Generic delimitations of *Schizostachyum* and its allies (Gramineae: Bambusoideae) inferred from GBSSI and *trnL-F* sequence phylogenies

Han-Qi Yang[1,2], Sheng Peng[1,2] & De-Zhu Li[1]

[1] *Key Laboratory of Biodiversity and Biogeography, Kunming Institute of Botany, Chinese Academy of Sciences, Heilongtan, Kunming, Yunnan Province 650204, People's Republic of China. dzl@mail.kib.ac.cn (author for correspondence)*

[2] *Graduate School of Chinese Academy of Sciences, Beijing 100039, People's Republic of China*

The monophyly of the woody bamboos *Schizostachyum* s.str., *Cephalostachyum, Dinochloa, Leptocanna, Melocanna, Melocalamus,* and *Pseudostachyum* was tested based on sequence data of the nuclear GBSSI gene and plastid *trnL-F* intergenic spacer, using maximum parsimony and Bayesian inference. *Schizostachyum* s.str., *Cephalostachyum, Leptocanna, Melocanna,* and *Pseudostachyum* were resolved as a monophyletic group while *Dinochloa* and *Melocalamus* were excluded and should be referred to another subtribe. *Schizostachyum* s.str. and *Cephalostachyum* were strongly supported as monophyletic in both the separate and combined analyses; their circumscriptions should be modified, however. *Leptocanna* and *Schizostachyum sanguineum* must be united with the *Cephalostachyum* subclade and *Cephalostachyum virgatum* and *C. pergracile* transferred to the *Schizostachyum* subclade. *Melocanna* and *Pseudostachyum* were strongly supported as good genera.

KEYWORDS: Bayesian inference, *Cephalostachyum*, GBSSI gene, Gramineae, parsimony, *Schizostachyum*, *trnL-F*

INTRODUCTION

The woody bamboos of the Old World tropics are generally divided into three subtribes in the modern woody bamboos systems, i.e., Bambusinae, Melocanninae, and Hickelinae (Soderstrom & Ellis, 1987; Dransfield & Widjaja, 1995), which obtains support from recent molecular studies (Clark & al., 1995; Kelchner & Clark, 1997; Zhang & Clark, 2000). Melocanninae, a small subtribe distributed in the Old World tropics with ca. 70–90 species, is characterized by bearing iterauctant inflorescences and producing pseudospikelets usually with 1 or 2 (3) perfect florets and with a distinctive glabrous ovary that bears an elongated and persistent style divided usually into three short stigmas (Clayton & Renvoize, 1986; Soderstrom & Ellis, 1987; Keng & Wang, 1996).

Schizostachyum Nees, the biggest genus in Melocanninae, was established by Nees in 1829 based on *Schizostachyum blumei* Nees and currently consists of ca. 40–50 species (Ohrnberger, 1999; Dransfield, 2000a). This genus was previously considered to have a wide distribution including Madagascar, South Asia and Malesia, and some Pacific islands (Clayton & Renvoize, 1986; Soderstrom & Ellis, 1987). However, according to Dransfield (2000b), the occurrence of *Schizostachyum* in Madagascar was due to incorrect identification, so the distribution of this genus was limited to tropical Asia. Systematically, *Schizostachyum* was classified as a member of Melocanninae in main woody bamboos classification systems (e.g., Holttum, 1956; Clayton & Renvoize, 1986; Soderstrom & Ellis, 1987; Tzvelev, 1989; Dransfield & Widjaja, 1995; Keng & Wang, 1996; Li, 1997; Ohrnberger, 1999). Holttum (1946) first found that the characters of the ovary were uniform within *Schizostachyum, Cephalostachyum* Munro, *Pseudostachyum* Munro, *Teinostachyum* Munro, *Neohouzeaua* A. Camus, and *Dendrochloa* Parkinson, and he suggested that these six genera be united into *Schizostachyum* s.l. This opinion had a great influence on later taxonomic and systematic studies of Melocanninae (e.g., Clayton & Renvoize, 1986; Soderstrom & Ellis, 1987; Xia, 1993; Dransfield & Widjaja, 1995). In the present study, *Schizostachyum* and its allies include *Schizostachyum* s.str. (as described by Nees), the genera merged into *Schizostachyum* by Holttum (1946), and some genera described later in Melocanninae, which are the core taxa of this subtribe.

In spite of several attempts to study the systematics of Melocanninae including *Schizostachyum* and its allies based on morphological characters, the circumscriptions and interrelationships in this group remain highly controversial. Munro (1868) separated the woody bamboos of the Old World tropics into two sections (= subtribes in other systems): Bambusae and Bacciferae, based on fruit characters. Bacciferae, which bore berry-like fruits, included eight genera, i.e., *Beesha* Rheede, *Cephalostachyum, Dendrocalamus* Nees, *Dinochloa* Büse,

Melocanna Trinius, *Pseudostachyum, Schizostachyum,* and *Teinostachyum*. Furthermore, Bacciferae was subdivided into two subdivisions, Bambusoidea and Schizostachyoidea; the latter subdivision, which was characterized by one floret in each spikelet, consisted of *Cephalostachyum, Melocanna, Pseudostachyum,* and *Schizostachyum*. Bentham (1883) modified Munro's scheme and split Bacciferae into two new tribes (= subtribes in other systems): Dendrocalameae (including *Cephalostachyum, Dendrocalamus, Melocalamus, Pseudostachyum,* and *Teinostachyum*) and Melocanneae (including *Dinochloa*, *Melocanna, Ochlandra* Thwaites, and *Schizostachyum*). This treatment was adopted by Gamble (1896) in his monograph of Indian bamboos. Holttum (1946) proposed *Schizostachyum* s.l. based on the uniform characters of the ovary, i.e., the top of ovary bore a long, hollow, stiff and tapering appendage. As a result, his system of Melocanneae consisted of *Dinochloa, Melocanna, Ochlandra,* and *Schizostachyum* s.l. With further studies on the ovary of *Dinochloa,* Holttum (1956) transferred this genus to Bambusaeae, another subtribe of the Old World tropics, based on their similar ovary characters. This treatment was adopted by Clayton & Renvoize (1986). Soderstrom & Ellis (1987) built a new system within *Schizostachyum* and its allies based on the comprehensive morphological, anatomical and developmental characteristics of ovary, floret and foliar blade. In their system, the subtribe Schizostachydinae (= Melocanninae) included *Cephalostachyum, Leptocanna* Chia & Fung, *Melocanna, Ochlandra, Pseudostachyum, Schizostachyum,* and *Teinostachyum*. This treatment was largely adopted by Dransfield & Widjaja (1995). However, in *Flora Reipublicae Popularis Sinicae* (*FRPS*), Keng & Wang (1996) followed Bentham (1883) and Gamble (1896) in treating *Dinochloa* and *Melocalamus,* which were placed in Bambusinae in other systems, as members of Melocanneae (= Melocanninae).

