Bamboo Taxonomy and Diversity in the Era of Molecular Markers

MALAY DAS,* SAMIK BHATTACHARYA,† PARAMJIT SINGH,‡
TARCISO S. FILGUEIRAS,§ AND AMITA PAL†

*US Environmental Protection Agency, National Health and Environmental Effects Research Laboratory, Western Ecology Division, 200 S.W. 35th Street, Corvallis 97333, Oregon
†Plant Molecular & Cellular Genetics, Bose Institute, Kolkata 700054, India
‡Botanical Survey of India, CGO Complex, Salt Lake, Kolkata 700064, India
§Reserva Ecológica do IBGE, C.P. 08770, Brasilia-DF 70312-970, Brazil

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ABSTRACT

A total of ~1400 species of bamboos are grouped under the sub-family Bambusoideae within the family Poaceae. The plant group harbours both herbaceous and woody members while the taxonomy has traditionally been dependent on morphological characters. Classification systems proposed to date need further support, and taxonomic delineation at lower levels often lack sufficient resolution. Infrequent flowering events and extensive genome polyploidization are an additional challenge for the woody group. The tremendous advancement of molecular marker technologies holds the promise to address different needs of bamboo taxonomy (systematics and identification) and diversity studies. One of the most important prerequisites is to apply the appropriate molecular tool at the proper taxonomic level. More studies are required to better understand the population level genetic diversity in bamboo.

I. INTRODUCTION

A. ORIGIN, SYSTEMATIC POSITION AND HABIT

Bamboos are members of the sub-family Bambusoideae within the grass family Poaceae. Grass family is monophyletic and the early diverging lineages recognized within the family are Anomochlooideae, Pharoideae and Puelioideae (Grass Phylogeny Working Group [GPWG], 2001). Anomochlooideae lacks a true spikelet and is sister to the rest of the family members. Pharoideae is the earliest lineage from the true spikelet-bearing group and was followed by Puelioideae. The earliest fossil evidence for grasses was reported

sometimes between Paleocene and Eocene ages (Crepet and Feldman, 1991). According to the fossil species of *Pharus*, the early diversification of the family started between late Eocene and early Oligocene (Poinar and Columbus, 1992) and extensive diversification occurred by Miocene (Thomasson, 1987). Most possibly, the major radiations of the grasses including Bambusoideae happened 40–50 million years ago (Malcomber *et al.*, 2006). Very recently the first petrified bamboo fossil, *Guadua zuloagae* sp. nov, was reported from the Pliocene age (Brea and Zucol, 2007).

Traditionally, the members of the group share some common features that include rhizomatous habit, hollow segmented culms, petiolate blade with tessellate venation, flowers with three or more lodicules, usually with six stamens, and fruit possess small embryo and linear hilum (Soderstrom, 1981). Few synapomorphic features which are unique for Bambusoideae were reported by GPWG (2001). Leaf blade is mainly constituted of mesophyll tissue with asymmetrically invaginated arm cells, while pseudo-petiole structures are secondary gain for the sub-family. It is broadly divided into two tribes, that is Bambuseae/woody bamboos and Olyreae/herbaceous bamboos depending on the presence (Bambuseae) or absence (Olyreae) of the abaxial ligule (GPWG, 2001; Zhang and Clark, 2000).

B. GEOGRAPHICAL DISTRIBUTION

Bamboos are distributed all over the world, but major species richness is found in Asia Pacific (China: 626, India: 102, Japan: 84, Myanmar: 75, Malaysia: 50 and few others) and South America (Brazil: 134, Venezuela: 68, Colombia: 56 and few others) while least (5) in Africa (Bystriakova *et al.*, 2003a,b). The herbaceous bamboos with ~110 species are mostly concentrated in the Neotropics of Brazil, Paraguay, Mexico, Argentina and West Indies (Judziewicz *et al.*, 1999, Fig. 1). Brazil is the most prominent place representing 89% of the genera and 65% of the species that are reported from the New World (Filgueiras and Goncalves, 2004). The largest natural bamboo forests, known as 'tabocais' in Brazil and 'pacales' in Peru, cover 600,000 ha across Brazil, Peru and Bolivia (Filgueiras and Goncalves, 2004). The woody bamboos are unique with complex branching patterns, woody culm and gregarious, monocarpic flowering (Fig. 2). There are ~1290 species and they are universally distributed except in Europe which has no native species. They are classified into three major groups: the paleotropical woody bamboos (distributed in tropical and sub-tropical regions of Africa, Madagascar, India,

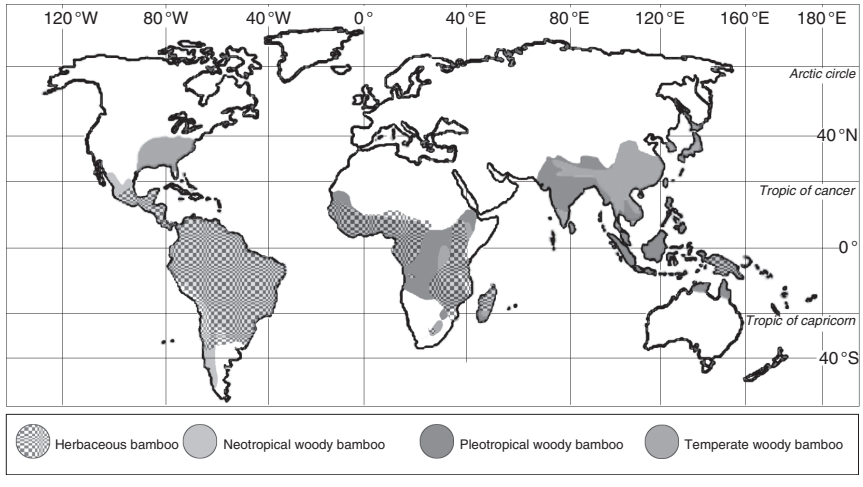


Fig. 1. World distribution of woody (paleotropical, neotropical, temperate) and herbaceous bamboos (modified and compiled from http://www.eeob.iastate.edu/research/bamboo/maps.html).

Fig. 2. An example of gregarious flowering in woody bamboo (*Thamnocalamus spathiflorus* subsp. *spathiflorus*) covering an area of ~3.5 km^2 at an altitude of 3000 m in Sikkim, India (recorded during August 2006).

Sri Lanka, Southern China, Southern Japan and Oceania, Fig. 1), the neotropical woody bamboos (Southern Mexico, Argentina, Chile, West Indies) and the north temperate woody bamboos (mostly in North temperate zone and few at high elevation habitats in Africa, Madagascar, India and Sri Lanka, http://www.eeob.iastate.edu/bamboo/maps.html).

C. USE

Bamboos are popularly known as poor man's timber for their multipurpose use in the rural life of many Asian countries. Thin culms with narrow cavities are popularly used as umbrella handles, fishing rods and flutes, while mature culms are widely used in constructing mud huts, mats, baskets and fences. Highly nutritious leaves (Khatta and Katoch, 1983) as well as young shoots are often used for preparing delicious soups and pickles. Adult culms are useful for the production of high quality charcoal (Park and Kwon, 1998) along with the fibres which are ideal for paper and pulp production. Because of a high growth rate (typically matures within 5–7 years) plus a number of important fuel characteristics such as low ash content, alkali index or heating value, bamboo is a promising energy crop for future (for details, see Scurlock *et al*., 2000). The fate of a number of endangered as well as wild species is intimately linked with bamboos (http://www.unep.org/cpi/briefs/Bamboo). For instance, leaves of *Sasa senanesis*, *S. kurilensis* and *S. nipponica* constitute a major part of the winter diet for Hokkaido voles (*Clethrionomys rufocanus*) when most other plants wither (Stenseth *et al*., 2003). In central Brazil, the wild stands of *Actinocladum verticillatum* and *Filgueirasia* spp. constitue a valuable fodder resource for both livestock and wildlife during the dry season when rest of the vegetation sheds their leaves (Filgueiras, 2002). The hollow bamboo culms provide refuge to many invertebrates and the inadvertent link to giant panda is well known.

D. CHROMOSOME NUMBER AND GENOME SIZE

The basic chromosome number for most woody members is $x = 12$, the same as rice, while for herbaceous bamboo it is synapomorphic ($x = 11$; GPWG, 2001). Occurrence of polyploidization has been reported in the woody bamboos (Pohl and Clark, 1992; Soderstrom, 1981). Cytological studies indicated the existence of two distinct sections, tropical (hexaploids, $2n = 6x = 72$) and temperate (tetraploids, $2n = 4x = 48$) within the woody group (Clark *et al*., 1995; Ghorai and Sharma, 1980; Kellogg and Watson, 1993). It was later supported by the flow cytometric estimation of the genomic DNA content (Gielis *et al*., 1997a). Temperate bamboos had higher DNA content (4.17–5.3 pg) than tropical ones (2.34–3.23 pg). According to the most recent estimate (Gui *et al*., 2007), the genome size of tetraploid *Phyllostachys pubescens* is ~2034 Mb, which is 5.4-fold larger than that of the diploid cultivated rice and 1.9-fold larger than that of tetraploid wild rice, while little less (86.92%) than that of maize genome. Their analysis utilizing 996 genome survey sequences (GSS) covering 0.92 Mb regions revealed that

23.28% of the genome consisted of repeat elements. Although it indicates that there might be some direct relationship between the higher genome size and the proportion of repeat elements present, more coverage is essential prior to confirming any such presumption. Soderstrom (1981) pointed out that Southeast Asia is a centre of distribution for tetraploid and hexaploid bamboos. Hsu (1967, 1972) reported one diploid species of *Phyllostachys* and one of *Arundinaria* from China. Ruiyang (2003) on the basis of an exhaustive chromosome analysis on 185 species from 33 genera and 6 subtribes has shown the variation in chromosome numbers for some species of *Bambusa* and *Dendrocalamus*.

II. IMPLICATIONS OF VARIOUS MORPHOLOGICAL FEATURES IN BAMBOO TAXONOMY

Bamboo taxonomy like all other plant groups has traditionally been built upon various morphological features and several classification systems have been proposed to date.

A. RHIZOMES

Rhizome is the horizontally grown underground plant part that often sends out roots and shoots (culms) from its nodes. The existence of two basic forms of rhizome was first indicated by Riviere and Riviere (1879) on the basis of their observation of two different growth habits in two bamboo genera. The caespitose habit was studied in *Gigantochloa* while the spreading habit was observed in *Phyllostachys*. These two different forms of rhizomes were later recognized as monopodial and sympodial type (McClure, 1925) and were further redefined as leptomorph and pachymorph type (McClure, 1966). The terms leptomorph and pachymorph were preferred over monopodial and sympodial as the later terms were more related to the branching patterns and clump forms than the actual morphological forms of the rhizome (McClure, 1966). In sympodial type, the culms usually grow in clumps and the rhizomes are usually short, thickened and spindle shaped (Fig. 3A). Sometimes the neck length (distance between the point of origin of two culms) in sympodial rhizome is relatively longer and thus generates less tufted culms that look like the monopodial rhizome as observed in *Melocanna* (Fig. 3B). In the monopodial or running type, the rhizome grows horizontally without frequent, upright culm repetition and hence culms always grow in isolation (Fig. 3C). They are usually slender and hollow (Wong, 2004). Sometimes a mixed situation of both monopodial and sympodial types is observed and known as amphipodial type (Fig. 3D). However, the taxonomic importance of

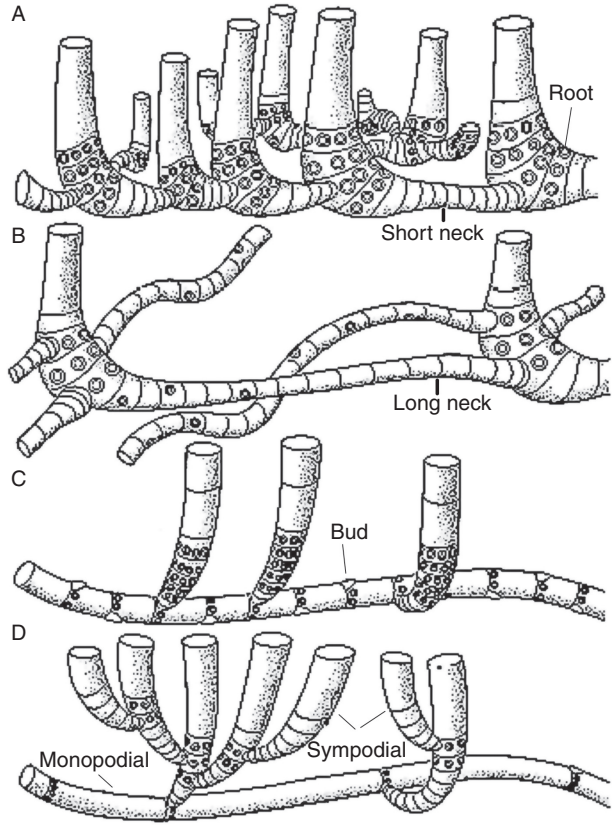


Fig. 3. Basic rhizome types in bamboo. (A) sympodial (pachymorph) rhizome with short necks, (B) sympodial (pachymorph) rhizome with long necks, (C) monopodial (leptomorph) rhizome with nodal bud and (D) amphipodial (amphimorph) rhizome with mixed sympodial and monopodial types (redrawn from Soderstrom and Young, 1983 with permission from Missouri Botanical Garden Press).

rhizome, at least for the old world bamboo at genera or supra-generic level, is well recognized (Stapleton, 1997).

B. BRANCHING, BUD AND LEAF CHARACTERS

The first extensive study to understand the importance of branch and bud characteristics was undertaken by Usui (1957) and was subsequently carried out by McClure (1966). A fundamental difference with respect to the branching pattern was noticed between the tropical and temperate groups. A basic and ancestral branching pattern was observed in most tropical genera, while the temperate group represented both basic (*Arundinaria and*

Thamnocalamus) and complex (*Fargesia, Yushania and Borinda*) branching patterns (Stapleton, 1994b). Branch complements are most informative at the mid culm position as they are not well developed at the lower culm body (Wong, 2004). Chao *et al*. (1980) have used branching characters to revise few Asian genera. Bud characteristics have been found to be useful for resolving generic level confusions. For instance, McClure (1966) treated *Pleioblastus* as a synonym of *Arundinaria*, although they were later differentiated by bud closure characteristics. In addition, the taxonomic and evolutionary significance of prophyll gained special attention. Modification of prophylls into protective bud-scale was a result of fusion events, while sheath reduction can explain insertion of multiple buds as one observed in *Chusquea culeou* (Stapleton, 1991).

Two functionally different forms of leaves are observed in bamboo. The culm leaves (culm-sheath) play protective roles for younger shoots, while the green foliage leaves are basically for photosynthetic purposes. The basal part of culm leaf surrounds the internode, while the upper part is usually free and known as the sheath blade. The juncture between the two parts is known as the ligule and is an important taxonomic character. Various other characteristic features such as adaxial plus abaxial hairs, auricle (tiny appendages at the base of lamina on both sides) and sheath blade are useful for quick identification of species in the field. Gamble (1896) was the first one to extensively use various culm leaf features at species level and were later employed at generic level too (Nakai, 1925). They are often very informative even for higher taxonomic rank such as sub-family. For instance, Bambuseae and Olyreae are clearly differentiated on the basis of presence or absence of the abaxial ligule.

