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Phylogeny of Aceraceae Based on ITS and *trn*L-F Data Sets

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Abstract: The nuclear encoded internal transcribed spacer (ITS) region and the plastid encoded *trn*L-F region were sequenced for 41 species of the Aceraceae, representing both genera *Acer* and *Dipteronia*, to reconstruct phylogeny of this family, especially within *Acer*. The analyses were performed in separate and combined sequence data sets, with the Sapindaceae and Hippocastanaceae being selected as outgroups. It was indicated that the Aceraceae was monophyletic and *D. sinensis* was basal to the rest of the family but the two genera of it might be not monophyletic because *Dipteronia dyerana* was nested within *Acer*. The result inferred from the combined data showed greater resolution within *Acer* than that from the two separate data sets. The monophyly of most sections in Xu's system (1996) were supported with high bootstrap values, and some relationships between (or among) sections were also inferred, such as sect. *Palmata* and sect. *Microcarpa*; sect. *Platanoidea*, sect. *Lithocarpa* and sect. *Macrophylla*; sect. *Integrifolia*, sect. *Trifoliata* and sect. *Pentaphylla*; and sect. *Acer*, sect. *Goniocarpa* and sect. *Saccharina* (sensu Ogata). However, the sectional status and circumscriptions of some of the above-mentioned sections should be further adjusted. It seemed that the Xu's delimitations of sect. *Rubra* and sect. *Saccharodendran* should be revaluated.

Key words: Aceraceae; phylogeny; ITS sequences; *trn*L-F sequences

The family Aceraceae, consisting of two genera, *Acer* and *Dipteronia*, is a constant member of the order Sapindales based on previous morphological analyses[1-3]. The close relationships between Aceraceae and Sapindaceae or Hippocastanaceae are supported by nucleotide sequences analyses of the chloroplast *rbc*L gene[4, 5]. Thorne[6] and APG[7] even included the Aceraceae in the Sapindaceae. The genus *Acer*, containing about 200 species, is widely distributed in the northern hemisphere[8]. China is the modern center of diversification because most sections of the genus and seventy percent of the species occur in this country[8]. The other genus, *Dipteronia*, with only two species, is endemic to China[9].

The genus *Acer* is characterized by their unique elongated winged fruits (samaras), while other morphological characters are highly diversified. For example, the leaf shapes vary from the 3-, 5- or 7-lobed, undivided leaves to trifoliate, 5-foliolate, or even pinnately compound leaves. Several different inflorescence types, including racemes, panicles, corymbs and spikes, occur in this genus. The three types of sexuality, andromonoecism (or andropolygamy), androdioecism and dioecism, are all represented in this genus. These variances make infrageneric divisions very difficult. The species delimitation and phylogenetic relationships within the genus *Acer* are also very controversial. Mainly on the basis of the relative position of stamens to discs, Pax divided the genus into 14 sections in 4 large groups in his first system of *Acer*[10], although he later recognized 13 sections[11]. Rehder[12] reduced all sections to the rank of series and placed them under two newly-circumscribed sections, which were mainly characterized by intrastaminal discs and extrastaminal discs, respectively. Fang[13] proposed a different system in which the genus was divided into two subgenera, mainly on the basis of simple versus compound leaves. In Ogata's[14] system, the genus was classified into 26 sections. In 1970, Murray[15] published his monograph of the Aceraceae with 7 subgenera, 24 sections and 35 series within *Acer*. Ogata's system was essentially followed by Xu[16], with some additions and amendments. More recently, de Jong[17] recognized only 19 series in 16 sections, providing a quite different arrangement from those of other authors. Some researchers[11, 14, 18-21] discussed the infrageneric phylogenetic relationships in the genus by analyzing gross morphology, seed proteins, fossils and geographic distributions, but the conclusions were not in consensus.

As reviewed above, the infrageneric systems and phylogenetic relationships in the genus *Acer* based on morphological characters were repeatedly proven to be not convincing. The second genus, *Dipteronia*, was seldom studied for its limited distribution. It was necessary to study the phylogeny of the Aceraceae, especially within the controversial genus *Acer* by other means and methods. Molecular data are different criteria on phylogenetic inferences from other phenotypic data[22]. Nucleotide sequences of a chloroplast gene, *rbc*L, have been extensively used to examine plant phylogenies at higher taxonomic levels, indicating the phylogenetic relationships of the Aceraceae among the angiosperms[4, 5, 7, 23]. However, to resolve phylogenetic relationships among closely

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related groups, fast-evolving DNA fragments are needed[24]. Taberlet *et al*[25] presumed that the intergenic spacer of *trn*L-F could be used for phylogenetic studies at the interspecific level in *Acer* based on comparison of two species of *Acer*. Recently, some phylogenetic analyses of *Acer* using nrDNA ITS data showed that this region was informative in resolving infrafamilial relationships in the Aceraceae[26-29]. However, the divergence on the delimitation of some sections was missed in the previously published data sets and was not addressed because of lack of sampling. In this study, we selected nrDNA ITS and cpDNA *trn*L-F regions with a much broader sampling (1) to reconstruct phylogeny of the Aceraceae, especially within *Acer*; (2) to evaluate previous classification systems of the genus *Acer* based mainly on morphological data; and (3) to address the sister relationship between *Acer* and *Dipteronia*.

1 Materials and Methods

1.1 Materials

Forty-one species of the Aceraceae were sampled which represent the two genera with both species of *Dipteronia* and all the sections except for sect. *Emeiensia* of *Acer* of Xu[16], which need verifying (Table 1). We followed Xu's[16] system in Table 1 and in discussions. To ensure the reliability of sampling, two accessions of *D. dyerana* from different sources were sampled. Some species of *Acer* were re-sequenced because the available sequences in the GenBank were with too many ambiguous sites. No dubious sampling was founded, such as in *D. sinensis* and *A. carpinifolium*. Three species, *Sapindus mukorossi*, *S. delavayi* (Sapindaceae), and *Aesculus wangii* (Hippocastanaceae) were used as outgroups. Vouchers are deposited in the herbarium of Kunming Institute of Botany (KUN). In the ITS separate data set, the sequences of additional 17 species of *Acer* plus *S. mukorossi* of Sapindaceae were downloaded from the GenBank.

1.2 Methods

1.2.1 DNA isolation Total genomic DNA was extracted from fresh or silica-gel-dried leaves with a modified CTAB procedure[30], although some leaf materials in the herbarium were used. Prior to DNA extraction, we followed Su *et al*[31] in using acetone to get rid of the stiff materials that interfere to the DNA's extraction.

1.2.2 PCR amplification Double-stranded DNA was directly amplified by symmetric PCR in GeneAmp 9600 (Perkin Elmer, Norfolk, Connecticut). Reaction was conducted in 0.2-mL thin-walled microcentrifuge tubes and contained 1.5 U Ampli*Taq* DNA polymerase, Replitherm TM buffer, 1.5 mmol/L $MgCl_2$, 0.4 mmol/L dNTP, 0.1 μmol/L primer, 5% dimethyl sulfoxide, 25 - 60 ng sample DNA. The procedures of PCR thermal cycle was conducted as follows: template pre-denaturation of 2 min at 97 ℃, then 30 cycles of 1 min at 94 ℃ for template denaturation, 1 min at 55 ℃ for primer annealing, 1 min at 72 ℃ for primer extension, followed by a final extension of 7 min at 72 ℃. We used the ITS4 and ITS5 primers of White *et al*[32] for the ITS regions for the PCR and *trn* "c" and *trn* "f"[25] for the *trn*L-F regions. PCR products were purified by Watson's purification kit prior to being sequenced.

