanging on the top of the central column (the preserved column) [8]. Mature seed in geranium is without endosperm or with a thin layer of endosperm [9]. The embryo fills completely inside the seed. The cotyledons are large, usually flat, folded and with edges on top of each other. They perform a storing function and contain oil and aleurone [10] (Tab. 1).

Table 1

Representative features of the genus *Geranium*

Species	Character of pubescent	Length of awn sepals, mm	Degree of petalse marginate	Carpels surface	Form of leaves plates	Seed surfase
<i>G. sanguineum</i>	long white deflexed pilose	3	emarginate	not deep pilose	roundish-nodulated	line-dotty
<i>G. columbinum</i>	touth, upward eglandular pilose	3	entire	smooth, without wrinkled pressed pilose	rounded or nodulated	with pits
<i>G. dissectum</i>	glandular pilose	0.5–2.0	emarginate	without wrinkled glandular pilose	rounded or nodulated	with pits
<i>G. ibericum</i>	long and soft pilose	1.5–2.5	deeply emarginate with barb	smooth pilose	roundish-pentagonal	–
<i>G. montanum</i>	long white remotod or deflexed pilose	2–3	deeply emarginate	thick pilose	pentagonal	–
<i>G. platypetalum</i>	long eglandular and short glandular pilose	3–4	deeply, narrowly emarginate	pressed upward pilose	broadly wedge-shaped segments	dotty

Table 1 Continuation

<i>G. sylvaticum</i>	remoted pilose	3	entire or weakly emarginate	pressed pilose, smooth	roundish-nodulated	thinly-dotted
<i>G. ruprechtii</i>	pressed pilose	3–4	entire or weakly emarginate	–	rounded or nodulated	–
<i>G. robertianum</i>	remoted pilose	5	entire	reticulated wrinkled, glabrous	pentagonal	thinly-dotted
<i>G. lucidum</i>	glabrous	0.2–0.5	entire	reticulated wrinkled	rounded	smooth
<i>G. palustre</i>	tough or glandular pilose	2–3	entire	pilose, without idges	fivefold	smooth
<i>G. collinum</i>	pressed sometimes glandular pilose	0.1–0.2	entire	smooth pilose	rounded	–
<i>G. albanum</i>	long remoted pilose	1	emarginate	scallop-thickened outgrowths	nodulated rounded	–
<i>G. pyrenaicum</i>	long eglandular and short glandular pilose	0.1–0.3	deeply, narrowly emarginate	upward pilose	nodulated rounded	glabrous
<i>G. pusillum</i>	eglandular and glandular pilose	0.1–0.2	emarginate	pressed pilose, without wrinkled	rounded or roundish-nodulated	smooth
<i>G. molle</i>	soft short pilose	0.1–0.2	deeply emarginate	glabrous, transversely wrinkled	roundish-nodulated	smooth
<i>G. rotundifolium</i>	soft uppermost glandular pilose	0.2–0.5	entire	smooth	roundish-nodulated	with pits
<i>G. divaricatum</i>	glandular and soft remoted eglandular pilose	1.5	emarginate	transversely wrinkled	pinnate-fivefold	smooth
<i>G. tuberosum</i>	short and thick pilose	1	weakly emarginate	smooth pilose	nearly rounded	smooth
<i>G. linearilobum</i>	short declinate pilose	1	weakly emarginate	smooth	nearly rounded	smooth

There are 20 species of the genus *Geranium* growing in the flora of Armenia, and karyologically were investigated following species: *G. columbinum*, $2n=18$ (sect. *Columbinum*); *G. ibericum*, $2n=28$, *G. sylvaticum*, $2n=28$ (sect. *Geranium*); *G. lucidum*, $2n=40$ (sect. *Robertiana*); *G. pyrenaicum*, $2n=26$; *G. pusillum*, $2n=26$; *G. molle*, $2n=26$; *G. rotundifolium*, $2n=26$; *G. divaricatum*, $2n=26$ (sect. *Rotundifolia*). According to the literature data, on the chromosome numbers of the species of the genus *Geranium*, presented in Tab. 1, the different basic chromosome numbers $x = 9, 10, 11, 12, 14, 15, 16, 17, 23, 25$ is revealed.

***G. columbinum* L., $2n=18$.** The species is karyologically well studied. According to the literature data, only the diploid cytotype is characteristic for *G. columbinum* with $2n=18$ [3, 11–13]. Our material also revealed a diploid cytotype for this species $2n=2x=18$ with basic chromosome number $x=9$. The karyotype of

G. columbinum is asymmetric, consisting of 2 pairs of submetacentric and 7 pairs of metacentric chromosomes. Karyotype formula is: $2n=18=4SM+14M$.

