Molecular phylogeny of the arthrostylidioid bamboos (Poaceae: Bambusoideae: Bambuseae: Arthrostylidiinae) and new genus *Didymogonyx*

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ABSTRACT

We present the first multi-locus chloroplast phylogeny of Arthrostylidiinae, a subtribe of neotropical woody bamboos. The morphological diversity of Arthrostylidiinae makes its taxonomy difficult and prior molecular analyses of bamboos have lacked breadth of sampling within the subtribe, leaving internal relationships uncertain. We sampled 51 taxa, chosen to span the range of taxonomic diversity and morphology, and analyzed a combined chloroplast DNA dataset with six chloroplast regions: ndhF, trnD-trnT, trnC-rpoB, rps16-trnQ, trnT-trnL, and rpl16. A consensus of maximum parsimony and Bayesian inference analyses reveals monophyly of the Arthrostylidiinae and four moderately supported lineages within it. Six previously recognized genera were monophyletic, three polyphyletic, and two monotypic; *Rhipidocladum* sect. *Didymogonyx* is here raised to generic status. When mapped onto our topology, many of the morphological characters show homoplasy.

1. Introduction

The bamboos, with about 1450 species, are the only grass subfamily (Poaceae: Bambusoideae) to diversify primarily in forests (BPG, 2006a, 2012; Bouchenak-Khelladi et al., 2010; GPWG, 2001). Our understanding of Bambusoideae taxonomy is in a state of flux, but the emerging picture based on molecular sequence data (Sungkaew et al., 2009) is that the bamboos evolved into three main lineages: woody, mainly north temperate species (Arundinarieae), woody tropical species (Bambuseae), and herbaceous bamboos (Olyreae). The Bambuseae, including nearly 800 described species, is usually classified into seven subtribes, of which four are paleotropical and three are neotropical (BPG, 2012; Judziewicz et al., 1999; Soderstrom and Ellis, 1987). The present work is focused on one morphologically diverse but understudied subtribe of neotropical woody bamboos.

The three neotropical subtribes of Bambuseae are Arthrostylidiinae (172 described species), Chusqueinae (160 described species) and Guaduinae (45 described species) (BPG, 2012; Fisher et al., 2009; Judziewicz et al., 1999). The Arthrostylidiinae plus Guaduinae are recovered as a moderately- to well-supported clade (BPG, in preparation; Clark et al., 2007; Sungkaew et al., 2009; Zhang and Clark, 2000). In recent studies, this clade is moderately supported as sister to Chusqueinae, thus forming a neotropical woody bamboo clade, which is sister to the paleotropical woody bamboo clade (BPG, in preparation; Sungkaew et al., 2009).

The results of the BPG (in preparation), Sungkaew et al. (2009) and Zhang and Clark (2000) support the monophyly of each subtribe; however, each is represented by only one to three exemplar taxa. Other molecular analyses included more species but discovered reciprocally misplaced taxa. Guala et al. (2000) generated a phylogeny that included seven Arthrostylidiinae and two Guaduinae species, and found that two presumed Guaduinae taxa were allied with Arthrostylidiinae. Conversely, Ruiz-Sanchez et al. (2008, 2011) found two species of Arthrostylidiinae that resolved within the Guaduinae based on plastid sequence data. Guala et al. (2000) recovered a topology with moderate support for the two subtribes as sister clades, whereas Ruiz-Sanchez et al. (2011) recovered a robustly supported Guaduinae sister to the Arthrostylidiinae, but without support. Clark et al. (2007) and Ruiz-Sanchez et al. (2008), however, recovered a robustly monophyletic Guaduinae derived from within a paraphyletic Arthrostylidiinae.

The Arthrostylidiinae can be distinguished from other woody bamboo subtribes using branch leaf micromorphology and anatomy (Soderstrom and Ellis, 1987). The leaf blades of Arthrostylidiinae possess a unique combination of intercostal sclerenchyma fibers in the blades and simple vasculature in the midrib, and the leaf blades are basically hypostomatic with papillae usually

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developed only on the abaxial epidermis. A green marginal stripe on the abaxial leaf epidermis (due to reduced or no wax deposition) is also characteristic of Arthrostylidiinae, although this is seen occasionally in other neotropical Bambuseae, Olyreae, and even some Arundinarieae (BPG, 2012; Judziewicz et al., 1999; Soderstrom and Ellis, 1987; pers. obs. by all authors). In several taxa of Arthrostylidiinae, stomates have been observed on the adaxial surface over the abaxial green stripe, but they appear to be restricted to this region and it is unknown how general this pattern is (Santos-Gonçalves, 2005). Arthrostylidiinae also have sympodial rhizomes, usually a single primary branch bud at each node, rudimentary florets terminating the spikelets or pseudospikelets, and reflexed pseudopetioles (except in *Glaziophyton* Franch., *Filgueirasia* Guala and some species of *Aulonemia* Goudot; BPG, 2012).

With 172 described species currently classified into 13 genera exhibiting a broad range of life forms, Arthrostylidiinae is arguably the most morphologically diverse subtribe of neotropical woody bamboo. In comparison, the Chusqueinae include nearly the same number of described species but only one genus, *Chusquea* Kunth, is recognized based on a uniform spikelet structure and papillate subsidiary cells (Fisher et al., 2009). The characters most commonly used to distinguish among genera of Arthrostylidiinae, however, often intergrade in a "mosaic pattern of variation" (Clark and Londoño, 1991) making taxonomy and phylogenetic inference difficult (Judziewicz et al., 1999). McClure (1973) notes that "such divergent attributes are connected by intermediate expressions to form clines, both from one species to another and (in some cases) within the same specimen." Notable morphological enigmas within the subtribe include internode length pattern, culm leaf blade orientation, vegetative bud prophyll shape, branching architecture, synflorescence form, the occurrence of pseudospikelets, and fruit anatomy. The observations summarized in the following paragraphs are based on decades of field work in the Neotropics and examination of thousands of herbarium specimens by Santos-Gonçalves, Londoño and Clark as well as on information from the literature, primarily the BPG (2012), Judziewicz et al. (1999), and McClure (1973).

Patterns of unequal culm internode lengths (length dimorphism) are a feature observed in species of five Arthrostylidiinae genera: *Arthrostylidium* Rupr., *Glaziophyton*, *Myriocladus* Swallen, *Aulonemia*, and *Rhipidocladum* McClure. The patterns vary in expression, but are sometimes species specific and thus diagnostic. *Arthrostylidium schomburgkii* Munro and *Glaziophyton* usually have one long basal internode (as much as 5 m long in the former) followed successively by much shorter internodes. A similar pattern is seen in *Myriocladus* which has one or two basal elongated internodes, and several short internodes near the culm apex or one elongated internode regularly alternating with a few shorter ones. Internodes of *Aulonemia queko*, *Au. herzogiana* (Henrard) McClure, and *Au. hirtula* alternate one to four short with one long (up to 2 m in length). The two species of *Rhipidocladum* sect. *Didymogonyx* demonstrate a regular alternation of one short and one long internode along the entire length of their culms (Clark and Londoño, 1991). The remaining species display uniformly subequal internode lengths.

All members of *Rhipidocladum*, *Filgueirasia*, *Alvimia* C.E. Calderón ex Soderstr. & Londoño, and *Glaziophyton* (monotypic), several species of *Arthrostylidium*, *Aulonemia ulei*, and two species of *Atractantha* McClure have erect culm leaf blades which are confluent with the sheath. The remaining species of the subtribe have culm leaf blades that are reflexed and constricted into a pseudopetiole where the blade meets the sheath. Reflexed culm leaf blades are generally deciduous, while erect blades tend to be persistent.

With the exception of the two species of *Filgueirasia*, all species in the subtribe have a single bud per node, each covered by a protective, two-keeled prophyll. In *R.* sect. *Didymogonyx* and *Myriocladus* the prophylls are cordate in shape. The remaining taxa have triangular shaped prophylls.

Arthrostylidioid bamboos typically have branch complements with one dominant branch that arises from the main culm often with several smaller branches arising laterally, a pattern commonly seen in other woody bamboos (e.g., *Bambusa* Schreb.). Yet the branch complements of several genera deviate from this arrangement. *Atractantha* and *Athroostachys* Benth. exhibit three to five subequal branches, which may or may not rebranch, arising from each node; *Aulonemia ulei* has five to seven subequal branches per node. *Actinocladum* McClure ex Soderstr., *Merostachys* Spreng., and *Rhipidocladum* share an unusual branching morphology superficially resembling a hand fan. This is termed apsidate branching (McClure, 1973), referring to the numerous subequal secondary branches that radiate in an apsidal or vault-like fashion from a flattened triangular surface (postulated to be a modification of the dominant branch); we here use the less technical term "fan-branching" to describe this morphology. In *Arthrostylidium*, *Alvimia*, *Atractantha*, *Aulonemia*, *Colanthelia* and *Elytrostachys* McClure, the branches arise from a raised, ellipsoidal base, called a promontory, which either continues as a dominant branch (often producing small lateral branches from its basal nodes) or separates into multiple subequal branches. If the latter, the branches are not orderly as in fan-branching. *Arthrostylidium merostachyoides* manifests a combination of promontory and fan-branching morphologies when mature.