1.2.3 DNA sequencing Double-stranded purified PCR products were sequenced by the dideoxy chain termination method with an ABI PRISM™ Bigdye Terminator Cycle Sequencing Ready Reaction Kit with Ampli*Taq* DNA polymerase FS (Perkin Elmer, Norfolk, Connecticut). Reactions and programs were chosen according to the recommendations of the manufacturer, with slight modification in some cases. Samples were electrophoresed in an ABI 310 Genetic Analyzer (Applied Biosystems Inc.). Primers for PCR were used to sequence all samples.

1.2.4 Phylogenetic analyses DNA sequences were edited and aligned with a DNASTAR Package, adjusted manually where necessary. Phylogenetic analyses by maximum-parsimony method were performed with PAUP 4.0b8[33] for the two data sets respectively as well as for the combined data set. In phylogenetic analyses, ambiguous sites were excluded from the matrix. Gaps were treated as missing, and inferred indels of unambiguous alignment were recoded as unordered separated characters. All unambiguous characters and character-state transformations were given equal weight. A heuristic search was performed for each data set, with RANDOM stepwise data addition (1 000 replications, start seed = 1) and TBR branch-swapping algorithm options. To assess the relative support for each clade, bootstrap values were calculated from 1000 replicate analyses with the heuristic search strategy and simple addition sequence of the taxa. The amount of phylogenetic information in the MP analysis was constructed with the consistency (CI), retention (RI) and rescaled consistency indices (RC). Distance trees were also constructed with the neighbor-joining (NJ) method (PAUP 4.0b8).

2 Results

All nucleotide sequences of the sampled species were deposited in the GenBank (Table 1). Sequence characteristics of the two DNA regions were summarized in Table 2.

2.1 Analysis of the ITS data set

The ITS1 and ITS2 sequences of the outgroup *S. mukorossi* were downloaded from the GenBank. The unknown 5.8S of it was treated as no answer in the data matrix. The length of the ITS region, including both spacers and the 5.8S subunit, ranged from 617 to 661 bp in all the 58 accessions of 57 species of the Aceraceae sampled and downloaded in this study. The aligned matrix consisted of 707 alignment positions with 6 recoded characters. After 97 ambiguous sites were excluded from the analysis, there were 190 potentially informative sites out of the 300 variable sites. A total of 1 617 most parsimonious trees was yielded from 1 000 replicates heuristic search with a

Table 1 Plant materials investigated in the analyses

Taxa (species)	Taxon abbr.	Section (sensu Xu)	Collection number	GenBank accessions	
				ITS	*trn*L-F
Acer crassum Hu & Cheng	CRAS	*Integrifolia*	Tian 9901	AF401135	AF401175
A. poliophyllum Fang & Wu	POLI		Tian 9903	AF401134	AF401174
A. paxii Franch.	PAXI		Tian 9915	AF401132	AF401172
A. buergerianum Miq.	BUER		Tian 9931	AF401133	AF401173
A. fabri Hance	FABR		See "Suh *et al.*, 2000"	AF241486 #	
A. palmatum Thunb. ex Murray	PALM	*Palmata*	Tian 9969	AF401123	AF401163
A. sieboldianum Miq.	SIEB		See "Ackerly & Donoghue, 1998"	AF020377 #	
A. shirasawanum Koidzumi	SHIR		See "Ackerly & Donoghue, 1998"	AF020376 #	
A. japonicum Thunb.	JAPO		See "Ackerly & Donoghue, 1998"	AF020374 #	
A. circinatum Pursh	CIRC		See "Ackerly & Donoghue, 1998"	AF020373 #	
A. takeshimense Nakai	TAKE		See "Suh *et al.*, 2000"	AF241504 #	
A. pseudosieboldianum Komarov	PSES		See "Suh *et al.*, 2000"	AF241501 #	
A. pubinerve Rehd. var. *apiferum* Fang & Chiu	PUBa		Tian 9933	AF401125	AF401165
A. miaoshanicum Fang	MIAO	*Microcarpa*	Tian 9934	AF401124	AF401164
A. cappadocicum Gled. var. *sinicum* Rehd.	CAPs		Tian 9942	AF401138	AF401178
A. platanoides L.	PLAT	*Platanoidea*	Li 119-74A	AF401136	AF401176
A. campestre L.	CAMP		Tian 19031010 * A	AF401158	AF401198
A. mono Maxim.	MONO		See "Suh *et al.*, 2000"	AF241491 #	
A. truncatum Bunge	TRUN		See "Suh *et al.*, 2000"	AF241507 #	
A. davidii Franch.	DAVI	*Macrantha*	Tian 9919	AF401144	AF401184
A. tegmentosum Maxim.	TEGM		Kim 99-1298	AF401145	AF401185
A. wardii Smith	WARD		Specimen from KUN 0587937	AF401159	AF401199
A. pensylvanicum L.	PENS		See "Ackerly & Donoghue, 1998"	AF020370 #	
A. rufinerve Sieb. & Zucc.	RUFI		See "Ackerly & Donoghue, 1998"	AF020371 #	
A. micranthum Sieb. & Zucc.	MICR		See "Ackerly & Donoghue, 1998"	AF020369 #	
A. tschonoskii Maxim.	TSCH		See "Ackerly & Donoghue, 1998"	AF020372 #	
A. tataricum L.	TATA	*Ginnala*	Li 002	AF401146	AF401186
A. ginnala Maxim.	GINN		Tian 9967	AF401147	AF401187
A. kungshanense Fang & Wu	KUNG	*Lithocarpa*	Tian 9946	AF401143	AF401183
A. diabolicum Blume	DIAB		See "Suh *et al.*, 2000"	AF241484 #	
A. macrophyllum Pursh	MACR	*Macrophylla*	Tian 19330500 * A	AF401156	AF401196
A. argutum Maxim.	ARGU	*Arguta*	Li 001	AF401153	AF401193
A. tetramerum Pax	TETR		Tian 9941	AF401154	AF401194
A. glabrum Torrey	GLAB	*Glabra*	Li 003	AF401139	AF401179
A. trautvetteri Medw.	TRAU	*Acer*	Li 135A-80A	AF401126	AF401166
A. caesium Wall. ex Brandis ssp. *giraldii* (Pax) Murry	CAEg		Tian 9939	AF406969	AF411087
A. pseudoplatanus L.	PSEP		See "Suh *et al.*, 2000"	AF241500 #	
A. monspessulanum L.	MONS	*Goniocarpa*	Li 004	AF401127	AF401167
A. opalus Mill.	OPAL		Li 12109A	AF401128	AF401168
A. saccharum Marshall	SACC	*Saccharodendron*	Tian 19460079 * A	AF401152	AF401192
A. saccharinum L.	SACI		Tian 19701662 * A	AF401151	AF401191
A. rubrum L.	RUBR	*Rubra*	Li 005	AF401150	AF401190
A. spicatum Lam.	SPIC		Li 007	AF401122	AF401162
A. carpinifolium Sieb. & Zucc.	CARP	*Carpinifolia*	Li 10959B	AF401148	AF401188
A. cissifolium (Sieb. & Zucc.) Koch.	CISS	*Cissifolia*	Li 006	AF401140	AF401180
A. henryi Pax	HENR		Tian 9901	AF401141	AF401181
A. negundo L.	NEGU	*Negundo*	Tian 9968	AF401142	AF401182
A. distylum Sieb. & Zucc.	DIST	*Distyla*	Tian 19481023 * A	AF401155	AF401195
A. nipponicum Hara	NIPP	*Parviflora*	Tian 19795193 * A	AF401157	AF401197
A. pentaphyllum Diels	PENT	*Pentaphylla*	Chen 2070	AF401137	AF401177
A. decandrum Merr.	DECA	*Hypsocarpa*	Specimen from KUN 0580004	AF401149	AF401189
A. laurinum Hasskarl	LAUR		See "Suh *et al.*, 2000"	AF241490 #	
A. triflorum Komarov	TRIF	*Trifoliata*	Liu 9962	AF401130	AF401170
A. griseum (Franch.) Pax	GRIS		Li 12488A	AF401131	AF401171
A. mandshuricum Maxim.	MAND		Liu 9962	AF401129	AF401169
Dipteronia sinensis Oliv.	SINE	Genus *Dipteronia*	Tian 9970	AF401121	AF401161
D. dyeriana Henry	DYER1		Tian 2064	AF401120	AF401160
	DYER2		Tian 2063	AF401120	AF401160
outgroups					
Aesculus wangii Hu	AESC	Hippocastanaceae	Tian 2100	AF406968	AF411085
Sapindus mukorossi Gaertn.	SAPI1	Sapindaceae	See "Cho *et al.*, 1997"	U89913(ITS1) # U95780(ITS2) #	
Sapindus delavayi (Franch.) Radlk.	SAPI2		Yang 2000		AF411086

