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Phylogenetic Evaluation of Series Delimitations in Section Palmata (Acer, Aceroideae, Sapindaceae) Based on Sequences of Nuclear and Chloroplast Genes

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PHYLOGENETIC EVALUATION OF SERIES DELIMITATIONS IN SECTION *PALMATA* (*ACER*, ACEROIDEAE, SAPINDACEAE) BASED ON SEQUENCES OF NUCLEAR AND CHLOROPLAST GENES

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ABSTRACT

Acer section *Palmata* (Japanese maples) is the largest section within the genus; however, series delimitations within section *Palmata* have not been evaluated in a phylogenetic context. Both maximum parsimony and maximum likelihood analyses of DNA sequence data of nuclear rDNA ITS and chloroplast genes (*ndh*F, *trn*L–*trn*F, and *psb*A–*trn*H) from 23 species of *Acer* section *Palmata* show that traditional series do not form individual clades. Results from this study support the most recent taxonomic treatment of *Acer* that does not recognize any series in section *Palmata*. Nuclear and chloroplast phylogenies are significantly incongruent, indicating that hybridization and introgression may be an important factor in the evolutionary history of section *Palmata*.

Key words: *Acer*, ITS, *ndh*F, nrDNA, *psb*A–*trn*H, section *Palmata*, series, *trn*L–*trn*F.

INTRODUCTION

Acer L. (the maple genus) is one of the most diverse tree genera in the Northern Hemisphere with approximately 129 species (Xu et al. 2008). The genus has been divided into sections with some sections being further divided into series (Pax 1902; Pojarkova 1933; Momotani 1962; Fang 1966; Ogata 1967; Xu 1996; de Jong 2004). Recent molecular phylogenetic studies of *Acer* recognize 16–22 sections including section *Palmata* (Ackerly and Donoghue 1998; Hasebe et al. 1998; Suh et al. 2000; Pfosser et al. 2002; Tian et al. 2002; Grimm et al. 2006; Li et al. 2006; Renner et al. 2007, 2008). As currently circumscribed (de Jong 2004; Xu et al. 2008), section *Palmata* includes species from traditional sections *Integrifolia*, *Microcarpa*, and *Palmata* (Pojarkova 1933; Ogata 1967; Xu 1996; Table 1). Within section *Palmata*, Ogata (1967) recognized the three series *Sinensia*, *Laevigata* (=*Penninervia* Metcalf), and *Palmata* while pointing out that there are morphological gradations between series *Sinensia* and *Palmata*. De Jong (2004) combined series *Sinensia* and *Palmata*, recognizing two series (*Palmata* and *Penninervia*). However, the most recent taxonomic treatment of *Acer* did not recognize any series in section *Palmata* (Xu et al. 2008).

Section *Palmata* is the largest clade within *Acer* with approximately 35 species (Xu et al. 2008). Previous phylogenetic analyses, however, have included only 5–13 species (Ackerly and Donoghue 1998; Hasebe et al. 1998; Suh et al. 2000; Pfosser et al. 2002; Tian et al. 2002; Grimm et al. 2006; Li et al. 2006; Renner et al. 2007, 2008). Therefore, the objective of this study was to evaluate series delimitations of section *Palmata* by increasing species sampling and using DNA sequence data from both nuclear and chloroplast genomes.

MATERIALS AND METHODS

Twenty-six taxa were included in the study representing 23 species of section *Palmata* and three outgroup taxa (*Aesculus glabra*, *Dipteronia dyerana*, and *D. sinensis*). Species from other sections of *Acer* were not used as outgroups because section *Palmata* is monophyletic and because it is unclear which section(s) is most closely related to section *Palmata* (Renner et al. 2008).

Procedures for DNA extraction and sequence editing have been described elsewhere (Li et al. 2006). Polymerase chain reactions (PCR) were conducted to amplify the DNA regions using primers "ITS4" (White et al. 1990) and "ITSLeu" (Baum et al. 1998) for the nrDNA ITS region, primers "c" and "e" of Taberlet et al. (1991) for the *trn*L intron and *trn*L–*trn*F intergenic spacer, primers of Davis et al. (2002) for the 3′-end of the *ndh*F gene, and of Kress et al. (2005) for the *psb*A–*trn*H intergenic spacer. PCR products were sequenced directly using a BigDye Fluorescent Chemistry Kit and automated Genetic Analyzers 3130 or 3730 (Applied Biosystems, Foster City, CA).

