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# EVOLUTIONARY STUDIES AND MOLECULAR PHYLOGENETICS OF ASCLEPIADOIDEAE, PERIPLOCOIDEAE AND SECAMONOIDEAE IN IRAQ

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## Abstract

The study was conducted on a level species phylogenetics of Asclepiadoideae, Periplocoideae and Secamonoideae were studied to explain relationships between patterns of close plant taxon and tissue use from species. The present work includes many taxa not previously. The topology of the tree is in accordance with several recent works. The improved sampling of taxa has improved the resolution of the clades especially in subfamily Asclepiadoideae. Due to unavailability of specimens, Secamonoideae could not be well resolved. Yet, there remains more work to be done, especially at the subtribal levels of Asclepiadeae which is a very big tribe.

***Keywords*:** Molecular Phylogenetic, plants, Asclepiadoideae

## Introduction

Molecular phylogenetics study evolutionary relationships between organisms or genes through stratify a combination of statistical and molecular methods.

Molecular phylogenetics uses function and structure molecules, how they modify with time to infer these evolutionary relationships. Studies indicated that there are probability of molecular technique and biological functions between organisms where species have the same ancestor. It did not start before 1960, with electrophoresis, PCR, protein sequencing, and other molecular biology techniques. When there is public access to genetic data, the growth of molecular phylogenetics continues and identifies new implications (Durbin *et al.,* 1998'Hall, 2004; Li 1997; Liò and Goldman, 1998; Patthy, 1998).

The purpose of molecular phylogenetic study is to recover the event order of symbolizing evolutionary trees that graphically explain the association between genes and species for a long time. This complicated process is made more complicated for approaching phylogenetic problems. Hundreds of species, each of which contains phylogenetic data sets can that change or influence evolutionary modification. There are stochastic and evolutionary models available for the analysis but the most optimum method relies on the research nature and the used data in the study (Ewens and Grant 2005; Linder and Warnow, 2005; Liò and Goldman, 1998).

## Materials and Methods

### Plant material

Twenty-Five species of in group taxa (Asclepiadoideae, Periplocoideae and Secamonoideae) were collected from various parts of Iraq. The vouchers specimens are available in the herbarium of University of Basrah, Basrah, Iraq. The names of plants used in this study, their location, Genbank accession numbers and voucher information.

### Extraction of DNA, Amplification and Sequencing of PCR

Extraction of DNA, amplification of PCR and extraction of DNA sequencing were the same as explained in Segment 4.2.2. The rbcL region was amplified using primers rbcL1F and rbcL1390R (Table 1.1). rbcL1F is a forward primer that corresponds to the first 20 base pairs of the rbcL exon and rbcL1390R is the reverse primer corresponding to the 24 nucleotides on the complementary strand from the 1390th position in reverse direction. The amplified product was about 1.4 kbp in length. For sequencing internal primers rbcL700F and rbcL800R were used. rbcL700F corresponds to nucleotides from 686 to 713 in the rbcL gene. rbcL800R corresponds to nucleotides between 791 and 812 in the complementary strand of rbcL gene. PCR amplifications and sequencing were similar to the information given in section 4.2.3.

**Table 1:** Primers for amplification of rbcL region

| Primer Name | Sequence |
|---|---|
| rbcL1F | ATG TCA CCA CAA ACA GAG AC |
| rbcL700F | CAT TAC TTG AAT GCT ACT GCA GGT AC |
| rbcL800R | AGC TCG ATA TTG CAG TGA ATC C |
| rbcL1390R | CTT TCC ATA CTT CAC AAG CAG CAG |

