

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



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### 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%).

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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  $3.64 \times 10^5$  km<sup>2</sup> (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.

Ploidy 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 *Lycotomonum* (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.

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

<sup>a</sup> 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  $\mu\text{m}$ . 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  $\mu\text{m}$ . 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  $\mu\text{m}$ . 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  $\mu\text{m}$ . 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  $\mu\text{m}$ . 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  $\mu\text{m}$ . 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  $\mu\text{m}$ . 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

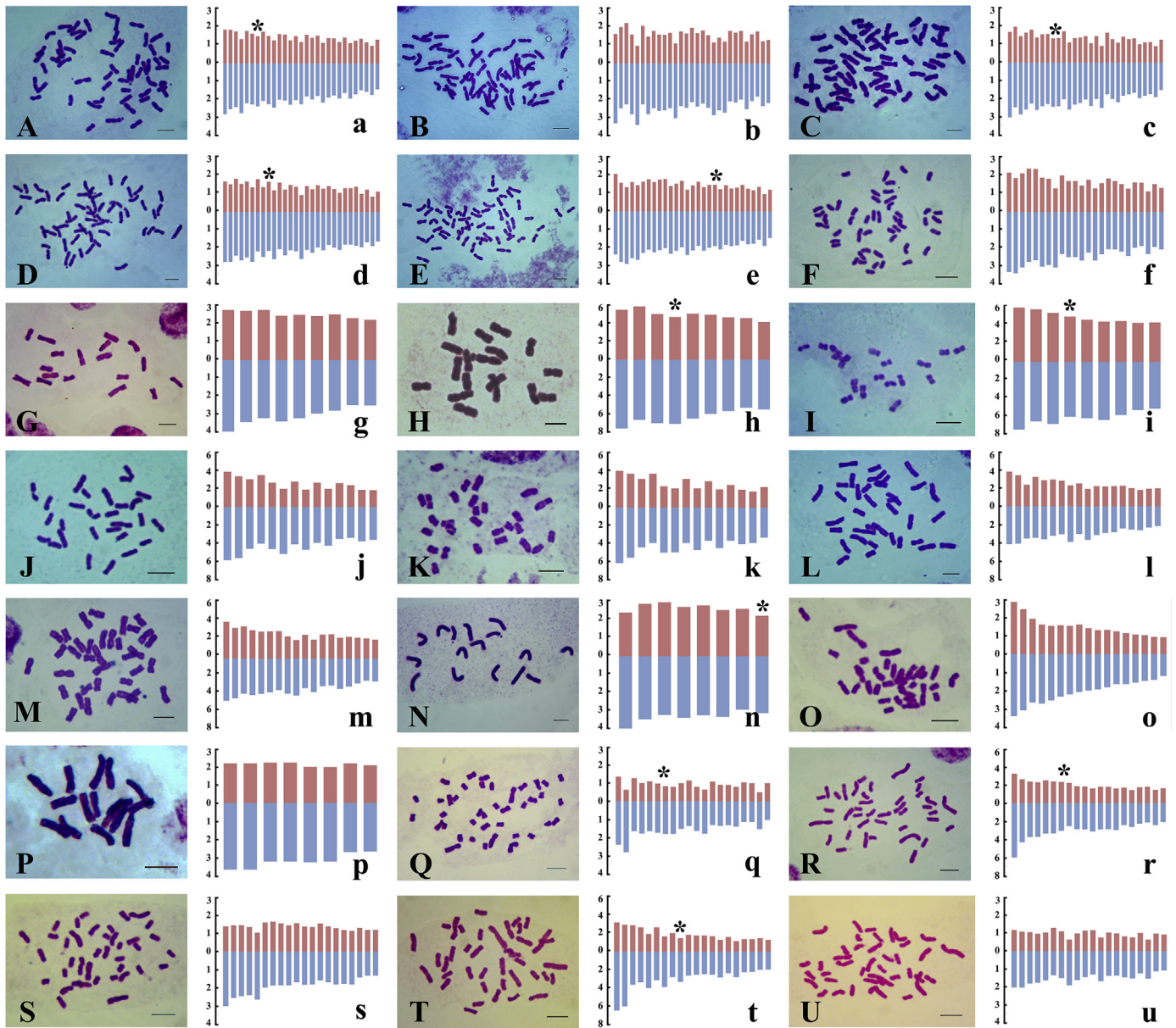
In this study, two populations of *Aster gouldii* (from Chayu and Bomi) were sampled. The karyotype of the Bomi population was formulated as  $2n = 2x = 18 = 18m$  (2SAT) (Table 2; Fig. 1: H, h). The chromosome length varied from 2.96 to 3.34  $\mu\text{m}$ . The ratio of the longest to the shortest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

