J. Plant Res. **114**: 315-322, 2001

Journal of Plant Research

Genetic Variation and Evolution of the Alpine Bamboos (Poaceae: Bambusoideae) using DNA Sequence Data

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Difficulties in phylogenetic reconstruction are common within the woody bamboos due to their unique life cycles, a lack of morphological characters and even an absence of molecular evidence. The genetic variability and population structure of the bamboos are also poorly understood which impedes their exploitation via breeding. In this paper, ITS sequence data were used to examine the degree of genetic variation within the alpine bamboos and to explore their phylogeny. Twenty-three species representing three genera, *Thamnocalamus*, *Fargesia* and *Yushania*, of the alpine bamboos and one species of *Ampelocalamus* as an outgroup were studied. The results indicated that *Thamnocalamus spathiflorus* var. *crassinodus* and the *Fargesia spathacea* clade form the basal groups but bootstrap support was weak. Among the rest of the species, including species previously placed in *Fargesia* (plus *Borinda*) and *Yushania*, the *F. yunnanensis* subclade and the *F. communis* subclade were recognized but internal support for such groups was again low. The result indicated that, *Fargesia* and *Yushania* as delimited by morphological characters, are not monophyletic in the ITS phylogeny and require further resolution. We revealed relatively high levels of genetic variability in the alpine bamboos and showed that the ITS region could be used to improve generic delimitation of the woody bamboos in general.

Key words: Alpine bamboos — Evolution — Genetic variation — ITS Phylogeny

The "alpine bamboos" as a phrase first appeared in some Chinese literature on bamboo taxonomy of *Fargesia*, *Sinarundinaria* and *Yushania* (Chao *et al.* 1980, Wang and Ye 1980). Although this phrase has been widely used ever since (Yi 1985, 1988, Hsueh and Li 1987, Keng 1992, Wang 1997), there is no clear circumscription for it. In this study, we restricted our analysis to three genera of subtribe Thamnocalaminae (Keng 1992), namely *Thamnocalamus*, *Fargesia* (including *Sinarundinaria* and *Borinda*) and *Yushania*. The alpine bamboos are distributed in mountainous areas, especially in southwest China. They produce pachymorph rhizomes, semelauctant synflorescences, and comprise the core of the *Thamnocalamus* group (Li 1997). In these bamboos, the length of culm-necks and the type of synflorescence are important characters for classification. However, delimitation among the three genera is not always clear because the length of culm necks and the degree of spathe subtending the synflorescence as observed in the field are variable. For these reasons, two main opinions are held by taxonomists: treat *Fargesia* as a synonym of *Thamnocalamus* and *Yushania* as a synonym of *Sinarundinaria* (Soderstrom 1979a, b, Soderstrom and Ellis 1982, Chao *et al.* 1980, Clayton and Renvoize 1986, Hsueh and Li 1987, Chao and Renvoize 1989); or recognize *Fargesia* and *Yushania* as genera (proposed by Wang and Ye 1980 and supported by Yi 1988 and Keng and Wang 1996). In addition, Stapleton (1994) split *Borinda* from *Fargesia* and transferred Himalayan species of *Fargesia* to this genus, but further studies are needed to investigate its status.

In spite of the controversy in classification, the alpine bamboos have important economic value and are the main food of giant pandas and many other animals, including the red panda. Unfortunately, we know little about the genetic variability and population structure of these species, although this information is vital for understanding evolution and ecology and for managing forests for harvest. Because the vegetative characters used for identification are likely to be subtle and variable in most cases, it is difficult to say how much they reflect the true evolutionary history of the organisms.

To overcome some of these problems, we applied modern molecular techniques to explore the genetic variability and evolution in alpine bamboos. Many molecular approaches, such as RAPD (Random Amplified Polymorphism DNA), AFLPs (Amplified Fragment Length Polymorphisms) and RFLPs (Restriction Fragment Length Polymorphisms) have been used in studies of bamboo genetic variability (Friar and Kochert 1994, Lai and Hsiao 1997, Hodkinson *et al.* 2000). In recent years, DNA sequencing has been used in phylogenetic studies as well as in analyses of polymorphisms for other purposes (Palumbi and Benzie 1991, Qiu *et al.* 2000). We selected the ITS (internal transcribed spacer) region

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between 26SrDNA and 18S rDNA since it has relatively high nucleotide sequence variability and can be used to resolve lower-level phylogenetic issues in angiosperms (Baldwin *et al.* 1995). The ITS region has also proved suitable for clarifying the systematic problems in Poaceae (Hsiao *et al.* 1994, 1995, 1999) and in bamboos (Renvoize and Hodkinson 1997, Hodkinson *et al.* 2000). In this paper, we explore the utility of this region in studies on genetic variation and evolution in the alpine bamboos.

