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Chromosome counts and karyotype analysis of nine taxa in *Sorbus* subgenera *Aria* and *Micromeles* (Rosaceae) from China

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Abstract. Chromosome numbers of nine taxa in *Sorbus* subgenera *Aria* (Pers.) Host and *Micromeles* Decne. were determined. All are diploid with 2n = 2x = 34. Eight out of them: *S. alnifolia* (Siebold and Zucc.) K. Koch var. *angulata* S.B. Liang Rehd., *S. alnifolia* (Siebold and Zucc.) K. Koch var. *lobulata* (C.K. Schneid.) Rehd., *S. dunnii* Rehd., *S. folgneri* (C.K. Schneid.) Rehd., *S. hemsleyi* (C.K. Schneid.) Rehd., *S. lushanensis* X. Chen & J. Qiu, *S. ochracea* (Hand.-Mazz.) J.E. Vidal and *S. yuana* Spongberg, which are endemic to China, were reported for the first time. One remaining Asian native *S. alnifolia* var. *alnifolia* (Siebold and Zucc.) K. Koch, 2n = 34 was confirmed here. The chromosomes size and the total haploid chromosome length varied from 0.91 µm to 3.51 µm and 23.43 µm to 50.04 µm respectively. A predominance of metacentric chromosomes were perceived. Satellites were observed only in taxa of subgenera *Micromeles*. Nine taxa were classified as 1A, 2A and 2B according to the Stebbins classification. *S. yuana* in subgenus *Aria* presents the most symmetrical karyotype according to all the 11 quantitative indices analyzed, while *S. dunnii* and *S. ochracea* in *Micromeles* present the most asymmetrical karyotypes according to different indices.

Keywords: chromosome number, karyotype asymmetry, *Sorbus*, subgenera *Aria* and *Micromeles*, China.

INTRODUCTION

The genus *Sorbus* L. (Rosaceae) comprises about from 67 (Lu and Spongberg 2003) to at least 111 species in China (Phipps *et al.*1990). Chinese native species were traditionally assigned to three subgenera (three sections in the same names by Yü and Kuan (1963)): *Aria* (Pers.) Host and *Micromeles* Decne. with simple leaved species, *Sorbus* with pinnately leaved species (Phipps *et al.* 1990).

Polyploidy together with hybridization and apomixis is an important evolutionary force in shaping plant diversification in *Sorbus* (Robertson *et al.* 2010; Ludwig *et al.* 2013; Lepší *et al.* 2019). The number of species recognized in the genus varied between authors mainly depending on the way that the

numerous polyploidy apomicts were treated (Aldasoro *et al.* 2004; McAllister 2005; Sennikov and Kurtto 2017).

Knowledge of chromosome numbers is highly important in *Sorbus* in understanding the species delimitation and relationship. Modern taxonomic studies (McAllister 2005; Sennikov and Kurtto 2017) and descriptions of new species (Rich *et al.* 2014; Lepší *et al.* 2015; Somlyay *et al.* 2017) are accompanied to count of chromosome numbers or DNA ploidy levels based on flow cytometry.

Polyploidy was reported only in subgenus Sorbus from China, which account for more than half of the subgenus' species richness and are distributed mainly in the mountains of southwest area, especially the Qinghai-Tibet Plateau (McAllister 2005; Li et al. 2021). For subgenus Aria, which is disjunctly distributed across Europe and Asia, with polyploidy species reported from only Europe (Sennikov and Kurtto 2017). For the Asian native subgenus Micromeles, the only chromosome number available is S. alnifolia (2n = 2x = 34; Sax 1931; Baranec and Murín 2003). Though Aldasoro et al. (2004) suggested that the great variability in leaf outline of S. alnifolia might reflect the presence of agamospermous individuals in populations, no evidence is available up to now. Agamospermy as a form of asexual reproduction is prevalent in Sorbus (Robertson et al. 2010; Hajrudinović et al. 2015) and other genera in Maloideae such as Amelanchier Medik. (Burgess et al. 2014), Cotoneaster Medik. (Rothleutner et al. 2016) and Crataegus L. (Talent and Dickinson, 2007) etc.

The aim of this study is to investigate the chromosome number, karyotype, idiogram and other detailed measurements of eight Chinese endemic taxa from Subgenus *Aria* and *Micromeles*, together with an Asian distributed species *S. alnifolia*, to find out whether there are polyploid and to analyze the species relationship.

