



A new genus and a major temperate bamboo lineage of the Arundinarieae (Poaceae: Bambusoideae) from Sri Lanka based on a multi-locus plastid phylogeny

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Abstract

Kuruna, a new temperate woody bamboo (Poaceae, Bambusoideae, Arundinarieae) genus from Sri Lanka, is recognized based on chloroplast sequence data from five markers (coding: *ndhF* 3' end; non-coding: *rps16-trnQ*, *trnC-rpoB*, *trnD-trnT*, *trnT-trnL*). This genus represents the twelfth major lineage of temperate woody bamboos and is characterized by pachymorph culm bases with short necks, unicaespitose clumps, culm leaf girdles ca. 1 mm wide, usually abaxially hispid culm leaves with non-irritating hairs, persistent foliage leaf sheaths, complete branch sheathing and acute to biapiculate palea apices. Maximum Parsimony, Bayesian Inference and Maximum Likelihood analyses of a combined data set consistently strongly supported the monophyly of this Sri Lankan temperate woody bamboo clade. Although the Kishino-Hasegawa test is unable to reject the alternative hypothesis of monophyly of the Sri Lankan clade plus *Bergbambos tessellata* from South Africa, *Kuruna* and *Bergbambos* are distinguishable by a combination of morphological characters. A few additional cpDNA markers not previously used in phylogenetic analyses of Arundinarieae were tested to evaluate their utility in this taxonomically difficult tribe.

Introduction

Bamboos are important components of forest and tropical high altitude grassland ecosystems worldwide (Soderstrom & Calderón 1979, Judziewicz *et al.* 1999). The bamboos (Poaceae subfamily Bambusoideae) include approximately 1,450 species (Bamboo Phylogeny Group [BPG] 2012) classified into two tribes of woody bamboos (the tropical Bambuseae and the temperate Arundinarieae) and one tribe of herbaceous bamboos (the Olyreae). Significant animal biodiversity is associated with bamboo-dominated ecosystems (Judziewicz *et al.* 1999, Bystrakova *et al.* 2003, Mutschler & Tan 2003 & others cited in BPG 2012) and bamboos play important roles in forest dynamics (e.g., Li & Xue 1997, Judziewicz *et al.* 1999). Despite the ecological and economic importance of bamboos, basic knowledge of the biology and genetics of woody bamboos is still lacking due in part to their unusual life cycle, with the vegetative phase ranging from a few to 120 years (McClure 1966). Furthermore, the generic classification of bamboos is in a fluctuating state, although the supra-generic classification of bamboo has been improved based on recent phylogenetic analyses (BPG 2012).

The indigenous flora of Sri Lanka has about 7,000 species of mosses, ferns and flowering plants (Abeywicrama 1986). Nearly one fourth of the angiosperms of Sri Lanka are endemic and highly concentrated in the humid southwestern quarter of the country, which includes moist low country and the montane zone (Gunatilleke & Gunatilleke 1990). Bamboos occur naturally in all three major climatic zones (wet, dry and intermediate) in Sri Lanka and no native bamboo is found in extremely dry areas (Kariyawasam 1998). Bamboo, in general, is an economically and culturally important plant for Sri Lanka (De Zoysa & Vivekanandan 1994, Gunatilleke *et al.* 1994) and a series of studies have been conducted mainly focusing on bamboo reproductive ecology (Ramanayake & Yakandawala 1995, 1998, Ramanayake & Weerawardene 2003), vegetative propagation (Ramanayake *et al.* 2006) and bamboo growth and development (Ramanayake *et al.* 2001). These studies were carried out before the widespread use of molecular sequence data to establish evolutionary relationships and confirm the generic classification of these species.

Nine species native to Sri Lanka have been documented in Bambuseae and Arundinarieae (Soderstrom & Ellis 1988), eight of which are reported to be endemic (Dassanayake & Fosberg 1994). Of the eight endemic bamboos, five species are classified within the temperate woody bamboo genus *Arundinaria* Michaux (1803: 73) and they are found in high elevation montane forests or grasslands in Sri Lanka. But these shrubby montane-type *Arundinaria* species are not well suited for utility purposes (Kariyawasam 1998). All the Sri Lankan *Arundinaria* species are wind pollinated perennials as far as is known, each with a very limited distributional range (Soderstrom & Ellis 1988) and some are important components of high elevation grasslands in Sri Lanka.

Arundinarieae include ca. 550 species worldwide and are characterized by the presence of leptomorph, monopodial rhizomes (pachymorph in some species), basipetal vegetative branch development and tetraploidy ($2n=48$) (BPG 2012); molecular evidence strongly supports the monophyly of the temperate woody bamboos (BPG 2012, Kelchner *et al.* 2013). Among the temperate bamboos, *Arundinaria* is the oldest generic name and over 400 species have at one time or another been classified within it. In the traditional sense, at least since McClure (1973), the genus also includes East Asian, African and Madagascan species. Soderstrom & Ellis (1988) treated *Arundinaria* in the broad sense and included the species from Sri Lanka, India and China. In addition, according to the Soderstrom & Ellis (1988) study, all the Sri Lankan *Arundinaria* species exhibited similarities with the Chinese and Indian *Arundinaria* species that had been placed by some authors (Yi 1983) in another segregate genus, *Fargesia* Franchet (1893: 1067). Other authors have included these species and similar taxa from Africa and Madagascar in the genus *Yushania* P.-C. Keng (1957: 355) (Majumdar 1989).

To date, 11 major lineages are found in the temperate woody bamboo clade, mainly based on cpDNA sequence analyses, but the relationships among these clades are still not resolved (Triplett & Clark 2010, Zeng *et al.* 2010, Yang *et al.* 2013). Based on recent phylogenetic studies, *Arundinaria* is now restricted to the three North American species: *A. gigantea* (Walter 1788: 81) Muhlenberg (1813: 14) (type species), *A. tecta* (Walter 1788: 81) Muhlenberg (1813: 14) and *A. appalachiana* Triplett, Weakley & Clark (2006: 88) (Triplett & Clark 2010). Thus, the generic classification of the Sri Lankan species must be updated in light of this recent work.

The primary objective of the current study was to conduct a molecular phylogenetic analysis of the native Sri Lankan *Arundinaria* species using chloroplast DNA sequencing, with a particular emphasis on testing the monophyly of this group and placing them in the correct genus or genera. A detailed morphological comparison of the native Sri Lankan *Arundinaria* species and putatively taxonomically related taxa within Arundinarieae was also conducted to identify distinguishing features for this group. In addition, several cpDNA markers not previously used in phylogenetic analyses of Arundinarieae were tested to evaluate their utility in resolving relationships among the major temperate bamboo clades.

Materials and methods

Taxon sampling and outgroup selection:—All five known species and a potentially new Sri Lankan *Arundinaria* species were sampled for this study. In addition, representatives from ten of the 11 currently recognized temperate clades were selected based on previous studies (Triplett & Clark 2010, Zeng *et al.* 2010) and their sequences downloaded from GenBank (<http://www.ncbi.nlm.nih.gov/genbank/>) (Appendix 1). Sequence data for Clade XI, which includes only *Ampelocalamus calcareus* C.-D. Chu & C.-S. Chao (Chao & Chu 1983: 204) (Yang *et al.* 2013), was not available when this study was conducted and therefore was not included. Several taxa were selected as outgroups based on prior studies (Triplett & Clark 2010, Zeng *et al.* 2010): *Brachyelytrum erectum* (Schreber 1789: 97) Palisot de Beauvois (1812: 155) (Pooideae), *Chusquea spectabilis* L.G. Clark (Fisher *et al.* 2009: 681) (neotropical woody bamboos), *Guadua angustifolia* Kunth (1822: 253) (neotropical woody bamboos) and *Bambusa vulgaris* Schrader ex Wendland (Wendland 1808: 26) (paleotropical woody bamboos).

Chloroplast DNA Marker Selection:—Based on prior (Triplett & Clark 2010) and preliminary studies, four intergenic regions (*rps16-trnQ*, *trnC-rpoB*, *trnD-trnT*, *trnT-trnL*) and one coding region (*ndhF* 3' end) that provided reasonable numbers of parsimony-informative characters for temperate species were selected. An additional set of cpDNA markers, three from domain IV [D4] of chloroplast group II introns (*petD*, *atpF*, *ndhA*) and three other intergenic regions (*psbD-trnT*, *psbJ-petA* and *ycf6-psbM*), based on previous studies (Shaw *et al.* 2005, Shaw *et al.* 2007, Watts *et al.* 2008) were selected to evaluate their potential utility in the temperate clade and in resolving the relationships of the Sri Lankan *Arundinaria* species.

TABLE 1. Chloroplast DNA primers used for amplification and sequencing. * indicates published primer sequences that were modified from Triplett & Clark (2010); underlined text indicates modified nucleotide sites. SEQ indicates primers used for sequencing reactions, if different from the PCR primers. § indicates regions that showed very low genetic variation for the temperate clade.

