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Cytological observation on some Chinese species of *Plagiochila* (Plagiochilaceae, Marchantiophyta)

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Abstract – Karyological studies were made on 12 species of the genus *Plagiochila* (Plagiochilaceae, Jungermanniopsida) from China. The chromosome number n = 9 was found in all *Plagiochila* species investigated. The chromosome numbers of five species, *Plagiochila fordiana* Steph., *P. gracilis* Lindenb. & Gottsche, *P. peculiaris* Schiffn., *P. salacensi*s Gottsche, and *P. wightii* Nees ex Lindenb., are reported for the first time. The results for *Plagiochila flexuosa* Mitt., *P. fruticosa* Mitt., *P. ovalifolia* Mitt., *P. pulcherrima* Horik., *P. sciophila* Nees ex Lindenb., *P. semidecurrens* (Lehm. & Lindenb.) Lindenb., and *P. trabeculata* Steph. confirmed previous records (n = 9). The length and width of the largest chromosomes and smallest ones are provided. The karyotypes of five species are proposed. The m-chromosome was found in the cytotypes of 10 out of 12 *Plagiochila* species investigated here, except *Plagiochila sciophila* and *Plagiochila wightii*.

China / chromosome number / Hepaticae / karyotype / m chromosome / *Plagiochila*

INTRODUCTION

Plagiochila is the largest genus of Hepaticae and about 400 species accepted (So, 2001). Eighty species of this genus are known in China (So, 2001). The species of *Plagiochila* have great environmental range and morphological plasticity and their taxonomy is thought to be difficult (Inoue, 1974a; So, 2001). Accumulation of cytological data in *Plagiochila* may provide some information for the intrageneric classification (Inoue, 1974a). Cytological work of *Plagiochila*, however, is rather laggard and till now only 31 species of this genus are reported for the chromosomes number (Fritsch, 1991).

In China, karyological data of this genus is extremely scarce, till now only four common species of *Plagiochila* are investigated karyologically (Söderström *et al.*, 1999; Sha *et al.*, 2003; Zhai *et al.*, 2006). This paper presents our results of karyological observation on 12 Chinese species, which aim to provide the chromosome number and karyotype for this difficult genus in Marchantiophyta. Owing to lack of enough data, the karyotypes are proposed only for five species, as shown in Tables 1-5 and Figs 13-17, based on our present study.

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MATERIAL AND METHODS

Materials of 26 species of *Plagiochila* for the cytological study were collected in Guangxi, Hainan, Shaanxi, and Zhejiang provinces of China during 2003-2006. Most of the specimens were pretreated and fixed directly in the field. Some fresh samples were brought to the laboratory in plastic bags, and transferred to a CLMACELL for further culture at 15ºC and ca. 4500 LUX with a regime of 12 hour's light and 12 hour's dark. The tips of living shoots were cut off and treated further for cytological observation, following the methods given in our earlier paper (Zheng *et al.*, 2005). The methods for representation of karyotypes and karyotypic analysis are the same as those described in Ramsay (1982) and Inoue *et al.* (1994).

RESULTS AND DISCUSSION

An alphabetical listing of the species of *Plagiochila* investigated in the present study is presented below. The mean length and width of the largest chromosome (L) and the smallest one (S) are given in the parenthesis after the chromosome number.

Plagiochila flexuosa Mitt. (Fig. 1)
n = 9 (L: 6.40 × 0.98 μm; S: 1.05 × 0.85 μm).

There is only one report of the chromosome number (n = 9 = H+7+h) for sterile materials of this species in Japan (Inoue, 1967). Two female populations of this species were checked under the present study and n = 9 was confirmed. The karyotypes is K(n) = 9 = 3V+2J+3v+m (Fig. 13; Table 1). The cytotype consists of five larger chromosomes and four smaller ones. The smallest chromosome is a m-chromosome.

Table 1. The parameters of mitotic metaphase chromosomes in *Plagiochila flexuosa* [K(n) = 9 = 3V+2J+3v+m].

