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EVALUATING RANGE GENETICS IN BLACK CHERRY (*PRUNUS SEROTINA*) AND THE  
GENETIC STATUS OF AN ENIGMATIC RELATIVE, ALABAMA CHERRY (*PRUNUS  
ALABAMENSIS*)

A Thesis by

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Bachelor of Science, Wichita State University, 2014

Submitted to the Department of Biological Sciences  
and the faculty of the Graduate School of  
Wichita State University  
in partial fulfillment of  
the requirements for the degree of  
Master of Science

May 2017

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EVALUATING RANGE GENETICS IN BLACK CHERRY (*PRUNUS SEROTINA*) AND THE  
GENETIC STATUS OF AN ENIGMATIC RELATIVE, ALABAMA CHERRY (*PRUNUS  
ALABAMENSIS*)

The following faculty members have examined the final copy of this thesis for form and content, and recommend that it be accepted in partial fulfillment of the requirement for the degree of Master of Science with a major in Biological Sciences.

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## CHAPTER 1

### GEOGRAPHIC STRUCTURE OF GENETIC VARIATION IN *PRUNUS SEROTINA* SUBSP. *SEROTINA*

#### **Abstract:**

*Premise of the study:* Isolation by distance (IBD) is a genetic pattern in which populations geographically closer to one another are more genetically similar to each other than populations which are further apart. Black cherry (*Prunus serotina* Ehrh.) (Rosaceae) is a forest tree species widespread in eastern North America, and found sporadically in the southwestern United States, Mexico, and Guatemala. This tree is both commercially important as timber for furniture and ecologically important as a source of nutrition for local wildlife. IBD has been studied in relatively few North American plant taxa, and no study has rigorously sampled across the range of such a widespread species. In this research we evaluated IBD and overall genetic structure in eastern black cherry (*Prunus serotina* Ehrh. subsp. *serotina*), the widespread black cherry subspecies of eastern North America.

*Methods:* Dense sampling across the eastern black cherry range was made possible by genotyping 15 microsatellite loci in 439 herbarium samples from all portions of the range. Mantel tests and STRUCTURE analyses were performed to evaluate the hypothesis of IBD and overall genetic structure.

*Key results:* Mantel tests demonstrated no isolation by distance at any distance class, and STRUCTURE analyses revealed no clear geographic pattern of genetic groups.

*Conclusions:* The lack of detectable large-scale genetic structure across the range of eastern black cherry suggests widespread gene flow in this taxon. This is consistent with *P. serotina*'s status as a disturbance-associated species, and for comparison further studies should include species characteristic of low-disturbance forests.

## **Introduction:**

Isolation by distance (IBD) is a fundamental biogeographic pattern in which a given population is most genetically similar to geographically proximate populations, with decreasing similarity to increasingly distant populations. This pattern is driven by an intuitive limit to gene flow involving a decreasing movement of pollen and seeds between populations as the distance between these populations increases (Wright 1943). Although evaluating this pattern is relatively straightforward analytically, IBD has been rigorously assessed in relatively few widespread North American plant taxa, among them forest trees.

The main obstacle to geographically rigorous tests of IBD is the difficulty in assembling a sample set representative of an entire species' range. While a few studies have analyzed large sets of samples, these are often drawn from a relatively small number (<100) of locations (Parker et al. 1997; Griffin and Barrett 2004; Mylecraine et al. 2004; Hoban et al. 2010; Campitelli and Stinchombe 2013; Waselkov and Olsen 2014; Thomson et al. 2015) and/or from a subset of the total species range (Yakimowski and Eckert 2008; Habziabdic 2010; Lloyd et al. 2011; Dennhardt et al. 2016). This lack of sampling is due to feasibility- although rewarding, field-collecting material across large spatial scales is an expensive and extremely time-consuming task (planning routes, obtaining permits, fieldwork, preparing voucher specimens, permit reporting). In reality, even sampling across a large range is beyond the scope of a dissertation, to say

nothing of a 2-year M.S. thesis. Due to these limitations, field-collecting is often done in a highly non-random manner, with much of the sampling confined to a few clusters of fairly proximate populations. For example, Hoban et al. (2010) sampled over 1000 trees, but these were largely obtained at ca. 10 clusters of geographically proximate populations. This study exemplifies many existing studies of IBD in North American plant taxa. As a result, what little we know about the geographic structure of genetic variation in widespread North American plants is based on highly uneven sampling designs.

Studies evaluating IBD in native North American plant species with a variety of genetic markers suggest that such structure is typically present. In herbaceous plants, Waselkov and Olsen (2014) demonstrated a significant ( $R^2 = 0.079$ ) relationship in waterhemp [*Amaranthus tuberculatus* (Moq. ex DC) J. D. Sauer] with a 10-locus microsatellite dataset, and Griffin and Barrett (2004) found significant IBD in *Trillium grandiflorum* (Michx.) Salisb. ( $R^2 = 0.17$ ) using allozymes. A study by Servick et al. (2015) used 10 microsatellite loci to find a significant signal of IBD in diploid *Galax urceolata* (Poir.) Brummitt ( $R^2 = 0.148$ ). Significant IBD has also been observed in studies of widespread North American woody plants. Hoban et al. (2010) reported significant IBD in butternut (*Juglans cinera* L.) with 11 microsatellite loci ( $R^2 = 0.128$ ) when calculated with  $F_{ST}$ ,  $R^2 = 0.067$  when calculated with  $R_{ST}$ , and Geraldts et al. (2014) reported this pattern using SNPs in black cottonwood (*Populus trichocarpa* Torr. & A. Gray ex Hook) ( $R^2 = 0.2873$ ). Victory et al. (2006) also found a significant, albeit very weak, signal of IBD in *Juglans nigra* using 12 microsatellite loci ( $P = 0.0485$ ,  $R^2 = 0.006$ ), as did Habzidic (2010) in a study of flowering dogwood (*Cornus florida*) ( $P = 0.0029$ ,  $R^2 = 0.01$ ). Many other studies have indirectly addressed the notion of IBD in North American plants, often through the visual interpretation of the geographic array of DNA sequence haplotypes or multivariate genetic

clusters. These studies also typically note geographically-structured genetic variation. Thirteen of the 19 native plant taxa reviewed in Soltis et al. (2006) noted some level of geographic structure, as have numerous subsequent studies (Tsai and Manos 2010; Thomson et al. 2015; Rodrigues and Stefanović 2016; Willyard et al. 2017).

In contrast, many exotic species in North America do not exhibit IBD in this non-native range. Using AFLP markers, Jorgensen and Mauricio (2004) found no signal of IBD in *Arabidopsis thaliana* (L.) Heynh. ( $P = 0.99$ ) in North America. Similarly, Kirk et al. (2011) and Stabile et al. (2016) found no signal of IBD in *Phragmites australis* (Cav.) Trin. ex Steud. ( $P = 0.979$  and  $0.62$ , respectively) using microsatellite markers. Additionally, Durka et al. (2005) found a significant but very weak signal of IBD in *Alliaria petiolata* Cavara & Grande ( $P = 0.002$ ,  $R^2 = 0.043$ ) using 8 microsatellite loci. This general lack of IBD in exotic species is perhaps not surprising given the potential for multiple, potentially geographically distant, introductions of genetically variable material to the non-native range, and the propensity for widespread non-native species to exhibit frequent long-range gene flow.

Taken as a whole then, previous research suggests that IBD is to be expected in native North American plants, and our research aims to test this basic hypothesis of genetic structure in eastern black cherry (*Prunus serotina* Ehrh. subsp. *serotina*), a widespread eastern North American forest tree that is important ecologically as a fruit-source for wildlife (Thompson 1979) and as a timber source (Auclair and Cottam 1971). Each year, over 7 million board feet of black cherry are harvested (Howard et al. 2013), largely for use as a veneer in furniture making (Gatchell 1971). Eastern black cherry has by far the largest range of the five *P. serotina* subspecies, found throughout the United States and southern Canada east of 96° west longitude

(McVaugh 1951), with rare occurrences in Mexico and Guatemala. The remaining subspecies have more restricted distributions. *Prunus serotina* Ehrh. subsp. *eximia* (Small) Little is restricted to the Edwards Plateau of Central Texas; *Prunus serotina* Ehrh. subsp. *alabamensis* (C. Mohr) Little is found in Georgia, Alabama, South Carolina, and Florida; *Prunus serotina* Ehrh. subsp. *capuli* (Cav. Ex Spreng) McVaugh is found in southern Mexico and Guatemala; and *Prunus serotina* Ehrh. subsp. *virens* (Wooton & Standl.) McVaugh from Texas to Arizona and south to Jalisco and Guanajuato (McVaugh 1951).

As with any widespread species, rigorously evaluating IBD in eastern black cherry requires a large geographically representative set of samples. Unfortunately, as discussed above densely field-collecting samples across this large range is simply not feasible in the time frame of most studies. This limitation can be bypassed, however, through the extensive use of museum tissue. Black cherry is commonly collected, and thousands of specimens are archived in North American herbarium collections. These specimens bear locality data, allowing researchers to easily choose specific locations from which to collect genetic data. Genetic data from a set of geographically representative specimens could therefore be used to evaluate the hypothesis of IBD in this widespread forest tree.

## **Materials and Methods:**

**Obtaining samples** - Our group personally visited 21 herbaria (obtaining 373 specimens) and requested tissue from 13 herbaria (131 specimens) in 2014, 2015, and 2016 (see acknowledgements and Appendix 1). Following visits to large national institutions, specific regional and local collections were targeted in order to fill in geographic gaps in our sample set. We attempted to maximize the spatial scope of our sampling by choosing only one specimen per

county, generally avoiding adjacent counties. All specimen identifications were verified, with a positive ID for *P. serotina* subsp. *serotina* involving two or more of the following characteristics when possible: small orange hairs at the base of the midrib, (hooked) serrated leaf margins, leaves more than 2.5× as long as wide, and persistent sepals when in fruit. One or two small leaves were removed when sufficient material was present, and acid-free labels noting that material was removed for DNA extraction were affixed to all sheets from which we sampled. All specimens were georeferenced using the specimen locality data with Google Earth (Google Inc. 2009) and EarthPoint (Bill Clark, 2015) software.

**Molecular methods** - Following a high-throughput DNA extraction (Beck et al. 2012), 15 microsatellite loci originally designed for other *Prunus* species were amplified (Table 1): *Prunus persica* (L.) Batsch (Cipriani et al. 1999; Downey and Iezzoni 2000; Testolin et al. 2000; Dirlewanger et al. 2002; Yamamoto et al. 2002), *Prunus avium* L. (Dirlewanger et al. 2002; Struss et al. 2003), *Prunus cerasus* L. (Downey and Iezzoni 2000), *Prunus virginiana* L. (Wang et al. 2012), and *Prunus geniculata* R. M. Harper (Germain-Aubrey et al. 2011). These loci were chosen on the basis of previous success in *Prunus serotina* subsp. *serotina* (Pairon and Jacquemart 2008; Pairon et al. 2010) or preliminary CAG-tag PCR screening (Beck et al. 2014). Ten of these 15 loci (Table 1) amplified at most two alleles per locus, presumably due to the allotetraploid nature of *P. serotina* (Pairon and Jacquemart 2005, 2008). Three to four locus sets (Table 1) were dye-labeled and simultaneously amplified in 8 μL reactions using the following reagents: 2.5μL Qiagen multiplex PCR Master mix (Qiagen, Germantown, MD), 2μM each primer, and 2μL 1:10 diluted DNA template. PCR cycling conditions included an initial denaturing step at 95°C for 15 minutes, 30 cycles of 94°C for 30 seconds/ 53°C for 1 minute and

30 seconds/ 72°C for 1 minute, followed by a final extension at 60°C for 30 minutes. Amplicons were sized at the University of Chicago Comprehensive Cancer Center DNA Sequencing and Genotyping Facility, and alleles were scored with GeneMarker 1.9 (SoftGenetics, State College, Pennsylvania, USA). As a quality-control measure, 20% of samples were both re-extracted and re-amplified, and an additional 14% were re-amplified from existing extractions.

**Genotyping success assessment** – To determine if specimen age or curatorial conditions affected genotyping success, we explored the relationship between the number of loci genotyped with both age of the specimen and its herbarium of origin. A generalized linear model implemented in R (R core team 2015) was used. For this analysis, we constructed a generalized linear model (“glm” function) with the “poisson” distribution argument due to the non-normal distribution of our dataset. A likelihood ratio test was then used to evaluate the fit of nested statistical models (“drop1” function with the “Chisq” test argument) to discover any significant relationship between specimen age, herbarium, and number of loci genotyped.

**Mantel tests and Structure analyses** - Isolation by distance was evaluated using Mantel tests (Mantel 1967), which assessed the overall correlation between matrices of genetic and geographic distances among samples. Mantel tests were performed in R v.3.2.3 (R Core Team 2015) using the package “vegan” (Oksanen et al. 2016) on three datasets: including only samples from which  $\geq 10$  loci were amplified ( $n = 464$ ), including only samples from which  $\geq 12$  loci were amplified ( $n = 439$ ), and including only samples from which  $\geq 14$  loci were amplified ( $n = 381$ ). Since Mantel tests on all datasets failed to detect IBD (see results), the  $\geq 12$ -locus dataset ("12-locus dataset") was chosen for the remainder of our analyses to strike a balance between locus

missingness and sample number. In addition to a standard Mantel analysis we also performed a Mantel correlogram (Oden and Sokal 1986), which seeks to find a correlation between genetic and geographic distances between samples separated by a given range of geographic distance. We specified 20 distance classes of 200 km each (e.g. all samples separated by 0–200 km, all samples separated by 201–400 km, etc.). Both standard Mantel and Mantel correlogram analyses utilized the Bruvo genetic distance measure (Bruvo et al. 2004) obtained in GenoDive v.20b27 (Merimans and Tienderen 2004). The Bruvo measure lends itself well to mixed ploidy levels by both applying a stepwise mutational process and allowing for distance calculations between mixed ploidy levels by adding “virtual alleles” to genotypes/individuals with lower ploidy levels. Finally, the presence and location of major genetic groups was evaluated with STRUCTURE v.2.3.4 (Hubisz et al. 2009). STRUCTURE attempts to identify populations that are in linkage equilibrium, and in the process assigns each sample to one of these populations or partially to multiple populations. We performed STRUCTURE analyses on the 12-locus dataset in two ways: first using only the 10 “diploid” loci, and then using all loci, but “diploidized,” where all loci are subsampled to include only two alleles per locus per individual. The diploidized dataset was constructed in GenoDive. Both analyses included five iterations each at  $K = 2$  to  $K = 10$ , with each iteration featuring 100,000 burnin generations followed by 500,000 data collection generations. All STRUCTURE analyses assumed the admixture and independent allele frequency models. The approach outlined in Evanno et al. (2005) and implemented in Structure Harvester (Earl and vonHoldt 2012) was used to find the most likely value of  $K$ .

## Results:

**Genetic dataset and genotyping success** - The 506 *P. serotina* subsp. *serotina* samples obtained from 34 herbaria represented 37 states/ provinces and 496 U.S. counties (Appendix 1, Fig. 1). Specimen collection years ranged from 1885–2015 (mean age = 41.66 years,  $\pm$  26.25 years). Overall genotyping success was high, as data was obtained at all 15 loci in 59.9% (303) of samples and  $\geq$ 12 loci in 86.8% (439) of samples. The generalized linear modeling analysis demonstrated a significant effect of specimen age ( $P = 3.887e-06$ ) but not of herbarium of origin ( $P = 0.1797$ ) on number of loci genotyped (Fig. 2).

**Mantel tests** – All Mantel tests ( $\geq$ 10,  $\geq$ 12, and  $\geq$ 14-locus datasets) were insignificant ( $P = 0.54$ ,  $P = 0.349$ ,  $P = 0.448$ , respectively). Likewise, no distance class was significant in the Mantel correlogram analysis of the 12-locus dataset (Fig. 3).

**Structure analyses** – The Evanno et al. (2005) summary of the STRUCTURE analysis identified an optimal  $K$  of 4 for both the diploid-only and diploidized versions of the 12-locus dataset. However, at  $K = 4$ , all samples exhibited nearly equal cluster assignments. In the diploid-only analysis, only 8.66% (38 samples) exhibited a cluster assignment of  $\pm$  25%, while all other samples exhibited an assignment of exactly 25% to each cluster. In the diploidized analysis, only 18% (79 samples) had a cluster assignment of  $\pm$  25%.

## Discussion:

**Isolation by distance** – No significant signal of IBD was found, regardless of dataset type or distance class. Consistent with this simple lack of a relationship between genetic similarity and

geographic distance, STRUCTURE suggests that few samples are strongly assigned to any of the most optimal set of genetic groups. Together these results suggest that gene flow is sufficiently powerful to randomize neutral genetic variation throughout the range of *P. serotina* subsp. *serotina*, at least at spatial scales at and above the population.

It is important to emphasize here that our dataset is different from most existing studies in that we utilized a "many sites, one individual per site" strategy, as opposed to a one featuring "few sites, many individuals per site." The standard dataset of multiple individuals per site considers variation among individuals within sites, but leads to a sparse and often geographically uneven array of sites given time and financial constraints. Our strategy instead emphasizes a geographically dense and even array of sites, but does not account for within-site variation. Eastern black cherry populations do harbor considerable within-site neutral genetic variation, as the seven populations surveyed in Beck et al. (2014) exhibited an average observed heterozygosity of 0.75 across five SSR loci, which was higher than the average value (0.65) reported for the 71 outcrossing plant taxa reviewed in Nybom et al. (2004). However, any limits to gene flow would tend to partition this variation between sites, and our single-individual per site strategy would therefore be misleading only if we were consistently analyzing individuals that were somehow genetically atypical for their site. Although we feel that this is unlikely, the role of sampling design on the detection of genetic structure should be explored through simulation studies (Prunier et al. 2013).

The overall lack of genetic structure we observed in *P. serotina* subsp. *serotina* is surprising given the numerous studies of North American plants that have revealed significant IBD or obvious geographically structured genetic variation (see introduction). Two factors may

underlie the lack of this pattern. The first is life history- eastern black cherry is a pollinated by generalist insects (Guitian et al. 1993; Fortuna et al. 2008; Jacobs et al. 2009; Lander et al. 2013) and has seeds that are potentially dispersed via bird feces (Marks 1974; Thompson 1979). It is also a fast grower (Auclair 1975) that easily exploits disturbance (Ladd and Thomas 2015). Ladd and Thomas used a modified version of the Floristic Quality Assessment (FQA) to evaluate the conservatism of all vascular plant species in Missouri. In this assessment, a species' conservatism level can be thought of as its affinity (or lack thereof) for naturally intact habitat (Ladd and Thomas 2015). Each species was given a conservatism score, with values ranging from 0 (no conservatism) through 10 (highly conservative). *Prunus serotina* subsp. *serotina* was given a score of two, indicating little conservatism in habitat preference. For context, other species with conservatism scores of two include *Juniperus virginiana* L. (red cedar), *Acer saccharinum* L. (silver maple), and *Gleditsia triacanthos* L. (honey locust) – all of which are well-known tree species associated with disturbed sites. In contrast, two *Juglans* species, *Juglans nigra* and *Juglans cinerea*, displayed significant IBD (Victory et al. 2006), receiving FQA scores of 4 and 7 respectively, and *Cornus florida* had a significant signal of IBD (Hadziabdic 2010) and a FQA score of 5. Taken together, these results suggest that the lack of genetic structure in eastern black cherry is due to high levels of gene flow resulting from aspects of its life history, namely high pollen/seed movement and an ability to quickly exploit man-made dispersal corridors.

