



Phylogeny of *Fargesia* (Poaceae: Bambusoideae) and infrageneric adaptive divergence inferred from three cpDNA and nrITS sequence data

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Abstract

Fargesia, a temperate woody bamboo genus, is one of the largest genera and constitutes a taxonomically problematic group due to unusual life cycles and the rarity of flowering. We explored phylogenetic relationships within *Fargesia* and its allies based on sequence data from three cpDNA regions (*matK*, *psbA-trnH* and *trnL-trnF*) and one nuclear region (nrITS). A representative sample of 49 species, including 36 *Fargesia* and nine *Yushania*, were sampled, and maximum parsimony, maximum likelihood and Bayesian inference were used to reconstruct the phylogeny of *Fargesia*. The results suggest that *Fargesia* is polyphyletic, with *F. crassinoda* and *F. damuniu* in the *Thamnocalamus* clade, *F. ampullaris*, *F. semiorbiculata*, *F. gyirongensis* and *F. collaris* in the *Drepanostachyum* + *Himalayacalamus* clade, and the rest of species of *Fargesia* and all sampled species of *Yushania* in a *Fargesia* + *Yushania* clade, which was further divided into weakly supported *Fargesia* spathe and non-spathe clades. Species in the *Fargesia* spathe clade have the derived “spathe-like leaf sheath syndrome,” which may have evolved as a result of an adaptive advantage of compressed inflorescences in colder temperatures.

Keywords Adaptive evolution · Divergence · *Fargesia* · Infrageneric phylogeny · *Yushania*

Introduction

The bamboos (Poaceae: Bambusoideae) have rich species diversity (Bamboo Phylogeny Group 2012). Based on molecular systematic studies, Bambusoideae is divided into three tribes: Arundinarieae, Bambuseae and Olyreae, representing temperate woody, tropical woody and herbaceous bamboos,

respectively (Bouchenak-Khelladi et al. 2008; Sungkaew et al. 2009; Bamboo Phylogeny Group 2012). Most members of the Bambusoideae are woody species (Bamboo Phylogeny Group 2012). The majority of the woody bamboo species have been frequently described without knowledge of the flowers (Wang and Ye 1980; Stapleton 1997; Li et al. 2006; Viana et al. 2011) due to the long blooming intervals from several to 120 or even 150 years (Janzen 1976; Campbell 1987; Geng and Wang 1996). Thus, vegetative features are significant in identifying these infrequently flowering plants (Soderstrom 1981; Keng and Wen 1989; Stapleton 1997; Stapleton et al. 2009). The current infrageneric taxonomy is mainly based on the varied vegetative features, especially for the woody bamboos distributed in China (e.g., Yi 1996; Li et al. 2006). Since the vegetative characters used for identification are likely to be unstable in most cases, it is difficult to argue if they truly reflect the evolutionary history of these species (Guo et al. 2001). Therefore, the roles of vegetative and reproductive characteristics in evolutionary relationships of the Chinese genera remain unclear (Zhang et al. 2012; Yang et al. 2013). *Fargesia* is a good system to address this issue as it is one of the largest alpine woody bamboo genera. Furthermore, *Fargesia* species have various morphological

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characteristics, wide distributions and an infrageneric classification based on vegetative features (Yi 1988a).

Fargesia, a member of the tribe Arundinarieae (Bamboo Phylogeny Group 2012; Kellogg 2015), comprises about 80–100 species (Yi 1996; Li et al. 2006; Yi et al. 2008; Kellogg 2015) and is distributed mainly in mountains from 1400 to 3800 m in the eastern Qinghai-Tibetan Plateau and southwest China, the eastern Himalayas, Burma and Vietnam (Yi 1996; Li et al. 2006; Yi et al. 2008).

Fargesia was first described by Franchet (1893) based on *F. spathacea* Franchet, and contained only one species until the 1980s, when Yi (1983a, b, c, 1985a, b, c, 1986, 1988a, b, 1989) and others (Lu 1981; Wang and Ye 1981; Keng 1987; Yi and Shao 1987; Wen 1984, 1989; Yi and Long 1989) published 70 new species in < 10 years, and nine species were transferred into *Fargesia* from other genera. Ninety to 100 *Fargesia* species are currently recognized in the literature (Li et al. 2006; Yi et al. 2008; Kellogg 2015).

After *Fargesia*, some allied genera, including *Sinarundinaria* (Nakai 1935), *Yushania* (Keng 1957) (ca. 80 species, Li et al. 2006), *Chimonocalamus* (Hsueh and Yi 1979), *Ampelocalamus* (Chen et al. 1981), *Drepanostachyum*, *Himalayacalamus* (Keng 1983) and *Borinda* (Stapleton 1994b), were described, among which *Sinarundinaria* and *Borinda* were later shown to be synonymous with *Fargesia* (Wang and Ye 1980; Keng 1984, 1987; Yi 1982, 1988a, 1996; Guo et al. 2001, 2002; Guo and Li 2004; Li et al. 2006). Although *Fargesia* was accepted by many authors (Wang and Ye 1980; Keng 1983; Yi 1982, 1988a, 1996; Yi et al. 2008; Bamboo Phylogeny Group 2012), the publication of these genera made the classification of *Fargesia* more controversial, with different opinions appearing. There were mainly three points of view. First, *Fargesia* was recognized as a separate genus (Keng 1982, 1983; Soderstrom and Ellis 1987; Yi 1988a, 1996; Yi et al. 2008), and at least part of the species in *Yushania*, *Chimonocalamus*, *Ampelocalamus*, *Drepanostachyum* and *Himalayacalamus* were considered to be included in *Fargesia* (Chao et al. 1980; Hsueh and Li 1987; Soderstrom and Ellis 1987; Chao and Renvoize 1989; Yi 1988a, 1996). Second, *Fargesia* was either included in *Thamnocalamus* (Soderstrom 1979a, b; Chao et al. 1980; Soderstrom and Ellis 1982; Clayton and Renvoize 1986; Chao and Renvoize 1989; Demoly 1991) or *Arundinaria* (Soderstrom and Ellis 1988). Demoly (2005) even moved the species of *Fargesia* into *Thamnocalamus*, *Drepanostachyum* or *Yushania*. The third opinion partly accepted *Fargesia*, with the removal of some species in Yi's (1988a) infrageneric classification into *Thamnocalamus*, *Drepanostachyum* or *Himalayacalamus* (Stapleton 1994c; Ohrnberger 1996; Li et al. 2006). The boundary of *Fargesia* is not well defined possibly because the identifications of those genera were based mainly on vegetative characteristics. It is well known that compared with the characteristics of flowers and inflorescences, vegetative characteristics are more affected by

environment factors, and some labile traits exist among genera (Guo et al. 2001). Because of the controversial classification of *Fargesia*, it is reasonable to doubt the monophyly of *Fargesia* as suggested by previous molecular studies (Guo and Li 2004; Triplett and Clark 2010; Zeng et al. 2010).

Yi (1988a) established an infrageneric classification of *Fargesia* based on vegetative characteristics including two sections and six series among which only two sections were accepted (Yi et al. 2008), but this infrageneric classification has not yet been tested by molecular phylogenetics. It is difficult to determine the position of those species due to the lack of description of the flowers, especially the species in section *Sphaerigemma* that were put in *Fargesia* (Yi 1996). Regardless of the inflorescences or floral characters, the vegetative character-based infrageneric classification might result in instable generic/infrageneric classifications because of extensive morphological parallelism or homoplasy in morphology.

Fargesia has been included in several molecular systematic studies (Kelchner and Clark 1997; Zhang and Clark 2000; Bouchenak-Khelladi et al. 2008; Peng et al. 2008; Hodkinson et al. 2010; Triplett and Clark 2010; Zeng et al. 2010; Zhang et al. 2012; Yang et al. 2013; Ma et al. 2014). Sequence data from the nuclear internal transcribed spacer region (nrITS) have shown that some species of *Fargesia* nested within *Yushania* (Guo et al. 2001, 2002) and that *Fargesia* might be polyphyletic (Guo et al. 2002). Based on nrITS and *GBSSI* sequence data, Guo and Li (2004) showed that *Fargesia* and *Yushania* species form a monophyletic group except for *F. fractiflexa*, and again *Fargesia* species nested with *Yushania* species in different clades. However, the focus of these studies has been to resolve the intergeneric relationships within tribe Arundinarieae, but not the infrageneric phylogeny of *Fargesia*. The infrageneric phylogeny of *Fargesia* has not been resolved because of limited sampling in previous studies.

Fargesia species play an important role in the ecosystem and also as the main food of giant pandas and some other rare animals (Yi 1985a, b; McNeely 1999). In this study, representative species from each series and section of *Fargesia* (sensu Yi 1988a) and *Yushania* were sampled. Chloroplast *matK*, *psbA-trnH*, *trnL-trnF* and nuclear nrITS sequence data were used to (1) evaluate the boundary of this genus; (2) provide an infrageneric phylogenetic framework; and (3) investigate the evolutionary pattern of floral characters.

Materials and methods

Taxon sampling

Thirty-six species of *Fargesia* from Yi's (1988a) infrageneric classification, including species transferred to

Drepanostachyum, *Himalayacalamus* or *Thamnocalamus* by other authors, and nine species of *Yushania* were selected as members of the ingroup. Two species of *Thamnocalamus* and two species of *Pleioblastus* were selected as the outgroup. Fresh leaves from each individual were sampled in the field and immediately placed on silica gel and stored at room temperature for DNA extraction.

All vouchers were deposited in the herbarium of Shaanxi Normal University (SANU, Xi'an, China). The identification of bamboo species was based on *Flora Republicae Popularis Sinicae* (Yi 1996), *Flora Yunnanica* (Li 2003), *Flora of China* (Li et al. 2006), *The Bamboos of Nepal and Bhutan* (Stapleton 1994a, b, c) and *Iconographia Bambusoidearum Sinicarum* (Yi et al. 2008).

DNA extraction, PCR amplification and DNA sequencing

Total DNA was extracted from silica gel-dried leaves (0.3 g) using a modified CTAB method (Doyle and Doyle 1987). Three cpDNA gene regions, *matK* (Bouchenak-Khelladi et al. 2008), *psbA-trnH* (Ruiz-Sanchez et al. 2008) and *trnL-trnF* (Taberlet et al. 1991), and the nuclear ribosomal ITS (White et al. 1990) were isolated in the accessions (Table 1).

