Aliso: A Journal of Systematic and Evolutionary Botany

Volume 23 | Issue 1 Article 26

2007

Phylogenetic Relationships Among the One-Flowered, Determinate Genera of Bambuseae (Poaceae: Bambusoideae)

Lynn G. Clark
Iowa State University, Ames

Soejatmi Dransfield
Royal Botanic Gardens, Kew, UK

Jimmy Triplett
Iowa State University, Ames

J. Gabriel Sánchez-Ken
Iowa State University, Ames

Aliso 23, pp. 315–332

PHYLOGENETIC RELATIONSHIPS AMONG THE ONE-FLOWERED, DETERMINATE GENERA OF BAMBUSEAE (POACEAE: BAMBUSOIDEAE)

LYNN G. CLARK,[1,3] SOEJATMI DRANSFIELD,[2] JIMMY TRIPLETT,[1] AND J. GABRIEL SÁNCHEZ-KEN[1,4]

[1]*Department of Ecology, Evolution and Organismal Biology, Iowa State University, Ames, Iowa 50011-1020, USA;*
[2]*Herbarium, Royal Botanic Gardens, Kew, Richmond, Surrey TW9 3AE, UK*
[3]*Corresponding author (lgclark@iastate.edu)*

ABSTRACT

Bambuseae (woody bamboos), one of two tribes recognized within Bambusoideae (true bamboos), comprise over 90% of the diversity of the subfamily, yet monophyly of the tribe is generally only moderately supported, and phylogenetic relationships within the tribe are poorly understood. In addition, there appears to be some level of conflict between morphological and molecular data within the tribe. We conducted a parsimony analysis of 43 species of Bambuseae, three of Olyreae (herbaceous bamboos), and two outgroup taxa using morphological and plastid *rpl*16 intron sequence data to (1) further test the monophyly of Bambuseae, (2) test the monophyly of Chusqueinae and Hickelinae (the two one-flowered, determinate subtribes), and (3) examine the apparent conflict between molecular and morphological data sets in the determinate, one-flowered genera of Bambuseae. We recovered a monophyletic Bambusoideae, Bambuseae, Olyreae, and Chusqueinae, although support for Bambuseae remained moderate. Our results suggest that the morphological similarities between Chusqueinae and Hickelinae are homoplasious, but robust resolution of relationships among the major lineages of woody bamboos is still wanting.

Key words: Bambuseae phylogeny, Chusqueinae phylogeny, one-flowered bamboos, *rpl*16 intron.

INTRODUCTION

Bambusoideae (true bamboos), including over 1400 species, represent one of the major lineages within the grass family (Poaceae), and are the only major grass lineage to diversify primarily in association with woody vegetation (Grass Phylogeny Working Group [GPWG] 2001). Bambusoideae are defined by the synapomorphy of strongly asymmetrically invaginated arm cells in the leaf mesophyll, and comprise two tribes, Olyreae (herbaceous bamboos) and Bambuseae (woody bamboos) (GPWG 2000, 2001; Zhang and Clark 2000). Olyreae are monoecious tropical understory plants with somewhat lignified culms, restricted vegetative branching, no specialized culm leaves, no outer (abaxial) ligules, unisexual spikelets, and usually a seasonal pattern of flowering. This tribe, which includes about 110 species, is primarily American, with one species in both tropical America and Africa, and one monotypic genus endemic to New Guinea (Judziewicz et al. 1999; Judziewicz and Clark 2007). Bambuseae are characterized by the presence of well-developed rhizomes, strongly lignified culms, new shoots with culm leaves specialized for the protection and support of immature tissue, foliage leaves with both inner and outer ligules, complex vegetative branching, and usually cyclical, gregarious, and monocarpic flowering. The woody bamboos include over 1300 species and are widely distributed in both tropical and temperate zones, with centers of diversity in the Neotropics, Southeast Asia, Madagascar, and Eastern Asia (Clark 1997*a*; Judziewicz et al. 1999; Judziewicz and Clark 2007).

Although support for the monophyly of both Bambusoideae and Olyreae generally has been strong (e.g., GPWG 2000, 2001; Zhang and Clark 2000; but see Soreng and Davis 1998 for differing results regarding the subfamily), monophyly of Bambuseae was robust only when morphological characters were included (Zhang and Clark 2000). Zhang and Clark (2000), using *ndh*F sequence and morphological data, recovered four relatively well-supported clades within Bambuseae (the North Temperate clade [Arundinariinae + Shibataeinae], the Paleotropical clade, Chusqueinae [Neotropical], and the Arthrostylidiinae + Guaduinae clade [Neotropical]), but relationships among these clades were unresolved. Zhang (2000), using *rpl*16 intron sequence data, inferred the same four clades. Relatively low rates of base substitution in Bambuseae, which appear to be correlated with the long generation times in the tribe (Gaut et al. 1997), likely contributed to the lack of resolution in both molecular data sets. Morphological observations and molecular data sets also appeared to be in conflict in several instances. We chose to examine phylogenetic relationships within Bambuseae and to further test monophyly of the tribe by focusing on one of these conflicts involving the determinate, one-flowered genera of woody bamboos.

Among the nine currently recognized subtribes of Bambuseae, Chusqueinae (Neotropical) and Hickelinae (= Nastinae; Paleotropical) comprise the determinate, one-flowered genera (Dransfield and Widjaja 1995). The name Nastinae has been used for the latter subtribe in much of the recent bamboo literature, but Hickelinae has priority for the subtribe containing *Hickelia* A. Camus, so we note this correction here. The affinities of *Greslania* Balansa, placed within Hickelinae, and several recently described one-flowered genera (e.g., *Temburongia* S. Dransf. & K. M. Wong, *Temochloa* S. Dransf., *Sirochloa* S. Dransf., and *Valiha* S. Dransf.) were uncertain (Dransfield 1998, 2000, 2002*a, b*; Dransfield and Wong 1996) and thus these were also included in our analysis.

[4] Present address: National Herbarium of Mexico, Departamento de Botánica, Instituto de Biología, U.N.A.M., Tercer Circuito s/n, Ciudad Universitaria, Delegación Coyoacán, Apartado Postal 70-233, 04510 México, D.F., Mexico.

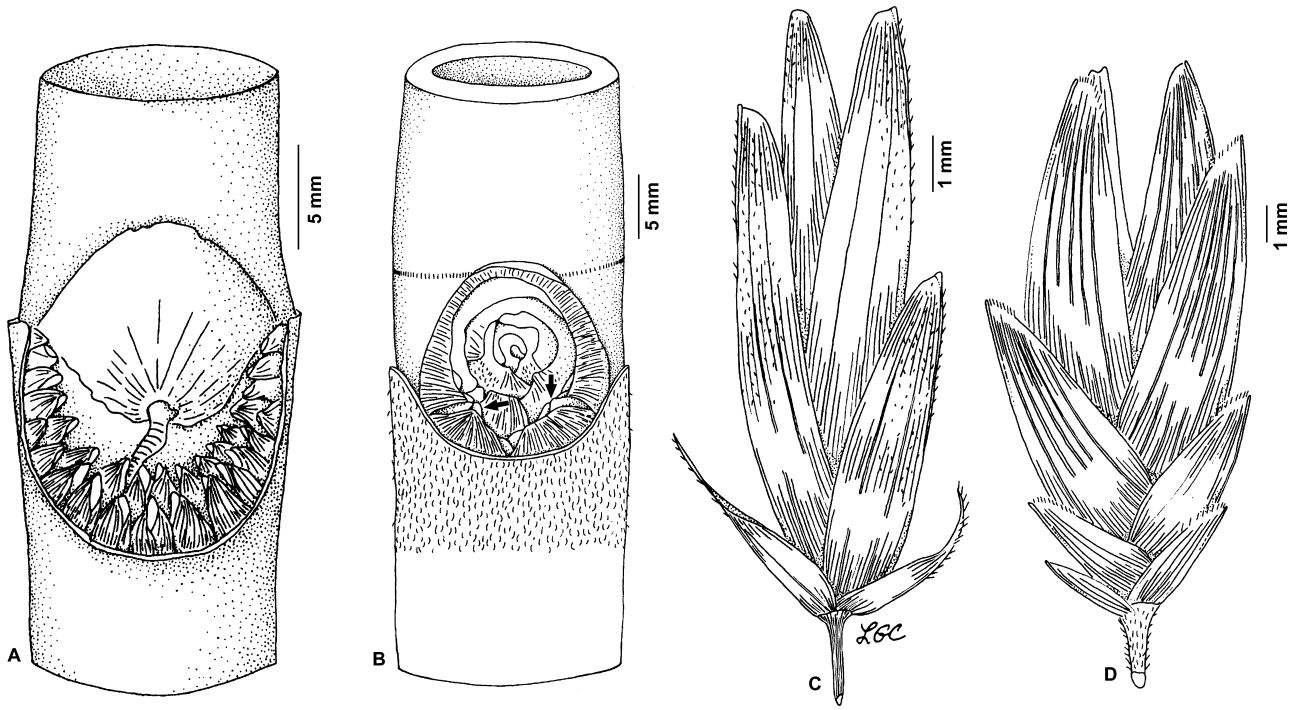

Fig. 1.—Comparison of bud and spikelet morphology in *Chusquea* subgen. *Rettbergia* and *Nastus.*—A. Bud complement of *C. capituliflora* Trin.—B. Bud complement of *Nastus* sp.; arrows indicate smaller secondary buds.—C. Spikelet of *C. bambusoides.*—D. Spikelet of *Nastus.* (After Clark 1997*b:* Fig. 2)

Morphologically, members of Chusqueinae and Hickelinae share a number of similarities, primarily in habit, buds and branching, and spikelets. Many members of both subtribes are moderate-sized, clambering or scandent bamboos with one larger and a few to many smaller branches per node. *Chusquea* subgen. *Rettbergia* is characterized in part by the presence of a dome-shaped central bud (Fig. 1A), an unusual bud morphology observed elsewhere only in a few species of *Chusquea* subgen. *Chusquea* (e.g., *C. liebmannii* E. Fourn.), *Nastus* Juss. (Fig. 1B), and a few other genera of Hickelinae (Dransfield 1994, 1997, 1998). In addition to determinate synflorescences and a single fertile floret per spikelet, members of these two subtribes share the presence of four to six glumes (except *Greslania,* which has two), unlike most woody bamboos, which have one to three (or no) glumes. Species of *Chusquea* subgen. *Rettbergia* and *Nastus* also tend to have obtuse lemmas and sometimes also obtuse glumes (Fig. 1C, D). The unbranched culms of *Greslania* recall those of the cauline-leaved species of *Neurolepis* Meisn. (the *N. aristata* complex) (Dransfield 2002*a*).

In contrast, prior analyses of plastid sequence data (*ndh*F, *rpl*16 intron) with sufficient sampling reflected a geographic set of relationships. Hickelinae (as Nastinae) consistently associated with Paleotropical Bambusinae and Melocanninae (Kelchner and Clark 1997; Zhang 2000; Zhang and Clark 2000), and Chusqueinae appeared either as a single lineage of a tetrachotomy (Zhang 2000; Zhang and Clark 2000) or associated with Neotropical Arthrostylidiinae and Guaduinae (Kelchner and Clark 1997). Branch lengths tended to be short, however, and support values moderate at best, although the grouping of Hickelinae with Bambusinae and Melocanninae received the strongest support. Ní Chonghaile (2002) did not sample Hickelinae, but consistently found a sister relationship between the Chusqueinae clade and the Arthrostylidiinae + Guaduinae clade in analyses of plastid sequence data (*trn*L–*trn*F, *rpl*16 intron); however, these two clades did not associate in analyses of ITS sequence data.

