

PHYLOGENETIC ANALYSIS OF THE GRAPE FAMILY (VITACEAE) BASED ON THREE CHLOROPLAST MARKERS¹

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Seventy-nine species representing 12 genera of Vitaceae were sequenced for the *trnL-F* spacer, 37 of which were subsequently sequenced for the *atpB-rbcL* spacer and the *rps16* intron. Phylogenetic analysis of the combined data provided a fairly robust phylogeny for Vitaceae. *Cayratia*, *Tetrastigma*, and *Cyphostemma* form a clade. *Cyphostemma* and *Tetrastigma* are each monophyletic, and *Cayratia* may be paraphyletic. *Ampelopsis* is paraphyletic with the African *Rhoicissus* and the South American *Cissus striata* nested within it. The pinnately leaved *Ampelopsis* form a subclade, and the simple and palmately leaved *Ampelopsis* constitutes another with both subclades containing Asian and American species. Species of *Cissus* from Asia and Central America are monophyletic, but the South American *C. striata* does not group with other *Cissus* species. The Asian endemic *Nothocissus* and *Pterisanthes* form a clade with Asian *Ampelocissus*, and *A. javalensis* from Central America is sister to this clade. *Vitis* is monophyletic and forms a larger clade with *Ampelocissus*, *Pterisanthes*, and *Nothocissus*. The eastern Asian and North American disjunct *Parthenocissus* forms a clade with *Yua austro-orientalis*, a species of a small newly recognized genus from China to eastern Himalaya. Vitaceae show complex multiple intercontinental relationships within the northern hemisphere and between northern and southern hemispheres.

Key words: *atpB-rbcL* spacer; chloroplast DNA; *rps16* intron; *trnL-F*; phylogeny; Vitaceae.

Vitaceae (the grape family) consist of approximately 14 genera and about 900 species (Table 1) primarily distributed in tropical regions in Asia, Africa, Australia, the neotropics, and the Pacific islands, with a few genera in temperate regions (*Vitis*, *Parthenocissus*, and *Ampelopsis*). *Ampelopsis* and *Parthenocissus* show a disjunct distribution in eastern Asia and eastern North America extending to Mexico. The family is well known economically for grapes, wine, and raisins (especially *Vitis vinifera*, as well as several other species and hybrids of *Vitis*).

The phylogenetic position of Vitaceae within the eudicots has been controversial. Vitaceae are most closely related to the monogeneric Leeaceae, and they share several important morphological synapomorphies including presence of “pearl” glands and raphides (Wen, in press). Most workers have now excluded *Leea* from Vitaceae and recognized the family Leeaceae (e.g., Planchon, 1887; Suessenguth, 1953b; Ridsdale, 1974; Shetty and Singh, 2000; Latiff, 2001a; Ren et al., 2003; Wen, in press), although APG (1998) and APGII (2003) placed *Leea* in Vitaceae. Several workers recognized *Leea* as comprising the subfamily Leoideae within Vitaceae (see Gilg, 1896; Gilg and Brandt, 1911). Vitaceae were usually placed in the order Rhamnales along with Rhamnaceae (e.g., Kirchheimer, 1939; Cronquist, 1981, 1988). Takhtajan (1997) recognized the order Vitales as consisting of Vitaceae and Leeaceae and considered the Vitales as highly isolated and as a sole

member of the superorder Vitanae in the Rosidae. Chase et al. (1993) reported that the Vitaceae-Leeaceae clade was sister to Dilleniaceae based on *rbcL* sequence data. The three-gene (*atpB*, *rbcL*, and 18S) analysis of Soltis et al. (2000) placed Vitaceae sister to the rest of the rosids, but did not confirm a close relationship between Vitaceae and Dilleniaceae. APG II (2003) added Vitaceae to the rosids, but left it unassigned to order.

Vitaceae are usually woody climbers or herbaceous vines or small succulents with leaf-opposed tendrils. These tendrils are considered to be modified shoots or inflorescences (Tucker and Hoefert, 1968; Gerrath et al., 2001). Leaves in Vitaceae commonly bear “pearl” glands, and these glands are usually small spherical epidermal structures with a short stalk. Inflorescences of Vitaceae are typically panicle systems (Troll, 1969). Flowers of Vitaceae are relatively uniform in morphology at maturity and not particularly informative in systematic studies. Nectary morphology is highly variable in Vitaceae and has been emphasized in defining genera (Suessenguth, 1953a; Gerrath et al., 2004). The floral disk is a typical nectariferous, saucer-like structure in *Ampelopsis*. In *Vitis*, the disk is morphologically evident at maturity, but is not known to produce nectar. In *Parthenocissus*, it is not morphologically recognizable, but there is some nectar production. The disk is initiated from the base of the ovary. Externally, the seeds are unusual in comparison with those of other angiosperms in that they have a cordlike raphe on the adaxial surface extending from the hilum to the seed apex and continuing onto the abaxial surface. A groove is commonly present on both sides of the raphe, and a chalazal knot (a depressed to raised region) is on the abaxial surface. The endosperm rumination is highly complex in Vitaceae (Periasamy, 1962). Detailed systematic vegetative and floral developmental studies have been conducted by Gerrath,

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TABLE 1. Generic diversity and distribution of Vitaceae (data extracted from Wen, in press).

Genus	No. of species	Distribution
<i>Acareosperma</i> Gagnepain	1	Laos
<i>Ampelocissus</i> Planch.	95	Africa, tropical Asia, and Australia with only four species in Central America and the Caribbean
<i>Ampelopsis</i> Michx.	25	Temperate to subtropical Asia (ca. 20 spp.) and North and Central America (3 spp.) and 2 in W Asia
<i>Cayratia</i> Juss.	63	Tropical and subtropical Asia, Africa, Australia, and the Pacific Islands
<i>Cissus</i> L.	350	All tropical regions with a few extending into the temperate zone
<i>Clematicissus</i> Planch.	1	Western Australia
<i>Cyphostemma</i> (Planch.) Alston	200	Mainly in Africa and Madagascar with a few species in India and Sri Lanka extending into Thailand
<i>Nothocissus</i> (Miq.) Latiff	5	Peninsular Malaysia, Sumatra, Bangka, Borneo, and Papua New Guinea.
<i>Parthenocissus</i> Planch.	15	12 in E Asia with one species extending into the western Ghats, India and three in North America.
<i>Pterisanthes</i> Blume	20	Malay Peninsula, Borneo, Sumatra, Java, Philippines, and peninsular Thailand.
<i>Rhoicissus</i> Planch.	12	Tropical and South Africa
<i>Tetrastigma</i> (Miq.) Planch.	95	Primarily in tropical and subtropical Asia with five species in Australia
<i>Vitis</i> L.	60	Mostly temperate regions of the northern hemisphere, 1 sp. extending into South America.
<i>Yua</i> C. L. Li	3	Subtropical China, India (Assam) and central Nepal

Poslusny, and their collaborators (e.g., Gerrath and Poslusny, 1988a, b, c; 1989a, b, c; Gerrath et al., 1998, 2001).

