

The cytology of *Actinidia*, *Saurauia* and *Clematoclethra* (Actinidiaceae)

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Meiosis and mitosis of six Chinese Actinidiaceae were studied: *Saurauia tristyla* DC., *S. miniata* C. F. Liang & Y. S. Wang, *Actinidia chinensis* Plach., *A. deliciosa* (Cheval.) C. F. Liang & A. R. Ferguson, *A. indochinensis* Merr. and *Clematoclethra lasioclada* Maxim. The chromosome numbers of *Saurauia tristyla* and *S. miniata* were $2n = 6x = 78$, establishing a base chromosome number of $x = 13$ in the genus, differing from the previous report of $x = 15$. The chromosome number of *Clematoclethra* was first reported to be $2n = 4x = 48$ ($x = 12$), while that of *Actinidia* was $x = 29$, consistent with previous reports. The base chromosome number of *Clematoclethra* ($x = 12$) was derived from an aneuploid decrease from *Saurauia* ($x = 13$). *Actinidia* ($x = 29$) was derived from the palaeotetraploid ($x = 14$), which was formed through the increase of the basic chromosome number $x = 13$ to $x = 14$ by aneuploidy and through the breakage of a centromere to add one more new chromosome. The chromosome data in *Actinidia* were consistent with the geographical and morphological evidence for the evolution of the three genera. The tropical American and Asian disjunct distribution pattern and the diversity of base chromosome numbers of *Saurauia* further support the probability that the genus was an early divergent from a common ancestor of *Actinidia* and *Clematoclethra*. © 2005 The Linnean Society of London, *Botanical Journal of the Linnean Society*, 2005, 147, 369–374.

ADDITIONAL KEYWORDS: chromosomal relationships – classification – evolution.

INTRODUCTION

Saurauia Willd., comprising about 250 species, is distributed widely in the tropics and subtropics (Melchior, 1964), with Taiwan and south-western China on the north-east edge of its distribution range, where 13 species are found (Liang, 1984). Its systematic position has long been debated. Some taxonomists have separated it from Actinidiaceae as a distinct family, Sauraiaceae (Hutchinson, 1959; Takhtajan, 1969), whereas others have placed it in Actinidiaceae (van Tieghem, 1899; Gilg & Werderman, 1925; Takhtajan, 1980; Cronquist, 1981; Dahlgren, 1983). The only other genera in Actinidiaceae are *Clematoclethra* Maxim. and *Actinidia* Lind. *Clematoclethra*, endemic to subtropical and temperate regions of central and western China, contains 20 species and four varieties according to Liang (1984), but one species according to Tang & Xiang (1989). *Actinidia*, comprising 66 species, has a wide geographical distribution in China, where 62 species are recognized.

Meiosis was studied in 15 species of *Saurauia* by Soejarto (1969, 1970), who found that 14 species were tetraploid with $n = 30$ chromosomes, and one species was hexaploid, $n = 45$, establishing a basic chromosome number of $x = 15$. Since then, there has been no further chromosome count for *Saurauia*. There are no cytological data for *Clematoclethra* available to date. Although there have been many chromosomal studies on *Actinidia* (Zhang & Beuzenberg, 1983; Xiong, Huang & Wu, 1985; Xiong & Huang, 1988; McNeilage & Considine, 1989; Yan *et al.*, 1997), none has dealt with the evolution of basic chromosome numbers or phylogenetic relationships in Actinidiaceae. Therefore, we considered that comparative cytological studies of *Saurauia*, *Clematoclethra* and *Actinidia* might contribute to knowledge of phylogenetic relationships and systematics in the family.

MATERIAL AND METHODS

The taxa examined are listed in Table 1. The voucher specimens were deposited in the Herbarium of Wuhan Botanical Garden (HIB).

