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# Tallapoosa Cane (*Arundinaria alabamensis*), a new species of temperate bamboo (Poaceae: Bambusoideae) from East Central Alabama

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## Abstract

***Arundinaria alabamensis*** (Tallapoosa Cane) is described as a new species of North American temperate woody bamboo. Recognition of this species is consistent with molecular genetic data that suggests an origin through hybridization and subsequent diversification, as it bears a unique chloroplast DNA haplotype most similar to *A. gigantea* (River Cane), nuclear DNA haplotypes that cluster with *A. appalachiana* (Hill Cane) and *A. tecta* (Switch Cane), and a distinct AFLP fingerprint. Tallapoosa Cane can be distinguished from the other North American *Arundinaria* by a combination of vegetative characters including larger leaf size, foliage sheath pubescence, and a distinctive branch complement. This Alabama endemic is currently known from eight populations across four counties: Cleburne (3), Lee (3), Macon (1), and Randolph (1). The new species is described and compared with the related species of *Arundinaria*, and an identification key is included along with a comparative table based on morphological characters.

**Keywords:** plant diversity, Southeastern United States, molecular phylogeny, hybridization

## Introduction

The cane bamboos (*Arundinaria* Michaux (1803: 73)) are north temperate woody grasses (Poaceae: Bambusoideae) endemic to the eastern United States (Ohrnberger 1999; McClure 1973; Li 1997; Judziewicz *et al.* 1999). *Arundinaria* represent the only bamboos native to North America and the only temperate bamboos (the North Temperate clade of Clark *et al.* 2007) native to the New World (Ohrnberger 1999; Wu *et al.* 2006; Sungkaew *et al.* 2009; Triplett and Clark 2010; Bamboo Phylogeny Group [BPG] 2012; Kellogg 2015; Vorontsova *et al.* 2016; Clark and Oliveira 2018). The group currently consists of three species: *A. appalachiana* Triplett, Weakley & L.G. Clark (2006: 88; Hill Cane), *A. gigantea* (Walter 1788: 81) Muhlenberg (1813: 14; River Cane or Giant Cane), and *A. tecta* (Walter 1788: 81) Muhlenberg (1813: 14; Switch Cane). River Cane and Switch Cane are sometimes recognized as subspecies of *A. gigantea*, although phylogenetic evidence demonstrates *A. gigantea* and *A. tecta* are as divergent from each other as either is from East Asian species in the *Arundinaria* clade (*Sasa* Makino & Shibata (1901: 18), *Sasamorpha* Nakai (1931: 180), and *Pleioblastus* Nakai (1925: 145); Triplett and Clark 2010; Burke *et al.* 2014). Divergence estimates for *Arundinaria* range from 3.6 to 4.9 mya, with post-dispersal evolution in North America including divergence events around 2.3 to 3.2 mya at the boundary of the Pliocene and Pleistocene (Burke *et al.* 2014). Estimates of the divergence of *A. appalachiana* and *A. tecta* from their ancestral lineage are more recent, ranging from 0.57 to 0.82 mya (Burke *et al.* 2014). Hybrids are known to occur amongst North American species of *Arundinaria*, and at least one author has recommended the name *A. gigantea* subspecies *macrosperma* (Michaux) McClure (1973: 28) for putative hybrids (McClure 1973); more recently, *A. macrosperma* Michx. was recognized as a synonym for *A. gigantea* (Triplett and Clark 2009). Based on intermediate morphotypes, bamboo expert Floyd McClure hypothesized that hybrids may be more common than pure lineages (McClure 1973), and evidence suggests that in some parts of the southeast, this is the case. For example, whereas pure stands of *A. tecta* occur in coastal populations, inland populations commonly identified as *A. tecta* were revealed to be F1-like hybrids, bearing cpDNA haplotypes from either *A. tecta* or *A. gigantea,* and equal AFLP contributions from both parental species (Triplett *et al.* 2010). As a point of comparison, natural hybridization is common among East Asian temperate bamboos, where intergeneric crosses have produced a diversity of lineages including *Sasaella* Makino (1929: 15), *Semiarundinaria* Makino ex Nakai (1925: 150), *Brachystachyum* Keng (1940: 151), and *Pseudosasa* Makino ex Nakai (1925: 150), among others (Triplett and Clark 2021).

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Thus, our current understanding of *Arundinaria* is based on field work complemented by molecular studies in the broader context of bamboo taxonomy (Judziewicz *et al.* 1999; Triplett *et al.* 2006; Triplett and Clark 2009; Triplett *et al.* 2014; Triplett and Clark 2021). Phylogenetic analysis of amplified fragment length polymorphism (AFLP) data (Triplett *et al.* 2010) demonstrated that species of *Arundinaria* represent distinct lineages with a sister relationship between *A. appalachiana* and *A. tecta* (the Switch Cane clade) and a relatively distant relationship between those two species and *A. gigantea*. That study revealed cpDNA haplotypes associated with *A. gigantea* (G.2, G.3, G.4) and *A. tecta/A. appalachiana* (A.1–A.5). The study also uncovered an Alabama population with a unique cpDNA haplotype (G.1), unusual morphological features (larger foliage leaves, densely long-pubescent foliage, brittle canes), and an AFLP fingerprint most similar to *A. tecta* and *A. appalachiana*. In that study, the Alabama specimen was characterized as putative hybrid (Triplett *et al.* 2010), along with other hybrids that were genetically (AFLP) and morphologically intermediate between *A. tecta* and *A. gigantea*, but had cpDNA haplotypes matching one or the other species. Subsequent fieldwork uncovered additional populations of the distinctive large-leaved "G.1" form of *Arundinaria*. In the current study, molecular and morphological analyses were used to test the hypothesis that plants from East Central Alabama are a phylogenetically cohesive unit.

## Materials & methods

**Taxon sampling:—**Field studies were conducted in 2010, 2012, 2013, 2018, 2019, and 2021. Standard bamboo collection procedures were followed (Soderstrom and Young 1983); bulky specimens of rhizomes, branch complements, and culm nodes and internodes were made for all collections.

For molecular studies, a total of 140 individuals representing 34 natural populations was sampled, including 8 populations of the large-leaved Alabama morphotype (hereafter referred to as Tallapoosa Cane or *Arundinaria alabamensis*), 6 populations of Hill Cane (*A. appalachiana*), 11 populations of River Cane (*A. gigantea*), 8 populations of Switch Cane (*A. tecta*), and one individual of *Sasamorpha borealis* (Hackel) Nakai (1931: 181) as an outgroup for DNA sequence analyses (Fig. 1, Tables 1–2). *Arundinaria* species were identified according to the key in the Flora of the Southeastern United States (Weakley 2022). Leaf tissue was collected in the field and lyophilized using silica gel (Chase and Hills 1991). Total genomic DNA was extracted from silica gel-dried specimens using DNeasy Plant Mini Kit (Qiagen, Valencia, California, USA) with the following protocol modifications: dry tissue mass was increased to 40 mg; lysis buffer (AP1) was increased to 500 µl; lysis time was increased to 30 min; and precipitated DNA was washed twice with 500 µl of ice cold 100% EtOH followed by 2 min spin to dry. Extracted DNA was eluted in water and stored at -20ºC. Nucleic acid quality was measured using a Nanodrop ND-1000 spectrophotometer (Thermo Fisher Scientific, Waltham, Massachusetts, USA), and concentrations were standardized to 250 ng/µl for AFLP enzyme digestions and 100 ng/µl for cpDNA PCR amplification by dilution with nuclease-free water.

Specimens were measured for a variety of morphological characters, including culm leaf length and width, foliage leaf length and width, and fimbriae length. Top knot (the cluster of leaves at the tip of new shoots) and foliage leaf blade lengths were measured from the base of the pseudopetiole to the tip of the blade. Leaf blade width was measured at the widest point. Primary branch length was measured from the point of origin at the node to the end of the branch axis.