The generic delimitation in Vitaceae has been problematic. Linnaeus (1753) only recognized two genera: *Cissus* and *Vitis* in the family. Hooker (1862) included *Vitis*, *Pterisanthes*, and *Leea* in the Ampelideae (=Vitaceae), treating *Cissus* as a synonym of *Vitis*. Baker (1871) and Lawson (1875) followed Hooker in merging *Cissus* with *Vitis*. Planchon (1887) provided a worldwide monograph of the family and defined most genera recognized today. He enumerated 10 genera in Ampelideae (=Vitaceae) and classified *Vitis*, *Ampelocissus*, and *Cissus* into subgeneric groups (sections and series for *Vitis* and sections for the latter two). Planchon's classification was largely followed by later workers (Viala, 1910; Suessenguth, 1953a; Brizicky, 1965; Latiff, 1982, 1983, 2001a, b; Li, 1998; Lombardi, 1997, 2000; Shetty and Singh, 2000). Several genera were described subsequently, e.g., *Acareosperma* (Gagnepain, 1919), *Pterocissus* (Urban, 1926, now treated under the synonymy of *Cissus*), *Cyphostemma* (Alston, 1931), *Nothocissus* (Latiff, 1982), and *Yua* (Li, 1990). *Cyphostemma* was included in *Cissus* by Suessenguth (1953a); however, Descoings (1960) argued for the recognition of *Cyphostemma* and pointed out the distinctions between the two, especially concerning the bud and corolla shape. The genus has been subsequently recognized by later workers (Mabberley, 1995; Li, 1998; Shetty and Singh, 2000; Latiff, 2001a). *Cissus* is characterized by its inflorescence as a leaf-opposite compound cyme, its four-merous flowers, and a continuous cupular floral disk, but was recently shown to be polyphyletic (Rossetto et al., 2002).

Ingrouille et al. (2002) sequenced the *rbcl* gene for 19 species of 10 genera in Vitaceae and one in Leeaceae. They showed that (1) Leeaceae are sister to Vitaceae s. str.; (2) *Ampelopsis* is basally branching, *Cissus*, *Ampelocissus*, and *Clematicissus* are intermediate, and *Vitis* most derived; (3) *Vitis* forms a clade with *Cayratia*, *Cyphostemma*, *Parthenocissus*, and *Tetrastigma*; (4) *Cayratia* and *Tetrastigma* form a weakly supported clade; and (5) *Vitis* is paraphyletic, and *Ampelopsis* is polyphyletic.

Rossetto et al. (2002) investigated 30 species belonging to five genera (*Ampelocissus*, *Cayratia*, *Cissus*, *Clematicissus*, and *Tetrastigma*), which mostly included taxa from Australia, a few species of *Vitis*, and a species of *Leea* (Leeaceae) using the chloroplast *trnL* intron and nuclear ribosomal ITS1 sequences. They showed that *Cissus* is polyphyletic and at

least five species should be separated from the genus. *Cissus opaca* is grouped with *Clematicissus*, four Australian species (*C. antarctica*, *C. hypoglaucia*, *C. oblonga*, and *C. sterculiifolia*) form a clade with *Vitis*. Other *Cissus* species form a large clade. *Cayratia* is paraphyletic and constitutes a well-supported clade with *Tetrastigma*.

Phylogenetic analyses with a broader sampling of taxa and markers are needed to further understand the relationships within Vitaceae and test the generic delimitation within the family. Objectives of our paper are to (1) construct the phylogeny of Vitaceae using three chloroplast markers and (2) test the generic delimitations in the family.

MATERIALS AND METHODS

Taxon sampling—A total of 108 accessions representing 79 species of Vitaceae and 12 outgroup taxa were sequenced for the *trnL* intron and the adjacent *trnL-F* spacer (Appendix). Our sampling well represents the taxonomic diversity of the family with 12 of the 14 recognized genera included. Only two genera, the monotypic *Acareosperma* from Laos (Gagnepain, 1919) and *Clematicissus* (Jackes, 1989) from Western Australia, were not sampled. The closely related Leeaceae plus several members of Rhamnaceae and Dilleniaceae were selected as outgroups due to the highly isolated position of the Vitaceae-Leeaceae clade and based on the recent *rbcl* and 18S data (Chase et al., 1993; Soltis et al., 2000).

Because the "backbone" of Vitaceae was poorly resolved in the *trnL-F* trees, a subset of 39 accessions (Appendix) was sequenced for the *atpB-rbcL* intergenic spacer and the *rps16* intron. The subset of samples covers the taxonomic diversity of each genus. In addition to *Dillenia*, three species of *Leea* (Leeaceae) were used as outgroups because the data of *trnL-F* sequences strongly supported the sister position of *Leea* to Vitaceae s. s. Several of the 39 accessions were not sequenced for one of the two additional chloroplast markers due to difficulties in PCR amplification, but all 39 samples were included in the phylogenetic analysis.

DNA extraction, amplification, and sequencing—DNAs of all samples were extracted from silica-gel-dried leaves following a modified CTAB buffer method (Doyle and Doyle, 1987). Leaves were ground into fine powder with sand at room temperature and incubated with 4× CTAB buffer, with 2% PVP (polyvinyl pyrrolidone), 2% PEG (polyethylene glycol), 1% sodium bisulfite, and 2% 2-mercaptoethanol at 60°C for 120 min. DNA was further purified with SEVAG (24 : 1 chloroform : isoamyl alcohol) twice, and was then precipitated with isopropanol once, followed by precipitation with 5.0 M NaCl and a wash with ethanol and a final precipitation with 3.0 M NaOAc (pH 4.8) and a wash with ethanol.

The *trnL* intron and *trnL-trnF* intergenic spacer were amplified using the primers of Taberlet et al. (1991). An additional primer, *trnL-F'* (5'-ATT TTC AGT CCT CTG CTC TAC C-3'), was designed for Vitaceae because many species failed to get amplified with the primer *f* (A50272) of Taberlet et al.,

1991. Amplification reactions were performed in a 20- μ L volume containing 1.5 mmol/L MgCl₂, 0.2 mmol/L of each dNTP, 0.2 μ mol/L each primer, 1 U of *Taq* polymerase, and about 25 ng of DNA template. PCR was done on a Peltier Thermal Cycler DNA engine DYAD (MJ Research Incorporated, Watertown, Massachusetts) starting at 94°C for 2 min, followed by 38 cycles of 1 min at 94°C, 1 min at 50°C, 2 min at 72°C, and ended with a final extension of 5 min at 72°C. PCR products were run in agarose gel. The gel containing desired fragments were cut and treated with gelase to digest the gel. Sequencing of both strands was done on an ABI 3100 Genetic Analyzer (Applied Biosystems, Foster City, California, USA) using ABI BigDye version 3.0. PCR profile of sequencing was 25 cycles of 30 s at 96°C, 15 s at 50°C, and 4 min at 60°C. RAMP was set at 1°C/s. The *atpB-rbcL* spacer and the *rps16* intron were amplified and sequenced based on protocols in Bremer et al. (2002) and Nie et al. (2005), respectively. DNA sequences were assembled using SEQUENCHER v3.1 (Gene Codes Corp., Ann Arbor, Michigan, USA). Sequence alignment was initially performed using Clustal X version 1.81 (Thompson et al. 1997) with the gap-opening penalty set at 10 and the gap extension penalty at 3. Sequence alignments were manually adjusted using BioEdit (Hall, 1999).

