

**SYSTEMATICS AND PHYLOGENY OF THE GENUS  
CALAMUS Linn. (ARECACEAE) IN THE WESTERN GHATS**

**THESIS**

**submitted to the**

**University of Calicut**

**in partial fulfillment of the degree of**

**DOCTOR OF PHILOSOPHY**

**by**

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## Certificate

This is to certify that the thesis entitled 'Systematics and phylogeny of the genus *Calamus* Linn. (Arecaceae) in the Western Ghats' is a record bonafide research carried out by Mr. Sreekumar V. B. under my guidance and supervision. No part of this work has been presented elsewhere for any degree, diploma, fellowship or other similar titles.

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## Declaration

I hereby declare that the thesis entitled '**Systematics and phylogeny of the Genus *Calamus* Linn. (Arecaceae) in the Western Ghats**' submitted by me in partial fulfillment for the Ph.D degree of the University of Calicut, incorporates the results of the work done by me. This thesis has not been submitted by me to any other University for the award of any other degree, diploma or any other titles and it represents the original work done by me.

Peechi

December 2004

  
Sreekumar V. B.

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## Abbreviations

$\mu\text{g}$	: microgram
$\mu\text{l}$	: micro litre
$\mu\text{m}$	: micromolar
AFLP	: Amplified Fragment Length Polymorphism
bp	: Base pair
CTAB	: Cetyl trimethyl ammonium bromide
DNA	: Deoxyribonucleic acid
EDTA	: Ethylene diamine tetra acetate
$\text{MgCl}_2$	: Magnesium chloride
NaCl	: Sodium chloride
ng	: nanogram
PAGE	: Poly acrylamide gel electrophoresis
PCR	: Polymerase chain reaction
PVP	: Polyvinyl pyrrolidone
QTL	: Quantitative trait loci
RAPD	: Randomly Amplified Polymorphic DNA
RFLP	: Restriction Fragment Length Polymorphism
rpm	: revolutions per minute
UPGMA	: Unweighted pair group mean algorithm

# Introduction

Sreekumar V.B “Systematics and phylogeny of the genus *calamus* linn. (arecaceae) in the western ghats ” Thesis. Kerala Forest Research Institute, University of Calicut, 2004

## *Introduction*

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# 1. Introduction

Rattans are spiny climbing palms with characteristic scaly fruits, belonging to the subfamily Calamoideae of Areaceae. The word “rattan” is derived from the local Malayan term ‘rotan’ commonly used for canes (Whitmore, 1973). Rattans comprise around 600 species belonging to 13 genera (Dransfield, 2000) which are concentrated solely in the old world tropics. They are one of the main Non Wood Forest Produces and provide livelihood for millions of tribal and rural people and raw material for industries in various parts of the world. It is estimated that more than half a million people are directly employed in harvesting and processing of rattans in the rural areas of South East Asia. The global trade and subsistence value of rattan and its products are estimated at over US\$ 7,000 million per annum (Pabuayan, 2000).

The genus *Calamus* L., locally known as Vetasa (Sanskrit), Betha (Kannada), Churel (Malayalam), Perambu (Tamil), Beth (Hindi) under the subfamily Calamoideae, include about 370 species (Uhl and Dransfield, 1987). The fossil pollen from the coal samples of Sarawak (Muller, 1979 and 1981) suggests that the genus is very ancient. Their distribution ranges from the Indian subcontinent and South China southwards and east through Malaysia and Indonesia to Fiji, Vanuatu and tropical and subtropical parts of eastern Australia. The greatest diversity is seen in Malay Peninsula and Borneo followed by New Guinea.

The genus is common in a wide geographic range matched with great ecological diversity in diverse forests and soil types. It is well adapted to different soil moisture regimes from swamps to dry ridges and the distribution extends from the sea level to

more than 3000 m elevation, from the equatorial rainforest to monsoon savannahs and the foothills of the Himalayas. Most of the species occur in closed forests and are early gap colonizers. They require light gaps for proper growth and development and adequate light is an essential factor for the enhanced growth of many species.

The genus, *Calamus* is represented by 46 species in India, distributed in three phyto-geographical regions viz. the Peninsular India, the Eastern Himalayas and the Andaman and Nicobar Islands (Renuka, 2001). In each of these regions, the genus shows high degree of endemism and only one species namely, *C. viminalis*, is found in all these regions. The distribution of some of the Indian species extends to like China, Myanmar and Sri Lanka.

### **Eastern Himalayas**

In the eastern Himalayas covering North Eastern States, the genus is mainly found in the evergreen forests of Assam Valleys, submontane forests of Arunachal Pradesh, Meghalaya, Manipur, Nagaland, Mizoram and Tripura. The distribution extends from the alluvial plains to the moist hill forests upto 2000 m altitude. The maximum concentration in terms of species diversity is found in Dumpui and Dumpa forest areas of Mizoram. The tropical evergreen forests of Tirap, Changland and Lohit districts of Arunachal Pradesh, Khasi and Garo hills in Meghalaya and the Lohar bandth forests in Cachar district of Assam also have rich diversity. With exception to other members, *C. tenuis* is found in the open and in the partially shaded swampy areas along human habitations. The genus is also found in the moist deciduous forests of Orissa, Chattisgarh and in the coastal swampy areas of West Bengal.

### **Andaman and Nicobar Islands**

In the Andaman and Nicobar Islands, the genus is distributed in Evergreen and Semi-evergreen forests with nine species. The South Andaman represents richest assemblage with six endemic species. The large diameter cane *C. andamanicus*, is widely distributed throughout the Andaman and Nicobar islands. *C. baratangensis* is so far known only from Baratang Islands in middle Andamans. *C. basui* is endemic to Little Andamans and *C. viminalis* is common along the denuded hill slopes in Southern and Northern Andaman.