**AFLP analysis:—**AFLP protocols followed Vos *et al.* (1995) with modifications for bamboos. DNA was digested with restriction enzymes EcoRI (10 units, New England Biolabs) and MseI (10 units, New England Biolabs) for 2 hours at 37ºC in a 20 µl volume, followed by ligation (20 units T4 DNA ligase [New England Biolabs], overnight at 16ºC) to double-stranded EcoRI and MseI adapters. Two rounds of PCR amplification followed. First, a preselective (+1) amplification was performed using primers MseI +C and EcoRI +A in a 50 µl reaction volume, with 10 µl of undiluted template. Second, the resulting +1 product was diluted 3-fold with water, and a selective (+3) amplification was performed using one MseI + 3 primer and two fluorescently labeled EcoRI +3 primers. Four primer combinations were chosen for this study based on previous research in this group (Triplett *et al.* 2010). FAM and HEX labeled +3 EcoRI primers were multiplexed in the following combinations: [set 1] mCAA, eACA (FAM), eAAC (HEX) and [set 2] mCAC, eAGC (FAM), eACG (HEX). Selective amplification products were separated electrophoretically at the Iowa State University DNA Sequencing Facility on a Perkin-Elmer 3100 capillary fragment analyzer (Applied Biosystems, Foster City, California, USA) with an internal standard (GeneScan 500 Rox, ABI).

**TABLE 1.** Population locality, cpDNA haplotypes, and vouchers. Vouchers used in nDNA analyses are noted by gene names in parentheses.

| Population | Lat/Lon | Elev. (m) | cpDNA | Voucher |
|---|---|---|---|---|
| *A. alabamensis* | | | | |
| Heflin, Cleburne Co., Al. | 33.645833, -85.629444 | 314 | G.1 | Triplett *et al*. 130621-2 |
| Chulafinnee, Hollis Crossroads, Cleburne Co., Al. | 33.544262, -85.648424 | 267 | G.1 | Triplett 180713-6 |
| Camp Sequoyah, Cleburne Co., Al. | 33.526317, -85.659330 | 280 | G.1 | Triplett 120627-1 |
| Wedowee, Randolph Co., Al. | 33.25307, -85.45384 | 326 | G.1 | Triplett & Jamison 120224-2 |
| Tsinia Wildlife Viewing Area, Macon Co., Al. | 32.4393, -85.65472 | 81 | G.1, G.2 | Triplett & Jamison 120224-3 |
| Chewacla Creek, Lee Co., Al. | 32.5451, -85.3885 | 175 | G.1 | Triplett & Ozaki 97 (*gpa1, pabp1, pvcel1*) |
| | | | | Triplett & Barger 180713-1 |
| Lochapoca Rd., Lee Co., Al. | 32.598765, -85.546022 | 203 | G.1 | Triplett & Barger 180713-4 |
| Moores Mill Rd., Lee Co., Al. | 32.574001, -85.419279 | 168 | G.1 | Triplett & Barger 180713-2 |
| *A. appalachiana* | | | | |
| Lost Falls Trail, DeSoto State Park, DeKalb Co., Al. | 34.4975, -85.631111 | 494 | AT.2 | Gregg *et al*. 130510-1 |
| Laurel Creek, DeSoto State Park, DeKalb Co., Al. | 34.502222, -85.631944 | 521 | AT.2 | Gregg *et al*. 130510-2 |
| West Fork, DeSoto State Park, DeKalb Co., Al. | 34.501667, -85.615 | 436 | AT.2 | Gregg *et al*. 130510-3 |
| Scout Trail, DeSoto State Park, DeKalb Co., Al. | 34.501012, -85.618650 | 448 | AT.2, AT.3, AT.4 | Triplett *et al*. 130424-1 |
| Lost Falls Trail, DeSoto State Park, DeKalb Co., Al. | 34.5005, -85.6352 | 521 | AT.2 | Triplett & Ozaki 99 (*pabp1, pvcel1*) |
| Firetower Road, Spring City, Rhea Co., TN | 35.7418, -84.8392 | 457 | AT.2 | Triplett 188 (*gpa1, pvcel1*) |
| *A. gigantea* | | | | |
| U.S. Route 72 Pull-Off, Jackson Co., Al. | 34.926389, -85.771389 | 186 | G.4 | Triplett *et al*. 130517-3 |
| Rockhouse Road, Limestone Co., Al. | 34.563056, -86.846389 | 171 | G.2 | Triplett *et al*. 130607-3 |
| Little River Canyon Mouth Park, Cherokee Co., Al. | 34.291944, -85.688333 | 183 | G.2 | Triplett *et al*. 130524-3 |
| Clyde Hermon West Bridge, Cherokee Co., Al. | 34.1275, -85.529444 | 175 | G.2 | Triplett *et al*. 130524-2 |
| Terrapin Creek, Cherokee Co., Al. | 33.971389, -85.597778 | 198 | G.2 | Triplett *et al*. 130524-4 |
| Broadwell Mill, Calhoun Co., Al. | 33.805278, -85.793611 | 183 | G.2 | Triplett *et al*. 130612-2 |
| Shoal Creek, St. Clair Co., Al. | 33.801667, -86.117778 | 158 | G.2 | Triplett *et al*. 130612-1 |
| Heflin, Cleburne Co., Al. | 33.645833, -85.629444 | 296 | G.2 | Triplett *et al*. 130621-1 |
| Moores Mill Rd. Lee Co., Al. | 32.5669, -85.3772 | 165 | G.4 | Triplett & Ozaki 98 (*pvcel1*) |
| Maxwell Loop Road, Tuscaloosa Co., Al. | 33.1146, -87.596 | 61 | G.4 | Triplett & Ozaki 96 (*pvcel1*) |
| Martin Road, Craig, Switzerland Co., In. | 38.7679, -85.1451 | 223 | G.2 | Triplett 197 (*gpa1, pabp1, pvcel1*) |
| *A. tecta* | | | | |
| Dauphin Island, Mobile Co., Al. | 30.248174, -88.086603 | 12 | AT.2, AT.3, AT.4 | Triplett 130510-1 |
| Wayne Co., NC | 35.4195, -78.0506 | 34 | AT.2, AT.3 | Triplett & Clark 22 |
| Craven Co., NC | 35.1078, -77.0153 | 10 | AT.2, AT.4 | Triplett & Clark 23 |
| Craven Co., NC | 35.2603, -77.1011 | 12 | AT.2 | Triplett & Clark 24 (*gpa1, pabp1, pvcel1*) |
| Suffolk City/Great Dismal Swamp, VA | 36.599, -76.5282 | 20 | AT.2, AT.4 | Triplett & Clark 25 |
| Suffolk City/Great Dismal Swamp, VA | 36.6214, -76.5403 | 25 | AT.2 | Triplett & Clark 26 |
| Chatham Co., GA | 31.999, -81.2682 | 18 | AT.2, AT.4 | Triplett & Clark 27 |
| Hollow Creek Road, Salley, Aiken Co., SC | 33.646, -81.2143 | 80 | AT.4 | Triplett 173 (*pvcel1*) |
| *Sasamorpha borealis* (Outgroup) | | | | |
| Kawazu, Shizuoka Prefecture, Japan | 34.82381, 138.93787 | 646 | n/a | Triplett 294 (*gpa1, pabp1, pvcel1*) |

