Chromosome numbers and polyploidy in Leontopodium (Asteraceae: Gnaphalieae) from the Qinghai-Tibet Plateau of S.W. China

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Leontopodium R. Br. ex Cass. is the second largest genus within the Asian Gnaphalieae (Asteraceae) and is most diversified on the Qinghai-Tibet Plateau and its adjacent areas of SW China. The chromosome numbers and karyomorphology of five species from this region were investigated for the first time: *Leontopodium sinense* ($2n = 28 = 22m + 6sm$), *L. muscoides* ($2n = 24 = 22m + 2sm$), *L. souliei* ($2n = 52 = 32m + 20sm$), *L. pusillum* ($2n = 72 = 52m + 20sm$), and *L. nanum* ($2n = 48 = 24m + 24sm$). The results suggest that the basic chromosome number of *Leontopodium* is $x = 14$, followed with dysploidy numbers (e.g. $x = 12, 13$). Polyploidization has also played an important role in the evolution of *Leontopodium* in this region.

Keywords: chromosome number; Gnaphalieae; *Leontopodium*; polyploidy; Qinghai-Tibet Plateau

Introduction

The tribe Gnaphalieae (paper daisies) of the family Asteraceae (or Compositae) contains *c.*185 genera and 1240 species distributed on most continents (Anderberg 1991; Bayer et al. 2007). They are most diverse in South Africa and Australia, with only two genera well diversified in Asia (*Anaphalis* DC. and *Leontopodium* R. Br. Ex Cass). *Anaphalis* is the largest genus within the Asian Gnaphalieae and *Leontopodium* is the second largest, comprising 30–41 species (Blöch et al. 2010; Safer et al. 2011). The main distribution of the genus is central and eastern Asia, including Russia, Japan, South Korea, Mongolia, China and the Himalayas to the borders of Afghanistan and Pakistan, with two species extending to Europe. Results based on **AFLP** (Amplified Fragment Length Polymorphism) suggest that the south-west of China is the centre of diversity and perhaps also the origin area of the genus (Safer et al. 2011). Molecular data also indicate that the monophyly *Leontopodium* (including *Sinoleontopodium*) is a characteristic Sino-Himalayan element that appears to have found its way into the mountains of Europe in geologically recent times (Anderberg 1991; Blöch et al. 2010).

Polyploidy is common in plants of cold climates with harsh and stressful environments (Löve and Löve 1949, 1967; Grant 1981). The Qinghai-Tibet Plateau has extreme elevation ranges compressed over short distances, largely because of the collision of India with Eurasia (Zhang et al. 1984; Dewey et al. 1988; Wen 1999). This alpine region has been designated a Himalayan biodiversity hotspot because of its high species richness and endemism (Myers et al. 2000). A high rate of polyploidy could be expected in this region because of the unusually high species diversity and endemism, the widespread alpine environment, and the importance of polyploidy in plant evolution. However, Nie et al. (2005) indicated that polyploidy may have played only a minor role in the evolutionary diversification in the region. More data need to be accumulated for a better understanding of chromosomal evolution of plants from this interesting region.

The chromosome number and karyology of six species in *Leontopodium* has been reported, mainly from the western Himalayan region, with the reported chromosome numbers of $n = 12, 14$ or $2n = 26, 48, 49, 52$ and 73 (Siljak 1977; Krogulevich 1978; Murin and Paclova 1979; Zhukova 1980; Nishikawa 1985; Vir Jee and Kachroo 1985; Khatoon and Ali 1988). However, no cytological reports have been performed on *Leontopodium* from the Sino-Himalayas, especially the Qianghai-Tibet Plateau. The present study aims at broadening our karyological knowledge of *Leontopodium* species of the Qinghai-Tibet Plateau and investigating whether polyploidy plays an important role in this genus.

Materials and methods

Seeds were collected representing five species of *Leontopodium* (Table 1). Voucher specimens are deposited in

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Table 1.   Locality, geographical position, altitude and voucher number of the investigated species of *Leontopodium*.

