

THE FIRST CHROMOSOME DATA DOCUMENTATIONS OF *MEGACODON* AND *LOMATOGONIOPSIS* AND THEIR SYSTEMATIC SIGNIFICANCE (GENTIANACEAE)*

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Abstract

In the present paper, the chromosome data of *Megacodon* and *Lomatogoniopsis* was firstly recorded. The chromosomes of *Megacodon stylophorus* at resting stage fall in the intermediate type between the diffuse and the complex chromocenter type and the mitotic-prophase chromosomes are of the interstitial type. The chromosomes of *Lomatogoniopsis alpina* at resting stage and mitotic-prophase stage are of the prochromosome type and the proximal type respectively. The karyotype and the complement of relative length are: $K(2n=28)=24m+4sm=12M_2+14M_1+2S$ for *M. stylophorus*; $K(2n=12)=12m=6M_2+6M_1$ for *L. alpina*. The possible basic chromosome number $X=7$ of *Megacodon* might infer its primitive position in the "Gentiana" line, representing a primitive knot taxon connecting two evolutionary lines in the Gentianinae. The chromosome data of *Lomatogoniopsis alpina* reveals its distinctness from both *Lomatogonium* and *Comastoma* and supports the separation of *Lomatogoniopsis* from *Lomatogonium*; furthermore, its lower asymmetrical indices might imply it might be a relic species, which might be a reason of its rarity in the special Qinghai-Xizang Plateau surrounding.

Key words: *Megacodon*; *Lomatogoniopsis*; Chromosome; Systematics

Megacodon is a small genus in Gentianaceae, which comprises two species: *M. stylophorus* (C. B. Clarke) H. Smith and *M. venosus* (Hemsl.) H. Smith; the former is distributed in west Sichuan, northwest Yunnan and north Sikkim while the latter endemic to the east of Sichuan. *Megacodon* was included in *Gentiana* subgenus *Gentianella* as a section (namely Sect. *Stylophora* C. B. Clarke or Sect. *Megacodon* Hemsl.) by early authors (Gilg, 1895). Smith (1936) raised it to generic rank and elucidated its morphological distinctness from *Gentiana* in his later paper (Smith, 1965). Nevertheless, Toyokuni (1965) reduced it as a subgenus *Stylophora* (C. B. Clarke) Toyokuni of the genus *Gentiana* and suggested its closer relationship to *Gentiana* section *Gentiana* than other groups. Based on their common gynoeceum nectars, Ho & Liu (1990) believed *Megacodon* and *Gentiana* are both at the same evolutionary line. However, ITS phylogeny showed it a distinct genus and belonged to the "Gentianella" evolutionary line with nectars at the

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corolla as a basal clade (Yuan et al., 1995). Morphologically *Megacodon* really shows its intermediate and ambiguous position between the "Gentiana" and "Gentianella" lines, because it has the "Gentiana" type of glands located on the base of ovary, but it does not have any plicae or folds on its corolla which suggests a close affinity with "Gentianella" line.

Lomatogoniopsis was newly established by Ho & Liu in 1985, which includes three species endemic to Qinghai-Xizang plateau. This genus was thought to have a close relationship to *Lomatogonium* (Liu, 1992; Ho & Liu, 1985). In our recent expedition in 1993 and 1996 to the flora of Qinghai, we carefully observed the fresh corolla of the type species *Lomatogoniopsis alpina* in the field. We found the corolla of *L. alpina* also with double nectars at the base of each lobe. The appendage on the corolla is not related to nectars as thought by earlier authors and might be similar to the fimbriate of *Comastoma*, which also scales the corolla when fully opened.

In addition to morphological and molecular information, chromosome data might aid in solving the systematic and taxonomic questions, and chromosome studies are often useful in suggesting taxonomic and phylogenetic relationships at genetic level (Stuessy, 1990; Raven, 1975.) However, information on chromosome numbers and karyomorphologies in *Megacodon* and *Lomatogoniopsis* is blank. In this paper, we firstly record the chromosome numbers of two genera and their chromosome appearances at metaphase, interphase and resting stage. The potential taxonomic and phylogenetic significance of the chromosome data is discussed.

