

CARYOLOGIA

**International Journal of Cytology, Cytosystematics
and Cytogenetics**

Founded by ALBERTO CHIARUGI



Published in Italy
by
the University of Florence

Karyological studies of ten *Ligusticum* species (Apiaceae) from the Hengduan Mountains Region of China

ZHOU^{1,2}, JING, FADING PU³, HUA PENG¹, YUEZHI PAN¹, XUN GONG^{1*}

¹ Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650204, Yunnan, China.

² Graduate University of Chinese Academy of Sciences, Beijing 100049, China.

³ Chengdu Institute of Biology, Chinese Academy of Sciences, Chengdu 610041, Sichuan, China.

Abstract — Karyological studies on ten species of the genus *Ligusticum* (Apiaceae) from the Hengduan Mountains Region of China were carried out. Five species of them are reported for the first time. All species have the same basic chromosome number, $x = 11$, but they could be differentiated by their karyotype formula and quantitative parameters of the karyotypes. *L. capillaceum* and *L. pteridophyllum* are tetraploid with the chromosome number of $2n = 4x = 44$, other species are diploid with the chromosome number of $2n = 2x = 22$. Our cytological results suggest that polyploidy did not play an important role in the chromosome evolution of plants from the Hengduan Mountains Region. Relationships between *Ligusticum* and its affinities are also discussed. *Ligusticum* has great diversification in northwestern Yunnan of China, so this region is probably the most diversified center for it.

Key words: chromosome, Hengduan Mountains region, karyotype, *Ligusticum*, polyploidy.

INTRODUCTION

The genus *Ligusticum* L., with about 60 species (PU and WATSON 2005), is probably one of the largest members of the family Apiaceae (PIMENOV and LEONOV 1993). It is widely distributed in the Eurasia continent and North-America, with Himalayas (including the Hengduan Mountains of western China) and North America as its two distribution centers (PU 1991). Forty species are distinguished in China (35 species are endemic), most of which are distributed in the alpine belt of southwestern China, with only a few species extending to northern region (PU and WATSON 2005).

Ligusticum is a large and widespread genus of complex taxonomy and its relationships with putatively allied genera *Cnidium* Cuss., *Hymenidium* DC., *Pachypleurum* Ledeb., *Paraligusticum* Tichom., *Rupiphila* Pimenov & Lavrova, *Selinum* L., *Tilingia* Regel & Tiling, and *Ligusticopsis* Leute are still being clarified (PU and WATSON 2005). LEUTE (1969; 1970) established an informal alliance "Verwandtschaftskreis der Gattung *Ligusticum*" to mark a group of genera presum-

ably related to *Ligusticum* and *Selinum*, which includes these genera - *Cortia* DC., *Cyatoseelinum*, *Cenolophium* W.D.J. Koch, *Endressia* J. Gay, *Heteroptylis*, *Ligusticopsis*, *Ligusticum* (incl. *Pachypleurum*), *Selinum* (incl. *Cnidium* and *Lithosciadium* Turcz.), and *Sphenosciadium* Gray. Among these genera, its relationships with *Tilingia* and *Ligusticopsis* are the most controversial. *Tilingia* was established by REGEL and TILING (1858). The diagnostic characters of it are the distinct calyx teeth and the carpels bearing a solitary vitta in each furrow (*T. ajanensis* is the type species). However, these characters could not differentiate it from *Ligusticum*, and it was transferred into *Ligusticum* by KOZO-POLJANSKY (1916). The genus *Ligusticopsis*, with 14 species occurring in China, was separated from *Ligusticum* based on the prominent calyx teeth of the former (LEUTE 1969). This treatment was not supported by the following botanists since its establishment (PU 1991). Based on their studies of pollen morphology, WANG *et al.* (1991) supported *Tilingia* and *Ligusticopsis* as the synonyms of *Ligusticum*. This treatment was also reflected in Flora of China (vol.14, PU and WATSON 2005). PU (1991) subdivided the *Ligusticum* into two sections: *L.* section *Ligusticum* L. and *L.* section *Pinnatibracteola* Pu. However, such a split was not reflected in our molecular analysis (ZHOU *et al.* 2008). All previ-

* Corresponding author: phone: +86-871-5223625; fax: +86-871-5223133; e-mail: gongxun@mail.kib.ac.cn

ous molecular studies have shown that *Ligusticum* is a polyphyletic heterogenous group in need of further revision (DOWNIE and KATZ-DOWNIE 1996; PLUNKETT *et al.* 1996; KATZ-DOWNIE *et al.* 1999; DOWNIE *et al.* 1998, 2000; VALIEJO-ROMAN *et al.* 2006; ZHOU *et al.* 2008).

The value of cytological data in the study of plant evolution and diversification has long been recognized, especially for taxa occurring in extreme habitats such as alpine mountains (STEBBINS 1950; 1971; HONG 1990; STACE 2000). Previous chromosome studies on *Ligusticum* were mainly limited to chromosome counts (GAGNIDZE and CHKHEIDZE 1974; CONSTANCE *et al.* 1976; ROSTOVTSOVA 1976; 1979; KROGULEVICH 1978; SILVESTRE 1978; SKALINSKA *et al.* 1978; UHRKOVA 1978; AHMAD and KOUL 1980; ARANO and SAITO 1980; VACHOVA and PACLOVA 1980; VASILYEVA *et al.* 1981; 1991; HAMAL *et al.* 1986; CHINNAPPA and CHMIELEWSKI 1987; PASHUK 1987; NISHIKAWA 1988; GE *et al.* 1989; TAMMARO 1989; NIKOLOV 1991; WETSCHNIG and LEUTE 1991; MESÍČEK and JAVŮRKOVÁ-JAROLÍMOVÁ 1992; ZHANG and FANG 1993; PIMENOV *et al.* 1998, 1999). All of these previous data showed a consistent basic chromosome number, $x=11$ (while in *L. sinense*, the basic chromosome number is $x=10$, ZHANG 1994), with B chromosome and polyploidy as rare exceptions. As aforementioned, Hengduan Mountains Region is one of the two distribution centers of *Ligusticum*. Moreover, according to the distribution of endemic species, this region is also thought to be its diversified center (PU 1991). However, the cytological data from this region is known insufficiently. Polyploidy has been considered to play an important role in plant speciation, especially in the harsh environments of alpine regions (HANELT 1966; LÖVE and LÖVE 1967; 1975; GRANT 1981). *Ligusticum* are largely restricted to such habitats in the Hengduan Mountains Region, so we expected to find relatively high levels of polyploidy among them. Therefore, the purpose