### **Peninsular India**

In Southern India, *Calamus* species are mainly distributed in evergreen, semi-evergreen and moist deciduous forests of the Western and the Eastern Ghats. In terms of diversity, greatest concentration with highest rate of endemism is found in the Western Ghats. Even though, Kadambi (1950) reported the presence of cane breaks in the windward western slopes of the ghat forests, however, such cane breaks could not be located during the present survey. *Calamus thwaitesii* is widely distributed throughout the Western Ghats. *Calamus dransfieldii* is endemic to Dhoni hills in Palakkad district of Kerala State and *C. nagbettai*, originally reported from the Subrahmanya forests of Karnataka, has recently been reported from Kollam District as well (Mukundakumar *et al.*, 1998). The distribution of *C. rivalis* is limited to the coastal areas of Kollam and Alapuzha districts. In Kerala part of the Western Ghats, Rosemala of the Shendurunii Wildlife Sanctuary represents maximum diversity with 10 species. The other main areas are Chemunchi of Wayanad, New Amarambalam Forest Reserve in Nilambur, Achenkovil of Kollam and Silent valley National Park. In Karnataka, the genus is distributed mainly in the forest areas of Thalakkaveri, Subramanya, Agumbae and

Coorg. *C. prasinus* and *C. stoloniferus* are known only from Karnataka state. In Tamilnadu, the genus is not very common but *C. thwaitesii* and *C. brandisii* are found in the forests of Thirunalvely, Trichy and Kanyakumari.

### **Outline of the problem**

Taxonomically, rattans are one of the neglected groups due to their spiny nature, and difficulty in collecting flowers and fruits. The size, texture and colouration of plants and plant parts especially the diagnostic features of the leaves, sheaths, inflorescences, fruits etc. do not yield to conventional herbarium techniques. Hence many species are poorly represented in herbaria, both in number of collections and completeness of specimens. Even if materials of all stages are available, by the sheer size of the plant parts, one cannot get all of them on the same sheet. Similarly the samples with the morphological variations are also rarely represented. These factors account for the confusion that have crept into rattan taxonomy.

The first systematic arrangement of the genus was proposed by Beccari (1908) who arranged the species into sixteen infra-generic groups. But Beccari found it almost impossible to assign to each division constant and precise characters due to the lack of enough representative materials. Subsequent to Beccari's work, several species were described and added (Renuka, 1992 and 1995b) and it was not possible to include them under any of the recognized groups, as they exhibit a mixture of defined characters among different groups. The systematic relationships of the newly described species with other members are also not clear at present. Another problem of the systematics of the genus is high degree of intra-specific variability which often led to treat morphologically closely related species as different ones (Renuka, 1994). A study on

the systematic position of the genus among the members of the subtribe Calaminae using 5 SsrDNA sequence data showed that the remaining five genera are nested within it (Baker *et al.*, 2000). Many species of the genus are described on the basis of fragmentary materials and in many cases the vegetative part of one taxon is mixed with the reproductive part of another as in the case of *C. pseudorivalis* (Renuka, 2001). So far 21 species have been reported from the Western Ghats of India, out of which 16 are endemic. Four species extend their distribution to Sri Lanka and one to North Eastern regions of India and to the Andaman Islands. Some of these reported species appear to be closely related and hence a phylogenetic regrouping and morphometric studies are needed. Since majority of the species are endemic, one tends to consider the species of the Western Ghats as monophyletic. This hypothesis also needs to be re-examined. In this context, the present study was undertaken with the following objectives.

1. To study the phylogenetic relationships among the *Calamus* species of the Western Ghats.
2. To check whether the species are monophyletic.
3. To verify the species delimitation through morphometric and genetic variation studies.

### **Systematics and phylogeny**

The term systematics was originally defined by Simpson (1961) as “the scientific study of the kinds and diversity of organisms and of any and all relationships among them”. Solbrig (1970a), as an advocate of this definition, recognized four major types of relationships between plants, based on descent (phylogeny), similarity (phenetics), space (geography) and trophic relations. Studies on the systematics have progressed

tremendously during recent years with a notable increase in diverse approaches. During the late 1950s and early 1960s of the twentieth century, phytochemical data were a major part of the taxonomic investigation. With the advancement of biological tools, new information is added continuously and taxonomists are now faced with the challenge of integrating and synthesising various types of available information.

The major aim of modern systematics is to generate a phylogenetic classification, which reflects the evolutionary relationships of different members of a group. Phylogenies are different from phenetics which produce tree like diagrams showing groups of organisms based on overall similarity. Phenetic methods are not designed to retrieve the evolutionary history. Phylogenies are hypothesis of relationship that are constructed by the analysis of shared derived character states or synapomorphies. A phylogeny has an implicit predictive value because in knowing the qualities of one member of a group defined in a phylogeny, one can predict that the other members of same group also will possess the same qualities. Thus phylogenies are powerful bio-informatic tools. The phylogenetic tree and corresponding classification predict the properties of newly discovered or poorly known organisms. In the case of higher-level groups, phylogenetic methods are used simultaneously to identify the main evolutionary units or clades and to estimate the relationships among them. The great advantage of phylogenetic classification is its repeatability.

### **Molecular markers**

Molecular markers have proved as an important tool for classification of taxonomic groups with high level of precision. Molecular markers provide ample coverage of genome and quick detection of DNA variation and the results are not influenced by spatial and temporal effects of genes (Wickneswary *et al.*, 1996). They were used in

conventional taxonomic lines in examining the genetic variability across species and in unravelling the evolutionary history or phylogeny of a species. The combination of both molecular and morphological data has provided the unique opportunity to understand the history of life and its evolution.