Phylogenetic analyses—Four data sets were analyzed to infer relationships of Vitaceae: (1) a *trnL* intron and *trnL-F* spacer matrix of 108 accessions with *Leea* and *Rhamnus* as the outgroups; (2) an *atpB-rbcL* spacer matrix with 28 species of Vitaceae and two species of *Leea* as the outgroups; (3) a *rps16* intron of 32 taxa including two of *Leea* and one of *Dillenia* species as the outgroups; and (4) the combined chloroplast data sets with 37 taxa with *Leea* and *Dillenia* as the outgroups. In each analysis, all the gaps are treated as new characters, except for ambiguous gaps. Ambiguous gaps were the ones located in regions with tandem repeats of one or a few nucleotides, which may cause difficulties in recognizing homology. Overlapping gaps were treated as independent characters; in that case, the position of smaller gaps was treated as missing data in the accessions possessing larger gaps. Furthermore, phylogenetic analyses with all the gaps treated as missing data were executed for all four data sets. The trees from the analyses with gaps as missing data were congruent with those with gaps treated as new characters, but the resolution was lower.

Parsimony analyses were conducted using PAUP* version 4.0b10 (Swofford, 2003) with heuristic search, random taxon addition, tree-bisection-reconnection (TBR) branch swapping, and the Mulpars and Steepest descent options. Bootstrap analyses (Felsenstein, 1985) were performed using 500 replicates, with the random taxon addition sequence limited to 10 and branch swapping limited to 10 000 000 rearrangements per replicate.

Nucleotide substitution model parameters were determined for the cpDNA data sets using MODELTEST version 3.0 (Posada and Crandall, 1998). A heuristic maximum likelihood search with TBR branch swapping was then conducted. Branches were collapsed (creating polytomies) if the branch length was less than or equal to 1e-08, and the random taxon addition sequence was limited to 100.

Bayesian analyses (Rannala and Yang, 1996; Mau et al., 1999) were carried out using MrBayes version 3.0b3 (Huelsenbeck and Ronquist, 2001) with the model parameters determined from the MODELTEST. Bayesian analyses started from random trees and employed four Markov chain Monte Carlo (mcmc) runs, monitored over one million generations, re-sampling trees every 100 generations. Runs were repeated twice to confirm results. The resulting log likelihood and number of generations were plotted to determine the point after which the log likelihoods had stabilized. After discarding the trees saved prior to this point as burn-in, the remaining trees were imported into PAUP* and a 50% majority-rule consensus tree was produced to obtain posterior probabilities of the clades.

RESULTS

The characteristics of the sequences are shown in Table 2. The aligned positions of the *trnL-F*, *atpB-rbcL*, and *rps16* intron data sets are 1189, 1050, and 1025 bp, respectively. The phylogenetically informative sites are 218 in *trnL-F*, 86 in *atpB-rbcL*, and 179 in *rps16* intron. The insertions-deletions, which are transformed into binary character in the analyses, are 41 in *trnL-F*, 21 in *atpB-rbcL*, and 34 in *rps16* intron.

Strict consensus trees of the most parsimonious trees of *trnL-F* (CI = 0.76, RI = 0.89), *atpB-rbcL* (CI = 0.87, RI = 0.90), *rps16* intron (CI = 0.81, RI = 0.86) are presented in Figs. 1–3. The trees produced by separate analyses of the three chloroplast DNA data sets and the combined data (CI = 0.84, RI = 0.86) (Fig. 4) are congruent with minor differences. The parsimony analysis supports the following relationships within Vitaceae: (1) a clade of *Cayratia*, *Cyphostemma*, and *Tetrastigma* in all trees (clade E in Fig. 4). (2) Within clade E, *Cyphostemma* and *Tetrastigma* are each monophyletic. (3) *Cayratia* is paraphyletic in *trnL-F*, *atpB-rbcL*, and combined trees (with gaps as new characters), but monophyletic in *rps16* tree (with gaps as new characters), and in the *atpB-rbcL* and combined tree (with gaps as missing data). (4) *Ampelopsis* may be paraphyletic with *Rhoicissus* and *Cissus striata* nested within it as shown in the *atpB-rbcL* and *rps16*, as well as the combined trees (clade A in Fig. 4). The pinnately leaved *Ampelopsis* species (*A. arborea*, *A. cantoniensis*, *A. chaffanjoani*, *A. hypoglauca*, and *A. megalophylla*) form a clade, and the simple or palmately leaved *Ampelopsis* (*A. brevipedunculata*, *A. aconitifolia*, *A. heterophylla*, *A. bodinieri*, *A. delavayana*, and *A. cordata*) constitutes another clade. (5) *Cissus* species sampled from Asia and Central America are monophyletic (clade D in Fig. 4), whereas *C. striata* from South America groups with *Ampelopsis* and *Rhoicissus* (clade A in Fig. 4). (6) *Vitis* is monophyletic in all trees except in the *trnL-F* tree with gaps treated as missing data. When gaps are treated as missing data, there are no synapomorphies for *Vitis* in the *trnL-F* data, and all the species of *Vitis* are unresolved. (7) *Vitis* forms a clade with *Ampelopsis*, *Pterisanthes*, and *Nothocissus* in the *atpB-rbcL*, *rps16* as well as the combined trees (clade C in Fig. 4). (8) Within clade C (Fig. 4), a clade of *Pterisanthes*, *Ampelocissus*, and *Nothocissus* is supported in the *atpB-rbcL* and the combined trees. But *A. javalensis* from Central America forms a weakly supported clade with *Parthenocissus* in the *atpB-rbcL* tree or with the *Ampelocissus-Pterisanthes-Vitis* clade in the *rps16* and the combined trees. (9) *Parthenocissus* forms a clade with *Yua austro-orientalis* in the *rps16* and the combined trees (clade B in Fig. 4). In the *trnL-F* tree, six species of *Parthenocissus* from Asia constitute a clade and are distinct from the North American *P. quinquefolia*-*P. inserta* clade. And (10) all species of *Leea* form a clade sister to Vitaceae.

TABLE 2. Characteristics of the four chloroplast data sets for Vitaceae.

Data set	Aligned positions	No. variable sites	No. informative sites	Indels	Tree length	CI	RI	No. MPTs
<i>trnL-trnF</i> region	1189	317	218	41	1119	0.76	0.89	206 700
<i>atpB-rbcL</i> spacer	1050	168	86	21	244	0.87	0.90	72
<i>rps16</i> intron	1025	268	179	34	561	0.81	0.86	54
Combined data	3264	753	483	96	1212	0.84	0.86	6

Note: CI = consistency index, RI = retention index, and MPT = most parsimonious trees.