of this paper is: (1) to present karyological data for ten *Ligusticum* species and investigate their possible systematic and evolutionary implications; (2) to test whether polyploidy take an important role in the evolution of *Ligusticum*.

MATERIALS AND METHODS

Living plants of 10 *Ligusticum* species were collected from Hengduan Mountains Region of southwestern China (Table 1). Voucher specimens are deposited at KUN (Herbarium of Kunming Institute of Botany, Chinese Academy of Sciences, Kunming, China).

Somatic chromosomes were studied in root meristems of transplanted plants. The root tips were pretreated in a mixture of 0.002mol/l 8-hydroxyquinoline and 0.1mol/L Colchicine (4:1) for 4-5 hours at room temperature, then fixed in Camoy's Fluid (95% ethanol: acetic acid=3:1) at 4°C for at least half an hour, before staining, the root tips were hydrolyzed with 1mol/l HCL and 45% acetic acid (1:1) for 5-8min at 60°C. Finally, samples were stained with carbolfuchsin and squashed. Slides were made permanent using neutral balsam (Shanghai reagent company, China) as the mounting medium.

Observations were made of somatic mitotic metaphase. The nomenclature used for the description of the chromosome morphology is that proposed by LEVAN *et al.* (1964): the abbreviations m, sm, and st designate metacentric, submetacentric, and subtelocentric chromosomes, respectively. Karyotype asymmetry was determined by centromeric terminalization value (the abbreviation, T.C., T.C% = sum of the long arms/total haploid length $\times 100\%$) according to ARANO (1963), and the categories of STEBBINS (1971).

Table 1 — Localities, altitudes, and voucher specimens of the investigated species of *Ligusticum*.

| Species | Localities, altitudes (m), and voucher (KUN) |
|--|---|
| <i>L. brachylobum</i> Franch. | China, Yunnan, Shangri-La, Napa Sea; 3500; ZJ0533 |
| <i>L. capillaceum</i> H. Wolff | China, Sichuan, Daocheng; 3740; ZJ0549 |
| <i>L. daucoides</i> (Franch.) Franch. | China, Sichuan, Daocheng--Litang; 4500; ZJ0557 |
| <i>L. delavayi</i> Franch. | China, Yunnan, Xiaozhongdian; 3257; ZJ810841 |
| <i>L. involucreatum</i> Franch. | China, Sichuan, Yajiang--Kangding; 4200; ZJ0572 |
| <i>L. pteridophyllum</i> Franch. | China, Yunnan, Wufengshan; 3500; ZJ810836 |
| <i>L. rechingianum</i> (Leute) R.H. Shan & F.D. Pu | China, Yunnan, Weixi, Lidiping; 3400; ZJ810838 |
| <i>L. scapiforme</i> H. Wolff | China, Yunnan, Shudu lake; 3400; ZJ0522 |
| <i>L. sikiangense</i> Hiroe | China, Sichuan, Daocheng--Litang; 4500; ZJ0563 |
| <i>L. tenuisectum</i> H.de Bossieu | China, Yunnan, Lijiang, Sandawan; 3100; ZJ0505 |

RESULTS

All species investigated have $2n = 2x = 22$, except for *L. capillaceum* and *L. pteridophyllum*, which have $2n = 4x = 44$. The karyotype asymmetry of all species is categorized as type 2A except for *L. rechingerianum* with the type 1A. The metaphase chromosomes of each species are shown in Fig. 1: A-J. Their detailed parameters and karyotype formulae are listed in Table 2. Brief descriptions of the cytological features of each species are as follows:

1. *L. brachylobum* - This species is endemic to China with the altitudes of 1600-4100 m, and has

entire bracteoles. It is diploid with the karyotype formula of $2n = 22 = 2M + 14 m + 6 sm$ (Fig. 1: A; Fig. 2: A). Its arm ratio (sum of the long arms/sum of the short arms) is 1.37. The karyotype asymmetry is of type 2A with the T.C. % of 57.84%.

2. *L. capillaceum* - This species is endemic to Hengduan Mountains Region of China with the altitudes of 2500-4000 m, and is characteristic of pinnate bracteoles. It is tetraploid with the karyotype formula of $2n = 44 = 4M + 28 m + 12 sm$ (Fig. 1: B; Fig. 2: B). Its arm ratio is 1.38. The karyotype asymmetry is categorized as 2A with the T.C. % = 57.92%.