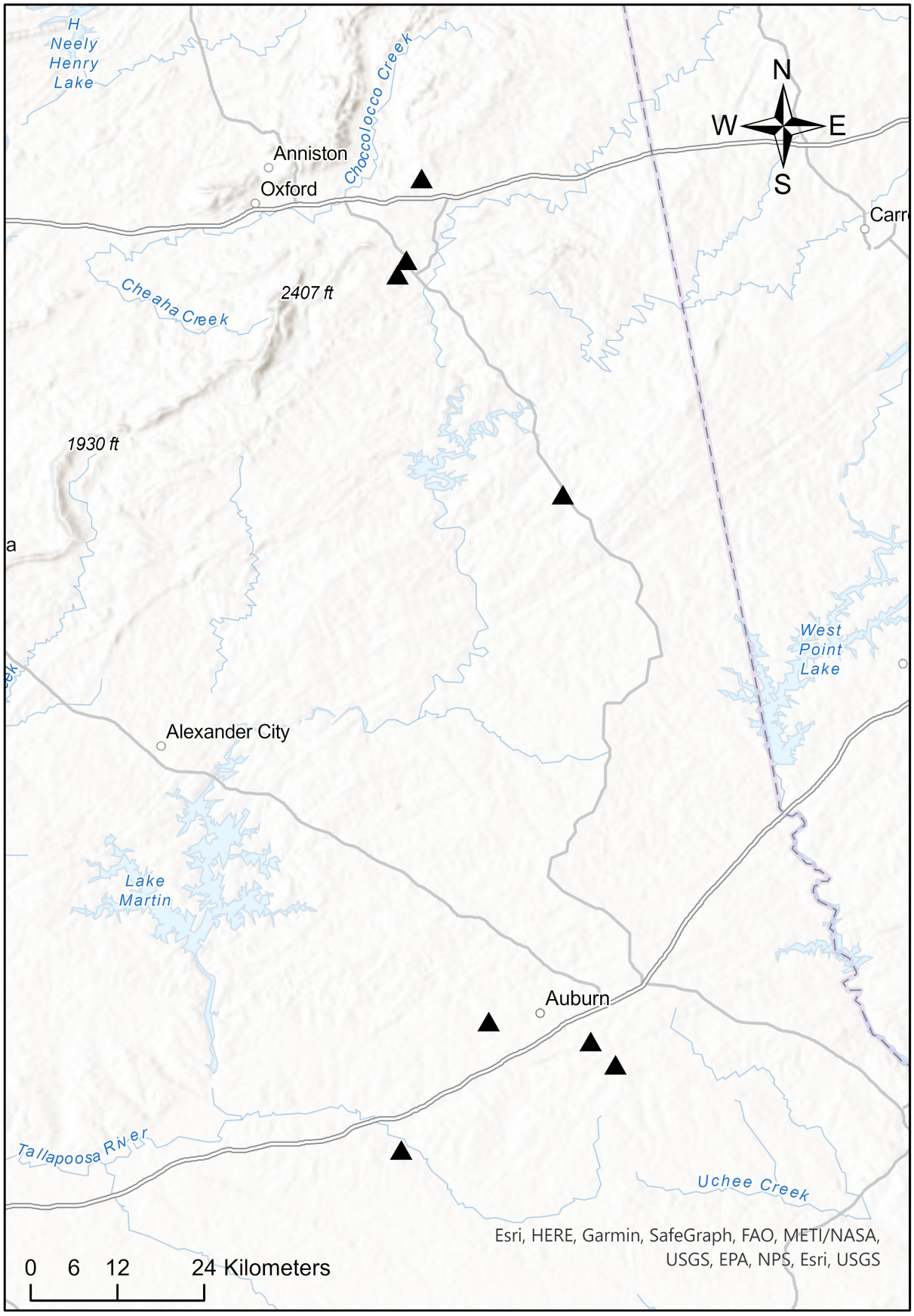


**FIGURE 1**. Known distribution of *Arundinaria alabamensis* in Eastern Alabama. Map by Ross H. Martin.

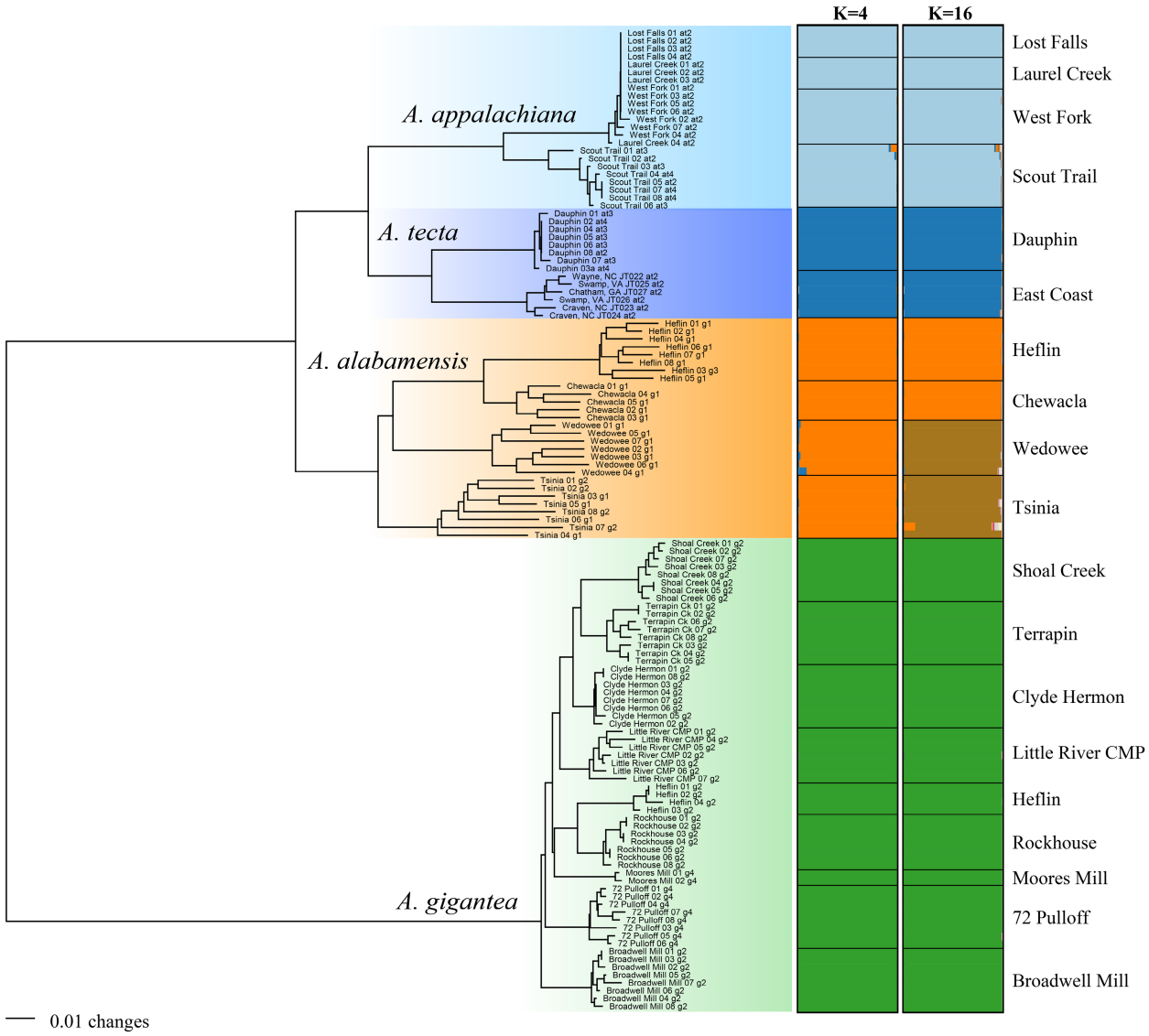


**FIGURE 2**. Results of the neighbor joining analysis of 288 AFLP loci, and the STRUCTURE analysis for K=4 and K=16. Chloroplast haplotypes (g1, g2, etc.) are indicated in the taxon labels. All branches receive 100% support in neighbor joining bootstrap analyses.