## **1.1. Study area**

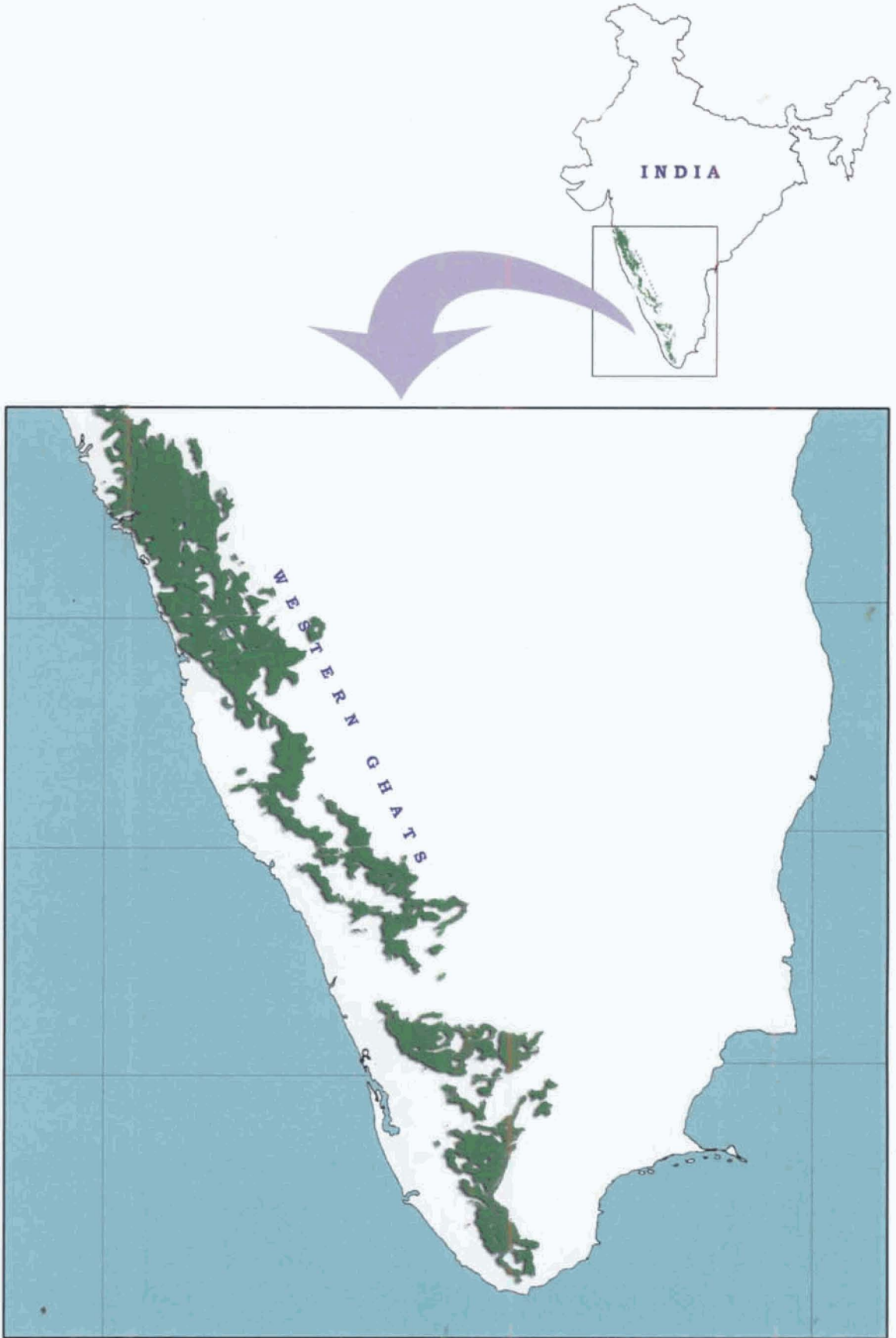
### **Location and physiography**

The Western Ghats, one of the 25 biodiversity hotspots of the world (Map1) consists of a chain of mountains of 1600 km long, running parallel to the west coast of peninsular India, from the river Tapti ( $21^{\circ}$  N) to Kanyakumari ( $8^{\circ}$  N) (Myers, 1988). The average elevation ranges from 900 to 1500 m and the highest mountain peaks are Anamudi (2695 m) and Doddabetta (2637 m). Along the entire length, the major discontinuity, called the Palghat gap, is about 30 km where the floor height is often less than 100 m above the sea level. The wide variation in the altitude, warm tropical climate, high rainfall and diverse topography create wide array of ecological niches and high rate of endemism.

### **Climate**

The climate of the Western Ghats is hot and humid tropical. The rainfall ranges from 3000 mm in the coast to 7500 mm in the crestline, but eastwards the rain decreases and the eastern foothill slopes are almost rain shadow areas. The rainfall received in the Western Ghats is primarily from the southwest monsoon and northeast monsoon is very short duration and brings much less rain. The peak rainy season in the western face is June to August, while it is October to December along the eastern edges. The mean temperature ranges from 20 to  $24^{\circ}$  C.

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**Map 1. Study Area**

## **Phytogeography and endemism**

Phytogeographically, the flora of the Western Ghats is palaeotropic derived from the original Gondwana land. Champion and Seath (1968) recognised five major forest types in the Western Ghats; Tropical wet evergreen forests, Tropical semi evergreen forests, Tropical moist deciduous forests, Littoral and Swamp forests and Tropical dry deciduous forests. Clarke (1904) included the Western Ghats under the phytogeographical unit “Malabar” based on soil types. Hooker and Thomson (1855) included the Western Ghats under ‘Malabar Province’ of the nine botanical provenances recognised in Indian region. The four phyto-geographical regions in the Western Ghats recognised by Subramaniyan and Nayar (1974) are the region from River Tapti to Goa, the Western Ghats from Kalinadi to Kodagu, the Nilgiris and the Anamalais, Palani and Cardomom hills including Tirunelveli and the highlands of Kerala.

