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 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. Qi, 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.
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; Lepši 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.

<table>
<thead>
<tr>
<th>Taxa</th>
<th>Locality</th>
<th>Collector/Voucher specimen</th>
</tr>
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<tbody>
<tr>
<td>S. hemsleyi</td>
<td>Wawushan Scenic Spot, Sichuan province, E102°57’06.82”, N29°39’54.35”, 2230 m, 25 September 2016</td>
<td>Xin Chen &amp; Zhongren Xiong/0745</td>
</tr>
<tr>
<td>S. yuana</td>
<td>Shennongjia Forestry District, Hubei province, E110°19’17.76”, N31°34’21.30”, 2173 m, 20 October 2016</td>
<td>Yun Chen &amp; Yang Zhao/0817</td>
</tr>
<tr>
<td>S. alnifolia var. alnifolia</td>
<td>Changbai Mountains, Jilin province, E127°49’24.85”, N42°02’04.85”, 867m</td>
<td>Xin Chen &amp; Dan Chen/2-1</td>
</tr>
<tr>
<td>S. alnifolia var. angulata</td>
<td>Lushan Mountain, Shandong province, E118°03’09.00”, N36°17’45.31”, 1047 m, 9 October 2015</td>
<td>Xin Chen &amp; Jing Qiu/0140</td>
</tr>
<tr>
<td>S. alnifolia var. lobulata</td>
<td>Laoshan Mountain, Shandong province, E120°37’26.58”, N36°10’40.04”, 942 m, 28 October 2014</td>
<td>Xin Chen &amp; Wan Du/0042</td>
</tr>
<tr>
<td>S. dunnii</td>
<td>Huangshan Mountain, Anhui province, E117°26’18.07”, N29°11’36.31”, 1603 m, September 2013</td>
<td>Xin Chen &amp; Dan Chen/4-7</td>
</tr>
<tr>
<td>S. folgneri</td>
<td>Badong, Hubei province, E110°17’19.75” N30°41’36.86”, 1543 m, 17 October 2016</td>
<td>Yun Chen &amp; Yang Zhao/0791</td>
</tr>
<tr>
<td>S. lushanensis</td>
<td>Lushan Mountain, Jiangxi province, E116°00’46.29”, N29°32’58.59”, 1310 m, 2 October 2015</td>
<td>Xin Chen, Weiqi Liu &amp; Mingwei Geng/0157</td>
</tr>
<tr>
<td>S. ochracea</td>
<td>Kunming Botanical Garden, Yunnan province, E102°44’17.54”, N25°8 ‘23.65”, 1936 m, 5 August 2020</td>
<td>Qin Wang/0256-2</td>
</tr>
</tbody>
</table>
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 (CVCI) and coefficient of variation of chromosome length (CVCL); the karyotype asymmetry index (AsK%); the total form percent (TF%); the index of karyotype symmetry (Syi%); the intra chromosomal asymmetry index (A1); the interchromosomal asymmetry index (A2); 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

| Table 2. Karyotype features of the nine studied Sorbus taxa. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Subgenus** | **Species** | **2n** | **Karyotype formula** | **VCL (µm)** | **THL (µm)** | **Lt/St** | **MAR** | **Stebbins’ type** | **CVCI** | **CVCL** | **MCA** | **AsK%** | **TF%** | **Syi%** | **A1** | **A2** | **A** | **DI** | **AI** |
| Aria | *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 | 2A | 9.31 | 14.2 | 12.06 | 57.02 | 42.98 | 75.38 | 0.23 | 0.14 | 0.14 | 5.73 | 1.32 |
| Micromeles | *S. alnifolia* var. *alnifolia* | 34 | 32m(2sat)+2sm | 1.48-2.49 | 32.91 | 1.68 | 1.34 | 1A | 9.41 | 14.2 | 13.8 | 49.5 | 50.5 | 79.9 | 0.19 | 0.11 | 0.11 | 5.73 | 1.32 |
| | *S. alnifolia* var. *angulata* | 34 | 26m+8sm(2sat) | 1.04-1.86 | 24.02 | 1.78 | 1.49 | 2A | 13.82 | 14.79 | 18.13 | 59.4 | 40.59 | 68.32 | 0.29 | 0.15 | 0.18 | 6.45 | 2.04 |
| | *S. alnifolia* var. *lobulata* | 34 | 32m(2sat)+2sm | 1.27-2.56 | 31.41 | 1.97 | 1.51 | 2A | 9.31 | 14.2 | 12.06 | 57.02 | 42.98 | 75.38 | 0.23 | 0.14 | 0.14 | 5.73 | 1.32 |
| | *S. dunnii* | 34 | 24m(2sat)+10sm | 2.14-3.51 | 50.04 | 1.64 | 1.51 | 2A | 14.57 | 15.68 | 14.10 | 56.99 | 43.01 | 68.32 | 0.28 | 0.14 | 0.14 | 6.45 | 2.04 |
| | *S. folgneri* | 34 | 30m(2sat)+4sm | 0.91-1.96 | 24.45 | 2.15 | 1.36 | 2B | 9.71 | 15.68 | 14.10 | 56.99 | 43.01 | 68.32 | 0.28 | 0.14 | 0.14 | 6.45 | 2.04 |
| | *S. lushanensis* | 34 | 30m(2sat)+4sm | 1.27-2.26 | 29.22 | 1.78 | 1.36 | 2A | 14.57 | 15.68 | 14.10 | 56.99 | 43.01 | 68.32 | 0.28 | 0.14 | 0.14 | 6.45 | 2.04 |
| | *S. ochracea* | 34 | 28m(2sat)+6sm | 1.26-2.5 | 29.22 | 1.98 | 1.36 | 2A | 14.57 | 15.68 | 14.10 | 56.99 | 43.01 | 68.32 | 0.28 | 0.14 | 0.14 | 6.45 | 2.04 |
size of the chromosomes varied from 0.91 μm (0.91–1.96 μm) in *S. folgneri* to 3.51 μm (2.14–3.51 μm) in *S. dunnii*. The total haploid chromosome length (THL) changed from 23.43 μm in *S. hemsleyi* (the THL value of *S. yuana* is 23.44 μm, nearly the same as *S. hemsleyi*) to 50.04 μm in *S. dunnii*.

Two species, *S. folgneri* and *S. hemsleyi* have both very small (≤ 1 μm) and small (> 1 μm and ≤ 4 μm) 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.

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

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 scatter 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

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**Figure 2.** Haploid idiograms of *Sorbus* taxa. Red arrows indicate satellites. Scale bar = 5 μm.
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%, CVCI, MCA, A and A1; *S. ochracea* with the highest values in CV CL, A2, 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 A1 and A2 (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.

ACKNOWLEDGEMENTS

We are grateful to Zhongren Xiong, Yun Chen, Yang Zhao, Jing Qiu, Wan Du, Mingwei Geng and Qin Wang for collecting samples; to Dan Chen, Haiying Peng and Weiqi Liu for their help during chromosome preparations. We also acknowledge the Priority Academic Program Development of Jiangsu Higher Education Institutions, Jiangsu Province, China (PAPD) for financial support.

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