The most common synflorescence forms in Arthrostylidiinae are paniculate or racemose with a straight axis, but this character exemplifies the mosaic variation seen in the subtribe. *Arthrostylidium venezuelae*, *Ar. sarmentosum* Pilg., *Ar. multispicatum*, *Ar. ecuadorense* Judz. & L.G. Clark, *Ar. virolinensis* Londoño & L.G. Clark, and *R. harmonicum* all have racemose synflorescences with a geniculate or zig-zag axis. This kinked morphology is the result of swollen pulvini at the base of the spikelets. Two other species, *Athroostachys capitata* Benth. and *Atractantha radiata*, have synflorescences that are contracted into prickly, globose clusters. The spikelets of most arthrostylidioid species arise bilaterally along the synflorescence axis. Those of *Merostachys*, however, are secund, inserted on one side only. This is also characteristic of *Arthrostylidium merostachyoides* and *Rhipidocladum maxonii*, and many other species of *Rhipidocladum* have nearly secund synflorescences.

Most arthrostylidioid species also have conventional spikelets, but pseudospikelets occur in three genera. Pseudospikelets differ from spikelets in that the former rebranch into other spikelet-like units from bud-bearing bracts within what appears to be a spikelet. Additionally, taxa with pseudospikelets also have subtending bracts and prophylls at the base of each branch. Pseudospikelets are observed in *Elytrostachys*, *Alvimia*, and *Atractantha* (except for *At. amazonica* Judz. & L.G. Clark). *Glaziophyton*, while lacking bud-bearing bracts, has the subtending bracts and prophylls typically found in taxa with pseudospikelets.

Most arthrostylidioid genera have a typical grass fruit, the grain-like basic caryopsis, characterized by a pericarp that is fully adnate to the seed coat (Sendulsky et al., 1987). *Actinocladum* and *Merostachys* both possess a fruit in which the pericarp is separable from the seed coat, known as a nucoid or nut-like caryopsis. Furthermore, the caryopsis of *Alvimia* is bacoid, having an enlarged, fleshy pericarp superficially resembling a berry (Soderstrom and Londoño, 1988).

Prior molecular analyses were not specifically focused on the Arthrostylidiinae and, given the morphological variation across the subtribe, lacked breadth of sampling. Thus, the monophyly of the subtribe has not been adequately tested and internal relationships remain ambiguous. Our objectives are to (1) generate a phylogenetic hypothesis for Arthrostylidiinae using multi-locus plastid sequence data and the broadest possible sampling of its taxonomic and morphological diversity; (2) test the monophyly of the subtribe and all genera for which multiple taxa could be sampled; and (3) examine the evolution of the apparently mosaic morphological characters that have been used to distinguish among genera in this subtribe using our phylogenetic hypothesis. We conclude with a discussion of the implications of our findings for generic circumscription and recommendations for future work in the subtribe.

2. Methods and materials

2.1. Taxon sampling

A total of 51 species, chosen to span a range of genera and morphologies, were analyzed (Table 1). Forty-five taxa represent 11 of the 13 recognized genera of Arthrostylidiinae and five taxa represent the five genera currently recognized in the Guaduinae. *Bambusa vulgaris* (paleotropical woody clade) is the designated outgroup based on Clark et al. (2007) and Ruiz-Sanchez et al. (2008). Leaf material was either collected in the field and silica-gel dried (Chase and Hills, 1991), obtained fresh from greenhouse specimens, or removed with permission from herbarium specimens.

2.2. DNA extraction, sequencing, alignment, and character coding

Total genomic DNA was extracted following CTAB-isopropanol precipitation protocols (Paterson et al., 1993) or using DNeasy® Plant Mini kits (July 2006 version; QIAGEN, Valencia, California, USA) with a modified protocol (Triplett and Clark, 2010). Six chloroplast DNA regions: one gene (ndhF 3′ half), four intergenic spacers (trnD-trnT, trnC-rpoB, rps16-trnQ, trnT-trnL), and an intron (rpl16), were amplified by polymerase chain reaction (PCR) on an MJ Research PTC-200 thermal cycler (Bio-Rad Laboratories, Inc., Hercules, CA USA) in 40 μL quantities. Amplification primers, internal sequencing primers, and thermal cycler protocols for ndhF, trnD-trnT, trnC-rpoB, rps16-trnQ, and trnT-trnL follow Triplett and Clark (2010). Amplification primers for rpl16 were F71 and R1661 of Jordan et al. (1996), internal sequencing primers were R1516 (Kelchner and Clark, 1997) and an unpublished primer, CCA TCC CAC CCA ATG AAG, developed by Kelchner. Thermal cycler protocol for rpl16 began with initialization at 95 °C for 2 m, then 35 cycles of 95 °C for 1 m denaturation, 50 °C for 10 s annealing, followed by a 50 s temperature ramp increasing 0.3 °C/s until elongation at 65 °C for 4 m. Final elongation was carried out at 65 °C for 5 m and products were then held at 4 °C. Amplification products were cleaned with Antarctic phosphatase (5 units, New England Biolabs, Ipswich, MA, USA) and exonuclease I (10 units, New England Biolabs) followed by ethanol precipitation. Asymmetric sequencing reactions were carried out using BigDye™ Terminators v3.1 (Applied Biosystems Inc., Foster City, CA USA) on a Perkin–Elmer Applied Biosystems GeneAmp PCR System 9600 thermal cycler, and cleaned using a Sephadex plate (Edge BioSystems, Gaithersburg, MD USA). Sequences were read on an automated 3730×l DNA Analyzer (Applied Biosystems) at the DNA Facility of the Iowa State University Office of Biotechnology.

Sequence electropherograms were verified and base-called using 4Peaks (Griekspoor and Groothuis, 2006). Contigs were manually assembled and aligned in Se-Al version 2.0a11 (Rambaut, 2002) using *Oryza sativa* L. (GenBank ID: NC001320) as a reference. Primer regions were trimmed from the final sequence. Biologically significant alignments were attempted using a parsimony concept (Morrison, 2006). Patterns for known molecular evolutionary events such as slip-strand mispairing, stem-loop secondary structures, and minute hairpins, were sought and aligned as recommended in Kelchner (2000) and Kelchner and Wendel (1996). Gaps of identical size and position were binary coded relative to the outgroup (Giribet and Wheeler, 1999) and appended to the DNA sequence matrix. Autapomorphic and parsimony-uninformative indels were not scored, and were excluded along with other gaps prior to analysis. Individual sequences are available in GenBank (Table 1).

2.3. Phylogenetic analysis

Separate analyses of the data, partitioned by locus, revealed little incongruence between phylogenetic topologies. Therefore, we assumed all loci track the same evolutionary history and combined regions into one dataset for use in analyses.

Unweighted maximum parsimony (MP) analysis on the data with binary coded gaps and indels was carried out in PAUP* (Swofford 2002) using full heuristic search with 1000 random addition sequences, tree bisection and reconnection (TBR) swapping, and no topological constraints. Bootstrap support (BS) values (Felsenstein, 1985) for MP analyses were estimated from 10,000 replicates with the above heuristic settings. Bremer (1994) decay index (DI) values were calculated using TreeRot version 3 (Sorenson and Franzosa, 2007) with PAUP*.

The GTR + Γ + I model of evolution was selected by the AIC criterion and hierarchical likelihood ratio test for each region and the combined matrix using MrModeltest version 2.3 (Nylander, 2004). Since the selected models were the same, the combined data were analyzed. Bayesian inference (BI) was performed in MrBayes version 3.1.2 (Ronquist and Huelsenbeck, 2003). BI was carried out using the chloroplast nucleotide data only, gap and indel coding was only used in parsimony analysis. Markov Chain Monte Carlo (MCMC) was implemented with eight chains in four runs over 5,000,000 generations, sampled every 1000th generation. We assessed the quality of the MCMC simulations by ensuring the average standard deviation of split frequencies was consistently below 0.01 and examining posterior probabilities of splits with AWTY (Wilgenbusch et al., 2004). We discarded the initial 10% of sampled generations as burn-in and used MrBayes to generate a majority rule consensus tree with posterior probabilities (PPs) for each recovered clade.

A consensus phylogeny of the shortest trees from ten parsimony ratchet searches was also constructed using PRAP (Müller, 2004) with 25% of the characters upweighted over 200 iterations. This topology agreed with the recovered trees from both the Bayesian and unratcheted parsimony analyses.

Given that branch support tends to be overestimated by Bayesian PP and underestimated by bootstrap values (Simmons et al., 2004), we adopted a conservative interpretation of clade support using the following criteria: well supported = 90–100% BS, 0.99–1.00 PP, and >5 DI; moderately supported = 80–89% BS, 0.95–0.98 PP, and 3–5 DI; poorly supported = <79% BS, <0.95 PP and <3 DI.

Several constrained topologies were constructed and used to test whether the combined data provided sufficient evidence to reject particular topological hypotheses of various taxa and morphological characters (e.g., monophyly of Arthrostylidiinae, *Arthrostylidium*, *Rhipidocladum*, *Aulonemia*, or a single origin of fan-branching, etc.). Two-tailed Kishino–Hasegawa (KH) and Templeton non-parametric tests were performed on parsimony scores as implemented in PAUP* (Table 2).