While important culm leaf characteristics were mostly restricted to macromorphology, the anatomical features of foliage leaves gained special attention in bamboo taxonomy. However, conflicts persisted and in many instances the taxonomic delineation based on the anatomical features was not fully supported by the morphological features. In one such study, 11 anatomical features of African and Asian bamboos were investigated (Soderstrom and Ellis, 1982). *Arundinaria tessellata* shared 10 out of 11 characters with *Thamnocalamus spathiflorus* and hence the new *Thamnocalamus tessellatus* was synthesized. However, the closeness between *Arundinaria* and *Thamnocalamus* was contradicted by their own datasets that revealed that only 5 out of 11 characters were overlapping between *A. tessellata* and *T. aristatus*. Similarly, studies on the leaf anatomy of Sri Lankan bamboo revealed close proximity between *Bambusa bamboos* and the members of Arundinariinae rather than other species of Bambusinae (Soderstrom and Ellis, 1988). It is possible that leaf anatomical characters

might not be a good choice for generic delineation but could have potential for lower taxonomic levels.

C. INFLORESCENCE, FLOWER AND FRUIT CHARACTERS

On the basis of the flowering cycle, bamboos have been categorized into three major groups (Brandis, 1899): annual flowering (*Indocalamus wightianus*, *Ochlandra* sp.), sporadic or irregular flowering (*Chimonobambusa* sp., *Dendrocalamus hamiltonii*) and gregarious flowering that occurs at long intervals with synchronized seeds production (*Bambusa bambos, B. tulda, Dendrocalamus strictus*). A majority of bamboos belong to the third category where the intermast period may range from 3 to 120 years (Janzen, 1976).

Like all other grasses, the flowers of Bambusoideae are arranged in bracteate units on the rachilla and are known as spikelets. Each spikelet is subtended by many empty (without flower) bracts called glumes followed by one to several specialized bracts called lemma which provides protection to the flowers (Fig. 4A). In addition to the lemma, each flower is covered by another bract, sometimes membranaceous, called the palea. Both lemma and palea are vegetative in origin (Stapleton, 1997). Flowers are sessile and a proper perianth is substituted by three lodicules. Bracts subtending to bamboo inflorescence followed a gradual reduction process for other grasses in general. It is believed that the fully bracteate inflorescence such as *Bambusa* had to loose bracts to develop the ebracteate grass panicle (Holttum, 1958). The spikelets of Olyreae are usually unisexual and one flowered. In Bambuseae it is bisexual and both spikelets and pseudo-spikelets are present. The later is often bracteate and re-branching (GPWG, 2001; Judziewicz *et al.*, 1999). Pseudo-spikelets are also characterized by the presence of specialized branch-bud bearing bracts at the base of the rachilla, which is not observed in a true spikelet (Fig. 4B). Another important fundamental difference is that true spikelets are always single, while pseudo-spikelets are in groups.

McClure (1934) first introduced the concept of different types of spikelets in bamboo. In addition to the idea of spikelets and pseudo-spikelets, he also conceptualized semelauctant or determinate and iterauctant or indeterminate inflorescence types (1966). The inflorescence type that had pseudo-spikelets as the basic unit was considered as indeterminate because it was capable of re-branching and producing new flowers for almost an indefinite period of time. On the other hand, the determinate inflorescence was primarily based on true spikelets that lack the capability of indefinite growth. Keng (1983) proposed two sub-tribes within the woody bamboos (Bambusoideae) primarily based on the determinate and indeterminate inflorescence types, which was subsequently adopted in the Flora Reipublicae Popularis Sinicae

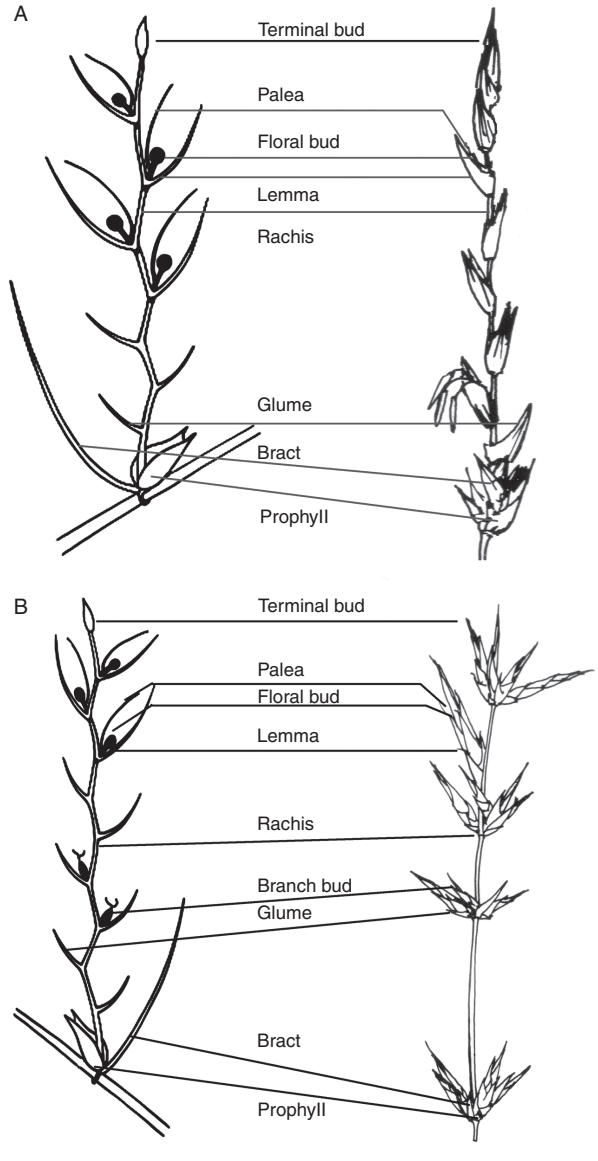


Fig. 4. A comparative account of (A) spikelet and (B) pseudo-spikelet inflorescences. Left panels represent schematic diagrams and right panels represent (A) spikelet of *Chimonobambusa callosa* and (B) pseudo-spikelet of *Bambusa tulda*.

(FRPS, Keng and Wang, 1996). Recently, a further enhanced version of FRPS has been published which includes several new taxa and provides a more detailed account of the Chinese bamboos (Flora of China, 2007).

It is generally accepted that typical bamboo flowers are monochlamydeous while the pseudo-spikelet of *Streptochaeta* was interpreted as a reduced form with a single terminal achlamydeous flower (Soderstrom, 1981). However, it is not always easy to define the inflorescence types in bamboos and several contradictory interpretations have been noticed in many cases. For instance, the inflorescence of *Racemobambos* was recognized as iterauctant by Chao and Renvoize (1989), while semelauctant by Dransfield (1992). Similar confusion has also been observed for its allied genus *Neomicrocalamus. N. prainii* was considered as semelauctant by Keng (1983) and Stapleton (1994c), while iterauctant by Wen (1986) and Dransfield (1992). The use of the term synflorescence (aggregation of spikelet) instead of inflorescence has also been proposed in bamboos (Stapleton, 1997). The various inflorescence types and floral features are of high taxonomic significance at all levels. For instance, presence or absence of lodicule is a key character for generic delineation. The number of stamens is often used to differentiate many closely related genera such as *Sinobambusa*/*Indosasa*, *Indocalamus*/*Sasa* and *Arundinaria*/*Acidosasa*. On the basis of the absence of ovary appendage, Holttum (1956) moved the Asiatic species of the genus *Oxytenanthera* to either *Dendrocalamus* or *Gigantochloa*. Soderstrom and Ellis (1988) described a new genus *Pseudoxytenanthera*, while Majumdar (1989) created a new genus *Pseudotenanthera* to accommodate some of the Indian and Sri Lankan species.

Bamboo fruits are usually one seeded, dry caryopsis structures, while in few cases (*Melocanna, Dinochloa, Ochlandra*) these are fleshy and pear shaped.

III. BAMBOO CLASSIFICATIONS BASED ON MORPHOLOGICAL FEATURES

Based on gross morphological features, many classification systems have been proposed till date. Munro's description (1868) was one of the earliest attempts that described 170 species under 20 genera. This classification system was primarily based on that of Nees von Esenbeck (1835). Bentham (1881) followed Nees and Munro with slight modifications and recognized four major groups. Gamble used important collections and notes of Wilhelm Sulpiz Kurz (1876), who was contemporary to Munro and had developed a comprehensive treatise on bamboos of British India (1896). Gamble's classification covered 15 genera and 115 species with elaborate descriptions and basically followed Bentham in placing the genera under four groups. Camus (1913) pooled the information from the work of Munro and Gamble and compiled 490 species

under 33 genera in his monograph '*Les Bambusées*'. McClure's description (1961) of the woody members of Bambusoideae was another landmark that was further improved by Parodi (1961) who included the herbaceous members in his treatment. He further divided the herbaceous members into three tribes: Olyreae, Phareae and Streptochaeteae. Soderstrom and Ellis (1987) considered a total of 11 tribes within Bambusoideae. Five of them were monophyletic and recognized as 'core' Bambusoideae with four herbaceous tribes (Olyreae, Anomochloeae, Streptochaeteae and Buergersiochloeae) and one woody tribe (Bambuseae). All the remaining six (Streptogyneae, Puelieae, Guaduelleae, Phareae, Oryzeae and Zizanieae) were considered as 'peripheral' tribes. The proposed 'core' Bambusoideae of Soderstrom and Ellis (1987) was similar to the circumscription proposed by Roshevits (1946) except for Parianeae. Prat (1960) only considered the woody members within Bambusoideae and moved the herbaceous taxa to Oryzoideae. Clayton and Renvoize (1986) and Renvoize and Clayton (1992) combined the 'core' and 'peripheral' Bambusoideae together. They also merged Guaduelleae and Puelieae to Bambuseae and Buergersiochloeae to Olyreae. They further subdivided tribe Bambuseae and recognized only 49 genera in three subtribes, that is Arundinariinae (20 genera), Bambusinae (25 genera) and Melocanniae (4 genera). Tzvelev (1989) recognized the members of 'core' Bambusoideae in a separate subfamily and all other grasses were placed under Pooideae. Watson and Dallwitz (1992) basically supported Clayton and Renvoize (1986) and Renvoize and Clayton (1992) except Centotheceae, which was included into Bambusoideae. Kellogg and Campbell (1987) considered Bambuseae as monophyletic based on the presence of woody culms and the herbaceous bamboos as either monophyletic or paraphyletic to Bambuseae. In a subsequent study, Kellogg and Watson (1993) also revised that the 'core' Bambusoideae as recognized by Soderstrom and Ellis (1987) were not monophyletic, but rather polyphyletic. Dransfield and Widjaja (1995) included 69 woody genera in their description that was further enhanced to 78 in Stapleton's description (1994a,b,c, 1997). However, one of the most extensive efforts to study grass phylogeny and sub-familial classification has been commenced (GPWG, 2001). The study based on 62 grasses recognized Poaceae as a monophyletic family (Fig. 5). The earliest diverging lineages were Anomochlooideae, Pharoideae and Puelioideae. Bambusoideae formed the clade 'BEP' along with Pooideae plus Ehrhartoideae and each of them was supported as monophyletic. One of the most significant conclusions is to abandon the long-standing belief that bamboos are the most primitive grasses as speculated by a large section of bamboo taxonomists

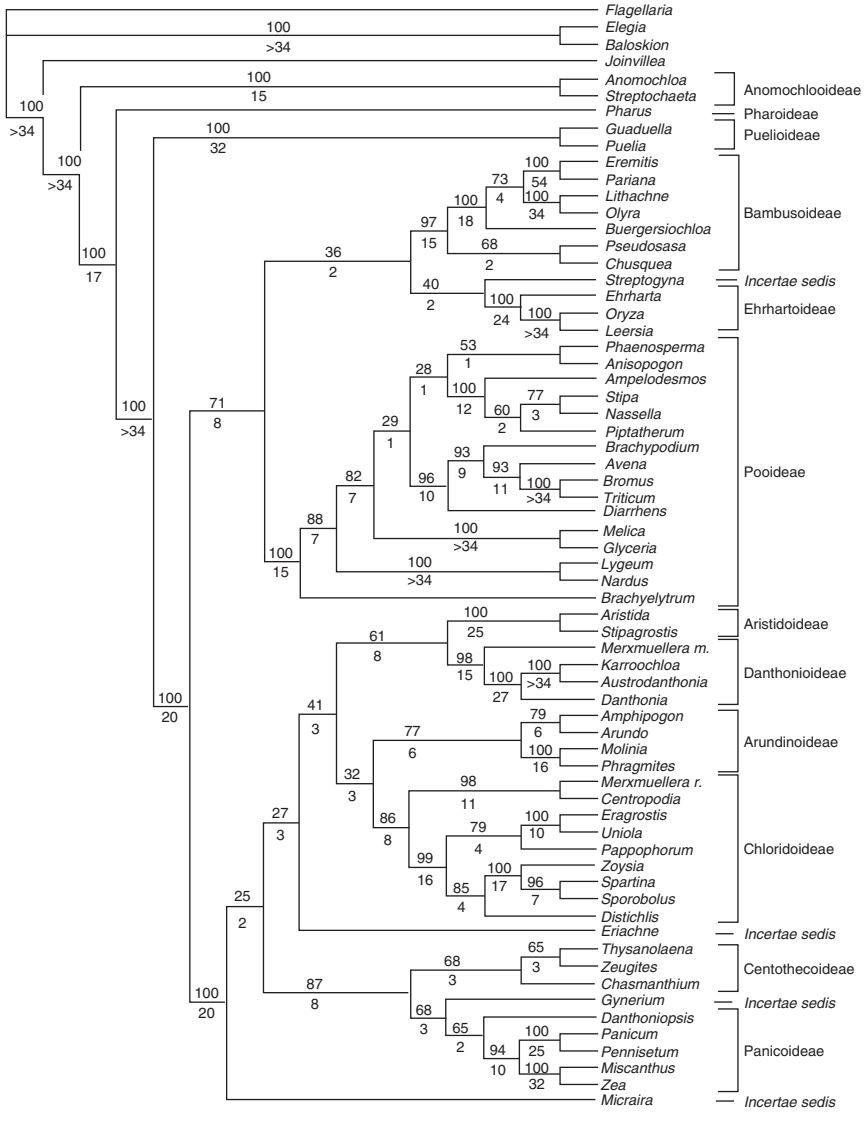


Fig. 5. The most recent grass classification system proposed by Grass Phylogeny Working Group (2001) (reproduced with permission from Missouri Botanical Garden Press).

(Clayton and Renvoize, 1986; Tateoka, 1957 and many others) based on the reproductive plesiomorphic characters such as bracteates, indeterminate inflorescence or the presence of spikelet like pseudo-spikelet structures.

IV. CONSPECTUS OF WOODY BAMBOO GENERA OF THE WORLD

Most of the recent classification systems (Dransfield and Widjaja, 1995; Li, 1997; Soderstrom and Ellis, 1987) placed 67 genera of woody bamboos in nine sub-tribes. These classification systems were largely dependent on various floral characters such as type of inflorescence or ovary appendages.