Taxon	Locality	Position	Altitude (m)	Voucher
Leontopodium sinense Hemsl.	Zuogong, Tibet	29°55′ N, 97°26′ E	4350	Sunh-07zx-0652
Leontopodium muscoides (Hook. f. et Thoms.) Hand.-Mazz.	Mangkang, Tibet	29°54′ N, 98°36′ E	4000	Sunh-07zx-1732
Leontopodium souliei Beauv.	Zuogong, Tibet	29°31′ N, 97°56′ E	3710	Sunh-07zx-2098
Leontopodium pusillum Hand.-Mazz.	Naqu, Tibet	30°46′ N, 90°57′ E	4715	Yangyp-Q-0152
Leontopodium nanum (Hook. f. et Thoms.) Hand.-Mazz.	Dangxiong, Tibet	30°49′ N, 91°08′ E	4653	Yangyp-Q-1000

the Herbarium of Kunming Institute of Botany (KUN). All cytological observations were made from root tips. Seeds were stored at 4°C, soaked overnight in distilled water at room temperature, and germinated on wet filter paper in Petri dishes. Fresh root tips *c*.1 cm long were cut, pretreated in 0.003 M 8-hydroxyquinoline solution at 22–23°C in the dark for 6–8 h, then fixed with Carnoy's fluid (1:3 glacial acetic acid/absolute alcohol) at approximately 4°C for 2–5 h. They were then placed in 70% ethanol for 10 min and rinsed in distilled water twice for *c*.20 min. Prior to staining, the root tips were hydrolysed in 1:1 1N HCl:45% acetic acid at 60°C for 30 s, stained with 1% aceto-orcein overnight, and squashed for cytological observation. Permanent slides were made using the standard liquid nitrogen method.

Observations were made of somatic mitotic interphase, prophase and metaphase. Karyomorphological classification of the mitotic interphase nuclei and prophase chromosomes follows Tanaka (1971, 1977, 1987), the designation of the centromeric position as median region (m) or submedian region (sm) follows Levan et al. (1964) and the karyotype asymmetry is classified according to Stebbins (1971).
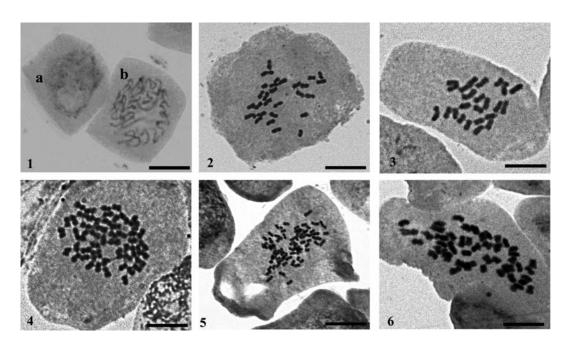
Results

The interphase nuclei of *Leontopodium* can be categorized based on their shapes and the distribution patterns of chromatin. The chromatin has an almost homogeneous distribution throughout the nucleus, forming a fibrous network of the chromonemata (Figure 1a). According to the system of Tanaka (1971, 1977), it can be categorized as a diffuse type.

The chromosomes at mitotic prophase stain darkly and homogeneously, showing several small elastic constrictions (Figure 1b). There were no conspicuously heterochromatic or euchromatic segments in five species. Using the system of Tanaka (1971, 1977, 1987), the prophase chromosomes of *Leontopodium* are classified as an interstitial type. Brief descriptions of the karyotypic features of each species are given below.

Leontopodium sinense Hemsl.

The species is endemic to China. Its chromosome number is $2n = 28$. It consists of 22 median (m), and six submedian (sm) chromosomes. The ratio of the longest to the shortest chromosomes is 2.21 and the karyotype symmetry is 2B (Tables 2 and 3; Figures 2 and 7A).

Figures 1–6.   Mitotic nuclei and metaphase chromosomes of *Leontopodium*. (1a) Mitotic interphase nuclei; (1b) mitotic prophase; (2) *L. sinense*, $2n = 28$; (3) *L. muscoides*, $2n = 24$; (4) *L. souliei*, $2n = 52$; (5) *L. pusillum*, $2n = 72$; (6) *L. nanum*, $2n = 48$. Scale bar = 5 μm.

