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Karyomorphology of *Biebersteinia* Stephan (Geraniaceae) and its systematic and taxonomic significance

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Abstract. The systematic and taxonomic position of *Biebersteinia* Stephan has long been in dispute. The present paper describes for the first time the karyomorphology of two species in *Biebersteinia* Stephan. Both species commonly showed the interphase nuclei of the simple chromocenter type and the mitotic prophase chromosomes of the interstitial type. The karyotype formulae of both *B. heterostemon* and *B. odora* were 2*n*=10=2m(2sec)+8sm(2sec), belonging to the 3A type of Stebbins' classification. The karyotype of this genus is recorded for the first time. The basic chromosome numbers of four of the five known species of *Biebersteinia* have been recorded as x=5. The combination of resting nuclei of the simple chromocenter type, mitotic prophase chromosomes of the interstitial type, two pairs of chromosomes with four obvious secondary constrictions at the mitotic prophase and metaphase stages, and the peculiar 3A karyotype in *Biebersteinia* can be regarded as the karymorphological marker of this genus. The karyomorphological data presented here do not support the traditional grouping of this genus in Geraniaceae. The unique karyomorphology of *Biebersteinia* justifies its familiar or ordinal status, which is congruent with embryological, anatomical, chemical and molecular data. The systematic position of *Biebersteinia* needs further study.

Keywords: *Biebersteinia*; Karyomorphology; Systematics.

Introduction

Biebersteinia Stephan, comprising five species: *B. heterostemon* Maxim, *B. odora* Stephan ex Fisch, *B. multifida* DC., *B. orphanidis* Boiss. and *B. leiosepala*, is distributed in mountainous, semi-arid areas of central and western-Asia to the eastern Mediterranean (Knuth, 1912; Yannistaros et al., 1996). The systematic and taxonomic position of *Biebersteinia* Stephan has long been in dispute. Stephan (1806) positioned *Biebersteinia* between *Grielum* L. (Neuradaceae) and *Suriana* L. (Simaroubaceae) when establishing the genus. Later, De Candolle (1924) suggested a position of *Biebersteinia* in 'Zygophylleae spuriae alternifoliae' and possible affiliations with Rosaceae, Geraniaceae and Rutaceae. It was Endlicher (1841) who raised *Biebersteinia* to a family status for the first time. But Hooker (1875) treated it as a tribe in Potentillae in the Rosaceae. However, Boissier (1867) included it as a genus of Geraniaceae. In the recent angiosperm systems, Thorne (1992) and Cronquist (1981, 1988) followed Boissier (1867) and Knuth (1912) and treated it as a genus of Geraniaceae while Dahlgren (1989) and Takhtajan (1987) recognized it as a family close to Geraniaceae. Takhtajan (1997) further raised it to an order near Geraniales. Most recently, Bakker et al. (1998) qualified its family status and implicated strong affiliation with Sapindales based on the molecular data. This result was positively accepted by APG (1998) in their new angiosperm ordinal system.

In addition to the gross-morphological and molecular evidence, other data such as the pollen morpholgy, embryology, anatomy, chemistry and karyomorphology can also be of great importance in clarifying the systematic position of a taxon (Takhtajan, 1997). Bortenschlager (1967) studied the pollen morphology of *Biebersteinia*, and suggested the genus should be recognized as a family near the Potentillae of Rosaceae. Nevertheless, the phanerothetic discoid nectaries of *Biebersteinia* were very similar to those found in most Geraniaceae (Link, 1990). But the presence of anacampylotropous ovules (Kamelina and Konnova, 1990), the lack of a persistent elongated central column in the ovary and a reduced tegmic seed coat (Boesewinkel, 1997), and other chemical data (Bate-Smith, 1973) distinguish *Biebersteinia* from all taxa assumed to be related to it.

In spite of the accumulating molecular data, chromosome information continues to be important in assessing phylogenetic relationships (Carr et al., 1999). Concerning the karyomorphological data of *Biebersteinia*, only the chromosome numbers of *B. multifida* DC. (Aryavand, 1975) and *B. orphanidis* Boiss. (Constantinidis, 1996) were reported to be x=5. The karyotype, the interphase nuclei, and the mitotic prophase chromosomes have never been reported. In the present paper, the chromosome numbers

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of two additional species in *Biebersteinia* were determined. Furthermore, the karyotype, the resting nuclei and the mitotic prophase chromosomes in both species are reported for the first time. Based on the karyomorphological data, the systematic position of *Biebersteinia* is further discussed.