We used maximum parsimony to analyze structural and plastid *rpl*16 intron sequence data sets for 43 species of Bambuseae, three of Olyreae, and two outgroup taxa to (1) further test the monophyly of Bambuseae, (2) test the monophyly of Chusqueinae and Hickelinae, and (3) examine the apparent conflict between molecular and morphological data sets in the determinate, one-flowered genera of Bambuseae. A number of bamboo *rpl*16 intron sequences were already available, and this intron has had a reasonable level of phylogenetic utility in bamboos (Kelchner and Clark 1997).

MATERIALS AND METHODS

Plant Materials

A total of 48 species in 29 genera were sampled for this study (Table 1). Based on prior studies, especially Zhang and Clark (2000) and GPWG (2001), one species each of Oryzeae and Streptogyneae were chosen as the most appropriate outgroups. The remaining 43 species of Bambuseae and three species of Olyreae were treated as the ingroup in order to test monophyly of Bambuseae and because placement of the taxa of uncertain affinities could not be predicted. All subtribes of Bambuseae except for Racemobambosinae were sampled; the North Temperate clade, well supported in all prior analyses (Kelchner and Clark 1997; Zhang and Clark

2000; Ní Chonghaile 2002), was represented by one species each of Arundinariinae and Shibataeinae, whereas the remaining subtribes were represented by at least two species each. Three taxa were represented by different species in the morphological and molecular analyses: *Arthrostylidium pubescens* Rupr., *Chusquea bahiana* L. G. Clark, and *Guadua paniculata* Munro were scored for morphological characters and paired with *rpl*16 intron sequences from *A. ecuadorense, C. arachniformis,* and *G. angustifolia,* respectively.

DNA Sequencing

Total genomic DNA was extracted from 27 species, following the standardized CTAB-isopropanol precipitation protocols (Paterson et al. 1993) and using the kits Nucleon Phytopure™ (Amersham Biosciences Corp., Piscataway, New Jersey, USA) and DNeasy® Plant (QIAGEN®, Valencia, California, USA). For most samples, DNA was extracted from silica-gel-dried leaf material (Chase and Hills 1991), with a few extracted from fresh material. Amplification reactions for the *rpl*16 intron sequences (ca. 1.2 kb) were conducted following known PCR protocols (Kelchner and Clark 1997; Zhang 2000). Amplified fragments were visualized and then cleaned with QIAEX II Gel Extraction Kit (QIAGEN®). Sequencing reactions were carried out using specific primers designed in prior studies (Kelchner and Clark 1997; Zhang 2000). Sequencing was performed by the Automated DNA Sequencer ABI 377 (Perkin-Elmer Applied Biosystems, Wellesley, Massachusetts, USA) at the Iowa State University DNA Sequencing and Synthesis Facility. Both strands were sequenced and assembled with Autoassembler (Perkin-Elmer Applied Biosystems).

The edited sequences were aligned manually with Se-Al vers. 2.09a (Rambaut 2001). The alignment of the sequences introduced gaps that later were treated as binary, presence/absence characters in the structural data set (Giribet and Wheeler 1999). A total of 27 *rpl*16 sequences were generated for this analysis.

Structural Characters

The structural data set included both morphological characters (Table 2) and nucleotide insertion/deletion (indel) characters (Table 3). A total of 98 morphological characters were generated, including 19 leaf anatomical and micromorphological characters. Gross morphological characters were scored primarily from herbarium specimens at ISC and K, but for a few taxa, living material was also available, and in a few cases data were taken from the literature. Preparation of hand cross-sections and epidermal scrapes followed Zhang and Clark (2000); preparation of paraffin-embedded sections followed Clark (1986) and March (2000). Specimens for scanning electron microscopy were prepared as described in Dávila and Clark (1990). Whenever possible, leaf material from the same vouchers as those for the molecular analyses was used. The 4-base-pair (bp) inversion identified by Kelchner and Clark (1997) in the *rpl*16 intron was ignored in this study, because it was likely to be homoplasious, the North Temperate clade already had extremely strong support, and only one of the three species of *Chusquea* known to have the inversion was included in this study.

The main criteria for inclusion of characters were presence in a majority of taxa, ability to be observed and scored, and potential phylogenetic signal. We included characters for which it was relatively easy to define discrete, non-overlapping states (e.g., meristic or binary characters) and quantitative characters for which non-overlapping states could be defined, either quantitatively when not widely disjunct (e.g., rhizome neck length) or in qualitative terms when widely disjunct (e.g., central or primary bud shape) (Stevens 1991; Thiele 1993). With few exceptions, characters were deconstructed to the fullest extent possible. Multistate characters were treated as unordered. Polymorphisms in terminal taxa were relatively few, and it was not obvious how to subdivide these to achieve monomorphic units, as recommended by Nixon and Davis (1991). For example, the number of stamens in *Buergersiochloa bambusoides* is two or three, and although this difference had been used to support the recognition of two species, it was later found that stamen number varied among male spikelets in a single inflorescence (Fijten 1975). We therefore retained these polymorphisms in the data set. For taxa with inapplicable characters, the characters were scored as missing.

We do not reject morphometric characters a priori, but for this study it was not feasible to incorporate them. Multiple measurements of some characters, e.g., leaf length or width, were available for many taxa, but not for others. For some species, sufficient material was available, but other species are known from very few collections (e.g., *Temochloa liliana, Nastus borbonicus*) or, due to the cyclical nature of flowering in woody bamboos, appropriate developmental stages were not available or flowering material was scarce. Characters that were clearly autapomorphic for the taxa in this sample were excluded. Characters 62–64 were excluded from the analyses because 81% of the 129 cells for these characters were scored as missing, a figure higher than the 75% missing data cut-off cited by Poe and Wiens (2000).

The unusual bud morphology of *Chusquea* posed problems for scoring the morphological characters relating to buds and branching. Under one interpretation (Hypothesis I), the large central bud is the primary bud, and is homologous to the single bud per node found in all other woody bamboos (with the exceptions of *Filgueirasia* G. F. Guala [Guala 2003] and perhaps *Chimonobambusa* Makino, neither of which is included in this analysis), making the multiple smaller buds that subtend or flank it truly supernumerary. Under the alternate interpretation (Hypothesis II), the bud complement is derived through fasciation of the primary axis (at least a number of its basal nodes and internodes) and loss of the main (primary) prophyll, making the multiple smaller buds truly secondary. Without additional independent evidence, these two hypotheses must be regarded as equally probable. We therefore scored a series of characters under each hypothesis for all taxa, and ran analyses excluding one character set or the other. Characters 9–15 were scored under Hypothesis I, and characters 85–90 were scored under Hypothesis II. The additional character under Hypothesis I (character 9) was required because the presence of multiple, independent buds per node is variable, whereas under Hypothesis II, the branching in all taxa is assumed to have been derived from a single bud per node, and is thus invariant.

Table 1. Species sequences for the *rpl*16 intron and vouchers. AF series GenBank numbers are from Zhang (2000) and U series GenBank numbers are from Kelchner and Clark (1997); these are presumed to be identical sequences that were submitted twice. The Pohl Conservatory is on the campus of Iowa State University. AC = Andre Carvalho; ER = Eduardo Ruiz; JD = John Dransfield; JGSK = J. Gabriel Sánchez-Ken; LC = Lynn Clark; LS = Luiz Sarahyba; PA = Patricio Asimbaya; SD = Soejatmi Dransfield; SK = Scot Kelchner; WZ = Wei-Ping Zhang; XL = Ximena Londoño.

Taxon	Voucher	Origin	GenBank number
BAMBUSOIDEAE			
Bambuseae			
Arthrostylidiinae			
Arthrostylidium ecuadorense Judz. & L. G. Clark	*LC et al. 1101* (ISC)	Ecuador	AY912189
Atractantha radiata McClure	*AC 4362* (ISC)	Brazil	AY912190
Aulonemia patula (Pilg.) McClure	*LC et al. 1075* (ISC)	Ecuador	AY912191
Glaziophyton mirabile Franch.	*LS et al. 1066* (ISC)	Brazil	AF133471; U54748
Arundinariinae			
Arundinaria gigantea (Walter) Muhl.	*WZ 8400703* (ISC)	USA	AF133465; U54742
Bambusinae			
Bambusa vulgaris Schrad. ex J. C. Wendl.	*JGSK 666* (ISC)	Cult. in Panama	AY912192
Oxytenanthera abyssinica (A. Richard) Munro	*Guala 1761* (FTG)	Malawi	AY912193
Chusqueinae			
Chusquea Kunth subgen. *Chusquea*			
C. coronalis Soderstr. & C. E. Calderón	*SK 19* (INB)	Costa Rica	U54759
C. exasperata L. G. Clark	*LC et al. 1093* (ISC)	Ecuador	U62784
C. ramosissima Lindm.	*AC 4358* (ISC)	Brazil	AF133472; U54751
C. scandens Kunth	*LC & XL 1235* (ISC)	Colombia	U62781
C. serpens L. G. Clark	*LC & XL 1253* (ISC)	Colombia	U54754
C. tomentosa Widmer & L. G. Clark	*Pohl 15802* (ISC)	Costa Rica	U62782
Chusquea subgen. *Rettbergia* (Raddi) L. G. Clark			
C. arachniformis L. G. Clark & Londoño	*LC & XL 1228* (ISC)	Colombia	U62787
C. bambusoides (Raddi) Hack.	*LC & XL 1029* (ISC)	Brazil	AY912194
C. oligophylla Rupr.	*LC & XL 1031* (ISC)	Brazil	U62785
Chusquea subgen. *Swallenochloa* (McClure) L. G. Clark			
C. culeou E. Desv.	*LC & ER 999* (ISC)	Chile	AY912195
C. pinifolia (Nees) Nees	*LC & PW 1056* (ISC)	Brazil	U54756
C. tessellata Munro	*LC et al. 1267* (ISC)	Colombia	U54752
Neurolepis aperta (Munro) Pilg.	*XL & LC 919* (ISC)	Colombia	U62793
N. aristata (Munro) A. Hitchc.	*LC & PA 1457* (ISC)	Ecuador	AY912196
N. nana L. G. Clark	*LC & PA 1453* (ISC)	Ecuador	AY912197
Guaduinae			
Guadua angustifolia Kunth	*XL & LC 931* (TULV)	Colombia	AY912198
Otatea acuminata (Munro) C. E. Calderón & Soderstr.	*LC et al. 1312* (ISC)	Mexico	AF133473; U54749
Hickelinae (= Nastinae)			
Cathariostachys capitata (Kunth) S. Dransf.	*SD 1334* (K)	Madagascar	AY912201
C. madagascariensis (A. Camus) S. Dransf.	*SD 1356* (K)	Madagascar	AY912202
Decaryochloa diadelpha A. Camus	*SD 1288* (K)	Madagascar	AY912203
Greslania circinata Balansa	*SD 1490* (K)	New Caledonia	AY912204
G. rivularis Balansa	*SD 1491* (K)	New Caldeonia	AY912205
Hickelia madagascariensis A. Camus	*SD 1292* (K)	Madagascar	AY912206
Nastus borbonicus J. F. Gmel.	*LC & SD 1656* (ISC)	Cult. in France (from Reunion Island)	AY912207
N. elatus Holttum	*SD s. n.* (K)	Cult. in Australia	AF133469; U54746
N. elegantissimus (Hassk.) Holttum	*Putut & SD 4* (K)	Java	AY912208
N. elongatus A. Camus	*SD 1343* (K)	Madagascar	AY912209
N. productus (Pilg.) Holttum	*Utteridge 438* (K)	Irian Jaya	AY912210
Perrierbambus madagascariensis A. Camus	*Randrimanampisoa s. n.* (K)	Madagascar	AY912211
Sirochloa parvifolia (Munro) S. Dransf.	*JD 7742* (K)	Madagascar	AY912212
Valiha diffusa S. Dransf.	*SD 1345* (K)	Madagascar	AY912213

Table 1. Continued.

