CONTRIBUTION TO THE KARYOMORPHOLOGY QF 7 SPECIES IN *GENTIANA* (GENTIANACEAE) *

Ho Tingnong　Liu Jianquan　Chen Shilong

(Northwest Plateau Institute of Biology, the Chinese Academy of Sciences, Xining, 810001)

Abstract

This paper reports the karyomorphological observations on seven species of *Gentiana*. The chromosomes of all the studied species at resting stage and mitotic-prophase are commonly of the complex chromocenter type and interstitial type, respectively. The karyotype formulae are as follows: *G. souliei* $2n=46=34m+10sm+2st=4L+14M_2+24M_1+4S$; *G. serra* $2n=34=12m+12sm+10s=2L+12M_2+20M_1$; *G. caeruleo-grisea* $2n=16=10m+6sm=10M_2+6M_1$; *G. siphonantha* $2n=26=26m(2SAT)=4L+6M_2+14M_1+2S$; *G. nubigena* $2n=24=24m=14M_2+10M_1$; *G. purdomii* $2n=24=20m+4sm=14M_2+10M_1$; *G. wasenensis* $2n=24=22m+2sm=16M_2+8M_1$.

Key words: *Gentiana*; Karyomorphology; Cytotaxonomy

Gentiana contains 361 species belonging to 15 sections (Ho & Liu 1990), widely distributed in the northern temperate zone. Its high diversity of species and sections occurred in west China (Ho et al. 1996). Although numerous reports on the chromosome numbers and karyotypes of *Gentiana* had been made (Yuan, 1993; Yuan & Kupfer 1993; Kupfer & Yuan, 1996), it is difficult to understand the mechanism of the chromosomal evolution in *Gentiana* since morphological and chromosomal homoplasy prevails in the genus. In order to detect the phylogenetic relationship of the genus *Gentiana*, a series of cytotaxonomical studies were carried out to answer some of the questions concerning chromosome data of Chinese species, especially the species from the high altitude regions of west China. We attempted, by observing as many species as possible, to find the key chromosome numbers and the links between them, in order to understand the chromosomal evolution of the genus. This paper is the fourth in a series of reports (Ho et al. 1997; Chen et al. 1997) dealing with chromosomal observations on Chinese gentians.

Materials and Methods

The materials and their localities are tabulated in Table 1. The voucher specimens are preserved in the

* The project was supported by the Key Project Foundation of the Chinese Academy of Sciences and the National Natural Science Foundation of China.

herbarium of Northwest Plateau Institute of Biology, the Chinese Academy of Sciences, The People's Republic of China (HNWP).

Table 1 Localities and chromosome numbers of the examined taxa in *Gentiana*

Taxon	Material	No.	Locality	Voucher
G. serra	Roots	34	Lijiang, Yunnan, 2400m	Liu Jianquan 337
G. souliei	Roots	46	Lijiang, Yunnan, 2400m	Liu Jianquan 349
G. caeruleo-grisea	Ovaries	16	Maqenm, Qinghai, 3500m	H.B.G. 544
G. purdomii	Ovaries	14	Darlag, Qinghai, 3800m	H.B.G. 1252
G. siphonantha	Roots	26	Tongren, Qinghai, 2600m	Liu Shangwu 3550
G. nubigena	Roots	24	Maqin, Qinghai, 4900m	H.B.G. 727
G. wasenensis	Roots	24	Baoxing, Sichuan, 3400m	Liu Jianquan 269

For the observations of ovaries, the method introduced by Liu (1996) was taken. For the growing root tips, the procedure was as same as our former reports (Ho et al., 1997; Chen et al. 1997). The karyotype formulae are estimated according to Leavan et al. (1964). The terminology used for chromosome complement of relative length (C.R.L.) is that defined by Kuo et al. (1972). The classification of karyotype asymmetry follows Stebbins (1971). The intrachromosomal asymmetry index (A_1) and the interchromosomal asymmetry index (A_2) proposed by Romero (1986) as well as index of the karyotypic asymmetry (As.K%) proposed by Arano (1963) were adopted.

Results

All of the plants of 7 species of Gentiana have commonly the resting nuclei in their root meristematic cells, which show several darkly stained, aggregated chromocenters and several large heteropycnotic bodies varying in size and number (Plate I: 1). Thus, the resting chromosomes of 5 species could be classified as the complex chromocenter type.