PCR amplifications were carried out using ABI Veriti™ 96-well Thermal cycler (Applied Biosystems, CA, USA) in total volumes of 25 µL. Each reaction contained 0.25 µL Takara LA *Taq*, 2.5 µL 10×PCR Buffer, 2.5 µL MgCl₂ (25 mM), 4 µL of dNTPs (Takara Biomedical Technology, Beijing, China), 1 µL of each primer (10 µM), 1 µL of template DNA (20–30 ng/µL) and 12.75 µL H₂O. The PCR products were subsequently purified through ethanol precipitation and sequenced by Beijing Dingguo Changsheng Biotechnology Co. Ltd. (Beijing, China). Several ITS sequences contained some nucleotide positions with double peaks, so we confirmed the sequences via cloning with pMD™19-T vector cloning kit (Takara Biomedical Technology, Beijing, China) following the manufacturer's protocols, and re-scanned and edited as necessary to accurately identify polymorphic positions.

Phylogenetic analyses

The sequences were assembled and manually edited in Geneious R9.0.2 (Kearse et al. 2012). To check whether the obtained sequence was the target sequence, we performed a homology search using BLAST (The National Center for Biotechnology Information, NCBI). Sequence divergences between taxa and base frequencies (G + C content) were determined using MEGA v.5.05 (Tamura et al. 2011).

Phylogenetic analyses were conducted using separate and combined data sets. Gaps were coded using simple indel coding (Simmons and Ochoterena 2000) in SeqState (Müller

2005) (Online Resource 1). Congruence between datasets was tested with the incongruence length difference test (ILD) (Darlu and Lecointre 2002) implemented in PAUP v4.0b10 (Swofford 2002) as the partition homogeneity test.

Maximum parsimony (MP) phylogenetic analyses were performed using PAUP v.4.0b10 (Swofford 2002). Maximum parsimony bootstrap (MPB) analyses (Felsenstein 1985) were carried out with 1000 random addition sequences and tree bisection-reconnection (TBR) branch swapping. A full heuristic bootstrap was conducted for maximum parsimony with 1000 bootstrap replicates, each with 100 random addition sequence replicates. Akaike information criterion (Posada 2008) calculations, implemented in jModelTest v.2.1.4 (Darriba et al. 2012), were used to select the appropriate model of sequence evolution for the different datasets. Bayesian inference (BI) was conducted using MrBayes v.3.2.6 (Ronquist and Huelsenbeck 2003). The Markov chain Monte Carlo algorithm was implemented with two parallel runs, each with four independent chains that were set to run for 4 million generations and to sample every 1000 generations. Convergence was assessed using the average standard deviation and the potential scale reduction factor (PSRF). Convergence of runs was also checked using Tracer v 1.6 (Rambaut et al. 2014) to assess the effective sample size (ESS) > 200 for all parameters. The first 25% of the trees was discarded as a burn-in. ML analysis was run in RA×ML-HPC ver.8 on XSEDE in the Cipres Science Gateway (Miller et al. 2010). Rapid bootstrap analysis with 1000 replications was performed using GTR-GAMMA estimation to assess branch support (Stamatakis 2006).

The approximately unbiased (AU) test was performed in CONSEL (Shimodaira and Hasegawa 2001; Shimodaira 2002) to test phylogenetic hypotheses (e.g., the monophyly of *Yushania*).

Reconstruction of character-state evolution

Vegetative features of *Fargesia*, *Thamnocalamus*, *Drepanostachyum* and *Yushania* are similar, but the inflorescences in these taxa vary. In particular, the inflorescences of some species are subtended by leaf sheaths, which expand to different levels. Inflorescence types were coded as follows: spathe-like leaf sheath syndrome (0: compressed and unilateral inflorescences are subtended by a series of spathe-like leaf sheaths, of which the topmost is longer than the inflorescence), non-expanding leaf sheaths (1: open inflorescences are subtended by non-expanding leaf sheaths) and slightly expanded leaf sheaths (2: open inflorescences are somewhat subtended but never spathe-like expanded leaf sheaths). Stigma types were coded as follows: pistil with three stigmas (0), pistil with two stigmas (1). To reconstruct ancestral states, we used the "trace character history" option with parsimony ancestral states in Mesquite v.2.75 (Maddison and Maddison 2011). These two

Table 1 Vouchers and GenBank accessions number for taxa included in the current study based on Yi (1996)

Taxa	Location/elevation (m)	Longitude and latitude	Voucher	GenBank accession no.			
				<i>matK</i>	<i>psbA-trnH</i>	<i>trnL-F</i>	nrITS
<i>Fargesia</i> Franch.							
I. Sect. <i>Ampullares</i> T.P.Yi (= Sect. <i>Sphaerigemma</i> T.P.Yi)							
1. Ser. <i>Collares</i> T.P.Yi							
<i>F. canaliculata</i> T.P.Yi	Mianning, Sichuan /1996	28.1983, 101.777	ZYQ C308	KP685605	KP685655	KP725367	KP725315
<i>F. collaris</i> T.P.Yi	Zhangmu, Tibet/2140	27.9745, 859535	ZYQ G650	KP685635	KP685685	KP725397	KP725333
2. Ser. <i>Ampullares</i> T.P.Yi							
<i>F. ampullaris</i> T.P.Yi	Zhangmu, Tibet/1752	27.9721, 85.9642	ZYQ G644	KP685601	KP685651	KP725363	KP725313
<i>F. fractiflexa</i> T.P.Yi	Lijiang, Yunnan/1978	27.1959, 100.0490	ZYQ D521	KP685603	KP685652	KP725364	KP725321
<i>F. gyirongensis</i> T.P.Yi	Jilong, Tibet/2183	28.2992, 85.3262	ZYQ G655	KP685636	KP685686	KP725398	KP725334
<i>F. membranacea</i> T.P.Yi	Mianning, Sichuan/1847	28.1877, 101.7913	ZYQ C317	KP685619	KP685669	KP725381	KP725324
<i>F. semiorbiculata</i> T.P.Yi	Cuona, Tibet/2660	27.8217, 91.7391	ZYQ G688	KP685602	KP685653	KP725365	KP725314
II. Sect. <i>Fargesia</i> Yi							
3. Ser. <i>Murielae</i> Yi							
<i>F. crassinoda</i> T.P.Yi	Jilong Tibet/3300	28.3663, 85.3461	ZYQ G664	KP685640	KP685690	KP725402	KP725337
<i>F. denudata</i> T.P.Yi	Pingwu, Sichuan/3180	32.8787, 104.0503	ZYQ C205	KP685609	KP685659	KP725371	KP725318
<i>F. grossa</i> T.P.Yi	Cuona, Tibet/2941	27.8745, 91.7947	ZYQ G684	KP685617	KP685667	KP725379	KP725322
<i>F. murielae</i> (Gamble) T.P.Yi	Fangxian, Hubei/2604	31.4477, 110.1927	ZYQ F622	KP685620	KP685670	KP725382	KP725325
<i>F. similis</i> Hsueh f. & T.P.Yi	Gongshan, Yunnan/3201	27.7824, 98.4834	ZYQ D584	KP685630	KP685680	KP725392	KP725343
<i>F. obliqua</i> T.P.Yi	Pingwu, Sichuan/3230	32.8393, 104.0531	ZYQ C210	KP685622	KP685672	KP725384	KP725327
<i>F. utilis</i> T.P.Yi	Dongchuan Yunnan/3140	26.1621, 103.0097	ZYQ D436	KP685633	KP685683	KP725395	KP725345
4. Ser. <i>Fargesia</i> T.P.Yi							
<i>F. dura</i> T.P.Yi	Zhongdian Yunnan/3031	27.3349, 99.8912	ZYQ D491	KP685611	KP685661	KP725373	KP725350
<i>F. nitida</i> (Mitford) Keng f. ex T.P.Yi	Lixian, Sichuan/2733	31.6588, 102.8074	ZYQ C280	KP685621	KP685671	KP725383	KP725326
<i>F. qinlingensis</i> T.P.Yi & J.X.Shao	Foping, Shaanxi/1142	32.6802, 106.7678	ZYQ A071	KP685624	KP685674	KP725386	KP725328
<i>F. rufa</i> T.P.Yi	Maoxian, Sichuan/2133	31.7415, 103.9361	ZYQ C214	KP685626	KP685676	KP725388	KP725330
<i>F. macclureana</i> (Bor) Stapleton	Linzi, Tibet/3135	29.7542, 94.1686	ZYQ G695	KP685629	KP685679	KP725391	KP725342
<i>F. spathacea</i> Franch.	Fangxian, Hubei/2849	31.4298, 110.2956	ZYQ F617	KP685631	KP685681	KP725393	KP725332
<i>F. subflexuosa</i> T.P.Yi	Deqin, Yunnan/3412	28.4828, 98.9107	ZYQ D558	KP685632	KP685682	KP725394	KP725344
5. Ser. <i>Angustissimae</i> T.P.Yi							
<i>F. altior</i> T.P.Yi	Tengchong, Yunnan/2260	25.7963, 98.5450	ZYQ D397	KP685604	KP685654	KP725366	KP725348
<i>F. communis</i> T.P.Yi	Yuling, Yunnan/2421	27.2131, 99.5105	ZYQ D540	KP685606	KP685656	KP725368	KP725347
<i>F. edulis</i> Hsueh f. & T.P.Yi	Lushui, Yunnan/2421	26.0331, 98.6104	ZYQ D418	KP685612	KP685662	KP725374	KP725353
<i>F. fungosa</i> T.P.Yi	Huize, Yunnan/2213	26.4025, 103.2528	ZYQ D444	KP685614	KP685664	KP725376	KP725322
<i>F. hygrophila</i> Hsueh f. & T.P.Yi	Dali, Yunnan/1862	25.6418, 100.1423	ZYQ D549	KP685618	KP685668	KP725380	KP725349
<i>F. praecipua</i> T.P.Yi	Gongshan Yunnan/2633	27.8481, 98.3033	ZYQ D578	KP685623	KP685673	KP725385	KP725340
<i>F. sagittatinea</i> T.P.Yi	Gongshan, Yunnan/2198	27.8679, 98.3192	ZYQ D574	KP685627	KP685677	KP725389	KP725341
<i>F. yuanjiangensis</i> Hsueh f. & T.P.Yi	Dali, Yunnan/2408	25.6787, 100.0551	ZYQ D563	KP685634	KP685684	KP725396	KP725346
6. Ser. <i>Yunnanenses</i> T.P.Yi							
<i>F. demissa</i> T.P.Yi	Yuzhong, Gansu/2930	35.8087, 103.9804	ZYQ B203	KP685608	KP685658	KP725370	KP725317
<i>F. decurvata</i> J.L.Lu	Yangxian, Shaanxi/1580	33.2618, 107.5583	ZYQ A120	KP685610	KP685660	KP725372	KP725319
<i>F. emaculata</i> T.P.Yi	Jiulong, Sichuan/3592	30.2464, 101.8639	ZYQ C334	KP685613	KP685663	KP725375	KP725320
<i>F. glabrifolia</i> T.P.Yi	Cuona, Tibet/3020	27.8583, 91.7858	ZYQ G683	KP685616	KP685666	KP725378	KP725352