Taxon	Voucher	Origin	GenBank number
Melocanninae			
Cephalostachyum pergracile Munro	*SD 1435* (K)	Thailand	AY912199
Schizostachyum brachycladum (Munro) Kurz	*Guala 2801* (FTG)	Cult. in USA	AY912200
Shibataeinae			
Phyllostachys pubescens Mazel ex J. Houz.	*LC 1289* (ISC)	Pohl Conservatory (seed from China)	AF133467; U54744
Olyreae			
Buergersiochloa bambusoides Pilg.	*SD 1382* (K)	Irian Jaya	AF133461
Pariana radiciflora Sagot ex Döll	*LC & WZ 1344* (ISC)	Pohl Conservatory (from Costa Rica)	AF133462; U54740
Sucrea maculata Soderstr.	*LC & WZ 1345* (ISC)	Pohl Conservatory (from Brazil)	AF133463; U54741
Incertae Sedis			
Temburongia simplex S. Dransf. & K. M. Wong	*JD 7498* (K)	Brunei	AY912214
Temochloa liliana S. Dransf.	*SD 1494* (K)	Thailand	AY912215
EHRHARTOIDEAE			
Oryzeae			
Oryza sativa L.		Shimada and Sugiura (1991)	NC001320
Streptogyneae			
Streptogyna americana C. E. Hubb.	*JGSK 657* (ISC)	Panama	AY912216

Phylogenetic Analyses

The morphological and *rpl*16 intron sequence data sets were analyzed individually. A separate analysis was also run for the sequence data plus indels data set. No well-supported clades in any of the data sets contradicted those found in the other data sets, so an analysis of the combined data sets (including indels) was also conducted. Phylogenetic analyses were performed by maximum parsimony using PAUP* vers. 4.0b10 for Macintosh (Swofford 2002). For all analyses, *Streptogyna americana* and *Oryza sativa* were defined as a monophyletic outgroup sister to the ingroup. Most-parsimonious trees were found using heuristic searches with 1000 random-addition sequence replicates, tree-bisection-reconnection (TBR) branch swapping, and the MulTrees option in effect. Character state transitions were optimized according to the ACCTRAN algorithm. All characters, including coded gap characters, were equally weighted.

Bootstrap values (bts; Felsenstein 1985) and Bremer support (brs; Bremer 1994) were calculated to infer the relative support for particular clades. Bootstrap analyses used 100–1000 replicates with either 10 random-addition sequences per replicate or simple taxon addition and a maximum of 100 trees held at each step, depending on the size of the analysis. All other options were as above (TBR, etc.). Decay analyses to calculate Bremer support were performed in conjunction with the heuristic option in PAUP* by searching for all trees up to five steps longer than the most-parsimonious tree and noting the number of steps required for each clade to collapse.

RESULTS

Data Matrices

All data sets included a total of 48 taxa. The morphological data set (Table 5) under Hypothesis I comprised 84 informative characters with 12.5% of the 4032 cells coded as missing. The morphological data set under Hypothesis II comprised 83 informative characters with 12.4% of the 3984 cells coded as missing. Percentages are based on informative characters only.

For the *rpl*16 intron, PCR products varied in length from 1034 bp in *Otatea acuminata* to 1230 bp in *Neurolepis nana.* Hand alignment resulted in the inference of numerous indel events ranging from 1 to 29 bp in length. Indels considered to be potentially phylogenetically informative are listed in Table 3, and the matrix character number assigned to each indel is indicated. Clearly autapomorphic indels were either removed from the alignment or not scored. One of these, a 35 bp deletion in *O. acuminata,* occurred at positions 770–805. Indel i (character 107) fell within this region and therefore could not be scored for this species. AT composition of the sequences was between 67.0 and 69.7%, a range that is typical of chloroplast introns and only slightly lower than that reported by Kelchner and Clark (1997) for this intron. Measures of percentage sequence divergence (*p* distances) were calculated with MEGA vers. 2.1 (Kumar et al. 2001) using the pairwise deletion of indels option. Sequence divergence was 0–10.9% within Bambusoideae and 4.3–5.2% within Olyreae (but only three species were sampled). Sequence divergence within Bambuseae as a whole was 0–

Table 2. Morphological character list and character states.

Life Cycle

1. Flowering: 0 = sporadic; 1 = continuous; 2 = gregarious and monocarpic at intervals; 3 = annual/seasonal.

Rhizomes

2. Rhizome branching (in adult/mature plants): 0 = sympodial; 1 = amphipodial; 2 = monopodial.
3. Rhizome neck length (of sympodium): 0 = short (neck ≤ 1/2 the length of the rhizome proper); 1 = long (neck > 1/2 the length of the rhizome proper).

Culms

4. Habit: 0 = erect; 1 = apically arching; 2 = clambering; 3 = twining; 4 = decumbent.
5. Culm internodes: 0 = solid; 1 = hollow, lacuna > 1/3 the diameter of the culm; 2 = hollow, lacuna ≤ 1/3 the diameter of the culm.
6. Culm branching: 0 = no aerial branching; 1 = aerial vegetative branching present.

Nodes and Branches

7. Nodal line position: 0 = horizontal; 1 = dipping slightly below bud(s); 2 = dipping markedly below bud(s).
8. Supranodal ridge: 0 = not prominent (a line); 1 = prominent (a ridge).
9. Primary buds per mid-culm node: 0 = one; 1 = two or more; 2 = none.
10. Multiple primary buds, relative size: 0 = buds subequal; 1 = central bud at least 2× the diameter of other primary buds (i.e., subsidiary buds).
11. Central bud shape: 0 = triangular; 1 = circular (dome-shaped).
12. Central bud prophyll: 0 = margins free (open); 1 = margins fused (closed).
13. Compression of 1° axis developing from the central bud: 0 = no compressed internodes at the base of the 1° axis; 1 = one to several compressed internodes at the base of the 1° axis, at least some bud-bearing; 2 = all bud-bearing internodes of the 1° axis compressed.
14. Relative sizes of 2° branches developing from the central axis: 0 = 2° axes subequal to the central axis; 1 = at least some of the 2° axes no more than one-half the diameter of the central axis.
15. Central branch size relative to main culm: 0 = ± equal in diameter; 1 = central branch smaller in diameter than the main culm.
16. Branching pattern: 0 = intravaginal; 1 = extravaginal; 2 = infravaginal.
17. Aerial root primordia: 0 = absent; 1 = present on the lower nodes only; 2 = present on lower to upper nodes.

Culm Leaves

18. Girdle: 0 = absent or poorly developed; 1 = present as a band at least 1 mm wide, no flap, prominent or not; 2 = prominent, with a flap covering the bud complement.
19. Culm leaf blade position: 0 = erect to slightly spreading; 1 = reflexed.
20. Culm leaf blade shape: 0 = broadly triangular; 1 = narrowly triangular; 2 = lanceolate (pseudopetiolate).
21. Culm leaf blade midrib abaxially: 0 = indistinguishable; 1 = visible.
22. Blade-derived appendages on the sheath summit: 0 = no true auricles or fimbriae; 1 = efimbriate auricles present; 2 = fimbriate auricles present; 3 = fimbriae only present.
23. Sheath summit extension: 0 = absent; 1 = present on one or both sides.
24. Abaxial sheath indument: 0 = stiff, dark, irritating hairs present; 1 = only soft hairs present; 2 = glabrous; 3 = scabrous.

Foliage leaves

25. Blade-derived appendages on the sheath summit: 0 = no true auricles or fimbriae (glabrous); 1 = efimbriate auricles present; 2 = fimbriate auricles present; 3 = fimbriae only present; 4 = cilia (or tufts of cilia) present.
26. Sheath summit extension: 0 = absent; 1 = present on one or both sides.
27. Sheath: 0 = rounded on the back; 1 = strongly keeled at least near the apex.
28. Foliage leaf blade: 0 = abaxial marginal green stripe absent; 1 = abaxial marginal green stripe present.
29. Midrib placement: 0 = centric; 1 = excentric (wider side of the blade ≥ 1.3 times as wide as the narrower side).

Synflorescence

30. Form: 0 = open paniculate (at least main axis elongated); 1 = capitate-paniculate; 2 = racemose; 3 = solitary spikelet; 4 = spicate.
31. Gemmiparous bracts subtending the spikelet proper: 0 = absent; 1 = present, buds developing subsequently or not.
32. Subtending bracts at the base of the first- (lowermost) and/or second-order paraclades: 0 = absent; 1 = present, as a scar/rim or scalelike, blade absent, a few mm long; 2 = present, well developed, with sheath and blade (modified).
33. Prophylls at the base of the first- or second-order paraclades: 0 = absent; 1 = present.
34. Prophylls: 0 = whole; 1 = at least some split lengthwise into two halves.
35. Spatheate bracts subtending the whole synflorescence: 0 = absent; 1 = one or more present.

Spikelets (for unisexual taxa, the characters refer to female-fertile spikelets)

36. Compression: 0 = terete; 1 = lateral; 2 = dorsal.
37. Number of glumes (in female-fertile spikelets or spikelets proper): 0 = absent; 1 = one; 2 = two; 3 = three; 4 = four; 5 = five or six.
38. Awns on the lower two glumes: 0 = absent; 1 = present.
39. Number of female-fertile florets per spikelet or spikelet proper: 0 = one; 1 = two or more.
40. Rachis extension (internode only, with or without rudimentary spikelet): 0 = absent; 1 = present and short (< floret); 2 = present and long (> floret).
41. Rachis extension (internode only): 0 = glabrous; 1 = hairy.
42. Lemma apex: 0 = margins/tip free; 1 = margins/tip connate.

Table 2. Continued.

43. Lemma texture: 0 = chartaceous (membrano-chartaceous); 1 = rigid, hardened.
44. Lemma indument: 0 = glabrous (glabrescent); 1 = scabrous; 2 = densely hispid; 3 = hispid only near the apex; 4 = pubescent (all or in part).
45. Palea apex: 0 = biapiculate (sinus shallow); 1 = tips long-divided (sinus deep); 2 = 1-keeled; 3 = acute, not divided.
46. Palea, margins at apex: 0 = free; 1 = connate.
47. Palea indument (excluding the sulcus): 0 = glabrous; 1 = scabrous; 2 = pubescent; 3 = hispid.
48. Sulcus: 0 = well developed for full length of palea; 1 = well developed only toward the apex; 2 = absent.
49. Sulcus indument: 0 = glabrous; 1 = pubescent; 2 = scabrous.

Flower

50. Lodicule number: 0 = absent; 1 = three; 2 = two.
51. Lodicule margin pubescence: 0 = ciliate (or ciliolate); 1 = glabrous (entire).
52. Stamen number: 0 = two; 1 = three; 2 = six; 3 = > six.
53. Stamen filaments: 0 = free; 1 = monadelphous; 2 = diadelphous (3 + 3).
54. Anther tip: 0 = lobed, no appendage; 1 = lobed, with an appendage.
55. Style base/ovary apex: 0 = ovary apex narrow and continuous with the style base (normal); 1 = ovary apex blunt, the style base forming an expanded cap (or hood) on top; 2 = ovary apex blunt, hood absent.
56. Style proper length: 0 = absent (including extremely short, <0.1 mm); 1 = elongated >0.1 mm up to the length of the ovary; 2 = elongated and greater than the length of the ovary.
57. Style proper pubescence: 0 = glabrous; 1 = pubescent.
58. Style proper core: 0 = hollow; 1 = solid.
59. Stigma number: 0 = three; 1 = two.
60. Stigma branching: 0 = very branched and plumose (2 or more orders of branching); 1 = limited branching/simple, hispid (1 order of branching).

Fruit

61. Caryopsis/ovary base: 0 = sessile; 1 = stalked.
62. Caryopsis apex: 0 = acute, no additional persistent structures; 1 = short style, style base (if style elongated) or short style plus stigma bases persistent; 2 = thickened style base persistent, often a slight constriction between the caryopsis apex and the style base evident or a distinct line or ridge present in this position; 3 = elongated style persistent; 4 = hood (cap) persistent.
63. Pericarp adnation (in mature fruit): 0 = strongly adnate to the seed coat; 1 = not adnate to the seed coat.
64. Pericarp texture: 0 = thin, papery and dull; 1 = thin, hardened and shiny; 2 = thickened, fleshy.
65. Embryo position (caryopsis in longitudinal side view): 0 = lateral at the base; 1 = central at the base.