As reviewed above, besides the systematic position of genera related to *Schizostachyum,* the generic delimitations within *Schizostachyum* and its allies were also in dispute. In summary, there were three different opinions. The first, proposed by Holttum (1946) and followed by Clayton & Renvoize (1986), was to unite those genera in *Schizostachyum* s.l. This opinion emphasized the significance of ovary structure in generic delimitation within *Schizostachyum* and its allies, and considered that the characters of florets and spikelets were not sufficient to support generic separation. The second and totally opposite opinion, based on Munro (1868), accepted many segregate genera including *Cephalostachyum, Dendrochloa, Leptocanna, Neohouzeaua, Pseudostachyum, Schizostachyum* s.str., and *Teinostachyum*. This treatment was supported by Keng (1982), Soderstrom & Ellis (1987), Tzvelev (1989), Stapleton (1994), Dransfield & Widjaja (1995), and Ohrnberger (1999). The third opinion, which was intermediate between the former two, was held by Xia (1993), who supported the combination of *Dendrochloa, Leptocanna, Neohouzeaua,* and *Teinostachyum* with *Schizostachyum*. On the other hand, he treated *Cephalostachyum* and *Pseudostachyum* as distinct genera. This treatment was supported by Keng & Wang (1996) and Li (1997), but the latter also separated *Leptocanna.*

Although it is generally emphasized that characters such as rhizomes, branches, structures of spikelets, florets and ovaries should be comprehensively taken into account when a genus of bamboos was delimited (e.g., Keng, 1982; Soderstrom & Ellis, 1987; Stapleton, 1994; Xia, 1993; Li, 1997), the interrelationships of *Schizostachyum* and its allies are too complicated to be solved based on morphological characters alone. In this paper, phylogenetic reconstruction is based on sequences of the nuclear GBSSI gene and the plastid *trnL-trnF* region (*trnL-F*). Divergence between *trnL-F* sequences have been extensively proven valuable for phylogenetic studies at low or high taxonomic levels (e.g., Fernandez & al., 2001; Zomlefer & al., 2001; Kong & al., 2002; Albach & Chase, 2004; Huang & al., 2005). *TrnL-F* has also demonstrated its suitability for clarifying the systematic problems in Gramineae (Hodkinson & al., 2002; Neves & al., 2005). The GBSSI gene exists in a single copy in the grass family and many other taxa in which it has been studied (Mason-Gamer & al., 1998), although it appears to be duplicated in the Rosaceae (Evans & al., 2000). Although the woody bamboos are presumably ancient polyploids (Soderstrom, 1981), GBSSI seems to be single-copy gene in the alpine woody bamboos (Guo & Li, 2004). In the current paper, we have randomly sequenced six to eight clones of each examined taxon (results not shown) and the results show that most of them are identical with only 1–3 variable sites. This indicates that the GBSSI gene probably exists in a single copy within paleotropical woody bamboos. Although this gene has been used in relatively few phylogenetic studies (Peralta & al., 1997; Mason-Gamer & al., 1998; Evans & al., 2000), it appears that the introns of the GBSSI gene show high genetic divergence among very closely related species. Guo & Li (2004) found that the GBSSI gene provided more variable and informative sites than ITS in the study of alpine woody bamboos, despite slightly lower genetic divergence.

In the current study, we selected the GBSSI and *trnL-F* DNA regions for examining the appropriateness of generic delimitations among *Schizostachyum* and some related genera. Our main focus is on *Schizostachyum* s.str. and *Cephalostachyum* which are the two largest genera in Melocanninae. Our main goals were to test the monophyly of the groups and to improve our understanding of their phylogeny.

MATERIAL AND METHODS

Taxon sampling. — A total of 30 taxa were examined. Among them were 25 species from seven genera which were treated as related to *Schizostachyum* or Melocanninae by previous authors (see Appendix). The ingroup taxa included 11 species from *Schizostachyum* s.str. (consisting of ca. 45 species), covering three groups of *Schizostachyum* recognized by Dransfield (1983); six species from *Cephalostachyum* (ca. 16 species), covering the two sections of *Cephalostachyum* described by Gamble (1896); two species from *Dinochloa* (ca. 27 species); three species from *Melocalamus* (ca. 9 species); one species from *Melocanna* (ca. 2 species); one species from *Pseudostachyum* (ca. 2 species); and one species from *Leptocanna* (1 species). Generic definitions follow Ohrnberger (1999). Leaf material of *Dinochloa malayana, Schizostachyum zollingeri,* and *S. gracile* were obtained from Professor Khoon Meng Wong (Malaysia) and total DNA of *D. scandens* and *S. blumei* from Dr. E.A. Widjaja (Indonesia). For practical reasons, we followed the classification scheme of *Melocanninae* and its related genera in *FRPS* (Keng & Wang, 1996) with a few exceptions, i.e., *Leptocanna chinensis, Melocalamus compactiflorus* var. *fimbriatus, Melocalamus scandens, Cephalostachyum mannii,* and *C. scandens* were treated as in *Flora Yunnanica* (Sun & al., 2003). Four species of woody bamboos from three genera of the North Temperate Zone, *Phyllostachys dulcis, P. nidularia, Shibataea kumasasa,* and *Pleioblastus gramineus* were used as outgroups. Because *Schizostachyum* and its allies are presumed to be closely related to subtribe Bambusinae, one species of *Bambusa, B. arundinacea,* was sampled to explore phylogenetic relationships between them. Vouchers from Malaysia and Indonesia are deposited in local herbaria (KLU, BO), all others in KUN.

DNA extraction, amplification, and sequencing. — Total DNA was extracted from silica-gel-dried or fresh leaves using a modified CTAB procedure (Doyle & Doyle, 1987). All leaf material was sterilized with 75% alcohol prior to DNA extraction.

In PCR amplification of GBSSI, the primers F′-for and M′-bac (Guo & Li, 2004) were used. Reaction volumes were 20μl and contained 1.5 U AmpliTaq DNA polymerase, Replitherm buffer, 1.5 mmol/L $MgCl_2$, 1.0 mmol/L dNTP, 0.2 μmol/L primer, 25–60 ng sample DNA. The PCR cycling consisted of an initial denaturation at 94°C for 5 min, followed by 5 cycles of 1.5 min at 94°C for template denaturation, 2 min at 52°C for primer annealing, 1 min at 72°C for primer extension, then additional 30 cycles of 94°C for 1 min, 52°C for 1 min, 72°C for 1 min, finally followed by an extension of 20 min at 72°C. PCR products were purified using Watson's purification kit. Cleaned PCR products were cloned into Promega's pGEM-T System vector. Ligation, transformation and plating were carried out following the recommendations of the manufacturer. One clone of each species was obtained and plasmid preparations were carried out follwing the Watson's plasmid mini-columns precipitation protocols. The plastid *trnL-F* was amplified with the c and f primers described by Taberlet & al. (1991). Reaction volumes were 20 μl and contained 1.5 U AmpliTaq DNA polymerase, Replitherm buffer, 1.5 mmol/L $MgCl_2$, 1.0 mmol/L dNTP, 0.2 μmol/L primer, 25–60 ng sample DNA. The thermal cycling comprised 30 cycles of 1 min at 95°C for template denaturation, 1 min at 55°C for primer annealing, 2 min at 72°C for primer extension, followed by a final extension of 10 min at 72°C. PCR amplifications mentioned above were performed in a T3 Thermocycler (Biometra). PCR products were purified with Watson's purification kit prior to being sequenced.