Sequences were aligned using MUSCLE (Edgar 2004), and the alignments were manually adjusted in MacClade (Maddison and Maddison 2000). Both maximum parsimony (MP) and maximum likelihood (ML) analyses were performed to reconstruct phylogenetic trees. MP analyses as implemented in PAUP* (Swofford 2002) were conducted using the heuristic search with the following options: simple sequence addition, tree bisection reconnection (tbr) branch swapping, maximum trees set to 10,000, steepest descent off, and Multrees on. Maximum likelihood analysis was performed with default settings in GARLI (Genetic Algorithm for Rapid Likelihood Inference) vers. 0.96 (Zwickl 2006) and the GTR + G + I model, as selected by MODELTEST with the Akaike information criterion (Posada and Crandall 1998). Nonparametric bootstrapping analyses (Felsenstein 1985) were used to estimate relative support for individual clades, and the tree search options were as in the MP and ML analyses. The numbers of replicates were 1000 for MP and 100 for ML. Both ILD (Incongruence Length Difference; Farris et al. 1995) and SH (Shimodaira and Hasegawa 1999) tests, as implemented in PAUP*, were conducted to test whether data sets were significantly incongruent.

RESULTS

Nuclear rDNA ITS Data Set

The amount of sequence divergence between section *Palmata* and outgroups is 7.0–17.5%, while within section

Table 1. Species used in this study, their series placements by different authors, and GenBank sequence accession numbers listed in the order of nrDNA ITS, *ndh*F, *trn*L–F, and *psb*A–*trn*H. Sequences obtained from GenBank are underlined. Sectional placement is indicated only where different from sect. *Palmata*. Dashes represent taxa that were not treated by the authors.