Region	Primer sequences (5'-3')	PCR Parameters	Reference
<i>ndhF</i> (3' end)	972F: GTCTCAATTGGGTTATATGATG	94°C, 1 min; 30x (94°C, 1 min 30 sec; touchdown 53–43°C, 2 min; 72°C, 3 min); 72°C 10 min.	Olmstead & Sweere (1994)
	2110R: CCCCCTAYATATTTGATACCTTCTCC		
	SEQ: 1318F*: GGATTA <u>ACT</u> GCGTTTTATATGTTTCG		
	1603R: GCATAGTATTTCCCGTTTCATGAGG		
5' end:	1F: GCACGTTGCTTTCTACCACA	95°C, 2 min; 35x (95°C, 1 min; 48°C, 10 sec; +17°C, 0.3°C/ sec; 65°C, 5 min); 65°C, 5 min.	Triplet & Clark (2010)
	929R: TTCTGTCTACTCGGCTTTTCG		
<i>rps16-trnQ</i> (1) (for temperate bamboos)	3' end: 538F: CGACTCGAATACCAAAAGAGG	Note: For some taxa, 628R was used to sequence the 5' amplicon [see <i>rps16-trnQ</i> (2) for primer sequence]	Triplet & Clark (2010)
	1574R: ATCCTTCCGTCACAGATTTT		
	SEQ: (5') 16Q 650R: GTTCGTTGGATAGAATGGATTC		
	(3') 16Q in-for: GCCGAGTAGACAGAATATATG		
<i>rps16-trnQ</i> (2) (for all other taxa)	(3') 16Q 1100R: GGCCAGATTAAGAATAGGAAG	95°C, 2 min; 35x (95°C, 1 min; 50°C, 10 sec; +15°C, 0.3°C/ sec; 65°C, 5 min); 65°C, 5 min.	Triplet & Clark (2010)
	1F: GCACGTTGCTTTCTACCACA		
	1574R: ATCCTTCCGTCACAGATTTT		
	SEQ: 334F: CGAGATGGTCAATCCTGAAATG		
<i>trnC-rpoB</i>	628R: CTTTTGGTATTCKAGTCGAAAG	94°C, 2 min; 35x (96°C, 1 min; touchdown 56–46°C, 2 min; 72°C, 3 min); 72°C, 5m.	PCR: Yamane & Kawahara (2005); SEQ: Triplet & Clark, 2010
	trnC: TGGGGATAAAGGATTTGCAG		
	rpoB*: ATTGTTGGACATTCCTCRIT		
	SEQ: jt400-for: CAGGTCCGAACAGCATT		
<i>trnD-trnT</i>	jt700-rev: CGTAGTAGTAGAATTGCTAG	94°C, 2 min; 35x (94°C, 45 sec; touchdown 58–48.5°C, 1 min; 72°C, 1 min 15 sec); 72°C, 5 min	trnD-for: Demesure <i>et al.</i> (1995); trnT-rev, trnY-rev: Triplet & Clark (2010); trnE-for: Doyle <i>et al.</i> (1992)
	trnD-for: ACCAATGAACTACAATCCC		
	trnT-rev: CCCTTTAACTCAGTGGTA		
	SEQ: trnY-rev: CTCTTTGCTTTGGATCTAG		
<i>trnT-trnL</i>	trnE-for: GCCTCCTTGAAAGAGAGATG	95°C, 2 min; 35x (95°C, 1 min; 48°C, 10 sec; +17°C, 0.3°C/ sec; 65°C, 5 min); 65°C, 5 min	Taberlet <i>et al.</i> (1991)
	trnT-L F: CATTACAAATGCGATGCTCT		
<i>atpF</i> intron D4	trnT-L R: TCTACCGATTTCCCATATC	80°C, 5min; 35x (65°C, 1min; 0.3°C/s, 50 °C, 1min; 65°C 1.5 min); 65°C, 4 min	Watts <i>et al.</i> (2008)
	sak21F: AAAGGGAGTGTGTGYGAGTT		
<i>ndhA</i> intron D4	sak22R: CCCGAACCAAAAYATGAATCTTTC	80°C, 5m; 35x (65°C, 1min; 0.3°C/s, 50 °C, 1min; 65°C 1.5 min); 65°C, 4 min.	Watts <i>et al.</i> (2008)
	sak26F: CAATATCTCTACGTGYGATTCG		
<i>petD</i> intron D4	sak28R: AACTGTTTRGATAATCATAGTCG	80°C, 5min; 35x (65°C, 1min; 0.3°C/s, 50 °C, 1min; 65°C, 1.5 min); 65 °C, 4 min.	Watts <i>et al.</i> (2008)
	sak17F: GGATTATGGGAGTGTRYGACTTG		
	sak18R: CTTTGTTATTGGGATAGGTGAA		
	SEQ: sak19F: GAGACRAYCCANAAAGCA		
<i>psbD-trnT</i>	sak18R: CTTTGTTATTGGGATAGGTGAA	80°C, 5min; 30x (95°C, 1min; 50°C, 0.3°C/ s, 50 °C, 1min; 65°C, 4 min); 65°C, 5 min	Shaw <i>et al.</i> (2007)
	psbD: CTC CGT ARC CAG TCA TCC ATA		
<i>psbJ-petA</i>	trnT(GGU)-R: CCC TTT TAA CTC AGT GGT AG	80°C, 5min; 30x (95°C, 1min; 50°C, 1 min, ramp of 0.3°C/s to 65°C; 65°C, 4 min); 65°C, 5 min	Shaw <i>et al.</i> (2007)
	psbJ: ATA GGT ACT GTA RY GGT ATT		
<i>ycf6-psbM</i>	petA: AAC ART TYG ARA AGG TTC AAT T	80°C, 5min; 35x (94°C, 1min; 50–55°C, 1 min, 72°C, 3.5 min); 72°C, 5 min	Shaw <i>et al.</i> (2005)
	ycf6F: ATG GAT ATA GTA AGT CTY GCT TGG GC		
	psbMR: ATG GAA GTA AAT ATT CTY GCA TTT ATT GCT		

DNA Extraction, Sequencing, Alignment, and Character Coding:—Total genomic DNA extractions were performed from silica gel-dried specimens using the Iowa State University DNA Facility's Autogenprep 740 DNA extraction robot. Primers for polymerase chain reactions (PCR) and sequencing protocols of all the markers are listed in Table 1. All PCR and cycle-sequencing reactions were performed in an MJ Research PTC-200 thermal cycler. PCR was performed in 25µL volumes. Amplification products were cleaned using polyethylene glycol (PEG) precipitation to remove unincorporated primers and dNTPs from the PCR products. Sequencing was performed on an ABI 3730xl

DNA Analyzer (Perkin-Elmer, Applied Biosystems Division, Norwalk, Connecticut) by the DNA Sequencing Facility at Iowa State University. Automated sequencing output was checked visually for correct automated base-calling. DNA sequences were aligned manually in Se-AL (Rambaut 2001). Gaps introduced with the sequence alignment were later treated as binary, presence/absence characters (Giribert & Wheeler 1999). Autapomorphic, parsimony uninformative indels were not scored, and they were excluded along with other gaps prior to analysis.

Phylogenetic Analyses:—All data were analyzed with Maximum Parsimony (MP), Maximum Likelihood (ML) and Bayesian Inference (BI) methods. MP analyses were conducted using PAUP* 4.0b10 (Swofford 2002), ML analyses were conducted using RAxML version 7.2.6 (Stamatakis 2006), and BI analyses were conducted using MrBayes 3.1 (Ronquist *et al.* 2005).

In MP analyses all characters were treated as unweighted and unordered. Heuristic tree searches were conducted with Tree Bisection and Reconnection (TBR) branch swapping. The initial trees for branch swapping were obtained by 1000 random stepwise taxa addition replicates. To assess the relative support for each node, bootstrap analysis was conducted from 1000 replicates with the heuristic search method and strict consensus trees were calculated. The Akaike information criterion (AIC) calculations, implemented in JmodelTest 0.1 (Guindon & Gascuel 2003, Posada 2008), were used to select the appropriate model of sequence evolution for each DNA partition and for the combined data set excluding indels. ML analyses were conducted using RAXML, version 7.2.6 (Stamatakis 2006), invoking a rapid bootstrap (1000 replicates) analysis and search for the best-scoring Maximum Likelihood Tree with the general time-reversible model of DNA sequence evolution with gamma-distributed rate heterogeneity (the GTRGAMMA model); this was performed for each DNA partition and for the combined data set. BI was conducted with flat priors. The Markov chain Monte-Carlo algorithm was executed for four chains for 10 million generations per run, sampling every 1,000 generations, and a chain-heating temperature of 0.2; this entire procedure was conducted twice. Posterior Probabilities (PP) were analyzed after a burn-in of 10,000 trees and then the remaining samples were summarized and a majority-rule consensus trees were constructed. The BI analyses were conducted for each DNA partition and for the combined dataset. When assessing conflicts among the resultant phylogenies, the threshold value for the bootstrap criterion for both MP and ML was 70% and posterior probability measure for BI was 0.95 (Mason-Gamer & Kellogg 1996, Wilcox *et al.* 2002). Values less than 70% MP Bootstrap/ML Bootstrap and less than 0.95 PP were considered as lacking support.