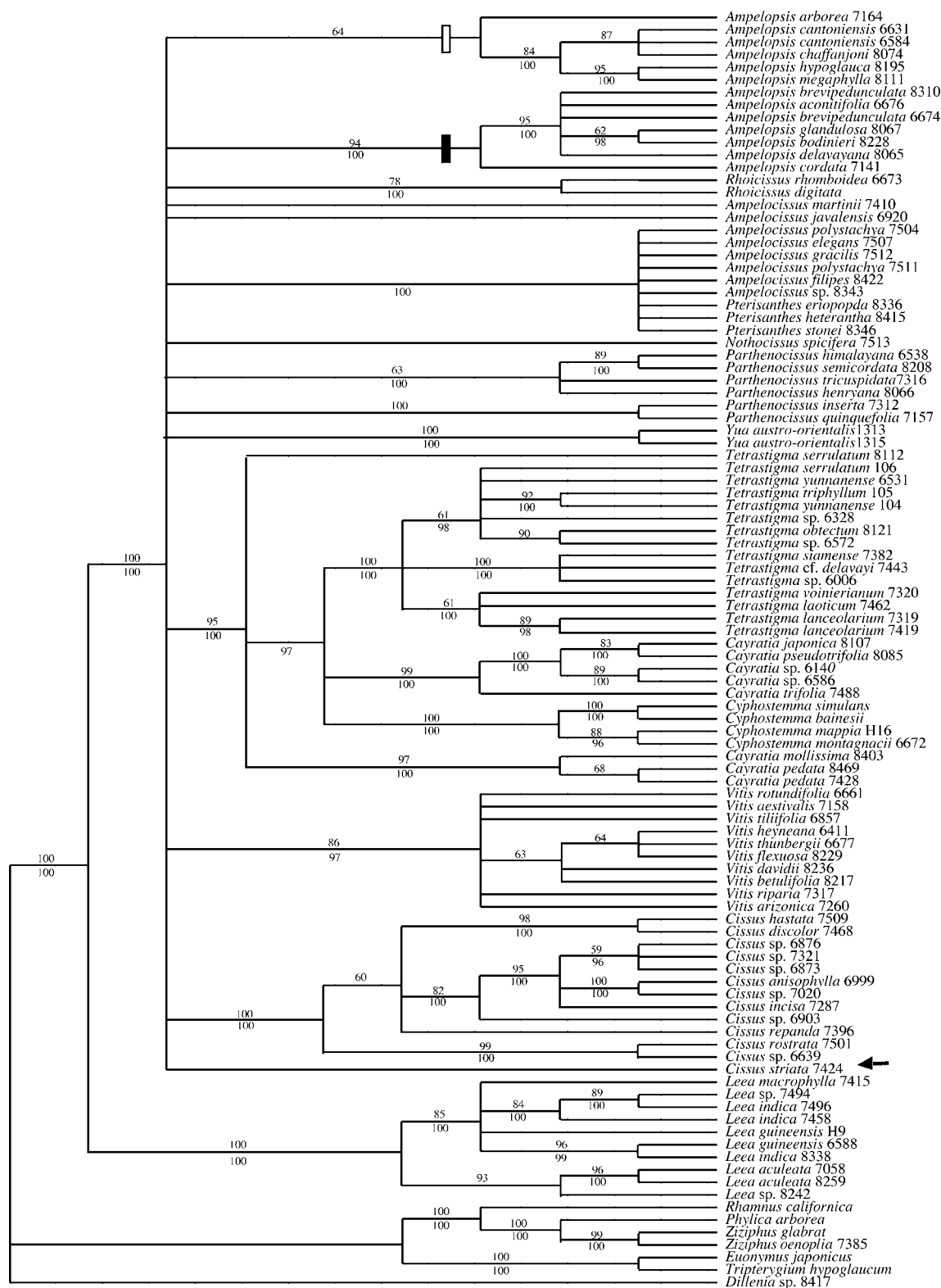


Fig. 1. The *trnL-F* strict consensus tree of Vitaceae with bootstrap values in 500 replicates above the branches and Bayesian posterior probabilities more than 95% below the branches. Open box = *Ampelopsis* taxa with pinnate leaves, closed box = *Ampelopsis* taxa with simple or palmate leaves. Arrow indicates the position of *Cissus striata*.

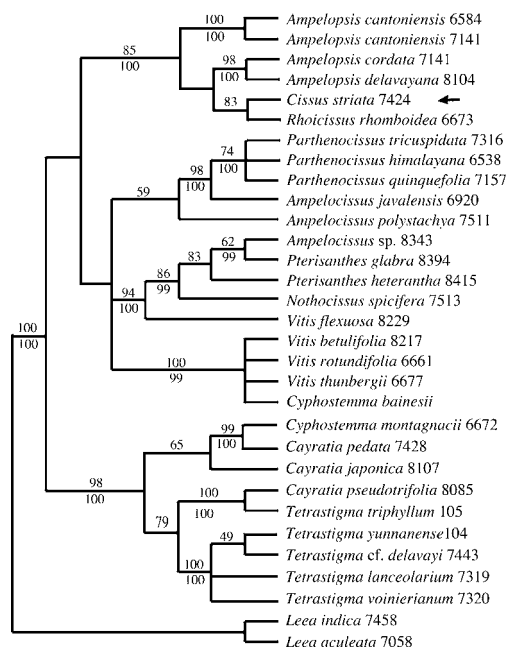


Fig. 2. The *atpB-rbcL* spacer strict consensus tree of Vitaceae with bootstrap values in 500 replicates above the branches and Bayesian posterior probabilities more than 95% below the branches. Arrow indicates the position of *Cissus striata*.

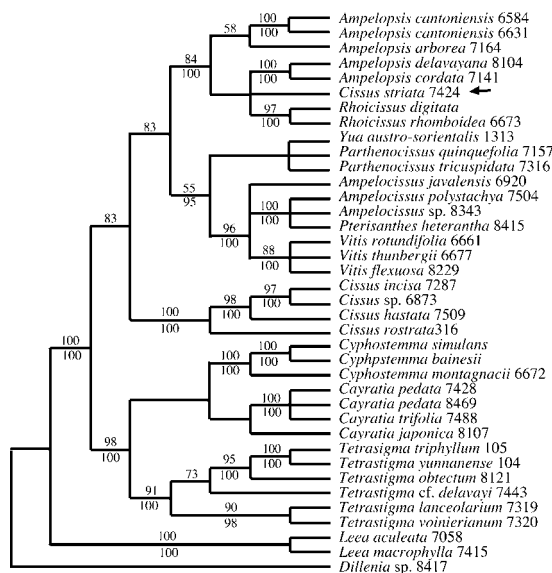


Fig. 3. The *rps16* intron strict consensus tree of Vitaceae with bootstrap values in 500 replicates above the branches and Bayesian posterior probabilities more than 95% below the branches. Arrow indicates the position of *Cissus striata*.

DISCUSSION

The results of this study revealed several relationships among genera of Vitaceae. They also indicated problems concerning generic delimitations of some genera.

