

Molecular Biology of Vitaceae using DNA Markers: A Review

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Abstract: *Vitaceae is the only family of the predominantly tropical order Vitales. The phylogenetic position of Vitaceae has been variously hypothesized prior to APG III (2009). Relationships of Vitaceae to other families have long been highly speculative and variable. Recently there are 14 accepted genera of Vitaceae with their species strength c. 978 (sensu APG III, 2009). The phylogeny of Vitaceae has been reconstructed with several markers which confines Leea within Vitaceae. The present study defines the review of Vitaceae phylogeny using several DNA markers.*

Keywords: Phylogeny, Vitaceae, DNA, Markers

1. Introduction

Vitaceae is the only family of the predominantly tropical order Vitales. The phylogenetic position of Vitaceae has been variously hypothesized prior to APG III (2009). Relationships of Vitaceae to other families have long been highly speculative and variable. According to Chase *et al.* (1993) Vitaceae + Leeaceae were sister to Dilleniaceae but subsequent analyses (Savolainen *et al.*, 2000a, b; Soltis *et al.*, 2000) with many more data have failed to confirm this placement. Furthermore there was little non-molecular evidence to support such a relationship. According to Chase *et al.* (1993) Vitaceae were not related to Rhamnaceae as thought earlier. Savolainen *et al.* (2000a), using *rbcL* and *atpB* (both plastid genes), placed Vitaceae/Leeaceae near the base of the large rosid clade, and Soltis *et al.* (2000) using three genes (the two plastid genes plus nuclear 18S rDNA) obtained Vitaceae (including *Leea*) as sister to the rest of the rosids with moderate jackknife support. Recent molecular analyses generally supported the Vitaceae clade (including Leeaceae) as sister to all other rosids (Soltis *et al.*, 2000, 2003, 2006; Jansen *et al.*, 2006; Wang *et al.*, 2009). Hillu *et al.* (2003) on the basis of *matK* data, suggested that Dilleniaceae is sister to Vitaceae, although support for this relationship varies with the methods applied (weak using MP, but 1.0 PP in BI). APG III (2009) placed Vitaceae as sister to the fabids + malvids clade (eurosids I + II) and recognized it in its own order Vitales following Takhtajan (1977). During early classifications genus *Leea* has been excluded from Vitaceae and treated as the monogeneric family Leeaceae by Vitaceae/Leeaceae specialists (e.g., Planchon, 1887; Suessenguth, 1953; Ridsdale, 1974; Shetty & Singh, 2000; Latiff, 2001; Ren *et al.*, 2003; Chen and Manchester, 2007; Wen, 2007a, b). Leeaceae has been supported as the closest relative of Vitaceae based on DNA molecular phylogenetic and morphological data (Soejima & Wen, 2006; Wen, 2007a, b; Wang *et al.*, 2009). The present study clearly defines that *Leea* lies within Vitaceae including the 14 genera are *Ampelocissus* Planch., *Ampelopsis* Michx., *Cayratia* Juss., *Cissus* L., *Clematicissus* Planch., *Cyphostemma* (Planch.) Alston, *Nothocissus* (Miq.) Latiff, *Parthenocissus* Planch., *Pterisanthes* Blume, *Rhoicissus* Planch., *Tetrastigma* (Miq.) Planch., *Vitis* L., *Yua* Li. and *Leea* (D.C) Dum. The work was carried out using molecular ITS sequences to estimate phylogenetic relationships in

Indian Vitaceae. The materials for the present study were collected from various localities.

The phylogeny of Vitaceae has been reconstructed with several markers (Rossetto *et al.*, 2001, 2002; Ingrouille *et al.*, 2002; Soejima & Wen, 2006; Wen *et al.*, 2007). With 37 taxa sampled in the combined analyses, Soejima & Wen (2006) reconstructed the phylogeny of Vitaceae based on three chloroplast markers (*trnL-F* region, *atpB-rbcL* spacer, the *rps16* intron), which supported three major clades: (1) the *Ampelopsis-Rhoicissus-Parthenocissus-Vitis-Nothocissus-Pterisanthes-Ampelocissus* clade; (2) the core *Cissus* clade (except the South American *Cissus striata* complex); and (3) the *Cayratia-Tetrastigma-Cyphostemma* clade. Wen *et al.* (2007) sampled eleven genera and 95 species and infra-specific taxa of Vitaceae to reconstruct the relationships within Vitaceae with the nuclear *GAIL* sequences. The three major clades formerly recognized by Soejima & Wen (2006) were strongly supported by the *GAIL* data. Particularly, the first clade was 100% supported by the *GAIL* data compared to a less than 50% bootstrap (BS) value in the three plastid markers, and a close relationship between the core *Cissus* clade and the 5-merous clade was well supported. Different from the plastid phylogeny, the *GAIL* data recognized *Ampelopsis* as the closest relative of *Parthenocissus* instead of *Vitis*, although the support values were low.