Materials and Methods

Collection

Twenty-three species representing three genera of the alpine bamboos and one species of *Ampelocalamus* as an outgroup were collected from natural populations in China, except two species collected in the UK (Table 1). Vouchers are deposited in the herbarium of Kunming Institute of Botany (KUN). Voucher numbers and Genbank accession numbers for sequences are shown in Table 1. We follow the classification scheme of alpine bamboos in *Flora Reipublicae Popularis Sinicae* (Keng and Wang 1996) except for *Thamnocalamus spathiflorus* var. *crassinodus* (=*Fargesia crassinoda*, see Stapleton 1994).

DNA isolation

We used silica-gel-dried or fresh leaves for most accessions although herbarium specimens were also used in some cases. Total DNA was extracted using a modified CTAB procedure of Doyle and Doyle (1987). The DNA was precipitated using 2-propanol for half an hour at −20 C, and washed with 70% ethanol two times and with 100% ethanol one time. The concentrated DNA was then stored in TE buffer (10 mM Tris-HCl; 1 mM EDTA; pH 8.0) at −20 C until use.

PCR amplification

Double-stranded DNA was directly amplified by PCR using the ITS5 and ITS4 primers of White *et al.* (1990). Reaction volumes (20 ul) contained 1.5 U of AmpliTaq DNA polymerase (Perkin-Elmer), Replitherm TM buffer, 1.5 mM $MgCl_2$, 0.4 mM dNTP, 0.1 mM primer, 5% DMSO and 25-60 ng of sample DNA. PCR reactions were performed in a GeneAmp 9600 (Perkin-Elmer, Applied Biosystems) and consisted of 30 cycles of 1.5 min at 94 C for template denaturation, 2 min at 55 C for primer annealing, and 1 min at 72 C for primer

Table 1. Taxa and vouchers used in study

Taxon[1]	Voucher number[2]	Source[3]	GenBank#
Ampelocalamus actinotrichus (Merr. et Chun) S.L.Chen *et al.*	DZL 199904	Hainan, China	AF280992
Thamnocalamus spathiflorus var. *crassinodus* (Yi) Stapleton	PT 199902	RBGE, UK	AF280989
Fargesia altior Yi	ZHG 008	Yunnan, China	AF280982
F. communis Yi	ZHG 010	Yunnan, China	AF280981
F. edulis Hsueh et Yi	ZHG 011	Yunnan, China	AF280978
F. fractiflexa Yi	ZHG 018	Yunnan, China	AF280972
F. frigida Yi	ZHG 004	Yunnan, China	AF280976
F. fungosa Yi	ZHG 016	Yunnan, China	AF280979
F. hygrophila Hsueh et Yi	ZHG 003	Yunnan, China	AF280983
F. lushuiensis Yi	ZHG 012	Yunnan, China	AF280994
F. murieliae (Gamble) Yi	DZL 19950106	Cambridge, UK	AF280986
F. nitida (Mitford) Keng f. ex Yi	TPY 99215	Sichuan, China	AF280990
F. porphyrea Yi	ZHG 144	Yunnan, China	AF280969
F. setosa Yi	DZL *et al.* 323	Xizang, China	AF280991
F. spathacea Franch.	JRX 96049	Sichuan, China	AF280985
F. sylvestris Yi	ZHG 017	Yunnan, China	AF280971
F. yuanjiangensis Hsueh et Yi	ZHG 002	Yunnan, China	AF280977
F. yulongshanensis Yi	ZHG 019	Yunnan, China	AF280973
F. yunnanensis Yi	ZHG 014	Yunnan, China	AF280987
Yushania bojieiana Yi	ZHG 040	Yunnan, China	AF280968
Y. falcatiaurita Hsueh et Yi	ZHG 006	Yunnan, China	AF280970
Y. niitakayamensis (Hayata) Keng f.	MZ 98-291	Taiwan, China	AF280980
Y. oblonga Yi	ZHG 019	Yunnan, China	AF280974
Y. polytricha Hsueh et Yi	ZHG No.6	Yunnan, China	AF280975

[1] Taxonomic names are based on the scheme in Flora Reipublicae Popularis Sinicae (Keng and Wang 1996) except *Thamnocalamus spathiflorus* var. *crassinodus* (=*Fargesia crassinodus*, see Stapleton 1994).

[2] Voucher name abbreviations: DZL stands for collection by De-Zhu Li, PT by Philip Thomas, ZHG by Zhen-Hua Guo, JRX by Jia-Rong Xue, TPY by Tong-Pei Yi, MZ by Mu Zang.

[2] RBGE represents Royal Botanical Garden Edinburgh.

extension, followed by a final extension for 7 min at 72 C. PCR products were purified on Watson's PCR mini-columns prior to sequencing.