Clade A: the *Ampelopsis*-*Rhoicissus*-*Cissus striata* clade—
 In all analyses except the *trnL-F* data, the eastern Asian and

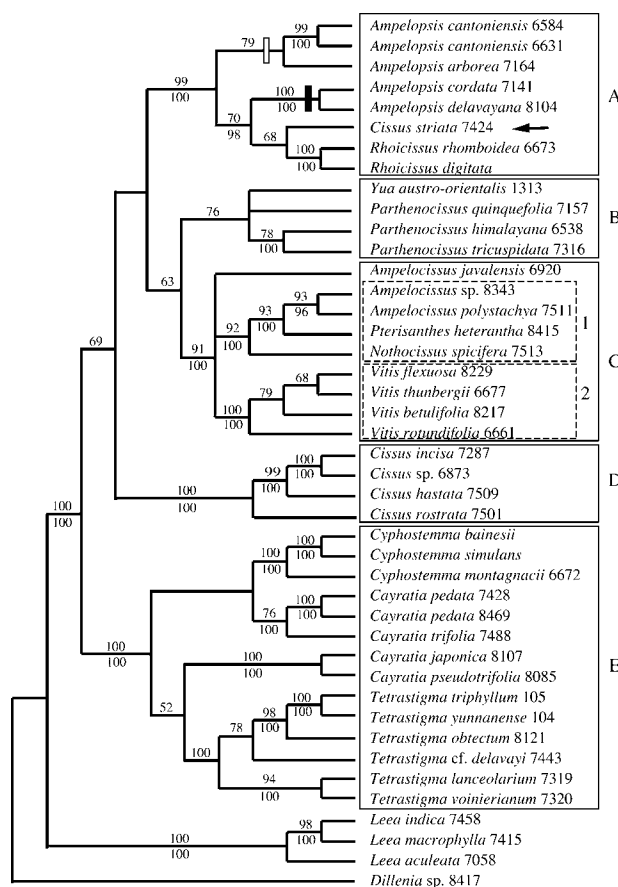


Fig. 4. The combined chloroplast (*trnL-F*, *atpB-rbcL* spacer, and *rps16* intron) strict consensus tree of Vitaceae with bootstrap values in 500 replicates above the branches and Bayesian posterior probabilities more than 95% below the branches. Open box = *Ampelopsis* taxa with pinnate leaves, closed box = *Ampelopsis* taxa with simple or palmate leaves. Arrow indicates the position of *Cissus striata*.

eastern North American *Ampelopsis*, the African *Rhoicissus*, and the South American *Cissus striata* are strongly supported to form a monophyletic group. The two species of *Rhoicissus* form a clade sister to *Cissus striata*. *Ampelopsis* is paraphyletic with a recognizable subclade of pinnately leaved species (e.g., *A. arborea* and *A. cantoniensis*) and another subclade of simple or palmately leaved species (e.g., *A. cordata* and *A. delavayana*) in the combined analysis (BS = 70, PP = 98, Fig. 4). Both subclades include species from eastern Asia and North America, suggesting that intercontinental disjunction has evolved at least twice in this genus. The pinnately leaved group was informally recognized as sect. *Leeaceifoliae* by Galet (1967) and the simple or palmately leaved group as sect. *Ampelopsis*. Bernard (1972–1973) examined buds in *Ampelopsis* and found that taxa in sect. *Leeaceifoliae* (with *A. bipinnata* Michx. = *A. arborea*, *A. chaffanjonii*, *A. macrophylla*, and *A. orientalis* Planch. examined) had complex axillary buds like *Vitis vinifera*, whereas those in sect. *Ampelopsis* (with *A. aconitifolia*, *A. bodinieri*, *A. brevipedunculata*, *A. citrullifolia* Lebaas, *A. delavayana*, *A. heterophylla* (Thunb.) Sieb. & Zucc. = *A. glandulosa*, and *A. micans* Rehder examined) all had serial accessory buds (J. Gerrath, University of Northern Iowa, personal communication). Both morpholog-

ical and phylogenetic data suggest that *Ampelopsis* needs to be redefined and the “Leeaceifoliae” group may need to be raised to the generic rank.

Geographically, this *Ampelopsis-Rhoicissus-Cissus striata* clade (clade A, Fig. 4) demonstrates an unusual distributional pattern. Most species of *Ampelopsis* are distributed in Asia and three species in North and Central America. *Rhoicissus* is an endemic genus to Africa with about 12 species, and *Cissus striata* is from South America. Although about 75 species of *Cissus* are known in South and Central America, only three (*C. palmata*, *C. striata*, and *C. sulcicaulis*) have their distribution range extending into the southern part of South America (Lombardi, 2000). Within the distributional area of *Cissus striata*, there are other South American-Asian disjunct plants such as *Lardizabalaceae*, *Hydrangea*, and *Berberis* (Good, 1974), as well as South American-African disjuncts (Goldblatt, 1995). This biogeographic relationship observed in Vitaceae clearly needs to be further explored with additional sampling in *Cissus* especially the taxa from Africa, Australia, and South America.

Clade C-1: the *Ampelocissus-Nothocissus-Pterisanthes* clade—Within clade C (Fig. 4), *Ampelocissus*, with *A. javalensis* from Central America excepted, *Nothocissus*, and *Pterisanthes* form a clade (BS = 92, PP = 100) in the combined tree. *Nothocissus* is a small Asian genus with five species distributed in Malaysia, Indonesia, and Papua New Guinea (Latiff, 1982; 2001a, b). It is a poorly defined genus, and its generic status needs to be critically examined. *Pterisanthes* is also an Asian genus with about 20 species distributed in Malaysia, Indonesia, Philippines, and Thailand (Wen, in press). Species of *Pterisanthes* are characterized by their leaf-opposed appanate or lamellate panicle with branched tendrils on the peduncle. *Ampelocissus* is a relatively large genus with c. 90 species distributed in Asia, Africa, and North and South America. Our results suggest that Asian *Ampelocissus* is more closely related to *Pterisanthes* and *Nothocissus* than to its congeneric species in Central America.

Ampelocissus is characterized by its 4–5-merous flowers in thyrses and inflorescences subtended by a tendril near the base. All species of *Ampelocissus* sampled in this study are from Asia except for *A. javalensis*, which represents one of the four New World species. *Ampelocissus javalensis* is sister to the clade of Asian *Ampelocissus*, *Nothocissus*, and *Pterisanthes*. This position is congruent with those of the trees of *trnL-F* and *atpB-rbcL*. This is a closely related and morphologically diverse clade showing geographic integrity. On the other hand, *Ampelocissus martinii* of southeast Asia, is separated from the other Asian *Ampelocissus*. Its taxonomic position needs to be reexamined with more taxa of the genus sampled. Our phylogenetic data clearly suggest the problematic generic circumscription of *Ampelocissus*.

Clade C-2: monophyly of *Vitis*—Within clade C, the Asian and North American *Vitis* species form a clade. *Vitis* is strongly supported as a monophyletic group in all analyses. Based on the *rbcL* data, Ingrouille et al. (2002) have, however, reported that *Vitis* is paraphyletic with *Cyphostemma* and *Parthenocissus* nested within it, but this relationship has no bootstrap support. In the present study, *Vitis* is monophyletic and forms a clade with *Ampelocissus*, *Pterisanthes*, and *Nothocissus* (BS = 91, PP = 100) (Fig. 4). Furthermore, the large *Vitis-Ampelocissus-Nothocissus-Pterisanthes* clade (clade C) is

sister to the clade of *Parthenocissus* and *Yua austro-orientalis* (clade B).

