Taxonomy and systematics of *Dendrocalamus* (Bambuseae; Poaceae)

Taxonomy and systematics of *Dendrocalamus* (Bambuseae; Poaceae)

Thesis submitted to the University of Dublin, Trinity College
for the
Degree of Doctor of Philosophy (Ph.D.)

by

Sarawood Sungkaew

School of Natural Sciences
Department of Botany
University of Dublin, Trinity College

2008

Research conducted under the joint supervision of
Dr. Trevor R. Hodkinson
School of Natural Sciences, Department of Botany
University of Dublin, Trinity College, Ireland
Dr. Soejatmi Dransfield
Herbarium, Royal Botanic Gardens, Kew, England
and
Dr. Chris M. A. Stapleton
Herbarium, Royal Botanic Gardens, Kew, England

Declaration

I hereby declare that this thesis has not been submitted for a degree at this or any other university. It is entirely my own work, unless otherwise stated. The Trinity College Library may lend or copy this thesis upon request.

Sarawood Sungkaew

Bamboo

'No plant is known in the tropical zone which could supply to man so many technical advantages as the bamboo. The strength of the culms, their straightness, smoothness, lightness combined with hardness and greater hollowness; the facility and regularity with which they can be split; the different sizes, various lengths and thickness of their joints make them suitable for numerous purposes to serve which other material would require much labour and preparation'

Kurz (1876)

Abstract

This thesis is concerned with the taxonomy and systematics of *Dendrocalamus* (Bambuseae; Poaceae). It aimed to assess phylogenetic relationships in the subfamily Bambusoideae and to focus on one highly economically, ecologically and culturally important genus, *Dendrocalamus*. It aimed to provide an account of *Dendrocalamus* for the Flora of Thailand project, to assess its infrageneric classification and to study the diversity and phylogeography of one of its most important species, *D. membranaceus*, in Thailand.

The taxonomy of Bambusoideae is in a state of flux and phylogenetic studies are required to help resolve taxonomic issues. Studies on a broad scale are also required to understand where *Dendrocalamus* is phylogenetically positioned within Bambusoideae. For the phylogenetic study of Bambusoideae, over 60 taxa, representing all the bamboo subtribes of the tribe Bambuseae and related non-bambusoid grasses were sampled. A combined analysis of five plastid DNA regions, *trnL* intron, *trnL-F* intergenic spacer, *atpB-rbcL* intergeneric spacer, *rps16* intron, and *matK*, was used to study the phylogenetic relationships among the bamboos in general and the woody bamboos in particular. The BEP (Bambusoideae, Ehrhartoideae, Pooideae) clade was resolved and Pooideae was supported as sister to Bambusoideae s.s.. Bambuseae as currently recognised, the woody bamboos, were not monophyletic. Olyreae, the herbaceous bamboos, were sister to tropical Bambuseae. Temperate Bambuseae were sister to the group consisting of tropical Bambuseae and Olyreae. Within the tropical Bambuseae, neotropical Bambuseae were sister to palaeotropical and Austral Bambuseae. Furthermore, Melocanninae were sister to the remaining palaeotropical and Austral Bambuseae. Bambuseae were therefore redefined and the use of the tribal name Arundinarieae was recommended to accommodate the temperate woody bamboos. Classification of the bamboos is discussed in relation to the commonly used classification systems and patterns of phylogenetic diversification interpreted in a biogeographic context.

To study the phylogenetics of *Dendrocalamus* and its close allies in detail, 55 plants from Bambuseae, including 20 *Dendrocalamus* species, were analyzed. The same five plastid DNA regions were sequenced and analyzed as the study that focussed on the phylogenetics of Bambusoideae. *Bambusa, Dendrocalamopsis, Gigantochloa,* and *Melocalamus* are genera closely related to *Dendrocalamus*. Based mainly on molecular data, the relationships of species within *Dendrocalamus* was not fully resolved and a review of the infraspecific classification based on molecular data not possible. However, the addition of morphological data in phenetic and phylogenetic analyses helped to resolve the problem. Thirteen representatives of Thai

Dendrocalamus species and a Chinese species were assessed with combined evidence from morphological and molecular characters and allowed classification of *Dendrocalamus* into two subgenera, subg. *Dendrocalamus* and subg. *Sinocalamus*. It also allowed for the grouping of species within subgenera and hence the review of currently recognized sections. A total of 12 morphological characters were included to study character evolution. Three characters, the mid-culm branch complement, pseudospikelet congestion (synflorescences), and number of fertile florets/spikelet, provided strong evidence to separate the two subgenera.

Little is known about the population genetics of bambooos and no studies have focussed on infraspecific population genetic variation in *Dendrocalamus*. Nine populations, and a total of 45 individuals, of an ecologically and economically important species of *Dendrocalamus* from Thailand, *D. membranaceus*, were sampled for diversity and phylogeographic studies. Using markers amplified from three plastid DNA microsatellite loci, a total of six haplotypes and three groups of nine populations were defined using Nei's unbiased genetic distance and UPGMA. The population from Phu Rue did not group closely with the other populations. All populations sampled except one, Suan Phung, contained more than one haplotype. This indicates that they are not stands of a singe genotype. In fact most populations had high haplotype diversity. Most variation was found within populations and the populations showed low population genetic differentiation (G_{ST} =8.6%). This indicates that seed mediated geneflow (or movement of vegetative propagules) between populations is high (and certainly not restricted). Evidence against monoclonal stands of plants is presented. The results are discussed in the context of the breeding biology of *Dendrocalamus,* its ecology and the physical geography of Thailand.

Until now, taxonomic/floristic knowledge of *Dendrocalamus* within Thailand was sparse. Floristic works of Thai *Dendrocalamus* for the Flora of Thailand project have been completed as part of this thesis. Thirteen species were reported to occur in Thailand, of which *D. copelandii* was recorded for the first time for Thailand while *D. khoonmengii* was described as a species new to science. Two keys to species, one based on fertile specimens and another based on sterile specimens and ecology, were constructed. Species descriptions, accompanied with synonyms, typifications, illustrations, distribution maps, and lists of specimens examined, are presented.

During the studies a new genus and species, *Phuphanochloa speciosa,* was described for the first time. Its closest relatives, on the basis of morphology and molecular evidence, are *Bambusa* species.

Acknowledgements

I wish to thank my great supervisor Dr. Trevor Hodkinson, and my two co-supervisors Drs. Soejatmi Dransfield and Chris Stapleton for their enthusiasm, support, and friendship throughout my research.

I also wish to thank Stephen Renvoize, my external examiner, and Dr. Stephen Waldren, my internal examiner, who have provided remarkably useful suggestions for my thesis.

I would also like to thank those who donated material to me or helped me in collecting bamboo specimens Drs. Soejatmi Dransfield, Ruth Kiew, Wang Hong, Trevor Hodkinson, Wong Khoon Meng, Chris Stapleton, and Miss Atchara Teerawatananon.

I would like to express my gratitude to Dr. David Simpson and Stephen Renvoize who helped me when I was working in the herbarium at the Royal Botanic Gardens, Kew, England, and to Dr. Vincent Savolainen and Laszlo Csiba when I was in The Jodrell Laboratory of Kew.

Thanks very much to all crew of the Department of Botany, Trinity College Dublin.

Special thanks to Dr. Duangchai Sookchaloem who helped me in a variety of ways from the beginning until the end of the thesis project.

Last, but certainly not least, a special thanks to Prof. John Parnell who never said "no" when I had any problems with my work.

This work was supported by the TRF/BIOTEC Special Program for Biodiversity Research and Training grant T_147003, a Trinity College Dublin, Ireland Postgraduate Studentship, the Trinity College Dublin Postgraduate Travel Reimbursement Fund, and Faculty of Forestry, Kasetsart University, Bangkok, Thailand.

Table of Contents

List of Tables

Chapter 1

Chapter 2

Chapter 3

Chapter 4

List of Figures

Chapter 1

Chapter 2

Chapter 3

Chapter 4

Chapter 5

Chapter 1

General introduction

1.1 What is the bamboo?

Bamboos are forest grasses belonging to the large monocotyledonous angiosperm family Poaceae (Gramineae). However, this is not the largest family in terms of the number of genera and species, coming after Asteraceae (Compositae), Fabaceae (Leguminosae), Orchidaceae and Rubiaceae (Clayton & Renvoize, 1986). The commonly known bamboos are generally woody but there are actually two major recognized groups of bamboos, namely woody and herbaceous (McClure, 1966; Clayton & Renvoize, 1986; Soderstrom & Ellis, 1987; Watson & Dallwitz, 1992; Dransfield & Widjaja, 1995a; Wong, 1995a; Clark, 1995, 1997a; Judziewicz *et al.*, 1999; Ohrnberger, 1999; GPWG, 2001).

Several morphological and anatomical characters of bamboos differ from common grasses. They generally have woody and usually hollow culms, complex rhizome and branching systems, relatively broad and pseudopetiolate leaves, fusoid and arm cells within leaf blades, prominent sheathing organs, external ligules, bracteate inflorescences and trimerous flowers (McClure, 1966; Clayton & Renvoize, 1986; Soderstrom & Ellis, 1987; Watson & Dallwitz, 1992; Dransfield & Widjaja, 1995a; Wong, 1995a; Judziewicz *et al.*, 1999; GPWG, 2001). The chromosome numbers of the Old World woody bamboos usually have a basic chromosome number (x) of 12 while those of the New World are divided between 10 and 12. Most woody bamboos are tetraploid or hexaploid (Soderstrom, 1981; Dransfield & Widjaja, 1995a; Judziewicz *et al.* (1999). The herbaceous bamboos have a diversity of base numbers including 7, 9, 10, 11, and 12, with 10 and 11 occurring most frequently (Judziewicz *et al.*, 1999).

For these reasons along with molecular DNA information, botanists or bambusologists generally group the woody bamboos and their allies as a subfamily known as Bambusoideae (McClure, 1966; Clayton & Renvoize, 1986; Soderstrom & Ellis, 1987; Watson & Dallwitz, 1992; Dransfield & Widjaja, 1995a; Wong, 1995a, 2004; Clark, 1995, 1997a; Judziewicz *et al.*, 1999; Ohrnberger, 1999; GPWG, 2000, 2001). The broad sense Bambusoideae *sensu lato* (s.l.) (Roshevits, 1946; Clayton & Renvoize, 1986; Watson & Dallwitz, 1992) includes woody bamboos, herbaceous bamboos, and other grasses such as rice (*Oryza* spp.; Ehrhartoideae), *Anomochloa, Streptochaeta* (Anomochlooideae), *Pharus* (Pharoideae), *Puelia* and *Guaduella* (Puelioideae) (Ohrnberger, 1999). However, in the strict sense Bambusoideae *sensu stricto* (s.s.)

consists of only two bamboo groups; the woody bamboos (tribe Bambuseae) and the herbaceous bamboos (tribe Olyreae) (Judziewicz *et al.*, 1999; GPWG, 2001).

The total number of accepted or provisionally accepted species reported in 'The Bamboos of the World' (Ohrnberger, 1999) is 1,575 plus several species still with incorrect names. In terms of the natural geographical distribution, bamboos occur in the tropical, subtropical and temperate regions of all continents except Europe, Antarctica, and western Asia, from lowland up to 4,000 m in altitude (Soderstrom & Ellis, 1987; Dransfield & Widjaja, 1995a; Ohrnberger, 1999), Figure 1.1 and Table 1.1.

Due to their species diversity, wide distribution, availability, rapid growth, and easy handling, bamboo is centrally involved in human daily life, especially in Asia, America, and Africa, where they primarily grow. Kurz (1876) emphasized that 'No plant is known in the tropical zone which could supply to man so many technical advantages as the bamboo. The strength of the culms, their straightness, smoothness, lightness combined with hardness and greater hollowness; the facility and regularity with which the can be split; the different sizes, various lengths and thickness of their joints make them suitable for numerous purposes to serve which other material would require much labour and preparation'. Bamboo has been designated as 'Poor Man's Timber' (Rashford, 1995). We can say that bamboos play a major role in the world's economy, its ecosystems and the lives of its people. They are not only used for many kinds of constructions but also for food, paper and countless articles of daily life including furniture and a most incredible variety of baskets and other containers, handicrafts, musical instruments, shelter, ornaments and so on (Kurz, 1876; Dransfield & Widjaja, 1995a; Rashford, 1995; Wong, 1995a, 2004).

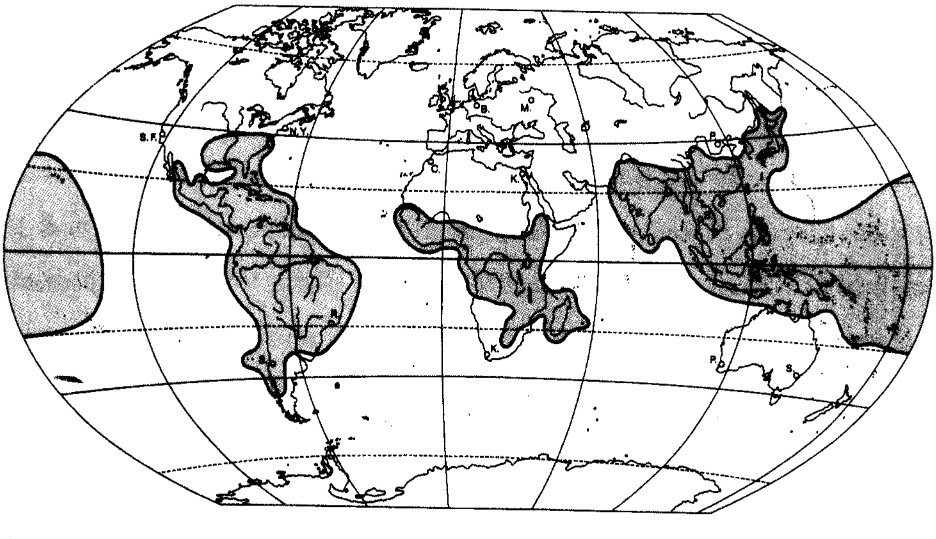


Figure 1.1 Map of the natural distribution of the subfamily Bambusoideae s.l.. Adapted from Ohrnberger (1999).

Table 1.1 Occurrence of Bambusoideae s.l. in the continents. From Ohrnberger (1999).

Continents	Area boundary	Approximate species number
America	North America: continental USA only.	2
	Central America: from Mexico to Panama, including Caribbean Islands.	115
	South America: from Colombia and Venezuela, including Trinidad and Tobago, to Chile and Argentina.	372
Africa	Tropical Africa: tropical West, Central, and East Africa.	16
	South Africa: Republic South Africa only.	1
	Madagascar, Comoro and Mascarene Islands.	37
Asia	South and South-East Asia (southern China and Tibet not included): from Pakistan and India to Philippines and Indonesia (excluding New Guinea).	311
	East Asia: from Sakhalin and Kuriles to and including China with Tibet.	748
Pacific	New Guinea and Pacific Islands (Melanesia, Micronesia, Polynesia).	39
Australia	Australia, northern part only.	3

1.2 Taxonomy and systematics of Bambusoideae

Bamboo taxonomy and systematics are thriving research fields but reliable taxonomic characters are difficult to find (one bamboo can look very much like another). Good taxonomic characters can be found in the flowers but finding flowering bamboos is difficult since they rarely flower and when they do so, the plants often die. Traditional methods have established systems of classification and allowed detailed floristic work, but these studies are sometimes limited due to this lack of characters (Stapleton, 1997). Furthermore, the herbarium specimens available do not represent the bamboos from all over the world and they are often inadequate to represent the species. One of the most important problems is that many collectors evade collecting bamboos because of their size or the difficulties in trying to make good scientific specimens (Holttum, 1958; McClure, 1966; Wong, 2004). In addition, gathering good information in the field to accompany the voucher specimen is usually time-consuming.

Linnaeus (1753) published the first valid name of bamboo, *'Arundo bambos'* in his 'Species Plantarum' but this was without its description in the first edition; a brief description has been given in the second edition (Linnaeus, 1761). It was a name which embraced more than one taxon at that time and it has been controversially discussed (especially its typification). It has later been considered as a basionym of *'Bambusa bambos'* (L.) Voss (Soderstrom, 1987; Xia & Stapleton, 1997b).

Soderstrom (1987) has summarized the principle steps in the history of bamboo classification as follows:

1623. Caspar Bauhin listed several kinds of plants under *Arundo,* of which it was a bamboo called *'Arundo arbor'* which is now a synonym of *B. bambos* (Soderstrom, 1987; Xia & Stapleton, 1997b).

1753. Linnaeus, in 'Species Plantarum', gave bamboos their first formal name in botanical nomenclature, *'Arundo bambos'*, a name that embraced more than one taxon.

1789. Retzius in Sweden and Schreber in Germany recognized the same bamboo that had been called *'Arundo arbor'* and decided that it represented a distinct genus from *Arundo.* Retzius called it *'Bambos'* (employing the specific name as his new genus name) whilst Schreber called it, using Latin form, *'Bambusa'.*

1815. Kunth recognized bamboos as one of his ten natural groups of grasses and thus conceptualized what we know today as the subfamily Bambusoideae.

1834. Nees von Esenbeck established the first system of classification for bamboos in his treatment of Brazilian bamboos, recognizing three groups – two of which were woody, as 'Tribus Bambusaceae' (with *Bambusa*), and as 'Series Arundinariae' (with *Arundinaria*) – and one of which was herbaceous, as *'Streptochaeta'* (by itself and not in a tribe).

1961. Parodi formalized the subfamily Bambusoideae in establishing a system of classification for the grasses of Argentina. In this system he included all of the woody bamboos in a single tribe Bambuseae, and allocated the herbaceous members to three tribes – Olyreae, Phareae and Streptochaeteae.

Since the first bamboo name was established, many bamboo classifications (which were usually part of more general grass family classifications) were published (eg. Nees von Esenbeck 1834; Roshevits, 1946; Tateoka; 1957; Prat, 1960; Caro, 1982; Clayton & Renvoize, 1986; Soderstrom & Ellis, 1987; Tzvelev, 1989; Watson & Dallwitz, 1992; Clark, 1995; Ohrnberger, 1999; GPWG, 2001). The several grass family classifications divided Poaceae into 3–12 subfamilies, depending on the type of classification systems or information they used. However, the taxonomic status of bamboos and related herbaceous grasses is still unclear.

Recent advances in molecular biology offer the opportunity to solve problems where classical methods have been of limited value (Stapleton, 1997). DNA sequencing and fingerprinting methods, in particular, are revolutionizing the field (e.g. *ndhF*, Clark *et al.* (1995); combined molecular and morphological data, GPWG (2001); *ITS*, Renvoize & Hodkinson (1997), Hodkinson *et al.* (2000), Sun *et al.* (2005); *ITS, trnL-F,* and *rps16,* Ni Chonghaile (2002); and DNA fingerprinting methods such as AFLP, Hodkinson *et al.* (2000) and Loh *et al.* (2000)). Several phylogenetic analyses of the bamboos have been published (Kellogg & Watson, 1993; Clark *et al.*, 1995; GPWG, 2001). We now know that the woody bamboos (Bambuseae) together with certain herbaceous bamboo genera (Olyreae, e.g. *Lithachne, Olyra, Pariana,* and two monotypic genera, *Buergersiochloa* and *Eremitis*) form a subfamily Bambusoideae s.s. The subfamily Bambusoideae s.l. is non-monophyletic (Clark *et al.* 1995; GPWG, 2001; Salamin *et al.*, 2002) and some of the previously recognized bambusoids (e.g. *Anomochloa, Guaduella, Pharus, Puellia, Streptochaeta*) are placed as sister to the rest of the grasses (they are among the earliest diverging lineages in the family and are not part of Bambusoideae s.s.), Figure 1.2.

1.3 Taxonomy and systematics of Bambuseae

Within Bambusoideae s.l., there are between 1–15 tribes depending on the intra-subfamilial classification adopted (Roshevits, 1946; Prat, 1960; Caro, 1982; Clayton & Renvoize, 1986; Soderstrom & Ellis, 1987; Tzvelev, 1989; Watson & Dallwitz, 1992; Clark, 1995; GPWG, 2001). Among those tribes, it is Bambuseae (woody bamboos) that are the most species rich. There are 3–10 subtribes of Bambuseae depending on intra-tribal classification adopted (Clayton & Renvoize, 1986; Soderstrom & Ellis, 1987; Dransfield & Widjaja, 1995a; Li, 1998; Ohrnberger, 1999). According to the molecular studies (Clark *et al.*,1995; Ni Chonghaile, 2002) there may be three major lineages within the woody bamboos; temperate, palaeotropical, and neotropical.

However there are few systematic studies on Bambuseae and those that are published are based mainly on the temperate lineage of woody bamboos (Hodkinson *et al.*, 2000; Ni Chonghaile 2002). Within woody bamboo, however, one of the most important subtribes, the Bambusinae could not be defined, either according to old or new classification systems (Clayton & Renvoize, 1986; Soderstrom & Ellis, 1987; Dransfield & Widjaja, 1995a; Clark, 1995; Li, 1998; Ohrnberger, 1999). It consists of highly useful bamboos and is also the most morphologically and taxonomically complex group of all bamboos. It contains 10–25 genera that are mostly naturally distributed in tropical Asia (Clayton & Renvoize, 1986; Soderstrom & Ellis, 1987; Dransfield & Widjaja, 1995a; Clark, 1995; Li, 1998; Ohrnberger, 1999).

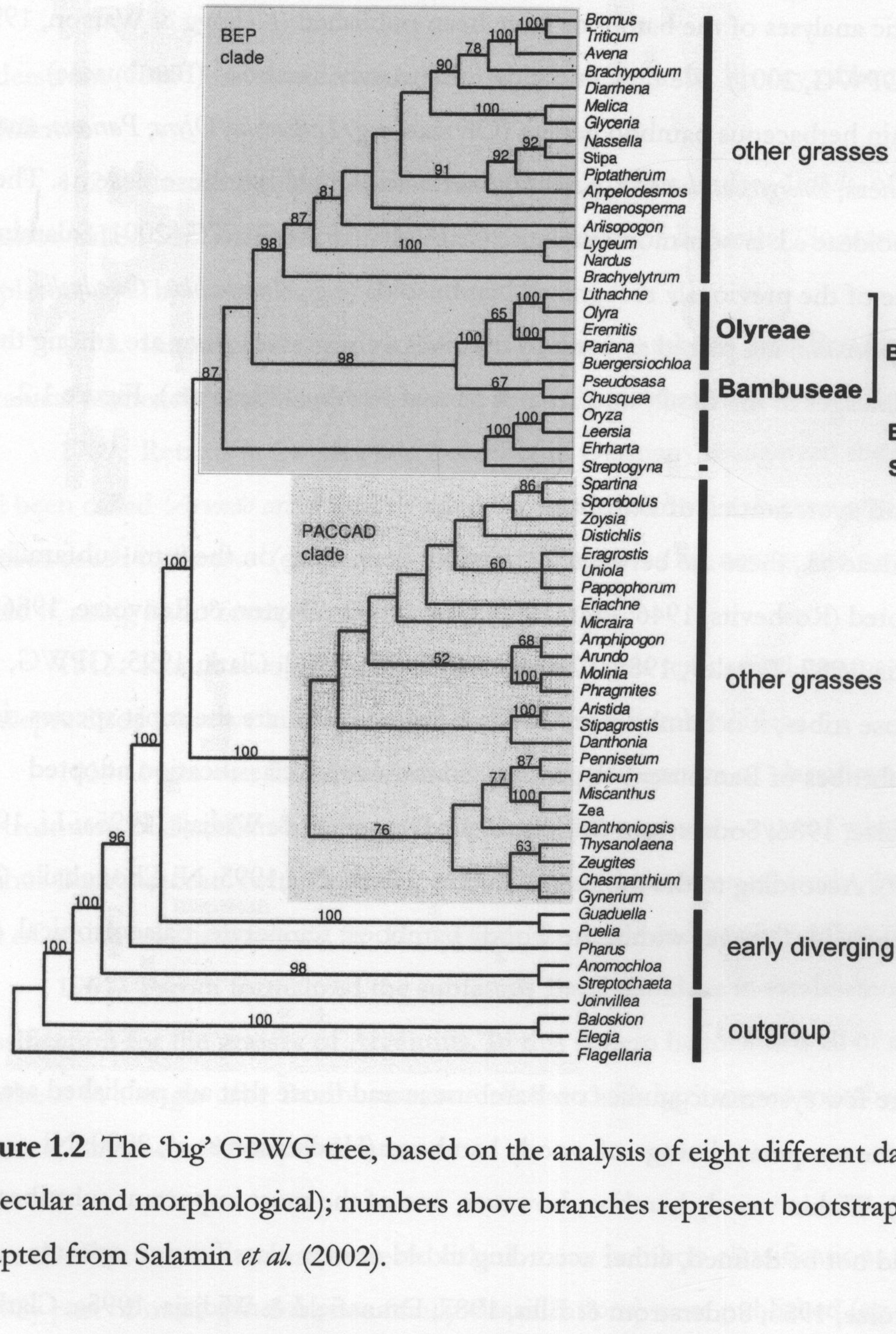


Figure 1.2 The 'big' GPWG tree, based on the analysis of eight different data sets (both molecular and morphological); numbers above branches represent bootstrap percentages. Adapted from Salamin *et al.* (2002).

1.4 Taxonomy and systematics of Asiatic bamboos

Ruprecht (1839) published a comprehensive treatment of the Old World bamboos which was then followed by Munro (1868) and these studies are still valuable today. Munro (1868) divided the Old World tropical woody bamboos into two sections (=subtribe of other systems), Bambusae and Bacciferae, based on fruit characters. The bamboo classification system of Bentham and Hooker (1883) who modified Munro's scheme, was adopted by Gamble (1896). Kurz (1876) studied bamboos of the Indian subcontinent and Malayan archipelago. This was the first time that field observations were provided. Gamble (1896) contributed excellent information on Asiatic bamboos which many tropical bambusologists are still using. His work, 'The Bambuseae of British India', covered the bamboos of India, Burma (Myanmar) and Malaya. Holttum (1958) published a classic account entitled 'The bamboos of the Malay Peninsula' and this is heavily cited and still used extensively for studying bamboos in Asia.

China is one of the centres of distribution of many bamboo genera (Ohrnberger, 1999). Soderstrom and Calderon (1979) reported that taxonomic accounts of bamboos of China appear in the many publications of F.A. McClure who spent most of the years between 1919 and 1941 in China. Other useful publications from several authors in different journals have also been published, such as in Acta Botanica Yunnanica, Acta Phytotaxonomica Sinica, Journal of Bamboo Research, Journal of South-Western Forestry College, and the Journal of South China Agricultural University, but these are often inaccessible or not translated into English (or other non-Chinese language). However Li (1997) reported that the revision of the Flora of China, *Flora Reipublicae Popularis Sinicae*, would have an English edition. Eventually this publication, that all bambusologists were awaiting, was published (Li *et al.*, 2006). From now on, studies on the taxonomy and systematics of Asiatic bamboos should be more satisfactory. Therefore many publications have been made (Gamble, 1896; Holttum, 1958; Dransfield & Widjaja, 1995a; Li, 1998; Wong, 1995a, 2004; Li *et al.*, 2006) and together they comprise a valuable resource for conducting bamboo research in this region.

Both traditional and phylogenetic classifications have been undertaken for whole genera or for bamboos within particular areas such as for the economically valuable *Bambusa* (Wong, 1991; 1993a), *Gigantochloa* (Widjaja, 1997) and *Phyllostachys* (Renvoize & Hodkinson, 1997; Hodkinson *et al.*, 2000). However, another taxonomically challenging and econonomically important genus, *Dendrocalamus*, is not well documented.

1.5 Taxonomy and systematics of *Dendrocalamus*

Dendrocalamus was first described by Nees von Esenbeck (1834). It provides not only the valuable timber for construction but also the young shoots for consumption. In addition, it contains *D. giganteus* which is probably the largest of all grasses, reaching heights of 30–35 metres (Holttum, 1958; Clayton & Renvoize, 1986; Dransfield & Widjaja, 1995a; Wong, 1995a; Sungkaew *et al.*, *unpublished*).

There are about 50 species of *Dendrocalamus* in the world distributed from southern China to India, Burma, Thailand, Laos, Cambodia, Vietnam, Malaysia to Papua New Guinea (Ohrnberger, 1999), Figure 1.3.

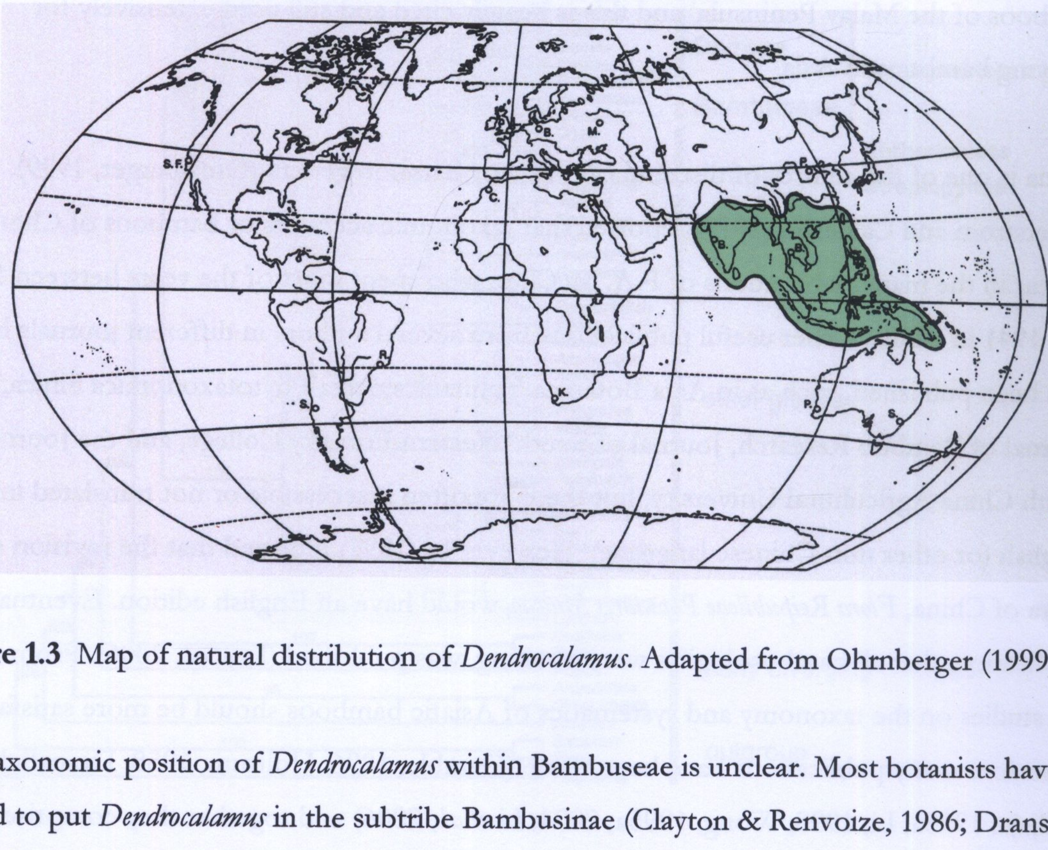


Figure 1.3 Map of natural distribution of *Dendrocalamus*. Adapted from Ohrnberger (1999).

The taxonomic position of *Dendrocalamus* within Bambuseae is unclear. Most botanists have agreed to put *Dendrocalamus* in the subtribe Bambusinae (Clayton & Renvoize, 1986; Dransfield & Widjaja, 1995a; Clark, 1995; Wong, 1995a; Li, 1998; Ohrnberger, 1999). *Bambusa* has also been put in the same subtribe. Occasionally, however, these two genera have been placed in different tribes (Li, 1997), where *Bambusa* was positioned in Bambuseae but *Dendrocalamus* was placed in Dendrocalameae. They have also been positioned in different subtribes of Bambuseae by Bentham and Hooker (1883), where *Bambusa* was in subtribe Eubambuseae and *Dendrocalamus* was in the subtribe Dendrocalaminae.

The generic delimitation of *Dendrocalamus* is also still unclear. Morphologically, it is believed to be closely allied to several genera including *Bambusa*, *Gigantochloa*, *Houzeaubambus*, *Klemaochloa*, *Oreobambos*, *Oxytenanthera*, *Sinocalamus*, and *Neosinocalamus* (Holttum, 1958; Clayton & Renvoize,1986; Soderstrom & Ellis, 1987; Wong, 1995a; Stapleton & Xia, 1997; Li, 1997; Li & Xue, 1997; Li, 1998; Ohrnberger, 1999). Wong (1995a) stated that the *Dendrocalamus* species enumerated in his 'The Bamboo of Penninsular Malaysia' may in fact represent more than one genus and also noted that "*Dendrocalamus* requires further critical study".

1.6 *Dendrocalamus* and Flora of Thailand project

Thailand covers approximately 515,000 km^2, and was once almost completely covered by forest; it has experienced one of the fastest rates of deforestation in the world (Santisuk *et al.*, 1991). Unfortunately, the forest cover has declined from 50% of the total land area in the 1950's to 25% in 2000 while the Thai flora remains poorly studied and has not yet been fully documented (Middleton, 2003). It is, therefore, unknown how many species may have gone extinct or been lost to Thailand. Many species new to science have been found in National Parks and Wildlife Sanctuaries which occupy 8% of the country's land area (Napompeth & Rodcharoen, 1998). However many remain to be discovered. New records of plant species previously unknown to exist within Thailand (new records), new species and even new genera are being documented, including woody bamboos, e.g. *Dendrocalamus copelandii* (Sungkaew *et al., accepted*), *D. khoonmengii* (Sungkaew *et al., accepted*), and *Phuphanochloa speciosa* (Sungkaew *et al., accepted*).

Due to the high species diversity of the Thai flora, approximately 10,000 species (equivalent to the total for all of Europe), it has been estimated that the Flora of Thailand publication would be completed within 100 years (Santisuk *et al.*, 1991; Middleton, 2003; Parnell, 2000), However, since the Flora of Thailand project was launched in 1967 (Middleton, 2003), the project's speed has increased and it is now hoped to be completed by 2030 (Parnell, 2000).

Currently, an account of the family Poaceae for the Flora of Thailand is being undertaken (Simpson, *personal communication*). The person responsible for leading the bamboo sections for the Flora of Thailand, is Dr. Soejatmi Dransfield, a bambusologist from the Royal Botanic Gardens Kew (KEW). *Dendrocalamus* is one of the largest genera (in terms of species number) of Thai bamboos and is being documented by myself. The floristic work for this genus is one major part of this thesis (Chapter 4).

1.7 Population genetic study on *Dendrocalamus* in Thailand

Many species of *Dendrocalamus* in Thailand are economically and ecologically valuable, for example, *D. asper*, *D. brandisii*, *D. latiflorus*, *D. membranaceus*, and *D. strictus* (Lin, 1968; Dransfield

& Widjaja, 1995a; Sungkaew *et al., unpublished*). However, none of them have been studied at a population genetic level or investigated for phylogeographic pattern. *Dendrocalamus strictus* has been heavily used for hundreds of years (Kurz, 1876). It can be found from India extending to Nepal, Bangladesh, and Burma to Thailand (Kurz, 1876; Ohrnberger, 1999). However, its distribution in Thailand is usually confined to dry areas of mixed deciduous forest along the bordering area between Thailand and Burma, from northern to southwestern part of Thailand, and never grows further to the peninsular (*personal observation*). There are another two species, *D. asper* and *D. membranaceus,* which are very interesting, and also economically and ecologically important in Thailand (Lin, 1968; Dransfield & Widjaja, 1995b; Duriyaprapan & Jansen, 1995a). Unlike *D. strictus,* the natural distribution of *D. membranaceus* in Thailand is much wider. It can be found almost throughout the whole country except in the southern part. *Dendrocalamus asper* has uncertain origin but is thought to be 'somewhere' in South-East Asia (Dransfield & Widjaja, 1995b) and has been cultivated throughout Thailand. Both species have been extremely well utilized, mainly for construction, furniture making, and for food. However, *Dendrocalamus membranaceus* is the selected species for the population genetic and phylogeography section in this thesis (Chapter 3). It is important to understand diversity within this species and how this genetic variation is partitioned across geographical space.

1.8 Morphology of bamboos

Every part of the bamboo plant, except the roots, consists of a ramifying system of segmented axes (McClure, 1966). Different patterns of branching of the segmented axes which have distinctive value for bamboo identification can be seen in both vegetative and reproductive parts of a bamboo plant. To be able to recognize a bamboo either in the field or in the laboratory it is important to know the fundamentals of bamboo morphology. Bamboo parts can be simply categorized into two, vegetative and reproductive.

1.8.1 Vegetative parts

The mechanism of waiting so long to flower, sometimes up to 150 years, is thought to be associated with ensuring regeneration by temporary satiation of grain predators (Clayton, 1990; Li et al., 2006b). Lack of flowering specimens makes day to day bamboo identification a difficult task. However, careful investigation on bamboo vegetative parts can provide enough diagnostic characters to identify a bamboo (Kurz, 1876; Holttum, 1958). The following sections describe the most important bamboo vegetative parts.

The vegetative bamboo plant body can be simply categorized into two main portions (Holttum, 1958; McClure, 1966; Dransfield & Widjaja, 1995a; Wong, 1995a, 2004; Stapleton, 1997, 1998; Judziewicz *et al.*, 1999; Sungkaew *et al.*, *unpublished*): an underground portion of the stem, called the 'rhizome' and an above-ground portion of the stem, called the 'culm'.

1.8.1.1 The rhizome

Bamboo rhizomes are taxonomically and horticulturally important (Stapleton, 1998). They serve to control where and when the culms develop, how the culms spread, and also provide vegetative propagation material. Thus interpretation of this part needs to be precise (Stapleton, 1998). Like the other segmented vegetative axes of a bamboo plant, especially the culm, a single rhizome is composed of many repeating units. Each of these units consists of a node, an internode, where each node bears a leaf, and usually a bud and one or more roots. Moreover, the individual rhizome may be seen as two recognizable parts: the rhizome proper and the rhizome neck, Figure 1.4. The difference between these two portions is that the rhizome neck lacks buds and roots while the rhizome proper has both. The rhizome neck is always more slender than the rhizome proper that produces it or originates from it. The leaf, whether in each node of the rhizome neck or the rhizome proper, is a scale-like, and may be called the rhizome sheath (McClure 1973) or rhizome bract (Judziewicz *et al.*, 1999). However rhizome leaves, culm leaves (culm sheaths), and foliage leaves, are homologous to each other (Holttum, 1958; McClure, 1966; Dransfield & Widjaja, 1995a; Wong, 1995a, 2004; Stapleton, 1997, 1998; Judziewicz *et al.*, 1999; Sungkaew *et al.*, *unpublished*).

By looking at their branching patterns or their appearances, the bamboo rhizomes can be classified into two basic forms, pachymorph and leptomorph (Holttum, 1958; McClure, 1966; Dransfield & Widjaja, 1995a; Wong, 1995a, 2004; Stapleton, 1997, 1998; Judziewicz *et al.*, 1999; Sungkaew *et al.*, *unpublished*). McClure (1966) stated that it was Rivière and Rivière (1878) who were the first to publish a clear distinction between the two basic forms. It was he who termed these two basic rhizome forms, pachymorph and the leptomorph (McClure, 1966). Stapleton (1998) later pointed out that Rivière and Rivière (1878) had actually been describing the clump habits rather than the rhizome forms.

1.8.1.1.1 Pachymorph rhizome

The word pachymorph is composed of two Greek syllables; 'pachy–' (meaning thick– or stout–) and '–morphus' (meaning –shaped) (Stearn, 1992). Thus, the pachymorph rhizome is typically short, thick, spindle-shaped, and usually solid, that grows horizontally at first, and then turns upwards to continue as a culm whose diameter is normally smaller than the diameter of its rhizome. The rhizome lateral buds, which usually develop every year, will form only new rhizomes and never form culms directly. The axis (the rhizome) is actually composed of many sections of separate axes that have branched repeatedly, this is why such rhizomes are said to have the pattern of branching as sympodial branching (McClure, 1966; Dransfield & Widjaja, 1995a; Wong, 1995a, 2004; Stapleton, 1997, 1998; Judziewicz *et al.*, 1999; Sungkaew *et al.*, *unpublished*), Figure 1.4. The Old World tropical bamboos in *Bambusa, Dendrocalamus,* and *Gigantochloa* or the New World tropical bamboos in *Guadua*, are examples of bamboo genera possessing pachymorph rhizomes.

1.8.1.1.2. Leptomorph rhizome

The word leptomorph is also composed of two Greek syllables; 'lepto–' (meaning slender–, thin– or narrow–) and '–morphus' (meaning –shaped) (Stearn, 1992). Hence, the leptomorph rhizome is typically long, slender, cylindrical, and usually hollow, that grows horizontally for an indefinite time. The rhizome lateral buds, when developed, will form either directly into culms (whose diameter is normally bigger than diameter of its rhizome) or into new rhizomes. Because the rhizomes branch rarely and can stay underground with indefinite apical growth, they are said to have monopodial branching (McClure, 1966; Dransfield & Widjaja, 1995a; Wong, 1995a, 2004; Stapleton, 1997, 1998; Judziewicz *et al.*, 1999; Sungkaew *et al.*, *unpublished*), Figure 1.4. The Asian temperate bamboo genus *Phyllostachys* is a good representative possessing leptomorph rhizomes.

There are some other terms involving bamboo rhizomes and culms which need to be briefly discussed. McClure (1966) used the term amphimorph or amphipodial to describe the rhizome form of *Chusquea fenderi* that possesses both typical pachymorph and leptomorph rhizomes in the same plant, Figure 1.5. Stapleton (1998) argued that such rhizomes are actually the leptomorph rhizomes which produce very swollen tillering culm bases, some having marked horizontal growth, and appearing very similar to pachymorph rhizomes. He rejected the terms amphimorph or amphipodial. Stapleton (1998) also suggested that the term metamorph I axis of McClure (1966), can actually be applied to either leptomorph or pachymorph rhizome that have tillering culm bases. The metamorph II axis of McClure (1966) is apparently peculiar to the pachymorph rhizomes of the bamboo genus *Vietnamosasa* (as *Arundinaria* in McClure (1966)), the genus found in Vietnam, Cambodia, Thailand, and possibly Laos (Ohrnberger, 1999). Stapleton (1998) suggested that this may be an adaptation to fire. However, Dransfield (2000a; *personal communication*) pointed out that this section is not a rhizome but it is part of lower portion of the culm which is buried in the soil and not because it is an adaptation to fire. She also suggested that the term metamorph II axis should be disregarded.

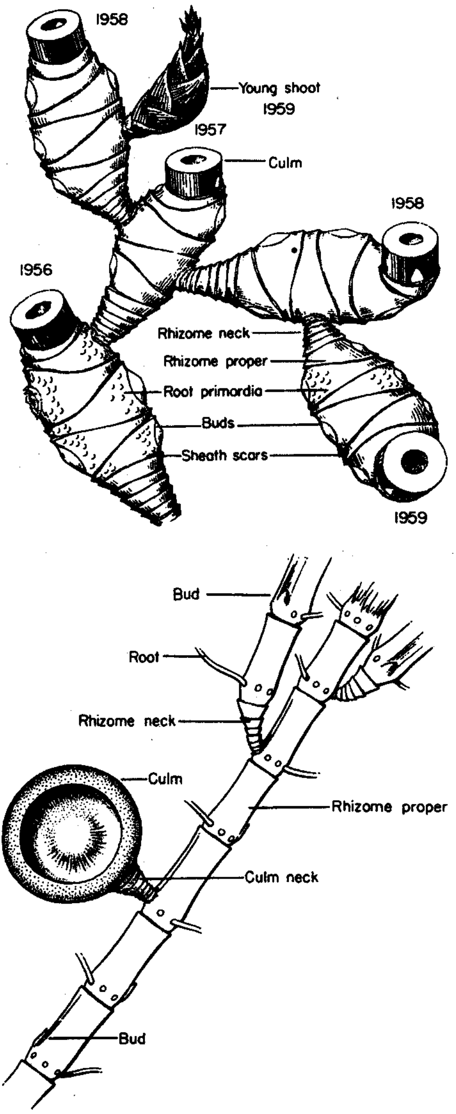


Figure 1.4 Two basic forms of rhizomes. Above, the pachymorph rhizomes; below, the leptomorph rhizomes. Adapted from McClure (1966).

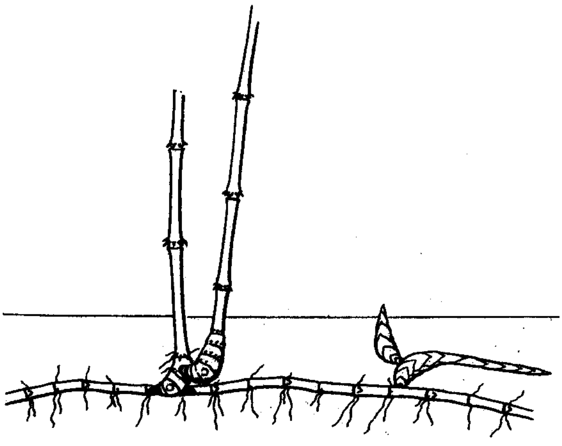


Figure 1.5 Rhizomes of *Chusquea fendleri*, where the term amphimorph or amphipodial was applied. Adapted from McClure (1966).

The growth of the rhizome can also be categorized into two types; determinate and indeterminate (McClure, 1966; Stapleton, 1998). This reflects the difference in the longevity of the apical meristems of different rhizome forms. The apical meristem of the determinate rhizome can stay underground for just a limit of time then it has to turn up to develop as a culm (equivalent to sympodial) while the apical meristem of the indeterminate rhizome can stay underground for indefinite time (equivalent to monopodial). Stapleton(1998) summarized that sympodial rhizomes are always pachymorph, and monopodial rhizomes are always leptomorph. Thus all the terms mentioned above are synonymous: pachymorph=sympodial=determinate, and leptomorph=monopodial=indeterminate.

Technical terms for describing culm or clump habit are also important. In many cases it is actually very risky to presume what the rhizome is like from the above-ground appearance. On the other hand, it is not always easy to predict how the culms will appear above the ground from the form of rhizome alone (Stapleton, 1998). There are three different terms for culm or clump habits; diffuse, unicaespitose, and pluricaespitose. It will be called 'culms diffuse' if the culms arise from the leptomorph rhizomes and in a consistently well-separated fashion (arising singly or isolated). Bamboos in the genus *Phyllostachys* are, generally, very good examples of bamboos having diffuse culms. In some bamboos with consistently long-necked pachymorph rhizomes, e.g. *Melocanna baccifera* from north-eastern part of India to Nepal, Bangaladesh, to Burma (McClure, 1966; Clayton & Renvoize, 1986; Stapleton, 1998; Ohrnberger, 1999) or *Pseudostachyum polymorphum* from north-eastern part of India to Bhutan, Burma, to southern China (Ohrnberger, 1999) expanding to north-eastern Thailand at high latitude (new record for species and distribution to Thailand, *personal investigation*), the culms also arise singly in isolated fashion. In this case, the term 'culms diffuse' may also be applied. *Valiha* and *Cathariostachys* from Madagascar also possess pachymorph rhizomes with long necks (Dransfield, 1998b; *personal communication*). Culms are called 'unicaespitose' (or caespitose, alternative spelling cespitose or unicespitose) if they arise from pachymorph rhizomes without long necks and always arise together in a clump manner. Species of bamboos in *Bambusa, Dendrocalamus,* and *Gigantochloa* are examples of bamboos having unicaespitose culms. The term 'pluricaespitose' (also called multicaespitose or compound-caespitose) can be applied if the culms arise from pachymorph rhizome where the neck length varies and a series of small separate clumps is produced, such as that found in species of *Yushania* (Stapleton, 1998). Many bamboos with leptomorh rhizomes whose culms often tiller at the base giving small separate clumps of culms, can also be called pluricaespitose (Stapleton, 1998). The precise terminology of four classic forms of rhizome and culm development has been discussed by Stapleton (1998), Figure 1.6.

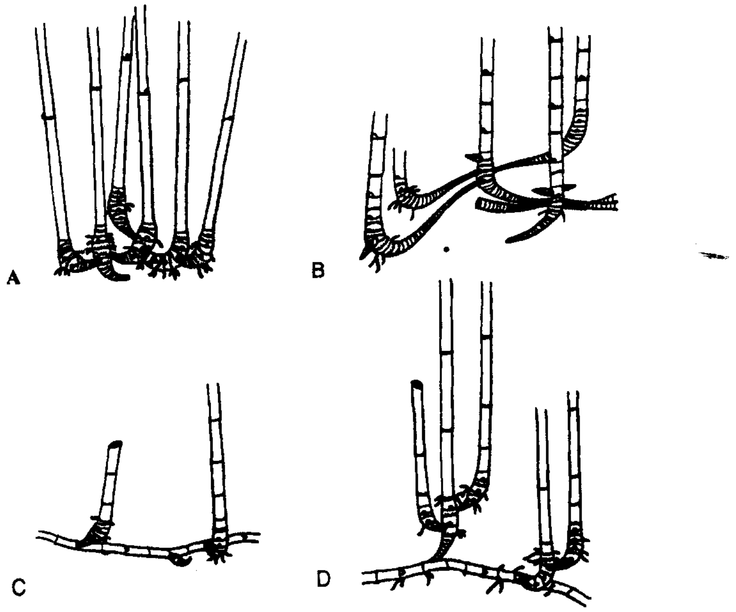


Figure 1.6 Terminology suggested by Stapleton (1998) for four classic forms of rhizome and culm development. A. rhizome pachymorph with short necks, culms unicaespitose; B. rhizome pachymorph with consistently long necks, culms diffuse; C. rhizome leptomorph, culms diffuse; D. rhizome leptomorph, culms pluricaespitose. Illustrations from But *et al.* (1985); cited after Stapleton (1998).

1.8.1.2 Culm shoot and culm

A culm shoot, or a bamboo shoot, is a young above-ground portion of the stem, Figures 1.7 and 1.8. Each culm shoot is commonly either an apical projection of a pachymorph rhizome or a lateral branch of a leptomorph rhizome or less commonly, a lateral bud of metamorph I or metamorph II axis (Holttum, 1958; McClure, 1966; Dransfield & Widjaja, 1995a; Wong, 1995a, 2004; Stapleton, 1997, 1998; Judziewicz *et al.*, 1999; Sungkaew *et al.*, *unpublished*). It is clothed with overlapping bracts, known as culm sheaths, on alternate sides of the axis at successive nodes. Wong (1995a) suggested that the very young stages of culm shoots provide comparatively few useful characters (e.g. colour of and materials covering culm sheaths, culm sheath blade orientation, details of auricles and ligules), but there are the culm shoots which have grown to 0.5–1 m high or higher which lend themselves well to identification. Sungkaew *et al.* (*unpublished*) further recommended that the culm shoots which reach from about 1 m high, up to about half the mature-culm height, are very useful for bamboo identification, especially Asian woody bamboos. This is because the height of 0.5 m of young shoots of some huge bamboos, e.g. *D. giganteus* or *D. asper*, is probably too young to provide useful characters for identification. Sungkaew *et al.* (*unpublished*) introduced the term 'flying shoot' to refer to it, because there are often culm sheath blades that stand off from the main axis and appear like wings Figure 1.8.

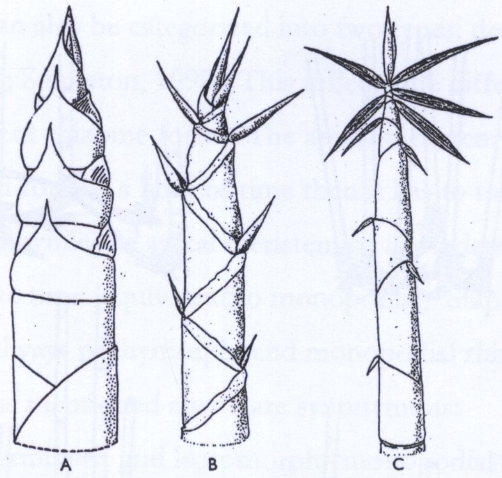


Figure 1.7 Tops of culm shoots, showing differences of shape and position of culm sheath blades. A. *Schizostachyum zollingeri* (blade erect, broad, convex); B. *Dendrocalamus pendulus* (blade narrow, partly reflexed (deflexed)); C. *Gigantochloa scortechinii* (blade leaf-like, spreading or reflexed). From Holttum (1958).

Figure 1.8 Flying shoots of different bamboo species. 1. *Bambusa vulgaris* 'vittata'; 2. *Cephalostachyum virgatum*; 3. *Dendrocalamus sinicus*; 4. *Phyllostachys edulis*; 5. *D. membranaceus*; 6. *D. asper*; 7. *D. latiflorus*; 8. *B. bambos*; 9. *Thyrsostachys siamensis*; 10. *Phyllostachys makinoi*; 11. *Gigantochloa albociliata*; 12. *B. beecheyana*. Adapted from Sungkaew *et al.* (*unpublished*).

The mature culms are the result of a process of primary growth through which culms reach their maximum diameter, and elongate their internodes in a telescopelike fashion with subsequent hardening through lignification (McClure, 1966; Liese, 1995; Judziewicz *et al.*, 1999).

The culms that develop from different forms of rhizomes differ in possessing the culm base (McClure, 1966). A culm arising from pachymorph rhizome does not have the culm base because it is taken by its rhizome while the one from leptomorh rhizome does (Figure 1.9).

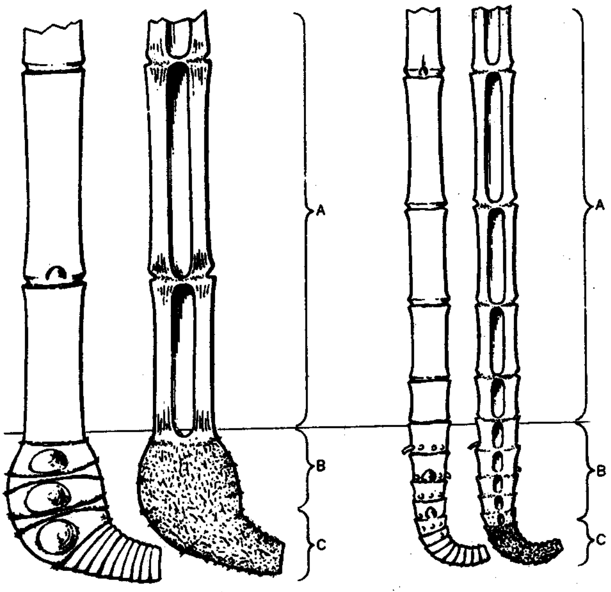


Figure 1.9 Lower part of a culm from two basic forms of rhizomes (both split in two). A. the culm proper in both; B. a pachymorph rhizome (left), and the culm base (right); C. a rhizome neck (left), and a culm neck (right). From McClure (1966).

From this point of view, the culm on the right, of *Chusquea fendleri* (Figure 1.5), would actually develop from the culm base of the culm on the left which was borne from the leptomorph rhizome. This would be a strong reason to support the idea of Stapleton (1998) by which he suggested that this bamboo should be better called 'rhizomes leptomorph; culms pluricaespitose'.

A bamboo plant consists of a ramifying system of segmented axes. The rhizomes, culms, and branches of a bamboo plant form segmented, vegetative axes (Judziewicz *et al.*, 1999). The repeating units that make up a culm, like those described for rhizomes, are a node, an internode, a bud, and a leaf. One or more adventitious roots may be present as well, as commonly seen at lower nodes of several species of *Dendrocalamus* such as *D. asper, D. brandisii,* *D. hamiltonii.* The nodes and internodes will be explained here, Figure 1.10, while the buds and the leaves will be discussed in next section.

Each internode is separated from adjacent internodes by the nodes which internally consist of solid, horizontal plates (sometimes also called septa or cross-walls or diaphragma) sealing off the internodal space (Wong, 1995a; Judziewicz *et al.*, 1999). Some node and internode characters are useful for bamboo recognition.

Typically, the bamboos have cylindrical or terete internodes but they may be partially quadrangular at lower internodes in several species of *Chimonobambusa*, e.g. *C. quadrangularis* (Li & Stapleton, 2006d). Basally inflated internodes can be seen in *B. vulgaris* 'wamin' (Wong, 1995a; Sungkaew *et al.*, *unpublished*).

The colour of the culms is normally green but some species can display some other colours, e.g. purple-brown or black in *Phyllostachys nigra* (Sungkaew *et al.*, *unpublished*; Wang & Stapleton, 2006), yellow with longitudinal green streaks such as in *B. vulgaris* 'vittata' (Wong, 1995a; Sungkaew *et al.*, *unpublished*) or in some *Phyllostachys* (Wang & Stapleton, 2006; Hodkinson, *personal communication*), entirely yellow or occasionally with a narrowly streaked green pattern in *Schizostachyum brachycladum* (Wong, 1995a; Sungkaew *et al.*, *unpublished*).

Most bamboos have hollow internodes, but many bamboos, especially a number of members of the bamboo genus *Chusquea* of Central and South America, have solid internodes (Clark, 1989, 1997b). However different people may have different ways to define the thickness of the walls of bamboo internodes. In this thesis, the mid-culm thickness will follow the definition given by Dransfield and Clark (*personal communication*) where as; 'solid or thick walls' means the lacuna (pith) diameter is smaller than 1/3 the diameter of the culm, while 'thin walls' means the lacuna is greater than 1/3 the diameter of the culm. Some Asian bamboos, such as *Thyrsostachys siamensis* and *D. strictus*, sometimes, show thick or solid walls (Sungkaew *et al.*, *unpublished*).

Most bamboos exhibit more or less glabrous culms, but puberulent internodes can be seen in *Phyllostachys edulis* (Sungkaew *et al.*, *unpublished*; Wang & Stapleton, 2006). The lower internodes of several species in *Dendrocalamus* are also hairy. Longitudinally grooved internodes above the point of insertion of the branch complement is quite unique in *Phyllostachys* (McClure, 1966; Wong, 1995a; Sungkaew *et al.*, *unpublished*; Wang & Stapleton, 2006). The presence or absence of white powder or wax, and types and colours of hairs on the culms, are also useful characters for recognizing some bamboos. However these characters will be obtained from only well-described specimens.

The nodes do not provide many useful characters for Old word tropical woody bamboos (Wong, 1995a), but they do for New world tropical woody bamboos (Judziewicz *et al.*, 1999). However, some species of temperate woody bamboos may show characteristic nodes. Some Chinese bamboos in *Chimonobambusa*, especially *C. tumidissinoda* (*Qiongzhuea*), can be distinguished by having prominently swollen nodes (Li & Stapleton, 2006d). Basal nodes of bamboos in *Chimonobambusa* (Wong, 1995a; Sungkaew *et al.*, *unpublished*; Li & Stapleton, 2006d), Figure 1.10, and *Chimonocalamus* (Li & Stapleton, 2006e) often have a ring of sparse or dense root-thorns. Nodes of two climbing bamboo genera, the Asian genus *Dinochloa* (Dransfield, 1981; Wong, 1986; Wong, 1995a) and the Australian genus *Mullerochloa* (Wong, 2005), have similar nodal characteristics where they both have a persistent rough basal portion of culm sheath at each node.

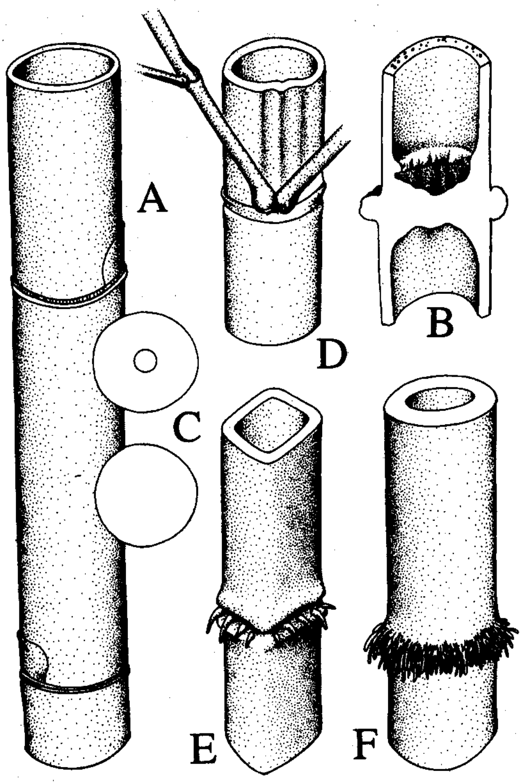
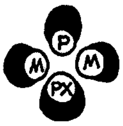
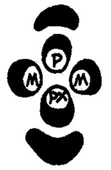


Figure 1.10 Culm nodes and internodes. A. internodal segment with two nodes; B. longitudinal section through a node, showing the septum of cross-wall; C. diagram of transverse section of culm internodes showing thick-walled internode (above) and solid internode (below), other transverse sections show thin-walled internodes; D. internode with longitudinal groove above branch insertion; E. four-angled internode with nodal root-thorns; F. node with verticil of roots. Adapted from Wong (1995a).

Several studies on bamboo culm anatomy, particularly on the vascular bundle types, have been conducted to help bamboo taxonomy (e.g. Metcalfe, 1960; Grosser & Liese, 1971; Wong 1995b; Liese, 1998). However, some aspects of the culm anatomy needed to be carefully considered. Metcalfe (1960) suggested that when cross (transverse) sections of bamboo culm are examined anatomically, the diameter of the culm should be noted, "since the sizes of the sclerenchyma strands increase with age". Grosser and Liese (1971) stated that species having two vascular bundle types will only show the simple one (i.e. without an additional supportive sclerenchyma bundle) in smaller culms. One bamboo species/genus can have vascular bundles of more than one type (pattern) and one type of vascular bundle can basically be found in more than one bamboo species/genera. Thus the bamboo culm anatomy is in fact only good enough to place a species/genus to one of the broad grouping (Grosser & Liese, 1971; Wong 1995b; Liese, 1998), Figure 1.11.

Table 1.2 Patterns of sclerenchyma associated with vascular bundles noted in culm internodes of various genera. P, phloem; PX, protoxylem; M, metaxylem; black represents sclerenchyma. From Wong 1995b.

Types of vascular bundle according to Grosser and Liese (1971)	**Forms of sclerenchyma associated with vascular bundle and genera in which they occur**
Vascular bundle 'consisting of one part'  	Sclerenchyma associated with vascular bundle as 'sheaths' in four positions: at the outer (phloem) pole, inner (protoxylem) pole, and as two lateral strands on either side of the metaxylem vessels. Type I: Sclerenchyma sheath at protoxylem pole small; tyloses present in the protoxylem space: genera with leptomorph rhizomes, *viz.*, *Arundinaria, Phyllostachys* (the only type found throughout the culm). Type II: Sclerenchyma sheath at protoxylem pole strikingly larger than other sclerenchyma sheaths; tyloses absent: genera with pachymorph rhizomes, *viz.*, *Cephalostachyum* (the only type found throughout the culm), *Melocanna, Schizostachyum, Teinostachyum* (the only type at midculm).
Vascular bundle 'consisting of two parts' 	Type III: Sclerenchyma sheaths as in Type I but with an additional, separate sclerenchyma strand inner to the protoxylem pole of the vascular bundle: genera with pachymorph rhizomes, *viz.*, *Melocanna, Schizostachyum, Teinostachyum* (in combination with Type II at the culm base), *Bambusa, Dendrocalamus, Gigantochloa, Thyrsostachys* (the only type at midculm), *Oxytenanthera* (the only type found throughout the culm).
Vascular bundle 'consisting of three parts' 	Type IV: Sclerenchyma sheaths as in Type I but with two additional, separate sclerenchyma strands, one inner to the protoxylem pole, and another outer to the phloem pole: genera with pachymorph rhizomes, *viz.*, *Bambusa, Dendrocalamus, Gigantochloa, Thyrsostachys* (always in combination with Type III and mostly at culm base).

1.8.1.2.1. Branch complement

The array of branches that develop at a single culm node is called a branch complement (McClure, 1966; Dransfield & Widjaja, 1995a; Wong, 1995a, 2004; Stapleton, 1997; Judziewicz *et al.*, 1999; Sungkaew *et al.*, *unpublished*). They develop from the branch-bud(s) that alternate from one side to another at successive nodes of any given culm. The branch-bud(s) are enclosed by the organ called a prophyll or bud scale (McClure, 1966; Wong, 1995a, Judziewicz *et al.*, 1999). The prophyll is known as a modified first leaf of the axis and its function is to protect a bud subtended by it. The prophyll has a distinctive basic structure where its back faces to the axis and its two inflexed edges enclose the branch in the bud stage. There are two keels formed by the folding forwards of these two inflexed edges. These inflexed edges can be different among bamboos, Figure 1.11. Some bamboos may have closed margins while other may have an open form. Morphology of culm-bud prophylls may be helpful to indicate genera or related groups of genera (McClure, 1966; Wong, 1995a, 2004; Judziewicz *et al.*, 1999). For example, the culm-bud prophylls of species of Peninsular Malaysian *Bambusa, Gigantochloa,* and *Schizostachyum* have fused margins, and resemble a hood (Wong, 1995a). Two species of *Dendrocalamus, D. hirtellus* and *D. pendulus* (Wong, 1995a), also have the same pattern of culm-bud prophylls to those three genera. However an intensive study for the whole genus on this issue would be needed. The culm-bud prophylls in the species of *Chimonobambusa, Dinochloa, Kinabaluchloa, Maclurochloa, Phyllostachys, Racemobambos, Soejatmia,* and *Thyrsostachys* have free margins (Wong, 1995a), Figure 1.11.

The culm-branch complements may develop while the culm is still growing or they develop after it reaches its full height (McClure, 1966; Dransfield & Widjaja, 1995a; Wong, 1995a, 2004; Judziewicz *et al.*, 1999). Generally in the Old World woody bamboos, the culm-branch complements develop from a single branch-bud (McClure, 1966; Dransfield & Widjaja, 1995a; Wong, 1995a, 2004) while in some of the New World woody bamboos, e.g. *Apoclada* and *Chusquea* (Judziewicz *et al.*, 1999), or the Old World woody bamboo genus *Holttumochloa* (Wong, 1995a), they develop from multiple branch-buds. Basically, the mid-culm branch complements (i.e. the branch complements from the middle portion of the culm) are the most fully developed and useful to be taken as 'typical' or 'representative' for given genus or species. The branch complements from lower nodes are generally less well developed than higher nodes and are not recommended for identification (McClure, 1966; Wong, 1995a). The patterns of mid-culm branch complements would be considered to be, at least, useful for recognition of bamboos at generic level (Wong, 1995a), Figure 1.12.

In *Holttumochloa*, the mid-culm branch complement develops from several to many individual, very small primary branch-buds, at a node. All the branches form a cluster of subequal, very small axes and do not re-branch distally (Wong, 1995a). The characteristics of branch complements in *Kinabaluochloa, Melocanna* and *Schizostachyum* are similar to that of *Holttumochloa* where all have slender, subequal branches clustering at each node. However the branch complement of these three genera actually develops from just one primary branch-bud which re-branches distally to several orders at its base (Wong, 1995a).

Bambusa, Dendrocalamus, Gigantochloa, and *Thyrsostachys* have similar patterns of branch complement. It develops from a solitary bud forming a clearly dominant, often quite central, primary branch axis. Typically it is accompanied with one or two subdominant secondary branches from its base, and a few higher-order branches from these main axes (Wong, 1995a).

Typically, the mid-culm branch complement of *Phyllostachys* is binary. It develops from just one branch-bud at each node, but the primary branch-bud typically produces a single secondary branch from its base. These two axes are usually more or less strongly unequal. Infrequently, however, the third, which is usually much smaller and from the base of the second branch, sometimes develops, and sits at a central position between the two divergent main branches (McClure, 1966; Wong, 1995a).

In *Chimonobambusa*, the branch complement consists of three main branches (from three buds) inserted at the same level, which are similar in size (Wong, 1995a), Figure 1.12.

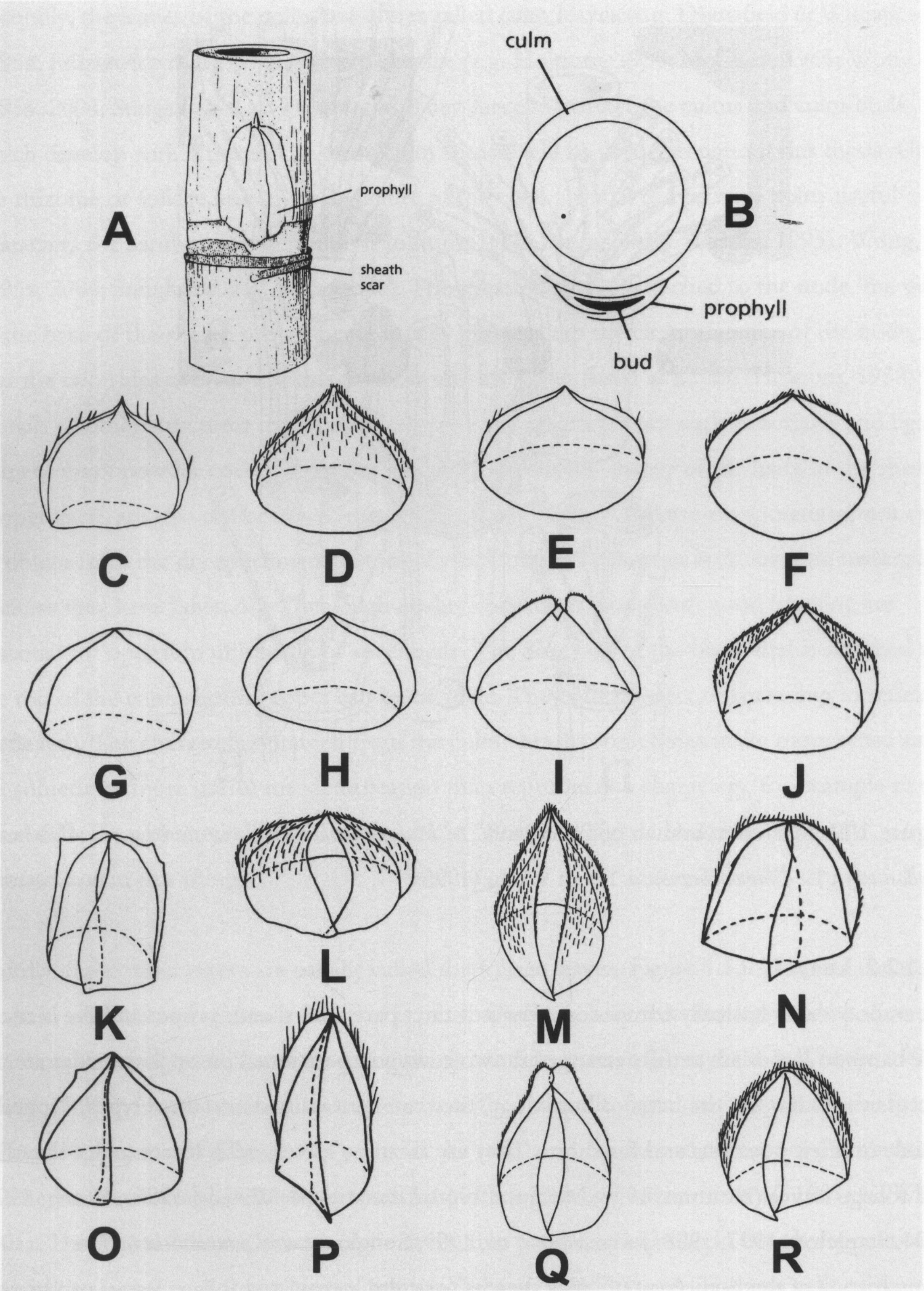


Figure 1.11 Culm-bud prophylls of some bamboos. A. a culm node, showing prophyll and culm-sheath scar; B. diagramatic cross section of culm through bud, showing prophyll; C. *Bambusa bambos*; D. *B. blumeana*; E. *Gigantochloa scortechchinii*; F. *G. rostrata*; G. *Schizostachyum grande*; H. *S. zollingeri*; I. *Dendrocalamus hirtellus*; J. *D. pendulus*; K. *Chimonobambusa quadrangularis*; L. *Dinochloa* sp.; M. *Kinabaluchloa wrayi*; N. *Maclurochloa montana*; O. *Phyllostachys viridis*; P. *Racemobambos setifera*; Q. *Soejatmi ridleyi*; R. *Thyrsostachys siamensis*. A and B, adapted from Judziewicz *et al.* (1999); C–R, adapted from Wong (1995a).

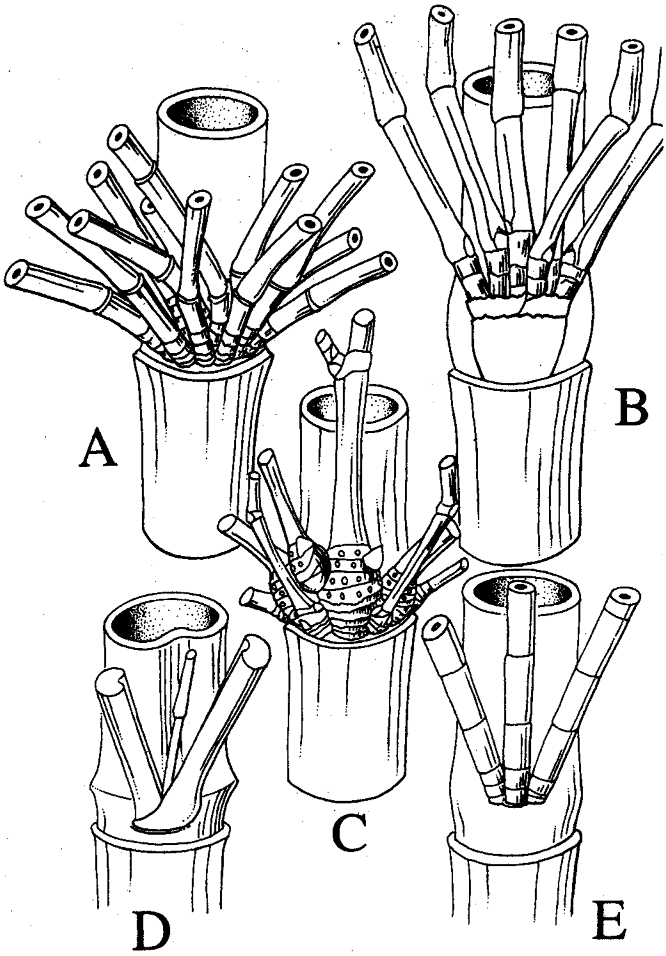


Figure 1.12 Mid-culm branch complements. A. *Holttumochloa*; B. *Schizostachyum*; C. *Bambusa*; D. *Phyllostachys*; E. *Chimonobambusa*. From Wong (1995a).

1.8.1.2.2. Leaves

A bamboo leaf is basically composed of two distinct parts, the sheath proper and the blade. The bamboo leaves are usually arranged in two rows and positioned on opposite sides at successive nodes. On the basis of homology, they can be classified into three types, depending mainly on their positions and functions. They are rhizome leaves, culm leaves (culm sheaths), and foliage leaves (Holttum, 1958; McClure, 1966; Dransfield & Widjaja, 1995a; Wong, 1995a, 2004; Stapleton, 1997, 1998; Judziewicz *et al.*, 1999; Sungkaew *et al.*, *unpublished*). The comparisons of the homology of culm sheaths (or culm leaves) and foliage leaves can be seen in Figure 1.13.

Firstly, rhizome leaves, sometimes called rhizome sheaths (McClure, 1966) or rhizome bracts (Judziewicz *et al.*, 1999), are often scale-like or ensheathing structures, papery in texture, and their function is to protect rhizome buds. Their blades are usually reduced or absent (McClure, 1966; Wong, 1995a; Judziewicz *et al.*, 1999). However they are generally not useful for identification (Wong, 1995a).

Secondly, the leaves of the culms are either called culm leaves (e.g. Dransfield & Widjaja, 1995a; Judziewicz *et al.*, 1999) or culm sheaths (e.g. Holttum, 1958; McClure, 1966; Wong, 1995a, 2004; Sungkaew *et al.*, *unpublished*). They serve to protect the culms and culm-buds which develop within them. The term 'culm sheath' will be used throughout this thesis. Unlike the rhizome or foliage leaves, the structure of the culm sheaths can provide many useful characters for bamboo identification (Holttum, 1958; Dransfield & Widjaja, 1995a; Wong, 1995a, 2004; Sungkaew *et al.*, *unpublished*). The sheath proper is attached to the node, the width of the base of the sheath proper being usually greater than the circumference of the node, so that the two sides overlap towards the base and are never fused as a tube (Holttum, 1958). Details of other structures that come along with the sheath proper such as auricles and ligule, hairs or waxy powder covering the back of the culm sheath, colour of the hairs of the sheath proper itself, are also characteristic of particular taxa. However, these are sometimes not easy to obtain from the dry specimens that usually lack original colour or lack covering materials because they have fallen off. Thus, high quality collections along with good labelling are essential for optimum utilization of specimens. The direction of the blade that is attached at the top of the culm sheath proper can be of value. They can be erect or spreading to reflexed (deflexed). The characters obtained from the culm sheaths sometimes make more sense and are sometimes more useful for identification than reproductive characters, for example in the case of *Dendrocalamus pendulus* versus *D. hirtellus* (Wong, 1995a) or *D. strictus* versus *D. membranaceus* in this thesis.

Thirdly, the normal leaves are usually called the foliage leaves, Figure 1.13. They are usually green in colour and their main function is photosynthesis. Like rhizome leaves, they show relatively few useful characters for recognizing bamboo species (Dransfield & Widjaja, 1995a; Wong, 1995a). However, some characters from the foliage leaf anatomy, especially the fusoid cells and the arm cells (Figure 1.14), can set the true bamboos (woody bamboos-Bambuseae and herbaceous bamboos-Olyreae) apart from other grasses (Judziewicz *et al.*, 1999; GPWG, 2001). The fusoid cells are characteristic in the true bamboos and also in the phylogenetically outlying grasses (e.g. Anomochloeae, Streptochaeteae, and Phareae). The arm cells of the outlying grasses are not lobed or are only weakly lobed while those of the true bamboos are strongly lobed. Complex midrib vasculature is also another prominent feature of bamboo foliage leaves that is poorly developed or absent in other grasses (Soderstrom & Ellis, 1987).

Apart from the sheath proper and the blade, there are another two important structures that can be often found in both culm sheaths and foliage leaves, i.e. the auricles and the ligules (Holttum, 1958; McClure, 1966; Dransfield & Widjaja, 1995a; Wong, 1995a, 2004; Judziewicz *et al.*, 1999; Sungkaew *et al.*, *unpublished*), Figure 1.13. The auricles, when developed, are usually lobe-like or ear-shaped structures occurring one on each side of the base of the blade. They are sometimes considered as lateral extensions of the base of the blade (Dransfield & Widjaja, 1995a; Wong, 1995a). The ligule is the extension of the adaxial surface of the sheath summit connecting to the blade.

Ideally the best representatives of the nodes and internodes, branch complements, foliage leaves, and culm sheaths, would be obtained from the mid point of the culm because at this position those parts are normally fully developed. This is especially the case for the culm sheaths; the ones from the very young shoots or from the lower culm nodes may not be fully developed. Also the ones from above the mid-culm towards the tip are not very useful as these appear very similar to the foliage leaves. Therefore, the best representative culm sheaths for identification should be obtained from the flying shoots or from the middle of the culms having fully developed and distinctive characters (Holttum, 1958; McClure, 1966; Dransfield & Widjaja, 1995a; Wong, 1995a, 2004; Sungkaew *et al.*, *unpublished*)

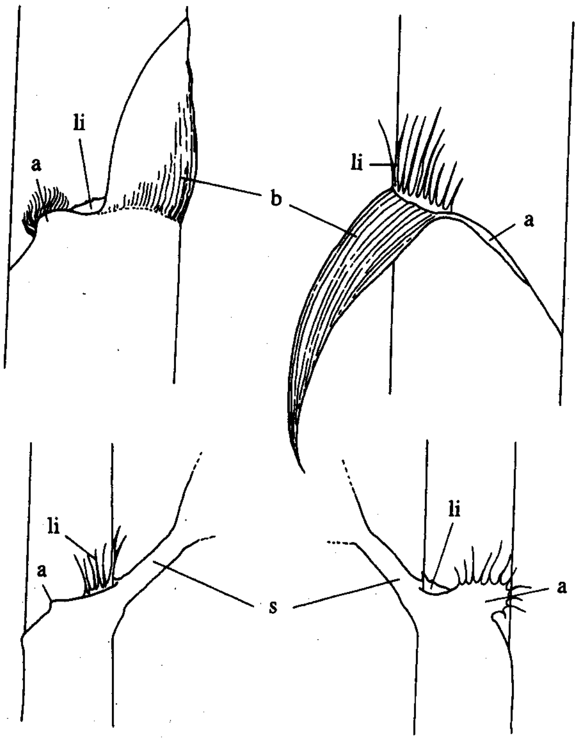


Figure 1.13 Culm sheaths (culm leaves) and foliage leaf bases. Top left, culm sheath with erect blade, a subentire ligule and a bristly lobe-like auricle; Top right, culm sheath with reflexed blade, a long-bristly ligule and a glabrous rim-like auricle; Bottom left, foliage leaf base with bristly ligule and an inconspicuous auricle; Bottom right, foliage leaf with rim-like ligule and a bristly lobe-like auricle. a, auricle; b, blade; li, ligule; s, leaf stalk or pseudo-petiole. From Wong (1995a).

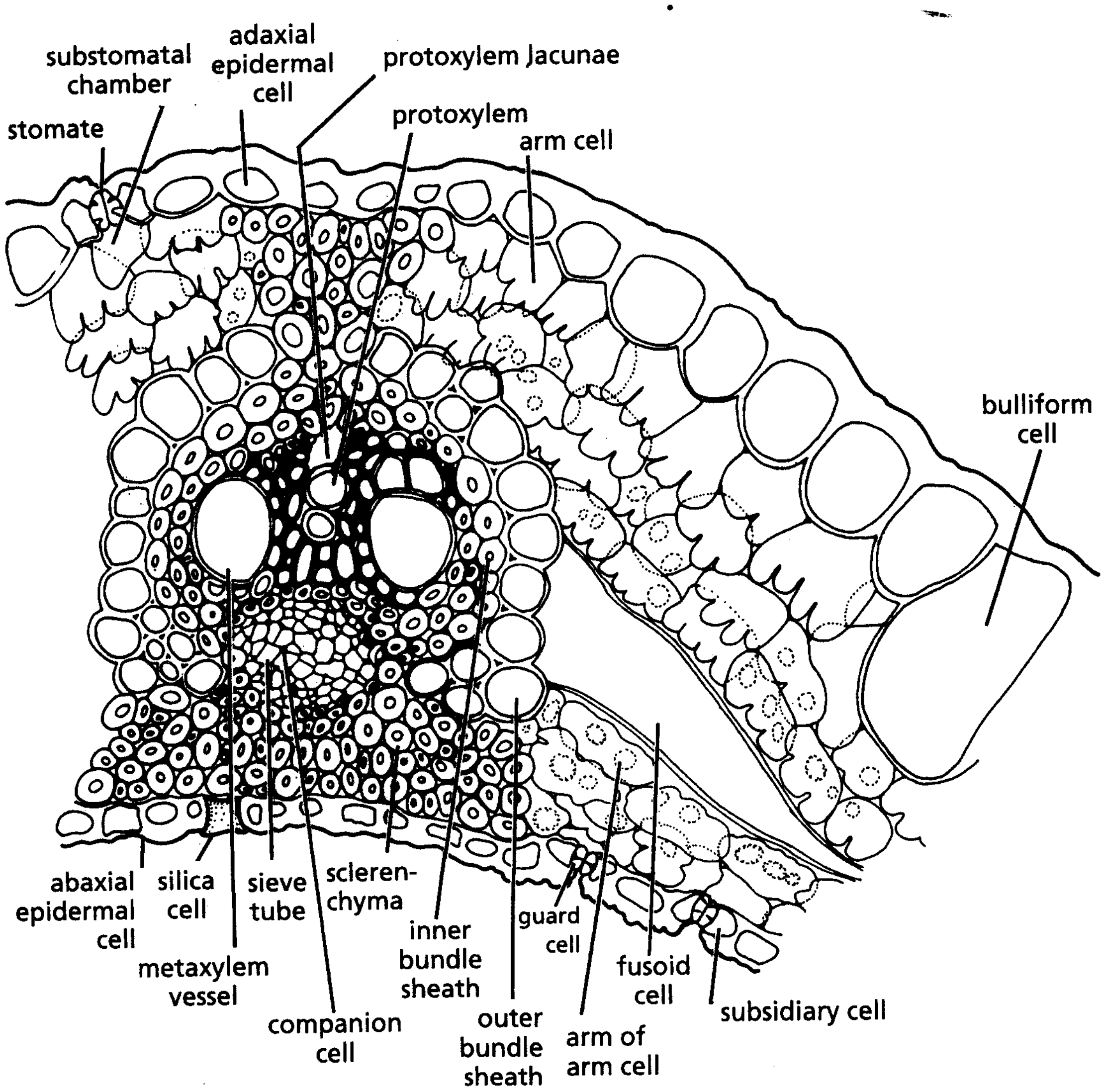


Figure 1.14 Schematic cross section through leaf blade of *Maclurolyra tecta.* The vascular bundle comprises the inner and outer bundle sheaths, the phloem (companion cells and sieve tube members), and the xylem. From Judziewicz *et al.* (1999).

1.8.2 Reproductive parts

Although desirable, it is not always possible to obtain reproductive parts (i.e. fertile specimens, with flowers or fruits) for bamboo identification, due to rarity of flowering in bamboos (Clayton, 1990). However, even in cases where fertile specimens have been collected, the vegetative parts are often poorly collected (especially the culm sheaths). This is because, particularly in the Old world tropical woody bamboos, the culm sheaths are usually long gone before a bamboo culm will flower and they normally stop producing the new culm shoots at least a season before flowering (*personal observation*). However it is very important to understand the reproductive parts of the bamboos as they are generally the most reliable characters to identify bamboos but different people interpret reproductive parts in different ways (e.g. Kurz, 1877; Gamble, 1896; Holttum, 1958; McClure, 1966; Weberling, 1989; Clayton, 1990; Bell, 1991; Dransfield & Widjaja, 1995a; Stapleton, 1997; Judziewicz *et al.*, 1999; Wong, 1995a). The following section describes the important terms for reproductive bamboo parts used in this thesis.

1.8.2.1 Inflorescences, synflorescences, spikelets, florets, and flowers

Like other grasses, the bamboo inflorescences are the specialized flower-bearing branches or axes and are regarded as complicated structures (Holttum, 1958; McClure, 1966; Dransfield & Widjaja, 1995a; Wong, 1995a). Unlike the normal grasses, however, it is difficult to distinguish the sharp distinction between the inflorescence and vegetative sections in most of the woody bamboos. A totally flowering bamboo clump was in the past sometimes called a single giant panicle (Stapleton, 1997).

The basic unit, whether in common grasses or bamboos, which is the most important part for identification, is called the 'spikelet' (Kurz, 1877; Gamble, 1896; Holttum, 1958; McClure, 1966; Clayton, 1990; Dransfield & Widjaja, 1995a; Stapleton, 1997; Judziewicz *et al.*, 1999; Wong, 1995a), Figures 1.14 and 1.15. A single spikelet consists of a series of overlapping bracts, some of which bear flowers in their axils. Typically, the lower bract(s) of a spikelet are empty (i.e. bearing neither floral axes nor buds), and are traditionally known as 'glumes' (Kurz, 1877; Gamble, 1896; Holttum, 1958; McClure, 1966; Clayton, 1990; Dransfield & Widjaja, 1995a; Stapleton, 1997; Judziewicz *et al.*, 1999; Wong, 1995a). The spikelet is not a flower and is best interpreted as a reduced inflorescence. Within a spikelet, there is one to several individual flowers, which are typically arranged in two rows on a comparatively short axis called a 'rachilla', Figure 1.16 (Kurz, 1877; Gamble, 1896; Holttum, 1958; McClure, 1966; Clayton, 1990; Bell, 1991; Dransfield & Widjaja, 1995a; Wong, 1995a); some interpretations (e.g. Stapleton, 1997; Judziewicz *et al.*, 1999) call it the 'rachis'.

In woody bamboos, the individual 'flower' (Clayton, 1990; Judziewicz *et al.*, 1999) is usually composed of three lodicules (but they are sometimes absent), 3–6 stamens, and a unilocular ovary bearing 1 to 3 stigmas (Dransfield & Widjaja, 1995a). A single flower is usually enclosed by two bracts, the outside one called the 'lemma' (the lemma is homologous to a foliar bract) and the inside one which is usually enveloped by the lemma, called the 'palea' (the palea is homologous to a prophyll). A flower, lemma, and palea, are collectively called the 'floret' (McClure, 1966; Clayton, 1990; Bell, 1991; Wong, 1995a; Judziewicz *et al.*, 1999). Thus, the whole unit, the glume(s) plus the floret(s), is termed the 'spikelet'. The aggregation of the spikelets is called a 'synflorescence' which refers to what is traditionally called the 'inflorescence' in bamboos (Stapleton, 1997; Judziewicz *et al.*, 1999). However, it is difficult to delimit the synflorescence or define where it starts.

Basically, there are two types of inflorecences (synflorescences), Figure 1.16. The first is called 'semelauctant' type, indicating a 'determinate' inflorescence with spikelet(s) borne in a raceme or a simple panicle, emerging and reaching maturity almost simultaneously (McClure, 1966; Dransfield & Widjaja, 1995a; Stapleton, 1997; Judziewicz *et al.*, 1999; Wong, 1995a). The second is 'iterauctant' type, indicating an 'indeterminate' inflorescence (McClure, 1966; Clayton, 1990; Dransfield & Widjaja, 1995a; Stapleton, 1997; Judziewicz *et al.*, 1999; Wong, 1995a). In this type of inflorescence, the basic unit is called a 'pseudospikelet' (McClure, 1934). The upper portion of a pseudospikelet is called the spikelet proper (usually from the first empty glume upwards) and the basal portion below the base of spikelet proper (below the lowest empty glume) always bears the prophyllate bud-bearing bract(s) (=gemmiparous bract(s)). A pseudospikelet, therefore, usually develops into a tuft of pseudospikelets as the buds in the bracts from its basal portion also develop into secondary pseudospikelets which in turn form tertiary pseudospikelets, and so on (Holttum, 1958; McClure, 1966; Clayton, 1990; Dransfield & Widjaja, 1995a; Stapleton, 1997; Judziewicz *et al.*, 1999; Wong, 1995a). In South-East Asia, the semelauctant type of inflorescence can be found in *Nastus, Racemobambos, Vietnamosasa,* and *Yushania* while *Bambusa, Dendrocalamus, Gigantochloa,* and *Schizostachyum* have the iterauctant type of inflorescences (Dransfield & Widjaja, 1995a; *personal observation*).

1.8.2.2 Fruits

Being a member of grass family, the bamboo fruit is also known as a caryopsis, and does not differ significantly from the caryopsis of other grasses. It consists of a pericarp enclosing the seed. The seed itself consists of endosperm and an embryo comprising a radicle, a plumule and a scutellum (Holttum, 1958; McClure, 1966; Clayton, 1990; Dransfield & Widjaja, 1995a; Judziewicz *et al.*, 1999; Wong, 1995a). The pericarp of four Asian genera, namely *Dinochloa,* *Melocalamus, Melocanna,* and *Sphaerobambos,* is thick and fleshy, the endosperm is much reduced and the embryo has a large scutellum containing a large number of starch grains functioning as food storage (Dransfield & Widjaja, 1995a).

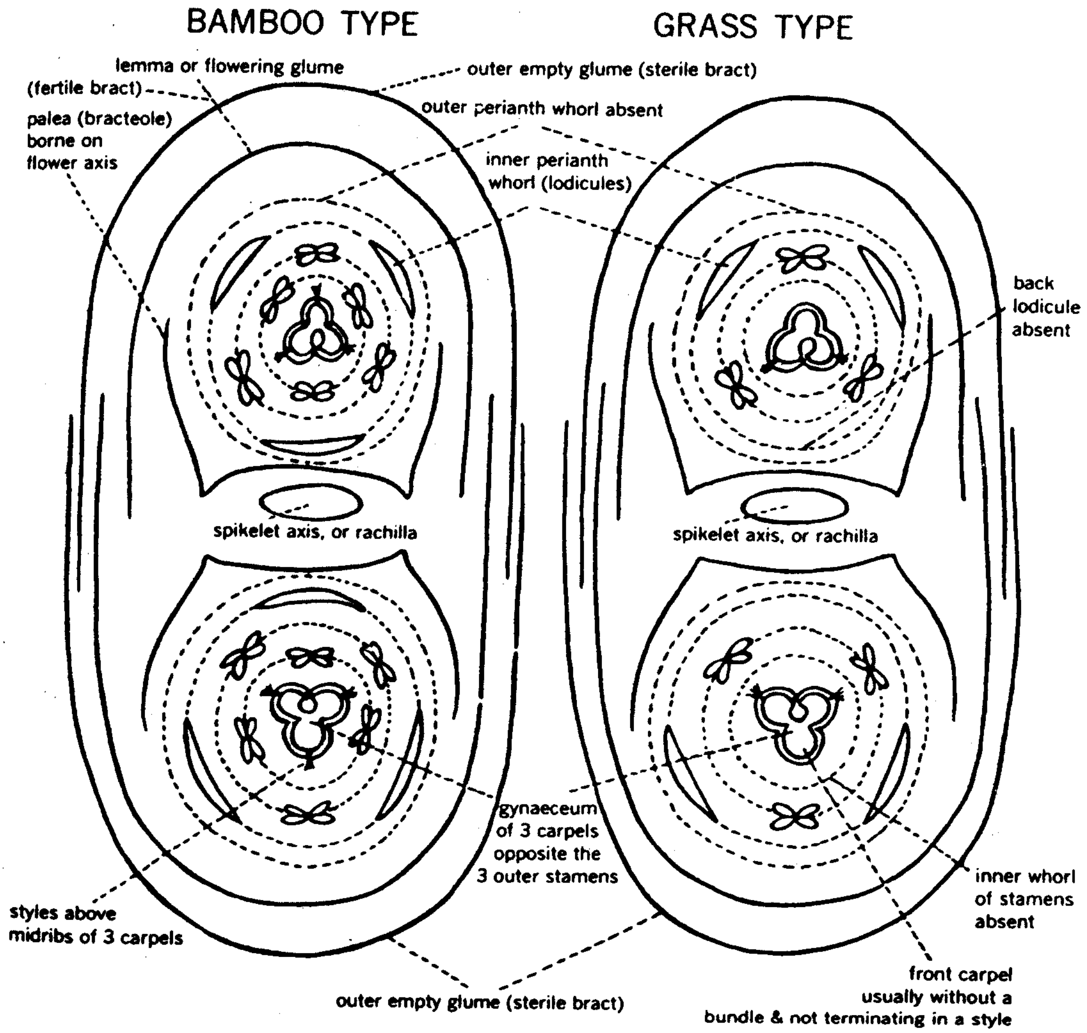


Figure 1.15 Diagramatic comparison of typical spikelet and floret structures in the bamboos (left) and the non-bambusoid grasses (right). From McClure (1966).