Myers (1988) recognized two main centres of diversity in the Western Ghats, *ie.*, the Agasthyimalai hills and Silent valley/New Amarambalam forest areas. Out of the 4,000 species of flowering plants, about 1,500 species are endemic restricted to this area (Nayar, 1989).

The Western Ghats is now an intrinsic mosaic of various degradation stages of the natural forests along with plantations of rubber, cardamum, tea, coffe, pepper, eucalypts etc. The high rate of deforestation, monoculture plantations and changes in the land use pattern resulted in soil erosion, forest fragmentation, alteration of habitats, prevention of gene flow and change in genetic structure which gradually lead to species extinction. Development activies like construction of dams, hydroelectric projects, tourism, intensive logging etc. had their impact on biodiversity. The distribution of rattans also is greatly affected by the degradation of forests. Majority of the species are in the threat category (Renuka, 2001) and a sound knowledge on the taxonomy of rattan is needed to start conservation activities.

# Review of literature

Sreekumar V.B “Systematics and phylogeny of the genus *calamus* linn. (arecaceae) in the western ghats ” Thesis. Kerala Forest Research Institute, University of Calicut, 2004

*Review of Literature*

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## 2. Review of literature

### 2.1. Systematic studies of the genus *Calamus*

The earliest account of Indian rattans was published in Rheede's *Hortus Malabaricus* (1693) where three species from the Western Ghats (Tsjeru-tsjurel, *C. travancoricus*; Katu-tsjurel, *Calamus* sp; Perim tsjurel, *C. thwaitesii*) were described and illustrated. In the early eighteenth century, Herman (1717) published description of *C. rotang* in *Musaeum Zeylanicum* and later by Burmann in 1737. Linnaeus (1747) included this species in his *Flora Zeylanica* based on the collection of Herman.

Otto Kuntze in "Revisio Generum Plantarum" used the generic name *Palmijuncus* Rumph. for the genus *Calamus* L., but later Beccari (1908) confirmed that *Palmijuncus calapparius* is a typical *Daemonorops* and he continued to use the generic name 'Calamus'. In Loureiro's (1790) *Flora of Cochinchinensis* detailed description of six species of *Calamus* from Cochin China were included. Martius (1823), Wallace (1853) and Richard Spruce (1871) made significant contributions towards the systematic studies of the genus. Beccari (1919) conducted detailed botanical explorations and subsequently, Fernando (1988) made an extensive survey and the rattan flora is now relatively well known in Philippine islands. The diversity of rattan is reported to be very high in Peninsular Malaysia. The systematic studies of Malayan species were first initiated by Furtado (1956) and he treated 72 species under the genus *Calamus*. Later on Dransfield (1979) conducted detailed studies on taxonomy, natural history and conservation aspects of Malayan rattans.

Dransfield (1982) initiated the taxonomic studies on rattans in Sabah, in which he validated the nomenclature and published several new species of *Calamus* which resulted in the publication of a field guide (1984) with keys, descriptions and

illustrations. “*Rattans of Sarawak*” was published by Dransfield (1992). Chang (1988) provided the morphology and taxonomic identity of three Taiwan species. In Sri Lanka, the earliest taxonomic documentation of the genus was made by Hermann (1717) followed by Thwaites (1864). Later, Trimen (1898) in *Handbook to the Flora of Ceylon* described some species but the detailed taxonomic work with description and illustrations were provided by De Zoysa and Vivekanandan (1994) and they reported that seven of the 10 species are endemic to Sri Lanka. The systematics of Chinese species were done by Pie and Chen (1991) followed by Xu *et al.* (2000) and they reported about 35 species of *Calamus*. In Bangladesh, Alam (1990) studied the taxonomy, distribution and reported 11 species under two genera.

The genus, *Calamus* is wide spread throughout west and central Africa with highest concentration of species diversity and endemism. The taxonomic studies on African species were published by Sunderland (2000). Brunei Darussalam has very good natural resources of rattans. Dransfield (1997) initiated the taxonomic studies and published an interactive database from this region. Evans *et al.* (2001) identified 38 species of *Calamus* from Lao PDR and released a detailed annotated nomenclature and taxonomic description. The detailed studies on rattans of the Papua region are lacking except a checklist of collection of *Calamus* published by John and Taurereko (1989). But recently the Royal Botanic Garden, Kew initiated a project in this region. The first authentic reference on the taxonomy of the Indian rattans is in Rheede’s *Hortus Malabaricus* (1693). Roxburgh (1832) described 4 new species of rattans from the eastern part of India. *The Palms of British East India* published in 1844 - 1845 by Griffith reported 11 new species. Beccari’s work on Indian rattans, published in the Hooker’s *Flora of British India* (1872 - 97) described 9 species of *Calamus*. The classical work by Beccari, the monograph on *Calamus*, between 1908 and 1918

included 12 southern Indian species. Gamble and Fischer (1931) reported 11 species. Renuka (1992 and 1995b) carried out detailed studies on the rattans of the Western Ghats and the Andaman and Nicobar islands and added several new species and published taxonomic manuals for the Western Ghats and the Andaman and Nicobar Islands.