Rossetto *et al.* (2007) constructed the phylogeny of Australian Vitaceae using plastid *trnL-F* and nuclear internal transcribed spacer sequences. Their data supported a robust sister relationship between *Clematicissus* and a clade of two South American *Cissus* (*Cissus tweediana* (Baker) Planch. and *Cissus striata* Ruiz & Pav.) and further supported the paraphyly of *Cayratia*. Vitaceae are most closely related with monogeneric Leeaceae (Ingrouille *et al.*, 2002) which share several important morphological synapomorphies including presences of pearl gland and raphides makes a reasonable reference point to start describing the trends culminating in *Vitis*. It is separated from Vitaceae only on the basis of its non-vining habit (Ingrouille *et al.*, 2002).

Traditionally it was considered in the order Rhamnales (Kirchheimer, 1939; Cronquist, 1981, 1988). Takhtajan (1977) recognized order Vitales consisting of Vitaceae as

highly isolated and the sole member of the superorder Vitanae in the Rosidae. Most workers excluded *Leea* from Vitaceae and recognized the family Leeaceae (Shetty and Singh, 2000; Lattif 2001a; Ingrouille *et al.*, 2002; Rossetto *et al.*, 2002c; Ren *et al.*, 2003; Soejima and Wen, 2006). Various important taxonomic studies of Vitaceae have been produced, emphasizing the taxa of particular regions. These include the treatments of Gagnepain (1911a, 1919), Lattif (1981, 1982, 1983, 1991, 2001a, 2001b), and Mabberley (1995) on species in southeast Asia/Malaysia; Jackes (1984, 1987a, 1988a, 1989a, 1989b). Jackes and Rossetto (2006) studied the Australian species. Wang *et al.* (1999, 2009), and Li's (1998) treatments of Vitaceae in China; Shetty and Singh's (2000) study of Indian species; Vassilczenko's (1970) treatment of Vitaceae in Iran; Gilg and Brandt's (1911), Dewit and Willems's (1960), Wild and Drummond's (1966), Descoings's (1967a) and Verdcourt's (1993) treatment of species in Africa and Madagascar; Lombardi's (2000) of South American species, Brizicky (1965), Galet (1967) and Moore (1987, 1991) worked on several species of temperate North America regions. In these taxonomic treatments, 77 genera were mainly differentiated based on floral structures like petal number, calyptras, styles, nectariferous disc, inflorescence, fruits and seed morphology. Species were often distinguished by variations in vegetative structures, mostly by different leaf forms and indument conditions and suction pads on the tips of the tendrils.

Comparative developmental morphology of floral and vegetative structures of Vitaceae have been extensively carried out by Gerrath and colleagues (Posluszny and Gerrath, 1986; Gerrath and Posluszny, 1988a, 1988b, 1989a, 1989b, 1989c; Lacroix and Posluszny, 1989a; Gerrath, Lacroix, and Posluszny, 1990; Gerrath and Posluszny, 1993; Gerrath and Lacroix, 1997; Gerrath, Lacroix, and Posluszny, 1998; Gerrath, Posluszny and Dengler, 2001; Wilson and Posluszny, 2003a, 2003b; Gerrath, Wilson and Posluszny, 2004; Gerrath and Posluszny, 2007; Timmons, Posluszny and Gerrath, 2007a, 2007b).

Besides this Kirchheimer 1939, and Tiffney and Barghoorn (1976) suggested that the fossil seed records of the family are easily recognized from a suite of unique and distinctive morphological characters like a pair of ventral infolds and a dorsal chalazal scar and are relatively common in tertiary floras. The infra-familial relationships are often reflected by the morphological variation among fossil seeds of the family. They considered that Vitaceous seeds can be differentiated at the generic level but with limited sampling of the extent species intrageneric variation has not been fully explored because of the lack of modern Vitaceous seed representatives. It is suggested that the evolutionary and phytogeographic history of the family influenced the seeds of the extent genera which provide a good basis for interpretation among basal rosid families.

