Phylogenetic relationships among the temperate bamboos (Poaceae: Bambusoideae) with an emphasis on *Arundinaria* and allies

by

Jimmy K. Triplett

A dissertation submitted to the graduate faculty
in partial fulfillment of the requirements for the degree of
DOCTOR OF PHILOSOPHY

Major: Botany (Systematics and Evolution)

Program of Study Committee:
Lynn G. Clark, Major Professor
Gregory W. Courtney
John D. Nason
Robert S. Wallace
Jonathan F. Wendel

Iowa State University
Ames, Iowa
2008

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ABSTRACT

The temperate clade is a diverse but poorly understood major lineage of woody bamboos (Poaceae: Bambusoideae: Bambuseae). Ongoing work is needed to reconstruct evolutionary relationships and establish nomenclatural stability for this difficult but ecologically and economically important group. We present the first robust multilocus plastid phylogeny for the temperate bamboos, assess relationships among key genera with an emphasis on *Arundinaria* and its allies, and discuss evolutionary phenomena potentially responsible for the taxonomic complexity of this group. Utilizing a total of twelve cp DNA regions (1 coding, 10 intergenic spacers, 1 intron), the temperate bamboos were resolved to include six major lineages: the Bergbamboes Lineage, the African Alpine Bamboo Clade, the *Chimonocalamus* Clade, the *Shibataea* Clade, the *Phyllostachys* Clade, and the *Arundinaria* Clade. The resulting chloroplast phylogeny is incongruent with current morphological classifications, rendering subtribes and many genera poly- or paraphyletic. Within the *Arundinaria* Clade, several lineages were identified including the *Sasa* Clade, the *Pleioblastus s.s. Clade*, and a clade containing Chinese species currently classified in *Acidosasa, Indosasa*, *Pleioblastus* sect. *Amari*, and *Pseudosasa* subg. *Sinicae* (the *Sinicae* Clade). The analysis also recovered a monophyletic *Arundinaria sensu stricto* and indicated *Sasa* and *Sasamorpha* as possible close relatives of the North America species, although results were equivocal.

We also report the results of a molecular phylogenetic analysis of *Arundinaria* in North America, including estimates of genetic variation and evidence of natural hybridization and introgression among all three native species. The study involved a comparative analysis of amplified fragment length polymorphisms (AFLPs) and chloroplast DNA sequences representing diversity within and among all three species of *Arundinaria sensu stricto* plus accessions with intermediate or unusual morphological characteristics (putative hybrids). Molecular evidence demonstrates that *A. tecta* and *A. appalachiana* are sister species, forming a clade that is significantly divergent from *A. gigantea*. All three species retain the potential for cross-fertilization, albeit presumably rare due to allopatry and infrequent flowering. Detected patterns of hybridization were relatively shallow, with the majority of hybrids being the apparent direct (F1) product of crosses between *A. gigantea* and *A. tecta*.

Several unusual genotypes were identified, presumably representing posthybridizational recombination and/or introgression. In conjunction with this molecular analysis, the newly recognized species from the southern Appalachian Mountains, *Arundinaria appalachiana,* is described, illustrated, and compared with the related species *A. gigantea* and *A. tecta*. This new species is distinguished by a combination of vegetative morphological characters including features of branching and leaf morphology, leaf anatomy, and ecology. A key for the identification of *Arundinaria* species in North America is included along with a comparative table based on morphology, leaf anatomy, and ecology.

Phylogenetic relationships among *Arundinaria* and its allies in East Asia were further explored using AFLP data in conjunction with a four-region plastid framework phylogeny, with an emphasis on species-level relationships in the genus *Pleioblastus sensu stricto*. Hybridization and introgression were detected both within and among genera, highlighting the significant role of reticulate evolution in temperate bamboo diversity. Molecular data confirmed the hybrid origin of *Hibanobambusa*, *Semiarundinaria*, and *Sasaella*, and also revealed the type species of *Pseudosasa* to be an intergeneric hybrid. Moreover, cryptic links were detected between *Sasa* and *Sasamorpha*, resulting in nothotaxa that have obscured the distinction between these genera. AFLP and chloroplast sequence data support the monophyly of *Pleioblastus s.s.* and reveal species-level resolution in section *Pleioblastus,* low genetic diversity among populations of the widespread *P. simonii* (section *Medakea*), and cryptic reticulation among species in sections *Nezasa* and *Medakea*. This analysis also provided additional evidence for the monophyly of North American genus *Arundinaria*, but failed to reveal its closest relative. A significant conclusion of this research is that reticulate evolution has had an important role in the evolution of the temperate bamboos, in spite of the rarity of flowering.

CHAPTER 1. OVERVIEW

ORGANIZATION OF THE THESIS

The focus of this dissertation is the molecular systematics of the temperate clade of the woody bamboos, with an emphasis on the genus *Arundinaria* and its closest allies. The initial motivation for this study was to better characterize diversity among *Arundinaria* in North America and one of its presumed closest relatives in East Asia, the genus *Pleioblastus*, with the goal of generating a monographic revision of the latter. In light of a number of surprising results, particularly concerning hybridization and reticulate evolution among genera in East Asia, it quickly became apparent that a fundamental investigation of relationships among a broader group of taxa would be necessary in order to address the complex evolutionary network in this group. Accordingly, the focus of this research shifted to a foundation-level study of the pattern and implications of reticulate evolution in the *Arundinaria* Clade.

The dissertation is organized into four main chapters, each dealing with a different level within the set of hierarchical relationships in the temperate clade. A literature review of the systematics of the temperate bamboos is presented in this introductory chapter. Chapter 2 consists of a manuscript entitled "Phylogeny of the temperate woody bamboos (Poaceae: Bambusoideae) with an emphasis on *Arundinaria* and allies." This investigation entailed a cladistic analysis of sequence data from twelve chloroplast (cp) DNA regions, and provides the first robust phylogeny for the temperate clade. The manuscript will be submitted to *Systematic Botany*. Chapter 3, entitled "Phylogenetic relationships within *Arundinaria* (Poaceae: Bambusoideae) in North America," presents a species- and population-level investigation of *Arundinaria sensu stricto* utilizing amplified fragment length polymorphisms (AFLPs) and cp DNA haplotype data, and discusses evidence of hybridization and introgression among species in the southeastern US. This manuscript will be submitted to *Evolution* or *American Journal of Botany.* Chapter 4, entitled "Hill Cane (*Arundinaria appalachiana*), a new species of bamboo (Poaceae: Bambusoideae) from the southern Appalachian Mountains," presents a new species of North American bamboo, including illustrations, a distribution map, and an identification key for *Arundinaria* in North America.

This chapter is based on a manuscript that has been published in *SIDA (*now JBRIT). Chapter 5, entitled "Reticulate evolution in the *Arundinaria* Clade (Poaceae: Bambusoideae)," targets one of the major lineages revealed in the cp DNA phylogeny and explores phylogenetic relationships among species and genera using a combination of cp DNA and AFLP markers. In this chapter, the broader impacts of hybridization and reticulate evolution are examined among ten genera, several of which are confirmed to be of hybrid origin. This chapter also provides a molecular phylogeny of the genus *Pleioblastus s.s.*, and identifies several important challenges for the systematics of that genus. This manuscript will be submitted to *New Phytologist*. The major findings of this dissertation are summarized in Chapter 6, along with some directions for future research.

LITERATURE REVIEW

The true bamboos (Poaceae: Bambusoideae) encompass over 1400 species worldwide and represent the single major grass lineage to diversify in association with forest habitats (Clark 1997; Grass Phylogeny Working Group [GPWG] 2001). Despite their ecological and economic importance, much remains to be learned about the evolution and natural history of these plants; even in North America, a new species has recently been discovered (Triplett *et al.* 2006), and the connection between our native bamboos and their East Asian relatives remains unclear. The Bambusoideae remain one of the most poorly understood subfamilies of grasses (Soderstrom and Ellis 1987; Clark *et al.* 1995; Li 1997).

Recent cladistic analyses of molecular and morphological data have revealed the broad outlines of bamboo phylogeny: the true bamboos are a monophyletic lineage (GPWG 2001; Zhang and Clark 2000; Clark *et al.* 2007) defined by the synapomorphy of strongly asymmetrically invaginated arm cells in the leaf mesophyll. The subfamily encompasses two major groups: the woody bamboos (tribe Bambuseae, ca. 1320 spp.) and the herbaceous bamboos (tribe Olyreae, ca. 115 spp.). The Bambuseae are characterized by the presence of well developed rhizomes, strongly lignified culms, specialized culm leaves for the protection and support of immature tissue, foliage leaves with both inner (adaxial) and outer (abaxial) ligules, complex vegetative branching, and usually cyclical, gregarious, and monocarpic flowering (Judziewicz *et al.* 1999). Woody bamboos are widely distributed in tropical and

temperate zones, where they are often a major component of forests; centers of diversity are in the Neotropics, SE Asia, Madagascar, and Eastern Asia (Clark 1997). The Olyreae are monoecious tropical understory plants with weakly lignified culms, restricted vegetative branching, no specialized culm leaves, no outer ligules, unisexual spikelets, and usually a seasonal pattern of flowering (Judziewicz *et al.* 1999). Herbaceous bamboos are primarily distributed in the Americas, with one species in both tropical America and Africa, and one monotypic genus endemic to New Guinea.

Cladistic support for the monophyly of Bambusoideae and the herbaceous bamboos is generally strong (e.g., Zhang and Clark 2000; GPWG 2000, 2001; Clark *et al.* 2007), but surprisingly, the monophyly of the woody bamboos is robust only when morphological characters are included (Zhang and Clark 2000). Within the woody bamboos, recent phylogenetic analyses recover a polytomy of four lineages (Kelchner and Clark 1997; Zhang 1996; Zhang and Clark 2000; Ní Chonghaile 2002; Clark *et al.* 2007): the Temperate clade (~500 spp.), a Paleotropical clade (~460 spp.), and two Neotropical clades (~360 spp.). These four clades accommodate several subtribes and many genera recognized on the basis of morphology (Soderstrom and Ellis 1987; Li 1997; Ohrnberger 1999), but generic concepts often rest on minor and controversial differences and intergeneric relationships remain poorly understood. In addition, morphological observations and molecular data sets appear to be in conflict in several instances. For the vast majority of woody bamboos, research is needed to answer fundamental questions regarding the deepest branches in their phylogeny as well as relationships at the generic level and below.

By all accounts, the taxonomy of the temperate clade is in the greatest confusion (Soderstrom and Ellis 1987; Li 1997; Zhang and Clark 2000). The clade represents a third of all bamboos and is highly diverse in Asia (~500 species); with *Arundinaria* (3 species) in the U.S., this group exhibits the classic disjunction pattern between East Asia and Eastern North America suggesting a prehistoric migration across the Bering Bridge (Zhu *et al.* 1994; Geng and Zheng 1995; Ohrnberger 1999). The temperate bamboos are very important in temperate forests, particularly in mountainous regions, but also in high altitude grasslands in some subtemperate montane systems. These species play a key role in forest dynamics due to their colonizing ability, and gregarious, monocarpic flowering permits forest to reoccupy

previously disturbed sites. Some of the longest flowering cycles among Angiosperms are found in this group.

Putative synapomorphies for the temperate bamboos include leptomorph rhizomes (with the exception of the group that contains *Fargesia*, *Yushania*, and *Thamnocalamus*: perhaps an evolutionary reversal to pachymorph rhizomes?), chromosome number (2n=48), and reduction in the number of stamens and stigmas (Soderstrom 1981; Soderstrom and Ellis 1987; Li 1997). Although the temperate bamboos receive strong support in molecular studies (Zhang 1996; Kelchner and Clark 1997; Zhang and Clark 2000; Ní Chonghaile 2002; Clark *et al.* 2007), DNA sequence data so far have provided little resolution within the clade and no support for subtribes or genera recognized on the basis of morphology (Guo *et al.* 2002; Stapleton *et al.*, unpubl.; Zhang and Clark 2000; Ní Chonghaile 2002). The poor resolution is presumed to be the result of recent and rapid diversification, compounded by the long generation times (*e.g.*, 120 years in one species!) that are characteristic of the temperate species (Gaut *et al.* 1997).

Among the various classification systems proposed for the woody bamboos, each has treated the temperate bamboos somewhat differently. Inflorescence morphology has been used as a key characteristic to recognize two major types of woody bamboos (Keng 1959; McClure 1966; Keng 1982a,b, 1983a,b): most old world tropical bamboos and some temperate species have bracteate, indeterminate (iterauctant) inflorescences with pseudospikelets (McClure 1966), in contrast to ebracteate, determinate (semelauctant) forms with true spikelets. In general, the bracteate inflorescence is assumed to be the primitive condition in the grass family, based on the presence of fully bracteate inflorescences in families closest to grass ancestors (Soderstrom 1981; GPWG 2001). However, the pseudospikelets of woody bamboos are distinctive structures, and the evolution of bamboo reproductive morphology is poorly understood.

As a consequence of divergent morphology among the temperate bamboos (*e.g.*, semelauctant vs. iterauctant synflorescences, pachymorph vs. leptomorph rhizomes), none of the traditional classifications treated these species as a unified group, instead placing them into two or more subtribes, and typically combining temperate and tropical genera. For example, Keng (1982) proposed a system of classification for the woody bamboos based on

inflorescence type, recognizing two supertribes (Arundinariatae and Bambusatae), both of which encompassed temperate and tropical bamboos. Similarly, Clayton and Renvoize (1986) divided woody bamboos into three subtribes (Melocanninae, Arundinariinae and Bambusinae) with temperate and tropical genera combined in the latter two. Keng and Wang (1996) retained the supertribal division, within which the temperate genera were placed into six subtribes, also combining temperate and tropical species. Some of these systems generated controversial conclusions; for example, certain temperate genera were interpreted to have 2-3 separate buds at the nodes, and this characteristic was used to place *Drepanostachyum* and *Chimonocalamus* of Southeastern Asia within the Chusqueinae ("*Chusqueae*") of Central and South America (Keng and Wang 1996).

Soderstrom and Ellis (1987) established the core Bambusoideae using a set of morpho-anatomical characters, recognizing a total of five subtribes including two (Arundinariinae and Shibataeinae) that exclusively accommodated temperate bamboos. Most recent classifications (Dransfield and Widjaja 1995; Li 1997) were essentially similar to Soderstrom and Ellis (1987) but recognized different generic boundaries. Li (1997) informally divided the Arundinariinae into two generic complexes on the basis of rhizome morphology and leaf anatomy: the *Arundinaria* group, with leptomorph rhizomes, and the *Thamnocalamus* group, with pachymorph rhizomes. The pachymorph species were further distinguished by large microhairs, and dumbbell-shaped silica bodies (Soderstrom and Ellis 1987), and this group is collectively placed in the subtribe Thamnocalaminae (Ohrhberger 1999) by some authors.

Intermediate forms of synflorescence are known, such as in *Arundinaria* and *Sasa*, which have inflorescence branches that are usually naked but sometimes subtended by tiny bracts. Other authors have identified putative intermediate taxa that may link the two inflorescence types (Wang and Ye 1980; Li 1999). Stapleton (1997) has indicated there are in fact three characters associated with synflorescence form (subtending bracts, prophylls, and buds at the base of the spikelet), each with several character states, and each potentially behaving independently. Moreover, the validity of inflorescence morphology as a fundamental distinction among lineages has been called into question in light of recent molecular studies (Ní Chonghaile 2002; Peng, in review).

The number of genera recognized in the temperate clade has been variable and problematic, with those related to *Arundinaria* posing the greatest difficulty. A few presumed genera are easily recognizable on the basis of charismatic features such as branching patterns (*e.g.*, *Phyllostachys*, with a characteristic pair of branches per node) and leaf type (*e.g.*, *Indocalamus* and *Sasa,* with large foliage leaves), and vegetative characters continue to be used extensively for taxonomy. However, many genera are indistinguishable except for one or two characters, such as stamen number or rhizome type. For example, the number of stamens provides the major difference between *Indocalamus* (3) and *Sasa* (6), or *Arundinaria s.l. (*3) and *Acidosasa* (6). Current classifications recognize 19-31 genera, but in the absence of an unambiguous phylogeny the taxonomy of the temperate bamboos remains tentative along with all inferences about the evolution of unique features in the lineage.

A key to resolving the taxonomy of the temperate clade is the position of *Arundinaria* and its allies (Li 1997; Soderstrom and Ellis 1987). *Arundinaria* is the earliest name for bamboos with leptomorph rhizomes, originally established for the formerly widespread and ecologically important giant cane of the Southeastern United States (Michaux, 1803). The genus has a long history of taxonomic confusion at various levels, notably arising from disagreement on the inclusion of East Asian taxa (McClure 1966; Chao *et al.* 1980; Clayton and Renvoize 1986; Li 1997). Early in bamboo taxonomy, *Arundinaria* became a catchall for an overabundance of unrelated leptomorph species. Almost all temperate and subalpine species in Asia and Africa, with the exception of *Phyllostachys*, were originally placed under this name, although most were later removed on the basis of suites of morphological characters. Most current treatments recognize a more narrowly defined genus (*Arundinaria sensu stricto)* endemic to the Southeastern United States and the only North American genus of tribe Bambuseae (woody bamboos). Even within this more restricted group, however, species delimitation has been controversial.

In the Southeastern US, canes (*Arundinaria spp.*) occupy circumneutral to highly acidic soils of alluvial and nonalluvial wetlands (West 1935), often creating dense thickets (including “canebrakes,” the once common, monospecific stands). Recent treatments of these plants recognized one species with two infraspecific taxa: *A. gigantea* subsp. *gigantea*, occurring primarily along river floodplains inland and in the Coastal Plain, and the smaller *A.*

gigantea subsp. *tecta*, more frequent in acidic swamps, seeps, and bogs of the Coastal Plain and readily distinguished by the presence of air canals in the rhizomes (McClure 1973). Species delimitation in this group has been controversial due to uncertainty regarding the nature of morphological variation including intermediate forms that have been recognized as putative hybrids (McClure 1973; Marsh 1977).

Based on similarity in vegetative characters (culm morphology, branching, leaf morphology and anatomy) and reproductive characters (inflorescence architecture, determinate spikelets, 3 stamens), a number of genera have long been considered close relatives of the North American species. Several widely recognized genera are defined on minor differences and continue to invoke taxonomic confusion. An especially difficult group is the *Arundinaria* group (Li 1997; Ní Chonghaile 2002), encompassing *Bashania, Ferrocalamus, Indocalamus*, *Oligostachyum*, *Pleioblastus*, and *Pseudosasa*. These genera share the essential characteristics of *Arundinaria* (determinate spikelets, 3 stamens) and numerous structural similarities with the North American taxa but are distinguished on the basis of bud and branch features that are notoriously difficult to interpret. Numerous authors have argued that the differences between these genera are too few, and combine all into the genus *Arundinaria* (Chao *et al.* 1980; Clayton and Renvoize 1986; Yang and Zhao 1993, 1994).

Of these, *Pleioblastus* (42+ species) is the one most often synonymized with *Arundinaria*. Nakai (1925) recognized *Pleioblastus* on the basis of branch buds, which he characterized as ramifying precociously before emerging from the prophyll. *Pleioblastus* is further characterized by a distinctive, reiterative branch complement and persistent culm leaves, and contains a striking diversity from arborescent species in the woodlands to diminutive groundcover species that create distinctive grasslands in montane communities. Flowering is unknown in several species, adding puzzlement from the perspective of traditional angiosperm taxonomy. In Japan alone, this genus has encompassed over 200 named species. *Pleioblastus* was revised by Suzuki (1978) and reduced to 21 species in three sections (*Medakea*, *Nezasa*, and *Pleioblastus*); current treatments recognize 19 species among these sections (Ohrnberger 1999). A fourth section (*Amari*) was recently erected to accommodate an assortment of putative congeners in China and Vietnam (Chen and Sheng

1991), but close examinations reveal these to differ from *Pleioblastus s.s.* in a suite of characters from branch architecture to leaf persistence. Moreover, some studies refute the original distinction between *Pleioblastus* and *Arundinaria* (Chao and Chu 1980; Soderstrom and Ellis 1987). Neither the internal relationships of *Pleioblastus* nor the long-standing problems with generic limits in the *Arundinaria* complex have been addressed with phylogenetic methods. As the largest of the narrowly defined genera in the *Arundinaria* species complex, the status of *Pleioblastus* and its relationship with the North American *Arundinaria gigantea* alliance is particularly important in efforts to reconstruct the phylogeny of temperate bamboos.

Molecular methods have been utilized with limited success to address taxonomic problems in the temperate bamboos, including DNA nucleotide sequencing (Clark *et al.* 1995; Zhang 1996; Guo *et al.* 2001, 2002; Ní Chonghaile 2002; Zhuge *et al.* 2004; Peng *et al.*, in review), micro- and minisatellite markers (Lai and Hsiao 1997), EST-SSRs (Barkley *et al.* 2005), and RFLPs (Friar and Kochert 1991, 1994; Watanabe *et al.* 1994; Kobayashi 1997). Recently, AFLP markers have been successfully used for species-level studies of bamboos (Hodkinson *et al.* 2000; Loh *et al.* 2000; Suyama *et al.* 2000), including temperate genera. However, with few exceptions, molecular studies have failed to resolve relationships among major lineages within the temperate bamboos, and most result in weakly supported assemblages that are inconsistent with morpology. Moreover, limited taxon sampling in earlier studies prevented them from providing a meaningful framework for temperate species. For example, Guo *et al.* (2002) argued that molecular data (internal transcribed spacers, ITS) supported the monophyly of the *Thamnocalamus* group, however the analysis failed to include diverse accessions from the temperate clade, such as *Phyllostachys*. Their result confirms that the taxa in the *Thamnocalamus* group are distant from *Arundinaria gigantea* and *Acidosasa purpurea* (the only included outgroup taxa) but provides no clear result on the monophyly of genera within the *Thamnocalamus* group, with the exception of *Ampelocalamus*.

Although higher-level studies in the temperate bamboos have largely failed to provide resolution, several studies have successfully addressed population-level questions, and these studies provide valuable information on the nature of genetic diversity in the clade. For

example, Hsiao and Rieseberg (1994) utilized RAPD markers to study genetic structure in a natural population of Yushan cane, *Yushania niitakayamensis*, in central Taiwan. This species rarely flowers, and yet exhibits a high level of morphological variation. High levels of genetic diversity were discovered among clones restricted to relatively small areas. The authors suggested that this apparently remnant diversity indicates a higher frequency of sexual reproduction than currently observed, or else a high somatic mutation rate. A number of studies have been conducted on the genus *Phyllostachys*, most revealing high variability among species but little variation within a given species (Friar and Kochert 1994; Gielis 1995; Gielis *et al.* 1997). For example, Lai and Hsiao (1997) studied genetic variation in recently naturalized populations of *Phyllostachys pubescens* utilizing microsatellites, minisatellites, and RAPDs. Their study identified 9 clones out of 23 populations throughout the distribution of *P. pubescens* in Taiwan, including one widespread, dominant clone. Similarly, Huh and Huh (2002) studied the population genetic structure of *Pseudosasa japonica* var. *koraiences* in Korea and found low levels of genetic variation consistent with clonal reproduction. Suyama *et al.* (2000) studied the clonal structure of a population of *Sasa senanensis* using AFLP polymorphisms, and surprisingly discovered a high number of genetically distinct clones within in a single population. AFLP markers have also been used to examine species-level relationships within the temperate clade, and were considered to be more useful than ITS for reconstructing phylogenetic relationships among closely related species of *Phyllostachys* (Hodkinson *et al.* 2000).

These observations reflect our best understanding of the temperate bamboos to date, and highlight the need for additional field and molecular systematic studies of a group that is scarcely understood in all aspects of its evolutionary biology. In light of the fundamental questions surrounding these species and the growing discordance between natural populations and cultivars (including the status of some species as "unknown in nature"), there is a pressing need for a cohesive phylogeny-based understanding of diversity within this lineage. Moreover, preliminary data suggests that hybridization and reticulation may be important forces guiding diversity in this group (Triplett and Clark, in prep; Chapter 5), consequently obscuring the correlation between morphology and phylogeny. Critical evaluation of multiple, independent molecular data partitions (*e.g.*, AFLPs, plastid nucleotide

sequence data) may help to elucidate cryptic patterns of speciation in this group, including reticulation. To this end, I seek to test generic boundaries and the phylogenetic position of taxa in the *Arundinaria* group while simultaneously examining evolutionary processes that may have been instrumental in their diversification, including large-scale phylogeographic patterns, hybridization and reticulation, and shifts in diversification rates.

LITERATURE CITED

BARKLEY, N.A., M.L. NEWMAN, M.L. WANG, M.W. HOTCHKISS, AND G.A. PEDERSON. 2005. Assessment of the genetic diversity and phylogenetic relationships of a temperate bamboo collection by using transferred EST-SSR markers. Genome 48: 731-737.

CHAO, C.S., AND C.D. CHU. 1980. *Arundinaria* Michaux and its distribution in China. Bamboo Res. 1: 1-23.

CHAO, C.S., C.D. CHU, AND W.Y. HSIUNG. 1980. A revision of some genera and species of Chinese bamboos. Acta Phytotax. Sin. 18: 20-36.

CHEN, S.L. AND G.Y. SHENG. 1991. New taxa and a new combination in Chinese bamboos. Bulletin of Botanical Research 11 (4): 41-51.

CLARK, L.G. 1997. Bamboos: the centrepiece of the grass family. In: Chapman, G.P. (ed.). The Bamboos. Linnean Society of London Symposium Series. Academic Press, London.

CLARK, L.G., S. DRANSFIELD, J. TRIPLETT, AND J.G. SÁNCHEZ-KEN. 2007. Phylogenetic relationships among the one-flowered, determinate genera of Bambuseae (Poaceae: Bambusoideae). Aliso 23: 315-332.

CLARK, L.G., W. ZHANG, AND J.F. WENDEL. 1995. A phylogeny of the grass family (Poaceae) based on *ndhF* sequence data. Syst. Bot. 20: 436-460.

CLAYTON, W.D. and S.A. RENVOIZE. 1986. Genera Graminum: Grasses of the World. Royal Botanic Gardens, Kew.

DRANSFIELD, S. AND E.A. WIDJAJA. 1995. Plant resources of South-East Asia No. 7 Bamboos. Leiden: Backhuys Publishers.

FRIAR, E. AND G. KOCHERT. 1991. Bamboo germplasm screening with nuclear restriction fragment length polymorphisms. Theor. Appl. Genet. 82: 697-703.

FRIAR, E. AND G. KOCHERT. 1994. A study of genetic variation and evolution of *Phyllostachys (*Bambusoideae: Poaceae) using nuclear restriction fragment length polymorphisms. Theor. Appl. Genet. 89: 265-270.

GAUT, B.S., L.G. CLARK, J.F. WENDEL, AND S.V. MUSE. 1997. Comparisons of the molecular evolutionary process at *rbcL* and *ndhF* in the grass family (Poaceae). Mol. Biol. Evol. 14: 769-777.

GENG, B. AND Z-P. ZHENG. 1995. Gramineae Bambusoideae. Flora Reipublicae Popularis Sinicae 9 (1). Beijing: Science Press.

GIELIS, J. 1995. Bamboo and biotechnology. European Bamboo Society Journal. May 6: 27-39.

GIELIS, J., I. EVERAERT, AND M. DE LOOSE. 1997. Genetic variability and relationships in *Phyllostachys* using random amplified polymorphic DNA. In: Chapman, G.P. (ed.). The Bamboos. Linnean Society of London Symposium Series. Academic Press, London.

GPWG (Grass Phylogeny Working Group). 2001. Phylogeny and subfamilial classification of the grasses (Poaceae). Ann. Miss. Bot. Gard. 88 (3): 373-457.

GUO, Z.-H, Y.-Y. CHEN, AND D.-Z. LI AND J.-B. YANG. 2001. Genetic variation and evolution of the Alpine Bamboos (Poaceae: Bambusoideae) using DNA sequence data. J. Plant Res. 114: 315-322.

GUO, Z.-H, Y.-Y. CHEN, AND D.-Z. LI. 2002. Phylogenetic studies on the *Thamnocalamus* group and its allies (Gramineae: Bambusoideae) based on ITS sequence data. Mol. Phyl. Evol. 22 (1): 20-30.

HODKINSON, T.R., S.A. RENVOIZE, G. NÍ CHONGHAILE, C.M.A. STAPLETON, AND M.W. CHASE. 2000. A comparison of ITS nuclear rDNA sequence data and AFLP markers for phylogenetic studies in *Phyllostachys* (Bambusoideae, Poaceae). J. Plant Res. 113: 259-269.

HSIAO, J.Y. AND L.H. RIESEBERG. 1994. Population genetic structure of *Yushania niitakayamensis* (Bambusoideae, Poaceae) in Taiwan. Molecular Ecology 3: 201-208.

HUH, H. W. AND M. K. HUH. 2002. Genetic diversity and population structure of *Pseudosasa japonica* (Bambusaceae) in Korea. Bamboo Science and Culture 16 (1): 9-17.

JUDZIEWICZ, E. J., L. G. CLARK, X. LONDOÑO AND M. J. STERN. 1999. American Bamboos. Washington, D. C.: Smithsonian Institution Press.

KELCHNER, S. A. AND L. G. CLARK. 1997. Molecular evolution and phylogenetic utility of the chloroplast *rpl16* intron in *Chusquea* and the Bambusoideae (Poaceae). Mol. Phyl. Evol. 8 (3): 385-397.

KENG, P.C. 1982a. A revision of genera of bamboos from the world. I. Journal of Bamboo Research 1 (1): 1-19.

KENG, P.C. 1982b. A revision of genera of bamboos from the world. II. Journal of Bamboo Research 1 (2): 31-46.

KENG, P.C. 1983a. A revision of genera of bamboos from the world. III. Journal of Bamboo Research 2 (1): 11-27.

KENG, P.C. 1983b. A revision of genera of bamboos from the world. IV. Journal of Bamboo Research 2 (2): 1-17.

KENG, P.C. AND Z.P. WANG, eds. 1996. *Flora Reipublicae Popularis Sinicae*, vol. 9 (1). Beijing. Science Press. [In Chinese]

KENG, Y.L. 1959. *Flora illustralis plantarum primarum sinicarum. Gramineae*. Beijing: Academia Sinica Press.

KOBAYASHI, M. 1997. Phylogeny of world bamboos analysed by restriction fragment length polymorphisms of chloroplast DNA. In: Chapman, G.P. (ed.). The Bamboos. Linnean Society Symposium Series. UK: Linnean Society of London.

LAI, C.C., AND J.Y. HSIAO. 1997. Genetic variation of *Phyllostachys pubescens* (Bambusoideae, Poaceae) in Taiwan based on DNA polymorphisms. Bot. Bull. Acad. Sin. 38: 145-152.

LI, D.Z. 1997. The Flora of China Bambusoideae Project: Problems and current understanding of bamboo taxonomy in China. In: Chapman, G.P. (ed.). The Bamboos. Linnean Society of London Symposium Series. Academic Press, London.

LOH, J. P., R. KIEW, O. SET, L. H. GAN AND Y-Y. GAN. 2000. A study of genetic

variation and relationships within the bamboo subtribe Bambusinae using amplified fragment length polymorphism. Ann. Bot. 85: 607-612.

MARSH, D.L. 1977. The taxonomy and ecology of cane, *Arundinaria gigantea* (Walter) Muhlenberg. Ph.D. dissertation. University of Arkansas.

MCCLURE, F.A. 1966. The Bamboos: A Fresh Perspective. Cambridge, MA: Harvard University Press.

MCCLURE, F.A. 1973. Genera of Bamboos Native to the New World (Gramineae: Bambusoideae). Ed. T.R. Soderstrom. Smithsonian Contr. Bot. 9: 1-148.

MICHAUX, A. 1803. Flora Boreali-Americana: sistens caracteres plantarum quas in America septentrionali collegit et detexit. Paris and Strasbourg.

NAKAI, T. 1925. Two new genera of Bambusaceae with special remarks on the related genera growing in eastern Asia. J. Arnold Arbor. 6 (3): 145-153.

NÍ CHONGHAILE, G. 2002. Systematics of the woody bamboos (Tribe Bambuseae). Unpublished Ph.D. thesis, University of Dublin, Trinity College, Ireland.

OHRNBERGER, D. 1999. The Bamboos of the World. Elsevier, Amsterdam, The Netherlands.

PENG, S., H.-Q. YANG, AND D.-Z. LI. Highly heterogeneous generic delimitation within the temperate bamboo clade (Poaceae: Bambusoideae): evidence from GBSSI and ITS sequences. In review.

RENVOIZE, S.A. AND T.R. HODKINSON. 1997. Classification of *Phyllostachys*. In: Chapman, G.P. (ed.). The Bamboos. Linnean Society of London Symposium Series. Academic Press, London.

SODERSTROM, T.R. 1981. Some evolutionary trends in the Bambusoideae (Poaceae). Ann. Miss. Bot. Gard. 68: 15-47.

SODERSTROM, T.R., AND R.P. ELLIS. 1987. The position of bamboo genera and allies in a system of grass classification. In: T.R. Soderstom, K.W. Hilu, C.S. Campbell, and M.E. Barkworth (eds.). Grass Systematics and Evolution. Random House (Smithsonian Inst. Press), New York.

STAPLETON, C.M.A. 1997. The morphology of woody bamboos. In: Chapman, G.P. (ed.). The Bamboos. Linnean Society Symposium Series. UK: Linnean Society of London.

SUYAMA, Y., K. OBAYASHI, AND I. HAYASHI. 2000. Clonal structure in a dwarf bamboo (*Sasa senanensis*) population inferred from amplified fragment length polymorphism (AFLP) fingerprints. Molecular Ecology 9: 901-906.

SUZUKI, S. 1978. Index to Japanese Bambusaceae. Gakken Co. Ltd., Tokyo.

TRIPLETT, J.K., A.S. WEAKLEY, AND L.G. CLARK. 2006. Hill cane (*Arundinaria appalachiana)*, a new species of bamboo (Poaceae: Bambusoideae) from the southern Appalachian Mountains. SIDA 22 (1): 79-85.

WATANABE, M, M. ITO, AND S. KURITA. 1994. Chloroplast DNA phylogeny of Asian bamboos (Bambusoideae, Poaceae) and its systematic implication. J. Plant Res. 107: 253-261.

WEST, E.M. 1935. Canebrakes of the Southeastern United States. Unpublished dissertation, Ohio State University.

WU, Z. Y., P. H. RAVEN AND D. Y. HONG (eds.). 2006. Flora of China. Vol. 22 (Poaceae). Science Press, Beijing, and Missouri Botanical Garden Press, St. Louis.

YANG, G.Y. AND Q.S. ZHAO. 1993. A revision of the genus *Arundinaria* Michaux in China (I). Journal of Bamboo Research 12 (4): 1-6.

YANG, G.Y. AND Q.S. ZHAO. 1994. A revision of the genus *Arundinaria* Michaux in China (II). Journal of Bamboo Research 13 (1): 1-23.

ZHANG, W.P. 1996. Phylogeny and classification of the bamboos (Poaceae: Bambusoideae) based on molecular and morphological data. Ph.D. dissertation. Iowa State University.

ZHANG, W.P. AND L.G. CLARK 2000. Phylogeny and classification of the Bambusoideae (Poaceae). In: S.W.L. Jacobs and J. Everett (eds.). Grasses: Systematics and Evolution: 35-42. CSIRO, Melbourne.

ZHU, S., N. MA AND M. FU. 1994. Compendium of Chinese Bamboos. China Forestry Publishing House.

ZHUGE, Q., Y.-L. DING, C. XU, H.-Y. ZOU, M.-R. HUANG, AND M.X. WANG. Phylogeny of the genus *Arundinaria* based on nucleotide sequences of nrDNA ITS region. Acta Genetica Sinica 31 (4): 349-356.

CHAPTER 2. PHYLOGENY OF THE TEMPERATE WOODY BAMBOOS (POACEAE: BAMBUSOIDEAE) WITH AN EMPHASIS ON *ARUNDINARIA* AND ALLIES

A manuscript to be submitted to *Systematic Botany*

JIMMY TRIPLETT[1,2] and LYNN G. CLARK[1]

[1]Department of Ecology, Evolution and Organismal Biology, Iowa State University, Ames, IA 50011-1020, USA

[2]Author for correspondence (triplett@iastate.edu)

Running title: Triplett and Clark: Phylogeny of the temperate bamboos

ABSTRACT

The temperate clade is a diverse but poorly understood major lineage of woody bamboos (Poaceae: Bambusoideae: Bambuseae). Ongoing work is needed to reconstruct evolutionary relationships and establish nomenclatural stability for this difficult but ecologically and economically important group. In the current study, we present the first robust multilocus plastid phylogeny for the temperate bamboos, assess relationships among key genera with an emphasis on *Arundinaria* and its allies, and discuss evolutionary phenomena potentially responsible for the taxonomic complexity of the group. The recovered phylogeny allows us to evaluate previous classifications while providing a framework to guide future research. Utilizing a total of twelve cp DNA regions (1 coding, 10 intergenic spacers, 1 intron), the temperate bamboos were resolved to include six major lineages: the Bergbamboes Lineage, the African Alpine Bamboo Clade, the *Chimonocalamus* Clade, the *Shibataea* Clade, the *Phyllostachys* Clade, and the *Arundinaria* Clade. Internal resolution varied among these six, in part reflecting sampling density and in part due to apparent rate heterogeneity. The resulting chloroplast phylogeny is incongruent with current

morphological classifications, rendering subtribes and many genera poly- or paraphyletic. Within the *Arundinaria* Clade, several lineages were identified including the *Sasa* Clade, the *Pleioblastus s.s.* Clade, and a clade containing Chinese species currently classified in *Acidosasa, Indosasa*, *Pleioblastus* sect. *Amari*, and *Pseudosasa* subg. *Sinicae* (the *Sinicae* Clade). The analysis also recovered a monophyletic *Arundinaria sensu stricto* and indicated *Sasa* and *Sasamorpha* as possible close relatives of the North America species, although results were equivocal. A significant conclusion of this analysis is that hybridization and reticulate evolution have likely had important roles in the evolution of this group in spite of the rarity of flowering.

KEYWORDS: temperate bamboos, phylogenetic framework, *Arundinaria*, *Pleioblastus*, plastid DNA, natural hybridization, reticulate evolution.

INTRODUCTION

The temperate woody bamboos (Poaceae: Bambusoideae: Bambuseae) are a diverse clade of 19-31 genera and ca. 500 species distributed primarily in forests of the northern temperate zone, but also found at high elevations in tropical regions of both hemispheres. Centers of diversity are in China and Japan (ca. 430 spp.), with areas of endemism in Southwestern China (ca. 180 spp.), Southeast Asia (ca. 60 spp.), eastern North America (3 spp.), Africa (2 spp.), and Madagascar (ca. 6 spp.). The group is economically important worldwide with over 200 species in cultivation, including several at the core of Asian timber industries and valued at over $7 billion annually (Stevens 1995). Temperate bamboos are strongly supported as monophyletic in molecular analyses (Zhang 1996; Kelchner and Clark 1997; Zhang and Clark 2000; Clark *et al.* 2007; Sungkaew *et al.*, in review), but unequivocal morphological synapomorphies have yet to be identified. Most species have monopodial, leptomorph rhizomes, reduced stamen and stigma numbers relative to tropical bamboos, and a chromosome number of 2n=48; however, none of these features is unique to the clade. Moreover, taxonomy of the temperate bamboos is highly controversial. Species and genera have sustained numerous revisions based on alternative sets of characters, while molecular data have provided little internal resolution and almost no support for morphological

classifications (Guo *et al.* 2002; Peng *et al.,* in review; Sungkaew *et al.*, in review). An overview of the vast and complex synonymy for the temperate bamboos can be found in Ohrnberger (1999).

Arundinaria Michx. is the earliest name for bamboos with monopodial, leptomorph rhizomes (Michaux, 1803), originally established for the formerly widespread and ecologically important river cane of the Southeastern United States. *Arundinaria* quickly became a catchall for temperate and subalpine species in Asia and Africa, most of which were subsequently partitioned into new genera on the basis of morphology. While most of these are justifiable on the basis of suites of characters, the presumed closest relatives of *Arundinaria s.s.* are defined on minor differences and continue to invoke taxonomic confusion. The *Arundinaria* group (Li 1997; Wu *et al.* 2006) includes *Pleioblastus* Nakai*, Pseudosasa* Makino ex Nakai, *Bashania* P.C. Keng and Yi, *Sarocalamus* Stapleton, *Indocalamus* Nakai, and *Oligostachyum* Z.P. Wang and G.H. Ye, and accounts for approximately 30% of the diversity in the temperate clade. These genera share essential characteristics of *Arundinaria s.s.* but are distinguished on the basis of bud and branch characters that can be difficult to interpret. Shared characters include leptomorph rhizomes, culms with (1-) 3-7 branches per node and 1-3 compressed internodes at the base of the branch complement, semelauctant (determinate) synflorescences that are usually racemose (sometimes paniculate or reduced to a single spikelet), spikelets with a pedicel and 2 glumes, and flowers with 3 (-5) stamens and 2-3 stigmas.

At least two other major categories of morphological diversity occur in the temperate clade, one encompassing species with indeterminate reproductive structures, and one surrounding species with sympodial, pachymorph rhizomes. The *Phyllostachys* group is distinguished from the *Arundinaria* group by virtue of reiterative units called pseudospikelets (McClure 1966; Stapleton 1997; Li 1997); these have a series of one or more bud-bearing bracts that in turn produce new pseudospikelets, and the synflorescence is thus iterauctant (indeterminate). Presumed relatives of *Phyllostachys* Siebold & Zuccarini include *Shibataea* Makino ex Nakai (ca. 9 spp.) and *Chimonobambusa* Makino (ca. 38 spp.). Although pseudospikelets in these genera present a striking contrast to the spikelets of *Arundinaria*, there are a number of intermediate forms that defy categorization.

The *Thamnocalamus* group (Li 1997) represents the other major source of diversity in the temperate bamboos, and comprises Sino-Himalayan, African, and Madagascan species with sympodial, pachymorph rhizomes. The group is species-rich and spans a vast diversity in vegetative and reproductive morphology. Two genera in particular, *Yushania* P.C. Keng (ca. 84 spp) in East and SE Asia, Africa, and Madagascar, and *Fargesia* Franchet (ca. 83 spp.) in East and SE Asia, encompass a third of the diversity in the temperate clade. The *Thamnocalamus* group is considered closer to *Arundinaria* than to *Phyllostachys* by virtue of their semelauctant inflorescences, although intermediate forms are known and the cohesiveness of the group is unclear.

Among these three broadly characterized morphological syndromes are a number of distinctive species complexes, including putative relictual lineages, transitional series, and intergeneric hybrids. For example, the Japanese genus *Sasa* Makino & Shibata differs from the *Arundinaria* group by virtue of six-stamened flowers (versus three), yet it shares other essential characteristics with the group as well as a presumed common ancestor. *Acidosasa* C.D. Chu & C.S. Chao ex P.C. Keng and *Indosasa* McClure, both with long, many-flowered spikelets and six-stamened flowers, align with *Arundinaria* and *Phyllostachys* respectively on the basis of inflorescence morphology, and have been interpreted as transitional genera. *Sinobambusa* Makino ex Nakai, *Semiarundinaria* Makino ex Nakai, *Brachystachyum* Keng, and *Oligostachyum* seem to combine features of *Arundinaria* and *Phyllostachys*, while other unusual taxa present morphological intergradation between *Phyllostachys* and *Sasa* (*i.e.*, *Hibanobambusa* Maruyama & H. Okamura) or *Pleioblastus* and *Sasa* (*i.e., Sasaella* Makino), and are variously interpreted as evolutionary links or intergeneric hybrids (Campbell, unpubl.; Maruyama *et al.* 1979; Suzuki 1987; Okamura 1991; Li 1999).

Juxtaposed on these morphological syndromes is a complex biogeography, particularly concerning the origin of species in North America, Africa, Madagascar, and Sri Lanka. For example, the South African mountain bamboos (*bergbamboes*) are currently placed in the Asiatic genus *Thamnocalamus* Munro, while alpine bamboos of East Africa are currently placed in the Asiatic genus *Yushania*, yet the validity of these placements is far from certain.

Current classifications recognize three subtribes based on the key differences outlined above: semelauctant, monopodial taxa belong to Arundinariinae Bentham; iterauctant, leptomorph taxa are placed in Shibataeinae (Nakai) Soderstrom & Ellis; and semelauctant, pachymorph taxa represent subtribe Thamnocalaminae P.C. Keng (Ohrnberger 1999; Li 1997, Wu *et al.* 2006). Arundinariinae includes *Acidosasa*, *Arundinaria, Bashania*, *Ferrocalamus* Hsueh & P.C. Keng, *Indocalamus* Nakai*, Oligostachyum, Pleioblastus, Pseudosasa, Sasa*, and *Sasaella*; Shibataeinae includes *Brachystachyum*, *Chimonobambusa*, *Hibanobambusa*, *Indosasa*, *Phyllostachys*, *Semiarundinaria*, *Shibataea*, and *Sinobambusa*; and Thamnocalaminae includes *Thamnocalamus, Borinda* Stapleton, *Fargesia*, and their allies, *Ampelocalamus* S.L. Chen, Wen & G.Y. Sheng*, Chimonocalamus* Hsueh & Yi, *Drepanostachyum* P.C. Keng, *Himalayacalamus* P.C. Keng, and *Gaoligonshania* D.Z. Li, Hsueh & N.H. Xia.

In spite of apparent taxonomic structure, all treatments based on morphological characters have proven inconsistent at the scale of genera and species, and it is unclear whether any morphologically-defined taxon accurately reflects a natural group. The morphological muddle surrounding this clade is compounded by a poorly resolved molecular phylogeny. Previous studies have failed to corroborate the proposed subtribal divisions (Kelchner and Clark 1997; Zhang and Clark 2000; Peng *et al.*, in review), providing minimal support for genera recognized on the basis of morphology, while weakly supporting groups that lack obvious morphological unity (Guo *et al.* 2002; Sungkaew *et al.*, in review). Poor resolution has been attributed to possibly recent or rapid divergence, compounded by long generation times (decades to 120 years in one species [Janzen 1976]) that are characteristic of temperate clade species (Gaut *et al.* 1997; Guo *et al.* 2001 2002; Hodkinson *et al.* 2000). In the absence of a comprehensive phylogenetic framework, all classifications of the temperate bamboos remain provisional along with all inferences about character evolution in the clade.

Problems encountered in sequence-based studies of the temperate clade fall into two categories: 1) insufficient informative characters from any single chloroplast region (*e.g.*, *rpl16* intron, *trnLF*; Ní Chonghaile 2002, Triplett and Clark, unpubl. data), and 2) internally incongruent signals and largely unalignable sequences from nuclear and ribosomal regions (*e.g.*, GBSSI, ITS; Guo *et al.* 2001, 2002; Guo and Li 2004; Zhuge *et al.* 2004; Peng *et al.*, in

review). In preliminary studies, our attempts to identify an unambiguous single-copy nuclear region were unsuccessful, revealing instead numerous sequence types in each species sampled (Triplett and Clark, unpubl. data). In contrast, our survey of non-coding plastid regions indicated the potential for sufficient informative characters from combined datasets. Thus, we chose to use an expanded, multi-region approach with plastid DNA on the assumption that a robust plastid phylogeny would provide a valuable starting place to address taxonomic problems in the temperate clade. However, as we emphasize later in this paper, the resulting maternal phylogeny must be interpreted with caution in the light of the apparent history of reticulate evolution in this clade.

The primary goals of this investigation were to 1) establish a phylogenetic framework for the temperate clade with an emphasis on *Arundinaria* and putative allies, 2) identify subclades for future, targeted phylogenetic studies with tools such as AFLPs, and 3) highlight evolutionary processes responsible for the complex pattern of morphological diversity in this clade. Problems encountered in previous molecular analyses led us to explore these issues via a two-staged approach. First, we utilized broad taxon sampling (94 spp.) and four cp DNA regions to identify major subclades within the temperate bamboos. Second, we expanded data sampling to twelve cp DNA regions for 30 exemplar taxa to seek increased resolution or branch support among major subclades, with an emphasis on *Arundinaria* and allies. As such, this study also provides a survey of cp DNA regions for phylogeny reconstruction in bamboos and related taxa.

MATERIALS AND METHODS

Taxon sampling. A total of 94 species in 40 genera were sampled for this study (Appendix 1). Based on prior studies (Zhang and Clark 2000; GPWG 2001), representatives of Ehrhartoideae, Pooideae, and Streptogyneae were chosen as the most appropriate outgroup taxa for the Bambusoideae. We included one representative from the early-diverging Pharoideae, primarily to polarize a ~400 bp portion of the *rps*16-*trn*Q region in the temperate clade that was missing in all other outgroups due to apparently independent deletions; the inclusion of *Pharus* also allow us to test the position of all other outgroup taxa relative to the Bambusoideae. Outgroups thus included *Pharus latifolius, Streptogyna americana*, *Oryza*

sativa, *Leersia oryzoides, Brachyelytrum erectum*, and *Diarrhena obovata*. Eighty-two accessions of temperate woody bamboos were analyzed in this study along with three tropical woody bamboos and three herbaceous bamboos (tribe Olyreae) representing the diversity in the grass subfamily Bambusoideae (the bamboos). Duplicate and alternate accessions were sequenced to verify unexpected placements and to test for variation introduced by PCR (Appendix 1). Silica-dried leaf tissues were obtained from natural populations in China, Japan, Madagascar, and North America. Additional materials were obtained from living collections in China (Kunming), Japan (Kyoto), and the US (California, Tennessee, and Washington). Vouchers of all collections are deposited in the Ada Hayden Herbarium of Iowa State University (ISC). When fresh material was unavailable, tissue samples were obtained from herbarium specimens (Appendix 1). In total, this paper reports phylogenetic relationships among 28 temperate genera (~95% of the clade) and 82 temperate species (16% of the clade), based on over 13,000 characters of DNA sequence data from 12 plastid loci.

cp DNA marker selection. We selected a preliminary set of potentially fast-evolving cp DNA regions based on a review of current literature and tested these on eight temperate species, representing all three subtribes plus several species of *Pleioblastus* in order to evaluate resolution at different taxonomic levels. Based on this survey, we identified 10 intergenic spacers and 1 intron that provided relatively high numbers of parsimony-informative characters for the temperate species (Table 1; Table 2). We also selected one coding region (*ndhF* 3' half) to use in conjunction with the eleven non-coding regions. From this set of 12 regions, we selected four to use in the analysis with expanded taxon sampling.

DNA Extraction, Sequencing, Alignment, and Character Coding. Total genomic DNA was extracted from all tissue samples using DNeasy Plant Mini Kits (Qiagen, Valencia, California) following the manufacturer's protocol, with the following modifications: 40-50 mg dry leaf tissue; 500 µl lysis buffer; 20-30 minute incubation; and a final wash with ice cold 100% EtOH. DNA was amplified and sequenced using primers and protocols listed in Table 1. All polymerase chain reactions (PCRs) and cycle-sequencing reactions were

Table 1. cp DNA primers and PCR parameters used for amplification and sequencing. Asterisk (*) indicates the published primer sequence was modified for this study. "SEQ:" indicates the list of primers used for sequencing reactions, if different than PCR primers.

Region	Primer Sets	PCR Parameters	Reference
atpI-atpH	atpI: CCG GTC ATG TTT CTT GGA TT atpH*: CAA TAA CRG AAG CAG CAG CA	94ºC, 2m; 25x (96ºC, 45s; 56ºC, 1m; 72ºC, 1m); 72ºC, 3m.	Yamane & Kawahara (2005); atpH revised for this study.
ndhF (3' end)	972F: GTC TCA ATT GGG TTA TAT GAT G 2110R: CCC CCT AYA TAT TTG ATA CCT TCT CC SEQ: 1318F*: GGA TTA ACT GCG TTT TAT ATG TTT CG 1603R: GCA TAG TAT TTC CCG TTT CAT GAG G	94ºC, 1m; 30x (94ºC, 1m 30s; touchdown* 53-43ºC, 2m; 72ºC, 3m); 72ºC 10m.	Olmstead & Sweere (1994); 1318F revised for this study.
psaA-ORF170	psaA: TCG AAA TCG TGA GCA TCA GC ORF170: TCT CAA GTA CGG TTC TAG G	95ºC, 2m; 35x (95ºC, 1m; 50ºC, 10s; +15ºC, 0.3ºC/s; 65ºC, 5m); 65ºC, 5m.	Saltonstal (2001)
rpl32-trnL	rpl32-F: CAG TTC CAA AAA AAC GTA CTT C trnL (UAG): CTG CTT CCT AAG AGC AGC GT	95ºC, 2m; 35x (95ºC, 1m; 48ºC, 10s; +17ºC, 0.3ºC/s; 65ºC, 5m); 65ºC, 5m	Shaw *et al.* (2007)
rps16-trnQ (1) For temperate bamboos and *Pharus.*	5' end: 1F: GCA CGT TGC TTT CTA CCA CA 929R: TTC TGT CTA CTC GGC TTT CG 3' end: 538F: CGA CTC GAA TAC CAA AAG AGG 1574R: ATC CTT CCG TCC CAG ATT TT SEQ: (5') 16Q 650R: GTT CGT TGG ATA GAA TGG ATT C (3') 16Q in-for: GCC GAG TAG ACA GAA TAT ATG (3') 16Q 1100R: GGC CAG ATT AAA GAA TAG GAA G	95ºC, 2m; 35x (95ºC, 1m; 48ºC, 10s; +17ºC, 0.3ºC/s; 65ºC, 5m); 65ºC, 5m. Note: For some taxa, also use 628R to sequence the 5' amplicon [see rps16-trnQ (2) for primer sequence]	Triplett 2008 (this study).
rps16-trnQ (2) For all except temperate clade and *Pharus*	1F: GCA CGT TGC TTT CTA CCA CA 1574R: ATC CTT CCG TCC CAG ATT TT SEQ: 334F: CGA GAT GGT CAA TCC TGA AAT G 628R: CTT TTG GTA TTC KAG TCG AAG	95ºC, 2m; 35x (95ºC, 1m; 50ºC, 10s; +15ºC, 0.3ºC/s; 65ºC, 5m); 65ºC, 5m.	Triplett 2008 (this study).
trnC-rpoB	trnC: TGG GGA TAA AGG ATT TGC AG rpoB*: ATT GTG GAC ATT CCC TCR TT SEQ: jt400-for: CAG GTC CGA ACA GCA TTA jt700-rev*: CGT AGT AGT AGA ATT GCT AG	94ºC, 2m; 35x (96ºC, 1m; touchdown* 56-46ºC, 2m; 72ºC, 3m); 72ºC, 5m. *For Olyreae & non-bamboo, use the "trnC" primer for SEQ 5' end.	PCR: Yamane & Kawahara (2005); rpoB revised for this study. SEQ: Triplett 2008 (this study).
trnD-trnT	trnD-for: ACC AAT TGA ACT ACA ATC CC trnT-rev: CCC TTT TAA CTC AGT GGT A SEQ: trnY-rev: CTC TTT GCT TTG GAT CTA G trnE-for: GCC TCC TTG AAA GAG AGA TG	94ºC, 2m; 35x (94ºC, 45s; touchdown* 58-48.5ºC, 1m; 72ºC, 1m15s); 72ºC, 5m	trnD-for: Demesure *et al.* (1995); trnT-rev, trnY-rev: Triplett 2008 (this study); trnE-for: Doyle *et al.* (1992)
trnG intron	5'trnG: GCG GGT ATA GTT TAG TGG TAA AA (52.5ºC) 3'trnG: GTA GCG GGA ATC GAA CCC GCA TC (62.1ºC)	95ºC, 2m; 35x (95ºC, 1m; 50ºC, 10s; +15ºC, 0.3ºC/s; 65ºC, 5m); 65ºC, 5m	Shaw *et al.* (2005)
trnH-psbA	trnH: CGC GCA TGG TGG ATT CAC AAT CC (61.1ºC) psbA*: GTW ATG CAY GAA CGT AAT GCT C (53.3ºC)	80ºC, 5m; 35x (94ºC, 30s; touchdown* 58-48ºC, 30s; 72ºC, 1m); 72ºC, 10m	trnH: Tate & Simpson (2003) psbA: Sang *et al.* (1997); revised for this study
trnK-rps16	trnK: TAC TCT ACC RTT GAG TTA GCA AC (53.1ºC) rps16: AAA GGK GCT CAA CCT ACA RGA AC (57.2ºC)	80ºC, 5m; 35x (94ºC, 30s; touchdown* 58-48ºC, 30s; 72ºC, 1m); 72ºC, 10m	Johnson & Soltis (1995); Kress *et al.* (2005)
trnT-trnL	trnT-L F: CAT TAC AAA TGC GAT GCT CT (51.0ºC) trnT-L R: TCT ACC GAT TTC GCC ATA TC (51.8ºC)	95ºC, 2m; 35x (95ºC, 1m; 48ºC, 10s; +17ºC, 0.3ºC/s; 65ºC, 5m); 65ºC, 5m	Taberlet *et al.* (1991)
trnV-ndhC	trnV (UAC) x2: GTC TAC GGT TCG ART CCG TA ndhC (for): TAT TAT TAG AAA TGY CCA RAA AAT ATC ATA TTC	95ºC, 2m; 35x (95ºC, 1m; 48ºC, 10s; +17ºC, 0.3ºC/s; 65ºC, 5m); 65ºC, 5m	Shaw *et al.* (2007)

Table 2. Statistics and evolutionary models for separate and combined analyses. Statistics for individual regions are based on the 12-region, 30-taxon data matrix. PIC = parsimony informative characters. PIC parenthetical indicates the number of parsimony informative characters within the temperate clade. MP = maximum parsimony; CI = consistency index, excluding uninformative characters; RI = retention index. Models are based on the Hierarchical Likelihood Ratio Test implemented in ModelTest.

Partition	DNA	Indels	Total char.	Char, no gaps	PIC	MP Trees	MP Length	CI	RI	Model
atpI-atpH	969	10	979	400	32 (1)	5	106	0.7556	0.8854	HKY
ndhF (3' end)	1135	1	1136	1111	62 (6)	10	259	0.595	0.6597	HKY+G
psaA-ORF170	1030	4	1034	845	48 (11)	14	152	0.6988	0.8512	K81uf+G
rpl32-trnL	1037	1	1038	438	43 (8)	8	174	0.6854	0.8272	HKY+G
rps16-trnQ	1945	13	1958	731	80 (13)	8	263	0.6715	0.8069	TVM+G
trnC-rpoB	1562	11	1573	802	59 (14)	4	198	0.7692	0.9023	TVM+G
trnD-trnT	1426	8	1434	1030	73 (7)	30	261	0.6984	0.839	HKY+G
trnG intron	777	2	779	659	32 (5)	36	116	0.6792	0.8661	HKY+G
trnH-psbA	698	2	700	610	19 (2)	12	69	0.6486	0.6977	HKY+I+G
trnK-rps16	740	3	743	448	39 (8)	5	146	0.6667	0.8182	HKY+G
trnT-trnL	992	5	997	561	46 (7)	18	162	0.7027	0.8406	TVM+G
trnV-ndhC	1211	7	1218	477	39 (10)	213	144	0.7385	0.8741	K81uf+G
12-region, all data	13522	67	13589	8161	582 (91)	6	2030	0.6718	0.8153	--
12-region, DNA	13522	--	13522	8045	518 (78)	4	1999	0.6593	0.7965	TVM+I+G
12-region, indels	--	67	67	--	54 (14)	1	71	0.9153	0.9784	--
4-region, all data	6255	57	6312	2784	439 (118)	768	1160	0.7031	0.8742	--
4-region, DNA	6255	--	6255	2727	383 (86)	576	1099	0.6846	0.8552	TVM+G
4-region, indels	--	57	57	--	56 (29)	18	60	0.9333	0.9853	--

performed in a Perkin-Elmer Applied Biosystems GeneAmp PCR System 9600 thermocycler or a MJ Research PTC-200 thermal cycler. Reactions were carried out in 40 µl volumes. Amplification products were cleaned using Antarctic phosphatase (5 units, NEB) and exonuclease I (10 units, NEB) followed by an ethanol precipitation. Sequencing reactions were carried out using BigDye v.2 to produce complementary strands, and sequence products were cleaned using Edge Biosystems clean-up plates. Sequencing was performed by the Automated 3730xl DNA Analyzer (Perkin-Elmer, Applied Biosystems Division) at the Iowa State University DNA Sequencing and Synthesis Facility. Sequences were assembled, verified, and manually aligned using the program Se-Al version 2.09a (Rambaut 2001). Sequence alignment introduced gaps that later were treated as binary, presence/absence characters (Giribert and Wheeler 1999). If a deletion occurred in a region that otherwise included informative point mutations, the missing nucleotide in the informative region was coded as a missing character. Autapomorphic, parsimony uninformative indels were not scored, and were excluded along with other gaps prior to analysis. All data matrices used in this study will be made available in TreeBASE and all individual sequences will be submitted to Genbank.