Species	Voucher specimen and source	Pojarkova (1933)	Momotani (1962)	Ogata (1967)	De Jong (1994)	Xu (1996)	GenBank accession numbers
A. calcaratum Gagnep.	*De Jong, P. s.n.* (HCHM); China	—	Ser. *Sinensia*	—	Ser. *Sinensia*	—	HM352651, HM352667, HM352690, HM352713
A. campbellii Hook.f. & Thomson ex Hiern	*De Jong, P. s.n.* (HCHM); China	Ser. *Sinensia*, sect. *Microcarpa*	Ser. *Sinensia*	Ser. *Sinensia*	Ser. *Sinensia*	Ser. *Microcarpa*, sect. *Microcarpa*	HM352652, HM352668, HM352691, HM352714
A. circinatum Pursh	*Li, J. 5663*, *3510* (HCHM); Arnold Arboretum 724-72A (A); Canada	Ser. *Palmata*	Ser. *Palmata*	Ser. *Palmata*	Ser. *Palmata*	Ser. *Palmata*	HM352653, HM352669 (3510), HM352692, HM352713 (A)
A. cordatum Pax	*De Jong, P. s.n.* (HCHM); China	Ser. *Oblonga*, sect. *Integrifolia*	Ser. *Integrifolia*, sect. *Integrifolia*	Ser. *Laevigata*	Ser. *Penninervia*	Ser. *Trinervia*, sect. *Integrifolia*	HM352654, HM352670, HM352693, —
A. erianthum Schwer.	Cornell University Plantation 87-490*B (BH); Hubei, China	Ser. *Sinensia*, sect. *Microcarpa*	Ser. *Sinensia*	Ser. *Sinensia*	Ser. *Sinensia*	Ser. *Microcarpa*, sect. *Microcarpa*	DQ238392, HM352671, HM352694, HM352716
A. erythranthum Gagnep.	*WWJ 1698; PW 150* (UBC); Vietnam	—	—	—	Ser. *Penninervia*	—	HM352655 (*WWJ 1698*) and HM352656 (*PW 150*), HM352672 (*WWJ 1698*) and HM352673 (*PW 150*), HM352695 (*WWJ 1698*) and HM352696 (*PW 150*), HM352717 (*WWJ 1698*) and HM352718 (*PW 150*)
A. fabri Hance	*Yi, Jiqin s.n.* (HCHM); Hunan, China	Ser. *Oblonga*, sect. *Integrifolia*	Ser. *Integrifolia*, sect. *Integrifolia*	Ser. *Laevigata*	Ser. *Penninervia*	Ser. *Penninervia*, sect. *Integrifolia*	AOU89910, HM352674, HM352697, HM352719
A. flabellatum Rehder	*De Jong, P. s.n.* (HCHM); China	—	Ser. *Sinensia*	Ser. *Sinensia*	Ser. *Sinensia*	Ser. *Microcarpa*, sect. *Microcarpa*	HM352657, HM352675, HM352698, HM352720
A. heptaphlebium Gagnep.	*WWJ 11716* (UBC); Vietnam	—	—	—	Ser. *Sinensia*	—	HM352658, HM352676, HM352699, HM352721
A. japonicum Thunb.	*Bill McNamara 112* (HCHM); Japan	Ser. *Palmata*	Ser. *Palmata*	Ser. *Palmata*	Ser. *Palmata*	Ser. *Palmata*	HM352659, HM352677, HM352700, DQ978602
A. laevigatum Wall.	—	Ser. *Oblonga*, sect. *Integrifolia*	Ser. *Integrifolia*, sect. *Integrifolia*	Ser. *Laevigata*	Ser. *Penninervia*	Ser. *Penninervia*, sect. *Integrifolia*	DQ238398, —, DQ978535, DQ978603
A. linganense W.P.Fang & P.L.Chiu	*Li, J. 4941* (HCHM); Zhejiang, China	—	—	—	Ser. *Sinensia*	Ser. *Palmata*	HM352660, HM352678, HM352701, HM352722
A. palmatum Thunb.	Arnold Arboretum 585-88B (A); Korea	Ser. *Palmata*	Ser. *Palmata*	Ser. *Palmata*	Ser. *Palmata*	Ser. *Palmata*	AY605426, HM352679, HM352702, HM352723
A. pauciflorum W.P.Fang	*De Jong, P. s.n.* (HCHM); Japan	—	Ser. *Palmata*	Ser. *Palmata*	Ser. *Palmata*	Ser. *Palmata*	HM352661, HM352680, HM352703, HM352724
A. pseudosieboldianum (Pax) Kom.	Arnold Arboretum 249-82A (A); China	Ser. *Palmata*	Ser. *Palmata*	Ser. *Palmata*	Ser. *Palmata*	Ser. *Palmata*	DQ238408, HM352681, HM352704, DQ978622

Table 1. Continued.

Species	Voucher specimen and source	Pojarkova (1933)	Momotani (1962)	Ogata (1967)	De Jong (1994)	Xu (1996)	GenBank accession numbers
A. robustum Pax	*De Jong, P. s.n.* (HCHM); China	Ser. *Sinensia*, sect. *Microcarpa*	Ser. *Palmata*	Ser. *Palmata*	Ser. *Palmata*	Ser. *Palmata*	HM352662, HM352682, —, HM352725
A. shirasawanum Koidz.	*Bill McNamara 062* (HCHM); Japan	Ser. *Palmata*	Ser. *Palmata*	Ser. *Palmata*	Ser. *Palmata*	Ser. *Palmata*	AY605428, HM352683, HM352705, HM352726
A. sieboldianum Miq.	*Bill McNamara 092* (HCHM); Japan	Ser. *Palmata*	Ser. *Palmata*	Ser. *Palmata*	Ser. *Palmata*	Ser. *Palmata*	AF020377, HM352684, HM352706, DQ978631
A. sinense Pax	*De Jong, P. s.n.* (HCHM); China	Ser. *Sinensia*, sect. *Microcarpa*	Ser. *Sinensia*	Ser. *Sinensia*	Ser. *Sinensia*	Ser. *Microcarpa*, sect. *Microcarpa*	HM352663, —, HM352707, DQ978586
A. takesimense Nakai	Arnold Arboretum 581-87B (A); Japan	—	Ser. *Palmata*	Ser. *Palmata*	Ser. *Palmata*	Ser. *Palmata*	ATU57777, HM352685, HM352708, HM352727
A. tonkinense Lecomte	*DJHV 6173* (UBC); Vietnam	—	—	—	Ser. *Sinensia*	Ser. *Tonkinensia*, sect. *Microcarpa*	HM352664, HM352686, HM352709, HM352728
A. wilsonii Rehder	South China Bot. Gard., s.n. (SCBG); China	Ser. *Sinensia*, sect. *Microcarpa*	—	Ser. *Sinensia*	Ser. *Sinensia*	Ser. *Microcarpa*, sect. *Microcarpa*	HM352665, HM352687, HM352710, HM352729
A. wuyuanense W.P.Fang & Y.T.Wu	Univ. of British Columbia 027764-0565-1989 (UBC); China	—	—	—	Ser. *Sinensia*	Ser. *Microcarpa*, sect. *Microcarpa*	HM352666, HM352688, HM352711, HM352730
Aesculus glabra Willd.	Arnold Arboretum 1221-79A (A); USA	n/a	n/a	n/a	n/a	n/a	AF406968, HM008542, AF411085, HM008566
Dipteronia dyerana A.Henry	*Li, J. 3363* (KUN); Yunnan, China	n/a	n/a	n/a	n/a	n/a	AF401120, HM008543, DQ978576, AM182900
Dipteronia sinensis Oliv.	*Li, J. 3086* (HCHM); Shaanxi, China	n/a	n/a	n/a	n/a	n/a	AF401121, HM008544, HM352689, HM352712