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Table 1. List of taxa studied, with information of locality of collection and voucher specimens

Species	Locality	Voucher
<i>Saurauia</i>		
<i>S. tristyla</i> DC.	Dinghushan Botanical Garden, Guangdong Province	Nian Liu 0001
<i>S. miniata</i> C. F. Liang & Y. S. Wang	Xianhu Botanic Garden, Shenzhen, Guangdong Province	Nian Liu 0002
<i>Clematoclethra</i>		
<i>C. lasioclada</i> Maxim.	Fangxian, Hubei Province	Zien Zhao 10005
<i>Actinidia</i>		
<i>A. chinensis</i> Planch.		
$2n = 4x = 116$ specimen	Yichang, Hubei Province	Zien Zhao 10089
$2n = 2x = 58$ specimen	Henan Province	Zien Zhao 1074
<i>A. deliciosa</i> (Cheval.) C. F. Liang & A. R. Ferguson	Yichang, Hubei Province	Zien Zhao 10125
<i>A. indochinensis</i> Merr.	Kunming Botanical Garden, Yunnan Province	Zien Zhao 10176

For mitotic studies, actively growing shoot tips and root tips were pretreated in 0.1% colchicine and 0.002 mol L⁻¹ 8-hydroxyquinoline mixture for 3 h at 25 °C, and fixed in Carnoy's fluid for 1 h. We used wall degradation and hypotonic treatment to prepare chromosome samples, both for mitotic and for meiotic studies. Samples were fixed in Carnoy's fluid (3 : 1 absolute ethyl alcohol to glacial acetic acid) for 30 min and then placed in refrigerated 70% alcohol for at least 30 min. After washing with distilled water three times, the samples were transferred to a 30 mol L⁻¹ (pH 4.5) citric acid buffer containing a mixed enzyme solution of 1.5% cellulase 'Onozuka' Rs (Yakult Honsha Co. Ltd) and 1.5% pectolyase Y23 (Sigma P3026) at 34 °C for 1 h. The digested tissues were then washed three times in distilled water for a total of 30 min and transferred to fresh Carnoy's fluid for 30 min. Samples were dispersed in 2–3 drops of Carnoy's fluid on a microscope slide. The slide was gently flamed until dry and then stained with Giemsa at pH 7.1 for 30 min. Each slide was washed for several seconds and examined using a Leitz compound microscope.

RESULTS

ACTINIDIA

Actinidia chinensis has somatic chromosome numbers of $2n = 2x = 58$ (Fig. 1) and $2n = 4x = 116$ (Fig. 3). The diploid individuals were collected in Henan Province, and the hexaploid individuals were collected in Hubei Province (Table 1). In the diploid, most of the meiotic configurations were bivalents, $n = 29\text{II}$ (Fig. 2). Dyads did not form after the first stage of meiosis, but normal tetrads formed after the second stage. *A. deliciosa* has $2n = 6x = 174$ (Fig. 4) and *A. indochinensis* has $2n = 2x = 58$ (Fig. 5). All three species examined here thus have the same basic chromosome number, $x = 29$.

CLEMATOCLETHRA

Clematoclethra lasioclada has $2n = 48$ (Fig. 6). This is the first chromosome count for this genus.