We tested whether the combined dataset provided sufficient evidence to reject particular hypotheses of relationships suggested by previous morphological studies (e.g., monophyly of the Sri Lankan *Arundinaria* and *Arundinaria* s.s. from North America, monophyly of Sri Lankan *Arundinaria* and other Indian and African temperate bamboo species). Constraint trees were generated in MacClade 4.08 (Maddison & 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. The Kishino-Hasegawa (K-H) test (Kishino & Hasegawa 1989) as implemented in PAUP* was then used to test the significance of differences in tree statistics amongst different topologies in comparison with the MP topologies.

In addition, the number of nucleotide substitutions, indels, and inversions (hereafter referred to collectively as Potentially Informative Characters or PICs) (Shaw *et al.* 2007) between the ingroup species and between either ingroup species and the outgroup species were tallied for each cpDNA region to evaluate the potential use of these cpDNA regions in the temperate clade (Shaw *et al.* 2007). The average number of PICs found within each cpDNA region was then computed.

Morphological Comparison:—A total of 21 vegetative and reproductive characters were examined in the morphological comparison across *Arundinaria* s.s., Sri Lankan *Arundinaria*, the *Thamnocalamus* Munro (1868: 157) clade, *Bergbambos* and *Oldeania* of the African alpine bamboo clade to understand the differences and similarities among these taxa. Even though the phylogenetic analyses include both *Oldeania alpina* and *Yushania ambositrensis* (Camus 1913:78) Ohrnberger (1999: 14), the two known taxa of the African alpine bamboo clade, the morphological comparison includes only *Oldeania alpina* due to the lack of good material of *Yushania ambositrensis*. The Sri Lankan *Arundinaria* species were also compared morphologically with *Yushania*, *Chimonobambusa* Makino (1914: 153) and *Indocalamus* Nakai (1925: 148), since some of the Sri Lankan species were included in these genera by previous authors (Nakai 1925, Majumdar 1989). In addition, *Fargesia* was included in this comparison, because Soderstrom & Ellis (1988) discussed morphological resemblances of these Sri Lankan species with the Chinese and Indian *Arundinaria* species that have been placed by some authors (Yi 1983) in *Fargesia*. As *Chimonobambusa* was easily distinguished from the other genera by the combination of leptomorph rhizomes, culms grooved above the basal branches, basal nodes with subequal multiple buds, more or less equal primary branches and pseudospikelets, this genus was not included in the comparison.

The choice of morphological characters for this comparison was based on Stapleton (2013), the characters in the Bamboo Biodiversity website (Bamboo Phylogeny Group 2005), some of which have been used for Bamboo Phylogeny Group morphological phylogenetic analyses, and direct examination of herbarium specimens (held at ISC, K, MO, PDA, US). For each genus, the entire genus was considered whenever possible. For *Fargesia* and *Indocalamus*, the type species of each genus has been included in molecular analyses along with other taxa from each but neither genus as currently delimited is demonstrably monophyletic (e.g., Yang *et al.* 2013). In addition, many species of *Fargesia* are unknown in flower (Li *et al.* 2006) and relatively little herbarium material is accessible for the majority of species in these two genera. We therefore used the type species for each genus as the most appropriate representatives.

Results

Figure 1 shows the average PIC (Potentially Informative Character) values found within each of the 11 tested cpDNA regions. Out of the 11 regions, only *rps16-trnQ*, *trnC-rpoB*, *trnD-trnT*, *trnT-trnL* and *ndhF* 3' gave enough variation in the temperate bamboo clade to provide resolution. The highest percentage average PIC value was 7.82% for *rps16-trnQ* while the second highest was 5% for *trnT-trnL*, whereas the regions *ndhA*, *ycf6-psbM*, *atpF*, *psbJ-petA* had percentage average PIC values less than 1.00% and *petD* and *psbD-trnT* showed no variation. In addition, *trnD-trnT*, *ndhF* (3' end) and *trnC-rpoB* had very similar percentage average PIC values: 3.37%, 3.33% and 3.30% respectively. The combined, aligned data matrix for *rps16-trnQ*, *trnC-rpoB*, *trnD-trnT*, *trnT-trnL* and *ndhF* 3' end was 4,885 bp long, with 49 indels. Only 223 characters (4.6%) were parsimony informative (PIC). A total of 172 sequences were used for this study and the data matrix was composed of 164,997 data points excluding the data scored as missing, which was 6.71% (11,823) of the total data. Table 2 summarizes statistics for each of the 11 regions selected for the temperate species. Relatively low genetic variation was found among the sequences for the chloroplast markers used. Maximum Parsimony analysis of the combined five-region dataset resulted in 1019 shortest trees of 967 steps, with a consistency index (CI) of 0.6014 (uninformative characters excluded) and a retention index of 0.7352.

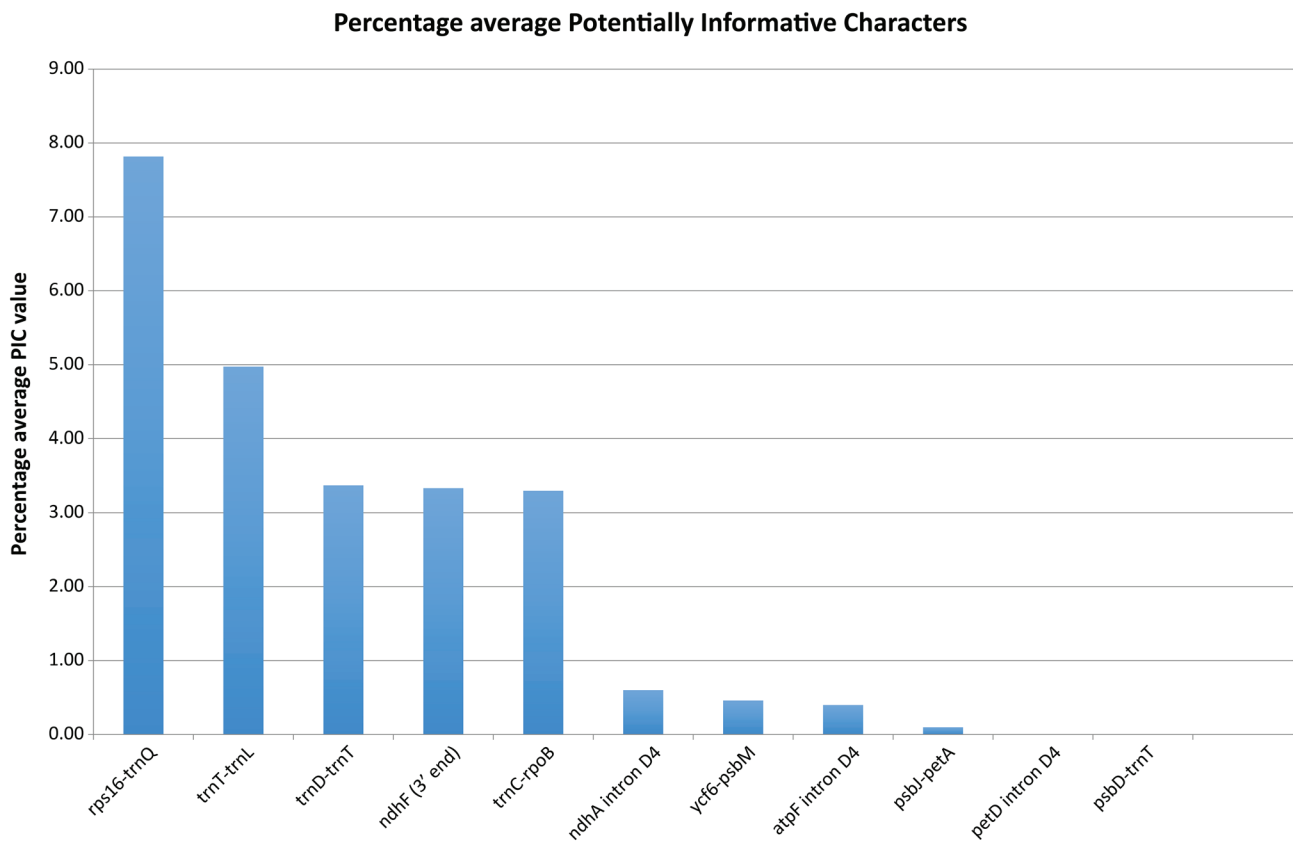


FIGURE 1. Percentage Potentially Informative Character values for all 11 chloroplast regions. For the regions *rps16-trnQ*, *trnC-rpoB*, *trnD-trnT*, *trnT-trnL* and *ndhF* 3' the PIC values include the outgroups. For the regions *psbD-trnT*, *psbJ-petA* and *ycf6-psbM* the PIC values include only the ingroup species.

