Untangling the passionflower vines: preliminary insights on the phylogeny of Passiflora subgenus Decaloba based upon trnL-F sequences.
Nicolle M. Siddall, Anna P. McLean, and Kristen Porter-Utley
Keene State College, 229 Main Street, Keene, N.H. 03435

Introduction
Passionflowers (genus Passiflora) are vines, lianas, and trees known for their incredible morphological diversity, stunningly beautiful flowers, and edible fruits. Passionflowers utilize a variety of different pollinators including bats, hummingbirds, butterflies, bees, and wasps (figure 2). Passionflower butterflies also use Passiflora as host plants, and the relationship between these two groups of organisms is a classic example of co-evolution. The genus is currently composed of five subgenera: Astrophytum, Decaloba, Deidamioides, Passiflora, and Tetrapatheae. Due to its economic importance, subgenus Passiflora has been the focus of most scientific research in the genus while the equally fascinating and species-rich subgenus Decaloba has been largely ignored. In addition, recently published revisionary studies of Passiflora have included at most 35 species from Decaloba. There are many rare species in the subgenus that are currently recognized as threatened or endangered, and it is the largest lineage in the genus possessing species found in both the New and Old World. Presented here is a preliminary phylogenetic analysis of the subgenus based upon the gene sequences of trnL-F (Jorgensen et al., 2006).

Methods
Total genomic DNA was extracted from silica dried fresh and herbarium leaf material using Doyle and Doyle’s (1998) CTAB method. Successful amplifications of trnL-F were cleaned using QIAQuick kits (Qiagen). Cycle sequencing and sequencing of the trnL-F region was completed in the DNA sequencing lab at Rancho Santa Ana Botanic Garden in Claremont, California. Sequences were initially assembled in Seqencher (GeneCodes, Ann Arbor, Michigan, USA). Sequences were then aligned in Clustal X (Thompson et al., 1994), and manually aligned in Se-Al (Rambaut, 2000). Simple gap coding (Simmons and Ochoterena, 2000) was applied to the data set using SeqState. Equally weighted parsimony analysis of the trnL-F (including gap characters) data was performed with WinClads (Nixon, 2002) running NONA (Goloboff, 1993) as a daughter process. A heuristic search for the shortest trees was performed using the parsimony ratchet (Nixon, 2002) in WinClada (200 iterations/replicate, one tree held/replicate, 20 ratchet runs). A heuristic search for the shortest trees was performed using the parsimony ratchet (Nixon, 2002) in WinClada (200 iterations/replicate, one tree held/replicate, 20 ratchet runs). A heuristic search for the shortest trees was performed using the parsimony ratchet (Nixon, 2002) in WinClada (200 iterations/replicate, one tree held/replicate, 20 ratchet runs). A heuristic search for the shortest trees was performed using the parsimony ratchet (Nixon, 2002) in WinClada (200 iterations/replicate, one tree held/replicate, 20 ratchet runs).

Discussion
Passiflora consists of four subgenera: Astrophytum, Deidamioides, Decaloba, and Passiflora; all subgenera were sampled. Subgenus Decaloba is currently organized into eight supersections: Pterosperma, Hahnipathanthus, Disemma, Multiflora, Auriculata, Cieca, Bryonioides, and Decaloba (MacDougal and Feuillet, 2006). All of these eight supersections were sampled in the analysis. Four monophyletic supersections are evident in our analysis of the trnL-F region: Hahnipathanthus, Multiflora, Bryonioides, and Cieca. Analysis of the ITS region currently supports four supersections as monophyletic (see poster by Erika Brooks, et al.).

To continue this study we will amplify and sequence additional taxa from the eight supersections. By increasing taxon sampling we hope to further resolve the relationships within the subgenus.

In this analysis we sequenced a 630 base pair segment of trnL-F for the majority of taxa; however, in Passiflora, trnL-F is 1350 base pairs in length. We will also optimize our PCR protocol so that the complete trnL-F region is successfully sequenced and used in future analyses.

Acknowledgments
We would like to thank our advisor, Dr. Kristen Porter-Utley, for her continual support. We would also like to thank the National Science Foundation, the Keene State College Student Conference Fund, and our colleagues at Rancho Santa Ana Botanic Garden; Shawn Krosnick, Lucinda McDade, Saeideh Mashayekhi, Maria Cristina Martinez-Habibe, and Connie Clarke.