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Systematics of Arundinelleae and Andropogoneae, subtribes Chionachninae, Dimeriinae and Germainiinae (Poaceae: Panicoideae) in Thailand

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

by

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2009

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Declaration

I hereby declare that the contents of this thesis are entirely my own work (except where otherwise stated) and that it has not been previously submitted as an exercise for a degree to this or any other university.

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Abstract

This thesis has provided a comprehensive taxonomic account of tribe Arundinelleae, and subtribes Chionachninae, Dimeriinae and Germainiinae of the tribe Andropogoneae in Thailand. Complete floristic treatments of these taxa have been completed for the Flora of Thailand project. Keys to genera and species, species descriptions, synonyms, typifications, illustrations, distribution maps and lists of specimens examined, are also presented. Fourteen species and three genera of tribe Arundinelleae, three species and two genera of subtribe Chionachninae, seven species of subtribe Dimeriinae, and twelve species and two genera of Germainiinae, were recorded in Thailand, of which *Gamotia ciliata* and *Jansenella griffithiana* were recorded for the first time for Thailand. Three endemic grasses, *Arundinella kerrii*, *A. kokutensis* and *Dimeria kerrii* were described as new species to science.

Phylogenetic relationships among major subfamilies in Poaceae and among major tribes within Panicoideae were evaluated using parsimony analysis of plastid DNA regions, *trnL-F* and *atpB-rbcL*, and a nuclear ribosomal DNA region, *ITS*. A total of 132 grasses from six subfamilies and representatives from five tribes of Panicoideae, Andropogoneae (11 subtribes), Arundinelleae (4 genera), Eriachneae (1 genus), Isachneae (2 genera), and Paniceae (4 subtribes), were analysed. The PACCMAD clade, including Panicoideae, Arundinoideae, Centothecoideae, Chloridoideae, Micrairoideae, Aristidoideae and Danthonioideae, was well resolved. A close relationship between Aristidoideae and Chloridoideae was found. The monophyly of Micrairoideae was resolved but the relationships of three tribes (Eriachneae, Isachneae and Micraireae) within Micrairoideae were not fully resolved, only *Eriachne* and *Isachne* were resolved as monophyletic.

Panicoideae s.s. were supported as monophyletic and sister to a clade of *Danthoniopsis* and *Tristachya*. Within Panicoideae, only a clade of tribe Andropogoneae + *Arundinella* + *Gamotia* was supported by bootstrapping. None of the analyses supported the monophyletic status of tribe Paniceae. Within Paniceae, the bristle clade (excluding *Cenchrus*) + a rare Australian non-bristle-bearing genus, *Alexsloidyia*, and the forest shade clade, were found, but their circumscription remains ambiguous. A sister relationship between *Homopholis* and *Walwhalleya*, which are endemic and rare Australian Paniceae grasses, was also resolved. Tribe Arundinelleae was found to be polyphyletic. This study supported the separation of *Arundinella* and *Gamotia* from the remaining Arundinelleae and the inclusion of both genera in their own subtribes (Arundinellinae Honda s.s. and Garnotiinae Pilger) within the
Andropogoneae. Tribe Arundinelleae should be abandoned as a taxonomic group within the centothecoid + panicoid clade. Tribe Andropogoneae were found to be monophyletic only if *Arundinella* and *Garnotia* were included. Only five out of a total of 11 subtribes from Andropogoneae, including Chionachninae, Coicinae, Dimeriinae, Germainiinae and Tripsacinae, were supported as monophyletic. This was the first time that Dimeriinae and Germainiinae were included in a molecular study. Five distinct groups within Andropogoneae corresponding to three genera from subtribe Rottboelliinae (*Hackelochloa, Hemarthria* and *Mnesithed*), an agamic complex (*Bothriochloa, Capillipedium* and *Dichanthium*), a sub-basal awn group (*Arthraxon* and *Thelephora*), the core Andropogoneae genera (*Hyparrhenia Cymbopogon, Schizachyrium* and *Andropogon*), and a *Themeda* group, were supported as monophyletic.

Plastid microsatellites or simple sequence repeat (SSR) DNA markers in conjunction with Nei's (1978) unbiased genetic distance and UPGMA were employed to assess the intrapopulational and interpopulational variations of two forest grasses with contrasting breeding system, *Arundinella setosa* and *Garnotia tenella*. A total of seven haplotype and four groups from 11 populations of *A. setosa*, and a total of 11 haplotypes and four groups from eight populations of *G. tenella*, were defined. The patterns of haplotype distributions of both grasses reflect patterns of plant dispersal via seed. The low population genetic differentiation among populations in both grasses (*Arundinella = 0.049; Garnotia = 0.155*) suggested that the most variation in haplotypes is distributed within populations and not among populations. The high value of genetic variation within *A. setosa* and *G. tenella* populations suggests that they consist of multiple genets. The high value of genetic diversity but low gene flow estimates of both grasses, found in Northern Thailand, can be affected by topographic barriers and habitat fragmentation. No obvious correlations of biogeographical distribution in *A. setosa* and *G. tenella* were found that were consistent with previously defined Thai floristic regions but groupings in the analysis indicated a general east-west divide.
Acknowledgements

First of all, I would like to thank my family for their support, encouragement, and unshakable faith in my abilities during the course of my studies.

I wish to thank my great supervisor Dr. Trevor Hodkinson for his enthusiasm, support, friendship and for all the encouragement he gave me during my Ph.D. study.

I also wish to thank those who helped me in collecting grass specimens Drs. Simon Laegaard, Surrey Jacobs, Trevor Hodkinson, Sarawood Sungkaew and Ms Narumon Kritsanachandee.

I am also grateful to thank 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 Mr. Laszlo Csiba when I was in the Jodrell Laboratory of Kew.

I would like to express my gratitude to Prof. Dr. Pranom Chantaranothai for his kindness, comments and useful suggestions and Drs. Pimwadee Pornpongrungrueng, Paweena Traiperm for their support of the work, and Prof. Dr. Benjamin Øllgaard for his kindly suggestions and for supplying the Latin diagnosis.

Many thanks to all curators and the staff of the following herbaria: AAU, ABD, B, BK, BKF, BKU, BM, C, CMU, E, GH, K, KKU, L, NY, QBG, SING and TCD for the use or loan of specimens.

Thanks very much to all members of the Botany Department from Trinity College. I will not forget Ireland!

Last, but not least, a special thanks to Prof. Dr. John Parnell, for his very kind help, encouragement, and support throughout my research.

This work was supported by the TRF/BIOTEC Special Program for Biodiversity Research and Training Grant BRT_148026, the Trinity College Dublin Postgraduate Travel Reimbursement Fund, Natural History Museum, National Science Museum, Technopolis, Pathum Thani, Thailand.

This thesis and the work carried out herein are dedicated to the memory of Dr. Jarujin Nabhitabhata (Director, Natural History Museum, Thailand) 1950-2008.
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1.1 General introduction

The monocotyledon grass family (Poaceae, nom. altern. Gramineae) is an extraordinarily successful evolutionary lineage. It is not only one of the largest families of flowering plants in terms of species number, including approximately 10,000 species in over 700 genera (Clayton and Renvoize, 1986; Tzvelev, 1989; Watson and Dallwitz, 1992; GPWG, 2001; Mabberley, 2008), but grasses are also ecologically dominant, covering large areas of ground in a wide variety of habitats throughout the world such as grasslands, steppes and savannahs. Moreover, grasses are the most economically important plants of the world. They provide the staple foods of humans and provide a great part of the food for grazing animals. Basically, Poaceae consist of bamboos and grasses. The bamboos are woody plants with complex rhizome and branching systems, relatively broad and pseudopetiolate leaves and disarticulating, fusoid and arm cells within leaf blades, prominent leaf-sheaths with rudimentary blade (culm-sheaths), bracteate inflorescences and trimerous flowers (e.g. Clayton and Renvoize, 1986; Watson and Dallwitz, 1992; GPWG, 2001). The grasses are herbaceous plants of which the above ground parts are annual or only short-lived (Clayton and Renvoize, 1986; Clark and Pohl, 1996). Morphologically, grasses are very distinctive from the most other plants, and grasses have evolved unique reproductive and vegetative features. In this introduction the basic morphology of grasses will be provided, explaining the detailed structural features which will be used to identify and describe taxa later in this study. The information presented here is mainly concerned with the common or herbaceous grasses, excluding the bamboos.

1.1.1 Morphology

Habit

Grasses (excluding bamboos) are generally herbaceous and can be both annual and perennial. They most commonly grow in a tuft consisting of several shoots or tillers clustered together at the base. In a tuft, the tillers are joined at the base by very short stems and this growth habit is also known as caespitose (Figure 1.1/A). In some grasses the outer tillers of a tuft may grow out horizontally at first and then curve upwards. This type of culm can be ascending or decumbent or semi-prostrate at the base (Figures 1.1/B, D). In perennial grasses, the shoots
can form a densely tufted growth habit, known as tussock. Many grasses produce horizontal stems which have culms arising at the nodes of the horizontal rooting stems. Such horizontal stems are called stolons (Figure 1.1/C) if they are above the ground or rhizomes if they are underground.

**Vegetative parts**

The aerial stems of grasses are known as culms and normally terminate in an inflorescence. Grass culms are made up of a series of nodes and internodes (Figure 1.1/E). Nodes are transverse septa which are thickened and serve to strengthen the culm. Most internodes are hollow or sometimes, solid, as in sugarcane (*Saccharum officinarum* L.) and maize (*Zea mays* L.). The outline of culms is usually terete or elliptic in transverse section. The culms may be simple or branched. The new branch may grow up within the sheath and emerge at its apex. This branching is described as intravaginal branching and results in tufted clumps. In other cases, the new branch may break through the base of the sheath and emerge and grow outward. This is called extravaginal branching. Rhizomes and stolons are the result of this branching (Dahlgren *et al.*, 1985; Clark and Pohl, 1996).

Grass leaves are borne singly and originate at the nodes in two ranks along the culm. In most grasses, leaves are always alternate, except in *Micraria*, where they are three-ranked. The leaves consist of three distinct parts: a sheath, a blade and a ligule. The sheath originates at the node and usually clasps the culms with overlapping margins. In some grasses the margins may be fused from the base upwards for varying distances. The surface of the sheath is usually ridged and very often hairy and/or scabrid or sometimes is armed with irritating hairs, as for instance *Polystota wallichiana* Benth. and *Rottboellia exalata* L.f. At the top of the sheath is the blade which is the chief organ of photosynthesis of the grasses. The leaf blade is generally flat, folded (conduplicate) or rolled lengthwise (involute or convolute). The rolling or folding is a response to water stress in some cases (Bor, 1960; McCusker, 2002). Grass leaf blades are generally narrow, but in a few grasses they may be relatively broad, either elliptical in outline or with a broad heart-shaped base. The vein pattern of the grass blade is composed of longitudinal veins of different orders. A large longitudinal midrib runs up the centre of the blade and is accompanied by several parallel veins on each side. In some (e.g. *Centaurea lappacea* Desv. and *Leptaspis ureolata* R. Br.) there are distinct cross-veins connecting the longitudinal veins. The cross nerves result in tessellate venation (Bor, 1960).
Figure 1.1 Grass habit. A. caespitose, erect; B. tufted, ascending; C. stoloniferous; D. tufted, semi-prostrate; E. culm of vegetative grass plant, showing nodes, leaf-sheaths and blades; F. ligule membranous, triangular; G. ligule a fringed membrane; H. ligule a row of long hairs; I. ligule a fringe of short hairs; J. Ligule membranous, showing auricles. A-D, F-I from McCusker (2002); E from Clayton et al. (1974); J from Dahlgren et al. (1985).

At the junction of the sheath and the blade, on the side facing the stem the inside or adaxial surface, is a structure called the ligule. The ligule of a grass is an important character for identification. It can be defined as a membrane-like tissue (Figures 1.1/F, G). Alternatively, it may be absent or may be a row of delicate hairs (Figures 1.1/H-I). In the same area of the
ligule, there are another two structures that can be found in some grasses: auricles and a collar. The collar is usually a line of demarcation which has an abscission line where the blade disarticulates (Bor, 1960; McCusker, 2002). The auricle is a claw-like appendage borne on either side at the base of the blade. Auricles are sometimes considered as the extensions of leaf margins (Figure 1.1/J) (Dahlgren et al., 1985; McCusker, 2002).

**Reproductive parts**

As in the other plants, the inflorescence of the grasses is the flowering part of a plant, or a flower cluster, or the arrangement of the flowers on the flowering axis. However, the structure of grass inflorescence is far more complex than those of plants due to the unusual floral morphology and complex structure (e.g. Dahlgren et al., 1985; Clayton and Renvoize, 1986; Gould, 1986; Clark and Pohl, 1996; Kellogg, 2000; Clark, 2004). Basically, the grass inflorescence is delimited at the base by the node of the uppermost leaf. The entire inflorescence of the grass is produced on a shoot which may be terminal or axillary. Although there are various types of inflorescences, the three most common types in grasses are spikes, racemes and panicles (Clayton and Renvoize, 1986; Clark and Pohl, 1996). A spike is an inflorescence with a single axis and spikelets (sessile spikelets) attached directly to the rhachis (Figure 1.2/A), while in a raceme, the spikelets are attached to the rhachis by their pedicels (Figure 1.2/B). A panicle is the most common type of grass inflorescence in which spikelets are borne on branches of the main rhachis or on further branches of these. Panicle branches may be spreading or diffuse, in which case the inflorescence is called as an open panicle (Figure 1.2/C), or the branches may be short and close to the rhachis, in which case the inflorescence is described as a compact or dense panicle (Figure 1.2/D). If the branches of panicle inflorescence are short and crowned together, the panicle is narrow and described as a spike-like panicle (Figure 1.2/E). However, many inflorescences are intermediate in type. Two or more racemes or spikes may be grouped in a panicle-like inflorescence. If the axis is very short, such a panicle is called subdigitate, or digitate (Clark and Pohl, 1996) (Figures 1.2/F, G). In some tropical grasses, the branches of the panicle axis are subtended by a leaf-like structure or a sheathing bract. This is called spatheate panicle or false-panicle (Bor, 1960; McCusker, 2002) (Figure 1.2/H).

The basic unit of the inflorescence is not a single flower but it is the unit called the spikelet. The spikelet consists of one or more flowers or florets arranged alternately on each side of a spikelet axis called the rhachilla (Figures 1.3/A, C). The base of the spikelet or floret is sometimes enlarged into a little knob or stalk called callus (Clayton and Renvoize, 1986;
According to its shape and texture of the callus which is often hard, sharp and penetrating and frequently bearded, it may have an important function in the dispersal of the propagule (Bor, 1960; McCusker, 2002). At the base of every spikelet are two empty bracts on each side known as the lower and upper glumes (Figures 1.3/A, B) (Bor, 1960; Gilliland, 1971; Dahlgren et al., 1985; Clayton and Renvoize, 1986; Clark and Pohl, 1996; McCusker, 2002). Typically, the flowers of grasses are very small and much reduced. The reduced flower of grasses consists of two or three stamens, a single ovule ovary with two free styles and plumose stigmas, and two tiny basal fleshy structures called lodicules (Figure 1.3/E). The lodicules are considered to represent a reduced perianth. At flowering time, lodicules swell and force open the enclosing flower bracts (Clayton and Renvoize, 1986; McCusker, 2002). The flower is immediately protected by two bracts; the lower one called the lemma, which is homologous to a subtending bract, and the upper one called the palea which is widely interpreted as a prophyll (Linder, 1987; Clayton, 1990; Soreng and Davis, 1998; GPWG, 2001; Clark, 2004). This tiny flower with its lemma and palea is called a floret (Figures 1.3/C-D) (Clayton and Renvoize, 1986; Clark and Pohl, 1996; McCusker, 2002). The spikelets of grasses are very variable in their structures. The diversity of spikelet structure mainly depends on the number of florets, variation in size and shape of glumes, lemmas and paleas, and the sexuality of the florets (Figures 1.3/H-P). The glumes and lemmas may be modified and vary in size, shape, texture or may be absent, while the paleas are often of a different shape and texture from the lemma. The palea is mostly thin, hyaline and two-keeled with its margins clasped by the lemma (Bor, 1960; Gilliland, 1971; Dahlgren et al., 1985; Clayton and Renvoize, 1986; McCusker, 2002).

The grass fruit is one-seeded and indehiscent (not opening at maturity) and consists of the mature fertilized ovary or seed and the pericarp which is normally fused to the seed coat. This type of fruit is called a grain or caryopsis (Bor, 1960; Dahlgren et al., 1985; Clayton and Renvoize, 1986; McCusker, 2002). In some genera, Sporobolus R. Br. and Eleusine Geartn., the fruit is nutlet or achene in which a pericarp is separated from the seed coat (Dahlgren et al., 1985; McCusker, 2002). Grass seed consists of the endosperm, which provides nourishment for the seedling, and a small embryo lying at the base on one side of the caryopsis. The embryo is attached to the endosperm by a flat haustorial cotyledon which is the organ of nutrient transfer and known as the scutellum. On the other side, diametrically opposite, is the hilum which is considered as an attachment scar of the ovule to the pericarp wall (Clayton and Renvoize, 1986). The embryo comprises a special outer sheath or the coleoptile, which is a protective sheath protecting the shoot tip or the plumule, the coleorhiza and the epiblast (Figures 1.3/F, G) (Dahlgren et al., 1985; Clayton and Renvoize, 1986).
Figure 1.2 Inflorescence types. A. spike (*Lolium*); B. raceme (*Briza*); C. open panicle (*Cytococcum*); D. dense panicle (*Hymenachne*); E. spike like panicle (*Deyeuxia*); F. digitate (*Chloris*); G. subdigitate (*Microstegium*); H. spatheate panicle (*Themeda*). A–C, E from McCusker (2002); C and F from Gilliland (1971); G from Noltie (2000); H. from Pilger (1940).
Figure 1.3 Grass spikelets. A. diagram of a grass spikelet; B. spikelet; C. floret; D. floral diagram; E. grass flower, showing two lodicules, three stamens and two fused carpels. F. two views of grains (caryopsyes); G. section through the embryo; H. spikelet with single floret (Sporobolus); I. spikelets with glumes as long as spikelet, enclosing two florets (Isachne); J. spikelets with one sterile floret below one fertile floret (Panicum); K. spikelet with one fertile and two sterile florets (Chloris) L-M. spikelets with many florets (Catapodium and Eragrostis); N. dissimilar spikelet pair with one sessile and pedicelled spikelets (Hackelochloa); O. similar spikelet pair with one sessile and pedicelled spikelets (Sorghum); P. spikelet triad with one sessile and two pedicelled spikelets (Capillipedium). A, F from Clarke and Pohl (1959); B-D, G from Clayton and Renvoize (1986) E-P from McCusker (2002).
1.1.2 Major groups of grasses and their classification

Historically, Poaceae were considered to be closely related to Cyperaceae within the order Cyperales based on floral reduction and chemical characters (Engler, 1892; Cronquist, 1981). However, the phylogenetic studies, using morphological and molecular characters, have shown that many morphological similarities of these two families are convergent. Poaceae were placed in Poales with Anarthriaceae, Centrolepidaceae, Ecdieiocoleaceae, Flagellariaceae, Joinvilleaceae and Restionaceae (Dahlgren et al., 1985; Kellogg and Campbell, 1987; Linder, 1987; Doyle et al., 1992; Kellogg and Linder, 1995). These analyses have identified Joinvilleaceae as a possible sister group of Poaceae. In the most recent classification of the angiosperms, this group is known as the 'graminid clade or core Poales' (APG II, 2003; Soltis et al., 2005). Poales as circumscribed by APG II (2003), Chase (2005) and Soltis et al. (2005) now included 17 families, namely Anarthriaceae, Bromeliaceae, Centrolepidaceae, Cyperaceae, Ecdieiocoleaceae, Eriocaulaceae, Flagellariaceae, Hydatellaceae, Joinvilleaceae, Juncaceae, Mayacaceae, Poaceae, Rapateaceae, Restionaceae, Thurniaceae, Typaceae and Xyridaceae. Within Poales sensu APG II (2003), Poaceae are sister to the Australian family, Ecdieiocoleaceae (Bremer, 2002) and have Joinvilleaceae, Flagellariaceae, and the Restionaceae clade (Centrolepidaceae + Restionaceae, Anarthriaceae), as successive sister taxa (Chase, 2005; Soltis et al. 2005).

The history of Poaceae classification can be divided into three periods. The first period began from pre-Linnean days until about 1930 (Bor, 1960; Clayton, 1981b; Campbell, 1985). All of these studies were based on morphological characters. In 1814, it was Robert Brown who described the spikelet as a modified inflorescence and used the terms of an outer envelope or gluma (glumes) and the inner envelope (lemma and palea) and the squamae (lodicules) (Figure 1.3). He subdivided Poaceae into two groups: the Paniceae (Panicoideae of modern authors) and Poaceae (Festucoideae of Hitchcock and Chase, 1950 and Pooideae of modern authors) based on the spikelet composition and compression. This classification was formalized by Bentham (1878) and was retained by Bentham and Hooker (1883) and Hackel (1887) and used as a basis for the classification of Poaceae by later authors until the 20th century.

The second period of Poaceae classification, which lasted from 1930 to approximately 1990, expanded with the application of anatomy, cytology, embryology and biochemistry data in plant taxonomy (GPWG, 2001; Clark, 2005; Hilu, 2007). On the basis of these data, several systems of grass classification have been proposed to support different evolutionary perspectives. New subfamilies were recognised with the number varying from three to 13
subfamilies (e.g. Roshevits, 1946; Pilger 1940, 1956; Tateoka, 1957; Stebbins, 1956; Caro, 1982; Hilu and Wright, 1982; Clayton and Renvoize, 1986; Watson and Dallwitz, 1988; Tzvelev, 1989; Table 1, GPWG, 2001). The major change during this period was that Pooideae (Festucoideae) was divided into several subfamilies, while Panicoideae were retained almost without modification. By the end of this period, five to seven subfamilies were usually recognised based either on phenetic analyses or presumed evolutionary relationships (Hilu and Wright, 1982; Clayton and Renvoize, 1986; Watson and Dallwitz, 1988; Watson and Dallwitz, 1992). Among these extensive studies was the classification of Clayton and Renvoize (1986) in which Poaceae were divided into six subfamilies based on phenetic methods and evolutionary classification (Table 1.1). They also provided comprehensive diagrams of evolutionary relationships within and among the tribes of their subfamilies, especially at the tribal and generic levels (Figure 1.4), and also provided diagnostic generic descriptions for all genera of the family. Their hypotheses have been used as a starting point for much subsequent work.

The third period was from about 1990 onwards. It started with the development of increasingly powerful computers, the efficient software suitable for the analysis of large molecular and morphological datasets, and the ability to discover a phylogenetic signal in molecular sequence data. The first attempt to investigate phylogenetic trends in Poaceae was by Kellogg and Campbell (1987) using morphological and anatomical characters. Their result supported four main subfamilies, namely Pooideae, Panicoideae, Chloridoideae and Bambusoideae. Later on, three large monophyletic groups: Pooideae, Andropogoneae and Bambusoideae were tested by adding more data in order to examine their relationships within their clade by Kellogg and Watson (1993). Since then, the use of DNA sequence data became popular. Grass classification has been influenced by many phylogenetic studies in order to gain information on the evolutionary relationships using all three genomes, nuclear, plastid and mitochondrial (e.g. Hamby and Zimmer, 1988; Hsiao et al., 1999; Mathews and Sharrock, 1996; Mathews et al., 2000) and the plastid gene regions (e.g. Doebley et al., 1990; Davis and Soreng, 1993; Cummings, 1994; Nadot et al., 1994; Clark et al., 1995; Baker et al., 1995; Duvall and Morton, 1996; Liang and Hilu, 1996; Hilu et al., 1999). More recent phylogenetic studies proposed new classifications of Poaceae using the combined datasets either to produce comprehensive phylogenetic trees for the grasses or to revise the subfamilial classifications of Poaceae (e.g. GPWG, 2001; Hodkinson et al., 2007a; Sánchez-Ken et al., 2007; Bouchenak-Khelladi et al., 2008). The GPWG (2001) and Sánchez-Ken et al. (2007) have classified the grass family into 13 subfamilies (i.e. Anomochloideae, Pharoideae, Puelioideae, Bambusoideae, Ehrhartoideae, Pooideae, Panicoideae, Arundinoideae, Centothecoideae, Chloridoideae, Micrairoideae, Aristidoideae and Danthonioideae (Table 1.1) with families
Joinvilleaceae and Ecdieiocoleaceae as its sister groups. The earliest diverging lineages were recognized as subfamilies Anomochlooideae, Pharoideae and Puelioideae, respectively. The rest of the grasses could be split into two major lineages, the BEP clade and the PACCMAAD clade (Figure 1.5).

Figure 1.4 One of the approximation diagrams of Clayton and Renvoize (1986) showing the relationships among the Poaceae tribes and subfamilies.
Figure 1.5 A summary tree of showing subfamilial and intertribal relationships based on the analysis of three gene regions: ○ weak support (55–75 BP); ◆ moderate support (75–85 BP) and : ◉ strong support (85–100 BP). Adapted from Bouchenak-Khelladi et al. (2008).
Panicoideae

Subfamily Panicoideae, including approximately 3,270 species in 206 genera (GPWG, 2001), are most abundant in the tropics and subtropics, particularly in the mesic portions of such regions, but many species also grow in temperate regions of the world (Clayton and Renvoize, 1986; GPWG, 2001). The subfamily was first recognised as a group by Brown (1814) on the basis of spikelet structure having two florets per spikelet with the lower of which is staminate or neuter. In addition, all members of Panicoideae were found to have distinctive simple starch grains in the endosperm (Figure 1.6/E-G) (Tateoka, 1962; Clayton and Renvoize, 1986).

According to Clayton and Renvoize (1986), Panicoideae comprise eight tribes which are divided into two groups of tribes; the first is centered on the tribe Andropogoneae and associated tribe Arundineae, and the second is centered on the tribe Paniccae and its associated minor tribes Isachneae, Neurachneae, Hubbardiceae, Steyermarkochloeae and Eriachneae (Figure 1.4). The latter two tribes were placed in Arundinoideae by Watson and Dallwitz (1992) (Table 1.1).

The GPWG (2001) divided the Panicoideae into six tribes because the Eriachneae was recognised as incertae sedis. The monophyly of Panicoideae sensu GPWG (2001) has been supported by both morphological and molecular data from chloroplast and nuclear genes (e.g. Kellogg and Campbell, 1987; Davis and Soreng, 1993; Baker et al., 1995, 1999; Gomez-Martinez and Culham, 2000; GPWG, 2001; Mathews et al., 2000; Aliscioni et al., 2003). More recent phylogenetic studies showed that Panicoideae comprise five tribes because Eriachneae and Isachneae were placed within Micrairoideae (Sánchez-Ken et al., 2007). Furthermore, the subfamily has been found to form a monophyletic group with Gynerium and Centothecoideae (Sánchez-Ken and Clark, 2001; Sánchez-Ken and Clark, 2007; Bouchenak-Khelladi et al., 2008).
Figure 1.6 Comparison of spikelets and starch grains between pooid and panicoid grasses. A. diagram of a multi-flowered pooid (Pooideae) spikelet with apical reduction; B. compound starch grains (Festuca); C. compound starch grains (Eragrostis); D. spikelet (Festuca); E. diagram of two-flowered panicoid (Panicoideae) spikelet with basal reduction; F. simple starch grains (Sorghum); G. spikelet (Panicum). Dotted indicate structures that are usually absent. A, E from Clark (2004); B-C, F from http://www.fhsu.edu/biology/thomasson/starch.htm; D, E from Chapman (1996).

Arundinelleae

Arundinelleae comprise 12 genera and about 175 species. Most of them are distributed in tropical regions of the old world (Clayton and Renvoize, 1986). The tribe was established by Stapf (1898) in his account of Poaceae for the Flora Capensis. He realized the close relationship existing between Arundinella Raddi, Tristachya Nees and Trichopteryx Nees., and grouped them together in a new tribe. Arundinelleae are characterised by having two-flowered spikelets with persistent glumes, lower florets male or reduced and upper florets perfect and awned. Subsequently, this classification has been most generally accepted and developed by many studies such as Hubbard (1936), Keng (1936), Conert (1957), Clayton (1967) and Phipps (1967), including the phenetic studies of Phipps (1969), Clayton (1971) and Hilu and Wright (1982).
The most recent classification of Arundinelleae is the study of Clayton and Renvoise (1986) using morphological and anatomical characters that recognised 12 genera in the tribe, namely Arundinella Raddi, Chandrasekharania Nair, Danthoniopsis Stapf, Dilophotriche (C. E. Hubb.) Jac.-Fél., Garnotia Brongn., Gilgiochloa Pilger, Jansenella Bor, Loudetia Hochst. ex Steud., Loudetiopsis Conert, Trichopteryx Nees., Tristachya Nees. and Zonotriche (C. E. Hubb.) Phipps. They also found that two genera: Chandrasekharania and Jansenella, which were placed as the most primitive grasses within the tribe, are C₃ grasses, and the rest of the tribe are C₄ grasses (Figure 1.7). Additionally, many molecular studies have questioned the monophyly of Arundinelleae including studies with rbcL, ndhF, GBSSI and ITS sequences (Barker et al., 1995; Clark et al., 1995; Mason-Gamer et al., 1998; Hsiao et al., 1999; Spangler et al., 1999; Kellogg, 2000; Giussani, 2001; Aliscioni, 2003). In these studies, members of this group appear in different and distantly related clades and it is clear that further studies are required to recircumscribe this tribe.

Figure 1.7 A diagram of relationships in tribe Arundinelleae, showing the diversity of spikelet morphology in the tribe. Adapted from Clayton and Renvoise (1986). Spikelet pictures obtained from Hubbard (1943), Clayton et al. (1974) and Gould (1972).
Andropogoneae

Tribe Andropogoneae contains 85 genera and about 1,000 species (Clayton and Renvoize, 1986; GPWG, 2001). It also includes the important crop plants such as maize, sugarcane and sorghum (*Sorghum bicolor* (L.) Moench). The grasses of this tribe are for the most part tropical or subtropical, although a few are found in warm temperate regions (Clayton and Renvoize, 1986). The members of Andropogoneae are highly diverse in inflorescence and spikelet structures (Figure 1.9). This extensive morphological variation among members of Andropogoneae has been documented by many studies (e.g. Bentham, 1882; Hackel, 1889; Pilger, 1940; Roberty, 1960; Clayton, 1972, 1973; Clayton and Renvoize, 1986; Watson and Dallwitz, 1992).

Andropogoneae can be described as a ‘natural group’ based mainly on the presence of paired spikelets (one sessile and the other pedicellate) and the fragile racemes (Clayton, 1972; Clayton and Renvoize, 1986). According to anatomical characters, all genera in the tribe have C4 photosynthesis, and use the NADP-malic enzyme for decarboxylating the 4-carbon product of photosynthetic carbon fixation (Clayton and Renvoize, 1986; Hattersley and Watson, 1992; Watson and Dallwitz, 1992). They also have a single sheath of cells around the vascular bundle, reflecting a reduction in the amount of light harvesting chlorophyll-α, and chlorophyll-β binding proteins (Sheen and Bogorad, 1986; Sinha and Kellogg, 1996).

Within the tribe, Hackel (1889) recognised five subtribes in his monograph of the Andropogoneae (Figure 1.8). This subtribal classification has been the most widely used for Andropogoneae by many studies, e.g. Pilger (1940), Roberty (1960), Clayton (1972, 1973, 1981a) and Clayton and Renvoize (1986). In most accounts, the monoecious taxa are included in the Maydeae, and the tribe was excluded from the Andropogoneae, except in the phenetic studies of Clayton (1973, 1981a) and Clayton and Renvoize (1986). According to Clayton (1972), Andropogoneae were previously informally classified into two major groups based on the presence or absence of awns of the upper lemma. After that, Clayton and Renvoize (1986) classified the tribe into 11 subtribes, whereas Watson and Dallwitz (1992) recognised Andropogoneae as the supertribe and divided it into two tribes: the Andropogoneae, comprising the awned and the awnless upper lemma species, and the Maydeae including all monoecious taxa from three subtribes, according to Clayton and Renvoize (1986) (Table 1.1).

The first phylogenetic study of Andropogoneae was undertaken by Kellogg and Watson (1993) using morphological characters for all its genera. Their result revealed significant differences between awned and awnless taxa and supported Andropogoneae and Maydeae.
sensu Watson and Dallwitz (1992) as monophyletic. However, they found that most of the subtribes defined by Clayton and Renvoize (1986) were not monophyletic. Recent molecular studies using chloroplast and nuclear genes found that the monophyly of the tribe and its sister group relationship with *Arundinella* are strongly supported (e.g. Manson-Gamer et al., 1998; Spangler et al., 1999; Gomez-Martinez and Culham, 2000; Kellogg, 2000; Mathews et al., 2002; Rondeau et al., 2005; Skendzic et al., 2007), but did not find support for the subtribal designations of Clayton and Renvoize (1986).

**Chionachninae**

Chionachninae is a Southeast Asian and Australian subtribe of Andropogoneae comprising about four genera and 12 species (Clayton, 1981a; Jannink and Veldkamp, 2002). The members of this subtribe were considered to belong to tribe Maydeae due to their unisexual spikelets in which upper lemmas are unawned (Bentham, 1882; Hackel, 1889; Henrard, 1931; Pilger, 1940; Nirodi, 1955; Bor, 1960; Watson and Dallwitz, 1988, 1992). Maydeae were divided into three subtribes: Coicinae, Tripsacinae and Chionachninae by Clayton (1973, 1981). Among these subtribes, Chionachninae are characterised in that sessile female spikelets enclose the inflorescence rachis and are accompanied by more or less reduced male spikelets. Chionachninae has been presumed to be a derivation from Rottboellinae (Clayton and Renvoize, 1986). Chionachninae were found to be polyphyletic based on morphological phylogenetic analysis (Kellogg and Watson, 1993) in which the members of the tribe were grouped together with other monoecious taxa. However, molecular data are available only on genus *Chionachne* (e.g. Spangler et al., 1995; Hsiao et al., 1999; Lukens and Doebley, 2001; Mathews et al., 2002; Aliscioni et al., 2003; Bomblies and Doebley, 2005; Skendzic et al., 2007). The analyses showed that *Chionachne* was placed with *Tripsacum + Zea* clade as sister to the member of Rottboellinae. However, the support for these relationships was low (Lukens and Doebley, 2001; Bomblies and Doebley, 2005; Skendzic et al., 2007).

**Dimeriinae**

The monogeneric subtribe Dimeriinae, consisting of approximately 40 species, is distributed from India, China to Indonesia, Australia and Madagascar (Clayton and Renvoize, 1986; Clayton et al., 2006). Previously, *Dimeria* were grouped together with *Apocotis* and *Arthracon* in subtribe Arthraxeae (Bentham, 1882). Later on, *Dimeria* was recognised as subtribe Dimeriinae by Hackel (1887, 1889) based on its single spikelet with no trace of the pairing (Figure 1.9). This character isolated the subtribe from all other Andropogoneae (Clayton, 1972). Clayton
and Renvoize (1986) suggested that Dimeriinae were derived from subtribe Ischaeminae based on the suppression of the sessile spikelet. The phylogenetic analysis of Kellogg and Watson (1993), based on morphological data, showed that *Dimeria* was sister to *Cleistachne*, with both genera nested in a Saccharinae clade. Nevertheless, no molecular analysis has been conducted for this monotypic subtribe.

**Germainiinae**

Germainiinae is a small subtribe comprising three genera and 27 species. *Apocopis* and *Germainia* are found in Asia and Australia only and *Trachypogon* is found in Africa and Tropical America (Clayton and Renvoize, 1986). Bentham (1882) treated *Germainia* and *Trachypogon* as members of subtribe Euandropogoneae, while *Apocopis* was placed in subtribe Arthraxeae. *Apocopis* was later treated as a member of subtribe Ischaeminae by Hackel (1889). Pilger (1940) subsequently recognised *Apocopis* and *Sclerandrium* (now *Germainia*) as belonging to subtribe Saccharinae, but treated *Germainia* and *Trachypogon* as belonging to subtribe Andropogoninae. According to numerical analysis, *Germainia*, including *Chumsriella* (now *Germainia*) and *Sclerandrium* were placed with *Trachypogon* in the new subtribe Germainiinae by Clayton (1972) on the basis of a shared reduction in the sessile spikelet. Later on, *Apocopis* was separated from subtribe Saccharinae and was placed in the Germainiinae by Clayton and Renvoize (1986), even though the sessile spikelets of *Apocopis* are well developed and pedicelled spikelets usually reduced. Germainiinae were considered to have arisen from a 'Eulalia-like' ancestor based on the truncate lower glumes (Clayton, 1972; Clayton and Renvoize, 1986). Phylogenetic study of morphological data showed that Germainiinae were polyphyletic (Kellogg and Watson, 1993). No molecular studies have been carried out to test this.
Figure 1.8 A diagram of classification for the tribe Andropogoneae as proposed by Hackel (1889). Subtribes are denoted by dashed lines with subtribal names in bold boxes. Arrows indicate putative direction of evolution and size of arrow indicates certainty of relatedness. From Spangler et al. (1999).
Figure 1.9 The evolution diagram of tribe Andropogoneae based on development of raceme-segment and inflorescence axes. Adapted from Clayton and Renvoize (1986). Inflorescences and spikelets pictures obtained from Hitchcock (1935), Pilger (1940), Bor (1968), Gilliland (1971) and Noltie (2000).
1.2 Poaceae and the Flora of Thailand project

One of the first preliminary accounts of Poaceae of the Asian region was conducted by William Munro who collected and identified grasses including bamboos from India for the Royal Botanic Gardens Kew and completed the Bambuseae in 1866. The next major comprehensive study of Indian grasses was the Flora of British India which was written by Sir Joseph Hooker (1897). He also studied the grasses of Ceylon and his work appeared as the fifth volume of Trimen's Handbook of the Flora of Ceylon (Hooker, 1900). Further study of Asian grasses was carried out by the next generation botanists from different parts of Asia such as Flore Générale de l'Indo-Chine (Camus and Camus, 1923); Flora of the Malay Peninsula (Ridley, 1925); Grasses of China (Keng, 1933); The grasses of Burma (Burma is the old name of 'Myanmar') (Rhind, 1945); The Grasses of Burma, Ceylon, India and Pakistan (Bor, 1960); Flora of Japan (Ohwi, 1965); Flora of Java (Becker and Bakhuizen van Den Brink, 1986); Flora of Malaya (Gilliland, 1971); Grasses of the Soviet Union (Tsvelev, 1976); Flora of Taiwan (Hsu, 1978); The Tropical Grasses of Southeast Asia (Lazarides, 1980); Grasses of Japan and its Neighboring Region (Koyama, 1987); The concise Flora of Singapore (Keng et al., 1998) and Field Guide to the Grasses of Singapore (Duistermaat, 2005).

In Thailand, an intensive study of Poaceae was initiated in 1957 at the same time that general Thai-Danish Botanical projects began. The field expeditions were undertaken during 1957 to 1960 by Thai and Danish botanists (Middleton, 2003; BKF, 2008). Large numbers of flowering plants from all over Thailand were collected and distributed to several herbaria around the world. During the Thai-Danish botanical expedition, many grass specimens from Thailand were collected and sent to the herbarium at Kew and were identified by Dr. N. L. Bor who published the studies of Poaceae for the Flora of Thailand in Dansk Botanisk Arkiv (Bor, 1962, 1965, 1966, 1968) in which many new species were described, e.g. Chumsriella thailandica (now Germainia thailandica), Coelachne sorenseii, Cymbopogon siamensis. Some specimens from these expeditions were used in cytological studies by Larsen (1963). At the same time, another study of Thai grasses was carried out by Hambananda (1963) who is one of the leading Thai agrostologists. She constructed the key to genera focusing on Thai Andropogoneae. Approximately 120 species of andropogonoid grasses were reported. She continued her work on Thai grasses by publishing an account of genus Germainia (Chaianan, 1972). After that, only a few studies on Poaceae were carried out, e.g. Nanakorn (1990) in his unpublished Ph.D. thesis studied on subtribe Andropogoninae, Anthiiriinae and Saccharinae of tribe Andropogoneae. Yenying (1990) and Poonpan (1990) in their M.Sc. theses studied the genus Arundinella and subtribe Maydeae, respectively.
Knowledge of the Thai Poaceae was improved by Lazarides (1980) who recognised approximately 400 species of Thai grasses and published an account of the tropical grasses of Southeast Asia in which he constructed the key to subfamilies, tribes and genera. Recently, Nanakorn and Norsangsri (2001) published the Species Enumeration of Thai Gramineae. This book is a preliminary check list and survey of Thai grasses produced by collecting specimens in Thailand and also studying publications and herbarium specimens from major botanical libraries and herbaria. In this volume, 501 species of Thai grasses in 133 genera have been reported.

The Flora of Thailand project is a collaborative project between Thailand, Europe, Japan and the United States of America. Its main aim is to produce a complete floristic treatment of the flowering plants and ferns (Middleton, 2003; BKF, 2008). Thailand is situated at the junction of the Indo-Burmese, Indo-Chinese and Malesian regions and locates between latitudes 5° 37' to 20° 27' N and longitudes 97° 22' to 105° 37' E, covering an area of 513,115 km² (TMD, 2002) and this helps explain the high species diversity of the Thai flora (ca. 10,000 species; Parnell, 2000; Middleton, 2003) and fauna. Since, the Flora of Thailand project was launched in 1967, about 30 % of the total number of species have been published (ca. 850 species, Middleton, 2003). In recent years, the Flora of Thailand Project was accelerated to revise the species-rich families of which three species-rich families were completed, e.g. Apocynaceae (125 species), Cyperaceae (248 species) and Euphorbiaceae (ca. 400 species) (BKF, 2008). In 2004, Poaceae became a priority for the flora account of the Flora of Thailand Project. This project is being coordinated by David Simpson at Kew under Thai-European collaboration (Simpson, pers. comm.). In this Ph.D., tribe Arundinelleae and subtribes Chionachninae, Dimeriinae and Germainiinae from subfamily Panicoideae have been chosen for systematic and taxonomic research in order to provide a complete account of the aforementioned genera and also to resolve phylogenetic relationships of the tribes and subtribes within Panicoideae.

1.3 Aims of this thesis

The general aim of this thesis was to complete the floristic account of the tribe Arundinelleae and subtribes Chionachninae, Dimeriinae and Germainiinae for the Flora of Thailand project using classical herbarium techniques (Chapter 2). It is important to note that in the present floristic treatment, the taxonomy of Panicoideae has been based on morphological data. Thus, Arundinelleae are still treated as a separate tribe in its traditional circumscription, following the classification of Clayton and Renvoize (1986), though molecular studies indicated that the tribe is polyphyletic. In order to study how the tribes of the subfamily Panicoideae and
subtribes of the tribe Andropogoneae relate to one another, molecular data have been used to
investigate the phylogenetic relationships of the aforementioned taxa. The phylogeny of 132
taxa of Poaceae in Chapter 3 was inferred from plastid \((\text{trnL-F}, \text{atpB-rbcL})\) and nuclear gene
sequence regions \((\text{nrITS})\) under the principle of maximum parsimony. Due to time constraints
and the effect of slower convergence of MrBayes runs, the Bayesian analysis was not
examined in this study. However, the preliminary result of the analysis seemed to be more
useful to provide additional evidence of many relationships in the MP analyses. The last aspect
was to understand plant distribution patterns of selected Thai native grasses, and plastid
microsatellite DNA analyses were applied to assess population genetic variation of the selected
native species (Chapter 4). More specific objectives for each chapter are as follows:

**Chapter 2:**

- Construct keys to species, for genera of Thai Arundinelleae and subtribes Chionachninae,
  Dimeriinae and Germainiinae.
- Provide species descriptions including details on synonymy and typification, illustrations and
distribution maps.
- Provide the basis for the future completion of accounts of these genera for the Flora of
  Thailand project.

**Chapter 3:**

- Resolve major groupings within Panicoideae and elucidate the phylogenetic placement of
  most tribes of Panicoideae sensu Clayton and Renvoize (1986) and assess the monophyly of
  existing taxa.
- Revise the subtribal classification of Andropogoneae and to study how the subtribes relate to
  one another and other genera.
- Study molecular variation in different plastid \((\text{trnL-F}, \text{atpB-rbcL})\) and nuclear gene sequence
  regions \((\text{nrITS})\) to assess their use in phylogenetic study. The \(\text{atpB-rbcL}\) spacer was used for the
  first time to study relationships between taxa within this group of plants.

**Chapter 4:**

- Amplify polymorphic microsatellite regions within the chloroplast genome of \(A. \text{ setosa}\) and
  \(G. \text{ tenella}\) collected from Thailand and test the variability of the markers developed by
  McGrath *et al.* (2006) on these two genera.
- Investigate the extent of haplotype diversity of *A. setosa* and *G. tenella* in Thailand to determine if the diversity was structured into geographically meaningful patterns.

- Investigate distribution patterns and genetic diversity between and within the populations of *A. setosa* and *G. tenella* in Thailand.
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CHAPTER 2

Floristic treatments

2.1 Introduction

2.1.1 Taxonomic history of Arundinelleae

Previously, the genera of the tribe Arundinelleae have been referred to various tribes. Link (1827) proposed Tristeginae as the suborder for the genera *Acratherum* (now *Arundinella* Raddi), *Tristegis* Nees (now *Melinis* Beauv.), *Arrhenatherum* Beauv. and *Holcus* L. Nees von Esenbeck (1836) reduced Link's (1827) suborder to be the tribe Tristeginae which was later adopted by many botanists such as Bentham (1882) who recognised 13 genera in this tribe including *Arundinella* and *Garnotia* Brongn., while Hackel (1887) recognised only seven genera in this tribe, but placed *Garnotia* in tribe Agrostideae.

The tribe Arundinelleae was established by Stapf (1898) in Flora Capensis based mainly on three genera, *Arundinella*, *Tristachya* Nees and *Trichopteryx* Nees. Arundinelleae were later accepted by many notable botanists, including the anomalous systems of Janowski and Mez (1921) who nominated the tribe Arundinelleae containing three genera, *Arundinella*, *Thysanolaena* Nees (now in Centothecoideae) and *Garnotia*, and Janowsky’s (1922) study in which Arundinelleae consisted of *Arundinella*, *Thysanolaena*, *Phaenosterma* Munro ex Benth. (now in Pooideae) and *Beckera* Fresen (now in Paniceae). It was, however, Stapf’s (1889) system, that was developed by Hubbard (1936) who added three more genera, *Danthoniopsis* Stapf, *Loudetia* Hochst. ex Steud., and *Gilgiochloa* Pilg. to the tribe. Bor (1955a) described *Jansenella* as a new genus for Arundinelleae. Conert (1957) enumerated eight genera in his monograph of Arundinelleae and also described *Loudetiopsis* as a new genus separated from *Loudetia*. Phipps (1966) in his check-list of Arundinelleae proposed a new generic classification of the tribe. Twenty two genera were recognised in this study. He also noted that there still remained some doubts as to the unity of the tribe and the generic circumscription of the tribe was still unclear. Therefore, Phipps (1969) later used numerical taxonomy to resolve the confusion of generic classification of the tribe. However, none of his results were novel, except that three main groups, an *Arundinella* group, a Loudetioid group and a Tristachyoid group, were found. This approach had also been used by Clayton (1967, 1971) who recognised Arundinelleae with nine genera. However, according to Phipps (1966, 1969) and Clayton (1967, 1971), the genus *Garnotia* was not included in their studies. Clayton and Renvoize (1986) using morphological
and anatomical characters recognised 12 genera in the tribe, namely *Arundinella*, *Chandrasekharania* Nair, *Danthoniopsis*, *Dilophotriche* (C. E. Hubb.) Jac.-Fé!, *Garnotia*, *Gilgiochloa*, *Jansenella*, *Loudetia*, *Loudetioptis*, *Trichopteryx*, *Tristachya* and *Zonotriche* (C. E. Hubb.) Phipps. Two of these genera: *Arundinella* and *Garnotia* have been reported to occur in Thailand (Lazarides, 1980; Yenying, 1990; Nanakorn and Norsangsri, 2001).

**Arundinella**

The genus *Arundinella* was established by Raddi (1823) using *Arundinella brasiliaensis* (now *Arundinella hispida* (Wild.) Kuntze), collected from Brazil, as the type species. Its name is the diminutive of the Latin word “arundo” and means a “little reed”. This genus is distinguished from the rest of the tribe by having two-flowered spikelets, a scabrous upper lemma, a punctiform hilum and a short membranous ligule. However, before the name *Arundinella* was proposed, *Goldbachia* was the first generic name that had been used, when Trinius (1821) described *Goldbachia mikani* (now *A. hispida*). Unfortunately, this generic name was already preoccupied by De Candolle (1821), who had described the genus *Goldbachia* in the family Brassicaceae. Since the work of Raddi (1823), many additional species were published for this genus, e.g. Steudel (1854), Hooker (1897, 1900) and Janowski and Mez (1921).

Keng (1936) published the account for Asiatic taxa where 40 species were recognised, three of which were regarded as new species. He also divided *Arundinella* into 4 subgenera: *Psilachne*, *Chalynochlamis* Franch., *Arundinella* proper and *Miliosaccharum* Nees based on the characters of fertile lemmas. Bor (1955b) recognised 23 species and proposed a new status for some of Hooker’s (1897) taxa in his account of Poaceae for India, Sri Lanka and Myanmar. Conert (1957) in his monograph of Arundinelleae also recognised 23 species of *Arundinella*. Phipps (1966) in his checklist and key to genera of Arundinelleae, reported 47 species of *Arundinella*, and later divided the genus into 15 series (Phipps, 1967). Clayton and Renvoize (1986) reported that there are about 50 species of *Arundinella* distributed in tropical areas, while Sun and Phillips (2006) suggested that there are approximately 60 species, distributed mainly in Asia. In Thailand and neighbouring countries, several works relating to *Arundinella* have been published. Approximately 15 species have been recognised (Camus and Camus, 1922; Rhind, 1945; Schmid, 1958; Gilliland, 1971; Lazarides, 1980) of which nine species had been reported to occur in Thailand (Bor, 1962, 1965; Lazarides, 1980; Yenying, 1990; Nanakorn and Norsangsri, 2001).
The genus *Gamotia* was established by Brongniart (1832) based on a specimen of *Gamotia stricta* collected from Tahiti by Captain L. I. Duperrey during the expedition of the La Coquille ship. The genus was named in honour of Mr. P. Garnot, the ship’s doctor and naturalist. In addition, Brongniart (1832) also provided a perfect line drawing of the entire plant and of the spikelet and its parts. After Brongniart’s (1832) work, Arnott and Nees von Esenbeck (1843) described the genus *Miquelia* based on three grasses collected from India. However, the generic name *Miquelia* of Arnott and Nees von Esenbeck (1843) was invalid, having been applied earlier to a genus of the Icacinaceae by Meisner (1838). Later, Endlicher (1843) renamed *Miquelia* as *Berghausia* but without mentioning any species. *Berghausia* was adopted by Miquel (1851). Later, Bentham (1861) treated *Miquelia* and *Berghausia* to be synonyms of *Gamotia*. However, almost all of the species of *Berghausia* were later transferred to *Gamotia* by Janowski and Mez (1921). Additional species of *Gamotia* were published by Santos (1950), who recognised 73 species, 46 varieties and 24 forms, describing 35 new species, 41 new varieties and 23 new forms. He also divided the genus into two major sections: *Erectae* and *Deflexae* based mainly on characters of upper lemma awns. Gould (1972), in his treatment of *Gamotia*, disagreed with the Santos’s (1950) classification. He revised the genus and recognised 29 species of *Gamotia* which were separated into two sections: *Gamotia* and *Scoparia*. He also described a new endemic species from Thailand, *Gamotia thailandica*.

*Gamotia* was first characterised by having two-flowered spikelets, with suppressed lower glumes and lower florets represented by a lemma only (Brongniart, 1832). However, many early works, e.g. Gould (1972), Clayton and Renvoize (1986) and Watson and Dallwitz (1992) considered the genus *Gamotia* as a member of the tribe Arundinelleae. They believed that it has two well-developed glumes and a single floret with a suppressed second floret. They separated this genus from the rest of the tribe by its solitary singled-flowered spikelets which disarticulate below the glumes. There are about 30 species, distributed from India, Nepal, Sikkim, Sri Lanka, through Southern China, Southeast Asia, Queensland Australia, New Guinea, Fiji to the Hawaiian Islands and only one species is found in Africa (Gould, 1972; Clayton and Renvoize, 1986; Watson and Dallwitz, 1992; Clayton *et al.*, 2006; Wu and Phillips, 2006). Five species have been reported from Thailand (Bor, 1962; Larsen, 1963; Lazarides, 1980; Nanakorn and Norsangsri, 2001).
**Jansenella**

The monotypic genus *Jansenella* was proposed by Bor (1955a) based on the specimen collected from Khasia, India by W. Griffith. The genus was named in honour of Dr. P. Jansen, the well known Dutch agrostologist. Previously, the account of *Jansenella* first appeared in the Notulae ad Plantas Asiaticas (Griffith, 1851a) in which it was named as *Aira* sp. Subsequently, Griffith (1851b) provided an illustration of this plant in Icones Plantarum Asiaticarum under the name *Airoideum* sp. on plate 146, figure 3, but referred to the plate as *Airae* sp. in the index. Müller (1856) described a new species, *Danthonia griffithiana* using this specimen of Griffith, as the type specimen. This species was later transferred to be under genus *Arundinella* as *A. griffithiana* by Bor (1938) in which he also mentioned *Arundinella avenacea* Munro ex Thwaites, as a synonym. However, this species was considered to belong to *Danthoniopsis* and the combination in *Danthoniopsis* was made by Bor (1940). Finally, Bor (1955a) reconsidered the status of this species and proposed the new generic name, *Jansenella*, to accommodate this species. *Jansenella* differs from *Danthoniopsis* by having a membranous ligule, a punctiform hylum and distinctive one-celled hairs on the back of the upper palea. It was further distinguished by the remarkable one-celled hairs on a palea of the upper floret (Bor, 1955).

### 2.1.2 Taxonomic history of Chionachninae

The subtribe Chionachninae was proposed by Clayton (1981a), and four genera were recognised in it: *Chionachne* R. Br., *Sclerachne* R. Br., *Polytoca* R. Br. and *Trilohachne* Schenck ex Henrard. Chionachninae was traditionally grouped together with subtribes Coicinae and Tripsacinae in the Maydeae (Bentham, 1883; Henrard, 1931; Nirodi, 1955; Bor, 1960; Watson and Dallwitz, 1988, 1992). Maydeae was later separated into three subtribes: Chionachninae, Coicinae and Tripsacinae by Clayton (1973, 1981a) based largely on the characters of the inflorescence. Chionachninae are distinguished by having male and female spikelets in different parts of the same inflorescence with male spikelets normally in the proximal or the distal part of the racemes. The sessile female spikelets often enfold the rachis and the pedicelled companion spikelets are somewhat reduced and neuter (Clayton, 1981a; Jannink and Veldkamp, 2002). Two genera: *Sclerachne* and *Polytoca*, had been reported to occur in Thailand (Bor, 1962, 1965; Larsen, 1963; Lazarides, 1980). Jannink and Veldkamp (2002) revised this subtribe and recognised Chionachninae with four genera, *Chionachne*, *Cytborbachis* Steud., *Polytoca* and *Trilohachne*. 

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**Chionachne**

The genus *Chionachne* was established by Brown (1838) based on the specimen of *Chionachne barbata* (Roxb.) R. Br. ex Aitch. (now *Chionachne gigantea* (J. Koenig.) Veldkamp) collected from India. This genus is characterised by having the spikelets which are arranged in a solitary or a paniculate mixed spike. Each spike bears pairs of sessile female and neuter pedicelled spikelets at the base and pairs of male spikelets distally (Jannink and Veldkamp, 2002). Its name is presumably derived from the Greek word “chion or chioni” meaning “snow” and “achne” meaning “chaff or scale” alluding to the pale-coloured glumes (Watson and Dallwitz, 1992). There are nine species, distributed from India, Sri Lanka to South China, Indo-China, Indonesia to Polynesia and Australia (Clayton and Renvoize, 1986; Watson and Dallwitz, 1992; Clayton et al., 2006).

When Brown (1838) published *Chionachne*, he mentioned this genus in a note following the genus description of the genus *Sclerachne*. The differences between both genera were explained in the same book by Bennett (1838). Subsequently, many new species were published for *Chionachne*, e.g. Bentham (1878) published an additional species of *Chionachne* for the Flora of Australia, Balansa (1890) published a new species for Indo-China and Hackel (1906) published a new species for Malesia taxa. Henrard (1931) published an account of Indian Maydeae and recognised four species of *Chionachne*. Nirodi (1955), in her cytological analysis of Asian Maydeae, seven species of *Chionachne* were recognised. More recently, Jannink and Veldkamp (2002) in their revision of subtribe Chionachninae recognised nine species of *Chionachne*. They also placed the monotypic genus *Sclerachne* under *Chionachne* as *C. punctata* (R. Br.) Jannink.

**Polytoca**

The genus *Polytoca* was published by Brown (1838) based on the specimen of *Polytoca bracteata* R. Br. (now *Polytoca digitata* (L.f.) Druce) collected from India. This genus is recognised by its digitate inflorescence, lateral branches with only male spikelets and terminal branch with male or neuter spikelets below the female spikelets; the most distal spikelets of the terminal branch are male or neuter. Its name is presumably derived from the Greek word “polys” meaning “many” and “tokos” meaning “offspring” alluding to numerous offspring (Quattrocchi, 2000). The first specimen for this genus was described by Linné (1781). However, he named it as *Apluda digitata* which was later regarded as a member of *Polytoca* by Druce (1917).
Since the genus was proposed, the limits of *Polytoca* have been increasing widely by various studies to accommodate species resembling the type species, e.g. Steudel (1854) described a new genus *Cyathorhachis* which was later transferred to be under *Polytoca* by Bentham (1882). Henrard (1931) in his account of Indian Maydeae, recognised five species of *Polytoca*. Lazarides (1980) recognised four species of *Polytoca* in his account of Poaceae for Southeast Asia. Clayton and Renvoize (1986) described the new subtribe Chionachninae and proposed a new generic delimitation for *Polytoca* that included only two species, *P. digitata* and *P. wallichiana*. Both were reported as native in Thailand (Nanakorn and Norsangsri, 2001). More recently, Jannink and Veldkamp (2002) in their revision of subtribe Chionachninae suggested a new definition of *Polytoca* with only one species, *P. digitata* and placed *P. wallichiana* under *Cyathorhachis* as *C. wallichiana* Nees ex Steud.

### 2.1.3 Taxonomic history of Dimeriinae

The subtribe Dimeriinae is distinguished by the tough rhachis and strongly flattened single spikelets without trace of pairing. It contains only a single genus, *Dimeria* which was established by Brown (1810). Its name is derived from the Greek word, “dis” means “double” and “meros” means “part” referring to the two racemes of the type species, *Dimeria acinaciformis* R. Br. There are about 40 species in the world, distributed from India, Sri Lanka, China to Indonesia and Australia, including three species in Madagascar (Clayton and Renvoize, 1986; Clayton *et al.*, 2006).

The subtribe Dimeriinae was first proposed by Hackel (1887) as ‘Dimerieae’. Later, Hackel (1889) published a full account of Dimerieae in his monograph of tribe Andropogoneae. He recognised 12 species, two subspecies and 10 varieties of which five species were described as new. He also divided the subtribe into three major groups, using the number of racemes in the inflorescence. Hooker (1897) published an account of Poaceae for the Flora of British India and recognised 12 species of *Dimeria* of which two species were newly described, namely *Dimeria alata* and *Dimeria kurzii*. Later, *Dimeria trimeni* a new species from Sri Lanka was published by Hooker (1900). Bor (1952b) elevated two of Hackel’s (1887) subspecies, *Dimeria leptorhachis* subsp. *velutina* and *Dimeria leptorhachis* subsp. *genuina* to specific rank: *Dimeria gracilis* Nees ex Steud. and *Dimeria velutina* (Hack.) Bor (now *Dimeria leptorhachis* Hack.), respectively. Bor (1953) published an account of the genus for India and Myanmar in which he divided 25 species of *Dimeria* into 3 sections based on rhachis and pedicel characters: sections *Capillares, Loriformes* and *Annulares*. He also described seven new species and four new varieties from India based on the length of spikelets and stamens. Clayton (1994) in his account of *Dimeria*
for Sri Lanka recognised nine species. He placed *Dimeria velutina* (Hack.) Bor, as a synonym of *Dimeria leptorrhachis* Hack., and also placed *Dimeria ceylanica* Bor and *D. trimeni* to be synonyms of *Dimeria pubescens* Hack. In South East Asia, approximately 14 species were recognised from Indo-China, Malaysia, Java and China (Camus and Camus, 1922; Schmid, 1958; Ridley, 1925; Backer and Bakhuizen van den Brink, 1968; Gilliland, 1971; Lazarides, 1980; Chen and Phillips, 2006). In Thailand, preliminary checklists on *Dimeria* were presented by Lazarides (1980) and Nanakorn and Norsangsri (2001) in which eight species were reported.

### 2.1.4 Taxonomic history of Germainiinae

The tribe Germainiinae was proposed by Clayton (1972) with four genera recognised: *Germainia* Balansa & Poitrass, *Trachypogon* Nees, *Sclerandrium* Stapf & C. E. Hubb., and *Chumsriella* Bor, the latter two are now considered as synonyms of *Apocopis* and *Germainia*, respectively (Chai-Anan, 1972). All members of this subtribe were characterised by having fertile pedicelled spikelets and male or neuter sessile spikelets, except genus *Apocopis* Nees which was later added to this tribe by Clayton and Renvoize (1986). Two genera were found in Thailand: *Apocopis* and *Germainia* (Lazarides, 1980; Nanakorn and Norsangsri, 2001).

**Apocopis**

The genus *Apocopis* was established by Nees von Esenbeck (1841) to include *A. royleanus* (now *Apocopis paleacea* (Trin.) Hochr.), using the specimen of Dr. J. F. Royle, as the type specimen (see Bor, 1952 for detail). Since the genus *Apocopis* was proposed, several taxa of *Apocopis* were subsequently published, e.g. Munro (1860) published *Apocopis wrightii* a new species from Hong Kong. Hackel (1889) recognised two species, *Apocopis royleana* and *Apocopis wightii* Nees (now *A. courtablumensis* (Steud.) Henrard) in his monograph of Andropogoneae. Hooker (1897) recognised three *Apocopis* in his account of Poaceae for Flora of British India, including a new species, *Apocopis pallida* (now *Dichanthium pallidum* (Hook.f.) Stapf ex C. E. C. Fisch.). The most important account of this genus is Bor (1952a). He revised the genus *Apocopis* enumerating 11 species based mainly on the lower glume characters.

*Apocopis* is distinguished by the inflorescence being composed of spike-like racemes consisting of a fragile rhachis that bears the imbricate fertile sessile spikelets with broadly truncate lower glumes and pedicelled spikelets which are normally suppressed. Its name presumably came from the Greek word “apokope” meaning “to cut off” referring to the truncate lower glume of the sessile spikelet (Bor, 1952). There are about 15 species, distributed chiefly in India,
China and Southeast Asia to Polynesia (Bor, 1952; Clayton and Renvoize, 1986; Watson and Dallwitz, 1992; Clayton et al., 2006).

In South East Asia, several new species have been published. In total, five new species from Myanmar were recognised by Bor (1949, 1951, 1957). Four endemic species from Indo-China were described by Balansa (1890) and Camus (1914, 1919, 1957). Ridley (1910) described *Apocopis borneensis* (now *Apocopis collinus* Balansa) which was the first record of *Apocopis* from the Malay Peninsula. Chai-Anan (1972) considered *Sclerandrium intermedium* (A. Camus) C. E. Hubb., as a synonym of *Apocopis intermedius* (A. Camus) Chai-Anan. Chen and Phillips (2006) published an account of Chinese taxa and recognised four species of *Apocopis*. In Thailand, seven species have been reported (Lazarides, 1980; Nanakorn and Norsangsri, 2001).

There is a confusion of the generic name *Amblyachyrum*, which is a synonym of *Apocopis*. *Amblyachyrum* Hochst. ex Steud., was published by Steudel (1854) to accommodate the specimen of *Hohenacker* Ind. nr. 231b collected from India. Subsequently, Hochstetter (1856), who is credited with the authorship of the generic name, later published the full account for this genus and distanced himself from Steudel's (1854) publication. He also stated the specimen of *Hohenacker* 131a, as the type specimen for *Amblyachyrum*. Finally, *Amblyachyrum mangalorense* sensu Steudel's (1854) was illegitimate because it was a superfluous name for *Dimeria hohenackeri* Hochst. ex Miq., while, *Amblyachyrum mangalorensense* sensu Hochstetter (1856) was later transferred to *Apocopis* by Henrard (1941).

**Germainia**

The genus *Germainia* was established by Balansa and Poitrasson (1873) based on the specimen of *Germainia capitata*, collected from Saigon, Vietnam. The genus was named in honour of the collector, R. Germain. In the following years several new taxa were added, e.g. Hackel (1891) published *Germainia khayana*, a new species from Khasia Hills. Hooker (1897) described *Germainia lanipes* as a new species from Myanmar. Camus (1919, 1957) described two more species from Indo-China, *Germainia thorelli* and *Germainia schmidiana*, respectively. The most important account of this genus was the revision of Chai-Anan (1972). She proposed the new generic circumscription of *Germainia*. The genus is distinguished by the presence of basal pairs of homogamous involucral spikelets surrounding the central fertile awned spikelets and tough rhachis. In addition, she described *Germainia pilosa* as a new endemic species to Thailand and placed a generic name *Chumsriella* as a synonym of *Germainia*. In total, nine species were
recognised and distributed mainly in India, Myanmar, China, Indo-China, Indonesia, Papau New Guinea and Australia. Six species were reported to occur in Thailand.