Table 2. Somatic chromosome number (2*n*), karyotype formula (2*n*), karyotype asymmetry (KA), locality, and data source of *Leontopodium* examined in this and previous reports.

Taxon	Number	Karyotype formula (2*n*)	KA	Locality	Data source
L. alpinum	48/2*n*	—	—		Siljak (1977)
	52/2*n*	—	—		Murin and Paclova (1979)
L. hayachinense	73/2*n*	—	—	Hokkaido, Japan	Nishikawa (1985)
L. jacotianum	12,14/*n*	—	—	Pakistan	Khatoon and Ali (1988)
L. kurilense	48/2*n*	—	—	Chukotka, Russia	Zhukova (1980)
L. leontopodium	26/2*n*	—	—	Kashmir Himalaya	Vir Jee and Kachroo (1985)
L. ochroleucum	49/2*n*	—	—	Eastern Sayan Mt.	Krogulevich (1978)
L. sinense	28/2*n*	22m + 6sm	2B	Zuogong, Tibet	Present study
L. muscoides	24/2*n*	22m + 2sm	1A	Mangkang, Tibet	Present study
L. souliei	52/2*n*	32m + 20sm	1A	Zuogong, Tibet	Present study
L. pusillum	72/2*n*	52m + 20sm	2B	Naqu, Tibet	Present study
L. nanum	48/2*n*	24m + 24sm	2B	Dangxiong, Tibet	Present study

Leontopodium muscoides Handel-Mazzetti.

The species is endemic to the Qinghai-Tibet Plateau with chromosome number firstly reported as 2*n* = 24. The ratio of the longest to the shortest chromosomes is 1.79 and the karyotype symmetry is type 1A. It consists of 22 m and two sm chromosomes (Tables 2 and 3; Figures 3 and 7B).

Leontopodium souliei Beauverd.

The species is endemic to the Qinghai-Tibet Plateau with chromosome number firstly reported as 2*n* = 52. The ratio of the longest to the shortest chromosomes is 1.75 and the karyotype symmetry is type 1A. It consists of 32 m and 20 sm chromosomes (Tables 2 and 3; Figures 4 and 7C).

Leontopodium pusillum Hand.-Mazz.

This is the first time that 2*n* = 72 has been reported for this species, which is mainly distributed in the Qinghai-Tibet Plateau, extending to the north of Sikkim. The ratio of the longest to the shortest chromosomes is 2.35 and the karyotype symmetry is type 2B. It consists of 52 m and 20 sm chromosomes (Tables 2 and 3); Figures 5 and 7D).

Leontopodium nanum (Hook. f. et Thoms.) Hand.-Mazz.

This species is restricted into the Qinghai-Tibet Plateau with a chromosome number of 2*n* = 48. The ratio of the longest to the shortest chromosomes is 2.00 and the karyotype symmetry is type 2B. It consists of 24 m and 24 sm chromosomes (Tables 2 and 3; Figures 6 and 7E).

Discussion

Most genera of the Gnaphalieae have chromosome numbers that are multiples of 7 (Merxmüller et al. 1977; Anderberg 1994; Watanabe et al. 1999). However, *n* = 14 or 2*n* = 28 is the most frequent number in the tribe and Watanabe et al. (1999) suggested *x* = 14 as the possible base number of the tribe. In *Leontopodium*, the chromosome number were reported as *n* = 12, 14 or 2*n* = 26, 48, 49, 52, 73 (Siljak 1977; Krogulevich 1978; Murin and Paclova 1979; Zhukova 1980; Krasnikova et al. 1984; Nishikawa 1985; Vir Jee and Kachroo 1985; Khatoon and Ali 1988), and our results are 2*n* = 24, 28, 48, 52 and 72. Three species have the basic chromosome number *x* = 12, with *L. muscoides* being diploid, *L. nanum* tetraploid, and *L. pusillum* hexaploid. Different chromosome basic numbers are also observed in another two species, *L. sinense* (2*n* = 2*x* = 28) and *L. souliei* (2*n* = 4*x* = 52), with *x* = 14 and 13. The molecular data support *Leontopodium*, *Filago*, *Antennaria* and *Gamochaeta* forming the "FLAG clade" with a base chromosome number of *x* = 14, which have an allopolyploid origin (Galbany-Casals et al. 2010; Smissen et al. 2011). The other number, *x* = 12 and 13 probably are the results of secondary reduction. This is consistent with previous suggestions of secondary chromosome number reduction in Gnaphalieae (Watanabe et al. 1999) particularly *Podolepis* Labill. (Konishi et al. 2000), in which *n* = 12, 11, 10, 9, 8, 7 and 3 were reported.