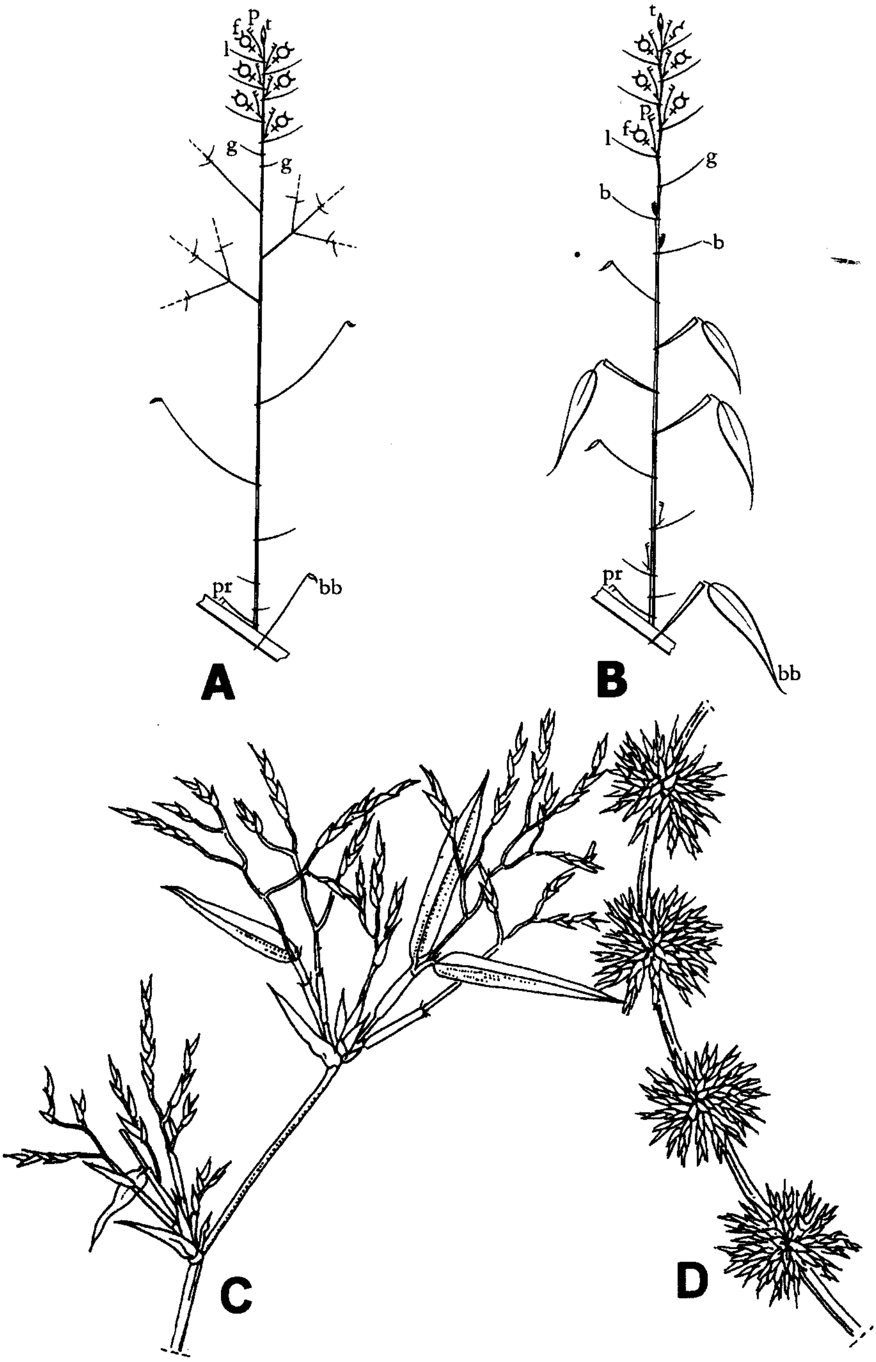


Figure 1.16 Inflorescences. A. and B. diagramatic comparison of a branch of (A) a determinate inflorescence (semelauctant) with a branch of (B) an indeterminate inflorescence; C. the determinate inflorescences; D. the indeterminate inflorescences; b, bracts subtending prophyllate buds (the latter shown as dark objects with double-barred tips); f, flower; g, glume; l, lemma; p, palea; t, vestigial terminal floret; bb, foliar organ or bract subtending the synflorescence unit; pr, the associated prophyll at the base of that unit.. The broken lines in the inflorescence proper in (A) indicate fully developed spikelets like the apical one shown. The two dark objects at the base of the inflorescence proper in (B) are prophyllate buds (each bud is capable of duplicating the pseudospikelet of which they are a part). A and B, adapted from McClure (1966); C and D, from Dransfield and Widjaja (1995a).

1.9 Research objectives

The aim of this thesis is to evaluate the phylogenetic position of *Dendrocalamus* within the whole subfamily Bambusoideae, to assess the inter-relationships of its species, and to document its species for the Flora of Thailand project. Furthermore, it aimed to assess population genetic variation in one of its species, *D. membranaceus*. These aims were achieved through comparative morphological and DNA sequence analysis. More specifically the thesis aimed to:

1. Assess relationships between *Dendrocalamus* and other genera in Bambusoideae and other related grass genera, and also to assess subtribal relationships within the tribe Bambuseae.

2. Study the generic boundary of *Dendrocalamus* and to find its most closely related genera.

3. Study the infrageneric classification of *Dendrocalamus* and devise a new one if necessary.

4. Study population genetics, clonality and phylogeography of *D. membranaceus* using plastid DNA markers.

5. Contribute to the floristic work for the Flora of Thailand for the genus *Dendrocalamus*.

Chapter 2

A multi-gene region phylogenetic analysis of the woody bamboos (Bambuseae; Bambusoideae; Poaceae)

2.1 Introduction

There are approximately 80–90 genera and about 1,000–1,500 bamboo species in the world (McClure, 1966; Dransfield & Widjaja, 1995a; Clark, 1995; Judziewicz *et al.*, 1999; Ohrnberger, 1999). The natural range of the genus includes all continents except Antarctica and Europe, from lowlands up to about 4,000 metres in altitude (Soderstrom & Ellis, 1987; Dransfield & Widjaja, 1995a; Clark, 1995; Judziewicz *et al.*, 1999; Ohrnberger, 1999). About 90% of Bambusoideae s.s. are woody bamboos (Ohrnberger, 1999). Biogeographically, woody bamboos (Bambuseae) can be simply categorized into two groups, tropical or temperate (Ohrnberger, 1999). However, molecular evidence (Clark *et al.*, 1995; Ni Chonghaile, 2002) has suggested that they could be divided into at least three major lineages. These can be defined as temperate, palaeotropical, and neotropical.

The bamboos have been treated as a taxonomic group since the grass family (Poaceae; Gramineae) was recognised by Barnhart in 1895. Woody and herbaceous bamboos, accompanied with rice (*Oryza* spp.) and their allies, were classified as subfamily Bambusoideae *sensu lato* (s.l.). The bamboos were generally regarded as a markedly heterogeneous and primitive subfamily, believed by some to be sister to the rest of the grasses. Different numbers of tribes have been included in this subfamily varying from one to 15 depending on the sorts of characters used (Roshevits, 1946; Prat, 1960; Caro, 1982; Clayton & Renvoize, 1986; Soderstrom & Ellis, 1987; Tzvelev, 1989; Watson & Dallwitz, 1992). Among those, Clayton and Renvoize (1986) published a widely used monograph 'Genera graminum: grasses of the world', that was based on presumed evolutionary relationships using mainly plant habit, inflorescence, spikelet and leaf-blade characters. They divided Bambusoideae s.l. into 13 tribes; namely Anomochloeae, Bambuseae (incl. Puelieae and Guaduelleae), Brachyelytreae, Diarrheneae, Ehrharteae, Olyreae (incl. Buergersiochloeae), Oryzeae, Parianeae, Phaenospermateae, Phareae, Phyllorachideae, Streptogyneae, and Streptochaeteae. Watson and Dallwitz (1992) in 'The Grass Genera of the World', phenetically classified bamboos and their allies into similar groups to Clayton and Renvoize (1986) with the exception of treating Centotheceae as another tribe within Bambusoideae s.l.. Clayton and Renvoize (1986) placed this tribe in the small subfamily Centothecoideae.

Soderstrom and Ellis (1987) relied greatly on ten characters of leaf anatomy, and those from spikelet, flower, and fruit, to delimit their 'core' Bambusoideae, which they considered to be monophyletic. They divided five tribes sharing all ten characters, into two supertribes; Olyrodae and Bambusodae. Olyrodae contained herbaceous members of four tribes: Anomochloeae, Buergersiochloeae, Olyreae (incl. Parianeae), and Streptochaeteae. Bambusodae had a single tribe (Bambuseae) and contained all the woody members of Bambusoideae. In this study, there were five genera with an uncertain placement, *Apoclada, Glaziophyton, Hitchcockella, Oligostachyum* and *Racemobambos* (*Neomicrocalamus*). According to the classification of Soderstrom and Ellis (1987), the Bambusoideae s.l. included six other non-core tribes (Guaduelleae, Oryzeae, Phareae, Puelieae, Streptogyneae, and Zizanieae). These were called 'peripheral tribes' by Clark *et al.* (1995), because they shared at least five to eight bambusoid character states. Clark *et al.* (1995) identified 'five core tribes' and excluded five other tribes (Brachyelytreae, Centotheceae, Diarrheneae, Ehrharteae, and Phaenospermateae) from Bambusoideae because they shared only three to five of the bambusoid character states.

Cladistic analysis using morphological and anatomical characters by Kellogg and Campbell (1987) proposed that Bambusoideae s.l. were associated with either tribe Ehrharteae or subfamily Panicoideae. However, the bambusoid clade was not sister to the rest of the grasses. Kellogg and Watson (1993) studied Bambusoideae s.l. in more detail and suggested that the 'core Bambusoideae' of Soderstrom and Ellis (1987) and the Bambuseae of Clayton and Renvoize (1986), were polyphyletic. They also emphasized that it is likely that Bambusoideae are neither monophyletic nor sister to the rest of the grasses.

There have been a number of molecular studies that have provided additional information for grass phylogenetics (Hamby & Zimmer, 1988; Doyle *et al.*, 1992; Davis & Soreng, 1993; Cummings *et al.*, 1994; Nadot *et al.*, 1994; Barker *et al.*, 1995; Clark *et al.*, 1995; Duvall & Morton 1996; Liang & Hilu, 1996; Hilu *et al.*, 1999; Zhang, 2000; GPWG, 2000, 2001; Hodkinson *et al.*, 2007a; Bouchenak-Khelladi *et al., in press*). However they have given some conflicting results on how Bambusoideae relates to the remaining grasses. Duvall and Morton (1996) suggested that outgroup selection affected the topology of the early diverging (outlying) lineages in grass phylogenetic trees. From their results, that used a dicot as the outgroup, the pooids were sister to the rest of the grasses. When the outgroup was Joinvilleaceae or Restionaceae, Bambusoideae (*Lithachne, Phyllostachys, Chusquea,* and *Bambusa*) were resolved as sister to the rest of the grasses. Analyses using only a single representative of bamboos inferred from ribosomal RNA sequences (Hamby & Zimmer, 1988), *rbcL* sequences (Barker *et al.*, 1995), and *matK* sequences (Liang & Hilu, 1996), have shown Bambusoideae to be sister to the rest of the grass family. However, restriction site data (Davis & Soreng, 1993), *ndhF* sequences (Clark *et al.*, 1995), *matK* sequences (Hilu *et al.*, 1999), *rpl16* sequences (Zhang, 2000), combined data sets (one morphological, four plastid DNA sequence regions, and three nuclear DNA sequence regions; GPWG, 2000, 2001), and large supertree analyses (Salamin *et al.* 2002; Hodkinson *et al.*, 2007a) have indicated that the woody bamboos were not an outlying group of the family. Clark *et al.* (1995) treated a group including the neotropical herbaceous bamboo tribes Anomochloeae and Streptochaeteae, as the earliest diverging lineage within the family, and the pantropical herbaceous bamboo, Phareae, as the next earliest diverging lineage. They also established the weakly supported 'BOP' clade (Bambusoideae, Oryzoideae, and Pooideae), as one of the two primary groups in the family, the other was the PACC clade (Panicoideae, Arundinoideae, Chloridoideae, and Centhothecoideae). Zhang (2000) showed with *rpl16* plastid DNA sequences that *Puelia* was sister to the rest of the BOP clade and agreed with Clark *et al.* (1995) that Anomochloeae, Streptochaeteae, and Phareae are sister to the rest of the extant grasses. He also found that the bambusoid clade was sister to the pooids while the oryzoids were supported as a robust monophyletic group which was well separated from pooid and bambusoid clades. The combined analysis of the GPWG (2001) allowed re-evaluation of intrafamilial classification of the grasses and helped clarify the classification of Bambusoideae s.l.. They found that Joinvilleaceae were sister to Poaceae. Anomochlooideae including two tribes, Anomochloeae and Streptochaeteae, were found to be sister to the rest of Poaceae. However this subfamily may not be monophyletic and two subfamilies may be needed to serve them (GPWG, 2001), as there is no morphological synapomorphy for this grouping. However, they would still represent outlying lineages if they were not combined, and they would be successively sister to the rest of the grasses. The next two diverging lineages were Pharoideae and Puelioideae (incl. tribes Puelieae and Guaduelleae), respectively. The subfamily Pharoideae includes a single redundant tribe Phareae (excl. *Olyra*; GPWG (2001)). Contrary to Zhang (2000) *Puelia* was not positioned in the BOP (=BEP) clade. The monophyletic Bambusoideae *sensu stricto* (s.s.) was proposed. This subfamily contained only two tribes, the woody Bambuseae and the herbaceous Olyreae. Olyreae also includes Buergersiochloeae and Parianeae, following Zhang and Clark (2000). From this study, the GPWG (2001) transformed the BOP clade into the 'BEP' clade including Bambusoideae s.s., Ehrhartoideae (=Oryzoideae), and Pooideae. Their subfamily Ehrhartoideae included three tribes, Ehrharteae, Oryzeae, and Phyllorachideae, even if they did not sample Phyllorachideae for their analysis (but based its placement on morphological similarity). Due to missing data, however, the tribe Streptogyneae could not be put in any of the 12 subfamilies of Poaceae and was therefore treated as incertae sedis GPWG (2001). The thirteenth subfamily, Micrairoideae has since been proposed but is unrelated to the bamboos (Sánchez-Ken *et al.*, 2007).

Phylogenetic relationships of tribal and subtribal levels within Bambusoideae are still unclear. Several classifications of woody bamboos are incongruent (Clayton & Renvoize, 1986; Soderstrom & Ellis, 1987; Dransfield & Widjaja, 1995a; Li, 1998; Ohrnberger, 1999), see Table 2.1. There is therefore a need for taxonomic stability and it is hoped that molecular data may help identify major groupings of bamboos. Some studies on genera of woody bamboos based on molecular phylogenetic analyses have been undertaken such as for 16 Asian genera using restriction site mutations of cpDNA (Watanabe *et al.*, 1994), *Chusquea* using chloroplast *rpl16* sequence data (Kelchner & Clark (1997) and *Phyllostachys* using RFLP markers (Friar & Kochert, 1994), RAPD markers (Gielis *et al.*, 1997), and ITS nuclear rDNA sequence data and AFLP markers (Renvoize & Hodkinson, 1997; Hodkinson *et al.*, 2000). Combined data-set analyses to study phylogenetic relationships among woody bamboos are rare. There is particularly a need for large multigene/sequence phylogenetic trees of Bambusoideae. Plastid DNA variation within the subfamily is low in comparison to several of the other grass subfamilies (Ni Chonghaile 2002) and single gene analyses of plastid DNA have been found to be insufficient to adequately resolve phylogenetic pattern needed for detailed classification. Few phylogenetic studies have attempted to combine sequences. Ni Chonghaile (2002) applied *trnL* intron, *trnL-trnF* intergenic spacer, *rpl16* intron sequence data, and ITS nuclear rDNA sequences to study relationships among woody bamboos. However her study focused mainly on temperate woody bamboos.

In this chapter, representatives from all subtribes of Bambuseae according to Clayton and Renvoize (1986) and Ohrnberger (1999) were sequenced for five plastid DNA regions (*trnL* intron, *trnL-F* intergenic spacer (the names of these two regions are hereafter combined and called *trnL-F* as they are continuous tandemly arranged sections of DNA sequence), *atpB-rbcL* intergenic spacer, *rps16* intron, and *matK* gene region) for combined analysis (see Table 2.2). The *trnL-F* and *atpB-rbcL* are commonly used for phylogenetic study of plants (from species to family levels; Soltis & Soltis, 1998), while *matK* is used for species to order levels. Several studies have shown these genes to be useful for phylogenetic study of grasses and bamboos: *trnL-F* (e.g. Ni Chonghaile, 2002); *matK* (e.g. Liang & Hilu, 1996; Hilu *et al.*, 1999). The *atpB-rbcL* and *rps16* have not been used to study bamboo phylogenetics before. However, *rps16* has been proved to be useful for plant molecular systematics for both dicots and monocots (such as Caryophyllaceae (Oxelmann *et al.*, 1997), Palmae (Asmussen *et al.*, 2000), and Marantaceae (Andersson & Chase, 2001)). Combined analyses of plastid DNA regions are often useful for improving phylogenetic resolution and support (Reeves *et al.*, 2001). Plastid DNA is generally non-recombining and maternally inherited in most angiosperms. Different sequences found on the plastid genome should therefore share the same evolutionary history and provide congruent phylogenetic trees. The justification to combine datasets in the analyses in this thesis was based on an examination of groupings (and support for these) found in the single gene analyses. No major and well supported incongruences were found between the results from single gene region analyses and it was deemed appropriate to combine datasets.

The aim of this chapter was to resolve major groupings within Bambusoideae and evaluate the currently used classifications. More specifically, it aimed to 1) define major bamboo groups and assess the monophyly of existing taxa, 2) study molecular variation in different plastid gene sequence regions to assess their use in bamboo phylogenetics, 3) study the relationships of woody bamboos in comparison with other closely related bamboos and grasses especially to examine the relationship between Bambuseae and Olyreae and 4) study the subtribe classification of Bambuseae and their biogeography.

Table 2.1 Subtribes of woody bamboos according to previous five classifications and this study*

Subtribe	Clayton & Renvoize (1986)	Soderstrom & Ellis (1987)	Dransfield & Widjaja (1995a)** (=Clark, 1995)	Li (1998)	Ohrnberger (1999)	This study
	Tribe adopted as BAMBUSEAE S.L.	Tribe adopted as BAMBUSEAE S.L.	Tribe adopted as BAMBUSEAE S.L.	Tribe adopted as BAMBUSEAE S.L.	Tribe adopted as BAMBUSEAE S.L.	Tribe treated as BAMBUSEAE S.S.
1. Arthrostylidiinae (subtribe established 1929)		***11/confined to the New World	13/confined to the New World	13/New World	13/New World	13/New World
	In Bambusinae	**Actinocladum**	**Actinocladum**	**Actinocladum**	**Actinocladum**	**Actinocladum**
	in Bambusinae	**Arthrostylidium**	**Arthrostylidium**	**Arthrostylidium**	**Arthrostylidium**	**Arthrostylidium**
	in Bambusinae	**Athroostachys**	**Athroostachys**	**Athroostachys**	**Athroostachys**	**Athroostachys**
	in Bambusinae	**Atractantha**	**Atractantha**	**Atractantha**	**Atractantha**	**Atractantha**
	in Arundinariinae	**Aulonemia**	**Aulonemia**	**Aulonemia** (incl. Matudacalamus)	**Aulonemia** (incl. Matudacalamus)	**Aulonemia** (incl. Matudacalamus)
	in Arundinariinae	**Colanthelia**	**Colanthelia**	**Colanthelia**	**Colanthelia**	**Colanthelia**
	in Bambusinae	**Elytrostachys**	**Elytrostachys**	**Elytrostachys**	**Elytrostachys**	**Elytrostachys**
	under Aulonemia, Arundinariinae,	**Matudacalamus**	?	under Aulonemia	under Aulonemia	under Aulonemia
	in Bambusinae	**Merostachys**	**Merostachys**	**Merostachys**	**Merostachys**	**Merostachys**
	in Arundinariinae	**Myriocladus**	**Myriocladus**	**Myriocladus**	**Myriocladus**	**Myriocladus**
	in Bambusinae	**Rhipidocladum**	**Rhipidocladum**	**Rhipidocladum**	**Rhipidocladum**	**Rhipidocladum**
	-	-	**Alvimia** genus established 1988	**Alvimia**	**Alvimia**	**Alvimia**
	in Bambusinae	UNCERTAIN PLACEMENT-1	**Apoclada**	**Apoclada**	**Apoclada**	**Apoclada**
	in Arundinariinae	UNCERTAIN PLACEMENT-2	**Glaziophyton**	**Glaziophyton**	**Glaziophyton**	**Glaziophyton**

* All previous five classifications recognized all woody bamboos as a single tribe, Bambuseae s.l. while this study recognized two tribes; Arundinarieae and Bambuseae s.s.

** the classification system based on Soderstrom and Ellis (1987), with some modifications developed as a collaborative effort by C.M.A. Stapleton, S. Dransfield, L.G. Clark, and K.M. Wong

*** below each classification system is the number of genera and their distribution

? not recognized

Subtribe	Clayton & Renvoize (1986)	Soderstrom & Ellis (1987)	Dransfield & Widjaja (1995a)* (=Clark, 1995)	Li (1998)	Ohrnberger (1999)	This study
	Tribe adopted as BAMBUSEAE S.L.	Tribe adopted as BAMBUSEAE S.L.	Tribe adopted as BAMBUSEAE S.L.	Tribe adopted as BAMBUSEAE S.L.	Tribe adopted as BAMBUSEAE S.L.	Tribe treated as ARUNDINARIEAE
2. Arundinariinae (subtribe established 1834)	20/Pantropic	12/essentially Old World (1 extending to N. America)	14/Old World (1 extending to N. America)	14/essentially Old World	16/S. & SE. Asia, E. Asia (1 confined to south-eastern N America)	16/essentially Old World (1 confined to N. America)
	Acidosasa	**Acidosasa**	**Acidosasa**	**Acidosasa**	**Acidosasa**	**Acidosasa**
	Arundinaria (incl. Pleioblastus, Oligostachyum, Bashania, Butania, Omeiocalamus Clavinodum)	**Arundinaria** (incl. Pleioblastus Bashania, Omeiocalamus, Clavinodum, Pseudosasa)	**Arundinaria**	**Arundinaria**	**Arundinaria**	**Arundinaria** (endemic to N America)
	Aulonemia (incl. Matudacalamus)	in Arthrostylidiinae	in Arthrostylidiinae	in Arthrostylidiinae	in Arthrostylidiinae	in Arthrostylidiinae
	Chimonobambusa (incl. Oreocalamus, Qiongzhuea)	**Chimonobambusa** (incl. Oreocalamus, Qiongzhuea, Tetragonocalamus)	in Shibataeinae	in Shibataeinae	in Shibataeinae	in Shibataeinae
	Chusquea (incl. Rettbergia, Swallenochloa)	in Chusqueinae	in Chusqueinae	in Chusqueinae	in Chusqueinae	in Chusqueinae
	Colanthelia	in Arthrostylidiinae	in Arthrostylidiinae	in Arthrostylidiinae	in Arthrostylidiinae	in Arthrostylidiinae
	Glaziophyton	UNCERTAIN PLACEMENT-2	in Arthrostylidiinae	in Arthrostylidiinae	in Arthrostylidiinae	in Arthrostylidiinae
	Guaduella (incl. Microbambus)	in Guaduelleae, Bambusoideae s.l.	?	?	herbaceous bamboo tribe Guaduelleae	herbaceous bamboo tribe Guaduelleae
	Hitchcockella	UNCERTAIN PLACEMENT-3	in Hickeliinae (Nastinae)	in Hickeliinae (Nastinae)	in Hickeliinae (Nastinae)	in Hickeliinae (Nastinae)
	Indocalamus (incl. Gelidocalamus, Ferrocalamus)	**Indocalamus** (incl. Ferrocalamus)	**Indocalamus**	**Indocalamus**	**Indocalamus**	**Indocalamus**
	Indosasa	under Shibataeinae	**Indosasa**	under Shibataeinae	under Shibataeinae	under Shibataeinae

Table 2.1 (continued)

Subtribe	Clayton & Renvoize (1986)	Soderstrom & Ellis (1987)	Dransfield & Widjaja (1995a)* (=Clark, 1995)	Li (1998)	Ohrnberger (1999)	This study
	Myriocladus	in Arthrostylidiinae	in Arthrostylidiinae	in Arthrostylidiinae	in Arthrostylidiinae	in Arthrostylidiinae
	Neurolepis	in monotypic subtribe Neurolepidinae	in Chusqueinae	in Chusqueinae	in Chusqueinae	in Chusqueinae
	Olmeca	in Guaduinae	in Guaduinae	in Guaduinae	in Guaduinae	in Guaduinae
	Perrierbambus	**Perrierbambus**	in Hickeliinae (Nastinae)	in Hickeliinae (Nastinae)	in Hickeliinae (Nastinae)	in Hickeliinae (Nastinae)
	Pseudosasa	under Arundinaria	**Pseudosasa**	**Pseudosasa**	**Pseudosasa**	**Pseudosasa**
	Sasa (incl. Sasaella, Sasamorpha, Neosasamorpha)	**Sasa** (incl. Sasaella, Sasamorpha, Neosasamorpha,	**Sasa**	**Sasa**	**Sasa**	**Sasa**
	Sinarundinaria (incl. Yushania, Chimonocalamus. Otatea, Ampelocalamus, Burmabambus, Drepanostachyum)	under Fagesia	?	?	under Fagesia, Thamnocalaminae	under Fagesia, Thamnocalaminae
	Sinobambusa (incl. Neobambus)	in Shibataeinae	in Shibataeinae	in Shibataeinae	in Shibataeinae	in Shibataeinae
	Thamnocalamus (incl. Fargesia, Himalayacalamus)	**Thamnocalamus**	**Thamnocalamus**	**Thamnocalamus**	in Thamnocalaminae	in Thamnocalaminae
	under Sinarundinaria	**Ampelocalamus**	**Ampelocalamus**	**Ampelocalamus**	in Thamnocalaminae	in Thamnocalaminae
	-	-	**Borinda** genus established 1994	under Fargesia	in Thamnocalaminae	in Thamnocalaminae
	under Sinarundinaria	**Chimonocalamus**	**Chimonocalamus**	**Chimonocalamus**	in Thamnocalaminae	in Thamnocalaminae
		Drepanostachyum (incl. Himalayacalamus)	**Drepanostachyum**	**Drepanostachyum** (incl. Himalayacalamus)	in Thamnocalaminae	in Thamnocalaminae
	under Indocalamus	**Gelidocalamus**	?	**Gelidocalamus**	**Gelidocalamus**	**Gelidocalamus**

Subtribe	Clayton & Renvoize (1986)	Soderstrom & Ellis (1987)	Dransfield & Widjaja (1995a)* (=Clark, 1995)	Li (1998)	Ohrnberger (1999)	This study
	under Thamnocalamus	**Fargesia** (incl. Burmabambus, Butania, Sinarundinaria, Yushania)	**Fargesia**	**Fargesia** (incl. Borinda, Yushania)	in Thamnocalaminae	in Thamnocalaminae
	under Thamnocalamus	under Drepanostachyum	**Himalayacalamus**	under Drepanostachyum	in Thamnocalaminae	in Thamnocalaminae
	under Sinarundinaria	under Fargesia	**Yushania**	under Fargesia	in Thamnocalaminae	in Thamnocalaminae
	under Indocalamus	under Indocalamus	?	**Ferrocalamus**	**Ferrocalamus**	**Ferrocalamus**
	-	-	?	**Gaoligongshania** genus established 1995	**Gaoligongshania**	**Gaoligongshania**
	under Arundinaria	UNCERTAIN PLACEMENT	?	**Oligostachyum**	**Oligostachyum**	**Oligostachyum**
	under Arundinaria	under Arundinaria	?	?	**Bashania**	**Bashania**
	-	-	?	?	**Menstruocalamus** genus established 1992	**Menstruocalamus**
	-	-	?	?	**Metasasa** genus established 1988	**Metasasa**
	under Arundinaria	under Arundinaria	?	?	**Pleioblastus**	**Pleioblastus**
	-	-	?	?	**Polyanthus** genus established 1991	**Polyanthus**
	under Sasa	under Sasa	?	?	**Sasaella**	**Sasaella**
	-	-	?	?	**Vietnamocalamus** genus established 1991	**Vietnamocalamus**

Table 2.1 (continued)

Subtribe	Clayton & Renvoize (1986)	Soderstrom & Ellis (1987)	Dransfield & Widjaja (1995a)* (=Clark, 1995)	Li (1998)	Ohrnberger (1999)	This study
	Tribe adopted as BAMBUSEAE S.L.	Tribe adopted as BAMBUSEAE S.L.	Tribe adopted as BAMBUSEAE S.L.	Tribe adopted as BAMBUSEAE S.L.	Tribe adopted as BAMBUSEAE S.L.	Tribe treated as BAMBUSEAE S.S.
3. Bambusinae (subtribe established 1830)	25/Pantropic	6/Old World tropics	13/mostly Tropical Asia	10/Old World tropics	17/S. & SE. Asia, E. Asia (*Bambusa* also found in Madagascar, Pacific, and Australia; *Dendrocalamus* also found in Pacific; *Oreobambos* and *Oxytenanthera* only found in Tropical Africa)	21/Old World tropics and Australia (*Oreobambos* and *Oxytenanthera* endemic to Tropical Africa; *Mullerochloa* endemic to Australia)
	Actinocladum	in Arthrostylidiinae	in Arthrostylidiinae	in Arthrostylidiinae	in Arthrostylidiinae	in Arthrostylidiinae
	Apoclada	UNCERTAIN PLACEMENT	in Arthrostylidiinae	in Arthrostylidiinae	in Arthrostylidiinae	in Arthrostylidiinae
	Arthrostylidium	in Arthrostylidiinae	in Arthrostylidiinae	in Arthrostylidiinae	in Arthrostylidiinae	in Arthrostylidiinae
	Athroostachys	in Arthrostylidiinae	in Arthrostylidiinae	in Arthrostylidiinae	in Arthrostylidiinae	in Arthrostylidiinae
	Atractantha	?	in Arthrostylidiinae	in Arthrostylidiinae	in Arthrostylidiinae	in Arthrostylidiinae
	Bambusa (incl. Guadua, Bonia, Leleba, Tetragonocalamus, Lingania, Dendrocalamopsis)	**Bambusa** (incl. Bonia, Dendrocalamopsis, Leleba, Lingania, Neosinocalamus, Sinocalamus)	**Bambusa**	**Bambusa** (incl. Dendrocalamopsis)	**Bambusa** (incl. Leleba, Tetragonocalamus, Lingania, Dendrocalamopsis, Neosinocalamus)	**Bambusa** (*B. moreheadiana* of Ohrnberger (1999) is accepted as *Mullerochloa moreheadiana* following Wong (2005))
	Dendrocalamus (incl. Klemachloa, Sinocalamus, Neosinocalamus)	**Dendrocalamus** (incl. Gigantochloa, Houzeaubambus, Oreobambos, Oxytenanthera)	**Dendrocalamus**	**Dendrocalamus** (incl. Klemachloa, Oreobambos, Oxytenanthera, Sinocalamus)	**Dendrocalamus** (incl. Sinocalamus)	**Dendrocalamus** (incl. Sinocalamus)
	Decaryochloa	in Hickeliinae (Nastinae)	in Hickeliinae (Nastinae)	in Hickeliinae (Nastinae)	in Hickeliinae (Nastinae)	in Hickeliinae (Nastinae)
	Dinochloa	**Dinochloa**	**Dinochloa**	**Dinochloa**	**Dinochloa**	**Dinochloa**
	Elytrostachys	in Arthrostylidiinae	in Arthrostylidiinae	in Arthrostylidiinae	in Arthrostylidiinae	in Arthrostylidiinae
	Gigantochloa	under Dendrocalamus	**Gigantochloa**	**Gigantochloa**	**Gigantochloa**	**Gigantochloa**

Subtribe	Clayton & Renvoize (1986)	Soderstrom & Ellis (1987)	Dransfield & Widjaja (1995a)* (=Clark, 1995)	Li (1998)	Ohrnberger (1999)	This study
	Greslania	in Hickeliinae (Nastinae)	in Hickeliinae (Nastinae)	in Hickeliinae (Nastinae)	in Hickeliinae (Nastinae)	in Hickeliinae (Nastinae)
	Hickelia	in Hickeliinae (Nastinae)	in Hickeliinae (Nastinae)	in Hickeliinae (Nastinae)	in Hickeliinae (Nastinae)	in Hickeliinae (Nastinae)
	Melocalamus	**Melocalamus**	**Melocalamus**	**Melocalamus**	**Melocalamus**	**Melocalamus** Note: This genus, together with Dendrocalamus, requires further phylogenetic studies to see their relationships.
	Merostachys	in Arthrostylidiinae	in Arthrostylidiinae	in Arthrostylidiinae	in Arthrostylidiinae	in Arthrostylidiinae
	Nastus (incl. Chloothamnus, Oreiostachys)	in Hickeliinae (Nastinae)	in Hickeliinae (Nastinae)	in Hickeliinae (Nastinae)	in Hickeliinae (Nastinae)	in Hickeliinae (Nastinae)
	Phyllostachys	in Shibataeinae	in Shibataeinae	in Shibataeinae	in Shibataeinae	in Shibataeinae
	Oreobambos	under Dendrocalamus	**Oreobambos**	under Dendrocalamus	**Oreobambos**	**Oreobambos**
	Pseudocoix	under Hickelia, Hickeliinae (Nastinae)	?	?	under Hickelia, Hickeliinae (Nastinae)	under Hickelia, Hickeliinae (Nastinae)
	Puelia	in Puelieae, Bambusoideae s.l.	?	?	herbaceous bamboo tribe Puelieae	herbaceous bamboo tribe Puelieae
	Racemobambos (incl. Microcalamus=Neomicrocalamus)	UNCERTAIN PLACEMENT-5 (incl. Neomicrocalamus)	in Racemobambosinae	in Racemobambosinae	in Racemobambosinae	in Racemobambosinae
	Rhipidocladum	in Arthrostylidiinae	in Arthrostylidiinae	in Arthrostylidiinae	in Arthrostylidiinae	in Arthrostylidiinae
	Semiarundinaria (Brachystachyum)	in Shibataeinae	in Shibataeinae	in Shibataeinae	in Shibataeinae	in Shibataeinae
	Shibataea	in Shibataeinae	in Shibataeinae	in Shibataeinae	in Shibataeinae	in Shibataeinae
	Thyrsostachys	**Thyrsostachys**	**Thyrsostachys**	**Thyrsostachys**	**Thyrsostachys**	**Thyrsostachys**
	under Dendrocalamus	**Klemachloa**	?	under Dendrocalamus	**Klemachloa**	**Klemachloa**

Table 2.1 (continued)

Subtribe	Clayton & Renvoize (1986)	Soderstrom & Ellis (1987)	Dransfield & Widjaja (1995a)* (=Clark, 1995)	Li (1998)	Ohrnberger (1999)	This study
	-	-	**Holttumochloa** genus established 1993	**Holttumochloa**	**Holttumochloa**	**Holttumochloa**
	-	-	**Kinabaluchloa** genus established 1993	**Kinabaluchloa** (incl. Maclurochloa, Soejatmia)	**Kinabaluchloa**	**Kinabaluchloa**
	-	-	**Maclurochloa** genus established 1993	under Kinabaluchloa	**Maclurochloa**	**Maclurochloa**
	in Melocanninae (=Schizostachydinae)	under Dendrocalamus	**Oxytenanthera**	under Dendrocalamus	**Oxytenanthera**	**Oxytenanthera**
	-	-	**Soejatmia** genus established 1993	under Kinabaluchloa	**Soejatmia**	**Soejatmia**
	-	-	**Sphaerobambos** genus established 1989	**Sphaerobambos**	**Sphaerobambos**	**Sphaerobambos**
	under Bambusa	under Bambusa	?	**Bonia** (incl. Monocladus)	**Bonia** (incl. Monocladus)	**Bonia** (incl. Monocladus)
	-	-	?	?	**Pseudobambusa** genus established 1991	**Pseudobambusa** genus established 1991
	-	-	?	?	**Pseudoxytenanthera** genus established 1988	**Pseudoxytenanthera** genus established 1988
	-	-	-	-	-	**Cyrtochloa** genus established 1998 (see Dransfield, 1998a)
						Mullerochloa genus established 2005 (see Wong, 2005)
	-	-	-	-	-	**Temochloa** genus established 2000
	-	-	-	-	-	**Phuphanochloa** genus newly described (Sungkaew *et al*, *accepted*)

Subtribe	Clayton & Renvoize (1986)	Soderstrom & Ellis (1987)	Dransfield & Widjaja (1995a)* (=Clark, 1995)	Li (1998)	Ohrnberger (1999)	This study
4. Chusqueinae (subtribe established 1929)		1/New World tropics	2/New World tropics	2/New World tropics	2/New World tropics	2/New World tropics
	in Arundinariinae	**Chusquea** (incl. Rettbergia, Swallenochloa	**Chusquea**	**Chusquea**	**Chusquea** (incl. Rettbergia, Swallenochloa	**Chusquea** (incl. Rettbergia, Swallenochloa
	in Arundinariinae	in monotypic subtribe Neurolepidinae	**Neurolepis**	**Neurolepis**	**Neurolepis**	**Neurolepis**
5. Guaduinae (subtribe established 1987)		5/New World tropics	5/New World tropics	5/New World tropics	5/New World tropics	5/New World tropics
	-	**Criciuma** genus established 1987	**Criciuma**	**Criciuma**	**Criciuma**	**Criciuma**
	-	**Eremocaulon** genus established 1987	**Eremocaulon**	**Eremocaulon**	**Eremocaulon**	**Eremocaulon**
	under Bambusa, Bambusinae	**Guadua**	**Guadua**	**Guadua**	**Guadua**	**Guadua**
	in Arundinariinae	**Olmeca**	**Olmeca**	**Olmeca**	**Olmeca**	**Olmeca**
	under Sinarundinaria, Arundinariinae	**Otatea**	**Otatea**	**Otatea**	**Otatea**	**Otatea**
6. Hickeliinae (subtribe established 1924=Nastinae; subtribe established 1987)		4/E. Africa (Tanzania) and Madacascar to Indonesia and New Guinea	6/mostly in southern hemisphere of the Old World tropics	6/Troical Africa and Madagascar to Tropical Asia	7/Tropical Africa to Madacascar and Pacific to S. Asia	9/Tropical Africa to Madacascar and Pacific to S. Asia Note: More studis required for phylonenetic relationships between this subtribe and the rest of palaeotropical and Austral woody bamboos.

Table 2.1 (continued)

Subtribe	Clayton & Renvoize (1986)	Soderstrom & Ellis (1987)	Dransfield & Widjaja (1995a)* (=Clark, 1995)	Li (1998)	Ohrnberger (1999)	This study
	in Bambusinae	**Decaryochloa**	**Decaryochloa**	**Decaryochloa**	**Decaryochloa**	**Decaryochloa**
	in Bambusinae	**Greslania**	**Greslania**	**Greslania**	**Greslania**	**Greslania**
	in Bambusinae	**Hickelia** (incl. Pseudocoix)	**Hickelia**	**Hickelia**	**Hickelia** (incl. Pseudocoix)	**Hickelia** (incl. Pseudocoix)
	in Bambusinae	**Nastus** (incl. Chloothamnus, Oreiostachys)	**Nastus**	**Nastus**	**Nastus** (incl. Chloothamnus, Oreiostachys)	**Nastus** (incl. Chloothamnus, Oreiostachys)
	in Arundinariinae	?	**Hitchcockella**	**Hitchcockella**	**Hitchcockella**	**Hitchcockella**
	in Arundinariinae	in Arundinariinae	**Perrierbambus**	**Perrierbambus (?)**	**Perrierbambus**	**Perrierbambus**
	-	-	-	UNCERTAIN PLACEMENT	**Temburongia** genus established 1996	UNCERTAIN PLACEMENT
	-	-	-	-	-	**Cathariostachys** genus established 1998 (see Dransfield, 1998b; *personal communication*)
	-	-	-	-	-	**Sirochloa** genus established 2002 (see Dransfield, 2002; *personal communication*)
	-	-	-	-	-	**Valiha** genus established 1998 (see Dransfield, 1998b; *personal communication*)
7. Melocanninae (subtribe established 1881=Schizostachydinae; subtribe established 1987)	4/Old World tropics	7/Old World tropics	8/mostly tropical Asia	9/Old World tropics	9/S. & SE. Asia, E. Asia (*Cephalostachyum* and *Ochlandra* also found in Madagascar; *Schizostachyum* also found in Madagascar and Pacific)	9/S. & SE. Asia, E. Asia
	Melocanna	**Melocanna**	**Melocanna**	**Melocanna**	**Melocanna**	**Melocanna**

Subtribe	Clayton & Renvoize (1986)	Soderstrom & Ellis (1987)	Dransfield & Widjaja (1995a)* (=Clark, 1995)	Li (1998)	Ohrnberger (1999)	This study
	Ochlandra	**Ochlandra**	**Ochlandra**	**Ochlandra**	**Ochlandra**	**Ochlandra**
	Oxytenanthera (incl. Houzeaubambus)	under Dendrocalamus, Bambusinae	in Bambusinae	under Dendrocalamus, Bambusinae	in Bambusinae	in Bambusinae
	Schizostachyum (incl. Cephalostachyum, Pseudostachyum, Neohouzeaua, Dendrochloa, Leptocanna, Teinostachyum)	**Schizostachyum** (incl. Dendrochloa, Neohouzeaua (?))	**Schizostachyum**	**Schizostachyum**	**Schizostachyum** (incl. Leptocanna)	**Schizostachyum** (incl. Leptocanna)
	under Schizostachyum	**Cephalostachyum**	**Cephalostachyum**	**Cephalostachyum**	**Cephalostachyum**	**Cephalostachyum**
	under Schizostachyum	**Leptocanna**	?	**Leptocanna**	under Schizostachyum	under Schizostachyum
	under Schizostachyum	**Pseudostachyum**	**Pseudostachyum**	**Pseudostachyum**	**Pseudostachyum**	**Pseudostachyum**
	under Schizostachyum	**Teinostachyum**	**Teinostachyum**	**Teinostachyum**	**Teinostachyum**	**Teinostachyum**
	-	-	**Davidsea** genus established 1988	**Davidsea**	**Davidsea**	**Davidsea**
	under Schizostachyum	under Schizostachyum	**Neohouzeaua**	**Neohouzeaua**	**Neohouzeaua**	**Neohouzeaua**
	under Schizostachyum	under Schizostachyum	?	?	**Dendrochloa**	**Dendrochloa**
8. Neurolepidinae (subtribe established 1987)		New World at high elevation from Costa Rica to Peru				
	in Arundinariinae	**Neurolepis**	in Chusqueinae	in Chusqueinae	in Chusqueinae	in Chusqueinae
9. Racemobambosinae (subtribe established 1994)			**3/Himalayas and Tropical Asia**	**1/Tropical Asia**	**3/S. & SE. Asia, E. Asia** (1 also found in Pacific)	**3/S. & SE. Asia, E. Asia** (1 also found in Pacific) Note: This subtribe requires further phylogenetic studies. It is probably better placed under Bambusinae.
	under Racemobambos, Bambusinae	under Racemobambos	**Neomicrocalamus**	under Racemobambos	**Neomicrocalamus**	**Neomicrocalamus**
	in Bambusinae	UNCERTAIN PLACEMENT-5 (incl. Neomicrocalamus)	**Racemobambos**	**Racemobambos** (incl. Neomicrocalamus, Vietnamosasa)	**Racemobambos**	**Racemobambos**
	-	-	**Vietnamosasa** genus established 1990	under Racemobambos	**Vietnamosasa**	**Vietnamosasa**

Table 2.1 (continued)

Subtribe	Clayton & Renvoize (1986)	Soderstrom & Ellis (1987)	Dransfield & Widjaja (1995a)* (=Clark, 1995)	Li (1998)	Ohrnberger (1999)	This study
	Tribe adopted as BAMBUSEAE S.L.	Tribe adopted as BAMBUSEAE S.L.	Tribe adopted as BAMBUSEAE S.L.	Tribe adopted as BAMBUSEAE S.L.	Tribe adopted as BAMBUSEAE S.L.	Tribe treated as ARUNINARIEAE
10. Shibataeinae (subtribe established 1987)		**7/Western Himalayas to Japan**	**5/Temperate Asia**	**7/E. Asia forests**	**8/S. & SE. Asia, E. Asia**	**8/S. & SE. Asia, E. Asia**
	under Semiarundinaria, Bambusinae	**Brachystachyum**	?	under Semiarundinaria	**Brachystachyum**	**Brachystachyum**
	Hybrid genus X Hibanobambusa (perp form: Sasa x Semiarundinaria)	**Hibanobambusa (?)**	?	?	**Hibanobambusa**	**Hibanobambusa**
	in Arundinariinae	**Indosasa (?)**	in Arundinariinae	**Indosasa**	**Indosasa**	**Indosasa**
	in Bambusinae	**Phyllostachys**	**Phyllostachys**	**Phyllostachys**	**Phyllostachys**	**Phyllostachys**
	in Bambusinae	**Semiarundinaria**	**Semiarundinaria**	**Semiarundinaria** (incl. Brachystachyum)	**Semiarundinaria**	**Semiarundinaria**
	in Bambusinae	**Shibataea**	**Shibataea**	**Shibataea**	**Shibataea**	**Shibataea**
	in Arundinariinae	**Sinobambusa** (incl. Neobambus)	**Sinobambusa**	**Sinobambusa**	**Sinobambusa**	**Sinobambusa**
	in Arundinariinae	in Arundinariinae	**Chimonobambusa**	**Chimonobambusa**	**Chimonobambusa** (incl. Oreocalamus, Qiongzhuea, Tetragonocalamus	**Chimonobambusa** (incl. Oreocalamus, Qiongzhuea, Tetragonocalamus
	under Chimonobambusa, in Arundinariinae	under Chimonobambusa, in Arundinariinae	?	**Qiongzhuea**	under Chimonobambusa	under Chimonobambusa

Subtribe	Clayton & Renvoize (1986)	Soderstrom & Ellis (1987)	Dransfield & Widjaja (1995a)* (=Clark, 1995)	Li (1998)	Ohrnberger (1999)	This study
11. Thamnocalaminae (subtribe established 1992)					8/S. & SE. Asia, E. Asia (2 also found in Africa and Madagascar)	8/S. & SE. Asia, E. Asia (2 also found in Africa and Madagascar)
	under Sinarundinaria, Arundinariinae	in Arundinariinae	in Arundinariinae	in Arundinariinae	**Ampelocalamus**	**Ampelocalamus**
	-	-	in Arundinariinae	under Fargesia, Arundinariinae	**Borinda** genus established 1994	**Borinda** genus established 1994
	under Sinarundinaria, Arundinariinae	in Arundinariinae	in Arundinariinae	in Arundinariinae	**Chimonocalamus**	**Chimonocalamus**
	under Sinarundinaria, Arundinariinae	in Arundinariinae	in Arundinariinae	in Arundinariinae	**Drepanostachyum**	**Drepanostachyum**
	under Thamnocalamus, Arundinariinae	in Arundinariinae	in Arundinariinae	in Arundinariinae	**Fargesia** (incl. Sinarundinaria)	**Fargesia** (incl. Sinarundinaria)
	under Thamnocalamus, Arundinariinae	under Drepanostachyum, Arundinariinae	in Arundinariinae	under Drepanostachyum, Arundinariinae	**Himalayacalamus**	**Himalayacalamus**
	in Arundinariinae	in Arundinariinae	in Arundinariinae	in Arundinariinae	**Thamnocalamus**	**Thamnocalamus**
	under Sinarundinaria, Arundinariinae	under Fargesia, Arundinariinae	in Arundinariinae	under Fargesia, Arundinariinae	**Yushania**	**Yushania**

2.2 Materials and methods

Plant materials

Plant material was collected in silica gel to rapidly desiccate the material and minimize DNA degradation (Hodkinson *et al.*, 2007b). Most of the fresh leaf materials preserved in silica gel (following Chase & Hills, 1991) were collected during fieldwork in several regions of Thailand, mainly in the areas bordering Burma, Malaysia, Cambodia and Laos. Some specimens were collected from living collections in the Singapore Botanic Gardens, Singapore and Xishuangbanna Tropical Botanical Garden, China, via collaboration of Dr Ruth Kiew and Dr Wang Hong, respectively. Some private collections made by Drs Soejatmi Dransfield, Trevor Hodkinson, Wong Khoon Meng, and Chris Stapleton, were also used. Some samples were obtained from herbarium specimens. Sixty-four taxa from three subfamilies sensu Clayton and Renvoize (1986), Bambusoideae, Pooideae, and Panicoideae, were sampled for the study, see Table 2.2. Panicoid grasses were selected as the outgroup because they lie outside the BEP clade and because I wanted to determine the closest relatives to the bamboos. Because of the incongruence between classifications of woody bamboos, all representatives from all bamboo subtribes according to Clayton and Renvoize (1986) and Ohrnberger (1999) were included. Details of sampled species and voucher specimens (numbers and herbaria) are presented in Table 2.2. *Phuphanochloa* is a bamboo genus new to science (Sungkaew *et al.*, *accepted*).

Isolation of total genomic DNA

Total genomic DNA (tDNA) was extracted using the modified CTAB method (Doyle & Doyle, 1987; Hodkinson *et al.*, 2007b) (see Protocol 1, Appendix, page 281). All crude tDNA samples were precipitated in isopropanol prior to washing and purification and were stored in the -20°C freezer overnight or longer to further precipitate the DNA. Long precipitation periods (a week or longer) have been shown to improve DNA extractions (T. Hodkinson, *personal communication*). All DNA samples were extracted by me, except for *Oreobambos buchwaldii* that was done by Ni Chonghaile (2002).

DNA washing and purification

The crude tDNA was washed and purified using wash buffer (70% ethanol) (see Protocol 2, Appendix, page 283) and JETQUICK Spin Columns respectively (see Protocol 3, Appendix, page 284). Each clean tDNA sample was then assessed for quality and quantity using gel electrophoresis (see Protocol 4, Appendix, page 286).

Amplification of chloroplast DNA using PCR

The polymerase chain reaction (PCR) was used to amplify each of the five gene regions (see Protocol 5, Appendix, page 287). The amplification primers used are provided in Table 2.3. The PCR amplification protocol of *trnL-F* consisted of a pre-heat treatment of 95° C for 1 min., and 30 cycles of the following: 95°C for 45 sec. of denaturation, 50°C for 45 sec. of annealing, 72°C for 2 min. of extension. A final extension of 72°C for 7 min. was also included. The protocols for *atpB-rbcL* and *rps16* were similar to *trnL-F* but used an increased annealing temperature (52°C). The PCR amplification protocol of *matK* consisted of a pre-heat treatment of 94° C for 3 min., and 30 cycles of the following: 94°C for 1 min. of denaturation, 52°C for 1 min. of annealing, 72°C for 2.5 min. of extension. A final extension of 72°C for 7 min. was also employed. The PCR products were then quantified by gel electrophoresis (see Protocol 4, Appendix, page 286) and stored in a -20°C freezer before further purification for sequencing.

Purification of PCR products

All successful PCR products were purified prior to sequencing (cycle sequencing). Purification of PCR products was similar to the procedure described in Protocol 3: Total genomic DNA purification. The only difference to Protocol 3 was that the elution buffer was ultra-pure sterile water and not TE buffer (see Protocol 6, Appendix, page 289).

Cycle sequencing and purification

Cycle sequencing used the same primers as the PCRs, but they were diluted 20 times (5 ngμl^{-1} instead of 100 ngμl^{-1}) (see Protocol 7, Appendix, page 290). The cycle sequencing products were then purified by ethanol and sodium acetate (see Protocol 8, Appendix, page 292). The purified sequencing products were then dried at room temperature overnight between tissue papers to remove any traces of ethanol and sodium acetate. Subsequently, the dried cycle sequencing samples were denatured prior to sequencing on the DNA sequencer (see Protocol 9, Appendix, page 293).

DNA sequencing

DNA sequencing was performed on an ABI Prism^{TM} 310 Genetic Analyzer (Applied Biosystems) using the ABI Prism^{TM} 310 module SEQ POP6 RAPID (1.0-mL) E. The sequences were then processed using ABI Prism^{TM} DNA Sequencing Analysis Software, version 3.4.1. The full sequences of all taxa listed in Table 2.2 were obtained. Only a partial sequence of one taxon, *Olyra latifolia*, was used due to difficulties with the *matK* 19F primer during sequencing.

DNA sequence editing, assembly and phylogenetic analysis

Successful DNA sequences were edited and assembled using AutoAssembler Software, version 2.1. The sequences were then imported to PAUP 4.0* Beta 2 (Swofford, 1998) for alignment. Sequences were aligned by eye. Gaps were scored as additional binary characters (scoring gaps of identical size and position only). The resulting sequences were subjected to maximum parsimony analysis using the heuristic search options in PAUP 4.0* Beta 2 (Swofford, 1998). Searches included 1,000 replicates of random stepwise addition saving no more than 100 trees for tree bisection reconstruction (TBR) branch swapping per replicate. Bootstrapping included 1,000 replicates and the same heuristic search settings as the individual searches except that simple addition sequence was used instead of random stepwise addition.

Table 2.2 Taxa and vouchers of all sequences

Taxon	Voucher/Herbarium	Origin
<u>**Bambusoideae**</u>		
Bambuseae		
Arundinariinae		
Borinda sp.[1]	Stapleton 1347/KEW	USA*, cultivated
Chimonobambusa quadrangularis (Fenzi) Makino	SS&AT 105/THNHM&KUFF	Thailand, cultivated
Chimonocalamus pallens Hsueh & Yi[2]	Stapleton 1340/KEW	USA*, cultivated
Chimonocalamus sp.[2]	Piya s.n./KUFF	Thailand
Chusquea patens L.G. Clark	Hodkinson 571/TCD	Costa Rica
Menstruocalamus sichuanensis (Yi) Yi[3]	Stapleton 1319/KEW	USA*, cultivated
Oligostachyum glabrescens (Wen) P.C. Keng & Z.P. Wang[4]	Stapleton 1302/KEW	USA*, cultivated
Pseudosasa cantorii (Munro) P.C. Ken	Stapleton 1/KEW	USA*, cultivated
Bambusinae		
Arthrostylidium glabrum R.W. Pohl	Hodkinson 572/TCD	Costa Rica
Bambusa bambos (L.) Voss	SS&AT 030704-16/THNHM&KUFF	Thailand
Bambusa beecheyana Munro	Stapleton 1313/KEW	USA*, cultivated
Bambusa malingensis McClure	Stapleton 1332/KEW	USA*, cultivated
Bambusa oliveriana Gamble	Stapleton 1321/KEW	USA*, cultivated
Bambusa oldhamii Munro [*Sinocalamus oldhamii* (Munro) McClure]	SS&AT 111/THNHM&KUFF	Thailand, cultivated
Bambusa tulda Roxburgh	Stapleton 1328/KEW	USA*, cultivated
Dendrocalamus asper (J.H. Schultes) Backer ex K. Heyne	BAM[5] 1	Malaysia, cultivated
Dendrocalamus giganteus Munro	BAM[5] 45	Malaysia, cultivated
Dendrocalamus latiflorus Munro [*Sinocalamus latiflorus* (Munro) McClure]	SS&AT 113/THNHM&KUFF	Thailand, cultivated
Dendrocalamus membranaceus Munro	SS&AT 020704-4/THNHM&KUFF	Thailand
Dendrocalamus minor (McClure) Chia & H.L. Fung	Stapleton 1317/KEW	USA*, cultivated
Dendrocalamus strictus (Roxburgh) Nees	SS&AT 718/THNHM&KUFF	Thailand
Dendrocalamopsis valida Q.H. Dai[6]	SS&AT 625/THNHM&KUFF&TCD	China, cultivated
Dinochloa malayana S. Dransfield	SD 1412/KEW	Thailand
Gigantochloa ligulata Gamble	SS&AT 090704-4/THNHM&KUFF	Thailand
Gigantochloa scortechinii Gamble	SS&AT 309/THNHM&KUFF	Singapore, cultivated
Guadua angustifolia Kunth subsp. *chacoensis* (N. Rojas Acosta) S.M. Young & W. Judd	Stapleton1308/KEW	USA*, cultivated
Melocalamus compactiflorus (Kurz) Bentham	SS&AT 175/THNHM&KUFF	Thailand
Neosinocalamus affinis (Rendle) P.C. Keng[7]	SS&AT 624/THNHM&KUFF&TCD	China, cultivated
Oreobambos buchwaldii K. Schumann	Kare s.n./TCD	Uganda
Phuphanochloa speciosa Sungkaew & A. Teerawatananon[8]	SS&AT 191/THNHM&KUFF&TCD	Thailand
Phyllostachys nigra (Loddiges ex Lindley) Munro	SS&AT 106/THNHM&KUFF	Thailand, cultivated
Phyllostachys edulis (Carriere) Houzeau De Lehaie	SS&AT 107/THNHM&KUFF	Thailand, cultivated
Rhipidocladum racemiflorum (Steudel) McClure	Hodkinson 576/TCD	Costa Rica
Temburongia simplex S. Dransfield & K.M. Wong[9]	Ahmed *et al.*, 20038/(dnaBank no. 21774)	Brunei

Table 2.2 (Continued)

Taxon	Voucher/Herbarium	Origin
Temochloa liliana S. Dransfield[10]	SS&AT 100704-15/THNHM&KUFF	Thailand
Thyrsostachys siamensis Gamble	SS&AT 020704-3/THNHM&KUFF	Thailand
Vietnamosasa ciliata (A. Camus) Nguyen[11]	SS&AT 208/THNHM&KUFF	Thailand
Vietnamosasa pusilla (A. Chevalier & A. Camus) Nguyen[11]	SD 1466/KEW	Thailand
Melocanninae		
Cephalostachyum pergracile Munro[12]	SD 1435/KEW	Thailand
Neohouzeaua fimbriata S.Dransf., Pattan. & Sungkaew[12]	SS&RP 12/KEW&BKF	Thailand
Neohouzeaua kerriana S.Dransf., Pattan. & Sungkaew[12]	SS&RP 13/KEW&BKF	Thailand
Oxytenanthera abyssinica (A. Richard) Munro	Stapleton 1307/KEW	USA*, cultivated
Pseudostachyum polymorphum Munro[12]	SS&AT 176/THNHM&KUFF	Thailand
Schizostachyum grande Ridley	SS&AT 100704-6/THNHM&KUFF	Thailand
Schizostachyum jaculans Holttum	SS&AT 307/THNHM&KUFF	Singapore, cultivated
Schizostachyum zollingeri Steudel	SS&AT 090704-1/THNHM&KUFF	Thailand
Mullerochloa moreheadiana (F.M. Bailey) K.M. Wong[13]	Hodkinson NSW33/TCD	Australia**
Neololeba atra (Lindley) Widjaja[14]	Hodkinson NSW32/TCD	Australia**
Olyreae		
Cryptochloa granulifera Swallen	Hodkinson 554/TCD	Costa Rica
Lithachne pauciflora (Swartz) Palisot De Beauvois ex Poiret	Mejia s.n./TCD	Herbarium specimen
Olyra latifolia Linnaeus	Hodkinson 614/TCD	Guyana
Piresia sp.	Hodkinson 601/TCD	Guyana
Oryzeae		
Leersia hexandra Sw.	Hodkinson 636/TCD	Guyana
Oryza rufipogon Griff.	AT&SS 164/THNHM	Thailand
Oryza sativa L.	Hodkinson 46/TCD	Ireland, cultivated
Ehrharteae		
Ehrharta calycina Sm.	Hodkinson G25/TCD	New Zealand
<u>Pooideae</u>		
Lygeeae		
Lygeum spartum Loefl. ex L.	Hodkinson 18/TCD	Spain
Nardeae		
Nardus stricta L.	Hodkinson & Curran 5/TCD	Ireland
Poeae		
Lolium perenne L.	Hodkinson 29/TCD	Ireland
Aveneae		
Alopecurinae		
Alopecurus pratensis L.	Hodkinson 30/TCD	Ireland
Aveninae		
Arrhenatherum elatius (L.) P. Beauv. ex J. Presl & C. Presl	Hodkinson 27/TCD	Ireland

Table 2.2 (Continued)

Taxon	Voucher/Herbarium	Origin
Panicoideae		
Paniceae		
Setariinae		
Panicum virgatum L.	Hodkinson 120/TCD	England, cultivated
Andropogoneae		
Saccharinae		
Saccharum officinarum L.	Hodkinson & Renvoize 104/KEW	England, cultivated
Miscanthus sinensis Anderss.	Hodkinson & Renvoize 5/KEW	England, cultivated

Abbreviations are as follows; KEW, Kew herbarium, England; KUFF, Herbarium of Faculty of Forestry, Kasetsart University, Bangkok, Thailand; TCD, Herbarium, School of Botany, Trinity College, Dublin, Ireland; THNHM, Thailand Natural History Museum, National Science Museum, Techno Polis, Pathum Thani, Thailand; AT, A. Teerawatananon; RP, R. Pattanavibool; SD, S. Dransfield; SS, S. Sungkaew

* California, United States of America

** Sydney Botanic Garden, cultivated

[1] genus established in 1994, in Thamnocalaminae in Ohrnberger (1999), but *Thamnocalamus* in Arundinariinae in Clayton and Renvoize (1986)

[2] genus under *Sinarundinaria*, Arundinariinae in Clayton and Renvoize (1986)

[3] genus established in 1992, in Arundinariinae in Ohrnberger (1999)

[4] genus under *Arundinaria*, Arundinariinae in Clayton and Renvoize (1986)

[5] Bambusetum, Rimba Ilmu Botanic Garden, University of Malaya, Kuala Lumpur, Malaysia; specimen collected by K.M. Wong

[6] genus under *Bambusa* (Clayton & Renvoize, 1986)

[7] genus under *Dendrocalamus* in Clayton and Renvoize (1986), but under *Bambusa* in Ohrnberger (1999)

[8] new genus and a new species (Sungkaew *et al.*, *accepted*)

[9] genus established in 1996, in Hikeliinae in Ohrnberger (1999), but *Hickelia* in Bambusinae in Clayton and Renvoize (1986)

[10] genus established in 2000, no subtribe applied

[11] genus establisted in 1990, not present in Clayton and Renvoize (1986), but in Racemobambosinae in Ohrnberger (1999), but *Racemobambos* placed in Bambusinae by Clayton and Renvoize (1986)

[12] genus under *Schizostachyum* in Clayton and Renvoize (1986)

[13] genus established in 2005, no subtribe applied

[14] genus established in 1997, no subtribe applied

Table 2.3 Gene regions and primers used for this study

Target region	Primer base sequence (F-forward; R-reverse)	Reference
trnL-F	F: c: 5'-CGAAATCGGTAGACGCTACG-3' R: f: 5'-ATTGAACTGGTGACACGAG-3'	Taberlet *et al.* (1991)
atpB-rbcL	F: 2R: 5'-GAAGTAGTAGGATTGATTCTC-3' R: 1R: 5'-GTTTCTGTTTGTGGTGACAT-3'	Samuel *et al.* (1997)
rps16	F: 16F: 5'-GTGGTAGAAAGCAACGTGCGACTT-3' R: 2R: 5'-TCGGGATCGAACATCAATTGCAAC-3'	Oxelmann *et al.* (1997)
matK	F: 19F: 5'-CGTTCTGACCATATTGCA-3'	Molvray *et al.*(2000)
	R: 9R: 5'-GCTAGAACTTTAGCTCGTA-3'	Hilu *et al.* (1999)
	F: 390F: 5'-CGATCTATTCATTCAATA-3'	Cuènoud *et al.* (2002)
	R: *trnK*2R: 5'-AACTAGTCGGATGGAGTAG-3'	Johnson & Soltis (1994)

2.3 Results

2.3.1 Phylogenetic analysis of the woody bamboos (Bambuseae; Bambusoideae; Poaceae) inferred from *trnL-F* sequences

The aligned *trnL-F* matrix was 1,369 bp long. 110 characters were excluded and of the remaining 1,259 characters, 810 were constant, 198 were variable but parsimony-uninformative and 251 were parsimony informative.

The tree search using maximum parsimony found 18 equally most parsimonious trees (Figures 2.1 and 2.2), each consisting of 741 steps. Consistency and retention indices (CI and RI) were 0.68 and 0.75 respectively. Bootstrap (BS) percentages (≥50%BS) are described as low (50–74%), moderate (75–84%), and high (85–100%). One of the equally most parsimonious trees is shown as a phylogram in Figure 2.1 and as a cladogram, with bootstrap values and strict consensus information, in Figure 2.2.

The BEP clade including Bambusoideae s.s., Ehrhartoideae (=Oryzoideae), and Pooideae, is highly supported (99%BS). However, there is only low bootstrap support for the monophyly of herbaceous bamboos (62%BS) and there is no support either from strict consensus or from bootstrap analysis for the monophyly of the woody bamboos. The sister group relationship of Olyreae to Bambuseae is also not supported. Pooideae (81%BS) are sister to Bambusoideae s.s. (78%BS) but with only low support (63%BS).

Within Bambuseae it is possible to geographically divide woody bamboos into three lineages, temperate (T, Figure 2.2), palaeotropical (P) and neotropical (N). The temperate woody bamboo clade is highly supported (100%BS) while the palaeotropical woody bamboo plus *Mullerochloa moreheadiana* and *Neololeba atra* receives only low support (62%BS). The neotropical woody bamboos are not supported as monophyletic but they do outly (as a grade) the palaeotropical group. The relationships within neotropical woody bamboos are unclear as there is little support for any subgroup divisions except a grouping of *Arthrostylidium* and *Rhipidocladum* (87%BS), Figure 2.2.

Melocanninae (Schizostachydinae) and Arthrostylidiinae, according to Ohrnberger (1999), are the only formally recognized subtribes to be supported by this analysis (Figure 2.2). The other subtribes are either not resolved or not supported by bootstrapping.

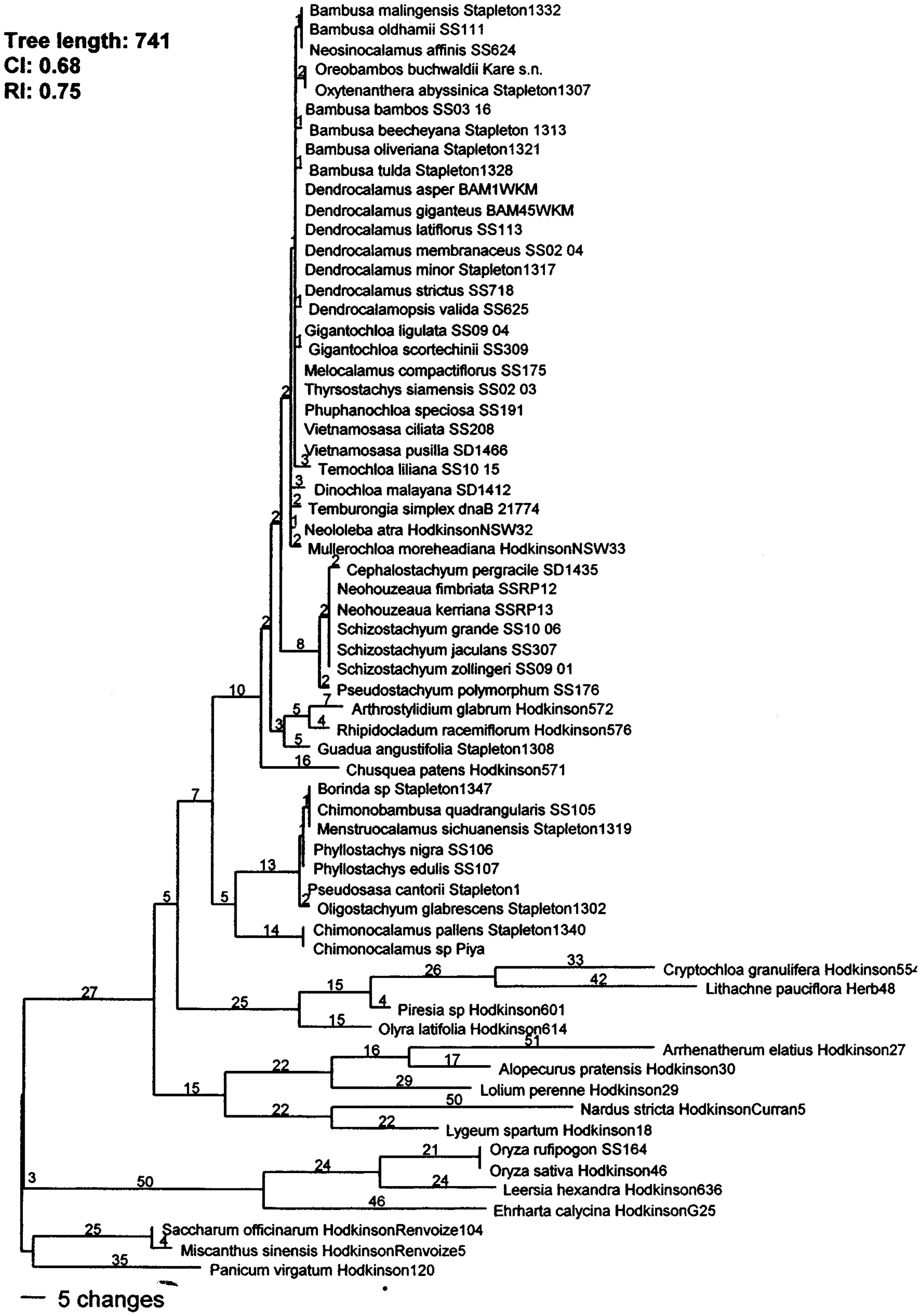


Figure 2.1 One of 18 equally most parsimonious trees shown as a phylogram obtained from comparative sequence analysis of the *trnL-F* sequence data. Values above branches represent the number of steps supporting each branch.

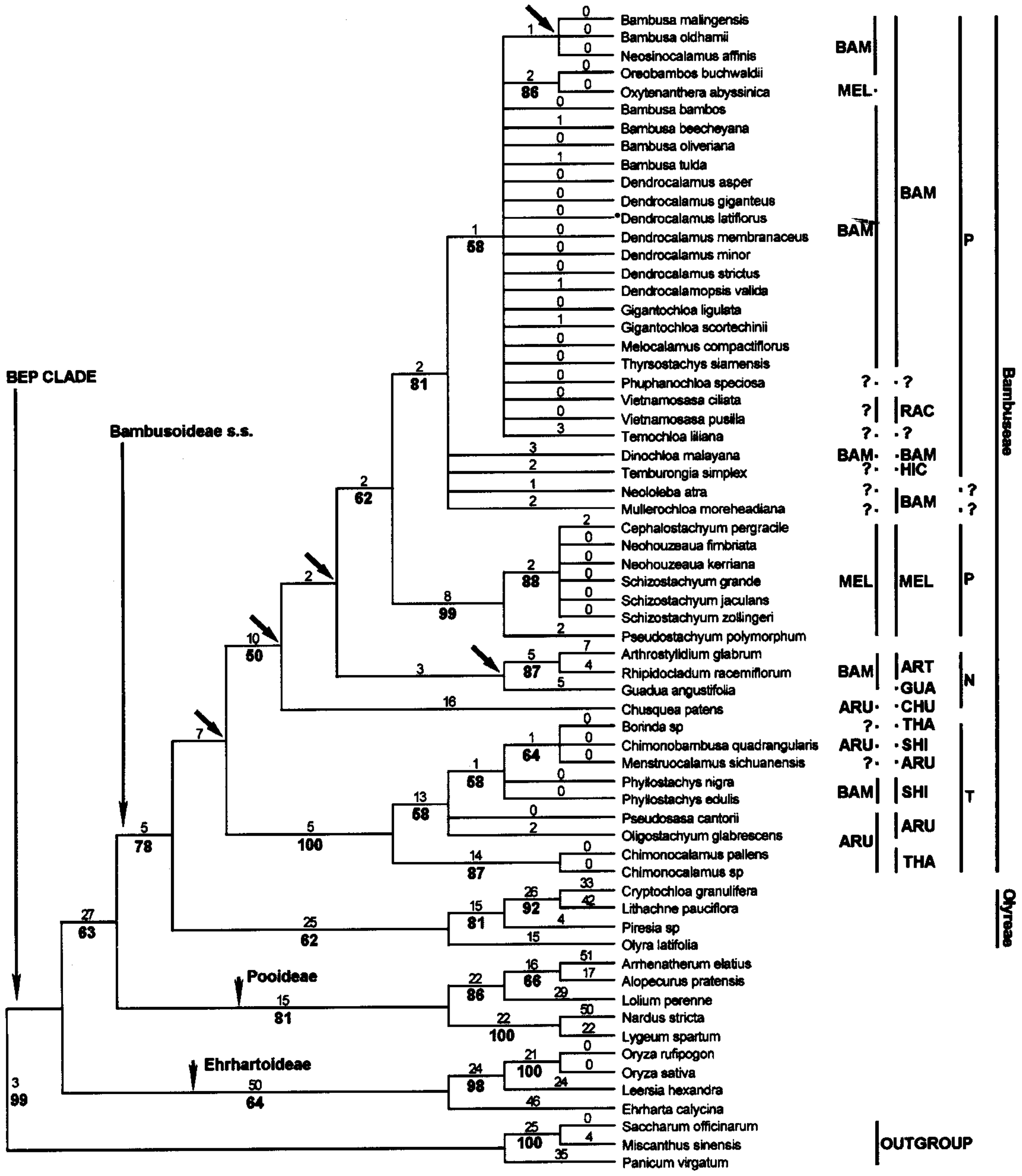


Figure 2.2 Same tree as Figure 2.1 (*trnL-F*), shown as a cladogram. Values above branches represent the number of steps supporting each branch. Values below branches represent the percentages of bootstrap supporting each branch. Arrow heads represent nodes not supported by the strict consensus. The BEP clade, the subfamial classification, and the tribal classification (first column on far right) shown are according to GPWG (2001). The geographical classification of woody bamboos (second column from far right) is shown according to Clark *et al.* (1995) and Ni Chonghaile (2002). The two subtribal classifications shown (blue and red columns from far right) are according to Ohrnberger (1999) and Clayton and Renvoize (1986) respectively. N=Neotropical woody bamboos; P=Palaeotropical woody bamboos; T=Temperate woody bamboos; ART=Arthrostylidiinae; ARU=Arundinariinae; BAM=Bambusinae; CHU=Chusqueinae; GUA=Guaduinae; HIC=Hickeliinae; MEL=Melocanninae; RAC=Racemobambosinae; SHI=Shibataeinae; THA=Thamnocalaminae.

2.3.2 Phylogenetic analysis of the woody bamboos (Bambuseae; Bambusoideae; Poaceae) inferred from *atpB-rbcL* sequences

The aligned *atpB-rbcL* matrix was 948 bp long. 32 characters were excluded and of the remaining 916 characters, 615 were constant, 142 were variable but parsimony-uninformative and 159 were parsimony informative.

The tree search using maximum parsimony found 39,485 equally most parsimonious trees (Figures 2.3 and 2.4), each consisting of 429 steps. CI and RI were 0.79 and 0.83 respectively. One of the equally most parsimonious trees is shown as a phylogram in Figure 2.3 and as a cladogram, with bootstrap values and strict consensus information, in Figure 2.4.

The BEP clade is highly supported (100%BS), Figure 2.4. Bambusoideae s.s. receives low support (65%BS). Olyreae are highly supported (99%BS) as monophyletic but there is no support either from strict consensus or from the bootstrap analysis for the monophyly of Bambuseae. Pooideae (100%BS) are sister to Bambusoideae s.s. (65%BS) with high bootstrap support (95%BS). There is also no support for the sister group status of Olyreae to Bambuseae. The geographical classification for woody bamboos into temperate, neotropical and palaeotropical groups cannot be applied to this analysis due to uncertain relationships especially between neotropical and palaeotropical woody bamboos, and also between temperate and palaeotropical woody bamboos, Figure 2.4. None of subtribes of Clayton and Renvoize (1986) or Ohrnberger (1999) can be regarded as monophyletic from evidence in this study.

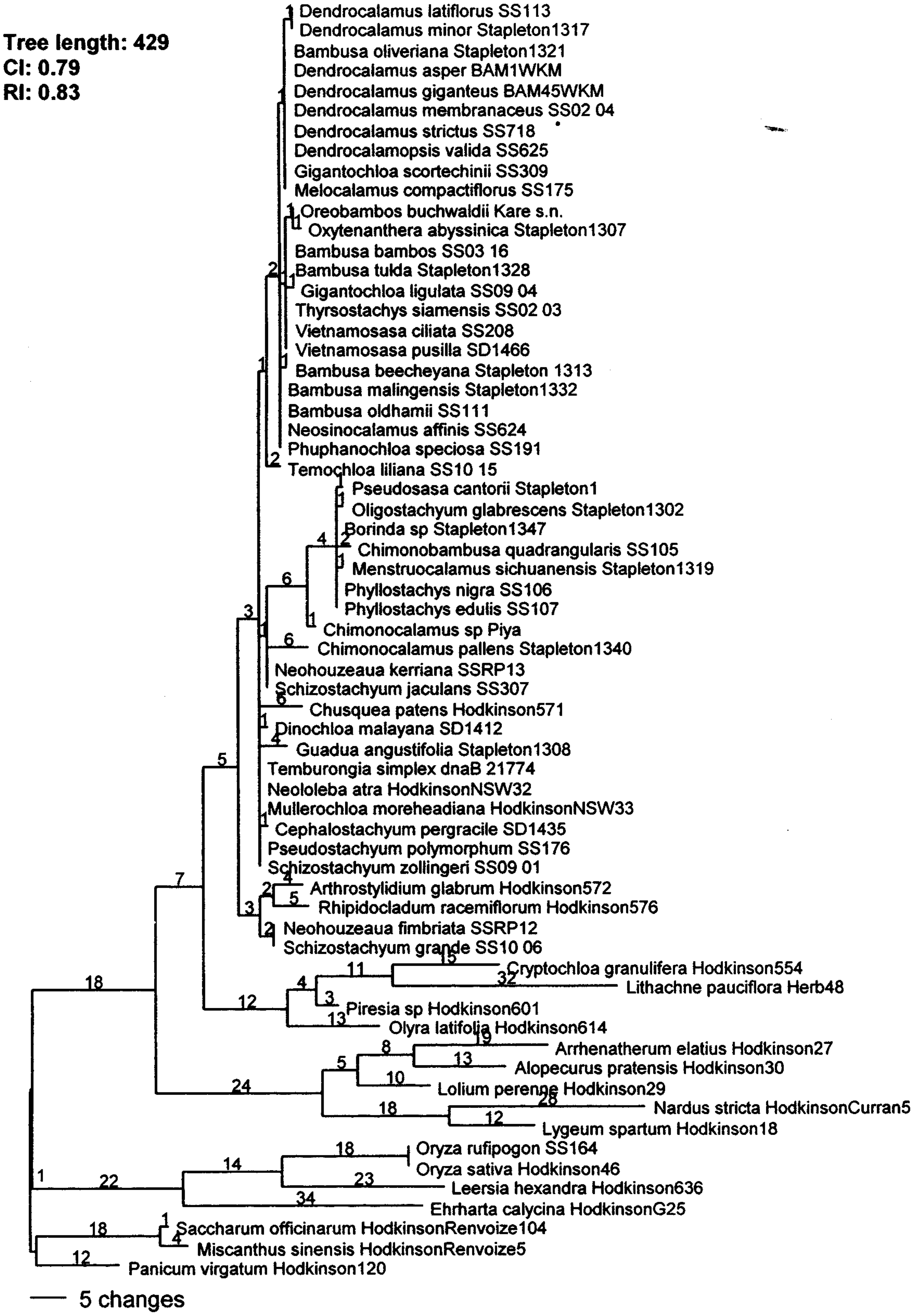


Figure 2.3 One of 39,485 equally most parsimonious trees shown as a phylogram obtained from comparative sequence analysis of the *atpB-rbcL* sequence data. Values above branches represent the number of steps supporting each branch.

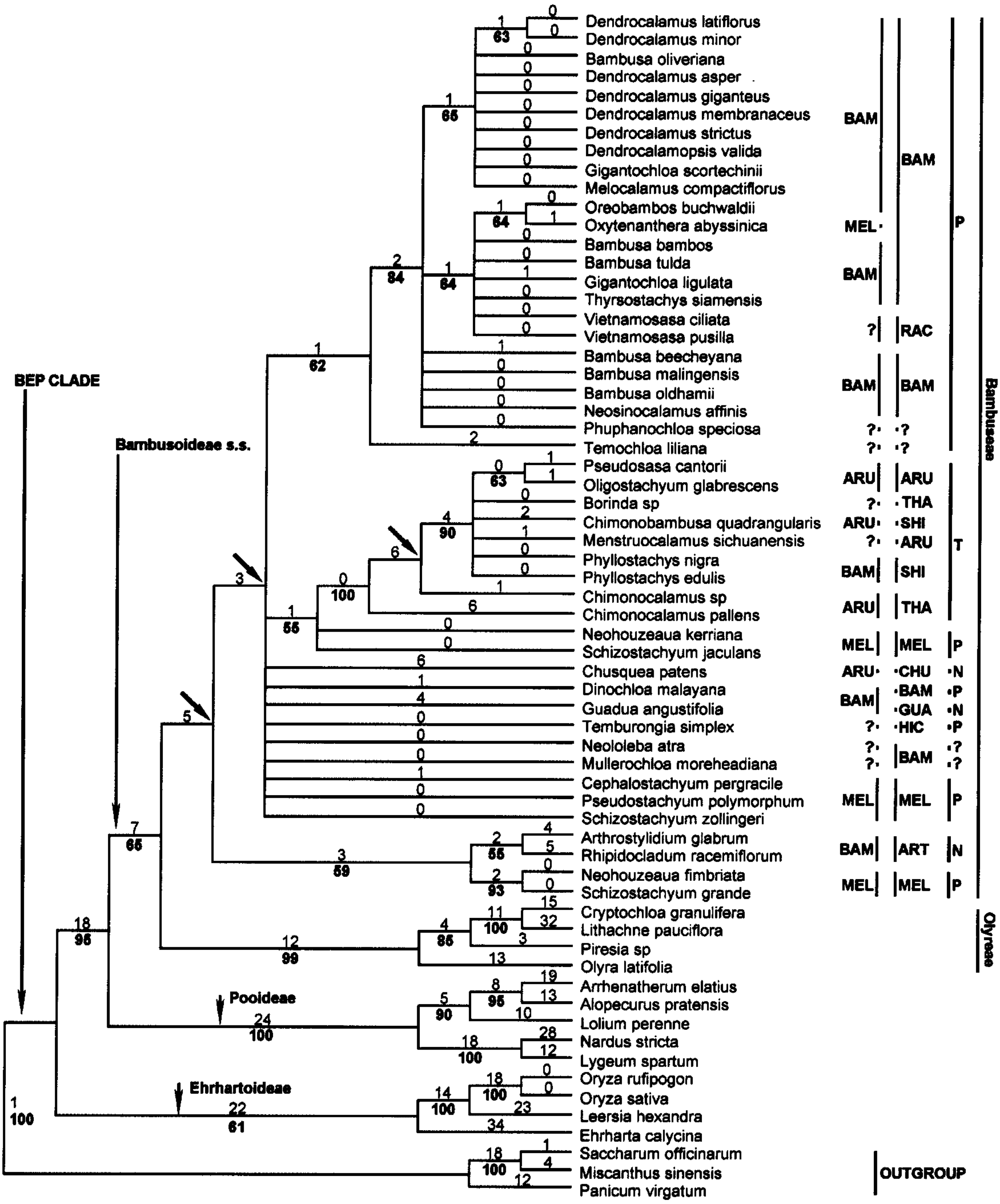


Figure 2.4 Same tree as Figure 2.3 (*atpB-rbcL*), shown as a cladogram. Values above branches represent the number of steps supporting each branch. Values below branches represent the percentages of bootstrap supporting each branch. Arrow heads represent nodes not supported by strict consensus. The BEP clade, the subfamial classification, and the tribal classification (first column on far right) are according to the GPWG (2001). The geographical classification of woody bamboos (second column from far right) is shown according to Clark *et al.* (1995) and Ni Chonghaile (2002). The two subtribal classifications shown (blue and red columns from far right) are according to Ohrnberger (1999) and Clayton and Renvoize (1986) respectively. N=Neotropical woody bamboos; P=Palaeotropical woody bamboos; T=Temperate woody bamboos; ART=Arthrostylidiinae; ARU=Arundinariinae; BAM=Bambusinae; CHU=Chusqueinae; GUA=Guaduinae; HIC=Hickeliinae; MEL=Melocanninae; RAC=Racemobambosinae; SHI=Shibataeinae; THA=Thamnocalaminae.

2.3.3 Phylogenetic analysis of the woody bamboos (Bambuseae; Bambusoideae; Poaceae) inferred from *rps16* sequences

The aligned *rps16* matrix was 1,021 bp long. 71 characters were excluded and of the remaining 950 characters, 652 were constant, 126 were variable but parsimony-uninformative, and 172 were parsimony informative.

The tree search using maximum parsimony found 12 equally most parsimonious trees (Figures 2.5 and 2.6), each consisting of 515 steps. CI and RI were 0.71 and 0.79 respectively. One of the equally most parsimonious trees is shown as a phylogram in Figure 2.5 and as a cladogram, with bootstrap values and strict consensus information, in Figure 2.6.

The relationships of subfamilies within the BEP clade were not resolved. There is no support from strict consensus for the grouping of the pooids with the erhartoids (Figure 2.6) and they are part of a polytomy (a trichotomy of three subfamilies) in the bootstrap analysis. Therefore, it is not evident which subfamily is sister to Bambusoideae s.s.. However, these three branches, Ehrhartoideae, Pooideae, and Bambusoideae s.s. are grouping together with a high bootstrap support (100%BS), Figure 2.6. Bambusoideae s.s. are highly supported (92%BS) as monophyletic, Figure 2.6.
Unlike the results from *trnL-F* and *atpB-rbcL*, the results obtained from *rps16* sequence analysis show that the herbaceous bamboo tribe Olyreae (98%BS) is not sister to the whole of the Bambuseae, but it is grouped with the tropical woody bamboos with low bootstrap support (70%BS), Figure 2.6. However, it is possible to divide Bambuseae into the three geographical lineages, temperate, palaeotropical and neotropical. The temperate woody bamboo clade is highly supported (100%BS) but there is no bootstrap support for the palaeotropical woody bamboos plus *Mullerochloa moreheadiana* and *Neololeba atra* (but this clade is present in the strict consensus). The relationships within neotropical woody bamboos are unclear because *Chusquea* is in a polytomy with the remaining neotropical woody bamboos and the palaeotropical bamboos, Figure 2.6.

There are only two subtribes, Melocanninae (Schizostachydinae) and Arthrostylidiinae, according to Ohrnberger (1999), that can be regarded as monophyletic in the strict consensus results. However, there is no bootstrap support for Melocanninae. Only two Arthrostylidiinae genera have been included but they are grouped with 96%BS. None of the remaining subtribes can be defined as a monophyletic, see Figure 2.6 and Table 2.1.

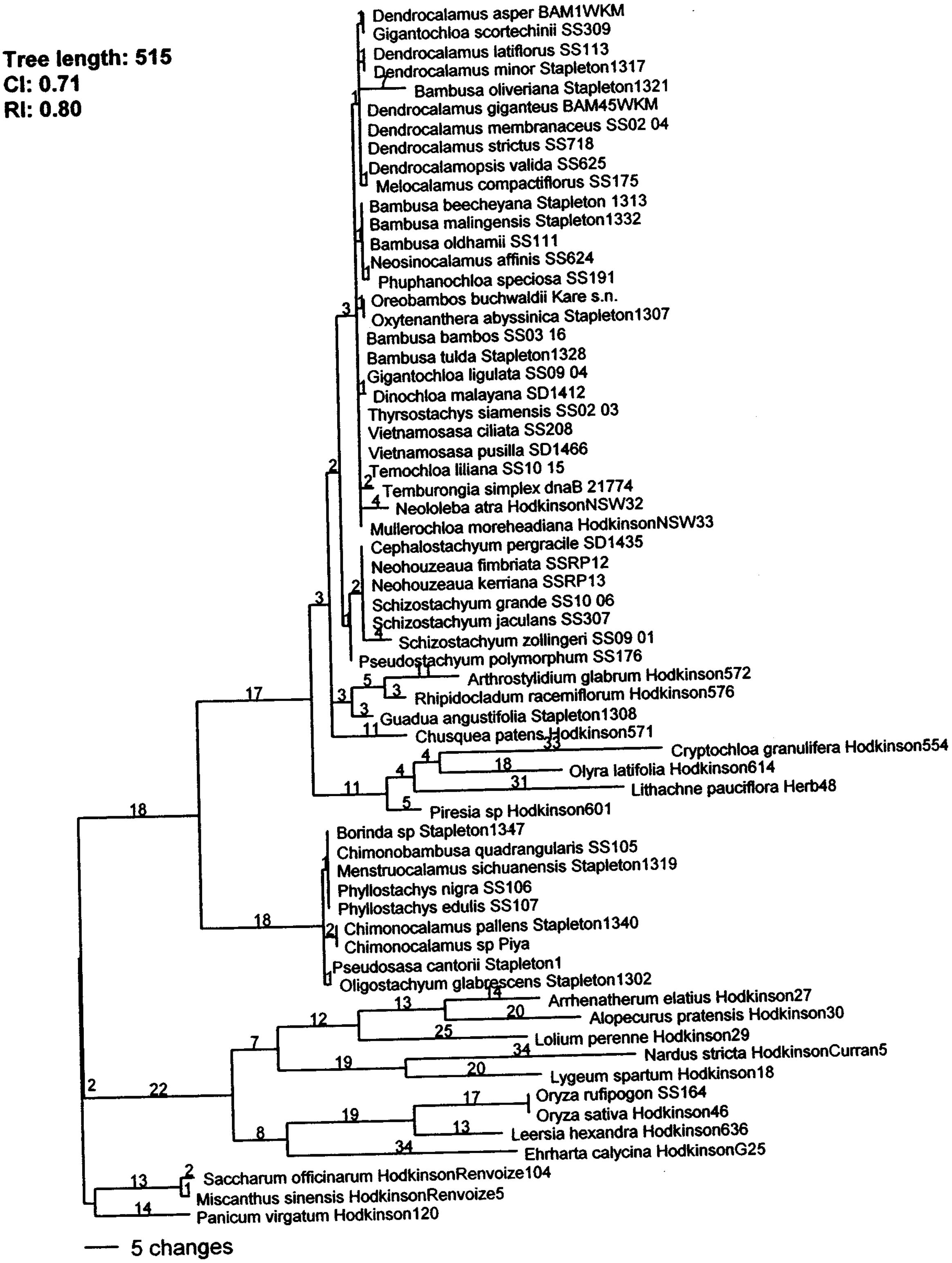


Figure 2.5 One of 12 equally most parsimonious trees shown as a phylogram obtained from comparative sequence analysis of the *rps16* sequence data. Values above branches represent the number of steps supporting each branch.

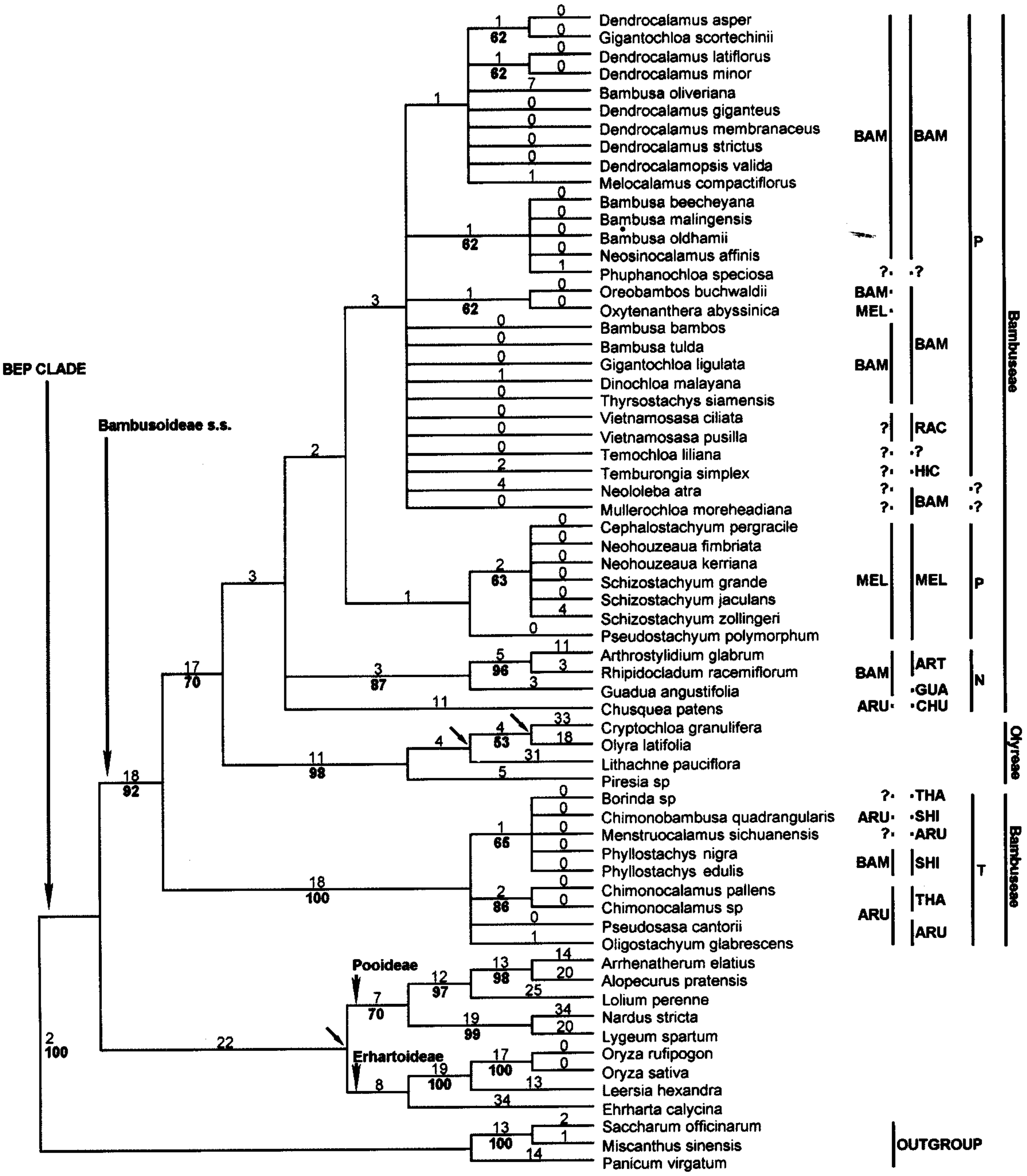


Figure 2.6 Same tree as Figure 2.5 (*rps16*), shown as a cladogram. Values above branches represent the number of steps supporting each branch. Values below branches represent the percentages of bootstrap supporting each branch. Arrow heads represent nodes not supported by strict consensus. The BEP clade, the subfamial classification, and the tribal classification (first column on far right) are according to GPWG (2001). The geographical classification of woody bamboos (second column from far right) is shown according to Clark *et al.* (1995) and Ni Chonghaile (2002). The two subtribal classifications shown (blue and red columns from far right) are according to Ohrnberger (1999) and Clayton and Renvoize (1986) respectively. N=Neotropical woody bamboos; P=Palaeotropical woody bamboos; T=Temperate woody bamboos; ART=Arthrostylidiinae; ARU=Arundinariinae; BAM=Bambusinae; CHU=Chusqueinae; GUA=Guaduinae; HIC=Hickeliinae; MEL=Melocanninae; RAC=Racemobambosinae; SHI=Shibataeinae; THA=Thamnocalaminae.

2.3.4 Phylogenetic analysis of the woody bamboos (Bambuseae; Bambusoideae; Poaceae) inferred from *matK* sequences

The aligned *matK* matrix was 1,984 bp long. 126 characters were excluded and of the remaining 1,858 characters, 1,249 were constant, 272 were variable but parsimony-uninformative, and 337 were parsimony informative.

The tree search using maximum parsimony retrieved a single most parsimonious tree (Figures 2.7 and 2.8), of 997 steps. CI and RI were 0.72 and 0.80 respectively. The most parsimonious tree is shown as a phylogram in Figure 2.7 and as a cladogram, with bootstrap values and strict consensus information, in Figure 2.8.

The BEP clade is highly supported (100%BS), Figure 2.8. Bambusoideae s.s. is highly supported (98%BS) as monophyletic consisting of Olyreae (100%BS) as sister to Bambuseae, Figure 2.8. However, Bambuseae only have low bootstrap support (59%BS). Pooideae (98%BS) is sister to Bambusoideae s.s. with high bootstrap support (85%BS). Geographical classification of woody bamboos into temperate, palaeotropical and neotropical groups can be generally applied. There is 100%BS for the temperate Bambuseae, but no bootstrap support for both the neotropical and palaeotropical Bambuseae plus *Mullerochloa moreheadiana* and *Neololeba atra*, Figure 2.8.

Similar to the results obtained from *trnL-F* and *rps16*, there are only two subtribes, Melocanninae (Schizostachydinae) and Arthrostylidiinae, according to Ohrnberger (1999), that can be regarded as monophyletic. There is no bootstrap support for Melocanninae and 85%BS for Arthrostylidiinae, Figure 2.8. None of sub-tribal classification groups recognized by Clayton and Renvoize (1986) were supported as monophyletic, Figure 2.8.

The results from *matK* analysis seem to give more resolution at a generic level than those from the other single gene analyses.