DNA Sequencing

Double-stranded purified PCR products were sequenced using the Dideoxy Chain Termination method with an ABI PRISM™ Bigdye Terminator Cycle Sequencing Ready Reaction Kit and AmpliTaq DNA polymerase FS (Perkin-Elmer, Norfolk, Connecticut). Reactions and programs were chosen according to the recommendations of the manufacturers, with slight modification in some cases. Samples were eletrophoresed on an ABI 310 automated sequencer. Primers ITS5 and ITS4 Primers were used to sequence all samples, and in the case of ambiguous sequences, a third primer N18L18 (Wen and Zimmer 1996) was also used.

Phylogenetic analyses

Base determination was complete and unambiguous in nearly all cases. DNA sequences were edited and aligned using SeqMan and Megalign (DNASTAR), and adjusted manually where necessary. The alignment of the entire ITS region of the 23 species sampled in this study is available upon request. In all phylogenetic analyses, characters were weighted equally. Maximum parsimony (MP) analysis was performed using PAUP version. 4.0 b5 (Swofford 2000) treating gaps as missing data using the heuristic search options with 1000 random replications of stepwise data addition and TBR branch-swapping. Tree fit measures from the MP analysis were calculated using consistency (CI), retention (RI) and re-scaled consistency (RC) indices. A genetic distance tree was also constructed using the neighbor-joining (NJ) method (Swofford 2000). *Ampelocalamus actinotrichus* was used as an outgroup in both analyses.

Results

Polymorphism analysis

The entire ITS region including both spacers and the 5.8S subunit of 23 species of alpine bamboos was obtained and ranged from 592 bp to 596 bp in length, similar to the published sequences of Poaceae (Hsiao *et al*. 1994, 1995, 1999, Renvoize and Hodkinson 1997). In 599 alignment characters, we detected 54 polymorphic nucleotide sites (9.02%) and 22 different genotypes (*Fargesia yulongshanensis* and *F. hygrophila* had the same genotype). The ITS1 region ranged from 213 bp to 215 bp. There were 25 polymorphic sites (11.6%) in 216 alignment characters. The ITS2 region ranged from 216 bp to 219 bp and had 26 polymorphic sites (11.8%) in 220 alignment characters. The ITS1 region was thus 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 with 3 polymorphic sites (1.8%).

In the two ITS spacers, the sequence divergence (% difference) between genera ranged from 0 (between *Fargesia porphyrea* and *Yushania falcatiaurita*, and between *F. yulongshanensis* and *F. hygrophila*) to 4.2% (between *Thamnocalamus spathiflorus* var. *crassinodus* and *F. yuanjiangensis*), while divergence within genera ranged from 0 (between *F. yulongshanensis* and *F. hygrophila*) to 3.5% (between *F. yuanjiangensis* and *F. setosa*) (Table 2).

MP analysis

Nineteen variable sites (3.2%) were parsimony-informative in the entire ITS region with seven sites (3.2%) in ITS1, ten sites (4.5%) in ITS2, and two sites (1.2%) in 5.8S rDNA. ITS2 had more parsimony-informative sites than ITS1 and 5.8S rDNA.

The 50% majority consensus of all 170 most parsimonious trees is shown in Fig. 1. Each of these trees had a minimal length of 71 steps, a CI of 0.718, an RI of 0.770 and an RC of 0.553. The ITS phylogenetic tree indicated that *Thamnocalamus spathiflorus* var. *crassinodus* was sister to all the other alpine bamboos sampled in this study. *Fargesia spathacea*, *F. murieliae* and *F. nitida* formed a separate monophyletic group (the *Fargesia spathacea* clade). However, the basal position of both the *Thamnocalamus spathiflorus* var. *crassinodus* and the *Fargesia spathacea* clade are not supported by bootstrap analysis (bootstrap values less than 38). Among the rest of the species of alpine bamboos, *Fargesia fractiflexa* was sister to a further clade, which was divided into two groups. The first group included *Fargesia altior*, *F. communis*, *F. hygrophila* and *F. yulongshanensis* (the *Fargesia communis* subclade). In the second group, *F. sylvestris*, *Yushania polytricha*, *F. yuanjiangensis*, *F. yunnanensis*, *F. edulis*, *F. lushuiensis* and *F. fungosa* were monophyletic (the *Fargesia yunnanensis* subclade). Within this group, *Yushania bojieiana* grouped with the *Fargesia yunnanensis* subclade but the bootstrap support is very low; *Yushania niitakayamensis* grouped with *Yushania oblonga*, while *Fargesia porphyrea*, *Yushania falcatiaurita*, *F. frigida* and *F. setosa* were unresolved. Bootstrap support for both the *Fargesia communis* and *Fargesia yunnanensis* subclades is, however, weak indicating that these groupings may not be real. The clades and/or subclades with bootstrap values higher than 50 included the *F. spathacea* clade and *F. yunnanensis* subclade. For details of the value of support, refer to Fig. 1 (numbers below the branches).