Phylogenetic analysis. All data were analyzed with maximum parsimony (MP), maximum likelihood (ML), and Bayesian Inference (BI). MP and ML analyses were conducted using PAUP* 4.0b10 (Swofford 2002), and BI was conducted using MrBayes 3.1 (Ronquist *et al.* 2005). Before combining cp DNA datasets, we implemented a Partition Homegeneity test (ILD, Farris *et al.* 1994) in PAUP* to evaluate congruence among regions, using 1,000 iterations in a full heuristic search and random taxon addition. Maximum parsimony analyses used the heuristic search option with 1,000 random-addition-sequence (RAS) replicates and tree bisection and reconnection (TBR) branch swapping. Strict consensus trees were calculated for all MP analyses, and branch support was estimated with 1,000 bootstrap replicates (Felsenstein 1985) using heuristic searches as described above. The hierarchical likelihood ratio test, as implemented in Modeltest 3.6 (Posada and Crandall 1998), was employed to determine the appropriate model of sequence evolution for each DNA partition and for each combined dataset, excluding indels (Table 2). Maximum Likelihood (ML) parameter values were optimized using a BioNJ tree as a starting point

(Gascuel 1997) with the appropriate model parameters for the combined datasets. ML analyses used the heuristic search option with 1,000 RAS replicates and TBR branch swapping. ML bootstrap analyses (MLBS) comprised 100 replicates, each with two RAS replicates. Bayesian Inference (BI) was conducted using MrBayes 3.0b4 with flat priors. Nucleotide data from different DNA regions were treated as separate partitions, and binary coded indels were combined into a single partition. In all searches, three heated chains and a single cold chain were used, and runs were initiated with random trees. Chains were run for 10 million generations, and trees were sampled every 1000 generations. A majority-rule consensus of the remaining trees was calculated to obtain a topology and posterior probabilities (PP). BI searches were repeated three times in order to confirm that searches converged on the same topology.

Conflict among the resultant phylogenies was assessed according to a 70% bootstrap criterion for both MP and ML, and a 0.95 posterior probability measure for BI (Mason-Gamer and Kellogg 1996; Wilcox *et al.* 2002). Values less than 70% MPBS/MLBS and less than 0.95 PP were regarded as lacking support.

Additionally, we undertook two distance analyses using the neighbor-joining (NJ) algorithm (Saitou and Nei 1987) as implemented in PAUP*. The first analysis included nucleotide and binary indel data, and standard distances were calculated. The second analysis was restricted to nucleotides and distances were computed using the general time reversible (GTR) model. For both analyses, bootstrap values were calculated using 10,000 replications.

We tested whether the combined datasets provided sufficient evidence to reject particular hypotheses of relationship suggested by previous classifications (*e.g.*, monophyly of subtribes Arundinariinae, Thamnocalaminae, and Shibataeinae, monophyly of Bambuseae, etc.). Constraint trees were constructed in MacClade 4.08 (Maddison and Maddison 2005) by forcing test groups to be monophyletic, but otherwise allowing taxa to "float," and MP analyses were performed in PAUP* using each constraint in turn. Both Templeton and the Kishino-Hasegawa tests, as implemented in PAUP*, were then used to test the significance of differences in tree statistics amongst different topologies in comparison with the MP topologies.

After completing the four-region analysis of the large dataset, we established a criterion-based checklist to select taxa for the twelve-region analysis: (1) include the type species for each subtribe (Arundinariinae, Thamnocalaminae, and Shibataeinae); (2) choose one taxon to represent each major cp DNA type (essentially a consensus sequence); (3) include representatives of each major *Arundinaria* subclade; and (4) include other taxa of interest as revealed by the four-region analysis (*e.g.*, putative hybrids; novel placements).

RESULTS

A total of 716 sequences were generated for this study, including duplicates and alternate accessions to test the unexpected placement of certain species. Table 2 summarizes statistics for each of the twelve regions and combined data sets (PIC, trees, length, CI, RI, models of evolution, etc.). Additional information about each of the 12 regions will be discussed as part of a larger study on 20 non-coding cp DNA regions in Bambusoideae (Triplett *et al.,* in prep.). The ILD test indicated that cp DNA regions were highly congruent ($p = 0.76$ in the four-region dataset, $p = 0.67$ in the twelve region data set). The chloroplast genome is highly conserved within the temperate clade, and divergence values are particularly low. The maximum divergence between two taxa within the temperate clade was 0.79%, in contrast to 3.25% between two Neotropical bamboos [*Guadua angustifolia* Kunth and *Neurolepis elata (*Kunth) Pilger], and 4.70% between two herbaceous bamboos (*Pariana radiciflora* Sagot ex Doell and *Sucrea maculata* Soderstrom).

Sequences/Matrices. The four-region combined alignment was 6255 base pairs in length and required 57 indels. 383 of the nucleotide characters were parsimony informative (6.1% of the total) and 56 of the indels were parsimony informative (98.2%), for a total of 439 parsimony informative characters. Pairwise comparisons of the nucleotide sequences among temperate species indicated a range of sequence divergence values from 0% to 0.79%. The twelve-region combined alignment was 13522 base pairs in length and required 67 indels. 518 of the nucleotide characters were parsimony informative (3.8% of the total), and 54 of the indels were parsimony informative (80.6%). Pairwise comparisons among the temperate species indicated a range of divergence values from 0% to 0.66%.

Four-region analyses. MP analysis of the combined four-region data set recovered 768 shortest trees of 1160 steps, with a consistency index (CI) of 0.7031 (uninformative characters excluded) and a retention index (RI) of 0.8742. The strict consensus tree is shown in Fig. 1. The ML analysis found a single optimal tree (-lnL = 10487.30010). The majority-rule consensus trees from the Bayesian analysis were consistent with the MP and ML analyses with respect to robust lineages, but also provided robust support for several weakly supported clades in the MP and ML analyses. Maximum parsimony bootstrap (MPBS), maximum likelihood bootstrap (MLBS), and Bayesian posterior probability (PP) values are shown on the MP strict consensus tree in Fig. 1.

In the four-region analysis, Ehrhartoideae plus *Streptogyna americana* C.E. Hubb was recovered as monophyletic (86% MPBS, 100% PP) and sister to Pooideae + Bambusoideae, while Pooideae was resolved as sister to the bamboos (86% MPBS, 100% PP). Within the true bamboos, herbaceous bamboos (Olyreae) paired with neotropical and paleotropical woody bamboos (98% MPBS, 100% PP), and this clade was sister to the temperate species (99% MPBS, 100% PP), resulting in a paraphyletic woody bamboo tribe (Bambuseae). Consistent with previous studies, the temperate bamboos were strongly supported as monophyletic (100% MPBS, 100% PP). Although branching order is unresolved, six principal lineages can be discerned within the temperate clade, four of which are relatively narrow in scope while two represent the majority of diversity in the temperate clade. The six lineages are designated the Bergbamboes Lineage [*i.e.*, *Thamnocalamus tessellatus* (Nees) Soderstrom & Ellis], the African Alpine Bamboo Clade, the *Chimonocalamus* Clade, the *Shibataea* Clade, the *Phyllostachys* Clade, and the *Arundinaria* Clade (Fig. 1). *Thamnocalamus tessellatus* was resolved as a monotypic lineage, with insufficient resolution to support an association with any of the remaining taxa in the temperate clade. *Yushania alpina* (K. Schumann) Lin (East Africa) and *Yushania ambositrensis* (A. Camus) Ohrnb. (Madagascar) represent a distinct lineage (98% MPBS, 100% PP), as does *Chimonocalamus* of Southwestern China (100% MPBS, 100% PP). *Shibataea*, *Ferrocalamus*, *Pseudosasa gracilis* S.L. Chen & G.Y. Sheng and Chinese accessions of *Sasa* cluster in a moderately well-supported clade (86% MPBS, 100% PP). The remaining accessions fall within two large subclades: one encompassing *Phyllostachys* and

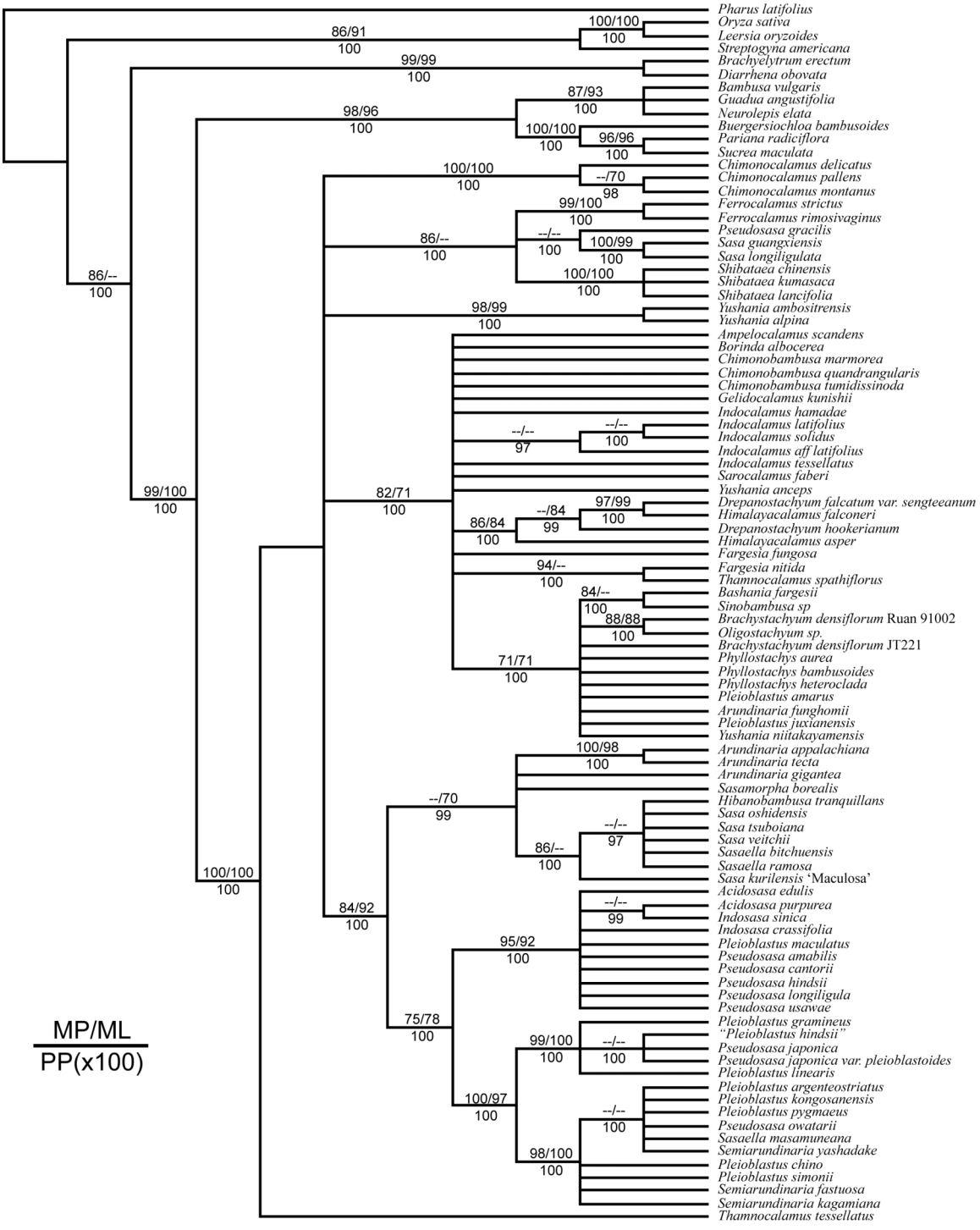

Figure 1. Strict consensus of 768 equally most parsimonious trees based on the 4-region dataset (*rps16-trnQ, trnC-rpoB, trnD-trnT, trnT-trnL).* Numbers above lines indicate bootstrap values (MP/ML). Numbers below the line indicate posterior probabilities from the Bayesian analysis.

17 allied genera (82% MPBS, 100% PP), and one containing *Arundinaria* and nine allied genera (84% MPBS, 100% PP).

Little resolution is apparent within the *Phyllostachys* Clade, reflecting cp DNA sequences that are very similar among all members of this group. However, several clusters can be identified. A clade for three accessions of *Indocalamus* was recovered (97% PP), leaving the positions of *I. hamadae* (Hatushima) Stapleton and *I. tessellatus* (Munro) P.C. Keng unresolved. *Drepanostachyum* and *Himalayacalamus* form a clade with moderate support (86% MPBS, 100% PP), although the recovered topology suggests that neither of these is monophyletic. The cp DNA haplotype of *Fargesia nitida* (Mitf.) P.C. Keng was revealed to be more similar to *Thamnocalamus spathiflorus* (Trin.) Munro than to *F. fungosa* Yi. The genus *Phyllostachys* is resolved as part of a subclade with *Bashania*, *Sinobambusa*, *Brachystachyum*, *Oligostachyum*, *Arundinaria sensu lato*, *Pleioblastus*, and *Yushania* (71% MPBS, 100% PP). DNA sequences among these accessions are nearly identical, however a single point mutation unites *Bashania fargesii* (Camus) P.C. Keng & Yi and *Sinobambusa sp.* (82% MPBS, 100% PP). Similarly, the wild accession of *Brachystachyum densiflorum* (Rendle) Keng and *Oligostachyum sp*. [cultivated in the US, collected as *P. oleosus* Wen] form a pair (82% MPBS, 100% PP), to the exclusion of *B. densiflorum* from material in cultivation.

Several moderate- to well-supported clades can be identified within the *Arundinaria* Clade. Three primary clusters represent the following subclades: (1) the *Sasa*-Cane Clade (99% PP), containing *Arundinaria sensu stricto* (N. America) plus *Sasa s.s.* (Japan) and allies (*Sasamorpha* Nakai, *Sasaella*, and *Hibanobambusa*); (2) the *Sinicae* Clade (95% MPBS, 100% PP), with *Acidosasa*, *Indosasa,* and Chinese representatives of *Pleioblastus* and *Pseudosasa*; and (3) the *Pleioblastus s.s.* Clade (100% MPBS, 100% PP), containing *Pleioblastus s.s.* (Japan archipelago), within which were nested the Japanese taxa *Pseudosasa japonica (*Siebold & Zuccarini ex Steudel) Makino ex Nakai, *P. owatarii* (Makino) Makino ex Nakai, *Semiarundinaria*, and one accession of *Sasaella*. Additional relationships can be discerned within each of these subclades. *Sasa s.s.* is moderately supported as a monophyletic group with *Sasa kurilensis* (Ruprecht) Makino & Shibata sister to the other species and *Hibanobambusa tranquillans* (Koidzumi) Maruyama & H. Okamura plus two

accessions of *Sasaella* nested within. *Arundinaria tecta* (Walt.) Muhl. and *A. appalachiana* Triplett, Weakley & L.G. Clark are resolved as sister species (the Switchcane Clade), however, relationships among *A. gigantea (*Walt.) Muhl.*,* the Switchcane Clade, *Sasamorpha*, and the *Sasa s.s.* Clade were unresolved. Relationships within the *Sinicae* Clade are largely unresolved, with the exception of several species pairs indicating identical cp DNA haplotypes [*e.g.*, *Acidosasa purpurea* (Hsueh & Yi) P.C. Keng and *Indosasa sinicae* C.D. Chu & C.S Chao]. The *Pleioblastus* s.s. Clade is resolved with two robust lineages: one consisting of *P. linearis (*Hackel) Nakai and allies (the Ryūkyū Clade, 99% MPBS), and one consisting of the *P. argenteostriatus* (Regel) Nakai*, P. simonii* (Carrière) Nakai and allies (the *Nezasa/Medakea* Clade, 98% MPBS).

Combined 12-region analyses. The MP analysis of the twelve-locus combined data set recovered 6 most parsimonious trees (2130 steps, CI= 0.6718, RI=0.8153), while ML found seven optimal trees (-lnL = 22650.48025) and BI resulted in a well-resolved majority-rule consensus tree. Maximum parsimony bootstrap (MPBS), maximum likelihood bootstrap (MLBS), and Bayesian posterior probability (PP) values are shown on the MP strict consensus tree in Fig. 2.

Relationships revealed for the major clades within Bambusoideae are consistent with the four-region analysis and provide clear evidence for the monophyly of temperate bamboos (100% MPBS, 100% PP) as well as the sister relationship between Olyreae and tropical woody bamboos (100% MPBS, 100% PP). The expanded sequence dataset provides robust support for the *Phyllostachys*, *Shibataea*, and *Arundinaria* clades (100% MPBS and PP for all), and reinforces the distinctiveness of lineages represented by *T. tessellatus*, *Y. alpina*, and *C. pallens*. However, increased sampling from 6255 bp to 13534 bp was unsuccessful at resolving branching order among the six major lineages within the temperate clade. Putative associations are indicated in some topologies (*e.g.*, a sister relationship between *Chimonocalamus* and the *Yushania alpina* clade in the MP and BI consensus trees), but these relationships receive inconsistent support. Comparing the six shortest MP trees indicates conflicting placements for *T. tessellatus* and the *Shibataea* Clade, such that if one is resolved as sister to the remainder of the Temperate Clade the other is sister to the *Arundinaria* Clade,

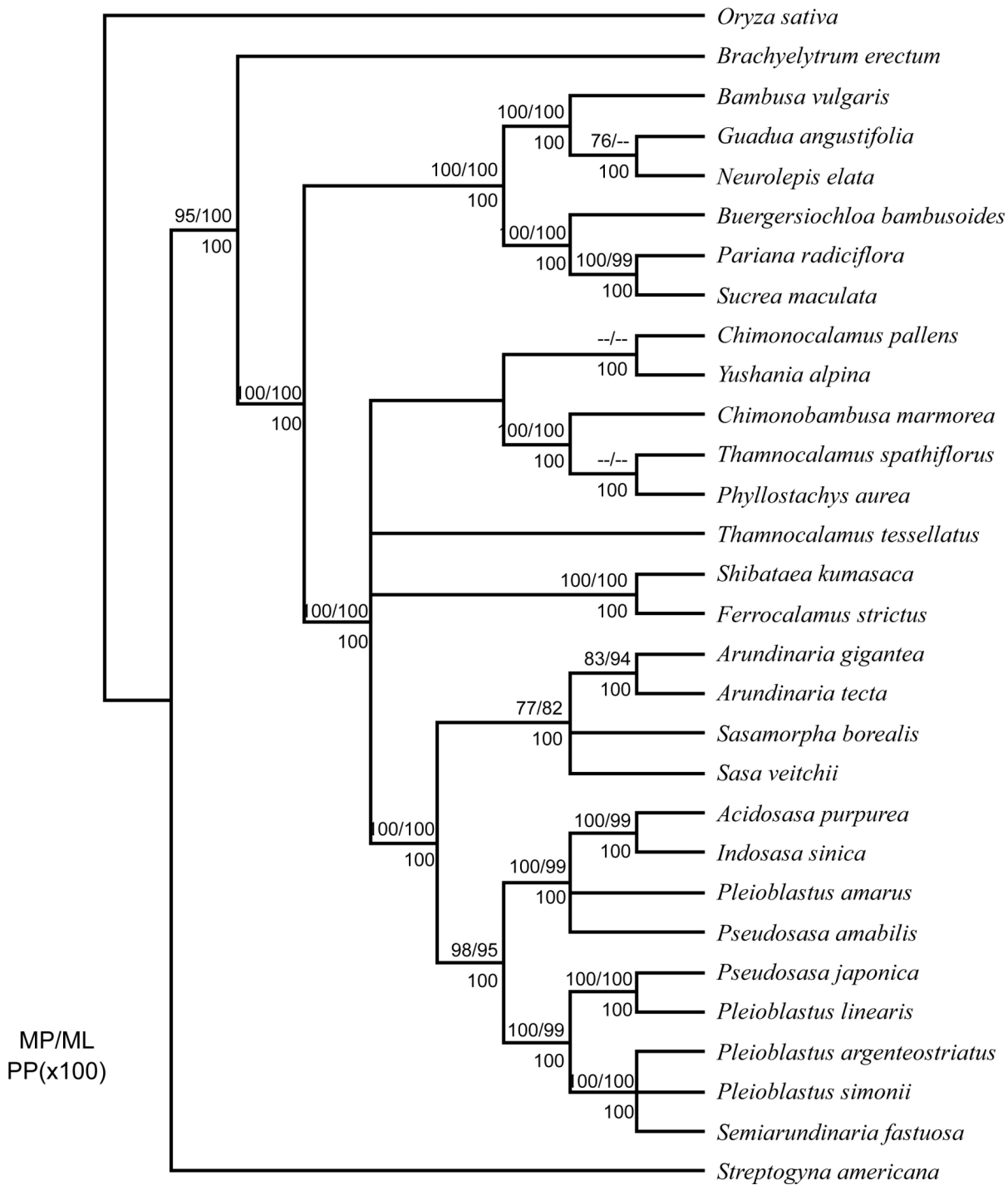

Figure 2. Strict consensus of 6 equally most parsimonious trees based on the 12-region dataset. Numbers above lines indicate bootstrap values (MP/ML), and numbers below lines indicate posterior probabilities from the Bayesian analysis.

or else both occur at the base of the *Arundinaria* Clade. Tests in which either lineage was excluded failed to provide a robust placement for the other lineage.

Several subclades received increased support in the twelve-region analysis. In particular, relationships within the *Arundinaria* Clade are more robust. The *Arundinaria/Sasa* group received increased support (77% MPBS, 100% PP), and the monophyly of *Arundinaria sensu stricto* is also supported (83%MPBS, 100% PP). The placement of *Sasamorpha borealis* (Hackel) Nakai is variable among MP trees, either sister to *Arundinaria s.s.* or in a polytomy with *Arundinaria s.s.* and the *Sasa s.s.* Clade. Robust support was revealed for the relationship between the *Sinicae* Clade and the *Pleioblastus* s.s. Clade (98% MPBS, 100% PP), as well as the monophyly of each group (100% MPBS and PP). Within the *Pleioblastus* group, Ryūkyū and *Nezasa/Medakea* clades both receive strong support (100% MPBS and PP), and the nested placements of *Pseudosasa japonica* and *Semiarundinaria* are confirmed.

Hypothesis Testing. The results of the Kishino-Hasegawa tests are summarized in Table 3. In both four-region and twelve-region analyses, our data reject the monophyly of the woody bamboos, *i.e.,* the hypothesis that Olyreae is sister to Bambuseae. Data also reject the monophyly of Arundinariinae, Shibataeinae, and Thamnocalaminae, including the putative sister relationship between Arundinariinae and Thamnocalaminae. Our data reject the monophyly of *Pleioblastus sensu lato (*including sect. *Amari*) and *Pleioblastus sensu stricto*. It is not possible to reject the hypothesis that the *Sasa s.s.* Clade (with or without *Sasamorpha*) is sister to all other members of the *Arundinaria* Clade.

DISCUSSION

The molecular topology recovered in the current study provides a number of new insights on bamboo evolution. Among these, several important observations can be noted regarding the closest living relatives of bamboo. Controversy has surrounded the identity of the sister group of the Bambusoideae. Early results suggested Ehrhartoideae (Rice and allies; GPWG 2001), although data were equivocal, suggesting a possible relationship with Pooideae. In our analysis, the sampled representatives of Pooideae (*Brachyelytrum* and *Diarrhena*) were clearly resolved as sister to Bambusoideae. Further analysis with additional

taxa is necessary, but this conclusion can be considered robust based on the increased number of parsimony informative characters (PICs).

Table 3. Hypotheses regarding clades and relationships among them. All hypotheses were tested under Maximum Parsimony using the Kishino-Hasegawa test. We report the difference between the MP trees and those consistent with the constraint (percent difference is calculated relative to lengths of the MP trees).

Hypothesis	Results of Test
Olyreae and *Bambuseae* are sister taxa	4-region: Reject (+13 steps, 1.1%, t = 3.1580, $p = 0.0016$) 12-region: Reject (+20 steps, 0.9%, t = 3.9258, $p = 0.0001$)
*Arundinariinae (*A), *Shibataeinae* (S), and *Thamnocalaminae (*T) are each monophyletic	4-region: Reject (+51 steps, 4.4%, t = 6.3706, $p < 0.0001$) 12-region: Reject (+78 steps, 3.7%, t = 8.7610, $p < 0.0001$)
Arundinaria + *Thamnocalaminae* sister to *Shibataeinae* (and each subtribe is monophyletic)	4-region: Reject (+51 steps, 4.4%, t = 6.2734, $p < 0.0001$) 12-region: Reject (+79 steps, 3.7%, t = 8.8190, $p < 0.0001$)
Pleioblastus sensu lato is monophyletic	4-region: Reject (+35 steps, 3.0%, t = 5.7874, $p < 0.0001$)
Pleioblastus sensu stricto is monophyletic	4-region: Reject (+11 steps, 0.9%, t = 3.3226, $p = 0.0009$)
The *Sasa s.s. Clade* is sister to all other members of the *Arundinaria* Clade	4-region: Cannot reject (+1 steps, 0.1%, t = 0.5773, $p = 0.5638$) 12-region: Cannot reject (+1 steps, 0.0%, t = 0.4472, $p = 0.6547$)
The *Sasa s.s. Clade* + *Sasamorpha* is sister to all other members of the *Arundinaria* Clade	4-region: Cannot reject (+1 steps, 0.1%, t = 0.5773, $p = 0.5638$) 12-region: Cannot reject (+2 steps, 0.0%, t = 1.4143, $p = 0.1573$)

This investigation confirms earlier molecular studies demonstrating the monophyly of the temperate bamboos (Zhang 1996; Kelchner and Clark 1997; Zhang and Clark 2000; Ní Chonghaile 2002; Clark *et al.* 2007). Moreover, the current study provides evidence that the temperate clade is sister to other members of Bambusoideae, including the herbaceous bamboos, thus confirming the paraphyly of the woody bamboos (tribe Bambuseae). The position of Olyreae has been controversial since the earliest molecular analyses of Bambusoideae. In combined analyses of morphological and molecular data, herbaceous bamboos are resolved outside of the woody bamboos as a consequence of the derived morphological characters that define the woody bamboos. However, molecular studies were equivocal and hinted at the possible position of Olyreae within the woody bamboos. The current analysis provides the strongest support to date for this nested relationship, with full

support for each branch in the topology of these groups. Accordingly, herbaceous bamboos (tribe Olyreae) can be interpreted as a group that secondarily lost the character states that define the woody bamboos. This relationship is currently under investigation with broader taxon sampling (Bamboo Phylogeny Group, in prep.).

Six major lineages within the temperate bamboos were revealed in the current study. As such, this marks the first analysis to provide a robust framework for the temperate clade. Surprisingly, the species examined in this study assemble into lineages that are largely incongruent with historic taxonomic circumscriptions, providing evidence that current subtribes and many genera are not natural groups. That the recovered phylogeny bears no resemblance whatsoever to published classifications highlights the problematic nature of morphology in this group.

Neither Arundinariinae nor Shibataeinae formed a monophyletic group in the cp DNA phylogeny. Moreover, the *Thamnocalamus* group (subtribe Thamnocalaminae) does not appear to be a cohesive lineage. None of the species placed in the Thamnocalaminae or any of its genera appear to be close to *Arundinaria*, contrary to classifications that subsume the *Thamnocalamus* group under the Arundinariinae. No published molecular evidence contradicts our findings regarding Arundinariinae or Shibataeinae, however, Guo *et al.* (2002, 2004) concluded that the *Thamnocalamus* group (*i.e.*, Thamnocalaminae) was monophyletic based on ITS and GBSSI. However, those studies included only two outgroup taxa, and the apparent monophyly of the group may be an artifact of the long branch separating the selected outgroup taxa from the ingroup. The topology of the ingroup was largely unresolved. The classification system of Keng and Wang in FRPS (1996) recognizes more subtribes within the woody bamboos, including separate groups for *Phyllostachys* (Phyllostachydinae P.C. Keng) and *Shibataea* (Shibataeinae). However, their system was based on over-arching supertribes that separate semelauctant and iterauctant bamboos, resulting in tropical and temperate bamboos within the same groups; these conclusions are not supported by the current study or previous molecular analyses. Based on the recovered phylogeny, we recommend that current subtribal classifications be abandoned within the temperate bamboos.

Prior to this analysis, only morphological evidence has been available to infer relationships among the temperate bamboos, although no cladistic analysis has been conducted using morphology. This molecular study suggests that charismatic characters used for higher-level taxonomy of the temperate bamboos (*i.e.*, subtribes, genera) may not track phylogeny directly (but see below, regarding implications of reticulate evolution). In fact, this conclusion is consistent with the problems associated with classifying taxa according to strict interpretations of morphological variation, as demonstrated by the conflicting opinions regarding the placement of certain species with hard-to-interpret structures, and the complex synonymy of taxa that are vegetatively similar but differ in reproductive morphology. For example, *Chimonobambusa, Indosasa, Phyllostachys, Semiarundinaria,* and *Shibataea* are interpreted by some authors to have bracteate inflorescences, and have been interpreted as a natural group (Shibataeinae) for this reason. However, *Chimonobambusa* is interpreted by some authors as being either semelauctant (McClure 1966; Soderstrom and Ellis 1987; Stapleton 1994), or weakly iterauctant (Clayton and Renvoize 1986), while Li (1999) interpreted the bracts on this genus to represent an intermediate state between the two inflorescence types. *Acidosasa* and *Indosasa* are very similar in vegetative morphology (Li 1999), but belong to different subtribes on the basis of reproductive morphology. *Arundinaria sensu lato* (*i.e., Pleioblastus*, *Pseudosasa*) and *Sinobambusa* are also similar vegetatively, but fall in different groups based on reproductive structures. Morphological characters are certainly of value from the perspective of taxonomy, but first it may be necessary to remove confounding factors of reticulate evolution, convergent evolution, and other sources of apparent homoplasy so that homologous structures can be accurately interpreted.

The current data provide tests of the monophyly of 22 temperate genera (*i.e.*, those for which at least two species were sampled). Of these, four genera were strongly supported as monophyletic, while two were resolved as conditionally monophyletic. Data clearly support the monophyly of *Chimonocalamus* (MPBS/4 = 100%), *Ferrocalamus* (MPBS/4 = 100%), *Shibataea* (MPBS/4 = 100%), and *Arundinaria s.s.* (MPBS/12 = 83%). The Japanese *Pleioblastus s.s.* is the core of a lineage that includes *Semiarundinaria*, *Sasaella*, and *Pseudosasa* subgenus *Pseudosasa*. Similarly, *Sasa s.s.* is the core of a lineage containing

Hibanobambusa and *Sasaella*. Depending on the status of the nested genera, *Pleioblastus s.s.* and *Sasa s.s.* may emerge as monophyletic genera. With the exception of *Pseudosasa*, each of these nested taxa has been suggested to be of hybrid origin; if they are hybrids, their placement in the cp DNA topology indicates their maternal lineage. Additional work is currently underway to test these hypotheses using AFLP data (Triplett and Clark, in prep.; Ch. 5). Another monophyletic group is indicated by taxa currently classified as *Sasa guangxianesis, S. longiligulata,* and *Pseudosasa gracilis* from Mainland China. Flowers are unknown in the three sampled species, and their taxonomic placements are tentative. Based on the current results, the generic names for these plants are inappropriate, and this cluster represents a monophyletic group that likely warrants recognition as a new genus. This group is currently under investigation by Chun-Xia Zeng and D.Z. Li at the Kunming Institute of Botany, China.

Approximately ten genera are indicated to be polyphyletic or paraphyletic in this study. For example, *Indocalamus* is represented by several lineages within the *Phyllostachys* clade, including unresolved species in the *Phyllostachys* polytomy; it is possible that additional data will resolve this genus as a monophyletic group. *Drepanostachyum* and *Himalayacalamus* cluster in a well-resolved clade, although branching order indicates that these genera are paraphyletic as currently circumscribed. Other examples of polyphyly involving *Fargesia, Thamnocalamus, Yushania, Acidosasa, Indosasa, Pseudosasa,* and *Pleioblastus* are discussed below.

A number of surprising results emerged with strong support; for example, *Shibataea* and *Phyllostachys*, two taxa frequently paired in recent morphological classifications, are here resolved as divergent lineages, while the unusual taxon *Ferrocalamus*, often synonymized with *Indocalamus*, pairs with *Shibataea*. *Thamnocalamus* and presumed relatives are clearly not monophyletic; most are close to *Phyllostachys*, and not *Arundinaria*, while a few form distinct lineages (*e.g.*, *Thamnocalamus tessellatus* in Africa, *Yushania* in Africa, *Chimonocalamus* in southwestern China). These are among some of the more surprising relationships revealed in this study; each is discussed in more detail below. The complex pattern suggested by these results indicates that other phenomena must be

considered and resolved before morphological evolution can be fully appreciated and understood.

Below we discuss the major clades resolved in this study. Each clade can be interpreted to represent a distinct cp DNA lineage, and the current study permits the characterization of these lineages to greater (*e.g.*, the *Arundinaria* Clade) or lesser extents (*e.g.*, the *Phyllostachys* clade). Each lineage has accumulated synapomorphic molecular changes since diverging, but branching order remains a mystery. Accumulated changes have the potential to create long-branch attraction among the six lineages; a few weakly supported associations may prove to be of phylogenetic significance (*e.g.*, *Chimonocalamus* and the African Alpine Bamboo clade), but the nature of the data, with relatively few PIC and the high potential impact of homoplasy, has warranted a conservative interpretation.

Bergbamboes Lineage— [1 spp.] The plants known as *bergbamboes* (Afrikaans) or South African Mountain bamboos (*Arundinaria tessellata, Thamnocalamus tessellatus*) occur in mountains spanning Cape, Natal, Orange Free State, and Lesotho at elevations of 1200-2700 m, where they are common along stream edges or in sheltered ravines (Ohrnberger 1999). This species was tentatively placed in *Thamnocalamus* by Soderstrom and Ellis (1982) on the basis of rhizome morphology, vegetative branch architecture, and bracteate spikelets. Other features of their gross morphology, including inflorescence architecture, and more recent studies of leaf anatomy suggest these taxa are not particularly close (Stapleton 1991; Campbell, unpubl.), and the current molecular results confirm that Bergbamboes are a distinct lineage. Bergbamboes have a disjunct distribution among temperate bamboos, and cp DNA sequence data suggest a relatively high divergence from other lineages, although insufficient informative molecular characters are available to determine the closest relatives among the remaining lineages. The position of *T. tessellatus* in the *Thamnocalamus* group was tested by Guo and Li (2004) using nuclear ITS and GBSSI, but those regions provided insufficient resolution. It is possible that this species is related to *A. ibityensis* from Madagascar, and features of its rhizomes (including air canals) and spikelets suggest a relationship with *A. densifolia* and allies in Sri Lanka and South India (Campbell, unpubl.) but these associations have yet to be tested in a molecular phylogenetic context. Current DNA evidence combined with biogeography and a preliminary review of

morphology warrant the recognition of a distinct taxon, and research is currently underway to describe the new genus (Triplett *et al.*, in prep.).

African Alpine Bamboo Clade— [2+ spp.] Members of the "*Arundinaria*" (or "*Yushania*") *alpina* clade are a dominant constituent of the highlands of Central and East Africa (1800-3900 m) and Madagascar (1300-1400 m). Other allies may include *A. madagascariensis* A. Camus. The taxonomic history of *A. alpina* parallels that of *T. tessellatus*, having a disjunct distribution and unclear affinities among other temperate taxa. *Arundinaria alpina* was associated with the *Thamnocalamus* group (as *Yushania* by Lin 1974; as *Sinarundinaria* by Chao and Renvoize 1989) on the basis of rhizome morphology and inflorescence architecture. Its position in the *Thamnocalamus* group was examined in the analysis of ITS and GBSSI sequences (Guo and Li 2004), which suggested a possible affiliation with the monotypic genus *Gaoligonshania* of northwestern Yunnan, China; however, results were considered equivocal by the authors on the basis of conflicting signal and low support values. The African Alpine Bamboo lineage clearly emerges as distinct in the chloroplast phylogeny. The association between *A. alpina* and *A. ambositrensis* of Madagascar receives robust support in the molecular phylogeny (MPBS = 98%), although chloroplast sequences between these two are moderately divergent, suggesting a genetically diverse group in spite of the narrow taxonomic range (2-4 spp). The East African and Madagascan species are united by their spikelet architecture, but differ in vegetative features such as leaf size, pubescence, auricles and fimbriae (Campbell, unpubl.) loosely consistent with generic-level diversity. *Yushania (Arundinaria) alpina* differs substantially from the other African lineage, the Bergbamboes Clade, in spikelet and inflorescence structure, although similar leaf anatomical features have been noted (Soderstrom and Ellis 1987). Additional research is needed to fully characterize diversity in this clade.

***Chimonocalamus* Clade**— [11 spp.] The Sino-Himalayan genus *Chimonocalamus* emerges as a distinctive monophyletic lineage in the chloroplast phylogeny, reinforcing previous evidence for monophyly based on the nuclear phylogeny (Guo *et al.* 2002; Guo and Li 2004), which recovered a sister relationship with the other members of the *Thamnocalamus* group. *Chimonocalamus* encompasses 11 species in subtropical to warm temperate zones of Southwest China (S. Yunnan), the Eastern Himalayas, and Myanmar.

Additional sampling is necessary to determine affinities among all of the species, particularly the morphologically divergent *C. griffithiana*, but as a whole the group is morphologically coherent. *Chimonocalamus* shares a number of vegetative characters with *Chimonobambusa*, while its reproductive morphology is similar to *Yushania s.s.*; however, the cp DNA phylogeny clearly resolves the latter two genera within the *Phyllostachys* clade. Additional work is needed to characterize the distinctive vegetative branching of *Chimonocalamus*, which appears to initiate from multiple buds per node. The plants are further distinguished by fragrant shoots rich in sesquiterpenoids (Hsueh and Yi 1979) and foliage leaf blades that lack tessellation, unlike most temperate species. Culms often have spiny aerial roots, which are especially dense at lower nodes. *Chimonocalamus* represents an important branch of diversity in the temperate clade and warrants special attention for conservation given its limited distribution. Interestingly, Guo and Li (2004) observed a weak association between *A. alpina* and *Chimnocalamus* in the GBSSI phylogeny; that weak association is also represented in the 12-region cp DNA phylogeny (MPBS = 60%; PP = 100%).

***Shibataea* Clade**— [~15 spp.] Perhaps the most surprising result of this study was the distinct position of *Shibataea* and its relationship with *Ferrocalamus* in Southwestern China and *Sasa*-like species from Southeastern China. The *Shibataea* clade unites an extremely diverse group of plants, many of which merit special attention due to their threatened status. Within this clade, a polytomy of three subclades was recovered: the *Ferrocalamus* subclade (MPBS = 99%), the "*Sino-sasa"* subclade (Chinese *Sasa* plus *Pseudosasa gracilis*), and the *Shibataea* subclade (MPBS = 100%). All three are monophyletic on the basis of DNA evidence, and each is distinguished by unique morphological characters. From a molecular perspective, the *Shibataea* Clade is perhaps the most diverse. Moreover, these plants represent interesting departures from the more characteristic bamboo morphology.

Among temperate lineages, *Shibataea* appears to have the highest number of molecular synapomorphies. Numerous accumulated changes in the chloroplast genome can be interpreted to indicate a relatively long period of separation, perhaps compounded by rate heterogeneity due to a shorter life cycle. In most morphological classifications, *Shibataea* was considered a close relative of *Phyllostachys* based on inflorescence morphology and

vegetative similarities (*e.g.*, flattened rhizome internodes). Before Makino recognized a new genus for these plants (Makino 1912; validly published by Nakai 1933), Munro (1868) and other authors placed these species in *Phyllostachys*. However, in many respects *Shibataea* is very distinct. For example, its foliage leaves lack sheaths, and instead appear to have a true petiole and blade. Keng and Wang (1996) separated *Shibataea* from *Phyllostachys* on the basis of the number of buds (*i.e.*, branches) at mid-culm nodes, with 2 in *Phyllostachys* versus 3-5 in *Shibataea*. Peng *et al.* (in review) found support for a relationship between *Phyllostachys* and *Shibataea* in the ITS and GBSSI phylogeny, but no association between *Shibataea* and *Ferrocalamus*. Moreover, RFLP studies by Watanabe *et al.* (1994) supported a close relationship between *Phyllostachys* and *Shibataea*, however, that clade also included *Sasa veitchii*, which is shown by this study to be unrelated to either lineage. Furthermore, Hodkinson *et al.* (2000) examined these genera using ITS and found no relationship between *Shibataea* and *Phyllostachys*. Clearly, additional work is needed before nuclear regions can be interpreted with confidence.

***Phyllostachys* Clade**.— [~17 genera; ~330 (?) spp.] The clade containing *Phyllostachys* and allies is surprising for a number of reasons. First, the clade is morphologically diverse and species rich, comprising ca. 50% of the temperate genera and, by extension, upwards of 70% of its species. The clade unites members of all three morphology-based subtribes, bringing together plants with true spikelets vs. pseudospikelets, bracteate vs. ebracteate inflorescences, and sympodial, pachymorph rhizomes vs. monopodial, leptomorph rhizomes. In fact, most of the morphological variation used for taxonomy in the temperate bamboos is represented within this one lineage. The clade includes representatives of at least four genera from Shibataeinae (*Phyllostachys*, *Chimonobambusa*, *Sinobambusa*, and *Brachystachyum*, with bracteate iterauctant inflorescences); seven from Thamnocalaminae (*Ampelocalamus*, *Borinda*, *Drepanostachyum*, *Fargesia*, *Himalayacalamus*, *Thamnocalamus*, and *Yushania*, with ebracteate to bracteate semelauctant inflorescences and sympodial, pachymorph rhizomes); and five from the Arundinariinae (*Gelidocalamus*, *Indocalamus*, *Sarocalamus*, *Bashania*, and *Pleioblastus* sect. *Amari*, with semelauctant inflorescences). As such, the clade accommodates most of the genera in Shibataeinae, with the exception of *Hibanobambusa*,

Indosasa, Semiarundinaria, and *Shibataea*, and all of the diversity in Thamnocalaminae with the exception of *Chimonocalamus* and the African/Madagascan species. The combination of Thamnocalaminae and Shibataeinae is especially unexpected, contradicting the predicted relationship between Arundinariinae and the *Thamnocalamus* group. Other surprises concern the placement of *Pleioblastus amarus* within this clade, and the absence of *Semiarundinaria.*

Because this group received less attention in this study (with only 3 species sampled in the 12 region analysis) than the next clade, only broad generalizations can be made about internal relationships. Based on the results of the four-region analysis, the group is remarkable for the constrast of high morphological diversity and low genetic diversity. Sequences in this group are nearly identical, differing by only a few point mutations and indels, most of which are autapomorphic. However, although resolution is poor, sufficient phylogenetic signal allows some subclades to emerge. Two general conclusions can be drawn from the data: (1) the available evidence rejects the monophyly of certain genera as currently defined (*i.e.*, *Fargesia*, *Drepanostachyum*, *Himalayacalamus,* and *Yushania*); and (2) insufficient information is available to support or reject the monophyly of other genera (*i.e.*, *Ampelocalamus*, *Bashania*, *Borinda*, *Chimonobambusa*, *Gelidocalamus*, *Indocalamus, Oligostachyum, Phyllostachys*, *Sinobambusa*, or *Thamnocalamus sensu stricto*). In other words, the available phylogenetic signal in the data is clearly incongruent with morphological taxonomy.

Within this clade, a subclade containing *Phyllostachys* and its closest allies was recovered with moderate support (MPBS = 82%). This cluster includes *Bashania fargesii*, *Sinobambusa sp.*, *Brachystachyum densiflorum*, *Oligostachyum sp.*, *Yushania niitakayamensis*, *Pleioblastus amarus*, and two species from cultivation in the US, currently identified as *Arundinaria funghomii* and *Pleioblastus juxianensis*. Many of these species have morphology that can be interpreted as intermediate between *Phyllostachys* and *Arundinaria sensu lato*. For example, vegetative branching is more complex than *Phyllostachys* with additional compressed internodes and secondary branching, but suggestive of the overall form of *Phyllostachys*. Moreover, these species have more or less sulcate or flattened culms, and intermediate combinations of inflorescence bracts. Could it be that one or more of these taxa represents a hybrid between *Phyllostachys* and an

Arundinaria-like parent? An especially interesting case is presented by the placement of *Brachystachyum* and *Semiarundinaria* in different major clades, as the former is often synonymized under *Semiarundinaria*. Molecular evidence demonstrates that *Brachystachyum* is close to *Phyllostachys*, while *Semiarundinaria* is close to *Pleioblastus*. It thus seems plausible that *Semiarundinaria* and *Brachystachyum* represent natural hybrids between these two parent genera, with the plastid data indicating the maternal lineage of each hybrid. A similar argument could be made regarding the placement of *P. amarus* in this clade, while most of its putative allies are in the *Arundinaria* Clade.

Sampling in the current study was primarily designed to test the position of putative relatives of *Arundinaria,* and the data clearly demonstrate the polyphyly of the *Arundinaria* group and the nested position of the *Thamnocalamus* group within the *Phyllostachys* Clade. Because of the large scope of the *Phyllostachys* Clade, only a small portion of its diversity has been sampled, making it difficult to generalize. Beyond establishing a guide towards further research on this clade, little can be determined about generic level relationships. However, even though resolution is too low to reconstruct internal branching, it is clear that the constituent taxa are closer to *Phyllostachys* than previously recognized.

***Arundinaria* Clade—** [~10 genera; ~130 (?) spp.] Morphology-based taxonomy of *Arundinaria gigantea* and its closest relatives has been especially problematic, and published classifications represent a number of conflicting schemes (Li 1997). Although some authors have advocated a wide interpretation of the genus *Arundinaria* to include *Pleioblastus*, *Bashania*, *Oligostachyum*, and *Sarocalamus* (Chao *et al. 19*80; Clayton and Renvoize 1986; Watson and Dallwitz 1992 onwards; Yang and Zhao 1993, 1994), others recommend narrower generic treatments, and close relationships among segregate genera. The molecular phylogeny that has emerged in this analysis establishes a compelling new hypothesis that is largely inconsistent with published classifications, providing a clear framework for the group but generating a number of new questions.

This study found robust support for a clade consisting of *Arundinaria s.s.*, *Sasa*, *Sasamorpha, Sasaella, Hibanobambusa, Acidosasa, Indosasa, Pleioblastus, Pseudosasa,* and *Semiarundinaria*. Of these, only *Pleioblastus* and *Pseudosasa* were formerly considered close to *Arundinaria*, although *Sasa*, *Sasaella*, *Sasamorpha*, and *Acidosasa* are allied taxa in

the subtribe Arundinariinae (sharing ebracteate, semelauctant inflorescences). The placement of *Semiarundinaria*, *Hibanobambusa* and *Indosasa* within this clade is somewhat surprising, since these taxa have iterauctant inflorescences and corresponding suites of characters that suggested they were closer to *Phyllostachys* and presumed allies.

Within the diverse *Arundinaria* Clade are two major subclades: one encompassing *Arundinaria s.s.*, *Sasa* and allies, and *Sasamorpha (*the *Sasa*/Cane Clade), and the other encompassing *Pleioblastus* and allies in Japan and China (the *Pleioblastus* Clade). Results are inconclusive regarding the branching order among *Arundinaria*, *Sasa*, and *Sasamorpha*, but molecular data clearly distinguish *Arundinaria* from *Pleioblastus*, the genus most often synonymized with *Arundinaria*.

Four distinct lineages were identified within the *Sasa*/Cane Clade, each characterized by unique point mutations and indels: (1) the *A. gigantea* lineage; (2) the *A. appalachiana* + *A. tecta* lineage (the Switchcane Clade); (3) the *Sasa* s.s. Clade (including *Sasa s.s.* in Japan, *Sasaella*, and *Hibanobambusa*); and (4) the *Sasamorpha* lineage. While insufficient informative characters were available in the 4-region analysis to resolve branching order, the 12-region analysis revealed *Arundinaria s.s*. to be monophyletic. Chloroplast DNA evidence suggested a relatively high divergence between *A. gigantea* and the Switchcane Clade, which is especially surprising considering that many authors recognize only a single species of North American cane (Vines 1960; Voight and Mohlenbrock 1964; Radford *et al.* 1968; McClure 1973). In fact, the divergence between *A. tecta* (or *A. appalachiana)* and *A. gigantea* is equivalent to the divergence between *A. gigantea* and the *Sasa s.s.* Clade (0.29%), whereas the maximum divergence values among species of *Sasa* is 0.07%. *Arundinaria appalachiana* and *A. tecta* share a number of morphological features that clearly distinguish them from *A. gigantea*, including terete culm internodes, persistent culm leaves, and 2-5 unexpanded internodes at the base of primary branches (Triplett *et al.* 2006). These observations suggest that *A. gigantea* and the Switchcane Clade represent a relatively ancient divergence.

Sasa is distinguished from most Arundinarioid species by six-stamened flowers and single branches with relatively large foliage leaves. Our analysis recovered a *Sasa s.s.* Clade (excluding *Sasamorpha* and Chinese *Sasas*), within which the putatively hybrid genera

Sasaella and *Hibanobambusa* are nested. *Sasa kurilensis* is recovered as sister to all other sampled taxa in the *Sasa s.s.* Clade, but no additional information on relationships is available. *Sasamorpha* shares a number of key features with *Sasa*, including six-stamened flowers and relatively large leaves, but this genus is distinguished by a number of other differences in habit and vegetative morphology, and there is significant molecular differentiation between *Sasa* and *Sasamorpha*, enough to warrant the recognition of the latter as a distinct genus. *Sasamorpha* has relatively few informative characters that associate it with either *Sasa s.s.* or *Arundinaria*, and sequence variation suggests these three represent divergent lineages. Many of the distinguishing features of *Sasa* are absent in *Sasamorpha*, including prominent, swollen nodes and scabrous fimbriae. However, some taxa in *Sasa* are morphologically intermediate between the extremes of form found in *Sasamorpha* and *Sasa (e.g., Sasa* section *Lasioderma*), blurring the distinction between these two woodland genera. Additional work is needed to fully characterize *Sasamorpha*, especially given its distinct chloroplast haplotype and its potential link with the North American species.

Nakai (1925) recognized *Pleioblastus* to accommodate *Arundinaria*-like species in East Asia, particularly in Japan. The name (Gk. *pleios*, many; Gk. *blastos*, buds) derives from the branch primordium at each node, which ramifies precociously before rupturing the bud scale (prophyll), giving rise to a characteristic branch complement with extreme internodal compression and 3-7 branches per node, each with reiterative higher-order branching. Suzuki (1978) made major contributions to the taxonomy of *Pleioblastus*. Prior to his revision, the genus encompassed 110 Japanese species; he recognized 20, and described three sections on the basis of vegetative morphology. Section *Pleioblastus* contains many of the larger species found in southern Japan, with tillering culms and relatively long leaves. Sections *Medakea* and *Nezasa* contain Japanese species with the monopodial rhizomes and with shorter leaves; the two sections are distinguished by differences in the upper margins of leaf sheaths. A fourth section, *Amari*, has been established to accommodate Chinese species recently transferred to *Pleioblastus*, with approximately 15 species currently assigned to it. The genus currently comprises 42 species and more than 260 named entities (Ohrnberger 1999).

Within the *Pleioblastus s.l.* Clade, our results indicate two major lineages, which are correlated with geography. The *Pleioblastus s.s.* Clade encompasses plants primarily in Japan

(from the Ryūkyū Islands north to Hokkaido) and the *Sinicae* Clade includes a diverse group of arundinarioid or pleioblastoid species in Mainland China and Taiwan.

Pleioblastus s.s. encompasses the three sections recognized by Suzuki (1978). One of these emerges as a distinct lineage (the *Ryūkyū* Clade) with the inclusion of *Pseudosasa japonica*. The other two sections are united in a single clade with limited internal resolution and the nested placement of *Pseudosasa owatarii*, *Semiarundinaria (*6 spp.), and at least one species from the polyphyletic genus *Sasaella (S. masamuneana)*. Within the *Nezasa/Medakea* clade, there is weak support for a clade containing all samples representing sect. *Nezasa* except *P. chino*, which is indistinguishable from *P. simonii* (sect. *Medakea*).

Morphology unites the species of *Pleioblastus* in Japan, but what is the explanation for the nested positions of *Semiarundinaria*, *Sasaella*, and *Pseudosasa*? The two most likely explanations are (1) hybridization or (2) divergent evolution. The suite of characters exhibited by *Semiarundinaria*, including bracteate inflorescences and late-deciduous culm leaves, suggest divergence within this group of species, and yet cp DNA sequences exhibit no variation from their closest relatives in *Pleioblastus*. Based on morphology and results of artificial hybridization experiments, *Semiarundinaria* is considered by some authors to represent a hybrid between *Pleioblastus* and *Phyllostachys*. What is particularly interesting is that minor sequence variation was detected within the *Nezasa/Medakea* group indicating two chloroplast haplotypes, and *Semiarundinaria* has both, consistent with multiple hybrid origins. Interestingly, *Semiarundinaria* sometimes includes *Brachystachyum* of China. Both genera are intermediate between *Phyllostachys* and *Arundinaria*, but current evidence demonstrates that *Brachystachyum* belongs in the *Phyllostachys* clade; if *Semiarundinaria* is a hybrid between *Pleioblastus* and *Phyllostachys* in Japan, perhaps *Brachystachyum* represents a similar a reciprocal cross between a pleioblastoid species and *Phyllostachys* in China.

Reconciling *Pseudosasa* within *Pleioblastus* is more difficult. *Pseudosasa japonica* shares a number of morphological characters with *P.* section *Pleioblastus*, and its placement is not necessarily inconsistent with morphology. At least one other species in section *Pleioblastus* exhibits reduced secondary branching (*P. gozadakensis*, adapted to harsh environments at higher elevations), and the morphology exhibited by *P. japonica* may

represent reductions relative to the typical forms in *Pleioblastus*. Other characters exhibited by *P. japonica*, such as stamen number (3-5), are more divergent. The spectrum of intergrading morphological traits exhibited in this species is also consistent with the hypothesis of hybridization, although such a relationship has never been proposed for *Pseudosasa japonica*.

Hibanobambusa and *Sasaella* in the *Sasa* Clade pose similar problems. The cp DNA of these species is identical to *Sasa veitchii*, yet morphologically these taxa are intermediate between divergent genera (*Hibanobambusa* is intermediate between *Phyllostachys* and *Sasa*; *Sasaella* is intermediate between *Pleioblastus* and *Sasa*). If the hybridization hypothesis is correct, the intergrading morphology (and apparent polyphyly of *Sasaella, Pseudosasa, and Semiarundinaria*) is explained by genetic admixture.

The Chinese representatives of *Pleioblastus* and *Pseudosasa* were demonstrated to be a polyphyletic group, with several species of *Pleioblastus* nested within the *Phyllostachys* clade, and one species of *Pseudosasa* nested in a novel group with *Shibataea* and *Ferrocalamus*. Other representatives of these taxa nested together with *Acidosasa* and *Indosasa* in the *Sinicae* Clade, which forms the sister group to *Pleioblastus* and allies in Japan. The *Sinicae* Clade thus presents a complex and fascinating group, deserving special attention. Moreover, the placement of certain species within the *Phyllostachys* Clade suggests a situation as compelling as the placement of *Semiarundinaria* in *Pleioblastus*, and is likely due to the same underlying cause (hybridization, convergent evolution, divergent evolution?). Additional work is needed to characterize the molecular diversity in this group. In particular, the relationships among members of the *Sinicae* clade and other taxa that are similar to *Arundinaria* (*e.g.*, *Bashania, Sarocalamus,* and *Oligostachyum*) need careful reevaluation.

***Incertae sedis*—** A number of morphologically distinct genera were unavailable for this analysis, and thus their positions remain unknown. Most notable among these are *Gaoligonshania*, a monotypic genus from Yunnan, China, and several temperate species in Sri Lanka, currently classified in *Arundinaria*.

Hybridization and reticulate evolution in the temperate clade. Hybridization has emerged as a likely explanation for at least some portion of the complex pattern of

morphological variation in the temperate clade. The emerging account of evolution in this group suggests that several major lineages diverged in isolation, subsequently overlapping in distribution and undergoing reticulate evolution, resulting in the complex pattern of variation observed today. The potential influence of hybridization warrants a comprehensive reappraisal of variation in this group.

It is not readily apparent whether reticulation has had a shallow or deep impact on the evolution of the temperate clade. In all likelihood, if hybridization has occurred at all, then it has probably occurred throughout the history of the group, with some hybrid taxa contributing to the gene pool via introgression. Intermediacy among certain lineages, such as *Phyllostachys*, *Hibanobambusa*, and *Sasa*, are suggestive of recent hybridization events, with no apparent influence of the putative hybrid (*Hibanobambusa*) on parent species, whereas the complex pattern of intergradation in the *Sinicae* Complex (*Pleioblastus* sect. *Amari*, *Pseudosasa* subg. *Sinicae*, *Indosasa*, and *Acidosasa*) could be indicative of a more elaborate history of hybridization and introgression. This study has identified a number of putative hybrid relationships that can be tested with additional data, for example AFLP markers and nuclear sequences that reveal bidirectional inheritance. By investigating putative reticulate relationships, it is possible that fundamental differences among major lineages (*e.g.,* the *Phyllostachys* Clade vs. the *Arundinaria* Clade) will become apparent.

Taxonomic Implications. Although the current study represents significant progress towards a revised classification of the temperate clade, additional molecular and morphological analyses are necessary before extensive taxonomic revisions are attempted. The current results suggest that we are approximately two steps away from being in a position to reconstruct major events in the evolution of the temperate clade. First, we need a more robust backbone for major lineages; such a phylogeny can possibly be obtained through a phylogenetic analysis of whole chloroplast genomes for at least one representative of each of the major lineages. Second, we need a cohesive understanding of the impact of reticulate evolution in this group, which will require additional tests of species complexes. One such study is currently underway for the *Arundinaria* Clade (Triplett and Clark, in prep.; Chapter 5). Only after hypotheses of reticulate evolution and other possible sources of incongruence have been explored can a meaningful taxonomic revision be attempted across the temperate

clade. However, it seems clear that the current subtribal classification of the temperate bamboos should be abandoned, and in its place a provisional framework based on the six lineages outlined herein can be utilized for ongoing research.

Clades that emerged in the current analysis need to be examined more closely using sources of molecular information appropriate for phylogeny reconstruction among closely related organisms, such as AFLP analysis and sequencing of nuclear introns. These markers may help to resolve species-level taxonomic questions, including those concerning hybridization and reticulation. Many temperate species have yet to be sampled, and many more need to be examined before we can fully characterize the phylogeny of this group. Moreover, morphological taxonomy should be considered in conjunction with the molecular framework in order to identify taxa to include in targeted studies; for example, a study of *Pleioblastus* is currently underway utilizing not only exemplars of *Pleioblastus s.s.* and allies revealed in the cp DNA study, but also *Phyllostachys* and *Sasa* to test the putative hybrid origins of *Semiarundinaria*, *Sasaella*, and *Pseudosasa*. Similarly, any study of *Oligostachyum*, *Brachystachyum*, and *Sinobambusa* should also include representatives of both the *Phyllostachys* clade and the *Arundinaria* Clade.