Species of *Vitis* are morphologically characterized by their polygamodioecious reproductive biology, calyptrate petals, and five-merous flowers. Two subgenera are commonly recognized in the genus: subg. *Vitis* and subg. *Muscadinia*. Species of subg. *Vitis* usually have shreddy bark on old stems, lenticels inconspicuous (vs. prominent in subg. *Muscadinia*), pith interrupted by diaphragms within the nodes (vs. continuous through nodes in subg. *Muscadinia*), and tendrils 2–3-forked (vs. simple in subg. *Muscadinia*). Subgenus *Muscadinia* consists of only 2–3 species from the USA, the West Indies, and Mexico (Brizicky, 1965), whereas subg. *Vitis* has a wide distribution in the northern hemisphere. We sampled *V. arizonica* and *V. rotundifolia* of subg. *Muscadinia* and the other species belonging to subg. *Vitis*. With our present data, we cannot evaluate this infrageneric classification of *Vitis* due to our limited taxon sampling in this genus.

Clade E: the *Cayratia-Cyphostemma-Tetrastigma* clade—

The three genera, *Cayratia*, *Cyphostemma*, and *Tetrastigma* form a strongly supported clade in all trees (Figs. 1–4). Within the clade, *Cyphostemma* and *Tetrastigma* are each monophyletic, but *Cayratia* is paraphyletic except in the *rps16* tree (cf. Figs. 1–4). *Cayratia* is distributed in tropical-subtropical regions in Asia, Africa, Australia, and Pacific Islands (Jackes, 1987). Within *Cayratia*, a close relationship between *C. japonica* and *C. trifolia* is suggested based on morphology (Latiff, 1983) and molecular ITS data (Rossetto et al., 2002). One of the two *Cayratia* clades in the *trnL-F* tree includes both *C. japonica* and *C. trifolia* (BS = 99, PP = 100, Fig. 1), supporting their close affinity.

The clade of *Cayratia* and *Tetrastigma* has been reported by previous studies (e.g., Ingrouille et al., 2002; Rossetto et al., 2002). Our study supports their results, but the position of *Cyphostemma* is different in these analyses. In the *rbcL* tree, *Cyphostemma juttae* is in a clade with *Vitis* and *Parthenocissus*, which is then sister to the *Cayratia* and *Tetrastigma* clade (Ingrouille et al., 2002). Our analyses from all three chloroplast markers, however, strongly support the clade of *Cayratia*, *Cyphostemma*, and *Tetrastigma* (clade E).

Alston (1931) raised *Cissus* sect. *Cyphostemma* Planch. to the generic rank. *Cyphostemma* was, however, treated as a synonym of *Cissus* by Suessenguth (1953a). Descoings (1960) argued for the separation of *Cyphostemma* from *Cissus* and recognized the genus in several floristic treatments in Africa (e.g., Descoings, 1967a, b, 1975). *Cyphostemma* has been accepted recently by several workers (e.g., Mabberley, 1995; Shetty and Singh, 2000; Latiff, 2001a; Wen, in press). Although only three of the approximately 200 species of this genus are included in this study, *Cyphostemma* is shown to be distinct from the polyphyletic *Cissus* (Figs. 1–4; also see Rossetto et al., 2002). Morphologically, *Cyphostemma* is characterized by its unique flask-shaped floral buds and its floral disk of four free glands (Descoings, 1960). The morphological synapomorphies of the strongly supported *Cayratia-Cyphostemma-Tetrastigma* clade need to be documented.

Clade B: *Parthenocissus* and *Yua*—*Parthenocissus* forms a clade with *Yua austro-orientalis* in the combined tree (BS = 76). *Yua* was recently established by Li (1990) and includes

three species from central and South China, Nepal, and northern India. Taxa of *Yua* were previously included in *Parthenocissus* (Planchon, 1887; Rehder, 1945). Li (1990) argued that species of *Yua* differed in their tendril and inflorescence morphology and should be separated as a distinct genus. *Yua* possesses bifurcate (vs. 3-12 branched in *Parthenocissus*) tendrils and leaf-opposed (vs. terminal or nearly so in *Parthenocissus*) inflorescence. Morphologically, the digitate leaf form, the fall color change of leaves from green to red, the five-merous flower, and the inconspicuous floral discs of *Yua* support its close relationship with *Parthenocissus* (Wen, in press). The generic status of *Yua* still needs further evaluation with additional sequence data of other congeneric species.

Clade D: *Cissus*—Two groups were recognized within the 13 species of *Cissus* analyzed in our study, one consisting of *C. striata*, and the other composed of taxa from Asia and Central America (clade D in Fig. 4). *Cissus* is a large genus with about 350 species distributed throughout the tropics. It has remarkable morphological diversity (Jackes, 1988). Rossetto et al. (2001, 2002) have recently shown that *Cissus* is polyphyletic using chloroplast *trnL* intron and nuclear ribosomal ITS sequences. Our study also supports that *Cissus* is at least biphyetic based on our sampling alone. Our study also shows that the South American *C. striata* occupies an unusual position in Vitaceae. It is supported to be closely related to the African *Rhoicissus*, and the Asian-North American disjunct *Ampelopsis*. A broader sampling is required to further test this relationship.

Conclusions—Phylogenetic analyses of 12 genera and 79 species of Vitaceae provided a fairly well-supported phylogeny of the family. The *trnL-trnF* tree alone was poorly resolved concerning intergeneric relationships. When two additional chloroplast markers, *atpB-rbcL* spacer and the *rps16* intron, were included, much higher resolution was obtained. Several closely relationships between genera are suggested: *Ampelopsis*, *Rhoicissus*, and *Cissus striata* in clade A; *Yua* and *Parthenocissus* in clade B; *Ampelocissus*, *Nothocissus*, *Pterisanthes*, and *Vitis* in clade C; *Cayratia*, *Cyphostemma*, and *Tetrastigma* in clade E. Within clade C, *Vitis* forms a subclade (C2). *Ampelopsis* and *Parthenocissus* each demonstrate an Asian-New World disjunct distribution, suggesting multiple intercontinental migrations in this family. Furthermore, The clade of the Asia-North American *Ampelopsis*, South American *Cissus striata*, and African *Rhoicissus* shows an unusual biogeographical relationship among Asia, North and South America, and Africa. The *Cayratia-Cyphostemma-Tetrastigma* clade have a close biogeographic relationship of southeastern Asia, Australia, and Africa including Madagascar. Vitaceae thus have complex multiple intercontinental relationships within the northern hemisphere and between northern and southern hemispheres.

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APPENDIX. Taxa and accessions used for analysis of Vitaceae with their GenBank numbers. Voucher specimens are deposited at the Field Museum Herbarium (F).

Taxon; **Voucher;** Locality; GenBank accession no.: *trnL-trnF*; *atpB-rbcL*; *rps16*.