Palmata the sequences show much less variation (0–5.6%). MP analysis of the nrDNA ITS data set generated 3320 trees of 282 steps (CI = 0.81, RI = 0.64; Fig. 1). ML analysis produced a single tree with a −ln likelihood of 2401.65 (tree not shown). MP and ML trees are nearly identical. *Acer erianthum*, *A. campbellii*, and *A. flabellatum* diverge early, with *A. campbellii* sister to *A. flabellatum* (bs = 98% in MP and 81% in ML). The remaining species in section *Palmata* form a weakly supported clade (bs = 61% in the MP tree and 52% in the ML tree). Another clade, albeit weakly supported (bs = 63–66%), contains *A. calcaratum*, *A. erythranthum*, *A. fabri*, *A. heptaphlebium*, *A. linganense*, *A. sinense*, *A. wilsonii*, *A. tonkinense*, and *A. wuyuanense*.

Chloroplast Data Set

Both pairwise and three-way ILD tests indicate that sequences of the chloroplast genes are not significantly incongruent ($P = 0.1$). Therefore, they were concatenated to generate a combined data set comprising 2705 sites, 60 of which are parsimony informative. MP analysis of the combined data set produced 11,264 trees of 347 steps (CI = 0.87, RI = 0.79; Fig. 2). There are three clades with bootstrap support. *Acer cordatum* and *A. linganense* form a well-supported clade (bs = 99%), while *A. campbellii*, *A. erythranthum*, *A. flabellatum*, and *A. tonkinense* form a weak clade (bs = 52%). *Acer calcaratum*, *A. fabri*, *A. heptaphlebium*, *A. pauciflorum*, *A. wilsonii*, and *A. wuyuanense* are grouped together in a clade (bs = 81%), within which *A. calcaratum*, *A. fabri*, and *A. heptaphlebium* form a clade (bs = 68%), and so do *A. pauciflorum* and *A. wuyuanense* (bs = 85%). ML analysis generated one tree (−ln likelihood = 5776.51; tree not shown), which is nearly identical to that in Fig. 2.

Incongruence Between Nuclear and Chloroplast Genes

The ILD test suggests that nuclear and chloroplast genes are significantly incongruent ($P = 0.01$), and the SH tests also indicate that nuclear and chloroplast trees differ significantly ($P < 0.001$). Therefore, phylogenetic analyses were not performed based on the combined data set of nuclear and chloroplast genes.