2.2 Aims

The aims of this chapter were to:

1) Construct keys to genera and species of Thai Arundinelleae and subtribes Chionachninae, Dimeriinae and Germainiinae.
2) Provide species descriptions including details on synonymy and typification, illustrations and distribution maps.
3) Provide the basis for the future completion of accounts of these genera for the Flora of Thailand project.

2.3 Materials and methods

This floristic work was undertaken at the Herbarium, Department of Botany, School of Natural Science, Trinity College Dublin (TCD). There were two main sources of specimens used in this study: specimens collected from fieldwork in Thailand and herbarium specimens obtained from the collaborating institutions of the Flora of Thailand project. Four field trips, totalling a period of five months were made to Thailand during 2005 and 2006. In total, approximately 500 specimens were collected. Most of the grass specimens collected from fieldwork are housed in the Herbarium, Department of Botany, Trinity College Dublin (TCD) and the Herbarium, Thailand Natural History Museum, National Science Museum, Technopolis, Pathum Thani, Thailand.

Approximately 1,000 herbarium specimens were examined from the following herbaria using classical herbarium techniques: University of Aarhus, Aarhus, Denmark (AAU); Herbarium, Plant and Soil Science Department, University of Aberdeen, Aberdeen, Scotland, U.K. (ABD); Botanic Garden and Botanical Museum Berlin-Dahlem, Freie Universität Berlin, Germany (B) (digital images); Forest Herbarium, National Park, Wildlife and Plant Conservation Department, Bangkok, Thailand (BKF); Herbarium, Department of Botany, Kasetsart University, Bangkok, Thailand (BKU); Herbarium, Botany Department, The Natural History Museum, London, England, U.K. (BM); Herbarium, Royal Botanic Garden, Edinburgh, Scotland, U.K. (E); Harvard University Herbaria, Cambridge, Massachusetts, U.S.A. (GH); Herbarium, Royal Botanic Gardens, Kew, England, U.K. (K) (including digital images, photos and microfiches); Herbarium, Department of Biology, Khon Kaen University, Khon Kaen,
Thailand (KKU); Rijksherbarium, National Herbarium Nederland, Leiden University, Netherlands (L) (digital images); Herbarium, New York Botanical Garden, Bronx, New York, U.S.A. (NY); Herbarium, Parks and Recreation Department, Botanic Gardens, Cluny Road, Singapore (SING); Herbarium, Department of Botany, School of Natural Science, Trinity College Dublin (TCD); Herbarium, Thailand Natural History Museum, National Science Museum, Technopolis, Pathum Thani, Thailand (THNHM); United States National Herbarium (Smithsonian Institution), Washington D.C., U.S.A. (US) (including digital images). Other herbaria were also visited as follows and their specimens were studied, namely Herbarium, Botany Section, Botany and Weed Science Division, Department of Agriculture, Bangkok, Thailand (BK); Herbarium, Department of Biology, Chiang Mai University, Chiang Mai, Thailand (CMU); Herbarium, Queen Sirikit Botanic Garden, Botanic Garden Organization, Chiang Mai, Thailand (QBG). 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 and Holmgren, 1998 [continuously updated]) (published on the internet: http://sweetgum.nybg.org/ih/). The following regional herbaria: BKU and THNHM are not in Index Herbariorum and the abbreviations have been coined for use in this study.

Dimensions given in the description are mainly based on herbarium specimens, spirit material and are supplemented by observations made of living plants in the field. For dry specimens, spikelets were softened in water containing a small amount of detergent (ca. 1% of washing-up liquid), and measured using a stereomicroscope (Leica MZ-12) with graticule. The data on quantitative characters in most cases are taken of the smallest and largest exemplar for each organ (i.e. ligule, leaf, spikelet) at least are based on 3–5 measurements per specimen. Several specimens per species were measured if available. When both length and width are used, the measurements are given as length by width. A range of measurement is separated by an-dash (—). Discontinuous states within the taxon are separated by the word “or”. Exceptional measurements or character states are in parentheses (). Taxa are arranged alphabetically. Most of the major Floras and publications in SE Asia, surrounding areas and important monographs are cited where appropriate. The synonym given is based on close examination of excepted name and synonyms listed in major SE Asia floras. At the generic level, synonyms widely used in the literature are included. At the specific and infraspecific levels, only the main synonyms, especially those listed in the floras of the works mentioned above, are included.

Almost all types of correct names and synonyms have been seen and are indicated by exclamation mark (!) after the herbarium abbreviation. For the corrected names, when no
holotype or previous lectotypification, a lectotype or an epitype has been chosen and the reasons of typification are given in the species notes, following Article 9 (Art. 9.1-holotype; Art. 9.2-lectotype and Art. 9.7-epitype) of the International Code of Botanical Nomenclature (Vienna Code) 2006 (McNeill et al., 2006). In general, where lectotypifications have been made previously these are cited with 'fide', followed by a reference to the author and place of publication, when a given specimen is designated as a holotype, all duplicates of the specimen become isotypes. If the holotype is not designated by the author or if it is lost or destroyed, the lectotype will be selected from the material which was cited or consulted by the author (i.e. isotype, syntype) in order of preference. If no original material is extant or has been validated by lectotypification, literature research will be carried out to locate the main collections of the author, mainly based on the data provided in the "Taxonomic Literature" Stafleu and Cowan (1976–1988). Subsequently, the appropriate herbarium collections are searched to locate the specimens which are likely to have been used by the author in the first description. If an appropriate specimen is found, this is designated as the lectotype.

All specimens cited under each species have been examined and are alphabetically arranged by the name of the countries, major political divisions, localities, the dates of collections, collector names, and collector numbers. Vernacular names and distributional information in Thailand were presented 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. The global distribution of species, genera, subtribes and tribes 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 Clayton and Renvoize (1986). Cited publication titles and author abbreviations follow The International Plant Names Index (2005) (published on the internet: http://www.ipni.org/index.html). Abbreviations of publications which are not included in the website above, follow those commonly used in botanical literature or are abbreviated according to the recommendations in Botanico-Periodicum-Huntianum; alternatively publication names are written in full.

2.4 Species concept

In order to study biological classification, the criteria being used to delimit species should be clearly set forth in any paper in which a species is described (Luckow, 1995). However, it is well known that the species concept continues to be the subject of much debate (Cracraft, 2000). As, there are many different concepts proposed, a single species concept, universally used among botanists has yet to emerge (Mishler and Donoghue, 1982; Luckow, 1995). The
The morphological species concept of Davis and Heywood (1963) was used in this thesis. This concept defines a species as:

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

In this study, comparative morphological differences were used to delimit taxa. Taxonomic decisions were based mainly on morphological information gathered from the large number of herbarium specimens examined (approximately 1,500 herbarium specimens were examined), living plant materials when available, and information observed from several fieldwork expeditions by the author. From a practical standpoint in the preparation of floras, the circumscription of species based on easily observable morphological features is the sensible approach (Stuessy, 2009). In order to provide a practicable and communicable flora account, the morphological species concept appeared to be suitable and practical as reference concept for this account.

There is a need to recognise intraspecific taxa when morphological variation occurs within the species and discontinuities exist. According to Stace (1989) and Stuessy (2009), two infraspecific ranks are commonly employed (i.e. subspecies and variety) using geographical pattern as the most important component in the formal recognition. Subspecies are considered if the distributions are largely allopatric or taxa process clear geographical or ecological distinctions. No subspecies are in fact recognised in this account because there are no taxa which fall into the categories mentioned above. Varieties are recognised when the taxa possess less clear cut geographical (are more or less sympatric) and/or ecological distinctions. In these cases the difference in morphology are minor when compared to taxa at subspecific or specific ranks but the variation is consistent. The variety category is the only infraspecific rank officially recognised in this study.
2.5 Results

TRIBE ARUNDINELLEAE


Type genus: Arundinella Raddi.

Annual or perennial. Ligate usually a line of hairs or an eciliate membrane or a ciliate membrane. Inflorescence a panicle. Spikelets often in triads or in pairs (in Thailand), similar, slightly laterally or dorsally compressed; florets 1 or 2, without rachilla extension (except Garnotia), falling entirely or breaking up at maturity; glumes membranous to coriaceous, mostly persistent, the upper as long as spikelet, the lower usually shorter, often covered with tubercle-based hairs, rarely awned, 3–5-nerved; lower florets male or neuter, lemmas resembling the upper glume, often persistent, 3–9-nerved, usually with a narrow palea; upper florets hermaphrodite, subterete, lemma membranous or coriaceous to cartilaginous, often with hair tuft, entire to bifid, awned from sinus, awns geniculate with twisted columns or straight, sometimes caducous. Caryopsis with linear or punctiform hilum.

There are 12 genera including 175 species in the tribe, distributed in the Tropics, mainly in Africa and Asia (Clayton and Renvoize, 1986). Three genera and 14 species are found in Thailand, of which three species are endemic.

Key to genera of Thai Arundinelleae

1. Spikelets with 1 floret, falling entire ........................................... 2. Garnotia (p. 65)
1. Spikelets with 2 florets, usually breaking up
   2. Upper lemmas pilose, with 2 hair tufts ......................... 3. Jansenella (p. 82)
   2. Upper lemmas scabrous, without hair tufts ..................... 1. Arundinella (p. 41)
ARUNDINELLA


Type: *Arundinella brasiliensis* Raddi (= *Arundinella hispida* (Willd.) Kuntze.)

— *Goldbachia* Trin. in Spreng., Neue Entdeck. 2: 42. 1821. non DC. 1821. Type: *Goldbachia makani* Trin. (= *Arundinella hispida* (Willd.) Kuntze.)

— *Acratherum* Link, Hort. Berol. 1: 230. 1827. Type: *Acratherum miliaceum* Link (see later, under *Arundinella nepalensis* Trin.).


— *Branditia* Kunth, Révis. Gramin. 2: 511, tab. 170. 1830. Type: *Branditia holcoides* Kunth (see later, under *Arundinella holcoides* (Kunth) Trin.).


Perennial or annual. Culms slender to robust, erect or ascending or shortly decumbent; nodes glabrous or bearded. Leaf-blades linear or linear-lanceolate or oblong-lanceolate. Ligule a short membrane or a ciliate membrane. Inflorescence an open or a contracted panicle. Spikelets in pairs, one short and one long-pedicelled, similar, laterally compressed; florets 2, without rachilla extension, breaking up at maturity, disarticulating below each fertile floret; glumes persistent, membranous, shorter or reaching or exceeding apex of florets in the spikelets, 3–5-nerved; lower florets male or neuter; lower lemmas membranous, almost glabrous, acute, edges fringed, 3–5(–7)-nerved; lower paleas membranous, edges fringed, slightly ciliate on the upper half of the keels; upper florets hermaphrodite; callus hairy or glabrous; upper lemmas
coriaceous, scaberulous to scabrid on dorsal surface on the upper half, acute or bifid, usually awned from the sinus or awnless, awns geniculate with brown twisted columns (except *A. kerrii*), scaberulous, 3–5-nerved; upper paleas the same texture as upper lemmas, glabrous to scaberulous on dorsal surface, edges fringed, upper half of the keels ciliate or glabrous, 2-nerved; lodicules 2; stamens 3 (2 in *A. kokutensis*), anthers yellow to purple; styles 2, stigmas purple to reddish purple. *Caryopsis* with adherent pericarp, oblong, hilum punctiform.

About 60 species, distributed in Africa, Temperate Asia, Tropical Asia, Australasia, North America and South America. Eight species are found in Thailand of which two are endemic: *Arundinella kerrii* C. Hambananda ex A. Teerawat. & Sungkaew (Teerawatananon et al., in prep.) and *Arundinella kokutensis* Teerawat. & Sungkaew (Teerawatananon et al., submitted).
Key to species of Thai Arundinella

1. Upper lemmas deeply bifid, lateral awns 0.5–3 mm long, awned from the sinus; pedicels often with stiff setose hairs at apex ................................................................. 8. A. setosa

1. Upper lemmas without lateral awns or awnless or with a single awn; pedicels without stiff setose hairs at apex

2. Upper lemmas awnless or with a very short awn, less than 2.5 mm long
   3. Spikelets hispid with tubercle-based hairs on nerves; awn of upper lemmas absent or a straight mucro up to 0.6 mm ................................................................. 5. A. kerrii

   3. Spikelets glabrous or scabrous on nerves; awn of upper lemmas caducous and geniculate, 1–2.5 mm
      4. Panicle (25–)40–70 cm long; branches 9–15 cm long ................................................................. 3. A. decempedalis

      4. Panicle 10–30 cm long; branches 2–7 cm long ......................................................................... 1. A. bengalensis

2. Upper lemmas awned, awns conspicuous

   5. Spikelets hairy with tubercle-based hairs
      6. Rhachis almost glabrous. Lower glumes almost glabrous; stamens 2 ................................................................. 6. A. kokutensis

      6. Rhachis scabrous and pilose with tubercle-based hairs. Lower glumes hairy; stamens 3 ................................................................. 4. A. holcoides

5. Spikelets glabrous or scaberulous

   7. Panicles 40–65 cm long; branches erect, concealing the stout main axis; spikelets imbricate ................................................................. 2. A. cochinchinensis

   7. Panicles 20–40 cm long; branches spreading ascending; spikelets distant ................................................................. 7. A. nepalensis


— *Panicum strictum* Roxb. in Carey & Wall., F. Ind. 1: 306. 1820. non R. Br. 1810. Type: India, Bengal, Native of Bengal, *Unknown collector* [holotype, not found].


Perennial, rhizomes creeping and scaly. *Culms* 0.7—1.5(—2.5) m tall, erect or short ascending; nodes hirsute rarely glabrous. *Leaf-sheaths* 5–30 cm long, glabrous or pilose on the back, margins densely hairy. *Ligule* a short membrane, 0.3–0.5 mm long, with a dense row of hairs behind the ligule. *Leaf-blades* linear-lanceolate, 15–60(–70) cm by 0.8–1.2(–2) cm, hirsute on both surfaces, margins scabrous. *Panicles* contracted, 10–30 cm long; racemes 2–5(–7) cm, racemes alternate or sometimes whorled, rhachis scabrous. *Spikelets* green to purple, ovate-oblong, 3–4 mm by 1–1.2 mm; pedicels 1–5 mm long, scabrous; lower glumes ovate, 2–3 mm by 0.8–1.2 mm, acute, 3–5-nerved, scabrous on nerves, glabrous or with a few tubercle-based hairs on or between the lateral nerves; upper glumes ovate-oblong to ovate-lanceolate, 3–4 mm by 0.5–1 mm, acuminate, 5-nerved, finely scabrous on the nerves, glabrous or with a few tubercle-based hairs on or between the lateral nerves; lower florets male or neuter; lower lemmas ovate-oblong to ovate-lanceolate, 2.8–3 mm long, 3–5-nerved; lower paleas ovate-lanceolate, 2–2.8 mm long; upper lemmas ovate-oblong, 1.8–2.2 mm long, apex entire to minutely bifid, awnless or awned, awns 1.4–2.7 mm long, caducous, 3-nerved; upper paleas ovate-oblong, 1.8–2.2 mm long; callus hairy, hairs 0.2–0.5 mm long; stamens 3, anthers yellow to purple, 0.8–1.2 mm long; styles 2, stigmas purple, ca. 1 mm long. *Caryopsis* ovate to ovoid-elliptic, 1.2–1.3 mm long.
Thailand. — NORTHERN: Chiang Mai (Ban Yang, Chiang Dao, Doi Ang Khang, Doi Inthanon, Doi Pai, Doi Suthep, Khun Wang, Mae Rim, Mae Sa, Mae Tang); Chiang Rai (Chieng Kien, Phaya Mengrai); Mae Hong Son (Pang Oong); SOUTH-WESTERN: Uthai Thani (Ban Rai).

Distribution. — India, Bangladesh, Nepal, Bhutan, China, Myanmar and Vietnam.

Ecology. — Open areas, along roadsides, in deciduous dipterocarp-oak, oak-pine, grassy-pine and hill evergreen forests, marshy places and rice fields, 400–2,500 m altitude.

Vernacular. — Ya Khai Yai (Ya Khaei Yai), from Soradej; Ya Sae Ma (Ya Sae Ma), from Yenying (1990).

Notes. — Yenying (1990) reported Soradej 190 as *Arundinella hispida* (Willd.) Kuntze ‘Arundinella hispida Hack’. However, I have examined this specimen and found that it is *Arundinella bengalensis*.

Typification notes. — Steudel (1854) first published the name *Arundinella wallichii* Nees ex Steud based on the specimen “*Arundinella* nr. 8669 A. B. Wallich cat.”, which were collected from Nepal, as the type specimens, but he did not mention where they were kept. Later, Bor (1955b) mentioned *Wallich* 8669, collected from Silhet, de Silva, India, as the type of *A. wallichii*. However, Bor did not state where it was kept and what kind of type it was. I have found several specimens of *Wallich* 8669, in BM, C, CAL, E and K, but I have been unable to find any evidence to confirm which specimen was cited by Steudel (1854) or by Bor (1955b). According to Stafleu and Cowan (1985), and Stafleu and Mennega (1993), some of Steudel’s types may be kept in K where Bor (1955b) was the assistant director when he published his work. I have found that the sheet of K 245491 is the only one that has a label “*Arundinella*, A. Nepalia 1821, B. Silhet, de Silva, *Wallich* 8669” which correspondes to Steudel (1854).

Therefore, I assume that the sheet, K 245491, would carry Steudel’s (1854) type specimens. However, in selecting a lectotype, only one of specimens must be selected as the lectotype. In addition, I also found that only the specimens of *Wallich* 8669A were collected from Nepal, while *Wallich* 8669B were collected from Sylhet, Bangladesh. Therefore, I hereby designate the specimen *Wallich* 8669A (on the left hand side of sheet K 245491) to be the lectotype of *A. wallichii* and regard the duplicates of *Wallich* 8669A in BM, C, CAL and E, as isolectotypes.
Specimens examined.


*Mae Hong Son* [Pang Oong, alt. 900 m, 11 Nov. 1999, Suksathan 2044 (QBG)]; [Pa Sing, 7 Dec. 1957, Walker 8003 (US)].

SOUTH-WESTERN: *Uthai Thani* [Ban Rai, Ban Dong, alt. ca. 1,000 m, 25 Oct. 1974, Sutheesoen & Sangkhachand 3087 (BK)].

Perennial. **Culms** 2–2.5 m tall, erect; nodes swollen, dense appressed-pubescent hairs. **Leaf-sheaths** 12–20 cm long, mostly longer than internodes, glabrous or pilose, margins shortly ciliate. **Ligule** a short membrane, 0.3–0.5 mm long, with a dense row of hairs behind the ligule, hairs 5–8 mm long. **Leaf-blades** linear-lanceolate, 50–80 cm by 1.2–2 cm, papillos-hispid on both surfaces, margins scabrous. **Panicles** dense, narrowly oblong in outline, 40–65 cm long; racemes numerous, 6–25 cm long, whorled or alternate, concealing the stout main axis, rhachis scaberulous. **Spikelets** ovate-lanceolate, 3–5 mm by 1–1.5 mm; pedicels 0.5–5 mm long, scaberulous; lower glumes ovate-oblong, 3–4 mm by 1-1.2 mm, acuminate, 3- or 5-nerved, scabrous on nerves; upper glumes ovate-lanceolate, 4–5 mm by 1–1.2 mm, usually recurved, 3–5-nerved, glabrous or scaberulous on nerves; lower florets male; lower lemmas ovate-oblong, 3–3.5 mm long, 3–5-nerved; lower paleas ovate-oblong to ovate-lanceolate, 2.5–3 mm long; upper lemmas ovate-oblong, 2–2.5 mm long, minutely bifid, awned from the sinus, awns 4–5 mm long, 3-nerved; upper paleas ovate-oblong to ovate-lanceolate, 2–2.5 mm long, dentate, minutely bifid; callus hairy, hairs 0.5–1.2 mm long; stamens 3, anthers 1–1.8 mm long; styles 2, stigmas 0.8–1 mm long. **Caryopsis** not seen.

**Distribution.** — China and Vietnam.

**Ecology.** — Open grassy-pine and evergreen forests, 1,000–1,500 m altitude.

**Notes.** — The specimen of *Sørensen et al. 5282* (K) was reported as *Arundinella cochinchinensis* Keng by Bor (1965). I have examined this specimen and found that it is a young form of *Arundinella bengalensis* (Spreng.) Druce and differs from *A. cochinchinensis* in having smaller inflorescences and racemes, shorter spikelets with short and deciduous awns.

**Specimens examined.**

**Thailand: NORTHERN:** *Chiang Mai* [Mae Sa, alt. 1,300–1,400 m, 18 Sept. 1995, *K. Larsen et al.* 46623 (L)].

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— *Arundinella clarkei* Hook.f., Fl. Brit. India 7: 75. 1897. Type: India, Sikkim, alt. 152 m, 2 Dec. 1875, *Clarke* 26483A [lectotype K! (K 245486), designated here; isolectotype K! (K 245959)].

Perennial. **Culms** 1.5–2.5(–3) m tall, erect; nodes densely pubescent. **Leaf-sheaths** 20–27 cm long, usually glabrous, hirsute with tubercle-based hairs at the upper part, margins short ciliolate. **Ligule** a ciliolate membrane, 0.3–0.5 mm long, with a dense row of hairs behind the ligule hairs up to 1 cm long. **Leaf-blades** linear-lanceolate, 50–120 cm by 1–1.5(–3) cm, glabrous or hispid with tubercle-based hairs on both surfaces, margins scabrous. **Panicles** subcontracted, (25–)40–50(–70) cm long; racemes 9–15 cm long, whorled or alternate, rhachis scabrous. **Spikelets** ovate-oblong to ovate-lanceolate, 3–4 mm by 1–1.2 mm; pedicels 0.5–3.5 mm long, scabrous; lower glumes ovate-oblong, 2.5–3 mm by 0.8–1.2 mm, acute, 3- or 5-nerved, scabrous on nerves; upper glumes ovate-oblong, 3–3.5 mm by ca. 1 mm, acute, 3- or 5-nerved, scaberulous on the nerves; lower florets male or neuter; lower lemmas ovate-oblong, 2–2.5(–3) mm long, 3- or 5-nerved; lower paleas ovate-oblong, 2–2.7 mm long; upper lemmas ovate-oblong to ovate-lanceolate, 1.8–2.2 mm long, minutely bifid, awned or awnless, awns 1–2(–2.5) mm long, caducous, 3-nerved, 3-nerved; upper paleas ovate-oblong to ovate-lanceolate, 1.8–2 mm long, acute; callus hairy, hairs 0.3–0.5 mm long; stamens 3, anthers 0.8–1.5 mm long; styles 2, stigmas 0.8–1 mm long. **Caryopsis** ovoid-elliptic, 1.3–1.4 mm long.

Thailand. — NORTHERN: Chiang Mai (Doi Suthep); Chiang Rai (Doi Tung).

Distribution. — China and India.

Ecology. — Open grassy fields, 600–1,200 m altitude.
Notes. — This type is on two sheets: the upper part of culm and inflorescence on NY 414089 and the lower culm and leaves on NY 414090. It was previously reported to occur in Thailand by Keng (1936).

Typification notes. — When Hooker (1897) published the species called *Arundinella clarkei* Hook.f., the specimen of Clarke collected from “Sikkim Terai”, was cited. However, he did not provide the collector name nor mention where it was kept. Later, Bor (1955b) stated that the collection of Clarke 26483 housed in K was the type specimen of *A. clarkei*. However, I have found two sheets of *Clarke* 26483 in K. They are *Clarke* 26483A and *Clarke* 26483C which have the same data label (i.e. date, altitude, location). I think, they are duplicates of each other and can be the type specimens sensu Bor (1955b). Therefore, I hereby designate *Clarke* 26483A (K 245486) as the lectotype and regard *Clarke* 26483C (K 245959) as an isolectotype.

Specimens examined.

**Thailand:** NORTHERN: *Chiang Mai* [Doi Suthep (Sootep), Camp Hoi Chang Kiang, alt. 600 m, 1920, Kock 110 (US)]; *Chiang Rai* [Doi Tung, alt. 1,200–1,450 m, 25 Sept. 1967, Iwatsuki et al. 11141 (GH)].


— Arundinella agrostoides Trin., Sp. Gram. 3, tab. 265. 1836. Type: Philippines, Manila (Manillense) Unknown collector s.n. [lectotype K (digital image K 290215!), designated here; isolecotype K (digital image, the right-hand specimen of K 290214!)].

Annual, loosely tufted. Culms slender, 20—45 cm tall, erect, shortly decumbent at base, branching at lower nodes; nodes hirsute. Leaf-sheaths 1—6 cm long, pilose with tubercle-based hairs. Ligule a ciliolate membrane, 0.5—0.8 mm long. Leaf-blades oblong-lanceolate, 2—15 cm by 0.3—0.8 cm, pilose with tubercle-based hairs on both surfaces. Panicles narrow, 3—15(—20) cm long; racemes 1—3 cm long, alternate, raceme base pilose, rhachis scabrous and pilose with tubercle-based hairs. Spikelets ovate-oblong, (2—)3—3.5 mm by 1 —1.2 mm; pedicels 0.5—3(—6) mm long, glabrous to scabrous, sometimes with sparsely pilose; lower glumes ovate to ovate-oblong, 1.8—2(—3) mm by 0.5—1 mm, acuminate, 3- or 5-nerved, pilose with tubercle-based hairs on nerves and sometimes between the nerves; upper glumes ovate to ovate-oblong, 2.8—3.5 mm by 0.8—1 mm, pilose with tubercle-based hairs, caudate, 5-nerved; lower florets male or neuter; lower lemmas ovate-oblong, 1.8—2.5 mm long, 3-nerved; lower paleas ovate to ovate-oblong, 1.5—2 mm long; upper lemmas ovate, 1—1.3 mm long, minutely bifid, awned from the sinus, awns 4—4.5 mm long, 3-nerved; upper paleas ovate, 1—1.2 mm long, acute; callus hairy, hairs 0.2—0.3 mm long; stamens 3, anthers 0.3—0.5 mm long; styles 2, stigmas 0.4—0.5 mm long. Caryopsis elliptic-oblong, 0.8—1 mm long.

Thailand. — PENINSULAR: Ranong.

Distribution. — India, Myanmar, Indonesia (Java, Sulawesi) and Philippines.

Ecology. — Open grasslands, ca. 10 m altitude.

Typification notes. — When Trinius (1836) described Arundinella agrostoides Trin., he cited a specimen collected from Manila (Manillense) as the type specimen and also provided an illustration of the whole plant in tab. 265. However, he did not mention the collector's name.
and number. According to Bor (1955b), the type sheet of *A. agrostoides* was housed at K, where I found two specimens, having the same label "*Arundinella agrostoides* Trin. Manilla". They are the sheets K 290214 and K 290215 which, I think, are duplicates of each other and can be the type specimens sensu Bor (1955b). However, on the sheet of K 290214, there are two specimens; the left hand one has a label in Wallich’s handwriting reading “8671 *Arundinella?*, Tavoy, W. Gomez, Nov. 1827” and the right hand one has the label as “*Arundinella agrostoides* Trin., Manilla” without the collector name. In order to avoid any confusion of mixing specimens, I hereby designate K 290215 as the lectotype of *A. agrostoides* and regard the specimen on the right-hand side of K 290214 as an isolectotype.

Specimens examined.

**Thailand**: PENINSULAR: *Ranong* [Nok Nang, alt. ca. 10 m, 14 Jan. 1929, Kerr 16726 (BK, BM, K)].

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Perennial, loosely tufted. *Culms* 30–120 cm tall, erect; nodes hirsute, basal sheaths becoming fibrous. *Leaf-sheaths* 9–16 cm long, almost glabrous, margins hispid with tubercle-based hairs. *Ligule* a ciliolate membrane, 0.5–0.6 mm long, with a dense row of hairs behind the ligule. *Leaf-blades* narrowly linear, (8–)20–50(–)70 cm by 0.3–0.8 cm, glabrous on both surfaces, margins scabrous and hispid with tubercle-based hairs. *Panicles* contracted, 5–15 cm long; racemes 1.5–5 cm long, alternate, rhachis scabrous. *Spikelets* greyish-green, ovate-oblong, 3–3.6 mm by 1–1.5 mm; pedicels 0.2–2 mm long, scabrous and hispid; lower glumes ovate, 2.5–3 mm by 0.8–1.2 mm, acute, 3–5-nerved, hispid with tubercle-based hairs on nerves; upper glumes ovate to ovate-oblong, 2.5–3.5 mm by 1–1.2 mm, acuminate, 5-nerved, hispid with tubercle-based hairs on nerves; lower florets male; lower lemmas ovate to ovate-oblong, 2.5–3 mm long, upper margins fringed, 5- or 7-nerved; lower paleas ovate-oblong, 2.5–2.7 mm long; upper lemmas ovate-oblong, 1.8–2 mm long, acute or mucronate or minutely bifid, shortly awned from the sinus, awns 0.5–0.6 mm long, 3-nerved; upper paleas ovate-oblong, 1.8–2 mm long, acute; callus hairy, hairs 0.2–0.3 mm long; stamens 3, anthers 0.9–1.6 mm long; styles 2, stigmas ca. 1 mm long. *Caryopsis* not seen.

Thailand. — NORTH-EASTERN: Nakhon Phanom (Chaiyaburi, Tha Uthen).

Distribution. — Endemic to Thailand.

Ecology. — Open grassy ground areas, ca. 200 m altitude.

Notes. — This species is similar to *Arundinella hirta* (Thunb.) Tanaka and *Arundinella fluviatilis* Hand.-Mazz., from which it differs in having fibrous basal leaf sheaths, smaller size of glumes and upper lemma and shorter callus hairs, ca. 1/5 the length of the lemma. The differences among *A. fluviatilis*, *A. hirta*, and *A. kerrii* are summarized in Table 2.1.

Typification notes. — This species was originally described by Hambananda in Yenying (1990), but without a Latin diagnosis. The name was therefore not validly published.
Specimens examined.

Thailand: NORTH-EASTERN: *Nahkon Phanom* [Chaiyaburi, alt. ca. 200 m, 1 May 1932, *Kerr* 21330 (BK, BM, K)]; [Tha Uthen, alt. ca. 200 m, 16 Feb. 1924, *Kerr* 8474 (BK, BM, K)].

Table 2.1 Comparision on habitats and vegetative morphological characters of *Arundinella fluviatilis*, *A. hirta* and *A. kerrii*.

<table>
<thead>
<tr>
<th>Species/Characters</th>
<th><em>A. fluviatilis</em></th>
<th><em>A. hirta</em></th>
<th><em>A. kerrii</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Nodes</td>
<td>glabrous</td>
<td>glabrous or bearded</td>
<td>hirsute</td>
</tr>
<tr>
<td>Leaf-sheaths</td>
<td>glabrous, margins ciliate</td>
<td>glabrous to pilose, margins ciliate or hispid with tubercle-based hairs</td>
<td>almost glabrous, basal sheath fibrous, margins hispid with tubercle-based hairs</td>
</tr>
<tr>
<td>Inflorescence</td>
<td>an open panicle, 10–20 cm long</td>
<td>an open or contracted panicle, 8–45 cm long</td>
<td>a contracted panicle, 5–15 cm long</td>
</tr>
<tr>
<td>Pedicels</td>
<td>scabrous</td>
<td>scabrous</td>
<td>scabrous and hispid</td>
</tr>
<tr>
<td>Lower glumes</td>
<td>2.6–3.5 mm long, 5-nerved, scabrous on nerves</td>
<td>2.5–4 mm long, 3–5-nerved, scabrous on nerves (pilose with tubercle-based hairs on nerves in var. <em>bondana</em>)</td>
<td>2.5–3 mm long, 3–5-nerved, hispid with tubercle-based hairs on nerves</td>
</tr>
<tr>
<td>Upper glumes</td>
<td>4.5–5 mm long, scabrous on nerves</td>
<td>3.5–4.8 mm long, scabrous on nerves (pilose with tubercle-based hairs on nerves in var. <em>bondana</em>)</td>
<td>2.5–3.5 mm long, hispid with tubercle-based hairs on nerves</td>
</tr>
<tr>
<td>Upper lemmas</td>
<td>2.6–3.4 mm long</td>
<td>2.5–3.5 mm long</td>
<td>1.8–2 mm long</td>
</tr>
<tr>
<td>Upper lemma apex</td>
<td>awned, awns 0.3–1.5 mm long</td>
<td>acute to short mucronate</td>
<td>acute or mucronate or minutely bifid, shortly awned from the sinus, awns 0.5–0.6 mm long</td>
</tr>
<tr>
<td>Callus hairs</td>
<td>1.3–1.7 mm (ca. 1/2 of length of upper lemma)</td>
<td>0.8–1.5 mm (1/3–1/2 of length of upper lemma)</td>
<td>0.2–0.3 mm long (ca. 1/5 length of upper lemma)</td>
</tr>
</tbody>
</table>

Annual. Culms slender, 5–80 cm tall, erect, shortly decumbent at base; nodes sparsely pubescent. Leaf-sheaths 2–8 cm long, hispid with tubercle-based hairs especially along margins. Ligule a ciliate membrane, 0.8–1.3 mm long. Leaf-blades oblong-lanceolate, (1–)5–30 cm by (0.3–)0.6–1.3 cm, hispid with tubercle-based hairs on both surfaces, margins sometimes scaberulous. Panicles open, sometimes drooping, (3–)10–25 cm long; racemes (2–)4–10 cm long, alternate, rhachis almost glabrous rarely scabrous. Spikelets green, ovate-lanceolate, 3–4 mm by 0.6–1 mm; pedicels 0.5–6 mm long, glabrous; lower glumes ovate-lanceolate, 2–2.5 mm by 0.5–1 mm, almost glabrous, sometimes scabrous near apex, acuminate to aristate, 3–5-nerved; upper glumes ovate-lanceolate, 3–3.5(–4) mm by 0.5–1 mm, glabrous to sparsely hispid with tubercle-based hairs, caudate, recurved, 5-nerved; lower florets neuter; lower lemmas ovate-lanceolate, 2–2.5 mm long, 5-nerved; lower paleas ovate-oblong to ovate-lanceolate, 1.5–2 mm long; upper lemmas ovate-oblong, 1–1.5 mm long, minutely bifid, awned from the sinus, awns 5–6.5 mm long, 3-nerved; upper paleas ovate-lanceolate, 1.2–1.4 mm long, acute; callus hairy, hairs 0.3–0.5 mm long; stamens 2 rarely 3, anthers yellow, 0.3–0.5 mm long; styles 2, stigmas purple, 0.5–0.7 mm long. Caryopsis ovoid-elliptic, 1–1.2 mm long.

Thailand. — SOUTH-EASTERN: Trat (Ko Kut).

Distribution. — Endemic to Thailand.

Ecology. — Growing on moist mossy rocks along the streams in evergreen forests.

Etymology. — This species is named after the island called Ko Kut (‘Ko’ as Thai for Island), Trat Province, south-eastern Thailand, where this plant was collected for the first time.

Note. — This species has only two stamens instead of three as normally found in the other members of the genus (Bor, 1955; Watson and Dallwitz, 1992; Clayton et al., 2006). Although florets having three stamens have been found, their occurrence is extremely rare. This species is otherwise similar to Arundinella metziæ Hochst. ex Miq. and Arundinella holcoïdi (Kunth) Trin.,
but can be distinguished by its glabrous pedicels, awn of upper lemma which is 5–6.5 mm long and the florets that normally have only two stamens, rarely three. The differences between *A. holcoides*, *A. kokutensis* and *A. metzii* are summarized in Table 2.2.

Specimens examined.


Table 2.2 Comparision of habitats and vegetative morphological characters of *Arundinella holcoides*, *A. kokutensis* and *A. metzii*.

<table>
<thead>
<tr>
<th>Species/Characters</th>
<th><em>A. holcoides</em></th>
<th><em>A. kokutensis</em></th>
<th><em>A. metzii</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Nodes</td>
<td>hirsute</td>
<td>sparsely pubescent</td>
<td>glabrous or pubescent</td>
</tr>
<tr>
<td>Inflorescence</td>
<td>3–15(–20) cm long, a narrow</td>
<td>(3–)10–25 cm long, an open panicle, sometimes drooping</td>
<td>up to 50 cm, an open panicle, usually much shorter and narrower</td>
</tr>
<tr>
<td>Racemes</td>
<td>up to 3 cm long</td>
<td>(2–)4–10 cm long</td>
<td>5–15 cm</td>
</tr>
<tr>
<td>Rhachis</td>
<td>scabrous and pilose with tubercle-based hairs</td>
<td>almost glabrous, rarely scabrous</td>
<td>scabrous</td>
</tr>
<tr>
<td>Pedicels</td>
<td>glabrous, sometimes with sparsely pilose</td>
<td>glabrous</td>
<td>scabrous</td>
</tr>
<tr>
<td>Spikelets</td>
<td>(2–)3–3.5 mm long</td>
<td>3–4 mm long</td>
<td>3–4.5 mm long</td>
</tr>
<tr>
<td>Upper lemmas</td>
<td>1–1.3 mm long</td>
<td>1–1.5 mm long</td>
<td>ca. 1 mm long</td>
</tr>
<tr>
<td>Callus hairs</td>
<td>0.2–0.3 mm long</td>
<td>0.3–0.5 mm long</td>
<td>0.4–0.5 mm long</td>
</tr>
<tr>
<td>Awns</td>
<td>4–4.5 mm long</td>
<td>5–6.5 mm long</td>
<td>ca. 3 mm long</td>
</tr>
<tr>
<td>Stamens</td>
<td>3</td>
<td>2 extremely rarely 3</td>
<td>3</td>
</tr>
</tbody>
</table>

— *Acratherum miliaceum* Link, Hort. Berol. 1: 230. 1827. Type: Nepalia, *Unknown collector* [holotype, not found].


Perennial, tufted. *Calms* 1.2–1.8 up to 2 m tall, erect or short ascending, sometimes branched; nodes pubescent. *Leaf-sheaths* 6–13 cm long, glabrous, margins short ciliate. *Ligule* a short ciliolate membrane, 0.2–0.5 mm long, with a dense row of hairs behind the ligule. *Leaf-blades* linear-lanceolate, 20–60 cm by 0.8–1.5 cm, hirsute with tubercle-based hairs on both surfaces, margins scabrous. *Panicles* open, 20–40(–50) cm long; racemes 8–20 cm long, alternate or whorled, rachis almost glabrous or scaberulous. *Spikelets* green to purple, ovate-oblong to ovate-lanceolate, 3.5–4(–5.5) mm by 0.8–1 mm; pedicels 0.5–4 mm long, scabrous; lower glumes ovate-oblong to ovate-lanceolate, 2.5–3.5 mm by 0.5–0.8 mm, acuminate, 3-nerved, almost glabrous or sometimes scaberulous on nerves; upper glumes ovate-lanceolate, 3.5–4(–5) mm by 0.5–0.8 mm, acuminate, 5-nerved, glabrous or scaberulous on the nerves; lower florets male or neuter or sometimes hermaphrodite; lower lemma ovate-oblong to ovate-lanceolate, 2.5–3 mm long, 5-nerved; lower paleas ovate-lanceolate, 2.2–2.5 mm long; upper lemmas ovate-lanceolate, (1.5–)2–2.5 mm long, minutely bifid, awned from the sinus, awns 4.5–6 mm long, 3-nerved; upper paleas ovate-oblong,
(1.2—)1.8—2.5 mm long, minutely bifid; callus hairy, hairs 0.4—0.8 mm long; stamens 3, anthers purple, 0.8—1.5 mm long; styles 2, stigmas purple, 0.5—1.2 mm long. Caryopsis ovoid to ovoid-elliptic, 0.9—1.3 mm long.

Thailand. — NORTHERN: Chiang Mai (Chiang Doi Hills, Doi Suthep, Fang, Mae Taeng); Mae Hong Son (Mae Ngao, Pang Ma Pha); Phitsanulok (Thung Salaeng Luang); NORTH-EASTERN: Loei (Phu Kradueng).


Ecology. — Open humid areas on hill slopes and in deciduous dipterocarp-oak, pine-dipterocarp forests and tropical grasslands, 500—1,700 m altitude.

Note. — Specimens of Sorensen et al. 5732 and Usa 53 (which were collected from Doi Suthep, Chiang Mai have been identified as Arundinella rupestris A. Camus by Bor (1962) and Yenying (1990), respectively. These two specimens have been examined by me and were identified to be a young plant of Arundinella nepalensis Trin.

Specimens examined.


NORTH-EASTERN: **Loei** (Phu Kradueng, Samkae, 23 Mar. 1954, Smitinand 1790 (BKF, K)].


— *A. setifera* Steud., Syn. Pl. Glumac. 1: 115. 1854. Type: India, Nilagri (Nilagiri) 1851, *Hohenacker* 920 [holotype K! (K 245473), fide Bor (1955b); isotypes C!, TCD!].


Key to the varieties of *A. setosa*

1. Upper lemmas with 2 lateral awns .................................................. **var. setosa**
1. Upper lemmas without or with vestigial lateral awns .......................... **var. esetosa**

**var. setosa** Figures 2.5 and 2.9.

Perennial, tufted with short rhizome. **Culms** up to 2.5 m tall, erect; nodes glabrous. **Leaf-sheaths** 5–15 cm long, glabrous or pilose or sometimes with tubercle-based hairs. **Ligule** a ciliolate membrane, 0.5–1 mm long. **Leaf-blades** linear, 10–60 cm by 0.5–1.2 cm, almost glabrous or pilose with tubercle-based hairs on both surfaces, margins scabrous. **Panicles** open, 10–35(–55) cm long; racemes 5–15(–30) cm, alternate or sometimes whorled, rhachis scabrous. **Spikelets** green to purple, ovate-lanceolate, 4.5–6(–8) mm by 1–1.5 mm; pedicels 1–8(–15) mm long, scabrous, with a few stiff setose hairs at apex; lower glumes ovate-lanceolate 3–6 mm by 0.8–1.2 mm, acuminate, glabrous or with a few tubercle-based hairs, 3-nerved, scabrous on nerves; upper glumes ovate-lanceolate, 4–6(–8) mm by 1–1.2 mm, glabrous or sparsely hispid with tubercle-based hairs, acuminate to caudate, 5-nerved, scabrous on nerves; lower florets male or neuter; lower lemmas ovate-oblong to ovate-lanceolate, 3–4.5 mm long, 5-nerved; lower paleas ovate-lanceolate, 3–4 mm long; upper lemmas ovate-lanceolate, 2–3 mm long, deeply bifid, lateral awns 0.5–3 mm, awned from the sinus, awns 6–10(–12) mm long, 3- or 5-nerved; upper paleas ovate-oblong, 2–3 mm long, acute; callus hairy, hairs 0.5–1 mm long; stamens 3, anthers yellow to purple, 1–2 mm long; styles 2, stigmas reddish-purple, 1–1.5 mm long. **Caryopsis** ovoid, 1.7–1.8 mm long.

Thailand. — NORTHERN: Chiang Mai (Doi Ang Khang, Doi Buak Ha, Doi Chiang Dao, Doi Chom Cheng, Doi Inthanon, Doi Saget, Mae Rim, Tintok, Thoen); Chiang Rai (Khun Chae National Park); Kamphaeng Phet; Lampang (Doi Palad); Lamphun (Doi Khun Tan); Mae Hong Son (Huai Hee, Khun Yuam); Phitsanulok (Phu Hin Rong Kla, Thung Salaeng Luang National Park); Tak (Doi Musoe, Umphang); NORTH-EASTERN: Loei (Phu Kradueng); Phetchabun (Nam Nao National Park); Sakon Nakhon (Phu Phan National Park); EASTERN: Nakhon Ratchasima (Bua Yai, Chan Tuk, Khao Yai National Park); SOUTH-WESTERN: Kanchanaburi (Khao Salap National Park, Sai Yok Noi Waterfall); Phetchaburi (Bo Fai, Thung Luang); Prachuap Khiri Khan (Ban Kan Kradai, Khao Sai, Khao Sung); Ratchaburi (Suan Phung); Uthai Thani (Huai Kha Khaeng Wildlife); CENTRAL: Nakhon Nayok (Nang Rong Waterfall); SOUTH-EASTERN: Chon Buri (Ban Bueng); PENINSULAR: Krabi (Ko Lanta).
Distribution. — India, Nepal, Bhutan, Myanmar, China, Laos, Cambodia, Vietnam, Malesia and Australia.

Ecology. — In deciduous dipterocarp, mixed deciduous, pine-oak forests and open grassy-pine forests, grassy hillsides and tropical grasslands, on rocks by the sea, 50–2650 m altitude.

Vernacular. — Ya Lian Nong (Ya Lien Nong) (Marcan 2727), Ya Ma Lai (Ya Ma Lai), Ya Hang Nok Yung (Ya Hang Nok Yung) (Yenying, 1990).

Note. — This species is morphologically very variable. The height of the plant in Thailand can be 80–250 cm tall. The colours of the spikelets are greenish yellow to purplish to dark violet. The indumentum on culms and the sheaths varies too as they are occasionally hairy. The size of the inflorescences varies from 10–50 cm long; though most are compact, the specimens collected from deciduous dipterocarp forest, Doi Suthep National Park area, at altitude 300–500 m are not (Langaard & Norsanger 21620; Maxwell 88-1265; Teerawatananon 121001-2; Teerawatananon & Sungkaew 558; Sorensen et al. 4591 and 4810), having large, dropping effuse inflorescences. The lateral awns of the upper lemma are also variable in length being either long, short or absent. Specimens with short or without lateral awns, sampled in this study, are herein separated at the varietal rank following Phillips and Chen (2005).

Typification notes. — Nees von Esenbeck (1850) first described Arundinella stricta Nees and cited Cuming 1415 collected from Philippines (Ins. Philippinae) as the type specimen, but he did not indicate where it was kept. I have found three sheets of Cuming 1415 housed in E and K (K 209218, K 209219). Therefore, I hereby designate Cuming 1415 (K 209218) housed in K as the lectotype and regard its duplicates in K (K 209219) and E as isolectotypes.

When Steudel (1854) established Arundinella mutica Nees ex Steud., he cited a species identified as Andropogon capillaris Heyn., Hrbr. Wall. nr. 8665A, collected from Nepal, as the type specimen, but did not mention where it was kept. Later, Bor (1955b) treated A. mutica, as a synonym of A. setosa. He also mentioned that there are actually two specimens cited by Steudel (1854), which are housed in K. Steudel's (1854) citation can be interpreted into two ways: either he referred to only one collection or two. Actually, there are two specimens of Wallich 8665A mounted on the same sheet (K245475); one of which (the specimen on the left hand side specimen) (ex Herb. Hookerianum, 1867) bears the following label “8665 Arundinella, A. Andropogon capillaris Hb. Heyn., B. Hb. Wight”. The other is on the right hand side (ex Herb.
Benthamianum, 1854) was cut and pasted on from another sheet and is labelled as "Wall. cat. 8665A, Andropogon capillaris Hb. Heyn". Whether or not these two duplicates were both cited by Steudel (1854) is unknown. To make typification clearer, I hereby designate Wallich 8665A on the right hand side of K 245475 sheet (ex Herb. Benthamianum, 1854), as the lectotype of A. mutica and regard its duplicates in K and CAL as isoelectotypes.

When Steudel (1854) published Danthonia neuroeljtrum Steud., he cited the specimen of Fortune Hrbr. nr. 5 as the type specimen without mentioning the herbarium name. According to Stafleu and Cowan (1976), Fortune’s main herbarium collections are available in K where I have found two sheets of Fortune 5. Therefore, I hereby designate Fortune 5 (ex Herb. Hookerianum, 1867), housed in K, as the lectotype and regard the duplicate (ex Herb. Benthamianum, 1854) as an isoelectotype.

When Rendle (1904) first described Arundinella sinensis Rendle, three collections of Ford (Ford 125, 201 and 212) which were collected from China, were cited. However, only the latter two were examined by Rendle (1904). I have examined these three collections at K and also found that the collection Ford 212 has more potential to be selected as the lectotype than the others. In addition, I have also found two duplicates of Ford 212 in BM and US (USNH 865381). Therefore, I hereby designate the specimen of Ford 212, housed in K, as the lectotype and regarded the duplicates housed in BM and US (USNH 865381) as isoelectotypes.

Specimens examined.

Thailand: NORTHERN: Chiang Mai [Doi Ang Khang, alt. ca. 1,400 m, 29 Apr. 2005, Teerawatanan & Sungkaew 548 (THNHM)]; [l.c., alt. 1,000 m, 24 Nov. 2005, Teerawatanan & Sungkaew 800 (K, TCD, THNHM)]; [Doi Buak Ha, West of Chiang Mai, alt. 1,575 m, 30 Nov. 1965, Hennipman 3173 (BKF, C)]; [Doi Chiang Dao, alt. 1,400 m, 7 Nov. 1922, Kerr 6642 (ABD, BK, K)]; [Doi Intan, alt. 1,500-1,600 m, 26 Oct. 1920, Rock 231 (US)]; [Doi Inthanon, alt. 2,650 m, 22 Sept. 2001, Laegaard 21597 (US, K)]; [l.c., Kew Mae Paan, 21 Dec. 1997, Niyomdbam 5255 (BKF)]; [l.c., 16 Nov. 2005, Teerawatanan & Sungkaew 729 (TCD, THNHM)]; [l.c., Kew Mae Paan, 21 Nov. 2005, Teerawatanan & Sungkaew 779 (TCD, THNHM)]; [Doi Saget, alt. 500 m, 8 Nov. 1993, Suwanaratana 28 (L)]; [Doi Surat, alt. 560 m, 24 Nov. 1996, Kafle 11 (L)]; [l.c., 10 Oct. 1909, Kerr 824 (BM, E, K)]; [l.c., 11 Nov. 1911, Kerr 2230, (BM, 3 sheets K)]; [l.c., alt. 1,100 m, 15 Nov. 1914, Kerr 3471 (BM, K)]; [l.c., Pah Laht area, alt. 575 m, 2 Nov. 1988, Maxwell 88-1265 (BKF)]; [l.c., Wang Bau Bahn area, alt. 500
m, 21 Oct. 1987, Maxwell 87-1216 (BKF, CMU)]; [l.c., Doi Suthep Temple, alt. 950 m, 14 Oct. 1987, Maxwell 87-1176 (BKF)]; [l.c., alt. 1120 m, 25 July 1958, Sorensen et al. 4395 (C)]; [l.c., alt. 450 m, 29 Aug. 1958, Sorensen et al. 4591 (C)]; [l.c., alt. 500 m, 7 Sept. 1958, Sorensen et al. 4810 (C)]; [l.c., alt. 850 m, 3 Oct. 1958, Sorensen et al. 5415 (C)]; [l.c., alt. 1,500 m, 9 Oct. 1958, Sorensen et al. 5541 (E, K)]; [l.c., alt. 1,150 m, 23 Oct. 1958, Sorensen et al. 5848 (C, IQ)]; [l.c., alt. ca. 1,500 m, 9 Mar. 1965, Smitinand & Phengkhai 8693 (K)]; [Thoen & Lee, alt. ca. 600 m, 29 Nov. 1959 Smitinand & Thengklai 6167 (BKF, K)]; Chiang Rai
Khun Chae National Park, alt. 2,030 m, 21 Nov. 1997, Maxwell 97-1397 (CMU, GH, L)];
Kamphaeng Phet [alt. 50 m, 30 July 1925, Kerr 15990 (BM, K)]; Lampang [Doi Palad, alt. 600 m, 26 Sept. 1967, Shimizu et al. T-10836 (BKF, C, E, K)]; Lamphun [Doi Khun Tan, alt. 1,200–1,374 m, 5 Sept. 1967, Tagawa et al. T-9264 (BKF, E)]; Mae Hong Son [Huai Hee, 20 Nov. 2005, Teerawatananon & Sungkaw 765 (THNHM)]; Khun Yuam, alt. 600–700 m, 5 Sept. 1974, K. Larsen & S. Larsen 34150 (NY)]; Phitsanulok [Phu Hin Rong Kla, alt. ca. 1,700 m, 25 Nov. 2005, Teerawatananon & Sungkaw 810 (THNHM, SING, US)]; Phuching [Doi Hua Mod, alt. ca. 1,300 m, 28 July 1966, K. Larsen et al. 994 (K, SING)]; [Thung Salaeng Luang National Park, Nong Mae Na, alt. ca. 500 m, 30 Nov. 2005, Teerawatananon & Sungkaw 852 (THNHM)]; Tak [Doi Musoe, alt. 1,000 m, 7 Dec. 1960, Smitinand 7083 (BFK)]; Umphang, Doi Hua Mod, alt. ca. 900 m, Teerawatananon & Sungkaw 707 (TCD, THNHM)].

NORTH-EASTERN: Loei [Phu Krudueng, alt. 1,300 m, 26 Dec. 1971, Bensekom et al. 4610 (BFK, C, K)]; [l.c., alt. 1150–1250 m, 1 Nov. 1984, Murata et al. T-42534 (L)]; [l.c., alt. 1,300 m, 1 Nov. 1984, Murata et al. T-42557 (L)]; [l.c., alt. 1,300 m, 2 Sept. 1954, Smitinand 1895 (BFK, K)]; [l.c., alt. 1,300 m, 19 Mar. 1958, Sorensen et al. 2308 (BFK, C)]; [l.c., alt. 1,300 m, 28 Nov. 2005, Teerawatananon & Sungkaw 828/1 (BFK, TCD)]; [l.c., alt. 1,300 m, 28 Nov. 2005, Teerawatananon & Sungkaw 831 (TCD, THNHM)]; [l.c., alt. 1,300 m, 28 Nov. 2005, Teerawatananon & Sungkaw 839 (TCD, THNHM)]; Phetchabun [Nam Nao National Park, alt. ca. 1,000 m, 10 Apr. 2005, Teerawatananon & Sungkaw 465 (TCD)]; Sakon Nakhon [Phu
Phan National Park, alt. 500 m, *Teerawatananon & Sungkaew* 475 (TCD); [l.c., alt. 500 m, *Teerawatananon & Sungkaew* 478 (K)].


PENINSULAR: *Krabi* [Ko Lanta Yai, alt. 20 m, 12 Nov. 1966 *Hansen & Smitinand* 12251 (C)].

Culms nodes glabrous. Leaf-sheaths and blades glabrous or pilose or sometimes with tubercle-based hairs. Ligule a ciliolate membrane. Panicles 18–50 cm long. Spikelets 4–7 mm long; pedicels scabrous, with a few stiff setosa hairs at apex; upper lemmas with lateral awns wanting or with very short lateral awns, up to 0.5 mm long, central awns 6–9 mm long.

Thailand. — NORTHERN: Chiang Mai (Doi Ang Khang, Mae Ngon, Mae Rim, Mae Soi); Phitsanulok (Thung Salaeng Luang National Park).

Distribution. — India, Nepal, Myanmar, China, Vietnam, Malaysia and Sumatra.

Ecology. — Pine-oak forests, grassy hillsides and tropical grasslands, 500–1,350 m altitude.

Specimens examined.


NORTH-EASTERN: Locii [Phu Kradueng, alt. 1,300 m, 8 July 1959, Floto 7352 (K)].
GARNOTIA


Type: *Garnotia stricta* Brongn.

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Type: *Miquelia courtallensis* Arn. & Nees (= *Garnotia courtallensis* (Arn. & Nees) Thwaites.)

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**Berghausia** Endl., Gen. Suppl. 3: 57. 1843. Type: as for *Miquelia* Arn. & Nees.

Perennial or annual. **Culms** geniculate or ascending and branched or erect and unbranched; nodes usually pubescent sometimes glabrous. **Leaf-blades** linear or linear-lanceolate or oblong-lanceolate. **Ligule** a ciliate membrane. **Inflorescence** an open or contracted panicle. **Spikelets** in pairs, one short and one long-pedicelled, similar, laterally compressed; florets 1, disarticulating below spikelet; spikelet callus hairy; glumes membranous, reaching or exceeding apex of florets in the spikelets, acute or acuminate to minutely bifid, awned from the sinus, 3(−5)-nerved, scabrous on nerves; lemmas membranousacut e or bifid, usually awned from the sinus, awns straight or geniculate with brown twisted columns at base, scaberulous, 3-nerved, usually scabrous on nerves; paleas the same texture as upper lemmas, almost glabrous, edges fringed, keels usually ciliate, 2-nerved; lodicules 2; stamens 3, anthers yellow to purple; styles 2, stigmas plumose. **Caryopsis** with adherent pericarp, oblong, hilum punctiform.
About 30 species, distributed in Africa, Temperate Asia, Tropical Asia, Australasia and Pacific. Five species are found in Thailand of which one species is endemic: *Gamotia thailandica* Gould.

**Key to species of Thai *Gamotia***

1. Lemmas awnless or with straight awns
   2. Panicles with stiffly spreading branches ......................................................... 3. *G. patula*
   2. Panicles with appressed or loosely erect-spreading branches, not stiffly spreading

1. *G. acutigluma*

1. Lemmas with geniculate awns
   4. Spikelets 2–4 mm long ................................................................. 4. *G. tenella*
   4. Spikelets 4.5–6 mm long
      5. Perennial. Panicles 40–80 cm long. Glumes scabrous on nerves. Leaf-blades 20–40 cm long ......................................................... 5. *G. thailandica*
      5. Annual. Panicles 10–30 cm long. Glumes often hirsute or hispid with tubercle-based hairs. Leaf-blades 5–12 cm long ................................. 2. *G. ciliata*


Perennial, caespitose. Culms 20–40(−75) cm tall, erect or decumbent at base; nodes typically pubescent (Gould, 1972) to glabrous. Leaf-sheaths 2–10 cm long, hirsute or glabrous, margins scarious. Collar pilose or hirsute. Ligule a ciliate membrane, 0.2–1.3 mm long. Leaf-blades linear, 4–20 cm by 0.2–0.6(−0.8) cm, scabrous or hispid on both surfaces, margins scabrous. Panicles contracted, 5–20 cm long; racemes 2-4 cm long, appressed or loosely erect-spreading branches, rachis scabrous. Spikelets green, ovate-lanceolate, 3–4.5 mm by 0.5–0.6 mm; pedicels 0.5–4.5 mm long, scabrous; spikelet callus hairy, hairs 0.3–0.6(−1) mm long; lower glumes ovate-lanceolate, 3–4.5 mm by 0.5–0.6 mm, acuminate to awned, awns 0.5–0.6 mm long; upper glumes ovate-lanceolate, 3–4 mm by ca. 0.5 mm, acuminate to shortly awned; lemmas ovate-lanceolate, 2.5–4 mm long, awned, awns 8–12(−20) mm long, straight or undulant; paleas ovate-lanceolate, 2–2.8 mm long; stamens 3, anthers ca. 1 mm long; styles 2, stigmas ca. 1 mm long. Caryopsis ovoid-elliptic, 2–2.2 mm long, glabrous.
Thailand. — NORTH-EASTERN: Loei (Phu Kradueng).


Ecology. — Semi-shady areas, on sandstone rocks, along streams in evergreen forests, 1,100—1,300 m altitude.

Notes. — Most of the specimens from Thailand are without nodal hairs and the margins of the leaf-sheath are glabrous.

Typification notes. — Santos (1950) cited a specimen from Herb. Hort. Bot. Singap. num. 13854 (K) collected from Pahang, Malaysia, as the holotype of *Gamotia erecta* Santos. Gould (1972) synonymised this species with *Gamotia acutigluma* (Steud.) Ohwi, and stated the "without collector SFN 13854" (K) as the holotype of this species. I found that the bottom part of the label of Santos's (1950) holotype in K (K 290154) where the collector name should be, was missing. I also found that there are two more duplicates of this collection in BM and SING. Both have the same label which indicated that Ridley was the collector. Therefore, I concluded that the collector of Unknown collector 13854 of *G. erecta* Santos should be Ridley 13854.

When Santos (1950) published *Gamotia flexuosa* Santos, he cited *East India Company 6780* in US (USNH 999393) as the holotype. However, I have found that his holotype sheet bears the following labels "6780, *Gamotia griffithiana* Munro, Locality India, Collector Griffith, 1863-1864". Therefore, I concluded that the collector of this specimen should be Griffith.

Santos (1950) stated that Kuntze s.n. in Herb. Hort. Bot. Novebor. collected from Sikkim on 5 Dec. 1875, was the holotype of *Gamotia himalayensis* Santos. However, the specimen that he labelled as the type sheet is actually kept in NY. Moreover, it was collected on 5 Nov (not Dec.) 1875.

Santos (1950) stated *Clarke 25234* (K) to be the holotype of *Gamotia himalayensis* var. *sikkimensis* Santos. However, the specimen that he labelled as the type sheet was *Clarke 25234A*.

Santos (1950) designated *Clarke 45539C* in US (USNH 1257830) as the holotype of *Gamotia khasiana* Santos and regarded *Clarke 45539E* in K as the isotype. However, the specimen of *Clarke 45539C* is actually deposited in K and *Clarke 45539E* is in US.
Santos (1950) stated that Clarke 15510 in K is the holotype of *Gamotia khasiana* var. *clarkei*. Santos, but the specimen that he labelled as the type sheet was Clarke 15510D. To make the typification clearer, Clarke 15510D should be regarded as the holotype and the rest of Clarke 15510 collections, which I think are duplicates of each other and should be regarded as isotypes.

Santos (1950) stated Clarke 19242 (K) to be the holotype of *Gamotia khasiana* var. *hemitrichophylla*. Santos, but the specimen that he labelled as the type sheet was Clarke 19242A.

**Specimens examined.**

**Thailand:** NORTH-EASTERN: *Loei* [Phu Kradueng, alt. 1,300 m, 24 Dec. 1971, Beusekom et al. 4581 (BKF, C, K)]; [l.c., 31 Oct. 1984, Mitsuta et al. 42259 (BKF)]; [l.c., alt. 1,150–1,250 m, 1 Nov. 1984, Murata 42493 (BKF)]; [l.c., alt. 1,100–1,200 m, 28 Nov. 1965, Tagawa et al. 522 (BKF)]; [l.c., alt. 1,100–1,200 m, 28 Nov. 1965, Tagawa et al. 582 (BKF)]; [l.c., alt. 1,300 m, 25 Mar. 2006, Teerawatananon & Sungkaew 894 (TCD, THNHM)]; [l.c., alt. 1,300 m, 25 Mar. 2006, Teerawatananon & Sungkaew 895 (AAU, BKF, BM, K, TCD, THNHM)].

— G. ciliata var. conduplicata Santos, J. Arnold Arbor. 25: 89. 1944. Type: China, Guangdong (Kwangtung), Loh Fau Mountain (Lofaushan), 26-29 Oct. 1921, Hitchcock 19009½ [holotype US (digital image USNH 1106724!); isotype K! (fragment of US)].

— G. ciliata var. glabriuscula Santos, J. Arnold Arbor. 25: 139. 1944. Type: China, Guangdong (Kwangtung), Loh Fau Mountain (Lofaushan), 26-29 Oct. 1921, Hitchcock 19009B [holotype UC, not seen].


Annual. Culms 20–70 cm tall, erect or ascending or decumbent, often branched; nodes hispid. Leaf-sheaths 4–9 cm long, hispid with tubercle-based hairs. Collar almost glabrous. Ligule a ciliate membrane, 0.5–0.6 mm long. Leaf-blades linear-lanceolate, 5–12 cm by 0.5–1 cm, pilose or hispid with tubercle-based hairs on both surfaces especially at lower part, margins scabrous. Panicles open, 10–30 cm long; racemes 2–15 cm long, rhachis scabrous. Spikelets ovate-lanceolate, 4–6 mm by 0.6–1 mm; pedicels 0.2–2(–4) mm long, scabrous; spikelet callus hairy, hairs 0.2–0.5 mm long; lower glumes ovate-lanceolate, 4–6.5 mm by 0.6–1 mm, sparsely hirsute or hispid with tubercle-based hairs to glabrous, acute to minutely bifid, awned from the sinus, awns 1–2 mm long; upper glumes ovate-lanceolate, 4–6 mm by 0.6–0.8 mm, sparsely hirsute or hispid with tubercle-based hairs to glabrous, acuminate to awned, awns 0.5–2.5 mm long; lemmas ovate-lanceolate, 3.5–5.5 mm long, minutely bifid, awned from the sinus, awns 6–12 mm long, geniculate with brown twisted columns, columns 0.5–2.5 mm long; paleas ovate-lanceolate, 3.5–4.5 mm long; stamens 3, anthers 0.8–1.2 mm long; styles 2, stigmas 0.8–1 mm long. Caryopsis ovoid to ovoid-elliptic, ca. 2.5 mm long.

Thailand. — NORTHERN: Chiang Mai (Doi Inthanon).

Distribution. — China.

Ecology. — In ditches along main roads, moist places on rocky slopes, 1,300–2,300 m altitude.
Notes. — The specimens of *Laegaard & Norsangsri* 21672 and 21736 collected from Doi Inthanon, Chiang Mai differ from all the other specimens seen in having longer and wider spreading racemes, especially the first raceme and having less hairy glumes, though Gould (1972) mentioned that the spikelets of *G. ciliata* could also be found to be glabrous like *Hitchcock* 19009 ⅓.