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

Taxon	Chromosome length range ( $\mu\text{m}$ )	Ratio LC/SC	<2:1	As.K%	M <sub>CA</sub>	CV <sub>CL</sub>	CV <sub>CI</sub>	THL	2n/x/ploidy level	Karyotype formula	KA Type	Figure
<i>Cremanthodium lineare</i> var. <i>eligulatum</i> <sup>a</sup>	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
<i>C. angustifolium</i> <sup>a</sup>	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
<i>C. principis</i> <sup>a</sup>	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
<i>C. helianthus</i> <sup>a</sup>	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
<i>C. campanulatum</i> var. <i>campanulatum</i> <sup>a</sup>	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
<i>Leontopodium souliei</i>	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
<i>Aster pekinensis</i>	4.68–6.62	1.41	0	55.10	11.62	11.91	3.90	31.06	18/9/2x	18m	1A	G, g
<i>A. gouldii</i> (BM) <sup>a</sup>	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
<i>A. gouldii</i> (CY) <sup>a</sup>	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
<i>Anaphalis xylorhiza</i>	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
<i>A. spodiophylla</i> <sup>a</sup>	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
<i>Myriactis nepalensis</i>	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
<i>M. wightii</i> <sup>a</sup>	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
<i>Syncalathium roseum</i> <sup>a</sup>	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
<i>Pertya phyllicoides</i> <sup>a</sup>	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
<i>Dubyaea tsarongensis</i> <sup>a</sup>	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
<i>Carpesium cernuum</i> (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
<i>C. cernuum</i> (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
<i>C. lipskyi</i> <sup>a</sup>	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
<i>C. scapiforme</i> <sup>a</sup>	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
<i>C. velutinum</i> <sup>a</sup>	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<sub>CA</sub>: Mean Centromeric Asymmetry; CV<sub>CL</sub>: the relative variation in chromosome length; CV<sub>CI</sub>: 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.

<sup>a</sup> 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. *eligitatum*; 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 phlycooides*; 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  $\mu$ m; 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  $\mu$ m. 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  $\mu$ m. 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  $\mu$ m. 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  $\mu$ m. 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  $\mu$ m. 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  $\mu\text{m}$ . 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 phyllicoides* Jeffrey