NJ analysis

Neighbor-joining analysis of ITS sequences yielded overall topologies (Fig. 2) that were similar to those from MP analysis. The NJ tree differed from MP tree in the following aspects. Firstly, *Thamnocalamus spathiflorus* var. *crassinodus* and the *Fargesia spathacea* clade were resolved as a monophyletic basal clade. Secondly, *Yushania bojieiana* separated from the *Fargesia yunnanensis* subclade. Thirdly, *Fargesia porphyrea*, *Yushania falcatiaurita*, *F. frigida* and *F. setosa* were grouped as a monophyletic group but with little internal support. This group, together with *Yushania niitakayamensis* and *Y. oblonga*, was sister to the *Fargesia communis* subclade. It was comprised of the *Fargesia communis* subclade *sensu* lato with no bootstrap support, to which *Yushania bojieiana* was sister. The bootstrap values of the NJ tree were generally higher for the NJ tree than the

Table 2. ITS pairwise distances between alpine bamboos. Total character differences are indicated at lower diagonal, and mean character differences (adjusted for missing data) at upper diagonal.

Taxon[1)]	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
1 FALTI	—	0.005	0.023	0.016	0.012	0.021	0.005	0.026	0.021	0.021	0.007	0.016	0.019	0.030	0.033	0.005	0.019	0.035	0.012	0.007	0.009	0.009	0.033
2 FCOMM	2	—	0.021	0.014	0.009	0.019	0.002	0.023	0.019	0.019	0.005	0.014	0.016	0.028	0.030	0.002	0.016	0.033	0.009	0.005	0.007	0.007	0.030
3 FEDUL	10	9	—	0.026	0.021	0.002	0.019	0.014	0.026	0.026	0.016	0.026	0.019	0.021	0.023	0.019	0.019	0.035	0.023	0.016	0.019	0.019	0.019
4 FFRAC	7	6	11	—	0.014	0.023	0.012	0.023	0.019	0.016	0.009	0.019	0.016	0.033	0.033	0.012	0.019	0.033	0.012	0.009	0.012	0.012	0.033
5 FFRIG	5	4	9	6	—	0.019	0.007	0.023	0.019	0.019	0.005	0.014	0.016	0.033	0.030	0.007	0.016	0.033	0.012	0.005	0.007	0.007	0.030
6 FFUNG	9	8	1	10	8	—	0.016	0.012	0.023	0.023	0.014	0.023	0.016	0.019	0.021	0.016	0.016	0.033	0.021	0.014	0.016	0.016	0.016
7 FHYGR	2	1	8	5	3	7	—	0.021	0.016	0.016	0.002	0.012	0.014	0.026	0.028	0.000	0.014	0.030	0.007	0.002	0.005	0.005	0.028
8 FLUSH	11	10	6	10	10	5	9	—	0.026	0.023	0.019	0.028	0.023	0.007	0.005	0.021	0.007	0.035	0.021	0.019	0.021	0.021	0.002
9 FMURI	9	8	11	8	8	10	7	11	—	0.005	0.014	0.023	0.007	0.033	0.035	0.016	0.021	0.026	0.021	0.014	0.016	0.016	0.030
10 FNITI	9	8	11	7	8	10	7	10	2	—	0.014	0.023	0.007	0.030	0.033	0.016	0.019	0.026	0.019	0.014	0.016	0.016	0.028
11 FPORP	3	2	7	4	2	6	1	8	6	6	—	0.009	0.012	0.028	0.026	0.002	0.012	0.028	0.007	0.000	0.002	0.002	0.026
12 FSETO	7	6	11	8	6	10	5	12	10	10	4	—	0.021	0.037	0.035	0.012	0.021	0.033	0.016	0.009	0.012	0.012	0.035
13 FSPAT	8	7	8	7	7	7	6	10	3	3	5	9	—	0.030	0.033	0.014	0.023	0.026	0.019	0.012	0.014	0.014	0.028
14 FSYLV	13	12	9	14	14	8	11	3	14	13	12	16	13	—	0.019	0.026	0.019	0.040	0.028	0.028	0.030	0.030	0.014
15 FYUAN	14	13	10	14	13	9	12	2	15	14	11	15	14	8	—	0.028	0.014	0.042	0.023	0.026	0.028	0.028	0.009
16 FYULO	2	1	8	5	3	7	0	9	7	7	1	5	6	11	12	—	0.014	0.030	0.007	0.002	0.005	0.005	0.028
17 FYUNN	8	7	8	8	7	7	6	3	9	8	5	9	10	8	6	6	—	0.030	0.014	0.012	0.014	0.014	0.014
18 TSPAV	15	14	15	14	14	14	13	15	11	11	12	14	11	17	18	13	13	—	0.035	0.028	0.030	0.030	0.040
19 YBOJI	5	4	10	5	5	9	3	9	9	8	3	7	8	12	10	3	6	15	—	0.007	0.009	0.009	0.028
20 YFALC	3	2	7	4	2	6	1	8	6	6	0	4	5	12	11	1	5	12	3	—	0.002	0.002	0.026
21 YNIIT	4	3	8	5	3	7	2	9	7	7	1	5	6	13	12	2	6	13	4	1	—	0.002	0.028
22 YOBLO	4	3	8	5	3	7	2	9	7	7	1	5	6	13	12	2	6	13	4	1	1	—	0.028
23 YPOLY	14	13	8	14	13	7	12	1	13	12	11	15	12	6	4	12	6	17	12	11	12	12	—