Materials and Methods

M. stylophorus was collected from Baima snow mountains in Degin county of Yunnan province. The voucher specimen is Liu Jianquan 339. The materials for chromosome observation are growing roots. *L. alpina* was collected from Dari county of Qinghai province. The voucher specimen is Liu Jian-quan 253. The materials for chromosome observation are young ovaries. The voucher specimens are preserved in the herbarium of Northwest Plateau Institute of Biology, the Chinese Academy of Sciences, The People's Republic of China (HNWP).

For the young ovaries of *L. alpina*, the methods introduced by Liu (1997) were adopted. For the roots of *M. stylophorus*, the methods were as same as those used in our former reports (Ho et al. 1997; Chen et al. 1997). The terminology used for chromosome complement of relative length (C.R.L.) is that defined by Kuo et al. (1972). The classification of karyotype asymmetry follows Stebbins (1971). The intrachromosomal asymmetry index (A_1) and the interchromosomal asymmetry index (A_2) proposed by Romero (1986) as well as index of the karyotypic asymmetry (As.K%) proposed by Arano (1963) were adopted.

Results

1. *Megacodon stylophorus* (C. B. Clarke) H. Smith

The interphase nuclei contain irregular-shaped heteropycnotic bodies, which stain darkly and vary in size, some aggregating large chromocenters while the remaining nuclear regions are granular and stain lightly (Plate I: 4.). Thus, the resting chromosomes fall in the intermediate type between the diffuse and the complex chromocenter type as described by Tanaka (1971).

Mitotic-prophase chromosomes contain heterochromatic segments in the interstitial regions of most chromosomes of each complement (Plate I: 5). Thus, the mitotic-prophase chromosomes could be classified as

the interstitial type.

The chromosome number of this species is $2n=28$ at mitotic-metaphase (Plate I: 6). The chromosome complement consists of 24 median-centromeric and 4 submedian-centromeric chromosomes. The complement of relative length exhibits 14 longer ($12M_2$), 12 shorter ($14M_1$) and 2 small (2S) chromosomes. The chromosome length range from 2.46 to 4.95 μm and the ratio of the longest to the shortest chromosome is 1.98. The proportion of the chromosomes of which the arm ratios are more than 2 in karyotype is 0.07. Therefore, the karyotype was estimated as the Stebbins' 2A type. The chromosomes of the complement show mostly gradual decrease in lengths from the longest to the shortest with no obvious bimodality. The karyotype asymmetry indices were estimated to be $A_1 = 0.24$, $A_2 = 0.20$ and $As.K\% = 57.23$. The chromosome number and karyomorphology of this species is reported here for the first time.

2. *Lomatogniopsis alpina* T. N. Ho

The ovary meristematic cells of *L. alpina* show several round or rod chromocenters which vary in size and shape (Plate I: 1.). The boundary among chromocenters is distinct. The rest regions are staining lightly without obvious granular chromatin. The resting chromosomes is similar to the prochromosome type of Tanaka (1971).

At mitotic prophase (Plate I: 2), most chromosomes have early condensed segments at the proximal region of one or both arms. Early and late condensed segments of chromosomes show rather sudden transition between. This type of prophase chromosomes is referred to as the proximal type of Tanaka (1971).

The chromosome number of this species is $2n=12$ at mitotic-metaphase (Plate I: 3). The chromosome complement consists of 12 median-centromeric chromosomes. The complement of relative length exhibits 6 longer ($6M_2$) and 6 shorter ($6M_1$) chromosomes. The chromosome length range from 2.44 to 3.67 μm and the ratio of the longest to the shortest chromosome is 1.51. The proportion of the chromosomes of which the arm ratios are more than 2 in karyotype is 0.007. Therefore, the karyotype was 1A type according to the Stebbins' classification. The chromosomes of the complement show mostly gradual decrease in length from the longest to the shortest with no obvious bimodality. The karyotype asymmetry indices were estimated to be $A_1 = 0.18$, $A_2 = 0.16$ and $As.K\% = 55.59$. The chromosome number and karyomorphology of this species is reported here for the first time.

