Plant Diversity 39 (2017) 194–201

Contents lists available at ScienceDirect

Plant Diversity

journal homepage: http://www.keaipublishing.com/en/journals/plant-diversity/
http://journal.kib.ac.cn

Karyotypes of nineteen species of Asteraceae in the Hengduan Mountains and adjacent regions

Wenguang Sun [a, b, c, 1], Xiangguang Ma [b, 1], Jianwen Zhang [b], Fuming Su [a], Yonghong Zhang [a], Zhimin Li [a, *]

[a] School of Life Science, Engineering Research Center of Sustainable Development and Utilization of Biomass Energy, Yunnan Normal University, Yunnan, Kunming 650500, China
[b] Key Laboratory for Plant Diversity and Biogeography of East Asia, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming, 650201, China
[c] University of Chinese Academy of Sciences, Beijing, 100049, China

ARTICLE INFO

Article history:
Received 11 April 2017
Received in revised form
2 August 2017
Accepted 4 August 2017
Available online 24 August 2017

(Editors: David Boufford, Zhekun Zhou)

Keywords:
Polyploidy
Karyotype morphology
Asteraceae
Chromosome number
Hengduan Mountains

ABSTRACT

The Hengduan Mountains region is a biodiversity hotspot. In this study, we report the karyotypes of 19 species (21 populations) of Asteraceae from this region, 14 of which are reported for the first time. We also examined polyploidy in Asteraceae plants and summarized karyotype data in the literature for 69 congeneric taxa. In these genera, there were five different ploidy levels in the region, though the most dominant was diploid (73.08%). There is no direct evidence that ploidy level and karyotype asymmetry are associated with the distribution of recorded Asteraceae species from the Hengduan Mountains. This suggests that polyploidy (26.92%) may not play an important role in the evolutionary history of these plants, even though, among these genera, the ratio of paleopolyploidy was high (46.15%).

The Hengduan Mountains region (HDM), which is located in southwest China on the southeast portions of the Qinghai-Tibet Plateau (25°–34°N, 96°–105°E), covers an area of 3.64×10^5 km^2 (Li, 1987). This region is a very interesting floristic area in view of the fact that Quaternary glaciations covered only a small part of the Hengduan Mountains and the region was likely a core refugium for temperate species of East Asia during glacial periods (Li et al., 1991). Perhaps partly because of these glacial episodes, the HDM became a center for the rapid evolution of new species (Liu et al., 2006; Sun, 2002; Wang and Liu, 2004; Wu, 1988). It is also one of the major centers of endemism in China (Li, 1994). The flora of HDM comprises more than 8590 species belonging to 1348 genera, 2783 of which are endemic (32.4% of the native flora) (Li and Li, 1993; Wu, 1988; Zhang et al., 2009b). Moreover, it is known as one of the 25 most significant biodiversity hotspots in the world (Myers et al., 2000; Olson and Dinerstein, 2002). However, reports of chromosome number and karyotype are relatively few for the HDM flora.

Polyploidy has long been recognized as an important evolutionary force in plants (Grant, 1981; Jiao et al., 2011; Levin, 2002; Stebbins, 1940, 1950, 1971), however the role of ploidy level on rapid speciation is complex, with different lines of evidence often yielding conflicting inferences. Statistical analyses of chromosome numbers of 552 taxa from the HDM show that polyploidy may have played only a minor role in the evolutionary diversification of plants in this region (Nie et al., 2005). Although this point of view is somewhat supported by some highly diversified genera in the region, such as *Cremanthodium* (Liu et al., 2001), *Cyananthus* (Chen et al., 2014), *Dolomiaea* (Wang et al., 2013), *Delphinium* (Yuan and Yang, 2008) and *Ligularia* (Liu, 2004), investigations on other groups have drawn different conclusions. *Anaphalis* and *Leontopodium* from the Hengduan Mountains suggest that polyploidization has played a relatively important role in the chromosome evolution (Meng et al., 2012, 2014). Research on *Aconitum* subgenus *Lycoctonum* (Yuan and Yang, 2006) and *Buddleja* (Chen and Sun, 2006) from HDM show similar results. All reported chromosome numbers from HDM have been thus far scattered among different families and genera. Therefore, selecting an appropriate group to evaluate the role of polyploidy in speciation is important.

* Corresponding author.
E-mail address: lizhimin_vip@163.com (Z. Li).
Peer review under responsibility of Editorial Office of Plant Diversity.
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.pld.2017.08.001

Asteraceae has a series of ploidy levels from di-, tri-, tetra-, hexa-, to octaploid. Therefore, we chose this family to study the role of polyploidy in the evolution of plants in the HDM region. Asteraceae is one of the largest families in the world. It contains approximately 1700 genera and 24,000 species distributed all over the world (Shi et al., 2011). There are about 833 taxa of Asteraceae which belong to 137 genera in the HDM (Shi et al., 2011; Wang, 1994; Zhang et al., 2009a, 2009b). They show extreme diversification in morphology, and their geographical distribution extends from dry-hot valley to the alpine subnival belt (from 700 to 5500 m) (Shi et al., 2011; Wang, 1994; Xu et al., 2014a, 2014b).

In this study we report the chromosome numbers and chromosome morphology of 19 Asteraceae species, and calculate the frequency of polyploidy of these genera in the Hengduan Mountains region.

1. Material and methods

1.1. Plant materials and cytological studies

All of the nineteen species (21 populations) were collected from the Hengduan Mountains (HDM) region of SW China (Table 1). Voucher specimens have been stored in the Herbarium of the Kunming Institute of Botany (KUN).

Root tips from the germination of seeds were used to examine somatic chromosomes. Seeds were stored at 4 °C for one month. Seeds were then germinated using wet double filter paper in glass culture dishes at 24 °C. Exuberant root tips (1–2 cm in length) were excised from the seedlings and pretreated in 0.002 or 0.003 mol/L 8-hydroxyquinoline solution at 24 °C in darkness for 1–3 h, and then immobilized with Carnoy's solution (1 glacial acetic acid: 3 absolute alcohol, v/v) for at least 24 h at 4 °C. The fixed roots were hydrolyzed in 1 mol/L HCl at 60 °C for 10–15 min, and then washed with distilled water, dyed with carbolfuchsin and squashed for observation.

1.2. Karyotype analysis

Karyotype formulae were based on measurements of mitotic–metaphase chromosomes taken from photographs. To standardize the procedure, chromosome metaphase plates from at least six cells were measured. The terminology used for the karyotype position of chromosome composition followed Levan et al. (1964). The degree of karyotype asymmetry was calculated following Arano (1963) "As.K%". The equation: index of Karyotypic Asymmetry (As.K%) = the total of the longest chromosome length/the total of the all chromosome length × 100. Stebbin's asymmetry category was described by karyotypic symmetry division according to Stebbins (1971) and marked as KA. To evaluate karyotype asymmetry, we calculated two parameters suggested by Paszko (2006), Peruzzi et al. (2009), Peruzzi and Eroğlu (2013) and Peruzzi and Altınordu (2014): M_{CA} (Mean Centromeric Asymmetry) and CV_{CL} (Coefficient of Variation of Chromosome Length). Karyotype asymmetry was compared to the CV_{CI} (Coefficient of Variation of Chromosome Index) and karyotype total haploid length (THL) was calculated. To account for these parameters, we used Karyo-Type software (Altınordu et al., 2016), a cytogenetic tool which can measure karyotype asymmetry indices automatically and efficiently.