Foliar Anatomy

66. Vasculature of the midrib: 0 = complex (superposed bundles); 1 = simple (one bundle or an arc of bundles).
67. Intercostal sclerenchyma in mesophyll: 0 = absent; 1 = present.
68. Arm cells (transverse section, 1–2 rows directly beneath the adaxial epidermis): 0 = weakly invaginated; 1 = rosette; 2 = asymmetrically invaginated.
69. Fusoid cells: 0 = absent; 1 = present.
70. Abaxial sclerenchyma girder of primary bundles: 0 = ± straight-sided (narrow to wide); 1 = dilated.
71. Adaxial sclerenchyma girder of primary bundles: 0 = narrow to slightly dilated (one or a few columns wide); 1 = anchor-shaped (surface between bulliform cell groups lined with sclerenchyma cells).

Foliar Micromorphology

72. Papillae on the long cells in the stomatal zone (abaxial): 0 = absent; 1 = present.
73. Papillae on the long cells in the stomatal zone (abaxial): 0 = simple; 1 = branched; 2 = simple and branched.
74. Papillae on the long cells in the interstomatal zone (abaxial): 0 = absent; 1 = present.
75. Papillae on the long cells in the interstomatal zone (abaxial): 0 = simple; 1 = branched.
76. Papillae on the adaxial surface: 0 = absent; 1 = present on the bulliform cells only; 2 = present on the long cells only; 3 = present on both bulliform and long cells.
77. Papillae on the subsidiary cells of the stomatal apparatus: 0 = absent; 1 = present and simple; 2 = present and branched.
78. Papillae associated with the stomates: 0 = not overarching; 1 = overarching the stomates.
79. Distribution of stomates on foliage leaf blades: 0 = present and common on the abaxial surface only; 1 = present and common on both surfaces.
80. Vertically tall and narrow silica bodies (abaxial, intercostal): 0 = present; 1 = absent.
81. Saddle-shaped silica bodies (abaxial, intercostal): 0 = present; 1 = absent.
82. Vertically tall and narrow silica bodies (abaxial, costal): 0 = present; 1 = absent.
83. Saddle-shaped silica bodies (abaxial, costal); 0 = present; 1 = absent.
84. Horizontal dumbbell-shaped silica bodies (abaxial, costal): 0 = present; 1 = absent.

Buds and Branching (Hypothesis II)

85. Primary (main) bud prophyll: 0 = present; 1 = absent.
86. Primary bud shape: 0 = triangular; 1 = circular (dome-shaped).
87. Primary bud prophyll: 0 = margins free (open); 1 = margins fused (closed).
88. Compression of 1° axis developing from the central bud: 0 = no compressed internodes at the base of the primary axis; 1 = one to several compressed internodes at the base of the 1° axis, at least some bud-bearing; 2 = all bud-bearing internodes of the 1° axis compressed.

Table 2. Continued.

89. Relative sizes of 2° branches developing from the central axis: 0 = 2° axes subequal to the central axis; 1 = at least some of the 2° axes no more than one-half the diameter of the central axis.
90. Central branch size relative to main culm: 0 = ± equal in diameter; 1 = central branch smaller in diameter than the main culm.

Additional Characters for the Herbaceous/Outgroup Taxa

91. Life span: 0 = perennial; 1 = annual.
92. Leaf position/culm elongation: 0 = leaves basal or mostly basal; 1 = cauline, not basally aggregated.
93. Leaf differentiation: 0 = foliage/branch leaves (including cataphylls) only; 1 = leave differentiated into foliage/branch leaves and culm leaves.
94. Foliage leaf blade pseudopetiole: 0 = present; 1 = absent.
95. Outer (abaxial) ligule of foliage leaf: 0 = present; 1 = absent.
96. Spikelet sexuality: 0 = fully bisexual; 1 = functionally unisexual, plants monoecious.
97. Crenate silica bodies (intercostal): 0 = present; 1 = absent.
98. Cross-shaped silica bodies (costal): 0 = present; 1 = absent.

6.4%; within Chusqueinae alone it was 0–5.1%, compared with the 0–2.5% found by Kelchner and Clark (1997) for their *Chusquea* matrix.

The *rpl*16 intron sequences with indels intact had a total aligned length of 1100 bp. This data set comprised 133 informative characters with 5.0% of the 6384 cells coded as missing, that is, coded as gaps. After removal of indel sequences corresponding to those rescored as binary data, the *rpl*16 sequences had an aligned length of 978 bp. We analyzed both alignments, but used only the latter in the reported analyses. It comprised 124 potentially informative characters with 2.8% of the 5952 cells coded as gaps and 1.4% coded as missing due to incomplete (partial) sequences (primarily at the beginning or end of the sequences). The binary indel matrix comprised a total of 20 indels: 14 informative characters with one cell coded as missing where the indel could not be scored in *Otatea acuminata* (see above) (Table 3) and six indels that could be interpreted as autapomorphic for *Oryza* or as supporting monophyly of *Streptogyna americana* + Bambusoideae (not shown in Table 3). The *rpl*16 sequence + binary indel data set comprised 138 informative characters with 3.8% of the cells coded as missing. The complete matrix of aligned *rpl*16 intron sequences is deposited in TreeBASE (M2145), or is available upon request from the authors Clark or Triplett. Combining the morphological data set with *rpl*16 sequences and the binary indel data resulted in the following data sets: the combined data sets under Hypothesis I included 1087 characters, of which 222 were potentially phylogenetically informative and with 7.1% of the 10,656 cells scored as missing; the combined data sets under Hypothesis II included 1086 characters, of which 221 were informative and with 7.0% of the 10,608 cells scored as missing.

Phylogenetic Analyses

Tree statistics for the five separate phylogenetic analyses are shown in Table 4. For morphology-only analyses, Hy-

Table 3. Phylogenetically informative length mutations in the *rpl*16 intron. Six indels interpreted as autapomorphic for *Oryza* are not shown.

Number	Character letter	Position	Size (bp)	Type	Taxa
99	a	146–150	5	deletion	*Aulonemia, Bambusa, Oxytenanthera*
100	b	179–207	29	insertion	*Neurolepis aristata, N. nana*
101	c	425–435	11	deletion	*Chusquea, Neurolepis*
102	d	472–476	5	insertion	*Arundinaria, Phyllostachys*
103	e	528	1	deletion	*Arundinaria, Phyllostachys*
104	f	533	1	insertion	*Arundinaria, Phyllostachys*
105	g	685–689	5	insertion	*All except the outgroups, Arundinaria, Phyllostachys,* and *Temochloa*
106	h	738–760	23	deletion	*Chusquea, Neurolepis*
107	i	779	1	deletion	Olyreae, *Arundinaria, Glaziophyton, Nastus elatus, Oryza, Phyllostachys*
108	j	804–809	6	insertion	*Chusquea arachniformis, C. bambusoides, C. oligophylla, Oryza*
109	k	922	1	deletion	*Glaziophyton, Nastus elatus, Sucrea*
110	l	952–957	6	deletion	*Chusquea arachniformis, C. bambusoides, C. oligophylla*
111	m	966–970	5	insertion	*Nastus productus, Neurolepis aristata, N. nana*
112	n	981–985	5	insertion	*Neurolepis aristata, N. nana*

Table 4. Tree statistics for the individual and combined analyses.

Tree statistics	Morphology (Hypothesis I)	Morphology (Hypothesis II)	*rpl*16 intron + binary indels	Combined (Hypothesis I)	Combined (Hypothesis II)
Total number of characters	89	88	998	1087	1086
Number of informative characters	84	83	138	222	221
Number of trees	169	8	3425	36	30
Length, most-parsimonious trees	488	485	598	1139	1136
Consistency index (CI)	0.273	0.270	0.739	0.505	0.504
Retention index (RI)	0.561	0.551	0.731	0.593	0.588

pothesis II consistently produced trees three steps shorter than Hypothesis I, and significantly fewer trees were obtained (8 vs. 169, respectively). In the analyses of combined data sets, the same difference in tree length was noted, but the difference in number of trees was not as great (30 for Hypothesis II vs. 36 for Hypothesis I). With respect to the molecular data, only the statistics for the sequence data (gaps deleted) plus binary indel characters are shown; an analysis of sequence data without the binary indel data produced 10,184 trees of 625 steps, with CI = 0.736 and RI = 0.703. The trees shown in Fig. 2–4 are deposited in TreeBASE (S1233).

In the morphology-only analyses employing Hypothesis II (Fig. 2), *Oryza sativa* was embedded within Bambusoideae, but Bambuseae (arrow) and Olyreae were each supported as monophyletic with moderate levels of support. Within Bambuseae, Chusqueinae were paraphyletic to a relatively weakly supported (bts <50%, brs 2; bts 53% and brs 3 under Hypothesis I) clade containing all of the other woody bamboos. A majority of branches received Bremer support values of 1 or 2, indicating generally weak support for the topology. Of the subtribes sampled with two or more taxa, only Arthrostylidiinae and Melocanninae were recovered as monophyletic. *Cathariostachys* S. Dransf., *Greslania,* and *Nastus* were each resolved as monophyletic genera; *Cathariostachys,* however, with bootstrap support of 99% and Bremer support >5, was the only clade in the tree to receive greater than 85% bootstrap or Bremer support >3.

Analysis of the sequence data plus indels provided greater resolution overall relative to the morphological data, but this was mostly concentrated in Chusqueinae (Fig. 3). Neither Bambusoideae nor Bambuseae were recovered as monophyletic, although Olyreae were well supported. *Temochloa,* a Paleotropical woody bamboo, appeared as sister to *Oryza* + (North Temperate clade + [Olyreae + tropical Bambuseae]). The North Temperate clade was strongly supported (bts 99%, brs >5; indels d, e, f, i), but it was placed sister to the tropical Bambuseae (minus *Temochloa*) + Olyreae. With the exception of *Temburongia,* which was sister to the Arthrostylidiinae + Guaduinae clade, the remaining tropical woody bamboos were divided geographically into two weakly supported clades, the Neotropical bamboos (Chusqueinae and the Arthrostylidiinae + Guaduinae clade) and the Paleotropical bamboos (Bambusinae, Hickelinae, and Melocanninae). Of the subtribes sampled with two or more taxa, Bambusinae, Chusqueinae, Guaduinae, and Melocanninae were each resolved as monophyletic. Chusqueinae received moderate bootstrap (58%) and Bremer (3) support, but were also supported by two unambiguous deletions (c, h). *Chusquea* and *Greslania* both received support as monophyletic genera, but both *Cathariostachys* and *Nastus* were polyphyletic, and *Neurolepis* was paraphyletic to *Chusquea.* The sister relationship between *Neurolepis aristata* and *N. nana* was very strongly supported (bts 100, brs >5; indels b, m, n), as was the monophyly of *Chusquea* subgen. *Rettbergia* (bts 100, brs >5; indels j, l).