**Implications and future directions** – If the lack of IBD we observed indeed results from eastern black cherry's life history, this result requires context before any generalization is made about widespread North American forest trees. Fortunately, similar studies could be easily

performed on other taxa given the efficiency of the herbarium sampling strategy and availability of microsatellite markers. For example, a set of loci developed for *Liriodendron tulipifera* L. (tulip tree – FQA = 7; loci from Xu et al. 2006) is available. Other candidate species include *Aesculus glabra* Willd. (American buckeye – FQA = 5; Minami et al. 1998); *Cornus florida* L. (flowering dogwood – FQA = 5; Wang et al. 2008); *Quercus rubra* L. (red oak – FQA = 5; Aldrich et al. 2002); and *Lindera benzoin* (L.) Blume (spice bush – FQA = 5; Edwards and Niesenbaum 2007). All of these trees/shrubs are widespread and occur in mature, higher quality forests. Like *P. serotina*, the following species exhibit lower FQA values: *Cercis canadensis* L. (redbud – FQA = 3; Wadl et al. 2012); *Sassafras albidum* (Nutt.) Nees (sassafras – FQA = 2; Chung et al. 2011). These studies would lend context to the lack of IBD in *P. serotina* subsp. *serotina* and help shape our understanding of gene flow across North American forests.

## CHAPTER 2

### IS THE RARE ALABAMA CHERRY (*PRUNUS ALABAMENSIS*) GENETICALLY DISTINCT?

#### **Abstract:**

*Premise of the study:* Black cherry (*Prunus serotina* Ehrh.) is morphologically variable across its large range, and numerous subspecies have been recognized in portions of this distribution. One such taxon, *Prunus serotina* Ehrh. subsp. *alabamensis* (C. Mohr) Little (*Prunus alabamensis* Mohr. - "Alabama cherry"), is completely sympatric with, but morphologically distinct from the widespread *Prunus serotina* Ehrh. subsp. *serotina*. Our study seeks to assess the genetic distinctiveness of *P. alabamensis* and attribute any genetic distinctiveness to climatic variables and/or elevation.

*Methods:* Dense geographic sampling was made possible by genotyping 15 microsatellite loci in 89 herbarium samples (30 *P. alabamensis*, 59 *P. serotina* subsp. *serotina*) across all portions of the Alabama cherry range. Redundancy, distance-based redundancy, and STRUCTURE analyses were performed.

*Key Results:* Redundancy analysis demonstrated a significant effect of taxon on the variation in allele frequencies among samples. Distance-based redundancy analysis identified no significant effects at  $P < 0.05$ , although taxon approached significance. STRUCTURE analyses revealed no consistent distinction between *P. alabamensis* and *P. serotina* subsp. *serotina*, although a trend in group assignment was evident.

*Conclusions:* Our results support the hypothesis that *P. alabamensis* is a distinct genetic entity, although this distinction appears to be quite subtle. This distinction was not

strongly related to climatic variables, although the coarse nature of our environmental data limited our ability to make this assessment.

## **Introduction:**

Alabama cherry [*Prunus serotina* Ehrh. subsp. *alabamensis* (C. Mohr) Little] is a regionally endemic, morphologically distinctive subspecies of *Prunus serotina* Ehrh. which most authors treat as a separate species (*Prunus alabamensis* Mohr). No clear distinction between Alabama cherry and eastern black cherry were seen in a recent phylogenetic analysis of plastid and nuclear ITS sequence data (Liu et al. 2013). Although the remaining three *Prunus serotina* subspecies [*Prunus serotina* Ehrh. subsp. *eximia* (Small) Little; *Prunus serotina* Ehrh. subsp. *capuli* (Cav. Ex Spreng) McVaugh; and *Prunus serotina* Ehrh. subsp. *virens* (Wooton & Standl.)] formed a clade in the ITS topology, the single analyzed *P. alabamensis* individual was placed sister to one of the two *P. serotina* subsp. *serotina* individuals, albeit with low support. Alabama cherry exhibits a range that is completely nested within that of the widespread eastern black cherry (*Prunus serotina* Ehrh. subsp. *serotina*) (Figs. 4 and 5), and where they co-occur the habitat differences between them are subtle. In these areas *P. alabamensis* is found in forests that are less disturbed and/or more xeric, and although *P. serotina* subsp. *serotina* can be found in these same areas, eastern black cherry is more common in disturbed and/or more mesic areas (McVaugh 1951, A. Diamond pers. comm.). Despite their proximity and close phylogenetic relationship, Alabama cherry is quite morphologically distinctive among the *P. serotina* subspecies (McVaugh 1951), with *P. alabamensis* displaying distinctive blunt-tipped leaves that are less than twice as long as wide and a generally pubescent lower leaf surface lacking tufts at

the base of the midrib. Rogers McVaugh (1951), when writing about the distinctiveness of *P. alabamensis* stated, "... the gap between it [*P. alabamensis*] and the other [*P. serotina*] subspecies is much greater than any gap which exists between any two of the others." This distinction notwithstanding McVaugh (1951) nevertheless noted occasional morphologically intermediate individuals.

Taken together, these phylogenetic, biogeographic, ecological, and morphological details point to two alternatives. In the first, the distinctive morphology of *P. alabamensis* is the result of heritable genetic variation maintained in the face of likely frequent gene flow with its more common relative. In the second, the morphological distinctiveness of *P. alabamensis* is the result of environmentally induced phenotypic plasticity (Schlichting 1986). Answering this question begins with a rigorous assessment of the overall genetic distinctiveness of *P. alabamensis*, a study that to date has not been conducted. Here we compare neutral genetic variation in Alabama cherry specimens to that of eastern black cherry from the same region. Specifically, we ask if genetic variation in these samples can be attributed to basic factors such as taxonomic identity, climate, and elevation.

## **Materials and Methods:**

**Obtaining samples** – During the course of our larger *P. serotina* range genetics project we visited or had tissue sent to us from multiple herbaria (see Chapter 1). This overall strategy involved visits to large national institutions (e.g. MO, NYBG, US) followed by visits to or specimen requests from targeted regional and local collections in order to fill in geographic gaps in our sample set. Several of these herbaria sent us samples from both *P. serotina* subsp. *serotina* and *P. alabamensis*. We then chose tissues for this study based on the distribution of

the *P. alabamensis* samples as well as the 12-locus data threshold adopted in that study (see Chapter 1). These tissues represented 5 herbaria that we visited (55 specimens) and 9 herbaria that sent us tissue (34 specimens) in 2014, 2015, and 2016 (see acknowledgements and Appendix 2). Interestingly, some of the *P. alabamensis* tissues originated from specimens cited in McVaugh's 1951 paper (McVaugh 8938, 8941, 8943, 8948, 8953, 8956, 8968, and 8969; Biltmore Herbarium 6038a and 9282k). All specimen identifications were verified, with a positive ID for *P. alabamensis* involving distinctive blunt-tipped leaves that are less than twice as long as wide and a generally pubescent lower leaf surface lacking tufts at the base of the midrib. A positive ID for *P. serotina* subsp. *serotina* involved two or more of the following characteristics when possible: small orange hairs at the base of the midrib, (hooked) serrated leaf margins, leaves more than 2.5× as long as wide, and persistent sepals when in fruit (McVaugh 1951). One or two small leaves were removed when sufficient material was present, and acid-free labels were affixed to all sheets from which we sampled noting that material was removed for DNA extraction. All specimens were georeferenced using the specimen locality data with Google Earth (Google Inc. 2009) and EarthPoint (Clark 2015) software. We drew the smallest rectangle possible in ArcMap (Fig. 6) (ESRI 2015) to encompass all *P. alabamensis* samples we obtained as well as nearby *P. serotina* subsp. *serotina* samples (Fig. 6).

**Molecular methods** – Following a high-throughput DNA extraction (Beck et al. 2012), 15 microsatellite loci originally designed for other *Prunus* species were amplified: *Prunus persica* (L) Batsch (Cipriani et al. 1999; Testolin et al. 2000; Downey and Iezzoni 2000; Dirlewanger et al. 2002; Yamamoto et al. 2002), *Prunus avium* L. (Dirlewanger et al. 2002; Struss et al. 2003), *Prunus cerasus* L. (Downey and Iezzoni 2000), *Prunus virginiana* L. (Wang et al. 2012), and

*Prunus geniculata* R. M. Harper (Germain-Aubrey et al. 2011). These loci were chosen based on previous success in *Prunus serotina* subsp. *serotina* (Pairon and Jacquemart 2008) or CAG-tag PCR screening (Beck et al. 2014). Due to the allotetraploid nature of *P. serotina*, 10 of these loci only amplified in half of the genome, thus causing them to appear be "diploid," exhibiting no more than two alleles per locus (Table 1). Three to four loci were dye-labeled and simultaneously amplified (Table 1) using the reagents and amplification conditions outlined in Chapter 1. Amplicons were sent to the University of Chicago Comprehensive Cancer Center DNA Sequencing and Genotyping Facility for genotyping, and alleles were scored with GeneMarker 1.9 (SoftGenetics, State College, Pennsylvania, USA).

**Variable extraction and multicollinearity** – Nineteen climatic variables were obtained from the BioClim database (Hijmans et al. 2005) and elevation was obtained from the National Elevation Dataset (NED) (USGS 2009). Values for all variables were extracted at the 89 sample locations using ArcMap (ESRI 2015, Redlands, California, USA). These variables were then tested for multicollinearity using the “vif” (Variance Inflation Factor) function, which quantifies the factor by which the variance is inflated in the analysis when including a specific variable or combination of variables. This function was conducted in the “usdm” package (Naimi 2015) in R (R Core Team 2015) with a maximum threshold score of 10. Variables were excluded from the analysis sequentially by eliminating those variables exhibiting the highest VIF scores. We were satisfied that we had eliminated severe multicollinearity among explanatory variables when the model only included variables with a VIF of <10.

**Redundancy analysis** – Redundancy analysis seeks to find the significance of any correlation between a table of allele frequencies and a set of explanatory variables (Legendre and Legendre 2012). Redundancy analysis was conducted in R (R Core Team 2015) using the package “vegan” (Oksanen et al. 2016). Following the removal of certain variables based on multicollinearity, the remaining variables along with the variable “taxon” (with levels *P. serotina* subsp. *serotina* and *P. alabamensis*) were considered. A full model including all independent variables was fitted, and then reduced models were re-fitted after discarding non-significant explanatory variables to obtain a model including significant explanatory variables only. A subsequent redundancy analysis was performed on those significant variables only. In all analyses, the effects of spatial autocorrelation were removed by using a “condition” statement, which has as its input a table of latitudes and longitudes for the samples. An ANOVA table (999 permutations) was then extracted.

**Distance-based redundancy analysis** – Distance-based redundancy analysis seeks to find the significance of any correlation between a matrix of genetic distances and a set of explanatory variables (Legendre and Anderson 1999). The matrix of genetic distances was built using the Bruvo distance metric (Bruvo et al. 2004) in GenoDive (Merimans and VanTienderen 2004), and the Distance-based redundancy analyses were conducted in R (R Core Team 2015) using the package “vegan” (Oksanen et al. 2016). The variables considered were those used in the redundancy analysis above. As in the redundancy analysis, spatial autocorrelation was conditioned out by taking into account latitude and longitude. An ANOVA table (999 permutations) was then extracted.

**Structure analysis** – The presence and location of major genetic groups was evaluated with STRUCTURE (Hubisz et al. 2009). STRUCTURE attempts to identify populations that are in linkage equilibrium, and in the process assigns each sample to one of these populations or partially to multiple populations. We performed STRUCTURE analyses on both a diploid dataset (only the 10 “diploid” loci used in the analysis) and a "diploidized" dataset, which included all loci but randomly subsampled those loci to two alleles per locus per individual. The diploidized dataset was constructed in GenoDive (Merimans and VanTienderen 2004). Both analyses included 5 iterations each at  $K = 2$  to  $K = 10$ , with each iteration featuring 100,000 burn-in generations followed by 500,000 generations. All STRUCTURE analyses assumed the admixture and independent allele frequency models. The approach outlined in Evanno et al. (2005) and implemented in Structure Harvester (Earl and vonHoldt 2012) was used to find the most likely value of  $K$ . The analysis was visualized in STRUCTURE PLOT v.2.0 (Ramasamy et al. 2014).

## **Results:**

**Data** – The 99 *P. serotina* subsp. *serotina* and *P. alabamensis* samples obtained from the Alabama cherry range represented 14 herbaria, 5 states and 76 counties. This included *P. alabamensis* specimens from 24 of the 51 counties where *P. alabamensis* is known to occur. Data was obtained at all 15 loci in 64.6% (64) of samples, and at  $\geq 12$  loci in 89.9% (89) of samples. Only those samples with at least 12 loci were included in redundancy and STRUCTURE analyses. See Table 2 for information regarding locus variability.

**Multicollinearity** – Eight of 20 variables met the requirements for a lack of multicollinearity (Table 3). These values ranged from 1.97 (mean diurnal range) to 7.89 (precipitation of the driest month).

**Redundancy analysis** – Of the eight non-multicollinear explanatory variables identified in our VIF analysis, two were individually significant or approached significance (mean temperature of the wettest quarter and taxon- Table 4), and a separate redundancy analysis was performed on these two variables simultaneously (Table 5). Only taxon was significant ( $P = 0.003$ ) in this two-variable analysis. Spatial autocorrelation accounted for 1.038% of the variation in allele frequencies in this two-variable analysis, while the variation due to the constrained axes was 5.947%. Unconstrained variation accounted for 93.015% of the total variation in allele frequencies. A subsequent redundancy analysis including only taxon was performed and visualized using the “vegan3d” package (Oksanen et al. 2016). In the plot of this ordination, clear separation can be seen between *P. alabamensis* and the *P. serotina* subsp. *serotina* samples (Fig. 7).

**Distance-based redundancy analysis** – Of the eight non-multicollinear explanatory variables identified in our VIF analysis, two were significant or approached significance (mean temperature of the driest quarter and taxon- Table 6), and a separate distance-based redundancy analysis was performed on these variables simultaneously (Table 7). Neither was individually significant ( $P = 0.202$  and  $P = 0.084$ , respectively) at  $P < 0.05$  in this two-variable analysis, although taxon approached significance. Spatial autocorrelation accounted for 2.666% of variation in genetic distance in this two-variable analysis, while the variation due to the

constrained axes was 2.849%. Unconstrained variation accounted for 94.485% of the total variation in genetic distance.

**Structure analysis** - The Evanno et al. (2005) approach identified a  $K$  of 4 for both the diploid-only and diploidized versions of the microsatellite dataset. In the diploid-only dataset all samples exhibited nearly equal cluster assignments at  $K = 4$ , with only 2.25% (two samples) exhibiting a cluster assignment of  $\pm 25\%$ , while all other samples exhibited a cluster assignment of exactly 25%. In the diploidized analysis, however, cluster assignment was less even, and a subtle pattern of taxonomic structure is evident (Fig. 8). For example, in *P. alabamensis* specimens, majority (cluster assignment  $> 50\%$ ) yellow cluster assignments are common ( $n = 20$ , 67% of samples), while in *P. serotina* subsp. *serotina* specimens, the majority green cluster assignment is common ( $n = 41$ , 69.5% of samples). However, both patterns can be seen in several specimens from both taxa.

## **Discussion:**

**Attribution of genetic variation** – Taken together, the redundancy analyses established that taxon assignment explains a portion of the genetic variation among Alabama cherry and regional eastern black cherry individuals. This factor, however, explain only a modest portion of this genetic variation. This suggests that *P. serotina* subsp. *serotina* and *P. alabamensis* are indeed genetically differentiated, but either A) frequently exchange genes or B) do not exchange genes but are only recently diverged from one another. The high assignment of variation in allele frequencies and genetic distances to unconstrained axes ( $>90\%$ ) suggests that most of the genetic

variation among cherry trees in this region is due to sources of variability that we have yet to identify.

**Implications and future directions** – Although *P. serotina* subsp. *serotina* and *P. alabamensis* are modestly genetically differentiated at our 15 neutral loci, they maintain obvious morphological distinctiveness. This implies that these two taxa are either recently diverged or that the genetic architecture of these alternative phenotypes are being maintained in the face of ongoing gene flow. Indeed, unconstrained axes of our redundancy analysis harbor ca. >90% of variation in allele frequencies, and future studies should investigate the factors underlying the maintenance phenotypic distinctiveness, such as habitat and life history disparity. For instance, it has been observed that Alabama cherry and eastern black cherry populations exhibit at least partially non-overlapping flowering times north-central Alabama (Ross Clark, personal communication). Such studies will require fine scale spatial data or, more likely, ecological and life history data gathered from *P. serotina* s. l. populations in this region. Such data could include, but is not limited to: phenology, pollinator preference, soil, aspect, and mycological pathogen (Reinhart et al. 2005) data. Ultimately, the genetic architecture underlying the ecological and morphological differences between these two taxa is also of interest, information that could be obtained through a genome-wide association study (Glazier et al. 2002).