[1)] Taxon abbreviations: FALTI, *Fargesia altior*; FCOMM, *F. communis*; FEUL, *F. edulis*; FFRAC, *F. fractiflexa*; FFRIG, *F. frigida*; FFUNG, *F. fungosa*; FHYGR, *F. hygrophila*; FLUSH, *F. lushuiensis*; FMURI, *F. murieliae*; FPORP, *F. porphyrea*; FSETO, *F. setosa*; FSPAT, *F. spathacea*; FSYLV, *F. sylvestris*; FYUAN, *F. yuanjiangensis*; FYULO, *F. yulongshanensis*; FYUNN, *F. yunnanensis*; TSPAV, *Thamnocalamus spathiflorus* var. *crassinodus*; YBOJI, *Yushania bojieiana*; YFALC, *Y. falcatiaurita*; YNIIT, *Y. niitakayamensis*; YOBLO, *Y. oblonga*; YPOLY, *Y. polytricha*.

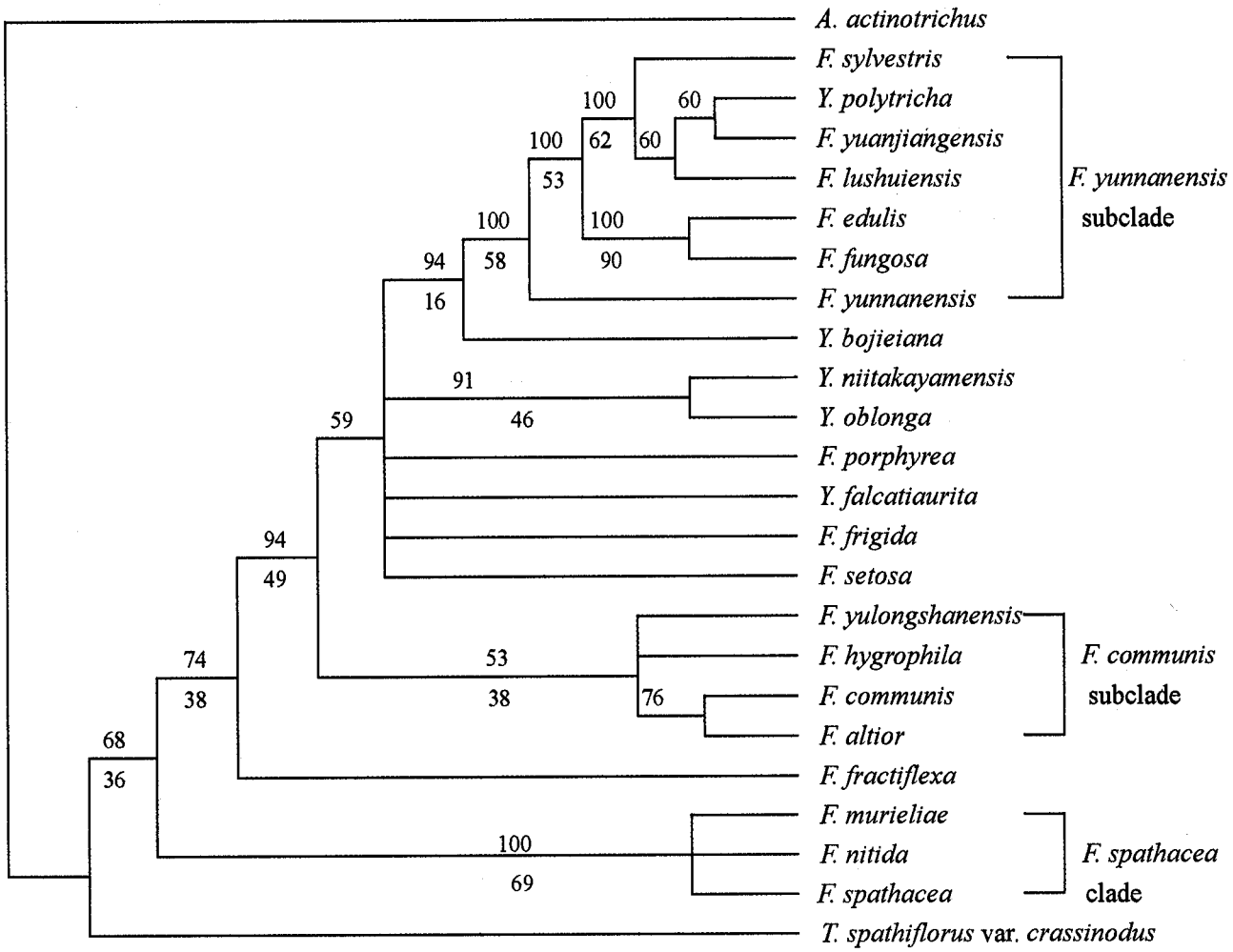

Fig. 1. 50% majority consensus tree of the 170 most parsimonious trees of the alpine bamboos, resulting from a heuristic search. Values above branches are combinable component percentages; numbers below branches are bootstrap values (CI=0.718, RI=0.770, RC=0.553).

MP tree. The clades and/or subclades with bootstrap values higher than 50 included the *F. spathacea* clade, the *F. communis* and *F. yunnanensis* subclades, and the *Y. niitakayamensis* and *Y. oblonga* group (Fig. 2).

Discussion

Genetic variation in three genera of alpine bamboos

In this paper, we revealed relatively abundant genetic variability in the alpine bamboos. Among 599 alignment sites of the entire ITS region, there were 54 polymorphic nucleotide sites (9.05%) and 22 different genotypes. The divergence of ITS spacers ranged from 0 to 4.2%. The divergence within *Fargesia sensu Flora Reipublicae Popularis Sinicae* (Keng and Wang 1996) ranged from 0 to 3.5%. This is similar to that of the *rpl*16 intron within the Chusquea (0-2.5%) or within the Bambusoideae (0-8.2%) (Kelchner and Clark 1997). However, compared with the *ndh*F and *phy*B genes (Gaut *et al.* 1997), the ITS region shows a higher level of base substitution and provided more parsimony-informative sites. Through the statistical analysis of the ITS region of angiosperms collected from the international DNA database, Qu and Chen (1999) calculated that the divergence between angiosperm species was 1.2%-10.2% and between genera was 9.6%-28.8%. In our studies of alpine bamboos, of 253 comparison pairs, 56 had a divergence lower than 1.2% (Table 2). The largest divergence between these closely related genera was 4.2%, which was still lower than 9.6%. The reasons for the low sequence divergence are unknown. One explanation is that they may be of recent origin and have undergone very little molecular evolution relative to each other (Renvoize and Hodkinson, 1997), or that the bamboos have a special life cycle (Zhang 1996, Gaut *et al.* 1997). Therefore, the average degree of molecular variation revealed in other angiosperms is not necessarily representative of the woody bamboos. However, exploring molecular data for generic delimitation of the woody bamboos is undoubtedly of value since the woody bamboos rarely flower and the present classifications are based largely on vegetative characters, which are variable in some cases. The rapid concerted evolution in the ITS region makes it a good molecular target for the delimitation of species, how-