Download from GenBank

Table 2 Sequence characteristics of ITS and *trn*L-F, separated and combined, in the Aceraceae

Comparison	ITS	*trn*L-F	Combined (ITS and *trn*L-F)
Number of sampling (within Aceraceae)	58	42	41
Length range (bp)	617 – 661	852 – 949	1495 – 1598
Aligned length (bp)	707	1005	1706
Recoded numbers	6	13	18
Number of excluded sites	97	0	56
G + C content range (%)	60.67	35.15	45.56
Sequence divergence (%)	0 – 16 64	0 – 4.83	0 – 6.96
Number of variable sites	300	245	486
Number of potentially informative sites	190	124	247
Number of most parsimonious trees	1617	555	4
Tree length	813	341	1002
Consistency index (CI)	0.540	0.824	0.601
Retention index (RI)	0.664	0.789	0.617
Rescaled consistency indices (RC)	0.359	0.651	0.371

length of 813 steps, CI = 0.540, RI = 0.664, and RC = 0.359. GC average content was high (60.67%, Table 2). Except for the sequences downloaded from GenBank with too many ambiguous sites, the pairwise sequence divergence ranged from 0 to 16.64% within the family and from 0 to 13.60% within *Acer*. The two sampled species of sect. *Microcarpa*, *A. miaoshanicum* and *A. pubinerve* var. *apiferum*, as well as the two accessions of *D. dyerana* shared the same sequence in the ITS region, respectively.

The strict consensus tree (Fig. 1) indicated that the Aceraceae was a monophyletic group with a bootstrap value of 96%. Within the family, the resolution was generally low. *Dipteronia* was resolved as paraphyletic and the whole *Acer* was monophyletic, but the internal support for this topology was low. Within *Acer*, the monophyly of most sections were strongly supported, except that within the sect. *Macrantha*, *A. tschonoskii*, *A. wardii* and *A. microanthum* of ser. *Micrantha* formed a strongly supported monophyletic group, which separated from the other four species of the section. Sect. *Rubra*, sect. *Acer*, sect. *Saccharodendron*, sect. *Trifoliata* and sect. *Intergrifolia*, as defined by Xu, were also not resolved as monophyletic. Some infrageneric relationships were also supported with high bootstrap values. Sect. *Palmata* and sect. *Microcarpa* formed a monophyletic clade with a bootstrap support of 96%, while sect. *Palmata* became paraphyletic if the two sampled species of sect. *Microcarpa* were excluded. *A. rubrum* (sect. *Rubra*) and *A. saccharinum* (sect. *Saccharodendron*) formed a monophyletic group with a bootstrap support of 99%. It appeared that sect. *Integrifolia*, sect. *Trifoliata* and sect. *Pentaphylla* were closely related, but the internal support was relatively low (51%). Sect. *Lithocarpa*, sect. *Macraphylla* and sect. *Platanoidea* might be also interrelated, but the bootstrap support was low, indicating that the clade may collapse.

2.2 Analyses of the *trn*L-F data set

The length of the *trn*L-F region ranged from 920 to 949 bp in the 42 samples of the Aceraceae, except for *Acer kungshanense* with 852 bp. The aligned data matrix (including 13 recoded indel characters) had 245 variable characters and 124 potentially informative characters out of a total of 1 018 characters. The heuristic search produced 555 most parsimonious trees with 341 steps, CI = 0.824, RI = 0.789, RC = 0.651. GC average content was low (35.14%), which was the character of cpDNA sequences (Table 2). Pairwise sequence divergence ranged from 0 to 4.03% within the genus *Acer* and from 0 to 4.83% within the family. Three species pairs of *Acer* showed the same sequences for *trn*L-F region, i.e. *A. miaoshanicum* vs. *A. pubinerve* var. *apiferum*, *A. monspessulanum* vs. *A. opalus*, and *A. davidii* vs. *A. wardii*, respectively. The sequences of the two accessions of *Dipteronia dyerana* were also completely coincident.

The strict consensus tree (Fig. 2) also strongly supported the monophyly of the Aceraceae with a bootstrap value of 100%. *D. sinensis* was basal to the rest of the Aceraceae. The two genera may not be monophyletic because *D. dyerana* nested within *Acer*, which was different from the tree based on ITS data. *A. carpinifolium*, which had been regarded as a very specialized taxon (even being treated as a monotypic subgenus), formed a second basal clade within the clade of *Acer* plus *D. dyerana*. However, the bootstrap support for this topology was relatively low (60%). Again, most sections were supported. Comparing with the ITS phylogeny, the bootstrap values were generally lower. There were also a few exceptions. *A. paxii* was not resolved within the sect. *Intergrifolia*. The monophyly of sect. *Trifoliata*, sect. *Rubra*, sect. *Saccharodendron* and sect. *Acer* was also not achieved. It appeared that sect. *Macrantha* may be monophyletic, but the relationship between *A. tegmentosum* and the strongly supported *A. davidii* and *A. wardii* clade may collapse. The clade with sect. *Palmata* and sect. *Microcarpa* was strongly supported with a bootstrap value of 99%. *A. rubrum* (sect. *Rubra*) and *A. saccharinum* (sect. *Saccharodendron*) again formed a monophyletic group, but the bootstrap support was relatively low (60%). However, the relationships among most sections were not resolved.