## REFERENCES

## REFERENCES

- Aldrich, P.R., Michler, C.H., Sun, W., Romero-Severson, J.R. 2002. Microsatellite markers for northern red oak (Fagaceae: *Quercus rubra*). *Molecular Ecology Notes* 2:472 – 474.
- Auclair, A.N. 1975. Sprouting response in *Prunus serotina* Ehrh.: Multivariate analysis of site, forest structure and growth rate relationships. *The American Midland Naturalist* 94:72–87.
- Auclair, A.N., and Cottam, G. 1971. Dynamics of black cherry (*Prunus serotina* Ehrh.) in southern Wisconsin oak forests. *Ecological Monographs* 41:153 – 177.
- Beatty, G.E., Brown, J.A., Cassidy, E.M., Finlay, C.M.V., McKendrick, L., Montgomery, W.I., Reid, N., Tosh, D.G., Provan, J. 2015. Lack of genetic structure and evidence for long-distance dispersal in ash (*Fraxinus excelsior*) populations under threat from an emergent fungal pathogen: implications for restorative planting. *Tree Genetics and Genomes* 11:53.
- Beck, J.B., Alexander, P.J., Allphin, L., Al-Shehbaz, I.A., Rushworth, C., Bailey, C.D., Windham, M.D. 2012. Does hybridization drive the transition to asexuality in diploid *Bochera*? *Evolution* 66:985 – 995.
- Beck, J.B., Semple, J.C., Brull, J.M., Lance, S.L., Phillips, M.M., Hoot, S.B., Meyer, G.A. 2014. Genus-wide microsatellite primers for the goldenrods (*Solidago*; Asteraceae). *Applications in Plant Sciences* 2:1300093.
- Bruvo, R., Michiels, and N.K., D'Souza, T.G., Schulenburg, H. 2004. A simple method for the calculation of microsatellite genotype distances irrespective of ploidy level. *Molecular Ecology* 13:2101–2106.
- Campitelli, B.E., and Stinchcombe, J.R. 2013. Natural selection maintains a single-locus leaf shape cline in ivyleaf morning glory, *Ipomea hederacea*. *Molecular Ecology* 22:552 – 64.
- Chung, K., Lin, T., Tsai, Y., Lin, S., Peng, C. 2011. Isolation and characterization of microsatellite loci in *Sassafras randaiense* (Lauraceae). *American Journal of Botany* e326 – e329.
- Cipriani, G., Lot, G., Huang, W.-G., Marrazzo, M.T., Peterlunger, E., Testolin, R. 1999. AC/GT and AG/CT microsatellite repeats in peach [*Prunus persica* (L) Batsch]: isolation, characterisation and cross-species amplification in *Prunus*. *Theoretical Applied Genetics* 99:65 – 72.
- Clark, B. 2015. EarthPoint [Software].

- Dennhardt, L. A., DeKeyser, E.S., Tennefos, S.A., Travers, S.E. 2016. There is no evidence of geographical patterning among invasive Kentucky Bluegrass (*Poa pratensis*) populations in the northern Great Plains. *Weed Science Society of America* 64(3): 409 – 420.
- Dirlewanger, E., Cosson, P., Tavaud, M., Aranzana, M.J., Poizat, C., Zanetto, A., Arus, P., Laigret, F. 2002. Development of microsatellite markers in peach [*Prunus persica* (L.) Batsch] and their use in genetic diversity analysis in peach and sweet cherry (*Prunus avium* L.). *Theoretical Applied Genetics* 105:127 – 138.
- Downey, S.L., and Iezzoni, A.F. 2000. Polymorphic DNA markers in black cherry (*Prunus serotina*) are identified using sequences from sweet cherry, peach, and sour cherry. *Journal of the American Society of Horticultural Science* 125:76 – 80.
- Durka, W., Bossdorf, O., Prati, D., Auge, H. 2005. Molecular evidence for multiple introductions of garlic mustard (*Alliaria petiolata*, Brassicaceae) to North America. *Molecular Ecology* 14:1697 – 1706.
- Earl, D.A. and vonHoldt, B.M. 2012 STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conservation Genetics Resources* 4:359 – 361.
- Edwards, M.J. and Niesenbaum, R.A. 2007. Eleven polymorphic microsatellite loci in *Lindera benzoin*, Lauraceae. *Molecular Ecology Notes* 7:1302 – 1304.
- Environmental Systems Research Institute. 2015. ArcMap [Software].
- Evanno, G., Regnaut, S., Goudet, J. 2005. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Molecular Ecology* 14:2611–2620.
- Fortuna, M. A., García, C., Guimarães Jr, P. R., Bascompte, J. 2008. Spatial mating networks in insect-pollinated plants. *Ecology letters* 11(5):490 – 498.
- Gatchell, C.J. 1971. Black Cherry (*Prunus serotina* Ehrh.) *American Woods* – FS 229.
- Geraldes, A., Farzaneh, N., Grassa, C.J., McKown, A.D., Guy, R.D., Mansfield, S.D., Douglas, C.J., Cronk, Q.C.B. 2013. Landscape genomics of *Populus trichocarpa*: the role of hybridization, limited gene flow, and natural selection in shaping patterns of population structure. *Evolution* 68:3260 – 3280.
- Germain-Aubrey, C.C., Soltis, P.S., Soltis, D.E., Gitzendanner, M.A. 2011. Microsatellite marker development for the federally listed *Prunus geniculata* (Rosaceae). *American Journal of Botany* e58-e60.
- Glazier, A.M., Nadeau, J.H., Aitman, T.J. 2002. Finding genes that underlie complex traits. *Science* 298:2345 – 2349.

- Google Inc. (2009). Google Earth (Version 5.1.3533.1731) [Software]
- Griffin, S. R., and Barrett S.C.H. 2004. Post-glacial history of *Trillium grandiflorum* (Melanthiaceae) in eastern North America: inferences from phylogeography. *American Journal of Botany* 91:465 – 73.
- Guitian, J., Guitian, P., Sanchez, J.M. 1993. Reproductive biology of two *Prunus* species (Rosaceae) in the Northwest Iberian Peninsula. *Plant Systematics and Evolution* 185:153 – 165.
- Hadziabdic, D. 2010. Evaluation of genetic diversity of flowering dogwood (*Cornus florida* L.) in the eastern United States using microsatellites. PhD diss., University of Tennessee.
- Hijmans, R.J., Cameron, S.E., Parra, J.L., Jones, P.G., Jarvis, A. 2005. Very high resolution interpolated climate surfaces for global land areas. *International Journal of Climatology* 25:1965-1978.
- Hoban, S.M., Borkowski, D.S., Brosi, S.L., Mcclary, T.S., Thompson, L.M., Mclachlan, J.S., Pereira, M.A., Schlarbaum, S.E., Romero-Severson, J. 2010. Range-wide distribution of genetic diversity in the North American tree *Juglans cinerea*: a product of range shifts, not ecological marginality or recent population decline. *Molecular Ecology* 19:4876-891.
- Howard, J.L., and Westby, R.M. 2013. U.S. timber production, trade, consumption and price statistics 1965-2011. Research Paper FPL-RP-676. 91p.
- Hubisz, M.J., Falush, D., Stephens, M., Pritchard, J.K. 2009. Inferring weak population structure with the assistance of sample group information. *Molecular Ecology Resources* 9:1322 – 332.
- Jacobs, J.H., Clark, S.J., Denholm, I., Goulson, D., Stoaate, C., Osborne, J.L. 2009. Pollination biology of fruit-bearing hedgerow plants and the role of flower-visiting insects in fruit-set. *Annals of Botany* 104:1397–1404.
- Jorgensen, S., and Maruicio, R. 2004. Neutral genetic variation among wild North American populations of the weedy plant *Arabidopsis thaliana* is not geographically structured. *Molecular Ecology* 13:3403 – 3413.
- Kartesz, J.T. 2015. The Biota of North America Program (BONAP). Floristic Synthesis of North America, Version 1.0. (in press).
- Kirk, H., Paul, J., Straka, J., Freeland, J.R. 2011. Long-distance dispersal and high genetic diversity are implicated in the invasive spread of the common reed, *Phragmites australis* (Poaceae), in northeastern North America. *American Journal of Botany* 98(7):1180 – 1190.

- Ladd, D., Thomas, J.R. 2015. Ecological checklist of the Missouri flora for floristic quality assessment. *Phytoneuron* 12:1–274.
- Lander, T.A., Klein, E.K., Stoeckel, S., Mariette, S., Musch, B., Oddou-Muratorio, S. 2013. Interpreting realized pollen flow in terms of pollinator travel paths and land-use resistance in heterogeneous landscapes. *Landscape Ecology* 28:1769–1783.
- Legendre, P. and Anderson, M.J. 1999. Distance-based redundancy analysis: testing multispecies responses in multifactorial ecological experiments. *Ecological Monographs* 69(1):1-24.
- Legendre, P., and Legendre, L. F. 2012. *Numerical ecology* Vol. 24. Elsevier.
- Liu, X., Wen, J., Nie, Z., Johnson, G., Liang, Z., Chang, Z. 2013. Polyphyly of the *Padus* group of *Prunus* (Rosaceae) and the evolution of biogeographic disjunctions between eastern Asia and eastern North America. *Journal of Plant Resources* 126:351-361.
- Lloyd, M.W., Roche, B., Roberts, R.P. 2011. Genetic variation and population structure of *Arabidopsis lyrata* ssp. *lyrata* (Brassicaceae) along the eastern seaboard of North America. *Castanea* 76:28 – 42.
- Mantel, N. 1967. The detection of disease clustering and a generalized regression approach. *Cancer Research* 27:209 – 220.
- Marks, P.L. 1974. The role of pin cherry (*Prunus pensylvanica* L.) in the maintenance of stability in northern hardwood ecosystems. *Ecological Monographs*. 44(1):73 – 88.
- McVaugh, R. 1951. A revision of the North American black cherries (*Prunus serotina* Ehrh., and relatives). *Brittonia* 7:279 – 315.
- Merimans, P.G., and Van Tienderen, P.H. 2004. GENOTYPE and GENODIVE: two programs for the analysis of genetic diversity of asexual organisms. *Molecular Ecology Notes* 4:792 – 794.
- Minami, E., Isagi, Y., Kaneko, Y., Kawaguchi, H. 1998. Polymorphic microsatellite markers in Japanese horse chestnut *Aesculus turbinata* Blume. *Molecular Ecology* 7:1616.
- Mylecraine, K.A., Kuser, J.A., Smouse, P.E., Zimmermann, G.L. 2004. Geographic allozyme variation in Atlantic white-cedar, *Chamaecyparis thyoides*. (Cupressaceae). *Canadian Journal of Forest Research* 34:2443 – 454.
- Naimi, B. 2015. usdm: Uncertainty analysis for species distribution models. R package version 1.1-15.
- Oden, N.L. and Sokal, R.R. 1986. Directional Autocorrelation: An Extension of Spatial Correlograms to Two Dimensions. *Systematic Zoology* 35:608 – 617.

- Oksanen, J.F., Blanchet, G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P.R., O'Hara, R.B., Simpson, G.L., Solymos, P., Stevens, M.H.H., Szoecs, E., Wagner, H. 2016. *vegan*: Community Ecology Package. R package version 2.4 – 1.
- Oksanen, J., Kindt, R., Simpson, G.L. 2016. *vegan3d*: Static and Dynamic 3D Plots for the 'vegan' Package. R package version 1.0-1.
- Pairon, M.C., and Jacquemart, A. 2005. Disomic segregation of microsatellites in the tetraploid *Prunus serotina* Ehrh. (Rosaceae). *Journal of the American Society of Horticultural Science* 130:729 – 34.
- Pairon, M., and Jacquemart, A. 2008. Detection and characterization of genome-specific microsatellite markers in the allotetraploid *Prunus serotina*. *Journal of the American Society of Horticultural Science* 133:390 – 395.
- Parker, K.C., Hamrick, J.L., Parker, A.J., Stacy, E.A. 1997. Allozyme diversity in *Pinus virginiana* (Pinaceae): intraspecific and interspecific comparisons. *American Journal of Botany* 84:1372.
- Prunier, J.G., Kaufmann, B., Fenet, S., Picard, D., Pompanon, F., Joly P., Lena, J.P. 2013. Optimizing the trade-off between spatial and genetic sampling efforts in patchy populations: towards a better assessment of functional connectivity using an individual-based sampling scheme. *Molecular Ecology* 22:5516 – 5530.
- R Core Team. 2015. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL <https://www.R-project.org/>.
- Ramasamy, R.K., Ramasamy, S., Bindroo, B.B., Naik, V.G. 2014. STRUCTURE PLOT: a program for drawing elegant STRUCTURE bar plots in user friendly interface. *SpringerPlus* 3:341.
- Reinhart, K.O., Royo, A.A., Van Der Putten, W.H., Clay, K. 2005. Soil feedback and pathogen activity in *Prunus serotina* throughout its native range. *Journal of Ecology* 93:890 – 898.
- Rodrigues, A. and Stefanovic, S. 2016. Present-day genetic structure of the holoparasite *Conopholis americana* (Orobanchaceae) in eastern North American and the location of its refugia during the last glacial cycle. *International Journal of Plant Science* 177:132 – 144.
- Schlichting, C.D. 1986. The evolution of phenotypic plasticity in plants. *Annual Review of Ecology and Systematics* 17:667-693.
- SoftGenetics. 2012. Gene Marker: The biologist friendly software v. 1.9.

- Soltis, D.E., Morris, A.B., McLachlan, J.S., Manos, P.S., Soltis, P.S. 2006. Comparative phylogeography of unglaciated eastern North America. *Molecular Ecology* 15:4261 – 4293.
- Stabile, J., Lipus, D., Maceda, L., Maltz, M., Roy, N., Wirgin, I. 2016. Microsatellite DNA analysis of spatial and temporal population structuring of *Phragmites australis* along the Hudson River Estuary. *Biological Invasions* 18:2517 – 2529.
- Struss, D., Ahmad, R., Southwick, S.M., Boritzki, M. 2003. Analysis of sweet cherry (*Prunus avium* L.) cultivars using SSR and AFLP markers. *Journal of the American Society for Horticultural Science* 128(6):904 – 909.
- Testolin, R., Marrazzo, T., Cipriani, G., Quarta, R., Verde, I., Dettori, M.T., Pancaldi, M., Sansavini, S. 2000. Microsatellite DNA in Peach (*Prunus persica* L. Batsch) and its use in fingerprinting and testing the genetic origin of cultivars. *Genome* 43:512 – 520.
- Thomson, A.M., Dick, C.W., Dayanandan, S. 2015. A similar phylogeographical structure among sympatric North America birches (*Betula*) is better explained by introgression than by shared biogeographical history. *Journal of Biogeography* 42:339 – 350.
- Thomson, A.M., Dick, C.W., Pascoini, A.L., Dayanandan, S. 2015. Despite introgressive hybridization, North American birches (*Betula* spp.) maintain strong differentiation at nuclear microsatellite loci. *Tree Genetics and Genomes* 11:101.
- Thompson, J.N., and Willson, M.F. 1979. Evolution of temperate fruit/bird interactions: phenological strategies. *Evolution* 33:973 – 982.
- Tsai, Y.E., and Manos, P.S. 2010. Host density drives the postglacial migration of the tree parasite, *Epifagus virginiana*. *PNAS* 107(39):17035 – 17040.
- U.S. Geological Survey (USGS). 2009. National Elevation Dataset (NED). 2<sup>nd</sup> Edition.
- Victory, E.R., Gloubitz, J.C., Rhodes, O.E., Woeste, K.E. 2006. Genetic homogeneity in *Juglans Nigra* (Juglandaceae) at nuclear microsatellites. *American Journal of Botany* 93:118 – 126.
- Wang, H., Walla, J.A., Zhong, S., Huang, D., Dai, W. 2012. Development and cross-species/genera transferability of microsatellite markers discovered using 454 genome sequencing in chokecherry (*Prunus virginiana* L.). *Plant Cell Rep* DOI 10.1007/s00299-012-1315-z.
- Waselkov, K.E., and Olsen, K.M. 2014. Population genetics and origin of the native North American agricultural weed waterhemp (*Amaranthus tuberculatus*; Amaranthaceae). *American Journal of Botany* 101:1726 – 736.

- Willyard, A., Gernandt, D.S., Potter, K., Hipkins, V., Marquardt, P., Mahalovich, M.F., Langer, S.K., Telewski, F.W., Cooper, B., Douglas, C., Finch, K., Karemera, H.H., Lefler, J., Lea, P., Wofford, A. 2017. *Pinus ponderosa*: A checkered past obscured four species. *American Journal of Botany* 104:161 – 181.
- Wright, Sewall. 1943. Isolation by Distance. *Genetics* 28:114 – 38.
- Xu, M., Li, H., Zhang, B. 2006. Fifteen polymorphic simple sequence repeat markers from expressed sequence tags of *Liriodendron tulipifera*. *Molecular Ecology Notes* 6:728 – 730.
- Yakimowski, S.B., and Eckert, C.G. 2008. Populations do not become less genetically diverse or more differentiated towards the Northern limit of the geographical range in clonal *Vaccinium stamineum* (Ericaceae). *New Phytologist* 180(2):534 – 544.
- Yamamoto, T., Mochida, K., Imai, T., Shi, Y.Z., Ogiwara, I., Hayashi, T. 2002. Microsatellite markers in peach [*Prunus persica* (L.) Batsch] derived from an enriched genomic and cDNA libraries. *Molecular Ecology Notes* 2:298 – 301.

## APPENDIXES

Table 1. The 15 microsatellite loci used in this study.

<b>Locus Name</b>	<b>Locus set</b>	<b>Original Publication</b>	<b>Diploid</b>	<b>Dye</b>
UDP96-005	1	Cipriani et al. 1999	Yes	6-FAM
UDP98-405	1	Cipriani et al. 1999	Yes	HEX
UCD-CH14	1	Struss et al. 2003	Yes	6-FAM
M4c	2	Yamamoto et al. 2002	No	6-FAM
PceGA34	2	Downey and Iezzoni 2000	Yes	6-FAM
M12a	2	Yamamoto et al. 2002	No	HEX
UDP96-001	3	Testolin et al. 2000	No	NED
pchpgms3a	3	Downey and Iezzoni 2000	Yes	6-FAM
pchpgms3b	3	Downey and Iezzoni 2000	Yes	6-FAM
pchgms2	3	Downey and Iezzoni 2000	No	HEX
UDP98-025	3	Testolin et al. 2000	Yes	6-FAM
C3292	4	Wang et al. 2012	Yes	HEX
BPPCT-002a	4	Dirlewanger et al. 2002	Yes	6-FAM
BPPCT-002b	4	Dirlewanger et al. 2002	Yes	6-FAM
BPPCT-017	4	Dirlewanger et al. 2002	No	NED

Table 2. Comparative locus information for *P. serotina* subsp. *serotina* and *P. alabamensis*

<b>Locus</b>	<b><i>P. alabamensis</i></b>		<b><i>P. serotina</i> subsp. <i>serotina</i></b>	
	<b>Number of alleles</b>	<b>Private alleles</b>	<b>Number of alleles</b>	<b>Private alleles</b>
96005	6	1	7	0
CH14	24	7	18	1
98405	7	1	6	0
M4c	12	0	15	3
GA34	18	1	24	7
M12a	36	4	34	2
98025	11	0	16	5
gms3a	13	1	16	4
gms3b	12	0	13	1
gms2	15	4	15	4
96001	8	0	11	3
BPPCT-002A	6	2	5	1
BPPCT-002B	7	1	9	3
C3292	6	0	7	1
BPPCT-017	19	2	29	11

Table 3. VIF values for eight variables included in the redundancy analysis. Values were sequentially dropped from the VIF analysis until all VIF scores were <10.