## **2.2. Phenetic/morphometric studies**

The phenetic or morphometric studies involve the application of multivariate techniques in systematics. The phenetics is a method of organising data which helps to understand the relationship among species. The phenetic taxonomy is variously defined as taxometrics (Rogers, 1963), taxometrics (Mayr, 1966), mathematical taxonomy (Jardane and Sibsen, 1971) and multivariate morphometrics (Blackith and Reyment, 1971). The philosophy of modern phenetic method is based on ideas proposed by Adanson (1763), a French naturalist and the principles of phenetics were further developed by Sneath and Sokal (1963) based on the modern interpretation of adansonian principles which are known as neo-adansonian principles. Jardane and Sibsen (1971) described the methods and applications of phenetic taxonomy followed by McNeill (1974). The publication of Sneath and Sokal's (1963), "Principle of Numerical Taxonomy" epitomizes the aims, objectives and purposes of phenetics. According to Sneath and Sokal (1963), the phenetic classifications have generally been constructed using overall similarity but it has been argued that better phenetic classification can be made using other methods (Farris, 1980).

Phenetic analyses at the higher taxonomic level were done by Prance *et al.* (1969), McNeill (1978) and Baum (1977). In the lower level the earliest work includes Olsson (1967); Stearn (1968); Banks and Hillis (1969); Bemis *et al.* (1970) and Baum (1974).

Later on Wilson *et al.* (1990) conducted phenetic comparison of some *Fumaria* spp. (Fumariaceae) using cluster analysis and principal component analysis. Phenetic and cladistic analysis were used for analysing different species of *Eucalyptus* from eastern Victoria for clarifying hybrid origin and for assessing species boundaries (Kottek, 1990). Maxted (1993) conducted phenetic analysis of *Vicia* section *Atossa* series *Truncatulae* (Leguminosae) based on 40 herbarium samples and found 2 species within the series. The Infra-generic phenetic relationships of 23 sections of new world *Panicum* (Zuloaga *et al.*, 1993) clustered into five major groups. The species boundaries of *Solanum* series *Demissa* and potentially related species in series *Acaulia* and series *Tuberosa* were studied by Spooner *et al.* (1995). Sasikala *et al.* (1998) conducted phenetic evaluation of trichome characters in certain members of Asteraceae. The relationships of 37 species belonging to 18 genera of Bignoniaceae growing in Argentina were investigated by cluster analysis and by ordination using 48 morphological characters (Rivera, 1999).

The problem of intra-specific variability has been addressed by multivariate techniques in various plants such as *Polyalthia hypoleuca* complex (Rogsted, 1989), Australian waratahs (Crisp and Weston, 1993), *Cyphomeris* of Nyctaginaceae (Mahrt, 1993), *Leptospermum trinervium* of Myrtaceae (Krauss, 1994), *Epipactis helleborine* of Orchidaceae (Tyteca and Dufrene, 1994), *Tasmannia* of Winteraceae (Raleigh, *et al.*, 1995), the genus *Poa* (Rua, 1996), *Ernodea* of Rubiaceae (Negron-Ortiz and Hickey 1996), *Lobelia cardinalis* complex of Campanulaceae (Thompson and Lammers 1997), *Eucalyptus flocktoniae* complex of Myrtaceae (Nicolle and Conran, 1999), *Echinocactus polycephalus* complex of Cactaceae (Chamberland, 1997), *Boronia grandisepala* group under Rutaceae (Duretto and Ladiges, 1997), *Daviesia ulicifolia*

complex of Fabaceae (Chandler and Crisp, 1998), *Salvia* sect. *Ekmania* of Lamiaceae (Torke, 2000), *Datura* (Cavazos *et al.*, 2000), *Quercus acutifolia* and *Q. conspersa* of Fagaceae by Romero *et al.* (2000a) and pines by Romero *et al.* (2000b). Kim *et al.* (2003) conducted phenetic analysis of *Typha* in Korea and far east Russia. Morphometric and Randomly Amplified Polymorphic DNA data were successfully used for the classification of different subspecies of *Cardamine amara* (Lihova *et al.*, 2000) in the Siberian Peninsula. In the case of palms, Borchsenius (1999) studied the local variation within *Genoma cuneata* which is the first application of multivariate techniques in palm systematics, however he examined only a small portion of the range of the complex, using population level data taken directly from living plants. The intra-specific variation in *Licuala glabra* (Loo *et al.*, 2001) based on herbarium specimens, found clearly defined infra-specific groups associated with certain geographic areas. Henderson (2002) studied the variation within the genus *Reinhardia*, providing details on the range variation within this complex. Henderson and Ferreira (2002) using univariate, bivariate and multivariate statistical methods analysed morphological variation between and within the palm genus *Synechanthus* based on the morphological characters taken from herbarium specimens. Cupido (2003) reassessed the species boundaries of the genus *Merciera* and studied the pattern of morphological variation.

### **2.3. Phylogenetic studies**

The goal of phylogenetic systematics is to identify and discard the non-monophyletic taxa. Cain and Harrison (1960) used the term "cladistics" to refer the relationship expressing recency of common ancestry. According to Sneath and Sokal (1963) cladistic relationship refers to the path of ancestral lineages and therefore describes the