Data extraction was done manually from trace files using the GeneMarker (v2.4.0) software package (SoftGenetics, State College, Pennsylvania). AFLP bands were scored as present (1) or absent (0). Only robust, unambiguous DNA fragments ranging from 50 to 400 bp in size and above 200 relative fluorescent units were scored. Fragment data represent an anonymous sampling of the genome, and it is likely that some fragments of a given size represent different loci; however, the impact of homoplasy is assumed to be negligible when a strong enough phylogenetic signal is present in the data, indicating numerous independent loci supporting a given relationship. Nevertheless, a conservative approach to scoring and interpretation of AFLP data was used in order to minimize the potential problems associated with homoplasy. Bands were scored by hand using a reiterative approach to confirm that scored peaks were similar in trace size, shape, and intensity, and data were comparatively analyzed using distance, cladistic, and ordination methods to detect and evaluate all possible sources of signal conflict (Koopman *et al.* 2001; Lara-Cabrera & Spooner 2004). The resulting AFLP data matrix is available from the author on request.

Pairwise genetic distances were calculated in PAUP* v4b10 (Swofford 2003) using the Nei-Li dissimilarity coefficient (Nei & Li 1979). This algorithm is appropriate for dominantly-inherited AFLP markers because it gives greater weight to the information content of presence data and is less sensitive to the potentially homoplastic absence of bands (i.e., absence due to different mutations). Thus, it emphasizes the similarities between individuals rather than their dissimilarities. Phylogenetic relationships were reconstructed using neighbor-joining (NJ) analysis (Saitou & Nei 1987) as implemented in PAUP*, with ties broken randomly. The NJ tree was midpoint-rooted and statistical support was estimated based on 10 000 bootstrap replicates.

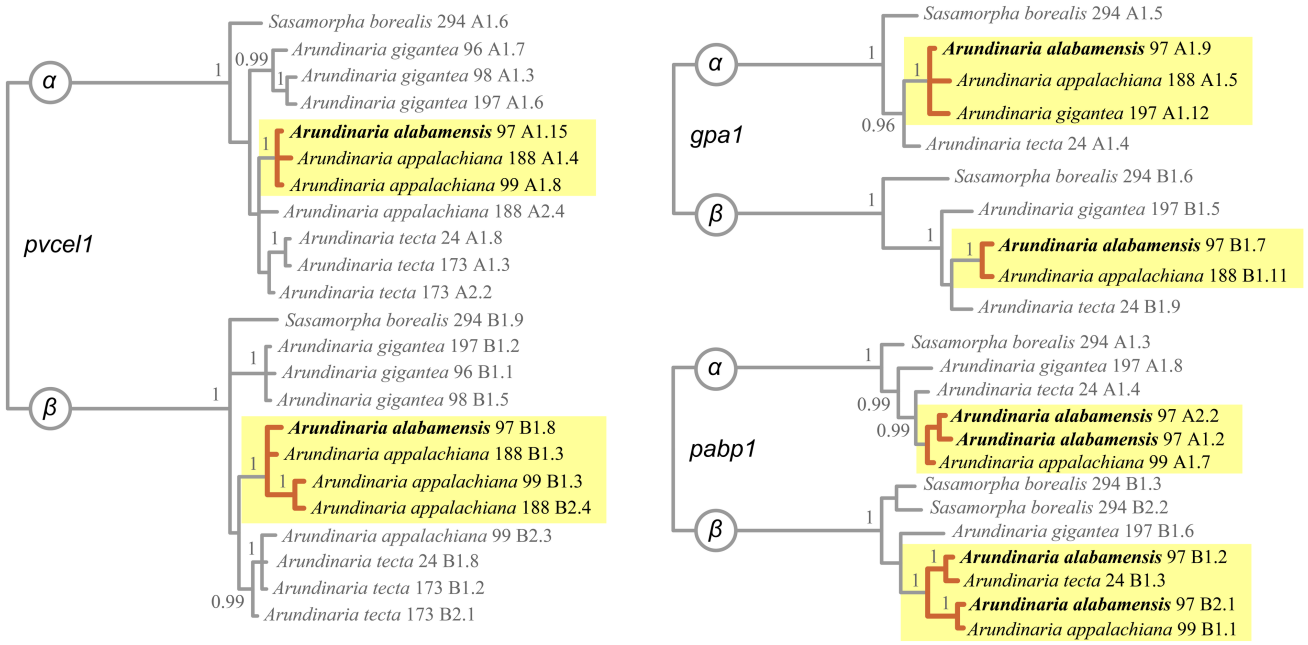


**FIGURE 3**. Results of the Bayesian analyses of nuclear loci, highlighting haplotypes that are the most similar to those recovered in *A. alabamensis*.

AFLP data were also analyzed using the program STRUCTURE 2.3.4, which assigns individuals to clusters based on their multilocus genotype using a Markov chain Monte Carlo (MCMC) algorithm (Pritchard *et al.* 2000; Falush *et al.* 2007) and provides a Bayesian method of calculating genome contributions that maximizes the likelihood of a given genotype of mixed ancestry. The program accommodates the genotypic uncertainty of dominant markers such as AFLPs (Falush *et al.* 2007). The analysis assumes that ancestral populations were at Hardy—Weinberg equilibrium and linkage equilibrium. Data were treated as haploid to relax the modeling assumption regarding statistical independence of alleles at a given locus within an individual. STRUCTURE was used to test whether the data would group *Arundinaria* individuals into clusters corresponding to species, populations, or other biologically meaningful groups. The program was run using no prior knowledge of sampling origin, under the Admixture model and correlated allele frequencies, with all other settings at their default values. The Admixture model assumes that the genome of an individual is a mixture of genes originating from K unknown ancestral populations. Under this model, the STRUCTURE algorithm estimates the proportion of genome ancestry of each individual from each of the K ancestral populations. Admixed individuals are indicated by having substantial proportion of their alleles from two or more clusters. Posterior probabilities were calculated for each value of K = 1 to 20, and the optimum K determined following Pritchard *et al.* (2000). Each value of K was evaluated using 10 independent MCMC replicates consisting of a burn-in of 50 000 iterations followed by a run of 250 000 iterations.

**cpDNA haplotype analysis:**—Based on a previous study of *Arundinaria* (Triplett *et al.* 2010), the *trnT-trnL* intergenic spacer region was used to provide diagnostic chloroplast DNA (cpDNA) haplotypes. A total of 134 new sequences were generated for this analysis (GenBank accession numbers: OQ468307-OQ468440). Amplification reactions for the *trnT-trnL* sequences (~800 bp) were conducted using the primers *trnT* F (TabA): 5′-CAT TAC AAA TGC GAT GCT CT-3′ and 5’ *trnL* R (Tab B): 5′-TCT ACC GAT TTC GCC ATA TC-3′ (Taberlet *et al.* 1991) with the following PCR parameters: 95ºC for 2 min; 35 (95ºC, 1 min; 48ºC, 10 sec; +17ºC, 0.3ºC/sec; 65ºC, 5 min); 65ºC, 5 min (40 µl reaction volumes). PCR products were sequenced directly in both directions at ETON Bioscience Inc., Research Triangle Park, NC, using Sanger sequencing. Strands were assembled, edited, and aligned manually using MEGA-X (Kumar *et al.* 2018), and sequences were compared to determine chloroplast haplotypes.

**nDNA haplotype analysis:**—Based on a previous study of bamboos (Triplett *et al.* 2014), three regions were used for an analysis of nuclear haplotypes and allelic variation: *cellulase1 (pvcel1)*, *G protein α subunit 1 (gpa1)*, and *poly-A binding protein1 (pabp1)*. The sampled regions are present as two copies (homoeologs) in the Temperate Bamboos (Triplett *et al.* 2014), designated as α (or A) and β (or B), arising from the ancestral allopolyploid origin of the temperate bamboos. A total of 27 new sequences were generated for this analysis (*gpa1*: GenBank OQ468286-OQ468289; *pabp1*: GenBank OQ468441-OQ468446; *pvcel1*: GenBank OQ468290-OQ468306) using primers and

cloning methods described in Triplett *et al.* 2014; these were combined with previously published sequences to build data sets for each region (Table 2).