The phylogeny of the temperate bamboos can best be described as puzzling. Morphological studies lead to seemingly endless problems parsing variation into meaningful groups, and no single character has emerged that avoids controversy or internal contradiction. Molecular methods offer promising tools, yet most studies have only revealed new mysteries to rival the old. Previous DNA-based studies have failed to recover a bifurcating topology, instead providing support for seemingly spurious relationships with no clear indication of an underlying pattern (Ní Chonghaile 2002; Guo and Li 2004; Zhuge *et al.* 2004). The phylogenetic hypotheses generated in this study improve our understanding of relationships among the temperate bamboos and provide a new framework with which to investigate the evolutionary history of these plants. These results provide a much clearer concept of phylogenetic relationships in this clade and will greatly aid the future taxonomic revision. Re-examination of clade members will allow for the identification of synapomorphies that may provide useful characters for recognition of relatives and for the generation of identification keys. Solving the riddle of bamboo evolution will require the synthesis of

several lines of evidence with contributions from the broad toolbox of molecular and morphological phylogenetics.

ACKNOWLEDGEMENTS.

Field work was supported by a research grant from the NSF/JPSP (EAPSI) and grants from the American Bamboo Society to JT as well as a National Geographic Society Grant (#7336-02) to LC. Support for JT and for the final preparation of this manuscript came from a National Science Foundation Grant (DEB-0515712) to LC. We are indebted to Adam and Sue Turtle (Earth Advocates Research Farm, Summertown, TN), Jennifer York and Jesus Mora (Bamboo Sourcery, Sebastopol, CA), Dr. Michael Hotchkiss (USDA, Byron, GA), Marc Hilt (Bambu-u, Chimacum, WA), Erika Harris (Clinton Bamboo, Seattle, WA), Stan Andreason (Beauty & the Bamboo, Seattle, WA), and Dr. Masatoshi Watanabe (Rakusai Bamboo Gardens, Kyoto, Japan) for providing access to bamboos in cultivation, and to Dr. Julian Campbell (Lexington, KY), Dr. Michael Hotchkiss, and Dr. Alan Weakley (UNC, Chapel Hill, NC) for help with field work in the US. We are especially grateful to Dr. Dezhu Li, Yuxiao Zhang, and colleagues at Kunming Botanical Institute, China, for help with field work in Yunnan, China, and for valuable discussions on the temperate clade. We thank the curators of the herbaria that loaned specimens for this study. We also thank Dr. Jonathan Wendel, Ryan Percifield, Dr. Corrine Grover, Gabriel Sánchez-Ken, Chi-sing Ho, and Dr. Robert Wallace of Iowa State University and the lab of Dr. Noriaki Murakami of Kyoto University for technical assistance and access to laboratory facilities.

LITERATURE CITED

CHAO, C.S., AND S.A. RENVOIZE. 1989. Revision of species described under *Arundinaria* (Gramineae) in South-east Asia and Africa. Kew Bull. 44: 349-367.

CHAO, C.S., C.D. CHU, AND W.Y. HSIUNG. 1980. A revision of some genera and species of Chinese bamboos. Acta Phytotax. Sin. 18: 20-36.

CLARK, L.G., S. DRANSFIELD, J. TRIPLETT, AND J.G. SÁNCHEZ-KEN. 2007. Phylogenetic relationships among the one-flowered, determinate genera of Bambuseae (Poaceae: Bambusoideae). Aliso 23: 315-332.

CLAYTON, W.D. AND S.A. RENVOIZE. 1986. Genera Graminum: Grasses of the World. Royal Botanic Gardens, Kew.

DEMESURE, B., N. SODZI, AND R.J. PETIT. 1995. A set of universal primers for amplification of polymorphic non-coding regions of mitochondrial and chloroplast DNA in plants. Molecular Ecology 4:129-131.

DOYLE, J.J., J.I. DAVIS, R.J. SORENG, D. GARVIN, AND M. ANDERSON. 1992 Chloroplast DNA inversions and the origin of the grass family (Poaceae). Proceedings of the National Academy of Sciences, USA 89: 7722–7726.

FARRIS, J.S. M. KÄLLERSJÖ, A.G. KLUGE AND C. BULT. 1994. Testing significance of incongruence. Cladistics 10: 315–319.

FELSENSTEIN, J. 1985. Confidence limits on phylogenies: An approach using the bootstrap. Evolution 39: 783-791.

GASCUEL. O. 1997. BIONJ: an Improved Version of the NJ Algorithm Based on a Simple Model of Sequence Data. Mol. Biol. Evol. 14 (7): 685-695.

GAUT, B.S., L.G. CLARK, J.F. WENDEL, AND S.V. MUSE. 1997. Comparisons of the molecular evolutionary process at rbcL and ndhF in the grass family (Poaceae). Mol. Biol. Evol. 14: 769-777.

GIRIBERT, G. AND W.C. WHEELER. 1999. On gaps. Mol. Phyl. Evol. 13: 132-143.

GPWG (Grass Phylogeny Working Group). 2001. Phylogeny and subfamilial classification of the grasses (Poaceae). Ann. Miss. Bot. Gard. 88 (3): 373-457.

GUO, Z.-H. AND D.-Z. LI. 2004. Phylogenetics of the *Thamnocalamus* group and its allies (Gramineae: Bambusoideae): inference from the sequences of GBSSI gene and ITS spacer. Mol. Phyl. Evol. 30: 1-12.

GUO, Z.-H, Y.-Y. CHEN, AND D.-Z. LI AND J.-B. YANG. 2001. Genetic variation and evolution of the Alpine Bamboos (Poaceae: Bambusoideae) using DNA sequence data. J. Plant Res. 114: 315-322.

GUO, Z.-H., Y.-Y. CHEN, AND D.-Z. LI. 2002. Phylogenetic studies on the *Thamnocalamus* group and its allies (Gramineae: Bambusoideae) based on ITS sequence data. Mol. Phyl. Evol. 22 (1): 20-30

HODKINSON, T.R., S.A. RENVOIZE, G. NÍ CHONGHAILE, C.M.A. STAPLETON, AND M.W. CHASE. 2000. A comparison of ITS nuclear rDNA sequence data and AFLP markers for phylogenetic studies in *Phyllostachys* (Bambusoideae, Poaceae). J. Plant Res. 113: 259-269.

HSUEH, C.J. AND T.P. YI. 1979. Two new genera of Bambusoideae from S.W. China, 1. : *Chimonocalamus* Hsueh et Yi; in: Acta Botanica Yunnanica 1 (2): 74-92.

JANZEN, D. 1976. Why bamboos wait so long to flower. Ann. Rev. Ecol. Syst. 7: 347-391.

JOHNSON, L.A. AND D.E. SOLTIS. 1995. Phylogenetic inference in Saxifragaceae *sensu stricto* and *Gilia* (Polemoniaceae) using matK sequences. Ann. Miss. Bot. Gard. 82: 149-175.

KELCHNER, S.A. AND L.G. CLARK. 1997. Molecular evolution and phylogenetic utility of the chloroplast *rpl16* intron in *Chusquea* and the Bambusoideae (Poaceae). Mol. Phyl. Evol. 8 (3): 385-397.

KRESS, W.J., K.J. WURDACK, E.A. ZIMMER, L.A. WEIGT, AND D.H. JANZEN. 2005. Use of DNA barcodes to identify flowering plants. Proc. Natl. Acad. Sci. 102 (23): 8369-8374.

LI, D.-Z. 1997. The Flora of China Bambusoideae Project: Problems and current understanding of bamboo taxonomy in China. In: Chapman, G.P. (ed.). The Bamboos. Linnean Society of London Symposium Series. Academic Press, London.

LI, D.-Z. 1999. Taxonomy and biogeography of the Bambuseae (Gramineae: Bambusoideae). In Rao, A.N. Rao V.R. [eds.], Bamboo – Conservation, Diversity, Ecogeography, Germplasm, Resource Utilization and Taxonomy. Proceedings of training course cum workshop. 10-17 May 1998, Kunming & Xishuangbanna, Yunnan, China. Published by IPGRI_APO, Serdang, Malaysia, pp. 14-23.

LIN, W.C. 1974. Bulletin of the Taiwan Forestry Research Institute 248: 49.

MADDISON, W.P. AND D.R. MADDISON. 2005. MacClade: Analysis of Phylogeny and Character Evolution. Version 4.08. Sinauer Assoc., Sunderland, Massachusetts.

MAKINO, T. 1912. *Take no ishi shinzoku* (A new genus of Bambusaceae), *Shibataea* Makino. Bot. Mag. Tokyo 26: 236-237.

MARUYAMA, I., H. OKAMURA, AND G. MURATA. 1979. On a new hybrid genus *Hibanobambusa*. Acta Phytotaxonomica et Geobotanica 30: 148-152 [In Japanese].

MASON-GAMER, R.J., AND E.A. KELLOGG. 1996. Chloroplast DNA analysis of the monogenomic Triticeae: phylogenetic implications and genome-specific markers. In: P.P. Jauhar (ed.). Methods of Genome Analysis in Plants, CRC Press, Boca Raton. Pp. 301-325.

MCCLURE, F.A. 1966. The Bamboos. Harvard University Press, Cambridge, Massachusetts.

MCCLURE, F.A. 1973. Genera of Bamboos Native to the New World (Gramineae: Bambusoideae). T.R. Soderstrom (ed.). Smithsonian Contr. Bot. 9: 1-148.

MICHAUX, A. 1803. Flora Boreali-Americana: sistens caracteres plantarum quas in America septentrionali collegit et detexit. Paris and Strasbourg.

MUNRO, W.M. 1868. A monograph of the bambusaceae, including descriptions of all the species. Trans. Linn. Soc. London. 26: 1-157.

NAKAI, T. 1925. Two new genera of Bambusaceae with special remarks on the related genera growing in eastern Asia. J. Arnold Arbor. 6 (3): 145-153.

NAKAI, T. 1933. Bambusaceae in Japan proper. J. Jap. Bot. 9 (2): 77-95.

NÍ CHONGHAILE, G. 2002. Systematics of the woody bamboos (Tribe Bambuseae). Unpublished Ph.D. thesis, University of Dublin, Trinity College, Ireland.

OHRNBERGER, D. 1999. The Bamboos of the World. Elsevier, Amsterdam, The Netherlands.

OKAMURA, H., Y. TANAKA, M. KONISHI AND H. KASHIWAGI. 1991. Illustrated Horticultural Bamboo Species in. Japan. Haato Limited, Shinzaiki Wakaiama.

OLMSTEAD, R.G. AND J.A. SWEERE. 1994. Combining data in phylogenetic systematics: an empirical approach using three molecular data sets in the Solanaceae. Systematic Biology 43: 467-481.

PENG, S., H.-Q. YANG, AND D.-Z. LI. Highly heterogeneous generic delimitation within the temperate bamboo clade (Poaceae: Bambusoideae): evidence from GBSSI and ITS sequences. In review.

POSADA, D. AND K.A. CRANDALL.1998. Modeltest: testing the model of DNA substitution. Bioinformatics 14 (9): 817-818.

RADFORD, A.E., H.E. AHLES, AND C.R. BELL. 1968. Manual of the vascular flora of the Carolinas. University of North Carolina Press, Chapel Hill.

RONQUIST, F., J.P. HUELSENBECK, AND P. VAN DER MARK. 2005. MrBayes 3.1 Manual. Draft 26/05/2005. Available at: http://mrbayes.csit.fsu.edu/manual.php Accessed 2/13/08

SAITOU N, M. NEI M. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol. Biol. Evol. 4 (4): 406-25.

SALTONSTALL, K. 2001. A set of primers for amplification of noncoding regions of chloroplast DNA in the grasses. Mol. Ecol. Notes 1 (1-2), 76-78

SANG T., D.J. CRAWFORD, AND T.F. STUESSY. 1997. Chloroplast DNA phylogeny, reticulate evolution, and biogeography of *Paeonia*. Am. J. Bot. 84: 1120–1136.

SHAW J, E.E. LICKEY, SCHILLING, AND R.L. SMALL. 2007 Comparison of whole chloroplast genome sequences to choose noncoding regions for phylogenetic studies in angiosperms: the tortoise and the hare III. Am. J. Bot. 94: 275-288

SHAW, J., E. LICKEY, J. BECK, S. FARMER, W. LIU, J. MILLER, K. C. SIRIPUN, C. WINDER, E. E. SCHILLING, AND R. SMALL. 2005. The tortoise and the hare II: relative utility of 21 noncoding chloroplast DNA sequences for phylogenetic analysis. Am. J. Bot. 92 (1): 142-166.

SODERSTROM, T.R., AND R.P. ELLIS. 1987. The position of bamboo genera and allies in a system of grass classification. In: T.R. Soderstom, K.W. Hilu, C.S. Campbell, and M.E. Barkworth (eds.). Grass Systematics and Evolution. Random House (Smithsonian Inst. Press), New York.

STAPLETON, C.M.A. 1991. A morphological investigation of some Himalayan bamboos with an enumeration of taxa in Nepal and Bhutan. Ph.D. thesis. University of Aberdeen.

STAPLETON, C.M.A. 1994. The bamboos of Nepal and Bhutan, Part III: *Drepanostachyum, Himalayacalamus, Ampelocalamus, Neomicrocalamus* and *Chimonobambusa* (Gramineae: Poaceae, Bambusoideae). Edinburgh Journal of Botany 51: 310-330.

STAPLETON, C.M.A. 1997. The morphology of the woody bamboos. In: Chapman, G.P. (ed.). The Bamboos. Linnean Society of London Symposium Series. Academic Press, London.

STEVENS, J. 1995. Bamboo is back. International Wildlife. (Jan/Feb): 38-44.

SUNGKAEW, S., C.M.A. STAPLETON, N. SALAMIN, AND T.R. HODKINSON. Non-monophyly of the woody bamboos (Bambuseae; Poaceae): a multi-gene region phylogenetic analysis of Bambusoideae s.s. In review.

SUZUKI, S. 1978. Index to Japanese Bambusaceae; 384 pp. Tokyo: Gakken Co. Ltd.

SUZUKI, S. 1987. New or noteworthy plants in Japanese Bambusaceae (5). J. Jap. Bot. 62 (9): 274-280.

SWOFFORD, D.L. 2002. PAUP*. Phylogenetic analysis using parsimony, ver. 4.0b10. Sunderland: Sinauer Associates.

TABERLET P, L. GIELLY, G. PAUTOU, AND J. BOUVET. 1991. Universal primers for amplification of three non-coding regions of chloroplast DNA. Plant Molecular Biology 17: 1105-1109.

TATE J.A. AND B.B. SIMPSON. 2003. Paraphyly of *Tarasa* (Malvaceae) and diverse origins of the polyploid species. Systematic Botany 28: 723-737.

VINES, R.A. 1960. Trees, shrubs, and woody vines of the Southwest. University of Texas Press, Austin.

VOIGHT, J.W., AND R.H. MOHLENBROCK. 1964. Plant communities in southern Illinois. Southern Illinois University Press, Carbondale.

WATANABE, M, M. ITO, AND S. KURITA. 1994. Chloroplast DNA phylogeny of Asian bamboos (Bambusoideae, Poaceae) and its systematic implication. J. Plant Res. 107: 253-261.

WATSON, L., AND M.J. DALLWITZ 1992 onwards. The grass genera of the world: descriptions, illustrations, identification, and information retrieval; including synonyms, morphology, anatomy, physiology, phytochemistry, cytology, classification, pathogens, world and local distribution, and references. Version: 6th June 2008. http://delta-intkey.com

WILCOX, T. P., D. J. ZWICKL, T. A. HEATH, AND D. M. HILLIS. 2002. Phylogenetic relationships of the dwarf boas and a comparison of Bayesian and bootstrap measures of phylogenetic support. Mol. Phyl. Evol. 25: 361-371.

WU, Z.Y., P.H. RAVEN AND D.Y. HONG, eds. 2006. Flora of China. Vol. 22 (Poaceae). Science Press, Beijing, and Missouri Botanical Garden Press, St. Louis.

YAMANE, K. AND T. KAWAHARA (2005). Intra- and interspecific phylogenetic relationships among diploid *Triticum-Aegilops* species (Poaceae) based on base-pair substitutions, indels, and microsatellites in chloroplast noncoding sequences. Am. J. Bot. 92 (1): 1887-1898.

YANG, G.Y. AND Q.S. ZHAO. 1993. A revision of the genus *Arundinaria* Michaux in China (I). J. Bamb. Res. 12 (4): 1-6.

YANG, G.Y. AND Q.S. ZHAO. 1994. A revision of the genus *Arundinaria* Michaux in China (II). J. Bamb. Res. 13 (1): 1-23.

ZHANG, W.P. 1996. Phylogeny and classification of the bamboos (Poaceae: Bambusoideae) based on molecular and morphological data. Ph.D. dissertation. Iowa State University.

ZHANG, W.P. AND L.G. CLARK. 2000. Phylogeny and classification of the Bambusoideae (Poaceae). In S.W.L. Jacobs and J. Everett (eds.). Grasses: Systematics and Evolution: 35-42. CSIRO, Melbourne.

ZHUGE, Q., Y.-L. DING, C. XU, H.-Y. ZOU, M.-R. HUANG, AND M.X. WANG. Phylogeny of the genus *Arundinaria* based on nucleotide sequences of nrDNA ITS region. Acta Genetica Sinica 31 (4): 349-356.

APPENDIX 1

List of taxa utilized in this study. Vouchers at ISC unless otherwise indicated. For published sequences, GenBank accession numbers are provided.

Acidosasa edulis (Wen) Wen, *Triplett 148*; ***Acidosasa purpurea*** (Hsueh & Yi) P.C. Keng, *Triplett 269*; ***Ampelocalamus scandens*** Hsueh & W.D. Li, *L.G. Clark 1291*; ***Arundinaria appalachiana*** Triplett, Weakley, & L.G. Clark, *Triplett 99*; ***Arundinaria funghomii*** McClure, *Triplett 10*; ***Arundinaria gigantea (***Walt.) Muhl., *Triplett 197*; ***Arundinaria tecta (***Walt.) Muhl., *Triplett 173*; ***Bambusa vulgaris*** Schrader ex Wendland, *Sánchez-Ken 666*; ***Bashania fargesii (***Camus) P.C. Keng & Yi, *Triplett 149*; ***Borinda albocerea*** (Hseuh & Yi) Stapleton, *Triplett 210*; ***Brachyelytrum erectum*** (Schreb. ex Spreng.) P. Beauv., *Triplett 199b*; ***Brachystachyum densiflorum (***Rendle) Keng, *Triplett 221* (Rendle) Keng, *Ruan 91002*; ***Buergersiochloa bambusoides*** Pilger, *S. Dransfield 1382, S. Dransfield 1365*; ***Chimonobambusa marmorea (***Mitford) Makino, *Triplett 69*; ***Chimonobambusa quandrangularis*** (Fenzi) Makino, *L.G. Clark 1214*; ***Chimonobambusa tumidissinoda*** Hsueh & Yi ex Ohrnb., *Triplett 212*; ***Chimonocalamus delicatus*** Hseuh & Yi, *Triplett 275*; ***Chimonocalamus montanus*** Hseuh & Yi, *Triplett 261*; ***Chimonocalamus pallens*** Hsueh & Yi, *Triplett 238*; ***Diarrhena obovata*** (Gleason) Brandenburg, *Triplett 290* ; ***Drepanostachyum falcatum*** (Nees) P.C. Keng ***var. sengteeanum*** Stapleton, *Triplett 234*; ***Drepanostachyum hookerianum (***Munro) P.C. Keng, *Triplett 237*; ***Fargesia fungosa*** Yi, *Triplett 251*; ***Fargesia nitida (***Mitf.) P.C. Keng, *Triplett 222*; ***Ferrocalamus rimosivaginus*** Wen, *Triplett 272*; ***Ferrocalamus strictus*** Hsueh & P.C. Keng, *J. Campbell 10*; ***Gelidocalamus kunishii*** (Hayata) P.C. Keng & Wen, *R. March 29*; ***Guadua angustifolia*** Kunth, *L.G. Clark & X. Londoño 931*; ***Hibanobambusa tranquillans (***Koidzumi) Maruyama & H. Okamura, *Triplett 72*; ***Himalayacalamus asper*** Stapleton, *Triplett 209*; ***Himalayacalamus falconeri (***J.D. Hooker ex Munro) P.C. Keng, *L.G. Clark 1324*; ***Indocalamus aff. latifolius*** (Keng) McClure, *Triplett 243*; ***Indocalamus hamadae*** (Hatusima) Stapleton, *Triplett 70*; ***Indocalamus latifolius*** (Keng) McClure, *Triplett 155*; ***Indocalamus solidus*** C.D. Chu & C.S. Chao, *Triplett 15*; ***Indocalamus tessellatus (***Munro) P.C. Keng, *Triplett 116*; ***Indosasa crassifolia*** McClure, *Triplett 218*; ***Indosasa sinica*** C.D. Chu & C.S. Chao, *Triplett 267*; ***Leersia oryzoides*** (L.) Sw., *Triplett 199a*; ***Neurolepis elata***

(Kunth) Pilger, *L.G. Clark & P.A. 1409*; ***Oligostachyum sp.***, *Triplett 44, Triplett 124*; ***Oryza sativa*** L., *Genbank: NC 001320*; ***Pariana radiciflora*** Sagot ex Doell, *L.G. Clark & W. Zhang 1344* ; ***Pharus latifolius*** L., *L.G. Clark 1302* ; ***Phyllostachys aurea*** Carrière ex A. & C. Rivière, *Triplett 120*; ***Phyllostachys bambusoides*** Siebold & Zuccharini, *Triplett 121* ; ***Phyllostachys heteroclada*** Oliver, *Triplett 119*; ***Pleioblastus amarus (***Keng) P.C. Keng, *Y. Zhang 07082 (KIB)*; ***Pleioblastus argenteostriatus (***Regel) Nakai, *Triplett 66*; ***Pleioblastus chino (***Franchet & Savatier) Makino, *Triplett 11*; ***Pleioblastus gramineus*** (Bean) Nakai, *Triplett 35*; ; ***Pleioblastus hindsii*** (Munro) Nakai, *Triplett 65*; ***Pleioblastus juxianensis*** Wen, C.Y. Yao & S.Y. Chen, *Triplett 43*; ***Pleioblastus juxianensis*** Wen, C.Y. Yao & S.Y. Chen, *Triplett 117*; ***Pleioblastus kongosanensis*** Makino, *Triplett 46*; ***Pleioblastus linearis*** (Hackel) Nakai, *Triplett 59*; ***Pleioblastus maculatus*** (McClure) C.D. Chu & C.S. Chao, *Triplett 252*; ***Pleioblastus pygmaeus*** (Miquel) Nakai, *Triplett 127*; ***Pleioblastus simonii (***Carrière) Nakai, *Triplett 42*; ***Pseudosasa amabilis (***McClure) P.C. Keng, *Triplett 16* (McClure) P.C. Keng, *Triplett 139*; ***Pseudosasa cantorii*** (Munro) P.C. Keng, *Triplett 114* (Munro) P.C. Keng, *Triplett 153*; ***Pseudosasa gracilis*** S.L. Chen & G.Y. Sheng, *Y. Zhang 06107*; ***Pseudosasa hindsii*** (Munro) S.L. Chen & G.Y. Sheng, *Y. Zhang 06078*; ***Pseudosasa japonica (***Siebold & Zuccarini ex Steudel) Makino ex Nakai, *Triplett 122*; ***Pseudosasa japonica var. pleioblastoides*** Muroi, *Triplett 52*; ***Pseudosasa longiligula*** Wen, *Triplett 152*; ***Pseudosasa owatarii*** (Makino) Makino ex Nakai, *Triplett 33*; ***Pseudosasa usawae*** (Hayata) Makino & Nemoto, *Triplett 206*; ***Sarocalamus faberi*** (Rendle) Stapleton, *Triplett 249*; ***Sasa guangxiensis*** C. D. Chu & C. S. Chao, *Zeng 06197 (KIB)*; ***Sasa kurilensis*** **'Maculosa'** (Ruprecht) Makino & Shibata, *Triplett 223*; ***Sasa longiligulata*** McClure, *Zeng 061213 (KIB)*; ***Sasa oshidensis*** Makino & Uchida, *Triplett 161*; ***Sasa tsuboiana*** Makino, *Triplett 133*; ***Sasa veitchii (***Carrière) Rehder, *Triplett 126*; ***Sasaella bitchuensis*** (Makino) Makino ex Koidzumi, *Triplett 128*; ***Sasaella masamuneana*** (Makino) Hatusima & Muroi, *Triplett 76*; ***Sasaella ramosa (***Makino) Makino, *Triplett 118*; ***Sasamorpha borealis (***Hackel) Nakai, *L.G. Clark 1327*; ***Semiarundinaria fastuosa (***Marliac ex Mitford) Makino ex Nakai, *Triplett 138*; ***Semiarundinaria kagamiana*** Makino, *Triplett 79*; ***Semiarundinaria yashadake*** (Makino) Makino, *Triplett 137*; ***Shibataea chinensis*** Nakai, *Triplett 13*; ***Shibataea kumasaca (***Zoll. ex Steud.) Makino, *L.G. Clark 1290*; ***Shibataea lancifolia*** C.H. Hu, *Triplett 132*; ***Sinobambusa***

sp., *Triplett 159*; ***Streptogyna americana*** C.E. Hubb, *J.G. Sánchez-Ken 657*; ***Sucrea maculata*** Soderstr., *L.G. Clark & W. Zhang 1345*; ***Thamnocalamus spathiflorus (***Trin.) Munro, *L.G. Clark 1319*; ***Thamnocalamus tessellatus (***Nees) Soderstr. & R.P.Ellis, *Triplett 202*; ***Yushania alpina*** (K.Schum.) W.C. Lin, *Faden et al. 96/413 (US)*; ***Yushania ambositrensis*** (A. Camus) Ohrnberger, *S. Dransfield 1353*; ***Yushania anceps (***Mitford) Lin, *Triplett 227*; ***Yushania niitakayamensis (***Hayata) Keng f., *R. March 28.*

CHAPTER 3. PHYLOGENETIC RELATIONSHIPS WITHIN *ARUNDINARIA* (POACEAE: BAMBUSOIDEAE) IN NORTH AMERICA

A manuscript to be submitted to *Evolution* or *American Journal of Botany*

JIMMY TRIPLETT[1], KIMBERLY A. OLTROGGE[2], and LYNN G. CLARK[1]

[1]Department of Ecology, Evolution and Organismal Biology, Iowa State University, Ames, IA 50011-1020, USA

[2]North Polk Junior-Senior High School, 315 NE 141st Ave., Alleman, IA 50007

ABSTRACT

We report the results of a molecular phylogenetic analysis of the North American woody bamboo genus *Arundinaria*, including estimates of genetic variation and evidence of natural hybridization and introgression among all three native species. In this study, we pursued two main goals: (i) reconstruction of evolutionary relationships among *Arundinaria* in North America, and (ii) investigation of the role of hybridization in this species complex. The study involves a comparative analysis of amplified fragment length polymorphisms (AFLPs) and chloroplast DNA sequences representing diversity within and among all three species of *Arundinaria sensu stricto* plus accessions with intermediate or unusual morphological characteristics (putative hybrids). Six AFLP primer combinations yielded 338 polymorphic bands that were used in a combination of hierarchical clustering, ordination methods, and AMOVA. Chloroplast DNA sequences from the *trn*TL region were used to characterize maternal haplotypes, which were subsequently mapped onto the AFLP network to determine the direction of hybridization. Molecular evidence demonstrates that *A. tecta* and *A. appalachiana* are sister species, forming a clade that is significantly divergent from *A. gigantea*. All three species retain the potential for cross-fertilization, albeit presumably rare due to allopatry and infrequent flowering. Detected patterns of hybridization were relatively shallow, with the majority of hybrids being the apparent direct (F1) product of crosses between *A. gigantea* and *A. tecta*. Several unusual genotypes were identified, presumably

representing posthybridizational recombination and introgression. The role of hybridization and reticulate evolution in the phylogenetic history of *Arundinaria* is discussed along with implications for the taxonomy of the temperate bamboos.

KEYWORDS: Bamboo phylogeny, natural hybridization, *Arundinaria*, temperate bamboos, amplified fragment length polymorphisms (AFLP), chloroplast DNA, *trn*TL

INTRODUCTION

Arundinaria Michaux (Poaeae: Bambusoideae) is the oldest generic epithet among the temperate lineage (ca. 500 species 19-31 genera) of woody bamboos, originally established for the cane bamboos of the southeastern United States (McClure 1973; Triplett *et al.* 2006). *Arundinaria gigantea* (Walt.) Muhl. (river cane) is the most widespread species in the genus, forming extensive colonies in low woods, moist ground, and along riverbanks from the lowlands east of the Appalachians, west to Missouri, up the Mississippi Valley to southern Illinois and up the Ohio River to southern Ohio. *Arundinaria tecta* (Walt.) Muhl. (switchcane) is primarily restricted to the Coastal Plain of the southeastern US, and forms colonies in non-alluvial swamps, moist pine barrens, live oak woods and along sandy margins of streams. *Arundinaria appalachiana* Triplett, Weakley & L.G. Clark (hill cane) is endemic to the southern Appalachians and upper Piedmont of northeastern Alabama, northern Georgia, southwestern North Carolina, northwestern South Carolina, and southeastern Tennessee, occurring primarily in upland oak-hickory-pine forests. River cane and switch cane were common and ecologically important components of eastern US flora as recently as the early twentieth century, where they established extensive monotypic stands known as canebrakes (West 1935). Primarily as a consequence of land use and agricultural practices in the past 150 years, these native bamboos are now relegated to scattered remnant populations throughout their natural ranges (Platt and Brantley 1997; Judziewicz *et al.* 1999), and recognized as a critically endangered ecosystem (Noss *et al.* 1995). The North American species present an intriguing phylogeographic mystery: *Arundinaria* is only distantly related to the tropical bamboos of Central and South America (ca. 352 spp.) but instead is a close relative of temperate species in East Asia (Triplett and Clark, in prep; Ch. 2). As such,

Arundinaria represents another example of the classic floristic disjunction between eastern North America and eastern Asia (Koyama and Kawano 1964; Wen 1999).

Current treatments consider *Arundinaria* to be a North American endemic (Triplett and Clark 2006; Clark and Triplett 2007). The genus has had a notably troubled taxonomy, owing not only to confusion over the number of species in North America but also to ongoing debate about its closest relatives among the temperate bamboos in Asia and Africa and their inclusion within *Arundinaria*. For example, the recently published Flora of China lists two Asiatic subgenera within *Arundinaria* (Stapleton 2006), while several species from Madagascar and Sri Lanka are still generally placed in *Arundinaria* (Soderstrom and Ellis 1988; Chao and Renvoize 1989; Ohrnberger 1999). Bamboo taxonomy is notoriously difficult due to the apparent shortage of distinguishing morphological characters, and contemporary molecular studies have brought only limited relief to this problem. The problems are particularly severe among temperate species, where long generation times (typically 40-120 year cycles) and apparently rapid diversification have minimized the molecular phylogenetic signal. Taxonomic problems within *Arundinaria* in North America are thus indicative of issues throughout the temperate clade, where species numbers ebb and flow with ever-changing opinions of morphological variation in the absence of a robust phylogenetic framework.

Based on morphology, *Arundinaria* has been allied with *Pleioblastus* Nakai and *Pseudosasa* Nakai in East Asia and *Bashania* P.C. Keng and T.P. Yi and *Sarocalamus* Stapleton in China (Clayton and Renvoize 1986; Chao and Renvoize 1989; Li 1997; Stapleton *et al.* 2004). These associations were based on inflorescence morphology and a suite of vegetative characters, especially branch architecture. *Arundinaria* and its allies were recently examined in the context of a larger molecular framework study of the temperate clade (Triplett and Clark, in prep.; Ch. 2). That study determined that many taxa associated with *Arundinaria s.s.* on the basis of morphology, such as *Bashania* [*Arundinaria* subg. *Bashania* (P.C. Keng and T.P. Yi) D.Z. Li], *Sarocalamus* [*A.* subg. *Sarocalamus* (Stapleton) D.Z. Li], *Indocalamus* Nakai, *Ferrocalamus* Hseuh and P.C. Keng, and *Gelidocalamus* T.H. Wen, are in fact closer to *Phyllostachys* Siebold & Zuccharini or *Shibataea* Makino ex Nakai, leaving a core of taxa in the *Arundinaria* Clade, including *Pleioblastus* in Japan and

related taxa in China (the *Sinicae* Clade, including *Pleioblastus s.l, Pseudosasa s.l.*, *Acidosasa* C.D. Chu & C.S. Chao ex P.C. Keng, and *Indosasa* McClure). Within the *Arundinaria* Clade, molecular data supported the monophyly of *Arundinaria s.s.* while taxa commonly synonymized under *Arundinaria*, such as *Pleioblastus* and *Pseudosasa*, were revealed to be phylogenetically distinct lineages. The study also revealed a weak but intriguing sister relationship with *Sasa* Makino & Shibata or *Sasamorpha* Nakai in Japan, two genera that are distinguished from *Arundinaria* on the basis of a number of diagnostic characters, including six-stamened flowers, single branches, and relatively large foliage blades.

Prior to recent revisions, *Arundinaria* in North America was treated as consisting of two species (Young and Haun 1961; Hitchcock 1971; Tucker 1988) or a single species with two or more subspecies (Vines 1960; Voight and Mohlenbrock 1964; Radford *et al.* 1968; McClure 1973). The earliest treatments had varying degrees of success characterizing diversity in this group or clarifying the fundamental morphological differences among the forms. In particular, it has been unclear whether intermediate forms of *Arundinaria* represent points on a continuum of population-level genetic variation or some complex pattern brought about by hybridization or ecological processes. Munro (1868) acknowledged a great diversity among *Arundinaria* in the US that encompassed two morphological types and many intermediate forms, and argued to include all of the forms in a single species (*A. gigantea*). This position was reinforced by Mitford (1896), who generalized the diversity as consisting of taller and shorter varieties. Based on morphology and distribution, West (1935) concluded that the different varieties of *Arundinaria* were ecological forms of a single species, which he recognized as *A. tecta*. Gilly (1943) recognized two types of cane: the "Atlantic-type" and the "Mississippi-type," without attempting to resolve their nomenclature, further suggesting that the differences were ecological rather than genetic. He documented the occurrence of specimens that were morphologically intermediate between the two types, and found in intermediate environments. However, his conclusions were based on an untested assumption that the morphological differences between these plants would vanish under cultivation in a common garden. Hughes (1951) noted that many of the descriptive characteristics used for the presumed species overlapped, and (justifiably, given the characters selected) came to the

conclusion that the criteria used to distinguish *A. tecta* from *A. gigantea* were of questionable validity.

In the most thorough taxonomic treatment of *Arundinaria* to date, McClure (1973) considered *Arundinaria* to consist of a single species (*A. gigantea*) with 3 subspecies, one of which [*A. gigantea* ssp. *macrosperma* (Michx.) McClure] he established to accommodate presumed hybrids between the other two subspecies. McClure balanced two main observations in deciding how to circumscribe *Arundinaria*. First, through careful analysis of a broader range of vegetative and reproductive characters, he clearly demonstrated that (subspecies) *gigantea* and *tecta* were set apart by strongly contrasting morphological and ontogenetic features in all aspects of their vegetative structure (branching, culm leaf persistence, internode form, fruit micromorphology, etc.). He also noted the unreliable features used in earlier attempts to differentiate the taxa, including maximum culm height, appendages of culm and foliage leaf sheaths, vestiture of vegetative structures, loci of insertion of inflorescences, transitional glumes incident at the base of each spikelet, lodicules, and the stylar axis. Moreover, McClure had cultivated representatives of the two types in a common garden for over 10 years, and confirmed that they retain their distinguishing features. Second, he recognized that *Arundinaria* consisted of a polymorphic array of populations with intermediate forms bridging the divide between the two strongly contrasted types. The occurrence of these intermediate forms was taken as evidence for the absence of a reproductive barrier and thus incomplete speciation between the divergent forms. Therefore, in spite of the pronounced morphological (and ecological) differences between the two forms, McClure used the biological species concept to justify placing these into a single species.

Current species-level taxonomy was based on a reevaluation of diagnostic morphological characters in the broader context of the temperate clade and on preliminary genetic (AFLP and cp DNA) data that suggested greater phylogenetic distance between *A. gigantea* and *A. tecta* than previously suspected (Triplett and Clark, unpubl. data). *Arundinaria* is now considered to consist of three species: *A. gigantea*, *A. tecta*, and *A. appalachiana* (Triplett *et al.* 2006; Clark and Triplett 2007).

In addition to defining the major lineages within the temperate clade, our recent molecular studies suggested that hybridization is potentially responsible for some of its numerous taxonomic problems (Triplett and Clark, in prep.; Ch. 2), but we lack a clear understanding of the importance of this phenomenon in bamboos. Hybridization is a major force in plant evolution (Grant 1981; Arnold 1997; Rieseberg 1997) and apparently common among grasses (Stebbins 1956, 1972, 1985), but remains poorly understood in the woody bamboos. Given the complex pattern of morphological intergradation among species and genera, perhaps it should not be surprising that reticulate evolution has contributed to diversity in the temperate bamboos, yet this aspect of their biology has largely been ignored due to the presumed temporal barriers among species. Woody bamboos flower only after long, species-specific intervals, commonly only once every 15 to 60 years but in some cases as long as 120 years (Janzen 1976). Natural hybridization is thus expected to occur only very rarely, even ignoring other barriers to hybridization (Clark *et al.* 1989). However, although rare, the mating system does appear to allow cross-pollination between species. Several morphological studies have suggested that certain species are the result of hybridization (Clark *et al.* 1989; Ohrnberger and Goerrhings 1986; Maruyama *et al.* 1979; Holttum 1958), and evidence from other taxa in the temperate clade suggests that hybridization may occur between phylogenetically distant species. For example, recent AFLP and cp DNA studies indicate that the monotypic genus *Hibanobambusa* is an intergeneric hybrid between *Phyllostachys* and *Sasa* (Triplett and Clark, in prep.; Ch. 5), and mounting evidence suggests that natural hybridization could be common throughout the temperate clade. Most if not all temperate bamboos appear to be tetraploids (2n=48) (Soderstrom 1981; Chen and Zong 1991), and may have no other reproductive barrier besides temporal and geographic isolation.

Based on distribution data and a suite of intermediate morphological characteristics, McClure (1973) argued that the assemblage of plants he recognized as *A. gigantea* subsp. *macrosperma* were the result of hybridization between subspecies *gigantea* and subspecies *tecta*, with subsequent introgression producing a phenotypically complex assemblage. Intermediate features in *A. gigantea* subsp. *macrosperma* occur in rhizomes (discontinuous air canals), midculm branch complements (variation in the constricted basal portion of the branches), and the number and size of foliage leaves. In at least one putative hybrid, foliage

leaf blades were noted to be larger than typical for subsp. *gigantea* or *tecta,* perhaps demonstrating heterosis. McClure kept a living specimen of subsp. *macrosperma* in cultivation; the plant eventually flowered and produced fruits, which he described as similar in shape to the fruits of subsp. *tecta* but larger in size and variable in the presence of an unciform rudimentary style branch (a feature associated with subsp. *tecta*). McClure concluded that genetic isolation has not yet fully been established between the two entities of *Arundinaria* in North America, thus permitting them to continue to cross in the wild.

Previous molecular studies have not resolved relationships within *Arundinaria s.s.* nor addressed the status of the intermediate forms. To date there has been no published test of species-level relationships and genetic variation within *Arundinaria s.s.* using molecular methods. Thus, numerous questions surround the North American bamboos: Do they constitute a single polymorphic species or several phylogenetically distinct lineages? Can the occurrence of hybridization be confirmed or refuted? If so, how does this fit with observed morphological variation within *Arundinaria*?

Amplified fragment length polymorphism (AFLPs) analysis is an anonymous DNA fingerprinting technique developed for plant breeding (Vos *et al.* 1995) that has been adapted to evolutionary studies (Mueller and Wolfenbarger 1999; Després *et al.* 2003; Althoff *et al.* 2007). AFLPs are robust and highly reproducible, and produce a large number of informative markers from loci dispersed throughout the genome. The technique produces taxon-specific bands that are potentially useful for resolving lower-level problems in phylogeny reconstruction (*e.g.*, Beardsley *et al.* 2003; Sullivan *et al.* 2004; Lara-Cabrera and Spooner 2004; Álvarez and Wendel 2006), making it a powerful tool for elucidating relationships that sequence data fail to recover. AFLPs have also been used to address putative reticulate relationships among closely related plants (*e.g.*, Lindqvist *et al.* 2003; Guo *et al.* 2005; Bleeker and Matthies 2005), since diagnostic bands allow the detection of parental contributions. AFLPs have been used to study species boundaries and species-level relationships in other grasses (Xu and Ban 2004; Saarela *et al.* 2003; Sasanuma *et al.* 2004; Massa and Larson 2005), and the approach has been used in the woody bamboos to assess relationships within *Phyllostachys* (Hodkinson *et al.* 2000) and *Guadua* (Marulanda *et al.* 2002), and in a broader study across the paleotropical woody bamboos (Loh *et al.* 2000). Our

preliminary studies of the *Arundinaria* complex suggested that the regions of the genome sampled by this method are sufficiently polymorphic to permit investigations at the generic level and below.

The current study utilizes AFLP and cp DNA sequence data to evaluate the nature and extent of genetic diversity in *Arundinaria* and the role of natural hybridization in an exemplar temperate bamboo taxon. With only three species, *Arundinaria sensu stricto* functions as a model system in which to test hypotheses on species-level genetic relationships in the temperate bamboos. The primary objectives were to (1) generate a population-level phylogenetic analysis of *Arundinaria* in order to test the current species-level taxonomy, and (2) investigate the potential role of hybridization and introgression in the evolution of *Arundinaria* and, by extension, temperate woody bamboos in general.

MATERIALS AND METHODS

Plant material. Field trips were conducted in 2003 and 2005 throughout the geographic range of each species (Fig. 1, Table 1). A total of eighty-three populations of *Arundinaria* were sampled, including 51 populations of river cane (*A. gigantea*), 8 populations of switch cane (*A. tecta*), 14 populations of hill cane (*A. appalachiana*), and 10 putative hybrids. Species were identified according to the key in the Flora of North America (Clark and Triplett 2007), and putative hybrids were identified on the basis of intermediate or otherwise unusual morphological features (McClure 1973). Representatives of the East Asian genus *Sasa*, obtained from specimens in cultivation, were included as outgroups. This choice was based on recent molecular analyses (Triplett and Clark, in prep.; Ch. 2) indicating *Sasa* to be a possible sister group of *Arundinaria*. Leaf tissue was collected in the field and lyophilized using silica gel (Chase and Hills 1991). Using the methods described below, we recovered consistent AFLP fingerprints and cp DNA sequences from leaf samples stored at room temperature for up to 4 years.

Total genomic DNA was extracted from silica gel-dried specimens using DNeasy Plant Mini Kit (QIAGEN, Inc., Valencia, California) 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 30m; and precipitated DNA was washed twice with 500

µl of ice cold 100% EtOH followed by 2m spin to dry. Extracted DNA was eluted in water and stored at –20ºC. Nucleic acid quality was measured using a Nanodrop ND-1000, and concentrations were standardized to 250ng/µl for AFLP enzyme digestions and 100 ng/µl for cp DNA PCR amplification.

cp DNA haplotypes. Chloroplast DNA sequences were obtained in order to corroborate AFLP results and to determine maternal haplotypes, which were subsequently mapped onto the AFLP network. Sequencing multiple representatives of each species also allowed us to evaluate population-level sequence variation in bamboos, for which limited

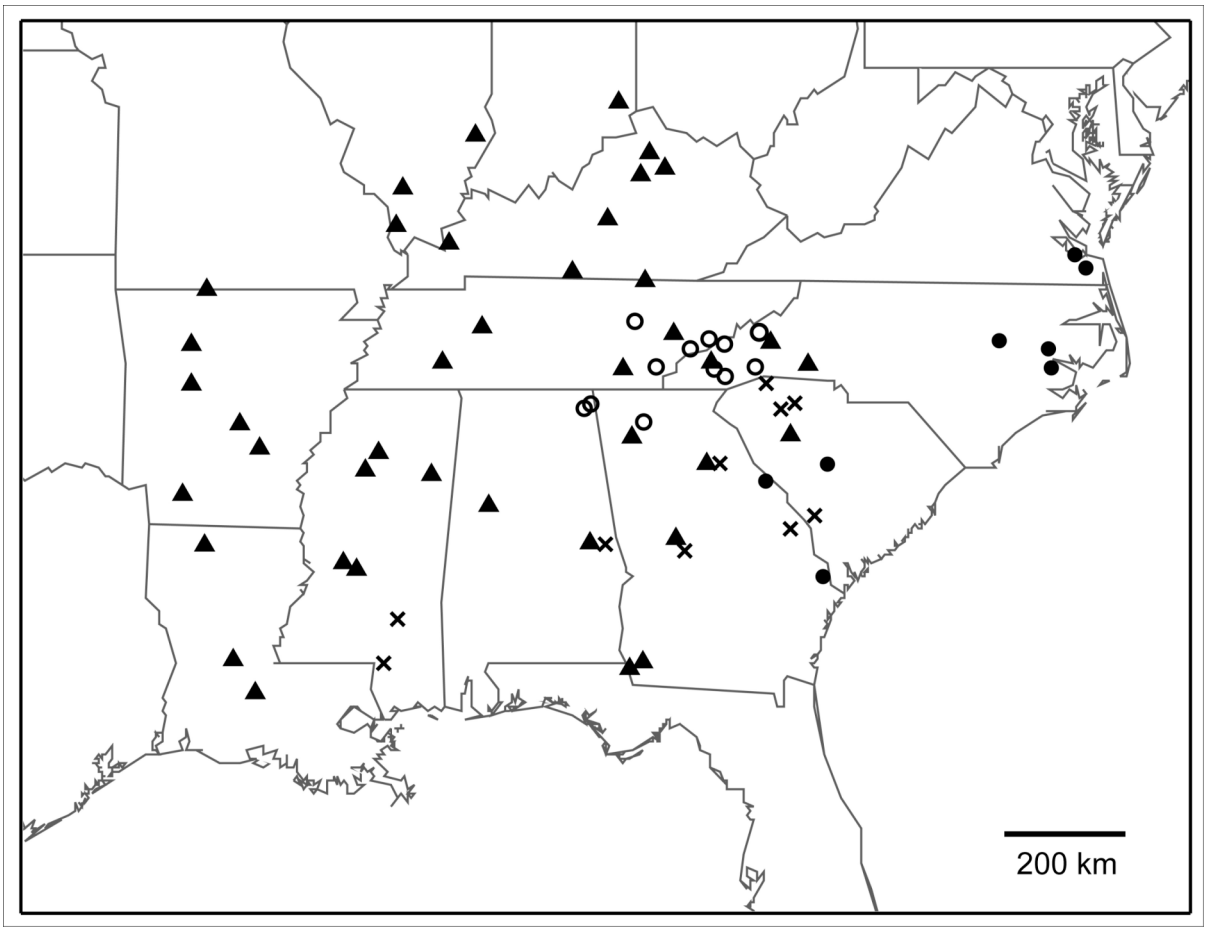

Figure 1. Map of southeastern North America showing geographical distribution of *Arundinaria* species. Black triangles = *A. gigantea*; open circles = *A. appalachiana*; black circles = *A. tecta*; x = putative hybrids.

Table 1. Voucher information for taxa used in this study. All vouchers at Ada Hayden Herbarium, Iowa State University (ISC). Collector abbreviations are as follows: JT = J. Triplett; LC = L.G. Clark; MO = M. Ozaki; JC = J. Campbell; YZ = Y. Zhang. In cult. = *Arundinaria* specimen obtained in cultivation; wild locality indicated in parentheses if confidently known.

Taxon	Voucher	Locality	Lat (N), Lon (W)
***Arundinaria* (N. America)**			
A. appalachiana	JT & LC 19	Macon, NC	35.0168, 83.3882
A. appalachiana	JT & LC 20	Rabun, GA	34.9576, 83.1806
A. appalachiana	JT & LC 21	Jackson, NC	35.3898, 83.1981
A. appalachiana	JT & MO 99	Dekalb, AL	34.5005, 85.6352
A. appalachiana	JT & MO 100	Dekalb, AL	34.4973, 85.6337
A. appalachiana	JT & MO 102	In cult.	n/a
A. appalachiana	JT & MO 103	In cult.	n/a
A. appalachiana	JT 165	Polk, TN	35.0421, 84.4514
A. appalachiana	JT 166	Bartow, GA	34.2456, 84.6753
A. appalachiana	JT 179	Greenville, SC	35.0851, 82.608
A. appalachiana	JT 182	Buncombe, NC	35.5673, 82.5683
A. appalachiana	JT 184	Swain, NC	35.4469, 83.4251
A. appalachiana	JT 185	Graham, NC	35.3827, 83.8623
A. appalachiana	JT 188	Rhea, TN	35.7418, 84.8392
A. gigantea	JT & LC 1	Gadsden, FL	30.6512, 84.8721
A. gigantea	JT & LC 2	Gadsden, FL	30.6512, 84.8721
A. gigantea	JT & LC 3	Jackson, FL	30.6995, 84.8607
A. gigantea	JT & LC 4	Decatur, GA	30.757, 84.7857
A. gigantea	JT & LC 5	Gadsden, FL	30.6977, 84.8497
A. gigantea	JT & LC 6	Taylor, GA	32.5426, 84.0184
A. gigantea	JT & LC 18	Macon, NC	35.1861, 83.3723
A. gigantea	JT & MO 80	Marion, AR	36.2421, 92.7667
A. gigantea	JT & MO 81	Pope, AR	35.4109, 93.1328
A. gigantea	JT & MO 82	Perry, AR	34.8698, 93.1093
A. gigantea	JT & MO 83	Grant, AR	34.3074, 92.2163
A. gigantea	JT & MO 84	Lincoln, AR	33.9545, 91.8439
A. gigantea	JT & MO 85	Columbia, AR	33.2695, 93.272
A. gigantea	JT & MO 86	Bienville Parish, LA	32.5054, 92.8744
A. gigantea	JT & MO 87	Evangeline Parish, LA	30.8003, 92.281
A. gigantea	JT & MO 88	St. Martin Parish, LA	30.2937, 91.918
A. gigantea	JT & MO 91	Rankin, MS	32.204, 90.0513
A. gigantea	JT & MO 92	Hinds, MS	32.2818, 90.183
A. gigantea	JT & MO 93	Grenada, MS	33.8065, 89.7415
A. gigantea	JT & MO 94	Carroll, MS	33.6364, 89.7963
A. gigantea	JT & MO 95	Clay, MS	33.5419, 88.6345
A. gigantea	JT & MO 96	Tuscaloosa, AL	33.1146, 87.596
A. gigantea	JT & MO 98	Lee, AL	32.5669, 85.3772
A. gigantea	JT & MO 101	In cult. (Macon, GA)	(36.4599, 85.9938)
A. gigantea	JT 108	Jackson, IL	37.627, 89.2087
A. gigantea	JT 109	Alexander, IL	37.1209, 89.2737
A. gigantea	JT 110	Marshall, KY	36.8565, 88.3156

Table 1. (continued)

A. gigantea	JT 111	Hickman, TN	35.6638, 87.6908
A. gigantea	JT 112	McNairy, TN	35.2119, 88.3949
A. gigantea	JT 164	Hamilton, TN	35.0775, 85.0394
A. gigantea	JT 167	Bartow, GA	34.149, 84.8443
A. gigantea	JT 169	Oconee, GA	33.724, 83.3849
A. gigantea	JT 174	Greenwood, SC	34.1586, 81.946
A. gigantea	JT 177	Cherokee, SC	35.1234, 81.588
A. gigantea	JT 180	Buncombe, NC	35.4644, 82.4586
A. gigantea	JT 181	Buncombe, NC	35.5634, 82.5622
A. gigantea	JT 183	Swain, NC	35.4337, 83.4388
A. gigantea	JT 186	Swain, NC	35.4501, 83.9407
A. gigantea	JT 187	Monroe, TN	35.565, 84.0933
A. gigantea	JT 189	Scott, TN	36.3145, 84.6271
A. gigantea	JT 190	Adair, KY	37.1932, 85.3459
A. gigantea	JT & JC 191	Garrard, KY	37.8083, 84.6568
A. gigantea	JT & JC 192	Jessamine, KY	37.8448, 84.5836
A. gigantea	JT & JC 195	Fayette, KY	38.073, 84.5363
A. gigantea	JT & JC 196	Fayette, KY	n/a
A. gigantea	JT 197	Switzerland, IN	38.7679, 85.1451
A. gigantea	JT 198	Gibson, IN	38.3477, 87.8024
A. gigantea	JT & YZ 291-00	Marion, AR	36.2436, 92.7694
A. gigantea	JT & YZ 291-10	Marion, AR	36.2436, 92.7694
A. gigantea	JT & YZ 291-14	Marion, AR	36.2436, 92.7694
A. gigantea	JT & YZ 291-20	Marion, AR	36.2436, 92.7694
A. tecta	JT & LC 22	Wayne, NC	35.4195, 78.0506
A. tecta	JT & LC 23	Craven, NC	35.1078, 77.0153
A. tecta	JT & LC 24	Craven, NC	35.2603, 77.1011
A. tecta	JT & LC 25	Suffolk City/GDS, VA	36.599, 76.5282
A. tecta	JT & LC 26	Suffolk City/GDS, VA	36.6214, 76.5403
A. tecta	JT & LC 27	Chatham, GA	31.999, 81.2682
A. tecta	JT & MO 89	Pearl River, MS	30.7114, 89.5553
A. tecta	JT 170	McDuffie, GA	33.4122, 82.3818
A. tecta	JT 172	Screven, GA	32.9401, 81.4955
A. tecta	JT 173	Aiken, SC	33.646, 81.2143
Putative hybrid	JT & LC 8	Macon, GA	32.4894, 83.9378
Putative hybrid	JT & MO 90	Forrest, MS	31.4156, 89.2806
Putative hybrid	JT & MO 97	Lee, AL	32.5451, 85.3885
Putative hybrid	JT 168	Greene, GA	33.7116, 83.3033
Putative hybrid	JT 171	Jenkins, GA	32.7734, 81.9227
Putative hybrid	JT 175	Laurens, SC	34.5031, 81.8121
Putative hybrid	JT 176	Laurens, SC	34.5028, 81.8115
Putative hybrid	JT 178	Greenville, SC	34.8649, 82.419
Outgroup Taxa (All: In cult.)			
Sasa kurilensis	JT 223	Bamboo Sourcery, Sonoma, CA	
Sasa palmata	JT & MO 113	Earth Advocates Research Farm, Summertown, TN	
Sasa palmata	JT 228	Bamboo Sourcery, Sonoma, CA	
Sasa senanensis	JT 146	Earth Advocates Research Farm, Summertown, TN	
Sasa oshidensis	JT 161	Earth Advocates Research Farm, Summertown, TN	
Sasa tsuboiana	JT 133	Earth Advocates Research Farm, Summertown, TN	
Sasa veitchii	JT 126	Earth Advocates Research Farm, Summertown, TN	

information is available. Based on studies of 12 non-coding regions in the temperate woody bamboos (Triplett and Clark, in prep.; Ch. 2), the *trn*TL region was determined to be sufficiently variable among the three *Arundinaria* species to provide diagnostic sequence haplotypes. A total of 88 *trn*TL sequences were generated for this analysis, representing all 83 *Arundinaria* accessions and one representative for each outgroup species. Amplification reactions for the *trn*TL sequences (~800 bp) were conducted using the primers *trn*T F (TabA): CAT TAC AAA TGC GAT GCT CT and 5' *trn*L R (Tab B): TCT ACC GAT TTC GCC ATA TC (Taberlet *et al.* 1991) with the following PCR parameters: 95ºC, 2m; 35x (95ºC, 1m; 48ºC, 10s; +17ºC, 0.3ºC/s; 65ºC, 5m); 65ºC, 5m (40 µl reaction volumes). Amplification products were cleaned using Antarctic phosphatase (5 units, NEB) and exonuclease I (10 units, NEB) followed by an ethanol precipitation. Sequencing reactions were carried out using the PCR primers and BigDye v.2 to produce complementary strands. Sequencing was performed by the Automated 3730xl DNA Analyzer (Perkin-Elmer, Applied Biosystems Division) at the Iowa State University DNA Sequencing and Synthesis Facility. Strands were assembled, edited and aligned manually using Se-Al version 2.09a (Rambaut 2001), and sequences were compared to determine chloroplast haplotypes. Sequences will be deposited in GenBank and the data matrix will be available from TreeBASE.

Phylogenetic relationships among the haplotypes were reconstructed using an exhaustive search under maximum-parsimony (MP) in PAUP* v4b10 (Swofford 2002) with one representative per haplotype. Indels were binary coded relative to outgroup taxa and gaps were excluded from the analysis. One poly-G region was excluded from the cladistic analysis because it could not be unambiguously aligned. Bootstrap support (Felsenstein 1985) was estimated from 1,000 heuristic search replicates as above with random taxon addition.

Amplified Fragment Length Polymorphisms (AFLPs). AFLP analysis followed the protocol of Vos *et al.* (1995) with modifications suggested by the J.F. Wendel lab at ISU (http://www.eeob.iastate.edu/faculty/WendelJ/aflp.htm) and additional optimization for bamboos. DNA was digested with restriction enzymes EcoRI (10 units, NEB) and MseI (10 units, NEB) for 2h at 37ºC in a 20 µl volume, followed by ligation (20 units T4 DNA ligase [NEB], overnight at 16ºC) to double-stranded 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. Out of a total of 16 tested primer combinations, 6 were chosen for this study based on banding patterns in representatives of the three *Arundinaria* species. This follows the recommendation of Ellis *et al.* (1997), which suggests at least 80% of the expected relatedness can be explained by choosing six best primer combinations. FAM and HEX labeled +3 EcoRI primers were multiplexed in the following combinations: [1.] mCAA, eACT (FAM), eACG (HEX); [2.] mCTG, eACA (FAM), eAAC (HEX); [3.] mCTC, eACA (FAM) eAAC (HEX). Selective amplification products were separated electrophoretically at the ISU DNA Facility on an Applied Biosystems Inc. 3100 capillary fragment analyzer (Perkin Elmer) with an internal standard (GeneScan 500 Rox, ABI) and read using GeneScan software.

AFLP Data Scoring. Data extraction was done manually from trace files using the program Genographer 1.6.0 (http://hordeum.oscs.montana.edu/genographer). AFLP bands were scored as present (1) or absent (0). Only robust, unambiguous DNA fragments ranging from 60 to 650 bp in size and above 200 relative fluorescent units were scored. Scored fragments represent 7 size classes: 60-100 (31; 9.2%), 101-200 (91; 26.9%); 201-300 (108; 32.0%); 301-400 (66; 19.5%): 401-500 (32; 9.5%), 501-600 (9; 2.7%), and 601-650 (1; 0.3%). Fragment data represent an anonymous sampling of the genome, and it is likely that some fragments of a given size represent different loci; however, we assume the impact of homoplasy is negligible when a strong enough phylogenetic signal is present in the data, indicating numerous independent loci supporting a given relationship. Nevertheless, we have adopted a conservative approach to scoring and interpretation of AFLP data 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 and cladistic methods to detect and evaluate all possible signal conflict (Koopman *et al.* 2001; Lara-Cabrera and Spooner 2004).