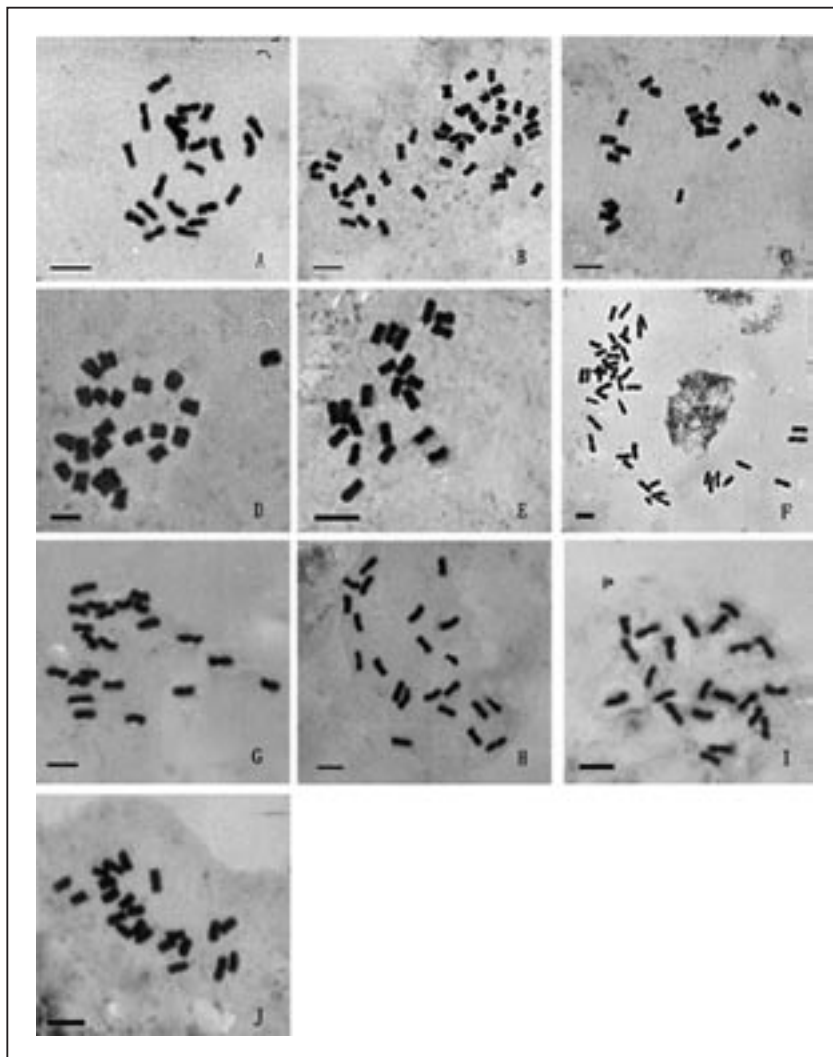


Fig. 1 — Mitotic metaphase of *Ligusticum*: A) *L. brachylobum*; B) *L. capillaceum*; C) *L. daucooides*; D) *L. delavayi*; E) *L. involucreatum*; F) *L. pteridophyllum*; G) *L. rechingerianum*; H) *L. scapiforme*; I) *L. sikiangense*; J) *L. tenuisectum*. Bar = 10 μ m.

Table 2 — The karyotype parameters of the 10 investigated species of *Ligusticum*: Arm ratio (AR); T.C.% = sum of the long arms/total haploid length \times 100%; Karyotype formula (KF); STEBBINS's asymmetry categories (ST).

| Taxon | AR | T.C% | KF | ST |
|--------------------------|------|-------|-----------------------|----|
| <i>L. brachylobum</i> | 1.37 | 57.84 | K (2n=2x)=2M+14m+6sm | 2A |
| <i>L. capillaceum</i> | 1.38 | 57.92 | K (2n=4x)=4M+28m+12sm | 2A |
| <i>L. daucoides</i> | 1.31 | 56.78 | K (2n=2x)=2M+16m+4sm | 2A |
| <i>L. delavayi</i> | 1.41 | 58.53 | K (2n=2x)=2M+12m+8sm | 2A |
| <i>L. involucratum</i> | 1.32 | 56.81 | K (2n=2x)=18m+4sm | 2A |
| <i>L. pteridophyllum</i> | 1.21 | 54.82 | K (2n=4x)=8M+32m+4sm | 2A |
| <i>L. rechingerianum</i> | 1.30 | 56.54 | K (2n=2x)=2M+18m+2sm | 1A |
| <i>L. scapiforme</i> | 1.37 | 57.72 | K (2n=2x)=2M+18m+2st | 2A |
| <i>L. sikiangense</i> | 1.25 | 55.65 | K (2n=2x)=2M+18m+2sm | 2A |
| <i>L. tenuisectum</i> | 1.34 | 57.34 | K (2n=2x)=18m+4sm | 2A |

3. *L. daucoides* - This species has pinnate bracteoles, and is distributed in Hubei, Sichuan, Xizang, Yunnan of China with the altitudes of 2600-4800 m. Its karyotype formula is $2n = 22 = 2M + 16m + 4sm$ (Fig. 1: C; Fig. 2: C) with the arm ratio of 1.31. The karyotype asymmetry is of type 2A with the T.C. % = 56.78%.

4. *L. delavayi* - This species is endemic to Hengduan Mountains Region of China with the altitudes of 2800-4500 m, and has entire bracteoles. Its karyotype formula is $2n = 22 = 2M + 12m + 8sm$ (Fig. 1: D; Fig. 2: D). Its arm ratio is 1.41. The karyotype symmetry is of type 2A with the T.C. % = 58.53%.

5. *L. involucratum* - This species is endemic to Hengduan Mountains Region of China with the altitudes of 2800-4900 m, and has pinnate bracteoles. It has the karyotype formula of $2n = 22 = 8m + 4sm$ (Fig. 1: E; Fig. 2: E). Its arm ratio is 1.32. The karyotype asymmetry is categorized as 2A with the T.C. % = 56.81%.