MATERIALS AND METHODS

Plant materials

Nine taxa, including two species in Sorbus subgenus Aria: S. hemsleyi (C.K. Schneid.) Rehd. and S. yuana Spongberg, and seven taxa in subgenus Micromeles: S. alnifolia (Siebold and Zucc.) K. Koch var. alnifolia, S. alnifolia (Siebold and Zucc.) K. Koch var. angulata S.B. Liang, S. alnifolia (Siebold and Zucc.) K. Koch var. lobulata Rehd., S. dunnii Rehd., S. folgneri (C.K. Schneid.) Rehd., S. lushanensis X. Chen and J. Qiu, S. ochracea (Hand.-Mazz.) J.E. Vidal, were investigated. One species, S. ochracea, was collected from the Kunming Botanical Garden, and the remaining eight taxa were collected from wild populations between 2012 and 2016. Voucher specimens are deposited at the herbarium of Nanjing Forestry University (NF; Table 1).

Chromosome counts

Root tips were immersed in a mixed solution of 0.05% aqueous colchicine and 0.002 mol/L 8-hydroxy quinolone (1:1) at 0-4 °C for 2 h, and then fixed in Carnoy's fixative (3:1 alcohol:glacial acid, v/v) for at least 24

Table 1. Localities and voucher specimens of Sorbus taxa examined in the present study.

Taxa	Locality	Collector/Voucher specimen
S. hemsleyi	Wawushan Scenic Spot, Sichuan province, E102°57'06.82", N29°39'54.35", 2230 m, 25 September 2016	Xin Chen & Zhongren Xiong/0745
S. yuana	Shennongjia Forestry District, Hubei province, E110°19'17.76", N31°34'21.30", 2173 m, 20 October 2016	Yun Chen & Yang Zhao/0817
S. alnifolia var. alnifolia	Changbai Mountains, Jilin province, E127°49'24.85", N42°02'04.85", 867m	Xin Chen & Dan Chen/2-1
S. alnifolia var. angulata	Lushan Mountain, Shandong province, E118°03'09.00", N36°17'45.31", 1047 m, 9 October 2015	Xin Chen & Jing Qiu/0140
S. alnifolia var. lobulata	Laoshan Mountain, Shandong province, E120°37'26.58", N36°10'40.04", 942 m, 28 October 2014	Xin Chen & Wan Du/0042
S. dunnii	Huangshan Mountain, Anhui province, E117°26'18.07", N29°11'36.31", 1603 m September 2013	'Xin Chen & Dan Chen/4-7
S. folgneri	Badong, Hubei province, E110°17'19.75" N30°41 '36.86", 1543 m, 17 October 2016	Yun Chen & Yang Zhao/0791
S. lushanensis	Lushan Mountain, Jiangxi province, E116°00'46.29", N29°32'58.59", 1310 m, 2 October 2015	Xin Chen, Weiqi Liu & Mingwei Geng/0157
S. ochracea	Kunming Botanical Garden, Yunnan province, E102°44'17.54", N25°8 '23.65",1936 m, 5 August 2020	Qin Wang/0256-2

h at room temperature. The root tips were hydrolyzed in 1 mol/L HCl at 60 °C for 10 min, then were washed with distilled water for 2-3 min. The fixed roots were stained with Carbol fuchsin for 3-4 h and were squashed on glass slides for observation.

No less than five cells per individual and three to five plants per taxon were examined. Photos were taken under a Nikon Eclipse Ci-S microscope.

Karyotype analysis

For the numerical characterization of the karyotypes, the following parameters were measured and calculated using KaryoType software (Altinordu *et al.* 2016): short arm length (S) and long arm length (L); mean length of the chromosome (CL); total haploid length of the chromosome set (THL); longest chromosome/shortest chromosome (Lt/St); ratio of mean long to short arm length (MAR); centromeric index (CI); coefficient of variation of the centromeric index (CV_{CI}) and coefficient of variation of chromosome length (CV_{CI}); mean centromeric asymmetry (M_{CA}); the karyotype asymmetry index (AsK%); the total form percent (TF%); the index of karyotype symmetry (Syi%); the intra chromosomal asymmetry index (A_1) ; the interchromosomal asymmetry index (A_2) ; the degree of karyotype asymmetry (A); the dispersion index (DI) and the asymmetry index (AI).

Karyotype formula was determined by chromosome morphology based on centromere position according to Levan classification: median point (M, AR = 1.00), median region (m, AR = 1.01–1.70), submedian (sm, AR = 1.71–3.00), subterminal (st, AR = 3.01-7.00) and terminal region (t, AR > 7.00). Satellite chromosomes were abbreviated as 'sat' (Levan *et al.* 1964). Idiograms were drawn using KaryoType based on length of chromosome size.

Statistical analysis was carried out by using SPSS 26.0.

RESULTS

All nine investigated Sorbus taxa are diploids with 2n = 2x = 34. New counts were reported for eight Chinese endemic taxa, S. alnifolia var. angulata, S. alnifolia var. lobulata, S. dunnii, S. folgneri, S. hemsleyi, S. lushanensis, S. ochracea and S. yuana. For the remaining S. alnifolia var. alnifolia, the previously reported chromosome numbers was confirmed here.

