

A karyomorphological study of the genera *Michelia* and *Manglietia* (Magnoliaceae)

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Abstract — A karyomorphological study on eleven and one hybrid belonging to *Michelia* L. and *Manglietia* Blume (Magnoliaceae, *sensu stricto*) was conducted. The structure of interphase nuclei and prophase chromosomes of representatives of the two genera were presented here for the first time. Three karyomorphological types at resting nuclei and two types of prophase chromosomes were recognized according to TANAKA's catalogue (1971; 1977; 1980) respectively. It might provide information to distinguish the intergeneric and infrageneric relationships in Magnoliaceae. An evolutionary trend is proposed that awaits verification by further study. All taxa studied have the same diploid number of chromosomes ($2n=38$) in somatic cells, three of them were reported for the first time, and others were confirmed. The karyotype analysis have shown a predominance of chromosomes with centromeres in a median position and a few submedian centromeres, except for *Michelia hypolampra* with subterminal and terminal point centromeres, which also showed high karyotypic symmetry. The karyotype data documented with $2n=38$ indicate that the speciation may have taken place predominantly at the diploid level in *Michelia* and *Manglietia* and that minor structural alternation of chromosomes may have resulted in distinct morphological divergence and probably played the most important role in the evolution of genus and species.

Key words: chromosome number, diploid, karyomorphology, karyotype evolution.

INTRODUCTION

The family Magnoliaceae is one of the most primitive groups of angiosperms, comprising deciduous or evergreen trees or shrubs, characterized by annular stipular scars around the nodes, and floral parts spirally arranged on an elongated receptacle. It consists of about sixteen genera and 300 species mainly distributed throughout East and Southeast Asia, Southeast North America, Central America and South America. Approximately 11 genera and more than 160 species of Magnoliaceae are found in China (LIU 2004).

The genus *Michelia* is the second largest genus in the family (LAW 1984) and comprises approximately 80 species distributed mainly in tropical and subtropical Asia. Approximately 70 species are found in China, mainly distributed Southwest and East in China (LIU 2004). It is a natural group of evergreen trees or shrubs distinguished from other members of subfamily Magnolioideae by ax-

illary flowers, stipitate gynoecea, and laterally dehiscent anthers. *Manglietia* was recognized as the most primitive position in the family Magnoliaceae (LAW 1984) and consists of approximately 55 species distributed in tropical and subtropical Asia. There are 47 species found in China, distributed in the southern area of Changjiang River (LIU 2004). The *Manglietia* species are usually evergreen, rarely deciduous trees, with sessile gynoecea, and introrsely dehiscent anthers. Many species of both genera *Michelia* and *Manglietia* have been introduced and cultivated as ornamental plants, and natural resources of stacte, a sweet spice used in making incense, and flavor (e.g. *Michelia hypolampra*).

Because of extensive homogeneity and confusion of generic delimitation, there have been persistent debates on the delimitation and classification of the family (DANDY 1927; 1964; 1978; LAW 1984; 1996; LIU 2000; NOOTEBOOM 1985; 2000; CHEN and NOOTEBOOM 1993; GONG *et al.* 2003; FIGLAR and NOOTEBOOM 2004; SUN and ZHOU 2004). It is generally accepted that Magnoliaceae could be divided into two subfamilies, Magnolioideae and Liriodendroideae. However, the relationships of tribes, genera and sections in a given genus within subfamily Magnolioideae are not

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well delimited. For example, the genus *Manglietia* is very closely allied to *Magnolia*, from which it differs mainly by having a great number of ovules in the carpels. It was included in *Magnolia* by some magnolia experts (BAILLON 1866; NOOTEBOOM 2000; GONG *et al.* 2003; SUN and ZHOU 2004; FIGLAR and NOOTEBOOM 2004). But, DANDY and LAW considered *Manglietia* to be a natural group because its members can be distinguished from other genera of Magnoliaceae by their general aspect as well as the number of ovules per carpel.

Cytological data are essential to the study of plant evolution and diversification (STEBBINS 1950; 1971; HONG 1990; STACE 2000). Due to investigations of karyological features of all the genera of Magnoliaceae contribute significantly to a better understanding of systematics and evolution of the primitive groups of higher plants, and a comparative karyomorphological study on intergenera within Magnoliaceae might also contribute to understanding the systematical classification of the family. The cytology of Magnoliaceae have already been thoroughly studied (JANAKI 1952; BISWAS 1979; BISWAS and SHARMA 1984; CHEN S Z *et al.* 1984a; 1984b; 1986; 1989; HUANG *et al.* 1985; CHEN R. Y. *et al.* 1985; 1989; GOLDBLATT 1988; CHEN Z. Y. *et al.* 1989; 1990; LI *et al.* 1997; 1998a; 1998b; ZHANG and XIA 2005; MENG *et al.* 2004; 2006), but only a few investigations are focused on the interphase nuclei and the mitotic prophase chromosomes of members in the family (OKADA 1975; WANG *et al.* 2004; WANG *et al.* 2004; MENG *et al.* 2006). So it needs to be explored karyomorphologically for a better understanding of the intergeneric and interspecies relationships.

The present study aims to: (1) investigate the structure of interphase nuclei, prophase chromosomes, karyotype and chromosome number of representatives of *Manglietia* and *Michelia*, including *Tsoongiodendron*, (2) discuss the karyotype evolution of *Michelia* and *Manglietia*, and (3) compare *Michelia* with its related genera *Manglietia* and *Tsoongiodendron* for systematic classifications based on combination of karyomorphological information and morphological data.