2.3 Analysis of the ITS and *trn*L-F combined data set

A total of 41 species of the Aceraceae (with one

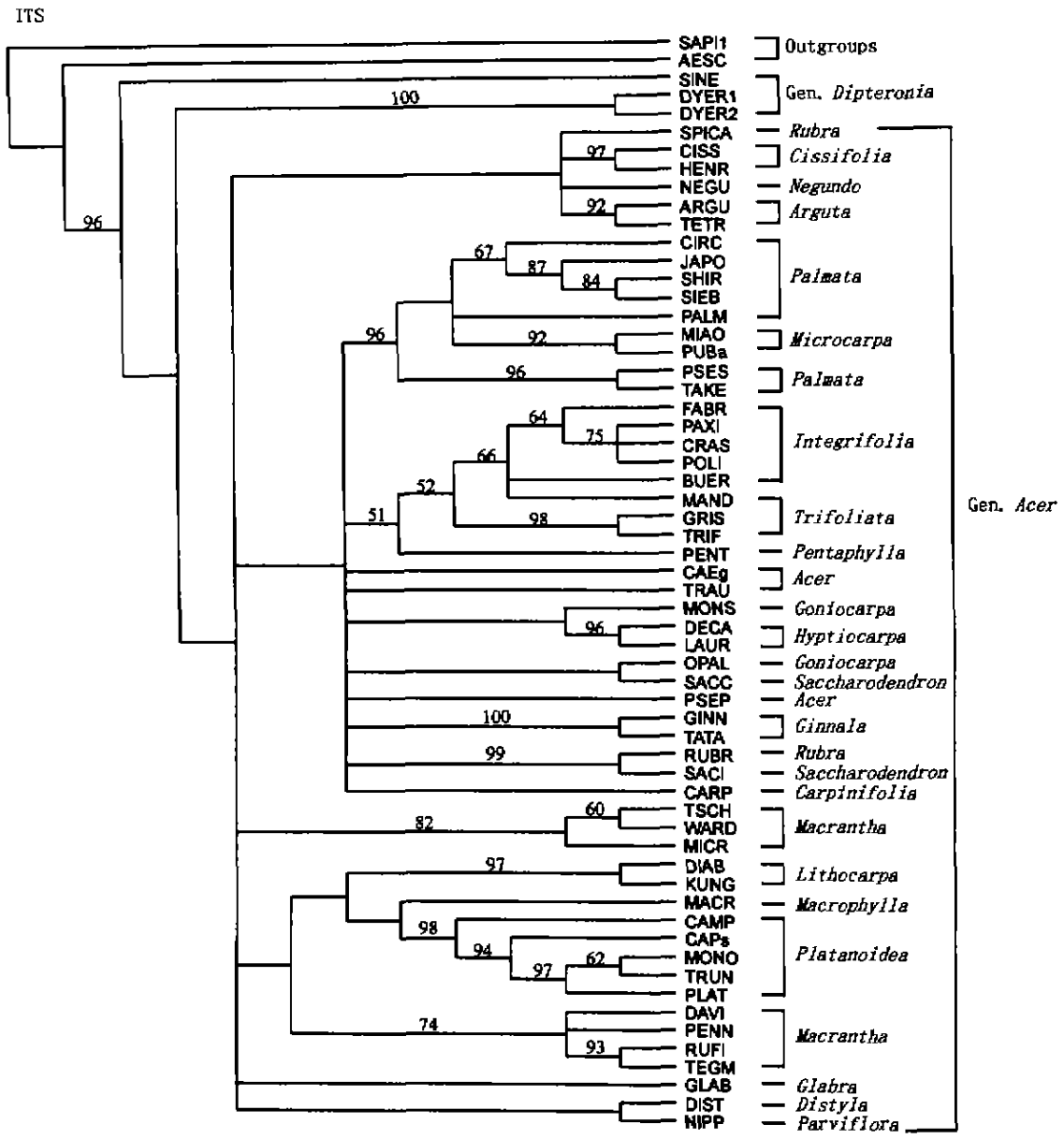

Fig.1. Strict consensus tree based on nuclear ITS data of Aceraceae. Length = 813, CI = 0.540, RI = 0.664, and RC = 0.359. Bootstrap values > 50 (%) are indicated above branches.

accession of *D. dyerana*) was analyzed based on the combined data set with *Aesculus wangii* (Hippocastanaceae) as outgroup. The length of the combined sequences ranged from 1 495 to 1 598 bp. Alignment of 42 sequences resulted in a matrix of 1 706 positions. Fifty-six ambiguous sites were excluded and 18 new scores of inferred gaps were recoded. There were 1 668 characters in the analysis of combined data set. Of 486 variable sites, 247 sites were potentially informative. The heuristic search produced 4 most parsimonious trees with 1 002 steps, CI = 0.601, RI = 0.617, and RC = 0.371. GC average content was 45.56%. Pairwise sequence divergence ranged from 0 to 6.96% within the family and from 0 to 5.97% within the genus *Acer* (Table 2).

The strict consensus tree (Fig. 3) and neighbor-joining (NJ) tree (Fig. 4) were generated. After bootstrap analyses, the discrepancies observed between the two trees were largely attributable to many poorly supported nodes. When these nodes (characterized by bootstrap values $\leq$50%) were treated as unresolved (i.e., they were collapsed to yield polytomies), the trees were consistent, with the only remaining area of discord being the relative positions of *A. carpinifolium*. In general, greater resolutions were achieved with higher bootstrap supports. Most sections were resolved as monophyletic with the exceptions of sect. *Acer*, sect. *Rubra*, sect. *Saccharodendron* and sect. *Trifoliata*. The relationships among the sections resolved better than the two trees based on separate data sets. The monophyly formed by sect. *Palmata* and sect. *Microcarpa* was strongly supported with a high bootstrap value (100%). *A. rubrum* (sect. *Rubra*) and *A. saccharinum* (sect. *Saccharodendron*) again formed a

