Molecular Phylogenetics and Evolution
Vol. 22, No. 1, January, pp. 20–30, 2002
doi:10.1006/mpev.2001.1039, available online at http://www.idealibrary.com on IDEAL®

Phylogenetic Studies on the *Thamnocalamus* Group and Its Allies (Gramineae: Bambusoideae) Based on ITS Sequence Data

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Received September 15, 2000; revised April 12, 2001; published online December 5, 2001

Phylogenetic analyses, using the parsimony method and the neighbor-joining method, of 31 species of the *Thamnocalamus* group and its allies based on internal transcribed spacer (ITS) sequences in nuclear ribosomal DNA are presented. Two species from *Arundinaria* and *Acidosasa* were used as outgroups. The ITS phylogeny strongly supports the monophyly of the *Thamnocalamus* group and its allies, which have pachymorph rhizomes and semelauctant synflorescences with three stamens. Within this clade, *Chimonocalamus pallens* was resolved in the basal position. The *Thamnocalamus* group, including species placed in *Thamnocalamus, Fargesia (Sinarundinaria, Borinda),* and *Yushania,* may be polyphyletic/paraphyletic according to the ITS phylogeny, but internal support was relatively low. All three sampled species of *Ampelocalamus* were resolved as a monophyletic group and may be related to *Drepanostachyum hookerianum*. Two taxa of *Thamnocalamus* and the species *Gaoligongshania megalothyrsa* were resolved as monophyletic despite their different morphological characters, but again with a low bootstrap support. Within the *Fargesia yunnanensis* subclade, the sister relationship of *Fargesia fungosa* and *Fargesia edulis* was supported. Another subclade, the *Fargesia communis* clade, was also weakly supported as monophyletic. However, further resolution within the *Thamnocalamus* group has not been provided by this sequence data. The results indicate that reevaluation of relationships within this group is necessary. **© 2001 Elsevier Science**

***Key Words: Thamnocalamus* group; allies; phylogenetic analyses; ITS.**

INTRODUCTION

The *Thamnocalamus* group and its allies are distributed in mountainous areas in China and the adjacent Himalayas, with some species in Africa, Sri Lanka, India, and eastwards to the islands of Taiwan and the Philippines (Li, 1997b), and are included in subtribe Arundinariinae which belongs to the temperate clade of woody bamboos (Clayton and Renvoize, 1986; Soderstrom and Ellis, 1987; Zhang, 1996; Clark, 1997). Wang (1997) included this generic complex and the New World *Arthrostylidium* in the Arthrostylidinae due to the similarity of morphological characters; however, molecular evidence proved that the New World bamboos and Old World bamboos likely evolved independently (Clark *et al.*, 1995; Zhang, 1996; Kelchner and Clark, 1997). Li (1997b) grossly divided the Arundinariinae into two generic complexes on the differentiation of rhizome types and leaf anatomy. One comprises the *Arundinaria* group, which produces leptomorph rhizomes and includes *Acidosasa (Metasasa), Arundinaria (Bashania, Pleioblastus), Ferrocalamus, Gelidocalamus, Indocalamus, Oligostachyum, Pseudosasa,* and *Sasa*. These genera, *Gaoligongshania,* and a few others are placed in the Arundinariinae s.s (Ohrnberger, 1999). The other consists of the *Thamnocalamus* group and its allies, which have pachymorph rhizomes and appear to have very large microhairs and dumbbell-shaped silica bodies, although some lack fusoid cells (Wu, 1960, 1962; Soderstrom and Ellis, 1982, 1987). The *Thamnocalamus* group comprises *Thamnocalamus, Fargesia (Borinda, Sinarundinaria)* and *Yushania* and is closely related to *Ampelocalamus, Chimonocalamus, Drepanostachyum (Himalayacalamus),* and *Gaoligongshania,* which are regarded as its allies. Collectively, these genera (with the exception of *Gaoligongshania*) are placed in the subtribe Thamnocalaminae Keng f. (Ohrnberger, 1999).

The classification, especially the generic delimitation in this group, is highly controversial but critical to the systematics of temperate woody bamboos. One opinion, expressed by Soderstrom (1979a,b), Soderstrom and Ellis (1982), and Chao *et al.* (1980), was to recognize two genera, *Thamnocalamus* with bracteate racemiform synflorescences and *Sinarundinaria* with open panicles, treating *Fargesia* and *Yushania* as synonyms for *Thamnocalamus* and *Sinarundinaria,* respectively. This treatment was supported by Clayton and Renvoize (1986), Hsueh and Li (1987), and Chao

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1055-7903/01 $35.00

and Renvoize (1989). One of the key issues in this opinion is the status of *Sinarundinaria*. In the original description of *Sinarundinaria,* two specimens of the type species, *S. nitida* (Mitford) Nakai (*Arundinaria nitida* Mitford), were cited; these proved to be different species (Li, 1997b) and thus *Sinarundinaria* should be treated as a synonym of *Fargesia* (Wang and Ye, 1980; Yi, 1985, 1988). Another totally different opinion was held by Wang and Ye (1980), who accepted *Fargesia* and *Yushania* as genera in addition to *Thamnocalamus,* with *Sinarundinaria* as a synonym for *Fargesia*. This opinion was supported by Keng (1983) and Yi (1985, 1988). However, the delimitation of *Fargesia* and *Yushania* was confused, especially when Yi (1988) greatly amended *Fargesia*. Stapleton (1994a) supported the opinion of Wang and Ye (1980) and erected a new genus, *Borinda,* which was somewhat intermediate between *Fargesia* and *Yushania*. Because of confusion surrounding these genera, Soderstrom and Ellis (1988) suggested a "very broad" *Arundinaria* Michaux which included members of the *Thamnocalamus* group.