Vitaceae

Ampelocissus elegans Gagnepain; *Wen 7507*; Singapore, Bukit Timah Nature Reserve; AB234981; —; —. *A. filipes* Planch.; *Wen 8422*; Malaysia, Negri Sembilan, Pasoh; AB234982; —; —. *A. gracilis* Planch.; *Wen 7512*; Singapore, Bukit Timah Nature Reserve; AB234983; —; —. *A. javalensis* (Seem.) W.D. Stevens & A. Pool; *Wen 6920*; Costa Rica, Limon; AB234984; AB234911; AB234943. *A. martinii* Planch.; *Wen 7410*; Thailand, Mae Hong Son; AB234985; —; —. *A. polystachya* Planch.; *Wen 7504*; Singapore, Bukit Timah Nature Reserve; AB234986; —; AB234944. *A. polystachya* Planch.; *Wen 7511*; Singapore, Bukit Timah Nature Reserve; AB234987; AB234912; —. *Ampelocissus* sp.; *Wen 8343*; Malaysia, Selangor; AB234988; AB234913; AB234945. *Ampelopsis aconitifolia* Bunge; *Wen 6676*; Cult. in MO Bot. Gard. USA; AB234989; —; —. *A. arborea* Koehne; *Wen 7164*; USA, Alabama; AB234990; —; AB234946. *A. bodinieri* (H. Lév. & Vaniot) Rehder; *Wen 8228*; China, Chongqing; AB234991; —; —. *A. brevipedunculata* Maxim. ex Trautv.; *Wen 6674*; Cult. in MO Bot. Gard. USA; AB234992; —; —. *A. brevipedunculata* Maxim. ex Trautv.; *Wen 8310*; Philippines, Luzon; AB234993; —; —. *A. cantoniensis* K. Koch; *Wen 6584*; China, Hainan; AB234994; AB234914; AB234947. *A. cantoniensis* K. Koch; *Wen 6631*; China, Hainan; AB234995; AB234915; AB234948. *A. chaffanjonii* (H. Lév.) Rehder; *Wen 8074*; China, Chongqing; AB234996; —; —. *A. cordata* Michx.; *Wen 7141*; Cult. in Illinois; AB234997; AB234916; AB234949. *A. delavayana* Planch. ex Franch.; *Wen 8104*; China, Chongqing; —; AB234917; AB234950. *A. delavayana* Planch. ex Franch.; *Wen 8065*; China, Chongqing; AB234998; —; —. *A. glandulosa* (I. Wall.) Momiyama; *Wen 8067*; China, Chongqing; AB234999; —; —. *A. hypoglaucula* (Hance) C.L. Li; *Wen 8195*; China, Chongqing; AB235000; —; —. *A. megalophylla* Diels & Gilg; *Wen 8111*; China, Chongqing; AB235001; —; —. *Cayratia japonica* (Thunb.) Gagnepain; *Wen 8107*; China, Chongqing; AB235002; AB234918; AB234951. *C. mollissima* Gagnepain; *Wen 8403*; Malaysia, Pahang; AB235003; —; —. *C. pedata* Gagnepain; *Wen 7428*; Thailand, Chiang Mai; AB235004; AB234919; AB234952. *C. pedata* Gagnepain; *Wen 8469*; China, Yunnan; AB235005; —; AB234953. *C. pseudotrifolia* W.T. Wang; *Wen 8085*; China, Chongqing; AB235006; AB234920; —. *C. trifolia* (L.) Domin; *Wen 7488*; Thailand, Chiang Mai; AB235007; —; AB234954. *Cayratia* sp.; *Wen 6586*; China, Hainan; AB235008; —; —. *Cayratia* sp.; *Wen 6140*; Vietnam, Lao Cai; AB235009; —; —. *Cissus anisophylla* Lombardi; *Wen 6999*; Costa Rica; AB235010; —; —. *C. discolor* Blume; *Wen 7468*; Thailand, Chiang Mai; AB235011; —; —. *C. hastata* Miq.; *Wen 7509*; Singapore, Bukit Timah Nature Reserve; AB235012; —; AB234955. *C. incisa* Desmoul.; *Wen 6671*; Cult. in MO Bot. Gard., USA; AB235013; —; —. *C. incisa* Desmoul.; *Wen 7287*; USA, Texas; AB235014; —; AB234956. *C. repanda* Vahl; *Wen 7396*; Thailand, Chiang Mai; AB235015; —; —. *C. rostrata* Korth. ex Planch.; *Wen 7501*; Singapore, Bukit Timah Nature Reserve; AB235016; —; AB234957. *C. striata* Ruiz & Pav.; *Wen 7424*; Chile, Valdivia; AB235017; AB234921; AB234958. *C. striata* Ruiz & Pav.; *Wen 7355*; Chile, Concepción; AB235018; —; —. *Cissus* sp.; *Wen 6639*; China, Hainan; AB235019; —; —. *Cissus* sp.; *Wen 6873*; Costa Rica; AB235020; —; AB234959. *Cissus* sp.; *Wen 7321*; Cult. in MO Bot. Gard. USA; AB235021; —; —. *Cissus* sp.; *Wen 7020*; Costa Rica, Puntarenas Prov.; AB235022; —; —. *Cissus* sp.; *Wen 6876*; Costa Rica; AB235023; —; —. *Cissus* sp.; *Wen 6903*; Costa Rica; AB235024; —; —. *Cyphostemma bainesii* (Hook.f) Descoings; *Gerrath s.n.*; Cult. in Iowa; AB235025; AB234922; AB234960. *C. mappia* (Lam.) Galet; *Wen H16*; Cult. in Hawaii; AB235026; —; —. *C. montagnacii* Descoings; *Wen 6672*; Cult. in MO Bot. Gard. USA; AB235027; AB234923; AB234961. *C.*

