

PHYLOGENETICS OF *ACER* (ACEROIDEAE, SAPINDACEAE) BASED ON NUCLEOTIDE SEQUENCES OF TWO CHLOROPLAST NON-CODING REGIONS

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Abstract. *Acer* is one of the most diverse woody genera in the Northern Hemisphere. Recent phylogenetic studies support the placement of *Acer* and *Dipteronia*—sole members of the traditional Aceraceae—in the Sapindaceae. However, the monophyly of *Acer* and its sections remain to be tested. In this study, sequences of two chloroplast non-coding regions, *psbM-trnD* and *trnD-trnT*, are used to elucidate phylogenetic relationships of *Acer* and *Dipteronia*. Our results support the monophyly of *Acer* and sects. *Arguta*, *Ginnala*, *Integrifolia*, *Lithocarpa*, *Macrantha*, *Palmata*, *Platanoidea*, and *Trifoliata*. In contrast, sects. *Acer*, *Goniocarpa*, *Parviflora*, *Saccharodendron*, and *Spicata* are not monophyletic. *Acer trautvetteri* and *A. opalus* of sect. *Acer* are more closely related to *A. monspessulanum* of sect. *Goniocarpa* and *A. saccharum* of sect. *Saccharina* than to *A. caesium* and *A. pseudoplatanus* of sect. *Acer*. *Acer distylium* of sect. *Parviflora* is more closely related to sect. *Platanoidea* than to *A. nipponicum* of sect. *Parviflora*. Morphological species pairs between eastern Asia and North America are not sister species, including *A. pycnanthum*—*A. rubrum* and *A. caudatum*—*A. spicatum*. *Acer ukurunduense* is a distinct species from *A. caudatum*. *Acer glabrum* is most closely related to *A. pseudoplatanus*, whereas *A. spicatum* may be more closely related to *A. carpinifolium* than to *A. caudatum*. Section *Hyptiocarpa* is most closely related to sect. *Rubra*, and the two North American species of sect. *Rubra* (*A. rubrum* and *A. saccharinum*) are more closely related to each other than they are to the Japanese species (*A. pycnanthum*). Sections *Integrifolia* and *Trifoliata* are closely related, and so are *Cissifolia* and *Arguta*. Nevertheless, more data are needed to fully resolve intersectional relationships of *Acer*.

Keywords: *Acer*, *Dipteronia*, chloroplast non-coding regions, *psbM-trnD*, *trnD-trnT*, Sapindaceae.

Maples (*Acer* L.) are one of the most important trees in the Northern Hemisphere, particularly in the temperate regions of eastern Asia, eastern North America, and Europe (van Gelderen et al., 1994). During the fall season, the colorful foliage of maples paints the landscape with red, yellow, and orange. Many species are also important sources of commercial products, for example, syrup from *Acer saccharum* Marshall and timber from *A. saccharum*, *A. rubrum* L., and *A. pseudoplatanus* L.

Traditionally, *Acer* and *Dipteronia* Oliver have been placed in Aceraceae. Recent phylogenetic analyses, however, suggest that these two genera be placed in Sapindaceae (Gadek et al., 1996; Harrington et al., 2005). *Dipteronia* consists of two species, both of which are endemic to China (Ying and Zhang, 1994). The most recent survey of *Acer* listed 156 species,

including more than 20 recently described taxa (de Jong, 2002). Although species of *Acer* occur mainly in temperate to subtropical areas, several species extend their distribution ranges to the tropics, such as *A. laurinum* Hassk. in Thailand and Vietnam, and *A. decandrum* Merr. in Hainan, China.

Various authors have proposed classification systems of *Acer* since the end of the 19th century (Table 1), including Pax (1885, 1886, 1902), Rehder (1905), Koidzumi (1911), Nakai (1915), Pojarkova (1933), Momotani (1962), Fang (1966), Ogata (1967), Murray (1970), de Jong (1976, 1994, 2002), Delendick (1981), Mai (1984), and Xu (1996).

In recent years, four maple phylogenies have been published, greatly improving our understanding of the evolutionary history of *Acer* (Huang et al., 2002). Hasebe et al.'s (1998) RFLP

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TABLE 1. Species sampled in this study, their placements in major classification systems of Acer, and GenBank accession numbers.

SPECIES & DNA NO.	SOURCE & VOUCHER	TRND-TRNT	PSBM-TRND	PAX (1902)	KOIZDZUMI (1911)
<i>Acer acuminatum</i> Wallich ex D. Don 3854	MA 94-009A (A)	DQ659781	DQ659841	Spicata Pax	—
<i>Acer argutum</i> Maximowicz 3596	AA 640-77B (A)	DQ659782	DQ659842	Lithocarpa Pax	Arguta
<i>Acer buergerianum</i> Miquel 4035	AA 421-90C (A)	DQ659783	DQ659843	—	—
<i>Acer campetre</i> L. 3557	AA 238-98A (A)	DQ659784	DQ659844	Campestris Pax	—
<i>Acer carpinifolium</i> Sieb. & Zucc. 3569	AA 10959B (A)	DQ659785	DQ659845	Indivisa Pax	Carpinifolia Koizdumi
<i>Acer caesium</i> Wall. ex Brand. 4277	Li, J. et al. 81906	DQ659833	DQ659893	Spicata Pax	—
<i>Acer caudatum</i> Wall. 4228	Morton Arboretum 320-2000	DQ659827	DQ659887	Spicata Pax	—
<i>Acer cinnamomifolium</i> Hayata 4181	SCBG; 2005-4-4. DMY-021	DQ659786	DQ659846	—	—
<i>Acer circinatum</i> Thunberg ex Murray 3510	AA 722-72C (A)	DQ659796	DQ659856	Palmata Pax	—
<i>Acer crataegifolium</i> Sieb. & Zucc. 4044	RBGE 19900885 (E)	DQ659787	DQ659847	Indivisa Pax	Indivisa
<i>Acer davidii</i> Franchet 3523	AA 692-94A (A)	DQ659788	DQ659848	Indivisa Pax	—
<i>Acer diabolicum</i> Blume ex Koch 3546	AA 2625A (A)	DQ659789	DQ659849	Lithocarpa Pax	Diabolica Koizdumi
<i>Acer distylium</i> Sieb. & Zucc. 3354	RBGE 19481023A (E)	DQ659790	DQ659850	Indivisa Pax	Indivisa
<i>Acer erianthum</i> Schwerin 3735	CP 87-490*B (A)	DQ659791	DQ659851	Spicata Pax	—
<i>Acer fabri</i> Hance 4095	Jiqin Yi, Hunan, China	DQ659792	DQ659852	—	—
<i>Acer flabellatum</i> Rehder 3856	MA 84-046A (A)	DQ659793	DQ659853	Palmata Pax	—
<i>Acer garrettii</i> Craib 3353	Tian 2079 (KUN)	DQ659794	DQ659854	—	—
<i>Acer ginnala</i> Maximowicz 3590	AA 902-85A (A)	DQ659795	DQ659855	Spicata Pax	Spicata
<i>Acer glabrum</i> Torr. 4275	Liston, A., OR	DQ659832	DQ659892	Glabra Pax	—
<i>Acer griseum</i> (Franchet) Pax 3522	AA 12488A (A)	DQ659797	DQ659857	Trifoliata Pax	—
<i>Acer henryi</i> Pax 3361	Tian 9901 (KUN)	DQ659798	DQ659858	Trifoliata Pax	—
<i>Acer lucidum</i> Metcalf 4124	SYI-03, Shenzhen, China (A)	DQ659799	DQ659859	—	—
<i>Acer macrophyllum</i> Pursh 3356	RBGE 19330500A (E)	DQ659800	DQ659860	Spicata Pax	—
<i>Acer maximowiczianum</i> Miquel 3555	AA 3337A (A)	DQ659801	DQ659861	—	—
<i>Acer micranthum</i> Sieb. & Zucc. 3732	CP 01-261*A (A)	DQ659802	DQ659862	Macrantha Pax	Macrantha
<i>Acer monspessulanum</i> L. 3511	AA 1188-82B (A)	DQ659803	DQ659863	Campestris Pax	—
<i>Acer negundo</i> L. 4041	JLI, Newton, Mass (A)	DQ659804	DQ659864	Negundo Pax	—
<i>Acer nipponicum</i> Hara 4045	RBGE 19795193 (E)	DQ659805	DQ659865	—	Parviflora Koizdumi
<i>Acer opalus</i> P. Miller 3756	CP 03-232B (A)	DQ659806	DQ659866	Campestris Pax	—
<i>Acer palmatum</i> Thunberg ex Murray 3553	AA 585-88B (A)	DQ659807	DQ659867	Palmata Pax	—
<i>Acer paxii</i> Franchet 3359	Tian 9915 (KUN)	DQ659808	DQ659868	Spicata Pax	—
<i>Acer pectinatum</i> Wall. 4260	Quarryhill 1990.139B	DQ659829	DQ659889	Macrantha Pax	—
<i>Acer pectinatum</i> Wall. 4278	Li, J. et al., 81905	DQ659834	DQ659894	Macrantha Pax	—