1.3. Source of karyotype data

We surveyed the literature to collect available chromosome number data for Asteraceae species distributed in the Hengduan Mountains region. The online database from Index to Plant Chromosome Numbers (IPCN, http://www.tropicos.org/NameSearch.aspx?projectid=9) was also consulted to confirm the diversity of chromosome number and basic number.

To confirm the distribution of a species in the HDM, we consulted the *The Vascular Plants of the Hengduan Mountains* (Wang, 1994), *Flora of China*, local flora and an online database of plants and fungi in south-central China (http://hengduan.huh.harvard.edu/fieldnotes). For a standardized database, we used the Taxonomic Name Resolution Service v.4.0 (TNRS: http://tnrs.iplantcollaborative.org/index.html) to examine our checklist for synonyms and illegitimate names.

2. Results

2.1. Karyotype of 19 species from Asteraceae

Chromosome numbers and karyotypes for 19 Asteraceae species are briefly described below. These species, which represent nine

Table 1
Location of Asteraceae species karyotyped.

Taxon	Origin	Location	Altitude/m	Voucher number
Cremanthodium lineare var. *eligulatum*[a]	Daocheng, Sichuan	100°02′36″E; 29°08′16″N	4715	SunH-07zx-3332
C. angustifolium[a]	Daocheng, Sichuan	100°02′36″E; 29°08′16″N	4715	SunH-07zx-3333
C. principis[a]	Shangri-La, Yunnan	99°57′23″E; 28°30′05″N	4600	PengDL-15
C. helianthus[a]	Shangri-La, Yunnan	99°57′23″E; 28°31′05″N	4500	PengDL-20
C. campanulatum var. *campanulatum*[a]	Shangri-La, Yunnan	99°55′40″E; 28°30′39″N	4542	PengDL-17
Leontopodium souliei[a]	Mangkang, Tibet	98°40′17″E; 29°44′37″N	3590	SunH-07zx-3383
Aster pekinensis	Batang, Sichuan	99°12′10″E; 30°34′13″N	3831	SunH-07zx-3389
A. gouldii (CY)[a]	Chayu, Tibet	97°10′34″E; 29°19′14″N	3640	SunH-07zx-2496
A. gouldii (BM)[a]	Bomi, Tibet	96°22′57″E; 29°36′46″N	3370	SunH-07zx-2544
Anaphalis xylorhiza[a]	Ganzi, Sichuan	100°02′36″E; 29°08′16″N	4715	SunH-07zx-3336
A. spodiophylla	Ganzi, Sichuan	99°37′50″E; 31°42′23″N	3739	SunH-07zx-3923
Myriactis nepalensis	Mianning, Sichuan	101°57′12″E; 28°20′17″N	2486	SunH-07zx-3788
M. wightii	Mianning, Sichuan	101°57′12″E; 28°20′17″N	2486	SunH-07zx-3789
Syncalathium roseum[a]	Zhanang, Tibet	91°25′0.5″E; 29°10′02.6″N	3750	ZhangJW-1054
Pertya phylicoides[a]	Deqin, Yunnan	99°15′26″E; 28°15′17″N	2686	PengDL-031
Dubyaea tsarongensis[a]	Gongshan, Yunnan	98°28′33″E; 27°46′56″N	3275	MS-025
Carpesium cernuum (MN)	Mianning, Sichuan	101°57′19″E; 28°20′00″N	2221	SunH-07zx-3781
C. cernuum (LJ)	Lijiang, Yunnan	99°38′27″E; 26°47′34″N	2550	SunH-07zx-3132
C. lipskyi[a]	Mianning, Sichuan	101°57′12″E; 28°20′17″N	2486	SunH-07zx-3790
C. scapiforme	Batang, Sichuan	99°12′10″E; 30°34′13″N	3831	SunH-07zx-3391
C. velutinum	Jiulong, Sichuan	101°26′31″E; 29°02′33″N	3125	SunH-07zx-3774

[a] Endemic to the Himalayan–Hengduan Mountains region.

genera, include 12 species endemic to the Himalayan–Hengduan Mountains.

2.1.1. ***Cremanthodium lineare var. eligulatum*** *Y. Ling & S. W. Liu*

C. lineare var. *eligulatum* was collected from Daocheng (Sichuan, China). The karyotype was formulated as 2n = 2x = 58 = 44m + 14sm (2SAT) (Table 2; Fig. 1: A, a). The chromosome length varied from 2.68 to 4.64 μm. The ratio of the longest to the shortest chromosome was 1.72, and As.K% = 59.12. KA belongs to Stebbins's–2A (Table 2). This is the first report of karyotype and chromosome numbers for *C. lineare* var. *eligulatum*.

2.1.2. ***Cremanthodium angustifolium*** *W. W. Smith*

C. angustifolium was collected from Daocheng (Sichuan, China). The karyotype was formulated as 2n = 2x = 58 = 34m + 22sm + 2st (Table 2; Fig. 1: B, b). The chromosome length varied from 3.43 to 4.84 μm. The ratio of the longest to the shortest chromosome was 1.55, and As.K% = 61.30. KA belongs to Stebbins's–2A (Table 2). This is the first report of karyotype and chromosome numbers of *C. angustifolium*.

2.1.3. ***Cremanthodium principis*** *(Franch.) R. D. Good*

C. principis was collected from Shangri-La (Yunnan, China). The karyotype was formulated as 2n = 2x = 58 = 32m + 26sm (2SAT) (Table 2; Fig. 1: C, c). The chromosome length varied from 2.69 to 4.56 μm. The ratio of the longest to the shortest chromosome was 1.69, and As.K% = 61.76. KA belongs to Stebbins's–2A (Table 2). This is the first report of karyotype and chromosome numbers of *C. principis*.

2.1.4. ***Cremanthodium helianthus*** *(Franch.) W. W. Smith*

C. helianthus was collected from Shangri-La (Yunnan, China). The karyotype was formulated as 2n = 2x = 58 = 36m + 22sm (2SAT) (Table 2; Fig. 1: D, d). The chromosome length varied from 2.65 to 4.30 μm. The ratio of the longest to the shortest chromosome was 1.62, and As.K% = 61.58. KA belongs to Stebbins's–2A (Table 2). This is the first report of karyotype and chromosome numbers of *C. helianthus*.

2.1.5. ***Cremanthodium campanulatum*** *(Franch.) Diels* ***var. campanulatum***

C. campanulatum var. *campanulatum* was collected from Shangri-La (Yunnan, China). The karyotypes were formulated as 2n = 2x = 58 = 44m (2SAT) + 14sm (Table 2; Fig. 1: E, e). The chromosome length varied from 2.96 to 4.96 μm. The ratio of the longest to the shortest chromosome was 1.68, and As.K% = 59.24. KA belongs to Stebbins's–2A (Table 2). This is the first report of karyotype and chromosome numbers of *C. campanulatum* var. *campanulatum*.

2.1.6. ***Leontopodium souliei*** *Beauverd*

L. souliei was collected from Mangkang (Tibet, China). The karyotype was formulated as 2n = 4x = 48 = 36m + 12sm + 0/2/3/4B (Table 2; Fig. 1: F, f). The chromosome length varied from 2.72 to 4.21 μm. The ratio of the longest to the shortest chromosome was 1.51 and As.K% = 59.09. KA belongs to Stebbins's–2B (Table 2).

2.1.7. ***Aster pekinensis*** *(Hance) F. H. Chen*

We formulated the karyotype of *Aster pekinensis* as 2n = 2x = 18 = 18m (Table 2; Fig. 1: G, g). The chromosome length varied from 4.68 to 6.62 μm. The ratio of the longest to the shortest chromosome length was 1.41, and the KA belongs to Stebbins's–1A (Table 2).

