Endangered *Endodeca serpentaria*: Living it Large in NYC!

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Introduction

Aristolochia* s.l.** is a genus found predominantly in **tropical and warm temperature** regions, particularly so in Asia and South America[1]. Morphological, molecular, and chromosomal data suggests recognition of the genus as four genera, ***Aristolochia* s.s.**, ***Pararistolochia, ***Isotrema***, and ***Endodeca***[1,2,3]. Historically, plants from Aristolochiaceae were used medicinally despite notoriety for containing **aristolochic acid**, which numerous studies associate with nephrotoxicity, carcinogenicity, and mutagenicity[4].

If recognized as a genus, ***Endodeca serpentaria*** (L.) Raf. represents a **monotypic herb** found across a wide swath of the **U.S.** from the East Coast, north to Michigan, west to Missouri, and south to Florida[5]. The same chemical that is fatal to humans acts a protectant to the larvae of **pipe swallowtail butterflies** that feed off its foliage, which sequester the toxin into adulthood making them unpalatable to insectivores[4,5]. In its **northern range**, several states consider it **endangered**. In **New York State**, it was considered **extinct** until recent sightings in the **Hudson Highlands** a decade ago[5]. Henning located the taxon in **Van Cortlandt Park** in 2012[6], its first recording in New York City since the early 1900s (see figure 1). **Pollination history** and **seed dispersal** of the genus is unclear[4].

Figure 1. *Endodeca* growing in Van Cortlandt Park, Bronx, on 8/4/18, (photo credit Acevedo).

Urban green spaces function as '**islands**' where the interruption of gene flow can allow trapped taxa to accumulate genetic mutations that may result in differentiation[7]. Given its **threatened status**, we commenced a **population genetics study of *Endodeca*** using **microsatellites** to elucidate the **VCP haplotype** for comparison against isolates from the tristate region from which it has been separated for decades, if not centuries. **We predict coastal populations will show more drift from inland sites** given distance and geographical separation. This study is important since it may show the emergence of **genetic structure** at the population level. Given the endangered status of *Endodeca* in the Northeast, homogenization of local gene pools might indicate the need for purposeful **transplants** to add genetic variability back into the lineage in its northern range.

Study Aims

- **Generate a current cladogram of our focal taxon, *Endodeca***
- **Conduct a literature review**
- **Relocate populations in Van Cortlandt Park for DNA work**
- **Locate additional tristate sites for collection and sequencing**
- **Create a haplotype map based on microsats**

Materials and Methods

A **literature search** was conducted for relevant material[1-5]. **NCBI**[8] search and BLAST were used to see what nucleotide material is currently available. A **FASTA** file was constructed using **matK cpDNA sequences** for ***Endodeca*** and three representatives of ***Aristolochia*** along with ***Asarum*** and ***Saruma*** (sister genera from Aristolochiaceae), and ***Saururus*** (Saururaceae), which was used as an outgroup. This was imported into **CLC Sequence Viewer 8** to construct a preliminary cladogram using a **neighbor-joining model**[9] (bs 1000, Jukes-Cantor).

Van Cortlandt Park was visited to relocate ***Endodeca***, collect tissue and **georeference** the site. **Virtual herbaria** were searched to compile a list of locations to visit for DNA sampling over the course of the next months awaiting plant collection approval. **DNA extractions** will be done using a **DNeasy Plant Mini Kit**[10] following standard protocol except for an extended incubation period to 30 min with product verification using a nanodrop. Sequencing will be outsourced to **Macrogen**[11] using the **microsat $_{cp}$primers AR-trnK-420F** and **AR-trnK-1302R** per Wanke *et al.*[12] (e.g. F: AAG TGA ATA AAT GGA TAG AGC; R: ATC GCT CTT TTG ACT TTG G). Chloroplasts are usually conservatively evolving although recent research suggests useable intronic variation exists in the **trnK gene** of *Aristolochia pallida*, which returned 21 haplotypes[3,12] (Table 1).

Table 1. Wanke *et al.*[12] report success using intronic microsats in trnK, whose differences in aligned lengths were coded as if they were 'indels', insertion and deletion events.

