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Cytological studies on the eastern Asian family Trochodendraceae

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The somatic chromosome numbers for *Trochodendron* and *Tetracentron* were determined as $2n = 38$ from shoot tip cells, from cultivated plants introduced from three places in Japan and southwest China. This number is consistent with one of the only two previous studies on the two genera and adds support for their membership of a distinct family Trochodendraceae. *Botanical Journal of the Linnean Society*, 2008, **158**, 332–335.

ADDITIONAL KEYWORDS: chromosome numbers – *Tetracentron* – *Trochodendron*.

INTRODUCTION

Trochodendraceae and Tetracentraceae were described as two monotypic families endemic to Eastern Asia (Wu & Wu, 1996), but are now generally treated as one family Trochodendraceae (e.g. APG, 1998; APG II, 2003) . *Trochodendron aralioides* P. F. Siebold & J. G. Zuccarini is distributed in Taiwan, South Japan, the Ryukyu Islands and South Korea (Mabberley, 1987; Fu & Endress, 2001). *Tetracentron sinense* Oliv. (previously Tetracentraceae) is found in mainland China (Gansu, Shaanxi, Tibet, Yunnan, Sichuan, Guizhou, Henan, Hubei and Hunan), Bhutan, northeast India, northwest Myanmar, Nepal and Vietnam (Doweld, 1998; Fu & Bartholomew, 2001; Qin, 2004).

The position of *Trochodendron* and *Tetracentron* is pivotal in angiosperm phylogeny and many different types of research have been conducted on them including phylogeography (Huang *et al.*, 2004; Huang & Lin, 2006), chemical components (Wu *et al.*, 2000), anatomy (Wu, Lin & Li, 1993; Chen *et al.*, 2007), embryology (Pan *et al.*, 1993) and allozymes (Wu *et al.*, 2001), but their systematic position is still disputed. As two independent families, the Tetracentraceae and Trochodendraceae were placed between the magnoliids and the hamamelids, although the two genera *Trochodendron* and *Tetracentron* were considered to be closely related (Chen *et al.*, 2007).

Because chromosome cytology is important evidence of relationships among angiosperms, the cytology of *Trochodendron* and *Tetracentron* has been emphasized in some studies (Whitaker, 1933; Raven, 1975), but there are only three conflicting records of chromosome numbers so far: $2n = 38$ for both *Trochodendron* and *Tetracentron* (Whitaker, 1933), $2n = 40$ for *Trochodendron* and $2n = 48$ for *Tetracentron* (Ratter & Milne, 1973, 1976). Characters such as idioblast oil cells, vessel less wood, chloranthoid-tooth leaves and chromosome number $2n = 38$ have been used to suggest that they are close to Magnoliaceae and *Cercidiphyllum* (with $x = 19$) (Whitaker, 1933; Smith, 1945; Carlquist, 1983; Endress, 1986; Carlquist, 1992; Takhtajan, 1997). Alternatively, their common characters such as valvate–dehiscent anthers, tri- or pluri-aperturate pollen, edged and winged seeds and chromosome number $2n = \pm 48$ for *Tetracentron* (Ratter & Milne, 1973, 1976), implied that they are related to Hamamelidaceae (with

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Table 1. Collection data and specimens deposited

Species	Original locality	Voucher
Tetracentron sinense	China, Yunnan, JinPin	XY2466 (KUN)
	China, Yunnan, Gongshan	XY2467 (KUN)
Trochodendron aralioides	Japan	XY2468 (KUN)

$x = 12$), in spite of the record of $2n = 40$ in *Trochodendron* (Ratter & Milne, 1976; Thorne, 1983). Inconsistent chromosome records have suggested different systematic positions for the two genera (Whitaker, 1933; Cronquist, 1981; Crane, 1984; Endress, 1986; Crane, 1989). With more data and analyses being presented, especially of flower structure and fossil evidence, *Trochodendron* and *Tetracentron* were generally regarded as being in the same family Trochodendraceae (Crane, 1984; Endress, 1986; Crane, 1989; Endress, 1993; Soltis *et al.*, 2005). The position of the Trochodendraceae, together with Buxaceae, Proteales, Sabiaceae and Ranunculales, as the basal group of core eudicotyledons was supported by molecular analysis in recent research (Chase *et al.*, 1993; APG; Doyle & Endress, 2000; Savolainen *et al.*, 2000; APG II, 2003.