MATERIAL AND METHODS

The taxa studied with voucher specimens are listed in Table 1. Investigated plants were introduced and cultivated in the Magnolia Garden of South China Botanical Garden, China.

Young stem tips were pretreated in a 0.002M 8-hydroxyquinoline solution at room temperature for 1.5 – 2.0 h, and then fixed in freshly prepared Carnoy's liquid fixative (ethanol alcohol: glacial acetic acid = 3: 1) at about 4°C for 2 h, lastly, transferred to 70% alcohol, and stored in a refrigerator. Wall degradation hypotonic method was utilized to prepare the chromosomes (CHEN *et al.* 1979). Slides were stained with 2% Giemsa (GUERRA 1983) for about 15-20 min.

The karyomorphological classification of resting nuclei and mitotic prophase chromosomes followed TANAKA's categories (1977; 1971; 1980). The karyotypes were analyzed following LI (1985). The symbols for the description of chromosomes followed LEVAN *et al.* (1964). The symmetry of karyotypes was classified according to STEBBINS (1971). Mean length (L) was calculated for each chromosome pair. These values were then used to calculate the total chromatin length

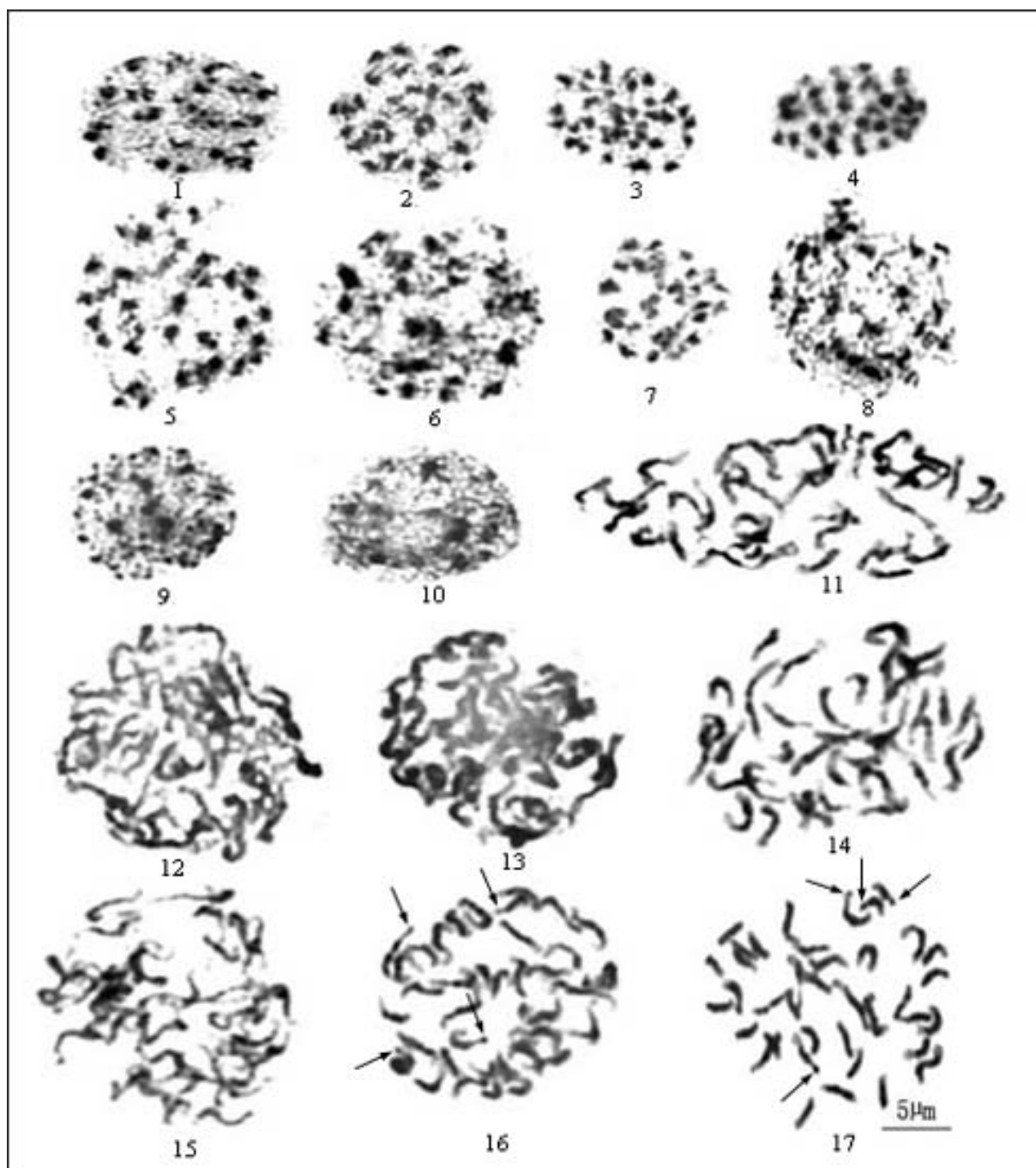
Table 1 — List of investigated taxa. Voucher specimens are deposited in the herbarium of South China Botanical Garden, the Chinese Academy of Sciences (IBSC).