Haplotype	Relative haplotype frequencies	Size (bp)	Indel matrix (sic)	EMBL Accession no.
01	0.0667	388.0	10??100?1??10?0?????1??????111	AM237617, AM237618
02	0.0667	393.0	10??100?1?10000????1??????111	AM237604, AM237605
03	0.0667	397.0	1000000111?1000000????1??????111	AM237606, AM237607
04	0.1	442.0	10?100001100001????1000?110111	AM237629, AM237628, AM237630
05	0.167	399.0	10?100001?1000000????1??????111	AM237608, AM237609, AM237610, AM237613, AM237615
06	0.0333	387.0	11??100?1??10?0?????1??????111	AM237603
07	0.0333	404.0	10?100001?10000000????1??????011	AM237614
08	0.0333	402.0	11???10???000001100000?1????111	AM237620
09	0.0333	442.0	11??100?1??10?0??10000011111111	AM237621
10	0.0333	399.0	11????1????000000????1??????100	AM237602
11	0.0333	392.0	10?100001???1?????????1??????111	AM237612
12	0.0333	445.0	10?100001??10?0????100000101111	AM237624
13	0.0333	401.0	10100000100001?0?????1??????111	AM237619
14	0.0333	447.0	10?100001000010????100000111111	AM237625
15	0.0333	420.0	00??100?0?1000000000001?????111	AM237611
16	0.0333	386.0	11????1????000000????1??????111	AM237601
17	0.0333	394.0	10?100001??10?0??????1??????111	AM237616
18	0.0333	441.0	10?100001??10?0????100000111111	AM237626
19	0.0333	436.0	10?100001??10?0????1000?111111	AM237622
20	0.0333	464.0	10?100001??10?0????100000011111	AM237623
21	0.0333	465.0	10?100001100001????1000?010111	AM237627

Alignment and **haplotyping** will be accomplished using **Structure**[13], a freely downloadable software package. **PAST ver. 3.2** software[14] will be used for ordination for visualization of genetic distance between populations and to construct a Shepard plot.

Results and Discussion

Our cladogram resolves Aristolochiaceae into **two major clades**, ***Aristolochia* s.s.** showing ***Endodeca*** as a unique **operational taxonomic unit** (**OTU**) sister to *A. macrophylla*, another taxon from Eastern USA. Another clade is made up of Asian *Asarum* and *Saruma*, with the nearest outgroup family, Saururaceae, resolving as root.

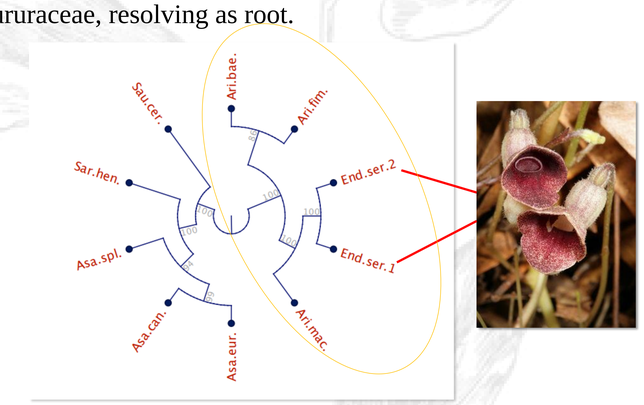

Figure 2. Cladogram emphasizing the molecular uniqueness of *Endodeca* within *Aristolochia* and Aristolochiaceae. Insert shows taxon in bloom (photo credit EOL[14].)

It took two visits to VCP to **relocate *Endodeca*** as **eight plants** in a single stand. The **population is increasing in size** indicating a lack of pipevine swallowtail larval predation, which typically reduces populations to a few plants at most[5]. A second small stand[6] has disappeared due to competition from poison ivy (*Toxicodendron radicans*) and Oriental bittersweet (*Celastrus orbiculatus*). A physical visit to the **Steere Herbarium** at New York Botanical Garden was combined with **virtual herbaria** reports[14] to identify **6-8 locations** in the tristate region for collection of comparison sample tissue. **DNA extractions** of VCP's population have commenced.

In preliminary **conclusion**, **a distance measure supports *Endodeca* as an OTU whose rare occurrence locally will be elucidated by haplotyping to understand its population structure in relation to distribution.**

References

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