SAURAUIA

Saurauia tristyla

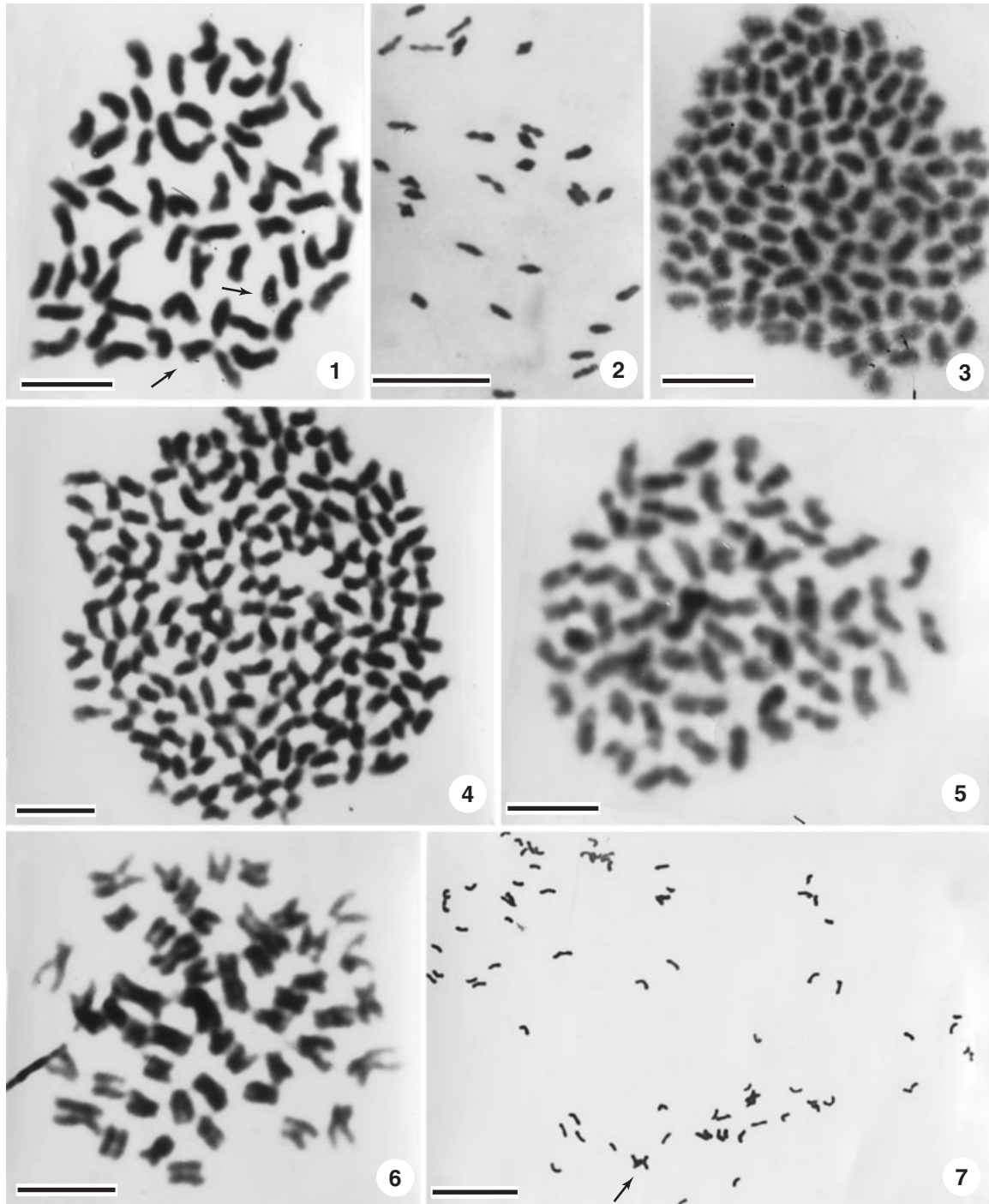
The chromosome number of *Saurauia tristyla* was determined as $2n = 78$ (Fig. 7).

At diakinesis and metaphase of meiosis, there are a few quadrivalents, trivalents and a relatively high frequency of univalents besides bivalents (Fig. 9). Typical chromosome associations observed were $2n = 6\text{III} + 23\text{II} + 14\text{I}$, $2n = 5\text{III} + 23\text{II} + 17\text{I}$ and $2n = 1\text{IV} + 6\text{III} + 21\text{II} + 14\text{I}$, indicating that the species is polyploid. At anaphase I (Figs 10, 11), there is often unequal chromosome segregation, with the separating groups of chromosomes ranging from $n = 34$ to 44, the total number remaining at 78 (Fig. 10). This phenomenon of unequal segregation at anaphase is associated with univalent and multivalent formation. Because univalents tend to have abnormal behaviour and form laggards at anaphase I, unequal segregation gives rise to unbalanced chromosome complements. In Figure 12, an arrow indicates a chromosome bridge at anaphase. After meiosis, many pollen grains developed abnormally (Fig. 13). Based on the cytogenetic data, particularly the chromosome pairing behaviour, *Saurauia tristyla* was found to be a hexaploid with the basic chromosome number $x = 13$, which differs from the previous report of $x = 15$ by Soejarto (1969, 1970).