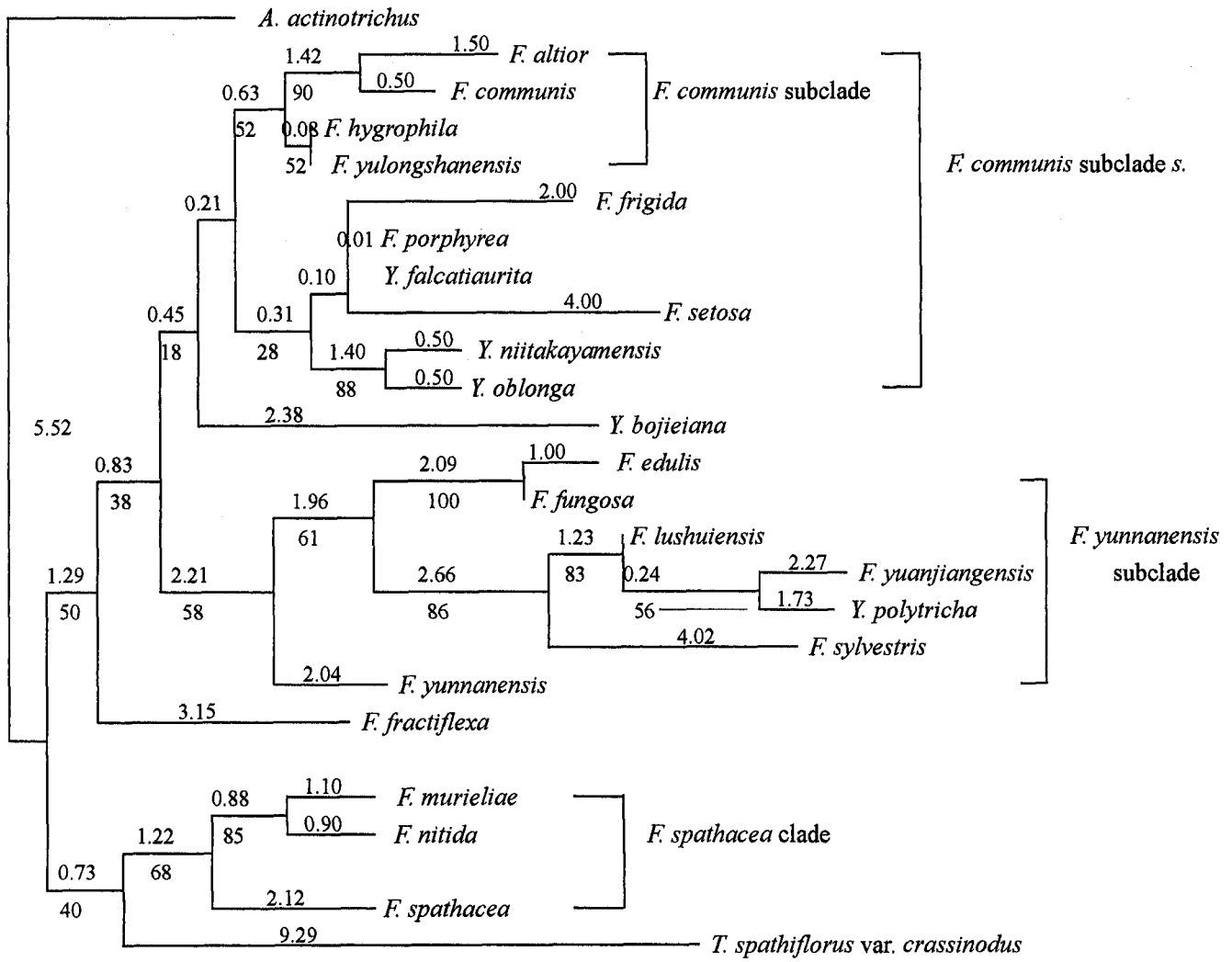

Fig. 2. Neighbor-joining tree of the alpine bamboos inferred from ITS sequence data. Numbers above branches are branch lengths; numbers below branches are bootstrap values (Distance measure=total character difference).

ever, ITS data should also be interpreted with care because the region is subject to recombination and because paralogous sequences can exist (Soltis *et al.* 1998). More information about the ITS region in the woody bamboos is required.

Phylogenetic relationships in the alpine bamboos

The ITS phylogenetic trees in both the MP and NJ analyses indicated that *Thamnocalamus spathiflorus* var. *crassinodus* and the *Fargesia spathacea* clade may form the basal groups in the alpine bamboos but bootstrap support for this conclusion is weak. *Thamnocalamus spathiflorus* var. *crassinodus* was grouped with the *Fargesia spathacea* clade in the NJ analysis but was paraphyletic with the latter in the MP analysis. This species was first published as *Fargesia crassinoda* by Yi (1983). Stapleton (1994) transferred it into *Thamnocalamus* as a variety of *Thamnocalamus spathiflorus* because the vegetative branching shows an affinity to *Thamnocalamus* rather than *Fargesia*. Its flowers of it are unknown but the ITS phylogeny suggests it may not be allied with *Fargesia*. More *Thamnocalamus* species are needed before a definite conclusion can be made regarding its affinities. Soderstrom (1979a, 1979b) treated *Fargesia* as a synonym of *Thamnocalamus* by making *F. spathacea*, the type species of Fargesia, as *Thamnocalamus spathaceus*. This treatment is supported by the NJ analysis, but not by the MP analysis. A wider sampling is needed to resolve their relationship. The *Fargesia spathacea* clade, including *F. spathacea*, *F. nitida* and *F. murieliae*, was resolved as monophyletic and distinct from the other species of *Fargesia* in both analyses. This result is supported by morphological characters. They all have bracteate racemiform synflorescences, short culm necks and very similar habitats.

Fargesia fractiflexa has many subequal culm branches with underdeveloped secondary branches, which is very different from other species of *Fargesia*. In the parsimony and NJ

analyses, it diverged as sister to the other alpine bamboos except for the basal groups but bootstrap support is again low (38 in MP tree and 50 in NJ tree). Further research based on more comprehensive data is also necessary to resolve its systematic position.