I. SUBTRIBE ARTHROSTYLIDIINAE:

1. *Actinocladum*, 2. *Alvimia*, 3. *Arthrostylidium*, 4. *Athroostachys*, 5. *Atractantha*, 6. *Aulonemia* (Matudacalamus), 7. *Colanthelia*, 8. *Elytrostachys*, 9. *Glaziophyton*, 10. *Merostachys*, 11. *Myriocladus*, 12. *Rhipidocladum*

II. SUBTRIBE ARUNDINARIINAE:

13. *Acidosasa*, 14. *Ampelocalamus*, 15. *Arundinaria*, 16. *Chimonocalamus*, 17. *Drepanostachyum* (*Himalayacalamus*), 18. *Fargesia* (*Borinda, Yushania*), 19. *Ferrocalamus*, 20. *Gaoligongshania*, 21. *Gelidocalamus*, 22. *Indocalamus*, 23. *Oligostachyum*, 24. *Pseudosasa*, 25. *Sasa*, 26. *Thamnocalamus*

III. SUBTRIBE BAMBUSINAE:

27. *Bambusa* (*Dendrocalamopsis*), 28. *Bonia* (*Monocladus*), 29. *Dendrocalamus* (*Klemachloa*, *Oreobambos*, *Oxynanthera*, *Sinocalamus*), 30. *Gigantochloa*, 31. *Dinochloa*, 32. *Holttumochloa*, 33. *Kinabaluchloa* (*Maclurochloa*, *Soejatmia*), 34. *Melocalamus*, 35. *Sphaerobambos*, 36. *Thyrsostachys*

IV. SUBTRIBE CHUSQUEINAE:

37. *Chusquea*, 38. *Nerolepis*

V. SUBTRIBE GUADUINAE:

39. *Apoclada*, 40. *Eremocaulon*, 41. *Filgueirasia*, 42. *Guadua*, 43. *Olmeca*, 44. *Otatea*

VI. SUBTRIBE MELOCANNINAE:

45. *Cephalostachyum*, 46. *Davidsea*, 47. *Leptocanna*, 48. *Melocanna*, 49. *Neohouzeaua*, 50. *Ochlandra*, 51. *Pseudostachyum*, 52. *Schizostachyum*, 53. *Teinostachyum*

VII. SUBTRIBE NASTINAE:

54. *Decaryochloa*, 55. *Greslania*, 56. *Hickelia*, 57. *Hitchcockella* (?), 58. *Nastus*, 59. *Perrierbambus* (?)

VIII. SUBTRIBE RACEMOBAMBOSINAE:

60. *Racemobambos* (*Neomicrocalamus*)

IX. SUBTRIBE SHIBATAEINAE:

61. *Chimonobambusa*, 62. *Indosasa*, 63. *Phyllostachys*, 64. *Qiongzhuea*, 65. *Semiarundianria* (*Brachystachyum*), 66. *Shibataea*, 67. *Sinobambusa*.

V. RELEVANCE OF MOLECULAR TAXONOMY IN BAMBOO

Two major objectives of any taxonomic study are (a) systematic grouping of the taxa of interest through generation of robust, natural classification system based on stable characters that reflect their true evolutionary history and (b) development of reliable identification key(s) for easy taxon determination. Most of the classifications proposed to date for bamboo are primarily dependent on various morphological features and one of the most immediate needs is to test how natural all these systems are. Stapleton (1997) has summarized few important limitations associated with the traditional morphological classifications: (1) Morphology-based classifications are often superficial as similarities have frequently gained priorities over dissimilarities. (2) Reproductive characters have often earned priority with an assumption of having higher evolutionary significance than the vegetative characters. The importance of many vegetative features such as rhizome or branch patterns was understood later and thus many of the early herbarium specimens were incomplete. (3) In many cases artificiality was enhanced as characters were frequently considered in isolation rather than considered in groups.

It is undeniable that vegetative features are quite essential for field identification of the woody members as flowering cycles are often erratic, which severely restricts the opportunity to study fresh reproductive materials. Even if the dried, herbarium samples are available, quite often these lack enough morphological resolution and thus create confusion in the real field condition. Hence, the identification keys are mostly dependent on various vegetative features that need further refinement and re-investigation. In particular, the taxonomic demarcation of woody bamboos at lower ranks, such as genera and species, are not well resolved to date. There are several species which are known only vegetatively, new species are constantly been described (Clark *et al*., 2007; Filgueiras and Londoño, 2006; Triplett *et al*., 2006) and several undescribed taxa are known to occur in the wild habitat of South and Central Americas.

Molecular data sets can provide useful information for addressing various aspects of plant taxonomy. Considerable progress has already been achieved in bamboo and this chapter is primarily aimed at reviewing the various molecular tools applied to date and also the potential pitfalls that need to be critically considered. The major challenge associated with any molecular method is to determine the appropriate taxonomic level at which it is most informative and to correlate it with morphologically definable taxonomic groupings.

VI. DNA FINGERPRINTING-BASED METHODS

A. RFLP

In restriction fragment length polymorphism (RFLP), differences in the restriction enzyme recognition site sequences between genomes are the basis of polymorphism. These markers are co-dominant in nature and are useful for marker assisted selection. The technique was introduced to bamboo by Friar and Kochert (1991, 1994) for phylogeny assessment of 61 accessions and 20 species of *Phyllostachys*. The study supported the earlier observations of the presence of two distinct sections (*Phyllostachys* and *Heteroclada*) in *Phyllostachys* species pool. However, they disagreed to place *P. nigra* under the section *Heteroclada* and thus contradicted a previous study (Wang *et al*., 1980).

The regular use of RFLP in plant genotyping as well as bamboo has been limited mainly due to the requirements of large amount of DNA along with the use of radioactive isotopes.

B. RAPD

In randomly amplified polymorphic DNA (RAPD, Williams *et al*., 1990) technology, a single and short arbitrary primer is used. RAPD was utilized to assess phylogenetic relationships among 73 genotypes of *Phyllostachys* (Gielis *et al*., 1997b). The resultant phylogeny neither supported the existence of two distinct sections in the *Phyllostachys*-species-complex nor the placement of *P. nigra* under *Phyllostachys*, hence deviated from the previous proposal by Friar and Kochert (1994). However, based on a combined application of RAPD and morphometry, it was confirmed that *P. nigra* belongs to the section *Phyllostachys* (Ding, 1998) and it was also confirmed by AFLP (Fig. 6A) and ITS sequence data (Fig. 6B) that two distinct sections, *Phyllostachys* and *Heteroclada*, do exist in the *Phyllostachys* species pool. The utility of RAPD was extended to the tropical group as well. *B. ventricosa* was found close to *B. vulgaris* var. *striata* (Nayak and Das, 2003) and was supported by a previous finding that *B. ventricosa* is a cultivated variety of *B. vulgaris* (Chua *et al*., 1996). Similarly, a high level genetic proximity

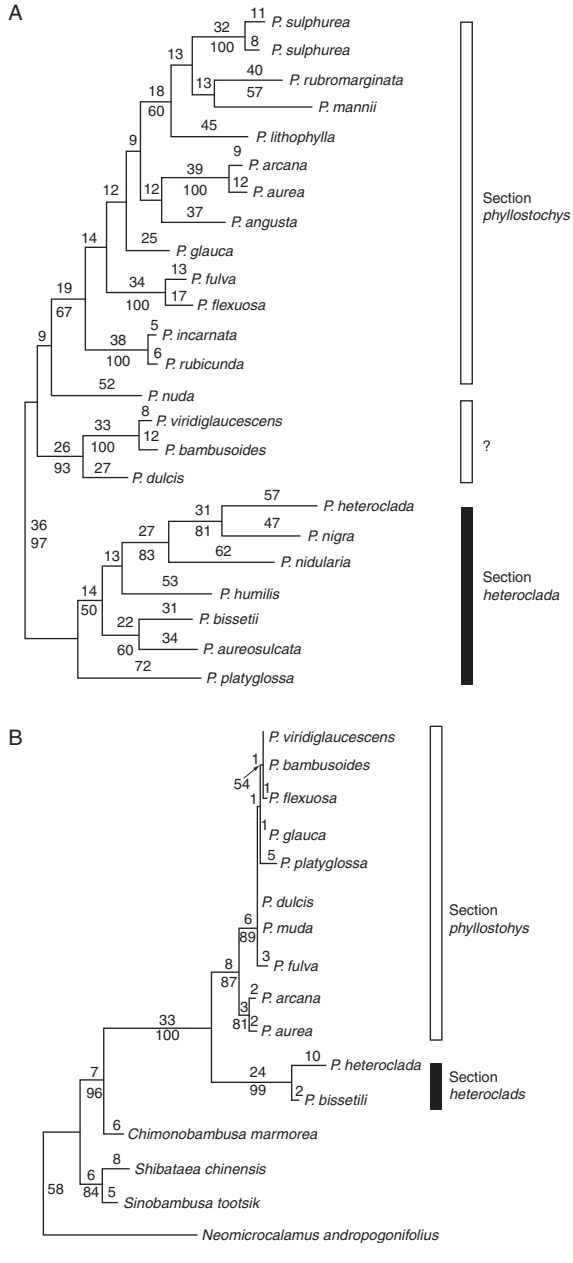


Fig. 6. The existence of two separate sections, *Phyllostachys* and *Heteroclada*, was confirmed by both (A) AFLP and (B) ITS sequence-based phylogeny (Hodkinson *et al*., 2000; reproduced with permission from The Botanical Society of Japan).

(0.91) was obtained between *B. striata* and *B. vulgaris* (Das *et al*., 2007) that was in compliance with the proposition that *B. striata* is a somatic mutant of *B. vulgaris* (Bennet and Gaur, 1990). RAPD-based neighbour joining tree

clearly separated the thorny core *Bambusa* group from the *Dendrocalamus* group (Sun *et al*., 2006). However, most of these studies considered limited number of species and hence the phylogenetic relationships need to be further validated by applying wider species and genera range. Studies on population variability are another area that could benefit from RAPD technology (Hsiao and Rieseberg, 1994). It was found more efficient than micro- or minisatellite to assess genetic variations among the clones of *P. pubescens* in Taiwan (Lai and Hsiao, 1997). Identification of only nine genotypes among a pool of 176 samples clearly suggested the existence of low population genetic variability. Likewise, applying selected primers which were found highly polymorphic at the rank of species (Das *et al*., 2007) could not detect any polymorphism among 17 geographically isolated populations of *B. tulda* (Bhattacharya *et al*., 2006). These two population level studies indicate the possible existence of limited genetic variability that could be attributed to the pre-dominant vegetative mode of propagation in bamboo. Nevertheless, it is necessary to emphasize that in spite of enormous promise, the reliability and reproducibility of RAPD technique is not beyond doubt.

C. SCARs

Sequence characterized amplified regions (SCARs) is an extension of the RAPD procedure (Paran and Michelmore, 1993), but with better reproducibility due to the use of higher annealing temperature. SCARs are co-dominant and have been proved useful for genotype/varietal identification. Particularly, they are useful at the seedling stage when key morphological features are indistinguishable. We have developed two species-specific SCAR markers for *B. balcooa* and *B. tulda* (Das *et al*., 2005) to aid the paper and pulp industry for accurate species diagnosis. To authenticate the utility of these markers at the population level, 80 individual plants collected from 16 eco-geographically diverse populations were screened.

D. AFLP

Amplified fragment length polymorphism (AFLP) is a method described as a combination of RFLP- and PCR-based techniques (Vos *et al*., 1995). It generates dominant markers like RAPD and is highly sensitive to detect polymorphisms among closely related genomes. It has already been demonstrated efficient in measuring genetic relationships among 15 bamboo species representing four different genera (Loh *et al*., 2000). Unique banding patterns were obtained in 13 out of 15 species and the cluster pattern helped reveal the polyphyletic nature of the genus *Bambusa*. However, separation of two *Dendrocalamus* species in two different clusters emphasized the need to re-examine

their status. This technique has also been employed to assess the genetic diversity of the woody American bamboos, namely, *Guadua angustifolia*, *G. amplexifolia*, *G. macrospiculata, G. superba* and *G. unicata* (Marulanda *et al.*, 2002). Distinct genetic differentiations were observed between species. At the accession level, higher genetic diversity was observed for *G. amplexifolia*, while it was low for *G. angustifolia*. Study of clonal structure is an integral part of bamboo biodiversity assessment and AFLP proved useful. A study on the population of the dwarf bamboo, *Sasa senanensis*, revealed high clonal diversity (Suyama *et al.*, 2000). The clonal distribution pattern over a 10 ha study plot indicated a possible relationship between the clone size and the site characteristics where they grow. For example, larger clones were found in the flat areas, while smaller sized clones were found in steep soil that might have interfered with proper rhizome growth. Another population level study re-confirmed 67 years of flowering interval in *P. pubescens* and enumerated that a population originating from the seeds of same flowering event may not necessarily have the same flowering interval (Isagi *et al.*, 2004). The temporal variations in flowering cycles among the siblings of *P. pubescens* reflect heterogeneity among seeds and are not unexpected in the perennial plant group.

AFLP has been proved useful in diverse aspects of bamboo systematics, population structure and variability studies (Gielis *et al.*, 2001) due to the high sensitivity of the technique. However, few limitations associated with the technique include high technical skill and difficulty in analysis of the large number of amplified bands in addition to the cost and time involved.

E. MICROSATELLITES (SSRs)

Microsatellites or simple sequence repeats (SSRs, Litt and Lutty, 1989) are short tandem repeated sequences of 1–6 nucleotides in length, highly polymorphic, co-dominant, multi-allelic, presumed selectively neutral and hence widely used in plant genetic diversity studies. Primers are designed from the conserved genomic regions flanking the repeat sequences and the detected polymorphism reflects variation in the number of repeats among genomes. However, the entire procedures that include construction and screening of genomic library prior to primer designing are cumbersome and cost intensive. This severely limits the wide application of the technique in non-crop plants like bamboo, as sufficient genomic information is not yet available in the database. In spite of that, they have already been successfully applied to *Phyllostachys* (Lai and Hsiao, 1997) and *Bambusa* (Nayak and Rout, 2005). Six microsatellites were isolated from *B. arundinacea* and their cross-species amplification was tested in 18 other bamboo species (Nayak and Rout, 2005). This proof of the principle study indicates that informative conserved

sequences across taxa could be successfully utilized for defining comparative systematic strategies, and thus reduces the efforts to develop microsatellites for individual bamboo species.

F. EXPRESSED SEQUENCE TAG DERIVED MICROSATELLITES (EST-SSR)

This is another chimeric marker technology, where SSRs are harvested *in silico* from EST sequences. It has been shown that EST-SSR markers derived from maize, wheat, sorghum and rice could be successfully utilized to evaluate genetic diversity among 92 temperate bamboo accessions (Barkley *et al*., 2005). The technique proved sensitive enough to detect contamination in a bamboo plot where *Phyllostachys rubromarginata* stands were mixed with either *P. flexuosa* or *P. glauca* stands. Thus EST-SSR holds the promise to extrapolate genomic information from crop to non-crop plants by exploiting genetic collinearity among the members of the grass family. Although EST-SSRs are less polymorphic than genomic SSRs, their easy transferability across species border-line is highly desirable (Yu *et al*., 2004), particularly in systems like bamboo where much genomic information is not yet available.

G. TRANSPOSON

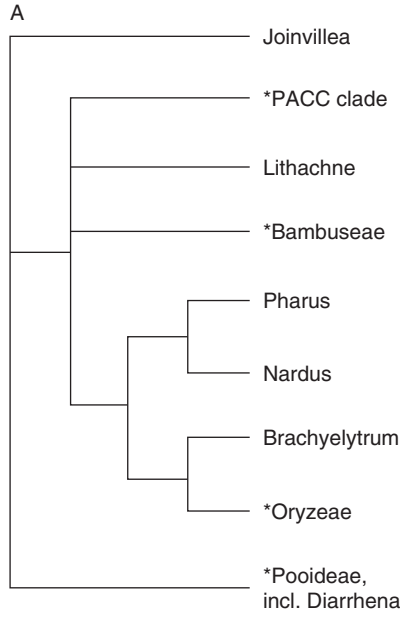
Miniature inverted-repeat transposable elements (MITEs) are an important member of the transposon family with high abundance in plants (Wessler *et al*., 1995). MITE-transposon display (MITE-TD) is a modification of the AFLP technique, where the conserved sequence stretches of the MITE transposons are targeted. It has been successfully recruited to assess genetic variations among *Oryza* species (Park *et al*., 2003). Sensitivity of another transposon family member, Rim2/Hipa-TD, has been tested positive to clearly differentiate *japonica* and *indica* ecotypes of rice (Kwon *et al*., 2005). Retro-elements like Wis-2 have been found conserved across grass genomes like wheat, barley, rye, oats and *Aegilops* and transcriptionally more active in grasses than in dicots (Vicient *et al*., 2001). The presence of Ac-like sequences was found in *Bambusa multiplex* (Huttley *et al.*, 1995), while partial Ac-like transposon elements were isolated from three bamboo species: *Bambusa vulgaris*, *Sasa veitchii* and *Phyllostachys edulis* (Gielis, 1998). The sequence obtained from *B. vulgaris* revealed considerable homology to the hAT superfamily of transposons (Keukeleire *et al*., 2004). A recent study indicates that 23.28% of *P. pubescens* genome is enriched with repeat elements and majority of them (18.89%) were LTR retro-transposons, mainly Gypsy/DIRS1 and Ty1/Copia type (Jie *et al*., 2007). The possible link between transposons and

flowering event in bamboo has been speculated over the years, although no direct evidence has yet been obtained.