2.1.8. ***Aster gouldii*** *C. E. C. Fischer*

In this study, two populations of *Aster gouldii* (from Chayu and Bomi) were sampled. The karyotype of the Bomi population was formulated as 2n = 2x = 18 = 18m (2SAT) (Table 2; Fig. 1: H, h). The chromosome length varied from 2.96 to 3.34 μm. The ratio of the longest to the shortest chromosome was 1.13, and the asymmetry of

Table 2
Karyotypes and chromosomal data of 19 species from the HDM.

Taxon	Chromosome length range (μm)	Ratio LC/SC	<2:1	As.K%	M_{CA}	CV_{CL}	CV_{CI}	THL	2n/x/ploidy level	Karyotype formula	KA Type	Figure
Cremanthodium lineare var. *eligulatum*[a]	2.68–4.64	1.72	0.14	59.12	18.26	14.45	10.80	100.03	58/29/2x	44m + 14sm(2sat)	2A	A, a
C. angustifolium[a]	3.43–4.84	1.55	0.24	61.30	22.60	7.73	18.30	116.08	58/29/2x	34m + 22sm + 2st	2A	B, b
C. principis[a]	2.69–4.56	1.69	0.21	61.76	23.38	14.33	13.95	99.63	58/29/2x	32m + 26sm(2sat)	2A	C, c
C. helianthus[a]	2.65–4.30	1.62	0.17	61.58	22.94	13.34	12.40	99.74	58/29/2x	36m + 22sm(2sat)	2A	D, d
C. campanulatum var. *campanulatum*[a]	2.96–4.96	1.68	0.10	59.24	18.73	13.26	11.19	101.21	58/29/2x	44m(2sat) + 14sm	2A	E, e
Leontopodium souliei	2.72–4.21	1.51	0.13	59.09	10.16	11.23	5.50	25.02	48/12/4x	36m + 12sm + 0/2/3/4B	2B	F, f
Aster pekinensis	4.68–6.62	1.41	0	55.10	11.62	11.91	3.90	31.06	18/9/2x	18m	1A	G, g
A. gouldii (BM)[a]	2.96–3.34	1.13	0	55.92	12.86	10.48	5.24	48.57	18/9/2x	18m(2sat)	1A	H, h
A. gouldii (CY)[a]	2.84–3.45	1.21	0	55.82	18.18	15.34	12.45	58.79	18/9/2x	18m(2sat)	1A	I, i
Anaphalis xylorhiza	2.25–3.16	1.40	0.36	62.20	25.21	17.48	14.55	37.98	28/14/2x	16m + 12sm	2A	J, j
A. spodiophylla[a]	2.68–4.05	1.51	0.43	62.42	24.80	18.25	16.58	36.56	28/14/2x	16m + 12sm	2A	K, k
Myriactis nepalensis	4.56–6.59	1.45	0.11	56.36	13.04	18.27	9.43	76.91	36/18/2x	32m + 4sm	2A	L, l
M. wightii[a]	3.84–5.34	1.39	0.06	56.08	12.13	17.4	9.29	79.27	36/18/2x	32m + 4sm	2A	M, m
Syncalathium roseum[a]	4.00–4.76	1.50	0	55.75	11.46	6.21	7.65	35.78	16/8/2x	14m + 2sm(2sat)	1A	N, n
Pertya phylicoides[a]	1.48–4.36	1.49	0	58.55	17.15	31.00	5.36	42.73	34/17/2x	30m + 4sm	1B	O, o
Dubyaea tsarongensis[a]	2.97–4.71	1.48	0	59.72	7.37	13.42	19.03	31.89	16/8/2x	14m + 2sm	1A	P, p
Carpesium cernuum (MN)	1.81–7.07	1.89	0.4	63.19	24.76	39.95	7.69	64.17	40/20/2x	26m + 10sm(2sat) + 4st	2B	Q, q
C. cernuum (LJ)	2.00–6.17	1.62	0.15	60.15	23.99	31.90	12.00	64.20	40/20/2x	26m + 10sm(2sat) + 4st	2B	R, r
C. lipskyi[a]	1.64–4.93	1.39	0.1	57.88	13.45	35.20	5.44	47.05	40/20/2x	8M + 26m + 6sm	2B	S, s
C. scapiforme[a]	2.03–6.05	1.75	0.35	62.14	21.26	32.30	7.46	61.83	40/20/2x	14sm + 26m(2sat)	2B	T, t
C. velutinum[a]	1.43–5.17	1.66	0.2	61.32	22.09	35.15	9.18	49.84	40/20/2x	2M + 22m + 16sm	2B	U, u

LC/SC: the proportion of the longest chromosome length to the shortest chromosome length; As.K%: index of karyotypic asymmetry; M_{CA}: Mean Centromeric Asymmetry; CV_{CL}: the relative variation in chromosome length; CV_{CI}: the relative variation in centromeric index; THL: karyotype total haploid length; M: median point; m: median; sm: submedian; st: subterminal region; sat: satellite chromosome; KA: karyotype asymmetry.

[a] Chromosome number and karyomorphology investigated for the first time.