Table 1 (continued)

Taxa	Location/elevation (m)	Longitude and latitude	Voucher	GenBank accession no.			
				<i>matK</i>	<i>psbA-trnH</i>	<i>trnL-F</i>	nrITS
<i>F. robusta</i> T.P.Yi Series unknown	Wolong, Sichuan/2297	31.0000, 103.1535	ZYQ C287	KP685625	KP685675	KP725387	KP725329
<i>F. funiushanensis</i> T.P.Yi*	Luanchuan, Henan/1470	33.7393, 111.6394	ZYQ E612	KP685615	KP685665	KP725377	KP725323
<i>F. damuniu</i> T.P.Yi* Yushania Keng f.	Nielamu, Tibet/3473	28.0578, 85.9937	ZYQ G660	KP685607	KP685657	KP725369	KP725316
I. Sect. <i>Brecipaniculatae</i> T.P.Yi							
<i>Y. brevipaniculata</i> (Hand.-Mazz.) T.P.Yi	Wolong, Sichuan/2013	31.1080, 103.3207	ZYQ C358	KP685642	KP685692	KP725404	KP725356
<i>Y. elevata</i> T.P.Yi	Tengchong, Yunnan/2418	25.5904, 98.3891	ZYQ D456	KP685644	KP685694	KP725406	KP725358
<i>Y. exilis</i> T.P.Yi	Dongchuan, Yunnan/3025	26.1698, 102.9849	ZYQ D469	KP685645	KP685695	KP725407	KP725359
<i>Y. lineolata</i> T.P.Yi	Meigu Sichuan/2623	28.6734, 102.9761	ZYQ C261	KP685647	KP685697	KP725409	KP725360
<i>Y. violascens</i> (Keng) T.P.Yi	Xianggelila, Yunnan/3276	28.0719, 99.7828	ZYQ D601	KP685650	KP685700	KP725412	KP725362
II. Sect. <i>Yushania</i> T.P.Yi							
<i>Y. confusa</i> (McClure) Z.P.Wang & G.H.Ye	Changyang, Hubei/1442	30.5475, 110.8513	ZYQ F642	KP685643	KP685693	KP725405	KP725357
<i>Y. glandulosa</i> Hsueh & T.P.Yi	Lushui, Yunnan/1963	26.0132, 98.6268	ZYQ D453	KP685646	KP685696	KP725408	KP725335
<i>Y. pachyclada</i> T.P.Yi	Tengchong, Yunnan/2963	25.8304, 98.5246	ZYQ D458	KP685648	KP685698	KP725410	KP725338
<i>Y. polytricha</i> Hsueh & T.P.Yi	Kunming, Yunnan/2180	25.0678, 102.6225	ZYQ D468	KP685649	KP685699	KP725411	KP725361
OUT groups							
<i>Thamnocalamus arista-</i> <i>tus</i> E. G. Camus	Zhangmu, Tibet/3220	28.0711, 85.9873	ZYQ G676	KP685639	KP685689	KP725401	KP725336
<i>T. unispiculatus</i> T.P.Yi & J.Y.Shi*	Zhangmu, Tibet/1875	32.6061, 104.1456	ZYQ G655	KP685641	KP685691	KP725403	KP725339
<i>Pleioblastus amarus</i> (Keng) Keng f.	Chengdu, Sichuan/489	30.6341, 104.0902	ZYQ C371	KP685637	KP685687	KP725399	KP725354
<i>P. gramineus</i> Nakai	Chengdu, Sichuan/489	30.6341, 104.0902	ZYQ C373	KP685638	KP685688	KP725400	KP725355

*Name followed *Iconographia Bambusoidearum Sinicarum* (Yi et al. 2008)

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reproductive characters were optimized on the ML phylogenetic tree obtained from the combined data.

Average temperature data were downloaded from WorldClim (<http://www.worldclim.org/>) for current conditions (interpolations of observed data, representative of 1960–1990) at 2.5-min grids and exported from DIVA-GIS v.7.5 (Hijmans et al. 2012) based on the latitude and longitude of the sampling point. The 3D-scatter line was made to reflect the fluctuation of average temperature along with the latitude and distribution elevation using Origin v 9.1 (Seifert 2014).

Results

Phylogenetic analyses

The characteristics of the alignment matrix of three plastid regions and nrITS sequences are summarized in Table 2. The ILD test indicated that cpDNA sequences were congruent ($P = 0.100$ between *matK* and *psbA-trnH*, $P = 0.080$ between *trnL-trnF* and the combined *matK* and

Table 2 Characteristics of the sequence datasets

Sequence characteristics	<i>matK</i>	<i>psbA-trnH</i>	<i>trnL-F</i>	nrITS
Aligned length	778	543	920	619
Variable sites (and %)	17 (2.2)	2 (2.9)	11 (1.2)	45 (7.3)
Parsimony informative sites	13	2	9	39
Numbers of indels	0	3	0	1
Pairwise distance in groups (%)	0–0.7	0–0.3	0–0.3	0–6.2
G + C content range (mean)	34.0	36.9	33.6	68.3

psbA-trnH), and likewise, the ILD test indicated the combined cpDNA and nrITS sequences ($P=0.50$) were also congruent. The best evolutionary model estimated for each dataset was TIM1 + I, TrN + I + G and TIM3 + I + G for the cpDNA, nrITS and combined dataset, respectively. Different methods of phylogenetic analysis were used based on the separate and the combined cpDNA and nrITS sequences.

In all the phylogenetic trees, *F. crassinoda* (= *T. spathiflorus* var. *crassinodus*) and *F. damuniu* together with two *Thamnocalamus* species formed the *Thamnocalamus* clade; four species, including *F. ampullaris* (= *D. ampullare*), *F. semiorbiculata* (= *D. semiorbiculatum*), *F. gyirongensis* (= *H. falconeri*) and *F. collaris* (= *H. collaris*) formed a clade in most of the trees, and all species in this clade were transferred to *Drepanostachyum* or *Himalayacalamus*, so the clade was accepted as the *Drepanostachyum* + *Himalayacalamus* clade in previous studies (Ohrnberger 1996; Demoly 2005; Stapleton et al. 2005; Li et al. 2006); and the remaining *Fargesia* species and all *Yushania* species formed a *Fargesia* + *Yushania* clade, except in the cpDNA tree, in which the *Drepanostachyum* + *Himalayacalamus* clade and *Fargesia* + *Yushania* clades were not well resolved. The species in the *Fargesia* + *Yushania* clade divided into the *Fargesia* spathe and the non-spathe clades, and each of them divided into smaller clades, but with weak or no bootstrap support (Fig. 1).

The *Fargesia* spathe clade also resolved as monophyletic based on nrITS sequences (Online Resource 2) but formed a polytomy based on plastid data (Online Resource 3). Two main subclades were resolved in the *Fargesia* spathe clade, one including *F. decurvata*, *F. robusta* and *F. rufa*, and the other including *F. demissa*, *F. obliqua* and *F. qinlingensis* (Fig. 1). The non-spathe clade was subdivided into subclades B1 and B2 (Fig. 1) with moderate statistical support (MPB = 62, MLB = 57, PP = 1.00). Subclades B1 consisted of the most species of *Fargesia* and all species of *Yushania*, but the statistical support was low. *Yushania* species did not form a monophyletic group but collapsed with *Fargesia* species in subclades B1 (Fig. 1). Subclades B2 consisted

of four species with moderate bootstrap values (MPB = 87, MLB = 74, PP = 1.00), including *F. canaliculata*, *F. membranacea*, *F. fractiflexa* and *F. fungosa*.

Based on the AU test, our data failed to reject the monophyly of the *Fargesia* spathe clade as sister to the non-spathe clade ($p=0.987$), but rejected the monophyly of the non-spathe clade + *Drepanostachyum* + *Himalayacalamus* clade ($p<0.001$) (Online Resource 3). The AU test also rejected the monophyly of *Yushania* as sister to the remainder of *Fargesia* ($p=0.013$).

Morphological characteristic and geographic distribution

Species in the *Thamnocalamus*, *Drepanostachyum* and *Fargesia* + *Yushania* clades are similar in their vegetative features, but differ in inflorescence features. In the *Thamnocalamus* clade, the racemes are lateral and terminal on the front nodes of the flowering branches and the leaf sheaths on the front nodes are spathe-like. In the *Drepanostachyum* + *Himalayacalamus* clade, the inflorescences are fascicled on the upper nodes of culms and branches, and the nodes in lower parts of inflorescences are compressed and wrapped by scaly sheaths at the base.

Species in the *Fargesia* + *Yushania* clade have inflorescences at the tips of leafy branches. Species in the *Fargesia* spathe clade have pistils with three stigmas (rarely two such as in *F. spathacea*) and the spathe-like leaf sheath syndrome where the inflorescences are compressed, unilateral and subtended by a series of leaf sheaths which expand as spathes, of which the uppermost spathe is generally longer than the inflorescence (Fig. 3). Bamboos in the *Fargesia* spathe clade bloom from March to May (rarely June, December) (Yi 1985b, 1996, 2000; Li et al. 2006; Yi et al. 2007; Lei et al. 2011), and occupy the northern part of the distribution of the *Fargesia* + *Yushania* clade, including Sichuan, southern Gansu, Shaanxi and Ningxia, northwestern Hunan, western Henan and Hubei and northern Chongqing (Geng and Wang 1996; Yi et al. 2008; Yi and Zhu 2012) (Fig. 2).