***G. ibericum* Cav., $2n=28$.** The species is poorly studied karyologically. According to the literature data, for the European species *G. ibericum*, mainly the tetraploid cytotype was found $2n=4x=56$ [14, 15], in addition, a diploid cytotype is known for this species $2n=28$ [16]. On our material diploid cytotype was also identified for this species $2n=2x=28$ with basic chromosomes number $x=14$. The karyotype of *G. ibericum* is asymmetric, consisting of 7 pairs of metacentric, 4 pairs of submetacentric and 3 pairs chromosomes with satellites: 2 pairs of metacentric and 1 pairs of submetacentric satellite chromosomes. Karyotype formula is: $2n=28=14M+8SM+4M^S+2SM^S$.

***G. sylvaticum* L., $2n=28$.** The species is karyologically well studied. According to the literature data, mainly the diploid cytotype is characteristic for *G. sylvaticum*, $2n=28$, with basic chromosomes number $x=14$ [16–19]. Only for the Belarusian material S.A. Dmitrieva gives the number of chromosomes $2n=24$ [20]. Our material also revealed a diploid cytotype for this species $2n=2x=28$ with basic chromosomes number $x=14$. The karyotype of *G. sylvaticum* is asymmetric, consisting of 6 pairs of metacentric, 5 pairs of submetacentric and 3 pairs of chromosomes with satellites: 2 pairs of metacentric and 1 pairs of submetacentric chromosomes with satellites. Karyotype formula is: $2n=28=12M+10SM+4M^S+2SM^S$.

***G. lucidum* L., $2n=40$.** The species is karyologically well studied. According to the literature data, for the species *G. lucidum*, in most cases, di- and tetraploid cytotypes were found $2n=20, 40$ [12, 13, 17, 18, 21–23]. In addition, for *G. lucidum*, a hexaploid cytotype $2n=60$ ($6x$) is given on Indian populations of this species [24], and for Belarusian populations, an octoploid chromosome number is also known, $2n=80$ [25]. For European populations of this species, chromosome numbers $2n=40–44$ were exhibited [13, 16]. In the studied specimens of the species *G. lucidum*, we found tetraploid cytotype $2n=4x=40$ with basic chromosomes number $x=10$. Karyotype of this species is asymmetric, consisting of 5 pairs of metacentric, 12 pairs of submetacentric and 3 pairs of submetacentric chromosomes with satellites. Karyotype formula is: $2n=40=10M+24SM+6SM^S$.

***G. pyrenaicum* Burm. F., $2n=26$.** The species is karyologically well studied. According to the literature data, mainly the diploid cytotype is characteristic for *G. pyrenaicum* $2n=26$ [11, 12, 16, 18, 21, 26–30], only for Indian populations is given $2n=2x=20$ chromosomes number with basic chromosomes number $x=10$ [31], and for European – $2n=28$ with basic number $x=14$ [32]. In the studied specimens of the species *G. pyrenaicum* also revealed a diploid cytotype $2n=2x=26$ with basic chromosomes number $x=13$, which confirms the previously presented numerous data. Karyotype of this species is asymmetric, consisting of 8 pairs of metacentric, 3 pairs of submetacentric chromosomes and on 1 pairs of meta- and submetacentric chromosomes with satellites. Karyotype formula is: $2n=26=16M+6SM+2M^S+2SM^S$.

***G. pusillum* L., $2n=26$.** The karyology of this species has been fairly well investigated. According to the literature data, for the species *G. pusillum* mainly the diploid cytotype is found $2n=26$ with basic chromosomes number $x=13$ [11, 13, 19, 26, 33, 34]. In the studied specimens of the species *G. pusillum* we found diploid cytotype $2n=2x=26$ with basic chromosomes number $x=13$, which confirms the

previously presented numerous data. The chromosomes number is given for the first time for the flora of Armenia. Karyotype of this species is asymmetry. Diploid set of chromosomes consists of 8 pairs of metacentric, 3 pairs of submetacentric chromosomes and 2 pairs of metacentric chromosomes with satellites. Karyotype formula is: $2n=26=16M+6SM+4M^S$.

***G. molle* L., $2n=26$.** The karyology of this species has been fairly well investigated. According to the literature data, for the species *G. molle* mainly the diploid cytorace is found $2n=26$ [12, 13, 35–37]. In the studied specimens of the species *G. molle* we found diploid cytorace $2n=2x=26$ with basic chromosomes number $x=13$, which confirms the previously presented numerous data. Karyotype of this species is asymmetric, consisting of 3 pairs of metacentric, 8 pairs of submetacentric chromosomes and 1 pairs of metacentric and 1 pairs of submetacentric chromosomes with satellite. Karyotype formula is: $2n=26=6M+16SM+2M^S+2SM^S$.