**TABLE 2.** GenBank accession numbers for the nDNA haplotype analysis.

| Species | Voucher | *gpa1* | *pabp1* | *pvcel1* |
|---|---|---|---|---|
| *A. alabamensis* | Triplett & Ozaki 97 | OQ468288, OQ468289 | OQ468442, OQ468443, OQ468445, OQ468446 | OQ468295, OQ468304 |
| *A. appalachiana* | Triplett & Ozaki 99 | | OQ468441, OQ468444 | OQ468292, OQ468300, OQ468301 |
| | Triplett 188 | OQ468286, OQ468287 | | OQ468290, OQ468291, OQ468298, OQ468299 |
| *A. gigantea* | Triplett & Ozaki 96 | | | OQ468293, OQ468302 |
| | Triplett & Ozaki 98 | | | OQ468294, OQ468303 |
| | Triplett 197 | KM209184, KM209165 | KM209011, KM208993 | KM209119, KM209088 |
| *A. tecta* | Triplett & Clark 24 | KM209185, KM209166 | KM209012, KM208992 | KM209120, KM209083 |
| | Triplett 173 | | | OQ468296, OQ468297, OQ468305, OQ468306 |
| *Sasamorpha borealis* | Triplett 294 | KM209187, KM209167 | KM209030, KM208995, KM208996 | KM209105, KM209075 |

Each data set was analyzed using Bayesian inference (BI) with MrBayes 3.2 (Ronquist *et al.* 2012). BI analyses were conducted using a partitioned GTR + I + G model for reasons outlined by Huelsenbeck and Rannala (2004), with all parameter values estimated during analysis. A Dirichlet prior was used for base frequencies and the rate matrix. A uniform prior was used for the shape parameter (α), proportion of invariable sites (I), and topology. Branch lengths were unconstrained. Partitions were designated for each data set and for the microstructural characters and all parameters were unlinked across partitions. Four separate MCMC runs were initiated, each with 10,000,000 generations. Runs were started from a random tree; the topology was sampled every 1,000 generations of the MCMC chain. Majority rule (50%) consensus trees were constructed after removing the first 10% of sampled trees ("burn-in"). Branch support was assessed according to a 0.95 posterior probability measure for BI (Mason-Gamer and Kellogg 1996; Wilcox *et al.* 2002).

## Results

**AFLP markers:—**A total of 288 markers (AFLP loci) were scored for the 4 AFLP primer combinations used in this study. The average number of scored bands per primer pair was 72, with a range of 58 to 80. The fragments represent 4 size classes: 50–100 (84; 29.2%), 101–200 (113; 39.2%), 201–300 (71; 24.7%), and 301–400 (20; 6.9%). 214 (74.3%) of the sites were polymorphic, and the average number of fragments per individual was 170.

Among the 28 individuals of *A. alabamensis*, 104 (36.1%) of the markers were polymorphic. Among the 60 individuals of *A. gigantea*, 45 (15.6%) of the markers were polymorphic. Among the 23 individuals of *A. appalachiana*, 27 (9.4%) markers were polymorphic, while among the 14 individuals of *A. tecta*, 32 (11.1%) markers were polymorphic. A total of 15 bands were diagnostic for *A. alabamensis*, while 60 were diagnostic for *A. gigantea* (including those

unique to at least one of the sampled populations), and 11 for *A. appalachiana.* No diagnostic bands were recovered for *A. tecta*. Seventy-seven diagnostic bands distinguished *A. alabamensis* and the Switch Cane Clade (*A. appalachiana* and *A. tecta*) from *A. gigantea*, whereas four diagnostic bands distinguished *A. alabamensis* and *A. gigantea* from the Switch Cane Clade.

**TABLE 3.** Morphological comparisons of *Arundinaria alabamensis*, *A. appalachiana*, *A. tecta,* and *A. gigantea*.

| Character | *A. alabamensis* | *A. appalachiana* | *A. tecta* | *A. gigantea* |
|---|---|---|---|---|
| Rhizome air canals | present | present or absent | present | absent |
| Sulcus | usually absent | usually absent | usually absent | usually present |
| Culm leaf duration | persistent | persistent | persistent | deciduous |
| Culm leaf auricles | present, deciduous | absent | present, deciduous | present, deciduous |
| Top knot number of leaves | 5–7(–9) | 6–12 | 9–12 | 6–8 |
| Top knot leaf blade length (cm) | 9–37 | 9–22.5 | 20–30 | 16–24 |
| Compressed basal internodes on primary branch | 3–5 | 2–5 | 2–4 | 0–1 |
| 1º branch basal nodes: 2º branches | present, subequal | absent | present, subequal | present, subequal |
| Primary branch length (cm) | 12–49 | 7–33 | usually >50 | 15–25 |
| Foliage leaf blade length (cm) | 5–30 | 5–20 | 7–23 | 8–15 |
| Foliage leaf blade width (cm) | 0.8–3.4 | 0.8–2 | 1–2 | 0.8–1.3 |
| Foliage leaf blade vestiture | densely pubescent or glabrous | pilose or glabrous | densely pubescent or glabrous | densely pubescent or glabrous |
| Foliage leaf sheath vestiture | densely pilose | glabrous, pilose, or pubescent | glabrous (sometimes densely pubescent, esp. when young) | glabrous (sometimes densely pubescent, esp. when young) |
| Foliage leaf duration | evergreen | deciduous | evergreen | evergreen |
| Foliage leaf texture | chartaceous to subcoriaceous | chartaceous | coriaceous | subcoriaceous |
| Foliage leaf abaxial tessellation | tessellate | weakly tessellate | strongly tessellate | strongly tessellate |

Within *A. alabamensis*, pairwise Nei-Li distances ranged from 0.01736 to 0.18056 (18% difference between the most divergent individuals). In contrast, pairwise differences within *A. gigantea* (8.7%), *A. appalachiana* (8.0%), and *A. tecta* (10%) were lower. Similarly, average pairwise differences among individuals were higher in *A. alabamensis* (11.6%) than in *A. gigantea* (4.7%), *A. appalachiana* (3.6%), or *A. tecta* (5.0%).

Pairwise differences between *A. appalachiana* and *A. tecta* individuals ranged from 12.2% to 18.1%. In contrast, Nei-Li distances between *A. alabamensis* and *A. appalachiana* ranged from 18.8% to 26.4%, and between *A. alabamensis* and *A. tecta* ranged from 13.9% to 22.6%. Pairwise differences between *A. gigantea* and the switch cane clade (*A. appalachiana* and *A. tecta*) ranged from 40.6% to 47.9%; similarly, pairwise differences between *A. gigantea* and *A. alabamensis* individuals ranged from 40.6% to 50.3%.

Neighbor-joining (NJ) analysis of the Nei-Li distance matrix derived from the AFLP data produced a phylogram (Fig. 2) with clusters that reflected populations within species. STRUCTURE analysis of the AFLP data identified 16 clusters (K = 16) having a posterior probability of greater than 0.999 relative to other evaluated values of K (1–20). However, although 16 ancestral populations were identified, STRUCTURE primarily assigned individuals to one of 4 (or 5) ancestors, corresponding to the four species (or two ancestors for *A. alabamensis*, parsing Heflin + Chewacla populations as distinct from Wedowee + Tsinia; Fig. 2). Small proportions (< 5%) of the genotypes of some individuals were attributed to other sources (clusters) that were not readily interpretable. Similarly, at K = 4, the inferred ancestors

corresponded to the recognized species, including *A. alabamensis*. Other values of K > 4 converged on similar results, with ancestry mostly corresponding to species assignments.