In the non-spathe clade, species which had descriptions of flowers and inflorescences share the following features: Open inflorescences are subtended by more or less expanded but never spathe-like leaf sheaths that are always shorter than the inflorescence, or part of the inflorescences on a culm are unilateral, such as in *F. edulis* (Yi 1988a, 1996), *F. fungosa* (Yi 1985c, 1996) and *F. subflexuosa* (Zhang and Ren 2016); the pistil has two stigmas (rarely two to three in *Y. violascens* or three in *F. grossa*) (Yi 1988a, 1996) (Fig. 3). Species in the non-spathe clade bloom from April to August (rarely to September) and occupy the southern part of the distribution of the *Fargesia* + *Yushania* clade, including southern Sichuan, Yunnan, southeastern Tibet and Taiwan, as well as India, Nepal, Bhutan, Burma, Vietnam, the

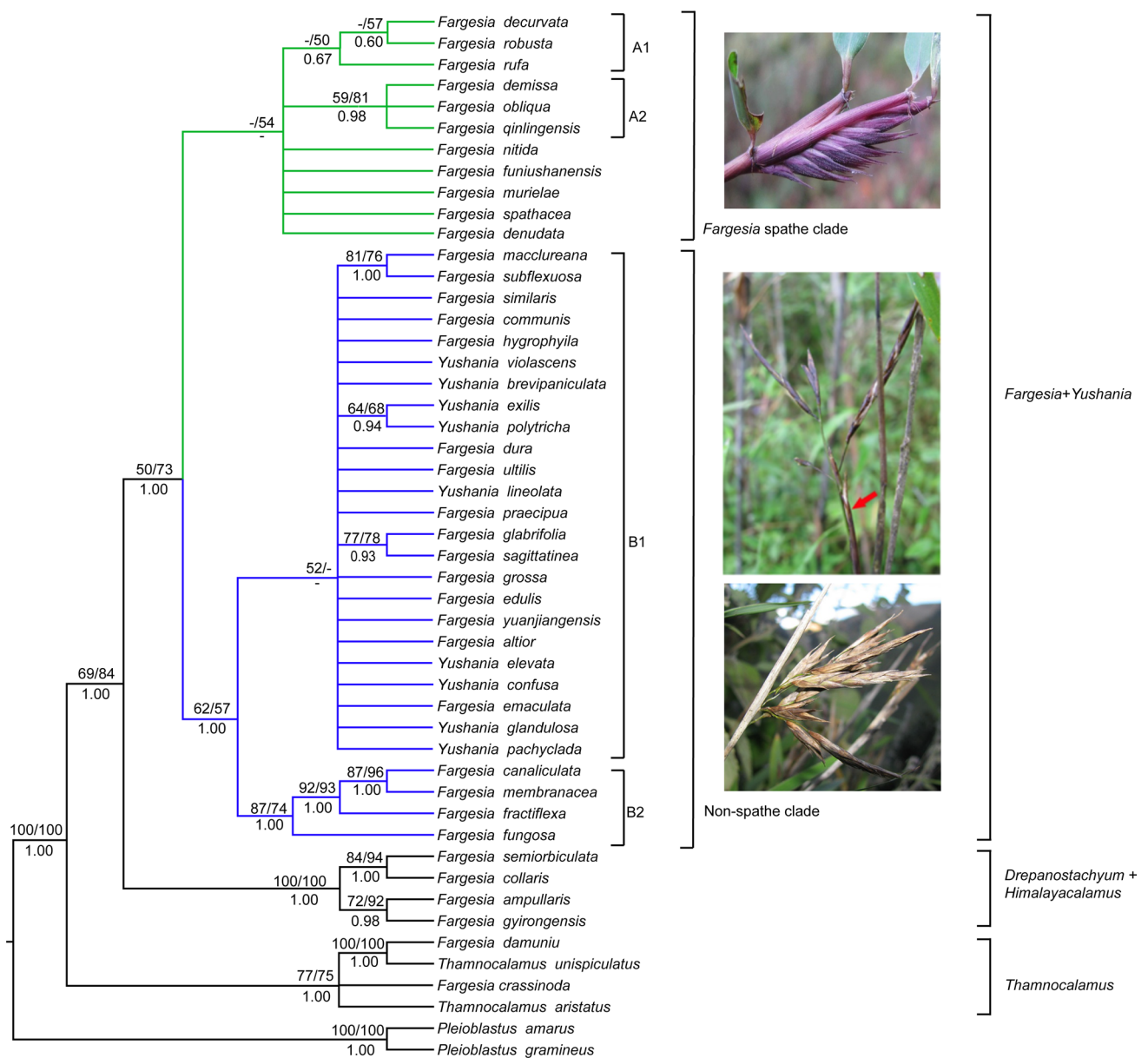


Fig. 1 50% majority-rule consensus tree based on the combined data of cpDNA (*matK*, *psbA-trnH*, *trnL-F*) and nrITS. Tree length = 164, CI = 0.610, RI = 0.829. The bootstrap values ($\geq 50\%$) from 1000 rep-

licates are plotted along the branches. Numbers above and below branches are bootstrap values of MP and ML analysis and PP of Bayesian analysis, respectively

Philippines, Malaysia and Sri Lanka (Stapleton 1994b; Yi 1996; Yi et al. 2008) (Fig. 2).

To better understand the evolution of inflorescences in *Fargesia*, floral types were mapped onto the MP tree (Fig. 3). The ancestral condition was inferred as open inflorescences subtended by non-expanding leaf sheaths. The spathe-like leaf sheath syndrome is the exclusive inflorescence type seen among the *Fargesia* spathe clade. Although we considered the distribution elevation of

sampling points, there were significant upward trends of average temperature with the decreasing latitude during the winter, namely from November to February (Fig. 4). Species in the *Fargesia* spathe clade tend to occupy the northern part of the distribution of the *Fargesia* + *Yushania* clade, where species are distributed at higher latitudes than species of the non-spathe clade, which are distributed in the southern part; the environment of the *Fargesia* spathe clade is colder than that of the non-spathe clade.

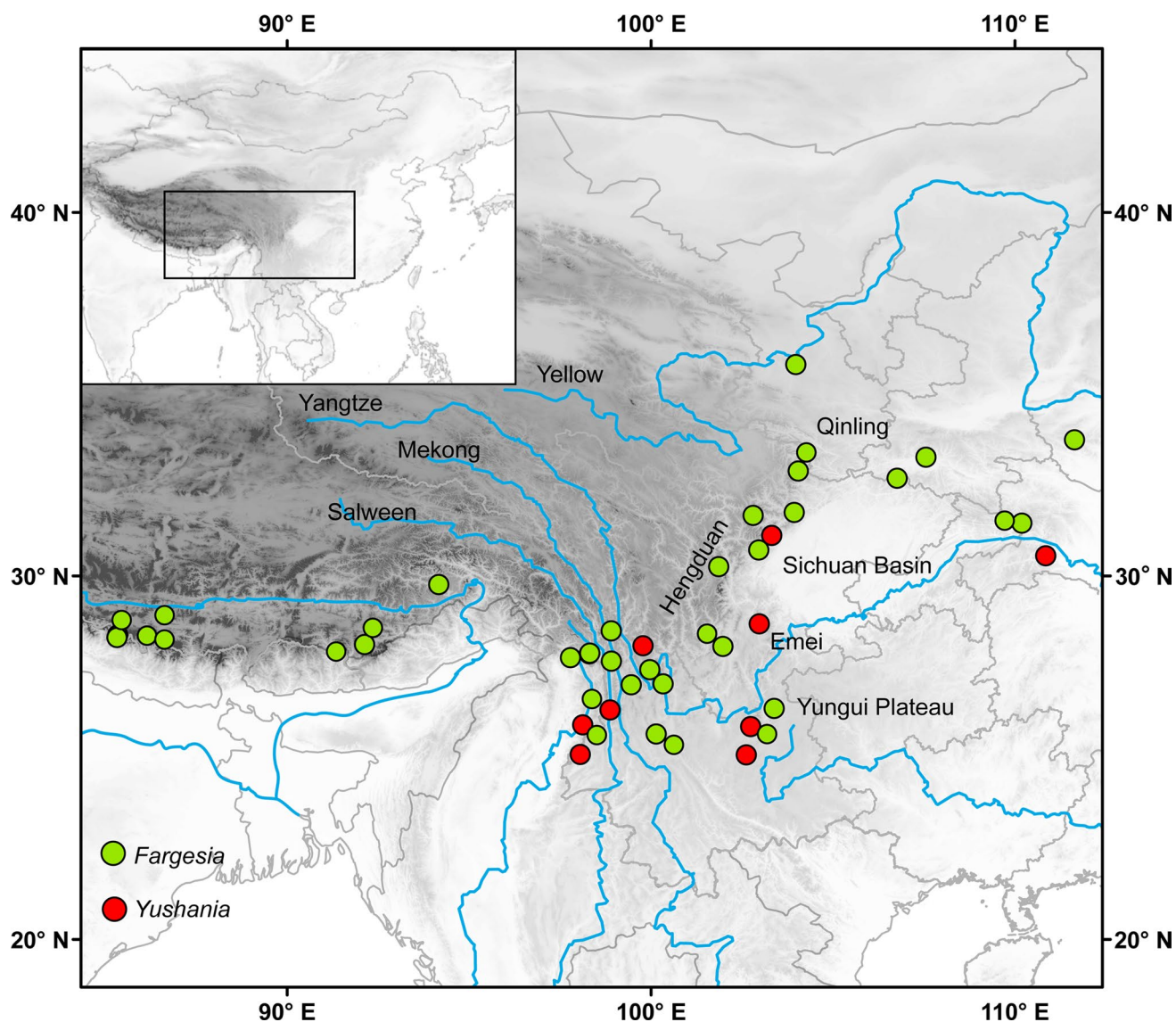


Fig. 2 Geographical distribution of *Fargesia* and *Yushania* specimens

Discussion

Fargesia (sensu Yi 1988a) as a polyphyletic group

Several phylogenetic studies have been conducted that the monophyly of *Fargesia* (sensu Yi 1988a) was problematic, as the boundary of *Fargesia* is confused with *Yushania* (Guo et al. 2002; Guo and Li 2004; Wang et al. 2017), *Chimonocalamus* (Yang et al. 2013; Wang et al. 2017), *Bashania* (Zhang et al. 2012; Wang et al. 2017) and *Phyllostachys* (Ma et al. 2014) based on limited taxon sampling and various gene markers. Both the current and previous molecular phylogenetic studies demonstrate that the classification of *Fargesia* will need to be re-evaluated.