AFLP Data Analysis. Relationships within *Arundinaria* were explored using a variety of distance and parsimony methods on the AFLP data. We used a comparative

approach in order to explore alternative topologies as potential sources of biologically meaningful information about population genetic and evolutionary relationships within *Arundinaria*, particularly with respect to hybridization. Because the results of analytical reiterations were fundamentally congruent, we report only a select subset, described below. Phylogenetic relationships were essentially investigated in two stages: (1) analyses were run with all available accessions, including the 10 putative hybrids, and (2) select analyses were repeated, excluding hybrids. This reiterative approach allowed us to test *a priori* assignments of morphological forms and to subsequently minimize noise introduced via the genetic mosaic of hybrids, which is particularly problematic for parsimony analysis (McDade 1992, 1997).

For distance-based analyses, pairwise genetic distances were calculated in PAUP* v4b10 (Swofford 2002) using the Nei-Li dissimilarity coefficient (Nei and 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 dissimilarity.

Genetic relationships were reconstructed using neighbor-joining (NJ) analysis as implemented in PAUP*. The NJ tree was rooted with the outgroup taxa, and bootstrap support was estimated based on 10,000 replicates. Dendrograms were also calculated using the unweighted pair group method with arithmetic averages (UPGMA) to explore other meaningful groups.

Subsequent to the identification of hybrid accessions, phylogenetic relationships among the three species were further explored under maximum parsimony (MP) using Wagner (Farris 1970) and Dollo (Le Quesne 1974; Farris 1977) criteria on the original presence/absence matrix. Wagner parsimony gives equal weight to fragment gain or loss, whereas Dollo parsimony makes the assumption that a fragment can be lost multiple times but gained only once. Because of the high level of internal conflict associated with AFLP data, trees were produced using a four-step heuristic search adapted from Olmstead and Palmer (1994): (1) 50,000 random replicates were performed with nearest-neighbor interchange (NNI) branch-swapping algorithm and MulTrees off; (2) the resulting trees were

used as starting trees under the TBR algorithm with MulTrees off; (3) these MP trees were used as starting trees in a heuristic search for multiple parsimonious trees with NNI branch swapping and MulTrees on; and finally (4) these trees were used as starting trees for a search using TBR with MulTrees on. The resulting MP trees were used to compute a strict consensus tree. Bootstrap values were obtained from 10,000 random replicates with NNI, MulTrees off.

Reticulate evolution cannot be reconstructed directly by either NJ or parsimony analysis. Therefore, we explored two other methods to characterize relationships among the three species and their putative hybrids. First, we produced a network diagram using SplitsTree4 (Huson and Bryant 2006), which provides a visual representation of conflicting signals in the data, highlighting the mosaic genotypes of hybrids. Networks were constructed using the NeighborNet algorithm (Bryant and Moulton 2004) on the Nei-Li pairwise distance matrix. Second, we conducted a non-metric multidimensional scaling (nMDS) analysis of the pairwise Nei-Li distance matrix using the software R version 2.6.2 and supplemental package, MASS. This method positions accessions in a reduced ordination space that best reflects their original genetic distances, and is especially useful for exploring nonhierarchical relationships associated with hybridization and introgression.

Hybrids are characterized by an additive combination of diagnostic bands from both parents (Raamsdonk *et al.* 2000). For each hybrid, the proportion of fragments contributed by different parental species was determined by manually tallying character states associated with particular clusters recovered in the NJ analysis. Because our goal was to associate bands in the hybrids with their potential parental source, we used a relaxed criterion for identifying diagnostic characters. For the purposes of this survey, a band was considered diagnostic if it occurred in at least one individual of a given group and was absent in other groups (Bleeker 2003; Bleeker and Matthies 2005). Fragments were assigned to one of five categories: 1, occurring in all species of *Arundinaria* (*i.e.*, absent in *Sasa*); 2, diagnostic for *A. gigantea*; 3, diagnostic of *A. tecta*; 4, diagnostic of *A. appalachiana*; and 5, diagnostic of the Switchcane Clade (*A. appalachiana* + *A. tecta*).

An analysis of molecular variance (AMOVA) was conducted to calculate variance components and their significance levels within and among groups (Excoffier *et al.* 1992), as

implemented in ARLEQUIN 3.0 (Excoffier *et al.* 2000) and AFLP-SURV 1.0 (Vekemans 2002; Vekemans *et al.* 2002). Levels of variability were estimated through the number and proportion of polymorphic loci (%P), Nei's gene diversity (Hj), average gene diversity (Hw), total gene diversity (Ht), and Nei's genetic differentiation (Dst). We also calculated Wright's fixation index (Fst, the proportion of total gene diversity that occurs among as opposed to within groups) as an additional estimate of the genetic structure within and among species. Significance of group partitioning was tested against alternate random distribution of individuals among groups through 1000 permutations of individuals among groups (Excoffier *et al.* 1992), and a bootstrap analysis of genetic distances was performed with 1000 replicates.

We used a Mantel test (Smouse *et al.* 1986) to evaluate the potential association between genotype and geographic distance, whereby a positive association would suggest migration rates between populations decrease with increasing distance as expected under an isolation by distance model of gene flow (Wright 1942). Latitude and longitude coordinates were converted into a matrix of geographic distances using the software Geographic Distance Matrix Generator 1.2.2 (Ersts 2008), and Mantel tests were conducted using R 2.6.2, with 10,000 permutations. Two accessions of *A. appalachiana* from material in cultivation were excluded because their original localities were unknown. Additional analyses were performed to test for correlations between (1) AFLP and cp DNA variation in *A. gigantea*, and (2) cp DNA variation and geographic distance in *A. gigantea*.

RESULTS

Chloroplast DNA sequence variation and haplotype assignment. *Arundinaria trn*TL sequences varied from 784 base pairs (bp) in one of the putative hybrids to 800 bp in *A. appalachiana*. Alignment among the 83 *Arundinaria* accessions and 5 outgroup taxa required 25 gaps, and the aligned region was 809 bp long. A total of 10 variable sites (4 point mutations, 5 indels, and 1 variable poly-G region) were identified that characterize 9 haplotypes within *Arundinaria (*Tables 2 and 3), including four haplotypes associated with *A. gigantea* (G.1-G.4) and five associated the *A. tecta* and *A. appalachiana* (AT.1-AT.5). The two most common forms among *A. gigantea* accessions, G.2 and G.4, differ by a 5 bp indel

Table 2. cp DNA haplotypes. Informative point mutations, insertions, and deletions in the *trnTL* intergenic spacer that distinguish among accessions of *Sasa* and *Arundinaria*.

Haplotype	Position									
	120	**245**	**361**	**378-388**	**431-435**	**502**	**503**	**547**	**610-614**	**615-620**
Sasa 1	-	A	G	-	-	A	A	-	G	TGGGGG
Sasa 2a	-	A	G	-	-	A	G	-	G	TGGGGG
Sasa 2b	-	A	G	-	-	A	G	-	-	TGGGGG
G.1	A	A	T	-	-	A	G	A	G	-
G.2	A	A	T	-	-	A	G	A	GGG	-
G.3	A	A	T	-	-	A	G	-	GGG	-
G.4	A	A	T	-	TTTTT	A	G	A	GGGG	-
AT.1	-	G	T	GGAGAAAATGC	-	G	G	-	G	TGGGGG
AT.2	-	G	T	GGAGAAAATGC	-	G	G	-	GG	TGGGGG
AT.3	-	G	T	GGAGAAAATGC	-	G	G	-	GGG	TGGGGG
AT.4	-	G	T	GGAGAAAATGC	-	G	G	-	GGGG	TGGGGG
AT.5	-	G	T	GGAGAAAATGC	-	G	G	-	GGGGG	TGGGGG

Table 3. cp DNA haplotype distribution among accessions of *Sasa* and *Arundinaria*. *Arundinaria* accessions are coded by species (A= *A. appalachiana,* G=*A. gigantea*, T= *A. tecta*; x = putative hybrid) and voucher numbers (*e.g., A. appalachiana* accession JT184 = "A184").

Haplotype	Accessions
S1	*S. kurilensis (*JT223)
S2a	*S. palmata (*JT113, JT228)*, S. tsuboiana (*JT133)*, S. veitchii (*JT126)
S2b	*S. oshidensis (*JT161)
AT.1	A184, A185
AT.2	A019, A020, A021, A099, A100, A102, A103, A166, A179, A182, A188, x008
AT.3	A165, T022
AT.4	T023, T025, T027, T170, T173, x171, x172, x089, x090, x168, x178
AT.5	T024, T026
G.1	x097
G.2	G001, G002, G003, G006, G018, G085, G086, G087, G092, G093, G101, G164, G167, G169, G174, G183, G186, G187, G190, G197, G198
G.3	G088
G.4	G004, G005, G080, G081, G082, G083, G084, G091, G094, G095, G096, G098, G108, G109, G110, G111, G112, G177, G180, G181, G189, G191, G192, G195, G196, G291, x175, x176

and 1 bp variation in the poly-G region. Both were found throughout the distribution of *A. gigantea*, but as a consequence of our sampling strategy it is unknown whether they co-occur within a single population. Form G.3 was recovered from a single accession (G088) and is distinguished from the widespread G.2 haplotype on the basis of a 2 bp deletion in the poly-G region. Form G.1 was recovered from a putative hybrid (x097) and is placed in haplotype class G on the basis of overall similarity to the G.2 haplotype, from which it differs by a single bp deletion. Five haplotypes (AT.1-AT.5) were associated with *A. appalachiana* and *A. tecta*. These five differ only in the poly-G region 610-614. The most common haplotype for *A. appalachiana* was AT.2, while that for *A. tecta* was AT.4. Haplotype AT.1 was found in two accessions of *A. appalachiana* in Western NC, while haplotype AT.5 occurred in two accessions of *A. tecta* in Eastern NC. Haplotype AT.3 was recovered from geographically distinct accessions of *A. tecta* (T022, Wayne Co., NC) and *A. appalachiana* (A165, Polk Co., TN). Four of the nine haplotypes (G1, G4, AT2, AT4**)** were recovered from putative hybrids, including a widespread *A. gigantea* haplotype, the common *A. tecta* haplotype, and one hybrid-specific form (see Table 3).

Comparing haplotypes of class G with class AT reveals a total of 5 characters (2 point mutations and 3 unambiguous indels) that collectively distinguish *A. gigantea* from *A. appalachiana*/*A. tecta*. In contrast, only 3 characters (1 pm and 2 indels) distinguish *A. gigantea* from *Sasa*, while 4 characters (3 pm and 1 indel) distinguish *A. appalachiana* and *A. tecta* from *Sasa*. Although the representative sample of *Sasa* species presumably represents a greater range of species-level diversity, fewer differences were detected in *Sasa* than in *Arundinaria*. *Sasa kurilensis* is distinguished from the common haplotype of *S. veitchii, S. palmata*, and *S. tsuboiana* on the basis of a single point mutation.

Phylogenetic relationships among the haplotypes are summarized in Fig. 2 The variable poly-G region 610-614, which differentiates among haplotypes, was excluded from the cladistic analysis because it could not be unambiguously aligned. The exhaustive search under maximum parsimony evaluated 654,729,075 trees and identified a single MP tree of length = 9, CI = 1.0, RI = 1. The cladogram reveals a well-defined split between *A. gigantea* (BS = 86%) and the Switchcane Clade (BS = 94%), but provides no resolution within the latter.

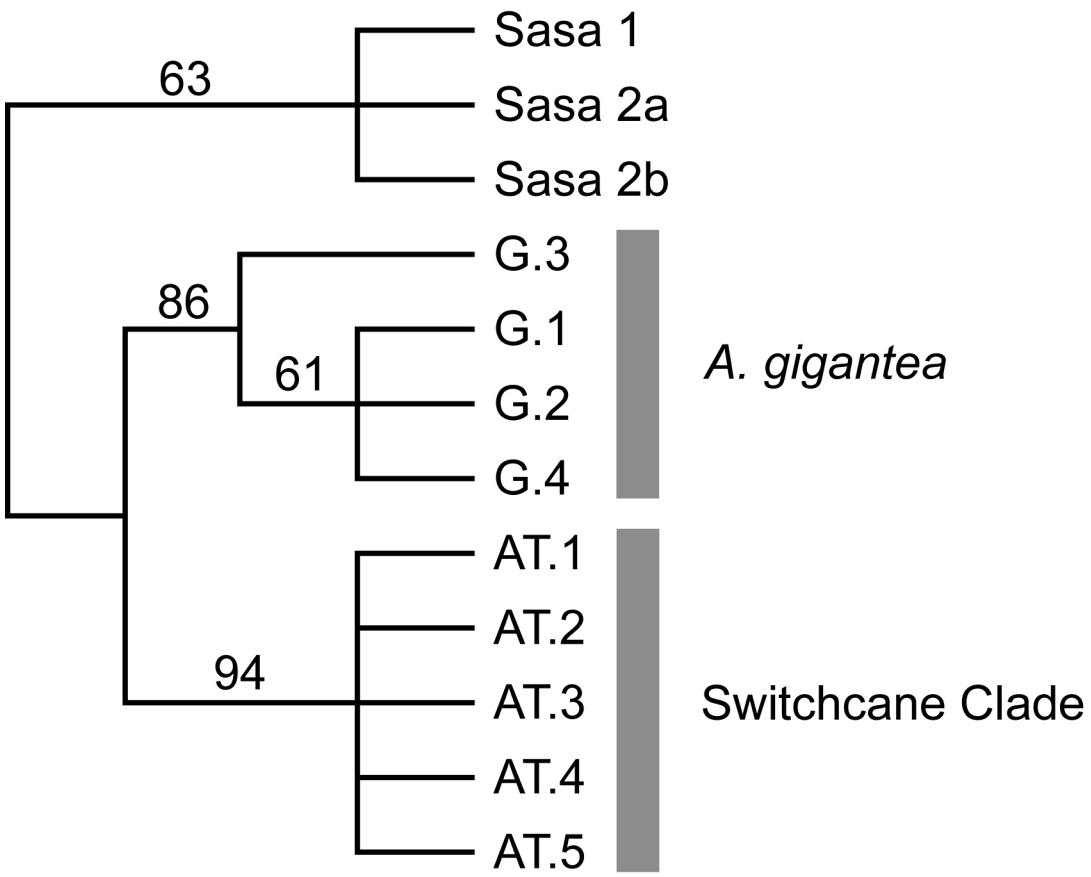

Figure 2. cp DNA haplotype phylogeny. Single most parsimonious tree (Length = 9, CI = 1.0, RI = 1.0) resulting from the analysis of *trnTL* and including indels recoded as binary present/absent characters. Bootstrap values greater than 50% are given above the branches. Haplotype accessions are summarized in Table 2.

AFLP markers. A total of 338 markers were scored for the 6 AFLP primer combinations used in this study (summarized in Table 4), representing a conservative sampling of the total fragment pool. The average number of scored bands per primer pair was 56.3, with a range of 37 to 95. Of the 338 scored fragments, 332 (98.2%) characters were polymorphic, and the average number of fragments per individual was 122.1. When the summary was restricted to *Arundinaria* species (*i.e.*, excluding putative hybrids and *Sasa*), the total number of polymorphic fragments was 214 (63.3%) and the average number of

Table 4. AFLP primer combinations used in the selective PCR and total number of scored fragments obtained with each combination.

EcoRI	Mse	Total
ACT (6-FAM)	CAA	95
ACG (HEX)	CAA	54
ACA (6-FAM)	CTG	61
AAC (HEX)	CTG	37
ACA (6-FAM)	CTC	47
AAC (HEX)	CTC	44

fragments per individual was 121.5. Pairwise Nei-Li distances among *Arundinaria* accessions ranged from 0.00245 between the two most similar individuals (A099 and A100) to 0.22792 between the two most divergent individuals (G195 and A103). Within the 51 accessions of *A. gigantea*, 42 (12.4%) of the markers were polymorphic. This is comparable to the observed values for the other two species, in spite of the fact that fewer accessions were available: within 8 accessions of *A. tecta*, 29 (8.6%) fragments were polymorphic, while in the 14 accessions of *A. appalachiana*, 36 (10.7%) characters were variable. Among the 10 putative hybrids 201 (59.5%) of the markers were polymorphic, presumably indicating the composite and additive nature of AFLP band patterns in hybrid taxa. Within the 7 accessions (5 species) of *Sasa*, 119 (35.4%) of the markers were polymorphic.

Phylogenetic relationships within Arundinaria sensu stricto. Neighbor-joining (NJ) analysis of the Nei-Li distance matrix derived from the AFLP data resulted in a dendrogram (Fig. 3a) with two major clusters: one containing all accessions of *A. gigantea* (the River Cane Clade, with 100% bootstrap support) and the other containing all accessions of *A. appalachiana* and *A. tecta* (the Switchcane Clade, with 89% bootstrap support). Each species formed a distinct cluster with robust bootstrap support. Within the Switchcane Clade, *A. appalachiana* (BS = 97%) and *A. tecta* (BS = 93%) are both revealed to be monophyletic.

Relationships among populations within each species were fully resolved but received poor bootstrap support, lending little confidence to the population-level associations suggested by the distance-based tree. For example, within the *A. gigantea* clade, AFLP data failed to reveal robust subclades with the exception of several accessions that occurred within

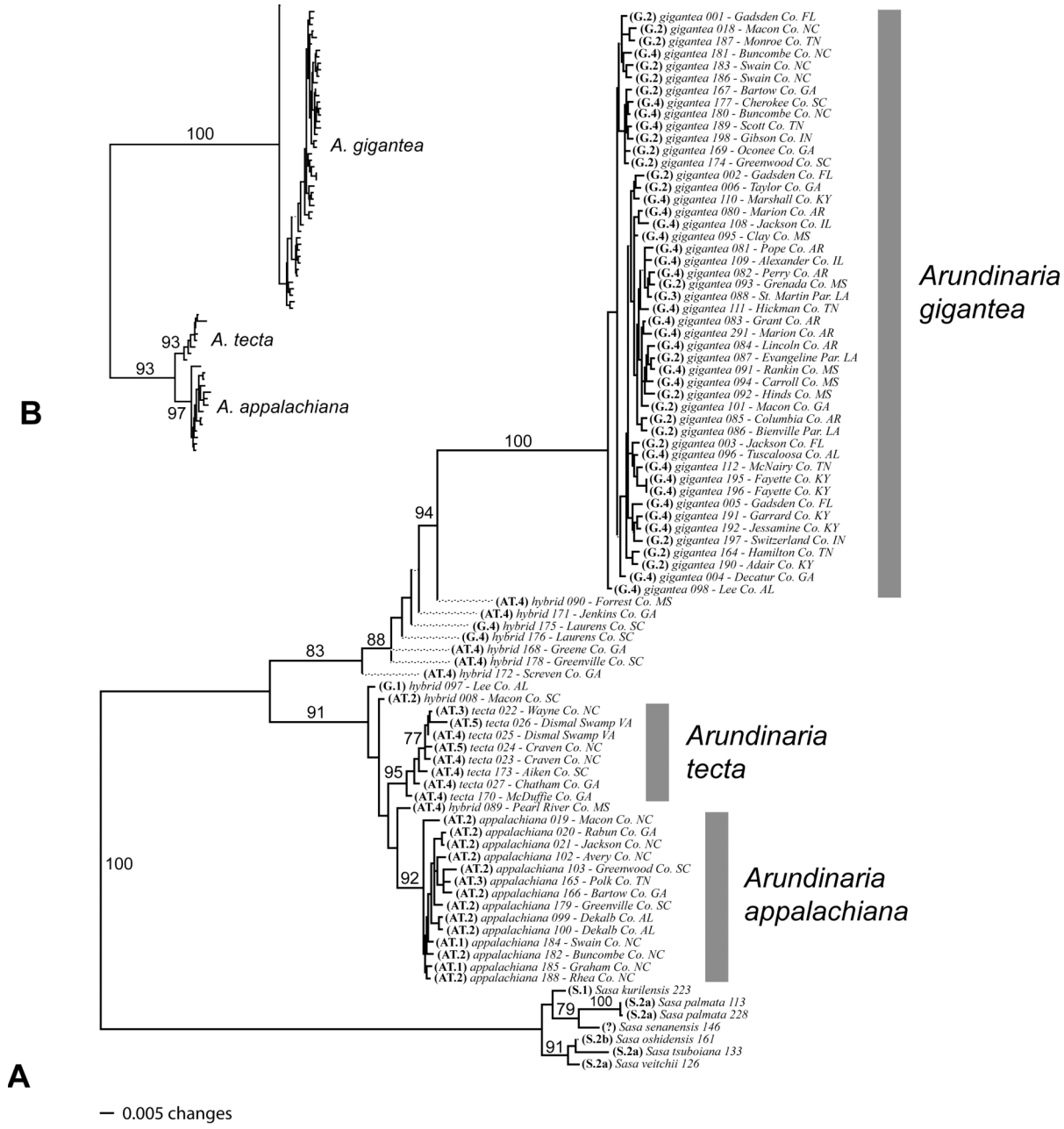

Figure 3. A. Genetic relationships among 83 populations of *Arundinaria* inferred from AFLP data using a neighbor-joining analysis of Nei-Li distances. Tree rooted with *Sasa*. Parenthetical codes designate *trnTL* haplotypes. Numbers above branches indicate bootstrap support values (>70%). B. Neighbor-joining phylogram, excluding hybrid accessions. (*Sasa* removed for clarity). Bootstrap values are indicated for major clusters only.

5 km of each other: accessions G195 and G196 from Fayette Co., KY cluster with 100% bootstrap support, while accessions G191 and G192 from Gerrard and Jessamine Cos., KY, respectively are supported in 76% of the bootstrap replicates. In general, the NJ dendrogram reveals relatively low levels of genetic diversity within each of the three species, in contrast to marked differences between species. The apparent paucity of genetic diversity is particularly striking for *A. gigantea*, which occurs over a relative broad geographic area.

Putative hybrids fell in two general areas of the neighbor-joining phylogram: one group (x090, x168, x171, x172, x175, x176, x178, hereafter referred to as type I hybrids) formed a grade at the base of the *A. gigantea* cluster, while the remaining accessions (x008, x089, x097; hereafter referred to as type II hybrids) nested at two different locations within the Switchcane cluster: at the base (x008 and x097), or sister to the *A. appalachiana* cluster (x089). UPGMA clustering (not shown) suggested an alternative topology, with type I hybrids forming a separate cluster that was sister to the *gigantea* cluster, while type II hybrids united in sister group to the *A. appalachiana* cluster. These results are identical in essence: type I hybrids cluster intermediate between the two major clades with a greater affinity for *A. gigantea*, while type II hybrids have an apparent association with the Switchcane Clade.

The phylogenetic network diagram generated in SplitsTree4 from the Nei-Li distances reveals distinct clusters for each of the three species, with putative hybrids occurring at intermediate locations between species clusters (Fig. 4). Consistent with the NJ tree (Fig. 3), the hybrids appear to be the result of more than one hybridization event. Based on their intermediate position between the River Cane and Switchcane clades and the parallel branch with *A. tecta*, accessions x168, x171, x172, x175, x176, and x178 are the result of crosses between *A. gigantea* and *A. tecta*. Moreover, these hybrids possess either the widespread *A. gigantea* cp DNA haplotype G.4 or haplotype AT.4, the most common form associated with *A. tecta*. Accession x090 is also positioned intermediate between the two major clades, but appears to share a greater portion of bands with *A. appalachiana* than with *A. tecta*. However, the haplotype recovered from x090 (AT.4) implicates *A. tecta* as the maternal parent. Type II hybrids x008, x089, x097 are essentially intermediate between the *A. appalachiana* and *A. tecta* clades. Accession (x097) possessed a distinct haplotype

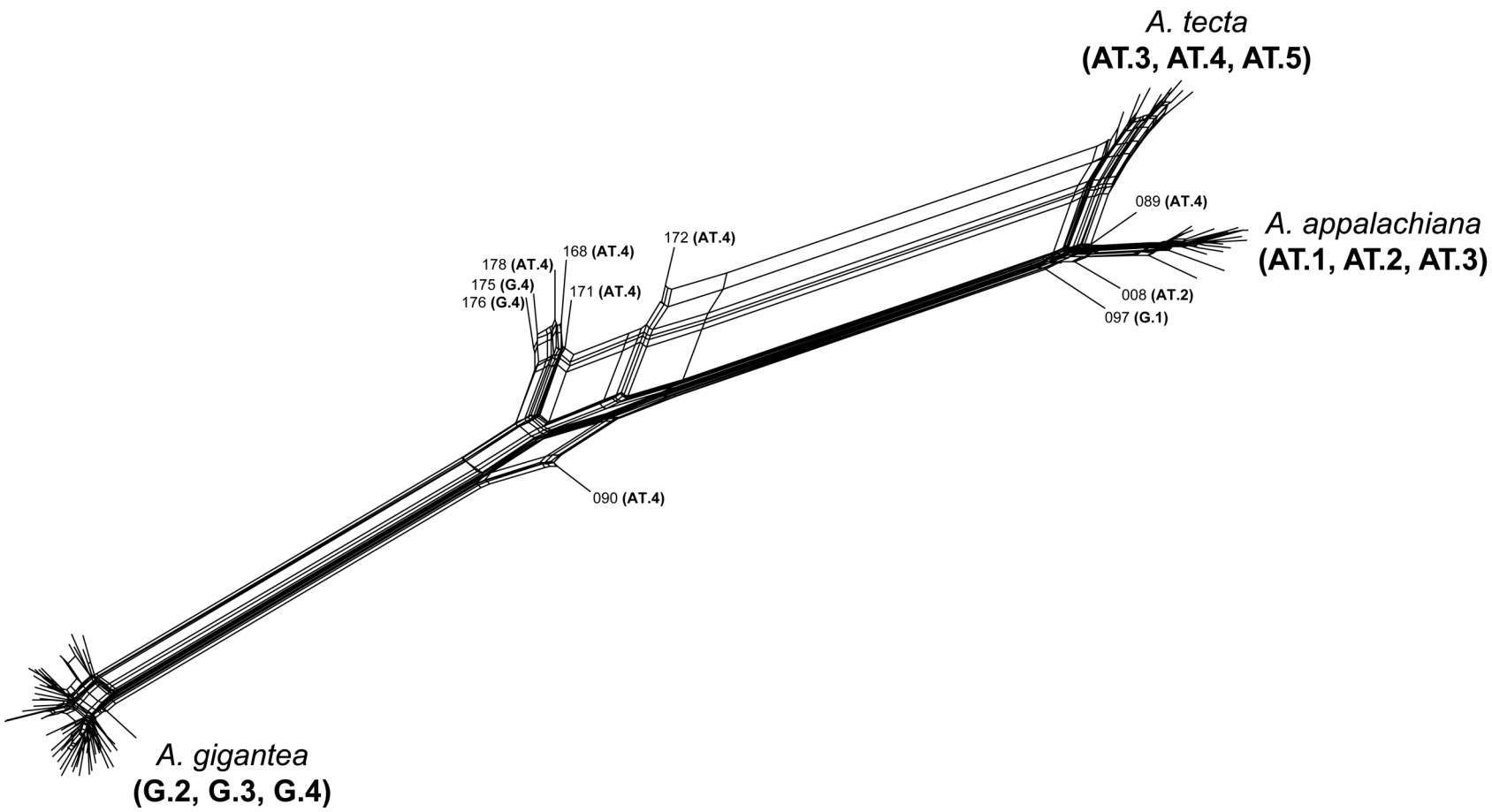

Figure 4. A SplitsTree 4 generated neighbor-net of AFLP genotypes based on Nei-Li distances, highlighting the positions of putative hybrid accessions relative to species of *Arundinaria*. cp DNA haplotypes of the three species and the putative hybrids are indicated in parentheses. (See Table 2 for explanation of haplotype codes).

associated with *A. gigantea* (G.1). This accession was also characterized by the greatest morphological divergence from other *Arundinaria* species, including large, chartaceous foliage leaf blades and long culm internodes.

NMDS Ordination of *Arundinaria* accessions shows each species to occupy a distinct region of coordinate space (Fig. 5). The first axis (nMDS1) clearly separates two groups,

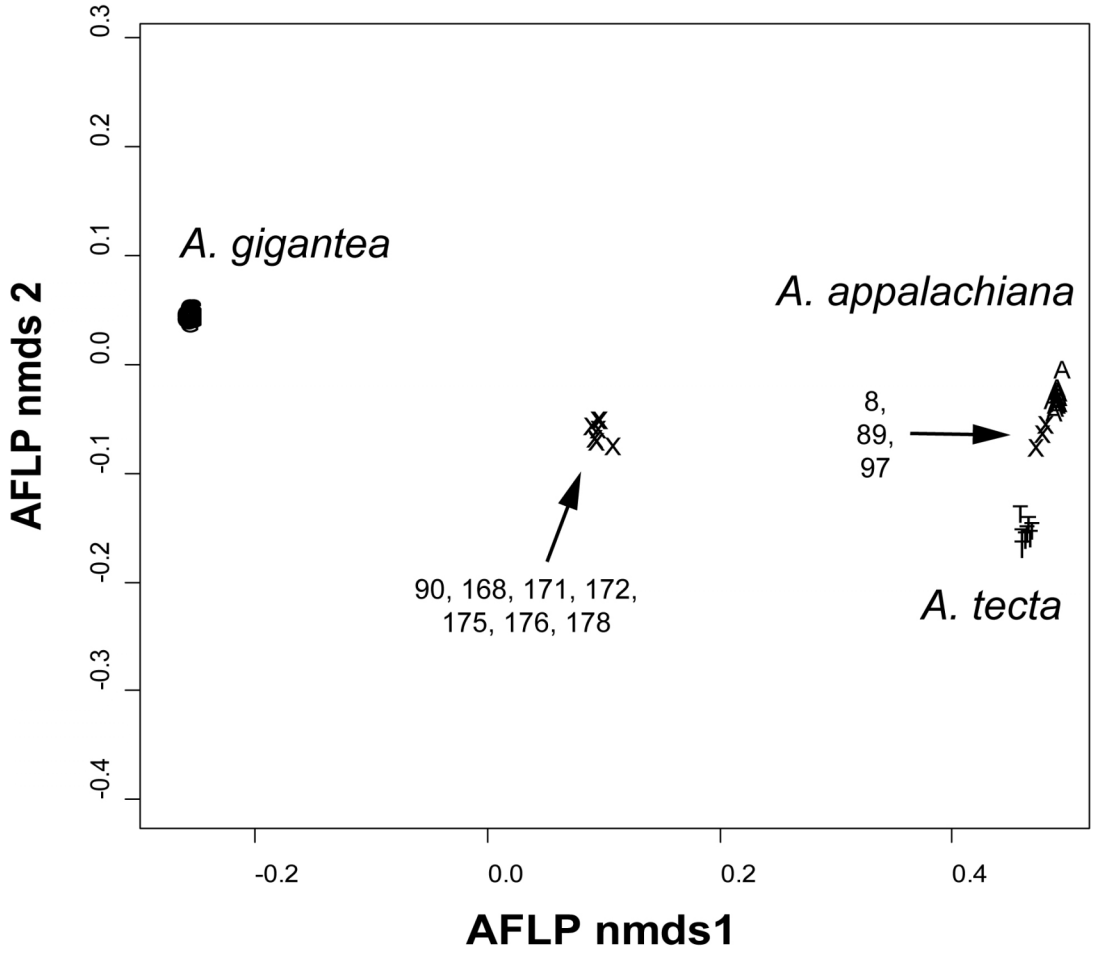

Figure 5. Two-dimensional ordination of accessions representing *Arundinaria gigantea (*G), *A. appalachiana (*A), *A. tecta (*T), and putative hybrids (indicated by voucher numbers). Ordination was conducted using nonmetric multidimensional scaling on a pairwise distance matrix calculated from AFLP genotypes using the Nei-Li dissimilarity coefficient. Final stress was reached after 50 iterations. Final stress = 1.169095.

corresponding to the River Cane Clade and the Switchcane clade. The second axis (nMDS2) provides resolution between *A. appalachiana* and *A. tecta*. Type I hybrids are located in an intermediate position between *A. gigantea* and *A. tecta* on both axes, while type II hybrids occur intermediate between *A. appalachiana* and *A. tecta*.

Parental contributions to Arundinaria hybrids. All 10 of the putative hybrids exhibited additive banding patterns of parental-specific AFLP markers, supporting the hypothesis that they originated via hybridization (Fig. 6). Type I hybrids were similar in their genetic composition, consisting of approximately equal contributions from *A. gigantea* and *A. tecta*. On average, 137 (78.7%) of the fragments discriminating between *A. gigantea* and

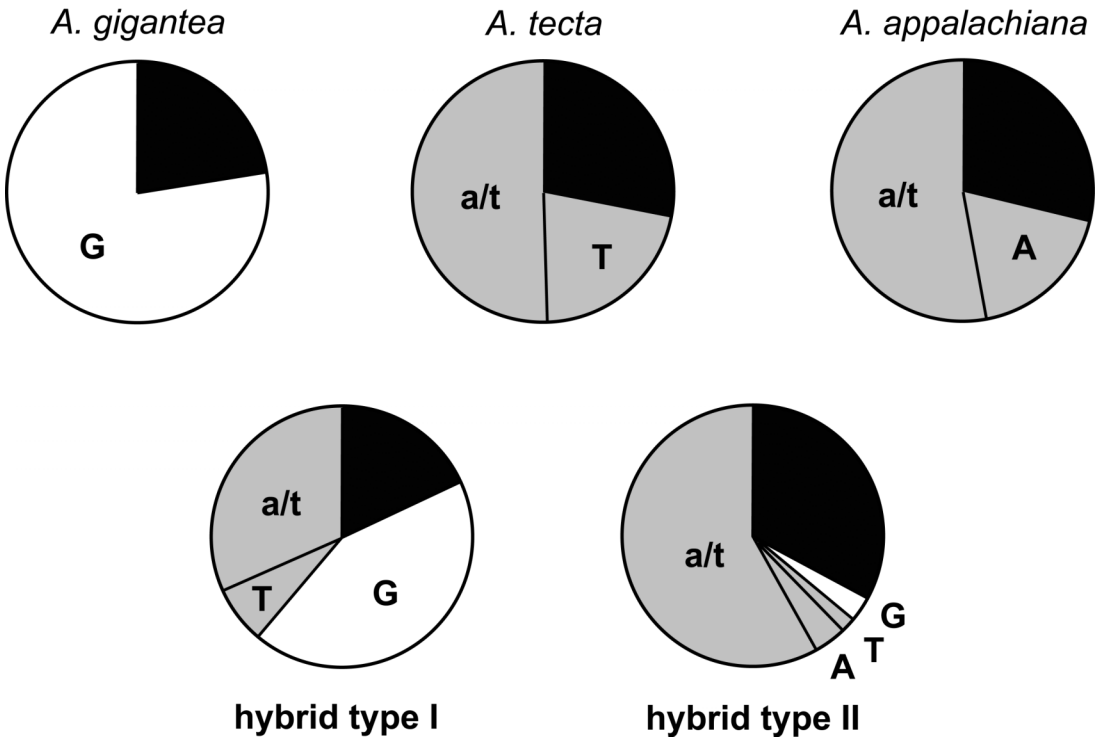

Figure 6. Contribution of AFLP fragments to *Arundinaria* species and hybrids. LETTER CODES – **G**: present only in *A. gigantea*; **T**: present only in *A. tecta*; **A**: present only in *A. appalachiana*; **a/t**: present in both *A. appalachiana* and *A. tecta.* COLOR CODES – **Black**: present in all three species; **White**: present only in *A. gigantea*; **Grey**: present in *A. appalachiana* and/or *A. tecta.*

the Switchcane Clade occur in the type I hybrids, which receive nearly equal contributions from *A. gigantea* and the Switchcane Clade (41.8% vs. 36.7%). Among the seven type I hybrids, the proportion of bands from *A. gigantea* ranged from 36.3% to 44.0%, while the proportion of bands diagnostic of the Switchcane Clade ranged from 31.9% to 42.0%.

The average proportion of bands diagnostic of *A. tecta* in the type II hybrids was 5.2%, versus 0.4% for *A. appalachiana*. Type II hybrids were a somewhat more variable group, each with a distinctive combination of parental bands. While all three of the type II hybrids received the majority of bands from the Switchcane Clade, each had a small and variable proportion of diagnostic bands from all three species. For example, while 57.4% of the bands in x008 were diagnostic of the Switchcane Clade, 5.3% were diagnostic of *A. gigantea*, 2.1% were diagnostic of *A. tecta*, and 1.1% were associated with *A. appalachiana*.

Additional analyses of species level relationships in Arundinaria. The parsimony analysis contained 332 variable characters, 331 of which were potentially parsimony informative. The 4-step heuristic analysis resulted in 7385 most parsimonious trees of 655 steps, with a CI of 0.5061 and an RI of 0.9364 (Fig. 7). The resulting topology was consistent with the NJ tree with regard to species-level relationships but lacked resolution within species. Parsimony analysis under both Wagner and Dollo optimization criteria recovered the same well-supported clades as the NJ analysis, but presented slightly different groups within clades (not shown). However, none of the internal associations were significant.

Genetic diversity within Arundinaria sensu stricto. Analysis of molecular variance (AMOVA) revealed significant genetic variation among species but relatively low population-level diversity, even among geographically distant populations (Table 5). When the data are structured taxonomically, treating each species as a separate group (and excluding putative hybrids and *Sasa* spp.), AFLPs reveal relatively low genetic variation within species (8.17%) as compared to variability partitioned among species (91.83%). The AMOVA-derived estimate of genetic differentiation (Fst) was high (0.8455), indicating significant divergence among the three species. Pairwise Fst values demonstrate greater differentiation between *A. gigantea* and the Switchcane Clade (0.8822) than between *A. appalachiana* and *A. tecta* (0.5822). Similarly, Nei's pairwise distances indicate greater

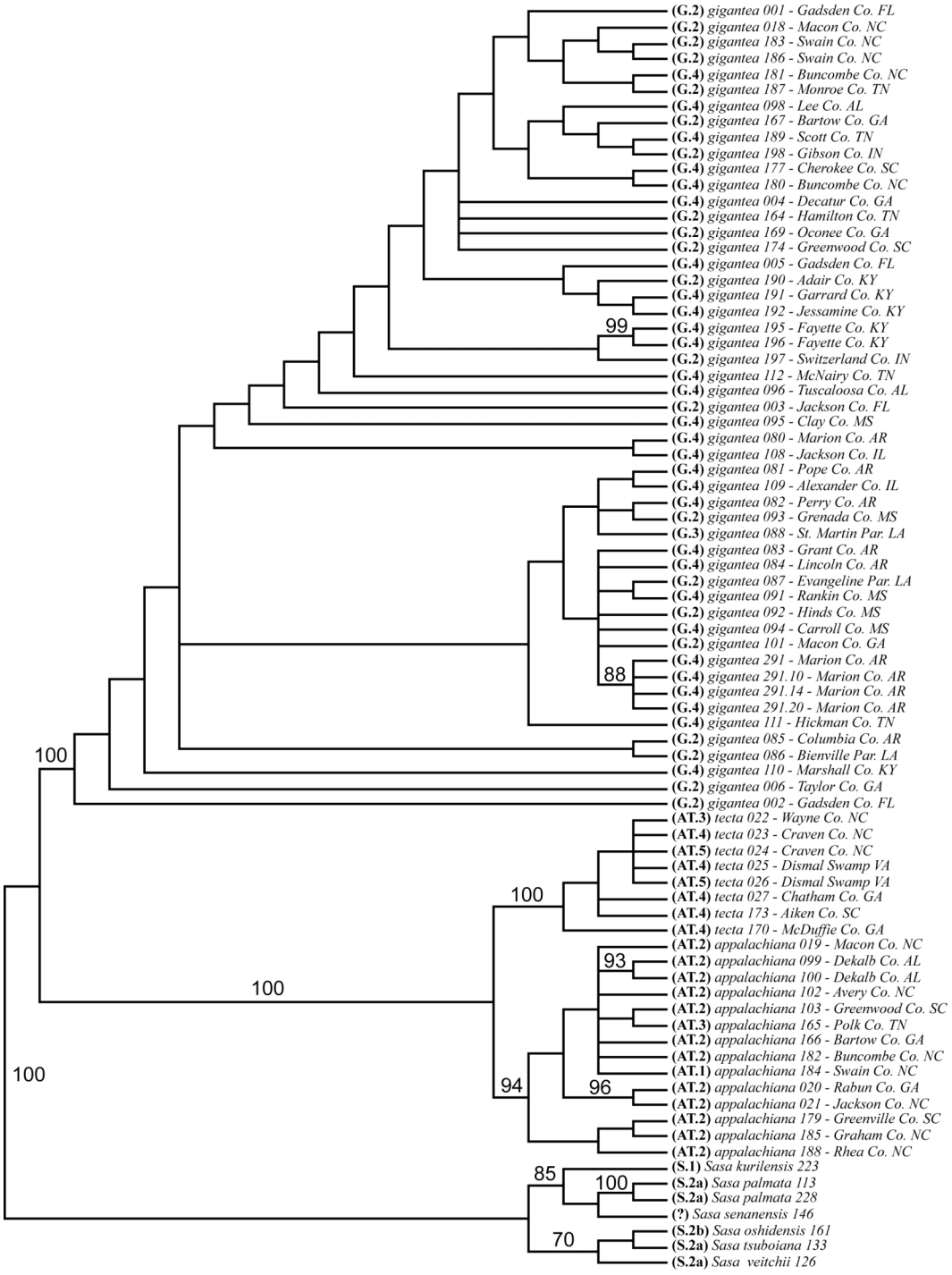

Figure 7. Strict consensus of 7385 equally most parsimonious trees obtained from the AFLP dataset, excluding putative hybrid accessions. Tree length (L) 655, CI = 0.5061, RI = 0.9364. Parenthetical codes designate *trnTL* haplotypes. Bootstrap values greater than 70% are given above the branches.

similarity between *A. tecta* and *A. appalachiana (*0.0809) than between *A. gigantea* and *A. appalachiana* (0.5399) or *A. tecta (*0.5658). Other measures of diversity reiterate the apparent paucity of genetic variation within species. For example, average gene diversity (Hw) within species was 0.0534 (+/-0.00067), while total gene diversity (Ht) was 0.3463 (+/-0.00067). Nei's gene diversity indices (Hj) were similar among the three species (*A. gigantea*, 0.05452 +/- 0.00677; *A. appalachiana*, 0.0522 +/- 0.00610; *A. tecta* 0.05360 +/- 0.00636), in each case indicating low levels of genetic variation.

Table 5. Analysis of Molecular Variance (AMOVA) of AFLP data among three species of *Arundinaria*.

Source	***df***	**SS**	**Est. var.**	**% total var.**
Among spp.	2	2414.285	71.03716	91.83
Within spp.	70	442.565	6.32235	8.17

The Mantel test revealed that AFLP data are geographically structured in the two widespread species, *A. gigantea* ($r=0.4142$, $p<0.0001$) and *A. tecta* ($r=0.7615$, $p<0.01$). Geographic structure was not detected in *A. appalachiana* ($r=0.1604$, $p=0.16$). Weak associations were revealed for cp DNA haplotype variation in *A. gigantea* in relation to geography ($r=0.03968$, $p<0.10$) and in correlation with the genetic diversity measured by AFLP ($r=0.0504$, $p<0.05$).

DISCUSSION

The expected consequence of including hybrids in a tree-building analysis is a general reduction in support values due to signal conflict among otherwise distinct groups (McDade 1992, 1997). The placement of the putative *Arundinaria* hybrids, with moderate to low bootstrap values and conflicting positions depending on method of analysis, supports the

hypothesis that these accessions are of hybrid or introgressed origin. The fundamental topology of the tree was well-supported even with the inclusion of putative hybrids, but excluding these accessions provided increased bootstrap support overall for the NJ analysis (Fig. 3b), including population-level associations.

Presumably, F1 hybrids are positioned between parents in a NJ phylogram. Thus, the NJ tree suggests that type I hybrids involved a cross between *A. gigantea* and a species in the Switchcane Clade. Since the mosaic pattern is expected to become increasingly complex with posthybridizational gene segregation and introgression, the relatively simple pattern observed here suggests type I hybrids are essentially clonal descendents of F1 hybrids, and have likely undergone limited sexual reproduction since hybridization. The parentage of type II hybrids is less clear, and can be interpreted as involving hybridization between *A. appalachiana* and *A. tecta* or else posthybridization introgression. Accession (x097) had a distinct haplotype that occurs in the *gigantea* haplotype clade (G.1). This accession was also characterized by the greatest morphological divergence from other *Arundinaria* species. The unusual position and the occurrence of the G.1 haplotype are thus suggestive of introgression.

Hybrid accession x090 from Mississippi had diagnostic bands from *A. gigantea* and the Switchcane clade, but none that identified the parental species from the Switchcane Clade. Since no accessions of *A. tecta* were collected west of Georgia, a possible explanation for this observation is that species-specific bands in *A. tecta* are also geography-specific, and thus in the absence of a local parental genotype, diagnostic bands were undetected.

Chloroplast DNA haplotype AT.3 was found only in *A. appalachiana* (A165) and *A. tecta* (A022). These two populations are not in close proximity, and the most likely explanation is that this haplotype was independently derived in these two species; alternatively, this may indicate incomplete lineage sorting (assuming cp DNA polymorphism in the ancestral population) or introgression.

Many of the bands detected in the hybrids showed a dosage effect, *i.e.*, a band present in one parent but absent in the other occurred in the hybrid at 50% the intensity noted in the donor parent. This suggests that the relative number of copies of a particular allele may be detectable by AFLP analysis. In such a case, it is reasonable to assume that the parent is

homozygous for the allele, while the hybrid is heterozygous. These observations helped to diagnose the hybrids but they were not explored further in the current study.

Genetic diversity and phylogenetic relationships within Arundinaria. Numerous taxonomic treatments have been recommended for *Arundinaria*, reflecting confusion over an enigmatic group of plants that is at once cohesive and polymorphic. McClure's (1973) circumscription represents an exceptional account of organisms too diverse to be considered the same taxon but nonetheless linked though morphological intergradation, thus guiding his decision to recognize subspecies. The result is a species (*A. gigantea sensu* McClure) that exhibits higher morphological and molecular diversity than any other among the temperate bamboos. Nevertheless, McClure's description of *Arundinaria* was extremely accurate regarding the complex evolutionary network that underlies this group.

Molecular data presented here provide clear evidence of three distinct lineages within *Arundinaria s.s.* and reconcile taxonomic difficulties by diagnosing a pattern of genetic intergradation consistent with interspecific hybridization. Moreover, AFLP data reveal a sharp divide between *A. gigantea* and the Switchcane Clade, reflecting greater divergence among these taxa than generally recognized. These results are corroborated by cp DNA sequence variation that reveals significant divergence between these clades (0.2936) of an order of magnitude above species-level differences in other well-defined genera (*e.g.*, divergence among species of *Sasa*: 0.0734; among species of *Phyllostachys*: 0.0367), and closer to the values observed among subgenera (*e.g.*, *Pleioblastus* section *Pleioblastus* vs. sect. *Nezasa*: 0.3670). The overall similarity among sequences in *A. appalachiana* and *A. tecta* (0.0367) suggests a more recent divergence.

Consistent with other studies on species- and population-level variation in bamboo (Friar and Kochert 1994; Gielis 1995; Gielis *et al.* 1997; Lai and Hsiao 1997; Marulanda *et al.* 2002; but see Suyama *et al.* 2000), the three species of *Arundinaria* exhibit low levels of genetic diversity. Moreover, the limited within-species variation appears to lack a hierarchical structure. The lack of within-species resolution is not unexpected assuming polymorphic AFLP markers represent independently segregating loci, whereby gene flow and/or incomplete lineage sorting among populations could result in signal conflict. Low MP

CI values (Fig. 7) demonstrate a relatively high level of homoplasy in the AFLP data, presumably resulting from polymorphism at population level.

This study provides validation of McClure's conjectures about hybridization in *Arundinaria* and indeed his characterization of *Arundinaria* diversity in general. The major exception is our decision to recognize phylogenetically distinct entities as species rather than subspecies. On its own, the ability to hybridize cannot be taken as evidence of incomplete speciation, since distant relatives among the temperate clade have been shown to hybridize in nature (Triplett and Clark, in prep; Ch. 5; Hosoyama *et al.* 2002; Ishii *et al.* 2003). Morphological differences (summarized in Triplett *et al.* 2006) clearly track the AFLP phylogeny and provide a straightforward interpretation of diversity in this genus. The combined lines of evidence from AFLPs, cp DNA, morphology, ecology, and phytogeography suggest that North American *Arundinaria* comprise divergent evolutionary lineages that are best recognized as distinct species.

Hybridization in Arundinaria s.s. AFLP data considered in conjunction with morphology and cp DNA haplotypes provide unequivocal evidence of hybridization among species of *Arundinaria*. The approximately additive nature of AFLP markers in morphologically intermediate or otherwise unusual accessions clearly supports the hypothesis of a hybrid origin for these plants. Furthermore, the occurrence of different *trn*TL haplotypes among these accessions indicates multiple and reciprocal hybridization events.

Hybridization clearly plays a role in cane biology, but it is unclear how important this phenomenon has been in the evolution of diversity in this genus. Based on current evidence, hybrid speciation does not seem to have occurred in *Arundinaria*. Rather, molecular data argue for a relatively shallow phylogenetic impact of hybridization. We hypothesize that hybrids exhibiting one-to-one complements of parental AFLP markers (type I hybrids) represent F1 offspring (Estabrook *et al.* 1996). These hybrids may in fact be very old, persisting clonally through vegetative reproduction, or they may represent recent events. Introgression via outcrossing appears to play a relatively minor role in this species complex, based on the current dataset. Why was greater evidence of introgression not detected? Perhaps hybrid populations are short-lived, or rarely flower; alternatively, introgression may be difficult to detect at the level currently sampled. Additional population-level studies may

reveal more complex patterns of introgression and hybridization, including the persistence and subsequent diversification of hybrid genomes.

Although this study does not provide direct evidence about the outcome of natural hybridization, our observations demonstrate that seemingly improbable hybridization events may be more common among bamboos than presently realized. Hybridization and introgression potentially generate significant genetic and phenotypic variability, including the transfer of ecological adaptations between species (Stutz and Thomas 1964; Arnold and Bennett 1993; Kim and Rieseberg 1999; Mahelka *et al.* 2007). Although hybrid fitness was not measured in this study, observed populations were apparently healthy and well established, suggesting they may be as fit as the parental plants and potentially better suited to the habitats in which they occur. Studies of hybridization in the neotropical bamboo genus *Chusquea* suggest that hybrids can be as fertile as parental species, as demonstrated by pollen fertility, lodicule development, and normal stigma and stamen exsertion (Clark *et al.* 1989). A better understanding of habitat type for the three species and hybrids is needed, along with additional information on the fate of hybrids. Are they reproductive dead ends, or can they successfully reproduce, crossing either with themselves or with parental species? How widespread are clones of hybrid origin? The detected hybrid populations appear to occur in isolation, and not in sympatry with either parent, although in the broad sense they occur in the area of overlapping ranges. What is the explanation for the persistence of the hybrid in the absence of the parental species? It could be strictly due to chance, or a selective advantage for the hybrids in a changing environment. More extensive sampling within populations, coupled with studies of morphology and ecology, would allow these questions to be more rigorously explored.

The observed hybridization in *Arundinaria* highlights the potential role of this phenomenon in the temperate bamboos, and indicates the need to review other examples of hybridization. For example, Hodkinson *et al.* (2000) suggested that unexpected patterns revealed by comparisons of ITS, AFLPs and morphology in *Phyllostachys* may be the result of hybridization. In particular, one group of species ("*Phyllostachys 1,"* including *P. bambusoides* Siebold & Zuccharini*, P. dulcis* McClure*, P. nuda* McClure, and *P. viridiglaucescens* (Carriere) A. & C. Riviere) are morphologically intermediate between the

two established sections of *Phyllostachys (Phyllostachys* and *Heterocladae* Z.P. Wang & G.H. Ye), and principal coordinates analysis of AFLP data also revealed an intermediate position.

In spite of their ability to hybridize, the three species of *Arundinaria* have retained species-specific morphologies and phylogenetic distinction. In the absence of a biological barrier to cross-fertilization, the most likely explanation for speciation in this genus is geographical isolation followed by ecological and phenological diversification. The observed level of genetic differentiation suggests that these species have had a long history of physical separation since colonizing North America, whereas hybridization may have a more recent history. Further research is needed to determine the fitness landscape of these three species, and the potential interaction between hybrids and parental species. A likely hypothesis is that species-level differences are maintained by selection, for example via adaptation to particular habitats, but selection may favor hybrid populations in intermediate or otherwise novel habitats.

Hybrid populations appear to be relatively common in southern Mississippi and east to southwestern Georgia. In contrast, stands of *A. tecta* are apparently not as common as expected. Further research is needed to determine whether hybrids occupy an intermediate habitat type, and whether the historical range of *A. tecta* has been impacted by either habitat loss or hybrid competition.

Phylogeography of Arundinaria. Recent analyses of *Arundinaria* and its putative allies support the monophyly of the North America genus (Triplett and Clark, in prep; Zhang *et al.*, in prep.; Ch. 2; Ch. 5), while the cp DNA phylogeny of the temperate clade demonstrates relatively high divergence among major lineages within *Arundinaria* (Triplett and Clark, in prep.; Ch. 2). Results support the hypothesis that New World temperate species form an exclusive lineage that is sister to a clade of East Asian taxa. Our analysis is consistent with the hypothesis that *Arundinaria* originated in East Asia and migrated across the Bering land bridge into North America (or that a *Sasa*-like population moved into North America and then diverged). A likely timetable for the introduction of *Arundinaria* or its immediate ancestor into North America would be during the early Miocene (ca. 16-23 mya),

when the climate was favorable for the migration of temperate species across the Bering land bridge (Tiffney 1985).

In light of the relatively high sequence divergence among these species, it is not possible to determine whether two lineages were extant prior to this migration, or whether diversification of *A. gigantea* and the Switchcane Clade occurred after occupying North America, but evidence suggests the phylogenetic split is relatively old. Diversification of *Arundinaria* in North America resulted in three distinct lineages, which have subsequently become intertwined due to historical events relating to biogeography and hydridization. The genetic similarity between *A. tecta* and *A. appalachiana* suggests a recent divergence.

The three species of *Arundinaria* have unique geographical distributions, with some areas of overlap along the borders of their respective ranges but separated ecologically (Fig. 1). The current distribution of *Arundinaria* in North America has likely been influenced by past climatic and geologic changes, and the current pattern of genetic diversity and cp DNA haplotype distribution suggests an interesting hypothesis on the biogeographic history of these species. If *Arundinaria s.s.* diversification represented a simple pattern of isolation by distance, the expected pattern of molecular diversity would follow a cline across the distribution. Distant populations would have the greatest differences, and intermediate populations would fall along a continuum. Observations on the widespread *A. gigantea* suggest a more complex pattern. First, AFLP data indicate low levels of genetic diversity and incomplete lineage sorting consistent with a recent diversification, yet also reveal an association between geographic and genetic distance. Second, the occurrence of at least four cp DNA haplotypes within *A. gigantea* suggests a relatively ancient plastid diversification within this species, yet these haplotypes are widespread, suggesting relatively recent gene flow among the populations. Perhaps *A. gigantea* was forced south into a much smaller area, latter to spread back into its wider distribution. Much of the current range of *A. gigantea* is north of the Wisconsinan glacial boundaries, and it is likely that the range of this species was compressed into suitable southern habitats during glacial maxima, subsequently expanding to obtain its current distribution. The occurrence of multiple haplotypes suggests that these chloroplast lineages predate the current distribution, while the narrow genetic variation indicated by AFLPs suggests the ancestral population experienced a bottleneck.

Implications for the evolution of temperate bamboos. Perhaps by virtue of the relatively narrow range of diversity (*i.e.*, only 3 species), the North American species have received greater scrutiny than species in other genera of the temperate clade, and the impact of hybridization has been better characterized. In contrast, the vast diversity among temperate bamboos in East Asia and the corresponding difficulty associated with parsing that variation into meaningful morphological groups has obscured the detection of hybrids among those taxa. But if hybridization is possible among sister species in North America, why are there so few accounts among the nearly 500 species in Asia? Most likely, the detection of hybridization has suffered as a consequence of high phylogenetic diversity paired with poorly-characterized phenotypic plasticity, plus the guiding opinion that rare flowering events negate the potential for hydridization.

The temperate bamboos have a long history of taxonomic difficulty, and earlier problems impacting traditional taxonomy have carried over into molecular systematics. Rapid radiation is generally invoked to explain the complex suites of overlapping, intergrading, or mosaic morphological features as well as the observed paucity of sequence divergence. Perhaps some portion of the morphological muddle is also due to reticulate evolution. The current study demonstrates that hybridization can explain morphological intergradation in *Arundinaria*, and more important, that the pattern it presents is not intractable. Removing the noise arising from hybridization and introgression makes it possible to improve phylogenetic resolution and to progress towards an improved understanding of morphological diversity.

Recent phylogenetic work has provided evidence that hybridization can occur between divergent lineages of temperate bamboos, *e.g.*, *Phyllostachys* and *Pleioblastus* (Triplett and Clark, in prep.; Ch. 5). Thus, rather than basing species boundaries in this group on the biological species concept, it is necessary to use a combination of phylogenetic and morphological species concepts, whereby evolutionarily divergent and morphologically distinct entities can be recognized. In light of the divergence between river cane, switchcane and hill cane, it seems clear that these lineages are more appropriately considered species. Such a treatment is consistent with diversity in the temperate clade and more accurately represents phylogenetic relationships and evolutionary history.

This study provides additional evidence that AFLPs are a valuable tool for the phylogenetic analysis of temperate bamboos. This study also provides a relatively course-grained study of population-level variation. Additional research with multiple accessions per population is needed to provide more accurate estimates of genetic diversity within species, particularly with reference to cp DNA variation within a local population. Indeed, this study serves as a call for phylogenetic research with narrower taxonomic scopes, targeting species complexes in order to address questions at the boundary between phylogenetics and population biology. Only with this additional level of detail will evolutionary processes influencing bamboo diversity be revealed. Future work is also needed to explore questions on the fate of bamboo hybrids. For example, are they capable of sexual reproduction, and do they have a genetic impact on the parental populations? Do they exist in distinct populations, or mixed with parents? A more detailed population-level analysis would be particularly valuable, especially if a hybrid zone encompassing sympatric parental populations were to be located.

Hybridization adds to a long list of factors adding difficulty for bamboo systematics, including low sequence divergence, unusual reproductive strategies, possibly recent origin, parallel or convergent evolution, phenotypic plasticity, and over-taxonomy. Natural hybridization has been noticeably understudied in bamboos, but may in fact be an important source of diversity. In view of current results, bamboo taxonomy needs to be carefully reevaluated.

ACKNOWLEDGEMENTS

Field work was supported by a Graduate Research Grant from the American Society of Plant Taxonomists and grants from the American Bamboo Society to JT as well as a National Geographic Society Grant (#7336-02) to LC. Support for JT and for the final preparation of this manuscript came from a National Science Foundation Grant (DEB-0515712) to LC. We are indebted to Angus Gholson (Chattahoochee, FL), Dr. Michael Hotchkiss (USDA, Byron, GA), Dr. Alan Weakley (UNC, Chapel Hill, NC), Gary Kauffman, Adam and Sue Turtle (Earth Advocates Research Farm, Summertown, TN), and Dr. Julian Campbell (Lexington, KY) for their assistance in locating populations of *Arundinaria*. JT is

grateful to Masayo Ozaki for assistance with fieldwork. We also thank the curators of the herbaria that loaned specimens for this study. We thank Ryan Percifield, Dr. Corrine Grover, and Chris Tyrrell for technical assistance and Dr. Jonathan Wendel and Dr. Robert Wallace for access to laboratory facilities.