MA, Morris Arboretum; AA, Arnold Arboretum; SCBG, South China Botanical Garden; RBGE, Royal Botanical Garden at Edinburgh; CP, Cornell Plantation

TABLE 1. CONT.

POJARKOVA (1933)	MOMOTANI (1962)	OGATA (1967)	DE JONG (1976)	XU (1996)	DE JONG (2002)
—	Arguta	Arguta	Glabra	Subg. Arguta	Arguta
Arguta	Arguta	Arguta	Glabra	Subg. Arguta	Arguta
—	Trifida Pojark.	Intergrifolia	Intergrifolia	Subg. Acer sect. Intergrifolia	Pentaphylla
Platanoidea	Platanoidea	Campestris	Platanoidea	Subg. Acer sect. Platanoidea	Platanoidea
Carpinifolia	Subg. Carpinifolia	Indivisa	Indivisa	Subg. Carpinifolia	Indivisa
Gemmata Pojarkova	—	—	—	Subg. Acer sect. Acer	Acer
Microcarpa Pojarkova	—	—	Palmata	Subg. Acer sect. Microcarpa	Spicata
Intergrifolia Pax	Intergrifolia	—	—	Subg. Acer sect. Intergrifolia	—
Palmata	Palmata	Palmata	Palmata	Subg. Acer sect. Palmata	Palmata
Macrantha	Macrantha	Macrantha	—	Subg. Acer sect. Macrantha	—
Macrantha	Macrantha	Macrantha	Macrantha	Subg. Acer sect. Macrantha	Macrantha
Lithocarpa	Lithocarpa	Lithocarpa	Lithocarpa	Subg. Acer sect. Lithocarpa	Lithocarpa
—	Spicata	Distyla Ogata	Parviflora	Subg. Acer sect. Distyla	Parviflora
Microcarpa Pojarkova	Palmata	Palmata	Palmata	Subg. Acer sect. Microcarpa	Palmata
Intergrifolia Pax	Intergrifolia	—	Palmata	Subg. Acer sect. Intergrifolia	Palmata
—	Palmata	Palmata	Palmata	Subg. Acer sect. Microcarpa	Palmata
Intergrifolia Pax	Intergrifolia	—	Hyptiocarpa Fang	Subg. Acer sect. Hyptiocarpa	Hyptiocarpa Fang
Trilobata Pojarkova	Trilobata	Trilobata	Ginnala	Subg. Acer sect. Ginnala	Ginnala
Glabra	Glabra	—	Glabra	Subg. Acer sect. Glabra	Glabra
Trifoliata	Trifoliata	Trifoliata	Trifoliata	Subg. Acer sect. Trifoliata	Trifoliata
Cissifolia	Subg. Negundo	Cissifolia	Negundo	Subg. Negundo sect. Cissifolia	Negundo
Intergrifolia Pax	Intergrifolia	—	Palmata	Subg. Acer sect. Intergrifolia	Palmata
Lithocarpa	Macrophylla	Macrophylla	Lithocarpa	Subg. Acer sect. Macrophylla	Macrophylla
—	—	Trifoliata	Trifoliata	—	Trifoliata
Macrantha	Macrantha	Macrantha	Macrantha	Subg. Acer sect. Macrantha	Macrantha
Goniocarpa Pojarkova	Platanoidea	Goniocarpa	Acer	Subg. Acer sect. Goniocarpa	Acer
Negundo	Subg. Negundo	Negundo	Negundo	Subg. Negundo sect. Negundo	Negundo
Macrantha	Spicata	Parviflora	Parviflora	Subg. Acer sect. Parviflora	Parviflora
Goniocarpa Pojarkova	Platanoidea	Goniocarpa	Acer	Subg. Acer sect. Goniocarpa	Acer
Palmata	Palmata	Palmata	Palmata	Subg. Acer sect. Palmata	Palmata
Trilobata Pojarkova	Trifida Pojark.	Intergrifolia	Intergrifolia	Subg. Acer sect. Intergrifolia	Pentaphylla
Macrantha	Macrantha	Macrantha	Macrantha	Subg. Acer sect. Macrantha	Macrantha
Macrantha	Macrantha	Macrantha	Macrantha	Subg. Acer sect. Macrantha	Macrantha

TABLE 1. c. CONT.