DISCUSSION

Prior to Ogata (1967), species of section *Palmata* had been placed in three different sections, *Microcarpa*, *Palmata*, and *Integrifolia*, based on leaf habit and inflorescence types (Pax 1902; Pojarkova 1933; Momotani 1962). Evergreen species with simple, unlobed leaves have been grouped in section *Integrifolia* (Pax 1902; Pojarkova 1933; Momotani 1962), while those with palmate leaves and corymbose inflorescences were placed in section *Palmata* (Pojarkova 1933). Ogata (1967) redefined section *Palmata* using the following characters: (1) 4-paired bud scales; (2) differentiated sepals and petals; and (3) incumbent and horizontally circinate cotyledons. His taxonomic treatment has been adopted by some authors (de Jong 1994) and received support from recent molecular analyses (Hasebe et al. 1998; Suh et al. 2000; Pfosser et al. 2002; Grimm et al. 2006; Li et al. 2006; Renner et al. 2007, 2008).

Within section *Palmata*, Ogata (1967) recognized three series: *Sinensia*, *Palmata*, and *Laevigata*. Species of series *Sinensia* possess elongated panicles or spike-like panicles with 1–3 pairs of leaves, 3–7-lobed and serrate leaves, and scaly leaves. Series *Palmata* differs from *Sinensia* in several features—terminal buds generally wanting, leaves 3–13-lobed and serrate, corymbose inflorescence with one pair of leaves, and scaly leaves rarely present. Nevertheless, there are species with intermediate forms between series *Sinensia* and *Palmata* in central and southern China, as pointed out by Ogata (1967). Recently, de Jong (2004) treated them as one series (*Palmata*). In both ITS (Fig. 1) and chloroplast trees (Fig. 2), species of series *Sinensia* are scattered throughout the trees, some being sister to species from series *Palmata* as well as *Laevigata*. Therefore, molecular data from this study support the merger of *Sinensia* and *Palmata* (de Jong 2004). Series *Laevigata* Ogata includes species with an inflorescence similar to that in series *Palmata* but shares the presence of scaly leaves with series *Sinensia*. However, its species are unique in having simple, entire or slightly serrate, and persistent leaves (Ogata 1967; de Jong 2004). Series *Laevigata* does not form a clade in either nuclear or chloroplast phylogenetic trees (Fig. 1, 2). Therefore, none of the three traditional series in section *Palmata* (Ogata 1967; de Jong 1994) forms individual clades. Molecular data from this study support the treatment by Xu et al. (2008) that does not recognize any series within section *Palmata*. This treatment is also consistent with the conclusion based on a survey of flavonoids in Aceraceae (Delendick 1990).

Nuclear and chloroplast data are significantly incongruent as indicated by ILD tests. Although few clades are strongly supported due to low levels of sequence variation in both nuclear and chloroplast markers, conflicting relationships between nuclear and chloroplast trees (Fig. 1, 2) are significant statistically as shown by SH tests. For example, *A. cordatum* is most closely related to *A. linganense* in the chloroplast tree (Fig. 2), whereas in the ITS tree (Fig. 1) *A. linganense* forms a weakly supported clade with *A. calcaratum*, *A. heptaphlebium*, *A. sinense*, *A. wilsonii*, and *A. wuyuanense*. In the ITS tree, *A. pauciflorum* is closely allied with *A. shirasawanum* (bs = 58%), but in the chloroplast trees it is embedded in the clade containing *A. calcaratum*, *A. fabri*, *A. heptaphlebium*, *A. wilsonii*, and *A. wuyuanense* (bs = 80–81%). Moreover, the two samples of *A. erythranthum*—though part of the same clade in the ITS tree (bs = 63–66%)—do not share a clade in the chloroplast tree, one of them forming a weakly supported clade (bs = 52%) with *A. campbellii*, *A. flabellatum* and *A. tonkinense*.

Because chloroplast genes are maternally inherited in *Acer* (Corriveau and Coleman 1988), while nuclear genes are biparental, and given that hybridization between species of *Acer* is frequent (de Jong 1994), it is probable that hybridization and introgression has occurred in the evolution of section *Palmata*. However, random lineage sorting cannot be ruled out as another explanation for the phylogenetic incongruence between nuclear and chloroplast genomes. Therefore, it is desirable in the future to use model-based analytical tools to tease apart hybridization, lineage sorting, and paralogy with additional data from multiple samples of individual species, and nuclear and chloroplast markers with higher levels of sequence variation.

