Identifying & DNA Barcoding Asteraceae of the Chicago Region

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INTRODUCTION AND OBJECTIVES

Asteraceae, commonly called the sunflower family, is the largest family of flowering plants in the world, and often difficult to identify to the species level. DNA barcoding is a process that uses a short piece of DNA sequence from a standard locus as a species identification tool. A DNA barcode region has been adopted for animals (1). Several regions have been suggested for plants, but no consensus has been reached (2, 3). We took a floristic approach in testing the utility of barcoding in Asteraceae. We attempted to sequence several suggested plant barcode regions of species found in the Chicago Region belonging to the former genus *Aster* to determine **1) if a floristic approach is able to uniquely identify each species and 2) if the barcode regions are phylogenetically informative and support the current classification of the former genus *Aster* which is now broken into several different genera (*Eurybia, Symphyotrichum, Oligoneuron*, and *Doellingeria*).**

We also explored other identification tools by working on the development of an interactive identification key for approximately 70 Asteraceae species found in the Dixon Prairie at the Chicago Botanic Garden (CBG). An interactive plant identification key is a beneficial tool because it allows users to navigate through a series of questions in whatever order they prefer. This key will be posted on the website discoverlife.org, and will be a useful tool for non-botanists and botanists alike, because of its user-friendly language and images.

MATERIALS AND METHODS

One hundred plant samples representing 23 of the 29 species formerly belonging to genus *Aster* found in the Chicago Region were collected from either live material in the Dixon Prairie at CBG or from herbarium specimens at CBG (Table 1). DNA was extracted from them using the Qiagen DNeasy Plant mini kit protocol. We attempted to PCR amplify four previously suggested plant DNA barcode regions (*trnH-psbA, rbcL, matK,* and ITS) on a subset of samples. Due to the low success rate of PCR amplification, *trnH-psbA* was not pursued for the remaining samples. Due to time constraints, only successful ITS amplifications were cleaned using a Qiagen PCR cleanup kit and subsequently sequenced on an ABI 3730 following standard protocol. Sequences from some individuals, as well as outgroup taxa, were downloaded from Genbank.

Sequences were edited in Codoncode Aligner and aligned using MUSCLE and Se-Al. Phylogenetic analyses to determine if the different species were monophyletic were performed using maximum parsimony and heuristic searches in PAUP (4). Pairwise distances between taxa were also calculated using PAUP.

RESULTS

Sequences were obtained and aligned for 110 individuals representing seven outgroup taxa and 103 ingroup samples. Example sequence alignments are shown in Figure 1. Results of pairwise distances are shown in Table 1. Levels of variation within species were not consistently lower than levels of variation between species. There were 123 parsimony informative characters and 33 most parsimonious trees (consistency index=0.60, retention index=0.91) of length 267 were found. The strict consensus tree (Figure 2) revealed that each of the four genera into which the *Aster* of the Chicago Region have been divided represent monophyletic groups.

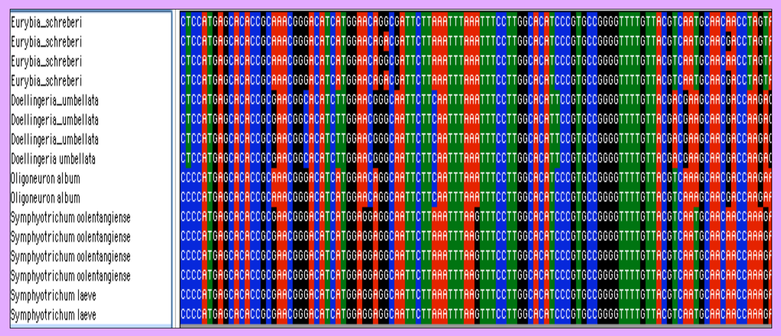

Figure 1. Portion of ITS sequence alignment.

Symphyotrichum novae-angliae

Symphyotrichum pilosum

DATA

Species	N	% intraspecific differences	% interspecific differences
Doellingeria umbellata	7	0	Interspecific differences ranged from 0 (e.g. *S. oolentangiense and S. laeve*) to over 20% (e.g. *S. ericoides and S. boreale*)
Eurybia macrophylla	1	NA	
E. schreberi	9	0-12.602	
E. furcata	3	0-2.21	
Oligoneuron album	2	0.833	
O. x lutescens	0	NA	
Symphyotrichum boreale	5	0-4.959	
S. ciliatum	1	NA	
S. urophyllum	7	0-2.596	
S. cordifolium	8	0-10.746	
S. dumosum	5	0-2.479	
S. ericoides	4	0-5.53	
S. laeve	2	2.011	
S. lanceolatum	3	1.695	
S. lateriflorum	0	NA	
S. lateriflorum	4	0.847-12.005	
S. novae-angliae	3	6.02-8.932	
S. oblongifolium	2	1.667	
S. ontarionis	0	NA	
S. parviceps	0	NA	
S. pilosum	4	0-1.695	
S. praealtum	3	2.542-5.932	
S. prenanthoides	0	NA	
S. puniceum	8	0-6.914	
S. sericeum	5	0-5.484	
S. shortii	4	0-4.361	
S. subulatum	7	0-5.311	
S. oolentangiense	6	0	

Table 1. List of former *Aster* species found in the Chicago Region. N refers to the number of individuals for each species that was included in the study.

CONCLUSIONS

The conclusions of this study are as follows:

1) Is a floristic approach able to uniquely identify each of the former *Aster* species in the Chicago Region?