SPECIES & DNA NO.	SOURCE & VOUCHER	TRND-TRNT	PSBM-TRND	PAX (1902)	KOIZUMI (1911)
<i>Acer pennsylvanicum</i> L. 3515	AA 521-80C (A)	DQ659809	DQ659869	Macrantha Pax	—
<i>Acer pentamomicum</i> 4241	Morton Arboretum 1115-90	DQ659828	DQ659888	Spicata Pax	—
<i>Acer pentaphyllum</i> Diels 3360	Yousheng Chen 2070, Beijing. Bot. Gard	DQ659810	DQ659870	—	—
<i>Acer platanoides</i> L. 3554	AA 12543A (A)	DQ659811	DQ659871	Platanoidea Pax	—
<i>Acer pseudoplatanus</i> L. 4197	JLI, AA (A)	DQ659812	DQ659872	Spicata Pax	—
<i>Acer pseudosieboldianum</i> (Pax) Komarov 3558	AA 581-87B (A)	DQ659821	DQ659881	Palmata Pax	—
<i>Acer pycnanthum</i> Koch 4046	RBGE 19781656F (E)	DQ659813	DQ659873	—	—
<i>Acer rubrum</i> L. 3565	AA 1196-84A (A)	DQ659814	DQ659874	Rubra Pax	Rubra
<i>Acer saccharinum</i> L. 3525	AA 1142-85B (A)	DQ659815	DQ659875	Rubra Pax	—
<i>Acer saccharum</i> Marshall 3594	AA 1353-84A (A)	DQ659816	DQ659876	Saccharina Pax	—
<i>Acer sinopurpurascens</i> Cheng 3644	Li, J. & P. Del Tridici, 20049331, China	DQ659818	DQ659878	—	—
<i>Acer sinense</i> Pax 3832	MA 83-097B (A)	DQ659817	DQ659877	Spicata Pax	—
<i>Acer spicatum</i> Lamarck 3516	AA 359-79D (A)	DQ659819	DQ659879	Spicata Pax	Spicata
<i>Acer stachyophyllum</i> Hiern 3855	MA 93-038A (A)	DQ659820	DQ659880	Indivisa Pax	—
<i>Acer tataricum</i> L. 3572	AA 239-98A (A)	DQ659822	DQ659882	Spicata Pax	—
<i>Acer tegmentosum</i> Maximowicz 3592	AA 525-77A (A)	DQ659823	DQ659883	Macrantha Pax	—
<i>Acer trautvetteri</i> Medvedev 3551	AA 1354-80A (A)	DQ659824	DQ659884	Spicata Pax	—
<i>Acer triflorum</i> Komarov 3532	AA 968-80A (A)	DQ659825	DQ659885	—	—
<i>Acer ukurunduense</i> Trautvetter & Meyer 3831	MA 97-284C (A)	DQ659826	DQ659886	Spicata Pax	Spicata
<i>Acer ukurunduense</i> Trautvetter & Meyer 4271	Quarryhill 2002.029A	DQ659830	DQ659890	Spicata Pax	Spicata
<i>Acer wardii</i> W. W. Sm. 4273	Tom Hudson, s.n.	DQ659831	DQ659891	—	—
<i>Aesculus glabra</i> Willd. 3774	AA 1221-79A (A)	DQ659780	DQ659840	—	—
<i>Dipteronia dyerana</i> Henry 3363	Tian, Lao Jun Shan, Yunnan, China	DQ659778	DQ659838	—	—
<i>Dipteronia sinensis</i> Oliv. 3086	JLI, Daping, Huayang, Shaanxi, China	DQ659779	DQ659839	—	—
<i>Koelreuteria paniculata</i> Laxm. 3805	AA 378-2001A (A)	DQ659775	DQ659835	—	—
<i>Sapindus drummondii</i> Hook. & Arn. 3808	AA 680-91A (A)	DQ659776	DQ659836	—	—
<i>Xanthoceras sorbifolium</i> Bunge 3809	AA 1293-79D (A)	DQ659777	DQ659837	—	—

MA, Morris Arboretum; AA, Arnold Arboretum; SCBG, South China Botanical Garden; RBGE, Royal Botanical Garden at Edinburgh; CP, Cornell Plantation

TABLE 1. CONT.

	POJARKOVA (1933)	MOMOTANI (1962)	OGATA (1967)	DE JONG (1976)	XU (1996)	DE JONG (2002)
Macrantha	Macrantha	Macrantha	Macrantha	Macrantha	Subg. Acer sect. Macrantha	Macrantha
	—	—	—	Platanoidea	Subg. Acer sect. Gontiocarpa	Pubescentia
	Trifoliata (?)	Pentaphylla	Pentaphylla	Integrifolia	Subg. Acer sect. Pentaphylla	Pentaphylla
Platanoidea	Platanoidea	Platanoidea	Platanoidea	Platanoidea	Subg. Acer sect. Platanoidea	Platanoidea
Gemmata Pojarkova	Acer	Acer	Acer	Acer	Subg. Acer sect. Acer	Acer
Palmata	Palmata	Palmata	Palmata	Palmata	Subg. Acer sect. Palmata	Palmata
Rubra	Rubra	Rubra	Rubra	Rubra	Subg. Acer sect. Rubra	Rubra
Rubra	Rubra	Rubra	Rubra	Rubra	Subg. Acer sect. Rubra	Rubra
Rubra	Rubra	Rubra	Rubra	Rubra	Subg. Acer sect. Rubra	Rubra
Saccharina	Saccharina	Saccharina	Saccharina	Acer	Subg. Acer sect. Saccharodendron	Rubra
Lithocarpa	Lithocarpa	Lithocarpa	Lithocarpa	Lithocarpa	Subg. Acer sect. Saccharodendron	Acer
					Subg. Acer sect. Lithocarpa	Lithocarpa
Microcarpa Pojarkova	Palmata	Palmata	Palmata	Palmata	Subg. Acer sect. Microcarpa	Palmata
Microcarpa Pojarkova	Spicata	Spicata	Spicata	Parviflora	Subg. Acer sect. Rubra	Spicata
Arguta	Arguta	Arguta	Arguta	Glabra	Subg. Arguta	Arguta
Trilobata Pojarkova	Trilobata	—	—	Ginnala	Subg. Acer sect. Ginnala	Ginnala
Macrantha	—	Macrantha	Macrantha	Macrantha	Subg. Acer sect. Macrantha	Macrantha
Gemmata Pojarkova	Acer	Acer	Acer	Acer	Subg. Acer sect. Acer	Acer
Trifoliata	Trifoliata	Trifoliata	Trifoliata	Trifoliata	Subg. Acer sect. Trifoliata	Trifoliata
Microcarpa Pojarkova	Spicata	Spicata	Spicata	Parviflora	Subg. Acer sect. Rubra	Spicata
Microcarpa Pojarkova	Spicata	Spicata	Spicata	Parviflora	Subg. Acer sect. Rubra	Spicata
Macrantha	—	Macrantha	Macrantha	Macrantha	Subg. Acer sect. Macrantha	Wardiana

(restriction fragment length polymorphism) study sampled 64 species of *Acer*, representing 17 sections of Ogata (1967), and generated 53 phylogenetically informative sites. Their data support the monophyly of sects. *Arguta*, *Cissifolia*, *Lithocarpa*, and *Spicata*, and further suggest that sections *Distyla* and *Parviflora* may be closely related. Nonetheless, sectional relationships are generally unresolved or weakly supported. In that study, Hasebe et al. (1998) did not sample outgroups from outside *Acer*, and instead chose sect. *Spicata* as a functional outgroup on the basis of the fossil record. This may not be a warranted assumption, given that the basal position of sect. *Spicata* has not been tested. Sequences of nuclear ribosomal (nr) DNA ITS (internal transcribed spacer) regions support the monophyly of sects. *Arguta*, *Cissifolia*, *Palmata*, *Integrifolia*, *Trifoliata*, *Ginnala*, *Macrantha*, *Lithocarpa*, and *Platanoides* (Ackerly and Donoghue, 1998; Suh et al., 2000). Pfosser et al. (2002)

used AFLP and chloroplast sequence data to test the origin of endemic species of *Acer* from Ullung Island off South Korea. Tian et al. (2002) included 2 species of *Dipteronia* and 39 species of *Acer* in their combined analyses of sequences of nrDNA ITS and chloroplast non-coding *trnL-trnF* regions. Their results suggest that *Dipteronia* may be paraphyletic with *D. deyerana* Henry embedded in *Acer* and that some sections sensu Xu (1996) need reevaluation. Nevertheless, sectional relationships are hardly resolved.

The objectives of this study were (1) to further test the monophyly of *Dipteronia*, *Acer*, and sections of *Acer*, and (2) to provide insights into sectional relationships of *Acer*. We chose to use sequences of two non-coding regions of the chloroplast genome including *psbM-trnD* and *trnD-trnT*, since these markers are among the most variable regions in the chloroplast genome (Kelchner and Clark, 1997; Shaw et al., 2005).