The rest of the alpine bamboos were resolved as a monophyletic clade in the 50% majority rule tree and in the NJ analyses but bootstrap values for this clade and its subgroups were low indicating that they may not be real. This clade included species of *Fargesia* (as well as *Borinda*) and *Yushania*, and was divided into two groups. In the MP analysis, this clade was composed of the *Fargesia yunnanensis* subclade, together with *Yushania niitakayamensis*, *Y. oblonga*, and *Fargesia porphyrea*, *Yushania falcatiaurita*, *F. frigida* and *F. setosa*, and the *Fargesia communis* subclade. In the NJ analysis, this clade was composed of the *Fargesia yunnanensis* subclade (excluding *Y. bojieiana*) and the *Fargesia communis* subclade *sensu lato* (including *Yushania niitakayamensis*, *Y. oblonga*, and *Fargesia porphyrea*, *Yushania falcatiaurita*, *F. frigida* and *F. setosa* as a monophyletic group). The *Fargesia yunnanensis* and *Fargesia communis* subclades were relatively consistent in both the MP analysis and the NJ tree. Species in the *Fargesia communis* subclade, that is, *F. altior*, *F. communis*, *F. hygrophila* and *F. yulongshanensis*, bear short pachymorph rhizomes and their synflorescences are unknown. However, in the *Flora Reipublicae Popularis Sinicae* (Keng and Wang, 1996), *F. yulongshanensis* was placed in Series *Yunnanenses*, and the other three in Series *Angustissimae*. *Yushania niitakayamensis* and *Y. oblonga* formed a monophyletic group in both analyses. This is consistent with their morphological characters. However, *Fargesia porphyrea* (Ser. *Yunnanenses*), *Yushania falcatiaurita*, *F. frigida* (Ser. Murielae) and *F. setosa* (as Ser. *Fargesia*, but placed in Borinda by Stapleton 1994) were grouped in a monophyletic group in the NJ analysis with little bootstrap support. They were placed in different genera or series.

The *Fargesia yunnanensis* subclade, including *F. sylvestris*, *Yushania polytricha*, *F. yuanjiangensis*, *F. lushuiensis*, *F. edulis*, *F. fungosa* and *F. yunnanensis*, is mostly heterogeneous in morphological characters. This subclade is composed of species placed by Keng and Wang (1996) in several series, i.e., Ser. *Fargesia* (*F. sylvestris*), Ser. *Angustissimae* (*F. fungosa*, *F. edulis*, and *F. yuanjiangensis*), and Ser. *Yunnanenses* (*F. yunnanensis* and *F. lushuiensis*). Morphologically, this subclade covers species having very open or condensed synflorescences, and very long (up to 30 cm) to very short (4–5 cm) culm necks. For example, *Fargesia yunnanensis* and *Yushania polytricha* have very open synflorescences without subtended bracts and long culm necks while *Fargesia edulis*, *Fargesia fungosa* and *Fargesia yuanjiangensis* have condensed synflorescences with some bractlets at the base and shorter culm necks. Stapleton (1998) transferred *F. edulis*, *F. fungosa*, *F. lushuiensis* and *F. frigida* (as frigidorum) into *Borinda*. This is also not supported by our molecular evidence. Incongruence exists between the molecular-based phylogenies and relationships emphasized by morphological characters. It appears that the vegetative characters used to delimit the genera of alpine bamboos, such as the degree of spathe subtending synflorescences and the length of culm necks, cannot reflect evolutionary relationships and the genera delimited based on these characters may not be monophyletic. However, the bootstrap values of the MP tree were generally low and are due to weak character support for these branches; base substitution of the ITS region is generally rare in woody bamboos (Renvoize and Hodkinson 1997; Hodkinson *et al.* 2000). To solve this problem, wider sampling and more comprehensive data from both morphological and molecular sources such as AFLPs (Amplified Fragment Length Polymorphisms) and SSR (Length Polymorphism of Simple Sequence Repeat) are required.

The phylogenetic value of ITS in closely related woody bamboos

The entire ITS region of 23 species of alpine bamboos provided useful information for phylogenetic reconstruction but further data will be needed for improved resolution and internal support of the trees. The woody bamboos show a slower rate of sequence evolution, particularly the temperate woody bamboos (Zhang 1996, Gaut *et al.* 1997). It is also possible that they radiated recently since South American woody bamboos show higher degrees of molecular variation (Hodkinson *et al.* 2000). The divergence in the ITS region within alpine bamboos ranged from 0 to 4.2%. These results support the utility of the ITS region for resolving taxonomic relationships of closely related woody bamboo genera.

We would like to acknowledge to the financial support of a Special Grant of the National Science Foundation of China (39725001 to De-Zhu Li) and through the Hundreds of Talents Program of the Chinese Academy of Sciences (CAS 1998-0054 to De-Zhu Li). We are grateful to Phillip Thomas of the Royal Botanical Garden Edinburgh (Scotland UK) for providing material of *Thamnocalamus spathiflorus* var. *crassinodus*, to Jia-Rong Xue of South-west Forest College, Kunming (China) for help in collecting materials of *Fargesia* and *Yushania*, to Su-Hua Shi of the Zhong Shan University (China) for providing the original amplification primers, and to Jing Luo of Kunming Institute of Zoology (China), for doing partial sequence analysis. We are also indebted to an anonymous reviewer for constructive comments.

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(Received September 18, 2000; accepted May 9, 2001)