The results from the present study suggest that *Fargesia* (Yi 1988a) is polyphyletic with *F. crassinoda* (= *Thamnocalamus spathiflorus* var. *crassinodus*) and *F. damuniu* in the *Thamnocalamus* clade, *F. semiorbiculata* (= *D. semiorbiculatum*), *F. collaris* (= *H. collaris*), *F. gyirongensis* (= *Himalayacalamus falconeri*) and *F. ampullaris* (= *Drepanostachyum ampullare*) in the *Drepanostachyum* + *Himalayacalamus* clade and the remaining *Fargesia* and all species of *Yushania* in the *Fargesia* + *Yushania* clade.

Fargesia crassinoda was described by Yi (1983b) without description of inflorescences and later transferred to *Thamnocalamus* as *T. spathiflorus* (Trin.) Munro var. *crassinodus* (Yi) Stapleton (Stapleton 1994b). This treatment was supported by previous molecular systematic studies (Guo et al. 2001, 2002; Guo and Li 2004) and

accepted by Ohrnberger (1999) and Li et al. (2006). *Fargesia damuniu* was described by Yi et al. (2007), but lacked description of flowers and inflorescences, and placed in section *Fargesia* (Yi et al. 2008). The present study suggests that both *F. crassinoda* and *F. damuniu* are in the same clade with the two *Thamnocalamus* species included in this investigation.

Because of a lack of description of flowers and inflorescences, *F. ampullaris*, *F. canaliculata*, *F. collaris*, *F. fractiflexa*, *F. gyirongensis*, *F. membranacea* and *F. semi-orbiculata* were placed in *Fargesia* section *Sphaerigemma* by Yi (1985a, b, 1988a, 1992, 1996). Later, *F. collaris* and *F. gyirongensis* were transferred into *Himalayacalamus* as *H. collaris* (T.P.Yi) Ohrnb. and *H. gyirongensis* (T.P.Yi) Ohrnb. (Ohrnberger 1996), respectively; *F. fractiflexa*, *F. ampullaris*, *F. semi-orbiculata* and *F. membranacea* were transferred into *Drepanostachyum* as *D. fractiflexum* (T.P.Yi) D.Z.Li (2003), *D. ampullare* (T.P.Yi) Demoly (Demoly 2005), *D. semi-orbiculatum* (T.P.Yi) Stapleton and *D. membranaceum* (T.P.Yi) D.Z.Li (Stapleton et al. 2005), respectively. Li et al. (2006) accepted all the transferred names except *H. gyirongensis*, which was treated as a synonym of *H. falconeri* (Hook.f. ex Munro) Keng f. in the *Flora of China* (Li et al. 2006). On the one hand, only *F. gyirongensis* (= *H. falconeri*) has had its flowers and inflorescences described (Keng 1983), and we collected the flowers and inflorescences of *F. semi-orbiculata* (= *D. semi-orbiculatum*); these flowers and inflorescences were different from *Fargesia* species and further supported the inclusion of *D. semi-orbiculatum* in *Drepanostachyum* (Zhang and Ren 2016). On the other hand, *Fargesia ampullaris*, *F. collaris*, *F. gyirongensis* and *F. semi-orbiculata* should be transferred into *Drepanostachyum* or *Himalayacalamus*, and other species in section *Sphaerigemma* should be retained in *Fargesia*. Our phylogenetic trees support the opinion that *Himalayacalamus* should be merged into *Drepanostachyum* (Soderstrom and Ellis 1987; Li 1997; Wang 1997), whereas others treated *Himalayacalamus* as a distinct genus closely related to *Drepanostachyum* (Stapleton 1994b; Ohrnberger 1999; Zhang et al. 2012). Additional phylogenetic research sampling more species in those genera will be necessary to disentangle their relationships and help make taxonomic revisions.

Consistent with morphological data, the present analyses corroborate the placement of the *Thamnocalamus* clade and the *Drepanostachyum* + *Himalayacalamus* clade, which is consistent with previous studies (Guo and Li 2004; Zhang et al. 2012; Yang et al. 2013; Ma et al. 2014; Attigala et al. 2016; Zhang et al. 2016; Wang et al. 2017). Several species which were treated as the members of *Fargesia* seems more reasonable to transfer into *Thamnocalamus* clade and the *Drepanostachyum* + *Himalayacalamus* clade. Thus, *Fargesia* (sensu Yi 1988a) is polyphyletic.

Phylogenetic relationships between *Fargesia* and *Yushania*

Yushania was considered the most closely allied genus to *Fargesia*, although it differs in having pachymorphic rhizomes with very long, symmetrical necks (Keng 1957; Wang and Ye 1980; Keng 1984, 1987; Stapleton 1994a; Yi 1982, 1985a, 1988a, 1996; Li et al. 2006) and inflorescences without subtending spathes (Wang et al. 2017). Chao et al. (1980) treated *Yushania* as a synonym of *Sinarundinaria*, which was subsequently shown to be a synonym of *Fargesia*, and this was also followed by Clayton and Renvoize (1986, 1989). The molecular systematic results on the relationship between these two genera are confusing. Neither *Fargesia* nor *Yushania* is monophyletic, and species in these two genera nest together in different clades with low to strong support under varying sampling strategies and gene markers (Guo et al. 2001, 2002; Guo and Li 2004; Wang et al. 2017). Several studies suggested that the phylogenetic relationships between these two genera were not resolved (Zeng et al. 2010; Zhang et al. 2012; Yang et al. 2013). Our results suggested that *Yushania* and *Fargesia* were indeed closely related. Further work including additional taxa is required to resolve relationships between the two genera.

Species in the *Fargesia* + *Yushania* clade share the following morphological features: rhizomes pachymorphic with short or long necks; inflorescences are on top of leafy branches, subtended by a series of leaf sheaths which expand to varying degrees, or even to spathes, and are not scaly, as in the *Drepanostachyum* + *Himalayacalamus* clade. Although the pulvini at the base of the spikelet stalks were considered an identifying characteristic of *Yushania* (Stapleton 1994b), they exist in most of *Yushania* species except *Y. lineolata*, and do not exist in *Fargesia* except in *F. yunnanensis* (Yi 1996). Therefore, this characteristic is labile in these two genera. The tessellation in the leaf venation was also considered a unique characteristic of *Yushania* (Stapleton 1994b), but this characteristic seems to not be critical to distinguish the two genera.

Our data also revealed that the *Fargesia* + *Yushania* clade is divided into a *Fargesia* spathe clade and a non-spathe clade. Previous molecular studies showed that species with the spathe-like leaf sheath syndrome formed a clade which is sister to other genera (Zhang et al. 2012, 2016; Wang et al. 2017), and some researchers treated *F. spathacea*, *F. nitida* and *F. murielae* as *Fargesia* (s.s.) (Guo and Li 2004). However, *Fargesia* species without the spathe-like leaf sheath syndrome nested with *Yushania*, *Phyllostachys*, *Chimonocalamus* and other genera (Zhang et al. 2012, 2016; Wang et al. 2017). Thus, in order to understand the phylogenetic relationships within the *Fargesia* + *Yushania* clade, more taxa from other related genera should be considered in further research before making any taxonomic conclusions.