Morphologically, the allies of the *Thamnocalamus* group have a simple, open, semelauctant synflorescence, without the enclosing spathes seen in *Thamnocalamus* and *Fargesia* or the pulvini common in *Yushania*. These characters, together with the subtropical to warm temperate habitats of their allies, are easily distinguished from the temperate genera of the *Thamnocalamus* group (Stapleton, 1994b). However, the interrelationships of these genera and their relationships to the *Thamnocalamus* group need to be elucidated. Soderstrom and Ellis (1987) recognized *Chimonocalamus, Ampelocalamus,* and *Drepanostachyum,* whereas Clayton and Renvoize (1986) and Chao and Renvoize (1989) included them in a broad interpretation of *Sinarundinaria*. *Himalayacalamus* is a monotypic genus split from *Thamnocalamus* and was recognized by Stapleton (1994b). However, it has been suggested by Soderstrom and Ellis (1987) and supported by Campbell (1991) that *Himalayacalamus* would be better merged with *Drepanostachyum*. *Gaoligongshania* was formerly included in *Arundinaria, Indocalamus, Monocladus,* and *Yushania,* but its several distinct morphological characters and climbing habit distinguish it from those genera and it is considered somewhat intermediate between *Indocalamus* of the *Arundinaria* group and the *Thamnocalamus* group (Li *et al.,* 1995).

As reviewed above, all treatments based on morphological characters have repeatedly proven to be confusing, so it was necessary to study phylogenetic relationships within these controversial genera through other means and methods. In this paper, we explore the molecular approach to provide a new option for clarifying systematic relationships within the *Thamnocalamus* group and its allies. In this study, we considered using the plastic *mat*K gene to reconstruct phylogenetic trees, but, unfortunately, the preliminary results from sequencing showed that this gene is highly conserved in this group and could not provide enough phylogenetic resolution. Therefore, ITS was a most practical option (Baldwin *et al.,* 1995). Divergence between ITS sequences has been used in phylogenetic studies of many families, such as Asteraceae (Baldwin, 1991, 1992; Kim and Jansen, 1994; Kim *et al.,* 1999; Ganders *et al.,* 2000; Vilatersana *et al.,* 2000), Hamamelidaceae (Shi *et al.,* 1999), Polemoniaceae (Ferguson *et al.,* 1999), Apiaceae (Lee and Downie, 1999), Cruciferae (Mayer and Soltis, 1999), and Orchidaceae (Cox *et al.,* 1997). In these families, ITS was proven valuable for examining relationships within genera and among the more closely related genera. ITS has also demonstrated its suitability for clarifying the systematic problems in Poaceae (Hsiao *et al.,* 1994, 1995, 1999) and in bamboos (Renvoize and Hodkinson, 1997; Hodkinson *et al.,* 2000). Zhang *et al.* (1997) used it in a systematic study of bamboo, but was impeded due to the contamination by fungal genomes. In this study, we sterilized the surface of leaves before DNA isolation and obtained ITS sequences of 33 species of bamboo. Our main objectives were (1) to examine the adequacy of ITS sequences for phylogenetic reconstruction in the *Thamnocalamus* group and its allies, (2) to test the monophyly of the *Thamnocalamus* group and explore phylogenetic relationships within it, and (3) to clarify the phylogenetic relationships between the *Thamnocalamus* group and its allies.

MATERIALS AND METHODS

A total of 33 species was sampled. Among them, 31 species represent three genera of the *Thamnocalamus* group, i.e., *Thamnocalamus, Fargesia,* and *Yushania,* and four genera of its allies [*Ampelocalamus, Chimonocalamus, Drepanostachyum* (including *Himalayacalamus*), and *Gaoligongshania*]; 2 species, *Arundinaria gigantea* and *Acidosasa purpurea,* were used as outgroups (Table 1). For practical reasons, we follow the classification scheme of the *Thamnocalamus* group in "Flora Reipublicae Popularis Sinicae" (as FRPS thereafter) (Keng and Wang, 1996) with a few exceptions, e.g., *Acidosasa purpurea* (as *A. hirtiflora*; see Li, 1997a), *Ampelocalamus scandens* and *A. patellaris* (as *Drepanostachyum scandens* and *Dendrocalamus patellaris*; see Stapleton, 1994b), and *Thamnocalamus spathiflorus* var. *crassinodus* (as *Fargesia crassinoda*; see Stapleton, 1994a). Twenty-two species of *Fargesia* represent five series of two sections and 5 species of *Yushania* represent two sections following the intrageneric subdivision of these two genera in FRPS (T. P. Yi in Keng and Wang, 1996).