**Phylogenetic analyses**

Sequence alignments were made using ClustalW (Thompson *et al.,* 1994) and edited with the BioEdit package (Hall, 1999). Some nucleotides in the beginning and the end of the rbcL exon were trimmed to obtain of matrix of uniform length. After trimming the there were 1266 base pairs which where in frame with protein translation starting from the 40th base to 1306th base of the rbcL gene. Phylogenetic analyses were performed using PAUP v4.0beta10 (Swofford, 2002), MrBayes (Ronquist and Huelsenbeck, 2003) and Garli (Zwickl, 2006). The best model of evolution for each reading frame was selected using MrModeltest (Nylander, 2004). The dataset

was partitioned into first, second and third codon positions, which were subjected to model selection according to Fern´andez et al. (2006). General time reversible (GTR) model (Tavare, 1986) was obtained for the dataset. GTR+I+G, GTR+I+G and GTR+G were the best models for the first, second and third reading frames respectively according to the Akaike information criterion (AIC). Bayesian posterior probabilities for the branches were calculated with a Metropolis-coupled Markov chain Monte Carlo (MCMCMC3) sampling method as implemented in the program MrBayes, v3.1.2. Two million repetitions, incorporating the obtained models were run, sampling every 100, resulting in 20000 trees and the first 20% considered as the burnin phase and eliminated. A majority rule consensus tree with the remaining 16000 trees was computed with the SUMT command in MrBayes. The resulting posterior probability support values for bipartitions were considered significant at 95%. Maximum-likelihood analysis was performed using Garli v0.95 implementing the GTR+I+G model. ML bootstrap (Davis *et al*., 2007) was obtained from 1000ML nonparametric bootstrap replicates using Garliv0.95 according to Kronauer *et al.* (2007). The bootstrap replicates were used to calculate a majority rule consensus tree in PAUP. Bootstrap frequencies above 50% are indicated at the nodes.

## Results and Discussion

The rbcL dataset comprised of 93 taxa with 1266 base pair length, with 959 characters constant. Of the 25 variable characters 68 characters were parsimony informative (nearly 10%). Since the dataset has numerous taxa, a parsimony based heuristic search would be time consuming and computationally intensive. Therefore a ML search was performed. With programs like Garli which implements the same algorithm as PAUP and can run faster, bootstrapping of ML trees can also be performed (Zwickl, 2006). The maximum likelihood tree obtained using the GTR+I+G model is shown in Figure 6.1. The branches with significant (0.95) Bayesian posterior probability (BPP) are thickened and sequences sequenced in this study are indicated in purple, whereas published sequences from Genbank are in black. ML bootstrap support (BS) (50%) is shown above the branches. *Holarrhena pubescens* which belongs to the Apocynoideae were used as the out group.

The ML tree shows that the subfamilies Periplocoideae, Secamonoideae and Asclepiadoideae

appear in separate clades. Periplocoideae is basal to Secamonoideae and Secamonoideae is basal to Asclepiadoideae (Fig. 1.1). The basal portion of the tree is shown in Fig. 1.1. The Secamonoideae taxa are not well resolved. The Asclepiadoideae clade shows the four tribes Fockeeae, Asclepiadeae, Marsdenieae and Ceropegieae (Fig. 1.1). The subfamilial clades, Periplocoideae and Asclepiadoideae are well supported by high BPP and fairly good bootstrap support (BS = 75% and 54% respectively for Periplocoideae and Asclepiadoideae). The taxa of the tribe Asclepiadeae are paraphyletic (Figs. 1.1). Marsdenieae and Ceropegieae are sister to the Asclepiadeae (Fig. 1.1).

The present work includes many taxa not previously studied by 140 Sennblad and Bremer (2002). The topology of the tree is in accordance with several recent works. The improved sampling of taxa has improved the resolution of the clades especially in subfamily Asclepiadoideae. Due to unavailability of specimens, Secamonoideae could not be well resolved. However, recent papers have addressed these issues to some extent (Livshultz *et al*., 2007; Lahaye *et al.,* 2007). Yet, there remains more work to be done, especially at the subtribal levels of Asclepiadeae which is a very big tribe.