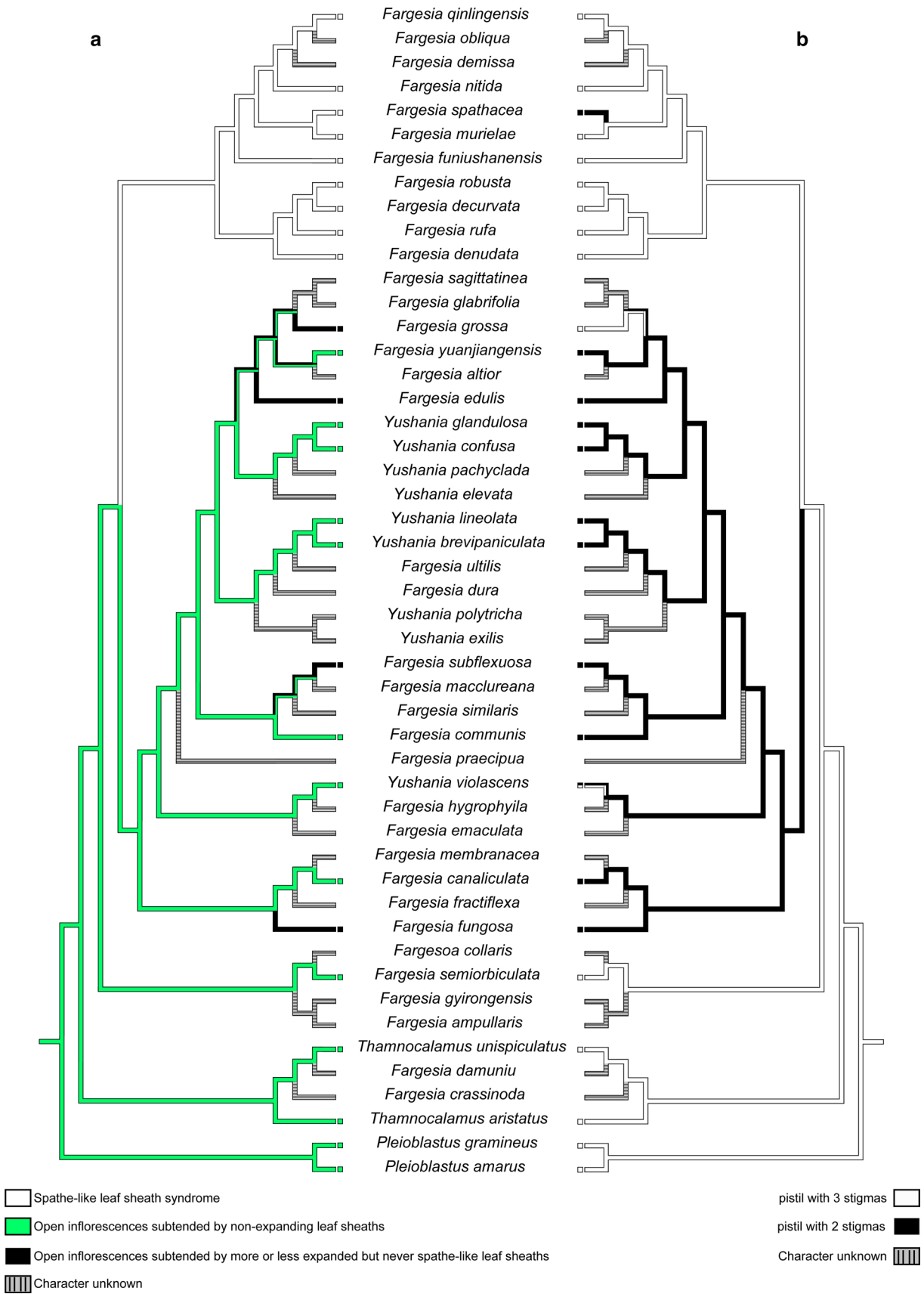


Fig. 3 Morphological character evolution optimized onto the tree from the analysis of combined data. **a** Evolution of the leaf sheaths of inflorescence and **b** the number of stigmas. Note that spathe-like leaf sheath syndrome represents the inflorescences are compressed, unilateral and subtended by a series of leaf sheaths which expand as spathes, of which the uppermost is generally longer than the inflorescence

Evolution of the spathe-like leaf sheath syndrome as an adaptation to low temperature

Yi (1988a) divided *Fargesia* into section *Sphaerigemma*, which have culm buds that are ovoid or pyramidal, thick and appressed with two early deciduous prophylls, and section *Fargesia*, which have culm buds that are long ovoid, thin and unappressed with two late deciduous prophylls. Section *Sphaerigemma* was divided into series *Collares*, which has unequal, i.e., thick and thin, branches at the same node and series *Ampullares*, which has equal branches. Section *Fargesia* was divided into four series: series *Murielae* with oblong or oblong-elliptical culm sheaths, while series *Fargesia*, series *Angustissimae* and series *Yunnanenses* have long triangular or oblong-triangular culm sheaths. This subdivision

was accepted by Yi (1996), and Yi et al. (2008) accepted only the subdivision of two sections.

Moreover, previous molecular studies showed that *Fargesia* (sensu Yi 1988a) might be a polyphyletic group with other closely related genera under limited sampling taxa and different markers (Guo et al. 2001, 2002; Guo and Li 2004; Zeng et al. 2010; Ma et al. 2014; Zhang et al. 2016; Wang et al. 2017). Our analyses with expanded *Fargesia* species found that the shape of the culm buds and the shape of the culm sheaths used by Yi (1988a) as the identity characteristic of sections and series, respectively, were inconsistent with the division of *Fargesia* (Fig. 1). Therefore, the morphological feature-based subdivision of *Fargesia* (sensu Yi 1988a) is not objective. These vegetative characteristics may not be a reliable diagnostic character for the delineation of the *Fargesia* + *Yushania* clade.

Interestingly, floral characters to some extent support the revision of the classification of the *Fargesia* + *Yushania* clade as indicated by molecular data. The ancestral inflorescences in the *Fargesia* + *Yushania* clade are open inflorescences with varying degrees of expansion but never spathe-like leaf sheaths, while the ancestral inflorescence types in the *Fargesia* spathe clade are compressed,

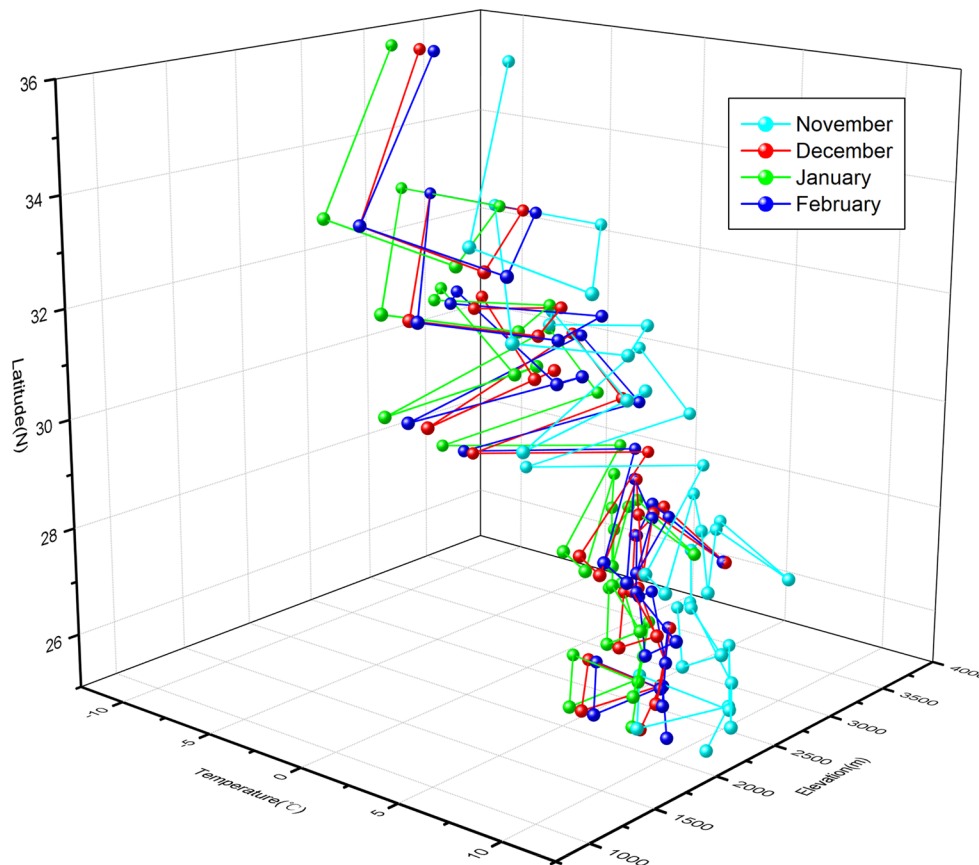


Fig. 4 Scatter diagram shows the relationship among average temperature in winter (X axes), elevation (Y axes) and latitude (Z axes)

unilateral and subtended by a series of persistent and delicate leaf sheaths that expand as spathes. Members of the *Fargesia* spathe clade occupy the northern part of the distribution, where it is colder in the spring than in the south (Figs. 2, 4). The temperature from November to February might influence flower bud differentiation. As shown in Lin et al. (2015), the flower buds of *Bambusa multiplex* initiated in October, differentiated into spikelets by March or April and the inflorescences bloomed from April to May (Lin et al. 2015). Changes in the size, color and structure of spathes have been reported as adaptive responses to temperature (Li et al. 2013). Furthermore, the structure of spathes could have influenced insect pollination as demonstrated in other plants (Ørgaard and Jacobsen 1998). Compared with the non-spathe clade, this derived spathe-like leaf sheath syndrome in the *Fargesia* spathe clade can be considered a “key innovation” or morphological novelty that allowed these species to adapt to the cold weather in the north, because this type of morphological novelty can open new adaptive zones (Nitecki 1990). In other words, the inflorescences of species in the *Fargesia* spathe clade might have proceeded via an acquisition of the spathe-like leaf sheath syndrome to protect young flowers from damage caused by low temperatures. Nevertheless, further studies are needed to test this hypothesis based on a broad taxonomic and geographic sampling.

In conclusion, our analyses suggested that recovered clades of *Fargesia* (Yi 1988a) and its closely related genera conflicted significantly with traditional morphological classification. All species of *Yushania* nested with most *Fargesia* species and formed *Fargesia* + *Yushania* clade. Only nine species of *Yushania* were examined in our study; therefore, more taxa will be required to be included to resolve relationships between the two genera. We also found that the spathe-like leaf sheath syndrome could be an adaptive advantage for species in the *Fargesia* spathe clade in cold environment. The tangled relationships recovered among these taxa demonstrate that the floral characters may be a reliable diagnostic character. Nevertheless, further analyses including more taxa from *Fargesia* and other related genera are required to understand the evolution of the different components of floral morphology.

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Compliance with ethical standards

Conflict of interest The authors of “Phylogeny of *Fargesia* (Poaceae: Bambusoideae) and infrageneric adaptive divergence inferred from three cpDNA and nrITS sequence data” declare that they have no conflict of interest.

Information on Electronic Supplementary Material

Online resource 1. Nucleotide sequence alignment matrix of 4 combined marker datasets (*psbA-trnH*, *matK*, *trnL-trnF*, nrITS) in the Nexus format.

Online resource 2. 50% majority-rule consensus tree based on the nrITS data.

Online resource 3. 50% majority-rule consensus tree based on the combined cpDNA data (*matK*, *psbA-trnH*, *trnL-F*).