MATERIALS AND METHODS

Plant Material

In this study, we sampled 2 species of *Dipteronia* and 52 species of *Acer*, representing all sections of previous classification systems (Pax, 1902; Rehder, 1905; Koidzumi, 1911; Nakai, 1915; Pojarkova, 1933; Momotani, 1962; Fang, 1966; Ogata, 1967; Murray, 1970; de Jong, 1976, 1994, 2002; Delendick, 1981; Mai, 1984; Xu, 1996). For rooting purposes we also included *Aesculus glabra* Willd., *Sapindus drummondii* Hook. & Arn., *Koelreuteria paniculata* Laxm., and *Xanthoceras semibifolia* Bunge as outgroup taxa (Table 1), which are representatives of the sister clade of *Acer* and *Dipteronia* (Harrington et al., 2005).

Molecular Techniques

DNAs were extracted from silica-gel dried leaves using a DNeasy Plant Mini Kit (Qiagen, CA). The chloroplast DNA region between *trnC* and *trnD* was amplified using primers designed by Lee and Wen (2004), and that between *trnD-trnT* was obtained using primers of Shaw et al. (2005). Polymerase chain reactions (PCR) were carried out using either an MJ-PT200 Thermocycler or an Eppendorff MasterCycler. Each 25- μ l PCR reaction contained 50–100 ng of genomic DNA, 4 μ l of DNTPs (2.5mM), 3 μ l of MgCl₂, 2.5 μ l of *Taq* polymerase buffer (10), 0.3 μ l of *Taq* (5 U/ μ l),

1 μ l of each primer (10 μ M), and an appropriate amount of sterilized distilled water. The PCR program included 3 m hotstart at 94°C and 35 cycles of 1 min denaturing at 94°C, 1.5 min annealing at 46–50°C, and 2 min extension at 72°C. The cycles were followed by an additional 7-min extension at 72°C. For most taxa we used PCR products as templates for sequencing. However, for several taxa direct sequencing PCR products did not work well, either because of the sequence variation within an individual or because of the long stretch of As or Ts that may have caused a polymerase slippage. In such circumstances, we cloned the PCR products using a standard pGEM-T Tail Vector System (Promega, Madison). Two or more clones were sequenced to detect sequence polymorphisms within each accession. Clones and PCR products were sequenced using the Dideoxy Terminator Chemistry with an ABI BigDye Cycle Sequencing Ready Kit. Sequences were analyzed using an ABI 3100 or 3730 Genetic Analyzer, and were edited using Sequencher (Version 4.1, GeneCode Inc., Ann Arbor, Mich.).

Phylogenetic Analyses

Sequence alignment of both non-coding regions were readily done manually across all taxa, including the outgroup taxa. Phylogenetic

analyses were conducted using neighbor-joining (NJ), maximum parsimony (MP), and maximum likelihood (ML) methods as implemented in PAUP* (Version 4.0b10; Swofford, 2002). For MP analyses, a heuristic tree search was used with the following options: maxtrees = 20,000, TBR (tree bisection reconnection) branch swapping, random sequence addition with 5000 replicates and 1 tree held in each replicate, and steepest descent off. Gaps were treated as missing data. Characters were equally

weighted and their states were unordered. For ML analyses, Modeltest (Version 3.06; Posada and Crandall, 1998) was used to select the best evolutionary model, and then the estimated parameters were used in the tree reconstruction. Bootstrap analyses of 100 replicates were carried out to estimate support for individual clades (Felsenstein, 1985), and tree search options for bootstrap analyses were the same as in parsimony analyses except for simple sequence addition.

RESULTS

Sequence Characteristics

PCR amplifications using primers *psbM2* and *trnD* produced a segment of 560–1200 base pairs (bp), whereas primers *trnD* and *trnT* amplified a region of about 1400 bp. The lengths of *psbM-trnD* ranged from 560 to 616 bp in *Acer* and from 781 to 1167 bp in the outgroup taxa, whereas those of *trnD-trnT* were from 1255 to 1431 bp in *Acer* and outgroup taxa. The alignment across all taxa generated 1424 and 1725 sites for the *psbM-trnD* and *trnD-trnT*, respectively. The alignment of *psbM-trnD* sequences across the outgroup taxa and *Dipteronia* and *Acer* required 18 indels ranging from 3 to 632 bp. Eight indels were parsimony informative. In the aligned data set of *trnD-trnT*, there were 42 indels ranging from 5 to 63 bp, and 14 of these indels were parsimony informative. A few indels were synapomorphies and will be discussed in the context of tree structures. Both chloroplast regions were AT rich, with an average of 65.6% and 64.8%, respectively. Sequence divergence of the *psbM-trnD* ranged from 5.9% to 10.2% between *Acer* and outgroup taxa, 1.4% to 3.8% between *Dipteronia* and *Acer*, and 0.2% to 3.1% within *Acer*. For the *trnD-trnT* region, sequences diverged from 4.6% to 10.6% between *Acer* and outgroup species, 0.9% to 3.2% between *Dipteronia* and *Acer*, and 0.1% to 2.5% within *Acer*.

Phylogenetic Analyses

Clones of *trnD-trnT* in *Acer palmatum* Thunberg ex Murray, *A. henryi* Pax, and *A. distylium* Sieb. & Zucc. had similar sequences; therefore, only one clone was used in the data set for phylogenetic analyses. Because chloroplast genes generally share a similar evolutionary history and the trees based on individual

data sets of *psbM-trnD* and *trnD-trnT* did not produce well-supported but conflicting clades (tree not shown), the two data sets were combined in phylogenetic analyses. The combined data set had 3150 sites, 281 of which were parsimony informative. NJ analyses produced a cladogram (Fig. 1), and MP analyses generated 20,000 trees of 1018 steps; the strict consensus is shown in Fig. 2 (CI = 0.83, RI = 0.79). Modeltest indicated that the best fitting model of evolution for the chloroplast regions was TVM+G with estimated parameters as follows: base frequencies (A = 0.3339, C = 0.1744, G = 0.1601, T = 0.3316), rate matrix (A–C = 1.2302, A–G = 1.4401, A–T = 0.2821, C–G = 0.6580, G–T = 1.4401), and Gamma shape parameter = 0.6461. ML analyses with the selected model and estimated parameters produced a single tree with likelihood of $-\ln = 10485.86$ (Fig. 3).

Two species of *Dipteronia* formed a clade with moderate support (bs = 66%) in the NJ tree (Fig. 1), whereas in both MP and ML trees these two species did not form a clade (Figs. 2–3). Species of *Acer* formed a clade in all trees and this clade received little support in the NJ tree, but in the MP tree it was moderately supported (71% and 3 base substitutions). Within *Acer*, there were 18 clades with moderate to strong support (>70%) in the NJ and/or MP trees, as well as in the ML tree (A–R). *Acer henryi* of sect. *Negundo* formed a clade (Clade A, bs = 80% in the NJ tree, bs = 75% and 3 base substitutions in the MP tree) with sect. *Arguta* (B, 81%, 89% and 4). *Acer distylium* of sect. *Parviflora* and *A. pentapomicum* Stewart & Brand. of sect. *Pubescentia* formed a clade (C, 92%, 90% and 4) with sect. *Platanoides* represented by *A. platanoides* L. and *A. campestre* L.