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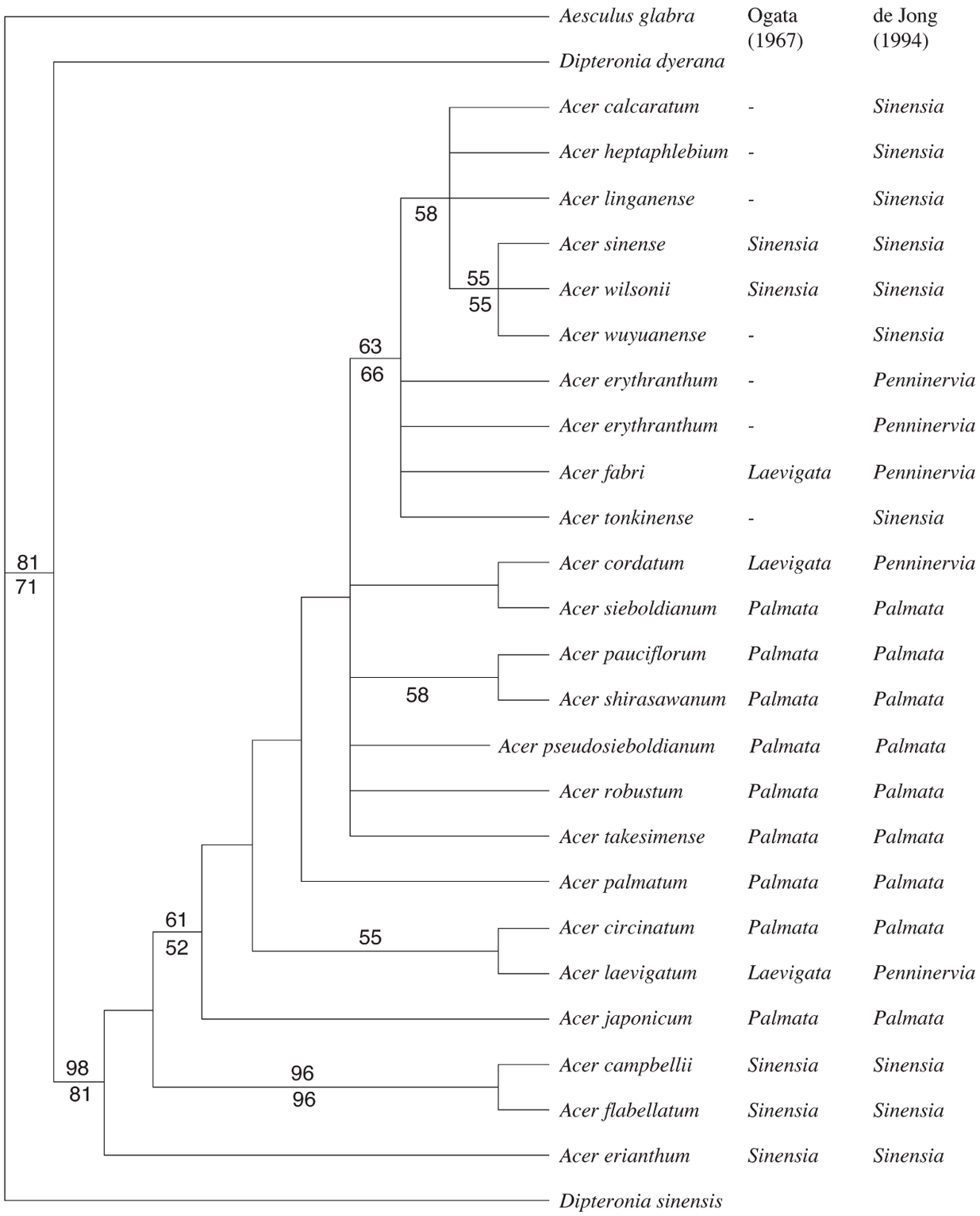

Fig. 1. Strict consensus of 3320 most parsimonious trees based on sequences of the nuclear ribosomal DNA ITS region (CI = 0.81, RI = 0.64). Numbers above and below branches represent bootstrap percentages for MP and ML analyses, resp. On the right are classification systems of Ogata (1967) and de Jong (1994).