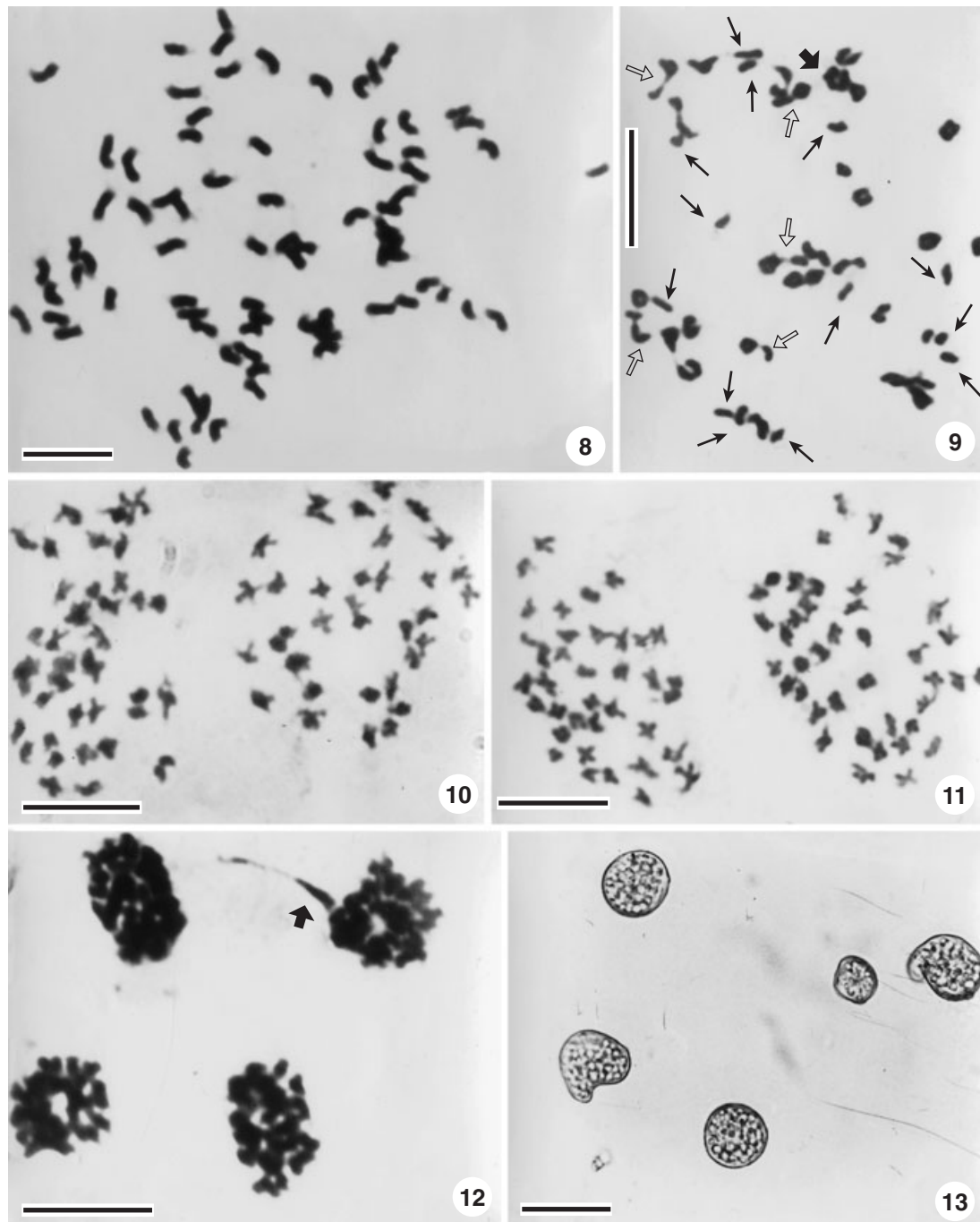
Saurauia miniata

The somatic number of *S. miniata* was also $2n = 78$ (Fig. 8). Thus, *S. miniata* is also a hexaploid species with $x = 13$.