Taxon	Locality	Voucher specimens
<i>Michelia sphaerantha</i> C. Y. Wu ex Law et Y. F. Wu	Jingdong, Yunnan, China	Zhang X. H. 051001
<i>Michelia longistamina</i> Law	Guaungdong, China	Huang X.X. and Zhang X. H. 050402
<i>Michelia coriacea</i> Chang et B. L. Chen	Malipo, Yunnan, China	Zhang X. H. 051003
<i>Michelia sirindhorniae</i> (Noot. & Chalermglin) N. H. Xia et X. H. Zhang	Thailand	Zhang X. H. 051004
<i>Michelia hypolampra</i> Dandy	Daqingshan, Guangxi, China	Huang X.X. and Zhang X. H. 050403
<i>Michelia elegans</i> Law et Y. F. Wu	Guangdong, China	Huang X.X. and Zhang X. H. 050404
<i>Michelia wilsonii</i> Finet et Gagnep.	Emei, Sichuan, China	Huang X.X. and Zhang X. H. 050405
<i>Michelia szechuanica</i> Dandy	Tianfu Forestry Centre, Sichuan, China	Huang X.X. and Zhang X. H. 050406
<i>Michelia maudiae</i> × <i>M. macclurei</i> var. <i>sublanea</i>	Hunan Institute of Forestry, China	Zhang X. H. 051005
<i>Tsoongiodendron odorum</i> Chun	Guangxi, China	Huang X.X. and Zhang X. H. 050408
<i>Manglietia pubipes</i> ined.	Xichou, Yunnan, China	Huang X.X. and Zhang X. H. 050407
<i>Manglietia garrettii</i> Craib	Thailand	Zhang X. H. 051006

(TCL) of each karyotype. Karyotypic asymmetry rate (As. k %), based on the long arm (L) and on each chromosome length (CL) As. k% = $100 \sum L \sum CL^{-1}$, were calculated together with the CI (centromeric index) for a better comparison between karyotypes.

RESULTS

The structure of interphase nuclei, mitotic prophase chromosomes or the prometaphase chromosomes of the taxa examined are shown in Figs. 1-17.



Figs. 1-17 — The interphase nuclei, prophase chromosomes and prometaphase chromosomes of taxa investigated. 1 *Michelia longistamina*; 2, 13 *Michelia sirindhorniae*; 3, 12 *Michelia sphaerantha*; 4 *Michelia coriacea*; 5 *Michelia elegans*; 6 *Michelia hypolampra*; 7, 11, 14 *Michelia maudiae* x *Michelia macclurei* var. *sublanca*; 8 *Tsoongiodendron odorum*; 10 *Manglietia pubipes*; 9, 15 -17 *Manglietia garrettii*, arrows pointing to satellites.

Interphase nuclei - At resting nuclei, three karyomorphological types were found according to TANAKA's catalogue (1971; 1977).

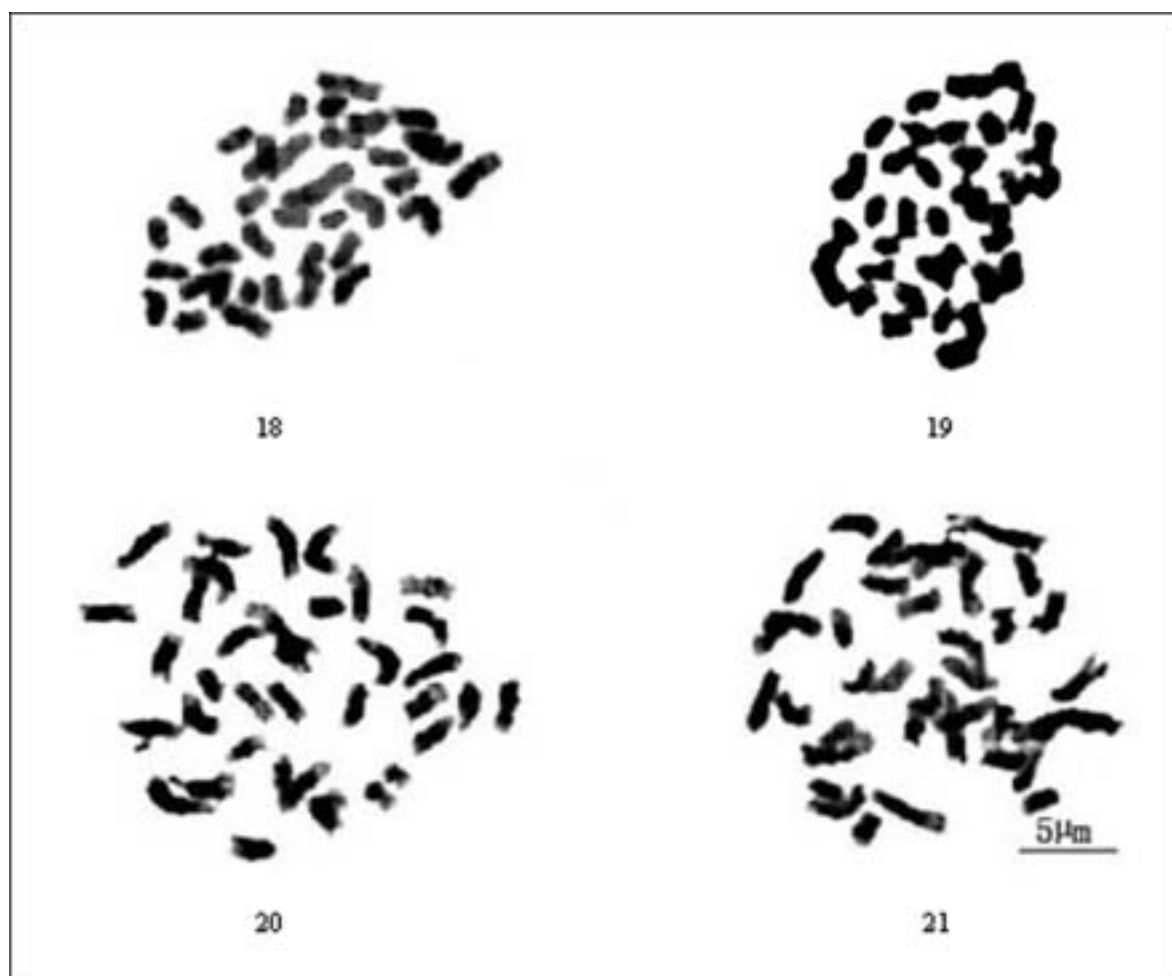
Type I. The nucleus was characterized by forming about 30-38 condensed and deeply stained round-shaped or spheroid equal granules evenly dispersed throughout the nuclei. Diffused chromatin in these nuclei showed lightly stained semireticular fibers (Figs. 1-5, 7). We categorized this pattern as the round prochromosome type (i.e., semireticular interphase nuclei with granulated reticulum).