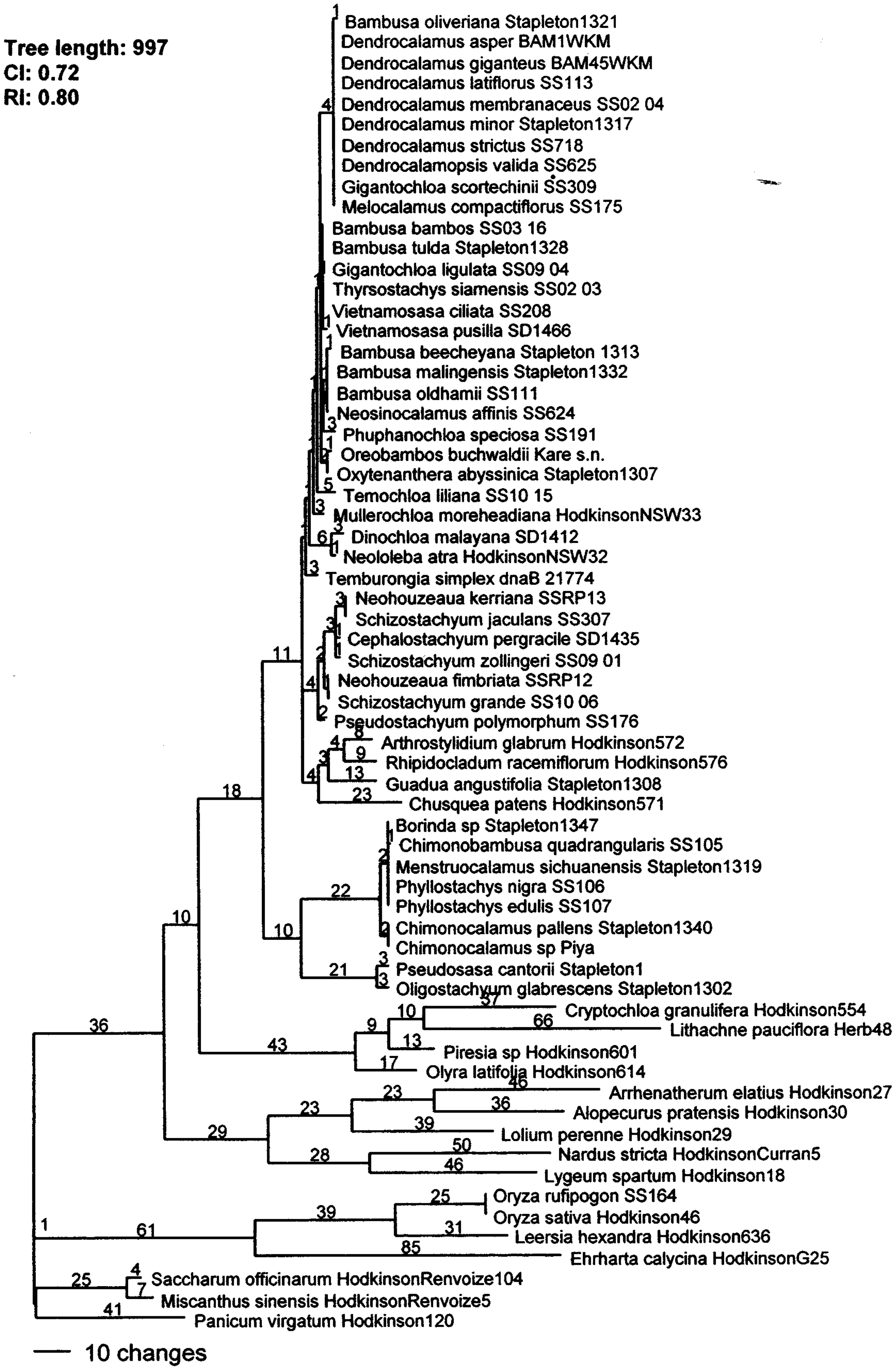


Figure 2.7 A single most parsimonious trees shown as a phylogram obtained from comparative sequence analysis of the *matK* sequence data. Values above branches represent the number of steps supporting each branch.

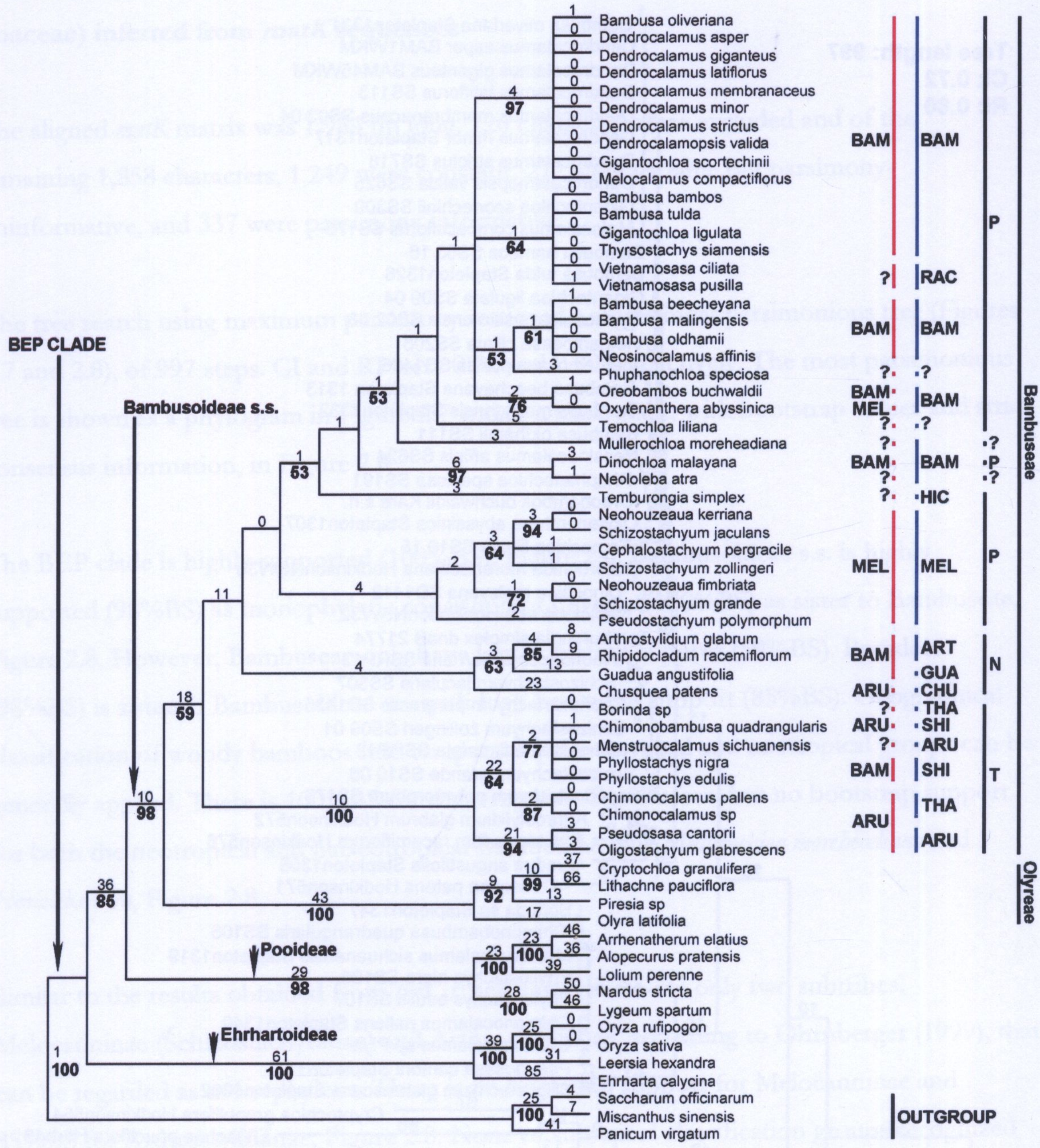


Figure 2.8 Same tree as Figure 2.7 (*matK*), shown as a cladogram. Values above branches represent the number of steps supporting each branch. Values below branches represent the percentages of bootstrap supporting each branch. The BEP clade, the subfamial classification, and the tribal classification (first column on far right) are according to GPWG (2001). The geographical classification of woody bamboos (second column from far right) is shown according to Clark *et al.* (1995) and Ni Chonghaile (2002). The two subtribal classifications shown (blue and red columns from far right) are according to Ohrnberger (1999) and Clayton and Renvoize (1986) respectively. N=Neotropical woody bamboos; P=Palaeotropical woody bamboos; T=Temperate woody bamboos; ART=Arthrostylidiinae; ARU=Arundinariinae; BAM=Bambusinae; CHU=Chusqueinae; GUA=Guaduinae; HIC=Hickeliinae; MEL=Melocanninae; RAC=Racemobambosinae; SHI=Shibataeinae; THA=Thamnocalaminae.

2.3.5 A multi-gene region phylogenetic analysis of the woody bamboos (Bambuseae; Bambusoideae; Poaceae)

The matrix used for the multi-gene analysis was obtained from *trnL-F, atpB-rbcL, rps16*, and *matK* sequences. The aligned matrix was 5,322 bp long. 339 characters were excluded and of the remaining 4,983 characters, 3,326 were constant, 738 were variable but parsimony-uninformative, and 919 were parsimony informative. The tree search using maximum parsimony found three equally most parsimonious trees (Figures 2.9 and 3.0), of 2,688 steps. CI and RI were 0.72 and 0.79 respectively. The most parsimonious tree is shown as a phylogram in Figure 2.9 and as a cladogram, with bootstrap values and strict consensus information, in Figure 2.10.

The BEP clade is highly supported (100%BS) as monophyletic Figure 2.10. The monophyly of each subfamily is also highly supported (100%BS). Pooideae (100%BS) are sister to Bambusoideae s.s. (100%BS) with 99%BS while Ehrhartoideae (100%BS) is sister to the lineage consisting of Pooideae and Bambusoideae s.s., (100%BS).

The combined analysis shows that Olyreae (a monophyletic group with 100%BS) are sister only to the tropical Bambuseae and not to the whole of Bambuseae. This suggests that Bambuseae are not a monophyletic group, Figure 2.10. However, there is only 57%BS for the sister group status of Olyreae to the tropical Bambuseae. Division of the Bambuseae into temperate, neotropical and palaeotropical groups is also evident. The temperate Bambuseae are highly supported (100%BS) as monophyletic and are sister to a group consisting of Olyreae and the tropical woody bamboos. The tropical woody bamboos are also highly supported (91%BS) as monophyletic, consisting of neotropical and the palaeotropical Bambuseae plus *Mullerochloa moreheadiana* from Australia and another species *Neololeba atra* from South Mindanao of the Philippines extending to Australia. The neotropical woody bamboos are moderately supported (77%BS). Arthrostylidiinae, represented by *Arthrostylidium* and *Rhipidocladum*, were monophyletic with high boostrap support (100%BS). *Guadua* (Guaduinae) is sister to Arthrostylidiinae with high bootstrap support (93%BS). *Chusquea* (Chusqueinae) is sister to the group consisting of Arthrostylidiinae and Guaduinae with moderate support (77%BS). The palaeotropical Bambuseae together with *Mullerochloa moreheadiana* and *Neololeba atra* are monophyletic with high support of 91%BS, Figure 2.10.

None of the subtribes of temperate woody bamboos, whether according to Clayton and Renvoize (1986) or Ohrnberger (1999), can be regarded as monophyletic, Figure 2.10, Table 2.1.

The combined analysis has shown only one subtribe, Melocanninae (Schizostachydinae) of Ohrnberger (1999), as highly supported (100%BS) as monophyletic. Furthermore, Melocanninae are sister to the palaeotropical Bambuseae together with *Mullerochloa moreheadiana* and *Neololeba atra* (a monophyletic group with 99%BS) with high bootstrap support (91%BS). Figure 2.10, Table 2.1.

Temburongia represents subtribe Hickeliinae according to Ohrnberger (1999) and it is sister to the remaining palaeotropical woody bamboos plus *Mullerochloa moreheadiana* and *Neololeba atra* (a group with 61%BS), with high bootstrap support (99%BS).

The subtribe Bambusinae according to Ohrnberger (1999) is not monophyletic because the genus *Vietnamosasa* (Racemobambosinae of Ohrnberger, 1999), is embedded within Bambusinae. Subtribe Bambusinae according to Clayton and Renvoize (1986) is also not monophyletic, Figure 2.10, Table 2.1.

Main groups supporting by strict consensus

Nine main groups found in the strict consensus (or single tree in the case of *matK*) obtained from individual and combined analyses of the five gene regions are shown in Table 2.4. The single most parsimonious tree obtained from *matK* supported the existance of all the defined groups while the strict consensus tree of *atpB-rbcL* supported the least number of defined groups.

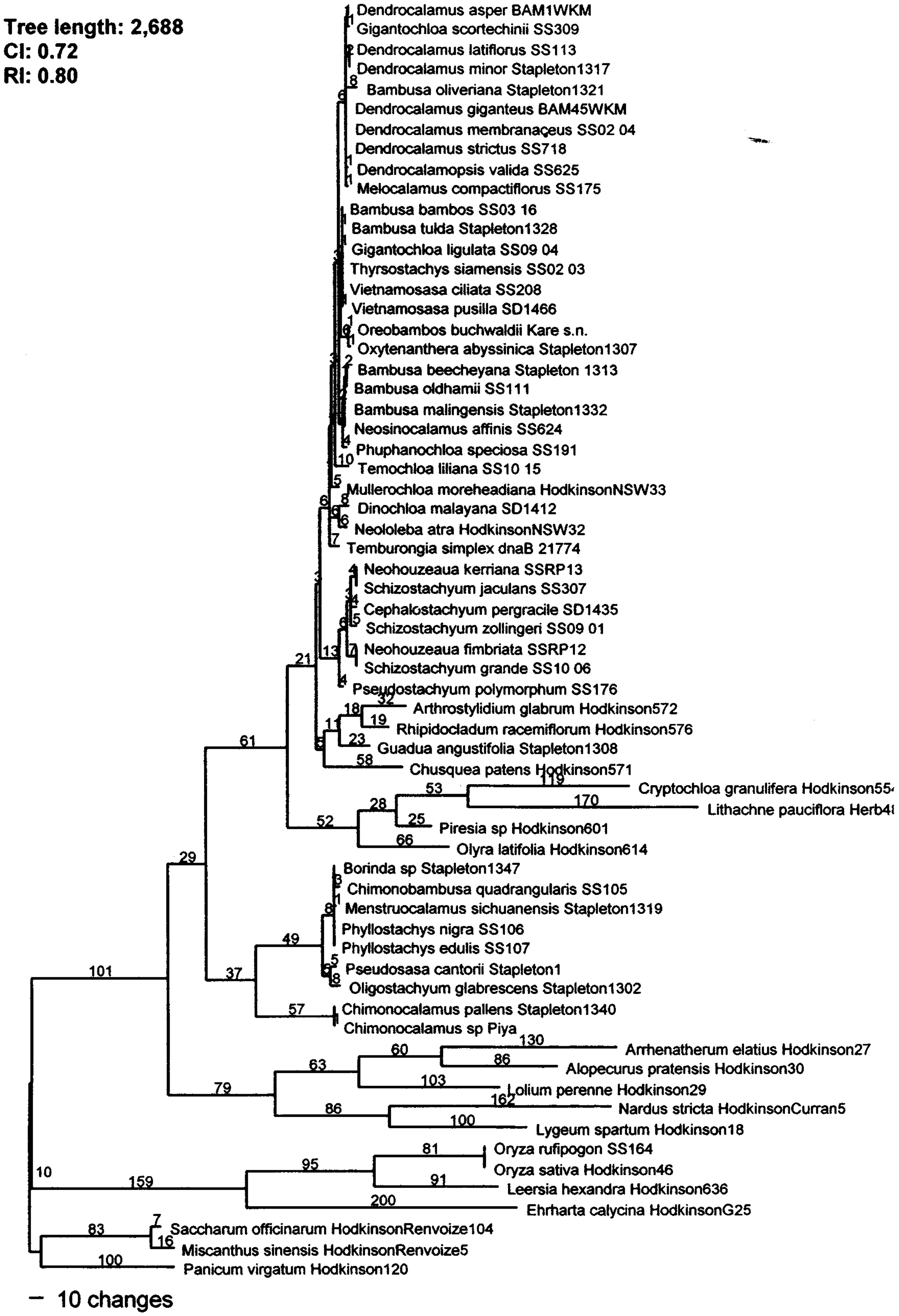


Figure 2.9 One of three equally most parsimonious trees shown as a phylogram obtained from comparative sequence analysis of combined *trnL-F, atpB-rbcL, rps16* and *matK* sequence data. Values above branches represent the number of steps supporting each branch.

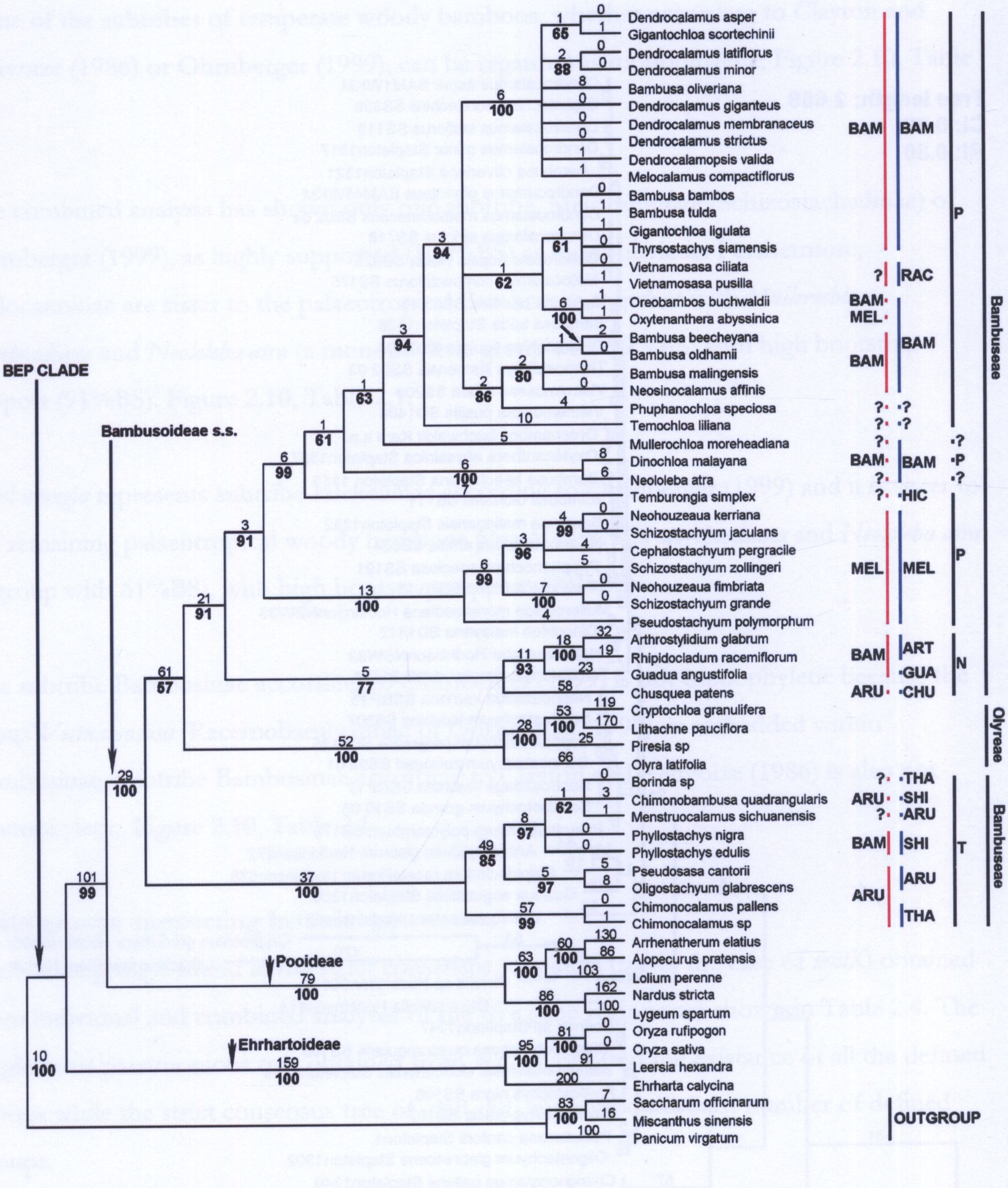


Figure 2.10 Same tree as Figure 2.9 (combined *trnL-F*, *atpB-rbcL*, *rps16* and *matK)*, shown as a cladogram. Values above branches represent the number of steps supporting each branch. Values below branches represent the percentages of bootstrap supporting each branch. The BEP clade, the subfamial classification, and the tribal classification (first column on far right) are according to GPWG (2001). The geographical classification of woody bamboos (second column from far right) shown according to Clark *et al.* (1995) and Ni Chonghaile (2002). The two subtribal classifications shown (blue and red columns from far right) are according to Ohrnberger (1999) and Clayton and Renvoize (1986) respectively. N=Neotropical woody bamboos; P=Palaeotropical woody bamboos; T=Temperate woody bamboos; ART=Arthrostylidiinae; ARU=Arundinariinae; BAM=Bambusinae; CHU=Chusqueinae; GUA=Guaduinae; HIC=Hickeliinae; MEL=Melocanninae; RAC=Racemobambosinae; SHI=Shibataeinae; THA=Thamnocalaminae.

Table 2.4 Comparison of the main groups obtained from individual and combined analyses of five gene regions. Groups present in the strict consensus tree (or the single most parsimonious tree in the case of *matK*) of the five analyses are indicated with ticks.

Groups/Analyses	***trnL-F***	***atpB-rbcL***	***rps16***	***matK***	**combined**
BEB clade	✓	✓	✓	✓	✓
Ehrhartoideae	✓	✓	✓	✓	✓
Pooideae	✓	✓	✓	✓	✓
Bambusoideae s.s.	✓	✓	✓	✓	✓
Olyreae	✓	✓	✓	✓	✓
Bambuseae	X	X	X	✓	X
Temperate Bambuseae	✓	✓	✓	✓	✓
Neotropical Bambuseae	X	X	X	✓	✓
Palaeotropical Bambuseae	✓	X	✓	✓	✓

2.4 Discussion

BEP clade

The BEP clade, including Bambusoideae s.s., Ehrhartoideae (=Oryzoideae), and Pooideae, as defined by the GPWG (2001) is monophyletic. This clade was found in the strict consensus trees from all the single gene analyses and the combined analysis of the five genes (*trnL-F* (*trnL* intron, *trnL-F* intergenic spacer), *atpB-rbcL*, *rps16*, and *matK*) have given high bootstrap support values for the monophyly of BEP, Table 2.5. This pattern of relationship is congruent with previous studies (Clark *et al.*, 1995, as BOP clade; GPWG, 2000; Zhang, 2000, Salamin *et al.*, 2002; Bouchenak-Khelladi *et al.*, *in press*).

Bambusoideae s.s.

The subfamily Bambusoideae s.s. as defined by the GPWG (2001) is clearly a monophyletic group, consisting of herbaceous bamboos (tribe Olyreae) and woody bamboos (tribe Bambuseae). All single gene analyses and the combined analysis of the five genes have given high support for its monophyly, except the individual analysis of *atpB-rbcL* and *trnL-F* (low and moderate support, 65%BS and 78%BS, respectively), Table 2.5. This confirms the monophyly of Bambusoideae s.s. as found in previous analyses (*matK* sequence data, Hilu *et al.*, 1999; *rpl16* sequence data, Zhang, 2000; one morphological, four plastid DNA sequence regions, and three nuclear DNA sequence regions, GPWG, 2000, 2001; supertree analysis , Salamin *et al.* 2002; combined *rbcL*, *trnL-F*, and *matK* sequence data, Bouchenak-Khelladi *et al.*, *in press*). The secondary gain of a pseudopetiole and the secondary loss of the lamina of the first seedling leaf were pointed by GPWG (2001) as synapomorphies for Bambusoideae s.s.. The presence of strongly asymmetrically invaginated arm cells, as found by Zhang and Clark (2000), may also be a potential synapomorphy for this clade. The very wide range of natural distritribution of Bambusoideae s.s. is approximately from 46°N to 47°S latitude and from sea level to as much as 4,300 m in equatorial highlands (Judziewicz *et al.*, 1999).

Olyreae and Bambuseae

One tribe of Bambusoideae s.s., Olyreae, is supported as monophyletic. All analyses produced high bootstrap support for Olyreae, except *trnL-F* that provided only low support, Table 2.5. However, most analyses (*trnL-F*, *atpB-rbcL*, *rps16*, and the combined analysis) have shown that the other tribe, Bambuseae, is non-monophyletic (it is paraphyletic). Only a single gene analysis, *matK*, has resolved it as monophyletic, but with only low bootstrap support, Table 2.5. The relationships of taxa classified as Olyreae and Bambuseae are highly important for the classification of the bambusoids. The non-monophyly of Bambuseae has also been found in a combined sequence (*rbcL*, *trnL-F*, and *matK*) analysis of taxa spanning the whole of the grass family (Bouchenak-Khelladi *et al.*, *in press*). This combined study and that of Bouchenak-Khelladi *et al.* (*in press*) show that Olyreae are monophyletic but are embedded in, or are sister to, the tropical Bambuseae. However, there is incongruence between single sequence analyses regarding its position within Bambusoideae (Table 2.5). Olyreae is sister to Bambuseae only in the *matK* analyses but is sister to tropical woody bamboos only in the *rps16* and combined gene analyses. It position is not resolved in the *trnL-F* and *atpB-rbcL* analyses. The combined analysis of Bouchenak-Khelladi *et al.* (*in press*) included 25 bambusoid taxa and found that Olyreae were sister to the tropical Bambuseae. It seems, therefore, that Bambuseae are not monophyletic and that Olyreae are the sister group of the tropical Bambuseae. Thus, there is a need for a revised taxonomic treatment within Bambusoideae s.s..

Taxonomic treatment of Bambusoideae s.s.

The intra-subfamily classification of Bambusoideae s.s. as defined by the GPWG (2001) requires revision because tribe Bambuseae is not monophyletic (based on evidence from this study and that of Bouchenak-Khelladi *et al.* (*in press*)). The Bambuseae can be divided into two clades based only on their molecular variation and geographical distribution. The tropical woody bamboos are sister to Olyreae (70%BS in *rps16*; 57%BS in the combined analysis, Figures 2.6 and 2.10 and Table 2.5) while the temperate woody bamboos are sister to the group consisting of Olyreae and tropical woody bamboos (92%BS in *rps16*; 100%BS in the combined analysis, Figures 2.6 and 2.10). To allow for a more natural classification, the Bambuseae needs to be re-defined and the temperate woody bamboos need to be accommodated by a new tribe. Fortunately, Ascherson and Graebner (1902) have validated the tribe Arundinarieae Nees ex Ascherson & Graebner. This tribe has a priority to be used to accommodate the temperate woody bamboos. A total of 14 genera (*Acidosasa, Ampelocalamus, Bashania, Fargesia, Ferrocalamus, Gelidocalamus, Indocalamus, Metasasa, Oligostachyum, Pleioblastus, Pseudosasa, Sasa, Thamnocalamus,* and *Yushania*) of Chinese bamboos were listed to be placed in this tribe by Li (1997), two of which, *Oligostachyum* and *Pseudosasa,* were also included in this study, Table 2.2. The type genus of the tribe Arundinarieae is *Arundinaria* Michaux. This study did not include *Arundinaria* in the analyses. However, each single gene and combined analyses (two chloroplast DNA genes, *trnL-F* and *rpl16* and a nuclear DNA gene, ITS (Ni Chonghaile, 2002); combined chloroplast; *rbcL*, *trnL-F*, and *matK* sequence data (Bouchenak-Khelladi *et al.*, *in press*), which included *Arundinaria* in their analyses have shown that this genus grouped closely together with other temperate genera. Thus, there is good evidence to use Arundinarieae to accommodate the temperate woody bamboos. The following classification is proposed to re-define the three tribes of Bambusoideae s.s. (see also Figure 2.11 and Table 2.1).

1. **Arundinarieae** Nees ex Ascherson & Graebner, Syn. Mitteleurop. Fl. 2(1): 770. 1902.

TYPE: *Arundinaria* Michaux

Included subtribes:

Tribe Arundinarieae includes three subtribes of temperate woody bamboos (Figure 2.12) as follows;

1. Arundinariinae Bentham
   TYPE: *Arundinaria* Michaux
2. Shibataeinae (Nakai) Soderstrom & Ellis
   TYPE: *Shibataea* Makino ex Nakai
3. Thamnocalaminae P.C. Keng
   TYPE: *Thamnocalamus* Munro

2. **Bambuseae** Kunth ex Dumort., Anal. Fam. Pl.: 63. 1829.

TYPE: *Bambusa* Schreber

Included subtribes:

Tribe Bambuseae s.s. includes seven subtribes of tropical woody bamboos (both Old World plus Australia (Figure 2.14) and New World (Figure 2.15)) as follows;

1. Bambusinae J.S. Presl
   TYPE: *Bambusa* Schreber
2. Hickeliinae A. Camus
   TYPE: *Hickelia* A. Camus
3. Melocanninae Bentham
   TYPE: *Melocanna* Trinius
4. Racemobambosinae Stapleton*
   TYPE: *Racemobambos* Holttum
5. Arthrostylidiinae Bews
   TYPE: *Arthrostylidium* Ruprecht
6. Chusqueinae Bews
   TYPE: *Chusquea* Kunth
7. Guaduinae Soderstrom & Ellis
   TYPE: *Guadua* Kunth

*probably better placed under Bambusinae.

3. Olyreae Kunth ex Spenner, Fl. Friburg. 1: 172. 1825.

TYPE: *Olyra* Linnaeus

Note: This tribe of herbaceous bamboos (Figure 2.13) also includes tribe Buergersiochloeae S.T. Blake, Blumea, Suppl. 3: 62. 1946 (TYPE: *Buergersiochloa* Pilger) and tribe Parianeae C.E. Hubbard, in Hutchinson, Fam. Flower. Pl. 2: 202, 219. 1934.

Geographical distribution of woody bamboos

The geographical division of woody bamboos into temperate, palaeotropical and neotropical groups, as found by Clark *et al.* (1995) and Ni Chonghaile (2002), could be generally applied to the results of this study. The temperate woody bamboos are highly supported (100%BS) as monophyletic by all analyses, Table 2.5. Palaeotropical woody bamboos plus *Mullerochloa moreheadiana* and *Neololeba atra* (palaeotropical and Austral woody bamboos), are not supported as monophyletic by the *atpB-rbcL* analysis. However, they were resolved as monophyletic by *rps16* and *matK* analyses (although without bootstrap support) and with low bootstrap support by the *trnL-F* analysis. In the combined analyses they are strongly supported as monophyletic, Table 2.5. This is the first time an Australian Bambuseae genus, *Mullerochloa,* and a genus extending to Australia, *Neololeba,* have been included in phylogenetic analyses. The topologies of having *Neololeba* sister to *Dinochloa* and *Mullerochloa,* are novel results. *Temburongia* is a monotypic genus (*T. simplex*) from Brunei (Dransfield & Wong, 1996), representing the subtribe Hickeliinae according to Ohrnberger (1999). It is the most outlying among outlying groups to the rest of palaeotropical and Austral woody bamboos. Thus it may be concluded that the three outlying groups, a lineage represented by *Temburongia,* a lineage represented by a group consisting of *Neololeba* and *Dinochloa,* and another lineage represented by *Mullerochloa,* are successively sister to the rest of the palaeotropical and Austral Bambuseae s.s..

There was no support for the monophyly of the neotropical woody bamboos in the *trnL-F*, *atpB-rbcL*, and *rps16* analyses. They were, however, resolved as monophyletic by the *matK* analyses (although without bootstrap support) and with moderate bootstrap support by the combined analysis (77%BS), Table 2.5. The previous phylogenetic analyses of this group (e.g. Clark *et al.*, 1995; Kelchner & Clark, 1997; Zhang, 2000; Ni Chonghaile, 2002; Bouchenak-Khelladi *et al.*, *in press*) also have given the similar results to this study. This would suggest that more studies on the monophyly of the neotropical woody bamboos and the relationships among them are required.

Sub-tribal and generic delimitation within woody bamboos

Temperate woody bamboos

The temperate woody bamboos were supported as monophyletic in all analyses, but none of their subtribes according to Clayton and Renvoize (1986) or Ohrnberger (1999) were resolved or supported. Ni Chonghaile (2002) applied *trnL-trnF*, *rpl16* intron sequences and ITS nuclear rDNA sequences to study relationships among a large number of temperate woody bamboos. However, her study could not resolve infratribal relationships among them or help evaluate the classification system of Clayton and Renvoize (1986) or Ohrnberger (1999).

Borinda, Chimonobambusa and *Menstruocalamus*

Borinda, Chimonobambusa and *Menstruocalamus* group together with low support in the *trnL-F* (64%BS) and the combined analysis (62%BS). Genus *Borinda* was erected by Stapleton (1994) using *Borinda macclureana* (Bor) Stapleton (transferred from *Arundinaria macclureana* Bor) as the type species. However, before transferring this species to *Borinda*, Stapleton (1993) treated it as a member of *Fargesia*, as *Fargesia macclureana* (Bor) Stapleton. *Borinda*, however was later placed as synonymy in *Fargesia* by Stapleton and his colleagues (Li *et al.*, 2006a). *Fargesia* was treated under *Thamnocalamus* in Arundinariinae by Clayton and Renvoize (1986) whilst recognized as a valid genus in Thamnocalaminae by Ohrnberger (1999), Table 2.1. *Menstruocalamus* was establised by Yi (1992) and placed in Arundinariinae by Ohrnberger (1999). It was, later, treated as synonymous with *Chimonobambusa* by Li and Stapleton (2006d). *Chimonobambusa* was placed in Arundinariinae by Clayton and Renvoize (1986) but in Shibataeinae by Ohrnberger (1999). Classifications of these three genera seem very incongruent. There is not enough evidence to solve the relationships of these three genera and their related ones from this study. Therefore it will be very important to intensively sample more representative species of each genus and each subtribe to study relationships not only for these three genera but for the whole of the temperate woody bamboos.

Neotropical woody bamboos

Arthrostylidiinae, Chusqueinae, Guaduinae

The three subtribal names used here follow the classification of Ohrnberger (1999), Table 2.1. Arthrostylidiinae, represented by *Arthrostylidium* and *Rhipidocladum*, were positioned in Bambusinae by Clayton and Renvoize (1986), and are resolved as a monophyletic group by *matK* and the combined analyses with no and high (100%BS) bootstrap support respectively, Figures 2.8 and 2.10. Members of Arthrostylidiinae were positioned either in Arundinariinae or Bambusinae by Clayton and Renvoize (1986) but none of the analyses from single sequence analyses or the combined analysis support this hypothesis, Figures 2.2, 2.4 and 2.6, and Table 2.1.

Guadua, representing Guaduinae, is sister to Arthrostylidiinae in *rps16*, *matK*, and combined analyses, with bootstrap supports of 87%, 63%, and 93%, repectively, Figures 2.6, 2.8 and 2.10. Results from *trnL-F* and *atpB-rbcL* do not support this relationship but neither do they contradict it. *Guadua* was treated under *Bambusa* in Bambusinae by Clayton and Renvoize (1986). There is no evidence from the results of single or combined analyses to support this placement. Other molecular studies, which was sampling *Otatea*, but also representing Guaduinae according to Ohrnberger (1999), have shown that it was also sister to Arthrostylidiinae (*rpl16* sequence data, Kelchner and Clark (1997), Zhang (2000), and Ni Chonghaile (2002); *trnL-F* sequence data, Ni Chonghaile (2002); combined *rbcL*, *trnL-F*, and *matK* sequence data, Bouchenak-Khelladi *et al.* (*in press*)). More genera from Guaduinae according to Ohrnberger (1999) should be sampled for analyses to study the monophyly of Guaduinae and assess its relationship with Arthrostylidiinae.

Chusqueinae, represented by *Chusquea*, are sister to the group consisting of Arthrostylidiinae and Guaduinae. This relationship is resolved by the *matK* analysis (with no bootstrap support) and by the combined analysis (77%BS), Figures 2.6, 2.8 and 2.10. This relationship is congruent with previous phylogenetic studies (*rpl16* sequence data, Kelchner and Clark (1997), Zhang (2000) , and Ni Chonghaile (2002); *trnL-F* sequence data, Ni Chonghaile (2002); combined *rbcL*, *trnL-F*, and *matK* sequence data, Bouchenak-Khelladi *et al.* (*in press*)). *Chusquea* was placed in Arundinariinae by Clayton and Renvoize (1986) but there is no evidence from the data to support this. From the data, its closest relatives are clearly members of Guaduinae and Artrhostylidiinae. All three of these subtribes are from Central and South America (Judziewicz *et al.*, 1999; Ohrnberger (1999).

In contrast, Soderstrom and Ellis (1987) suggested that Guaduinae were not closely related to either Arthrostylidiinae or Bambusinae, based on evidence from leaf-blade anatomy. Apart from geographical differences, there is no synapomorphic morphological or anatomical character to separate these three substribes (Arthrostylidiinae, Guaduinae, and Chusqueinae) from palaeotropical and Austral woody bamboos. Thus, relationships among these three require much further research. There are, however, plenty of molecular synapomorphies from for example, *ndhF* sequence and structural data (Clark *et al.*, 1995; Zhang & Clark, 2000), *rpl16* intron sequence data (Kelchner & Clark, 1997; Zhang, 2000), *trnL-trnF* and *rpl16* intron sequence data (Ni Chonghaile, 2002), combined *rbcL*, *trnL-F*, and *matK* sequence data

(Bouchenak-Khelladi *et al.*, *in press*), and combined *trnL-F*, *atpB-rbcL* intergeneric spacer, *rps16* intron, and *matK* sequence data, this study).

Unfortunately, *Neurolepis*, a woody bamboo genus from Central and South America, of Chusqueinae according to classification systems adopted by Dransfield and Widjaja (1995a), Clark (1995), Li (1998), Ohrnberger (1999) and Judziewicz *et al.* (1999), was not included in this study. Clayton and Renvoize (1986) placed this genus in Arundinariinae while Soderstrom and Ellis (1987) treated it in the monotypic subtribe Neurolepidinae. It would be interesting to include this genus in future analyses to see how it relates to Chusqueinae, Guaduinae, Arthrostylidiinae, and Arundinariinae. However, *Neurolepis* was sister to *Chusquea* (in *trnL-F*, ITS, and combined *trnL-F*, ITS of Ni Chonghaile (2002); *rpl16* analyses of Kelchner and Clark (1997) and of Ni Chonghaile (2002); morphological and plastid *rpl16* intron sequence data of Clark *et al.* (*in press*)). The group consisting of *Neurolepis* and *Chusquea* which was sister to the group consisting of *Otatea* (representing Guaduinae), *Aulonemia* and *Rhipidocladum* (representing Arthrostylidiinae according to Ohrnberger (1999) from *trnL-F* analysis of Ni Chonghaile (2002) is generally consistent to the combined analysis of this study (Figure 2.10).

Palaeotropical and Austral woody bamboos

Melocanninae

All analyses except *atpB-rbcL* resolved the subtribe Melocanninae (Schizostachydinae) according to Ohrnberger (1999). The analyses with *rps16* and *matK* retrieved this clade in the strict consensus but found no bootstrap support for it (Figures 2.6 and 2.8). However, the *trnL-F* and the combined analyses provided high support (99% and 100%BS, respectively) for Melocanninae, Figures 2.2 and 2.10. In addition, the analyses have shown that Melocanninae are sister to the palaeotropical and Austral woody bamboos. In the combined analysis a high bootstrap value (91%BS) was found for this position. However, the single gene analyses were less conclusive (*trnL-F* 62%BS; *atpB-rbcL*, *rps16* and *matK*, no support), Table 2.5. This suggests that Melocanninae is an outlying group among palaeotropical and Austral woody bamboos. This pattern of relationship is a novel result. Melocanninae are generally found from lower elevations of South, South-East, and East Asia (Soderstrom & Ellis, 1987 (as Schizostachydinae); Ohrnberger, 1999; Dransfield & Widjaja, 1995a), Table 2.1. Morphologically, this subtribe has the pseudo-spikelets with a distinctive glabrous ovary that bears an elongated and persistent style divided usually into 3 short stigmas (Soderstrom & Ellis, 1987; Dransfield & Widjaja, 1995a; Wong, 1995a). Anatomically, it differs from other subtribes in having larger microhairs, in the presence of refractive papillae, and in having the pronounced S-shaped keel with complex vasculature, in its leaf-blade. These would be the synapomorphic characters that set Melocanninae apart from the rest of palaeotropical and Austral woody bamboos.

Genus *Pseudostachyum* was placed under *Schizostachyum* by Clayton and Renvoize (1986). However, the results from all analyses except *atpB-rbcL* showed that *Pseudostachyum* is sister to the remaining Melocanninae. This novel sister group status is supported strongly by the *trnL-F* and combined analyses (99%BS and 100%BS, respectively) but *rps16* and *matK* analyses provide no support for this placement except that it is found in their strict consensus trees. The data support the generic status of *Pseudostachyum* as it does not group with *Schizostachyum* in any of the analyses. This finding is congruent with the classification adopted by Soderstrom & Ellis (1987), Dransfield & Widjaja (1995a), Clark (1995), Li (1998) and Ohrnberger (1999). *Pseudostachyum* is composed of two species, a well understood speicies *P. polymorphum* and a less well known and possibly doubtful species *P. wakha* (known only from upper part of Burma) (Ohrnberger, 1999). *Pseudostachyum polymorphum* is distributed at high latitudes (900–1,000 m from sea level) from India, Bhutan, Burma, and southern China to Vietnam (Ohrnberger, 1999; Xia & Stapleton, 2006b). *Pseudostachyum polymorphum* is a new record for Thailand (*personal observation*) and can be found at high elevation (ca 1,000 m) in Phu Luang Wildlife Sanctuary and Phu Kradung National Park, both of these protected areas are in northeastern Thailand.

Cephalostachyum, Neohouzeaua and *Schizostachyum,* are resolved as a monophyletic group by *trnL-F* (88%BS), *rps16* (63%BS), *matK* (no bootstrap support) and the combined analysis (99%BS), Figures 2.8 and 2.10. However, the relationships among these genera are still unclear. Soderstrom and Ellis (1987) placed *Neohouzeaua* under *Schizostachyum.* Clayton and Renvoize (1986) treated *Neohouzeaua* and *Cephalostachyum* to be synonymous with *Schizostachyum* while Dransfield and Widjaja (1995a), Clark (1995), Li (1998); and Ohrnberger (1999) treated them as separate genera. The results from this study indicate that the monophyletic status of *Schizostachyum* and *Neohouzeaua* is questionable. The results are more consistent with the *Schizostachyum* s.l. hypothesis of Clayton and Renvoize (1986).

It seems *Schizostachyum* (Figure 2.16) and its related genera can be arbitrarily divided into two groups, Figures 2.8, 2.10 and 2.16. One would be a group of *S. zollingeri*, *S. jaculans*, *Cephalostachyum pergracile* (Figure 2.16), and *Neohouzeaua kerriana*, (64%BS in *matK*; 96%BS in the combined analysis). Another one would be a group consisting of *Schizostachyum grande* and *Neohouzeaua fimbriata* (72%BS in *matK*; 100%BS in the combined analysis). *Schizostachyum jaculans* is sister to *Neohouzeaua kerriana* with high bootstrap support (94%BS in *matK*; 99% in the combined analyses). Dransfield (1983) compared the bamboos of *Schizostachyum* from Borneo and Sumatra with the others she has known, and categorized them into three groups. The first group included *S. brachycladum* (Munro) Kurz and its related species, *S. undulatum* S. Dransfield, have erect culms with a nodding tip, and the culm sheaths have a broadly triangular erect blade. The second group included *S. blumei* Nees and *S. jaculans* Holttum, and these usually have erect culms with a drooping tip and the culm sheaths have a long, narrow, deflexed or reflexed blade. The last group included *S. grande* Ridley, *S. pleianthemum* S. Dransfield, *S. pilosum* S. Dransfield, and *S. hantu* S. Dransfield. These have erect young culms that lean over when mature with a long drooping tip; the culm sheaths have a long blade with a broad base and tapering tip.

The results are generally consistent with the division of *Schizostachyum* defined by Dransfield (1983), even though the species in each group are not all the same. However, by including *Neohouzeaua* species in the analyses, the two groups resulting from this study appear to be intermediate to the three groups of Dransfield (1983). The group 2 of this study is generally consistent with the third group of Dransfield (1983), as they have the same species, *S. grande. Neohouzeau fimbriata* is a scrambling bamboo, its culm-sheath blades are ovate-lanceolate and deflexed (Dransfield *et al.*, 2003), which is more or less fitting with the second group. However, the group 1 of this study has members that have characters which are intermediate among the three groups of Dransfield (1983). *Schizostachyum jaculans* (group 1 of study) was in the second group of Dransfield (1983). Both *Schizostachyum zollingeri* and *C. pergracile* have erect culms with a nodding tip, and the culm sheaths have a broadly triangular erect blade, are in the sence of the first group of Dransfield (1983). *Neohouzeaua kerriana* has erect culms with a tip that arches almost to the ground or leans on nearby vegetation, and the culm sheaths having a blade that is lanceolate, tapering to long tips, spreading or deflexed (Dransfield *et al.*, 2003) which is intermediate between the second and the third groups of Dransfield (1983).

By sampling a great number of *Cephalostachyum* and *Schizostachyum* species, a species of *Pseudostachyum* (*P. polymorphum*) and other woody bamboos, but unfortunately no representative of *Neohouzeaua*, Yang *et al.* (2007) used combined *GBSSI* and *trnL-F* analyses to study phylogenetic patterns in these taxa. They found that *Cephalostachyum* and *Schizostachyum* could be recognized as distinct genera. However, they recommended that *Cephalostachyum pergracile* and *C. virgatum* (Munro) Kurz (Figure 2.16) shoud be transferred to *Schizostachyum.* Their analyses also detected three groups of *Schizostachyum.* Group A (the *S. brachycladum* group) consisted of *C. pergracile, C. virgatum, S. zollingeri,* and *S. brachycladum.* These share erect and arboreous culms, broadly triangular culm-sheath blades, and inflorescences composed of densely glomerate spikelets at the nodes of flowering branches. Group B (the *S. blumei* group) consisted of both arboreous and scrambling bamboos, they are *S. dumetorum, S. funghomii, S. gracile, S. pseudolima, S. xinwuense,* and *S. blumei.* These share the common characters of long, narrowly lanceolate and deflexed culm-sheath blades, and inflorescences consisting of sparsely panicled spikelets on the nodes of flowering branches. Group C (the *S. jaculans* group) was composed of two scrambling bamboos, *S. hainanense* and *S. jaculans,* that bear long, narrowly lanceolate and deflexed culm-sheath blades and inflorescences consisted of densely glomerate spikelets at the nodes of flowering branches.

The group 1 of this study is almost congruent with group A of Yang *et al.* (2007) except for the inclusion of *S. jaculans* which was in Group C of Yang *et al.* (2007). The group A of Yang *et al.* (2007) was congruent with the first group of Dransfield (1983). *Schizostachyum blumei* and *S. jaculans* were in the second group of Dransfield (1983) but in the group B and C respectively, of Yang *et al.* (2007).

As can be seen, all three systems from three studies, whether using morphological or molecular information (Dransfield, 1983; Yang *et al.*, 2007; and this study), contrast with each other. To date, neither morphological, molecular, nor combined morphological and molecular information could solve the relationships within *Schizostachyum.* However this study has helped understanding phylogenetic pattern in this group and it should be possible to find out consistent phylogenetic pattern with further study both within *Schizostachyum* and between *Schizostachyum* and its related genera. More species of *Neohouzeaua,* including the type species of the genus, *N. mekongensis* A. Camus, from Laos (Ohrnberger, 1999), should be included for better understanding the relationships among *Schizostachyum, Cephalostachyum* and *Neohouzeaua.*

Hickeliinae

Temburongia, a genus recently established from Ulu Temburong National Park, Brunei (Dransfield & Wong, 1996), was the only representative of the subtribe Hickeliinae according to Ohrnberger (1999) in this study. It is sister to the remaining palaeotropical and Austral woody bamboos (*matK,* 53%BS; 99%BS in the combined analysis), Figures 2.8 and 2.10. However, *Nastus,* a genus also represented Hickeliinae according to Ohrnberger (1999), is distributed from Madagascar, to Indonesia, Papua New Guinea, to Solomon Islands, embedded in the palaeotropical woody bamboos (*Bambusa* and *Schizostachyum*) in *rpl16* sequence analyses of Zhang (2000). This woud suggest that the relationships between Hickeliinae and the remaining palaeotropical and Austral woody bamboos are still unclear. Therefore, more genera from Hickeliinae (*Decaryochloa, Greslania, Hickelia, Hitchcockella, Nastus,* and *Perrierbambus*), especially the type genus *Hickelia* from Madagascar (Ohrnberger, 1999), shoud be included to further investigate its monophyly and position within palaeotropical and Austral woody bamboos. The geographical distribution of Hickeliinae ranges from Madagascar to New Guinea, Pacific Islands, and to Indonesia and Brunei (Soderstrom & Ellis, 1987 (as Nastinae); Ohrnberger (1999; Dransfield & Widjaja, 1995a), Table 2.1. The microhairs of leaf-blades have pointed apices and are wider and larger than in most bamboos, and may be unique to this subtribe (Soderstrom & Ellis, 1987). The sister group status of Hickeliinae to the rest of the palaeotropical and Austral Bambuseae is a novel result. If future analyses support this placement it will help interpret the evolutionary patterns within palaeotropical woody bamboos. It was one of the earliest lineages to diverge in the group and will help examine pre-historical biogeographic patterns of diversification.

Bambusinae and Racemobambosinae

None of the analyses resolved or supported the monophyly of subtribe Bambusinae according to Ohrnberger (1999) because the genus *Vietnamosasa*, which he positoned in Racemobambosinae, is embedded within Bambusinae. All analyses have shown that the subtribe Bambusinae according to Clayton and Renvoize (1986) is also not monophyletic. It has taxa distributed across the phylogenetic tree of the traditional Bambuseae and is hence polyphyletic.

However, if *Vietnamosasa* was included witin Bambusinae, then there is no evidence against the monophyly of Bambusinae sensu Ohrnberger (1999) from our analyses (84%BS, *atpB-rbcL*; 61%BS in the combined analysis; Bambusinae is unsupported in the other single gene analyses but *Vietnamosasa* is always embedded with Bambusinae taxa groups). It will be important to sample more genera from Racemobambosinae according to Ohrnberger (1999) to see whether Racemobambosinae is monophyletic and to establish how it relates to Bambusinae. However, the analysis of Ni Chonghaile (2002) using *trnL-F*, which included another two genera of Racemobambosinae according to Ohrnberger (1999), *Neomicrocalamus* and *Racemobambos*, has shown that these two species grouped with other genera (*Bambusa, Dendrocalamus*, and *Gigantochloa*) of Bambusinae according to Ohrnberger (1999). From this study together with that of Ni Chonghaile (2002), it would suggest that *Neomicrocalamus, Racemobambos*, and *Vietnamosasa* may be better placed in Bambusinae than in Racemobambosinae.

There are four recently established genera (*Mullerochloa, Neololeba, Phuphanochloa and Temochloa*) that can be included within Bambusinae according to Ohrnberger (1999). *Phuphanochloa* is a new genus from northeastern Thailand, composed of a single species, *P. speciosa* (Sungkaew *et* *al., accepted*). It can be included in Bambusinae because it morphologically similar to, and phylogenetically related to, *Bambusa* (Sungkaew *et al., accepted*; Sungkaew *et al., in prep.*). It is also related to *Dendrocalamus* and *Gigantochloa*. The results from the analyses have confirmed that *Phuphanochloa* is sister to, or embedded in, a group consisting of *Bambusa beecheyana, B. malingensis, B. oldhamii,* and *Neosinocalamus affinis* (=*B. affinis*). This pattern was resolved with *rps16* (62%BS), *matK* (53%BS), and the combined analysis (86%BS), Figures 2.6, 2.8 and 2.10.

Temochloa, a monotypic and endemic genus (*T. liliana*) from southern Thailand, which has no subtribe applied to it (Dransfield, 2000b), can be interpreted in two ways. *Temochloa* can simply be fitted within Bambusinae according to Ohrnberger (1999) as one of the outlying taxa within this subtribe. Alternatively, it can be treated under a new subtribe that is sister to the remaining palaeotropical woody bamboos (94%BS in the combined analysis, Figure 2.10). *Temochloa liliana* is a slender bamboo and is an endemic species that is only found confined to the limestone range of three adjacent provinces (Surat Thani, Phangnga, and Krabi) at the middle of peninsular Thailand (Dransfield, 2000b; *personal observation*). Dransfield (2000b) remarked that the spikelet characters of *Temochloa* approached those of *Temburongia* (of Hickeliinae of Ohrnberger (1999)) in having only one fertile flower that is borne on an elongated rachilla internode and in having a long rachilla extension with a rudimentary floret. However, she further explained that the branch complement of these two genera was very different because in *Temochloa* it originated from 2–4 single branch buds that were borne at the same level at a culm node while that of *Temburongia* developed from a solitary branch bud. Results from the *trnL-F, atpB-rbcL, matK,* and the combined analysis have shown that these two genera are not closely related. *Temochloa* may also superficially look similar to *Cephalostachyum* and *Schizostachyum* of Melocanninae in having a branch complement at each culm node that is composed of many slender, sub-equal branches that develop from a solitary branch bud (Dransfield & Widjaja, 1995a; Wong, 1995a; *personal observation*). However, the branch complement of *Temochloa* is obtained from 2–4 single branch buds that are borne at the same level at each culm node. These develop into slender, sub-equal, geniculate branches and each of them can re-branch a few more branches from its base that are also geniculate, sub-equal to or a bit smaller than those of the 2–4 primary branches (Dransfield, 2000b; *personal observation*). The branch complement pattern of *Temochloa* is also similar to that of *Holttumochloa* of Wong (1993b) but it has fewer single buds at each culm node; *Holttumochloa* can vary from several to many (Wong, 1993b; Wong, 1995a). *Holttumochloa* was positioned under an uncertain subtribe designation by Wong (1995a) while it was placed in Bambusinae by Dransfield and Widjaja (1995a), Clark (1995), and Ohrnberger (1999). It seems that *Temochloa* is better placed in Bambusinae as one of the outlying taxa within this subtribe rather than creating a new subtribe to accommodate it. It would be useful to put *Temochloa* and *Holttumochloa* in future analyses to see how they relate to each other and also to the remaining Bambusinae.

Mullerochloa is a monotypic and endemic genus (*M. moreheadiana*) (Wong, 2005) from Cooktown, Queensland, Australia (Ohrnberger, 1999; Wong, 2005). Before generic reclassification, this species was accepted as a member of *Bambusa* (Bailey, 1889; Ohrnberger, 1999), as *B. moreheadiana*, and placed in Bambusinae by Ohrnberger (1999). The results from *matK* and combined analyses show that it is best placed as the outlying taxon of the 'core' Bambusinae group (*Bambusa, Dendrocalamus, Gigantochloa, Melocalamus, Orebambos, Oxytenanthera, Phuphanochloa, Thyrsotachys,* and *Vietnamosasa* (if it can be placed in Bambusinae)), with (53%BS and 63%BS respectively). *Mullerochloa* can be interpreted in a similar way to *Temochloa*; it may be fitted within Bambusinae as one of the outlying taxa within this subtribe. Alternatively, it can be treated under a new subtribe that is sister to the remaining palaeotropical woody bamboos including *Temochloa.*

Neololeba is a relatively new genus established to accommodate five bamboos known from South Mindanao, North Sulawesi, Mollucas, New Guinea, Solomon Islands, and Australia (Queensland) (Widjaja, 1997). *Neololeba atra* (Lindley) Widjaja, which was transferred from *Bambusa,* is the type species of this genus, naturally distributed from Northern Sulawesi, Philippines (Mindanao, Davao), Moluccas, New Guinea, and Australia (Queensland) (Widjaja, 1997). Ohrnberger (1999), without referring to Widjaja (1997), kept it under *Bambusa* as *B. atra* Lindley and placed it in Bambusinae. Like the interpretation for *Temochloa* and *Mullerochloa,* this species accompanied with *Dinochloa* may either be fitted within Bambusinae as one of its outlying groups within this subtribe or treated under a new subtribe which is sister to the majority of palaeotropical and Austral woody bamboos including *Temochloa* and *Mullerochloa.* These two patterns of relationship between *Neololeba* and the remaining palaeotropical and Austral woody bamboos including *Temochloa* and *Mullerochloa,* are resolved by *matK* analysis in all equally most parsimonious trees (but with no bootstrap support) and by the combined analysis (61%BS), Figure 2.8 and 2.10. On the basis of morphology, we would expect to see *Neololeba* grouping with *Bambusa* taxa (Widjaja, 1997; Ohrnberger, 1999). *Dinochloa* has climbing culms and single-flowered spikelets while *Neololeba* has erect to scrambling culms and 3–12-perfect flowered spikelets (Dransfield, 1981; Widjaja, 1997). More *Neololeba* species should be studied, especially from the species that have never been transferred from *Bambusa,* e.g. *N. glabra* Widjaja and *N. inaurita* Widjaja, to establish the monophyly of *Neololeba* and determine its relationship with *Bambusa* and the remaining palaeotropical and Austral woody bamboos.

Dinochloa and *Melocalamus* were traditionally and are consistently placed in Bambusinae by most classification systems (Clayton & Renvoize, 1986; Soderstrom & Ellis, 1987; Dransfield & Widjaja, 1995a; Clark, 1995; Li, 1998; Ohrnberger, 1999). Surprisingly, *Dinochloa malayana* is sister to *Neololeba atra* with high bootstrap support from *matK* and combined analyses (97%BS and 100%BS, repectively), Figure 2.8 and 2.10. On the basis of morphology we might expect *Dinochloa* to group either with *Melocalamus* or *Mullerochloa,* rather than with *Neololeba.* This is because *Dinochloa* is similar to *Melocalamus* in having a berrylike caryopsis with thick and fleshy pericarp (McClure, 1966; Dransfield, 1981; Wong, 1995a; Li & Stapleton, 2006h) and in having a rugose basal zone of the culm sheath which is probably equivalent to the term 'girdle' of McClure (1966) and Judziewicz *et al.* (1999). The combined *GBSSI* and *trnL-F* analyses of Yang *et al.* (2007) have shown that *Bambusa, Dinochloa,* and *Melocalamus* group together. *Bambusa bambos* (as *B. arundinacea* (Retz.) Willd.) was sister to three *Melocalamus* species and a group of two *Dinochloa* species were sister to this group. However, Dransfield (1981) and Rudall & Dransfield (1989) remarked that *Melocalamus* differed from *Dinochloa* in having two perfect flowers in each spikelet (only a single perfect flower in *Dinochloa*) and by having the plumule and radicle apical in position (oblique to the vertical axis of the fruit in *Dinochloa*). In addition, *Dinochloa* is superficially similar to *Mullerochloa* by sharing an abrupt swelling at the very basal part of the culm internodes, and the presence a rugose basal zone of the culm sheath (Dransfield, 1981; Wong, 2005) but the spikelet of *Dinochloa* is composed of only a single perfect flower while there are 4–9 perfect flowers in *Mullerochloa.* This would suggest that the one reproductive character (berrylike caryopsis with thick and fleshy pericarp) and two vegetative characters (presence of an abrupt swelling of the very basal part of the culm internodes and presence of a rugose basal zone of the culm sheath) are homoplasious because they have evolved independently among these three genera.

Apart from *Temburongia,* the biogeographical range of another two outlying taxa, a group consisting of *Neololeba* and *Dinochloa* and *Mullerochloa,* is outside the biogeographical range of the core Bambusinae. *Mullerochloa* is a monotypic and endemic genus of Cooktown, Queensland, Australia (Ohrnberger, 1999; Wong, 2005) while *Neololeba* is a genus of five bamboos known from South Mindanao, North Sulawesi, Mollucas, New Guinea, Solomon Islands, and Australia (Queensland) (Widjaja, 1997). *Dinochloa* which was resolved from this study to be sister to *Neololeba,* is mainly distributed on the Malay Peninsula, in Borneo, Indonesia and Philippines, extending to southern Thailand and the Andaman and Nicobar Islands (Dransfield, 1981; Ohrnberger, 1999). This would suggest that the ancestors of Bambusinae were from somewhere in mainland Asia, possibly south China or India or even mainland Southeast Asia and two outlying lineages evolved separately from southern Thailand, Malaysia to northern Australia. The paucity of bamboo species in Australia would also support this hypothesis. If the origin of these bamboos had been near Australia we would expect much higher diversification in this area.

Oreobambos and *Oxytenanthera*

Both *Oreobambos* and *Oxytenanthera* are monotypic genera (*Oreobambos buchwaldii* and *Oxytenanthera abyssinica*), naturally distributed in tropical Africa (Ohrnberger, 1999). All analyses have grouped *Oreobambos* and *Oxytenanthera* as sister groups with different degrees of bootstrap support, as follows: *trnL-F* (86%BS); *atpB-rbcL* (64%BS); *rps16* (62%BS); *matK* (76%BS); and combined analysis (100%BS). Clayton and Renvoize (1986) treated these two genera in different subtribes, *Oreobambos* in Bambusinae and *Oxytenanthera* in Melocanninae, Table 2.1. However, it is geographically (Ohrnberger, 1999) and phylogenetically (this study) clear that they shoud be grouped together. They should also be positoned in Bambusinae according to the classification of Ohrnberger (1999); however this would be logical only when *Vietnamosasa* is placed in Bambusinae as mentioned in section 'Bambusinae and Racemobambosinae' of this thesis (page 84).

Dendrocalamus and its related genera

Morphologically, the genus *Dendrocalamus* is similar to several other genera e.g. *Bambusa, Dendrocalamopsis, Gigantachloa, Houzeaubambus, Klemaochloa, Oreobambos, Oxytenanthera, Sinocalamus,* and *Neosinocalamus* (Holttum, 1958; McClure, 1966; Clayton & Renvoize, 1986; Soderstrom & Ellis, 1987; Dransfield & Widjaja, 1995a; Wong, 1995a; Stapleton & Xia, 1997; Li, 1997; Li & Xue, 1997; Li, 1998; Ohrnberger, 1999; Li *et al.*, 2006b). Surprisingly, the results from *atpB-rbcL, rps16, matK,* and combined analyses, have shown that *Melocalamus* is another genus closely related to *Dendrocalamus*, Figure 2.4, 2.6., 2.8, and 2.10. It groups with *Dendrocalamus* species with 100%BS in the combined analysis. *Melocalamus* was expected to group together either with *Dinochloa* or *Mullerochloa* on the basis of morphology.This relationship has never been reported before. The results also confirmed that *Dendrocalamus* is closely related to *Bambusa, Dendrocalamopsis* (=*Bambusa*), and *Gigantochloa.*

Bambusa, Dendrocalamus, and *Gigantochloa* are geographically distributed primarily from southen China, India, to Burma, Thailand, Vietnam and extending to Malaysia, Indonesia, Borneo, Java, Sumatra and Papua New Guinea (Dransfield & Widjaja, 1995a; Wong, 1995a; Widjaja, 1997; Ohrnberger, 1999; Li & Stapleton, 2006f,g; Xia *et al.*, 2006a). *Melocalamus* has a similar range to those three genera but is not found further south than Thailand (Ohrnberger, 1999; Li & Stapleton, 2006h; *personal observation*). Vegetative and reproductive characters of *Bambusa,* *Dendrocalamus,* and *Gigantachloa* differ from that of *Melocalamus.* Those three genera are mostly arborescent bamboos possessing culm sheaths with no girdle, and a terete caryopsis with slightly thickened and dry pericarp (Dransfield & Widjaja, 1995a; Wong, 1995a; Widjaja, 1997; Ohrnberger, 1999; Li & Stapleton, 2006f,g; Xia *et al.,* 2006a). In contrast, *Melocalamus* is a climbing bamboo possessing culm sheaths with a girdle section, and its caryopsis is globose, berrylike, with thick and fleshy pericarp (McClure, 1966; Dransfield, 1981; Ohrnberger, 1999; Li & Stapleton, 2006h). This would suggest that *Melocalamus* with its climbing habit, culm sheath with girdle section, fleshy pericarp and berry like caryopsis, has evolved from within a group of Bambusa/Dendrocalamus/Gigantochloa-like plants that lack these features. These characters would not be good for grouping *Melocalamus* with *Dinochloa,* as they are homoplasious.

Bambusa* and *Neosinocalamus

Neosinocalamus was established in 1983 using *Neosinocalamus affinis* (Rendle) P.C. Keng as the type species (Ohrnberger, 1999). On the basis of morphology, Clayton and Renvoize (1986) treated *Neosinocalamus* as a synonym of *Dendrocalamus* while Ohrnberger (1999) classified it under *Bambusa,* Table 2.1. The results from *matK* and combined analyses have shown that *Neosinocalamus* groups with *Bambusa beecheyana, B. malingensis* and *B. oldhamii,* with 61%BS and 80%BS, respectively, Figure 2.8 and 2.10. This would suggest that *Neosinocalamus* is better placed under *Bambusa.* This is consistent with Ohrnberger (1999) and inconsistent with Clayton and Renvoize (1986). The name *Neosinocalamus affinis* (Rendle) P.C. Keng is currently recognized as a synonym of *Bambusa emeiensis* Chia &H.L. Fung of *Bambusa* subgenus *Lingnania* (Ohrnberger, 1999; Li & Stapleton, 2006c). The topology of the phylogenetic trees for four species of *Bambusa* in *matK* and combined analyses (Figure 2.8 and 2.10) is in general agreement with the subgeneric classification adopted by Xia *et al.* (2006a). They included *B. beecheyana* (*Bambusa* subg. *Dendrocalamopis*), *B. emeiensis* (*Neosinocalamus affinis*) (*Bambusa* subg. *Lingnania*), *B. malingensis* (*Bambusa* subg. *Bambusa*), and *B. oldhamii* (*Bambusa* subg. *Dendrocalamopis*). However, from the analyses, *B. bambos* (not naturally ocurring in China) and *B. malingensis* did not group together even though they would be predicted to group together as they are in the same subgenus (*Bambusa* subg. *Bambusa*), on the basis of shared morphological characters including thorny branchlets and persistent culm-sheath blades (Xia *et al.,* 2006a). Incongruence between the morphological classification of *Bambusa* and phylogenetic studies on this genus were preliminarily revealed by Sun *et al.* (2005). Sun *et al.* (2005) used the nuclear ITS gene region on *Bambusa* to show their phylogenetic relationships. The taxonomy of *Bambusa* is in a state of flux, it is a large genus with over 100 poorly understood species

(Ohrnberger, 1999; Xia *et al.*, 2006a). Much work remains to be done to establish the generic limits of *Bambusa* and the inter-relationships of its taxa.

Dendrocalamopsis validida, Bambusa oldhamii, and Bambusa oliveriana

Bambusa subgenus *Dendrocalamopsis* Chia & H.L. Fung was established in 1980, using *Bambusa oldhamii* Munro as the type species (Chia & Fung, 1980; Ohrnberger, 1999; Stapleton & Xia, 2004). In 1983, the subgenus *Dendrocalamopsis* was up-transferred to generic rank as *Dendrocalamopsis* (Chia & H.L. Fung) P.C. Keng, using the name *Dendrocalamopsis oldhamii* (Munro) P.C. Keng as the type species of the genus (which was transferred from *B. oldhamii* Munro) (Keng, 1983; Ohrnberger, 1999; Stapleton & Xia, 2004). However, before it was transferred to the generic level, the speceies *Dendrocalamopsis grandis* Q.H. Dai & X.L. Tao was published in 1982 (Dai, 1982; Ohrnberger, 1999; Stapleton & Xia, 2004). Ohrnberger (1999) stated that the name *D. grandis* Q.H. Dai & X.L. Tao was invalid because the genus name was not validly puplished. He accepted that the status of the genus and the species *D. grandis* had already been validated by Keng (1983) as *D. grandis* Q.H. Dai & X.L. Tao ex P.C. Keng. However, Ohrnberger (1999) accepted this species to be under *Bambusa* as *Bambusa grandis* (Q.H. Dai & X.L. Tao ex P.C. Keng) Ohrnb. as he transferred it since 1997. Stapleton and Xia (2004) clarified the status of the genus and the species *D. grandis* on the basis of Note 1 in Article 42 of the Sydney Code of Voss *et al.* (1983). They accepted that the publication of *D. grandis* Q.H. Dai & X.L. Tao could, nevertheless, be considered to have provided a '*descriptio generico-specifico*' and therefore the genus *Dendrocalamopsis* Q.H. Dai & X.L. could be considered as a validly published name, and therefore the name *Dendrocalamopsis* (Chia & H.L. Fung) P.C. Keng was a later illegitimate homonym (Stapleton & Xia, 2004). However, Ohrnberger (1999) and Stapleton and Xia (2004) have agreed to accept '*Dendrocalamopsis*' at subgeneric rank under *Bambusa,* as *Bambusa* subgenus *Dendrocalamopsis* Chia & H.L. Fung, using *Bambusa oldhamii* Munro as the type.

Dendrocalamopsis, based mainly on morphological characters, was treated as a synonym under the genus *Bambusa* (Clayton & Renvoize, 1986; Ohrnberger, 1999), Table 2.1. The results from this study were not conclusive about the status of *Dendrocalamopsis. Dendrocalamopsis valida* Q.H. Dai, a representative of this genus was imbedded in a polytomy in a group consisting of *Dendrocalamus, Gigantochloa, Bambusa,* and *Melocalamus* (65%BS in *atpB-rbcL*, no support in *rps16*, 97%BS in *matK*, and 100%BS in the combined analysis). This would suggest that the status of *Dendrocalamopsis valida* is still unclear and it is not be able to decide whether it should be treated as a species of *Bambusa* subgenus *Dendrocalamopsis* as suggested by previous works (Ohrnberger, 1999; Stapleton & Xia, 2004; Li & Stapleton, 2006b) or as a species of the genus

Dendrocalamopsis. It does not group with *B. oldhamii* (=*D. oldhamii*) in any analyses so there is no evidence to support the monophyly of a *B.oldhamii* and *D. valida* group. The results from *matK*, and combined analyses have shown that *Bambusa oldhamii* groups together with *Bambusa beecheyana, Bambusa malingensis* and *Neosinocalamus affinis (=Bambusa emeiensis),* with 61%BS and 80%BS, respectively, Figure 2.8 and 2.10. This would be consistent with the placement of *Bambusa oldhamii* in *Bambusa* as suggested by Clayton and Renvoize (1986) and Ohrnberger (1999). If this is right, it would further mean that the status of *Dendrocalamopsis* as a genus is no longer valid because its type spcies has been correctly placed as a member of *Bambusa.* Thus, this would taxonomically and systematically validify the treatment of *Dendrocalamopsis* as a subgenus of *Bambusa* as adopted by Ohrnberger (1999), Stapleton and Xia (2004), Xia *et al.* (2006a), and Li and Stapleton (2006b). Unfortunately, if this is right, then, this would further mean that the species *Dendrocalamopsis valida* may need a new genus to accommodate it. To date, based on morphological characters, this species was either treated under *Bambusa* as *Bambusa valida* (Q.H. Dai) W.T. Lin (Lin, 1990; Ohrnberger 1999) or it was placed as one of 'taxa incertae sedis' (Xia *et al.*, 2006a).

Bambusa oldhamii Munro has not only been treated under *Dendrocalamopsis* (as type species), but it is also treated under *Sinocalamus* as *Sinocalamus oldhamii* (Munro) McClure. However, the delimitation of *Sinocalamus* has proven extremely difficult (McClure, 1940; Raizada, 1948; Chia & Fung, 1980; Xia & Stapleton, 1997a). McClure (1940) erected this genus to primarily accommodate the intermediate species between *Bambusa* and *Dendrocalamus* (eg. *Sinocalamus affinis* (Rendle) McClure, *S. latiflorus* (Munro) McClure-as type species, *Sinocalamus oldhamii* (Munro) McClure), but later rejected it (McClure, 1966).I do not recognize *Sinocalamus* and prefer instead to include its species in *Bambusa* and *Dendrocalamus.* There is no evidence for its monophyly in my results.

Bambusa oliveriana Gamble is a little known species, distributed from Burma to northern and western Thailand (Dransfield, 1994; Ohrnberger 1999; *personal observation*). Like the placement of *Dendrocalamopsis valida* in this study, *B. oliveriana* was imbedded in a polytomy in a group consisting of *Dendrocalamus, Gigantochloa, Dendrocalamopsis,* and *Melocalamus* (65%BS in *atpB-rbcL*, no support in *rps16*, 97%BS in *matK*, and 100%BS in the combined analysis). It does not have molecular evidence to show whether it should be treated as a species of *Bambusa* or as a species of another genus in this group. Ohrnberger (1999) accepted it as a member of *Bambusa* but provided no infrageneric assignment. Having a broad culm-sheath blade and small fringed culm-sheath auricles, it would fit in *Bambusa* subgenus *Dendrocalamopsis* as suggested by Xia *et al.* (2006a).

Table 2.5 Comparison of strict consensus and bootstrap support values of the main taxa or the major groups resolved from individual and combined analyses of five gene regions

Main taxa or major resolved groups	*trnL-F*		*atpB-rbcL*		*rps16*		*matK*		combined	
	strict consensus	%BS	strict consensus	%BS	strict consensus	%BS	strict consensus	%BS	strict consensus	%BS
BEP clade	✓	99	✓	100	✓	100	✓	100	✓	100
Ehrhartoideae	✓	64	✓	61	✓	X	✓	100	✓	100
Pooideae	✓	81	✓	100	✓	70	✓	98	✓	100
Bambusoideae s.s.	✓	78	✓	65	✓	92	✓	98	✓	100
Pooideae sister to Bambusoideae s.s.	✓	63	✓	95	X	X	✓	85	✓	99
Olyreae	✓	62	✓	99	✓	98	✓	100	✓	100
Bambuseae	X	X	X	X	X	X	✓	59	X	X
Temperate woody bamboos	✓	100	✓	100	✓	100	✓	100	✓	100
Neotropical woody bamboos	X	X	X	X	X	X	✓	X	✓	77
Palaeotropical and Austral woody bamboos	✓	62	X	X	✓	X	✓	X	✓	91
Olyreae sister to Bambuseae	X	X	X	X	X	X	✓	98	X	X
Olyreae sister to only palaeotropical and Austral woody bamboos, not terperate ones (hence, non-monophyly Bambuseae)	X	X	X	X	✓	70	X	X	✓	57
Melocanninae	✓	99	X	X	✓	X	✓	X	✓	100
Melocanninae sister to palaeotropical and Austral woody bamboos	✓	62	X	X	✓	X	✓	X	✓	91

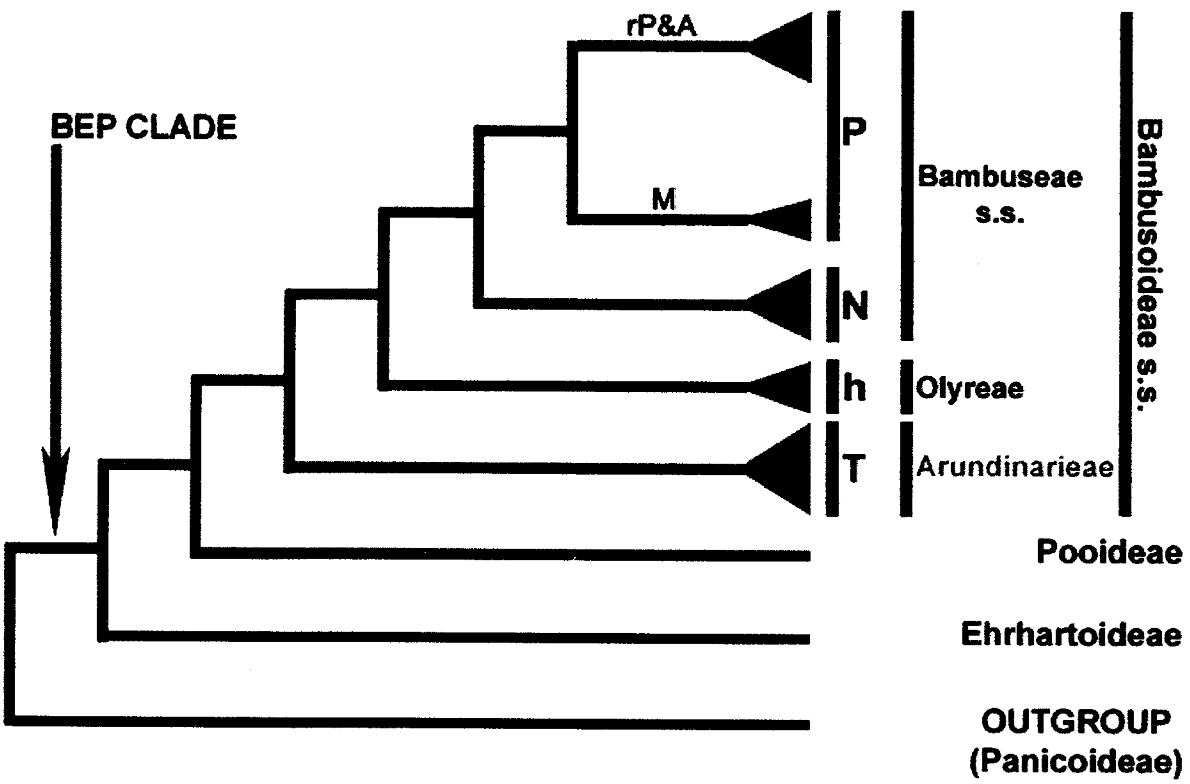


Figure 2.11 Cladogram showing relationships within Bambusoideae s.s. and other grasses. The BEP clade and the subfamial classification (column on far right) are according to GPWG (2001). The tribal classification (middle column) is according to this study where Arundinarieae and Bambuseae s.s. are re-defined. The geographical classification of woody bamboos; P, N, and T (column on far left) shown according to Clark *et al.* (1995) and Ni Chonghaile (2002). N=Neotropical woody bamboos (including Arthrostylidiinae, Chusqueinae, and Guaduinae); P=Palaeotropical plus Austral woody bamboos (Melocanninae (=M) according to Ohrnberger (1999) is sister to the rest of palaeotropical plus Austral woody bamboos (rP&A, including Bambusinae, Hickeliinae, and Racemobambosinae)); T=Temperate woody bamboos; h=Herbaceus bamboos distributed pantropically (see Figure 2.13 for detail). Size of triangular shapes reflex number of species of the taxa or groups

Figure 2.12 Distribution of Arundinarieae (temperate woody bamboos). From http://www.eeob.iastate.edu/research/bamboo/maps.html, with permission from Dr Lynn Clark. The distribution of temperate woody bamboos which found in tropical zone is from the high elevation, usually from 1,000 m to as high as 3,630 m (Ohrnberger, 1999).

Figure 2.13 Distribution of Olyreae (herbaceous bamboos). From http://www.eeob.iastate.edu/research/bamboo/maps.html, with permission from Dr Lynn Clark. The lighter shade of gold colour indicates the uncertainty whether it is truly native to these areas.

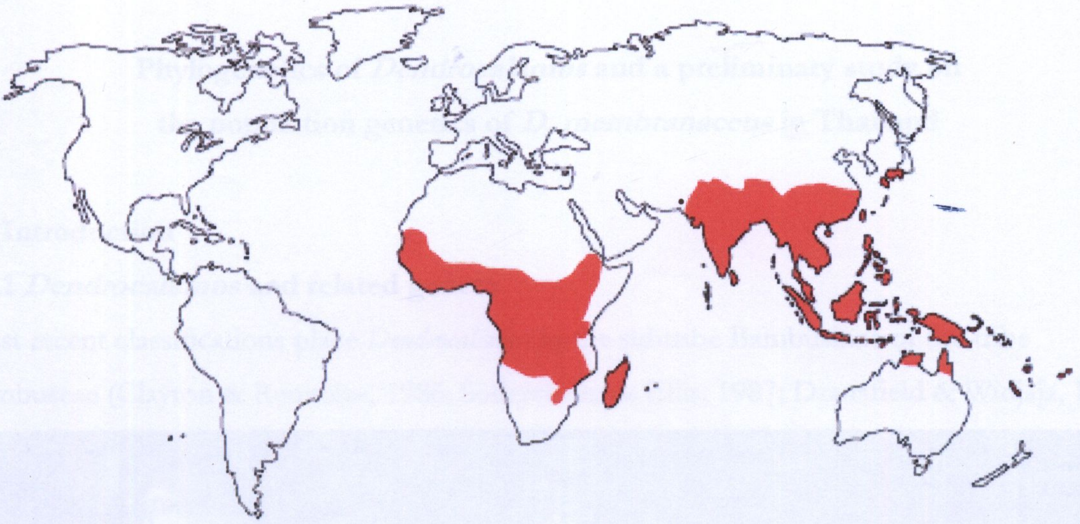


Figure 2.14 Distribution of palaeotropical and Austral Bambuseae. From http://www.eeob.iastate.edu/research/bamboo/maps.html, with permission from Dr Lynn Clark.

Figure 2.15 Distribution of neotropical Bambuseae. From http://www.eeob.iastate.edu/research/bamboo/maps.html, with permission from Dr Lynn Clark.

Figure 2.16 *Schizostachyum* and *Cephalostachyum*. From left to right, culm sheath of *C. pergracile*; flying shoot of *S. zollingeri*; flying shoot of *S. brachycladum*; flying shoot of *S. grande*; flying shoot of *C. virgatum*.

Chapter 3

Phylogenetics of *Dendrocalamus* and a preliminary study on the population genetics of *D. membranaceus* in Thailand

3.1 Introduction

3.1.1 *Dendrocalamus* and related genera

Most recent classifications place *Dendrocalamus* in the subtribe Bambusinae of the tribe Bambuseae (Clayton & Renvoize, 1986; Soderstrom & Ellis, 1987; Dransfield & Widjaja, 1995; Clark, 1995; Wong, 1995a; Li, 1998; Ohrnberger, 1999). *Dendrocalamus* and *Bambusa* are usually placed in the same subtribe but they have sometimes been treated in different tribes or subtribes. For example, Li (1997) included *Bambusa* in the Bambuseae and *Dendrocalamus* in Dendrocalameae. Gamble (1986), following Bentham and Hooker (1883) who modified the system of Munro (1868), placed them in different groups (what Gamble (1986) called 'Sub-tribes'), *Bambusa* in the Eubambuseae and *Dendrocalamus* in Dendrocalameae.

Morphologically, *Dendrocalamus* is close to several genera such as *Bambusa, Dendrocalamopsis, Gigantachloa, Houzeaubambus, Klemaochloa, Oreobambos, Oxytenanthera, Sinocalamus,* and *Neosinocalamus* (Holttum, 1958; Clayton & Renvoize,1986; Soderstrom & Ellis, 1987; Wong, 1995a; Stapleton & Xia, 1997; Li, 1997; Li & Xue, 1997; Li, 1998; Ohrnberger, 1999). McClure (1940) established the genus *Sinocalamus* to include several Chinese bamboos which were intermediate between *Dendrocalamus* and *Bambusa* (e.g. *Sinocalamus latiflorus, S. oldhamii*). Phylogenetically, *Dendrocalamus, Gigantochloa* and *Bambusa* are grouped closely together (Watanabe *et al.,* 1994).

Infrageneric classification of *Dendrocalamus* is unclear. Most recent classifications are incongruent (Hsueh & Li, 1988a; Ohrnberger, 1999; Li & Stapleton, 2006f), see Table 3.1. Hsueh and Li (1988a) recognized two major subgroups namely: subgenus *Dendrocalamus* (autonym) and subgenus *Sinocalamus* Hsueh & Li. Subgenus *Dendrocalamus* contained two sections, *Dendrocalamus* (autonym) and *Bambusoidetes.* Subgenus *Sinocalamus* contained three sections namely: *Sinocalamus* (McClure) Hsueh & Li; *Draconicalamus* Hsueh & Li; and *Patellares* Hsueh & Li. All these names were invalidly published (i.e. without basionym, latin description, or type). However, they were later validated (Hsueh & Li, 1988b, 1989). Ohrnberger (1999) and Li and Stapleton (2006f) did not recognize section *Patellares* (see Table 3.1). The infrageneric classification of Ohrnberger (1999) was almost congruent with Hsueh & Li (1988a), except that he recognized only section level taxa (except *Patellares*). Li and Stapleton

(2006f) also recognized only two subgenera. However the specific make up of each of these *Dendrocalamus* subgenera was in conflict (see Table 3.1).

A few studies based on molecular phylogenetic analyses of *Dendrocalamus* and its allies have been made. Watanabe *et al.* (1994) using restriction fragment length polymorphisms (RFLP) and Loh *et al.* (2000) using amplified fragment length polymorphism (AFLP), both found that *Dendrocalamus* was placed together with *Bambusa* and *Gigantochloa*. Ni Chonghaile (2002) applied *trnL-F* and *rpl16* sequence data and showed that *Dendrocalamus* is related at least to some degree with *Bambusa, Gigantochloa, Neomicrocalamus, Orebambos, Racemobambos,* and *Sinocalamus.* Sun *et al.* (2005) using ITS nuclear rDNA sequence data found that *Dendrocalamus* was closely related to, and nested in, a polyphyletic *Bambusa.* In addition, Loh *et al.* (2000) and Ni Chonghaile (2002) have found a very surprising result where a palaeotropical woody bamboo species, *Dendrocalamus giganteus,* was found to have the least genetic similarity to any of palaeotropical woody bamboos examined (Loh *et al.*, 2000) and that this was sister to temperate woody bamboos (Ni Chonghaile, 2002). There is therefore a need for taxonomic stability in *Dendrocalamus* and its allies and it is hoped that molecular sequence data may help to clarify such classifications.

In this study the representatives of *Dendrocalamus* and its allies according to the classification system of Ohrnberger (1999) (see Table 3.2) were sequenced for five plastid DNA regions (*trn*L intron, *trnL-F* intergenic spacer (*trn*L-*F*), *atpB-rbc*L intergeneric spacer, *rps16* intron, and *matK* gene region), (the same as Chapter 2, this thesis; Sungkaew *et al.*, *in prep.*). The sequences were, then, analyzed in combination.

Table 3.1 Comparison of infrageneric classification systems of *Dendrocalamus*

Hsueh & Li (1988a)	**Ohrnberger (1999)**	**Li & Stapleton (2006f)**	List of *Dendrocalamus* species in the world (Ohrnberger, 1999) Note: *italic*=without infrageneric rank assigned
Subgenus Dendrocalamus	under Sect. Dendrocalamus	**Subgenus Dendrocalamus**	1. D. asper
Section Bambusoidetes	**Section Bambusoidetes**	not recognized	2. D. bambusoides
D. bambusoides	”	In Subg. Dendrocalamus	3. D. barbatus -var. internodiiradicatus
Section Dendrocalamus	**Section Dendrocalamus**	not recognized	4. D. birmanicus
D. barbatus	”	in Subg. Dendrocalamus	5. D. brandisii
D. birmanicus	”	”	6. D. calostachyus
D. membranaceus	”*	”	7. *D. cinctus*
D. strictus	”	”	8. *D. collettianus*
Subgenus Sinocalamus	under Sect. Sinocalamus	**Subgenus Sinocalamus**	9. *D. dumosus*
Section Draconicalamus	**Section Draconicalamus**	not recognized	10. *D. elegans*
D. calostachyus	”	in Subg. Sinocalamus	11. *D. farinosus* -f. flavostriatus
D. fugongensis	”	”	12. D. fugongensis
D. giganteus	”	”	13. D. giganteus
D. sikkimensis	”	in Subg. Dendrocalamus	14. D. hamiltonii -var. edulis -var. undulates
D. sinicus	”	in Subg. Sinocalamus	15. *D. hirtellus*
D. tibeticus	”	in Subg. Dendrocalamus	16. *D. hookeri*
Section Sinocalamus	**Section Sinocalamus**	not recognized	17. *D. inermis*
D. asper	”	in Subg. Dendrocalamus	18. D. jianshuiensis
D. brandisii	”	”	19. D. latiflorus -var. magnus -'Mei-Nung' -'Subconvex'
D. hamiltonii	”	”	20. *D. liboensis*
D. jianshuiensis	”	in Subg. Sinocalamus	21. *D. longifimbriatus*
D. latiflorus	”	”	22. *D. longispathus*
D. pachystachyus	”	”	23. *D. membranaceus* -f. striatus -f. pilosus -f. fimbriligulatus
D. parishii	”	uncertain placement	24. *D. merrillianus*
D. peculiaris	”	in Subg. Sinocalamus	25. *D. messeri*
D. semiscandens	”	in Subg. Dendrocalamus	26. D. minor -f. amoenus
D. tomentosus	”	in Subg. Sinocalamus	27. *D. multispiculatus*
D. yunnanicus	”	”	28. *D. nudus*

Table 3.1 (Continued)

Section Patellares	under genus Ampellocalamus	not recognized	29. *D. ovatus*
D. farinosus	not assigned	in Subg. Dendrocalamus	30. D. pachystachyus
D. liboensis	not assigned	”	31. D. parishii
D. mianningensis	under Ampelocalamus mianningensis	under Ampelocalamus mianningensis	32. D. peculiaris
D. minor	in Sect. Sinocalamus	in Subg. Sinocalamus	33. *D. pendulus*
D. patellaris	under Ampelocalamus patellaris	under Ampelocalamus patellaris	34. *D. poilanei*
D. pulverulentus	not assigned	in Subg. Dendrocalamus	35. *D. pulverulentus*
D. tsiangii	not assigned	”	36. *D. ronganensis*
			37. *D. rongchengensis*
			38. *D. sahnii*
			39. *D. sapidus*
			40. D. semiscandens
			41. D. sikkimensis
			42. D. sinicus
			43. *D. sinuatus*
			44. *D. somdejvai*
			45. D. strictus -var. sericeus -‘Argenteus’
			46. *D. textilis*
			47. D. tibeticus
			48. D. tomentosus
			49. *D. tsiangii* -f. striatus
			50. *D. wabo* (invalid name)
			51. D. yunnanicus

* No infrageneric rank assigned because the copy dead-line of this publication (Ohrnberger, 1999) was before receiving research paper of Stapleton & Xia (1997) in which this species is shown to be a species of *Bambusa*, not *Dendrocalamus*

3.1.2 Phenetic and phylogenetic studies of Thai *Dendrocalamus*

To revise *Dendrocalamus* for the Flora of Thailand project it is important to know not only how *Dendrocalamus* fits within the tribe Bambuseae, but also how its species relate to one another. In this study the morphological and molecular characters of Thai *Dendrocalamus* have been analyzed to study the infrageneric classification of *Dendrocalamus* and to study morphological character evolution in the group.

3.1.3 Population genetics of *D. membranaceus*

Dendrocalamus membranaceus is one of the most ecologically and economically important species of bamboo in Thailand (Lin, 1968; Dransfield & Widjaja, 1995b; Duriyaprapan & Jansen, 1995a). However, neither the genetic diversity nor the phylogeography of this species has been documented before. *Dendrocalamus membranaceus* is distributed from Burma to Yunnan, southern China to Laos, and throughout Thailand mainly in northern and northeastern parts (Ohrnberger, 1999). Relatively little work has been done at the population level of woody bamboos. However, some studies have been made such as using randomly amplified polymorphic DNA (RAPD) fingerprinting for studying *Yushania niitakayamensis* (Hsiao & Rieseberg, 1994) and *Phyllostachys pubescens* (Lai & Hsiao, 1997) in Taiwan; using amplified fragment length polymorphisms (AFLPs) and nuclear DNA microsatellites for studying *Guadua* spp. in Colombia (Marulanda *et al.*, 2002, 2007). To my knowledge, there are no published papers on the use of cpDNA microsatellites for population genetic applications in bamboos. Fortunately, several universal primer pairs for variable cpDNA microsatellite marker amplification in grasses have been developed and suggested to be useful for studying the population genetics of a range of grasses including bamboos (McGrath *et al.*, 2006, 2007).

Microsatellites, or simple sequence repeats (SSRs), are tandemly repeated sequences between 2-5 bp in length (Page & Holmes, 1998; Goldstein & Schlotterer, 1999). These short DNA sequence motifs are highly polymorphic as a result of a high variation of repeat copy number and they are also highly abundant (Tautz & Renz, 1984; Goldstein & Schlotterer, 1999; Weising & Gardner, 1999). Microsatellite can be easily detected by the polymerase Chain Reaction (PCR) using specific primer pairs which are designed from unique sequences flanking the microsatellite-containing regions (Tautz, 1989; Weber & May 1989; McGrath *et al.*, 2006). Since microsatellites have been found at high frequency in every organism (Schlotterer & Pemberton, 1998; Li *et al.*, 2002), and show high mutation rates (Tautz, 1989; Weber & May, 1989), microsatellites have become highly useful markers for investigating the population genetic structure and phylogeography of plant populations (Condit and Hubbell, 1991; Avise, 1994; Goldstein and Pollock, 1997; Sun *et al.*, 1998; McGrath *et al.*, 2007).

In the population and phylogenetic study of Thai *Dendrocalamus membranaceus*, I use cpDNA microsatellites to assess genetic diversity in eight populations from Thailand and one from the Yunnan, China. I test several cpDNA markers developed for grasses in general (McGrath *et al.*, 2006) and evaluate their utility for studies of bamboo population genetics and phylogeography. I investigate maternally mediated geneflow and study clonality in stands of Thai *D. membranaceus*.

Aims of the chapter:

The aims of this chapter were to use combined plastid DNA sequences and morphological characters to resolve the phylogenetic relationships between *Dendrocalamus* and its allies (Bambusinae), to study morphological character evolution in the group, and to evaluate the currently used infrageneric classifications for *Dendrocalamus*. It also aimed to study the genetic diversity and phylogeography of a selected ecologically and economically important species, *D. membranaceus* using cpDNA SSRs.

3.2 Materials and methods

3.2.1 Materials and methods for studying phylogenetics of *Dendrocalamus* and related genera

Plant materials

A total of 55 individuals from Bambuseae, including 41 species of Bambusinae, seven species of Melocanninae, and two species of Racemobambosinae according to the classification system of Ohrnberger (1999) were sampled (Table 3.2). Seven species of the subtribe Melocanninae were selected as an outgroup because they have been shown to be closely related but distinct to the subtribe Bambusinae (Chapter 2, this thesis; Sungkaew *et al.*, *in prep.*). Racemobambosinae was chosen because two of its species were imbedded in Bambusinae in the phylogenetic analysis of the subfamily Bambusoideae (Chapter 2, this thesis; Sungkaew *et al.*, *in prep.*). Details of sampled species and voucher specimens (numbers and herbaria) are presented in Table 3.2. *Dendrocalamus khoonmengii* has recently been described as a new species (Sungkaew *et al.*, *accepted*). *Phuphanochloa speciosa* has been included as it has also been described as a new genus (and a new species) (Sungkaew *et al.*, *accepted*). All DNA was extracted from fresh leaves preserved in silica gel (following Chase & Hills, 1991). All DNA samples were extracted by me except *Oreobambos buchwaldii*, by Ni Chonghaile (2002).

All the methods used for preparing the samples for phylogenetic studies in this Chapter are the same as Chapter 2.