Interspecific polyploidy in the Gnaphalieae has played an important role in the evolution of many genera (Watanabe et al. 1999). In *Leontopodium*, polyploidy has been reported in four species (Siljak 1977; Krogulevich 1978; Murin and Paclova 1979; Zhukova 1980; Nishikawa 1985). Three out of the five counts are observed to be tetraploidy (*L. souliei*, *L. nanum*) or hexaploidy (*L. pusillum*) in this report (Table 2). It seems that polyploidy has probably played a relatively important role in the chromosome evolution of *Leontopodium*.

Dysploidy is observed in *Leontopodium*, and was supposed to take place in *Leontopodium* during its adaptation to different habitats. Dysploidy is common in many genera of Asteraceae, e.g. *Centaureinae*, *Artemisia* and *Cousinia* (Garcia-Jacas et al. 1996; Vallès et al. 2001; Vallès and Kapustina 2001; Zhao et al. 2009; Susanna et al. 2003; Ghaffari et al. 2006; Djavadi and Attar 2010).

Karyomorphology and karyotype are also reported for the first time for *Leontopodium*. All species show similar karyotypic asymmetry of 1A and 2B (Table 2). Karyotype asymmetry is one of the important standards

Table 3. Karyomorphological parameters of studied *Leontopodium* species. Chromosome pairs are assigned Arabic numerals.

Pair no.	RL	AR	CI	Type	Pair no.	RL	AR	CI	Type
Leontopodium sinense					*Leontopodium souliei*				
1	5.26	1.55	0.39	m	1	2.56	1.82	0.36	sm
2	3.92	2.17	0.32	sm	2	2.34	1.67	0.38	m
3	3.71	1.25	0.44	m	3	2.34	1.29	0.44	m
4	3.71	1.25	0.44	m	4	2.24	1.55	0.39	m
5	3.71	1.81	0.36	sm	5	2.08	1.36	0.42	m
6	3.71	2.00	0.33	sm	6	2.05	1.33	0.43	m
7	3.71	1.25	0.44	m	7	2.05	1.33	0.43	m
8	3.57	1.27	0.44	m	8	2.05	1.33	0.43	m
9	3.40	1.46	0.40	m	9	1.99	1.72	0.37	sm
10	3.30	1.50	0.40	m	10	2.03	1.48	0.40	m
11	3.30	1.67	0.38	m	11	2.05	1.33	0.43	m
12	3.30	1.20	0.46	m	12	1.78	1.91	0.34	sm
13	2.89	1.26	0.44	m	13	1.95	1.51	0.39	m
14	2.38	1.30	0.44	m	14	1.77	1.42	0.41	m
Leontopodium muscoides					15	1.99	1.27	0.44	m
1	5.91	1.93	0.34	sm	16	1.86	1.54	0.39	m
2	4.75	1.15	0.46	m	17	1.76	1.40	0.42	m
3	5.75	1.47	0.40	m	18	1.76	1.40	0.42	m
4	4.43	1.25	0.44	m	19	1.99	1.27	0.44	m
5	4.11	1.14	0.46	m	20	1.77	1.09	0.48	m
6	3.99	1.08	0.48	m	21	1.76	1.86	0.35	sm
7	3.89	1.36	0.42	m	22	1.65	1.82	0.35	sm
8	3.84	1.60	0.38	m	23	1.62	1.22	0.45	m
9	3.69	1.50	0.40	m	24	1.52	1.60	0.38	m
10	3.50	1.14	0.47	m	25	1.52	1.60	0.38	m
11	3.35	1.20	0.46	m	26	1.46	1.50	0.40	m
12	3.35	1.27	0.44	m	*Leontopodium nanum*				
Leontopodium pusillum					1	3.11	1.75	0.36	sm
1	2.12	1.40	0.42	m	2	2.63	2.10	0.32	sm
2	1.96	2.64	0.27	sm	3	2.52	1.23	0.45	m
3	1.86	2.50	0.29	sm	4	2.41	1.83	0.35	sm
4	1.93	1.73	0.37	sm	5	2.41	1.83	0.35	sm
5	1.77	1.86	0.35	sm	6	2.27	1.67	0.38	m
6	1.77	2.10	0.33	sm	7	2.27	1.67	0.38	m
7	1.63	1.31	0.43	m	8	2.27	1.67	0.38	m
8	1.63	1.47	0.40	m	9	2.27	1.67	0.38	m
9	1.50	1.43	0.41	m	10	2.27	2.20	0.31	sm
10	1.57	1.28	0.44	m	11	1.98	1.80	0.36	sm
11	1.43	1.61	0.38	m	12	1.98	1.33	0.43	m
12	1.41	1.67	0.38	m	13	1.98	1.33	0.43	m
13	1.41	1.67	0.38	m	14	1.98	1.33	0.43	m
14	1.41	1.29	0.44	m	15	1.98	1.33	0.43	m