sequence of branching of the ancestral lines. Several workers replaced the term "cladistics" by "phylogenetics" (Hennig, 1966; Wiley, 1981; Bremer and Wanntorp, 1982). Although, Nelson and Platnick (1901) provided some of the basic principles of phylogenetic analysis, the publication of "Phylogenetic Systematics" (Hennig, 1966) caused a major revolution in the systematic methodology. In cladistics various authors proposed different methods of analysis as transformed cladistic method by (Hennig, 1966); Wagner ground plan divergence method (Wagner, 1961); Branch and bound method (Foulds *et al.*, 1979; Penny, 1982) and character compatibility method (Estabrook *et al.*, 1975, 1976). The hennigian methods and principles were further developed and applied on various groups by Koponen (1973), Bremer (1978), Bremer and Wantorp (1978), Humphries (1981), Humphries and Richardson (1980), Wiley (1981), Bolick (1981), Churchill (1981), Sanders (1981) and Hill and Crane (1982). Humphries and Funk (1984) gave an account of the detailed methodology of cladistics and its applications in plant systematics. Doungue and Cantino (1988), Hamphries and Chappil (1988) upheld the virtues of cladism, even though criticised by Cronquist (1987). Whiffin and Biener (1972) developed a simplified method for the construction of wagner trees and discussed the type of characters used and applied it on genus *Helenium* L. The construction of tress using wagner method is further described by Farris (1970) Kluge and Farris (1969) and Solbrig (1970b). The criteria used for choosing characters for the analysis and its various definitions were advocated by Sneath and Sneath (1963), Jones and Luchsinger (1979) and Crowson (1970). Sneath and Sokal (1963) defined a "unit character" as a "taxonomic character of two or more states which cannot be further subdivided logically except for subdivision brought by changes in the method of coding the states." With this background, therefore, characters

to be used in this study were selected based on ease of observation, availability and discontinuity. Complex characters were broken down to simple unit characters. A good classification of objects will largely depend on the characters selected, how varied they are, whether they show discontinuities and also the way they are treated (Pankhurst, 1991). Good characters are not easily modified by environmental factors and have a genetic background such that they are unlikely to change readily (Heywood, 1967). These may be referred to as constant characters and are highly heritable. The best essays on characters are provided by Stevens (1980) and Pimental and Riggins (1987). In phenetic classification, it has been argued that as many characters as possible ought to be used when classifying organisms (Sneath and Sokal, 1963; Pankhurst, 1991). But in phylogenetic systematics it is argued that only characters which bear evolutionary significance must be used, and other characters must be ignored (Pankhurst, 1991). Stevens (1991) provided a review on character states, morphological variation and its application in phylogenetic analysis.

Parenti (1980) presented a phylogenetic reconstruction of land plants based upon Hennigian principles which was the first attempt at applying cladistics to land plants. Hussain and Paul (1988) analysed the cladistic relationships among the Indian species of *Ixora* (Rubiaceae). Zona (1990) conducted studies on phylogenetic relationship among different species of the New World genus *Sabal* and proposed phylogenetic hypothesis with remarkable congruence with the bio-geographic data. The taxonomy and systematic position of the genus *Sphinctospermum* (Leguminosae) inferred from cladistic analysis of traditional data (Lavin, 1990) suggests its systematic position between subtribes. Crisci and Berry (1990) conducted phylogenetic re-evaluation of the Old World species of *Fuchsia* (Onagraceae) based on 17 characters.

In a study conducted by Dransfield *et al.* (1990) on subfamily Coryphoideae based on both morphological and molecular data suggested the polyphyly of the group. Kramadibrata (1992) conducted phylogenetic analysis of Malayan rattans and concluded that they form a paraphyletic group. Sanderson (1991) studied 113 species of North American *Astragalus* L (Fabaceae) and identified three major groups. The systematics and biogeography of nine species of *Bauhinia* of the *Corymbosae* series (Leguminosae) based on phylogenetic analysis of 14 morphological characters were well established by Zhang and Chen (1992). Karis *et al.* (1992) conducted phylogenetic analysis of Cichorioideae (Asteraceae), with emphasis on the Multisieae. Welzen (1993) formulated the evolutionary relationship within the genus *Arthraxon* using Wagner parsimony and identified two infra-generic groups. The evolution in the Andean epiphytic Genus *Columnnea* (Gesneriaceae) with emphasis on morphological variation were studied by Smith and Systma (1994). The first cladistic study in the family level was initiated by Bremer (1994) on Asteraceae and he proposed a phylogenetic classification. The monophyly of the fern genera *Macrothamnium*, *Leptohymenium* and *Orontobryum* were confirmed by Chiang (1995). Salzman and Judd (1995) conducted taxonomic revision and cladistic studies on Greater Antillean species of *Bactris* (Bactridinae: Arecaceae) points that they form a well supported monophyletic group. The study by Schutte (1997) on taxonomic revision of the genus *Liparia* (Fabaceae) using cladistic analysis of 13 morphological characters produced a single partially resolved cladogram, with two distinct main clades. Cladistic analysis of the subtribe Sicyinae (Cucurbitaceae) (Lira *et al.*, 1997) resulted in two equally most parsimonious cladograms confirming monophyly of the group. Generic interrelationships of *Cardueae* (Asteraceae) were confirmed using 75 morphological character data by Petit (1997)

indicating paraphyly. The phylogenetic study (Castillo and Spooner, 1997) of wild potatoes, *Solanum* series *Conicibaccata* (Sect. Petota) suggests that occurrence of some species represent a combination of apospecies and plesiospecies. Morton *et al.* (1997) studied phylogenetic relationships of Lecythidaceae using *rbcL* sequence and morphological data indicated paraphyletic nature and potential outgroup relationships with Sapotaceae. Sidiyasa (1998) analysed the taxonomy, phylogeny, and wood anatomy of *Alstonia*. Barrow (1998) conducted phylogenetic analysis of the genus *Phoenix* using morphology and 5 SsrDNA sequence data which indicated high degree of character conflict between the data sets in supporting pattern of relationships within the genus. Mogeia (1999) assessed the relationships and phylogeny of 21 different species of *Arenga* based on morphological characters. Zona (1999) studied the relationships within the genus *Drymophloeus* by maximum parsimony and indicated that the closest sister group to the genus is *Balaka*. Systematic relationships within the *Litsea* complex on the basis of morphological and leaf cuticle data points that most of the genera are complex and polyphyletic (Li *et al.*, 2000). The cladistic analysis of the subfamily Calamoideae indicate the systematic position of the subtribe Calaminae and non-monophyly (Baker *et al.*, 2000). Using morphological and phylogenetic arguments, Ortiz (2001) conducted the reinstatement of the genus *Macledium* (Asteraceae, Multisieae). Bradford and John (2001) succeeded in applying morphological cladistic approach for the classification and identification of the *Weinmannia* in Madagascar and the Comoro Islands. Carine and Scotland (2002) evaluated the three different classifications of South Indian and Sri Lankan *Strobilanthisae* using morphological cladistic approach and the results was highly incongruent with earlier classification. Selvi *et al.* (2002) conducted phenetic and cladistic analysis of *Nonea* and recognized 2