*Garnotia ciliata* Merr. is similar to *Garnotia thailandica* Gould, *Garnotia tenella* (Arn. ex Miq.) Janowski and *Garnotia cortalluensis* (Arn. & Nees) Thwaites, but it differs from the *G. thailandica* in having shorter inflorescences, spikelets, leaf-blades and in its smaller habit. It differs from *G. tenella* and *G. cortalluensis* in having longer inflorescences and wider spreading racemes.

**Specimens examined.**


**var. patula** Figures 2.11 and 2.14.


Perennial, caespitose. *Culms* 60–150 cm tall, erect; nodes pubescent. *Leaf-sheaths* 10–23 cm long, glabrous to hirsute, both margins of uppermost part villous. *Collar* hispid. *Ligule* a ciliate membrane, 0.3–0.5 mm long, with a dense row of long hairs behind the ligule. *Leaf-blades* linear-lanceolate, 30–60 cm by 0.8–1.5 cm, glabrous to hirsute with tubercle-based hairs on both surfaces, margins scabrous. *Panicles* open, 25–50(–60) cm long; racemes 8–15 cm long, erect spreading or widely spreading, alternate or sometimes whorled, hairy at base, rhachis sub-glabrous to scaberulous. *Spikelets* purplish-green, ovate-lanceolate, 5–6 mm by 0.8–1 mm; pedicels 1–6 mm long, scabrous; spikelet callus hairy, hair 0.7–1.2 mm long; lower glumes ovate-oblong to ovate-lanceolate 3–5(–6) mm by 0.6–0.8 mm, aristate or awned, awns 1–7 mm long; upper glumes ovate-lanceolate, 3–6 mm by 0.7–0.8 mm, acuminate to awned, awns 1–3(–7) mm long; lemmas ovate-lanceolate, 3–5 mm long, awned, awns 6–12(–17) mm long, straight or undulant; paleas ovate-lanceolate, 3–4 mm long; stamens 3, anthers purple, 1.7–2 mm long; styles 2, stigmas purple, 1.7–1.8 mm long. *Caryopsis* ovoid-elliptic, 2–2.3 mm long.

Thailand. — NORTH-EASTERN: Loei (Phu Luang).
Distribution. — India, China, Vietnam and Malaysia.

Ecology. — Along streams or moist places on open area of dwarf oak forests, sandy soil areas, 1,300–1,500 m altitude.

Typification notes. — Munro (1860) cited Wright s.n., collected from Hong Kong, as the type specimen of Berghausia patula Munro, but did not mention where it was kept. Later, Bentham (1861) transferred this species to be under the genus Garnotia, as G. patula (Munro) Benth., and stated that no duplicates of the type specimen of Munro (1860) existed. Later, Gould (1972) revised Garnotia and mentioned the Hong Kong specimen of Wright s.n. in K, as the type specimen. However, he specified Wright s.n. collected from Hong Kong in GH as the holotype of B. patula in his specimen examined list. Therefore, the holotype of G. patula should be Wright s.n. (GH) and the isotype is Wright s.n. (K, ex Herb. Hookerianum, 1867).

Garnotia patula var. hainanensis Santos was first published by Santos (1950). He indicated that Hitchcock 12628 (US) was the holotype and Hitchcock 12628 (NY) was the isotype. He also stated the holotype's herbarium sheet number as USNH 1106186. However, the collector of sheet USNH 1106186 is actually Hitchcock 19628 (not 12628). This collector number (19628) is the same as what was written on the specimen sheet housed in NY. Thus I concluded that the Hitchcock 12628 of Santos was a mistake in printing and the correct number must be Hitchcock 19628.

Specimens examined.

Thailand: NORTH-EASTERN: Loei [Phu Luang, alt. 1,300 m, 15 Nov. 1968, Chermsirivathana 1095 (BK)]; [l.c., alt. 1,400 m, 16 Nov. 1968, Chermsirivathana 1095 (BK)]; [l.c., alt. 1,300–1,562 m, 5 Dec. 1965, Tagawa et al. 1575 (BKF, E, GH)]; [l.c., alt. 1,400 m, 26 Nov. 2005, Teerawatananon & Sungkaew 812 (AAU, BKF, GH, K, NY, TCD)]; [l.c., alt. 1,400 m, 26 Nov. 2005, Teerawatananon & Sungkaew 813 (THNHM, US)]; [l.c., alt. 1,400 m, 26 Nov. 2005, Teerawatananon & Sungkaew 815 (TCD, THNHM)].


— *G. fragilis* Santos, J. Arnold Arbor. 25: 89. 1944; Schmid, Fl. Agrostologique de l’Indochine 13(1): 479, fig. 89/2. 1958. Type: Vietnam (Indo-China), Sa Pa (Chapa), Lo Qui Ho, alt. 2,000 m, Sept. 1933, Petelot 4745 [holotype US (digital image USNH 1610035!); isotypes K! (fragment of US), NY! (NY 381162)].


— *G. asiatica* Santos, Nat. & Applied Sci. Bull., Univ. Philipp. 10: 125. 1950. Type: India, Parasnath, alt. 4,300 ft, 7 Oct. 1883, Clarke 33691B (not 33619) [holotype K! (K 245513); isotype K].


— *G. fragilis* var. *paritorta* Santos, Nat. & Applied Sci. Bull., Univ. Philipp. 10: 132. 1950. Type: Malaysia, Kedah, Kedah Peak, alt. 4,000 ft, 13 Nov. 1915, Haniff 628 (not 638) [holotype K! (K 290156); isotype SING! (SBG 68657)].


— *G. tenuis* var. *angustata* Santos, Nat. & Applied Sci. Bull., Univ. Philipp. 10: 118. 1950. Type: India, Travancore or Thiruvithaamkoor (Travencore), Pallode, Nov. 1901, Bourne s.n. [holotype K! (K 245520)].

Annual, tufted. Culms 15–60(–90) cm tall, erect or geniculately ascending or decumbent, branching, often rooting at lower nodes; nodes hispid. Leaf-sheaths 3–9 cm long, almost glabrous, occasionally pilose, both margins of uppermost part of sheath villous. Collar glabrous, occasionally ciliate. Ligule a ciliate membrane, 0.3–1.5 mm long. Leaf-blades linear-lanceolate, 3–18 cm by 0.5–1.5 cm, occasionally glabrous, mostly hispid and scabrous at one or both surfaces, sometimes with tubercle-based hairs especially in lower part, margins scabrous. Panicles contracted, 5–20(–30) cm long; racemes 5–10 cm long, appressed, alternate
or sometimes verticillate, rhachis scabrous. Spikelets ovate-lanceolate, (2–)3–3.5(–4) mm by 0.4–0.6 mm; pedicels 0.2–3 mm long, scabrous; spikelet callus hairy or absent, up to 0.5 mm long; lower glumes ovate-lanceolate, 2–4 mm by 0.4–0.6 mm, apex acuminate to minutely bifid, awned from the sinus, awns 0.2–3 mm long; upper glumes ovate-lanceolate, 2–4 mm by 0.4–0.6 mm, acuminate to minutely bifid, awned from the sinus, awns 0.2–5 mm long; lemmas ovate-lanceolate, 2–4 mm long, minutely bifid, awned from the sinus, awns 6–12 mm long, geniculate with brown twisted columns, columns 1–3 mm long; paleas ovate-lanceolate, 2–3 mm long; stamens 3, anthers yellow, 0.4–1 mm long; styles 2, stigmas purple, 0.5–1.2 mm long. Caryopsis ovoid to ovoid-elliptic, 1.8–2.5 mm long.

Thailand. — NORTHERN: Chiang Mai (Doi Angka, Doi Suthep, Doi Inthanon, Doi Ang Khang; Lampang (Doi Luang); Phitsanulok (Nakhon Thai); Tak (Um Phang); NORTH-EASTERN: Loei (Phu Kradueng); EASTERN: Nakhon Ratchasima (Khao Yai, Khao Lem; SOUTH-WESTERN: Kanchanaburi (Thong Pha Phum); Ratchaburi (Suan Phung); CENTRAL: Bangkok; Nakhon Nayok (Khao Yai, Nang Rawng, Nakhon Nayok Waterfall); SOUTH-EASTERN: Trat (Ko Chang, Khao Kuap); PENINSULAR: Nakhon Si Thammarat (Prom Lok); Phangnga (Khao Pawta Luang Kaeo); Phatthalung (Khao Chong); Trang (Yahndakao, Khao Chong (Chawng), Na-Yong).

Distribution. — India, Bhutan, Myanmar, China, Vietnam, Malasia, Indonesia (Java, West Sumbawa), Papua New Guinea.

Ecology. — In sunny or shady and moist areas, on tree trunks, on mossy rocks, rocky ground areas, along stream banks, hill slopes of evergreen and montane forests, 100–2,600 m altitude.

Notes. — Four specimens which were collected from Thailand during the Thai-Danish Botanical Expedition in 1958 have been reported as Gamotia stricta Brongn by Bor (1962) but he mentioned only the collector numbers and the details of each location. They are number 593, 2098, 5587 and 6169 collected from Phatthalung, Nakhon Nayok Province, Doi Suthep and Phu Kradueng, respectively. I have found that these specimens were actually collected by Th. Sorensen, K. Larsen and B. Hansen in 1958. They are Sorensen et al. 593, 2098, 5587 and 6169 (BKF, C, E, K, GH (fide Gould, 1972) and SING). I also examined all specimens and found that they are Gamotia tenella.

Typification notes. — Santos (1950) mentioned that the specimens of Clarke 33619 which was housed in K, as the holotype of Gamotia asiatica Santos but the specimen he labelled as the
type sheet is *Clarke* 33691B. To make the typification clearer, *Clarke* 33691B is actually the holotype and the rest of *Clarke* 33691 are regarded to be isotypes.

Santos (1950) stated *Haniff* 638 (K) to be the holotype of *Gamotia fragilis var. paritorta* Santos. However, the specimen he labelled as the type sheet is *Haniff* 628.

There are two specimens mounted on the sheet of *Griffith* 6781 at K (ex KH-3309/70-3), the one on the right hand side is *Gamotia tenella* (Arn. ex Miq.) Janowski and another on the left hand side is *Gamotia acutigluma* (Steud.) Ohwai.

**Specimens examined.**


NORTH-EASTERN: *Locii* [Phu Kradeung, alt. 1,100 m, 7 Sept. 1911, *Charoenpol* et al. 4732 (AAU, BKF, C, K)]; [l.c., 31 Oct. 1984, *Muras* & *Phengklai* 40371 (AAU)]; [l.c., alt. 1,150–1,250
m, 1 Nov. 1984, Murata et al. T-42504 (BKF); [l.c., alt.1,300 m, 16 Oct. 1954, Smitinand 2041 (BKF, K)]; [l.c., alt.1,300 m, 16 Oct. 1954, Smitinand 2079 (BKF, K)]; [l.c., alt.1,300 m, 16 Oct. 1954, Smitinand 2079A (BKF, K)]; [l.c., alt.1,200 m, 29 Oct. 1955, Smitinand 3097 (BKF, K)]; [l.c., alt. 1,300 m, 24 Nov. 1958, Sorensen et al. 6169 (C)]; [l.c., alt. 1,300 m, 28 Nov. 2005, Teerawatananon & Sungkaew 825 (BKF, TCD, THNHM)]; [l.c., alt. 1,300 m, 28 Nov. 2005, Teerawatananon & Sungkaew 826 (TCD)].


SOUTH-WESTERN: **Kanchanaburi** [Thong Pha Phum, alt. ca. 800 m, 3 Nov. 2005, Teerawatananon & Sungkaew 663 (E, SING, TCD, THNHM)]; [l.c., alt. 800 m, 3 Nov. 2005, Teerawatananon & Sungkaew 669 (AAU, SING, TCD, THNHM)]; **Ratchaburi** [Suan Phung, alt. 1,200–1,300 m, 10 Nov. 2004, Teerawatananon & Sungkaew 317 (TCD, THNHM)].


SOUTH-EASTERN: **Trat** [Khao Kuap, alt. 1,600 m, 23 Dec. 1929, Kerr 17719 (BK, BM, K)]; [Ko Chang, 22 Oct. 1972, Maxwell 72-517 (AAU)]; [l.c., alt. 20 m, 19 Feb. 1955, Smitinand 2225 (BKF, K)].

K, NY, US, TCD]); [l.c., alt. 100–200 m, 15 Mar. 2006, Teerawatananon & Sungkaew 877 (BM, TCD, THNHM)]; [Yahndakao, alt. 400 m, 26 Apr. 1987, Maxwell 87-433 (BKF)].
5. **Garnotia thailandica** Gould, Kew Bull. 27(3): 533, fig. 2. 1972. Type: Thailand, Trang, Khao Soi Dao, alt. 1,800 m, 28 Apr. 1930, *Kerr* 19197 [holotype **K**! (K 290151); isotypes **BK**, **BM**! (BM 928170), **K**! (K 290150, K 290152)]. Figures 2.12 and 2.14.

Perennial grass. **Culms** 1–1.5 m tall, decumbent at base; nodes hirsute. **Leaf-sheaths** 10–18 cm long, almost glabrous. **Collar** hirsute. **Ligule** a ciliate membrane, 0.3–1 mm long, with a row of hairs behind the ligule. **Leaf-blades** oblong-lanceolate, 20–40 cm by 1.2–2 cm, pilose with tubercle-based hairs on both surfaces especially at lower part, margins scabrous. **Panicles** open, 40–80 cm long; racemes 8–15 cm long, elongate, rhachis scabrous. **Spikelets** ovate-lanceolate, 4.6–6 mm by ca. 1 mm; pedicels 0.5–1.5 mm long, scabrous; spikelet callus hairy, hairs 0.2–0.6 mm long; lower glumes ovate-lanceolate 4–5.5 mm by 0.8–1 mm, acuminate to awned, awns 1.5–3 (–7) mm long; upper glumes ovate-lanceolate, 4.5–6 mm by 0.8–1 mm, acuminate to awned, awns 1.5–3(–10) mm long; lemmas ovate-lanceolate, 4.5–5 mm long, awned, awns 8–15 mm long, geniculate with brown twisted columns, columns 2–3 mm long; paleas ovate-lanceolate, 4–4.5 mm long; stamens 3, anthers 1.4–1.5 mm long; styles 2, stigmas ca. 0.5 mm long. **Caryopsis** ovoid-elliptic, 3 mm long.

Thailand. — PENINSULAR: Trang (Khao Soi Dao).

Distribution. — Endemic to Thailand.

Ecology. — In open patches of evergreen forests, 1,800 m altitude.

Specimens examined.

Thailand: PENINSULAR: *Trang* [Khao Soi Dao, alt. 1,800 m, 28 Apr. 1930, *Kerr* 19197 (BK, BM, K)].
JANSENELLA


Type: *Jansenella griffithiana* (Müll. Hal.) Bor (basionym = *Danthonia griffithiana* Müll. Hal.)


Annual. **Culms** ascending or decumbent. **Leaf-blades** lanceolate, firm or flaccid. **Ligule** a short membrane. **Inflorescence** a compact panicle. **Spikelets** in pairs, lanceolate, laterally compressed; florets 2, without rachilla extension, breaking up at maturity, disarticulating below each fertile floret; glumes persistent, membranous, exceeding apex of florets, glabrous or pilose with or without tubercle-based hairs on the dorsal surface, aristate, arista scaberulous, 3–5-nerved; lower florets male, female, hermaphrodite or neuter; lower lemmas membranous, aristate to shortly awned, awns scaberulous; lower paleas membranous, winged on the keels; upper florets hermaphrodite; callus hairy; upper lemmas chartaceous to coriaceous or sub-cartilaginous, pilose, with 2 hair tufts on the dorsal surface, deeply bifid, awned from the sinus, awns geniculate with brown twisted columns, scaberulous; upper paleas winged on keels, between keels covered with turgid hairs; stamens 3; stigmas 2, stigmas plumose. **Caryopsis** with adherent pericarp, oblong, hilum punctiform.

One species, distributed from India to Sri Lanka, Myanmar and peninsular Thailand.


Annual. **Culms** 10–30 cm tall, ascending or decumbent, branched; nodes glabrous, rooting from lower nodes. **Leaf-sheaths** 0.8–3 cm long, margin ciliate. **Ligule** a short membrane, 0.2–0.4 mm long. **Leaf-blades** lanceolate to ovate-acuminate, 1–6 cm by 0.2–0.8(–1.4) cm, sparsely pilose on both surfaces, veins prominent on upper surface and scabrous, pilose with tubercle-based hairs at base along the margin, base amplexicaul or rounded, margins thick and scabrous. **Panicles** compact, 1–5 cm long, hirsute below the panicle, rhachis hirsute. **Spikelets** green, ovate-lanceolate, 6–7(–9) mm by 0.8–1.5 mm; pedicels 0.5–3 mm long, scabrous; lower glumes lanceolate, 3.5–5 mm by 0.5–0.8(–1) mm, with or without few tubercle-based hairs on or sometimes between the nerves, acuminate to awned, awns ca. 1 mm long, 3-nerved, scabrous on centre nerve; upper glumes elliptic-oblong, 5–6 mm by 0.8–1(–1.5) mm, with or without a few tubercle-based hairs on dorsal surface, awned, awns up to 2 mm long,
3–5-nerved; lower lemmas oblong-ovate, 5–6 mm long, awned from midnerve, awns 0.5–1 mm long, 3–5-nerved; lower paleas elliptic-oblong, 3 mm long, very narrowly winged on the keels; upper lemmas elliptic-oblong, 2.5–3 mm long, deeply bifid, pilose, with a hair tufts on each side near the margins at the bases of the lobes, hair tufts 0.5–1.3 mm long, lateral awns 1.5–2 mm long, awned from the sinus, awns up to 15 mm long, nerves obscure; upper paleas ob lanceolate, 2–2.5 mm long, covered on the dorsal surface with 1-celled hairs, minutely bifid, narrowly winged on keels; callus hairy, hairs 0.5–1 mm long; stamens 3, anthers 0.5–1.75 mm long; stigmas ca. 0.5 mm long. Caryopsis oblong-obovate, 1–1.5 mm long.

Thailand. — PENINSULAR: Ranong (Khao Pawta Luang Kaeo).

Distribution. — India, Sri Lanka, Myanmar.

Ecology. — In open areas on the mountain ridges in montane forests, 1,000–1,300 m altitude.

Notes. — The specimens examined from Thailand tend to have smaller and more compact panicles and less hairy glumes than the specimens from India.

Typification notes. — When Müller (1856) described Danthonia griffithiana Müll. Hal., he cited the specimen Griffith legit, collected from Khasiya, India, as the type specimen, but he did not mention where it was kept. According to Stafleu and Cowan (1981), the main herbarium of Karl Müller is in B. However, there are two Griffith specimens in B, one of which is labelled Griffith 36 (B 100240400, from Röpert, 2000) and one of which has no number (B 100240395, from Röpert, 2000). Both are also labelled “Danthonia griffithiana n.sp.” in the same handwriting, possibly Müller’s. Later, Bor (1938) transferred this species to Arundinella and stated that the type sheet of D. griffithiana, which was made available to him, was deposited in B. In 1955, he finally transferred it to Jansenella, as J. griffithiana. Conert (1957) published a monograph of Arundinelleae and stated that Griffith 36 in B was the holotype of J. griffithiana. As there are at least two original specimens neither of them can be a holotype. However, as Bor (1938) stated the type was in Berlin, one of these two specimens must be designated as a lectotype even if further material were to be found in another herbarium. It would appear that the unnumbered Griffith specimen (B 100240395) was borrowed by Bor in Kew and was the one which he presumed to be the type. Unfortunately, Conert’s (1957) incorrect citation of the other specimen (Griffith 36, B 100240400) as the holotype did have the effect of establishing that specimen as the lectotype.
When Thwaites (1864) published *Arundinella avenacea* Munro ex Thaites, he cited the Sri Lanka specimen of *Thwaites c.p. 3471* as the type specimen but he did not mention where it was kept. I have found several duplicates of *Thwaites c.p. 3471* in BM, E, K and PDA. Therefore, I hereby designate the specimen of *Thwaites c.p. 3471* housed in K as lectotype and regard the remaining duplicates as isolectotypes.

Specimens examined.

SUBTRIBE CHIONACHNINAE


Type genus: Chionachne R. Br.

Annual or perennial, monoecious. Auricles triangular if present. Ligule usually an eciliate membrane, sometimes a ciliate membrane. Inflorescence of spatheate panicle, solitary or digitate racemes; racemes with female segments below and male segments above or basal male segments below and female segments above, distally with pairs of male spikelets or neuter spikelets. Spikelets in pairs, one sessile and another pedicelled, dissimilar; florets 2, without rachilla extension, falling entirely at maturity. Female part: sessile spikelets dorsally compressed; spikelet callus with central knob; lower glumes coriaceous to cartilaginous, upper margins usually winged; lower florets neuter; upper lemma oblong, entire, awnless. Caryopsis oblong to triangular, base emarginate with punctiform hilum (except Trilobachne). Pedicelled spikelets male or neuter, sometimes suppressed, arising laterally near the top of internode; pedicel fused to the joint. Male part: spikelets in pairs, more or less similar; pedicel free or fused with internode; glumes chartaceous to coriaceous; florets male.

There are three genera and about 12 species, distributed in the Old World tropics. Two genera and three species are found in Thailand.

Key to genera of Thai Chionachninae

1. Inflorescences with solitary raceme, in spatheate axillary. Racemes at base with pairs of female sessile and neuter pedicelled spikelets, distally with pairs of male spikelets. Lower glumes of female spikelets keeled ........................................... 1. Chionachne (p. 87)

1. Inflorescences digitate, lateral branched male, terminal branch mixed. Racemes at base with pairs of neuter or male spikelets, then with a zone with female sessile and neuter pedicelled spikelets, distally with pairs of male or neuter spikelets. Lower glumes of female spikelets not keeled ........................................... 2. Polyrroca (p. 91)
CHIONACHNE


Type: *Chionachne barbata* (Roxb.) R. Br. ex Aitch. (=*Chionachne gigantea* (J. Koenig.) Veldkamp.)


Perennial or annual, tufted or rhizomatous. **Culms** erect or decumbent or short ascending or prostate, branched; nodes glabrous to hairy, rooting at the lower nodes. **Culm internodes** solid or hollow. **Leaf-blades** linear, lanceolate or oblong, margins thick. **Ligule** an eciliate membrane or a ciliate membrane or a fringe of hairs. **Inflorescence** terminal and axillary, solitary or digitate racemes, often gathered into a spatheate panicle, each raceme usually subtended by a spatheole, racemes all mixed, with pairs of female sessile and neuter pedicelled spikelets at base, distally with pairs of male spikelets. **Female part**: sessile spikelets embedded in or enveloping the joints; spikelet callus truncate with central knob; lower glumes coriaceous to cartilaginous, pear-shaped or cylinder-shaped or oblong or ovate; upper glumes coriaceous, 3–17-nerved; lower florets neuter; lower lemmas hyaline, epaleate; upper floret female; upper lemmas hyaline, lanceolate to ovate; upper paleas hyaline, nerveless, keeled or not; styles 2, stigmas plumose. **Caryopsis** with adherent pericarp, reniform-ovate, hilum basal, punctiform. **Pedicelled spikelets** barren, reduced or suppressed. **Male part**: spikelets in pairs or triads, dissimilar. **Sessile spikelets**, lower glumes chartaceous to coriaceous, muticous; upper glumes membranous to chartaceous; florets male; lower lemmas membranous to hyaline, subequal to upper glume; paleas present, hyaline. **Pedicelled spikelets**, lower glumes oblique to symmetric, awnless; lodicules 2; stamens 3.

About nine species, distributed in Temperate Asia, Tropical Asia, Australasia and the Pacific. One species is found in Thailand.

— Polytoca massiei (Balansa) Schenck ex Henrard, Meded. Herb. Leid. 67: 9, plate 1, fig. 3. 1931, as 'massii'. Type: as for above.


Perennial or annual, tufted. **Culms** 20–75 cm tall, decumbent or ascending, branched; nodes pubescent, rooting at the lower nodes. **Leaf-sheaths** 1.3–5(–10) cm long, glabrous to sparsely pilose with tubercle-based hairs near margins. **Auricles** absent or triangular. **Ligule** an eciliate membrane, 0.5–1.5 mm long. **Leaf-blades** ovate-linear-lanceolate to linear-lanceolate, (3–)7–30(–39) cm by 0.7–1 cm, glabrous to pilose with tubercle-based hairs, margins thick and scaberulous. **Racemes** 1–3(–5), 0.8–2.1 cm long, at base with 1–3 female spikelets, distally with 1–2(–3) pairs of male spikelets; joints of rhachis 3–5 mm long, glabrous; spatheoles linear, 1–1.8 cm long; peduncles funnel-shaped with deep cupular apex, 1–2.5 cm long, glabrous. **Female part**: **sessile spikelets** enveloping the joints; lower glumes green, pear-shaped or cylinder-shaped with 2 conspicuous transverse constrictions, 7–10 mm by 4–5 mm, smooth and glabrous, margins and upper margin winged, nerves obscure; upper glumes broadly ovate or round, 4.5–5.25 mm by 3–4 mm, attenuate, 15–17-nerved; lower florets neuter; lower lemmas ovate-elliptic, 4.5–5 mm long, attenuate, 3–4-nerved; lower paleas absent; upper florets female; upper lemmas ovate-oblong, 4–4.5 mm long, acuminate, 1–2-nerved, nerves anastomosing; upper paleas ovate-oblong, 3–4 mm long, acuminate; styles 2, stigmas white, 5–6 mm long. **Caryopsis** reniform-ovate, 2–3 mm by 3–4 mm, hilum circular. **Pedicelled spikelets** neuter, reduced, ovate-lanceolate, 1.5–3.25 mm long; pedicels up to 1 mm long. **Male part**: **sessile spikelet** lower glumes ovate-elliptic, 2.5–4 mm by 2.5–3 mm, muticous or emarginate, margins winged, 6–9-nerved, nerves anastomosing; upper glumes ovate-oblong, 3–4 mm by ca. 1 mm, acute, 3–7-nerved, nerves anastomosing; lower florets male; lower lemmas ovate-oblong, 2.75–3.8 mm long, acute, 3-nerved, nerves anastomosing; lower paleas ovate-oblong, 2.5–3.5 mm long, acute, lower part keeled, keels scaberulous, 2-nerved; upper florets male; upper lemmas ovate-oblong, 2.5–3 mm long, acute, 1-nerved; upper paleas ovate-oblong,
2.5–3 mm long, acute; stamens 3, anthers yellow, 1–1.3 mm long. *Pedicelled spikelets*; lower glumes oblique, ovate-elliptic to ovate-oblong, 3.5–5 mm by 0.8–1 mm, truncate to acute, keeled, 5–7(–14)-nerved, nerves anastomosing, median nerve ridged on back, scaberulous on nerves; upper glumes ovate-oblong, 3–4.5 mm by 0.8–1 mm, acute, keeled or not, 3–7-nerved, nerves anastomosing, median nerve ridged on back, scaberulous on nerves; lower florets male; lower lemmas ovate-elliptic, 3–4 mm long, acute, 3-nerved, nerves anastomosing or not; lower paleas ovate-oblong, 2.75–3.8 mm long, acute, lower part keeled, 2-nerved; upper florets male; upper lemmas ovate-lanceolate, 3–3.5 mm long, acute, 1–3-nerved; upper paleas ovate-lanceolate, 3–3.5 mm long, acute; stamens 3, anthers yellow, 1–1.3 mm long.

Thailand. — NORTHERN: Chiang Mai (Doi Inthanon, San Kamphaeng); SOUTH-WESTERN: Ratchaburi (Ban Kao); CENTRAL: Bangkok; Saraburi (Phu Kae).

Distribution. — China (possibly introduced, fide Chen (1997) and Vietnam.

Ecology. — In moist and open mixed deciduous and dry evergreen forests, clayey soil or humid soil areas, rice fields, disturbed areas, 30–425 m altitude.

Vernacular. — Ya Kan Kluai (Ya Kan Kluai), from Poonpan (1990).

Notes. — The specimens of *Sorensen et al.* 5931 and *K. Larsen* 8106 were reported as *Sclerachne punctata* R. Br. by Bor (1962, 1965). I have examined them and found that they actually are *Chionachne massiei*.

Specimens examined.


SOUTH-WESTERN: *Ratchaburi* [Ban Kao, alt. 70 m, 10 Nov. 1961, *K. Larsen* 8106 (C, K)].
POLYTOCA


Type: Polytoca bracteata R. Br. (see later, under Polytoca digitata (L.f.) Druce)

Perennial, robust, tufted. Culms erect, branched; nodes hairy. Culm internodes solid, glabrous to setose. Leaf-blades linear-oblong, base attenuate, margins thick. Ligule a ciliate membrane. Inflorescence terminal and axillary spicate racemes; racemes 1–4; lateral racemes male, terminal raceme mixed, with pairs of neuter or male spikelets below, then with a zone with female sessile and neuter pedicelled spikelets, distally with pairs of male or neuter spikelets. Female part: sessile spikelets enveloping the joints; spikelet callus truncate with central knob; lower glumes coriaceous to sub-cartilaginous, cylinder-shaped or oblong; upper glumes chartaceous to coriaceous, 7–17-nerved; lower florets neuter, epealeate; upper florets female, epealeate; upper lemmas and paleas hyaline; styles 2, stigmas plumose. Caryopsis hilum basal, punctiform. Pedicelled spikelets neuter, reduced. Male part: sessile spikelets; lower glumes chartaceous to coriaceous, muticous or awned, winged on the upper half; upper glumes membranous to chartaceous; florets both male; lemmas membranous to hyaline; paleas present, hyaline. Pedicelled spikelets similar to the sessile except lower glumes; lower glumes oblique, keeled, awnless; lodicules 2; stamens 3.

Two species are distributed in Temperate Asia, Tropical Asia and Thailand.

Key to species of Thai Polytoca

1. Terminal raceme ending with pairs of male spikelets. Lower glume of male spikelets muticous ................................................................. 1. P. digitata

1. Terminal raceme ending with pairs of neuter spikelets. Lower glume of male spikelets awned ......................................................... 2. P. wallichiana

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Basionym: — *Apluda digitata* L.f., Suppl. Pl. 434. 1781. Type: India, *Thunberg s.n.* in Herb. Linn 1213.6 [holotype LINN (IDC microfiche at K)].


— *P. digitata* (L.f.) Henrard, Meded. Herbar. Leid. 67: 10, plate 2, fig. 2. 1931. non Druce. 1917. Type: as for *Apluda digitata* L.f.

Perennial, tufted. **Culms** up to 2.5(–3) m tall, erect, branched, glabrous to setose with tubercle-based hairs; nodes hirsute. **Leaf-sheaths** (4–)8–40 cm long, glabrous to setose with tubercle-based hairs. **Auricles** absent or triangular. **Ligule** an elicilate membrane, 2–3.5 mm long. **Leaf-blades** linear-oblong, (11–)20–90 cm by 0.7–2.5(–3.8) cm, glabrous to hirsute or setose with tubercle-based hairs, margins thick, scabrous to serrate. **Racemes** 2–4, 3–10 cm long, hirsute at base, terminal raceme mixed, distally with pairs of male spikelets, joints of rachis flat with deep cupular apex, 2–7(–11), margins hirsute. **Female part:** sessile spikelets; lower glumes green, sometimes purplish at apex, cylinder-shaped or oblong, (6–)9–11 mm by 3–4 mm, hirsute on the dorsal surface, upper part attenuated, setose at base of attenuated beak, emarginate to bifid, keeled, keels not overlapping the joint and winged, wings serrate, nerves obscure; upper glumes ovate-elliptic to oblong, (5–)8 mm by ca. 3 mm, glabrous, attenuate, 7–17-nerved, nerves anastomosing; lower lemmas sub-coriaceous, ovate-elliptic to oblong, 4–6.5 mm long, attenuate, 5–9-nerved, nerves anastomosing; upper lemmas ovate-elliptic to ovate-lanceolate, 4–6 mm long, acuminate, 1–2(–3)-nerved, nerves anastomosing or not; upper paleas broadly ovate to ovate-lanceolate, 3–5 mm long, acuminate; styles 2, stigmas purple, 2–3 cm long. **Caryopsis** ovoid-elliptic, 2–3 mm long. **Pedicelled spikelets** ovate-lanceolate, 11–15(–27) mm long, asymmetrical, broadly winged on one side; pedicels ca. 0.5 mm long. **Male part:** sessile spikelets green to purplish-green; lower glumes ovate-lanceolate, (5–)8–9(–12) mm by 2–3 mm, hirsute on dorsal surface, muticous to acute, keeled, keels winged, wings ciliate, 7–11-nerved, nerves anastomosing or not; upper glumes ovate-elliptic, (5–)6–8(–10)
mm by ca. 2 mm, sparsely ciliate, acute, 6–7(–9)-nerved, median nerve with scabrous apex; lower lemmas ovate-oblong, (4.5–)6.5–9 mm long, acute, 3- or 4-nerved, nerves anastomosing; lower glumes oblique, ovate-elliptic to ovate-lanceolate, (5–)8–9(–14) mm by 2–3 mm, hirsute on dorsal surface, acute to attenuate, keeled, keels winged, wings ciliate keeled, 8–9(–11)-nerved, nerves anastomosing; lodicules 2; stamens 3, anthers yellow to pinkish brown, 3–3.5 mm long.

Thailand. — NORTHERN: Chiang Mai (Doi Angka, Doi Chiang Dao, Doi Suthep, Mae Rim, Om Koi, Ob Luang); Chiang Rai (Wiang Pa Pao); Lamphun (Mae Tha); Phitsanulok (Thung Salaeng Luang); NORTH-EASTERN: Phetchabun (Lom Kao); Loei (Phu Kradueng); EASTERN: Chaiyaphum (Thung Kra Mang); Nakhon Ratchasima (Pak Chong); SOUTH-EASTERN: Prachin Buri (Kabin Buri); Sa Kaeo (Aranyaprathet); PENINSULAR: Satun (Tha Phae).

Distribution. — India, Sikkim, Bhutan, China, Myanmar, Vietnam, Indonesia (Java) and Philippines.

Ecology. — Open and shaded areas, disturbed areas, roadsides, deciduous dipterocarp, pine forests and tropical grasslands, 0–1,500 m altitude.

Vernacular. — Ya Kao Pote Phi (Ya Kao Pote Phi) (Poonpan, 1990; Smitinand, 2001).

Specimens examined.

Thailand: NORTHERN: Chiang Mai [Doi Angka, 18 July 1922, Kerr 6350 (ABD, BK, BM, K, TCD)]; [Doi Chiang Dao, alt. 900 m, 19 Jan. 1991, Maxwell 91-79 (GH)]; [l.c., alt. 950 m, 10 Oct. 1995, Maxwell 95-882 (2 sheets L)]; [l.c., 20 Nov. 1955, Phloenchit 888 (K)]; [Doi Suthep, alt. 1,800 ft, 25 Sept. 1910, Kerr 1437 (BM, 2 sheets K, 2 sheets TCD)]; [l.c., 11 Nov. 1911, Kerr 2219 (BM, E, K)]; [l.c., alt. 590–600 m, 12 Nov. 1911, Kerr 2226 ( BM, E, K)]; [l.c., alt. 950 m, 14 Oct. 1987, Maxwell 87-1171 (BKF)]; [l.c., alt. 1,300–1,400 m, 11 Dec. 1957, Smitinand 3954 (K)]; [l.c., alt. ca. 750 m, 12 Dec. 1957, Smitinand 3994 (K)]; [l.c., 18 Nov. 2005, Teerawatananom & Sungkaew 752 (THNHM)]; [Mae Rim, alt. 700–1,000 m, 5 Oct. 2001, Langaard 21678 (AAU)]; [l.c., 16 Nov. 2005, Teerawatananom & Sungkaew 737 (K, L, TCD,
THNHM]; [Om Koi, alt. 1,100 m, 3 July 1968, K. Larsen et al. 2020 (AAU, BKF, E, K)]; [Ob Luang, 11 June 1968, Beusekom & Phengklai 1136 (AAU, C, E, K); Chiang Rai [Wiang Pa Pao, alt. 1,025 m, 18 Nov. 1997, Maxwell 97-1376 (GH)]; Lamphun [Mae Tha, alt. 1,000 m, 23 Sept. 1993, Maxwell 93-1076 (GH, L)]; Phitsanulok [Thung Salaeng Luang National Park, alt. ca. 500 m, 30 Nov. 2005, Teerawatananon & Sungkaew 861 (AAU, BM, TCD, THNHM)].

NORTH-EASTERN: Locii [Phu Kradueng, alt. 1,300 m, 19 Mar. 1958, Sørensen et al. 2281 (C, K)]; [loc., alt. 1,300 m, 19 Mar. 1958, Sørensen et al. 2312 (C, K)]; [loc., alt. 600 m, 18 Aug. 1954, Smitinand 1861 (K)]; [loc., alt. 1,300 m, 29 Nov. 2005, Teerawatananon & Sungkaew 844 (THNHM)]; [loc., alt. 1,300 m, 26 Mar. 2006, Teerawatananon & Sungkaew 901 (NY, TCD)]; Phetchabun [Lom Kao, alt. ca. 1,000 m, 5 May 1955, Smitinand 2635 (K)].

EASTERN: Chaiyaphum [Thung Kra Mang, alt. 850 m, 31 May 1974, Geesink et al. 7103 (AAU, K)]; [loc., alt. 800 m, 9 Aug. 1972, K. Larsen et al. 31645 (AAU)]; Nakhon Ratchasima [Pak Chong, alt. ca. 200 m, 27 Dec. 1923, Kerr s.n. (BM)].

SOUTH-EASTERN: Prachin Buri [Kabin Buri, alt. 100 m, 27 Dec. 1924, Kerr 9784 (BK, BM, K)]; [loc., alt. 25 m, 10 Nov. 1930, Marcan 2595 (K)]; Sa Kaco [Aranyaprathet, alt. 50 m, 9 Aug. 1930, Kerr 19605 (2 sheets K)].

PENINSULAR: Satun [Tha Phae, alt. 25 m, 14 Nov. 1986, Maxwell 86-920 (BKF)]; [alt. 100 m, 10 Jan. 1924, Kerr 8253 (BM, 2 sheets K, TCD)]; [alt. 20 m, 30 Dec. 1927, Kerr 13771 (BK, BM, 2 sheets K)].


Perennial or annuals, tufted. **Culms** up to 3 m tall, erect, branched, glabrous to setose with tubercle-based hairs; nodes hirsute, rooting at lower nodes. **Leaf-sheaths** (5—)10—20 cm long, glabrous to setose with tubercle-based hairs. **Auricles** triangular. **Ligule** a ciliate membrane, 1—3 mm long. **Leaf-blades** linear-oblong, (10—)30—60 cm by 1.2—4 cm, base attenuate, glabrous to hirsute or setose with tubercle-based hairs, margins thick and scabrous. **Racemes** 1—9, 3—8 cm long, rhachis pubescent, terminal raceme mixed, distally with pairs of neuter spikelets, joints of rhachis flat with deep cupular apex, 3—6 mm long. **Female part:** sessile spikelets, hirsute; lower glumes green to purplish-green cylinder-shaped or oblong, (5—)8—9 mm by 3—3.5 mm, sometimes slightly constricted in the lower third, hirsute on the dorsal surface, upper part attenuated, setose at base of attenuated beak, emarginate to bifid, keeled, keels not overlapping the joint and winged, wings serrate; upper glumes ovate-oblong, 4—6 mm by ca. 3 mm, glabrous, attenuate, 7—9-nerved, nerves anastomosing; lower lemmas ovate-oblong, 4—5 mm long, attenuate, 5—9-nerved, nerves anastomosing; upper lemmas ovate-lanceolate, 2—3 mm long, acuminate, 1-nerved; styles 2, stigmas purple, 4—6 cm long. **Caryopsis** broadly ovoid to cuboid, 2—3 mm long. **Pedicelled spikelets** neuter, reduced, ovate-lanceolate, 14—25 mm long, asymmetrical, margins winged; pedicels ca. 0.5 mm long. **Male part:** sessile spikelets green to purplish-green; lower glumes ovate-oblong, 5—7 mm by 1.5—2 mm, scaberulous on dorsal surface, awned, awns up to 1.5 mm long, scabrous, 5—7-nerved, nerves anastomosing; upper glumes ovate-oblong, 5—6.5 mm by ca. 1.5 mm, scaberulous on dorsal surface, acute, 5—8-nerved; lower lemmas ovate-oblong, 4.5—6.5 mm long, acute, 3- or 5-nerved, nerves anastomosing; lower paleas ovate-oblong, 4—6 mm long, acute, keeled, keels scaberulous, 2-nerved; upper lemmas ovate-oblong, 3.5—5 mm long, acute, 1—(3)—nerved; upper paleas ovate-oblong, 3.5—5 mm long, acute. **Pedicelled spikelets**, pedicels ca. 3—4 mm long, fused with
the rhachis, hirsute; lower glumes oblique, ovate-oblong, 5—6 mm by 1.5—2 mm, scaberulous
on dorsal surface, awned, awns up to 1.5 mm long, scabrous, 5—7-nerved, nerves
anastomosing; lodicules 2; stamens 3, anthers yellow to orangish pink or pinkish brown, 2—2.5
mm long.

Thailand. — NORTHERN: Chiang Mai (Fang); Kamphaeng Phet; SOUTH-WESTERN:
Kanchanaburi (Huai Bankau, Kroeng Krawai, Lin Thin, Sai Yok, Thong Pha Phum);
CENTRAL: Chai Nat (Makam Tao); PENINSULAR: Surat Thani (Ban Kawp Kep).

Distribution. — India, Sikkim, Bhutan, Bangladesh and Myanmar.

Ecology. — Deciduous forests, edge of or in swamps, open areas or along banks of streams in
evergreen forests, 100—750 m altitude.

Notes. — Bor (1940) and Jannink and Veldkamp (2002) mentioned that the female spikelet of
Pobytaca wallichiana has only a lower floret while the upper floret is absent. However, I have
found that the existence of the upper floret of the female sessile spikelet appears to be
problematic. The two inner membranous bracts of the female sessile spikelet can either be
considered as a lower floret or as the lemmas of lower and upper florets (Clayton and
Renvoize, 1986; Noltie, 2000). In this study, I followed the latter interpretation that is
consistent with Clayton and Renvoize's (1986) and Noltie's (2000) views that P. wallichiana has
two florets. I used the term 'upper lemma' rather than that of 'lower palea' used by Jannink
and Veldkamp (2002).

Vernacular. — Ya Kao Pote Phi (น้ำข้าวโพธิ์), from Kerr 13164 (BM).

Specimens examined.

Thailand: NORTHERN: Chiang Mai [Fang, alt. 550 m, 26 July 1968, K. Larsen et al. 2747
sheets K)].

SOUTH-WESTERN: Kanchanaburi [Huai Bankau, alt. 750 m, 9 Nov. 1971, Bensekom et al.
3581 (BKF)]; [Kroeng Krawai, alt. 350 m, 3 Feb. 1962, K. Larsen 9524 (C, K)]; [Lin Thin, 2
Sept. 2001, Teerawatwananon 020901-2 (THNHM)]; [Lc, alt. 100—150 m, 19 July 1946,
Kostermans 1223 (GH)]; [Sai Yok, alt. 200 m, 6 Dec. 1961, K. Larsen 8559 (C, 2 sheets GH, K)];

CENTRAL: Chai Nat [Makam Tao, 21 Sept. 1930, Kerr 19696 (BK, BM, 3 sheets K)].

PENINSULAR: Surat Thani [Ban Kawp Kep, alt. ca. 20 m, Aug. 1927, Kerr 13164 (BK, BM)].
SUBTRIBE DIMERIIINAE


Type genus: Dimeria R. Br.

Annual or perennial. Ligule an eciilie membrane or a ciliate membrane. Inflorescence solitary or digitate racemes with tough rhachis. Spikelets single, strongly laterally compressed; florets 2, without rachilla extension, usually falling entirely at maturity (or persistent in D. woodrowii, the two mature racemes coil into a ball and shed together); glumes persistent, membranous to coriaceous, usually keeled, often winged; lower florets reduced to a neuter lemma; upper lemmas bilobed, often awned from the sinus. Caryopsis punctiform hilum.

Monogeneric subtribe, about 40 species, distributed in Africa, Temperate Asia, Tropical Asia, Australasia and Pacific. Seven species are found in Thailand.
**DIMERIA**


**Type:** *Dimeria acinaciformis* R. Br.


Annual or perennial, caespitose. **Culms** slender, erect or geniculate or ascending or decumbent; nodes usually pubescent or hirsute sometimes glabrous. **Leaf-blades** linear or linear-lanceolate, midrib prominent beneath. **Ligule** a ciliate membrane. **Inflorescence** of solitary or digitate racemes, racemes unilateral, rhachis triquetrous or flattened or filiform, winged or wingless. **Spikelets** reddish or greenish olive or reddish-purple, single, ovate-oblong or lanceolate, strongly laterally compressed, disarticulating below spikelet; pedicels usually short, flattened or clavate or cuneate; spikelet callus truncate, hairy; glumes chartaceous to coriaceous, acute or acuminate or awned, margins hyaline, strongly keeled, winged on keel or wingless, lower glumes usually narrower and shorter than the upper; florets 2; lower florets
reduced to a hyaline lemma; upper florets hermaphrodite, epaleate; upper lemmas hyaline, bifid, awned from the sinus, geniculate with brown twisted columns, scaberulous; lodicules 2, small, hyaline; stamens 2, anthers yellow; styles 2, stigmas plumose. Caryopsis with adherent pericarp, oblong or ellipsoid, laterally compressed; hilum punctiform.

Seven species are found in Thailand of which one is endemic: *D. kerrii* C. E. Hubb. ex Teerawat. & Sungkaew.

**Key to species of Thai *Dimeria***

1. Racemes with filiform rhachis ................................................................. 4. *D. leptorhachis*

1. Racemes with flattened or triquetrous rhachis
   2. Annual
   3. Racemes more than one
      4. Upper glumes winged below the apex or all along; apex recurved. Spikelets 4.5–5 mm long. Rhachis flattened, ciliate along the margins .......................................................... 6. *D. pubescens*
      4. Upper glumes wingless, apex not recurved. Spikelet 1–3.5 mm long. Rhachis usually triquetrous, scabrid along the margins .................. 5. *D. ornithopoda*

3. Raceme solitary
   5. Upper glumes broadly winged from base to apex .................. 7. *D. sinensis*
   5. Upper glumes wingless or winged only below the apex
      6. Spikelets 4.5–5 mm long; upper glumes winged just below the apex or all along; apex recurved; awns 8–11 mm long. .................. 6. *D. pubescens*
      6. Spikelets 3–3.5 mm long; upper glumes wingless; apex not recurved; awns 12–18 mm long .................................................................................. 3. *D. kurzii*

2. Perennial
   7. Upper glumes with a broad wing, wings rugose ....................... 2. *D. kerrii*
   7. Upper glumes with a narrow wing, wings not rugose ............ 1. *D. fuscescens*


Perennial. *Culms* up to 70 cm tall, often robust, erect or geniculate; nodes pubescent, usually short-noded at the base. *Leaf-sheaths* overlapping below, 4–8 cm long, glabrous to densely pilose with tubercle-based hairs, margins scarious. *Ligule* a ciliate membrane, 0.5–0.8 mm long. *Leaf-blades* linear, 3–15 cm by 2–5 mm, usually pilose with tubercle-based hairs on both surfaces, margins pilose with tubercle-based hairs, scabrous near the apex. *Racemes* (2–)3, 4–12 cm long, rachis flattened, 0.7–0.8 mm wide, straight or slightly zigzag, slightly convex or slightly ridged, ridge glabrous, narrowly winged, margins scabrous. *Spikelets* ovate-oblong, (4–)4.5–6(–7) mm by 1–1.3 mm; pedicels concave and swollen above, compressed but not flattened, 0.5–1 mm long, margins glabrous to ciliate; spikelet callus hairy, hairs up to 1 mm long; lower glumes linear-oblong, 4–6 mm long, more or less scabrous at both sides, acuminate, scabrous and ciliate on keel; upper glumes oblong-elliptic, 5–7 mm long, more or less scabrous at both sides, acute, keeled, keel scabrous and ciliate, narrowly winged all along the keel, not rugose; lower lemmas oblanceolate, 3–4 mm long, margins ciliate on the upper half, nerves obscure or 3-nerved; upper lemmas oblong-elliptic, 3–5 mm long, awns (8–)11–14 mm long, columns (1.5–)3–4 mm long; stamens 2, anthers yellow, 2–3.5 mm long; styles 2, stigmas yellowish green, ca. 1.5 mm long. *Caryopsis* oblong, 2.5–3 mm long.

Thailand. — NORTH-EASTERN: Loei (Phu Kradueng); EASTERN: Chaiyaphum (Phu Khiew); PENINSULAR: Satun.

Distribution. — India, Sri Lanka and Nepal

Ecology. — Open areas, on rocky places, clayey soil or sandstone areas, grassy areas, pine forests, 800–1,300 m altitude.
Notes. — Specimens of Beusekom et al. 4492 (C, K, L) and 4376 (L) collected from Loei and Chaiyaphum Provinces, respectively, have been misidentified as Dimeria falcatula Hack. I have examined them and found that they actually are Dimeria fuscescens.

Dimeria fuscescens is similar to Dimeria ballardii Bor and Dimeria falcata Hack. It differs from D. ballardii in having shorter spikelets and upper glumes with a narrow wing on the keel and can be separated from D. falcata by it larger spikelets and a concave and swollen pedicel tip.

Specimens examined.

Thailand: NORTH-EASTERN: Loei [Phu Kradueng, alt. 1,300 m, 23 Dec. 1971, Beusekom et al. 4492 (C, K, L)]; [l.c., alt. 1,300 m, 14 Sept. 1954, Smitinand 1932 (K)]; [l.c., alt. 1,300 m, 25 Nov. 1958, Smitinand 4974 (K)]; [l.c., alt. 1,300 m, 25 Nov. 1958, Sorensen et al. 6200 (C, K)]; [l.c., alt. 1,300 m, 28 Nov. 2005, Teerawatananon & Sungkaew 830 (AAU, BM, BKF, K, L, TCD, THNHM)].

EASTERN: Chaiyaphum [Phu Khiew, alt. 800 m, 16 Dec. 1971, Beusekom et al. 4376 (L)].

PENINSULAR: Satun [alt. ca. 5 m, 28 Dec. 1927, Kerr 13702A (K)].

Perennial. *Culms* up to 1.2 m tall, erect; nodes pubescent, stems waxy below nodes, usually short-noded at base. *Leaf-sheaths* overlapping below, 6–9 cm long, sparsely pilose at lower part, upper part tomentose, margins scarious. *Ligule* a ciliate membrane, ca. 0.6 mm long. *Leaf-blades* linear-lanceolate, 10–20 cm by 3–4.5 mm, tomentose on both surfaces, sparsely pilose with tubercle-based hairs especially near the margins, margins scaberulous near the apex. *Racemes* (2–)3, 8–16 cm long, rhachis flattened, 0.6–0.7 mm wide, slightly zigzagging, slightly ridged, ridge glabrous, narrowly winged, margins scaberulous; peduncles hirsute at top below the inflorescences. *Spikelets* ovate-oblong, 5–6 mm by 1.8–2 mm; pedicels compressed but not flattened, 0.8–1.2 mm long, margins glabrous, clavate; spikelet callus hairy, hairs up to 0.5 mm long; lower glumes oblong, 5–5.5 mm long, acuminate, keeled, ciliate on keel; upper glumes oblong-elliptic, 5.5–6 mm long, acute to acuminate, keeled, sparsely hirsute near margins, broadly winged all along the keel, wings rugose and ciliate; lower lemmas oblanceolate or clavate, 2.5–3 mm long, margins ciliate on the upper half; upper lemmas oblong-elliptic, ca. 4 mm long, awns 12–15 mm long, columns 2–3 mm long; stamens 2, anthers 1.8–2 mm long; styles 2, stigmas, 1–1.5 mm long. Caryopsis not seen.

Thailand. — PENINSULAR: Satun (Ban Tola Tai).

Distribution. — Endemic to Thailand.

Ecology. — In grasslands, ca. 50 m altitude.

Notes. — This species was first recognised by Dr. C. E. Hubbard of Kew based on *Kerr* 13868 (K). This name was later reported by Nanakorn and Norsangsri (2001). However, the name has never been validly published. It is distinguished from all other species of *Dimeria* by its rugose, broad wing on the keel of the upper glume.

Specimens examined.

**Thailand:** PENINSULAR: Satun [Ban Tola Sai (Tola), alt. ca. 50 m, 3 Jan. 1928, *Kerr* 13868 (BM, 2 sheets K)].

Annual. Culms up to 60 cm tall, slender, erect, branched; nodes pubescent. Leaf-sheaths 2–4 cm long, pilose with tubercle-based hairs, margins scarious. Ligule a ciliate membrane, 0.5–1 mm long. Leaf-blades linear, 4–8 cm by 2–3.5 mm, hirsute on both surfaces and pilose with tubercle-based hairs especially near the margins and upper surface, margins pilose with tubercle-based hairs, scabrous near the apex. Racemes solitary, 3–5.5 cm long, rhachis flattened, 0.6–0.7 mm wide, slightly ridged, ridge glabrous, narrowly winged, margins ciliate. Spikelets ovate-oblong, 2.5–3.5 mm by 0.5–0.7 mm; pedicels flattened, 0.2–0.5 mm long, margins ciliate; spikelet callus hairy, hairs up to 0.5 mm long; lower glumes oblong, ca. 2.5 mm long, acuminate to short aristate, keeled, densely ciliate on keel and at both sides on the lower half; upper glumes oblong-elliptic, 3–3.5 mm long, acuminate, keeled, 3-nerved, densely ciliate on keel and lateral nerves, wingless; lower lemmas narrow lanceolate, ca. 1.5 mm long; upper lemmas oblong-elliptic, 2–2.5 mm long, awns 12–18 mm long, columns 3–4 mm long; stamens 2, anthers yellow, 0.5–0.6 mm long; styles 2, stigmas purple, 0.5–0.6 mm long. Caryopsis oblong, 2.2–2.3 mm long.

Thailand. — NORTH-EASTERN: Loei (Phu Kradueng (Khao Krading); Nakhon Ratchasima (Ban Rai, Hui Reng); Ubon Ratchathani (Khong Chiam); SOUTH-EASTERN: Chanthaburi (Laem Sadet, Makham); PENINSULAR: Phangnga (Kopah–Pakok); Satun (Thung Nui); Songkhla (Khlong Hoi Khong).

Distribution. — Myanmar.

Ecology. — Common in open grassy areas, wet sandy soil areas, paddy fields, deciduous dipterocarp forests, 5–1,200 m altitude.
Specimens examined.


— *D. leptorhachis* subsp. *velutina* Hack, in A. DC. & C. DC., Monogr. Phan. 6: 90. 1889. Type: Myanmar, Tenasserim (Ind. Or. (Benglia v. Himalaya?)), *Griffith* 6799 [lectotype K! (K 245776), designated here; isolectotypes K! (K 245777), E!].


Perennial, caespitose. *Culms* 50—120 cm tall, erect; nodes glabrous to pubescent, stems waxy below the nodes, usually short-noded at the base, covered with overlapping sheaths in the lower half. *Leaf-sheaths* 4—9 cm long, sparsely pubescent to densely pilose with tubercle-based hairs, margins scarious. *Ligule* a ciliate membrane, 1(—2) mm long. *Leaf-blades* linear-lanceolate, 10—25 cm by 3—10 mm, sparsely pilose to densely pubescent with tubercle-based hairs on both surfaces, margins scabrous. *Racemes* 3—10, (5—)8—15 cm long, rhachis filiform, straight or slightly zigzag, glabrous. *Spikelets* linear-oblong, (4.5—)5—8 mm by 1—1.2 mm; pedicels cuneate, compressed but not flattened, 0.5—1 mm long, margins glabrous; spikelet callus hairy, hairs up to 1 mm long; lower glumes linear-oblong, 4.5—6.5 mm long, more or less scabrous and ciliate at both sides, acuminate, keeled, scabrous and ciliate on keel; upper glumes oblong-elliptic, 5—8 mm long, more or less ciliate at both sides, acuminate, keeled, scabrous and ciliate on keel, wingless; lower lemmas oblanceolate, 3—5 mm long, margins ciliate on the upper half, rarely awned; upper lemmas oblong-elliptic, 3.5—4 mm long, awns 7—12 mm long, columns 2.5—3.5 mm long; stamens 2, anthers 3—3.5 mm long; styles 2, stigmas ca. 1.5 mm long. *Caryopsis* not seen.

Thailand. — SOUTH-EASTERN: Trat (Khao Kuap).

Distribution. — Sri Lanka, Myanmar and Malaysia.

Ecology. — On open rocky areas, stony hillsides, ca. 600 m altitude.
Notes. — *Dimeria leptorhachis* Hack. is similar to *Dimeria gracilis* Nees ex Steud., from which it differs in having cuneate pedicels and larger spikelets.

Typification notes. — According to Hackel (1889), the type specimens of *Dimeria leptorhachis* Hack. are *Thwaites* c.p. 24 and 3261 collected from Sri Lanka. In selecting a lectotype, only one of specimens must be selected as the lectotype, and that *Thwaites* c.p. 24 has priority as the first specimen cited by Hackel (1889). In addition, according to Clayton (1994), the specimens of *Thwaites* c.p. 24 were housed in K and PDA. To ensure that the type is easily accessed, I hereby designate *Thwaites* c.p. 24, housed in K (K 2460009), as the lectotype and regard the remaining duplicate *Thwaites* c.p. 24 which I presume is in PDA, as an isolectotype.

Hackel (1889) stated that *Griffith* 6799, collected from India was the type specimen of *Dimeria leptorhachis* subsp. *velutina* Hack., but he did not indicate where it was kept. Later, Bor (1952b) elevated this taxon to be a species as *Dimeria velutina* Bor, and mentioned that two sheets of Hackel’s type specimens were kept in K. He also stated that Hackel (1889) gave the wrong location by mistake, as *Griffith* 6799 specimens were actually collected from Tenasserim, Myanmar. In addition, I found another duplicate of *Griffith* 6799 in E. According to Bor (1952b), one of the *Griffith* 6799 specimens should be selected as the lectotype. Therefore, *Griffith* 6799 (K 245776) is chosen here as the lectotype and the remaining duplicates (K 245777; E) are regarded as isolectotypes.

Specimens examined.

Thailand: SOUTH-EASTERN: *Trat* [Khao Kuap, alt. 600 m, 23 Dec. 1929, *Kerr* 17725 (BM, 2 sheets K)].

— *Andropogon filiformis* Roxb. in Carey & Wall., Fl. Ind. 1: 260. 1820. non Pers. 1805. Type: India, Kolkata (Calcutta), Unknown collector s.n. [holotype, not located]


— *D. filiformis* (Roxb.) Hochst. in Hohenacker, Plant. Indiae Or. no. 231, nomen.


Key to the varieties of *D. ornithopoda*

1. Spikelets 1.5–2.5 mm long; anthers (0.25–)0.5 mm long .................. **var. ornithopoda**

1. Spikelets 1–1.3 mm long; anthers up to 0.25 mm long .................. **var. gracillima**
var. ornithopoda  Figures 2.24, 2.26 and 2.28.

Annual, tufted. Culms slender, (4—)20—50 cm tall, erect or ascending, branched; nodes pubescent. Leaf-sheaths 1—4 cm long, margins scarious, glabrous to pilose with tubercle-based hairs. Légule a ciliate membrane, (0.3—)0.5 mm long. Leaf-blades linear-lanceolate, 2—5 cm by 1—3 mm, glabrous to hirsute or pilose with tubercle-based hairs on both surfaces, margins pilose with tubercle-based hair, scarious near the apex. Racemes 2—3(—5), 1—6(—8) cm long, rhachis triquetrous or flattened, 0.2—0.4 mm wide, slightly ridged, ridge scarious, narrowly winged or wingless, margins scarious. Spikelets linear-oblong or elliptic-oblong, (1—)1.5—3(—4.5) mm by 1—1.2 mm; pedicels ca. 0.1 mm long, compressed; spikelet callus hairy, hairs up to 0.4 mm long; lower glumes linear-oblong, 0.8—3 mm long, more or less shortly hairy at both sides, acute, scarious on keel; upper glumes oblong-elliptic, 1—3.5 mm long, scarious and ciliate on the dorsal surface, strongly compressed but not keeled, more or less shortly hairy at both sides, acuminate, wingless, sometimes upper glumes of basal spikelets narrowly winged; lower lemmas lanceolate, 0.5—1.5 mm long; upper lemmas oblong-elliptic, 0.8—2 mm long, awns (3—)6—9(—12) mm long, columns 1.5—2.5(—3.5) mm long; stamens 2, anthers yellow, (0.25—)0.5 mm long; styles 2, stigmas 0.3—0.6 mm long. Caryopsis oblong, 1—1.4 mm long.

Thailand. — EASTERN: Ubon Ratchathani (Khong Chiam, Kaeng Tana, Pha Tan, Warin Chamrap); SOUTH-WESTERN: Kanchanaburi (Thong Phaphum); CENTRAL: Nakhon Nayok (Khao Yai); SOUTH-EASTERN: Chanthaburi (Laem Sing); Trat (Khao Saming, Ko Chang); PENINSULAR: Chumphon (Bang Son); Phangnga; Ranong (Mang Len); Satun (Tarutao); Songkhla (Na Mom, Surin); Surat Thani (Tha Chang); Trang (Khao Chong).

Distribution. — India, Nepal, Bangladesh, Myanmar, China, Vietnam, Malaysia, Singapore, Indonesia (Java), Philippines and Australia.

Ecology. — Open grassy areas, secondary grasslands, wet sandy soil areas, rice fields, seasonally flooded flatlands, hill slopes, on rocks by the sea, along stream banks, secondary forests; 0—700 m altitude.

Notes. — This is a widespread and variable species, showing much variation in the height, the hairiness of the vegetative parts and spikelets, and the length of racemes. In Thailand, two varieties are recognised based on the length of their spikelets and stamens.
Typification notes. — When Trinius (1832) published *Dimeria tenera* Trin., he cited two specimens collected from Manila (ManUl.) and Nepal as the type specimens but did not mention the collector’s name and number. I have found a sheet of Trinius’s (1832) type specimen of *D. tenera* housed in LE (*Trinius* 1255.01, IDC microfiche at K). On the sheet, there are two specimens which were labelled in Trinius’s handwriting. One of which was labelled as “*D. tenera* Trin., Nepal”, another was labelled as “*D. tenera* m, [handwriting on label unreadable], Manilla [handwriting on label unreadable], de Chamisso, 1821”. It is possible that Trinius (1832) described *D. tenera* based on these specimens. According to Stafleu and Cowan (1976) and Liebersohn (1994), Chamisso was a French born German naturalist, who sailed as a botanist on the Russian vessel “Rurik” on a scientific circumnavigation of the world in 1815 and returned to St. Petersburg in 1818 via Manila. The first set of his collections on the voyage of the the *Rurik* is at LE. So, it is possible that one of the collectors of Trinius’s (1832) type specimen could be collected by Chamisso. Therefore, I hereby designate Chamisso s.n. m in LE, as the lectotype due to its better quality.

Specimens examined.


CENTRAL: *Nakhon Nayok* [Khao Yai, alt. 750 m, 17 Dec. 2001, Maxwell 01-726 (GH)].

9446 (BM, K); [Ko Chang, alt. ca. 500 m, 3 Oct. 1924, Kerr 9317 (BM, K)]; [l.c., 8 Nov. 2005, Teerawatananon & Sungkaew 685 (BKF, KKU, NY, TCD, THNHM)].

PENINSULAR: 

*Chumphon* [Bang Son, alt. 20 m, 9 May 1927, Kerr 11312 (ABD, 2 sheets K)]; [Muang, alt. 5 m, 15 Jan. 1987, Maxwell 87-41 (AAU, GH, L); *Phangnga* [alt. ca. 5 m, 6 Mar. 1929, Kerr 17355 (BM, K, TCD); *Ranong* [Muang Len, alt. 160 m, 11 Jan. 1966, Hansen & Smitinand 11894 (C, K, L)]; *Satun* [Tarutao, alt. 5 m, 27 Feb. 1966, Hansen & Smitinand 12454 (C, K, L)]; [l.c., 13 Jan. 1981, Congdon 1062 (AAU, GH)]; [alt. ca. 5 m, 28 Dec. 1927, Kerr 13720 (BM, 3 sheets K)]; *Songkhla* [Na Mom, alt. 50 m, 9 Apr. 1986, Maxwell 86-230 (GH, L)]; [Surin, alt. ca. 200 m, 13 Jan. 1924, Kerr 8275 (BM, K, TCD)]; *Surat Thani* [Tha Chang, 5 Feb. 1987, Maxwell 87-162 (GH, 2 sheets L)]; [alt. ca. 10 m, 6 Jan. 1927, Kerr 11293 (ABD, BM, 3 sheets K)]; *Trang* [Khao Chong (Kachawng), Feb. 1950, Williams 17250 (K)].
**var. gracillima** Bor, Kew Bull. 4: 576. 1953, Grasses Burma, Ceyl., Ind. & Pakist.: 144. 1960. Type: India, Jharkhand, Parasnath, alt. 4000 ft, 1 Oct. 1873 Clarke 21084B [lectotype K! (K 245790)]. Figure 2.28.

*Culms* up to 20 cm tall. *Leaf-sheaths and blades* densely pilose with tubercle-based hairs. *Spikelets* elliptic-oblong, 1(−1.3) mm long; glumes glabrous to sparsely scabrous on the dorsal surface, ciliate at both sides; *stamens* 2, *anthers* ca. 0.25 mm long.

Thailand. — NORTHERN: Chiang Mai (Doi Buak Ha, Doi Suthep).