VII. DNA SEQUENCE-BASED METHODS

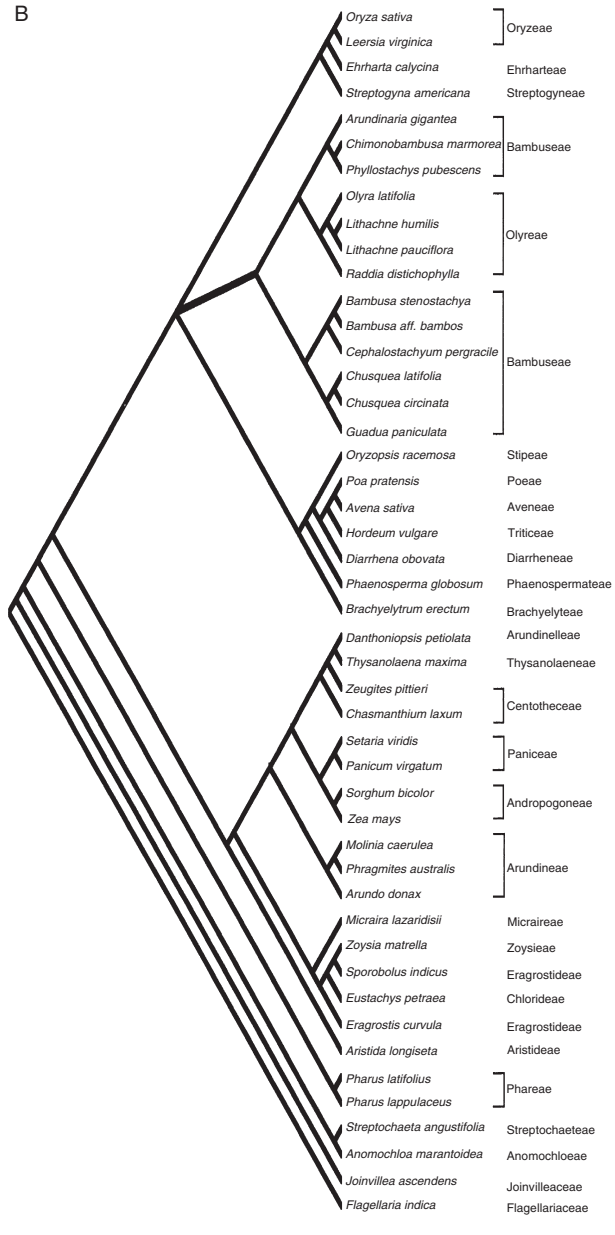
A. ORGANELLAR GENES

In the early era of plant molecular systematics, chloroplast DNA restriction site polymorphism was extensively utilized to discriminate plant taxa (Olmstead and Palmer, 1994, for detailed account) and grasses were no exception. In one such study, the phylogenetic relationships among 31 grass taxa selected from six different subfamilies were evaluated (Davis and Soreng, 1993). The analysis identified two main clades, one was the Pooideae and the other clade was PACC that included the woody Bambusoideae (Fig. 7A). This PACC clade



• Two major groups in grasses are PACC (Panicoideae, Arundinoideae, Chloridoideae and Centothecoideae) and Pooideae; Davis and Soreng (1993) based on chloroplast restriction polymporphism

Fig. 7. (*Continued*)

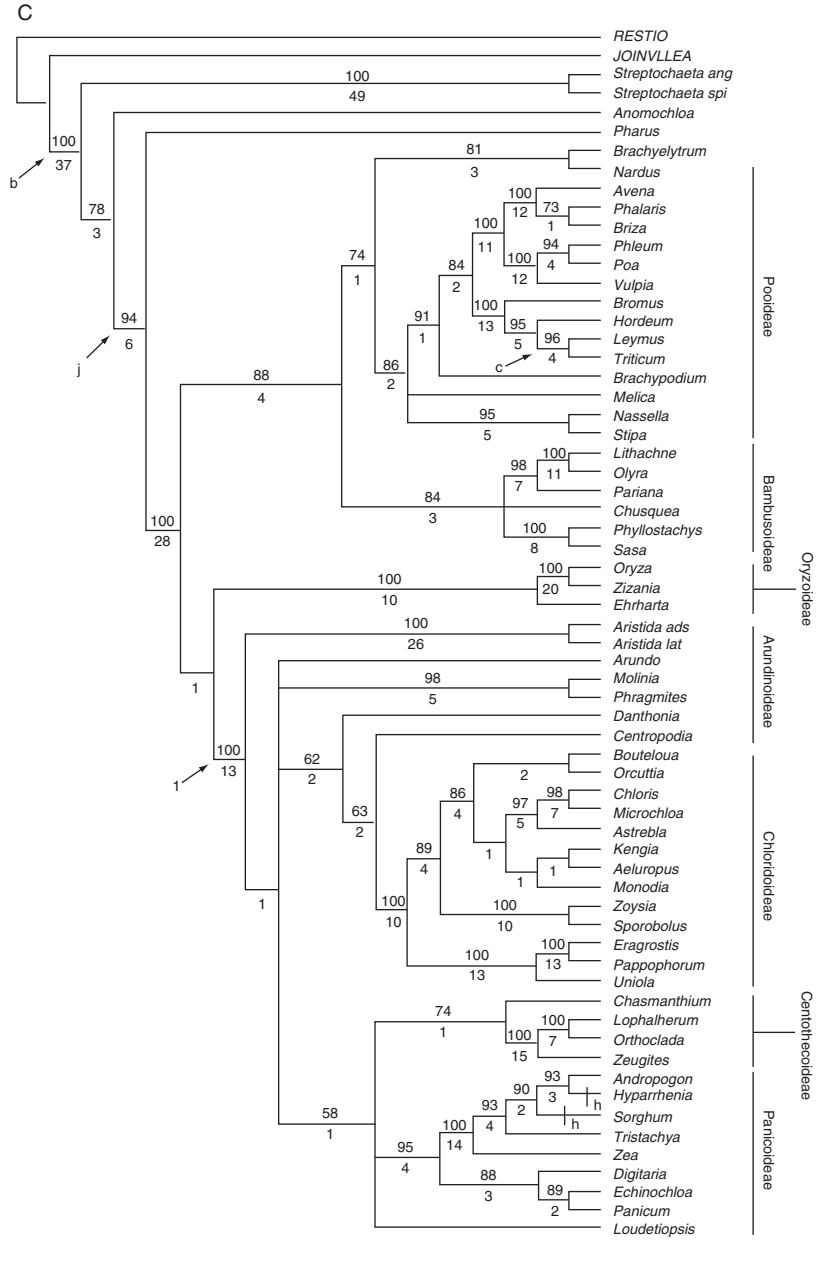


• Anomochloeae, Streptochaeteae and Phareae are basal lineages

• strong support for PACC and weak support for BOP

• core Bambusoideae (Olyreae and Bambuseae) momophyletic; Clark *et al.*, 1995 based on ndhF data

Fig. 7. (*Continued*)



• Bambusoideae is close to Pooideae, Hilu *et al.*, 1999 based on matK data

Fig. 7. The gradual progress of our current understanding of bamboo molecular phylogeny based on (A) chloroplast DNA restriction site polymorphism (Davis and Soreng, 1993, with permission to reproduce from *American Journal of Botany*),

(Panicoideae, Arundinoideae, Chloridoideae and Centothecoideae) was subsequently supported by others (Barker *et al.*, 1995; Cummings *et al.*, 1994). With the inclusion of DNA sequencing technology, employing coding sequences became a regular practice. The transcribed sequences of five 18S and three 26S rRNA were studied from nine grass species those were members of Bambusoideae, Pooideae and Panicoideae (Hamby and Zimmer, 1988). The study revealed *Arundinaria* as the basal lineage of the grasses. Chloroplast gene sequencing gained acceleration with the introduction of the rbcL gene encoding the large subunit of ribulose 1,5 bisphosphate carboxylase/oxygenase. Barker *et al.* (1995) on the basis of the rbcL data revealed monophyletic Bambusoideae related to Pooideae and PACC clade, while another study obtained a weakly supported basal position for Bambusoideae (Duvall and Morton, 1996). However, the utility of rbcL sequences is often restricted above family level and not sufficient for sub-familial resolutions in grasses (Doebly *et al.*, 1990). In particular, the longer generation time of the woody bamboos might cause slower nucleotide substitution rate compared to other grasses (Gaut *et al.*, 1997) and thus less preferable for lower taxonomic groups. Search for additional informative genes continued and new chloroplast genes encoding ribosomal protein S4 (rps4), NADH-plastoquinone oxidoreductase subunit 5 (ndhF), maturase K (matK) and RNA polymerase β subunit (rpoC2) have emerged. The rps4 was targeted to analyze 26 genera of grasses that included three woody bamboos (Nadot *et al.*, 1994). Their analyses showed paraphyly for the bambusoid group that was close to oryzoids and pooids. It was subsequently supported by the rbcL (Barker *et al.*, 1995) and matK (Liang and Hilu, 1996) sequence data.

The first extensive effort utilizing a wide sample of Bambusoid relied on the ndhF gene due to its higher evolution rate than rbcL (Clark *et al.*, 1995). The analyses based on 45 grass sequences resolved the three herbaceous bamboo tribes, Anomochloeae, Streptochaeteae and Phareae, as the basal lineage within the grass family (Fig. 7B). All the other members of Bambusoideae were clearly separated out. One of the two major clades was a weakly supported BOP clade (Bambusoids, Oryzoids and Pooids), while a strong support was obtained for the PACC clade. Monophyly for the core Bambusoid group (Olyreae and Bambuseae) was observed. It was also inferred that many features that are authentic to traditional Bambusoideae are possible synapomorphies for the family. The ndhF sequence data has also been utilized to confirm polyphyly for *Apoclada* (Guala *et al.*, 2000).

(B) ndhF sequence data (Clark *et al.*, 1995, with permission to reproduce from the American Society of Taxonomy) and (C) matK sequence data (Hilu *et al.*, 1999, with permission to reproduce from the Missouri Botanical Garden Press).

A consequence of this study was the description of a new genus (*Filgueirasia*) that was nested inside *Apoclada* S.L. (Guala, 2003). Nonetheless, the earliest divergence of *Streptochaeta* and *Anomochloa* followed by *Pharus* was subsequently supported by matK sequence analysis from 62 grass species covering 9 sub-families (Hilu *et al*., 1999). Bambusoideae was placed in a separate clade with Pooideae (Fig. 7C). By this time Clark and Judziewicz (1996) recognized that monophyly for Bambusoideae could not be retained if the basal lineages, that is Anomochlooideae and Pharoideae, were to be accommodated. Another chloroplast gene rpo C2 was found useful for grass phylogeny assessment for possessing an extra coding sequence that enhances the rate of substitution and insertion/deletion events (Cummings *et al*., 1994).

DNA sequence data from the non-coding regions of chloroplasts were simultaneously exploited, particularly for the lower taxonomic categories with the assumption that non-coding regions are under reduced functional constrain than are coding regions and thus exhibit higher level of sequence variations for enhanced phylogenetic resolutions (Gielly and Taberlet, 1994). The rpl 16 intron data was successfully utilized to study relationships among 23 species of *Chusquea* and 15 taxa from Bambusoideae (Kelchner and Clark, 1997). Monophyly for *Chusquea* was strongly supported as was also recognized for the herbaceous and woody bamboos within Bambusoideae. The woody bamboo was divided into temperate and tropical bamboos and the tropical group was further subdivided into New World and Old World clades. Zhang (2000) has demonstrated the successful utilization of rpl 16 sequences even for higher taxonomic level. His analysis based on 35 sequences from six major sub-families supported the existence of two major, monophyletic groups, BOP and PACC, within the grass family. Although Oryzoideae and Pooideae were strongly supported as monophyletic, support for Bambusoideae was weak. The basal lineage of Streptochaeteae, Anomochloeae and Phareae was also supported. An in-depth study of the clade Arthrostylidiinae and Guaduinae employing about 50 woody species from Brazil based on the rpl 16 intron as well as trnD-T and trnT-L sequences is currently under way by Santos-Gonçalves (personal communication by T.S.F.).

Various chloroplast sequences have contributed immensely to our current understanding of grass as well as bamboo systematics. Particularly at deeper levels, the relative ease of the plastid DNA sequencing makes it a powerful tool for phylogenetic reconstructions. However, several events such as recombination, heteroplasmy or haplotype polymorphism can confound these attempts (Wolfe and Randle, 2004, for detailed account) and hence plastid sequence data should always be combined with other sequences to achieve sufficient resolution for a robust phylogeny.

B. NUCLEAR GENES

Among the nuclear genes, internal transcribed spacers of 18S-5.8S-26S nuclear ribosomal cistron have gained rapid popularity for plant phylogenetic inference. The ITS regions, ~500–700 bp long in angiosperms (Baldwin *et al*., 1995), are flanked by highly conserved sequence stretches and thus amplified by universal primers (White *et al*., 1990) and sequenced. Taxon-specific character-state changes in the ITS regions are an outcome of concerted evolution and hence insertion-deletion polymorphisms (indels) are targeted for phylogenetic reexamination (Alvarez and Wendel, 2003).

Phylogenetic relationships among the members of *Thamnocalamus* and allied groups have been extensively studied with ITS sequence data. Monophyly for the *Thamnocalamus* group was revealed (Guo *et al*., 2002). It was subsequently supported by combined as well as individual application of the ITS and low copy granule bound starch synthase gene (GBSSI) sequence data (Guo and Li, 2004). However, the tree based on the combined data sets (GBSSI and ITS) had higher resolution than that based on individual data set. ITS sequence was also employed to study genetic variation and phylogeny assessment of 23 alpine bamboo species from three genera, *Thamnocalamus, Fargesia* and *Yushania*. It identified *T. spathiflorus* var. *crassinodus* and *F. spathacea* as the basal lineage of alpine bamboos, although the bootstrap support was weak (Guo *et al*., 2001). However, it did not observe monophyly for *Fargesia* and *Yushania* and suggested the need to re-investigate the delimiting morphological features. Sequence (ITS) and PCR markers (AFLP) were simultaneously applied to re-examine the phylogenetic relationships of *Phyllostachys* complex (Hodkinson *et al*., 2000). Monophyletic origin and existence of two distinct sections within the *Phyllostachys* species pool were supported (Fig. 6A and B). *Heteroclada* was further sub-divided into two groups and another group within the section *Phyllostachys* was strongly advocated. The application of ITS sequence data was also extended beyond temperate bamboos. In one such study encompassing 21 species of *Bambusa*, *Denrocalamus*, *Dendrocalamopsis*, *Guadua*, *Leleba* and *Lingnania*, the members of *Dendrocalamus* were shown as close relatives of *Bambusa* (Sun *et al*., 2005). In another study, monophyly of Olyreae and *Raddia* was strongly supported by either single or combined use of ITS and trnD-T sequence data (Oliveira, 2006). The basal position of *Streptochaeta* and *Pharus*, as already established by various chloroplast genes, has also been supported by ITS data, although *Anomochloa* was not included in this study (Hsiao *et al*., 1998). A search (as of October, 2007) in the NCBI-nucleotide database (www.ncbi.nlm.nih.gov) revealed that complete or partial sequence information is already available for 123 bamboo species spanning across 36

genera that reflects a wide acceptability of ITS sequence data to a broad section of bamboo taxonomists. Although nuclear markers were mostly dominated by spacer sequences, the use of low-copy nuclear genes is gaining popularity. For instance, Mathews *et al*. (2000) on the basis of the analysis of 51 PHYB sequences concluded *Anomochloa* and *Streptochaeta* as the first lineage of the grass family, followed by *Pharus* and *Puelia*. This is in gross agreements with many early reports based on various chloroplast regions. However, one of the most significant enhancements was to obtain a strong support for the BOP clade that was previously weakly supported by the ndhF data (Clark *et al*., 1995) and was not supported at all by other plastid sequences (Cummings *et al*., 1994; Davis and Soreng, 1993; Nadot *et al*., 1994). A high support for this clade had also been obtained by the combined application of three phytochrome loci (Mathews and Sharrock, 1996).