groups suggesting that the genus is paraphyletic. Henderson (2002) studied the phylogenetic relationship among different species of *Reinhardtia* which does not support infra-generic classification proposed by Moore (1957). Graham (2002) established phylogenetic relationships and biogeography of the endemic Caribbean genera *Crena*, *Ginoria* and *Haitia* (Lythraceae) using morphological characters.

## 2.4. Molecular markers

The advent of molecular techniques, especially the development of Polymerase Chain Reaction (PCR) for amplifying and sequencing of DNA resulted in solving various systematic problems, elucidating phylogenetic relationships and studying genetic variation up to species level. Molecular markers offer wide coverage of the genome resolution and provides detection of variation at DNA level which are less affected by the environmental plasticity. Hillis and Dixon (1992) provided detailed review on the importance of molecular markers in systematic studies. Molecular markers include co-dominant isozymes, RFLPs (Restriction fragment length polymorphism) and the dominant RAPDs and AFLPs (Amplified fragment length polymorphism).

### Isozymes

Isozymes are multi-molecular forms of enzymes that share a common substrate but differ in electrophoretic mobility. They appear on electrophoretic gels as multiple bands, which are coded by more than one gene locus (Moss, 1982). Isozymes were used for examining population structure (Legionnet and Lefevre, 1996; Affre and Thomson, 1997; Gillies *et al.*, 1999); systematics (Giannasi and Crawford, 1980; Gottlieb, 1981, 1984; Crawford, 1983; Buth, 1984; Hillis, 1987) to study gene flow, QTL mapping, species or varietal identification (Glaszmann, 1987; Davis and Manos, 1991) and for

studying phylogenetic relationships (Lambooy *et al.*, 1991; May, 1994; Jaaska, 1997; Mitton, 1998).

### **Restriction Fragment Length Polymorphism (RFLP)**

RFLP is the most conventional method and has played a major part in genetic mapping, comparative mapping and in elucidating phylogenetic relationships (Jansen *et al.*, 1990; Miller and Tanksley, 1990; Olmstead and Palmer, 1992; Devos *et al.*, 1992; Davis and Soreng 1993; Causse *et al.*, 1994; Neale and Harry 1994; Becker *et al.*, 1995; Jansen and Palmer 1998; Vivek and Simon 1999). Lu *et al.* (1999) assessed the taxonomic status of *Cunninghamia konishii* and *C. lanceolata* based on the RFLPs of a chloroplast trnD-trnT spacer. However, RFLPs detect relatively low amounts of genetic polymorphism when compared to the PCR based methods.

### **Randomly amplified polymorphic DNA (RAPD)**

In RAPDs, the reaction is performed on a genomic DNA template and is primed by 10 mer or 20 mers oligonucleotide primers resulting in amplification of discrete DNA products which are separated on agarose gels in the presence of ethidium bromide and visualised under ultraviolet light (Williams *et al.*, 1990). The polymorphism is detected as the presence or absence of a particular band in a specific locus. RAPDs have great potential in the study of genetic variation (Huff *et al.*, 1993; Vicario *et al.*, 1995; Nesbitt *et al.*, 1995; Nolan *et al.*, 1996; Sale *et al.*, 1996; Smith and Pham, 1996; Martin *et al.*, 1997; Gillies *et al.*, 1997; Maguire and Sedgley, 1997; Schierenbick *et al.*, 1997; Wolff *et al.*, 1997; Huff *et al.*, 1998; Cardoso *et al.*, 1998; Liao and Hsiao, 1998; Kwon and Morden, 2002), in molecular taxonomy and phylogeny (Hosaka *et al.*, 1984; Debener, *et al.*, 1990; Bandi *et al.*, 1995; Anzizar, *et al.*, 1998; Rath *et al.*, 1998; Gillies and

Abbott, 1998; Bartish *et al.*, 2000; Miaw *et al.*, 2000; Lihova *et al.*, 2000) in clonal and varietal fingerprinting, (Brunell and Whitkus, 1997) in construction of genetic maps (Bundock *et al.*, 2000) and in localization of important genes (Hedrick, 1992; Rafalski *et al.*, 1991, Caetano-Annoles *et al.*, 1991; Tingey and Deltufo, 1992).

Zona (1996) used RAPD markers to examine the species boundaries of the palm genus *Roystonea*. The study conducted by Anzizar *et al.* (1998) on various palm genera using RAPD's and ISTRs found utility identification within species level and failed above genus level. Changtragoon *et al.* (1995) obtained species-specific polymorphism on different species of Thailand rattans using RAPD markers. Low level of genetic polymorphism was uncovered in a study conducted by Dowe *et al.* (1997) of an endangered palm, *Carpoxyton macrospermum*, and suggested a history of inbreeding in natural populations.