<b>Variable</b>	<b>VIF</b>
Mean diurnal range	1.972151
Isothermality	4.268515
Mean temperature of the wettest quarter	5.04245
Mean temperature of the driest quarter	2.977184
Precipitation of the wettest month	3.014431
Precipitation of the driest month	7.891729
Precipitation of the driest quarter	7.776138
Elevation	2.671486

Table 4. Results of combined redundancy analysis.

<b>Variable</b>	<b><i>P</i>-value</b>
Elevation	0.921
Mean diurnal range	0.915
Isothermality	0.155
Mean temperature of the wettest quarter	0.066
Mean temperature of the driest quarter	0.914
Precipitation of the wettest month	0.518
Precipitation of the driest month	0.847
Precipitation of the driest quarter	0.904
Taxon	0.005**

Table 5. Results of combined redundancy analysis on two explanatory variables found to be significant or approach significance in the previous combined redundancy analysis.

<b>Variable</b>	<b><i>P</i>-value</b>
Mean temperature of the wettest quarter	0.149
Taxon	0.003**

Table 6. Results of distance-based redundancy analysis.

<b>Variable</b>	<b><i>P</i>-value</b>
Elevation	0.718
Mean diurnal range	0.348
Isothermality	0.682
Mean temperature of the wettest quarter	0.444
Mean temperature of the driest quarter	0.042*
Precipitation of the wettest month	0.847
Precipitation of the driest month	0.336
Precipitation of the driest quarter	0.145
Taxon	0.068

Table 7. Results of distance-based redundancy analysis on two explanatory variables found to be significant or approach significance in the previous distance-based redundancy analysis.

<b>Variable</b>	<b><i>P</i>-value</b>
Mean temperature of the driest quarter	0.202
Taxon	0.084

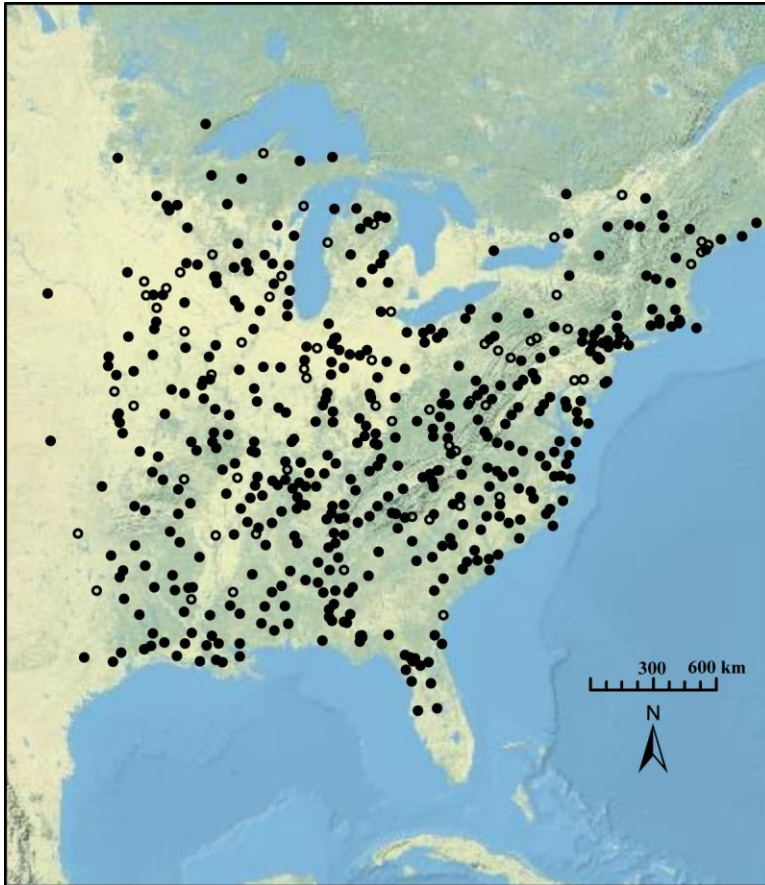


Figure 1. Locations of 506 *Prunus serotina* subsp. *serotina* tissue samples obtained from 34 herbaria (Appendix 1). Dark circles indicate the 439 samples from which  $\geq 12$ -loci were amplified.

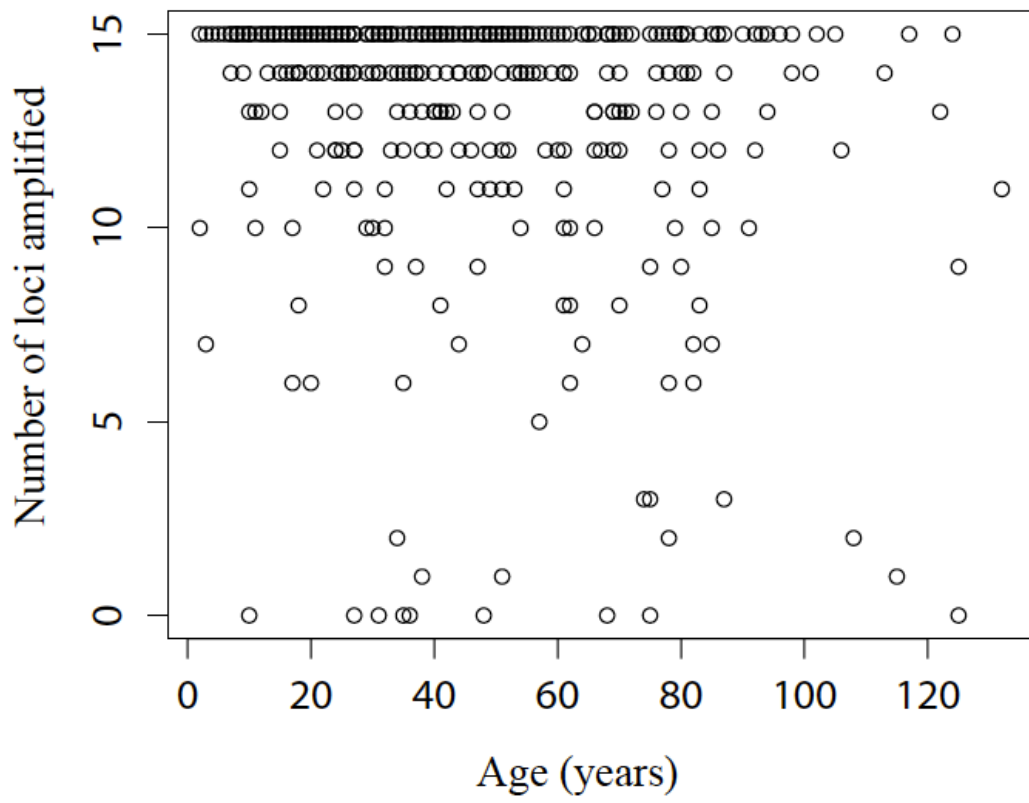


Figure 2. Plot of microsatellite genotyping success vs. specimen age in 506 *Prunus serotina* subsp. *serotina* samples.

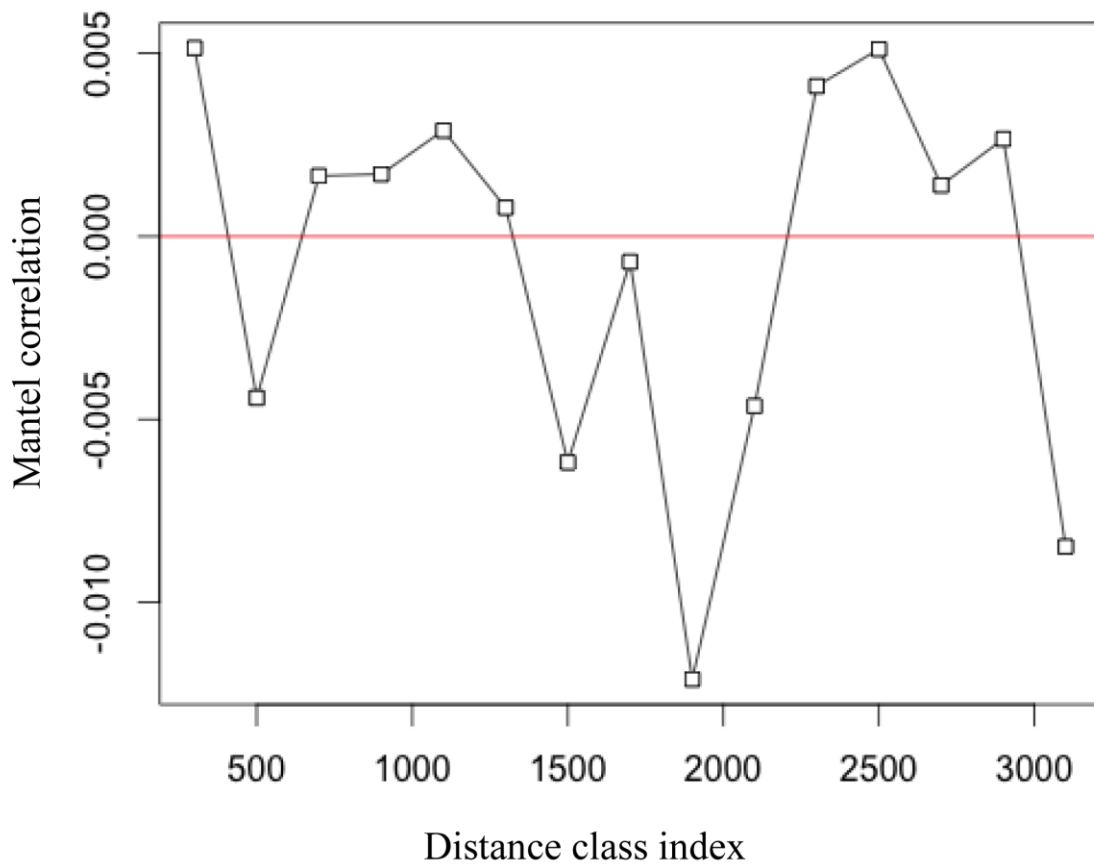


Figure 3. Mantel correlogram for 439 *P. serotina* subsp. *serotina* samples from which  $\geq 12$ -loci were amplified. The red line indicates zero correlation between genetic and geographic distance. All boxed points indicate a non-significant ( $P > 0.05$ ) Mantel test at that distance class.

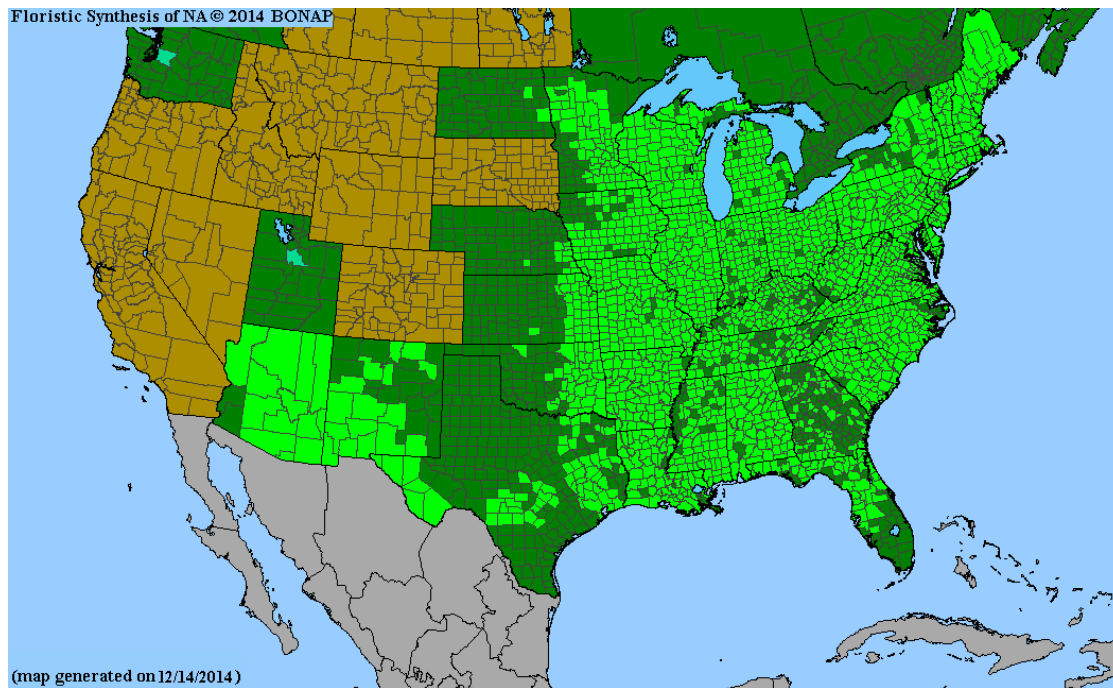


Figure 4. Range of *Prunus serotina* s.l. (bright green) (Kartesz 2015)

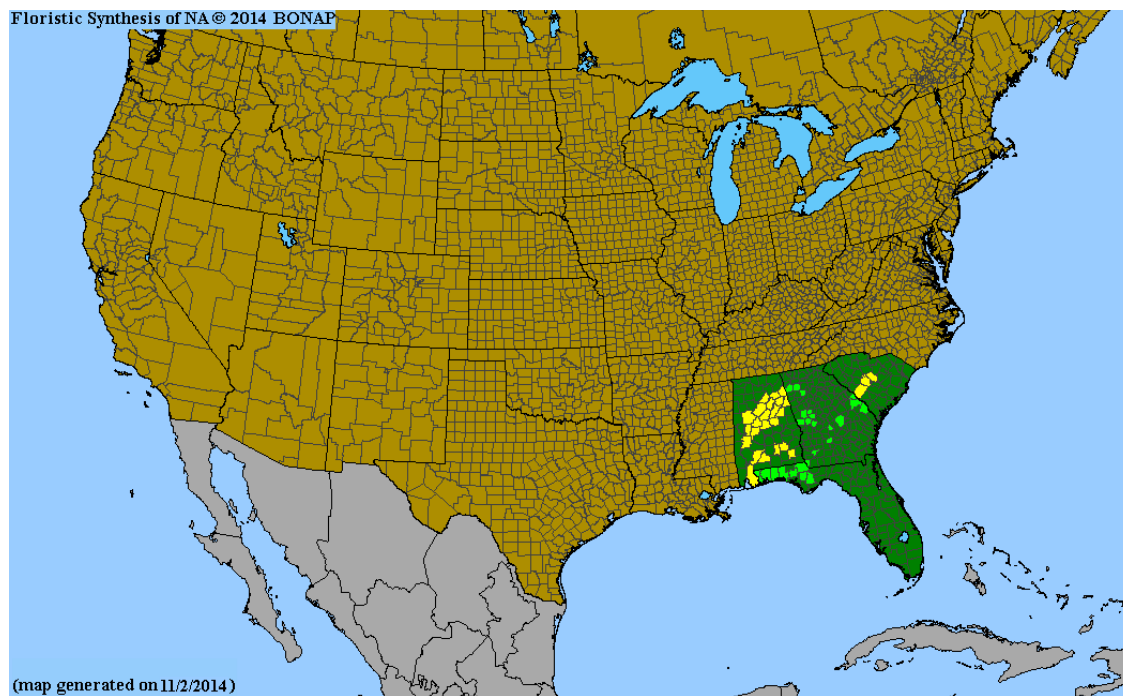


Figure 5. Range of *Prunus alabamensis* (bright green and yellow) (Kartesz 2015)

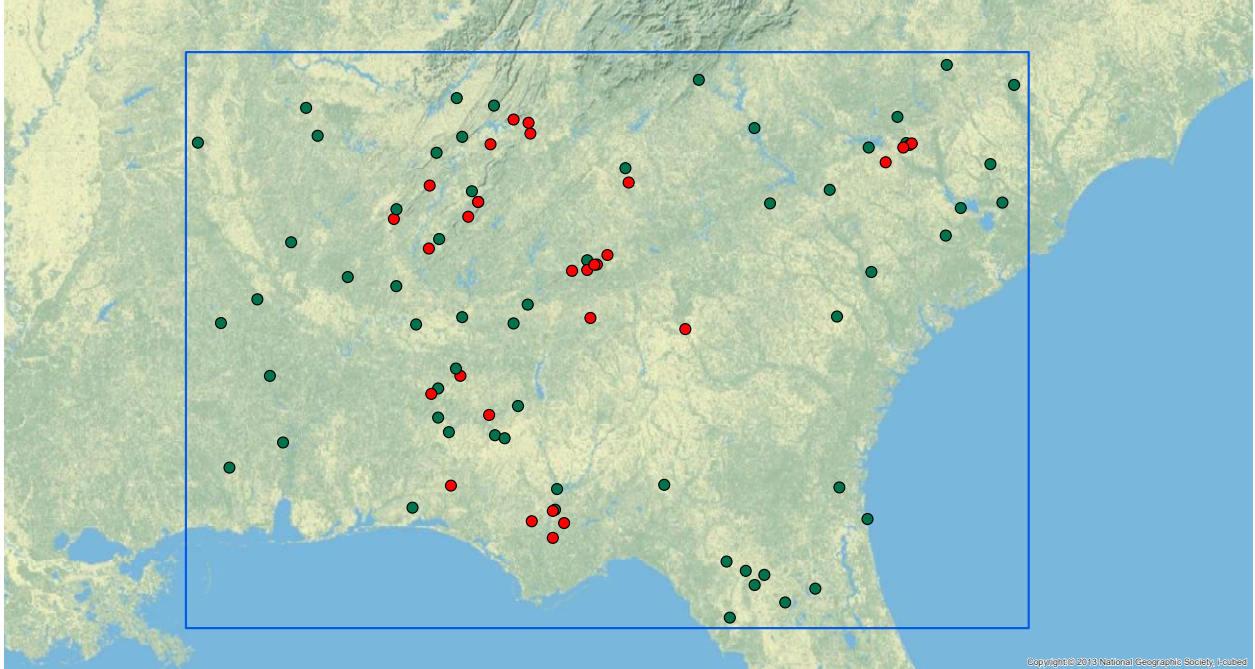


Figure 6. Location of analyzed samples (green points = *Prunus serotina* subsp. *serotina*, red points = *Prunus alabamensis*).