NO. Although several species can be uniquely identified, as revealed by their monophyly (Figure 1) and low levels of within species distances (table 1), many species could not be identified. In fact, in some cases the amount of differences *within* a species was greater than *between* species (table 1). For example, pairwise differences among *Eurybia schreberi* samples ranged from 0 - 12.6%, whereas there were absolutely no differences between certain species like *Symphyotrichum oolentangiense* and *S. laeve*.

2) Are the proposed barcode regions phylogenetically informative and do they support the current classification of the former genus *Aster,* which is now broken into several different genera (*Eurybia, Symphyotrichum, Oligoneuron*, and *Doellingeria*)?

YES. Using the ITS region (which has been proposed as a possible plant DNA barcode region) for phylogenetic reconstruction revealed *Eurybia, Symphyotrichum, Oligoneuron*, and *Doellingeria* all to be monophyletic, which supports the current classification of the former genus *Aster*.

3. Is ITS a useful plant DNA barcode region?

YES and NO. The ITS region was successful at differentiating among closely related genera, and among some species, but not all. Future work could focus on adding additional regions, such as *rbcL* and *matK*, to produce accurate species level identification.

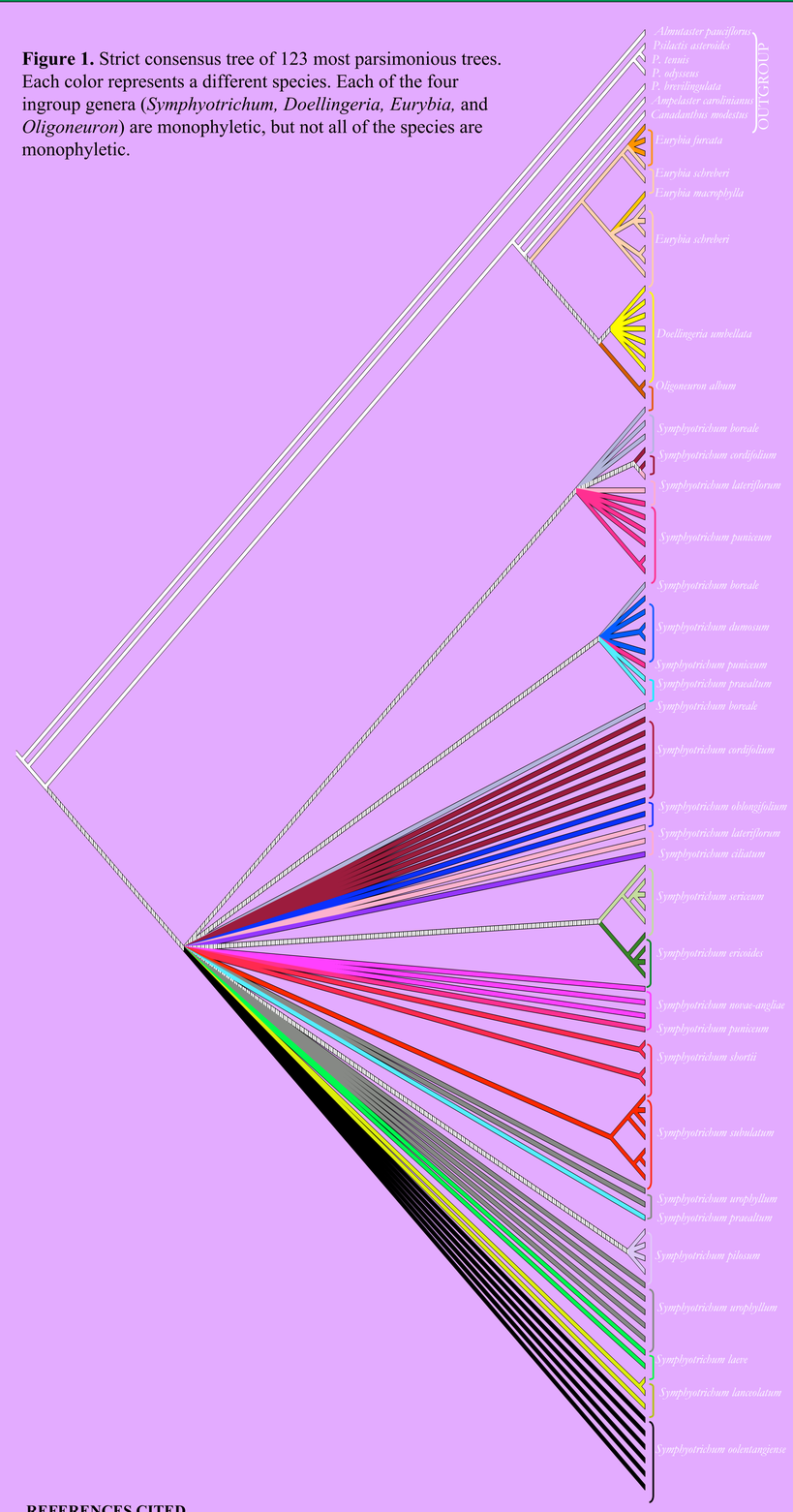

Figure 1. Strict consensus tree of 123 most parsimonious trees. Each color represents a different species. Each of the four ingroup genera (*Symphyotrichum, Doellingeria, Eurybia,* and *Oligoneuron*) are monophyletic, but not all of the species are monophyletic.

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