6. *L. pteridophyllum* - This species is endemic to China with the altitudes of 1800-3600 m, and has entire bracteoles. It is a tetraploid with the karyotype formula of $2n = 44 = 8M + 32m + 4sm$ (Fig. 1: F; Fig. 2: F). Its arm ratio is 1.21. The karyotype asymmetry is categorized as 2A with the T.C. % = 54.82%.

7. *L. rechingerianum* - This species is endemic to Hengduan Mountains region of China with the altitudes of 1500-4600 m, and has 2-3-pinnate bracteoles. It has the karyotype formula of $2n = 22 = 2M + 18m + 2sm$ (Fig. 1: G; Fig. 2: G). Its arm ratio is 1.30. The karyotype asymmetry is of type 1A with the T.C. % = 56.54%.

8. *L. scapiforme* - This species is endemic to Hengduan Mountains region of China with the altitudes of 2700-4800 m, and has 1-2-pinnate or apex 3-lobed bracteoles. Its karyotype formula is $2n = 22 = 2M + 18m + 2st$ (Fig. 1: H; Fig. 2: H).

Its arm ratio is 1.37. The karyotype asymmetry is of type 2A with the T.C. % = 57.72%.

9. *L. sikiangense* - Its bracteoles is various, with entire or pinnate types. This species is endemic to Hengduan Mountains region of China with the altitudes of 3400-4500 m. It has the karyotype formula of $2n = 22 = 2M + 18m + 2sm$ (Fig. 1: I; Fig. 2: I). Its arm ratio is 1.25. The karyotype asymmetry is of type 2A with the T.C. % = 55.65%.

10. *L. tenuisectum* - This species is endemic to China with the altitudes of 2000-4500 m, and has entire bracteoles. It has the karyotype formula of $2n = 22 = 18m + 4sm$ (Fig. 1: J; Fig. 2: J). Its arm ratio is 1.34. The karyotype asymmetry is of type 2A with the T.C. % = 57.34%.

DISCUSSION

Karyological aspects and polyploidy - The karyotypes of ten *Ligusticum* species investigated are shown in Fig. 2: A-J. Chromosome numbers and karyotypes of *L. brachylobum*, *L. delavayi*, *L. pteridophyllum*, *L. sikiangense*, *L. tenuisectum* are reported for the first time. The chromosome numbers of the remaining species agree with those published previously under synonymous *Ligusticopsis* (PIMENOV *et al.* 1999). All the species exhibited a karyotype asymmetry of type 2A except for *L. rechingerianum* which is of type 1A. As a whole, *Ligusticum* is characterized by symmetrical karyotypes, with a predominance of median centromeres (Table 2). The chromosome analysis of *Ligusticum* showed that the karyotypes of all species analyzed have very similar patterns (Fig. 2). Small differences in karyotype formulae and asymmetry indices found among species suggest that chromosome structure changes may have contributed to the diversification of the genus. Of the 30 species have been studied cytologically (including the 10 species in

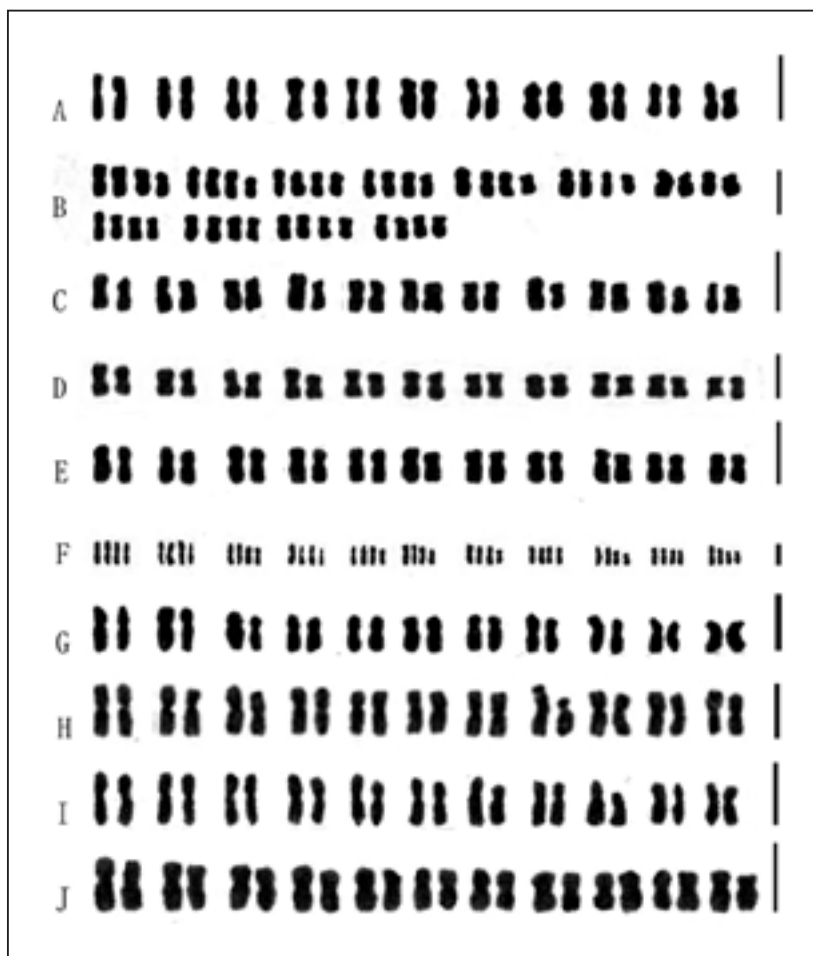


Fig. 2 — Karyotypes of *Ligusticum*: A) *L. brachylobum*; B) *L. capillaceum*; C) *L. daucooides*; D) *L. delavayi*; E) *L. involucreatum*; F) *L. pteridophyllum*; G) *L. rechingianum*; H) *L. scapiforme*; I) *L. sikiangense*; J) *L. tenuisectum*. Bar = 10 μ m.

present study), ten were analyzed under synonymous *Tilingia* and *Ligusticopsis* (three and seven, respectively).