Nonetheless, bi-parental, nuclear ITS regions are one of the most popular choices for phylogenetic inference at genus level or below due to higher rate of base substitution than most of the organellar genes. In addition their high copy numbers allow easy amplification by targeting the conserved priming sites surrounding 18S and 26S regions. However, there are associated molecular events that could always confound phylogenetic inference (Alvarez and Wendel, 2003) in addition to the limitation due to small number of informative features (Baldwin *et al*., 1995) and frequent difficulty in alignment due to length variations (Hsiao *et al*., 1998). One of the most important prerequisites is to target the true orthologous sequences in related taxa that are subjected to phylogenetic re-investigation. However, in absence of complete homogenization, unintended inclusion of paralogous counterpart is possible and can always delude the effort. Particularly, such chances are very high in woody bamboos where extensive genome polyploidization is a common occurrence. The other confounding phenomena discussed by Alvarez and Wendel (2003) are existence of large number of rDNA arrays, effect of secondary structure on base substitution and chances of contamination due to the use of universal primers. Of these, the contamination problem has already been experienced in the woody bamboo where fungal rDNA was inadvertently co-isolated and hence co-amplified with the target DNA (Zhang *et al*., 1997). Epiphyllous fungi are frequently associated with bamboo leaves. Hence fresh leaves should always be sufficiently surface sterilized prior to DNA extraction and to avoid any possible contamination. It is also preferable not to rely on a single PCR reaction, but to clone and sequence products amplified under various reaction conditions (Alvarez and Wendel, 2003) to avoid PCR bias or drift (Wagner *et al*., 1994). However, many of these issues could be equally associated with any rapidly evolving region that is essential for lower taxonomic or recently radiated groups. The use of low

copy, nuclear genes is gaining quick popularity as it combines the benefit of high substitution rate but lower chances of obtaining the paralogous counterparts (Small *et al.*, 1998). However, their extensive utilization is mainly restricted by experimental difficulties in isolation and characterization due to lack of sufficient sequence information available in the database.

VIII. SUMMARY AND FUTURE DIRECTIONS

A. MORPHOLOGICAL SYSTEMATICS AND IDENTIFICATION

Generation of enormous morphological and anatomical data over the years has built up a strong foundation for bamboo taxonomy studies to address both systematic and identification issues. However, one should always keep this in mind that vegetative morphology-only phylogenetic analyses often lack sufficient resolution and thus should always be compared with the outcome from other data sources. In an attempt to evaluate phylogenetic relationships among 15 tropical woody species, we obtained the dendrogram pattern based on 32 morphological descriptors that was not fully supported by the classification system of Gamble, while the cluster pattern computed from 120 polymorphic RAPD fragments was in gross agreement (Das *et al.*, 2007). Our follow-up study based on higher number of taxa (25) revealed similar discrepancy between morphological (Fig. 8A) and DNA polymorphism (Fig. 8B)-based dendrograms, while only the latter was in complete agreement with the classification system. It is surprising since most of the selected culm (Table I) and culm-sheath characters (Table II) used in this study are widely used for bamboo species characterization. The most probable explanation is that the classification system was developed using vegetative plus reproductive characters, while only vegetative characters were analyzed in the present study due to the unavailability of reproductive organs. This case study clearly shows that chances of potential errors exist for any phylogenetic interpretation in bamboo that is not based on a complete array of morphological features, that is vegetative plus reproductive.

Nonetheless, morphology-based identification keys are very useful for quick identification at the field, yet it needs further precision as morphological features are often influenced by environment due to the event of true parallelism (Kellogg and Watson, 1993). Particularly, the population level understanding of morphological variability needs to be enhanced in bamboo. We have identified a number of morphological variations in different *Bambusa* species that call for serious attention to reevaluate the identification keys applied in the field. For instance, in few cases striated culms were observed in *B. tulda*, which resembles that of *B. striata* (Fig. 9A and B), while bent culms and compressed,

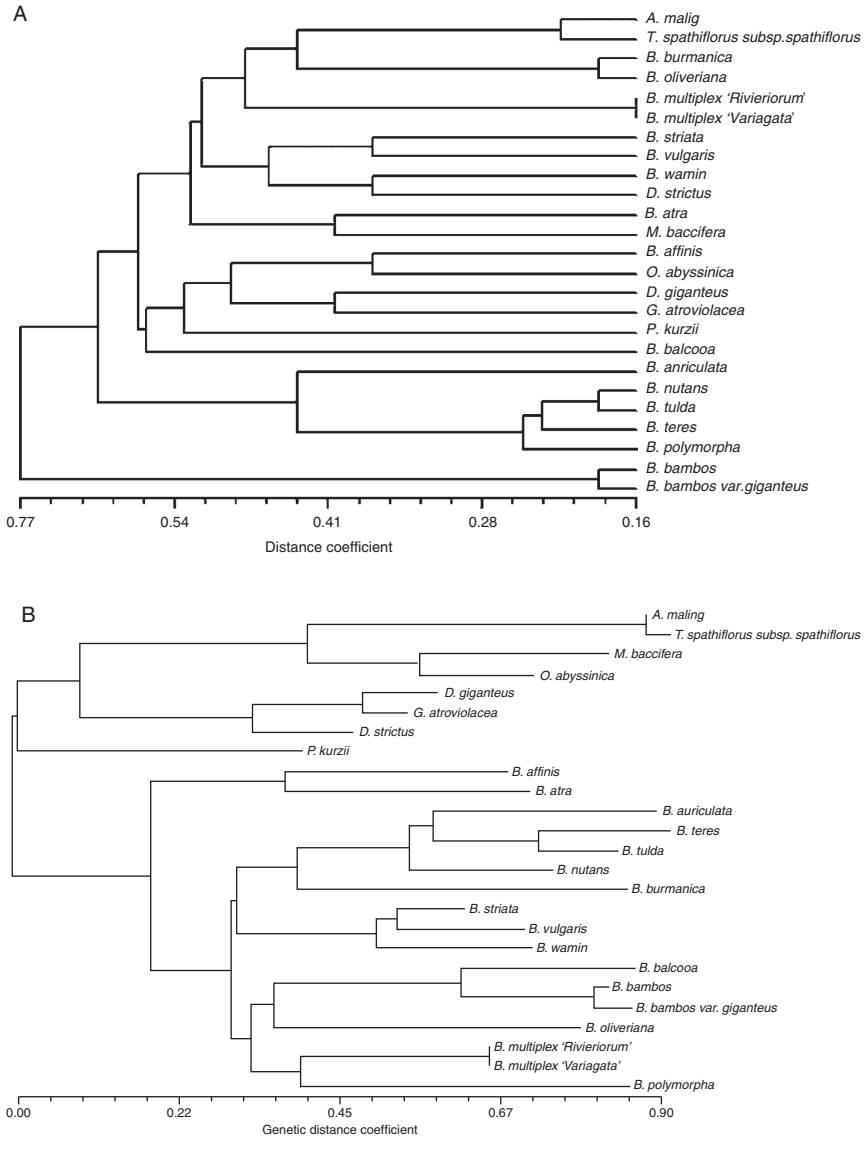


Fig. 8. Dendogram derived from UPGMA cluster analysis based on (A) 32 key morphological characters and (B) 244 polymorphic RAPD fragments of 25 woody bamboo species.

swollen internodes were recorded for *B. balcooa* and *B. tulda* (Fig. 9C and D). Therefore, further investigations covering diverse ecosystems and taxa are essential to confirm a set of morphological features stable across ecotypes.

TABLE I

A Comparison of the 15 Key Culm Descriptors Used to Evaluate Phylogenetic Relationships Among 25 Bamboo Species (OTUs): Mean Height (m), Diameter (mm), Length of Fifth Internode (mm), Ratio of Cavity Diameter to Total Culm Diameter, Internode Bending, Colour (Yellow with Striation = 0, Yellow-Green = 1, Gray-Green = 2, Pale Green = 3, Bright Green = 4, Glossy Green = 5, Dark Green = 6), Swollen Node, Nodal Ring (Absent = 0, Whitish = 1, Grayish = 2), Nodal Sheath Scar, Hairs at Nodal Ring, Branches Come Out Piercing Culm Sheath, Curved Lower Nodal Branches, Different Culm Leaf and Branch Leaf Size, Modification of Branches (None = 0, Thorns = 1, Spines = 2), Striation on Culm

OTUs	Height[a]	Diameter[a]	Internode[a]	Cavity[a]	Bending[b]	Colour	Swollen node[b]	Nodal ring	Sheath scar[b]	Hairs[b]	Branching[b]	Curved branches[b]	Leaf size[b]	Modifications	Striation[b]
1	4.2 ± 0.8	50 ± 2.0	110 ± 15	0.33 ± 0.05	0	1	1	1	1	1	0	0	0	0	0
2	6.5 ± 0.9	40.0 ± 6.0	500 ± 75.0	0.37 ± 0.06	0	2	0	1	1	0	0	0	1	0	1
3	7.0 ± 1.0	35.0 ± 5.2	600 ± 90.0	0.42 ± 0.06	0	6	0	1	1	0	0	0	0	0	0
4	18.3 ± 2.7	45.0 ± 6.7	400 ± 60.0	0.22 ± 0.03	0	3	0	2	1	1	1	0	1	0	0
5	20.0 ± 2.9	90.0 ± 13.5	200 ± 30.0	0.33 ± 0.05	0	4	1	1	1	1	1	0	1	2	0
6	25.0 ± 5.0	110 ± 20.0	300 ± 10.0	0.45 ± 0.05	0	2	1	0	0	0	0	1	1	1	0
7	15.8 ± 2.5	55.0 ± 8.8	230 ± 36.6	0.36 ± 0.06	0	2	1	0	0	0	0	1	1	1	0
8	15.5 ± 2.2	70.0 ± 5.5	300 ± 30.5	0.52 ± 0.05	0	5	1	1	1	1	0	0	0	0	0
9	3.0 ± 0.7	20.0 ± 4.6	200 ± 45.8	0.12 ± 0.03	0	3	1	2	0	0	0	0	0	0	0
10	2.5 ± 0.05	15.0 ± 3.6	180 ± 45.8	0.12 ± 0.03	0	3	1	2	0	0	0	0	0	0	0
11	12.5 ± 3.2	60.0 ± 8.7	400 ± 65.8	0.4 ± 0.07	0	4	0	1	1	0	1	0	1	0	1
12	14.0 ± 1.5	37.5 ± 4.1	350 ± 38.4	0.53 ± 0.06	0	5	1	1	1	1	0	0	0	0	0
13	20.2 ± 3.2	100 ± 8.7	500 ± 65.8	0.45 ± 0.07	0	1	0	1	1	0	1	0	1	0	1
14	16.5 ± 0.9	70.0 ± 9.4	190 ± 18.6	0.42 ± 0.06	0	0	1	2	1	0	0	0	0	0	1
15	15.5 ± 3.2	70.0 ± 8.7	500 ± 65.8	0.48 ± 0.07	0	6	0	1	1	0	1	0	1	0	1
16	18.3 ± 3.2	50.0 ± 8.7	380 ± 65.8	0.4 ± 0.07	0	6	0	1	1	0	1	0	1	0	1
17	19.8 ± 2.2	80.0 ± 8.9	230 ± 25.5	0.5 ± 0.06	0	4	1	2	1	0	0	0	0	0	1
18	7.0 ± 1.0	120 ± 17.0	120 ± 18.6	0.33 ± 0.05	0	6	1	1	1	0	0	0	0	0	0
19	30.5 ± 3.2	130 ± 13.4	330 ± 34.2	0.69 ± 0.07	0	2	0	2	1	1	0	0	1	0	0
20	14.0 ± 2.7	50 ± 9.5	200 ± 71.4	0.6 ± 0.11	0	1	1	0	1	0	0	1	0	0	0
21	21.0 ± 2.8	60 ± 8.0	375 ± 26.4	0.54 ± 0.07	0	6	0	0	0	0	0	0	1	0	0
22	15.0 ± 1.2	55 ± 5.0	220 ± 15.0	0.32 ± 0.05	0	4	0	0	0	0	0	0	0	0	0
23	8.0 ± 1.3	80 ± 5.0	180 ± 15.0	0.55 ± 0.06	0	4	0	1	1	0	0	0	1	0	0
24	9.14 ± 1.8	35 ± 7.0	220 ± 43.9	0.5 ± 0.09	1	2	0	1	1	0	0	0	0	0	0
25	5.0 ± 1.5	15 ± 5.0	120 ± 20.0	0.33 ± 0.05	0	1	1	1	1	1	0	0	0	0	0

OTU 1 = *Arundinaria maling*, 2 = *Bambusa affinis*, 3 = *B. atra*, 4 = *B. auriculata*, 5 = *B. balcooa*, 6 = *B. bambos* var. *giganteus*, 7 = *B. bambos*, 8 = *B. burmanica*, 9 = *B. multiplex* 'riviereorum', 10 = *B. multiplex* 'variagata', 11 = *B. nutans*, 12 = *B. oliveriana*, 13 = *B. polymorpha*, 14 = *B. striata*, 15 = *B. teres*, 16 = *B. tulda*, 17 = *B. vulgaris*, 18 = *B. wamin*, 19 = *Dendrocalamus giganteus*, 20 = *D. strictus*, 21 = *Gigantochloa atroviolacea*, 22 = *Melocanna baccifera*, 23 = *Oxytenanthera abyssinica*, 24 = *Pseudobambusa kurzii*, 25 = *Thamnocalamus spathiflorus* subsp. *spathiflorus*.

[a]Mean ± SE.

[b]Absent = 0, present = 1.