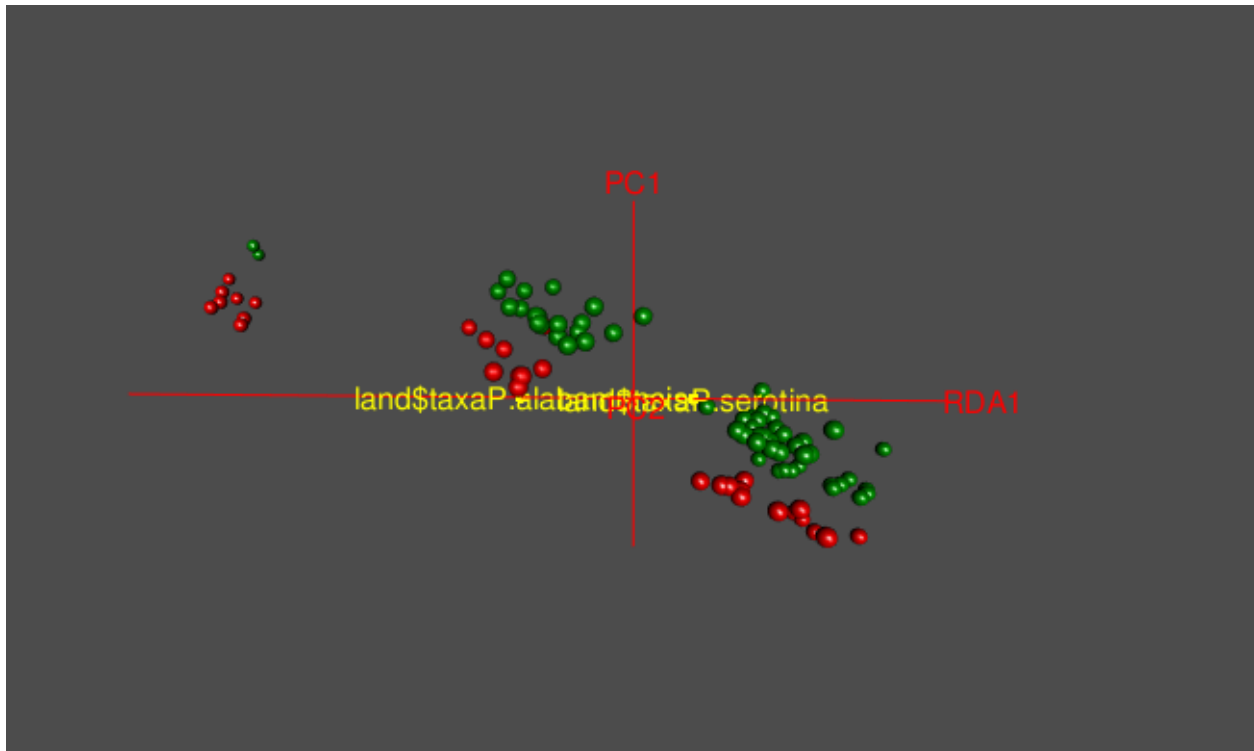


Figure 7. Plotted results of a redundancy analysis with taxon as the independent variable. (green points = *Prunus serotina* subsp. *serotina*, red points = *Prunus alabamensis*). RDA1 indicates the constrained axis, while PCA1 and PCA2 indicate unconstrained axes. Text in yellow indicates centroids for the two taxa.

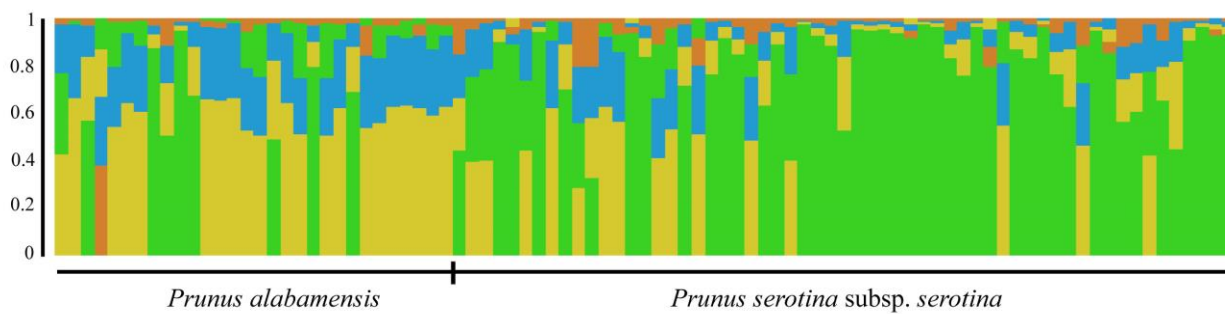


Figure 8. Bar graph indicating percent assignments of individual samples to one of four clusters chosen by STRUCTURE.

Appendix 1. Information for 506 *P. serotina* subsp. *serotina* samples

Extraction	State	County	Herbarium	Collector	Date	Latitude	Longitude	Locus Count
JB1980	Alabama	Cherokee	MO	Kral 59974	10-May-1977	34.39	-85.63	14
JB1981	Alabama	Crenshaw	MO	Kral 39467	1-Jun-1970	31.72	-86.26	15
JB1982	Alabama	Dale	MO	MacDonald 10239	8-Apr-1997	31.27	-85.62	15
JB1983	Alabama	Randolph	MO	Nixon 1287	7-Jun-1905	33.15	-85.62	9
JB1984	Alabama	Talladega	MO	Kral 46062	20-Apr-1972	33.14	-86.25	15
JB1985	Arkansas	Franklin	MO	Thompson C0328	8-Apr-1989	35.25	-94.14	15
JB1986	Arkansas	Searcy	MO	Demaree 70803	8-Apr-1976	35.99	-92.72	13
JB1987	Florida	Hillsborough	MO	Lakela 31336	22-Mar-1968	27.95	-82.46	15
JB1988	Florida	Jackson	MO	Hess 8496	27-Apr-1999	30.75	-84.92	15
JB1989	Florida	Okaloosa	MO	Miller 9482	3-Jun-1998	30.57	-86.55	15
JB1990	Georgia	Brooks	MO	McCarty s.n.	17-May-1973	30.79	-83.71	12
JB1991	Georgia	Meriwether	MO	McVaugh 8942	22-May-1948	32.94	-84.58	15
JB1992	Georgia	Stephens	MO	Spongberg 1789	14-May-1982	34.63	-83.32	15
JB1993	Illinois	Du Page	MO	Hess 7869	27-Aug-1997	41.77	-88.05	15
JB1994	Indiana	Fulton	MO	Nee 55473	6-Jun-2007	41.04	-86.03	15
JB1995	Iowa	Palo Alto	MO	Hayden 8513	8-Sep-1941	43.16	-94.90	13
JB1996	Kentucky	Woodford	MO	Semple 2592	21-Apr-1977	38.16	-84.68	13
JB1997	Minnesota	Anoka	MO	Smith 28908	5-Aug-2000	45.20	-93.21	15
JB1998	Minnesota	Goodhue	MO	Smith 28896	2-Aug-2000	44.53	-92.34	14
JB1999	Minnesota	Otter Tail	MO	Smith 27375	10-Jul-1998	46.63	-95.31	15
JB2000	Minnesota	Ramsey	MO	Smith 29018	20-Aug-2000	45.06	-93.12	15
JB2001	Minnesota	Sherburne	MO	Smith 29046	24-Aug-2000	45.50	-93.65	15
JB2002	Minnesota	Washington	MO	Smith 27957	30-Jun-1999	45.22	-92.77	15
JB2003	Mississippi	Forrest	MO	McDaniel 31439	10-May-1992	31.22	-89.17	14
JB2004	Missouri	Audrain	MO	Moe 05-19	11-Jun-2005	39.07	-91.64	15
JB2005	Missouri	Barry	MO	Hornberger 174	18-May-1979	36.59	-93.83	13
JB2006	Missouri	Barton	MO	Timme 14198	21-May-1997	37.30	-94.38	15
JB2007	Missouri	Butler	MO	Bornstein 463	9-Apr-1995	36.89	-90.30	14
JB2008	Missouri	Cape Girardeau	MO	Priest 22	17-Apr-2006	37.32	-89.57	15
JB2009	Missouri	Crawford	MO	Yatskievych 93-17	5-May-1993	37.94	-91.21	14
JB2010	Missouri	Dent	MO	Nee 27326	11-May-1983	37.63	-91.26	14
JB2011	Missouri	Harrison	MO	Summers 8756B	24-Jun-1998	40.51	-93.82	15
JB2012	Missouri	Lawrence	MO	Gibson 3537	25-Apr-2002	37.12	-93.58	15
JB2013	Missouri	Marion	MO	Davis 1225	8-May-1916	39.71	-91.36	14
JB2014	Missouri	Monroe	MO	Hinterthuer 396	20-Jun-1973	39.52	-91.72	12
JB2015	Missouri	Pettis	MO	McCauley 251	15-Jun-1998	38.57	-93.30	15
JB2016	Missouri	Pulaski	MO	Ovrebø W1331	26-Jul-1989	37.63	-92.17	12
JB2017	Missouri	Ralls	MO	Summers 8168	18-Jun-1997	39.67	-91.66	14
JB2018	Missouri	Randolph	MO	Pelton s.n.	10-Jun-1986	39.26	-92.45	15
JB2019	Missouri	Reynolds	MO	Ladd 13687	22-Jun-1989	37.42	-91.09	14
JB2020	Missouri	Ripley	MO	Rowland 12	25-Apr-2010	36.68	-90.83	15
JB2021	Missouri	St Francois	MO	Darigo 1341	13-May-1993	37.87	-90.59	13
JB2022	Missouri	St Louis	MO	Feltz 80	4-May-2009	38.54	-90.54	15
JB2023	Missouri	Scott	MO	Powell 8	14-Apr-2010	37.62	-89.48	15
JB2024	Missouri	Texas	MO	Freeman 21499	20-Apr-2006	37.33	-91.95	15
JB2025	Nebraska	Nemaha	MO	Churchill 5125	12-May-1975	40.45	-95.71	13
JB2026	New Hampshire	Belknap	MO	Bradley 1432	25-Apr-2004	43.61	-71.40	15
JB2027	North Carolina	Avery	MO	Solomon 3989	15-Sep-1978	36.08	-81.78	15
JB2028	North Carolina	Graham	MO	Miller 8956	21-Aug-1997	35.14	-83.40	14

JB2029	North Carolina	Watauga	MO	Crosby 17769	17-May-2001	36.28	-81.84	15
JB2030	Oklahoma	Sequoyah	MO	Little 36195	20-May-1980	35.41	-94.60	15
JB2031	Ontario	York	MO	Soper 4614	9-Jun-1950	43.83	-79.20	12
JB2032	Pennsylvania	Adams	MO	Myers 20	15-Jun-1990	39.71	-77.40	12
JB2034	Quebec		MO	Roy 3188	4-Jun-1934	45.53	-73.70	8
JB2035	Vermont	Caledonia	MO	Seymour 21435	7-Jul-1963	44.54	-71.90	14
JB2036	Vermont	Orleans	MO	Seymour 25170	24-Sep-1966	44.91	-71.98	15
JB2037	Wisconsin	Richland	MO	Nee 14503B	8-Aug-1976	43.30	-90.33	15
JB2038	Wisconsin	Vernon	MO	Ziegler 1746	14-Jul-1975	43.70	-91.26	11
JB2103	Alabama	Autauga	VDB	Haynes 9220	9-Jul-1987	32.69	-86.73	15
JB2104	Alabama	Calhoun	VDB	Hruska 829	25-Jul-1995	33.59	-85.88	15
JB2105	Alabama	Coffee	VDB	Kral 89583	8-May-2000	31.30	-86.14	15
JB2106	Alabama	Covington	VDB	Diamond 13015	6-Apr-2002	31.44	-86.26	15
JB2107	Alabama	De Kalb	VDB	Spaulding 5014	11-Jul-1993	34.46	-86.05	15
JB2108	Alabama	Etowah	VDB	Kral 92815	3-Jul-2002	34.10	-85.99	15
JB2109	Alabama	Franklin	VDB	Kral 26304	6-May-1966	34.37	-87.75	15
JB2110	Alabama	Henry	VDB	Diamond 19107	1-May-2008	31.55	-85.36	14
JB2111	Alabama	Houston	VDB	MacDonald 7638	10-Sep-1994	31.24	-85.51	15
JB2112	Alabama	Jackson	VDB	DiPietro s.n.	28-Apr-1993	34.98	-85.81	15
JB2113	Alabama	Jefferson	VDB	Williams 66	13-Apr-1963	33.42	-86.73	15
JB2114	Alabama	Lee	VDB	Kral 30769	9-May-1968	32.52	-85.25	15
JB2115	Alabama	Lowndes	VDB	Diamond 12961	17-Mar-2002	32.33	-86.51	14
JB2116	Alabama	Macon	VDB	Diamond 13009	1-Apr-2002	32.40	-85.99	15
JB2117	Alabama	Madison	VDB	Kral 43342	15-Jul-1971	34.96	-86.37	15
JB2118	Alabama	Perry	VDB	Kral 66736	28-Mar-1981	32.78	-87.28	15
JB2119	Alabama	Pickens	VDB	Kral 45265	28-Mar-1972	33.11	-87.92	15
JB2120	Alabama	Pike	VDB	Diamond 13803	23-Mar-2003	31.91	-86.06	15
JB2121	Alabama	Russell	VDB	Diamond 16283	24-Apr-2006	32.34	-85.41	15
JB2122	Alabama	St Clair	VDB	Keener 4419	11-May-2008	33.95	-86.28	15
JB2123	Alabama	Sumter	VDB	Jones 15560	9-Jun-1968	32.57	-88.30	15
JB2124	Alabama	Washington	VDB	Kral 37349B	22-Sep-1969	31.20	-88.01	15
JB2125	Alabama	Winston	VDB	Kral 23734	20-Apr-1965	34.11	-87.62	15
JB2126	Tennessee	Coffee	VDB	Blum 3517	25-May-1969	35.41	-86.12	15
JB2127	Tennessee	Cumberland	VDB	Shaffer 91	20-Jun-1979	35.96	-85.13	15
JB2128	Tennessee	Davidson	VDB	Kral 82613	13-Jun-1993	36.10	-86.82	15
JB2129	Tennessee	Dickson	VDB	Souza 86-651	4-Oct-1986	36.09	-87.27	15
JB2130	Tennessee	Franklin	VDB	Kral 30266	20-Apr-1968	35.16	-86.15	15
JB2131	Tennessee	Grundy	VDB	Patrick 353	21-Aug-1977	35.46	-85.62	15
JB2132	Tennessee	Hardin	VDB	Jones 2313	21-Apr-1980	35.22	-88.31	15
JB2133	Tennessee	Hickman	VDB	Estes 4711	28-May-2003	35.73	-87.63	15
JB2134	Tennessee	Humphreys	VDB	Kral 76313	19-May-1989	36.03	-87.83	15
JB2135	Tennessee	Lauderdale	VDB	Keiran 300	17-Apr-1972	35.69	-89.63	15
JB2136	Tennessee	Lewis	VDB	Howell 518	27-Jun-1991	35.47	-87.48	15
JB2137	Tennessee	Overton	VDB	Kral 82203	6-May-1993	36.35	-85.33	15
JB2138	Tennessee	Robertson	VDB	Blum 2910	15-Aug-1968	36.55	-87.11	15
JB2139	Tennessee	Sumner	VDB	Alcorn 291	4-May-1975	36.54	-86.47	15
JB2140	Tennessee	Wayne	VDB	Kral 31670	19-Jul-1968	35.33	-87.64	15
JB2141	Texas	Cass	SMU	McVaugh 7164	13-Jun-1945	33.30	-94.14	15
JB2142	Texas	Cherokee	BRIT	Ajilvsgi 5071	22-Jun-1977	32.11	-95.05	15
JB2143	Texas	Hardin	SMU	Cory 54921	28-Sep-1948	30.25	-94.18	13

JB2144	Texas	Harris	SMU	Traverse 1317	6-Sep-1959	29.80	-95.52	15
JB2145	Texas	Jasper	SMU	Cory 52743	8-Apr-1947	30.38	-93.90	12
JB2146	Texas	Kaufman	SMU	Shinners 15414	19-Jul-1953	32.41	-96.23	7
JB2147	Texas	Lamar	SMU	McVaugh 7146	10-Jun-1945	33.66	-95.60	13
JB2148	Texas	Leon	BRIT	Nixon 17652	3-Apr-1992	29.95	-96.75	15
JB2149	Texas	Montgomery	BRIT	Sanders 6015	19-Aug-2003	30.13	-95.18	15
JB2150	Texas	Nacogdoches	SMU	McVaugh 8407	21-May-1947	31.53	-94.39	15
JB2151	Texas	Newton	SMU	McVaugh 6860	13-May-1945	30.85	-93.82	15
JB2152	Texas	Red River	BRIT	Sanders 2040	11-Aug-1993	33.45	95.05	12
JB2153	Texas	Titus	SMU	Amerson 902	15-Jul-1971	33.13	-95.10	12
JB2154	Texas	Wood	BRIT	Wagnon 129	31-Mar-2000	32.89	-95.23	15
JH086	Wisconsin	Richland	WIS	Nee 24267	6-Jun-1982	43.31	-90.31	0
JH087	Wisconsin	Waukesha	WIS	Leitner 3714	21-Jun-1989	43.03	-88.30	0
JH088	Michigan	Cheboygan	MICH	McVaugh10875	8-Jul-1949	45.56	-84.67	0
JH264	Georgia	Seminole	FLAS	Gholson 3003	1-Nov-1970	30.78	-84.87	9
JH265	Florida	Gadsden	FLAS	Gholson 9498	1-Mar-1982	30.71	-84.85	6
JH266	Florida	Nassau	FLAS	Rider 180	1-Mar-2000	30.77	-81.73	15
JH267	Virginia	Prince William	FLAS	Keyser 708	1-Apr-1982	38.66	-77.25	12
JH268	Kentucky	Madison	FLAS	Abbott 2134	1-May-1992	37.90	-84.27	15
JH269	Virginia	York	FLAS	Kirkman 201	1-Jun-1975	37.29	-76.60	14
JH270	West Virginia	Upshur	FLAS	Rossbach 7255	1-May-1966	39.00	-80.21	1
JH271	West Virginia	Randolph	FLAS	Clendenning s.n.	1-May-1964	38.85	-79.56	11
JH272	Virginia	Accomack	FLAS	Ware 6654	1-May-1977	37.62	-75.69	15
JH273	South Carolina	Richland	FLAS	Nelson 514	1-Mar-1976	34.04	-80.98	15
JH274	Louisiana	West Felicene	FLAS	Urbatsch 2285	1-Mar-1976	30.75	-91.29	13
JH275	Louisiana	Temmeny	FLAS	Rylander 35	1-Aug-1963	30.48	-90.10	15
JH276	Louisiana	Ouchita	FLAS	Thomas 22580	1-Mar-1971	32.52	-92.10	14
JH277	Florida	Jackson	FLAS	Hess 8496	1-Apr-1999	30.75	-84.92	8
JH278	Florida	Hillsborough	FLAS	Lakela 26099	1-Jul-1963	28.00	82.46	15
JH280	Florida	Levy	FLAS	Golledge 525	20-Jun-1905	29.50	-82.97	15
JH281	Florida	Columbia	FLAS	Tan 411	7-Mar-1990	29.91	-82.58	15
JH282	Florida	Clay	FLAS	Ferguson 62	1-Jan-1996	29.78	-82.01	15
JH283	Florida	Alachua	FLAS	Lange 1396	1-Feb-2012	29.65	-82.35	15
JH284	Florida	Walton	FLAS	Perkins 16401	1-Mar-1977	30.46	-81.42	15
JH285	Florida	Suwanee	FLAS	Herring 291	1-Jun-1991	29.95	-82.79	15
JH286	Florida	Polk	FLAS	Conard s.n.	1-Mar-1966	28.04	-81.64	15
JH287	Florida	Liberty	FLAS	Sloan 1612	1-Apr-1985	30.55	-84.94	15
JH288	Florida	Marion	FLAS	George 23	1-May-2005	28.98	-81.90	15
LK1000	Arkansas	Garland	KANU	Demaree 36747	10-May-1955	34.51	-93.08	14
LK1001	Indiana	Blackford	BUT	Friesner 10942	26-Jun-1937	40.54	-85.38	15
LK1002	Indiana	Brown	BUT	RCF s.n.	28-Jul-1930	39.26	-86.34	14
LK1003	Indiana	Dubois	BUT	Friesner 5177	9-Sep-1932	38.31	-86.84	15
LK1004	Indiana	Fountain	BUT	Friesner 3973	21-May-1932	40.06	-87.34	7
LK1005	Indiana	Harrison	BUT	Friesner 2940	24-May-1931	38.30	-86.10	15
LK1006	Indiana	Kosciusko	BUT	Friesner 13437	10-Jun-1939	41.07	-85.96	12
LK1007	Indiana	Lawrence	BUT	Friesner 4941	6-Sep-1932	38.85	-86.53	13
LK1008	Indiana	Madison	BUT	Rothrock 2110	14-Jun-1990	40.10	-85.62	15
LK1009	Indiana	Marion	BUT	Crandall 43	12-Jun-1996	39.87	-86.19	15
LK1010	Indiana	Miami	BUT	Friesner 15478	28-Jun-1941	40.87	-86.14	15
LK1011	Indiana	Monroe	BUT	Friesner 3184	31-Jul-1931	39.06	-86.40	15