Discussion

1. The systematic position of *Megacodon*

As reviewed in the introduction, *Megacodon* has long been in dispute for its systematic position. In the Gentianinae, two evolutionary lines were recognized based on the position of the nectars. "Gentiana line" with glands located at the base of the ovary includes *Gentiana*, *Tripterospermum*, *Crawfordia*, *Latouchea* and *Megacodon* while "Gentianella" line with glands sited at the corolla comprises remained genera (Ho & Liu 1990; Gillet, 1957). The former three genera have other two synapomorphies delimiting from all other genera in Gentianinae that are corolla with plicae between lobes and calyx with continuous intracalycular membrane (Ho et al., 1996). In other words, *Megacodon* and *Latouchea* have the "Gentiana" type of glands located on the base of ovary, but they do not have any plicae or folder on its corolla and intracalycular membrane which suggests their close relationships to "Gentianella" line. Obviously, *Megacodon* and *Latouchea* are related to each other and represent a separated group based on the special combinations of nectar position, corolla plicae and intracalycular membrane if viewed from the gross morphology. It is regretting that ITS analyses didn't

include *Latouchea* (Yuan et al, 1995). ITS phylogeny showed *Megacodon* belonged to the "Gentianella" evolutionary line as a basal clade (Yuan et al., 1995), which means *Megacodon* differentiated from other genera of the "Gentianella" line very early.

The present paper reveals the chromosome numbers of *M. styphorus* are $2n=28$, inferring the possible basic number of the genus is $X=7$. In the "Gentiana" line, *Tripterospermum* and *Crawfordia* were found to have $2n=46$, $X=23$ (Wada, 1966; Shigehobu, 1984). *Gentiana* has polybasic numbers ranging from $X=6$ to $X=23$, but the primitive groups such as section *Pneumonanthe* mainly have $2n=26$, $X=13$ (Ho et al., 1996) while $2n=14$ and $X=7$ was found in the advanced biennial species such as *G. aristata* (in section *Chondrophylla*). The ITS phylogeny show robust assumptions that $2n=26$ and $X=13$ is unequivocally a plesiomorphic state (Yuan et al., 1996). Therefore, the primary chromosome basic number may be $X=13$. The chromosome number of *Megacodon* show no direct relationship to genera of the "Gentiana" line. However, in the "Gentianella" line, $2n=28$ was found in the genus *Swertia* section *Rugosa* and section *Swertia* (Shigenobu, 1983; our results to be published). The genus *Swertia* at least is a primitive group in the rotate genera of the "Gentianella" line (Liu & Ho, 1992). The other genera (e. g. *Gentianella*, *Comastoma* and *Gentianopsis*) may be at the advanced position because they have tubular corolla (Toyokuni, 1965) and may have secondary basic chromosome number ($X=9$, $X=8$ or 9 and $X=13$; Yuan & Kupfer, 1993b). Furthermore, sect. *Rugosa* and sect. *Swertia* are primitive groups in *Swertia* (Ho et al., 1994). So, if the position of *Swertia* is confirmed as primitive in the "Gentianella" line, the similarity of the chromosome number in *Megacodon* and *Swertia* indicates they maybe come from a common ancestor and they might represent the primitive taxon in Gentianinae, because the original basic chromosome number in Gentianaceae may be $X=7$ (Hong, 1990). Therefore, combined with gross morphology, we can guess that *Megacodon* and *Swertia* (with the primary basic chromosome number $X=7$) represent respectively the primitive taxon of two evolutionary lines suggested based on the sites of nectars. In the "Gentiana" line, *Megacodon* are primitive with primitive characters such as large flowers, tall and stout stems etc. The other genera especially *Gentiana*, *Tripterospermum* and *Crawfordia* might be at a more evolutionary stage for they share two synapomorphies of corolla with plicae between lobes and calyx with continuous intracalycular membrane (Ho et al., 1996). *Megacodon* may be a primitive taxon in "Gentiana" line, so it is not surprised that it might show some connections with "Gentianella" line as its out group as indicated as ITS analyses (Yuan & Kupfer, 1995).