Because the published chromosome records are contradictory, we intended to confirm the cytological data for *Trochodendron* and *Tetracentron* in this study, and to discuss the relationships of the two genera and their affinities within the angiosperm phylogenetic tree based on our result.

MATERIAL AND METHODS

The experimental material of *T. aralioides* and *T. sinense* from three populations is listed in Table 1. The plants were all grown in the Botanic Garden of the Kunming Institute of Botany, Chinese Academy of Sciences. Voucher specimens were deposited in KUN.

Shoot tips were pretreated in 0.002 M 8-hydroxyquinoline for 6–8 h in total darkness at 4 °C and then fixed in Carnoy's fluid (3 parts absolute ethanol : 1 part glacial acetic acid, v/v) for 1 h at room temperature 23 °C. The shoot tips were macerated in 1M hydrochloric acid at 60 °C for 5–6 min. After washing 3–5 times to eliminate residual hydrochloric acid and staining with carbol fuschin for 8 h, the material was squashed for observation in 45% acetic acid, sometimes after softening over an alcohol flame for 3–5 s (Li & Zhang, 1991). More than 30 chromosome micrographs were observed in each of the three accessions. Semi-permanent microscope slides and photographs of representative cells have been retained in our laboratory for inspection.

RESULTS

The interphase nuclei of *T. sinense* (Fig. 1) and *T. aralioides* (Fig. 3) were of the complex chromosome type (Type C) of Tanaka (1977). The chromosome number $2n = 38$ was demonstrated in both accessions of *T. sinense* (Fig. 2) and in *T. aralioides* (Fig. 4).

DISCUSSION

The finding of the same chromosome number ($2n = 38$) in all accessions of the two examined species agreed with the result of Whitaker (1933) rather than that of Ratter & Milne (1973, 1976).

The uniform chromosome number in *Tetracentron* and *Trochodendron* adds support for their retention in one family, Trochodendraceae, and the abolition of Tetracentraceae, a conclusion supported by studies of pollen (Praglowski, 1974), fossils (Crane, 1984, 1989), floral structure (Endress, 1986, 1993), carpology and seed structure (Doweld, 1998) and molecular analysis (Chase *et al.*, 1993; Angiosperm Phylogeny Group (APG) II, 2003; Soltis *et al.*, 2005), although the reports of $2n = 48$ in *Tetracentron* and $2n = 40$ for *Trochodendron* by Ratter & Milne (1973, 1976) undermine this judgment.

Over the years, *Euptelea* (with $2n = 28$) and *Cercidiphyllum* (with $x = 19$) have been regarded as having close affinity with *Tetracentron* and *Trochodendron*, especially *Cercidiphyllum*, but this relationship was not supported by molecular data and they have been treated as two families, Eupteleaceae and Cercidiphyllaceae in Ranunculales and Saxifragales, respectively (APG II, 2003). Trochodendraceae, comprising *Trochodendron* and *Tetracentron*, together with Sabiaceae, Buxaceae, Proteales and Ranunculales are early-branching members of the core eudicotyledons. However, their chromosome numbers, $x = 12$ and 14 for Buxaceae, 12 and 16 for Sabiaceae (IPCN, 2006), and different numbers for Proteales ($x = 21$ for Platanaceae; 7, 10, 11, 12, 13 and 14 for Proteaceae) (IPCN, 2006) and Ranunculales ($x = 7$, 8 for Ranunculaceae; 6, 8, 9, 14 and 21 for Berberidaceae; 15 for Circaeasteraceae; 14, 15 and 16 for Lardizabalaceae; 6 and 7 for Papaveraceae) (Yang, 2002; IPCN, 2006; Raven, 1975), do not demonstrate any evolutionary trends in the chromosomes of these taxa.