LK1012	Indiana	Parke	BUT	Daubenmire 2296	11-Aug-1930	39.76	-87.23	3
LK1013	Indiana	St. Joseph	BUT	Friesner 13560	17-Jun-1939	41.53	-86.28	15
LK1014	Indiana	Tipton	BUT	Friesner 11730	2-Oct-1937	40.29	-86.17	14
LK1015	Indiana	Wabash	BUT	Friesner 15290	14-Jun-1941	40.68	-85.83	14
LK1016	Indiana	Warren	BUT	Tonkovich 29	10-May-1991	40.34	-87.32	15
LK1017	Indiana	Washington	BUT	Friesner 5007	7-Feb-1932	38.65	-86.10	15
LK1018	Indiana	White	BUT	Loughridge 1713	19-May-1935	40.74	-86.78	6
LK1019	Iowa	Adams	ISC	Isely 73	5-Jul-1947	41.12	-94.87	15
LK1020	Iowa	Clayton	ISC	Paucel s.n.	6-Aug-1925	43.02	-91.18	15
LK1021	Iowa	Davis	ISC	Aikman s.n.	4-Sep-1925	40.75	-92.42	15
LK1022	Iowa	Delaware	ISC	LHP s.n.	6-Aug-1924	47.62	-91.56	15
LK1023	Iowa	Des Moines	ISC	Pammel 958	8-Aug-1925	40.83	-91.12	15
LK1024	Iowa	Floyd	KANU	Freeman 22431	26-Jul-2007	43.15	-92.64	0
LK1025	Iowa	Franklin	ISC	Monson 3645	29-Aug-1956	42.66	-93.24	8
LK1026	Iowa	Hamilton	ISC	Thompson s.n.	13-May-2002	42.45	-93.79	12
LK1027	Iowa	Hardin	KANU	Freeman 9738	8-Aug-1997	42.45	-93.38	14
LK1028	Iowa	Howard	ISC	Christiansen 861	28-Jul-1978	43.44	-92.38	15
LK1029	Iowa	Humboldt	ISC	Monson 3566	23-Aug-1956	42.87	-94.19	10
LK1030	Iowa	Lee	ISC	Fults 1219	1-Jul-1931	40.46	-91.40	12
LK1031	Iowa	Mahaska	ISC	Augustine 66	1-May-1938	41.28	-92.46	10
LK1032	Iowa	Page	ISC	Wilson 1047	4-May-1987	42.49	-98.32	15
LK1033	Iowa	Polk	ISC	Pammel 339	26-May-1925	41.58	-93.68	12
LK1034	Iowa	Story	ISC	Couch 016	1-May-1987	42.03	-93.65	10
LK1035	Iowa	Tama	ISC	LHP s.n.	30-May-1923	42.19	-92.47	13
LK1036	Iowa	Warren	ISC	Croat 25009	6-Jul-1973	41.35	-93.76	14
LK1037	Iowa	Webster	ISC	Niemann 308	23-Jul-1970	42.42	-94.10	11
LK1038	Iowa	Winneshiek	ISC	Norris 9869101	9-Jun-1998	43.40	-91.90	15
LK1039	Kansas	Anderson	KANU	Morse 8197	18-Jun-2002	38.27	-95.25	12
LK1040	Kansas	Brown	KANU	McGregor 17069	14-Aug-1961	39.85	-95.37	14
LK1041	Kansas	Franklin	KANU	Freeman 12837	17-Jul-1999	38.58	-95.27	14
LK1042	Kansas	Jefferson	KANU	Stehpens 89120	29-May-1976	39.32	-95.46	8
LK1043	Kansas	Johnson	KANU	Freeman 9144	11-Jun-1997	38.83	-94.64	6
LK1044	Kansas	Kingman	KANU	Stephens 53505	24-May-1972	37.66	-98.21	15
LK1045	Michigan	Alcona	MICH	Garlitz 837	25-Aug-1984	44.84	-83.83	15
LK1046	Michigan	Alger	MICH	Fruedenstein 1402	26-Jul-1984	46.65	-86.11	15
LK1047	Michigan	Antrim	MICH	Appel 232	14-Jun-1980	45.11	-85.10	14
LK1048	Michigan	Bay	MICH	Fruedenstein 1543	9-May-1985	43.69	-83.93	15
LK1049	Michigan	Crawford	MICH	Chittenden 478	1-Jun-1992	44.71	-84.59	15
LK1050	Michigan	Genesee	MICH	Merkle 70108	15-Jul-1970	42.84	-83.73	13
LK1051	Michigan	Gratiot	MICH	Fruedenstein 847	26-Jul-1983	43.26	-84.40	15
LK1052	Michigan	Ionia	MICH	Gereau 984	5-Jun-1982	42.84	-84.87	15
LK1053	Michigan	Leelanau A	MICH	Hazlett 1826	2-Sep-1982	45.10	-86.04	14
LK1054	Michigan	Lenawee	MICH	Smith 530	11-May-1985	41.91	-84.04	15
LK1055	Michigan	Marquette	MICH	McVaugh 11133	27-Aug-1949	46.54	-87.52	15
LK1056	Michigan	Mason	MICH	Hazlett 854	25-Aug-1979	44.08	-86.33	1
LK1057	Michigan	Mecosta	MICH	Ross 1180	19-May-2001	43.71	-85.33	15
LK1058	Michigan	Missaukee	MICH	Voss 3084	13-Jul-1956	44.51	-84.92	12
LK1059	Michigan	Monroe	MICH	Easterly 11609	22-May-1980	41.91	-83.60	9
LK1060	Michigan	Montmorency	MICH	Garlitz 863	25-Aug-1984	44.88	-84.15	15
LK1061	Michigan	Ontonagon	MICH	MacFarlane 4895	20-Jul-1986	46.77	-89.08	0

LK1062	Michigan	Oscoda	MICH	Zimmerman 210	16-Jul-1951	44.64	-84.34	10
LK1063	Michigan	Saginaw	MICH	Freudenstein 1290	20-Jun-1984	43.43	-84.06	12
LK1064	Missouri	Chariton	KANU	Freeman 23002	23-Jul-2008	39.38	-93.02	15
LK1066	Oklahoma	Murray	KANU	Goodman 7935	4-May-1969	34.44	-97.02	0
LK1067	Wisconsin	Crawford	WIS	Moore 48	12-Jul-1980	43.02	-91.11	14
LK1068	Wisconsin	Dane	WIS	Cochrane 11860	19-Aug-1989	43.21	-89.73	15
LK1069	Wisconsin	Dodge	WIS	Leitner 1264	10-Jul-1988	43.42	-88.70	15
LK1070	Wisconsin	Door	WIS	Judziewicz 13743	25-Jun-2000	45.19	-87.36	6
LK1071	Wisconsin	Grant	WIS	Anderson 60	26-May-1993	42.83	-91.07	15
LK1072	Wisconsin	Green Lake	WIS	Banks 166	10-Aug-1983	43.70	-89.01	15
LK1073	Wisconsin	Iron	WIS	Lucy 6001	18-May-1989	46.02	-90.01	15
LK1074	Wisconsin	Juneau	WIS	Freckmann 24661	15-Sep-1989	44.05	-90.17	15
LK1075	Wisconsin	Kenosha	WIS	Smith 735	23-Jun-1988	42.58	-89.95	14
LK1076	Wisconsin	Manitowoc	WIS	Moore 532	18-Aug-1986	44.28	-87.76	14
LK1077	Wisconsin	Ozaukee	WIS	Leitner 3081	21-Jul-1989	43.38	-87.99	14
LK1078	Wisconsin	Sauk	WIS	Cochrane 11820	29-Jul-1989	43.45	-89.82	14
LK1079	Wisconsin	Sawyer	WIS	Weshinsky 237	18-Sep-1993	46.11	-91.31	13
LK1080	Wisconsin	Shawano	WIS	De Stefano 153	14-Aug-1980	44.61	-88.49	14
LK1081	Wisconsin	Taylor	WIS	Fields 1109	31-May-1994	45.25	-90.62	15
LK1082	Wisconsin	Walworth	WIS	Cochrane 12445	7-Jul-1990	42.80	-88.62	14
LK1084	Kentucky	Allen	KANU	Hulbert3783	25-Jun-1959	37.91	-95.11	12
LK1085	Kentucky	Franklin	KANU	McGregor10473	18-Jun-1955	38.48	-95.34	6
LK1086	Nebraska	Richardson	KANU	McGregor19102	23-May-1965	40.14	-95.72	12
LK1089	Florida	Gilchrist	NYBG	Longbottom 18664	2-Mar-2013	29.82	-82.69	15
LK1090	Indiana	Jasper	NYBG	Welsh 63	16-Jun-1923	40.79	-87.23	15
LK1091	Kentucky	Fleming	NYBG	Wharton 3823f	12-May-1939	38.33	-83.56	6
LK1092	Kentucky	Henry	NYBG	Gentry 299	6-Jun-1962	38.42	-84.97	14
LK1093	Louisiana	Orleans	NYBG	Purrrington 16	21-Mar-1986	30.00	-90.10	15
LK1094	Maine	Washington	NYBG	Atha 8807	8-Aug-2010	44.68	-67.95	14
LK1095	Maryland	Allegany	NYBG	Longbottom 11447	25-May-2008	39.52	-78.91	15
LK1097	Maryland	Howard	NYBG	Longbottom 13180	24-Apr-2010	39.12	-76.80	14
LK1098	Maryland	Queen Annes	NYBG	Longbottom 13227	28-Apr-2010	38.97	-76.13	15
LK1099	Maryland	Talbot	NYBG	Longbottom 13182	24-Apr-2010	38.80	-76.06	15
LK1100	Maryland	Washington	NYBG	Longbottom 11415	25-May-2008	39.70	-77.94	15
LK1101	Maryland	Wicomico	NYBG	Longbottom 11814	28-Jul-2008	38.32	-75.61	15
LK1102	Maryland	Worcester	NYBG	Hill 13899	24-May-1984	38.24	-75.14	15
LK1104	Massachusetts	Dukes	NYBG	MacKeever 612	19-Aug-1963	41.38	-70.51	14
LK1105	New Jersey	Burlington	CHRB	Long 10697	26-Jul-2014	39.59	-74.45	15
LK1108	New Jersey	Essex	CHRB	Morton 6073	24-Jun-1976	40.77	-74.28	15
LK1109	New Jersey	Hunterdon	CHRB	Hough s.n.	13-May-1964	40.64	-75.06	14
LK1110	New Jersey	Mercer	CHRB	Kramer 1341	11-Jun-1966	40.38	-74.65	15
LK1112	New Jersey	Morris	NYBG	Atha 10445	25-May-2011	40.91	-74.56	15
LK1113	New Jersey	Ocean	NYBG	Atha 6539	31-Jul-2008	39.66	-74.35	15
LK1114	New Jersey	Passaic	CHRB	Barringer 9664	29-May-2003	40.98	-74.32	15
LK1115	New Jersey	Salem	CHRB	Vhryslr 1130	20-May-1935	39.71	-75.35	7
LK1116	New Jersey	Somerset	CHRB	Costich 17	14-May-1991	40.46	-74.75	15
LK1119	New Jersey	Warren	CHRB	Hanks s.n.	7-Aug-1966	40.89	-74.96	13
LK1120	New York	Bronx	NYBG	Nec 57867	11-May-2011	40.87	-73.89	15
LK1121	New York	Dutchess	CHRB	Ahles 66245	4-Jun-1967	41.79	-73.92	15
LK1122	New York	Putnam	NYBG	Atha 6543	2-Aug-2008	41.39	-73.91	15

LK1123	North Carolina	Cumberland	NYBG	Cruchfield 5612	16-Apr-1968	35.06	-78.96	15
LK1124	Ohio	Guernsey	NYBG	Nee 56389	9-Jun-2009	40.12	81.56	15
LK1125	Pennsylvania	Armstrong	PH	Wahl 4798	24-Sep-1947	40.88	-79.58	8
LK1126	Pennsylvania	Blair	PH	Skinner 43	5-Jun-1943	40.43	-78.49	3
LK1127	Pennsylvania	Centre	PH	Fogg 17873	15-Aug-1940	40.98	-77.63	11
LK1128	Pennsylvania	Clarion	PH	Wahl 5586	13-Jul-1948	41.02	-79.38	15
LK1129	Pennsylvania	Clinton	PH	Wahl 2136	5-Jun-1947	41.06	-77.37	8
LK1130	Pennsylvania	Crawford	PH	Thompson 05721	18-Jul-2005	41.70	-80.42	15
LK1131	Pennsylvania	Erie	PH	Phillips 78123	5-Aug-1919	41.99	-80.21	15
LK1132	Pennsylvania	Franklin	PH	Wahl 6664	10-Jun-1949	39.93	-77.54	15
LK1133	Pennsylvania	Huntingdon	PH	Wahl 3549	8-Aug-1947	40.33	-78.02	14
LK1134	Pennsylvania	Indiana	PH	Skinner 100	5-Jun-1942	40.66	-79.02	9
LK1135	Pennsylvania	Jefferson	PH	Wahl 3633	12-Aug-1947	41.13	-79.18	13
LK1137	Pennsylvania	Luzerne	PH	Wahl 15489	15-May-1955	41.36	-76.04	10
LK1138	Pennsylvania	Monroe	PH	Wherry s.n.	28-Jul-1952	40.95	-75.42	15
LK1139	Pennsylvania	Perry	CHRB	Adams 1405	1-Jul-1934	40.43	-77.20	12
LK1140	Pennsylvania	Pike	PH	DePue 403	26-Jul-1938	41.17	-74.91	15
LK1141	Pennsylvania	Potter	PH	Wahl 7930	8-Sep-1949	41.87	-77.72	14
LK1142	Pennsylvania	Schuylkill	PH	Wagner 4429	3-Jul-1937	40.65	-76.61	15
LK1143	Pennsylvania	Sullivan	NYBG	Penny 145	17-Jun-1942	41.41	-76.61	15
LK1145	Pennsylvania	Warren	PH	Wahl 11248	18-Jun-1951	41.73	-79.06	13
LK1146	Pennsylvania	Wayne	PH	Harper 2186	4-Aug-1946	41.24	-75.38	15
LK1149	Virgina	Northampton	CHRB	Small s.n.	11-Jul-1935	37.16	-75.98	14
LK1150	Virginia	Washington	NYBG	Britton s.n.	26-Jun-1892	36.64	-81.61	0
LK1151	West Virginia	Grant	NYBG	Atha 6627	9-Aug-2008	39.06	-79.28	15
LK1152	West Virginia	Wayne	CHRB	Gilbert 486	13-Jul-1936	38.32	-82.50	15
LK1153	Ontario	Leeds	NYBG	Dore 19936	4-Jun-1962	44.36	-76.01	15
LK1154	Quebec	Gatineau	NYBG	Cody 12375	21-Aug-1962	45.55	-76.09	15
LK1209	Arkansas	Arkansas	STAR	Richards 9516	19-Aug-1985	34.37	-91.12	10
LK1210	Arkansas	Baxter	STAR	Richards 9339	20-Apr-1985	36.34	-92.47	11
LK1211	Georgia	Bulloch	USF	Hill 27643	9-Apr-1996	32.40	-81.76	12
LK1212	Arkansas	Carroll	STAR	Atkins 131	18-May-1984	36.33	-93.39	15
LK1213	Arkansas	Clay	STAR	Hitt 2	27-Jun-1966	36.39	-90.20	11
LK1214	Arkansas	Cleburne	STAR	Babb 751	21-Jul-2012	35.50	-92.22	15
LK1215	Arkansas	Conway	STAR	Richards 10747	5-Jun-1990	35.13	-92.73	15
LK1216	Arkansas	Craighead	STAR	Scarborough 2	20-Jun-1965	35.85	-90.68	15
LK1217	Arkansas	Dallas	STAR	Richards 9665	14-Sep-1985	34.14	-92.67	15
LK1218	Arkansas	Drew	STAR	Richards 9299	6-Apr-1985	33.61	-91.81	15
LK1235	Mississippi	Adams	MISS	Temple 11259	14-Jun-1969	31.51	-91.37	14
LK1236	Mississippi	Alcorn	MISS	Pullen 66519	22-Jun-1966	34.82	-88.72	14
LK1237	Mississippi	Attala	MISS	Jones 16960	19-Jun-1969	32.99	-89.56	15
LK1238	Mississippi	Benton	MISS	Temple 5649	29-Jun-1967	34.64	-89.30	15
LK1239	Mississippi	Chickasaw	MISS	Pullen 66888	20-Jul-1966	34.04	-88.97	14
LK1240	Mississippi	Copiah	MISS	Herrington 014	27-Sep-1964	31.87	-90.50	15
LK1241	Mississippi	DeSoto	MISS	Ferrari 456	12-Sep-1969	34.85	-89.77	14
LK1242	Mississippi	George	MISS	Jones 6993	21-Jun-1966	30.96	-88.61	15
LK1243	Mississippi	Hinds	MISS	Temple 8099	9-Apr-1968	32.35	-90.39	11
LK1244	Mississippi	Jasper	MISS	Jones 13007	5-Jun-1967	31.83	-89.02	15
LK1245	Mississippi	Lafayette	MISS	Connolly 66	5-Jun-2000	34.43	-89.39	10
LK1246	Mississippi	Lauderdale	MISS	Jones 7072	23-Jun-1966	32.34	-88.71	12

