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An Examination of Speciation, Extinction, and Evolutionary Relationships in Plants from Two Continents

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Introduction

Aneilema is a genus in the subfamily Commelinaceae. The genus *Aneilema* is quite large with 62 described species (Faden, 1991), andis found primarily in tropical Africa with a few species in Australia (and nearby islands) and in the neotropics. The morphology of the genus is highly variable, and the species range from forested to open habitats(Faden, 1991). These characteristics make morphology-based phylogenies difficult to construct, as shared traits can result from either common ancestry or convergent evolution. Molecular data are thus required to further elucidate the evolutionary lineages within the genus.

In this study, DNA sequences from several chloroplast genes and spacers (*matK*, *rps16*, *psbA-trnH*, and *trnL-trnF*) were used to evaluate phylogenetic relationships within *Aneilema*. *matK*and *rps16* are coding regions that evolve relatively quickly (Crayn et al., 200). The *matK* region has previously been used in other monocot plant families, such as the Bromeliaceae(Crayn et al., 2000). The chloroplast non-coding intergenic spacer regions*psbA-trnH*and *trnL-trnF* were used in this study because they evolve faster than most coding regions. The non-coding intergenic spacer regions are under less genetic constraints,allowing them to undergo greater genetic change (Sang et al., 1997).

The purpose of this research is three-fold. First, it is intended to construct a phylogeny of *Aneilema* and closely related genera in the tribe Commelineae using the chloroplast-encoded regions *matK, rps16, psbA-trnH*, and *trnL-trnF*. Previous family-wide studies have cast doubt on the monophyly of *Aneilema*as currently delineated due to

the position of *Rhopalephora* within *Aneilema* and the placement of *A. brasiliense* apart from the remainder of the genus.

Second, this study is intended to examine relationships among the seven recognized sections (Faden 1991) of *Aneilema* using a phylogenetic approach. The seven sections that Faden (1991) described are *Aneilema, Amelina, Rendlei, Somaliense, Lamprodithyros, Brevibarbata,* and *Pedunculosa*. These sections were described using morphological characteristics, which define all species of known*Aneilema*. These sections were grouped using only morphological characteristics and no molecular data were included.

Third, this work attempts to clarify the position of *Aneilema* within the tribe Commelineae. Previous studies (Evans, unpublished)have indicated that *Aneilema* may be sister to *Pollia*, but those studies were based on relatively low taxon coverage. We believe that data obtained from multiple genes and more complete taxon sampling will further clarify relationships of *Aneilema* within the tribe.

Materials and Methods

Species Sampled- The genus *Aneilema* is composed of 62 species (Faden, 1991). We sampled 17 species within this genus. The species included were *Aneilemaacuminatum, Aneilemaaequnioctiale, Aneilemabeninense, Aneilemabrasiliense, Aneilemacalceolus, Aneilemaclarkei, Aneilemadregeanum, Aneilemagillettii, Aneilemahockii, Aneilemaindetiscens ssp. lilacinum, Aneilemajohnstonii, Aneilemaleiocaule, Aneilemaneocalidonicum, Aneilemarendlei,Aneilemasomaliense, Aneilematanaense,* and*Aneilemaumbrosumsspumbrosum.* Also 13 outgroups were selected to root the

phylogeny. These include *Buforrestiaobovata,Commelinacongesta, Commelinadiffusa, Commelinaerecta, Dicyospermum*sp.*, Floscopaafricana, Floscopascandens, Murdannia japonica,Polliamanni, Polliahasskarlii, Polyspathahirsuta, Rhopalephorascaberrima,*and *Stanfieldiellaimperforata.*

DNA Extraction, Gene Amplification and Sequencing- Every *Aneilema* sample had DNA that had been previously extracted by the Evans group except *A.tanaense* and *A.dregeanum*. DNA extraction was performed using the QiagenDNeasy Mini Kit and following the manufacturer directions.

Polymerase chain reaction (PCR) was used to carry out all gene amplification, using standard procedureswith the addition of 5% DMSO solution. The following parameters were set on the thermal cycler: 94º for 7 minutes (X1), then 30 cycles of 94º for 30 seconds, 42º for 1 minute, and 72º for 2 minutes. A final extension step of 72º for 5 minutes for one cycle followed.

Primers for amplification of *rps16* were taken from Crayn et al (2004), those used in amplification of the *matK* gene are described in Crayn et al. (2000), and those forthe *trnH-psbA* region are from Sang et al., 1997. All primer sequences can be found in Table 1.

The PCR reactions were were purified withQiaquick spin columns. Each purified PCR product then underwent sequencing reactions using either Big Dye Terminator Reaction v3.1 or v1.1, under normal parameters provide by Applied Biosystems. The sequencing products were subsequently sequenced on an ABI 3130 automated sequencer, following manufacturer's guidelines.