In analyses of combined data sets, Bambusoideae, Bambuseae (arrow), and Olyreae each were relatively well supported as monophyletic (Fig. 4). Bambusoideae received strong Bremer support (>5) but no bootstrap support under both hypotheses, whereas Olyreae received strong support from both indices under both hypotheses. Bambuseae, however, received stronger support under Hypothesis I (bts 58%, brs 4) than under Hypothesis II (Fig. 4; bts 64%, brs 1). Within Bambuseae, a major dichotomy between Chusqueinae and all other woody bamboos was resolved, although Chusqueinae received strong support (bts 90%, brs 4) and the other clade weak support (bts 51%, brs 1). Within Chusqueinae, *Neurolepis* was paraphyletic to a well-supported *Chusquea* (bts 96%, brs 5), and a dichotomy between *Chusquea* subgen. *Rettbergia* and the remainder of *Chusquea* was very strongly supported. Among the remaining Bambuseae, the North Temperate clade, Melocanninae, *Cathariostachys,* and *Greslania* all received strong support; the Arthrostylidiinae + Guaduinae clade, Guaduinae, *Chusquea* subgen. *Swallenochloa,* and a sister relationship between *Cathariostachys* and *Decaryochloa* A. Camus were moderately supported. The remaining clades were weakly supported. Although support was weak (brs 1), *Temochloa* was resolved as sister to the North Temperate clade. The geographic associations noted in the analysis of molecular data alone persisted, with the differences that the Arthrostylidiinae + Guaduinae clade was no longer sister to Chusqueinae, and a Madagascan Hickelinae clade was recovered within the Paleotropical bamboos. Aside from some variation in branch support measures, the major difference in the topologies derived from the combined analyses is the dissociation of *Temburongia* from the Paleotropical clade into a tetrachotomy under Hypothesis I (not shown).

DISCUSSION

Monophyly of Bambuseae

Consistent with the results of Zhang and Clark (2000), support for monophyly of the woody bamboos emerged in analyses of morphological data only or structural and sequence data combined (Fig. 2, 4), but in both analyses support for Bambuseae was moderate at best. Zhang (2000) did not calculate bootstrap support, but in his analysis Bambuseae had a Bremer support value of 1. The *ndh*F and structural analysis of Zhang and Clark (2000) produced much stronger support (brs 5) for the tribe. A preliminary analysis of 17 species (including two Olyreae and *Oryza*), for which *ndh*F and *rpl*16 intron sequences and morphology are available, revealed increased support for Bambuseae (87% bts),

Table 5 Morphological matrix for one-flowered genera. See Table 2 for explanations of characters and states. Explanation of symbols and letters: ? = character unknown or unobserved; - = character inapplicable; A = polymorphism of states 0 and 1; B = polymorphism of states 1 and 2; E = polymorphism of states,0, 1, 2, and 3; G = polymorphism of states 0 and 2.

	Character									
	0000000001	1111111112	2222222223	3333333334	4444444445	5555555556	6666666667	7777777778	8888888889	99999999
Species	1234567890	1234567890	1234567890	1234567890	1234567890	1234567890	1234567890	1234567890	1234567890	12345678
Streptogyna americana	300F00002-	------0---	----41A01G	000-012110	-000000001	2000020?01	03?0000000	00-0-00-01	0101------	00000011
Oryza sativa	3-0010002-	------?---	----211000	000-010-00	-00421G2-2	1200010?10	0??0000110	0101A31A11	0011------	10011011
Buergersiochloa bambusoides	3??010002-	------0---	----????10	000-022000	-0043022-1	0A10020?10	0????11001	A120-00101	0100------	0100?111
Pariana radiciflora	3??410002-	------0---	----301004	000-022000	-00???0??1	?3100?0?10	0???000200	0100-00?01	1000------	01001100
Sucrea maculata	300400002-	------0---	----011100	000-022000	-0103002-1	1100020?10	0???000201	0101000101	1000------	01001100
Temochloa liliana	000111000-	00????0002	000200000C	000-012002	000000000?	????010???	??????0200	0101020100	1101000???	01100011
Phyllostachys pubescens	22-1?1010-	0000110011	1202010000	1210?00-?1	0003003011	0100110?1?	0????00200	0101000?00	1101000001	01100011
Arundinaria gigantea	0B-111000-	0?101000A1	020220000G	010-01C110	-00400B021	010000--00	01?0010200	0101010100	1101000101	01100011
Bambusa vulgaris	000111010-	0011111000	0200100100	111A012010	-000000001	02A110--01	0???000200	0101020011?	0?01000111	01100011
Oxytenanthera abyssinica	?000G1000-	00???1?0??	?30231000A	1211002000	-100000100	-211021?01	0???000200	0101?00?00	1101000???	01100011
Temburongia simplex	000111000-	00???10101	0102100000	0111002002	0000000001	1200010100	0?0??00200	00-0-0?00?	????000???	01100011
Schizostachyum brachycladum	100111000-	0020110000	0201200?00	111?000AA2	000000?101	0200020001	0?1?000200	0121??0100	1011000201	01100011
Cephalostachyum pergracile	000111000-	002011?000	0200200A00	1110012012	0002003111	0201020001	0???000200	01?1??0?00	0001000201	01100011
Arthrostylidium pubescens	?002B1000-	0?11100101	0201200112	000-012012	0000000011	?100????1?	0????11000	0121?2010?	????00?111	01100011
Atractantha radiata	2002G1000-	0011100101	0002300111	1110020-02	0000000001	0100011?10	?????11200	0121000100	10110??111	01100011
Aulonemia patula	?00111010-	0011000112	1302300100	000-012112	10040000B1	0100011?10	0?????1200	0101?20000	1011000110	01100011
Glaziophyton mirabile	00A010000-	??----??01	1002300100	0B1001E012	0000000011	1100010?10	?????10110	01?1?20110	11010??---	00000011
Guadua paniculata	?0A1H1010-	0011100100	0C02300010	1B1001??11	1000000001	1200121?0?	0????10B00	010103A1?	0101000111	01100011
Otatea acuminata	?0A1H1000-	0010100001	0310F00000	0A0-012111	1004000011	0100010?10	0???010100	0101030A10	1100000001	01100011
Greslania circinata	G00010000-	0?----00--	----3001-0	0211002001	0000000001	020020--01	011?0-1100	0120-1010?	????00?---	01000011
G. rivularis	000011000-	0011000001	0002300100	021A012001	0000000001	120020--01	0112010B00	0101000100	0001000?00	01100011
Hickelia madagascariensis	201211100-	1011020212	0000000000	0210005001	1110000011	0200000100	0?0??00B00	0101000100	0101010110	01100011
Nastus borbonicus	001111000-	1??01100??	??02300000	010-015002	1000000001	120010--01	0???010200	0121000101	0101????01	01100011
N. elatus	000111000-	??11?10000	0002000000	000-005000	-000000101	020010--01	?????01?00	0101020010?	0?010??11?	01100011
N. elegantissimus	000121000-	0011?10112	0000300000	010-015000	-004002101	020010--01	040?0A0000	0100-0010?	????00011?	01100011
N. elongatus	0002B1200-	1?11?201??	?300300000	010-015002	0004002001	120010--01	?????AA0A0	0121000101	0101010011?	01100011
N. productus	000221200-	1?10?20001	0000300000	000-015002	0000000001	020010--00	?????00B00	0100-0100?	0?011--11?	01100011
Cathariostachys capitata	001111100-	1011010101	0200300101	02A1005001	1003101010	-201021101	110?1A0201	0101000010?	????010110	01100011
C. madagascariensis	001111100-	1011010111	0200200101	02A1015101	1003101010	-201021101	110?110201	0101000010?	????010110	01100011
Decaryochloa diadelpha	201111200-	1011020201	120020000C	0211004001	1012101010	-2G0011100	0?0??00200	0101000100	1001010110	01100011
Perrierbambus madagascariensis	00?2B1200-	??11?202??	?102200003	000-005001	0003002111	120000--01	0?1??10200	0101000010	00010??11?	01100011
Sirochloa parvifolia	0002G1000-	0011010102	0002200?02	0110004101	0000000000	-200010101	0110000201	0101000010?	?101000110	01100011
Valiha diffusa	001111200-	1011120202	0100200000	0111015001	1101100001	1201021101	0?1??00200	0101010010?	????010111	01100011
Neurolepis aperta	?00000000-	0?----10??	???2011010	010-014000	-000000101	?100????1?	0???000200	0101001100	000100?---	00100011
N. aristata	?00000002-	------0001	1001000000	0A0-014100	-000000101	0100????1?	0?????1100	1100-01101	0101------	01100011
N. nana	000000002-	------0001	1012011000	010-014100	-000000101	1100????1?	0????10100	1101001110?	????------	00100011
Chusquea bahiana	2??2012011	1?10020201	1003000001	010-114100	-101000121	010000--10	0????00200	0100-?1?0?	01011--110	01100011
C. bambusoides	???2012011	1?10020201	1003011000	010-114100	-10000A121	010000--1?	0?????0200	00-0-01?01	01011--110	01100011
C. oligophylla	G??2012011	1?10020201	1003001000	010-014000	-100000101	010000--1?	0????00B00	0121?01001	01011--110	01100011
C. scandens	G101010111	0010010101	0001011000	010-014000	-0000001B1	0100010?10	0???000?00	0101?11?0?	0?011--110	01100011
C. tomentosa	2001012111	0010021201	0002011000	010-014000	-001001021	010000--1?	0???010B00	0101001110?	????1--110	01100011

Table 5 Continued.

C. exasperata	???B012011	0010020201	0003011000	010-014A00	-001001021	0100010?10	0????00200	0121?31101	01111--110	01100011
C. coronalis	100B011011	00100101A1	000300000G	000-024000	-000000121	010000--11	0???010200	0101031001	01101--110	01100011
C. serpens	2??2011111	0010022201	A002011000	010-014100	-000000??1	01000???1?	0????00200	0121?0110?	01011--110	01100011
C. ramosissima	2??2011011	0?10020212	1012011000	010-014000	-000000101	0100010?1?	0????00B00	0101?1100?	0??01--110	01100011
C. tessellata	0000010010	0010100001	0002001000	010-014000	-0010001B1	010000--10	0????10100	1121?0110?	??011--111	01100011
C. pinifolia	0000010010	0010100001	0002011000	010-014000	-00100B121	0100010?10	0????10?00	1121?0?10?	??0?1--111	01100011
C. culeou	G000010010	0?101??001	0002011000	010-014000	-0040021B1	0100010?10	0???010100	0101?0100?	??011--111	01100011

the Arthrostylidiinae + Guaduinae clade (99% bts), and the Paleotropical clade (83% bts) compared with previous analyses and this study, but the relationships of these major clades remained ambiguous (L. G. Clark et al. unpubl. data). This suggests that additional sequence data are needed to improve resolution, although broader sampling, especially among the subtribes with multiple florets per spikelet, is also necessary.

Differentiation of the foliage and culm leaves (character 93) is an unambiguous synapomorphy for Bambuseae, with a reversal in *Glaziophyton* Franch. The presence of an outer ligule also supports Bambuseae, but as noted in Zhang and Clark (2000), this feature is homoplasious, occurring in *Streptogyna americana* among the taxa in this sample. The presence of highly lignified, perennating culms was not scored in this analysis, but would show up as an unreversed synapomorphy for the tribe. As noted by the GPWG (2001), however, a few other grasses do possess this character (e.g., *Arundo* L., *Gynerium* Willd. ex P. Beauv., *Phragmites* Adans.), but the derivation of ''woody'' culms in these taxa may not be homologous. Complex vegetative branching, here subdivided into three characters (characters 13–15 or 88–90), is nonetheless characteristic of Bambuseae. Likewise, the presence of true (determinate) spikelets vs. pseudospikelets is subdivided into three characters (characters 31–33), but using any one as a surrogate produces the result that pseudospikelets were derived independently a minimum of three or four times (Fig. 4, starred taxa). Based on this analysis, we hypothesize that the following are symplesiomorphic in Bambuseae: sympodially branching rhizomes, differentiation of culm and foliage leaves, complex vegetative branching, the presence of an outer ligule on the foliage leaf, and determinate spikelets. With regard to cyclical, gregarious, monocarpic flowering, observations are lacking for a number of species, but departures from this behavior in woody bamboos are relatively rare (Judziewicz et al. 1999) and found in relatively derived taxa. We infer that cyclical, gregarious, monocarpic flowering is also symplesiomorphic in Bambuseae, but this phenomenon requires further study.