LITERATURE CITED

ALTHOFF, D.M., M.A. GITZENDANNER AND K.A. SEGRAVES. 2007. The utility of amplified fragment length polymorphisms in phylogenetics: a comparison of homology within and between genomes. Syst. Biol. 56 (3): 477–484.

ÁLVAREZ, I. AND J.F. WENDEL. 2006. Cryptic interspecific introgression and genetic differentiation within *Gossypium aridum (*Malvaceae) and its relatives. Evolution 60 (3): 505-517.

ARNOLD, M.L. 1997. Natural Hybridization and Evolution, Oxford University Press, New York.

ARNOLD, M.L., AND B.D. BENNETT. 1993. Natural hybridization in Louisiana irises: genetic variation and ecological determinants. In: Hybrid Zones and the Evolutionary Processes—Harrison R.G., ed. Oxford: Oxford University Press. 115–139.

BEARDSLEY, P.M., A. YEN, AND R.G. OLMSTEAD. 2003. AFLP phylogeny of *Mimulus* section *Erythanthe* and the evolution of hummingbird pollination. Evolution 57 (6): 1397-1410.

BLEEKER, W. 2003. Hybridisation and *Rorippa austriaca* (Brassicaceae) invasion in Germany, Molecular Ecology 12: 1831–1841.

BLEEKER,W. AND A. MATTHIES. 2005. Hybrid zones between invasive *Rorippa austriaca* and native *R. sylvestris* (Brassicaceae) in Germany: ploidy levels and patterns of fitness in the field, Heredity 94: 664–670.

BRYANT, D. AND V. MOULTON. 2004. NeighborNet: an agglomerative algorithm for the construction of phylogenetic networks. Molecular Biology and Evolution 21 (2): 255-265.

CHAO, C.S., AND S.A. RENVOIZE. 1989. Revision of species described under *Arundinaria* (Gramineae) in South-east Asia and Africa. Kew Bull. 44: 349-367.

CHASE, M.W. AND H.H. HILLS. 1991. Silica gel: An ideal material for field preservation of leaf samples for DNA studies. Taxon 40: 215–220.

CHEN, R.Y. AND W.X. ZONG. 1991. Chromosome numbers of some scattered bamboos. Acta Phytotax Sin. 29 (5): 452-455.

CLARK, L.G., AND J.K. TRIPLETT. 2007. *Arundinaria*. In: Barkworth, M.E., K.M. Capels, S. Long, L.K. Anderton, and M.B. Piep (eds.). *Magnoliophyta commelinidae* (in part: Poaceae, part 1). Flora of North America north of Mexico, volume 24. Oxford University Press, New York, New York, USA.

CLARK, L.G., G. DAVIDSE, AND R.P. ELLIS. 1989. Natural hybridization in bamboos: evidence from *Chusquea* sect. *Swallenochloa (*Poaceae: Bambusoideae). Natl. Geog. Res. 5: 459-476.

CLAYTON, W.D. AND S.A. RENVOIZE. 1986. Genera Graminum: Grasses of the World. Royal Botanic Gardens, Kew.

DESPRÉS, L., L. GIELLY, B. REDOUTET, AND P. TABERLET. 2003. Using AFLP to resolve phylogenetic relationships in a morphologically diversified plant species complex when nuclear and chloroplast sequences fail to reveal variability. Molec. Phyl. Evol. 27: 185–196.

ELLIS, R.P., J.W. MCNICOL, E. BAIRD, A. BOOTH, AND P. LAWRENCE. 1997. The use of AFLPs to examine genetic relatedness in barley. Molecular Breeding 3: 359-369.

ERSTS, P.J. [Internet] Geographic Distance Matrix Generator (version 1.2.2). American Museum of Natural History, Center for Biodiversity and Conservation. Available from http://biodiversityinformatics.amnh.org/open_source/gdmg. Accessed on 2008-4-23.

ESTABROOK, G.F., N.L. GIL-AD, AND A.A. REZNICEK. 1996. Hypothesizing hybrids and parents using character intermediacy, parental distance, and equality. Taxon 45: 647-662.

EXCOFFIER, L. G. LAVAL, AND S. SCHNEIDER. 2005. Arlequin ver. 3.0: An integrated software package for population genetics data analysis. Evolutionary Bioinformatics Online 1: 47-50.

EXCOFFIER, L., P.E. SMOUSE and J.M. QUATTRO. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: applications to human mitochondrial DNA restriction data. Genetics 131: 479-491.

FARRIS, J.S. 1970. Methods for computing Wagner trees. Systematics Zoology 19: 83-92.

FARRIS, J.S. 1977. Phylogenetic analysis under Dollo's Law. Systematic Zoology 26: 77-88.

FELSENSTEIN, J. 1985. Confidence limits on phylogenies: An approach using the bootstrap. Evolution 39:783-791.

FRIAR, E. AND G. KOCHERT. 1994. A study of genetic variation and evolution of *Phyllostachys* (Bambusoideae: Poaceae) using nuclear restriction fragment length polymorphisms. Theor. Appl. Genet. 89: 265-270.

GIELIS, J. 1995. Bamboo and biotechnology. European Bamboo Society Journal. May 6: 27-39.

GIELIS, J., I. EVERAERT, AND M. DE LOOSE. 1997. Genetic variability and relationships in *Phyllostachys* using random amplified polymorphic DNA. In: Chapman, G.P. (ed.). The Bamboos. Linnean Society of London Symposium Series. Academic Press, London.

GILLY, C.L. 1943. A preliminary investigation of the North American Canes (*Arundinaria*). Bull. Torrey Club 70: 297-309.

GUO Y.P., J. SAUKEL, R. MITTERMAYR, AND F. EHRENDORFER. 2005 AFLP analyses demonstrate genetic divergence, hybridization, and multiple polyploidization in the evolution of *Achillea* (Asteraceae-Anthemideae). New Phytol. 166 (1): 273-89.

GRANT, V. 1981. Plant speciation. Columbia University Press, New York.

HITCHCOCK, A.S. 1971. Manual of the grasses of the United States. Dover Publ., New York

HODKINSON, T.R., S.A. RENVOIZE, G. NÍ CHONGHAILE, C.M.A. STAPLETON, AND M.W. CHASE. 2000. A comparison of ITS Nuclear rDNA sequence data and AFLP markers for phylogenetic studies in Phyllostachys (Bambusoideae, Poaceae). J. Plant Res. 113: 259-269.

HOLTTUM, R.E. 1958. The bamboos of the Malay Peninsula. Gardens' Bulletin, Straits Settlements 16: 1-35.

HOSOYAMA, Y., K. HOSHIDA, S. TAKEOKA, AND S. MIYATA. 2002. On the inter-generic hybrid *Sasaella ramosa*. Proceedings on the Institute of Natural Sciences, Nihon University. 37: 209-216. [In Japanese]

HUGHES, R.H. 1951. Observations of cane (*Arundinaria*) flowers, seed, and seedlings in the North Carolina coastal plain. Bull. Torrey Bot. Club. 78: 113-121.

HUSON, D. AND D. BRYANT. 2006. Application of Phylogenetic Networks in Evolutionary Studies. Molecular Biology and Evolution 23 (2) 254-267.

ISHII, A., S. MIYATA, AND Y. HOSOYAMA. 2003. The maternal species of hannouzasa (*Sasaella ramosa*). Bamboo J. 20: 12-18. [In Japanese]

JANZEN, D. 1976. Why bamboos wait so long to flower. Ann. Rev. Ecol. Syst. 7: 347-391.

JUDZIEWICZ, E.J., L.G. CLARK, X. LONDONO, AND M.J. STERN. 1999. American Bamboos. Smithsonian Institution Press, Washington.

KIM, S.C., AND L.H. RIESEBERG. 1999. Genetic architecture of species differences in annual sunflowers: implications for adaptive trait introgression. Genetics 153: 965-977.

KOOPMAN, W.J.M., M.J. ZEVENBERGEN, AND R.G. VAN-DEN-BERG. 2001. Species relationships in *Lactuca S. L.* (Lactuceae, Asteraceae) inferred from AFLP fingerprints. Am. J. Bot. 88: 1881-1887.

KOYAMA, T. AND S. KAWANO 1964: Critical taxa of grasses with North American and eastern Asiatic distribution. Canadian Journal of Botany 42: 859-884

LAI, C.C., AND J.Y. HSIAO. 1997. Genetic variation of *Phyllostachys pubescens* (Bambusoideae, Poaceae) in Taiwan based on DNA polymorphisms. Bot. Bull. Acad. Sin. 38: 145-152.

LARA-CABRERA, S.I. AND D.M. SPOONER. 2004. Taxonomy of North and Central American diploid wild potato (*Solanum* sect. *Petota*) species: AFLP data. Plant Syst. Evol. 248: 129-142.

LE QUESNE, W. J. 1974. The uniquely evolved character concept and its cladistic application. Systematic Zoology 23: 513-517.

LI, D.Z. 1997. The Flora of China Bambusoideae Project: Problems and current understanding of bamboo taxonomy in China. In: Chapman, G.P. (ed.). The Bamboos. Linnean Society of London Symposium Series. Academic Press, London.

LINDQVIST C., T.J. MOTLEY, J.J. JEFFREY, AND V.A. ALBERT. 2003. Cladogenesis and reticulation in the Hawaiian endemic mints (Lamiaceae). Cladistics 19: 480-495

LOH, G.P., R. KIEW, A. HAYS, A. KEE, L. HUAT, AND Y. YUEN. 2000. A study of genetic variation and relationships within the Bamboo subtribe using Amplified Fragment Length Polymorphism. Annals of Botany 85: 607-612.

MCDADE, L.A. 1992. Hybrids and phylogenetic systematics II. The impact of hybrids on cladistic analysis. Evolution 46 (5): 1329-1346.

MCDADE, L.A. 1997. Hybrids and phylogenetic systematics III. Comparison with distance methods. Syst. Bot. 22 (4): 669-683.

MAHELKA, V, J. FEHRER, F. KRAHULEC, AND V. JAROLÍMOVÁ. 2007. Recent natural hybridization between two allopolyploid wheatgrasses (*Elytrigia*, Poaceae): ecological and evolutionary implications. Ann Bot 100: 249–260.

MARULANDA, M.L., P. MÁRQUEZ, AND XIMENA LONDOÑO. 2002. AFLP analysis of *Guadua angustifolia* (Poaceae: Bambusoideae) in Colombia with emphasis on the Coffee Region. J. Am. Bamboo Soc. 16 (1): 32-42.

MARUYAMA, I., H. OKAMURA, AND G. MURATA. 1979. On a new hybrid genus *Hibanobambusa*. Acta Phytotaxonomica et Geobotanica 30: 148-152 (Japanese)

MASSA, A.N. AND S.R. LARSON. 2005. Phylogeography of North American mountain bromes. Native Plants Journal 6 (1): 29-35.

MCCLURE, F.A. 1973. Genera of Bamboos Native to the New World (Gramineae: Bambusoideae). Ed. T.R. Soderstrom. Smithsonian Contr. Bot. 9: 1-148.

MITFORD, A.B. 1896. The Bamboo Garden. London and New York: Macmillan and Co.

MUELLER, U. G., AND L.L. WOLFENBARGER. 1999. AFLP genotyping and fingerprinting. Trends Ecol. Evol. 14: 389-394.

MUNRO, W.M. 1868. A monograph of the Bambusaceae, including descriptions of all the species. Trans. Linn. Soc. London. 26: 1-157.

NEI, M., AND W.H. LI. 1979. Mathematical model for studying genetic variation in terms of restriction endonucleases. Proc. Natl. Acad. Sci. 76: 5269-5273.

NOSS, R. F., E.T. LAROE, III, AND J.M. SCOTT. 1995. Endangered ecosystems of the United States: a preliminary assessment of loss and degradation. U.S. Dept. Interior, Nat. Biol. Serv., Biol. Rep. 28.

OHRNBERGER, D. 1999. The Bamboos of the World. Elsevier, Amsterdam, The Netherlands.

OHRNBERGER, D., AND J. GOERRHINGS (eds). 1986. The Bamboos of the World. Odenthal, FRG.

OLMSTEAD, R.G. AND J.D. PALMER. 1994. Chloroplast DNA systematics: a review of methods and data analysis. Amer. J. Bot. 81: 1205-1224.

PLATT, S.G. AND C.G. BRANTLEY. 1997. Canebrakes: An ecological and historical perspective. Castanea 62: 8-21.

R DEVELOPMENT CORE TEAM (2008). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org.

RAAMSDONK, L.W.D.V., M.V.-V. GRINKEL, AND C. KIK. 2000. Phylogeny reconstruction and hybrid analysis in *Allium* subgenus *Rhizirideum*. Theoretical and Applied Genetics 100: 1000-1009.

RADFORD, A.E., H.E. AHLES, AND C.R. BELL. 1968. Manual of the vascular flora of the Carolinas. University of North Carolina Press, Chapel Hill.

RAMBAUT, A. 2001. Se-Al version 2.0a9: sequence alignment editor. Department of Zoology, University of Oxford, Oxford, UK.

RIESEBERG, L.H. 1997. Hybrid origins of plant species. Annual Review of Ecology and Systematics 28: 359-389.

SAARELA, J.M., P.M. PETERSON, R.J. SORENG, AND R.E. CHAPMAN. 2003. A taxonomic revision of the eastern North American and Eastern Asian disjunct genus Brachyelytrum (Poaceae): Evidence from morphology, phytogeography, and AFLPs. Systematic Botany 28 (4): 674-692.

SASANUMA, T., K. CHABANE, T.R. ENDO, AND J. VALKOUN. 2004. Characterization of genetic variation in and phylogenetic relationships among diploid *Aegilops* species by AFLP: incongruity of chloroplast and nuclear data. Theor. Appl. Genet. 108: 612-618.

SMOUSE, P.E., J.C. LONG, AND R.R. SOKAL. 1986. Multiple regression and correlation extensions of the Mantel test of matrix correspondence. Syst. Zool. 35: 627-632.

SODERSTROM, T.R. 1981. Some evolutionary trends in the Bambusoideae (Poaceae). Ann. Miss. Bot. Gard. 68: 15-47.

SODERSTROM, T.R., AND R.P. ELLIS. 1988. The Woody Bamboos (Poaceae: Bambuseae) of Sri Lanka: A morphological-anatomical study. Smithsonian Contr. Bot. 72: 1-75.

STAPLETON, C.M.A. (2006). Bambuseae. In: Flora of China, 22. Poaceae: 7–180. Science Press, Beijing and Missouri Botanical Garden Press, St. Louis.

STAPLETON, C.M.A., G.N. CHONGHAILE, AND T.R. HODKINSON. 2004. *Sarocalamus*, a new Sino-Himalayan bamboo genus (Poaceae: Bambusoideae). Novon 14: 345-349.

STEBBINS, G.L. 1956. Cytogenetics and evolution in the grass family. Amer. J. Bot. 43: 890-905.

STEBBINS, G.L. 1972. The evolution of the grass family. In: Youngner, V. B. and C. M. McKell (ed.). The Biology and Utilization of Grasses. Academic Press, Inc., New York and London.

STEBBINS, G.L. 1985. Polyploidy, hybridization, and the invasion of new habitats. Ann. Miss. Bot. Gard. 72: 824-832.

STUTZ, H.C., AND L.K. THOMAS. 1964. Hybridization and introgression in *Cowania* and *Purshia*. Evolution 18: 183-195.

SULLIVAN, J.P., S. LAVOUE, M.E. ARNEGARD, AND C.D. HOPKINS. 2004. AFLPs resolve phylogeny and reveal mitochondrial introgression within a species flock of African electric fish (Mormyroidea: Teleostei). Evolution 58: 825–841.

SUYAMA, Y., K. OBAYASHI, AND I. HAYASHI. 2000. Clonal structure in a dwarf bamboo (*Sasa senanensis*) population inferred from amplified fragment length polymorphism (AFLP) fingerprints. Molecular Ecology 9: 901-906.

SWOFFORD, D.L. 2002. PAUP*. Phylogenetic analysis using parsimony, ver. 4.0b10. Sunderland: Sinauer Associates.

TABERLET, P., L. GIELLY, G. PAUTOU, AND J. BOUVET. 1991. Universal primers for amplification of three non-coding regions of chloroplast DNA. Plant Mol. Biol. 17: 1105-1109.

TIFFNEY, B.H. 1985. The Eocene North Atlantic land bridge: its importance in Tertiary and modern phytogeography of the northern hemisphere. J. Arnold Arbor. 66: 243-273.

TRIPLETT, J.K., A.S. WEAKLEY, AND L.G. CLARK. 2006. Hill cane (*Arundinaria appalachiana)*, a new species of bamboo (Poaceae: Bambusoideae) from the southern Appalachian Mountains. SIDA 22 (1): 79-85.

TUCKER, G.C. 1988. The genera of Bambusoideae (Gramineae) in the southeastern United States. J. Arnold Arbor. 69: 239–273.

VEKEMANS, X. 2002. AFLP-SURV v1.0. Laboratoire de Génétique et Ecologie Végétale, Université Libre de Bruxelles, Belgium.

VEKEMANS, X., T. BEAUWENS, M. LEMAIRE, AND I. ROLDÁN-RUIZ. 2002. Data from amplified fragment length polymorphism (AFLP) markers show indication of size homoplasy and of a relationship between degree of homoplasy and fragment size. Molecular Ecology 11: 139-151.

VINES, R.A. 1960. Trees, shrubs, and woody vines of the Southwest. University of Texas Press, Austin.

VOIGHT, J.W., AND R.H. MOHLENBROCK. 1964. Plant communities in southern Illinois. Southern Illinois University Press, Carbondale.

VOS, P, R. HOGERS, M. BLEEKER, M. REIJANS, T. VAN DE LEE, M. HORNES, A. FRITJERS, J. POT, J. PELEMAN, M. KUIPER, AND M. ZABEAU. 1995. AFLP: a new technique for DNA fingerprinting. Nucleic Acids Research 23: 4407-4414.

WEN, J. 1999: Evolution of eastern Asian and eastern North American disjunct distributions in flowering plants. Annual Review of Ecology and Systematics 30: 421-455.

WEST, E.M. 1935. Canebrakes of the Southeastern United States. Unpublished dissertation, Ohio State University.

XU, D.H. AND T. BAN. 2004. Phylogenetic and evolutionary relationships between *Elymus humidus* and other *Elymus* species based on sequencing of non-coding regions. Theor. Appl. Genet. 108: 1443-1448.

YOUNG, R.A., AND J.R. HAUN. 1961. Bamboo in the United States: descriptions, culture, and utilization. United States Dept. Agric., Crops Res. Div., Agric. Handbook No. 193, Washington, D.C.

CHAPTER 4. HILL CANE (ARUNDINARIA APPALACHIANA), A NEW SPECIES OF BAMBOO (POACEAE: BAMBUSOIDEAE) FROM THE SOUTHERN APPALACHIAN MOUNTAINS

J.K. TRIPLETT,[1,3] A.S. WEAKLEY[2] and L.G. CLARK[1]

[1] Department of Ecology, Evolution and Organismal Biology, Iowa State University, Ames, Iowa, 50011-1020, U.S.A.

[2] University of North Carolina Herbarium (NCU), North Carolina Botanical Garden, University of North Carolina, Campus Box 3280, Chapel Hill, NC, 27599-3280, U.S.A.

[3] Author for correspondence (triplett@iastate.edu)

ABSTRACT

A newly recognized species of *Arundinaria* from the southern Appalachian Mountains is described, illustrated, and compared with the related species *A. gigantea* and *A. tecta*. ***Arundinaria appalachiana*** is distinguished by a combination of vegetative morphological characters including features of branching and leaf morphology, leaf anatomy, and ecology. Recognition of this species is consistent with genetic data that provide evidence for monophyly of the species and its sister relationship with *A. tecta*. A key for the identification of *Arundinaria* species in North America is included along with a comparative table based on morphology, leaf anatomy, and ecology.

KEYWORDS: bamboo, *Arundinaria*, eastern U.S., Appalachian Mountains.

INTRODUCTION

Arundinaria Michaux is a genus of north temperate woody bamboos (Poaceae: Bambusoideae) with a complex taxonomic history involving numerous entities that have been placed within it at one time or another over the past two hundred years (McClure 1973; Li 1997; Judziewicz *et al.* 1999), with older treatments including upwards of 400

heterogeneous species from Asia, Africa, and the Americas. Currently *Arundinaria* is treated in a restricted sense to include only those species endemic to the eastern United States (Ohrnberger 1999), but debate continues regarding the inclusion of certain Asiatic taxa (e.g., *Pleioblastus* Nakai, *Pseudosasa* Makino ex Nakai, *Bashania* P.C. Keng and Yi, and *Oligostachyum* Z.P. Wang and G.H. Ye) that share key morphological features with the North American species (Li 1997; Judziewicz *et al.* 1999). However, because *Arundinaria gigantea* (Walter) Muhl. is the type species for the genus, its generic placement is secure. As such, *Arundinaria* represents the only bamboos native to North America and the only temperate bamboos (the North Temperate clade of Clark *et al.*, in press; Zhang and Clark 2000; Kelchner and Clark 1997) native to the New World, and provides another example of the classic disjunction pattern in the flora of eastern Asia and eastern North America (Wen 1999). With a species richness ratio of approximately 20:1 up to 90:1, depending on which Asian group is used for comparison within the North Temperate bamboo clade, *Arundinaria* and allies also provide an example of the intriguing asymmetry within this disjunction discussed by Guo and Ricklefs (2000).

Arundinaria s.s. encompasses arborescent or subarborescent woody bamboos with leptomorphic (running) rhizomes, persistent to deciduous and mostly glabrous culm leaves, and leaves at the tip of new shoots crowded into a distinctive fan-shaped cluster or top knot with blades expanded as on foliage leaves. Branch complements typically have 1 primary branch and 0–2 subequal secondary branches arising from shortened internodes at the base of the primary branch, which rebranch to produce up to 40 or more secondary branches on older culms. The culm and foliage leaves bear fimbriae and usually also auricles. Synflorescences in this group are determinate, open, and racemose or paniculate, with 6–12 laterally compressed florets per spikelet. Spikelets have 1–2 glumes and 3 stamens per floret. Like most temperate bamboos, *Arundinaria* has a basic chromosome number of $x = 12$ and presents several enigmatic characteristics including delayed flowering and monocarpy; reproduction is primarily vegetative and seed production is infrequent and unpredictable. *Arundinaria s.s.* is confined to southeastern portions of the continental United States (specifically Alabama, Arkansas, Delaware, Florida, Illinois, Indiana, Kentucky, Louisiana, Maryland, Mississippi, Missouri, New Jersey, North Carolina, Ohio, Oklahoma, South

Carolina, Tennessee, Texas, Virginia, and West Virginia), occurring from the Coastal Plain from New Jersey south to Florida and west to eastern Texas, and inland through the Piedmont to moderate elevations in the Appalachian Mountains. *Arundinaria* once formed extensive and dense canebrakes (with or without tree canopy) covering vast areas of fertile river bottomland often described by early explorers in the pre-colonial U.S. (West 1935), but has been greatly reduced in extent and abundance from its historical range by grazing and fire suppression (Hughes 1951 1957 1966; Platt and Brantley 1997; Judziewicz *et al.* 1999).

Two species (*Arundo gigantea* Walter*, Arundo tecta* Walter*)* were originally described by Walter (1788) and represent extremes of morphological types found among inland and coastal forms, sometimes referred to as "Mississippi-type" and "Atlantic-type" (*sensu* Gilly 1943). *Arundinaria gigantea* (Walt.) Muhl. (river cane or giant cane) forms extensive colonies in low woods, moist ground, and along riverbanks from the lowlands east of the Appalachians, west to Missouri, up the Mississippi Valley to southern Illinois and up the Ohio River to southern Ohio. *Arundinaria tecta* (Walt.) Muhl. (switch cane) forms colonies in non-alluvial swamps, moist pine barrens, live oak woods and along sandy margins of streams, preferring moister sites than *A. gigantea*. It is restricted to the Coastal Plain of the southeastern U.S., from southern Maryland to Alabama and Mississippi (McClure 1973; Hitchcock 1951).

Within *Arundinaria s. s.*, complex population-level variation has continued to be problematic for taxonomists and field botanists (McClure 1973; Judziewicz *et al.* 1999; Platt and Brantley 1997). Phenotypic diversity among North American cane populations has inspired diverse taxonomic interpretations, generally with 1-3 taxa recognized at either specific or subspecific levels (Gilly 1943; Young and Haun 1961; Voight and Mohlenbrock 1964; Radford *et al.* 1968; Hitchcock 1971; McClure 1973; Campbell 1985; Tucker 1988; Platt and Brantley 1997). McClure (1973) published the most recent exhaustive treatment of *Arundinaria* and took a conservative approach, recognizing a single polymorphic species (*A. gigantea*) and three subspecies, one of which [*A. gigantea* ssp. *macrosperma (*Michx.) McClure] is a catch-all for putative hybrids derived from the introgression of the other two. However, McClure acknowledged that further studies, particularly genetic-based studies, were necessary to clarify the phylogeny and taxonomy of this group.

In the Southern Appalachians, astute botanical observers have long questioned the identity of a curious short-statured cane that typically occupied sites away from streams and rivers**.** Among the diverse North American populations of *Arundinaria*, a variety with deciduous foliage was recorded by botanist C.D. Beadle in Western North Carolina (Beadle 1914; see also Young 1945). Beadle recognized this form as *A. tecta* var. *decidua*, not ruling out the possibility that it might in fact be a distinct species. It is unclear why Beadle associated this deciduous variety with *A. tecta*, although perhaps it was because of the small stature typically associated with *A. tecta*. In the first half of the nineteenth century, two botanists affiliated with the University of North Carolina Herbarium, William Willard Ashe and William Chambers Coker, made notes on specimens collected near Highlands, North Carolina indicating that the short, delicate, deciduous cane of the mountains might be a distinct taxon. Roland Harper (1928) was also intrigued by cane specimens occurring on bluffs in northern Alabama and thought that an unrecognized taxon might be present. In the latter half of the twentieth century, botanists and ecologists have informally considered the small, upland cane as "hill cane" and have been unconvinced that it could be assigned to either *A. gigantea* or *A. tecta*. Hill cane is often common in mesic and submesic slopes and upland woodlands. Moreover, because of overlapping morphological characteristics, floristic descriptions of *A. tecta* and *A. gigantea* may be confounded by this distinct form of cane. For example, *Arundinaria tecta* has been described as occurring along river branches 450 to 580 feet above sea-level on the southeast slopes and along the courses of mountain streams and shady mesic hillsides in the foothill region, well back from water (Harper 1928, Peattie 1929), but these inland and upland habitats almost certainly are populated by hill cane, not switch cane. In our field work on *Arundinaria*, we located several widespread populations of hill cane along the southern Appalachian Mountain chain, extending the range of the form that Beadle described. Field work has been complemented by herbarium studies to produce our current understanding of the range for this entity.

Species limits within *Arundinaria s.s.* have not been examined previously in a phylogenetic framework or with molecular tools. Our investigation of hill cane is part of a larger study of the phylogenetic history of the North Temperate bamboo clade (in

collaboration with the Bamboo Phylogeny Group), and as part of that study we are reconstructing the phylogeny of river cane, switch cane, and hill cane utilizing Amplified Fragment Length Polymorphism (AFLP) data to test the monophyly of putative species and to correlate the results with morphological and geographical characters, with the goal of producing a revised treatment of the genus. Ordination analyses (PCA) of morphological characters and AFLP studies will be presented in a later publication.

Phylogenetic analysis of AFLP data (Triplett and Clark, in prep.; Ch. 3) demonstrated that the three types of cane form separate monophyletic lineages encompassing two previously recognized entities (river cane and switch cane) and one entity encompassing those plants recognized as hill cane. Moreover, hill cane specimens from a wide geographic range cluster as the sister clade of *A. tecta*, rejecting the hypothesis that hill cane is an ecologically induced form of *A. tecta* and instead suggesting that it is a distinct lineage with a unique evolutionary history. These results prompted a reevaluation of diagnostic characters within *Arundinaria s.s.* Ordination (nMDS) analyses of morphological characters similarly identified three non-overlapping entities and allowed us to recognize the most important diagnostic vegetative features. These groups correspond precisely with the three lineages derived from the AFLP data. We therefore propose the recognition of each entity at the species level: *A. gigantea* (river cane), *A. tecta* (switch cane) and a previously undescribed species (hill cane). In advance of the publication of the Flora of North America, we here describe and illustrate the new species, *A. appalachiana* Triplett, Weakley, and L.G. Clark, from the southern Appalachian Mountains, and compare and contrast it with its congeners *A. gigantea* and *A. tecta*.

MATERIALS AND METHODS

Field studies of natural populations were conducted in October 2003 and July-October 2005. 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.

Herbarium specimens from A, F, GA, GH, ISC, NCU, and US (herbarium acronyms following Holmgren and Holmgren 2006) were examined. While our taxonomic

circumscription of *Arundinaria s.s.* is based on an approach combining morphological and molecular data, we have relied upon morphological characters to provide identifications of the specimens examined. Complete specimens, including culm leaves, buds, branch complements with foliage leaves, and synflorescences were rarely available, and some herbarium specimens of *Arundinaria* could not be conclusively assigned to species. Only one flowering specimen was located among the specimens identified as *A. appalachiana*.

Specimens were measured for a variety of morphological characters, including foliage leaf length and width, inner ligule length, inflorescence length, spikelet length, and lengths of spikelet bracts (glumes I–IV, lemma and palea). Top knot (the cluster of leaves at the tip of new shoots) and foliage leaf lengths were measured from the base of the pseudopetiole to the tip of the blade. Leaf 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. Synflorescence length was measured from the base of the basalmost branch to the apex of the main axis. Spikelets were removed from specimens and softened using a modified Pohl's solution (Pohl 1965; 750 ml distilled water, 250 ml 1-propanol, 2 ml liquid dish soap), dissected, examined, and measured for floral characters using a dissecting microscope equipped with a micrometer. Anatomical characters of leaf blades (both epidermal micromorphology and cross sections) were obtained using light microscopy of sections made following standard protocols for free hand sectioning and epidermal peels (Clark 1986; Ellis 1976 1979).

RESULTS AND DISCUSSION

Our decision to recognize this taxon at the species level is based upon 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 and Marshall 2003). The features discussed below are those identified as the most diagnostic based on observations made during field work and morphological ordination analyses (PCA). The most consistent differences among the North American species are seen in vegetative

Table 1. Morphological comparisons of *Arundinaria appalachiana*, *A. tecta,* and *A. gigantea*.

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

characters including features of the rhizomes, culm internodes and culm leaves, branching, and top knot and foliage blades, described below and summarized in Table 1.

Distribution and ecology

Arundinaria appalachiana is indigenous to the southern Appalachian Mountains where it occurs in the southern Blue Ridge, Blue Ridge/Piedmont Escarpment, upper Piedmont, and Ridge and Valley physiographic provinces (Fig. 1). The full extent of its distribution is still poorly known, because of the infrequent collection of bamboos in eastern North America and the often poor quality of the existing specimens; for this reason we have chosen to supplement vouchered specimens with additional county records based on what we consider reliable sight records of this new species (these counties should be verified with vouchers). Hill cane is common in oak-hickory forests and woodlands on mesic, submesic,

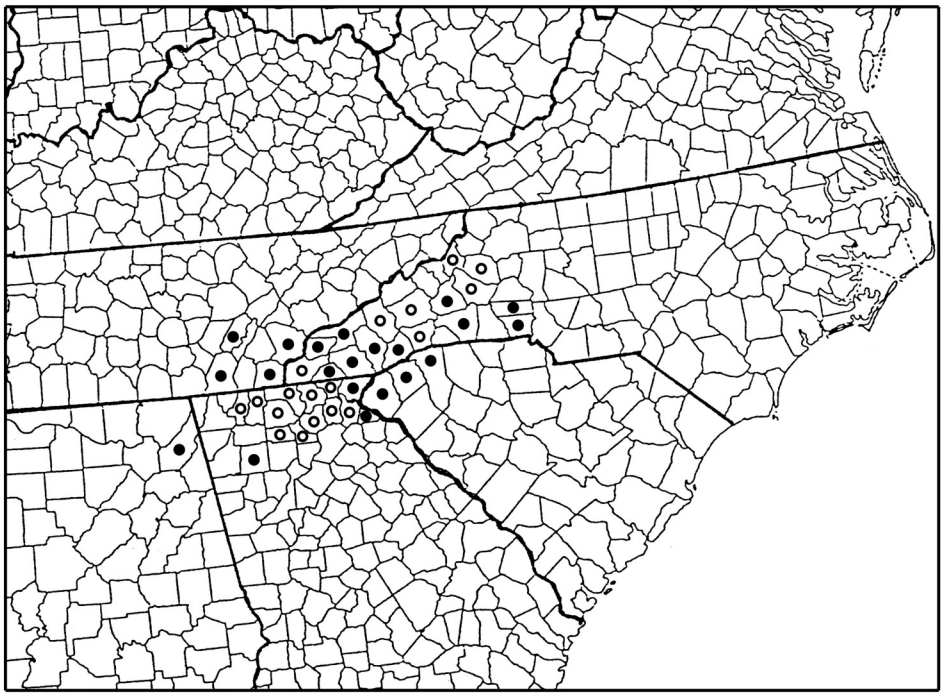

Figure 1. Distribution of *Arundinaria appalachiana* in the southeastern United States. Filled circles based on documented specimens; open circles based on unvouchered sight records.

and xeric slopes and uplands, sometimes occurring as well in hillside seepages, but nearly always on slopes, bluffs, and ridges away from perennial streams, in contrast to the geographically sympatric *A. gigantea*. Associated overstory species include *Quercus montana* Willd., *Q. coccinea* Münchh., *Q. alba* L., *Q. rubra* L., *Q. falcata* Michx., *Pinus echinata* Mill., *P. virginiana* Mill., *Carya alba (*L.) K. Koch, *Carya glabra* (Mill.) Sweet, and *Nyssa sylvatica* Marshall. Although these slope and ridge forests are well-drained, the annual rainfall amounts in this region are high and equably distributed. In the escarpment gorges of the Chattooga River, Whitewater River, Thompson River, Horsepasture River, Toxaway River, and Eastatoe Creek, the highest annual rainfalls in eastern North America (exceeding 80 inches a year) maintain higher then expected moisture levels even in topographic situations that tend to create xeric communities (ridgetops and convex upper slopes and side ridges) (Robinson 2000). Still, there is no question that hill cane occupies unusually dry and upland habitats compared to its congeners. *Arundinaria gigantea* typically occurs on the floodplains of large to small rivers, sometimes edging onto lower portions of mesic slopes, whereas *A. tecta* typically occurs along small to medium blackwater rivers, in swamps, on deep peat in pocosins, and in small seepages with organic soils.

A. appalachiana is sympatric (at least in the broadest sense) with both of its congeners, in that it occurs in the same counties and within a few kilometers of populations of both *A. gigantea* and *A. tecta*. Within the region occupied by *A. appalachiana* however, *A. gigantea* occurs only along the upper reaches of major rivers, notably the Little Tennessee and French Broad Rivers, while *A. tecta* also occurs at lower elevations and in different topographic and ecological situations.

The species biology of *A. appalachiana* is poorly understood. Its congeners are long-lived monocarpic perennials, and this appears to be the case with *A. appalachiana* as well. It has been seen flowering and fruiting even more infrequently than its congeners; judging from its habitat, its flowering and fruiting may be stimulated by fire (like *A. tecta*), and the paucity of fertile specimens may reflect the general suppression of fire in its habitat through the twentieth century. Field observations suggest that clones of *A. appalachiana* are slow-growing and very long-lived, certainly persisting for decades and likely for centuries.

Morphology

Rhizomes. The rhizomes of *A. appalachiana* are leptomorphic, a characteristic they share with other north temperate woody bamboos; however, in many 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. tecta* but has not been confirmed for *A. gigantea*. An interesting characteristic of this species is the variability in air canal development (McClure 1963). Air canals are present in some specimens but not others, and may in fact be longitudinally and peripherally discontinuous in rhizomes of *A. appalachiana*. Air canals are consistently present in *A. tecta* and are apparently continuous.

Culm internodes. Although the culm internodes of *A. appalachiana* can be somewhat flattened behind the branch complement, the internodes lack a prominent groove or sulcus. This is consistent with *A. tecta* but contrasts with *A. gigantea*, which typically has internodes that are prominently sulcate.

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. appalachiana* is characterized by 2–5 shortened or compressed internodes at the base of the primary branch, without rebranching in this basal area. The first elongated internode above the shortened ones is typically constrained to ~30% the length of distal internodes. In contrast, *A. tecta*, while having a similar pattern of compressed internodes, typically will produce buds and branches from the nodes in the area of compression, creating subequal branches from the base of the primary branch. *Arundinaria gigantea* typically has only one (or no) compressed basal internode, but if present, this node may produce a secondary branch. Primary branches in *A. appalachiana* are usually less than 35 cm long. In contrast, *A. tecta* produces long primary branches usually >50 cm.

Culm leaves. The culm leaves of *A. appalachiana* 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. *Arundinaria appalachiana* and *A. tecta* have persistent culm leaf sheaths, whereas *A. gigantea* has deciduous sheaths. The culm leaf sheaths of *A. appalachiana* are tessellate; however, their tessellation is not as pronounced as it is in *A. tecta*. The culm leaves lack well-developed, prominent auricles, unlike *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. The top knot leaf blades of *A. appalachiana* are typically 9–22.5 cm in length, while *A. tecta* typically has larger blades (20–30 cm long); those of *A. gigantea* are typically 16-24 cm in length. The foliage leaf blades of *A. appalachiana* are deciduous; bladeless branches persist on the plants after leaf drop, often with the older sheaths still intact. The blades are chartaceous; presumably, since the blades are deciduous, the plant invests less energy in producing them by producing less sclerenchyma. In contrast, the leaves of *A. gigantea* are persistent and subcoriaceous, while leaves of *A. tecta* are persistent and coriaceous. The abaxial surfaces of the leaf blades of *A. appalachiana* are weakly tessellate, whereas in *A. gigantea* and *A. tecta* they are strongly tessellate. The abaxial and adaxial leaf surfaces are typically sparsely to more or less densely pilose (glabrous in some populations) in *A. appalachiana*. Leaf blades of *A. gigantea* are typically densely pubescent with short, soft hairs on abaxial surfaces, while the blades of *A. tecta* are densely pubescent on both surfaces; neither of these pubescence patterns has been seen in *A. appalachiana*.

It is important to note that *Arundinaria gigantea* and *Arundinaria tecta* can both survive in deeper shade in the forest, albeit in stunted conditions, and thus it is possible to find diminutive or depauperate plants of both that superficially resemble hill cane, mostly in stature. However, a combination of characters such as the branch complement, leaf texture, and leaf pubescence are usually sufficient to distinguish these stunted forms of river and switch cane from hill cane populations and from each other.

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, basal nodes developing secondary branches; top knot blades 20–30 cm long ……*A. tecta*
   2. Foliage blades chartaceous, deciduous, abaxial surfaces pilose or glabrous, weakly tessellate; primary branches usually less than 35 cm long, basal nodes not developing secondary branches; top knot blades 9–22.5 cm long ……*A. appalachiana*

***Arundinaria* appalachiana** Triplett, Weakley and L.G. Clark, sp. nov. (Fig. 1-5). TYPE: UNITED STATES. ALABAMA: Dekalb Co., Desoto State Park by Rt. 89 (34º 30' N Lat.; 85º 38' W Long.), elev. ca. 515 m, 25 Jul 2005, *Triplett and Ozaki 99 (*Holotype: ISC; Isotypes: MO, NCU, UNA, US). *Nomen vul.* hill cane.

Rhizomata leptomorpha, strato cavernularum aëriarum interdum praesenti. Culmi 2–6 mm diametro, 0.5–1.8 m alti, omnino glabri, erecti. Internodia 4.5–12 cm longa, teretia (sine sulco). Vaginae culmorum 5.5–11 (–15) cm longae, persistentes, sine auriculis. Culmi juniores fasciculo terminali (5–) 6–12 foliorum, laminis (5–) 9–22.5 cm longibus, 1.4–2.8 cm latibus, linearibus vel lineari-lanceolatis vel ovati-lanceolatis, pilosis vel interdum glabris. Rami primarii ad nodos medianos culmorum 1, 7–33 cm longi, internodiis abbreviatis sine gemmis basi 2–5. Folia cujusquisque complementi 3–7. Laminae foliorum (3–) 5–20 cm longae, 0.5–2 cm latae, chartaceae, deciduae, pilosae vel interdum glabrae, abaxialiter infirme tessellatae. Synflorescentiae 7–11.5 cm longae, 2–5 cm latae, determinatae racemosae apertae; pedicelli 4–25 mm longi, spiculis 6–8. Spiculae 3–5.5 cm longae, glumis

Figure 2. *Arundinaria appalachiana*. A. Top knot of new shoot. B. Foliage leaf complement from midculm node. C. Foliage leaf, showing apex of sheath, fimbriae, pseudopetiole, and base of blade. D. Detail of abaxial surface of blade showing tessellation and pilose vestiture. E. Culm leaf at midculm node. F. Branch complement showing compressed basal internodes and reiterative secondary branch (arrow). Scale bar = 1 cm unless otherwise noted. All drawings based on *Triplett and Ozaki 99*. (Illustrations by J. Triplett)

Figure 3. *Arundinaria appalachiana*. A. Partially dissected spikelet showing two florets. B. Synflorescence with five spikelets. Scale bar = 1 cm. Drawings based on *Ahles and Leisner 15147*. (Illustrations by J. Triplett)

1 (–2), flosculis basilibus interdum sterilibus, 5–8 flosculis fertilibus continentibus et anthoeciis rudimentalibus terminalibus 1-3; glumae inaequales, 5-nervatae, attenuatae, glabrae; gluma I 3–6.5 mm longa; gluma II 5.5–9 mm longa; lemmata fertilia 11–16 mm longae, 7–11-nervata, apicibus acutis vel acuminatis, abaxialiter glabris; palea 10–13 mm longa, 8–10-nervata. Stamina 3; anthera 5–7 mm longa.

Woody bamboo. Plants of diffuse to (pluri-) caespitose habit. **Rhizomes** leptomorphic, usually horizontal for a only short distance before turning up at the apex to form a culm (therefore often presenting a sympodial branching pattern), hollow (with a small central lumen), peripheral air canals sometimes present (if so, apparently discontinuous longitudinally and/or peripherally). **Culms** 2–6 mm in diameter, 0.4–1.8 m tall, erect,

tillering; **internodes** 4.5–12 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.

Figures 4 and 5. *Arundinaria appalachiana*. 4. Habit, in Rhea Co., Tennessee. 5. Close-up of primary branch with compressed basal internodes, Dekalb Co., Alabama. (Photos by J. Triplett)

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** 5.5–11 (-15) cm long, shortest on lower nodes, becoming progressively longer towards the culm apex, glabrous, margins ciliate; **blades** 0.5–1.4 cm long, triangular to lanceolate, reflexed to erect, glabrous, deciduous, intergrading into top knot leaves; **auricles** absent; **fimbriae** 1–9 mm long, ascending to erect; **inner ligules** ca. 0.1

mm long, a fringe of short cilia; **outer ligule** absent. **Top knot leaves** in an apical cluster of (5–) 6–12; **sheaths** glabrous, margins ciliate; **auricles** absent; **fimbriae** 1–9 mm, ascending to erect; **blades** (5–) 9–22.5 cm long, 1.4–2.8 cm wide, L:W = 8.3–9.3, linear, linear-lanceolate or ovate-lanceolate, chartaceous, pilose or glabrous, abaxially weakly tessellate, apices acuminate, bases attenuate to cuneate, midrib ± centric. **Branching** infravaginal (rarely extravaginal); **primary branches** 1 per node, 7–33 cm long, with 2–5 compressed basal internodes, basal nodes not developing 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** 3–7 per complement; **sheaths** glabrous, margins ciliate, weakly tessellate; **auricles** absent; **fimbriae** 1–9 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, deciduous, surfaces pilose (sometimes glabrous), abaxially weakly tessellate, apices acuminate, bases attenuate to cuneate, midrib ± centric; primary branch foliage leaf blades (4–) 9–20 cm long, (0.5–) 0.8–2 cm wide; L:W = 10.7–11.7; higher order branch foliage leaf blades (3–) 5–17.5 cm long, (0.5–) 0.8–1.5 cm wide; terminal foliage leaf blade rarely unexpanded laterally, withering but persisting as a tail-like appendage. **Synflorescences** 7–11.5 cm long, 2–5 cm wide, determinate, open-racemose, apparently borne on specialized nonleafy shoots; peduncle 10–17 cm long (3 measured), glabrous, terete; rachis glabrous; pedicels 4–25 mm long; 6–8 spikelets per synflorescence. **Spikelets** 3–5.5 cm long, laterally compressed, disarticulating above the glumes and between the florets, consisting of 1 (–2) glumes, occasionally a basal sterile floret, 5–8 fertile florets and 1–3 progressively rudimentary apical sterile florets; **rachilla internodes** 3–4 mm long; **glumes** unequal, 5-nerved, attenuate, glabrous; glume I 3–6.5 mm long; glume II 5.5–9 mm long; **fertile lemmas** 11–16 mm long, 7–11-nerved, apex acute or acuminate, abaxially glabrous, transverse veinlets barely perceptible or not at all manifest, usually somewhat reddish-purple; **paleas** 10–13 mm long, 8–10-nerved, broadly sulcate and 2-keeled dorsally; **lodicules** and **ovary** not seen. **Stamens** 3; anthers 5–7 mm long. **Fruit** not seen.

*Distribution and Ecology. – (*Fig. 1) – Endemic to the southern Appalachians and upper Piedmont of northeastern Alabama, northern Georgia, southwestern North Carolina, northwestern South Carolina, and southeastern Tennessee, United States; 300 to 800 (1065) 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. Of the specimens cited below, only one confirmed flowering specimen of this species was identified, suggesting that flowering may be an even rarer event in this species than in most woody bamboos.

Etymology. – Arundinaria appalachiana is named for its distribution in the forests of the Appalachian Mountains.

Representative specimens examined – UNITED STATES. **Alabama: Dekalb Co.:** Little River Canyon National Preserve, plot PIEC 27 2002, *McDaniel s.n. (*NCU); in steep wooded bank of West Fork, Little River, across stream from overlook shelter, DeSoto State Park, 9 May 1959, *Sherman and Carter 25747 (*GH); Desoto State Park on trail by Laurel Creek (34º 30' N Lat; 85º 38' W Long.), elev. ca. 515 m, 25 Jul 2005, *Triplett and Ozaki 100 (*ISC, MO, US). **Georgia**: **Bartow Co.:** in woods on S side of Stamp Creek Rd. just E of Jones Mill Rd. (34º 14' N Lat; 84º 40' W Long.), elev. ca. 311 m, 26 Sept 2005, *Triplett 166 (*ISC, MO, US); **Rabun Co.:** maple-birch-magnolia association, Laurel Creek Olivine Deposit, 1.5 mi. E of Pine Mt., 21 Jun 1946, *Radford s.n.* (NCU); oak-hickory woods on Pine Mt., Bald Rd., 4 Jun 1952, *Radford 6134* (NCU); Warwoman Road and Overflow Creek bridge, N side 20 May 1996, *Stancil 950* (GA); in swamp near top of Oakey Mountain, SE of Nacoochee Reservoir, 25 May 1941, *Duncan 3283* (GA); on trail from 155 0.5 mi. S of Kattie Place, elev. ca. 790 m, 22 Oct 1995, *Milsted and Zhang 585* (GA); pine-oak woodland near roadside park, 1 mi. N of Tallulah Falls River, 9 May 1967, *Isely, Welsh, and Isely 10286* (ISC); 2 mi. N of Warwoman Rd. along GA 28, scattered throughout mature woods W of road (34º 57' N Lat; 83º 10' W Long.), elev. ca. 687 m, 25 Oct 2003, *Triplett and Clark 20 (*ISC, MO, US).

Stephens Co.: Deep gorge, Cedar Creek, Camp Mikell Rd. off GA 184, just W of Camp Mikell and N of Toccoa (34 08' 06" N. lat.; 83 20' 13" W. long), elev. ca. 300 m 19 Jun 1975, *Boufford and Wood 16501* (NCU); NW-facing ravines and ridges on the S side of Panther Creek, SW of Yonah Lake (Tugaloo River), N of Toccoa (34 43' 30" N. lat.; 83 21' 13" W. long.), 25 Jun 1975, *Boufford and Wood 16766* (NCU). **North Carolina: Clay Co.:** Oak-hickory woods, 4 mi NW of Hayesville, 5 Jun 1952, *Radford and Wood 6162 (*NCU). **Gaston Co.:** low woodland near the northern tip of Pasour Mt., about 3 mi. S-SW of High Shoals, 21 Jun 1956, *Ahles and Leisner 15147 (*NCU); oak-hickory woods on Crowder's Mt., 4 Jun 1953, *Radford 7084* (NCU). **Graham Co.:** small population on N side of Santeetlah Dam Rd. (SR 1146) just off Hwy 129 (35º 22' N Lat; 83º 51' W Long.), elev. ca. 537 m, 2 Oct 2005, *Triplett 185 (*ISC, MO, US). **Jackson Co.:** on highway between Dillsboro and Park Entrance, extensive clump on the top of bank by roadside, 26 Jul 1937, *Coker s.n.* (NCU); Cope Creek Rd. just off NC 23 out of Sylva, growing among *Polytrichum* sp., Pine, and sedge (35º 23' N Lat; 83º 11' W Long.), elev. ca. 675 m, 25 Oct 2003, *Triplett and Clark 21 (*ISC, MO, US). **Lincoln Co.:** rich deciduous forest and stream banks 0.4 mi. W of Cat Square, 28 Apr 1957, *Bell 6638* (NCU). **Macon Co.:** maple-birch-magnolia (cove) association, Corundum Hill Olivine Deposit, 1 1/2 mi. NW Gneiss, 15 Jun 1946, *Radford s.n.* (NCU); Horse Cove near Highlands, 3 Sept 1948, *Radford s.n.* (NCU); in deep, shaded ravine at top of pasture at foot of Whiterock Mountain, 7 mi. from Otto, NC on Tessentee Creek Rd., elev. ca. 1065 m, 7 August 1938, *Stewart and Hechenbleikner s.n.* (NCU); pine-broom-straw association, Corundum Hill Olivine Deposit, 1.5 mi. NW Gneiss, 15 August 1946, *Radford s.n.* (NCU); Mulberry Rd. ca. 0.5 mi. off 441, on steep hillside among oak, rhododendron, and maple (35º 01' N Lat; 83º 23' W Long.), elev. ca. 647 m, 25 Oct 2003, *Triplett, Clark, and Weakley 19* (ISC, MO, US). **McDowell Co.:** near Marion, 25 October 1915, *Ashe s.n.* (NCU). **Polk Co.:** 4 mi. W of Tyron, valley of Fall Creek in wet meadow on peninsula in reservoir 19 Jun 1942, *Walker 3469* (US). **Rutherford Co.:** wet ditch on CR 1721 2 mi. N of Sunshine, 24 Jun 1967, *Smith 74* (NCU). **Swain Co.:** Bryson City, by stream, 11 Jul 1927, *Hunnewell s.n. (*GH); Bryson City, private property on W side of Wiggins Rd., 0.3 mi. up from Betts Branch (SR 1343) (35º 26' N Lat; 83º 25' W Long.), elev. ca. 601 m, 1 Oct 2005, *Triplett 184 (*ISC, MO, US). **Transylvania Co.:** rocks and cliffs on

side of mountain, 100 to 200 feet above the bank of Davidson River, Pisgah Forest, 28 Sept 1915, *Ashe s.n.* (NCU); Pisgah Forest, road to Pink Beds, 23 Aug 1938, *Stewart s.n.* (NCU); Middle Bearcamp Creek area, roadside, Highlands, 2 Aug 1962, *Rodgers and Shake 62165a* (NCU); oak woods near Looking Glass Falls, 7 Jun 1952, *Radford and Wood 6192* (NCU); Horsepasture Gorge, roadside about 1 mi. W of crossing, elev. ca. 488 m, 8 Jun 1961, *Rodgers 6162a* (NCU). **South Carolina: Greenville Co.:** Cedar Mountain, 0.4 mi. down gravel road 3.8 mi. S of Caesar's Head SP Visitor Center (35º 05' N Lat; 82º 36' W Long.), elev. ca. 515 m, 30 Sept 2005, *Triplett 179 (*ISC, MO, US). **Oconee Co.:** oak-hickory woods, ridge above Walhalla Fish Hatchery, ca. 11 mi. N of junction of S.C. Routes 28 and 107 on Route 107, 9 Jun 1952, *Wood 7879 (*A); Hill property, NW side Old Rocky Gap Road, W side West Village Creek near creek, Mountain Rest, Blue Ridge province, elev. ca. 520 m., 24 Aug 1991, *Hill 22585 (*GH). **Pickens Co.:** mixed deciduous forest, 3 mi. N of Rocky Bottom near US 178, 22 Aug 1956, *Radford 16758* (NCU); Boggs' Rock, granite-gneiss outcrop N of Liberty, 3 Jun 1974, *Knox 407* (NCU); Rich wooded slope, 2.4 mi. S of NC line on US 178, 8 Jun 1956, *Ahles and Bell 14298, (*NCU). **Tennessee: Hamilton Co.:** Lookout Mt. Nat. Military Park, Chattanooga, 1 small plant at summit of mountain, in hardwood forest., 29 Jun 1957, *Pohl 7664A* (ISC). **Monroe Co.:** White Cliff Springs, Jul 1890, *Lamson-Scribner s.n. (*US). **Polk Co.:** Boyd Gap Overlook above Ocoee River, 4.2 mi. W of Hwy 68, on steep wooded hillside just beyond entrance to Boyd Gap Trail #331 (35º 02' N Lat; 84º 27' W Long.), elev. ca. 529 m, 26 Sept 2005, *Triplett 165 (*ISC, MO, US). **Rhea Co.:** Firetower Rd just off Hwy 68 next to Grandview Community Center and below intersection with Emergency Road (35º 44' N Lat; 84º 50' W Long.), elev. ca. 445 m, 3 Oct 2005, *Triplett 188 (*ISC, MO, US).

ACKNOWLEDGEMENTS

Field work was supported by a Graduate Research Grant from the American Society of Plant Taxonomists and grants from the American Bamboo Society to JT as well as a National Geographic Society Grant (#7336-02) to LC. Support for JT and for the final preparation of this manuscript came from a National Science Foundation Grant (DEB-0515712) to LC. We are indebted to Angus Gholson (Chattahoochee, FL), Dr. Michael

Hotchkiss (USDA, Byron, GA), Robert and Lauren Clark (Peachtree City, GA), Jim and Frankie Triplett (Winston-Salem, NC), Gary Kauffman, Linda Lee Leslie, Adam and Sue Turtle (Earth Advocates Research Farm, Summertown, TN), and Dr. Julian Campbell (Lexington, KY) for their hospitality or assistance in locating populations of *Arundinaria*. We also thank the curators of the herbaria that loaned specimens for this study. We are grateful to Gary Kauffman, Keith Langdon, Virginia McDowell, Tom Govus, and Mike Schafale for their interest and assistance in resolving the persistent taxonomic issue of the correct identity of the small upland mountain cane. Dr. Chris Stapleton (Royal Botanic Gardens, Kew) kindly pointed out to us the importance of compressed internodes in the taxonomy of this group and Dr. Emmet Judziewicz (Dept. of Biology, University of Wisconsin-Stevens Point) suggested the specific epithet.

LITERATURE CITED

BEADLE, C.D. 1914. Bamboo. Page 446 in Bailey, L.H. The Standard Cyclopedia of Horticulture, Vol. 1. New York: The Macmillan Company.

CAMPBELL, J.N. 1985. The land of cane and clover: presettlement vegetation in the so-called bluegrass region of Kentucky. Herbarium Report, University of Kentucky, Lexington.

CLARK, L.G. 1986. Systematics of *Chusquea* section *Chusquea*, section *Swallenochloa*, section *Verticillatae*, and section *Serpentes* (Poaceae: Bambusoideae). Unpublished Ph.D. dissertation, Iowa State University, Ames.

CLARK, L.G., S. DRANSFIELD, J. TRIPLETT, AND J.G. SÁNCHEZ-KEN. In press. Phylogenetic relationships among the one-flowered, determinate genera of Bambuseae (Poaceae: Bambusoideae). In: Columbus, J.T., E.A. Friar, C.W. Hamilton, J.M. Porter, L.M. Prince, and M.G. Simpson (eds.). Monocots: Comparative biology and evolution. 2 vols. Rancho Santa Ana Botanic Garden, Claremont, California, U.S.A.

ELLIS, R.P. 1976. A procedure for standardizing comparative leaf blade anatomy in the Poaceae. I. The leaf blade as viewed in transverse section. Bothalia 12:65–109.

ELLIS, R.P. 1979. A procedure for standardizing comparative leaf blade anatomy in the Poaceae. II. The epidermis as seen in surface view. Bothalia 12:641–672.

GILLY, C.L. 1943. A preliminary investigation of the North American canes (*Arundinaria)*. Bull. Torrey Bot. Club, 70:297–309.

GUO, Q. AND R. E. RICKLEFS. 2000. Species richness in plant genera disjunct between temperate eastern Asia and North America. Bot. J. Linn. Soc. 134:401–423.

HARPER, R.M. 1928. Economic Botany of Alabama. Part 2. Catalogue of the trees, shrubs, and vines of Alabama, with their economic properties and local distribution. Geol. Survey Alabama Monogr. 9:1–357.

HITCHCOCK, A.S. 1951. Manual of grasses of the United States. Rev. by Agnes Chase. 2nd ed. USDA Misc. Pub. 200, U.S. Government Printing Office, Washington, DC.

HITCHCOCK, A.S. 1971. Manual of the grasses of the United States. Dover Publ., New York.

HOLMGREN, P.K. AND N. H. HOLMGREN. 1998 onwards (continuously updated). Index Herbariorum. New York Botanical Garden. http://sciweb.nybg.org/science2/IndexHerbariorum.asp

HUGHES, R.H. 1951. Observations of cane (*Arundinaria)* flowers, seed, and seedlings in the North Carolina coastal plain. Bull. Torrey Bot. Club 78:113–121.

HUGHES, R.H. 1957. Response of cane to burning in the North Carolina coastal plain. North Carolina Agric. Exp. Stat. Bull. 402.

HUGHES, R.H. 1966. Fire ecology of canebrakes. Proceedings of the Fifth Annual Tall Timbers Fire Ecology Conference, March 24-25 1966. Mimeographed.

JUDZIEWICZ, E.J., L.G. CLARK, X. LONDONO, AND M.J. STERN. 1999. American Bamboos. Smithsonian Institute Press, Washington.

KELCHNER, S.A. AND L.G. CLARK. 1997. Molecular evolution and phylogenetic utility of the chloroplast *rpl*16 intron in *Chusquea* and the Bambusoideae (Poaceae). Mol. Phylogen. Evol. 8:385–397.

LI, D.Z. 1997. The Flora of China Bambusoideae Project: Problems and current understanding of bamboo taxonomy in China. In: Chapman, G.P., ed. The Bamboos. Linnean Society of London Symposium Series. Academic Press, London.

MCCLURE, F.A. 1963. A new feature in bamboo rhizome anatomy. Rhodora 65:134–136.

MCCLURE, F.A. 1973. Genera of bamboos native to the New World (Gramineae: Bambusoideae). Smithsonian Contr. Bot. 9. Smithsonian Institution, Washington, D.C.

OLMSTEAD, R.G. 1995. Species concepts and plesiomorphic species. Syst. Bot. 20:623–630.

OHRNBERGER, D. 1999. The Bamboos of the World. Elsevier, Amsterdam, The Netherlands.

PEATTIE, D.C. 1929. Flora of the Tyron region. J. Elisha Mitchell Sci. Soc 44:141–229.