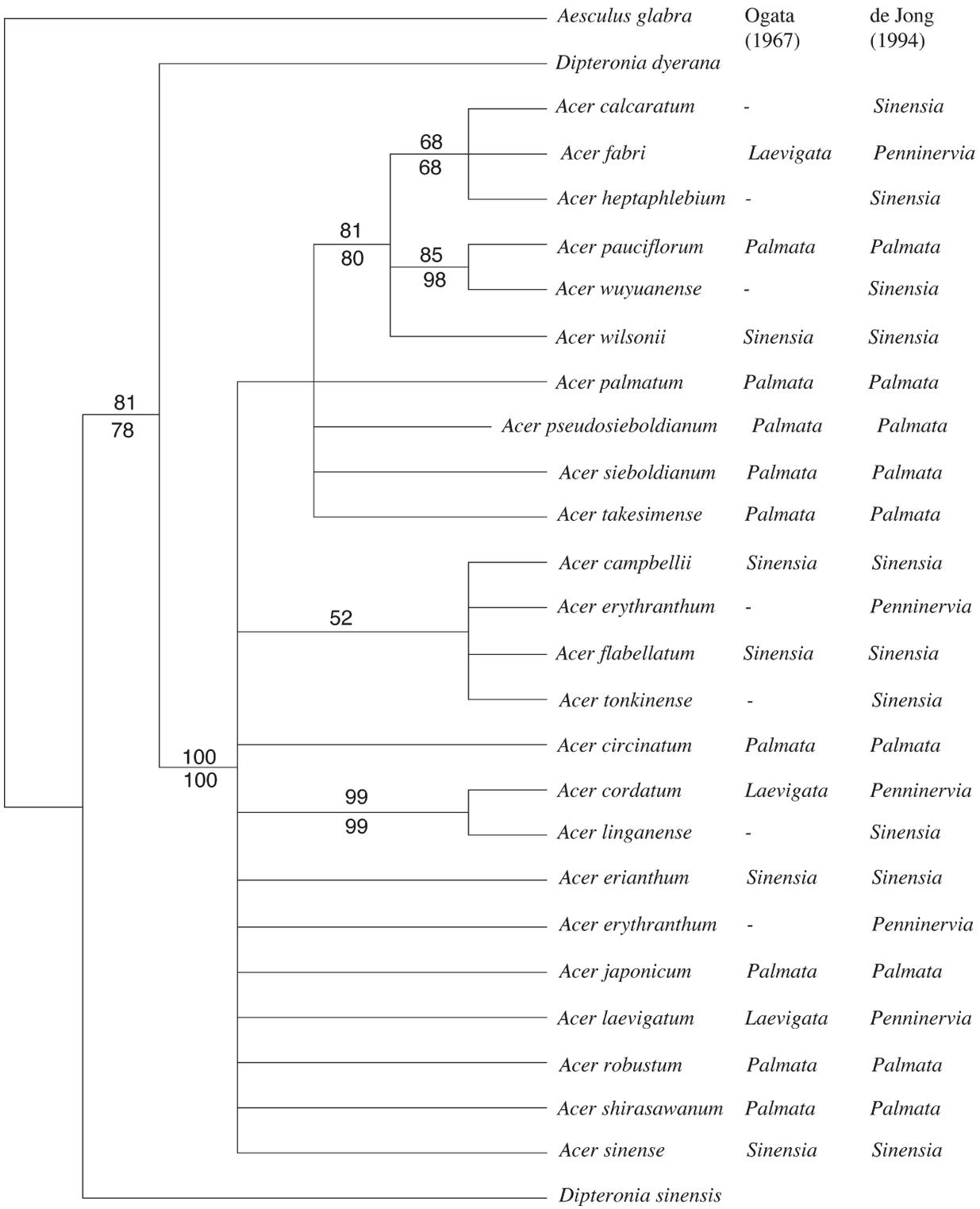

Fig. 2. Strict consensus of 11,264 most parsimonious trees based on sequences of chloroplast genes *ndh*F, *trn*L–*trn*F, and *psbA*–*trn*H (CI = 0.87, RI = 0.79). Numbers above and below branches represent bootstrap percentages for MP and ML analyses, resp. On the right are classification systems of Ogata (1967) and de Jong (1994).

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