**cpDNA haplotype assignment:—***Arundinaria trnT-trnL* sequences varied from 783 base pairs (bp) in *A. alabamensis* to 801 bp in *A. tecta*. Alignment among the 134 *Arundinaria* accessions required 28 gaps, and the aligned region was 809 bp long. A total of 9 variable sites (4 point mutations, 3 indels, 1 variable poly-A region, and 1 variable poly-G region) were identified that characterize six *Arundinaria* haplotypes in two classes, G and AT (Table 1, Fig. 2). These represent a subset of haplotypes found in the previous study of *Arundinaria* (Triplett *et al.* 2010), including one found exclusively in *A. alabamensis* (G.1), two associated with *A. gigantea* (G.2, G.4), and four associated with *A. appalachiana* and *A. tecta* (AT.2, AT.3, AT.4, AT.5). River cane haplotype G.2 was also recovered from 4 individuals of *A. alabamensis* (Tsinia population, Macon Co.), and the rare G.3 haplotype (recovered from a single population in Triplett *et al.* 2010) was recovered from a single individual of *A. alabamensis* (Heflin population, Cleburne Co.). None of the sampled River Cane populations were variable (i.e., G.2 and G.4 haplotypes did not co-occur within a single population). Three of the four hillcane populations were monomorphic (haplotype AT.2). However, three AT haplotypes (AT.2, AT.3, AT.4) were found in the hill cane population at DeSoto Scout trail. Similarly, these three haplotypes were found in the single population of switch cane in Southern Alabama (Dauphin Island, Mobile Co.). Although sample size was small, East Coast populations of *A. tecta* were found to be variable for AT haplotypes (AT.2, AT.3, AT.4, and AT.5). Chloroplast haplotypes are mapped on the NJ phylogram in Fig. 2.

**nDNA sequence variation and haplotype assignment:—***Gpa1* sequences varied from 1131 base pairs (bp) to 1179 bp. Alignment among the 10 accessions required 71 gaps, and the aligned region was 1194 bp long. For each of the sampled species, sequences representing homeologous copies α (or A) and β (or B) were recovered, and these form clades consistent with previous results (Triplett *et al.* 2014). Both of the homeologous sequences recovered from *Arundinaria alabamensis* clustered with *A. appalachiana* (plus *A. gigantea*, in the case of copy A). *Pabp1* sequences varied from 795 base pairs (bp) to 887 bp. Alignment among the 13 accessions required 123 gaps, and the aligned region was 888 bp long. For both homeologs (A and B), two haplotypes were recovered from *A. alabamensis*. Haplotypes A1.2 and A2.2 clustered with *A. appalachiana*. In the case of the B copy, one haplotype clustered with *A. appalachiana* and the other clustered with *A. tecta*. *Pvcel1* sequences varied from 930 base pairs (bp) to 941 bp. Alignment among the 23 accessions required 49 gaps, and the aligned region was 960 bp long. *PvCel1* homeologs recovered from *A. alabamensis* clustered with *A. appalachiana*. Of the 6 sampled loci, *A. gigantea* was homozygous at all 6 loci, whereas *A. alabamensis*, *A. appalachiana*, and *A. tecta* were each heterozygous at 2 out of 6 loci.

## Morphology

***Rhizomes***. The rhizomes of *A. alabamensis* are leptomorphic, a characteristic they share with other north temperate woody bamboos; however, in some cases the growing tips of new rhizomes travel only a short distance before turning up to form a new culm, thus presenting a sympodial branching pattern. This pattern also occurs in *A. appalachiana* and *A. tecta* but has not been observed in *A. gigantea*. Like *A. tecta*, this new species has air canals (McClure 1963).

***Culm internodes.*** Like *A. appalachiana* and *A. tecta,* the culm internodes of *A. alabamensis* lack a prominent groove (sulcus) and can be somewhat flattened behind the branch complement. This contrasts with *A. gigantea*, which typically has internodes that are prominently sulcate. A distinctive feature of the culms is that they are relatively brittle, especially in comparison with *A. gigantea*.

***Branching***. In bamboos, the morphology and architecture of the set of branches arising from culm nodes (the branch complement) is a source of numerous taxonomically useful characters. In *Arundinaria,* the pattern of shortened or compressed internodes at the base of primary branches and the extent and pattern of secondary branching are especially valuable. The branch complement of *A. alabamensis* most similar to *A. appalachiana*, with 3–5 shortened or compressed internodes at the base of the primary branch. The first elongated internode above the shortened ones is typically constrained to ~30% the length of distal internodes. Like *A. tecta*, *A. alabamensis* typically produces buds and branches from the nodes in the area of compression, creating subequal branches from the base of the primary branch. The first buds typically occur on nodes 4 and 5 (3–5). In contrast, *A. appalachiana*, while having a similar pattern of compressed internodes, lacks the rebranching in this basal area. *Arundinaria gigantea* typically has only one compressed basal internode (or none), but if present, this node may produce a secondary branch. Primary branches in *A. alabamensis* are 12–49 cm long ($\bar{x}$ = 24 cm). In contrast, *A. tecta* produces long primary branches usually >50 cm, and *A. appalachiana* has branches that are usually less than 35 cm long.

***Culm leaves.*** The culm leaves of *A. alabamensis* are typically shorter than their associated internodes at the base of the plant, becoming progressively longer towards the top knot. At midculm they are approximately the same length as the associated internode. In contrast, midculm culm leaves of *A. tecta* are longer than their associated internodes, and those of *A. gigantea* shorter. Like *Arundinaria appalachiana* and *A. tecta*, *A. alabamensis* has persistent culm leaf sheaths, whereas *A. gigantea* has deciduous sheaths. The culm leaf sheaths of *A. alabamensis* are tessellate; however, their tessellation is not as pronounced as it is in *A. tecta*. The culm leaves have well-developed, prominent auricles, unlike *A. appalachiana* but like *A. gigantea* and *A. tecta.*

***Top knot and foliage leaves***. In *Arundinaria*, leaves at the tip of new culms are crowded into a distinctive fan-shaped cluster or top knot, with their blades expanded as on foliage leaves. In mature stands, the top knot leaf blades of *A. alabamensis* are typically 9–37 cm in length, while those of *A. gigantea* are 16–24 cm, *A. appalachiana* 9–22.5 cm, and *A. tecta* 20–30 cm long. Foliage leaf blades of *A. alabamensis* are typically 5–30 cm in length (0.8–3.4 cm wide), while those of *A. gigantea* are 8–15 cm long (0.8–1.3 cm wide), *A. appalachiana* 5–20 cm long (0.8–2.0 cm wide), and *A. tecta* 7–23 cm long (1–2 cm wide). Thus, *A. alabamensis* is distinguished by having the largest leaves of the cane bamboos. Like *A. gigantea* and *A. tecta*, the foliage leaf blades of *A. alabamensis* are persistent. The blades are chartaceous (like those of *A. appalachiana*) to subcoriaceous; in contrast, the leaves of *A. gigantea* are subcoriaceous, while leaves of *A. tecta* are coriaceous. The abaxial surfaces of the leaf blades are tessellate as in *A. gigantea* and *A. tecta*, whereas *A. appalachiana* is weakly tessellate. Leaf blades of *A. alabamensis* are typically densely pubescent with short, soft hairs on abaxial surfaces, while the blades of *A. tecta* are densely pubescent on both surfaces. In contrast, *A. appalachiana* is typically sparsely to more or less densely pilose (or else glabrous). The foliage leaf sheaths of *A. alabamensis* are typically densely pilose, although this feature may be lost on individual culms that have persisted for one or more seasons. In contrast, foliage leaf sheaths of the other three species are typically glabrous, although sometimes pubescent to densely pubescent (especially on younger plants).