Double-stranded and purified PCR products were sequenced by the dideoxy chain termination method with an ABI PRISM Bigdye Terminator Cycle Sequencing Ready Reaction Kit with AmpliTaq DNA polymerase FS (Perkin-Elmer). Reactions and programs were chosen according to the recommendations of the handbook, with slight modification in some cases. Samples were electrophoresed in an ABI3700 automated sequencer. In DNA sequencing reactions, primers F′-for and M′-bac were used to sequence for GBSSI and primers c and f for *trnL-F.*

Alignment and gap coding. — Base determination was complete and unambiguous in all cases and no cells were treated as missing. DNA sequences were edited with SeqMan (DNASTAR Package), aligned by Clustal X, and adjusted manually where necessary. Substitution and indels were used as equally probable events. The potentially informative indels that were located in regions of unambiguous alignment of GBSSI and *trnL-F* sequences were scored following the "simple indel coding" method suggested by Simmons & Ochoterena (2000) and added to the matrix as extra gap characters (see Electronic supplement). The "simple indel coding" method is useful in utilizing indels as a source of phylogenetic information (Kawakita & al., 2003; Guo & Li, 2004).

Phylogenetic analyses. — Maximum parsimony (MP) analysis was performed with PAUP* 4.0b10 (Swofford, 2002). Searches were conducted on the separate GBSSI and *trnL-F* datasets, and a combined GBSSI + *trn*L-F dataset. The option of collapse branches if minimum length is zero ("amb-") was selected. The initial tree search was conducted under the equal and unordered weights criterion using the heuristic search option with stepwise data addition (1,000 random replications) and TBR branch-swapping, but permitting only 10 trees to be saved at each step. To assess the relative support for each clade, bootstrap values were calculated with equally weighted character matrices from 1,000 replicate analy-

ses with the heuristic search strategy and simple addition sequence of the taxa.

The aligned matrices were also analyzed by Bayesian inference (using MrBayes version 3.0, Huelsenbeck & Ronquist, 2001). The models for the analyses were selected with the likelihood-ratio test implemented in Modeltest 3.06 (Posada & Crandall, 1998). Each analysis was initiated from a random starting tree and the program was set to run four (three heated and one cold) Markov chain Monte Carlo iterations simultaneously for 2,000,000 generations and a tree was saved every 100 generations. The posterior probabilities for clades were estimated by a majority-rule consensus tree based on the saved trees which were used to indicate branch supports.

RESULTS

Tree statistics for each analysis are given in Table 1. Clades are referred to throughout the text by outmost genera of the clade, as they are found in the corresponding figures.

GBSSI. — The strict consensus tree from eight most parsimonious trees is shown in Fig. 1. Modeltest found the K81 + Γ model with α = 0.3723 to be the most appropriate model for Bayesian analysis, and analysis reached equilibrium after ca. 30,000 generations. The tree of Bayesian analysis was almost identical with that of MP. Like the results of ITS, *Dinochloa, Melocalamus,* and *Cephalostachyum* (including *Leptocanna chinensis, Schizostachyum sanguineum, Cephalostachyum pallidum, C. fuchsianum, C. scandens,* and *C. mannii*) gained strong supports in either MP or Bayesian inference. *Schizostachyum,* which included *Cephalostachyum pergracile, C. virgatum* and all examined species of *Schizostachyum* except *S. sanguineum,* received weak support (BP = 54, PP = 0.92). Likewise, *Cephalostachyum, Melocanna, Pseudostachyum,* and *Schizostachyum* formed a clade with strong support (BP = 97, PP = 1.00).

trnL-F. — The strict consensus tree from 75 most parsimonious trees is shown in Fig. 2. Modeltest found the HKY + Γ model with α = 0.2149 to be the most appropriate model for Bayesian analysis, and analysis reached equilibrium after ca. 25,000 generations. Although the topology of the Bayesian analysis was different from that of MP, the main branches in these two analyses were congruent. The resolution in *trnL-F*-based tree was very poor due to few informative characters. Only *Dinochloa* (BP = 91, PP = 1.00) and *Schizostachyum* (including *Cephalostachyum pergracile, C. virgatum* and all examined species of *Schizostachyum* except *S. sanguineum*) (BP = 71, PP = 0.97) were identified. *Dinochloa, Melocalamus,* and *Bambusa arundinacea* as a group were supported as monophyletic (BP = 85, PP = 0.98) while *Cephalostachyum, Leptocanna, Meclocanna, Pseudostachyum,* and *Schizostachyum* formed a clade with weak support in Bayesian inference.

Combined dataset. — We examined the feasibility of integrating the GBSSI and *trnL-F* datasets into a single matrix according to the congruence of the topologies and the incongruence length difference (ILD) test proposed by Farris et al. (1994). ILD value was computed by executing the "partition homogeneity test" command of PAUP on the combined matrix. The setting opinions used 1,000 replicates, heuristic searches, branch swapping with nearest-neighbor interchange (NNI), and five random addition sequences following the suggestions of Sjolin & al. (2005).

No conflicts among major clades of separate GBSSI and *trnL-F* analyses were identified (especially in Bayesian analysis clades were highly congruent), and the ILD-test showed congruency within the combined datasets (ILD = 0.223). Therefore, the GBSSI and *trnL-F* datasets were combined to explore the phylogeny of *Schizostachyum* and its allies.

The strict consensus tree from eight most parsimonious trees is shown in Fig. 3. Modeltest found the HKY + Γ + G model with α = 0.7764 to be the most appropriate model for Bayesian analysis; the analysis reached equilibrium after ca. 25,000 generations. Bayesian and MP topologies were almost identical. Resolution and support for the clades in the combined dataset were better or higher than in the separate analyses. *Dinochloa, Melo-*

Table 1. Statistics of separate and combined datasets.