Distribution. — India.

Ecology. — Open areas or hill slopes in montane forests, 1,500–1,700 m altitude.

Notes. — Specimens from Thailand housed in K and L have slightly longer spikelets than *Clarke* 21084B, *Clarke* 33719A.

Typification notes. — Bor (1953) cited three specimens of *Clarke* which are housed in K as the syntypes of *Dimeria ornithopoda var. gracillima* Bor. They are *Clarke* 21084B, *Clarke* 33719A and *Clarke* 33719C. However, in selecting a lectotype, only one of specimens must be selected as the lectotype. Therefore, *Clarke* 21084B (K 245790) is selected as the lectotype because it has more potential to be selected as the lectotype than the others.

Specimens examined.

**Thailand:** NORTHERN: *Chiang Mai* [Doi Buak Ha, alt. *ca.* 1,575 m, 30 Nov. 1965, Hennipman 3174 (C, K, L)]; [Doi Suthep, 12 Dec. 1904, Hosseus 212 (BM, K, L)]; [l.c., alt. 5,500 ft, 12 Nov. 1911, Kerr 1581B (BM, K)]; [l.c., alt. 1,700 m, 6 Dec. 1957, Richards 5462 (K)]; [l.c., alt. 1,600 m, 11 Dec. 1957, Smitinand 3963 (K)]; [l.c., alt. 1,550 m, 28 Nov. 1961, Smitinand & Anderson 7199 (2 sheets K)].


— *D. trimeni* Hook.f. in Trim., Fl. Ceylon 5: 196. 1900; Bor, Kew Bull. 4: 587. 1953, as ‘*trimenii*’, Grasses Burma, Ceyl., Ind. & Pakist.: 144. 1960, as *’trimenii’*. Type: Sri Lanka, Unknown collector s.n. [holotype PDA, not seen].

Annual. Culms up to 70 cm tall, erect; nodes hirsute. Leaf-sheaths distichous below, 2–4 cm long, usually pilose with tubercle-based hairs, margins scarious. Ligule a ciliate membrane, ca. 1 mm long. Leaf-blades linear, up to 20 cm by 2–4 mm, scabrous and pilose with tubercle-based hairs on both surfaces, margins pilose with tubercle-based hairs, scabrous near the apex. Racemes 1–2 racemes, 6–10 cm long, rhachis flattened, 0.8–1 mm wide, slightly ridged, ridge glabrous, narrowly winged, margins ciliate. Spikelets ovate-oblong, 4.5–5 mm by 0.8–1 mm; pedicels flattened, 0.5–0.7 mm long, shallowly concave at apex, margins ciliate; spikelet callus hairy, hairs up to 1 mm long; lower glumes oblong, 3.5–5 mm long, acute to acuminate, keeled, densely ciliate on keel and at both sides, especially on the lower half, winged at the apex; upper glumes oblong-elliptic, 4.5–5 mm long, acuminate to attenuate, slightly recurved, keeled, densely ciliate on keel and at both sides, narrowly to broadly winged at the apex or sometimes winged all along the keel; lower lemmas oblanceolate to clavate, 2.5–2.8 mm long, margins ciliate on the upper half; upper lemmas oblong-elliptic, 3–3.5 mm long, awns 8–11 mm long, columns 2–3 mm long; stamens 2, anthers up to 2 mm long; styles 2, stigmas 0.8–1 mm long. Caryopsis not seen.

Thailand. — NORTH-EASTERN: Loei (Phu Kradueng); EASTERN: Ubon Ratchathani (Kaeng Tana, Pha Taem, Warin Chamrap); PENINSULAR: Chumphon (Na Sak).

Distribution. — Sri Lanka.
Ecology. — Gregarious on wet rocks, silica soil areas, open sandy ground areas, open pine forests, 1,100–1,300 m altitude.

Typification notes. — According to Bor (1953), the type specimens of *Dimeria ceylanica* Bor are Sri Lanka specimens of *Thwaites c.p. 956 pro parte* which were housed in K and DD. He also mentioned that there is one sheet of *Thwaites c.p. 956* which contains two annual specimens, one is *D. pubescens* and another is *D. ceylanica*. However, there are five sheets of *Thwaites c.p. 956* in K. Two of which was labelled by Bor as *D. pubescens* and the other two are *D. trimeni* (now *D. pubescens*). Another sheet contains two annual specimens, the left hand side (K 98726) with a winged upper glume and the right hand side (K 98727) with a wingless upper glume. It seems that the latter closely match the description of type material sensu Bor (1953). To ensure that the type is easily accessed, I hereby designate *Thwaites c.p. 956 pro parte*, housed in K (K 98727), as the lectotype and regard the remaining duplicate in DD, as an isolectotype

Specimens examined.

**Thailand:** NORTH-EASTERN: *Loei* [Phu Kradueng, alt. 1,300 m, 29 Oct. 1954, Smitinand 2064 (K)]; [loc., alt. 1,100 m, 23 Nov. 1958, Sørensen et al. 6147 (C, K)]; [loc., 31 Oct. 1984, Murata & Phengklai T.42181 (AAU, GH, L)].


PENINSULAR: *Chumphon* [Na Sak, alt. -50 m, 6 Feb. 1927, Kerr 11860 (ABD, BM, 3 sheets K)].

Annual, caespitose. **Culms** 10–50 cm tall, slender, erect; nodes pubescent. **Leaf-sheaths** 1–4 cm long, pilose with tubercle-based hairs, margins scarious. **Ligule** a ciliate membrane, *ca.* 0.5 mm long. **Leaf-blades** linear, 1.5–10 cm by up to 5 mm, pilose with tubercle-based hairs on both surfaces and hirsute underneath, margins pilose with tubercle-based hairs, scabrous near the apex. **Racemes** solitary, 2–7 cm long, rhachis flattened, 0.4–0.6 mm wide, slightly ridged, ridge ciliate, narrowly winged, margins ciliate. **Spikelets** ovate-oblong, (2–)3–4 mm by 1–1.3 mm; pedicels flattened, 0.3–0.5 mm long, margins ciliate; spikelet callus hairy, hairs *ca.* 0.3 mm long; lower glumes linear to oblong, 3–4 mm long, acute to obtuse, keeled, densely ciliate on keel and at both sides on the lower half; upper glumes oblong-elliptic, 3.5–4 mm long, acute, keeled, densely ciliate on keel and near margins, broadly winged all along the keel; lower lemmas linear-oblong, 1.5–2 mm long, margins ciliate on the upper half; upper lemmas oblong-elliptic, 2.5–3 mm long, awns 12–18 mm long, columns 3–6 mm long; stamens 2, anthers yellow, 1–1.2 mm long; styles 2, stigmas purple, 0.5–0.7 mm long. **Caryopsis** oblong, 1–2.5 mm long.

Thailand. — **NORTHERN**: Phitsanulok (Nakhon Thai); **NORTH-EASTERN**: Nakhon Phanom (Tha Uthen); Nong Khai (Bueng Kan); **EASTERN**: Chaiyaphum (Chum Pae); Surin; **CENTRAL**: Bangkok; Nakhon Nayok; **SOUTH-EASTERN**: Chanthaburi (Makham); **PENINSULAR**: Phangnga (Kapoe).

**Distribution.** — China.

**Ecology.** — In open grassy areas, seasonally flooded flatlands, wet sandy soil areas, paddy fields, along the edge of rocky or sandstone areas, 0–1,100 m altitude.

**Notes.** — I have found four sheets of **Hancke** 1385 collected from China. Three of them are kept in BM and were collected from Guangzhou (Cantone or Canton) on Sept. 1869, Xiamen (Amoy) on Oct. 1857 and Hong Kong on Oct. 1859. The first one (from Guangzhou) is the holotype of **Dimeria sinensis** (Rendle, 1904), while the others are **Dimeria falcata** Hack. Another sheet of **Hancke** 1385 is kept in K (K433919). There are two specimens on it. The specimen on the right hand side was collected from Hong Kong in November, 1859, and the left hand side was collected from Amoy. I have examined them and found that they are acutually **D. falcata**.
Dimeria sinensis was a new record for Thailand, previously reported by Chen and Phillips (2006).

Specimens examined.

Thailand: NORTHERN: *Phitsanulok* [Nakhon Thai, alt. 1,100 m, 29 Oct. 2001, *Watthana & Suksathan* 1546 (AAU)].


EASTERN: *Chaiyaphum* [Chum Pae, 19 Dec. 1929, *Kerr* 17618A (K)]; *Surin* [alt. 100 m, 9 Jan. 1924, *Kerr* 8235 (BM, K, TCD)].


PENINSULAR: *Phangnga* [alt. ca. 5 m, 6 Mar. 1929, *Kerr* 17355 (BM, K, TCD)]; *Ranong* [Kapoe, alt. 0 m, 29 Nov. 1996, Maxwell 96-1553 (2 sheets GH)].
SUBTRIBE GERMAINEINAE


Type genus: Germainia Balansa & Poitrass.

Annual or perennial. Ligule usually an eciliate membrane, sometimes a ciliate membrane. Inflorescence of solitary or digitate racemes. Spikelets in pairs, dissimilar, usually dorsally compressed, florets 2, without rachilla extension, falling entirely at maturity. Sessile spikelets male or neuter, persistent (bisexual and caducous in Apocopsis), sometimes enlarged or involucral at base of raceme, lower glumes chartaceous to coriaceous or indurated, usually obtuse or truncate or reteuse, upper usually longer than the lower; florets male or neuter or reduced to a lemma or suppressed. Pedicelled spikelets fertile, sometimes suppressed at base of raceme or throughout, subterete; spikelet callus obtuse to pungent; lower glumes coriaceous, obtuse; lower florets reduced to a lemma or suppressed; upper lemmas linear, entire or bidentate, with a hairy awn.

Three genera, about 27 species, distributed in tropics, Australia, mainly in Asia. Two genera and 12 species are found in Thailand.

Key to genera of Thai Germainiinae

1. Inflorescence of digitate racemes (rarely solitary), not surrounded at base by an involucre of homogamous spikelets; rhachis fragile. Sessile spikelets awned (sometimes shortly awned or awnless in A. collinus). Pedicelled spikelets male, neuter or suppressed (fertile in A. intermedius) ........................................................................................................................................... 1. Apocopsis (p. 118)

1. Inflorescence of 1–2(–6) racemes, surrounded at base by an involucre of homogamous spikelets; rhachis tough. Sessile spikelets awnless. Pedicelled spikelets fertile  ................................................................................................................................................................ 2. Germainia (p. 132)
APOCOPIS


Type: Apocopis royleana Nees (= Apocopis paleacea (Trin.) Hochr.)

— Amblyachyrum Hochst., Flora 39: 25. 1856. Type: Amblyachyrum mangalorensis Hochst. (see later, under Apocopis mangalorensis (Hochst.) Henrard.)

Annual or perennial, tufted or shortly rhizomatous. Culms slender, erect or short ascending or prostate; nodes glabrous to hirsute. Leaf-blades lanceolate to linear-lanceolate or linear, usually hairy with tubercle-based hairs, rarely glabrous, margins thick. Ligule an eciliate membrane or a ciliate membrane. Inflorescence of (1—) 2 (—4) digitate racemes, rhachis internodes fragile, hairy at apex, margins hirsute. Spikelets in pairs, heteromorphous, basal pairs almost neuter, dorsally compressed; florets 2, without rachilla extension. Sessile spikelets usually fertile (sterile in A. intermedius); spikelet callus obtuse, attached transversely, hairy; lower glumes chartaceous to coriaceous, oblong-ovate, glabrous to hairy, truncate or emarginate, (5—)7—9-nerved, nerves parallel or arched, free or united in various patterns; upper glumes chartaceous, truncate or obtuse or emarginate, 3-nerved, the two outer flaps folded round the florets; lower florets male; lower lemmas hyaline, truncate or obtuse or emarginate to bifid, nerves obscure or 1-nerved; lower paleas hyaline, truncate or obtuse or bifid, upper margin ciliate, nerveless; upper florets neuter or male or female or hermaphrodite; upper lemmas narrowly oblong, nerves obscure or 1-nerved, awned from entire or bifid apex, awns geniculate with brown twisted columns, scaberulous; upper paleas hyaline, upper margin ciliate; lodicules absent; stamens 2; styles 2, stigmas plumose. Pedicelled spikelets male, neuter or suppressed, usually represented by the pedicel only (developed in A. intermedius); pedicels usually hirsute, partly adnate to the margin of the lower glume of sessile spikelet. Caryopsis with adherent pericarp, oblong, hilum punctiform.
reduced usually represented by the pedicel only, very rarely present (in \textit{A. intermedius})

About 15 species, distributed in Temperate Asia and Tropical Asia. Six species are found in Thailand of which one is endemic: \textit{Apocopis siamensis} A. Camus.

**Key to species of Thai \textit{Apocopis}**

1. Peduncles hirsute in uppermost part
   2. Pedicelled spikelets present. Sessile spikelets 6–9 mm long ............ 3. \textit{A. intermedius}
   2. Pedicelled spikelet absent. Sessile spikelets 4–5 mm long ................. 5. \textit{A. siamensis}

1. Peduncles glabrous in uppermost part
   3. Pedicelled spikelets present .................................................. 3. \textit{A. intermedius}
   3. Pedicelled spikelets absent
      4. Spikelets awnless or awned, awns less than 10 mm long
         .......................................................................................... 1. \textit{A. collinus}
      4. Spikelets awned; awns longer than 10 mm long
         5. Median nerve or/and side nerves reaching the upper margin of the lower glumes, and exerted as a small point ......................... 2. \textit{A. courtallumensis}
         5. All nerves ending below the upper margin of the lower glumes
         6. Annual. Spikelets up to 4 mm long ......................... 4. \textit{A. mangaloresensis}
         6. Perennial. Spikelets 4.5–6 mm long ......................... 6. \textit{A. wrightii}

— **A. borneensis** Ridl., Bot. Jahrb. Syst. 44: 519. 1910. Type: Indonesia, Kalimantan (Borneo), Martapura, 1908, Winkler 3392 [lectotype K! (K 290140), designated here; isolecotype L (digital image L 43626)]


Perennial, tufted. **Culms** 20–40(–50) cm tall, erect; nodes glabrous. **Leaf-sheaths** 2–5 cm long, glabrous to pilose with tubercle-based hairs especially on the upper part. **Ligule** a ciliate membrane, 0.5–1 mm long. **Leaf-blades** linear, 5–9 cm by 0.2–0.4 cm, pubescent or pilose with tubercle-based hairs on both surfaces, rarely glabrous, margins thick, scabrous. **Racemes** 1–2, 2–4 cm long, slightly swollen below the racemes; joints of rhachis 1.5–2 mm long; peduncles 6–15 cm long, glabrous. **Sessile spikelet** fertile except the lowest, greenish red or purplish with narrow brownish apex; oblong-obovate, 5–5.5 mm by 1.8–3 mm; spikelet callus hairy, hairs up to 1.5 mm long; lower glumes oblong-obovate, 5–5.5 mm by 1.8–3.5 mm, glabrous or granular on dorsal surface, round or truncate, upper margin ciliate, 7-nerved, nerves anastomosing, nerves not reaching upper margin; upper glumes oblong-lanceolate, 5–5.5 mm by ca. 1 mm, glabrous to pubescent on upper part, truncate, upper margin ciliate, 3-nerved; lower florets male; lower lemmas oblong to ovate-lanceolate, 4.5–5 mm long, truncate, upper margin ciliate, 1-nerved; lower paleas lanceolate, 4.5–5 mm long, margins ciliate, truncate; stamens 2, anthers purple, 2–3 mm long; upper florets female or hermaphrodite; upper lemmas oblong-lanceolate, 4.5–5 mm long, truncate, upper margin ciliate, minutely bifid, rarely awned from the sinus, awns up to 7 mm long, short geniculate, nerves obscure or 1-nerved; upper paleas oblong-lanceolate, 3.5–4 mm long, truncate; styles 2, stigmas purple, ca. 3.5 mm long. **Pedicelled spikelets** suppressed; pedicels 1.5–2 mm long. **Caryopsis** not seen.

Thailand. — NORTH-EASTERN: Udon Thani (Nong Han); EASTERN: Roi Et (Thung Kula Rong Hai); Ubon Ratchathani (Khong Chiam); SOUTH-EASTERN: Sa Kaeo (Aranyaprathet, Ta Phraya, Watthana Nakhon).
Distribution. — Cambodia, Malaysia and Indonesia (Kalimantan).

Ecology. — Open grassy and wet ground areas, fire prone deciduous dipterocarp forests, 50–200 m altitude.

Notes. — The specimens of Kerr 19624 housed in BM and K have lemmas with short awns or are awnless. Among the cited specimens examined, the Borneo specimens (Lugas 586 and Forster F23) have pedicelled spikelets and the lower glumes of the sessile spikelets are hirsute. The specimens of Kerr 8584 (BM, K) were misidentified as Apocops royleana Nees (now A. paleacea).

Typification notes. — When Ridley (1910) first published Apocops borneensis Ridl., he cited Winkler 3392 collected from Borneo as the type specimen but he did not indicate where it was kept. According to Stafleu and Cowan (1988), Winkler’s herbarium is deposited in B, BM, C, G, K, L, MO, NY, P, PC, PRE, WRSL and Z. I have found two sheets of Winkler 3392, one is kept in K (K 290140) and another is kept in L (L 43626). Therefore, I hereby designate Winkler 3392 in K as the lectotype, because it is of good quality and regard the remaining duplicate as an isolectotype.

Specimens examined.

Thailand: NORTH-EASTERN: Udon Thani [Nong Han, alt. 200 m, 26 Feb. 1924, Kerr 8584 (BM, K)].

EASTERN: Roi Et [Thung Kula Rong Hai, alt. 150 m, 22 June 1969, Smitinand 10756 (BKF)]; Ubon Ratchathani [Khong Chiam, alt. 157 m, 22 May 2001, Greijmans 60 (BKF)].

SOUTH-EASTERN: Sa Kaeo [Aranyaprathet, alt. 50 m, 10 Aug. 1930, Kerr 19624 (BM, 2 sheets K)]; [Ta Phraya, 8 July 2001, Teerawatananon s.n. (BKF, BM, K, THNHM)]; [Watthana Nakhon, 11 Nov. 2006, Teerawatananon & Kritsanachandee 977 (TCD)].


— *Apocopis wightii* Nees ex Hack. in A. DC. & C. DC., Monogr. Phan. 6: 258. 1889. *nom. inval.*

Type: as for above.

Perennial. Culms up to 80 cm tall, erect or short ascending, branched; nodes glabrous. Leaf-sheaths 5–11 cm long, densely pilose with tubercle-based hairs, margins scarious. Ligule a lacerate membrane or a ciliate membrane, up to 2 mm long. Leaf-blades linear-lanceolate, 5–15 cm by 0.5–0.6 cm, pilose with tubercle-based hairs on both surfaces, margins thick, scabrous and pilose with tubercle-based hairs. Racemes 2, 3–6 cm long; joints of rhachis 1.5–2 mm long; peduncles 10–15 cm long, glabrous. Sessile spikelet fertile, brownish-green to brown with reddish band across apex, oblong-obovate, 5–6 mm by 3–3.5 mm; spikelet callus hairy, hairs up to 3 mm long; lower glumes oblong-obovate, 4.5–5 mm by 3–3.5 mm, almost glabrous, slightly emarginate, upper margin ciliate, margins sometimes hirsute, 7(–9)-nerved, nerves anastomosing, the median nerve reaching the upper margin and exerted as a small point, sometimes the two intramarginal nerves almost reaching the upper margin; upper glumes lanceolate-oblong, 4.5–5 mm by 1–2 mm, sparsely pubescent on the dorsal surface, truncate to slightly emarginate, upper margin ciliate, 3-nerved, the two outer flaps folded round the florets; lower florets male; lower lemmas lanceolate-oblong, 4.5–5 mm long, almost glabrous, obtuse, upper margin ciliate, nerveless; lower paleas lanceolate-oblong, 4–4.5 mm long, bifid; stamens 2, anthers yellow to purple or reddish pink or greyish purple, 3–3.8 mm long; upper florets hermaphrodite or female; upper lemmas lanceolate-oblong, 4.5–5 mm long, pubescent along the margins, bifid, awned from the sinus, awns 1.8–2.5 cm long, columns 0.8–1.3 cm; upper paleas ovate-oblong, 3–3.5 mm long, slightly emarginate; styles 2, stigmas creamy white to old-rose colour, 4–5.5 mm long. Pedicelled spikelets suppressed; pedicels 1.8–2.5 mm long. Caryopsis oblong, 1.3–1.5 mm long.
Thailand. — NORTHERN: Chiang Mai (Doi Suthep); Mae Hong Son (Mae Ngao National Park); Phitsanulok (Thung Salaeng Luang National Park); SOUTHEASTERN: Sa Kaeo (Aranyaprathet).

Distribution. — India and Sri Lanka.

Ecology. — Open areas or partly shady places, deciduous dipterocarp-oak forests, tropical grasslands, 500–600 m altitude.

Vernacular. — Ya Khon Bung (Ya Kon Bung) (Smitinand, 2001).

Specimens examined.


Perennial, shortly rhizomatous. *Culms* up to 50 cm tall, erect; nodes glabrous. *Leaf-sheaths* 1—3 cm long, glabrous to densely hirsute. *Ligule* a ciliate membrane, 1—1.5 mm long, with a dense row of hairs behind the ligule. *Leaf-blades* linear-lanceolate, 5—20 cm by 0.3—0.5 cm, pilose to densely hirsute with tubercle-based hairs on both surfaces, margins thick, pilose with tubercle-based hairs. *Racemes* 2, 4—6.5 cm long; joints of rachis 2—2.5 mm long; peduncles 5—20 cm long, usually glabrous, sometimes hirsute at uppermost part. *Sessile spikelets* male, yellowish green to pale yellow with red or brown band across apex, oblong, 6—9 mm by 2.5—3 mm; spikelet callus hairy, hairs 2.5—3 mm long; lower glumes oblong-obovate, 6—8 mm by *ca*. 3 mm, glabrous to densely hirsute on the dorsal surface, truncate to slightly emarginate, upper margin ciliate, (5—)7-nerved, all nerves ending below the apex; upper glumes oblong, 7—9 mm by 1—1.5 mm, pubescent on the dorsal surface, truncate, upper margin ciliate, 3-nerved; lower florets male; lower lemmas lanceolate-oblong, 6—8 mm long, truncate to obtuse, upper margin ciliate, 1-nerved; lower paleas lanceolate-oblong, 6—8 mm long, truncate to obtuse; stamens 2, anthers brownish yellow, 3.5—5 mm long; upper florets usually neuter or male and hermaphrodite if pedicelled spikelet wanting; upper lemmas narrowly oblong, 5—7 mm long, bifid, awned from the sinus, awns up to 3.5 cm long, columns 0.6—1.5 cm; upper paleas ovate-oblong, 4—5 mm long, obtuse; styles 2, stigmas purple, 4—5 mm long. *Pedicelled spikelets* usually present, female, lanceolate, 4.5—5.5 mm by *ca*. 1 mm, caducous; pedicels 1.5—3.5 mm long; spikelet callus hairy, hairs up to 2 mm long; lower glumes lanceolate-oblong, 5—5.5 mm by 0.8—1 mm, hirsute, obtuse, upper margin ciliate, 3-nerved; upper glumes lanceolate-oblong, 4.5—5 mm by 0.5—0.8 mm, hirsute, truncate to obtuse, upper
margin ciliate, 3-nerved; lower florets suppressed; upper lemmas linear, 3.5–4 mm long, awned, awns up to 3 cm long, columns 6–1.5 cm long; upper paleas oblong, 3.5–4 mm long, obtuse, upper margin ciliate; styles 2, stigmas purple, up to 10 mm long. Caryopsis oblong, 2.5–3 mm long.

Thailand. — NORTHERN: Phitsanulok (Thung Salaeng Luang National Park); SOUTHEASTERN: Chanthaburi (Makham); PENINSULAR: Pattani; Songkhla (Khlong Hoi Khong).


Ecology. — Open sandy areas, tropical grasslands, 0–500 m altitude.

Typification notes. — When Camus (1919) published *Lophopogon intermedius* A. Camus, she cited *Balansa* 394, Mouret 542 and Godefroy 935 from Vietnam (Tonkin), as the syntypes. Later, Roberty (1960) transferred *L. intermedius* to *Apocopsis* as *A. tridentatus var. intermedius* (A. Camus) Roberty. He stated the specimen of *Balansa* 394, as the holotype, but did not mention where it was kept. This species was later transferred to *Apocopsis intermedius* (A. Camus) Chai-Anan by Chi-Anan (1972). According to Roberty (1960), his citation of *Balansa* 394 as the holotype did have the effect of establishing that specimen as the lectotype of *A. intermedius*. However, I have found four sheets of *Balansa* 394 kept in K and L. To ensure that the type is easily accessed, therefore, *Balansa* 394 in K (K 433917) is designated as the lectotype and the remaining duplicates regarded as isolectotypes.

Specimens examined.


Annual. Culms usually less than 30 cm tall, slender, short ascending, often prostrate, branched; nodes glabrous. Leaf-sheaths 1–3 cm long, inflated, slipping from the culm, glabrous to pilose with tubercle based hairs at the upper part. Ligule an eciliate membrane, ca. 0.5 mm long. Leaf-blades lanceolate, 1–3(–4) cm by up to 0.4 cm, pilose to densely hirsute with tubercle-based hairs on both surfaces, margins thick, pilose with tubercle-based hairs. Racemes 2, 4–6.5 cm long; joints of rhachis 1–1.5 mm long; peduncles up to 10 cm long, glabrous. Sessile spikelet fertile, oblong, 3–4 mm by ca. 3 mm; spikelet callus hairy, hairs up to 2 mm long; lower glumes oblong-obovate, 3–4 mm by 2–3 mm, truncate to slightly emarginate, upper margin ciliate, hyaline and often reddish, 7-nerved, all nerves not reaching the top of the glume; upper glumes lanceolate-oblong, ca. 4 mm by ca. 1 mm, with two lateral flaps folded round the florets, pubescent on the dorsal surface, truncate, upper margin ciliate, 3-nerved; lower florets male; lower lemmas lanceolate-oblong, 3–4 mm long, emarginate to bifid, upper margin ciliate, nerveless; lower paleas lanceolate-oblong, 3–4 mm long, bifid; stamens 2, anthers 2–2.25 mm long; upper florets female; upper lemmas narrowly oblong, 3.5–4.5 mm long, acutely bifid, awned from the sinus, awns 1.5–2 cm long, columns 0.6–0.8 cm; upper
paleas oblong, 2.5—3 mm long, acutely 2-lobed; styles 2, stigmas 3—3.5 mm long. Pedicelled spikelets suppressed; pedicels 1.5—2 mm long. Caryopsis not seen.

Thailand. — NORTHERN: Chiang Mai (Doi Suthep).

Distribution. — India and Sri Lanka.

Ecology. — Open gravel slopes, ca. 335 m altitude.

Notes. — Three sheets of Kerr 2745, which are housed in K and BM, were identified as Apocopis cochinchinensis A. Camus and as Apocopis wightii Nees (now Apocopis courtallumensis (Steud.) Henrard), respectively. I have carefully examined all of them and found that they actually are Apocopis mangaloresis.

Apocopis mangaloresis differs from A. cochinchinensis in having a prostrate culm and differs from A. courtallumensis in having an annual habit and geniculate culms often prostrate at base. It differs from both species in having lower glumes with no nerves reaching the upper margins.

Among the cited specimens examined, the Indian specimens are hairier than Kerr’s specimens. However, according to Bor (1952a), the lower glumes can be glabrous and hairy.

Specimens examined.

Thailand: NORTHERN: Chiang Mai [Doi Suthep, alt. 1,100 ft, 21 Oct. 1912, Kerr 2745 (BM, 2 sheets K)].

Annual, tufted. **Culms** slender, up to 45 cm tall, erect; nodes usually glabrous, sometimes 1–2 nodes at uppermost part hairy below the nodes. **Leaf-sheaths** 1–3 cm long, upper part pilose with tubercle-based hairs. **Ligule** an eciliate membrane, 0.5–0.8 mm long. **Leaf-blades** linear-lanceolate, 3–5 cm by 0.2–0.3 cm, pilose with tubercle-based hairs on both surfaces, margins thick, scabrous and pilose with tubercle-based hairs. **Racemes** 2, 2–4 cm long; joints of rhachis 1.5–1.8 mm long; peduncles 8–10 cm long, hirsute at uppermost part. **Sessile spikelets** fertile, brownish green, oblong, 4.5–5 mm by 1–1.8 mm; spikelet callus hairy, hairs 1–1.5 mm long; lower glumes oblong or slightly wider upwards, 3.5–4.3 mm by 1–1.8 mm, scabrous on the dorsal surface, dentate, upper margin ciliolate, 5–7-nerved, nerves reaching the upper margin and exserted as small points; upper glumes oblong, 4–4.5 mm by 0.8–1 mm, with two lateral flaps wrapped round the florets, scabrous on the dorsal surface, truncate to slightly emarginate, upper margin ciliate, 3-nerved; lower florets male; lower lemmas narrowly oblong, 3.8–4 mm long, obtuse, upper margin ciliate, nerveless; lower paleas narrowly oblong, 3.5–3.8 mm long, obtuse; stamens 2, anthers yellow to purple, 2–2.5 mm long; upper florets female or hermaphrodite; upper lemmas narrowly oblong, 4(−5.3) mm long, minutely bifid, awned from the sinus, awns 2.5–3 cm long, columns 1–1.4 cm; upper paleas ovate-oblong, 2.8–3.3 mm long, truncate; styles 2, stigmas white or creamy, up to 4 mm long. **Pedicelled spikelets** suppressed; pedicels 1.3–1.5 mm long. **Caryopsis** not seen.

Thailand. — NORTHERN: Tak (Rahaeng, Wang Chao); SOUTH-WESTERN: Prachuap Khiri Khan (Hua Hin); Ratchaburi (Thung Luang); SOUTH-EASTERN: Chon Buri (Siracha); Sa Kaeo (Watthana Nakhon).

Distribution. — Endemic to Thailand.

Ecology. — Open moist sandy areas, deciduous dipterocarp forests, 5-50 m altitude.
Typification notes. — When Camus (1914) described *Apocopis siamensis* A. Camus, she cited *Hosseus* s.n. collected from Thailand as the type specimen, but she did not mention where it was kept. Later, Bor (1952a) stated that *Hosseus* 145 was the type specimen of *A. siamensis*. Thus, *Hosseus* 145 must be considered as the lectotype. I have found six sheets of *Hosseus* 145 housed in K, L and US. According to Stafleu and Mennega (1993), Bor was the assistant director at Kew during 1948-1959, so, it is possible that he had used *Hosseus* 145 in K as the type specimen when he published his work. However, there are three sheets of *Hosseus* 145 in K (K 290134, K 290135, K 290136). Therefore, I hereby designate *Hosseus* 145 (K 290134) as lectotype due to its good quality, and regard the remaining duplicates as isolectotypes.

Specimens examined.

**Thailand:** NORTHERN: *Tak* [Rahaeng (Raheng), 8 Jan. 1904 *Lindhard* 20 (C)]; [Wang Chao, 14 Oct. 1904, *Hosseus* 145 (3 sheets K, L)].

SOUTH-WESTERN: *Prachuap Khiri Khan* [Hua Hin, alt. 5 m, 8 Nov. 1927, *Kerr* 13499 (BM, 2 sheets K)]; *Ratchaburi* [Thung Luang, alt. 50 m, 8 Nov. 1931, *Kerr* 20583 (BM, 2 sheets K)].


Perennial, tufted. **Culms** up to 100 cm tall, erect, branched; nodes glabrous to hirsute with white hairs. **Leaf-sheaths** 6–9 cm long, glabrous to densely hirsute, margins scarious. **Ligule** a ciliate membrane, up to 1 mm long. **Leaf-blades** linear-lanceolate, 10–20 cm by 0.3–0.7 cm, pilose to densely hirsute with tubercle-based hairs on both surfaces, uppermost very reduced, margins thick, pilose with tubercle-based hairs. **Racemes** 2, 2–4.5 cm long; joints of rhachis 1.5–2 mm long; peduncles 8–15 cm long, glabrous. **Sessile spikelet** fertile, oblong, 4.5–6 mm by ca. 3 mm; spikelet callus hairy, hairs up to 3 mm long; lower glumes oblong-obovate, 4.5–5.5 mm by 2.5–3 mm, almost glabrous, truncate to slightly emarginate, upper margin ciliate, 7–9-nerved, all nerves not reaching the upper margin; upper glumes lanceolate-oblong, 4.5–6 mm by 1.3–1.8 mm, pubescent on the dorsal surface, truncate to slightly emarginate, upper margin ciliate, 3-nerved; lower florets male; lower lemmas lanceolate-oblong, 4–6 mm long, pubescent on dorsal surface, acute to bifid, upper margin ciliate, 1-nerved; lower paleas lanceolate-oblong, 4–5 mm long, truncate to obtuse; stamens 2, anthers 2.5–3 mm long; upper florets hermaphrodite; upper lemmas lanceolate-oblong, 4.5–5.5 mm long, pubescent along the margins, bifid, awned from the sinus, awns 2–3 cm long, columns 1–1.5 cm; upper paleas ovate-oblong, 3–5 mm long, obtuse; styles 2, stigmas ca. 4 mm long. **Pedicelled spikelets** suppressed; pedicels 1.5–2.5 mm long. *Caryopsis* not seen.

Thailand. — NORTH-EASTERN: Nong Khai (Si Chiang Mai); SOUTH-WESTERN: Kanchanaburi (Ku Jae).

Distribution. — China.

Ecology. — Open areas, gravelly places, deciduous dipterocarp forests, 100–180 m altitude.
Notes. — Among the Kostermans’s 1281, only the specimen in GH seems different from the others in having a few lower glumes with median nerve reaching the apex.

Typification notes. — When Munro (1860) published Apocapis wrightii Munro, he cited Wright s.n. collected from Cum-sing-moon, Hong Kong, as the type specimen, but he did not mention where it was kept. According to Stafleu and Cowan (1988), corresponding general collections of Wright were kept in GH. However, I have found three duplicates of Wright s.n. specimens housed in GH, K and NY, all of which were labelled by Munro as “Apocapis wrightii n. sp. Munro”. However, only the specimen in GH bears a label with Munro’s handwriting indicating the reason why he recognised this plant as a new species. Therefore, I hereby designate Wright s.n. housed in GH (UHU 23071), as the lectotype and regard the remaining duplicates as isolectotypes.

Specimens examined.

Thailand: NORTH-EASTERN: Nong Khaï [Si Chiang Mai, alt. 180 m, 18 Aug. 1972, Smitinand 11640 (K, L)].

SOUTH-WESTERN: Kanchanaburi [Ku Jae, alt. 100–150 m, 20 July 1946, Kostermans 1281 (GH, K, 2 sheets L, US)].
GERMAINIA


Type: G. capitata Balansa & Poitrass.


— Balansochloa Kuntze in Post & Kuntze, Lexic.: 58. 1903. Type: as for above.

— Sclerandrium Stapf & C. E. Hubb. in Hook. Ic. Pl. 33, tab. 3262. 1935. Type: Sclerandrium truncatiglume (F. Muell. ex Benth.) Stapf & C. E. Hubb. (basionym = Ischaemum truncatiglume F. Muell. ex Benth.) (= Germainia truncatiglumis (F. Muell. ex Benth.) Chai-Anan.)


— Chumsriella Bor, Dansk Bot. Ark. 23: 467, fig. on page 468. 1968. Type: Chumsriella thailandica Bor (see later, under Germainia thailandica (Bor) Chai-Anan).

Perennial, tufted, rarely stoloniferous. Culms slender, erect; nodes glabrous to pubescent or pilose. Leaf-blades lanceolate to linear-lanceolate or linear, hairy to glabrous. Ligule an eciliate membrane or a ciliolate membrane. Inflorescence of 1–2(–6) racemes, capitate or elongate or subdigitate racemes, closely appressed to divergent, rachis internodes tough, short or almost reduced, with 2–14 sessile and pedicelled spikelets; peduncles usually exserted, rarely enclosed in uppermost sheath, espatheate, glabrous to hirsute. Spikelets in pairs, heteromorphous, sometimes basal pairs reduced to the sessile spikelets forming an involucre, dorsally compressed, 2 florets, without rachilla extension. Sessile spikelets male, persistent; lower glumes membranous or chartaceous (G. pilosa, G. thailandica) or coriaceous, glabrous to hairy, obliquely bifid, muticous, truncate or obtuse, 7–9(–11)-nerved, nerves sometimes

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anastomosing; upper glumes membranous, almost reaching or exceeding apex of lower glumes in spikelets, acute or obtuse or dentate or emarginate, 3–5-nerved; lower florets sometimes absent if present male or neuter; lower lemmas membranous, acute to obtuse, 1–(–3–)nerved; lower paleas membranous, acute or obtuse or bifid or dentate, 2-nerved; upper florets male or neuter; upper lemmas absent if present linear or filiform, obtuse or acute to mucronate, 1-nerved; upper paleas as the lower one; lodicules absent; stamens 2. Pedicelled spikelets fertile, caducous; spikelet callus linear to obtuse, attached obliquely or transversely, hairy with yellowish to reddish brown hairs; glumes coriaceous, subequal, acute or obtuse or muticous or dentate or truncate or muticous, 3–7-nerved; lower florets usually suppressed, if present neuter, epaleate; upper florets female; upper lemmas reduced to the narrow stipitate base of awn base, awns geniculate with brown twisted columns, hirsutulous, caducous, 1-nerved; upper paleas membranous, almost glabrous; styles 2, stigmas plumose, exserted. Caryopsis with adherent pericarp, oblong, hilum punctiform.

About nine species, distributed in Temperate Asia, Tropical Asia and Australasia. Six species are found in Thailand.
Key to species of Thai Germainia

1. Basal sheaths densely covered with white to pale yellow lanate hairs ............ 3. G. lanipes

1. Basal sheaths not covered with lanate hairs

2. Plant up to 15 cm tall. Lower glumes of sessile spikelets membranous to chartaceous

3. Inflorescence well exserted; uppermost sheath with reduced blade; racemes capitate. Lower glumes of sessile spikelets apex muticous or truncate or slightly denticulate. Ligule an eciliate membrane ....................................................... 4. G. pilosa

3. Inflorescence enclosed in uppermost sheath with well developed blade; racemes elongate. Lower glumes of sessile spikelets apex dentate. Ligule a ciliate membrane .................................................................................. 5. G. thailandica

2. Plant more than 20 cm tall. Lower glumes of sessile spikelets coriaceous

4. Sessile spikelets more than 4 per raceme. Leaf-blade linear, greater than 7 cm long .................................................................................................................................................. 1. G. capitata

4. Sessile spikelets 2–3 per raceme. Leaf-blade linear-lanceolate, up to 5 cm long

5. Two sessile and one pedicelled spikelet per raceme; lower glumes of sessile spikelets truncate with lateral caudate teeth ......................... 6. G. thorelii

5. Three sessile and three pedicelled spikelets per raceme; lower glumes of sessile spikelets truncate to muticous without lateral teeth ............ 2. G. khasyana


Perennial, tufted. Culms 30—60(—90) cm tall, erect; nodes glabrous to pubescent. Leaf-sheaths 5—13 cm long, basal sheaths tomentose, densely covered with white to pale yellow hairs to glabrous, upper sheath shorter than internodes. Ligule a ciliate membrane, up to 1.5 mm long, with a dense row of hairs behind the ligule. Leaf-blades linear, 7—40 cm by 0.5—1 cm, tomentose to sparsely pubescent or glabrous on both surfaces, margins thick, tomentose to scabrous. Racemes 2, 2—4 cm long, capitate to elongate, usually with one main axis rarely with two, very closely appressed, composed of 4—14 sessile and pedicelled spikelets, basal homogamous 1—4; peduncles 20—30 cm long, hirsute below the inflorescences. Sessile spikelets yellowish-green to green, oblong, 15—23 mm by 2.5—4.5 mm; lower glumes coriaceous, oblong, 13—22 mm by 3—4.5 mm, pubescent to glabrous, obliquely bifid to muticous, upper margin ciliate, 7—9-nerved, sometimes outer two nerves anastomosing; upper glumes linear-lanceolate, 16—23 mm by 3—4 mm, upper part pubescent, acute, upper margin ciliate, 3-nerved; lower lemmas linear-lanceolate, 15—20 mm long, acute and ciliate, 1(—3)-nerved; lower paleas ovate-lanceolate, 13—19 mm long, pubescent on nerves, bifid, upper margin ciliate; upper lemmas linear-lanceolate, 15—20 mm long, upper part pubescent, acute, upper margin ciliate; upper paleas lanceolate, 15—20 mm long, pubescent on nerves, acute, upper margin ciliate; stamens 2, anthers reddish-purple, 9—12 mm long. Pedicelled spikelets lanceolate, 8—11 mm by 1.2—2.5 mm, caducous; pedicels 5—7 mm long, hirsute; spikelet callus linear, 3—3.5 mm long, attached obliquely, hairy, hairs 0.5—2 mm long; lower glumes lanceolate to ovate-lanceolate, 8—11 mm by 1.5—2.5 mm, pubescent, obtuse to muticous, upper margin ciliate, 3-nerved; upper glumes ovate-lanceolate, 7—9 mm by 1—1.8 mm, pubescent, obtuse to truncate, upper margin ciliate, 3(—5)-nerved; lower florets neuter or suppressed, lower lemmas linear,
6–7 mm long, truncate, upper margin ciliate, nerves obscure; lower paleas absent; upper
lemmas linear, 3–4 mm long, awns 6–9(–11.5) cm long, columns 4–6 cm long; upper paleas
oblong, 5–7 mm long, truncate and dentate, upper margin ciliate; styles 2, stigmas reddish-
purple, ca. 1.5 mm long. Caryopsis oblong, 3–4 mm long.

Thailand. — NORTH-EASTERN: Loei (Phu Kradueng, Phu Tong); EASTERN:
Chaiyaphum (Phu Khiew); Si Sa Ket (Kantaralak); SOUTH-EASTERN: Chanthaburi
(Makham); PENINSULAR: Satun (Thung Nui); Songkhla (Had Yai, Khlong Hoi Khong).


Ecology. — Wet and open sandy and clayey soil areas in tropical grasslands, pine forests,
0–1,300 m altitude.

Notes. — Cha-Anan (1972) divided this species into two groups of high (1,000–2,000 m) and
low (5–50 m) altitudes. Plants from the high altitude group are pubescent to nearly glabrous.
The raceme axes of these plants are closely appressed, their inflorescences have 6–9 or up to
11 sessile spikelets and their lower glumes of the sessile spikelet are glabrous to pubescent.
Plants from the low altitude group are less pubescent. The raceme axes of plants in this group
are mostly reduced and their inflorescences have 4–6 sessile spikelets with more or less
glabrous lower glumes.

However, based on the specimens examined in this study, only the specimens collected from
the summit of Phu Kradueng (1,000–1,300 m) are assignable to a group of high altitude
plants, while the specimens collected from the other places (0–800 m) are assignable to a
group of low altitude plants. I also found that the distinction between the two forms is
insufficient for specific recognition and some characters are inconsistent, e.g. the shape of the
apex of the lower glume of sessile spikelet which is variable and intermediate from obliquely
bifid to muticous glumes which can be found in both groups of plants, although most
specimens from low altitude tend to have obliquely bifid apices, while the specimens from
high altitude tend to have muticous apices. I agree with Chai-An (1972) in keeping them as
the same taxon. More material for further study is needed to clarify the status of the taxon.

Vernacular. — Ya Kamong (Ya Kamong), from Bunpheng 898 (BKF).

Specimens examined.
Thailand: NORTH-EASTERN: *Loci* [Phu Kradueng, alt. 1,300 m, 12 July 1956, Bunpheng 898 (BKF)]; [l.c., 7 Mar. 1979, O'Connor & Niyomdham 15679 (BKF)]]; [l.c., 23 Mar. 1954, Smitinand 1794 (BKF, K)]; [l.c., 9 May 1951, Smitinand 367 (K)]; [l.c., alt. 1,300 m, 8 July 1959, Smitinand 5856 (BKF)]; [l.c., alt. 1,300 m, 20 Mar. 1958, Sorensen et al. 2369 (BKF, C)]; [l.c., alt. 1,300 m, 28 Nov. 2005, Teerawatananon & Sungkaew 834 (TCD, THNHM)]; [l.c., alt. 1,300 m, 23 Mar. 2006, Teerawatananon & Sungkaew 888 (BM, GH, TCD, THNHM)]; [l.c., alt. 1,300 m, 23 Mar. 2006, Teerawatananon & Sungkaew 889 (ABD, BK, BKF, K, SING, TCD, THNHM, US)]; [l.c., alt. 1,300 m, 25 Mar. 2006, Teerawatananon & Sungkaew 898 (AAU, L, NY, TCD)]; [l.c., 9 May 1951, Tem & Ploenchit 367 (BKF)]; [l.c., alt. 1,300 m, 17 Mar. 1974, Unknown collector 32 (BKF)]; [l.c., alt. 1,300 m, 18 Mar. 1974, Unknown collector 57 (BKF)]; [Phu Tong, alt. 1,000 m, 27 Mar. 1924, Kerr 8831 (BM, K, L)].

EASTERN: *Chaiyaphum* [Phu Khiew, alt. 800 m, 14 Dec. 1971, Beusekom et al. 9265 (BKF, C, K, L)]; *Si Sa Ket* [Kantaralak, alt. 600 m, 19 Aug. 1976, Maxwell 76-548 (AAU, L)].

SOUTH-EASTERN: *Chanthaburi* [Makham, alt. ca. 20 m, 9 Dec. 1924, Kerr 9583 (BM, 3 sheets K)]; [l.c., alt. 50 m, 25 Oct. 1956, Smitinand 3598 (BKF)]; [25 Nov. 1970, Lazarides 7468 (K, L)].


Perennial. *Culms* slender, 25—40(—75) cm tall, erect, branched; nodes pilose. *Leaf-sheaths* 1.5—3 cm long, pilose with tubercle-based hairs, margins scarious. *Ligule* an eciliate membrane, 1—1.5 mm long. *Leaf-blades* lanceolate to linear-lanceolate, 2—4.5 cm by 0.3—0.4 cm, upper surface and margins pilose with tubercle-based hairs, margins thick. *Racemes* solitary, capitate, 1—1.7 cm long, composed of (2—)3(—4) sessile and pedicelled spikelets; peduncles slender, 6—14 cm long, waxy. *Sessile spikelets* yellowish-green, oblong, 10—17 mm by 3—4 mm; lower glumes coriaceous, oblong, 6.5—12 mm by 3—4 mm, glabrous, truncate to muticous, upper margin ciliate, 7—9(—11)-nerved, nerves not anastomosing; upper glumes lanceolate to linear-lanceolate, 8—17 mm by 2—3 mm, pubescent on dorsal surface on the upper half, obtuse, upper margin ciliate, 3-nerved; lower lemmas linear-lanceolate, 6—14 mm long, obtuse, upper margin ciliate, 1-nerved; lower paleas linear-lanceolate, 6—14 mm long, obtuse, upper margin ciliate; upper lemmas absent; upper paleas similar to the lower one; stamens 2, anthers purple, 4—7 mm long. *Pedicelled spikelets* lanceolate, 6—6.5 mm by 0.8—1(—2) mm, caducous; pedicels 2.5—4 mm long, sparsely hispid; spikelet callus linear, *ca.* 1.5 mm long, attached obliquely, hairy, hairs 1—2 mm long; lower glumes lanceolate to ovate-lanceolate, 6—6.5 mm by 1—2 mm, pubescent, truncate or obtuse, upper margin ciliate, 5—7-nerved; upper glumes lanceolate to ovate-lanceolate, 5—5.5 mm by 1—1.8 mm, pubescent, truncate or obtuse, upper margin ciliate, 3-nerved; lower florets suppressed; upper lemmas linear, 2.5—4 mm long, awns 3.5—5.5 cm long, columns 2—4 cm long; upper paleas oblong, 2.5—3 mm long, truncate and dentate, upper margin ciliate; styles 2, stigmas white, 5—7 mm long. *Caryopsis* oblong, 3—3.2 mm long.


Distribution. — India and Myanmar.

Ecology. — Open swampy areas, tropical grasslands, 150—1,200 m altitude.

Typification notes. — Hackel (1891) cited *Clarke 44830* and *Clarke 42558* as the type specimens of *Germainia khasyana* Hack. However, he did not mention where they were kept.
Chai-Anan (1972), in her revision of *Germainia*, stated that *Clarke* 44830 was housed in K and BM as the hololectotype. I have found three sheets of *Clarke* 44830 in K and BM: *Clarke* 44830A, 44830C and 44830D which I think, are duplicates of each other and can be type specimens sensu Chai-Anan (1972). According to McNeill *et al.* (2006), Chai-Anan’s (1972) citation of *Clarke* 44830A did have the effect of establishing that specimen as the lectotype and I regard the remaining duplicates as isolectotypes.

**Specimens examined.**


Perennial. **Culms** 20—45(—60) cm tall, erect, branched; nodes pubescent, sometimes pilose. **Leaf-sheaths** clasping, 4—10 cm long, basal sheaths densely covered with white to pale yellow lanate hairs, upper sheath shorter than internodes and sparsely pubescent at base and at throat, to glabrous. **Ligule** an eciliate membrane, 0.2—0.5 mm long, basal leaves with a dense row of hairs behind the ligule. **Leaf-blades** linear, 20—45 cm by 0.2—0.3(—0.5) cm, lower surface covered with white wax, margins thick, pilose at base, margins spinulose-scabrous. **Racemes** 1 or 2, 1.5—2(—3) cm long, elongate, closely appressed, composed of 4—11 sessile and pedicelled spikelets, basal homogamous 2(—3); peduncle 8—15(—20) cm long. **Sessile spikelets** lanceolate, 10—15 mm by 1—1.5 mm, 3—4 dentate, 3-nerved, pubescent on nerves; lower florets male; lower lemmas linear-lanceolate, 9—12 mm long, acute, upper margin ciliate, 3 dentate; upper paleas linear-lanceolate, 2.8—3.2 mm long, truncate and dentate; styles 2, stigmas white or rufous-red, 6—10 mm long. **Caryopsis** oblong, ca. 3.5 mm long.

Thailand. — NORTHERN: Tak (Um Phang); SOUTH-WESTERN: Kanchanaburi (Saiyok, Thong Pha Phum); Ratchaburi (Tapoh).

Distribution. — Myanmar.

Ecology. — Open grassy *Shorea-Phoenix humilis* vegetation areas, dry hill sides in deciduous dipterocarp forests, open sandy soil areas on limestone hills, 150-900 m altitude.
Vernacular. — Ya Da Ru Nee (เย้ายอดนี้), from Phengklai et al. 12559 (BKF).

Specimens examined.

Thailand: NORTHERN: Tak [Um Phang, Doi Hua Mod, 16 Nov. 2006, Teerawatananon & Kritsanachandee 978 (AAU, ABD, BK, BKF, BM, E, GH, K, KKU, L, NY, SING, TCD, THNHM, US)].


Annual, tufted. Culms slender, up to 15 cm tall, erect, branched; nodes glabrous. Leaf-sheaths 0.5—1 cm long, pilose with tubercle-base hairs, margins scarious and glabrous. Ligule an eciliate membrane, 0.5—0.8 mm long. Leaf-blades lanceolate to linear-lanceolate, 1—2 cm by 0.2—0.25 cm, pilose with tubercle-based hairs on both surfaces, margins scabrous. Racemes 1, 6—7 mm long, capitate, composed of 2—3(—4) pairs of spikelets; peduncles slender, 3—6 cm long, waxy. Sessile spikelets yellowish-green to purplish, oblong, 4—4.5 mm by ca. 2 mm; lower glumes chartaceous, oblong, 3.8—4 mm by 2—2.2 mm, glabrous, muticous or truncate or slightly denticulate, 7—9-nerved, nerves anastomosing below apex; upper glumes lanceolate, 4—4.5 mm by 0.8—1.5 mm, pubescent on the upper half, obtuse, 3-nerved; lower lemmas lanceolate, 3.5—3.8 mm long, pubescent on the upper half, obtuse, 1-nerved; lower paleas lanceolate, 3.5—3.8 mm long, pubescent on dorsal surface on the upper half, obtuse; upper lemmas absent; upper paleas similar to the lower palea; stamens 2, anthers yellow to purple, 2—3 mm long. Pedicelled spikelets lanceolate, 3.5—4 mm by 0.2—0.6 mm, caducous; pedicels 1—2 mm long, glabrous; spikelet callus linear, 0.5—1 mm long, attached obliquely, hairy, hairs 0.5—0.8 mm long; lower glumes chartaceous, lanceolate, 3.5—4(—5.5) mm by 0.3—0.6 mm, pubescent, apex hispid, acute to obtuse, 3-nerved; upper glumes lanceolate, 3—4(—5) mm by 0.2—0.5 mm, pubescent, acute to obtuse, 3-nerved; lower florets suppressed; upper lemmas linear, 2—2.5 mm long, awns 3—4.5 cm long, columns 1—2.5 cm long; upper paleas lanceolate, 2.8—3 mm long, dentate; styles 2, stigmas pale brown, 1.4—1.5 mm long. Caryopsis oblong, 1.3—1.5 mm long.

Thailand. — NORTH-EASTERN: Mukdahan (Phu Pha Tub National Park); Sakon Nakhon (Phu Phan National Park).

Distribution. — Endemic to Thailand.

Ecology. — On moist sandy soil in open areas, along stream banks, deciduous dipterocarp and mixed deciduous forests, open and moist rocky plain areas, 500—800 m altitude.

Notes. — Chai-Anan (1972) mentioned that the width of the lower glume of the pedicelled spikelet was 1 to 1.4 mm. However, I have examined the holotype and isotype from BKF, L
including the fresh specimens from the fieldwork and found that their lower glumes are not wider than 0.6 mm.

**Specimens examined.**

5. **Germainia thailandica** (Bor) Chai-Anan, Thai Forest Bull. Bot. 6: 37, fig. 2. 1972; Lazarides, The Tropical Grasses of Southeast Asia: 42. 1980. Figures 2.39 and 2.40.

Basionym: — **Chumsriella thailandica** Bor, Dansk Bot. Ark. 23: 467, fig. on page 468. 1968. Type: Thailand, Chiang Mai, Doi Inthanon, *Boonchuay* 1436 [holotype K! (K 290143); isotypes AAU!, BKF! (BKF 42243), BM! (BM 928266), L! (L 44454)].

Annual, tufted. **Culms** slender, up to 10 cm tall, erect, branched; nodes glabrous. **Leaf-sheaths** 0.8—1.5 cm long, upper part pilose with tubercle-base hairs, margins scarious and glabrous. **Ligule** a ciliate membrane, 0.8—1 mm long. **Leaf-blades** lanceolate to linear-lanceolate, 1—2.5 cm by 0.2—0.3 cm, densely patently pilose with tubercle-based hairs on both surfaces, margins scabrous. **Racemes** 2, 0.8—1.5 cm long, elongate, more or less divergent, composed of 3—4 pairs of spikelets on each, basal homogamous solitary or absent; peduncle slender, 1—2 cm long, enclosed in the uppermost sheath with well developed blade. **Sessile spikelets** oblong, 4—5 mm by 1—2 mm; lower glumes membranous to chartaceous, oblong, 3.5—4.5 mm by 1—2 mm, glabrous, apex with 3—4 dentate, the mid-lobe shorter than the laterals, upper margin ciliate, 7—9-nerved, nerves anastomosing below apex; upper glumes lanceolate, 4—5 mm by 1—1.2 mm, glabrous, truncate to emarginate, upper margin ciliate, 1-nerved; lower florets absent, if present neuter; lower lemmas lanceolate, 2.7—3.7 mm long, obtuse, upper margin ciliate, nerves obscure; lower paleas absent; upper lemmas filiform, 4—4.5 mm long; upper paleas lanceolate, 4—4.2 mm long, glabrous, obtuse, upper margin ciliate; stamens not seen.

**Pedicelled spikelets** lanceolate, 3.5—4 mm by 0.5—1.2 mm, caducous; pedicels 1—1.5 mm long, sparsely pubescent; spikelet callus obtuse, attached transversely, hairy, hairs 0.3—0.5 mm long; lower glumes lanceolate, 3.5—4.3 mm by 0.8—1.2 mm, densely hirsute, 2—3 dentate, upper margin ciliate, 3-nerved, anastomosing; upper glumes lanceolate, 2.8—3.2 mm by 0.8—1 mm, densely hirsute, obtuse with 2—3 dentate, 3-nerved; lower florets suppressed; upper lemmas linear, 1.5—2 mm long, awns 2.5—4.5 cm long, columns 1—1.2 cm long; upper paleas oblong, 1—1.7 mm long, acute, glabrous. **Caryopsis** oblong, 1.3—1.5 mm long.

Thailand. — NORTHERN: Chiang Mai (Doi Inthanon).

Distribution. — Endemic to Thailand.

Ecology. — Evergreen forests.

Vernacular. — Ya Hang Ma (ဗုးမော်ဟူ), from *Boonchuay* 1436 (BKF).
Typification notes. — Bor (1968) stated that the specimen of *K. Boongobeng* 1436 housed in K was the holotype of *Chumsriella thailandica* Bor and regarded the duplicates in BKF, BM and AAU as isotypes. However, the label on the holotype sheet indicated that “Booncheuy 1436” was the collector. The isotype in BM was labelled as “Boocheng 1436”, while the isotype in BKF was labelled using the signature of a collector 1436 written in Thai but can be spelled either “Booncheuy” or “Boonchuay”. As this species was transferred to *Germainia* by Chai-Anan (1972), a Thai grass-taxonomist, she stated the collector name of the holotype was “Boonchuay”. The collector’s name can be spelled in English in different ways. Thus, the correct name should be “Boonchuay” as Chai-Anan (1972) indicated.

Specimens examined.

**Thailand: NORTHERN: Chiang Mai** [Doi Inthanon, 8 Dec. 1964, Boonchuay 1436 (AAU, BKF, BM, K, L)].


Annual. Culms slender, 25–50 cm tall, erect, branched; nodes glabrous. Leaf-sheaths 1–2.5 cm long, glabrous to appressed pubescent, margins scarious. Ligule an eciliate membrane, 0.4–1.2 mm long. Leaf-blades lanceolate to linear-lanceolate, 1–2.5 cm by 0.2–0.3 cm, strigose and pilose with tubercle-based hairs especially on the upper surface, margins thick. Racemes 1, 1–1.4 cm long, capitate, composed of 2 sessile and 1 pedicelled spikelets; peduncles slender, 9–14 cm long, glabrous. Sessile spikelets oblong, 10–14 mm by 2–3 mm; lower glumes coriaceous, oblong, 9–12 mm by 2–3 mm, glabrous, truncate with 2 lateral caducous; upper margin ciliate, 7–9-nerved, nerves not anastomosing; upper glumes lanceolate to linear-lanceolate, 10–14 mm by 1.8–2 mm, pubescent on dorsal surface on the upper half, obtuse, upper margin ciliate, 3-nerved; lower lemmas linear-lanceolate, 8–12 mm long, obtuse, upper margin ciliate, 1-nerved; lower paleas linear-lanceolate, 8–9 mm long, obtuse, upper margin ciliate, nerves obscure; upper lemmas absent; upper paleas as the lower one; stamens 2, anthers, 3.5–6.5 mm long. Pedicelled spikelets lanceolate, 6–7 mm by 0.6–2 mm, caducous; pedicels 2–3.2 mm long, sparsely pubescent; spikelet callus linear, 2–2.5 mm long, attached obliquely, hairy, hairs 1–1.5 mm long; lower glumes lanceolate to ovate-lanceolate, 6–7 mm by 1–2 mm, hirsute on dorsal surface on the upper half, truncate or obtuse, upper margin ciliate, 3-nerved; upper glumes lanceolate to ovate-lanceolate, 5–6.5 mm by 1–1.8 mm, hirsute on dorsal surface on the upper half, truncate or obtuse, upper margin ciliate, 3-nerved; lower florets suppressed; upper lemmas linear, 3–4 mm long, awns 6.5–10.5 cm long, columns 3–4 cm long; upper paleas oblong, 3–3.5 mm long, glabrous, 3–4 dentate, upper margin ciliate; styles 2, stigmas 4–5 mm long. Caryopsis oblong, 3–3.5 mm long.

Thailand. — EASTERN: Ubon Ratchathani (Khong Chiam).

Distribution. — Laos.

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Ecology. — On sloping sandstone with seeping water areas, grasslands on limestone areas, 150–900 m altitude.

Specimens examined.

2.6 Discussion

Tribe Arundinelleae in Thailand

Fourteen species and three genera of tribe Arundinelleae in Thailand were reported by this study, including eight species of *Amndinella*, namely *A. bengalensis*, *A. cochinshinensis*, *A. decempedalis*, *A. holcoides*, *A. kerrii*, *A. kokutensis*, *A. nepalensis* and *A. setosa*, five of *Garnotia*, namely *G. acutigluma*, *G. ciliata*, *G. patula*, *G. tenella* and *G. thailandica*, and one species of *Jansenella: J. griffithiana*. A comparison of the number of species recorded in previous works (Lazarides, 1980; Yenying, 1990; Nanakom and Norsangsri, 2001) and the current study, including the distribution of each species is given in Table 2.3.

Two new species of tribe Arundinelleae were discovered and described, *A. kerrii* and *A. kokutensis*. Both species are endemic to Thailand. I have tried to collect more samples of both species, but only *A. kokutensis* was found and collected from the same site as previous collections. Morphological comparisons to similar taxa of *A. kerrii* and *A. kokutensis* have been made in Tables 2.1 and 2.2, respectively. *Amndinella kerrii* was first described by Hambananda in Yenying (1990) in Thai based on the specimen Kerr 8474 (BK) but the name was not validly published (Teerawatananon et al., in prep.; see manuscript 2; Appendix 1). Many specimens of *A. kokutensis* were previously misidentified as *A. metzii* Hochst. ex Miq. However, *A. kokutensis* differs from *A. metzii* in having an almost glabrous rhachis, a glabrous pedicel, a longer awn and two stamens instead of three as normally found in other species of the genus (Keng, 1936; Bor, 1955; Watson and Dallwitz, 1992; Sun and Phillips, 2006). Occasionally florets with three stamens can be found but the occurrence is extremely rare within an inflorescence that predominantly contains florets with two stamens (Teerawatananon et al., submitted; see manuscript 1; Appendix 1).

There are two new records for Thai Arundinelleae, namely *Garnotia ciliata* and *Jansenella griffithiana*. Three collections of *G. ciliata* were taken from the high altitude areas (1,600–2,650 m) of Doi Inthanon, Chiang Mai Province, Northern Thailand. They are Koyama et al. 48646 (BKF), Laegaard & Norsangsri 21672 and 21736 (AAU, K, US). The first one was identified as *G. tenella* by H. Koyama (without date, 1988). However, it differs from *G. tenella* in having longer inflorescences and wider spreading racemes and longer spikelets. The latter two were misidentified as *G. thailandica* by Laegaard (without date, 2002) and then they were re-identified as *G. ciliata* by Veldkamp (without date, 2003). I have examined those three collections and compared them with the type specimens of *G. thailandica* (Kerr 19197, BK, BM,
K) and *G. ciliata* (Merrill 10701, NY, US). I found that all three collections are similar to *G. ciliata* but differ from *G. thailandica* in having shorter inflorescences, smaller spikelets and leaf-blades and smaller plant size. Although there are some characters that are slightly different from typical *G. ciliata*, e.g. having longer and wider spreading racemes, shorter twisted columns and more glabrous glumes, these three collections are considered here as *G. ciliata*. There is a spectrum of variation and no clear discontinuity to divide the species into infraspecific taxa.

*Jansenella griffithiana* was a new record from Thailand based on *Kerr 16947* from Khao Pawta Luang Kaeo, Ranong Province, Peninsular Thailand (Teerawatanananon and Hodkinson, 2008).

**Subtribe Chionachninae in Thailand**

In this study three species and two genera of subtribe Chionachninae were found in Thailand, comprising two species of *Polytoca*, namely *P. digitata* and *P. wallichiana* and one of *Chionachne*, *C. massiei*. A comparison of the number of species recorded by previous work (Lazarides, 1980; Nanakom and Norsangsri, 2001; Jannink and Velkamp, 2002) and the current study including the distribution of each species are given in Table 2.4.

*Cyathorhachis wallichiana* was proposed by Steudel (1854) based on *Wallich 8629B* from Myanmar. This species was then transferred to be under *Polytoca* as *P. wallichiana* by Bentham (1882). Recently, *P. wallichiana* was placed under genus *Cyathorhachis* by Jannink and Velkamp (2002) based on the following three main reasons: 1) The distal end of the terminal racemes is neuter or not with well-developed pairs of spikelets. 2) The upper floret of the female sessile spikelet is absent, while the lower palea is present. 3) The apex of the lower glume of the male spikelet is awned.

However, relying mainly on those three characters may not be good enough to separate *Cyathorhachis* from *Polytoca*. In addition, molecular studies using nuclear ribosomal DNA (*ITS1-ITS2*) sequences have shown that *P. wallichiana* grouped with *P. digitata* and the analyses of chloroplast DNA and nuclear DNA gene regions (*atpB-rbcL* and *ITS*, Chapter 3) also confirmed that *P. wallichiana* should be placed within *Polytoca* rather than *Cyathorhachis*.