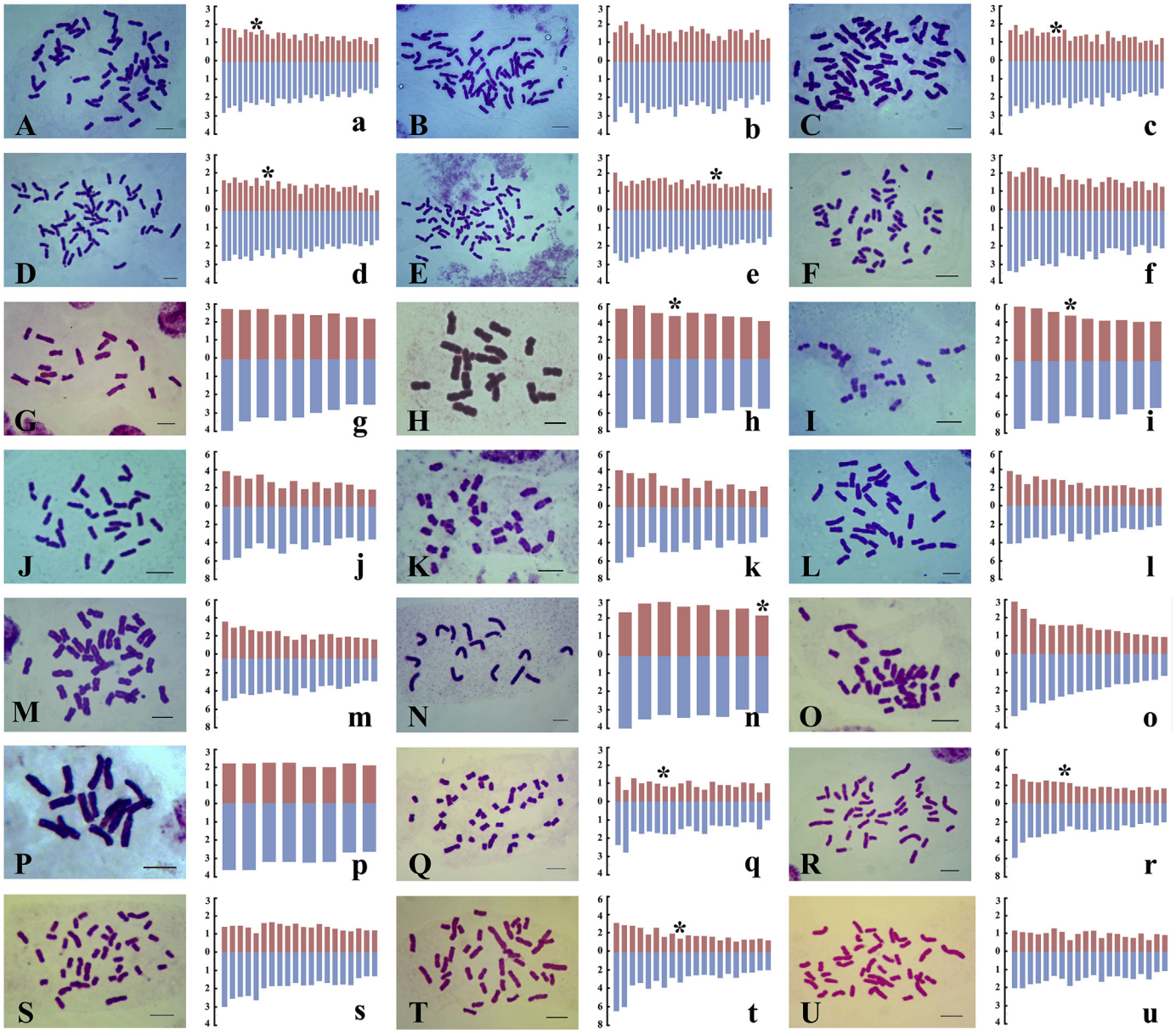

Fig. 1. Mitotic metaphase chromosomes and ideograms of 19 species from the HDM. A, a: *Cremanthodium lineare* var. *eligulatum*; B, b: *C. angustifolium*; C, c: *C. principis*; D, d: *C. helianthus*; E, e: *C. campanulatum* var. *campanulatum*; F, f: *Leontopodium souliei*; G, g: *Aster pekinensis*; H, h: *A. gouldii* (BM); I, i: *A. gouldii* (CY); J, j: *Anaphalis xylorhiza*; K, k: *A. spodiophylla*; L, l: *Myriactis nepalensis*; M, m: *M. wightii*; N, n: *Syncalathium roseum*; O, o: *Pertya phylicoides*; P, p: *Dubyaea tsarongensis*; Q, q: *Carpesium cernuum* (MN); R, r: *C. cernuum* (LJ); S, s: *C. lipskyi*; T, t: *C. scapiforme*; U, u: *C. velutinum* (Scale bar = 5 μm; Red: relative length of short arm; Blue: relative length of long arm; *: satellite chromosome).

the karyotype belongs to Stebbins's–1A (Table 2). The karyotype of the Chayu population was also formulated as 2n = 2x = 18 = 18m (2SAT) (Table 2; Fig. 1: I, i). The chromosome length varied from 2.84 to 3.45 μm. The ratio of the longest to the shortest chromosome was 1.21, and KA belongs to Stebbins's–1A (Table 2).

2.1.9. ***Anaphalis xylorhiza*** *Schultz Bipontinus ex J. D. Hooker*

In this study, the sample from Ganzi (Sichuan) has a formula of 2n = 2x = 28 = 16m + 12sm (Table 2; Fig. 1: J, j). The chromosome length varied from 2.25 to 3.16 μm. The ratio of the longest to the shortest chromosome was 1.40, the As.K% = 62.20. KA belongs to Stebbins's–2A (Table 2).

2.1.10. ***Anaphalis spodiophylla*** *Y. Ling & Y. L. Chen*

The karyotype of *Anaphalis spodiophylla* was formulated as 2n = 2x = 28 = 16m + 12sm (Table 2; Fig. 1: K, k). The chromosome length varied from 2.68 to 4.05 μm. The ratio of the longest to the shortest chromosome length was 1.51 and the AsK. % = 62.42. KA belongs to Stebbins's–2A (Table 2).

2.1.11. ***Myriactis nepalensis*** *Lessing*

The karyotype of *Myriactis nepalensis* was formulated as 2n = 2x = 36 = 32m + 4sm (Table 2; Fig. 1: L, l). The chromosome length varied from 4.56 to 6.59 μm. The ratio of the longest to the shortest chromosome length was 1.45 and the AsK. % = 56.36. KA belongs to Stebbins's–2A (Table 2).

2.1.12. ***Myriactis wightii*** *Candolle*

We formulated the karyotype of *Myriactis wightii* as 2n = 2x = 36 = 32m + 4sm (Table 2; Fig. 1: M, m). The chromosome length varied from 3.84 to 5.34 μm. The ratio of the longest to the

shortest chromosome length was 1.39 and the AsK. % = 56.08. KA belongs to Stebbins's–2A (Table 2).

2.1.13. ***Syncalathium roseum*** *Y. Ling*

We collected seeds of *Syncalathium roseum* from Zhanang at the type locality. The karyotype of *S. roseum* was formulated as 2n = 2x = 16 = 14m + 2sm(2sat) (Table 2; Fig. 1: N, n). The chromosome length varied from 4.00 to 4.76 μm. The proportion of the longest to the shortest chromosome length was 1.50 and the AsK. % = 55.75. KA belongs to Stebbins's–1A (Table 2).

2.1.14. ***Pertya phylicoides*** *Jeffrey*

The karyotype of *Pertya phylicoides* was formulated as 2n = 2x = 34 = 30m + 4sm (Table 2; Fig. 1: O, o) in the present study. The chromosome length varied from 1.48 to 4.36 μm. The ratio of the longest to the shortest chromosome length was 1.49 and the AsK. % = 58.55. KA belongs to Stebbins's–1B (Table 2).

2.1.15. ***Dubyaea tsarongensis*** *(W. W. Smith) Stebbins*

We formulated the karyotype of *Dubyaea tsarongensis* as 2n = 2x = 16 = 14m + 2sm (Table 2; Fig. 1: P, p). The chromosome length varied from 2.97 to 4.71 μm. The ratio of the longest to the shortest chromosome length was 1.48 and the AsK. % = 59.72. KA belongs to Stebbins's–1A (Table 2).

2.1.16. ***Carpesium cernuum*** *Linnaeus*

Two populations of *Carpesium cernuum* (from Mianning and Lijiang) were sampled. The karyotype of the Mianning population was formulated as 2n = 2x = 40 = 26m + 10sm(2sat) + 4st (Table 2; Fig. 1: Q, q). The chromosome length varied from 1.81 to 7.07 μm. The ratio of the longest to the shortest chromosome was 1.89, and the asymmetry of the karyotype belongs to Stebbins's–2B (Table 2). The karyotype of the Lijiang population was also formulated as 2n = 2x = 40 = 26m + 10sm(2sat) + 4st (Table 2; Fig. 1: R, r). The chromosome length ranged from 2.00 to 6.17 μm. The ratio of the longest to the shortest chromosome was 1.62, and KA belongs to Stebbins's–2B (Table 2).

2.1.17. ***Carpesium lipskyi*** *C. Winkler*

C. lipskyi was collected from Mianning (Sichuan, China). The karyotype was formulated as 2n = 2x = 40 = 8M + 26m + 6sm (Table 2; Fig. 1: S, s). The chromosome length varied from 1.64 to 4.93 μm. The ratio of the longest to the shortest chromosome was 1.39, and As.K% = 57.88. KA belongs to Stebbins's–2B (Table 2).

2.1.18. ***Carpesium scapiforme*** *F. H. Chen & C. M. Hu*

C. scapiforme was collected from Batang (Sichuan, China). The karyotype was formulated as 2n = 2x = 40 = 14sm + 26m(2sat) (Table 2; Fig. 1: T, t) in this study. The chromosome length varied from 2.03 to 6.05 μm. The ratio of the longest to the shortest chromosome was 1.75, and As.K% = 62.14. KA belongs to Stebbins's–2B (Table 2).