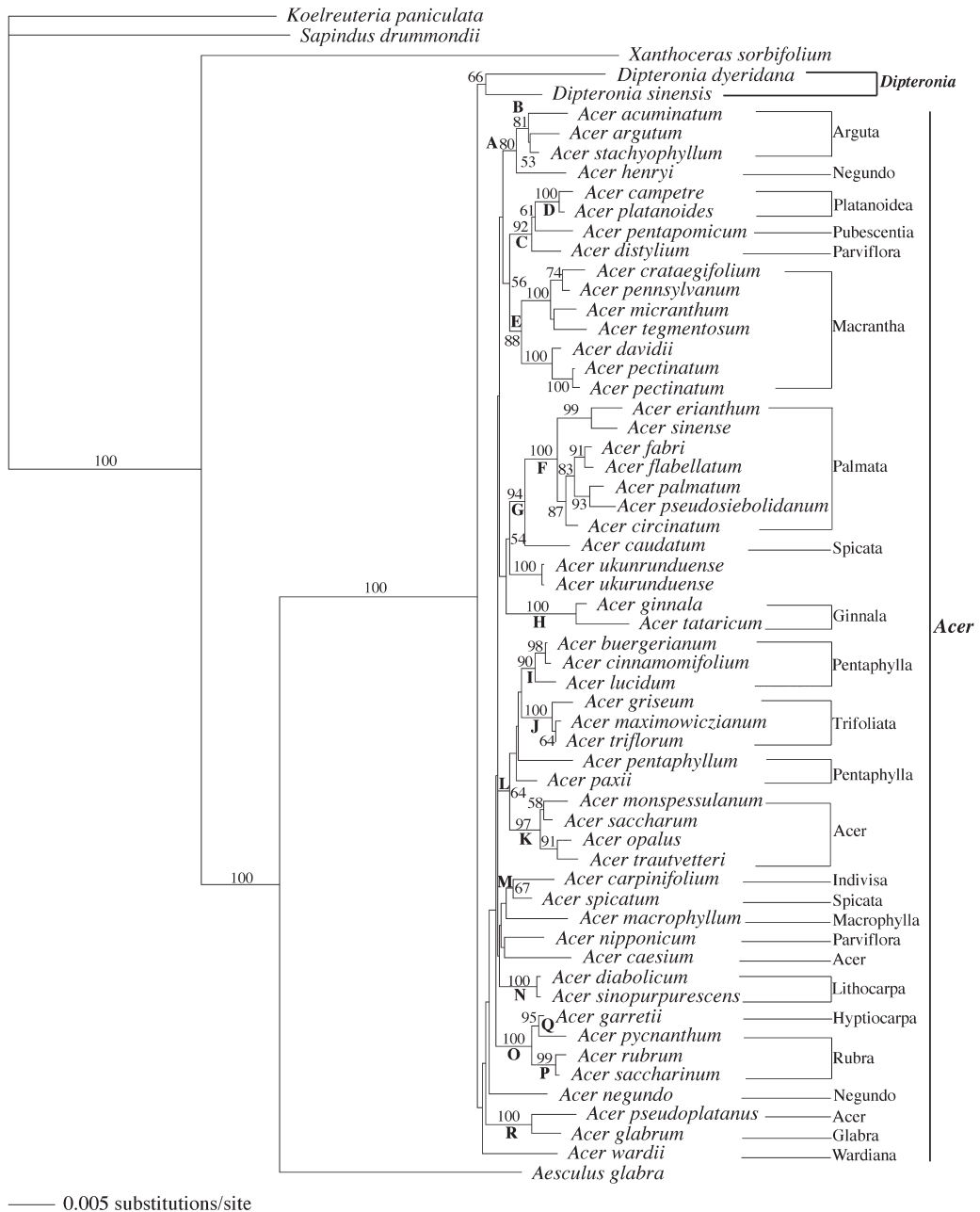


FIGURE 1. Neighbor-joining cladogram based on sequences of chloroplast non-coding regions of *psbM-trnD* and *trnD-trnT*. Numbers at branches are bootstrap percentages of 1000 replicates. Letters A–R indicate clades discussed in text. Section designations on the right follow de Jong (2002).

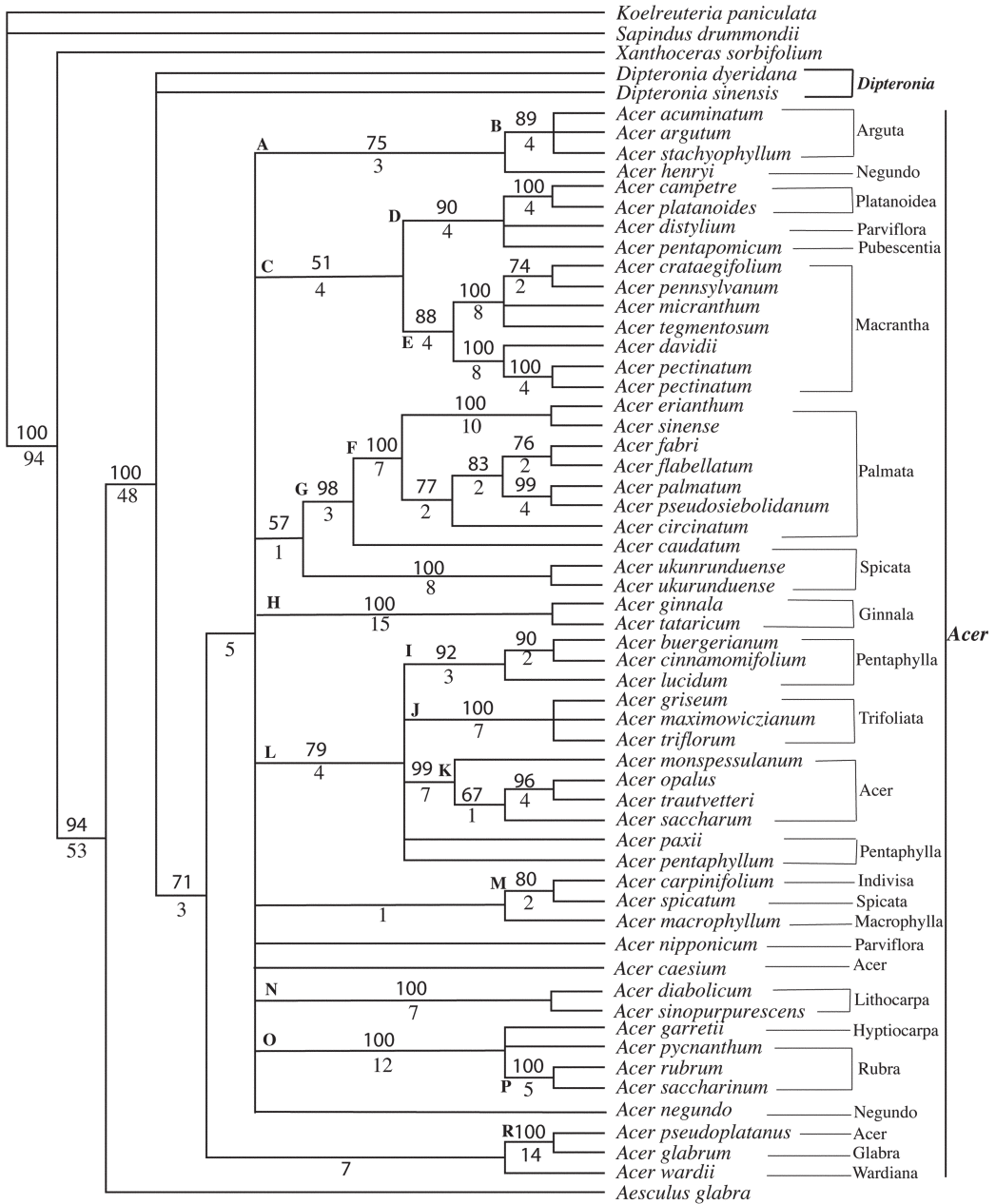


FIGURE 2. Strict consensus of 20,000 parsimonious trees of 1018 steps based on sequences of chloroplast non-coding regions of *psbM-trnD* and *trnD-trnT* (CI = 0.83 and RI = 0.79). Numbers above and below branches are bootstrap percentages and numbers of base substitutions. Letters A–R indicate clades discussed in text. Section designations on the right follow de Jong (2002).