TABLE 2. Statistics and evolutionary models for each region and the combined analyses. Evolutionary models and phylogenetic analyses were conducted only for the first five regions *ndhF* (3' end), *rps16-trnQ*, *trnC-rpoB*, *trnD-trnT* and *trnT-trnL*, which showed reasonable PIC values. Statistics for the first five regions are based on the five region, 36 taxon data matrix. * indicates the combined data set of five regions including all the indels. § indicates the markers that showed very low genetic variation for the temperate clade and amplified for a subset of species (~7 species). bp = base pairs, CI = Consistency Index, excluding uninformative characters, MP = Maximum Parsimony, PIC = Parsimony Informative Characters, RI = Retention Index. Models are based on the Akaike information criterion (AIC) calculations implemented in JmodelTest 0.1.

Partition	Length (bp)	Indels	Total char.	Char., no gaps	PIC	MP trees	MP length	CI	RI	Model
<i>ndhF</i> (3' end)	1,170	0	1,170	1,120	39	195	174	0.6324	0.6753	TVM+G
<i>rps16-trnQ</i>	1,740	14	1,754	1,140	136	193	235	0.6383	0.7862	GTR+G
<i>trnC-rpoB</i>	1,395	14	1,409	965	46	174	191	0.6533	0.8452	TVM+I+G
<i>trnD-trnT</i>	1,335	13	1,348	960	45	194	193	0.6883	0.7838	TVM+G
<i>trnT-trnL</i>	925	8	933	650	46	168	170	0.6118	0.7381	GTR+I
5-region, all data*	6,565	49	6,614	4,835	223	1019	967	0.6014	0.7352	TVM+G
§ <i>atpF</i> intron D4	755	2	757	---	3	---	---	---	---	---
§ <i>ndhA</i> intron D4	999	1	1,000	---	6	---	---	---	---	---
§ <i>petD</i> intron D4	765	0	765	---	0	---	---	---	---	---
§ <i>psbD-trnT</i>	1,500	0	1,500	---	0	---	---	---	---	---
§ <i>psbJ-petA</i>	1,020	0	1,020	---	1	---	---	---	---	---
§ <i>ycf6-psbM</i>	870	0	870	---	4	---	---	---	---	---

As shown in Figure 2, MP, ML and BI analyses of the combined, 5-region dataset all recovered 11 major temperate bamboo lineages: *Bergbambos* (Clade I), African alpine bamboos (Clade II), *Chimonocalamus* J.R. Xue & T. P. Yi (1979: 76) (Clade III), *Shibataea* Makino ex Nakai (Makino 1912: 236) clade (Clade IV), *Phyllostachys* Siebold & Zuccarini (Muroi 1963: 13) clade (Clade V), *Arundinaria* clade (Clade VI), *Thamnocalamus* (Clade VII), *Indocalamus wilsonii* (Rendle 1914: 63) Chao & Chu (1981: 43) (Clade VIII), *Gaoligongshania* D.-Z. Li, C.-J. Hsueh & N.-H. Xia (1995: 598) (Clade IX), *Indocalamus sinicus* (Hance 1876: 336) Nakai (1925: 148) (Clade X) (Triplett & Clark 2010, Zeng *et al.* 2010), and the Sri Lankan *Arundinaria* clade (Clade XII). In the current study, the monophyly of the temperate woody bamboo clade was highly supported, with 100% Maximum Parsimony Bootstrap, 100% Maximum Likelihood Bootstrap and 1.00 PP. The Sri Lankan *Arundinaria* clade received maximal support (Maximum Parsimony Bootstrap 100%; Maximum Likelihood Bootstrap 100%; PP 1.0). Relationships within the Sri Lankan *Arundinaria* clade were unresolved, as was the case for the other major lineages, except for the *Arundinaria* clade. Further, the African alpine bamboo (Clade II), *Shibataea* (Clade IV) and *Arundinaria* (Clade VI) clades each received moderate MP support, but strong support from the BI (all 1.00 PP). Only the African alpine bamboos and the *Arundinaria* clades received strong support from the ML analysis (95% and 95% respectively) whereas the *Shibataea* clade received no MLBS support. In addition, five lineages were represented by a single species: *Bergbambos tessellata* (Nees von Esenbeck 1834: 482) Stapleton (2013: 99), *Indocalamus wilsonii*, *Thamnocalamus spathiflorus* (Trin.) Munro (1868: 34), *Gaoligongshania megalothyrsa* (Handel-Mazzetti 1936: 1271) D.Z. Li, J.R. Xue & N.H. Xia (Li, Hsueh & Xia 1995: 601) and *Indocalamus sinicus*. However, *Chimonocalamus pallens* J.R. Xue & T.P. Yi (1979: 78) (the type species of *Chimonocalamus*) did not cluster with *Chimonocalamus montanus* J.R. Xue & T.P. Yi (1979:80) and thus *Chimonocalamus* was resolved as polyphyletic.

Results of the K-H test are summarized in Table 3. Based on the K-H test, our data reject the monophyly of a group consisting of Sri Lankan *Arundinaria*, *Arundinaria* s.s. (Clade VI), *Thamnocalamus* (Clade VII), *Bergbambos tessellata* (Clade I), and the African alpine bamboos (Clade II), i.e., the hypothesis that the Sri Lankan *Arundinaria* species belong to *Arundinaria* in the broad sense. Data also reject the monophyly of Sri Lankan *Arundinaria* + *Arundinaria* s.s. and monophyly of Sri Lankan *Arundinaria* + the *Thamnocalamus* clade. Further, monophyly of the Sri Lankan *Arundinaria* + African alpine bamboos is rejected by the K-H test. Despite the lack of resolution among lineages, the K-H test could not reject the monophyly of the Sri Lankan *Arundinaria* species (Clade XII) plus the South African mountain bamboo *Bergbambos tessellata*.