Analysis	Characteristics	GBSSI	*trnL-F*	Combined analysis
Maximum Parsimony (MP)	No. of characters	1,293	953	2,246
	Pairwise distance of ingroups (%)	0.17–4.80	0–1.51	0.56–3.48
	Variable sites (%)	270 (20.9)	68 (7.1)	338 (15.0)
	Informative sites (%)	138 (10.7)	38 (4.0)	176 (7.8)
	No. of most parsimony trees (M. P. T.)	8	75	8
	Minimal length of M. P. T.	364	79	451
	CI of strict consensus tree	0.694	0.826	0.812
	RI of strict consensus tree	0.865	0.948	0.870
Bayesian Inference (BI)	No. of nodes (PP > 0.85)	17	9	20

calamus, Cephalostachyum (including *Leptocanna chinensis, Schizostachyum sanguineum, Cephalostachyum pallidum, C. fuchsianum, C. scandens,* and *C. mannii*) and *Schizostachyum,* which included *Cephalostachyum pergracile, C. virgatum* and all examined species of *Schizostachyum* except *S. sanguineum*, gained strong support in either MP or Bayesian inference. All of the ingroup taxa were resolved as two monophyletic groups. *Cephalostachyum, Melocanna, Pseudostachyum,* and *Schizostachyum* were strongly supported as monophyly (BP = 100, PP = 1.00). *Dinochloa, Melocalamus,* and *Bambusa arundinacea* were supported as monophyletic with moderate support (BP = 71, PP = 0.94).

DISCUSSION

As in previous analyses of woody bamboos (e.g., Zhang, 1996; Kelchner & Clark, 1997; Guo & Li, 2004), analysis of each of the separate datasets in this study resulted in less resolved trees in comparison with that of combined dataset. Therefore, only the results from combined analyses will be further discussed here.

Systematics. — Delimitations of *Schizostachyum* and its allies in the past were mainly based on the characters of fruits, ovary, or inflorescence, especially the structures of spikelets and florets (e.g., Munro, 1868; Holttum, 1946; Soderstrom & Ellis, 1987; Xia, 1993). Unfortunately, it was difficult to find common diagnos-

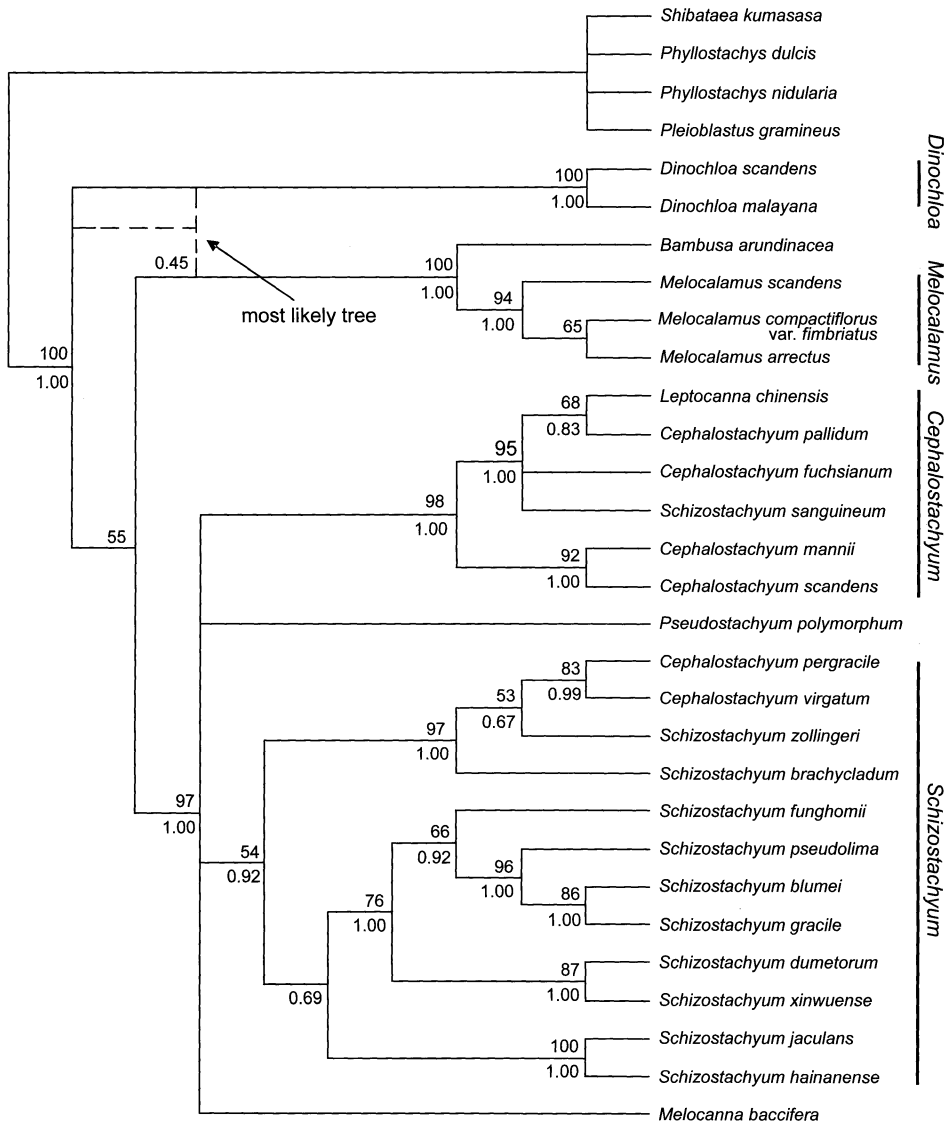

Fig. 1. Strict consensus trees of eight most parsimonious trees based on GBSSI sequences (tree length = 364 steps, CI = 0.694, RI = 0.865). Numbers on the branches indicate bootstrap percentage (above branch). Posterior probability >0.60 from Bayesian analysis is shown below branches.

Fig. 2. Strict consensus tree of 75 most parsimonious trees based on *trnL-F* sequences (tree length = 79, CI = 0.826, RI = 0.948). Numbers on the branches indicate bootstrap percentage (above branch). Posterior probability >0.60 from Bayesian analysis is shown below branches.

tic features for this group because these characteristics were often identical or continuous (Xia, 1993). The current study is the first to address this problem by explicitly examining phylogenetic relationships within this group, emphasizing the two largest genera in Melocanninae, *Cephalostachyum* and *Schizostachyum,* which are also the core taxa of this subtribe. Within the sampled taxa, *Cephalostachyum, Leptocanna* (better treated as part of *Cephalostachyum*), *Melocanna, Pseudostachyum,* and *Schizostachyum* s.str. were resolved as a monophyletic group while *Dinochloa* and *Melocalamus* were clearly excluded from this group and should be placed in another subtribe. The latter two groups are morphologically distinguished by characters of the ovary (Holttum, 1956).