The chromosome data comparison of the three genera can be summarized as follows:



Figures 1–7. Fig. 1. *Actinidia chinensis* somatic chromosomes, $2n = 2x = 58$. Arrows indicate telocentric chromosomes. Scale bar = 5 μm . Fig. 2. *Actinidia chinensis*. PMC at metaphase I, $2n = 29\text{II}$. Scale bar = 10 μm . Fig. 3. *Actinidia chinensis* somatic chromosomes, $2n = 4x = 116$. Scale bar = 5 μm . Fig. 4. *Actinidia deliciosa* somatic chromosomes, $2n = 6x = 174$. Scale bar = 5 μm . Fig. 5. *Actinidia indochinensis* somatic chromosomes, $2n = 2x = 58$. Scale bar = 5 μm . Fig. 6. *Clematiclethra lasioclada* somatic chromosomes, $2n = 2x = 48$. Scale bar = 10 μm . Fig. 7. *Saurauia tristyla* somatic chromosomes, $2n = 6x = 78$. Arrow shows chromosome stickiness. Scale bar = 10 μm .



Figures 8–13. Fig. 8. *Saurauia miniata* somatic chromosomes, $2n = 6x = 78$. Scale bar = 10 μm . Figs 9–12. Meiosis in *Saurauia tristyla*. Fig. 9. Metaphase I, $11\text{V} + 6\text{III} + 21\text{II} + 14\text{I}$. Open arrows show trivalents; thin arrows show univalents; thick arrow shows a quadrivalent. Scale bar = 10 μm . Figs 10, 11. Asymmetrical separation at anaphase I, 43–35 in Fig. 10 and 41–37 in Fig. 11. Scale bars = 10 μm . Fig. 12. Chromosome bridge at anaphase II. Scale bar = 10 μm . Fig. 13. Pollen grains of *Saurauia tristyla*. Scale bar = 50 μm .

1. Their basic chromosome numbers differ; $x = 29$ for *Actinidia*, $x = 12$ for *Clematoclethra* and $x = 13$ for *Saurauia*.
2. There are interspecific and intraspecific polyploid series ranging from $2x$ to $8x$ in *Actinidia* (Zhang & Beuzenberg, 1983; Xiong *et al.*, 1985; Xiong & Huang, 1988; McNeilage & Considine, 1989; Yan *et al.*, 1997). It was suggested that *Actinidia* might be a palaeopolyploid derivation, its $x = 29$ being derived from $x = 14$. *Clematoclethra*, with $n = 24$, might also be of palaeopolyploid derivation. So far, most of the species of *Saurauia* have been reported as tetraploid with $2n = 60$, only one being hexaploid (Soejarto, 1969, 1970). Reported here, both species native to China are hexaploid. No diploid species has been found to date. The above data show that polyploidization has played an important role in the evolution of the three genera.

DISCUSSION

The phylogenetic relationship of *Actinidia*, *Clematoclethra* and *Saurauia* has long been under debate. Airy-Shaw (1936) suggested that *Clematoclethra* originated from the temperate species of *Saurauia*. The pollen morphology of *Actinidia* and *Clematoclethra* was found to be very similar, but different from that of *Saurauia* (Zhang, 1987). Furthermore, considering the morphological characteristics and distribution pattern of the three genera, Tang & Xiang (1989) proposed that *Actinidia* and *Clematoclethra* might be a sister group, while *Saurauia* was their sister group. Wang (1992) further suggested that the three genera might have been derived from a common ancestor distributed in the Yunnan–Guizhou plateau of China. Li, Cai & Huang (2000) reported that *Saurauia* could be monoecious or functionally dioecious, intermediate between *Actinidia* and *Clematoclethra*, which are monoecious and dioecious, respectively. *Saurauia* and *Clematoclethra* were always assumed to be an outgroup in the molecular systematics studies of *Actinidia* in recent years (Testolin, Huang & Cipriani, 1999; Li, Huang & Sang, 2002). The micromorphological characters of foliar trichomes have been observed in *Actinidia* and it has been suggested that those of *Actinidia* were more specialized (He *et al.*, 2000).