Bud/Branch Evolution within Bambuseae

McClure (1973) regarded the bud complement in *Chusquea* as consisting entirely of primary buds, with one usually much larger than the others (Hypothesis I). Even from the earliest stages of development, these buds are distinct (L. G. Clark pers. obs.), whereas in virtually all other woody bamboos, the branch complement (even those with numerous branches) is derived from a single bud per node. Stapleton (1997), based on detailed observations of the bud complement of *C. culeou,* proposed that extensive loss or reduction of prophylls was consistent with condensation of a single primary axis as a pathway for the evolution of the bud complement in *Chusquea* (Hypothesis II). Results of the present study support Hypothesis II as the more parsimonious explanation, but lack of resolution among major lineages means that we cannot reject either hypothesis at this time. Observations of additional species of *Chusquea,* especially from *Chusquea* subgen. *Rettbergia,* and analyses including *Filgueirasia* and *Chimonobambusa* would be desirable.

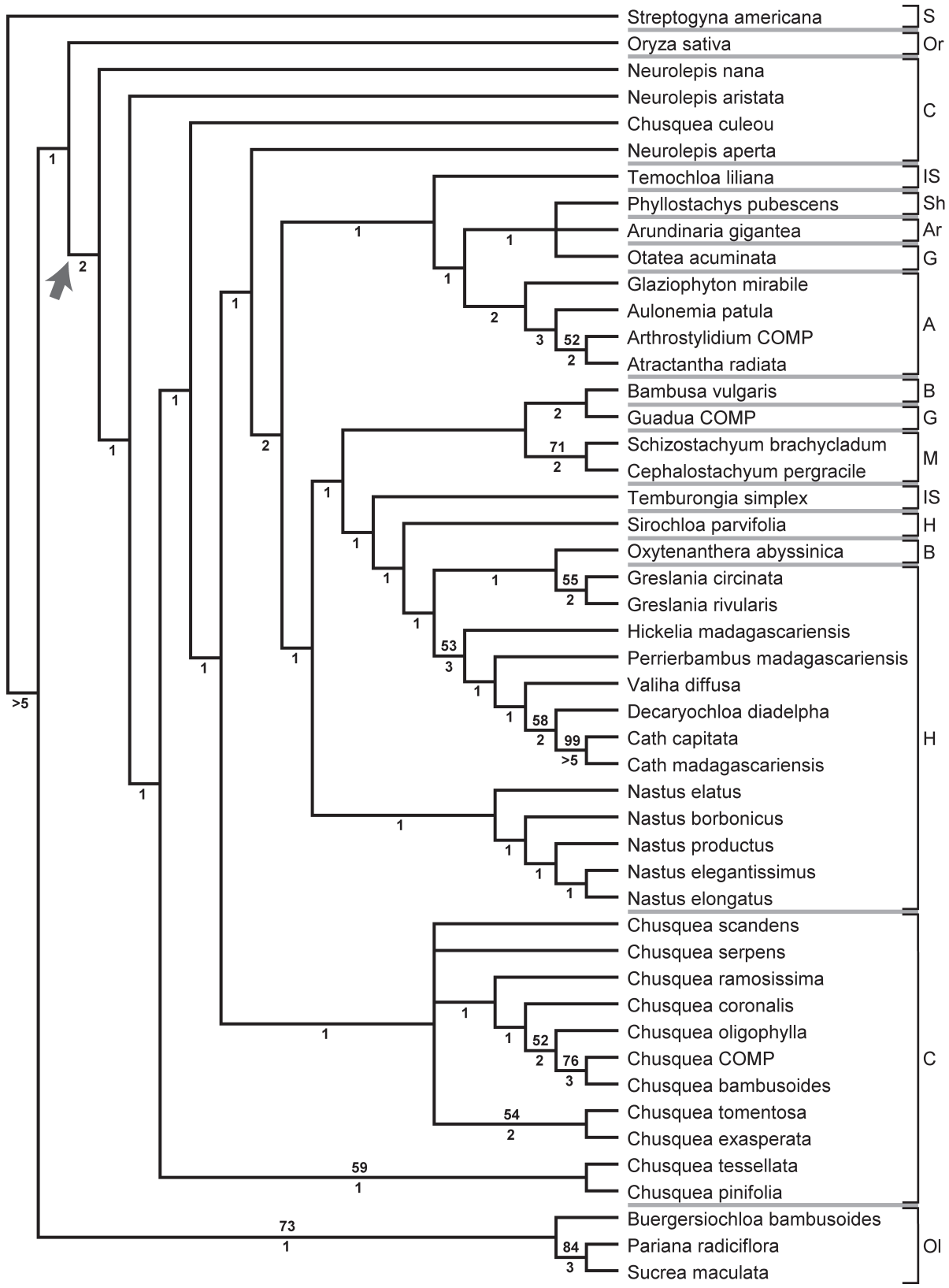

Fig. 2.—Strict consensus of eight most-parsimonious trees inferred from analysis of the morphological data set under Hypothesis II; node supporting Bambuseae indicated by arrow. Bootstrap support values >50% are above the branches, Bremer support below. A = Arthrostylidiinae; Ar = Arundinariinae; B = Bambusinae; C = Chusqueinae; Cath = *Cathariostachys*; COMP = composite taxon; G = Guaduinae; H = Hickelinae; IS = incertae sedis; M = Melocanninae; Ol = Olyreae; Or = Oryzeae; S = Streptogyneae; Sh = Shibataeinae.

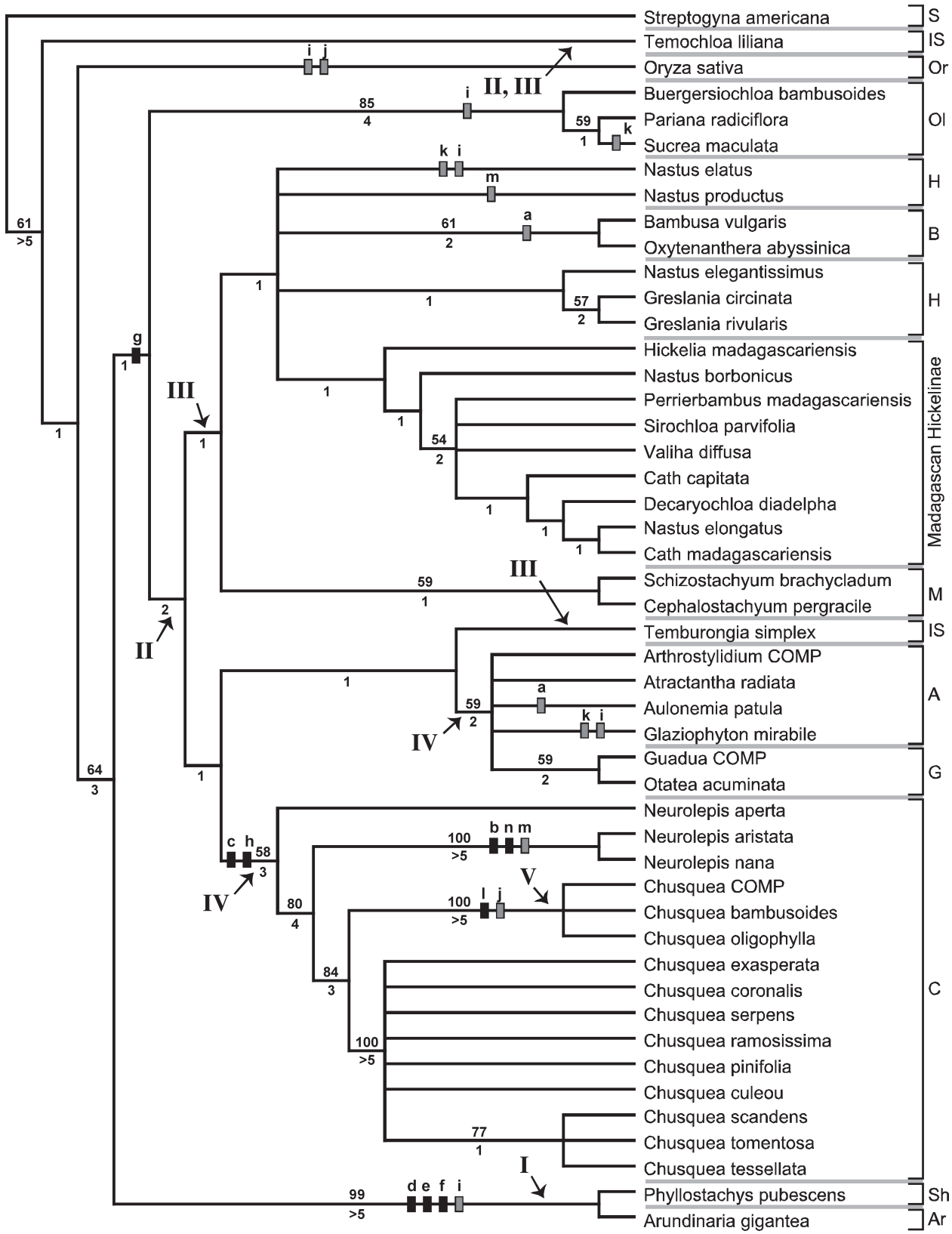

Fig. 3.—Strict consensus of 3425 most-parsimonious trees inferred from analysis of the *rpl*16 intron sequence and binary indel (Table 3) data sets. Bootstrap support values >50% are above the branches, Bremer support below. A = Arthrostylidiinae; Ar = Arundinariinae; B = Bambusinae; C = Chusqueinae; Cath = *Cathariostachys*; COMP = composite taxon; G = Guaduinae; H = Hickelinae; IS = incertae sedis; M = Melocanninae; Ol = Olyreae; Or = Oryzeae; S = Streptogyneae; Sh = Shibataeinae. I = North Temperate clade; II = Tropical Bambuseae; III = Paleotropical Bambuseae; IV = Neotropical Bambuseae; V = *Chusquea* subgen. *Rettbergia.* Closed bars = unambiguous indels; shaded bars = homoplasious indels.