All species investigated are diploid except for *L. capillaceum* and *L. pteridophyllum*, which are tetraploid with $2n = 4x = 44$. In total, 9.4% polyploidy occurred in all studied populations of *Ligusticum*. Cytological statistical analysis on plants of the Hengduan Mountains Region showed that polyploidy, especially infrageneric polyploidy, has played a minor role in the evolution of the Hengduan Mountain flora (NIE *et al.* 2005). Our result was consistent with their inference that polyploidization is a minor factor in the species diversification of *Ligusticum*. Several other studies on karyological researches from Hengduan Mountains also indicated similar inference and inferred that chromosome evolution mainly oc-

curred at diploid level (LIU 2000; GONG *et al.* 2001; NIE *et al.* 2002; CAI *et al.* 2004; PAN *et al.* 2004; YANG *et al.* 2004; ZHANG *et al.* 2007). Therefore, the lower levels of polyploidy maybe a main character for the evolution of Hengduan Mountain flora, unlike the global situation, where it is far more common.

Systematic position of L. delavayi - Karyotypes evolved from symmetry to asymmetry (STEBBINS 1971). According to ARANO (1963), the karyotype asymmetry of *Ligusticum delavayi* is the largest (T.C. % = 58.53%). Due to its membranous margined bracts and slightly dorsally compressed fruits, *L. delavayi* has been transferred into *Hymenidium* (PIMENOV and KLJUYKOV 1999). Molecular results confirmed this treatment in which *L. delavayi* comprises a well-supported clade with *Pleurospermum bookeri* var. *thomsonii* and *P. yunnanense* (ZHOU

et al. 2008), both of which were also referred to *Hymenidium* by the same authors (PIMENOV and KLJUYKOV 2000). *L. delavayi* is obviously more closely related to *Hymenidium*. However, as its basic chromosome number ($x=11$) is different from that of *Hymenidium* ($x=9$, PIMENOV and KLJUYKOV 2000), and as *Hymenidium* is not monophyletic as presently circumscribed (PIMENOV and KLJUYKOV 2000; ZHOU *et al.* 2008), so the position of *L. delavayi* within *Hymenidium* deserve further study.

Systematic affinities for *Ligusticum* - PU (1991) inferred an evolutionary trend of morphological characters for *Ligusticum*. That is, bracteoles evolved from entire to pinnate, and mericarps evolved from lateral-compressed to dorsal-compressed. He also arranged the species of *Ligusticum* into two sections: *L.* section *Ligusticum* *L.* with entire bracteoles, and *L.* section *Pinnatibracteola* Pu with pinnate bracteoles. In our study, *L. brachylobum*, *L. delavayi*, *L. pteridophyllum*, *L. tenuisectum* have entire bracteoles, while bracteoles of *L. capillaceum*, *L. daucoides*, *L. involucreatum*, *L. rechingerianum*, *L. scapiforme* are pinnate (*L. sikiangense* has entire or pinnate bracteoles). As indicated in Table 2, no major differences were detected in karyotypes formulae between these two sections, both with M, m and sm centromeres. A molecular analysis on *Ligusticum* showed that species with entire bracteoles did not constitute a monophyletic group, while those with pinnate ones fell into the tribe Selineae (ZHOU *et al.* 2008). Considering *Ligusticum* is not monophyletic (PIMENOV and LEONOV 1993), this infrageneric

subdivision is in need of further revision.

The genus *Ligusticopsis*, with 14 species occurring in China, was separated from *Ligusticum* based on the prominent calyx teeth of the former (LEUTE 1969). These prominent calyx teeth, however, actually occur in both genera. Other characters, e.g. basic chromosome number ($x=11$), centromere types (M, m, sm) and pollen morphology (WANG *et al.* 1991), also could not differentiate *Ligusticopsis* from *Ligusticum*. All species investigated in this study have been referred to *Ligusticopsis* save the three (*L. delavayi*, *L. involucreatum* and *L. sikiangense*). Molecular analysis indicated that species referred to *Ligusticopsis* (*Ligusticum daucoides*, *L. oliverianum*, *L. scapiforme*, and *L. acuminatum*) fell into two distant clades-- Selineae and *Sinodielsia* clade (ZHOU *et al.* 2008). Therefore, *Ligusticopsis* is not monophyletic and deserves further study. For *Tilingia*, it has consistent basic chromosome number ($x=11$) with *Ligusticum*, but more studies need be done to clarify their relationships. Therefore, until a final revision and a general consensus reached, we adopt traditional classification regarding *Ligusticopsis* and *Tilingia* as synonyms of *Ligusticum*.

In the Flora Reipublicae Popularis Sinicae, *Ligusticum* was placed near *Selinum* and *Cnidium* (CHANG 1979). Molecular studies also confirmed its close relationship to *Cortia*, *Cortiella* C. Norman, *Conioselinum scopulorum* Coult. & Rose and the monotypic genus *Trochiscanthes* W.D.J. Koch (PAPINI and MOSTI 2006; VALIEJO - ROMAN *et al.* 2006; ZHOU *et al.* 2008). LEUTE (1969; 1970) estab-

Table 3 — A comparison of the basic chromosome numbers for the genera that are presumably related to *Ligusticum* - “*Ligusticum* affinity circle” as defined by VALIEJO-ROMAN *et al.* (2006). Note: ‘?’ indicated that no cytological materials were encoded for this genus).