Data Analysis- The forward and reverse DNA fragments were assembled using the computer program DNA Baser. The sequences were then aligned using ClustalX, followed by manual optimization. Bayesian analysis were conducted using computer program MrBayes, and parsimony analyses were conducted using PAUP. Parsimony analysis consisted of a multiple islands search and TBR branch swapping. Bootstrap analysis was conducted to evaluate the support for each branch of the phylogeny.

Results

Previous results- Some data for the *trnL-trnF*regions were collected previously in the Evans lab. Sequences were available for seven species each for *psbA-trnH* and *trnL-trnF* (Table 2).

matK- Thirty-two species of *Aneilema* and its out groups were sampled in this study. Of these 32 species, 22 samples were successfully amplified for the *matK* gene. There were 16 samples that sequence was successfully obtained for both *matK* primers. Only one of these 16 did not produce a complete contig sequence (i.e. we were unable to combine the different fragments into a single sequence). For two species, *Aneilemarendeli* and *Aneilemaumbrosum*ssp. *Umbrosum*, only one *matK* primer (primer 5F) worked.

rps16- Of the 32 species, 20 were successfully amplified and sequenced for this gene. Eight species were successfully amplified but did not produce a successful sequence. Three species, *Aneilemaclarkei, Commelinadiffusa,* and *Dictyospermum*,produced a weak reverse primer sequence and a reliable forward primer

sequence. And all three produced a successful contig sequenceeven with the marginal reverse primer.

trnL-trnF- Seven species had been previously sequenced for this intergenic spacer. We successfully amplified 16 of the remaining species, but only collected complete sequences from eight of them.

psbA-trnH- Seven species had been previously sequenced for this intergenic spacer. Only seven additional species produced a successful amplification product, and of those seven only four species resulted in a successful *psbA* primer sequence.

Phylogenetic analysis- The combined alignment (containing each DNA region) consisted of 2,391 nucleotides, where 224 sites were phylogentically informative. Bayesian analysis produced a tree that was almost fully resolved and had a high posterior probability support (Fig. 1). Parsimony analysis yielded six most parsimonious trees of 638 evolutionary steps. The consistency index(CI) was 0.80, and the retention index RI was equal to 0.81. Bootstrap values were also taken and attributed where values were greater than 50. CI, RI, and bootstrap values indicate the level of support for the trees, and they tend to be relatively high in this study, showing good support. It was found that the genus *Aneilema*was not monophyletic due to the positions of *Rhopalephora* and *Aneilemabrasiliense*. *Rhopalephora* is nested within *Aneilema* and *A.brasiliense* is sister to *Polyspatha*(Fig. 1).

Discussion

The Bayesian tree that was created for genus *Aneilema* and its outgroups provide several interesting findings. First, the South American species *A. brasiliense* is more closely related to *Polyspatha* and *Pollia*than any of the other species of *Aneilema* (Fig 1.). *A. brasiliense* is biogeographically unusual in the genus, as it is the only species of *Aneilema* with a strictly New World distribution. Additionally it differs from other *Aneilema* species in its morphology. Clarke (1881) and laterFaden (1991) noted that the divergent morphology might warrant elevation of *A. brasiliense* to generic status.

Second, we found that with the inclusion of *Rhopalephora* (which contains 4 species) and the exclusion of *Aneilemabrasiliense*, the genus *Aneilema* is monophyletic (Fig. 1). This is in agreement with previous results, such as Evans et al. (2003), who found that based on the marker *rbcL*,*Rhopalephora* was also nested within *Aneilema*. Also it is understood that apart from a basic chromosome number of *x*=29, *Rhopalephora* cannot be distinguished from *Aneilema* by any single character, but only by a combination of characters (Faden 1991).

Third, our results show that sections*Aneilema, Brevibarbata* and *Lamprodithyros* are all monophyletic. Section *Aneilema* was supported with a bootstrap value of 80 and a posterior probability of 100, whereas*Brevibarbata* has a bootstrap value of 78 and a posterior probability of 82. The section *Laprodithyrous* has a bootstrap value of 77 and a posterior probability of 100. These high values demonstrate strong supported for monophyly.

Fourth, our data indicate that section *Ameilina* is polyphyletic. Figure 1 shows that *A.aequinoctiale* and *A. hockii* form a strongly supported clade, with a bootstrap and posterior probability of 100, whereas*A.gillettii* is outside of this clade and grouped with *A. pedunculosa*.

Finally, our data indicate that*A. johnstonii* is sister to the rest of the entire genus *Aneliema*. Knowledge of the earliest-diverging lineage of a group will be instrumental in determining the early evolutionary trends within that group.

While the phylogeny obtained here is supported by relatively strong bootstrap and posterior probability values, more work needs to go into this study to achieve completion. First we wish to obtain data from more more species of *Aneilema*. We sampled only 17 species, while there are 64 species in the genus. Also additional outgroupsprovide stronger support for rooting our tree. We also plan to obtain data using one or more nuclear encoded genes to compliment the chloroplast DNA study.

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