Table 3.2 Taxa and vouchers of all sequences

Taxon	Voucher/Herbarium	Origin
Bambusinae		
Bambusa bambos (L.) Voss	SS&AT 030704-16/THNHM&KUFF	Thailand
Bambusa beecheyana Munro	Stapleton 1313/KEW	USA*, cultivated
Bambusa chungii McClure	Stapleton 1320/KEW	USA*, cultivated
Bambusa dolichomerithalla Hayata	Stapleton 1343/KEW	USA*, cultivated
Bambusa malingensis McClure	Stapleton 1332/KEW	USA*, cultivated
Bambusa oldhamii Munro	SS&AT 111/THNHM&KUFF	Thailand, cultivated
Bambusa oliveriana Gamble	Stapleton 1321/KEW	USA*, cultivated
Bambusa pachinensis Hayata	Stapleton 1333/KEW	USA*, cultivated
Bambusa tulda Roxburgh	Stapleton 1328/KEW	USA*, cultivated
Bambusa tuldoides Munro	Stapleton 1327/KEW	USA*, cultivated
Dendrocalamus asper (J.H. Schultes) Backer ex K.Heyne	BAM1[1]	Malaysia, cultivated
Dendrocalamus asper (J.H. Schultes) Backer ex K.Heyne	SS&AT 110704-1/THNHM&KUFF	Thailand, cultivated
Dendrocalamus asper (J.H. Schultes) Backer ex K.Heyne**	SS&AT 130704-5/THNHM&KUFF	Thailand, cultivated
Dendrocalamus asper (J.H. Schultes) Backer ex K.Heyne	SS&AT 378/THNHM&KUFF	Thailand, cultivated
Dendrocalamus barbatus Hsueh & D.Z. Li**	SS&AT 123/THNHM&KUFF	Thailand, cultivated
Dendrocalamus birmanicus A.Camus	SS&AT 622/THNHM&KUFF&TCD	China, cultivated
Dendrocalamus brandisii (Munro) Kurz**	SS&AT 260903-8/THNHM&KUFF	Thailand
Dendrocalamus copelandii (Gamble ex Brandis) N.H.Xia & Stapleton**	SS&AT 20/THNHM&KUFF&TCD	Thailand
Dendrocalamus dumosus (Ridley) Holttum**	SS&AT 389/THNHM&KUFF&TCD	Thailand
Dendrocalamus giganteus Munro**	BAM45[1]	Malaysia, cultivated
Dendrocalamus hamiltonii Nees & Arnott ex Munro**	SS&AT 787/THNHM&KUFF	Thailand
Dendrocalamus khoonmengii Sungkaew, A. Teerawatananon & Hodk.[2], **	SS&AT 257/THNHM&KUFF&TCD	Thailand
Dendrocalamus latiflorus Munro**	SS&AT 113/THNHM&KUFF	Thailand, cultivated
Dendrocalamus longispathus (Kurz) Kurz	Rashid s.n./TCD	Bangladesh
Dendrocalamus membranaceus Munro**	SS&AT 020704-4/THNHM&KUFF	Thailand
Dendrocalamus membranaceus Munro	SS&AT 612-1/THNHM&KUFF	Thailand
Dendrocalamus minor (McClure) Chia & H.L. Fung**	Stapleton 1317/KEW	USA*, cultivated
Dendrocalamus peculiaris Hsueh & D.Z. Li	SS&AT 618/THNHM&KUFF&TCD	China, cultivated
Dendrocalamus pendulus Ridley**	SS&AT 231/THNHM&KUFF	Thailand
Dendrocalamus sapidus Q.H. Dai & D.Y. Huang	SS&AT 628/THNHM&KUFF&TCD	China, cultivated
Dendrocalamus semiscandens Hsueh & D.Z. Li	SS&AT 620/THNHM&KUFF&TCD	China, cultivated
Dendrocalamus sinicus Chia & J.L. Sun**	SS&AT 127/THNHM&KUFF	Thailand, cultivated

Table 3.2 (Continued)

Taxon	Voucher/Herbarium	Origin
Dendrocalamus sinicus Chia & J.L. Sun	SS&AT 128/THNHM&KUFF	Thailand, cultivated
Dendrocalamus strictus (Roxburgh) Nees**	SS&AT 718/THNHM&KUFF	Thailand
Dendrocalamus yunnanicus Hsueh & D.Z. Li	Stapleton 1304/KEW	USA*, cultivated
Dendrocalamopsis valida (Q.H. Dai) W.T. Lin	SS&AT 625/THNHM&KUFF&TCD	China, cultivated
Gigantochloa albociliata Munro	SD 1436/KEW	Thailand
Gigantochloa atroviolacea Widjaja	Stapleton 1311/KEW	USA*, cultivated
Gigantochloa ligulata Gamble	SS&AT 090704-4/THNHM&KUFF	Thailand
Gigantochloa scortechinii Gamble	SS&AT 309/THNHM&KUFF	Singapore, cultivated
Melocalamus compactiflorus (Kurz) Bentham	SS&AT 175/THNHM&KUFF	Thailand
Neosinocalamus affinis (Rendle) P.C. Keng	SS&AT 624/THNHM&KUFF	China, cultivated
Oreobambos buchwaldii K. Schumann	Kare s.n./TCD	Uganda
Oxytenanthera abyssinica (A. Richard) Munro	Stapleton 1307/KEW	USA*, cultivated
Phuphanochloa speciosa Sungkaew & A. Teerawatananon[3]	SS&AT 191/THNHM&KUFF&TCD	Thailand
Thyrsostachys siamensis Gamble	SS&AT 020704-3/THNHM&KUFF	Thailand
Racemobambosinae		
Vietnamosasa ciliata (A. Camus) Nguyen	SS&AT 208/THNHM&KUFF	Thailand
Vietnamosasa pusilla (A. Chevalier & A. Camus) Nguyen	SD 1466/KEW	Thailand
Melocanninae		
Cephalostachyum pergracile Munro	SD 1435/KEW	Thailand
Neohouzeaua fimbriata S. Dransf., Pattan. & Sungkaew	SS&RP 12/KEW&BKF	Thailand
Neohouzeaua kerriana S. Dransf., Pattan. & Sungkaew	SS&RP 13/KEW&BKF	Thailand
Pseudostachyum polymorphum Munro	SS&AT 176/THNHM&KUFF	Thailand
Schizostachyum grande Ridley	SS&AT 100704-6/THNHM&KUFF	Thailand
Schizostachyum jaculans Holttum	SS&AT 307/THNHM&KUFF	Singapore, cultivated
Schizostachyum zollingeri Steudel	SS&AT 090704-1/THNHM&KUFF	Thailand

Remarks; Abbreviations are as follows; KEW, Kew herbarium, England; KUFF, Herbarium of Faculty of Forestry, Kasetsart University, Bangkok, Thailand; THNHM, Thailand Natural History Museum, National Science Museum, Techno Polis, Pathum Thani, Thailand; TCD, Herbium, School of Botany, Trinity College, Dublin, Ireland; SS, S. Sungkaew; AT, A. Teerawatananon; SD, S. Dransfield.

* California, United States of America

** Species and sequences, also used in phenetic and phylogenetic studies of Thai *Dendrocalamus*

[1] Bambusetum, Rimba Ilmu Botanic Garden, University of Malaya, Kuala Lumpur, Malaysia; specimen collected by K.M. Wong

[2] Currently described as a new species (Sungkaew *et al., accepted*)

[3] Being described as a new genus and a new species (Sungkaew *et al., accepted*)

Phylogenetic analysis

Successful DNA sequences were edited and assembled using AutoAssembler Software, version 2.1. The sequences were then imported to PAUP 4.0* Beta 2 (Swofford, 1998). Sequences were aligned by eye. Gaps were scored as additional binary characters (scoring gaps of identical size and position only). The resulting sequences were subjected to parsimony analysis using PAUP 4.0* Beta 2 (Swofford, 1998) using heuristic search options. Searches included 1,000 replicates of random stepwise addition saving no more than 100 trees for tree bisection reconstruction (TBR) branch swapping per replicate. Bootstrapping included 1,000 replicates and the same heuristic search settings as the individual searches (except that SPR branch swapping was used; following recommendations in Salamin *et al.* 2002).

3.2.2 Materials and methods for studying phenetic and phylogenetic relationships of Thai *Dendrocalamus* using morphological and molecular data

In the phenetic study, 12 morphological characters of 13 Thai *Dendrocalamus* and a Chinese species (*D. minor*) were used (see Table 3.3). *Dendrocalmus minor* is particularly included in order to test the validity of the *Dendrocalamus* subg. *Sinocalamus* sect. *Patellares* according to Hsueh and Li (1988a), see Table 3.1. All these characters were used in the species descriptions of Thai *Dendrocalamus* (Chapter 4). Each character was coded as a two-state character, Table 3.3. The binary matrix (Table 3.4) was then used in a PCO analysis (principal co-ordinates analysis; Gower, 1966) in the 'R package' (Legendre & Vaudor, 1991). This was performed using Jaccard's coefficient (Jaccard, 1908) in which shared presence of a character is weighted more heavily than shared absence. Other similarity or distance measures were tested such as Dice similarity and Euclidean distances. Varying the similarity or distance statistics did not change the conclusions obtained from the analyses with Jaccard similarity and are therefore not presented.

For the phylogenetic studies, matrices of both morphological characters (see Tables 3.3 and 3.4) and combined morphology and DNA sequences of five plastid DNA regions (*trnL-F*, *atpB-rbcL*, *rps16*, and *matK*), were used (see Table 3.2). The matrices were subjected to parsimony analysis using PAUP 4.0* Beta 2 (Swofford, 1998) using the same heuristic search parameters as those used for studying the phylogenetics of *Dendrocalamus* and related genera (see section 3.2.1).

Table 3.3 Characters and character states used in morphological phenetic and phylogenetic analyses of Thai *Dendrocalamus*

Characters	Character states
1# Clump habit	(0) loosely tufted (culms more or less well-spaced)
	(1) densely tufted (culms compact)
2# Mid-culm branch complement	(0) 1—3 branches dominant
	(1) more or less subequal branches
3# Lower culm internodes hairy or scurfy	(0) absent (glabrous)
	(1) present (hairy or scurfy)
4# Verticils of roots at lower culm nodes	(0) absent
	(1) present
5# Culm sheath deciduousness	(0) deciduous
	(1) tardily deciduous to persistent
6# Culm-sheath auricles with oral setae	(0) absent (without oral setae)
	(1) present (with oral setae)
7# Pseudospikelets congestion (synflorescences)	(0) globose clusters
	(1) stellate clusters
8# Pseudospikelets spiny to the touch	(0) absent (not spiny to the touch)
	(1) present (spiny to the touch)
9# Spikelets yellow-green	(0) absent (not yellow green)
	(1) present (yellow green)
10# Spikelet compression	(0) sub-terete
	(1) laterally compressed
11# Number of fertile florets/spikelet	(0) 1—4(—6), usually less than 4
	(1) 4—8, usually more than 4
12# Floret separability	(0) not separable (not patent or not open)
	(1) separable (more or less patent or open)

Table 3.4 Binary matrix of character states for morphological phenetic and phylogenetic analyses of Thai *Dendrocalamus*

Taxa/Characters*	1	2	3	4	5	6	7	8	9	10	11	12
1 *D. dumosus*	0	0	0	0	0	1	0	1	1	0	0	0
2 *D. khoonmengii*	0	0	0	0	0	1	?	?	?	?	?	?
3 *D. pendulus*	1	0	0	1	0	1	0	1	1	0	0	0
4 *D. strictus*	1	0	0	1	0/1	0/1	0	1	1	0	0	0
5 *D. barbatus*	0	0	1	1	0	1	0	1	1	0	0	0
6 *D. membranaceus*	0	0	0	0	0	1	0	1	1	0	0	0
7 *D. asper*	1	0	1	1	0	1	0	0	0	1	0	1
8 *D. brandisii*	0/1	0	1	1	0	1	0	0	0/1	1	0	1
9 *D. hamiltonii*	1	0	1	1	0/1	0	0	0	0	1	0	1
10 *D. latiflorus*	0/1	1	0	1	0	1	1	0	0	1	1	1
11 *D. copelandii*	1	1	0	0	1	0	1	0	0	1	1	0
12 *D. sinicus*	0/1	1	1	1	0/1	0	1	0	0	1	1	0
13 *D. giganteus*	1	1	0	1	0	0	1	0	0	1	1	0
14 *D. minor*	?	1	0	?	0	?	?	0	0	1	1	1

*Character states of each species based on species description of Thai *Dendrocalamus* (Chapter 4), except of *D. minor* were obtained from Li and Stapleton (2006f) and Clayton *et al.* (2006 onwards) (published on the internet: http://www.kew.org/data/grasses-db.html). The character state '0/1' is polymorphic and was either: 1) treated as 'missing data', or 2) treated separately so as to lead to the inclusion of multiple entries for individual species, in the phenetic analysis.

3.2.3 Materials and methods for studying the genetic diversity and phylogeography of *D. membranaceus*

Plant materials

45 samples from nine natural populations of *D. membranaceus* (Table 3.5 and Figure 3.11) were collected in silica gel following Chase & Hills (1991) to rapidly desiccate the material and minimize DNA degradation (Hodkinson *et al.*, 2007b). Five individuals of each population were sampled. Eight of nine populations were from Thailand and one was from Yunnan, China. Total genomic DNA was extracted using the same technique as used for the phylogenetic study (the modified CTAB method) (Doyle & Doyle, 1987; Hodkinson *et al.*, 2007b) (see Protocol 1). The crude tDNA was then washed, purified, and quantified, respectively (see Protocols 2, 3, and 4).

Microsatellite (SSR) amplification

The purified tDNA was then amplified using the polymerase chain reaction (PCR), carried out on an Applied Biosystems GeneAmp® PCR System 9700 (Protocol 5). Three loci of chloroplast SSRs were amplified using three primer pairs as described by McGrath *et al.* (2006) (see detail in Table 3.6). The PCR amplification protocol of all loci consisted of a pre-heat of 95°C for 5 min., and 35 cycles of the following: 95°C for 1 min of denaturation, 60°C for 1 min of annealing, 72°C for 1 min of extension. A final extension of 72°C for 10 min was also included.

Microsatellite genotyping

A 20 times dilution (by water) of each PCR product was made. 2 µl of this diluted sample was then combined with 0.25 µl of Gene Scan™ 500 Rox™ size standard and 24 µl of Hi-Di™ Formamide. Formamide is used to denature the DNA samples prior to genotyping on an ABI Prism™ 310 Genetic Analyzer (Applied Biosystems) (see Protocol 9) using POP4 polymer (Applied Biosystems). The samples were loaded on the machine using the run module GS STR POP4 (1.0-mL) A. After the samples were genotyped, the Applied Biosystems Genescan® Analysis Software version 3.1 was used to read and size each sample according to the internal lane size standard (ROX) and then processed using the Genotyper® Software Version 3.7 (Applied Biosystems).

Data analysis

The Genotyper® Software Version 3.7 was used to examine and label the fragments. The microsatellite peaks (alleles) from each locus were scored according to their size (base pairs). The scores were then transferred into a Microsoft Excel spreadsheet and formatted to produce a presence/absence matrix for the further analysis. The haplotypes were constructed by combining the allele data from the three loci *TeaSSR3, TeaSSR4, and TeaSSR5*. Haplotype sizes (in base pairs) were recorded and proportions of haplotypes from each population were detected and illustrated using pie charts. The geographic pattern of haplotype proportions for each population were represented by pie charts superimposed on a map of Thailand (including part of Yunnan Province, China).

Analysis into the extent of diversity within and among the populations from each location was carried out using the statistical software Popgene32 (Version 1.3.1) for population genetics (Yeh *et al.*, 1999). The diversity estimates included the percentage of polymorphic loci (*P*), observed number of alleles (*Na*), effective number of alleles (*Ne*), and the mean expected heterozygosity (*h*) (Nei, 1973). For estimating population diversity and differentiation, total gene diversity (H_t), gene diversity within populations (H_s), coefficients of gene differentiation (G_{st}), and estimates of gene flow (N_m) were made. The differentiation among populations was also illustrated by a UPGMA dendrogram based on Nei's unbiased genetic distances (1978).

Table 3.5 Details of sampled populations of *D. membranaceus*

Population numbers	Voucher specimens*	Locations
1	SS&AT 020704-4 to 020704-8	Suan Phung, Ratchaburi Provice, southwestern Thailand
2	SS&AT 50 to 54	Lan Sang National Park, Tak Province, northern Thailand
3	SS&AT 131 to 135	Wat Chan, Chiang Mai Province, northern Thailand
4	SS&AT 178 to 182	Phu Rue, Loei Province, northeastern Thailand
5	SS&AT 201 to 205	Nam Nao National Park, Phechabun Province, northeastern Thailand
6	SS&AT 209 to 213	Sakaerat Biosphere Reserve, Nakhon Ratchasima Province, eastern Thailand
7	SS&AT 271 to 275	Kui Buri, Prachuap Khiri Khan Province, southwestern Thailand
8	SS&AT 328 to 332	Phu Pieng, Nan Province, northern Thailand
9	SS&AT 612/1 to 612/5	Yunnan Province, China

* All housed in the Herbarium of the Thailand Natural History Museum, National Science Museum, Techno polis, Pathum Thani, Thailand (THNHM)

Abbreviations are as follows; AT, A. Teerawatananon; SS, S. Sungkaew

Table 3.6 Size ranges, position, and chloroplast genome region of SSR makers and allele number (from McGrath *et al.,* 2006)

cpSSR maker	Chloroplast genome region	Primer sequence F (5'-3') and R (5'-3')	Fluorescent dye	Repeat motif	Size range	Allele no.
TeaSSR3	*trn-L* intron and *trn-F* intergenic spacer region	AGGGACTTGAACCCTCACAA GCAAACGATTAATCATGGAACC	JOE	A_9	305-318	10
TeaSSR4	*23S-5S* internal transcribed spacer	ACGAACGAACGATTTGAACC TGAAGCCCCAATTCTTGACT	JOE	A_8	185-200	9
TeaSSR5	Herbicide binding protein D1 (*psbA*)	GCTATGCATGGTTCCTTGGT TTCCTACTACAGGCCAAGCAG	TAMRA	$(CTT)_3$	209-212	2

3.3 Results

3.3.1 Phylogenetics of *Dendrocalamus* and related genera (Bambuseae; Bambusoideae; Poaceae)

The aligned combined *trnL-F, atpB-rbcL, rps16*, and *matK* matrix was 4,243 bp long. Thirteen characters were excluded and of the remaining 4,230 characters, 4,136 were constant, 30 were variable but parsimony-uninformative and 64 were parsimony informative.

The tree search, using maximum parsimony, found 12 equally most parsimonious trees (Figure 3.1), each consisting of 101 steps. Consistency and retention indices (CI and RI) were 0.95 and 0.99 respectively. Bootstrap (BS) percentages (≥50%BS) are described as low (50–74%), moderate (75–84%), and high (85–100%). One of the equally most parsimonious trees is shown as a cladogram in Figure 3.1 and as a phylogram, with bootstrap values and strict consensus information, in Figure 3.2.

A group of *Dendrocalamus* species including other bamboos, *Bambusa oliveriana, Dendrocalamopsis valida, Gigantochloa atroviolacea, G. scortechinii,* and *Melocalamus compactiflorus*, is highly supported (100%BS). A group consisting of *Bambusa bambos, B. tulda, G. albociliata, G. ligulata, Thyrsostachys siamensis,* and two species of *Vietnambosasa* (*V. ciliata* and *V. pusilla*), has low support (62%BS). A group consisting of two sister genera, *Oreobambos* and *Oxytenanthera,* is highly supported (100%BS). *Phuphanochloa* is highly supported as sister (87%BS) to a group consisting of eight *Bambusa* species (86%BS; *N. affinis=B. emeiensis*).

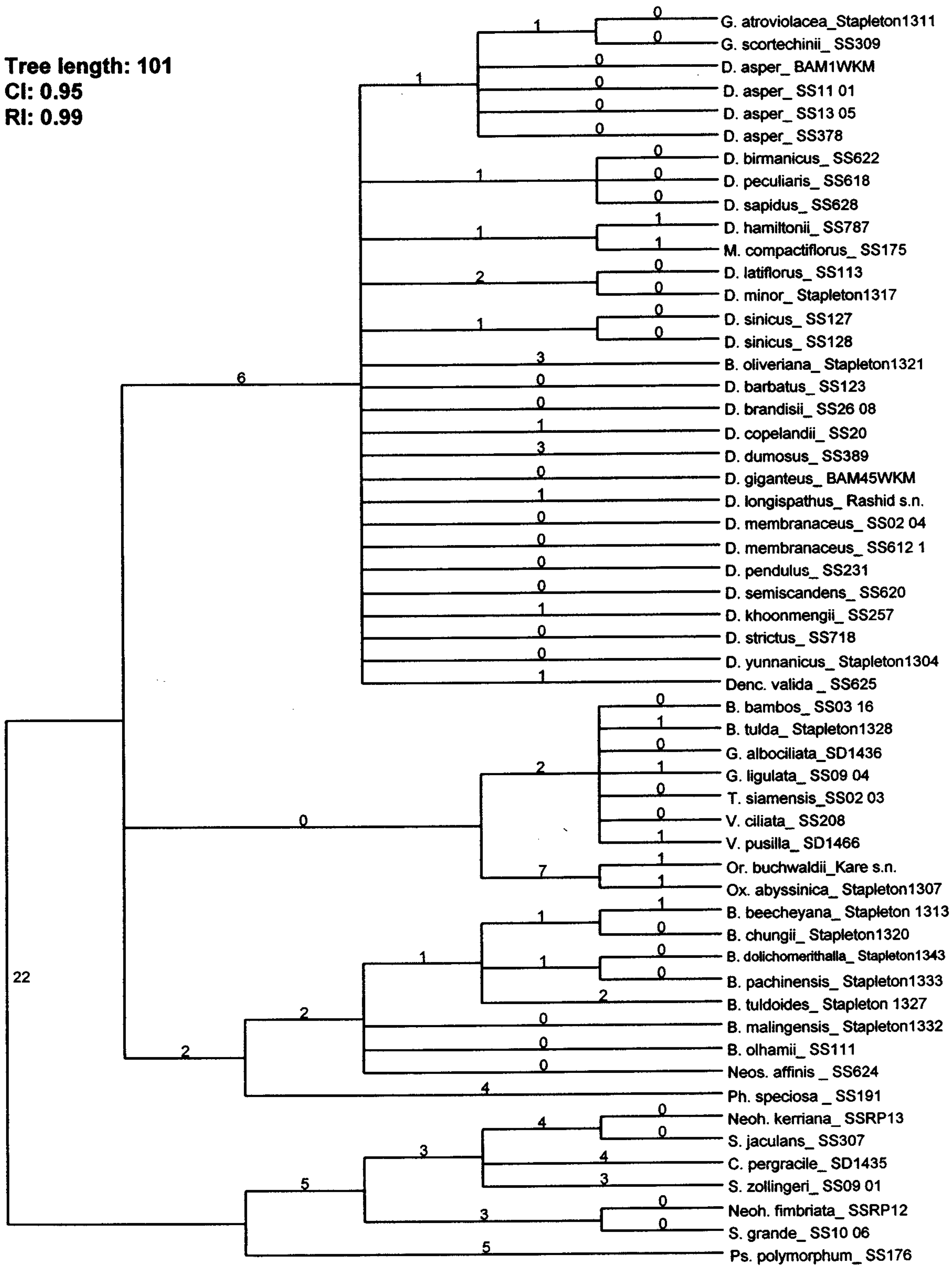


Figure 3.1 One of 12 equally most parsimonious trees shown as a cladogram obtained from comparative sequence analysis of the combined *trnL-F*, *atpB-rbcL*, *rps16*, and *matK* sequence data. Values above branches represent the number of steps supporting each branch. B=*Bambusa*; C=*Cephalostachyum*; D=*Dendrocalamus*; Denc=*Dendrocalamopsis*; G=*Gigantochloa*; M=*Melocalamus*; Neoh=*Neohouzeaua*; Neos=*Neosinocalamus*; Or=*Oreobambos*; Ox=*Oxytenanthera*; Ph=*Phuphanochloa*; Ps=*Pseudostachyum*; S=*Schizostachyum*; T=*Thyrsostachys*; V=*Vietnamosasa*; *D=Dendrocalamus*; *Drac=Draconicalamus*; *Pat=Patellares*; *Sino=Sinocalamus*.

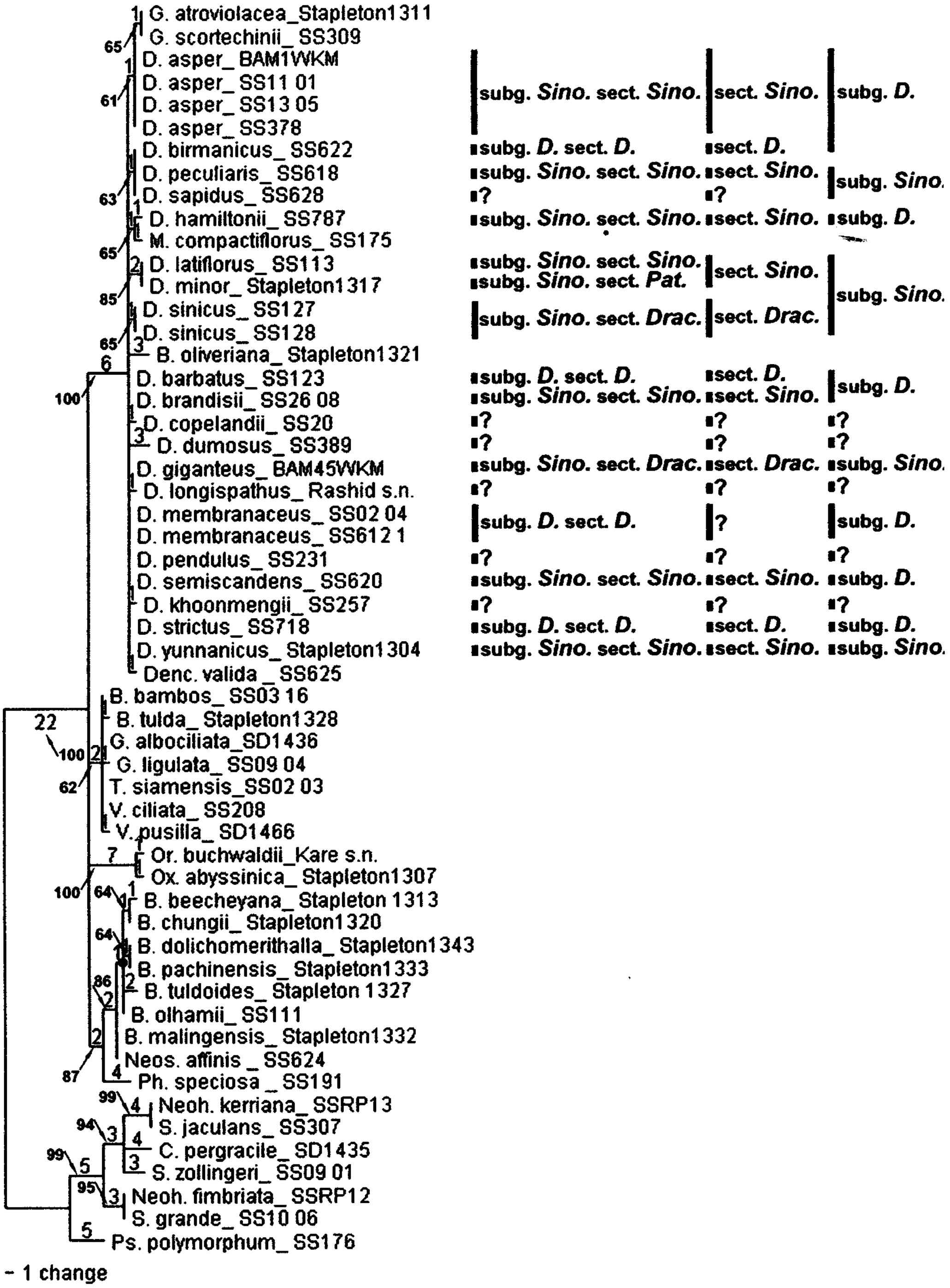


Figure 3.2 One of 12 equally most parsimonious trees shown as a phylogram obtained from comparative sequence analysis of the combined *trnL-F*, *atpB-rbcL*, *rps16*, and *matK* sequence data. Values above branches represent the number of steps supporting each branch. Values followed the arrows represent the percentage of bootstrap supporting each branch. Bold ball represents node not supported by the strict consensus. The three classification systems of *Dendrocalamus* according to Li and Stapleton (2006f), Ohrnberger (1999), and Hsueh and Li (1988a), are shown as the columns from right to left, respectively. B=*Bambusa*; C=*Cephalostachyum*; D=*Dendrocalamus*; Denc=*Dendrocalamopsis*; G=*Gigantochloa*; M=*Melocalamus*; Neoh=*Neohouzeaua*; Neos=*Neosinocalamus*; Or=*Oreobambos*; Ox=*Oxytenanthera*; Ph=*Phuphanochloa*; Ps=*Pseudostachyum*; S=*Schizostachyum*; T=*Thyrsostachys*; V=*Vietnamosasa*; *D=Dendrocalamus*; *Drac=Draconicalamus*; *Pat=Patellares*; *Sino=Sinocalamus*.

3.3.2 Phenetic and phylogenetic studies of Thai *Dendrocalamus*

3.3.2.1 Phenetic study of Thai *Dendrocalamus*

When the character state '0/1' (polymorphic, see Table 3.4) was treated as 'missing data', the Eigenvalues and the Eigenvectors from PCO analysis (Gower, 1966) using Jaccard's coefficient (Jaccard, 1908) are shown in the Tables 3.7 and 3.8 respectively. A scatter plot of the Eigenvectors of axis 1 against axis 2 is shown in Figure 3.3. Four groups of Thai *Dendrocalamus* species can be detected from the PCO analysis (Figure 3.3). The first is a '*D. strictus* Group', consisting of three species namely *D. barbatus, D. pendulus,* and *D. strictus.* The second is a '*D. membranaceus* Group' including three species, *D. dumosus, D. khoonmengii,* and *D. membranaceus.* The third is a '*D. asper* Group', consisting of three species namely *D. asper, D. brandisii,* and *D. hamiltonii.* The fourth group is a '*D. giganteus* Group', composed of five species, *D. copelandii, D. giganteus, D. latiflorus, D. minor,* and *D. sinicus.*

Scatter plots of the Eigenvectors of axis 1 against axis 3 and axis 2 against axis 3 are also provided in Figures 3.4 and 3.5, respectively. These do not provide any further clarification of the relationships among these taxa.

When the character state '0/1' (polymorphic) was treated separately so as to lead to the inclusion of multiple entries for individual species, the Eigenvalues and the Eigenvectors from the PCO analysis are shown in the Tables 3.9 and 3.10 respectively. A scatter plot of the Eigenvectors of axis 1 against axis 2 is shown in Figure 3.6. Another two scatter plots of the Eigenvectors of axis 1 against axis 3 and axis 2 against axis 3 are also provided in Figures 3.7 and 3.8, respectively; as before, only the plot of axis 1 and 2 provides clarification of the relationships among the taxa. In this case, there are only two groups of Thai *Dendrocalamus* species can be recognized from the PCO analysis (Figure 3.6). The first is Group A which is equal to the '*D. strictus* Group' plus the '*D. membranaceus* Group' of Figure 3.3. The second is Group B which is equal to the '*D. asper* Group' plus the '*D. giganteus* Group' of Figure 3.3.

Table 3.7 Axes, Eigenvalues, percentage of variance, and cumulative percentage of variance from PCO of morphological data of Thai *Dendrocalamus*

Axis	Eigenvalues	% of variance	cumulative %
1	2.11749	34.87092	34.87%
2	0.76873	15.10506	49.98%
3	0.69394	14.00909	63.99%
4	0.17114	6.34757	70.33%
5	0.1061	5.39438	75.73%
6	0.08237	5.04664	80.77%
7	0	3.83947	84.61%
8	0	3.67796	88.29%
9	-0.01102	3.43225	91.72%
10	-0.02779	3.15462	94.88%
11	-0.04673	2.68532	97.56%
12	-0.07876	2.43672	100.00%
13	-0.09572	0	100.00%
14	-0.26199	0	100.00%

Note: the character state '0/1' (polymorphic) was treated as 'missing data'.

Table 3.8 Taxa and Eigenvectors of each axis from the PCO analysis of the morphological matrix of Thai *Dendrocalamus*

Taxa	Axis 1	Axis 2	Axis 3
1	-0.1246	-0.2925	0.2177
2	0.3747	-0.1946	-0.0295
3	-0.0677	-0.2769	0.3376
4	-0.423	0.2853	-0.2412
5	0.5514	0.251	-0.026
6	-0.4272	0.1151	-0.2212
7	-0.2685	-0.3993	0.0773
8	-0.3042	0.1766	0.1553
9	0.543	0.1981	-0.026
10	-0.5011	0.1689	0.0843
11	0.3649	-0.1302	-0.1904
12	-0.4285	0.0528	-0.0868
13	0.4162	0.345	0.3874
14	0.2862	-0.2263	-0.4384

Note: the character state '0/1' (polymorphic) was treated as 'missing data'.

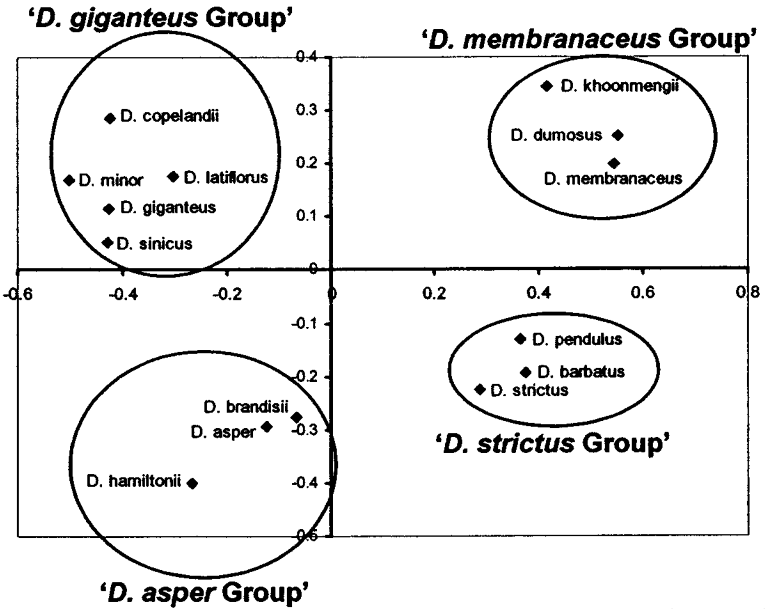


Figure 3.3 Scatter plot showing the relationship of Eigenvectors of axis 1 against axis 2. Four groups of Thai *Dendrocalamus* species can be detected.

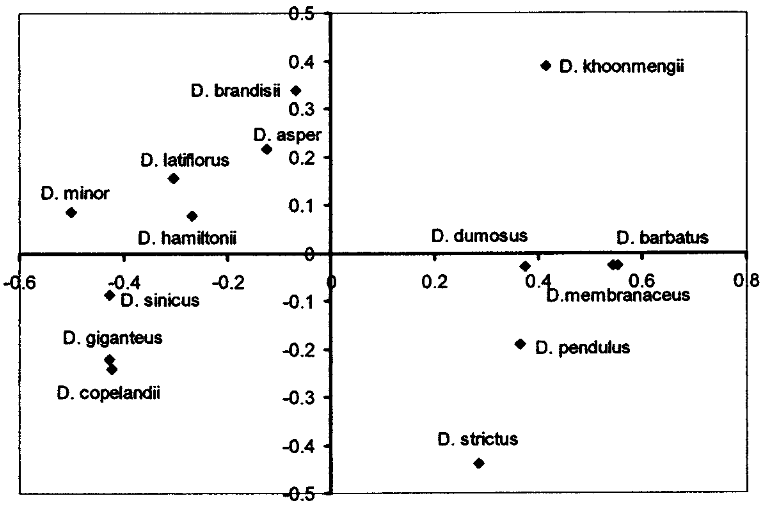


Figure 3.4 Scatter plot showing the relationship of Eigenvectors of axis 1 against axis 3.

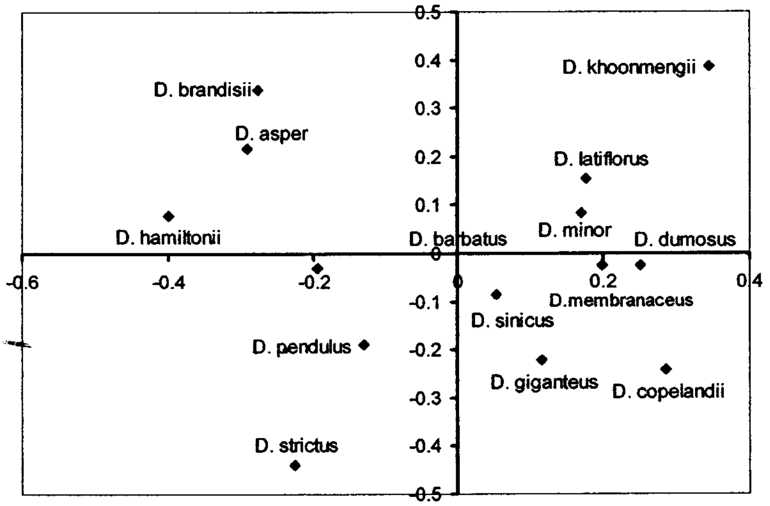


Figure 3.5 Scatter plot showing the relationship of Eigenvectors of axis 2 against axis 3.

Table 3.9 Axes, Eigenvalues, percentage of variance, and cumulative percentage of variance from PCO of morphological data of Thai *Dendrocalamus*

Axis	Eigenvalues	% of variance	cumulative %
1	3.01932	27.75045	27.75%
2	1.28811	13.08975	40.84%
3	1.13074	11.75708	52.60%
4	0.38395	5.43289	58.03%
5	0.29508	4.68027	62.71%
6	0.16694	3.59512	66.31%
7	0.09465	2.98298	69.29%
8	0.03422	2.47122	71.76%
9	0	2.18143	73.94%
10	0	2.18143	76.12%
11	0	2.18143	78.30%
12	0	2.08317	80.39%
13	-0.0116	2.07091	82.46%
14	-0.01305	2.02015	84.48%
15	-0.01905	1.96677	86.45%
16	-0.02535	1.91392	88.36%
17	-0.03159	1.89766	90.26%
18	-0.03351	1.86431	92.12%
19	-0.03745	1.82498	93.95%
20	-0.04209	1.69462	95.64%
21	-0.05748	1.59927	97.24%
22	-0.06874	1.50123	98.74%
23	-0.08032	1.25895	100.00%
24	-0.10893	0	100.00%
25	-0.25759	0	100.00%

Note: the character state '0/1' (polymorphic) was treated separately as 'multiple entries'.

Table 3.10 Taxa and Eigenvectors of each axis from the PCO analysis of the morphological matrix of Thai *Dendrocalamus*

Taxa	Axis 1	Axis 2	Axis 3
1	0.0778	-0.3092	-0.1494
2	-0.3942	-0.0631	-0.0358
3	0.0762	-0.4377	-0.0154
4	-0.0643	-0.3527	-0.035
5	0.0778	-0.3092	-0.1494
6	-0.0491	-0.246	-0.1682
7	0.3721	0.367	0.1402
8	-0.5726	0.0598	0.2903
9	0.369	0.2687	0.0654
10	0.2015	-0.2275	-0.3157
11	0.1975	-0.1268	-0.3349
12	0.2745	-0.0674	0.2971
13	0.2664	-0.0006	0.1445
14	-0.5726	0.0598	0.2903
15	0.4812	-0.0405	0.2233
16	-0.429	0.1103	-0.089
17	0.4039	0.0959	0.1172
18	0.3844	0.1706	0.071
19	0.3765	0.1428	-0.0136
20	0.3599	0.2089	-0.0484
21	-0.3959	-0.2432	0.5194
22	-0.3456	0.2852	-0.2768
23	-0.429	0.1103	-0.089
24	-0.2929	0.3491	-0.2974
25	-0.3735	0.1922	-0.1407

Note: the character state '0/1' (polymorphic) was treated separately as 'multiple entries'.

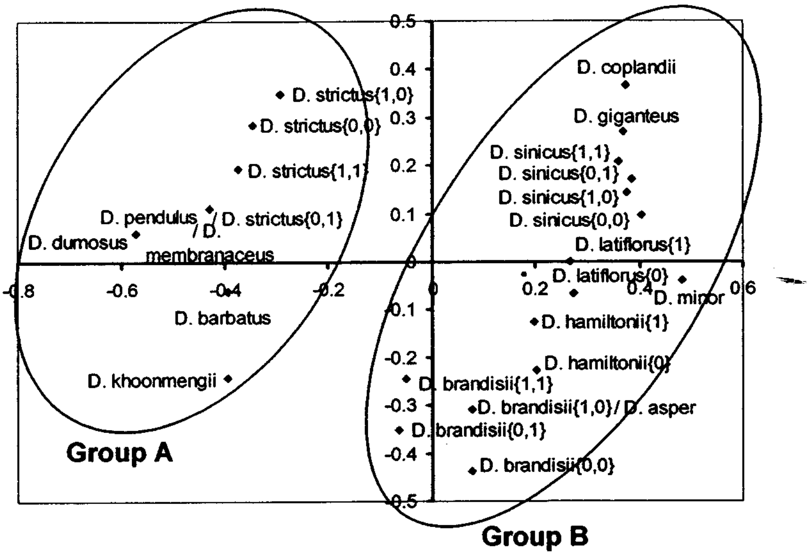


Figure 3.6 Scatter plot showing the relationship of Eigenvectors of axis 1 against axis 2. Two groups of Thai *Dendrocalamus* species can be detected.

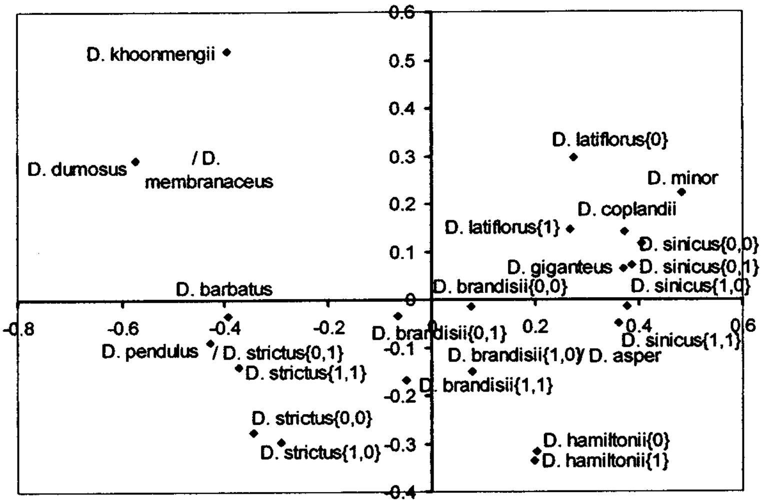


Figure 3.7 Scatter plot showing the relationship of Eigenvectors of axis 1 against axis 3.

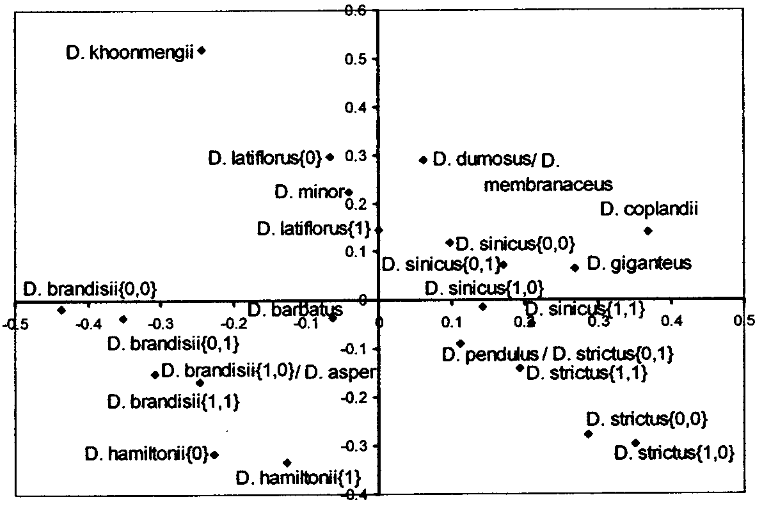


Figure 3.8 Scatter plot showing the relationship of Eigenvectors of axis 2 against axis 3.

3.3.2.2 Phylogenetic study of Thai *Dendrocalamus*

3.3.2.2.1 Phylogenetic study of Thai *Dendrocalamus* based on the morphological characters

The matrix of 12 morphological characters was analyzed. One character was variable but parsimony-uninformative and 11 were parsimony informative.

The tree search using maximum parsimony found six equally most parsimonious trees, each consisting of 17 steps. Consistency and retention indices (CI and RI) were 0.70 and 0.87 respectively. Bootstrap (BS) percentages (≥50%BS) are described as low (50–74%), moderate (75–84%), and high (85–100%). One of the equally most parsimonious trees is shown as an unrooted cladogram in Figure 3.9 with bootstrap values and strict consensus tree information.

Similar to the PCO analyses, parsimony analyses using morphological characters only revealed three groups of *Dendrocalamus* (Figure 3.9). The '*D. strictus* Group' together with the '*D. membranaceus* Group', as found in the PCO analysis, is supported by strict consensus and with high support (90%BS). The '*D. asper* Group', is present in the strict consensus tree but has no bootstrap support. The '*D. giganteus* Group' is supported by strict consensus and with 75%BS. The '*D. membranaceus* Group' is found in the strict consensus but with low bootstrap (65%BS). The '*D. strictus* Group' is unresolved because *D. pendulus* and *D. strictus* appeared to be polytomies and are the two outlining taxa to a group consisting of *D. barbatus* and the '*D. membranaceus* Group'. *Dendrocalamus barbatus* grouped with the '*D. membranaceus* Group' with 52% bootstrap support. Within the '*D. giganteus* Group', a subgroup consisting of two sister species, *D. latiflorus* and *D. minor*, is not present in strict consensus tree.

3.3.2.2.2 Phylogenetic study of Thai *Dendrocalamus* based on the combined morphological and molecular characters

The aligned combined *trnL-F*, *atpB-rbcL*, *rps16*, and *matK* and 12 morphological character matrix was 4,193 characters in total. 4,172 characters were constant, 8 were variable but parsimony-uninformative and 13 were parsimony informative. There was only two additional parsimony informative characters from the previous analysis.

The tree search using maximum parsimony found 2 equally most parsimonious trees, each consisting of 26 steps. Consistency and retention indices (CI and RI) were 0.81 and 0.88 respectively. Bootstrap (BS) percentages (≥50%BS) are described as low (50–74%), moderate (75–84%), and high (85–100%). One of the equally most parsimonious trees is shown as an unrooted cladogram in Figure 3.10 with bootstrap values and strict consensus information.

The results either using morphological characters alone or from the combined analysis divided *Dendrocalamus* species into three identical groups. The tree obtained from the combined analysis gave similar results to the PCO analyses. The '*D. strictus* Group' combined with the '*D. membranaceus* Group' possesses high bootstrap (87%BS). The '*D. asper* Group' is present in the strict consensus but has no bootstrap support. The '*D. giganteus* Group' is supported by strict consensus and with moderate bootstrap (74%BS). The '*D. membranaceus* Group' is also found in the strict consensus but with low bootstrap (64%BS). However, the '*D. strictus* Group' is unresolved because *D. pendulus* and *D. strictus* are the two outlining taxa to a group consisting of *D. barbatus* and the '*D. membranaceus* Group'. In addition, within the '*D. giganteus* Group', the sister group relationship between *D. latiflorus* and *D. minor*, is highly supported by 92%BS.

CI and RI values are the same for morphological tree and combined morphological and molecular tree as the two trees have the same topology (but differing branch lengths). Mapping of some important morphological and habit character transitions onto one of the two equally most parsimonious combined morphological and molecular tree is shown in Figure 3.11. The two equally most parsimonious trees only differed in a minor way from each other (from *D. giganteus*, in the second tree, it was one step before leading to a group consisting of *D. copelandii* and *D. sinicus*).

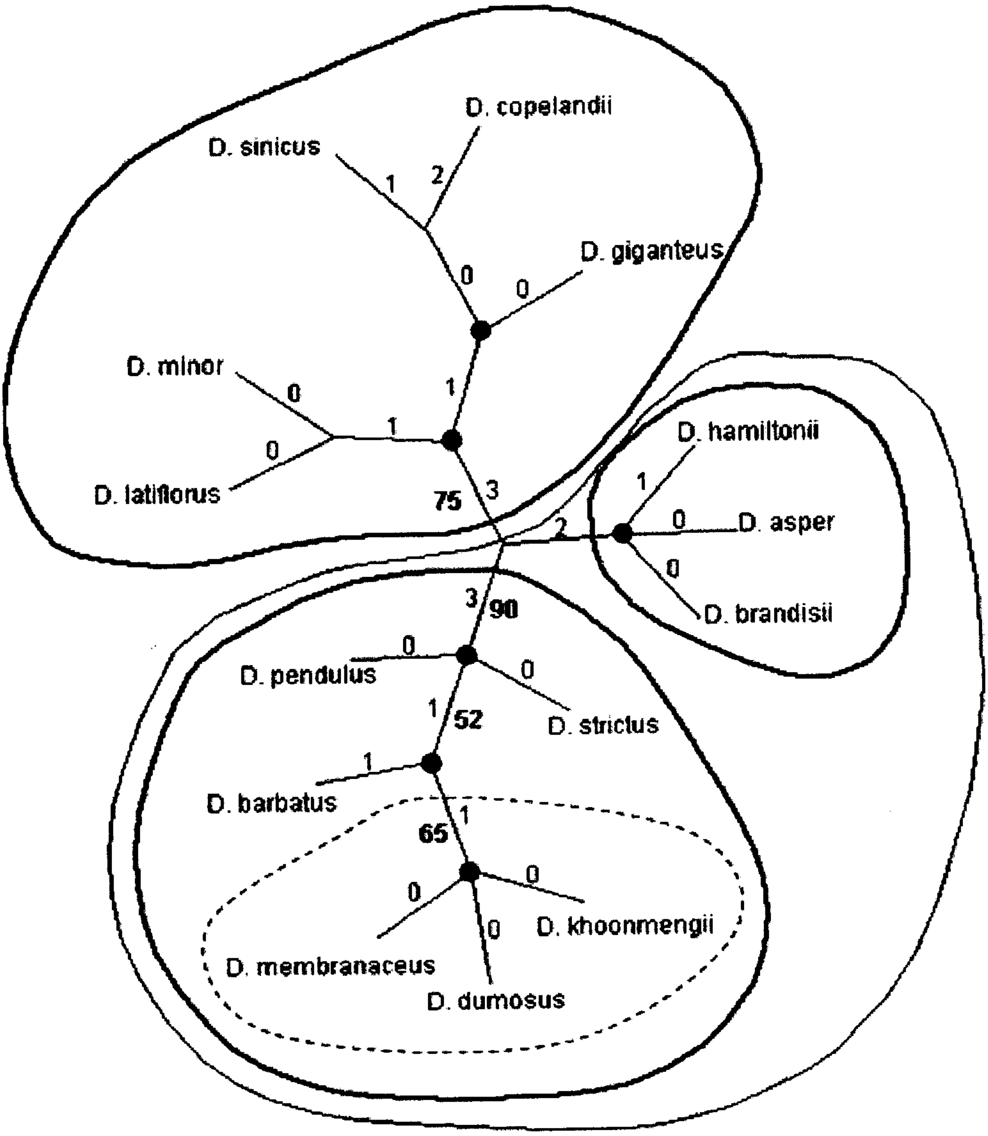


Figure 3.9 One of 6 equally most parsimonious trees shown as an unrooted cladogram obtained from the morphological data matrix. Values above branches represent the number of steps supporting each branch. Values below branches (bold figures) represent the percentages of bootstrap supporting each branch. Bold balls represent nodes supported by the strict consensus. Three groups are defined by this study. The dashed loop represents the '*D. membranaceus* Group'. The group demarcated by the thin line includes the '*D. strictus* Group' and '*D.* asper Group' and represents *Dendrocalamus* subg. *Dendrocalamus* according to Li and Stapleton (2006f)

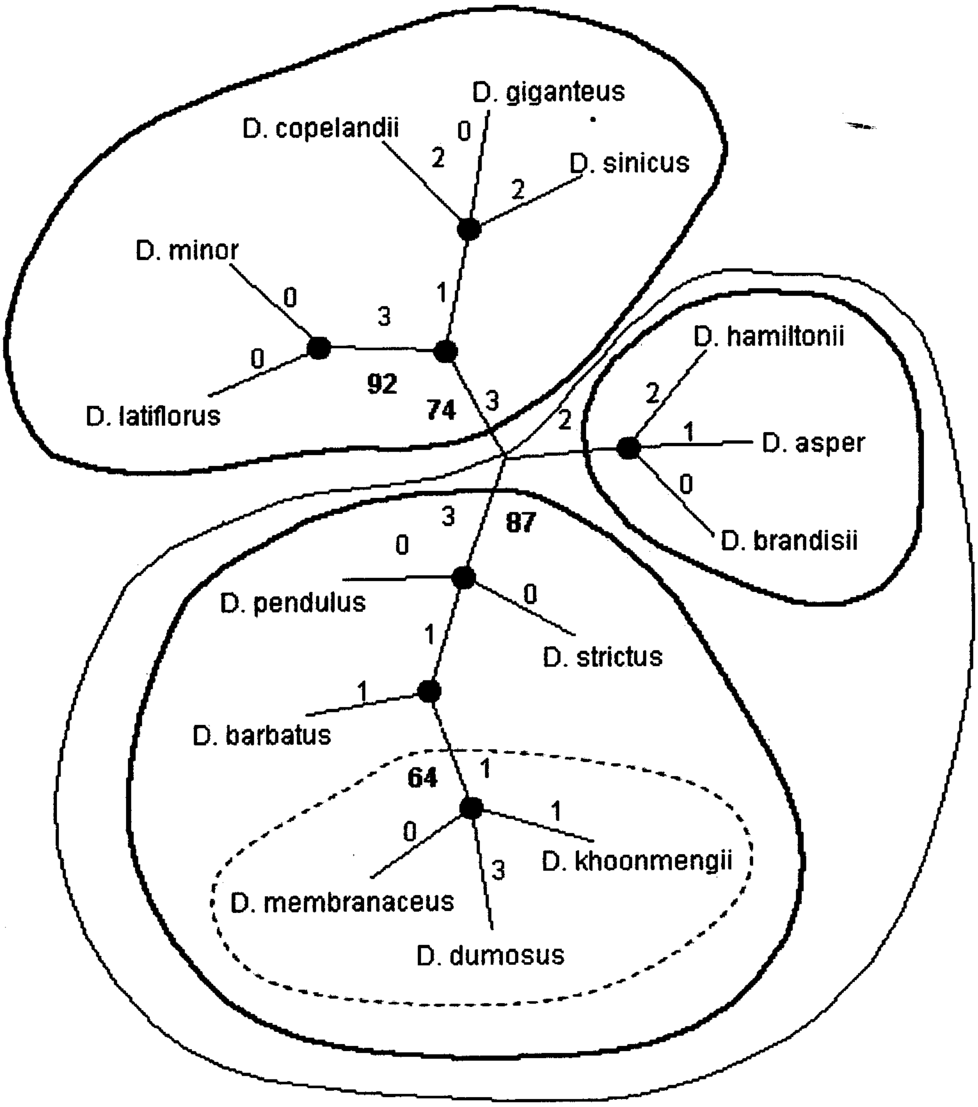


Figure 3.10 One of 2 equally most parsimonious trees shown as an unrooted cladogram obtained from the morphological and molecular sequence data. Values above branches represent the number of steps supporting each branch. Values below branches (bold figures) represent the percentages of bootstrap supporting each branch. Bold balls represent nodes supported by the strict consensus tree. Three major groups are identified. The dashed loop represents the '*D. membranaceus* Group'. The thin line demarcating the '*D. strictus* Group' and '*D.* asper Group' also represents *Dendrocalamus* subg. *Dendrocalamus* (Li & Stapleton, 2006f)

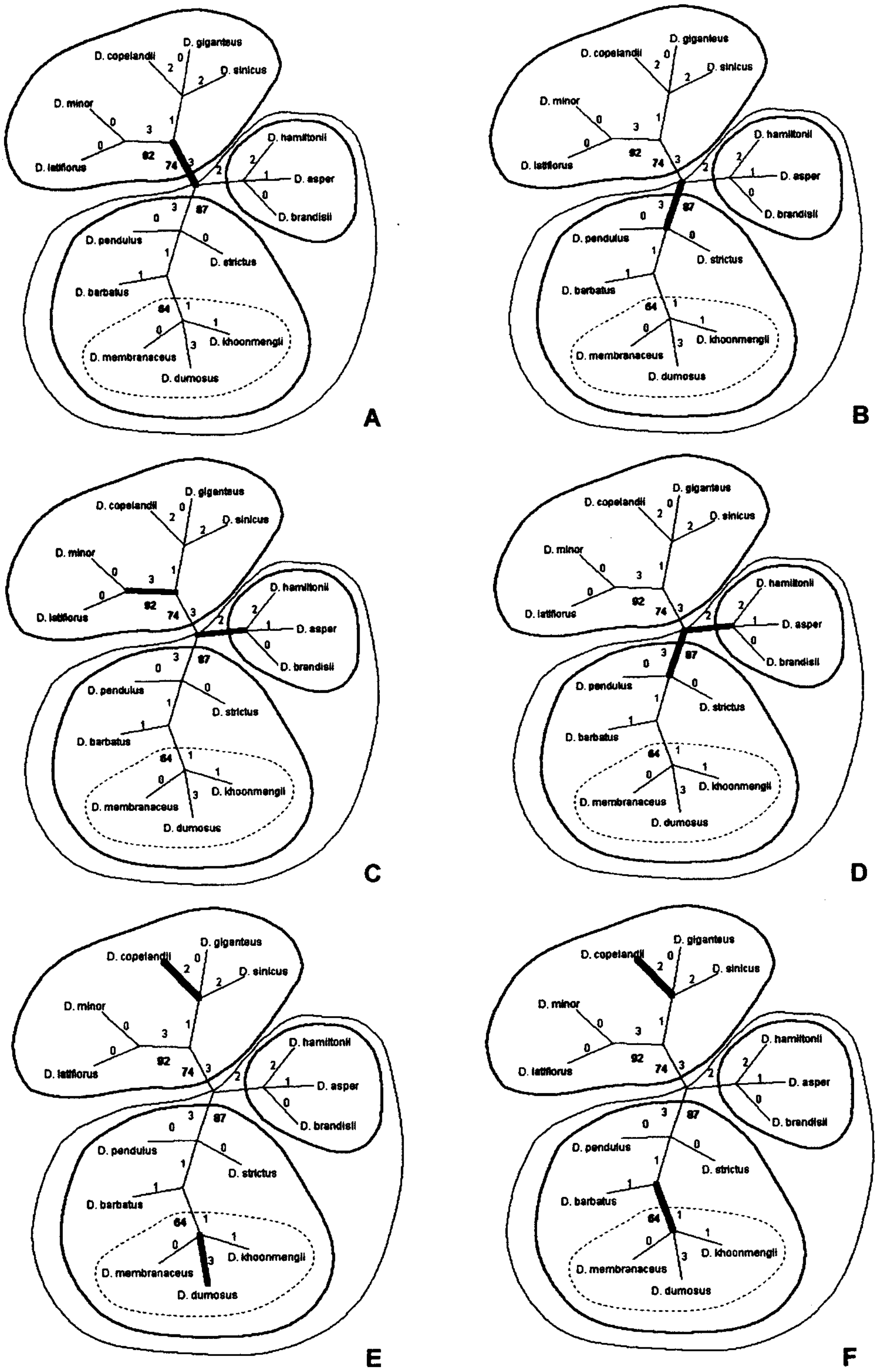


Figure 3.11 Morphological and habitat character states mapped onto a single most parsimonious combined morphological and molecular tree. Thick bars represent character state changes. A. mid-culm branch complement [changing from 1—3 branches dominant→more or less subequal branches]; B. pseudospikelets spiny to the touch [changing from not spiny to the touch→spiny to the touch]; C. floret separability [changing from not patent or not open→more or less patent or open)]; D. mid-culm branch complement [changing from more or less subequal branches→1—3 branches dominant]; E. plants distributed on limestone areas [changing from other kinds of habitat→confined to the limestone areas]; F. lower culm internodes hairy or scurfy [changing from hairy or scurfy→glabrous]

3.3.3 Population genetics of *D. membranaceus*

Assessment of allele lengths at three loci of microsatellites amplified using the three primer pairs of McGrath *et al.* (2006), *TeaSSR3, TeaSSR4,* and *TeaSSR5* (see Table 3.6), were made to study plastid genome variation of nine populations (a total of 45 individuals) of *D. membranaceus.*

A total of six different alleles were found in *D. membranaceus* populations. Two alleles at each locus were detected, see Table 3.11. At *TeaSSR3*, the most frequent allele was Allele 2, which was found in all nine populations at frequency of 0.956. At *TeaSSR4,* the most frequent allele was Allele 4, which had a frequency of 0.689, and this was also present in all populations. For *TeaSSR5*, the most frequent allele was Allele 5, with the frequency of 0.622, and this was detected in all populations except the Phu Rue population (see Table 3.5 and Figure 3.17).

Table 3.11 Alleles detected from three chloroplast microsatellite loci over all nine populations of *D. membranaceus*, including size range in base pairs and frequency.

Locus		Size in base pairs	Frequency
		(*trn*-L intron and *trn-F* intergenic spacer region)	
TeaSSR3	Alleles 1	301	0.044
	Alleles 2	302	0.956
		(*23S-5S* spacer)	
TeaSSR4	Alleles 3	195	0.311
	Alleles 4	196	0.689
		(Herbicide binding protein D1 (*psbA*))	
TeaSSR5	Alleles 5	212	0.622
	Alleles 6	213	0.378

The overall gene diversity within *D. membranaceus* populations detected using each of the alleles was estimated, according to Nei (1973), to be 32.8%. *TeaSSR3* detected 8.5% diversity, *TeaSSR4* detected 42.9% diversity and *TeaSSR5* detected 47% (Table 3.12).

Table 3.12 Overall descriptive statistics and mean gene diversity estimates (Nei, 1973) per locus from nine *D. membranaceus* populations.

Locus	Sample size	Allele Number	Range of Sizes Detected (base pairs)	Gene Diversity (*h*)
TeaSSR3	45	2	301-302	0.085
TeaSSR4	45	2	195-196	0.429
TeaSSR5	45	2	212-213	0.470

A summary of the genetic diversity parameters is given in Table 3.13. Based on the number of alleles and the estimated gene diversity from three microsatellite regions, Nam Nao was the most diverse population among the nine populations, followed by Phu Rue. For comparisons between *D. membranaceus* groups, Group 1 was the most diverse (Table 3.13; 100% polymorphic loci; gene diversity 0.33).

Table 3.13 Summary of genetic variation, based on three SSR loci within populations and groups of *D. membranaceus*

Population	Observed number of alleles (*Na*) averaged across the three loci	Effective number of alleles (*Ne*)	Gene Diversity (*h*)	Percentage of polymorphic loci (*P*)
ID1=Suan Phung	1.00	1.00	0	0
ID2=Lan Sang	1.33	1.31	0.16	33.33
ID3=Wat Chan	1.33	1.31	0.16	33.33
ID4=Phu Rue	1.67	1.46	0.27	66. 67
ID5=Nam Nao	1.67	1.61	0.32	66. 67

Table 3.13 (Continued)

Population	Observed number of alleles (*Na*) averaged across the three loci	Effective number of alleles (*Ne*)	Gene Diversity (*h*)	Percentage of polymorphic loci (*P*)
ID6=Sakaerat	1.33	1.16	0.11	33.3
ID7=Kui Buri	1.33	1.16	0.11	33.3
ID8=Phu Phieng	1.33	1.16	0.11	33.3
ID9=Yunnan	1.33	1.31	0.16	33.3
Mean	**1.37**	**1.28**	**0.15**	**22.21**
*Group1=un-isolated Thai population	2.00	1.58	0.33	100
Group2=isolated Thai population	1.33	1.16	0.11	33.33
Group3=Yunnan population	1.33	1.31	0.16	33.33
Mean	**1.55**	**1.35**	**0.20**	**55.55**

* The three groups were defined arbitrarily based on geography.

A total of six haplotypes were detected using the combined allelic information from the three microsatellite loci. The frequency of each of the haplotypes detected within each of the *D. membranaceus* populations is given in Table 3.14. The haplotypes detected have been geographically mapped and are represented in Figure 3.12. All populations, except a population from Suan Phung, were polymorphic. The most common haplotype within the population was found to be 'Haplotype 1' which occurred at frequency of 0.38. Haplotype 1 was found to occur within all locations except Phu Rue, while Suan Phung was found to be monomorphic for this haplotype. 'Haplotype 3' and 'Haplotype 4' were the rarest two haplotypes, these two can be found only in the Phu Rue population.

Table 3.14 Haplotypes detected of *D. membranaceus* in Thailand.

Haplotype	Allele combination for the three loci (*TeaSSR3, TeaSSR4, TeaSSR5)*	Frequency
1	302, 196, 212	0.38
2	302, 196, 213	0.29
3	301, 196, 213	0.02
4	301, 195, 213	0.02
5	302, 195, 213	0.04
6	302, 195, 212	0.24

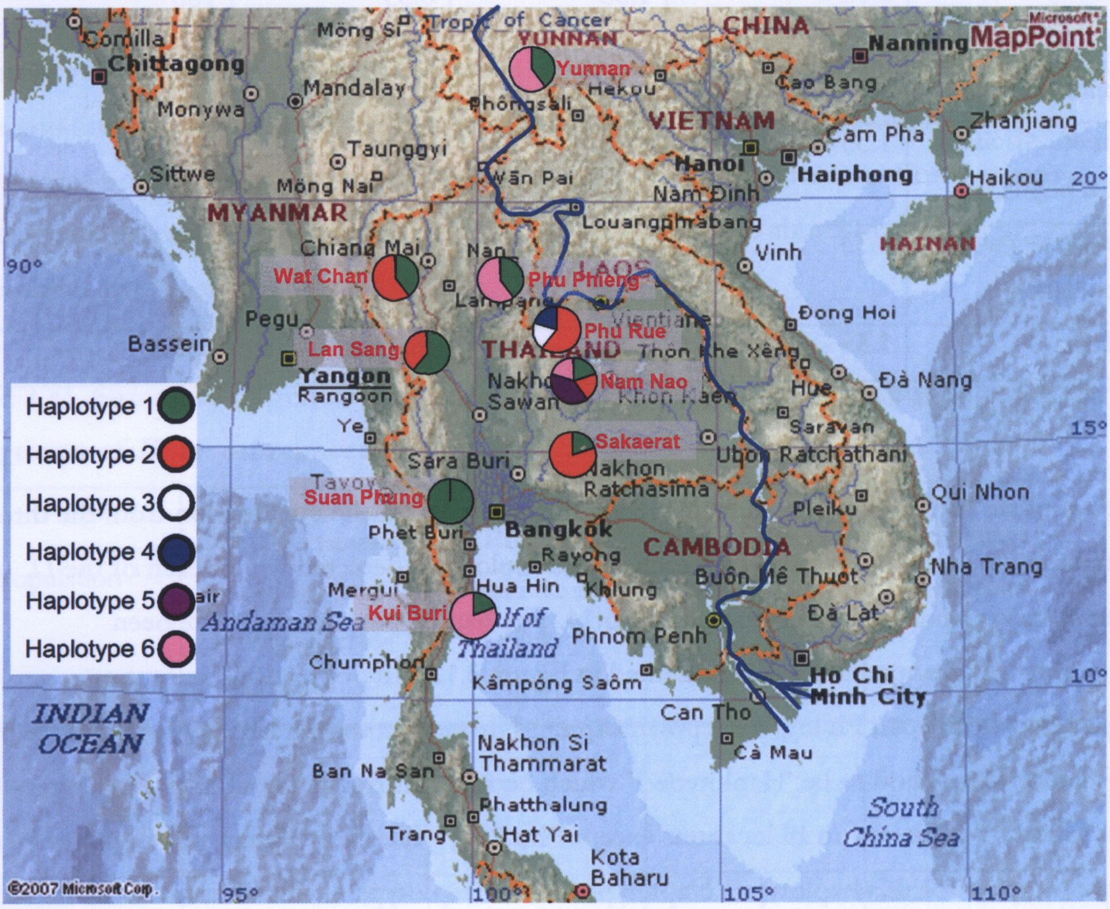


Figure 3.12 Frequency of chloroplast DNA haplotypes of *D. membranaceus* in Thailand. The blue line represents the Mekong River. See Figure 4.15 (Chapter 4, this thesis) for a map of the total geographical range of this species. It is also known from a few sites outside Thailand including Burma, China and northern Vietnam.

The extent of chloroplast diversity within and among *D. membranaceus* populations estimated using H_T (total gene diversity), H_s (within population gene diversity) and G_{ST} (gene differentiation among the populations) is shown in Table 3.15. The mean value of the within population gene diversity was 29% and the total genetic diversity was 32.8 % respectively, while the mean estimation of the coefficient of gene differentiation (G_{ST}) was 0.086 and the mean estimation of gene flow was 14.922.

Table 3.15 Nei (1987) genetic diversity statistics and estimates of gene flow.

Locus	H_T	H_S	G_{ST}	N_M
TeaSSR3	0.085	0.084	0.013	37.125
TeaSSR4	0.429	0.389	0.093	4.849
TeaSSR5	0.470	0.399	0.152	2.792
Mean	**0.328**	**0.290**	**0.086**	**14.922**

Remarks: within population genetic diversity (H_S), total genetic diversity (H_T), proportion of the total diversity among populations (G_{ST}), estimate of gene flow (N_M)

According to the UPGMA analysis based on Nei's unbiased genetic distance (1978), a clustering of *D. membranaceus* populations in Thailand was made. The UPGMA dendrogram is shown in Figure 3.13. Three groups were chosen (Table 3.16) because they represent splits in the tree at a deeper level (i.e. more molecular divergence). The Phu Rue population (Group 2) was separated from the other populations (constuting the Group 1) although it groups with the Group 1 it shows a high level of divergence from the Group 1. The groupings of the populations have been illustrated on a map of Thailand map using different colours (Figure 3.14).

Table 3.16 Groupings of populations according to UPGMA with Nei's unbiased genetic distance measure (1978).

Group 1	Group 2	Group 3
ID1=Suan Phung	ID4=Phu Rue	ID5=Nam Nao
ID2=Lan Sang		ID7=Kui Buri
ID3=Wat Chan		ID8=Phu Phieng
ID6=Sakaerat		ID9=Yunnan

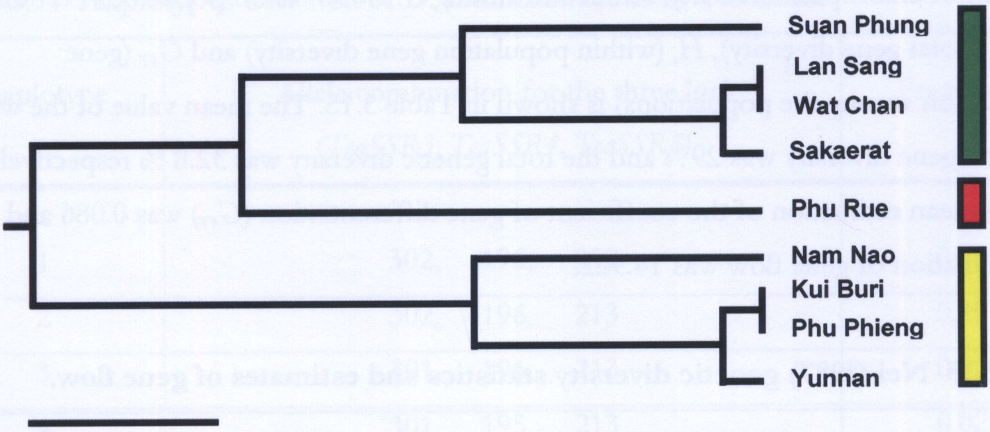


Figure 3.13 UPGMA dendrogram of *D. membranaceus* populations based on Nei's unbiased genetic distance (1978). Green colour=Group 1; Red colour=Group 2; Yellow colour=Group 3. The bar below the tree indicates a Nei unbiased genetic distance of 5

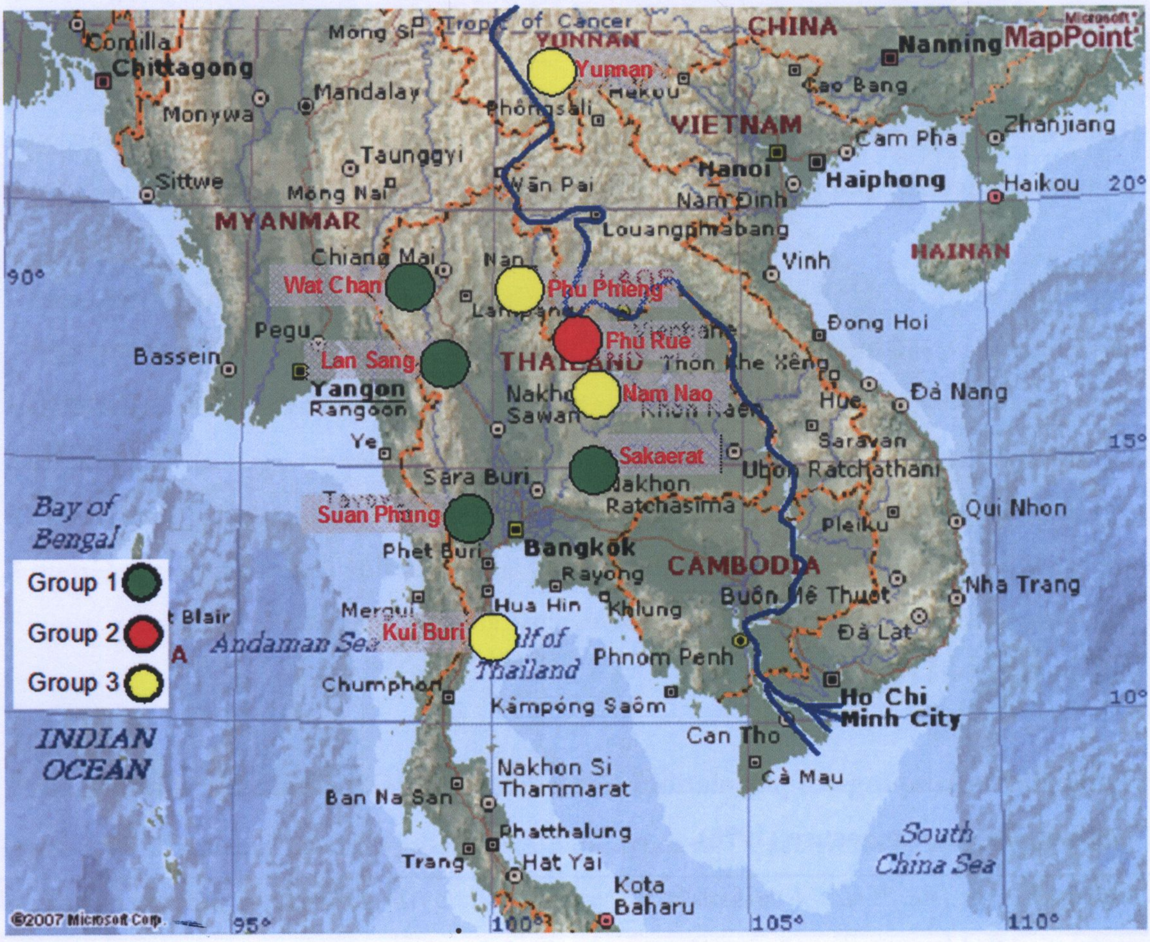


Figure 3.14 Overall patterns of population grouping of *D. membranaceus* in Thailand according to Nei's unbiased genetic distance (1978). The blue line represents the Mekong River

3.4 Discussion

3.4.1 Phylogenetics of *Dendrocalamus* and related genera

Dendrocalamus and its related genera

The results showed that *Dendrocalamus* is not strictly monophyletic because *Bambusa, Dendrocalamopsis, Gigantochloa,* and *Melocalamus* are embedded in the strongly supported *Dendrocalamus* group (100%BS, Figure 3.2). The placement of these taxa requires further investigation especially in the case of *Melocalamus* as it shows a high degree of morphological differentiation from *Dendrocalamus.* The positioning of the other three genera within *Dendrocalamus* is less surprising because they are morphologically similar to *Dendrocalamus.* The phylogenetic tree is a plastid gene tree. The gene tree does not necessarily indicate the species tree. It is possible that chloroplast capture events could explain the grouping of these taxa with *Dendrocalamus.* In this scenario, past hybridisation between taxa could have resulted in the transfer of the plastid genome (Hodkinson *et al.*, 2002). Nuclear DNA and morphological characteristics can be representative of one parent and plastid DNA type representative of the other parent. This is because the plastid DNA does not recombine. Relationships among these four genera and *Dendrocalamus* have already been discussed in Chapter 2. Besides this, *D. asper* groups with two species of *Gigantochloa* (*G. atroviolacea, G. scortechinii*). However, this relationship only had low bootstrap support (61%BS), see Figure 3.2. The sister group relationship between *D. hamiltonii* and *M. compactiflorus* is of interest even though it only received low support (65%BS). Morphologically, *D. hamiltonii* is extremely similar to *D. semiscandens* and the former is widely distributed and morphologically variable (Li & Stapleton, 2006f). However, the DNA sequence evidence does not support a close relationship between these two species. Using morphology, Li and Stapleton (2006f) recognized two varieties of *D. minor* (*D. minor* var. *minor* and *D. minor* var. *amoenus* (Q.H. Dai & C.F. Huang) Hsueh & D.Z. Li) and treated *D. sapidus* as a synonym of *D. minor* var. *minor.* However, Ohrnberger (1999) accepted *D. minor* var. *amoenus* to be only at a 'forma' (f.) taxonomic level, as *D. minor* f. *amoenus* (Q.H. Dai & C.F. Huang) Ohrnb. and recognized *D. sapidus* as a extant species. However, the sequence data results show that *D. minor* is a sister species of *D. latiflorus* (85%BS) and does not have a close relationship with *D. sapidus.*

Infrageneric classification

The phylogenetic results inferred from five plastid DNA sequences were not statistically robust enough to make firm conclusions about the infrageneric relationships among *Dendrocalamus* species. None of infrageneric classifications, according to Hsueh and Li (1988a), Ohrnberger (1999), and Li and Stapleton (2006f), can be interpreted as congruent with the phylogenetic results of this study. Morphologically, *Dendrocalamus* species are distinct from one another (Dransfield & Widjaja, 1995; Wong, 1995a; Li & Stapleton, 2006f) and easy to identify using even only vegetative characters (Chapter 4, this thesis). However, Holttum (1958) stated that 'in fact bamboos with quite different vegetative characters may bear almost identical spikelets'. However, the sister group relationship found in the phylogenetic analysis between *D. latiflorus* and *D. minor* would suggest that these two species should be placed in the same taxon in infrageneric classification. Li and Stapleton (2006f) placed *D. latiflorus* and *D. minor* in subg. *Sinocalamus,* while Hsueh and Li (1988a) placed them in the same subgenus, *Sinocalamus,* but different sections. *Dendrocalamus latiflorus* was placed in sect. *Sinocalamus* and *D. minor* placed in sect. *Patellares* (see Table 3.1). Ohrnberger (1999) positioned them in the same section, *Sinocalamus.* From the molecular point of view, treating *D. latiflorus* and *D. minor* in the same taxon of infrageneric classification should make the classification more phylogenetically meaningful. Placing these two species in different sections, as suggest by Hsueh & Li (1988a), would not be recommended. It would further mean that the section *Patellares* of Hsueh and Li (1988a) which contained seven species (*D. farinosus, D. liboensis, D. mianningensis, D. minor, D. patellaris, D. pulverulentus,* and *D. tsiangii*) needs to be redefined. Li and Stapleton (2006f) treated these seven species differently: *D. farinosus, D. liboensis* and *D. pulverulentus* in subg. *Dendrocalamus*; *D. minor* in subg. *Sinocalamus. Dendrocalalmus patellaris,* the type species of section *Patellares* of Hsueh & Li (1988a) and *D. mianningensis* are now accepted as *Ampelocalamus patellaris* (Gamble) Stapleton and *A. mianningensis* (Q. Li & X. Jiang) D.Z. Li & Stapleton, respectively (Ohrnberger, 1999; Li & Stapleton, 2006f). This would further mean that the section *Patellares* of Hsueh & Li (1988a) is no longer systematically legitimate as its type species was transferred to the genus *Ampelocalamus.*

Bambusa

The taxonomy of *Bambusa* is in a state of flux as discussed in Chapter 2 of this thesis. Phylogenetic analyses of *Bambusa* and allies by Sun *et al.* (2005) using ITS sequence data also showed the incongruence between the morphological and phylogenetic classifications. Almost all of *Bambusa* species included in this study can be found in China (Ohrnberger, 1999; Xia *et al.,* 2006a). The results of the phylogenetic analyses presented here showed that *Bambusa* species can superficially be divided into three groups (Figure 3.2). Group 1 is *B. oliveriana,* which is imbedded in a group of *Dendrocalamus* species, with 100%BS. Group 2 consists of eight species (*B. beecheyana, B. chungii, B. dolichomerithalla, B. malingensis, B. oldhamii, B. pachinensis, B. tuldoides, Neosinocalamus affinis* (=*B. emeiensis* as discussed in Chapter 2)), with 86%BS. This group is sister to *Phuphanochloa* with 87%BS. Group 3 consists of two *Bambusa* (*B. bambos* and *B. tulda*) and other three other genera, *Gigantochloa, Thyrsostachys,* and *Vienamosasa,* but with low support (62%BS). However, these three groups are not consistent with the infrageneric classification of *Bambusa* into three subgenera, subg. *Bambusa*, subg. *Dendrocalamopsis*, subg. *Leleba*, and subg. *Lingnania*, as adopted by Xia *et al.* (2006a). The species of *Bambusa* included in the three subgenera of Xia *et al.* (2006a) are spread over the three groups of this study. For example, *B. malingensis* and *B. bambos*, which should according to Xia *et al.* (2006a) be in the same subgenus, subg. *Bambusa* (as discussed in Chapter 2), are in Group 2 and 3 of this study. According to Xia *et al.* (2006a) and Li and Stapleton (2006b), *Bambusa oliveriana* should be placed within subg. *Dendrocalamopsis*, together with *B. beecheyana* and *B. oldhamii*, as they have broad culm-sheath blades and small fringed culm-sheath auricles. However, *B. oliveriana*, was placed in Group 1 of this study and *B. beecheyana* and *B. oldhamii*, in group 2. *Bambusa pachinensis*, *B. tuldoides*, and *B. tulda* were placed in subg. *Leleba* by Xia *et al.* (2006b). However, the former two species are placed in Group 2 of this phylogenetic analysis and the latter species is in Group 3. Only the combination of members of subg. *Lingnania*, *B. chungii* and *B. emeiensis*, as recognized by Li and Stapleton (2006c), is consistent with this study, as they are in the Group 2. There is no infrageneric classification for the placement of *B. dolichomeritballa* in recent publications (Ohrnberger, 1999; Xia *et al.*, 2006a) and there is not enough information from this molecular study to decide which subgenus it is best placed in.

Gigantochloa

There were only four species of *Gigantochloa* included in this study. However, it may be possible to arbitrarily divide *Gigantochloa* into two groups. Group A consists of *G. atroviolacea* and *G. scortechinii* (65%BS), which is embedded in a group of *Dendrocalamus* species and is sister to *D. asper* (61%BS, Figure 3.2). Group B consists of *G. albociliata* and *G. ligulata* which group together with *Bambusa*, *Thyrsostachys*, and *Vietnamosasa* (62%BS). This would appear to offer strong evidence against the monophyly of *Gigantochloa*. Holttum (1958) and Wong (1995a) classified *Gigantochloa* into two groups based on the culm-sheath blades at mid-culm; spreading or deflexed (reflexed) versus erect. *Gigantochloa atroviolacea* and *G. scortechinii* typically have spreading to deflexed culm-sheath blades (Holttum, 1958; Wong, 1995a; Clayton *et al.*, 2006 onwards). This would be congruent with Group A of this study. *Gigantochloa ligulata* has erect culm-sheath blades (Holttum, 1958; Dransfield, 1995; Wong, 1995a) but *G. albociliata* has erect (Li & Stapleton, 2006g) or spreading culm-sheath blades (Duriyaprapan & Jansen, 1995b; *personal observation*). This is not congruent with Group B of this study as *G. albociliata* seems to fit better with Group A on the basis of morphological characters. Holttum (1958) stated that 'the species of *Gigantochloa* are difficult to characterize clearly; and there is no doubt that their area of great abundance is in Lower Burma and Peninsular Siam (now Thailand)'. Soderstrom and Ellis (1988) erected a new genus called *Pseudoxytenanthera* to accommodate a single type species, *P. monadelpha* (Thwaites) Soderstrom & Ellis, which was transferred from

Dendrocalamus monadelphus Thwaites. *Pseudoxytenanthera monadelpha* is straggling or sub-scandent, and distributed in high latitudes (1,000 to 1,800 m) of the central part of Sri Lanka and the southern part of India (Soderstrom & Ellis, 1988; Ohrnberger, 1999). Its flowers have 'fused filaments' (even though in the genus description it describes them as free filaments (Soderstrom & Ellis (1988)). Fused filaments can also be found in *Gigantochloa* (Holttum, 1958; Dransfield & Widjaja, 1995; Wong, 1995a; Li & Stapleton, 2006g). *Pseudoxytenanthera monadelpha* has culms that are first stout and erect but become thin and whip-like at the tip and bend far over. These are similar to those of *G. albociliata* and *G. ligulata* (*personal observation*). I, personally, would suggest that the genus *Pseudoxytenanthera* may be best treated at subgenus rank under the *Gigantochloa*, and those species of *Gigantochoa* that relate to *P. monadelpha*, *G. albociliata* and *G. ligulata* would also fit to this subgenus.

Phuphanochloa, *Vietnamosasa*, and *Thyrsostachys*

There is strong evidence that *Phuphanochloa* is sister to (with 87%BS) a group consisting of eight species of *Bambusa* (Figure 3.2). Detail of this genus and its related genera can be seen in Chapter 2 and Chapter 4 (MANUSCRIPT 4.2).

Vietnamosasa is a genus under the subtribe Racemobambosinae according to the several relatively recent classification systems (Dransfield & Widjaja, 1995; Clark, 1995; Li, 1998; Ohrnberger, 1999). This genus and other genera in Racemobambosinae were always embedded in Bambusinae (Ni Chonghaile; 2002; Chapter 2, this thesis; this Chapter, Figure 3.2), but with low support (62%BS, using *trnLF*, Ni Chonghaile (2002); 61%BS, using combined five plastid DNA sequences, Chapter 2 (this thesis, Figure 2.10); 62%BS, this Chapter, Figure 3.2). In order to make Bambusinae monophyletic, Racemobambosinae needs to be merged with Bambusinae.

Thyrsostachys is a genus of two known species (*T. oliveri* Gamble and *T. siamensis* Gamble) distributed from south China, Burma, Thailand to Vietnam (Holttum, 1958; Dransfield & Widjaja, 1995; Wong, 1995a; Li & Stapleton, 2006i; Ohrnberger, 1999). In this study, *Thyrsostachys* groups with *Bambusa*, *Gigantochloa*, and *Vietnamosasa*, as a polytomy (62%BS, Figure 3.2). *Thyrsostachys* is distinguished greatly from these three genera in having paleas of the lower florets that are deeply 2-cleft, with narrow tail-like divisions (Holttum, 1958; Wong, 1995a; Li & Stapleton, 2006i; *personal observation*). However, *Thyrsostachys* is morphologically close to *Bambusa* in having the same type of ovary except that the swollen top is almost glabrous instead of hairy as usually found in *Bambusa* (Holttum, 1958).

Oreobambos and *Oxytenanthera*

Oreobambos is sister to *Oxytenanthera* (100%BS, Figure 3.2) and found in a polytomy among others of Bambusinae according to several classifications (Soderstrom & Ellis, 1987; Dransfield & Widjaja, 1995; Clark, 1995; Li, 1998; Ohrnberger, 1999). Clayton and Renvoize (1986) also placed *Oreobambos* in Bambusinae but positioned *Oxytenanthera* in Melocanninae. From this study and that of Chapter 2, it is obvious that these two monotypic genera from tropical Africa should be placed in the same subtribe, Bambusinae and not Melocanninae, as also already discussed in Chapter 2.

3.4.2 Phenetic and phylogenetic studies of Thai *Dendrocalamus*

3.4.2.1 Phenetic study of Thai Dendrocalamus using PCO analysis

Four groups of Thai *Dendrocalamus*; '*D. strictus* Group', '*D. membranaceus* Group', '*D. asper* Group', and '*D. giganteus* Group', can be detected from the scatter plot (Figure 3.3) of the PCO analysis when the character state '0/1' (polymorphic) was treated as 'missing data'. However, when the polymorphic characters were treated separately as the 'multiple entries', only two groups of Thai *Dendrocalamus* species can be recognized from the PCO analysis (Figure 3.5); the Group A (=the '*D. strictus* Group' plus the '*D. membranaceus* Group') and the Group B (=the '*D. asper* Group' plus the '*D. giganteus* Group'). The three existing subgeneric classifications of *Dendrocalamus* (Hsueh & Li, 1988a; Ohrnberger, 1999; Li & Stapleton, 2006f) can be applied to these groupings in different degrees.

The '*D. strictus* Group' (consisting of three species, *D. barbatus, D. pendulus,* and *D. strictus*) together with '*D. membranaceus* Group' (including three species, *D. dumosus, D. khoonmengii,* and *D. membranaceus*) represents *Dendrocalamus* subg. *Dendrocalamus* sect. *Dendrocalamus* of Hsueh and Li (1988a); *Dendrocalamus* sect. *Dendrocalamus* of Ohrnberger (1999), and *Dendrocalamus* subg. *Dendrocalamus* of Li and Stapleton (2006f), see Table 3.1. Morphologically, having the verticils of roots at the lower culm nodes would separate the '*D. strictus* Group' from the '*D. membranaceus* Group'.

The '*D. asper* Group' including three species namely *D. asper, D. brandisii,* and *D. hamiltonii,* can be fitted, in some degree, in *Dendrocalamus* subg. *Sinocalamus* sect. *Sinocalamus* of Hsueh and Li (1988a) or in *Dendrocalamus* sect. *Sinocalamus* of Ohrnberger (1999), see Table 3.1. However, having *D. latiflorus* outside '*D. asper* Group' as found by this study, makes it inconsistent with *Dendrocalamus* infrageneric classifications of Hsueh and Li (1988a) as they treated these two species in the same taxon. Actually, the '*D. asper* Group' can be seen as a subset of *Dendrocalamus* subg. *Dendrocalamus* of Li and Stapleton (2006f), see Figure 3.3 and Table 3.1.

Furthermore, if '*D. asper* Group' and '*D. strictus* Group' can be bounded together, they then will be equivalent to *Dendrocalamus* subg. *Dendrocalamus* of Li and Stapleton (2006f).

The '*D. giganteus* Group' is composed of five species, *D. copelandii, D. giganteus, D. latiflorus, D. minor,* and *D. sinicus.* These are placed into the *Dendrocalamus* subg. *Sinocalamus* of Li and Stapleton (2006f). However, this grouping disagrees with the classification systems of Hsueh and Li (1988a) and Ohrnberger (1999) because *D. latiflorus* and *D. minor* were in *Dendrocalamus* sect. *Sinocalamus* while *D. giganteus* and *D. sinicus* were in *Dendrocalamus* sect. *Draconinalamus* (Ohrnberger, 1999). Ohrnberger also accepted *D. copelandii* as *Bambusa copelandii* Gamble ex Brandis. Hsueh and Li (1988a) treated *D. latiflorus* and *D. minor* in different sections of *Dendrocalamus* subg. *Sinocalamus, D. latiflorus* in sect. *Sinocalamus,* and *D. minor* in sect. *Patellares;* they did not recognize *D. copelandii* (see Table 3.1).

3.4.2.2 Phylogenetic study of Thai *Dendrocalamus*

3.4.2.2.1 Phylogenetic study of Thai *Dendrocalamus* based on morphological characters

As shown in Figure 3.9, Thai *Dendrocalamus* species can be phylogenetically divided into three groups based on morphological characters. These three major groups, '*D. strictus* Group' plus '*D. membranaceus* Group', '*D. asper* Group', and '*D. giganteus* Group', are resolved, and are congruent with the PCO analysis.

The six species of the '*D. strictus* Group' plus the '*D. membranaceus* Group', are supported with 90%BS (Figure 3.9) and shared four characters: 8#, pseudospikelets that are spiny to the touch; 9#, yellow-green spikelets; 10#, spikelets that are sub-terete (see Tables 3.3 and 3.4, see also Figure 3.11). There was no flower information available for *D. khoonmengii* (it is most parsimonious to assume that it also shares these characters). Of the two groups (the '*D. strictus* Group' and the '*D. membranaceus* Group') found in the PCO analysis, only the '*D. membranaceus* Group' is resolved. Its three species shared only one character, the character 4#, lower culm nodes are usually with no verticils of roots, resulting in the low support value for this grouping (65%BS). However, this character is homoplasious as it is also independently present in *D. copelandii* (of '*D. giganteus* Group'), see Tables 3.3 and 3.4. The '*D. strictus* Group' is not present in the strict consensus tree. In addition, a species of its group, *D. barbatus,* was sister to the '*D. membranaceus* Group' (but with only 52% bootstrap support) as they shared the character 1#, Clump habit that is loosely tufted.

The three species of the '*D. asper* Group' resolved together but had no bootstrap support (Figure 3.9). They shared two characters in common, 3#, lower culm internodes are hairy; 12#, Florets are separable. These two characters are, however, homoplasious among the *Dendrocalamus* species as the same character state of 3# can be also found in *D. sinicus* of '*D. giganteus* Group' and *D. barbatus* of '*D. strictus* Group' (as scurfy). The same behavior of the character 12# is also present in *D. latiflorus* and *D. minor* of '*D. giganteus* Group' (Tables 3.3 and 3.4, see also see Figure 3.11). In this study, *Dendrocalamus latiflorus* was placed outside the '*D. asper* Group' because it differs in having a mid-culm branch complement of the branches that are more or less subequal in size, pseudospikelets that are congested as a stellate cluster, and spikelets that are usually composed of 4—8 fertile florets (character 2#, 7#, and 11# respectively). As discussed for the PCO analyses, the '*D. asper* Group' is a part of *Dendrocalamus* subg. *Dendrocalamus* of Li & Stapleton (2006f), but is in *Dendrocalamus* sect. *Sinocalamus* of Ohrnberger (1999), see Figure 3.9 and Table 3.1, since they did not share the same character states (of characters 8#, 9#, and 10#) to '*D. strictus* Group'. In combination, the '*D. asper* Group' and the '*D. strictus* Group' constitute *Dendrocalamus* subg. *Dendrocalamus* as adopted by Li and Stapleton (2006f) (as encompassed in Figure 3.9).

The five species of the '*D. giganteus* Group' shared three characters: character 2#, 7#, and 11# respectively (as discussed earlier) with 75%BS). This group can be seen as the *Dendrocalamus* subg. *Sinocalamus* of Li and Stapleton (2006f) but it is inconsistent with the infrageneric classification systems of Hsueh and Li (1988a) and Ohrnberger (1999) as discussed in the phenetic section above. The sister relationship between *D. latiflorus* and *D. minor*, is not present in strict consensus.

3.4.2.2.2 Phylogenetic study of Thai *Dendrocalamus* based on the combined morphological and molecular characters

The phylogenetic study using combined morphological and molecular data yielded the results consistent to the phenetic (PCO) and phylogenetic parsimony analyses based on only morphological characters. Three groups of Thai *Dendrocalamus* can also be detected from this study. They are '*D. strictus* Group' plus the '*D. membranaceus* Group', '*D. asper* Group', and '*D. giganteus* Group' (Figure 3.10). The combined morphological and molecular differed from morphological tree only in a minor way.

The '*D. strictus* Group' is resolved with high support (87%BS, Figure 3.10). The '*D. strictus* Group' is not present in the strict concensus while the '*D. membranaceus* Group' does occur with 64%BS. *Dendrocalamus barbatus* groups with (present in strict consensus analysis but with no bootstrap support, Figure 3.10) the '*D. membranaceus* Group'. The '*D. asper* Group' was found in the strict consensus tree but has no bootstrap support (Figure 3.10). The '*D. giganteus* Group' is moderately supported (74%BS, Figure 3.10). Unlike the morphological tree, the combined tree showed that within '*D. giganteus* Group' the sister relationship between *D. latiflorus* and *D. minor*, is highly supported (92%BS). Only a single morphological character, character 12# (which is homoplasious, as discussed earlier), would support this relationship. However two more molecular characters (1,186, G→T; 2,283, A→C) would help to support this relationship that has high bootstrap percentage (Figures 3.9 and 3.10, this Chapter; see also Figure 2.10, Chapter 2).

Habitat characters are not often included in phylogenetic or phenetic analyses but I have tried to include two characters to see the effect of the Kra Isthmus and the influence of calcareous substrate. Analyses with the habitat characters included yielded identical branching patterns to analyses excluding the habitat characters except that in the '*D. membranaceus* Group', *D. membranaceus* was grouped with, but with no bootstrap support, a group consisting of *D. dumosus* and *D. khoonmengii*. *Dendrocalamus dumosus* and *D. khoonmengii* both occur naturally below the Kra Isthmus (see detail in the Chapter 4). But this character is homoplasious as it is also present in *D. pendulus* (of '*D. strictus* Group'). The habitat character, plants usually restricted to the limestone areas, can be seen in *D. dumosus* and *D. copelandii*. However, this character is, again, homoplasious because the former species is in the '*D. membranaceus* Group' whilst the latter species is in '*D. giganteus* Group'.

3.4.3 Population genetics and phylogeography of *D. membranaceus*

In this study, only two alleles, differing by only a single nucleotide in length, from each locus were found (Table 3.11) in the chloroplast microsatellite analysis of nine populations (each contained 5 individuals) of *D. membranaceus* using three primer pairs. The loci *TeaSSR3* and *TeaSSR4* contain a mononucleotide adenine repeat (A_8-A_9 in *Lolium perenne* (McGrath *et al.*, 2006) from which the universal primers were developed). It is likely that the single base pair indel occurred in this region. However, the locus *TeaSSR5* has a trinucleotide repeat (CTT_3) in the grass species *Lolium perenne* from which the universal primers were developed. Loss or gain of a repeat at this locus would result in a length variants of at least 3 bp. Therefore the single base pair length variation of alleles detected at this locus in *Dendrocalamus* may not have occurred at this microsatellite. It could have occurred anywhere between the primer sites.

Allele 2 (302bp) of *TeaSSR3*; Allele 4 (196bp) of *TeaSSR4*; and Allele 5 (212bp) of *TeaSSR5*, were found to be the dominant alleles in most *D. membranaceus* populations. However, Allele 5 was not found in Phu Rue population. The locus *TeaSSR5* was found to provide the most gene diversity (h=47%) among the three loci, followed by *TeaSSR4* (h=42.9%) and *TeaSSR3* (h=8.5%), respectively (Table 3.12). *Dendrocalamus membranaceus* from the Nam Nao population, with h=32%, P=66.67% and average Na=1.67, was the most diverse population among the nine populations studied (Table 3.13.) followed by the Phu Rue population (h=27%; P=66.67%; Na=1.67). The Suan Phung population was the least diverse and was monomorphic at all loci (h=0%; P=0%; Na=1).