2.4. Morphological character mapping

We coded a matrix of morphological character states for each taxon based on the voucher and conspecific herbarium specimens and observations recorded in the literature as cited in the introduction (Table 3). Because this is an initial assessment, we focused

Table 1

Taxa, vouchers, and GenBank IDs for six chloroplast DNA regions used to infer phylogeny. AC, André Carvalho; AS, Ana Paula Santos-Gonçalves; CT, Christopher Tyrrell; ER, Eduardo Ruiz-Sanchez; FE, Francisca Ely; FZ, Fernando Zuloaga; GR, Gary Reiners; JS, J. Gabriel Sánchez-Ken; JT, Jimmy Triplett; LC, Lynn Clark; LS, Luiz Sarahyba; PA, Patricio Asimbaya; PV, Pedro Viana; RP, Richard Pohl; TF, Tarciso Filgueiras; WO, Walter de Oliveira; WZ, Wei-Ping Zhang; XL, Ximena Londoño. Taxa that failed to amplify for a region are indicated by a dash.

Taxon	Voucher	Origin	GenBank ID					
			ndhF	rpL16 Intr.	trnC-rpoB	trnD-trnT	rps16-trnQ	trnT-trnL
Bambuseae Kunth ex Dumort.								
Arthrostylidiinae Soderstr. & R.P.Ellis								
Actinocladum verticillatum (Nees) McClure ex Soderstr.	TF s.n.	Brazil	JQ408524	JQ408570	JQ408485	JQ408623	JQ408445	JQ408586
Alvimia auriculata Soderstr. & Londoño	AS 598	Brazil	JQ408525	–	JQ408486	JQ408624	JQ408446	JQ408587
Alvimia gracilis Soderstr. & Londoño	AC 4389	Brazil	JQ408526	JQ408571	JQ408487	JQ408625	JQ408447	JQ408588
Arthrostylidium cubense Rupr.	XL 957	Cuba	JQ408529	JQ408572	–	JQ408628	JQ408450	JQ408590
Arthrostylidium merostachyoides R.W.Pohl	CT et al. 36	Costa Rica	JQ408531	–	JQ408491	–	JQ408452	–
Arthrostylidium multispicatum Pilg.	LC & GR 1508	Puerto Rico	JQ408532	JQ408574	–	JQ408630	JQ408453	JQ408592
Arthrostylidium pubescens Rupr.	CT et al. 42	Costa Rica	JQ408533	JQ408575	–	JQ408631	JQ408454	–
Arthrostylidium urbanii Pilg.	XL 851	Cuba	JQ408534	JQ408576	JQ408492	JQ408632	JQ408455	JQ408594
Arthrostylidium venezuelae (Steud.) McClure	CT et al. 43	Costa Rica	JQ408535	–	JQ408493	JQ408633	–	–
Arthrostylidium sp.	LC et al. 1101	Ecuador	JQ408530	JQ408573	JQ408490	JQ408629	JQ408451	JQ408591
Arthrostylidium sp.	XL 900	Colombia	JQ408528	–	JQ408489	JQ408627	JQ408449	JQ408593
Atractantha aureolanata Judz.	AS 596	Brazil	JQ408536	–	JQ408494	JQ408634	–	JQ408595
Atractantha cardinalis Judz.	AS 591	Brazil	JQ408537	–	JQ408495	JQ408635	JQ408456	JQ408596
Atractantha radiata McClure	AC 4362	Brazil	JQ408538	JQ408577	JQ408496	JQ408636	JQ408457	JQ408597
Aulonemia amplissima (Nees) McClure	AS 492	Brazil	JQ408539	–	JQ408497	JQ408637	–	JQ408598
Aulonemia hirtula (Pilg.) McClure	LC & PA 1412	Ecuador	JQ408540	–	JQ408498	JQ408638	–	–
Aulonemia patriae R.W.Pohl	CT et al. 44	Costa Rica	JQ408541	–	JQ408499	JQ408639	JQ408458	–
Aulonemia patula (Pilg.) McClure	LC et al. 1075	Ecuador	JQ408542	JQ408578	JQ408500	JQ408640	JQ408459	JQ408599
Aulonemia queko Goudot	LC & PA 1445	Ecuador	JQ408543	JQ408579	JQ408501	JQ408641	JQ408460	JQ408600
Aulonemia ulei (Hack.) McClure & L.B.Sm.	LC & XL 1048	Brazil	JQ408544	–	JQ408502	JQ408642	JQ408461	JQ408601
Colanthelia cingulata (McClure & L.B.Sm.) McClure	TF s.n.	Brazil	JQ408545	–	–	JQ408643	–	JQ408602
Colanthelia distans (Trin.) McClure	PV s.n.	Brazil	JQ408546	–	JQ408503	JQ408644	–	JQ408603
Colanthelia intermedia (McClure & L.B.Sm.) McClure	AS 553	Brazil	–	–	–	JQ408645	JQ408462	JQ408604
Elytrostachys clavigera McClure	RP 13572	Costa Rica	JQ408547	–	–	–	–	–
Elytrostachys typica McClure	XL 985	Colombia	JQ408548	–	–	JQ408646	JQ408463	–
Filgueirasia arenicola (McClure) Guala	TF s.n.	Brazil	JQ408550	–	JQ408505	JQ408648	JQ408465	JQ408605
Filgueirasia cannavieira (Silveira) Guala	TF s.n.	Brazil	JQ408551	JQ408580	JQ408506	JQ408649	JQ408466	JQ408606
Glaziophyton mirabile Franch.	LS et al. 1066	Brazil	JQ408552	U54748	JQ408507	JQ408650	JQ408467	JQ408607
Merostachys ternata Nees	LC & WO 1023	Brazil	JQ408555	–	JQ408510	JQ408653	JQ408471	JQ408610
Merostachys sp.	AC 4366	Brazil	JQ408553	–	JQ408508	JQ408651	JQ408469	JQ408608
Merostachys sp.	AC 4378	Brazil	JQ408554	–	JQ408509	JQ408652	JQ408470	JQ408609
Rhipidocladum sect. *Rhipidocladum*								
Rhipidocladum harmonicum (Parodi) McClure	LC et al. 1128	Ecuador	JQ408563	JQ408582	JQ408517	JQ408661	JQ408478	JQ408615
Rhipidocladum sp.	LC 1464	Ecuador	JQ408561	JQ408581	JQ408516	JQ408659	JQ408476	JQ408622
Rhipidocladum sect. *Didymogonyx* L.G.Clark & Londoño								
Rhipidocladum geminatum (McClure) McClure	FE 2	Venezuela	JQ408562	–	–	JQ408660	JQ408477	JQ408614
Rhipidocladum aff. *longispiculatum* Londoño & L.G.Clark	XL & ER 995	Colombia	JQ408558	–	JQ408513	JQ408656	JQ408473	–
Rhipidocladum sect. *Racemiflorum* L.G.Clark & Londoño								
Rhipidocladum bartlettii (McClure) McClure	LC 1168	Mexico	JQ408559	–	JQ408514	JQ408657	JQ408474	JQ408613
Rhipidocladum clarkiae R.W.Pohl	CT et al. 47	Costa Rica	JQ408560	–	JQ408515	JQ408658	JQ408475	–
Rhipidocladum maxonii (Hitchc.) McClure	CT et al. 46	Costa Rica	JQ408564	–	JQ408518	JQ408662	–	–
Rhipidocladum neumannii Sulekic, Rúgolo & L.G.Clark	LC & FZ 1019	Argentina	JQ408565	JQ408583	JQ408519	JQ408663	JQ408479	JQ408616
Rhipidocladum pacuarense R.W.Pohl	CT et al. 41	Costa Rica	JQ408566	–	JQ408520	JQ408664	JQ408480	–
Rhipidocladum parviflorum (Trin.) McClure	TF s.n.	Brazil	JQ408567	–	JQ408521	JQ408665	JQ408481	JQ408617
Rhipidocladum pittieri (Hack.) McClure	LC & WZ 1349	Costa Rica	JQ408568	JQ408584	JQ408522	JQ408666	JQ408482	JQ408618
Rhipidocladum racemiflorum (Steud.) McClure	CT & JT 26	Costa Rica	JQ408569	JQ408585	JQ408523	JQ408667	JQ408483	JQ408619
Rhipidocladum sibilans Davidse, Judz. & L.G.Clark	LC et al. 1524	Venezuela	–	–	–	JQ408668	JQ408484	JQ408620
Rhipidocladum sp.	TF s.n.	Brazil	JQ408557	–	JQ408512	JQ408655	JQ408472	JQ408621

(continued on next page)

Table 1 (*continued*)

Taxon	Voucher	Origin	GenBank ID					
			ndhF	rpL16 Intr.	trnC-rpoB	trnD-trnT	rps16-trnQ	trnT-trnL
Guaduinae Soderstr. & R.P.Ellis								
Apoclada simplex McClure & L.B.Sm.	LC & WO 1027	Brazil	JQ408527	–	JQ408488	JQ408626	JQ408448	JQ408589
Eremocaulon aureofimbriatum Soderstr. & Londoño	AC 4393	Brazil	JQ408549	EF589616	JQ408504	JQ408647	JQ408464	–
Guadua angustifolia Kunth	XL & LC 931	Colombia	FJ643714	FJ751664	FJ643910	FJ644003	FJ643817	FJ644154
Olmeca recta Soderstr.	LC 1313	Mexico	JQ408556	–	JQ408511	JQ408654	JQ408468	JQ408611
Otatea acuminata (Munro) C.E.Calderón & Soderstr.	LC 1312	Mexico	AF182350	U54749	FJ751705	FJ751732	FJ751678	JQ408612
Bambusinae J.Presl in C.Presl								
Bambusa vulgaris Schrad.	JS 666	Cultivated	FJ643709	AY912192	FJ751706	FJ643982	FJ643796	FJ644133

only on characters traditionally used to distinguish genera in this subtribe. Characters and coded states are listed in the legend on Fig. 2. Only taxa with subtending bracts, prophylls and bud-bearing bracts were scored as having pseudospikelets. The states were parsimony optimized on the combined consensus tree using MacClade (Maddison and Maddison, 2005). Alternative reconstructions resolved by accelerated and delayed transformation (ACCTRAN/DELTRAN) were explored. Fig. 2 graphically depicts state changes under delayed reconstruction. We also inferred ancestral states by likelihood reconstruction using the Markov k-state 1 parameter model (Mk1) in Mesquite (Maddison and Maddison, 2011); these results are available as electronic Supplementary Material.