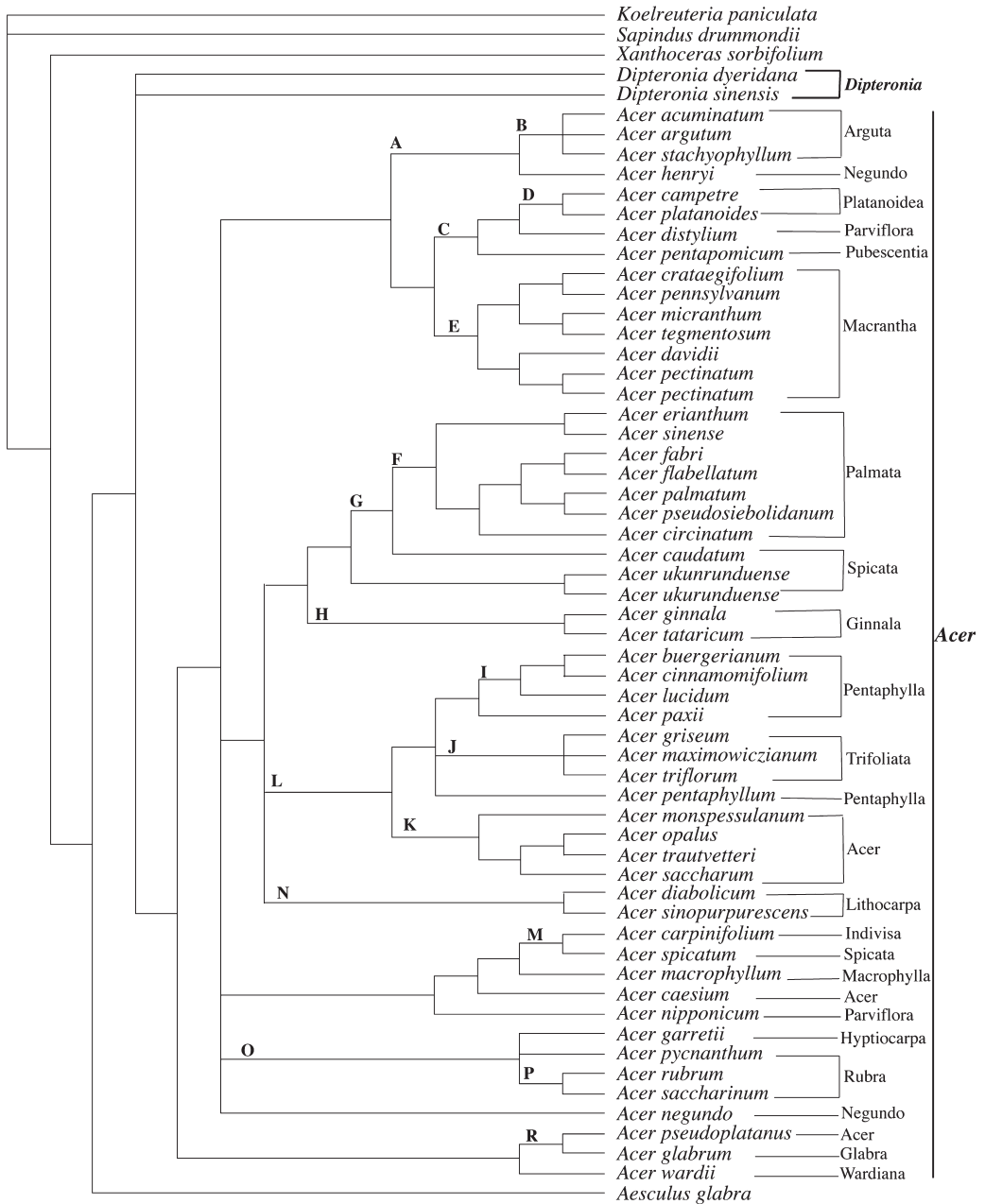


FIGURE 3. Maximum likelihood tree with a likelihood score of $-\ln = 10485.86$ based on sequences of chloroplast non-coding regions of *psbM-trnD* and *trnD-trnT*. Letters A–R indicate clades discussed in text. Section designations on the right follow de Jong (2002).

(D, 100%, 100% and 4). Section *Macrantha* formed a clade (E, 88%, 88% and 4), and so did sect. *Palmata* (F, 94%, 100% and 7). *Acer caudatum* Wall. clustered with sect. *Palmata* (G, 54%, 98% and 3), and together they were weakly allied with *A. ukurunduense* (54%, 57% and 1). Section *Ginnala* was well supported as a clade (H, 100%, 100% and 15). Four sampled species of sect. *Pentaphylla* formed a clade in the ML tree (Fig. 3), but in the NJ and MP trees (Figs. 1–2), *A. paxii* Franchet did not cluster with the other three species of the clade (I, 90%, 92% and 3). Section *Trifoliata* formed a clade (J, 100%, 100% and 7) and so did sect. *Acer* (K, 97%, 99% and 7), excluding *A. pseudoplatanus* and *A. caesium* Wall. ex Brand. Clades I–K, *A. paxii*, and *A. pentaphyllum*

Diels formed a clade (L, Figs. 1–3) with weak (64%, Fig. 1) or moderate (79%, Fig. 2) support. The monotypic sect. *Indivisa* clustered with *A. spicatum* Lamarck (M, 67%, 80% and 2). Section *Lithocarpa* was well supported as a clade (N, 100%, 100% and 7). Section *Rubra* formed a clade (O, 100%, 100% and 12), which also contained *A. garrettii* Craib. of sect. *Hyptiocarpa*. Whereas *A. saccharinum* L. and *A. rubrum* of sect. *Rubra* formed a group (P, 99%, 100% and 5), *A. pycnanthum*, another species of sect. *Rubra*, clustered with *A. garrettii* in the NJ tree (Q, 95%). However, this cluster was absent in both MP and ML trees (Figs. 2–3). *Acer pseudoplatanus* of sect. *Acer* and *A. glabrum* Torr. of sect. *Glabra* formed a clade (R, 100%, 100% and 14).