PLATT, S.G. AND C.G. BRANTLEY. 1997. Canebreaks: An ecological and historical perspective. Castanea 62:8-21.

POHL, R.W. 1965. Dissecting equipment and materials for the study of minute plant structures. Rhodora 67:95–96.

RADFORD, A.E., H.E. AHLES, AND C.R. BELL. 1968. Manual of the vascular flora of the Carolinas. University of North Carolina Press, Chapel Hill.

ROBINSON, P.J. 2000. Weather and climate. In: D.M. Orr, Jr. and A.W. Stuart. The North Carolina atlas: portrait for a new century. University of North Carolina Press, Chapel Hill. Pp. 21–30

SITES, J.W. JR., AND J.C. MARSHALL. 2003. Delimiting species: a renaissance issue in systematic botany. Trends in Ecol. Evol. 18:462–470.

SODERSTROM, T.R., AND S.M. YOUNG. 1983. A guide to collecting bamboos. Ann. Miss. Bot. Gard. 70:128–136.

TUCKER, G.C. 1988. The genera of Bambusoideae (Gramineae) in the southeastern United States. J. Arnold Arbor. 69:239–273.

VOIGHT, J.W., AND R.H. MOHLENBROCK. 1964. Plant communities in southern Illinois. Southern Illinois University Press, Carbondale.

WALTER, T. 1788. Flora Caroliniana. 263 pp. London: J. Fraser.

WEN, J. 1999. Evolution of eastern Asian and eastern North American disjunct distributions in flowering plants. Ann. Rev. Ecol. Syst. 30:421–455.

WEST, E.M. 1935. Canebrakes of the southeastern United States. The Ohio State University Press. Abstracts of Doctors' Dissertations, number 16:253–265.

YOUNG, R.A. 1945. Bamboos for American horticulture (I). Natl. Hort. Mag. 24: pp. 195–196.

YOUNG, R.A., AND J.R. HAUN. 1961. Bamboo in the United States: descriptions, culture, and utilization. United States Dept. Agric., Crops Res. Div., Agric. Handbook No. 193, Washington, D.C.

ZHANG, W.P., AND L.G. CLARK. 2000. Phylogeny and classification of the Bambusoideae (Poaceae). In: Jacobs, S.W.L., and J. Everetts (eds.). Grasses: Systematics and Evolution: 35–42. CSIRO, Melbourne.

CHAPTER 5. RETICULATE EVOLUTION IN THE *ARUNDINARIA* CLADE (POACEAE: BAMBUSOIDEAE)

A manuscript to be submitted to *New Phytologist*

Jimmy Triplett[1,2] and Lynn G. Clark[1]

[1]Department of Ecology, Evolution and Organismal Biology, Iowa State University, Ames, IA 50011-1020, USA

[2]Author for correspondence (triplett@iastate.edu)

ABSTRACT

Monophyly of the *Arundinaria* Clade was demonstrated in a recent phylogenetic analysis of temperate bamboos, but relationships among its major lineages are still unresolved and a number unexpected associations were revealed, including putative hybrid links with other major clades. In this study, phylogenetic relationships among *Arundinaria* and allied genera were further explored using AFLP data in conjunction with a four-region plastid framework phylogeny, with an emphasis on species-level relationships in the genus *Pleioblastus sensu stricto*. Analyses involved a total of 248 individuals in 10 genera and 57 species. Hybridization and introgression were detected both within and among genera, demonstrating the important role of reticulate evolution in temperate bamboo diversity. Molecular data confirmed the hybrid origin of *Hibanobambusa*, *Semiarundinaria*, and *Sasaella*, and also revealed the type species of *Pseudosasa* to be of hybrid origin. Moreover, cryptic links were detected between *Sasa* and *Sasamorpha*, resulting in nothotaxa that have obscured the distinction between these genera. AFLP and cp DNA sequence data support the monophyly of *Pleioblastus s.s.* and reveal species-level resolution in section *Pleioblastus,* low genetic diversity among populations of the widespread *P. simonii* (section *Medakea*), and cryptic reticulation among species in sections *Nezasa* and *Medakea*. AFLP data also

provided evidence for the monophyly of North American genus *Arundinaria*, but failed to reveal the closest relative of this genus.

KEYWORDS: *Arundinaria*, *Pleioblastus*, Bambusoideae, AFLPs, phylogeny, hybridization, reticulate evolution

INTRODUCTION

The temperate bamboo genera *Arundinaria* Michx., *Pleioblastus* Nakai, *Sasa* Makino & Shibata, and *Sasamorpha* Nakai form the core of a taxonomically difficult group of species in Eastern Asia and Eastern North America. Taxonomic delimitation in this group has been challenging due to the lack of both straightforward morphological characters and a cohesive evolutionary hypothesis. Recent phylogenetic research with an expanded DNA sequence dataset (Triplett and Clark, in prep; Chapter 2) produced a framework phylogeny for the temperate bamboos and highlighted a number of possible evolutionary phenomena contributing to the taxonomic complexity of this group, including intergeneric hybridization and reticulate evolution. Six major lineages were identified in the 12-region molecular analysis, none of which were predicted by current morphology-based classifications. Previously recognized subtribes Shibataeinae, Arundinariinae, and Thamnocalaminae were revealed to be polyphyletic complexes, indicating that features such as bracteate inflorescences, pseudospikelets, and pachymorph rhizomes are not diagnostic of broad phylogenetic trends. The plastid phylogeny revealed unexpected combinations of taxa: *Phyllostachys* Siebold & Zuccharini formed a clade with the *Thamnocalamus* group in China, including *Fargesia* Franchet, *Yushania* P.C. Keng, and *Ampelocalamus* S.L. Chen, Wen & G.Y. Sheng. *Shibataea* Makino ex Nakai segregated from *Phyllostachys* (its presumed sister genus), forming a clade with the morphologically unusual genus *Ferrocalamus* Hsueh & P.C. Keng from Western China plus Chinese species currently placed in *Sasa*. The analysis also revealed many widely recognized genera to be para- or polyphyletic, including *Pleioblastus sensu lato*, *Yushania*, and *Thamnocalamus* Munro.

Arundinaria was identified to belonging to a clade that includes *Pleioblastus s.s., Pseudosasa* Makino ex Nakai, *Sasa*, and *Sasamorpha*, while excluding a number of genera

previously assumed to be its closest relatives, including *Bashania* P.C. Keng & Yi, *Indocalamus* Nakai, *Oligostachyum* Z.P. Wang & G.H. Ye, and *Sarocalamus* Stapleton. Chloroplast sequences revealed the general topology of the *Arundinaria* Clade but failed to identify the closest relatives of *Arundinaria s.s.* or to resolve genera- and species-level relationships. Moreover, recovered lineages represented novel and unexpected combinations of taxa, such as *Acidosasa* C.D. Chu & C.S. Chao ex P.C. Keng and *Indosasa* McClure in the sister group to *Pleioblastus*, *Semiarundinaria* Makino ex Nakai nested within *Pleioblastus,* and polyphyletic placements for *Sasaella* Makino and *Pseudosasa*. *Pleioblastus sensu stricto* clustered into two main subclades: one corresponding to section *Pleioblastus*, native to the Ryūkyū Islands, and one that was inclusive of sections *Medakea* Koidzumi and *Nezasa* Koidzumi. Both subclades also contained species from other genera: *Pseudosasa japonica* (Siebold & Zuccarini ex Steudel) Makino ex Nakai (*Pseudosasa* subg. *Pseudosasa)* nested within the Ryūkyū Clade, while *Sasaella*, *Semiarundinaria*, and *Pseudosasa owatarii* (Makino) Makino ex Nakai (the only other member of *Pseudosasa* subgenus *Pseudosasa*) aligned with the *Medakea*/*Nezasa* Clade. Similarly, the *Sasa s.s.* Clade was discovered to include *Hibanobambusa* Maruyama & H. Okamura, a presumed relative of *Phyllostachys,* plus the polyphyletic *Sasaella*. A number of Chinese species currently placed in *Acidosasa*, *Indosasa*, *Pleioblastus*, and *Pseudosasa* formed a sister group (the *Sinicae* Clade) to *Pleioblastus s.s.*, while other members of these genera were resolved within the *Phyllostachys* Clade.

What is the explanation for these surprising and unexpected results? Do they reflect fundamental misinterpretations of morphology, or could underlying evolutionary phenomena such as convergent evolution, lineage sorting, or reticulate evolution be responsible for the complex phylogenetic patterns? Reticulate evolution has been recognized as a major force in plant evolution, with hybridization and introgression providing an important source of new genetic variation, allowing plants to adapt to ever-changing environments (Stebbins 1950; Grant 1981; Rieseberg 1997). The role of hybridization in the evolution of grasses is well known (Knobloch 1968; Stebbins 1956, 1972, 1985), but little evidence is available regarding this phenomenon in bamboos, and hybridization is generally expected to be rare as a consequence of temporal isolation (*i.e.*, long flowering cycles). Accordingly, bamboo

biodiversity is generally interpreted with little consideration of hybridization, but the growing list of putative hybrid links is compelling. For example, putative hybrids have been detected among species of *Chusquea* sect. *Swallenochloa* (McClure) L.G. Clark in the cloud forests of Central and South America (Clark *et al.* 1989). Based on patterns of morphological intermediacy, *Chusquea subtessellata* Hitchc.*, C. amistadensis* L.G. Clark, Davidse & Ellis*, C. tessellata* Munro, *C. vulcanalis* (Soderstrom & C.E. Calderón) L.G. Clark and *C. spencei* Ernst were hypothesized to be involved in pairwise hybrid complexes. Within the temperate clade, hybrid origins have been hypothesized for several genera on the basis of morphological intermediacy. For example, *Semiarundinaria* is hypothesized to be a cross between *Phyllostachys* and *Pleioblastus* (Okamura and Tanaka 1986), the monotypic genus *Hibanobambusa* is presumed to be a cross between *Sasa* and *Phyllostachys (*Okamura and Tanaka 1986), and *Sasaella* is considered a putative hybrid between *Sasa* and *Pleioblastus (*Suzuki 1987). Alternatively, these taxa are interpreted as phylogenetically distinct lineages, and their intermediate morphologies may indicate transitional taxa or evolutionary throwbacks (Cambpell, unpubl.). In a recent investigation of *Arundinaria* in North America using AFLPs and cp DNA data, hybrids and introgressants were identified among all three species (Triplett *et al.*, in prep; Chapter 3), and the detected hybrids were morphologically intermediate between parental species. The recent cp DNA phylogeny of the temperate bamboos (Triplett and Clark, in prep.; Ch. 2) is consistent with the hypothesis of hybridization, but provides insufficient evidence to exclude other possible explanations. Putative hybrid taxa were revealed to have DNA haplotypes matching putative parents, rather than having divergent sequences suggesting relictual lineages.

Phylogeny reconstruction within the temperate clade is problematic due to markedly low rates of nucleotide substitution. For similar groups in which sequence data have failed to provide sufficient resolution, amplified fragment length polymorphisms (AFLPs) have emerged as one of the best resources for phylogenetic studies (Després *et al.* 2003; Guo *et al.* 2005). AFLPs have been used to study genetic variation, species limits, and phylogenetic relationships in several genera of grasses (Ridout and Donini 1999; Peterson *et al.* 2002; Loh *et al.* 2000; Saarela *et al.* 2003), and proved to be an excellent tool for reconstructing phylogenetic relationships among closely related species of bamboo (Triplett *et al.*, in prep,

Ch. 3; Hodkinson *et al.* 2000). AFLPs provide a large number of independent markers from across the genome, the majority of which represent nuclear rather than plastid loci (Álvarez and Wendel 2006). As such, AFLPs have the advantage of tracking biparental inheritance, making it possible to detect hybridization and introgression (Bleeker and Matthies 2005). Hybridization results in an additive (mosaic) AFLP banding pattern, such that diagnostic bands from each parent can be recovered in hybrids (Raamsdonk *et al.* 2000). The expected impact of composite genotypes in tree-based searches is the loss of resolution and the recovery of grades rather than clades (McDade 1992, 1997); however, using a combination of ordination and quantitative methods it is possible to parse the noise generated by hybrids into meaningful patterns.

In this study, we explore the phylogenetic utility of AFLP data to resolve relationships within one major clade of temperate bamboos in light of potential hybrid networks. The primary goals of this study are to (1) investigate species-level relationships within the *Arundinaria* Clade, with an emphasis on the Japanese genus *Pleioblastus s.s.,* and (2) examine putative reticulate relationships within the *Arundinaria* Clade, especially as they impact on the interpretation of subclades and genera.

Taxonomic History of *Pleioblastus* and allies

As the largest of the narrowly defined genera in the *Arundinaria* Clade, the status of *Pleioblastus* and its relationship with the North American type species of *Arundinaria* is particularly important in efforts to reconstruct the phylogeny of the temperate bamboos. *Pleioblastus s.s.* occurs in the Japanese archipelago from the Ryūkyū Islands north to Hokkaido, and is the genus most often synonymized with *Arundinaria* on the basis of morphology, although recent molecular evidence indicate it is no closer than a number of other Asian genera (Triplett and Clark, in prep; Ch. 2). Moreover, recent morphological studies suggest that *Pleioblastus* shares characteristics of prophyll structure with *Sasa* and *Pseudosasa* (Stapleton 1997), further widening the gap with *Arundinaria*. Nakai (1925) recognized *Pleioblastus* to accommodate leptomorph bamboos with branch primordia that ramify precociously to produce a characteristic branch complement with 3-9 principal branches per node. *Pleioblastus* contains a striking diversity from arborescent species in the

woodlands to diminutive groundcover species in monodominant montane grassland communities. During the early twentieth century, *Pleioblastus* swelled to over 100 species and as many subspecific taxa. This nomenclaturally complex group was revised by Suzuki (1978), who reduced it to 21 species in 3 sections: *Pleioblastus*, *Medakea*, and *Nezasa*. At least two types of rhizomes are present in this genus, which served as the primary basis for intrageneric assignments. One type is described as amphipodial (leptomorph with tillering culms): the rhizomes are short and thick, with short internodes and the tips always turning upward to produce culms that are close together in a dense clump. The other type is more typical monopodial (leptomorph), with new culms arising from lateral buds at intervals along the rhizome. Section *Pleioblastus* contains many of the larger species with tillering culms, relatively long foliage leaf blades, and long inner ligules. Sections *Medakea* and *Nezasa* contain species with monopodial rhizomes, relatively shorter foliage leaf blades, and short inner ligules; these two are primarily distinguished by minor differences in the upper margins of leaf sheaths, which are oblique in *Medakea* and horizontal in *Nezasa*. Taxonomically, section *Nezasa* is the most problematic: many of its species are only known in cultivation, and several are unknown in flower.

Recently, *Pleioblastus* section *Amari* S.L. Chen & G.Y. Sheng was established to accommodate putative congeners of *Pleioblastus* from China and Vietnam (Chen and Sheng 1991). While some species in this group are morphologically similar to the taxa in the Ryūkyū Islands, others differ in a suite of characters from branch architecture to leaf persistence, resembling *Oligostachyum* and *Semiarundinaria*. Moreover, the morphological distinction between this group and Chinese species placed in *Pseudosasa* subgenus *Sinicae* S.L Chen & G.Y. Sheng is unclear (Wu *et al.* 2006), and most taxonomic placements are considered provisional. The recent cp DNA framework phylogeny demonstrated both *Pleioblastus* section *Amari* and *Pseudosasa* subgenus *Sinicae* to be polyphyletic, with a core of species forming the sister clade to *Pleioblastus s.s.* (the *Sinicae* Clade, Triplett and Clark, in prep.; Ch. 2) while others are apparently closer to *Phyllostachys* and allies. Within this heterogeneous group is a core of plants that resemble *Pleioblastus sensu stricto*, and likely represent a link between species in the Japanese archipelago and Mainland China.

MATERIALS AND METHODS

Taxon sampling. A total of 248 accessions representing 10 genera and 57 species were utilized for this study (Appendix 1). Samples were obtained from natural populations in China, Japan, and North America, and from living collections in China (Kunming), Japan (Kyoto), and the US (California, Tennessee, and Washington). Additional samples were obtained as gifts. Leaf tissue was collected in the field and lyophilized using silica gel (Chase and Hills 1991). Vouchers of all collections are deposited in the Ada Hayden Herbarium of Iowa State University (ISC) unless otherwise indicated. Sampling emphasized *Pleioblastus sensu stricto* and taxa implicated in putative hybrid associations with this genus. Representatives of *Pleioblastus* section *Amari* and *Pseudosasa* subgenus *Sinicae* were included to further test the placement of the *Sinicae* Clade and the monophyly of *Pleioblastus sensu stricto.* Exemplars of *Phyllostachys* were selected to represent major taxonomic diversity within that genus and the most widespread species in Japan (Suzuki 1978). Two species of *Chimonocalamus* Hsueh & Yi were included as outgroups in the cp DNA sequence analysis based on previous results that demonstrate this genus to be phylogenetically distinct from both the *Arundinaria* Clade and the *Phyllostachys* Clade.

A number of specimens tested in this study could not be placed satisfactorily according to current taxonomy, either due to missing characters (*e.g.*, young culm leaves) or because they had intermediate or otherwise unusual morphology. These were labeled "*aff.*" (*e.g.*, *P. aff. chino* 402) to indicate insufficient information or "putative hybrid." (*e.g.*, *Pl. putative* HYB 360) based on intergrading morphologies. One such plant (*Pl. putative* HYB 60) collected as *Pleioblastus hindsii* (Munro) Nakai from Rakusai Bamboo Garden in Kyoto, Japan, had characters suggestive of both the Ryūkyū clade and the *Medakea* clade. Another plant (Unknown 51, 78, and 115) was collected under three different names: *S. borealis* (Hackel) Nakai (Japan), *P. virens* Makino (USA), and *P. kiushianus* Makino (Japan), but does not key out to any recognized species. A similar plant was collected in Central Honshu. These could not be satisfactorily identified to species, although they appeared to belong to *Pleioblastus* on the basis of branch architecture and foliage leaf fimbriae.

DNA extraction. For AFLP reactions, total genomic DNA was extracted from silica gel-dried samples according to the modified 2× CTAB procedure of Doyle and Doyle (1987).

Preliminary tests comparing CTAB and DNeasy Plant Mini Kit (QIAGEN, Inc., Valencia, California) extractions recovered essentially identical AFLP genotypes. Some cp DNA sequences were generated from extractions done using QIAGEN DNeasy Plant Mini Kits, as described in Chapter 2. Nucleic acid quality was measured using a Nanodrop ND-1000, and concentrations were standardized to 250ng/µl for AFLP enzyme digestions and 100 ng/µl for cp DNA PCR amplification.

Sequencing. A subset of taxa were sequenced for *rps16-trnQ, trnC-rpoB, trnD-trnT,* and *trnT-trnL* to complement available sequences (Triplett and Clark, in prep.; Ch. 2), using primers and protocols described previously (Triplett and Clark, in prep.; Ch. 2). All polymerase chain reactions (PCRs) and cycle-sequencing reactions were performed in a Perkin-Elmer Applied Biosystems GeneAmp PCR System 9600 thermocycler or a MJ Research PTC-200 thermal cycler. PCR reactions were carried out in 40 µl volumes. Amplification products were cleaned using Antarctic phosphatase (5 units, NEB) and exonuclease I (10 units, NEB) followed by an ethanol precipitation. Sequencing reactions were carried out using BigDye v.2 to produce complementary strands, and sequence products were cleaned using Edge Biosystems clean-up plates. Sequencing was performed by the Automated 3730xl DNA Analyzer (Perkin-Elmer, Applied Biosystems Division) at the Iowa State University DNA Sequencing and Synthesis Facility. Sequences were assembled, verified, and manually aligned using the program Se-Al version 2.09a (Rambaut 2001). Sequence alignment introduced gaps that later were treated as binary, presence/absence characters (Giribert and Wheeler 1999). Autapomorphic, parsimony uninformative indels were not scored, and were excluded along with other gaps prior to analysis. All data matrices used in the study will be made available in TreeBASE and all individual sequences will be available in Genbank.

Amplified Fragment Length Polymorphisms (AFLPs). AFLP analysis followed the protocol of Vos *et al.* (1995) with modifications suggested by the J.F. Wendel lab at ISU (http://www.eeob.iastate.edu/faculty/WendelJ/aflp.htm) and additional optimization for bamboos. DNA was digested with restriction enzymes EcoRI (10 units, NEB) and MseI (10 units, NEB) for 2h at 37ºC in a 20 µl volume, followed by ligation (20 units T4 DNA ligase [NEB], overnight at 16ºC) to double-stranded 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. Out of a total of 16 tested primer combinations, 6 were chosen for this study based on banding patterns in the *Arundinaria* Clade. FAM and HEX labeled +3 EcoRI primers were multiplexed in the following combinations: [1.] mCAA, eACT (FAM), eACG (HEX); [2.] mCTG, eACA (FAM), eAAC (HEX); [3.] mCTT, eACT (FAM) eACG (HEX). Selective amplification products were separated electrophoretically at the ISU DNA Facility on an Applied Biosystems Inc. 3100 capillary fragment analyzer (Perkin Elmer) with an internal standard (GeneScan 500 Rox, ABI) and read using GeneScan software. Data extraction was done manually from trace files using the program Genographer 1.6.0 (2004). Bands were scored as present (1) or absent (0).

AFLP markers represent an anonymous sampling of the genome, and it is likely that some co-migrating fragments are from different loci (Bussell *et al.* 2005; Althoff *et al.* 2007); however, nonhomologous bands are distributed randomly and thus more likely to reduce resolution than to produce robust pseudogroups (Adams and Rieseberg 1998). Nevertheless, we have adopted a conservative approach to scoring and interpretation of AFLP data 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. Only robust, unambiguous DNA fragments ranging from 100 to 550 bp in size and above 200 relative fluorescent units were scored, and we avoided areas of high band density. Data were analyzed comparatively using ordination and network analyses as well as distance and cladistic tree-based methods in order to detect and evaluate all possible signal conflict (Lara-Cabrera and Spooner 2004; Koopman *et al.* 2001).

Phylogenetic analysis – cp DNA. Sequence datasets were analyzed with maximum parsimony (MP), maximum likelihood (ML), and Bayesian Inference (BI). MP and ML analyses were conducted using PAUP* 4.0b10 (Swofford 2002), and BI was conducted using MrBayes v3.1 (Ronquist *et al.* 2005). Prior to combining cp DNA datasets, we implemented a Partition Homegeneity test (ILD, Farris *et al.* 1994) in PAUP* to evaluate congruence

among regions, using 1,000 iterations in a full heuristic search and random taxon addition. Maximum parsimony analyses used the heuristic search option with 1,000 random-addition-sequence (RAS) replicates and tree bisection and reconnection (TBR) branch swapping. Strict consensus trees were calculated for all MP analyses, and branch support was estimated with 1,000 bootstrap replicates (Felsenstein 1985) using heuristic searches as described above. The hierarchical likelihood ratio test, as implemented in Modeltest 3.6 (Posada and Crandall 1998), was employed to determine the appropriate model of sequence evolution for each DNA partition. Maximum Likelihood (ML) parameter values were optimized using a BioNJ tree as a starting point (Gascuel 1997) with the appropriate model parameters for the combined datasets. ML analyses used the heuristic search option with 1,000 RAS replicates and TBR branch swapping. ML bootstrap analyses (MLBS) comprised 100 replicates, each with two RAS replicates. Bayesian Inference (BI) was conducted using flat priors. Nucleotide data from different DNA regions were treated as separate partitions, and binary coded indels were combined into a single partition. In all searches, three heated chains and a single cold chain were used, and runs were initiated with random trees. Chains were run for 10 million generations, and trees were sampled every 1000 generations. A majority-rule consensus of the remaining trees was calculated to obtain a topology and posterior probabilities (PP). BI searches were repeated three times in order to confirm that searches converged on the same topology. Branch support was assessed according to a 70% bootstrap criterion for MP and ML and a 0.95 posterior probability measure for BI (Mason-Gamer and Kellogg 1996; Wilcox *et al.* 2002).

Phylogenetic analysis – AFLPs. We used a reiterative approach to explore the phylogenetic structure of the AFLP data, investigating alternative topologies as potential sources of biologically meaningful information, particularly with respect to hybridization. Selected iterations are reported below to highlight key steps in data exploration.

Relationships in the *Arundinaria* Clade were investigated in three stages: (1) first, we analyzed a subset of the data that was parallel to the cp DNA matrix in order to look for major trends and to test for congruence; (2) next, analyses were run with all available samples (the “Large Dataset”), including putative hybrids, and broad patterns were reconciled with the results of the first iteration; and (3) targeted analyses were conducted on

closely related taxa revealed in the first two stages. This approach allowed us to test *a priori* taxonomic assignments and to subsequently minimize noise introduced via distant relatives or genetically mosaic taxa.

Pairwise genetic distances were calculated in PAUP* v4b10 (Swofford 2002) using the Nei-Li dissimilarity coefficient (Nei and Li 1979). This algorithm is appropriate for dominantly inherited AFLP markers because it gives greater weight to the shared presence of fragments (presumably due to common descent) and is less sensitive to the potentially homoplastic absence of bands. Genetic relationships were reconstructed using neighbor-joining (NJ) analyses as implemented in PAUP*. Bootstrap support for the NJ tree was estimated based on 10,000 replicates. Dendrograms were also calculated using the unweighted pair group method with arithmetic averages (UPGMA) on the Nei-Li distances. UPGMA assumes clock-like evolution, and hybridization, with its sudden dramatic change of the AFLP genotype, strongly violates this assumption. UPGMA were specifically utilized to assist the visual inspection of trends in the data, and not interpreted to indicate natural groups *per se*. UPGMA is expected to be more sensitive to posthybridization changes in the hybrid genotype, or apparently unequal contributions by the parents, shifting the hybrid towards the more derived parent. Hybrids are predicted to cluster in intermediate positions between parents, or else sister to the more derived parent, consistent with results obtained using morphological characters (McDade 1992, 1997).

Subsequent to the identification and removal of hybrids, phylogenetic relationships among subsets of taxa were explored under maximum parsimony (MP) using Wagner (Farris 1970) and Dollo (Le Quesne 1974; Farris 1977) criteria on the original presence/absence matrix. Wagner parsimony gives equal weight to fragment gain or loss, whereas Dollo parsimony makes the assumption that a fragment can be lost multiple times but gained only once. Because of the high level of internal conflict associated with AFLP data, trees were produced using a four-step heuristic search adapted from Olmstead and Palmer (1994): (1) First, 50,000 random replicates were performed with nearest-neighbor interchange (NNI) branch-swapping algorithm and MulTrees off; (2) the resulting MP tree was used as the starting tree under the TBR algorithm with MulTrees off; (3) this MP tree was used as starting tree in a heuristic search for multiple parsimonious trees (MulTrees on) with NNI

branch swapping; and finally (4) these trees were used as starting trees for a search using TBR with MulTrees on. The resulting MP trees were used to compute a strict consensus tree. Bootstrap values were obtained from 1,000 random replicates with TBR, MulTrees On. AFLP topologies were also explored using Bayesian Inference, as implemented in MrBayes for standard data, using the heuristic search methods described above.

We explored three additional methods to characterize relationships among select species. We conducted non-metric multidimensional scaling (nMDS) analyses of the pairwise Nei-Li distance matrix using the software R version 2.6.2 and the supplemental package MASS in order to obtain two-dimensional ordinations of the data. Network diagrams of relationships among select species and hybrids were produced using SplitsTree4 (Huson and Bryant 2006), which provides a visual representation of signal conflict in the data. We also produced networks using the NeighborNet algorithm (Bryant and Moulton 2004) on the Nei-Li pairwise distances matrix. We used STRUCTURE 2.2 (http://pritch.bsd.uchicago.edu/structure.html) to estimate the number of structured groups (K clusters) within the *Pleioblastus Medakea + Nezasa* clade. Program settings used the admixture ancestry and correlated marker frequency models. The graphs of L (K) were used to determine the number of clusters used for estimating admixture. The length of burn-in was set at 10000 followed by 30000 iterations. Three replications were performed at each proposed K. The proportion of markers from each cluster was calculated for each accession, and we used proportions to establish classifications of admixture: an accession receiving 6-35% from another cluster was labeled INT (putative introgressant), while accessions receiving 35-65% from two clusters were labeled putative HYB, or indicated by the likely species pair if additional information was available (*i.e.*, morphological intermediacy).

Determining Parental contributions. Hybrids are identifiable by mosaic banding patterns that combine diagnostic bands from different parents. We used a simple quantitative approach to identify putative parents by searching for the minimum pairwise distance between a hybrid and each of its putative parents. Because this method is limited to the included samples, it is not guaranteed to identify the true parent taxa. Moreover, band inheritance is likely to be susceptible to other sources of stochastic and systematic error, and therefore must be interpreted with caution. For representative hybrid accessions, we

determined the proportion of fragments attributable to parental species. Fragments were assigned to one of four categories: (1) diagnostic of parent "A"; (2) diagnostic of parent "B"; (3) occurring in the hybrid and both parents; and (4) unique to the hybrid.

RESULTS

Chloroplast sequences. Statistics for the aligned data matrices are presented in Table 1. Sequences contained no missing data for any of the four datasets, and alignments were unambiguous with the exception of three areas: an indel region in *rps16-trnQ* (aligned position 505-520), a poly-A region in *trnTL* (aligned position 617-619), and a poly-G region in *trnTL* (aligned position 681-686). Ambiguous regions were excluded in all analyses. Partition homogeneity tests indicated no significant conflicts in phylogenetic signal among datasets (P-value = 0.13), and analyses of separate datasets did not reveal statistically different topologies. The combined four-region data matrix consisted of 5009 aligned positions plus 21 binary-coded indels. A total of 81 characters (60 nucleotide, 21 structural) were parsimony informative.

AFLPs. A total of 810 markers were scored for the 6 AFLP primer combinations. Scored fragments represent 7 size classes: 101-200 (240; 30%); 201-300 (284; 35%); 301-400 (183; 23%): 401-500 (93; 11%) and 501-527 (10; 1%). The average number of scored bands per primer pair was 135, with a range of 75 to 238, and the average number of fragments per individual was 114.3. Of the 810 scored fragments, 801 characters (98.9%) were polymorphic, reflecting the fact that monomorphic bands were largely ignored.

Part I: cp DNA vs. AFLP subset

The cp DNA phylogeny is summarized in Fig. 1. Topologies of the strict consensus of the equally most parsimonious trees (6 trees of 96 steps), the strict consensus of 2 ML trees (-ln L = 7226.09059), and the BI phylogeny were highly congruent. In all analyses, a polytomic clade containing *Phyllostachys, Arundinaria funghomii* McClure, and four accessions of *Pleioblastus* section *Amari* [*P. amarus* (Keng) P.C. Keng, *P. intermedius* S.Y. Chen, *P. juxianenesis* Wen, C.Y. Yao & S.Y. Chen, and *P. solidus* S.Y. Chen] received strong support (MPBS = 100%). Sequences in this group were identical (or nearly so). The

Table 1. Statistics and evolutionary models for separate and combined analyses. PIC = parsimony informative characters. MP = maximum parsimony; CI = consistency index, excluding uninformative characters; RI = retention index. Models are based on the Hierarchical Likelihood Ratio Test implemented in ModelTest.

Partition	**DNA**	**Indels**	**Total char.**	**Char, no gaps**	**PIC**	**MP Trees**	**MP Length**	**CI**	**RI**	**Model**
rps16-trnQ	1648	6	1654	1542	20	1	27	0.9524	0.9940	TVM+G
trnC-rpoB	1273	6	1279	1114	17	1	24	0.9444	0.9522	TVM+G
trnD-trnT	1207	2	1209	1030	11	47	19	0.9286	0.9881	HKY+G
trnT-trnL	881	7	888	777	15	1	24	1.0000	1.0000	TVM+G
All data	5009	21	5030	4593	63	6	96	0.9296	0.9909	--
DNA	5009	--	5009	4572	48	6	75	0.9107	0.9880	TVM+G
indels	--	21	21	--	15	1	21	1.0000	1.0000	--

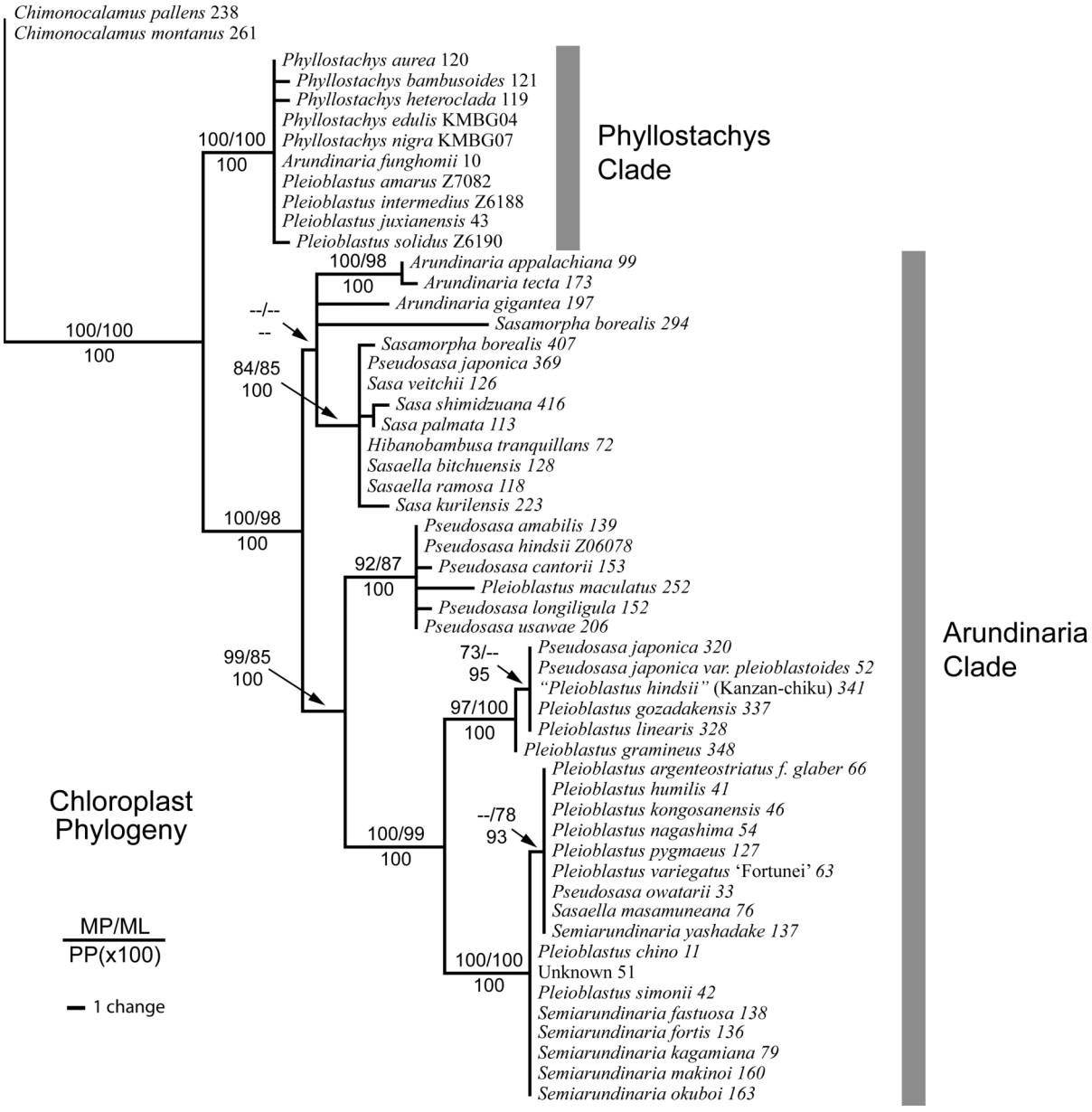

Figure 1. Strict consensus of 6 equally most parsimonious trees based on the 4-region dataset (*rps16-trnQ, trnC-rpoB, trnD-trnT, trnT-trnL).* Numbers above lines indicate bootstrap values (MP/ML). Numbers below lines indicate posterior probabilities from the Bayesian analysis.

remaining taxa formed a monophyletic group (the *Arundinaria* Clade) with robust subclades but little species-level resolution. All topologies suggested a weak affinity among *Sasa*, *Sasamorpha*, and *Arundinaria s.s.*, but relationships among these genera cannot be confidently resolved with the current cp DNA data nor when sampling was expanded to 12 regions (Triplett and Clark, in prep., Ch. 2). *Arundinaria appalachiana* Triplett, Weakley & L.G. Clark and *A. tecta (*Walt.) Muhl. are strongly supported as sister species (MPBS = 100) but a relationship with *A. gigantea* is not indicated. *Sasa* formed a moderately supported clade (MPBS = 84) with *Hibanobambusa tranquillans* (Koidzumi) Maruyama & H. Okamura, *Sasaella*, and one accession each of *Pseudosasa japonica* and *Sasamorpha borealis*. Other accessions of *Pseudosasa japonica* nested with "*Pleioblastus hindsii*," while other accessions of *Sasamorpha borealis* from Central Honshu were resolved as a distinct lineage. *Pleioblastus* and allies form a robust clade (MPBS = 99) including the *Sinicae* Clade (MPBS = 92) of Mainland China and *Pleioblastus sensu stricto* and allies (MPBS = 100) of the Japanese archipelago. The *Sinicae* Clade is here represented by *Pseudosasa* subgenus *Sinicae* [including *Pseudosasa hindsii* (Munro) S.L. Chen & G.Y. Sheng] and one species from *Pleioblastus* section *Amari* [*Pleioblastus maculatus* (McClure) C.D. Chu & C.S. Chao]. Two robust clades were recovered within *Pleioblastus s.s.*: the Ryūkyū Clade (MPBS = 97), including *"Pleioblastus hindsii,*" and the *Nezasa* + *Medakea* Clade (MPBS = 100), including *P. argenteostriatus (P.* section *Nezasa)* and *P. simonii (P.* section *Medakea)*. *Pseudosasa owatarii* (as cultivated in US and Japan) nested within the *Nezasa* + *Medakea* Clade (likely representing a misidentification or taxonomic misplacement).

As demonstrated previously, the cp DNA phylogeny contains several taxon placements consistent with hypothesized intergeneric hybridizations. *Hibanobambusa tranquillans,* the putative hybrid between *Sasa* and *Phyllostachys*, nested within the *Sasa* Clade. *Sasaella*, the putative nothogenus of *Sasa* and *Pleioblastus,* was revealed to be polyphyletic, with *S. masamuneana* in the *Pleioblastus Nezasa/Medakea* Clade and the other species in the *Sasa* Clade. All accessions of *Semiarundinaria,* the presumptive hybrid between *Phyllostachys* and *Pleioblastus*, nested within the *Nezasa* + *Medakea* Clade. Sequence variation resulted in a weakly supported cluster containing *S. yashadake* (Makino) Makino and *Pleioblastus* section *Nezasa* (MLBS = 78) to the exclusion of *P. chino* (Franchet

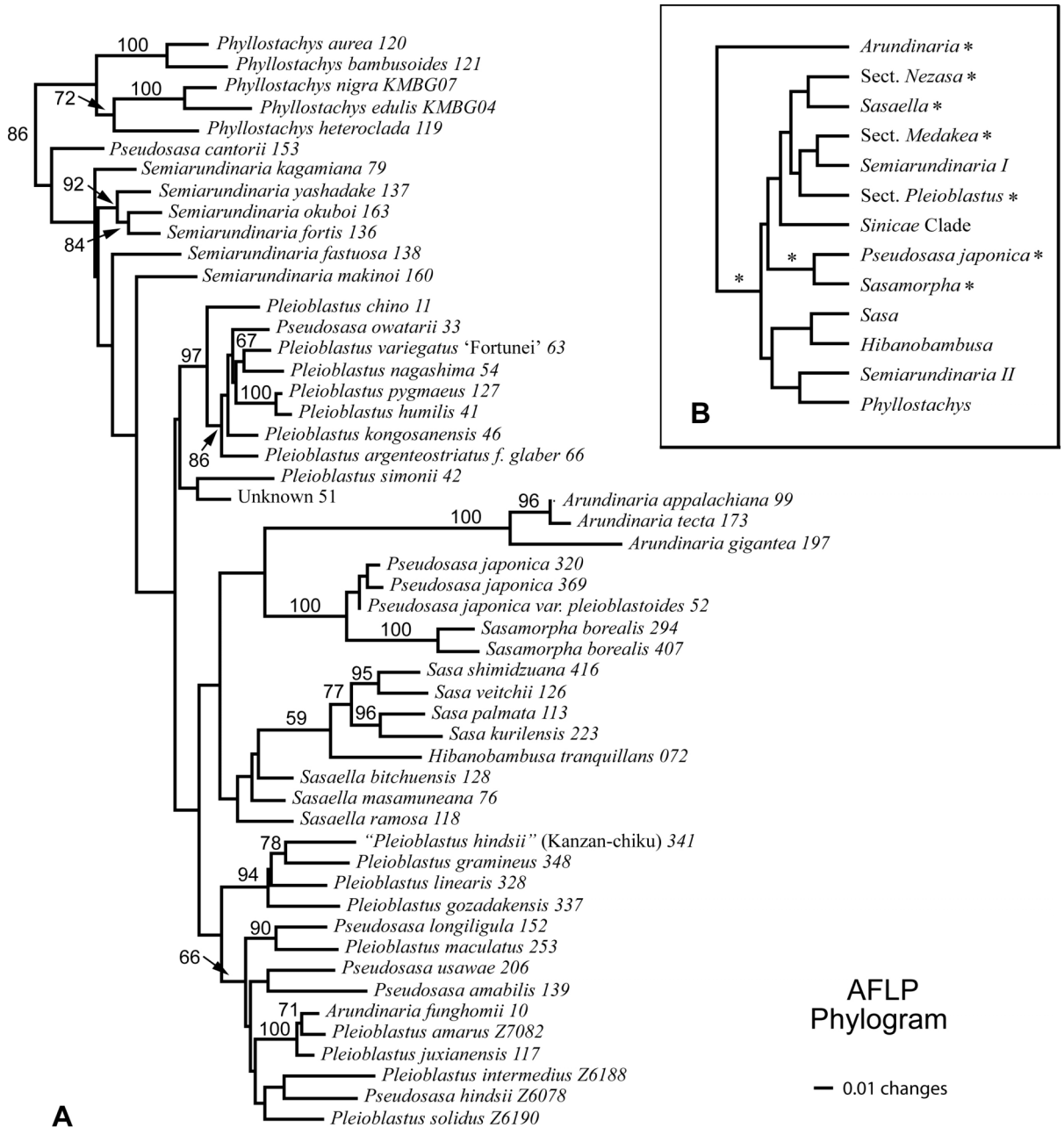

Figure 2. A. Genetic relationships among 52 accessions of the *Arundinaria* Clade inferred from AFLP data using a neighbor-joining analysis of Nei-Li distances. Tree rooted with *Phyllostachys*. Numbers above branches indicate bootstrap support values (>65%). B. Summary of the UPGMA dendrogram for the same dataset. Clusters that received bootstrap support over >70% are indicated by asterisks.

& Savatier) Makino*, P.* section *Medakea,* and other species of *Semiarundinaria*, suggesting multiple origins of *Semiarundinaria*.

Analyses of the corresponding AFLP data subset (limited to the species in the cp DNA phylogeny and excluding *Chimonocalamus*) produced topologies that were inconsistent with the cp DNA framework (Fig. 2a,b). The fundamental differences were the relative positions of major groups and the positions of putative hybrids. Moreover, recovered trees lacked support for nodes along the backbone. Several taxa received moderate to robust support in NJ analysis, including *Arundinaria s.s.* (NJBS = 100), *Sasa* (NJBS = 77), *Sasamorpha* (NJBS = 100), *Pleioblastus* section *Pleioblastus* (NJBS = 93) and *Pleioblastus* section *Nezasa* (NJBS = 97). Species-level resolution is evident in some of these taxa. For example, AFLP data supported the sister-species relationship of *A. appalachiana* and *A. tecta*, and *Sasa sensu stricto* (excluding *Sasamorpha*) was resolved to have two robust clades.

The putative nothogenus *Semiarundinaria* grouped close to *Phyllostachys*, forming a grade in the NJ phylogeny and a nested cluster in the UPGMA dendrogram. *Hibanobambusa* and *Sasaella* formed a grade at the base of *Sasa* in the NJ analysis, whereas *Sasaella* clustered with *Pleioblastus* section *Nezasa* in the UPGMA tree. *Pseudosasa cantorii* (Munro) P.C. Keng clustered adjacent to *Phyllostachys* in the NJ analysis, while all other Chinese species (*Pleioblastus* section *Amari* and *Pseudosasa* subgenus *Sinicae)* formed a weakly supported cluster. In the NJ analysis, *Pseudosasa japonica* formed a well-supported cluster (NJBS = 100) within which *Sasamorpha borealis* (NJBS = 100) was nested, while the UPGMA topology clustered these as sister taxa.

NMDS ordination analysis of the AFLP distance matrix for select taxa is illustrated in Fig. 3. In order to more clearly visualize relationships among putative hybrid taxa in the Japanese archipelago, *Arundinaria* and the Chinese taxa were excluded from the analysis used to generate the plot in Fig. 3. *Arundinaria* shared relatively few AFLP bands with other taxa, and its inclusion in the ordination produced a highly skewed plot (not shown). Chinese allies of *Pleioblastus* (*Pleioblastus* section *Amari* and *Pseudosasa* subgenus *Pseudosasa*) formed a weak cluster close to *Pleioblastus s.s.*, with the exception of *Pseudosasa cantorii*, which clustered in an intermediate position between *Phyllostachys* and the other Chinese

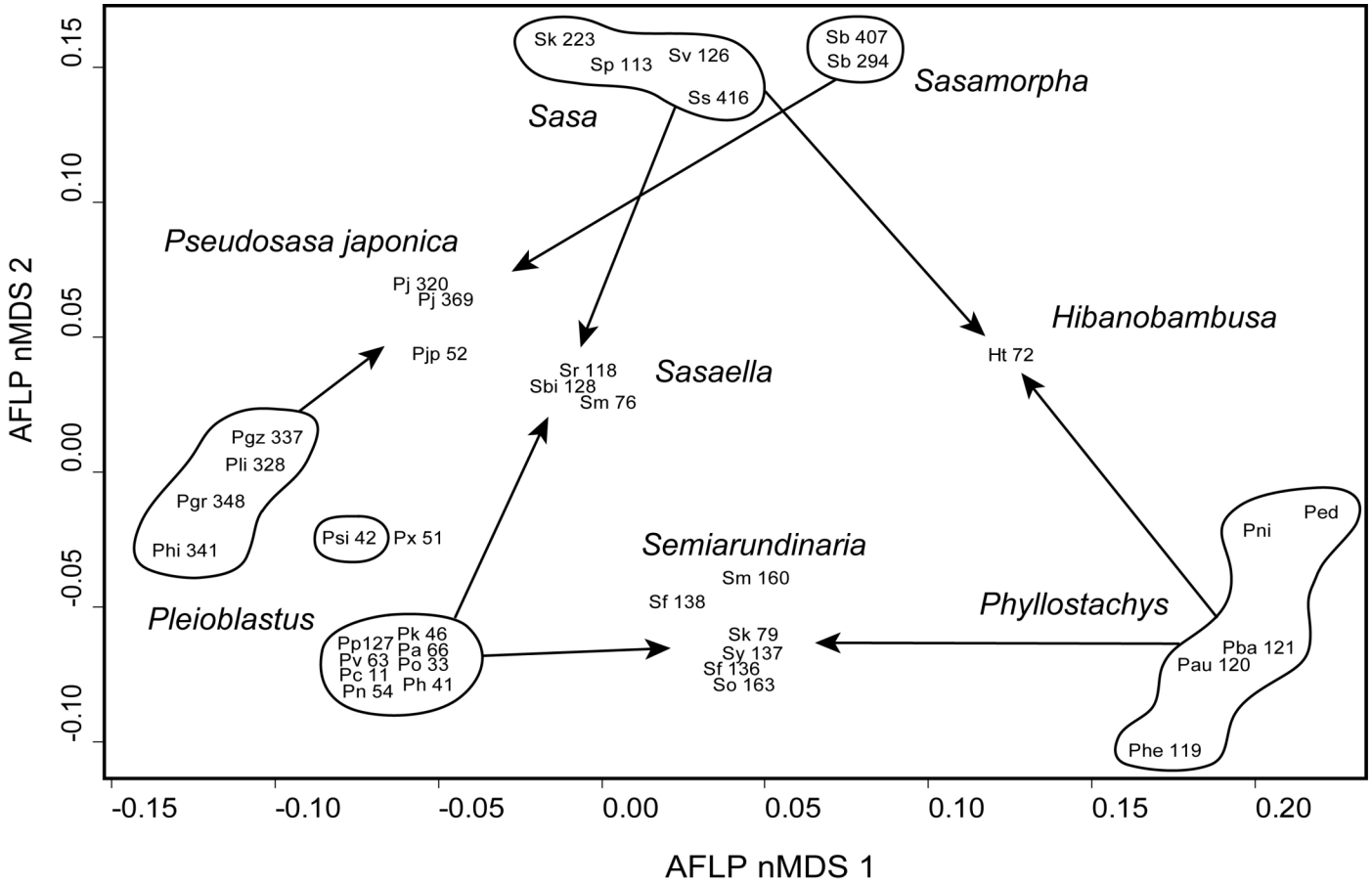

Figure 3. Two-dimensional ordination of accessions representing the *Arundinaria* Clade. Putative hybrid associations are indicated between parent genera (circled) and nothogenera (indicated by arrows). See Fig. 1 for species names (by voucher number). Ordination was conducted using nonmetric multidimensional scaling on a pairwise distance matrix calculated using the Nei-Li dissimilarity coefficient. Final stress was reached after 5 iterations. Final stress = 16.059440.

species (not shown). Excluding these did not alter the relative positions of the remaining taxa.

Recovered clusters corresponded to morphologically defined groups (genera or sections), and putative nothogenera were positioned intermediate between presumed parent taxa: *Semiarundinaria* between *Phyllostachys* and *Pleioblastus*; *Sasaella* between *Pleioblastus* (*Nezasa/Medakea*) and *Sasa*; and *Hibanobambusa* between *Sasa* and *Phyllostachys*. *Pseudosasa japonica* was intermediate between *Pleioblastus* section *Pleioblastus* and *Sasa*/*Sasamorpha*.

Part II: Large Dataset

Summary trees of the large AFLP dataset are presented in Figure 4, and the full phylogram is presented in Figure 5. The overall pattern recapitulates the results of the framework analysis above, whereby clusters were recovered for groups defined by morphology and relative positions of groups varied depending on the method of analysis. Clusters were recovered for *Arundinaria s.s.*, *Sasa* + *Sasamorpha* (NJ only), *Pleioblastus* sections *Nezasa*, *Medakea,* and *Pleioblastus*, and the *Sinicae* Clade (*Pseudosasa* subgenus *Sinicae* and *Pleioblastus* section *Amari*), with bootstrap support ranging from robust (*e.g.*, *Arundinaria s.s., Sasamorpha, Pseudosasa, Pleioblastus* section *Pleioblastus*) to weak (*e.g.*, *Sasa sensu stricto*, the *Sinicae* Clade, *Pleioblastus* sections *Nezasa* and *Medakea, Sasaella*). All higher-order clusters had bootstrap values below 70%. Putative hybrids *Sasaella*, *Semiarundinaria*, *Hibanobambusa, Pseudosasa japonica,* and members of the *Sinicae* group (*e.g., Pseudosasa cantorii*) were resolved in variable positions depending on clustering method. For example, all fourteen accessions of *Sasaella* formed a grade in the NJ phylogram intermediate between *Sasa* and *Pleioblastus*, while in the UPGMA dendrogram *Sasaella* nested within *Sasa*. Accessions of *Semiarundinaria* collapsed near *Phyllostachys* in the NJ phylogram, but formed a weakly supported cluster adjacent to *Pleioblastus* sections *Nezasa* and *Medakea* in the UPGMA tree. *Hibanobambusa tranquillans* was positioned close to *Phyllostachys* in both the NJ and UPGMA trees, in contrast to its nested position within *Sasa* in the smaller analysis and in the cp DNA framework.

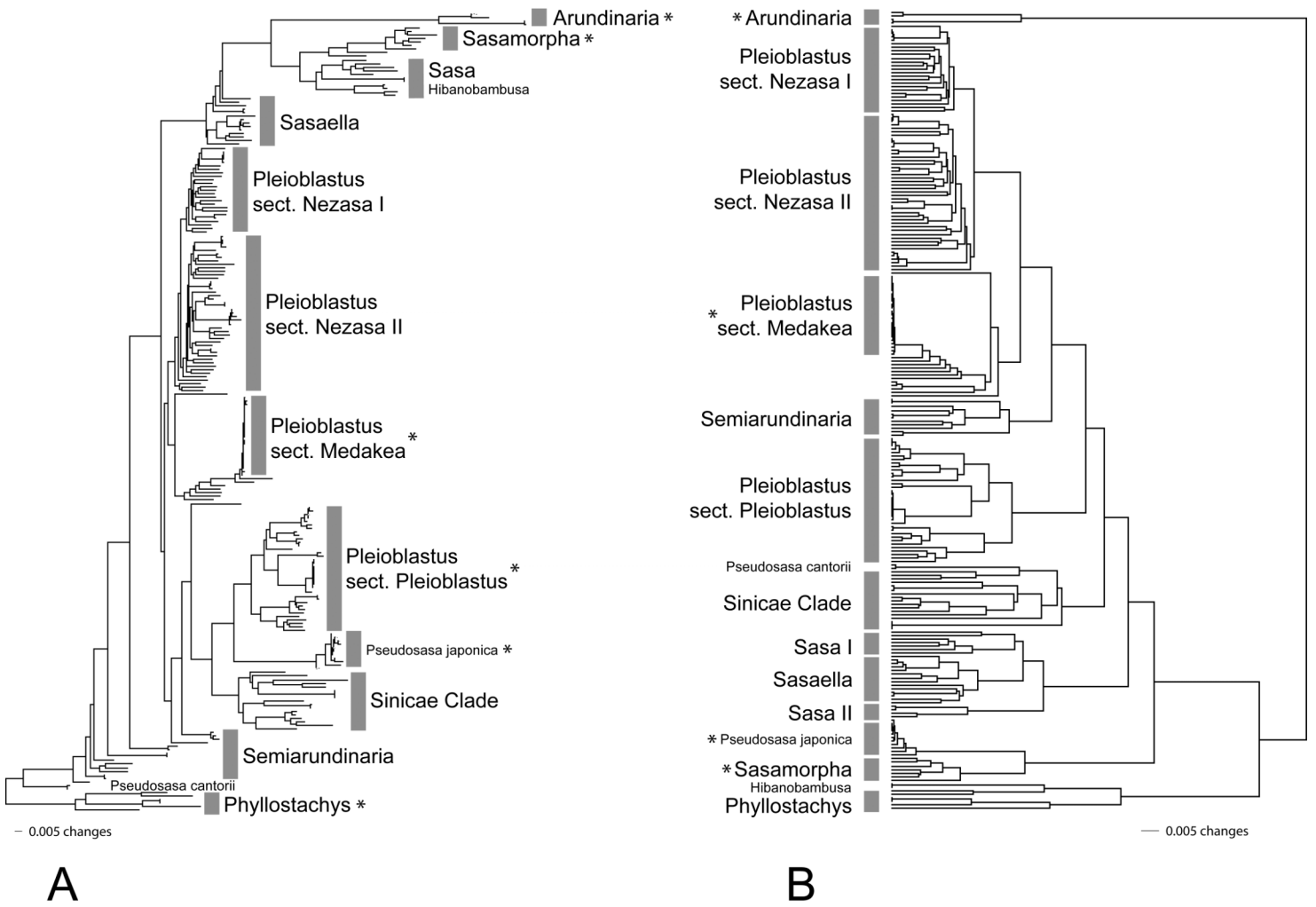

Figure 4. A. Summary phylogram of genetic relationships among 248 accessions of the *Arundinaria* Clade inferred from AFLP data using a neighbor-joining analysis of Nei-Li distances. Tree rooted with *Phyllostachys*. B. Summary UPGMA dendrogram for the same dataset. Major clusters that received bootstrap support over >70% are indicated by asterisks.

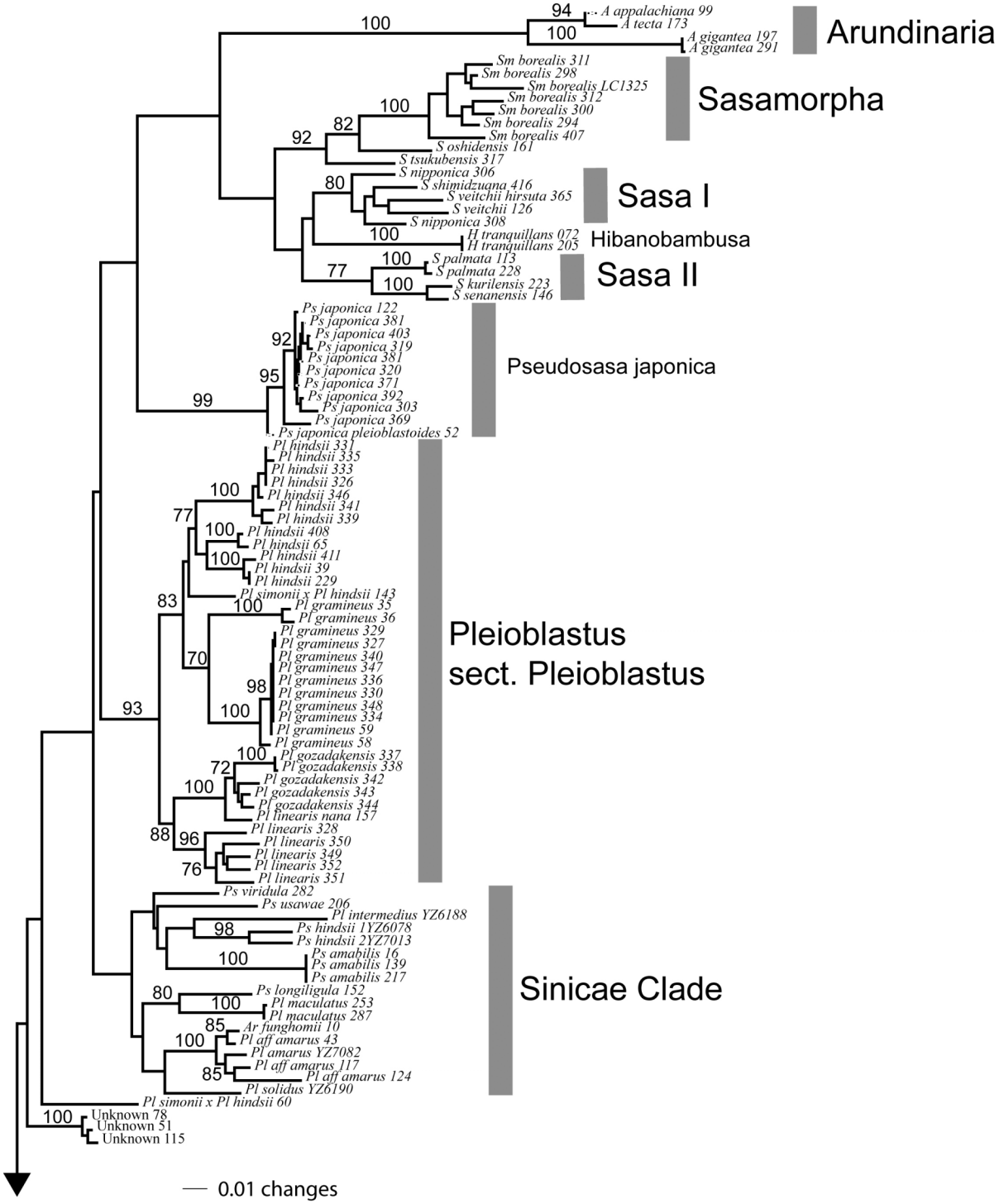

Figure 5. A (continued on 5B). Mid-point rooted phylogram of genetic relationships among 248 accessions of the *Arundinaria* Clade inferred from AFLP data using a neighbor-joining analysis of Nei-Li distances. Numbers above branches indicate bootstrap support values (>70%).