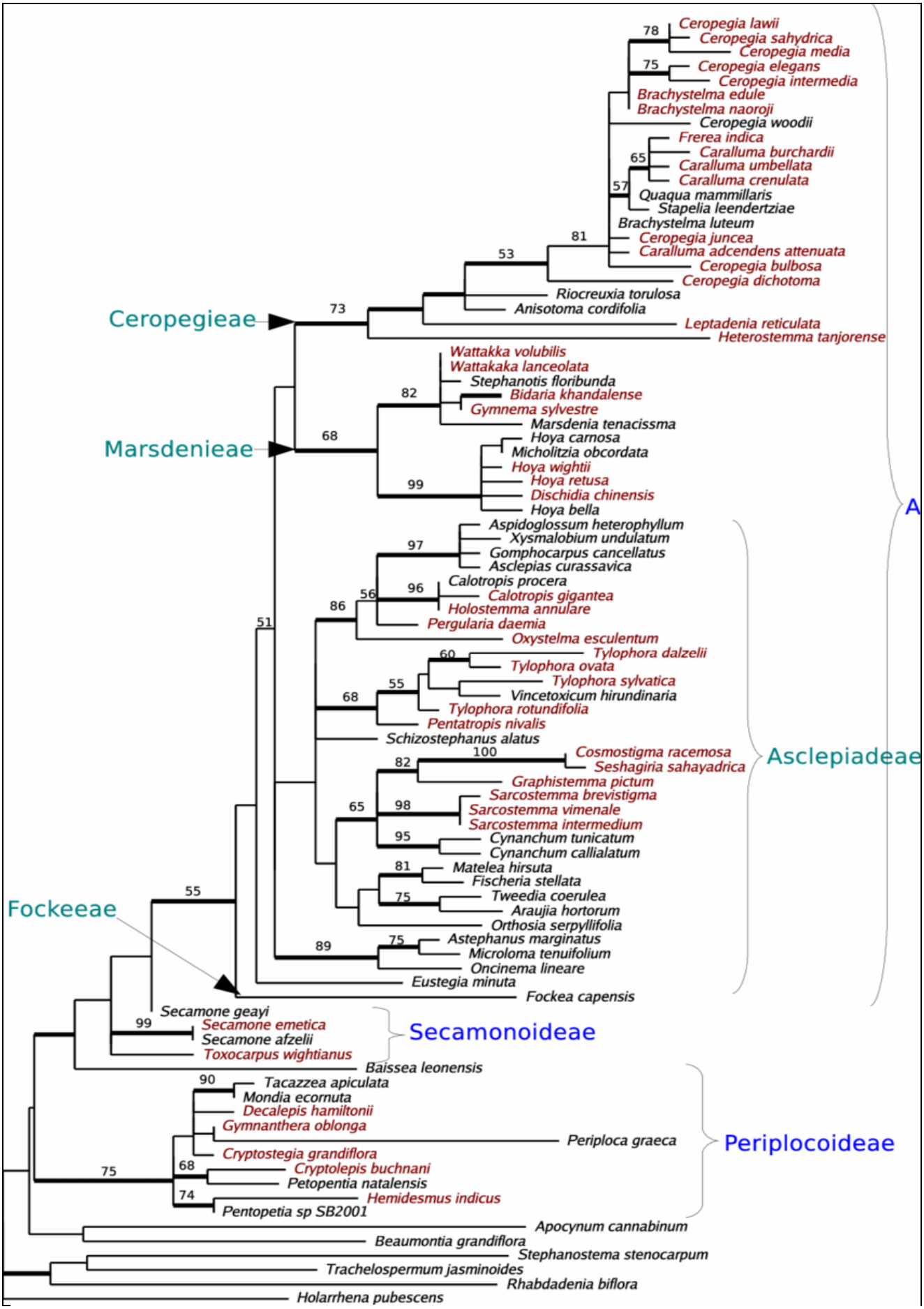


**Fig. 1.1 :** Maximum likelihood tree of rbcL sequences. Branches with 0.95 Bayesian support are thickened. Bootstrap values (50%) are shown above the branches. Sequences from this study are indicated in purple.

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