Phylogeny of the Celastreae (Celastraceae) inferred using chloroplast and nuclear loci

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Gymnosporia divaricata


Gymnosporia divaricata


Tripterygium wilfordii


Maytenus boaria

Introduction

The phylogenetic inference presented of the Celastreae (Celastraceae) is based on four loci: ***matK*** and ***trnL-F*** from the chloroplast genome and ITS and 26S rDNA from the nuclear genome. The species sampled are combined with taxa sampled from previous studies to better test the monophyly of the genera along with the intergeneric relationships within this tribe. The main purpose is to give an overview of the laboratory procedures used and the results from the sampling. The procedures include DNA isolation, DNA amplification, DNA purification, construction of contiguous sequences, sequence alignment, and lastly phylogenetic inference.


Maytenus disticha

DNA Isolation

The DNA was isolated from dried leaf tissue from all seven taxa (Table 1) using a Qiagen DNEasy plant mini kit The tissue was ground using the "Machadora," a reciprocating saw that macerated the material. After the addition of lysis buffer containing an enzyme for RNA digestion, the lysate was heated and centrifuged to remove polysaccharides, proteins and larger leaf solids. Plant cell debris was separated from the decant by filtration and centrifugation. Unfiltered proteins and polysaccharides within the mixture were removed in a series of filtrations and washings via centrifugation through a DNA-binding silica-gel-based membrane. DNA was then eluted into a low-salt solution to obtain optimal yield and concentration. The resulting isolations were kept at -20°C.

DNA Amplification

The DNA was amplified using PCR (Figure 1), a method which uses RNA primers and free nucleotides to create replicates of a target gene. The PCR cycle includes three temperature dependent stages: denaturation, annealing, and extension. Denaturation created single strands of DNA that allowed the primers to anneal to them; extension was accomplished by employing *TAQ* polymerase and the free nucleotides. Denaturation was run at 94ºC, Annealing at 50ºC for all primers except to obtain the 3' end of trnL-F which ran at 53ºC and extension was carried out at 72ºC. A total of 35 cycles were run and each time the amount of the target gene doubled creating millions of copies.

Gel electrophoresis was the method used to determine the success of the PCR by forcing the DNA to migrate through an agarose gel based on its negative charge, which caused it to separate based on the size of the fragments amplified. The gel contained ethidium bromide, which binds to DNA and fluoresces in U.V. light. Digital pictures were taken of all results (Figure 2).

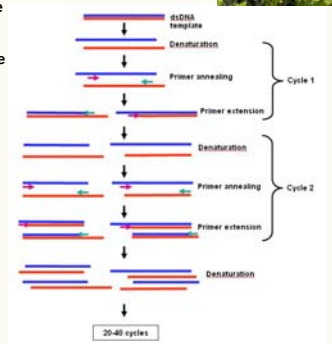

Figure 1. The three steps of the Polymerase chain reaction (PCR)

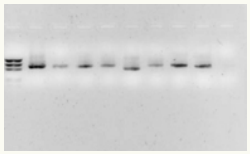
Figure 2. PCR results of the 5' end of the *trnL-F* gene

DNA Purification

The PCR product was purified using the QIAgen QIAquick PCR Purification Kit. A high salt buffer was combined with the PCR product in a spin column to bind the DNA to a silica-gel membrane. The column was centrifuged and then the membrane was washed to remove excess salts. The DNA was then eluted from the membrane with a low salt buffer. The resulting purified product was shipped to Macrogen, Inc. in South Korea for sequencing.

Creating Contiguous Sequences

After sequences were obtained from Macrogen, the raw sequences were compared with known sequences in GenBank to check for contamination. They are then edited in the program *CodonCode Aligner* (Figure 3; CodonCode Corporation) to make manual corrections to automated base calls and delete ambiguous regions at the beginnings and ends of sequences. Forward and reverse-complement sequences were then aligned to create a contiguous gene sequence.

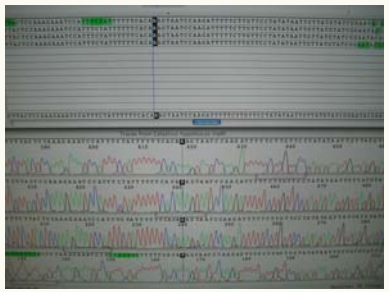
Figure 3. CodonCode Aligner, aligns sequences and shows the electropherograms associated with each sequence. This screen shows the process of making one contiguous matK sequence from the 3' and 5' ends with internal primers.

Sequence alignment

Contiguous sequences, each corresponding to a particular species, were imported into the program *Clustal X* (Figure 4; Thompson et al. 1997), and sequences of each gene were aligned using the default alignment parameters (transition cost = 0.5; gap opening cost = 10.0; gap extension cost = 0.2). This multiple alignment was performed using a series of pairwise alignments, with the most similar sequences aligned first.

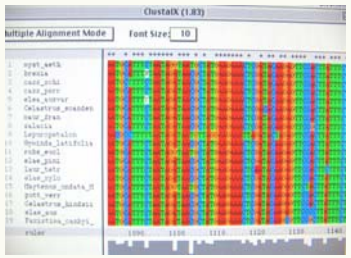

Figure 4. Sequences of *matK* aligned in Clustal X. Each color and letter represents one of the four nucleotides.