2. The taxonomical and systematic position of *Lomatogoniopsis*

Lomatogoniopsis was thought to have a close relationship to *Lomatogonium* and more evolutionary than it (Liu & Ho, 1992; Ho & Liu, 1985). The chromosome numbers of *Lomatogonium* were reported to have $2n=16$, 32 and 48 , rarely $2n=10$ and its primary basic chromosome number should be $X=8$ (Yuan & Kupfer, 1993). In case of the chromosome number, *Lomatogoniopsis* is distinct from *Lomatogonium* and it is better to treat it as a separate genus. If viewed from the gross morphology, *Lomatogoniopsis alpina* might be related to *Comastoma* for they commonly share double nectars at the base of each lobe and the appendage of the corolla in *L. alpina* is similar to the fimbriate of *Comastoma* as observed in the field. The chromosome numbers of *Comastoma* show a great variation with $X=5$, 7 , 8 , 9 , 10 ; and its primitive basic chromosome numbers might be $X=9$ and $X=8$ (Our results to be published). The chromosome basic number of *L. alpina* is also different from those found in *Comastoma*.

As for the karyotype of *L. alpina*, it has lower asymmetrical indices $A_1=0.18$, $A_2=0.16$, $A_s.K\% =$

55. 59, conforming to 1A type of Stebbins (1971) and without bimodality. The karyotypes of *Lomatogonium* and *Comastoma* mainly are 2B, 2C and 3C, and most species of them with obvious bimodality. According to Stebbins (1971), there is a predominant trend in flowering plants towards increasing asymmetry of the karyotype asymmetry. The karyotype indexes of *L. alpina* might imply this species might be a relic species. However, *Lomatogoniopsis* was thought as a "new endemic genus" with the rise of Qinghai-Xizang Plateau (Liu & Ho, 1992). *L. alpina* is a rare species with a small distribution. If the genus had originated with the Plateau, it should have adapted the habits of the Plateau and extended its distribution. Also, the reasons for rarity were caused by many factors. But some species (e.g. Ginkgo) are connected with phylogeny. Certainly, the origin, systematics and rarity reasons of *L. alpina* need more investigation. The present chromosome data supports the separation of *Lomatogoniopsis* from *Lomatogonium* and do not agree with its close relationship to *Lomatogonium* as suggested by Ho (1985) and Liu (1992). About its origin and systematics, *Lomatogoniopsis* is really related to *Comastoma* if viewed from gross morphology, anatomy and embryology (to be published).