Mitotic-prophase chromosomes in the 7 species contain heterochromatic segments in the interstitial regions of most chromosomes of each complement (Plate I: 2). Thus, the mitotic-prophase chromosomes could be classified as the interstitial type.

1. *Gentiana souliei* Franch. (Sect. *Stenogyne*) Table 2; Plate I: 3, 4

All plants studied show the mitotic-metaphase chromosome number of $2n = 46$ and the chromosome complement consists of 34 median-centromeric, 10 submedian-centromeric and 2 subterminal-centromeric chromosomes. Chromosome bimodality is indistinct. However, the complement of relative length of the plants from population 1 exhibits 4 long (4L), 14 longer ($14M_2$), 24 shorter ($24M_1$) and 4 short (4S) chromosomes. The total length of the chromosome complement is 59.03 μm with the lengths ranging from 1.76 to 4.03 μm. The mean length is 2.57 μm. The karyotype was estimated to be the 2B type according to Stebbins' classification. The karyotype asymmetry indices are $A_1 = 0.30$, $A_2 = 0.16$ and As.K% = 60.03. The chromosome number and karyomorphology of this species are reported here for the first time.

Table 2 The chromosome parameters in *G. serra* and *G. souliei*

G. serra						*G. souliei*					
No.	RL	RS	RT	AR	PC	No.	RL	RS	RT	AR	PC
1	6.09	1.81	7.90	3.36	st	1	5.32	1.51	6.83	3.52	st
2	5.30	1.92	7.22	2.75	sm	2	3.40	2.49	5.89	1.37	m
3	3.88	3.12	7.00	1.24	m	3	3.34	1.52	4.86	2.19	sm
4	3.77	2.97	6.74	1.27	m	4	2.46	2.39	4.85	1.03	m
5	4.28	2.21	6.49	1.93	m	5	3.59	1.22	4.81	2.94	sm
6	4.86	1.60	6.46	3.04	sm	6	2.51	2.27	4.78	1.10	m
7	4.17	1.96	6.13	2.13	st	7	3.12	1.64	4.76	1.90	sm
8	3.99	1.74	5.73	2.29	sm	8	3.18	1.51	4.69	2.11	sm
9	4.03	1.56	5.59	2.58	sm	9	2.45	2.24	4.69	1.10	m
10	4.17	1.05	5.22	3.96	sm	10	2.25	2.05	4.30	1.10	m
11	2.97	2.21	5.18	1.34	st	11	2.66	1.57	4.23	1.69	m
12	3.19	1.99	5.18	1.60	m	12	2.32	1.88	4.20	1.23	m
13	3.99	1.16	5.15	3.43	m	13	2.12	2.08	4.20	1.02	m
14	3.48	1.67	5.15	2.09	st	14	2.35	1.81	4.16	1.30	m
15	3.08	2.07	5.15	1.49	sm	15	2.20	1.95	4.15	1.13	m
16	4.17	0.91	5.08	4.60	st	16	2.24	1.57	3.99	1.54	m
17	2.61	1.96	4.57	1.33	m	17	2.08	1.85	3.93	1.13	m
						18	2.18	1.73	3.91	1.26	m
						19	2.08	1.81	3.89	1.15	m
						20	2.42	0.92	3.34	2.65	sm
						21	1.85	1.46	3.31	1.27	m
						22	1.69	1.52	3.21	1.11	m
						23	1.57	1.41	2.98	1.12	m

RL = relative length of long arm; RS = relative length of short arm; RT = relative total length of chromosome; AR = arm ratio; PC = position of centromere; m = median; sm = submedian; st = subterminal.

2. *Gentiana serra* Franch. (Sect. *Stenogyne*) Table 2; Plate Ⅰ: 5, 6.

The chromosome number of this species is 2n = 34 at mitotic-metaphase. The chromosome complement consists of 12 median-centromeric, 12 submedian-centromeric and 10 subterminal-centromeric chromosomes. The complement of relative length exhibits 2 long (2L), 12 longer ($12M_2$) and 20 shorter ($20M_1$) chromosomes. The total length of the chromosome complement is 27.56 μm with the lengths ranging from 1.26 to 2.18 μm. The mean length is 1.62 μm. Chromosome bimodality is indistinct. The karyotype was estimated to be the 2A type of Stebbins (1971). The karyotype asymmetry indices are $A_1 = 0.50$, $A_2 = 0.16$ and As.K% = 68.07. The chromosome number and karyomorphology of this species are reported here for the first time.