2.1.19. ***Carpesium velutinum*** *C. Winkler*

C. velutinum was collected from Jiulong (Sichuan, China). The karyotype was formulated as 2n = 2x = 40 = 2M + 22m + 16sm (Table 2; Fig. 1: U. u). The chromosome length varied from 1.43 to 5.17 μm. The ratio of the longest to the shortest chromosome was 1.66, and As.K% = 61.32. KA belongs to Stebbins's–2B (Table 2). This is the first report of karyotype and chromosome numbers of *C. velutinum*.

2.2. The recorded data of chromosome numbers

We surveyed published papers for the recorded chromosome numbers of species that are both distributed in the Hengduan Mountains and are congeners of the species we sampled (Table 3). By comprehensively surveying these data, the chromosome number or cytogenetics of 69 taxa (include 78 populations) were recorded. We calculated the frequency of polyploidy in these genera in the Hengduan Mountains region, and found that the recorded frequency of Asteraceae polyploidy was only 26.92% (21/78).

From the survey data of Asteraceae species from the Hengduan Mountains, patterns and trends in ploidy level can be seen: (1) Five ploidy levels (x) have been recorded, including 2x (n = 57, 73.08%), 3x (n = 1, 1.28%), 4x (n = 17, 21.79%), 6x (n = 2, 2.56%), and 8x (n = 1, 1.28%). (2) The majority of genera were diploid, while a notable number were tetraploid. A number of Asteraceae species in the HDM had multiple haploid chromosome numbers (n). About four types of n with the largest numbers as 9 (16 species, 20.51%), 14 (10 species, 12.82%), 28 (14 species, 17.95%) and 29 (12 species, 15.38%), respectively; (3) Following Lewis (1980), which defines paleopolyploidy as n ≥ 11, we found that 46.15% (36/78) of Asteraceae species in the HDM could be classified as paleopolyploids. Somatic chromosome number (2n) of the recorded Asteraceae species showed more than 17 patterns of 2n types in HDM. The major patterns of 2n included 18 (16 species, 20.51%), 28 (10 species, 12.82%), 56 (14 species, 17.95%) and 58 (12 species, 15.38%).

3. Discussion

In this study, we examined the karyotypes of 19 species of Asteraceae from the Hengduan Mountains. We also surveyed previously reported chromosome numbers of congeneric species from the HDM and adjacent regions.

The genus *Cremanthodium* consists of 69 identified species occurring primarily in the Himalayas–Hengduan Mountains region and all species (46 endemics) can be found in China (Shi et al., 2011). This genus is the largest endemic genus of the Himalayas (Zhang et al., 2009b). All the species of this genus grow almost exclusively on alpine scree or meadow areas at an altitude ranging from 3000 to 5500 m (Shi et al., 2011). Previous studies have shown that *Cremanthodium* species have one of two chromosome numbers, the most common being 2n = 58, with some species 2n = 60 (Liu et al., 2001). In this study, we examined the karyotypes of five species of *Cremanthodium* and all of which were examined for the first time. Our results indicate that the basic chromosome numbers are x = 29 and 2n = 58, which is similar to Liu et al. (2004). Combined with the molecular evidence, our chromosome numbers support the treatment of *Ligularia–Cremanthodium–Parasenecio* as a complex (Liu et al., 2006). Chromosome numbers varied from 2n = 48, 58, 60 in this complex of species (Liu, 2004). The evolution of chromosome number in this genus may represent a good case study to explore the radiation and diversification of species within the uplift of the Qinghai-Tibet Plateau (Liu et al., 2006).

The genus *Leontopodium* has about 58 taxa in Eurasia, and 37 species (17 endemics) in China (Shi et al., 2011). *Leontopodium souliei* is endemic to the Himalayas–Hengduan Mountains region and it grows naturally in grasslands or thickets at altitudes mostly ranging from 2700 to 4500 m (Shi et al., 2011). The previously reported chromosome number of *L. souliei* (Zuogong, Tibet) is 2n = 4x = 52 (Meng et al., 2012). In our study, *L. souliei* was collected from Mangkang (Tibet, China) and the karyotype was formulated as 2n = 4x = 48 = 36m + 12sm + 0/2/3/4B. Meng et al. (2012) suggested that the basic chromosome number of

Table 3
Chromosome data of Asteraceae from the HDM using this and previous reports.

Original taxon	2n	x	Ploidy	KA	Data source
Anaphalis aureopunctata	28, 56	14	2x, 4x	2A, 2B	Meng et al. (2014)
A. bicolor	28, 56	14	2x, 4x	2A, 1B, 2B	Meng et al. (2014)
A. deserti	56	14	4x	1B	Meng et al. (2010)
A. flavescens	28	14	2x	2A	Meng et al. (2014)
A. latialata	56	14	4x	2B	Meng et al. (2014)
A. likiangensis	56	14	4x	2B	Meng et al. (2014)
A. margaritacea	28, 56	14	2x, 4x	1B, 2B	Meng et al. (2010)
A. nepalensis var. *corymbosa*	28, 84	14	2x, 6x	2A, 2B	Meng et al. (2014)
A. nepalensis var. *nepalensis*	28, 56	14	2x, 4x	2A, 2B	Meng et al. (2014)
A. pachylaena	28	14	2x	2A	Meng et al. (2014)
A. pannosa	56	14	4x	2A	Meng et al. (2014)
A. plicata	56	14	4x	2B	Meng et al. (2010)
A. rhododactyla	56	14	4x	2B	Meng et al. (2010)
A. royleana	28	14	2x	2B	Meng et al. (2010)
A. spodiophylla	28	14	2x	2A	Present study
A. stenocephala	56	14	4x	2A, 2B	Meng et al. (2014)
A. surculosa	56	14	4x	2A	Meng et al. (2014)
A. virens	56	14	4x	2B	Meng et al. (2014)
A. xylorhiza	28	14	2x	2A	Present study
A. yunnanensis	56	14	4x	2B	Meng et al. (2014)
Aster albescens var. *glabratus*	18	9	2x	2A	Meng et al. (2016)
A. albescens var. *gracilior*	18	9	2x	2A	Meng et al. (2016)
A. altaicus var. *hirsutus*	18	9	2x	1A	Meng et al. (2016)
A. auriculatus	18	9	2x	1A	Meng et al. (2016)
A. crenatifolius	18	9	2x	1A	Chen et al. (2010) and Meng et al. (2016)
A. diplostephioides	18	9	2x		Liu (1999)
A. gouldii	18	9	2x	1A	Present study
A. oreophilus	18	9	2x	1A	Meng et al. (2016)
A. pekinensis	18	9	2x	1A	Present study
A. poliothamnus	18	9	2x	1A	Meng et al. (2016)
A. pycnophyllus	18, 36	9	2x, 4x	1A, 2A	Meng et al. (2016)
A. salwinensis	18	9	2x	1A	Meng et al. (2016)
A. souliei	18, 54	9	2x, 6x	2A	Liu (1999) and Meng et al. (2016)
A. techinensis	18	9	2x	1A	Meng et al. (2016)
A. trinervius subsp. *ageratoides*	72	9	8x	2A	Meng et al. (2016)
A. yunnanensis var. *labrangensis*	18	9	2x		Liu (1999)
A. yunnanensis var. *yunnanensis*	18	9	2x	2A	Meng et al. (2016)
Carpesium cernuum	40	20	2x	2B	Present study
C. lipskyi	40	20	2x	2B	Present study
C. scapiforme	40	20	2x	2B	Present study
C. velutinum	40	20	2x	2B	Present study
Cremanthodium angustifolium	58	29	2x	2A	Present study
C. brunneo-pilosum	58	29	2x	2A	Liu et al. (2001)
C. campanulatum	58	29	2x	2A	Present study
C. discoideum	58	29	2x	2A	Liu et al. (2001)
C. ellisii	58	29	2x	2A	Liu et al. (2001)
C. helianthus	58	29	2x	2A	Present study
C. humile	58, 60	29, 30	2x	2A	Liu et al. (2001)
C. lineare	58	29	2x	2A	Liu et al. (2001)
C. lineare var. *eligulatum*	58	29	2x	2A	Present study
C. microglossum	58	29	2x	2A	Liu et al. (2001)
C. principis	58	29	2x	2A	Present study
C. stenoglossum	58	29	2x	2A	Liu et al. (2001)
Dubyaea glaucescens	34, 51	17	2x, 3x		Liu et al. (2014)
D. tsarongensis	16	8	2x	1A	Present study
Leontopodium artemisiifolium	26	13	2x		Russell et al. (2013)
L. dedeckensii	26	13	2x		Russell et al. (2013)
L. himalayanum	24	12	2x		Russell et al. (2013)
L. sinense	48	12	4x		Russell et al. (2013)
L. souliei	48	12	4x	2B	Present study
L. stracheyi	26	13	2x		Russell et al. (2013)
Myriactis nepalensis	36	18	2x	2A	Present study
M. wightii	36	18	2x	2A	Present study
Pertya berberidoides	32	16	2x	1B	Chen et al. (2013)
P. phylicoides	34	17	2x	1B	Present study
Syncalathium chrysocephalum	16	8	2x	1A	Zhang et al. (2009c)
S. disciforme	16	8	2x	1A	Zhang et al. (2009c)
S. kawaguchii	16	8	2x	1A	Zhang et al. (2009c)
S. roseum	16	8	2x	1A	Present study