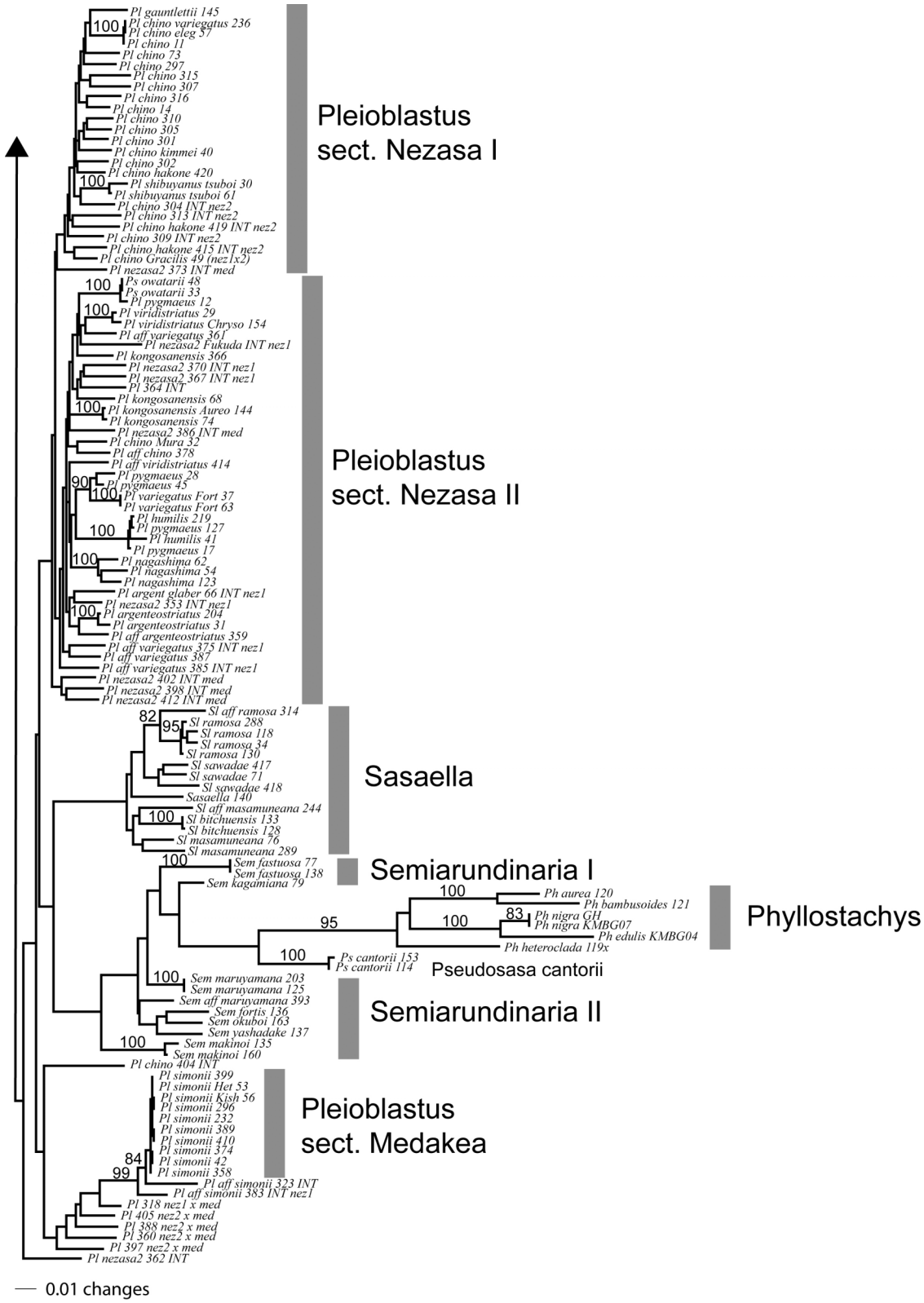

Figure 5. B (continued from 5A)

Varying degrees of resolution are revealed within clusters (Fig. 5). For example, groups within *Pleioblastus* section *Pleioblastus* correspond to defined species (*P. gozadakensis* Nakai, *P. gramineus* (Bean) Nakai, *P. linearis* (Hackel) Nakai, "*P. hindsii*") whereas most clusters in section *Nezasa* have weak support and for the most part do not correspond to species. Branch lengths within the *Sinicae* clade and *Sasa* + *Sasamorpha* cluster suggest relatively high levels of variation in these groups. The widespread *P. simonii*, for which N samples from throughout Japan were screened, was revealed to be genetically homogenous over its entire range. Putative hybrid taxa collected during fieldwork in Japan or obtained from living collections were scattered throughout the cluster diagrams in positions that are largely consistent with the hypothesis of hybridization. In particular, one group of specimens appears to represent hybrids between the *Nezasa* and *Medakea* clades. Other putative hybrids were detected in this analysis, including two apparent *Sasa* x *Sasamorpha* hybrids (*Sasa oshidensis* Makino & Uchida and *Sasa tsukubensis* Nakai; both currently classified in *Sasa* section *Lasioderma* Nakai*)*.

Results of the STRUCTURE analysis of *Pleioblastus* sections *Nezasa* and *Medakea* were used to supplement taxon labels (Fig. 5). The analysis revealed five clusters within *Pleioblastus* sections *Nezasa* + *Medakea*, corresponding to *Nezasa* I, *Nezasa* II, *Medakea* (*P. simonii*), and an unidentified source (15-19%), restricted to unknowns 51, 78, 115.

Putative parental taxa— Table 2 summarizes putative parent taxa suggested by minimum pairwise Nei-Li distances between hybrids and the two closest non-hybrid taxa from divergent groups, and Fig. 6 presents pie charts of the relative contributions from each parent for a representative group of putative hybrids. This survey produced a number of striking results. For example, the suggested parents of *Sasaella sawadae* (Makino) Makino ex Koidzumi (*n.v.* Hakone-medake) are *Sasa shimidzuana* Makino (*n.v.* Hakone-nambu-suzu) and *Pleioblastus chino* var. *vaginatus* (Hackel) S. Suzuki (*n.v.* Hakone-dake); all three species are indigenous to the Hakone region of central Honshu. Similarly, *S. ramosa* was found to be the hybrid between *Sasa nipponica* (Makino) Makino & Shibata and *Pleioblastus chino*, all collected in central Izu Peninsula of Japan. The inferred parents of *Hibanobambusa tranquillans* are *Sasa veitchii* (Carrière) Rehder and *Phyllostachys bambusoides* Siebold & Zuccarini [not *P. nigra (*Loddiges ex Lindley) Munro, as suggested in the literature).

Table 2. Putative hybrids and possible parent taxa suggested by minimum pairwise Nei-Li distances. P1, P2 = putative parents; P1 dist = Nei-Li distance between hybrid and P1. P2 dist = Nei-Li distance between hybrid and P2. P1-P2 = Nei-Li distance between putative parents.

Putative Hybrid	**P1**	**P1 dist**	**P2**	**P2 dist**	**P1-P2**
***Sinicae* Clade Allies**					
Pl amarus 7082	*Ps usawae 206*	0.0860	*Ph heteroclada 119*	0.2042	0.2390
Ps cantorii 114	*Ps viridula 282*	0.0745	*Ph aurea 120*	0.1150	0.2419
Hibanobambusa					
H tranquillans 205	*Sasa veitchii 126*	0.0929	*Ph bambusoides 121*	0.0974	0.2729
***Pleioblastus* hybrids**					
Pl 360 nez2 x med	*Pl chino Gracilis 49*	0.0620	*Pl simonii 232*	0.0486	0.0874
Pl 362 nezasa2 INT	*Pl pygmaeus 28*	0.0578	*Pl simonii 299*	0.0641	0.1031
Pl chino 404 INT	*Pl aff variegatus 387*	0.0687	*Pl simonii 299*	0.0700	0.0967
Pl 60 simonii x gramineus	*Pl simonii 379*	0.0724	*Pl gramineus 59*	0.0818	0.1013
Unknown 51	*Pl kongosanensis 366*	0.0672	*Pl simonii 232*	0.0706	0.1010
Pseudosasa japonica					
Ps japonica 403	*Pl hindsii 39*	0.1127	*Sm borealis 407*	0.0876	0.2805
Sasa					
Sasa tsukubensis 317	*Sasa shimidzuana 416*	0.0714	*Sm borealis 298*	0.0923	0.1931
Sasa oshidensis 161	*S veitchii hirsuta 365*	0.0955	*Sm borealis 311*	0.0801	0.1976
Sasaella					
Sl bitchuensis 128	*Sasa shimidzuana 416*	0.0996	*Pl aff variegatus 353*	0.0875	0.2194
Sl masamuneana 244	*Sasa shimidzuana 416*	0.0961	*Pl argenteostriatus 204*	0.0920	0.2166
Sl masamuneana 289	*Sasa nipponica 308*	0.0936	*Pl aff variegatus 353*	0.0894	0.2194
Sl ramosa 118	*Sasa shimidzuana 416*	0.0869	*Pl chino 309*	0.0905	0.2251
Sl ramosa 288	*Sasa shimidzuana 416*	0.0825	*Pl chino 307*	0.0837	0.2227
Sl ramosa 314	*Sasa nipponica 308*	0.0723	*Pl chino 307*	0.0928	0.2088
Sl sawadai 71	*Sasa nipponica 308*	0.0671	*Pl shibuyanus tsuboi 61*	0.0868	0.2117
Sl sawadai 417	*Sasa shimidzuana 416*	0.0705	*Pl chino 415*	0.0879	0.2117
Semiarundinaria					
Se fastuosa 138	*Pl simonii 399*	0.0772	*Ph bambusoides 121*	0.0969	0.3261
Se fortis 136	*Pl aff variegatus 387*	0.0899	*Ph bambusoides 121*	0.0904	0.3314
Se kagamiana 79	*Pl chino 305*	0.0986	*Ph bambusoides 121*	0.0804	0.3425
Se makinoi 160	*Pl chino 313*	0.0756	*Ph aurea 120*	0.1048	0.2633
Se maruyamana 203	*Pl chino 301*	0.0930	*Ph bambusoides 121*	0.0828	0.3130
Se okuboi 163	*Pl kongosanensis 74*	0.0929	*Ph bambusoides 121*	0.1024	0.3166
Se yashadake 137	*Pl kongosanensis 74*	0.0999	*Ph bambusoides 121*	0.0928	0.3166

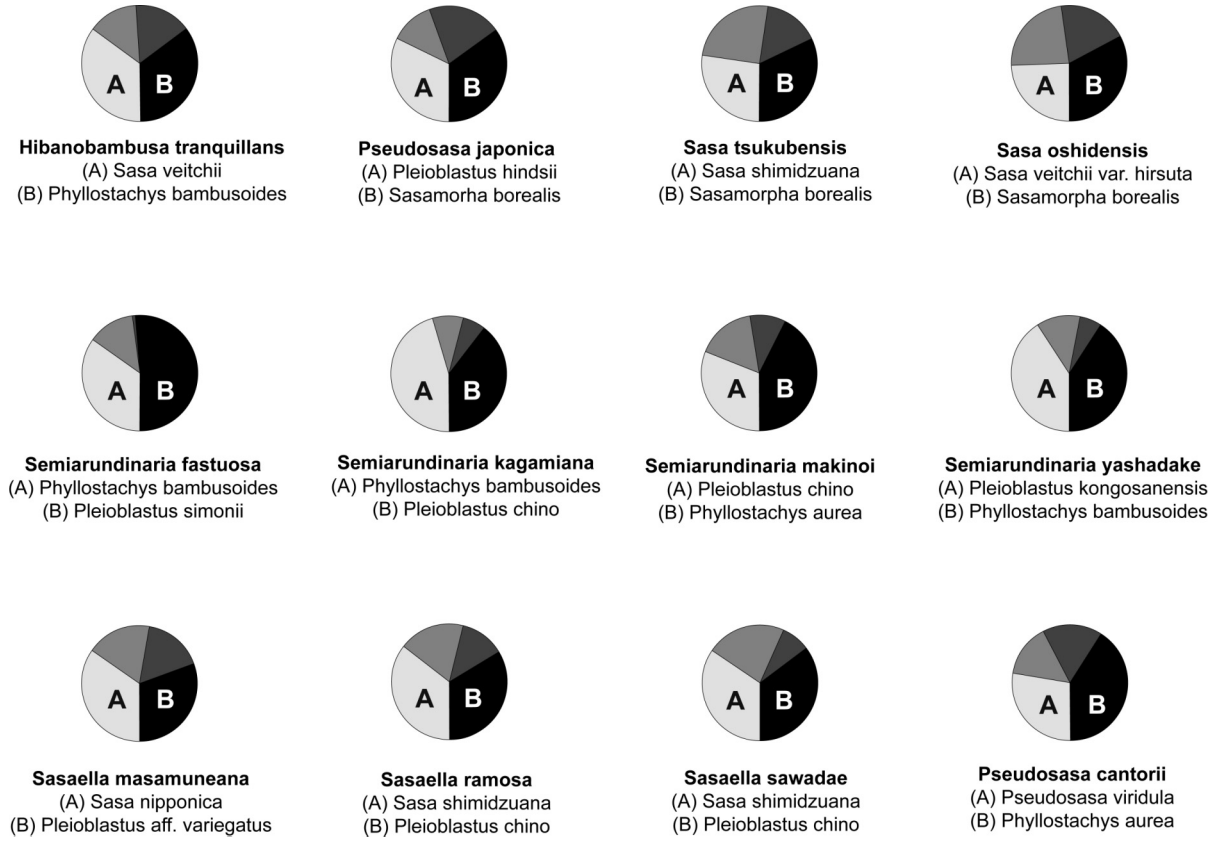

Figure 6. Contribution of amplified fragment length polymorphism (AFLP) fragments to putative hybrid taxa. Putative parents are indicated under the name of the hybrid. LETTER CODES – **A**, **B**: proportion of bands diagnostic of parent A or B, respectively. SHADING – **White**: parent A; **Black**: parent B; **Light Grey** (upper left): bands present in both parents and the hybrid; **Dark Grey:** bands unique to the hybrid.

Semiarundinaria is revealed to be of multiple origins, rather than a single hybridization event followed by diversification. For example, *Semiarundinaria fastuosa* (Marliac ex Mitford) Makino ex Nakai is most similar to *Pleioblastus simonii* (Carrière) Nakai and *Phyllostachys bambusoides*, while *Semiarundinaria yashadake* is most similar to *Pleioblastus kongosanensis* Makino and *Phyllostachys bambusoides. Phyllostachys bambusoides* was the putative pollen parent of all species of *Semiarundinaria* except *S. makinoi* Hisauti & Muroi, for which *P. aurea* was indicated.

Based on its position in the phylogram and the pattern of shared bands, *Sasa tsukubensis* was determined to be a likely hybrid between *Sasa shimidzuana* Makino and *Sasamorpha borealis*. Several Chinese species of *Pleioblastus* and *Pseudosasa* appear to have hybrid origins. Surprisingly, the five accessions that nest in the *Phyllostachys* clade (*i.e.*, having cp DNA haplotypes of *Phyllostachys*) were not similar to *Phyllostachys* based on AFLP bands. In contrast, *Pseudosasa cantorii* was revealed to have a relatively large contribution of bands from *Phyllostachys*.

A neighbor-net diagram was produced for *Pseudosasa japonica* and putative relatives *Sasa*, *Sasamorpha*, and *Pleioblastus* section *Pleioblastus* (Fig. 7). Robust branch support was found for each genus except *Pseudosasa*. The diagram reveals the expected signature of reticulate relationships, with more or less equally weighted but contradictory splits for the relationship between *Pseudosasa, Sasamorpha*, and *Pleioblastus* section *Pleioblastus*. Neighbor-net diagrams for other putative hybrids produced similar patterns (not shown) consistent with the hypothesis of hybridization.

Based on the results of the prior analyses, we selected exemplars of each major group within the *Arundinaria* Clade (*Arundinaria, Sasa*, *Sasamorpha*, the *Sinicae* Clade, and *Pleioblastus sensu stricto*) for a targeted analysis excluding those taxa identified to be of hybrid origin (*Hibanobambusa, Pseudosasa, Sasaella, Semiarundinaria, Pseudosasa cantorii, Sasa oshidensis, Sasa tsukubensis,* etc). For *Arundinaria*, *Sasa*, and the *Sinicae* Clade, we included one accession per species. For *Pleioblastus s.s.* and *Sasamorpha*, we included more than one accession per species as warranted by the availability of the following: (1) geographically divergent populations (e.g., *P. simonii* from Kyushu, Shikoku, and Honshu; "*P. hindsii*" from Iriomote, Ishigaki, Kyushu, and cultivated in the US) or (2) polyphyletic species (*e.g.*, *P. pygmaeus* (Miquel) Nakai]. One unidentified species of *Pleioblastus* (Unknown 51, Unknown 78) was included because results of previous analysis were unclear as to whether this was a hybrid or a distinct lineage within *Pleioblastus*.

The core dataset consisted of 54 taxa and was analyzed using MP, NJ, and BI. Results are summarized in Fig. 8. The reduced taxon set produced relatively robust topologies that are largely congruent with the cp DNA phylogeny (ignoring hybrids). Four major subclades were resolved: *Arundinaria*, *Sasa*, *Sasamorpha*, and *Pleioblastus* + the *Sinicae* Clade. All

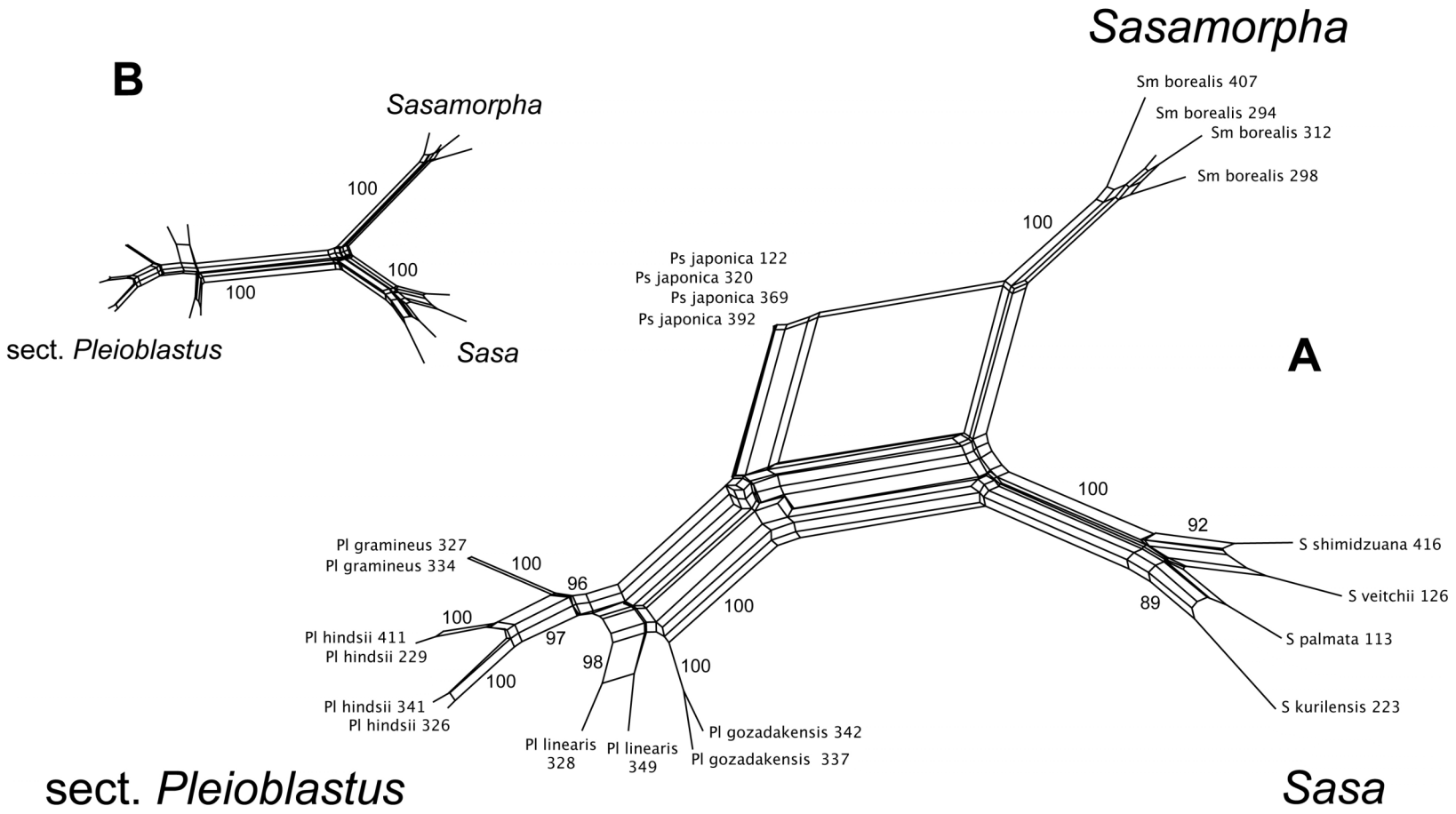

Figure 7. A. Neighbor-net of AFLP data based on Nei-Li distances, highlighting the positions of *Pseudosasa japonica* accessions relative to species of *Pleioblastus* section *Pleioblastus, Sasa*, and *Sasamorpha*. B. Neighbor-net of AFLP data for the same dataset, excluding *Pseudosasa japonica*. In both diagrams, numbers at splits are bootstrap values (>70%) from a neighbor-joining analysis.

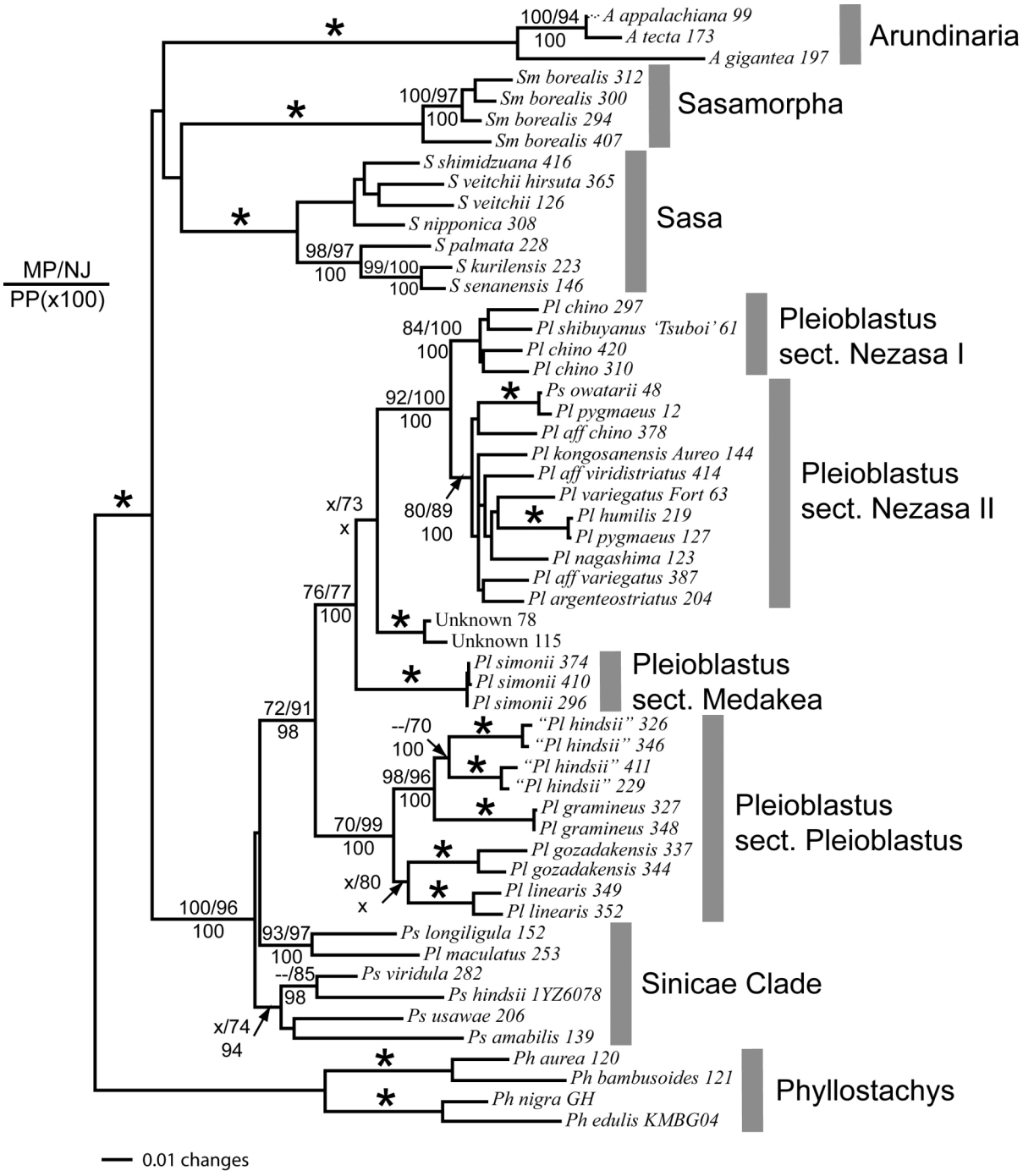

Figure 8. Neighbor-joining phylogram from the analysis of the core *Arundinaria* Clade AFLP data subset (excluding putative hybrid taxa), rooted with *Phyllostachys*. Numbers above lines indicate bootstrap values (MP/ML). Numbers below the line indicate posterior probabilities from the Bayesian analysis.

four groups are strongly supported as monophyletic (MPBS = 100, PP = 1.00). However, consistent with the cp DNA phylogeny, branching order is unresolved among these groups. Analyses reveal a monophyletic *Pleioblastus s.s*. (consistent with the chloroplast phylogeny) and a polyphyletic *Sinicae* Clade (in contrast to the chloroplast phylogeny). Within *Pleioblastus s.s.,* the three sections received moderate to robust support. Section *Nezasa* was strongly supported in each analysis (MP = 92, NJ = 100, PP = 1.0), and was indicated to have two subclades (*Nezasa* I and II), although internal resolution mostly lacked branch support. The unknown species (51 and 78) was resolved in different locations depending on method of analysis: BI placed this species sister to *P. simonii* (PP= 1.0) while NJ placed it sister to *Nezasa* (NJBS = 73) and MP created a polytomy with *Nezasa* and *Medakea*. Section *Pleioblastus* included a robust clade for *Pl. gramineus* plus "*P. hindsii,*" but relationships between this clade, *P. linearis,* and *P. gozadakensis* were incongruent among the three analyses due to conflicting placements of *P. gozadakensis*.

Pleioblastus sensu stricto

The NJ analysis of *Pleioblastus sensu stricto* is summarized in Fig. 9. For this analysis, seven accessions from the *Sinicae* Clade were included as outgroups, and the total number of ingroup taxa was minimized by retaining only a single accession for taxa with Nei-Li distances = 0. Two rounds of analyses were conducted: (1) including all ingroup taxa, and (2) excluding those taxa determined in STRUCTURE 2.2 to have genetic characteristics from multiple sources. Two major clusters were recovered in the first iteration: one consisting of sect. *Pleioblastus* (98%) and the other of *Medakea* + *Nezasa* (86%). The topology for sect. *Pleioblastus* was essentially identical to results for the large dataset (described above). Within *Nezasa* + *Medakea,* robust support was limited to duplicate accessions of species in cultivation [*e.g., P. viridistriatus (*Regel) Makino: 100%; *P. nagashima* (Mitford) Nakai: 100%], a cluster uniting accessions of *P. pygmaeus* and *P. humilis* (Mitford) Nakai (100%), and *P. simonii* + allied taxa (99%). Putatively admixed taxa nested intermediate between *Nezasa* and *Medakea* or were scattered within *Nezasa* I and II. Excluding these taxa resulted in increased support for *Medakea* + *Nezasa* (93%), *Nezasa*

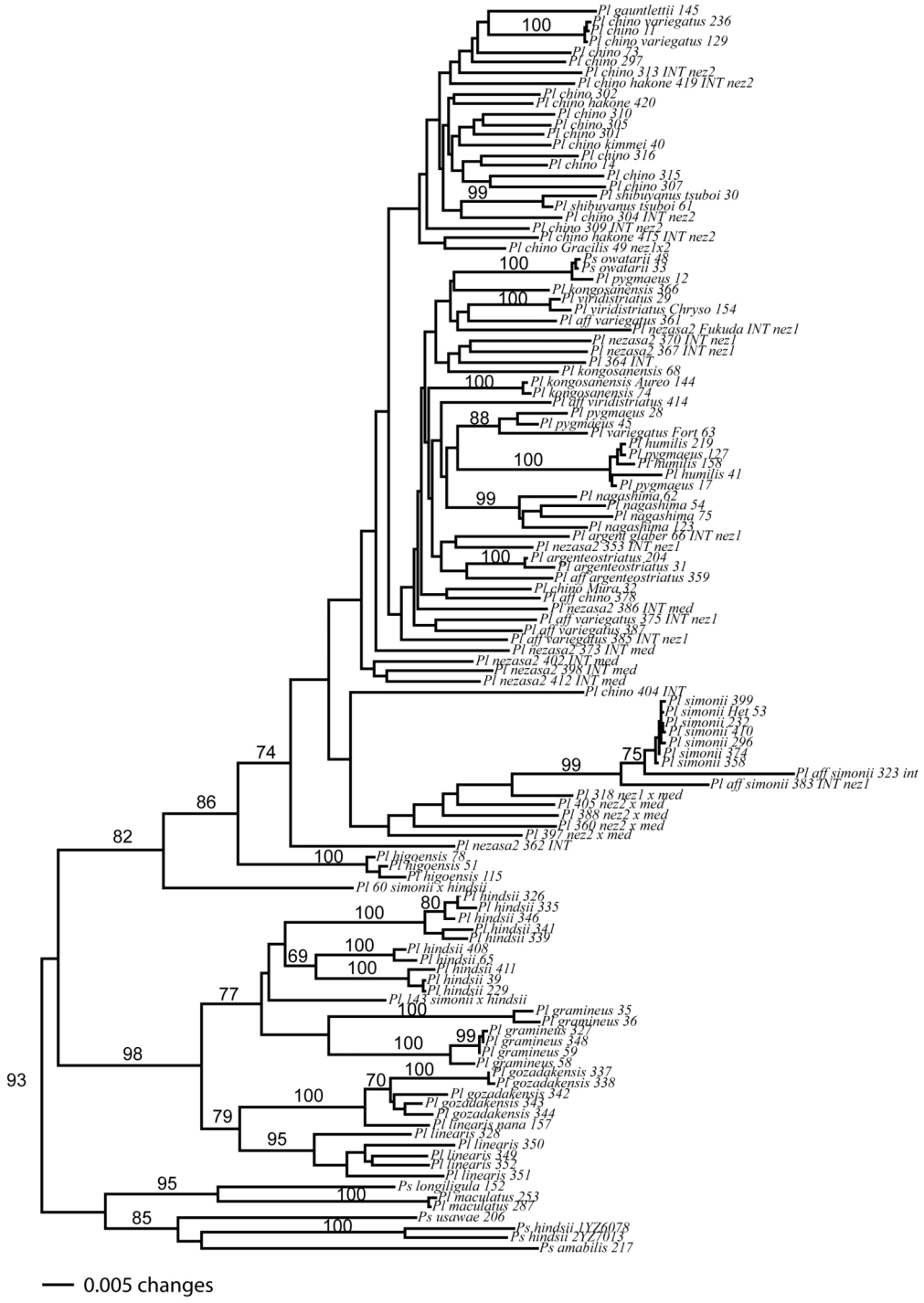

Figure 9. Neighbor-joining phylogram from the analysis of the *Pleioblastus sensu stricto* AFLP data subset, including all putative hybrids originating from within this group, and rooted with representatives from the *Sinicae* Clade. Numbers above lines indicate bootstrap values (>65%).

(100%), *Nezasa* I (76%), *Nezasa* II (100%), *Pleioblastus simonii* (100%), but did not significantly improve branch support within these clusters (not shown).

The neighbor-net analysis of *Nezasa* + *Medakea* accessions (including all admixed accessions except two putative *P. simonii* × *P. hindsii* hybrids) is presented in Fig. 10, and highlights the high level of signal conflict within this group. Relationships within *Nezasa* in particular are noisy. Accessions in this group form a complex network pattern consistent with ongoing gene flow, incomplete lineage sorting, hybridization, or some combination of the above.

DISCUSSION

Utility of AFLPs in the Temperate Clade.

Our results demonstrate that AFLPs are a valuable tool for exploring relationships in the temperate bamboos, providing sufficient variation to reconstruct lower-level relationships while also revealing underlying reticulation. This group of species provides a valuable test of the phylogenetic application of AFLP data. The appropriateness of AFLPs for phylogeny reconstruction is correlated with taxonomic distance (Álvarez and Wendel 2006; Koopman 2005), and is generally presumed to be appropriate at the generic level or below. This study represents a test of AFLP data across a phylogenetically diverse group, among different genera and potentially different subtribes. Given the low levels of genetic variation detected by DNA sequences in the temperate bamboos, this method seemed appropriate at a relatively higher level than for plant groups exhibiting greater molecular divergence. Imposing a limit on the applicability of AFLPs based on taxonomic rank seems less important than limits based on genetic variation in the group under study. Relationships among genera in the *Arundinaria* Clade appear to be sufficiently conservative to permit phylogenetic analysis with AFLPs. Results indicate that AFLPs work remarkably well, and reveal phylogenetic signal that can be reconciled with cp DNA data (*i.e.*, allowing for incongruence due to hybridization to be resolved). Subsequent to parsing the genetic overlap due to hybridization, the scale at which AFLP data are most powerful and accurate can be better estimated. Clearly AFLP bands are valuable at the species level in this group, however their use at the generic

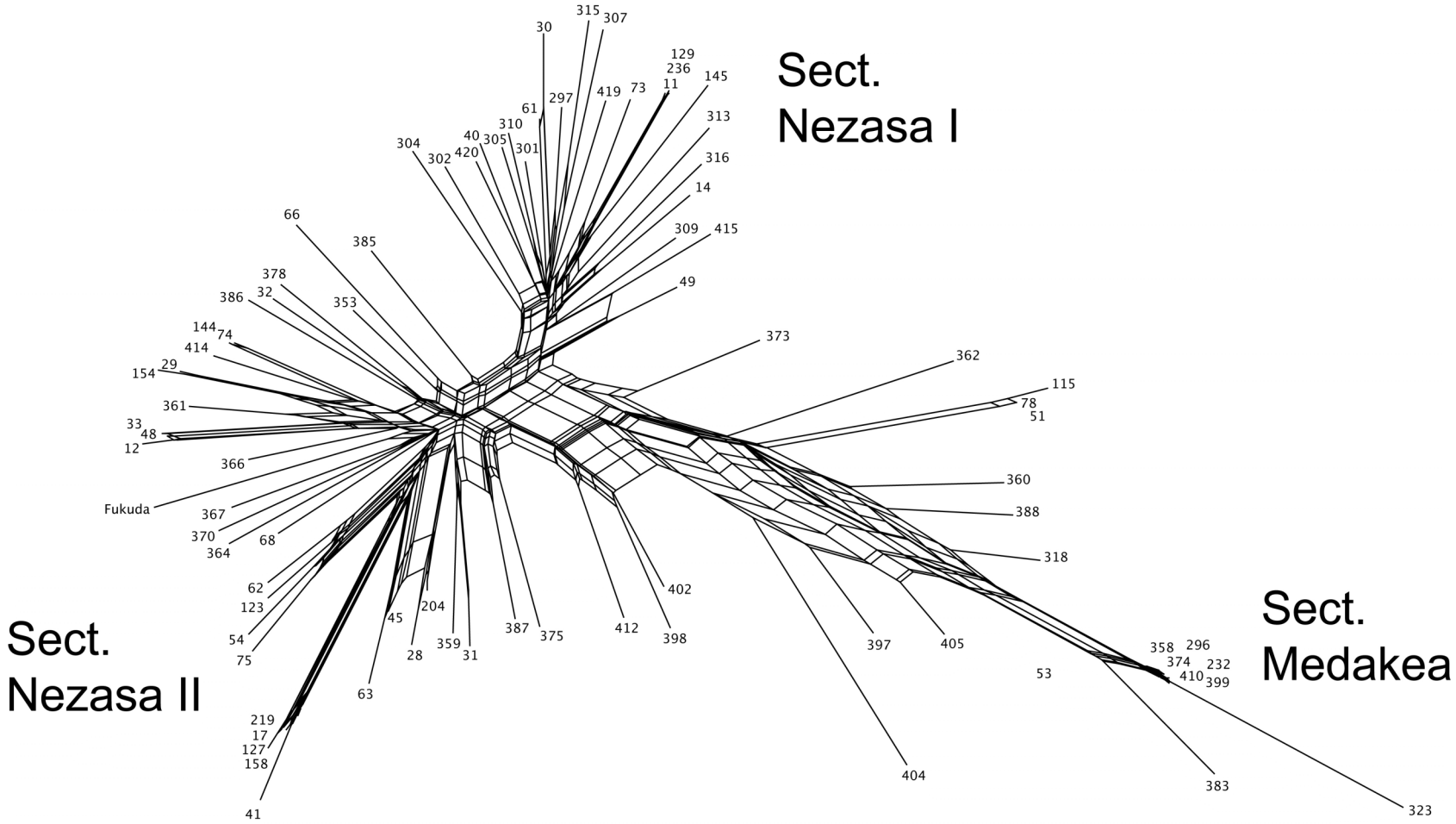

Figure 10. Neighbor-net of the AFLP data subset restricted to *Pleioblastus* sections *Medakea* and *Nezasa*, indicating clusters for *Nezasa* I, *Nezasa* II, and *Medakea*. All accessions are identified by voucher numbers (see Fig. 9 for IDs).

level is less clear, and results must be interpreted with caution. For example, *Arundinaria sensu stricto* is highly divergent from relatives in Japan, and as a result the AFLP analysis is susceptible to long branch attraction.

Hybridization in the *Arundinaria* Clade.

The pattern of relationships revealed by complementary AFLP and cp DNA data strongly support the hypothesis of widespread hybridization and reticulation among temperate bamboos, in spite of temporal barriers imposed by long flowering cycles. AFLP data provide an independent test of phylogenetic relationships in this group and make it possible to explain unexpected taxon placements in the chloroplast phylogeny. Conflicting positions and reduced levels of support in AFLP dendrograms were symptomatic of hybridization, and hybrid band patterns were strongly suggestive of genomic mergers between divergent taxa.

Many of the hybrids detected in this study were predicted by previous researchers based on morphological intergradation and combinations of characters from divergent taxa. Presumably, if hybridization has occurred at higher taxonomic levels, it is also occurring at lower levels but may be more difficult to detect based on morphology. In the current AFLP analysis, a number of taxa were revealed to have intermediate positions between robust clades within genera. These results support the occurrence of hybrids between closely related taxa, for example *Pleioblastus* sections *Nezasa* and *Medakea*. Moreover, the morphology of these taxa is consistent with hybridization, albeit less obviously so.

The genera identified as hybrids in this study have been problematic in bamboo systematics. Our results suggest that a portion of the taxonomic difficulties in the temperate clade is likely correlated with the high level of reticulation, resulting in intermediate and intergrading morphologies that have troubled morphological classifications. Moreover, the current results suggest that it should be possible to disentangle the complicated morphological patterns in this group that are ultimately due to hybridization. A discussion of the major hybrid taxa follows.

***Hibanobambusa tranquillans*—** This species was discovered in 1932 on Mt. Hiba in Shimane Prefecture, S. Honshu (Ohrnberger 1999). It was originally described in the genus

Semiarundinaria by Koidzumi (1941) and transferred to *Sinoarundinaria* and *Phyllostachys* before being recognized as a new genus (Maruyama *et al.* 1971). Based on its intermediate features, Maruyama *et al.* (1971) also speculated on a hybrid origin, and proposed an alternate, nothogeneric name *(×Hibanobambusa)*. Maruyama and Okamura (1979) postulated a natural hybridization between *Sasa veitchii* (Carrière) Rehder var. *hirsuta* and a species of *Semiarundinaria* Makino. Most recently, Okamura and Tanaka (1986) hypothesized the parents to be *S. veitchii* f. *tyuhgokensis* (Makino) Muroi and *Phyllostachys nigra* f. *henonis (*Mitford) Muroi. Demoly (1995) established the name ×*Phyllosasa* Demoly to reflect this most recent hypothesis. Other botanists speculated that the intermediate features of this plant could indicate it to be a relictual lineage, with transitional features between the subtribes Shibataeinae and Arundinariinae (Campbell, unpubl.).

Based on AFLP genotype data, *Hibanobambusa tranquillans* appears to be a hybrid between *Sasa veitchii* (*n.v. kuma-zasa*) and *Phyllostachys bambusoides* (*n.v. madake*), the Japanese timber bamboo. *Madake* is the most widely cultivated bamboo in Japan, covering vast acres in commercial bamboo groves. *Phyllostachys bambusoides* was introduced very early into Japan from China (Ohrnberger 1999). What is particularly surprising is that *madake* has one of the longest known flowering cycles (120 years; Janzen 1976). Prior to the discovery of *H. tranquillans*, *madake* flowered gregariously in the 1840s, which presents a possible time of origin, however it is possible that the plant had persisted from an earlier hybridization event, or that a more recent, out-of-cycle flowering event generated the cross with *Sasa*.

Hibanobambusa exhibits a number of intermediate features between *Phyllostachys* and *Sasa*: the bracteate inflorescences are comprised of pseudospikelets, like *Phyllostachys*; the flowers usually have six stamens (rarely 3 or 5), like *Sasa*; culm internodes are mostly terete like *Sasa*, but flattened proximal to branches, suggesting *Phyllostachys*; culm leaves are deciduous, as in *Phyllostachys*, while the thick, solitary branches and large leaves are characteristic of *Sasa*. Several other features suggest hybridization; for example, glumes and lemmas are much larger than in either *Sasa* or *Phyllostachys (*heterosis?*),* and the plants produce few viable seed (Ohrnberger 1999).

***Semiarundinaria*—** Plants known as *Semiarundinaria sensu stricto* are restricted to Honshu, Shikoku, and Kyushu, Japan, where they are widely cultivated and locally naturalized, but their original localities are unknown. Approximately seven species are recognized in Japan (including *S. fastuosa, S. fortis* Koidzumi, *S. kagamiana* Makino, *S. makinoi, S. maruyamana* Muroi, *S. okuboi* Makino, *S. yashadake*). *Semiarundinaria fastuosa* is the most widespread member of this group, and is cultivated worldwide for its graceful features. All members of this genus are morphologically intermediate between *Phyllostachys* and *Pleioblastus*, having inflorescences with leafy bracts and occasional pseudospikelet branching, culm leaves that are tardily deciduous (in contrast to persistent in *Pleioblastus* and early-decidous in *Phyllostachys*). Culm internodes are flattened or grooved above branches, weakly suggesting the internodes of *Phyllostachys*. In contrast to the diagnostic pair of mid-culm branches produced by *Phyllostachys*, *Semiarundinaria* produces 3-9 (13), resembling *Pleioblastus*. *Semiarundinaria* is apparently incapable of producing fruits.

Japanese botanists speculated on the origin of this genus, concluding that it was a hybrid between *Phyllostachys* and *Pleioblastus*. Moreover, Muramatsu (1981) demonstrated that plants resembling *Semiarundinaria* can be produced from crosses between *Phyllostachys bambusoides* and *Pleioblastus simonii*. As with *Hibanobambusa*, the alternative hypothesis suggests that this species is a relictual group, and the intermediate features represent evolutionary transitions between major clades.

AFLP evidence clearly supports the hybridization hypothesis. In each case it appears that *Phyllostachys* was the pollen donor and *Pleioblastus* was the maternal plant, as demonstrated by the cp DNA phylogeny (Fig. 1). Furthermore, the recognized species appear to have at least three different combinations of parent species. *Semiarundinaria kagamiana, S. maruyamana, S. fortis, S. okuboi,* and *S. yashadake* are apparently hybrids between *Phyllostachys bambusoides* and a member of the Nezasa clade, while *S. fastuosa* is a hybrid between *Phyllostachys bambusoides* and *Pleioblastus simonii* (supporting the hypothesis of Muramatsu). *Pleioblastus kongosanensis* (Nezasa II) was indicated as a likely maternal parent of *S. maruyamana*, *S. okuboi*, and *S. yashadake*, while *P. chino* (Nezasa I) was indicated to be the maternal plant of *S. kagamiana* and *S. maruyamana*. The only species that

appears to have *Phyllostachys aurea* Carrière ex A. and C. Rivière as a parent is *Semiarundinaria makinoi*.

Three plants in China have been assigned to *Semiarundinaria*: *S. sinica* Wen, *S. densiflora* (Rendle) T.H. Wen, and *S. shapoensis* McClure. With the recognition of the hybrid status of the Japanese plants, the species in China will require further study to determine whether they are nothotypes or perhaps belong in a distinct genus. One species [*S. densiflora*, as *Brachystachyum densiflorum* (Rendle) Keng] was examined in the cp DNA phylogeny (Triplett and Clark, Ch. 2), and was determined to belong to the *Phyllostachys* Clade. Thus, a likely hypothesis is that *S. densiflora* represents hybridization between *Phyllostachys* and a pleioblastoid species in Mainland China, but with *Phyllostachys* as the maternal plant. *Semiarundinaria sinica* of China, like the species in Japan, is only known in cultivation. The material of *S. shapoensis* is imperfectly known (Wu *et al.* 2006). Other Chinese species that were formerly placed in *Semiarundinaria* have been transferred to *Oligostachyum*, *Pseudosasa*, and *Pleioblastus*, but should receive additional scrutiny in light of current results.

***Sasaella*—** The genus *Sasaella* comprises approximately 13 species (Suzuki 1978), although more than 100 have been described based on minor differences since the genus was first recognized by Makino (1929). *Sasaella* is primarily found in Honshu, though not as far North as *Sasa*, and extending south and west to Shikoku, Kyushu, and Yakushima Island. Fewer than half of the species are known in flower. The genus is morphologically intermediate between *Pleioblastus* and *Sasa* in stamen number [(2)3-5 (6)], branches per node (sometimes 2-3, unlike the single branch in *Sasa*), foliage leaf blade size, lemma size, and rhizome growth (Ohrnberger 1999; Suzuki 1978). *Sasaella* is further characterized by fimbriae that are scabrous only at the base, versus fully scabrous in *Sasa* and glabrous in *Pleioblastus*. Thus plants in this group have been particularly challenging for botanists, with disagreement as to whether they belong in *Sasa*, *Pleioblastus* (*Arundinaria*), or a distinct genus altogether. More recently, Japanese botanists established the alternate hypothesis of hybridization when a *Sasaella*-like plant was produced from an artificial cross between *Pleioblastus* and *Sasa* (Suzuki 1987; Okamura *et al.* 1991; Ohrnberger 1999). Recent molecular analyses support the hybridization hypothesis. Hosoyama *et al.* (2002) examined

the putative hybrid origins of *S. hannouensis* (Makino) Makino and *S. sawadae* [both synonyms of *S. ramosa* (Makino) Makino sensu Suzuki] using cp DNA sequences, and determined that the maternal genus of the former was *Sasa* while that for the latter was *Pleioblastus*. Ishii *et al.* (2003) subsequently concluded that the maternal parent of *S. hannouensis* was *Sasa nipponica*, based on cp DNA sequence comparisons among likely parents (*S. veitchii*, S. *nipponica*, and *Pleioblastus chino*).

In our investigation, two cp DNA haplotypes were identified among *Sasaella* accessions. *Sasaella ramosa* and *S. bitchuensis* (Makino) Makino ex Koidzumi have the *Sasa* haplotype, while *S. masamuneana* has that of *Pleioblastus* section *Nezasa*. All fourteen accessions of *Sasaella* clustered together in AFLP analyses, and revealed a mosaic of diagnostic bands from *Sasa* and *Pleioblastus* (Table 2). Moreover, results demonstrate that *Sasaella* species originate in areas of sympatry between parents. As described above, the parents of *Sasaella sawadae* in Hakone were *Sasa shimidzuana* and *Pleioblastus chino* var. *vaginatus* (Hackel) S. Suzuki, both native to the Hakone region, while *S. ramosa* was found to be a likely hybrid between *Sasa nipponica* and *Pleioblastus chino*, all found in Central Honshu. Rather than sharing a common ancestor, results suggest that *Sasaella* species originated independently from reciprocal *Sasa* x *Pleioblastus* crosses, and even a single species of *Sasaella* may in fact represent more than one hybridization event.

***Pseudosasa japonica*—** Perhaps the biggest surprise in this analysis was the apparent hybrid origin of *Pseudosasa japonica*. The genetic background of this species is potentially complex, and current evidence suggests multiple origins, possibly involving up to three genera. AFLP data indicate a complement of diagnostic bands from *Pleioblastus hindsii* (~ 35%) and *Sasamorpha borealis* (~ 32%). Most accessions of *P. japonica* have cp DNA sequences matching the Ryūkyū Clade of *Pleioblastus (*section *Pleioblastus*), but at least one population (JT 369, Shikoku) has the cp DNA of the *Sasa* clade. Accessions of *P. japonica* were genetically homogeneous in the AFLP analysis. Among the accessions of *Sasamorpha borealis*, *P. japonica* was most similar to JT 407, from Kyushu. Additional research is needed to fully characterize the compelling and potentially complex history of this species.

Pseudosasa japonica possesses a number of morphological features that are consistent with its hybrid origin. For example, the lack of secondary branches is a

characteristic of *Sasamorpha* (and *Sasa*), as are the long culm leaf sheaths; the foliage leaves generally lack fimbriae, but when present they are white and smooth like *Pleioblastus* (Suzuki 1978); flowers have 3-5 stamens, which is intermediate between *Pleioblastus* (3) and *Sasamorpha (*6). This species can apparently produce viable fruits (Eppner 2002).

Pseudosasa was first described as a new genus by Makino (1920; Nakai 1925), and is widely recognized as a distinct lineage (McClure 1973; Clayton and Renvoize 1986; Chao 1989; Keng and Wang 1996). Japanese species include *Pseudosasa japonica* and *P. owatarii* (Suzuki 1978). These two are classified in subgenus *Pseudosasa*, which sometimes includes *P. usawae* from Taiwan. Current DNA and morphological evidence suggest that *P. owatarii* belongs in *Pleioblastus* section *Nezasa*, although additional evidence from wild populations on Yakushima is needed to confirm the identity of plants in cultivation. cp DNA evidence places *P. usawae* (Hayata) Makino & Nemoto with the Chinese species of *Pseudosasa* in the *Sinicae* Clade.

Pseudosasa japonica is one of the most widely cultivated bamboos in the world, and a historically important plant in Japan, although its original wild locality is unknown (Suzuki 1978; Ohrnberger 1999). It apparently occurs wild on remote islands in the south of Japan (Ohrnberger 1999), but it is uncertain whether these represent native or naturalized populations. A likely scenario suggests that *Pseudosasa japonica* originated relatively recently in Southern Japan, where *P. hindsii* and other species from the Ryūkyū Islands were cultivated since the early history of Japan. It is likely that clones of *Pseudosasa japonica* were distributed widely in the early history of Japan, as this was one of the most useful plants (Bess 2001). Among many other applications, these plants were used for arrowmaking and a number of other traditional crafts. Along with *Pleioblastus simonii*, this species provides a natural historic landmark, indicating the former locations of villages in rural Japan (Triplett, pers. obs.).

Additional examples of hybrids in the Arundinaria Clade. An example of the impact that a plant of unsuspected hybrid origin can have on bamboo horticulture is provided by an unusual specimen that may represent a cross between *P. simonii* and an unidentified parent in *Pleioblastus* section *Nezasa*, with possible introgression from other sources. Accessions of this plant (unknown 51, 78, 115) were obtained in cultivation under the names *Sasamorpha*

borealis (Japan), *Pleioblastus virens* Makino (USA), and *Pleioblastus kiusianus* Makino (Japan). All three have essentially identical morphological features, which intergrade between *Pleioblastus* and *Sasamorpha*. In particular, the branch complement is consistent with section *Nezasa* (but typically constrained to three subequal branches), the habit and leaf morphology suggest *P. simonii*, and the long inner ligules and weakly developed fimbriae suggest *Pseudosasa s.s*. These three accessions clustered together in AFLP analyses, and cp data indicate they have identical sequences, differing by a single base pair from *P. simonii* and *P. argenteostriatus* (Regel) Nakai. AFLP genotyping indicates that this plant shares bands with both Nezasa II (*e.g.*, *P. kongosanensis*) and *P. simonii*, and possibly represents an introgressed form of *P. simonii*. It is unknown whether these three originate from a single locality or represent independently derived hybrids, but the fact that they share a distinct cp haplotype suggests they are from a single source. No information is available regarding wild localities for this species, however a plant with similar morphological features was collected in Mie Prefecture (JT 362). This accession also demonstrated a mosaic AFLP genotype (implicating *P.* aff. *variegatus* (Siebold ex Miquel) Makino and *P. simonii* as the likely parents), but did not cluster with the unknown species.

Phylogeny of the *Arundinaria* Clade

The pattern of relationships suggested by AFLP fingerprint data considered against the phylogenetic framework revealed by cp DNA sequence data provide substantial new information on the evolution of the *Arundinaria* Clade. Data indicate a core group consisting of *Arundinaria*, *Sasa*, *Sasamorpha*, *Pleioblastus*, and the *Sinicae* clade, and excluding *Hibanobambusa*, *Semiarundinaria*, *Sasaella*, and *Pseudosasa japonica* as intergeneric hybrids. AFLP evidence further demonstrate that *Arundinaria sensu stricto* is monophyletic, although its sister group remains unknown. Branches weakly supporting a relationship with *Sasa* and *Sasamorpha* must remain doubtful, since a number of explanations can be postulated to explain this connection as spurious (*e.g.*, long branch attraction).

Given the range of morphological variation in each of the five major subclades, it is possible to hypothesize any number of evolutionary links without a satisfactory argument favoring any particular hypothesis. Floral morphology suggests links between *Sasa* and

Sasamorpha and between *Arundinaria* and *Pleioblastus*, and numerous vegetative characters reinforce these links. Other features support conflicting hypotheses, for example, *Sasamorpha* and the Switchcane Clade (*A. tecta* and *A. appalachiana*) are united by a number of features such as branch architecture. The current molecular datasets cannot exclude possible links among major lineages within the *Arundinaria* Clade. As such, it is impossible to evaluate morphological hypotheses, such as 6 stamens being the plesiomorphic condition for the *Arundinaria* Clade.

Pleioblastus sensu stricto has emerged as a monophyletic group, with significant support in both cp and AFLP datasets (Fig. 1, Fig. 9). A number of interesting problems remain, however. The genus appears to consist of three subclades, corresponding to the sections defined by Suzuki. Section *Nezasa* is relatively diverse, and yet AFLP data do not support subclades, instead suggesting ongoing gene flow. Perhaps insufficient characters were provided by the current AFLP datasets, or perhaps there are in fact fewer species than currently defined. Analyses searching for structure within the data suggest two major groups, and gene flow occurring between these. Clearly additional work is needed to characterize this group, which may represent as few as two species or as many as nine (or more). Plants representing section *Medakea* were collected throughout much of Japan, and virtually every plant had a nearly identical AFLP genotype (*P. simonii*, Fig. 5). As such, this plant is similar to *A. gigantea* (Walt.) Muhl. in the US, which was also found to have low genetic diversity throughout its broad distribution (Triplett *et al.*, in prep; Chapter 3). In contrast, a significant amount of variation was revealed in section *Pleioblastus*, which encompasses at least four species, and may reveal additional morphological forms. Additional characteristics of *Pleioblastus s.s*. and each major subclade within the *Arundinaria* Clade are discussed below.

Arundinaria sensu stricto— *Arundinaria* is native to the warm-temperate eastern and southeastern United States, from New Jersey south to Florida and west to Texas, extending inland through the Piedmont to moderate elevations in the Appalachian Mountains (Judziewicz *et al.* 1999; McClure 1973). In the past, these bamboos formed extensive canebrakes over vast areas of fertile river bottomland, but are now greatly reduced from their historical range by grazing and fire suppression (Platt and Brantley 1997; Hughes 1966). *Arundinaria gigantea* (river cane) occurs in low woods and along riverbanks from the

lowlands east of the Appalachians, west to Missouri, up the Mississippi Valley to southern Illinois and up the Ohio River to southern Ohio. *Arundinaria tecta* (switch cane) is found primarily along non-alluvial swamps, moist pine barrens, live oak woods and along sandy margins of streams, preferring moister sites than *A. gigantea* and restricted to the Coastal Plain of the southeast from southern Maryland to Alabama and Mississippi. *Arundinaria appalachiana* (hill cane) occurs on slopes and in upland woodlands in the Southern Appalachians, away from streams and rivers (Triplett *et al.* 2006; Ch. 4).

Sasa, *Sasamorpha*, and *Pleioblastus sensu lato* are allies of the North American *Arundinaria*, however, previously reported cp DNA evidence and current AFLP data are unable to identify the closest relatives among these taxa. Nevertheless, current AFLP data and previous cp DNA evidence (Triplett and Clark, in prep; Ch. 2) support the hypothesis that *Arundinaria sensu stricto* is monophyletic. Moreover, *Arundinaria* is morphologically and genetically diverse relative to many other bamboo genera (Triplett and Clark, in prep; Ch. 3). Differences in the chloroplast genomes of *A. gigantea* and the Switchcane Clade suggest divergence occurred very early in the history of these two lineages, while *A. tecta* and *A. appalachiana* have diverged more recently. Most likely, the common ancestor of *Arundinaria sensu stricto* migrated across the Bering Strait land bridge from Asia as recently as 5.4 million years ago (Gladenkov *et al.* 2002).

***Sasa*—** The genus *Sasa sensu stricto* is distributed in Japan, Sakhalin, the Kurile Islands, and Korea. *Sasa* represents a morphologically cohesive taxon, although a number of species intergrade with *Sasamorpha*, and the latter is sometimes subsumed within *Sasa* [section *Sasamorpha* (Nakai) Muroi]. The combination of large foliage leaf blades and solitary branches on prominently thickened nodes makes this one of the easiest genera to identify. The genus is further distinguished by its six-stamened flowers, which may reflect the ancestral state for the group. Multitudes of species have been described on very minor differences (with upwards of 400 epithets), including culm and foliage leaf pubescence, culm leaf sheath length, and foliage leaf blade size, thickness, and surface luster. Suzuki (1978) reduced *Sasa* to 35 species, which he further classified in five sections based mostly on branching patterns, from those with little or no branching (sect. *Crassinodi* Nakai), to those branching mainly at lower nodes (sect. *Sasa*), versus those branching mainly at the upper

culm nodes (sects. *Monilicladae* Nakai, *Macrochlamys* Nakai, and *Lasioderma*) (Suzuki 1978). Several species in China are morphologically suggestive of *Sasa sensu stricto*, and eight of these have been placed in *Sasa*; however, molecular data suggest these are unrelated to *Sasa sensu stricto (*Triplett and Clark, Ch. 2). The survey included in the current study is cursory, and primarily designed test the position of *Sasa* relative to other taxa in the *Arundinaria* Clade, but includes some of the major diversity and distributions. An extensive phylogenetic investigation of *Sasa* is needed.