Chromosome No.	*Total length (μm)*	*Short arm (μm)*	*Form (%)*	*Type*
1	6.400000	2.382684	37.23%	J
2	4.850000	2.016579	41.58%	V
3	3.750000	1.426056	38.03%	V
4	3.475000	1.106852	31.85%	J
5	3.175000	1.473200	46.40%	V
6	2.700000	1.191892	44.14%	v
7	2.175000	0.998724	45.92%	v
8	1.650000	0.965854	58.54%	v
9	1.050000			m

Voucher specimens. China. Guangxi, Maoershan Nature Reserve, 1950 m, on tree trunks, 5 Sep 2004, *R.-L. Zhu et al. 2004095505* (♀, HSNU); *ibid*, 2000 m, on rocks, 9 Sep 2004, *R.-L. Zhu et al. 200409904* (♀, HSNU).

Plagiochila fordiana Steph. (Fig. 2)

n = 9 (L: 2.65 × 0.67 μm; S: 0.50 × 0.67 μm).

The largest chromosome is v-shaped and one m-chromosome can be observed (Fig. 2). Although *Plagiochila fordiana* is not rare in China, Japan, India, Vietnam and Thailand (So, 2001), this is the first cytological report of this species.

Voucher specimen. China. Hainan, Bawangling Nature Reserve, 660 m, on rocks, 29 Nov 2003, *R.-L. Zhu 2003112904* (HSNU).

Plagiochila fruticosa Mitt. (Fig. 3)

n = 9 (L: 4.53 × 0.94 μm; S: 0.89 × 0.68 μm).

Segawa (1965a) studied the karyotype in *Plagiochila fruticosa* using female material, and proposed that the Chromosome 1 is the structural sex chromosome X. Inoue (1977) reported the same chromosome number from the sterile materials and the karyotype is n = 9 = H+7+h. The chromosome number n = 8 of this species was reported from China (Sha *et al.*, 2003). Our results confirmed the chromosome number n = 9. The cytotype of this species consists of two larger chromosomes, six medium ones, and one m-chromosome. Chromosome 1 and 2 are larger than the other chromosomes and Chromosome 1 has a secondary constriction in the larger arm as described by Segawa (1965a). The karyotype available here is K(n) = 9 = 4V+2j+2v+m (Fig. 14; Table 2).

Voucher specimens. China. Guangxi, Maoershan Nature Reserve, 2090 m, on rocks, 3 Sep 2004, *R.-L. Zhu et al. 20049312* (HSNU); *ibid*, 1950 m, on tree trunks, 5 Sep 2004, *R.-L. Zhu et al. 20049511* (HSNU).

Plagiochila gracilis Lindenb. & Gottsche (Fig. 4)

n = 9 (L: 4.76 × 0.77 μm; S: 1.06 × 0.74 μm).

The karyotype is represented by K(n) = 9 = 4V+2j+2v+m (Fig. 15; Table 3), based on four populations from Guangxi. Four larger chromosomes are

Table 2. The parameters of mitotic metaphase chromosomes in *Plagiochila fruticosa* [K(n) = 9 = 4V+2j+2v+m)].

Chromosome No.	*Total length (μm)*	*Short arm (μm)*	*Form (%)*	*Type*
1	4.533333	1.725591	38.1%	V
2	3.222222	1.435354	44.5%	V
3	2.377778	0.956280	40.2%	V
4	2.200000	0.846154	38.5%	V
5	1.946667	0.726368	37.3%	j
6	1.711111	0.624691	36.5%	j
7	1.488889	0.638095	42.9%	v
8	1.324444	0.613169	46.3%	v
9	0.890000			m

Table 3. The parameters of mitotic metaphase chromosomes in *Plagiochila gracilis* [K(n) = 9 = 4V+2j+2v+m)].

Chromosome No.	*Total length (μm)*	*Short arm (μm)*	*Form (%)*	*Type*
1	4.766667	1.941975	40.74%	V
2	3.466667	1.446217	41.72%	V
3	3.000000	1.086957	36.23%	J
4	2.766667	0.893176	32.28%	J
5	2.400000	1.077966	44.92%	V
6	2.133333	0.802446	37.61%	V
7	1.666667	0.607639	36.46%	J
8	1.466667	0.751220	51.22%	v
9	1.066667		48.39%	m

similar in shape, and the smallest chromosome is a m-chromsome. Although *Plagiochila gracilis* is a highly variable taxon with a wide distribution in East and Southeast Asia, from as far north as Korea to as far south as Java (So, 2001), it has never been investigated cytologically.