**Subtribe Dimeriinae in Thailand**

Seven species of *Dimeria* were reported for Thailand in this study, they are *D. fusescens*, *D. kerrii*, *D. kurzii*, *D. leptorhachis*, *D. ornithopoda*, *D. pubescens* and *D. sinensis*. A comparison of the
number of species recorded by previous work (Lazarides, 1980; Nanakorn and Norsangsri, 2001) and the current study, including the distribution of each species, is given in Table 2.5.

*Dimeria kerrii* is a new species for Thailand. It is endemic to Peninsular Thailand, known only from the type collection, which was collected from Ban Tola Sai, Satun Province (see Manuscript 2, Appendix 1). More fieldwork will be required to evaluate its conservation status. This species is distinguished from all other species of *Dimeria* by its rugose, broad wing on the keel of the upper glume.

**Subtribe Germainiinae in Thailand**

Twelve species and two genera of subtribe Germainiinae have been recorded in Thailand. There are six species of *Apocopis*, namely *A. collinus, A. courtallumensis, A. intermedius, A. mangalorenis, A. siamensis* and *A. wrightii* and six of *Germainia*, namely *G. capitata, G. khasyana, G. lanipes, G. pilosa, G. thailandica* and *G. thorelii*. A comparison of the number of species recorded by previous work (Lazarides, 1980; Nanakorn and Norsangsri, 2001) and the current study, including the distribution of each species, is given in Table 2.6.

**Problematic taxa in Thailand**

In total, twelve species from tribe Arundinelleae and subtribes Chionachninae, Dimeriinae and Germaininae, which were previously reported to occur in Thailand (Chai-Ann, 1972; Lazarides, 1980; Yenying, 1990; Nanakorn and Norsangsri, 2001; Jannink and Veldkamp, 2002), were not found in this study. They are *Arundinella birmanica* Hook.f, *Arundinella ciliata* Nees & Miq.; *Arundinella hispida* (Willd.) Kuntze, *Arundinella rugulata* A. Camus, *Garnotia stricta* Brongn.; *Chionachne koenigii* (Spreng.) Thwaites, *Sclerachne punctata* R. Br.; *Dimeria ciliata* Merr.; *Dimeria falcata* Hack., *Apocopis cochinchinensis* A. Camus, *Apocopis paleacea* Hochr., and *Germainia tenax* (Balansa) Chai-Anan. I have examined the type specimens of *Arundinella hurmanica* [Burma, Kurz 3161 (K)]; *Arundinella rupestris* [Vietnam, Balansa 1694, 4912 (K)]; *Garnotia stricta* [Tahiti, Duperrey s.n. (plate 21, illustration at K, L), Philippines, Merrill s.n. (AAU, BM, E, K)]; *Sclerachne punctata* (now *Chionachne punctata* (R. Br.) Jannink) [Java, Massie s.n. (L)]; *Dimeria ciliata* [Philippines, Merrill 9320 (US); *Dimeria falcata* [China, T. Sampson 1051 (K) and Hanse 1385 (BM, K)], *Apocopis cochinchinensis* [Vietnam, Pierre s.n. (K, BM, E)], *Apocopis paleacea* [Nepal, Wallich s.n. in Herb. Trinmus 68.01 (IDC microfiche at K); Nepal, 1821, Wallish cat. nr. 8843 (NY, E)] and *Germainia tenax* [Laos, Harmand s.n. (L)]. However, none of the specimens collected from Thailand corresponded to authentic *Arundinella burmanica, Arundinella rupestris,*
Garnotia stricta, Chionachne punctata, Dimeria ciliata, Dimeria falcata, Apocopis cochinchinensis, Apocopis paleacea and Germainia tenax. I also found that some specimens of other species sometimes have been misidentified as one of these species, e.g. the specimens Sorensen et al. 5732 (K, BKF) and Usa 53 (BKU) of Arundinella nepalensis were misidentified as Arundinella replestri by Bor (1962) and Yenying (1990), the specimens Smitinand 3097, 4088 and 6994 (BKF, GH, K) and Sorensen et al. 593, 2098, 5587 and 6169 (BKF, C, E, K, SING) of Garnotia tenella were misidentified as Garnotia stricta by Bor (without date, 1958 and 1959) and Bor (1962), the specimens of Beusekom et al. 4492 (C, K, L) and 4376 (L) of Dimeria fuscescens have been misidentified as Dimeria falcata, and the specimens Kerr 8584 (BM, K) of Apocopis collinus and Kerr 2745 (BM, K) of Apocopis mangalorensis were misidentified as Apocopis royleana Nees (now Apocopis paleacea) and Apocopis cochinchinensis, respectively.

Unfortunately, there are three species which their type specimens were not available for this study, Arundinella ciliata, A. hispida and Chionachne koenigii. The name A. ciliata was reported by Yenying (1990) based on Kerr s.n. from Ranong Province. However, I have found that Kerr s.n. is actually Kerr 16726 which is kept in BK (Yenying, pers. comm.) and it actually is A. holoides. The name A. hispida was reported to occur in Thailand from two studies, Camus and Camus (1922 and Yenying (1990). I have found that the specimens Kerr 2228 (from Doi Sutep, Chiang Mai, Northern Thailand) of A. nepalensis was misidentified as A. hispida by Camus and Camus (1922) and the specimen Soradej 190 (BKF) of A. bengalensis was misidentified as A. hispida by Yenying (1990). So, it is possible that Lazarides (1980) and Nanakorn and Norsangsri (2001) only presumed that A. ciliata and A. hispida could be found in Thailand according to Camus and Camus (1922) and Yenying (1990). As for C. koenigii, I also found that the specimens Weatherwax-Th-67 (GH, US) of C. massiei, which were collected from Saraburi Province, Central Thailand, were misidentified as C. koenigii. Thus, it is possible that C. koenigii sensu Lazarides (1980) and Nanakorn and Norsangsri (2001) could be C. massiei in this study.
Table 2.3 Comparision of the species numbers recorded in previous work and the current study and the geographical distribution of tribe Arundinelleae occurring in Thailand.

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<tbody>
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<td></td>
<td>✓</td>
<td>✓</td>
<td></td>
<td>✓</td>
<td>N</td>
</tr>
<tr>
<td>1. <em>A. bengalensis</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>✓</td>
</tr>
<tr>
<td>2. <em>A. birmanica</em></td>
<td>–</td>
<td>–</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3. <em>A. ciliata</em></td>
<td>–</td>
<td>✓</td>
<td>–</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4. <em>A. cochinchinensis</em></td>
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<td>–</td>
<td>✓</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5. <em>A. decempedalis</em></td>
<td>–</td>
<td>–</td>
<td>–</td>
<td></td>
<td>✓</td>
</tr>
<tr>
<td>6. <em>A. hispida</em></td>
<td>–</td>
<td>✓</td>
<td>✓</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7. <em>A. boloides</em></td>
<td>✓ as <em>A. boloides</em> var. <em>ciliata</em></td>
<td>–</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>8. <em>A. kerri</em></td>
<td>–</td>
<td>✓</td>
<td>✓</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9. <em>A. kokutensis</em></td>
<td>–</td>
<td>–</td>
<td>–</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10. <em>A. nepalensis</em></td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td></td>
<td>✓</td>
</tr>
<tr>
<td>11. <em>A. rapestris</em></td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td></td>
<td>✓</td>
</tr>
<tr>
<td>12. <em>A. setosa</em></td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td></td>
<td>✓</td>
</tr>
<tr>
<td>13. <em>G. acutigluma</em></td>
<td>✓</td>
<td>–</td>
<td>✓</td>
<td></td>
<td>✓</td>
</tr>
<tr>
<td>2. <em>G. ciliata</em></td>
<td>–</td>
<td>–</td>
<td>✓ new record</td>
<td></td>
<td>✓</td>
</tr>
<tr>
<td>3. <em>G. patula</em></td>
<td>–</td>
<td>–</td>
<td>✓ new record</td>
<td></td>
<td>✓</td>
</tr>
<tr>
<td>4. <em>G. stricta</em></td>
<td>✓ as <em>G. stricta</em> var. <em>longiseta</em></td>
<td>–</td>
<td>✓</td>
<td>✓</td>
<td>= <em>G. tenella</em></td>
</tr>
<tr>
<td>5. <em>G. tenella</em></td>
<td>✓</td>
<td>–</td>
<td>✓ new record</td>
<td></td>
<td>✓</td>
</tr>
<tr>
<td>6. <em>G. thailandica</em></td>
<td>✓</td>
<td>–</td>
<td>✓</td>
<td></td>
<td>✓</td>
</tr>
<tr>
<td>1. <em>J. griffithiana</em></td>
<td>–</td>
<td>–</td>
<td>✓ new record</td>
<td></td>
<td>✓</td>
</tr>
</tbody>
</table>

Remarks: Abbreviations: A = *Arundinella*, G = *Gamotia*, J = *Jansenella*, N = Northern; NE = North-eastern; E = Eastern; SW = South-western; C = Central; SE = South-eastern; PEN = Peninsular; syn. = synonym; SNF = Specimen not found.
Table 2.4 Comparison on the species numbers recorded in previous work and the current study and the geographical distribution of subtribe Chionachninae species occurring in Thailand.

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<td>1. <em>Chi. massiei</em></td>
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<td>–</td>
<td>✓ ✓ ✓ ✓</td>
<td></td>
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<tr>
<td>2. S. punctata</td>
<td>✓</td>
<td>✓</td>
<td>–</td>
<td>= <em>Chi. massiei</em></td>
<td></td>
</tr>
<tr>
<td>3. <em>Chi. koenigii</em></td>
<td>✓</td>
<td>✓</td>
<td>syn. of <em>Chi. gigantea</em></td>
<td>= <em>Chi. massiei</em></td>
<td></td>
</tr>
<tr>
<td>4. P. digitata</td>
<td>✓</td>
<td>✓</td>
<td>–</td>
<td>✓ ✓ ✓ ✓ ✓ ✓ ✓ ✓</td>
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</tr>
<tr>
<td>5. <em>P. wallichiana</em></td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓ ✓ ✓ ✓ ✓ ✓ ✓ ✓</td>
<td></td>
</tr>
</tbody>
</table>

Remarks: Abbreviations; *Chi* = *Chionachne*, *S* = *Sclerachne*, *P* = *Polystoa*, *N* = Northern; *NE* = North-eastern; *E* = Eastern; *SW* = South-western; *C* = Central; *SE* = South-eastern; *PEN* = Peninsular; *syn.* = synonym.

Table 2.5 Comparison on the species numbers recorded in previous work and the current study and the geographical distribution of subtribe Dimeriinae species occurring in Thailand.

<table>
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<tr>
<th>Species</th>
<th>Lazarides (1980)</th>
<th>Nanakom and Norsangsri (2001)</th>
<th>This study</th>
<th>Distribution</th>
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<tr>
<td>1. <em>D. ciliata</em></td>
<td>✓</td>
<td>✓</td>
<td>SNF</td>
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<tr>
<td>2. <em>D. cylonica</em></td>
<td>✓</td>
<td>✓</td>
<td>syn. of <em>D. pubescens</em></td>
<td></td>
</tr>
<tr>
<td>3. <em>D. falcata</em></td>
<td>–</td>
<td>✓</td>
<td>= <em>D. fuscescens</em></td>
<td></td>
</tr>
<tr>
<td>4. <em>D. fuscescens</em></td>
<td>✓</td>
<td>✓</td>
<td>✓ ✓ ✓ ✓ ✓ ✓ ✓ ✓</td>
<td></td>
</tr>
<tr>
<td>5. <em>D. kerrii</em></td>
<td>–</td>
<td>✓ nom. nud.</td>
<td>✓ sp. nov.</td>
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<td>6. <em>D. kurzii</em></td>
<td>✓</td>
<td>✓</td>
<td>✓ ✓ ✓ ✓ ✓ ✓ ✓ ✓</td>
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<tr>
<td>7. <em>D. leptorhachis</em></td>
<td>–</td>
<td>–</td>
<td>✓ ✓ ✓ ✓ ✓ ✓ ✓ ✓</td>
<td></td>
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<tr>
<td>8. <em>D. ornithopoda</em></td>
<td>✓</td>
<td>✓</td>
<td>✓ ✓ ✓ ✓ ✓ ✓ ✓ ✓</td>
<td></td>
</tr>
<tr>
<td>9. <em>D. pubescens</em></td>
<td>–</td>
<td>–</td>
<td>✓ ✓ ✓ ✓ ✓ ✓ ✓ ✓</td>
<td></td>
</tr>
<tr>
<td>10. <em>D. sinensis</em></td>
<td>–</td>
<td>–</td>
<td>✓ ✓ ✓ ✓ ✓ ✓ ✓ ✓</td>
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<tr>
<td>11. <em>D. velutina</em></td>
<td>✓</td>
<td>✓</td>
<td>syn. of <em>D. leptorhachis</em></td>
<td></td>
</tr>
</tbody>
</table>

Remarks: Abbreviations; *D* = *Dimeria*, *N* = Northern; *NE* = North-eastern; *E* = Eastern; *SW* = South-western; *C* = Central; *SE* = South-eastern; *PEN* = Peninsular; *syn.* = synonym; SNF = Specimen not found.
Table 2.6 Comparison on the species numbers recorded in previous work and the current study, and the geographical distribution of subtribe Germainiinae species occurring in Thailand.

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<td>1. <em>Ap. collinus</em></td>
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<tr>
<td>2. <em>Ap. cochinchinensis</em></td>
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<td>= <em>Ap. mangalorensis</em></td>
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<td>3. <em>Ap. courtallamensis</em></td>
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<td>5. <em>Ap. mangalorensis</em></td>
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<td>6. <em>Ap. paleacea</em></td>
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<td>✓</td>
<td>= <em>Ap. mangalorensis</em></td>
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<td>7. <em>Ap. siamensis</em></td>
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<tr>
<td>8. <em>Ap. wrightii</em></td>
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<td>1. <em>Ger. capitata</em></td>
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<td>2. <em>Ger. khayana</em></td>
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<td>3. <em>Ger. lanipes</em></td>
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<td>4. <em>Ger. pilosa</em></td>
<td>✓</td>
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<td>5. <em>Ger. tenax</em></td>
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<td>6. <em>Ger. thailandica</em></td>
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Remarks: Abbreviations; *Ap.* = *Apocoris*; *Ger.* = *Germainia*; N = Northern; NE = North-eastern; E = Eastern; SW = South-western; C = Central; SE = South-eastern; P = Peninsular; syn. = synonym; ? = probably may be found in Thailand; SNF = Specimen not found.
Figure 2.1 *Amndinella bengalensis*. Left, plant habit and habitat along the margin of hill evergreen forest at Doi Inthanon National Park, Chiang Mai Province, Northern Thailand; Right, inflorescences showing the arrangement of racemes.
Figure 2.2 *Arundinella kerrii*. A. habit; B. inflorescence; C. spikelets; D. lower glumes; E. upper glumes; F. lower lemmas; G. lower paleas; H. upper lemmas; J. upper paleas. A-B *Kerr* 21330; C-J. *Kerr* 8474 (BK, K). Drawn by A. Teereawatananon.
Figure 2.3 *Arundinella kokutensis*. A. habit; B. inflorescence; C. spikelets; D. lower glumes; E. upper glumes; F. lower lemmas; G. lower paleas; H. upper lemmas; J. upper paleas. All drawn from Charoenphol et al. 5104, by A. Teerawatananon.
Figure 2.4 *Arundinella nepalensis*. Top, plant habit and habitat on hill slope, along the margin of deciduous dipterocarp forest at Mae Ngao National Park, Mae Hong Son Province, Northern Thailand; Bottom, inflorescence showing the arrangement of racemes.
Figure 2.5 *Arundinella setosa*. Top left, plant habitat on hill slopes at Doi Hua Mod, Um Phang, Tak Province, Northern Thailand; Top right, habit in deciduous dipterocarp forest at Suan Phung District, Ratchaburi Province, South-Western Thailand; Bottom left, inflorescence showing the arrangement of racemes; Bottom right, raceme showing flowering spikelets and stamens.
Figure 2.6 Distribution map of *Arundinella bengalensis*. The circles represent the distribution obtained from specimens examined.
Figure 2.7 Distribution map of *Arundinella cochinchinensis*, *A. decempedalis*, *A. holcoïdes*, *A. kerrii*, and *A. kokutensis*. The circles represent the distribution obtained from specimens examined.
Figure 2.8 Distribution map of *Arundinella nepalensis*. The circles represent the distribution obtained from specimens examined.
Figure 2.9 Distribution map of *Arundinella setosa* var. *setosa* and *A. setosa* var. *esetosa*. The circles represent the distribution obtained from specimens examined.
Figure 2.10 *Garnotia acutigluma*. Top and bottom left, plant habit and habitats on the rocks, in semi-shady areas along the streams at Phu Kradueng National Park, Loei Province, North-Eastern Thailand; Bottom right, inflorescence and spikelets.
**Figure 2.11** *Garnotia patula*. Top, inflorescences showing the arrangement of racemes; Bottom left, plant habit and habitat in dwarf oak forest at Phu Luang Wildlife Sanctuary, Loei Province, North-Eastern Thailand; Bottom right, racemes showing the arrangement of spikelets.
Figure 2.12 Garnotia thailandica. A. habit; B. spikelet; C. lower glume; D. upper glume; E. lemma; F. palea; All modified from Gould (1972).
Figure 2.13 *Garnotia tenella*. Top left, plant habitat on rocky ground area at Phu Hin Rong Kla National Park, Phitsanulok Province, Northern Thailand; Top right, habit on mossy rock in montane forest at Khao Yai National Park, Nakhon Ratchasima Province, Eastern Thailand; Bottom left, inflorescences; Bottom right, inflorescences showing the arrangement of racemes.
Figure 2.14 Distribution map of all species of Thai *Gamotia*. The circles represent the distribution obtained from specimens examined.
Figure 2.15 *Jansenella griffithiana*. A. habit; B. leaf-sheath and blade; C. spikelets; D. lower glumes; E. upper glumes; F. lower lemmas; G. lower paleas; H. upper lemmas; J. upper paleas; K. grains. All drawn from *Teerawatananon & Sungkaew* 2001-1 by A. Teerawatananon.
Figure 2.16 Distribution map of *Jansenella griffithiana*. The circles represent the distribution obtained from specimens examined.
Figure 2.17 *Chionochne massiei*. A. habit; B. inflorescence; C. raceme showing a spatheole; D. female sessile spikelets; E. upper glumes of female sessile spikelet; F. lower lemmas of the same; G. upper lemmas of the same; H. upper palea of the same. All drawn from *Teerawatanan on* 151001-15 by A. Teerawatanan on.
Figure 2.18 *Chionachne massiei*. Top, plant habit along the margin of deciduous dipterocarp forest at Inthanon National Park, Chiang Mai Province, Northern Thailand; Bottom left, inflorescence showing the arrangement of racemes; Bottom right, raceme showing the flowering spikelets.
Figure 2.19 *Polystea digitata*. Top, inflorescences showing the arrangement of racemes; Bottom left, inflorescence showing the arrangement of male and female spikelets in the terminal raceme, and lateral male raceme; Bottom right, plant habit and habitat in deciduous dipterocarp forest at Doi Suthep National Park, Chiang Mai Province, Northern Thailand.
Figure 2.20 *Polytoca wallichiana.* Top left, plant habit in deciduous forest at Lin Thin, Kanchanaburi Province, South-Western Thailand; Top right, flowering female raceme; Bottom left, inflorescence showing the arrangement of male racemes and male and female spikelets in terminal raceme; Bottom right, flowering male racemes.
Figure 2.21 Distribution map of all species of Thai Chionachninae. The circles represent the distribution obtained from specimens examined.
Figure 2.22 *Dimeria fuscescens*. Top, inflorescence showing the arrangement of racemes and flowering spikelets and their stamens; Bottom, plant habit and habitat on rocky ground area at Phu Kradueng National Park, Loei Province, North-Eastern Thailand.
Figure 2.23 *Dimeria kerrii*. A. habit; B. inflorescences; C. spikelets; D. lower glume; E. upper glume; F. lemma; G. palea. All drawn from *Kerr* 13868 by A. Teerawatananon.
Figure 2.24 *Dimeria ornithopoda* var. *ornithopoda*. Top, plant habits on rocky ground area at Khong Chiam District, Ubon Ratchathani Province, Eastern Thailand; Bottom left and right, inflorescences showing the arrangement of racemes.
Figure 2.25 *Dimeria sinensis*. Left, plant habits and habitat in paddy field at Tha Uthen District, Nakhon Phanom Province, North-Eastern Thailand; Right, inflorescences.
Figure 2.26 *Dimeria* spikelet-silhouettes: A. *D. fuscescens* (Wallich 8841, K); B. *D. kerrii* (Kerr 13868, K); C. *D. kurzii* (Teerawatananon & Kritsanachande 962, TCD); D. *D. leptorhachis* (Kerr 17725, K); E. *D. ornithopoda* var. *ornithopoda* (Teerawatananon & Kritsanachande 967, TCD); F. *D. pubescens* (Teerawatananon & Kritsanachande 968, TCD); G. *D. sinensis* (Teerawatananon & Kritsanachande 946, TCD). All drawn by A. Teerawatananon.
Figure 2.27 Distribution map of *Dimeria pubescens*, *D. kurzii*, *D. leptorrhachis* and *D. sinensis*. The circles represent the distribution obtained from specimens examined.
Figure 2.28 Distribution map of *Dimeria fusescens*, *D. kerrii*, *D. ornithopoda* var. *ornithopod* and *D. ornithopoda* var. *gracillima*. The circles represent the distribution obtained from specimens examined.
Figure 2.29 *Apocapis intermedius* and *A. collinus*. Top and bottom left (*A. intermedius*), inflorescences showing the arrangement of spikelets. Bottom right (*A. collinus*), inflorescence showing flowering spikelets and stigmas.
Figure 2.30 *Apocopsis courtallumensis*. Top, inflorescence showing flowering spikelets and stamens; Bottom left, plant habit and habitat in tropical grassland at Thung Salaeng Luang National Park, Phitsanulok Province, Northern Thailand; Bottom right, inflorescence showing flowering spikelets and stigmas.
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Figure 2.40 Distribution map of all species of Thai Germainia. The circles represent the distribution obtained from specimens examined.
CHAPTER 3

Molecular evolution of the grass subfamily Panicoideae (Poaceae): based on chloroplast and nuclear DNA sequences

3.1 Introduction

The phylogeny of Poaceae

Biological systematics is the study of the diversity of life that exists on Earth, and its evolutionary history. It aims to detect, describe and explain biological diversity (Moritz and Hillis, 1996). One aim of systematics is to reconstruct phylogenetic relationships among living and extinct (fossil) organisms and hence to infer the tree of life which reflects the hierarchical classification of life within and between the groups (Hodkinson and Parnell, 2007). Another fundamental activity of systematic research is the identification, naming and classification of organisms. Classification is a basic method to describe the structure of living organisms by grouping species or infraspecific taxa in a defined way, while identification involves the referral of an individual specimen to a previously classified and named group (Judd et al., 1999; Stuessy, 2009). Phenetic and the phylogenetic approaches have been applied to classification. The numerical or phenetic approach is based on the concept that relationships between organisms are ascertained by their overall degree of similarity without any implication as to their phylogeny or evolutionary relationships (Sokal and Sneath, 1963; Stuessy, 2009). The cladistic or phylogenetic approach groups species according to how recently they share a common ancestor. It therefore aims to directly represent evolutionary relationships of the species (Ridley, 2004; Stuessy, 2009). In practice, the two approaches are both used successfully and are often used in combination by systematists.

The grasses (Poaceae) are one of the largest and most important plant families to humankind and the ecology of the world, consisting of approximately 10,000 species in over 700 genera (Clayton and Renvoize, 1986; Mabberley, 1987; Watson and Dallwitz, 1992). The first effective classification of Poaceae was developed in 1814 by Robert Brown. Subsequent to Brown (1814), many comprehensive classifications have been proposed, such as Bentham (1878), Bentham and Hooker (1883), Hackel (1887), Pilger (1956), Tateoka (1957), Stebbins (1956), Bor (1960) and Caro (1982). All these classifications were mainly based on morphology and anatomy including the more recent phenetic analyses of Hilu and Wright (1982), Clayton and Renvoize (1986) and Watson and Dallwitz (1992). However, the classification of grasses is
still unclear (and hence unstable) because of the conflict between these phenetic classifications. To try and overcome these problems, application of phylogenetic methodology has been undertaken. The study of Kellogg and Watson (1993) was one of the first attempts to use cladistic analysis based on morphological and anatomical characters in order to produce phylogenetic trees of the grass family. However, the morphological characteristics are often difficult to study and require extensive developmental and anatomical investigation to establish appropriate comparisons. More recently studies have used DNA sequences to determine relationships among organisms (Kellogg, 2001) as these characters are simple to compare and almost unlimited in potential availability (the rice genome, for example, is 410 mbp in size; Feng et al., 2002).

The first phylogenetic study of grass family using DNA sequences was by Hamby and Zimmer (1988) using nuclear ribosomal RNA sequences from nine species representing four subfamilies of Poaceae. After that, many studies used plastid (chloroplast) genome DNA sequences, such as rbcL (the gene for the large subunit of ribulose biphosphate caboxylase), Doebley et al. (1990), Baker et al. (1995) and Duvall and Morton (1996); plastid DNA restriction site variation, Davis and Soreng (1993); rps4 (ribosomal protein small subunit 4), Nadot et al. (1994); ndhF (NADH dehydrogenase) gene, Clark et al. (1995); madK sequence gene, Liang and Hilu (1996), Hilu et al. (1999), Hilu and Alice (1999); the grass-specific insert in the chloroplast rsaC2 gene, Cummings et al. (1994) and Barker et al. (1999); chloroplast non-coding rpl16 intron gene, Zhang (2000). The phylogenetic analyses of plastid DNA showed that the neotropical herbaceous bamboos, including tribes Anomochlocae and Streptochaetaeae, were the most outlying clades within the family and the next most outlying clade was the pantropical herbaceous bamboo tribe Phareae. Furthermore, two main clades in grass family were proposed: the BOP-clade of Clark et al. (1995), consisting of Bambusoideae, Oryzoideae and Pooideae, and the PACC clade of Davis and Soreng (1993), consisting of Panicoideae, Arundinoideae, Chloridoideae and Centothecoideae.

Nuclear DNA has also been used to construct phylogenetic trees among the grasses, e.g. phytochrome genes (Mathews and Sharrock, 1996; Mathews et al., 2000) and the internal transcribed spacer (ITS) of nuclear ribosomal DNA (Hsiao et al., 1999). The results from nuclear DNA resolved the PACC clade with high support. However, the result from Hsiao et al. (1999) found that Pooideae were not placed in the BOP clade but were sister to the PACC clade.
In order to resolve the ambiguous relationships and the conflicts between the single-gene analyses of grass family, combined analyses or multigene analyses became increasingly popular in molecular phylogenetic studies, e.g. Soreng and Davis (1998), Clark et al. (2000); GPWG (2001) and Salamin et al. (2002). One of the most significant combined data analysis of the grass family was that of Grass Phylogeny Working Group, GPWG, (2001) using three chloroplast gene regions (ndhF, rbcL, rpoC2), three nuclear gene regions (GBSSI or waxy, ITS2, PHYB), plastid restriction site data, morphological and anatomical data from the previous study for 62 grass species of commonly recognised subfamilies, and four genera from families Flagellariaceae, Restionaceae (two genera) and Joinvilleaceae, as outgroups. The analysis of the combined data showed that Joinvillea was sister to a monophyletic Poaceae. The earliest diverging subfamilies were Anomochlooideae, Pharoidae and Puelioideae. The remaining grasses were split into two major groups, the BEP clade (BOP clade sensu Clark et al., 1995) and PACCAD clade. The BEP clade consisted of subfamilies Bambusoideae sensu stricto (including tribes Olyreae and Bambuseae), Ehrhartoideae (= Oryzoideae) and Pooideae. The PACCAD clade contained subfamilies Panicoideae, Arundinoideae sensu stricto, Chloridoideae sensu lato, Centothecoideae, Aristidoideae, and Danthonioideae. This has been enlarged in a recent analysis to become the PACCMAAD clade because of the inclusion of a new subfamily Micrairoideae (Sánchez-Ken et al., 2007).

The phylogeny of the subfamily Panicoideae

Panicoideae are one of the largest subfamilies in Poaceae, comprising approximately 3000 species and 200 genera. Although Panicoideae are the most diverse group, all members were recognised as a natural group by the presence of two-flowered spikelets with the lower floret male or sterile (Brown, 1810, 1814; Clayton and Renvoize, 1986; Kellogg, 2000) and the simple type of starch grain in the endosperm (Tateoka, 1962). These spikelet and starch grain types were indicated as the uniquely derived-characters by Kellogg and Campbell (1987) based on cladistic analysis which supported the Panicoideae as a monophyletic group.

The monophyly of Panicoideae has been verified by many molecular studies using chloroplast DNA and nuclear genes, e.g. Davis and Soreng (1993) based on an analysis including just three genera of Panicoideae (Miscanthus, Zea from Andropogoneae and Pennisetum from Paniceae). In their analysis, Panicoideae formed a sister group to the rest of the PACC clade. Analyses by Barker et al. (1995, 1999) supported the monophyly of Panicoideae. In their work seven genera from three tribes were sampled. Panicoideae were divided into two lineages: Paniceae and Andropogoneae + Tristachya (Arundinellae). However, Clark et al. (1995)
showed that Panicoideae were paraphyletic because *Danthoniopsis* (Arundinelleae) nested in a clade comprised of tribes Thysanolaenaeae and Centothecaeeae sensu Clayton and Renvoize (1986) but this clade was sister to Panicoideae. Hilu et al. (1999) also found that Panicoideae were paraphyletic because *Loudetiopsis* (Arundinelleae) was sister to the Centothecoideae-Panicoideae in their neighbour joining (NJ) tree. However, the other genera of Panicoideae formed a strongly supported clade that was divided into two lineages: the Paniceae and the paraphyletic Andropogoneae, due to the inclusion of *Tristachya* (Arundinelleae). Hsiao et al. (1999) confirmed the monophyly of Panicoideae sensu Clayton and Renvoize (1986) excluding *Eriachne* (Eriachneae), because *Eriachne* was placed in an Arundinoideae clade and sister to tribe Micraireae. They also found that Panicoideae were sister to Chloridoideae instead of Centothecoideae. Within Panicoideae, Paniceae were sister to the clade of Andropogoneae + *Arundinella*. Mathews et al. (2000) supported the monophyletic status of Panicoideae and resolved them sister to *Danthoniopsis* (Arundinelleae). Gomez-Martinez and Culham (2000) resolved the monophyletic Panicoideae that were sister to Centothecoideae. Giussani et al. (2001) showed that Panicoideae were strongly supported as a monophyletic group in all analyses with Centothecoideae and *Danthoniopsis* as outgroups. The result suggested that Panicoideae were divided into three strongly supported clades: Andropogoneae with an *Arundinella* clade and two clades of Paniceae, but the relationships among the three clades were unclear. The GPWG (2001) also confirmed monophyly of Panicoideae (excluding *Danthoniopsis* and *Eriachne*) but the relationships among the major lineages in the PACCAD clade were not resolved.

In the widely accepted classification system of Clayton and Renvoize (1986), Panicoideae comprised of seven tribes: Andropogoneae, Paniceae, Arundinelleae, Isachneae, Hubbardiaeae, Steyermarkochloaeae and Eriachneae. Later on, Panicoideae were divided into two groups by two studies using phenetic analysis: the Andropogoneae + Garnotieae group and the Paniceae + Isachneae + Arundinelleae group of Hilu and Wright (1982) and the supertribes Panicodae and Andopogonodae of Watson and Dallwitz (1992). More recent phylogenetic analyses have resolved five tribes in Panicoideae because two tribes, Eriachneae and Isachneae, were placed together with Micraireae in a new subfamily Micrairoideae (Sánchez-Ken et al., 2007).

**The phylogeny of the tribe Arundinelleae**

Over the past few decades, the members of tribe Arundinelleae have been included in several phylogenetic analyses. The morphological phylogenetic reconstruction of Kellogg and Watson (1993) found that Arundinelleae (excluding *Garnotia*) were monophyletic and sister to *Polytrias*
(subtribe Saccharinae). However, DNA sequence evidence from the rbcL gene (Baker et al., 1995, 1999) and multi-gene analyses (Bouchenak-Khelladi et al., 2008) have positioned Tristachya within Andropogoneae. This was consistent with the matK gene analysis of Hilu et al. (1999) which also found that another genus of Arundinelleae, Lendetiopsis, was nested outside an Andropogoneae and Paniceae clade. Moreover, the studies of ndhF (Clark et al., 1995) and combined data (GPWG, 2001; Bouchenak-Khelladi et al., 2008) found that Danthoniopsis was separated from the Andropogoneae and Paniceae clade. This result also appeared in many studies which also supported the position of Arundinella as a sister group to Andropogoneae (Mason-Gamer et al., 1998; Spangler et al., 1999; Mathews et al., 2000; Giussani et al., 2001; Mathews et al., 2002; Aliscioni et al., 2003) or within Andropogoneae (Hsiao et al., 1999). The results from the rbcL gene analysis of Baker et al. (1995, 1999), ndhF gene analysis of Spangler et al. (1999, 2000) and phylogenetic study of Kellogg (2000) recommended that Arundinella and Tristachya should be placed in the Andropogoneae. Danthoniopsis and Rattraya (now Danthoniopsis) should be placed in Paniceae and the remaining genera treated as incertae sedis. Later, Danthoniopsis was placed within Centothecoideae, though the position of Danthoniopsis in the Centothecoideae clade is unstable (Giussani et al., 2001; GPWG, 2001). More recent studies showed that the genus Danthoniopsis and related genera should be considered as a new tribe within the Panicoideae plus Centothecoideae clade (Sánchez-Ken et al., 2007). It seems that representative members of this group were resolved in different and distantly related clades which corresponded to the confused generic classification within the tribe (Clayton, 1971). This suggested that further molecular data and taxa of Arundinelleae need to be added to analyses in order to clarify the phylogeny of this tribe.

The phylogeny of the tribe Andropogoneae

Andropogoneae are a well-defined tribe of tropical grasses containing more than 900 species with extensive morphological variation among its members. All members have the C₄ pathway with NADP-malic enzyme as the primary decarboxylating enzyme and have a single sheath of cells around the vascular bundle. Two wildly used classifications of Andropogoneae come from the studies of Clayton and Renvoize (1986) and Watson and Dallwitz (1992). Clayton and Renvoize (1986) divided the tribe into 11 subtribes, while Watson and Dallwitz (1992) recognised Andropogoneae as the supertribe Andropogonanae and divided it into two tribes: Andropogoneae and Maydeae (Table 3.3).

Andropogoneae were found to be non-monophyletic in the morphology based phylogenetic reconstruction of Kellogg and Watson (1993). They sampled 49 genera of Andropogoneae
using tribes Arundinelleae and Neurachneae as outgroups. The final tree showed that *Garnotia* (Arundinelleae) was placed in Andropogoneae and *Polytrias* was placed as a sister to Arundinelleae. Molecular data, on the other hand, consistently supported the monophyly of Andropogoneae (e.g. Doebley et al., 1990; Davis and Soreng, 1993; Nadot et al., 1994; Clark et al., 1995; Duvall and Morton, 1996). In addition, more extensive studies employing greater taxon sampling as well as larger amounts of DNA sequence data have confirmed the monophyly of the tribe as circumscribed by Clayton and Renvoize (1986) and also supported the sister relationship of Andropogoneae to *Arundinella* (Manson-Gamer et al., 1998; Spangler et al., 1999; Giussani et al., 2001; Lukens and Doebley, 2001; Mathews et al., 2002; Bomblies and Doebley, 2005).

Even though many molecular results have supported the monophyly of the tribe the identity and relationships of its subtribes are not well understood. Most recent phylogenetic analyses suggested that the short branches along the backbone of the tree and the concentration of nucleotide changes on terminal branches in Andropogoneae clade were caused by a rapid evolutionary radiation near the base of the clade. Many of these studies suggested that better sampling of lineages within the tribe, or additional of phylogenetic characters (more genes) may help to resolve the relationships (Kellogg, 2000; Spangler, 2000; Mathews et al., 2002; Skendzic et al., 2007).

**Gene regions implemented in grass phylogenetic study**

Molecular data offer great potential to study the evolutionary relationships of plants and especially species rich groups where morphological analyses have provided insufficient resolution (Hodkinson and Parnell, 2007). The plastid (chloroplast) genome has been used in many evolution and phylogeny studies because it is a relatively abundant component of plant total DNA, thus allowing successful extraction and analysis. In addition, it has a conservative rate of nucleotide substitution and a relatively slow rate of plastid protein-coding gene mutation which has made chloroplast DNA useful tool for elucidating plant phylogenetic relationships at higher levels (e.g. Clegg and Zurawshi, 1992; Downie and Palmer, 1992; Soltis and Soltis, 1998). In this chapter, three regions of non-coding chloroplast DNA have been used: the *trnL* intron and the *tmF* intergenic spacer gene region (hereafter combined and called *trnL-trnF*) and the *atpB-riboL* intergenic gene region.

The *trnL-trnF* gene region contains two large non-coding components: the *trnL* (UAA) intron [between *trnL* (UAA) 5'exon and *trnL* (UAA) 3'exon] and the *trnL-F* intergenic spacer
[between trnL (UAA) 3' exon and trnF (GAA) exon] (Fig. 4.1). These non-coding regions are relatively small, with the trnL intron ranging from 350–600 base pairs (bp) and the trnL-trnF spacer ranging from 120 to 350 bp in the monocots and dicots (Soltis and Soltis, 1998). They are easily amplified and sequenced and also useful for phylogenetic studies at several taxonomic levels in Poaceae (Taberlet et al., 1991; Hodkinson et al., 2002a, 2002b). The non-coding intergenic region between the 3' ends of atpB and rbcL is approximately 900 bp in length (Fig. 4.2) (Manen et al., 1994; Soltis and Soltis, 1998), although the length may vary considerably due to insertions and deletions. Many studies have demonstrated that the atpB-rbcL region may be particularly useful for systematic studies at the genus and species level (Golenberg et al., 1993; Manen et al., 1994; Natali et al., 1995; Savolainen et al., 1995, 2000; Soltis and Soltis, 1998).

Apart from chloroplast DNA, the sequences from nuclear ribosomal DNA have been used to reconstruct phylogenies because it comprises of conserved regions (i.e. 18S and 26S genes) that can be used to infer phylogeny at higher taxonomic levels and also contains more rapidly evolving segments (i.e. ITS and IGS) that may be useful at the generic and specific levels (Soltis and Soltis, 1998). The internal transcribed spacer (ITS) region of 18S–26S nuclear ribosomal DNA contains three important components: 5.8S rDNA, an evolutionarily highly conserved sequence and two spacers: ITS-1 and ITS-2 (Baldwin et al., 1995; Soltis and Soltis, 1998) (Fig. 4.3). These two spacer segments of the ribosomal DNA transcript are not incorporated into mature ribosomes. Instead, ITS-1 and ITS-2 regions appear to function, at least in part, in the maturation of ribosomal DNA (see Baldwin et al., 1995). The ITS-1 and ITS-2 regions are highly repeated in the plant nuclear genome and the repeat unit can therefore become a mosaic of nucleotides from both parental types such that the original types are not easily distinguished (Wendel and Doyle, 1998; Soltis et al., 2008). The ITS region has proven useful for phylogenetic studies at the species and generic levels in many plant groups and within the grass family (Sun et al., 1994; Buckler and Holtsford, 1996; Hodkinson et al., 1997, 2000, 2002a, 2002b; Hsiao et al., 1999; Soltis et al., 2008).
Figure 3.1 A schematic diagram of the *trnL-F* regions. Arrows indicate orientation and approximate position of primer sites (adapted from Soltis and Soltis, 1998).

Figure 3.2 A schematic diagram of the *atpB-rbcL* regions. Arrows indicate orientation and approximate position of primer sites (adapted from Soltis and Soltis, 1998).

Figure 3.3 A schematic diagram of the *ITS* regions. Arrows indicate orientation and approximate position of primer sites (adapted from Baldwin *et al.*, 1995 and Sun *et al.*, 1994).
3.2 Aims

The broad aim of this chapter is to improve the phylogenetic understanding within the subfamily Panicoideae and among subfamilies in the PACCMA clade by increasing the sampling of taxa and by using plastid and nuclear DNA sequences separately and in combination. It also aims to apply the results to taxonomy by testing the infrasubfamilial classification proposed by several authors. More specifically, it aims to:

1) Resolve major grouping within Panicoideae and elucidate the phylogenetic placement of most tribes of Panicoideae sensu Clayton & Renvoize (1986) and assess the monophyly of existing taxa.
2) Revise the subtribal classification of Andropogoneae and to study how the subtribes relate to one another and other genera in the tribe.
3) Study molecular variation in different plastid gene regions (trnL-F, atpB-rbcL) and nuclear gene sequence regions (nr ITS) to assess their use in phylogenetic study. The atpB-rbcL spacer was used for the first time to study inter-relationships of taxa within this group of plants.

3.3 Materials and methods

Plant materials

The majority of the materials used in this study were collected during fieldwork in several regions of Thailand. Leaf tissue was dried with silica gel in order to rapidly desiccate the material and reduce DNA degradation before extraction (Chase and Hills 1991; Hodkinson et al., 2007b). Some samples were collected in the wild from Australia by Dr Trevor Hodkinson and Dr Surrey Jacobs (Royal Botanic Gardens, Sydney) using the alternative preservative solution of saturated CTAB to reduce degradative changes affecting the quality of DNA (Thomson, 2002). Three samples were taken from herbarium specimens and were successfully amplified and sequenced: Arundinella berteroniana (Schult.) Hitchc. & Chase, A. hispida (Humb. & Bonpl. ex Willd.) Kuntze, and Danthoniopsis dinteri (Pilg.) C.E. Hubb.

In total, 132 taxa from six subfamilies sensu Clayton and Renvoize (1986) were sampled. There are 115 taxa of subfamily Panicoideae, including sequences of tribes Andropogoneae (11 subtribes/42 genera), Paniceae (4 subtribes/18 genera), Arundinelleae (4 genera), Isachneae (2 genera) and Eriachneae (1 genus). In addition, 17 other taxa of subfamilies Centothecoideae, Arundinoideae and Chloridoideae were included in the analysis. All
classifications followed Clayton and Renvoize (1986). Outgroup selection was based on the results of GPWG (2001) and Bouchenak-Khelladi *et al.* (2008). For the *trnL-F* gene region, 13 sequences out of a total of 129 sequences were downloaded from GenBank (http://www.ncbi.nlm.nih.gov). For the *atpB-rbcL* gene region, 122 out of a total of 127 samples were sequenced by myself, five sequences were obtained from Trevor Hodkinson. Five grasses from Bambusoideae and Pooideae were chosen as outgroups. For the *ITS1-ITS2* region, 127 taxa were sampled, including 27 sequences from GenBank. All steps of DNA extraction, purification, amplification and cycle sequencing were carried out by the author at the molecular laboratory, Department of Botany, TCD. Voucher information is provided in Table 3.3.

**DNA extraction**

Total genomic DNA (tDNA) was extracted using a modified hot CTAB (hexadecyltrimethylammonium bromide) method (see Protocol 1, Appendix 3) (Doyle and Doyle, 1987; Hodkinson *et al.*, 2007b). The extractions used 0.2 g of dried leaf or 0.3 g of material obtained from herbarium specimens. The extract was precipitated using isopropanol and kept at -20°C overnight, or 2–3 weeks to increase precipitation in the case of herbarium specimens (Hodkinson, pers. comm.). Each of the crude total DNA samples was then washed and purified by ethanol (70% ethanol) washing (see Protocol 2, Appendix 3) and further purified by using JETQUICK Spin Columns (see Protocol 3, Appendix 3). Each of the clean DNA samples was then tested for quality and quantity using gel electrophoresis (see Protocol 4, Appendix 3). The DNA was then transferred into a 1.5 ml micro-centrifuge tube and stored at -20°C until used, or at -80°C for longer periods.

**DNA amplification and purification of PCR product**

The Polymerase Chain Reaction (PCR) was used to amplify two regions of chloroplast genome DNA, *trnL-trnF, atpB-rbcL* and one region of nuclear ribosomal DNA, *ITS*. All of the amplifications were carried out in an Applied Biosystems GeneAmp® PCR System 9700 thermal cycler (see Protocol 5, Appendix 3). The amplification of the target fragment began with an initial pre-melt at 94°C for 1 minute, followed by 29 cycles of denaturation at 95°C for 45 sec, annealing at 50°C for *trnL-F* or 52°C for *atpB-rbcL* and *ITS* for 45 sec, and extension at 72°C for 2 minutes. A final extension of 72°C for 7 minutes was also included. Sequences of all primers used are shown in Table 3.1. The quality and quantity of PCR products were checked by gel electrophoresis (see Protocol 4, Appendix 3) and then purified.
using JETQUICK Spin Columns and stored in a -20°C freezer prior to sequencing (see Protocol 6, Appendix 3). Each of the amplified double-stranded fragments was purified by the procedure in Protocol 3 to remove the PCR reagents, especially the primers, but in this case using sterile ultra pure water as the elution buffer (see Protocol 6, Appendix 3).

**Cycle sequencing and purification**

Cycle sequence reactions were carried out on the amplified PCR products, using BigDye Terminator v. 1.1 cycle-sequencing kits (Applied Biosystems) and an Applied Biosystems GeneAmp ® PCR System 9700 thermal cycler. The sequencing reaction consisted of 1 to 4 µl of purified PCR product, 1 µl of Big Dye Cycle Sequencing Mix, 1.8 µl of sequencing buffer, 0.7 µl of primer (at 5 ng µl⁻¹) and sterile ultra pure water (see Protocol 7, Appendix 3). The cycle sequencing products were purified using an ethanol precipitation technique (see Protocol 8, Appendix 3) to remove unincorporated dye-labelled terminators and other unwanted reaction components and then left to dry at room temperature overnight to remove any traces of ethanol and sodium acetate.

**DNA sequencing**

The dried cycle sequencing products were resuspended using 25 µl Hi-Di™ Formamide and denatured prior to loading on the ABI Prism™ 310 Genetic Analyzer machine (Applied Biosystems) using POP6 polymer (Applied Biosystems). Samples were analysed using the run module SEQ POP6 RAPID (1.0-ml) E (see Protocol 9, Appendix 3). Forward and reverse strands were sequenced in separate reactions. The raw sequence data were analyzed using ABI Prism™ DNA Sequencing Analysis Software, version 3.4.1., and assembled using Auto Assembler Software, version 2.1 (Applied Biosystems).

**Data analysis**

The obtained sequence chromatograms of *tmL*-F and *atpB-rbcL* regions were checked and aligned by inserting gaps manually using Se-Al v. 2.0a11 (Rambaut, 1996) following the guidelines of Kelchner (2000). Sequences from *ITS* region were aligned with CLUSTAL X (Thompson et al., 1997) and then manually edited in Se-Al v. 2.0a11. The obtained sequences were imported into PAUP* 4.0b10 (Swofford, 2002). Gaps smaller than 10 bp were coded as missing data, unless they were caused by regions where there was an obvious tandemly arranged duplication in one sequence that was clearly due to a single mutation (a duplication).
Such duplications were only given a weight of one by including only the first base of the duplication instead of the entire duplication in the subsequent phylogenetic analyses. Gaps larger than 10 bp were excluded from analyses. Two combined datasets were analysed. These were the combined matrix of chloroplast DNA gene regions and the combined matrix of nuclear and chloroplast DNA gene regions. For the 129 taxa combined chloroplast matrix, two key taxa for \textit{atpB-rbcL}, \textit{Arundinella birta} (Thunb.) Tanaka and \textit{Tristachya leucothrix} Trin. ex Nees, were included even though they were lacking one respective DNA region. For the combined nuclear and chloroplast matrix, 124 taxa were presented, that had two missing sequences for \textit{atpB-rbcL}.

All data were then subjected to maximum parsimony (MP) using PAUP* 4.0b10 (Swofford, 2002) to provide phylogenetic trees. Maximum parsimony analyses of the final matrix were performed using the heuristic search algorithms of PAUP with 1,000 replicates of random addition sequence (saving no more than 100 trees per replicate to reduce time spent swapping large islands of tree) and with tree bisection reconnection (TBR) branch swapping on multiple trees and maxtrees set to automatically increase by 100 (Salamin \textit{et al.}, 2003). Clade support was evaluated using 1,000 bootstrap replicates of a heuristic search (Felsenstein, 1985) with simple addition sequence and TBR branch swapping.

\textbf{Table 3.1} Gene regions and primers used for this study.

<table>
<thead>
<tr>
<th>Target region</th>
<th>Primer base sequence (\text{F-forward}; \text{R-reverse})</th>
<th>Reference</th>
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<tbody>
<tr>
<td>\textit{trnL}-F</td>
<td>F: \text{GC}: 5'-CGAAATCGGTAGACGCTACG-3'\text{'}&lt;br&gt; R: \text{F}: 5'-ATTGAACTGGTGACACGAG-3'\text{'}</td>
<td>Taberlet \textit{et al.} (1991)</td>
</tr>
<tr>
<td>\textit{atpB-rbcL}</td>
<td>F: 2\text{R}: S'-GAAGTATAGTAGATTCTTC-3'\text{'}&lt;br&gt; R: 1\text{R}: S'-GTTTCTGTTTGTGGTGACAT-3'\text{'}</td>
<td>Samuel \textit{et al.} (1997)</td>
</tr>
<tr>
<td>\textit{ITS-1} and \textit{ITS-2}</td>
<td>F: \text{AB101}(S'-CGAAATTTCATGGTGCCGTAGTGTTCTC-3'\text{'}&lt;br&gt; R: \text{AB102}(S'-AGAATTTCGTTTGCTCGCCGTT-3'\text{'})</td>
<td>Sun \textit{et al.} (1994)</td>
</tr>
</tbody>
</table>
3.4 Results

3.3.1 The *trnL*-F dataset

The total length of the *trnL* intron and the *trnL*-*trnF* intergenic spacer were confirmed using a comparative alignment of *Miscanthus nepalensis* (Trin.) Hack. (Panicoideae, Poaceae), obtained from GenBank and NCBI website (AY116252; Hodkinson et al., 2002b). The final aligned matrix was 1,546 bp long. Eight hundred and eight characters were excluded and of the remaining 738 included characters, 412 characters were constant, 124 were variable but parsimony-uninformative and 202 characters were parsimony-informative.

Parsimony analysis of the matrix generated 95,900 equally most parsimonious trees of 725 steps with a consistency index (CI) of 0.639 and a retention index (RI) of 0.807. 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 3.4 and as a cladogram, with bootstrap values and strict consensus information, in Figure 3.5.

The resolution of phylogenetic analysis of *trnL*-F data was low, in general terms. The PACCMAD clade, including subfamilies Panicoideae sensu GPWG (2001), Arundinoideae sensu stricto, Chloridoideae, Centothecoideae sensu GPWG (2001), Micrairoideae sensu Sánchez-Ken et al. (2007), Aristidoideae and Danthonioideae, received low support (70% BS). Within this clade, a monophyletic Chloridoideae (81% BS) was sister to *Arundo. Danthonia* was sister to a strongly supported clade (100% BS) consisting of Isachneae plus *Eriachne. Aristida, Lophatherum* and the rest of Centothecoideae plus *Tristachya* were successively sister groups to Panicoideae but there was no support from the bootstrap analysis for these sister group relationships. Tribe Isachneae were not supported as monophyletic because a monophyletic *Isachne* (100% BS) was sister to a monophyletic *Eriachne* (92% BS) with high support (96% BS) but *Coelachne* (also classified in Isachneae) was sister to this *Isachne + Eriachne* group.

Subfamily Panicoideae sensu GPWG (2001) (including *Danthoniopsis*, excluding *Tristachya*) were monophyletic with low support (52% BS). Within Panicoideae, Paniceae were found to be paraphyletic. *Paspalum, Cenchrus* and *Panicum auritum* were grouped together with 57% BS. *Walwhalleya* was sister to *Homopholis* with 66% BS. *Pennisetum* and *Sassiolepis* were found to be monophyletic with 82% and 99% BS, respectively.
A group consisting of Andropogoneae plus *Danthoniopsis* was monophyletic (71% BS) and grouped in a trichotomy with a *Gamotia* clade (95% BS) and an *Arundinella* clade (99% BS), with moderate support (76% BS). However, the relationships between the clades were unclear. Within Andropogoneae, a few phylogenetic relationships were resolved at the terminal branches, though with low support for most clades, except for *Apoopis, Arthraxon, Hemarthria* and Chionachninae which were strongly supported as monophyletic (92%, 98%, 99% and 86% BS, respectively). Subtribe Germainiinae were not supported as monophyletic because *Eremochloa* was united with *Germainia capitata* (63% BS). However, this clade and the rest of Germainiinae formed a moderately supported group with 79% BS. Subtribe Chionachninae, according to Clayton and Renvoize (1986), was the only formally recognised subtribes to be supported by this analysis. The other subtribes were either not resolved or not supported by bootstrapping.
Figure 3.4 One of 95,900 equally most parsimonious trees shown as a phylogram obtained from comparative sequence analysis of the tmL-F sequence data. Values above branches represent the number of steps supporting each branch.
Figure 3.5 Same tree as Figure 3.4 (\(mL-F\)), shown as a cladogram. Values above branches represent the number of steps supporting each branch. Values below branches represent the bootstrap support above 50%. Arrow heads represent nodes not found in strict consensus. The PACCMAD clade, the subfamilial and the tribal classifications (the column on far right) are according to GPWG (2001) and Clayton and Renvoize (1986), respectively.
3.3.2 The *atpB-rbcL* dataset

The total length of the *atpB-rbcL* intergenic region was confirmed using a comparative alignment of *Paspalum dilatatum* Poir. (Panicoideae, Poaceae), obtained from GenBank and the NCBI website (DQ104279). The final aligned matrix was 1,123 bp long. Four hundred and twenty characters were excluded and of the remaining 703 included characters, 380 characters were constant, 122 were available but parsimony-uninformative and 201 characters were parsimony-informative.

Analysis of the matrix generated 1,300 equally most parsimonious trees of 779 steps with a consistency index (CI) of 0.576 and a retention index (RI) of 0.774. One of the equally most parsimonious trees is shown as a phylogram in Figure 3.6 and as a cladogram, with bootstrap values and strict consensus information in Figure 3.8.

The phylogenetic relationships of the *atpB-rbcL* sequences were resolved only near the terminal branches of the tree. The spine of the tree was largely unresolved. The monophyly of the PACCMAD clade and Chloridoideae were resolved with 84% and 100% BS, respectively. The data also showed that Isachneae were monophyletic (79% BS) and sister to an *Eriachne* clade (99% BS) with high support (94% BS). This clade was sister to *Arundo* in strict consensus tree but was not supported by bootstrapping. There was no bootstrap support for the relationships between the subfamilies.

Panicoideae sensu GPWG (2001) (excluding *Danthoniopsis*) were monophyletic in the strict consensus tree but were not supported by bootstrapping. Within Panicoideae, *Arundinella* and Paniceae were successively sister to Andropogoneae plus *Garnotia* in the strict consensus tree but these relationships were not supported by bootstrapping. Paniceae were monophyletic in the strict consensus tree and were divided into two groups: a *Paspalum, Cenchrus, Panicum auritum* and *Ichnanthus* clade (57% BS) and a clade consisting of the rest of Paniceae (53% BS). Tribe Arundinellae were not supported as monophyletic because *Danthoniopsis* was grouped with Centothecoidae in the strict consensus tree, while the rest of Arundinellae were grouped within Panicoideae. *Arundinella* was monophyletic in the strict consensus tree and most of the species were supported as monophyletic in the bootstrap analysis (only *A. berteroniana* was not supported by bootstrapping as being part of this group). However, *Garnotia* was monophyletic with 100% BS.

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None of the subtribes of Clayton and Renvoize (1986) from either Andropogoneae or Paniceae were resolved as monophyletic except Chionachnininae, Dimeriinae and Tripsacinae which were monophyletic with 99%, 69% and 74% BS, respectively. The results from \textit{atpB-rbcL} tree also supported the grouping of subtribe Germainiinae with \textit{Eremochloa} (66% BS). \textit{Capillipedium} grouped with \textit{Bothriochloa} and \textit{Dichanthium} (86% BS).
Figure 3.6 One of 1,300 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.

Tree length: 779
CI: 0.576
RI: 0.774
Figure 3.7 Same tree as Figure 3.6 (atpB-rbcL), shown as a cladogram. Values above branches represent the number of steps supporting each branch. Values below branches represent the bootstrap support above 50%. Arrow heads represent nodes not found in the strict consensus. The PACCMAD clade, the subfamilial and the tribal classifications (the column on far right) are according to GPWG (2001) and Clayton and Renvoize (1986), respectively.
3.3.3 The combined chloroplast \((trnL-F\) and \(atpB-rbcL\)) dataset

The matrix used for the combined chloroplast DNA analysis was obtained from \(trnL-trnF\) and \(atpB-rbcL\) sequences. The aligned matrix was 2,669 bp long. One thousand two hundred and twenty eight characters were excluded and of the remaining 1,441 included characters, 797 characters were constant, 246 were variable but parsimony-uninformative and 398 characters were parsimony-informative.

Parsimony analysis of the matrix generated 13,100 equally most parsimonious trees of 1,532 steps with a consistency index (CI) of 0.589 and a retention index (RI) of 0.776. One of the equally most parsimonious trees is shown as a phylogram in Figure 3.8 and as a cladogram, with bootstrap values and strict consensus information, in Figure 3.9.

The monophyly of the PACCMAD clade and Chloridoideae was highly supported (97% and 100% BS, respectively). Within the PACCMAD clade, a number of relationships were resolved with varying degrees of bootstrap support. \(Eriachne\) was monophyletic (99% BS) and sister to a monophyletic \(Isachne\) (100% BS), with 77% BS. This clade \((Eriachne + Isachne)\) was sister to the genus \(Coelachne\) with high support (100% BS).

Subfamily Panicoideae sensu GPWG (2001) (including \(Danthoniopsis\), but excluding \(Tristachya\)) were monophyletic (85% BS) and grouped with a polyphyletic Centothecoideae sensu GPWG (2001) plus \(Tristachya\) with low support (61% BS). Within Panicoideae, Paniceae were not retrieved in the strict consensus tree and did not receive bootstrap support (>50%). However, there was no evidence against its monophyly. \(Pennisetum\) was found to be monophyletic (88% BS) and had \(Setaria\) with \(Spinifex\) (71% BS) and \(Alexfloydia\) as its successive sister groups with 67% and 99% BS, respectively. \(Saccolepis\) was also found to be monophyletic with 100% BS and was sister to \(Panicum notatum\) with 72% BS. The clade consisting of \(Pastpalum, Cenchrus\) and \(Panicum auritum\) were grouped together with 95% BS. \(Walwhalleya\) and \(Homopholis\) were grouped together with 68% BS. \(Alloteropsis\) was found to group with \(Ottochloa\) with 64% BS. A sister relationship between \(Cyrtococcum\) and \(Pseudoechinolaena\) (86% BS) was also found.

Andropogoneae were found to be monophyletic (62% BS) and sister to \(Danthoniopsis\) in the strict consensus tree. This clade was grouped together with a monophyletic \(Arundinella\) (99% BS) and a monophyletic \(Garnotia\) (100% BS) with low support (69% BS) but their interrelationships are not resolved in the strict consensus. Within Andropogoneae, subtribes Chionachiniae, Dimeriniae and Tripsacinae, according to Clayton and Renvoize (1986), were
the only formally recognised subtribes to be supported by this analysis with 100%, 88% and 87% BS, respectively. The other subtribes were not resolved in either the strict consensus tree or by bootstrapping. However, the clade containing subtribe Germainiinae and Eremochloa was monophyletic with high support (89% BS). Pogonatherum and Imperata were successively sister taxa to this group with 83% and 58% BS, respectively. The analysis also supported the monophyly of Arthroxon with high support (100% BS). Schizachyrium was also monophyletic (67% BS). A monophyletic Hyparrhenia (56% BS) was united with Andropogon gerardii (50% BS). The clade with Capillipedium plus Bothriochloa plus Dichanthium was also resolved with moderate support (84% BS). Hemarthria was also found to be monophyletic (98% BS) and united with Hackelochloa (72% BS).
Figure 3.8 One of 13,100 equally most parsimonious trees shown as a phylogram obtained from comparative sequence analysis of combined chloroplast DNA sequences. Values above branches represent the number of steps supporting each branch.
Figure 3.9 Same tree as Figure 3.8 (combined chloroplast data), shown as a cladogram. Values above branches represent the number of steps supporting each branch. Values below branches represent the bootstrap support above 50%. Arrow heads represent nodes not found in the strict consensus. The PACCMAD clade, the subfamilial and the tribal classifications (the column on far right) are according to GPWG (2001) and Clayton and Renvoize (1986), respectively.
3.3.4 The ITS dataset

The total length of the ITS region, were confirmed using a comparative alignment of *Miscanthus nepalensis* (Trin.) Hack., obtained from GenBank and the NCBI website (AY116292; Hodkinson et al., 2002b). The aligned matrix was 678 bp long. One hundred and eighty one characters were excluded and of the remaining 497 included characters, 154 characters were constant, 58 were variable but parsimony-uninformative and 285 characters were parsimony-informative. Analysis of the matrix generated 3,100 equally most parsimonious trees of 2,792 steps with a consistency index (CI) of 0.252 and a retention index (RI) of 0.575. One of the equally most parsimonious trees is shown as a phylogram in Figure 3.10 and as a cladogram, with bootstrap values and strict consensus information, in Figure 3.11.

Similar to the results obtained from chloroplast DNA gene regions, the monophyly of PACCMAD clade was resolved with high support (85% BS). Within the PACCMAD clade, an *Eriachne* clade (100% BS) was sister to an Isachneae clade (62% BS) with 79% BS, but there was no bootstrap support for the sister relationship between this clade and *Micraira*. In the strict consensus tree, Chloridoideae (97% BS) were united within Paniceae with *Alloteropsis*, but this position was not supported by bootstrapping. Two taxa of Centothecoideae were placed but not supported as the most outlying lineage of the PACCMAD clade. No support values from the bootstrap analysis for the relationships between the subfamilies within the PACCMAD clade were found.

Subfamily Panicoideae sensu GPWG (2001) (excluding *Danthoniopsis* and *Tristachya*) were monophyletic (53% BS). Within Panicoideae, a monophyletic Chloridoideae was sister to genus *Alloteropsis* within the Paniceae clade in the strict consensus. In the same tree, Paniceae plus Chloridoideae clade formed a sister clade to the rest of Panicoideae. However, no bootstrap values were found for these relationships. In addition, three well supported clades were formed within Paniceae: a *Paspalum, Cenchrus, Panicum auritum* and *Ichnanthus* clade (94% BS); an *Aelclaydia, Spinifex, Setaria* plus paraphyletic *Pennisetum* clade (81% BS), and a *Sacciolepis* clade (100% BS). Andropogoneae were grouped with *Garnotia* and *Arundinella* with high support (89% BS). *Arundinella* was monophyletic (99% BS) and sister to the clade consisting of *Arthroacum* and *Thelepolon* in the strict consensus tree, while *Garnotia* was paraphyletic and grouped with subtribe Tripsacinae in strict consensus tree. *Hyparrhenia* was monophyletic (63% BS) but there was no bootstrap support for its relationship with *Andropogon gerardii*. *Hemarthria* was also found to be monophyletic (74% BS) and united with *Hackelochloa* but only found in the strict consensus tree. No bootstrap support for the relationships between
Bothriochloa plus Capillipedium plus Dichanthium was retrieved, but the latter two taxa were grouped together with low support (58% BS). None of subtribes of Paniceae and Andropogoneae, according to Clayton and Renvoize (1986), were resolved as monophyletic, except subtribes Coicinae, Dimeriinae, Germainiinae and Tripsacinae (90%, 100%, 89% and 100% BS, respectively).
Figure 3.10 One of 3,100 equally most parsimonious trees shown as a phylogram obtained from comparative sequence analysis of the ITS DNA sequences. Values above branches represent the number of steps supporting each branch.
Figure 3.11 Same tree as Figure 3.10 (ITS), shown as a cladogram. Values above branches represent the number of steps supporting each branch. Values below branches represent the bootstrap support above 50%. Arrow heads represent nodes not found in the strict consensus. The PACCMAD clade, the subfamilial and the tribal classifications (the column on far right) are according to GPWG (2001) and Clayton and Renvoize (1986), respectively.
3.3.5 The combined *trnL-F, atpB-rbcL* and *ITS* dataset

The matrix used for the combined analysis was obtained from *trnL-trnF, atpB-rbcL* and *ITS* sequences. The final aligned matrix was 3,348 bp long. One thousand four hundred and nine characters were excluded and of the remaining 1,939 included characters, 969 characters were constant, 288 were variable but parsimony-uninformative and 682 characters were parsimony-informative. Analysis of the matrix generated 687 equally most parsimonious trees of 4,330 steps with a consistency index (CI) of 0.365 and a retention index (RI) of 0.626. One of the equally most parsimonious trees is shown as a phylogram in Figure 3.12 and as a cladogram, with bootstrap values and strict consensus information, in Figure 3.13.

The PACCMAD clade was highly supported (99% BS). Aristidoideae were sister to Chloridoideae (81% BS) with strong support (100% BS). This clade was sister to a strongly supported clade (100% BS) containing a monophyletic Eriachneae (100% BS) and a paraphyletic Isachneae, in strict consensus tree. The result also supported *Danthonia* as sister to *Arundo* but no bootstrap support was found for this relationship.