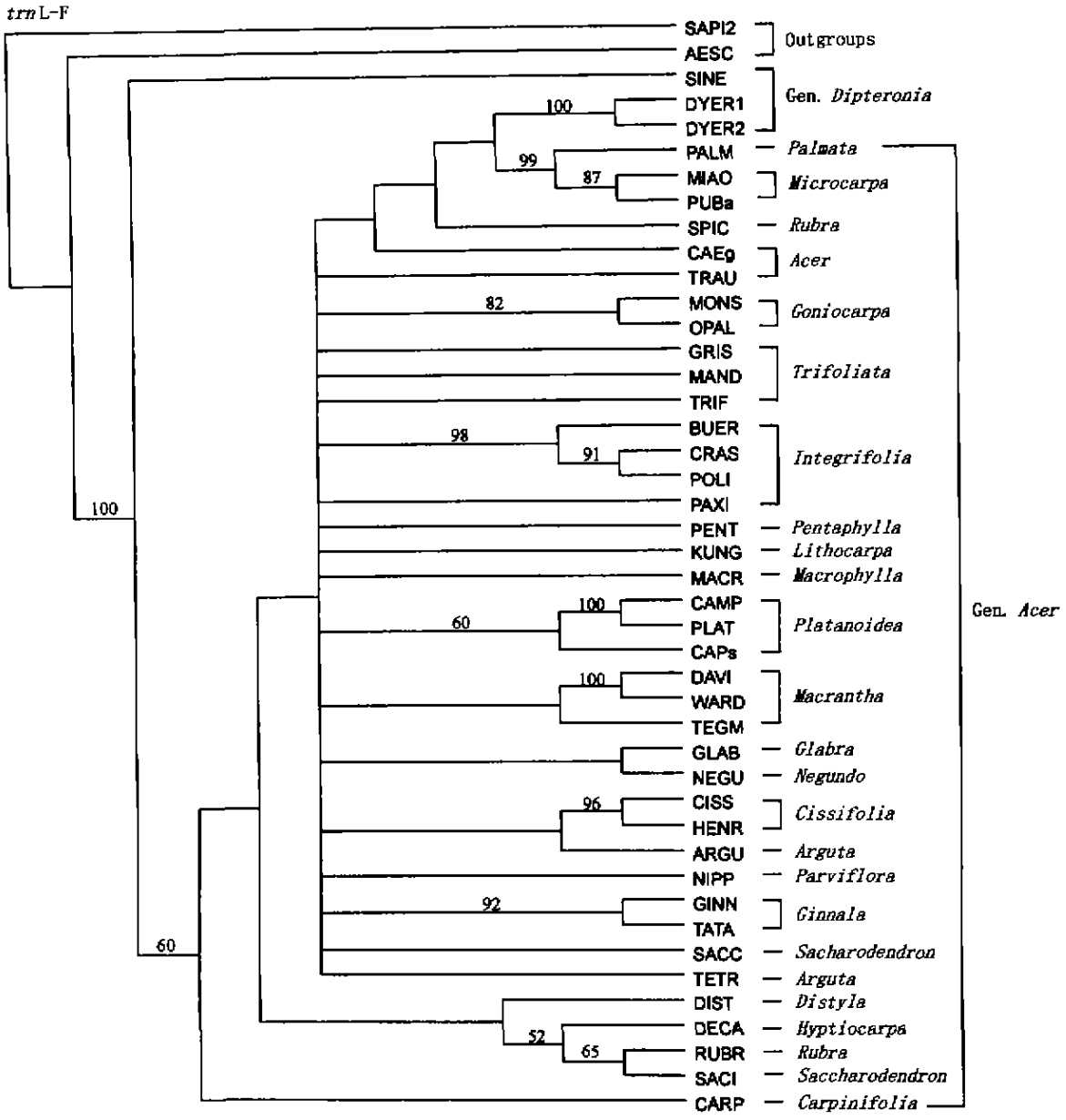

Fig.2. Strict consensus tree based on plastid *trn*L-F data of Aceraceae. Length = 341, CI = 0.824, RI = 0.789, RC = 0.651. Bootstrap values > 50 (%) are indicated above branches.

monophyletic group with a high bootstrap support (100%) in both trees. The close relationship between sect. *Macrophylla* and sect. *Platanoidea* was supported with a high bootstrap value (80%) in the MP tree. However, sect. *Platanoidea* was sister to sect. *Lithocarpa* with a high bootstrap support (80%) in the NJ tree. Within the sect. *Macrantha*, *A. davidii* and *A. tegmentosum* formed a monophyletic clade in both trees with high internal supports (71% – 96%), but their relationship with *A. wardii* was not supported by bootstrap analyses. In both trees, sect. *Trifoliata* was paraphyletic, yet together with sect. *Integrifolia* and sect. *Pentaphylla*, they formed a strongly supported clade with bootstrap value of 95% – 97%.

3 Discussion

3.1 Comparison of the two individual data sets and the combined data set

In the Aceraceae, the sequence divergence of ITS was much higher than that of *trn*L-F. The length of *trn*L-F region was nearly 1.5 times longer than that of ITS region, but the potentially informative sites of the *trn*L-F region (124 sites,) were 2/3 less than those of ITS data (190 sites, excluding 97 ambiguous sites). This indicates that the ITS region might have a faster evolutionary rate (approximately 3 – 4 times) than *trn*L-F in the Aceraceae. However, the ability of ITS to assess the infrafamilial phylogenetic relationships was not evidently higher

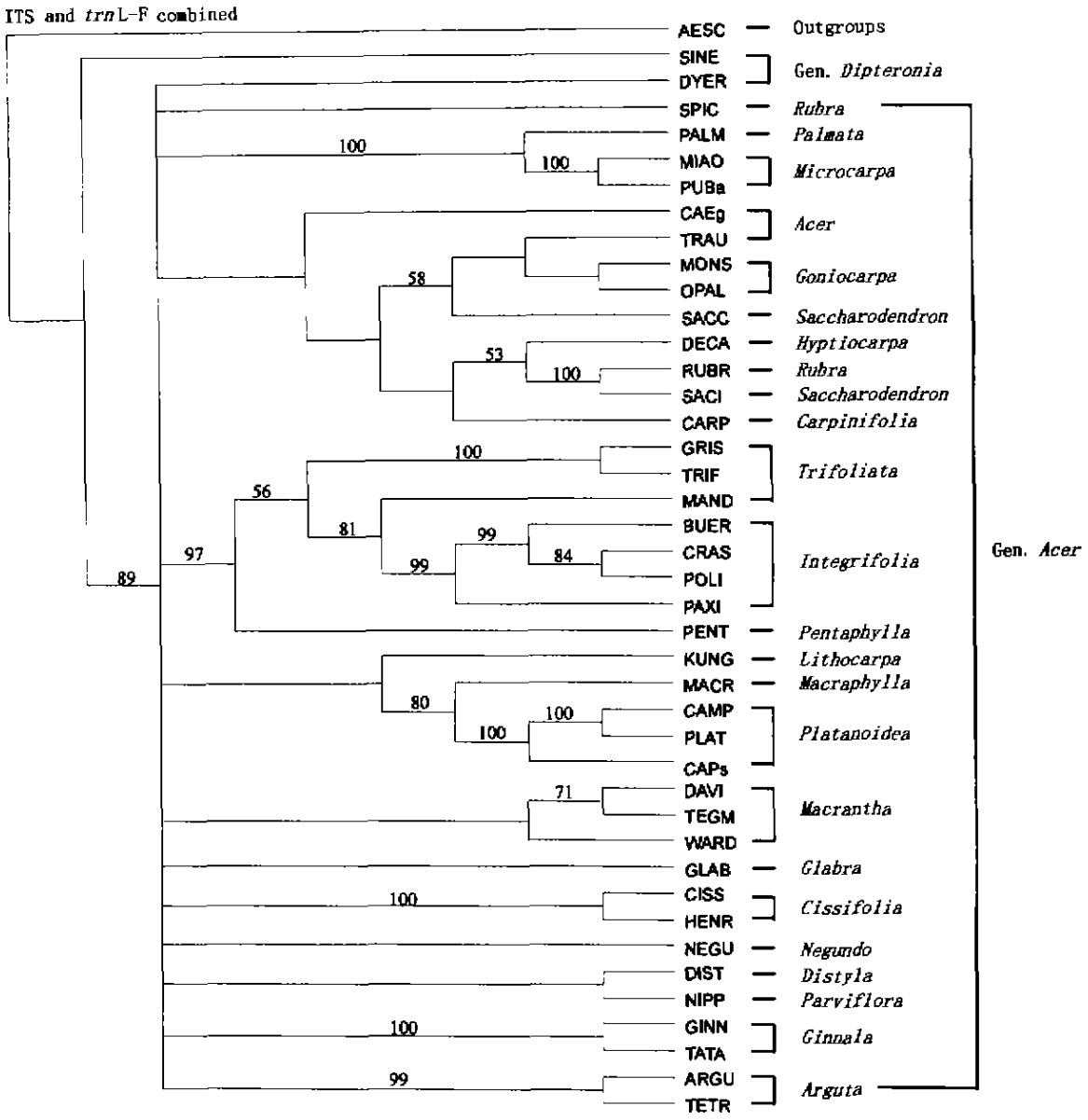

Fig.3. Strict consensus tree based on the combined ITS and *trn*L-F data. Length = 1002, CI = 0.601, RI = 0.617, and RC = 0.371. Bootstrap values > 50 (%) are indicated above branches.

than that of *trn*L-F, which may be because of the higher homoplasy of ITS sequences (CI = 0.540) than that of *trn*L-F sequences (CI = 0.824). In the combined data analysis, greater resolutions were achieved because of the more potentially informative sites (247 sites) and lower homoplasy (CI = 0.601).