| Genus | Basic number | Genus | Basic number |
|-------------------------|--------------|-----------------------|--------------|
| <i>Arafoe</i> | ? | <i>Ligusticopsis</i> | 11 |
| <i>Cenolophium</i> | 10,11 | <i>Ligusticum</i> | 11 |
| <i>Cnidium</i> | 11 | <i>Lithosciadium</i> | 11 |
| <i>Cnidiocarpa</i> | ? | <i>Lomatocarpa</i> | 11 |
| <i>Cortia</i> | 11 | <i>Macrosciadium</i> | 11 |
| <i>Cyatoselinum</i> | ? | <i>Magadania</i> | ? |
| <i>Dimorphosciadium</i> | 11 | <i>Mutellina</i> | ? |
| <i>Endressia</i> | ? | <i>Notopterygium</i> | 11 |
| <i>Hansenia</i> | 11 | <i>Oreocome</i> | ? |
| <i>Haloscistrum</i> | ? | <i>Pachypleurum</i> | 11 |
| <i>Heteroptylis</i> | ? | <i>Paraligusticum</i> | ? |
| <i>Hymenidium</i> | ? | <i>Rupiphila</i> | 11 |
| <i>Kadenia</i> | 11 | <i>Selinum</i> | 11 |
| <i>Kailashia</i> | ? | <i>Sphaenolobium</i> | 11 |
| <i>Karatavia</i> | 11 | <i>Sphenosciadium</i> | 11 |
| | | <i>Tilingia</i> | 11 |

lished an informal alliance “Verwandtschaftskreis der Gattung *Ligusticum*” to mark a group of genera presumably related to *Ligusticum* and *Selinum*. Molecular and immunochemical analysis on this informal alliance confirm the separate status of some genera, e.g. *Magadania* Pimenov & Lavrova, *Sphaenolobium* Pimenov, *Dimorphosciadium* Pimenov, *Lomatocarpa* Pimenov, *Paraligusticum*, and *Arafoe* Pimenov & Lavrova, also point that *Ligusticum* is a heterogeneous assemblage of not very closely related plants in need of further taxonomical revision (VALIEJO-ROMAN *et al.* 2006). As indicated in Table 3, the basic chromosome number was $x=11$ for almost all genera of the *Ligusticum* affinity circle (VALIEJO-ROMAN *et al.* 2006). That is, these genera could not be differentiated by the basic chromosome number. The revision for *Ligusticum* will only be achieved by expanded sampling from its allies and by multidisciplinary methods.

Diversity center - *Ligusticum* is a genus typical of temperate distribution. It has two main distribution centers, one in North America, and the other in the Himalayas (including the Hengduan Mountains of western China, PU 1991). Among the 40 species distinguished in China, more than 20 species are concentrated in northwestern Yunnan (PU and WATSON 2005). Species in this region have highly diversified morphological characters, e.g., of the 13 species with pinnate bracteoles, twelve occur in this region. Species of this region also hold all of the four types of petioles anatomy of the genus (PAN *et al.* 1992). In this study, they also presented some variations on karyotypes. So we can infer that the northwest of Yunnan is probably the most diversified centre of *Ligusticum*.

Acknowledgements — We are grateful to Dr. ZHEN-WEN LIU (Kunming Institute of Botany, Yunnan) for valuable help in data analysis, to Ms. LI-QIN FANG (Shanghai Botanical Garden, Shanghai) for field works and to anonymous reviewers for helpful comments on the manuscript. This work is supported by grants from the National Basic Research Program of China (973 Program 2007CB411600).

REFERENCE

- AHMAD I. and KOUL A.K., 1980 — *Chromosome number reports LXVIII*. Taxon, 29: 538-542.
- ARANO H., 1963 — *Cytological studies in subfamily Carduoideae (Compositae) of Japan*. Botanical Magazine (Tokyo), 76:32-39.
- ARANO H. and SAITO H., 1980 — *Cytological studies in family Umbelliferae 5. Karyotypes of seven species in subtribe Seselinae*. La Kromosomo II, 17: 471-480.
- CAI J., WANG H., GU Z.J., MILL R.R. and LI D.Z., 2004 — *Karyotype morphology of thirteen species of Pedicularis (Orobanchaceae) from the Hengduan Mountains Region, NW Yunnan, China*. Caryologia, 57: 337-347.
- CHANG H.Z., 1979 — *Ligusticum* L. In R.H. SHAN and M.L. SHEH (Eds), Flora Reipublicae Popularis Sinicae, vol. 55. Science Press, Beijing.
- CHINNAPPA C.C. and CHMIELEWSKI J.G., 1987 — *Documented plant chromosome numbers 1987: I. Miscellaneous counts from western North America*. SIDA Contributions to Botany, 12: 409-417.
- CONSTANCE L., CHUANG T.I. and BYE R.A., 1976 — *Chromosome numbers in Chihuahuan Umbelliferae*. Botanical Museum Leaflets Harvard University, 24: 241-247.
- DOWNIE S.R. and KATZ-DOWNIE D.S., 1996 — *A molecular phylogeny of Apiaceae subfamily Apioideae: evidence from nuclear ribosomal DNA internal transcribed spacer sequences*. American Journal of Botany, 83: 234-251.
- DOWNIE S.R., RAMANATH S., KATZ-DOWNIE D.S. and LLANAS E., 1998 — *Molecular systematics of Apiaceae subfamily Apioideae: phylogenetic analyses of nuclear ribosomal DNA internal transcribed spacer and plastid rpoC1 intron sequences*. American Journal of Botany, 85: 563-591.
- DOWNIE S.R., KATZ-DOWNIE D.S. and WATSON M.F., 2000 — *A phylogeny of the flowering plant family Apiaceae based on chloroplast DNA rpl16 and rpoC1 intron sequences: towards a suprageneric classification of subfamily Apioideae*. American Journal of Botany, 87: 273-292.
- GAGNIDZE R.J. and CHKHEIDZE P.B., 1974 — *The chromosome numbers of some species of Caucasian flora*. Soobsc Akademii Nauk Gruzinski SSR, 75: 681-684.
- GE C.J., LI Y.K., WAN P. and HSU P.S., 1989 — *Chromosome numbers of 31 medicinal plants from Shandong Province*. Plant Chromosome Research, 267-272.
- GONG X., GU Z.J., LU Y.X. and ZHANG C.Q., 2001 — *The karyotypes of seven species in Ligularia*. Acta Botanica Yunnanica, 23: 216-222.
- GRANT V., 1981 — *Plant speciation, 2nd edn*. Columbia University Press, New York.
- HAMAL I.A., LANGER A. and KOUL A.K., 1986 — *Nucleolar organizing region in the Apiaceae (Umbelliferae)*. Plant Systematics and Evolution, 154: 11-30.
- HANELT P., 1966 — *Polyploidie-Frequenz und geographische Verbreitung bei höheren Pflanzen*. Biologische Rundschau, 4:183-196.
- HONG D.Y., 1990 — *Plant cytotaxonomy*. Beijing: Science Press.
- KATZ-DOWNIE D.S., VALIEJO-ROMAN C.M., TERENTIEVA E.I., TROITSKY A.V., PIMENOV M.G., LEE B. and DOWNIE S.R., 1999 — *Towards a molecular phylogeny of Apiaceae subfamily Apioideae: additional information from nuclear ribosomal DNA ITS sequences*. Plant Systematics and Evolution, 216: 167-195.