(*Continued*)

Table 3. (*Continued*).

Pair no.	RL	AR	CI	Type	Pair no.	RL	AR	CI	Type
15	1.41	2.64	0.28	sm	16	1.98	1.50	0.40	m
16	1.41	1.67	0.38	m	17	1.76	1.82	0.35	sm
17	1.37	1.21	0.45	m	18	1.76	1.82	0.35	sm
18	1.25	1.35	0.43	m	19	1.84	1.60	0.38	m
19	1.29	1.43	0.41	m	20	1.70	2.00	0.33	sm
20	1.36	1.57	0.39	m	21	1.70	2.00	0.33	sm
21	1.29	1.43	0.41	m	22	1.70	2.00	0.33	sm
22	1.25	1.35	0.43	m	23	1.70	1.40	0.42	m
23	1.24	1.33	0.43	m	24	1.56	1.75	0.36	sm
24	1.24	1.33	0.43	m					
25	1.24	1.33	0.43	m					
26	1.18	1.23	0.45	m					
27	1.11	2.15	0.32	sm					
28	1.28	1.42	0.42	m					
29	1.15	1.17	0.46	m					
30	1.24	1.80	0.36	sm					
31	1.13	1.56	0.39	m					
32	1.15	1.17	0.46	m					
33	1.06	1.40	0.42	m					
34	1.06	2.00	0.33	sm					
35	0.97	1.75	0.36	sm					
36	0.90	1.43	0.41	m					

RL, relative length; AR, arm ratio L/S (length of long arm/length of short arm); CI, centrometic index 100S/TL (100 × length of short arm/total chromosome length); M, median point; m, median region; sm, sub-median region; st, acrocentric region.

Figure 7.   Karyotypes of *Leontopodium*. (A) *L. sinense*, $2n = 28$; (B) *L. muscoides*, $2n = 24$; (C) *L. souliei*, $2n = 52$; (D) *L. pusillum*, $2n = 72$; (E) *L. nanum*, $2n = 48$. Scale bar = 5 μm.

for evaluating evolutionary relationship (Stebbins 1971; Li and Chen 1985). There is a general assumption that, within the angiosperms, asymmetrical karyotypes are derived from symmetrical ones (Stebbins 1971; Stace 1989). However, the change in ploidy levels and karyotype asymmetry in *Leontopodium* is not necessarily coincident. It suggests not only that polyploidy and dysploidy play important roles during the karyotype evolution and the speciation, but also that ecological selection should be an alternative important factor for karyotype asymmetry. There is a great diversity of karyotypes in Gnaphalieae genera (Watanabe et al. 1999). In our study, however, karyotypes of all *Leontopodium* species seem to be unimodal with no obvious gaps among all their chromosome length (Figure 7, Table 3). More cytological studies on this genus as well as from the tribe are needed to better understand their chromosomal evolution.