## Distribution and ecology

*Arundinaria alabamensis* is native to east central Alabama where it occurs primarily in the Piedmont Upland physiographic province (Fig. 1). The full extent of its distribution is unknown, but it appears to be the second most common species of *Arundinaria* in east central Alabama, after *A. gigantea*. Tallapoosa Cane occurs in oak-hickory forests and woodlands on mesic, submesic, and xeric slopes and uplands, sometimes occurring as well in hillside seepages, and sometimes along perennial streams. This contrasts with *A. gigantea*, which typically occurs on the floodplains of large to small rivers, sometimes edging onto lower portions of mesic slopes.

*Arundinaria alabamensis* is sympatric with *A. gigantea*, and populations of that species can be found in the same counties and, in some cases, within a few kilometers of populations of Tallapoosa Cane. In Alabama, *A. tecta* is only known from the coastal plain, south of Jefferson County, while *A. appalachiana* appears to be restricted to Dekalb, Jackson, and Marshall Counties in Northeastern Alabama. Additionally, putative hybrids of *Arundinaria* are found throughout Alabama.

*Arundinaria alabamensis* is not known in flower. *Arundinaria* species in general are long-lived monocarpic perennials, and this appears to be the case with *A. alabamensis*. Field observations suggest that *A. alabamensis* is slow-growing and long-lived. Its clones likely persist for decades if not centuries.

## Taxonomic treatment

### Key to the Species of *Arundinaria sensu stricto*

1. Primary branches with 0–1 compressed basal internodes; culm internodes usually sulcate; culm leaves deciduous ..... *A. gigantea*
1. Primary branches with 2–5 compressed basal internodes; culm internodes usually terete; culm leaves persistent to tardily deciduous.
2. Foliage blades coriaceous, persistent, abaxial surfaces densely pubescent or glabrous, strongly tessellate; primary branches usually more than 50 cm long ..... *A. tecta*
2. Foliage blades chartaceous to subcoriaceous, deciduous, abaxial surfaces glabrous or pubescent, weakly tessellate; primary branches usually less than 50 cm long.
3. Primary branch basal nodes not developing secondary branches; primary branches usually less than 35 cm long; foliage blade

abaxial surfaces sparsely pilose or glabrous; auricles absent; top knot blades 9–22.5 cm long ..... *A. appalachiana*

3. Primary branch basal nodes developing secondary branches; primary branches 12–49 cm long; foliage blade abaxial surfaces long pubescent; auricles present; top knot blades 9–37 cm long ..... *A. alabamensis*

***Arundinaria alabamensis*** Triplett, *spec. nov.* (Fig. 4–5). TYPE: UNITED STATES. ALABAMA: Lee Co., near Auburn; Mailpost 1541 on Co Rd. 112, near Chewacla Creek. Woodland, with sandy, loamy soil, 32.5451, -85.3885, elev. ca. 170 m, 24 Jul 2005, *Triplett & Ozaki 97* (Holotype: JSU; Isotypes: AUA, ISC, MO, UNA, US, UWAL). *Nomen vul.* Tallapoosa Cane, Alabama Cane, Brittle Cane.


**FIGURE 4**. Holotype of *Arundinaria alabamensis*. Photo by J.K. Triplett.

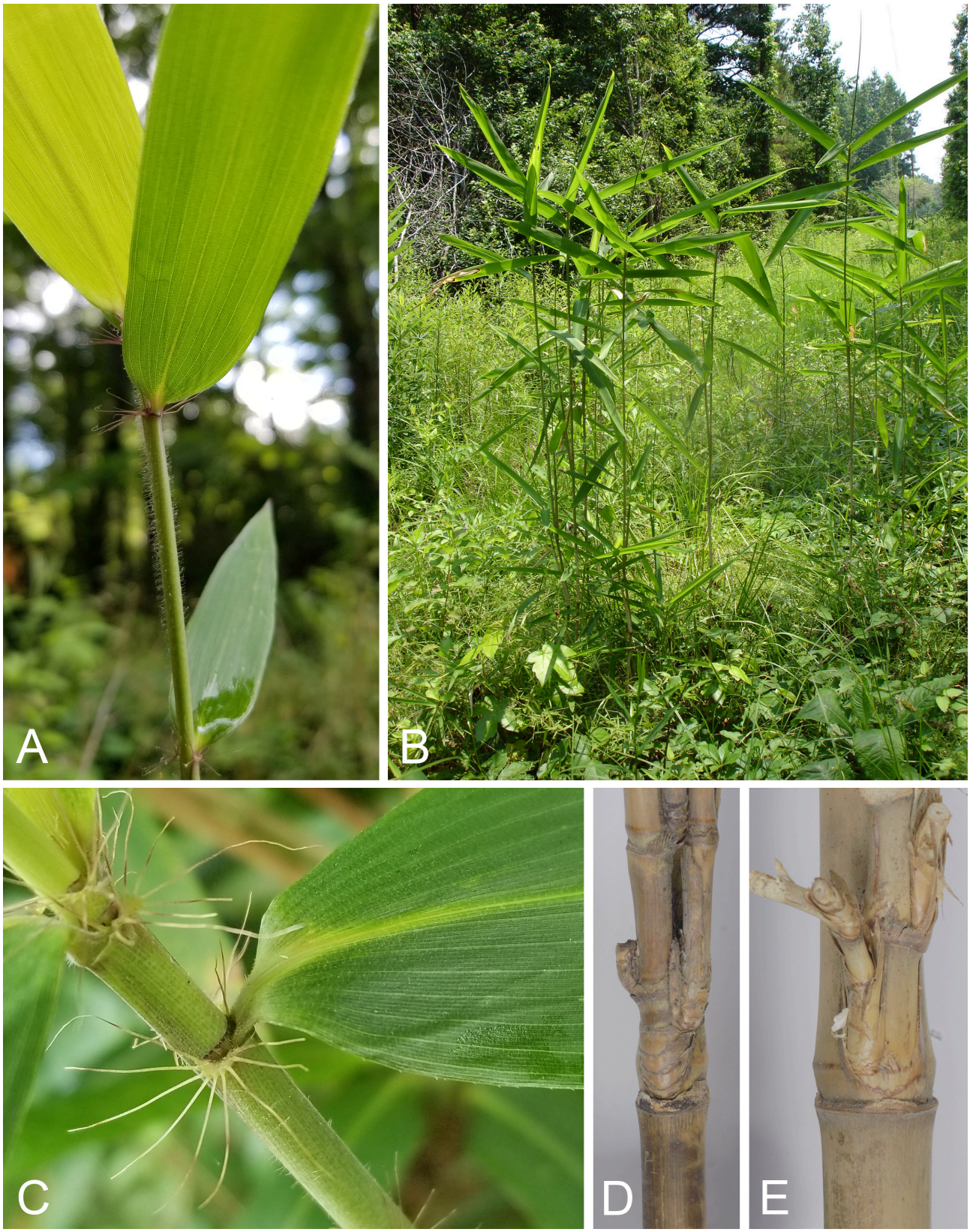


**FIGURE 5**. *Arundinaria alabamensis*. **A.** Region of top knot cluster showing leaf tessellation, fimbriae, and sheath pubescence. **B.** Habit, in Lee County, Alabama. **C.** Foliage leaves, highlighting auricles and fimbriae. **D.** Branch complement. **E.** *Arundinaria gigantea* branch complement, for comparison. (Photos by J.K. Triplett).