3.2 Phylogeny of the Aceraceae

In all the analyses, the Aceraceae formed a robust monophyletic group with high bootstrap values, but, strangely, the monophyly of *Diperonia* was failed to achieve. In the published studies of the Aceraceae based on ITS data[26-29], as only one species of *Dipteronia*, *D. sinensis* was selected as outgroup, all sampled species of *Acer* seemed to be a monophyly. In the current study, both species of *Dipteronia* were included in the analyses, *D. dyerana* thus nested in *Acer*, although the internal support was generally low, indicating that the relationship may collapse. *D. sinensis* was basal to the rest of the Aceraceae in all analyses, and the independence of it was strongly supported by the three data sets. The position of *D. dyerana* was slightly different in the trees based on the two separate and combined data sets. In the ITS phylogeny, it formed a basal clade in Acearceae and this made the sampled species of *Acer* monophyletic. In the other analyses, *D. dyerana* was nested with sect. *Palmata* plus sect. *Microcarpa* (*trn*L-F tree), or with *A. spicata* (sect. *Rubra*) (combined analysis, NJ tree). Although these relationships did not have internal support, it made *Dipteronia* a possible paraphyletic genus, and this was quite contradictory. Morphologically, the two genera were obviously different from each other, such as the outline of samaras, the presence or absence of bud scales,

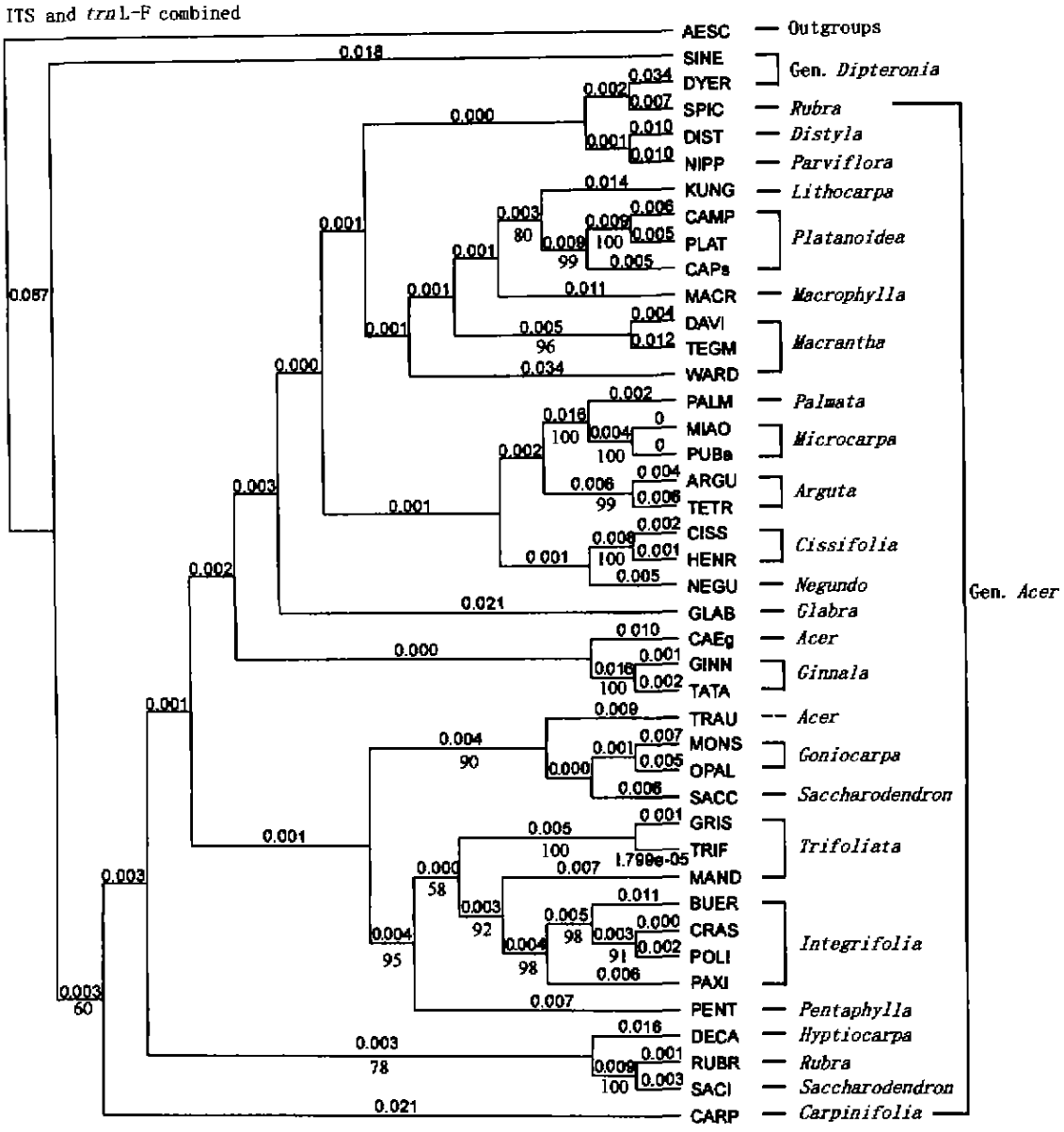

Fig.4. NJ tree based on the combined ITS and *trn*L-F data. Length = 1002, CI = 0.601, RI = 0.617, and RC = 0.371. Base substitutions and bootstrap values > 50 (%) are indicated above and below branches, respectively.

the arrangement of leaves and the chromsome number, while the two species of *Dipteronia* were very similar and distinguished only in glabrous or hairy of inflorescences and sizes of leaves and nutlets. Therefore, the relationship of the two species of *Dipteronia* may be hinted by the molecular analyses, but more evidence is needed.

Sect. *Palmata* and *Microcarpa* formed a monophyletic group in all analyses with strong internal support. In the ITS tree, sect. *Palmata* became paraphyletic with the exclusion of sect. *Microcarpa*. The two sections share many morphological characters such as typically palmate leaves, 4-paired bud scales, and corymbose inflorescences. Ogata[14] and de Jong[17] combined them into one section, sect. *Palmata*. However, Ogata's and de Jong's treatments by putting *A. fabri* and *A. crassum* in sect. *Palmata* as a third series[14, 17] were not supported here. In the ITS tree, both *A. fabri* and *A. crassum* were nested within species of sect. *Integrifolia* with relatively high bootstrap support (64% - 75%). In the ITS and *trn*L-F combined analyses, *A. crassum* was also nested within species of sect. *Integrifolia* with high bootstrap support (98% - 99%). This was also backed by previous pollen analysis[34]. Morphologically, sect. *Integrifolia* was characterized by the unlobed oblong or obovate leaves[13, 16], and *A. fabri* and *A. crassum* nicely fitted within it.

Sect. *Macrantha* was divided into three series, ser. *Tegmentosa* (i.e., *Macrantha*), ser. *Crataegifolia* (i.e., *Rufinervia*) and ser. *Micrantha* by some authors[14, 16, 19]. Ogata[14] inferred that ser. *Micrantha*,

with patent perianths, apiculate anthers and shallowly hollowed disc, was in a relatively remote relationship to the other two series. This was strongly supported by the ITS analysis. The section with seven species sampled, representing the three different series, was resolved as paraphyletic. Ser. *Micrantha* represented by three species (*A. tschonoskii*, *A. micranthum* and *A. wardii*) formed a monophyletic clade with high internal support. However, in the *trn*L-F analysis, *A. wardii* and *A. davidii* (ser. *Crataegifolia*) showed no difference in their sequence divergence. de Jong[17] divided *A. wardii* as a monotypic section, and this was supported by the combined analysis to some extant. More evidence would be needed to resolve a better affinity of this species.