Type II. The secondary type was characterized by several darkly stained chromocenters with an irregularly protruding rough surface which gradually transformed into diffuse chromatin (Figs. 9, 10). The interphase nuclei were categorized to be simple chromocenter type.

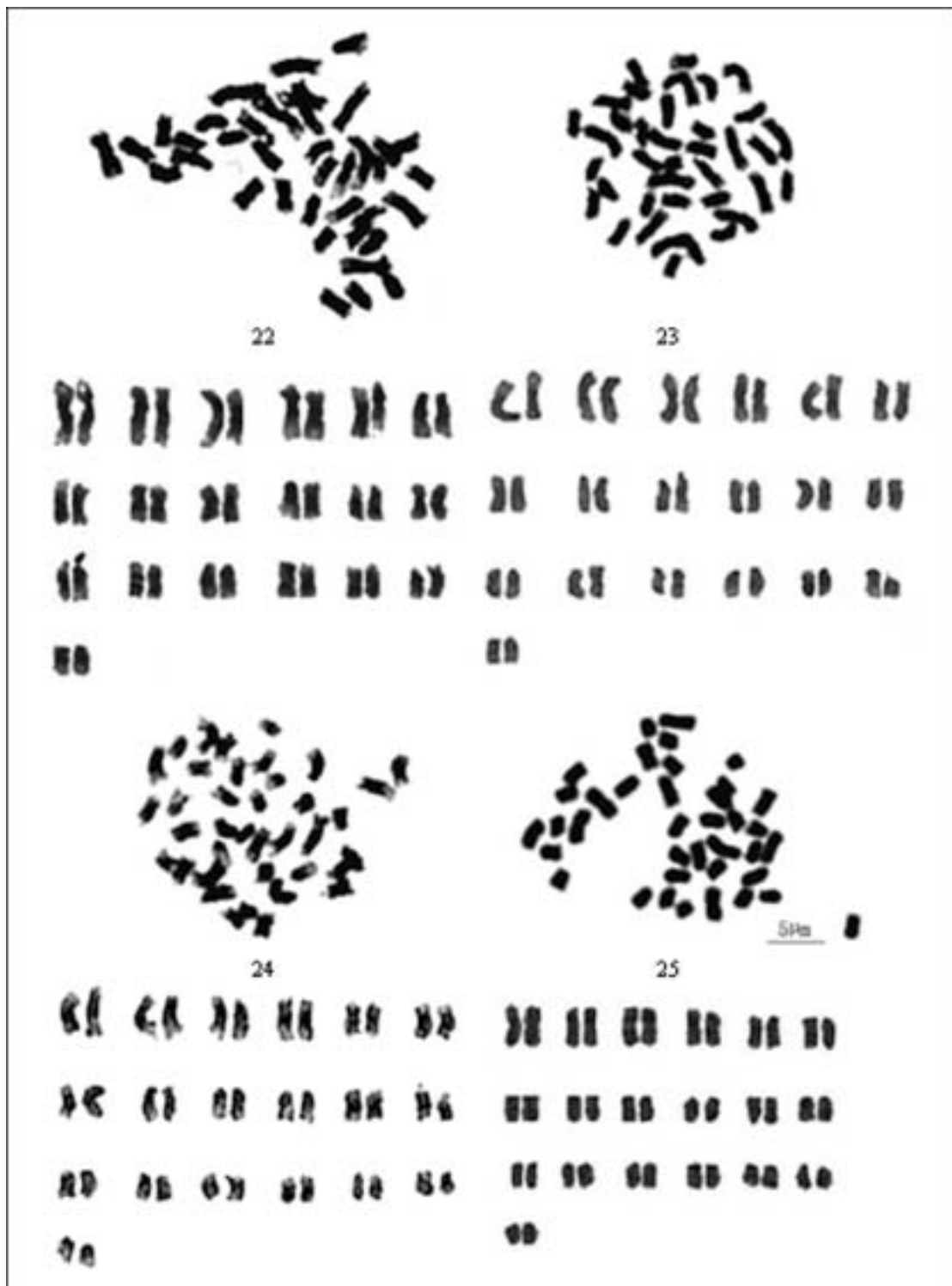
Type III. The nucleus contains many similar round-shaped, spheroid or irregular-shaped het-

eropycnotic bodies, which were distinguished by 4-5 prominently much larger granulated bodies from the first type (Figs. 6, 8). We categorized to be the complex chromocenter type.