By combining the alleles from the three microsatellite loci, six of haplotypes were detected (Table 3.14 and Figure 3.12). Apart from Suan Phung, the remaining eight populations were polymorphic. Haplotype 1 (base pair combination: 302, 196, 212), with the frequency of 0.38, was the most common haplotype within the *D. membranaceus* populations. This haplotype can be found in all populations, except the one from Phu Rue. The population at Suan Phung was found to be monomorphic for this haplotype. Haplotype 3 and Haplotype 4 were the rarest and were found only in the Phu Rue population. There was an overall high degree of intra-population variation (H_s=29%) in comparison to total diversity (H_T=32.8%). This meant that most variation was distributed within rather than among populations and resulted in a low G_{ST} value (8.6%) and high estimated geneflow (N_m=14.92).

Dendrocalamus disperses by seed and vegetative growth/propagules. It is not known how genetically diverse populations of *Dendrocalamus* are in Thailand. For example, no studies have tried to determine the proportion of ramets (genetically identical individuals; clones) from genets (different genotypes). It is theoretically possible, and even likely, that some stands of *Dendrocalamus* that appear as a large population of individuals are actually made up of a single genet (all individuals are ramets of one genet). It is well known that many bamboos, including *Dendrocalamus*, rarely flower (some bamboos may sometimes take up to 150 years, Li *et al.* (2006b)). Therefore populations could have had over 100 years to spread vegetatively. The results presented in this population genetic study have allowed us to help determine the incidence of genets and ramets in the populations. Only one of the nine populations, Suan Phung, was monomorphic. The Suan Phung population is located in southwestern Thailand. Along this region up to northern Thailand is where the bamboo flora is rich and diverse (Dransfield, 1994; Dransfield & Widjaja, 1995; *personal observation*). Bamboo forests of almost pure stands can be found as a vast area along this belt, especially the populations of *Cephalostachyum pergracile, Dendrocalamus brandisii, D. membranaceus*, and *Thyrsostachys siamensis* (*personal observation*). It is possible that *D. membranaceus* from Suan Phung population is a single genet. However, all other populations contained more than one haplotype (they were not all ramets of one genet). The average number of haplotypes per population was 2, and the maximum was 4 in Nam Nao (out of a possible 6).

These results therefore confirm a high plastid diversity within *Dendrocalamus* populations and suggest that the populations are not entirely clonal. They appear to be have generated plastid variation via high levels of seed mediated geneflow (there are only six haplotypes in total but these are widely distributed across Thailand). It is possible that clonally reproducing populations have accumulated mutations in their cpDNA and have not gained these via immigration of seed or rhizome. However, this scenario is less likely, given the close geographical distance between populations and the relatively slow rate of mutation recorded in the plastid genome ($0.86\text{-}1.2 \times 10^{-9}$ substitutions per year at synonymous sites in cpDNA markers compared to 3.5×10^{-9} in biparentally inherited nuclear DNA markers) (Wolfe *et al.*, 1987; Lowe *et al.*, 2004). Evoking somaclonal variation as an explanation would suggest that there is homoplasy in the data, the same mutation has occurred independently in separate populations (in parallel). Given the relatively low level of allelic variation detected, it is more likely that populations share haplotypes because of common descent and not because of homoplasy. The low G_{ST} values recorded (0.086, Table 3.15) suggest that most variation in haplotypes is distributed within populations and not among populations. This contrasts with some other plant species such as oaks in Ireland where high G_{ST} values (0.73, for both species, *Quercus petraea* and *Q. robur*) were found for plastid genome markers over a smaller geographical distance (Kelleher *et al.*, 2004). In oak, seeds are heavy and not easily distributed leading to high G_{ST} values (high population differentiation). In *Lolium perenne* (perennial ryegrass) ecotypes, an extremely high plastid diversity was recorded by McGrath *et al.* (2007) and low G_{ST} values found (0.238 in Irish ecotypes) suggesting that seed movement between populations was relatively high. In the same study G_{ST} values between European ecotypes were higher at 0.431 (probably due to the higher geographical distance between populations; lower amounts of seed mediated geneflow). In *Dendrocalamus* in Thailand the results are more similar to those of *Lolium* and this would suggest that there are high degrees of seed mediated geneflow or high influx of vegetative propagules (e.g. rhizomes) into populations.

The UPGMA dendrogram based on Nei's unbiased genetic distance (1978) revealed three possibly major groups among the nine *D. membranaceus* populations (Table 3.16 and Figures 3.13 and 3.14). It was surprising that the Phu Rue population (Group 2) was sister to Group 1. On the basis of geographical and ecological isolation we may, in contrast, expect to see the *D. membranaceus* population from Sakaerat to be the most genetically distinct among these populations, see Figures 3.15 and 3.17. Sakaerat forest is surrounded by residential areas, agricultural areas, Lam Phra Ploeng Dam, and the Road no. 304. Samples of *D. membranaceus* from Sakaerat were collected from a very small population that consisted of less than ten clumps. This population was located at the transition zone between deciduous dipterocarp forest and dry evergreen forest. There was no record whether this species in that area was native or cultivated. It may be a naturalized population as there was also a bamboo living collection in that area (Sakaerat Living Bamboo Collection). However, this population had two different haplotypes suggesting that it was not simply a vegetative spread from the living collection. Morphologically, the flying shoots of *D. membranaceus* from Sakaerat looked distinctly different from those found in other places of the country. Instead of having orange culm sheaths covered with brown hairs as found in the other populations, it had white culm sheaths covered with black hairs (Figure 3.10). This white form was also present in the living collection.

It was also interesting to see the populations from Nam Nao, Kui Buri Phu, and Phieng grouping together with the Yunnan (China) population in Group 3 ((Table 3.16 and Figures 3.13 and 3.14). We may expect to see the Yunnan population genetically isolated from the populations within Thailand as the Mekong River may act as a geographical barrier. This would suggest that having a huge river as a barrier may not be able to significantly restrict seed mediated gene flow from one population to another. The chloroplast microsatellite markers used in this study cannot infer pollen mediated geneflow as they are maternally inherited. Therefore it is not possible to suggest that pollen of *Dendrocalamus* can cross the Mekong. However, the markers are useful for tracking seed mediated gene flow (seed movement). The results are consistent with high seed mediated geneflow as the populations are not strongly differentiated from each other (neither do they show strong spatial geographical structuring). Both seed and vegetative organ movement, especially by rhizomes, could account for such patterning. The Mekong River does not appear to be a barrier to their movement.

Apart from Sakaerat, all the individual of the remaining populations were collected from the mixed deciduous forest as described by Santisuk (2006) and Dr. Utis Kutintara (*personal communication*). There is no obvious broad-scale biogeographic pattern in the cpDNA haplotypes. This could be because there are few major geographical or ecological barriers to geneflow in Thailand (see Figure 3.17). The other important reason would be because of the small number of samples per population used in this study (S. Waldren, *personal communication*).

3.4.4 Conclusions

The analyses using the combined *trnL-F, atpB-rbcL*, *rps16*, and *matK* sequence data (Figure 3.2) could not resolve the infrageneric classifications of *Dendrocalamus* according to Hsueh and Li (1988a), Ohrnberger (1999), and Li and Stapleton (2006f) (Table 3.1). There is a hope that the AFLP fingerprinting, a method that has proven useful for infrageneric classification of *Phyllostachys* (Hodkinson *et al*, 2002) can help to solve this problem. However, morphological characters (and combined molecular and morphological analyses) have been shown to be good enough to resolve inter-relationships within *Dendrocalamus* and largely agree with the subgenera according to a Li and Stapleton (2006f) (Figures 3.3, 3.9, and 3.10). The three loci of cpDNA microsatellites revealed some patterns of differentiation among *D. membranaceus* populations in Thailand. In combination they detected six different haplotypes. It is clear that many populations are made up of several genets and that much seed/or vegetative propagule mediated geneflow has occurred even across considerable geographical distance and major physical barriers such as the Mekong River. This study is the first one that investigated plastid DNA variation in bamboos at population level in Thailand. More microsatellite loci, AFLP fingerprinting, and more samples per population may be needed for future studies.

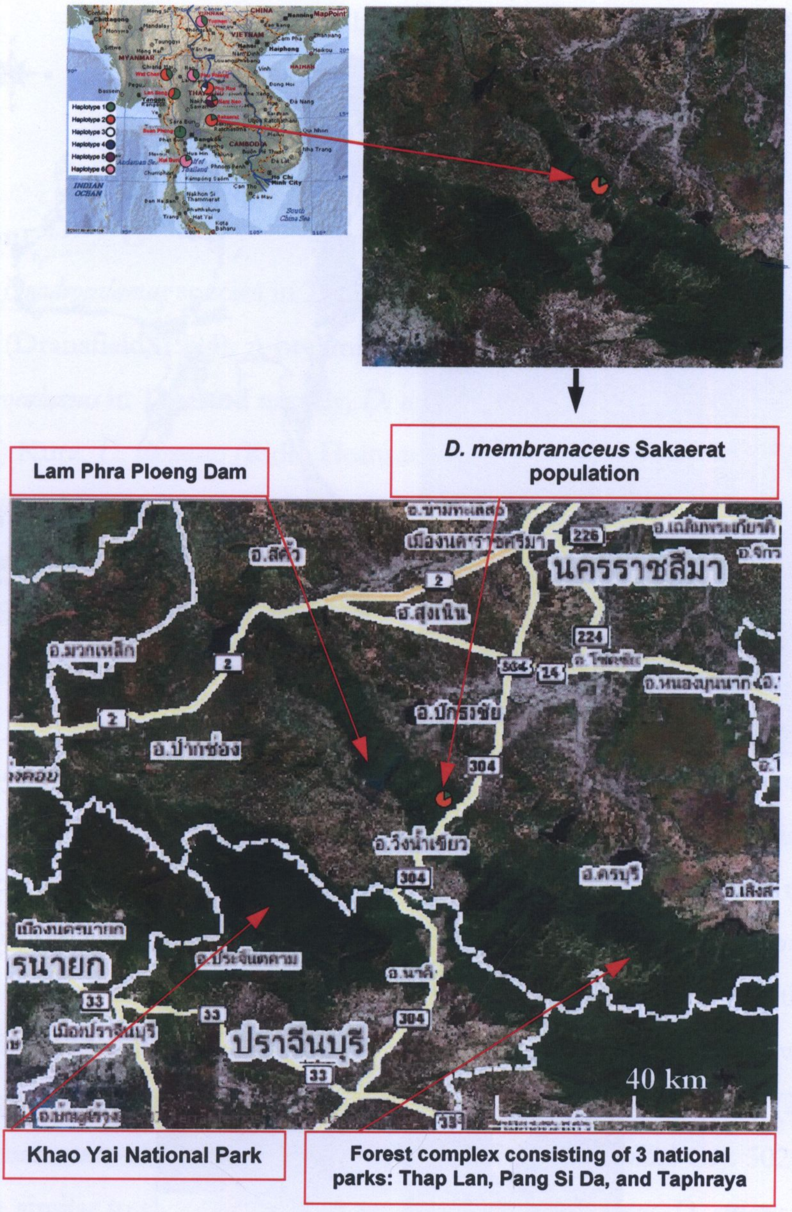


Figure 3.15 Locality of *D. membranaceus* from Sakaerat. Map obtained from http://maps.google.com/maps?t=h.

Figure 3.16 Comparison of flying shoots of *D. membranaceus* from Sakaerat (left) and the typical one that is usually found in other places (right).

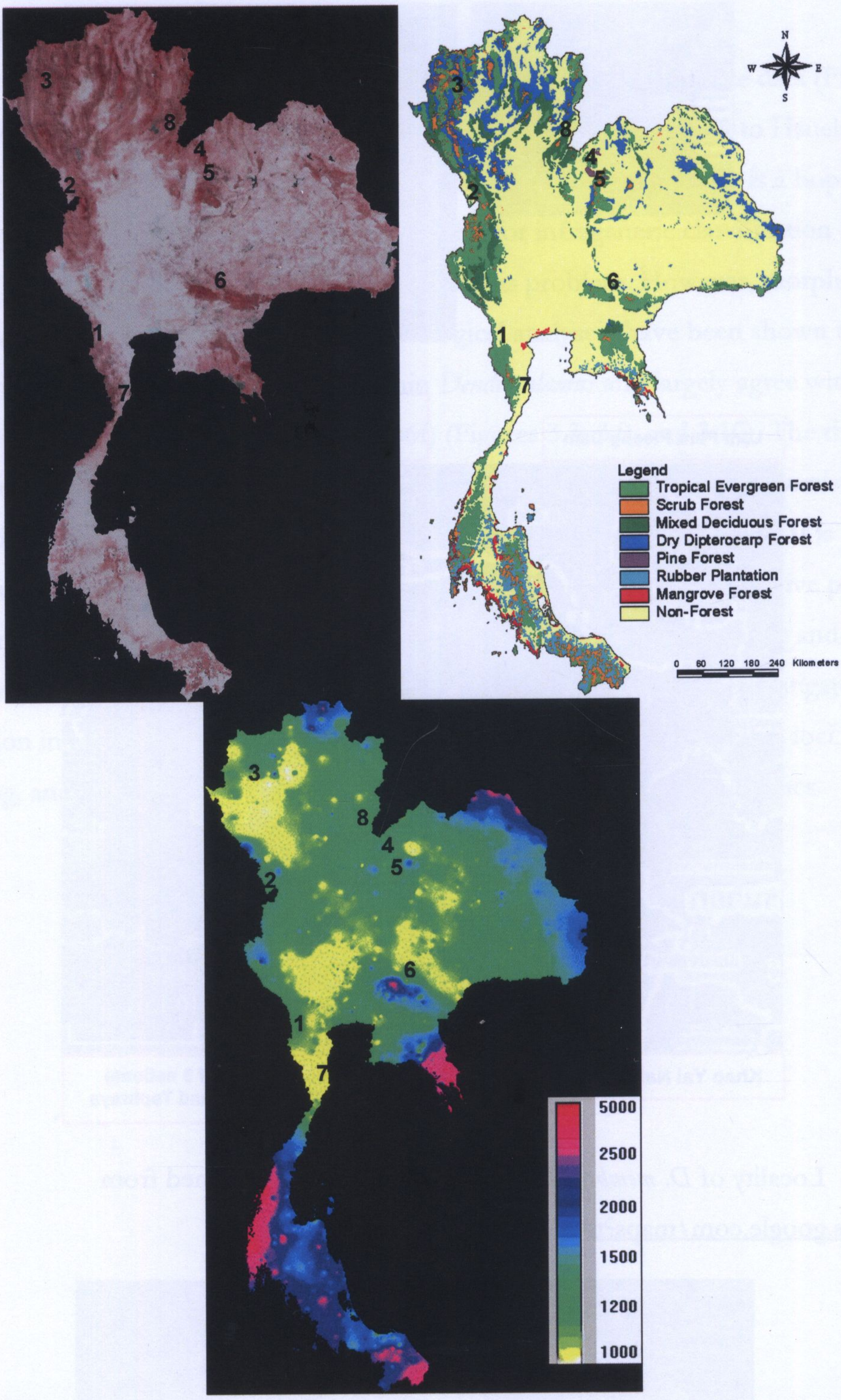


Figure 3.17 Maps of Thailand; showing relief (top left), vegetation types (top right), and annual rainfall (mm) between 1950 and 1997 (bottom). Reddish elements show the areas of more or less high elevation where the natural vegetation still exist. Two maps from the top obtained from http://www.rrcap.unep.org; the bottom one from http://tiwrm.hpcc.nectec.or.th/GIS/iso_rainfall/main.html. Numbers represent the eight locations of Thai *D. membranaceus* populations: 1=Suan Phung; 2=Lan Sang; 3=Wat Chan; 4=Phu Rue; 5=Nam Nao; 6=Sakaerat; 7=Kui Buri; 8= Phu Phieng. The Yunnan population is not shown.

Chapter 4

Floristic treatments for Thai *Dendrocalamus*

4.1 Introduction

The number of *Dendrocalamus* species in Thailand is probably the largest among Southeast Asian countries (Dransfield, 1994). A preliminary investigation, by Dransfield (1994), listed 14 species of *Dendrocalamus* in Thailand namely, *D. asper* (Schult. f.) Backer ex K. Heyne, *D. brandisii* (Munro) Kurz, *D. dumosus* (Ridl.) Holttum, *D. elegans* (Ridl.) Holttum, *D. giganteus* Munro, *D. hamiltonii* Nees & Arn. ex Munro, *D. latiflorus* Munro, *D. longifimbriatus* Gamble, *D. membranaceus* Munro, *D. nudus* Pilg., *D. pendulus* Ridl., *D. sericeus* Munro, *D. strictus* (Roxb.) Nees, and an unidentified species (*Dendrocalamus* sp.). However, only seven of these 14 species had formerly been reported by Lin (1968) and eight species recognized by Smitinand (2001).

The exact number of *Dendrocalamus* species occurring in Thailand is still, therefore, unclear (Lin, 1968; Dransfield, 1994; Smitinand, 2001) and there is a need to resolve this problem. Moreover, the species status of several taxa appears problematic. For example, the widespread and economically important and taxonomically controversial species, *D. membranaceus*, has been transferred to *Bambusa*, as *B. membranacea*, by Stapleton and Xia (1997) on the grounds that the leaf-blades are small and the synflorescence prophylls are 2-keeled. However, a few years later, this species was included in *Dendrocalamus* by Dransfield and Wong (2004). They argued that the characters of the spikelets as found in specimen *Wallich* Cat. 5029, the type of this species, were similar to those of the type species of *Dendrocalamus, D. strictus.* Furthermore, they indicated that *D. membranaceus* is not the only species in this genus possessing small leaf-blades, as *D. elegans* also has small leaf-blades. Therefore, Dransfield and Wong (2004) stated that the separation of *Bambusa* from *Dendrocalamus*, relying upon the broad, 2-keeled prophyll subtending the synflorescence, may require further substantiation.

There is a need to document, in a floristic treatment of the genus, the Thai *Dendrocalamus* species. An up to date account of the *Dendrocalamus* species in Thailand will be published in the Flora of Thailand and is essential for the Flora of Thailand project (Parnell, 2000; Middleton, 2003). It provides an account of the species found in Thailand and their taxonomy/synonymy, a description of their characteristics (so they can be accurately identified by others), details of specimens that have been examined and also information on species distributions. Fieldwork and herbarium work are essential for these accounts and, in the case of bamboos, fieldwork is particularly important.

Importance of fieldwork for studying bamboos

Describing a plant based mainly on herbarium specimens may be sufficient for some other plant groups. However, it is not the case with bamboos, especially the genus *Dendrocalamus*. Some of the difficulties arise because of a lack of fieldwork on *Dendrocalamus* in Thailand which therefore make the interpretation of herbarium material problematic. Certainly, fieldwork is critically required. Important bamboo characters, especially vegetative characters, that could only be gained from the fieldwork, have been commented on many previous works (e.g. Holttum, 1958; McClure, 1966; Dransfield & Widjaja, 1995a; Wong, 1995a, 2004; Sungkaew *et al.*, *unpublished*; Li *et al.*, 2006b). Making a good herbarium specimen of bamboo is time-comsuming but very worthwhile, indeed essential. Sufficient labelling of the characters that cannot be investigated on a herbarium specimen is very important; these include for example, habitat, type of rhizome, habit of clump and culms, branch complement, colour of organs, and the covering of all these organs.

In this PhD thesis, taxonomic, ethnobotanical and ecological information was gathered during fieldwork. Information was also taken from herbarium specimens I borrowed from several herbaria and during visits to the regional herbaria that have excellent collections, but do not have the facility for sending material on loan. In addition, field study within the region was extremely important in order to increase the understanding of the poorly known bamboos. Four field trips, totalling a period of 5 months were made to Thailand and neighboring countries during the years between 2003 and 2006. From the fieldwork, a number of new findings were found including: 1) three new records for Thailand, *Dendrocalamus copelandii* (Sungkaew *et al.*, *accepted*), see Manucript 4.1, *Pseudostachyum polymorphum* (Sungkaew *et al.*, *in prep.*), *Chimonocalamus* sp. (Sungkaew *et al.*, *in prep.*), 2) a new species, *Dendrocalamus khoonmengii* (Sungkaew *et al.*, *accepted*), see Manucript 4.2 and 3) a new genus, *Phuphanochloa* (Sungkaew *et al.*, *accepted*), see Manucript 4.3. Such kinds of new finding without field work would be almost impossible. Although it is theoretically possible to describe new species based on only herbarium specimens, any person so doing may end up standing under a clump of bamboo that s/he decribed as a new taxon but be unable to identify the same taxon in the field.

Most of the bamboo herbarium specimens collected from my field expeditions were housed in BKF, KUFF, TCD, and THNHM[1].

---

[1] See Materials and methods section 4.2 for details about these acronyms.

Species concepts

To do a taxonomic revision, it is important that the species concept should be clear. It is well known that there are a number of different concepts currently in use. However, there is no universal acceptance of a single species concept (Luckow 1995).

In the case of this work, comparative morphological differences were used to delimit taxa and to produce the account of Thai *Dendrocalamus*. Taxonomic decisions were based mainly on morphological information gathered from the large number of herbarium specimens examined (approximately 950 herbarium specimens), living plant materials when available, and information observed from several field expeditions by the author. Stuessy (1990) suggested that from a practical standpoint in the preparation of Floras, the circumscription of species based upon easily observable morphological features is the sensible approach. Providing a usable and communicable Flora account, using this approach was adopted in this thesis. The morphological species concept or taxonomic species concept as stated by Davis & Heywood (1963, p. 91), is probably the closest to the species concept used in this account. It states that species are:

"assemblages of individuals with morphological features in common and separable from other such assemblages by correlated morphological discontinuities in a number of features".

The aims of this chapter were to:

1) Provide keys to species of Thai *Dendrocalamus*
2) Provide species descriptions including details on synonymy and typification, illustrations and distribution maps
3) Provide the complete account on Thai *Dendrocalamus* for the Flora of Thailand project
4) Discuss the findings of the floristic account in the context of previous taxonomic work, biogeography and conservation status of the taxa.

4.2 Materials and methods

This floristic work was undertaken at the Herbarium, Department of Botany, Trinity College Dublin (**TCD**[1]). Approximately 950 herbarium specimens were examined from the following herbaria using classical herbarium techniques; Herbarium Jutlandicum, Botanical Institute, University of Aarhus, Aarhus, Denmark (**AAU**); Herbarium, Plant and Soil Science Department, University of Aberdeen, Aberdeen, Scotland, U.K. (**ABD**); Herbarium, Botany Department, The Natural History Museum, London, England, U.K. (**BM**); Forest Herbarium, National Park, Wildlife, and Plant Conservation Department, Bangkok, Thailand (**BKF**); Harvard University Herbaria, Cambridge, Massachusetts, U.S.A (**GH**); Rijksherbarium, National Herbarium Nederland, Leiden University, Netherlands (**L**); Herbarium, Botanische Staatssammlung, München, Germany (**M**); Herbarium, Department of Botany, Trinity College Dublin (**TCD**); Herbarium, Thailand Natural History Museum, National Science Museum, Techno polis, Pathum Thani, Thailand (**THNHM**).

Other herbaria were also visited as follows and their specimens studied; Herbarium, Botany Section, Botany and Weed Science Division, Department of Agriculture, Bangkok, Thailand (**BK**); Forest Herbarium, National Park, Wildlife, and Plant Conservation Department, Bangkok, Thailand (**BKF**); Herbarium, Department of Biology, Chiang Mai University, Chiang Mai, Thailand (**CMU**); Herbarium, Royal Botanic Garden, Edinburgh, Scotland, U.K. (**E**); Herbarium, Royal Botanic Gardens, Kew, London, England, U.K. (**K**); Herbarium of Faculty of Forestry, Kasetsart University, Bangkok, Thailand (**KUFF**); Herbarium, Department of Biology, Prince of Songkhla University, Haad Yai, Songkhla, Thailand (**PSU**); Herbarium, Queen Sirikit Botanic Garden, Botanic Garden Organization, Chiang Mai, Thailand (**QBG**); Herbarium, Parks and Recreation Department, Botanic Gardens, Cluny Road, Singapore (**SING**); Herbarium, Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences. Menglun, Mengla, Yunnan, China (**XTBG**).

[1] All international herbarium acronyms used here follow 'Index Herbariorum' (Holmgren *et al.*, 1990) and 'Index Herbariorum: A Global Directory of Public Herbaria and Associated Staff' (Holmgren & Holmgren, 1998 [continuously updated]) (published on the internet: http://sweetgum.nybg.org/ih/). The following regional herbaria; **KUFF**, **THNHM**, and **XTBG**, are not in Index Herbariorum, and the abbreviations have been coined for use in this study.

To examine dry specimens, spikelets were softened in water containing a small amount of detergent (c. 1% of washing-up liquid) before measurements were taken using a stereomicroscope (Leica MZ12) with graticule..

In the species descriptions that follow, taxa are arranged systematically (and species ordered by position in the key). All specimens cited under each species have been seen (examined in detail) and are alphabetically arranged by the name of the countries, localities, years of collections, collector names and collector numbers, repectively. The species descriptions were based on herbarium specimens, spirit materials and on living plants, supplemented by observations of plants made in the field. However, the characters of the caryopses were based mainly on 'GrassBase-The Online World Grass Flora' (Clayton *et al.*, 2006 onwards) (published on the internet: http://www.kew.org/data/grasses-db.html) and the global distribution of species was based primarily on Ohrnberger (1999). Vernacular names and distributional information in Thailand were constructed on the basis of fieldwork, herbarium specimen labels and from the literature. Ecological information was taken from label information of the herbarium specimens and from field observations.

Publication title and author abbreviations cited follow The International Plant Names Index (2004) (published on the internet: http://www.ipni.org/index.html.). Abbreviations of publications that are not included in the web-site above, follow those commonly used in botanical literature. Sometimes journals have not been abbreviated for clarity and because no commonly used abbreviation exists.

Almost all types of accepted species and synonyms have been seen and are indicated by '!' after the herbarium's abbreviation. For the accepted species, when there is no holotype or previous lectotypification, a lectotype or an epitype has been designated and the reasons of typification are given, following Article 9 (Art. 9.1-holotype; Art. 9.2-lectotype; Art. 9.7-epitype) of 'International Code of Botanical Nomenclature (Vienna Code) 2006 (McNeill *et al.*, 2006).

4.3 Results

DENDROCALAMUS

Dendrocalamus Nees, Linnaea 9: 476. 1834; Munro, Trans. Linn. Soc. 26: 146. 1868; Kurz, Forest Fl. Burma 2: 558. 1877; Gamble, Ann. Bot. Gard. Calc. 7: 77. 1896; Ridl., Fl. Malay. Penin. 5: 265. 1925; Backer & Brink, Fl. Java (Spermatoph.) 3: 633. 1968; Brandis, Indian Trees (Fourth Impression): 675. 1921; Holttum, Gard. Bull. Singapore 16: 86. 1958; W.C. Lin in H.L. Li *et al.*, Fl. Taiwan 5: 772. 1978; K.M. Wong, Malayan Forest Records 41: 106. 1995; Ohrnb., The Bamboos of the World 282. 1999; D.Z. Li & Stapleton, Fl. China 22: 39. 2006.

Type: *Dendrocalamus strictus* (Roxb.) Nees

— *Sinocalamus* McClure, Lingnan Univ. Sci. Bull. No. 9: 66. 1940. Type: *Sinocalamus latiflorus* (Munro) McClure (see later, under *Dendrocalamus latiflorus* Munro)

Arborescent, un-armed bamboos. *Rhizomes* short necked, pachymorph. *Culms* unicaespitose, straight and erect, occasionally scrambling (sometimes found in *D. dumosus*), never climbing, forming a dense or loose clump; internodes terete. *Culm sheaths* usually deciduous, occasionally tardily deciduous; blades erect, spreading (patent) to deflexed (reflexed or re-curved); auricles ranging from lobelike and with oral setae (fimbriae) to low or inconspicuous and glabrous. *Buds* at each culm node solitary. *Mid-culm branch complements* with several branches at each node, a dominant primary branch and 1-several sub-dominant secondary branches and usually several smaller branchlets from its base, or all branches more or less subequal in size, always un-armed. *Leaves* shortly pseudo-petiolate, variable in size, sometimes very broad, with no transverse veinlets (tessellate), but frequently with pellucid glands instead. *Inflorescence* iterauctant (indeterminate), fully bracteate, usually borne on leafless branches, occasionally terminating a leafy branch (sometimes found in *D. pendulus*). *Pseudospikelets* congested as globose or stellate clusters. *Spikelets* yellow-green, yellow-brown, or purple-brown (sometimes found in *D. hamiltonii*), reddish purple or dark purple (usually found in *D. latiflorus*), occasionally yellow-green (occasionally found in *D. brandisii*); consisting of 0-several small empty bracts, 1–several bracts subtending prophyllate buds, 1–9 transitional (empty) glumes (shorter than the lowest lemma), 1–8 perfect flowers, with or without a vestigial terminal floret; rachilla internodes between flowers short and not disarticulating; palea 2-keeled except in uppermost or sole flower (then only slightly to not keeled), apex acute, blunt, to shortly bifid; lodicules none, or varying from 1–3; stamens 6; filaments free, anther apices mucronate to penicillate (the connective prolonged beyond the anther apex), glabrous or covered with miniscule spines; ovary tubinate to elliptic, summit thickened and hairy; stigma usually 1, occasionally divided into 2–3 (sometimes found in *D. hamiltonii*), slightly to firmly plumose, arising on a long, slender, hairy style. *Caryopses* small, seed surrounded by a crustaceous or hardened pericarp.

A genus distributed from the southern part of China, extending to western and eastern parts; India: almost throughout India including the Himalayas and Andaman Islands; Nepal; Bhutan; Bangladesh; Sri Lanka; Burma (Myanmar); Thailand; Laos; Cambodia; Vietnam; Malaysia: Malay Peninsula and Borneo; Philippines; Indonesia; Papua New Guinea. There are 13 species found in Thailand.

Key to subgenera and species of Thai *Dendrocalamus*

(based primarily on fertile specimens)

D. khoonmengii (species number 2) cannot be fitted into this key as no flowering specimens were available.

1. Pseudospikelets congested as globose clusters; fertile florets usually 1—4(—6). Mid-culm branch complement with 1—3 branches dominant..***D.* subg. *Dendrocalamus***

2. Pseudospikelets spiny to the touch; spikelets yellow-green, sub-terete

3. Fertile florets 1—2. Native plants, only found in southern part of the country

4. Paleas 0–2-nerved between keels. Plants restricted to limestone or rocky, open vegetation. Diameter of culm internodes usually less than 3 cm; walls thick to culms solid..**1. *D. dumosus***

4. Paleas 2–4(–6)-nerved between keels. Plants usually found in valleys in tropical rain forest. Diameter of culm internodes usually more than 3 cm; walls relatively thin, culms hollow......................................**3. *D. pendulus***

3. Fertile florets 2—4(—6). Exotic or native or plants only found in cultivation, if native, then never occuring in wild in the southern part of the country

5. Culm sheaths covered with tawny or golden brown hairs on the back or glabrous; auricles usully absent or inconspicuous..**4. *D. strictus***

5. Culm sheaths covered with dark brown to black hairs on the back, occasionally glabrous; auricles always present, conspicuous

6. Lower internodes covered with scurf or appressed white hairs below nodes or upper half of internodes. Exotic bamboo, only found in cultivation..**5. *D. barbatus***

6. Lower internodes usually glabrous. Native bamboo, found in the wild..........................**6. *D. membranaceus***

2. Pseudospikelets not spiny to the touch; spikelets yellow-brown, purple-brown (sometimes found in *D. hamiltonii*), reddish purple or dark purple (usually found in *D. latiflorus*), occasionally yellow-green (occasionally found in *D. brandisii*), laterally compressed

7. Spikelets yellow-brown, occasionally yellow-green. Culm-sheath auricles lobelike, never a triangular protuberance, with oral setae

8. Spikelets yellow-brown; fertile florets (3—)4—5. Margin of culm-sheath ligule lacerate or dentate, each division further divided into fine bristles..**7. *D. asper***

8. Spikelets yellow-brown, occasionally yellow-green; fertile florets 2—4(—5). Margin of culm-sheath ligule lacerate, each division not divided into fine bristles..**8. *D. brandisii***

7. Spikelets purple-brown. Culm-sheath auricles a triangular protuberance, without oral setae..................**9. *D. hamiltonii***

1. Pseudospikelets congested as stellate clusters; fertile florets usually 4—8-flowered. Mid-culm branch complement with more or less subequal branches..***D.* subg. *Sinocalamus* (McClure) Hsueh & D.Z. Li**

9. Spikelets purplish, tip of lemma more or less patent (spikelets appearing rough); fertile florets 6–8. Margins of culm-sheath auricles fringed with few bristles (readily deciduous)..**10. *D. latiflorus***

9. Spikelets yellow-brown, tip of lemma not patent (spikelets appearing smooth); fertile florets 4–6. Margins of culm-sheath auricles not fringed

10. Spikelets ≥ 2 cm long. Paleas membranous or sub-chartaceous. Lower culm nodes without verticils of roots; roots if present, then, developing from the very basal nodes

11. Spikelets 2–2.8 cm long; inflexed edges of palea narrow, not nerved. Lower culm internodes not hairy ....**11. *D. copelandii***

11. Spikelets 3–3.7 cm long; inflexed edges of palea wide, usually 1-nerved. Lower culm internodes densely hairy ....**12. *D. sinicus***

10. Spikelets < 2 cm long. Paleas chartaceous. Lower culm nodes with verticils of roots..................**13. *D. giganteus***

Key to subgenera and species of Thai *Dendrocalamus*

(based on sterile specimens and ecology)

1. Mid-culm branch complement with 1—3 branches dominant..***D.* subg. *Dendrocalamus***

2. Native plants, only found in southern part of the country

3. Culm sheaths coriaceous, usually covered with dark brown to black hairs; blades narrowly lanceolate; auricles more or less falcate lobes

4. Plants restricted to limestone or rocky, open vegetation, on thin soil. Walls of culm internodes usually relatively thick to internode solid. Culm-sheath auricles not twisted...**1. *D. dumosus***

4. Plants not restricted to limestone or rocky areas, usually occur on steep slopes along streams in tropical rainforest, on deep soil. Walls of culm internodes usually relatively thin, internode hollow. Culm-sheath auricles slightly twisted..**2. *D. khoonmengii***

3. Culm sheaths largely coriaceous or rather rigid but brittle, but margins in upper half sheath papery, usually covered with felted white to pale brown, readily deciduous hairs; blades broadly lanceolate; auricles with low rims..**3. *D. pendulus***

2. Exotic or native or plants only found in cultivation, if native, then never occuring in wild in the southern part of the country

5. Lower culm internodes usually not hairy (except *D. barbatus*, lower ones covered with scurf or appressed white hairs below nodes or upper half of internodes, occasionally becoming glabrous). Mid-culm branch complements usually without aerial roots

6. Culm sheaths covered with tawny or golden brown hairs on the back to glabrous; auricles tiny or absent ....**4. *D. strictus***

6. Culm sheaths with dark brown to black hairs on the back, occasionally glabrous; auricles obviously present

7. Lower internodes covered with scurf or appressed white hairs above nodes or lower half of internodes. Exotic bamboo, only found in cultivation..**5. *D. barbatus***

7. Lower internodes not scurfy or hairy. Native bamboos, found in the wild.......................**6. *D. membranaceus***

5. Lower culm internodes always densely hairy. Mid-culm branch complements usually with aerial roots.

8. Culm-sheath auricles lobelike, never a triangular protuberance, with oral setae

9. Margin of culm-sheath ligule lacerate or dentate, each division further divided into fine bristles ....**7. *D. asper***

9. Margin of culm-sheath ligule lacerate, each division not divided into fine bristles.........................**8. *D. brandisii***

8. Culm-sheath auricles a triangular protuberance, without oral setae...**9. *D. hamiltonii***

1. Mid-culm branch complement with more or less subequal branches.***D.* subg. *Sinocalamus* (McClure) Hsueh & D.Z. Li**

10. Margins of culm-sheath auricles fringed with few bristles (readily deciduous)................................**10. *D. latiflorus***

10. Margins of culm-sheath auricles not fringed

11. Lower culm nodes without verticils of roots, if present, then developing from the very basal nodes.

12. Lower culm internodes not hairy..**11. *D. copelandii***

12. Lower culm internodes densely hairy..**12. *D. sinicus***

11. Lower culm nodes with verticils of roots..**13. *D. giganteus***

1. **Dendrocalamus dumosus** (Ridl.) Holttum, Gard. Bull. Singapore 11(4): 296. 1947, *l.c.*, 16: 96. 1958; K.M. Wong, Malayan Forest Records 41: 109. 1995; Ohrnb., The Bamboos of the World 285. 1999. Type: Rawi Island (Thailand, Satun Province, west of Tarutao National Park), fertile, April 1911, *Ridley* 15903 [lectotype **BM!**, designated here; isolectotypes **K!**, **SING**, designated here]. Fig. 4.1 & 4.2.

— *Schizostachyum dumosum* Ridl., Journ. As. Soc. Straits 61: 64. 1912. Type: as for above.

— *Schizostachyum elegans* Ridl., Journ. As. Soc. Straits 73: 146. 1916. Type: Pinang [cult. (origin Langkawi), fertile, 5 April 1915, *Burkill* SFN 785 [lectotype **BM!**, designated here; isolectotypes **K!**, **SING**, designated here], ***synon. nov.***

— *Dendrocalamus elegans* (Ridl.) Holttum, Gard. Bull. Singapore 11(4): 296. 1947, *l.c.*, 16: 95. 1958. Type: as for *Schizostachyum elegans* Ridl., ***synon. nov.***

Culms rather well-spaced, straight, occasionally scrambling, upper part out-arched to drooping, to 7 m long, 1–5(–6) cm in diameter; internodes 15–40 cm long, lower ones not hairy; nodes sometimes slightly swollen, lower ones usually without verticils of roots; walls relatively thick, culms often solid. *Branches* developing from all nodes or nearly so; mid-culm branch complements with several branches at each node, only the primary one dominant, or accompanied by two sub-dominant branches, without aerial roots. *Culm sheaths* deciduous, coriaceous, 14.5–30 cm long by 5–12.5 cm wide, tops shallowly concave or truncate to broadly convex, back covered with dark brown to black hairs, sometimes glabrous; blades linear-lanceolate, spreading to deflexed, 2–13 cm long, about 0.3–1.2 cm wide near the base, sparsely hairy on adaxial surface, more densely hairy towards the base; auricles falcately lobed, fragile, 2–3 mm tall, 0.5–1 cm long, margins fringed with bristles 3–7 mm long; ligule 2–3 mm high, margin irregularly toothed in the middle, usually drawn into fine bristles up to 7 mm long at either end of the ligule. *Leaves* 5–9 per branch; blades 10–17 cm long by 0.8–2.5 cm wide, sparsely hairy underneath to glabrous, bases rounded to acute, pseudo-petiole 0.5–3 mm long; leaf sheaths 2.5-7 cm long; auricles with inconspicuous to small falcate lobes, fringed with bristles up to 7 mm long, sometimes glabrous; ligule 0.2–1 mm high, margin irregularly toothed at the middle, usually drawn into fine bristles up to 2.5 mm long at either end of the ligule; secondary veins 4–10 pairs, intermediate veins 5–8. *Pseudospikelets* congested as more or less densely globose clusters 0.6–2 cm in diameter, spiny to the touch; synflorescence axes hairy to glabrous, internodes 0.5–2 cm long. *Spikelets* light yellow-green, ovate-lanceolate, sub-terete, 7–11.5 mm long by 1.3–2.5 mm wide; glumes 2–3(–4), 2.2–10.5 mm long, 7–15-nerved; fertile florets 1–2, not patent between florets, terminal vestigial floret absent; lemmas chartaceous, 6–11.3 mm long, back sparsely hairy near apex to glabrous, 9–15-nerved, apex drawn out into a short or long stiff point, up to 1.5 mm long; paleas chartaceous, 5.2–9.7 mm long by 1–1.5 mm wide, 2-keeled on the back of the lower floret, not keeled in the uppermost floret or in solitary floret, keels and edges fringed, both sides sparsely pubescent near apex to glabrous, apex acute to acuminate, 0–2-nerved between keels, 0–1-nerved on each inflexed edge; lodicules none; anthers 2.5–5 mm long, yellow, tips mucronate to apiculate, usually glabrous, occasionally covered with a few minuscule spines; ovary turbinate, summit sparsely hairy or spiny to glabrous, stigma 1, slightly to firmly plumose. *Caryopsis* with adherent pericarp, about 5.5 mm long.

Thailand.— PENINSULAR: Chumphon (Langsuan District); Krabi (Muang District, Tham Sua Temple); Nakhon Si Thammarat (Thung Song District); Satun (La Ngu District, Tarutao National Park; Khuan Don District); Songkhla (Sadao District, Kao Roop Chang; Rattaphum District, Ton Nga Chang Wildlife Sanctuary); Surat Thani (Takhun District, Khlong Saeng Wildlife Sanctuary, Chiew Larn Dam; Kanchanadij District, Tambon Tha U-tae).

Distribution.— From peninsular Thailand to Malay Peninsula (Kedah, Langkawi Island), Fig. 4.3.

Ecology.— Confined to limestone or rocky areas in peninsular Thailand.

Vernacular.— Pai Ruak (ไผ่รวก), from *Kerr* 11872; Pai Khao (ไผ่เขา).

Notes.— Holttum (1958) and Wong (1995a) mentioned that *Dendrocalamus elegans* (Ridl.) Holttum appeared to be very close to *Dendrocalamus dumosus* (Ridl.) Holttum. I, also, could not find any characters to separate them from each other. According to the priority of the publications, I, therefore, treat *D. elegans* as a synonym of *D. dumosus.*

Typification notes.— When Ridley (1912) first described *Schizostachyum dumosum*, no specimen was cited, but he stated that this species could be found in '**Rawi** Island on a dry rock, face of the island forming dense thickets, the stems usually short 6 or 7 feet but sometimes much longer'. Holttum (1958), in his 'The Bamboos of the Malay Peninsula', later said that the original collection was from **Rawei** Island, in the Adang group, west of Tarutau (Siamese territory), where Ridley reported it **"on a dry rock, face of the island forming dense thickets, the stems usually short 6 or 7 feet but sometimes much longer"**. Holttum (1958) then made a conclusion about the type of this species by citing '**Rawei** Island, Ridley 15903 (S, K, Type collection)' but did not say what kind of types they were. I have seen two duplicates of this collection in **BM** and **K** (=K of Holttum (1958)). I also visited and studied bamboos specimens in **SING** (=S of Holttum (1958)), but have not seen its duplicate as referred to by Holttum (1958). Holttum (1958) tried to repeat what Ridley (1912) said but used **Rawei** Island instead of Ridley's **Rawi** Island. This is possibly because Holttum would (I believe) have seen the original label with the typed heading paper 'Flora of Kedah' and '**Rawei** Island' written in to indicate where the specimen was collected (as on the one currently housed in **BM**). I am sure that the two different spellings, **Rawi** Island and **Rawei** Island, are the same place which is now in Tarutao National Park, La Ngu District, Satun Province, southern Thailand but the name should be phonetically spelled '**Rawi** (or **Rawee**)'. I have also made collections of this species from that place. 'Kedah' is now a state of Malaysia, located in the northwestern part of Malay Peninsula which is very close to where **Rawi** Island is.

Whether or not the collection *Ridley 15903* was used by Ridley (1912) is unknownable. However, Holttum, a British botanist, who was working in Malay Peninsula during 1921-1954, and who was for part of this period the Director of the Singapore Botanical Garden (Stafleu & Cowan, 1979), and who worked at **K** later on (S. Dransfield, *personal communication*), must have known Ridley, who was also a British botanist, who lived between 1855 and 1956, and who directed the Singapore Botanical Garden during 1888-1912, and was living at **K** in his retirement (Stafleu & Cowan, 1983). So, it is possible that Holttum would also have had a clue which collection was used by Ridley (1912) to describe *S. dumosum*. I agreed with Holttum (1958) and, therefore, designate here *Ridley 15903*, housed in **BM** (code: BM000929632), as the lectotype and regard the duplicates in **K** (code: K000290648) and **SING** (if the duplicate is found) as isolectotypes.

When *Schizostachyum elegans* was first published, Ridley (1916) stated that this species was common in Lankawi Islands, Kedah and this locality was where it flowered in March 1915. However, he did not cite any type specimens. Holttum (1958) cited two collections, 'Burkill SFN 785, S, K and Henderson SFN 29097, S, K', both are from Langkawi and fertile, as the specimens examined for *Dendrocalamus elegans* (Ridl.) Holttum (transferred from *Schizostachyum elegans* Ridl.). I have seen *Burkill* SFN 785 in **BM** and **K** (but not in **SING**) and *Henderson SFN 29097* in **SING** (but not in **K**). To ensure that the type is easily accessed, therefore, one of the fertile specimens of *Burkill* SFN 785, housed in **BM** (code: BM000929636), which was collected from a cultivated plant in Pinang (origin Langkawi), is here designated as the lectotype, and the remaining duplicates (one in **K**, code: K000290646; one in **SING**, if the duplicate is found) are regarded as isolectotypes, respectively.

Specimens examined.

Thailand: PENINSULAR: ***Chumphon*** [Langsuan District, Tako, limestone hill, alt. ca. 100 m, fertile, 8 Feb. 1927, *Kerr* 11872 (**BK, K**)]; [Langsuan District, Tha Tako Subdistrict, Tham Sai, sterile, 25 Aug. 2004, *Sungkaew & Teerawatananon* 264 (**KUFF, THNHM**), 268 (**KUFF, TCD, THNHM**)]; ***Krabi*** [Muang District, Tham Sua Temple, fertile, 22 Feb. 2001, *Sungkaew & Pattanavibool* 6 (**BKF, K**)]; [*l.c.,* alt. ca. 200 m, sterile, 9 July 2004, *Sungkaew & Teerawatananon* 090704-5 (**KUFF, TCD, THNHM**)]; ***Nakhon Si Thammarat*** [Thung Song District, Tambon Cha-mai, alt. ca. 38 m, fertile, 24 March 2005, *Sungkaew & Teerawatananon* 390 (**KUFF, TCD, THNHM**), 395 (**KUFF, THNHM**)]; ***Satun*** [Adang Island, alt. ca. 5 m, fertile, 17 Jan. 1928, *Kerr* 14154 (**BM, K**)]; [La Ngu District, Tarutao National Park, Ko Rawi, sterile, 20 March 2005, *Sungkaew & Teerawatananon* 358 (**KUFF, TCD, THNHM**)]; [Tarutao National Park, Adang Island, south side, Laem Sone area, base of Chado Cliff, alt. sea level, fertile, 15 April 1987, *Maxwell* 87-349 (**BKF, GH, L**)]; [Tarutao National Park, Ko Adang, base of Chado Cliff, sterile, 20 March 2005, *Sungkaew & Teerawatananon* 368 (**KUFF, TCD, THNHM**)]; [Tarutao National Park, Rawi Island, open rocky areas, fertile, 8 Feb. 1981, *Congdon* 1179 (**AAU, GH**)]; [west of Tarutao National Park, Rawei Island, fertile, April 1911, *Ridley* 15903 (**BM, K**)]; [Khuan Don District, Tambon Wang Prajan, Ban Thung Maprang, sterile, 22 March 2005, *Sungkaew & Teerawatananon* 379 (**KUFF, THNHM**)]; ***Songkhla*** [Sadao District, Kao Roop Chang, alt. 125 m, fertile, 25 June 1986, *Maxwell* 86-413 (**BKF, GH, L, PSU**)]; [Rattaphum District, Ton Nga Chang Wildlife Sanctuary, sterile, 22 Aug. 2004, *Sungkaew & Teerawatananon* 238 (**KUFF, TCD, THNHM**)]; ***Surat Thani*** [Ratchaprapa Dam, sterile, 20 Feb. 2001, *Sungkaew & Pattanavibool* 2 (**BKF, K**)]; [Takhun District, Khlong Saeng Wildlife Sanctuary, Chiew Larn Dam, Khao Hna Daeng, sterile, 10 July 2004, *Sungkaew & Teerawatananon* 100704-9 (**KUFF, TCD, THNHM**)]; [Kanchanadij District, Tambon Tha U-tae, Ban Khao Mhon, alt. ca. 20 m, sterile, 25 March 2005 *Sungkaew & Teerawatananon* 396 (**KUFF, TCD, THNHM**)].

Malaysia: ***Langkawi*** [S. of Kuah, on ancient weathered black limestone, alt. 5–10 m, fertile, 15 Nov. 1979, *Stone* 14341 (**GH, K, L**)]; [S. of Kuah, near Country Club, coastal limestone, fertile, 15 Nov. 1979, *Chin* 2161 (**L**)]; [Pulau Timan, alt. sea level, fertile, 23 Nov. 1934, *Henderson* SFN 29097 (**SING**)]; ***Kedah*** [Gunong Baling, half way up, sterile, 25 Nov. 1941, *Nauen* s.n. (**L**)]; ***Pinang*** [cult. (origin Langkawi), fertile, 5 April 1915, *Burkill* SFN 785 (**BM, K**)],

2. **Dendrocalamus khoonmengii** Sungkaew, A. Teerawatananon, & Hodk., Thai Forest Bull., Bot. (in press). Typus: Thailand, Nakhon Si Thammarat, Lansaka District, Khaoluang National Park, *Wong, Thapyai, & Roisungnern* WKM 2868 [holotypus, **BKF!**; isotypi, **K**, **KLU**]. Fig. 4.4 & 4.5.

Related to *D. pendulus* Ridley, *D. hirtellus* Ridley and *D. dumosus* (Ridley) Holttum, from which it differs primarily in its projecting, curved and lightly twisted culm sheath auricles, and its abaxially glaucous leaf blades.

Culms loosely tufted, straight and erect, up to 13 m long, 3–4 cm in diameter, tips strongly arching over; internodes 35–50 cm long, lower ones not hairy; nodes not swollen, lower ones without verticils of roots; walls relatively thin (mid-culm portion). *Branches* developing from around mid culm or from lower quarter upwards; mid-culm branch complements with several branches at each node, the primary one dominant, long and slender, reaching 3–5 m long, accompanied by two sub-dominant branches, much shorter than the dominant one, aerial roots absent. *Culm sheaths* deciduous, coriaceous, 15–25 cm long by 10–15 cm wide, tops convexly truncate, back covered with black hairs, mainly on lower half; blades narrowly lanceolate, spreading to deflexed, 15–20 cm long, about 2 cm wide near the base, adaxial base sparsely hairy; auricles lobe-like, about 2–7 mm tall, 1–1.5 cm long, somewhat curved, slightly twisted, and free at the ends, margins fringed with pale brown bristles 7–10 mm long; ligule 3–12 mm high, margin irregularly toothed, some divisions further divided into fine bristles about 5 mm long. *Leaves* 5–10 per branchlet; blades 5–12 cm long by 0.4–1.2 cm wide, upper surface glabrous to sparsely hairy, lower surface glaucous and covered with short and fine hairs and scattered with long hairs to 1 mm long, bases rounded to obliquely cuneate, sessile or with pseudo-petiole to 1 mm long; leaf sheaths 2–3 cm long; auricles absent or just tiny rims, margins fringed with few wavy bristles to 3.5 mm long; ligule 0.1–0.3 mm high, margin irregularly toothed; secondary veins 2–4 pairs, intermediate veins 4–7. *Pseudospikelets* unknown.

Thailand.— So far known only from Nakhon Si Thammarat (Lansaka District), southern Thailand.

Distribution.— Possibly endemic to Thailand.

Ecology.— Steep slope along stream in tropical rain rainforest, deep soil of granite parent material.

Etymology.— This species is named after Professor Dr. Wong Khoon Meng, a plant taxonomist of Malaysia, who has been working on bamboos for more than 20 years and by whom the type specimen was collected.

Notes.— Wong Khoon Meng and his colleagues mentioned on the label that this species is similar to *D. pendulus* Ridley and *D. hirtellus* Ridley. However, it is also superficially similar to *D. dumosus* (Ridley) Holttum. The differences between these three species and *D. khoonmengii*, are summarized in Table 4.1.

The manuscript describing this species has been accepted for publication in the Thai Forest Bulletin (Botany), see Manuscript 4.2, following this section.

Specimens examined.

Thailand: PENINSULAR: ***Nakhon Si Thammarat*** [Lansaka District, Khaoluang National Park, steep slope beside Kra Rom Waterfall, alt. ca. 250 m, sterile, 11 July 2000, *Wong, Thapyai, & Roisungnern* WKM 2868 (**BKF**); [*l.c.*, sterile, 28 Aug. 2004, *Sungkaew & Teerawatananon* 257 (**KUFF, TCD, THNHM**)].

Table 4.1 Comparative table of habitats and vegetative morphological characters between *D. dumosus*, *D. hirtellus*, *D. khoonmengii*, and *D. pendulus*

Habitats and characters	***D. dumosus***	***D. hirtellus***	***D. khoonmengii***	***D. pendulus***
Habitat	confined to limestone vegetation, thin soil	open places and forest fringes	steep slope along stream in tropical rain rainforest, deep soil of granite parent material	thrives in foothills and valleys of the main mountain ranges or in logged or disturbed forest and forest fringes
Young culm nodes	glabrous	with a band of silvery brown hairs above and below node	with a band of black hairs only below node	with a band of silvery brown hairs above and below node
Culm length	to 7 m	to 15 m	to 13 m	to 30 m
Culm internodes	15–40 cm	40–50 cm	35–50 cm	40–50 cm
Culm diameter	1–2.5 cm	Commonly 6–8 cm	3–4 cm	commonly 6–9 cm
Culm-wall thickness	relatively thick (lacuna ≤ 1/3 the diameter of the culm), sometimes solid at lower internodes	relatively thin (lacuna ≥ 1/3 the diameter of the culm)	relatively thin	relatively thin
Verticils of roots at lower nodes	absent	present	absent	present
Culm sheaths	with black hairs mixed with thin white wax, to glabrous	with dense, but caducous, pale brown hairs mixed with copious white wax	with black hairs mixed with copious white wax	with dense, but caducous, pale brown hairs mixed with copious white wax
Culm-sheath auricles	readily deciduous, lobe-like, spreading, sometimes crisped, free at the end, somewhat curved, not twisted, bristly on margin, bristles to 7 mm long	spreading crisped lobes, not free at the ends, bristly on margin, bristles to 12–24 mm long	lobe-like, spreading, sometimes crisped, free at the ends, somewhat curved and slightly twisted, bristly on margin, bristles to 7–10 mm long	low rims, sometimes crisped, not free at the ends, bristly on margin, bristles to 7–10 mm long
Lower surface of leaf blade	not glaucous, short-hairy to glabrous	not glaucous, short-hairy	glaucous, short-hairy mixed with scattered long hairs	not glaucous, glabrous

Etymology.— This species is named after Professor Dr. Wong Khoon Meng, a plant taxonomist of Malaysia, who has been working on bamboos for more than 20 years and by whom the type specimen was collected.

Notes.— Wong Khoon Meng and his colleagues mentioned on the label that this species is similar to *D. pendulus* Ridley and *D. hirtellus* Ridley. However, it is also superficially similar to *D. dumosus* (Ridley) Holttum. The differences between these three species and *D. khoonmengii*, are summarized in Table 4.1.

The manuscript describing this species has been accepted for publication in the Thai Forest Bulletin (Botany), see Manuscript 4.2, following this section.

Specimens examined.

Thailand: PENINSULAR: ***Nakhon Si Thammarat*** [Lansaka District, Khaoluang National Park, steep slope beside Kra Rom Waterfall, alt. ca. 250 m, sterile, 11 July 2000, *Wong, Thapyai, & Roisungnern* WKM 2868 (**BKF**); [*l.c.*, sterile, 28 Aug. 2004, *Sungkaew & Teerawatananon* 257 (**KUFF, TCD, THNHM**)].

Table 4.1 Comparative table of habitats and vegetative morphological characters between *D. dumosus*, *D. hirtellus*, *D. khoonmengii*, and *D. pendulus*

Habitats and characters	***D. dumosus***	***D. hirtellus***	***D. khoonmengii***	***D. pendulus***
Habitat	confined to limestone vegetation, thin soil	open places and forest fringes	steep slope along stream in tropical rain rainforest, deep soil of granite parent material	thrives in foothills and valleys of the main mountain ranges or in logged or disturbed forest and forest fringes
Young culm nodes	glabrous	with a band of silvery brown hairs above and below node	with a band of black hairs only below node	with a band of silvery brown hairs above and below node
Culm length	to 7 m	to 15 m	to 13 m	to 30 m
Culm internodes	15–40 cm	40–50 cm	35–50 cm	40–50 cm
Culm diameter	1–2.5 cm	Commonly 6–8 cm	3–4 cm	commonly 6–9 cm
Culm-wall thickness	relatively thick (lacuna ≤ 1/3 the diameter of the culm), sometimes solid at lower internodes	relatively thin (lacuna ≥ 1/3 the diameter of the culm)	relatively thin	relatively thin
Verticils of roots at lower nodes	absent	present	absent	present
Culm sheaths	with black hairs mixed with thin white wax, to glabrous	with dense, but caducous, pale brown hairs mixed with copious white wax	with black hairs mixed with copious white wax	with dense, but caducous, pale brown hairs mixed with copious white wax
Culm-sheath auricles	readily deciduous, lobe-like, spreading, sometimes crisped, free at the end, somewhat curved, not twisted, bristly on margin, bristles to 7 mm long	spreading crisped lobes, not free at the ends, bristly on margin, bristles to 12–24 mm long	lobe-like, spreading, sometimes crisped, free at the ends, somewhat curved and slightly twisted, bristly on margin, bristles to 7–10 mm long	low rims, sometimes crisped, not free at the ends, bristly on margin, bristles to 7–10 mm long
Lower surface of leaf blade	not glaucous, short-hairy to glabrous	not glaucous, short-hairy	glaucous, short-hairy mixed with scattered long hairs	not glaucous, glabrous

3. Dendrocalamus pendulus Ridl., Journ. As. Soc. Straits 44: 210. 1905, Fl. Malay. Penin. 5: 266. 1925; Holttum, Gard. Bull. Singapore 16: 90. *fig. 22, 23 (A-C)*. 1958; S. Dransf. in S. Dransfield & E.A. Widjaja, Pl. Resources S.E. Asia 7: 92. *illustration on page 92.* 1995; K.M. Wong, Malayan Forest Records 41: 115. *fig. 58-61.* 1995; Ohrnb., The Bamboos of the World 290. 1999. Type: Malaysia, Selangor, Pahang Track, alt. 2,000 feet, fertile, *Ridley* 8482 [holotype **K!**]. Fig. 4.6 & 4.7.

— *Cephalostachyum malayense* Ridl., Journ. As. Soc. Straits 57: 118. 1911 (not 1910). Type: Malaysia, Perak, Temango, fertile (with galled inflorescences), *Ridley* 14350 [lectotype **K!**, designated here; isolectotype **SING**, designated here].

— *Schizostachyum subcordatum* Ridl., Journ. As. Soc. Straits 82: 204. 1920. Type: as for *Dendrocalamus pendulus* Ridl., *pro parte*

Culms closely tufted, straight, upper part arching over with pendulous tips, 7–30 m long, 2.5–9 cm in diameter; usually young culms and often old culms copiously covered with white wax; internodes 40–50 cm long, lower ones not hairy; nodes of young culm with a band of silvery brown hairs below and above each node, becoming glabrous when old, lower nodes usually with verticils of roots; walls relatively thin, about 0.5–1 cm thick (mid-culm portion). *Branches* developing from lower quarter or from around mid culm upwards; mid-culm branch complements with several branches at each node, the primary one dominant, long and slender, pendulous, aerial roots occasionally present. *Culm sheaths* deciduous, coriaceous or rather rigid but brittle, but margins in upper half of sheath papery, 15–25 cm long by 20–30 cm wide, tops shallowly convex to truncate, back covered with thick, white wax and felted white to pale brown, hairs readily deciduous; blades broadly lanceolate, spreading to deflexed, 7–25 cm long, about 2.5–8 cm wide near the base, adaxial base sparsely hairy; auricles with low rims, slightly pleated, 1.5–5 mm tall, 1–2 mm long, margins fringed with slightly flexuous pale bristles, about 0.5–2 cm long; ligule 0.3–1 cm high, margin irregularly toothed in the middle, becoming lacerate at either end of the ligule, up to 0.3 cm high. *Leaves* 5–17 per branchlet; blades 10–35 cm long by 1–4.5 cm wide, glabrous, occasionally sparsely hairy underneath near the base, bases rounded to acute or attenuate, occasionally sub-cordate, pseudo-petiole 0.2–1 cm long; leaf sheaths 3.5-9 cm long; auricles absent or inconspicuous ridges to small falcate lobes, glabrous or sometimes fringed with bristles 3–5 mm long; ligule 0.5–1 mm high, margin irregularly toothed; secondary veins 5–13 pairs, intermediate veins 5–7. *Pseudospikelets* usually congested as densely globose clusters 1.5–2.5 cm in diameter, spiny to the touch; synflorescence axes hairy to glabrous, internodes 1.5–3.5(–7) cm long. *Spikelets* light yellow-green, ovate-lanceolate, sub-terete, 5.5–9.5 mm long by 1.5–2 mm wide; glumes 2–3, 2.3–6 mm long, 7–15-nerved; fertile florets 1(–2), not patent between florets, terminal vestigial floret absent; lemmas chartaceous, 6–8.8 mm long, glabrous, 11–17-nerved, apex drawn out into a stiff point; paleas chartaceous, 5.5–7.5 mm long by 1–2 mm wide, 2-keeled on the back of lower floret, not keeled in sole or uppermost floret, keels not fringed, outside glabrous, inside sparsely pubescent to glabrous, apex acute to acuminate, 2–4(–6)-nerved between keels, 0–1-nerved on each inflexed edge; lodicules none; anthers 3–4 mm long, yellow or purple, tips mucronate or apiculate; ovary turbinate, summit sparsely to densely hairy, stigma 1, slightly plumose. *Caryopsis* with adherent pericarp, ca. 4 mm long.

Thailand.— PENINSULAR: Songkhla (Rattaphum District, Ton Nga Chang Wildlife Sanctuary); Yala (Bannang Sata; Betong).

Distribution.— Usually found on slope of hillsides in tropical rain forest in southern Thailand to the Main Range, Malay Peninsula, Fig. 4.8.

Ecology.— On hillsides, along steep banks in tropical rain forest.

Typification notes.— When *Cephalostachyum malayense* Ridl. was first described in 'Journ. As. Soc. Straits 57: 118. 1911 (not 1910)', no type was designated. Holttum (1958) stated that 'Perak, Temango, Ridley 14350 (with galled infl., type of *Cephalostachyum malayense* (as '*malayanum*'), S, K)' was the type. However, he did not specify what kind of type it is. I have seen this collection in **K** but have not seen its duplicate when I visited **SING**. Thus, I designate here *Ridley 14350*, housed in **K** as the lectotype, and if a duplicate is found in **SING** would regard it as an isolectotype.

Holttum (1958) stated that 'the type specimen of *Schizostachyum subcordatum* Ridl. was part of the original collection of *Dendrocalamus pendulus* (Ridley no. 8482) sent to Kew unnamed, later discovered by Ridley when he was preparing his Flora and re-described'. However, Holttum (1958) did not specify which part of that specimen was used by Ridley for describing *S. subcordatum*. Thus, the type of *S. subcordatum* is still questionable.

Specimens examined.

Thailand: PENINSULAR: ***Songkhla*** [Rattaphum District, Ton Nga Chang Wildlife Sanctuary, 22 Aug. 2004, *Sungkaew & Teerawatananon* 230 (fertile) (**KUFF, TCD, THNHM**), 231 (sterile) (**KUFF, THNHM**), 232 (sterile) (**KUFF, TCD, THNHM**)]; [Ton Nga Chang Wildlife Sanctuary, sterile, 23 March 2005, *Sungkaew & Teerawatananon* 381 (**KUFF**,

THNHM)]; [Ton Nga Chang Wildlife Sanctuary, sterile, 29 Oct. 2005, *Sungkaew & Teerawatananon* 632 (**KUFF, THNHM**)]; ***Yala*** [Bannang Sata, fertile, 20 Dec. 1965, *Sangkhachand* 1456 (**BKF**)]; [Betong, alt. 1,150 m, sterile, 22 Feb. 2000, *Niyomdham et al.*, 6022 (**BKF, K**)]; [Khao Han Kut, alt. 1,200 m, sterile, 28 March 1998, *Niyomdham* 5400 (**BKF, K**)].

Malay Peninsula: ***Kedah*** [Gunong Jerai, 3,200 feet, fertile, 15 Jan. 1964, *Burkill* HMB 3313 (**GH**)]; [Peak of Gunong Jerai, alt. 3,950 feet, fertile, 10 Sept. 1979, *Rao et al.* 38 (**AAU, L**)]; [Gunong Jerai (Kedah Peak), alt. 3,000 feet, sterile, 30 March 1982, *Wong* FRI 32374 (**L**)]; ***Negri Sembilan*** [Ulu Bendul, sterile, 30 Nov. 1922, *Holttum* SFN 9796 (**GH, K, SING**)]; ***Selangor*** [Pahang Track, alt. 2,000 feet, fertile, *Ridley* 8482 (**K**)]; [Ulu Gombak, fertile, 6 Dec. 1922, *Burkill* SFN 9973 (**GH, K, SING**)]; [Ulu Gombak VJR, alt. 2,000 feet, fertile, 12 Oct. 1970, *Kochummen* FRI 16227 (**L**)]; [Hulu Perak, S. Perak, near Fort Tapong, K. Kendrong, Ridge crest over shale, alt. 1,100 feet, fertile, 6 Jan. 1971, *Whitmore* FRI15791 (**L**)]; [Kanching, fertile, 11 Jan. 1982, *Wong* FRI 32341 (**GH, L**)].

Singapore: [cult. at Lawn Y, Botanic Gardens, fertile, 1 Oct. 1957, *Sinclair* 9361 (**E, K, L, SING**)].

4. **Dendrocalamus strictus** (Roxb.) Nees, Linnaea 9: 476. 1834; Munro, Trans. Linn. Soc. 26: 147. 1868; Kurz, Forest Fl. Burma 2: 558. 1877; Gamble, Ann. Bot. Gard. Calc. 7: 78. *pl. 68, 69* (except A). 1896; Brandis, Indian Trees (Fourth Impression): 675. 1921; Holttum, Gard. Bull. Singapore 16: 98. *fig. 24.* 1958; Backer & Brink, Fl. Java (Spermatoph.) 3: 634. 1968; W.C. Lin, Special Bul. Taiwan For. Res. Inst. 6: 30. *fig. 23* (not clear). 1968; W.C. Lin in H.L. Li *et al.*, Fl. Taiwan 5: 776. *pl. 1517.* 1978; P.C.M. Jansen & S. Duriyaprapan in S. Dransfield & E.A. Widjaja, Pl. Resources S.E. Asia 7: 93. *illustration on page 94.* 1995; K.M. Wong, Malayan Forest Records 41: 117. 1995; Ohrnb., The Bamboos of the World 291. 1999; D.Z. Li & Stapleton, Fl. China 22: 40. 2006. Type: Roxburgh's illustration in Pl. Corom. 1: 58. *t.* 80. (excluded stigma part). 1798 [lectotype (illustration) **K!**, designated here]; India, Ladwa? Gunti, fertile, *Wallich Cat.* 5038A [epitype **K!** (Herb. Hookerianum), only two leafy branches and a flowering branch on the main herbarium sheet (and not the other two attached), designated here]. Fig. 4.9 & 4.10.

— *Bambos stricta* Roxb., Pl. Corom. 1: 58. *t. 80.* (excluded stigma part). 1798. Type: as for above.

— *Bambusa stricta* (Roxb.) Roxb., Hort. Bengal. 25. 1814. Type: as for above.

— *Bambusa tanaea* Buch.-Ham. ex Wall. Cat. n. 5038A. *nom. nud.*

— *Bambusa pubescens* Lodd. ex Loudon, Hort. Brit. (Laudon): 124. 1830, *nom. nud.*

— *Bambusa pubescens* Lodd. ex Lindl., Penny Cyclop. 3: 357. 1835. Type: not located.

— *Dendrocalamus strictus* var. *prainiana* Gamble, Ann. Bot. Gard. Calc. 7: 80. *pl. 69* (only A). 1896. Type: Great Cocos Island, 'Table I', fertile, 2 Dec. 1889, *Prain* s.n. [lectotype **TCD!**, designated here).

Culms densely tufted, straight and erect, about 5–15 m long, 2.5–7.5 cm in diameter, tips much out-arched; internodes 30–45 cm long, lower ones not hairy; nodes somewhat swollen, lower ones usually with verticils of roots; walls relatively thick, culms often solid. *Branches* developing from all nodes or nearly so; mid-culm branch complements with several branches at each node, the primary one dominant, accompanied by two sub-dominant branches, the rest smaller, the primary branch from lower nodes usually curved downwards, without aerial roots. *Culm sheaths* deciduous, lower ones sometimes tardily deciduous, thickly papery to coriaceous, 11.5–30 cm long by 13.7–18.5 cm wide, tops convexly truncate, back covered with tawny or golden brown hairs to glabrous; blades broadly triangular, erect, 3–3.5 cm long, about 3.8–4.5 cm wide near the base, adaxial base with sparsely hairs, becoming glabrous; auricles absent or sometimes comprising inconspicuous tiny lobes 0.2–0.5 mm tall, about 3 mm long, margins or shoulders glabrous or with wavy bristles up to 4 mm long; ligule

1–2 mm high, margin irregular-dentate or erose. *Leaves* 3–10 per branchlet; blades 7–18.5 cm long by 0.8–2.2 cm wide, hairy both sides, usually denser below, sometimes nearly glabrous, bases rounded to acute, pseudo-petiole 2–4 mm long; leaf sheaths 2.5-8 cm long; auricles absent, shoulders bristly, bristles 1–5 mm long; ligule 0.5–0.7 mm high, margin somewhat dentate; secondary veins 3–6 pairs, intermediate veins 4–8. *Pseudospikelets* usually congested as densely globose clusters 1.5–4 cm in diameter, spiny to the touch; synflorescence axes hairy to glabrous, internodes 3–7 cm long. *Spikelets* light yellow-green, ovate-oblong, sub-terete, 5.6–16.5 mm long by 2–4.5 mm wide; glumes 1–2, 3.2–8.5 mm long, 5–15-nerved; fertile florets 2–3, not patent between florets, terminal vestigial floret absent; lemmas chartaceous, 5–15 mm long, back hairy to nearly glabrous, 11–19-nerved, apex drawn out into a long stiff point, up to 3.5 mm long; paleas chartaceous, 4.5–9.7 mm long by 1.3–1.5 mm wide, 2-keeled on the back, keels and edges long-fringed, the uppermost one not keeled or slightly keeled near the apex, outside pubescent, inside glabrous, apex blunt to shortly bifid, 2–3(–6)-nerved between keels, 1-nerved on each inflexed edge; lodicules usually none, occasionally 1–2, hyaline; anthers 2.9–6 mm long, yellow, tips mucronate; ovary turbinate, summit hairy, stigma 1, more or less plumose. *Caryopsis* with adherent pericarp, ovoid, or orbicular, 6–8 mm long, hairy at apex.

Thailand.— NORTHERN: Kamphaeng Phet (Me Klawng); Tak (Mae Sod District, Ban Hui Hin Fon).

Distribution.— Almost throughout India, Nepal, Bangladesh, Burma, Thailand (Ohrnberger, 1999), Fig. 4.11.

Ecology.— In dry mixed deciduous forest.

Vernacular.— Pai Sang (ไผ่ซาง).

Typification notes.— I noted incongruence between the detail of the stigma (which was two-cleft) on Roxburgh's illustration in Pl. Corom. 1: 58. *t.* 80. 1798. and that of all specimens of *Dendrocalamus strictus* (Roxb.) Nees examined by me (which has single stigma). No specimen of *Bambos stricta* Roxb. (basionym of *Dendrocalamus strictus* (Roxb.) Nees) has yet been designated as the type (C. Stapleton, *personal communication*). Therefore, I designate here the Roxburgh's illustration in Pl. Corom. 1: 58. *t.* 80. 1798., kept in **K** (currently in the library), as the lectotype. The specimen of *Wall. Cat.* 5038A, housed in **K**, was designated here as the epitype to serve as an interpretative type as the lectotype (Roxburgh's illustration in Pl. Corom. 1: 58. *t.* 80. 1798., which has two-cleft stigma) is demonstrably ambiguous and cannot be critically identified for purposes of the precise application of the name of the taxon (McNeill *et al.*, 2006).

When Gamble (1896) first described *Dendrocalamus strictus* var. *prainiana* Gamble, without citing any type, he stated that the drawing on Plate 69 was "A, spikelet of the variety (var. *prainiana*) from Table Island, Great Cocos", (see p. 80). There are two possibilities for the type of this variety. The first comprises two specimens (one in **ABD**, the other in **L**), both are fertile, collected by *Prain* s.n., were collected in 'Nov. 1890', from 'Great Coco' (spelling with no 's'), and the word 'Little' is crossed-off in both labels. The second is a specimen in **TCD**, also collected by *Prain* s.n., labelled as collected on 'Dec. 2, 1889', from 'Great Cocos' (spelling with 's') 'Island, [Table I]' which I presume means 'Table Island', the smallest island of the **Coco Islands** located at the north end of **Great Coco Island**. I am quite sure that these two different spellings, 'Coco' and 'Cocos', refer to the same place which is the **Coco Islands**, belonging to Burma, in the Indian Ocean, which islands are separated from North Andaman Island (belonging to India) by the Coco Channel. With the Alexandra Channel between them, the Coco Islands consist of the main **Great Coco Island** (Coco Island) and the smaller **Little Coco Island**. Therefore, I designate the **TCD** specimen as the lectotype, because it is more congruent with Gamble (1986).

Specimens examined.

Thailand: NORTHERN: ***Kamphaeng*** Phet [Me Klawng, alt. ca. 600 m, fertile, 16 June 1922, *Kerr* 6142 (**ABD, BK, BM, K**)]; ***Tak*** [Mae Sod District, Ban Hui Hin Fon, fertile, 6 May 2005, *Sungkaew & Teerawatananon* 582 (**KUFF, TCD, THNHM**)]; [*l.c.*, sterile, 6 May 2005, *Sungkaew & Teerawatananon* 583 (**KUFF, TCD, THNHM**)]; [*l.c.*, fertile, 15 Nov. 2005, *Sungkaew & Teerawatananon* 717 (**KUFF, TCD, THNHM**)]; [*l.c.*, fertile, 15 Nov. 2005, *Sungkaew & Teerawatananon* 718 (**KUFF, THNHM**)].

Bahama Islands: ***Nassau*** [cult. on grounds of Sheraton British Colonial Hotel, fertile, 31 Dec. 1975, *Gillis* 12785 (**GH**)].

Burma: ***Great Coco Island*** [fertile, Nov. 1890, *Prain* s.n. (**ABD, L**)]; ['Table I', fertile, 2 Dec. 1889, *Prain* s.n. (**TCD**)]; ***Mandalay*** [Hills N. of Mandalay, alt. 1,000-2,000 feet, fertile, Jan. 1884, *Oliver* s.n. (**K**)]; ***Shan State*** [fertile, 1893, *King* s.n. (**L**)]; [southern Shan State, Shan Plateau, Taunggyi, alt. 3,300-3,600 feet, fertile, 15 Jan. 1953, *Vogt* BU 519 (**K**)]; ***Shingyedi?*** [fertile, Jan. 1903, *Mokim* 1114 (**GH**)].

Cuba: ***Habana*** [cult. at Rio Cristal, fertile, 25 Nov. 1954, *Venning* 19479 (**GH**)].

India: ***Bombay*** [fertile, *Dalzell* s.n. (**GH**)]; ***Canara*** [fertile, 1851, *Hohenacker* 748 (**L, M**); [fertile, *Hohenacker* 165a (**GH**)]; [fertile, *Hohenacker* 700 (**M**)]; ***Central province*** [near Chiandgarh?, fertile, 26 Jan. 1889, *Duthie* 8544 (**K**)]; ***Dehra Dun*** [Kaulagone, fertile, May 1894. *Birbal* s.n. (**L**)]; ***Himalaya range*** [Kumaon, The Bhabar, alt. 1,000 feet, fertile (excl. sheath and lvs. samples), *Strachey & Winterbottom* s.n. (**GH**)]; ***'Himal. Bor. Oce.'*** [fertile, *Thomson* s.n. (**GH, L, TCD**)]; ***West Himalaya*** [fertile, *Griffith* s.n. (**L**)]; [Garhwal, fertile, 27 May 1902, *Duthie* 26011 (**K**)]; ***Kotdwara*** [District Bijnor, fertile, Feb. 1911, *Unknown collector* (**GH**)]; **Ladwa? Gunti** [fertile, *Wallich Cat.* 5038A (Herb. Hookerianum)]; ***Malabar*** [Concan, fertile, *Stocks* s.n. (**L**)]; ***Nagapatam*** [fertile, *Wight* s.n. (**GH**)]; ***Nagargali*** [Nagargali Forest, fertile, 20 March 1951, *Fernandes* 2287 (**GH**)]; ***Orissa*** [Shillong, Bhatipathar, fertile, 3 Nov. 1959, *Panegraaf* 20800 (**L**)]; ***'Plan. Ganget. Inf.'*** [fertile, *Thomson & Hooker* s.n. (**GH, L, TCD**)]; ***Rajasthan*** [Mount Abu, fertile, 27 April 1917, *Blatter* 2535 (**K**)]; ***Tamil Nadu*** [Mont. Nilghiri & Kurg, fertile, *Thomson* s.n. (**GH, L**)]; [Dindigul, Palni Hills, Thevankarai-Palani hill path, alt. 900 m, fertile, 9 March 1987, *Matthew & Rajendran* RTH 48611 (**AAU**)]; ***'Ind. or.'*** [fertile, *Wallich* 5037 (**TCD**)]; *No locality* [Herbarium of the late East India Company, fertile, *Falconer* 1241 (**M**)].

Indonesia: ***Bogor*** [cult. in Bogor Botanical Garden, fertile, +- 1927, *Unknown collector* (**L**)]; [cult., *l.c.*, alt. 250 m, fertile, 1924, *D.T.H.* 433 (**L**)].

Malaysia: ***Penang*** [cult. in Residency Garden, fertile, 29 Dec. 1938, *Kadir* SFN 36176 (**L**)].

New Caledonia: [cult.?, fertile, *Catala* 7460 (**L**)].

Singapore: [cult. in Botanic Gardens Singapore, Lawn F., near Lake, fertile, 15 Dec. 1965, *Tassim* 703 (**L, SING**)].

Trinidad: [cult. in Royal Botanic Garden, fertile, Feb./March 1921, *L.H. Bailey & E.Z. Bailey* s.n. (**GH**)].

Vietnam: ***Saigon*** (Ho Chi Minh City) [cult. in Jadin Botanique, fertile, July 1909, *Alleizette* 8294 (**L**)].

No locality: [fertile, 1859, *Griffith* s.n. (**L**)]; [fertile, *Unknown collector*, (**L**, code: L0412680)].

5. **Dendrocalamus barbatus** Hsueh & D.Z. Li, J. Bamboo Res. 7(4): 4. *fig. 1.* 1988; Ohrnb., The Bamboos of the World 284. 1999; D.Z. Li & Stapleton, Fl. China 22: 40. 2006. Types: Yunnan, Mengla, fertile, June 1975, *Hsueh* 928 [holotype **SWFC**]. Fig. 4.12.

— *Dendrocalamus barbatus* var. *internodiiradicatus* Hsueh & D.Z. Li, J. Bamboo Res. 7(4): 6. 1988. Type: Yunnan, Mengla, the Tropical Botanical Garden, fertile, 27 Feb. 1981, *Y.L. Huang* 11151. [holotype **YNTBI**], *syn. nov.*

Culms more or less well-spaced, straight and erect, about 15–20 m long, 8–15 cm in diameter, tips curved; internodes 30–40 cm long, lower ones covered with scurf or appressed white hairs above nodes or lower half of internodes, occasionally becoming glabrous; nodes not swollen, lower ones with verticils of roots; walls relatively thin, about 1.5 cm thick (mid-culm portion). *Branches* developing from lower quarter upwards, occasionally from almost all nodes; mid-culm branch complements with several branches at each node, the primary one dominant, accompanied by two sub-dominant branches, usually without aerial roots. *Culm sheaths* deciduous, coriaceous, 25–30 cm long by 30–37 cm wide, tops convexly truncate, back covered with sparsely dark brown or black hairs to glabrous, occasionally with longitudinal yellowish or white streaks; blades narrowly lanceolate, spreading to deflexed, 5–10 cm long, about 2–3 cm wide near the base, adaxial base glabrous to densely bearded; auricles low lobes 1–3 mm tall, about 1–1.5 cm long, margins with few bristles 2–3 mm long; ligule 1–1.3 cm high, margin divided into lacerations, each division 0.5–0.7 cm high. *Leaves* 6–7 per branchlet; blades 15–20 cm long by 1–2 cm wide, glabrous or hairy beneath near the base, bases somewhat truncate or rounded, acute to obliquely acute, pseudo-petiole 2–3 mm long; leaf sheaths 4-5.5 cm long; auricles absent or inconspicuous, shoulders glabrous or with few bristles 1–2 mm long; ligule about 0.5 mm high, margin irregularly toothed; secondary veins 5–6 pairs, intermediate veins 7–8. *Pseudospikelets* congested as more or less densely globose clusters 1–1.5 cm in diameter, spiny to the touch; synflorescence axes glabrous, internodes 1.5–2.5 cm long. *Spikelets* usually light yellow-green, elliptic-oblong, sub-terete, 6–8.5 mm long by 2.5–3 mm wide; glumes 1–2, 3.5–5.5 mm long, 5–11-nerved; fertile florets 2–3, not patent between florets, terminal vestigial floret absent; lemmas chartaceous, 6.5–7.7 mm long, back sparsely hairy to glabrous, 15–19-nerved, apex shortly mucronate; paleas chartaceous, 6–7 mm long by 1.3–1.5 mm wide, 2-keeled on the back, keels and edges fringed, the uppermost one not keeled, both sides pubescent to glabrous, apex blunt to acute, 1–2-nerved between keels, 0–1-nerved on each inflexed edge; lodicules none; anthers 3–3.5 mm long, yellow, tips mucronate; ovary turbinate, summit hairy, with long slender style, stigma 1, more or less plumose. *Caryopsis* unknown.

Thailand.— Found only in cultivation in the north. NORTHERN: Chiang Mai (Mae Taeng District; Mae Wang District).

Distribution.— Of uncertain origin, but possibly native in Yunnan, China (Ohrnberger, 1999).

Vernacular.— Pai Sang-khiew (ไผ่ซางเขียว), Pai Sang-hmon (ไผ่ซางหม่น).

Specimens examined.

Thailand: NORTHERN: ***Chiang Mai*** [cult.?, Mae Taeng District, Ban Pa-Pae, alt. ca. 800 m, fertile, 25 April 2005, *Sungkaew & Teerawatananon* 524 (**KUFF**, **THNHM**)]; [cult., Mae Wang District, Thung Luang Center, Royal Project, alt. ca. 1,000 m, sterile, 5 Aug. 2004, *Sungkaew & Teerawatananon* 122 (**KUFF**, **THNHM**)]; [cult., Mae Wang District, Ban Thung Luang, alt. ca. 1,000 m, fertile, 5 Aug. 2004, *Sungkaew & Teerawatananon* 123 (**KUFF**, **TCD**, **THNHM**)].

China: Yunnan [cult., Xishuanbanna Tropical Garden, fertile, 18 May 2005, *Sungkaew & Teerawatananon* 615 (**KUFF**, **THNHM**)].

6. **Dendrocalamus membranaceus** Munro, Trans. Linn. Soc. 26: 149. 1868; Kurz, Forest Fl. Burma 2: 560. 1877; Gamble, Ann. Bot. Gard. Calc. 7: 81. *pl. 71.* 1896; Brandis, Indian Trees (Fourth Impression): 676. 1921; W.C. Lin, Special Bul. Taiwan For. Res. Inst. 6: 29. *fig. 21, 22* (not clear). 1968; S. Duriyaprapan & P.C.M. Jansen in S. Dransfield & E.A. Widjaja, Pl. Resources S.E. Asia 7: 90. *illustration on page 91.* 1995; Ohrnb., The Bamboos of the World 288. 1999; S. Dransf. & K.M. Wong, Thai Forest Bull., Bot. 32: 74. 2004. D.Z. Li & Stapleton, Fl. China 22: 40. 2006. Types: Burma, Martaban, Trogla, fertile, 12 March 1827, *Wallich Cat.* 5029, [Lectotype **K**!, selected by Stapleton & N.H. Xia, Kew Bull. 52(1): 238. 1997.]. Fig. 4.13 & 4.14.

— *Dendrocalamus longifimbriatus* Gamble, Ann. Bot. Gard. Calc. 7: 92. *pl. 81* (excl. 1, leaf-branch). 1896. Types: Burma, Milwon Forest, flowering branches only, *unknown collector* s.n. [lectotype **K**!, selected by S. Dransf. & K.M. Wong, Thai Forest Bull., Bot. 32: 74. 2004.].

— *Sinocalamus longifimbriatus* (Gamble) T.Q. Nguyen, Bot. Zhurn., 74(11): 1662. 1989. Types: as for *Dendrocalamus longifimbriatus* Gamble.

— *Bambusa membranacea* (Munro) Stapleton & N.H. Xia, Kew Bull. 52(1): 238. 1997. Types: as for *Dendrocalamus membranaceus* Munro.

Culms usually well-spaced, straight and erect, about 15–23 m long, 6–10(–18) cm in diameter, tips more or less arching; internodes 23–38(–50) cm long, lower ones not hairy; nodes not swollen, lower ones usually without verticils of roots, occasionally present from the very low nodes; walls relatively thin, about 1 cm thick (mid-culm portion). *Branches* developing from all nodes or nearly so or from mid culm upwards; mid-culm branch complements with several branches at each node, the primary one dominant, accompanied by two sub-dominant branches, the rest smaller, the primary branch from lower nodes usually curved downwards, without aerial roots. *Culm sheaths* deciduous, coriaceous, 28.2–45 cm long by 11–24 cm wide, tops shallowly concave or rounded to convexly truncate, back covered with dark brown to black hairs, occasionally glabrous; blades linear-lanceolate, deflexed, 10–40 cm long, about 1–6 cm wide near the base, covered with brown hairs on both sides, denser towards adaxial base, occasionally glabrous; auricles 1–5 mm tall, 0.5–1.5 cm long, wavy or pleated, margins fringed with bristles 0.5–1.5 cm long; ligule 0.2–1 cm high, margin divided into broad lacerations or roughly serrate, about 0.5 cm high. *Leaves* 5–7 per branchlet; blades 12–15 cm long by 1–1.7 cm wide, usually hairy both sides, denser below, sometimes glabrous, bases rounded, acute to cuneate, pseudo-petiole 1–2 mm long; leaf sheaths 2.5-6.5 cm long; auricles tiny falcate lobes, margins fringed with bristles up to 1 cm long, sometimes absent but bristly; ligule 0.5–1 mm high, margin irregularly dentate to lacerate, each division sometimes drawn into fine bristles 1–

2 mm long; secondary veins 3–6 pairs, intermediate veins 5–7. *Pseudospikelets* congested as more or less densely globose clusters 1.2–3 cm in diameter, spiny to the touch; synflorescence axes hairy to glabrous, internodes 1.5–7(–9) cm long. *Spikelets* light yellow-green, ovate-oblong, sub-terete, 7–13.5 mm long by 2.5–3.5 mm wide; glumes 1–2, 4.5–8.4 mm long, 8–17-nerved; fertile florets 2–3, not patent between florets, terminal vestigial floret absent; lemmas chartaceous, 5.5–12.5 mm long, back sparsely hairy in upper part to glabrous, 13–21-nerved, apex drawn out into a long stiff point, up to 2 mm long; paleas chartaceous, 5.5–10.2 mm long by 1.4–2 mm wide, 2-keeled on the back, keels and edges long-fringed, the uppermost one not keeled, outside pubescent, inside glabrous, apex acute or blunt, rarely shortly bifid, 3(–4)-nerved between keels, (0–)1-nerved on each inflexed edge; lodicules none; anthers 3–6 mm long, yellow or purple or yellow becoming purple when dry, tips mucronate; ovary turbinate, summit hairy, stigma 1, plumose. *Caryopsis* with adherent pericarp, ovoid, sulcate on hilar side, 5–8 mm long.

Thailand.— EASTERN: Nakhon Ratchasima (Wang Nam Khiew District, Sakaerat Biosphere Reserve); NORTHERN: Chiang Mai (Chiang Dao District, Doi Chiang Dao Wildlife Sanctuary; Geut Chang Subdistrict; Mae Dtang District; Mae Jam District; Mae Rim District); Lampang (Che Hom); Lamphun (Doi Khun Tan National Park); Mae Hong Son (Mae Sariang); Nan (Phu Pieng Subdistrict); Phrae (Mae Kray?; Sa-iab Subdistrict, Mae Yom National Park); Tak (Lansang District, Lansang National Park); NORTHEASTERN: Loei (Kao Krading; Muang District, Ban Hui Kra Thing; Ta-li District); Phetchabun (Lomsak; Nam Nao District, Nam Nao National Park); SOUTHWESTERN: Kanchanaburi (Thong Pha Phum District; Srinakarin National Park; Sirindhorn Genebank); Phetchaburi (Tung Luang); Prachuap Khiri Khan (Kan Kradai; Kao Yai; Kui Buri District, Khao Soong); Ratchaburi (Suan Phung).

Distribution.— From Burma to Yunnan, southern China to Laos, throughout Thailand except southern part, Fig. 4.15.

Ecology.— Mainly in mixed deciduous forest.

Vernacular.— Pai Sang Nuan (ไผ่ซางนวล).

Specimens examined.

Thailand: EASTERN: ***Nakhon Ratchasima*** [Wang Nam Khiew District, Sakaerat Biosphere Reserve, sterile, 16 Aug. 2004, *Sungkaew & Teerawatananon* 209 (**KUFF, THNHM**)]; NORTHERN: ***Chiang Mai*** [Chiang Dao District, Doi Chiang Dao, east side, Pa Blawng cave area, alt. 550 m, fertile, 29 Jan. 1989, *Maxwell* 89-121 (**BKF, CMU, L**)]; [Doi Chiang Dao Wildlife Sanctuary, Ban Yang Toong Bong Forest Station, alt. 450 m, fertile, 10 March 1990, *Maxwell* 90-305 (**E, CMU, GH**)]; [Geut Chang Subdistrict, Ban Dton Kahm, alt. 475 m, fertile, 31 March 1990, *Maxwell* 90-377 (**CMU, GH**)]; [Mae Dtang District, near Mae Dtang River, alt. 500 m, fertile, 10 Jan. 1997, *Maxwell* 97-22 (**CMU, GH**)]; [Doi Sutep-Pui National Park, east side above Pha Ngerb, sterile, 4 Aug. 2004, *Sungkaew & Teerawatananon* 121 (**KUFF, TCD, THNHM**)]; [Mae Jam District, route from Wat Chan to Pai, fertile, 6 Aug. 2004, *Sungkaew & Teerawatananon* 131 (**KUFF, TCD, THNHM**)]; [Mae Rim District, fertile, 13 March 2005, *Sungkaew & Teerawatananon* 327 (**KUFF, THNHM**)]; [Route from Chiang Mai to Chiang Rai, fertile, 4 Jan. 1922, *Rock* 1856 (**E, GH, K**)]; [Chiang Dao, alt. ca. 700 m, 10 Nov. 1922, *Kerr* 6659 (**ABD, BK, K**)]; ***Kamphaeng Phet*** [Mae Wong National Park, alt. 300-500 m, sterile, 15 June 1995, *Niyomdham et al.*, 4416 (**BKF**)]; ***Lampang*** [Che Hom, alt. ca. 350 m, fertile, 5 Feb. 1921, *Kerr* 4785 (**BK, BM, K**)]; ***Lamphun*** [Doi Khun Tan National Park, trail to Ban Kukrit, alt. 950 m, fertile, 19 May 1993, *Maxwell* 93-448 (**CMU, GH**)]; [Doi Khun Tan National Park, Tah Goo Station area, alt. 650 m, fertile, 28 Dec. 1994, *Maxwell* 94-1316 (**CMU, GH**)]; ***Mae Hong Son*** [Mae Sariang, about 50 km east of Mae Sariang, alt. ca 50? m, sterile, 27 Oct. 1997, *Dransfield et al.*, SD 1446 (**K**)]; ***Nan*** [Phu Pieng Subdistrict, Tambon Fai Kaew, near Nakorn Nan forest plantation, alt. ca. 500 m, sterile, 14 March 2005, *Sungkaew & Teerawatananon* 328 (**KUFF, THNHM**)]; ***Phrae*** [Mae Kray?, alt. 700 m, fertile, 9 Jan. 1972, *Beusekom et al.* 4764 (**L, K**)]; [Sa-iab Subdistrict, Mae Yom National Park, Kaht Hoy area, alt. 250 m, fertile, 5 Nov. 1991, *Maxwell* 91-949 (**E, GH, CMU**)]; ***Tak*** [Lansang District, Lansang National Park, alt. ca. 300 m, sterile, 29 July 2004, *Sungkaew & Teerawatananon* 50 (**KUFF, THNHM**)]; NORTHEASTERN: ***Loei*** [Kao Krading, alt. ca. 700 m, fertile, 10 Feb. 1931, *Kerr* 20036 (**BK, BM, K**)]; [Muang District, Ban Hui Kra Thing, alt. 400 m, sterile, *Sungkaew & Teerawatananon* 178 (**KUFF, THNHM**)]; [Phu Krading, Sam Kok-phai, alt. ca. 800 m, fertile (excl. lvs.), 15 Dec. 1954, *Smitinand* 2120 (**BKF, K**)]; [Ta-li District, route from Ta-li to Dan Sai, sterile, 9 April 2005, *Sungkaew & Teerawatananon* 450 (**KUFF, THNHM**)]; ***Phetchabun*** [Lomsak, Nam Nao National Park, alt. 600-800 m, sterile, 8 Nov. 1997, *Dransfield et al.*, SD 1480 (**BKF, K**)]; [Nam Nao District, Nam Nao National Park, alt. ca. 580 m, sterile, 15 Aug. 2004, *Sungkaew & Teerawatananon* 201 (**KUFF, THNHM**)]; SOUTHWESTERN: ***Kanchanaburi*** [N.E. of Sai Yok, alt. 200 m, fertile, 8 Dec. 1961, *Larsen* 8612 (**GH, K**)]; [Sai Yok, alt. 170 m, fertile, 8 Feb. 1962, *Larsen & Smitinand* 9634 (**AAU, BKF, L**)]; [Thong Pha Phum District, Tha Ka-nun, fertile, 21 April 2005, *Sungkaew & Teerawatananon* 504 (**KUFF, TCD, THNHM**)]; [Thong Pha Phum District, Lin Thin, sterile, 19 July 2004, *Sungkaew & Teerawatananon* 2 (**KUFF, TCD, THNHM**)]; [Srinakarin National Park, Pha-tad Waterfall, sterile, 24 July 2004, *Sungkaew & Teerawatananon* 48 (**KUFF, TCD, THNHM**)]; [Sirindhorn Genebank, sterile, 24 July 2004, *Sungkaew & Teerawatananon* 49 (**KUFF, THNHM**)]; [Sisawat, fertile, 12 Jan. 1926, *Kerr* 10199 (**BK, K, TCD**)]; ***Phetchaburi*** [Tung Luang, alt. under 50 m, fertile, 8 Nov. 1931, *Kerr* 20594 (**BM, K**), *Kerr* 20594A (**BM, K, L**)]; ***Prachuap Khiri Khan*** [Kan Kradai, fertile, 15 Jan. 1929, *Put* 2310 (**BK, BM, K**)]; [Kao Yai, alt. ca. 50-300 m, fertile, 8 Nov. 1927, *Kerr* 13504 (**BM, K**)]; [Kui Buri District, Khao Soong, sterile, 25 Aug. 2004, *Sungkaew & Teerawatananon* 271 (**KUFF, THNHM**)]; [Kui Buri District, Khao Soong, fertile, 26 March 2005, *Sungkaew & Teerawatananon* 418, 425 (**KUFF, THNHM**)]; ***Ratchaburi*** [Suan Phung, Kao Jone Waterfall, sterile, 2 July 2004, *Sungkaew & Teerawatananon* 020704-4 (**KUFF, THNHM**)]; [Suan Phung, Khao Krajom, fertile, 27 March 2005, *Sungkaew & Teerawatananon* 426 (**KUFF, THNHM**)].