Leontopodium is x = 12, 13, 14. The unstable B chromosome range from 0 to 4 differs from Meng et al. (2012). This phenomenon may be due to the different basic chromosome number of *Leontopodium* with different ploidy in different populations.

The genus *Aster* is one of the largest genera of Asteraceae. This genus includes about 152 species in the Northern Hemisphere, 123 species (82 endemics) are distributed in China (Shi et al., 2011). *A. pekinensis* is widely distributed in China and mainly grows in forest margins, thickets, mountain slopes, riverbanks or roadsides from sea level to 1600 m (Shi et al., 2011). Previous studies have shown that *A. pekinensis* has two chromosome numbers, 2n = 2x = 16 and 2n = 4x = 32 (Gu, 1989). Additional studies have reported that the most common chromosome number in the genus is x = 9 (Meng et al., 2016; Liu, 1999). Our results show that *A. pekinensis* has the karyotype formula 2n = 2x = 18 = 18m. The two populations of *A. gouldii* had similar karyotypes, 2n = 2x = 18 = 18m. This is the first report of the karyotype morphology for this species.

Anaphalis is one of the largest genera of Asteraceae and is the most diverse in the Himalayas–Hengduan Mountains region. It comprises about 110 species with 54 species (40 endemics) in China (Shi et al., 2011). More than half of the species occur in the Hengduan Mountains. *Anaphalis xylorhiza* is widely distributed in alpine grasslands and lichen-covered areas at altitudes from 3800 to 4000 m (Shi et al., 2011). Previous cytology of this species collected from Rikaze (Tibet) showed the formula as 2n = 2x = 28 = 2M + 14m + 12sm (Meng et al., 2010). In this study, the sample from Ganzi (Sichuan) was formulated as 2n = 2x = 28 = 16m + 12sm. *A. spodiophylla* is endemic to the Hengduan Mountains region and restricted mainly to sunny roadsides at altitudes mostly ranging from 3000 to 3800 m (Shi et al., 2011). Here, we show that the karyotype formula of *A. spodiophylla* is 2n = 2x = 28 = 16m + 12sm. This is the first report of the karyotype and chromosome number of *A. spodiophylla* and it is similar to *A. xylorhiza*.

The genus *Myriactis* comprises 12 to 16 species, five species (one endemic) in China (Shi et al., 2011). The basic chromosome number of the genus *Myriactis* has been reported x = 18 and 2n = 36 (Razaq et al., 1994). *M. nepalensis* is widely distributed in moist or humid areas at altitudes from 700 to 3700 m (Shi et al., 2011). Our results show that the karyotype formula of the *M. nepalensis* is 2n = 2x = 36 = 32m + 4sm. Previous chromosome number data for *M. nepalensis* was reported as 2n = 2x = 36 (Gupta et al., 1989). We found a diploid population of *M. nepalensis*. *M. wightii* is endemic to the Hengduan Mountains region and is distributed on slopes, mixed forests, grasslands or stream sides at 1900–3600 m (Shi et al., 2011). Our results show that the karyotype formula of *M. wightii* is 2n = 2x = 36 = 32m + 4sm. The karyotype and chromosome numbers of *M. wightii* are reported here for the first time and are similar to *M. nepalensis*.

The genus *Syncalathium* is a small genus with five identified species endemic to alpine scree of the Sino-Himalayan region, all found in China (Shi et al., 2011; Zhang et al., 2011). All species are restricted mainly to altitudes ranging from 3700 to 5400 m (Shi et al., 2011). It is most likely that 2n = 16 was a common chromosome number in the genus (Zhang et al., 2007, 2009c). To our knowledge, this study is the first to examine karyotype and chromosome number data for *S. roseum*, a species restricted to sandy riverbanks at altitudes from 3700 to 3800 m (Xizang, Zhanang) (Shi et al., 2011). We collected seeds of *S. roseum* from Zhanang at the type locality. The karyotype formula of *S. roseum* is 2n = 2x = 16 = 14m + 2sm(2sat).

The genus *Pertya* has about 25 species in Eurasia, and 17 species (16 endemics) in China (Shi et al., 2011). *P. phylicoides* is endemic to the Hengduan Mountains regions and is distributed on dry-hot valley at 2400–3100 m (Shi et al., 2011). In this study, the karyotype of *P. phylicoides* was formulated as 2n = 2x = 34 = 30m + 4sm. A previous study reported that the karyotype formula for *Pertya berberidoides* in this area was 2n = 2x = 32 = 28m + 4sm (Chen et al., 2013). These two competing results suggest that *Pertya* may be an aneuploid or experience unstable structural changes in chromosomes.

The genus *Dubyaea* has about 15 species, 12 species (8 endemics) in China, with the endemics distributed to the Qinghai-Tibetan Plateau and adjacent regions (Shi et al., 2011). Our results show that the karyotype formula of the *D. tsarongensis* is 2n = 2x = 16 = 14m + 2sm. Liu and Yang (2014) reported the cytology of *Dubyaea glaucescens* with two chromosome numbers, 2n = 34 (diploid) and 2n = 51 (triploid), based on x = 17. The same study transferred *D. glaucescens* to *Faberia* after karyological data corroborated evidence from morphology, habitat preference and geographic distribution transferred of *D. glaucescens* to *Faberia* from strongly corroborated with karyological characters (). It is most likely that 2n = 16 was the basic chromosome number in this genus (Babcock et al., 1937). Here we show the karyotype and chromosome number of *D. tsarongensis* for the first time.