Mitotic prophase chromosomes - At the mitotic prophase, two types of prophase chromosomes can be recognized. The first one is all chromosomes have early condensed segments at proximal region of one or both arms. Some of them have early condenses segments not only at the proximal regions of one or both arms but also at the interstitial and terminal regions of one arm. Early and late condensed segments of chromosomes show a gradual transition between them (Figs. 11-14). This type of prophase chromosomes is referred to the gradient type of TANAKA (1980). On the other hand, hetero- and euchromatic segments were distinguishable, but their boundaries were not clear, and the heterochromatic segments were distrib-



Figs. 18-21 — Somatic metaphase chromosomes of taxa investigated. 18 *Michelia szechuanica*; 19 *Michelia wilsonii*; 20 *Manglietia pubipes*; 21 *Tsoongiodendron odorum*.



Figs. 22-25 — Karyograms and somatic mitotic metaphase of taxa investigated. 22 *Michelia sphaerantha*; 23 *Michelia maudiae* x *Michelia macclurei* var. *sublanaea*; 24 *Michelia hypolampra*; 25 *Michelia coriacea*.

uted in the distal and interstitial regional as well as the proximal regions (Figs. 15). According to TANAKA, the prophase chromosomes belonged to the interstitial type.

Furthermore, late prophase and premetaphase chromosomes of *Manglietia garrettii* are shown in Figs. 16, 17. Two pairs of large and obvious satellites were observed.



Figs. 26-29 — Karyograms and somatic mitotic metaphase of taxa investigated. 26 *Michelia sirindhorniae*; 27 *Michelia longistamina*; 28 *Manglietia garrettii*; 29 *Michelia elegans*.

Metaphase chromosomes - Metaphase chromosomes and idiograms of somatic metaphase chromosomes of taxa surveyed are shown in Figs. 18-37, with detailed parameters listed in Table 2 and 3.

The chromosome numbers of twelve taxa were reported to be $2n=38$, three of which were documented here for the first time (Table 3). Our findings also have confirmed previous investigations, with $2n=38$ chromosomes and a basic number of

Table 2 — Relative length, arm ratio and classification of the chromosome of eight taxa investigated of *Michelia* and *Manglietia*.

Abbreviations: No.: chromosome pair number; RL: relative length; AR: arm ratio; PC: position of centromere; m: median chromosome; sm: submedian chromosome; st: subterminal chromosome; t: terminal chromosome; *: satellite-chromosomes.

No.	RL	AR	PC	RL	AR	PC	RL	AR	PC	RL	AR	PC
	<i>Michelia sphaerantha</i>			<i>Michelia coriacea</i>			<i>Michelia sirindhorniae</i>			<i>Michelia longistamina</i>		
1	8.17	2.08	sm	7.19	1.29	m	7.86	1.20	m	7.52	1.17	m
2	7.40	2.13	sm	6.89	1.43	m	7.77	1.48	m	7.23	1.44	m
3	6.91	1.25	m	6.79	1.39	m	6.38	1.43	m	7.09	1.06	m
4	6.63	1.57	m	6.28	1.58	m	6.29	1.48	m	7.01	1.06	m*
5	6.56	1.47	m	5.88	1.42	m	5.76	1.45	m	6.36	1.26	m
6	5.59	3.44	st	5.67	1.33	m	5.59	1.67	m	5.78	1.35	m
7	5.38	1.27	m	5.47	1.45	m	5.33	1.54	m	5.06	1.36	m
8	4.89	1.50	m	5.37	1.30	m	5.33	1.35	m	4.84	1.31	m
9	4.82	1.31	m	5.07	1.38	m	5.07	2.06	sm	4.84	1.48	m*
10	4.68	1.49	m	4.96	1.33	m	4.98	1.48	m	5.06	2.67	sm
11	4.68	1.16	m	4.96	1.45	m	4.80	1.90	sm	5.13	1.37	m
12	4.61	1.28	m	4.66	1.42	m	4.63	1.43	m	5.06	1.20	m
13	4.40	1.25	m*	4.66	1.30	m	4.54	1.17	m	4.56	1.34	m
14	4.40	1.42	m	4.66	1.30	m	4.45	1.22	m	1.95	1.30	m
15	4.40	1.42	m	4.56	1.37	m	4.45	1.24	m	4.41	1.29	m
16	4.33	1.30	m	4.46	1.93	sm	4.45	1.32	m	4.12	1.85	sm
17	4.19	1.22	m	4.46	1.94	sm	4.45	2.06	sm	3.83	1.31	m
18	4.19	1.31	m	4.15	1.16	m	4.10	1.36	m	3.69	1.83	sm
19	3.77	1.25	m	3.85	1.71	sm	3.76	2.32	sm	3.90	1.16	m
	<i>Michelia hypolampra</i>			<i>Michelia elegans</i>			<i>M. maudiae</i> × <i>M. macclurei</i> var. <i>sublanaea</i>			<i>Manglietia garrettii</i>		
1	7.26	1.37	m	8.99	1.15	m	7.86	1.12	m	7.59	1.09	m
2	7.07	1.47	m	7.81	1.11	m	7.77	2.08	sm	6.97	1.08	m
3	6.70	1.40	m	6.78	1.26	m	6.38	1.02	m	6.70	1.40	m
4	6.60	1.45	m	5.68	1.19	m	6.29	1.33	m	6.08	1.47	m*
5	5.77	1.39	m	5.52	1.34	m	5.76	1.26	m	6.63	1.23	m
6	5.58	1.32	m	5.52	1.80	sm	5.59	1.61	m	6.02	2.14	sm
7	5.58	1.40	m	5.44	1.23	m	5.33	1.36	m	5.60	1.34	m
8	5.49	2.28	sm	5.44	1.09	m	5.33	1.26	m	5.40	1.20	m
9	5.21	3.67	st	5.13	1.51	m	5.07	1.38	m*	5.13	1.31	m
10	5.21	3.33	st	4.97	1.43	m	4.98	1.36	m	4.85	1.37	m
11	5.02	1.70	m	4.89	1.21	m	4.80	1.26	m	4.85	1.61	m
12	4.37	1.38	m*	4.89	1.50	m	4.63	1.42	m	4.72	1.56	m
13	4.47	1.29	m	4.42	1.34	m	4.54	1.19	m	4.65	1.33	m*
14	4.47	2.21	sm	4.42	1.43	m	4.45	1.28	m	4.72	1.38	m
15	4.47	1.41	m	4.26	1.57	m	4.45	1.62	m	4.58	1.24	m
16	4.47	1.41	m	4.10	1.89	sm	4.45	2.00	sm	4.44	1.83	sm
17	4.37	1.36	m	3.79	1.19	m	4.45	1.38	m	4.37	1.91	sm
18	3.91	1.80	sm	4.02	1.43	m	4.10	1.43	m	3.62	1.65	m
19	3.90	15.00	t	3.94	2.63	sm	3.76	1.45	m	3.08	1.50	m