Centothecoideae were not monophyletic in the combined analysis due to the exclusion of *Chasmanthium*. However, support for the monophyly of a *Centotheca* plus *Thysanolaena* clade was very high (99% BS). This centothecid clade was sister to a lineage, consisting of Panicoideae plus Arundinelleae, in the strict consensus tree. Arundinelleae was polyphyletic due to *Arundinella* and *Garotaia* that were united within Panicoideae (69% BS), while *Danthoniopsis* and *Tristachya* were grouped together with low support (55% BS) and placed outside of these as a sister group to the rest of Panicoideae with 52% BS.

Within Panicoideae, Paniceae were not supported as monophyletic or retrieved in the strict consensus tree. None of the subtribes of Paniceae were found to be monophyletic. The combined nuclear and chloroplast tree supported two genera of Paniceae as monophyletic: *Pennisetum* (74% BS) and *Sacciolepis* (100% BS). The *Pennisetum* clade was sister to *Setaria* (51% BS). This clade was sister to the clade consisting of *Alexfloydia* and *Spinifex* (90% BS) with high support (100% BS). *Cyrtococcum* was sister to *Pseudoechinolaena* (52% BS). This clade was united with *Acroceras* (57% BS). The clades consisting of *Paspalum, Cenchrus, Panicum auritum* and *Ichnanthus* was also resolved with high support (100% BS).

Andropogoneae were grouped (99% BS) with the *Garotaia* clade (100% BS) and *Arundinella* clade (100% BS) but no bootstrap support values were found for their inter-relationships.
Within Andropogoneae, five subtribes: Chionachninae, Coicinae, Dimeriinae, Germainiinae and Tripsacinae (according to Clayton and Renvoize, 1986), were monophyletic with 74%, 92%, 100%, 99%, 100% BS, respectively. The relationships between the monophyletic subtribes and the rest of Andropogoneae were unclear, except subtribe Dimeriinae which had *Ischaemum indicum* and *Ischaemum muticum* as its successively sister taxa with 54% and 66% BS, respectively. Three out of a total of seven taxa from subtribe Rottboelliiinae (*Hemarthria longiflora*, *Hemarthria partensis* and *Hackelochloa*) were grouped with 69% BS. *Hemarthria* was monophyletic (100% BS) and was sister to *Hackelochloa* with 69% BS. The monophyly of *Arthroxon* was resolved (100% BS) and was united with *Theleptagon* with 50% BS. The monophyletic *Hyparrhenia* (88% BS) was sister to *Andropogon gerardii* with 67% BS. This clade was grouped together with *Cymbopogon*, *Schizachyrium* and *Andropogon ascinodis* (76% BS). The clade consisting of *Capillipedium*, *Bothriochloa* and *Dichanthium* was resolved (91% BS). The analysis also resolved the monophyly of *Themeda* with 53% BS.
Figure 3.12 One of 687 equally most parsimonious trees shown as a phylogram obtained from comparative sequence analysis of the combined chloroplast and nuclear sequence data. Values above branches represent the number of steps supporting each branch.
Tree length: 4330
Cl: 0.365
RI: 0.626

**Figure 3.13** Same tree as Figure 3.12 (the combined chloroplast and nuclear sequence data), shown as a cladogram. Values above branches represent the number of steps supporting each branch. Values below branches represent the bootstrap support above 50%. Arrow heads represent nodes not found in the strict consensus. The PACC MAD clade, the subfamilial and the tribal classifications (the column on far right) are according to GPWG (2001) and Clayton and Renvoize (1986), respectively.
4.4 Discussion

PACCMAD clade

The sample of species presented in this study relied greatly on the Old World grasses especially the panicoid group. Additional taxa of other subfamilies within the PACCMAD clade from the New World and Australasia were also included. Five out of a total of seven tribes of Panicoideae sensu Clayton and Renvoize (1986) were sampled. In total, 67 genera of Panicoideae, including 42 genera in 11 subtribes of Andropogoneae, 4 genera of Arundinelleae, 1 genus of Eriachneae, 2 genera of Isachneae, and 18 genera in 4 subtribes of Paniceae, were analysed. The sample of panicoid species presented here is considerably larger than any molecular study to date. However, representatives of the two small tribes, Steyermarkochloaeae and Hubbardieae, were not included here due to the lack of material suitable for DNA extraction.

It is clear that the PACCMAD clade, including Panicoideae sensu stricto, Arundinoideae, Centothecoideae sensu GPWG (2001), Chloridoideae, Micrarioideae sensu Sánchez-Ken et al. (2007), Aristidoideae and Danthonioideae, is monophyletic. The support values for this clade were always high (84% BS in \textit{atpB-rbcL}; 85% BS in \textit{ITS}; 97% BS in combined chloroplast and 99% BS in combined nuclear and chloroplast), except the result from the \textit{trnL-F} analysis in which the support was lower (70% BS) (Table 3.3). The PACCMAD clade is robustly supported based on molecular data by previous studies (e.g. Davis and Soreng, 1993; Baker et al., 1995, 1999; Clark et al., 1995; Hilu et al., 1999; Hsiao et al., 1999; Mathews et al., 2000; Giussani et al., 2001; GPWG, 2001; Sánchez-Ken et al., 2007). Not all of these studies included representatives of each of the PACCMAD subfamilies, but in combination they consistently grouped these taxa together. All members of this clade also have several apparently synapomorphic morphological or anatomical characters, such as the presence of an elongated mesocotyl internode and the loss of the epiblast (GPWG, 2001).

Within the PACCMAD clade, the relationships among the six subfamilies were largely unresolved. There is evidence supporting the sister group relationship between Aristidoideae (\textit{Aristida}) and Chloridoideae (\textit{Tripogon, Microchloa} and \textit{Sporobolus}). This sister group relationship was found in the combined nuclear and chloroplast analysis with high support (100% BS) (Figure 3.12). This finding is consistent with the study of GPWG (2001), but congruent with Bouchenak-Khelladi et al. (2008) and Pirie et al. (2008) which suggested that Danthonioideae were the most closely related subfamily to Chloridoideae. It is noteworthy that in the \textit{ITS}
analysis, Chloridoideae were united within Paniceae as sister to *Alloteropsis*, but this position was not supported by bootstrapping. However, Chloridoideae are on a very long branch which can lead to erroneous phylogenetic trees because of long-branch attraction or the “Felsenstein zone” where the two long branched unrelated taxa grouped together rather than with their sister group (Felsenstein, 1978; Huelsenbeck and Hillis, 1993; Anderson and Swofford, 2004; Soltis et al., 2004; Bergsten, 2005). This occurs because the numerous convergent changes along the two long branches are interpreted as false synapomorphies (Philippe et al., 2005).

Four representatives of subfamily Centothecoideae (*Centotheca, Chasmanthium, Lophatherum* and *Thysanolaena*) did not form a monophyletic group. Only *Centotheca* and *Thysanolaena* are grouped together with high support in both combined analyses. This relationship was also found in the study of Bouchenak-Khelladi et al. (2008). In addition, this clade was placed as the sister to the clade consisting of Panicoideae + Arundinoideae in the combined nuclear and chloroplast analysis with no bootstrap support (Figure 3.13). The members of Centothecoideae were grouped with the monophyletic Panicoideae in all chloroplast analyses. This result is consistent with previous studies (Sánchez-Ken and Clark, 2001; Sánchez-Ken and Clarke, 2007; Bouchenak-Khelladi et al., 2008). However, this relationship was poorly resolved (< 50% BS in single chloroplast analyses and 61% BS in combined chloroplast analysis) and no support was found for other relationships within the clade.

**Micrairoideae**

Micrairoideae were first established as a monogeneric subfamily by Pilger (1956). Later, Micrairoideae were transferred to subfamily Arundinoideae as tribe Micraireae (Clayton and Renvoize, 1986; Watson and Dallwitz, 1992). Micrairoideae were reinstated by Sánchez-Ken et al. (2007) using evidence from two chloroplast gene regions and morphological data. They revealed that *Micraira, Eriachne* and *Isachne* were grouped together with high support but failed to resolve the relationships among the subfamilies. Their strict consensus tree also showed that Micrairoideae was sister to Arundinoideae (Figure 2; Sánchez-Ken et al., 2007). In addition, the result from the Bayesian analysis by Duvall et al. (2007) placed Micrairoideae as sister to Arundinoideae with high support. Surprisingly, the result of the large multi-gene tree by Bouchenak-Khelladi et al. (2008) supported only *Eriachne* and *Micraira* in the Micrairoideae clade. This clade was sister to a chloridoid + danthonioid, aristidoid group but the support was low in their Bayesian tree and no support was found in their bootstrap analysis.
On the basis of morphology, these three tribes were placed together in subtribe Milieae by Bentham (1878). Later, he transferred all genera to be under tribe Isachneae (Bentham, 1882). After that, Coelachne, Eriachne and Micraira were placed in tribe Aveneae, while Isachne was placed in tribe Paniceae (Bentham and Hooker, 1883). Pilger (1956) established subfamily Micrairoideae including only Micraira based mainly on the unique character of the spirally-arranged leaves. He also grouped Coelachne together with Isachne in tribe Isachneae under subfamily Panicoideae. This classification was adopted by Clayton and Renvoize (1986), except that they placed Micraireae in Arundinoideae. The genus Eriachne has been placed in many tribes e.g. Danthonieae (Bor, 1960; Gilliland, 1980), Aristideae (Brown, 1977) and Eriachneae (Clayton and Renvoize, 1986). Eriachneae were also placed within subfamily Panicoideae due to their two-flowered spikelets. Simultaneously, Micraira was isolated as monotypic tribe Micraireae and was placed in Arundinoideae because of its spirally-arranged leaves and arundinoid leaf-blade anatomy (Clayton and Renvoize, 1986). According to Sánchez-Ken et al. (2007), all members of Micrairoideae have indurated lemmas with a germination flap. However, these characters are homoplastic because they are also shared by the other panicoid grasses; thus no morphological synapomorphy has been found for this group.

In this study, the monophyly of Micrairoideae, with Eriachneae, Isachneae and Micraireae, is not well supported (< 50% BS) in the ITS analysis (Figure 3.1). Micraira was discarded for the combined (all regions) analysis because the DNA data of Micraira was only available for ITS (GenBank, AF 019859). However, Micrairoideae, with Eriachneae and Isachneae, is monophyletic with high support in all analyses (100% BS in trnL-F, 94% BS in atpB-rbcL, 79% BS in ITS; 100% BS in combined chloroplast; 100% BS in combined nuclear and chloroplast). Due to the lack of chloroplast DNA of Micraira, it is difficult to study the relationships within subfamily Micrairoideae. However, we can deduce some patterns from the single gene analyses. A strict consensus tree of these would reveal ((Eriachne + (Isachne + Coelachne)) + Micraira). The position of Micrairoideae within the PACCMAD clade is not clear in this study because the sister group relationship between Micrairoideae and chloridoid plus aristidoid clade is found only in the strict consensus tree.

Eriachne, tribe Eriachneae, was consistently resolved and supported as monophyletic. All single gene analyses and the combined data supported its monophyly (92% BS in trnL-F; 99% BS in atpB-rbcL; 100% BS in ITS; 99% BS in combined chloroplast; 100% BS in combined nuclear and chloroplast) (Table 3.2). This confirms the monophyly of Eriachne as found in the previous study by Sánchez-Ken et al. (2007). Morphologically, Clayton and Renvoize (1986)
noted that Eriachneae, consisting of *Eriachne* and *Pheidochloa*, closely resembles Isachneae, in the number of fertile florets and in the induration of lemmas with inrolled margins, but differs in having awned lemmas and Kranz anatomy.

Tribe Isachneae, represented by *Coelachne* and *Isachne*, are monophyletic in ITS and atpB-rbcL analyses in which *Coelachne* is sister to an *Isachne* clade with low to moderate support (62% BS, 79% BS, respectively) (Table 3.2; Figures 3.7 and 3.11). However, most analyses (trnL-F and two combined analyses) showed that Isachneae are non-monophyletic due to the exclusion of *Coelachne*. *Isachne* was monophyletic with high support in all analyses (100% BS) and was sister to *Eriachne* in the combined nuclear and chloroplast analysis (no bootstrap) (Figure 3.13). Morphologically, Isachneae, including *Coelachne*, *Heteranthoecia*, *Isachne* *Limnopoa* and *Sphaerocaryum*, are characterised by having two fertile disarticulating florets and by their non-Kranz anatomy. Clayton and Renvoize (1986) suggested that Isachneae were most likely to be derived from *Panicum* based upon close morphological similarities of spikelets between *Isachne* and *Panicum* sect. *Verruculosa*. However, these similarities were found to be homoplasies by Sánchez-Ken et al. (2007).

**Panicoideae**

Panicoideae sensu stricto (including *Arundinella* and *Garnotia*, but excluding Isachneae and Eriachneae) were supported as monophyletic in all analyses, except the atpB-rbcL analysis in which this relationship did not receive bootstrap support (> 50% BS). This clade was sister to a *Danthoniopsis* + *Tristachya* clade in the combined nuclear and chloroplast analysis. However, the support for this relationship was low (52% BS) (Figure 3.13). Within Panicoideae, only the clade consisting of tribe Andropogoneae plus *Arundinella* and *Garnotia* is highly supported (99% BS). Paniceae were separated into two main groups in the strict consensus tree but these groupings were not supported by bootstrapping (Figure 3.13). This finding is inconsistent with previous studies in which Panicoideae were made up of three major clades (e.g. Gomez-Martinez and Culham, 2000; Giussani et al., 2001; Aliscioni et al., 2003).

For this study, no DNA samples from two small tribes (Hubbardieae and Steyermarkochloeae) were available. The monotypic tribe Hubbardieae is an endemic taxon and was only known from Gersoppa Falls, Karnataka, Southwestern India. *Hubbardia* was reported as probably extinct after dam construction on the Sharavati River (Clayton and Renvoize, 1986; Renvoize and Clayton, 1992). However, it was rediscovered again in Tillari Ghat, Maharastra (Potdar et al., 2002). Based on morphological data, *Hubbardia*, which was
distinguished by the absence of paleas, is apparently derived from Isachneae (Clayton and Renvoize, 1986) sharing similarities in spikelet structure, the disarticulation of the florets above the glumes and the C₃ photosynthetic pathway.

The tribe Steyermarkochloeae was established by Davidse and Ellis (1984) with unique characters such as dimorphic culms and leaves, a solitary developed leaf with a cylindrical sheath lacking a ligule, and polygamous breeding system. It comprises two extraordinary genera, *Steyermarkochloa* and *Arundoclaytonia* which are found in isolated localities in the savannas and white sand caatingas of the northern and south-central Amazon region (Renvoize and Clayton, 1992). The phylogenetic implications obtained from leaf blade anatomy suggested that *Steyermarkochloa* was close to an arundinoid grass such as *Gynerium* (now Panicoideae + Centothecoideae clade; Sánchez-Ken and Clark, 2001), *Arundo*, *Phragmites* and *Thysanolaena* (now Centothecoideae; GPWG, 2001) (Davidse and Ellis, 1984), but *Steyermarkochloa* is morphologically distinct in almost all characters of the leaves, inflorescences, spikelets and flowers. Steyermarkochloeae were grouped in Panicoideae as controversial with the acceptance of arundinoid leaf anatomy type because its spiciform panicle and two-flowered spikelets (which fall entirely) are more in accord with the Paniceae, especially with *Hymenachne* (Clayton and Renvoize, 1986; Renvoize and Clayton, 1992). Although *Arundoclaytonia* shows no anatomical resemblance with the panicoid grasses, it was included in Steyermarkochloeae on the basis of spikelet similarity (Davidse and Ellis, 1987). Recent phylogenetic study showed that *Arundoclaytonia* was grouped outside the Panicoideae clade. It appeared as sister to the PACCAD clade (Sánchez-Ken and Clark, 2007).

**Paniceae**

None of the analyses can resolve or support the monophyly of tribe Paniceae or the relationships within the tribe. On the basis of molecular data, Gomez-Martinez and Culham (2000), Giussani et al. (2001) and Aliscioni et al. (2003) divided tribe Paniceae into two well-resolved clades corresponding to basic chromosome number. One clade representing the taxa with $x = 9$ taxa, whereas another clade comprised the taxa with $x = 10$. They also found that the clade corresponding to a basic chromosome number $x = 10$, was weakly supported as sister group to Andropogoneae. However, this relationship was not retrieved in this study because two taxa of $x = 10$ Paniceae (*Ichnanthus*, Pohl and Davise, 1971 and *Paspalum notatum*, Burton, 1942) were grouped together with * Panicum auritum* (no chromosome data available) and *Cenchrus incertus* ($x = 9$, Pohl and Davise, 1971) by *atpB–rbcL* (57% BS), ITS (94% BS) and combined nuclear and chloroplast (100% BS) analyses, except in the *trnL–F* analysis in which
only Ichnanthus was closely related to the Andropogoneae + Arundinella and Garnotia clade in the strict consensus tree. Although no support was found for the relationships within the tribe, the relationships among some of the terminal taxa are available.

A clade consisting of Alexfloydia and three bristle clade taxa (Setaria, Pennisetum and Spinifex) was found to be monophyletic with high support in all analyses (no bootstrap in trnL-F, 97% BS in atpB-rbcL, 81% BS in ITS; 99% BS in combined chloroplast; 100% BS in combined nuclear and chloroplast). Interestingly, Alexfloydia, a non bristle-bearing genus and is a rare Australian grass (Simon, 1992), which has not been included in any analyses before, was placed in the bristle clade. Some previous studies have also found non-bristle bearing taxa in the bristle clade (Gomez-Martinez and Culham, 2000; Giussani et al., 2001; Aliscioni et al., 2003; Bess et al., 2005; Doust et al., 2007; Morzone, pers. comm.). In these, the genus Zuloagaea bulbosum, formerly known as Panicum bulbosum, a species that lacks the synapomorphic bristles in its inflorescence, was found as a morphologically anomalous member of the bristle clade. Surprisingly, one of bristle-bearing genera, Cenchrus, was not grouped within this clade but was grouped within the clades consisting of Paspalum and Panicum auritum with low support in all single gene analyses (57% BS in trnL-F, 69% BS in atpB-rbcL, 51% BS in ITS) and with high support in both combined analyses (95% BS in combined chloroplast and 100% BS in combined nuclear and chloroplast). Within the bristle clade, Pennisetum was monophyletic with low to moderate support (82% BS in trnL-F, 88% BS in combined chloroplast and 74% BS in combined nuclear and chloroplast). However, previous studies have found that Pennisetum was paraphyletic and always forms a monophyletic assemblage with Cenchrus (Giussani et al., 2001; Doust and Kellogg, 2002; Ibrahim et al., 2009). The bristle clade was found to be monophyletic in several morphological and molecular studies (Zuloaga et al., 2000; Gomez-Martinez and Culham, 2000; Duvall et al., 2001; Giussani et al., 2001; Doust and Kellogg, 2002; Doust et al., 2007). Morphologically, the members of this clade have inflorescences that range from elongate and diffuse to highly branched and condensed, and usually have setae or bristles which are converted from inflorescence branch meristems (Doust and Kellogg, 2002). However, in some members, the bristles are less obvious occurring only at the tip of inflorescence branches, or singly under some spikelets, or wanting (Bess et al., 2005; Doust et al., 2007).

The forest shade clade as defined by Giussani et al. (2001), Christin et al. (2008) and Ibrahim et al. (2009) was also found in this study with Acroceras, Pasendoechinolaena and a new member, Cyrtococcum. However, the monophyly of forest shade clade remains ambiguous because Ottochloa was grouped with the core forest shade clade only in the strict consensus tree of the
combined nuclear and chloroplast analysis, and *Alloteropsis* was placed outside the forest shade clade and grouped together with *Digitaria* with no support (Figure 3.13). Morphologically, all members of the forest shade clade have lanceolate leaf blades. Their primary inflorescence branches are more or less racemose with the spikelets borne close together on short pedicels. Their glumes are herbaceous and their paleas and upper lemmas are crustaceous, with the margins of the upper lemma tucked into the palea (Giussani et al., 2001).

*Homopholis* and *Walwhalleya*, the endemic grasses from Queensland, Australia, were grouped together in all analyses (66% BS in *trnL-F*; no bootstrap in *ITS*; 68% BS in combined chloroplast and 57% BS in combined nuclear and chloroplast), except in the *atpB-rbcL* analysis. This monophyletic clade is consistent with previous molecular study (Hodkinson, unpublished data), but is inconsistent with the morphological phylogenetic trees of Wills et al. (2000) in which three members of *Walwhalleya* were grouped monophyletically and sister to the clade consisting of *Digitaria* and *Panicum*, while *Homopholis* was well-supported as the most outlying member of the ingroup. A monotypic and endangered genus, *Homopholis* was traditionally placed within section *Digitariastrae* under tribe *Paniceae* by Hubbard (1934). It was considered to be closely related to *Digitaria*, but differing in its well developed lower glumes and comparatively small fertile florets (Hubbard, 1934; Clayton and Renvoize, 1986). However, this relationship is not supported by the cladistic analysis using morphological data (Wills et al., 2000) nor by present molecular data. *Walwhalleya*, another Australian endemic genus of three species, was named by Bruhl et al. (2006). Before generic reclassification, the members of *Walwhalleya* were accepted as members of *Panicum* by Mueller (1855) and Domin (1915), as *Panicum prolatum* and *P. subxerophilum*, respectively, and were transferred to be under *Walwhalleya* by the cladistic study of Wills et al. (2000) in which a novel species, *Walhalleya pungens*, was proposed. However, *Walhalleya* sensu Wills et al. (2000) was later renamed as *Walwhalleya*, as it was an illegitimate later homonym of a fungal genus *Walhalleya* sensu Rogers et al. (1997).

*Sacciolepis* was consistently resolved as monophyletic by *trnL-F* (99% BS), *atpB-rbcL* (96% BS), *ITS* (100% BS), combined chloroplast (100% BS) and combined nuclear and chloroplast (100% BS), but its position is uncertain (Figure 3.13). Previously, *Sacciolepis*, represented by *S. indica* was nested within the *Panicum* sections *Monticola* clade-*Parvifolia* clade-*Verrucosa* clade (Aliscioni et al., 2003; Ibrahim et al., 2009). Aliscioni et al. (2003) suggested that the inclusion of *Sacciolepis* within this *Panicum* clade is doubtful because no apparent morphological relationship exists between *Sacciolepis* and *Monticola* clade-*Parvifolia* clade-*Verrucosa* clade. *Sacciolepis*, comprises of 30 species, is widely distributed in tropics, especially in Africa. It is a distinctive
genus and differs from the rest of Paniceae by the presence of spiciform panicle, with the ribbed glumes and the gibbous upper glumes (Clayton and Renvoize, 1986).

**Arundinelleae**

Arundinelleae were consistently found to be polyphyletic with *Arundinella, Danthoniopsis, Garnotia* and *Tristachya* failing to group together in any of the single gene or multi-gene region analyses. Previous studies (e.g. Mason-Gamer et al., 1998; Hihu et al., 1999; Spangler et al., 1999; Mathews et al., 2000; Giussani et al., 2001; Mathews et al., 2002; Sánchez-Ken and Clark, 2007) have also indicated the polyphyly of Arundinelleae. In this study, three main clades of Arundinelleae were found by the combined nuclear and chloroplast analysis: *Arundinella, Garnotia*, which were grouped together with Andropogoneae (99% BS), and a clade of *Danthoniopsis* plus *Tristachya* (55% BS) which was sister to Panicoideae (52% BS) (Figure 3.13). Based on morphological characters, Arundinelleae are grouped within Panicoideae as an early divergence from the ancestral panicoid line together with Andropogoneae as its descendants. They have the unique feature of the two-flowered spikelets with male or barren lower florets and bisexual upper florets. However, Arundinelleae differ from other panicoids in having spikelets with persistent glumes (except *Garnotia*) (Clayton and Renvoize, 1986; Renvoize and Clayton, 1992).

Within Arundinelleae, the classification at generic level of Arundinelleae is inconsistent due to the over-abundance of potentially significant generic characters and reticulate character distributions (Phipps, 1966; Clayton, 1967; Clayton and Renvoize, 1986). However, on the basis of anatomy, Arundinelleae was divided into two groups, Non–Kranz or C₃ pathway in *Chandrasekharania* and *Jansenella*, which were regarded as the most primitive members of Arundinelleae, and Kranz MS type or C₄ pathway in the rest of the tribe (Clayton and Renvoize, 1986). It would be interesting to include those two putative primitive genera in further analysis, especially *Jansenella* which was found as an intermediate taxon between *Arundinella* and *Danthoniopsis* (Clayton, 1967; Clayton, 1972; Clayton and Renvoize, 1986; Teerawatanaanon and Hodkinson, 2008), in order to see how they relate to *Arundinella*, Andropogoneae and the rest of Arundinelleae.

**Arundinella and Garnotia**

The results from all single genes and combined analyses support the separation of *Arundinella* and *Garnotia* from the remaining Arundinelleae and support the inclusion of both genera in
Andropogoneae. These results also suggest that *Arundinella* and *Garnotia* could better be placed in their own subtribes (Arundinellinae Honda sensu stricto and Garnotiinae Pilger, respectively) within the Andropogoneae and that the tribe Arundinelleae should be abandoned as being a taxonomic group. Although the appearance of *Garnotia* within Andropogoneae was previously demonstrated by the cluster analysis of Hilu and Wright (1982), the inclusion of *Garnotia* in Andropogoneae is a novel grouping overlooked by most previous phylogenetic studies (Manson-Gamer et al., 1998; Spanglex et al., 1999; Kellogg, 2000; Lukens and Doebley, 2001; Mathews et al., 2002; Rondeau et al., 2005) probably due to lack of *Garnotia* DNA samples. However, the positions of *Arundinella* and *Garnotia* within Andropogoneae, relative to other subtribes are still unclear. In this study, *Arundinella* was found to be monophyletic. This finding is consistent with previous studies (e.g. Mason-Gamer et al., 1998; Kellogg, 2000; Mathews et al., 2002; Skendzic et al., 2007). All analyses provided high support (99% BS in *trnL-F*; 99% BS in ITS; 99% BS in combined chloroplast and 100% BS in combined nuclear and chloroplast) for the *Arundinella* clade, except the *atpB-rbcL* analysis which provided no support (Figure 3.7). These data support the generic status of *Garnotia* as it does not group with *Arundinella* in any of analyses. The novel status of monophyletic *Garnotia* is strongly supported by the *trnL-F*, *atpB-rbcL*, combined chloroplast and combined nuclear and chloroplast analyses (95%, 100%, 100% and 100% BS, respectively).

However, the result obtained from the ITS analysis does not support *Garnotia* as monophyletic (Figure 3.11). In the combined all gene regions analysis, the *Garnotia* clade was found to be sister to genus *Eremochloa* with 83% BS (Figure 3.13) but there is no obvious morphological character to arrange these taxa together. Morphologically, *Arundinella* differs from the rest of tribe Arundinelleae in having a membranous ligule, a scabrid upper lemma and a punctiform hilum, while *Garnotia* has been distinguished by its single-flowered spikelets which disarticulate below the glumes (Clayton, 1967; Renvoize, 1982b; Clayton and Renvoize, 1986). Both genera differ from the rest of the tribe by having a punctiform hilum and a membranous ligule (Clayton and Renvoize, 1986). Anatomically, *Arundinella* and *Garnotia* appear to share certain characteristics and could have formed an independent line of development from the rest of the Arundinelleae taxa with the C₄ pathway by having isolated vascular bundle sheath cells (which were called distinctive cells or auxillary bundle cells), and auriculate paleas (Tateoka, 1958; Renvoize, 1982a, 1982b; Clayton and Renvoize, 1986; Renvoize and Clayton, 1992). However, the results presented in this thesis suggest that this shared character is homoplasious as *Arundinella* and *Garnotia* were not grouped together.
Danthoniopsis and Tristachya

The combined nuclear and chloroplast analysis resolved a grouping of Danthoniopsis and Tristachya. This clade was sister to Panicoideae. However, the bootstrap support for these relationships was low (55% and 52% BS, respectively) (Figure 3.13). The sister group relationship between Danthoniopsis and Tristachya is consistent with Sánchez-Ken et al. (2007), while the sister group relationship between Danthoniopsis plus Tristachya clade and the panicoid clade can be interpreted as a novel result. However, this finding should be interpreted with care because some species of Tristachya have been placed within the Andropogoneae with high support (Baker et al., 1995; Hilu et al., 1999; Bouchenak-Khelladi et al., 2008). It would be interesting to add more taxa from Tristachya and its related genera in future analysis to test its monophyly and see how it relates to Andropogoneae and the other genera of Arundinelleae. Morphologically, Danthoniopsis and Tristachya are similar in having bilobed upper lemmas with 5–9 nerves, ligules with a line of hairs and a linear hilum (Bor, 1955; Clayton, 1971; Clayton and Renvoize, 1986).

Andropogoneae and subtribal classification

Subtribe Andropogoneae were found to be monophyletic only if Arundinella and Garnotia were included. This result was supported by atpB-rbcL (in strict consensus but no bootstrap), ITS (89% BS) and combined nuclear and chloroplast (99% BS) analyses (Figures 3.7, 3.11 and 3.12). Only the result form the combined chloroplast analysis shows that Andropogoneae itself is monophyletic with low support (62% BS) and sister to Danthoniopsis in the strict consensus tree (no bootstrap) (Figure 3.9). Within the tribe, none of the phylogenetic analyses are consistent with the awned/awnless classification proposed by Clayton (1972, 1973). This hypothesis was supported by the molecular study of Mathews et al. (2002), but no strong evidence for this clade was found. It is clear that the subtribal classification of Clayton and Renvoize (1986) required considerable revision as also mentioned in previous studies (Kellogg, 2000; Mathews et al., 2002) even though some subtribes (Chionachninae, Coicinae, Dimeriinae, Germariniinae and Tripsacinae) are supported as monophyletic. More studies are needed to find a better way of subdividing the remaining members of Andropogoneae into subtribal taxa and to better determine their relationships.
Chionachninae, Coicinae and Tripsacinae

All monoecious taxa of Andropogoneae were traditionally placed in the Maydeae (e.g. Bentham, 1882; Hackel, 1889; Pilger, 1960; Bor, 1960; Watson and Dallwitz, 1992; Kellogg and Watson, 1993). However, Maydeae were divided into three subtribes, Chionachninae, Coicinae and Tripsacinae by Clayton (1973, 1981a) and Clayton and Renvoise (1986) using the difference of the inflorescences and spikelets. Previously, three members of Chionachninae (Chionachne, Polytoca and Triobachne) were accepted as members of subtribe Coicinae by Clayton (1973) using the bead-like feature of female spikelets. Later, Clayton (1981a) proposed the new subtribe name Chionachninae for Chionachne, Polytoca and Triobachne based on the different origin of the bead-like feature of female spikelets. In Chionachninae this structure is formed by a lower glume, while in Coicinae it is modified from a spatheole. These two subtribes completely differed from Tripsacinae in having male and female spikelets on the same inflorescences and their inflorescence rachis are narrower than spikelets.

In this study, Chionachninae, represented by Chionachne massiei, Polytoca digitata and P. wallichiana, were monophyletic in all analyses (86% BS in trnL-F; 99% BS in atpB-rbcL; 100% BS in combined chloroplast and 74% BS in combined nuclear and chloroplast) (Table 3.2), except the ITS analysis in that Chionachninae clade is not recovered as Chionachne, represented by C. cyathopoda and C. massiei, are not grouping together (Figure 3.11). However, almost every analysis, except the trnL-F analysis, has supported the monophyly of Polytoca, with P. digitata and P. wallichiana (50% BS in atpB-rbcL; 100% BS in ITS; 60% BS in combined chloroplast and 100% BS in combined nuclear and chloroplast). Polytoca wallichiana was first proposed under the name Cyathorhachis wallichiana by Steudel (1854). It was transferred to Polytoca by Bentham (1882). Recently, the name Cyathorhachis has been reinstated by Jannink and Veldkamp (2002). However, P. digitata and P. wallichiana are morphologically similar in many respects. The low level of genetic divergence between these two taxa also confirms that P. wallichiana should be placed within Polytoca rather than Cyathorhachis. Morphologically, subtribe Chionachninae were found to be polyphyletic by Kellogg and Watson (1993), in which Polytoca was grouped together with Tripsacinae, while Chionachne was placed outside this clade. Clayton and Renvoise (1986) and Renvoise and Clayton (1992) suggested that Chionachninae are linked to Rottboelliiinae by the appearance of the peg and the socket callus joints of sessile spikelets.

This relationship is not supported by molecular study of Lukens and Doebley (2001) in that Chionachne, represented by Chionachne koenigii, was found to be sister to genus Coelorachis, and is inconsistent with the study of Bomblies and Doebley (2005) in which C. koenigii was sister to genus Apluda mutica.
The monophyly of *Coix* (Coicinae) was demonstrated by the Bayesian phylogenetic analysis of *FLORICAULA/LEAFY* genes (Bombles and Doebley, 2005) and is resolved by the *ITS* (90% BS) and the combined nuclear and chloroplast (92% BS) analyses in this study (Table 3.2; Figures 3.11 and 3.13). Morphologically, the highly modified inflorescence of the monotypic subtribe Coicinae is composed of paired unisexual racemes. Female racemes are hidden in an indurated utricle which is derived from a spatheole. These extraordinary modifications confirmed the separation of Coicinae from the remaining monoecious taxa and indicated a possible link with genus *Apluda* and Coicinae (Clayton and Renvoize, 1986; Renvoize and Clayton, 1992). However, this debate remains unresolved based on molecular data.

Tribe Tripsacinae, including *Zea mays* and wild species of *Zea* and *Tripsacum*, are restricted to the New World, but have been introduced all over the world (Clayton and Renvoize, 1986). On the basis of morphology, *Tripsacum* and *Zea* were grouped together by Clayton (1973) using the unique character of inflorescence structures in which female spikelets are solitary in all wild species (sometimes accompanied by a rudiment spikelet in *Tripsacum*) (Clayton and Renvoize, 1986; Watson and Dallwitz, 1992). In *Zea*, female and male inflorescences are in separate inflorescences, whereas in *Tripsacum*, female and male flowers are borne on the same inflorescence. For this study, the monophyly of Tripsacinae was also resolved in all analyses with different degrees of bootstrap support (74% BS in *atpB-rbcL*, 100% BS in *ITS*; 87% BS in combined chloroplast and 100% BS in combined nuclear and chloroplast). The combined nuclear and chloroplast analysis shows that Tripsacinae are grouped with the clade consisting of *Garnotia* clade plus *Eremochloa* with no bootstrap support, but there is no obvious morphological character to arrange these taxa together. Tripsacinae were found to be monophyletic and closely related to Rottboellinae and Chionachninae in all molecular studies to date (e.g. Spangler et al., 1999; Spangler, 2000; Lukens and Doebley, 2001; Hodkinson et al., 2002b; Mathews et al., 2002; Bomblies and Doebley, 2005). Their results are consistent with the morphological studies of Clayton and Renvoize (1986) and Renvoize and Clayton (1992) in which Chionachninae and Tripsacinae are derived from Rottboellinae. The relationships between Tripsacinae and Rottboellinae were not, however, recovered in this study.

**Germainiinae**

Subtribe Germainiinae, represented by *Apocapis* and *Germainia*, were resolved as monophyletic by the *ITS* and the combined nuclear and chloroplast analyses with high support (89% BS and 99% BS, respectively) (Table 3.2; Figures 3.11 and 3.13). This relationship has never been
reported before. However, the monophyly of Germainiinae is not supported by the results obtained from chloroplast data due to the inclusion of *Eremochloa* (Figures 3.5, 3.7 and 3.9). This novel monophyletic clade is incongruent with the morphological phylogenetic trees of Kellogg and Watson (1993). Within Germainiinae, *Apocopsis* is monophyletic in the combined nuclear and chloroplast analysis (98% BS). Within this clade, the awned upper lemma taxa form a clade with moderate support (84% BS) and have the awnless upper lemma *A. collinus* as the most outlying species to the rest of *Apocopsis* (Figure 3.13). However, this hypothesis can be rejected because of the lack of ITS sequence of *Germainia lanipes*. Besides, the monophyly of *Apocopsis* was not supported by the combined chloroplast tree due to the inclusion of *Germainia lanipes* (Figure 3.9). For *Germainia*, three representative species were consistently paraphyletic in all analyses. The combined nuclear and chloroplast analysis showed that *Germainia khapiana* was sister to *G. pilosa* (94% BS), while *G. capitata* was the next most outlying branch to the rest of Germainiinae.

**Dimeriinae**

The monotypic subtribe Dimeriinae (*Dimeria* spp.) were resolved as monophyletic in all analyses (no bootstrap in *trnL-F*; 69% BS in *atpB-rbcL*; 100% BS in ITS; 88% BS in combined chloroplast and 100% BS in combined nuclear and chloroplast) (Table 3.2). A sister group relationship between Dimeriinae and Ischaeminae was demonstrated by the combined nuclear and chloroplast analysis in which *Ischaemum indicum* and *I. muticum* are successively sister taxa to a Dimeriinae clade with 54% and 66% support (Figure 3.13). These relationships are congruent with the study of Clayton and Renvoize (1986), but inconsistent with the study of Kellogg and Watson (1992). Based on morphological data, the monotypic subtribe Dimeriinae is unlike the remaining Andropogoneae in having a single pedicelled spikelet with no trace of the pairing (Clayton, 1972). Renvoize and Clayton (1992) suggested that the absence of a sessile spikelet appears to be derived from the orthodox arrangement of paired spikelets with one sessile and the other pedicelled. Dimeriinae is linked to *Pogonachne* in the Ischaeminae through *Dimeria leptorhachis* but differs by its espatheate inflorescences, racemes with tough rhachis, epaleate florets and the presence of two stamens (Clayton and Renvoize, 1986).

**Monophyly of other genera**

Although most intergeneric relationships within Andropogoneae were not fully clarified, some genera in the tribe were found as monophyletic as far as can be concluded on the basis of a limited sampling for some of the genera.
*Hackelochloa, Hemarthria and Mnesithea*

Although subtribe Rottboelliae, with *Eionurus, Eremochloa, Hackelochloa, Hemarthria, Mnesithea* and *Phacelurus*), were not found to be monophyletic in this study, three genera, *Hackelochloa, Hemarthria* and *Mnesithea*, often grouped together. The monophyly of *Hemarthria* was confirmed by the combined analyses (98% BS in combined chloroplast and 100% BS in combined nuclear and chloroplast) and the single gene analyses (99% BS in trnL-F and 74% BS in ITS), except the *atpB-rbcL* analysis. Although the results from the ITS and both combined analyses showed that *Hemarthria* is closely related to *Hackelochloa*, this sister group relationship was not strongly supported (no support in ITS; 72% BS in combined chloroplast and 69% BS in combined nuclear and chloroplast) and no obvious morphological relationship exists for this relationship. The relationship between *Hemarthria* and *Hackelochloa* in this study is inconsistent with the morphological studies of Clayton and Renvoize (1986) and Kellogg and Watson (1993). Morphologically, both genera are placed in subtribe Rottboelliae based on the characters of awnless upper lemmas, thickened internodes and the fused pedicel to internode (Clayton, 1973). *Hemarthria* can be distinguished from the rest of subtribe in possessing tough rhachis and an oblique basal callus, while *Hackelochloa* is the only genus in the subtribe having globose sessile spikelets with wingless lower glumes (Clayton and Renvoize, 1986).

*Bothriochloa, Capillipedium and Dichanthium*

Three genera, *Bothriochloa, Capillipedium* and *Dichanthium*, were found to form a monophyletic group by the *atpB-rbcL* (86% BS), combined chloroplast (94% BS) and combined nuclear and chloroplast (91% BS) analyses. The *Bothriochloa, Capillipedium* and *Dichanthium* clade was also resolved as monophyletic by previous molecular studies (Manson-Gamer et al., 1998; Spangler et al., 1999; Mathews et al., 2002; Skendzic et al., 2007). These three genera are known as an agamic complex and have produced a large number of interspecific and some intergeneric hybrids (Harlan and De Wet, 1963; De Wet and Harlan, 1970). Cytologically, *Bothriochloa, Capillipedium* and *Dichanthium* have been combined and could be recognised as section of *Dichanthium* (De Wet and Harlan, 1966; De Wet and Harlan, 1970). However, this relationship is not supported by previous morphological studies (Clayton and Renvoize, 1986; Watson and Dallwitz; 1992; Kellogg and Watson, 1993). Morphologically, most studies (e.g. Clayton and Renvoize, 1986; Watson and Dallwitz, 1992) preferred to keep these three genera separate. *Dichanthium* is related closely to *Bothriochloa* in having sub-digitate racemes, but can be distinguished by its pedicels and rhachis internodes being solid and lacking a translucent
median line. The members of *Capillipedium* are often confused with members of *Bothriochloa*, but the former differs in having paniculate inflorescences and short racemes often reduced to triads. In this study, the *Bothriochloa, Capillipedium* and *Dichanthium* clade was found to be grouped together within the core Andropogoneae clade as sister to the clade consisting of *Andropogon, Cymbopogon, Hyparrhenia, Schizachyrium* and *Sporiochloa*, but there is no support (>50% BS) for this relationship (Figure 1.13). In previous analyses, *Bothriochloa, Capillipedium* and *Dichanthium* clade was grouped within the core Andropogoneae clade. This clade, comprising *Andropogon, Caix, Cymbopogon, Heteropogon, Hyparrhenia, Schizachyrium* and *Sporiochloa*, was informally named by Spangler *et al.* (1999) corresponding to the chromosome number ($\times$) of 20. The core Andropogoneae were later found to be non-monophyletic by Mathews *et al.* (2002) and Skendzic *et al.* (2007) due to the exclusion of *Caix*. In this study, however, the core Andropogoneae were not monophyletic due to the exclusion of *Heteropogon* and *Caix*.

**Arthraxon**

Morphologically, the genus *Arthraxon* is distinct from all other Andropogoneae by its lemmas with a sub-basal awn. The results from all analyses confirm the isolation of *Arthraxon* from most Andropogoneae with high support (98% BS in *trnL-F*, no support in *atpB-rbcL*, 100% BS in *ITS* and two combined analyses) (Figures 3.5, 3.7, 3.9, 3.11 and 3.13). Surprisingly, the result from the combined nuclear and chloroplast analysis shows that *Thelepocon* is closely related to *Arthraxon*. However, the support is low (50% BS). This relationship has never been found before although the two genera have the same general characters such as barren pedicels, speculate glumes, sub-basal awns and cordate amplexicaul leaves.

**Hyparrhenia**

*Hyparrhenia* was resolved as a monophyletic group by the *trnL-F* (no bootstrap support), *ITS* (63% BS, combined chloroplast (56% BS) and combined nuclear and chloroplast analyses (88% BS). *Hyparrhenia* is a dominant grass species of the African savannas. Clayton (1969) suggested that *Hyparrhenia* appears to have evolved from *Andropogon* and *Cymbopogon*. This study also found that the *Hyparrhenia* clade is sister to *Andropogon gerardii*. This relationship was found in the analyses of *ITS* (<50% BS), combined chloroplast (50% BS) and combined nuclear and chloroplast (67% BS). This clade is grouped together with three other taxa, *Cymbopogon, Schizachyrium* and *Andropogon ascinodis* with moderate support (76% BS).
The genus *Themeda* was supported as monophyletic in *trnL-F* (61% BS) and combined nuclear and chloroplast (53% BS) analyses. *Themeda* was sister to the core Andropogoneae (including *Spodiopogon*, but excluding *Heteropogon* and *Coix*), but the support was lacking (<50% BS).

Morphologically, *Themeda* is distinctive among the sampled Anthistiriinae in that its racemes have two large homogamous pairs at the base and upper lemmas are entire. According to Clayton and Renvoize (1986), the position of *Themeda* within subtribe Anthistiriinae should be between *Heteropogon* and *Iseilema*. The relationship between *Iseilema* and *Themeda* was also found in the study of Kellogg and Watson (1993). However, none of analyses in this study support this hypothesis.

**Rapid radiation in Andropogoneae and future prospects of resolving phylogenetic inter-relationships within the tribe**

The phylogenetic trees resolved by previous molecular studies (Spangler et al., 1999; Kellogg, 2000; Mathews et al., 2002) found extremely short internal branch lengths within the tribe Andropogoneae relative to branch lengths in the rest of grasses sampled. These short branches can reflect a small number of highly consistent mutations and suggest that rapid evolutionary radiation has occurred during the evolution of Andropogoneae (Kellogg, 2000; Mathews et al., 2002). In addition, the short branches along the backbone of the tree suggest that radiation may occur at an early stage of evolution rather than the recent stage (Kellogg, 2000).

In this study, 42 out of 85 genera of Andropogoneae, representing 11 subtribes, were sampled and sequenced using three non-coding markers from both chloroplast and nuclear ribosomal DNA. Interrelationships resolved within the Andropogoneae were broadly consistent with other analyses (e.g. Manson-Gamer et al., 1998; Spangler et al., 1999; Mathews et al., 2002; Skendzic et al., 2007), except some novel clades which are the benefits of adding taxa to a phylogenetic analysis. However, the present matrix with three non-coding markers (*trnL-F*, *atpB-rbcL* and *ITS*) was insufficient to provide enough phylogenetically informative characters to resolve evolutionary relationships at the intergeneric level in Andropogoneae. There are several reports of phylogenetic analyses within the angiosperms that have encountered similar difficulties with resolution due to short lengths of internal branches relative to terminal branches (e.g. Fishbein et al., 2001; Wortley et al., 2005; Jian et al., 2008). These patterns have been explained by suggesting that the groups have undergone rapid phylogenetic radiation.
(and that the phylogenetic signal to resolve the inter-relationships of the lineages has been lost). However, the theory behind such empirical observations and deductions is not well developed or reported. A recent study by Moore et al. (2007) has highlighted this difficulty in the phylogenetic reconstruction of early angiosperm lineages that are particularly difficult to resolve (mesangiosperms; *Ceratophyllum*, Chloranthaceae, Eudicots, Magnoliids and Monocots). They suggested that the inability to resolve relationships with confidence is due to an early and potentially rapid diversification of the mesangiosperms in combination with a loss of phylogenetic signal at rapidly evolving nucleotide sites. This could be the problem with the non-coding gene regions used with the grasses in this study. There could have been too much molecular evolution at phylogenetically informative nucleotide sites since the divergence of the major taxa (hence a loss of phylogenetic signal). Adding more data and more taxa are the only ways of resolving these difficult groups (Hillis et al., 2003; Hodkinson et al., 2007a; Jian et al., 2008; Pirie et al., 2008). Choice of gene is also critical and it might be worthwhile sequencing a large number of more slowly evolving genes to recover phylogenetic patterns at these deeper regions of the Panicoideae (Hodkinson et al., 2007a; Moore et al., 2007; Pirie et al., 2008).
Table 3.2 Comparison of strict consensus and bootstrap support values of the main taxa or major groups resolved from single gene region and combined analyses.

<table>
<thead>
<tr>
<th>Main taxa or major resolved groups</th>
<th>trnL-F</th>
<th>atpB-rbcL</th>
<th>ITS</th>
<th>combined chloroplast</th>
<th>combined chloroplast and nuclear</th>
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<td>strict consensus</td>
<td>%BS</td>
<td>strict consensus</td>
<td>%BS</td>
<td>strict consensus</td>
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<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
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<td>62</td>
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Table 3.3 Species sequenced for \textit{trnL-trnF}, \textit{atpB-rbcL} and \textit{ITS1-ITS2}, with vouchers and GenBank accession numbers.

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<td>Tribe Oryzaceae</td>
<td>Tribe Oryzaceae</td>
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Table 3.1 (continued)

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</tbody>
</table>

Abbreviations are as follows:  
AT, A. Teerawatanaanon.  
KEW, Kew Herbarium, England, U.K.  
N/A, not applicable.  
SL, S. Laegaard.  
SJ, S.W.L. Jacobs.  
SS, S. Sungkaew.  
TCD, Herbarium, School of Botany, Trinity College, Dublin, Ireland.  
THNHM, Thailand Natural History Museum, Techno Polis, Pathum Thani, Thailand.  
TRH, T. R. Hodkinson.  
WK, W. Kowarat.
CHAPTER 4

Population genetic structure of two forest grasses with contrasting life forms, 
*Arundinella setosa* Trin. and *Garnotia tenella* (Arn. ex Miq.) Janowski 
(Poaceae) in Thailand

4.1 Introduction

Thailand is located in the Southeast Asia area. The country has a total area of 513,115 km² of which 136,698 km² is forest (Boontawee *et al.*, 1995, TMD, 2002). The range of elevation is from about sea level up to 2,200 m and hence the climate varies from the hot humid tropical conditions of the lowlands to the cooler sub-alpine and subtropical conditions of higher altitudes. Thus, this has resulted in a diversity of habitat types, including sub-alpine, dry and mixed deciduous forests, tropical rain forests and mangrove forests (Smitinand, 1977; Boontawee *et al.*, 1995; Kutintara, 1999). In addition, Thailand is also a bio-resource rich country because of its location which sits at the junction of the three broadly defined biogeographic regions, the Indo-Burmese, Indo-Chinese and Malesian regions (Santisuk *et al.*, 1991; Boontawee *et al.*, 1995). However, the biodiversity of Thailand is rapidly being eroded due to a number of factors including forest alteration, wetland drainage, pollution and coastal development. The forest cover in Thailand has declined from over 50% of the total land area in the 1950’s to approximately 25% in 2000 (Boontawee *et al.*, 1995; Middleton, 2003). As the forest area has been depleted, the existing protected areas are now divided into small fragmented areas. Such habitat fragmentation of natural populations can potentially alter the spatial distribution of genetic diversity. Fragmentation can decrease gene flow between populations and increase genetic differentiation among the fragmented areas (Hanski and Gilpin, 1991; Yung *et al.* 1996). In addition, genetic variation within a fragmented area may decrease if the remaining population is small because of the effects of genetic drift and inbreeding (Young *et al.* 1996; Lienert 2004).

However, in order to plan the conversation of plant biodiversity, it is important to understand the variation in genetic composition and population genetic structure, especially the variation in genetic composition within and among populations (Changtragoon and Szmidt, 1993; Henry, 2005, Zhang *et al.*, 2006). Genetic diversity assessments can help conservation managers to choose protected areas by providing genetic information about rare plant populations (Hopper and Coates, 1990; Zaghloul *et al.*, 2006). Therefore, the assessments of
genetic variation can assist in developing effective conservation strategies (Holsinger and Gottlieb, 1991; Newton et al., 1999).

In Thailand, several studies have been carried out on the genetic diversity of some forest tree species and over the last few decades work has been undertaken to develop molecular markers. For example, Soonhuae et al. (1994), Changtragoon and Finkeldey (1995) and Leingsiri et al. (1995) used isozymes as genetic markers on Thai forest tree species. Recently, DNA markers such as microsatellites have been developed and tested as alternatives to isozymes for estimating genetic diversity, mating system dynamics and gene flow in plants especially forest trees (e.g. Pakkad et al., 2004; Blakesley et al., 2004). However, little work has focused on Thai grasses. Research on the population genetics of grasses has, in contrast, been extensive in other areas of the world and on a broad sample of their subfamilies. For example, Ukoskit (2004) used amplified fragment length polymorphisms (AFLP) to study genetic diversity of wild rice (Oryza spp.) in the Northern region of Thailand. Hodkinson et al. (2002a) used AFLP to study genetic variation in the panicoid grass genus Miscanthus from Southeast Asia. Wu et al. (2004) used random amplification of polymorphic DNA (RAPD) and inter-simple sequence repeat (ISSR) to study genetic diversity in Oryza granulata Nees from China and McGrath et al. (2007) studied genetic diversity in pooid grass Lolium perenne L. in Europe using plastid microsatellite markers.

Microsatellites, or simple sequence repeats (SSRs), are tandemly repeated sequences between 2-5 base pairs (bp) in length and co-dominantly inherited (Page and Holmes, 1998; Goldstein and Schlotterer, 1999). These short DNA sequence motifs are highly polymorphic as a result of a high variation of repeat copy number (Tautz and Renz, 1984; Goldstein and Schlotterer, 1999; Weising and Gardner, 1999) and also have high abundance. Microsatellites can be easily detected by the Polymerase Chain Reaction (PCR) (Weber and May, 1989; Weising and Gardner, 1999) using specific primer pairs which are designed from unique sequences flanking the microsatellite-containing regions (Tautz, 1989; Weber and May, 1989). Since microsatellites have been found at high frequency in every organism analysed to date (Schotterer and Pemberton, 1994; Li et al., 2002), and because they show high levels of polymorphism and high mutation rates (Litt and Luty, 1989; Weber and May, 1989; Tautz, 1989), microsatellites have become useful markers for investigating the population genetic structure and genetic variation within populations and between geographically widely separated populations (Condit and Hubbell, 1991; Avise, 1994; Goldstein and Pollock, 1997; Sun et al., 1998; Collevatti et al., 2003). Microsatellite regions are found in all three genomes of plant cells (plastid, mitochondrial and nuclear). However, nuclear and plastid microsatellites
are most frequently used for population genetic research. Plastid DNA is maternally inherited without recombination in most angiosperms including grasses (Hodkinson et al., 2002a; McGrath et al., 2007), hence seeds will carry the plastid DNA type of their maternal parent. Plastid microsatellites are common in plants (Provan et al., 2001) and have proven to be useful for population genetic applications such as diversity, differentiation, phylogeography and gene flow studies (Provan et al., 2001; McGrath et al., 2007).

The grasses *Arundinella setosa* and *Garnotia tenella* are very variable in morphology and are widespread Asiatic species, usually found growing natively from temperate to tropical Asia (Clayton et al., 2006). Forest grasses are important to the forest ground cover plant community and also provide food and habitat for small animals such as insects, birds and small mammals which can attract larger predators. Native grasses often co-exist with species of native non-woody herbaceous plants such as legumes and other wildflowers which are the main source of food for living animals. High diversity of native ground cover plants means high food diversity and high biodiversity in native forests. In Thailand, *A. setosa* and *G. tenella* are native forest grasses. *Arundinella setosa* is a perennial grass in deciduous dipterocarp forests, deciduous dipterocarp with pine forests and tropical grasslands at high elevation, while *G. tenella* is an annual and is distributed in hill evergreen and tropical rain forests.

### 4.2 Aims

*A. setosa* var. *setosa* (hereafter *A. setosa*) and *G. tenella* were chosen as model species to investigate the biogeographical patterns of population genetic structure. These two grasses have contrasting life forms: *Arundinella setosa* is perennial, while *G. tenella* is annual. The study of genetic variation at the intrapopulational level as well as between geographically widely separated populations was analysed using the plastid microsatellite markers developed and tested for grasses by McGrath et al. (2006) and McGrath et al. (2007). More specifically, it aims to:

1) Amplify polymorphic microsatellite regions within the chloroplast genome of *A. setosa* and *G. tenella* collected from Thailand and test the variability of the markers developed by McGrath et al. (2006) on these two genera.

2) Investigate the extent of haplotype diversity of *A. setosa* and *G. tenella* in Thailand to determine if the diversity was structured into geographically meaningful patterns.

3) Investigate distribution patterns and genetic diversity between and within the populations of *A. setosa* and *G. tenella* in Thailand.
4.3 Materials and Methods

Plant material and DNA extraction

Plant material was obtained from a number of sources listed in Table 4.1. A total of 55 samples were collected from 11 populations of *A. setosa* and 40 samples from eight populations of *G. tenella*. Five individuals of each population were sampled from natural habitats distributed across Thailand (Figures 4.9 and 4.10). Plants were randomly sampled, depending on availability, over a geographical range of 100 km for each population and the distance between the sampled individuals in each population was at least 5 m to prevent collecting ramets from a single genet. Fresh leaves were quickly-dried with silica gel in order to rapidly desiccate the material and reduce DNA degradation (Chase and Hills 1991; Hodkinson *et al.*, 2007b). Total genomic DNA (tDNA) was extracted using modified hot CTAB method (see Protocol 1, Appendix 3) (Doyle and Doyle, 1987; Hodkinson *et al.*, 2007b).

Microsatellite amplification

Five chloroplast simple sequence repeats (SSR; microsatellite) primers (*TeaSSR1, TeaSSR2, TeaSSR3, TeaSSR4* and *TeaSSR5*) as described in McGrath *et al.* (2006) (Table 4.2) were used. Reactions were carried out in an Applied Biosystems GeneAmp® PCR System 9700 (thermal cycler). The amplification of the target fragment began with an initial premelt at 95°C for 5 minutes, followed by 35 cycles of denaturation at 95°C for 1 minute, annealing at 60°C for 1 minute and extension at 72°C for 1 minute. A final extension at 72°C for 10 minutes was also included. The components of the chloroplast microsatellite amplification master mix and thermal cycling protocol are given in Table 4.3. The quality and quantity of PCR products were checked by gel electrophoresis (see Protocol 4, Appendix 3). The successfully amplified products were diluted in sterile ultra pure water at 1: 20 to 1: 40 dilutions prior to genotyping.
### Table 4.1 Plant materials used in the microsatellite study.

<table>
<thead>
<tr>
<th>Pop. no.</th>
<th>Voucher specimens*</th>
<th>Locations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Thailand</td>
</tr>
<tr>
<td>A1</td>
<td>AT &amp; SS 293 to 297</td>
<td>Suan Phung, Ratchaburi Province, South-Western</td>
</tr>
<tr>
<td>A2</td>
<td>AT &amp; SS 419 to 423</td>
<td>Khao Sung, Prachuap Khiri Khan Province, South-Western</td>
</tr>
<tr>
<td>A3</td>
<td>AT &amp; SS 435 to 439</td>
<td>Khao Kiew, Chon Buri Province, South-Western</td>
</tr>
<tr>
<td>A4</td>
<td>AT &amp; SS 465 to 470</td>
<td>Nam Tao National Park, Phetchabun Province, North-Eastern</td>
</tr>
<tr>
<td>A5</td>
<td>AT &amp; SS 474 to 478</td>
<td>Phu Phan National Park, Sakol Nakhon Province, North-Eastern</td>
</tr>
<tr>
<td>A6</td>
<td>AT &amp; SS 522/1 to 522/5</td>
<td>Sai Yok Noi, Kanchanaburi Province, South-Western</td>
</tr>
<tr>
<td>A7</td>
<td>AT &amp; SS 535 to 537, 539 to 540</td>
<td>Wat Chan, Chiang Mai Province, Northern</td>
</tr>
<tr>
<td>A8</td>
<td>AT &amp; SS 547 to 551</td>
<td>Ang Khang, Chiang Mai Province, Northern</td>
</tr>
<tr>
<td>A9</td>
<td>AT &amp; SS 555 to 558, 560</td>
<td>Mae Rim, Chiang Mai Province, Northern</td>
</tr>
<tr>
<td>A10</td>
<td>AT &amp; SS 589 to 593</td>
<td>Ban Tak, Tak Province, Northern</td>
</tr>
<tr>
<td>A11</td>
<td>AT &amp; SS 595 to 599</td>
<td>Huai Kha Khaeng, Uthai Thani Province, South-Western</td>
</tr>
<tr>
<td>G1</td>
<td>AT &amp; SS 162/0 to 162/4, 162/6</td>
<td>Khao Yai, Nakhon Ratchasima Province, Eastern</td>
</tr>
<tr>
<td>G2</td>
<td>AT &amp; SS 316/1 to 316/4, 316/6 to 7</td>
<td>Khao Kra Jome, Ratchaburi Province, South-Western</td>
</tr>
<tr>
<td>G3</td>
<td>AT &amp; SS 700 to 704</td>
<td>Thong Pha Phum, Kanchanaburi Province, South-Western</td>
</tr>
<tr>
<td>G4</td>
<td>AT &amp; SS 700 to 704</td>
<td>Um Phang, Tak Province, Northern</td>
</tr>
<tr>
<td>G5</td>
<td>AT &amp; SS 741 to 746</td>
<td>Doi Suthep, Chiang Mai Province, Northern</td>
</tr>
<tr>
<td>G6</td>
<td>AT &amp; SS 804 to 806, 808 to 809</td>
<td>Phu Hin Rong Kla, Phitsanulok Province, Northern</td>
</tr>
<tr>
<td>G7</td>
<td>AT &amp; SS 822 to 826, 827</td>
<td>Phu Kradueng, Loei Province, North-Eastern</td>
</tr>
<tr>
<td>G8</td>
<td>AT &amp; SS 872 to 876</td>
<td>Na Yong, Trang Province, Peninsular</td>
</tr>
</tbody>
</table>

* All housed in the herbarium of the Thailand Natural History Museum, National Science Museum, Technopolis, Pathum Thani, Thailand. Abbreviations are as follows: A = *Arundinella setosa*, G = *Gamotia tenella*, AT = A. Teerawatananon, SS = S. Sungkaew.
Table 4.2 Size ranges, position and chloroplast genome region of SSR makers and allele number recorded during primer testing (from McGrath et al., 2006).

<table>
<thead>
<tr>
<th>cpSSR makers</th>
<th>Chloroplast genome regions</th>
<th>Primer sequence F (5'-3') and R (5'-3')</th>
<th>Fluorescent dye</th>
<th>Repeat motif</th>
<th>Size range</th>
<th>Allele no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>TeaSSR1</td>
<td>atpB-rbcL intergenic spacer region</td>
<td>ATTGATTTGGTTGCGCTAT TCATTAAAGAAAAATTGAGGCATA</td>
<td>VIC</td>
<td>(AAC)3</td>
<td>227-230</td>
<td>4</td>
</tr>
<tr>
<td>TeaSSR2</td>
<td>trn-L intron and trn-F intergenic spacer region</td>
<td>GGGTTGGGGTATAAGGAGCACT TCATTCCAATTGAAATTTTGT</td>
<td>NED</td>
<td>(CT)4</td>
<td>196-202</td>
<td>3</td>
</tr>
<tr>
<td>TeaSSR3</td>
<td>trn-L intron and trn-F intergenic spacer region</td>
<td>AGGGACTGAAACCTCAAA GCAAGGTTAACATTGGAAGC</td>
<td>JOE</td>
<td>A9</td>
<td>305-318</td>
<td>10</td>
</tr>
<tr>
<td>TeaSSR4</td>
<td>23S-5S internal transcribed spacer</td>
<td>AGAAGGCAAGATTTGAAACC TGAAGCCCCAATCTTGACT</td>
<td>JOE</td>
<td>A9</td>
<td>185-200</td>
<td>9</td>
</tr>
<tr>
<td>TeaSSR5</td>
<td>Herbicide binding protein D1 (pshA) (D1 protein of Photosystem II)</td>
<td>GCTATGCAAGTTCTTGTGT TGCTACTACAGGCCAGCACG</td>
<td>TAMRA</td>
<td>(CT)3</td>
<td>209-212</td>
<td>2</td>
</tr>
</tbody>
</table>

Table 4.3 The components of the chloroplast microsatellite amplification master mix and thermal cycling protocol.

Master mix

<table>
<thead>
<tr>
<th>Materials</th>
<th>Volume (µl)</th>
<th>Amount/ Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eluted tDNA</td>
<td>2</td>
<td>c. 100 ng</td>
</tr>
<tr>
<td>Master mix reagents</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sterile ultra pure water</td>
<td>36.75</td>
<td>-</td>
</tr>
<tr>
<td>Promega 10x Reaction buffer*</td>
<td>5</td>
<td>1x</td>
</tr>
<tr>
<td>dNTPs (10 mM each)</td>
<td>1</td>
<td>0.2 mM each</td>
</tr>
<tr>
<td>Forward primer 100 ng/µl</td>
<td>0.5</td>
<td>50 ng</td>
</tr>
<tr>
<td>Reverse primer 100 ng/µl</td>
<td>0.5</td>
<td>50 ng</td>
</tr>
<tr>
<td>MgCl₂ (25 mM)</td>
<td>4</td>
<td>2 mM</td>
</tr>
<tr>
<td>Promega Tæq DNA polymerase</td>
<td>0.25</td>
<td>1.25 units</td>
</tr>
<tr>
<td>(5 units/µl)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TOTAL</td>
<td>50</td>
<td></td>
</tr>
</tbody>
</table>

* contains 10 mM Tris-HCl (pH 9.0 at 25°C), 50mM KCl and 0.1% Triton® X-100.

Thermal cycling protocol

<table>
<thead>
<tr>
<th>Cycle phase</th>
<th>Temperature (°C)</th>
<th>Duration (minutes)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-melt</td>
<td>95</td>
<td>5</td>
</tr>
<tr>
<td>Standard cycle</td>
<td>1. Denaturation</td>
<td>95</td>
</tr>
<tr>
<td>(34 cycles)</td>
<td>2. Annealing</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td>3. Extension</td>
<td>72</td>
</tr>
<tr>
<td>Final cycle</td>
<td>1. Denaturation</td>
<td>95</td>
</tr>
<tr>
<td></td>
<td>2. Annealing</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td>3. Extension</td>
<td>72</td>
</tr>
<tr>
<td></td>
<td>4. Final extension</td>
<td>72</td>
</tr>
<tr>
<td></td>
<td>5. Soak</td>
<td>4</td>
</tr>
</tbody>
</table>
Microsatellite genotyping

The diluted microsatellite amplification products were genotyped using the following quantities with 0.25 μl Gene-Scan™ 500 Rox™ size standard and 24 μl Hi-Di™ Formamide (which is used to denature the DNA samples). The samples were then loaded on to a ABI Prism™ 310 Genetic Analyzer machine (Applied Biosystems) (see Protocol 10) and run using POP4 polymer (Applied Biosystems) and the run module GS STR POP4 (1.0-mL) A. The Applied Biosystems Genescan® Analysis Software version 3.1 was used to read and size each sample according to the internal lane size standard (GeneScan 500 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 the labelled fragments. The microsatellite peaks (alleles) from each locus were scored according to their size individually for each sample. An internal size standard facilitates the determination of the exact size of each allele. The scores were then converted 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 haplotype from each population were detected and illustrated using pie charts. The geographic patterns of haplotype proportions of each population are also represented by a pie chart on a map of Thailand to show their geographical sources.