3. Results

3.1. Data matrix

The combined, aligned data matrix was 6969 bp long, with 37 binary characters. Only 265 characters (3.8%) were parsimony informative (PIC). Alignments for all regions averaged 1.15 kb in length: ndhF, 1163 bp (68 PIC); rpl16, 1055 bp (23 PIC); rps16-trnQ, 1217 bp (52 PIC); trnC-rpoB, 1343 bp (43 PIC); trnD-trnT, 1292 bp (37 PIC); and trnT-trnL, 862 (31 PIC). Regions were missing for some taxa (Table 1) resulting in 23.3% of the combined matrix being coded as missing.

3.2. Phylogeny

MP analysis of the combined data inferred 808 equally most parsimonious trees, each with a length of 766 steps, consistency index (CI) = 0.6521 and retention index (RI) = 0.8424. The most likely BI trees had a −log score of 17037.93. The BI analysis produced trees with topologies similar to the MP analysis. Fig. 1 illustrates a consensus of these two analyses.

The monophyly of each subtribe was highly supported (100% BS, 1.00 PP, 7 + DI). We recovered several moderately supported lineages within Arthrostylidiinae, of which we highlight four, designating them by their oldest generic name (Fig. 1): (I) the Glaziophyton clade (82% BS, 1.00 PP, 3 DI); (II) the Arthrostylidium clade (66% BS, 1.00 PP, 2 DI); (III) the Aulonemia clade (86% BS, 1.00 PP, 5 DI); (IV) the Merostachys clade (88% BS, 1.00 PP, 3 DI). The Aulonemia clade (III) is sister to the Merostachys clade (IV) with overall moderate support (74% BS, 1.00 PP, 2 DI). The Arthrostylidium clade (II) is moderately supported (99% BS, 0.97 PP, 1 DI) as sister to the Merostachys + Aulonemia clade and the Glaziophyton clade (I) is strongly supported as sister to the Arthrostylidium + (Merostachys + Aulonemia) clade. We chose to name these four clades (as opposed to other possible groupings) based on a combination of phylogenetic divergence and approximate equivalence of diversity (clades II–IV) simply for ease of reference.

Three lineages form a polytomy within the Arthrostylidium clade (II). The type species of *Arthrostylidium*, *Ar. cubense*, falls within a relatively well-supported lineage (90% BS, 1.00 PP, 5 DI) including most sampled species of *Arthrostylidium* and *Rhipidocladum*. Within this grouping, *Ar. pubescens* is sister (72% BS, 1.00 PP, 1 DI) to a lineage representing much of *Rhipidocladum* section *Racemiflorum* (100% BS, 1.00 PP, 6 DI). *Rhipidocladum* section *Rhipidocladum*, including the type species *R. harmonicum* and an undescribed species from Ecuador (LC 1464) is strongly supported as monophyletic (100% BS, 1.00 PP, 7 DI). The second lineage is formed by the two species of *Elytrostachys*, a strongly supported lineage (99% BS, 1.00 PP, 5 DI), which appear as sister to the *Ar. merostachyoides* + *R. maxonii* clade (100% BS, 1.00 PP, 5 DI), but without significant support. *R. maxonii* is currently classified within *R.* sect. *Racemiflorum*. The two species of *R.* sect. *Didymogonyx*

Table 2
Results of Kishino-Hasegawa and Templeton tests for topologies with taxonomic and morphological test groups constrained to be monophyletic against the consensus topology recovered by Bayesian inference and maximum parsimony methods for Arthrostylidiinae using a combined chloroplast DNA data set.

Constraint	Length overall	Kishino–Hasegawa test				Templeton test			
		Length difference	s.d.	t	p-Value	Rank sums	N	z	p-Value
Taxonomic									
Arthrostylidiinae	766	0	–	–	–	1.5 −1.5	2	–	–
Arthrostylidium	799	33	6.228	5.299	<0.0001	630.0 −36.0	36	−5.284	<0.0001
Aulonemia	795	29	6.232	4.654	<0.0001	680.0 −100.0	39	−4.644	<0.0001
Rhipidocladum	791	25	5.557	4.499	<0.0001	378.0 −28.0	28	−4.490	<0.0001
Morphological									
Apsidate branching	789	23	4.99	4.61	<0.0001	312.0 −13.0	25	−4.600	<0.0001
Erect culm leaves	792	26	5.465	4.758	<0.0001	434.0 −31.0	30	−4.747	<0.0001
Nucoid caryopsis	766	0	–	–	–	0	–	–	–
Geniculate synflorescence form	781	15	4.118	3.643	0.0003	144.0 −9.0	17	−3.638	0.0003
Internodes with variable lengths	801	35	6.69	5.232	<0.0001	819.0 −84.0	42	−5.218	<0.0001
Pseudospikelets	782	16	4.685	3.415	0.0006	218.5 −34.5	22	−3.411	0.0006

comprise the third strongly supported lineage (100% BS, 1.00 PP, 12 DI). Two undescribed species from the high Andes of Ecuador and Colombia presumed to belong to *Arthrostylidium* (*Ar.* sp. LC 1101 and *Ar.* sp. XL 900) form a robust monophyletic group (100% BS, 1.00 PP, 11 DI), sister to the remainder of the Aulonemia clade (III) with 86% BS, 1.00 PP, 5 DI.

Four of the six sampled *Aulonemia* species, including the type species (*Au. queko*), form a moderately-well supported clade (86% BS, 1.00 PP, 4 DI) within the Aulonemia clade (III). The Brazilian *Au. amplissima* also falls within the Aulonemia clade (III), but as sister (97% BS, 1.00 PP, 4 DI) to the weakly monophyletic *Colanthelia* McClure & E.W. Sm. (70% BS, 0.91 PP, 1 DI). The lineage consisting of *Colanthelia* plus *Au. amplissima* is strongly supported (100% BS, 1.00 PP, 14 DI) as sister to the formerly mentioned clade of four species of *Aulonemia*. One species of *Aulonemia*, *Au. ulei*, is resolved within the Glaziophyton clade (I) with 82% BS, 1.00 PP, and 3 DI.

The Merostachys clade (IV) consists of three robustly supported lineages, two of which associate in a rather poorly supported clade. The two species of *Filgueirasia* are unequivocally supported as a monophyletic group (100% BS, 1.00 PP, 14 DI). Likewise, *Alvimia* (99% BS, 1.00 PP, 5 DI) and *Atractantha* (100% BS, 1.00 PP, 5 DI) are each well supported as is their sister relationship (100% BS, 1.00 PP, 6 DI); together they form a clade with *Filgueirasia* but with support only from the BI analysis (0.97 PP). The third lineage (98% BS, 1.00 PP, 4 DI) comprises the monotypic *Actinocladum* sister to a strongly monophyletic *Merostachys* (100% BS, 1.00 PP, 16 DI).

We resolved six genera as monophyletic: *Merostachys*, *Alvimia*, *Atractantha*, *Filgueirasia*, *Colanthelia*, and *Elytrostachys*. Both *Actinocladum* and *Glaziophyton* are monotypic, but both were recovered external to the other genera. The polyphyly of *Arthrostylidium*, *Aulonemia*, and *Rhipidocladum* is corroborated by the KH and Templeton tests which indicate a significant difference (p-value < 0.0001) between the recovered topology (Fig. 1) and topologies where each genus is constrained to be monophyletic (Table 2).

3.3. Morphology

Morphological characters (Table 3) mapped onto our phylogenetic hypothesis by parsimony and likelihood methods show very similar results. The delayed parsimony optimization summarized in Fig. 2 highlights several characters with homoplasious tendencies, but also demonstrates consistency in a few characters. Culm leaf blade orientation, branching architecture, synflorescence form, and spikelet structure have equivocal regions in the reconstruction, but examination of both accelerated and delayed optimizations suggests multiple origins or reversals for each, as well as for internode length dimorphism as a whole.

Culm leaf orientation is mostly consistent within genera or lineages. Erect, non-pseudopetiolate culm leaves are found in the Glaziophyton clade (I) and throughout the Arthrostylidium clade (II) except for *Elytrostachys*. The Aulonemia clade (III) evolved reflexed, pseudopetiolate culm leaf blades with no reversals. Within the Merostachys clade (IV), reflexed culm leaf blades also evolved in *Actinocladum* + *Merostachys* and in two species of *Atractantha*, but the trichotomy in *Atractantha* makes the number of origins or reversals ambiguous. Erect culm leaf blades are also consistently present in the Guaduinae, except in *Er. aureofimbriatum*.

Two of the three genera with fan-branching, *Merostachys* and *Actinocladum*, form a well-supported clade. Within the Arthrostylidium clade (II), fan-branching is optimized as having one origin with three reversals (in *Elytrostachys*, the *Ar. cubense–Ar. venezuelae* clade and *Ar. pubescens*). As coded, the branching configuration with 2–5 dominant branches arising from each mid-culm node putatively originated twice within the subtribe, once within *Atractantha* and once within *Filgueirasia*. A branch complement of 5–7 subequal branches arose once in *Aulonemia ulei*. The remaining lineages exhibit one dominant branch with or without lateral secondary branches.