Our study supports generic recognition of *Cephalostachyum* (e.g., Munro, 1868; Gamble, 1896; Soderstrom & Ellis, 1987; Xia, 1993; Ohrnberger, 1999), but the previous circumscription of this genus should be modified. *Leptocanna chinensis* and *S. sanguineum* should be merged with *Cephalostachyum* (Yang & Li, in press) while *C. pergracile* and *C. virgatum* should be transferred to *Schizostachyum* (Majumder, 1989). *Leptocanna chinensis*, described by Chia & Fung (1981) as endemic to southwestern China, has been regarded a member of *Schizostachyum* s.str. (e.g., Clayton & Renvoize, 1986; Xia, 1993; Keng & Wang, 1996; Ohrnberger, 1999) or a monotypic genus (e.g., Chia & Fung, 1981; Soderstrom & Ellis, 1987; Tzvelev, 1989; Li, 1997; Sun & al., 2003). In this study, however, it was strongly embedded within *Cephalostachyum*. Although *Leptocanna* is similar to *Schizostachyum* s.str. in vegetative appearance, both *Leptocanna* and *Cephalostachyum* have two glumes in

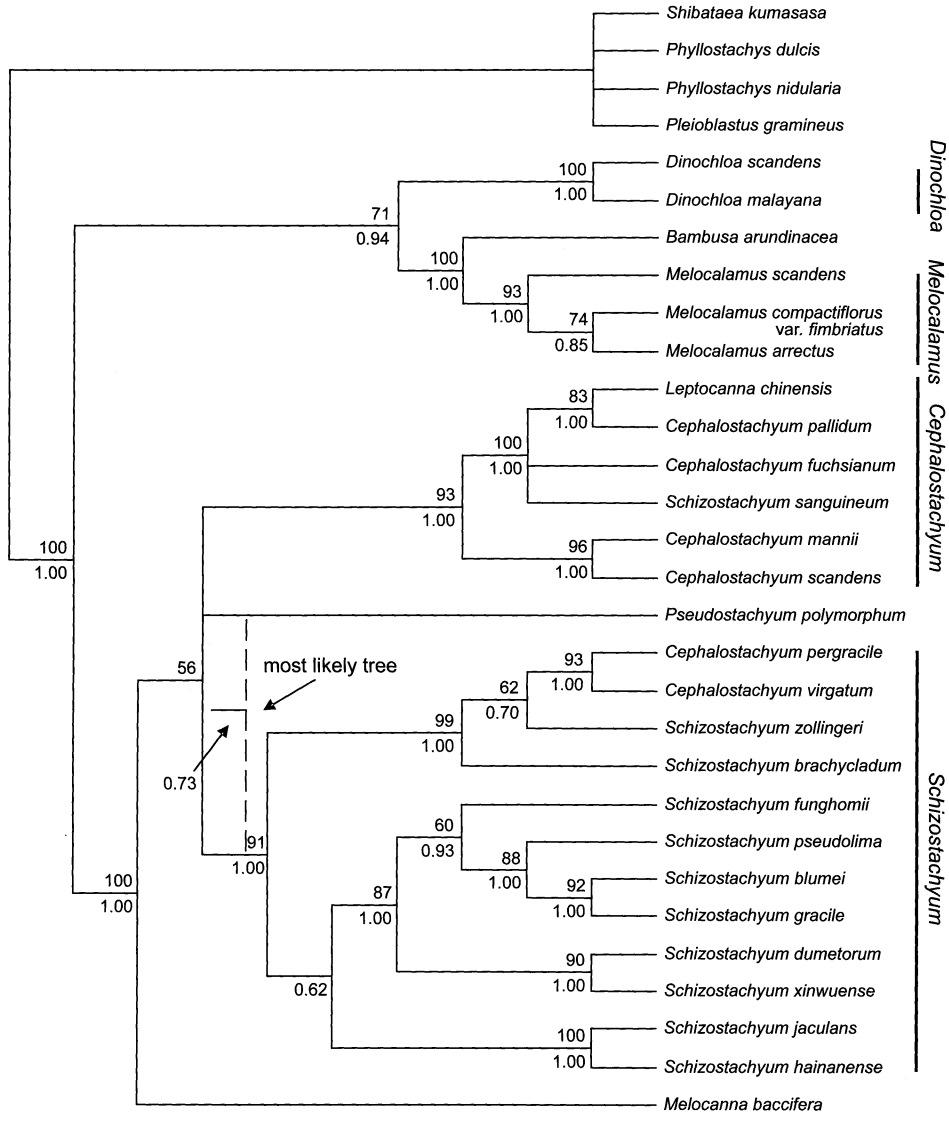

Fig. 3. Strict consensus tree of eight most parsimonious trees based on GBSSI + *trnL-F* sequences (tree length = 451 steps, CI = 0.812, RI = 0.870). Numbers on the branches indicate bootstrap percentage (above branch). Posterior probability >0.60 from Bayesian analysis is shown below branches.

the spikelets and three lodicules in the florets. In addition, *Leptocanna* has a more or less scrambling habit and occurs at higher elevations (1,500–2,500 m) and cooler habitats similar to most of the sampled *Cephalostachyum* species including the type species, *C. capitatum,* but excluding *C. virgatum* and *C. pergracile.* Therefore, we propose to include this genus in a newly circumscribed *Cephalostachyum. Schizostachyum sanguineum,* described by Zhang (1989) based on specimens without inflorescences and fruits, is a small climbing bamboo distributed at higher elevations of 1,600 m and endemic to the southwestern China. Gamble (1896) divided *Cephalostachyum* into two sections according to the structure of spikelets. Section I included the type species *C. capitatum* and species such as *C. fuchsianum* and *C. pallidum,* which were characterized by "spikelets in single terminal globose heads". Section II, which was described as having "spikelets in heads in interrupted paniculate spikes", included *C. pergracile* and *C. virgatum.* Majumder (1989) combined *C. pergracile* in *Schizostachyum,* but this treatment was overlooked by most authors. Morphologically, *Cephalostachyum pergracile* and *C. virgatum* can be distinguished from the other species of *Cephalostachyum* also by other characters including an erect habit; florets with three stigmas; and distribution at lower elevation (usually below 1,200 m) (Keng & Wang, 1996; Sun & al., 2003). The last two characters and the inflorescences of *C. pergracile* and *C. virgatum* are similar to *Schizostachyum* s.str. Gamble's section II seems more appropriate to be separated from *Cephalostachyum* based on molecular and morphological evidence. Thus, our newly circumscribed *Cephalostachyum* is limited to

the members that (1) have a shrubby or sub-arborescent habit; (2) occur in habitats at elevations of ca. 1,200–2,000 m; (3) bear terminal inflorescences (capitulum or panicle); (4) have spikelets with two or three glumes; and (5) have florets with two stigmas and two or three lodicules.