As *Actinidia* has a high basic chromosome number, $x = 29$, it was generally inferred to be a re-diploidized palaeopolyploid genus (Stebbins, 1971; Grant, 1975; Raven, 1975; Goldblatt, 1980; Yan *et al.*, 1997). Based on the basic number of $x = 15$ in *Saurauia* (Soejarto, 1970), McNeilage & Considine (1989) suggested that if the genus *Actinidia* has developed at a stabilized tetraploid level from ancestors with $x = 15$ and/or $x = 14$, then at least one aneuploid event must have preceded or followed the original tetraploidization. The study of

microsatellites (or SSR, simple sequence repeats) also supported that diploid *Actinidia* was of polyploid derivation from taxa with the basic chromosome number $x = 14$ or 15 (Testolin *et al.*, 1999).

The karyotype of diploid *Actinidia chinensis* is $2n = 2x = 58 = 38m + 18sm(2SAT) + 2t$. The 8th and 9th, 11th and 12th pairs of the chromosome are similar in length, Lt/St value and morphology (He, Huang & Zhong, 2003). It was concluded that diploid *Actinidia chinensis* was probably a palaeotetraploid (He *et al.*, 2003). In addition, it was observed that *Actinidia chinensis* has two telocentric chromosomes, an obvious secondary structure character, formed by centromere fission of a metacentric chromosome. We therefore speculate that the progenitor of *Actinidia*, a palaeotetraploid, may have had a basic number $x = 14$. With chromosome number doubling, followed by genomic differentiation, diploidization and a single chromosome fission, the current basic number of $x = 29$ has arisen.

We also found $x = 12$ in *Clematoclethra* and $x = 13$ in *Saurauia*, different from the previous report of $x = 15$ by Soejarto (1969, 1970), so the evolution trend of the three genera is accompanied by basic chromosome number change.

Saurauia is distributed mainly in tropical America and Asia, with only one species in tropical China. As it was dispersed to the subtropics, it differentiated and gave rise to the genera *Clematoclethra* and *Actinidia*.

This first chromosome count of the genus *Clematoclethra* suggests that *C. lasioclada* might be an ancient polyploid derivative of *Actinidia*. An *Actinidia* species with a basic chromosome number of $x = 12$ that served as the diploid progenitor might have become extinct. From its basic chromosome number of $x = 12$, we propose that *Clematoclethra* was derived from an aneuploid decrease from the $x = 13$ of an extinct diploid *Saurauia*, and the *Actinidia* with $x = 29$ was derived from a palaeotetraploid of $x = 14$, which was also derived from an aneuploid increase from $x = 13$ in an extinct diploid *Saurauia*. The doubling was to $n = 2x = 28$, subsequently giving rise to one more chromosome by centromere fission. This hypothesis is in agreement with the opinion that both *Actinidia* and *Clematoclethra* were derived from a common recent ancestor (Tang & Xiang, 1989).

Another genus, *Sladenia* Kurz is closely related to Actinidiaceae and has been reported recently to have $2n = 48$ with no telocentric chromosomes. This might also be of palaeotetraploid derivation (Li, Liang & Peng, 2003). The above data suggest that all of the three genera in Actinidiaceae are palaeopolyploids, the diploid progenitors of which have probably become extinct. The tropical Asian and American disjunct distribution of *Saurauia* and the variation in its base chromosome numbers ($x = 13, 15$) further imply that

Saurauia diverged from the common ancestor of *Actinidia* and *Clematoclethra*.

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