simulans (C.A.Sm.) Wild & R.B. Drumm.; *Gerrath s.n.*; Cult. in Iowa; AB235028; —; AB234962. *Nothocissus spicifera* (Griff.) A. Latiff; *Wen 7513-3*; Singapore Botanic Garden; AB235029; AB234924; —. *Parthenocissus henryana* Graebn. ex Diels; *Wen 6655*; Cult. in Beijing; AB235030; —; —. *P. henryana* Graebn. ex Diels; *Wen 8066*; China, Chongqing; AB235031; —; —. *P. henryana* Graebn. ex Diels; *Wen 8227*; China, Chongqing; AB235032; —; —. *P. himalayana* Planch.; *Wen 6464*; China, Yunnan; AB235033; AB234925; —. *P. himalayana* Planch.; *Wen 6538*; China, Yunnan; AB235034; AB234926; —. *P. inserta* (A. Kern.) Fritsch; *Wen 7312*; USA, Illinois; AB235035; —; —. *P. quinquefolia* (L.) Planch.; *Wen 7157*; USA, Illinois; AB235036; AB234927; AB234963. *P. quinquefolia* (L.) Planch.; *Wen 6657*; Cult. in Beijing; AB235037; —; —. *P. semicordata* Roxb.; *Wen 7377*; Thailand, Chiang Mai; AB235038; —; —. *P. semicordata* Roxb.; *Wen 8208*; China, Chongqing; AB235039; —; —. *P. tricuspidata* Planch.; *Wen 7148*; Cult. in Illinois; AB235040; —; —. *P. tricuspidata* Planch.; *Nie 2003107*; Cult. in Kunming, China; AB235041; —; —. *P. tricuspidata* Planch.; *Wen 7316*; Cult. in Illinois, USA; AB235042; AB234928; AB234964. *P. tricuspidata* Planch.; *Wen 6656*; Cult. in Beijing; AB235043; —; —. *Pterisanthes eriopoda* Planch.; *Wen 8336*; Malaysia, Kuala Lumpur; AB235044; —; —. *P. glabra* Ridl.; *Wen 8394*; Malaysia, Selangor; —; AB234929; —. *P. heterantha* M. Laws; *Wen 8415*; Malaysia, Negri Sembilan, Pasoh; AB235045; AB234930; AB234965. *P. stonei* A. Latiff; *Wen 8346*; Malaysia, Selangor; AB235046; —; —. *Rhoicissus digitata* Gilg & Brandt; *Gerrath s.n.*; Cult. in Iowa; AB235047; —; AB234966. *R. rhomboidea* Planch.; *Wen 6673*; Cult. in MO Bot. Gard. USA; AB235048; AB234931; AB234967. *R. rhomboidea* Planch.; *Wen H26*; Cult. in Hawaii; AB235049; —; —. *Tetrastigma cf. delavayi* Gagnepain; *Wen 7443*; Thailand, Chiang Mai; AB235050; AB234932; AB234968. *T. lanceolarium* Planch.; *Wen 7319*; Cult. in Missouri Bot. Gard. USA; AB235051; AB234933; AB234969. *T. lanceolarium* Planch.; *Wen 7419*; Thailand, Mae Hong Son; AB235052; —; —. *T. lanceolarium* Planch.; *Wen 8342*; Malaysia, Kuala Lumpur; AB235053; —; —. *T. laoticum* Gagnepain; *Wen 7462*; Thailand, Chiang Mai; AB235054; —; —. *T. oblectum* Planch. ex Franch.; *Wen 6104*; Vietnam, Lao Cai; AB235055; —; —. *T. oblectum* Planch. ex Franch.; *Wen 6522*; China, Yunnan; AB235056; —; —. *T. oblectum* Planch. ex Franch.; *Wen 6572*; China, Hainan; AB235057; —; —. *T. oblectum* Planch. ex Franch.; *Wen 8121*; China, Chongqing; AB235058; —; AB234970. *T. serrulatum* Planch.; *Wen 8112*; China, Chongqing; AB235059; —; —. *T. serrulatum* Planch.; *Nie 2003106*; Cult. in Kunming, China; AB235060; —; —. *T. siamense* Gagnepain & Craib; *Wen 7382*; Thailand, Chiang Mai; AB235061; —; —. *T. triphyllum* (Gagnepain) W.T. Wang; *Nie 2003105*; Cult. in Kunming, China; AB235062; AB234934; AB234971. *T. triphyllum* (Gagnepain) W.T. Wang; *Nie 2003108*; Cult. in Kunming, China; AB235063; —; —. *T. voinierianum* Pierre ex Gagnepain; *Wen 6666*; Cult. in MO Bot. Gard. USA; AB235064; —; —. *T. voinierianum* Pierre ex Gagnepain; *Wen 7320*; Cult. in MO Bot. Gard. USA; AB235065; AB234935; AB234972. *T. yunnanense* Gagnepain; *Wen 7384*; Thailand, Chiang Mai; AB235066; —; —. *T. yunnanense* Gagnepain; *Wen 5993*; Vietnam, Lao Cai; AB235067; —; —. *T. yunnanense* Gagnepain; *Wen 6531*; China, Yunnan; AB235068; —; —. *T. yunnanense* Gagnepain; *Nie 2003104*; Cult. in Kunming, China; AB235069; AB234936; AB234973. *Tetrastigma* sp.; *Wen 6328*; China, Yunnan; AB235070; —; —. *Tetrastigma* sp.; *Wen 7465*; Thailand, Chiang Mai; AB235071; —; —. *Tetrastigma* sp.; *Wen 6006*; Vietnam, Lao Cai; AB235072; —; —. *Vitis aestivalis* Michx.; *Wen 7158*; USA, Illinois; AB235073; —; —. *V. arizonica* Engelm.; *Wen 7260*; USA, Texas; AB235074; —; —. *V. betulifolia* Diels & Gilg; *Wen 8217*; China, Chongqing;

AB235075; AB234937; —. *V. davidii* (Roman. Du Caill.) Föex.; *Wen* 8236; China, Chongqing; AB235076; —; —. *V. flexuosa* Thunb.; *Wen* 8229; China, Chongqing; AB235077; AB234938; AB234974. *V. heyneana* Roem. & Schult.; *Wen* 6411; China, Yunnan; AB235078; —; —. *V. riparia* Michx.; *Wen* 7147; USA, Illinois; AB235079; —; —. *V. riparia* Michx.; *Wen* 7317; USA, Wisconsin; AB235080; —; —. *V. rotundifolia* Michx.; *Wen* 6661; Cult. in MO Bot. Gard. USA; AB235081; AB234939; AB234975. *V. thunbergii* Siebold & Zucc.; *Wen* 6677; Cult. in MO Bot. Gard. USA; AB235082; AB234940; AB234976. *V. tiliifolia* Humb. & Bonpl.; *Wen* 6857; Costa Rica; AB235083; —; —. *Vitis* sp.; *Wen* 8007; China, Gansu; AB235084; —; —. *Yua austro-orientalis* (Metcalf) C.L. Li; *Bond* 1313; China, Guangdong; AB235085; —; AB234977. *Yua austro-orientalis* (Metcalf) C.L. Li; *Bond* 1315; China, Guangdong; AB235086; —; —.

Leeaceae

Leea aculeata Blume; *Wen* 7058; Cult. in Hawaii, originally from Java; AB235087; AB234941; AB234978. *L. aculeata* Blume; *Wen* 8259; Philippines, Luzon; AB235088; —; —. *L. guineensis* G. Don; *Wen* 6588; China, Hainan; AB235089; —; —. *L. guineensis* G. Don;

Wen H9; Cult. in Hawaii, originally from Madagascar; AB235090; —; —. *L. herbacea* Buch.-Ham.; *Wen* 7494; Thailand, Chiang Mai; AB235091; —; —. *L. indica* Merrill; *Wen* 7496; Thailand, Chiang Mai; AB235092; —; —. *L. indica* Merrill; *Wen* 7458; Thailand, Chiang Mai; AB235093; AB234942; —. *L. indica* Merrill; *Wen* 8338; Malaysia, Kuala Lumpur; AB235094; —; —. *L. macrophylla* Roxb. ex Hornem. & Roxb.; *Wen* 7415; Thailand, Mae Hong Son; AB235095; —; AB234979. *Leea* sp.; *Wen* 8242; Philippines, Laguna; AB235096; —; —.

Celastraceae

Euonymus japonicus Thunb.; AF534670; —; —. *Tripterygium hypoglaucum* Hutchinson; AF534684; —; —.

Rhamnaceae

Phyllica arborea Thou.; AF327603; —; —. *Rhamnus californica* Eschsch.; AF348565; —; —. *Ziziphus glabrata* Heyne ex Roth; ZGJ225799; —; —. *Z. oenoplia* Mill.; *Wen* 7385; Thailand, Chiang Mai; AB235097; —; —.

Dilleniaceae

Dillenia sp.; *Wen* 8417; Malaysia, Negri Sembilan, Pasoh; AB235098; —; AB234980.