The promontory has at least seven independent origins, one within the Guaduinae and six within the Arthrostylidiinae. Lack of resolution, especially within the Arthrostylidium clade (II), obviously makes it possible to hypothesize fewer origins and more reversals.

Table 3
Character matrix of coded states for each taxon used to map morphological features on consensus tree of combined chloroplast DNA data set. Internode length patterns are abbreviated as follows: L-s-s-s-L = alternation of one to four short with one long; L-s-s-s-s = long basal internode followed by shorter internodes; L-s-L-s = alternation of one short with one long.

Taxon	Spikelets	Caryopsis	Culm leaf blade	Prophyll shape	Synflorescence form	Internode pattern	Dominant branches	Branch leaf tessellate	Promontory
Actinocladum verticillatum	Conventional	Nucoid	Reflexed	Triangular	Raceme	All equal	Fan	Absent	Absent
Alvimia auriculata	Pseudo-	Bacoid	Erect	Triangular	Panicle	All equal	1	Absent	Present
Alvimia gracilis	Pseudo-	Bacoid	Erect	Triangular	Panicle	All equal	1	Absent	Present
Apoclada simplex	Conventional	Typical	Erect	Triangular	Panicle	All equal	2–5	Absent	Absent
Arthrostylidium sp. LC 1101	Conventional	Typical	Reflexed	Triangular	Geniculate	All equal	1	Absent	Present
Arthrostylidium sp. XL 900	Conventional	Typical	Reflexed	Triangular	Raceme	All equal	1	Absent	Present
Arthrostylidium cubense	Conventional	Typical	Erect	Triangular	Raceme	All equal	1	Absent	Present
Arthrostylidium merostachyoides	Conventional	Typical	Erect	Triangular	Raceme	All equal	Fan	Absent	Present
Arthrostylidium multispicatum	Conventional	Typical	Erect	Triangular	Geniculate	All equal	1	Absent	Present
Arthrostylidium pubescens	Conventional	Typical	Erect	Triangular	Raceme	All equal	1	Absent	Present
Arthrostylidium urbanii	Conventional	Typical	Erect	Triangular	Raceme	All equal	1	Absent	Present
Arthrostylidium venezuelae	Conventional	Typical	Erect	Triangular	Geniculate	All equal	1	Absent	Present
Atractantha aureolanata	Pseudo-	Typical	Reflexed	Triangular	Panicle	All equal	2–5	Absent	Present
Atractantha cardinalis	Pseudo-	Typical	Reflexed	Triangular	Panicle	All equal	2–5	Absent	Present
Atractantha radiata	Pseudo-	Typical	Erect	Triangular	Head	All equal	2–5	Absent	Present
Aulonemia amplissima	Conventional	Typical	Reflexed	Triangular	Panicle	All equal	1	Absent	Present
Aulonemia hirtula	Conventional	Typical	Reflexed	Triangular	Panicle	L-s-s-s-L	1	Absent	Present
Aulonemia patriae	Conventional	Typical	Reflexed	Triangular	Panicle	All equal	1	Absent	Present
Aulonemia patula	Conventional	Typical	Reflexed	Triangular	Panicle	All equal	1	Absent	Present
Aulonemia queko	Conventional	Typical	Reflexed	Triangular	Panicle	L-s-s-s-L	1	Absent	Present
Aulonemia ulei	Conventional	Typical	Erect	Triangular	Raceme	All equal	5–7	Present	Present
Bambusa vulgaris	Pseudo-	Typical	Erect	Triangular	Panicle	All equal	1	Absent	Absent
Colanthelia cingulata	Conventional	Typical	Reflexed	Triangular	Panicle	All equal	1	Absent	Present
Colanthelia distans	Conventional	Typical	Reflexed	Triangular	Raceme	All equal	1	Absent	Present
Colanthelia intermedia	Conventional	Typical	Reflexed	Triangular	Panicle	All equal	1	Absent	Present
Elytrostachys clavigera	Pseudo-	Typical	Reflexed	Triangular	Raceme	All equal	1	Absent	Present
Elytrostachys typica	Pseudo-	Typical	Reflexed	Triangular	Raceme	All equal	1	Absent	Present
Eremocaulon aureofimbriatum	Pseudo-	Typical	Reflexed	Triangular	Panicle	All equal	1	Absent	Absent
Filgueirasia arenicola	Conventional	Typical	Erect	Triangular	Raceme	All equal	2–5	Absent	Absent
Filgueirasia cannavieira	Conventional	Typical	Erect	Triangular	Raceme	All equal	2–5	Absent	Absent
Glaziophyton mirabile	Conventional	Typical	Erect	Triangular	Panicle	L-s-s-s-s	1	Present	Absent
Guadua angustifolia	Pseudo-	Typical	Erect	Triangular	Panicle	All equal	1	Absent	Absent
Merostachys sp. AC 4366	Conventional	Nucoid	Reflexed	Triangular	Raceme	All equal	Fan	Absent	Absent
Merostachys sp. AC 4378	Conventional	Nucoid	Reflexed	Triangular	Raceme	All equal	Fan	Absent	Absent
Merostachys ternata	Conventional	Nucoid	Reflexed	Triangular	Raceme	All equal	Fan	Absent	Absent
Olmeca recta	Conventional	Bacoid	Erect	Triangular	Panicle	All equal	1	Absent	Present
Otatea acuminata	Conventional	Typical	Erect	Triangular	Panicle	All equal	2–5	Absent	Present
Rhipidocladum sp. TF s.n.	Conventional	Typical	Erect	Triangular	Raceme	All equal	Fan	Absent	Absent
Rhipidocladum aff. *longispiculatum*	Conventional	Typical	Erect	Cordate	Panicle	L-s-L-s	Fan	Absent	Absent
Rhipidocladum bartlettii	Conventional	Typical	Erect	Triangular	Raceme	All equal	Fan	Absent	Absent
Rhipidocladum clarkiae	Conventional	Typical	Erect	Triangular	Raceme	All equal	Fan	Absent	Absent
Rhipidocladum sp. LC 1464	Conventional	Typical	Erect	Triangular	Geniculate	All equal	Fan	Absent	Absent
Rhipidocladum geminatum	Conventional	Typical	Erect	Cordate	Panicle	L-s-L-s	Fan	Absent	Absent
Rhipidocladum harmonicum	Conventional	Typical	Erect	Triangular	Geniculate	All equal	Fan	Absent	Absent
Rhipidocladum maxonii	Conventional	Typical	Erect	Triangular	Raceme	All equal	Fan	Absent	Absent
Rhipidocladum neumannii	Conventional	Typical	Erect	Triangular	Raceme	All equal	Fan	Absent	Absent
Rhipidocladum pacuarense	Conventional	Typical	Erect	Triangular	Raceme	All equal	Fan	Absent	Absent
Rhipidocladum parviflorum	Conventional	Typical	Erect	Triangular	Raceme	All equal	Fan	Absent	Absent
Rhipidocladum pittieri	Conventional	Typical	Erect	Triangular	Raceme	All equal	Fan	Absent	Absent
Rhipidocladum racemiflorum	Conventional	Typical	Erect	Triangular	Raceme	All equal	Fan	Absent	Absent
Rhipidocladum sibilans	Conventional	Typical	Erect	Triangular	Raceme	All equal	Fan	Absent	Absent

With respect to synflorescence form evolution, most of the sampled species bear spikelets bilaterally along straight axes in racemose synflorescences. Paniculate synflorescences map to the Aulonemia + Colanthelia clade, with a reversal to racemose synflorescences in *C. distans*, but also occur in Glaziophyton, *Alvimia + Atractantha* and in *R.* sect. *Didymogonyx*.