Tripterygium wilfordii

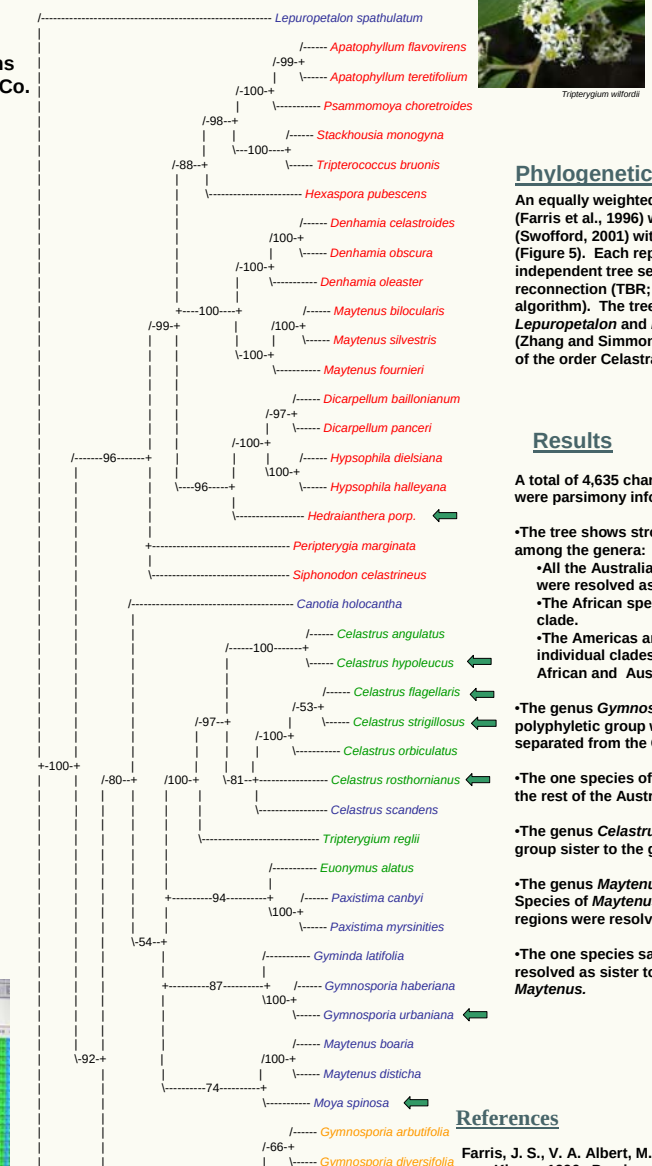

Figure 5. Jackknife Tree. The colors represent the geographic regions that species are found in, Red represents Australia and New Caledonia, Blue represents the Americas, Green represents Asia, and Orange represents Africa. Arrows denote species personally sampled.

Phylogenetic Inference

An equally weighted parsimony jackknife analysis (Farris et al., 1996) was performed using ***PAUP**** (Swofford, 2001) with 1,000 jackknife replicates (Figure 5). Each replicate comprised ten independent tree searches using tree-bisection-reconnection (TBR; a thorough tree-swapping algorithm). The tree was rooted using ***Lepuropetalon*** and ***Mortonia*** as outgroups, following (Zhang and Simmons' (2006) phylogenetic analysis of the order Celastrales.

Results

A total of 4,635 characters were used of which 1,061 were parsimony informative.

- The tree shows strong biogeographic relationships among the genera:
  - All the Australian and New Caledonian species were resolved as one clade.
  - The African species were also resolved as one clade.
  - The Americas and Asia were not resolved as individual clades, but were resolved from the African and Australian/New Caledonian clades.
- The genus ***Gymnosporia*** was resolved as a polyphyletic group with the New World species separated from the Old World species.
- The one species of ***Hedrianathera*** was resolved with the rest of the Australian and New Caledonian genera.
- The genus ***Celastrus*** was resolved as a monophyletic group sister to the genus ***Tripterygium***.
- The genus ***Maytenus*** was found to be polyphyletic. Species of ***Maytenus*** from different geographical regions were resolved as separate lineages.
- The one species sampled from the genus ***Moya*** was resolved as sister to the New World species of ***Maytenus***.

Table 1. Species sampled

Taxon	Source
Celastrus flagellaris Rupr.	***M.P. Simmons 1782,*** cult., Arnold Arboretum (BH)
Celastrus hypoleucus (Oliv.) Warb. ex Loes.	***M.P. Simmons 1785,*** cult., New York Botanical Garden (BH)
Celastrus rosthornianus Loes.	***M.P. Simmons 1783***, cult. Arnold Arboretum (BH)
Celastrus strigillosus Nakai	***M.P. Simmons 1786,*** cult. New York Bot. Garden (BH)
Gymnosporia urbaniana (Loes.) Liesner	***R. Seidel et al. 9169***, Bolivia (MO)
Hedraianthera porphyropetala F. Muell.	***A. Ford 4544,*** Australia (CS)
Moya spinosa Griseb.	***M. Nee 51170,*** Bolivia (NY)

References

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