**Woody bamboo.** Plants of diffuse to (pluri-) caespitose habit. **Rhizomes** leptomorphic, usually horizontal for only short distance before turning up at the apex to form a culm (therefore often presenting a sympodial branching pattern), hollow (with a central lumen), peripheral air canals present. **Culms** 3–7 mm in diameter, 1.0–2.5 m tall, erect, tillering;

**internodes** 13–26 cm long (progressively shorter towards culm apex), terete, hollow, glabrous, flattened behind the branch complement on larger culms but the sulcus not prominent; **nodes** solitary, the nodal line horizontal, supranodal ridge not prominent; bud one per node (single) on a slight promontory, triangular, the shoulders of the prophyll ciliate. **Culm leaves** persistent, approximately equaling associated internodes at midculm, typically shorter than associated internodes at the culm base, becoming proportionally longer towards the culm apex; **sheaths** 10–19 cm long, shortest on lower nodes, becoming progressively longer towards the culm apex, densely pilose, margins ciliate; **blades** 2–14.5 cm long, triangular to linear-lanceolate, reflexed to erect, pilose, persistent (evergreen), intergrading into top knot leaves; **auricles** present, well-developed, deciduous; **fimbriae** 5–10 mm long, ascending to erect; **inner ligules** 4–7 mm long, a fringe of long cilia; **outer ligule** absent. **Top knot leaves** in a loose apical cluster of 5–7(–9); **sheaths** pilose (becoming glabrous with age), margins ciliate; **auricles** present; **fimbriae** 4–14 mm, ascending to erect; **blades** 9–37 cm long, 2–5.9 cm wide, L:W = 6.3–8.5, linear, linear-lanceolate or ovate-lanceolate, chartaceous to subcoriaceous, pubescent (hairs short or long), abaxially (weakly) tessellate, apices acuminate, bases attenuate to cuneate, midrib ± centric. **Branching** intravaginal (rarely extravaginal); **primary branches** 1 per node, 12–49 cm long, with (2–) 3 (–4) compressed basal internodes, basal nodes developing 1–2 secondary branches; first elongated internode shorter than subsequent ones (~30%); **higher order branches** present on older plants, reiterating the 1º branch (*i.e.*, with the same pattern of compressed basal internodes and branching). **Foliage leaves** 5–8 per complement; **sheaths** pilose (becoming glabrous with age), margins ciliate, (weakly) tessellate; **auricles** present; **fimbriae** 4–14 mm, ascending to erect; **inner ligule** glabrous or ciliate, fimbriate or lacerate; **outer ligule** present as a minute rim; **blades** linear, linear-lanceolate, or ovate-lanceolate, chartaceous to subcoriaceous, deciduous, surfaces pilose (sometimes glabrous), abaxially (weakly) tessellate, apices acuminate, bases attenuate to cuneate, midrib ± centric; primary branch foliage leaf blades 5–30 cm long, 0.8–3.4 cm wide; L:W = 8.7–11.4; higher order branch foliage leaf blades 5–28.5 cm long, 0.8–3.3 cm wide. **Flowers and Fruit** not seen.

*Distribution and Ecology:*—(Fig. 1) Endemic to the Piedmont Upload section of Eastern Alabama, United States, from the Central Uplands of the Northern Piedmont to the Greenville Slope and Pine Mountain terrane of the Southern Piedmont; 81 to 326 m. In upland oak-hickory-pine forests on slopes, less typically in more mesic sites, seeps, or along small streams.

*Phenology:*—Lack of specimens in flower or information on the extent of blooming makes it impossible to determine flowering behavior in this species at present.

*Etymology:*—*Arundinaria alabamensis* is named for its distribution in Alabama.

*Representative specimens examined:*—UNITED STATES. **Alabama: Cleburne Co.**: Heflin, roadside along Highway 78, 33.645833, -85.629444, elev. ca. 314 m, 21 June 2012 *Triplett et al. 130621-2* (JSU); Chulafinnee, Hollis Crossroads, 33.544262, -85.648424, elev. ca. 267 m, 13 July 2018, *Triplett 180713-6* (JSU); Camp Sequoyah, 33.526317, -85.659330, elev. ca. 280 m, 27 June 2012 *Triplett 120627-1* (JSU). **Lee Co.**: Mailpost 1541 on Co. Rd. 112, near Chewacla Creek, 32.5451, -85.3885, 175, elev. ca. 175 m, 24 July 2005, *Triplett & Ozaki 97* (JSU); Chewacla Creek, 32.5451, -85.3885, elev. ca. 175 m, 13 July 2018 *Triplett & Barger 180713-1* (JSU); Lochapoca Rd., 32.598765, -85.546022, elev. ca. 203 m, 13 July 2018 *Triplett & Barger 180713-4* (JSU); Moores Mill Rd., 32.574001, -85.419279, elev. ca. 168 m, 13 July 2018 *Triplett & Barger 180713-2* (JSU). **Macon Co.**: Tsinia Wildlife Viewing Area, 32.4393, -85.65472, elev. ca. 81 m, 24 February 2012 *Triplett & Jamison 120224-3* (JSU). **Randolph Co.**: Wedowee, 33.25307, -85.45384, elev. ca. 326 m, 24 February 2012, *Triplett & Jamison 120224-2* (JSU).

## Discussion

The decision to recognize this taxon at the species level is based on the combination of phylogenetic and morphologic analyses with careful consideration of the decisions made in the past regarding the North American *Arundinaria* species complex and the ability to diagnose monophyletic units. This interpretation follows from morphological (*i.e.*, diagnostic characters) and phylogenetic (*i.e.*, unique ancestry) species concepts (Olmstead 1995; Sites & Marshall 2003).

Consistent with previous results that revealed a genetic similarity with the Switch Cane clade and an origin involving River Cane as the chloroplast donor, the molecular phylogenetic analysis of *A. alabamensis* reveals that it has a stronger association with *A. tecta* and *A. appalachiana* (maximum divergence: 26.4%) than with *A. gigantea* (maximum divergence: 50.3%). A relatively large number (77) of diagnostic bands unite *A. alabamensis* with the Switch Cane clade, versus a small number (4) that unite *A. alabamensis* and *A. gigantea*. Regarding the six sampled

nuclear genes, in every case *A. alabamensis* has an allele (nuclear haplotype) that is most similar to the Switch Cane clade (rather than *A. gigantea*). Although this sample of genes is relatively small, these observations suggest that subsequent introgression has occurred to fix the genetics into the Switch Cane lineage.

The co-occurrence of G.1 and G.2 cpDNA haplotypes might indicate a progenitor/derivative relationship for the G.1 haplotype; however, it is also possible that the Tsinia population has experienced backcrossing with River Cane. The STRUCTURE analysis suggests that the ancestry of *A. alabamensis* is more complex than the other sampled species; in general, *A. alabamensis* appears to harbor more genetic diversity than any of the other sampled species and may derive from two or more ancestral gene pools. This is consistent with a hybrid origin and suggests that *A. alabamensis* is still in the process of attaining genetic stability or homogeneity. The current data do not provide an estimate of the date of origin of *A. alabamensis*, but future phylogenomic studies could resolve this question in the broader context of the evolution of the *Arundinaria* clade of temperate bamboos.

*Arundinaria alabamensis* is relatively widespread in Alabama. Most likely, additional populations will be found in neighboring counties. In particular, the gap between Macon and Lee Counties at the southern end of the distribution and Cleburne to the north will likely be filled in with additional field work.

Hybridization is apparently common in temperate woody bamboos in spite of their long periods between flowering events (Triplett & Clark 2021). Based on previous research in this group, it is clear that hybridization occurs in *Arundinaria* (McClure 1973; Triplett *et al.* 2010). *Arundinaria alabamensis* potentially provides a good model system for hybrid speciation and the ongoing evolution of hybrids (Mallet 2007). Although hybridization in *Arundinaria* is widespread, the potential consequences are unclear. These processes can induce a substantial amount of genetic variation in the hybrid lineage, which may later display a higher evolutionary potential than that found in non-hybrid species (Abbott *et al.* 2013). Introgressive hybridization in the early stages of a radiation may play a central role in both speciation (Grant & Grant 2019; Abbott *et al.* 2013, Taylor & Larson 2019). Thus, this species has a valuable role in our understanding of the evolutionary history of woody bamboos.

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