- KOZO-POLJANSKY B.M., 1916 — *Sciadopnytorum systematis lineamenta*. Bulletin of Moscow Society of Naturalists, 29: 93-222.
- KROGULEVICH R.E., 1978 — *Karyological analysis of the species of the flora of eastern Sayana*. In L.I. MALYSHEV and G.A. RESHLCOVA (Eds.), *Flora of the Prebailak*, p. 19-48, Novosibirsk.
- LEVAN A., FREDG A. A. and SANDBERG A.A., 1964 — *Nomenclature for centromeric position on chromosome*. Hereditas, 52: 201-220.
- LEUTE G.H., 1969 — *Untersuchungen über den Verwandtschaftskreis der Gattung Ligusticum L. (Umbelliferae)*. Teil I. Annalen des Naturhistorischen Museums in Wien, 73: 55-98.
- LEUTE G.H., 1970 — *Untersuchungen über den Verwandtschaftskreis der Gattung Ligusticum L. (Umbelliferae)*. Teil II. Annalen des Naturhistorischen Museums in Wien, 74: 457-519.
- LIU J.Q., 2000 — *Karyotypes of 4 species in Sinacalia and Parasenecio*. Acta Botanica Yunnanica, 22: 447-450.
- LÖVE A. and LÖVE D., 1967 — *Polyploidy and altitude: Mt. Washington*. Biologische Zentralblatt (Suppl.), 86: 307-312.
- LÖVE A. and LÖVE D., 1975 — *Cytotaxonomical atlas of the Arctic flora*. Cramer J., Vaduz.
- MESÍČEK J. and JAVŮRKOVÁ-JAROLÍMOVÁ V., 1992 — *List of Chromosome Numbers of the Czech Vascular Plants*. Academia, Praha.
- NIE Z.L., GU Z.J. and SUN H., 2002 — *Cytological study of Tibetia (Fabaceae) in the Hengduanshan Mountains region of China*. Journal of Plant Research, 115: 17-22.
- NIE Z.L., WEN J. and GU Z.J., 2005 — *Polyploidy in the flora of the Hengduan Mountains hotspot, southwestern China*. Annals of the Missouri Botanical Garden, 92: 275-306.
- NIKOLOV N.A., 1991 — *Chromosome numbers of Bulgarian angiosperms from North Pirin Mountain: Reserve "Bajuv Dupki-Dzindzirica"*. Fitologija (Sofia), 41: 70-75.
- NISHIKAWA T., 1988 — *Chromosome counts of flowering plants of Hokkaido (11)*. Journal of Hokkaido University of Education: Section IIB, 38: 33-40.
- PAN Y.Z., GONG X. and YIN Q., 2004 — *The karyotypes of five species in Ligularia*. Acta Botanica Yunnanica, 26: 65-72.
- PAN Z.H., WU Z.J. and PU F.D., 1992 — *Anatomical studies of petiole in Ligusticum from China*. Acta Botanica Yunnanica, 14(2): 143-149.
- PAPINI A. and MOSTI S., 2006 — *Notes on Trochiscantes Koch. (Apiaceae) on the basis of ITS rDNA sequence*. Webbia, 61(2): 217-225.
- PASHUK K.T., 1987 — *Chromosome numbers in species of subalpine belt of Chernogora (Ukrainian Carpatians)*. Botaničeskij Žurnal, 72: 1069-1074.
- PIMENOV M.G., ALEXEEVA T.V., ARTEM'eva G.M. and KLJUYKOV E.V., 1998 — *IOPB chromosome data 13*. International Organization of Plant Biosystematists Newsletter, 29: 3-24.
- PIMENOV M.G., ALEXEEVA T.V., KLJUYKOV E.V., BOKOVA O.M. and LIU Q.X., 1999 — *IOPB chromosome data 15*. International Organization of Plant Biosystematists Newsletter, 31: 13-16.
- PIMENOV M.G. and KLJUYKOV E.V., 1999 — *New nomenclatural combinations for Chinese Umbelliferae*. Feddes Repertorium, 110: 481-491.
- PIMENOV M.G. and KLJUYKOV E.V., 2000 — *Taxonomic revision of Pleurospermum Hoffm. and related genera of Umbelliferae. III: The genera Physospermopsis and Hymenidium*. Feddes Repertorium, 111: 535-552.
- PIMENOV M.G. and LEONOV M.V., 1993 — *The genera of the Umbelliferae: a nomenclator*. Royal Botanic Gardens, Kew, UK.
- PLUNKETT G.M., SOLTIS D.E. and SOLTIS P.S., 1996 — *Evolutionary patterns in Apiaceae: Inferences based on matK sequence data*. Systematic Botany, 21: 477-495.
- PU F.D., 1991 — *A revision of the genus Ligusticum L. (Umbelliferae) in China*. Acta Phytotaxonomica Sinica, 29(5): 385-393.
- PU F.D. and WATSON M.F., 2005 — *Ligusticum L.* In M.L. SHEH, F.D. PU, Z.H. PAN, M.F. WATSON, J.F.M. CANNON, I. HOLMES-SMITH, E.V. KLJUYKOV, L.R. PHILLIPPE and M.G. PIMENOV (Eds.), *Apiaceae*, in *Flora of China Editorial Committee (Ed.), Flora of China*, vol. 14, p. 140-151. Missouri Botanical Garden, St. Louis, Missouri, USA.
- REGEL E. and TILING H., 1858 — *Florula ajanensis*, vol.1, p. 97.
- ROSTOVTSOVA T.S., 1976 — *Chromosome numbers of some species of the family Apiaceae in South Siberia*. Botaničeskij Žurnal, 61(1): 93-99.
- ROSTOVTSOVA T. S., 1979 — *Chromosome numbers of some species of the family Apiaceae*. Lindl. II. Botaničeskij Žurnal, 64 (2): 227-232.
- SILVESTRE S., 1978 — *Contribucion al estudio cariologico de la familia Umbelliferae en la peninsula Iberica*. 11. Lagasclia, 7: 163-172.
- SKALINSKA M., POGAN E. and CZAPIK R., 1978 — *Further studies in chromosome numbers of Polish angiosperms. XII*. Acta Biologica Cracoviensia Series Botanica, 21: 31-63.
- STACE C.A., 2000 — *Cytology and cytogenetics as a fundamental taxonomic resource for the 20th and 21st centuries*. Taxon, 49: 451-477.
- STEBBINS G.L., 1950 — *Variation and evolution in plants*. Columbia University Press, New York.
- STEBBINS G.L., 1971 — *Chromosomal evolution in higher plants*. Edward Arnold Ltd., London.
- TAMMARO F., 1989 — *Re-identification and characterization of Ligusticum cuneifolium Guss. Umbelliferae (gr. L. lucidum Miller), a neglected endemic entity from central Italy*. Annali di Botanica, 47: 215-225.
- UHRKOVA A., 1978 — *In Index of chromosome numbers of Slovakian flora (Part 6)*. Acta Facultatis Rerum Naturalium Universitatis Comenianae, Botanica, 26: 1-42.
- VACHOVA M. and PACLOVA L., 1980 — *In Chromosome number reports LXIX*. Taxon, 29: 721.