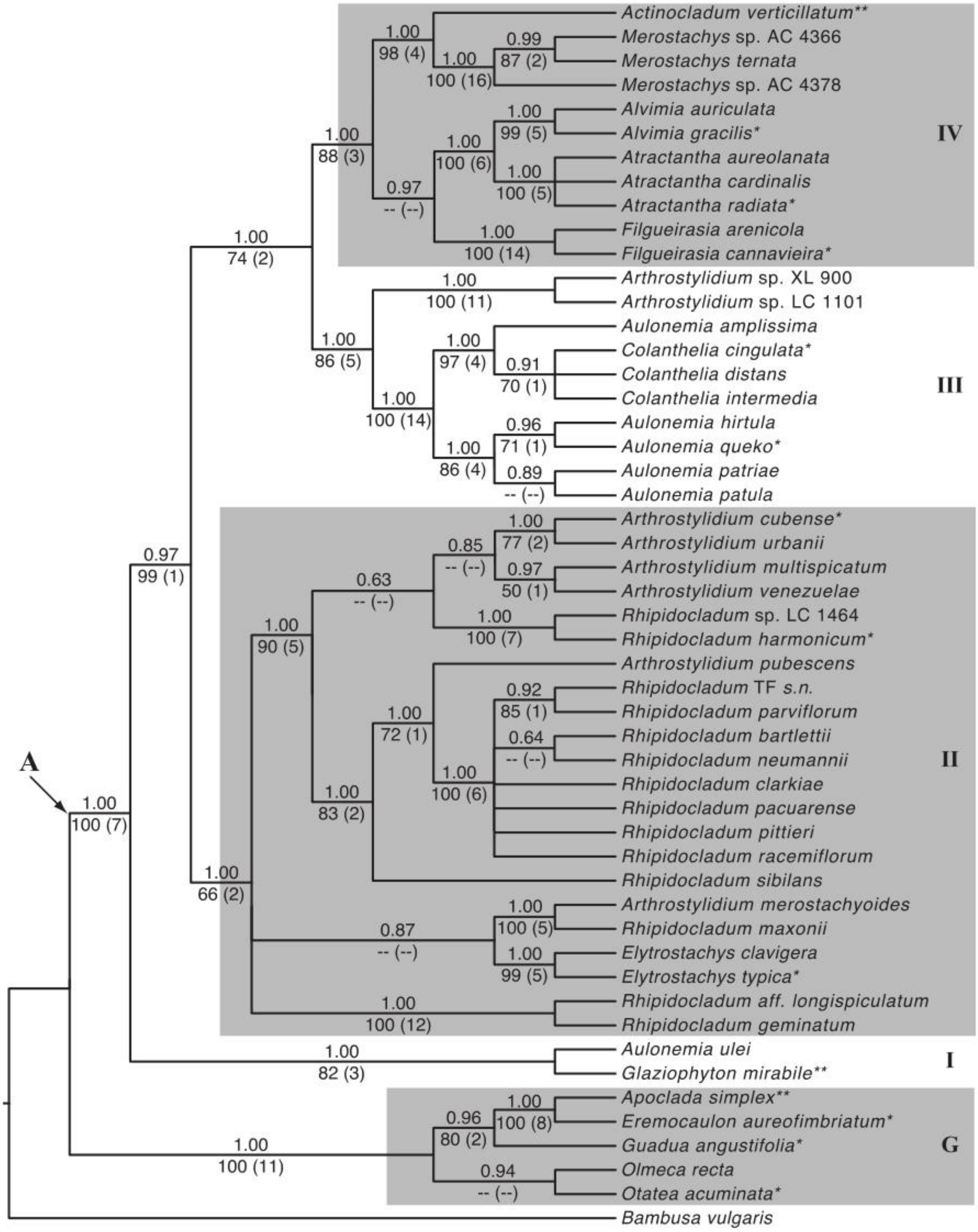

Fig. 1. Fifty percent majority consensus tree of Bayesian inference (BI) and strict consensus of maximum parsimony trees inferred from a combined chloroplast DNA dataset of trnD-trnT, rps16-trnQ, trnC-rpoB intergenic spacers, rpl16 intron, and the 3′ half of the ndhF gene. BI posterior probabilities appear above the branches; below the branches the first value indicates percentage bootstrap support (>50%) and the value following in parentheses is the Bremer decay index. Newly resolved arthrostylidioid clades are indicated with Roman numerals: I = Glaziophyton clade; II = Arthrostylidium clade; III = Aulonemia clade; IV = Merostachys clade; subtribes are indicated with letters: A = Arthrostylidiinae; G = Guaduinae; * = type species; ** = monotypic genus.

The geniculate synflorescence form is found in *R.* sect. *Rhipidocladum*, four described species of *Arthrostylidium* (two represented on our tree – *Ar. venezuelae* and *Ar. multispicatum*), and two undescribed species of *Arthrostylidium* (LC 1101 and XL 900). *R.* sect. *Rhipidocladum*, *Ar. venezuelae* and *Ar. multispicatum* are members of the Arthrostylidium clade (II). Our findings show these taxa forming a clade, along with *Ar. cubense* and *Ar. urbanii*, but with no statistical support. The presence of geniculate synflorescences in the undescribed Andean *Arthrostylidium* species in the Aulonemia clade (III) shows unquestionably that the geniculate synflorescence form had more than one origin or reversal.

Pseudospikelets evolved twice within the Arthrostylidiinae, once in *Elytrostachys* and again in *Alvimia* + *Atractantha*. In the Guaduinae, pseudospikelets are known from *Guadua* and *Eremocaulon*.

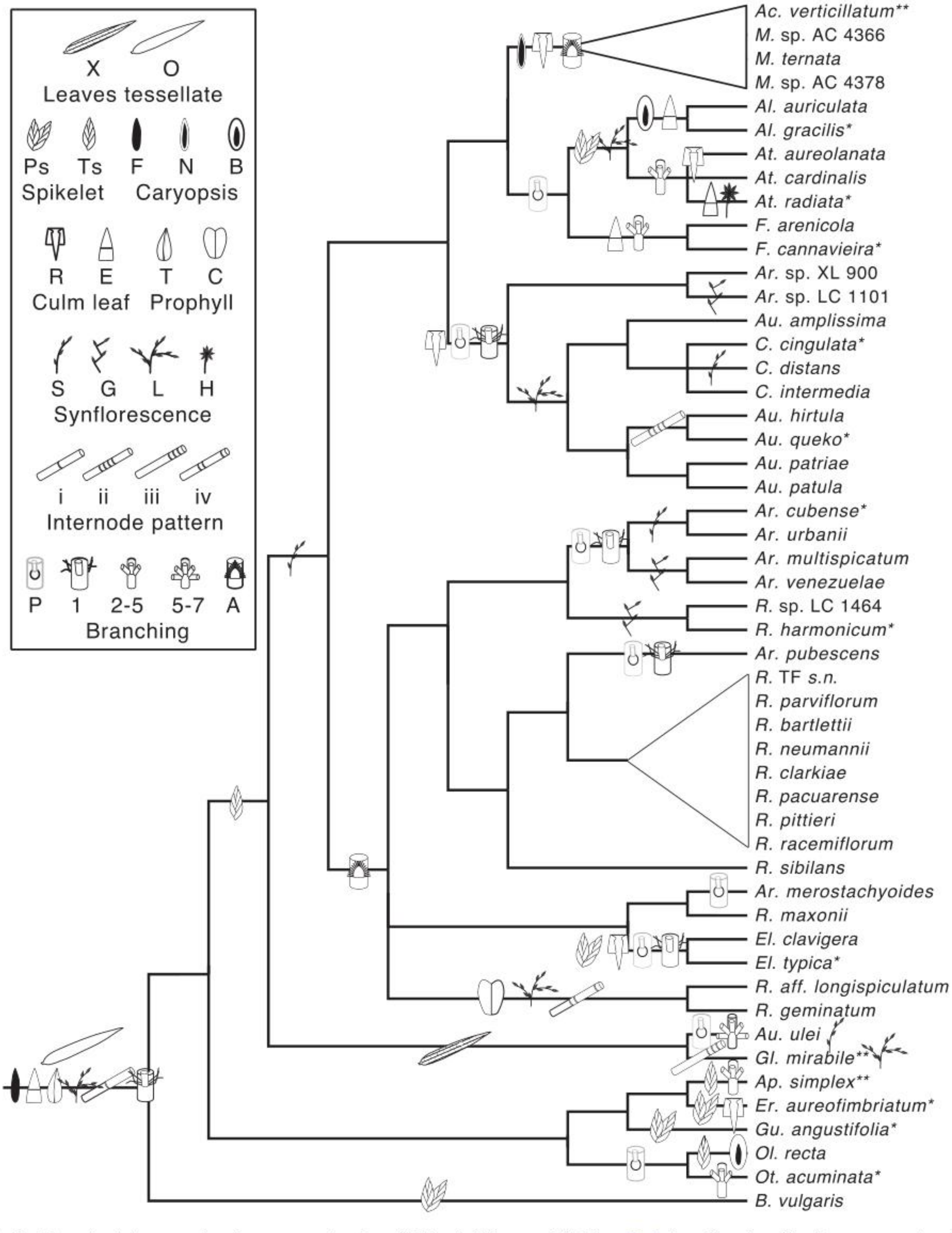

Fig. 2. Morphological character states mapped on the consensus topology of Arthrostylidiinae modified from Fig. 1. Branching: P = arising from a promontory, 1 = 1 dominant branch, 2–5 = 2–5 subequal branches, 5–7 = 5–7 subequal branches, A = fan-branching (apsidate); Caryopsis: F = fused pericarp (basic), N = nucoid, B = bacoid; Culm leaf blade position: R = blade reflexed, E = blade erect; Internode pattern: i = all subequal, ii = alternating 1 long with 2–4 short, iii = basalmost 1 elongated then 3–4 short, iv = entire culm alternating 1 long and 1 short; Prophyll shape: T = triangular, C = cordate; Spikelet: Ps = pseudospikelets, Ts = true spikelets; Synflorescence: S = racemose, straight axis, G = racemose, geniculate (zig-zag) axis, L = panicle, H = head; Branch leaves tessellate: O = absent, X = present. * = type species; ** = monotypic genus.

Both the parsimony and likelihood model optimizations are ambiguous as to the ancestral state for this character, therefore we cannot determine the number of origins or reversals in the Guaduinae.

Internodes of two different lengths (internode dimorphism) appear multiple times in our results, but each pattern of length differences may be independently derived. *Glaziophyton*, with its long basal internode followed by several very short ones, is monotypic. The *Aulonemia* species allied to *Glaziophyton* in our phylogeny has subequal internodes. The two *Aulonemia* species we sampled that consistently alternate 1–4 short internodes with one long were resolved in a sister relationship within the Aulonemia clade (III), indicating a single origin of that pattern. And the other internode length pattern, regular alternation of one short and one long internode, was recovered as potentially synapomorphic for *R.* sect. *Didymogonyx*.