The genus *Carpesium* contains about 20 species in Asia and Europe, 16 (6 endemics) in China (Shi et al., 2011). In this study, we report the karyotype of four species of *Carpesium*, three for the first time (*C. lipskyi*, *C. scapiforme*, *C. velutinum*). Two populations of *C. cernuum* (from Mianning and Lijiang) were sampled and the results show that they have the same formula 2n = 2x = 40 = 26m + 10sm(2sat) + 4st. The chromosome number of *C. cernuum* was previously reported as 2n = 2x = 40 = 24m + 12sm + 2T (Wang et al., 1999). The two populations sampled in our study had similar karyotypes and karyotype morphology as previous reports of *C. cernuum*.

Polyploidy has long been recognized as an important evolutionary force in plants (Grant, 1981; Jiao et al., 2011; Levin, 2002; Stebbins, 1940, 1950, 1971), especially in Asteraceae, one of the largest angiosperm families (Semple and Watanabe, 2009). Polyploidy has been reported in ca. 570 genera of the family (58.3% of the 978 genera with chromosome counts), and ploidy levels have been found to vary from 2x to 48x (Semple and Watanabe, 2009). Recently, Huang et al. (2016) found that whole-genome duplications (WGDs) in Asteraceae were related to environmental changes and species radiations, providing a possible scenario for polyploids to overcome the disadvantages of WGDs and to evolve into lineages with high biodiversity. The diversity of chromosome evolution patterns in Asteraceae from the HDM may be related to the high level of species richness and endemism. Our findings suggest that polyploidy may not play an important role in how plants from this region have adapted to the environment, although statistical analysis of chromosome number in Asteraceae species indicates that the number of paleopolyploids is large (46.15%). Further studies are needed to discover the relationship between genome polyploidy and genome evolution in Asteraceae and more detailed research in some groups may provide more information about the mechanisms of polyploidization and speciation. Undertaking a full-scale investigation of chromosome databases may potentially reveal the relationship between ploidy level and the evolution of species diversity in the Hengduan Mountains, SW China.

Author contributions

ZML conceived and designed the experiments. WGS and FMS collected the data and performed the experiments. WGS and XGM analyzed the data. WGS and XGM wrote the manuscript. JWZ, YHZ and ZML revised the manuscript.

Acknowledgements

This study was supported by the National Natural Science Foundation of China (31670206, 31360049) to Zhi-Min Li, and major Program of NSFC (grant 31590823, 31590820) to Hang Sun, and NSFC (31370004, 31570213) to Jian-Wen Zhang. We gratitude to the Alpine Plant Diversity Group of KIB for help during field trips, we also thank Hui-Xian Yang, Xu-Rui Zhao, Yun Xu, Hui-Ying Li, Ping-Fu Yang and Yan Xu for their help with the experiments.