LK1247	Mississippi	Lawrence	MISS	Jones 7747	6-Jul-1966	31.57	-90.10	15
LK1248	Mississippi	Leflore	MISS	Bryson 17226	11-Jun-1999	34.41	-90.34	15
LK1250	South Carolina	Abbeville	NYBG	Credle 1164	1-Aug-1979	34.18	-82.69	15
LK1251	Illinois	Knox	NYBG	Chase 10626	10-Aug-2014	40.93	-90.01	7
LK1252	Illinois	Jo Daviess	NYBG	Nee 22011	26-Sep-1981	42.26	-90.29	15
LK1253	South Carolina	Richland	NYBG	Nelson 17928	17-Mar-1997	34.00	-81.40	15
LK1254	South Carolina	Aiken	NYBG	Hill 22373	23-Jun-1991	33.60	-81.84	15
LK1255	Virginia	Virginia Beach City	NYBG	Egler 40-108	20-Jul-1940	36.88	-76.10	15
LK1256	New York	Suffolk	NYBG	Pace 252	15-Jun-2008	40.83	-73.43	15
LK1257	North Carolina	Beaufort	NYBG	Atha 9983	3-May-2011	35.31	-76.80	15
LK1258	New York	Westchester	NYBG	Doody s.n.	1-Jun-1958	41.13	-73.79	14
LK1260	New York	Franklin	NYBG	Britton s.n.	15-Sep-1900	44.56	-74.33	15
LK1261	Virginia	Arlington	NYBG	Harriman s.n.	13-May-1994	38.89	-77.07	15
LK1262	Ontario	La Haute-Yamaska	NYBG	Habuis 612	7-Dec-1946	45.43	-72.70	13
LK1263	Ontario	Kingston	NYBG	Fowler s.n.	11-Jun-1902	44.24	-76.61	1
LK1264	Kentucky	Harlan	NYBG	Kearney s.n.	Aug-1893	36.81	-83.31	15
LK1265	Georgia	De Kalb	NYBG	Small s.n.	May-1895	33.81	-84.15	13
LK1266	Illinois	Adams	NYBG	Evers 61	13-Sep-1939	39.87	-91.30	2
LK1267	Rhode Island	Providence	HUH	Robinson s.n.	29-May-1904	41.97	-71.43	14
LK1268	Connecticut	Tolland	HUH	Mehrhoff 14329	18-May-1991	41.99	-72.41	15
LK1269	Vermont	Chittenden	HUH	Cook 480	8-Dec-1958	44.56	-72.94	15
LK1270	Vermont	Windham	HUH	Wheeler s.n.	12-Jun-1912	43.04	-72.66	15
LK1271	Connecticut	Fairfield	HUH	Green 67/63	19-Sep-1963	41.02	-73.63	10
LK1272	New Hampshire	Cheshire	HUH	Batchelder	9-Jul-1919	42.94	-72.24	14
LK1273	New Hampshire	Hillsborough	HUH	Batchelder s.n.	15-Aug-1921	42.82	-71.63	15
LK1274	Connecticut	New London	HUH	Anderson 930	27-May-1975	41.53	-72.11	15
LK1275	Florida	Levy	FLAS	Abbott 9118	18-Jun-1905	29.07	-82.72	15
LK1276	Kentucky	Madison	BEREA	Thompson 10809	8-Jul-2010	37.58	-84.30	14
LK1277	Tennessee	Blount	BEREA	Thompson 09535	12-Aug-2009	35.01	-83.02	15
LK1278	Missouri	Warren	FLAS	Abbott 26078	11-Jun-2012	38.73	-91.14	15
LK1279	Maine	Cumberland	FLAS	Abbott 25568	5-Aug-2010	43.86	-70.11	15
LK1280	Rhode Island	Newport	FLAS	Abbott 25493	3-Aug-2010	41.58	-71.21	15
LK1281	New Jersey	Sussex	FLAS	Abbott 25420	31-Jul-2010	41.35	-74.68	15
LK1282	Georgia	Whitfield	FLAS	Abbott 25360	10-Jul-2010	34.83	-85.04	15
LK1283	Florida	Suwannee	FLAS	Abbott 22568	4-Jun-2007	30.04	-83.01	15
LK1293	Connecticut	Middlesex	HUH	Richardson s.n.	19-Jun-1964	41.47	-72.48	15
LK1294	Connecticut	Windham	HUH	Mehrhoff 19324	4-Jun-1997	41.66	-72.10	15
LK1295	Maine	Androscoggin	HUH	R.C.B.1	29-Aug-1932	44.10	-70.30	10
LK1296	Maine	Cumberland	HUH	Furbish	1-Jun-2015	43.78	-70.31	10
LK1297	Maine	Franklin	HUH	Laferriere 3914	26-May-2006	44.01	-70.00	10
LK1298	Maine	Hancock	HUH	Hill 2443	2-Sep-1915	44.27	-68.57	15
LK1299	Maine	Lincoln	HUH	Brierly 1226a	7-Jun-1931	44.18	-69.47	15
LK1300	Maine	Oxford	HUH	Wheeler 62/203	6-Jun-1938	44.49	-70.80	15
LK1301	Maine	York	HUH	True 223	28-Aug-1934	43.41	-70.75	11
LK1302	Rhode Island	Bristol	HUH	Countryman 22206	29-Aug-1964	41.64	-71.26	15
LK1303	Rhode Island	Washington	HUH	Bill s.n.	11-Jun-1927	41.43	-71.58	15
LK1304	North Carolina	Cabarrus	NCU	Ahles 15914	5-Jul-1956	35.43	-80.63	11
LK1305	North Carolina	Camden	NCU	Ahles 44418	25-Jun-1958	36.46	-76.33	15
LK1307	North Carolina	Currituck	NCU	Ahles 44491	25-Jun-1958	36.36	-75.92	14
LK1309	North Carolina	Nash	NCU	Ahles 11730	1-May-1956	36.02	-78.12	15

LK1310	North Carolina	Randolph	NCU	Downs 13255	25-Apr-2015	35.61	-79.83	15
LK1311	South Carolina	Allendale	NCU	Ahles 10667	6-Apr-1956	32.83	-81.37	15
LK1312	South Carolina	Cherokee	NCU	Ahles 11283	22-Apr-1956	35.12	-81.77	14
LK1313	South Carolina	Clarendon	NCU	Radford 21114	20-Apr-1957	33.84	-80.03	12
LK1314	South Carolina	Horry	NCU	Bell 6166	20-Apr-1957	33.69	-79.01	15
LK1315	South Carolina	Lancaster	NCU	Ahles 27470	6-Jun-1957	34.77	-80.52	15
LK1316	South Carolina	Marlboro	NCU	Radford 9291	2-Apr-1956	34.58	-79.76	15
LK1317	Louisiana	Acadia	ULM	Allen 12961	18-Mar-1984	30.47	-92.44	15
LK1318	Louisiana	Assumption	ULM	Thomas 117546	23-May-1990	29.87	-91.10	15
LK1319	Louisiana	Avoyelles	ULM	Thomas 118893	14-Jun-1990	30.86	-92.15	15
LK1320	Louisiana	Beuregard	ULM	Bruce 119	8-Sep-1985	30.50	-93.31	15
LK1321	Louisiana	Bienville	ULM	Thomas 100242	17-Jun-1987	32.42	-92.88	14
LK1322	Louisiana	Caddo	ULM	Lewis 3475	15-Mar-1982	32.52	-93.75	12
LK1323	Louisiana	Caldwell	ULM	Marx 463	20-Mar-1973	32.09	-92.17	7
LK1324	Louisiana	Cameron	ULM	Thomas 90684	17-Aug-1984	30.01	-92.78	14
LK1325	Louisiana	Claiborne	ULM	Lewis 1328	27-May-1978	32.95	-92.97	15
LK1326	Louisiana	Grant	ULM	Thomas 150887	11-Aug-1996	31.64	-92.47	15
LK1327	Louisiana	Iberia	ULM	Thomas 132576	22-Oct-1992	29.81	-91.79	15
LK1328	Louisiana	Iberville	ULM	Lewis 1130	16-Jul-1977	30.40	-91.51	15
LK1329	Louisiana	Lafouche	ULM	Guidroz 164	17-May-1973	29.78	-90.82	14
LK1330	Louisiana	Livingston	ULM	Thomas 130440	11-Jul-1992	30.46	-90.99	15
LK1334	Ohio	Summit	US	Adreas 3422	20-May-1979	41.08	-81.64	12
LK1335	Virginia	Rappahannock	US	Walker 2254	1-May-1938	38.79	-78.16	15
LK1336	Virginia	Page	US	Fosberg 42383	27-May-1962	38.53	-78.44	15
LK1337	Virginia	Bath	US	Morton 1957	11-Jun-1930	37.97	-79.60	15
LK1338	Kentucky	Pendleton	US	Braun 4485	2-May-1942	38.83	-84.27	3
LK1339	Virginia	Albermarle	US	Fosberg 36419	29-May-1955	38.23	-78.72	15
LK1340	New York	Tompkins	US	Coville s.n.	4-Jun-1885	42.44	-76.50	11
LK1341	Georgia	McDuffie	US	Bartlett 2593	7-Jun-1911	33.48	-82.52	12
LK1342	Ohio	Lorain	US	Ricksecker s.n.	27-May-1895	41.24	-82.22	13
LK1343	New York	Onondaga	US	Brown 8022	18-Jun-1934	43.05	-75.97	15
LK1354	Ohio	Ashland	KSC	Culler s.n.	24-May-1905	40.92	-82.16	15
LK1355	West Virginia	Pocahontas	KSC	Holland 9618	25-May-1999	38.37	-79.88	14
LK1356	Oklahoma	Ottowa	KSC	Grannerman s.n.	9-Mar-1974	39.99	-94.65	15
LK1372	Georgia	McIntosh	USF	Smith 2268	8-Jun-1909	31.51	-81.37	2
LK1373	Indiana	Jay	USF	Franck 3966	7-Aug-2015	40.50	-84.94	15
LK1374	North Carolina	Carteret	USF	Scarboro s.n.	11-Jul-1965	34.72	-76.67	15
LK1375	Ohio	Shelby	USF	Franck 556	2-Aug-2007	40.35	-84.42	11
LK1376	Oklahoma	McCurtain	USF	Stanford 3133	19-Apr-1969	34.03	-94.57	14
LK1377	South Carolina	Charleston	USF	Hill 29423	17-Apr-1993	33.15	-79.40	15
LK1378	Tennessee	Cocke	USF	Genelle 2748	16-May-1977	36.00	-83.10	13
LK1379	Virginia	Smyth	USF	Kral 11691	21-Sep-1960	36.66	-81.54	14
LK1380	Illinois	Ogle	USF	Sorenson 1206	22-Apr-2012	41.94	-89.23	15
LK1381	Illinois	Alexander	ILLS	Phillippe 25868	21-Sep-1994	37.26	-89.41	15
LK1382	Illinois	Bond	ILLS	Evers 39335	10-Jul-1953	38.98	-89.35	15
LK1383	Illinois	Boone	ILLS	Evers 47220	6-Jul-1955	42.38	-88.81	8
LK1384	Illinois	Bureau	ILLS	Evers 9273	29-Apr-1948	41.36	-89.49	13
LK1385	Illinois	Carroll	ILLS	Robertson 4423	12-May-1987	42.06	-90.10	15
LK1386	Illinois	Cass	ILLS	Phillippe 24060	17-May-1994	40.02	-90.11	15
LK1387	Illinois	Champaign	ILLS	Layden 16	12-Jun-1977	40.11	-88.34	15

LK1388	Illinois	Chrisitan	ILLS	Evers 107936	5-Jun-1972	39.41	-89.46	15
LK1389	Illinois	Clay	ILLS	Evers 112542	26-Apr-1974	38.77	-88.43	15
LK1390	Illinois	Cook	ILLS	Evers 34835	24-Jul-1952	42.14	-88.04	15
LK1391	Illinois	Dewitt	ILLS	Evers 64336	8-Jun-1960	40.12	-89.08	15
LK1392	Illinois	Edwards	ILLS	Edgin 4749	28-Aug-2001	38.61	-88.12	15
LK1393	Kentucky	Campbell	MU	Buddell 259	28-Apr-1981	39.11	-84.47	14
LK1394	Kentucky	Jackson	MU	Taylor 3540	24-May-1983	37.54	-84.19	13
LK1395	Kentucky	Mercer	MU	Taylor 15761	22-Jun-1991	37.82	-84.74	15
LK1396	Kentucky	Wolfe	MU	Vincent 13590	26-Jun-2007	37.77	-83.43	15
LK1397	Ohio	Cuyahoga	MU	Georgius 42	18-Sep-2002	41.35	-81.93	15
LK1398	Ohio	Franklin	MU	Schutte 03	3-May-2003	40.07	-83.01	15
LK1399	Ohio	Hamilton	MU	White 5591-1	5-May-1991	39.23	-84.51	14
LK1400	Ohio	Highland	MU	Sulgrove 90071334	13-Jul-1990	39.10	-83.43	14
LK1401	Ohio	Logan	MU	McCormac 3674	3-Jun-1991	40.30	-83.79	15
LK1402	Ohio	Mercer	MU	Trisel 111	10-May-1990	40.68	-84.64	13
LK1403	Ohio	Montgomery	MU	Dister s.n.	5-May-2001	39.77	-84.19	14
LK1404	Ohio	Portage	MU	Stewart 193	14-Sep-1985	41.23	-81.38	15
LK1405	Ohio	Seneca	MU	Jones 69-5-23-367	23-May-1969	41.24	-82.93	14
LK1406	West Virginia	Boone	WVA	Craig s.n.	25-May-1905	38.07	-81.82	15
LK1407	West Virginia	Preston	WVA	Grafton s.n.	8-Sep-1997	39.38	-79.64	15
LK1408	West Virginia	Monongalia	WVA	Dawson s.n.	15-May-1979	39.67	-79.96	14
LK1409	West Virginia	Barbour	WVA	Grafton s.n.	20-May-2001	39.19	-79.89	15
LK1410	West Virginia	Ohio	WVA	Grafton s.n.	28-Sep-2003	40.15	-80.71	15
LK1411	West Virginia	Wood	WVA	Grafton s.n.	14-May-1999	39.24	-81.29	15
LK1412	West Virginia	Fayette	WVA	Grafton s.n.	2-May-2004	38.14	-81.10	15
LK1413	West Virginia	Berkeley	WVA	Grafton s.n.	27-Jun-2004	39.51	-78.17	15
LK1414	West Virginia	Upshur	WVA	Chapman 5	3-Sep-2005	38.89	-80.30	13
LK1416	West Virginia	Roane	WVA	Bartholomew 14	24-Aug-1977	38.91	-81.43	12
LK1417	Virginia	Mecklenburg	GMUF	Belden 1839	19-Aug-1999	36.60	-78.37	14
LK1418	Virginia	Lancaster	GMUF	Stanley and Miller s.n.	8-Jul-1973	37.64	-76.37	15
LK1419	New York	Hamilton	PLAT	Keelan 111	1-Jul-1995	43.67	-74.70	15
LK1420	New York	Clinton	PLAT	Kretchman s.n.	11-Jul-1968	44.62	-73.42	12
LK1421	Tennessee	Shelby	TENN	Browne 70M5.7	4-Sep-1970	35.32	-90.05	14
LK1422	Tennessee	Crockett	TENN	Heineke 2271	13-Apr-1981	35.78	-89.13	13
LK1423	Tennessee	Blount	TENN	Thomas s.n.	12-Aug-1964	35.74	-83.72	15
LK1424	Tennessee	Obion	TENN	Guthrie 1487	22-Sep-1986	36.43	-89.32	14
LK1425	Tennessee	Dyer	TENN	Deneke 1672	13-Oct-1979	36.13	-89.43	15
LK1426	Tennessee	Carter	TENN	Pyne 93-272	1-Oct-1993	36.41	-82.24	12
LK1427	Tennessee	Johnson	TENN	Evans 43122	13-Jun-1969	36.52	-81.93	14
LK1428	Tennessee	Monroe	TENN	Maltev 53569	28-Jun-1977	35.40	-84.18	15
LK1429	Tennessee	Gibson	TENN	Chester 14650	24-May-2004	36.30	-88.69	15
LK1430	Tennessee	Marion	TENN	Blyveis 40	29-May-2009	35.01	-85.61	15
LK1431	Tennessee	Polk	TENN	Jacobs 83	7-May-1983	35.05	-84.54	13
LK1432	Tennessee	Carroll	TENN	Thompson 558	9-Jul-1972	36.14	-88.45	15
LK1433	West Virginia	Calhoun	MUHW	Dowell 109	1-Aug-1992	38.86	-81.12	12
LK1434	West Virginia	Kanawha	MUHW	Strickland 210	18-Apr-1998	38.46	-81.50	15
LK1435	West Virginia	Logan	MUHW	Bowen 18	16-Apr-2000	37.80	-81.80	15
LK1436	West Virginia	Raleigh	MUHW	Dobson 241	28-Apr-1989	37.76	-81.18	15
LK1437	West Virginia	Mercer	MUHW	Brumfield 199	28-Jun-1983	37.50	-81.12	2
LK1438	West Virginia	Mason	MUHW	West 134	29-Jul-1981	38.72	-81.97	0