$x=19$ in the two genera. The chromosomes were small in length ranging from 1.4 μm to 3.8 μm , differing slightly from species to species, and the ratio of the longest to the shortest chromosome arranged from 1.92 to 2.64. The chromosome morphology at mitotic metaphase has exhibited to be similar (Figs. 18-29). The karyotype analysis has showed a predominance of chromosomes with centromeres in a median position and a few submedian centromeres, and being of primitive and symmetry type. The karyotype types of all taxa in-

vestigated were of 2B type, except that of *Michelia coriacea* being 1A type and *Michelia maudiae* × *M. macclurei* var. *sublanaea* being 2A type. Asymmetry index arranged from 57.48 to 59.98 except that of *Michelia hypolampra* being 62.33. It was noteworthy that the nineteen pair of chromosome of *Michelia hypolampra* has terminal centromeres. Furthermore, there were differences in the number of m-, sm-, and st-chromosome, satellites as well as chromosomal location with satellite among taxa studied.

Table 3 — The parameters of mitotic metaphase chromosome of eleven species and one hybrid of Magnoliaceae. Abbreviations: As. k%: karyotypic asymmetry rate; P. C. A%: percentage of chromosomes with arm ratio > 2; Lt: longest arm; St: shortest arm; N. F.: number fundamental; * chromosome numbers are reported here for the first time; **: karyotype was investigated by CHEN *et al.* (1998); -: absent data.

Taxon	Chromosome numbers	Karyotype formula	Lt/St	As. k%	P. C. A%	Type	N. F.
<i>Michelia sphaerantha</i>	2n=2x=38	32m (2SAT) + 4sm + 2st	2.19	59.98	0.16	2B	74
<i>Michelia coriacea</i>	2n=2x=38	32m + 6sm	1.92	58.73	0	1A	76
<i>Michelia sirindhorniae</i>	2n=2x=38	30m (2SAT) + 8sm	2.07	59.65	0.16	2B	76
<i>Michelia hypolampra</i>	2n=2x=38	30m(2SAT) + 2sm + 4st + 2t	2.00	62.33	0.26	2B	70
<i>Michelia elegans</i>	2n=2x=38*	32m + 6sm	2.28	57.57	0.05	2B	76
<i>Michelia longistamina</i>	2n=2x=38	32m (2SAT) + 6sm	2.13	57.48	0.05	2B	76
<i>Michelia szechuanica</i>	2n=2x=38*	-	-	-	-	-	-
<i>Michelia wilsonii</i>	2n=2x=38	30m + 8sm (2SAT) **	2.67	58.21	0.21	2B	76
<i>Tsoongiodendron odorum</i>	2n=2x=38	32m + 6sm (2SAT) **	2.16	55.93	0.16	2B	76
<i>Michelia maudiae</i> × <i>Michelia macclurei</i> var. <i>sublanaea</i>	2n=2x=38	34m (2SAT) + 4sm	1.93	57.84	0.11	2A	76
<i>Manglietia garrettii</i>	2n=2x=38	32m (4SAT) + 6sm	2.64	57.96	0.05	2B	76
<i>Manglietia pubipes</i>	2n=2x=38*	-	-	-	-	-	-