DNA isolation. Total DNA was extracted from leaves with a modified CTAB procedure (Doyle and

TABLE 1

Taxa and Vouchers for Species Sequenced

Taxon	Voucher No.	GenBank Accession No.
Arundinaria		
Ar. gigantea (Walter) Muhlenberg	ZHG20001	AF305726
Acidosasa		
Ac. purpurea (Hsueh and Yi) P. C. Keng	ZHG109	AF305727
Ampelocalamus		
Am. scandens C. J. Hsuch and W. D. Li	ZHG013	AF280993
Am. patellaris (Gamble) C. M. A. Stapleton	ZHG170	AF280984
Am. actinotrichus (Merr. and Chun) S. L. Chen *et al.*	DZL199904	AF280992
Chimonocalamus		
C. pallens C. J. Hsueh and T. P. Yi	ZHG039	AF305724
Drepanostachyum		
D. hookerianum (Munro) P. C. Keng	DZL199902	AF305725
Gaoligongshania		
G. megalothyrsa (Hand.-Mazz) D. Z. Li *et al.*	JRX9401	AF305728
Thamnocalamus		
T. spathiflorus (Trin.) Munro	Mcbeath19901722	AF305729
T. tessellatus (Nees) T. R. Soderstrom and R. P. Ellis	DZL199901	AF280988
T. spathiflorus var. *crassinodus* (Yi) C. M. A. Stapleton	DZL199902	AF280989
Fargesia		
F. altior T. P. Yi	ZHG008	AF280982
F. porphyrea T. P. Yi	ZHG144	AF280969
F. yunnanensis T. P. Yi	ZHG 014	AF280987
F. sylvestris T. P. Yi	ZHG 017	AF280971
F. fractiflexa T. P. Yi	ZHG 018	AF280972
F. yulongshanensis T. P. Yi	ZHG 019	AF280973
F. frigida T. P. Yi	ZHG 004	AF280976
F. yuanjiangensis C. J. Hsuch and T. P. Yi	ZHG 002	AF280977
F. edulis C. J. Hsueh and T. P. Yi	ZHG 011	AF280978
F. fungosa T. P. Yi	ZHG 016	AF280979
F. communis T. P. Yi	ZHG 010	AF280981
F. hygrophila C. J. Hsueh and T. P. Yi	ZHG 003	AF280983
F. spathacea Franch.	JRX 96049	AF280985
F. murieliae (Gamble) T. P. Yi	DZL19950106	AF280986
F. nitida (Mitford) P. C. Keng ex T. P. Yi	TPY99215	AF280990
F. lushuiensis T. P. Yi	ZHG012	AF302723
F. setosa T. P. Yi	YF & HS323	AF280991
Yushania		
Y. bojieiana T. P. Yi	ZHG040	AF280968
Y. falcatiaurita C. J. Hsueh and T. P. Yi	ZHG 006	AF280970
Y. oblonga T. P. Yi	ZHG 019	AF280974
Y. polytricha C. J. Hsueh and T. P. Yi	ZHG 6	AF280975
Y. niitakayamensis (Hayata) P. C. Keng	MZ 98–291	AF280980

Note. Voucher name abbreviations as follows: DZL stands for collection by De-Zhu Li, ZHG by Zhen-Hua Guo, JRX by Jia-Rong Xue, TPY by Tong-Pei Yi (deposit in SIFS), MZ by Mu Zang, YF by Yong Fei, HS by Hong Sun. Vouchers collected by Jia-Rong Xue are in SWFC, those collected by Tong-Pei Yi are in SIFS, the others are all in KUN.

Doyle, 1987). We used silica-gel-dried or fresh leaves for most accessions, although leaf materials from herbarium specimens were used in some cases. We sterilized the surface of leaves prior to DNA isolation.

PCR amplification. Double-stranded DNA was directly amplified by symmetric PCR amplification with the ITS5 and ITS4 primers of White *et al.* (1990). Reaction volumes were 20 μl and contained 1.5 U Ampli*Taq* DNA polymerase, Replitherm buffer, 1.5 mmol/L $MgCl_2$, 0.4 mmol/L dNTP, 0.1 μmol/L primer, 5% dimethyl sulfoxide, 25–60 ng sample DNA. PCR was performed in a GeneAmp 9600 thermal cycler (Perkin–Elmer, Norolk, CT) and consisted of 30 cycles of 1.5 min at 94°C for template denaturation, 2 min at 55°C for primer annealing, 1 min at 72°C for primer extension, followed by a final extension of 7 min at 72°C. PCR products were purified with Watson's purification kit prior to being sequenced.

DNA sequencing. Double-stranded purified PCR products were sequenced by the dideoxy chain termination method with an ABI PRISM Bigdye Terminator Cycle Sequencing Ready Reaction Kit with Ampli*Taq* DNA polymerase FS (Perkin–Elmer). Reactions and programs were chosen according to the recommenda-

tions of the handbook, with slight modification in some cases. Samples were electrophoresed in an ABI310 automated sequencer. Primers ITS5 and ITS4 were used to sequence all samples, and in case of potential nucleotide site polymorphisms or ambiguous sequences, primer N18L18 (Wen *et al.*, 1996) was also used.

Phylogenetic analysis. 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. In all phylogenetic analyses characters were weighted equally. Maximum-parsimony (MP) analysis was performed with PAUP version 4.0b7 (Swofford, 2000). Because it is now generally accepted that sequence gaps can sometimes provide significant phylogenetic signals, we carried out two different analyses. One treated gaps as missing data and the other as a fifth base. We used the heuristic search option with stepwise data addition (1000 random replications, start seed = 1) and TBR branch-swapping; the shortest trees found were used as input trees for TBR branch-swapping with 1000 random-addition replicates to search for shorter "islands" of trees. To assess the relative support for each clade, bootstrap values were calculated from 1000 replicate analyses with the heuristic search strategy and simple addition sequence of the taxa. A distance tree was constructed with the neighbor-joining (NJ) method (PAUP 4.0).