Voucher specimens. China. Guangxi, Maoershan Nature Reserve, 1960 m, on dead wood, 4 Sep 2004, *R.-L. Zhu et al. 20049406* (HSNU); *ibid*, 1960 m, on rocks, 4 Sep 2004, *R.-L. Zhu et al. 20049416* (HSNU); *ibid*, 2000 m, on rocks, 9 Sep 2004, *R.-L. Zhu et al. 20049902* (HSNU); *ibid*, 2000 m, on rocks, 9 Sep 2004, *R.-L. Zhu et al. 20049903* (HSNU).

Plagiochila ovalifolia Mitt. (Fig. 5)

n = 9 (L: 6.40 × 0.60 μm; S: 1.27 × 0.70 μm).

The chromosome number (n = 9) and the structural sex chromosomes are previously reported by Segawa (1957, 1965a). Chromosome 1, the structural sex chromosome, is similar in size and shape in male and female materials, but it has a larger quantity of heterochromatin in male materials than that in female materials (Segawa, 1965a). The karyotype [K(n) = 9 = 5V+3J+m] was reported by Inoue *et al.* (1985). We checked the chromosome number from two populations and got the same chromosome number as the earlier records. Chromosome 1 is v-shaped and has a secondary constriction in the larger arm. Eight large chromosomes and one m-chromosome are observed (Fig. 5).

Voucher specimens. China. Zhejiang, Tianmushan Nature Reserve, 900 m, on soil, 25 Apr 2005, *R.-L. Zhu et al. 2005042505* (HSNU); *ibid*, 1000 m, on soil, 13 May 2004, *R.-L. Zhu et al. 2004051307* (HSNU).

Plagiochila peculiaris Schiffn. (Fig. 6)

n = 9 (L: 3.90 × 0.80 μm; S: 0.80 × 0.50 μm).

The chromosome number n = 9 is counted in this species. The largest chromosome is v-shape and has a secondary constriction in the larger arm (Fig. 6). Seven medium chromosomes and a smaller chromosome are observed (Fig. 6). The smallest chromosome is a m-chromosome as seen in other species. *Plagiochila peculiaris* is immediately distinguished from other members of *Plagiochila* in

Table 4. The parameters of mitotic metaphase chromosomes in *Plagiochila pulcherrima* [K(n) = 9 = V+4J+2v+j+m)].

Chromosome No.	*Total length (μm)*	*Short arm (μm)*	*Form (%)*	*Type*
1	4.660000	1.812222	38.89%	V
2	3.440000	1.179429	34.29%	J
3	2.800000	1.044068	37.29%	J
4	2.532000	0.949500	37.50%	J
5	2.320000	0.773333	33.33%	J
6	2.200000	0.995238	45.24%	v
7	1.960000	0.825263	42.11%	v
8	1.700000	0.623333	36.67%	j
9	1.150000			m

China by the peculiar vermiculate, flexuous leaf cells. It is widely distributed in southeastern Asia, but the chromosome has never been counted.

Voucher specimen. China. Zhejiang, Fengyangshan Nature Reserve, 1220 m, on rocks, 28 Jul 2006, *R.-L. Zhu et al. 2006072809* (HSNU).

Plagiochila pulcherrima Horik. (Fig. 7)

n = 9 (L: 4.66 × 1.03 μm; S: 1.15 × 0.78 μm).

Segawa (1965a) reported that the chromosome number was n = 8 for the female gametophytes of this species from Japan. It lacks a m-chromosome but has a satellite on Chromosome 5 (Segawa, 1965a). Inoue (1968) confirmed the chromosome number n = 8 in female gametophyte from Taiwan and reported the karyotype of this species [K(n) = 8 = H+4V+3J]. The chromosome number (n = 8) of female gametophyte lacks one chromosome in comparison with the chromosome number (n = 9) of male gametophyte (Inoue, 1989). Our results agree with their counts n = 9 of the male collections, and the karyotype of male gametophyte is K(n) = 9 = V+4J+2v+j+m (Fig. 16; Table 4). The cytotype of the male gametophyte has a m-chromosome, compared with the cytotype of female gametophyte of this species.