Of the characters we examined, the three most consistent with the phylogeny are prophyll shape, fruit type, and tessellate leaf blades. Within the Arthrostylidiinae, nucoid caryopses are potentially synapomorphic for *Merostachys* plus *Actinocladum*, and bacoid caryopses are potentially synapomorphic for *Alvimia*. Cordate prophylls, here, are a putative synapomorphy for *R.* sect. *Didymogonyx*, and the Glaziophyton clade (I) is supported by the potential synapomorphy of tessellate leaf blades.

4. Discussion

4.1. Lineages and taxonomy

The novel topology recovered in this study provides the first multi-locus chloroplast phylogeny of Arthrostylidiinae. Previously, only cursory relationships between the genera could be suggested based on morphology (Judziewicz et al., 1999; McClure, 1973) and the complicated morphology made it difficult to even speculate about higher level relationships. Each of the four clades revealed in our work represents a phylogenetically divergent chloroplast DNA lineage. Lack of data from nuclear DNA and low numbers of informative characters supporting some the branches, however, warrant a conservative interpretation of these groups.

4.1.1. Guaduinae

In agreement with other studies (Clark et al., 2007; Ruiz-Sanchez et al., 2011; Sungkaew et al., 2009; Zhang and Clark, 2000), we also recover a monophyletic Guaduinae. Even with the small number of representative guaduoid species in this study, the potential for good support of internal structure using these six DNA regions is evident. We expect that individual taxa will continue to be sifted between Arthrostylidiinae and Guaduinae, but these would represent misclassifications remedied by examination of leaf anatomy and micromorphology or sequencing.

4.1.2. Arthrostylidiinae

By including 11 of 13 genera and a substantial range of morphologies, we confidently demonstrate a monophyletic Arthrostylidiinae based on chloroplast DNA sequence data. Another important finding is the resolution, with reasonable support, of the Glaziophyton clade (I) as sister to the remainder of the subtribe. Tessellate leaf blades provide a potential synapomorphy for this clade, although this feature is known from other groups of bamboos characteristic of high altitudes (e.g., *Chusquea* sect. *Swallenochloa*; Clark, 1989) or high latitudes (many Arundinarieae; McClure, 1966). It is perhaps noteworthy that both members of the Glaziophyton clade (I) are from southeastern Brazil; once better internal resolution is achieved within the other three lineages and the remaining two genera sampled, a comprehensive biogeographic analysis should be undertaken.

The presence of reflexed branch leaf blades (pseudopetioles) is an apparent synapomorphy for the clade comprising the rest of the subtribe (II–IV); this is an unusual feature among woody bamboos and gives the members of this large group a characteristic appearance in the field. The erect leaf blades characteristic of the Glaziophyton clade (I) and *Filgueirasia* may be correlated with the open habitats these taxa occupy, but this remains to be investigated. None of the three other major arthrostylidioid clades (II, III or IV) we identified have well defined synapomorphies and most lack any recognized, shared morphological features. It is possible that a detailed study of leaf anatomy and micromorphology within Arthrostylidiinae will provide diagnostic characters for these clades or genera. Stomatal position has already proven useful in *Colanthelia* (Santos-Gonçalves, 2005).

Although this is the most comprehensive phylogenetic analysis of the Arthrostylidiinae to date, lack of internal resolution or support in parts of the tree hampers our understanding of its morphological evolution and biogeography. Whole plastome sequence data may help resolve some of these relationships, but plastid DNA alone is unlikely to provide a complete picture due to phenomena such as lineage sorting, chloroplast capture and hybridization (BPG, 2012 and references cited therein). Long generation times in woody bamboos may be responsible for their slower rates of molecular evolution relative to other grasses, which is evident in plastid sequence data (Gaut et al., 1997; Janzen, 1976). This is yet another reason why additional plastid sequence data may not be very informative, even though arthrostylidioid bamboos have shorter flowering cycles, on the order of 15–40 years (Pohl, 1980), than many woody bamboos. Phylogenetic reconstruction for this group (and other woody bamboos) should also be carried out using nuclear markers, recognizing that this is non-trivial given nearly universal polyploidy in woody bamboos (Judziewicz et al., 1999).

4.1.3. Actinocladum + Merostachys

Actinocladum verticillatum was originally described in *Rhipidocladum* based on its fan-branching, but it differed from its congeners in spikelet and synflorescence morphology (Soderstrom, 1981) as well as caryopsis type. The flowering structure is paniculate to racemose. *Actinocladum* also bears reflexed culm leaves, a trait shared with many Arthrostylidiinae genera including *Aulonemia* and *Merostachys*. The sequence data unambiguously support a sister relationship between *Actinocladum* and *Merostachys*, with the presence of fan-branching and the nucoid caryopsis providing diagnostic morphological characters for the clade.

Merostachys is the second largest genus in the subtribe and is ecologically important in South America, but remains poorly known (Judziewicz et al., 1999). It was thought to be closely related to *Rhipidocladum* (Judziewicz et al., 1999; McClure, 1973), sharing fan-branching, but differing in culm leaf orientation, spikelet features, and caryopsis type. We have shown *Merostachys* to belong to a clade distinct from that containing the type of *Rhipidocladum*, indicating no close relationship. Our current state of knowledge suggests that both *Actinocladum* and *Merostachys* should continue to be recognized as distinct genera until more detailed morphological and molecular analyses of both can be completed.

4.1.4. Alvimia + Atractantha and Filgueirasia

The known distribution of *Alvimia* is completely within the range of *Atractantha* in the Atlantic forests of Bahia, Brazil (Judziewicz, 1992). These genera were suspected to be related based on similarities in leaf anatomy, the presence of pseudospikelets, and their scrambling habit (Soderstrom and Londoño, 1988). The pseudospikelets of *Alvimia* are long and bear many florets, whereas those of *Atractantha* are short and include only one spindle-shaped fertile floret in the spikelet proper. Even though the pseudospikelets of *Atractantha radiata* are usually arrayed in dense heads, teratological specimens proliferate into long, spicate branches not unlike the pseudospikelets of *Alvimia*. Despite some similarities and their sister relationship, morphological differences clearly support the continued recognition of both *Alvimia* and *Atractantha* as genera.

The branching morphology of *Filgueirasia* is clearly non-homologous to that of *Atractantha*, as the usually 2–5 branches per node of the former genus are derived from multiple buds (Guala, 1995) and all other members of Arthrostylidiinae have a single bud per node (Judziewicz et al., 1999). The erect leaves, presence of white fimbriae on the foliage leaf sheaths, and cerrado habitat of *Filgueirasia* also support its generic status (Guala, 2003), although its relationship to the rest of the Merostachys clade (IV) remains unclear.

4.1.5. The Aulonemia clade

Branching architecture and occasional paniculate synflorescences in species of *Colanthelia* suggest an affinity with *Aulonemia*

(McClure, 1973). Recent revisions of both *Colanthelia* (Santos-Gonçalves, 2005) and *Aulonemia* (Judziewicz et al., in preparation; Viana et al., in review) as part of the Bamboo Phylogeny Project (BPG, 2006b) are nearing completion. These works will address our intermingled results of *Aulonemia* with *Colanthelia* and the Glaziophyton clade (I) from a morphological perspective. A separate manuscript (Judziewicz et al., in preparation) will evaluate the systematics of the Andean group of *Arthrostylidium* (*Ar.* sp. LC 1101 and *Ar.* sp. XL 900) in relation to *Colanthelia* and *Aulonemia*.

4.1.6. *Arthrostylidium* + *Rhipidocladum*

Arthrostylidium has long been a taxonomic catch-all for aberrant neotropical woody bamboos and its polyphyly in our results reflects that. Though progress has been made toward delineating the true entity deemed *Arthrostylidium*, it is clear that this imbroglio cannot be sorted out without greater taxon sampling, in-depth morphological analysis, and sequencing of nuclear markers. A number of the species currently classified as *Arthrostylidium* are morphological anomalies which may prove to be new genera or may not even belong in the subtribe.

The fate of *Rhipidocladum* is intimately tied to *Arthrostylidium*, and hinges on the blurred distinction between promontory development and fan-branching. *R.* sect. *Rhipidocladum* has strong molecular support and is morphologically distinct from *R.* sect. *Racemiflorum*, but beyond branch complement morphology, there are few characters that distinguish either section from *Arthrostylidium*. Furthermore, there is uncertainty surrounding the monophyly of *R.* sect. *Racemiflorum* as one species of *Arthrostylidium* is embedded in this section. Davidse and Pohl (1992) admit the generic placement of *Ar. merostachyoides* was difficult as it shares characters with *Merostachys*, *Rhipidocladum*, and *Arthrostylidium*. They eventually placed the taxon in *Arthrostylidium* on the basis of the branch complement morphology, which we have now shown to be unconserved among lineages. The *Ar. merostachyoides* + *R. maxonii* lineage is strongly supported as monophyletic, but does not exhibit a combination of features that might support its removal from a concept of *Arthrostylidium* that includes *Rhipidocladum*. This problem will be treated in more detail in a separate manuscript (Tyrrell and Clark, in preparation).