Xu[16] put *A. saccharum* of sect. *Saccharina* and *A. sacchrinum* of sect. *Rubra*, which were established by Pax[10] and followed by some authors[14, 18, 19], in his sect. *Saccharodendron*. He also transferred *A. spicatum* from sect. *Spicata* (sensu Ogata) to sect. *Rubra*. These treatments were quite different from others[10, 14, 17-19]. *A. saccharinum* (sect. *Saccharodendron*) and *A. rubrum* (sect. *Rubra*) were recognized as a strongly supported clade in both ITS, *trn*L-F separate or/and combined analyses. This was not in consensus with Xu[16]. Actually, their close relationship was recognized[10, 14, 17-19]. These two species may be distinguished from other sections of *Acer*, remarkably in their completely sessile umbels and early-ripen fruits. In all analyses, *A. spicatum* was not grouped within sect. *Rubra*. In fact, this species does not share any common features with sect. *Rubra*. It seemed that Xu's sect. *Saccharodendron* and sect. *Rubra* were ill-defined.

A. saccharum was treated as a sole member of sect. *Saccharina* by Pax[10], Ogata[14] and Koidzumi[19]. In gross morphology, it is very similar to sect. *Acer* and sect. *Goniocarpa*, especially in the shape of multiseriate xylem rays. de Jong[17] united the three sections into sect. *Acer*. The relationships among *A. saccharum*, the two sampled species of sect. *Goniocarpa* and *A. trautvetteri* of sect. *Acer* were somewhat supported by the combined data analyses, with bootstrap values of 58% - 90%. In all analyses, the two sampled species of sect. *Acer* (*A. trautvetteri* and *A. caesium* ssp. *giraldii*) failed to form a monophyletic group. The clear sequence divergence between them may be caused by the geographical disjunction. *A. caesium* and its subspecies *giraldii* are endemic to the Himalaya, and other species of sect. *Acer* are distributed from W. Asia to S. Europe.

There was another robust clade, constituted of taxa of sect. *Integrifolia*, sect. *Trifoliata* and sect. *Pentaphylla*, although the validities of the three sections, as currently defined, were generally not supported. On the one hand, the three sections are mainly defined by their leaf shapes, which are, however, very variable; on the other hand, there are many common characters among them, such as imbricate bud scales, andromonoecious, 5-merous flower, and terminal compound inflorescences. After more sampling and analyses together with *trn*L-F data, sect. *Integrifolia* and sect. *Pentaphyllum* did not form a sister relationship. Thus de Jong's treatment of putting them into one section[17] was not supported, although this was supported by Suh *et al*[29]. Sect. *Trifoliata* also failed to form a monophyletic clade, as *A. mandshuricum* showed a closer relationship with sect. *Integrifolia* (as sister to it in the combined analyses). The molecular trees identified the two series, *Grisea* (with *A. triflorum* and *A. griseum* sampled) and *Mandshurica* (with *A. mandshuricum* sampled).

Morphologically, sect. *Lithocarpa*, sect. *Macraphylla* and sect. *Platanoidea* were characterized by the presence of latex in leaves and young twigs. Their relationships were somewhat supported in the ITS phylogeny but with low bootstrap value. The sister relationship between sect. *Macrophylla* and sect. *Platanoidea* was strongly supported in the MP analysis of the combined data, while the sister relationship between sect. *Platanoidea* and sect. *Lithocarpa* was also indicated with high bootstrap value in the NJ analysis. Therefore, the combination of sect. *Lithocarpa* and *Macraphylla*, as posed by de Jong[17], should be carefully evaluated. *A. campestre* was treated in its own section, sect. *Campestria*[11, 12, 14, 18]. The close relationship between sect. *Campestria* and sect. *Platanoidea* was supported by the resemblance of general morphology[14], cambial peroxidase isoenzymes[35], seed protein[19] and DNA RFLP[22]. These more or less supported the combination of the two sections. Our analyses showed that sect. *Platanoidea* (including species of sect. *Campestria* sensu Ogata) formed a robust clade.

A. carpinifolium is exclusively characterized by penninerved leaves, remarkably big wood rays, finely striate-reticulate pollen and special protein components of the seed[14, 16, 19, 34], which make it separate from all the other species in *Acer* as a specialized monotypic group. This independence of it was supported by the *trn*L-F data and the NJ analysis of the combined data, but the internal supports were relatively low (60%).

Comparing with the published ITS studies of Suh *et al*[29], the close relationship between sect. *Distyla* and sect. *Parviflora*, and between sect. *Hyptiocarpa* and sect. *Rubra* were also supported by our molecular data sets, which were alike to the results of Suh *et al*[29], but the bootstrap values were generally very low, except that the monophyly formed by sect. *Hyptiocarpa* and sect. *Rubra* (sensu Ogata) was supported with a bootstrap value of 78% in the NJ analysis of the combined data. Unlike the previous ITS analysis, the sister relationships between sect. *Cissifolia* and sect. *Negundo*, and between sect. *Macrantha* and sect. *Arguta*, were not detected in our analyses.

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References:

[1] Cronquist A. The Evolution and Classification of Flowering Plants. 2nd ed. New York: The New York Botanical Garden, 1988.

[2] Dahlgren R. The last Dahlgrenogram, system of classification of the dicotyledons. Tan K. The Davis and Hedge Festschrift: Plant Taxonomy, Phytogeography and Related Subjects. Edinburgh: Edinburgh University Press, 1989. 249 – 260.

[3] Takhtajan A L. Diversity and Classification of Flowering Plants. New York: Columbia University Press, 1997.

[4] Gadek P A, Fernando E S, Quinn C J, Hoot S B, Terrazas T, Sheahan M C, Chase M W. Sapindales: Molecular delimitation and infraordinal groups. *Amer J Bot*, 1996, **83**: 802 – 811.

[5] Fay M F, Bayer C, Alverson W S, de Bruijn A Y, Chase M W. Plastid *rbc*L sequence data indicate a close affinity between *Dugedendron* and *Bixa*. *Taxon*, 1998, **47**:43 – 50.

[6] Thorne R F. An updated phylogenetic classification of the flowering plants. *Aliso*, 1992. **13**:365 – 389.

[7] The Angiosperm Phylogeny Group (APG). An ordinal classification for the families of flowering plants. *Ann MO Bot Gard*, 1998. **85**:531 – 553.

[8] Xu T-Z (徐廷志). Phytogeography of the family Aceraceae. *Acta Bot Yunnan* (云南植物研究), 1996, **18**:43 – 50. (in Chinese with English abstract)

[9] Fang W-P (方文培). Aceraceae. Fang W-P (方文培), Bao S-Y (包士英), Zhuang X (庄璇), Xu T-Z (徐廷志). Flora Reipublicae Porulasis Sinicae. Beijing: Science Press, 1981, **46**:66 – 273.

[10] Pax F. Monographie der Gattung *Acer*. *Bot Jahrb Engler*, 1885 – 1886, **6**: 287 – 373 (1885); **7**: 177 – 263 (1886).