References

Altınordu, F., Peruzzi, L., Yu, Y., He, X.J., 2016. A tool for the analysis of chromosomes: KaryoType. Taxon 65, 586–592.
Arano, H., 1963. Cytological studies in subfamily Carduoideae (Compositae) of Japan IX. Bot. Mag. 76, 32–39.
Babcock, E.B., Stebbins, G.L., Jenkins, J.A., 1937. Chromosomes and phylogeny in some genera of the Crepidinae. Cytologia 188–210.
Chen, G., Sun, W.B., 2006. Karyotype of four species in *Buddleja* (Loganiaceae). Acta Bot. Yunnan 28, 37–40.
Chen, G.F., Ba, L.J., Sun, W.G., Lou, X., Zhang, J.W., Li, Z.M., 2013. Karyotypes and chromosome numbers of eight species from the family Asteraceae in the Hengduan Mountains and the adjacent regions. Plant Divers. Resour. 35, 367–374.
Chen, G.F., Sun, W.G., Hong, D.Y., Zhou, Z., Niu, Y., Nie, Z.L., Sun, H., Zhang, J.W., Li, Z.M., 2014. Systematic significance of cytology in *Cyananthus* (Campanulaceae) endemic to the Sino-Himalayan region. J. Syst. Evol. 52, 260–270.
Chen, J.G., Xu, B., Li, Z.M., Sun, H., 2010. Karyological Studies on Two Species of Compositae from the Hengduan Mountains, SW China. Guihaia 1, 009.
Grant, V., 1981. Plant Speciation. Columbia University Press, New York xii, 563pp. -illus., maps, chrom. nos. En 2nd edition. Maps, Chromosome numbers. General (KR, 198300748).
Gu, H.Y., 1989. On chromosome numbers of *Kalimeris* (Astereae, Asteraceae) and some related taxa. Cathaya 1, 1–16.
Gupta, R.C., Gill, B.S., Garg, R.K., 1989. Chromosomal Conspectus of Western Himalayan Compositae. Plant Science Research in India: Present Status and Future Challenges. Part 1.
Huang, C.H., Zhang, C.F., Liu, M., Hu, Y., Gao, T.G., Qi, J., Ma, H., 2016. Multiple polyploidization events across Asteraceae with two nested events in the early history revealed by nuclear phylogenomics. Mol. Biol. Evol. 33, 2820–2835.
Jiao, Y.N., Wickett, N.J., Ayyampalayam, S., Chanderbali, A.S., Landherr, L., Ralph, P.E., Tomsho, L.P., Hu, Y., Liang, H., Soltis, P.S., 2011. Ancestral polyploidy in seed plants and angiosperms. Nature 473, 97–100.
Levan, A., Fredga, K., Sandberg, A.A., 1964. Nomenclature for centromeric position on chromosomes. Hereditas 52, 201–220.
Levin, D.A., 2002. The Role of Chromosomal Change in Plant Evolution. Oxford University Press on Demand.
Lewis, W.H., 1980. Polyploidy in Angiosperms: Dicotyledons. Springer, Polyploidy, pp. 241–268.
Li, B.Y., 1987. On the boundaries of the Hengduan mountains. Mt. Res. 5, 74–82.
Li, X.W., 1994. Two big biodiversity centres of Chinese endemic genera of seed plants and their characteristics in Yunnan Province. Acta Bot. Yunnan 16, 221–227.
Li, X.W., Li, J., 1993. A preliminary floristic study on the seed plants from the region of Hengduan Mountain. Acta Bot. Yunnan 15, 217–231.
Li, Z.W., Chen, Z.R., Wang, M.L., 1991. Classification and correlation of the quaternary glacial epoch in the Hengduan (Transverse) mountains. Geol. Rev. 37, 125–132.
Liu, J.Q., 1999. Karomorphological characteristics of three *Aster* species from southern Qing Hai. Bull. Bot. Res. 19 (4), 392–396.
Liu, J.Q., 2004. Uniformity of karyotypes in *Ligularia* (Asteraceae: Senecioneae), a highly diversified genus of the eastern Qinghai–Tibet Plateau highlands and adjacent areas. Bot. J. Linn. Soc. 144, 329–342.
Liu, J.Q., Liu, S.W., Ho, T.N., Lu, A.M., 2001. Karyological studies on the Sino-Himalayan genus, *Cremanthodium* (Asteraceae: Senecioneae). Bot. J. Linn. Soc. 135, 107–112.
Liu, J.Q., Wang, Y.J., Wang, A.L., Hideaki, O., Abbott, R.J., 2006. Radiation and diversification within the *Ligularia–Cremanthodium–Parasenecio* complex (Asteraceae) triggered by uplift of the Qinghai-Tibetan plateau. Mol. Phylogen. Evol. 38, 31–49.
Liu, Y., Yang, Q.E., 2014. Cytotaxonomy of *Dubyaea glaucescens* (Compositae-Cichorieae). Nord. J. Bot. 32, 871–874.
Meng, Y., Nie, Z.L., Sun, H., Yang, Y.P., 2012. Chromosome numbers and polyploidy in *Leontopodium* (Asteraceae: Gnaphalieae) from the Qinghai-Tibet plateau of SW China. Caryologia 65, 87–93.
Meng, Y., Sun, H., Yang, Y.P., Nie, Z.L., 2010. Polyploidy and new chromosome counts in *Anaphalis* (Asteraceae: Gnaphalieae) from the Qinghai-Tibet plateau of China. J. Syst. Evol. 48, 58–64.
Meng, Y., Yang, Y.P., Deng, T., Nie, Z.L., 2016. New chromosome counts, polyploidy, and karyotype evolution in *Aster* L. (Asteraceae: Astereae) from the Qinghai-Tibet plateau. Caryologia 69, 370–378.
Meng, Y., Yang, Y.P., Sun, H., Deng, T., Nie, Z.L., 2014. Chromosome numbers, karyotypes, and polyploidy evolution of *Anaphalis* species (Asteraceae: Gnaphalieae) from the Hengduan mountains, SW China. Caryologia 67, 238–249.
Myers, N., Mittermeier, R.A., Mittermeier, C.G., Da Fonseca, G.A., Kent, J., 2000. Biodiversity hotspots for conservation priorities. Nature 403, 853–858.
Nie, Z.L., Wen, J., Gu, Z.J., Boufford, D.E., Sun, H., 2005. Polyploidy in the flora of the Hengduan Mountains hotspot, southwestern China. Ann. Mo. Bot. Gard. 92, 275–306.
Olson, D.M., Dinerstein, E., 2002. The Global 200: priority ecoregions for global conservation. Ann. Mo. Bot. Gard. 89, 199–224.
Paszko, B., 2006. A critical review and a new proposal of karyotype asymmetry indices. Plant Syst. Evol. 258, 39–48.
Peruzzi, L., Altınordu, F., 2014. A proposal for a multivariate quantitative approach to infer karyological relationships among taxa. Comp. Cytogen. 8, 337.
Peruzzi, L., Eroğlu, H.E., 2013. Karyotype asymmetry: again, how to measure and what to measure? Comp. Cytogen. 7 (1), 1–9.
Peruzzi, L., Leitch, I.J., Caparelli, K.F., 2009. Chromosome diversity and evolution in Liliaceae. Ann. Bot. 103, 459–475.
Razaq, Z.A., Vahidy, A.A., Ali, S., 1994. Chromosome numbers in Compositae from Pakistan. Ann. Mo. Bot. Gard. 81, 800–808.
Russell, A., Safer, S., Weiss-Schneeweiss, H., Temsch, E., Stuppner, H., Stuessy, T.F., Samuel, R., 2013. Chromosome counts and genome size of *Leontopodium* species (Asteraceae: Gnaphalieae) from south-western China. Bot. J. Linn. Soc. 171, 627–636.
Semple, J.C., Watanabe, K., 2009. In: Funk, V.A., Susanna, A., Stuessy, T.F., Bayer, R.J. (Eds.), A Review of Chromosome Numbers in Asteraceae with Hypotheses on Chromosomal Base Number Evolution. Systematics, Evolution, and Biography of Compositae. IAPT, Vienna, pp. 61–72.
Shi, Z., Chen, Y.L., Chen, Y.S., Lin, Y.R., Liu, S.W., Ge, X.J., Gao, T.G., Zhu, S.X., Liu, Y., Christopher, J.H., Yang, Q., Raab-Straube, E.V., Gilbert, M.G., Nordenstam, B., Kilian, N., Brouillet, L., Illarionova, I.D., Hind, D.J.N., Jeffrey, C., Bayer, R.J., Kirschner, J., Greuter, W., Anderberg, A.A., Semple, J.C., Stěpánek, J., Freire, S.E., Martins, L., Koyama, H., Kawahara, T., Vincent, L., Sukhorukov, A.P., Mavrodiev, E.V., Gottschlich, G., 2011. Astereae. In: Wu, Z.Y., Raven, P.H., Hong, D.Y. (Eds.), Flora of China, pp. 545–652. Science Press (Beijing) & Missouri Botanical Garden Press (St. Louis).
Stebbins, G.L., 1940. The significance of polyploidy in plant evolution. Am. Nat. 74, 54–66.
Stebbins, G.L., 1950. Variation and Evolution in Plants. Columbia University Press, New York.
Stebbins, G.L., 1971. Chromosomal Evolution in Higher Plants. Addison-Wesley, London.
Sun, H., 2002. Evolution of arctic-tertiary flora in Himalayan-Hengduan mountains. Acta Bot. Yunnan 24, 671–688.
Wang, B., Yang, H.L., Zheng, T.S., Yen, H.B., Xu, X.J., 1999. Karyotype analysis of *Carpesium cernuum*. Zhong Yao Cai 22, 163–165.
Wang, W.T., 1994. Vascular Plants of the Hengduan Mountains, vol. 2. Science Press, Beijing.
Wang, X., Liu, B.B., Ma, Y.Z., Xie, P.H., He, X.Y., Shang, B.L., Wang, Y.J., 2013. Chromosomal studies on the alpine genus *Dolomiaea* (Asteraceae: Cardueae) from the Qinghai-Tibet plateau and adjacent regions. Caryologia 66, 186–193.
Wang, Y.J., Liu, J.Q., 2004. A preliminary investigation on the phylogeny of *Saussurea* (Asteraceae: Cardueae) based on chloroplast DNA trn LF sequences. Acta Phytotax. Sin. 42, 136–153.
Wu, Z.Y., 1988. Hengduan mountain flora and her significance. J. Jap. Bot. 63, 297–311.
Xu, B., Li, Z.M., Sun, H., 2014a. Plant diversity and floristic characters of the alpine subnival belt flora in the Hengduan Mountains, SW China. J. Syst. Evol. 52, 271–279.
Xu, B., Li, Z.M., Sun, H., 2014b. Seed Plants of the Alpine Subnival Belt from the Hengduan Mountains, SW China. Science Press, Beijing.
Yuan, Q., Yang, Q.E., 2006. Polyploidy in *Aconitum* subgenus *Lycoctonum* (Ranunculaceae). Bot. J. Linn. Soc. 150, 343–353.
Yuan, Q., Yang, Q.E., 2008. Low incidence of polyploids and high uniformity of karyotypes displayed by *Delphinium* (Ranunculaceae) in the Hengduan Mountains region of south-west China. Bot. J. Linn. Soc. 158, 172–188.
Zhang, D.C., Boufford, D.E., Ree, R.H., Sun, H., 2009a. The 29°N latitudinal line: an important division in the Hengduan mountains, a biodiversity hotspot in southwest China. Nord. J. Bot. 27, 405–412.
Zhang, D.C., Zhang, Y.H., Boufford, D.E., Sun, H., 2009b. Elevational patterns of species richness and endemism for some important taxa in the Hengduan Mountains, southwestern China. Biodivers. Conserv. 18, 699–716.
Zhang, J.W., Nie, Z.L., Sun, H., 2009c. Cytological study on the genus *Syncalathium* (Asteraceae-Lactuceae), an endemic taxon to alpine scree of the Sino-Himalayas. J. Syst. Evol. 47, 226–230.
Zhang, J.W., Nie, Z.L., Wen, J., Sun, H., 2011. Molecular phylogeny and biogeography of three closely related genera, *Soroseris, Stebbinsia*, and *Syncalathium* (Asteraceae, Cichorieae), endemic to the Tibetan plateau, SW China. Taxon 60, 15–26.
Zhang, J.W., Sun, H., Nie, Z.L., 2007. Karyological studies on the Sino-Himalayan endemic *Soroseris* and two related genera of tribe Lactuceae (Asteraceae). Bot. J. Linn. Soc. 154, 79–87.