In the APG III (2009) classification, Soltis *et al.* (2000) placed Vitaceae sister to the rest of the rosids. This current account of Angiosperm Phylogeny shows 978 species distributed under 14 genera of the family. These 14 genera identified excluded *Acareosperma* and included *Leea* in APG III, 2009.

2. Material and Methods

The present work includes genomic DNA extraction, PCR amplification and sequencing of amplified products and phylogenetic analysis. Of all the 14 identified genera, the sampling includes sequences of 6 genera and 15 species which well represent the taxonomic diversity of the family. Sequences were aligned using ClustalX vers. 2.0.11 (Thompson *et al.* 1997) followed by manual adjustments in ClustalW (Thompson *et al.* 1994).

3. Observation and Results

The present analyses involved 128 nucleotide sequences (including outgroups). Sequences comprised of ITS1, 5.8S and ITS2 regions. Forward and reverse sequences were edited and assembled using the computer program Codon Code Aligner vers. 3.7.1. (2002-2009, Codon code corp.) and DNA Baser (vers. 3) (2011). The combined length of the entire ITS region (ITS1, 5.8S and ITS2) from taxa sampled in the present study ranged from 594-658 nucleotides. The ITS1 region ranged from 200-245 nucleotides in length, the 5.8S gene was of 175 nucleotides, and the ITS2 region ranged from 220-285 nucleotides. All positions containing gaps and missing data were eliminated. The insertions and deletions (indels) were necessary to align the sequences. The sizes of the indels ranged from 1 to 20 bp. There were a total of 1142 positions in the final dataset. Phylogenetic analyses were done using Bayesian (Maximum Posterior Probability, MPP), Maximum Parsimony (MP) and Neighbour-Joining (NJ) methods. Bayesian analyses were done using MrBayes 3.1. Analyses were run for 30,00,000 generations by which stage the stationary state was achieved (standard deviation below 0.01). An analysis of a total of 58 accessions of DNA sequences representing 9 genera and 33 species of Vitaceae has been done. The present work reviews the data on different markers used in phylogenetic analysis of Vitaceae (Table 1). The results may be used as evidence to support the phylogenetic conflicts.

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Table 1: Vitaceae genera with DNA data including Leeaceae. (key to the type of molecular data 1= *rbcl*, 2= *matK*, 3=SSR, 4= *rps16*, 5= *rpl2-trnH*, 6=ITS, 7= *GAIL*, 8= *trnR-rnn5* spacer, 9= *trnC-petN*, 10=26S rRNA, 11= *rps16*, 12=*trnL-trnF*, 13= *rpl32-trnL*, 14=18S rRNA, 15=*trnK-rps16*, 16= *atpB*, 17= *atpB-rbcl*, 18= *rps16-trnQ*, 19= *trnK-matK*, 20= *rpoB*, 21= *rps2*, 22=*trnR-atpA*, 23= *rpoC1*, 24= *accD*, 25=ITS1-5.8S 26= ITS1, 27=*trnS-trnG*, 28=*rpl20-rps12*, 29=*trnL*, 30=*trnH-psbA*, 31=*trnH-psbA-trnK*, 32=*trnF-nahJ*, 33=18S-26S rRNA-*atpB-matK-ndhF-psbBTNH-rbcl-rpoC2-rps4-atp1-matR-nad5-rps3*).

Genera	Reference	Type of Data
<i>Ampelopsis</i>	Ingrouille <i>et al.</i> (2002)	1
	Soejima and Wen (2006)	4, 12, 17
	Jackes & Rossetto (2006)	11, 12, 17
	Wen <i>et al.</i> (2007)	7
	Li, <i>et al.</i> (2011)	26
	Ren <i>et al.</i> (2011)	9,12, 30
	Fatma, <i>et al.</i> (2015)	6
<i>Ampelocissus</i>	Rossetto <i>et al.</i> , (2001a, 2001b, 2002, 2007)	3, 26, 29
	Ingrouille <i>et al.</i> (2002)	1
	Jackes & Rossetto (2006)	11, 12, 17
	Wen <i>et al.</i> (2007)	7
	Ren <i>et al.</i> (2011)	9,12, 30
	Fatma, <i>et al.</i> (2015)	6
<i>Cayratia</i>	Rossetto, <i>et al.</i> (2001a, 2001b, 2002, 2007)	3, 12, 25, 26, 29
	Ingrouille <i>et al.</i> (2002)	1
	Soejima and Wen (2006)	4, 12, 17
	Jackes & Rossetto (2006)	11, 12, 17
	Wen <i>et al.</i> (2007)	7
	Ren <i>et al.</i> (2011)	9,12, 30
	Fatma, <i>et al.</i> (2015)	6