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大钟花属和辐花属染色体资料的首次记载及其系统学意义

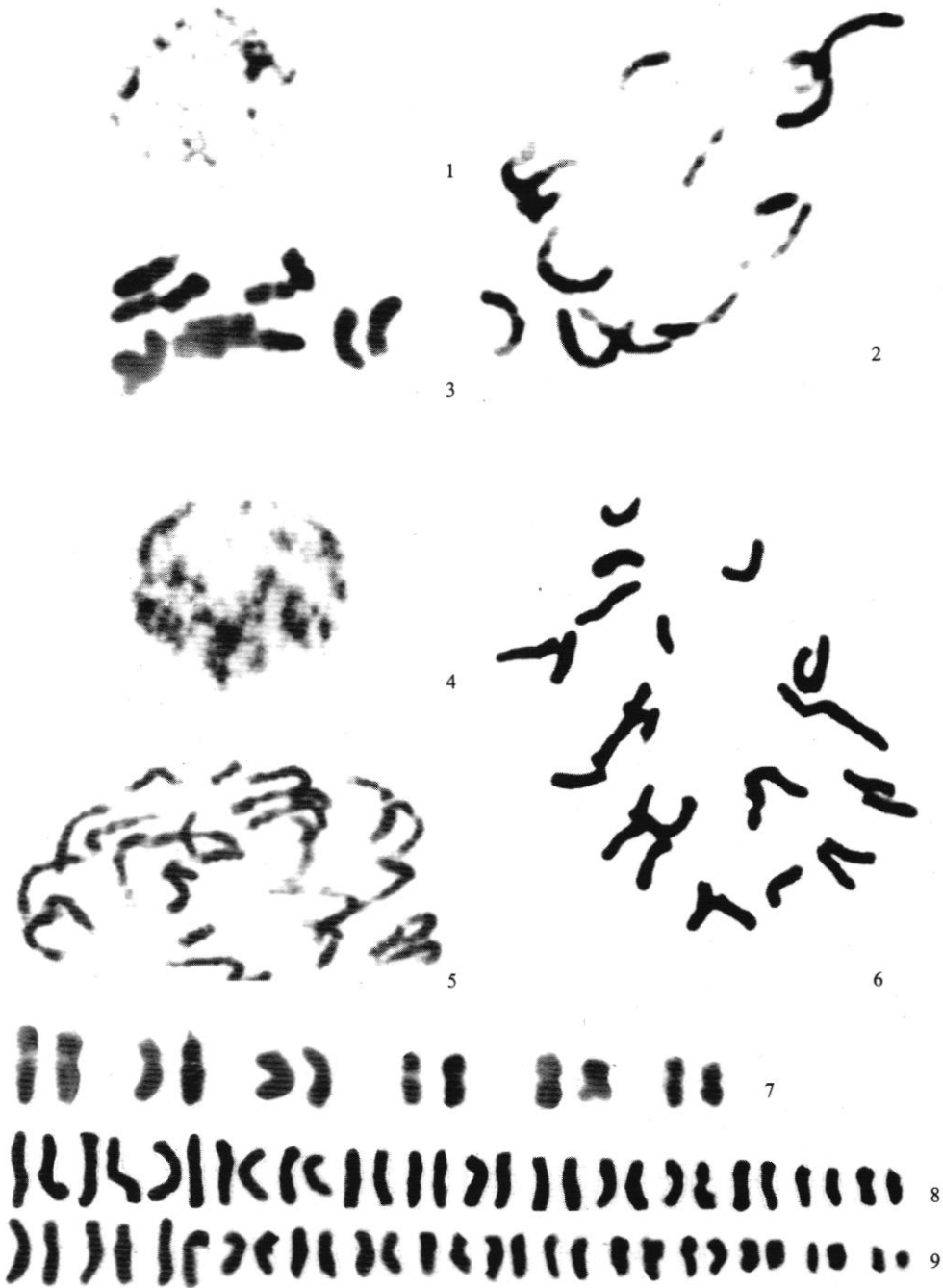
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摘 要

本文首次记载了大钟花属和辐花属的染色体资料。大钟花的间期染色体为扩散-复杂型的过渡型,而前期染色体为中间型。辐花的间期和前期染色体均分别为前染色体型与近基型。它们染色体中期核和相对长度组分分别是:大钟花为 $K(2n=28) = 24m + 4sm = 12M_2 + 14M_1 + 2S$; 辐花为 $K(2n=12) = 12m = 6M_2 + 6M_1$ 。大钟花可能的染色体基数为 $X=7$,由此可推测它可能是龙胆亚族中“龙胆”支的原始类群,并且可能是连接“龙胆”和“假龙胆”两条演化路线的原始节点。辐花在染色体性状方面明显不同于肋柱花属和喉毛花属,支持将其独立为属,其系统位置有待进一步研究;此外,它较低的染色体不对称系数似乎暗示它可能是一残遗种,这有可能是该种在青藏高原这一特殊生境处于濒危的原因之一。

关键词: 大钟花属; 辐花属; 染色体; 系统学



1, 2, 3, 7. Somatic chromosomes of *Lomatogoniopsis alpina*. 1. Resting stage. 2. Mitotic prophase.
3. Mitotic metaphase. 7. Karyotypes.

4, 5, 6, 8, 9. Somatic chromosomes of *Megacodon stylophorus*. 4. Resting stage. 5. Mitotic prophase.
6. Mitotic metaphase. 8, 9. Karyotypes. - Bar = 10 μ m