DISCUSSION

Monophyly of Acer

Dipteronia differs distinctively from *Acer* in its unique combination of pinnately compound leaves and circular fruit wings. In the nrDNA tree, *D. dyeriana* is sister to the monophyletic *Acer*. However, in the *trnL-trnF* phylogeny, this species is embedded within *Acer*, indicating that *Acer* is paraphyletic (Tian et al., 2002). *Dipteronia* species do not form a clade in either the MP or ML tree (Figs. 2–3), whereas in the NJ tree the two species cluster together with weak support (bs = 66%). In all trees (Figs. 1–3), *Acer* species form a clade, and in the MP tree this clade has moderate support (bs = 71% and 3 base substitutions). Thus, our results support the monophyly of *Acer*, which is consistent with the unique fruit morphology of *Acer*, that is, samaras, each with 2 one-seeded mericarps. Nevertheless, more data are needed to strengthen the support for the monophyly of *Dipteronia* and *Acer*.

Sections of Acer

The genus *Acer* has been divided into sections and series on the basis of morphological, anatomical, and chemical characters by various authors (Pax, 1902; Rehder, 1905; Koidzumi, 1911; Nakai, 1915; Pojarkova, 1933; Momotani, 1962; Fang, 1966; Ogata, 1967; Murray, 1970; de Jong, 1976, 1994, 2002; Delendick, 1981; Xu, 1996).

There are 4 species in sect. *Arguta* (de Jong, 2002), which is defined by dioecy and raceme inflorescence with 4-merous flowers. Three sampled species of sect. *Arguta* form a clade

with strong support (B, bs = 81%–84%, Figs. 1–2). This is consistent with previous phylogenetic studies (Hasebe et al., 1998; Tian et al., 2002).

Pax (1902) put *Acer henryi* in sect. *Trifoliata* on the basis of leaf morphology. Later, Koidzumi (1911) separated this species from *Trifoliata* and recognized it as a new sect. *Cissifolia*. This taxonomic treatment has generally been accepted (Pojarkova, 1933; Momotani, 1962; Ogata, 1967). *Cissifolia* may be closely related to *A. negundo* because they share similar morphology in buds and leaves (de Jong, 1976, 2002; Xu, 1996). However, in Figs. 1–3, *A. henryi* forms a moderately supported clade (A, bs = 75%–80%) with species of sect. *Arguta* (B) and is remotely related to *A. negundo* L. Morphologically, *A. negundo* differs from sect. *Arguta* in having apetalous flowers (vs. petalous flowers in sect. *Arguta*). Nevertheless, the systematic position of *A. negundo* remains unresolved.

Section *Platanoidea*, with 13 species, is unique in having a milky sap in the leaf petiole. Pax (1902) placed *Acer campestre* and *A. monspessulanum* L. in sect. *Campestris* and separated them from sect. *Platanoidea*. However, this treatment has not gained wide support. Instead, *A. campestre* has been put in sect. *Platanoidea* (Pojarkova, 1933; Momotani, 1962; Ogata, 1967; de Jong, 1976, 2002; Xu, 1996). The placement is supported by recent molecular evidence (Hasebe et al., 1998; Tian, 2002). Our results provide further evidence for

the close relationship of *A. campestre* and *A. platanooides* (Fig. 1). *Acer pentapomicum* also has the milky sap in the leaf petiole and has been recognized as a series in sect. *Platanoidea* (de Jong, 1976), as a member of sect. *Goniocarpa* (Xu, 1996), or as constituting a separate section (de Jong, 2002). Here, it forms a clade with sect. *Platanoidea* and *A. distylium* (D, Figs. 1–3), supporting the inclusion of *A. pentapomicum* in *Platanoidea* (de Jong, 1976).

Acer distylium is unique in linden-like leaves and has been placed in sects. *Indivisa* (Pax, 1902; Koidzumi, 1911) or *Spicata* (Momotani, 1962). It has also been treated as its own sect. *Distyla* (Ogata, 1967; Xu, 1996). De Jong (1976), however, recognized the close relationship of *A. distylium* and *A. nipponicum* Hara and considered both species as belonging in sect. *Parviflora*. This treatment is weakly supported by two RFLP markers (Hasebe et al., 1998) and nrDNA ITS sequence data (Suh et al., 2000; Tian et al., 2002). Sequences of *trnL-trnF*, however, did not support this relationship (Tian et al., 2002). Our chloroplast sequence data place *A. distylium* in a clade with sect. *Platanoidea* with strong support (D, bs = 90%, Fig. 1), whereas the relationship of *A. nipponicum* is not resolved. Here, we do not attempt to explain the contrasting hypotheses of relationships of *A. distylium* and *A. nipponicum*, since more accessions are needed to account for potential sequence polymorphisms within species, and we need more data from additional chloroplast and nuclear markers to generate a robust phylogeny.

Section *Macrantha* consists of 21 species distributed in eastern Asia and North America. Six sampled species form a clade (E, bs = 88%, Figs. 1–2). This clade is characterized by raceme inflorescences, horizontally spreading fruit wings, and buds with stalks (de Jong, 1976). Previous phylogenetic analyses also recognized this section (Hasebe et al., 1998; Ackerly and Donoghue, 1998; Suh et al., 2000; Tian et al., 2002; Pfosser et al., 2002).

Section *Palmata* is the largest section, with 41 species, and is characterized by a few potential synapomorphies including 4-pair bud scales and abortive terminal buds. Our data support the monophyly of the section (F, bs > 98%) as did previous molecular studies (Hasebe et al., 1998; Suh et al., 2000; Tian et al., 2002; Pfosser et al., 2002; Ackerly and Donoghue, 1998). In addition, species of sect.

Palmata share three indels, two (9 and 17 bp) in the *psbM-trnD* region and one (6 bp repeat) in the *trnD-trnT*.

Pojarkova (1933) placed *Acer spicatum* in her sect. *Microcarpa* with *A. erianthum* Schwerin and *A. sinense* Pax. In Figs. 1–2, *A. erianthum* and *A. sinense* are closely allied within sect. *Palmata*, whereas *A. spicatum* forms a moderately supported clade with *A. carpinifolium* Sieb. & Zucc. (M). Therefore, our data do not support sect. *Microcarpa*. *Acer caudatum* has been treated as a species of sect. *Spicata* (Pax, 1902; de Jong, 2002), *Microcarpa* (Pojarkova, 1933), and *Parviflora* (de Jong, 1976). *Acer caudatum* and *A. spicatum* have been considered as a species pair between eastern Asia and North America (de Jong, 1976, 2002), and *A. ukurunduense* as a subspecies or variety of *A. caudatum* (de Jong, 1994). However, in Figs. 1–2, *A. caudatum* is sister to sect. *Palmata*, and together they are weakly allied with *A. ukurunduense* (bs = 51%–54%). *Acer spicatum*, however, clusters with *A. carpinifolium*. Therefore, *Acer caudatum* and *A. spicatum* may not be as closely related as was previously thought (de Jong, 2002), and *A. ukurunduense* is probably a distinct species from *A. caudatum*.

Acer carpinifolium is a unique species with simple and serrate leaves. Pax (1902) placed it together with other simple-leaved species (e.g., *A. crataegifolium* Sieb. & Zucc., *A. davidii* Franchet, *A. distylium*, and *A. stachyophyllum* Hiern). However, it has generally been accepted as the sole species of sect. *Indivisa* (Koidzumi, 1911; Ogata, 1967; Pojarkova, 1933; van Gelderen et al., 1994), or subgenus *Carpinifolia* (Momotani, 1962; Xu, 1996). Our results indicate that *A. carpinifolium* may be closely related to *A. spicatum*. This relationship has not been proposed before and needs rigorous test from additional data.