3. *Gentiana caeruleo-grisea* T. N. Ho (Sect. *Dolichocarpa* T. N. Ho). Tabe 3, Plate Ⅱ: 7, 8.

The chromosome number of this species is 2n = 16 at mitotic-metaphase. The chromosome complement consists of 10 median-centromeric and 6 submedian-centromeric chromosomes. The complement of relative

length exhibits 10 longer (10 M_2) and 6 shorter (6 M_1) chromosomes. The total length of the chromosome complement is 25.56 μm. The lengths range from 2.49 to 3.59 μm and the mean length is 3.20 μm. Chromosome bimodality is indistinct. The karyotype was estimated to be the 2A type of Stebbins (1971). The karyotype asymmetry indices are $A_1 = 0.26$, $A_2 = 0.11$ and As.K% = 58.21. The chromosome number and karyomorphology of this species is reported here for the first time.

Table 3 The chromosome parameters of *G. caeruleo-grise*

G. caeruleo-grisea										
No.	RL	RS	RT	AR	PC	No.	RL	RS	RT	AR	PC
1	7.52	6.55	14.07	1.15	m	5	6.68	6.24	12.92	1.09	m
2	8.50	4.87	13.27	1.75	sm	6	7.80	4.32	12.12	1.81	sm
3	8.91	4.18	13.09	2.13	sm	7	6.82	4.87	11.69	1.40	m
4	6.69	6.27	12.96	1.07	m	8	5.29	4.46	9.75	1.19	m

See Table 2 for the explanation of abbreviations.

4. *Gentiana purdomii* Marq. (Sect. *Frigida*) Table 4, Plate Ⅱ: 9, 10.

The chromosome number at mitotic-metaphase is 2n = 24; 20 median centromeric and 4 submetacentric chromosomes. The complement of relative length shows 10 longer (10M_2) and 14 shorter (14M_1) chromosomes. The chromosome complement has a total of 30.54 μm and a mean length of 2.55 μm. The lengths range from 2.28 to 2.96 μm. Chromosome bimodality is indistinct. The karyotype classification belongs to Stebbins' 1A type. The karyotype asymmetry indices are $A_1 = 0.24$, $A_2 = 0.08$ and As.K% = 57.34. The chromosome number and karyomorphology of this species are reported here for the first time.

Table 4 The chromosome parameters in *G. wasanensis* and *G. purdomii*

G. wasanensis						*G. purdomii*					
No.	RL	RS	RT	AR	PC	No.	RL	RS	RT	AR	PC
1	5.08	4.42	9.50	1.15	m	1	6.18	3.50	9.68	1.77	m
2	5.06	4.31	9.37	1.18	m	2	4.66	4.55	9.21	1.02	m
3	4.97	4.20	9.17	1.18	m	3	5.24	3.50	8.74	1.50	m
4	5.64	3.32	8.96	1.70	m	4	4.66	3.96	8.62	1.18	m
5	4.97	3.87	8.84	1.29	m	5	5.01	3.38	8.39	1.48	m
6	4.42	4.31	8.73	1.03	m	6	4.20	4.08	8.28	1.03	m
7	5.41	3.32	8.73	1.63	m	7	4.20	3.96	8.16	1.06	m
8	4.75	3.87	8.62	1.23	m	8	4.66	3.50	8.16	1.33	m
9	3.98	3.65	7.63	1.09	m	9	4.90	3.15	8.05	1.56	m
10	3.76	3.32	7.08	1.13	m	10	4.20	3.50	7.70	1.20	m
11	3.65	3.21	6.86	1.14	m	11	4.66	2.91	7.57	1.60	m
12	3.32	3.21	6.53	1.03	m	12	4.78	2.68	7.46	1.78	sm

See Table 3 for the explanation of abbreviations.

5. *Gentiana siphonantha* Maxim. ex Kusn. (Sect. Cruciata) Table 5; Plate Ⅱ: 11, 12.

This species shows the chromosome number of 2n = 26. The mitotic-metaphase complement consists of 26

median-centromeric chromosomes. The third pair of chromosome show a secondary constriction. The complement of relative length shows 4 long (4L), 6 longer ($6M_2$), 14 shorter ($14M_1$) and 2 short (2S) chromosomes. The total length of the chromosome complement is 30.29 μm with the lengths ranging from 1.69 to 3.19 μm and a mean length of 2.33 μm. Chromosome bimodality is indistinct. The karyotype was categorized to be the 1A type according to Stebbins (1971). The karyotype asymmetry indices are $A_1 = 0.16$, $A_2 = 0.19$ and As.K% = 54.67. The chromosome number and karyomorphology of this species are reported here for the first time.