Schizostachyum s.str. was also supported as a distinct genus in this study. As discussed above, *Cephalostachyum virgatum* and *C. pergracile* should be merged into *Schizostachyum* s.str. and *Schizostachyum sanguineum* should be transferred into *Cephalostachyum*. So our new *Schizostachyum* is restricted to species that (1) occur in habitats at elevations usually below ca. 1,200 m; (2) bear panicles consisting of densely or slightly glomerate spikelets on the nodes of flowering branches; (3) have the glumes usually absent; and (4) bear florets with three stigmas and usually without lodicules. In the current study, the examined species of the modified *Schizostachyum* could be divided into three strongly supported subclades. First, *Cephalostachyum pergracile, C. virgatum, Schizostachyum zollingeri,* and *S. brachycladum* were supported as a subclade (the *S. brachycladum* group) (BP = 99, PP = 1.00). Morphologically, this subclade can be identified by the characters of having erect and arboreous culms; broadly triangular blades of culm-sheaths, and inflorescences composed of densely glomerate spikelets at the nodes of flowering branches. Second, *Schizostachyum funghomii, S. pseudolima, S. blumei, S. gracile, S. dumetorum,* and *S. xinwuense* were resolved as monophyletic (the *S. blumei* group) (BP = 87, PP = 1.00). These arboreous or scrambling *Schizostachyum* shared the common characters of long, narrowly lanceolate and reflexed blades of culm-sheaths and inflorescences consisting of sparsely panicled spikelets on the nodes of flowering branches. Third, *Schizostachyum jaculans* and *S. hainanense* formed a monophyletic group (the *S. jaculans* group) (BP = 100, PP = 1.00). These two scrambling *Schizostachyum* bear long, narrowly lanceolate and reflexed blades of culm-sheaths and inflorescences consisted of densely glomerate spikelets at the nodes of flowering branches. Dransfield (1983) recognized *Schizostachyum* as three groups based on their habit and the structure of culm-sheaths. The first group included *Schizostachyum brachycladum* and related species with erect culms and broadly triangular erect blades on the culm-sheaths. The second group included *Schizostachyum blumei, S. jaculans* and others with erect culms with drooping tips and long, narrow, deflexed blades on the culm-sheaths. The last group included *Schizostachyum grande* and its relatives with erect culms when young, but scrambling when mature as well as long blades with a broad base and tapering tips on the culm-sheaths. However, this treatment was not supported in the current analyses. It seems that, with the support of molecular evidence, our scheme of dividing *Schizostachyum* into three groups based on the structures of inflorescence and culm-sheaths could be more appropriate.

Dinochloa and *Melocalamus* were resolved as a clade with *Bambusa arundinacea*. This implied that *Dinochloa* and *Melocalamus* were very closely related to *Bambusa*. Morphologically, the ovary of *Dinochloa* and *Melocalamus* are very similar to that of *Bambusa,* i.e., there is a short and solid style at the top of the ovary (Holttum, 1956). In addition, *Dinochloa* and *Melocalamus* occurred in different subclades, which reflected a relationship between them that was not as close as that proposed by McClure (1936), who suggested that these two genera be united. In fact, *Dinochloa* has one perfect floret without lodicules and a rudimentary floret per spikelet; and its rachilla is not extended. On the other hand, *Melocalamus* has two or three perfect florets with three lodicules per spikelet, and its rachilla is extended. As for the fruit structure, the embryo of *Melocalamus* is basal, but lateral in *Dinochloa* (Rudall & Dransfield, 1989).

We suggest a broader sampling in future studies to help establish the natural delimitations of *Schizostachyum* and its allies. These studies should include *Dendrochloa, Neohouzeaua, Ochlandra* and *Teinostachyum*, which are not analyzed in the current paper but are also recognized as members of Melocanninae (e.g., Holttum, 1956; Clayton & Renvoize, 1986; Soderstrom & Ellis, 1987; Tzvelev, 1989; Ohrnberger, 1999).

Taxonomic implication. — Our results strongly supported the ovary character as a good criterion in defining major clades of the Old World tropical woody bamboos. On the contrary, characters previously used to divide main subtribes (or tribes) of woody bamboos such as the characters of fruits and spikelets (e.g., Munro, 1868; Bentham, 1883; Gamble, 1896; Keng & Wang, 1996), seem to be homoplastic (Holttum, 1956; Soderstrom & Ellis, 1987; Clark, 1997), and unsuitable for delimitation of main clades of woody bamboos. In general, our results supported the "small genus" perspective in the delimitation of genera within *Schizostachyum* and its allies (e.g., Soderstrom & Ellis, 1987; Xia, 1993; Dransfield & Widjaja, 1995; Li, 1997; Ohrnberger, 1999).

At lower levels of classification, molecular markers can provide useful information for taxonomy, for taxa that are morphologically unclear. Thus, in the current study it appeared appropriate to place *Leptocanna chinensis* and *Schizostachyum sanguineum* in *Cephalostachyum* and to merge *C. virgatum* and *C. pergracile* into *Schizostachyum*. In addition, *Schizostachyum dumetorum* and *S. xinwuense* were resolved as a monophyletic group (BP = 100, PP = 1.00). These two semi-scrambling *Schizostachyum* are greatly similar in vegetative appearance and our results support the treatment of *Schizostachyum xinwuense* as a variety of *S. dumetorum* (Xia, 1993).

ACKNOWLEDGMENTS

Comments and suggestions from Lynn G. Clark and Victoria C. Hollowell are sincerely appreciated. We are grateful to Professor Khoon Meng Wong of Institute of Biological Sciences, University of Malaya; to Dr. E.A. Widjaja, Dean of the Herbarium of Bogoriense; to Drs. Nian-He Xia and Yun-Fei Deng of the South China Institute of Botany, Chinese Academy of Science; and to Dr. Chao-Mao Hui of the Southwest Forest College (Kunming); to Dr. S. Dransfield of Royal Botanic Gardens, Kew, England for help in collecting experimental materials. We would also like express our thanks to Dr. T.R. Hodkinson of the Trinity College, Dublin, Ireland for suggestions regarding PCR; to Dr. Jian-Jun Gao of Yunnan University (Kunming, Yunnan) and Dr. Lian-Ming Gao of the Kunming Institute of Botany, Chinese Academy of Sciences, for help in data analyses, and to Jun-Bo Yang and other colleagues of Key Laboratory of Biodiversity and Biogeography of the Kunming Institute of Botany, Chinese Academy of Sciences, for assistance. This work was funded by the National Natural Science Foundation of China (grant no. 30200015 to Z.H. Guo), Yunnan Provincial Natural Science Foundation (grant no. 2002C0016Q to Z.H. Guo), Yunnan Provincial Government for Prominent Contributions in Science and Technology award to Prof. Wu Zheng-Yi in 2001 (grant no. KIB-WU-2001-03), and the Ministry of Science and Technology of China (2004DKA30430).

LITERATURE CITED

Albach, D.C. & Chase, M.W. 2004. Incongruence in Veroniceae (Plantaginaceae): evidence from two plastid and a nuclear ribosomal DNA region. *Molec. Phylog. Evol.* 32: 183–197.

Bentham, G. 1883. Gramineae. Pp. 1074–1215 in: Bentham, G. & Hooker, J.D. (eds.), *Genera Plantarum,* 3: pt. 2. Reeve, London.

Chia, L.C. & Fung H.L. 1981. *Leptocanna*, a new genus of Bambusoideae from China. *Acta Phytotax. Sin.* 19: 211–214.

Clark, L.G. 1997. Bamboos: the centrepiece of the grass family. Pp. 33–44 in: Chapman, G.P. (ed.), *The Bamboos.* Academic Press, London.