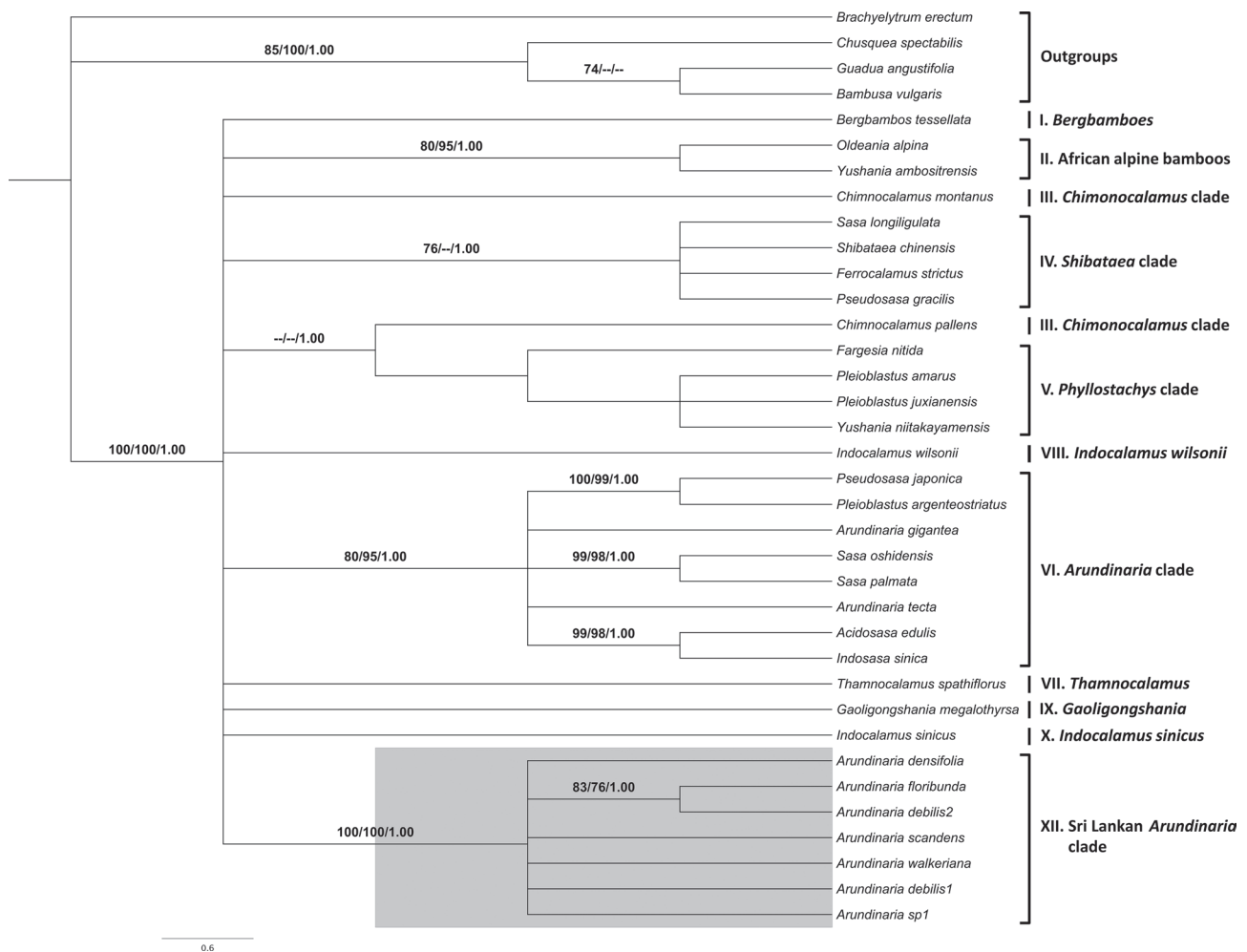


FIGURE 2. Strict consensus of 1019 most parsimonious trees based on the five-region cpDNA dataset (*rps16-trnQ*, *trnC-rpoB*, *trnD-trnT*, *trnT-trnL*, *ndhF 3'*). Shaded region indicates the well supported Sri Lankan *Arundinaria* clade. Numbers indicate bootstrap values $\geq 70\%$ from MP and ML analyses and posterior probabilities ≥ 0.95 from the BI analyses, respectively. Note that Clade XI is not shown in the tree because it was unsampled.

Results of the morphological comparison are reported in Table 4. Some of the characters, such as culm leaf auricles and palea apex (Figure 3), were variable within the Sri Lankan *Arundinaria* clade. Culm leaf blade position was quite variable within the Sri Lankan *Arundinaria*, *Arundinaria* s.s., *Thamnocalamus spathiflorus* and *Yushania* clades, whereas other characters listed in Table 4 were much more consistent.

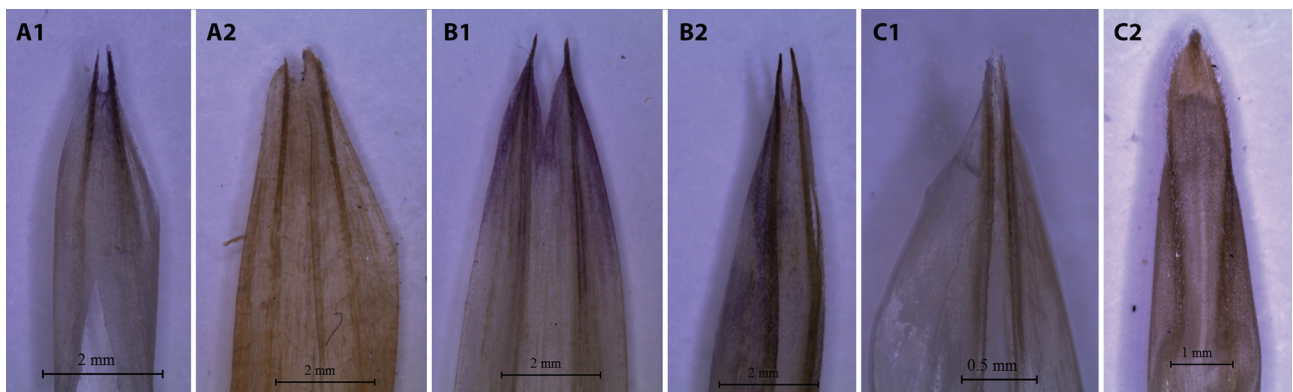


FIGURE 3. The three different types of palea apices. **A1**—biapiculate (sinus shallow) palea apex of *Yushania nitakayamensis* (Hayata) P.-C. Keng (1957: 357) and **A2**—biapiculate (sinus shallow) palea apex of *Bergbambos tessellata*; **B1**—long-divided tips (sinus deep) palea of *Arundinaria gigantea* and **B2**—long-divided tips (sinus deep) palea of *Fargesia spathacea* Franchet (1893: 1067); **C1**—acute, undivided palea apex of *A. debilis* and **C2**—acute, undivided palea apex of *Oldeania alpina* (K. Schum.) Stapleton (2013: 100).

TABLE 3. Hypotheses regarding clades and relationships among them. All hypotheses were tested under MP using the Kishino-Hasegawa test. The difference between the MP trees and those consistent with the constraint were reported. * indicates $p < 0.05$

Hypothesis	Results of K-H Test
Sri Lankan <i>Arundinaria</i> , <i>Arundinaria</i> s.s, <i>Thamnocalamus</i> , <i>Bergbambos</i> , and African Alpine bamboos are monophyletic	Reject (+7 steps, $p=0.000^*$)
Sri Lankan <i>Arundinaria</i> and <i>Arundinaria</i> s.s are monophyletic	Reject (+8 steps, $p=0.000^*$)
Sri Lankan <i>Arundinaria</i> and <i>Thamnocalamus</i> are monophyletic	Reject (+7 steps, $p=0.000^*$)
Sri Lankan <i>Arundinaria</i> and <i>Bergbambos</i> are monophyletic	Cannot reject (-2 steps, $p=0.078$)
Sri Lankan <i>Arundinaria</i> and African Alpine bamboos are monophyletic	Reject (+1 step, $p=0.000^*$)

Discussion

As predicted, the chloroplast genome appears to be conserved within the temperate clade, consistent with prior studies (Triplett *et al.* 2010, Zeng *et al.* 2010). Even though 11 relatively variable markers were tested in the current study, only five markers were selected for further phylogenetic analyses due to the lack of molecular variation within six of the markers as seen in a subset of seven species from across the temperate woody bamboo clade. These five markers, *ndhF* (3' end), *rps16-trnQ*, *trnC-rpoB*, *trnD-trnT* and *trnT-trnL*, were successfully utilized in previous studies to understand the relationships among Arundinarieae (Triplett & Clark 2010, Zeng *et al.* 2010, Yang *et al.* 2013).

The six markers that did not provide enough molecular variation for further use in this study had not been previously utilized in the temperate woody bamboo clade. Watts *et al.* (2008) successfully amplified and sequenced the markers *petD*, *atpF* and *ndhA* from domain IV (D4) of chloroplast group II introns, on a sample of closely related species in *Chusquea* Kunth (1822: 151), a genus of Neotropical woody bamboos. Shaw *et al.* (2007) utilized *psbD-trnT*, *psbJ-petA* and *ycf6-psbM* in a wide sample of flowering plants, both eudicots and monocots (including Poaceae) and revealed that these markers could be potential candidates to resolve genus and species-level molecular phylogenetic questions. However, none of these six markers showed enough variation in the temperate woody bamboo clade to resolve phylogenetic relationships due to their relatively low level of sequence divergence.

Lineages within the Arundinarieae:—The current study is the third analysis sampling almost all of the known temperate bamboo clades (Triplett & Clark 2010, Zeng *et al.* 2010) and the first to include the Sri Lankan *Arundinaria* species. Even though the monophyly of the temperate clade is always highly supported, relationships within the clade have been difficult to resolve based on cpDNA markers alone or cpDNA plus one or two nuclear markers (Triplett & Clark 2010, Zeng *et al.* 2010, Yang *et al.* 2013). Our results, based on five cpDNA markers, are consistent with these previous findings, but the molecular topology we obtained revealed a new, robustly supported major temperate bamboo lineage, designated as Clade XII, that includes all the Sri Lankan species previously recognized as *Arundinaria* by Soderstrom & Ellis (1988).

We were unable to resolve relationships among the 11 lineages of temperate woody bamboos sampled here, consistent with other analyses (Triplett & Clark 2010, Zeng *et al.* 2010, Yang *et al.* 2013). Several factors could account for the poor resolution among the lineages of temperate woody bamboos. An obvious explanation is a lack of phylogenetic signal, reflecting the relatively conserved nature of the plastid genome in these bamboos (Triplett & Clark 2010). The long generation times (up to 120 years) characteristic of many temperate woody bamboos may also affect the rate of molecular evolution in this group (Janzen 1976, Gaut *et al.* 1997, Smith & Donoghue 2008), although more study is needed to explore this in woody bamboos generally. Triplett *et al.* (2010) also suggested that reticulate evolution is much more significant in the temperate bamboos than previously predicted, but Triplett & Clark (2010) noted that incomplete lineage sorting could be an additional factor. Further, some recent studies have proposed that relatively recent and rapid diversification within Arundinarieae could be another cause for poorly resolved phylogenetic patterns, especially at the generic level (Stapleton *et al.* 2009, Hodkinson *et al.* 2010, Stapleton 2013).

As noted previously, Clade XI (consisting of the single species *Ampelocalamus calcareus*) was not included in this analysis due to its recent discovery (Yang *et al.* 2013) and the lack of material for comparable sequencing.

TABLE 4. Comparative table of morphological characters for the “*Arundinaria*” groups. “?” indicates unknown material.