Karyotype characters of the nine taxa were reported for the first time (Table 2; Figure 1, 2). The

genus Species	2n	Karyotype formula	VCL (µm) THL (µm)	Lt/St	MAR	Stebbins's type	CV _{CI}	CV _{CL}	$M_{\rm CA}$	AsK%	TF%	Syi%	A_1	A_2	A	DI	AI
t S. hemsleyi	34	29m+5sm	0.93-1.83 23.43	1.97	1.33	2A	11.51	18.56	12.92	56.7	43.3	76.35	0.22	0.19	0.13	7.51	2.14
S. yuana	34	32m+2sm	1.15-1.78 23.44	1.55	1.27	1A	8.36	11.29	11.06	55.59	44.41	79.9	0.19	0.11	0.11	5.08	0.97
romeles S. alnifolia vi	ar. alnifolia 34	32m(2sat)+2sm	1.48-2.49 32.91	1.68	1.34	1A	9.31	14.2	13.8	57.02	42.98	75.38	0.23	0.14	0.14	5.73	1.32
S. alnifolia vi	ar. angulata 34	26m+8sm(2sat)	1.04-1.86 24.02	1.78	1.49	2A	13.82	14.79	18.13	59.41	40.59	68.32	0.29	0.15	0.18	6.45	2.04
S. alnifolia vi	ar. lobulata 34	32m(2sat)+2sm	1.27-2.56 31.41	2.01	1.46	2B	9.92	17.72	17.66	58.8	41.2	70.06	0.29	0.18	0.18	7.14	1.76
S. dunnii	34	24m(2sat)+10sm	2.14-3.51 50.04	i 1.64	1.51	2A	14.57	14.58	18.49	59.53	40.47	67.97	0.30	0.15	0.18	5.75	2.12
S. folgneri	34	30m(2sat)+4sm	0.91-1.96 24.45	3 2.15	1.36	2B	9.71	15.68	14.10	56.99	43.01	75.47	0.24	0.16	0.14	6.95	1.52
S. lushanensı	is 34	30m(2sat)+4sm	1.27-2.26 29.2	1.78	1.39	2A	12.05	16.45	14.94	57.93	42.07	72.61	0.25	0.16	0.15	6.72	1.98
S. ochracea	34	28m(2sat)+6sm	1.26-2.5 29.22	1.98	1.36	2A	11.46	19.92	13.71	57.11	42.89	75.11	0.23	0.20	0.14	9.38	2.28

Cable 2. Karyotype features of the nine studied Sorbus taxa

Sub

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Figure 1. Somatic chromosomes at the metaphase stage in root tip cells of *Sorbus* taxa. A- S. alnifolia; B- S. alnifolia var. angulata; C- S. alnifolia var. lobulata; D- S. dunnii; E- S. folgneri; F- S. hemsleyi; G- S. lushanensis; H- S. ochracea; I- S. yuana. Scale bar = 5 μm.

size of the chromosomes varied from 0.91 μ m (0.91–1.96 μ m) in *S. folgneri* to 3.51 μ m (2.14–3.51 μ m) in *S. dunnii*. The total haploid chromosome length (THL) changed from 23.43 μ m in *S. hemsleyi* (the THL value of *S. yuana* is 23.44 μ m, nearly the same as *S. hemsleyi*) to 50.04 μ m in *S. dunnii*.

Two species, S. folgneri and S. hemsleyi have both very small ($\leq 1 \ \mu m$) and small (> 1 μm and $\leq 4 \ \mu m$)

chromosome. The remaining taxa had only small chromosome.

Two satellites were observed in seven subgenus *Micromeles* taxa, whereas no satellites in two subgenus *Aria* species. Karyotypes of the analyzed species exhibit a predominance of metacentric chromosomes with 2–10 submetacentric chromosomes detected in different taxon.



Figure 2. Haploid idiograms of *Sorbus* taxa. Red arrows indicate satellites. Scale bar = $5 \mu m$.

The karyotype asymmetry was assessed based on Stebbins classification and 11 different quantitative indices (Table 2).

According to the symmetry classification of Stebbins, *S. alnifolia* var. *alnifolia* and *S. yuana* were classified as category 1A, *S. alnifolia* var. *lobulata* and *S. folgneri* as category 2B, the other five taxa as category 2A.