***G. rotundifolium* L., $2n=26$.** The karyology of this species has been fairly well investigated. According to the literature data, mainly the diploid cytorace is characteristic for the species *G. rotundifolium* $2n=26$ [12, 18, 21, 34, 36]. In the studied specimens of this species we also found diploid cytorace $2n=2x=26$ with basic chromosomes number $x=13$. Karyotype of this species is asymmetric, consisting of 8 pairs of metacentric, 3 pairs of submetacentric chromosomes and 2 pairs meta-, 2 pairs submetacentric chromosomes with satellites. Karyotype formula is: $2n=26=16M+6SM+2M^S+2SM^S$.

***G. divaricatum* Ehrh., $2n=26$.** The species is poorly studied karyologically. According to the literature data, for the Slovakian *G. divaricatum* species mainly was found chromosomes number $2n=26$ with basic number $x=13$ [11], for the European species is given diploid cytorace $2n=28$ with basic number $x=14$ [13, 16, 38]. For our material also revealed a diploid cytorace for this species $2n=2x=26$ with basic chromosomes number $x=13$. The chromosomes number is given for the first time for the flora of Armenia. Karyotype of this species is asymmetry, consisting of 8 pairs of metacentric, 4 pairs of submetacentric chromosomes and 1 pairs of metacentric chromosomes with satellites. Karyotype formula is: $2n=26=16M+8SM+2M^S$.

The representatives of genus *Erodium* are annual, biennial or perennial herbs. The stems of the majority are ascending, simple or scarcely, or else they have rosette forming. Often there is a distinction between stem and basal leaves. Basal leaves are ovate, shortly 3–5 lobed, crenate or crenate-dentate. Leaf size and petiole length are steadily declining over the stem. Upper cauline leaves are usually binary and opposite, more acutely toothed or crenate-lobed, pinnatifid or dissected with stipules. The inflorescence is umbel with bracts, sometimes reduced to 1–2 flowers. The flowers of genus representatives are actinomorphic and consist of five sepals, five petals and five nectaries. Dorsal part of sepals is covered with 3 or 5 (7) veins and awn at apex. Petals are integral or emarginate at apex, with or without short claw, pubescent or without pubescence at the base. Five fertile stamens and five staminodes enclose the gynoecium. The fruit consists of 5 carpels and has a long beak-like extension. Mericarps hairy with stiff separate from the stylar column (rostrum) and awn spirally twisted towards the base, falcate and not twisted above, stiffly plumose along the adaxial surface, reactive to atmospheric humidity thus

working the mericarp into the soil [5, 39]. The seeds are differentiated with elongated shape (Tab. 2).

Table 2

Representative features of the genus *Erodium*

Species	Character of pubescent	Length of awn sepals, mm	Degree of petalae marginate	Length of carpels beak, cm	Form of leaves plates
<i>E. oxyrhynchum</i>	small pressed pilose	3	entire	8.0–9.5	oblong ovate
<i>E. armenum</i>	longe white declinate pilose and small glandular pilose	0.5–1.0	entire	8	oblong
<i>E. sosnowskianum</i>	small glandular and longe eglandular pilose	without own	entire	3.5–4.0	oblong
<i>E. hoefftianum</i>	glandular pilose	3	weakly emarginate	6–8	oblong ovate
<i>E. ciconium</i>	eglandular lammelar and weakly glandular pilose	0.2–0.5	weakly emarginate	7–10	ovate oblonge
<i>E. cicutarium</i>	lammelar or glandular pilose	0.2–0.5	entire	4–5	oblong

According to the literature data, on the chromosome numbers of the species of the genus *Erodium*, the different basic chromosome numbers $x=9, 10, 14, 18, 19$ is revealed, where the basic number of chromosomes $x=10$ predominates. Unfortunately, due to the poor germination of seeds, difficulties arose for the karyological study of species of the genus *Erodium* in the Armenian flora.

Conclusion. For genera and species differentiation we used main morphological characteristics of *Geraniaceae* family. The structure of fruits is distinctive for *Geranium* and *Erodium* genera differentiation. The mature fruits beak is bald and bends in *Geranium* genus, while it is hairy and spiral twisted in *Erodium*. The leaves of *Geranium* representatives are finger-lobed or divided, the length not exceeding its width, while leaves of *Erodium* are dentate- or crenate-lobed, pinnately divided or dissected, the length always exceeding its width. The degree of leaves dissection is a good character for the sectional division. Diagnostic characters for species of the family *Geraniaceae* are the degree of hairiness, types of hair, their position and length; leaf morphology and their location on the stem; shape, texture, color and pubescence of stipules; pubescence and position of pedicels; size, pubescence and number of veins on the sepals, as well as the size of the awn; the size of the clawed or petals; color of stamens; the nature of the valves of the fetus, the size of the elongated part of the beak; the nature of the surface of the seeds.