Acknowledgements

References

Anderberg AA. 1991. Taxonomy and phylogeny of the tribe Gnaphalieae (Asteraceae). Opera Botanica. 104:1–195.

Anderberg AA. 1994. Tribe Gnaphalieae. In: Bremer K, editor. Asteraceae: Cladistics and Classification. Oregon: Timber Press. p. 304–364.

Bayer MB, Breitweiser I, Ward J, Puttock CF. 2007. Tribe Gnaphalieae. In: Kubitzki K. editor. The Families and Genera of Flowering Plant, Vol. VIII: Flowering Plants, Eudicots, Asterales. Berlin: Springer. p. 246–283.

Blöch C, Dickoré WB, Samuel R, Stuessy TF. 2010. Molecular phylogeny of the Edelweiss (Leontopodium, Asteraceae – Gnaphalieae). Edinburgh J Botany. 67:235–264.

Dewey JF, Shackleton RM, Chang C, Sun Y. 1988. The tectonic evolution of Tibetan Plateau. Philos T Roy Soc A. 327:379–413.

Djavadi SB, Attar F. 2010. New chromosome counts in the genus Cousinia (Asteraceae-Cardueae) from Iran. Willdenowia. 40:351–357.

Galbany-Casals M, Andrés-Sánchez S, Garcia-Jacas N, Susanna A, Rico E, Martínez-Ortega MM. 2010. How many of Cassini anagrams should there be? Molecular systematics and phylogenetic relationships in the Filago group (Asteraceae, Gnaphalieae), with special focus on the genus Filago Taxon. 59:1671–1689.

Garcia-Jacas N, Susanna A, Ilarslan R. 1996. Aneuploidy in Centaureinae (Compositae): is n = 7 the end of the series? Taxon. 45:39–42.

Ghaffari SM, Garcia-Jakas N, Susanna A. 2006. New chromosome counts in the genus Cousinia (Asteraceae) from Iran. Bot J Linn Soc. 151:411–419.

Grant V. 1981. Plant speciation, 2nd ed. New York: Columbia University Press.

Khatoon S, Ali SI. 1988. Chromosome numbers in Compositae from Pakistan. Candollea. 43:455–465.

Konishi N, Watanabe K, Kosuge K. 2000. Molecular systematics of Australian Podolepis (Asteraceae: Gnaphalieae): evidence from DNA sequences of the nuclear ITS region and the chloroplast matK gene. Aust Syst Bot. 13:709–727.

Krasnikova SA, Krogulevich RE, Rostovtseva TS. 1984. In: Krogulevich RE, Rostovtseva TS, editors. Khromosomnye Chisla Tsvetkovykh Rastenii i Sibiri Dal'nego Vostoka. Izdatel'stvo "Nauka", Sibirskoe Otdelenie, Novosibirsk.

Krogulevich RE. 1978. Kariologicheskij analiz vidov flory Vostochnogo Sajana. Novosibirsk: V Flora Pribajkal'ja. Nauka.

Levan A, Fedga K, Sandberg AA. 1964. Nomenclature for centromeric position on chromosomes. Hereditas. 52:201–220.

Li MX, Chen RY. 1985. The standardization about the karyotype analysis. J Wuhan Bot Res. 3:297–302.

Löve A, Löve D. 1949. The geobotanical significance of polyploidy. I. Polyploidy and latitude. Portugaliae Acta Biologica Series *A*. 273–352.

Löve A, Löve D. 1967. Polyploidy and altitude: Mt. Washington. Biologische Zentralblatt (Suppl.). 86:307–312.

Merxmüller H, Leins P, Roessler H. 1977. Inuleae systematic review. In: Heywood JB, Harborne JB, Turner BL, editors. The Biology and Chemistry of the Compositae (Vol. 1). London: Academic Press. p. 577–602.