	Sri Lankan <i>Kuruna</i> clade [Clade XII]	<i>Arundinaria</i> s.s. [Clade VI]	<i>Bergbambos tessellata</i> [Clade I]	<i>Thamnochlamus spathiflorus</i> [Clade VIII]	<i>Yushania</i> [Clade V]	<i>Fargesia</i> (<i>F. spathacea</i> -type) [Clade V]	<i>Indocalamus</i> (<i>I. sinicus</i> -type) [Clade X]	<i>Oldeania alpina</i> [Clade II]
Vegetative Characters								
Rhizomes	Pachymorph culm bases with short necks	Leptomorph rhizomes	Pachymorph culm bases with short necks (2.5–30 cm)	Pachymorph culm bases with short necks	Pachymorph culm bases often with long necks	Pachymorph culm bases with short necks	Leptomorph rhizomes	Pachymorph culm bases with long necks
Clump form	Unicaespitose	Unicaespitose or pluricaespitose	Unicaespitose	Unicaespitose	Pluricaespitose	Unicaespitose	Pluricaespitose	Culms solitary
Culm grooves	Absent	Present in <i>A. gigantea</i>	Absent	Absent	Absent	Absent	Absent	Present (sulcate)
Supranodal ridge diameter	Prominent, wider than nodes (<i>A. debilis</i> & <i>A. floribunda</i>) or more or less the same diameter as the adjacent internodes (other 4 spp.)	More or less the same diameter as the adjacent internodes	More or less the same diameter as the adjacent internodes	More or less the same diameter as the adjacent internodes	More or less the same diameter as the adjacent internodes	More or less the same diameter as the adjacent internodes	More or less the same diameter as the adjacent internodes	Well developed, its diameter greater than the adjacent internodes
Culm leaf girdle	Present as a band at least 1mm wide	Absent or poorly developed	Absent or poorly developed	Present as a band at least 1mm wide	Present as a band at least 1mm wide	Absent or poorly developed	Present and usually yellow brown	Present as a band at least 1mm wide
Culm leaves abaxial surface	Mostly hispid	Mostly glabrous	Glabrous	Glabrous	Hispid with appressed brown hairs, becoming glabrous when mature	Hispid with dark brown irritating hairs or glabrous	Mostly glabrous	Hispid
Culm leaf auricle / fimbriae	Absent (except <i>A. debilis</i> with minute fimbriate auricles)	Usually with well-developed fimbriate auricles	Small fimbriate auricles present	Small fimbriate auricles present	Fimbriate auricles present	Absent	Auricles absent, summit fimbriate	Small fimbriate auricles present
Culm leaf blade position	Reflexed (except erect in <i>A. densifolia</i>)	Erect or becoming reflexed	Reflexed	Erect to slightly spreading	Erect to slightly spreading	Reflexed	Reflexed	Usually reflexed

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TABLE 4. (Continued)

	Sri Lankan <i>Kuruna</i> clade [Clade XII]	<i>Arundinaria</i> s.s. [Clade VI]	<i>Bergambos</i> <i>tessellata</i> [Clade I]	<i>Thamnochlamus</i> <i>spathiflorus</i> [Clade VII]	<i>Yushania</i> [Clade V]	<i>Fargesia</i> (<i>F.</i> <i>spathacea</i> -type) [Clade V]	<i>Indocalamus</i> (<i>I.</i> <i>sinicus</i> -type) [Clade X]	<i>Oldenania alpina</i> [Clade II]
Foliage leaf sheath	Strongly keeled at least near the summit	Rounded on the back	Rounded on the back	Strongly keeled at least near the summit	Strongly keeled at least near the summit	Narrowly rounded to strongly keeled at least near the summit	Rounded on the back	Strongly keeled at least near the summit
Foliage leaf sheath persistence	Persistent	Persistent	Persistent	Deciduous	Persistent	Persistent	?	Deciduous
Number of shortened internodes at the base of the branch complement	2–9	0–1 in <i>A. gigantea</i> ; 2– several in <i>A. tecta</i> and <i>A. applachiana</i>	4–5	2–3	3–5	2–9	2–5	2–3
Node	Without roots	Without roots	Without roots	Without roots	Without roots	Without roots	Without roots	With ring of thorn like roots at the nodes
Branch sheathing	Complete	Complete	Reduced	Complete	Reduced	Reduced	?	Reduced
Branch orientation	Initially erect, then spreading	Erect to spreading	Erect	Erect to slightly spreading	Erect to spreading	Erect	?	Spreading
Secondary branch initiation area	2nd or 3rd compressed internode	2nd, 3rd or 4th compressed internode	4th compressed internode	2nd compressed internode	2nd or 3rd compressed internode	2nd or 3rd compressed internode	?	2nd compressed internode
Reproductive Characters								
Synflorescence type	Open racemes or panicles, not unilateral	Open racemes or panicles, not unilateral	Contracted raceme, not unilateral	Contracted panicle, not unilateral	Open panicle, not unilateral	Densely contracted unilateral raceme	Raceme or an open panicle, not unilateral	Panicle, not unilateral
No. of fertile florets per spikelet	2–6 (except <i>A. densifolia</i> , with one)	7–12	1–3	2–7	1–7	3–4	3–4	2–11

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TABLE 4. (Continued)

	Sri Lankan <i>Kuruna</i> clade [Clade XII]	<i>Arundinaria</i> s.s. [Clade VI]	<i>Bergbambos tessellata</i> [Clade I]	<i>Thamnocalamus spathiflorus</i> [Clade VII]	<i>Yushania</i> [Clade V]	<i>Fargesia</i> (F. <i>spathacea</i> -type) [Clade V]	<i>Indocalamus</i> (I. <i>sinicus</i> -type) [Clade X]	<i>Oldenania alpina</i> [Clade II]
Presence of spatheate empty bracts at the base of the synflorescence	Absent	Absent	One or more present	One well-developed spatheate empty bract present	One or sometimes none	Two present	Absent	Absent
Presence of subtending bracts	Absent	Absent	Present at each node, well developed with a sheath and a blade	Present at each node, variable in size and development, but some well developed	Present as a very small bract only at the first node, otherwise absent	Present at each node except the terminal node, variable in size	Usually very small bracts present	Absent
Palca apex	Biapiculate (sinus shallow) (<i>A. floribunda</i> & <i>A. densifolia</i>) or acute, undivided (other 3 spp.)	Tips long-divided (sinus deep)	Biapiculate (sinus shallow)	Biapiculate (sinus shallow)	Biapiculate (sinus shallow)	Tips long-divided (sinus deep)	Biapiculate (sinus shallow)	Acute, undivided

Hypothesis testing:—The K-H test could not reject the possibility of the monophyly of the Sri Lankan *Arundinaria* species (Clade XII) plus the South African mountain bamboo *Bergbambos tessellata*, although morphological characters clearly differentiate Sri Lankan *Arundinaria* species from *Bergbambos tessellata* (Table 4), suggesting that these two clades are distinct. Linder *et al.* (1997) proposed an Indian – Sri Lankan – Madagascan – Southern African biogeographical pattern for *Crinipes* Hochstetter (Fürrohr 1855: 279) (Poaceae: Arundinoideae). The possible monophyly of Sri Lankan *Arundinaria* species (Clade XII) and *Bergbambos tessellata* revealed by the K-H test of the current study suggests the possibility of a similar biogeographical pattern in this group, but additional sampling of Indian and Madagascan *Arundinaria* species as well as better resolution among temperate bamboo lineages are both needed before this question can be addressed.

Generic status of the Sri Lankan *Arundinaria* clade:—The morphological comparison eliminated the possibility that any of these Sri Lankan *Arundinaria* species or other “*Arundinaria*” groups should be classified within *Fargesia*, *Indocalamus*, *Chimonobambusa* or *Chimonocalamus*. *Fargesia* has a densely contracted unilateral raceme with long divided (deep sinus) palea apices that separates it from the Sri Lankan *Arundinaria*, *Thamnocalamus*, *Bergbambos tessellata*, African alpine bamboos and *Yushania*. In addition, both *Indocalamus* and *Chimonobambusa* have leptomorph rhizomes while all the Sri Lankan *Arundinaria*, *Thamnocalamus*, *Bergbambos tessellata*, African alpine bamboo and *Yushania* clades have pachymorph culm bases (= pachymorph rhizomes as usually described in bamboo literature). Further, the presence of basally grooved culms and synflorescences with pseudospikelets distinguish *Chimonobambusa* from *Arundinaria* s.s., Sri Lankan *Arundinaria*, *Thamnocalamus*, *Bergbambos tessellata*, African alpine bamboos and *Yushania*. Both *Chimonobambusa* and *Chimonocalamus* have subequal multiple (or apparently multiple) buds per node which also provide evidence that these two genera are distinct from the *Arundinaria* s.s., Sri Lankan *Arundinaria*, *Thamnocalamus*, *Bergbambos tessellata*, African alpine bamboo and *Yushania* clades.