Burma: ***Lower Thaungyin*** [alt. 500 feet, sterile, 26 April 1923, *Ogilvie* 5 (**GH, K**)]; ***Mandalay*** [Maymyo, alt. 3,500 feet, sterile, 25 June 1919, *Rogers* 874 (**GH, K**)]; ***Martaban*** [Trogla, fertile, 12 March 1827, *Wallich* Cat. 5029 (**K**)]; ***Milwon Forest*** [flowering branches only, *unknown collector* s.n. (**K**)]; ***Shan State*** [Keng Tung Territory, between Pang Sop Lao and Ban Yang Kha, valley of the Meh Len, alt. 660-930 m, fertile, 29 Jan. 1922, *Rock* 2154 (**GH, K**)].

China: ***Yunnan*** [Nan-Chiao, 1180 m, fertile, July 1936, *Wang* 73445 (**GH**)]; [route from Jing Hong to Meng Yang, about 5 km away from Jing Hong, alt. 650 m, sterile, 17 May 2005, *Sungkaew & Teerawatananon* 612-1 (**KUFF, THNHM**)].

No locality: *locality illegible* [near Ban Meh Bak?, 1,000 feet, fertile, 21 Feb. 1910, *Kerr* 996 (**BM, K, TCD**)].

7. **Dendrocalamus asper** (Schult. f.) Backer ex K. Heyne, Nutt. Pl. Ned.-Ind. ed. 2, 1: 301. 1927; Holttum, Gard. Bull. Singapore 16: 100. *fig. 25.* 1958; W.C. Lin, Special Bul. Taiwan For. Res. Inst. 6: 24. *fig. 19.* 1968; S. Dransf. & Widjaja in S. Dransfield & E.A. Widjaja, Pl. Resources S.E. Asia 7: 80. *illustration on page 81.* 1995; K.M. Wong, Malayan Forest Records 41: 107. *fig. 51.* 1995; Ohrnb., The Bamboos of the World 283. 1999; D.Z. Li & Stapleton, Fl. China 22: 43. 2006. Type: cult.?, Penang, Rd. to Batu Feringy, fertile, March 1901, *Curtis* 3566 [lectotype **K!**, designated here]. Fig. 4.16.

— *Bambusa aspera* Schult. f., in J.A. Schultes & J.H. Schultes, Syst. Veg. 7(2): 1352. 1830. Type: as for above.

— *Dendrocalamus flagellifer* Munro, Trans. Linn. Soc. 26: 150. 1868. Type: Malacca, fertile, *Griffith* s.n. [lectotype **TCD!**, designated here].

— *Bambusa flagellifera* Griff. ex Munro, Trans. Linn. Soc. 26: 150. 1868. *nom. nud.*, as synonym of *Dendrocalamus flagellifer* Munro.

— *Schizostachyum loriforme* Munro, Trans. Linn. Soc. 26: 150. 1868. *nom. nud.*, as synonym of *Dendrocalamus flagellifer* Munro.

— *Gigantochloa aspera* (Schult. f.) Kurz, Indian Forester 1(3): 221. 1876. Type: as for *Dendrocalamus asper* (Schult. f.) Backer ex K. Heyne.

— *Gigantochloa merrilliana* Elmer, Leafl. Philipp. Bot. 1: 273. 1908. Type: Philippines, Leyte, fertile, Jan. 1906, *Elmer* 7283 [not located].

— *Dendrocalamus merrillianus* (Elmer) Elmer, Leafl. Philipp. Bot. 7: 2675. 1915, as '*merrilliana*'.

— *Sinocalamus flagellifer* (Munro) T.Q. Nguyen, Bot. Zhurn., 74(11): 1662. 1989. Type: as for *Dendrocalamus flagellifer* Munro.

Culms densely tufted, straight and erect, about 15–20 m long, 10–15 cm in diameter, tips slightly pendulous; internodes 30–60 cm long, lower ones densely covered with brown velvety hairs; lower nodes more or less swollen, developing conspicuous verticils of roots; walls relatively thin, about 1–2 cm thick (mid-culm portion). *Branches* developing from around mid culm or from lower quarter upwards; mid-culm branch complements with several branches at each node, the primary one dominant, usually accompanied by 1–4 sub-dominant branches, aerial roots present. *Culm sheaths* deciduous, coriaceous, 27–55 cm long by 27.5–50 cm wide, tops convexly truncate, back covered with pale brown hairs on upper part and dark brown hairs on lower part; blades linear-lanceolate, spreading to deflexed, 20–25 cm long, about 3–5 cm wide near the base, adaxial base hairy; auricles lobelike, 2–10 mm tall, about 1–3 cm long, inner side covered with branched bristles 1–4 mm long, margins with pale bristles, bristles 3–10 mm long; ligule 3–5 mm high, margin divided into broad lacerations or dentate, each division further divided into fine bristles 2–5 mm high. *Leaves* 5–13 per branchlet; blades 15–35 cm long by 2–4 cm wide, glabrous or sparsely hairy underneath, bases acute to obliquely cuneate, occasionally rounded, pseudo-petiole 2–7 mm long; leaf sheaths 6.3–11.2 cm long; auricles absent or as inconspicuous ridges, margins or shoulders glabrous, occasionally bristly, bristles 2–5 mm long; ligule 0.2–1 mm high, margin shallowly dentate to sub-entire; secondary veins 5–10 pairs, intermediate veins 5–8. *Pseudospikelets* congested as globose clusters 0.8–2 cm in diameter, not spiny to the touch; synflorescence axes hairy, becoming glabrous, internodes 1.5–2.5(–5) cm long. *Spikelets* yellow-brown, ovate, laterally compressed, 6–8 mm long by 3.5–4.5 mm wide; glumes (1–)2(–3), 2.8–4.5 mm long, (7–)9–17(–21)-nerved; fertile florets (3–)4–5, slightly patent between florets, terminal vestigial floret sometimes present; lemmas chartaceous, 3.8–7 mm long, back pubescent, mainly upper part, 11–27-nerved, apex pointed; paleas chartaceous, 3.5–6 mm long by 1.2–2 mm wide, 2-keeled on the back, keels and edges fringed, the uppermost one not keeled, outside pubescent, inside sparsely pubescent to nearly glabrous, apex acute to shallowly retuse, 1–3-nerved between keels, (0–)1–2(–3)-nerved on each inflexed edge; lodicules usually none, sometimes 1–2, hyaline; anthers 3–4.4 mm long, yellow, tips mucronate to penicillate, usually covered with miniscule spines; ovary turbinate, summit hairy, stigma 1, plumose. *Caryopsis* unknown.

Thailand.— Cultivated all over the country.

Distribution.— Uncertain origin. Ohrnberger (1999) stated that "it is supposed to originate somewhere in South-East Asia, probably northern Malaysia (an apparently wild plant was found near Cameron Highlands)". In southern Thailand, there were at least two possibly wild populations of this species, which is locally called "Pai Chiang Roon", in tropical rain forest in Klong Panom National Park, Surat Thani Province. However, this protected area was a logged area in the past with a camping area which used to be settled. It is possible that it may have been planted by man. Thus it is hard to say whether these two populations are wild or naturalized.

Ecology.— Only found in cultivation.

Vernacular.— Pai Tong, Pai Chiang Roon (ไผ่ตง, ไผ่เฉียงรุน).

Typification notes.— When *Bambusa aspera* Schult. f. was described in J.A. Schultes & J.H. Schultes, Syst. Veg. 7(2): 1352. 1830., no single specimen was designated as a type. Thus a fertile specimen, *Curtis* 3566 in **K**, was chosen here as the lectotype. Holttum, (1958) also cited this sample as one of his specimens examined for *Dendrocalamus asper* (Schult. f.) Backer ex K. Heyne.

When *Dendrocalamus flagellifer* Munro was published in Trans. Linn. Soc. 26: 150. 1868., a specimen from the herbarium **TCD**, (Malacca, *Griffith* s.n.), was cited. However, it was not entirely clear what Munro (1868) meant by stating that the type specimen was '*Hab.* in Malacca, *Griffith.* v.s.'. 'v.s.' indicates either that he has seen this herbarium specimen from **TCD** (Malacca, *Griffith* s.n.) or that he has got another duplicate with him. I, therefore, lectotypified the species on the basis of this specimen.

Specimens examined.

Thailand: CENTRAL: ***Nakhon Nayok*** [cult., Ong Karak District, Klong 15, sterile, 11 Aug. 2004, *Sungkaew & Teerawatananon* 160, 161 (**KUFF, THNHM**)]; ***Saraburi*** [cult., Muang District, alt. 75 m, sterile, 2 Nov. 1974, *Maxwell* 74-958 (**AAU, BK, L**)]; EASTERN: ***Surin*** [cult., Pra-sat District, sterile, 12 April 2005, *Sungkaew & Teerawatananon* 483 (**KUFF, THNHM**)]; ***Ubon Ratchathani*** [cult., Na Jalui District, fertile, 12 April 2005, *Sungkaew & Teerawatananon* 482 (**KUFF, THNHM**)]; NORTHERN: ***Chiang Mai*** [cult., Mae Jam District, Ban Pang Ung, alt. ca. 1,300-1,400 m, 7 Aug. 2004, *Sungkaew & Teerawatananon* 153 (fertile), 156 (sterile) (**KUFF, THNHM**)]; NORTHEASTERN: ***Kalasin*** [cult. as plantation, Namon District, sterile, 11 April 2005, *Sungkaew & Teerawatananon* 481 (KUFF, THNHM)]; PENINSULAR: ***Chumphon*** [cult., Lamae District, Ban Khuan Thang, sterile, 13 July 2004, *Sungkaew & Teerawatananon* 130704-5 (**KUFF, THNHM**)]; ***Krabi*** [cult., Nawng Le?, alt. ca. 50 m, sterile, 20 March 1930, *Kerr* 18638 (**BK, BM, K**)]; ***Satun*** [cult.?, La Ngu District, Tarutao National Park, Ko Tong, alt. sea level, sterile, 20 March 2005, *Sungkaew & Teerawatananon* 351 (**KUFF, THNHM**)]; [cult.?, Muang District, Hnong Plak Praya non-hunting area, sterile, 22 March 2005, *Sungkaew & Teerawatananon* 378 (**KUFF, THNHM**)]; ***Songkhla*** [cult., Rattaphum District, route from Had Yai to Ton Nga Chang Wildlife Sanctuary, sterile, 23 March 2005, *Sungkaew & Teerawatananon* 382 (**KUFF, THNHM**)]; ***Surat Thani*** [cult.?, Klong Panom National Park, alt. 100 m, 11 July 2004, *Sungkaew & Teerawatananon* 110704-1 (**KUFF, THNHM**)]; SOUTHWESTERN: ***Kanchanaburi*** [Thong Pha Phum District, near Thong Phaphum Plantation Station, sterile, 21 April 2005, *Sungkaew & Teerawatananon* 505 (**KUFF, THNHM**)].

Ecuador: ***Los Rios*** [cult., at the Pichilingue Agri. Exp. Sta., alt. 50 m, sterile, 14 Feb. 1980, *Young* 62 (**AAU**)].

Indonesia: ***C. Java*** [cult., Kudus, Piji Wetan, Lau village, sterile, 29 June 1982, *Widjaja* 1716 (K, L)]; ***E. Java*** [probably cult., Sidhoardjo, Kremboong, alt. under 50 m, fertile, 8 Nov. 1938, *Moll* 38.35 (L)]; ***W. Java*** [cult. in Hort. Bot. Bog., fertile, *Balansa* s.n. (L)]; [probably cult., Buitenzorg, sterile, *collector Illegible* (L, code: L0412537)]; [parobably cult., Batavia (Jakarta), Buitenzorg (Bogor), sterile, 21 March 1899, *Koorders* 37245b (L)]; [probably cult., fertile, *Dorgelo* 39382 (L)]; [probably cult., Pondok Gede, fertile, Nov. 1912, *Olivier* s.n. (L)]; [cult., Bogor, fertile, 3 July 1982, *Widjaja* 1719 (K, L)]; ***E. Kalimantan*** [Bulungan District, Apo Kayan, Long sungai Barang village, sterile, 14 May 1982, *Widjaja* 1469 (K, L)]; ***Lesser Sunda Islands*** [probably cult., W. Flores-Manggarai, alt. 350 m, fertile, 29 Dec. 1980, *Schmutz* SVD 4721 (L)]; [probably cult., W. Flores-Manggarai, Mborong, alt. 50 m, fertile, 30 Dec. 1980, *Schmutz* SVD 4722 (L)]; ***Pajakumbuh region*** [probably cult., Si-kabu, alt. ca. 750 m, fertile, 24 March 1957?, *Meijer* 7696 (L)]; [near Pajakumbuh, probably cult., Si-kabu2-Laku Dammar, alt. 650 m, fertile, 24 March 1957, *Meijer* 5664 (L)]; [near Pajakumbuh, probably cult., base of Mt. Sago, along road, alt. ca. 700 m, fertile, 22 Jan. 1958?, *Meijer* 7577 (L)]; ***Sumatra*** [probably cult., Kayu tanam, secincin-Sumbar, sterile, 14 Oct. 1982, *Panggabean* 54 (K, L)]; [cult.?, Belgulu? Selatan District, Gelumbang Village, alt. 10 m, fertile, 31 Dec. 1991, *Widjaja* 4740 (K)]; ***Ternate*** [probably cult., alt. ca. 300 m, sterile, 6 May 1920, *Beguin* 644 (L)]; [probably cult., alt. ca. 350 m, sterile, 23 Dec. 1920, *Beguin* 1260 (L)].

Malaysia: ***Malacca*** [fertile, *Griffith* s.n. (TCD)]; ***Penang*** [cult.?, Batu Feringhi, fertile, Jan. 1897, *Ridley & Curtis* 8363 (K)]; [cult.?, Rd. to Batu Feringy, fertile, March 1901, *Curtis* 3566 (K)]; ***Perak*** [cult., Parit District, Layang-layang Kanan, Kampung Kobah, sterile, 7 Oct. 1980 *Wong* FRI 28974 (K)]; ***Sarawak*** [cult., Kelabit Highlands, Pa Dalih, alt. 950 m, sterile, 30 July, 1993, *Christensen & Apu* 21 (AAU)].

Singapore: [cult.?, Adam Road, fertile, 23 Dec. 1932, *Kiah* s.n. (**BK, K**)]; [cult., Deer Shed, Botanic Gardens, sterile, 1894, Ridley, 5603 (K)]; [cult. Lower Garden, fertile, 1899, Ridley, 10644 (**SING**)]; [cult., Sungei Gedong Road, about 0.5 mile from seashore, sterile, 13 Nov. 1949, *Sinclair* s.n. (**L, SING**)]; [probably cult., off Yio Chu Kang Road, neglected village area, alt. 25 m, fertile, 5 March 1983, *Maxwell* 83-20 (**AAU, L, SING**)]; *No locality* [fertile, Ridley, 2929 (K)].

8. **Dendrocalamus brandisii** (Munro) Kurz, Prelim. Rep. For. Veg. Pegu, App. B. 94. 1875, Forest Fl. Burma 2: 560. 1877; Gamble, Ann. Bot. Gard. Calc. 7: 90. *pl. 79.* 1896; Brandis, Indian Trees (Fourth Impression): 678. 1921; W.C. Lin, Special Bul. Taiwan For. Res. Inst. 6: 27. 1968; Alam in S. Dransfield & E.A. Widjaja, Pl. Resources S.E. Asia 7: 83. *illustration on page 83.* 1995; Ohrnb., The Bamboos of the World 284. 1999; D.Z. Li & Stapleton, Fl. China 22: 43. 2006. Type: Burma, Pegu, alt. up to 4,000 feet, fertile, *Brandis* 2 [holotype, not located]; hills between Sitang and Beeling, fertile, March 1861, *Brandis* 361 [neotype **M!**, code: M0124730; isolectotype **M!**, code: M0124729, designated here]. Fig. 4.17 & 4.18.

— *Bambusa brandisii* Munro, Trans. Linn. Soc. 26: 109. 1868. Type: as for above.
— *Dendrocalamus nudus* Pilg., Repert. Spec. Nov. Regni Veg. 3: 117. 1906. Type: Thailand, Chiang Mai, alt. 300 m, fertile, 3 Jan. 1905, *Hosseus* 290a [lectotype **M!**; isolectotype **K!**, designated here], ***syn. nov.***
— *Sinocalamus nudus* (Pilg.) T.Q. Nguyen, Bot. Zhurn., 74(11): 1662. 1989. Type: as for *Dendrocalamus nudus* Pilg.

Culms densely or loosely tufted, straight and erect, about 15–30 m long, 8–20 cm in diameter, tips arching to drooping; internodes 30–65 cm long, lower ones densely covered with pale brown hairs; lower nodes developing conspicuous verticils of roots; walls relatively thin, about 1–2 cm thick (mid-culm portion). *Branches* developing from around mid culm upwards; mid-culm branch complements with several branches at each node, the primary one dominant, aerial roots present. *Culm sheaths* deciduous, coriaceous, 25–50 cm long by 25–65 cm wide, tops convex, back usually scattered by pale brown or golden brown hairs, occasionally with dark brown hairs or becoming glabrous; blades broadly lanceolate, usually deflexed, sometimes erect, 10–45 cm long, about 8–15 cm wide near the base, adaxial base densely hairy, often somewhat narrowed at the base, and here shortly waved-decurrent, covered with hispid hairs inside; auricles continuing from base of culm-sheath blade, lobelike, more or less pleated, often not reach its edge, varying from tiny lobe to about 1.5 cm tall, 5 cm long, inner side covered with un-branched hairs only or mixed with branched hairs, margins fringed with bristles 5-10 mm long, readily deciduous; ligule 2–10 mm high, middle portion the highest, margin broadly lacerate, up to 10 mm high. *Leaves* 6–9 per branchlet; blades 20–40 cm long by 2.5–5 cm wide, sparsely hairy both sides or upper surface glabrous, bases acute or cuneate to oblique-attenuate, occasionally rounded, pseudo-petiole 2–7 mm long; leaf sheaths 8–10 cm long; auricles absent or as inconspicuous ridges, margins or shoulders glabrous; ligule 1–2 mm high, margin irregularly dentate to sub-entire at the middle, lacerate outwards, each division drawn into a bristle 1–3 mm long; secondary veins 8–11 pairs, intermediate veins 5–9.

Pseudospikelets usually congested as densely globose clusters 1–2 cm in diameter, not spiny to the touch; synflorescence axes hairy, occasionally glabrous, internodes 1–3.5 cm long. *Spikelets* yellow-brown, occasionally yellow-green, ovate to obovate-oblong, more or less laterally compressed, 4.5–8.5 mm long by 2.8–4.3 mm wide; glumes 1–3, 2.2–5.5 mm long, 5–17-nerved; fertile florets 2–4(–5), more or less patent between florets, terminal vestigial floret absent; lemmas chartaceous, 3–7.6 mm long, back pubescent, mainly upper part, 9–23-nerved, apex pointed; paleas chartaceous, 2.9–6 mm long by 1–2 mm wide, 2-keeled on the back, keels and edges fringed, the uppermost one not keeled or slightly keeled near apex, both sides pubescent, apex acute to shallowly retuse, 1–3-nerved between keels, 1–2-nerved on each inflexed edge; lodicules usually none, sometimes 1–2, hyaline; anthers 2.5–5 mm long, yellow, tips mucronate to penicillate, usually covered with miniscule spines, occasionally glabrous; ovary turbinate to elliptic, summit hairy, stigma 1, slightly to firmly plumose. *Caryopsis* with adherent pericarp, ovoid, 2.5–4 mm long.

Thailand.— EASTERN; Nakhon Ratchasima (Pak Chong District, Khao Yai National Park); NORTHERN: Chiang Mai (cult., Fang District, Ang Khang Royal Project); Chiang Rai (near the Akha village of Meh Yao)); Nan (cult., Muang Pong; Mae Jarim District, Mae Jarim National Park); Mae Hong Son (Pang Tong Palace); Phitsanulok (cult. in bamboo living collection, opposite to Thung Salaeng Luang National Park headquarters); Sukhothai (Kirimat District, Ramkhamhaeng National Park ; cult.?, Muang Gow District, Sahwang Ahrome Temple area; Tak (cult., Mae Sod District, Tambon Dan Mae Lamao; Phop Phra District, Pa-Charoen National Park, Tambon Kirirat); NORTHEASTERN: Phetchabun (Nam Nao District, Nam Nao National Park); SOUTHEASTERN: Chon Buri (Sri Racha District, Kow Kieo); SOUTHWESTERN: Kanchanaburi (Thong Pha Phum District, Greng Grawia; Ta Kanun; Wangka; Srinakarin National Park, Pa Tad Waterfall; Ban Ti Pu-ye; Sungkhla Buri District); Phetchaburi (Kaeng Krajan); Ratchaburi (Suan Phung District).

Distribution.— From Yunnan, China to north-eastern part of India, Andaman Islands, Burma, Thailand, Laos and Vietnam (Ohrnberger, 1999), Fig. 4.19.

Ecology.— In moist mixed deciduous forest, dry evergreen forest, to lower montane forest.

Vernacular.— Pai Bong Yai (ไผ่บงใหญ่), Pai Tong Pa (ไผ่ตงป่า), sometimes also called Pai Hok (ไผ่หก).

Typification notes.— When Munro (1968) first described *Bambusa brandisii* (basionym of *Dendrocalamus brandisii* (Munro) Kurz), he cited only a single specimen, *Brandis* 2. This specimen is accepted as the holotype. However, Munro (1968) did not mention where it was kept. I have not seen this specimen either amongst the specimens I borrowed from several hebaria or the specimens I studied in the herbaria I visited. Therefore, I designate here a duplicate of *Brandis* 361, housed in **M** (code: M0124730), as the neotype and another duplicate (also in **M**, code: M0124729) is regarded as isoneotype.

The type of *Dendrocalamus nudus* Pilg., *Hosseus* 290a, was long believed to comprise only a single specimen, the one in **M**. Apparently, there are two duplicates of this collection, one in **M**, another one in **K**. Actually, Hosseus's herbarium specimens from Thailand were kept in several herbaria including **K** and **M** (Stafleu & Cowan, 1979). Thus it is also possible that there may be more duplicates of this collection (*Hosseus* 290a) in other herbaria, possibly at **B** (Herbarium, Botanischer Garten und Botanisches Museum Berlin-Dahlem, Berlin, Germany) and **P** (Herbier, Laboratoire de Phanérogamie, Muséum National d' Histoire Naturelle, Paris, France). When Pilger (1906) first described *D. nudus*, only a single collection, *Hosseus* 290a, was cited. However, he did not mention which duplicate was the holotype or isotype. Whether or not these two duplicates (*Hosseus* 290a in **K** and **M**) were both used by Pilger (1906) is not known. However, as Pilger, a German botanist, worked mainly in Germany (Stafleu & Cowan, 1983), I here, therefore, designated the specimen *Hosseus* 290a, housed in **M** (code: M0124727), as the lectotype, and the one in **K** (code: K000290643) is regarded as an isolectotype.

Specimens examined.

Thailand: EASTERN: ***Nakhon Ratchasima*** [Pak Chong District, Khao Yai National Park, 17 March 2005, *Sungkaew & Teerawatananon* 344 (fertile), 345 (sterile) (**KUFF, TCD, THNHM**)]; NORTHERN: ***Chiang Mai*** [alt. 300 m, fertile, 3 Jan. 1905, *Hosseus* 290a (**K, M**)]; [between Fang and Chiang Rai, alt. ca. 900 m, fertile, 28 Feb. 1958, *Sorensen et al.,* 1805 (**BKF, K**)]; [cult., Fang District, Ang Khang Royal Project, sterile, *Sungkaew & Teerawatananon* 260903-8 (26 Sep. 2003), 114 (3 Aug. 2004) (**KUFF, THNHM**)]; [Khun Awn, alt. ca. 1,000 m, fertile, 30 Jan. 1921, *Kerr* 4731 (**AAU, ABD, BM, K**)]; ***Chiang Rai*** [near the Akha village of Meh Yao, alt. 850 m, sterile, 19 Dec. 1983, *Anderson* 5360 (**GH**)]; ***Nan*** [cult., Muang Pong, alt. ca. 250 m, fertile, 24 Feb. 1921, *Kerr* 4892 (**ABD, K, TCD**)]; [Mae Jarim District, Mae Jarim National Park, Tambon Nam Pang, Ban Rom Klao, sterile, 15 March 2005, *Sungkaew & Teerawatananon* 341 (**KUFF, TCD, THNHM**)]; ***Mae Hong Son*** [Pang Tong, fertile, 1 May 1981, *Put* 3848 (**BK, BM, L, K**)]; [Pa Thong, Phaa Sua Waterfall National Park, alt. 500 m, fertile, 26 Oct. 1997, *Dransfield et. al.*, SD 1440 (**BKF, K**)]; [Muang District, Pang Tong Palace, alt. ca. 950 m, fertile, 26 April 2005, *Sungkaew & Teerawatananon* 533 (**KUFF, TCD, THNHM**)]; ***Phitsanulok*** [cult. in Bamboo living collection, opposite to Thung Salaeng Luang National Park headquarters, sterile, 10 April 2005, *Sungkaew & Teerawatananon* 464 (**KUFF, TCD, THNHM**)]; ***Sukhothai*** [Kirimat District, Ramkhamhaeng National Park, below the summit of Khao Luang, east side, 'caves' area, alt. 975 m, fertile, 28 Jan. 1995, *Maxwell* 95-30 (**BKF, CMU, GH**)]; [cult.?, Muang Gow District, Sahwang Ahrome Temple area, alt. 900 m, fertile, 23 Jan. 1974, , *Maxwell* 74-81 (**AAU, BK**]; ***Tak*** [Doi Mussor, alt. ca. 900 m, fertile, 6 Dec. 1960, *Smitinand* 7015 (**BKF**)]; [Maesaut, alt. ca. 800? m, fertile, 22 July 1959, *Smitinand* 5997 (**BKF, K**)]; [cult., Mae Sod District, Tambon Dan Mae Lamao, sterile, 29 July 2004, *Sungkaew & Teerawatananon* 62 (**KUFF, THNHM**)]; [Phop Phra District, Pa-Charoen National Park, Tambon Kirirat, fertile, 5 May 2005, *Sungkaew & Teerawatananon* 577 (**KUFF, TCD, THNHM**)]; NORTHEASTERN: ***Phetchabun*** [Nam Nao District, Nam Nao National Park, alt. ca. 1,100 m, sterile, 15 Aug. 2004, *Sungkaew & Teerawatananon* 207 (**KUFF, THNHM**)]; SOUTHEASTERN: ***Chon Buri*** [Sri Racha District, Kow Kieo, alt. ca. 800 m, fertile, 19 Jan. 1976, *Maxwell* 76-24 (**AAU, BK**)]; [Sri Racha District, near the summit of the mountain along Chan Ta Taen Waterfall, sterile, 2 April 2005, *Sungkaew & Teerawatananon* 431, 433 (**KUFF, TCD, THNHM**)]; SOUTHWESTERN: ***Kanchanaburi*** [Thong Pha Phum District, Greng Grawia, alt. 400 m, fertile, 5 Feb. 1962, *Larsen* 9565 (**BKF, GH, K**)]; [Ta Kanun, alt. ca. 300 m, fertile, 19 Jan. 1926, *Kerr* 10269 (**ABD, BK, BM, K, TCD**)]; [Wangka, alt. ca. 200 m, fertile, 29 Jan. 1926, *Kerr* 10326 (**ABD, BM, K, TCD**)]; [Thong Pha Phum District, Srinakarin National Park, Pa Tad Waterfall, alt. ca. 350 m, 21 July 2004, *Sungkaew & Teerawatananon* 13 (fertile), 15 (sterile) (**KUFF, THNHM**)]; [*l.c.*, fertile, 23 April 2005, *Sungkaew & Teerawatananon* 520 (**KUFF, TCD, THNHM**)]; [Thong Pha Phum District, Ban Ti Pu-ye, fertile, 21 July 2004, *Sungkaew & Teerawatananon* 19 (**KUFF, THNHM**)]; [Thong Pha Phum District, Ban Ti Pu-ye, fertile, 22 July 2004, *Sungkaew & Teerawatananon* 19, 33 (**KUFF, THNHM**)]; [Thong Pha Phum District, Khao Laem Dam National Park, sterile, *Sungkaew & Teerawatananon* 42 (**KUFF, THNHM**)]; [Sungkhla Buri District, Tambon Prang Plae, Ban Lijia, fertile, 19 April 2005, *Sungkaew & Teerawatananon* 484 (**KUFF, TCD, THNHM**)]; [Sungkhla Buri District, fertile, 19 April 2005, *Sungkaew & Teerawatananon* 489 (**KUFF, TCD, THNHM**)]; ***Phetchaburi*** [Kaeng Krajan, above the dam, alt. ca. 200 m, *Korawat* 69, (**TCD, THNHM**)]; ***Ratchaburi*** [Suan Phung, Kao Krajome, Kao Khiew foothill, sterile, 3 July 2004, *Sungkaew & Teerawatananon* 030704-2 (**KUFF, THNHM**)].

Burma: ***Mandalay*** or ***Pegu*** [hills between Sitang and Beeling, fertile, March 1861, *Brandis* 361 (**M**)]; ***Mon State*** [Thaton, Thaungyin valley, alt. 2,500 feet, fertile, 27 Feb. 1909, *Lace* 4659 (**E, K**)]; ***Rangoon*** [cult., Insein District, fertile, 28 Dec. 1924, *Parker* 2376 (**GH**)]; ***Ruby Mines*** [Ruby Mines road, alt. 2,000-4,000 feet, sterile, 3 Feb. 1894, *Oliver* s.n. (**K**)]; ***West Salween Division*** [fertile, 20 Jan. 1922, *Unknown collector* (**GH**)]; ***Lower Thaungyin*** [Meke, fertile, 22 Jan. 1923, *Min* 2, 3 (**GH**)]; ***Toungoo*** [hill, above Tawzit? stream, fertile, 28 Jan. 1907, *Brauthwaite*? s.n. (**E, GH**)]; ***North Toungoo Div.*** [fertile, 31 May 1921, *Unknown collector* (**GH**)].

India: Andaman and Nicobar Islands [S. Andaman, fertile, 1897, *Heinig* s.n. (**GH, M**)].

9. **Dendrocalamus hamiltonii** Nees & Arn. ex Munro, Trans. Linn. Soc. 26: 151. 1868; Gamble, Ann. Bot. Gard. Calc. 7: 84. *pl. 74*. 1896; Brandis, Indian Trees (Fourth Impression): 676. 1921; W.C. Lin, Special Bul. Taiwan For. Res. Inst. 6: 28. 1968; Stapleton, Edinburgh J. Bot. 51(1): 23. 1994; S. Dransfield & E.A. Widjaja, Pl. Resources S.E. Asia 7: 149. 1995; Ohrnb., The Bamboos of the World 286. 1999; Stapleton, Flora of Bhutan 3(2): 492. 2000; D.Z. Li & Stapleton, Fl. China 22: 42. 2006. Type: Assam, Goalpara, 7 (not 17) July 1808, *Buch.-Ham.* 882 [lectotype, E!, selected by Stapleton, Edinburgh J. Bot. 51(1): 23. 1994.]. Fig. 4.20 & 4.21.

— *Sinocalamus hamiltonii* (Nees & Arn. ex Munro) T.Q. Nguyen, Bot. Zhurn., 74(11): 1662. 1989. Type: as for above.

Culms densely tufted, straight and erect, about 12–15 m long, 5–12 cm in diameter, tips usually drooping; internodes 25–55 cm long, lower ones densely covered with pale brown hairs; lower nodes developing conspicuous verticils of roots; walls about 1.5–3 cm thick (basal-culm portion). *Branches* developing from around mid culm or from lower quarter upwards; mid-culm branch complements with several branches at each node, only the primary one dominant, or accompanied by 1-3 sub-dominants, aerial roots present; branchlets usually dense at clump base. *Culm sheaths* deciduous, occassionally tardily deciduous, often decaying on the culm, coriaceous, 30–45 cm long by 15–37 cm wide, tops convexly truncate to somewhat rounded, back scattered with patches of black hairs to glabrous; blades broadly lanceolate, usually erect, sometimes slightly spreading, 15–35 cm long, about 8.5–12 cm wide near the base, adaxial base sparsely hairy to glabrous; auricles a triangular protuberance, about 0.5–1 cm tall and long, fragile, without oral setae; ligule 2–8 mm high, middle portion the highest, margin dentate to sub-entire. *Leaves* 7–10 per branchlet; blades 20–35 cm long by 2–5 cm wide, hairy underneath, bases rounded to acute, pseudo-petiole 2–5 mm long; leaf sheaths 6–10 cm long; auricles absent; ligule 3.5–6 mm high, margin irregularly dentate; secondary veins 8–11 pairs, intermediate veins 6–9. *Pseudospikelets* usually congested as densely globose clusters 1–3 cm in diameter, not spiny to the touch; synflorescence axes hairy, internodes thick, 2.5–6 cm long. *Spikelets* yellow brown or purple-brown, ovate-oblong to bell-shaped, laterally compressed, 9–14 mm long by 4–10 mm wide; glumes (1–)2, 4.5–7 mm long, 11–23-nerved; fertile florets 2–4(–6), more or less patent between florets, terminal vestigial floret absent; lemmas chartaceous, 6.5–12.5 mm long, back sparsely hairy to glabrous, 19–25-nerved, apex pointed; paleas chartaceous, 6–10 mm long by 1.7–3 mm wide, 2-keeled on the back, keels and edges fringed, the uppermost one not keeled, both sides pubescent, inside denser, apex acute to shallowly retuse, 1–3-nerved between keels, 1–2-nerved on each inflexed edge;

lodicules absent; anthers 5.5–6.5 mm long, yellow, purplish-yellow to reddish-purple, tips apiculate to penicillate, usually covered with miniscule spines; ovary turbinate, summit hairy, stigma 1, sometimes divided into 2–3, plumose. *Caryopsis* with adherent pericarp, ovoid, glabrous, or hairy at apex.

Thailand.— NORTHERN: Chiang Mai (Fang District, Tambon Mae Ngon, route to Angkhang Royal Project); Chiang Rai (Wiang Kaen District, Huai Laeng Royal Project Station).

Distribution.— Tropical Himalaya and N.E. India, Bhutan, Nepal, south China (Yunnan) (Ohrnberger, 1999), Burma to Thailand.

Ecology.— Usually found in lower montane forest.

Vernacular.— Pai Hok (ไผ่หก), Pai Nuan Yai (ไผ่นวลใหญ่).

Specimens examined.
Thailand: NORTHERN: ***Chiang Mai*** [Fang District, Tambon Mae Ngon, route to Angkhang Royal Project, alt. ca. 1,400 m, 2 Aug. 2004, sterile, *Sungkaew & Teerawatananon* 102 (**KUFF, TCD, THNHM**)]; [*l.c.*, alt. ca. 1,280 m, 23 Nov. 2005, sterile, *Sungkaew & Teerawatananon* 790 (**KUFF, THNHM**)]; ***Chiang Rai*** [Wiang Kaen District, Huai Laeng Royal Project Station, sterile, 22 Nov. 2005, *Sungkaew & Teerawatananon* 787 (**KUFF, TCD, THNHM**), 788 (**KUFF, THNHM**)].

Burma: ***Upper Burma*** [Fort Stedman, fertile, Dec. 1892, *Abdul Huk* s.n. (**GH**)]

India: ***Assam*** [Kamrup District, fertile, 13 June 1964, *Rao* 38800 (**L**)]; [Nongpoh, fertile, 16 March 1943, *Bor* 16056 (**GH**)]; [fertile, 25 May 1951, *Bot. Survey of India* 9593 (**M**, code: M0124740)]; [cult., Bot. Gard. Calcutta & Serampore, fertile, 1834-1841, *Voigt* s.n. (**GH**)]; [cult. in Roy. Bot. Gard. Calcatta, fertile, 27 April 1901, *unknown collector* (**L**, code: L0412595; **TCD**)]; ***E. Himalaya*** [Peshok, alt. 3,000 feet, fertile, *Biswas* 5477 (**GH**)]; ***Sikkim*** [fertile, June 1899, *Rogers* s.n. (**M**)].

Nepal: ***West Nepal*** [Bansa Pani, fertile, 22 March 1929, *Bisram* 47, (**GH**)]

10. Dendrocalamus latiflorus Munro, Trans. Linn. Soc. 26: 152. *t. 6.* 1868; Gamble, Ann. Bot. Gard. Calc. 7: 131. *pl. 117.* 1896; Brandis, Indian Trees (Fourth Impression): 678. 1921; W.C. Lin, Special Bul. Taiwan For. Res. Inst. 6: 28. 1968; W.C. Lin in H.L. Li *et al.*, Fl. Taiwan 5: 774. *pl. 1516.* 1978; C.A. Roxas in S. Dransfield & E.A. Widjaja, Pl. Resources S.E. Asia 7: 87. *illustration on page 88.* 1995; The Bamboos of the World 287. 1999; D.Z. Li & Stapleton, Fl. China 22: 45. 2006. Type: Hongkong, fertile, *Wright* s.n. [lectotype **L!**, designated here]. Fig. 4.22 & 4.23.

— *Bambusa latiflora* (Munro) Kurz, Journ. As. Soc. Beng. 42(2): 250. 1873. Type: as for above.
— *Sinocalamus latiflorus* (Munro) McClure, Lingnan Univ. Sci. Bull. 9: 67. 1940. Type: as for above.

Misapplied name: *Bambusa verticillata sensu* Benth., Fl. Hongkong 434. 1861 (excl. type), ***non sensu*** Willd., Sp. Pl. ii. 245. 1799 (=basionym of *Gigantochloa verticillata* (Willd.) Munro, Trans. Linn. Soc. 26: 123. 1868).

Culms loosely or densely tufted, straight and erect, about 15–25 m long, 8–20 cm in diameter, tips arching; internodes 20–60 cm long, lower ones not hairy; lower nodes developing conspicuous verticils of roots; walls relatively thin, about 0.7–1.5 cm thick (mid-culm portion). *Branches* developing from around mid culm or from lower quarter upwards; mid-culm branch complements with several branches at each node, the primary one dominant but not so different in size from the remaining ones, aerial roots sometimes present. *Culm sheaths* deciduous, coriaceous, 15–40 cm long by 25–60 cm wide, tops concavely truncate, slightly raised up at the middle, back scattered with dull brown to black hairs, often glabrous; blades ovate to lanceolate, deflexed, 10–15 cm long, about 3–5 cm wide near the base, adaxial base sparsely hairy; auricles small, continuing from base of culm-sheath blade, more or less plicate, lobelike, 1–2 mm tall, about 0.5–1.5 cm long, inner side densely covered with short hairs, margins fringed with few bristles 0.5–3.5 mm long, readily deciduous; ligule 1.5–3 mm high, margin toothed. *Leaves* 5–12 per branchlet; blades 20–30 cm long by 2–4.5 cm wide, upper surface glabrous, lower surface sparsely hairy to glabrous, bases more or less rounded or obliquely acute to obliquely attenuate, pseudo-petiole 3–6 mm long; leaf sheaths 9–13 cm long; auricles absent or as inconspicuous ridges, margins glabrous or fringed with few short bristles 0.5–3 mm long; ligule 1–2 mm high, margin irregularly dentate to sub-entire; secondary veins 10–11 pairs, intermediate veins 5–7. *Pseudospikelets* congested as stellate, not spiny to the touch; synflorescence axes hairy, internodes 1.5–5.5 cm long. *Spikelets* reddish purple to dark purple, broadly ovate, laterally compressed, 12–17 mm long by 5–12 mm wide;

glumes 2, 3.3–7 mm long, 13–25-nerved; fertile florets 6–8, more or less patent between florets, terminal vestigial floret absent; lemmas chartaceous, 9–14 mm long, back pubescent, 23–29-nerved, apex pointed, margins long-fringed, margins and lower part usually membranous and wrinkled or wavy; paleas chartaceous, 8.5–11 mm long by 1.5–3 mm wide, 2-keeled on the back, keels and edges long-fringed, the uppermost one not keeled or slightly keeled near apex, both sides densely pubescent, apex acute, 2–3-nerved between keels, 2-nerved on each inflexed edge; lodicules none; anthers 5–7 mm long, yellow, tips penicillate, usually covered with numerous miniscule spines; ovary turbinate, summit hairy, stigma 1, plumose. *Caryopsis* with adherent pericarp, 8–12 mm long, 4–6 mm in diameter.

Thailand.— Cultivated mostly in northern Thailand.

Distribution.— Origin is not known precisely but possibly native in southern China including Taiwan (Ohrnberger, 1999).

Ecology.— Mostly found in cultivation.

Vernacular.— Pai Hma Joo (ไผ่หม๋าจู๋).

Typification notes.— When *Dendrocalamus latiflorus* Munro was first described, four collections were cited (Munro, 1868). They are three collections from Hongkong, *Wright* s.n., *Hance* 1050 and *Harland* 740; and one collection from Formosa (Taiwan), *Oldham* 649. I have examined the fertile specimen of this species collected by *Wright* s.n. (also from 'Hong Kong', but spelling with a space), housed in **L** (code: L0412613). There are two labels on this sheet. The first one is the label with the paper head typing 'Herbarium of the U.S. North Pacific Exploring Expedition under Commanders Ringgold and Rodgers, 1853-56', the determination for this specimen as *Bambusa verticillata* Willd. was hand-written, and 'C. Wright Coll. Hong Kong' was typed. The second label is the determination slip by J. Th. Henrard in 1925, determinating the specimen as *Dendrocalamus latiflorus* Munro. As *Wright* (Charles(Carlos) Wright), an American botanical collector, lived between 1811-1885 (Stafleu & Cowan, 1988), these two labels would suggest that this specimen was collected before or at least in 1853, which was before *D. latiflorus* was first described in 1868. It would also be possible that this specimen or at least its duplicate would have been used for describing the species by Munro (1868). I also examined the fertile specimen of *Hance* 1050, housed in **GH**, which also cited by Munro (1868). Both of these two specimens, *Wright* s.n. and *Hance* 1050, are of good quality and have the potential to be selected as a lectotype. However, I designate here the specimen of *Wright* s.n. to be the lectotype because it was the first specimen cited by Munro (1868).

Specimens examined.

Thailand: NORTHERN: ***Chiang Mai*** [cult., Fang District, Ang Khang Royal Project, sterile, 3 Aug. 2004, *Sungkaew & Teerawatananon* 113 (**KUFF, TCD, THNHM**)]; [cult., Muang District, Mae Hia Royal Project, fertile, 2003, *Sungkaew & Teerawatananon* s.n. (**KUFF, THNHM**).

Austria: ***Innsbruck*** [cult., fertile, *Unknown collector* (**L**)].

China: ***Hainan*** [Bak Sa, fertile, 25 April 1936, *Lau* 26499 (**GH**)]; [cult.?, Nam Fung, U Tau Ti, sterile, 30 Nov. 1921, *McClure* C.C.C. 8294 (**L**)]; [cult.?, Yai-hsien District, Yeung Lam Shan, near Yeung Lam Village, fertile, 4-24 May 1935, *Lau* 6286 (**GH**)]; ***Hong Kong*** [cult, Lower Albanny Nursery, fertile, 12 Nov. 1908, *Dunn* 5386 (**GH**)]; [fertile, *Hance* 1050 (**GH**); [fertile, *Wright* s.n. (**L**, code: L0412613)]; [fertile, *Babington* s.n. (**TCD**)].

Japan: Probably Japan [cult.?, Tainoku, fertile, June 1913, *Simada* W 220, (**GH**)].

Taiwan: [cult.?, Suido-tyo, Taihoku-si, fertile, 27 March 1934, *Tanaka & Shimada* 17774 (**E, L, GH, M**)]; [cult.?, Takow?, fertile, *Henry* 1803 (**GH**)]; [cult., Formosa, 1930, *Suzuki* s.n. (**L**)]; [probably cult., Taichung City, Peitun area, Takeng mountain hiking trail, Mixed orchards, alt. ca. 260 m, fertile, 23 Jan. 1996, *Liu et al.* 318 (**GH**)].

Vietnam: ***Hanoi*** [probably cult., fertile, May 1887, *Balansa* 1581 (**L**)]; [(Tonkin), probably cult., fertile, Sept. 1908, *Alleizette* s.n. (**L**)].

11. Dendrocalamus copelandii (Gamble ex Brandis) N.H. Xia & Stapleton, Kew Bull. 52(2): 484. 1997. Type: cult., India, Dehra Dun, 2,200 feet, sterile, *Gamble* 27166 [lectotype, **K!**, selected by N.H. Xia & Stapleton, Kew Bull. 52(2): 484. 1997.]. Fig. 4.24 & 4.25.

— *Bambusa copelandii* Gamble ex Brandis, Indian Trees (Fourth Impression): 671. 1921, as '*copelandi*'; Ohrnb., The Bamboos of the World 258. 1999. Type: as for above.

— *Sinocalamus copelandii* (Gamble ex Brandis) Raizada, Indian Forester 74(1): 10. *pl. 1*. 1948, as '*copelandi*'. Type: as for above.

— *Dendrocalamopsis copelandii* (Gamble ex Brandis) Keng f., J. Bamboo Res. 2(1): 12. 1983, as '*copelandi*'. Type: as for above.

Culms densely tufted, straight and erect, about 15–30 m long, 8–20 cm in diameter, tips more or less arching; internodes 20–48 cm long, lower ones not hairy; nodes not swollen, lower ones usually without verticils of roots; walls relatively thin, about 1 cm thick (mid-culm portion). *Branches* developing from around mid culm or from lower quarter upwards; mid-culm branch complements with several branches at each node, the primary one dominant but not so different in size from the remaining ones, aerial roots usually absent. *Culm sheaths* tardily deciduous to persistent, especially the ones from lower nodes, coriaceous, 15–46.5 cm long by 25–65 cm wide, tops convexly truncate, back covered with golden brown to dark brown hairs, occasionally glabrous; blades lanceolate, erect to spreading, 10–25.5 cm long, about 5–7 cm wide near the base, adaxial base hairy; auricles continuing from base of culm-sheath blade, lobes, thick and fleshy, crisped, 2–5 mm tall, 1–4 cm long, margins wavy or pleated, glabrous; ligule 3–10 mm high, margin irregularly dentate to sub-entire. *Leaves* 4–6 per branchlet; blades 10–27 cm long by 2.4–4 cm wide, hairy underneath, bases rounded to acute, pseudo-petiole 2–10 mm long; leaf sheaths 6.2–11 cm long; auricles absent, or present as inconspicuous lines or ridges, glabrous; ligule 1–1.2 mm high, margin dentate; secondary veins 7–10 pairs, intermediate veins 5–9. *Pseudospikelets* congested as stellate clusters, not spiny to the touch; synflorescence axes hairy, internodes 1.5–2.5 cm long. *Spikelets* yellow-brown, ovate-oblong to ovate-lanceolate, laterally compressed, 2–2.8 cm long by 5.5–7.3 mm wide; glumes 2–9, 2–12 mm long, 7–35-nerved; fertile florets 5–6, not patent between florets, terminal vestigial floret present; lemmas chartaceous, 1.2–2.5 cm long, back pubescent, 23–37-nerved, apex acute to pointed; paleas membranous or sub-chartaceous, 9.2–22 mm long by 1.3–3 mm wide, 2-keeled on the back, keels and edges fringed, the uppermost one not keeled, outside pubescent, inside sparsely pubescent to glabrous, apex shortly bifid, 2–3(–5)-nerved between keels, inflexed edges very narrow, not nerved; lodicules usually 3, occasionally none, hyaline; anthers 5–11 mm long, yellow, tips mucronate to apiculate, usually covered with miniscule spines; ovary turbinate, summit hairy, stigma 1, slightly plumose. *Caryopsis* 12-16 mm long, contracted towards the apex, almost bottled-shaped, slightly grooved on one side, pericarp rather thick and loose.

Thailand.— NORTHERN: Mae Hong Son (Muang District, route from Pai to Mae Hong Son, before Pa-sua Waterfall; Pang Ma Pa District, Lum Nam Pai Wildlife Sanctuary, Nam Rin-Mae Um Ong Station); Tak (Tha Song Yang District, route from Mae Sod to Mae Sa Riang, Ban Nam Ok Roo); SOUTHWESTERN: Kanchanaburi (Sai Yok District, near Sai Yok Arboretum; Thong Pha Phum District, Kreng Krawia, Sunyataram Temple).

Distribution.— From upper Burma (cult.?) to limestone mountain range along bordering areas from northern to southwestern Thailand, Fig. 4.26.

Ecology.— Confined to mixed deciduous or dry evergreen forest of limestone mountain.

Vernacular.— Pai Man Moo (ไผ่มันหมู).

Notes.— *Dendrocalamus copelandii* is similar to *D. giganteus* Munro and *D. sinicus* L.C. Chia & J.L. Sun. It differs from *D. giganteus* in having longer spikelets, membranous or sub-chartaceous paleas, and in having no verticil of roots at the lower culm nodes. It differs from *D. sinicus* in having shorter spikelets, paleas with un-nerved inflexed edges, and in having usually glabrous lower internodes.

This species is newly recorded for Thailand. A manuscript has been submitted to Thai Forest Bulletin (Botany), see Manuscript 4.1, following section.

Specimens examined.

Thailand: NORTHERN: ***Mae Hong Son*** [Muang District, route from Pai to Mae Hong Son, before Pa-sua Waterfall, sterile, 6 Aug. 2004, *Sungkaew & Teerawatananon* 139 (**KUFF, TCD, THNHM**)]; [Pang Ma Pa District, Lum Nam Pai Wildlife Sanctuary, Nam Rin-Mae Um Ong Station, sterile, 25 April 2005, *Sungkaew & Teerawatananon* 527 (**KUFF, TCD, THNHM**)]; ***Tak*** [Tha Song Yang District, route from Mae Sod to Mae Sa Riang, Ban Nam Ok Roo, sterile, 4 May 2005, *Sungkaew* & *Teerawatananon* 570 (**KUFF, TCD, THNHM**)]; SOUTHWESTERN: ***Kanchanaburi*** [Sai Yok District, near Sai Yok Arboretum, 95 km northwest of Kanchanaburi, alt. 160 m, fertile, 6 Nov. 1979, *Shimizu et al.* T-21752 (**L**)];

[Thong Pha Phum District, Kreng Krawia, Sunyataram Temple, alt. ca. 500 m, sterile, 22 July 2004, *Sungkaew & Teerawatananon* 20, 25, 29 (**KUFF, TCD, THNHM**)].

Burma: ***Northern Shan States*** [cult.?, fertile (only seeds), May 1896, *Copeland* s.n. (**K**)].

India: ***Dehra Dun*** [cult., Kuanli Garden, fertile, March 1944, *Raizada* s.n. (**K**)]; [cult., alt. 2200 feet, sterile, Sept. 1898, *Gamble* 27166 (**K**)]; [cult., Forest School Garden, sterile, June 1898, *Gamble* s.n. (**K**)].

12. Dendrocalamus sinicus L.C. Chia & J.L. Sun, J. Bamboo Res. 1(1): 10. *pl. on page 13.* 1982; Ohrnb., The Bamboos of the World 290. 1999; D.Z. Li & Stapleton, Fl. China 22: 44. 2006. Type: Yunnan, Mengla, Mengpeng, alt. 625 m, fertile, *Nan-Zhu 2570* [holotype, **SCBI**]. Fig. 4.27.

— *Sinocalamus sinicus* (L.C. Chia & J.L. Sun) W.T. Lin, J. Bamboo Res. 42: 6. 1990. Type: as for above.

Culms loosely or densely tufted, straight and erect, about 15–30 m long, 10–30 cm in diameter, tips more or less arching; internodes 15–35(–40) cm long, lower ones hairy; nodes usually not swollen, sometimes somewhat swollen (very old culms), lower ones usually without verticils of roots, occasionally present from the very basal nodes; walls relatively thin, about 1.5 cm thick (mid-culm portion); old culms sometimes curved or slightly twisted. *Branches* developing from lower quarter upwards; mid-culm branch complements with several branches at each node, the primary one dominant but not so different in size from the remaining ones, aerial roots usually absent. *Culm sheaths* deciduous or tardily deciduous especially from basal nodes, coriaceous, 15–35 cm long by 32–80 cm wide, tops slightly convexly truncate, back covered by dark brown to black hairs; blades lanceolate, erect to spreading, 10–30 cm long, about 5 cm wide near the base, adaxial base sparsely hairy; auricles continuing from base of culm-sheath blade, lobes, thick and fleshy, crisped, about 5 mm tall, 5–10 mm long, margins wavy or pleated, glabrous; ligule 5–8 mm high, margin dentate or sub-entire, sometimes lacerate at both ends. *Leaves* 5–9 per branchlet; blades 25–30 cm long by 2.5–4.5 cm wide, glabrous or hairy underneath near the base, bases rounded to acute, pseudo-petiole 3–10 mm long; leaf sheaths 8–12.3 cm long; auricles absent, or present as inconspicuous lines or ridges, glabrous; ligule 1–2 mm high, margin dentate or erose; secondary veins 9–11 pairs, intermediate veins 7–9. *Pseudospikelets* congested as stellate clusters, not spiny to the touch; synflorescence axes hairy, internodes 1.5–2 cm long. *Spikelets* yellow-brown, ovate-oblong to ovate-lanceolate, laterally compressed, 3–3.7 cm long by 6–8 mm wide; glumes 2–6, 3.5–15 mm long, 15–33-nerved; fertile florets 4–6, not patent between florets, terminal vestigial floret present; lemmas chartaceous, 1.5–3.5 cm long, back pubescent, 19–41-nerved, apex acute to pointed; paleas membranous or sub-chartaceous, 1–3 cm long by 2.3–3.5 mm wide, 2-keeled on the back, keels and edges fringed, the uppermost one not keeled or slightly keeled upper half, outside pubescent, inside sparsely pubescent, apex shortly bifid to acute, 3–5-nerved between keels, 1-nerved on each inflexed edge; lodicules usually 2, occasionally none, hyaline; anthers 9.5–13 mm long, yellow, tips mucronate to penicillate, glabrous or with few miniscule spines; ovary turbinate, summit hairy, stigma 1, slightly plumose. *Caryopsis* unknown.

Thailand.— Only found in cultivation. NORTHERN: Chiang Mai (Mae Jam District, Ban Wat Chan).

Distribution.— Yunnan, China.

Ecology.— Natural habitat unknown.

Vernacular.— Pai Pok, Mai Pok (ไผ่โปก, ไม้โปก).

Specimens examined.

Thailand: NORTHERN: ***Chiang Mai*** [cult., Mae Jam District, Ban Wat Chan, sterile, 5 Aug. 2004, *Sungkaew & Teerawatananon* 127 (**KUFF, THNHM**)]; [cult., Mae Jam District, Wat Chan Royal Project, sterile, 5 Aug. 2004, *Sungkaew & Teerawatananon* 128 (**KUFF, TCD, THNHM**)].

China: Yunnan [cult., Xishuanbanna Tropical Garden, fertile, 18 May 2005 *Sungkaew & Teerawatananon* 616 (**KUFF, TCD, THNHM**)].

13. Dendrocalamus giganteus Munro, Trans. Linn. Soc. 26: 150. 1868; Ridl., Fl. Malay. Penin. 5: 265. 1925; Gamble, Ann. Bot. Gard. Calc. 7: 87. *pl.* 76. 1896; Brandis, Indian Trees (Fourth Impression): 678. 1921; Holttum, Gard. Bull. Singapore 16: 103. *fig.* 26. 1958; W.C. Lin, Special Bul. Taiwan For. Res. Inst. 6: 27. *fig.* 20 (not clear). 1968; Widjaja in S. Dransfield & E.A. Widjaja, Pl. Resources S.E. Asia 7: 85. *illustration* on page 85. 1995; K.M. Wong, Malayan Forest Records 41: 111. *fig.* 54, 55. 1995; Ohrnb., The Bamboos of the World 285. 1999; D.Z. Li & Stapleton, Fl. China 22: 44. 2006. Type: cult., Calcutta Bot. Gard., fertile, *Herb. Munro* [lectotype, **K!**, selected by Stapleton, Edinburgh J. Bot. 51(1): 23. 1994.]. Fig. 4.28 & 4.29.

— *Bambusa gigantea* Wall., Cat. Bot. Gard. Calc. 79. 1840, *nom. nud.*
— *Bambusa gigantea* (Munro) Wall. ex A. & C. Riviere, Bull. Soc. Acclim. ser. 3 (5): 685. 1878. Type: as for above.
— *Sinocalamus giganteus* (Munro) A. Camus, Rev. Int. Bot. Appl. Agric. Trop. 29:551. 1949. Type: as for above.

Culms densely tufted, straight and erect, about 20–30 m long, 15–30 cm in diameter, tips more or less arching; internodes 30–45 cm long, lower ones not hairy; nodes not swollen, lower ones with verticils of roots; walls relatively thin, about 1–1.5 cm thick (mid-culm portion). *Branches* developing from around mid culm upwards; mid-culm branch complements with several branches at each node, the primary one dominant but not so different in size from the remaining ones, aerial roots usually absent. *Culm sheaths* deciduous, coriaceous, 20–35.5 cm long by 45–85 cm wide, tops slightly convexly truncate, back scattered with pale brown to dark brown hairs; blades broadly lanceolate, spreading to deflexed, 10–30 cm long, about 5–10 cm wide near the base, adaxial base hairy; auricles continuing from base of culm-sheath blade, lobes, thick and fleshy, crisped, about 5 mm tall, 5–10 mm long, margins wavy, glabrous; ligule 7–10 mm high, margin lacerate. *Leaves* 5–13(–19) per branchlet; blades 18–35.2 cm long by 2–4.5 cm wide, glabrous or hairy underneath, bases rounded to cuneate or attenuate, pseudo-petiole 2–5 mm long; leaf sheaths 9–11.5 cm long; auricles absent, or present as inconspicuous lines or ridges, glabrous; ligule 0.5–1.7 mm high, margin lacerate; secondary veins 6–12 pairs, intermediate veins 5–9. *Pseudospikelets* congested as stellate clusters, not spiny to ~~the~~ touch; synflorescençe axes hairy to glabrous, internodes 1.5–2.5 cm long. *Spikelets* yellow-brown, ovate-oblong, laterally compressed, 1.4–1.8 cm long by 4.5–6.2 mm wide; glumes 2–4, 4.7–7 mm long, 13–25-nerved; fertile florets 4–6, not patent between florets, terminal vestigial floret present; lemmas chartaceous, 7–16 mm long, back pubescent, mainly upper part, 19–29-nerved, apex acute to pointed; paleas chartaceous, 6.5–14 mm long by 1.5–3.5 mm wide, 2-keeled on the back, keels and edges fringed, the uppermost one not keeled, outside pubescent, inside glabrous, apex blunt to acute, un-divided, 2–3-nerved between keels, 1–2(–3)-nerved on each inflexed edge; lodicules usually none, occasionally 1–2, hyaline; anthers 5.5–9 mm long, yellow, tips penicillate, usually covered with miniscule spines; ovary turbinate, summit hairy, stigma 1, plumose. *Caryopsis* 7-8 mm long, hairy at apex.

Thailand.— So far, known only in cultivation. Lin (1968) reported (with the figure shown, but not clear enough to identify) that there was a natural grove of this species at altitude of 800 m in Lan Sang National Park, Tak Province.

Distribution.— Origin is not known precisely but it is believed to be native in Burma and adjacent regions in north-eastern India, southern China and north-western Thailand (Ohrnberger, 1999).

Ecology.— Natural habitat unknown.

Vernacular.— Pai Yak, Pai Pok, Mai Po (ไผ่ยักษ์, ไผ่โปก, ไม้โปก).

Specimens examined.

Thailand: NORTHERN: ***Chiang Mai*** [cult., alt. 300 m, fertile, 4 March 1914, *Kerr* 3150 (**BM**)]; [cult., alt. 300 m, fertile, 6 March 1915, *Kerr* 3542 (**BM, K**)]; [cult. Mae Rim District, Queen Sirikit Botanic Garden, sterile, *Veeraya* s.n. (**THNHM**); [cult., *l.c.*, sterile, 7 Aug. 2004, *Sungkaew & Teerawatananon* 157 (**KUFF, THNHM**)]; ***Chiang Rai*** [cult. in the Lahu Na village of Goshen on Doi Tung, 940 m, sterile, 10 Feb. 1991, *Anderson* 6183 (**GH**)]

India: ***Assam*** [cult.?, Lakhempur, sterile, Oct. 1890, *Mann* s.n. (**E, K**)]; [cult.?, Sadiya, sterile, 25 March 1894, *Gammie* 272 (**K**)]; ***Calcutta*** [cult., Bot. Gard., fertile, *Herb. Munro* (**K**)]; [cult. in Hort. Bot. Cal., fertile, *Herb. Gamble* (**K**)]; [*l.c.*, fertile, 11 Feb. 1898, *Lane* s.n. (**L, M**)]; [cult. in Hort. Bot. Cal., N. of Picnic Banyan, fertile, 29 Jan. 1898, *Prain* s.n. (**GH**)]; [cult. in Hort. Bot. Cal., fertile, 21 Feb. 1899, *Lane* s.n. (**ABD, E, K, TCD**)]; [cult. in Hort. Bot. Cal., sterile, *Unknown collector* (**L**, code: L0412574, -75; **M**, code: M0124731, -32, -33)].

Indonesia: ***Bogor*** [cult. in Hort. Bog., fertile, *Unknown collector* (**L**, code: L0412577 (cult. in Hort. Bog., XIV. B. Iia); code: L0412578 (cult. in Hort. Bog., XIV. B. II.); code: L0412579, -80 (not specified where); code: L0412581, -82 (cult. in Hort. Bog., XIII. B. II.))].

Trinidad: [cult. in Botanic Gardens, fertile, 2 Feb. 1916, *Broadway* s.n. (**GH**)].

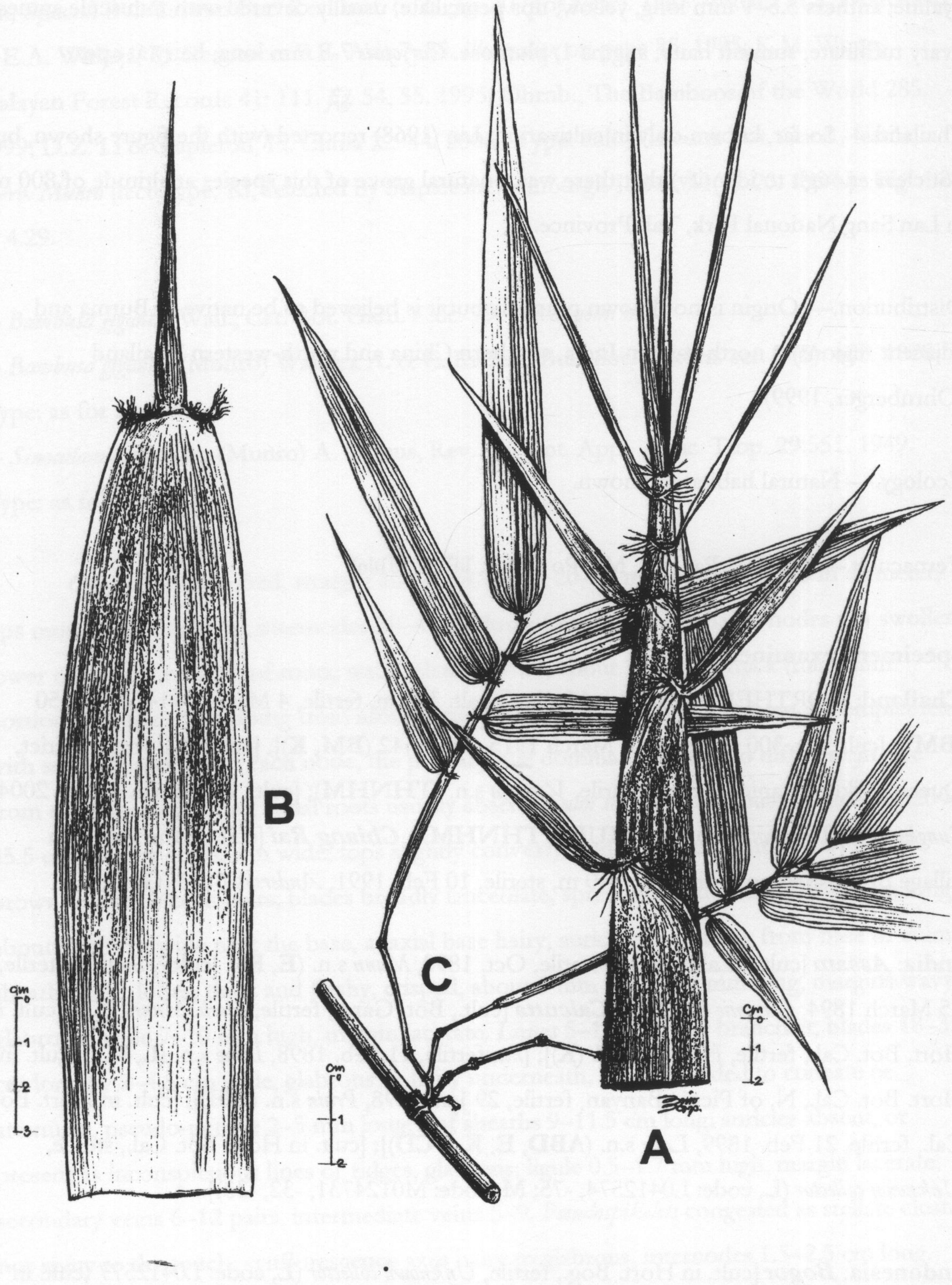


Figure 4.1 *Dendrocalamus dumosus*. A. flying shoot, showing auricles; B. culm sheaths (abaxial view), showing auricles; C. leafy branch. All from *Sungkaew & Teerawatananon* 264. All drawn by Pairach Payangkul.

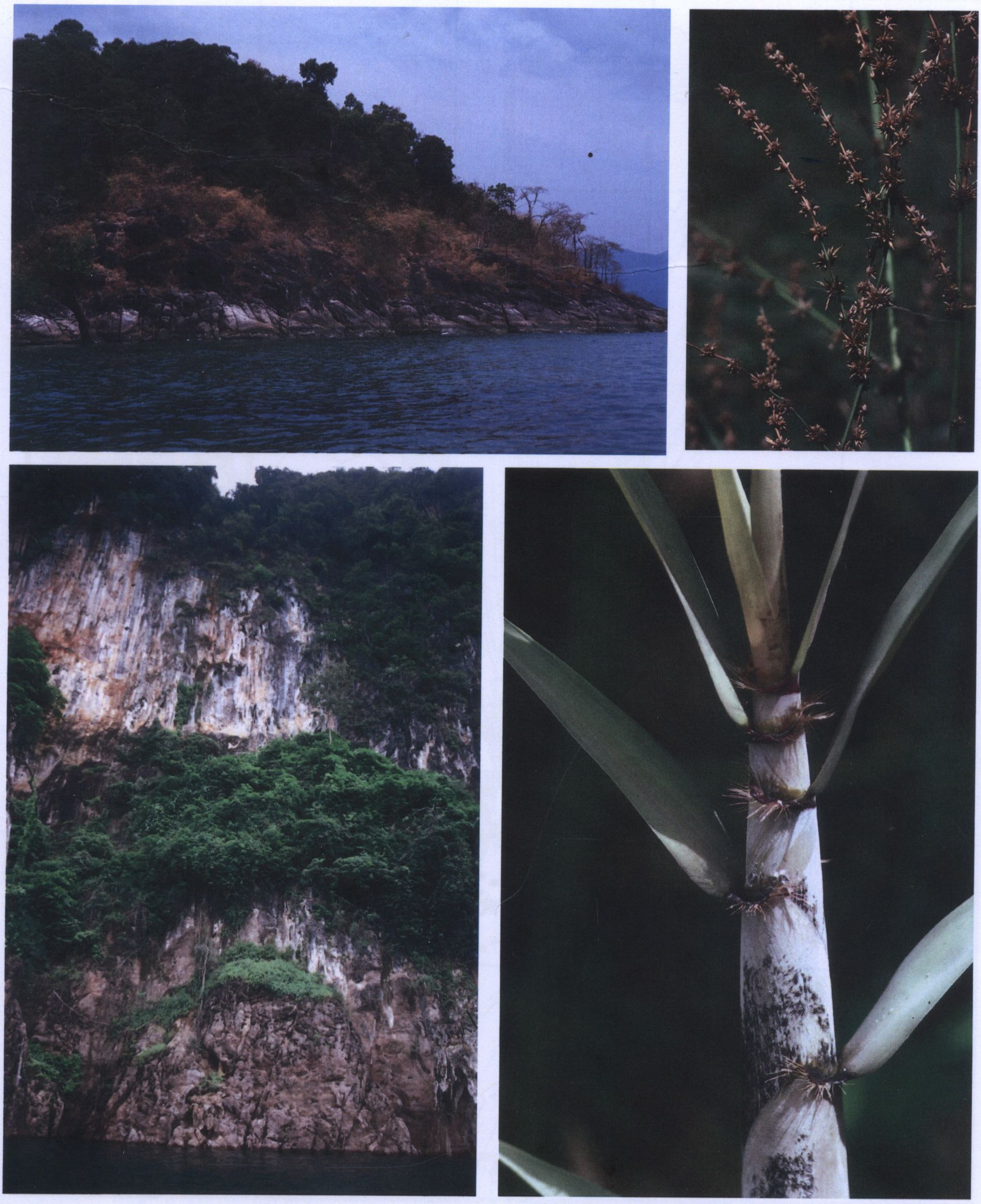


Figure 4.2 *Dendrocalamus dumosus*. Top left, habitat and habit in dry season on limestone rocks at Rawi Island, Tarutao National Park, Satun Province, southern Thailand, the same island where the type was collected; Top right, flowering branches (from Thung Song District, Nakhon Si Thammarat Province, southern Thailand); Bottom left, habitat and habit on limestone mountains in rainy season at Ratchaprapa Dam, Surat Thani Province, southern Thailand; Bottom right, a flying shoot, showing auricles (also from Ratchaprapa Dam).

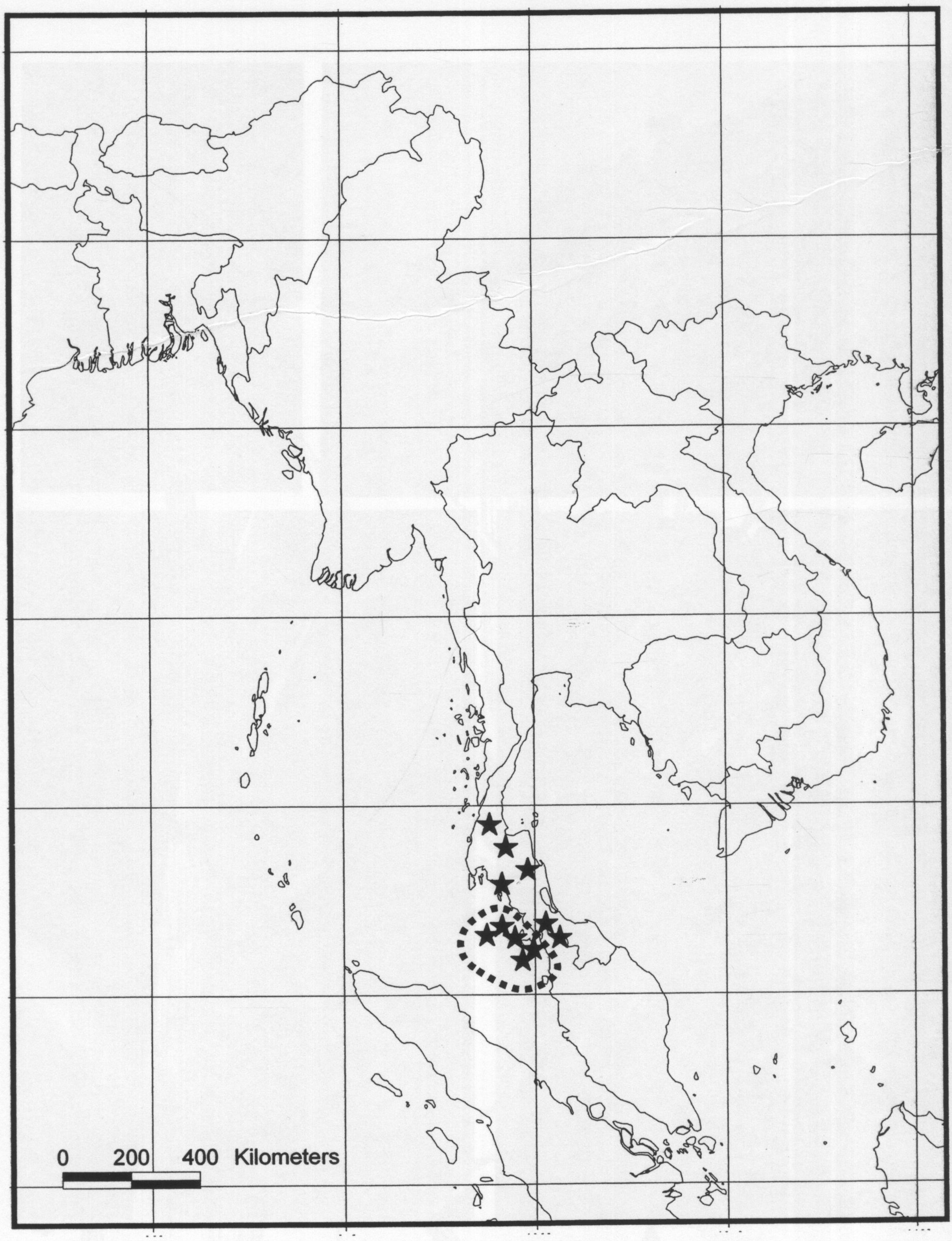


Figure 4.3 Distribution map of *D. dumosus.* The stars represent the distribution as obtained from specimen examination; the dashed loop represents the distribution as obtained from Ohrnberger (1999).

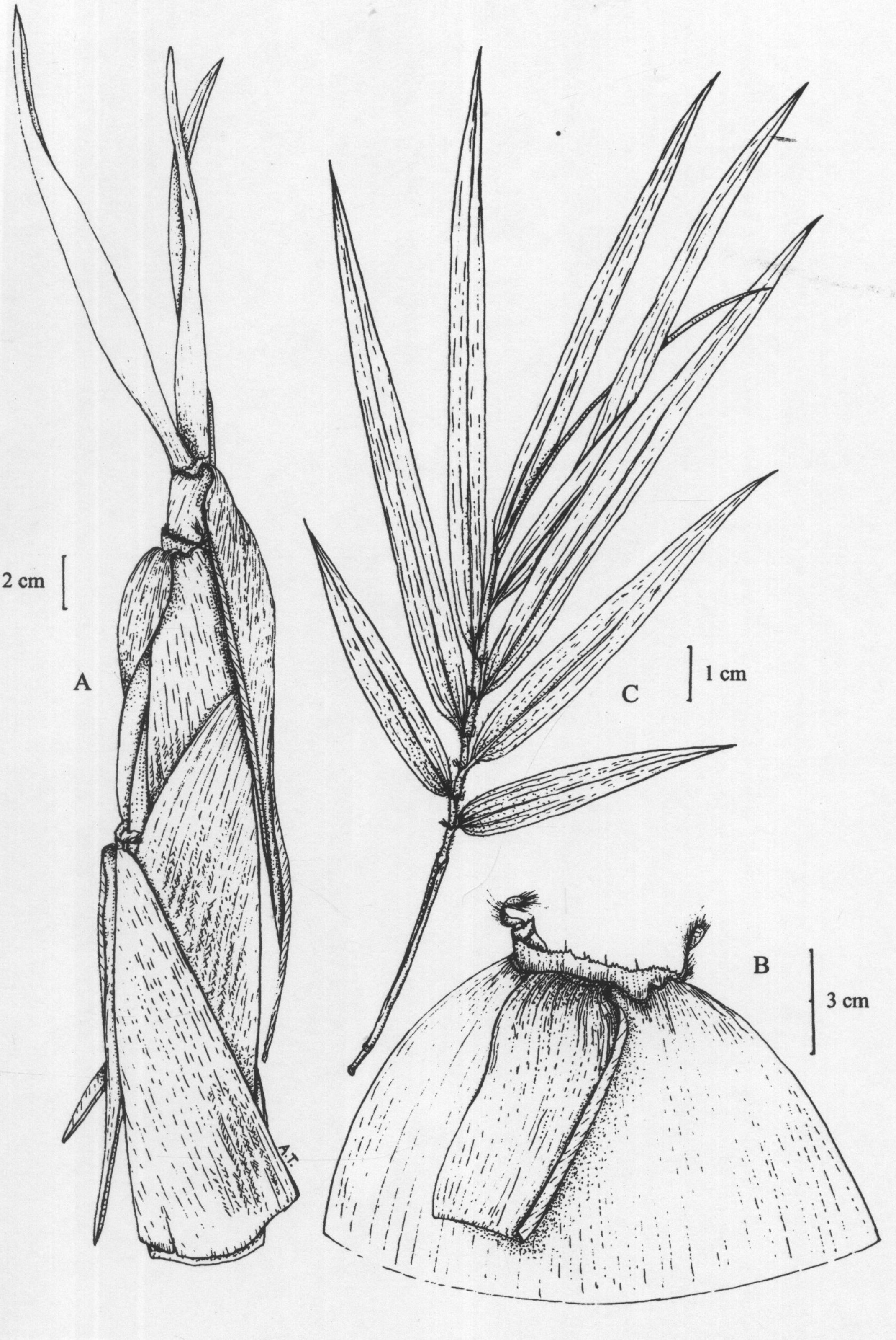


Figure 4.4 *Dendrocalamus khoonmengii*. A. flying shoot; B. part of culm sheath from A, showing auricles and ligule; C. leafy branchlet. All from *Wong, Thapyai, & Roisungnern* WKM 2868. All drawn by Atchara Teerawatananon.

Figure 4.5 *Dendrocalamus khoonmengii*. A flying shoot, photo taken from where the type was collected (Khaoluang National Park, Nakhon Si Thammarat Province, southern Thailand), showing the curved and twisted auricles.

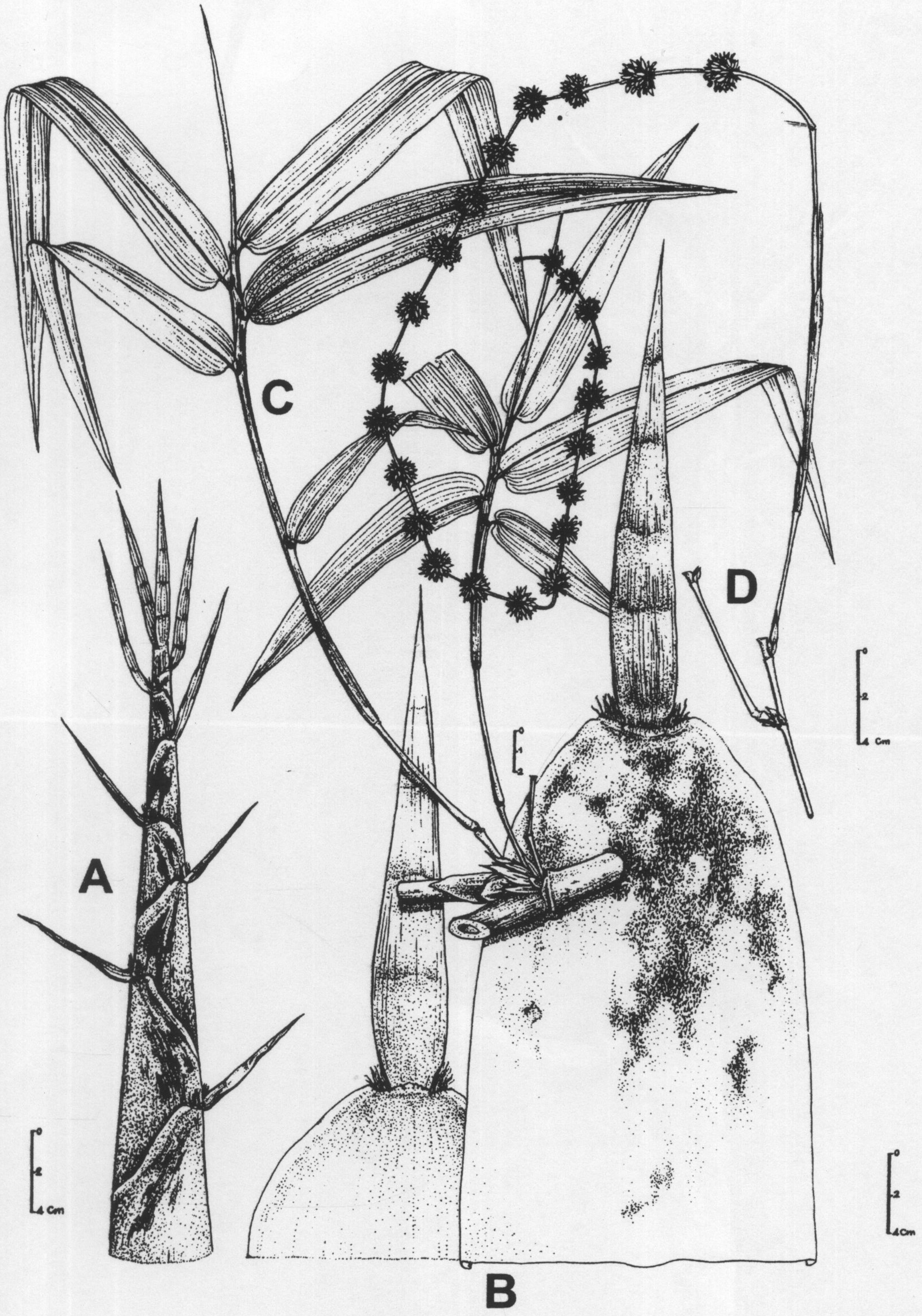


Figure 4.6 *Dendrocalamus pendulus.* A. flying shoot; B. culm sheath, part of culm sheath showing ligule (left, adaxial view) and full culm sheath showing auricles and covering on the back (right, abaxial view); C. leafy branch; D. flowering branch. A, from *Sungkaew & Teerawatananon* 232; B, from *Sungkaew & Teerawatananon* 381; C, from *Sungkaew & Teerawatananon* 231; D, from *Sungkaew & Teerawatananon* 230. All drawn by Pairach Payangkul.

Figure 4.7 *Dendrocalamus pendulus.* Top left and bottom, flying shoots, copiously covered with white wax; Top right, a flowering branch, showing globose pseudospikelet clusters (synflorescences) and purple anthers. All from Ton Nga Chang Wildlife Sanctuary, Songkhla Province, southern Thailand.

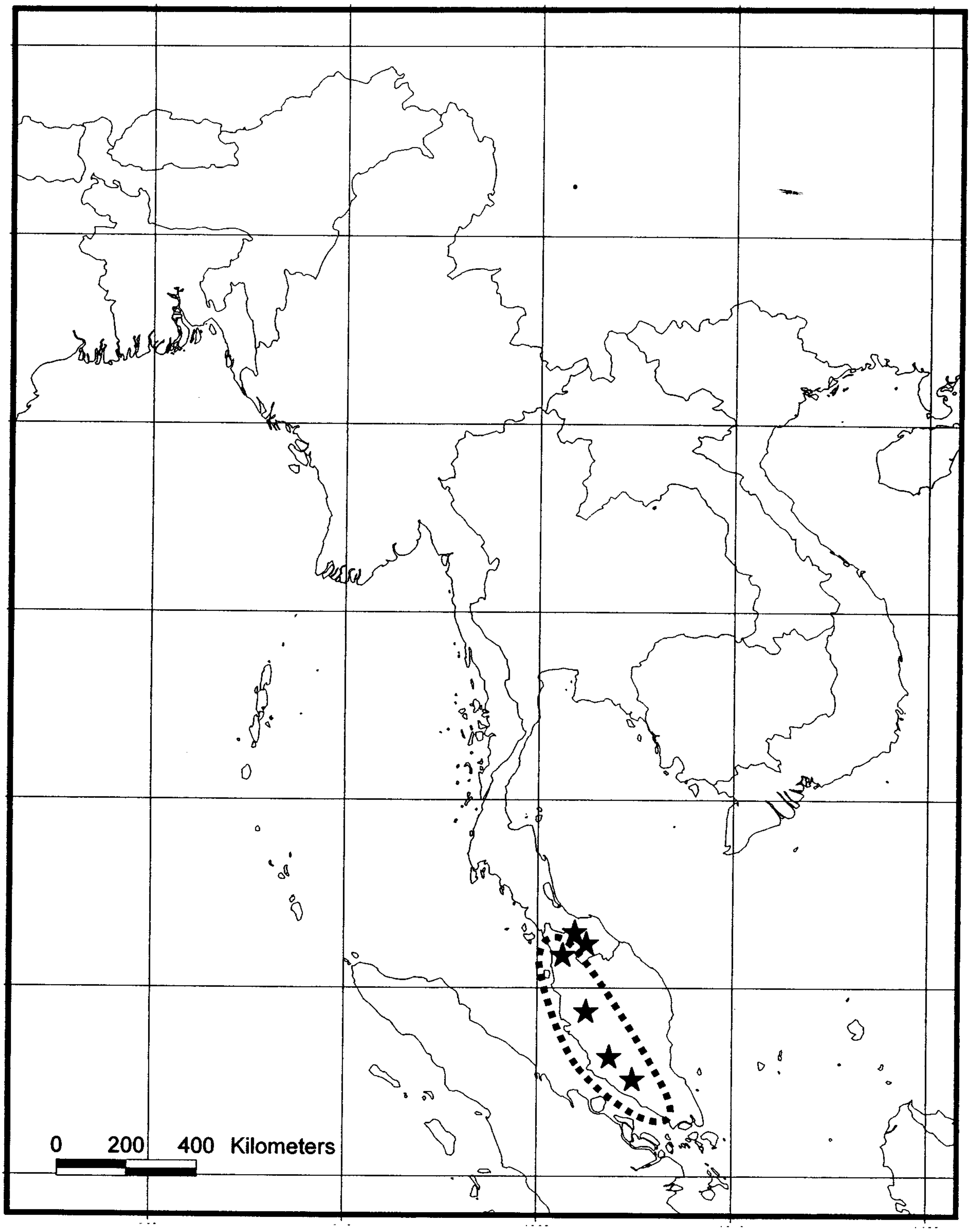


Figure 4.8 Distribution map of *D. pendulus*. The stars represent the distribution as obtained from specimen examination; the dashed loop represents the distribution as obtained from Ohrnberger (1999).

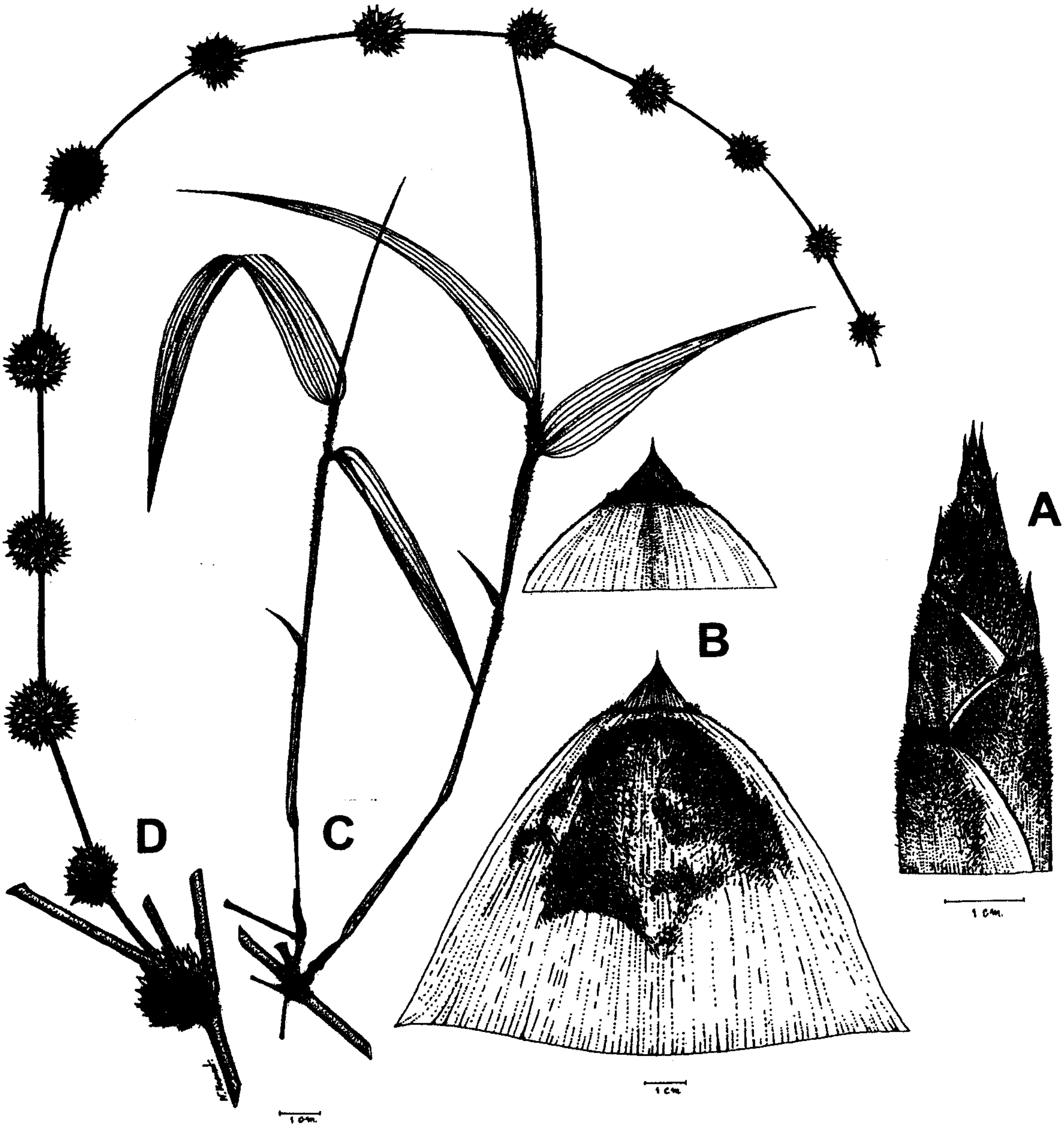


Figure 4.9 *Dendrocalamus strictus*. A. young shoot (collected from dead young shoot); B. culm sheath, part of culm sheath showing ligule (above, adaxial view) and full culm sheath showing tiny auricles and the covering on the back (below, abaxial view); C. leafy branch; D. flowering branch. A and B, from *Sungkaew & Teerawatananon* 718; C, from *Sungkaew & Teerawatananon* 583; D, from *Sungkaew & Teerawatananon* 582. All drawn by Weerapong Korawat.

Figure 4.10 *Dendrocalamus strictus.* Left, a culm sheath showing the covering on the back; Right, a flowering branch, showing two globose pseudospikelet clusters (synflorescences) and yellow anthers. All from Mae Sod District, Tak Province, northern Thailand.

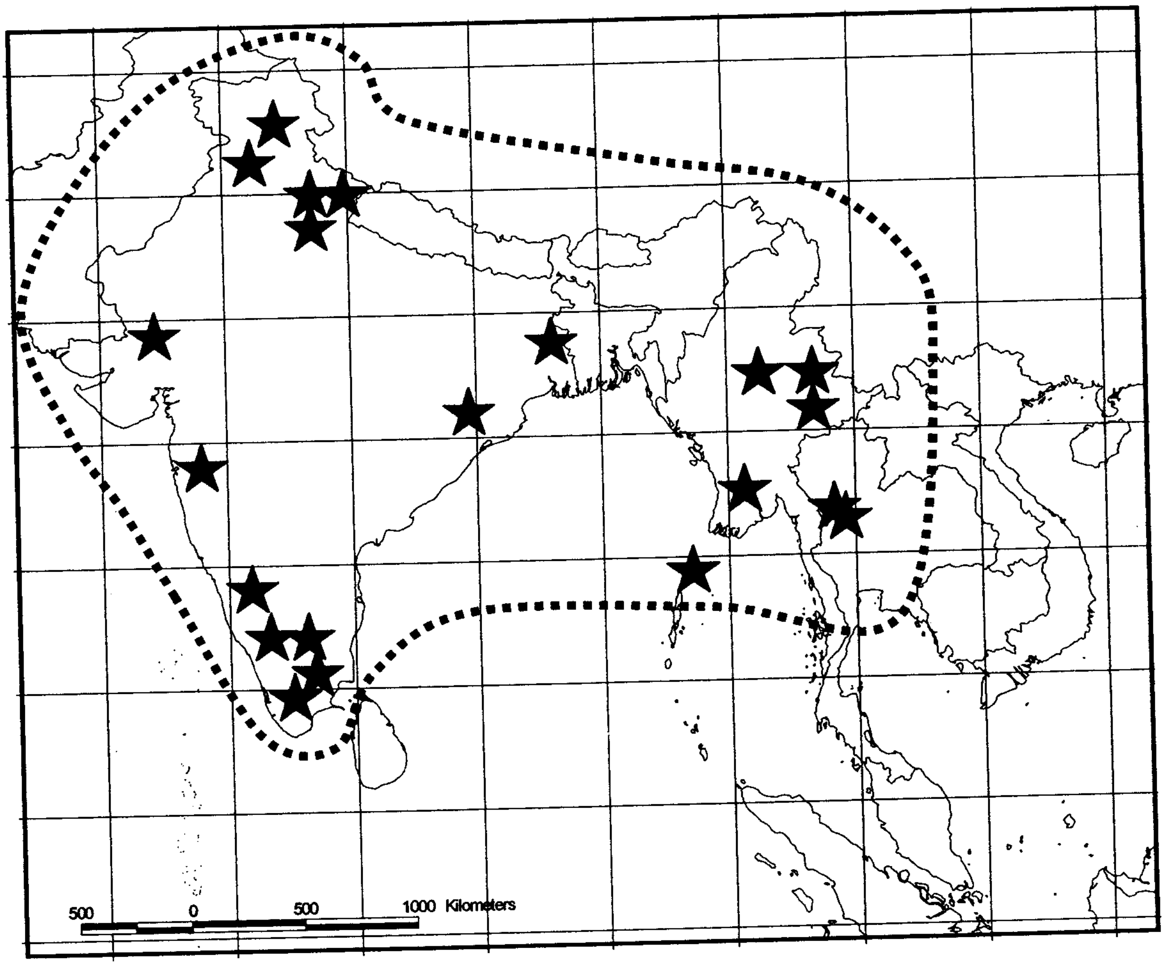


Figure 4.11 Distribution map of *D. strictus*. The stars represent the distribution as obtained from specimen examination; the dashed loop represents the distribution as obtained from Ohrnberger (1999).

Figure 4.12 *Dendrocalamus barbatus*. Top, clump habit, showing lower culm internodes covered with scurf or appressed white hairs and showing flying shoots; Bottom, flowering branches. All from Mae Wang District, Chiang Mai Province, northern Thailand (cultivated).

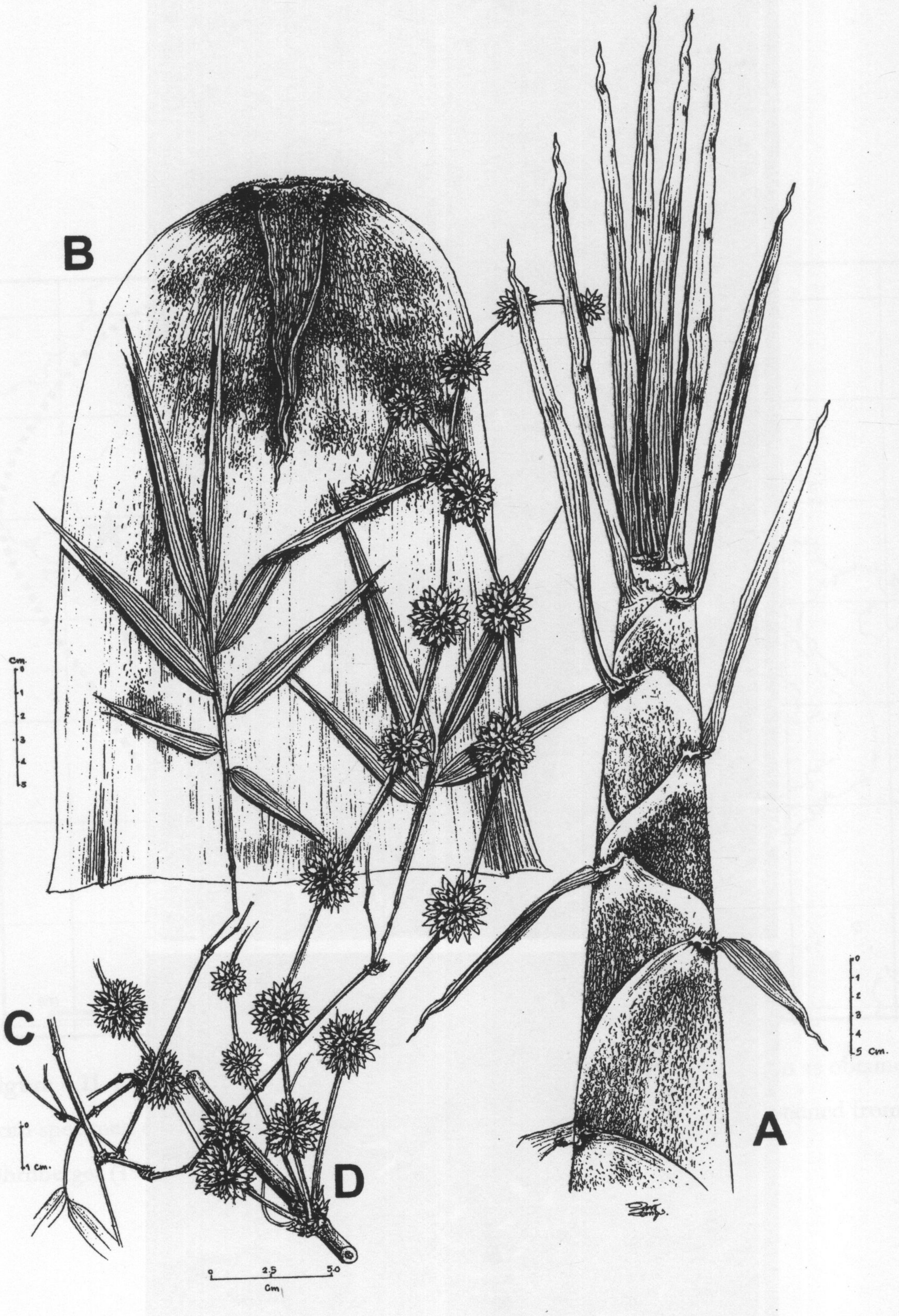


Figure 4.13 *Dendrocalamus membranaceus*. A. flying shoot; B. culm sheath; C. leafy branch; D. flowering branch. A-C, from *Sungkaew & Teerawatananon* 50; D, from *Sungkaew & Teerawatananon* 583; D, from *Sungkaew & Teerawatananon* 327. All drawn by Pairach Payangkul.