RESULTS

Sequence Analysis

The entire ITS region, including both spacers and the 5.8S subunit, of 33 species ranged from 588 bp in *Arundinaria gigantea* to 597 bp in *Acidosasa purpurea, Ampelocalamus patellaris,* and *Am. scandens* and is similar to the published sequences of the Poaceae (Hsiao *et al.*, 1994, 1995, 1999; Renvoize and Hodkinson, 1997; Hodkinson *et al.*, 2000). The ITS1 region ranged from 209 bp in *Ar. gigantea* to 217 bp in *Ac. purpurea*. The ITS2 region ranged from 215 bp in *Gaoligongshania megalothyrsa* to 220 bp in three species of *Ampelocalamus*. The ITS1 region was slightly shorter than the ITS2 region. The 5.8S subunit sequence was the most conserved region and was 163 bp long in all species sequenced.

Most indels in the sequences are small (1~3 bp); the largest indels (4~8 bp) were found in *Ar. gigantea, Ac. purpurea, Drepanostachyum hookerianum,* and three species of *Ampelocalamus*. In direct pairwise comparisons of unambiguous positions, sequence divergence between outgroup and ingroup ranged from 2.89% (between *Chimonocalamus pallens* and *Ac. purpurea*) to 5.34% (between *F. yuanjiangensis* and *Ar. gigantea*); divergence within the ingroup ranged from 0 (*F. yulongshanensis* and *F. hygrophila; F. porphyrea* and *Yushania falcatiaurita*) to 4.45% (between *C. pallens* and *G. megalothyrsa*); divergence in the *Thamnocalamus* group ranged from 0 (*F. yulongshanensis* and *F. hygrophila; F. porphyrea* and *Y. falcatiaurita*) to 3.38% (between *F. sylvestris* and *T. spathiflorus*) (Table 2).

TABLE 2

Sequence Characteristics of ITS in 33 Species

Sequence character	ITS
Length variation (bp)	588~597
Length mean (bp)	592.5
No. total aligned positions	607
No. unambiguous indels	15
Sequence divergence between outgoup and ingroup	2.89–5.34%
Sequence divergence within ingroup	0–4.45%
Sequence divergence within *Thamnocalamus* group	0–3.38%

Alignment of 33 sequences resulted in a matrix of 607 alignment positions with introduction of 15 indels. The characteristics of the ITS region of the *Thamnocalamus* group and its allies are shown in Table 2. No evidence of obvious ITS length variants, repeating multiple rDNA repeat types, in any of the accessions analyzed was observed. Four sequence polymorphisms at individual nucleotide sites within individual samples were observed in four samples. The polymorphisms of these sites were unambiguous, so they were included in the analysis. The aligned ITS matrix is available upon request from the corresponding author.

Phylogenetic Analysis

Two different analyses were constructed with different treatments of the unambiguous gaps in MP analysis.

First analysis. The strict consensus of all 20 most parsimonious trees when gaps were treated as missing data is shown with bootstrap values in Fig. 1. Each of these trees had a minimal length of 142 steps, a CI of 0.613, and an RI of 0.701 (Table 3). The *Thamnocalamus* group and its allies were resolved as monophyletic with a bootstrap value of 92, and the basal position of *C. pallens* within the ingroup was resolved. The internal support within the ingroup excluding *C. pallens* was generally low, indicating that the relationships may collapse. A couple exceptions were (1) a clade consisting of all three species sampled in *Ampelocalamus* with a bootstrap value of 91 and (2) the sister relationship of *F. edulis* and *F. fungosa* with a bootstrap value of 84. As shown in Fig. 1, three closely related species of *Fargesia, F. spathacea, F. nitida,* and *F. murieliae,* nested in the basal position of the *Thamnocalamus* group and allies, but they would probably not be monophyletic. The remaining species were divided into two groups, one consisting of *Thamnocalamus spathiflorus* and its variety together with *Gao-*