In this study, two robust subclades were found within *Sasa*. Clade I includes *S. veitchii (*sect. *Sasa*), *S. shimidzuana (*sect. *Lasioderma*), and *S. nipponica* (sect. *Crassinodi*); Clade II includes *S. palmata (*Mitford) Camus (sect. *Sasa*), *S. senanensis* (Franchet and Savatier) Rehder (sect. *Sasa*), and *S. kurilensis (*sect. *Macrochlamys*). These associations demonstrate that the sections are not monophyletic. This observation is consistent with a study of *Sasa* using cp DNA sequences of the *rbcL-ORF106* region (Sasaki *et al.* 2007), which found little correspondence between sequence types and taxonomic classification. Additionally, two species of *Sasa (S. oshidensis* and *S. tsukubensis; Sasa.* sect. *Lasioderma*) were associated with the *Sasamorpha* clade in the AFLP analysis, having diagnostic bands from both genera. And as previously noted, *Sm. borealis* from Shikoku was found to have a *Sasa* haplotype, indicating a possible history of introgression between *Sasa* and *Sasamorpha*.

Sasamorpha— *Sasamorpha* is native to the northern Japanese island of Hokkaido, and extends as far south as Southern Kyushu. The plants are important understory species in forests, and provide an important resource for herbivores. Three putative species of *Sasamorpha* are endemic to China, while populations of *Sasamorpha borealis* apparently occur in Korea and E. Russia as well as Japan. A full evaluation of these entities is needed before diversity can be accurately characterized, however, it is clear that *Sasamorpha borealis* deserves special attention in phylogenetic studies by virtue of its distinctive chloroplast haplotype and apparent hybrid links. The current study provides a minimal survey of this genus, and highlights a few interesting questions.

Sasamorpha is morphologically distinct from *Sasa*. In habit, *Sasamorpha* is diffuse (rhizomes monopodial, not tillering), while *Sasa* is caespitose (rhizomes amphipodial, tillering); unlike *Sasa*, the nodes are not prominently swollen above the sheath scar, and culm

leaves are generally longer than internodes, whereas in *Sasa* they are generally shorter. Unlike *Sasa*, *Sasamorpha* lacks auricles and fimbriae, and foliage leaf blades are medium-sized in comparison to *Sasa*; like *Sasa*, *Sasamorpha* is reported to have 6 stamens, however some references report 3 (Ohwi 1965).

Taxonomists have differed over the status of *Sasamorpha*, and current treatments consider it to be a subgenus of *Sasa* (Sugimoto 1961; Okamura *et al.* 1991). Molecular evidence demonstrates that *Sasamorpha* is phylogenetically distinct, although its relationship with *Sasa* is unclear. Chloroplast sequences for *Sasamorpha* (excluding a possible example of cp DNA capture) are relatively divergent from *Sasa*, and share no derived characters with that genus. AFLP data further indicate a split between these taxa, and morphological differences reinforce the recognition of *Sasamorpha*. The relationship between *Sasamorpha* and *Sasa* is further obscured by the apparent chloroplast capture among populations in Kyushu. Additional research is need on this genus, particularly as it potentially has a closer relationship with *Arundinaria* than previously recognized.

Sasamorpha is especially problematic in cultivation due to misidentification. For example, the plant introduced to the US via the PI station in Washington, DC was in fact a species of *Indocalamus* from China.

Sinicae Clade— The *Sinicae* Clade was identified in the cp DNA phylogeny of the temperate bamboos (Triplett and Clark, in prep.; Ch. 2), and unites divergent taxa in China including *Acidosasa*, *Indosasa*, *Pleioblastus* section *Amari*, and *Pseudosasa* subgenus *Sinicae*. This lineage was only minimally sampled in the current study, primarily to test the monophyly of *Pleioblastus sensu stricto* and to examine higher-level relationships within the *Arundinaria* Clade. Additional work is currently underway to explore relationships within this clade (Zhang and Li, Kunming Institute of Botany, China).

The current study further demonstrates that these putative allies of *Pleioblastus sensu stricto* are a phylogenetically heterogeneous group. *Pleioblastus amarus*, the type species of *Pleioblastus* section *Amari*, is apparently of hybrid origin. The chloroplast genome places this species in the *Phyllostachys* Clade, while AFLP data indicate a strong association with the *Arundinaria* Clade. The pattern is consistent with the hypothesis of chloroplast capture from the *Phyllostachys* Clade by a Chinese Pleioblastoid lineage. *Arundinaria funghomii,*

Pleioblastus juxianensis, and *Pleioblastus oleosus* (as cultivated in the US) are apparently close to *P. amarus*, and perhaps conspecific. *Pleioblastus solidus* and *P. intermedius* also appear to represent introgressed lineages, having cp DNA from the *Phyllostachys* Clade and an AFLP fingerprint matching the *Arundinaria* Clade. Sequence variation detected in these lineages suggests that the sampled species of *Phyllostachys* are not the parent species. *Pseudosasa cantorii* is also apparently of hybrid origin, but unlike *P. amarus*, its AFLP signature suggests an equal contribution from the *Arundinaria* Clade and the *Phyllostachys* clade.

A putative Pleioblastoid core of the *Sinicae* group can be inferred by excluding taxa with introgression from the *Phyllostachys* Clade. Of the taxa sample here, *Pseudosasa amabilis, Ps. hindsii, Ps. longiligula, Ps. viridula, Ps. usawae,* and *Pleioblastus maculatus* have cp DNA haplotypes and AFLP genotypes that clearly place them in the *Arundinaria* Clade and sister to *Pleioblastus s.s. Pseudosasa amabilis* was placed in *Pseudosasa*, but it is not particularly close to the type species (*Pseudosasa japonica*). *Pseudosasa usawae* (Hayata) Makino & Nemoto from Taiwan was originally described in *Arundinaria* and transferred to *Pleioblastus* by Ohki (1928), and finally placed in *Pseudosasa* by Makino and Nemoto (1931). This species is often synonymized with *Pseudosasa japonica*, but represents a distinct lineage. Morphologically, this species is similar to taxa in the Ryūkyū Islands, with persistent culm leaves, long inner ligules, and a habit similar to that of *P. linearis*. Additional work is necessary to clarify relationships among taxa in the *Sinicae* Clade. For example, are *Acidosasa* and *Indosasa* indicative of divergent lineages within the *Sinicae* Clade, or do they represent a hybrid cluster analogous to the Japanese *Pleioblastus/ Semiarundinaria/ Sasaella/ Pseudosasa* complex?

Pleioblastus sensu stricto— In both cp and AFLP analyses, *Pleioblastus s.s.* was recovered as a robust lineage with subclades corresponding to three sections defined by morphology (Suzuki 1978). *Pleioblastus* is characterized by a suite of morphological features including persistent culm sheaths, extensive secondary branching, and glabrous fimbriae; however, none of these are exclusive or provide clear synapomorphies. Thus, molecular data currently provide the only synapomorphies for this genus. Patterns of genetic and morphological diversity vary within the group, with section *Pleioblastus* exhibiting the

greatest divergence among four well-defined species, section *Nezasa* with an intermediate level of diversity, and section *Medakea* exhibiting the least genetic variation. AFLP analyses confirm a sister relationship between section *Medakea* and section *Nezasa* and provide resolution that was unavailable from cp sequence data. However, these data fail to provide a straightforward solution to taxonomic problems in section *Nezasa*, instead highlighting a complex genetic network of incomplete speciation or cryptic introgression.

Section *Pleioblastus*— Molecular data provide strong support for section *Pleioblastus*, the group defined by tillering culms and long inner ligules. AFLP data resolve three clusters and six subclusters within this section, essentially corresponding to four monophyletic species. Two subclades were recovered for kanzan-chiku ("*Pleioblastus hindsii"*), one consisting of plants collected on the Ryūkyū Islands of Iriomote and Ishigaki (PP = 1.0; Fig 8), and the other representing plants naturalized in Kyushu and cultivated in the US (PP = 1.0). These data may indicate divergence that has occurred since plants native to the Ryūkyū Islands were introduced into cultivation in Kyushu within the last 2000 years. Alternatively, source populations for the cultivated material were not sampled in the Ryūkyū Islands in this study. Data revealed a similar split within *P. gramineus*: plants collected in Iriomote, Ishigaki, and in cultivation in Honshu have nearly identical genotypes, while plants cultivated in the US and China have a different ancestry. The other major subclades in section *Pleioblastus* represent *P. linearis* and *P. gozadakensis*. Some botanists consider *P. gozadakensis* to be an ecotype of *P. linearis* (Walker 1976; Okamura *et al.* 1991); however, AFLP data demonstrate a significant level of divergence between these two, and morphological variation is consistent with this phylogenetic split. *Pleioblastus gozadakensis* is a high elevation endemic, found on mountains in Iriomote and Ishigaki, while *Pleioblastus linearis* is found at lower elevations. Clones of *P. gozadakensis* transplanted to lower elevations in Iriomote have retained their distinctive morphology, including restricted secondary branching, non-tillering rhizomes, and yellow culm coloration (Triplett, pers. observation). AFLP data indicate that specimens in cultivation in the US under the name *Pleioblastus linearis* 'Nana,' are more closely related to *P. gozadakensis.* Moreover, plants currently available in the US as *P. gozadakensis* are misidentified relatives of *Phyllostachys*, sharing none of the diagnostic morphological characters of *Pleioblastus*.

Results clearly indicate that plants known as *Arundinaria hindsii* in China [*Pseudosasa hindsii* (Munro) S.L. Chen & G.Y. Sheng] and Japan [*Pleioblastus hindsii* (Munro) Nakai] are unrelated species, thus highlighting a long-standing problem of misidentification dating to the introduction of Japanese bamboos into Europe in the 1800s. The Chinese species belongs to the *Sinicae* Clade (Fig. 1; Fig. 5), while the Japanese species (*n.v. Kanzan-chiku*) is related to *Pleioblastus gramineus*. When plants from the Ryūkyūs were first cultivated in England, they were mistakenly associated with the type specimen of *Arundinaria hindsii*, a plant collected in the vicinity of Hong Kong (Bean 1894) and occurring wild in the coastal hills and mountains of Fujian, Guangdong, Guangxi, Hunan, Jiangxi, and Zhejiang Provinces (Wu *et al.* 2006), China. *Kanzan-chiku* is thus an unnamed species that escaped recognition due to misidentification. A new species description is currently being prepared for publication to rectify this oversight (Triplett, in prep).

Section *Medakea*— Section *Medakea* encompasses six species distinguished primarily on the basis of foliage vestiture and culm coloration, including one widespread species (*P. simonii*) and five that are narrow endemics. A total of 36 wild accessions of *P. aff. simonii* from throughout Japan plus 5 cultivars in the US and Japan were included in the AFLP study, and these had virtually identical genotypes. The sample was reduced to 23 for the purposes of this manuscript, representing 18 geographically distinct wild populations and all five cultivars. The lack of genetic variation within this species is striking, but reminiscent of low diversity observed among North American populations of *A. gigantea* (Triplett and Clark *in prep.*; Chapter 3). Interestingly, both species are known by similar common names (river cane, *kawa-dake* in Japanese), and their wide distribution and correlated low diversity could be connected with the successful exploitation of a habitat favorable to clonal growth. Alternatively, anthropogenic interaction could account for the genetic structure of *P. simonii*. Historically, this species was widely used for thatched roofing and other purposes in rural Japan, and current populations may represent widespread clones from the original source population.

The current analysis calls into question the taxonomic diversity of section *Medakea*. Plants matching the description of *P. kodzumae* were collected from a number of localities in Japan, mostly correlated with harsh or disturbed habitats including river basins and exposed

hillsides. All of these had AFLP genotypes matching *P. simonii*. Plants collected from the type locality were also morphologically and genetically identical to *P. simonii*. Populations of *Pleioblastus kodzumae* putatively occur in Izu Peninsula, western Shikoku, central and southern Kyushu, and in several isolated localities on the northern side of Honshu. For such a distribution, the plant would have been widespread in the past, with subsequent habitat fragmentation. However, none of the plants collected from putative *P. kodzumae* localities were genetically different from *P. simonii*, in spite of morphological variation. Current data thus support the recognition of *P. kodzumae* as an ecotype of *P. simonii*.

Four additional species from section *Medakea* could not be located in the wild. *Pleioblastus nabeshimanus*, *P. matsunoi*, and *P. higoensis* are each considered relatively rare, and most are locally endemic. These are distinguished from *P. simonii* primarily on vestiture (*i.e.*, culm leaf sheaths puberulous or pilose vs. glabrous; foliage leaf sheaths puberulous or pilose vs. glabrous). *Pleioblastus simonii* was common in the vicinity of the type localities and other reported locations of these plants. One other member of section *Medakea*, *Pleioblastus pseudosasaoides*, is only known from two locations, and has not been studied in molecular or morphological analyses. The type specimen reveals a very distinctive plant with a single branch per node and other morphological features consistent with *Sasa*, and further research is needed to characterize this rare and unusual species. It is possible that this species represents a distinctive hybrid association: hybrid links have been established between *Sasamorpha* and *Pleioblastus* sect. *Pleioblastus* (*i.e.*, *Pseudosasa*) and between *Sasa* and *Pleioblastus* sect. *Nezasa* (*i.e. Sasaella*), but none are currently documented between sect. *Medakea* (*P. simonii*) and *Sasa* or *Sasamorpha*, and *P. pseudosasaoides* suggests a putative connection between these taxa.

Section *Nezasa*— Section *Nezasa* encompasses nine species, two of which (*P. pygmaeus*, *P. viridistriatus*) are known only in cultivation, while three of the seven wild species are unknown in flower. The group is important in Japan and the West as the source of popular groundcover and decorative species, represented by upwards of forty well-known cultivars (Ohrnberger 1999). Several species, including *P. chino* and *P. argenteostriatus*, are ecologically important, forming monocultures on mountains and dense groundcover in woodlands. The *Nezasa* group is morphologically cohesive, and species overlap in diagnostic

features such as flower morphology, inflorescence architecture, vegetative branching, and leaf morphology, with few fundamental differences. Species vary in relatively minor features such as vestiture, leaf size, and coloration, some of which are likely to be environmentally dependent. A better understanding of phenotypic plasticity in this group is greatly needed.

Negligible cp sequence variation was recovered within this group. Among eight sampled taxa, all except *P. chino* were distinguished from *P. simonii* only by a single point mutation in *trnDT*, resulting in a weak cluster in the MP analysis.

The AFLP study included all recognized species (Suzuki 1978 1991) with the exception of *P. hattorianus*, a rare species in central Honshu. AFLP data indicated two groups (*Nezasa* I and *Nezasa* II; Fig. 5), with intermediate forms blurring the morphological and phylogenetic distinction between these two. *Nezasa* I consists primarily of *P. chino*, endemic to central Honshu, plus one cultivar of *P. variegatus* ('Tsuboii'), also originating from central Honshu. *Nezasa* II consists of all other sampled species (*P. argenteostriatus*, *P. humilis*, *P. nagashima*, *P. kongosanensis*, *P. viridistriatus*, and *P. variegatus*) plus one cultivar of *P. chino* ('Murakamianus') and plants known in cultivation as *Pseudosasa owatarii*. *Nezasa* II is represented by wild accessions primarily from western Honshu, Shikoku, and Kyushu, and groundcover species widely cultivated in Japan and the west. Most species in Nezasa II failed to form monophyletic clusters, while several robust clusters result in polyphyletic species (*e.g.*, *P. pygmaeus*).

Representatives of the plants known only in cultivation (*P. pygmaeus* and *P. viridistriatus*) have weak genetic affinities for wild species with which they overlap in morphology. *Pleioblastus pygmaeus* clusters in three different groups, associating primarily with *P. humilis* and *P. variegatus,* while *P. pygmaeus* var. *distichus* clusters with *Pseudosasa owatarii.* These species are primarily distinguished by surface vestiture (*i.e.*, whether foliage leaf sheaths and blades are glabrous, puberulous or pilose). Thus, current results suggest that *P. owatarii* is a form of *P. pygmaeus* var. *distichus*. *Pseudosasa owatarii* is endemic to subalpine grasslands on Yakushima Island of southern Japan. Fieldwork is needed to determine whether the specimens in cultivation are in fact the same as plants from the type locality. *Pleioblastus viridistriatus* clusters with *P. kongosanensis*, a wild plant that is morphologically consistent with the species known only in cultivation. The fundamental

feature of both is dense pubescence over most plant surfaces. The species differ in reported stature, and most likely *P. viridistriatus* is a diminutive form of *P. kongosanensis*.

The neighbor-net plot of this group plus representatives of section *Medakea* (*P. simonii*) highlights significant internal signal conflict (Fig. 10). AFLPs are expected to fail to resolve relationships among closely related, cross-fertile species (Despres *et al.* 2003; Koopman *et al.* 2001), and the lack of robust resolution in section *Nezasa* may be evidence of ongoing gene flow or incomplete lineage sorting among recently diverged species. AFLP data provide evidence of putative hybridization between *Nezasa* and *Medakea*, with diagnostic bands from *P. simonii* occurring in intermediate *Nezasa*-like species. For example, accession JT 318 from Chiba Prefecture has diagnostic bands from neighboring species, *P. simonii* JT 322 and *P. chino* JT 316, suggesting a possible hybrid zone. Similarly, accession JT 388 from Yamaguchi Prefecture shares diagnostic bands from *P. simonii* JT389 and *P. aff. variegatus* JT 387, two populations within 5 km of each other.

Section *Nezasa* is a taxonomically complex group and will require additional work at the population level to reconcile species boundaries with the apparent reticulate relationships. The pattern of genetic diversity recovered in this study could be interpreted to indicate one widespread species (*Nezasa* I, *P. chino*) in central Honshu and a widespread species complex (*Nezasa* II) of one or more species in western Honshu, Shikoku, and Kyushu. Further work is needed to determine the accuracy of the current evaluation of diversity with respect to natural species boundaries, but results considered in light of other recent evaluations of genetic diversity in the temperate clade suggest that a wider circumscription of species in this group in warranted.

Conclusions

Reticulate evolution has not been previously documented to have an important role in the evolution of woody bamboos. However, current results provide numerous examples of both major and minor impacts of hybridization, from the origin of new genera to the breakdown of species barriers and the blurring of taxonomic boundaries. Temperate bamboos appear to have few if any reproductive barriers except those imposed by geography and time. Thus, the potential for genetic admixture is constrained only by a lack of opportunity.

AFLP patterns in hybrid bamboo genera are largely consistent with recent hybridization. Some natural hybridizations may ultimately be attributable to human causes; for example, *Semiarundinaria* is probably less than 2000 years old, subsequent to the introduction of *Phyllostachys* into Japan, where it has become common genus. But what of ancient reticulation events? Given the prevalence of hybridization among divergent extant lineages, hybridization was likely involved in the early history of this group. Additional molecular research may provide a window on genomic mergers that occurred deeper in evolutionary time.

Current results have important implications for the interpretation of characters used to delimit species, genera, and higher-level taxa in the temperate clade. Results indicate the need for a critical reevaluation of morphology in light of reticulating lineages. Moreover, the results suggest that the complex taxonomic history of the temperate bamboos can be employed to provide clues regarding other putative hybrids. For example, plants currently placed in the genus *Oligostachyum* (Wang and Ye 1980, 1982) exhibit features that bridge morphological diversity in phylogenetically disparate genera *Acidosasa*, *Phyllostachys*, and *Arundinaria*, including variable numbers of stamens (3-5), culms flattened on one side, and branch complements suggestive of *Semiarundinaria.* Other genera with troubled taxonomic histories and intergrading morphology include *Sinobambusa*, *Indosasa*, and *Brachystachyum*.

The current analysis sets the stage for a revision of *Pleioblastus sensu stricto*. Sections *Medakea* and *Pleioblastus* have well-defined morphological features, and molecular data support monophyletic species. However, additional population-level work is needed to clarify relationships within the *Nezasa* clade. Are there multiple species within this group? Morphology seems to argue yes, while molecular data present a more complicated answer. Phylogenetic analyses with low copy nuclear data scrupulously cloned to capture all possible copies may help to resolve relationships in this group.

Reticulate evolution in bamboos likely encompasses a suite of evolutionary outcomes from the origin of new species, to introgressive mergers that facilitate habitat expansion, to events with negligible impacts on parental species. For example, *Sasaella* apparently represents an ecologically advantageous merger of two divergent taxa, while *Sasamorpha* provides an example of chloroplast capture with an undetectable impact on phenotype. Little

is known regarding the fate of natural bamboo hybrids, but current distributions of *Sasaella* suggest that some are very successful, at least vegetatively. *Hibanobambusa* and *Semiarundinaria* are apparently infertile, but other hybrids, including *Pseudosasa japonica*, do produce viable seeds.

This study creates the need for additional research on the biology of these complex forest grasses, with questions spanning ecology, genetics, and evolutionary biology: How common are natural bamboo hybrids, and how long do they persist in the wild? Do reciprocal hybrids from a given parental pair have similar morphological traits? What impact does hybridization have on the internal clock regulating flowering cycles in bamboo? And are any bamboo lineages actually pure? Clearly, the *Arundinaria* Clade is complex from the perspectives of morphological taxonomy and genetics. The current study provides a new framework for evaluating biodiversity in this group, and provides a pressing need for further research on the impact of reticulate evolution in this diverse and fascinating group of plants.

ACKNOWLEDGEMENTS

Field work was supported by grants from the American Bamboo Society and a research grant from the NSF/JPSP (EAPSI) to JT as well as a National Geographic Society Grant (#7336-02) to LC. Support for JT and for the final preparation of this manuscript came from a National Science Foundation Grant (DEB-0515712) to LC. We are indebted to Adam and Sue Turtle (Earth Advocates Research Farm, Summertown, TN), Jennifer York and Jesus Mora (Bamboo Sourcery, Sebastopol, CA), Dr. Michael Hotchkiss (USDA, Byron, GA), Marc Hilt (Bambu-u, Chimacum, WA), Erika Harris (Clinton Bamboo, Seattle, WA), Stan Andreason (Beauty & the Bamboo, Seattle, WA), and Dr. Masatoshi Watanabe (Rakusai Bamboo Gardens, Kyoto, Japan) for providing access to bamboos in cultivation. JT is especially grateful to Masayo Ozaki for assistance with fieldwork in Japan and the US, and to Michihiro and Yoko Ozaki for their hospitality and assistance in Japan, including the generous loan of a vehicle. We thank Mr. Choken Ishigaki of the Tropical Biosphere Research Center (University of the Ryukyus) for assistance with fieldwork in Iriomote and Ishigaki, Japan. We are especially grateful to Dr. Dezhu Li, Yuxiao Zhang, and colleagues at Kunming Botanical Institute, China, for help with field work in Yunnan, China, and for

valuable discussions on the temperate clade. We thank Dr. Jonathan Wendel, Ryan Percifield, Dr. Corrine Grover, and Dr. Robert Wallace of Iowa State University for technical assistance and access to laboratory facilities, and Chris Tyrrell for statistical assistance. We also thank the lab of Dr. Noriaki Murakami of Kyoto University for valuable assistance in Japan.

LITERATURE CITED

ADAMS, R.P. AND L.H. RIESEBERG. 1998. The effects of non-homology in RAPD bands on similarity and multivariate statistical ordination in *Brassica* and *Helianthus*. Theoretical and Applied Genetics 97: 323–326.

ALTHOFF, D.M., M.A. GITZENDANNER, AND K.A. SEGRAVES. 2007. The utility of amplified fragment length polymorphisms in phylogenetics: a comparison of homology within and between genomes. Systematic Biology 56: 477-484.

ÁLVAREZ, I. AND J.F. WENDEL. 2006. Cryptic interspecific introgression and genetic differentiation within *Gossypium aridum (*Malvaceae) and its relatives. Evolution 60 (3): 505-517.

BEAN, W. J. 1894. A classification of hardy bamboos. Gardeners' Chronicle 15: 238–239, 301–302.

BESS, N.M. 2001. Bamboo in Japan. Kodansha International. Tokyo, New York, London.

BLEEKER,W. AND A. MATTHIES. 2005. Hybrid zones between invasive *Rorippa austriaca* and native *R. sylvestris* (Brassicaceae) in Germany: ploidy levels and patterns of fitness in the field, Heredity 94: 664–670.

BRYANT, D. AND V. MOULTON. 2004. NeighborNet: an agglomerative algorithm for the construction of phylogenetic networks. Molecular Biology and Evolution 21 (2): 255-265.

BUSSELL, J.D., M. WAYCOTT, AND J.A. CHAPPILL. 2005. Arbitrary amplified DNA markers as characters for phylogenetic inference. Perspectives in Plant Ecology, Evolution, and Systematics 7: 3-26.

CHAO, C.S. 1989. A Guide to Bamboos Grown in Britain. Royal Botanic Gardens, Kew.

CHASE, M.W. AND H.H. HILLS. 1991. Silica gel: An ideal material for field preservation of leaf samples for DNA studies. Taxon 40: 215-220.

CHEN, S.L. AND G.Y. SHENG. 1991. New taxa and a new combination in Chinese bamboos. In: Bulletin of Botanical Research 11 (4): 41-51.

CLARK, L.G., G. DAVIDSE, AND R.P. ELLIS. 1989. Natural hybridization in bamboos: evidence from *Chusquea* sect. *Swallenochloa (*Poaceae: Bambusoideae). Natl. Geog. Res. 5: 459-476.

CLAYTON, W.D. AND S.A. RENVOIZE. 1986. Genera Graminum: Grasses of the World. Royal Botanic Gardens, Kew.

DEMOLY, J.P. 1995. Nouveautés nomenclaturales pour des bambous cultivés en France. Bambou, Assoc. Europ. Bamb. 21: 14-15.

DESPRÉS, L., L. GIELLY, B. REDOUTET, P. TABERLET. 2003. Using AFLP to resolve phylogenetic relationships in a morphologically diversified plant species complex when nuclear and chloroplast sequences fail to reveal variability. Molec. Phylog. Evol. 27: 185–196.

DOYLE, J.J. AND J.L. DOYLE. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochem. Bull. 19: 11-15.

EPPNER, H.T. 2002. *Poaceae* in the greenhouses of the Botanic Garden of the Institute of Botany in Graz (Austria, Europe). Fritschiana (Graz) 31: 1-42.

FARRIS, J. S. 1970. Methods for computing Wagner trees. Systematics Zoology 19: 83-92.

FARRIS, J. S. 1977. Phylogenetic analysis under Dollo's Law. Systematic Zoology 26: 77-88.

FARRIS, J.S. M. KÄLLERSJÖ, A. G. KLUGE AND C. BULT. 1994. Testing significance of incongruence. Cladistics 10: 315–319.

FELSENSTEIN, J. 1985. Confidence limits on phylogenies: An approach using the bootstrap. Evolution 39: 783-791.

GASCUEL. O. 1997. BIONJ: an Improved Version of the NJ Algorithm Based on a Simple Model of Sequence Data. Mol Biol Evol. 14 (7): 685-695.

GIRIBERT, G. AND W.C. WHEELER. 1999. On gaps. Mol. Phylogenet. Evol. 13: 132-143.

GLADENKOV, A.Y., A.E. OLEINIK, L. MARINCOVICH JR. AND K.B. BARINOV. 2002. A refined age for the earliest opening of Bering Strait. Palaeogeogr. Palaeoclimatol. Palaeoecol. 183: 321-328.

GRANT, V. 1981. Plant Speciation. Columbia University Press: New York.

GUO Y.P., J. SAUKEL, R. MITTERMAYR, AND F. EHRENDORFER. 2005 AFLP analyses demonstrate genetic divergence, hybridization, and multiple polyploidization in the evolution of *Achillea* (Asteraceae-Anthemideae). New Phytol. 166 (1): 273-89.

HODKINSON, T.R., S.A. RENVOIZE, G. NÍ CHONGHAILE, C.M.A. STAPLETON, AND M.W. CHASE. 2000. A comparison of ITS nuclear rDNA sequence data and AFLP markers for phylogenetic studies in *Phyllostachys* (Bambusoideae, Poaceae). J. Plant Res. 113: 259-269.

HOSOYAMA, Y., K. HOSHIDA, S. TAKEOKA, AND S. MIYATA. 2002. On the inter-generic hybrid Sasaella ramosa. Proceedings on the Institute of Natural Sciences, Nihon University. 37: 209-216. [In Japanese]

HUGHES, R.H. 1966. Fire ecology of canebrakes. Proceedings of the Fifth Annual Tall Timbers Fire Ecology Conference, March 24-25 1966. Mimeographed.

HUSON, D. AND D. BRYANT. 2006. Application of phylogenetic networks in evolutionary studies. Molecular Biology and Evolution 23 (2) 254-267.

ISHII, A., S. MIYATA, AND Y. HOSOYAMA. 2003. The maternal species of hannouzasa (*Sasaella ramosa*). Bamboo J. 20: 12-18. [In Japanese]

JANZEN, D. 1976. Why bamboos wait so long to flower. Ann. Rev. Ecol. Syst. 7: 347-391.

JUDZIEWICZ, E.J., L.G. CLARK, X. LONDONO, AND M.J. STERN. 1999. American Bamboos. Smithsonian Institute Press, Washington.

KENG, P.C. AND Z.P. WANG, EDS. 1996. *Flora Reipublicae Popularis Sinicae*, vol. 9 (1). Beijing. Science Press. [In Chinese]

KITAMURA, S., AND G. MURATA. 1979. Colored Illustrations of Woody Plants of Japan, Vol. II. Hokuryukan, Tokyo.

KNOBLOCH, I.W. 1968. A checklist of crosses in the Graminae. Department of Botany and Plant Pathology, Michigan State University, East Lansing, MI.

KOIDZUMI, G. 1941. *Semiarundinaria tranquillans* Koidz. *nov. sp.* in: Acta Phytotax. Geobot. 10: 317.

KOOPMAN, W.J.M. 2005. Phylogenetic signal in AFLP data sets. Systematic Biology 54: 197–217.

KOOPMAN, W.J.M., M.J. ZEVENBERGEN, AND R.G. VAN-DEN-BERG. 2001. Species relationships in *Lactuca S. L.* (Lactuceae, Asteraceae) inferred from AFLP fingerprints. Am. J. Bot. 88: 1881–1887.

LARA-CABRERA, S.I. AND D.M. SPOONER. 2004. Taxonomy of North and Central American diploid wild potato (*Solanum* sect. *Petota*) species: AFLP data. Plant Syst. Evol. 248: 129-142.

LE QUESNE, W. J. 1974. The uniquely evolved character concept and its cladistic application. Systematic Zoology 23: 513-517.

LOH, J.P., R. KIEW, O. SET, L.H. GAN, AND Y. GAN. 2000. A study of genetic variation and relationships within the bamboo subtribe Bambusinae using amplified fragment length polymorphism. Ann. Botany 85: 607-612.

MAKINO, T. 1920. A contribution to the knowledge of the flora of Japan. J. Jap. Bot. 2 (4): 15-16.

MAKINO, T. 1929. A contribution to the knowledge of the flora of Japan. J. Jap. Bot. 6 (7): 5-26.

MARUYAMA, I., AND H. OKAMURA. 1971. *Hibanobambusa*. Report of the Fuji Bamb. Garden 16: 30.

MARUYAMA, I., AND H. OKAMURA. 1979. On a new hybrid genus *Hibanobambusa*. Acta Phyto. Geobot. 30 (4-6) 148-152.

MASON-GAMER, R.J., AND E.A. KELLOGG. 1996. Chloroplast DNA analysis of the monogenomic Triticeae: phylogenetic implications and genome-specific markers. In: P.P. Jauhar (ed.). Methods of Genome Analysis in Plants, CRC Press, Boca Raton.

MCCLURE, F.A. 1973. Genera of Bamboos Native to the New World (Gramineae: Bambusoideae). Ed. T.R. Soderstrom. Smithsonian Contr. Bot. 9: 1-148.

MCDADE, L.A. 1992. Hybrids and phylogenetic systematics II. The impact of hybrids on cladistic analysis. Evolution 46 (5): 1329-1346.

MCDADE, L.A. 1997. Hybrids and phylogenetic systematics III. Comparison with distance methods. Syst. Bot. 22 (4): 669-683.

MURAMATSU, M. 1981. Hybridization among Bambusaceae species: in Bamboo Production and Utilization. Proceedings of the Congress Group 5.3A, Production and Utilization of Bamboo and Related Species, XVII IUFRO [International Union of Forestry Research Organization] World Congress Kyoto, Japan, Sept. 6-17 1981.

NAKAI, T. 1925. Two new genera of Bambusaceae with special remarks on the related genera growing in eastern Asia. J. Arnold Arbor. 6 (3): 145-153.

NEI, M., AND W.H. LI. 1979. Mathematical model for studying genetic variation in terms of restriction endonucleases. Proc. Natl. Acad. Sci. 76: 5269-5273.

NEMOTO, K. 1931. *Pseudosasa usawae*; in: Flora of Japan, ed. 2, p. 1390.

OHKI, K. 1928. On the systematic importance of spodograms of the leaves of the Bambusaceae: II-V. Botanical Magazine 42: 514-520.

OHRNBERGER, D. 1999. The Bamboos of the World. Elsevier, Amsterdam, The Netherlands.

OHWI, J. 1960. Flora of Japan, revised ed., Shibundo, Tokyo.

OKAMURA, H, AND Y. TANAKA. 1986. The Horticultural Bamboo Species in Japan: The characteristic and utilization of ornamental bamboo species with illustration. Kobe: Hata Okamura.

OKAMURA, H., Y. TANAKA, M. KONISHI AND H. KASHIWAGI. 1991. Illustrated Horticultural Bamboo Species in. Japan. Haato Limited, Shinzaiki Wakaiama.

OLMSTEAD, R. G. AND J. D. PALMER. 1994. Chloroplast DNA systematics: a review of methods and data analysis. Am. J. Bot. 81: 1205-1224.

PETERSON, P.M., J. CAYOUETTE, Y.S.N. FERDINANDEZ, B. COULMAN, AND R.E. CHAPMAN. 2002. Recognition of *Bromus richardsonii* and *B. ciliatus*: evidence from morphology, cytology, and DNA fingerprinting (Poaceae: Bromeae). Aliso 20: 21-36.

PLATT, S.G. AND C.G. BRANTLEY. 1997. Canebrakes: An ecological and historical perspective. Castanea 62: 8-21.

POSADA, D. AND K.A. CRANDALL.1998. Modeltest: testing the model of DNA substitution. Bioinformatics 14 (9): 817-818.

R DEVELOPMENT CORE TEAM (2008). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org.

RAAMSDONK, L.W.D.V., M.V.-V. GRINKEL, AND C. KIK. 2000. Phylogeny reconstruction and hybrid analysis in *Allium* subgenus *Rhizirideum*. Theoretical and Applied Genetics 100: 1000-1009.

RAMBAUT, A. 2001. Se-Al version 2.0a9: sequence alignment editor. Department of Zoology, University of Oxford, Oxford, UK.

RIDOUT, C.J. AND P. DONINI. 1999. Use of AFLP in cereals research. Trends in Plant Science 4: 76-79.

RIESEBERG, L.H. 1997. Hybrid origins of plant species. Annual Review of Ecology and Systematics. 28: 359-389.

RONQUIST, F., J.P. HUELSENBECK, AND P. VAN DER MARK. 2005. MrBayes 3.1 Manual. Draft 26/05/2005. Available at: http://mrbayes.csit.fsu.edu/manual.php Accessed 2/13/08

SAARELA, J.M., P.M. PETERSON, R.J. SORENG, AND R.E. CHAPMAN. 2003. A taxonomic revision of the eastern North American and Eastern Asian disjunct genus *Brachyelytrum* (Poaceae): Evidence from morphology, phytogeography, and AFLPs. Systematic Botany 28 (4): 674-692.

SAITOU N, M., AND M. NEI. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol. Biol. Evol. 4 (4): 406-25.

SASAKI, Y., K. KOMATSU, M. TAKIDO, K. TAKESHITA, H. KASHIWAGI, AND S. NAGUMO. 2007. Genetic profiling of *Sasa* species by analysis of chloroplast intron between *rbcL* and *ORF106* and partial *ORF106* regions. Biol. Pharm. Bull. 30 (8): 1511-1515.

STAPLETON, C.M.A. 1997. The morphology of the woody bamboos. In: Chapman, G.P. (ed.). The Bamboos. Linnean Society of London Symposium Series. Academic Press, London.

STEBBINS, G. L. 1972. The evolution of the grass family. In: Youngner, V. B. and C. M. McKell (ed.), The Biology and Utilization of Grasses. Academic Press, Inc., New York and London.

STEBBINS, G.L. 1950. Variation and Evolution in Plants. Columbia University Press: New York.

STEBBINS, G.L. 1956. Cytogenetics and evolution in the grass family. Am. J. Bot. 43: 890–905.

STEBBINS, G.L. 1985. Polyploidy, Hybridization, and the Invasion of New Habitats. Ann. Miss. Bot. Gard. 72: 824-832.

SUGIMOTO, J. 1961. Keys to Woody Plants of Japan. Rokugatsusha, Osaka.

SUZUKI, S. 1978. Index to Japanese Bambusaceae. Tokyo: Gakken Co. Ltd.

SUZUKI, S. 1987. New or noteworthy plants in Japanese Bambusaceae (5). J. Jap. Bot. 62 (9): 274-280.

SUZUKI, S. 1991. New or noteworthy plants in Japanese Bambusaceae (8). J. Jap. Bot. 66 (4): 194-198.

SWOFFORD, D.L. 2002. PAUP*. Phylogenetic analysis using parsimony, ver. 4.0b10. Sunderland: Sinauer Associates.

TRIPLETT, J.K., A.S. WEAKLEY, AND L.G. CLARK. 2006. Hill cane (*Arundinaria appalachiana)*, a new species of bamboo (Poaceae: Bambusoideae) from the southern Appalachian Mountains. SIDA 22 (1): 79-85.

VEKEMANS, X. (2002). AFLP-SURV v1.0. Laboratoire de Génétique et Ecologie Végétale, Université Libre de Bruxelles, Belgium.

VEKEMANS, X., T. BEAUWENS, M. LEMAIRE, AND I. ROLDÁN-RUIZ. 2002. Data from amplified fragment length polymorphism (AFLP) markers show indication of size homoplasy and of a relationship between degree of homoplasy and fragment size. Molecular Ecology 11: 139-151.

VOS, P., R. HOGERS, M. BLEEKER, M. REIJANS, T. VAN DE LEE, M. HORNES, A. FRIJTERS, J. POT, J. PELEMAN, M. KUIPER AND M. ZABEAU. 1995. AFLP: a new technique for DNA fingerprinting. Nucl. Acids Res. 23: 4407-4414.

WALKER, E. H. 1976. Flora of Okinawa and the southern Ryukyu Islands. Smithsonian Institution Press, Washington, DC.

WANG, C.P. AND G.H. YE. 1982. *Oligostachyum*: a new genus of Chinese Bambusoideae. J. Nanjing Univ. Nat. Sci. No. 1: 95-101.

WANG, C.P. AND G.H. YE. 1980. On the problems of the classification of Chinese bamboos with creeping rhizomes. Acta Phytotaxonomica Sinica 18: 283-291.

WILCOX, T.P., D.J. ZWICKL, T.A. HEATH, AND D.M. HILLIS. 2002. Phylogenetic relationships of the dwarf boas and a comparison of Bayesian and bootstrap measures of phylogenetic support. Mol. Phylogenet. Evol. 25: 361-371.

WU, Z.Y., P.H. RAVEN AND D.Y. HONG, EDS. 2006. Flora of China. Vol. 22 (Poaceae). Science Press, Beijing, and Missouri Botanical Garden Press, St. Louis.

APPENDIX 1

List of taxa utilized in this study. Vouchers at ISC unless otherwise indicated.

Arundinaria appalachiana Triplett, Weakley & L.G. Clark*, Triplett 99*; ***Arundinaria funghomii*** McClure*, Triplett 10*; ***Arundinaria gigantea*** (Walt.) Muhl*, Triplett 197, Triplett 291*; ***Arundinaria tecta*** (Walt.) Muhl*, Triplett 173*; ***Hibanobambusa tranquillans*** (Koidzumi) Maruyama & H. Okamura*, Triplett 72, Triplett 205*; ***Phyllostachys aurea*** Carrière ex A. & C. Rivière*, Triplett 120*; ***Phyllostachys bambusoides*** Siebold & Zuccarini*, Triplett 121*; ***Phyllostachys edulis (***Carrière) Houzeau de Lehaie*, Y. Zhang KMBG04 (KIB)*; ***Phyllostachys heteroclada*** Oliver*, Triplett 119*; ***Phyllostachys nigra*** (Loddiges ex Lindley) Munro*, Triplett s.n., Y. Zhang KMBG07 (KIB)*; ***Pleioblastus amarus*** (Keng) P.C. Keng*, Y. Zhang 7082 (KIB)*; ***Pleioblastus aff. amarus*** (Keng) P.C. Keng*, Triplett 117, Triplett 124, Triplett 43*; ***Pleioblastus argenteostriatus*** (Regel) Nakai*, Triplett 204, Triplett 31*; ***Pleioblastus argenteostriatus* f. *glaber*** **(**Makino) Murata*, Triplett 66*; ***Pleioblastus aff. argenteostriatus*** (Regel) Nakai*, Triplett 359*; ***Pleioblastus chino (***Franchet & Savatier) Makino*, Triplett 11, Triplett 14, Triplett 297, Triplett 301, Triplett 302, Triplett 304, Triplett 305, Triplett 307, Triplett 309, Triplett 310, Triplett 313, Triplett 315, Triplett 316, Triplett 404, Triplett 415, Triplett 419, Triplett 420, Triplett 73*; ***Pleioblastus aff. chino (***Franchet & Savatier) Makino*, Triplett 318, Triplett 360, Triplett 364, Triplett 378, Triplett 388, Triplett 397, Triplett 405, Triplett 60*; ***Pleioblastus chino* f. *elegantissimus (***Makino ex Tsuboi) Muroi & H. Okamura*, Triplett 57*; ***Pleioblastus chino* 'Gracilis'***, Triplett 49*; ***Pleioblastus chino* 'Kimmei'***, Triplett 40*; ***Pleioblastus chino* 'Murakamianus'***, Triplett 32*; ***Pleioblastus chino* 'Variegatus'***, Triplett 236, Triplett 129*; "***Pleioblastus gauntlettii***" (In Cult.)*, Triplett 145*; ***Pleioblastus gozadakensis*** Nakai*, Triplett 337, Triplett 338, Triplett 342, Triplett 343, Triplett 344*; ***Pleioblastus gramineus*** (Bean) Nakai*, Triplett 327, Triplett 329, Triplett 330, Triplett 334, Triplett 336, Triplett 340, Triplett 347, Triplett 348, Triplett 35, Triplett 36, Triplett 58, Triplett 59*; ***Pleioblastus hindsii*** (Munro) Nakai*, Triplett 229, Triplett 326, Triplett 331, Triplett 333, Triplett 335, Triplett 339, Triplett 341, Triplett 346, Triplett 39, Triplett 408, Triplett 411, Triplett 65*; ***Pleioblastus humilis (***Mitford) Nakai *, Triplett 158, Triplett 219, Triplett 41*; ***Pleioblastus intermedius*** S.Y. Chen*, Y. Zhang 6188 (KIB)*; ***Pleioblastus kongosanensis*** Makino*, Triplett 366, Triplett 68, Triplett 74*; ***Pleioblastus***

kongosanensis* 'Aureostriatus'**, *Triplett 144, Triplett 46*; ***Pleioblastus linearis (Hackel) Nakai, *Triplett 328, Triplett 349, Triplett 350, Triplett 351, Triplett 352*; ***Pleioblastus linearis* 'Nana'**, *Triplett 157*; ***Pleioblastus maculatus*** (McClure) C.D. Chu & C.S. Chao, *Triplett 253, Triplett 287*; ***Pleioblastus nagashima*** (Mitford) Nakai, *Triplett 123, Triplett 54, Triplett 62, Triplett 75*; ***Pleioblastus pygmaeus*** (Miquel) Nakai, *Triplett 12, Triplett 127, Triplett 17, Triplett 28, Triplett 45*; ***Pleioblastus shibuyanus f. tsuboi (***Makino) S. Suzuki, *Triplett 30, Triplett 61*; ***Pleioblastus simonii (***Carrière) Nakai, *Triplett 232, Triplett 292, Triplett 293, Triplett 295, Triplett 296, Triplett 299, Triplett 322, Triplett 324, Triplett 325, Triplett 354, Triplett 355, Triplett 356, Triplett 357, Triplett 358, Triplett 363, Triplett 368, Triplett 372, Triplett 374, Triplett 376, Triplett 377, Triplett 379, Triplett 380, Triplett 384, Triplett 389, Triplett 391, Triplett 394, Triplett 395, Triplett 396, Triplett 399, Triplett 400, Triplett 401, Triplett 406, Triplett 409, Triplett 410, Triplett 413, Triplett 42, Triplett 9*; ***Pleioblastus aff. simonii*** (Carrière) Nakai , *Triplett 323, Triplett 382, Triplett 383*; ***Pleioblastus simonii*** (Carrière) Nakai x ***Pleioblastus hindsii*** (Munro) Nakai, *Triplett 143* ; ***Pleioblastus simonii* 'Heterophyllus'**, *Triplett 53*; ***Pleioblastus simonii*** 'Kishino', *Triplett 56*; ***Pleioblastus solidus*** S.Y. Chen, *Y. Zhang 6190 (KIB)*; ***Pleioblastus sp.*** **(**"unknown"), *Triplett 115, Triplett 51, Triplett 78*; ***Pleioblastus aff. variegatus*** (Siebold ex Miquel) Makino, *Triplett 361, Triplett 375, Triplett 385, Triplett 387, Triplett 353, Triplett 362, Triplett 367, Triplett 370, Triplett 373, Triplett 386, Triplett 398, Triplett 402, Triplett 412, T. Fukuda, s.n.*; ***Pleioblastus variegatus*** (Siebold ex Miquel) Makino 'Fortunei', *Triplett 37, Triplett 63*; ***Pleioblastus viridistriatus*** (Regel) Makino, *Triplett 29*; ***Pleioblastus aff. viridistriatus*** (Regel) Makino, *Triplett 414*; ***Pleioblastus viridistriatus* 'Chrysophyllus'**, *Triplett 154*; ***Pseudosasa amabilis*** (McClure) P.C. Keng, *Triplett 139, Triplett 16, Triplett 217*; ***Pseudosasa cantorii*** (Munro) P.C. Keng, *Triplett 114, Triplett 153*; ***Pseudosasa hindsii*** (Munro) S.L. Chen & G.Y. Sheng, *Y. Zhang 6078 (KIB), Y. Zhang 7013 (KIB)*; ***Pseudosasa japonica*** **(**Siebold & Zuccarini ex Steudel) Makino ex Nakai, *Triplett 122, Triplett 303, Triplett 319, Triplett 320, Triplett 369, Triplett 371, Triplett 381, Triplett 392, Triplett 403*; ***Pseudosasa japonica* var**. ***pleioblastoides*** Muroi, *Triplett 52*; ***Pseudosasa longiligula*** Wen, *Triplett 152*; ***Pseudosasa owatarii*** (Makino) Makino ex Nakai, *Triplett 33, Triplett 47, Triplett 48*; ***Pseudosasa usawae*** (Hayata) Makino & Nemoto, *Triplett 206*; ***Pseudosasa***

viridula S.L. Chen & G.Y. Sheng*, Triplett 282*; ***Sasa kurilensis*** (Ruprecht) Makino*, Triplett 223*; ***Sasa nipponica*** (Makino) Makino*, Triplett 306, Triplett 308*; ***Sasa oshidensis*** Makino and Uchida*, Triplett 161*; ***Sasa palmata (***Mitford) Camus*, Triplett 113, Triplett 228*; ***Sasa senanensis*** (Franchet and Savatier) Rehder*, Triplett 146*; ***Sasa shimidzuana*** Makino*, Triplett 416*; ***Sasa*** *tsukubensis* Nakai*, Triplett 317*; ***Sasa veitchii*** (Carrière) Rehder*, Triplett 126*; ***Sasa veitchii*** **(**Carrière) Rehder **var. *hirsuta****, Triplett 365*; ***Sasaella bitchuensis*** (Makino) Makino ex Koidzumi*, Triplett 128, Triplett 133*; ***Sasaella masamuneana*** (Makino) Hatusima & Muroi*, Triplett 289, Triplett 76*; ***Sasaella aff. masamuneana*** (Makino) Hatusima & Muroi*, Triplett 244*; ***Sasaella ramosa*** (Makino) Makino*, Triplett 118, Triplett 130, Triplett 288, Triplett 34*; ***Sasaella aff. ramosa*** (Makino) Makino*, Triplett 140, Triplett 314*; ***Sasaella sawadae*** (Makino) Makino ex Koidzumi*, Triplett 417, Triplett 418, Triplett 71*; ***Sasamorpha borealis*** (Hackel) Nakai*, Triplett 294, Triplett 298, Triplett 300, Triplett 311, Triplett 312, Triplett 407, L.G. Clark 1325*; ***Semiarundinaria fastuosa*** (Marliac ex Mitford) Makino ex Nakai*, Triplett 138, Triplett 77*; ***Semiarundinaria fortis*** Koidzumi*, Triplett 136*; ***Semiarundinaria kagamiana*** Makino*, Triplett 79*; ***Semiarundinaria makinoi*** Hisauti and Muroi*, Triplett 135, Triplett 160*; ***Semiarundinaria maruyamana*** Muroi*, Triplett 125, Triplett 203*; ***Semiarundinaria aff. maruyamana*** Muroi*, Triplett 393*; ***Semiarundinaria okuboi*** Makino*, Triplett 163*; ***Semiarundinaria yashadake*** (Makino) Makino *, Triplett 137.*

CHAPTER 6. GENERAL CONCLUSIONS

My research concerns the evolutionary history of the temperate woody bamboos, a diverse group of 19-31 genera and over 500 species in Asia, Africa, Madagascar, and North America (Ohrnberger 1999). The temperate bamboos are ecologically important in mountain forests and high elevation grasslands and have major roles in agriculture and horticulture with over 200 species in cultivation worldwide. Previous molecular studies demonstrated the monophyly of this group, yet its taxonomy has remained a source of great confusion, in part due to an incomplete understanding of natural variation and in part due to inadequate tools for lower-level phylogeny reconstruction (Li 1997; Ní Chonghaile 2002). Numerous classifications have been proposed on alternative interpretations of vegetative and reproductive features, while published molecular studies (Hodkinson *et al.* 2000; Zhang and Clark 2000; Guo *et al.* 2002; Guo and Li 2004) provide scant information and little or no support for genera or even subtribes defined by morphology.

Taxonomic problems in the temperate bamboos have thus involved poorly understood species complexes and a poorly resolved evolutionary framework. In light of the fundamental taxonomic questions surrounding these species, the growing discordance between natural populations and cultivars, and the status of many endemics as threatened or endangered, there is a pressing need for a cohesive phylogeny-based understanding of diversity in this important grass lineage. To this end, my research integrates traditional taxonomic and modern molecular methods to test the taxonomic position of genera while simultaneously examining species-level relationships and population-level genetic diversity.

This dissertation targeted the *Arundinaria* complex, a poorly characterized assemblage of East Asian genera presumed to be the closest extant links to the cane bamboos in the Southeastern US. Using a toolbox of fast-evolving molecular markers (plastid non-coding regions, AFLPs), I produced the first well-supported molecular phylogeny of this group in the context of the temperate bamboos, and provided species-level analyses of several subclades. The emerging phylogeny is at odds with current morphology-based classifications but provides strong support for key lineages as well as several surprising associations. Among the results of my analyses:

- Two major lineages emerge: one consisting of *Arundinaria* and allies (the *Arundinaria* Clade; ~10 genera, including *Pleioblastus* and *Sasa*), and another with *Phyllostachys* and allies (the *Phyllostachys* Clade; ~17 genera).
- Four unusual, phylogenetically isolated lineages were revealed: the *Shibataea* Clade (including *Shibataea* and *Ferrocalamus*; ~15 spp.), the Bergbamboes Lineage (*Thamnocalamus tessellatus*, in Africa), the African Alpine Bamboo Clade (~2 spp. in Central and East Africa and Madagascar), and the *Chimonocalamus* Clade (~11 spp; Southwest China, the Eastern Himalayas, and Myanmar). These taxa encompass relatively few species but a significant portion of the morphological diversity among temperate bamboos.
- Fieldwork, morphological studies, and DNA analyses (nucleotide sequences and AFLPs) were utilized to revise the taxonomy of *Arundinaria*, now recognized as a monophyletic North American endemic with 3 species, including the newly described *A. appalachiana* Triplett, Weakley & L.G. Clark. AFLP evidence also confirmed the occurrence of hybridization among these species.
- Surprisingly, the closest relatives of the North American species of *Arundinaria* may belong to *Sasa* and its allies, not the oft-synonymized genus *Pleioblastus*, although results were equivocal and additional work is needed.
- Several widely recognized genera were revealed to be intergeneric hybrids, including *Hibanobambusa*, *Sasaella*, and *Semiarundinaria*. Moreover, this investigation provided compelling evidence that the well-known species *Pseudosasa japonica* is also of hybrid origin, most likely involving *Sasamorpha borealis* and *Pleioblastus hindsii*.
- Many of the Chinese species tentatively but controversially placed in *Pleioblastus* (section *Amari*) and *Pseudosasa (*subgenus *Sinicae*) form a subclade with *Acidosasa* and *Indosasa*. This newly discovered assemblage, the *Sinicae* Clade, is currently under investigation in collaboration with Yuxiao Zhang (Ph.D. student; advisor, Dr. Dezhu Li) at the Kunming Institute of Botany, China. Other taxa in

this group were revealed to represent hybrids between the *Phyllostachys* Clade and the *Sinicae* Clade.

- Japanese *Pleioblastus* represent a distinct lineage (*Pleioblastus sensu stricto)* encompassing three subclades that are essentially congruent with the current morphology-based taxonomy of this group.
- Within *Pleioblastus* section *Pleioblastus*, one well-known plant in Southern Japan (nv. *Kanzan-chiku*) was revealed to be phylogenetically distinct from the plants in SE China of the same name. Because the type of this name rests with the Chinese entity, the Japanese species will require a new scientific name (Triplett, in prep.).
- *Pleioblastus* sections *Nezasa* and *Medakea* were revealed to be a genetically heterogeneous species complex, and may be involved in ongoing cryptic hybridization.

These observations reflect our best understanding of the temperate bamboos to date and establish a framework in which we can begin to explore evolutionary processes that may have been instrumental in their diversification, including large-scale phylogeographic patterns, hybridization and reticulation, shifts in diversification rates, and perplexing reproductive strategies. The framework also facilitates a re-evaluation of morphology and character evolution across the Temperate Clade. Most important, the results highlight the need for additional field and molecular systematic studies of the unusual and phylogenetically divergent groups, all of which are currently poorly represented in herbarium collections and scarcely understood in all aspects of their evolutionary biology.

FUTURE DIRECTIONS

Additional research is needed on at least two levels within the *Arundinaria* Clade. First, we need to obtain greater resolution among major subclades and genera. One possible method to resolve this issue would be to sequence the entire chloroplast genome for exemplar taxa from each major subclade. This approach has recently become both practical and affordable from a technical standpoint, and will likely facilitate major insights on bamboo phylogeny in the near future. Second, additional work is needed at lower taxonomic levels,

among species and within populations, utilizing methods that can address phylogenetic relationships in the context of cryptic hybridization. Now that a relatively robust phylogenetic framework is in place for a number of groups, it would be interesting to utilize nuclear introns to explore relationships in this group. Moreover, any studies of the population genetics of temperate bamboos would be of value simply because there is currently so little published.

Comparative studies of the unusual, phylogenetically isolated lineages can provide valuable information regarding the ancestry and diversification of the temperate bamboos. These divergent lineages harbor incredible diversity in vegetative form and ecology, yet phylogeographic studies of their morphological, molecular, and ecological characteristics are lacking. It is likely that detailed information on their phylogeny and natural history would dramatically improve our understanding of bamboo evolution by revealing both fundamental distinctions and unifying ancestral features.

Additional research is needed on taxa that have not been well studied so far, including species in Sri Lanka that are currently classified in the genus *Arundinaria*. Though the current taxonomic placement of these species is likely to be incorrect, their position is extremely interesting.

This project is complementary to recent revisionary work in the grass family in general and the woody bamboos in particular, and provides information that will facilitate future research in grass evolutionary biology. Modern monographs of the taxa revealed in this study, such as *Pleioblastus sensu stricto*, *Sasa s.s.,* and *Sasamorpha*, will provide baseline data for floristic studies, and morphological analyses combined with revised understanding of phylogeny will allow a better understanding of complex structures such as secondary branches and modified leaves to emerge.

LITERATURE CITED

GUO, Z.-H., Y.-Y. CHEN, AND D.-Z. LI. 2002. Phylogenetic studies on the *Thamnocalamus* group and its allies (Gramineae: Bambusoideae) based on ITS sequence data. Mol. Phyl. Evol. 22: 20-30.

GUO, Z.-H. AND D.-Z. LI. 2004. Phylogenetics of the *Thamnocalamus* group and its allies (Gramineae: Bambusoideae): inference from the sequences of GBSSI gene and ITS spacer. Mol. Phyl. Evol. 30: 1-12.

HODKINSON, T.R., S.A. RENVOIZE, G. NI CHONGHAILE, C.M.A. STAPLETON, AND M.W. CHASE. 2000. A comparison of ITS nuclear rDNA sequence data and AFLP markers for phylogenetic studies in *Phyllostachys* (Bambusoideae, Poaceae). J. Plant Res. 113: 259-269.

LI, D.Z. 1997. The Flora of China Bambusoideae Project: Problems and current understanding of bamboo taxonomy in China. In: Chapman, G.P. (ed.). The Bamboos. Linnean Society of London Symposium Series. Academic Press, London.

NÍ CHONGHAILE, G. 2002. Systematics of the woody bamboos (Tribe Bambuseae). Unpublished Ph.D. thesis, University of Dublin, Trinity College, Ireland.

OHRNBERGER, D. 1999. The Bamboos of the World. Elsevier, Amsterdam, The Netherlands.

ZHANG, W.P. AND L.G. CLARK 2000. Phylogeny and classification of the Bambusoideae (Poaceae). In S.W.L. Jacobs and J. Everett (eds.). Grasses: Systematics and Evolution: 35-42. CSIRO, Melbourne.

ACKNOWLEDGEMENTS

It has been my great honor to conduct the research described in this dissertation while a student in the lab of Dr. Lynn Clark at Iowa State University. Her guidance was a major inspiration for the work described herein, and the virtues of the research are largely a reflection of her excellence as a mentor. I am also grateful to the other members of my dissertation committee (Dr. Gregory Courtney, Dr. John Nason, Dr. Robert Wallace, and Dr. Jonathan Wendel), who provided valuable counsel and were excellent role models as faculty members in the biological sciences. I am especially grateful to Drs. Wallace and Wendel for providing access to their research facilities at ISU, and for the generosity of researchers in their labs. In particular, I am indebted to Ryan Percifield and Dr. Corrine Grover for valuable technical assistance. Many generous people have helped me in my travels to study and collect bamboo, including Dr. Dezhu Li, Yuxiao Zhang, Choken Ishigaki, Sue and Adam Turtle, Chris Tyrrell, Dr. Alan Weakley, Angus Gholson, Dr. Julian Campbell, Dr. Noriaki Murakami, Dr. Masatoshi Watanabe, Tomoko Fukuda, Marc Hilt, Jennifer York, Jesus Mora, Erika Harris, Stan Andreason, Chanda and Zack Skelton, Kousuke Takaki, Keiko Sakano, and my parents, Jim and Frankie Triplett. Lastly, significant portions of this research would not have been possible without the support of my wife, Masayo Ozaki, who not only provided encouragement and inspiration for my work at every step along the way but also contributed long hours helping me with field work in the US and Japan; the success of this research would not have been possible without her wisdom, patience, resourcefulness, and friendship.