Materials and Methods

Roots of *B. heterostemon* Maxim. were taken directly from plants in the field at Xining, Qinghai, China. Five seeds of *B. odora* Steph. ex Fisch. were collected from Tibet and germinated in the laboratory. Voucher specimens are deposited in the Northwest Plateau Institute of Biology, the Chinese Academy of Sciences (HNWP). The roots were pretreated with a mixture of 0.05% colchicine and 0.002 mol/L hydroxyquinoline for 5 h, and then fixed overnight in Carnoy's fluid (absolute alcohol:glacial acetic acid=3:1). After being macerated in 1 mol/L hydrochloric acid at 60°C for five min, the roots were stained and squashed with Carbol Fuchsin. The karyomorphological classifications of the resting and mitotic prophase followed Tanaka (1977, 1971). The chromosomes of more than 20 cells were counted. The parameters of the chromosomes were based on the measurements of 5 cells. The symbols for the description of karyotypes followed Levan et al. (1964). The symmetry of karyotypes was classified according to Stebbins (1971).

Results

The two species studied were basically similar in karyomorphology of the interphase nuclei and the mitotic prophase chromosomes. In the interphase nuclei (Figure 1A), several darkly stained chromocenters were observed. The chromocenters showed an irregularly protruded rough surface, which gradually transformed into diffused chromatin. The interphase nuclei were categorized as the simple chromocenter type (Tanaka, 1977, 1971). In the mitotic prophase stage (Figure 1B), hetero- and euchromatic segments were distinguishable, but their boundaries were not clear, and the heterochromatic segments were distributed in the distal and interstitial regions as well as the proximal regions. Therefore, the prophase chromosomes belonged to the interstitial type (Tanaka, 1977). Four chromosomes had secondary constrictions, and the resulted satellites consisted mainly of heterochromatic segments that were obvious at this stage (Figure 1B, arrows).

Metaphase chromosomes of *B. heterostemon* (Figure 1C) were determined to be 2*n*=10, ranging in length from 5.20 µm to 6.10 µm. The karyotype (Figure 1D) was formulated as 2*n*=10=2m(2sec)+8sm(2sec), belonging to Stebbins' 3A type. Secondary constrictions were found in the distal region of the third and fifth pairs of chromosomes (Figure 1C-D), and these often resulted in obvious satellites. Metaphase chromosomes of *B. odora* (Figure 1E) were also determined to be 2*n*=10, ranging in length from 4.81 µm to 5.90 µm. The karyotype (Figure 1F), similar to that of *B. heterostemon*, was formulated as 2*n*=10=2m (2sec)+8sm (2sec), and was also categorized as a 3A type by Stebbins (1971). In some metaphase cells, chromosomes were so contracted that secondary constrictions were not readily visible (Figure 1E, G). However, secondary constrictions were found in the distal region of the third and fifth pairs of chromosomes in most cells (Figure 1H-I). Parameters of mitotic metaphase chromosomes of 2 species in *Biebersteinia* are listed in Table 1. The arm ratios of more than half of the chromosomes in the two species exceeded 2.0, which indicated the intrachromosomal asymmetry of *Biebersteinia* was very high. However, the chromosome lengths differed little, and the interchromosomal asymmetry was very low. Both the above-mentioned characteristics determined the peculiar karyotype classification of 3A in *Biebersteinia*.

Table 1. Parameters of mitotic metaphase chromosomes of 2 species in *Biebersteinia*.

No.	Relative length	Arm ratio	Type
B. heterostemon			
1	7.60+17.27=23.87	2.27	sm
2	7.46+15.19=22.65	2.04	sm
3	8.29+13.81=22.10	1.67	m*
4	6.51+15.58=22.09	2.39	sm
5	7.18+14.91=22.09	2.08	sm*
B. odora			
1	6.98+14.37=21.35	2.06	sm
2	6.59+13.97=20.56	2.12	sm
3	7.98+12.18=20.16	1.53	m*
4	5.98+12.77=18.75	2.14	sm
5	6.36+12.38=18.74	1.95	sm*

*Indicating chromosomes with secondary constrictions.

Discussion and Conclusion

Five species of *Biebersteinia* have very similar morphology. Four species (*B. multifida*, *B. orphanidis*, *B. heterostemon* and *B. odora*) are now known to have the same chromosome number of 2*n*=10 (Aryavand, 1975; Constantinidis, 1996 and the present research). Therefore, the basic chromosome number of *Biebersteinia* is apparently x=5.