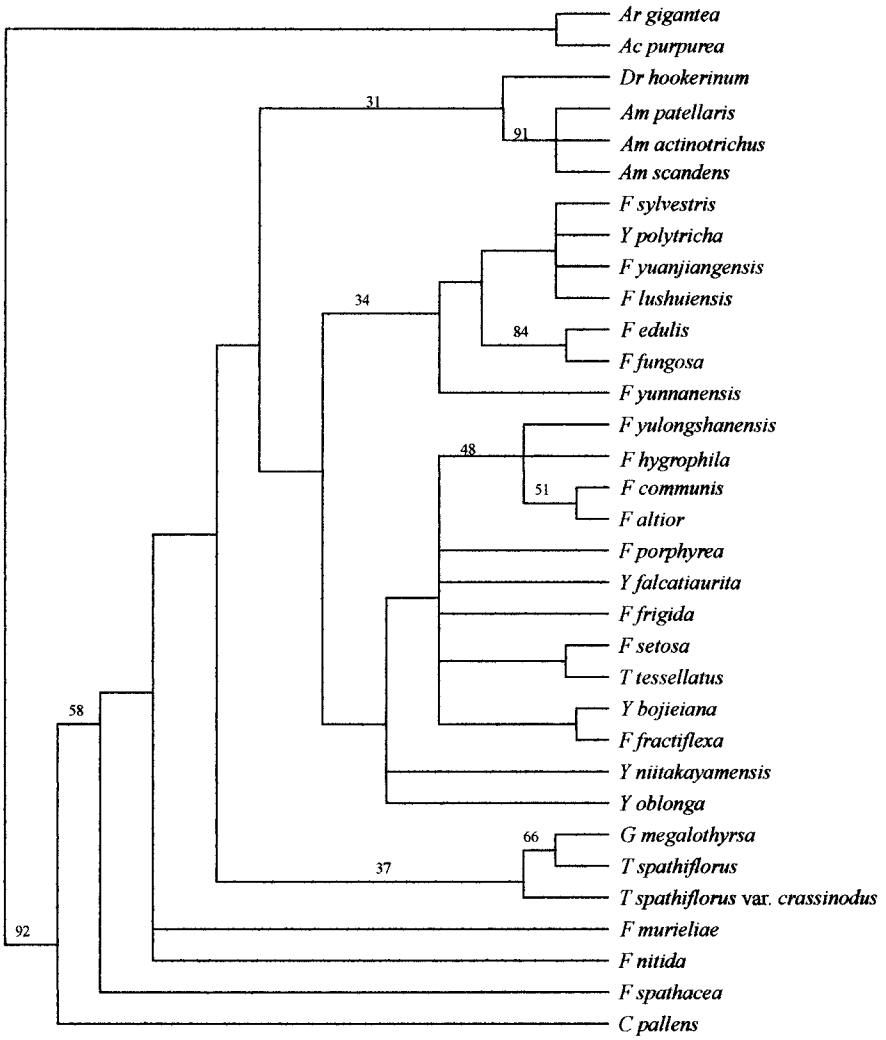

FIG. 1. Strict consensus tree of the 20 most parsimonious trees resulting from a heuristic search with PAUP 4.0, with gaps treated as missing data. Numbers below branches are bootstrap values. (CI = 0.613, RI = 0.701).

ligongshania and the other assembling the *Drepanostachyum hookerianum* and *Ampelocalamus* clade and a heterogeneous clade, which included the African *T. tessellatus*, all species sampled in *Yushania*, and the majority of the *Fargesia* species. Two subclades may be recognized within this clade. One includes *F. yulongshanensis*, *F. hygrophila*, *F. communis*, and *F. altior* with a bootstrap value of 48, named here the *Fargesia communis* subclade; the other includes *F. sylvestris*, *Y. polytricha*, *F. yuanjiangensis*, *F. lushuiensis*, *F. edulis*, *F. fungosa*, and *F. yunnanensis* with a bootstrap value of 34, named here the *Fargesia yunnanensis* subclade.

TABLE 3

Sequence Variation in the ITS Region of 33 Species When Gaps Were Treated as Missing Data

Characteristic	ITS	ITS1	5.8S	ITS2
Constant sites (%)	530 (87.3)	186 (83.8)	159 (97.5)	185 (83.3)
Variable sites (%)	77 (12.7)	36 (16.2)	4 (2.45)	37 (16.7)
Informative sites (%)	39 (6.4)	20 (9.0)	3 (1.84)	16 (7.2)
No. most parsimonious trees	20	—	—	—
Minimal length of most parsimonious tree	142	—	—	—
CI of consensus tree	0.613	—	—	—
RI of consensus tree	0.701	—	—	—

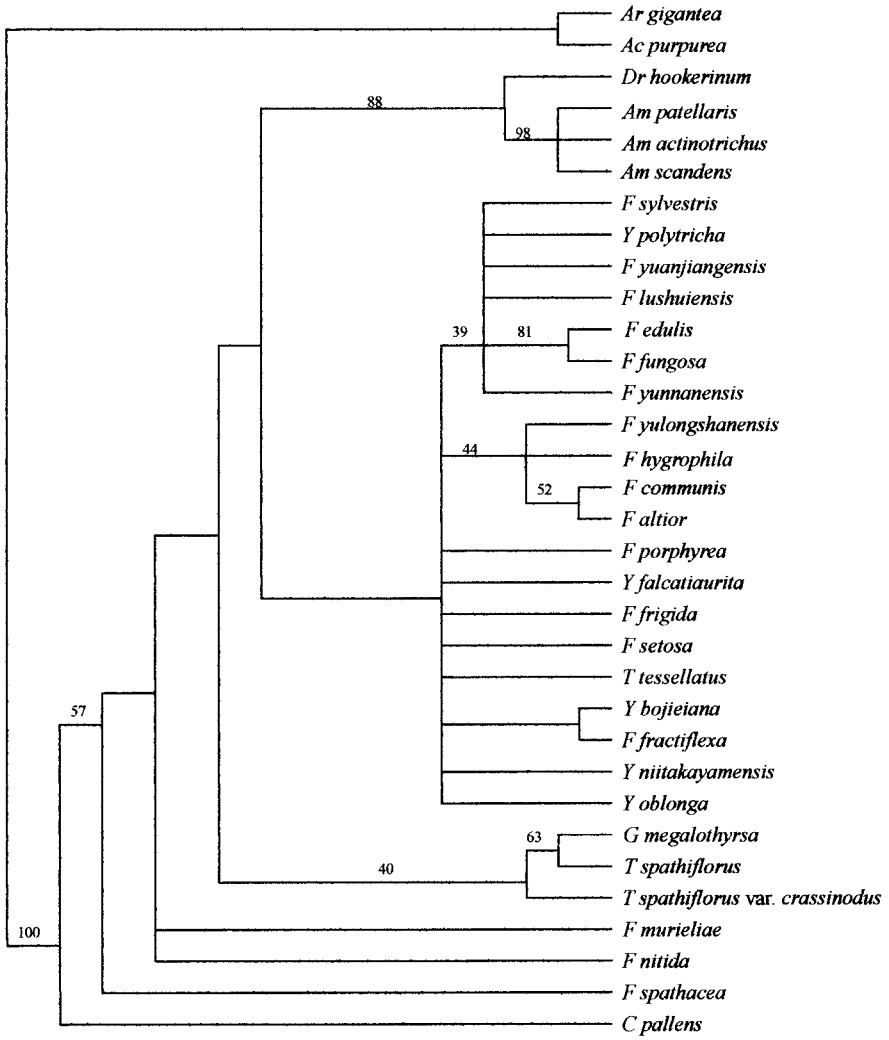

FIG. 2. Strict consensus tree of the 92 most parsimonious trees resulting from a heuristic search with PAUP 4.0, with gaps treated as a fifth base. Numbers below branches are bootstrap values. (CI = 0.646, RI = 0.716).