Clark, L.G., Zhang, W. & Wendel, J.F. 1995. A phylogeny of the Grass Family (Poaceae) based on *ndhF* sequence data. *Syst. Bot.* 20: 436–460.

Clayton, W.D. & Renvoize, S.A. 1986. *Genera Graminum.* Her Majesty's Stationery Office, London.

Doyle, J.J. & Doyle, J.L. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf material. *Phytochem. Bull.* 19: 11–15.

Dransfield, S. 1983. Notes on *Schizostachyum* (Gramineae-Bambusoideae) from Borneo and Sumatra. *Kew Bull.* 38: 321–332.

Dransfield, S. 2000a. *Schizostachyum khoonmengii,* a new species of bamboos (Poaceae-Bambusoideae) from Brunei Darussalam. *Kew Bull.* 55: 491–494.

Dransfield, S. 2000b. Woody Bamboos (Gramineae-Bambusoideae) of Madagascar. Pp. 43–50 in: Jacobs, S.W.L. & Everett, J. (eds.), *Grasses: Systematics and Evolution.* CSIRO, Melbourne.

Dransfield, S. & Widjaja, E.A. (eds.). 1995. *Plant Resources of South-East Asia,* No 7., *Bamboos.* Backhuys Publishers, Leiden.

Evans, R.C., Alice, L.A., Campbell, C.S., Kellogg, E.A. & Dickson, T.A. 2000. The granule-bound starch synthase (GBSSI) gene in the Rosaceae: multiple loci and phylogenetic utility. *Molec. Phylog. Evol.* 17: 388–400.

Farris, J.S, Källersjö, M., Kluge, A.G. & Bult, C. 1994. Testing sigficance of incongruence. *Cladistics* 10: 315–319.

Fernandez, I.A., Aguilar, J.F., Panero, J.L. & Feliner, G.N. 2001. A phylogenetic analysis of *Doronicum* (Asteraceae, Senecioneae) based on morphological, nuclear ribosomal (ITS), and chloroplast (*trnL-F*) evidence. *Molec. Phylog. Evol.* 20: 41–64.

Gamble, J.S. 1896. The Bambuseae of British India. *Ann. Roy. Bot. Gard. (Calcutta)* 7: 1–133.

Guo, Z.H. & Li, D.Z. 2004. Phylogenetics of the *Thamnocalamus* group and its allies (Gramineae: Bambusoideae) inference from the sequences of GBSSI gene and ITS spacer. *Molec. Phylog. Evol.* 30: 1–12.

Hodkinson, T.R., Chase, M.W., Lledo, M.D., Salamin, N. & Renvoize, S.A. 2002. Phylogenetics of *Miscanthus, Saccharum* and related genera (Saccharinae, Andropogoneae, Poaceae) based on DNA sequences from ITS nuclear ribosomal DNA and plastid *trnL* intron and *trnL-F* intergenic spacer. *J. Pl. Res.* 115: 381–392.

Holttum, R.E. 1946. The classification of Malayan bamboos. *J. Arnold Arbor.* 27: 340–346.

Holttum, R.E. 1956. The classification of bamboos. *Phytomorphology* 6: 73–90.

Huang, J.L., Giannasi, D.E. & Price, R.A. 2005. Phylogenetic relationships in *Ephedra* (Ephedraceae) inferred from chloroplast and nuclear DNA sequences. *Molec. Phylog. Evol.* 35: 48–59.

Huelsenbeck, J.P. & Ronquist, F.R. 2001. MrBayes: Bayesian inference of phylogenetic trees. *Bioinformatics* 17: 754–755.

Kawakita, A., Sota, T., Ascher, J.S., Ito, M., Tanaha, H. & Kato, M. 2003. Evolution and phylogenetic utility of alignment gaps within intron sequences of three nuclear genes in Bumble Bees (*Bombus*). *Molec. Biol. Evol.* 20: 87–92.

Kelchner, S.A. & Clark, L.G. 1997. Molecular evolution and phylogenetic utility of the chloroplast rpL16 intron in *Chusquea* and the Bambusoideae (Poaceae). *Molec. Phylog. Evol.* 8: 385–397.

Keng, P.C. 1982. A revision of the genera of bamboos from the world. *Int. J. Bamboo Res.* 1: 1–19.

Keng, P.C. & Wang, C.P. 1996. *Flora Reipublicae Popularis Sinicae,* vol. 9. Science Press, Beijing.

Kong, H.Z., Chen, Z.D. & Lu, A.M. 2002. Phylogeny of *Chloranthus* (Chloranthaceae) based on nuclear ribosomal ITS and plastid *trnL-F* sequence data. *Amer. J. Bot.* 89: 940–946.

Li, D.Z. 1997. The flora of China Bambusoideae project-problems and current understanding of bamboo taxonomy in China. Pp. 61–81 in: Chapman, G.P. (ed.), *The Bamboos.* Academic Press, London.

Majumder, R.B. 1989. Bambusoideae. Pp.272–283 in: Karthikeyan, S., Jain S.K., Nayar M.P. & Sanjappa M. (eds.),

Florae Indicae Enumeratio: Monocotyledonae. Botanical survey of India, Calcutta. [*Flora of India* 4]

Mason-Gamer, R.J., Well, C.F. & Kellogg, E.A. 1998. Granule-bound starch synthase: structure, function, and phylogenetic utility. *Molec. Biol. Evol.* 15: 1658–1673.

McClure, F.A. 1936. The bamboo genera *Dinochloa* and *Melocalamus. Kew Bull. Misc. Inform.* 1936: 251–254.

Munro, W. 1868. A monograph of the Bambusaceae. *Trans. Linn. Soc. London, Bot.* 26: 1–156.

Neves, S.S., Swire-Clark, G., Hilu, K.W. & Baird, W.V. 2005. Phylogeny of *Eleusine* (Poaceae: Chloridoideae) based on nuclear ITS and plastid *trnT-trnF. Molec. Phylog. Evol.* 35: 395–419.

Ohrnberger, D. 1999. *The Bamboos of the World: Annotated Nomenclature and Literature of the Species and the Higher and Lower Taxa.* Elsevier, Amsterdam.

Peralta, I.E., Ballard, J.R. & Spooner, D.M. 1997. GBSSI gene intron phylogeny of tomatoes, *Solanum* subsect. *Lycopersicum* (Solanaceae) (Abstract). *Amer. J. Bot.* 84: 222.

Posada, D. & Crandall, K.A. 1998. Modeltest: testing the model of DNA substitution. *Bioinformatics* 14: 817–818.

Rudall, P. & Dransfield, S. 1989. Fruit structure and Development in *Dinochloa* and *Ochlandra* (Gramineae-Bambusoideae). *Ann. Bot. (Oxford)* 63: 29–38.

Simmons, A.M. & Ochoterena, H. 2000. Gaps as characters in sequence-based phylogenetic analysis. *Syst. Biol.* 49: 369–381.