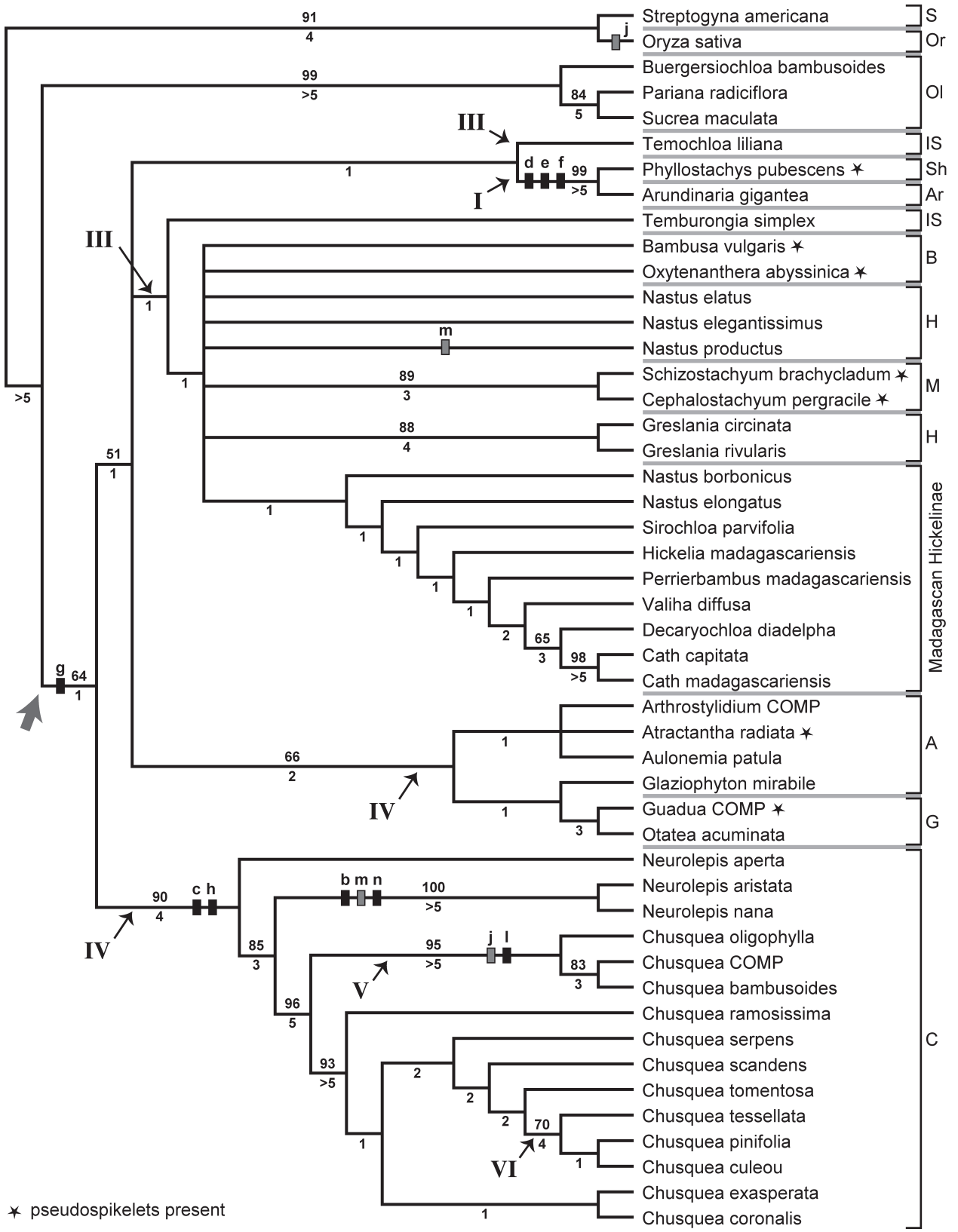

Fig. 4.—Strict consensus of 30 most-parsimonious trees inferred from analysis of the combined *rpl*16 intron and structural data sets under Hypothesis II; node supporting Bambuseae indicated by arrow. Bootstrap support values >50% are above the branches, Bremer support below. A = Arthrostylidiinae; Ar = Arundinariinae; B = Bambusinae; C = Chusqueinae; Cath = *Cathariostachys*; COMP = composite taxon; G = Guaduinae; H = Hickelinae; IS = incertae sedis; M = Melocanninae; Ol = Olyreae; Or = Oryzeae; S = Streptogyneae; Sh = Shibataeinae. I = North Temperate clade; III = Paleotropical Bambuseae; IV = Neotropical Bambuseae; V = *Chusquea* subgen. *Rettbergia*; VI = *Chusquea* subgen. *Swallenochloa.* Closed bars = unambiguous indels; shaded bars = homoplasious indels.

Major Clades within Bambuseae

Chusqueinae.—Molecular data provide moderate support for Chusqueinae, as well as good resolution within the subtribe (Fig. 3). Morphological data provide some resolution within Chusqueinae, but the subtribe itself is not resolved as monophyletic (Fig. 2). Analysis of combined data provides complete resolution within the subtribe, with moderate to strong support for most subclades (Fig. 4). Monophyly of *Neurolepis* is not supported, although a sister relationship between two members of the *N. aristata* complex is. The *N. aristata* complex includes all of the species with cauline leaves, although some members (e.g., *N. nana*) have the leaves in a basal cluster due to shortened internodes (Clark 1996), but no obvious potential morphological synapomorphies separate this complex from the remainder of *Neurolepis.* Additional sampling within the genus, especially of species similar in morphology to *N. aperta,* is required before any changes in classification or nomenclature can be considered.

Monophyly of *Chusquea* and the dichotomy between *Chusquea* subgen. *Rettbergia* and the remainder of the genus (hereafter referred to as the Euchusquea clade) are both extremely well supported and agree with the results of Kelchner and Clark (1997). The presence of dome-shaped central buds (character 11) and connate lemma tips (character 42) support *Chusquea* subgen. *Rettbergia,* but are independently derived in various members of Hickelinae. Recognition of two genera, *Chusquea* (type species: *C. scandens*; ca. 125 described species) and *Rettbergia* Raddi (type species: *C. bambusoides*; ca. 12 described species) could be justified, but no morphological synapomorphies for the Euchusquea clade have been identified and *Rettbergia* has not been recognized as a separate genus since Nees (1835) placed it in synonymy with *Chusquea.* Additionally, all species of *Chusquea* subgen. *Rettbergia* except for the type have binomials in *Chusquea* but not *Rettbergia.* The most conservative option is to retain a single genus readily diagnosed by the presence of multiple, dimorphic, (apparently) independent buds, solid culms, and a base chromosome number (x) of 10. Within the Euchusquea clade, monophyly of *Chusquea* subgen. *Swallenochloa* (*C. culeou, C. pinifolia, C. tessellata*) is moderately supported, whereas *Chusquea* subgen. *Chusquea* is paraphyletic. *Chusquea ramosissima,* previously classified within *Chusquea* subgen. *Rettbergia* (Clark 1997*b*; Judziewicz et al. 1999), clearly belongs within the Euchusquea clade although its affinities there remain to be established. More detailed analysis of the Euchusquea clade, with more extensive sampling among the sections, is needed.

Arthrostylidiinae + Guaduinae clade.—We recovered a moderately supported Arthrostylidiinae + Guaduinae clade in the combined analyses with a moderately supported Guaduinae embedded in a paraphyletic Arthrostylidiinae (Fig. 4); these relationships were also found in analyses of molecular data alone (Fig. 3). In contrast, neither of these clades was recovered in the morphology-only analyses, but a weakly to moderately supported Arthrostylidiinae was (Fig. 2). Three leaf anatomical characters usually cited as diagnostic for Arthrostylidiinae (simple midrib vasculature, presence of intercostal sclerenchyma, and presence of an abaxial marginal green stripe; Soderstrom and Ellis 1987; Judziewicz et al. 1999) are consistently present in the subtribe, but in the present study were homoplasious. Simple vasculature of the midrib (character 66) provided the best local support for this clade, but it had a minimum of six other occurrences in the analysis. We did not test the presence of refractive papillae as a potentially informative character due to difficulties in defining and scoring it.

In analyses of *ndh*F sequence data combined with morphology, or *ndh*F data alone, Arthrostylidiinae and Guaduinae were each supported as monophyletic, Guaduinae usually strongly so, and a sister relationship between the two subtribes was moderately well supported (Zhang 1996; Guala 2000; Zhang and Clark 2000). Kelchner and Clark (1997), using *rpl*16 intron data, resolved the Arthrostylidiinae + Guaduinae clade, with Guaduinae paraphyletic to the single sampled species of Arthrostylidiinae. As noted by Londoño and Clark (2002), patterns of character distribution within this clade are complex, and additional study with more extensive sampling from both subtribes is needed.

North Temperate clade + Temochloa.—The association of *Temochloa,* a Paleotropical bamboo of unknown affinities, and the North Temperate clade in the morphology-only and combined analyses is a striking, but perhaps not wholly unexpected, result (Fig. 2, 4). Ní Chonghaile (2002) recovered an association between *Dendrocalamus giganteus* Munro, a Paleotropical bamboo, and the North Temperate clade based on *trn*L–*trn*F spacer sequence data, although bootstrap support was only 54%. In our analyses, the association of *Temochloa* with the North Temperate clade received comparably weak support (Fig. 2, 4). While this strongly suggests that broader sampling within Paleotropical Bambuseae is critical, this placement may also be due to an unusually divergent *rpl*16 intron sequence or an error or contamination in sequencing. An analysis of *ndh*F Bambuseae sequences places *Temochloa* in a clade with *Racemobambos* Holttum and *Bambusa* Schreb. (bts 85%; L. G. Clark et al. unpubl. data), supporting its Paleotropical affinity. It should also be noted that some critical morphological data for *Temochloa* are missing due to the developmental stage at which the sole collection of flowering material was discovered (Dransfield 2000), perhaps leading to its ambiguous placement in the morphological analysis (Fig. 2).

The North Temperate clade, on the other hand, has been very strongly supported in all molecular analyses to date (Zhang 1996; Kelchner and Clark 1997; Zhang and Clark 2000; Ní Chonghaile 2002), and our results agree with that finding. The presence of monopodial rhizome branching is a potential synapomorphy, but it must be noted that amphipodial branching evolved in the Euchusquea clade, and within the North Temperate clade, one group (Thamnocalaminae of some authors) apparently reverted to sympodial rhizome branching.

Paleotropical Bambuseae.—Paleotropical Bambuseae, with the exception of *Temochloa,* formed a weakly supported clade that was part of a trichotomy with the Arthrostylidiinae + Guaduinae clade and the North Temperate clade + *Temochloa* in the combined analysis (Fig. 4). Support values, especially along the backbone of this clade, were mostly weak. Melocanninae were consistently recovered with moderate to strong support in our analyses (Fig. 2–4) and in all

prior analyses with sufficient sampling, but only in the *trn*L–*trn*F analyses of Ní Chonghaile (2002) were more than two species included. Our inclusion of *Greslania* represented the first sampling of this genus in any phylogenetic analysis of bamboos, but despite strong support for its monophyly its position remained ambiguous and its classification in Hickelinae could neither be confirmed nor rejected. Although Bambusinae were supported as monophyletic in the molecular analysis (Fig. 3), its two representative taxa resolved as part of an extensive polytomy within Paleotropical Bambuseae in the combined analysis (Fig. 4). Ní Chonghaile (2002), however, did find moderate support for Bambusineae (with taxa of Racemobambosinae embedded within it) in *trn*L–*trn*F (bts 62%) and *rpl*16 (bts 74%) analyses, although the latter did not include Melocanninae.

With sufficient sampling, Melocanninae have been consistently recovered with moderate (Fig. 2, 4) to strong support (e.g., the *trn*L–*trn*F analysis of Ní Chonghaile [2002]). Compression of all bud-bearing internodes of the primary axis (character 13 under Hypothesis I or character 88 under Hypothesis II) provided unambiguous support for Melocanninae in this sample set, but *Rhipidocladum* McClure and *Actinocladum* Soderstrom (both Arthrostylidiinae) have similar branch complements. Morphological similarities between Melocanninae in the Paleotropics and Arthrostylidiinae in the Neotropics constitute another apparent conflict between morphological and molecular data within Bambuseae, comparable to that involving the determinate, one-flowered genera. Our results tend to suggest that these morphological similarities are homoplasious, but resolution in this analysis is insufficient to allow a firm conclusion.

Hickelinae appeared to be polyphyletic (Fig. 2, 3) or possibly paraphyletic (Fig. 4), but aside from a weakly supported Madagascan Hickelinae clade (Fig. 3, 4), resolution was lacking. Monophyly of the Madagascan Hickelinae presents an interesting hypothesis that needs to be further tested. With regard to *Nastus* (type species: *N. borbonicus*), our results suggest that the Southeast Asian species might constitute a separate genus (for which the name *Chloothamnus* Buse is available), but we did not recover any support for such a lineage. As noted by Holttum (1955), the species of *Nastus* s.l. share a hooded ovary (character 55, although hooded ovaries also occur in *Phyllostachys* Siebold & Zucc. and *Bambusa*) and five or six glumes. A rachilla extension is present in the Madagascan species of *Nastus,* whereas it is lacking (except for *N. productus*) among the Southeast Asian species of *Nastus.* Consistent with these characters, the monophyly of *Nastus* received weak support on morphological grounds in our analysis (Fig. 2). All Madagascan Hickelinae except for *N. elongatus* and *Sirochloa* share long rhizome necks (character 3); all but *Cathariostachys* and *Decaryochloa* share pseudopetiolate culm leaf blades (character 20); and all but *N. borbonicus* and *Sirochloa* share dome-shaped primary buds (character 11 or 86). In all cases, however, there was some homoplasy in these characters. Within Madagascan Hickelinae, a sister relationship between *Cathariostachys* and *Decaryochloa* was supported (Fig. 2, 4), and even in the molecular-only analysis, the three species of these two genera still formed a clade (Fig. 3). Unequivocal morphological support for this clade came from the presence of scabrous paleas (character 47), and the monophyly of *Cathariostachys* was unambiguously supported by the presence of a stalked ovary (character 61). The position of *Valiha* as sister to *Cathariostachys* + *Decaryochloa* was also unambiguously supported by the presence of long-divided palea tips (character 45).