Second analysis. The strict consensus of all 92 most parsimonious trees when gaps were treated as a fifth base is shown in Fig. 2. Each of these trees had a minimal length of 175 steps, a CI of 0.646, and an RI of 0.716 (Table 4). This topology is generally similar to that generated in the first analysis but with relatively higher internal support. The monophyly of the *Thamnocalamus* group and its allies were supported by a bootstrap value of 100. The basal position of *Chimonocalamus pallens* was again supported. The monophyly of *Ampelocalamus* were resolved with a bootstrap value of 98 and its sister relationship with *Drepanos-*

TABLE 4

Sequence Variation in the ITS Region of 33 Species When Gaps Were Treated as a Fifth Base

Characteristic	ITS	ITS1	5.8S	ITS2
Constant sites (%)	506 (83.4)	168 (75.7)	159 (97.5)	179 (80.6)
Variable sites (%)	101 (16.6)	54 (24.3)	4 (2.45)	43 (19.3)
Informative sites (%)	48 (7.9)	25 (11.3)	3 (1.84)	20 (9.0)
No. most parsimonious trees	92	—	—	—
Minimal length of most parsimonious tree	175	—	—	—
CI of consensus tree	0.646	—	—	—
RI of consensus tree	0.716	—	—	—

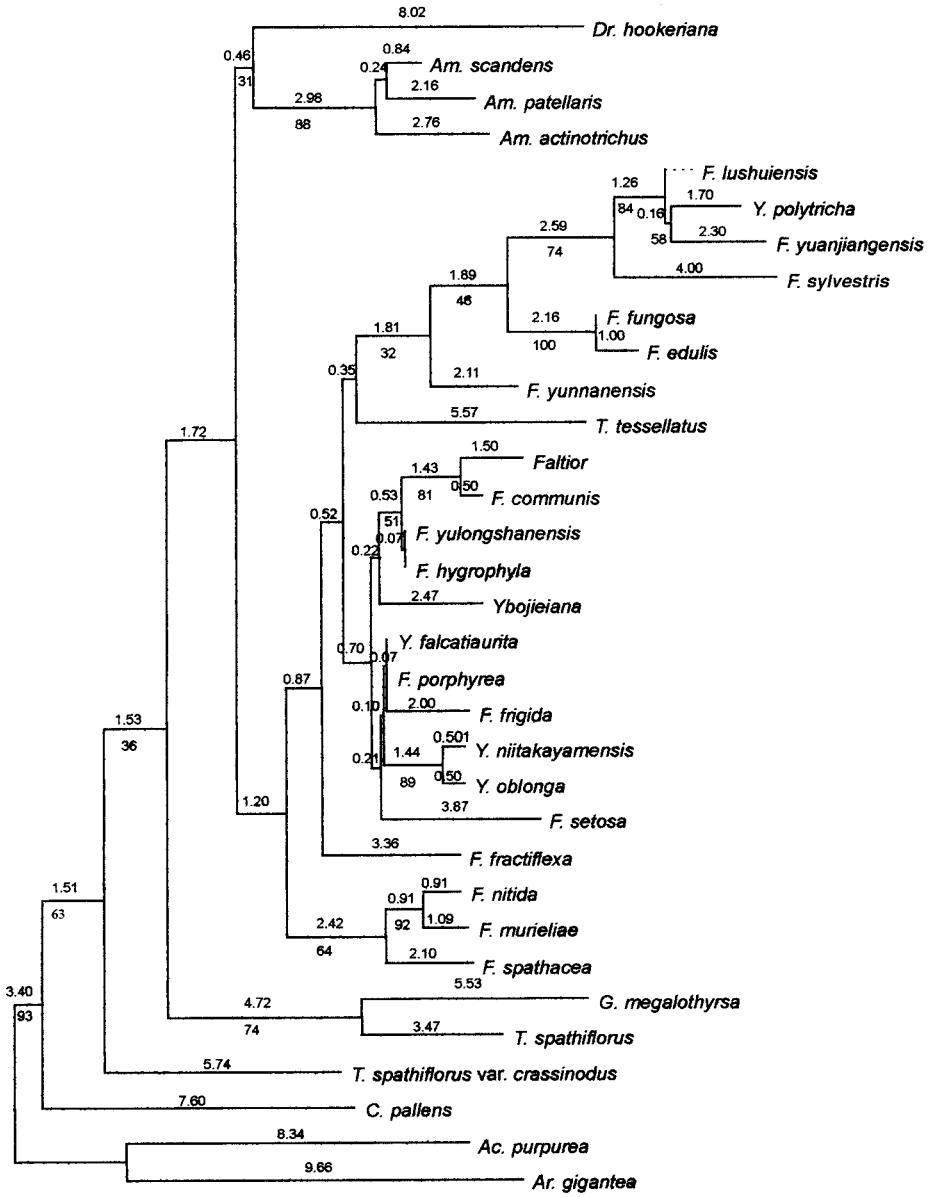

FIG. 3. Neighbor-joining tree inferred from ITS sequence data. Numbers above branches are genetic distance; numbers below branches are bootstrap values.

tachyum hookerianum was supported with a bootstrap value of 88. The the *Fargesia yunnanensis* and *F. communis* subclades also appeared.