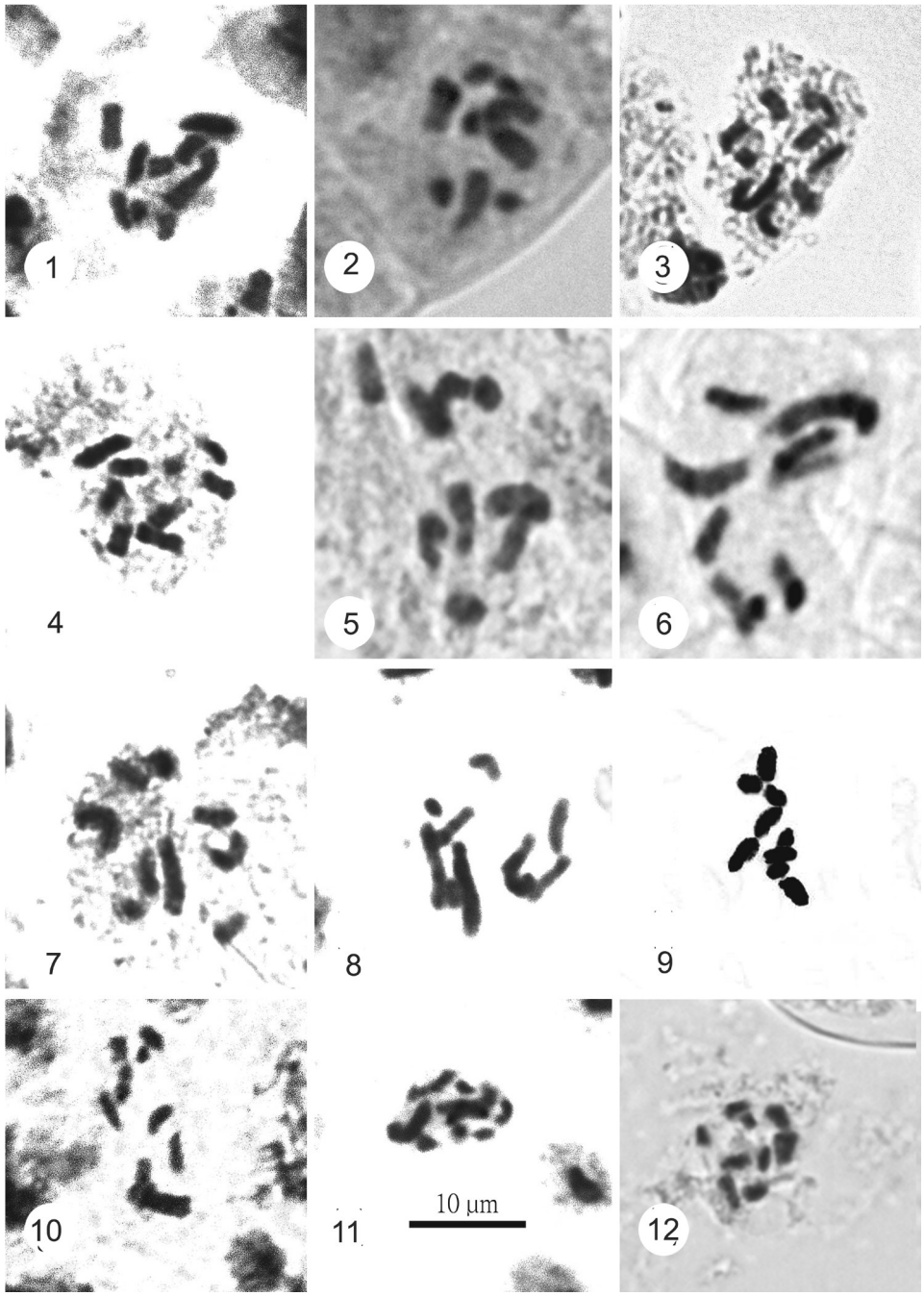
Voucher specimen. China. Guangxi, Maoershan Nature Reserve, 1960 m, on dead wood, 4 Sep 2004, *R.-L. Zhu et al. 20049405* (♂, HSNU).