TABLE II

A Comparative Account of the 17 Key Culm-Sheath Descriptors Used to Evaluate Phylogenetic Relationships Among 25 Bamboo Species (OTUs): Ratio of Total Length to Breadth at Base, Ciliate Margin, Pubescent Adaxial Side, Pubescent Abaxial Side, Hair Colour (None = 0, Golden Brown = 1, Brown = 2, Dark Brown = 3, Black = 4), Number of Hairs (Absent = 0, Scanty = 1, Profuse = 2), Ratio of Total Length versus Blade Length, Shape of Blade (Triangular = 0, Acuminate = 1, Lanceolate = 2, Ovate = 3), Blade Reflexed, Hairy Margin of Blade, Ligule Margin (Entire = 0, Dentate = 1, Serrate = 2), Hairs on Ligule, Auricle, Auricle Continuous with Blade, Bristles on Auricle, Auricle Fringed, Variable Culm Sheath Size at Different Culm Height

OTUs	Length/Breadth[a]	Margin[b]	Pub. adax[b]	Pub. abax[b]	Hair colour	Hair number	Total/Blade[a]	Shape	Blade reflexed[b]	Hairy margin[b]	Ligule margin	Hairs on ligule[b]	Auricle[b]	Auricle cont.[b]	Bristles[b]	Auricle fringed[b]	Sheath size[b]
1	3.0 ± 0.85	0	0	0	0	0	4.2 ± 0.55	1	1	1	2	1	1	0	1	0	0
2	1.55 ± 0.23	0	0	1	4	1	4.43 ± 0.66	3	1	1	0	0	0	0	0	0	0
3	3.33 ± 0.49	0	0	1	1	1	2.0 ± 0.29	3	0	0	0	1	1	1	1	0	0
4	1.5 ± 0.22	1	0	1	4	2	2.45 ± 0.37	3	0	1	1	1	1	1	1	1	1
5	1.59 ± 0.24	1	0	1	4	1	4.15 ± 0.64	0	0	0	2	1	0	0	0	0	0
6	2.8 ± 0.37	1	1	0	4	1	3.66 ± 0.62	1	0	0	1	1	1	1	1	1	0
7	2.92 ± 0.47	1	1	0	4	1	3.88 ± 0.62	1	0	0	1	1	1	1	1	1	0
8	2.60 ± 0.30	0	0	1	4	1	2.43 ± 0.26	1	0	0	2	0	1	0	1	0	0
9	2.87 ± 0.66	0	0	0	0	0	2.87 ± 0.56	2	0	0	0	0	1	0	1	0	0
10	2.75 ± 0.55	0	0	0	0	0	2.77 ± 0.55	2	0	0	0	0	1	0	1	0	0
11	1.15 ± 0.18	0	0	1	4	2	3.42 ± 0.64	3	0	0	2	0	1	1	1	1	1
12	2.69 ± 0.30	0	0	1	4	1	2.33 ± 0.26	1	0	0	2	0	1	0	1	0	0
13	1.20 ± 0.18	0	0	1	4	2	3.50 ± 0.64	3	1	0	2	0	1	1	1	1	1
14	1.78 ± 0.22	0	0	1	3	2	2.5 ± 0.31	3	0	0	2	1	1	0	0	0	0
15	1.25 ± 0.18	0	0	0	4	2	3.20 ± 0.64	3	0	0	2	0	1	1	1	1	1
16	1.04 ± 0.18	0	0	1	4	2	3.67 ± 0.64	3	0	0	2	0	1	1	1	1	1
17	1.65 ± 0.15	1	0	1	3	2	2.75 ± 0.30	1	0	0	0	1	1	0	1	0	1
18	0.87 ± 0.13	1	0	1	2	2	2.08 ± 0.31	3	0	1	2	1	1	0	0	0	0
19	1.3 ± 0.13	0	0	1	2	2	3.36 ± 0.35	2	1	0	2	1	0	0	0	0	1
20	1.39 ± 0.26	1	0	1	1	2	3.85 ± 0.73	0	0	1	2	1	1	1	0	0	0
21	1.31 ± 0.17	0	0	1	1	1	3.0 ± 0.39	2	1	0	0	1	0	0	0	0	1
22	1.16 ± 0.15	0	0	1	1	1	1.25 ± 0.42	2	0	0	1	0	1	1	1	1	0
23	2.1 ± 0.25	1	0	1	3	1	1.55 ± 0.22	2	0	1	0	0	0	0	0	0	0
24	3.37 ± 0.67	1	0	1	4	2	1.8 ± 0.36	2	1	1	1	1	1	1	0	0	1
25	3.6 ± 0.85	0	0	0	0	0	3.6 ± 0.55	1	1	1	2	1	0	0	0	0	0

OTU 1 = *Arundinaria maling*, 2 = *Bambusa affinis*, 3 = *B. atra*, 4 = *B. auriculata*, 5 = *B. balcooa*, 6 = *B. bambos* var. *giganteus*, 7 = *B. bambos*, 8 = *B. burmanica*, 9 = *B. multiplex* 'riviereorum', 10 = *B. multiplex* 'variagata', 11 = *B. nutans*, 12 = *B. oliveriana*, 13 = *B. polymorpha*, 14 = *B. striata*, 15 = *B. teres*, 16 = *B. tulda*, 17 = *B. vulgaris*, 18 = *B. wamin*, 19 = *Dendrocalamus giganteus*, 20 = *D. strictus*, 21 = *Gigantochloa atroviolacea*, 22 = *Melocanna baccifera*, 23 = *Oxytenanthera abyssinica*, 24 = *Pseudobambusa kurzii*, 25 = *Thamnocalamus spathiflorus* subsp. *spathiflorus*.

[a]Mean ± SE.

[b]Absent = 0, present = 1.

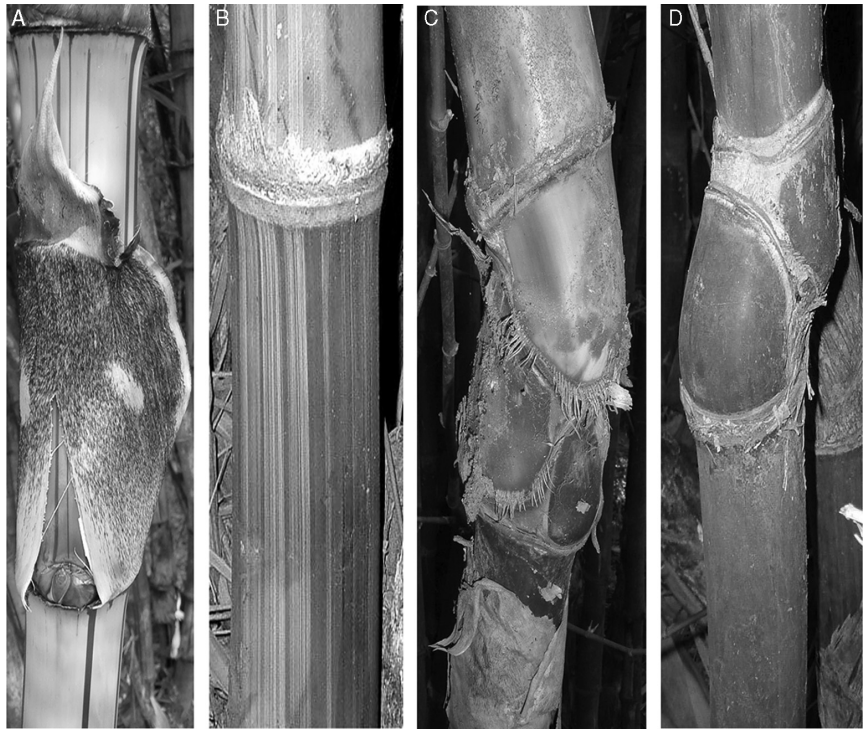


Fig. 9. Striations on the culm of (A) *Bambsa striata* and (B) *B. tulda*; bent culm with compressed internodes in (C) *B. tulda* and (D) *B. balcooa*.

B. MOLECULAR SYSTEMATICS AND IDENTIFICATION

Applications of the various molecular tools have enormous potential, but need judicial application based on the objectivity of the study and taxonomic rank under consideration. Utility of the sequence-based molecular markers over PCR-based markers for phylogenetic reconstructions is now well established. However, considerable disagreements still exist regarding the number(s) and nature (gene vs. spacer, or organellar gene vs. nuclear gene) of the target sequence(s). It is quite apparent that identifying one universal barcode, like that of mitochondrial CO1 in animals, is a distant hope for plant taxa (Pennisi, 2007). The slow evolution rate of the mitochondrial genes and low copy number of the nuclear genes led the plant taxonomists to focus mainly on various chloroplastic regions in recent years. Five candidate genes (rbcL, matK, rpoC1, rpoB, atpF/H) and two spacers (trnH-psbA, psbK/I) are in the centre of all interests (Pennisi, 2007). Also a consensus has been developed to use a combination of these target sequences to avoid the inherent problems associated with each one of them. Similar consensus needs

to be developed among the bamboo taxonomists to decide suitable target regions that could be universally sequenced, but should reveal enough sequence diversity to differentiate closely related taxa and lower ranks. In parallel the high copy, bi-parental, ITS regions could be exploited since sequence data are publicly available for many species. However, one of the major limitations of bamboo ITS sequences is their considerable length variation that constrain multiple alignment and phylogenetic tree development. We found that in *Bambusa* the length varied from 599 bp to 707 bp (Fig. 10). Even for the same species (*B. bambos*, *B. chungii*, *B. intermedia*, *B. membranacea*, *B. sinospinosa*, *B. surrecta*), considerable length variation was observed across different groups [represented by bar lines showing standard deviation (SD) in Fig. 6]. It is not clear at this stage whether this is due to differential evolution rate that generates this infra-species heterogeneity. In addition we also propose to set a stringent standard for ITS-based phylogeny development, similar to MIAME used for gene expression studies, to enhance resolution and reproducibility.

Another practical challenge for bamboo molecular taxonomy is to provide tools for rapid and accurate taxon determination. Particularly, species level questions are always critical for the woody group and development of specific DNA tags might be useful for commercial purposes. For instance, bamboo constitute a major non-wood fibre source for the paper and pulp

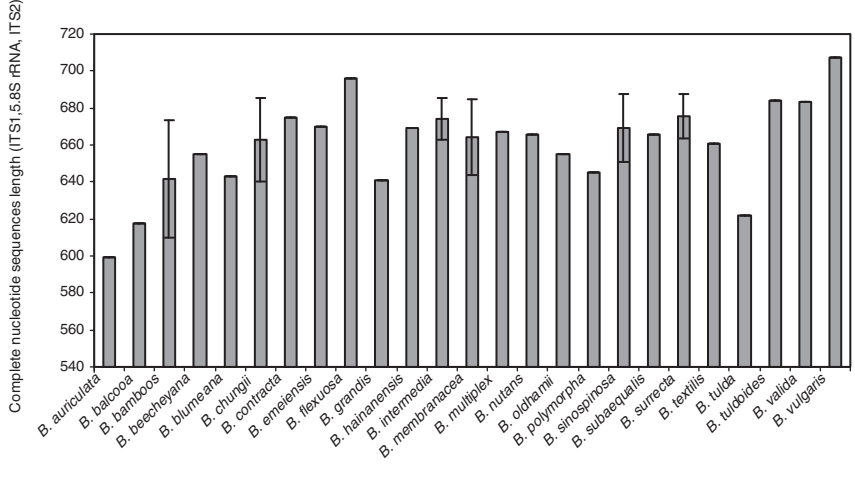


Fig. 10. Considerable length variations of spacers and 5.8S rRNA sequences among the members of *Bambusa* based on the data retrieved from www.ncbi.nlm.nih.gov as of October 2007. Where more than one submission was found for the same species, the mean value was used as the representative and SD represents variations.

production in India and only 12 species have been identified for their suitability based on certain physical and chemical properties (Ganapathy, 1997). One such candidate is *Bambusa balcooa*, which was preferred by the pulp and paper industries because of its mechanical strength attributable to the high specific gravity (Bhatt *et al*., 2003). Our identified strategy of developing species-specific SCAR-marker (Das *et al*., 2005) is quite effective to identify species, even at the seedling stage when traditional morphological characters often lack enough resolution.

C. FUTURE SCOPE OF COMPARATIVE GENOMICS

One of the potentially emerging areas for bamboo biology is the comparative genomic studies, wherein available genomic information of other well-characterized cereal crops could be extrapolated to initiate functional genomics in bamboo. In this respect, *Arabidopsis* would certainly not be a good choice since extensive genome duplications in bamboo, at least for the woody group, might hinder the possibility of obtaining true one-to-one orthologs. However, in absence of sufficient genomic information, a reasonable starting point would be to target the collinear regions of the well-characterized grass genomes and to search for their homologous regions in bamboos. Based on similar principle, EST-SSR markers derived from maize, wheat, sorghum and rice have already been applied to bamboo (Barkley *et al*., 2005). The scope of the comparative genomics research in bamboo exists beyond taxonomy. One of the intriguing questions for future is to study the genes and mechanisms that control the unique flowering behaviour in bamboo. In particular floral genes, such as *FEA2*, BA1/LAX1, FUL, IDS1, KN1 or RCN1/2 that have already been characterized and connected to phenotypes in other grasses (Malcomber *et al*., 2006, for further details), should be targeted. It is possible to identify their homologous counterparts in bamboo by utilizing the conserved regions and subsequent functional characterization by expressing those genes in rice or maize deletion mutant lines. It would also provide some fundamental knowledge on the level of orthology existing between bamboo and other domesticated grass genomes. Another important area for future research is to characterize the genes regulating unique flowering event and/or long vegetative phase in bamboo. This could be achieved by performing suppression subtractive hybridization analysis where RNA from a flowering clone could be used as a tester and a non-flowering clone as a driver or vice versa. The differentially expressed genes could be partially sequenced to generate ESTs and predicted by sequence alignment with available grass ESTs. A similar approach has recently been undertaken to identify the nuclear-encoded non-photosynthesis related genes in an albino mutant of

Bambusa edulis (Lin *et al*., 2006). However, such efforts are very scanty and the absence of genetic map(s) or mapping population is quite apparent. An NCBI search for bamboo revealed only 329 (mostly *Bambusa oldhamii*) and 998 (mostly *Phyllostachys edulis*) hits against publicly available EST and GSS collections. In addition databases that provide resources for different bamboo genotypes are also essential since without morphological connections it is always hard to characterize a set of genes. We have summarized a list of web-sites which are available so far and contain useful information regarding various aspects of bamboo biology (Table III).

D. GENETIC DIVERSITY AND ECOLOGY

In contrast to the vast majority of studies done to date on bamboo taxonomy and systematics, investigations on genetic diversity at the population level are in its infancy. Substantial work has been done to develop comprehensive maps that describe the richness and distribution of woody bamboo species in Asia Pacific, Africa, Madagascar and in America (Bystriakova *et al*., 2003a,b; http://www.ourplanet.com/wcmc, index 14 and 19). The most alarming fact is the finding that ~400 species are potentially threatened by destruction of natural forest cover in the Asia Pacific region. Studying distribution patterns is an important component of bamboo biodiversity, while estimation of population genetic diversity is equally essential for designing effective conservation strategies. Multi-loci PCR markers such as RAPD or AFLP are useful at the population level due to relatively low cost, fast assay time and their ability to depict polymorphism among closely related genomes. Our studies on *B. tulda* (Bhattacharya *et al*., 2006) and *Thamnocalamus spathiflorus*

TABLE III
Important Web-Resources for Bamboo Biologists

Web-resource	Information available
http://www.eeob.iastate.edu/research/bamboo/index.html	Distribution maps, key characters, methods, useful literatures
http://bamboo-identification.co.uk	Description of important identifying keys, classification, nomenclature, useful literatures
http://www.americanbamboo.org/	General bamboo information
http://www.oprins.be	Plantations, tissue culture, bio-energy
http://www.ars-grin.gov/cgi-bin/npgs/html/crop.pl/bamboo	Evaluation data on bamboo accessions
http://www.inbar.int/	Sustainable social, economic and environmental benefits of bamboo

(unpublished data from S.B) at different eco-geographical regions of eastern India indicated a low level of population genetic diversity for these two species. Similar trend was identified in *P. pubescens* from Taiwan (Lai and Hsiao, 1997) and *Guadua angustifolia* from Colombia (Marulanda *et al*., 2002). It is quite possible that only a few clones of individual species acted as the genetic donor within a particular geographic area and thus resulted in low level among population genetic variability. On the other hand, relatively higher clonal variation was found in *Sasa senanensis* from Japan (Suyama *et al*., 2000) and *G. amplexifolia* from Colombia (Marulanda *et al*., 2002). It indicates that the differential reproductive systems might have influence on population genetic diversity in different bamboo species, since it is expected that the allogamous species are usually more diverse than the autogamous ones. However, further studies are required to better understand emphatically the level of population genetic diversity and clonal structure in bamboo.