Neighbor-joining analysis of the ITS sequences yielded topologies that were somewhat congruent with those from the MP analysis (Fig. 3). In the NJ analysis, the position of *C. pallens* was the same as that in the MP analysis. *Gaoligongshania megalothyrsa*, *Thamnocalamus spathiflorus*, and its variety formed the second phenon rather than allying in the *Fargesia spathacea* phenon. Among the rest, *Drepanostachyum hookerianum* and the *Ampelocalamus* clade diverged from the other species first; second, *Fargesia spathacea*, *F. murieliae*, and *F. nitida* diverged as a monophyletic phenon; third, in the remaining set, *F. fractiflexa* separated first and the other species divided into two phenons, which are similar to those in the first MP analysis except for the exclusion of *F. fractiflexa* from the *Fargesia communis* subclade *s.l* and the inclusion of *Thamnocalamus tessellatus* in the *Fargesia yunnanensis* subphenon.

DISCUSSION

The ITS sequence data of the *Thamnocalamus* group and its allies provided some valuable information,

given the lack of resolution obtained in other studies with plastid genes or introns. However, the bootstrap values of the trees were generally low. This may be due to low character support for these branches; base substitution of the ITS region is generally low in woody bamboos (Hodkinson *et al.*, 2000).

The Monophyly of the Thamnocalamus Group and Its Allies

The ITS phylogeny in this study supported the *Thamnocalamus* group and its allies as a monophyletic clade with a bootstrap value of 92–100 when *Arundinaria gigantea* and *Acidosasa purpurea* were used as outgoups. This clade is also distinguished by the presence of pachymorph rhizomes; other genera of the Arundinariinae have leptomorph rhizomes. Though both types of rhizomes are produced in the New World genus of *Chusquea,* and may be an ecological strategy for habitats (Clark, 1997), rhizome type may be a good criterion for the definition of some bamboo genera or clades.

The Basal Position of the Chimonocalamus

Chimonocalamus Hsueh et Yi (1979) bears three branches at each node with a ring of spiny roots, which is similar to *Chimonobambusa* of the *Shibataeinae,* and bears the same synflorescence type as *Yushania*. Soderstrom and Ellis (1987) and FRPS (Keng and Wang, 1996) recognized it as a good genus, whereas Chao and Renvoize (1989) treated it as a section of *Sinarundinaria*.

The ITS phylogenetic trees indicate that it diverged earliest from all sampled species with pachymorph rhizomes and appears as a remotely related sister to the remainder of the *Thamnocalamus* group and its allies. It is therefore better to treat it as a distinct genus. The results of the ITS sequence analysis may cause us to reevaluate the phylogenetic position of *Chimonocalamus*. It is similar vegetatively to *Chimonobambusa* and to some genera in the *Arundinaria* group, such as *Arundinaria* and *Acidosasa*. These genera all have leptomorph rhizomes. The simple open semelauctant synflorescence of *Chimonocalamus* is also similar to those of *Arundinaria* and *Yushania*. However, *Chimonocalamus* is distributed at higher elevations and has warm temperate habitats similar to those of some genera in the *Arundinaria* group.

The Monophyly of Ampelocalamus and Its Relationship with Drepanostachyum

Ampelocalamus has a lax synflorescence and sometimes scrambling culms with a reiterative central branch, which is able to replace the main culm. Soderstrom and Ellis (1987) and Stapleton (1994b) recognized *Ampelocalamus,* although Clayton and Renvoize (1986) and Chao and Renvoize (1989) included it in *Sinarundinaria*. Keng (1986) and Yi (1993) restricted *Ampelocalamus* to the type and *Am. calcareus,* referring the other six species to *Drepanostachyum*. ITS phylogenetic trees supported the three species sampled in *Ampelocalamus* as monophyletic and confirmed *Ampelocalamus* as a good genus. *Ampelocalamus patellaris* certainly belongs to *Ampelocalamus* in the ITS phylogeny, although it differs in vegetative characters from the other scrambling species.

Drepanostachyum has a lax falcate synflorescence and occurs in habitats similar to those of *Ampelocalamus,* which is regarded as a related genus (Li *et al.*, 1996). In the ITS phylogeny, the sister relationship between *Drepanostachyum hookerianum* and the *Ampelocalamus* clade was constant in three analyses, although the internal support for the group was generally low.

The Polyphyly and/or Paraphyly of the Thamnocalamus Group and Genera in the Group

The ITS phylogeny indicated that the *Thamnocalamus* group as currently defined is polyphyletic and heterogeneous, although internal support of the relationships were generally low. In this group, four more or less similar assemblages were resolved by different methods. The relationships among the four clades were uncertain because there was low bootstrap support, but the two assemblages with bracteate racemiform synflorescence may be basal according to the topologies yielded by the different methods.