Genera	Reference	Type of Data
Cissus	Rossetto <i>et al.</i> (2001a, 2001b, 2002, 2007) Ingrouille <i>et al.</i> (2002) Jackes & Rossetto (2006) Wen <i>et al.</i> (2007) Ren <i>et al.</i> (2011) Fatma, <i>et al.</i> (2015)	3, 12, 25, 26, 29 1 11, 12, 17 7 9,12, 30 6
<i>Parthenocissus</i>	Ingrouille <i>et al.</i> (2002) Soejima and Wen (2006) Jackes & Rossetto (2006) Wen <i>et al.</i> (2007) Nie <i>et al.</i> (2010) Ren <i>et al.</i> (2011)	1 4, 12, 17 11, 12, 17 7 4, 7, 12, 17 9,12, 30
Tetrastigma	Rossetto <i>et al.</i> (2001a, 2002, 2007) Ingrouille <i>et al.</i> (2002) Soejima and Wen (2006) Jackes & Rossetto (2006) Wen <i>et al.</i> (2007) Ren <i>et al.</i> (2011) Fatma, <i>et al.</i> (2015)	3, 12,25, 26, 29 1 4, 12, 17 11, 12, 17 7 9,12, 30 6
<i>Cyphostemma</i>	Ingrouille <i>et al.</i> (2002) Soejima and Wen (2006) Jackes & Rossetto (2006) Wen <i>et al.</i> (2007) Ren <i>et al.</i> (2011)	1 4, 12, 17 11, 12, 17 7 9,12, 30
<i>Clematicissus</i>	Ingrouille <i>et al.</i> (2002) Rossetto <i>et al.</i> (2002, 2007) Wen <i>et al.</i> (2007)	1 3, 12, 25, 26 7
Rhoicissus	Ingrouille <i>et al.</i> (2002) Soejima and Wen (2006) Jackes & Rossetto (2006) Wen <i>et al.</i> (2007) Ren <i>et al.</i> (2011)	4, 12, 17 1 11, 12, 17 7 9,12, 30
Yua	Wen <i>et al.</i> (2007) Ren <i>et al.</i> (2011)	7 9,12, 30
Nothocissus	Soejima and Wen (2006) Jackes & Rossetto (2006) Wen <i>et al.</i> (2007) Ren <i>et al.</i> (2011)	4, 12, 17 11, 12, 17 7 9,12, 30
<i>Pterisanthes</i>	Soejima and Wen (2006) Jackes & Rossetto (2006) Wen <i>et al.</i> (2007) Ren <i>et al.</i> (2011)	4, 12, 17 11, 12, 17 7 9,12, 30
<i>Vitis</i>	Trondle <i>et al.</i> (2010) Ingrouille <i>et al.</i> (2002) Rossetto <i>et al.</i> (2002, 2007) Hillu <i>et al.</i> (2003) Soejima and Wen (2006) Wen <i>et al.</i> (2007) Peros <i>et al.</i> (2011) Ren <i>et al.</i> (2011) Zecca <i>et al.</i> (2012) Fatma, <i>et al.</i> (2015)	12, 31 1 4, 12, 17,29 2 1, 11, 12, 17 7 29, 31 9,12, 30 13,15, 30, 32 6
<i>Leea</i>	Chase <i>et al.</i> (1993) Ingrouille <i>et al.</i> (2002) Soejima and Wen (2006) Ren <i>et al.</i> (2011) Rossetto <i>et al.</i> (2002) Savolainen <i>et al.</i> (2000a) Soltis <i>et al.</i> 2000, 2011 Wen <i>et al.</i> (2007) Fatma, <i>et al.</i> (2015)	1 1 4, 12, 17 9,12, 30 25, 26 17 14, 17, 33 7 6