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REFERENCES

Alvarez, I. and Wendel, J. F. (2003). Ribosomal ITS sequences and plant phylogenetic inference. *Molecular Phylogenetics and Evolution* **29**, 417–434.

Baldwin, B. G., Sanderson, M. J., Porter, J. M., Wojciechowski, M. F., Campbell, C. S. and Donoghue, M. J. (1995). The ITS region of nuclear ribosomal DNA: A valuable source of evidence on angiosperm phylogeny. *Annals of the Missouri Botanical Garden* **82**, 247–277.

Barker, N. P., Linder, H. P. and Harley, E. H. (1995). Polyphyly of Arundinoideae (Poaceae): Evidence from rbcL sequence data. *Systematic Botany* **20**, 423–435.

Barkley, N. A., Newman, M. L., Wang, M. L., Hotchkiss, M. W. and Pederson, G. A. (2005). Assessment of the genetic diversity and phylogenetic relationships of a temperate bamboo collection by using transferred EST-SSR markers. *Genome* **48**, 731–737.

Bennet, S. S. R. and Gaur, R. C. (1990). "Thirty Seven Bamboos Growing in India." pp. 17–18. Controller of Publications, Government of India, New Delhi, India.

Bentham, G. (1881). Notes on Gramineae. *Journal of Linnean Society, Botany* **19**, 14–134.

Bhatt, B. P., Singh, L. B., Singh, K. and Sachan, M. S. (2003). Some commercial edible bamboo species of North East India: Production, indigenous uses, cost-benefit and management strategies. *Bamboo Science and Culture* **17**, 4–20.

Bhattacharya, S., Das, M., Bar, R. and Pal, A. (2006). Morphological and molecular characterization of *Bambusa tulda* with a note on flowering. *Annals of Botany* **98**(3), 529–535.

Brandis, D. (1899). Biological notes on Indian bamboos. *Indian Forester* **25**, 1–25.

Brea, M. and Zucol, A. F. (2007). *Guadua zuloagae* sp. nov., the first petrified bamboo culm record from the Ituzaingo formation (Pliocene), Parana basin, Argentina. *Annals of Botany* **100**(4), 711–723.

Bystriakova, N., Kapos, V., Lysenko, I. and Stapleton, C. (2003a). Distribution and conservation status of forest bamboo biodiversity in the Asia-Pacific region. *Biodiversity and Conservation* **12**, 1833–1841.

Bystriakova, N., Kapos, V., Stapleton, C. and Lysenko, I. (2003b). "Bamboo Biodiversity: Information for Planning Conservation and Management in the Asia-Pacific Region." pp. 1–71. UNEP-World Conservation Monitoring Center and International Network for Bamboo and Rattan, Cambridge, UK.

Camus, E. G. (1913). "Les Bambusees-Monographie, Biologie, Culture, Principaux Usages." pp. 1–215. Lechevalier, Paris.

Chao, C. S. and Renvoize, S. A. (1989). Revision of species described under *Arundinaria* (Gramineae) in Southeast Asia and Africa. *Kew Bulletin* **44**, 349–367.

Chao, C. S., Chu, C. D. and Hsiung, W. Y. (1980). A revision of some genera and species of Chinese bamboos. *Acta Phytotaxonomica Sinica* **18**, 20–36.

Chua, K. S., Soong, B. C. and Tan, H. T. W. (1996). "The Bamboos of Singapore." pp. 30–32. IPGRI, Regional Office for Asia Pacific and Oceanic, Singapore.

Clark, L. G. and Judziewicz, E. J. (1996). The grass subfamilies Anomochlooideae and Pharoideae (Poaceae). *Taxon* **45**(4), 641–645.

Clark, L. G., Zhang, W. and Wendel, J. F. (1995). A phylogeny of the grass family (Poaceae) based on ndhF sequence data. *Systematic Botany* **20**, 436–460.

Clark, L. G., Judziewicz, E. J. and Tyrrel, C. D. (2007). *Aulonemia ximenae* (Poaceae: Bambusoideae), a new northern Andean species with fimbriate sheath margin. *Bamboo Science and Culture* **20**, 1–6.

Clayton, W. D. and Renvoize, S. A. (1986). "Genera Graminum, Grasses of the World." Her Majesty's Stationery Office, London.

Crepet, W. L. and Feldman, G. D. (1991). The earliest remains of grasses in the fossil record. *American Journal of Botany* **78**, 1010–1014.

Cummings, M. P., King, L. M. and Kellogg, E. A. (1994). Slipped-strand mispairing in a plastid gene: rpoC2 in grasses (Poaceae). *Molecular and Biological Evolution* **11**, 1–8.

Das, M., Bhattacharya, S. and Pal, A. (2005). Generation and characterization of SCARs by cloning and sequencing of RAPD products: A strategy for species specific marker development in bamboo. *Annals of Botany* **95**, 835–841.

Das, M., Bhattacharya, S., Basak, J. and Pal, A. (2007). Phylogenetic relationships among the bamboo species as revealed by morphological characters and polymorphism analyses. *Biologia Plantarum* **51**(4), 667–672.

Davis, J. I. and Soreng, R. J. (1993). Phylogenetic structure in the grass family (Poaceae) as inferred from chloroplast DNA restriction site variation. *American Journal of Botany* **80**, 1444–1454.

Ding, Y. (1998). ''Systematic Studies on *Phyllostachys.*'' pp. 1–120. Ph. D. Dissertation. Nanjing University, China.

Dransfield, S. (1992). A new species of *Racemobambos* (Gramineae: Bambusoideae) from Sulawesi with notes on generic delimitation. *Kew Bulletin* **47**, 707–711.

Dransfield, S. and Widjaja, E. A. (1995). ''Plant Resources of Southeast Asia PROSEA no: 7-Bamboos.'' Backhuys Publishers, Leiden, Holland.

Doebley, J., Durbin, M., Golenberg, E. M., Clegg, M. T. and Ma, D. P. (1990) Evolutionary analysis of the large subunit of carboxylase (rbcL) nucleotide sequence data among the grasses (Poaceae). *Evolution* **44**, 1097–1108.

Duvall, M. R. and Morton, B. R. (1996). Molecular phylogenetics of Poaceae: An expanded analysis of rbcL sequence data. *Molecular Phylogenetics and Evolution* **5**, 353–358.

Filgueiras, T. S. (2002). Herbaceous plant communities. *In* ''The Cerrados of Brazil'' (P. Oliveira and R. J. Marquis, eds.), pp. 121–139. Columbia University Press, New York.

Filgueiras, T. S. and Goncalves, A. P. S. (2004). A checklist of the basal grasses and bamboos in Brazil. *Bamboo Science and Culture* **18**(1), 7–18.

Filgueiras, T. S. and Londoño, X. (2006). A giant new *Guadua* (Poaceae:Bambusoideae) from central Brazil. *In* ''Seminário Nacional de Bambus'' (J. G. de Almeida and A. A. Teixeira, eds.), pp. 27–32. Universidade de Brasília, Brasília.

Flora of China (2007). Poaceae. *Journal of the Torrey Botanical Society* **22**, 1–733.

Friar, E. and Kochert, G. (1991). Bamboo germplasm screening with nuclear restriction fragment length polymorphisms. *Theoretical and Applied Genetics* **82**, 697–703.

Friar, E. and Kochert, G. (1994). A study of genetic variation and evolution of *Phyllostachys* (Bambusoideae: Poaceae) using nuclear restriction fragment length polymorphisms. *Theoretical and Applied Genetics* **89**, 265–270.

Gamble, J. S. (1896). ''Bambuseae of British India.'' Annals of the Royal Botanical Garden, Calcutta, India.

Ganapathy, P. M. (1997). Sources of non wood fiber for paper, board and panels production: Status, trends and prospects for India. *In* ''Asia-Pacific Forestry Sector Outlook Study Working Paper Series, Working Paper No: APFSOS/WP/10.'' pp. 1–59. Forestry Policy and Planning Division, Rome Regional Office for Asia and the Pacific, Bangkok.

Gaut, B. S., Clark, L. G., Wendel, J. F. and Muse, S. V. (1997). Comparisons of the molecular evolutionary process at rbcL and ndhF in the grass family (Poaceae). *Molecular Biology and Evolution* **14**(7), 769–777.

Ghorai, A. and Sharma, A. (1980). Cyto taxonomy of Indian Bambuseae II: Dendrocalameae and Melocanneae. *Acta Botanica Indica* **8**(2), 134–138.

Gielis, J. (1998). Upstream fundamental research in bamboo-possibilities and directions. Keynote lecture at Vth International Bamboo Congress, San Jose, Costa Rica.

Gielis, J., Valente, P., Bridts, C. and Verbelen, J. P. (1997a). Estimation of DNA content of bamboos using flow cytometry and confocal laser scanning microscopy (CLSM). *In* ''The Bamboos'' (G. Chapman, ed.), pp. 215–223. Linnean Society Symposium Series 19, Academic press, London.

Gielis, J., Everaert, I. and De, L. M. (1997b). Analysis of genetic variability and relationships in *Phyllostachys* using random amplified polymorphic DNA. *In* ''The Bamboos'' (G. Chapman, ed.), pp. 107–124. Academic Press, London.

Gielis, J., Peeters, H., Gillis, K., Oprins, J. and Debergh, P. (2001). Tissue culture strategies for genetic improvement of bamboo. *Acta Horticulturae* **552**, 195–203.

Gielly, L. and Taberlet, P. (1994). The Use of Chloroplast DNA to Resolve Plant Phylogenies: Noncoding versus rbcL Sequences. *Molecular Biology and Evolution* **11**(5), 769–777.

Grass Phylogeny Working Group (2001). Phylogeny and sub-familial classification of the grasses. *Annals of the Missouri Botanical Garden* **88**, 373–457.

Guala, G. F. (2003). A new genus of bamboos from the Cerrados of Brazil. *Bamboo Science and Culture* **17**, 1–3.

Guala, G., Bogler, D., Sadle, J. and Francisco-Ortega, J. (2000). Molecular evidence for polyphyly in the genus *Apoclada* (Poaceae: Bambusoideae). *Bamboo Science and Culture* **14**(1), 15–20.

Gui, Y. J., Wang, S., Quan, L. Y., Zhou, C. P., Long, S. B., Zheng, H. J., Jin, L., Zhang, X. Y., Ma, N. X. and Fan, L. J. (2007). Genome size and sequence composition of moso bamboo: A comparative study. *Science in China Series C: Life Science* **50**(5), 1–6.

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

Guo, Z. H., Chen, Y. Y., Li, D. Z. and Yang, J. B. (2001). Genetic variation and evolution of the Alpine bamboos (Poaceae: Bambusoideae) using DNA sequence data. *Journal of Plant Research* **114**, 315–322.

Guo, Z. H., Chen, Y. Y. and Li, D. Z. (2002). Phylogenetic studies on *Thamnocalamus* group and its allies (Bambusoideaae: Poaceae) based on ITS sequence data. *Molecular Phylogenetics and Evolution* **22**, 20–30.

Hamby, R. K. and Zimmer, E. A. (1988). Ribosomal RNA sequences for inferring phylogeny within the grass family (Poaceae). *Plant Systematics and Evolution* **160**, 29–37.

Hilu, K. W., Alice, L. A. and Liang, H. (1999). Phylogeny of Poaceae inferred from matK sequences. *Annals of the Missouri Botanical Garden* **86**, 835–851.

Hodkinson, T. R., Renvoize, S. A., Chonghaile, G. N., Stapleton, C. M. A. and Chase, M. W. (2000). A comparison of ITS nuclear rDNA sequence data and AFLP markers for phylogenetic studies in *Phyllostachys* (Bambusoideae, Poaceae). *Journal of Plant Research* **113**, 259–269.

Holttum, R. E. (1956). The classification of bamboos. *Phytomorphology* **6**(1), 73–90.

Holttum, R. E. (1958). Bamboos of Malaya peninsula. *Garden's Bulletin,* Singapore **16**, 1–135.

Hsiao, J. Y. and Rieseberg, L. H. (1994). Population genetic structure of *Yushania niitakayamensis* (Bambusoideae, Poaceae) in Taiwan. *Molecular Ecology* **3**, 201–208.

Hsiao, C., Jacobs, S. W. L., Barker, N. P. and Chatterton, N. J. (1998). A molecular phylogeny of the subfamily Arundinoideae (Poaceae) based on sequences of rDNA. *Australian Systematic Botany* **11**, 41–52.

Hsu, C. C. (1967). Preliminary chromosome studies on the vascular plants of Taiwan (1). *Taiwania* **13**, 117–130.

Hsu, C. C. (1972). Preliminary chromosome studies on the vascular plants of Taiwan (5). *Taiwania* **17**, 48–62.

Huttley, G. A., McRae, A. F. and Clegg, M. T. (1995). Molecular evolution of the Ac/Ds transposable element family in pearl millet and other grasses. *Genetics* **139**, 1411–1419.

Isagi, Y., Shimada, K., Kushima, H., Tanaka, N., Nagao, A., Ishikawa, T., OnoDera, H. and Watanabe, S. (2004). Clonal structure and flowering traits of a bamboo [*Phyllostachys pubescens* (Mazel) Ohwi] stand grown from a simultaneous flowering as revealed by AFLP analysis. *Molecular Ecology* **13**(7), 2017–2021.

Janzen, D. H. (1976). Why bamboos wait so long to flower. *Annual Review of Ecology and Systematics* **7**, 347–391.

Jie, Y. G., Sheng, W., Yan, L. Q., Ping, C. Z., Bao, S. L., Jun, H. Z., Liang, J., Yin, X. Z., Xun, N. M. A. and Jiang, L. F. (2007). Genome size and sequence composition of moso bamboo: a comparative study. *Science in China Series C: Life Science*, **50**(5), 1–6.

Judziewicz, E. J., Clark, L. G., Londono, X. and Stern, M. J. (1999). ''American Bamboos.'' Smithsonian Institution Press, Washington, D.C.

Kelchner, S. A. and Clark, L. G. (1997). Molecular evolutions and phylogenetic utility of the chloroplast rpl 16 intron in *Chusquea* and the Bambusoideae (Poaceae). *Molecular Phylogenetics and Evolution* **8**, 385–397.

Kellogg, E. A. and Campbell, C. S. (1987). Phylogenetic analyses of the Gramineae. *In* ''Grass Systematics and Evolution'' (T. R. Soderstrom, K. W. Hilu, C. S. Campbell and M. E. Barkworth, eds.), pp. 217–224. Smithsonian Institution, Washington, DC, USA.

Kellogg, E. A. and Watson, L. (1993). Phylogenetic studies of a large data set. Bambusoideae, Andropogonodae, and Pooideae (Gramineae). *Botanical Review* **59**(4), 273–343.

Keng, P. C. (1983). A revision of genera of bamboos from the old world. IV. *Journal of Bamboo Research* **2**(2), 1–17.

Keng, P. C. and Wang, Z. P. (1996). ''Flora Reipublicae Popularis Sinicae Tomus 9(1).'' Science Press, Beijing, China.