The Fargesia spathacea, F. nitida, and F. murieliae assemblage. The three species are very closely related and their interrelationship was confusing (see Soderstrom, 1979b; Li, 1997b). The monophyly of *F. nitida* and *F. murieliae* was resolved in the NJ analysis with a bootstrap support of 92. The three species appeared to be monophyletic in the NJ analysis with a bootstrap support of 64 and basal in the MP analyses with low support. Morphologically, all three species have bracteate racemiform synflorescence and three stigmas, and they differ from the general condition of two stigmas in other species of *Fargesia* (or *Borinda*) and *Yushania*. Soderstrom (1979b) regarded *F. spathacea* and *F. murieliae* conspecific and named them *Thamnocalamus spathaceus* because of their similarity of synflorescence and pachymorph rhizome. In addition, Stapleton (1995) considered *F. nitida* conspecific with *F. spathacea* when it flowered in 1993. Wang and Ye (1980) and Yi (1988) recognized three species and *Fargesia* as a genus due to its series of bracts and terminal synflorescence as opposed to one large bract and lateral synflorescence at the nodes of flowering branches of *Thamnocalamus*. The ITS phylogeny indicates that this clade and the *Thamnocalamus* clade are potentially basal in the *Thamnocalamus* group, but the relationships between them have low bootstrap values, which supports the opinions of Wang and Ye (1980) and Yi (1988) to some extant. However, the delimita-

tion of *Fargesia* and *Yushania* and the relationships between the *Fargesia spathacea* clade and the *Thamnocalamus* clade also remain to be resolved.

The Thamnocalamus spathiflorus and Gaoligongshania assemblage. This assemblage includes *Thamnocalamus spathiflorus, T. spathiflorus* var. *crassinodus,* and the monotypic *Gaoligongshania* and appeared to be basal in the NJ analysis and to be monophyletic in a basal position in the MP analyses. The relationship of African *T. tessellatus* was variable in the three topologies but its relationship with the Himalayan *T. spathiflorus,* the type species, as suggested by Soderstrom and Ellis (1982), was not resolved. The Himalayan *Thamnocalamus* plus *Gaoligongshania* may be a natural group but it has some relationships with the *Fargesia yunnanensis* and *Fargesia communis* assemblages.

Gaoligongshania is a newly recognized monotypic genus distributed in northwest Yunnan. Its semelauctant synflorescences, pachymorph rhizomes, solitary midculm branch, which is as thick as the culm, and epiphytic habitat distinguish it from the other genera. However, in the ITS phylogenetic trees, *Gaoligongshania* was unexpectedly resolved as sister to *Thamnocalamus spathiflorus* in all our analyses, with a comparatively high (63–74) bootstrap support. *Thamnocalamus* has five to nine subequal branches and a bracteate racemiform synflorescence rather than the single branch and open paniculate synflorescence in *Gaoligongshania*. Incongruence existing between the molecular-based phylogenies and the relationships inferred from morphological characters is frequently encountered in studies of bamboo (Zhang *et al.*, 1995; Zhang, 1996). Zhang (1996) examined this problem in more detail, but one obstacle is the lack of good morphological and anatomical data for a number of bamboo taxa. As to the position of *Gaoligongshania,* more molecular and morphological studies are required.

The Fargesia yunnanensis assemblage. This assemblage includes *F. sylvestris, Y. polytricha, F. yuanjiangensis, F. yunnanensis, F. edulis, F. lushuiensis,* and *F. fungosa* and is recovered in all analyses, but with a low bootstrap support. However, the sister relationship of *F. edulis* and *F. fungosa* was resolved in all analyses with high internal support. If this assemblage represents a true monophyletic subclade, it would be very heterogeneous in morphological characters. *F. yunnanensis* and *Y. polytricha* have very open paniculate synflorescences (with no bract) and long-necked rhizomes, whereas *F. edulis, F. fungosa,* and *F. yuanjiangensis* have slightly open paniculate synflorescences (with some bracts at the base) and shorter-necked rhizomes. The common character that this subclade has is two stigmas, regardless of whether they were placed in *Fargesia, Borinda,* or *Yushania*. This result suggests that the number of stigmas may be a useful character for the delimitation of *Fargesia* and *Yushania*.

The Fargesia communis assemblage. This includes *F. altior, F. communis, F. yulongshanensis,* and *F. hygrophila* in all analyses and was supported by a bootstrap value of 51 in the NJ analysis. The subclade has short pachymorph rhizomes but the synflorescence type is unknown.

The *F. yunnanensis* and *F. communis* subclades include species of *Fargesia, Yushania,* or *Borinda,* which have confusing morphological characters. In these species, the length of rhizomes and synflorescence are the main characters for classification; however, there are many intermediate species between them. Yi (1986, 1988) and Stapleton (1994a) delimit *Fargesia* as having rhizome necks shorter than 20–25 cm, synflorescence substended by a series of large or small bracts at the base, and no pulvini, whereas *Yushania* has rhizome necks longer than 20–25 cm, synflorescence without bracts, and many pulvini. *Borinda* is intermediate between *Fargesia* and *Yushania*. Although this result remains to be confirmed by other data, it is suggested that the delimitation between Yi's (1988) *Fargesia* and *Yushania,* based on length of culm necks and between Stapleton's (1994) *Borinda* and *Yushania,* on the same basis, were questionable.

ACKNOWLEDGMENTS

We are grateful to Phillip Thomas of Royal Botanic Garden Edinburgh for providing materials of *Thamnocalamus spathiflorus* and *Thamnocalamus spathiflorus* var. *crassinodus,* Jia-Rong Xue of Southwest Forest University, Kunming for help in collecting materials of *Fargesia* and *Yushania,* and Su-Hua Shi of Zhongshan University, Guangzhou for providing the original amplification primers. We are also indebted to two anonymous reviewers for revising an early version of the paper. This work was supported by a special grant from the National Natural Science Foundation of China (NSFC 39725001 to De-Zhu Li) and through Hundreds of Talents Program of the Chinese Academy of Sciences (1998-0054).

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