Analysis of the extent of diversity within and among the populations from each location was carried out using a statistical software program Popgene 32 (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) and the mean expected heterozygosity (h) (genetic diversity; Nei, 1973). In order to estimate population diversity and differentiation, the genetic diversity measures total gene diversity (Ht), gene diversity within population (Hd), gene differentiation among the populations (Gst) and gene flow (Nm) were made. The differentiation among populations was illustrated by a UPGMA dendrogram based on Nei's (1978) genetic distances.
4.4 Results

Five regions of simple sequence repeat (amplified using TeaSSR1, TeaSSR2, TeaSSR3, TeaSSR4 and TeaSSR5 primers; Table 4.2) were analysed to reveal microsatellite variation in 55 individuals from 11 populations of *A. setosa* and 40 individuals from eight populations of *G. tenella*. Three out of the five marker loci: TeaSSR3, TeaSSR4 and TeaSSR5, were successfully amplified and genotyped.

**Genetic diversity within and among the *Arundinella setosa* populations**

The genetic diversity within and among populations was assessed using the three microsatellite loci. A total of seven alleles were found in the *A. setosa* populations, three alleles were found in *trnL-trnF* intergenic spacer region (TeaSSR3), while the *23S-5S* internal transcribed spacer (TeaSSR4) and Herbicide binding protein D1 (*psbA*) regions (TeaSSR5) each generated two alleles. The frequencies of alleles detected over the three loci are given in Table 4.4.

**Table 4.4** Alleles detected from three chloroplast microsatellite loci overall populations of *Arundinella setosa*, including size range in base-pairs and frequency.

<table>
<thead>
<tr>
<th>Locus</th>
<th>Size in base pairs</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>trnL</em> intron and <em>trnF</em> intergenic spacer region</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Allele 1</td>
<td>301</td>
<td>0.055</td>
</tr>
<tr>
<td>TeaSSR3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Allele 2</td>
<td>302</td>
<td>0.836</td>
</tr>
<tr>
<td>Allele 3</td>
<td>303</td>
<td>0.109</td>
</tr>
<tr>
<td><em>23S-5S</em> internal transcribed spacer</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Allele 4</td>
<td>199</td>
<td>0.146</td>
</tr>
<tr>
<td>TeaSSR4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Allele 5</td>
<td>200</td>
<td>0.855</td>
</tr>
<tr>
<td>Herbicide binding protein D1 (<em>psbA</em>)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Allele 6</td>
<td>212</td>
<td>0.746</td>
</tr>
<tr>
<td>TeaSSR5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Allele 7</td>
<td>213</td>
<td>0.255</td>
</tr>
</tbody>
</table>

At TeaSSR3, the dominant allele was *Allele 2*, which was found in all populations at frequency of 0.836. For TeaSSR4, the dominant allele was *Allele 5*, which had a frequency of 0.855 and was presented in all populations except Mae Rim. At TeaSSR5, the dominant allele was *Allele 6*, which was occurred in all populations except Ban Tak, at a frequency of 0.746 (Table 4.4;
Figure 4.1). Overall three of the loci, the gene diversity within each locus was estimated using Nei's (1973) diversity statistic (Table 4.5). The locus *TeaSSR3* was found to be the most variable with gene diversity ($b = 38\%$), followed by *TeaSSR3* ($b = 28.6\%$) and *TeaSSR4* ($b = 24.9\%$).

![Figure 4.1](image)

**Figure 4.1** Frequency of *Arundinella setosa* alleles detected in Thailand. Each pie chart shows allele proportions from three microsatellite loci, the first third of the pie chart represents the proportion of alleles at *TeaSSR3* (alleles 1-3), the second third represents *TeaSSR4* (alleles 4-5) and the final third represents *TeaSSR5* (alleles 6-7).

A summary of the genetic diversity parameters is given in Table 4.6. Based on the number of alleles and the estimated gene diversity from three microsatellite regions, Phu Phan (North-Eastern) was the most diverse population, with gene diversity ($b = 26.7\%$), percentage of
polymorphic loci ($P$) = 66.7% and mean observed number of alleles per locus ($Na$) = 1.67) among the 11 populations, followed by Nam Nao (North-Eastern) and Huai Kha Khaeng (South-Western) populations with $b$ = 21.3%, $P$ = 66.7% and $Na$ = 1.67. Ang Khang and Mae Rim populations (Northern) were the least diverse and monomorphic at all loci ($b$ = 0%, $P$ = 0% and $Na$ = 1). The results of gene diversity between *A. setosa* groups (i.e. Northern, North-Eastern, North-Eastern and South-Western; Table 4.6), indicated that *A. setosa* from Northern Thailand was the most diverse group with $b$ = 33.8%, $P$ = 100% and $Na$ = 2.33, compared to the South-Western group with $b$ = 29.8%, $P$ = 100% and $Na$ = 2.33.

**Table 4.5** Overall descriptive statistics and mean gene diversity estimates (Nei, 1973) per locus from 11 populations of *Arradinella setosa*.

<table>
<thead>
<tr>
<th>Locus</th>
<th>Sample size</th>
<th>Allele number</th>
<th>Range of sizes detected (base-pairs)</th>
<th>Gene diversity ($h$)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>TeaSSR3</em></td>
<td>55</td>
<td>3</td>
<td>301-303</td>
<td>0.286</td>
</tr>
<tr>
<td><em>TeaSSR4</em></td>
<td>55</td>
<td>2</td>
<td>199-200</td>
<td>0.249</td>
</tr>
<tr>
<td><em>TeaSSR5</em></td>
<td>55</td>
<td>2</td>
<td>212-213</td>
<td>0.380</td>
</tr>
</tbody>
</table>

The extent of chloroplast gene diversity within and among *A. setosa* populations estimated using diversity within populations ($H_i$), total diversity ($H_T$) and gene differentiation among the populations ($G_{ST}$) is shown in Table 4.7. The mean value of $H_i$ was 0.291 and $H_T$ was 0.305. The mean value of $G_{ST}$ was 0.049 and the mean estimation of gene flow ($N_{m}$) was 10.65.

In total, seven haplotypes were detected using the combined data from three microsatellite loci. The frequency of each haplotype detected within the *A. setosa* populations is given in Table 4.8. The haplotypes detected have been mapped and are illustrated in Figure 4.2. Nine of 11 populations were polymorphic. The most common haplotype within the population was found to be 'Haplotype 1' which occurred at a frequency of 0.491. Haplotype 1 was found to occur within all locations with the exception of the populations from Ban Tak and Mae Rim. Ang Khang was found to be monomorphic for this haplotype. Haplotype 2 was found in Suan Phung and Mae Rim, the latter population was fixed for Haplotype 2. Haplotype 3 was restricted in Khao Sung. Haplotype 6 was unique in Nam Nao. Haplotype 7 was found to occur only in Ban Tak and Huai Kha Khaeng.
Table 4.6 Summary of genetic variation based on three SSR loci within populations and groups of *Arundinella setosa* (Groups 1-4).

<table>
<thead>
<tr>
<th>Population</th>
<th>Mean observed number of alleles per locus</th>
<th>Gene diversity (h)</th>
<th>Percentage of polymorphic loci (P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1=Suan Phung</td>
<td>1.33</td>
<td>0.160</td>
<td>33.3</td>
</tr>
<tr>
<td>A2=Khao Sung</td>
<td>1.67</td>
<td>0.187</td>
<td>33.3</td>
</tr>
<tr>
<td>A3=Khao Khieo</td>
<td>1.33</td>
<td>0.160</td>
<td>33.3</td>
</tr>
<tr>
<td>A4=Nam Nao</td>
<td>1.67</td>
<td>0.213</td>
<td>66.7</td>
</tr>
<tr>
<td>A5=Phu Phan</td>
<td>1.67</td>
<td>0.267</td>
<td>66.7</td>
</tr>
<tr>
<td>A6=Sai Yok Noi</td>
<td>1.33</td>
<td>0.107</td>
<td>33.3</td>
</tr>
<tr>
<td>A7=Wat Chan</td>
<td>1.33</td>
<td>0.160</td>
<td>33.3</td>
</tr>
<tr>
<td>A8=Ang Khang</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>A9=Mae Rim</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>A10=Ban Tak</td>
<td>1.33</td>
<td>0.107</td>
<td>33.3</td>
</tr>
<tr>
<td>A11=Huai Kha Khaeng</td>
<td>1.67</td>
<td>0.213</td>
<td>66.7</td>
</tr>
<tr>
<td><strong>Mean</strong></td>
<td><strong>1.39</strong></td>
<td><strong>0.143</strong></td>
<td><strong>24.22</strong></td>
</tr>
<tr>
<td>Group 1=Northern</td>
<td>2.33</td>
<td>0.338</td>
<td>100</td>
</tr>
<tr>
<td>Group 2=North-Eastern</td>
<td>1.67</td>
<td>0.246</td>
<td>66.67</td>
</tr>
<tr>
<td>Group 3=South-Eastern</td>
<td>1.33</td>
<td>0.160</td>
<td>33.33</td>
</tr>
<tr>
<td>Group 4=South-Western</td>
<td>2.33</td>
<td>0.298</td>
<td>100</td>
</tr>
<tr>
<td><strong>Mean</strong></td>
<td><strong>1.95</strong></td>
<td><strong>0.26</strong></td>
<td><strong>75</strong></td>
</tr>
</tbody>
</table>

Table 4.7 Nei's (1987) genetic diversity statistics and estimates of gene flow.

<table>
<thead>
<tr>
<th>Locus</th>
<th>Within population genetic diversity (Hs)</th>
<th>Total genetic diversity (Ht)</th>
<th>Estimate of the genetic differentiation among the populations (Gst)</th>
<th>Estimate of gene flow (Nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TeaSSR3</td>
<td>0.269</td>
<td>0.286</td>
<td>0.058</td>
<td>8.14</td>
</tr>
<tr>
<td>TeaSSR4</td>
<td>0.229</td>
<td>0.249</td>
<td>0.078</td>
<td>5.87</td>
</tr>
<tr>
<td>TeaSSR5</td>
<td>0.375</td>
<td>0.380</td>
<td>0.013</td>
<td>37.77</td>
</tr>
<tr>
<td><strong>Mean</strong></td>
<td><strong>0.291</strong></td>
<td><strong>0.305</strong></td>
<td><strong>0.049</strong></td>
<td><strong>10.65</strong></td>
</tr>
</tbody>
</table>
Table 4.8 Haplotypes detected of *Arundinella setosa* in Thailand.

<table>
<thead>
<tr>
<th>Haplotype</th>
<th>Base pair Combination (TeaSSR1, TeaSSR4, TeaSSR5)</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>302 200 212</td>
<td>0.491</td>
</tr>
<tr>
<td>2</td>
<td>302 199 212</td>
<td>0.145</td>
</tr>
<tr>
<td>3</td>
<td>301 200 212</td>
<td>0.018</td>
</tr>
<tr>
<td>4</td>
<td>303 200 212</td>
<td>0.091</td>
</tr>
<tr>
<td>5</td>
<td>302 200 213</td>
<td>0.200</td>
</tr>
<tr>
<td>6</td>
<td>303 200 213</td>
<td>0.018</td>
</tr>
<tr>
<td>7</td>
<td>301 200 213</td>
<td>0.036</td>
</tr>
</tbody>
</table>

Figure 4.2 Haplotype map of *Arundinella setosa* in Thailand using the combined chloroplast SSR data.
The *A. setosa* populations were divided into four groups according to floristic region (Northern, North-Eastern, South-Eastern and South-Western; Smitinand, 1958) (Table 4.9). The diversity within populations ($H_s$), total diversity ($H_t$) and gene differentiation among the populations ($G_{st}$) were estimated and compared among the groups to examine if the diversity produced any meaningful patterns from a geographical perspective (Table 4.10).

**Table 4.9** Grouping of *Arundinella setosa* populations from floristic regions of Thailand.

<table>
<thead>
<tr>
<th>Northern</th>
<th>North-Eastern</th>
<th>South-Eastern</th>
<th>South-Western</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mae Rim</td>
<td>Nam Nao</td>
<td>Khao Khieo</td>
<td>Suan Phung</td>
</tr>
<tr>
<td>Wat Chan</td>
<td>Phu Phan</td>
<td></td>
<td>Khao Sung</td>
</tr>
<tr>
<td>Ang Khang</td>
<td></td>
<td></td>
<td>Huai Kha Khaeng</td>
</tr>
<tr>
<td>Ban Tak</td>
<td></td>
<td></td>
<td>Sai Yok Noi</td>
</tr>
</tbody>
</table>

**Table 4.10** Nei’s (1987) genetic diversity statistics and estimates of gene flow from five groups of *Arundinella setosa*.

<table>
<thead>
<tr>
<th>Population</th>
<th>Within populations genetic diversity ($H_s$)</th>
<th>Total genetic diversity ($H_t$)</th>
<th>Estimate of the genetic differentiation among the populations ($G_{st}$)</th>
<th>Estimate of gene flow ($N_{m0}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Northern</td>
<td>0.067</td>
<td>0.338</td>
<td>0.803</td>
<td>0.122</td>
</tr>
<tr>
<td>North-Eastern</td>
<td>0.240</td>
<td>0.247</td>
<td>0.027</td>
<td>18</td>
</tr>
<tr>
<td>South-Eastern</td>
<td>0.160</td>
<td>0.160</td>
<td>0*</td>
<td>0*</td>
</tr>
<tr>
<td>South-Western</td>
<td>0.167</td>
<td>0.298</td>
<td>0.441</td>
<td>0.633</td>
</tr>
</tbody>
</table>

* Differentiation and gene flow are 0 for this group as it only contains one population and hence differentiation and gene flow cannot be calculated.

The structuring of *A. setosa* populations in Thailand was examined using a UPGMA analysis based on Nei’s (1978) unbiased genetic distance. This analysis was carried out to determine if there was an association with genetic distance and the geographic origins of the populations. The UPGMA tree is shown in Figure 4.2. Four groups were formed (Table 4.11). The geographic patterns of these groupings are illustrated in Figure 4.3.
Table 4.11 Division of *Arundinella setosa* populations according to Nei's (1978) unbiased genetic distance measure.

<table>
<thead>
<tr>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>Group 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>(blue)</td>
<td>(green)</td>
<td>(red)</td>
<td>(yellow)</td>
</tr>
<tr>
<td>Suan Phung</td>
<td>Khao Sung</td>
<td>Khao Khieo</td>
<td>Sai Yok Noi</td>
</tr>
<tr>
<td>Mae Rim</td>
<td>Wat Chan</td>
<td>Huai Kha Khaeng</td>
<td>Ban Tak</td>
</tr>
<tr>
<td></td>
<td>Nam Nao</td>
<td>Ang Khang</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Phu Phan</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figure 4.3 UPGMA dendrogram of *Arundinella setosa* populations based on Nei’s (1978) unbiased genetic distance. The scale bar below the tree indicates Nei's unbiased genetic distance of 5.
Figure 4.4 Overall patterns of *Arundinella setosa* population groupings in Thailand according to Nei’s (1978) unbiased genetic distance.
Genetic diversity within and among the *Gamotia tenella* populations

The genetic diversity within and among populations of *Gamotia tenella* was also assessed using the three plastid microsatellite loci used for *Arundinella*. In total, nine different alleles were found in *G. tenella* in Thailand. Three alleles were found at each locus. The frequencies of alleles detected over the three loci are given in Table 4.12.

A total of nine alleles were found in *G. tenella* populations. *Allele 2* (302 bp) of *TeaSSR3*, *Allele 5* (200 bp) of *TeaSSR4* and *Allele 8* (212 bp) of *TeaSSR5* were found to be the dominant alleles in most *G. tenella* populations at a frequency of 0.625, 0.90 and 0.40, respectively. Most of the dominant alleles were found in eight populations except *Allele 8* which was not found in the Um Phang population (Table 4.12; Figure 4.5). Overall three loci, the gene diversity within each locus was estimated using Nei's (1973) diversity statistic. The locus *TeaSSR5* was found to be the most variable (*b* = 65.9 %) among the three loci, followed by *TeaSSR3* (*b* = 52.3 %) and *TeaSSR4* (*b* = 18.4 %) (Table 4.13).

**Table 4.12** Alleles detected from three chloroplast microsatellite loci overall populations of *Gamotia tenella*, including size range in base-pairs and frequency.

<table>
<thead>
<tr>
<th>Locus</th>
<th>Size in base pairs</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>tm</em>-L intron and <em>tm</em> F intergenic spacer region</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>TeaSSR3</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Allele 1</td>
<td>301</td>
<td>0.275</td>
</tr>
<tr>
<td>Allele 2</td>
<td>302</td>
<td>0.625</td>
</tr>
<tr>
<td>Allele 3</td>
<td>303</td>
<td>0.100</td>
</tr>
<tr>
<td><em>23S</em>-5S internal transcribed spacer</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>TeaSSR4</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Allele 4</td>
<td>199</td>
<td>0.075</td>
</tr>
<tr>
<td>Allele 5</td>
<td>200</td>
<td>0.900</td>
</tr>
<tr>
<td>Allele 6</td>
<td>201</td>
<td>0.025</td>
</tr>
<tr>
<td>Herbicide binding protein D1 (<em>psbA</em>)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>TeaSSR5</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Allele 7</td>
<td>211</td>
<td>0.325</td>
</tr>
<tr>
<td>Allele 8</td>
<td>212</td>
<td>0.400</td>
</tr>
<tr>
<td>Allele 9</td>
<td>213</td>
<td>0.275</td>
</tr>
</tbody>
</table>
Figure 4.5 Frequency of *Garnotia tenella* alleles detected in Thailand. Each pie chart shows alleles proportions from three microsatellite loci, the first third represents the proportion of alleles at *TeaSSR3* (alleles 1-3), the second third represents *TeaSSR4* (alleles 4-6) and the final third represents *TeaSSR5* (alleles 7-9).

Table 4.13 Overall descriptive statistics and mean gene diversity estimates (Nei, 1973) per locus from eight populations of *Garnotia tenella*.

<table>
<thead>
<tr>
<th>Locus</th>
<th>Sample size</th>
<th>Allele number</th>
<th>Range of sizes detected (base-pairs)</th>
<th>Gene diversity</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>TeaSSR3</em></td>
<td>40</td>
<td>3</td>
<td>300-302</td>
<td>0.523</td>
</tr>
<tr>
<td><em>TeaSSR4</em></td>
<td>40</td>
<td>3</td>
<td>199-201</td>
<td>0.184</td>
</tr>
<tr>
<td><em>TeaSSR5</em></td>
<td>40</td>
<td>3</td>
<td>211-213</td>
<td>0.659</td>
</tr>
</tbody>
</table>
A summary of the genetic diversity parameters is shown in Table 4.14. Based on the number of alleles and the estimated gene diversity from three microsatellite loci, Phu Kradueng was the most diverse population among the eight populations (\( h = 37.3 \% \), \( P = 100 \% \) and \( Na = 2 \)), followed by Khao Yai and Na Yong populations with \( h = 32 \% \), \( P = 66.7 \% \) and \( Na = 1.67 \). The results of gene diversity values between \( G. \text{tenella} \) groups (i.e. Northern, North-Eastern, Eastern, South-Western and Peninsular; Table 4.14), showed that \( G. \text{tenella} \) from the Northern group was the most diverse, with \( h = 52 \% \), \( P = 100 \% \) and \( Na = 2.67 \), compared to the North-Eastern with \( h = 37.3 \% \), \( P = 100 \% \) and \( Na = 2.33 \).

**Table 4.14** Summary of genetic variation based on three SSR loci within populations and broad geographic groups of *Garnotia tenella* (Groups 1-5).

<table>
<thead>
<tr>
<th>Population</th>
<th>Mean observed number of alleles per locus</th>
<th>Gene diversity</th>
<th>Percentage of polymorphic loci</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(Na)</td>
<td>(h)</td>
<td>(P)</td>
</tr>
<tr>
<td>G1=Khao Yai</td>
<td>1.67</td>
<td>0.320</td>
<td>66.67</td>
</tr>
<tr>
<td>G2=Khao Kra Jome</td>
<td>1.67</td>
<td>0.267</td>
<td>66.67</td>
</tr>
<tr>
<td>G3=Thong Pha Phum</td>
<td>1.67</td>
<td>0.213</td>
<td>66.67</td>
</tr>
<tr>
<td>G4=Um Phang</td>
<td>1.67</td>
<td>0.213</td>
<td>33.33</td>
</tr>
<tr>
<td>G5=Doi Suthep</td>
<td>1.67</td>
<td>0.267</td>
<td>66.67</td>
</tr>
<tr>
<td>G6=Phu Hin Rong Kla</td>
<td>1.33</td>
<td>0.107</td>
<td>33.33</td>
</tr>
<tr>
<td>G7=Phu Kradueng</td>
<td>2</td>
<td>0.373</td>
<td>100.00</td>
</tr>
<tr>
<td>G8=Na Yong</td>
<td>1.67</td>
<td>0.320</td>
<td>66.67</td>
</tr>
<tr>
<td><strong>Mean</strong></td>
<td><strong>1.67</strong></td>
<td><strong>0.26</strong></td>
<td><strong>62.5</strong></td>
</tr>
<tr>
<td>Group 1=Northern</td>
<td>2.67</td>
<td>0.520</td>
<td>100.00</td>
</tr>
<tr>
<td>Group 2=North-Eastern</td>
<td>2.33</td>
<td>0.373</td>
<td>100.00</td>
</tr>
<tr>
<td>Group 3=Eastern</td>
<td>1.67</td>
<td>0.320</td>
<td>66.67</td>
</tr>
<tr>
<td>Group 4=South-Western</td>
<td>2.00</td>
<td>0.327</td>
<td>66.67</td>
</tr>
<tr>
<td>Group 5=Peninsular</td>
<td>1.67</td>
<td>0.320</td>
<td>66.67</td>
</tr>
<tr>
<td><strong>Mean</strong></td>
<td><strong>2.07</strong></td>
<td><strong>0.37</strong></td>
<td><strong>80.00</strong></td>
</tr>
</tbody>
</table>

The extent of chloroplast gene diversity within and among *G. tenella* populations also estimated using diversity within populations (\(H_0\)), total diversity (\(H_T\)) and gene differentiation among the populations (\(G_{ST}\)) is given in Table 4.15. The mean value of \(H_0\) was 0.385 and \(H_T\) was 0.455. The mean value of \(G_{ST}\) was 0.155 and the mean estimation of gene flow (\(N_{M} \)) was 2.73.
Table 4.15 Nei’s (1987) genetic diversity statistics and estimates of gene flow.

<table>
<thead>
<tr>
<th>Locus</th>
<th>Within populations genetic diversity ($H_s$)</th>
<th>Total genetic diversity ($H_t$)</th>
<th>Estimate of the genetic differentiation among the populations ($G_{ST}$)</th>
<th>Estimate of gene flow ($N_m$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TeaSSR3</td>
<td>0.440</td>
<td>0.524</td>
<td>0.160</td>
<td>2.627</td>
</tr>
<tr>
<td>TeaSSR4</td>
<td>0.160</td>
<td>0.184</td>
<td>0.129</td>
<td>3.368</td>
</tr>
<tr>
<td>TeaSSR5</td>
<td>0.555</td>
<td>0.659</td>
<td>0.158</td>
<td>2.675</td>
</tr>
<tr>
<td>Mean</td>
<td>0.385</td>
<td>0.455</td>
<td>0.155</td>
<td>2.734</td>
</tr>
</tbody>
</table>

Table 4.16 Haplotypes detected of *Garnotia tenella* in Thailand.

<table>
<thead>
<tr>
<th>Haplotype</th>
<th>Base pair combination (TeaSSR3, TeaSSR4, TeaSSR5)</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>300 200 211</td>
<td>0.150</td>
</tr>
<tr>
<td>2</td>
<td>301 200 212</td>
<td>0.275</td>
</tr>
<tr>
<td>3</td>
<td>300 200 211</td>
<td>0.100</td>
</tr>
<tr>
<td>4</td>
<td>301 200 213</td>
<td>0.150</td>
</tr>
<tr>
<td>5</td>
<td>300 200 213</td>
<td>0.050</td>
</tr>
<tr>
<td>6</td>
<td>301 199 211</td>
<td>0.050</td>
</tr>
<tr>
<td>7</td>
<td>301 201 211</td>
<td>0.025</td>
</tr>
<tr>
<td>8</td>
<td>302 200 213</td>
<td>0.075</td>
</tr>
<tr>
<td>9</td>
<td>302 200 212</td>
<td>0.025</td>
</tr>
<tr>
<td>10</td>
<td>300 200 212</td>
<td>0.075</td>
</tr>
<tr>
<td>11</td>
<td>301 199 212</td>
<td>0.025</td>
</tr>
</tbody>
</table>

In total, 11 haplotypes were identified using the combined data from three microsatellite loci. The frequency and the distribution of each haplotype are given in Table 4.16 and Figure 4.6. All populations were polymorphic. The most common haplotype within the population was found to be ‘Haplotype 2’ which occurred at a frequency of 0.275. Haplotype 2 was found within all locations except Um Phang and Phu Kradueng. Haplotype 6 and Haplotype 7 were found to be unique to Um Phang. Haplotype 8 and Haplotype 9 were found only in Doi Suthep. Haplotype 11 was restricted to Phu Kradueng.
Figure 4.6 Haplotype map of *Garnotia tenella* in Thailand using the combined chloroplast SSR data.

The *G. tenella* populations were divided into five groups according to floristic region (Northern, North-Eastern, Eastern, South-Western and Peninsular; Smitinand, 1958) (Table 4.17). The diversity within populations ($H_s$), total diversity ($H_T$) and gene differentiation among the populations ($G_{ST}$) were estimated and compared among the groups to examine if the diversity produced any meaningful patterns from a geographical perspective (Table 4.18).
Table 4.17 Groupings of *Garnotia tenella* populations from floristic region of Thailand.

<table>
<thead>
<tr>
<th>Northern</th>
<th>North-Eastern</th>
<th>Eastern</th>
<th>South-Western</th>
<th>Peninsular</th>
</tr>
</thead>
<tbody>
<tr>
<td>Um Phang</td>
<td>Phu Hin Rong Kla</td>
<td>Khao Yai</td>
<td>Khao Kra Jome</td>
<td>Na Yong</td>
</tr>
<tr>
<td>Doi Suthep</td>
<td>Phu Kradueng</td>
<td></td>
<td>Thong Pha Phum</td>
<td></td>
</tr>
</tbody>
</table>

Table 4.18 Nei’s (1987) genetic diversity statistics and estimates of gene flow from five groups of *Garnotia tenella*.

<table>
<thead>
<tr>
<th>Population</th>
<th>Within populations genetic diversity ($H_s$)</th>
<th>Total genetic diversity ($H_t$)</th>
<th>Estimate of the genetic differentiation among the populations ($G_{ST}$)</th>
<th>Estimate of gene flow ($N_m$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Northern</td>
<td>0.240</td>
<td>0.520</td>
<td>0.539</td>
<td>0.429</td>
</tr>
<tr>
<td>North-Eastern</td>
<td>0.240</td>
<td>0.373</td>
<td>0.357</td>
<td>0.900</td>
</tr>
<tr>
<td>Eastern</td>
<td>0.320</td>
<td>0.320</td>
<td>0*</td>
<td>-</td>
</tr>
<tr>
<td>South-Western</td>
<td>0.240</td>
<td>0.327</td>
<td>0.265</td>
<td>1.38</td>
</tr>
<tr>
<td>Peninsular</td>
<td>0.320</td>
<td>0.320</td>
<td>0*</td>
<td>-</td>
</tr>
</tbody>
</table>

* Differentiation and gene flow are 0 for this group as it only contains one population and hence differentiation and gene flow cannot be calculated.

The geographic structuring of *G. tenella* populations in Thailand was examined using a UPGMA analysis based on Nei’s (1978) unbiased genetic distance. This analysis was carried out to determine if there was an association with genetic distance and the geographic origins or ecological attributes of the populations. The UPGMA dendrogram is shown in Figure 4.7. Four groups were formed (Table 4.19). The geographic patterns of these groupings are illustrated in Figure 4.8.
Table 4.19 Division of *Garnotia tenella* populations according to Nei's (1978) unbiased genetic distance measure (1978).

<table>
<thead>
<tr>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>Group 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>(green)</td>
<td>(red)</td>
<td>(yellow)</td>
<td>(blue)</td>
</tr>
<tr>
<td>Khao Yai</td>
<td>Thong Pha Phum</td>
<td>Doi Suthep</td>
<td>Um Phang</td>
</tr>
<tr>
<td>Phu Kradueng</td>
<td>Na Yong</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Khao Kra Jome</td>
<td>Phu Hin Rong Kla</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figure 4.7 UPGMA dendrogram of *Garnotia tenella* populations based on Nei's (1978) unbiased genetic distance. The scale bar below the tree indicates Nei's unbiased genetic distance of 5.
Figure 4.8 Overall patterns of *Garnoria tenella* population grouping in Thailand according to Nei’s unbiased genetic distance.
4.5 Discussion

The genetic diversity within and between two native grass species in Thailand was examined in this study using three chloroplast microsatellite regions. Three and two alleles, differing by only a single nucleotide in length were found (Table 4.4 and 4.12) in the chloroplast microsatellite analysis of 11 populations (each contained five individuals) of *A. setosa*, while three alleles were found in eight populations of *G. tenella*.

*TeaSSR3* and *TeaSSR4* are mononucleotide repeat markers (poly A markers) and it is likely that the range in allele sizes recorded for these markers are due to losses or gains of an A nucleotides at this site. For *TeaSSR 5*, the repeat type reported by McGrath et al. (2006) was CTT. For this marker the length variation may have occurred elsewhere in the amplification product. Sequencing of the product would be required to obtain such information.

**Genetic diversity of Arundinella setosa**

Population genetic theory predicts that larger populations tend to maintain higher allelic diversity (Hedrick, 1985; Ellstrand and Elam, 1993), the highest levels of genetic diversity based on the chloroplast microsatellite evidence were found in the Northern, South-Western and North-Eastern regions, especially the Phu Phan and Nam Nao areas and would suggest that these might represent the largest effective population sizes of the species. However, within the Northern group, Ang Khang and Mae Rim populations were the least diverse containing only one haplotype. Generally, species with small geographic ranges tend to maintain less genetic diversity than geographically widespread species (Hamrick and Godt, 1989). Population genetic theory also predicts that small, isolated populations will lose genetic diversity and become increasingly differentiated from other populations (e.g. Young et al. 1996; Young and Brown, 1999; Buza et al., 2000). Ang Khang and Mae Rim are small isolated fragment areas. Ang Khang is located in an ecotone of deciduous dipterocarp forest and lower montane oak forest. The Mae Rim population is located in a small and isolated forest fragment of deciduous dipterocarp forest (personal observation). It is possible that the lack of genetic diversity in these small and more isolated populations could result from lack of gene flow (via seed dispersal) and increased genetic drift.

In addition, the investigated populations of *A. setosa* population in the Northern group showed low estimated values of genetic variation within the populations (*Hs*) with 6.7 %, high estimated value of genetic variation among the populations (*Gst*) with 80.3 % and low
estimated value of gene flow ($N_{m0}$) with 0.122. The results therefore revealed a low level of seed migration between the populations. But according to the summary of genetic diversity, the Northern group was the most diverse group. It may be because Northern Thailand is the largest forest area (Figures 4.9 and 4.10). At the same time, low gene flow estimates in the Northern group and high levels of population differentiation indicated that a considerable amount of geographical population genetic structuring has occurred in this region. Gene flow between populations is restricted and this is a critical parameter for explaining genetic differentiation among populations (Wright, 1931; Slatkin, 1985) and understanding processes of local adaptation (Barton, 2001). The level of gene flow can be affected by many factors (e.g. mutation, genetic drift, vagility and dispersal, natural selection, physical barriers, environmental selection factors, Slatkin, 1987; Hamrick and Godt, 1989; Lowe et al., 2004).

There are two possibilities that may account for the low gene flow in the Northern group: topographic barriers such as mountains (Figures 4.9 and 4.10) and habitat fragmentation. Although the presence of topographic barriers in Northern Thailand has an effect on the gene flow in some local regions, it has no effect on the overall gene flow. In Northern Thailand, many areas were first fragmented over 50 years ago due to shifting cultivation and hunting and other forms of development (e.g. roads and human settlements; Fox et al., 1995; Dearden, 1996). Although many areas in Northern Thailand are protected, the remaining forests are highly fragmented and isolated (Figures 4.9 and 4.10). The effects of habitat fragmentation on wildlife in Northern Thailand were studied by Pattanavibool and Dearden (2002) and Pattanavibool et al. (2004). The studies indicated that the variation of species abundance and species composition of wildlife, including birds and animals, is affected by forest fragmentation. Although there is no direct evidence, field observations strongly suggest that natural dispersal of *A. setosa* seed by animals, especially birds, rodents and mammals tends to be very high (personal observation). In addition, many animals such as ants, termites, birds and deer are considered as means of distributing grasses (Ridley, 1930; Bor, 1960; Chapman, 1996). The awn bearing lemma remains attached to the seed in *A. setosa* and forms an important part of the dispersal unit. Seeds can attach themselves to feathers, wool and hoofs mechanically by bristles, awns or trichomes (Razi, 1950) and can be transferred to a new area. Therefore, habitat fragmentation can reduce the dispersal ability of seed via wildlife species and hence this may be a limiting factor for seed mediated gene flow.

Interestingly, *Arundinella setosa* was reported to show polyploidy variation by Larsen (1963). He discovered that there are three races of chromosomes within the species. The 32-chromosomed race is the group of specimens which were collected from the dry deciduous
dipterocarp at altitudes between 450 and 500 m of Doi Sutep (Sørensen et al. no. 4591 and 4810, housed in Herbarium, University of Copenhagen, Denmark (C). The inflorescences are large diffuse with dark violet spikelets. The others are 48- and 54-chromosomed races. Both races are represented by specimens which were collected from higher altitude (e.g. Sørensen et al. no. 4395 and 5415, also housed in Copenhagen). The inflorescences are shorter and less diffuse than 32-chromosomed race and the spikelets are brown or light brown. He also suggested that more work was required before the 32-chromosomed plants could be separated into a new species. However, A. setosa has been shown in this thesis (chapter 2) and other works (Keng, 1936; Bor, 1955; Sun and Phillips, 2006) to be a variable species in terms of morphology (for example the morphological key to species reveals that A. setosa is a variable species). The differences in the inflorescences and spikelets are not good enough to separate taxa (floristic treatment, in Chapter 2). In this study, the Mae Rim population is the only population which has the characters of the 32-chromosomed plant and is monomorphic at three loci. However, another population from Ang Khang, which has the characters of 48- and 54-chromosomed plant, is also a monomorphic, but is a different haplotype. Although the result from chloroplast microsatellites might not be variable enough to obtain high exclusion probabilities for parentage assignment because their uniparental mode of inheritance, they can represent a useful adjunct to studies using nuclear microsatellites. It is not known what breeding barriers exist between the chromosome races reported by Larsen (1963) but it would be interesting to obtain plants for the future chromosome counts or ploidy estimation via flow cytometry.

A total of seven haplotypes were detected using the combined data of three chloroplast microsatellite loci (Table 4.8; Figure 4.2). These haplotypes are widely distributed across Thailand. The pattern of haplotype distribution reflects the pattern of plant dispersal via seed. The most common haplotype within A. setosa populations is Haplotype 1 with a frequency of 0.49. This haplotype occurred in all populations except Ban Tak and Mae Rim. The average number of haplotypes per population was two and the maximum was three in Phu Phan and Khao Sung populations (out of a possible seven).

The investigated population of A. setosa showed the estimated value of genetic variation within the populations ($H_0$) with 29.1 % and the estimated value of genetic variation among the populations ($G_{ST}$) with 4.9 %. The overall diversity ($H_T$) was estimated to be 30.5 % and the estimates of gene flow ($N_{m}$) was 10.65 (Table 4.7). The results revealed a high level of migration between the populations. The low $G_{ST}$ value suggested that the most variation in haplotypes is distributed within populations and not among populations. The high value of genetic variation within Arundinella populations suggests that they consist of multiple genets.
The populations are not, therefore, composed of predominantly asexually reproducing individuals. McGrath et al. (2007), used chloroplast microsatellite markers to examine the genetic diversity of *Lolium perenne* (perennial ryegrass) ecotypes, and recorded a low value of $G_{ST}$ (23.8 %) in Irish ecotypes. Their result indicated a high level of gene flow, via seed, had occurred between the ecotypes. In this study, the results are similar to the study on *Lolium*. This would suggest that the movement of seed between the populations was relatively high. Nevertheless the high gene flow can occur through pollination mechanisms which are also one of the first factors to determine gene flow levels in plant populations (Govindaraju, 1988), the chloroplast microsatellite markers used in this study cannot infer pollen-mediated gene flow as they are maternally inherited.

### Genetic diversity of *Gamotia tenella*

The total gene diversity values of *G. tenella* showed that the Northern group is the most diverse and also indicated that Phu Kradueng, Khao Yai and Na Yong populations are particularly variable (Table 4.14). Indeed, they could, on the basis of population genetic theory, represent the populations with the largest effective population size. A total of 11 haplotypes were detected using the combined data of the three chloroplast microsatellite loci (Table 4.16; Figure 4.6). The most common haplotype within the *G. tenella* populations is Haplotype 2 with a frequency of 0.275. This haplotype occurred in all populations except the Um Phang population. The average number of haplotypes per population was three and the maximum was four in Na Yong populations (out of a possible 11). All 11 haplotypes are widely distributed across Thailand with no obvious geographic trend in their distribution. The pattern of the *G. tenella* haplotype distribution also reflects the pattern of plant dispersal via seed (as in *Arundinella* discussed above).

The extent of haplotype diversity within *G. tenella* populations ($H_o$) was found to be 26 %. The overall diversity ($H_T$) was estimated to be 45.5 % and the estimated value of genetic variation among the populations ($G_{ST}$) was estimated to be 15.5 % and the estimate of gene flow ($N_{m0}$) was 2.74 (Table 4.15). The results revealed a high level of migration via seed between the populations. The low $G_{ST}$ value suggested that the most variation in haplotypes is distributed within populations and not among populations. In *Gamotia*, the high value of genetic variation within the populations suggested that the populations are not entirely clonal. According to field observation, seed of *G. tenella* is dispersed by wind, water, animal (personal observation) especially birds (Carlquist, 1967). The estimation of the total gene diversity of *G. tenella* from Northern, North-Eastern, Eastern, South-Western and Peninsular Thailand is given in Table 281.
4.18. The Northern group had the lowest estimate of gene flow \( (N_{sb}) \) in Thailand with 0.429. Although gene flow in the Northern group was the lowest, the genetic diversity was high and this pattern is likely to be explained by the same factors discussed for \( A. \ setosa \) (above).

The overall \( G_{ST} \) values were three times higher for \( Garnotia \) than \( Arundinella \) (15.5 % vs. 4.9 %, respectively). This would suggest that there was less gene flow between \( Garnotia \) populations or that they were more susceptible to the effects of genetic drift. Plant life form also affected the \( G_{ST} \) value. Long-lived perennials tend to have lower levels of \( G_{ST} \) than shorter-lived perennial or annuals (Hamrick and Godt, 1996; Godt and Hamrick, 1998, Nybom,2004). This may explain why the annual \( Garnotia \) has a higher \( G_{ST} \) value than a perennial \( Arundinella \).

Insufficient data are available regarding the breeding system, dispersal mechanisms and population sizes of these two species to determine the major reason for this difference in population differentiation.

Geographic distribution of \( Arundinella \ setosa \) and \( Garnotia \ tenella \) chloroplast haplotypes

In order to clarify the complicated patterns of \( A. \ setosa \) and \( G. \ tenella \) in Thailand, the UPGMA dendrogram was constructed using Nei's (1978) unbiased genetic distance based on their similarity to each other at the three chloroplast microsatellite loci. The analysis indicated four major groups among 11 populations of \( A. \ setosa \) (Table 4.11; Figure 4.3) and four major groups among eight populations of \( G. \ tenella \) (Table 4.19; Figure 4.7). The overall patterns of \( A. \ setosa \) and \( G. \ tenella \) populations have been compared to their geographic locations (Figures 4.4 and 4.8). In this study, there is no obvious correlation of biogeographical distribution in \( A. \ setosa \) and \( G. \ tenella \) which would be consistent with the Thai floristic regions (Smitinand, 1958) (Figure 1, Appendix 2). This could be because of the small number of samples per population used in this study (Waldren, pers. comm.). The other possibility would be because there are only a few major geographical or ecological barriers to gene flow in Thailand (Figures 4.9 and 4.10). The studies by McGrath et al. (2007) on \( Lolium \) indicated that gene flow via seed was extensive over the geographic scale of Europe. The data presented here indicate that such gene flow is also likely in \( Arundinella \) and \( Garnotia \). These two species do not appear to be seriously affected by the processes of genetic drift that might be associated with population genetic bottlenecks caused by habitat fragmentation. These two species are not restricted to Thailand and have a wide geographic distribution. This also supports the hypothesis that they are good dispersers which can tolerate a broad range of ecological conditions.
However, the grouping in the analysis indicated a general East-West divide, with only Group 2 of *A. setosa* and Group 1 of *G. tenella* predominant in North-Eastern populations. On the other hand some haplotypes were restricted in Northern, South-Western and Peninsular Thailand and were not found in the North-Eastern region (Groups 1 and 4 of *A. setosa* populations; Group 2 of *G. tenella* populations). Although, the populations from the North-Eastern region were in the same group with Northern and South-Western populations, the North-Eastern populations were always grouped together and separated from the others. This may be because Nam Nao, Phu Phan, Khao Yai and Phu Kadueng populations are located in the Khorat plateau basin which is a large area of Mesozoic continental sedimentary rocks of the Khorat group which covers approximately 200,000 km². This area consists of North-Eastern and Eastern Thailand, Laos, Cambodia, some parts of Vietnam and some parts of Malaysia (Figure 4.11) (Hite, 1973; Metcalfe, 1996; Bunopas, 1981; Singharajwarapan and Berry, 2000; Charusiri et al., 2006). The local environmental conditions on the Khorat plateau may have had an impact on the genetic structure of *A. setosa* and *G. tenella* because environmental conditions can influence genetic drift and selection (Slatkin, 1985). However, more sites from Thailand and neighbouring countries and more number of samples per population used in the study need to be tested using these primers or additional primers in order that the proposed genetically distinct populations of *A. setosa* and *G. tenella* can be clarified.

**Conservation Considerations**

In order to determine the conservation strategies for plants, measures of genetic variation between and within populations are important considerations because they provide useful information about the preservation of genetic diversity (Hamrick *et al.*, 1991; Hamrick and Godt, 1996). In this study, the result from chloroplast microsatellite of the two grass species from Thailand detected a reduction of genetic diversity especially in Northern Thailand. Evidence indicated that habitat fragmentation might be adversely influencing the genetic diversity of *A. setosa* and *G. tenella* populations. They are grasses associated with forests and forests are becoming more fragmented. However, the reaction of populations to habitat fragmentation can differ strongly between the species. The severity of effects depends on many factors such as a species breeding system, capacity of survival, immigration and colonization, dependency on pollinators and on the time since fragmentation (Lienert, 2004).
Figure 4.9 Maps of Thailand; showing topography (top left), vegetation types (top right), and annual rainfall (mm) between 1950 and 1997 (bottom). Maps obtained from http://www.rrcap.unep.org; the bottom one from http://tiwrm.hpcc.nectec.or.th/GIS/iso rainfall/main.html. A1-A11 represent the locations of *Arundinella setosa* populations; G1-G8 represent the locations of *Garnotia tenella* populations (see Table 4.1).
Figure 4.10 Maps of Thailand; showing protected area (left) in Thailand and main landforms (bottom right) in Thailand. Maps obtained from www.mekong-protected-areas.org and http://www.ldd.go.th/FAO/zh/th/th.html. A1-A11 represent the locations of *Arundinella setosa* populations; G1-G8 represent the locations of *Gamotia tenella* populations (Abbreviations, see Table 4.1).
Figure 4.11 Map of Khorat plateau, North-Eastern Thailand and parts of Laos (top). Maps obtained from http://www.wikipedia.org; schematic geological maps of Khorat Plateau (bottom), showing the regional map of mainland Southeast Asia with Khorat Plateau (shaded). Map obtained from Charusiri et al. (2006). A4 and A5 represent the locations of *Arundinella setosa* populations; G1, G7 and G8, represent the locations of *Garnotia tenella* populations (Abbreviations, see Table 4.1).
Members of Poaceae are so different from most other angiosperms that they require many technical terms to describe their structures. Understanding their morphology is important for taxonomy, systematics and many other areas of comparative biology. Thus the basic morphological characters of grasses, which are necessary to understand for identification, classification and floristics (Chapter 2), were discussed already in Chapter 1 of this thesis.

The Flora of Thailand project is an International project aiming to produce a complete floristic treatment of the entire vascular flora. Species-rich families such as Poaceae are a priority and this thesis has provided complete floristic treatments of tribe Arundinelleae, and subtribes Chionachninae, Dimeriinae and Germainiinae of tribe Andropogoneae for that project (Chapter 2). The tribal and subtribal classifications used in these treatments are based on Clayton and Renvoize (1986). Keys to genera and species were also constructed. Species descriptions comprising synonyms, typifications, illustrations, distribution maps and lists of specimens examined have been provided. Fourteen species and three genera of tribe Arundinelleae in Thailand were reported, including eight species of *Arundinella*, namely *A. bengalensis*, *A. cochinchinensis*, *A. decempedalis*, *A. boleoides*, *A. kerrii*, *A. kokutensis*, *A. nepalensis* and *A. setosa* (Figures 2.1-2.9), five species of *Garnotia*, namely *G. acutigluma*, *G. ciliata*, *G. patula*, *G. tenella* and *G. thailandica* (Figures 2.10-2.14), and one species of *Jansenella*, *J. griffithiana* (Figures 2.15 and 2.16, see also Table 2.3).

Three species and two genera of subtribe Chionachninae were found. They are *Polytoca digitata*, *P. wallichiana* and *Chionachne massiei* (Figures 2.17-2.21 and Table 2.4). Seven species of *Dimeria* were recognised in subtribe Dimeriinae, namely *D. fuscescens*, *D. kerrii*, *D. kurzii*, *D. leptorhachis*, *D. ornithopoda*, *D. pubescens* and *D. sinensis* (Figures 2.22-2.28 and Table 2.5). For Germainiinae, twelve species from two genera were recorded, namely *Apocops collinus*, *A. courtallumensis*, *A. intermedius*, *A. mangalorensis*, *A. siamensis* *A. wrightii*, *Germainia capitata*, *G. khayana*, *G. lanipes*, *G. pilosa*, *G. thailandica* and *G. thorelii* (Figures 2.29-2.34 and Table 2.6). Two new records for Thailand were found, *G. ciliata* from Northern Thailand and *J. griffithiana* from Peninsular Thailand. The new distribution data of these species in Thailand extends their recorded geographic range from previous reports. Three names, *Arundinella kerrii* C. Hambananda ex A. Teerawat. & Sungkaew, *Arundinella kokutensis* Teerawat. & Sungkaew and *Dimeria kerrii* C. E.
Hubb. ex Teerawat. & Sungkaew, were described as taxa new to science (Teerawatananon et al., submitted, see manuscript 1, Appendix 1; Teerawatananon et al., in prep., see manuscript 2, Appendix 1). All species are also endemic to Thailand. Two species, *Arundinella kerrii* and *Dimeria kerrii*, are known only from the type collection made in 1924 and 1928, and may be now extinct, probably because of loss of habitat or lack of recruitment from other areas. *Arundinella kokutensis* is also only known from the type locality on Kut Island. It is extremely restricted in distribution and specific habitat, only occurring in small populations on Kut Island. It should therefore be given high conservation priority.

Phylogenetic relationships among major subfamilies in Poaceae and among major tribes within subfamily Panicoideae were evaluated using parsimony analysis of maternally inherited plastid *trnL-F* and *atpB-rbcL* sequences and biparentally inherited *ITS* of nuclear ribosomal DNA (Chapter 3). A total of 132 plants were analysed, representing six subfamilies and representatives from five tribes of Panicoideae sensu Clayton and Renvoize (1986), which included 42 genera in 11 subtribes of Andropogoneae, four genera of Arundinelleae, one genus of Eriachneae, two genera of Isachneae, and 18 genera in four subtribes of Paniceae (Table 3.1). A comparison of strict consensus and bootstrap support values of the main taxa or the major groups, obtained from individual (Figure 3.4-3.7 and 3.10-3.11) and combined (Figures 3.3-3.9 and 3.12-3.13) phylogenetic analyses of the three gene regions, is shown in Table 3.2. A summary diagram inferred from these findings is provided in Figure 5.1. The PACCMAclade, consisting of Panicoideae sensu stricto, Arundinoideae, Centothecoideae sensu GPWG (2001), Chloridoideae, Micrairoideae sensu Sánchez-Ken et al. (2007), Aristidoideae and Danthonioideae, was well resolved but the relationships among the seven subfamilies were largely unresolved. However, this study provided supporting evidence for the close relationship between Aristidoideae (*Aristida*) and Chloridoideae (*Tripogon, Microchloa* and *Sporobolus*). Subfamily Micrairoideae, with Eriachneae and Isachneae, was clearly monophyletic, but monophyly of Micrairoideae with the three tribes, Eriachneae, Isachneae and Micraireae was not well supported. Unfortunately, this study could not resolve the relationships among these tribes. However, the results in this study provided more support for the monophyly of tribe Eriachneae but did not support the monophyly of tribe Isachneae (as it did not consistently form a clade). The monophyly of *Isachne* was, however, supported consistently. Reclassification of subfamily Micrairoideae was previously undertaken by Sánchez-Ken et al. (2007).

Panicoideae sensu stricto, comprising of tribes Andropogoneae + *Arundinella* + *Carnoria* and Paniceae, were supported as monophyletic and sister to a clade represented by *Danthoniopsis*.
and *Tristachya*. Within Panicoideae, only the clade consisting of tribe Andropogoneae + *Arundinella* + *Garnotia* was supported by bootstrapping. None of the analyses supported the monophyletic status of tribe Paniceae. Paniceae has been shown to be paraphyletic in some previous studies (Gomez-Martinez and Culham, 2000; Giussani et al., 2001), but the results from this study do not support either the separation of tribe according to the basic chromosome number (e.g. Gomez-Martinez and Culham, 2000; Giussani et al., 2001) or the subtribal classification of Clayton and Renvoize (1986). Two Paniceae clades, according to morphological characters (Gomez-Martinez and Culham, 2000; Giussani et al., 2001; Aliscioni et al., 2003), were supported in this study. One contained the bristle clade (excluding *Cenchrus*) and a rare Australian non bristle-bearing genus, *Alexfloydia*. Another contained the members of the forest shade clade as defined by Giussani et al. (2001), including a new member, *Cyrtococcum*. However, the monophyly of forest shade clade remains ambiguous because *Ottochloa* and *Alloteropsis*, which also are forest shade loving grasses (Christin et al., 2008; Ibrahim et al., 2009), were not formed a monophyletic group with the remaining forest shade clade. Furthermore, this study resolved a sister relationship between two endemic and rare Australian Paniceae *Homopholis* and *Walwhalleya*.

Tribe Arundinelleae was found to be polyphyletic. This is consistent with the results of previous studies (e.g. Mason-Gamer et al., 1998; Hilu et al., 1999; Spangler et al., 1999; Mathews et al., 2000; Giussani et al., 2001; Mathews et al., 2002; Sánchez-Ken and Clark, 2007). This study supported the separation of *Arundinella* and *Garnotia* from the remaining Arundinelleae and the inclusion of both genera in their own subtribes (Arundinellinae Honda sensu stricto and Garnotiinae Pilger) within the Andropogoneae. The results suggest that the tribe Arundinelleae should be abandoned as a taxonomic group within the centothecoid + panicoid clade. However, the positions of these two taxa within Andropogoneae, relative to other subtribes are still unclear.

Tribe Andropogoneae were found to be monophyletic only if *Arundinella* and *Garnotia* were included. Within the tribe, the separation of monoecious taxa of Andropogoneae, comprising Chionachninae, Coicinae and Tripsacinae, was supported. However, no evidence was found for the relationships among these subtribes. Chionachninae, represented by two genera *Chionachne* and *Polytoca*, were found to be monophyletic in all analyses, except the ITS analysis due to the separation of *Chionachne*, represented by *C. cyathopoda* and *C. massiei*. Furthermore, the monophyly of *Polytoca*, represented by *P. digitata* and *P. wallachiana*, was strongly supported. The low level of genetic divergence between the two taxa indicates that *P. wallachiana* should be placed within *Polytoca* rather than *Cyathorhachis* as adopted by Jannink and Veldkamp (2002).
The monophyly of Coicinae as demonstrated by previous studies (Bomblies and Doebley, 2005) was confirmed in the ITS study but not in the trnL-F and atpB-rbcL analyses. The monophyly of Tripsacinae, including Zea and Tripsacum, was also resolved. Surprisingly, this clade was grouped with the clade consisting of the Garnotia clade plus Eremochloa, but there is no obvious morphological character to arrange these taxa together. These relationships are inconsistent with all molecular studies to date (e.g. Spangler et al., 1999; Spangler, 2000; Lukens and Doebley, 2001; Hodkinson et al., 2002b; Mathews et al., 2002; Bomblies and Doebley, 2005). However, the hypothesis of the inclusion of Tripsacinae and Garnotia clade plus Eremochloa only received low bootstrap support.

Subtribe Germainiinae, represented by Apoapis and Germainia, were resolved as monophyletic. This relationship has never been reported before by phylogenetic analysis. The monophyly of samples of the monotypic subtribe Dimeriinae was also resolved and Ischaeminae was found to be its sister group. In addition, five distinct groups within Andropogoneae corresponding to three genera from subtribe Rottboelliinae (Hackelochloa, Hemarthria and Mnesithea), an agamic complex (Bothriochloa, Capillipedium and Dichanthium), a sub-basal awn group (Arthraxon and Theleptagon), the core Andropogoneae genera (Hyparrhenia Cymbopogon, Schizachyrium and Andropogon), and a Themeda group, were supported as monophyletic.

In general, the relationships among many genera within tribe Andropogoneae remain unresolved due to morphological complexity of the tribe, perhaps as a result of hybridization, polyploidy and apomixis which were found in many genera by previous studies (e.g. Harlan and De Wet, 1963; De Wet and Harlan, 1970; Carman and Hatch, 1982; Hodkinson et al., 2002b). Discrepancies between the results from this molecular study and morphological studies of Clayton and Renvoize (1986), Renvoize and Clayton (1992) and Kellogg and Watson (1993) also reflect the numerous convergences in most morphological characters, especially spikelet structure. More studies are needed to find a better way of subdividing the remaining members of Andropogoneae into subtribal taxa and to better determine their relationships.

In this study, including a large number of Andropogoneae taxa and using three non-coding markers from both chloroplast and nuclear ribosomal DNA (trnL-F, atpB-rbcL and ITS) we have encountered difficulties with resolution due to short branch lengths of internal branches relative to terminal branches of the trees. The short branches along the backbone of the tree and poor branch support may be due to an insufficient quantity of data or might be explained by rapid radiation (Spangler et al., 1999; Kellogg, 2000; Fishbein et al., 2001). Although the
rapid radiation hypothesis has previously been proposed by several phylogenetic analyses (e.g., Spangler et al., 1999; Kellogg, 2000; Fishbein et al., 2001; Mathews et al., 2002; Wortley et al., 2005; Moore et al., 2007; Jian et al., 2008), the theory behind such empirical observations is not well developed or reported. These patterns have been explained by suggesting that the phylogenetic signal to resolve the inter-relationships of the lineages has been lost during evolution. These short branches along the backbone and poor branch support may be resolved by adding more data and more taxa, including further non-coding gene regions which can help to assign species to clades due to its high ability to resolve phylogeny at the intrageneric level (Gielly and Taberlet, 1994; Soltis and Soltis, 1998; Kelchner, 2000) and also coding region genes which have been shown to be sufficiently variable to be useful for phylogeny reconstruction at relatively low taxonomic levels (Soltis and Soltis, 1998; Bremer et al., 2002).

Very little is known about the phylogeography of Thai plant species and, to our knowledge, no studies have assessed population genetic variation across geographical space for Thai grasses. Two native species Arundinella setosa and Gamotia tenella which are very variable in morphology and are geographically widespread were chosen as model species to investigate the intrapopulational and interpopulation variation using the plastid microsatellite markers (Chapter 4). A total of seven alleles were detected and four groups from 11 populations were defined in A. setosa (Figures 4.1, 4.2 and 4.4), while in G. tenella, a total of nine alleles and four groups from eight populations were found (Figures 4.5, 4.7 and 4.8). The patterns of haplotype distributions (Figures 4.2 and 4.6) of both grasses reflect patterns of plant dispersal via seed. All populations of A. setosa, except Ang Khang and Mae Rim, contained more than one haplotype. In addition, the low population genetic differentiation among populations in both grasses (Arundinella = 0.049; Gamotia = 0.155) suggested that the most variation in haplotypes is distributed within populations and not among populations. The high value of genetic variations within Arundinella and Gamotia populations suggests that they consist of multiple genets. The populations are not, therefore, composed of predominantly asexually reproduced individuals. However, only a single haplotype was found in Ang Khang and Mae Rim populations (Table 4.6 and Figure 4.2). The lack of genetic diversity in these small and more isolated populations could result from lack of gene flow via seed dispersal and increased genetic drift. The highest value of genetic diversity from both grass species was found in Northern Thailand, while the population of A. setosa from Phu Phan (North-Eastern) and G. tenella from Phu Kradueng population (North-Eastern) could, from the data, be predicted to have the largest effective population size. At the same time, low gene flow estimates in the Northern group and high levels of population differentiation indicated that a considerable
amount of geographical population genetic structuring has occurred in this region. Two possibilities, which may cause the low gene flow in the Northern group, are topographic barriers and habitat fragmentation. Although the presence of topographic barriers in Northern Thailand has an effect on the gene flow in some local regions, it has no effect on the over all gene flow. In this study, there was no obvious correlation of genetic structure in \textit{A. setosa} and \textit{G. tenella} populations which would be consistent with the Thai floristic regions. However, the groupings in the analysis indicated a general East-West divide. It seems that the local environmental conditions may have had an impact on the genetic structure of \textit{A. setosa} and \textit{G. tenella}. However, in order to clarify the phylogeography of \textit{A. setosa} and \textit{G. tenella}, which have wide geographic distributions, more sites from Thailand and neighboring countries need to be tested using these and additional markers.

**Conclusions**

This thesis has provided a comprehensive taxonomic account of tribe Arundinelleae sensu lato, and subtribes Chionachninae, Dimeriinae and Germainiinae of tribe Andropogoneae, in Thailand which will be used directly for the Flora of Thailand project. The phylogenetic relationships inferred from the \textit{trnL-F}, \textit{atpB-rbcL}, \textit{ITS} and combined datasets in this thesis were generally in agreement with previous molecular studies at the basic subfamilial and tribal levels. The molecular phylogeny presented here indicated that \textit{Arundinella} and \textit{Garnotia} should be placed in the Andropogoneae as their own subtribes although patterns of intrageneric relationships within this tribe remained obscure. It is clear that the subtribal classification of Clayton and Renvoize (1986) requires considerable revision but some of their subtribes were supported as monophyletic. In addition, population level variation of two native grasses \textit{Arundinella setosa} and \textit{Garnotia tenella} was assessed to investigate the biogeographical patterns of population genetic structure and the genetic variation at the intrapopulational level as well as between geographically widely separated populations. The data suggest that most variation in haplotypes is distributed within populations and not among populations.

The following subtribes and genera are recognised in the taxonomic treatment of the thesis (Chapter 3).


Arundinellinae as treated here includes only its type genus: \textit{Arundinella}. This subtribe was first established with three genera: \textit{Arundinella}, \textit{Phaenosperma} and \textit{Thysanolaena} (Honda, 1930).
However, recent systematic treatment of Poaceae placed *Phaenosperma* in Pooideae and grouped *Thysanolaena* within Centrocoideae (GPWG, 2001).


Included genus: *Garnotia*.

Manuscripts resulting from work in this thesis that are published, submitted for publication, or in preparation are listed below:

3) Teerawatananon, A., S. Sungkaew and T. R. Hodkinson. Two new endemic grasses (Poaceae, Panicoideae) from Thailand *Arundinella kerrii* and *Dimeria kerrii* [in prep.].  
Figure 5.1 Cladogram showing relationships within PACCMA clade. Summary is inferred from the \text{trnL-F}, \text{atpB-rbcL}, \text{ITS} and combined analyses. Examples of spikelets of each group are shown. A. diagram of a multi-flowered pooid spikelet; B. longitudinal section of a pooid embryo showing presence of an epiblast; C. longitudinal section of a panicoid embryo showing presence of a scutellar tail and the elongated mesocotyl internode; D. diagram of a two-flowered panicoid spikelet; E. simple starch grain of \textit{Sorghum}. c-coleoptile; co-coleorhiza; ep-epiblast; mi-mesocotyl; s-scutellum; st-scutellar. Spikelet pictures obtained from Hitchcock (1935), Gilliland (1971), Clayton \textit{et al.} (1974), Welzen (1981) and Noltie (2000). A, D from Clark (2004); B, C from GPWG (2001); E. from \url{http://www.fhsu.edu/biology/thomasson/starch.htm}. Bold lines indicate branches supported by all data combined (bootstrap>50).
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Appendix 1

MANUSCRIPT 1
[Submitted to Kew Bulletin]

A new species of *Arundinella* Raddi (Poaceae, Arundinellae) from south-eastern Thailand

Atchara Teerawatananon¹,², Sarawood Sungkaew², and Trevor R. Hodkinson³

Summary. *Arundinella kokutensis* Teerawat. & Sungkaew is described and illustrated here as a new species from Kut Island, Trat Province, Thailand.

Key words. *Arundinella*, Gramineae, Poaceae, Thailand.

INTRODUCTION

The genus *Arundinella* was established by Raddi (1823) based on the type species *Arundinella brasiliensis* Raddi collected from Brazil but now recognised as *Arundinella hispida* (Willd.) Kuntze. This genus is distinguished by its paired, 2-flowered spikelets which disarticulate above the glume, scabrid upper lemmas, punctiform hilums and short membranous ligules. Clayton & Renvoize (1986) reported that there are about 50 species of *Arundinella*, distributed chiefly in Asia, an estimate that has now been raised to approximately 60 species (Sun & Phillips, 2006).

Among the specimens of the genus *Arundinella* collected from Thailand, a very distinctive collection, Charoenphol et al. 5104 from Kut Island, Trat Province, Southeastern Thailand was previously labelled as *Arundinella metzii* Hochst. ex Miq. After re-collecting and critically

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examining both living and herbarium specimens, from the same locality, their morphology
could not be matched with the species descriptions or type specimens of *A. metzii* (Hohenacker
297 (K)) nor any other *Arundinella* species. Neither do they fit within the natural range of
morphological variation found within those other species. Therefore, a new species *A.*
kokutensis* Teerawat. & Sungkaew, is described here.

**Arundinella kokutensis** Teerawat. & Sungkaew *sp. nov.* Species *A. metzii* Hochst. ex Miq.
et *A. holcoidi* (Kunth) Trin. affinis, sed ab illis ramis et pedicellis fere glabris, glumis imus
glabris, aristis lemmatium superiorium 5 – 6.5 mm longis, staminibus 2 differt. Typus:
Charoenphol et al. 5104 (holotypus K!; isotypi BKF!, C!, E!, NY!). Fig. 1.

Annual. *Cuims* slender, 5 – 80 cm tall, erect, shortly decumbent at base; nodes sparsely
pubescent. *Leaf-sheaths* 2 – 8 cm long, hispid with tubercle-based hairs especially along
margins. *Ligule* a ciliate membrane, 0.8 – 1.3 mm long. *Leaf-blades* oblong-lanceolate, (1 –)5 –
30 x (0.3 –)0.6 – 1.3 cm, hispid with tubercle-based hairs on both surfaces, margins
sometimes scaberulous. *Panicles* open, sometimes drooping, (3 –)10 – 25 cm long; racemes (2 –)4 – 10 cm long, alternate, rhachis almost glabrous, rarely scabrous. *Spikelets* green, ovate-
lanceolate, 3 – 4 x 0.6 – 1 mm; pedicels 0.5 – 6 mm long, glabrous; lower glumes ovate-
lanceolate, 2 – 2.5 x 0.5 – 1 mm, almost glabrous, sometimes scabrous at apex, acuminate to
aristate, 3 – 5-nerved; upper glumes ovate-lanceolate, 3 – 3.5(– 4) x 0.5 – 1 mm, glabrous to
sparsely hispid with tubercle-based hairs, caudate, recurved, 5-nerved; lower florets barren;
lower lemmas ovate-lanceolate, 2 – 2.5 mm long, acute, 5-nerved; lower paleas ovate-oblong
to ovate-lanceolate, 1.5 – 2 mm long; upper lemmas ovate-oblong, 1 – 1.5 mm long, minutely
bifid, awned from the sinus, awns 5 – 6.5 mm long, 3-nerved; upper paleas ovate-lanceolate,
1.2 – 1.4 mm long, acute; callus hairy, hairs 0.3 – 0.5 mm long; stamens 2 rarely 3, anthers
yellow, 0.3 – 0.5 mm long; styles 2, stigmas purple, 0.5 – 0.7 mm long. *Caryopsis* ovoid-elliptic,
1 – 1.2 mm long.