The species of *R.* sect. *Didymogonyx* are robustly supported as a monophyletic group and they do share a suite of characters unique within the subtribe. This section is easily distinguished from the other sections of *Rhipidocladum* and all currently recognized genera in the subtribe by the combination of cordate prophylls, alternating long and short internodes along the culm, the presence of culm leaf fimbriae, spikelets in pairs or triads in a contracted synflorescence, and the unbranching long-cell extensions overarching the stomates (Clark and Londoño, 1991). We here elevate *R.* sect. *Didymogonyx* to generic status:

Didymogonyx (L.G. Clark & Londoño) C.D. Tyrrell, L.G. Clark & Londoño gen. et. stat. nov. *Rhipidocladum* section *Didymogonyx* L.G. Clark & Londoño, American Journal of Botany 78(9): 1271. 1991. TYPE: *Didymogonyx geminatum* (McClure) C.D. Tyrrell, L.G. Clark & Londoño.

1. *Didymogonyx geminatum* (McClure) C.D. Tyrrell, L.G. Clark, & Londoño comb. nov. *Arthrostylidium geminatum* McClure, Journal of the Washington Academy of Sciences 32(6): 169. 1942. *Rhipidocladum geminatum* (McClure) McClure, Smithsonian Contributions to Botany 9: 105. 1973. TYPE: VENEZUELA. Trujillo: Páramo de La Cristalina, on the border of the State of Trujillo, 20 October 1910, *A. Jahn 11* (holotype: VEN; isotype: US).
2. *Didymogonyx longispiculatum* (Londoño & L.G. Clark) C.D. Tyrrell, L.G. Clark & Londoño comb. nov. *Rhipidocladum longispiculatum* Londoño & L.G. Clark, American Journal of Botany 78(9): 1272. 1991. TYPE: COLOMBIA. Cundinamarca: Mun. Cabrera: vereda Nuñez, Hda. Pto. Nuñez, west slope of páramo of Sumapaz, 3°54′N 74°31′W, 2490–2500 m, 14 March 1989, *X. Londoño 429* (holotype: COL; isotypes: ISC, K, MO, TULV, US).

4.1.7. *Elytrostachys*

Elytrostachys is a seldom collected genus previously thought to be related to *Alvimia* and *Atractantha* based on the shared diffuse or capitate synflorescence form, reflexed culm leaves and pseudospikelets (Judziewicz et al., 1999). Our molecular results show *Elytrostachys* to be distinctly separate from the latter two genera and allied with the *Arthrostylidium–Rhipidocladum* complex. Neither *Arthrostylidium* nor *Rhipidocladum* have any of the above mentioned characters, nor do they exhibit the slender, straight fimbriae 2–8 cm long on the culm and foliage leaf sheaths unique to *Elytrostachys* (Judziewicz et al., 1999) The only obvious common feature among these genera is the development of a weak promontory in *Elytrostachys*, but otherwise its morphology clearly supports its recognition as at the generic level.

4.1.8. Other genera

Our phylogenetic hypothesis is still missing representatives of two genera: *Myriocladus* and *Athroostachys*. Given the level of homoplasy shown among the morphological characters, it is difficult to predict where these taxa are likely to be placed based on gross anatomy or morphology. McClure (1973) noted similarities between *Myriocladus* and *Glaziophyton* in the elongated first internode and spikelet morphology. We predict that sequence data will place *Myriocladus* (at least in part) in the Glaziophyton clade (I) based on these similarities and the shared presence of erect, usually tessellate leaf blades. *Athroostachys* is possibly related to *Merostachys*, based on superficial similarities in the fimbriae and reproductive apparatus (McClure, 1973); it should be noted that McClure did not consider these similarities enough to suggest a close phylogenetic relationship. It is perhaps more likely that *Athroostachys* will resolve within the *Alvimia* + *Atractantha* clade based on its typically three branches per node and bracteate synflorescences.

4.2. Morphology

We hypothesize that subequal internodes, triangular prophylls, branch complements with one dominant branch, culm leaves with erect blades, non-tessellate leaf blades, paniculate synflorescences and basic caryopses are plesiomorphic for the taxa sampled in this analysis. Polarity of character states for development of the promontory and spikelet structure cannot be unambiguously inferred from the current analysis, although conventional spikelets are inferred to be plesiomorphic for the Arthrostylidiinae. The presence of reflexed branch leaf blades diagnoses a major clade within the Arthrostylidiinae. Although the nucoid and bacoid caryopsis types are unambiguous synapomorphies in this analysis for the *Actinocladum* + *Merostachys* clade and *Alvimia*, respectively, these fruit types are not unique among woody bamboos, as examples of both are found in several other genera in the Old and New World bamboo floras (Judziewicz et al., 1999; Ruiz-Sanchez et al., 2011; Yang et al., 2008). However, the homologies of these fruit types across bamboos have not been adequately investigated.

Cordate prophylls are known from *R.* sect. *Didymogonyx* and *Myriocladus*. Without knowing the position of *Myriocladus*, however, little more can be inferred regarding prophyll evolution.

Most branching types can either possess a promontory or not. Based upon our phylogeny, fan-branching, however, appears to be mostly mutually exclusive with promontory morphology, with the exception of *Ar. merostachyoides*, at least based on adult morphology. McClure (1973) argued that the apsidate branch complement readily distinguished *Rhipidocladum* from the raised promontory that typifies *Arthrostylidium*. Increased collections, however, have provided numerous specimens with intermediate branch complements representing a continuum from a well-developed promontory to fan-branching. This is recapitulated ontogenetically, as the branching on young shoots of *Rhipidocladum* resemble a promontory while the mature culms of the same individual will have a distinctly apsidate form. On the other hand, a promontory is present in genera such as *Atractantha*, *Aulonemia* and *Colanthelia*, which lack fan-branching, and no obvious promontory is associated with the fan-branching characteristic of *Actinocladum* and *Merostachys*. In keeping with a conservative approach, we therefore treat the promontory as a separate character in the present analysis and make no inferences about a possible developmental connection between fan-branching and the presence of a promontory in the Arthrostylidium clade (II).

Capitate synflorescences cannot be evaluated as we are missing one (*Athroostachys*) of the two taxa in which this arrangement occurs. Occasional flowering specimens of *Elytrostachys* have been known to display synflorescences that appear capitate, but this is not consistent. The paired spikelets found in *R.* sect. *Didymogonyx* and *Ar. merostachyoides* could be interpreted as condensed from a paniculate arrangement (Clark and Londoño, 1991; Davidse and Pohl, 1992).

Pseudospikelets originate twice on our tree in the Arthrostylidiinae, but appear to be conserved within the three genera where they occur. The absence of pseudospikelets in *At. amazonica* is here interpreted as a loss, but this species needs to be included in a molecular analysis. A possible third origin could be in *Glaziophyton*, given the presence of subtending bracts and prophylls in its synflorescences (McClure, 1973), but the absence of gemmiparous bracts would then have to be interpreted as a loss.

The distribution of morphological variation on our phylogeny for a number of characters indicates that many gross morphologies may be the result of convergent evolution or lineage sorting. Still, features such as culm leaf blade orientation and pseudospikelets are largely conserved among currently delineated genera, but may not be synapomorphic. Both synflorescence form and branch complement architecture work moderately well at separating genera, but our current interpretations of these characters may be too simplified. Within the subtribe, fruit anatomy, prophyll shape, and tessellate branch leaves also appear to be a good characters for separation, but we were only able include two species with cordate prophylls.

5. Conclusions

Clearly, we have only begun to elucidate the evolutionary history of the arthrostylidioid bamboos. By generating a phylogeny that is taxonomically focused on the subtribe and its morphological variability, we have a better understanding of the internal relationships among genera and a foundation for future work. The results presented here will guide taxonomic investigations within the subtribe and should serve as molecular evidence for the putative monophyly of *Merostachys*, *Alvimia*, *Atractantha*, *Filgueirasia*, *Colanthelia*, and *Elytrostachys*. Based on our findings, we recommend a critical assessment of morphological and foliar anatomical characters including those used to distinguish Arthrostylidiinae genera. Promontory development and fan-branching deserve special attention, as these will have ramifications for the circumscriptions of *Arthrostylidium* and *Rhipidocladum*. We also recommend that nuclear markers be sequenced to improve our understanding of the phylogeny of this complex subtribe of neotropical woody bamboos.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ympev.2012.05.033.

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Glosssary

Apsidate: having branches arrayed in a vault-like or fan-shaped fashion

Bacoid: having a caryopsis (fruit) in which the seed coat and/or pericarp become enlarged and fleshy, berry-like

Capitate: a synflorescence that is contracted into a head or ball-shaped cluster

Caryopsis: the fruit of the grass family; hard, dry, indehiscent with pericarp fused to seed coat; a grain

Culm: aerial stem of a grass including bamboo

Floret: the basic unit of a grass inflorescence, consisting of a flower enclosed by two bracts (the lemma and the palea)

Nucoid: having a caryopsis (fruit) in which the seed coat is free from the pericarp, achene-like

Panicle: an indeterminate, branching synflorescence

Promontory: the swollen, unsegmented base of a bamboo branch complement

Prophyll: bud scale, the adaxial first branch leaf modified for protection of the meristem

Pseudopetiole: a constriction at the base of the leaf blade separating it from the apex of the sheath, similar to a eudicotyledon petiole

Pseudospikelet: a complex spikelet-like structure in which unit inflorescences develop from the lower bracts of the first-order spikelet and then rebranch in turn

Pulvinus (plural, pulvini): the swollen base of a synflorescence branch

Raceme: an indeterminate synflorescence with spikelets borne directly on the main axis

Spikelet: the unit inflorescence of the grass family; consists of one or more florets subtended by usually two empty bracts (glumes)

Synflorescence: the aggregation of spikelets (unit inflorescences) terminal to a culm or branch