For the two species in subgenus *Aria*, *S. hemsleyi* and *S. yuana*, the latter presented the most symmetrical karyotype of all the analyzed species as shown in scat-

ter diagram (Figure 3), which with the highest values in Syi% and TF% and the smallest values in the other nine asymmetrical indices (Table 2). Two species in subgenus *Aria* were more symmetrical than those taxa in subgenera *Micromeles* based on two indices A_1 and M_{CA} . In general, *S. hemsleyi* was more asymmetrical than *S. yuana* with two more submetacentric chromosomes and with values of asymmetrical parameters among the taxa in subgenus *Micromeles* (Table 2; Figures 3).

For subgenera Micromeles, the seven analyzed taxa



Figure 3. Scatter plots of studied taxa based on karyotype parameters. A- A1 versus A1; B- AI versus DI; C- CVCI versus CVCL; D- MCA versus CVCL.

displayed considerable differences in karyotypic parameters (Table 1). The two species: *S. dunnii* with the smallest values in Syi% and TF% and the highest values in AsK%, CV_{CI} , M_{CA} , A and A_1 ; *S. ochracea* with the highest values in CV_{CL} , A_2 , AI and DI. The most asymmetrical karyotype was observed in *S. ochracea* according to the scatter diagram based on AI and DI (Figures 3B). However, the two species, *S. dunnii* and *S. ochracea*, presented the most asymmetrical karyotypes respectively according to different indices as shown in scatter diagram based on A_1 and A_2 (Table 2; Figures 3A, C, D).

DISCUSSION

The chromosome base number in *Sorbus* is x = 17, and it is common to all members of Maloideae, Rosaceae. Four ploidy levels (di-, tri-, tetra- and pentaploid with 2n = 34, 51, 68 and approximately 87, respectively) were reported in the genus (Nelson-Jones *et al.* 2002; Bailey *et al.* 2008).

Only diploids in subgenera *Aria* and *Micromeles* were found from China in this and previous studies (Sax 1931; Baranec and Murín 2003), though a large number of polyploidy species (tri-, tetra and pentaploids) appeared in subgenus *Aria* in Europe (Sennikov and Kurtto 2017).

Polyploidy is an important evolutionary mechanism in *Sorbus* and it is particularly widespread in subgenus *Sorbus* native to China (McAllister 2005; Li *et al.* 2021). Whether there are polyploids in subgenera *Aria* and *Micromeles* in China, especially in the mountainous area in southwest where a great quantity of polyploidy species were reported from subgenus *Sorbus*, ploidy-level determination of more species and on more populations are required.

Sorbus subgenus Aria is considered the most primitive and *Micromeles* is more derived (Yü and Kuan 1963; Phipps et al. 1990). However, the taxonomic delimitation between Aria and Micromeles is complex. These two subgenera are easy to distinguish morphologically: Aria species with a persistent upper part of the hypanthium, and Micromeles species with a deciduous calyx and distinct annular scar at the apex of fruit (Yü and Kuan 1963). However, Micromeles is included within Aria by Robertson et al. (1991) and Aldasoro et al. (2004) based on comprehensive morphological characteristics. A merge (Campbell et al. 2007) or separate (Zhang et al. 2017) of the two is supported by molecular evidence in different phylogenetic studies of Maloideae. Not only the subgeneric concept changed, the delimitation of species varied greatly between authors. For Chinese native subgenera Aria and Micromeles, 20 species out of the total 31 species and 7 varieties recognized in Flora of China (Lu and Spongberg 2003) were accepted by Aldasoro et al. (2004) in the latest revision of subgenera Aria and Torminaria.

In this study, the two subgenera could be distinguished easily by the existence of satellites. Both the two species of subgenus *Aria* with much small chromosomes had more symmetrical karyotype, as showed by M_{CA} and A (Table 1). Not the same as *S. yuana*, which with all the indices indicating its primitive, *S. hemsleyi* showed status of taxonomically complicated with values of some parameters among species of subgenus *Micromeles. S. hemsleyi* was assigned to *Micromeles* by Yü and Kuan (1963) and was assigned to *Aria* by Phipps *et al.* (1990). The inconsistent in values of asymmetry indices just strengthened its complex classification status or a support factor for the merge of the two subgenera need to be further studied. For the limited sampling (two species in *Aria* and seven taxa in *Micromeles*), the comparison of karyotype data could not solve the taxonomic problems related to the two subgenera. Whether or which Karyotype parameters in *Sorbus* are useful for distinguishing subgenus and species, additional karyotype analysis of a larger number of species are needed.

CONCLUSION

The chromosome numbers, karyotypes, idiograms and karyotype asymmetry degrees of nine Chinese native taxa in *Sorbus* subgenera *Aria* and *Micromeles* were investigated in this study. The chromosome numbers (2n = 2x = 34) of eight Chinese endemic taxa were firstly reported. In general, the karyotypes of species in subgenus *Aria* were quite symmetric than that in Subgenus *Micromeles*.

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