References

- Attigala L, Wysocki WP, Duvall MR, Clark LG (2016) Phylogenetic estimation and morphological evolution of Arundinarieae (Bambusoideae: Poaceae) based on plastome phylogenomic analysis. *Molec Phylog Evol* 101:111–121. <https://doi.org/10.1016/j.ympev.2016.05.008>
- Bamboo Phylogeny Group (2012) An updated tribal and subtribal classification for the Bambusoideae (Poaceae). In: Gielis J, Potters G (eds) Proceedings of the 9th World Bamboo congress. World Bamboo Organization, Antwerp, pp 3–27
- Bouchenak-Khelladi Y, Salamin N, Savolainen V, Forest F, Van der Bank M, Chase MW, Hodkinson TR (2008) Large multi-gene phylogenetic trees of the grasses (Poaceae): progress towards complete tribal and generic level sampling. *Molec Phylog Evol* 47:488–505. <https://doi.org/10.1016/j.ympev.2008.01.035>
- Campbell JJN (1987) The history of sino-himalayan bamboo flowering, droughts and sun-spots. *J Bamboo Res* 6:1–15
- Chao CS, Renvoize SA (1989) A revision of the species described under *Arundinaria* (Gramineae) in Southeast Asia and Africa. *Kew Bull* 44:349–367. <https://doi.org/10.2307/4110809>
- Chao CS, Chu CD, Hsiung WY (1980) A revision of some genera and species of Chinese bamboos. *Acta Phytotax Sin* 18:20–36
- Chen SL, Sheng GY, Wen TH (1981) *Ampelocalamus*-A new genus of Chinese Bamboo. *Acta Phytotax Sin* 19:332–334
- Clayton WD, Renvoize SA (1986) Genera Graminum, grasses of the world. *Kew Bull Addit Ser XIII Her Majesty's Stationery Office, London*
- Darlu P, Lecointre G (2002) When does the incongruence length difference test fail? *Molec Biol Evol* 19:432–437. <https://doi.org/10.1093/oxfordjournals.molbev.a004098>
- Darriba D, Taboada GL, Doallo R, Posada D (2012) jModelTest 2: more models, new heuristics and parallel computing. *Nat Meth* 9:772. <https://doi.org/10.1038/nmeth.2109>
- Demoly JP (1991) Recensement des bambous cultivés en Europe. *Bambou Bull Assoc Europeene du Bambou Section France* 8:20–28
- Demoly JP (2005) Nouvelles combinaison nomenclaturales. *Bambou Bull Assoc Europeene du Bambou Section France* 46:6–8
- Doyle JJ, Doyle JL (1987) A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochem Bull* 19:11–15
- Felsenstein J (1985) Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* 39:783–791. <https://doi.org/10.2307/2408678>
- Franchet MA (1893) *Fargesia*, nouveau genre de Bambusées de la Chine. *Bull Mens Soc Linn Paris* 2:1067–1069
- Geng PC, Wang CP (1996) *Flora Reipublicae Popularis Sinicae*. Science Press, Beijing
- Guo ZH, Li DZ (2004) Phylogenetics of the *Thamnocalamus* group and its allies (Gramineae: Bambusoideae): inference from the sequences of *GBSSI* gene and nrITS spacer. *Molec Phylog Evol* 30:1–12. [https://doi.org/10.1016/S1055-7903\(03\)00161-1](https://doi.org/10.1016/S1055-7903(03)00161-1)

- Guo ZH, Chen YY, Li DZ, Yang JB (2001) Genetic variation and evolution of the alpine bamboos (Poaceae: Bambusoideae) using DNA sequence data. *J Pl Res* 114:315–322. <https://doi.org/10.1007/PL00013993>
- Guo ZH, Chen YY, Li DZ (2002) Phylogenetic Studies on *Thamnochalamus* group and its allies (Poaceae: Bambusoideae) based on nrITS sequence data. *Molec Phylogen Evol* 22:20–30. <https://doi.org/10.1006/mpev.2001.1039>
- Hijmans RJ, Guarino L, Mathur P (2012) DIVA-GIS, Version 7.5, Manual. Available at: <http://www.diva-gis.org>
- Hodkinson TR, Chonghaile GN, Sungkaew S, Chase MW, Salamin N, Stapleton CMA (2010) Phylogenetic analyses of plastid and nuclear DNA sequences indicate a rapid late Miocene radiation of the temperate bamboo tribe Arundinarieae (Poaceae, Bambusoideae). *Pl Ecol Diversity* 3:109–120. <https://doi.org/10.1080/17550874.2010.521524>
- Hsueh CJ, Li DZ (1987) New taxa of Bambusoideae from Sichuan and Yunnan, with discussion on concepts of related genera. *J Bamboo Res* 6:16–19
- Hsueh CJ, Yi TP (1979) Two new genera of Bambusoideae from S. W. China, 1. *Chimonocalamus* Hsueh et Yi. *Acta Bot Yunnan* 1:74–81
- Janzen DH (1976) Why bamboos wait so long to flower. *Annual Rev Ecol Syst* 7:347–391. <https://doi.org/10.1146/annurev.ev.07.110176.002023>
- Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Buxton S, Cooper A, Markowitz S, Duran C, Thierer T, Ashton B, Mentjies P, Drummond A (2012) Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* 28:1647–1649. <https://doi.org/10.1093/bioinformatics/bts199>
- Kelchner SA, Clark LG (1997) Molecular evolution and phylogenetic utility of chloroplast *rpl16* intron in *Chusquea* and the Bambusoideae (Poaceae). *Molec Phylogen Evol* 8:385–397. <https://doi.org/10.1006/mpev.1997.0432>
- Kellogg EA (2015) Flowering plants, monocots, Poaceae. In: Kubitzki K (ed) *The families and genera of vascular plants*. Springer, Basel, pp 1–416. <https://doi.org/10.1007/978-3-319-15332-2>
- Keng PC (1957) One new genus and two new species of Chinese bamboos. *Acta Phytotax Sin* 6:355–360
- Keng PC (1982) A revision of the genera of bamboos from the world. I. *J Bamboo Res* 1:1–19
- Keng PC (1983) A revision of the genera of bamboos from the world. III. *J Bamboo Res* 2:11–27
- Keng PC (1984) A revision of the genera of bamboos from the world. V. *J Bamboo Res* 3:22–42
- Keng PC (1987) On the nomenclature of the high-alpine bamboos from China. *J Bamboo Res* 6:11–17
- Keng PC, Wen TH (1989) A preliminary study on bamboo classification according to the vegetative characters. *J Bamboo Res* 8:17–29
- Lei KM, Zhang Y, Xiao WY, Cai YS, Ze J, Liu Y, Sun HG, Zeng T (2011) Characteristics of the flowering *Fargesia nitida* populations in Jiuzhaigou. *J Sichuan Forest Sci Technol* 32:92–95
- Li DZ (1997) The flora of China Bambusoideae project-problems and current understanding of bamboo taxonomy in China. In: Chapman GP (ed) *The Bamboos*. Academic Press, London, pp 61–81
- Li DZ (2003) *Flora yunnanica*, vol. 9. Science Press, Beijing
- Li DZ, Guo ZH, Stapleton CMA (2006) *Fargesia*. In: Wu ZY, Raven PH (eds) *Flora of China* (Poaceae). Science Press, Beijing, pp 74–96
- Li CH, Huang SR, Wang LL, Wang C, Yang GS (2013) Relationship between temperature, accumulation of mineral elements and “green ear” of anthurium spathe. *Chin J Trop Agric* 33:3–7
- Lin SY, Li J, Zhao R, Xu Q, Ding YL (2015) A research on the flowering biological characteristics of *Bambusa multiplex* in Nanjing City. *J Nanjing Forest Univ* 39:52–56
- Lu JL (1981) The new species of Bambusoideae from China. *J Henan Coll Agric* 74:70–79
- Ma PF, Zhang YX, Zeng CX, Guo ZH, Li DZ (2014) Chloroplast phylogenomic analyses resolve deep-level relationships of an intractable bamboo Tribe Arundinarieae (Poaceae). *Syst Biol* 63:933–950. <https://doi.org/10.1093/sysbio/syu054>
- Maddison WP, Maddison DR (2011) Mesquite: a modular system for evolutionary analysis, version 2.75. Available at: <http://mesquiteproject.org>. Accessed 30 Sept 2011
- McNeely JA (1999) Biodiversity and bamboo genetic resources in Asia: in situ, community-based and ex situ approaches to conservation. *Chin Biodivers* 7:38–51
- Miller MA, Pfeiffer W, Schwartz T (2010) Creating the CIPRES Science Gateway for inference of large phylogenetic trees. In: *Proceedings of the gateway computing environments workshop (GCE)*. New Orleans, US, pp 1–8
- Müller K (2005) SeqState. *Appl Bioinformatics* 4:65
- Nakai T (1935) Novitates Bambusacearum in imperio Japonico recentissime detectae III. *J Jap Bot* 11:1
- Nitecki MH (1990) *Evolutionary innovations*. University of Chicago Press, Chicago
- Ohrnberger D (1996) *The bamboos of the world: introduction to the work*. Blackwell Ltd., Beaver House, England, p 14
- Ohrnberger D (1999) *The bamboos of the world: annotated nomenclature and literature of the species and the higher and lower taxa*. Elsevier Science, Amsterdam
- Ørgaard M, Jacobsen N (1998) SEM study of surface structures of the spathe in *Cryptocoryne* and *Lagenandra* (Araceae: Aroideae: Cryptocoryneae). *Bot J Linn Soc* 126:261–289. <https://doi.org/10.1006/boj.1997.0136>
- Peng S, Yang HQ, Li DZ (2008) Highly heterogeneous generic delimitation within the temperate bamboo clade (Poaceae: Bambusoideae): evidence from *GBSSI* and ITS sequences. *Taxon* 57:799–810
- Posada D (2008) jModelTest: phylogenetic model averaging. *Molec Biol Evol* 25:1253–1256. <https://doi.org/10.1093/molbev/msn083>
- Rambaut A, Suchard MA, Xie D, Drummond AJ (2014) Tracer, version 1.6. Available at: <http://beast.bio.ed.ac.uk/Tracer>
- Ronquist F, Huelsenbeck JP (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19:1572–1574. <https://doi.org/10.1093/bioinformatics/btg180>
- Ruiz-Sanchez E, Sosa V, Mejía-Saules MT (2008) Phylogenetics of *Otatea* inferred from morphology and chloroplast DNA sequence data, and circumscription of Guaduinae (Poaceae: Bambusoideae). *Syst Bot* 33:277–283. <https://doi.org/10.1600/036364408784571644>
- Seifert E (2014) Origin Pro 9.1: scientific data analysis and graphing software-software review. *J Chem Inf Model* 54(5):1552. <https://doi.org/10.1021/ci500161d>
- Shimodaira H (2002) An approximately unbiased test of phylogenetic tree selection. *Syst Biol* 51:492–508. <https://doi.org/10.1080/10635150290069913>
- Shimodaira H, Hasegawa M (2001) CONSEL: for assessing the confidence of phylogenetic tree selection. *Bioinformatics* 17:1246–1247. <https://doi.org/10.1093/bioinformatics/17.12.1246>
- Simmons MP, Ochoterena H (2000) Gaps as characters in sequence-based phylogenetic analyses. *Syst Biol* 49:369–381. <https://doi.org/10.1093/sysbio/49.2.369>
- Soderstrom TR (1979a) The bamboozling *Thamnochalamus*. *Garden* 3:22–27
- Soderstrom TR (1979b) Another name for the umbrella bamboo. *Brittonia* 31:495. <https://doi.org/10.2307/2806007>