Keukeleire, P. D., Schepper, S. D., Gielis, J. and Gerats, T. (2004). A PCR-based assay to detect hAT-like transposon sequences in plants. *Chromosome Research* **12**, 117–223.

Khatta, V. and Katoch, B. S. (1983). Nutrient composition of some fodder tree leaves available in sub-mountainous region of Himachal Pradesh. *Indian Forester* **109**, 17–24.

Kurz, S. (1876). Bamboo and its use. *Indian Forester* **1**(3), 219–269.

Kwon, S. J., Park, K. C., Kim, J. H., Lee, J. K. and Kim, N. S. (2005). Rim 2/Hipa CACTA transposon display a new genetic marker technique in *Oryza* species. *BMC Genetics* **6**, 15.

Lai, C. C. and Hsiao, J. Y. (1997). Genetic variation of *Phyllostachys pubescens* (Bambusoideae, Poaceae) in Taiwan based on DNA polymorphisms. *Botanical Bulletin of Academia Sinica* **38**, 145–152.

Li, D. Z. (1997). The Flora of China Bambusoideae project: Problems and current understanding of bamboo taxonomy in China. *In* ''The Bamboos'' (G. P. Chapman, ed.), pp. 61–81. Academic Press, London, United Kingdom.

Liang, H. and Hilu, W. (1996). Applications of the matK gene sequences to grass systematics. *Canadian Journal of Botany* **74**, 125–134.

Lin, C. S., Lai, Y. H., Sun, C. W., Liu, N. T., Tsay, H. S., Chang, W. C. and Chen, J. J. W. (2006). Identification of ESTs diffrentially expressed in green and albino mutant bamboo (*Bambusa edulis*) by suppressive subtractive hybridization (SSH) and microarray analysis. *Plant Cell Tissue and Organ Culture* **86**, 169–175.

Litt, M. and Lutty, J. A. (1989). A hypervariable microsatellite revealed by *in vitro* amplification of a dinucleotide repeat within the cardiac muscle actin gene. *American Journal of Human Genetics* **44**, 397–401.

Loh, J. P., Kiew, R., Set, O., Gan, L. H. and Gan, Y. Y. (2000). A study of genetic variation and relationships within the bamboo subtribe Bambusinae using amplified fragment length polymorphism. *Annals of Botany* **85**, 607–612.

Majumdar, R. (1989). Bambusoideae. *In* "Florae Indicae Enumeratio: Monocotyledonae" (S. Karthikeyan, S. K. Jain, M. P. Nayar and M. Sanjappa, eds.), pp. 274–254. Botanical Survey of India, Calcutta.

Malcomber, S. T., Preston, J. C., Reinheimer, R., Kossuth, J. and Kellog, E. A. (2006). Developmental gene evolution and the origin of grass inflorescence diversity. *Advances in Botanical Research* **44**, 426–481.

Marulanda, M. L., Marquez, P. and Londono, X. (2002). AFLP analysis of *Guadua angustifolia* (Poaceae: Bambusoideae) in Columbia with emphasis on the coffee region. *Bamboo Science and Culture* **16**(1), 32–42.

Mathews, S. and Sharrock, R. A. (1996). The phytochrome gene family in grass (Poaceae): A phylogeny and evidence that grasses have a subset of the loci found in dicot angiosperms. *Molecular Biology and Evolution* **13**, 1141–1150.

Mathews, S., Tsai, R. C. and Kellogg, E. A. (2000). Phylogenetic structure in the grass family (Poaceae): Evidence from the nuclear gene phytochrome B. *American Journal of Botany* **87**(1), 96–107.

McClure, F. A. (1925). Some observations on the bamboos of Kwangtung. *Lingnan Agricultural Review* **3**, 40–47.

McClure, F. A. (1934). The inflorescence of *Schizostachyum* Nees. *Journal of the Washington Academy of Sciences* **24**, 541–548.

McClure, F. A. (1961). Toward a fuller description of the Bambusoideae (Gramineae). *Kew Bulletin* **15**(2), 321–324.

McClure, F. A. (1966). "The Bamboos." Harvard University Press, Cambridge, Massachusetts.

Munro, W. (1868). A monograph of the Bambusaceae, including description of all the species. *Transactions of the Linnean Society of London* **26**(1), 1–157.

Nadot, S., Bajon, R. and Lejeune, B. (1994). The chloroplast gene rps4 as a tool for the study of Poaceae phylogeny. *Plant Systematics and Evolution* **191**, 27–38.

Nakai, T. (1925). Two new genera of Bambusaceae, with special remarks on the related genera growing in eastern Asia. *Journal of Arnold Arboretum* **6**, 151.

Nayak, S. G. R. and Das, P. (2003). Evaluation of the genetic variability in bamboo using RAPD markers. *Plant, Soil and Environment* **49**(1), 24–28.

Nayak, S. and Rout, G. R. (2005). Isolation and characterization of microsatellites in *Bambusa arundinacea* and cross species amplification in other bamboos. *African Journal of Biotechnology* **4**(2), 151–156.

Nees von Esenbeck, C. G. D. (1835). Bambuseae brasilienses: Recensuit et alias in India Orientalis provenientes adjecit. *Linnaea* **9**, 461–494.

Oliveira, R. P. (2006). Estudos taxonômicos, filogenéticos e biossistemáticos em Raddia Bertol. (Poaceae:Bambusoideae:Olyreae), Ph. D. dissertation-Universidade Estadual de Feira de Santana, Bahia, Brazil[Taxonomic, phylogenetic and biosystematic studies on *Raddia*].

Olmstead, R. G. and Palmer, J. D. (1994). Chloroplast DNA systematics: A review of methods and data analysis. *American Journal of Botany* **81**(9), 1205–1224.

Paran, I. and Michelmore, R. W. (1993). Development of reliable PCR based markers linked to downy mildew resistance genes in lettuce. *Theoretical and Applied Genetics* **85**, 985–993.

Park, S. B. and Kwon, S. D. (1998). Development of new uses of bamboos (II): Development of carbonization kiln and schedule investigation for bamboo charcoal making. *FRI Journal of Forest Science* **59**, 17–24.

Park, K. C., Lee, J. K., Kim, N. H., Shin, Y. B., Lee, J. H. and Kim, N. S. (2003). Genetic variation in *Oryza* species detected by MITE-AFLP. *Genes and Genetic Systems* **78**, 235–243.

Parodi, L. R. (1961). La taxonomica de las Gramineae Argentinas a la luz de las investigaciones mas recientes. *In* "Recent Advances in Botany." pp. 125–130. University of Toronto Press, Toronto.

Pennisi, E. (2007). Wanted: A barcode for plants. *Science* **318**, 190–191.

Pohl, R. W. and Clark, L. G. (1992). New chromosome counts for *Chusquea* and *Aulonemia* (Poaceae: Bambusoideae). *American Journal of Botany* **79**(4), 478–480.

Poinar, G. O. and Columbus, J. T. (1992). Adhesive grass spikelet with mammalian hair in Dominican amber. First fossil evidence of epizoochory. *Experientia* **48**, 906–908.

Prat, H. (1960). Vers une classification naturelle des graminhes. *Bulletin de la Societe Botanique de France* **107**, 32–79.

Renvoize, S. A. and Clayton, W. D. (1992). Classification and evolution of grasses. *In* "Grass Evolution and Domestication" (G. P. Chapman, ed.), pp. 3–37. Cambridge University Press, Cambridge, England.

Riviere, A. and Riviere, C. (1879). "Les Bambous.: au Siege de la Societe d'Acclimatation." Paris.

Roshevits, R. Y. (1946). Sistema zlakov v svyazi s ikh evolyutsiei. Leningard: Komarav Botanical Institute, U.S.S.R. Academy of Sciences. Leningrad [Portuguese translation by T. Sendulsky, Instituto de Botlnica, Slo Paulo, Brazil, 1969. Evolucao e sistemitica das gramineas. Boletim do Instituto de Botlnica No. 5, 1–20.

Ruiyang, C. (2003). "Chromosome Atlas of Major Economic Plants Genome in China Vol. 4: Chromosome Atlas of Various Bamboo Species." pp. 646. Science Press, Beijing.

Scurlock, J. M. O., Dayton, D. C. and Hames, B. (2000). Bamboo: An overlooked biomass resource? *Biomass and Bioenergy* **19**, 229–244.

Small, R. L., Ryburn, J. A., Cronn, R. C., Seelanan, T. and Wendel, J. F. (1998). The tortoise and the hare: Choosing between noncoding plastome and nuclear ADH sequences for phylogeny reconstruction in a recently diverged plant group. *American Journal of Botany* **85**, 1301–1315.

Soderstrom, T. R. (1981). Some evolutionary trends in the Bambusoideae (Poaceae). *Annals of the Missouri Botanical Garden* **68**, 15–47.

Soderstrom, T. R. and Ellis, R. P. (1982). Taxonomic status of the endemic South African bamboo. *Thamnocalamus tessellatus. Bothalia* **14**(1), 53–67.

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

Soderstrom, T. R. and Ellis, R. P. (1988). The woody bamboos (Poaceae: Bambusoideae) of Sri Lanka: A morphological anatomical study. *Smithsonian Contributions to Botany* **72**, 1–75.

Soderstrom, T. R., and Young, S. T. (1983). A guide to collecting bamboo. *Annals of the Missouri Botanical Garden*, **70**, 128–136.

Stapleton, C. M. A. (1991). "A Morphological Investigation of Some Himalayan Bamboos with an Enumeration of Taxa in Nepal and Bhutan." Ph.D. thesis, University of Aberdeen, Aberdeen, UK.

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

Stapleton, C. M. A. (1994b). The bamboos of Nepal and Bhutan, Part II: *Arundinaria, Thamnocalamus, Borinda* and *Yushania* (Gramineae: Poaceae, Bambusoideae). *Edinburgh Journal of Botany* **51**, 275–295.

Stapleton, C. M. A. (1994c). The bamboos of Nepal and Bhutan, Part III: *Drepanostachyum, Himalayacalamus, Ampelocalamus, Neomicrocalamus* and *Chimnobambusa* (Gramineae: Poaceae, Bambusoideae). *Edinburgh Journal of Botany* **51**, 310–330.

Stapleton, C. M. A. (1997). The morphology of woody bamboos. *In* ''The Bamboos'' (G. P. Chapman, ed.), pp. 251–267. Academic Press, London.

Stenseth, N. C., Viljugrein, H., Saitoh, T., Hansen, T. F., Kittilsen, M. O., Bolviken, E. and Glockner, F. (2003). Seasonality, density dependence, and population cycles in Hokkaido voles. *Proceedings of the National Academy of Sciences USA* **100**(20), 11478–11483.

Sun, Y., Xia, N. and Lin, R. (2005). Phylogenetic analysis of *Bambusa* (Poaceae: Bambusoideae) based on internal transcribed spacer sequences of nuclear ribosomal DNA. *Biochemical Genetics* **43**(11/12), 603–612.

Sun, Y., Xia, N. and Stapleton, C. M. A. (2006). Relationships between *Bambusa* species (Poaceae, Bambusoideae) revealed by random amplified polymorphic DNA. *Biochemical Systematics and Ecology* **34**, 417–423.

Suyama, Y., Obayashi, K. and Hayashi, I. (2000). Clonal structure in a dwarf bamboo (*Sasa senanensis*) population inferred from amplified fragment length polymorphism (AFLP) fingerprints. *Molecular Ecology* **9**, 901–906.

Tateoka, T. (1957). Miscellaneous papers on the phylogeny of Poaceae (10). Proposition of a new phylogenetic system of Poaceae. *Journal of Japanese Botany* **32**, 275–287.

Thomasson, J. R. (1987). Fossil grasses: 1820–1986 and beyond. *In* ''Grass Systematics and Evolution'' (T. R. Soderstrom, K. W. Hilu, C. S. Capmbell and M. E. Barkworth, eds.), pp. 159–167. Smithsonian Institution Press, Washington, D.C.

Triplett, J. K., Weakley, A. S. and Clark, L. G. (2006). Hill cane (*Arundinaria appalachiana*) a new species of bamboo from the southern Appalachian Mountains. *Sida* **22**, 79–85.

Tzvelev, N. (1989). The system of grasses (Poaceae) and their evolution. *The Botanical Review* **55**, 141–203.

Usui, H. (1957). Morphological studies on the prophyll of Japanese bamboos. *The Botanical Magazine, Tokyo* **70**, 223–227.

Vicient, C. M., Jaaskelainen, M. J., Kalendar, R. and Schulman, A. H. (2001). Active transposons are common feature of grass genomes. *Plant Physiology* **125**, 1283–1292.

Vos, P., Hogers, R., Bleeker, M., Reijans, M., van de Lee, T., Hornes, M., Frijters, A., Pot, J., Peleman, J., Kuiper, M. and Zabeau, M. (1995). AFLP, a new technique for DNA fingerprinting. *Nucleic Acids Research* **23**, 4407–4414.

Wagner, A., Blackstone, N., Cartwright, P., Dick, M., Misof, B., Snow, P., Wagner, G. P., Bartels, J., Murtha, M. and Pendleton, J. (1994). Surveys of gene families using polymerase chain reaction: PCR selection and PCR drift. *Systematic Biology* **43**, 250–261.

Wang, C. P., Yu, Z. H., Ye, G. H., Chu, C. D. and Chao, C. S. (1980). A taxonomic study of *Phyllostachys* in China. *Acta Phytotaxonomica Sinica* **18**(15–19), 168–193.

Watson, L. and Dallwitz, M. J. (1992). ''The Grass Genera of the World.'' CAB International, Wallingford, Oxon, England.

Wen, T. H. (1986). Some issues of taxa of Bambusoideae in China. *Journal of Bamboo Research* **5**(2), 10–27.

Wessler, S. R., Bureau, T. and White, S. (1995). LTR-retrotransposons and MITEs: Important players in the evolution of plant genomes. *Current Opinion in Genetics and Development* **5**, 814–821.

White, T. J., Bruns, T., Lee, S. and Taylor, J. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *In* "PCR Protocols: A Guide to Methods and Applications" (M. Innis, D. Gelfand, J. Sninsky and T. White, eds.), pp. 315–322. Academic Press, San Diego.

Williams, J. G. K., Kubelik, A. R., Livak, K. J., Rafalski, J. A. and Tingey, S. V. (1990). DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Research* **18**, 6531–6535.

Wolfe, A. D. and Randle, C. P. (2004). Recombination, heteroplasmy, haplotype polymorphism, and paralogy in plastid genes: Implications for plant molecular systematics. *Systematic Botany* **29**(4), 1011–1020.

Wong, K. M. (2004). Bamboo—the amazing grass. A guide to the diversity and study of bamboos in Southeast Asia. International Plant Genetic Resources Institute (IPGRI) and Kuala Lumpur, University of Malaya, Malaysia.

Yu, J. K., Rota, M. L., Kantety, R. V. and Sorrells, M. E. (2004). EST derived SSR markers for comparative mapping in wheat and rice. *Molecular Genetics and Genomics* **271**, 742–751.

Zhang, W. (2000). Phylogeny of the grass family (Poaceae) from rpl 16 intron sequence data. *Molecular Phylogenetics and Evolution* **15**(1), 135–146.

Zhang, W. and Clark, L. G. (2000). Phylogeny and classification of the Bambusoideae (Poaceae). *In* "Grasses: Systematics and Evolution" (S. W. L. Jacobs and J. E. Everett, eds.), pp. 35–42. CSIRO Publishing, Collingwood, Victoria.

Zhang, W., Wendel, J. F. and Clark, L. G. (1997). Bamboozled again! inadvertent isolation of fungal rDNA sequences from bamboos (Poaceae: Bambusoideae). *Molecular Phylogenetics and Evolution* **8**(2), 205–217.