Murin A, Paclova L. 1979. In IOPB chromosome number reports LXIV. Taxon. 28:403–405.

Myers N, Mittermeier RA, Mittermeier GC, Da Fonseca GAB, Kent J. 2000. Biodiversity hotspots for conservation priorities. Nature. 403:853–858.

Nie ZL, Wen J, Gu ZJ, Boufford DE, Sun H. 2005. Polyploidy in the flora of the Hengduan mountains hotspot, southwestern China. Ann MO Bot Gard. 92:275–306.

Nishikawa T. 1985. Chromosome counts of flowering plants of Hokkaido (9). J Hokkaido Univ Education, Section IIB. 36:25–40.

Safer S, Tremetsberger K, Guo YP, Kohl G, Samuel MR, Stuessy TF, Stuppner H. 2011. Phylogenetic relationships in the genus Leontopodium (Asteraceae: Gnaphalieae) based on AFLP data. Bot J Linn Soc. 165:364–377.

Siljak S. 1977. In IOPB chromosome number reports LVII. Taxon. 26:443–452.

Smissen RD, Galbany-Casals M, Breitwieser I. 2011. Ancient allopolyploidy in the everlasting daisies (Asteraceae: Gnaphalieae): Complex relationships among extant clades. Taxon. 60:649–662.

Stace CA. 1989. Plant taxonomy and biosystematics, 2nd ed. London: Edward Arnold.

Stebbins GL. 1971. Chromosomal evolution in higher plants. London: Edward Arnold.

Susanna A, Garcia-Jacas N, Valles J, Ghaf-fari SM. 2003. New chromosome counts in the genus Cousinia and the related genus Schmalhausenia (Asteraceae, Cardueae). Bot J Linn Soc. 143:411–418.

Tanaka R. 1971. Types of resting nuclei in Orchidaceae. Bot Mag (Tokyo). 84:118–122.

Tanaka R. 1977. Recent karyotype studies. In: Ogawa K, Koike S, Kurosumi I, Sato M, editors. Plant cytology. Tokyo: Asakura. p. 293–326.

Tanaka R. 1987. The karyotype theory and wide crossing as an example in Orchidaceae. In: Hong DY, editor. Plant chromosome research. Hiroshima: Proceedings of the Sino-Japanese Symposium on Plant Chromosomes. p. 1–10.

Vir Jee DU, Kachroo P. 1985. Chromosomal conspectus of some alpine-subalpine taxa of Kashmir Himalaya. Chromosome Information Service. 39:33–35.

Vallès J, Torrell M, Garcia-Jacas N. 2001. New or rare chromosome counts in Artemisia L. (Asteraceae, Anthemideae) and related genera from Kazakhstan. Bot J Linn Soc. 137:399–407.

Vallès J, Kapustina L. 2001. New or rare chromosome counts in the genera Artemisia L. and Mausolea Bunge (Asteraceae, Anthemideae) from Uzbekistan. Bot J Linn Soc. 135:391–400.

Watanabe K, Short PS, Denda T, Konishi N, Ito M, Kosuge K. 1999. Chromosome numbers and karyotypes in the Australian Gnaphalieae and Plucheeae (Asteraceae). Aust Syst Bot. 12:781–802.

Wen J. 1999. Evolution of eastern Asian and eastern North American disjunct distributions in flowering plants. Annu Rev Ecol Syst. 30:421–455.

Wu YH. 2008. The vascular plants and their eco-geographical distribution of the Qinghai-Tibetan Plateau. Beijing: Science Press. p. 947–952.

Zhang ZM, Liu JG, Coleman RG. 1984. An outline of the plate tectonics of China. Bull Geol Soc Am. 95:295–312.

Zhao HB, Li C, Tang FP, Chen FD, Chen SM. 2009. Chromosome numbers and morphology of eighteen Anthemideae (Asteraceae) taxa from China and their systematic implications. Caryologia. 62:288–302.

Zhukova PG. 1980. Chromosome numbers of some Southern Chukotka plant species. Botanicheskii Z. 65(1):51–59.