The karyotype of *Pertya phyllicoides* 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  $\mu\text{m}$ . 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  $\mu\text{m}$ . 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  $\mu\text{m}$ . 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  $\mu\text{m}$ . 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  $\mu\text{m}$ . 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  $\mu\text{m}$ . 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  $\mu\text{m}$ . 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  $n \geq 11$ , 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
<i>Anaphalis aureopunctata</i>	28, 56	14	2x, 4x	2A, 2B	Meng et al. (2014)
<i>A. bicolor</i>	28, 56	14	2x, 4x	2A, 1B, 2B	Meng et al. (2014)
<i>A. deserti</i>	56	14	4x	1B	Meng et al. (2010)
<i>A. flavescens</i>	28	14	2x	2A	Meng et al. (2014)
<i>A. latialata</i>	56	14	4x	2B	Meng et al. (2014)
<i>A. likiangensis</i>	56	14	4x	2B	Meng et al. (2014)
<i>A. margaritacea</i>	28, 56	14	2x, 4x	1B, 2B	Meng et al. (2010)
<i>A. nepalensis</i> var. <i>corymbosa</i>	28, 84	14	2x, 6x	2A, 2B	Meng et al. (2014)
<i>A. nepalensis</i> var. <i>nepalensis</i>	28, 56	14	2x, 4x	2A, 2B	Meng et al. (2014)
<i>A. pachylaena</i>	28	14	2x	2A	Meng et al. (2014)
<i>A. pannosa</i>	56	14	4x	2A	Meng et al. (2014)
<i>A. plicata</i>	56	14	4x	2B	Meng et al. (2010)
<i>A. rhododactyla</i>	56	14	4x	2B	Meng et al. (2010)
<i>A. royleana</i>	28	14	2x	2B	Meng et al. (2010)
<i>A. spodiophylla</i>	28	14	2x	2A	Present study
<i>A. stenocephala</i>	56	14	4x	2A, 2B	Meng et al. (2014)
<i>A. surculosa</i>	56	14	4x	2A	Meng et al. (2014)
<i>A. virens</i>	56	14	4x	2B	Meng et al. (2014)
<i>A. xylophiza</i>	28	14	2x	2A	Present study
<i>A. yunnanensis</i>	56	14	4x	2B	Meng et al. (2014)
<i>Aster albescens</i> var. <i>glabratus</i>	18	9	2x	2A	Meng et al. (2016)
<i>A. albescens</i> var. <i>gracilior</i>	18	9	2x	2A	Meng et al. (2016)
<i>A. altaicus</i> var. <i>hirsutus</i>	18	9	2x	1A	Meng et al. (2016)
<i>A. auriculatus</i>	18	9	2x	1A	Meng et al. (2016)
<i>A. crenatifolius</i>	18	9	2x	1A	Chen et al. (2010) and Meng et al. (2016)
<i>A. diplostephioides</i>	18	9	2x		Liu (1999)
<i>A. gouldii</i>	18	9	2x	1A	Present study
<i>A. oreophilus</i>	18	9	2x	1A	Meng et al. (2016)
<i>A. pekinensis</i>	18	9	2x	1A	Present study
<i>A. poliothamnus</i>	18	9	2x	1A	Meng et al. (2016)
<i>A. pycnophyllus</i>	18, 36	9	2x, 4x	1A, 2A	Meng et al. (2016)
<i>A. salwinensis</i>	18	9	2x	1A	Meng et al. (2016)
<i>A. souliei</i>	18, 54	9	2x, 6x	2A	Liu (1999) and Meng et al. (2016)
<i>A. techinensis</i>	18	9	2x	1A	Meng et al. (2016)
<i>A. trinervius</i> subsp. <i>ageratoides</i>	72	9	8x	2A	Meng et al. (2016)
<i>A. yunnanensis</i> var. <i>labrangensis</i>	18	9	2x		Liu (1999)
<i>A. yunnanensis</i> var. <i>yunnanensis</i>	18	9	2x	2A	Meng et al. (2016)
<i>Carpesium cernuum</i>	40	20	2x	2B	Present study
<i>C. lipskyi</i>	40	20	2x	2B	Present study
<i>C. scapiforme</i>	40	20	2x	2B	Present study
<i>C. velutinum</i>	40	20	2x	2B	Present study
<i>Cremanthodium angustifolium</i>	58	29	2x	2A	Present study
<i>C. brunneo-pilosum</i>	58	29	2x	2A	Liu et al. (2001)
<i>C. campanulatum</i>	58	29	2x	2A	Present study
<i>C. discoideum</i>	58	29	2x	2A	Liu et al. (2001)
<i>C. ellisii</i>	58	29	2x	2A	Liu et al. (2001)
<i>C. helianthus</i>	58	29	2x	2A	Present study
<i>C. humile</i>	58, 60	29, 30	2x	2A	Liu et al. (2001)
<i>C. lineare</i>	58	29	2x	2A	Liu et al. (2001)
<i>C. lineare</i> var. <i>eligulatum</i>	58	29	2x	2A	Present study
<i>C. microglossum</i>	58	29	2x	2A	Liu et al. (2001)
<i>C. principis</i>	58	29	2x	2A	Present study
<i>C. stenoglossum</i>	58	29	2x	2A	Liu et al. (2001)
<i>Dubyaea glaucescens</i>	34, 51	17	2x, 3x		Liu et al. (2014)
<i>D. tsarongensis</i>	16	8	2x	1A	Present study
<i>Leontopodium artemisiifolium</i>	26	13	2x		Russell et al. (2013)
<i>L. dedeckensii</i>	26	13	2x		Russell et al. (2013)
<i>L. himalayanum</i>	24	12	2x		Russell et al. (2013)
<i>L. sinense</i>	48	12	4x		Russell et al. (2013)
<i>L. souliei</i>	48	12	4x	2B	Present study
<i>L. stracheyi</i>	26	13	2x		Russell et al. (2013)
<i>Myriactis nepalensis</i>	36	18	2x	2A	Present study
<i>M. wightii</i>	36	18	2x	2A	Present study
<i>Pertya berberidoides</i>	32	16	2x	1B	Chen et al. (2013)
<i>P. phyllicoides</i>	34	17	2x	1B	Present study
<i>Syncalathium chrysocephalum</i>	16	8	2x	1A	Zhang et al. (2009c)
<i>S. disciforme</i>	16	8	2x	1A	Zhang et al. (2009c)
<i>S. kawaguchii</i>	16	8	2x	1A	Zhang et al. (2009c)
<i>S. roseum</i>	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. phyllicoides* 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. phyllicoides* 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.

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