- VALIEJO-ROMAN C.M., SHNEYER V.S., SAMIGULLIN T.H., TERENTIEVA E.I. and PIMENOV M.G., 2006 — *An attempt to clarify taxonomic relationships in "Verwandtschaftskreis der Gattung Ligusticum" (Umbelliferae-Apioideae) by molecular analysis*. Plant Systematics and Evolution, 257: 25-43.
- VASILYEVA M.G., ALEXEEVA T.V. and PIMENOV M.G., 1991 — *IOPB chromosome data 3*. International Organization of Plant Biosystematists Newsletter, 17: 10-13.
- VASILYEVA M.G., RETINA T.A. and PIMENOV M.G., 1981 — *Chromosomal figures of some Umbelliferae of the Caucasus and the south of the european part of the USSR*. Biologicheskije Nauki, 207 (3): 60-65.
- WANG P.L., PU F.D. and MA J.S., 1991 — *Pollen morphology of the genus Ligusticum from China and its systematic significance*. Acta Phytotaxonomica Sinica, 29(3): 235-245.
- WETSCHNIG W. and LEUTE G.H., 1991 — *Chromosomenzahlen Kärntner Gefäßflanzen (Teil 2, Doldenblütler-Apiaceae = Umbelliferae)*. Linzer Biologische Beiträge, 23: 457-481.
- YANG Z.Y., GONG X. and PAN Y.Z., 2004 — *Cytological study of six Salvia species (Lamiaceae) from the Hengduanshan Mountains region of China*. Caryologia, 57(4): 360-366.
- ZHANG H.X. and FANG S.M., 1993 — *The origin of Chinese traditional drug "shuigaoben" from Hubei*. Acta Phytotaxonomica Sinica, 31: 281-285.
- ZHANG J.W., SUN H. and NIE Z.L., 2007 — *Karyological studies on the Sino-Himalayan endemic Soroseris and two related genera of tribe Lactuceae (Asteraceae)*. Botanical Journal of the Linnean Society, 154: 79-87.
- ZHANG Y.X., 1994 — *Studies on chromosomes of some plants from Guandi Mountain, Shanxi*. Journal of Wuhan Botanical Research, 12(2): 201-206.
- ZHOU J., PENG H., DOWNIE S.R., LIU Z.W. and GONG X., 2008 — *A molecular phylogeny of Chinese Apiaceae subfamily Apioideae inferred from nuclear ribosomal DNA internal transcribed spacer sequences*. Taxon, 57: 402-416.

Received January 5th 2008; accepted March 5th 2008