6. *Gentiana nubigena* Edgew. (Sect. Frigida) Table 5, Plate Ⅱ: 13, 14.

The chromosome number at mitotic metaphase is 2n = 24. The chromosome complement consists of 20 median-centromeric and 4 submedian-centromeric chromosomes and the complement of relative length shows 14 longer ($14M_2$) and 10 shorter ($10M_1$) chromosomes. Chromosome bimodality is indistinct. The total length of the chromosome complement is 28.91 μm with lengths ranging from 1.96 to 2.71 μm. The mean length is 2.41 μm. The karyotype was categorized to be the 2A type of Stebbins (1971). The karyotype asymmetry indices are $A_1 = 0.23$, $A_2 = 0.09$ and As.K% = 56.89. The chromosome number and karyomorphology of this species are reported here for the first time.

Table 5 The chromosome parameters in *G. nubigena* and *G. siphonantha*

G. nubigena						*G. siphonantha*					
No.	RL	RS	RT	AR	PC	No.	RL	RS	RT	AR	PC
1	4.93	4.43	9.36	1.11	m	1	5.48	5.05	10.53	1.09	m
2	5.30	3.94	9.24	1.35	m	2	6.40	3.93	10.33	1.63	m
3	4.56	4.44	9.00	1.03	m	3	4.95	4.02	8.97	1.23	m
4	5.17	3.69	8.86	1.40	m	4	4.13	3.93	8.06	1.05	m
5	5.54	3.09	8.63	1.80	sm	5	4.32	3.50	7.82	1.23	m
6	4.56	3.94	8.50	1.16	m	6	4.03	3.20	7.23	1.26	m
7	5.91	2.46	8.37	2.40	sm	7	4.13	3.07	7.20	1.35	m
8	4.80	3.45	8.25	1.39	m	8	3.93	3.17	7.10	1.24	m
9	4.43	3.57	8.00	1.24	m	9	3.73	3.30	7.03	1.13	m
10	4.31	3.33	7.74	1.29	m	10	3.80	3.20	7.00	1.19	m
11	3.82	3.57	7.39	1.07	m	11	3.53	3.27	6.80	1.08	m
12	3.57	3.20	6.79	1.12	m	12	3.24	3.10	6.34	1.05	m
						13	3.00	2.58	5.58	1.16	m

See Table 3 for the explanation of abbreviations.

7. *Gentiana wasenensis* Marq. (Sect. Frigida) Table 4. Plate Ⅱ: 15, 16.

This species has the chromosome number of 2n = 24 at mitotic-metaphase: 22 median-centromeric and 2 submedian-centromeric chromosomes. The complement of relative length shows 16 longer ($16M_2$) and 8 shorter ($8M_1$) chromosomes. The total length of the chromosome complement is 32.21 μm. The mean length is 2.69 μm. The lengths range from 2.1 to 3.06 μm. Chromosome bimodality is indistinct. The karyotype was estimated to be the 1A type of Stebbins (1971). The karyotype asymmetry indices are $A_1 = 0.17$, $A_2 = 0.12$ and As.K% = 55.00. The chromosome number and karyomorphology of this species are reported here for the first time.

Discusion

According to the available chromosome data (Shigenobu, 1984; Yuan, 1993; Yuan & Kupfer 1993; Kupfer & Yuan 1996; Ho et al. 1997; Chen et al., 1997), a wide range of chromosome number variation was found in the genus *Gentiana* from 2n = 12 to 96. As pointed out by Kupfer & Yuan (1996), great variations exist among some sections. Some sections are restricted to a certain chromosome number while some sections show much higher variations. That means the chromosome data are usually incongruent with the morphological data. Therefore, it is still difficult and sometimes impossible to determine a basic number for a specific section since the karyological mechanism and polarities of chromosomal variation is not clear yet. It is too early to specialize the ancestral basic number of *Gentiana*. Furthermore, as indicated in the present paper and the former reports (Shigenobu, 1984; Yuan, 1993; Yuan & Kupfer 1993; Kupfer & Yuan 1996; Ho et al. 1997) the karyotype structures among the taxa of *Gentiana* are very similar, mainly consisting of median-centromeric and submedian-centromeric chromosomes without bimodality. The differentiation of the karyotypes among species or sections is indistinct. The karyotype results add more puzzles to understand the chromosomal mechanisms of this genus. New experimental paths to detect the chromosomal evolution of *Gentiana* are badly needed.