[11] Pax F. Aceraceae. Engler's Pflanzenreich IV, 1902, **163**: 1 – 89.

[12] Rehder A. Aceraceae. Rehder A. Bibliography of Cultivated Trees and Shrubs. 1949. 412 – 429.

[13] Fang W-P (方文培). Revisio Taxorum Aceracearum Sunicarum. *Acta Phytotax Sin* (植物分类学报), 1966, **11**: 139 – 189. (in Chinese with English abstract)

[14] Ogata K. A systematic study of the genus *Acer*. *Bull Tokyo Univ Forests*, 1967, **63**:89 – 206.

[15] Murray E. A monograph of the *Acer*. Ph. D. dissertation. Pennsylvania State University, 1970.

[16] Xu T-Z (徐廷志). A new system of *Acer*. *Acta Bot Yunnan* (云南植物研究), 1996, **18**:277 – 292. (in Chinese with English abstract)

[17] De Jong P C. Taxonomy and reproductive biology of maples. van Gelderen D M, de Jong P C, Oterdoom H J. Maples of the World. Portland: Timber Press, 1994. 69 – 103.

[18] Koidzumi G. Revisio Averacearum Japonicarum. *J Coll Sci Imp Univ Tokyo*, 1911, **32**:1 – 75.

[19] Momotani Y. Taxonomic study of the genus *Acer*, with special reference to the seed proteins. Ⅲ. System of Aceraceae. *Mem Coll Sci Univ Kyoto* Ser. B, 1962, **29**:177 – 189.

[20] Wolfe J A, Tanai T. Systematics, phylogeny and distribution of *Acer* (Maples) in the Cenozoic of Western North America. *J Fac Sci, Hokkaido Univ* Ser. IV, 1987, **22**:1 – 246.

[21] Xu T-Z (徐廷志). The systematic evolution and distribution of the Genus *Acer*. *Acta Bot Yunnan* (云南植物研究). 1998, **20**:383 – 393. (in Chinese with English abstract)

[22] Hasebe M, Ando T, Iwatsuki K. Intrageneric relationships of maple trees based on the chloroplast DNA restriction fragment length polymorphisms. *J Plant Res*, 1998, **111**:441 – 451.

[23] Chase M W, Soltis D E, Olmstead R G, Morgan D, Les D H, Mishler B D, Duvall M R, Price R A, Hills H G, Qiu Y-L, Kron K A, Rettig J H, Conti E, Palmer J D, Manhart J R, Sytsma K J, Michaels H J, Kress W J, Karol K G, Clark W D, Hedrén M, Gaut B S, Jansen R K, Kim K-J, Wimpee C F, Smith J F, Furnier G R, Strauss S H, Xiang Q-Y, Plunkett G M, Soltis P S, Swensen S M, Williams S E, Gadek P A, Quinn C J, Eguiarte L E, Golenberg E, Learn G H Jr, Graham S W, Barrett S C H, Dayanandan S, Albert V A. Phylogenetics of seed plants: an analysis of nucleotide sequences from plastid gene *rbc*L. *Ann Missouri Bot Gard*, 1993, **80**:528 – 580.

[24] Tian X (田欣), Li D-Z (李德铢). Application of DNA sequences in plant phylogenetic study. *Acta Bot Yunnan* (云南植物研究), 2002. **24**:170 – 184. (in Chinese with English abstract)

[25] Taberlet P, Gielly L, Pautou G, Bouvet J. Universal primers for amplification of three non-coding regions of chloroplast DNA. *Plant Mol Biol*, 1991, **17**:1105 – 1109.

[26] Ackerly D D, Donoghue M J. Leaf size, sapling allometry and Corner's rules: a phylogenetic study of correlated evolution in maples (*Acer*). *Am Nat*, 1998, **152**:767 – 791.

[27] Cho H-J, Kim S, Suh Y, Park C-W. ITS sequences of some *Acer* species and phylogenetic implication (in Korean). *Sigmul Buntyu Hag-hoeji*, 1997, **26**:271 – 291.

[28] Suh Y, Cho H-J, Kim S, Park C-W. Comparative analysis of ITS sequences from *Acer* species (Aceraceae) in Korea. *J Plant Biol*, 1996, **39**:1 – 8.

[29] Suh Y, Heo K, Park C-W. Phylogenetic relationships of maples (*Acer* L.; Aceraceae) implied by nuclear ribosomal ITS sequences. *J Plant Res*, 2000, **113**:193 – 202.

[30] Doyle J J, Doyle J L. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochem Bull*, 1987, **19**:11 – 15.

[31] Su Y-J (苏应娟), Wang T (王艇), Huang C (黄超), Zhu J-M (朱建明), Zhou Q (周勤). RAPD analysis of different population of *Dacydium pierrei*. *Acta Sci Nat Univ Sunyatseni* (中山大学学报), 1999, **38**:98 – 101. (in Chinese with English abstract)

[32] White T J, Bruns T, Lee S, Taylor J. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. Innis M, Gelfand D, Sninsky J, White T. PCR Protocols: A Guide to Methods and Application. San Diego: Academic Press, 1990. 315 – 322.

[33] Swofford D L. Paup: Phylogenetic analysis using parsimony, ver. 4. 0b8. Sinauer, Assiciates, Massachusetts, USA, 2001.

[34] Tian X (田欣), Jin Q-J (金巧军), Li D-Z (李德铢), Wei Z-X (韦仲新), Xu T-Z (徐廷志). Pollen morphology of Aceraceae and its systematic implications. *Acta Bot Yunnan* (云南植物研究), 2001, **23**:457 – 465. (in Chinese with English abstract)

[35] Santamour F S. Cambial peroxidase isoenzymes in relation to systematics of *Acer*. *Bull Torrey Bot Club*, 1982, **109**:152 – 161.

基于 ITS 与 *trn*L-F 序列探讨槭树科的系统发育

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摘要： 报道了槭树科 41 种(其中槭属 39 种)植物的 *trn*L-F 和 ITS 序列(其中部分种的 ITS 序列为重新测定)，以期通过分子手段对槭树科内部尤其是复杂的槭属的系统发育关系进行重建。以无患子科和七叶树科为外类群，基于对 57 个种单独的 ITS 序列(包括从 GenBank 下载的 16 种的序列)、41 种 *trn*L-F 序列及 41 种两者序列的联合数据，分别采用最大简约法(Maximum Parsimony Method)和邻接法(Neighbor-Joining Method)对槭树科的系统发育进行了分析。结果显示，整个槭树科为一单系类群；金钱槭位于槭树科的基部；但由于云南金钱槭(*Dipteronia dyerana*)聚在了槭属内部，认为金钱槭属和槭属均可能是非单系类群；槭属内组间关系的支持率普遍较低，但多数组的组内关系得到了较好的支持。将两个片段结合比单独的 ITS 或 *trn*L-F 分析能更好地解决槭属内部的系统关系，其中 sect. *Palmata* 和 sect. *Microcarpa*、sect. *Platanoidea*、sect. *Lithocarpa* 和 sect. *Macrophylla*、sect. *Integrifolia*、sect. *Trifoliata* 和 sect. *Pentaphylla*，以及 sect. *Acer*、sect. *Goniocarpa* 和 sect. *Saccharina* (sensu Ogata)的组间亲缘关系得到了一定的支持，但对其中部分组的划分可能应做进一步调整。重新评价了徐廷志系统中对 sect. *Rubra* 和 sect. *Saccharodendron* 的处理。

关键词： 槭树科；系统发育；ITS；*trn*L-F

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