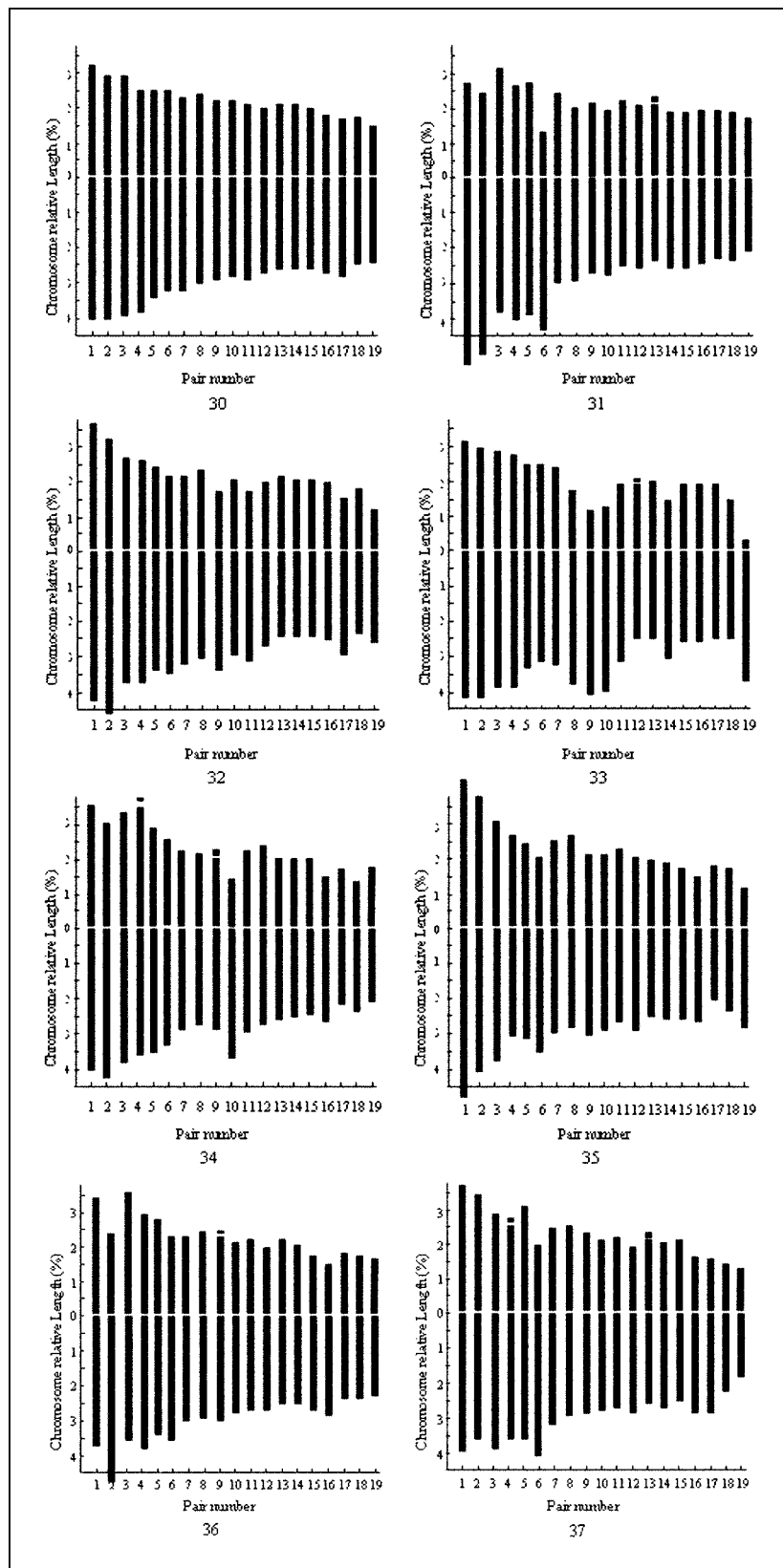
DISCUSSION

The twelve taxa of *Michelia* and *Manglietia* investigated had a consistent somatic chromosome number of 2n=38, with a basic number of x=19. Employing chromosome data documented, the two genera *Michelia* and *Manglietia* show constancy in chromosome number (2n=38) and no polyploidy has been recorded in both previous and present investigations, so it is concluded that *Michelia* and *Manglietia* are diploid.

Chromosome size and morphology may help indicate evolutionary relationships among plant species (CLARK and WALL 1996). In the present study, the chromosomes were small in length and the ratio of the longest to the shortest chromosome arranged from 1.92 to 2.64, indicating that the taxa have a relative low interchromosomal asymmetry and a monomodal karyotype. Moreover, the arm ratios of a few of chromosomes pairs exceeded 2.0, which indicated the intrachromosomal symmetry in *Michelia* and *Manglietia* was very high. LI *et al.* (1998) pointed out in Magnoliaceae the karyotypes of *Liriodendron* and *Michelia* belonged to 2A (except *M. floribunda* to 1A), and the other genera belong to 2B. In contrast, the karyotype types of all taxa here investigated were of 2B type, except that of *Michelia coriacea* being 1A type and *Michelia maudiae* × *M. macclurei* var. *sublanaea* being 2A type. The result indicates that the karyotype of *Michelia* was not absolutely uniform. Combinations of morphological characters suggested that *Michelia* was a complex taxon, which comprised not only the primitive taxa, but also the advanced taxa (LAW 1984). As OKADA (1975), BISWAS and SHARMA (1984)

and LI *et al.* (2003), proposed, in Magnoliaceae, some minute heteromorphosis structural alternation occur and they probably play a very important role in the evolution of genus or species. To some extent, karyotype diversity often provides indications of on-going speciation events. Nevertheless, it would appear that the relative little difference and homogeneity of *Michelia* and *Manglietia* chromosome morphology and invariable number mirror its relative evolutionary stasis. Compared with other members investigated of the Magnoliaceae, the chromosomal evolution in the family was quite slow. The karyotype data provide no remarkable support to intergeneric relationships and infrageneric classification within the family.