Unlike the K-H test, which was unable to reject the monophyly of the Sri Lankan *Arundinaria* clade + *Bergbambos tessellata*, the morphological characters indicate consistent differences. Both vegetative and reproductive morphological characters clearly differentiate the Sri Lankan *Arundinaria* species from *Bergbambos tessellata*. The presence of usually hispid culm leaves and the general absence of both culm leaf auricles and foliage leaf fimbriate auricles separate the Sri Lankan *Arundinaria* clade from *Bergbambos tessellata*, which has glabrous culm leaves and fimbriate auricles in both culm and foliage leaves. As discussed in Stapleton (2013), branch sheathing is an important character that could be used to differentiate these clades. Compared to *Bergbambos tessellata*, the Sri Lankan *Arundinaria* clade has a complete set of sheaths at the nodes (complete sheaths), while *Bergbambos tessellata* has lost some of the sheaths at the nodes (reduced sheaths). Even though both of these clades possess non-unilateral synflorescences, the Sri Lankan *Arundinaria* species have open racemes or panicles, but *Bergbambos tessellata* has contracted racemes. In addition, the presence of one or more spatulate bracts at the base of the synflorescence and subtending bracts at the synflorescence nodes in *Bergbambos tessellata* further supports the difference between these two clades, as these structures are completely absent in the Sri Lankan clade. Finally, the shape of the palea apex (Figure 3) is one of the best characters to differentiate among these temperate woody bamboo clades, particularly between the Sri Lankan clade (acute, undivided tips) and *Bergbambos* (biapiculate with a shallow sinus). We note that Stapleton (2013) reported a single fertile floret per spikelet in *Bergbambos tessellata*, which could serve as an additional distinction between *Bergbambos* and the Sri Lankan clade. However, based on our observations and the morphological analysis of Soderstrom & Ellis (1982), *Bergbambos tessellata* has one to three fertile florets plus at least one apical reduced or rudimentary floret, so floret number is not a useful character in this case. But overall, the molecular evidence combined with the morphology supports the recognition of a new genus that will accommodate all the Sri Lankan temperate woody bamboo species, which we here describe.

Although we were unable to examine any material of *Ampelocalamus calcareus* (Clade XI) for the morphological analysis, the strongly arching to hanging culms of this species, along with the well-developed, fimbriate auricles on its culm leaves and foliage leaves (Yi *et al.* 2008), mean it is unlikely to have a close relationship to the Sri Lankan *Arundinaria* clade. We therefore exclude it from further consideration.

According to the molecular analyses and morphological comparison all the reported native Sri Lankan *Arundinaria* species, namely *Arundinaria debilis*, *A. densifolia*, *A. floribunda*, *A. scandens* and *A. walkeriana*, belong to this new Sri Lanka temperate woody bamboo genus. However, based on morphology, especially of the spikelets, previous studies suggest a possible relationship between *Arundinaria densifolia* and allies in Sri Lanka and South India (Campbell, unpubl.). Seethalakshmi & Muktesh Kumar (1998) described *A. floribunda* and *A. walkeriana* as being distributed in both South India and Sri Lanka in very similar habitats. These taxa of “*Arundinaria*” from India were not sampled for the current analysis, so we could not confirm their identification. Furthermore, *A. wightiana* Nees von Esenbeck (1834: 482) is also reported to occur in both South India and Sri Lanka (Seethalakshmi & Muktesh Kumar 1998), but

in our field work this species could not be located in Sri Lanka. Further, no previous studies relating to the Flora of Ceylon (Soderstrom & Ellis 1988, Dassanayake & Fosberg 1994) indicated any distribution of *A. wightiana* in Sri Lanka. Morphological comparison of *A. wightiana* and the Sri Lankan *Arundinaria* species shows some resemblance of the Indian taxon mainly with *A. debilis* and *A. floribunda* from Sri Lanka. Presence of a well-developed supranodal ridge, abaxially hispid culm leaves, fimbriate culm leaf sheath summits and foliage leaves with fimbriae are the main similarities among these three species. However, the very small culm leaf blade, relatively long fimbriae on both culm leaf sheath summits and on the foliage leaf auricles distinguish *A. wightiana* from the Sri Lankan *Arundinaria* species. In addition, the abaxial culm leaf surfaces of *A. wightiana* are covered by dark brown irritating hairs, while the abaxial culm leaf surfaces of Sri Lankan *Arundinaria* species are covered with non-irritating whitish brown hairs. Thus, morphological comparison suggests that *A. wightiana* could belong to the Sri Lankan *Arundinaria* clade, but this species needs further study and it has not yet been sampled in a molecular analysis. In addition, Dransfield (2003) reported six *Arundinaria* species endemic to Madagascar, but only one (*Yushania ambositrensis*) has been sampled in this and previous molecular analyses (Triplett & Clark 2010), and it clusters with *Oldeania alpina*. Stapleton (2013) suggests that temperate bamboos radiated from India to Asia, Africa, and North America. Therefore, it is possible that one or more of these Madagascan *Arundinaria* species will ultimately be shown to belong to the Sri Lankan *Arundinaria* clade, but these species are poorly known and more work is needed before they can be assigned to an appropriate genus.

Conclusions

A major finding of this investigation is the resolution of a robustly supported twelfth lineage, the Sri Lankan *Arundinaria* clade (Clade XII), within the temperate woody bamboos (Arundinarieae). Although the position of this clade with respect to the other ten sampled clades of temperate woody bamboos is unresolved, alternate hypothesis testing rejects monophyly of the Sri Lankan *Arundinaria* in combination with *Arundinaria* s.s. (restricted to North America) or other clades considered as *Arundinaria* in the broad sense, with the exception of *Bergbambos tessellata* (Clade I) from South Africa. A morphological analysis of these clades, however, provides characters to distinguish the Sri Lankan *Arundinaria* clade from *Bergbambos*.

The current study therefore provides robust molecular and morphological support for the recognition of the Sri Lankan *Arundinaria* clade as a new genus, here named *Kuruna*. We also make new combinations in *Kuruna* for the five described species in Sri Lanka; a complete taxonomic revision for this group is currently in preparation. We emphasize that the south Indian species of *Arundinaria* have not yet been sampled in a molecular study, and therefore concepts of *Bergbambos*, *Kuruna*, African alpine bamboos or other temperate woody genera may ultimately change, and we also cannot rule out the recognition of additional lineages within the temperate woody bamboos.

We suggest the use of other markers such as low copy nuclear markers and plastid genome sequences to better understand phylogenetic relationships within the highly complex, taxonomically difficult temperate woody bamboo clade. Though the topology that we obtained was imperfectly resolved, it can still serve as a foundation for testing biological or biogeographic hypotheses. The integration of more polymorphic markers into molecular analyses is necessary to obtain better resolution in order to critically examine divergence times, biogeography and morphological evolution within temperate woody bamboos.

Taxonomic Treatment

Kuruna Attigala, Kaththriarachchi & L. G. Clark, *gen. nov.*

TYPE: *Arundinaria debilis* Thwaites (1864: 375).

Kuruna debilis (Thwaites) Attigala, Kaththriarachchi & L. G. Clark

Diagnosis:—Differs from *Arundinaria* s.s. by its pachymorph culm bases with short necks, culm leaves usually abaxially hispid, culm leaf girdles ca. 1 mm wide, culm leaf auricles absent and the palea apex biapiculate to acute. The following characters differentiate *Kuruna* from other “*Arundinaria*” groups (*Bergbambos*, African alpine bamboos,

Thamnocalamus and *Yushania*): pachymorph culm bases with short necks, unicaespitose clumps, culm leaf girdles present as a band ca. 1 mm wide, usually abaxially hispid culm leaves with non-irritating hairs, persistent foliage leaf sheaths and complete branch sheathing, palea apex biapiculate (sinus shallow) to acute and undivided, and both spathe empty bracts at the base of the synflorescence and subtending bracts absent.

Description:—*Culm bases* pachymorph, short necked, two or more tillers per culm base present. *Culms* woody, erect, shrubby or scandent; midculm internodes usually hollow, terete to flattened or shallowly sulcate above the branches, smooth, wall thickness (ratio of 2 times wall thickness: culm diameter) moderate (ratio 0.31–0.45) to thick (ratio 0.46–0.61), lacuna greater than 1/3 of the diameter of the culm; nodal lines horizontal; supranodal ridge mostly conspicuous. *Culm leaves* clearly differentiated from the foliage leaves; girdle present as a band at least 1 mm wide; sheath usually abaxially hispid with non-irritating hairs, sheath apex usually symmetrically concave, fimbriate, sheath summit extension present; blade usually reflexed, sessile, more or less narrowly triangular. *Branching pattern* intravaginal. *Branch complement* derived from one bud per node and born on a promontory; bud prophyll margins unitary, free; two to several compressed proximal internodes at the base of the primary axis; secondary branches subequal to the primary axis, developing from the second or third compressed internodes and above; central primary branch smaller in diameter than the main culm; three or more leaves per leafy branch and leaf branch apex growth indeterminate. *Foliage leaves* fimbriate, erect, all leaves with a sheath and a blade; sheath usually strongly keeled at least near the summit. *Synflorescences* paniculate or racemose, with 1–2 orders of branching, open, terminating the leafy branches, both spathe bracts at the base and subtending bracts absent. *Spikelets* pedicellate, laterally compressed, consisting of two glumes, usually two to six female-fertile florets per spikelet (in *A. densifolia* only one); rachilla extension hairy, bearing a rudimentary floret, shorter than or equal to about half the length of a fertile floret; glumes shorter than the spikelet, unawned; lemmas unawned; paleas 2-keeled, the keels winged, apex biapiculate or acute, sulcus well developed for the full length. Lodicules 3, ciliate; stamens 3, filaments free, anther apex lobes rounded, anther connective lower than the apical anther lobes; stigmas 2 or 3, plumose. Fruit a basic caryopsis with a linear hilum as long as the fruit.