As stated in the introduction, *Biebersteinia* was traditionally classified in the Geraniaceae or Geraniales, but molecular data supported its position in Sapindales (Bakker et al., 1998). The basic chromosome number is of importance to determine the systematic position of a taxon at high taxonomic levels (Raven, 1975). The basic chromosome numbers of main taxa in Geraniales and Sapindales were summarized and compared with that of *Biebersteinia* in Table 2 (Table 2, summarized from Darlington and Wylie, 1955; Cave, 1958-1965; Ornduff, 1967-1969; Moore, 1970-1977; Goldblatt, 1981, 1984, 1985, 1988; Goldblatt and Johnson, 1990, 1991, 1994, 1996). In the Geraniales, only Limnanthaceae shares the same basic chromosome num-

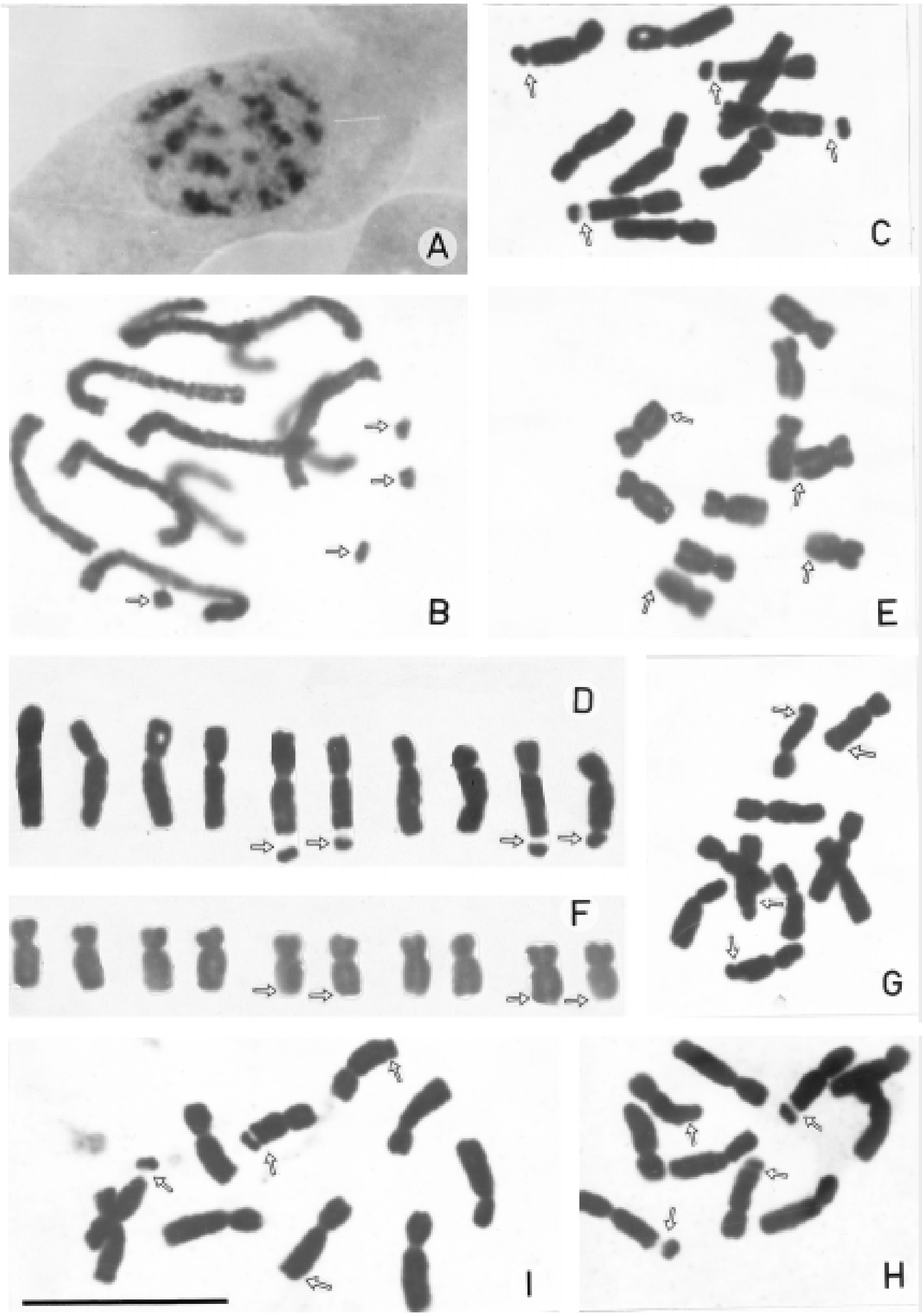

Figure 1. Microphotographs of chromosomes of *Biebersteinia*. A-D, *B. heterostemon*; E-I, *B. odora*. A, Resting nucleus; B, Prophase chromosomes; C, Metaphase chromosomes; D, F, Karyotype; E, G, H, Metaphase chromosomes. Arrows indicating secondary constrictions on the long arms. Bar=10 μm

ber of x=5 with *Biebersteinia*. But they are well distinguished in gross morphology, greatly different in embryology, pollen morphology and anatomy (see Takhtajan, 1997). Up to now, the basic chromosome number of x=5 has not been found for the taxa in Sapindales. As viewed by Raven (1975) and Hong (1990), the primitive or basic chromosome number of Geraniales at the ordinal level might be x=7 or 12 while that of Sapindales might also be x=7. From the standpoint of basic chromosome numbers, *Biebersteinia* is related neither to Geraniales nor to Sapindales. In addition, the karyomorphological combination—such as the resting nuclei of the simple chromocenter type, the mitotic prophase chromosomes of the interstitial type, two pairs of chromosomes with obvious secondary constrictions at the mitotic prophase and metaphase stages, and the peculiar 3A karyotype found in *Biebersteinia*—have never been reported for either Geraniales or Sapindales.