Pax (1902) placed all species with undivided simple leaves in sect. *Indivisa*. Pojarkova (1933) revised sect. *Indivisa* by transferring *A. crataegifolium* to sect. *Macrantha* and *A. stachyophyllum* to sect. *Arguta*. Nevertheless, she considered the rest of the species as belonging to sect. *Integrifolia* (e.g., *A. fabri* Hance, *A. garrettii*, *A. cinnamomifolium* Hayata, and *A. lucidum* Metcalf). De Jong (1976) moved *A. fabri* from sect. *Integrifolia* to sect. *Palmata*, whereas Fang (1966) established sect. *Hyptiocarpa* for *A. garrettii*. Molecular data

support the separation of *A. fabri* and *A. garrettii* from *Integrifolia* (Hasebe et al., 1998; Suh et al., 2000; this study). However, results from Tian et al. (2002) suggest that *A. fabri* belongs in sect. *Integrifolia*, which might have resulted from species misidentification. *Acer lucidum* has been placed in sect. *Palmata* (de Jong, 2002). But, our data recognize it as a species of sect. *Integrifolia* (Figs. 1-3). All molecular evidence supports the close relationship of sect. *Trifoliata* and *Integrifolia* (Hasebe et al., 1998; Suh et al., 2000; Tian et al., 2002; this study). One potential synapomorphy for this clade is the potential bud with multiple pairs of imbricate scales.

Acer pentaphyllum is characterized by its compound leaf with five leaflets and has been placed in sect. *Trifoliata* (Momotani, 1962) or sect. *Integrifolia* (de Jong, 1976). Ogata (1967), however, recognized it as its own sect. *Pentaphylla*. Our results support the close relationship of *A. pentaphyllum* with sects. *Trifoliata* and *Integrifolia* (Suh et al., 2000; Tian et al., 2002). Nevertheless, the support for the relationship is weak.

Pojarkova (1933) established sect. *Goniocarpa* for *Acer monspessulanum* and *A. opalus* P. Miller, and sect. *Gemmata* for *A. caesium*, *A. pseudoplatanus*, and *A. trautvetteri* Medvedev. However, Momotani (1962) placed *A. monspessulanum* in sect. *Platanoidea*. This treatment has not received wide support (Ogata, 1967; de Jong, 1976; Xu, 1996). Instead, *A. caesium*, *A. monspessulanum*, *A. pseudoplatanus*, and *A. trautvetteri* have been placed in sect. *Acer* (de Jong, 1976). In Fig. 1, *A. monspessulanum*, *A. opalus*, *A. trautvetteri*, and *A. saccharum* form a well-supported clade (K, bs = 97%), excluding *A. caesium* and *A. pseudoplatanus*. Section *Acer*, therefore, is not monophyletic. *Acer saccharum* is a species complex with five subspecies in North America (de Jong, 2002). Pax (1902) erected sect. *Saccharina* for *A. saccharum* and its varieties. This treatment has been followed (Pojarkova, 1933; Momotani, 1962; Ogata, 1967). However, de Jong (1976) put *A. saccharum* in sect. *Acer*, whereas Xu (1996) recognized sect. *Saccharodendron* consisting of *A. saccharum* and *A. saccharinum*. In Fig. 1, *A. saccharum* is embedded in a well-supported clade containing sect. *Goniocarpa*, and *A. monspessulanum* and *A. trautvetteri* of sect. *Acer* (de

Jong, 2002). Our results, therefore, reject sects. *Saccharodendron* of Xu (1996), *Goniocarpa* (Pojarkova, 1933), and *Acer* (de Jong, 2002). Sections *Integrifolia*, *Trifoliata*, and *Acer* (excluding *A. caesium* and *A. pseudoplatanus*) form a moderately supported clade (L, bs = 64%–79%, Figs. 1–2). These sections also share an indel (14 bp).

The phylogenetic position of *Acer caesium* is unclear, whereas *A. pseudoplatanus* forms a clade with *A. glabrum* (R, bs = 100%). *Acer glabrum* is unique in having variable leaves from 3- to 5-lobed to partly trifoliate. It has been treated as its own sect. *Glabra* (Pax, 1902; Pojarkova, 1933; Momotani, 1962), or as a section also containing ser. *Arguta*. Sequences of nrDNA ITS suggest a close but weakly supported affinity of *A. glabrum* with *A. ginnala* (Suh et al., 2000). The alliance of *A. glabrum* with *A. pseudoplatanus* (Figs. 1–2) is new and thus requires further tests with more data. Nevertheless, our data do not support the close relationship of *A. glabrum* with sect. *Arguta* or *Macrantha*, as suggested by de Jong (1976).

Section *Hyptiocarpa* consists of two species, *A. garrettii* and *A. laurinum*. However, some authors have treated them as a single species (van Gelderen et al., 1994). On the basis of chemical and morphological characters, Delendick (1981) and de Jong (1976) considered *Hyptiocarpa* as closely related to sect. *Rubra*, which has three species (two in eastern North America and one in Japan). The suggestion gets support from sequence data of nrDNA and *trnL-trnF* (Tian et al., 2002). *Acer rubrum* of eastern North America and *A. pycnanthum* K. Koch of Japan have been considered as a sister species pair (de Jong, 1976; Barnes et al., 2004). This implies that these two species are more closely related to each other than either is to *A. saccharinum*, the other eastern North American species of sect. *Rubra*. However, our data indicate that the two North American species, *A. saccharinum* and *A. rubrum*, are phylogenetically closer to each other than either is to the Japanese species (Figs. 1–3).

Section *Ginnala* has one species with four subspecies (de Jong, 2002). Previous molecular studies have suggested that it may be closely related to *Rubra* (Hasebe et al., 1998) or *Glabra* (Suh et al., 2000). Nevertheless, the support for the relationships is weak. Our data do not resolve the relationship of sect. *Ginnala*

either (Figs. 1–2). Therefore, more data are needed.

Section *Lithocarpa* consists of eight Asian species (de Jong, 2002) and has been considered to be closely related to sect. *Macrophylla*, which has a single species from western North America. Two sampled species of sect. *Lithocarpa* form a clade (N, bs = 100%), supporting the monophyly of the section. However, the relationships of sect. *Macrophylla* remain unresolved (Figs. 1–2).

In summary, our data from two non-coding chloroplast regions support the monophyly of the genus *Acer* and sects. *Arguta*, *Integrifolia*, *Trifoliata*, *Platanioidea*, *Macrantha*, *Lithocarpa*, *Palmata*, *Rubra*, and *Ginnala*. In contrast, sects. *Goniocarpa*, *Spicata*, *Acer*, *Parviflora*, and *Saccharodendron* are not

monophyletic. Section *Cissifolia* is more closely related to sect. *Arguta* than to *A. negundo*. *Acer caudatum* is sister to sect. *Palmata*, and *A. saccharum* clusters with sect. *Acer* (excluding *A. caesium* and *A. pseudoplatanus*). *Acer carpinifolium* is closely related to *A. spicatum*, whereas *A. pseudoplatanus* is allied with *A. glabrum*. Section *Hyptiocarpa* is closely related to sect. *Rubra*, and *A. saccharinum* and *A. rubrum* are more closely related to each other than either is to *A. pycnanthum*. Nevertheless, intersectional relationships are generally weakly supported. More data are needed to obtain a fully resolved phylogeny of *Acer*, which will then provide a backdrop for better understanding of evolutionary and biogeographic history of the genus.

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