Etymology:—The generic name *Kuruna* is derived from the common name in Sinhalese of the native Sri Lankan *Arundinaria* group, “KuruUna”. “Kuru” means dwarf, and “Una” means bamboo.

Distribution:—Warm temperate and montane regions (both forests and open grasslands) of Sri Lanka (Soderstrom & Ellis, 1988).

Following are the new combinations for all the native *Arundinaria* species in Sri Lanka:

1. *Kuruna debilis* (Thwaites) Attigala, Kaththriarachchi & L. G. Clark, *comb. nov.*

Basionym:—*Arundinaria debilis* Thwaites (1864: 375).

2. *Kuruna densifolia* (Munro) Attigala, Kaththriarachchi & L. G. Clark, *comb. nov.*

Basionym:—*Arundinaria densifolia* Munro (1868: 32).

3. *Kuruna floribunda* (Thwaites) Attigala, Kaththriarachchi & L. G. Clark, *comb. nov.*

Basionym:—*Arundinaria floribunda* Thwaites (1864: 375).

4. *Kuruna scandens* (Soderstrom & Ellis) Attigala, Kaththriarachchi & L. G. Clark, *comb. nov.*

Basionym:—*Arundinaria scandens* Soderstrom & Ellis (1988: 20).

5. *Kuruna walkeriana* (Munro) Attigala, Kaththriarachchi & L. G. Clark, *comb. nov.*

Basionym:—*Arundinaria walkeriana* Munro (1868: 21).

Acknowledgments

Field work was supported by National Science Foundation grant DEB-0515712 to LGC and support for LA and for the final preparation of this manuscript came from National Science Foundation grant DEB-1120750 to LGC. We are especially grateful to the Forest Department, Department of Wildlife Conservation of Sri Lanka and Sri Lanka Customs for providing the necessary permits to collect and export both herbarium material and silica dried leaf material from Sri

Lanka. We would also like to thank Nuwan De Silva and Abayapala De Silva for their generous help during field work. We thank the curators of the herbaria (ISC, K, MO, PDA, US) that loaned specimens for this study. We also thank Kara Grupp, Hathairat Chokthaweeapanich, Chakkrapong Rattamanee and the laboratory of Dr. Jonathan Wendel, Iowa State University for technical assistance, laboratory assistance and access to laboratory facilities.

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Appendix 1. Alphabetical list of voucher specimens utilized in the cpDNA analysis. All the vouchers are deposited at ISC unless otherwise indicated

Taxon	Voucher	Source	GenBank accession no.				
			<i>ndhF</i> (3' end)	<i>rps16-trnQ</i>	<i>trnC-rpoB</i>	<i>trnD-trnT</i>	<i>trnT-trnL</i>
<i>Acidosasa edulis</i>	Triplet148	GenBank	---	FJ643789	FJ643882	FJ643975	FJ644126
<i>Arundinaria debilis</i> 1	Attigala123	Horton Plains, Sri Lanka	KJ638186	KJ638172	KJ638200	KJ638191	KJ638179
<i>A. debilis</i> 2	Attigala147	Hayes-Gongala, Sri Lanka	---	KJ638173	KJ638201	KJ638192	KJ638180
<i>A. densifolia</i>	Attigala126	Horton Plains, Sri Lanka	KJ638187	KJ638174	KJ638197	KJ638193	KJ638181
<i>A. floribunda</i>	Attigala139	Amanawala-Ampane, Sri Lanka	---	KJ638175	---	---	KJ638182
<i>A. gigantea</i>	Triplet197	GenBank	FJ643707	FJ643794	FJ643887	FJ643980	FJ644131
<i>A. scandens</i>	Attigala166	Pidurutalagala summit, Sri Lanka	KJ638188	KJ638176	KJ638202	KJ638194	KJ638183
<i>A. tecta</i>	Triplet173	GenBank	FJ643708	FJ643795	FJ643888	FJ643981	FJ644132
<i>A. walkeriana</i>	Attigala162	Adam's Peak, Sri Lanka	KJ638189	KJ638177	KJ638198	KJ638195	KJ638184
<i>Arundinaria</i> sp1	Attigala146	Hayes-Gongala, Sri Lanka	KJ638190	KJ638178	KJ638199	KJ638196	KJ638185
<i>Bambusa vulgaris</i>	Sánchez-Ken 666	GenBank	FJ643709	FJ643796	FJ643889	FJ643982	FJ644133
<i>Brachyleyrum erectum</i>	Triplet199b	GenBank	U22005	FJ643799	FJ643892	FJ643985	FJ644136
<i>Chinnocalamus montanus</i>	Triplet261	GenBank	---	FJ643807	FJ643900	FJ643993	FJ644144
<i>Chinnocalamus pallens</i>	Triplet238	GenBank	FJ643712	FJ643808	FJ643901	FJ643994	FJ644145
<i>Chusquea spectabilis</i>	Clark 919	GenBank	AF182355	FJ751698	FJ751725	FJ751752	---
<i>Fargesia nitida</i>	Triplet222	GenBank	---	FJ643813	FJ643906	FJ643999	FJ644150
<i>Ferrocatalamus strictus</i>	Campbell 10	GenBank	FJ643713	FJ643815	FJ643908	FJ644001	FJ644152
<i>Gaoligongshania megalolhyrsa</i>	Xue 9401 (KUN)	GenBank	---	GU354641	GU354481	GU354801	GU354961
<i>Guadua angustifolia</i>	Clark & Londoño 931	GenBank	FJ643714	FJ643817	FJ643910	FJ644003	FJ644154
<i>Indocalamus sinicus</i>	Zeng & Zhang 06081 (KUN)	GenBank	---	GU354673	GU354513	GU354833	GU354993
<i>Indocalamus wilsonii</i>	Zhang 07088	GenBank	---	GU354626	GU354466	GU354786	GU354945
<i>Indosasa sinica</i>	Triplet267	GenBank	FJ643715	FJ643827	FJ643920	FJ644013	FJ644164
<i>Pleioblastus amarus</i>	Zhang 07082 (KUN)	GenBank	---	FJ643836	FJ643929	FJ644022	FJ644173
<i>Pleioblastus argenteostriatus</i>	Triplet66	GenBank	---	FJ643837	FJ643930	FJ644023	FJ644174
<i>Pleioblastus justianensis</i>	Triplet117	GenBank	---	FJ643841	FJ643934	FJ644027	FJ644178
<i>Pseudosasa gracilis</i>	Zhang 06107 (KUN)	GenBank	---	FJ643849	FJ643942	FJ644035	FJ644186
<i>Pseudosasa japonica</i>	Triplet122	GenBank	FJ643723	FJ643851	FJ643944	FJ644037	FJ644188
<i>Sasa longiligulata</i>	Zeng 061213 (KUN)	GenBank	---	FJ643859	FJ643952	FJ644045	FJ644196
<i>Sasa oshidenis</i>	Triplet161	GenBank	---	FJ643860	FJ643953	FJ644046	FJ644197
<i>Sasa palmata</i>	Triplet228 (KUN)	GenBank	---	GU354661	GU354501	GU354821	GU354981
<i>Shibataea chinensis</i>	Triplet13	GenBank	---	FJ643870	FJ643963	FJ644056	FJ644207
<i>Thamnocalamus spathiflorus</i>	Clark 1319	GenBank	FJ643728	FJ643876	FJ643969	FJ644062	FJ644213
<i>Bergambos tessellata</i>	Triplet202	GenBank	FJ643729	FJ643877	FJ643970	FJ644063	FJ644214
<i>Oldenania alpina</i>	Fadenet al. 96/413 (US)	GenBank	FJ643730	FJ643878	FJ643971	FJ644064	FJ644215
<i>Yushania ambositrensis</i>	Dransfield 1353	GenBank	---	FJ643879	FJ643972	FJ644065	FJ644216
<i>Yushania nitakayamensis</i>	March 28	GenBank	---	FJ643881	FJ643974	FJ644067	FJ644218