Plagiochila salacensis Gottsche (Fig. 8)

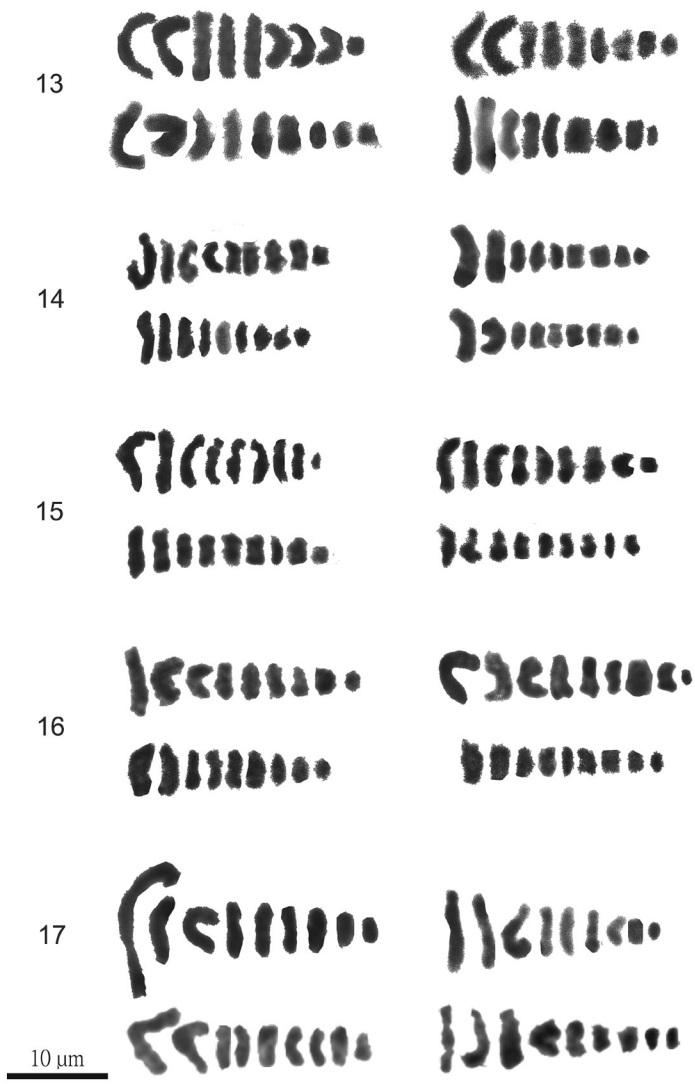
n = 9 (L: 5.90 × 0.71 μm; S: 1.23 × 0.84 μm).

The largest chromosome is rather variable in size among one population of this species. The length of Chromosome 1 is measured from 3.2 to 11.4 μm, but its shape is always v-shape. The karyotype is K(n) = 9 = 2V+2J+2j+2v+m (Fig. 17; Table 5), and an obvious secondary constriction occurred in the larger arm of Chromosome 1. *Plagiochila salacensis* is widely distributed in India, Thailand, the Philippines, Sumatra, Bali, Sulawesi, and Java, but in China it is known only from Guangxi and Yunnan (So, 2001). This is the first record of cytological study on this species.

Voucher specimen. China. Guangxi, Maoershan Nature Reserve, 1150 m, on rocks, 7 Sep 2004, *R.-L. Zhu et al. 20049707* (HSNU).



Figs 1-12. Photomicrographs of mitotic metaphase chromosomes in gametophytes of *Plagiochila*. **1.** *Plagiochila flexuosa,* n = 9; **2.** *P. fordiana*, n = 9; **3.** *P. fruticosa*, n = 9; **4.** *P. gracilis*, n = 9; **5.** *P. ovalifolia*, n = 9; **6.** *P. peculiaris*, n = 9; **7.** *P. pulcherrima*, n = 9; **8.** *P. salacensis*, n = 9; **9.** *P. sciophila*, n = 9; **10.** *P. semidecurrens*, n = 9; **11.** *P. trabeculata*, n = 9; **12.** *P. wightii*, n = 9.