Figure 4.14 *Dendrocalamus membranaceus.* Top, a vast area of natural grove of this species (from Mae Moei National Park, Tak Province, northern Thailand); Bottom left, a mid-culm branch complement (from Suan Phung District, Ratchaburi Province, southwestern Thailand); Bottom middle, a flying shoot (from Lansang National Park, Tak Province); Bottom right, a flowering branch, showing spiny pseudospikelets (from Mae Rim District, Chiang Mai Province, northern Thailand).

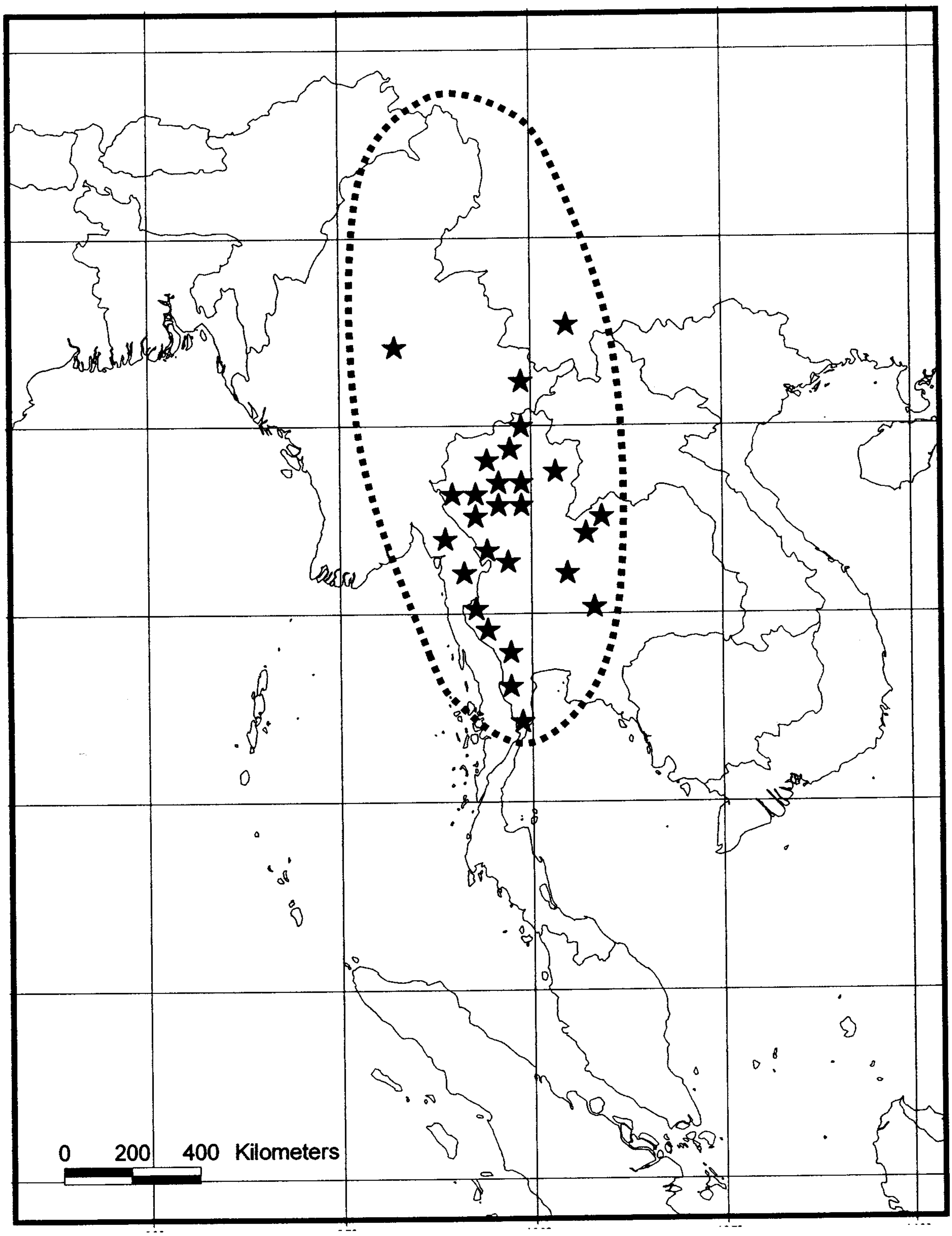


Figure 4.15 Distribution map of *D. membranaceus.* The stars represent the distribution as obtained from specimen examination; the dashed loop represents the distribution as obtained from Ohrnberger (1999).

Figure 4.16 *Dendrocalamus asper*. Top left, clump habit of 'Pai Tong', the popular cultivated one; Top right, clump habit of 'Pai Chiang Roon', the one found in the tropical rain forest in Klong Panom National Park, Surat Thani Province, southern Thailand; Bottom left, a flying shoot of 'Pai Tong'; Bottom middle, young shoots of 'Pai Chiang Roon'; Bottom right, a flowering branch of 'Pai Tong'. All of 'Pai Tong' from Mae Jam District, Chiang Mai Province, northern Thailand; all of 'Pai Chiang Roon' from Klong Panom National Park.

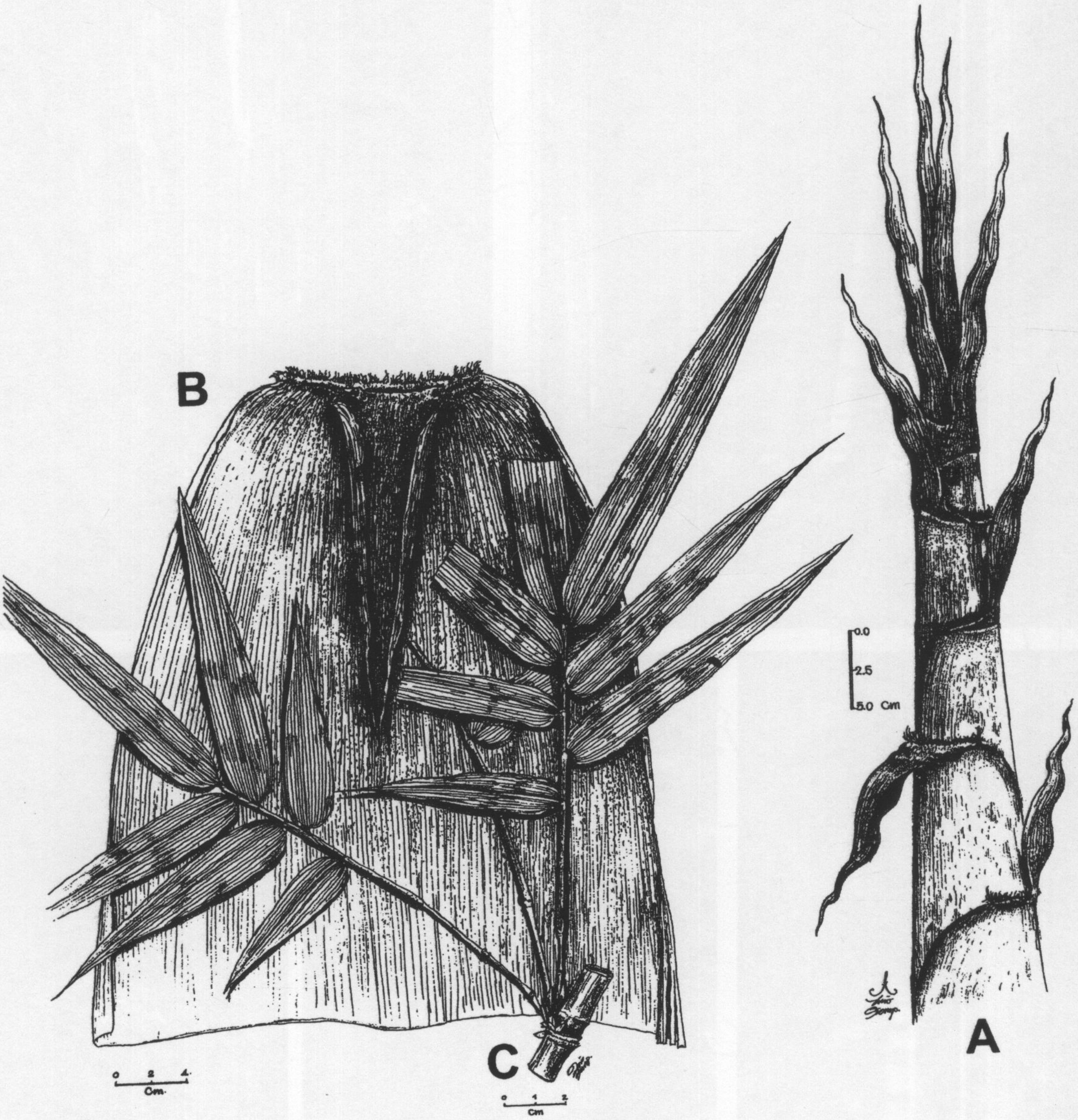


Figure 4.17 *Dendrocalamus brandisii*. A. young shoot; B. culm sheath (abaxial view), showing ligule; C. leafy branch. All from *Sungkaew & Teerawatananon* 114. All drawn by Pairach Payangkul.

Figure 4.18 *Dendrocalamus brandisii.* Left, a young shoot; Top, a leafy branchlet, both from Ang Khang Royal Project, Chiang Mai Province, northern Thailand (cultivated); Bottom, a flowering branch, showing globose pseudospikelet clusters (synflorescences) (from Pang Tong Palace, Mae Hong Son Province, northern Thailand).

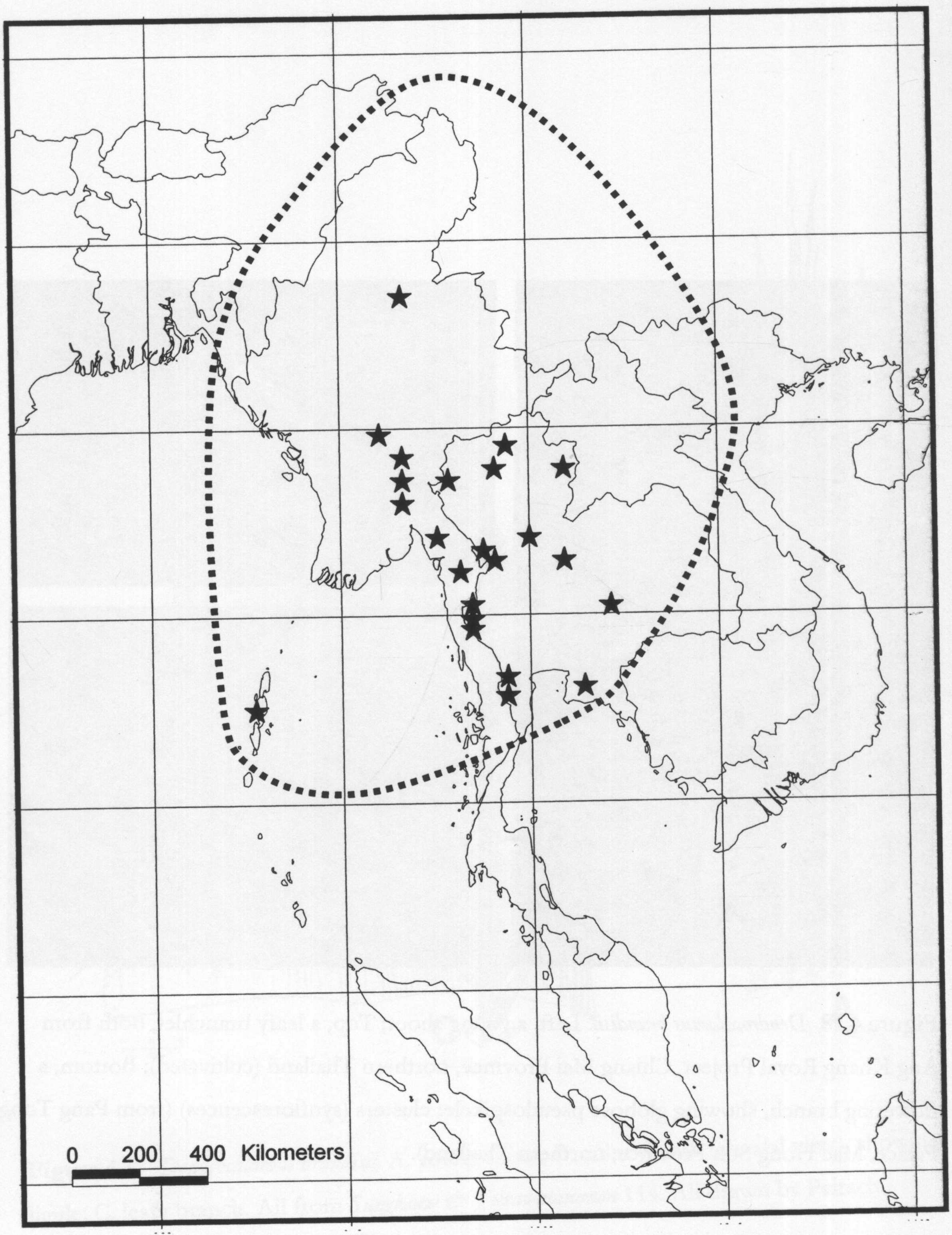


Figure 4.19 Distribution map of *D. brandisii.* The stars represent the distribution as obtained from specimen examination; the dashed loop represents the distribution as obtained from Ohrnberger (1999).

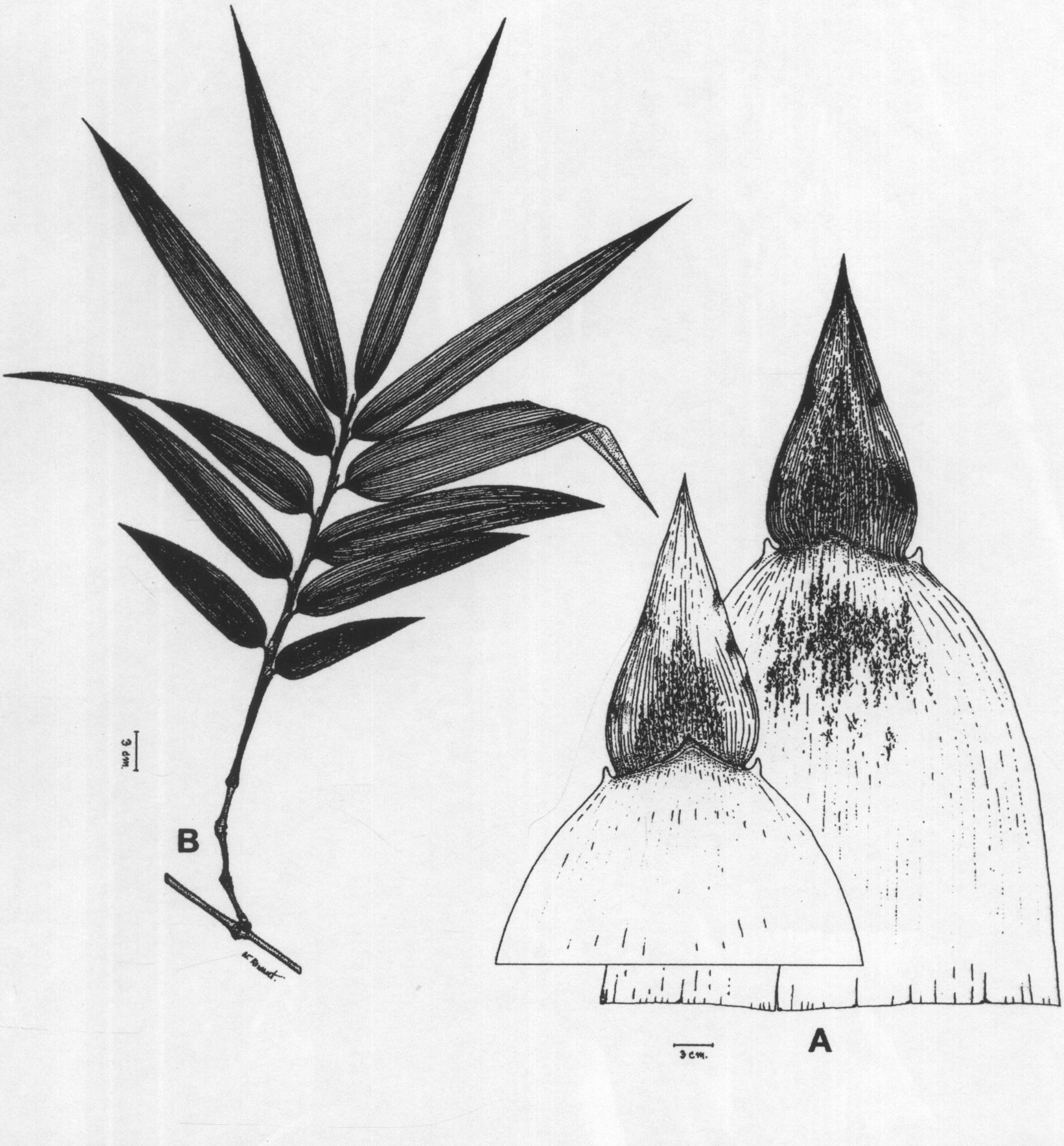


Figure 4.20 *Dendrocalamus hamiltonii*. A. culm sheath, full culm sheath showing auricles (back, abaxial view) and upper part of culm sheath showing auricles and ligule (front, adaxial view); B. leafy branchlet. All from *Sungkaew & Teerawatananon* 787. All drawn by W. Korawat.

Figure 4.21 *Dendrocalamus hamiltonii*. Young culms, showing culm sheaths; Inset left, two culm sheaths (abaxial view); Inset right, part of culm sheath, showing ligule. All from Wiang Kaen District, Chiang Rai Province, northern Thailand.

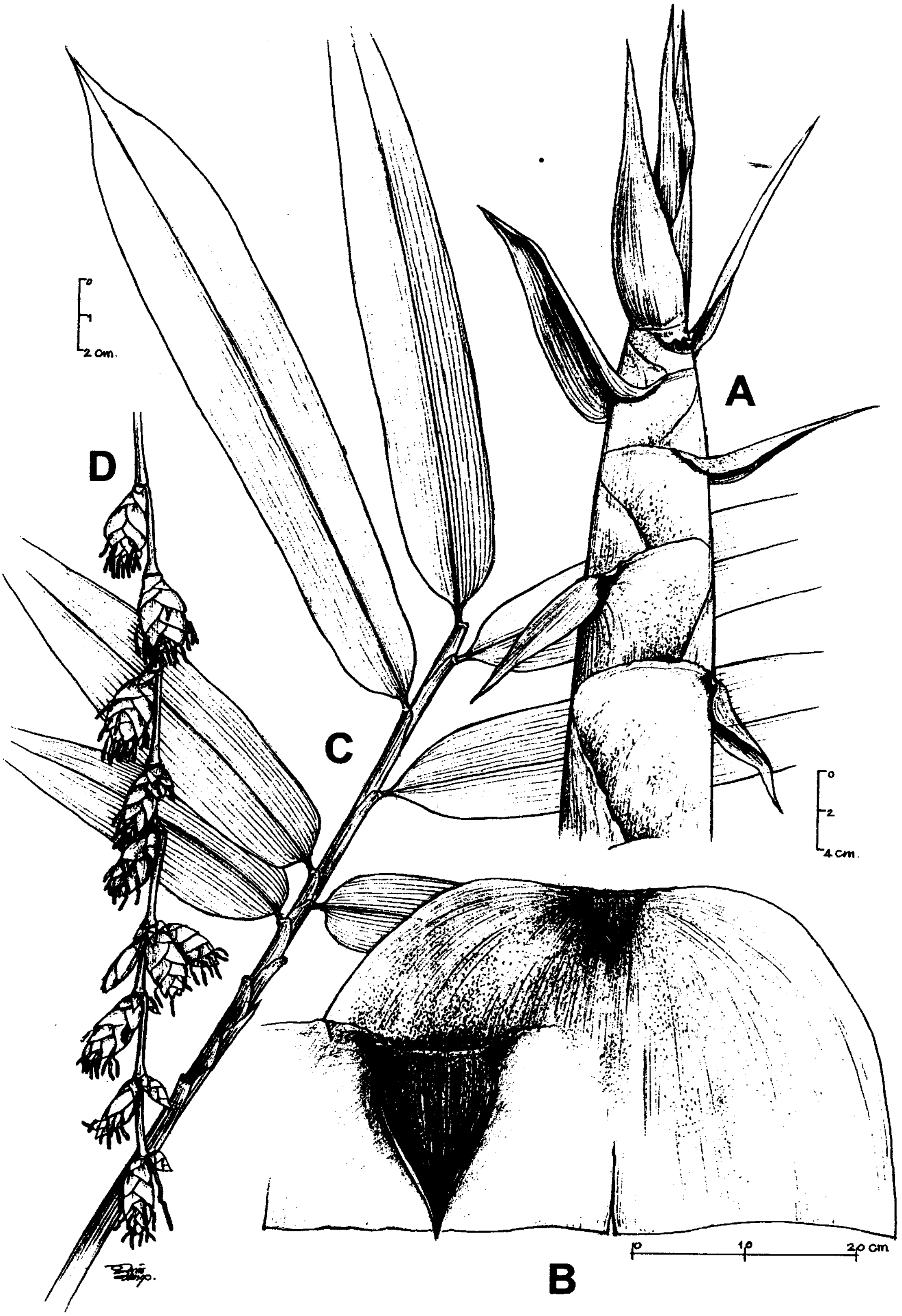


Figure 4.22 *Dendrocalamus latiflorus*. A. flying shoot; B. culm sheath, part of culm sheath showing ligule (front, abaxial view); C. leafy branchlet; D. flowering branch, showing stellate pseudospikelet clusters. A-C, from *Sungkaew & Teerawatananon* 113; D, from *Sungkaew & Teerawatananon* s.n.. All drawn by Pairach Payangkul.

Figure 4.23 *Dendrocalamus latiflorus.* Top left, habit of a young clump, showing two young shoots and a flying shoot (far right), from Samoeng District, Chiang Mai Province, northern Thailand (cultivated); Top right, a flying shoot (from Ang Khang Royal Project, Chiang Mai Province, cultivated); Bottom, a flowering branch, showing reddish purple spikelets (from Muang District, Chiang Mai Province, cultivated).

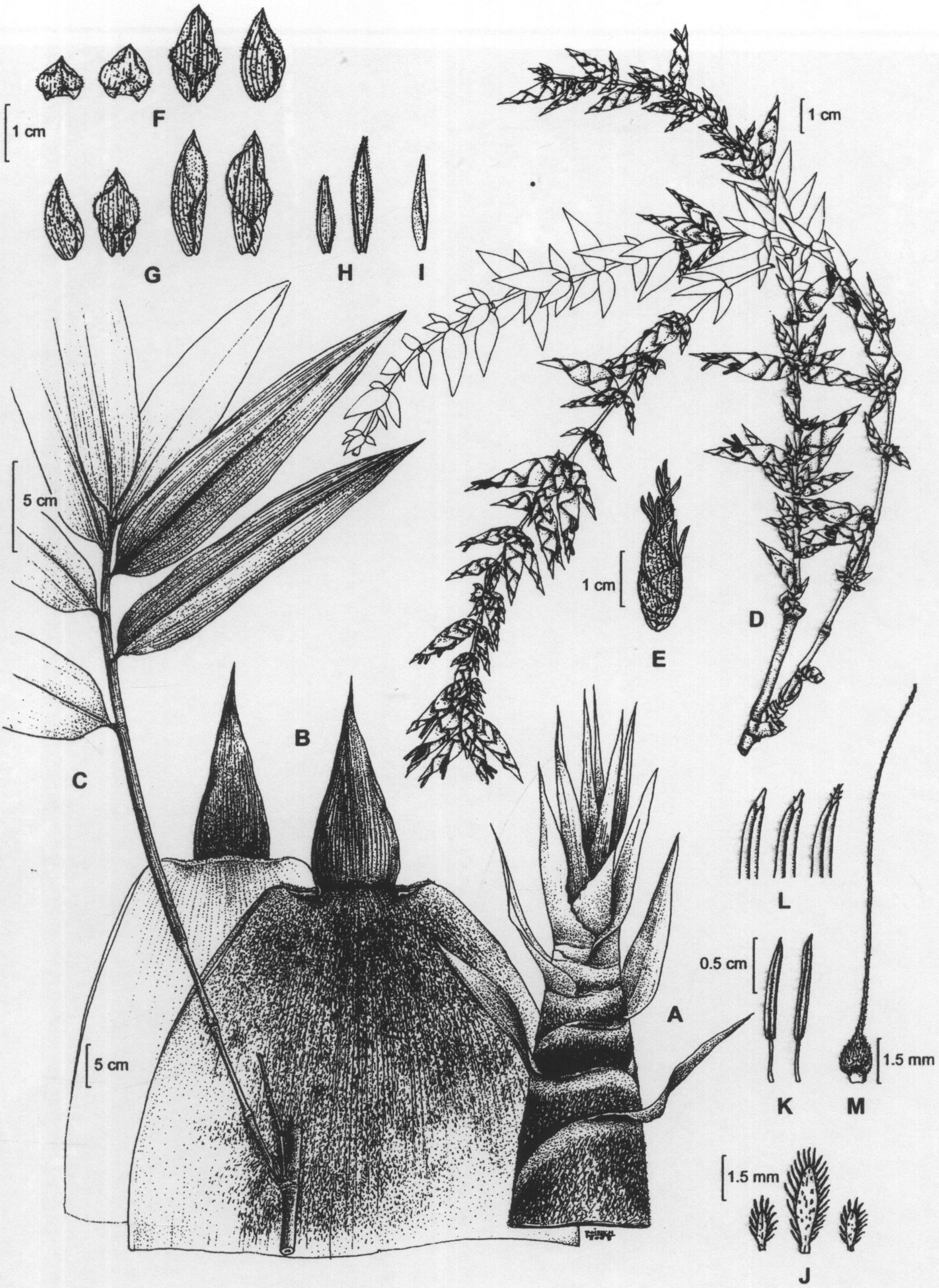


Figure 4.24 *Dendrocalamus copelandii*. A. top of flying shoot, showing auricles and covering on the back of the sheaths; B. culm sheaths, showing auricles (front, abaxial view) and ligule (back, adaxial view); C. leafy branch; D. flowering branch; E. spikelet; F. glumes; G. lemmas; H. paleas; I. empty lemma representing terminal vestigial floret; J. lodicules; K. stamens; L. variation of anther tips; M. pistil. A, from *Sungkaew & Teerawatananon* 29; B and C, from *Sungkaew & Teerawatananon* 20; D-M, from *Raizada* s.n.. A-C, drawn by Pairach Payangkul; D-M, drawn by Atchara Teerawatananon.

Figure 4.25 *Dendrocalamus copelandii*. A flying shoot (from Thong Pha Phum District, Kanchanaburi Province, southwestern Thailand).

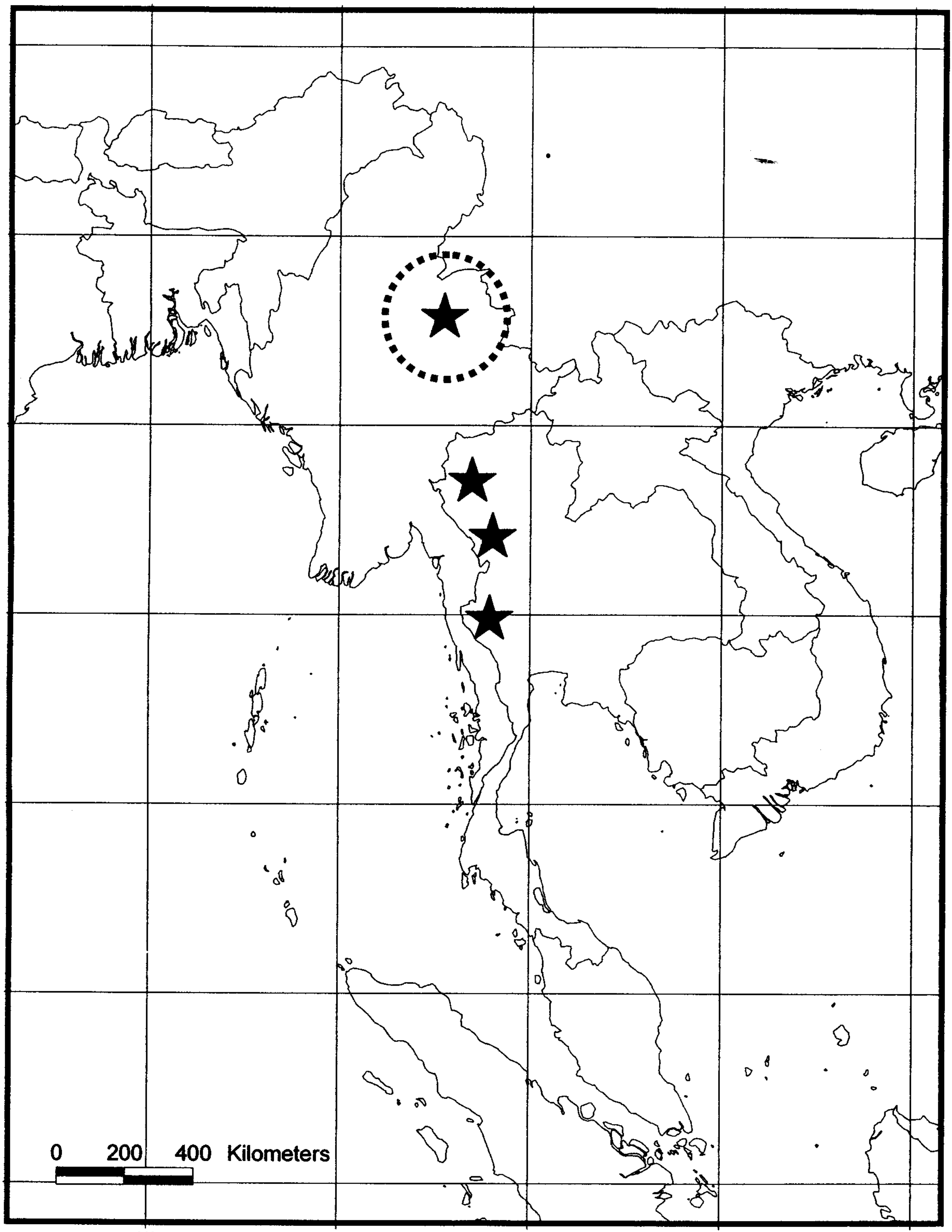


Figure 4.26 Distribution map of *D. copelandii.* The stars represent the distribution as obtained from specimen examination; the dashed loop represents the distribution as obtained from Ohrnberger (1999), but it is uncertain whether or not this species was native in that area.

Figure 4.27 *Dendrocalamus sinicus*. Top, clump habit, showing the tardily deciduous culm sheaths and showing the hairy lower culm-internodes (top right), from Mae Jam District, Chiang Mai Province, northern Thailand (cultivated); Bottom, a flowering branch, showing stellate pseudospikelet clusters (synflorescences), from Xishuanbanna Tropical Garden, Yunnan, China (cultivated).

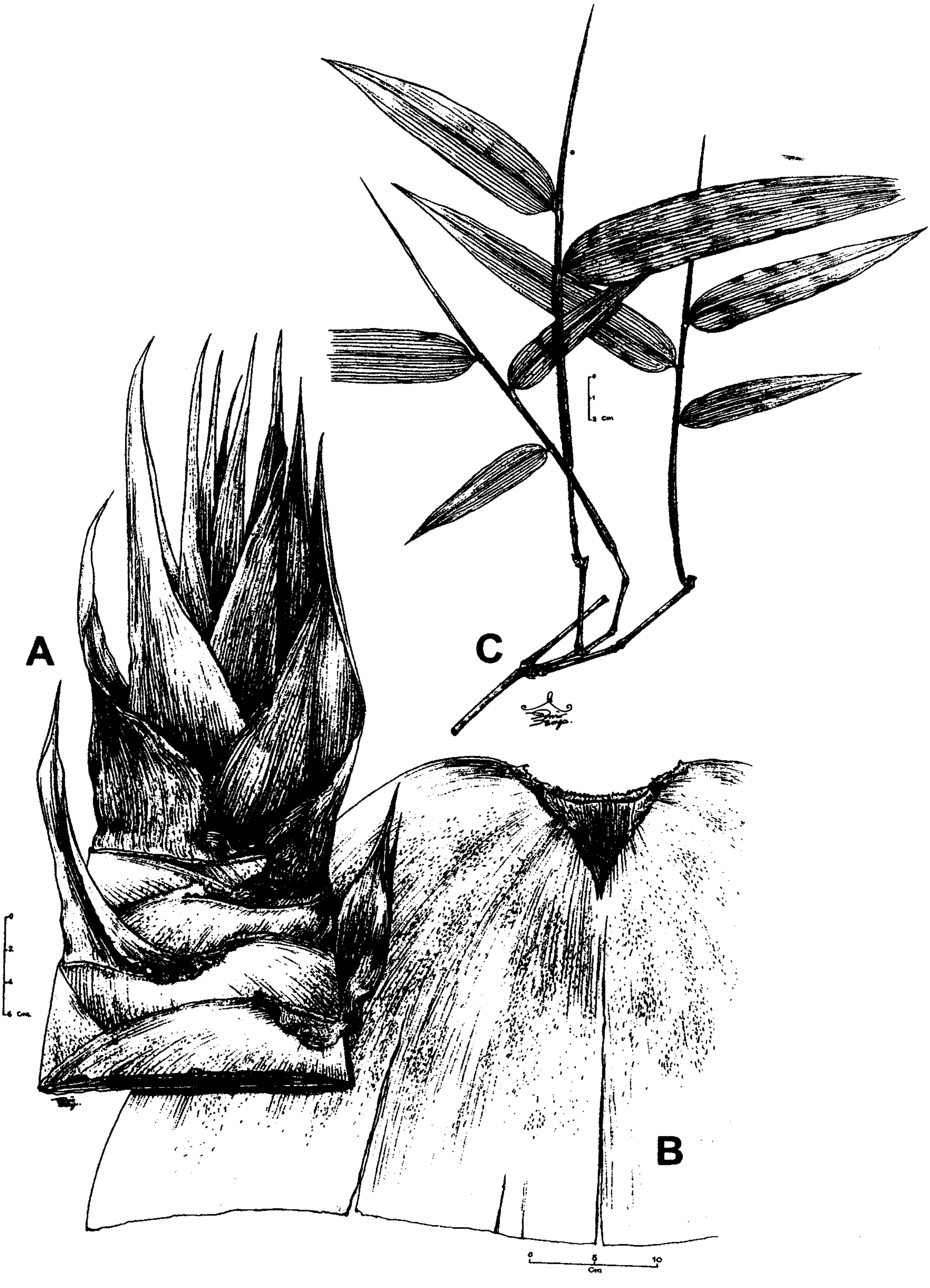


Figure 4.28 *Dendrocalamus giganteus*. A. top of flying shoot, showing auricles; B. culm sheath (abaxial view), showing auricles, ligule, and covering on the back; C. leafy branch. All from *Sungkaew & Teerawatananon* 157. All drawn by Pairach Payangkul.

Figure 4.29 *Dendrocalamus giganteus*. Top, a top of flying shoot, showing auricles; Bottom, clump habit, showing muture culms and flying shoots (compared to the size of two (quite) big people). All from Queen Sirikit Botanic Garden, Chiang Mai Province, northern Thailand.

4.3 Discussion

4.3.1 *Dendrocalamus* species found in Thailand

Thirteen species of *Dendrocalamus* have been recorded in Thailand namely, *D. asper*, *D. barbatus*, *D. brandisii*, *D. copelandii*, *D. dumosus*, *D. giganteus*, *D. hamiltonii*, *D. khoonmengii*, *D. latiflorus*, *D. membranaceus*, *D. pendulus*, *D. sinicus*, and *D. strictus*. A comparison of the number of species recorded in previous works (Lin, 1968; Dransfield, 1994; Smitinand, 2001) and the current study has been made (Table 4.2). The distributions of each species are also given in Table 4.2.

Two species that were previously reported to occur in Thailand, *D. sericeus* Munro (Dransfield, 1994; Smitinand, 2001) and *D. longispathus* (Kurz) Kurz (Lin, 1968; Smitinand, 2001), were not found in this study. I have examined the type specimens of both species, both housed in **K** (*D. sericeus*, Bengal, Parasnath, alt. 4,000 feet, fertile, *Thomson* s.n.; *D. longispathus*, Burma, Pegu, fertile, *Kurz* s.n.). However, none of specimens collected from Thailand correspond to authentic *D. sericeus* or *D. longispathus*. Some specimens of other species have sometimes been misidentified as one of these two species. The specimen *Smitinand* 2120 (**BKF**) of *D. membranaceus* which was collected on 15 December 1954, was misidentified by Bor (24 October 1955) as *D. sericeus*. *Dendrocalamus sericeus* has been reduced to varietal rank and is now recognized as *D. strictus* var. *sericeus* (Munro) Brandis (Ohrnberger, 1999). The specimens of *D. asper* (e.g. *Maxwell* 74-958 (**AAU**, **BK**, **L**)) and *D. brandisii* (e.g. *Maxwell* 76-24 (**AAU**, **BK**)) were misindentified as *D. longispathus*.

A species reported as *Dendrocalamus* sp. by Dransfield (1994) has no specimen cited. However, it is possible that it could be any of the 13 species reported in this study.

Dendrocalamus copelandii, one of the giant bamboo species, is a new record for Thailand. It is usually confined to mixed deciduous or dry evergreen forest of limestone mountains in northern and southwestern Thailand. It was previously unrecorded possibly because of two reasons. Firstly, it looks vegetatively similar to the other two species, *D. giganteus* and *D. sinicus* and secondly because no flowers were available. Fertile specimens of this species were collected only once in Thailand, in 1979 (*Shimizu et al.* T-21752). Surprisingly, I have not seen this collection housed anywhere in Thai herbaria I visited. The species description of the reproductive parts given in this study was based mainly on the collection of *Shimizu et al.* T-21752 housed at **L** and those descriptions of Raizada (1948) and Clayton *et al.* (2006 onwards). Reproductively, *D. copelandii* differs from *D. giganteus* in having longer spikelets, membranous or sub-chartaceous paleas, and in having no verticil of roots at the lower culm nodes.

Table 4.2 Comparative table of the species numbers recorded in previous works and the current study, and the distribution of *Dendrocalamus* species occurring in Thailand

Species	Lin (1968)	Dransfield (1994)	Smitinand (2001)	This study	Distribution								
					native							exotic	uncertain
					C	E	N	NE	P	SE	SW		
1. ***D. dumosus***	-	✓	-	✓					✓^{2}				
2. ***D. khoonmengii***	-	-	-	✓, new species					✓^{2}				
3. ***D. pendulus***	-	✓	-	✓					✓^{2}				
4. ***D. strictus***	✓	✓	✓	✓			✓^{1}				?		
5. ***D. barbatus***	-		-	✓								✓	
6. ***D. membranaceus***	✓	✓	under *Bambusa*	✓		✓^{1}	✓^{1}	✓^{1}			✓^{1}		
7. ***D. asper***	✓	✓	✓	✓									✓
8. ***D. brandisii***	✓	✓	✓	✓		✓^{1}	✓^{1}	✓^{1}		✓^{1}	✓^{1}		
9. ***D. hamiltonii***	✓	✓	✓	✓			✓^{1}						
10. ***D. latiflorus***	✓	✓	✓	✓								✓	
11. ***D. copelandii***	-	-	-	✓, new record			✓^{1}				✓^{1}		
12. ***D. sinicus***	-		-	✓								✓	
13. ***D. giganteus***	✓	✓	✓	✓							?		✓
14. *D. elegans*	-	✓	-	syn. of *D. dumosus*									
15. *D. longifimbriatus*	-	✓	-	syn. of *D. membranaceus*									
16. *D. nudus*	-	✓		syn. of *D. brandisii*									
17. *D. sericeus*	-	✓	✓	-									
18. *D. longispathus*	✓	-	✓	-									
19. *Dendrocalamus* sp.	-	✓	-	-									

Remarks: Abbreviations; C=Central; E=Eastern; N=Northern; NE=Northeastern; P=Peninsular; SE=Southeastern; SW=Southwestern; syn.=synonym; ?=may be found in the wild in that area; ^{1}=species grows above Kra Isthmus; ^{2}=species grows below Kra Isthmus

It differs from *D. sinicus* in having shorter spikelets, paleas with un-nerved inflexed edges, and in having usually glabrous lower internodes.

Dendrocalamus khoonmengii is a new species which is probably endemic in tropical rain forest in Khaoluang National Park, Nakhon Si Thammarat Province, southern Thailand. More fieldwork will be required to evaluate its conservation status, whether it is common, rare, threatened, or endangered. No use of this bamboo has been recorded. Vegetatively, it is similar to *D. dumosus, D. pendulus,* and *D. hirtellus* Ridley. Comparisons of its vegetative morphological characters to other *Dendrocalamus* have already been provided in Table 4.1.

Eight *Dendrocalamus* species are obviously native to Thailand, as they can be found in natural habitats, namely, *D. brandisii, D. copelandii, D. dumosus, D. hamiltonii, D. khoonmengii, D. membranaceus, D. pendulus,* and *D. strictus. Dendrocalamus khoonmengii* is, so far, the only endemic species to Thailand. Three species are introduced to Thailand, *D. barbatus, D. latiflorus,* and *D. sinicus. Dendrocalamus barbatus* is of uncertain origin, but possibly native in Yunnan, as is *D. sinicus* (Ohrnberger, 1999; Li & Stapleton, 2006f). *Dendrocalamus latiflorus* is possibly native in southern China including Taiwan (Ohrnberger, 1999; Li & Stapleton, 2006f). All three species were introduced to Thailand for their culms for construction and young shoots for food. *Dendrocalamus latiflorus,* in particular, is now being promoted as a crop to farmers in northern Thailand by a Royal Project (the project originated from His Majesty the King's private study) mainly for its young shoots (Sungkaew *et al., unpublished*). Its culms can also be used for several purposes such as for housing and temporary constructions, agricultural implements, water pipes, furniture making and woven wares.

The origins of two species, *D. asper* and *D. giganteus,* are not known precisely. *Dendrocalamus asper* has been cultivated throughout tropical Asia for its culms and shoots (Holttum, 1958; Dransfield & Widjaja, 1995; Wong, 1995a; Ohrnberger, 1999). It is believed that it was introduced from either China or Malaysia to Thailand in the 1910's (Ohrnberger, 1999). I have seen at least two possibly wild populations of this species, which is locally called "Pai Chiang Roon", in the tropical rain forest of Klong Panom National Park, Surat Thani Province, southern Thailand. Each population consisted of at least 20 clumps, covering a vast area. However, this protected area was once a site of logging where the camping ground used to be settled. It is possible, therefore, that it may have been planted by man. Thus it is hard to say whether these two populations are wild or naturalized. More investigations on this topic would be useful and challanging.

Dendrocalamus giganteus is believed to be native in Burma and adjacent regions in north-eastern India, southern China and north-western Thailand (Ohrnberger, 1999) and perhaps it also originated somewhere in this broad area. Lin (1968) reported (with the figure shown, but not clear enough to identify) that there was a natural grove of this species at an altitude of 800 m in Lan Sang National Park, Tak Province, northern Thailand. However, almost all specimens examined by me are stated to be collected from cultivations. Therefore, the origin and the native status of this species are still uncertain. More fieldwork is required to confirm this occurrence.

Dendrocalamus copelandii, D. giganteus, and *D. sinicus,* are the biggest bamboos in this genus, and probably in the world. With suitable environmental factors, I believe they can grow up to at least 30 m long and with a culm diameter of 30 cm. All can potentially reach similar hights. In contrast, *Dendrocalamus dumosus* is the smallest bamboo in the genus. It is usually confined to limestone mountains in southern Thailand to Langkawi Island, Kedah, Malay Peninsula. It can grow up to 7 m long, with a culm diameter of 1–5(–6) cm.

Dendrocalamus asper is the only species that has been cultivated throughout Thailand for its culms and shoots. It is planted commercially, especially in Prachin Buri Province, southeastern Thailand. Unfortunately, no up-to-date official annual data on its economics and production are available. In 1984, with production area of 4,465 ha, a shoot production of 37,975 tons was harvested from this province. The price was 2–8 Baht/kg (0.04–0.18 euro/kg), depending on the time of the year (Dransfield & Widjaja, 1995a). *Dendrocalamus brandisii* and *D. hamiltonii* are also used by local people where these species grow in the wild in the same ways as *D. asper* is used. These two species also have the potential to be alternative options for the farmers. *Dendrocalamus giganteus* also has potential for the farmers for its culm and shoot productions (Sungkaew *et al.*, *unpublished*). In general, its bigger culm size and sweeter, juicier and less fibrous shoots than *D. asper*, may make this species more popular. However, it may require higher soil moisture content and more spacing for plantations than *D. asper.*

The widespread and economically important species, *D. membranaceus,* has been transferred to *Bambusa,* as *B. membranacea* by Stapleton and Xia (1997) based on the small leaf-blades and the 2-keeled synflorescence prophylls. However, relying mainly on these two characters may not be enough to seperate *Bambusa* from *Dendrocalamus.* Otherwise other species that possess such characters, *D. barbatus, D. dumosus, D. khoonmengii,* and *D. pendulus,* may have to be transferred to *Bambusa* too. Molecular studies using nuclear ribosomal DNA (ITS) sequences have shown that *D. membranaceus* was sister to *D. strictus* (Sun *et al.*, 2005). Analyses of five plastid DNA regions (*trnL* intron, *trnL-F* intergenic spacer, *atpB-rbcL* intergenic spacer, *rps16* intron, and *matK* gene region (Chapter 2, this thesis; Sungkaew *et al.*, *in prep.*) have also confirmed that *D. membranaceus* should be placed within *Dendrocalamus* rather than *Bambusa*. Stapleton and Xia now also treat this species under *Dendrocalamus* (Li & Stapleton, 2006f; Li *et al.*, 2006b).

4.3.2 Infrageneric classification of Thai *Dendrocalamus*

Using both vegetative and reproductive characters and DNA sequence data (Chapter 3), Thai *Dendrocalamus* can be classified into two
subgenera as follows:

Dendrocalamus subg. *Dendrocalamus*: Pseudospikelets congested as globose clusters; fertile florets usually 1—4(—6). Mid-culm branch complement with 1—3 branches dominant. There are nine species in this subgenus, namely; *D. asper*, *D. barbatus*, *D. brandisii*, *D. dumosus*, *D. hamiltonii*, *D. khoonmengii* (flower unknown), *D. membranaceus*, *D. pendulus*, and *D. strictus*.

Dendrocalamus subg. *Sinocalamus* (McClure) Hsueh & D.Z. Li: Pseudospikelets congested as stellate clusters; fertile florets usually 4—8-flowered. Mid-culm branch complement with more or less subequal branches. Four species can be assigned to this subgenus, *D. copelandii*, *D. giganteus*, *D. latiflorus*, and *D. sinicus*.

This scheme of classification is congruent to that suggested by Li and Stapleton (2006f). However, to use the two vegetative characters adopted by them, habit of culm tips (nodding or penduluous) and basal nodes (with or without branches) to help differentiate subg. *Dendrocalamus* and subg. *Sinocalamus*, repectively, seems arbitrary. This is because different people would have different standards of interpreting the habit of a culm tip. Moreover, *D. hamiltonii*, which usually has drooping or very pendulous culm tips, would be an exceptional species to be included in subg. *Dendrocalamus*. In addition, the basal nodes of many bamboos in subg. *Sinocalamus*, especially from the old clumps, can sometimes be found to produce branches.

4.3.3 Distribution of subgenera and species of *Dendrocalamus*

The natural distribution of almost all native Thai *Dendrocalamus* recorded from this study is congruent to that reported by Ohrnberger (1999). Three species, however, have shown new localities beyond previous reports. *Dendrocalamus dumosus* (Figure 4.3) and *D. elegans* (treated as a synonym of *D. dumosus* in this study) have been reported to be found growing wild only in Rawi Island, Tarutao National Park, southern Thailand (where the type of *D. dumosus* was collected) and Langkawi Island, Kedah, Malay Peninsular (near Rawi Island) (Ridley, 1912, 1916; Holttum, 1958; Ohrnberger, 1999; Wong, 1995a). In this study, *D. dumosus* has been found further north up to Tha Tako Subdistrict, Langsuan District, Chumphon Province, southern Thailand.

Dendrocalamus pendulus was first reported to occur in Thailand by Dransfield (1994). It was previously known mainly from the foothills of the Main Range, Malay Peninsular (Ridley, 1905; Holttum, 1958; Ohrnberger, 1999; Wong, 1995a). However, we now know that it extends to southernmost Thailand as well (Figure 4.3).

Unfortunately, I could not make fieldtrips to the three provinces in the farthest south (Pattani, Yala, and Narathiwat) of Thailand due to continuing political problems in these areas. This means that it may still be possible to find some Malaysian species especially the species that grow in bordering areas (Kedah, Perak, Kelantan, and Terengganu) such as *D. hirtellus* Ridl. or *D. sinuatus* (Gamble) Holttum (Wong, 1995a).

It is not precisely known whether *D. copelandii* is native to Burma or not (Raizada, 1948; Xia & Stapleton 1997a; Ohrnberger, 1999). However as it is a new bamboo record for Thailand, this species has been found growing wild in the limestone mountains from northern to southwestern Thailand (Figure 4.26).

It seems that the distributions of the native Thai *Dendrocalamus* species are limited by a well-known biogeographical boundary. They can be geographically divided into two groups, the species that grow north and those that grow south of the 'Kra Isthmus' (see Figure 4.30).

The Kra Isthmus is located at about latitude 10° 11' 0" N and longitude 98° 53' 0" E. It is the narrow landbridge which connects the Malay Peninsula with the mainland of Asia (Chaimanee, 1997; Pooma, 2003; Woodruff, 2003; Hughes *et al.*, 2003). The east part of the landbridge belongs to Thailand and the west part belongs to the Tanintharyi division of Burma. To the west of the Isthmus is the Andaman Sea and to the east is the Gulf of Thailand. The narrowest part between the estuary of the Kra River (in Kra Buri District, Ranong Province) and the bay of Sawi (Sawi District, Chumphon Province) has a width of 44 km, and has a maximum altitude of 75 m above sea level. The Isthmus is named after the Kra Buri District which is located at the west side of the narrowest part. The Isthmus of Kra marks the boundary between two parts of the central cordillera, the mountain chain which runs from Tibet through all of the Malay peninsula. The southern part is called the Phuket chain, the northern part is the Tenasserim chain, which continues for 400 km until the Three Pagodas Pass (='Dan Jedi Sam Ong', in Sungkhla Buri District, Kanchanaburi Province, southwestern Thailand).

Five species are found growing north of the Kra Isthmus namely *D. brandisii, D. copelandii, D. hamiltonii, D. membranaceus,* and *D. strictus.* Three species are found below Kra Isthmus are *D. dumosus, D. khoonmengii,* and *D. pendulus.*

In general, this hypothesis can also be applied to all *Dendrocalamus* species (*i.e.* including those not found in Thailand), see the map in Figure 4.30. This map is constructed by gathering the distribution information from Ohrnberger (1999), Wong (1995a), Li and Stapleton (2006f), and from this study.

Dendrocalamus subg. *Dendrocalamus* (possessing the mid-culm branch complement having 1—3 branches dominant; globose pseudospikelet clusters that are spiny to the touch; spikelets yellow-green, sub-terete), has a distribution boundary more or less the same to the distribution of the whole genus, this is from southern China (excluding Taiwan), to India, Nepal, Bhutan, Bangladesh, Sri Lanka, Burma, Thailand, Laos, Cambodia, Vietnam, and Malaysia and to Papua New Guinea (the green loop, Figure 4.30). *Dendrocalamus strictus* and *D. membranaceus* have wide ranging distributions (see Figure 4.11 and Figure 4.15, repectively) but never grow further south than the Kra Isthmus. It may be further hypothesized that species that usually have only 1—2 fertile florets/spikelet rarely grow further north than the Kra Isthmus. Species so confined are, for example, *D. dumosus* and *D. pendulus* from this study, *D. cinctus* R.B. Majumdar ex Soderstr. & R.P. Ellis from Sri Lanka (endemic) (Soderstrom & Ellis, 1988), *D. hirtellus* from Peninsular Malaysia (Wong, 1995a), and *D. buar* Widjaja from Indonesia (Widjaja, 1997). However, there are the exceptions, for example, a well known species, *D. sinuatus* from Peninsular Malaysia, has 2—3 fertile florets/spikelet (Wong, 1995a) and a poorly known species, *D. poilanei* A. Camus from Vietnam, has 2 fertile florets/spikelet (Camus, 1925). Wong (1995a) reported that *D. longispathus* was newly recorded in Peninsular Malaysia but its flowers were not known. If this record is correct, then, it will be an exception for the bamboos in this group in that it grows south of the Kra Isthmus. *Dendrocalamus longispathus* can be recognized as a member of subg. *Dendrocalamus* possessing globose pseudospikelet clusters that are not spiny to the touch. However, I think there is no *D. longispathus* naturally growing further south than the Kra Isthmus.

Dendrocalamus subg. *Sinocalamus* (with mid-culm branch complement having more or less subequal branches; stellate pseudospikelet clusters, can be found from southern China, to N.E. India, Nepal, Bhutan, Bangladesh, Burma, upper parts of Thailand (above the Kra Isthmus), Laos, Cambodia, Vietnam, and to Taiwan (above the red dashed line, Figure 4.30). *Dendrocalamus copelandii*, the representative of subg. *Sinocalamus*, is probably the only species that grows southwards down to Thong Pha Phum District, Kanchanaburi Province, southwestern Thailand. It is the southernmost species of this subgenus.

In Thailand, the trend of a species gradient above/below the Kra Isthmus can be seen in some other plant groups, epecially in the family Dipterocarpaceae (Pooma, 2003; *personal observation*). The dominant species in the top layer of the forests that are found further north beyond the Kra Isthmus tend to have less species diversity and are dominated by the 'large-leaf dipterocarps' of the genus *Dipterocarpus* while those found growing further south beyond the Kra Isthmus tend to have more diversity and are dominated by the 'small-leaf dipterocarps' from several genera, viz. *Cotylelobium, Hopea, Neobalanocarpus, Shorea,* and *Parashorea.* A rather widely distributed species of Horse-chestnut, *Aesculus assamica* Griff. (Hippocastanaceae), generally considered to occur from Sikkim to southern China and northern Vietnam (Turland & Xia, 2005), can actually be found southwards to at least Ban Lin Thin, Thong Pha Phum District, Kanchanaburi Province, southwestern Thailand, which is not so far above where the Kra Isthmus is located (*personal observation*). Takhtajan (1986), in his biogeographical scheme, placed Thailand in the Indochinese region comprising South Burmese, Andamanese, South Chinese, North Indochinese, Annamese, South Indochinese, and Thailandian provinces. From this study and that of other previous works, the Kra Isthmus is actually acting not only as the phytogeographical transition between 'Indochinese' and 'Sundaic' provincial floras (van Steenis, 1950; Whitmore, 1984; Ashton, 1992; Pooma, 2003) but also as the zoogeographical transition between Indochinese and Sundaic faunas (Chaimanee, 1997; Hughes *et al.*, 2003; Woodruff, 2003).

4.3.4 Conclusions

This chapter has provided a comprehensive and up to date floristic treatment of Thai *Dendrocalamus* species. It has provided keys to species, detailed descriptions and synonymy information, the specimens examined according to the species, illustrations, and distribution maps. The work will be modified to fit the publishing format for the Flora of Thailand project and submitted for publication. It will hopefully provide an invaluable resource for taxonomists and biologists who wish to identify and study Thai *Dendrocalamus*.

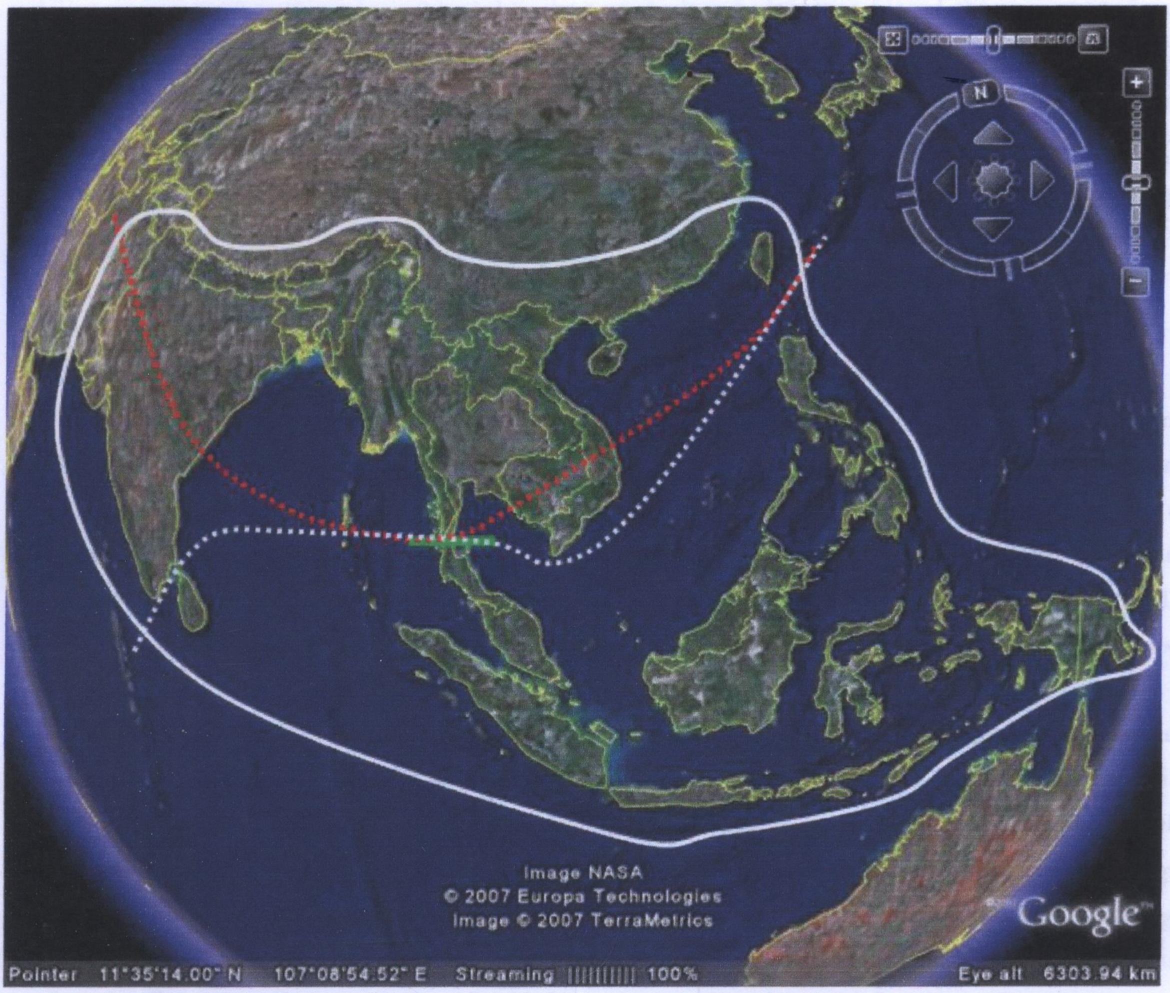


Figure 4.30 Distribution map of *Dendrocalamus.* The white loop represents the distribution of the genus, and also represents the distribution of subg. *Dendrocalamus* possessing pseudospikelets that are spiny to the touch; spikelets yellow-green, sub-terete. The red dashed line represents the lowermost distribution of subg. *Sinocalamus.* The white dashed line represents the lowermost distribution of subg. *Dendrocalamus* possessing pseudospikelets that not spiny to the touch; spikelets yellow-brown, reddish purple or dark purple, occasionally yellow-green (sometimes found in *D. brandisii*), laterally compressed. The green bar represents the location of the Kra Isthmus. The background map is taken from http://earth.google.com.

MANUSCRIPT 4.1

[Accepted for publication in the Thai Forest Bulletin (Botany)]

Dendrocalamus copelandii, a new giant bamboo record for Thailand

Sarawood Sungkaew*,**, Atchara Teerawatananon*, Weerapong Korawat**, and Trevor R. Hodkinson*

ABTRACT. *Dendrocalamus copelandii* (Gamble ex Brandis) N.H. Xia & Stapleton is newly recorded for Thailand. A species description and an illustration are provided.

INTRODUCTION

The genus *Dendrocalamus* Nees (Bambuseae: Poaceae) comprises about 50 species and is distributed from southern China to India, Burma, Thailand, Indo-China, and Malaysia through Papua New Guinea (Ohrnberger, 1999). Eight species have been reported to occur in Thailand (Smitinand, 2001). During revision of this genus for the Flora of Thailand project, sterile specimens of *D. copelandii* from Kanchanaburi, Mae Hong Son, and Tak provinces were collected by the authors. A fertile specimen, also from Kanchanaburi, was collected by Tatemi Shimizu and his colleagues in 1917. Those sterile and fertile specimens matched the lectotype and other specimens at Kew. This allowed us to record this species for the first time for Thailand.

Dendrocalamus copelandii (Gamble ex Brandis) N.H. Xia & Stapleton, Kew Bull. 52(2): 484. 1997. Type: India, Dehra Dun, *Gamble* 27166 (lectotype, K!, selected by N.H. Xia & Stapleton).— *Bambusa copelandii* Gamble ex Brandis, Indian Trees: 671. 1906, as "*copelandi*".— *Sinocalamus copelandii* (Gamble ex Brandis) Raizada, Indian Forester 74(1): 10. *pl.* 1. 1948, as "*copelandi*".— *Dendrocalamopsis copelandii* (Gamble ex Brandis) Keng f., J. Bamboo Res. 2(1): 12. 1983, as "*copelandi*". Fig. 1.

Sympodial bamboo. *Culms* straight and erect, about 15–30 m long, 8–20 cm in diameter, walls relatively thin, about 1–2 cm thick, culm tips more or less arching; internodes 20–48 cm long, lower ones without hairs, covered with copious white wax when young; nodes not swollen, lower ones without verticils of roots. *Branches* several at each node, the primary one dominant, without aerial roots. *Culm-sheaths* tardily deciduous to persistent, coriaceous, 28–46.5 cm long by 42–55 cm wide, top convexly horizontal, covered with golden brown to dark brown hairs, occasionally glabrous; blades lanceolate, erect to spreading, 10–25.5 cm long, about 5 cm wide near the base, hairy near the adaxial base; auricles continuing from the base of culm-sheath blade, lobes fleshy crisped, 2–5 mm tall, 10–40 mm long, margin wavy or pleated, glabrous; ligule 3–10 mm high, margin dentate to sub-entire, fringed with cilia up to 0.5 mm long. *Leaf-blades* 10–27 cm long by 2.4–4 cm wide, hairy underneath, apex acuminate, base rounded to acute, pseudo-petiole 2–10 mm long; auricles absent; ligule 1–1.2 mm high, margin dentate, fringed with cilia 0.1–0.2 mm long. *Inflorescences* iterauctant, borne on leafless branches, spikelets congested as stellate clusters subtended by 1-keeled prophyll and 1-keeled matching bract, axis hairy, internodes 1.5–2.5 cm long.

---

*Department of Botany, School of Natural Sciences, Trinity College Dublin, University of Dublin, Dublin 2, Ireland.

**Department of Forest Biology, Faculty of Forestry, Kasetsart University, Bangkok, Thailand.

Pseudospikelets ovate-oblong to ovate-lanceolate, laterally compressed, 20–28 mm long by 5.5–7.3 mm wide; transitional (empty) glumes 2–9, shorter than the lowest lemma, 7–35-nerved; fertile florets 5–6, terminal vestigial floret present, shorter than florets; rachilla internodes between florets short, not disarticulating below each floret; lemmas chartaceous, 12–25 mm long, minutely pubescent on the back, 23–37-nerved, apex acute to pointed; paleas membranous, 9.2–22 mm long, 2-keeled on the back, keels and edges long-fringed, the uppermost one not keeled, outside minutely pubescent, inside sparsely and minutely pubescent to glabrous, apex shortly bidentate, 2–3(–5)-nerved between keels, edges very narrow, not nerved; lodicules usually 3, occasionally none, hyaline, pubescent; stamens 6, filaments free, anthers 5–11 mm long, yellow, tips pointed, usually with minute spines; ovary umbonate, summit hairy, with long slender style, stigma 1, slightly plumose. *Caryopsis* 12–16 mm long, contracted towards the apex, almost bottled-shaped, slightly grooved on one side; pericarp rather thick and loose.

Thailand.— NORTHERN: Mae Hong Son (Pang Ma Pa District), Tak (Tha Song Yang District); SOUTHWESTERN: Kanchanaburi.

Distribution.— Upper Burma.

Ecology.— Confined to limestone regions and vegetation.

Vernacular.— Pai Man Moo (ไผ่มันหมู).

Specimens examined.

Thailand.— NORTHERN: Mae Hong Son [Pang Ma Pa District, Mae Um Ong-Nam Rin Forest Station, sterile, 25 April 2005, *Sungkaew & Teerawatananon* 527 (Herbarium of Faculty of Forestry, Kasetsart University, Thailand, TCD, Herbarium of Thailand Natural History Museum, National Science Museum)]; Tak [Tha Song Yang District, Ban Nam Ok Roo, sterile, 4 May 2005, *Sungkaew* & *Teerawatananon* 570 (Herbarium of Faculty of Forestry, Kasetsart University, Thailand, TCD, Herbarium of Thailand Natural History Museum, National Science Museum)]; SOUTHWESTERN: Kanchanaburi [Thong Pha Pum District, Sunyataram Temple, alt. ca 500 m, sterile, 22 July 2004, *Sungkaew & Teerawatananon* 20, 25, and 29 (Herbarium of Faculty of Forestry, Kasetsart University, Thailand, TCD, Herbarium of Thailand Natural History Museum, National Science Museum); Sai Yok District, near Sai Yok Arboretum, 95 km northwest of Kanchanaburi, alt. 160 m, fertile, 6 Nov. 1979, *Shimizu et al.* T-21752 (L)].

Burma: Northern Shan States [cult.?, fertile, May 1896, *Copeland* s.n. (K)].

India: Dehra Dun [cult., Kuanli Garden, fertile, March 1944, *Raizada* s.n. (K); cult., alt. 700 m (2200ft), sterile, Sept. 1898, *Gamble* 27166 (K); cult., Forest School Garden, sterile, June 1898, *Gamble* s.n. (K)].

Notes.— This species is similar to *Dendrocalamus giganteus* Munro but differs in having no verticil of roots at lower culm-nodes and membranous paleas with much narrower edges.

ACKNOWLEDGEMENTS

This work was supported by the TRF/BIOTEC Special Program for Biodiversity Research and Training grant T_147003, the Trinity College, Dublin, Ireland Postgraduate Studentship, and the Trinity College Postgraduate Travel Reimbursement Fund.

REFERENCES

Ohrnberger, D. 1999. The Bamboos of the World: annotated nomenclature and literature of the species and the higher and lower taxa. Elsevier Science B.V., Amsterdam, The Netherlands. 585 pp.

Smitinand, T. 2001. Thai plant names (revised edition). The Forest Herbarium, Royal Forest Department. Bangkok, Thailand. 812 pp.

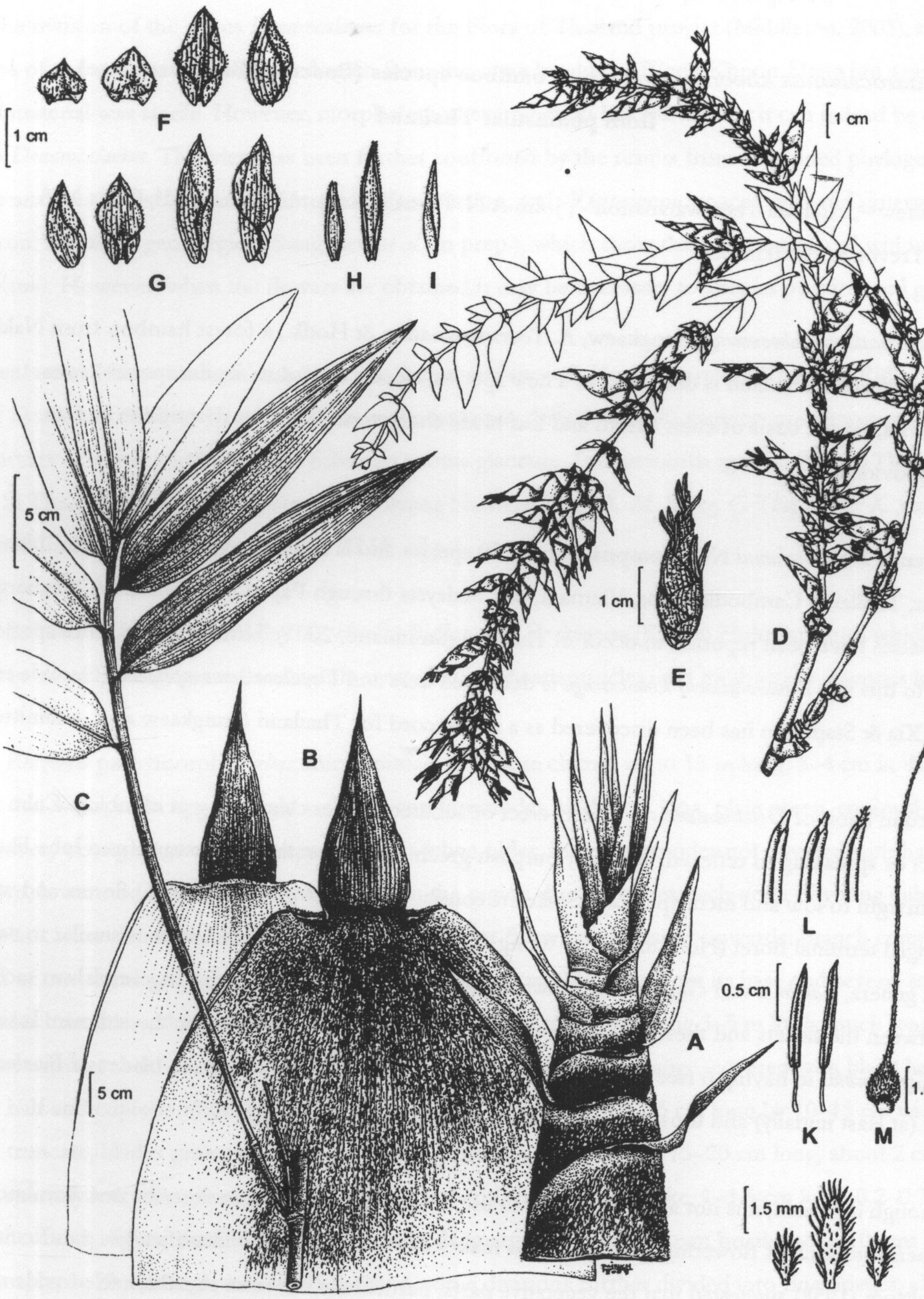


Figure 1. *Dendrocalamus copelandii* (Gamble ex Brandis) N.H. Xia & Stapleton: A. culm shoot; B. culm sheaths, showing auricles on dorsal view (right) and ligule on ventral view (left); C. leafy branch; D. flowering branch; E. spikelet; F. empty glumes; G. lemmas; H. paleas; I. empty lemma representing terminal vestigial floret; J. lodicules; K. stamens; L. variation of anther tips; M. pistil. A from *Sungkaew & Teerawatananon* 29; B, C from *Sungkaew & Teerawatananon* 20; D-M from *Raizada* s.n.. A-C drawn by Pairach Payangkul; D-M drawn by Atchara Teerawatananon.

MANUSCRIPT 4.2

[Accepted for publication in the Thai Forest Bulletin (Botany)]

Dendrocalamus khoonmengii, a new bamboo species (Poaceae: Bambusoideae) from peninsular Thailand

Sarawood Sungkaew[1,2], Atchara Teerawatananon[1,3], John A.N. Parnell[1], Soejatmi Dransfield[4], Chris M.A. Stapleton[4], and Trevor R. Hodkinson[1]

ABTRACT. *Dendrocalamus khoonmengii* Sungkaew, A. Teerawatananon & Hodk., a forest bamboo from Nakhon Si Thammarat, peninsular Thailand is described as a new species, closely related to similar species from peninsular Malaysia, on the basis of culm sheath and leaf blade characters. A species description and an illustration are provided.

The genus *Dendrocalamus* Nees comprises about 50 species and is distributed from southern China to India, Myanmar, Thailand, Cambodia, Laos, Vietnam, and Malaysia through Papua New Guinea (Ohrnberger, 1999). Eight species have been reported to occur in Thailand (Smitinand, 2001). However, two more species can now be added to this list. *Dendrocalamus khoonmengii* is described here and *Dendrocalamus copelandii* (Gamble ex Brandis) N.H. Xia & Stapleton has been discovered as a new record for Thailand (Sungkaew *et al.*, submitted).

The culm habit of *Dendrocalamus* is usually erect or suberect, never clambering or climbing. Culm sheath blades are erect or spreading to reflexed, generally purplish-green. Culm sheath auricles vary from lobe-like and bristly on the margin to low and inconspicuous. Spikelets consist of (1–)2–4 glumes, 1–3(–6) florets and with or without a vestigial terminal floret (Holttum, 1958; Wong, 1995). Superficially, *Dendrocalamus* is similar to two closely related genera, *Bambusa* and *Gigantochloa*. However, it differs from *Bambusa* in having very short rachilla internodes between the florets and these rachilla internodes are not disarticulating below the lemmae. It simply differs from *Gigantochloa* in having 6 free filament stamens. Furthermore, the culm sheath blades of *Bambusa* are typically erect (at least initially) and those of *Gigantochloa* are always green (Wong, 1995).

Although desirable, it is not always possible to use flowering specimens to describe new bamboo species. Furthermore, lack of flowering specimens also makes day to day bamboo identification a difficult task. However, Holttum (1958) suggested that the vegetative parts, particularly the culm sheaths, can provide many diagnostic characters to identify bamboos and there are numerous examples where this is the case. For example, 18 Indonesian bamboo species have been described without access to flowering material (Widjaja, 1997).

---

[1] Department of Botany, School of Natural Sciences, Trinity College Dublin, University of Dublin, Dublin 2, Ireland.

[2] Department of Forest Biology, Faculty of Forestry, Kasetsart University, Bangkok, Thailand.

[3] Thailand Natural History Museum, National Science Museum, Techno polis, Pathum Thani, Thailand.

[4] The Herbarium, Royal Botanic Gardens, Kew, Richmond, Surrey, TW9 3AB, England.

In 2000, Dr Wong Khoon Meng and his colleagues collected perfect sterile specimens of a bamboo from Nakhon Si Thammarat, southern Thailand. They labelled the specimens as '*Dendrocalamus* sp. nov. ined'. During the revision of the genus *Dendrocalamus* for the Flora of Thailand project (Middleton, 2003), the specimens of this species were collected again from the same locality as Wong Khoon Meng but again all collected material was sterile. However, morphological examination has shown that it can indeed be correctly placed in *Dendrocalamus*. This view has been further confirmed by the results from combined phylogenetic sequence analyses of five plastid DNA regions (*trnL* intron, *trnL-F* intergenic spacer, *atpB-rbcL* intergenic spacer, *rps16* intron, and *matK* gene region; Sungkaew *et al.*, in prep.), which show that it is best placed within *Dendrocalamus*. However, when the flowers are obtained it may be necessary to alter its position and genus.

Dendrocalamus khoonmengii Sungkaew, A. Teerawatananon & Hodk. sp. nov. *D. pendulus* Ridley, *D. hirtellus* Ridley et *D. dumosus* (Ridley) Holttum affinis, a qua imprimis differt auriculis vaginorum culmorum projectis, leviter curvata et leviter tortilis; laminis foliorum subtus glaucum. Inflorescentia ignota. Typus: Thailand, Nakhon Si Thammarat, Lansaka District, Khaoluang National Park, *K. M. Wong, C. Thapyai, & K. Roisungnern* WKM 2868 (holotypus, BKF!; isotypi, K, KLU). Fig. 1.

Related to *D. pendulus* Ridley, *D. hirtellus* Ridley and *D. dumosus* (Ridley) Holttum, from which it differs primarily in its projecting, curved and lightly twisted culm sheath auricles, and its abaxially glaucous leaf blades.

Rhizomes pachymorph. *Culms* unicaespitose, in a loose clump, up to 13 m long, 3–4 cm in diameter, with relatively thin walls, culm tips strongly arching over; internodes 35–50 cm long, plain green, copiously white-waxy all over in very young stages, glabrous and waxless when older, lower internodes not covered with hairs; nodes not swollen, lower nodes without verticils of roots, the region directly below each node of young culm with a band of black hairs. *Branches* developing from mid-culm or from lower quarter upwards; branch complement at mid-culm of one dominant primary axis with two secondary, weaker axes from its base and several smaller higher-order branchlets, the dominant primary axis long and slender, reaching 3–5 m long, much longer than other axes; without aerial roots. *Culm-sheaths* pale pinkish to greenish to orange, covered with black hairs mainly on lower half and copious white wax all over, deciduous, coriaceous, 15–25 cm long by 10–15 cm wide, top convexly truncate; blades green, narrowly lanceolate, spreading to reflexed, 15–20 cm long, about 2 cm wide near the base, sparsely hairy near the adaxial base; auricles purplish-black, lobe-like, 1–1.5 cm long, 0.2–0.7 cm wide, somewhat curved, slightly twisted, and free at the ends, margin with pale brown bristles to 7–10 mm long; ligule pinkish purple, 3–12 mm high, irregularly toothed, some divisions further divided into fine bristles, about 5 mm long. *Leaves* 5–10 per branchlet; leaf blades narrow, 5–12 cm long by 0.4–1.2 cm wide, upper surface glabrous to sparsely hairy, lower surface glaucous and covered with short and fine hairs and scattered with long hairs to 1 mm long, apex acuminate, margin serrate, base rounded to oblique-cuneate; sessile or with pseudo-petiole to 1 mm long; auricles absent or just tiny rims, fringed with few wavy bristles to 3.5 mm long; ligule about 0.1–0.3 mm high, irregularly toothed; secondary veins 2–4 pairs, intermediate veins 4–7; leaf sheaths 2–3 cm long, hairy. *Inflorescences* unknown.

Wong Khoon Meng and his colleagues mentioned on the label that this species is similar to *D. pendulus* Ridley and *D. hirtellus* Ridley. However, the authors have found that it is also superficially similar to *D. dumosus* (Ridley) Holttum. The differences between these three species and *D. khoonmengii*, are summarized in Table 1.

Table 1 Comparative table of habitats and vegetative morphological characters between *D. dumosus*, *D. hirtellus*, *D. khoonmengii*, and *D. pendulus*

Habitats and characters	***D. dumosus***	***D. hirtellus***	***D. khoonmengii***	***D. pendulus***
Habitat	confined to limestone vegetation, thin soil	open places and forest fringes	steep slope along stream in tropical monsoon rainforest, deep soil of granite parent material	thrives in foothills and valleys of the main mountain ranges or in logged or disturbed forest and forest fringes
Young culm nodes	glabrous	with a band of silvery brown hairs above and below node	with a band of black hairs only below node	with a band of silvery brown hairs above and below node
Culm length	to 7 m	to 15 m	to 13 m	to 30 m
Culm internodes	15–40 cm	40–50 cm	35–50 cm	40–50 cm
Culm diameter	1–2.5 cm	Commonly 6–8 cm	3–4 cm	commonly 6–9 cm
Culm-wall thickness	relatively thick (lacuna $\leq 1/3$ the diameter of the culm), sometimes solid at lower internodes	relatively thin (lacuna $\geq 1/3$ the diameter of the culm)	relatively thin	relatively thin
Verticils of roots at lower nodes	absent	present	absent	present
Culm sheaths	with black hairs mixed with thin white wax, to glabrous	with dense, but caducous, pale brown hairs mixed with copious white wax	with black hairs mixed with copious white wax	with dense, but caducous, pale brown hairs mixed with copious white wax
Culm-sheath auricles	easily deciduous, lobe-like, spreading, sometimes crisped, free at the end, somewhat curved, not twisted, bristly on margin, bristles to 7 mm long	spreading crisped lobes, not free at the ends, bristly on margin, bristles to 12–24 mm long	lobe-like, spreading, sometimes crisped, free at the ends, somewhat curved and slightly twisted, bristly on margin, bristles to 7–10 mm long	low rims, sometimes crisped, not free at the ends, bristly on margin, bristles to 7–10 mm long
Lower surface of leaf blade	not glaucous, short-hairy to glabrous	not glaucous, short-hairy	glaucous, short-hairy mixed with scattered long hairs	not glaucous, glabrous

Thailand.— PENINSULAR: Nakhon Si Thammarat [Lansaka District, Khaoluang National Park, steep slope beside Kra Rom Waterfall, alt. ca. 250 m, sterile, 11 July 2000, *K. M. Wong, C. Thapyai, & K. Roisungnern* WKM 2868 (BKF, K, KLU); *l. c.*, sterile, 28 Aug. 2004, *Sungkaew & Teerawatananon* 257 (Herbarium of Faculty of Forestry, Kasetsart University, Thailand, TCD, Herbarium of Thailand Natural History Museum, National Science Museum)].

Distribution.— So far known only from Nakhon Si Thammarat (Lansaka District).

Ecology.— Steep slope along stream in tropical monsoon rainforest, deep soil of granite parent material.

Etymology.— This species is named after Professor Dr. Wong Khoon Meng, a plant taxonomist of Malaysia, who has been working on bamboos for more than 20 years and by whom the type specimen was collected.

ACKNOWLEDGEMENTS

This work forms part of a PhD research project by the first author and was supported by the TRF/BIOTEC Special Program for Biodiversity Research and Training grant T_147003, a Trinity College, Dublin, Ireland Postgraduate Studentship, and the Trinity College Postgraduate Travel Reimbursement Fund.

REFERENCES

Holttum, R. E. 1958. The bamboos of the Malay Peninsula. Gardens' Bulletin Singapore 16: 1-135.

Middleton, D. J. 2003. Progress on the Flora of Thailand. Telopea 10: 33-42.

Ohrnberger, D. 1999. The Bamboos of the World: annotated nomenclature and literature of the species and the higher and lower taxa. Elsevier Science B.V., Amsterdam, The Netherlands.

Smitinand, T. 2001. Thai plant names (revised edition). The Forest Herbarium, Royal Forest Department, Bangkok, Thailand.

Widjaja, E. A. 1997. New taxa in Indonesian bamboos. Reinwardtia 11(2): 57-152.

Wong, K. M. 1995. The bamboos of Peninsular Malaysia. Malayan Forest Records No. 41, Forest Research Institute Malaysia (FRIM), Kuala Lumpur, Malaysia.

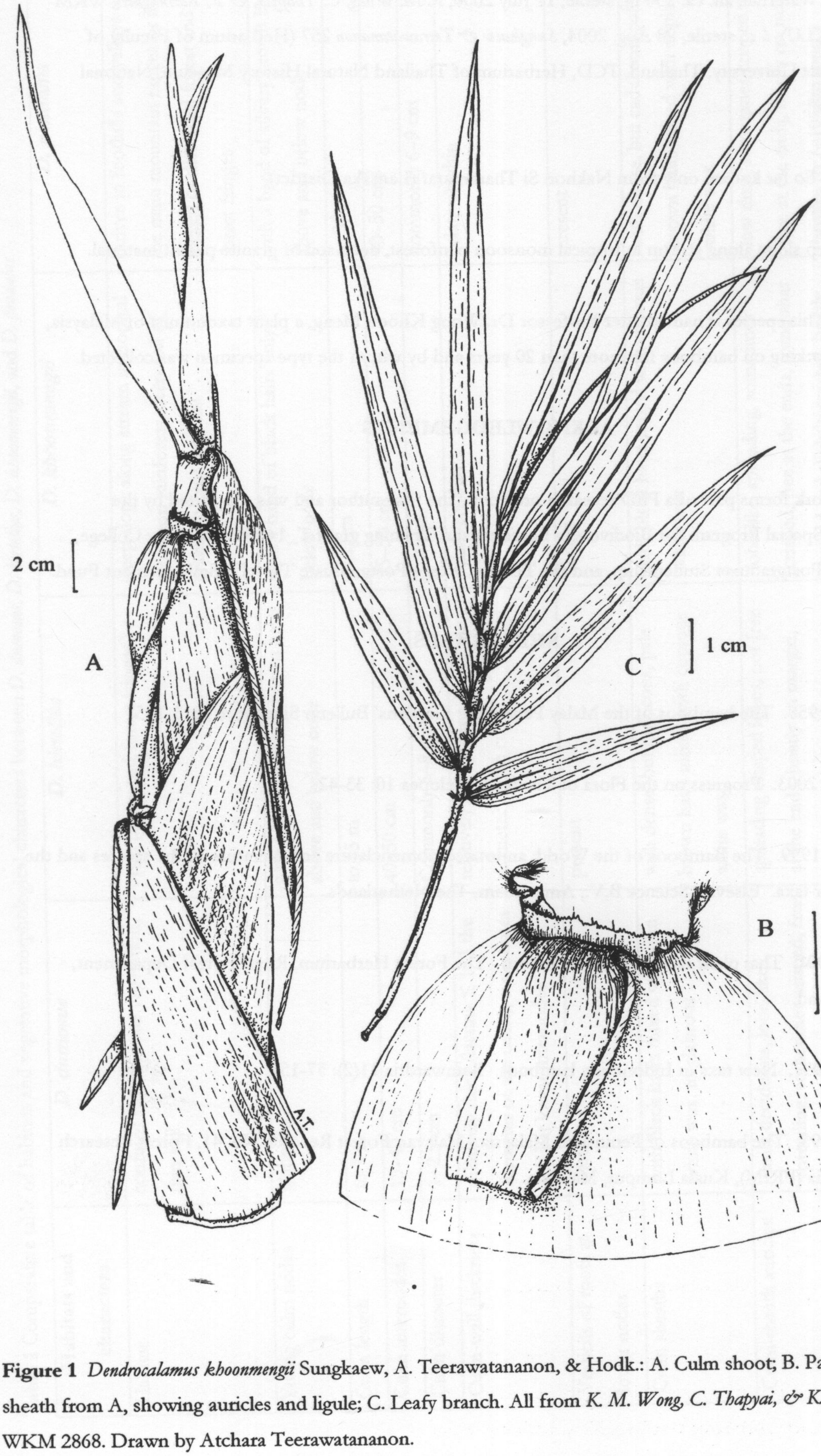


Figure 1 *Dendrocalamus khoonmengii* Sungkaew, A. Teerawatananon, & Hodk.: A. Culm shoot; B. Part of culm sheath from A, showing auricles and ligule; C. Leafy branch. All from *K. M. Wong, C. Thapyai, & K. Roisungnern* WKM 2868. Drawn by Atchara Teerawatananon.

MANUSCRIPT 4.3

[Submitted to Kew Bulletin]

Phuphanochloa, a new bamboo genus (*Poaceae*: *Bambusoideae*) from Thailand

Sarawood Sungkaew[1,2], Atchara Teerawatananon[1,3], John A.N. Parnell[1], Chris M.A. Stapleton[4] & Trevor R. Hodkinson[1]

Summary. A new monotypic bamboo genus *Phuphanochloa* (*Poaceae*: *Bambusoideae*) from north-eastern Thailand is described, together with a new species, *P. speciosa*.

Key words. *Bambuseae*, *Bambusoideae*, *Phuphanochloa*, *Poaceae*, Thailand

INTRODUCTION

During field work for the revision of the genus *Dendrocalamus* for the Flora of Thailand, vegetative and flowering specimens of a distinctive new bamboo were found. This bamboo was included in a comprehensive DNA sequence analysis (Sungkaew *et al.* submitted), and can now be described as a new genus *Phuphanochloa*, on the basis of morphology and molecular phylogenetics.

PHUPHANOCHLOA

Phuphanochloa *Sungkaew & A. Teerawatananon*, **gen. nov.** Poacearum-Bambusoidearum rhizomatibus sympodialibus brevicollibus. Culmus unicaespitosus erectus. Inflorescentia indeterminata; spicula 1-4 gluma, 7–9 flores et 1–2 flores terminales vestigiales ferenti, internodio basalis rachillae longo, 3 lodiculis, staminibus filamentis liberis 6, ovario apice pubescenti, stylo solitarium, stimatibus 3. Typus: *P. speciosa* Sungkaew, A. Teerawatananon

Rhizomes short-necked pachymorph. *Culms* unicaespitose, straight and erect; internodes smooth; nodes slightly swollen. *Branch bud* solitary at each node, developing into several branches, the primary one dominant. *Culm sheaths* deciduous, occasionally tardily deciduous at basal nodes, coriaceous; blades spreading to deflexed; auricles absent, or inconspicuous rims, margins entire, occasionally slightly wavy, glabrous; ligule short, margin toothed to bristly. *Leafy branchlets* bearing 6–8 moderate sized leaves. *Leaves* hairy underneath, pseudo-petioles short; sheaths hairy; auricles absent or of tiny rims bearing oral setae; ligule short, margin toothed. *Inflorescences* indeterminate, bracteate, borne on leafless branches; pseudospikelets clustered in spicate mass, 0–few small empty bracts, 2–4 bracts subtending prophyllate buds; internodes glabrous.

---

[1] Department of Botany, School of Natural Sciences, Trinity College Dublin, University of Dublin, Dublin 2, Ireland.

[2] Department of Forest Biology, Faculty of Forestry, Kasetsart University, Bangkok, Thailand.

[3] Thailand Natural History Museum, National Science Museum, Techno polis, Pathum Thani, Thailand.

[4] The Herbarium, Royal Botanic Gardens, Kew, Richmond, Surrey, TW9 3AB, England.

Spikelets large, ovate to elliptic in outline, purple, laterally compressed; glumes 1–4, shorter than the lowest lemma, coriaceous or somewhat rigid; fertile florets 7–9, with 1–2 terminal vestigial florets, shorter than fertile florets; rachilla internodes between florets (from the second floret upwards) short, disarticulating below each floret, rachilla internode distal to the lowermost floret frequently longer; lemmas chartaceous, the basal ones distally more rigid; paleas 2-keeled; lodicules usually 3; stamens 6, filaments free; ovary hairy at the top, style more or less hairy and flattened upwards, stigma 3, slightly plumose. *Caryopsis* hirsute at apex, beaked with the persistent base of the style.

Phuphanochloa can be included in subtribe *Bambusinae* in the classification of Soderstrom & Ellis (1987). It shares several characters in common with *Bambusa*, including similar vegetative appearance and branching, indeterminate spicate inflorescences with six stamens and free filaments. There are also few glumes, many (7–9) fertile florets which have variably elongated rachilla internodes that disarticulate below each floret. However, in *Phuphanochloa* the spikelets usually break up at maturity in one of two ways (both of which are usually present on any single individual); either the spikelet totally breaks up above the glume(s) leaving only the elongated rachilla internode (0.5–2 cm long), or it breaks up above the glume(s) and above the lowest floret leaving the upper part of the elongated rachilla internode (to 0.5 cm long) along with the glume(s) and intact lowest floret (this is not the case in *Bambusa*). In the length of the basal rachilla internodes, the genus shows similarities to *Bonia* Balansa from S China (Xia & Stapleton 2006), but the spikelets of *Phuphanochloa* are broader, and culm branching is not solitary. In addition, in *Bambusa* there are 1–3 distinctly plumose stigmas while in *Phuphanochloa* there are usually three, and they are only slightly plumose. *Phuphanochloa* can be easily distinguished from *Dendrocalamus* by its elongated and disarticulating rachilla internodes (very short and not disarticulating in *Dendrocalamus*) and the less globose inflorescences. *Phuphanochloa* differs from *Gigantochloa*, in having free filaments instead of fused filaments. The differences between these four genera and *Phuphanochloa* are summarised in Table 1.

The results from combined analysis of five plastid DNA regions (*trnL* intron, *trnL-F* intergenic spacer, *atpB-rbcL* intergenic spacer, *rps16* intron, and *matK* gene region, Fig. 1) (Sungkaew *et al.* submitted) show that *Phuphanochloa* is sister to a group comprising *Bambusa* species (*B. beecheyana* Munro, *B. emeiensis* L.C. Chia & H.L. Fung (as *Neosinocalamus affinis* in the tree), *B. malingensis* McClure and *B. oldhamii* Munro) with parsimony bootstrap support of 86% and a Bayesian posterior probability (PP) value of 1.00. These four species of *Bambusa* group together with bootstrap support of 80% (1.00PP). They represent three of the four subgenera of *Bambusa* according to Xia *et al.* (2006). *Bambusa malingensis* represents subg. *Bambusa*; *B. beecheyana* and *B. oldhamii* represent subg. *Dendrocalamopsis*; and *B. emeiensis* represents subg. *Lingnania*. The results of the molecular analysis show that *Phuphanochloa* cannot be included in any of these three subgenera. *Phuphanochloa* cannot be considered as a member of the fourth subgenus of *Bambusa* (subg. *Leleba*) either. This is because subg. *Leleba* typically has a broad culm-sheath blade (base of culm-sheath blade 1/2–3/4 width of sheath apex) as remarked by Xia *et al.* (2006); it is much smaller than 1/2 in *Phuphanochloa*. Its culm-sheath blade, in contrast, is more similar to those of *Dendrocalamus* or *Gigantochloa*. In addition, subg. *Leleba* has a thick papery culm sheath with a deciduous culm-sheath blade (Xia *et al.*, 2006) while *Phuphanochloa* has a coriaceous culm sheath with a persistent culm-sheath blade. *Phuphanochloa* is therefore clearly distinct from *Bambusa*.

The genus is named after Phu Phan National Park, in Sakon Nakhon Province, north-eastern Thailand, where the species *P. speciosa* grows.

Phuphanochloa speciosa *Sungkaew & A. Teerawatananon*, **sp. nov.**

Culmi unicaespitosi, compacti, c. 5–10 m longi et 3–5 cm diametri, internodia 25–30 cm longa; vaginis culmi pubescens; laminis foliorum pubescens infra; specula purpura, 1–4 gluma, 7–9 flores et 1–2 flores terminales vestigiales ferenti, lemmata hirsuta dorsi prope basin; styli plus minusve pubescens, stigmatum 3 parum plumosus. Typus: Thailand, *Sungkaew & Teerawatananon* 472 (holotypus TCD!; isotypi BKF!, K!, Herbarium of Faculty of Forestry, Kasetsart University!, Herbarium of Thailand Natural History Museum, National Science Museum!).