References

Arano H., 1963. Cytological studies in Subfamily Carduoideae (Compositae) of Japan IX. The karyotype analysis and phylogenic considerations on Perta and Ainsliaea (2). *Bot. Mag. Tokyo* 76: 32~39.

Chen S L, Ho T N, Liu J Q, 1997. The chromosome numbers of eight species in Gentiana (Gentianaceae) from alpine mountains of the western China. *Acta Bot Oreal-Occid Sin* 17 (4): 547~550.

Ho T N, J Q L, Zhang D C, 1997. The karyotypes and chromosome numbers in nine species of Gentiana from alpine mountains of western China. *Acta Biol Plateau Sin* 13: 9~21.

Ho T N, Liu S W, 1990. The infrageneric classication of Gentiana (Gentianaceae). *Bull Br Mus (Nat Hist), Bot* 20: 169~192.

Ho T N, Liu S W, Lu X F, 1996. A phylogenetic analysis of *Gentiana* (Gentianaceae). *Acta Phytotax. Sin* 34 (5): 505~530.

Hong D Y, 1990. Plant Cytotaxonomy. Beijing: Science Press, 333.

Kuo S. R, 1972. Karyotype analysis of some Formosan Gymnosperms. *Taiwania* 17(1): 66~80.

Kupfer P, Yuan Y M, 1996. Karyomorphological studies on Gentiana Sect. Chondrophyllae (Gentianaceae) from China. *Pl. Syst. Evol.* 200: 161~176.

Levan A., Fredga K., Sandberg A. A., 1964. Nomenclature for centromeric position on chromosomes. *Hereditas* 52: 201~220.

Liu J Q, 1997. The application of alcoholic hydrochloric acid carmine stain the plant chromosome squash preparations. *Chinese Bull Bot*, 1996; 13(2): 63~64.

Romero Z C, 1986. A new method for estimating karyotype asymmetry. *Taxon* 35: 526~530.

Shigenobu Y, 1984. Karyomorphological studies in some genera of Gentianaceae. II. Gentiana and its allied four genera. *Bull Coll Child Developm Kochi Womens Univ*, 8: 55~104.

Stebbins G. L, 1971. Chromosomal evolution in higher plants. London (Edward Arnold Ltd.).

Tanaka R, 1974. Organization system of mitotic division and development of reproductive cells in higher plants. The cell 6: 22~25.

Tanaka R, 1974. Recent karyotype studies. In: K Ogawa et al., Eds., Plant cytology. Tokyo: Asakura Shoten. 293~326.

Yuan Y M, 1993. Karyological studies on Gentiana Sect. Cruciata (Gentianaceae) from China. Caryologia 46: 99～114.
Yuan Y M, Kupfer Ph, 1993. Karyological studies on *Gentiana* Sect. *Frigida* s.l. and Sect. *Stenogyne* (Gentianaceae) from China. *Bull Soc Neuchatel Sci Nat* 116 (2): 65～78.
Zhukova P G, 1967. Chromosome numbers in some species of plants from the northern part of the USSR. II. *Bot Zhurn*, 51: 1511～1516.