The karyological research of the genus *Geranium*, growing in Armenian flora, has revealed the following: for 8 species we discovered diploid cytoraces – *G. columbinum* $2n=18$ (sect. *Columbinum*), *G. ibericum* $2n=28$, *G. sylvaticum* $2n=28$ (sect. *Geranium*), *G. pyrenaicum* $2n=26$, *G. pussilum*, $2n=26$, *G. molle*, $2n=26$, *G. rotundifolium*, *G. divaricatum*, $2n=26$ (sect. *Rotundifolia*) $2n=26$ with different basic chromosome numbers $x=9, 13, 14$. For the species *G. lucidum* (sect. *Robertiana*)

a tetraploid cytorace $2n=40$ was discovered with the basic chromosome number $x=10$. The different basic chromosome numbers $x=9, 10, 13, 14$ (Tab. 3) shows up in the genus *Geranium*.

Table 3

Basic chromosome numbers and polyploid series of the representatives of the genus *Geranium* of the flora of Armenia

X	9	10	13	14
	←			
2x	18		26	28
3x	–	–	–	–
4x		40		

The species of the section *Rotundifolia*, growing on the territory of Armenia (*G. pyrenaicum*, $2n=26$; *G. pusillum*, $2n=26$; *G. molle*, $2n=26$; *G. rotundifolium*, $2n=26$; *G. divaricatum*, $2n=26$), are characterized with the basic main chromosome number of $x=13$.

The main basic chromosome number of $x=9$ is typical for the species of the section *Columbinum* growing on the territory of Armenia (*G. columbinum*, $2n=18$).

The main basic chromosome number of $x=10$ is typical for the species of the section *Robertiana*, growing on the territory of Armenia (*G. lucidum*, $2n=40$).

The main basic chromosome number of $x=14$ is typical for the species of the section *Geranium*, growing on the territory of Armenia (*G. ibericum*, $2n=28$; *G. sylvaticum*, $2n=28$).

Asymmetric karyotype is observed in all the karyologically investigated species of the genus *Geranium*.

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ՀԱՅԱՍՏԱՆԻ ՖԼՈՐԱՅԻ *GERANIACEAE* JUSS. ԸՆՏԱՆԻՔԻ
ՏԵՍԱԿՆԵՐԻ ՄՈՐՖՈԼՈԳԻԱԿԱՆ ԵՎ ԿԱՐԻՈԼՈԳԻԱԿԱՆ
ՈՒՍՈՒՄՆԱՍԻՐՈՒԹՅՈՒՆ

Կատարվել է Հայաստանի ֆլորայի *Geraniaceae* ընտանիքի ներկայացուցիչների տարսոնոմիական վերլուծություն: Տեսակների նույնականացման համար կարևորվել են արմատային համակարգի կառուցվածքը, կենսաձևը, տերևի մորֆոլոգիան, ծաղկի կառուցվածքը, ծաղկաբույլը, պտղի կառուցվածքը: Հայաստանի ֆլորայում աճող *Geranium* ցեղի 8 տեսակների կարիոլոգիական ուսումնասիրման արդյունքում հայտնաբերվել են դիպլոիդ և տետրապլոիդ ցիտոռասաներ: *Geranium* ցեղում առկա է հիմնական քրոմոսոմային թվերի հետևյալ շարքը $x=9, 10, 13, 14$:

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МОРФОЛОГИЧЕСКОЕ И КАРИОЛОГИЧЕСКОЕ ИССЛЕДОВАНИЕ
ВИДОВ СЕМЕЙСТВА *GERANIACEAE* JUSS. ФЛОРЫ АРМЕНИИ

В связи с таксономической обработкой представителей сем. *Geraniaceae* флоры Армении проведен анализ диагностических признаков. При идентификации видов обычно учитываются строение корневой системы, жизненная форма растения, морфология листа, строение цветка, соцветие, строение плодов. В результате кариологического исследования видов рода *Geranium* (сем. *Geraniaceae*), произрастающих в Армении, для 8 видов обнаружена диплоидная и тетраплоидная циторасса. В роде *Geranium* вырисовывается анеуплоидный ряд основных хромосомных чисел $x=9, 10, 13, 14$.