Figs 13-17. Karyotypes of five species of *Plagiochila.* **13.** *Plagiochila flexuosa*. **14.** *P. fruticosa*. **15.** *P. gracilis*. **16.** *P. pulcherrima*. **17.** *P. salacensis*.

Plagiochila sciophila Nees ex Lindenb. (Fig. 9)

n = 9 (L: 2.10 × 0.60 μm; S: 1.04 × 0.50 μm).

Plagiochila sciophila is a highly variable species with a large number of synonyms. Its chromosome number (n = 9) was reported as *P. japonica* Sande Lac. by Segawa (1957,1965b), Masubuchi (1973), Tanaka & Inoue (1982), and Inoue et al. (1985). The karyotype was reported as K(n) = 9 = 6V+3J (Inoue *et al.*, 1985). Our results confirmed the chromosome number n = 9. The nine chromosomes are

Table 5. The parameters of mitotic metaphase chromosomes in *Plagiochila salacensis* [K(n) = 9 = 2V+2J+2j+2v+m)].

Chromosome No.	*Total length (μm)*	*Short arm (μm)*	*Form (%)*	*Type*
1	5.900000	2.212500	37.50%	J
2	4.800000	1.802299	37.55%	J
3	3.966667	1.494975	37.69%	V
4	3.433333	1.312745	38.24%	V
5	3.200000	1.141401	35.67%	j
6	2.866667	1.121739	39.13%	v
7	2.400000	0.870796	36.28%	j
8	1.600000	0.688172	43.01%	v
9	1.233333			m

similar in size (Fig. 9). Unfortunately, we had not enough data to propose the karyotype based on the present material examined.

Voucher specimen. China. Hainan, Bawangling Nature Reserve, 700 m, on rocks, 29 Nov 2003, *R.-L. Zhu 2003112906* (HSNU).

Plagiochila semidecurrens (Lehm. & Lindenb.) Lindenb. (Fig. 10)

n = 9 (L: 3.73 × 0.89 μm; S: 0.84 × 0.51 μm).

The female gametophyte of this species was reported by Inoue (1974) with the chromosome number n = 9 and karyotype K(n) = H+4V+3J+h. Our results confirmed the earlier records of chromosome number (Inoue, 1974). Chromosome 1 is larger than the other chromosomes and Chromosome 9 is a m-chromosome (Fig. 10).

Voucher specimens. China. Guangxi, Maoershan Nature Reserve, 2100 m, on rocks, 3 Sep 2004, *R.-L. Zhu et al. 20049305* (HSNU); *ibid*, 2100 m, on rocks, 3 Sep 2004, *R.-L. Zhu et al. 20049306* (HSNU).

Plagiochila trabeculata Steph. (Fig. 11)

n = 9 (L: 4.12 × 0.84 μm; S: 1.03 × 0.74 μm).

The chromosome number (n = 9) of this species was reported by Inoue (1977, 1980). Our results obtained from two Chinese populations corroborated the previous reports. The cytotype consists of four larger, v-shaped chromosomes, four median ones and one m-chromosome (Fig. 11).

Voucher specimens. China. Guangxi, Maoershan Nature Reserve, 2100 m, on base of bamboo, 3 Sep 2004, *R.-L. Zhu et al. 20049310* (HSNU); *ibid*, 1960 m, on rocks by road, 4 Sep 2004, *R.-L. Zhu et al. 20049401* (HSNU).

Plagiochila wightii Nees ex Lindenb. (Fig. 12)

n = 9 (L: 3.00 × 0.80 μm; S: 1.40 × 0.70 μm).

Plagiochila wightii was known only from southwestern China (Guangxi, Sichuan, Yunnan) and India (So, 2001). It has not been investigated cytologically before. The chromosome number of this species (n = 9) was established in our collections from Hainan which is the southernmost locality of the species. The

cytotype is composed of five larger chromosomes similar in shape and size, and four median ones. No m-chromosome was found (Fig. 12).

Voucher specimen. China. Hainan, Bawangling Nature Reserve, 1130 m, on tree trunks, 27 Nov 2003, *R.-L. Zhu 2003112713* (HSNU).

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