Table 2. A comparison of the primitive or basic chromosome numbers of *Biebersteinia* and main taxa of Geraniales and Sapindales.

Taxon	Basic chromsome number
Biebersteinia	**x=5**
Geraniales	x=7 or x=12
Oxalidaceae	x=12
Averrhoa	x=12, 11
Biophytum	x=9
Oxalis	x=5-12
Geraniaceae	x=12 or x=7
Sarcocaulon	x=22
Monsonia	x=12
Erodium	x=10
Geranium	x=14
Pelargonium	x=11
Balbasia	x=9
Vivianiaceae	x=7
Limnanthaceae	x=5
Ledocarpaceae	x=9
Tropaeolaceae	x=12-14
Balsaminaceae	?
Hydrocera	x=8
Impatiens	x=6-11
Sapindales	x=7
Staphyleaceae	x=13
Melianthaceae	x=19
Greyiaceae	x=17 or 16
Sapindaceae	x=11-16
Hippocastanaceae	x=20
Aceraceae	x=13
Burseraceae	x=11, 12, 23
Anacardiaceae	x=14-16
Podoaceae	x=7
Simaroubaceae	x=12-14
Rutaceae	x=9
Chorilaena	x=14
Geleznowia	x=14
Growea	x=19
Rhabdodendron	x=10
Meliaceae	x=11-14
Cneoraceae	x=18
Zygophyllaceae	x=11, 13

Morphologically, *Biebersteinia* is related more closely to Sapindales than to Geraniales, because *Biebersteinia* has compound leaves, a diplostemonous flower, and one ovule in each locule. However, the phanerotherothetic-discoid nectaries in *Biebersteinia* are similar to those of Geraniaceae (Link, 1990) while a distinct nectariferous disc exists in Sapindales, which is a potentially important morphological synapomorphy for the Sapindales clade as circumscribed by Gadek et al. (1996). Other than the distinct difference between *Biebersteinia* and *Geranium* found by Bate-Smith (1973) through a simple comparison of the chemical constituents, Zhang et al. (1995) separated two new natural products: quercimeritrin and *N*-3-methyl-2-butenyl urea from *B. heterostemon*. The *N*-3-methyl-2-butenyl urea or similar chemical products occurring in *B. Heterostemon* show hypotensive, analgesic, and immunity stimulating effects and have not been previously reported for the angiosperms. The presence of an acampylotropous ovule (Kamelina and Konnova, 1990), the lack of a persistent elongated central column in the ovary, and a reduced tegmic seed coat (Boesewinkel, 1997) in *Biebersteinia* also distinguish this genus from Geraniales and Sapindales. Although molecular data indicated a position in Sapindales, *Biebersteinia* has many autapomorphic substitutions (Bakker et al., 1998). The karyomorphological peculiarity found in the present study, congruent with the above-mentioned data, supports the isolated position of *Biebersteinia* and justifies its familiar or ordinal status. The systematic position of this family or order needs further study.

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薰倒牛屬的核形態及其系統與分類學意義

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薰倒牛屬（*Biebersteinia*）的系統位置及其分類長期存在爭議。本文首次報導了該屬 2 個種的核形態。染色體間期為簡單型，前期染色體為中間型。薰倒牛（*B. heterostemon* Maxim.）和羽裂薰倒牛（*B. odora* Stephan ex Fisch）的核型公式均為 $2n=10=2m+(2sec)+8sm(2sec)$，核型類型屬於 3A 型。到目前為止，本屬的 5 個種中已有 4 個種的染色體被計數，其染色體基數可斷定為 X=5。該屬具一致的核形態特徵，如染色體間期為簡單型，前期為中間型，在有絲分裂前期和中期，二對染色體具有明顯的 4 個次縊痕，及3A的核型，這些均可作為薰倒牛屬的核形態標誌。核形態資料不支持把薰倒牛屬放在傳統的牻牛兒苗科（Geraniaceae）中。本屬獨特之核形態以及來自胚胎學、解剖學、化學和分子生物學的証據均支持該屬為一科或目的等級，至於其系統位置仍需進一步研究。

關鍵詞：薰倒牛屬；核形態；系統學。