- Soderstrom TR (1981) Some evolutionary trends in the Bambusoideae (Poaceae). *Ann Missouri Bot Gard* 68:15–47. <https://doi.org/10.2307/2398809>
- Soderstrom TR, Ellis RP (1982) Taxonomic status of the endemic South African bamboo, *Thamnocalamus tessellatus*. *Bothalia* 14:53–67. <https://doi.org/10.4102/abc.v14i1.1135>
- Soderstrom TR, Ellis RP (1987) The position of bamboo genera and allies in a system of grass classification. In: Soderstrom TR, Hilu KW, Campbell S, Barkworth ME (eds) *Grass systematics and evolution*. Institution Press, Washington, DC, pp 225–238
- Soderstrom TR, Ellis RP (1988) The woody bamboos (Poaceae: Bambuseae) of Sri Lanka: a morphological–anatomical study. *Smithsonian Contr Bot* 72:1–75. <https://doi.org/10.5479/si.0081024X.72>
- Stamatakis A (2006) RA×ML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* 22:2688–2690. <https://doi.org/10.1093/bioinformatics/btl446>
- Stapleton CMA (1994a) The bamboos of the Nepal and Bhutan Part I: *Bambusa*, *Dendrocalamus*, *Melocanna*, *Cephalostachyum*, *Teinostachyum*, and *Pseudostachyum* (Gramineae: Poaceae, Bambusoideae). *Edinburgh J Bot* 51:1–32. <https://doi.org/10.1017/S0960428600001682>
- Stapleton CMA (1994b) The bamboos of the Nepal and Bhutan Part II: *Arundinaria*, *Thamnocalamus*, *Borinda*, and *Yushania* (Gramineae: Poaceae, Bambusoideae). *Edinburgh J Bot* 51:275–295. <https://doi.org/10.1017/S0960428600000883>
- Stapleton CMA (1994c) The bamboos of the Nepal and Bhutan Part III: *Drepanostachyum*, *Himalayacalamus*, *Ampelocalamus*, *Neomicrocalamus* and *Chimonobambusa* (Gramineae: Poaceae, Bambusoideae). *Edinburgh J Bot* 51:301–330. <https://doi.org/10.1017/S0960428600001815>
- Stapleton CMA (1997) Morphology of woody bamboos. In: Chapman GP (ed) *The Bamboos*. Academic Press, London, pp 167–251
- Stapleton CMA, Li DZ, Xia NH (2005) New combinations for Chinese bamboos (Poaceae, Bambuseae). *Novon* 15:599–601
- Stapleton CMA, Chonghaile GN, Hodkinson TR (2009) Molecular phylogeny of Asian woody bamboos: Review for the Flora of China. *Bam Sci Cult: J Amer Bamboo Soc* 22:5–25
- Sungkaew S, Stapleton CMA, Salamin N, Hodkinson TR (2009) Non-monophyly of the woody bamboos (Bambuseae; Poaceae): a multi-gene region phylogenetic analysis of Bambusoideae. *J Pl Res* 122:95–108. <https://doi.org/10.1007/s10265-008-0192-6>
- Swofford DL (2002) PAUP*: phylogenetic analysis using parsimony (*and other methods), version 4.0 Beta. Sinauer Ass. Inc., 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. <https://doi.org/10.1007/BF00037152>
- Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S (2011) MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molec Biol Evol* 28:2731–2739. <https://doi.org/10.1093/molbev/msr121>
- Triplett JK, Clark LG (2010) Phylogeny of the temperate bamboos (Poaceae: Bambusoideae: Bambuseae) with an emphasis on *Arundinaria* and allies. *Syst Bot* 35:102–120. <https://doi.org/10.1600/036364410790862678>
- Viana PL, Figueiras TS, Paiva EAS (2011) A new combination in *Aulonemia* (Poaceae: Bambusoideae: Bambuseae) based on floral analysis, anatomical features, and distribution. *Brittonia* 63:102–112. <https://doi.org/10.1007/s12228-010-9138-0>
- Wang CP (1997) A proposal concerning a system of classification of Bambusoideae from China. *J Bamboo Res* 16:1–6
- Wang CP, Ye GH (1980) On the problems of the classification of Chinese bamboos with creeping rhizomes. *Acta Phytotax Sin* 18:283–291
- Wang ZP, Ye GH (1981) Miscellaneous notes on Chinese Bambusoideae. *J Nanjing Univ (Nat Sci)* 1:91–108
- Wang XQ, Ye XY, Zhao L, Li DZ, Guo ZH, Zhuang HF (2017) Genome-wide RAD sequencing data provide unprecedented resolution of the phylogeny of temperate bamboos (Poaceae: Bambusoideae). *Sci Rep* 7:11546. <https://doi.org/10.1038/s41598-017-11367-x>
- Wen TH (1984) New taxa of Bambusoideae in China (I). *J Bamboo Res* 3:23–47
- Wen TH (1989) Some new bamboos from Southern Yangtze River. *J Bamboo Res* 8:13–24
- White TJ, Bruns T, Lee S, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis M, Gelfand D, Sninsky J, White T (eds) *PCR protocols: a guide to methods and applications*. Academic Press, San Diego, pp 315–322
- Yang HM, Zhang YX, Yang JB, Li DZ (2013) The monophyly of *Chimonocalamus* and conflicting gene trees in Arundinarieae (Poaceae: Bambusoideae) inferred from four plastid and two nuclear markers. *Molec Phylogenet Evol* 68:340–356. <https://doi.org/10.1016/j.ympev.2013.04.002>
- Yi TP (1982) A revision of the genera of *Fargesia* group in China. *J Sichuan Forest Sci Technol* 2:54–59
- Yi TP (1983a) New taxa of Bambusoideae from Xizang (Tibet), China. *J Bamboo Res* 2:28–46
- Yi TP (1983b) New species of *Fargesia* Franchet and *Yushania* Keng f. from Tibet. *J Bamboo Res* 2:18–53
- Yi TP (1983c) A new species of bamboo from island Hainan, China. *Bull Bot Res* 3:151–154
- Yi TP (1985a) Classification and distribution of the food bamboos of the Giant Panda (I). *J Bamboo Res* 4:11–27
- Yi TP (1985b) Classification and distribution of the food bamboos of the Giant Panda (II). *J Bamboo Res* 4:20–45
- Yi TP (1985c) New taxa of bamboo from China. *Bull Bot Res* 5:121–137
- Yi TP (1986) A new species of *Fargesia* from Sichuan. *Acta Bot Yunnan* 8:48–50
- Yi TP (1988a) A study on the genus *Fargesia* from China. *J Bamboo Res* 7:1–119
- Yi TP (1988b) Four new species of bamboo from South Yunnan, China. *Acta Bot Yunnan* 10:437–443
- Yi TP (1989) Two new species of bamboo from Southwest Sichuan. *Acta Bot Yunnan* 11:35–38
- Yi TP (1992) New bamboos of *Fargesia* and *Chimonobambusa* from Sichuan. *Acta Bot Yunnan* 14:135–138
- Yi TP (1996) *Fargesia*, *Yushania*. In: Geng PC, Wang ZP (eds) *Flora reipublicae popularis sinicae*. Science Press, Beijing, pp 387–560
- Yi TP (2000) A new species of *Fargesia* from northeastern Sichuan, China. *Acta Bot Yunnan* 22:251–254
- Yi TP, Long TL (1989) Two new species of bamboos for Giant Panda. *J Bamboo Res* 8:30–36
- Yi TP, Shao JX (1987) New taxa of *Fargesia* from Shaanxi. *J Bamboo Res* 6:42–45
- Yi TP, Zhu XB (2012) A new species and two combinations of Bambusoideae (Poaceae). *J Sichuan Forest Sci Technol* 33:8–11
- Yi TP, Shi JY, Yang L (2007) Alpine new bamboos from Sichuan, Tibet and Chongqing, China. *Bull Bot Res* 27:515–520
- Yi TP, Shi JY, Ma LS (2008) *Iconographia bambusoidearum sinicarum*. Science Press, Beijing
- Zeng CZ, Zhang YX, Triplett JK, Yang JB, Li DZ (2010) Large multi-locus plastid phylogeny of the tribe Arundinarieae (Poaceae: Bambusoideae) reveals ten major lineages and low rate of

- molecular divergence. *Molec Phylogen Evol* 56:821–839. <https://doi.org/10.1016/j.ympev.2010.03.041>
- Zhang WP, Clark LG (2000) Phylogeny of classification of the Bambusoideae (Poaceae). In: Jacobs SWL, Everett JE (eds) *Grasses: systematics and evolution*. CSIRO, Collingwood, pp 35–42
- Zhang YQ, Ren Y (2016) Supplementary description of flower and flowering branch for four *Fargesia* species and one *Drepanostachyum* species (Bambusoideae, Poaceae), and notes on their taxonomy. *Nordic J Bot* 34:565–572. <https://doi.org/10.1111/njb.00975>
- Zhang YX, Zeng CX, Li DZ (2012) Complex evolution in Arundinarieae (Poaceae: Bambusoideae): incongruence between plastid and nuclear *GBSSI* gene phylogenies. *Molec Phylogen Evol* 63:777–797. <https://doi.org/10.1016/j.ympev.2012.02.023>
- Zhang XZ, Zeng CX, Ma PF, Haevermans T, Zhang YX, Zhang LN, Guo ZH, Li DZ (2016) Multi-locus plastid phylogenetic biogeography supports the Asian hypothesis of the temperate woody bamboos (Poaceae: Bambusoideae). *Molec Phylogen Evol* 96:118–129. <https://doi.org/10.1016/j.ympev.2015.11.025>