Rhizomes short-necked pachymorph. *Culms* self-supporting, unicaespitose in a dense clump, straight and erect, about 5–10 m long, 3–5 cm in diameter, walls 0.5–1 cm thick, smaller culms sometimes solid at basal internodes, culm tips more or less arched outwards; internodes 25–30 cm long; young culms glabrous, white-waxy, mature culms, green; nodes slightly swollen, all nodes without verticils of roots. *Branches* developing at all nodes to near base, several branches at each node, the primary one dominant, without aerial roots. *Culm sheaths* deciduous, occasionally tardily deciduous at basal nodes, coriaceous, 20–25 cm long by 12–17 cm wide, top obtuse, scattered with short pale hairs on the back, sometimes with a slight distal abaxial wrinkle; blades lanceolate, spreading to deflexed, 2–3.5 cm long, about 0.5–1 cm wide near the base, hairy at the adaxial base; auricles absent, or small rims, about 1 mm high, margins usually entire, occasionally slightly wavy, glabrous; ligule 2–3 mm high, margin toothed to bristly, bristles 2–5 mm long. *Leaves* 6–8 per branchlet; leaf-blades 10–15 cm long by 1–1.3 cm wide, abaxially hairy, apex acuminate, base acute, pseudo-petioles 0.5–2 mm long, secondary veins in 3–4 pairs, intermediate veins 5–6; auricles absent or of tiny rims bearing oral setae 1–3 mm long; ligule 1–2 mm high, apex obtuse, margin toothed; sheaths 4–5 cm long, hairy. *Inflorescences* indeterminate, bracteate, borne on leafless branches; pseudospikelets clustered in spicate mass, 2–4 cm in diameter, 0–few small empty bracts, 2–4 bracts subtending prophyllate buds; internodes 2–7 cm long, glabrous. *Spikelets* ovate to elliptic (from the second floret upwards), 1–2 cm long by 0.5–1 cm wide, purple, laterally compressed; glumes 1–4, shorter than the lowest lemma, coriaceous or somewhat rigid, 11–17-nerved; fertile florets 7–9, with 1–2 terminal vestigial florets, shorter than fertile florets; rachilla internodes between florets (from the second floret upwards) short, 1–1.5 mm long, disarticulating below each floret, rachilla internode distal to the lowermost floret frequently longer, to 0.5 cm; lemmas chartaceous, the basal ones distally more rigid, 6–11.5 mm long, with a tuft of brown hairs on the back near the base, 13–21-nerved, apex acute; paleas coriaceous, 5–10 mm long, 2-keeled on the back, slightly winged, wings 0.5–0.7 mm wide, keels and margins ciliate, the uppermost slightly winged, keeled but only sparsely or not ciliate, outside glabrous, inside scattered with short and minute hairs, apex acute or slightly cleft, 2–3-nerved between keels, 1–2-nerved between keel and margin; lodicules usually 3, hyaline, fringed with fine cilia, distally shortly pubescent; stamens 6, filaments free, anthers 3–4 mm long, yellow, tips shortly apiculate; ovary ovate-umbonate, summit hairy, style more or less hairy and flattened upwards, stigma 3, flat and thin, slightly plumose. *Caryopsis* oblong-elliptic, 5–6 mm long, apex hirsute, beaked with the persistent base of the style, furrowed on one side. Fig. 2

DISTRIBUTION. Endemic to north-eastern Thailand.

THAILAND. NE21, Sakhon Nakhon: Phu Phan National Park, base of Nang Mern cliff, sterile, 14 Aug. 2004, *Sungkaew & Teerawatananon* 191 (TCD, Herbarium of Faculty of Forestry, Kasetsart University; Herbarium of Thailand Natural History Museum, National Science Museum), fertile, 11 April 2005, *Sungkaew & Teerawatananon* 472 (holotype TCD; isotypes BKF, K, Herbarium of Faculty of Forestry, Kasetsart University, Herbarium of Thailand Natural History Museum, National Science Museum), fertile, 20 March 2006, *Sungkaew & Teerawatananon* 884 (TCD, Herbarium of Faculty of Forestry, Kasetsart University, Herbarium of Thailand Natural History Museum, National Science Museum).

HABITAT. Deciduous dipterocarp forest; c. 500 m.

CONSERVATION STATUS. Uncertain but probably rare; known from only a single locality.

NOTES. Spikelets break up at maturity in one of two ways (both of which are usually present on any single individual); either the spikelet totally breaks up above the glume(s) leaving only the elongated rachilla internode (0.5–2 cm long), or it breaks up above the glume(s) and above the lowest floret leaving the upper part of the elongated rachilla internode (to 0.5 cm long) along with the glume(s) and intact lowest floret.

Table 1 Comparative table of habit and morphological characters between *Bambusa, Bonia, Dendrocalamus, Gigantochloa* and *Phuphanochloa*

Characters	***Bambusa***	***Bonia***	***Dendrocalamus***	***Gigantochloa***	***Phuphanochloa***
Habit	usually erect	scrambling	usually erect	usually erect	usually erect
Branch number at mid-culm branch complement	several	single	several	several	several
Culm-sheath auricles; oral setae/ Culm-sheath blade	usually conspicuous; always present/ usually erect	usually conspicuous, occasionally inconspicuous or small; usually present, occasionally absent/ erect to deflexed	conspicuous, but often small to absent; present or absent/ erect to deflexed	usually absent or small; usually absent, occasionally present/ erect to deflexed	usually absent or small; always absent/ spreading to deflexed, never erect
Number of glumes per spikelet	0–3	0–2	(1–)2–4(–9)	1–5	1–4
Number of fertile florets per spikelet	2–13	3–9	1–8	(1–)2–5	7–9
Rachilla internodes	distinct and disarticulating	distinct and disarticulating	obscure and not disarticulating	obscure and not disarticulating	distinct and disarticulating
Stigma	typically (1–)3, plumose	typically 3, plumose	typically 1(–3), plumose	typically 1, plumose	typically 3, slightly plumose
Filaments	typically free	typically free	typically free	always fused into a firm tube	typically free
Breaking up at maturity of spikelets	either break up above the glume(s) or between the florets	unknown	usually break up above the glume(s)	usually break up above the glume(s)	usually break up in one of two ways (both of which are usually present on any single individual); either above the glume(s) or above the lowest floret

ACKNOWLEDGEMENTS

We are grateful to Drs Soejatmi Dransfield and Wong Khoon Meng for their valuable suggestions and constructive comments on this paper. We also thank the two anonymous reviewers for their comments on the manuscript and their suggestions for improvement. This work forms part of a PhD research project by the first author and was supported by the TRF/BIOTEC Special Program for Biodiversity Research and Training grant T_147003, a Trinity College Dublin, Ireland Postgraduate Studentship, and the Trinity College Dublin Postgraduate Travel Reimbursement Fund.

REFERENCES

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

Xia, N. H. & Stapleton, C.M.A. (2006). *Bonia* Balansa. *Flora of China* 22: 49-50.

Xia, N. H., Chia, L. C., Li, D. Z. & Stapleton, C.M.A. (2006). *Bambusa* Schreber. *Flora of China* 22: 9-38.

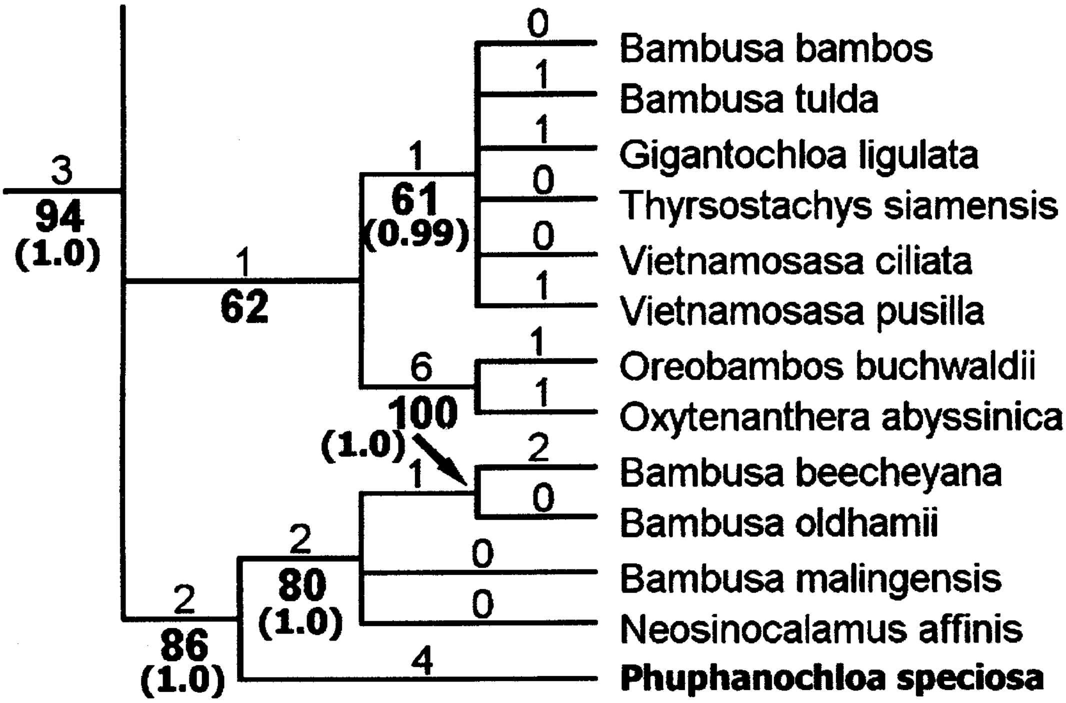


FIG. 1. Part of a multigene region phylogenetic tree (*trn*L intron, *trn*L-*F* intergenic spacer, *atp*B-*rbc*L intergenic spacer, *rps16* intron, and *mat*K gene region) of Bambusoideae (from Sungkaew *et al.* submitted). Values above branches represent branch length, values below branches represent parsimony bootstrap support and bracketed numbers represent Bayesian posterior probability value (PP). *Phuphanochloa* is sister to a group of *Bambusa* species (*B. beecheyana* Munro, *Neosinocalamus affinis* (=*B. emeiensis* L.C. Chia & H.L. Fung), *B. malingensis* McClure and *B. oldhamii* Munro) and does not group closely with another two *Bambusa* species (*B. bambos* (L.) Voss and *B. tulda* Roxb.).

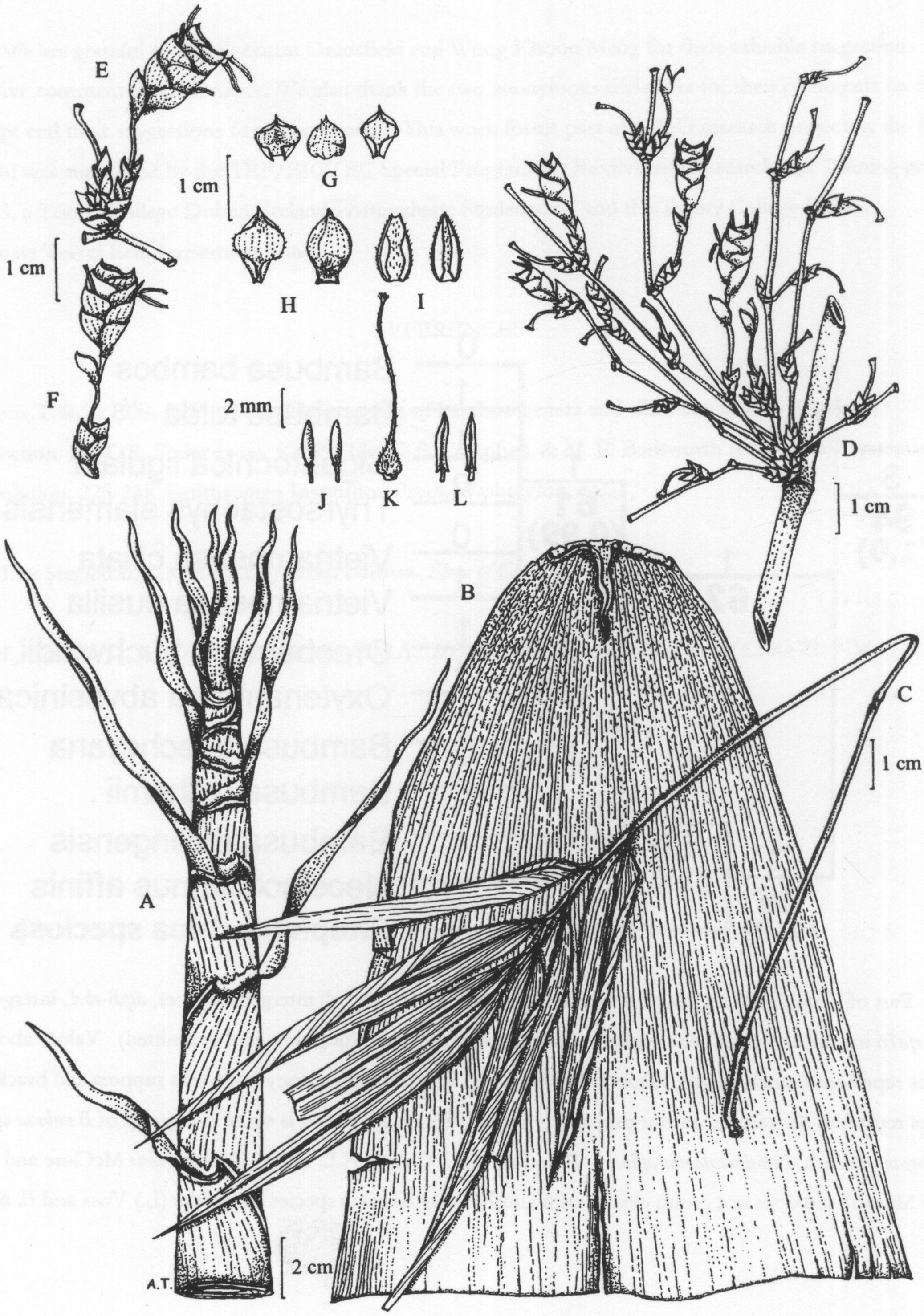


FIG. 2. *Phuphanochloa speciosa*. **A** culm shoot; **B** culm sheath; **C** leafy branch; **D** flowering branch; **E** flowering branch, showing 2 mature pseudospikelets (the one on the left with floret parts fallen); **F** a pseudospikelet, showing (from the base) an empty bract, 3 bracts subtending prophyllated buds (gemmiferous bracts), 2 glumes, 9 florets with a hidden vestigial floret; **G** glumes; **H** lemmas, showing ventral view (left) and dorsal view (right); **I** paleas, intermediates are found; **J** terminal vestigial florets; **K** pistil; **L** stamens. **A-C** from *Sungkaew & Teerawatananon* 191; **E-L** from *Sungkaew & Teerawatananon* 472. Drawn by A. Teerawatananon.

Chapter 5

General discussion and conclusions

Bamboos are among the most notoriously difficult groups of plants to identify and classify because their morphological characters are not clearly understood and are difficult to interpret (Stapleton, 1997). Thus, to further the taxonomy and systematics of bamboos, it is important to understand fundamental bamboo morphology. The most important morphological characters, both vegetative and reproductive, to identify and classify bamboos, are described in Chapter 1 of this thesis. Although desirable, it is not always possible to use flowering specimens to describe a new bamboo species. This is because sometimes it is necessary to wait as long as 150 years for a bamboo to flower (Li *et al.*, 2006b). This mechanism is thought to be associated with ensuring regeneration by temporary satiation of grain predators (Clayton, 1990). Even if we could have a fertile specimen in our hands, sometimes it is not that easy to identify what species that bamboo is. Bamboos with quite different vegetative characters may show almost identical spikelets (Holttum, 1985). However, Holttum (1958) suggested that the vegetative parts, particularly the culm sheaths, can provide many diagnostic characters and there are numerous examples where this is the case, such as Dransfield and Widjaja (1995a), Wong (1995a), Widjaja (1997), Li *et al.* (2006b), and Chapter 4 of this thesis.

Many advances have been made in bamboo phylogenetics (e.g. Clark *et al.*, 1995; Zhang, 2000; Ni Chonghaile, 2002). However, further progress is needed before a stable system of classification can be proposed. To this end, the BPG (Bamboo Phylogeny Group) has been set up to coordinate and focus attention on this topic (BPG, 2006; http://www.eeob.iastate.edu/research/bamboo/index.html). This is an international group of bamboo specialists, coordinated by Dr. Lynn Clark (Iowa State University, USA) that has agreed to work towards a common goal of improving the knowledge of bamboo systematics and taxonomy. I have recently been nominated to join this group. I hope this thesis will contribute significantly to the group's goals.

To understand how bamboos fit phylogenetically within the grass family (Poaceae) and how they relate to one another, a multi-gene region phylogenetic analysis was performed and reported in Chapter 2. A total of 64 taxa representing three subfamilies (Bambusoideae, Panicoideae, and Pooideae) sensu Clayton and Renvoize (1986), and representatives from all bamboo subtribes according to Clayton and Renvoize (1986) and Ohrnberger (1999), were included. Five plastid DNA regions, *trnL* intron, *trnL-F* intergenic spacer, *atpB-rbcL* intergeneric spacer, *rps16* intron, and *matK*, were sequenced for each of these taxa and used to study the phylogenetic relationships among the bamboos in general and the woody bamboos in particular. The individual (Figures 2.2, 2.4. 2.6, and 2.8) and combined (Figure 2.10, see also Figures 2.11 and 5.1) analyses of these studies have been presented. A comparison of the main groups obtained from individual and combined phylogenetic analyses of the five gene regions was shown in Table 2.4. A comparison of strict consensus and bootstrap support values of the main taxa or the major groups resolved from individual and combined analyses of five gene regions has been given in Table 2.5.

From the summary diagram provided in Figure 5.1, we can see that the BEP (Bambusoideae, Ehrhartoideae, Pooideae) clade adopted by the GPWG (2001) was resolved. Ehrhartoideae, traditionally sometimes treated under Bambusoideae s.l., were shown to be sister to a group consisting of Pooideae and Bambusoideae s.s.. The pooids were sister to the bambusoids. This was also found by Clark *et al.* (1995, as BOP clade), GPWG (2000), Zhang (2000), Salamin *et al.* (2002), and Bouchenak-Khelladi *et al.* (*in press*).

Bambusoideae s.s. were composed of herbaceous bamboos (Olyreae) and woody bamboos (Bambuseae s.l.). The results from this study were almost consistent with tribal and subtribal classification of Ohrnberger (1999). However, Bambuseae, as currently known, were not monophyletic because Olyreae were sister to tropical Bambuseae. Temperate Bambuseae were sister (outlying) to the group consisting of tropical Bambuseae and Olyreae. The monophyly of Bambuseae was generally not well supported in phylogenetic analyses (Clark *et al.*, 1995; Zhang, 2000; GPWG, 2001; Salamin *et al.*, 2002) and here I present evidence against its monophyly. This pattern was also found in a multi-gene analysis by Bouchenak-Khelladi *et al.* (*in press*) who analyzed phylogenetic pattern across the whole of the grass family. It seems therefore that evidence is building against the monophyly of the woody bamboos. This will have profound repercussions regarding the taxonomy of the Bambusoideae and our understanding of character evolution within the group.

Within the tropical Bambuseae, neotropical Bambuseae were sister to palaeotropical and Austral Bambuseae. In addition, Melocanninae were found to be sister to the remaining palaeotropical and Austral Bambuseae. Bambuseae were in Chapter 2 (and Sungkaew *et al.*, *in prep.*), therefore, redefined and the use of the tribal name Arundinarieae was recommended to accommodate the temperate woody bamboos. I feel that three tribes (Arundinarieae, Bambuseae ss. and Olyreae) better represent the variation present within the Bambusoideae than the commonly used two tribe system (Bambuseae, Olyreae). Bambuseae s.s. in this new system consists of seven subtribes of tropical woody bamboos, both palaeotropical and Austral Bambuseae (Figure 2.14) and neotropical Bambuseae (Figure 2.15), namely; Bambusinae, Hickeliinae, Melocanninae, Racemobambosinae, Arthrostylidiinae, Chusqueinae, and Guaduinae. Arundinarieae possesses three subtribes of temperate woody bamboos (Figure 2.12), they are Aruninariinae, Shibataeinae, and Thamnocalaminae. The delimitation of Olyreae was accepted as defined by GPWG (2001).

Although the results from this study were largely consistent with the subtribal classification system of Ohrnberger (1999) they showed little support for the subtribal classification of Clayton and Renvoize (1986). The Bambusinae of Clayton and Renoize (1986) were distributed in groups across the phylogenetic trees, whether in the trees of the single gene analyses or the combined gene region tree. The temperate woody bamboos were supported as monophyletic in all analyses, but none of their subtribes according to Clayton and Renvoize (1986) or Ohrnberger (1999) were resolved or supported. More work with intensive sampling of representative from all subtribes of temperate woody bamboos would be needed to resolve relationships among them. Furthermore, longer stretches of DNA may need to be sequenced or combined to provide additional characters for phylogenetic reconstructions. This will hopefully be one of the achievements of the BPG.

As for the neotropical woody bamboos, the relationship of having Chusqueinae sister to a group consisting of Guaduinae and Arthrostylidiinae is congruent with previous work (e.g. Kelchner & Clark, 1997; Zhang, 2000; Ni Chonghaile, 2002; and Bouchenak-Khelladi *et al., in press*). Within palaeotropical and Austral woody bamboos, Hickeliinae represented by only one genus in this thesis, *Temburongia*, was found to be sister to the remaining palaeotropical and Austral woody bamboos. However, it is probably not good enough to represent the whole subtribe by a single genus. Thus, more genera from Hickeliinae according to Ohrnberger (1999) (*Decaryochloa, Greslania, Hickelia, Hitchcockella, Nastus,* and *Perrierbambus*), especially the type genus *Hickelia*, and also three relatively new genera (*Cathariostachys* and *Valiha* Dransfiled (1998); *Sirochloa*, Dransfield (2002)), should be included to further investigate their monophyly and position within palaeotropical and Austral woody bamboos.

Subtribe Bambusinae according to Ohrnberger (1999) is not monophyletic as the genus *Vietnamosasa* of Racemobambosinae was embedded within it. However, if *Vietnamosasa* could be included within Bambusinae, then there is no evidence against the monophyly of Bambusinae sensu Ohrnberger (1999). The analysis of Ni Chonghaile (2002) using *trnL-F* sequences, including two genera of Racemobambosinae, *Neomicrocalamus* and *Racemobambos*, has shown that these two species grouped with other genera of Bambusinae (*Bambusa, Dendrocalamus,* and *Gigantochloa*). This could be strong evidence to suggest that *Neomicrocalamus, Racemobambos,* and *Vietnamosasa* may be better placed in Bambusinae than in Racemobambosinae; hence Racemobambosinae could be merged with Bambusinae.

Besides *Temburongia,* there are another three lineages which were successively outlying to the remaining palaeotropical and Austral woody bamboos, they are; 1) *Mullerochloa,* 2) a group consisting of *Neololeba* and *Dinochloa,* and 3) *Temochloa. Temochloa* is a monotypic and endemic genus from southern Thailand. *Dinochloa* is mainly distributed from southern Thailand to the Malay Peninsular, Indonesia and the Philippines, to Borneo (Dransfield, 1981; Ohrnberger, 1999). Apart from *Temochloa,* the biogeographical range of another two outlying lineages, a group consisting of *Neololeba* and *Dinochloa,* and *Mullerochloa,* is outside the biogeographical range of the core Bambusinae. *Mullerochloa* is a monotypic and endemic genus from Queensland, Australia (Ohrnberger, 1999; Wong, 2005) while *Neololeba* is a genus distributed from South Mindanao, North Sulawesi, Mollucas, New Guinea, Solomon Islands, and Australia (Queensland) (Widjaja, 1997). This would suggest that the ancestors of Bambusinae were from somewhere in mainland Asia, possibly south China or India or even mainland Southeast Asia and two outlying lineages evolved separately from southern Thailand, Malaysia to northern Australia. This hypothesis could be supported by considering the paucity of bamboo species in Australia.

Dendrocalamus was found to be closely related to *Bambusa, Dendrocalamopsis, Gigantochloa,* and *Melocalamus* (Figure 2.10). It is therefore placed in the palaeotropical Bambuseae clade, subtribe Bambusinae. *Bambusa, Dendrocalamus,* and *Gigantochloa* possess many taxonomically, ecologically and economically important bamboo species in Asia. The taxonomy of *Bambusa* is in a state of flux as it is a large genus with over 100 poorly understood species (Ohrnberger, 1999; Xia *et al.,* 2006a). Phylogenetic results from this thesis and that of Sun *et al.* (2005) have shown a huge incongruence between classifications based on molecular and morphological characters by Xia *et al.* (2006a). Much work remains to be done to establish the generic delimitation of *Bambusa* and the inter-relationships of its taxa. Moreover, intensive sampling for the representative species of *Bambusa, Dendrocalamopsis, Dendrocalamus, Gigantochloa,* and *Melocalamus* for phylogenetic studies is critically needed to resolve relationships among these five genera.

To revise *Dendrocalamus* for the Flora of Thailand project it is important to know, not only how *Dendrocalamus* fits phylogenetically within the Bambusoideae and more specifically tribe Bambuseae (Chapter 2) but also, how its species relate to one another. In this thesis (Chapter

3) the morphological and molecular characters of Thai *Dendrocalamus* were analyzed to establish patterns of infrageneric relationships. A total of 55 individuals from Bambuseae, including 20 *Dendendrocalamus* species, were sampled to study the phylogenetics of *Dendrocalamus* and its related genera. The study included the same five plastid DNA regions for sequence data as the analyses for Chapter 2. *Bambusa, Dendrocalamopsis, Gigantochloa,* and *Melocalamus* were shown to be the genera closely related to *Dendrocalamus.* However, based on morphological (Chapter 4 this thesis, and that of Holttum, 1985; Dransfield & Widjaja, 1995a; Wong, 1995a; Li *et al.,* 2006b; Li & Stapleton, 2006f) and molecular characters (Chapters 2 and 3), *Bambusa* and *Gigantochloa* are most likely to be the two most closely related genera to *Dendrocalamus.*

Unfortunately analyses based on molecular data alone could not resolve the infrageneric classification of this genus (Figure 3.2). The *trnL-F* and *atpB-rbcL* regions were found to be useful for phylogenetic study of plants from family to species levels (Soltis & Soltis, 1998; Hodkinson *et al.*, 2002; McGrath *et al.*, 2006) while *matK* has been used from order to species levels (Soltis & Soltis, 1998; Hilu *et al.*, 1999). Several other studies have also shown that these gene regions are useful for phylogenetic study of grasses and bamboos: *trnL-F* (e.g. Ni Chonghaile, 2002; Chapter 2; Sungkaew *et al.*, *in prep.*); *matK* (e.g. Liang & Hilu, 1996; Hilu *et al.*, 1999; Chapter 2; Sungkaew *et al.*, *in prep.*). However, there are no previous published papers of the use of *atpB-rbcL* and *rps16* for phylogenetics in Bambusoideae (Chapter 2; Sungkaew *et al.*, *in prep.*). For Bambusoideae and related grasses, however, the results from Chapter 2 have shown that *trnL-F* and *atpB-rbcL* regions (Figures 2.2 and 2.4 respectively) have provided well-resolved trees at sub-familial and tribal levels. The *rps16* and *matK* have been shown to be useful from sub-familial to sub-tribal levels (Figures 2.6 and 2.8 respectively) and also to some degree at generic level. Neither single gene nor combined genes analyses, however, can reveal the relationships at the specific level for *Dendrocalamus* (Chapter 3, Figure 3.2). AFLP fingerprinting, a method that has proven useful for infrageneric classification of *Phyllostachys* (Hodkinson *et al,* 2002) may help to solve this problem. However, morphological characters have also been shown to be good enough to classify *Dendrocalamus* into subgenera (Chapter 3). In Chapter 3, phenetic (PCO) and phylogenetic analyses (maximum parsimony) studies, using combined evidence from morphological and molecular characters, including 13 representative species of Thai *Dendrocalamus* and one Chinese species (*D. minor*) provided sufficient evidence to support the classification of *Dendrocalamus* into two subgenera, subg. *Dendrocalamus* and subg. *Sinocalamus* according to Li and Stapleton (2006f), see Figures 3.3, 3.9, and 3.10.

Results from phenetic and phylogenetic studies (based on combined morphological and molecular evidence) revealed three major groups of *Dendrocalamus* that could be practically identified (Figures 3.3, 3.9, and 3.10, see also Chapter 4):

1) The '*D. strictus* Group' (including '*D. membranaceus* Group') namely *D. barbatus, D. dumosus, D. khoonmengii, D. membranaceus, D. pendulus,* and *D. strictus.* The morphological synapomorphies for this group are: pseudospikelets that are spiny to the touch; yellow-green spikelets; spikelets that are sub-terete (see Tables 3.3 and 3.4, see also Figure 3.11). Morphologically, having the verticils of roots at the lower culm nodes would separate the '*D. strictus* Group' from the '*D. membranaceus* Group'.

2) The '*D. asper* Group' namely *D. asper, D. brandisii,* and *D. hamiltonii.* They shared two characters in common; lower culm internodes are hairy and florets are separable. However, these two characters are homoplasious among the *Dendrocalamus* species as the hairy lower culm internodes can also be seen in *D. sinicus* of the '*D. giganteus* Group' and *D. barbatus* of the '*D. strictus* Group'. The separable florets are also independently present in *D. latiflorus* and *D. minor* of the '*D. giganteus* Group'.

3) The '*D. giganteus* Group' namely *D. copelandii, D. giganteus, D. latiflorus, D. minor,* and *D. sinicus.* Three characters that they shared in common are: the mid-culm branch complement of the branches that are more or less subequal in size; pseudospikelets that are congested as stellate clusters (though this is not always the case in *D. minor*); and the spikelets that are usually composed of 4—8 fertile florets.

The '*D. strictus* Group' (including '*D. membranaceus* Group') and the '*D. asper* Group' constituted subg. *Dendrocalamus* while the '*D. giganteus* Group' represented subg. *Sinocalamus.* The vegetative character, mid-culm branch complement (that is composed of more or less subequal branches), was the most useful character to support the separation of subg. *Sinocalamus* from subg. *Dendrocalamus* (Figure 3.11).

Population genetic studies of bamboos are not common. None are known for *Dendrocalamus* species. For this reason I examined nine populations, and a total of 45 individuals, of an economically and ecologically important species of *Dendrocalamus* from Thailand, *D. membranaceus.* These were assessed for diversity, differentiation, evidence of clonality (against clonality) and phylogeography. Using three plastid microsatellite loci, six haplotypes were detected and three groups from the nine populations defined (Figures 3.12, 3.13 and 3.14 respectively). The population from Phu Rue did not group closely with any of the other populations. All nine populations sampled, except Suan Phung, contained more than one haplotype. This surely indicates that they are not stands of a singe genotype. In fact most populations had high haplotype diversity. Furthermore, most variation was found within populations and the populations showed low population genetic differentiation (G_{ST} =8.6%). This indicates that seed mediated geneflow (or movement of vegetative propagules) between populations is high (and certainly not restricted). The population from Sakaerat was expected to be well differentiated from the other populations as it is isolated from nearby forests, see Figure 3.15. However, the results showed that this population grouped together with populations from Suan Phung, Lan Sang, and Wat Chan. AFLP fingerprinting would be a means to give more information on genetic variation of those populations. AFLP has been used successfully by Hodkinson *et al.* (2000) to study inter-specific relationships of the bamboo genus *Phyllostachys* but it is not known how much infra-specific variation AFLPs would detect. However, studies on other grasses such as *Miscanthus* (Hodkinson *et al.*, 2002), would suggest that they are suitable for this purpose. In addition, some other studies have recently assessed infraspecific variation of bamboos and other grasses such as *Guadua angustifolia* (Marulanda *et al.*, 2002); *Chloris gayana* (Rhodes grass) (Ubi et al., 2003); *Phalaris aquatica* (Harding grass) (Mian *et al.*, 2005), and showed useful results with a range of marker systems.

Floristic treatments of Thai *Dendrocalamus* for the Flora of Thailand project have been done in Chapter 4. Two keys to species, one based on fertile specimens and another based on sterile specimens and ecology, were constructed. Species descriptions accompanied with synonyms, typifications, illustrations, distribution maps, and lists of specimens examined have been provided. Thirteen species were reported, namely *D. asper, D. barbatus, D. brandisii, D. copelandii, D. dumosus, D. giganteus, D. hamiltonii, D. khoonmengii, D. latiflorus, D. membranaceus, D. pendulus, D. sinicus*, and *D. strictus.* Distributions of each species in Thailand and patterns of subgenus distribution involving with the Kra Isthmus can be seen in Table 4.2 and Figure 4.30. Eight species are obviously native to Thailand, as they can be found in natural habitats and three species are introduced to Thailand as they have never been found in the wild (Table 4.2). The origins of *D. asper* and *D. giganteus*, are not known precisely. *Dendrocalamus copelandii*, one of the biggest bamboos in this genus, was recorded for the first time for Thailand (Sungkaew *et al.*, *accepted*; see Manuscript 4.1, Chapter 4). *Dendrocalamus khoonmengii* Sungkaew, A. Teerawatananon & Hodk., a possibly endemic bamboo to southern Thailand, was described as a species new to science (Sungkaew *et al.*, *accepted*; see Manuscript 4.2, Chapter 4).

During thesis production, *Phuphanochloa* Sungkaew & A. Teerawatananon, a genus of bamboo from Phuphan National Park, northeastern Thailand, was described for the first time (Sungkaew *et al., accepted*). It currently consists of only a single species, *P. speciosa* Sungkaew & A. Teerawatananon. *Phuphanochloa* can be included in Bambusinae because it is morphologically similar to, and phylogenetically related to, *Bambusa* (Sungkaew *et al., in prep.*). It is also morphologically similar to *Dendrocalamus* and *Gigantochloa*. The results from the phylogenetic analyses have confirmed that *Phuphanochloa* is sister to, or embedded in, a group consisting of *Bambusa beecheyana, B. malingensis, B. oldhamii,* and *Neosinocalamus affinis* (=*B. affinis*), see Figures 2.6, 2.8 and 2.10, Chapter 2 and Manuscript 4.3, Chapter 4).

Conclusions

In conclusion this thesis has advanced taxonomic, systematic and population genetic understanding of *Dendrocalamus* (Bambuseae; Poaceae). It has established phylogenetic relationships in the subfamily Bambusoideae and focussed attention on one economically, ecologically and culturally very important genus, *Dendrocalamus*. The phylogenetic position of *Dendrocalamus* within the Bambusoideae and more specifically within the Bambuseae of a newly defined three-tribe classification of Bambusoideae (Bambuseae, Arundinarieae, Olyreae) has been established. The thesis has also assessed the infrageneric classification of *Dendrocalamus* and advanced understanding of the intra-specific variation and phylogeography of one of its most important species, *D. membranaceus*, in Thailand. Last but not least, this thesis has provided a comprehensive taxonomic account of *Dendrocalamus* in Thailand that will be used directly for the Flora of Thailand project.

Manuscripts resulting from work in this thesis that are accepted for publication or in preparation are listed below:

1) A multi-gene region phylogenetic analysis of the woody bamboos (Bambuseae; Bambusoideae; Poaceae [*in prep.*]
2) *Phuphanochloa,* a new bamboo genus (Poaceae: Bambusoideae) from Thailand [*accepted*; Kew Bulletin]
3) *Dendrocalamus copelandii,* a new giant bamboo record for Thailand [*accepted*; Thai Forest Bulletin (Botany)]
4) *Dendrocalamus khoonmengii,* a new bamboo species (Poaceae: Bambusoideae) from peninsular Thailand [*accepted*; Thai Forest Bulletin (Botany)]

Long live bamboos!

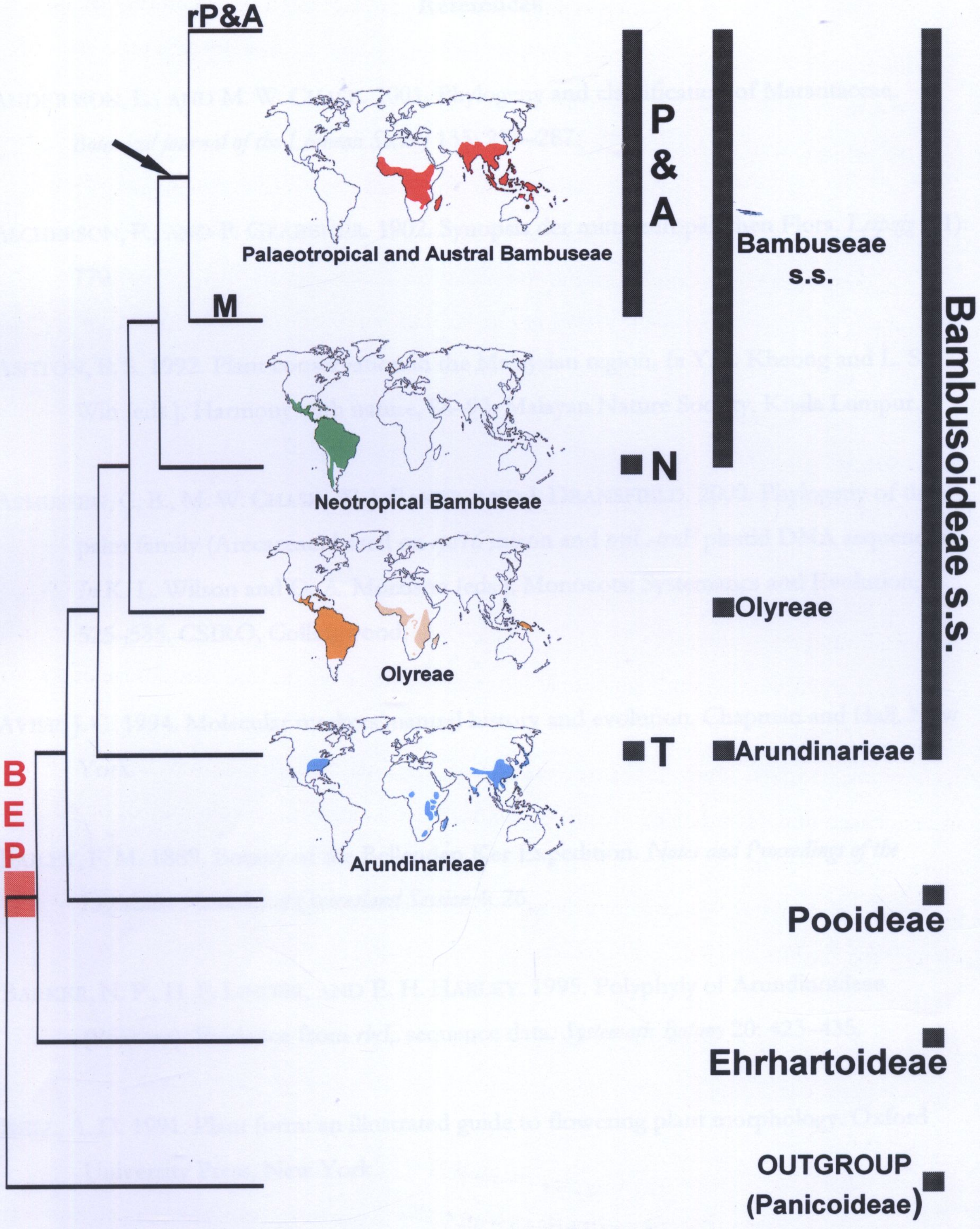


Figure 5.1 Cladogram showing relationships within Bambusoideae s.s. and other grasses. The red box indicates the BEP clade (Bambusoideae, Ehrhartoideae, Pooideae) according to GPWG (2001). The tribal classification (middle column) is according to this study where Arundinarieae and Bambuseae s.s. are redefined while Olyeae (herbaceous bamboos) is as defined by GPWG (2001). The geographical classification of woody bamboos; P&A, N, and T (column on far left) is shown according to Clark *et al.* (1995) and Ni Chonghaile (2002). N=Neotropical woody bamboos; P&A=Palaeotropical and Austral woody bamboos (arrowed). M=Melocanninae according to Ohrnberger (1999) is sister to rP&A (=the rest of Palaeotropical plus Austral woody bamboo subtribes). For the distribution of Olyreae, the lighter shade of gold colour indicates the uncertainty whether it is truly native to these areas. The distribution of Arundinarieae found in tropical zone is from the high elevation, usually from 1,000 m to as high as 3,630 m (Ohrnberger, 1999). All the distribution maps obtained from http://www.eeob.iastate.edu/research/bamboo/maps.html, with permission from Dr Lynn Clark.

References

ANDERSSON, L., AND M. W. CHASE. 2001. Phylogeny and classification of Marantaceae. *Botanical journal of the Linnean Society* 135: 275–287.

ASCHERSON, P., AND P. GRAEBNER. 1902. Synopsis der mitteleuropäischen Flora. *Leipzig* 2(1): 770.

ASHTON, P. S. 1992. Plant conservation in the Malaysian region. *In* Y. S. Kheong and L. S. Win [eds.], Harmony with nature, 86–93. Malayan Nature Society, Kuala Lumpur.

ASMUSSEN, C. B., M. W. CHASE, W. J. BAKER, AND J. DRANSFIELD. 2000. Phylogeny of the palm family (Arecaceae) based on *rps16* intron and *trnL-trnF* plastid DNA sequences. *In* K. L. Wilson and D. A. Morrison [eds.], Monocots: Systematics and Evolution, 525–535. CSIRO, Collingwood.

AVISE, J. C. 1994. Molecular markers, natural history and evolution. Chapman and Hall, New York.

BAILEY, F. M. 1889. Botany of the Bellenden Ker Expedition. *Notes and Proceedings of the Legislative Assembly of Queensland Session* 4: 26.

BARKER, N. P., H. P. LINDER, AND E. H. HARLEY. 1995. Polyphyly of Arundinoideae (Poaceae): Evidence from *rbcL* sequence data. *Systematic Botany* 20: 423–435.

BELL, A. D. 1991. Plant form: an illustrated guide to flowering plant morphology. Oxford University Press, New York.

BENTHAM, G., AND J. D. HOOKER. 1833. Gramineae. *In* G. Bentham and J. D. Hooker [eds], Genera Plantarum, 3(2): 1094–1096, 1207–1215. L. Reeve and Co., London.

BPG (Bamboo Phylogeny Group). 2006. The Bamboo Phylogeny Project. *BAMBOO, The Magazine of the American Bamboo Society*, December 2006, Vol. 27 (6): 11–14.

BOUCHENAK-KHELLADI, Y., N. SALAMIN, V. SAVOLAINEN, F. FOREST, M. VAN DER BANK, M. W. CHASE, AND T. R. HODKINSON. *in press.* Large multi-gene phylogenetic trees of the grasses (Poaceae): progress towards complete tribal and generic level sampling. *Molecular Phylogenetics and Evolution.*

BRANDIS, D. 1921. Indian trees: an account of trees, shrubs, woody climbers, bamboos and palms indigenous or commonly cultivated in the British Indian Empire (fourth impression). Constable and Company Ltd., London.

BUT, P. P. H., L. C. CHIA, AND H. L. FUNG. 1985. Hong Kong Bamboos. Urban Council, Hong Kong.

CAMUS, A. 1925. Graminées nouvelles d'Extrême-Orient. *Bulletin du Museum National d'Histoire Naturelle* 31: 205–208.

CARO, J. A. 1982. Sinopsis taxonomica de las gramineas argentines. *Dominguezia* 41–51.

CHAIMANEE, Y. 1997. Plio-Pleistocene rodents of Thailand. PhD thesis, Montpellier University, Montpellier.

CHASE, M. W., AND H. H. HILLS. 1991. Silica gel: An ideal material for field preservation of leaf samples for DNA studies. *Taxon* 40: 215–220.

CHIA, L. C., AND H. L. FUNG. 1980. On the validity of the genera *Sinocalamus* McClure and *Lingnania* McClure. *Acta Phytotaxonomica Sinica* 18: 211–216.

CLARK, L. G. 1989. Systematics of *Chusquea* section Swallenochloa, section Verticillatae, section Serpentes, and section Longifoliae (Poaceae: Bambusoideae). *Systematic Botany Monographs* 27: 1–127.

CLARK, L. G. 1995. Bamboo systematics today. *European Bamboo Society Journal* 6: 40–46.

CLARK, L. G. 1997a. Bamboo; the centerpiece of the grass family. *In* G.P. Chapman [ed.], The Bamboos: *Linnaean Society Symposium Series* 19: 237–248.

CLARK, L. G. 1997b. Diversity, biogeography and evolution of *Chusquea*. *In* G. P. Chapman [ed.], *The Bamboos. Linnaean Society Symposium Series* 19: 33–44.

CLARK, L. G., S. DRANSFIELD, J. TRIPLETT, AND J. G. SÁNCHEZ-KEN. *in press*. Phylogenetic relationships among the one-flowered, determinate genera of Bambuseae (Poaceae: Bambusoideae). *In* J. T. Columbus, E. A. Friar, C. W. Hamilton, J. M. Porter, L. M. Prince, and M. G. Simpson [eds.], Proceedings of the 4th International Conference on Grass Diversity and Evolution, Rancho Santa Ana Botanic Garden, Claremont, California.

CLARK, L. G., W. ZHANG, AND J. F. WENDEL. 1995. A phylogeny of the grass family (Poaceae) based on *ndhF* sequence data. *Systematic Botany* 20: 436–460.

CLAYTON, W. D. 1990. The spikelet. *In* G. P. Chapman [ed], Reproductive versatility in the grasses, 32–51. Cambridge University Press, New York.

CLAYTON, W. D., AND S. A. RENVOIZE. 1986. Genera Graminum, grasses of the world. Kew Bulletin Additional Series XIII.

CLAYTON, W. D., K. T. HARMAN, AND H. WILLIAMSON. 2006 onwards. GrassBase-The Online World Grass Flora. http://www.kew.org/data/grasses-db.html. [accessed 05 June 2007; 08:00 GMT].

CONDIT, R., AND S. P. HUBBELL. 1991. Abundance and DNA sequence of two-base repeats in plant genomes. *Genome* 34: 66–71.

CUÈNOUD, P., V. SAVOLAINEN, L. W. CHATROU, M. POWELL, R. J. GRAYER, AND M. W. CHASE. 2002. Molecular phylogenetics of Caryophyllales based on nuclear *18S* rDNA and plastid *rbcL*, *atpB*, and *matK* DNA sequences. *American Journal of Botany* 89: 132–144.

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

DAI, Q. H. 1982. New species of Bambusoideae from Guangxi. *Acta Phytotaxonomica Sinica* 20: 210–215.

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

DAVIS, P. H., AND V. H. HEYWOOD. 1963. Principles of angiosperm taxonomy. D. Van Nostrand Company, New York.

DOYLE, J. J., AND J. L. DOYLE. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemical Bulletin for the Botany Society of America* 19: 11–15.

DOYLE, J. J., J. I. DAVIS, R. J. SORENG, D. GARVIN, AND M. J. ANDERSON. 1992. Chloroplast DNA inversions and the origin of the grass family (Poaceae). *Proceedings of the National Academy of Sciences, U.S.A.* 89: 7722–7726.

DRANSFIELD, S. 1981. The genus *Dinochloa* (Gramineae-Bambusoideae) in Sabah. *Kew Bulletin* 36: 613–633.

DRANSFIELD, S. 1983. Notes on *Schizostachyum* (Gramineae-Bambusoideae) from Borneo and Sumatra. *Kew Bulletin* 38(2): 321–332.

DRANSFIELD, S. 1994. Bamboo resources in Thailand: How much do we know? *In* Bamboo in Asia & the Pacific. Proceedings of the 4th International Bamboo Workshop, Chiangmai, Thailand: 1–6. FAO, Bangkok.

DRANSFIELD, S. 1995. *Gigantochloa ligulata. In* S. Dransfield & E.A. Widjaja [eds], Plant resources of South-East Asia No. 7: Bamboos, 111–113. Backhuys Publishers, Leiden.

DRANSFIELD, S. 1998a. *Cyrtochloa*, a new genus of bamboo (Gramineae-Bambusoideae) from the Philippines. *Kew Bulletin* 53(4): 857–873.

DRANSFIELD, S. 1998b. *Valiha* and *Cathariostachys*, two new bamboo genera (Gramineae-Bambusoideae) from Madagascar. *Kew Bulletin* 53(2): 375–397.

DRANSFIELD, S. 2000a. Notes on 'Pek' and 'Chote', members of the genus *Vietnamosasa* (Poaceae-Bambusoideae) in Thailand. *Thai Forest Bulletin (Botany)* 28: 163–176.

DRANSFIELD, S. 2000b. *Temochloa*, a new bamboo genus (Poaceae-Bambusoideae) from Thailand. *Thai Forest Bulletin (Botany)* 28: 179–182.

DRANSFIELD, S. 2002. *Sirochloa*, a new bamboo genus from Madagascar (Poaceae-Bambusoideae). *Kew Bulletin* 57(4): 963–970.

DRANSFIELD, S., AND E. A. WIDJAJA, (eds.). 1995a. Plant resources of South-East Asia No. 7: Bamboos. Backhuys Publishers, Leiden.

DRANSFIELD, S., AND E. A. WIDJAJA. 1995b. *Dendrocalamus asper. In* S. Dransfield & E.A. Widjaja [eds], Plant resources of South-East Asia No. 7: Bamboos, 80–83. Backhuys Publishers, Leiden.

DRANSFIELD, S., AND K. M. WONG. 1996. *Temburongia*, a new genus of bamboo (Gramineae: Bambusoideae) from Brunei. *Sandakania* 7: 49–58.

DRANSFIELD, S., AND K. M. WONG. 2004. Notes on *Dendrocalamus longifimbriatus* (Poaceae-Bambusoideae) from Myanmar. *Thai Forest Bulletin (Botany)* 32: 73–75.

DRANSFIELD, S., R. PATTANAVIBOOL, AND S. SUNGKAEW. 2003. Two new species of *Neohouzeoua* (Gramineae-Bambusoideae) from Thailand and Myanmar. *Thai Forest Bulletin (Botany)* 31: 27–33.

DURIYAPRAPAN, S., AND P. C. M. JANSEN. 1995a. *Dendrocalamus membranaceus. In* S. Dransfield & E.A. Widjaja [eds], Plant resources of South-East Asia No. 7: Bamboos, 90–92. Backhuys Publishers, Leiden.

DURIYAPRAPAN, S., AND P. C. M. JANSEN. 1995b. *Gigantochloa albociliata. In* S. Dransfield & E.A. Widjaja [eds], Plant resources of South-East Asia No. 7: Bamboos, 98–100. Backhuys Publishers, Leiden.

DUVALL, M. R., AND B. R. MORTON. 1996. Molecular phylogenetics of Poaceae: an expanded analysis of *rbcL* sequence data. *Molecular Phylogenetics and Evolution* 5(2): 352–358.

FRIAR, E., AND G. KOCHERT. 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. The Bambuseae of British India. *Annals of the Royal Botanic Garden, Calcutta* 7: 1–133, plates 131–119.

GIELIS, J., I. EVERAERT, AND M. DE LOOSE. 1997. Genetic variability and relationships in *Phyllostachys* using random amplified polymorphic DNA. *In* G. P. Chapman [ed.], The bamboos. *Linnaean Society Symposium Series* 19: 107–124.

GOLDSTEIN, D. B., AND D. D. POLLOCK. 1997. Launching microsatellites: a review of mutation processes and methods of phylogenetic inference. *Journal of Heredity* 88: 335–342.

GOLDSTEIN, D. B., AND C. SCHLOTTERER. 1999. Microsatellites: evolution and applications. Oxford University Press, Oxford.

GOWER, J. C. 1966. Some distance properties of latent root and vector methods used in multivariate analysis. *Biometrika* 53: 325–338.

GPWG (Grass Phylogeny Working Group). 2000. A phylogeny of the grass family (Poaceae), as inferred from eight character sets. *In* S. W. L. Jacobs and J. Everett [eds.], Grasses: Systematics and Evolution, 1–7. CSIRO, Melbourne.

GPWG. 2001. Phylogeny and subfamily classification of the grasses (Poaceae). *Annals of the Missouri Botanical Garden* 88(3): 373–430.

GROSSER, D., AND W. LIESE. 1971. On the anatomy of Asian bamboos, with special reference to their vascular bundles. *Wood Science and Technology* 5: 290–312.

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

HILU, K. W., L. A. ALICE, AND H. P. LIANG. 1999. Phylogeny of Poaceae inferred from *matK* sequences. *Annals of the Missouri Botanical Garden* 86(4): 835–851.

HODKINSON, T. R., S. A. RENVOIZE, G. NÍ CHONGHAILE, C. M. A. STAPLETON, AND M. W. CHASE. 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.

HODKINSON, T. R., M. W. CHASE, C. TAKAHASHI, I. J. LEITCH, M. D. BENNETT, AND S. A. RENVOIZE. 2002. The use of DNA sequencing (*ITS* and *trnL-F*), AFLP, and fluorescent *in situ* hybridization to study allopolyploid *Miscanthus* (Poaceae). *American Journal of Botany* 89(2): 279–286.

HODKINSON, T. R., N. SALAMIN, M. W. CHASE, Y. BOUCHENAK-KHELLADI, S. A. RENVOIZE, AND V. SAVOLAINEN. 2007a. Large trees, supertrees, and diversification of the grass family. *Aliso* 22: 1–25.

HODKINSON, T. R., S. WALDREN, J. A. N. PARNELL, C. T. KELLEHER, K. SALAMIN, AND N. SALAMIN. 2007b. DNA banking for plant breeding, biotechnology and biodiversity evaluation. *Journal of Plant Research* 120(1): 17–29.

HOLMGREN, P. K., AND N. H. HOLMGREN. 1998 [continuously updated]. Index Herbariorum: a global directory of public herbaria and associated staff. New York Botanical Garden's Virtual Herbarium. Published on the internet http://sweetgum.nybg.org/ih/ [accessed 17 July 2007].

HOLMGREN, P. K., N. H. HOLMGREN, AND L. C. BARNETT. 1990. Index Herbariorum, Part I: The Herbaria of the World (eighth edition). New York Botanical Garden.

HOLTTUM, R. E. 1958. The bamboos of the Malay Peninsula. *Gardens' Bulletin Singapore* 16: 1–135.

HSIAO, J. Y., AND L. H. RIESEBERG. 1994. Population genetic structure of *Yushania niitakayamensis* (Bambusoideae, Poaceae) in Taiwan. *Molecular Ecology* 3: 201–208.

HSUEH, C. J., AND D. Z. LI. 1988a. A study on the genus *Dendrocalamus* Nees from China. *Journal of Bamboo Research* 7(3): 1–19.

HSUEH, C. J., AND D. Z. LI. 1988b. A study on the genus *Dendrocalamus* Nees from China. *Journal of Bamboo Research* 7(4): 1–19.

HSUEH, C. J., AND D. Z. LI. 1989. A study on the genus *Dendrocalamus* Nees from China. *Journal of Bamboo Research* 8(1): 25–43.

HUGHE J. B., ROUND P. D., AND D. S.WOODRUFF. 2003. The Indochinese-Sundaic faunal transition at the Isthmus of Kra: An analysis of resident forest bird species distributions. *Journal of Biogeography* 30: 569–580.

JACCARD, P. 1908. Nouvelles recherches sur la distribution florale. Bulletin de la Societé Vaud. Sciences Naturelles 44: 223–270.

JOHNSON, L. A., AND D. E. SOLTIS. 1994. *matK* DNA sequences and phylogenetic reconstruction in Saxifragaceae sensu stricto. *Systematic Botany* 19: 143–156.

JUDZIEWICZ, E. J., L. G. CLARK, X. LONDOÑO, AND M. J. STERN. 1999. American bamboos. Smithsonian Institution Press, Washington and London.

KELCHNER, S. A., AND L. G. CLARK. 1997. Molecular evolution and phylogenetic utility of the chloroplast *rpl16* intron in *Chusquea* and the Bambusoideae (Poaceae). *Molecular Phylogenetics and Evolution* 8(3): 385–397.

KELLEHER, C. T., T. R. HODKINSON, D. L. KELLY, AND G. C. DOUGLAS. 2004. Characterisation of chloroplast DNA haplotypes to reveal the provenance and genetic structure of oaks in Ireland. *Forest Ecology and Management* 189: 123–131.

KELLOGG, E. A., AND C. S. CAMPBELL. 1987. Phylogenetic analyses of the Gramineae. *In* T. R. Soderstrom, K. W. Hilu, C. S. Campbell, and M. E. Barkworth [eds.], Grass systematics and evolution, 310-322. Smithsonian Institution Press, Washington, D.C.

KELLOGG, E. A., AND L. WATSON. 1993. Phylogenetic studies of a large data set. I. Bambusoideae, Andropogonodae, and Pooideae (Gramineae). *Botanical Review* 59: 273–343.

KENG, P. C. 1983. A revision of the genera of bamboos from the world, III. *Journal of Bamboo Research* 2(1): 11–27.

KURZ, S. 1876. Bamboo and its use. *The Indian Forester* 1: 219–269.

KURZ, S. 1877. Gramineae. *Forest Flora of Burma* 2: 547–571.

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

LEGENDRE, P., AND A. VAUDOR. 1991. The R Package: multidimensional analysis, spatial analysis. Department of Biological Sciences, University of Montreal.

LI, D. Z. 1997. The Flora of China Bambusoideae Project-problems and current understanding of bamboo taxonomy in China. *In* G. P. Chapman [ed.], The Bamboos. Linnaean Society Symposium Series 19: 61–81.

LI, D. Z. 1998. Taxonomy and biogeography of the Bambuseae (Gramineae: Bambusoideae). *In* A. N. Rao & V. R. Rao [eds], Proceedings of training course/workshop 10–17 May 1998, Kunming and Xishuanbanna, Yunnan, China, 14–23.

LI, D. Z., AND C. M. A. STAPLETON. 2006a. *Ampelocalamus* S. L. Chen, T. H. Wen & G. Y. Sheng. *Flora of China* 22: 99–102.

LI, D. Z., AND C. M. A. STAPLETON. 2006b. *Bambusa* subg. *Dendrocalamopsis* L.C. Chia & H.L. Fung. *Flora of China* 22: 35–38.

LI, D. Z., AND C. M. A. STAPLETON. 2006c. *Bambusa* subg. *Lingnania* (McClure) L.C. Chia & H.L. Fung. *Flora of China* 22: 31–35.

LI, D. Z., AND C. M. A. STAPLETON. 2006d. *Chimonobambusa* Makino. *Flora of China* 22: 152–161.

LI, D. Z., AND C. M. A. STAPLETON. 2006e. *Chimonocalamus* Hsueh & T.P. Yi. *Flora of China* 22: 103–105.

LI, D. Z., AND C. M. A. STAPLETON. 2006f. *Dendrocalamus* Nees. *Flora of China* 22: 39–46.

LI, D. Z., AND C. M. A. STAPLETON. 2006g. *Gigantochloa* Kurz ex Munro. *Flora of China* 22: 46–48.

LI, D. Z., AND C. M. A. STAPLETON. 2006h. *Melocalamus* Bentham. *Flora of China* 22: 48–49.

LI, D. Z., AND C. M. A. STAPLETON. 2006i. *Thyrsostachys* Gamble. *Flora of China* 22: 38–39.

LI, D. Z., AND J. R. XUE. 1997. The biodiversity and conservation of bamboos in Yunnan, China. *In* G. P. Chapman [ed.], The Bamboos. Linnaean Society Symposium Series 19: 83–94.

LI, D. Z., Z. H. GUO, AND C. M. A. STAPLETON. 2006a. *Fargesia* Franchet. *Flora of China* 22: 74–96.

LI, D. Z., Z. P. WANG, Z. D. ZHU, N. H. XIA, L. Z. JIA, Z. H. GUO, G. Y. YANG, AND C. M. A. STAPLETON 2006b. Tribe Bambuseae. *Flora of China* 22: 11–180.

LI, Y. C., A. B. KOROL, T. FAHIMA, A. BEILES, AND E. NEVO. 2002. Mircosatellites: genomic distribution, putative functions and mutational mechanisms. *A review, Molecular Ecology* 11: 2453–2465.

LIANG, H., AND K. W. HILU. 1996. Application of the *matK* gene sequences to grass systematics. *Canadian Journal of Botany* 74: 125–134.

LIESE, W. 1995. Anatomy and utilization of bamboos. *European Bamboo Society Journal* (May): 5–12.

LIESE, W. 1998. The anatomy of bamboo culms. Technical Report 18, International Network for Bamboo and Rattan, China.

LIN, W. C. 1968. The bamboos of Thailand (Siam). Special bulletin of Taiwan Forestry Research Institute No. 6. Taiwan Forestry Research Institute, Taipei, Taiwan.

LIN, W. T. 1990. New materials for Chinese bamboos. *Guihaia* 10(1): 15–20.

LOH, J. P., R. KIEW, O. SET, L. H. GAN, AND Y. Y. GAN. 2000. A study of genetic variation and relationships within the bamboo subtribe Bambusinae using Amplified Fragment Length Polymorphism. *Annals of Botany* 85: 607–612.

LOWE, A., S. HARRIS, AND P. ASHTON. 2004. Ecological genetics: design, analysis, and application. Blackwell Science, Oxford, UK.

LUCKOW, M. 1995. Species concepts: assumptions, methods, and applications. *Systematic Botany* 20(4): 589–605.

MARULANDA, M. L., A. M. LOPEZ, AND J. L. CLAROZ. 2007. Analyzing the genetic diversity of *Guadua* spp. in Colombia using rice and sugarcane microsatellites. *Crop Breeding and Applied Biotechnology* 7: 43–51.

MARULANDA, M. L., P. MÁRQUEZ, AND X. LONDOÑO. 2002. AFLP analysis of *Guadua angustifolia* (Poaceae: Bambusoideae) in Colombia with emphasis on the coffee region. *Journal of the American Bamboo Society* 16: 32–42.

MCCLURE, F. A. 1934. The inflorescence in *Schizostachyum* Nees. *Journal of the Washington Academy of Sciences* 24: 541–548.

MCCLURE, F. A. 1940. New genera and species of Bambusaceae from eastern Asia. *Lingnan University Science Bulletin* 9: 66–67.

MCCLURE, F. A. 1966. The bamboos; a fresh perspective. Harvard University Press, Cambridge, Massachusetts, United States.

MCCLURE, F. A. 1973. Genera of bamboos native to the New World. Smithsonian Contributions to Botany 9. Smithsonian Institution, Washington, D. C.

MCGRATH, S., T. R. HODKINSON, AND S. BARTH. 2007. Extremely high cytoplasmic diversity in natural and breeding populations of *Lolium* (Poaceae). *Journal of Heredity*.

McGRATH, S., T. R. HODKINSON, N. SALAMIN, AND S. BARTH. 2006. Development and testing of novel chloroplast microsatellite markers for *Lolium perenne* and other grasses (Poaceae) from *de novo* sequencing and *in silico* sequences. *Molecular Ecology Notes* 6: 449–452.

McNEILL, J., F. R. BARRIE, H. M. BURDET, V. DEMOULIN, D. L. HAWKSWORTH, K. MARHOLD, D. H. NICOLSON, J. PRADO, P. C. SILVA, J. E. SKOG, J. H. WIERSEMA, AND N. J. TURLAND. 2006. International Code of Botanical Nomenclature (Vienna Code); adopted by the Seventeenth International Botanical Congress Vienna, Austria, July 2005. A.R.G. Gantner Verlag, Ruggell, Liechtenstein.

METCALFE, C. R. 1960. Anatomy of the Monocotyledons I. Gramineae. Oxford University Press, USA.

MIAN, M. A. R., J. C. ZWONITZER, Y. CHEN, M. C. SAHA, AND A. A. HOPKINS. 2005. AFLP Diversity within and among Hardinggrass Populations. *Crop Science* 45: 2591–2597.

MIDDLETON, D. J. 2003. Progress on the Flora of Thailand. *Telopea* 10: 33–42.

MOLVRAY, M., P. J. KORES, AND M. W. CHASE. 2000. Polyphyly of mycoheterotrophic orchids and functional influences on floral and molecular characters. *In* K. L. Wilson and D. A Morrison [eds.], Monocots: systematics and evolution, 441–448. CSIRO Publishing, Collingwood, Victoria, Australia.

MUNRO, W. 1868. A monograph of the Bambusaceae, including descriptions of all the species. *Transactions of the Linnean Society of London* 26: 1–157.

NADOT, S., R. BAJON, AND B. LEJEUNE. 1994. The chloroplast gene *rps4* as a tool for the study of Poaceae phylogeny. *Plant Systematics and Evolution* 191: 27–38.

NAKAI, T. 1943. Ordines, familiae, tribi, genera, sectiones, species, varietates, formae et combinationes novae a Prof. Nakai ad huc ut novis edita; Tokyo.

NAPOMPETH, B., AND J. RODCHAREON. 1998. Environmental and biodiversity issues in Thailand. *In* G. Ismail & M. Mohamed [eds], Biodiversity conservation in ASEAN: emerging issues and regional needs, 233–246. (ASEAN) Academic Press, London, UK.

NEES VON ESENBECK, C. G. D. 1834. Bambuseae brasilienses: recensuit et alias in India Orientalis provenientes adjecit. *Linnaea* 9: 461–494.

NEI, M. 1973. Analysis of gene diversity in subdivided populations. *Proceedings of the National Academy of Science U.S.A.* 70: 3321–3323.

NEI, M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics* 89: 583–590.

NI CHONGHAILE, G. 2002. Molecular systematics of the woody bamboos (Bambuseae). PhD Thesis, University of Dublin, Trinity College, Dublin, Ireland.

OHRNBERGER, D. 1999. The bamboos of the world: annotated nomenclature and literature of the species and the higher and lower taxa. Elsevier Science B.V., Amsterdam, The Netherlands.

OXELMANN, B., M. LIDEN, AND D. BERGLUND. 1997. Chloroplast *rps16* intron phylogeny of the tribe Sileneae (Caryophyllaceae). *Plant Systematics and Evolution* 206: 257–271.

PAGE, R. D. M., AND E. C. HOLMES. 1998. Molecular evolution: a phylogenetic approach. Blackwell Science, Oxford, UK.

PARNELL, J. A. N. 2000. The conservation of biodiversity: aspects of Ireland's role in the study of tropical plant diversity with particular reference to the study of the Flora of Thailand and *Syzygium*. *In* B.S. Rushton [ed.], Biodiversity: the Irish dimension. Royal Irish Academy, Dublin.

PILGER, R. 1906. Zwei neue Bambuseae aus Siam. *Repertorium Novarum Specierum Regni Vegetabilis* 3: 116–117.

POOMA, R. 2003. Dipterocarpaceae in Thailand: taxonomic and biogeographical analysis. PhD Thesis, Kasetsart University, Bangkok, Thailand.

PRAT, H. 1960. Vers une classification naturelle des graminees. *Bulletin de la Société Botanique de France* 107: 32–79.

RAIZADA, M. B. 1948. A little-known Burmese bamboo. *The Indian Forester* 74(1): 7–10.

RASHFORD, J. H. 1995. The past and present use of bamboo in Jamaica. *Economic Botany* 49(4): 395–405.

REEVES, G., M. W. CHASE, P. GOLDBLATT, P. RUDALL, M. F. FAY, A. V. COX, B. LEJEUNE, AND T. SOUSA-CHIES. 2001. Molecular systematics of Iridaceae: evidence from four plastid DNA regions. *American Journal of Botany* 88: 2074–2087.

RENVOIZE, S., AND T. HODKINSON. 1997. The classification of *Phyllostachys* (Poaceae). *In* G. P. Chapman [ed.], The bamboos. *Linnaean Society Symposium Series* 19: 95–106.

RIDLEY, H. N. 1905. New and little known Malayan plants, Series II. *Journal of the Straits Branch of the Royal Asiatic Society* 44: 189–211.

RIDLEY, H. N. 1912. A botanical excursion to Pulau Adang. *Journal of the Straits Branch of the Royal Asiatic Society* 61: 45–65.

RIDLEY, H. N. 1916. New and rare Malayan plants, Series VIII. *Journal of the Straits Branch of the Royal Asiatic Society* 73: 139–146.

RIVIÈRE, A., AND C. RIVIÈRE. 1878. Les bambous. Socièté d' Acclimination. Paris.

ROSHEVITS, R. Yu. 1946. Sistema zlakov v svyazi s ikh evolyutsiei. Leningrad: Komarav Botanical Institute, U.S.S.R. Academy of Sciences. [Evolução e sistemática das gramíneas. Boletim do Instituto de Botânica No. 5: 1–20. Portuguese translation by T. Sendulsky, Instituto de Botânica, São Paulo, Brazil, 1969.]

RUDALL, P., AND S. DRANSFIELD. 1989. Fruit structure and development in *Dinochloa* and *Ochlandra* (Gramineae-Bambusoideae). *Annals of Botany* 63: 29–38.

RUPRECHT, F. J. 1839. Bambuseas monographice exponit. St. Petersburg: Typis Caesareae Scientiarum.

SALAMIN, N., T. R. HODKINSON, AND V. SAVOLAINEN. 2002. Building supertrees: an empirical assessment using the grass family (Poaceae). *Systematic Biology* 51(1): 136–150.

SAMUEL, R., W. PINSKER, AND M. KIEHN. 1997. Phylogeny of some species of *Cyrtandra* (Gesneriaceae) inferred from the *atpB/rbcL* cpDNA intergene region. *Botanica Acta* 110(6): 503–510.

SÁNCHEZ-KEN, J. G., L. G. CLARK, E. A. KELLOGG, AND E. E. KAY. 2007. Reinstatement and emendation of subfamily Micrairoideae (Poaceae). *Systematic Botany* 32: 71–80.

SANTISUK, T. 2006. Vegetation types in Thailand. Office of the Forest Herbarium; National Park, Wildlife and Plant Conservation Department, Bangkok, Thailand. (in Thai)

SANTISUK, T., T. SMITINAND, W. HOAMUANGKAEW, P. S. ASHTON, S. H. SOHMER, AND J. R. VINCENT. 1991. Plants for our future: botanical research and conservation needs in Thailand. Research publication by USAID/RFD/WWF-US: printed in Bangkok.

SCHLOTTERER, C., AND J. PEMBERTON. 1994. The use of microsatellites for genetic analyses of natural populations. *In* B.S. Schierwater, G. P. Wagner, and R. DeSalle [eds.], Molecular ecology and evolution: approaches and applications, 203–214. Birhauser Verlag, Basel.

SMITINAND, T. 2001. Thai plant names (revised edition). The Forest Herbarium, Royal Forest Department. Bangkok, Thailand.

SODERSTROM, T. R. 1981. Some evolutionary trends in the Bambusoideae. *Annals of the Missouri Botanical Garden* 68: 15–47.

SODERSTROM, T. R. 1987. Bamboo systematics: yesterday, today, and tomorrow. *Journal of the American Bamboo Society* 6: 4–16.

SODERSTROM, T. R., AND C. E. CALDERON. 1979. A commentary on the bamboos (Poaceae: Bambusoideae). *Biotropica* 11(3): 161–172

SODERSTROM, T. R., AND R. P. ELLIS. 1987. The position of bamboo genera and allies in a system of grass classification. *In* T. R. Soderstrom, K. W. Hilu, C. S. Campbell, and M. E. Barkworth [eds.], Grass systematics and evolution, 225–238. Smithsonian Institution Press, Washington, D.C.

SODERSTROM, T. R., AND R. P. ELLIS. 1988. The woody bamboos (Poaceae: Bambuseae) of Sri Lanka: a morphological-anatomical study. Smithsonian Contributions to Botany 72. Smithsonian Institution, Washington, D. C.

SOLTIS, D. E., AND P. S. SOLTIS. 1998. Choosing and approach and an appropriate gene for phylogenetic analysis. *In* D. E. Soltis, P. S. Soltis, and J. J. Doyle [eds.], Molecular systematics of Plants II, DNA sequencing, 1–41. Kluwer Academic Publishers.

STAFLEU, F. A., AND R. S. COWAN. 1979. Taxonomic literature: a selective guide to botanical publications and collections with dates, commentaries and types, Volume II: H-Le (second edition). Bohn, Scheltema & Holkema, Utrecht.

STAFLEU, F. A., AND R. S. COWAN. 1983. Taxonomic literature: a selective guide to botanical publications and collections with dates, commentaries and types, Volume IV: P-Sak (second edition). Bohn, Scheltema & Holkema, Utrecht.

STAFLEU, F. A., AND R. S. COWAN. 1988. Taxonomic literature: a selective guide to botanical publications and collections with dates, commentaries and types, Volume VII: W-Z (second edition). Bohn, Scheltema & Holkema, Utrecht.

STAPLETON, C. M. A. 1993. *Fargesia macclureana*, a Tibetan bamboo in Europe. Bamboo Society (Great Britain) Newsletter 17: 17.

STAPLETON, C. M. A. 1994. The bamboos of Nepal and Bhutan, Part II: *Arundinaria, Thamnocalamus, Borinda,* and *Yushania* (Gramineae: Poaceae, Bambusoideae). *Edinburgh Journal of Botany* 51(2): 275–295.

STAPLETON, C. M. A. 1997. The morphology of woody bamboos. *In* G. P. Chapman [ed.], The Bamboos. *Linnaean Society Symposium Series* 19: 251–267.

STAPLETON, C. M. A. 1998. Form and function in the bamboo rhizome. *Journal of the American Bamboo Society* 12(1): 21–29.

STAPLETON, C. M. A., AND N. H. XIA. 1997. A new combination in *Bambusa* (Gramineae-Bambusoideae). *Kew Bulletin* 52(1): 235–238.

STAPLETON, C. M. A., AND N. H. XIA. 2004. *Qiongzhuea* and *Dendrocalamopsis* (Poaceae-Bambusoideae): publication by *descriptio generico-specifico* and typification. *Taxon* 53(2): 526–528.

STEARN, W. T. 1992. Botanical Latin; history, grammar, syntax, terminology and vocabulary (fourth edition). Redwood Press Ltd, England.

STUESSY, T. F. 1990. Plant taxonomy: the systematic evaluation of comparative data. Columbia University Press, New York.

SUN, Q., Z. NI, Z. LIU, J. GAO, AND T. HUANG. 1998. Genetic relationships and diversity among Tibetan wheat, common wheat and European spelt wheat revealed by RAPD markers. *Euphytica* 99: 205–211.

SUN, Y., N. H. XIA, AND R. LIN. 2005. Phylogenetic analysis of *Bambusa* (Poaceae: Bambusoideae) based on Internal Transcribed Spacer sequences of nuclear ribosomal DNA. *Biochemical Genetics* 43: 603–612.

SUNGKAEW, S., A. TEERAWATANANON, K. JINDAWONG, AND B. THAIUTSA. *unpublished.* Diversity of highland bamboos. unpublished manuscript.

SUNGKAEW, S., A. TEERAWATANANON, W. KORAWAT, AND T. R. HODKINSON. *accepted. Dendrocalamus copelandii*, a new giant bamboo record for Thailand. *Thai Forest Bulletin (Botany).*

SUNGKAEW, S., A. TEERAWATANANON, J. A. N. PARNELL, S. DRANSFIELD, C. M. A. STAPLETON, AND T. R. HODKINSON. *accepted. Dendrocalamus khoonmengii*, a new bamboo species (Poaceae: Bambusoideae) from peninsular Thailand. *Thai Forest Bulletin (Botany).*

SUNGKAEW, S., A. TEERAWATANANON, J. A. N. PARNELL, C. M. A. STAPLETON, AND T. R. HODKINSON. *accepted. Phuphanochloa*, a new bamboo genus (Poaceae: Bambusoideae) from Thailand. *Kew Bulletin.*

SWOFFORD, D. L. 1998. Phylogenetic analysis using Parsimony (PAUP) version 4.0. Sinauer Associates, Sunderland MA.

TABERLET, P., L. GIELLY, G. PAUTOU, AND J. BOUVET. 1991. Universal primers for amplification of three non-coding regions of chloroplast DNA. *Plant Molecular Biology* 17(5): 1105–1109.

TAKHTAJAN, A. 1986. Floristic Regions of the World. University of California Press, Berkeley and Los Angelis, California.

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.

TAUTZ, D. 1989. Hypervariability of simple sequences as a general source for polymorphic DNA markers. *Nucleic Acids Research* 17: 6463–6471.

TAUTZ, D., AND M. RENZ. 1984. Simple sequences are ubiquitous repetitive components of eukaryotic genomes. *Nucleic Acids Research* 12: 4127–4138.

THE INTERNATIONAL PLANT NAMES INDEX. 2005. Published on the internet http://www.ipni.org/index.html [accessed 07 July 2007].

TURLAND, N. J., AND N. H. XIA. 2005. A new combination in Chinese *Aesculus* (Hippocastanaceae). *Novon* 15: 488–489.

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

UBI, B. E., R. KÖLLIKER, M. FUJIMORI, AND T. KOMATSU. 2003. Genetic diversity in diploid cultivars of Rhodesgrass determined on the basis of Amplified Fragment Length Polymorphism markers. *Crop Science* 43: 1516–1522.

VAN STEENIS, C. G. G. J. 1950. The delimitation of Malesia and its main plant geographical divisions. *Flora Malesiana Series* 1: 70–75.

VOSS, E. G., H. M. BURDET, W. G. CHALONER, V. DEMOULIN, P. HIEPKO, J. MCNEILL, R. D. MEIKLE, D. H. NICOLSON, R. C. ROLLINS, P. C. SILVA, AND W. GREUTER, (eds). 1983. International Code of Botanical Nomenclature. Adopted by the Thirteenth International Botanical Congress, Sydney, August 1981. Bohn, Scheltema & Holkema, Utrecht & Antwerpen; W. Junk, The Hague & Boston.

WANG, Z. P., AND C. M. A. STAPLETON. 2006. *Phyllostachys* Siebold & Zuccarini. *Flora of China* 22: 163–180.

WATANABE, M., M. ITO, AND S. KURITA. 1994. Chloroplast DNA phylogeny of Asian Bamboos (Bambusoideae, Poaceae) and its systematic implication. *Journal of Plant Research* 107: 253–261.

WATSON, L., AND M. J. DALLWITZ. 1992. The grass genera of the world. CAB International, Wallingford, Oxon, U.K.

WEBER, J., AND P. MAY. 1989. Abundant class of human DNA polymorphisms which can be typed using the polymerase chain reaction. *American Journal of Human Genetics* 44: 388–396.

WEBERLING, F. 1989. Morphology of flowers and inflorescences (transl. R.J. Pankhurst). Cambridge University Press, Cambridge.

WEISING, K., AND R. C. GARDNER. 1999. A set of conserved PCR primers for the analysis of simple sequence repeat polymorphisms in chloroplast genomes of dicotyledonous angiosperms. *Genome* 42: 9–19.

WHITMORE, T. C. 1984. Tropical rainforests of the Far East (second edition). Oxford University Press, Oxford.

WIDJAJA, E. A. 1997. New taxa in Indonesian bamboos. *Reinwardtia* 11(2): 57–152.

WOLFE, K. H., W. H. LI, AND P. M. SHARP. 1987. Rates of nucleotide substitution vary greatly among plant mitochondrial, chloroplast and nuclear DNA. *Proceedings of the National Academy of Science U.S.A.* 84: 9054–9058.

WONG, K. M. 1986. The growth habits of Malayan bamboos. *Kew Bulletin* 41: 703–720.

WONG, K. M. 1991. Taxonomy of the *Bambusa* complex in Peninsular Malaysia and aspects of its comparative morphology, culm anatomy and flowering behaviour. Ph.D. Thesis, University of Malaya, Kuala Lumpur.

WONG, K. M. 1993a. A revision of *Bambusa* (Gramineae: Bambusoideae) in the Malay Peninsula, with two new species. *Sandakania* 3: 17–41.

WONG, K. M. 1993b. Four new genera of bamboos (Gramineae: Bambusoideae) from Malaysia. *Kew Bulletin* 48(3): 517–532.

WONG, K. M. 1995a. The bamboos of Peninsular Malaysia. Malayan Forest Records No. 41, Forest Research Institute Malaysia (FRIM), Kuala Lumpur, Malaysia.

WONG, K. M. 1995b. The morphology, Anatomy, Biology and Classification of Peninsular Malaysian Bamboos. *University of Malaya Botanical Monographs* 1: 1–189.

WONG, K. M. 2004. Bamboo; the amazing grass. International Plant Genetic Resources Institute (IPGRI), Selangor Darul Ehsan, Malaysia and University of Malaya, Kuala Lumpur, Malaysia.

WONG, K. M. 2005. *Mullerochloa*, a new genus of bamboo (Poaceae: Bambusoideae) from north-east Australia and notes on the circumscription of *Bambusa*. *Blumea* 50: 425–441.

WOODRUFF, D. S. 2003. Neogene marine transgressions, palaeogeography and biogeographic transitions on the Thai–Malay Peninsula. *Journal of Biogeography* 30: 551–567.

XIA, N. H., AND C. M. A. STAPLETON. 1997a. A new combination in *Dendrocalamus* (Gramineae: Bambusoideae). *Kew Bulletin* 52(2): 483–485.

XIA, N. H., AND C. M. A. STAPLETON. 1997b. Typification of *Bambusa bambos* (L.) Voss (Gramineae: Bambusoideae). *Kew Bulletin* 52(3): 693–698

XIA, N. H., AND C. M. A. STAPLETON. 2006a. *Bonia* Balansa. *Flora of China* 22: 49–50.

XIA, N. H., AND C. M. A. STAPLETON. 2006b. *Pseudostachyum* Munro. *Flora of China* 22: 55–56.

XIA, N. H., L. C. CHIA, D. Z. LI, AND C. M. A. STAPLETON. 2006a. *Bambusa* Schreber. *Flora of China* 22: 9–38.

XIA, N. H., L. C. CHIA, D. Z. LI, AND C. M. A. STAPLETON. 2006b. *Bambusa* subg. *Leleba* (Rumphius ex Nakai) P.C. Keng ex L.C. Chia & X.L. Feng. *Flora of China* 22: 19–31.

YANG, H. Q., S. PENG, AND D. Z. LI. 2007. Generic delimitations of *Schizostachyum* and its allies (Gramineae: Bambusoideae) inferred from *GBSSI* and *trnL-F* sequence phylogenies. *Taxon* 56(1): 45–54.

YEH, F. C., R. C. YANG, AND T. BOYLE. 1999. POPGENE: Microsoft Windows-based freeware for population genetic analysis. Release 1.31. University of Alberta, Edmonton.

YI, T. P. 1992. A new genus of Chinese bamboo-*Menstruocalamus*. *Journal of Bamboo Research* 11(1): 38–41.

YOUNG, S. M., AND W. S. JUDD. 1992. Systematics of the *Guadua angustifolia* complex (Poaceae: Bambusoideae). *Annals of the Missouri Botanical Garden* 79: 737–769.

ZHANG, W. 2000. Phylogeny of the grass family (Poaceae) from *rpl16* intron sequence data. *Molecular Phylogenetics and Evolution* 15(1): 135–146.

ZHANG, W., AND L. G. CLARK. 2000. Phylogeny of classification of the Bambusoideae (Poaceae). *In* S. W. L. Jacobs and J. E. Everett [eds.], Grasses: systematics and evolution, 35–42. CSIRO Publishing, Collingwood, Victoria.

Appendix

Molecular protocols

Caution: gloves should be worn at all times.

Protocol 1

Isolation of total genomic DNA using CTAB

Materials	Equipment
Silica gel dried leaf material or material obtained from herbarium specimens 2xCTAB buffer (100 mM Tris-HCl pH 8.0 (use Tris base and set pH using HCl), 1.4 M NaCl, 20 mM EDTA, 2% CTAB w/v) 2-mercaptoethanol CI (24: 1 chloroform: isoamyl alcohol) isopropanol **Remarks;** EDTA= ethylenediaminetetraacetate CTAB= hexadecyltrimethyl ammonium bromide	30 ml re-useable chloroform resistant capped centrifuge tubes Pipette (P200) Tips Mortars and pestles Water bath Weighing boats and balance Scissors Fume hood Centrifuge 3.5 ml transfer pipettes Measuring cylinder 50 ml capped tubes

Procedure

1. 10 ml of 2xCTAB extraction buffer and 40 µl of 2-mercaptoethanol (in a labelled 30 ml re-useable capped centrifuge tube) were preheated to 65°C in the water bath prior to use. Mortars and pestles were also pre-heated in the water bath to the same temperature.

2. Approximately 0.2 g of silica gel dried leaf material or 0.3 g of material obtained from herbarium specimens was cut up into small pieces using clean scissors for every individual.

3. The leaf material, accompanied with a small amount of the extraction buffer, was ground in a pre-heated mortar and pestle into a paste. This material was then combined with the remaining extraction buffer, ground further and swirled gently to suspend the slurry (all work took place in the fume hood).

4. The slurry was then poured back into the centrifuge tube, the lid replaced and the tube incubated at 65°C for at least 10 minutes with occasional mixing.

5. 10 ml of CI was added into the centrifuge tube, the lid replaced and the tube mixed thoroughly. Then the lid was released briefly to release gas and tightened prior to horizontal shaking on a mechanical shaker for approximately 30 minutes.

6. The lids of each tube were then loosened to release gas and balanced by CI to 0.1 g prior to centrifugation at 4,000 relative centrifugal force (rcf) for 10 minutes to separate the layers.

7. The aqueous (upper) phase containing the DNA was removed using a transfer pipette (taking care not to disturb the separation) into a fresh labelled 50 ml tube. The aqueous phase is often clear and colorless but this is not always the case, depending on material quality and type.

8. An equal volume of isopropanol (-20°C) was added into the tube and the tube inverted gently to mix the solutions and precipitate the DNA. All sample tubes were kept in the -20°C freezer for two days or longer to further precipitate the DNA.

Protocol 2

Total genomic DNA washing

Materials	Equipment
Crude tDNA from Protocol 1	Centrifuge
Wash buffer (70% ethanol)	Measuring cylinder
	Fume hood
TE buffer (10 mM Tris-HCl pH 8.0, 1 mM EDTA)	3.5 ml transfer pipettes
	1.5 ml microcentrifuge tubes

Procedure

1. The crude tDNA in isopropanol (-20°C) from the Protocol 1 was centrifuged at 2,000 rcf for 10 minutes to pellet the DNA and then the supernatant was poured off.

2. 3 ml of wash buffer was added with gentle mixing and the sample centrifuged again at 2,000 rcf for 5 minutes to pellet the DNA. The supernatant was again poured off.

3. The sample tube was then gently placed upside down for 5 minutes, or longer, on tissue papers to drain the wash buffer. The sample tube was then turned the right way up for 20 minutes or longer in the fume hood to let the pellet dry further and to evaporate all traces of wash buffer.

4. The pellet was then re-suspended in 0.5 ml of TE buffer and then transferred into a labelled 1.5 ml microcentrifuge tube and stored in freezer overnight or continued immediately for further clean up using the JETQUICK spin column technique.

Protocol 3

Total genomic DNA purification (JetQuick PCR* product purification kit; GenoMed)

Materials	Equipment
Washed tDNA from Protocol 2	Centrifuge
Solution H1 (Binding) stored at room temperature (contains concentrated guanidine hydrochloride, EDTA, Tris/HCl and isopropanol)	1.5 ml microcentrifuge tubes Pipettes (P200, P1000)
Solution H2 (Wash, reconstituted) stored at room temperature (contains ethanol, NaCl, EDTA and Tris/HCl; diluted with 96–100% ethanol prior to use)	Tips JetQuick spin columns and 2 ml receiver tubes
TE buffer (10 mM Tris-HCl pH 8.0, 1 mM EDTA)	

*PCR purification spin columns were used, in preference to genomic DNA kits, for clean up of total genomic DNA as they have been shown by previous researchers to purify total genomic DNA efficiently while maintaining high fragment sizes in the extract. They are much cheaper than commercially available total genomic DNA spin columns (Hodkinson, *personal communication*).

Procedure

1. For the JetQuick (GenoMed) spin column technique, 100 µl of the washed tDNA was transferred into a new labelled 1.5 ml microcentrifuge tube. 400 µl of solution H1 was added and mixed thoroughly.

2. The mixture was then transferred into the prepared JETQUICK spin column that was placed in a 2 ml receiver tube.

3. The column was spun at 12,000 rcf for 1 minute. The solution in the receiver tube was then discarded.

4. The spin column was reinserted into the receiver tube and then 500 µl of solution H2 was added. Then the sample was centrifuged at 12,000 rcf for 1 minute and the solution in the receiver tube poured away. Spinning at 12,000 rcf for 1 minute was needed one more time to make sure the residual solution H2 was completely removed.

5. The spin column was then inserted into a new labelled 1.5 ml microcentrifuge tube. 50 µl of pre-heated (65°C) TE buffer was pipetted directly onto the centre of the silica matrix for DNA elution.

6. The sample was then centrifuged at 12,000 rcf for 2 minutes. In this step, the DNA was already transferred into a new tube. Each eluted tDNA sample was checked for quality and DNA quantity on a 1.5% agarose gel (see Protocol 4) and then stored at -20°C.

Protocol 4

Quality assessment and quantification of total DNA and PCR products using agarose gel electrophoresis

Caution: Ethidium bromide is a mutagen. Gloves must be worn at all times.

Materials	Equipment
tDNA or PCR product	Parafilm
80 ml of 1.5% of agarose gel (1.5 g of agarose gel, 100 ml of 1xTBE, 2 µl of Ethidium Bromide)	Pipette (P2~20) Tips
Loading Dye (Bromophenol Blue 0.25% w/v and Sucrose 40% w/v)	Gel tank
1xTBE buffer solution	Power pack UV light box and Kodak EDAS camera system
Remark; TBE= 0.89 M Tris, 0.89 M Boric acid, 0.02 M NaEDTA	

Procedure

Prior to work, make sure the gel is completely covered by the 1xTBE buffer solution.

1. A 2 µl drop of loading dye was placed on small piece of parafilm.

2. 6 µl of tDNA or 4 µl of PCR product was mixed thoroughly with the loading dye.

3. The mixture was then loaded into the well on the gel.

4. Only 1 µl of the molecular weight ladder (e.g. the 1 KB ladder) was also loaded onto the gel to quantify the DNA fragments.

5. Once all samples were loaded, the tank lid was replaced and the tank connected to the power pack. All samples were then run at about 125 V for 30 minutes.

6. The agarose gel was then placed on the UV light box and the bands of the DNA samples were visualized. The photograph of the bands of DNA on the gel was taken for keeping as the reference using a Kodak EDAS camera system. Samples were quantified by comparing intensity of fluorescence of DNA to known standards or relative degree of fluorescence noted.

Protocol 5

Amplification of target regions of plastid DNA using PCR

Materials	Volume (µl)	Amount/ Concentration	Equipment
Eluted tDNA	2	c. 100 ng	1.5 ml and 0.5 ml (dome lid) microcentrifuge tubes
Master Mix			
Ultra pure sterile water	36.75 (34.75 for *matK*)	-	
10x Reaction Buffer	5	1x	Ice
dNTPs (10 mM each)	1	0.2 mM each	
Forward primer	0.5	50 ng	Pipettes (P2, P10, P20, P200)
Reverse primer	0.5	50 ng	
Bovine Serum Albumin Acetylated (BSA)	1 (only for *matK*)	1 mgml^{-1}	
MgCl$_2$ (25 mM)	4 (5 for *matK*)	2 mM	Tips
Promega *Taq* DNA Polymerase (5 units/µl)	0.25	1.25 units	
			Centrifuge
TOTAL	50		
Remarks; 10x Reaction Buffer= 500 mM KCl, 100 mM Tris-HCl (pH 9.0 at 25°C) and 1.0% Triton® X-100 dNTPs each at 10 mM Primers conc.= 100 ngµl^{-1} The forward and reverse primers for all target regions see Table 2.3 (Chapter 2)			Applied Biosystems GeneAmp® PCR System 9700

Procedure

1. All eluted tDNA samples from Protocal 3 and reagents were placed at room temperature for thawing and then kept on ice. 2 µl of each eluted tDNA sample was transferred in the labelled 0.5 dome lid microcentrifuge tube.

2. The master mix was prepared in the order stated above in a labelled 1.5 ml microcentrifuge tube. The master mix tube was vortexed briefly prior to use.

3. 48 µl of the master mix was aliquoted into individual tubes of tDNA.

4. 50 ml of total reaction volume per sample was centrifuged briefly before being placed on an Applied Biosystems GeneAmp® PCR System 9700. Each cycle (of 30 cycles) of the PCR was carried with the following thermal cycling parameters;

   a. 95°C for 45 sec. of denaturation (94°C for 1 min. for *matK*).

b. 50°C for 45 sec. of annealing (52°C for *atpB-rbcL* and *rps16*; 52°C for 1 min. for *matK*).

c. 72°C for 2 min. of extension (2.5 minutes for *matK*).

A pre-melt of 95°C for 1 min. (94°C for 3 min. for *matK*) and a final extension of 72°C for 7 min. were also included. Upon completion the samples were kept at 4°C until analyzed further.

4. The PCR products were checked on a 1.5% agarose gel (see Protocol 4) and then kept at -20°C for further cleaning.

Protocol 6

Purification of PCR products (JetQuick PCR product purification kit; GenoMed)

Materials	Equipment
PCR product	1.5 ml microcentrifuge tubes
Solution H1 (Binding) (contains concentrated guanidine hydrochloride, EDTA, Tris/HCl and isopropanol)	Pipettes (P200, P1000) Tips
Solution H2 (Wash, reconstituted) (contains ethanol, NaCl, EDTA and Tris/HCl; diluted with 96–100% ethanol before use)	Centrifuge JETQUICK spin columns and 2 ml receiver tubes
Pure water	

Procedure

1. 100 µl of the successful PCR product was transferred into a new labelled 1.5 ml microcentrifuge. 400 µl of solution H1 was added and the samples mixed thoroughly.

2. The mixture was then transferred into the prepared JETQUICK spin column that was placed in a 2 ml receiver tube.

3. The sample was spun at 12,000 rcf for 1 minute. The solution in the receiver tube was then drained.

4. The spin column was reinserted into the receiver tube and 500 µl of solution H2 was added and then the tube was spun at 12,000 rcf for 1 minute and then poured off the solution. Spinning at 12,000 rcf for 1 minute was needed one more time to make sure the residual solution H2 was completely removed.

5. The spin column was then inserted into a new labelled 1.5 ml microcentrifuge tube. 50 µl of pre-heated to 65°C of pure water was pipetted directly onto the center of the silica matrix.

6. The sample was then centrifuged at 12,000 rcf for 2 minutes. In this step, the PCR product was already transferred into a new tube. All clean PCR products were then stored at -20°C.

rotocol 7

ycle sequencing (BigDye terminator kits v.1.1; Applied Biosystems)

Materials	Volume (µl)	Amount/ Concentration	Equipment
Pink mix (Big Dye™ Sequencing Mix version 1.1)	1	-	0.2 ml microcentrifuge tubes (dome lid) 0.5 ml microcentrifuge tubes Ice Pipettes (P2, P10) Tips Centrifuge Applied Biosystems GeneAmp® PCR System 9700
Sterile ultra pure water	1.8	-	
Sequencing buffer	3.5	70 mM Tris, 1.75 MgCl$_2$	
Forward primer	0.7	3.5	
Reverse primer	0.7	3.5	
Clean PCR product	3	-	
TOTAL	<u>10</u>		
Remark; Primers conc.= 5 ngµl^{-1}			

rocedure

. All clean PCR products and sequencing reagents were thawed and then kept on ice until sed.

2. 1 µl of the pink mix was transferred into a labelled 0.2 ml dome lid microcentrifuge tube.

3. A master mix of water, sequencing buffer and the forward primer was made up in a labelled 0.5 ml microcentrifuge tube and then 6 µl of it was added into the reaction tube from above.

4. 3 µl of PCR product for forward primer was then added. Thus, the total reaction volume of each tube was 10 µl.

5. The same procedures were employed with the PCR product for the reverse primer.

6. All sequencing samples were spun down briefly prior to placing on the thermal cycler machine, Applied Biosystems GeneAmp® PCR System 9700. The conditions used in the reactions were as follows;

   a. Denaturation at 96°C for 10 sec.

   b. Annealing at 50°C for 5 sec.

   c. Sequence extension at 60°C for 4 min.

d. All steps were repeated 27 cycles

e. Sequence products were then incubated at 4°C for indefinite time

7. The cycle sequencing products were then kept in a -20°C freezer or directly purified.

Protocol 8

Purification of cycle sequencing product

Materials	Equipment
Cycle sequencing product	0.5 ml microcentrifuge tubes
100% ethanol	Pipettes (P2, P200, P500)
3 M sodium acetate (NaOAc)	Ice
70% ethanol	Tips
	Centrifuge

Procedure

1. The cycle sequencing product was transferred to a labelled 0.5 ml microcentrifuge tube.

2. 50 µl of 100% ethanol and 2 µl of sodium acetate were added into the tube.

3. The sample tube was left at room temperature for 5–10 minutes and then put on ice for 30 minutes.

4. The sample tube was centrifuged at 12,000 rcf for 25 minutes and then the supernatant was drained off.

5. The sample was washed with 300 µl of 70% ethanol and then spun at 12,000 rcf for 15 minutes and also the supernatant then was drained off.

6. The sample was washed with 300 µl of 70% ethanol one more time and then spun at 12,000 rcf for 15 minutes. The supernatant was then completely drained off.

7. All purified cycle sequencing products were laid horizontally between tissue papers at room temperature (overnight) to let the pellets dry and then stored in a -20°C freezer.

Protocol 9

Denaturing of dried cycle sequencing samples prior to sequencing using the ABI Prism™ 310 Genetic Analyzer

Materials	Equipment
Dried cycle sequencing samples Template suppression reagent (TSR) or Hi-Di™ Formamide	Pipette (P200) Tips WhirliMixer™ (Fisons Scientific Equipment) Ice Septa for 0.5 ml microcentrifuge tubes Perkin Elmer DNA Thermo Cycler 480 Centrifuge

Procedure

1. 25 µl of TSR or Hi-Di™ Formamide was added to each dried cycle sequencing sample.

2. The samples were then vortexed slightly and heated up for 5 minutes at 95°C.

3. The samples were then chilled on ice for 5 minutes. The old lids were replaced by the septa needed for the automated sequencer. The samples were vortexed once more and then spun down briefly.