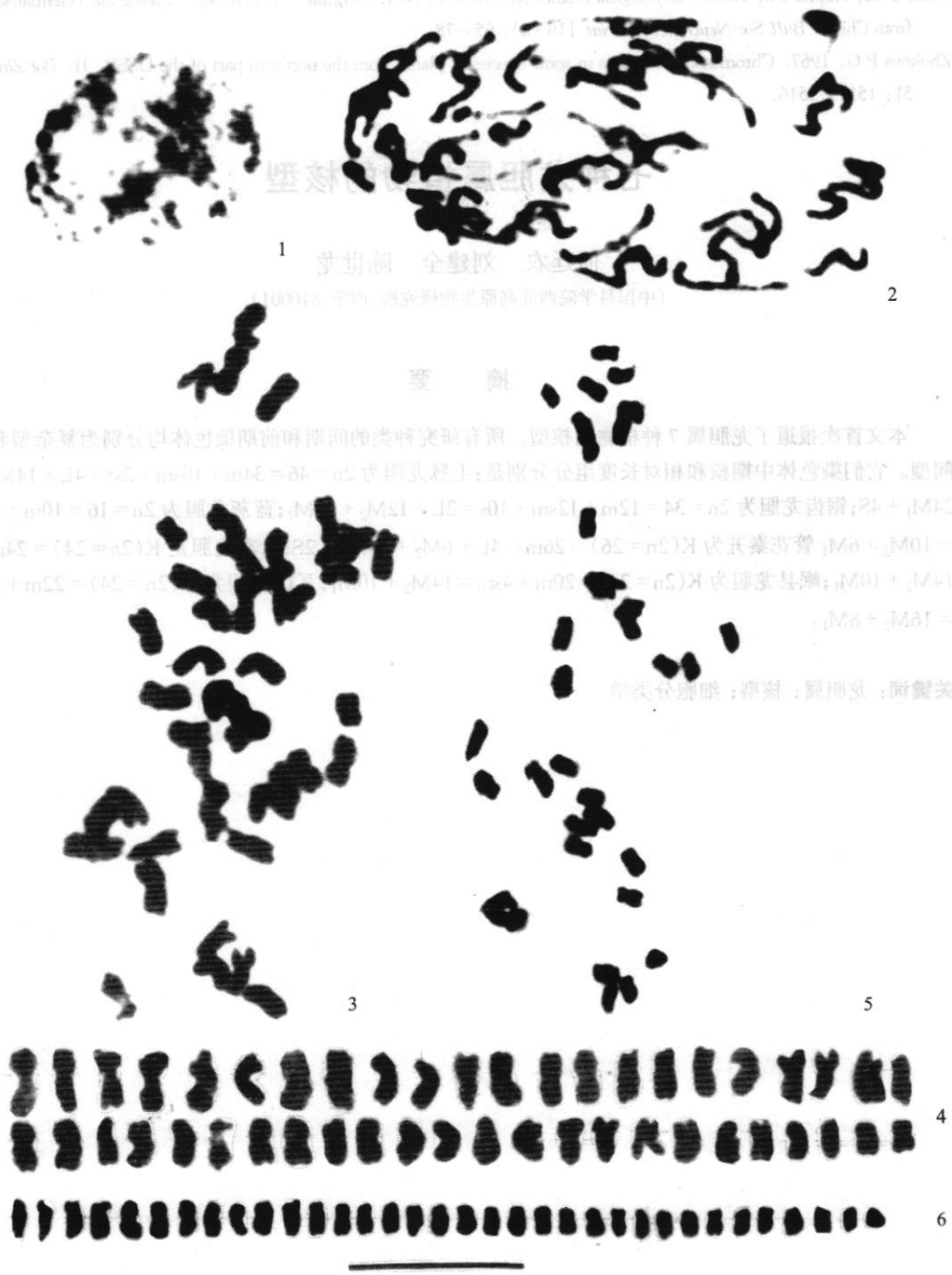
七种龙胆属植物的核型

何廷农　刘建全　陈世龙
（中国科学院西北高原生物研究所，西宁，810001）

摘　要

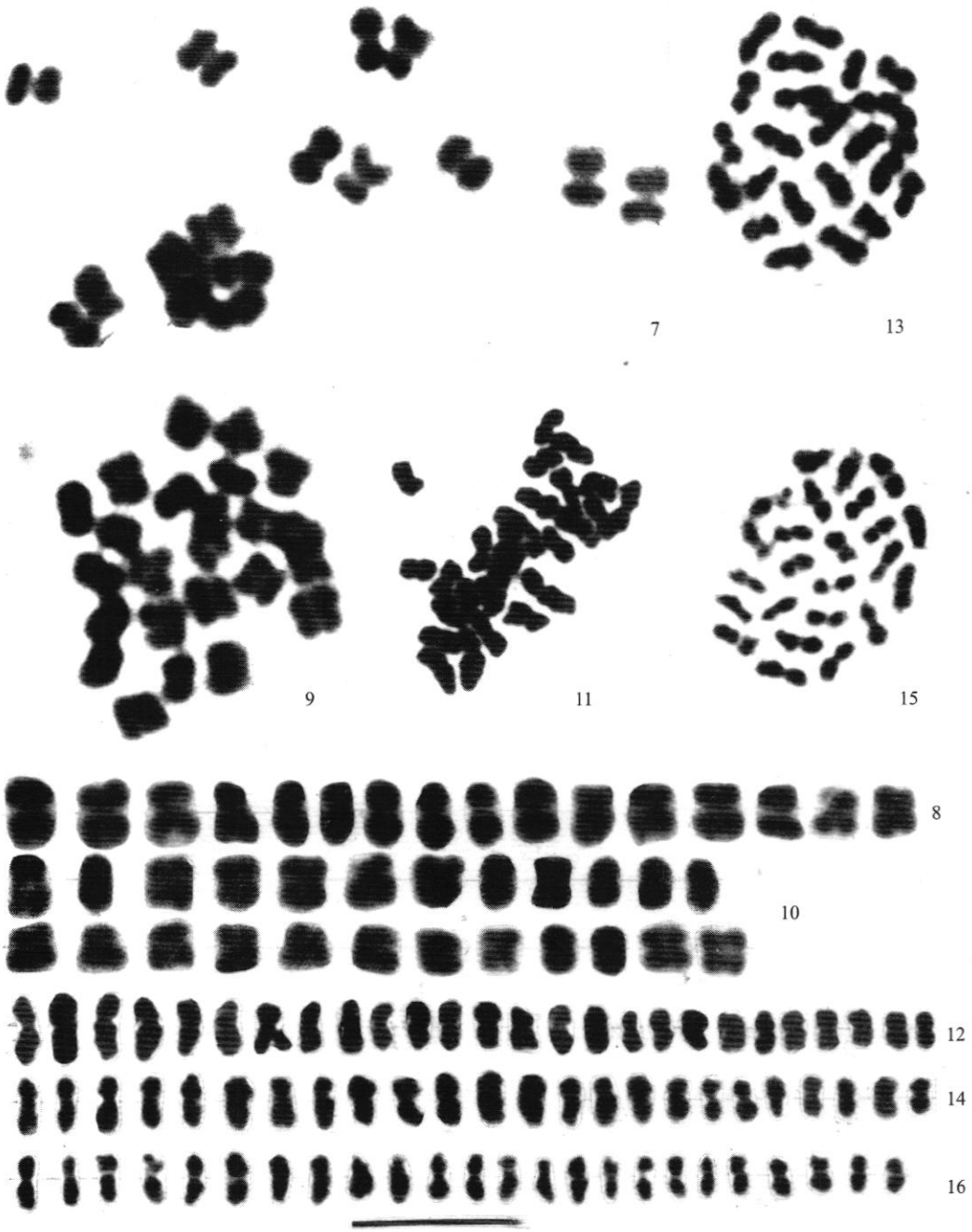
本文首次报道了龙胆属7种植物的核型。所有研究种类的间期和前期染色体均分别为复杂型和中间型。它们染色体中期核和相对长度组分分别是：毛脉龙胆为 $2n=46=34m+10sm+2st=4L+14M_2+24M_1+4S$；锯齿龙胆为 $2n=34=12m+12sm+10s=2L+12M_2+20M_1$；蓝灰龙胆为 $2n=16=10m+6sm=10M_2+6M_1$ 管花秦艽为 $K(2n=26)=26m=4L+6M_2+14M_1+2S$；云雾龙胆是 $K(2n=24)=24m=14M_2+10M_1$；岷县龙胆为 $K(2n=24)=20m+4sm=14M_2+10M_1$；瓦山龙胆为 $K(2n=24)=22m+2sm=16M_2+8M_1$。

关键词：龙胆属；核型；细胞分类学



1, 2, 3, 4. Somatic chromosomes of *Gentiana souliei*: 1. Resting stage. 2. Mitotic prophase. 3. Mitotic metaphase. 4. Karyotypes.

5, 6. Somatic chromosomes of *G. Serra*: 5. Mitotic metaphase. 6. Karyotypes.



7, 8. Somatic chromosomes of *G. caeruleo-grisea*: 7 Mitotic metaphase. 8. Karyotypes. 9, 10. Somatic chromosomes of *G. purdomii*: 9 Mitotic metaphase. 10. Karyotypes. 11, 12. Somatic chromosomes of *G. siphonantha*: 11 Mitotic metaphase. 12. Karyotypes. 13, 14. Somatic chromosomes of *G. nubigena*: 13 Mitotic metaphase. 14. Karyotypes. 15, 16. Somatic chromosomes of *G. wasenensis*: 15 Mitotic metaphase. 16. Karyotypes. Scale bars = 10 μm