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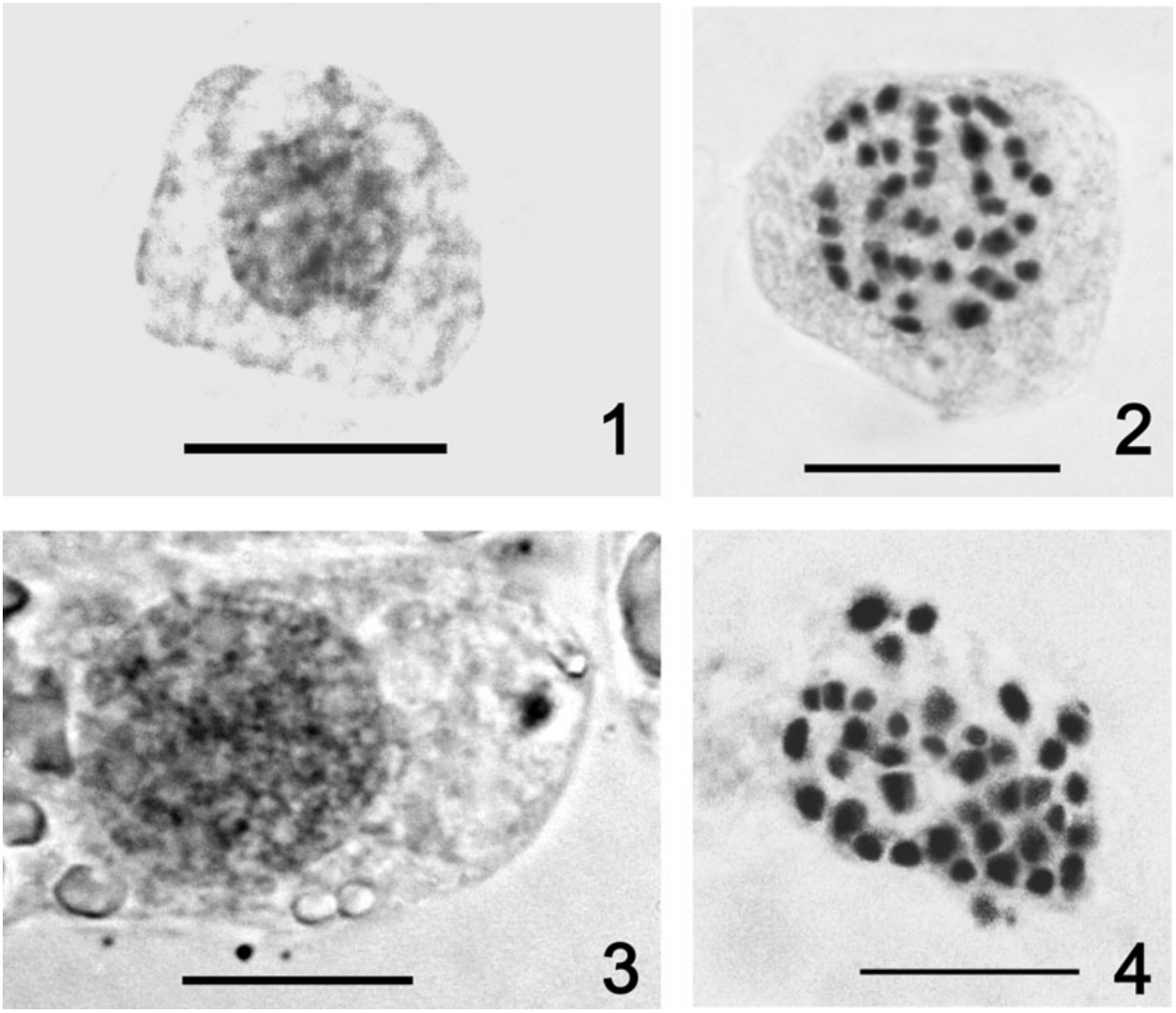

Figures 1–4. Mitotic phases in somatic cells of *Trochodendron aralioides* and *Tetracentron sinense*. Fig. 1. Interphase of *Tetracentron sinense* (Type C). Fig. 2. Metaphase of *Tetracentron sinense* $2n = 38$. Fig. 3. Interphase of *Trochodendron aralioides* (Type C). Fig. 4. Metaphase of *Trochodendron aralioides* $2n = 38$. Scale bars, 10 μm.

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