Sjolin, E., Erseus, C. & Kallersjo, M. 2005. Phylogeny of *Tubificidae* (Annelida, Clitellata) based on mitochondrial and nuclear sequence data. *Molec. Phylog. Evol.* 35: 431–441.

Soderstrom, T.R. 1981. Some evolutionary trends in the Bambusoideae (Gramineae). *Ann. Missouri Bot. Gard.* 68: 15–47.

Soderstrom, T.R. & Ellis, R.P. 1987. The position of bamboo genera and allies in a system of grass classification. Pp. 225–238 in: Soderstrom, T.R., Hilu, K.W., Campbell, C.S. & Barkworth, M.E. (eds.), *Grass Systematics and Evolution.* Smithsonian Institution Press, Washington, D.C.

Stapleton, C.M.A. 1994. The bamboos of the Nepal and Bhutan, Part I: *Bumbusa, Dendrocalamus, Melocanna, Cephalostachyum, Teinostachyum,* and *Pseudostachyum* (Gramineae: Poaceae, Bambusoideae). *Edinburgh J. Bot.* 51: 1–32.

Sun, B.X., Li, D.Z. & Hsueh, C.J. 2003. *Flora Yunnanica,* Tomus 9. Science Press, Beijing.

Swofford, D.L. 2002. *PAUP* Phylogenetic Analysis Using Parsimony (* and Other Methods),* version 4.0b 10. Sinauer, Associates, Sunderland.

Taberlet, P., Gielly, L., Pautou, G. & Bouvet, J. 1991. Universal primers for amplification of three non-coding regions of chloroplast DNA. *Pl. Molec. Biol.* 17: 1105–1109.

Tzvelev, N.N. 1989. The system of Grass (Poaceae) and their evolution. *Bot. Rev.* 55: 141–204.

Xia, N.H. 1993. Studies on the genus *Schizostachyum* and other bamboos from China. *J. Trop. Subtrop. Bot.* 1: 1–10.

Yang, H.Q. & Li, D.Z. In press. Two new combinations in *Cephalostachyum* (Gramineae: Bambusoideae) from Yunnan, China. *Ann. Bot. Fenn.* 44.

Zhang, W.P. 1989. A new species of *Schizostachyum* Nees. *sanguineum. Bamboo Res.* 8: 12–14.

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

Zhang, W. & Clark, L.G. 2000. Phylogeny and classification of the Bambusoideae (Poaceae). Pp. 35–42 in: Jacobs, S.W.L. & Everett, J. (eds.), *Grass: Systematics and Evolution.* CSIRO, Melbourne.

Zomlefer, W.B., Williams, N.H., Whitten, W.M. & Judd, W.S. 2001. Generic circumscription and relationships in the tribe Melanthieae (Liliales, Melanthiaceae), with emphasis on *Zigadenus:* evidence from ITS and *trnL-F* sequence data. *Amer. J. Bot.* 88: 1657–1669.

Appendix. Taxa and vouchers for species sequenced.

Species; country; collector, collection number and herbarium; GBSSI GenBank no.; *trnL-F* GenBank no.

Bambusa arundinacea (Retz.) Willd; Ghana; *De-Zhu Li 200202* (KUN); DQ137292; DQ137349. *Cephalostachyum fuchsianum* Gamble & Hook. f.; China; *Han-Qi Yang 009* (KUN); DQ137316; DQ137373. *C. mannii* (Gamble) Stapleton & D.Z. Li; China; *Han-Qi Yang 008* (KUN); DQ137317; DQ137374. *C. pallidum* Munro; China; *Han-Qi Yang 018* (KUN); DQ137318; DQ137375. *C. pergracile* Munro; China; *Han-Qi Yang 023* (KUN); DQ137319; DQ137376. *C. scandens* Bor; China; *Han-Qi Yang 011* (KUN); DQ137315; DQ137372. *C. virgatum* (Munro) Kurz; China; *Han-Qi Yang 014* (KUN); DQ137320; DQ137377. *Dinochloa malayana* S. Dransfield; Malaysia; *Khoon Meng Wong 200503* (KLU); DQ137298; DQ137355. *D. scandens* (Blume) Kuntze; Indonesia; *E.A. Widjaja 200502* (BO); DQ137299; DQ137356. *Leptocanna chinensis* (Rendle) Chia & H.L. Fung; China; *Han-Qi Yang 042* (KUN); DQ137302; DQ137359. *Melocalamus arrectus* Yi; China; *Han-Qi Yang 015* (KUN); DQ137328; DQ137385. *M. compactiflorus* var. *fimbriatus* (Hsueh & C.M. Hui) D.Z. Li & Z.H. Guo; China; *Han-Qi Yang 017* (KUN); DQ137329; DQ137386. *M. scandens* Hsueh & Hui; China; *Han-Qi Yang 039* (KUN); DQ137330; DQ137387. *Melocanna baccifera* (Roxb.) Kurz; China; *Han-Qi Yang 053* (KUN); DQ137303; DQ137360. *Phyllostachys dulcis* McClure; China; *Sheng Peng 013* (KUN); DQ137275; DQ137332. *Ph. nidularia* Munro; China; *Sheng Peng 053* (KUN); DQ137276; DQ137333. *Pleioblastus gramineus* (Bean) Nakai; China; *Sheng Peng 047* (KUN); DQ137277; DQ137334. *Pseudostachyum polymorphum* Munro; China; *Han-Qi Yang 016* (KUN); DQ137301; DQ137358. *Schizostachyum blumei* Nees; Indonesia; *E.A. Widjaja 200501* (BO); DQ137209; DQ137366. *S. brachycladum* (Kurz) Kurz; Ghana; *De-Zhu Li 200201* (KUN); DQ137311; DQ137368. *S. dumetorum* (Hance) Munro; China; *Han-Qi Yang 050* (KUN); DQ137304; DQ137361. *S. funghomii* McClure; China; *Han-Qi Yang 028* (KUN); DQ137305; DQ137362. *S. gracile* (Munro) Holttum; Malaysia; *Khoon Meng Wong 200502* (KLU); DQ137310; DQ137367. *S. hainanensis* Merr. ex McClure; China; *Han-Qi Yang 058* (KUN); DQ137314; DQ137371. *S. jaculans* Holttum; China; *Han-Qi Yang 059* (KUN); DQ137306; DQ137363. *S. pseudolima* McClure; China; *Han-Qi Yang 041* (KUN); DQ137307; DQ137364. *S. sanguineum* Hsueh & W.P. Zhang; China; *Jin-Mei Lu 164* (KUN); DQ137312; DQ137369. *S. xinwuense* Wen & J.Y. Chin; China; *Han-Qi Yang 047* (KUN); DQ137308; DQ137365. *S. zollingeri* Steudel; Malaysia; *Khoon Meng Wong 200501* (KLU); DQ137313; DQ137370. *Shibataea kumasasa* (Zoll. ex Steud.); China; *Han-Qi Yang 054* (KUN); DQ137274; DQ137331.