DISTRIBUTION. Endemic to Thailand.

SPECIMENS EXAMINED. THAILAND. Trat: Ko Kut, (12° 35'N 101° 31'E), 21 Nov.
914 (BKF, TCD, Herbarium of Thailand Natural History Museum, National Science
Museum); *(l.c., 3 April 2006, *Teerawatananon & Sungkaew* 917 (TCD, Herbarium of Thailand
Natural History Museum, National Science Museum).
HABITAT. Growing on the shaded, moist and mossy granite rocks, along the streams in tropical monsoon rainforest.

ETYMOLOGY. This species is named after the island ('Ko' in Thai) called Kut, Trat Province, southeastern Thailand, where this plant was collected for the first time.

CONSERVATION STATUS. *Arundinella kokutensis* is extremely restricted in distribution and specific habitat, occurring in small populations. It is very likely an endemic to Ko Kut. We recommend treating this species as vulnerable until more data are obtained.

NOTES. *Arundinella kokutensis* has only 2 stamens instead of 3 as normally found in other species of the genus (Keng, 1936; Bor, 1955; Watson & Dallwitz, 1992; Clayton et al., 2006; Sun & Phillips, 2006). Occasional florets with 3 stamens can be found but the occurrence is extremely rare within an inflorescence that predominantly contains florets with 2 stamens. This species is similar to *A. bolcoides* and *A. metzii* but can be distinguished from them by its almost glabrous rhachis, a glabrous pedicel, an awn of upper lemma which is longer (5 – 6.5 mm long), and its stamen number which is only 2. The differences between *A. kokutensis*, *A. bolcoides* and *A. metzii* are summarized in Table 1.

Table 1. Morphological variation in *A. kokutensis* and its close relatives, *A. bolcoides* and *A. metzii*

<table>
<thead>
<tr>
<th>Species/Character</th>
<th><em>A. kokutensis</em></th>
<th><em>A. bolcoides</em></th>
<th><em>A. metzii</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Nodes</td>
<td>sparsely pubescent</td>
<td>hirsute</td>
<td>glabrous or pubescent</td>
</tr>
<tr>
<td>Inflorescence</td>
<td>(3 –)10 – 25 cm long, an open panicle, sometimes drooping</td>
<td>3 – 15(– 20) cm long, a narrow panicle</td>
<td>up to 50 cm, an open panicle</td>
</tr>
<tr>
<td>Rhachis</td>
<td>almost glabrous, rarely scabrous, rarely glabrous</td>
<td>scabrous and pilose with tubercle-based hairs</td>
<td>scabrous</td>
</tr>
<tr>
<td>Pedicels</td>
<td>glabrous</td>
<td>glabrous, sometimes sparsely pilose</td>
<td>scabrous</td>
</tr>
<tr>
<td>Upper lemmas</td>
<td>1 – 1.5 mm long</td>
<td>1 – 1.3 mm long</td>
<td>1 ± 1 mm long</td>
</tr>
<tr>
<td>Callus hairs</td>
<td>0.3 – 0.5 mm long</td>
<td>0.2 – 0.3 mm long</td>
<td>0.4 – 0.5 mm long</td>
</tr>
<tr>
<td>Awns</td>
<td>5 – 6.5 mm long</td>
<td>4 – 4.5 mm long</td>
<td>3 ± 3 mm long</td>
</tr>
<tr>
<td>Stamens</td>
<td>2 (extremely rarely 3)</td>
<td>3</td>
<td>3</td>
</tr>
</tbody>
</table>
ACKNOWLEDGMENTS

The authors are grateful to the curators and the staff of the following herbaria: BKF, C, E, K, and NY for the use or loan of specimens. Thanks to Prof. Dr. Pranom Chantaranothai for his kind comments and useful suggestions, to Prof. Dr. John Parnell and Dr. Pimwadee Pornpongurueng for their support of the work, and Prof. Dr. Benjamin Øllgaard for help with the Latin diagnosis. This work was supported by the TRF/BIOTEC Special Program for Biodiversity Research and Training Grant T_148026.

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FIG. 1. *Arundinella kokutensis*. A habit; B inflorescence; C spikelets; D lower glumes; E upper glumes; F lower lemmas; G lower paleas; H upper lemmas; I upper paleas. All drawn Charoenphol *et al.* 5104. DRAWN BY ATCHARA TEERAWATANANON.
Two new endemic grasses (Poaceae, Panicoideae) from Thailand, *Arundinella kerrii* and *Dimeria kerrii*

Atchara Teerawatananon¹, Sarawood Sungkaew², and Trevor R. Hodkinson³

ABSTRACT. *Arundinella kerrii* C. Hambananda ex A. Teerawatananon & S. Sungkaew and *Dimeria kerrii* C. E. Hubbard ex A. Teerawatananon & S. Sungkaew are validly published for the first time. Species descriptions and illustrations are provided.

Key words: *Arundinella, Dimeria*, IUCN Red List, Thailand, Panicoideae, validations.

While preparing the account of some grasses genera for Flora of Thailand, two overlooked grass taxon names were found to require validation. They are *Arundinella kerrii* C. Hambananda, and *Dimeria kerrii* C. E. Hubbard, which were first reported by Hambananda in Yenying (1990) and Nanakorn & Norsangsri (2001), respectively. However, according to Articles 30.5 and 36.1 (McNeill et al., 2006), the names are invalid, because *A. kerrii* was described in Thai, but without a Latin diagnosis, while *D. kerrii* was recognised from the specimen label of A. F. G. Kerr 13868 (K) and neither description nor a Latin diagnosis were provided. The names are validated here. Illustrations, full descriptions, Latin descriptions and type information are provided.

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Arundinella kerrii C. Hambananda ex A. Teerawatnanon & S. Sungkaew, sp. nov. TYPE: Thailand. Nakhon Phanom: Tha Uthen, ca. 200 m, 16 Feb. 1924, A. F. G. Kerr (holotype, BK; isotypes, BM, K). Figure 1.

Arundinella hirta (C. P. Thunberg) T. Tanaka and A. fluviatilis E. R. E. Handel-Mazzetti similis sed ad illa vaginae fibrosae, minor gluma et superiore lemma, callus floris superioris pilis longis 1/5 lematis aequanribus.

Perennial, loosely tufted. Culms 30–120 cm tall, erect; nodes hirsute, basal sheaths becoming fibrous. Leaf-sheaths 9–16 cm long, almost glabrous, margins hispid with tubercle-based hairs. Ligule a ciliolate membrane, 0.5–0.6 mm long, with a dense row of hairs behind ligule. Leaf-blades narrowly linear, (8–)20–50(--70) × 0.3–0.8 cm, glabrous on both surfaces, margins scabrous and hispid with tubercle-based hairs. Panicles contracted, 5–15 cm long; racemes 1.5–5 cm long, alternate, rhachis scabrous. Spikelets grayish-green, ovate-oblong, 3–3.6 × 1–1.5 mm; pedicels 0.2–2 mm long, scabrous and hispid; lower glumes ovate, 2.5–3 × 0.8–1.2 mm, acute, 3–5-nerved, hispid with tubercle-based hairs on nerves; upper glumes ovate to ovate-oblong, 2.5–3.5 × 1–1.2 mm, acuminate, 5-nerved, hispid with tubercle-based hairs on nerves; lower florets male; lower lemmas ovate to ovate-oblong, 2.5–3 mm long, acute, upper margins fringed, 5- or 7-nerved; lower paleas ovate-oblong, 2.5–2.7 mm long; upper lemmas ovate-oblong, 1.8–2 mm long, acute or mucronate or minutely bifid, shortly awned from the sinus, awns 0.5–0.6 mm long, 3-nerved; upper paleas ovate-oblong, 1.8–2 mm long, acute; callus hairy, hairs 0.2–0.3 mm long; stamens 3, anthers 0.9–1.6 mm long; styles 2, stigmas ca. 1 mm long. Caryopsis not seen.

Distribution and habitat. Endemic to Thailand. This species is known only from Nakhon Phanom Province, North-Eastern Thailand occurring in open grasslands, at an altitude of ca. 200 m.

Discussion. This species is similar to Arundinella birta (C. P. Thunberg) T. Tanaka and Arundinella fluviatilis E. R. E. Handel-Mazzetti, but differs from them in having fibrous basal leaf sheaths, smaller glumes and upper lemma and shorter callus hairs, ca. 1/5 the length of lemma. Arundinella kerrii was originally described by Hambananda in Yenying (1990), with the description in Thai, but without a Latin diagnosis. The name was therefore not validly published.
IUCN Red List category. Using the IUCN Red list criteria (IUCN, 2001), *Arundinella kerrii* could be considered as either extinct or endangered as it is known only from collections last made in Nahkon Phanom Province, North-Eastern, Thailand in 1924, despite attempts by the authors to recollect it. However, insufficient data exists regarding its conservation status so we provisionally assign the species status as Data Deficient (DD).

*Additional specimens examined.* THAILAND. Nahkon Phanom, Chaiyaburi, A. F. G. Kerr 21330 (BK, BM, K).

*Dimeria kerrii* C. E. Hubbard ex A. Teerawatananon & S. Sungkaew, sp. nov. TYPE: Thailand. Satun: Ban Tola Tai (Tola), ca. 50 m, 3 Jan. 1928, A. F. G. Kerr 13868 (holotype K (ex H2005/01944-92); isotypes, K (ex H2008/00159-110), BM (BM 928281)). Figure 2.

Perennis. Vaginae quoad laminae tomentosae. Racemi 8--16 cm longi, rhachis compressa, pedunculus superne hirsutus, pedicellus 0.8--1.2 mm longus, glabrat us, clavatus; gluma supera valde compressa, carina late alata, rugosa.

Perennial. Culms up to 1.2 m tall, erect; nodes pubescent, stems waxy below nodes, usually short-noded at base. Leaf-sheaths overlapping below, 6--9 cm long, sparsely pilose at lower part, upper part tomentose, margins scarious. Ligule a ciliate membrane, ca. 0.6 mm long. Leaf-blades linear-lanceolate, 10--20 × 3--4.5 mm, tomentose on both surfaces, sparsely pilose with tubercle-based hairs especially near the margins, margins scaberulous near the apex. Racemes (2--)3, 8--16 cm long, rhachis flattened, 0.6--0.7 mm wide, slightly zigzag, slightly ridged, ridge glabrous, narrowly winged, margins scaberulous; peduncles hirsute at top below the inflorescences. Spikelets ovate-oblong, 5--6 × 1.8--2 mm; pedicels compressed but not flattened, 0.8--1.2 mm long, margins glabrous, clavate; spikelet callus hairy, hairs up to 0.5 mm long; lower glumes oblong, 5--5.5 mm long, acuminate, keeled, ciliate on keel; upper glumes oblong-elliptic, 5.5--6 mm long, acute to acuminate, keeled, sparsely hirsute near margins, broadly winged all along the keel, wings rugose and ciliate; lower lemmas oblanceolate or clavate, 2.5--3 mm long, margins ciliate on the upper half; upper lemmas oblong-elliptic, ca. 4 mm long, awns 12--15 mm long, columns 2--3 mm long; stamens 2, anthers 1.8--2 mm long; styles 2, stigmas, 1--1.5 mm long. Caryopsis not seen.

*Distribution and habitat.* Endemic to Thailand. Known only from the type locality (Satun Province, Peninsular Thailand), at an altitude of ca. 50 m.
Discussion. This species was first named, on the specimens of A. F. G. Kerr 13868 (K), by Dr. C. E. Hubbard of Kew, but he never published a description. This species is distinguished from all other species of Dimeria by its rugose, broad wing on the keel of the upper glume.

IUCN Red List category. Using the IUCN Red list criteria (IUCN, 2001), Dimeria kerrii could be considered as either extinct or endangered as it is known only from collections last made in Satun Province, Peninsular Thailand in 1928, despite attempts by the authors to recollect it. However insufficient data exists regarding its conservation status so we provisionally assign the species status as Data Deficient (DD).

Acknowledgments. We thank the curators and the staff of the following herbaria: BKF, C, E, K and NY for the use or loan of specimens. Thanks to Prof. Dr Pranom Chantaranothai and Prof. Dr. John Parnell for their kind comments on the manuscript, to Dr. Pimwadee Pornponggrungrueng for her support of the work, and Prof. Dr. Benjamin Øllgaard for the Latin diagnoses. This work was supported by the TRF/BIOTEC Special Program for Biodiversity Research and Training Grant T_148026.

Literature Cited


Appendix 2

A list of some non-Thai specimens examined. The specific sites are plotted only when they can be located precisely.

Tribe Arundinelleae

*Arundinella bengalensis* (Spreng.) Druce

**Bhutan:** *Punakha* [alt. 1,280 m, 22 Sept. 1998, Noltie et al. 308 (E)].

**India:** *Assam* [July 1857, Jenkins 219 (ABD)]; *Jashpur* [Sardih, alt. 2,900 ft, 30 Sept. 1941, Mooney 1907 (K)]; *Jharkhand* [Parasnath, alt. 4,300 ft, 7 Oct. 1883, Clarke 33718 (E)]; *Meghalaya* [Lohit valley, alt. 4,500 ft, 3 Aug. 1950, Kingdom-Ward 20100 (K)]; [Khasia, alt. 4,000 ft, 2 Sept. 1886, Clarke 44803A (K)]; [l.c., 4,000 ft, 2 Sept. 1886, Clarke 44803B (BM)]; [l.c., Griffith s.n. (TCD)]; [l.c., alt. 2–4,000 ft, Hooker & Thomson s.n. (E, TCD)]; [l.c., alt. 3–6,000 ft, Hooker & Thomson s.n. (K)]; [l.c., Kurz s.n. (E)]; [l.c., alt. 6,000 ft, 8 Sept. 1954, Thakur Tup Chand 8141 (K)]; [l.c., alt. 5,500 ft, 15 Sept. 1954, Thakur Tup Chand 8197 (K)]; [Pynursla, alt. 4,000 ft, 12 Aug. 1949, Koelz 23496 (K)]; [l.c., alt. 4,000 ft, 11 Aug. 1949, Thakur Tup Chand 1972 (K)]; [Shillong, alt. 3,000 ft, 20 Aug. 1886, Clarke 44627 (K)]; [l.c., alt. 5,500 ft, 20 Sept. 1954, Thakur Tup Chand 8258 (K)]; [Theria, alt. 1,500 ft, 19 Oct. 1871, Clarke 15506 (K)]; *Orissa* [Koraput, 10 July 1950, Mooney 3838 (K)]; *Sikkim* [alt. 4,000 ft, Hooker & Thomson s.n. (GH, TCD)]; [alt. 4,000 ft, Hooker & Thomson s.n. (GH)]; [1810, Wallich 8700 (2 sheets K)]; [Wallich 8700 (TCD)]; **No locality** [Griffith 1391 (BM, E)]; [Jenkins s.n. (TCD)]; [Aug. 1985, Haines 543 (ABD)].

**Bangladesh:** *Bengal* [July 1810, Wallich 8700 a, B (K)]; *Sylhet* **"Silhet"** [Silhet de Silva, Wallich 8669B (2 sheets BM, 2 sheets E)]; [Wallich 8669B (C, E)]; [1821, Wallich 8669B (K)].

**Myanmar:** *Kachin* [16 Oct. 1945, Belcher s.n. (K)]; *Mandalay* [Maymyo, alt. 1,050 m, 29 Aug. 1908, Law 4242 (E)]; [l.c., alt. 3,500 ft, Aug. 1912, Law s.n. (E)].

**Nepal:** *Kathmandu* [Pokhara, alt. 3,500 ft, 10 Aug. 1954, Stainton et al. 6717 (K)]; [l.c., alt. 3,500 ft, 10 Aug. 1954, Stainton et al. 6735 (K)]; [l.c., alt. 2,500 ft, 8 Aug. 1954, Stainton et al. 6783 (K)]; [alt. 1,350 m, 3 Sept. 1954, Zimmermann 1008 (K)]; *Parsta* [Narayani, alt. 190–200 m, 9 Oct. 1995, Mikage et al. 9552778 (GH)]; **No locality** [Nepalia, Wallich 8669a (C, K)]; [Wallich 8669a (E)]; [1821, Wallich 8669 (left hand side of the same sheets with Wallich s.n. (E))]; [Wallich s.n. (right hand side of the same sheets with Wallich 8669 (E)].


**No locality:** [Wallich 6789 (3 sheets K)] [Wallich 8666 (E)].

*Arundinella cochinchinensis* Keng
Vietnam: Sapa [alt. 1,500 m, Sept. 1927, Petelot 5066 (2 sheets US, NY)].

*Arundinella decempedalis* (Kuntze) Janowski

India: Assam [Goalpara, 25 May 1912, Hole s.n. (K)]; Sikkim [Dulkajhar, alt. 500, 16 Oct. 1886, Clarke 36658A (K)]; [Terray vor Sikkim 15 Nov. 1875, Kunte 6581 (K, 2 sheets NY, US)]; [26 Oct. 1968, Kurz s.n. (K)]; [alt. 300 ft, 1898, Wood 173 (K)]; West Bengal [Dooars (Duars), Oct. 1895, Haines 565 (2 sheets K)]; [Darjeeling, alt. 500, 2 Dec. 1875, Clarke 26483A (K)]; [l.c., alt. 500, 2 Dec. 1875, Clarke 26483C (K)]; [l.c., alt. 1,000 ft, Nov. 1879, Gamble 7478 (K)].

*Arundinella holcoides* (Kunth) Trin.

India: Madhya Pradesh [10 Oct. 1969, Naik 550 (AAU)]; Orissa [Kalanhandi, alt. 3,000–4,000 ft, 28 Dec. 1939, Mooney 1214 (K)]; No locality [Griffith 6790 (2 sheets E, 3 sheets K)].

Indonesia: Java [Jakarta, alt. 250 m, 2 July 1894, Schiffer 1546 (K)]; [Megamendon, 24 May 1875, Kunte s.n. (K)]; Sulawesi (Celebes) [S.W. Celebes, alt. 1,400 m, 1 July 1921, Bunnemeijer 12650 (K)]; [16 June 1875, Kunte 4805 (NY)]; [18 Oct. 1875, Kunte 6200 (NY)]; [18 Oct. 1875, Kunte 6214 (NY)].

Myanmar: Mandalay [Kanbauk, alt. 25 m, 26 Oct. 1998, Maxwell 98-1385 (2 sheets CMU, L)]; Tenasserim [Kyaikkami (Amherst), alt. 200 ft, 11 Jan. 1927, Parkinson 5011 (K)]; [Dawei (Tavoy), Nov. 1827, Gomes in Wallich list 8671 (2 sheets K)]; [Mergui, Herb. Mergui, Griffith 334 (2 sheets K)]; [l.c., Griffith 698 (K)]; [Helfer s.n. (K)].

Philippines: Luzon [Abra, alt. 250 m, 18 Nov. 1996, Rosa 38620 (K)]; [Benguet, Lober 1873 (K)]; [Benguet, Merrill 4328 (K, NY)]; [l.c., Oct. 1921, Ramos & Edano 40487 (BM, SING)]; [l.c., 8 Nov. 1904, Williams 1961 (2 sheets; NY)]; [Manila, Lober 7217 (K)]; [l.c., Unknown collector (2 sheets K)]; [Zambales, Nov. to Dec. 1924, Ramos & Edano 44647 (NY)]; [l.c., Nov. to Dec. 1924, Ramos & Edano 44756 (NY)]; [8 Dec. 1892, Lober 1871 (K)]; [alt. 1,000 m, 30 Nov. 1953, Steenis 17922 (BM, SING)].

*Arundinella nepalensis* Trin.

Australia: Northern Territory [Kakadu National Park, 21 April 1990, Dunlop & Munns 8544 (K)].

Bhutan: Tongsa [alt. 1,950 m, 19 Sept. 1998, Nottie et al. 264 (E)].

China: Guangdong (Kwangtung) [Hwei-Yang, Oct. 1935, Tsang 25957 (E)]; [Mei (Kaying), 4 Aug. 1932, Tsang 21399 (K)]; [Swatow, Thai-Yong, alt. 2,000 ft, July 1901, Datziei s.n. (E)]; [Tapu, Sept. 1932, Tsang 21692 (K)]; [Guangzhou (Canton), 16 Oct. 1885, Simpson s.n. (K)]; Hainan [Ching Mai, 18 Oct. 1932, Lei 147 (K)]; [Oct. 1921, Macfie 7809 (K)]; Hong Kong [Fau Tan Valley, 21 Oct. 1972, Shiu Ying Hu 12300 (K)]; [High Island, 11 Nov. 1969, Shiu Ying Hu 8689 (K)]; [Jubilee Reservoir, 25 Oct. 1969, Shiu Ying Hu 8397 (K)]; [Sai Kong, 4 Nov. 1969, Shiu Ying Hu 8544 (K)]; Yunnan [Mentze, alt. 4,600 ft, Henry 9139 (E, K)]; [l.c., alt. 5,000 ft, Henry 10170 (K)].

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India: **Karnataka** [Hassan, alt. 740 m, 24 Oct. 1970, *Jarrett & Ramamoorthy* HFP 983 (K)]; **Meghalaya** [Khasia Hills, alt. 4,000 ft, 9 Oct. 1951, *Koelz* 28752 (K)]; [Shillong, alt. 6,000 ft, 7 Sept. 1954, *Thakur Rap Chand* 8136 (K)].

**Indo-China:** **No Locality** [alt. 1,100 m, April 1932, Unknown collector 4387 (US)].


**Nepal:** **Mayangdi Khola** [alt. 3,000 ft, 24 Dec. 1971, i0tq;3160 (SING)]; **West Nepal** [Koshi Zone, 30 Aug. 1997, *Noshiro* 9770394 (GH); [i.e., alt. 1770 m, 12 Aug. 1997, *Noshiro* 9770023 (GH); [i.e., alt. 2,310 m, 3 Aug. 1999, *Omori et al.* 9920001 (GH)]; **West Nepal** [Dhawalagiri Zone, alt. 1,370 m, 18 Sept. 1995, *Mikage et al.* 9552251 (K)]; **No locality** [Wallich 8666AB (2 sheets K)].


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*Arundinella setosa* Trin. var. *setosa*

**Australia:** **Northern Territory** [Kakadu National Park, 21 April 1990, *Dunlop & Munns* 8544 (K)]; **Queensland** [Almaden, 10 March 1980, *Simon & Clarkson* 3603 (K)].

**Cambodia:** **Kompong Thom** [Santuk, 18 April 2003, *Davidson* 008KH (K)].


**India:** **Chattisgarh** [Surguja, 28 Sept. 1947, *Koelz* 19007 (K)]; **Meghalaya** [Khasia hills, alt. 6,000 ft, 14 Aug 1954, *Thakur Rap Chand* 7988 (K)]; **Tamil Nadu** [Nilgiri (Nilagiri) 1851, *Hohenacker* 920 (K, I, by mistake, determined it as lectotype of *A. setifera* Steud.)]; [Kodaikanal, alt. 1,800 m, 25 Aug. 1936, *Stewart* 15586 (NY)]; **Uttarakhand** [Mussoorie, alt. 7,500 feet, 28 Aug. 1936, *Stewart* 15586 (NY)]; **N.W. India** [Reyle s.n. (K)]; **No locality** [Thomson s.n. (K)].


**Laos:** **Champasak** [Khong Island, alt. 125—225 m, 11 Sept. 1998, *Maxwell* 98-889 (L)].

**Malaysia:** **Kedah** [Bukit Wang, 17 Nov. 1915, *Haniff* 650 (K, SING)].

**Myanmar:** **Mandalay** [Mandalay, alt. 4,000 ft, 12 Sept. 1938, *Rhind* 2610 (K)]; [Pyin U Lwin, Maymyo plateau, alt. 3,500 ft, 21 Sept. 1911, *Lace* 5428 (K)]; **Tenasserim** [Kyai khami (Amherst), alt. 6,300 ft, 3 Feb. 1927, *Parkinson* 5162 (K)].

**Nepal:** **Maikot** [alt. 8,500 ft, 8 Oct. 1954, *Stainton et al.* 4751 (K)].

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Papua New Guinea: *Babjara* [alt. 100 ft, 6 Sept. 1954, *Saunders* 96 (BM)]; *Eastern Highlands* [alt. 1,370 m, 2 Nov. 1959, *Brass* 32358 (K)]; *Central* [Rona, Laloki river, alt. 450 m, *Brass* 3563 (BM, NY)]; *Morobe* [alt. 100 m, 29 April 1959, *Brass* 29337 (K)]; [2 Feb. 1950, *Furr* 3642 (SING)].


Vietnam: *Adong Hoi* [North of Dong Hoi, 3 Oct. 1921, *Hitchcock* 19456 (US)]; *Dac Lac* [Buon Don, alt. 250 m, 10 Nov. 1998, *Hacker* 1582 (L)]; *Lam Dong* [South of Dalat, alt. 1,200 m, *Hacker* 1608 (L)]; [Aug. 1885, *Balansa* 360 (K)].

*Arundinella setosa* Htin. var. esetosa *Bor* ex *S. M. Phillips* & *S.-L. Chen*


Indonesia: *Sumatra* [April to May 1933, *Rahmat Si Toroes* 4111 (GH, specimen on the left hand side NY)].

Malaysia: *Kelantan* [May 1948, *Unknown collector* 38440 (SING)].


*Gamotia acutigluma* (Steud.) Ohwai

Bhutan: *Deothang* [alt. 1,400 m, 18 Oct. 1987, *Wood* 5974 (K)].


China: *Guangdong* [Kweichow, 18 Oct. 1930, *Tsian* 4718 (NY)].

India: *Meghalaya* [Khasia, alt. 4,000 ft, 7 Oct. 1867, *Clarke* 5647 (K)]; [l.c., alt. 4,000 ft, 19 Oct. 1871, *Clarke* 15510B (E)]; [l.c., alt. 4,000 ft, 19 Oct. 1871, *Clarke* 15510D (K)]; [l.c., alt. 4,000 ft, 19 Oct. 1871, *Clarke* 15510G (K)]; [l.c., alt. 4,000 ft, 19 Oct. 1871, *Clarke* 15510H (K)]; [l.c., alt. 5,000 ft, 16 Oct. 1872, *Clarke* 19242A (K)]; [l.c., alt. 4,200 ft, 5 Nov. 1872, *Clarke* 19450A (K)]; [l.c., alt. 4,200 ft, 5 Nov. 1872, *Clarke* 19450E (K)]; [l.c., alt. 4,000 ft, 17 Sept. 1886, *Clarke* 45593C (K)]; [l.c., alt. 4,200 ft, 5 Nov. 1872, *Clarke* 19450G (K)]; [l.c., alt. 5,500 ft, 26 Sept. 1886, *Clarke* 45593C (K)]; [l.c., 17 Sept. 1899, *Koez* 23938 (K)]; [l.c., alt. 6,000 ft, 10 Nov. 1953, *Thakur Rap Chand* 7310 (K)]; *Sikkim* [Yoksun, alt. 6,000 ft, 11 Oct. 1875, *Clarke* 25234A (K)]; [alt. 1,100 m, 1811, *King* s.n. (ABD)]; [5 Dec. 1875, *Kuntze* s.n. (NY)]; *West Bengal* [Darjeeling, alt. 3,000 ft, 24 Sept. 1875, *Clarke* 26738A (K)]; [Aug. 1885, *Balansa* 360 (K)].

June 1921, Bakuit van den Brink 5188 (K, SING); **Lesser Sunda** [Flores, 30 May 1970, Verheijen 2868 (NY)]; **West Sumbawa** [Batudulang, alt. 500 m, 15 April 1961, Kostermans 18198 (C, GH, K, SING)].

**Malaysia:** **Pahang** [Cameron Highlands, 6 Apr., Henderson 23465 (SING)]; [Kuala Teka, Feb. 1921, Seimund 380 (K)]; [Kuala Teka, Feb. 1921, Seimund 540 (SING)]; [alt. 3,900 ft, 18 May 1936, Holttum 31332 (SING)]; [30 Mar. 1909, Ridley 13854 (BM, K, SING)]; **Sabah** [Tawau, 21 Mar. 2001, Laegaard et al. 151285 (AAU)]; **Sarawak** [alt. 50–100 m, 18 Jan. 1978, Nielsen 55 (AAU)]; [South Tapin, alt. 90 m, 4 Feb. 1978, Nielsen 247 (AAU)].

**Myanmar:** **Bago (Pegu)** [South of Bago, 10 Nov. 1939, U Thein Twin 3170 (K)]; **Kachin State** [Hkinlum, North Triangle, alt. 4,000 ft, 12 Aug. 1953, Kingdom-Ward 21256 (K)]; [l.c., alt. 4,000 ft, 19 Sept. 1953 Kingdom-Ward 21343 (K)].

**Nepal:** **Kosi** [Sankhuwasabha, alt. 1,850 m, 21 Aug. 1997, Nosbiro et al. 977022 (GH)]; [l.c., alt. 1800 m, 30 Aug. 1997, Nosbiro et al. 9770389 (GH)].

**Papau New Guinea:** **Central Highland** [alt. 7,500 ft, 22 Nov. 1954, Womersley & Floyd 6915 (SING)]; **New Britain** [alt. 3,000 m, 21 Mar. 1966, Henty & Frodin 27300 (NY)]; [23 Mar. 1966, Henty & Frodin 27347 (NY)].

**Philippines:** **Luzon** [Benguet, Apr. 1904, Elmer 6210 (K, NY)]; [l.c., Mar. 1907, Elmer 8898 (K, 2 sheets NY)]; [Laguna, 6 Mar. 1913, Gate 6237 (BM, K)]; [l.c., Mar. 1910, Robinson 9763 (NY)]; [San Mariang, Feb.-Mar. 1926, Ramos & Edano 47127 (BM, NY)].

**Vietnam:** **Lao Cai** [Ta Yang Ping, Sept. 1942, Petelot 8900 (US)]; **Sa Pa (Chapa)** [alt. 1,500 m, Jan. 1928, Petelot 3253 (NY)].

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**Garnotia ciliata** Merr.

**China:** **Guangdong (Kwangtung)** [Loh Fau Moutain (Lofaushan), Aug. 1917, Merrill 10701 (NY, US)]; [l.c., 26-29 Oct. 1921, Hitchcock 19009 ½ (K)].

**Garnotia patula** (Munro) Benth.

**China:** **Hong Kong** [Fau Tan Valley, 21 Oct. 1972, Shiu Ying Hu 12314 (K)]; [Dec. 1858, Hance 1009 (2 sheets K)]; [Nov. 1862, Hance 9668 (K)]; [15 Feb. 1857, Wright s.n. (K)]; [1853-1856, Wright s.n. (K)]; **Kwangsi** [Tang Lung, 20 Sept. 1934, Tsang 24301 (NY)]; [Nam She, Oct. 1934, Tsang 24562 (NY)]; **Guangdong (Kwangtung)** [Ting Wu Shan, alt. 70–80 m, 1978, Chow 78015 (AAU, E, K)].

**Malaysia:** **Pahang** [Sungai Teka, 24 July 1936, Kiab 31792 (K, SING)].

**Vietnam:** **Tien Yen** [Ho Yung Shan, Oct.-Nov. 1940, Tsang 30691 (C, E, GH, K, SING)]; **Tonkin** [5-19 Nov. 1885, Balansa 331 (K)]; [1888, Balansa 1595 (K)]; **Saigon** [20 Dec. 1946, Petelot 8827 (US)]; **No Locality** [alt. 1,000 m, Nov. 1930, Petelot 3839 (NY)].

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**Garnotia tenella** (Arn. ex Miq.) Janowski
India: *Anamallais (Anamallays)* [Unknown collector (C, K)]; *Bihar* [Parasnath, alt. 4,000 ft, 1 Oct. 1873, Clarke 20766 (K)]; *Chhattisgarh* [Bastar, alt. 4,000 ft, 5 Oct. 1940, Mooney 1447 (K)]; *Jharkhand* [Parasnath, alt. 4,300 ft, 7 Oct. 1883, Clarke 33691B (K)]; *Karnataka* [Castle Rock, alt. 2,000 ft, Oct. 1908, Meebold 10773 (K)]; [l.c., alt. 2,000 ft, Oct. 1908, Meebold 10774 (K)]; *Kerala* [Kollam (Quilon), 24 Nov. 1893, Unknown collector 126 (K)]; *Maharashtra* [Konkan, 6 Sept. 1896, Woodrow s.n. (K)]; *Meghalaya* [Sohra (Cherra), alt. 4,000 ft, 12 Oct. 1872, Clarke 17449C (K)]; [Khasia, Griffith 304 (K)]; *Orissa* [Keonihar, alt. 2,500 ft, 2 Oct. 1946, Moon^ 2776 (K)]; *Tamil Nadu* [Chennai (Madras), Jolpad, 13 Nov. 1900, Barber 22] yi]; [Kodaikanal, 7 Dec. 1898, Bume 1956 (2 sheets ^]); [l.c., 15 Dec. 1989, Mathew & Sebastian 53902 (AAU)]; *Travancore or Thiruvithamkoor (Travencore)* [Pallode, Nov. 1901, Bourne s.n. (K)]; A7o/affp [Peninnsula Ind. orientalis, Wight 2599 (E)]; [/.ir., 1837, Wight 52A4 (K)]; [l.c., Griffith 6781 (2 sheets K, NY)]; [Wallich 8827 (K)]; [Wight 52A5 (K)]-

Indonesia: *Java* [Wanajasa, 26 July 1920, Backhuizen van der Brink 4687 (2 sheets K, SING)]; *West Sumbawa* [Mt. Batulanteh, alt. 700—1,000 m, 27 Apr. 1961, Kostermans 18528 (C, GH)].

Malaysia: *Kedah* [Kadah Peak, alt. 3,900 ft, Dec. 1915, Robinson & Kloss 6043 (K, SING)]; [l.c., alt. 4,000 ft, 13 Nov. 1915, Haniff 628 (K, SING)]; *Pahang* [Kuala Teku, Feb. 1921, Seimund 455 (K)].


Papua New Guinea: *Southern Highland* [Waren, alt. 700—1,000 m, 27 Apr. 1961, Kostermans 18528 (GH)].

Vietnam: *Sa Pa (Chapa)* [Lo Qui Ho, alt. 2,000 m, Sept. 1933, Petelot 4745 (K, NY)]; *Tonkin* [25 Aug. 1943, Petelot 8553 (US)]; No locality [alt. 1,600 m, Sept. 1927, Petelot 5068 (C, NY)].

**Jansenella griffithiana** (Müll. Hal.) Bor

India: *Chhattisgarh* [Bastar, alt. 3,000—3,700 ft, 15 Oct. 1940, Mooney 1426 (K, SING)]; *Karnataka* [Castle Rock, alt. 2,000 ft, Oct. 1908, Meebold 10572 (E)]; [l.c., alt. 2,000 ft, Oct. 1908, Meebold 10576 (K)]; [Kodagu (Coorg), 1873, Beddome s.n. (K)]; [Kulhuttu, alt. 6,000 m, Oct. 1908, Meebold 10571 (K)]; [North Kanara, 15 Jan. 1890, Talbot 2255 (K)]; *Kerala* [Cochin, alt. 3,000 ft, Nov. 1910, Meebold 12179 (K)]; [Nilgiris, alt. 4,000 ft, Nov. 1884, Gamble 15449 (K)]; [l.c., 1886, Gamble 18306 (BM, K)]; [Peermade, Beddome s.n. (BM)]; [l.c., alt. 1,500 m, 1964, Guyer 8 (K)]; *Mahabeshwar* [com. Lixoa (K)]; *Maharashtra* [Lonaivala (Lanavli), 1 Oct. 1898, Woodrow s.n. (E)]; *Meghalaya* [Khasia, alt. 5,000 ft, 22 Sept. 1886, Clarke 45595 (E)]; [l.c., Hooker & Thomson s.n. (E, TCD)]; [l.c., Griffith 36 (B, K, TCD)]; [l.c., Griffith 6785 (2 sheets K)]; [l.c., Griffith s.n. (B)]; [l.c., Griffith s.n. (E)]; [Mausmai, alt. 3750 ft, 10 Oct. 1886, Clarke 45866E (BM)]; [l.c., alt. 3750 ft, 10 Oct. 1886, Clarke 45866D (K)]; [Pynursla, Khasi Hills, alt. 4,000 ft, 5 Sept. 1949, Thakur Rup Chand 2167 (K)]; [Sohra (Cherra), alt. 4,000 ft, 19 Oct. 1871, Clarke 15537 (K, BM)]; [l.c., alt. 4,000 ft, 22 Oct. 1871, Clarke 15667 (K)]; [l.c., alt. 4,000 ft, 11 Sept. 1885, Clarke 40376B (BM)]; [l.c., alt. 4,000 ft, 11 Sept. 1885, Clarke 40376C (K)]; [l.c., Griffith 6784 (2 sheets K)]; *Orissa*, [Koraput, alt. 5,200 ft, 10 Oct. 1950, Mooney 4082 (GH, K)].
Myanmar: **Bogo** (Pegu) [27 Dec. 1970, Kurz 3158 (GH)]; **Mon State** [Mawlamyaing (Mulmein), Griffith 328 (K)].

Sri Lanka: **Saffragam** [Thwaites c.p. 3471 (2 sheets BM, E, 2 sheets K)].

No locality: [Gamble s.n. (K)]; [Griffith 6780 (BM, K)].

**SUBTRIBE CHIONACHNINAE**

*Chionachne massiei* Balansa


*Polytoca digitata* (L.f.) Druce

China: **Hainan** [Ta Han, alt. 250 m, 1935, Linsley Gressitt 927 (BM, E)]; [1 Nov. 1921, McClure 7795 (BM, E, K)].

India: **Manipur** [Karong, alt. ca. 3,500 ft, Oct. 1950, Koelz 26421 (K)]; **Meghalaya** [Khasia, alt. 5,000 ft, 16 Oct. 1872, Clarke 19251G (K)]; [c., alt. 5,400 ft, 23 Aug. 1885, Clarke 4004OC (E)]; [c., alt. 4,550 ft, 25 Aug. 1885, Clarke 40111A (BM)]; [c., alt. 4,500 ft, 26 July, Hooker & Thomson 1932 (E, K)]; [c., Hooker & Thomson s.n. (BM)]; **Sikkim** [Sikkim Terai, 30 Sept. 1968, Kurz 235 (GH)]; **West Bengal** [Dooars (Duars), Oct. 1895, Haines 566 (2 sheets K)]; [Darjeeling, alt. 500 ft, 14 Dec. 1876, Clarke 31737B (K)]; [c., 15 Oct. 1884, Clarke 36578 (E)]; [No locality] [Wight 2601, 3318 (3 sheets E)].

Indonesia: **Java** [Horsfield 113 (BM)]; [1802-1818, Horsfield s.n. (BM)].


Philippines: **Mindanao** [Bukidnon, alt. 1,600 ft, Dec. 1932, Franco 31502 (NY)]; [c., 1920, Ramos & Edano 39226 (K)]; [Davao, June 1909, Elmer 11026 (NY)]; [c., alt. 600 ft, 9 Apr. 1905, Williams 2616 (NY)]; [c., alt. 600 ft, 11 July 1905, Williams 3028 (NY)].


*Polytoca wallichiana* (Nees ex Steud.) Benth.

Myanmar: **Bago** (Pegu) [Sitaug, Kurz 1136 (K)]; **Mon State** [Mawlamyaing (Mulmein), Lobb s.n. (K)]; [c., Wallich 8629B (BM, 2 sheets K)]; **Yangon** (Rangoon) [Golden Valley, 20 Oct. 1947, U Thein Lwin 281 (K)]; [Kokkine, 21 Sept. 1932, Parkinson 15064 (2 sheets K)].
**SUBTRIBE DIMERIINAE**

*Dimeria fuscescens* Trin.

**India:** *Chhattisgarh* [Bastar, alt. 3,100 ft, 9 Oct. 1940, *Mooney* 1533 (K)]; *Meghalaya* [Khasia, alt. 5,000 ft, 10 Sept. 1886, *Clariske* 45723A (K)]; [*lc., Griffith* s.n. (K)]; [*lc., alt. 3–4,000 ft, Hooker & Thomson* s.n. (TCD)]; [Pynursla, alt. 4,000 ft, 31 Aug. 1949, *Thakur Ram Chand* 2134 (K)]; *Orissa* [Pal Lahara, alt. ca. 3,800 ft, 20 Apr. 1950, *Mooney* 3772 (K)].

**Nepal:** *No locality* [*Wallich* 8839B (TCD)]; [Oct. 1827, *Wallich* 8839ABC (TCD)]; [1821, *Wallich* 8841 (C, E, K)].

**Sri Lanka:** *No locality* [*Gardner* 1028 (TCD)].

*Dimeria kurzii* Hook.f.

**Myanmar:** *Bago (Pegu)* [Irrawady and Sittang Valley, *Kurz* 2741 (2 sheets K)]; [Shawdon, 28 Nov. 1939, *U Thein Twin* s.n. (K)]; *Tanintharyi* [Mergui, *Griffith* 6798 (K)].

*Dimeria leptorhachis* Hack.


**Myanmar:** *Tenasserim* [*Griffith* 6799 (2 sheets E, 2 sheets K)]; [*Helfer* s.n. (2 sheets K)].

**Sri Lanka:** *Sabaragamuwa* [Adam’s Peak, *Thwaites* cp. 24 (K)]; *No locality* [*Thwaites* cp. 3261 (2 sheets K)].

*Dimeria ornithopoda* Trin. var. *ornithopoda*

**Australia:** *Northern Territory* [Port Darwin, *Schultz* 321 (K)].

**Bangladesh:** *Bengal (East Bengal)* [*Griffith* s.n. (K)].

**India:** *Karnataka* [Mangalore, 1847, *Hobenacker* 231 (BM, K, TCD)]; *Meghalaya* [Sohra (Cherra), alt. 4,000 ft, 6 Oct. 1867, *Clarke* 5624 (K)].

**Indonesia:** *Java* [Tjikoya, 27 July 1842, *Zollinger* 351 (BM)]; [1802, *Horsfield* 135 (BM)]; [*Lobb* 135 (BM, TCD)].

**Malaysia:** *Kedah* [Langkawi, Feb. 189?, *Ridley* 15508 (BM)]; *Sabah* [Sadankan, 1920, *Ramos* 1301 (K)].

Nepal: **No locality** [1821, *Wallich 8841 (TCD)*]; *[Wallich s.n. (K)*].


Singapore: **No locality** [29 Dec. 1989, *Ridley 1703 (BM)*].

Vietnam: **Nha Trang** [Mar. 1911, *Robinson 1265 (BM)*] **No locality** [Pierre s.n. (BM)].

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**Dimeria ornithopoda Trin. var. gracillima Bor**

India: **Jharkhand** [Parasnath, alt. 4000 ft, 1 Oct. 1873 *Clarke 21084B (K)*]; [Lc., alt. 4,300 ft, 7 Oct. 1883 *Clarke 33719A (K)*]; [Lc., alt. 4,300 ft, 7 Oct. 1883 *Clarke 33719C (K)*].

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**Dimeria pubescens** Hack.

Sri Lanka: **No locality** [Gardner 1008 (TCD)]; [Sept. 1804, *Thwaites c.p. 956 (K)*]; *[Thwaites c.p. 956 (3 sheets K)*].

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**Dimeria sinensis** Rendle


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**SUBTRIBE GERMAINIINAE**

**Apocopis collinus** Balansa

Cambodia: **Pursat** [June, *Godfrey s.n. (K)*].

Indonesia: **Kalimantan** [Banjarmasin (Bangarmassing), 1857-58, *Motley 302 (K)*]; [Martapura, 1908, *Winkler 3392 (K)*].


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**Apocopis courtallumensis** (Steu.d.) Henrard

Dec. 1889, Gamble 21722 (K); Tamil Nadu [Kutttalarn, Aug. 1835, Wright s.n. (K)]; [l.c., Aug. 1835, Wright 2352, 1048 (E)]; [l.c., Aug. 1835, Wright 2352 (K)]; No locality [Wright 2352 (BM)].

Ceylon: Mysore [Hassan, 27 Oct. 1969, Saldanha 15376 (K)]; Jaffna [West of Paranthan, alt. 0 m, 6 Dec. 1974, Devidse & Sumithraarachchi 9134 (K)].

Apocopis intermedius (A. Camus) Chai-Anan
China: Hong Kong [Ma On Shan, 1969, Shiu Ying Hu 7769 (K)]; [Wah Shan Kuek, 24 Aug 1970, Paul But 114 (K)]; [1801, Hance 7432 (K)].
Vietnam: Quang Ninh [Ouonbi, 1969, Shiu Ying Hu 7769 (K)].

Apocopis mangalorensis (Hochst.) Henrard
India: Karnataka (Terr. Canara) [Mangalore (Mangalor), 1847, Hohenacker 231a (2 sheets E, BM, TCD)]; [Mysore (Maior & Carnatic), Apocopis 4, Thomson s.n. (BM, TCD)].
Sri Lanka: No locality [Thwaites 3959 (BM)].

Apocopis wrightii Munro

Germainia khasyana Hack.
India: Meghalaya [Khasia (Khasiana), alt. 4,500 ft, 17 Oct. 1867, Clarke 6447 (BM)]; [l.c., alt. 4,500 ft, 15 Dec. 1885, Clarke 42558A (K)]; [l.c., alt. 4,500 ft, 15 Dec. 1885, Clarke 42558D (BM)]; [l.c., alt. 4,500 ft, 15 Dec. 1885, Clarke 42558J (E)]; [l.c., alt. 4,000 ft, 1 Sept. 1886, Clarke 44830A (K)]; [l.c., alt. 4,000 ft, 1 Sept. 1886, Clarke 44830C (K)]; [l.c., alt. 4,000 ft, 1 Sept. 1886, Clarke 44830D (BM)].
Myanmar: Kachin State [Bhamo, 27 Nov. 1912, Lace 6054 (K)].

Germainia lanipes Hook.f.
Myanmar: Tenasserim [Hefler s.n. (K)].
Appendix 3

Protocol 1

Isolation of total genomic DNA using CTAB

<table>
<thead>
<tr>
<th>Materials</th>
<th>Equipment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Silica gel dried leaf material or material obtained from herbarium specimens</td>
<td>30 ml re-useable chloroform resistant capped centrifuge tubes</td>
</tr>
<tr>
<td>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)</td>
<td>Pipette (P200), Tips</td>
</tr>
<tr>
<td>2-mercaptoethanol</td>
<td>Mortars and pestles</td>
</tr>
<tr>
<td>CI (24:1 chloroform: isoamyl alcohol)</td>
<td>Water bath</td>
</tr>
<tr>
<td>isopropanol</td>
<td>Weighing boats and balance</td>
</tr>
<tr>
<td>Remarks: EDTA= Ethylenediaminetetraacetate</td>
<td>Scissors</td>
</tr>
<tr>
<td>CTAB= Hexadecyltrimethyl ammonium bromide</td>
<td>Fume hood</td>
</tr>
<tr>
<td>3.5 ml transfer pipettes</td>
<td>Centrifuge machine</td>
</tr>
<tr>
<td>Measuring cylinder</td>
<td>50 ml capped tubes</td>
</tr>
</tbody>
</table>

Procedure

1. Preheat 10 ml of 2xCTAB extraction buffer and add 40 µl of 2-mercaptoethanol (in a labelled 30 ml re-useable capped centrifuge tube) to 65°C in a water bath for 15-20 minutes prior to use. Preheat mortars and pestles in the water bath at the same temperature.

2. Prepare approximately 0.2-0.3 g of dried leaf tissue (0.2 g of silica gel dried leaf material and 0.3 g of material obtained from herbarium specimens). Cut leaf into small pieces using clean scissors.
3. Put leaf fragments in a pre-heated mortar and add a small portion of the extraction buffer, use the pestle to grind leaf material. In this process the material was combined with extraction buffer, ground further and added to the buffer and swirled gently to suspend the slurry (all work took place in the fume hood).

4. Return the slurry to the centrifuge tube and then place the tube in water bath to incubate at 65 °C for 1 hour.

5. Add 10 ml of CI into the centrifuge tube. Replace lid and mix and then release lid briefly to release gas. Place on the shaker machine for approximately 30 minutes, make sure all caps are on tightly prior to horizontal shaking.

6. Centrifuge samples at 4,000 revolutions per minute (rpm) for 10 minutes to separate the layers. Balance the sample by CI and loosen lid prior to centrifugation.

7. Remove the upper layer (aqueous phase which contains the DNA) using a transfer pipette (taking care not to disturb the separation) into a fresh labelled 50 ml tube. Ideally the upper phase will be clear and colourless, but this is not always the case, depending on material quality and type.

8. An equal volume of isopropanol (-20°C) and mix gently to precipitate DNA. Place sample into the -20°C freezer to further precipitate the DNA (sometimes it is necessary to leave the sample for a week or more).
Protocol 2
Total genomic DNA washing

<table>
<thead>
<tr>
<th>Materials</th>
<th>Equipment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude tDNA from protocol 1</td>
<td>Centrifuge machine</td>
</tr>
<tr>
<td>Wash buffer</td>
<td>Measuring cylinder</td>
</tr>
<tr>
<td>(70% ethanol)</td>
<td>Fume hood</td>
</tr>
<tr>
<td>TE buffer</td>
<td>Pipettes (P1000), 3.5 ml transfer pipettes</td>
</tr>
<tr>
<td>(10 mM Tris-HCl pH 8.0, 1 mM EDTA)</td>
<td>1.5 ml microcentrifuge tubes</td>
</tr>
</tbody>
</table>

Procedure

1. Centrifuge the sample (DNA in isopropanol, from protocol 1) at 2,000 rpm for 10 minutes to pellet the DNA and then pour off the supernatant.

2. Add 3 ml of the wash buffer. Mix gently and centrifuge again at 2,000 rpm for 5 minutes to pellet the DNA. The supernatant was again poured off.

3. Pour off the supernatant and then gently place the tube upside down for 5 minutes on tissue paper to drain the wash buffer.

4. Turn the tube 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 ethanol.

5. Resuspend the pellet in 0.5 ml of TE buffer and then transfer into a labelled 1.5 ml microcentrifuge tube and store in the -20°C freezer overnight or continued immediately for further clean up using the JETQUICK spin columns technique.
Protocol 3
Total genomic DNA purification

<table>
<thead>
<tr>
<th>Materials</th>
<th>Equipment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Washed tDNA from protocol 2</td>
<td>Centrifuge machine</td>
</tr>
<tr>
<td>Solution H1 (binding) stored at room temperature (contains concentrated</td>
<td>1.5 ml microcentrifuge tubes</td>
</tr>
<tr>
<td>guanidine hydrochloride, EDTA, Tris/HCl and isopropanol)</td>
<td>Pipettes (P200, P1000)</td>
</tr>
<tr>
<td>Solution H2 (wash, reconstituted) stored at room temperature (contains</td>
<td>Tips</td>
</tr>
<tr>
<td>ethanol, NaCl, EDTA and Tris/HCl; diluted with 96-100% ethanol prior to</td>
<td>JETQUICK spin columns and 2 ml receiver tubes</td>
</tr>
<tr>
<td>use)</td>
<td></td>
</tr>
<tr>
<td>TE buffer (10 mM Tris-HCl pH 8.0, 1 mM EDTA)</td>
<td></td>
</tr>
</tbody>
</table>

Procedure

1. Transfer 100 µl of the washed DNA sample into labeled 1.5 ml microcentrifuge tube and add 400 µl of H1-solution. Mix thoroughly.

2. Transfer the solution into labelled JETQUICK spin column that was placed in a 2 ml receiver tube. Centrifuge the sample at 12,000 rpm for 1 minute. Discard the solution in the receiver tube.

3. Add 500 µl of H2-solution into JETQUICK spin column and then centrifuge at 12,000 rpm for 1 minute. Pour off the solution in the receiver tube and centrifuge at 12,000 rpm for 1 minute to remove the H2-solution once more.

4. Place the spin column into a new labelled 1.5 ml micro-centrifuge tube. Add 50 µl of preheated (65°C) TE buffer into the spin column at the centre of the silica matrix for DNA elution.

5. Centrifuge the sample at 12,000 rpm for 2 minutes to transfer the cleaned-DNA 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 store in the -20°C freezer until use.
**Protocol 4**

**Quality assessment and quantification of total DNA and PCR products using agarose gel electrophoresis**

<table>
<thead>
<tr>
<th>Materials</th>
<th>Equipment</th>
</tr>
</thead>
<tbody>
<tr>
<td>tDNA or PCR product</td>
<td>Parafilm</td>
</tr>
<tr>
<td>80 ml of 1.5% of agarose gel</td>
<td>Pipettes (P2~20)</td>
</tr>
<tr>
<td>(1.5 g of agarose gel, 100 ml of 1xTBE, 2 μl of Ethidium Bromide)</td>
<td>Tips</td>
</tr>
<tr>
<td>Loading dye</td>
<td>Gel tank</td>
</tr>
<tr>
<td>(Bromophenol Blue 0.25% w/v and Sucrose 40% w/v)</td>
<td>Power pack</td>
</tr>
<tr>
<td>1xTBE buffer solution</td>
<td>UV light box and Kodak EDAS camera system</td>
</tr>
<tr>
<td>Remark;</td>
<td></td>
</tr>
<tr>
<td>TBE= 0.89 M Tris, 0.89 M Boric acid, 0.02 M NaEDTA</td>
<td></td>
</tr>
</tbody>
</table>

**Procedure**

(Prior to work, make sure the gel is completely covered by the 1xTBE buffer solution.)

1. Place a small drop (2 μl) of loading dye onto parafilm for each DNA sample.

2. Mix 6 μl of tDNA or 4 μl of PCR product with the loading dye by gently expelling and withdrawing the mixture into the pipette tip.

3. Load the sample into the well on the gel.

4. Connect the tank to the power pack and run the samples at about 125 V for 30 minutes.

5. Place the agarose gel onto the UV light box and visualize the bands of DNA samples. Take the photograph of the bands of DNA using a Kodak EDAS camera system. Samples were quantified by comparing intensity of fluorescence of DNA to known standards or relative degree of fluorescence.
Protocol 5
Amplification of targeted regions of plastid DNA and nuclear ribosomal DNA using PCR

<table>
<thead>
<tr>
<th>Materials</th>
<th>Volume (μl)</th>
<th>Amount/Concentration</th>
<th>Equipment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eluted tDNA</td>
<td>2</td>
<td>c. 100 ng</td>
<td>1.5 ml and 0.5 ml</td>
</tr>
<tr>
<td>Master mix reagents</td>
<td></td>
<td></td>
<td>(dome lid)</td>
</tr>
<tr>
<td><em>trnL</em>-F and <em>atpB-rbcL</em></td>
<td></td>
<td></td>
<td>microcentrifuge</td>
</tr>
<tr>
<td>Sterile ultra pure water</td>
<td>36.75</td>
<td></td>
<td>tubes</td>
</tr>
<tr>
<td>10x Reaction buffer</td>
<td>5</td>
<td>1x</td>
<td>Ice</td>
</tr>
<tr>
<td>dNTPs (10 mM each)</td>
<td>1</td>
<td>0.2 mM each</td>
<td>Pipettes (P2, P10,</td>
</tr>
<tr>
<td>Forward primer 100 ng/μl (e.g. c, 2R)</td>
<td>0.5</td>
<td>50 ng</td>
<td>P20, P200, P1000)</td>
</tr>
<tr>
<td>Reverse primer 100 ng/μl (e.g. f, 1R)</td>
<td>0.5</td>
<td>50 ng</td>
<td></td>
</tr>
<tr>
<td>MgCl₂ (25 mM)</td>
<td>4</td>
<td>2 mM</td>
<td></td>
</tr>
<tr>
<td>Promega <em>Taq</em> DNA polymerase (5 units/μl)</td>
<td>0.25</td>
<td>1.25 units</td>
<td>Tips</td>
</tr>
<tr>
<td>TOTAL</td>
<td>50</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

T/T Mix

| Sterile ultra pure water                       | 37.75       | -                    | Centrifuge machine |
| 10x Reaction buffer                            | 5           | 1x                   | Applied Biosystems |
| dNTPs (10 mM each)                             | 1           | 0.2 mM each          | GeneAmp® PCR System 9700 |
| Forward primer 100 ng/μl (e.g. *AB101*)        | 0.5         | 50 ng                |                    |
| Reverse primer 100 ng/μl (e.g. *AB102*)        | 0.5         | 50 ng                |                    |
| MgCl₂ (25 mM)                                  | 3           | 2 mM                 |                    |
| Promega *Taq* DNA polymerase (5 units/μl)      | 0.25        | 1.25 units           |                    |
| 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

Procedure

1. Place DNA samples from Protocol 3 and reagents at room temperature for thawing and immediately place on ice. Transfer 2 μl of each DNA sample into the labelled 0.5 dome lid microcentrifuge tubes.
2. Prepare the master mix into a labelled 1.5 ml microcentrifuge tube by combining the reagents and then vortex gently.

3. Add 48 μl of the master mix into individual tubes of cDNA for a total reaction mixture of 50 μl and then briefly centrifuge the tubes to collect all drops from the walls of the tube.

4. Place the tube in an Applied Biosystems GeneAmp® PCR System 9700.

The following thermal cycling conditions are used for amplification:

<table>
<thead>
<tr>
<th>Cycle phase</th>
<th>Temperature (°C)</th>
<th>Duration (minutes)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-melt</td>
<td>94</td>
<td>1</td>
</tr>
<tr>
<td>Standard cycle (29 cycles)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. Denaturation</td>
<td>95</td>
<td>0.45</td>
</tr>
<tr>
<td>2. Annealing</td>
<td>50 or 52 (atpB-rbcL and ITS)</td>
<td>0.45</td>
</tr>
<tr>
<td>3. Extension</td>
<td>72</td>
<td>2</td>
</tr>
<tr>
<td>Final cycle</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. Denaturation</td>
<td>95</td>
<td>0.45</td>
</tr>
<tr>
<td>2. Annealing</td>
<td>50 or 52 (atpB-rbcL and ITS)</td>
<td>0.45</td>
</tr>
<tr>
<td>3. Extension</td>
<td>72</td>
<td>2</td>
</tr>
<tr>
<td>4. Final extension</td>
<td>72</td>
<td>7</td>
</tr>
<tr>
<td>5. Soak</td>
<td>4</td>
<td>∞ (hold)</td>
</tr>
</tbody>
</table>

5. Check the PCR products on a 1.5% agarose gel (see Protocol 4) and then keep at -20° for further cleaning.
Protocol 6
Purification of PCR products

<table>
<thead>
<tr>
<th>Materials</th>
<th>Equipment</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCR product</td>
<td>1.5 ml microcentrifuge tubes</td>
</tr>
<tr>
<td>Solution H1 (binding)</td>
<td>Pipettes (P200, P1000)</td>
</tr>
<tr>
<td>(contains concentrated guanidine hydrochloride, EDTA, Tris/HCl and isopropanol)</td>
<td>Tips</td>
</tr>
<tr>
<td>Solution H2 (wash, reconstituted)</td>
<td>Centrifuge machine</td>
</tr>
<tr>
<td>(contains ethanol, NaCl, EDTA and Tris/HCl; diluted with 96-100% ethanol before use)</td>
<td>JETQUICK spin columns and 2 ml receiver tubes</td>
</tr>
<tr>
<td>Sterile ultra pure water</td>
<td></td>
</tr>
</tbody>
</table>

Procedure

1. Transfer 50 μl of the successful PCR product into a labelled 1.5 ml microcentrifuge tube and add 400 μl of H1-solution. Mix thoroughly.

2. Transfer the solution into labeled JETQUICK spin column that was placed in a 2 ml receiver tube. Centrifuge the sample at 12,000 rpm for 1 minute. Discard the solution in the receiver tube.

3. Add 500 μl of H2-solution into JETQUICK spin column and then centrifuge at 12,000 rpm for 1 minute. Pour off the solution in the receiver tube and centrifuge at 12,000 rpm for 1 minute to remove H2-solution once more.

4. Place the spin column into a new labelled 1.5 ml microcentrifuge tube. Add 50 μl of preheated (65°C) pure water into the spin column at the centre of the silica matrix for DNA elution.

5. Centrifuge the sample at 12,000 rpm for 2 minutes to transfer the cleaned-DNA into a new tube and then store in the -20°C freezer until further use.
Protocol 7
Cycle sequencing

<table>
<thead>
<tr>
<th>Materials</th>
<th>Volume (μl)</th>
<th>Amount/Concentration</th>
<th>Equipment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pink mix (Big dye™ Sequencing Mix version 1.1)</td>
<td>1</td>
<td>-</td>
<td>0.2 ml microcentrifuge tubes</td>
</tr>
<tr>
<td>Sterile ultra pure water</td>
<td>1.8</td>
<td>-</td>
<td>0.5 ml microcentrifuge tubes</td>
</tr>
<tr>
<td>Sequencing buffer</td>
<td>3.5</td>
<td>70 mM Tris, 1.75 Mγ₂</td>
<td>Ice</td>
</tr>
<tr>
<td>Forward primer</td>
<td>0.7</td>
<td>3.5</td>
<td>Pipettes (P2, P10)</td>
</tr>
<tr>
<td>Reverse primer</td>
<td>0.7</td>
<td></td>
<td>Tips</td>
</tr>
<tr>
<td>Clean PCR product</td>
<td>3</td>
<td>3.5</td>
<td>Centrifuge machine</td>
</tr>
<tr>
<td>TOTAL</td>
<td>10</td>
<td>-</td>
<td>Applied Biosystems GeneAmp® PCR System 9700</td>
</tr>
</tbody>
</table>

Remark; Primers conc. = 5 ng/μl

Procedure

1. Place PCR products from Protocol 7 and sequencing reagents at room temperature for thawing and immediately place on ice

2. Add 1 μl of pink mix into a labelled 0.2 ml domed lid microcentrifuge tube.

3. Prepare the master mix into a labelled 1.5 ml microcentrifuge tube by combining the reagents for each primer and vortex gently.
4. Add 6 µl of the master mix into a domed lid microcentrifuge tube and then add 3 µl of each of the PCR product for the forward primer into the tube. The total reaction mixture volume of each tube is 10 µl.

5. The same procedures were employed with the PCR product for the reverse primer and then briefly centrifuge each of the tubes to collect all drops from the walls of the tube.

6. Place the tube in an Applied Biosystems GeneAmp® PCR System 9700 using the following thermo cycling conditions:

<table>
<thead>
<tr>
<th>Cycle phase</th>
<th>Temperature (°C)</th>
<th>Duration (minutes)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard cycle</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(24 cycles)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. Denaturation</td>
<td>96</td>
<td>0.10</td>
</tr>
<tr>
<td>2. Annealing</td>
<td>50</td>
<td>0.05</td>
</tr>
<tr>
<td>3. Sequence extension</td>
<td>60</td>
<td>4</td>
</tr>
<tr>
<td>Final cycle</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. Denaturation</td>
<td>96</td>
<td>0.10</td>
</tr>
<tr>
<td>2. Annealing</td>
<td>50</td>
<td>0.05</td>
</tr>
<tr>
<td>3. Sequence extension</td>
<td>60</td>
<td>4</td>
</tr>
<tr>
<td>4. Soak</td>
<td>4</td>
<td>∞(hold)</td>
</tr>
</tbody>
</table>

7. Keep the cycle sequencing products at -20°C or directly purify.
Protocol 8
Ethanol precipitation

<table>
<thead>
<tr>
<th>Materials</th>
<th>Equipment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cycle sequenced product</td>
<td>0.5 ml microcentrifuge tubes</td>
</tr>
<tr>
<td>100% ethanol</td>
<td>Pipettes (P2, P200, P500)</td>
</tr>
<tr>
<td>3 M sodium acetate (NaOAc)</td>
<td>Ice</td>
</tr>
<tr>
<td>70% ethanol</td>
<td>Tips</td>
</tr>
<tr>
<td></td>
<td>Centrifuge machine</td>
</tr>
</tbody>
</table>

Procedure

1. Transfer the cycle sequencing product into a labelled 0.5 ml microcentrifuge tube.

2. Add 50 µl of 100% ethanol and 2 µl of sodium acetate into the tube.

3. Leave the sample tube at room temperature for 10 minutes and then place on ice for 30-60 minutes.

4. Centrifuge the sample tube at 13,000 rpm for 25 minutes and then pour off the supernatant.

5. Add 300 µl of 70% ethanol and then spin at 13,000 rpm for 15 minutes and also pour off the supernatant again.

6. Add 300 µl of 70% ethanol one more time and spin at 13,000 rpm for 15 minutes. Completely drain off the supernatant.

7. Let the sample tube dry at room temperature (overnight) to dry the pellets and then store at -20°C.
Protocol 9
Denaturing of dried cycle sequencing samples prior to sequencing using the ABI Prism™ 310 Genetic Analyzer

<table>
<thead>
<tr>
<th>Materials</th>
<th>Equipment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dried cycle sequencing samples</td>
<td>Pipette (P200)</td>
</tr>
<tr>
<td>Template suppression reagent (TSR) or</td>
<td>Tips</td>
</tr>
<tr>
<td>Hi-Di™ Formamide</td>
<td>WhirliMixer™ (Fisons Scientific Equipment)</td>
</tr>
<tr>
<td></td>
<td>Ice</td>
</tr>
<tr>
<td></td>
<td>Septa for 0.5 ml microcentrifuge tubes</td>
</tr>
<tr>
<td></td>
<td>Perkin Elmer DNA Thermo Cycler 480</td>
</tr>
<tr>
<td></td>
<td>Centrifuge</td>
</tr>
</tbody>
</table>

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.