However, it is noteworthy that the structure of interphase nuclei, mitotic prophase chromosomes might provide information to distinguish the intergeneric and infrageneric relationships in the family. Multiple evidence indicates that *Tsoongiodendron* (CHUN 1963), which is monotypic genus with its single species, *Tsoongiodendron odorum*, and distributed in South China and North Vietnam, is more advanced group in Magnoliaceae (DANDY 1927; CHUN 1963; PRAGLOWSKI 1974; LAW 1984; XU 1995; LI *et al.* 1998; HONG *et al.* 1998a; 1998b; WANG *et al.* 2000; XIONG *et al.* 2001). CHEN and NOOTEBOOM (1993) placed *Michelia hypolampra* in *Michelia* sect. *Anisochlamys* which only contains the species because of its membranous and narrow outer 3 tepals. It suggests that the species presumably had a relatively advanced position in *Michelia*. In the present study, the karyotypic analysis indicate that its asymmetry index was higher than that of other



Figs. 30-37 — Idiograms of somatic metaphase chromosomes of taxa studied. 30 *Michelia coriacea*; 31 *Michelia sphaerantha*; 32 *Micheliasirindhorniae*; 33 *Michelia hypolampra*; 34 *Michelia longistamina*; 35 *Michelia elegans*; 36 *Michelia maudiae* x *M. macclurei* var. *sublanea*; 37 *Manglietia garrettii*. ■: satellite

members investigated, which coincides with STEBBINS' viewpoint (1971) that the karyotypic evolution, generally, is from symmetry to asymmetry in higher plants, and found subterminal and terminal centromere chromosomes for the first time in *Michelia*. At interphase nuclei, *Tsoongiodendron odorum* and *Michelia hypolampra* possess the complex chromocenter type (Figs. 7, 8), while the other species in *Michelia* have the round prochromosome type. Taking the morphological and karyomorphological information together into consideration, a hypothesis can be developed for the possible evolutionary trend of the structure of interphase nuclei within the family Magnoliaceae as follows: the simple chromocenter type → the round prochromosome type → the complex chromocenter type, which the first type was only found in *Manglietia* species and considered as the primitive type. Further work on the additional samples is necessary, however, to test this hypothesis. In addition, the chromosomal morphology of *Michelia* at the mitotic prophase was distinguished from that of *Manglietia*. On this point, we support that *Manglietia* should be considered as a distinct genus as defined by Dandy, and is the most primitive genus in the family. The recognition of *Manglietia* are supported by leaf epidermal evidence (BARANOVA 1972), foliar sclereids (TUCKER 1977), molecular data (SHI *et al.* 2000; UEDA *et al.* 2000; KIM *et al.* 2001), and a morphological cladistic analysis (LI and CONRAN 2003).

The karyomorphological data has provided limited evidence to sharpen the genus *Michelia* boundaries (CHEN and NOOTEBOOM 1993). In their revision of Chinese Magnoliaceae, CHEN and NOOTEBOOM (1993) reduced *Tsoongiodendron* to *Michelia*. *Tsoongiodendron* and *Michelia* are distinguished from each other by fruit. In *Michelia*, the torus elongates after fertilization and the fruiting-carpels are free and longitudinally dehiscent. In *Tsoongiodendron*, the carpels are concrescent and form a syncarp, and the mature carpels are woody and 2-valved dehiscent. The karyomorphological features of *Tsoongiodendron odorum* at resting nuclei are similar to those of *Michelia hypolampra*, and the chromosome number and karyotype are same as those of *Michelia* species. The evidences of morphology, anatomy, molecular data, and morphological cladistic analysis (ZHANG 1974; 1984; CHEN *et al.* 1984; 1985; 1989; LI *et al.* 1998; HONG *et al.* 1998a; 1998b; WANG *et al.* 2000; SHI *et al.* 2000; ZENG *et al.* 2000; XIONG *et al.* 2001; KIM *et al.* 2001; LI and CONRAN 2003) strongly suggest that *Tsoongiodendron* should be reduced to a synonym of *Michelia*.

The hybrid *Michelia maudiae* × *Michelia macclurei* var. *sublanea* was diploid with a chromosome number of $2n=2x=38$, which equals the total chromosome number of its parents. So, the hybrid was confirmed to be crossbreeding filial generation of *Michelia maudiae* and *Michelia macclurei* var. *sublanea*, which indicated that the species *Michelia maudiae* was related to the variation *Michelia macclurei* var. *sublanea*. Polyploids or aneuploids were not found, indicating that pericentric inversions or chromosome fragmentation in *Michelia* seldom occur. Comparing the homologue pairs only by their relative length and the centromer-index, practically no differences were visible. Logical would be, that the current sizes in the hybrid species would be between them or close to the size of one parent.

All in all, although the chromosomal differentiation was quite low in Magnoliaceae, it has come into being a plenty of biodiversity. It could be possible that many morphological features exhibit extensive phenotypic plasticity that is influenced environmentally rather than genetically. So, we suggest that the taxonomic placement of the systematic position of a given genus and the infrageneric phylogeny in the family need to consider environmental factors.

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