Relationships of the One-Flowered, Determinate Genera

Despite the strong morphological similarities between members of Chusqueinae and Hickelinae, there was no support for a sister relationship between the two subtribes. Based on these results, we conclude that the morphological similarities are probably homoplasious. The geographic relationships seen using *ndh*F data (Zhang 1996, 2000; Zhang and Clark 2000) were to some extent supported by this study, but lack of resolution among the major lineages of the woody bamboos continues to indicate that we cannot reject either set of relationships at this time.

Summary and Future Work

Although support for Bambuseae remains moderate, our results support a monophyletic Bambusoideae, Bambuseae, and Olyreae. Several morphological synapomorphies support Bambuseae, but as in other studies, analysis of molecular data alone does not recover a monophyletic Bambuseae. Our results suggest that the morphological similarities between Chusqueinae and Hickelinae are homoplasious, but robust resolution of relationships among the major lineages of woody bamboos is still wanting.

In order to obtain more robust results, which would allow us to better understand both the biogeography of the bamboos and character evolution within the group, broader sampling and additional sequence data (including nuclear markers) are clearly needed. In terms of sampling, Paleotropical Bambuseae and the Arthrostylidiinae + Guaduinae clade must be studied in more detail. Additional sequence data for the plastid *rbc*L, *ndh*F, and *trn*L–*trn*F loci are already available, so filling in sampling gaps for these markers for the same set of taxa would be a logical starting point. Some *waxy* (GBSSI) sequences are available for bamboos, and appear to have some utility (Guo and Li 2004), but use of nuclear markers will require some care due to the polyploid nature of the woody bamboos. The North Temperate clade will be particularly challenging due to the low amount of sequence divergence within it, even relative to other woody bamboos (Guo et al. 2002; Guo and Li 2004; Ní Chonghaile 2002), so morphological data take on added importance for this group.

ACKNOWLEDGMENTS

National Science Foundation Grant DEB-9806877 to LGC supported sequencing and fieldwork by LGC and SD, and travel by LGC to Kew. J.-Y. Lesouf of the Conservatoire Botanique National de Brest, France, graciously provided access to material of *Nastus borbonicus.* We thank J. F. Wendel (Iowa State University) for access to laboratory facilities. G. F. Guala (Fairchild Tropical Garden) and J. Dransfield and T. Utteridge (Royal Botanic Gardens, Kew) graciously collected material for certain taxa. We also thank S. Renvoize (Grass Section, Royal Botanic Gardens, Kew) for as-

sistance and access to specimens. A. Gardner (Iowa State University) provided invaluable assistance with the figures. E. J. Judziewicz, G. F. Guala, and an anonymous reviewer provided helpful comments.

LITERATURE CITED

BREMER, K. 1994. Branch support and tree stability. *Cladistics* **10**: 295–304.

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

CLARK, L. G. 1986. Systematics of *Chusquea* section *Chusquea,* section *Swallenochloa,* section *Verticillatae,* and section *Serpentes* (Poaceae: Bambusoideae). Ph.D. dissertation, Iowa State University, Ames, Iowa, USA. 370 p.

———. 1996. Six new species of *Neurolepis* (Poaceae: Bambusoideae: Bambuseae) from Ecuador and Peru. *Novon* **6**: 335–350.

———. 1997*a.* Bamboos: the centerpiece of the grass family, pp. 237–248. *In* G. P. Chapman [ed.], The bamboos. Academic Press, London, UK.

———. 1997*b.* Diversity, biogeography and evolution of *Chusquea,* pp. 33–44. *In* G. P. Chapman [ed.], The bamboos. Academic Press, London, UK.

DÁVILA, P., AND L. G. CLARK. 1990. A scanning electron microscopy study of leaf epidermes in *Sorghastrum* (Poaceae: Andropogoneae). *Amer. J. Bot.* **77**: 499–511.

DRANSFIELD, S. 1994. The genus *Hickelia* (Gramineae: Bambusoideae). *Kew Bull.* **49**: 429–443.

———. 1997. Notes on the genus *Decaryochloa* (Gramineae: Bambusoideae) from Madagascar. *Kew Bull.* **52**: 593–600.

———. 1998. *Valiha* and *Cathariostachys,* two new bamboo genera (Gramineae: Bambusoideae) from Madagascar. *Kew Bull.* **53**: 375–397.

———. 2000. *Temochloa,* a new bamboo genus (Poaceae–Bambusoideae) from Thailand. *Thai Forest Bull., Bot.* **28**: 177–180.

———. 2002*a. Greslania circinata* and *Greslania rivularis* (Poaceae–Bambusoideae) from New Caledonia. *Bamboo Science and Culture* **16**: 18.

———. 2002*b. Sirochloa,* a new bamboo genus from Madagascar (Poaceae–Bambusoideae). *Kew Bulletin* **57**: 963–970.

———, AND E. WIDJAJA (editors). 1995. Plant resources of South-East Asia. No. 7, Bamboos. Backhuys Publishers, Leiden, The Netherlands. 189 p.

———, AND K. M. WONG. 1996. *Temburongia,* a new genus of bamboo (Gramineae: Bambusoideae) from Brunei. *Sandakania* **7**: 49–58.

FELSENSTEIN, J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**: 783–791.

FIJTEN, F. 1975. A taxonomic revision of *Buergersiochloa* Pilg. (Gramineae). *Blumea* **22**: 415–418.

GAUT, B. S., L. G. CLARK, J. F. WENDEL, AND S. V. MUSE. 1997. Comparison of the molecular evolutionary process at *rbc*L and *ndh*F in the grass family (Poaceae). *Molec. Biol. Evol.* **14**: 769–777.

GIRIBET, G., AND W. C. WHEELER. 1999. On gaps. *Molec. Phylogen. Evol.* **13**: 132–143.

GRASS PHYLOGENY WORKING GROUP [GPWG]. 2000. A phylogeny of the grass family (Poaceae), as inferred from eight character sets, p. 37. *In* S. W. L. Jacobs and J. Everett [eds.], Grasses: systematics and evolution. CSIRO Publishing, Collingwood, Victoria, Australia.

———. 2001. Phylogeny and subfamilial classification of the Poaceae. *Ann. Missouri Bot. Gard.* **88**: 373–457.

GUALA, G. F. 2000. Molecular evidence for polyphyly in the genus *Apoclada* (Poaceae: Bambusoideae). *Bamboo Science and Culture* **14**: 15–20.

———. 2003. A new genus of bamboos from the cerrados of Brazil. *Bamboo Science and Culture* **17**: 13.

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

———, AND D-Z. LI. 2004. Phylogenetics of the *Thamnocalamus* group and its allies (Gramineae: Bambusoideae): inference from the sequences of GBSSI and ITS spacer. *Molec. Phylogen. Evol.* **24**: 1–12.

HOLTTUM, R. E. 1955. The bamboo genera *Nastus* and *Chloothamnus. Kew Bull.* **4**: 591–594.

JUDZIEWICZ, E. J., AND L. G. CLARK. 2007. Classification and biogeography of New World grasses: Anomochlooideae, Pharoideae, Ehrhartoideae, and Bambusoideae, pp. 303–314. *In* J. T. Columbus, E. A. Friar, J. M. Porter, L. M. Prince, and M. G. Simpson [eds.], Monocots: comparative biology and evolution—Poales. Rancho Santa Ana Botanic Garden, Claremont, California, USA.

———, ———, X. LONDOÑO, AND M. J. STERN. 1999. American bamboos. Smithsonian Institution Press, Washington, D.C., USA. 392 p.

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

KUMAR, S., K. TAMARU, I. B. JAKOBSEN, AND M. NEI. 2001. MEGA2: molecular evolutionary genetics analysis software. *Bioinformatics* **17**: 1244–1245.

LONDOÑO, X., AND L. G. CLARK. 2002. A revision of the Brazilian bamboo genus *Eremocaulon* (Poaceae: Bambuseae: Guaduinae). *Syst. Bot.* **27**: 703–721.

MARCH, R. H. 2000. A morphological and anatomical analysis of *Yushania niitakayamensis* (Poaceae: Bambusoideae). M.S. thesis, Iowa State University, Ames, Iowa, USA. 65 p.

MCCLURE, F. A. 1973. Genera of bamboos native to the New World (Gramineae: Bambusoideae). *Smithsonian Contr. Bot.* **9**: 1–148.

NEES VON ESENBECK, C. G. D. 1835. Bambuseae brasilienses: recensuit, et alias in India orientali provenientes adjecit. *Linnaea* **9**: 484–493.

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

NIXON, K., AND J. I DAVIS. 1991. Polymorphic taxa, missing values and cladistic analysis. *Cladistics* **7**: 233–241.

PATERSON, A. H., C. L. BRUBAKER, AND J. F. WENDEL. 1993. A rapid method for extraction of cotton (*Gossypium* spp.) genomic DNA suitable for RFLP and PCR analysis. *Pl. Molec. Biol. Reporter* **11**: 122–127.

POE, S., AND J. J. WIENS. 2000. Character selection and the methodology of morphological phylogenetics, pp. 20–36. *In* J. J. Wiens [ed.], Phylogenetic analysis of morphological data. Smithsonian Institution Press, Washington, D.C., USA.

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

SHIMADA, H., AND M. SUGIURA. 1991. Fine structural features of the chloroplast genome: comparison of the sequenced chloroplast genomes. *Nucl. Acids Res.* **19**: 983–995.

SODERSTROM, T. R., AND R. P. ELLIS. 1987. The position of bamboo genera and allies in a system of grass classification, pp. 225–238. *In* Soderstrom, T. R., K. W. Hilu, C. S. Campbell, and M. Barkworth [eds.], Grass systematics and evolution. Smithsonian Institution Press, Washington, D.C., USA.

SORENG, R. J., AND J. I. DAVIS. 1998. Phylogenetics and character evolution in the grass family (Poaceae): simultaneous analysis of morphological and chloroplast DNA restriction site character sets. *Bot. Rev. (Lancaster)* **64**: 1–84.

STAPLETON, C. M. A. 1997. The morphology of woody bamboos, pp. 251–267. *In* Chapman, G. P. [ed.], The bamboos. Academic Press, London, UK.

STEVENS, P. F. 1991. Character states, morphological variation, and phylogenetic analysis: a review. *Syst. Bot.* **16**: 553–583.

SWOFFORD, D. L. 2002. PAUP*: phylogenetic analysis using parsimony (*and other methods), vers. 4.0b10. Sinauer Associates, Inc., Sunderland, Massachusetts, USA.

THIELE, K. 1993. The holy grail of the perfect character: the cladistic treatment of morphometric data. *Cladistics* **9**: 275–304.

ZHANG, W-P. 1996. Phylogeny and classification of the bamboos (Poaceae: Bambusoideae) based on molecular and morphological data. Ph.D. dissertation, Iowa State University, Ames, Iowa, USA. 161 p.

———. 2000. Phylogeny of the grass family (Poaceae) from *rpl*16 intron sequence data. *Molec. Phylogen. Evol.* **15**: 135–146.

———, AND L. G. CLARK. 2000. Phylogeny and classification of the bamboos (Poaceae: Bambusoideae), pp. 35–42. *In* S. W. L. Jacobs and J. Everett [eds.], Grasses: systematics and evolution. CSIRO Publishing, Collingwood, Victoria, Australia.