LK1442	Oklahoma	Tulsa	EKY	Clark 23383	23-Jun-1996	36.09	-95.99	14
LK1443	Kentucky	Barren	EKY	Lapham 21	6-May-1993	36.89	-85.98	15
LK1444	Kentucky	Marion	EKY	Clark 23604	31-Aug-1997	37.59	-85.06	15
LK1445	Kentucky	McCreary	EKY	Shaw 391	18-Aug-1999	36.63	-84.53	15
LK1446	Kentucky	Trigg	EKY	Mowrer 4	27-Jun-1995	36.68	-88.05	11
LK1447	Kentucky	Henderson	EKY	Hannan 3606	1-May-1980	37.72	-87.76	14
LK1448	Kentucky	Edmonson	EKY	Elmore 781	3-May-1969	37.17	-86.32	15
LK1449	Kentucky	Whitley	EKY	Carter 67	21-Apr-1994	36.81	-84.19	15
LK1450	Kentucky	Laurel	EKY	Allen 94	2-Oct-1999	37.05	-84.02	15
LK1451	Kentucky	Union	EKY	Dreier s.n.	16-Apr-1976	37.63	-87.87	15
LK1452	Virginia	Buckingham	VPI	Little 106155	28-Jun-1951	37.49	-78.55	12
LK1453	Virginia	Giles	VPI	Williams 10388	28-Jun-1988	37.32	-80.83	10
LK1454	Virginia	Pittsylvania	VPI	Massey 1188	15-Jun-1937	36.74	-79.47	15
LK1455	Virginia	Amelia	VPI	Lewis 813	7-Jul-1937	37.32	-77.97	13
LK1456	Virginia	Bland	VPI	Kral 12881	28-Jun-1961	37.22	-81.04	15
LK1457	Virginia	Floyd	VPI	Uttal 11893	27-May-1976	36.99	-80.36	15
LK1458	Virginia	Prince George	VPI	Bailey s.n.	19-Apr-1905	37.14	-77.23	14
LK1459	Virginia	Amherst	VPI	Massey 2942	10-May-1939	37.59	-79.05	14
LK1460	Kentucky	Christian	APSC	Francis s.n.	5-May-1968	36.92	-87.48	15
LK1461	Kentucky	Lyon	APSC	Ellis 1895	13-Jul-1966	36.95	-88.13	15
LK1462	Tennessee	Houston	APSC	Chester 2855	6-Jul-1962	36.23	-87.53	15
LK1463	Tennessee	Henry	APSC	Chester 9068	20-Apr-1990	36.33	-88.13	15
LK1464	Louisiana	Ouachita	APSC	Englandand 142	8-May-1967	32.51	-92.29	15
LK1465	Alabama	Choctaw	APSC	Harrell 114	23-Mar-2007	31.84	-88.16	13
LK1466	Tennessee	Maury	APSC	Estes 58	27-Apr-1999	35.45	-87.27	15
LK1467	South Carolina	Orangeburg	DUKE	Wilbur 73355	21-May-2001	33.43	-80.36	15
LK1468	South Carolina	Pickens	DUKE	Rodgers 531	4-May-1942	35.05	-82.70	0
LK1469	South Carolina	Spartanburg	DUKE	Etters 17	22-Apr-1960	34.93	-81.97	5
LK1470	Virginia	Rockbridge	DUKE	Wyatt 676	10-Jul-1974	37.80	-79.46	13
LK1471	Virginia	Southampton	DUKE	Wilbur 75931	30-May-2002	36.81	-76.96	15
LK1472	Virginia	Halifax	DUKE	Thomas 953	9-Apr-1988	36.62	-79.07	15
LK1473	Virginia	Washington	DUKE	Small s.n.	28-Jun-1892	36.64	-81.61	9
LK1474	North Carolina	Alamance	DUKE	Baim 125	25-Oct-1992	36.10	-79.38	14
LK1475	North Carolina	Macon	DUKE	Mark s.n.	6-Jun-1956	35.18	-83.56	14
LK1476	North Carolina	Hyde	DUKE	Wilbur 65855	6-Jun-1996	35.60	-76.23	15
LK1477	North Carolina	Harnett	DUKE	Wilbur 52070	18-Apr-1989	35.47	-78.93	15
LK1478	North Carolina	Richmond	DUKE	Wilbur 56064	28-Jun-1990	35.05	-79.52	12
LK1479	North Carolina	Rowan	DUKE	Batson 893	28-Aug-1951	35.73	-80.65	15
LK1480	North Carolina	Surry	DUKE	Correll 14656	16-Jul-1948	36.51	-80.59	12
LK1481	North Carolina	Wake	DUKE	Wilbur 52181	23-Apr-1989	35.72	-78.95	11
LK1482	North Carolina	Pamlico	DUKE	Wilbur 60880	2-Jun-1992	35.14	-76.96	15
LK1483	North Carolina	Mecklenburg	DUKE	Daggy 7656	23-Apr-1951	35.47	-80.94	13
LK1484	North Carolina	Person	DUKE	Wilbur 47667	4-Jun-1988	36.44	-78.83	15
LK1485	North Carolina	Pitt	DUKE	Wilbur 60187	24-Apr-1992	35.68	-77.49	15
LK1486	North Carolina	Gates	DUKE	Wilbur 62285	25-May-1994	36.45	-76.61	15
LK1487	North Carolina	Edgecombe	DUKE	Wilbur 67810	15-Jul-1997	35.93	-77.61	15
LK1488	North Carolina	Duplin	DUKE	Wilbur 55073	24-May-1990	34.94	-78.07	15
LK1489	North Carolina	Brunswick	DUKE	Wilbur 63974	11-May-1995	34.27	-78.11	15
LK1490	North Carolina	Bladen	DUKE	Wilbur 58427	25-Apr-1991	34.82	-78.56	15
LK1491	South Carolina	Dorchester	DUKE	Wilbur 75163	23-Apr-2002	33.17	-80.53	13
LK1492	South Carolina	Farifield	DUKE	Nelson 5361	24-Apr-1987	34.29	-81.08	15
LK1495	South Carolina	Georgetown	DUKE	Wilbur 77129	22-Apr-2004	33.55	-79.40	15
LK1496	South Carolina	Williamsburg	DUKE	Wilbur 77167	22-Apr-2004	33.48	-79.89	14
LK1497	Delaware	Sussex	DOV	Tucker s.n.	6-Jul-1984	38.62	-75.65	15
LK1498	Delaware	New Castle	DOV	Baereurodt s.n.	18-May-1937	39.69	-75.75	9
LK1499	Delaware	Kent	DOV	McAvoy 4841	12-Jul-2000	38.99	-75.48	15
LK1500	Rhode Island	Newport	APSC	Laferriere 3691	11-Jun-2003	41.54	-71.27	15

Appendix 2. Specimen information for 89 *P. alabamensis* and *P. serotina* subsp. *serotina* samples

Extraction	Taxon	State	County	Herbarium	Collector	Date	Latitude	Longitude
JB1973	<i>P. alabamensis</i>	Alabama	Cherokee	MO	McVaugh 8969	30-May-48	34.03	-85.67
JB2094	<i>P. alabamensis</i>	Alabama	Clay	VDB	Haynes 9069	30-Apr-87	33.35	-85.92
JB1974	<i>P. alabamensis</i>	Alabama	Cleburne	MO	McVaugh 8953	25-May-48	33.49	-85.81
LK1503	<i>P. alabamensis</i>	Alabama	Coosa	AUA	Schotz 1781	7-May-00	33.05	-86.37
LK1504	<i>P. alabamensis</i>	Alabama	Crenshaw	AUA	Diamond 10375	5-Jul-96	31.67	-86.34
LK1332	<i>P. alabamensis</i>	Alabama	Dale	US	Biltmore Herbarium 9282K	2-Jun-02	31.46	-85.68
JB1972	<i>P. alabamensis</i>	Alabama	Pike	MO	Kral 33152	10-Sep-68	31.84	-86.01
JB1976	<i>P. alabamensis</i>	Alabama	Shelby	MO	Kral 53151B	29-May-74	33.33	-86.76
LK1511	<i>P. alabamensis</i>	Alabama	St. Clair	TROY	Kral 64904	3-May-80	33.64	-86.36
JB2095	<i>P. alabamensis</i>	Alabama	Talladega	VDB	Kral 53104	28-May-74	33.49	-85.81
LK1288	<i>P. alabamensis</i>	Florida	Calhoun	FLAS	Godfrey 80618	May-83	30.44	-85.20
JB2096	<i>P. alabamensis</i>	Florida	Liberty	VDB	Godfrey 84093	9-Apr-91	30.42	-84.84
LK1289	<i>P. alabamensis</i>	Florida	Liberty	FLAS	Gholson 9695	May-82	30.54	-84.97
LK1291	<i>P. alabamensis</i>	Florida	Liberty	FLAS	Gholson 9513	Mar-82	30.28	-84.97
LK1287	<i>P. alabamensis</i>	Florida	Walton	FLAS	Gholson 12109	Apr-89	30.78	-86.12
JB2097	<i>P. alabamensis</i>	Georgia	Floyd	VDB	McVaugh 8968	28-May-48	34.13	-85.22
JB2098	<i>P. alabamensis</i>	Georgia	Floyd	VDB	Lipps s.n.	17-May-67	34.23	-85.24
JB2099	<i>P. alabamensis</i>	Georgia	Floyd	VDB	Kral 72490	16-May-85	34.26	-85.41
JB1979	<i>P. alabamensis</i>	Georgia	Harris	MO	McVaugh 8945	22-May-48	32.84	-84.75
LK1292	<i>P. alabamensis</i>	Georgia	Marion	FLAS	Gholson 8466	Sep-80	32.39	-84.54
LK1286	<i>P. alabamensis</i>	Georgia	Meriwether	FLAS	Jones 22005	Jun-72	32.85	-84.58
JB1978	<i>P. alabamensis</i>	Georgia	Pike	MO	McVaugh 8938	21-May-48	32.99	-84.35
LK1331	<i>P. alabamensis</i>	Georgia	Pulaski	US	Biltmore Herbarium 6038a	2-Aug-01	32.29	-83.47
LK1501	<i>P. alabamensis</i>	Georgia	Rockdale	TTRS	Godfrey 05023	14-Sep-80	33.67	-84.11
JB2102	<i>P. alabamensis</i>	Georgia	Taylor	VDB	McVaugh 8941	22-May-48	32.90	-84.47
LK1333	<i>P. alabamensis</i>	Georgia	Upson	US	Harper 1267	29-Aug-01	32.90	-84.50
LK1506	<i>P. alabamensis</i>	South Carolina	Lexington	CLEMS	B.J. Disney 104	12-Oct-84	33.86	-81.21
LK1505	<i>P. alabamensis</i>	South Carolina	Richland	CLEMS	Townsend 1212	5-Sep-96	34.02	-80.94
LK1507	<i>P. alabamensis</i>	South Carolina	Richland	CLEMS	J.F. Townsend 1296	20-Sep-66	34.04	-80.92
LK1508	<i>P. alabamensis</i>	South Carolina	Richland	CLEMS	R.B. Efrid E-35	4-Oct-85	34.00	-81.01
JB2103	<i>P. serotina serotina</i>	Alabama	Autauga	VDB	Haynes 9220	9-Jul-87	32.69	-86.73
JB2104	<i>P. serotina serotina</i>	Alabama	Calhoun	VDB	Hruska 829	25-Jul-95	33.59	-85.88
JB1980	<i>P. serotina serotina</i>	Alabama	Cherokee	MO	Kral 59974	10-May-77	34.39	-85.63
LK1465	<i>P. serotina serotina</i>	Alabama	Choctaw	APSC	Harrell 114	23-Mar-07	31.84	-88.16
JB2105	<i>P. serotina serotina</i>	Alabama	Coffee	VDB	Kral 89583	8-May-00	31.30	-86.14
JB2106	<i>P. serotina serotina</i>	Alabama	Covington	VDB	Diamond 13015	6-Apr-02	31.44	-86.26
JB1981	<i>P. serotina serotina</i>	Alabama	Crenshaw	MO	Kral 39467	1-Jun-70	31.72	-86.26
JB1982	<i>P. serotina serotina</i>	Alabama	Dale	MO	MacDonald 10239	8-Apr-97	31.27	-85.62
JB2107	<i>P. serotina serotina</i>	Alabama	De Kalb	VDB	Spaulding 5014	11-Jul-93	34.46	-86.05
JB2108	<i>P. serotina serotina</i>	Alabama	Etowah	VDB	Kral 92815	3-Jul-02	34.10	-85.99
JB2109	<i>P. serotina serotina</i>	Alabama	Franklin	VDB	Kral 26304	6-May-66	34.37	-87.75
JB2110	<i>P. serotina serotina</i>	Alabama	Henry	VDB	Diamond 19107	1-May-08	31.55	-85.36
JB2111	<i>P. serotina serotina</i>	Alabama	Houston	VDB	MacDonald 7638	10-Sep-94	31.24	-85.51
JB2113	<i>P. serotina serotina</i>	Alabama	Jefferson	VDB	Williams 66	13-Apr-63	33.42	-86.73
JB2114	<i>P. serotina serotina</i>	Alabama	Lee	VDB	Kral 30769	9-May-68	32.52	-85.25
JB2115	<i>P. serotina serotina</i>	Alabama	Lowndes	VDB	Diamond 12961	17-Mar-02	32.33	-86.51
JB2116	<i>P. serotina serotina</i>	Alabama	Macon	VDB	Diamond 13009	1-Apr-02	32.40	-85.99
JB2118	<i>P. serotina serotina</i>	Alabama	Perry	VDB	Kral 66736	28-Mar-81	32.78	-87.28
JB2119	<i>P. serotina serotina</i>	Alabama	Pickens	VDB	Kral 45265	28-Mar-72	33.11	-87.92

JB2120	<i>P. serotina serotina</i>	Alabama	Pike	VDB	Diamond 13803	23-Mar-03	31.91	-86.06
JB2121	<i>P. serotina serotina</i>	Alabama	Russell	VDB	Diamond 16283	24-Apr-06	32.34	-85.41
JB2122	<i>P. serotina serotina</i>	Alabama	St Clair	VDB	Keener 4419	11-May-08	33.95	-86.28
JB2123	<i>P. serotina serotina</i>	Alabama	Sumter	VDB	Jones 15560	9-Jun-68	32.57	-88.30
JB1984	<i>P. serotina serotina</i>	Alabama	Talladega	MO	Kral 46062	20-Apr-72	33.14	-86.25
JB2124	<i>P. serotina serotina</i>	Alabama	Washington	VDB	Kral 37349B	22-Sep-69	31.20	-88.01
JB2125	<i>P. serotina serotina</i>	Alabama	Winston	VDB	Kral 23734	20-Apr-65	34.11	-87.62
JH283	<i>P. serotina serotina</i>	Florida	Alachua	FLAS	Lange 1396	12-Feb	29.65	-82.35
JH282	<i>P. serotina serotina</i>	Florida	Clay	FLAS	Ferguson 62	Jan-96	29.78	-82.01
JH281	<i>P. serotina serotina</i>	Florida	Columbia	FLAS	Tan 411	Mar-90	29.91	-82.58
LK1089	<i>P. serotina serotina</i>	Florida	Gilchrist	NYBG	Longbottom 18664	2-Mar-13	29.82	-82.69
JB1988	<i>P. serotina serotina</i>	Florida	Jackson	MO	Hess 8496	27-Apr-99	30.75	-84.92
JH280	<i>P. serotina serotina</i>	Florida	Levy	FLAS	Golledge 525	1998	29.50	-82.97
JH287	<i>P. serotina serotina</i>	Florida	Liberty	FLAS	Sloan 1612	Apr-85	30.55	-84.94
JH266	<i>P. serotina serotina</i>	Florida	Nassau	FLAS	Rider 180	Mar-00	30.77	-81.73
JB1989	<i>P. serotina serotina</i>	Florida	Okaloosa	MO	Miller 9482	3-Jun-98	30.57	-86.55
JH285	<i>P. serotina serotina</i>	Florida	Suwanee	FLAS	Herring 291	Jun-91	29.95	-82.79
LK1283	<i>P. serotina serotina</i>	Florida	Suwanee	FLAS	Abbott 22568	4-Jun-07	30.04	-83.01
JH284	<i>P. serotina serotina</i>	Florida	Walton	FLAS	Perkins 16401	Mar-77	30.46	-81.42
JB1990	<i>P. serotina serotina</i>	Georgia	Brooks	MO	McCarty s.n.	17-May-73	30.79	-83.71
LK1211	<i>P. serotina serotina</i>	Georgia	Bulloch	USF	Hill 27643	9-Apr-96	32.40	-81.76
LK1265	<i>P. serotina serotina</i>	Georgia	De Kalb	NYBG	Small s.n.	May 1895	33.81	-84.15
LK1341	<i>P. serotina serotina</i>	Georgia	McDuffie	US	Bartlett 2593	7-Jun-11	33.48	-82.52
JB1991	<i>P. serotina serotina</i>	Georgia	Meriwether	MO	McVaugh 8942	22-May-48	32.94	-84.58
JB1992	<i>P. serotina serotina</i>	Georgia	Stephens	MO	Spongberg 1789	14-May-82	34.63	-83.32
LK1239	<i>P. serotina serotina</i>	Mississippi	Chickasaw	MISS	Pullen 66888	20-Jul-66	34.04	-88.97
LK1242	<i>P. serotina serotina</i>	Mississippi	George	MISS	Jones 6993	21-Jun-66	30.96	-88.61
LK1246	<i>P. serotina serotina</i>	Mississippi	Lauderdale	MISS	Jones 7072	23-Jun-66	32.34	-88.71
LK1250	<i>P. serotina serotina</i>	South Carolina	Abbeville	NYBG	Credle 1164	1-Aug-79	34.18	-82.69
LK1254	<i>P. serotina serotina</i>	South Carolina	Aiken	NYBG	Hill 22373	23-Jun-91	33.60	-81.84
LK1311	<i>P. serotina serotina</i>	South Carolina	Allendale	NCU	Ahles 10667	6-Apr-56	32.83	-81.37
LK1313	<i>P. serotina serotina</i>	South Carolina	Clarendon	NCU	Radford 21114	20-Apr-57	33.84	-80.03
LK1491	<i>P. serotina serotina</i>	South Carolina	Dorchester	DUKE	Wilbur 75163	23-Apr-02	33.17	-80.53
LK1492	<i>P. serotina serotina</i>	South Carolina	Farfield	DUKE	Nelson 5361	24-Apr-87	34.29	-81.08
LK1315	<i>P. serotina serotina</i>	South Carolina	Lancaster	NCU	Ahles 27470	6-Jun-57	34.77	-80.52
LK1316	<i>P. serotina serotina</i>	South Carolina	Marlboro	NCU	Radford 9291	2-Apr-56	34.58	-79.76
LK1467	<i>P. serotina serotina</i>	South Carolina	Orangeburg	DUKE	Wilbur 73355	21-May-01	33.43	-80.36
JH273	<i>P. serotina serotina</i>	South Carolina	Richland	FLAS	Nelson 514	Mar-76	34.04	-80.98
LK1253	<i>P. serotina serotina</i>	South Carolina	Richland	NYBG	Nelson 17928	17-Mar-97	34.00	-81.40
LK1496	<i>P. serotina serotina</i>	South Carolina	Williamsburg	DUKE	Wilbur 77167	22-Apr-04	33.48	-79.89