### **Amplified Fragment Length Polymorphism (AFLP)**

AFLP proposed by Vos *et al.*, (1995), which uses selective amplification of restriction fragments and complex-banding patterns, DNA fingerprints, of up to at least 100 DNA fragments in each reaction can be generated using this method. AFLP is, therefore, potentially very useful for the study of assessing genetic diversity (Negi *et al.*, 2000), biosystematic studies (Jouke *et al.*, 1998; Zhu *et al.*, 1998; Kahn and Second, 1999) and in genetical and physical mapping studies (Becker *et al.*, 1995).

# Phenetic and phylogenetic studies

Sreekumar V.B “Systematics and phylogeny of the genus *calamus* linn. (arecaceae) in the western ghats ” Thesis. Kerala Forest Research Institute, University of Calicut, 2004

*Phenetic and phylogenetic studies*

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## 3. Phenetic and phylogenetic studies

### 3.1. Introduction

All the organisms on earth are the result of evolution. The evolutionary theory of Darwin, especially after the publication of "*Origin of species*" in 1859 had profound effect on its application in the classification of plant and animal groups. Systematics aims at developing classification based on different criteria with distinct methodologies. Phenetics and phylogenetics are the two methods for establishing relationships between the organisms based on overall similarity and ancestor-descendant relationships respectively. Phenetics or taxometrics is the application of multivariate numerical techniques in systematics. Cain and Harrison (1960) proposed the term phenetic relationship to elucidate the relationship among species based on overall similarity. Phylogenies are hypothesis of relationship constructed by analysis of shared derived character states, which can predict the properties of newly discovered or poorly known organisms. Phylogenetic methods are largely based on the principles of parsimony according to which most likely evolutionary route is the shortest hypothetical pathway of change that explains the pattern under observation.

Phenetics and phylogenetics were widely applied in elucidating relationships among different palm genera like *Arenga* (Mogea, 1999); *Bactris* (Ferreira, 1999); *Phoenix* (Barrow, 1998); *Reinhardia* (Henderson, 2002); *Sabal* (Zona, 1990) and *Drymopholeus* (Zona, 1999). The preliminary studies of Malayan species shows that the genus, *Calamus* is paraphyletic (Kramadibrata, 1992) which was further confirmed by Baker *et al.* (2000) using DNA sequence data.

The present study deals with the following aspects:

1. To study the phenetic relationships among species using overall morphological similarity
2. To understand phylogenetic relationships among species with a view to the concept of monophyly.

## **3.2. Materials and methods**

### **3.2.1. Plant material**

The samples consists of 31 species of *Calamus* (Operational Taxonomic Units-OTUs) representing all the three major centers of India, were used for the purpose of both phenetic and phylogenetic analysis. Phytogeographical distribution of these taxa are shown in Table 3.1 and the grouping according to Beccari (1908) in Table 3.2. Most of these species were collected from throughout their geographical range and herbarium specimens were prepared. All the voucher specimens are deposited in Kerala Forest Research Institute (KFRI) herbarium.

### **3.2.2. Characters**

Characters were selected from all parts of the plant, both from vegetative and reproductive parts. A total of forty-seven morphological characters were selected (Table.3.3). All the specimens used in the analysis were at their mature stage of development. The characters which showed significant variations among OTUs were selected. For the character selection, guidelines by Sokal and Sneath (1963) were adopted. For each species ten to 15 separate measurements were taken to determine the mean of quantitative characters included in the analysis. Quantitative characters were

measured to the nearest centimeter using calibrated tapes and they were transformed into binary state followed by Almeida and Bisby (1984).

### **3.2.3. Phenetic analysis**

Principal component analysis and cluster analysis were conducted using NTSYS pc (Rolf, 2000). Principal component analysis was used to detect the groups of specimens and to estimate the contribution of each variable in the analysis. From the correlation matrix both eigen value and eigen vectors were extracted. Cluster analysis was used to assess the grouping found in principal component analysis. For this the standardised data were used to compute a distance matrix based on average taxonomic distance and this was subjected to unweighted pair group method, arithmetic average (UPGMA) clustering algorithm. Then the cophenetic correlation was computed by means of Mantel T test (Rolf, 2000).

### **3.2.4. Phylogenetic analysis**

The same forty-seven morphological characters used for the phenetic analysis were used for the phylogenetic analysis also. The characters were coded as binary and the polarity of the characters were assessed according to Uhl and Dransfield (1987) and Baker *et al.* (1999). In some cases, it was necessary to assume the character state, which appeared to be most common within the rest of the genus as primitive. The phylogenetic analysis was conducted on MIX algorithm from the PHYLIP Version 3.2 (Phylogeny Inference Software Package; Felsenstien, 1989). The strict consensus tree was obtained using CONSENCE option. The program Treeview 1.5 (Page, 1996) was used to display the tree.

**Table 3.1. List of species included for phenetic and phylogenetic analysis and its phyto - geographical distribution**

No.	Name of species	Phyto-geographical area.
1	<i>C. acanthospathus</i> Griff.	North - Eastern States
2	<i>C. andamanicus</i> Kurz.	Andaman and Nicobar Islands
3	<i>C. brandisii</i> Becc.	Western Ghats
4	<i>C. delessertianus</i> Becc.	Western Ghats
5	<i>C. dilaceratus</i> Becc.	Andaman and Nicobar Islands
6	<i>C. dransfieldii</i> Renuka	Western Ghats
7	<i>C. erectus</i> Roxb.	North - Eastern States
8	<i>C. flagellum</i> Griff.	North - Eastern States
9	<i>C. gamblei</i> Becc.	Western Ghats
10	<i>C. gracilis</i> Roxb.	North - Eastern States
11	<i>C. hookerianus</i> Becc.	Western Ghats
12	<i>C. huegelianus</i> Mart.	Western Ghats
13	<i>C. karnatakensis</i> Renuka & Lakshmana	Western Ghats
14	<i>C. khasianus</i> Becc.	North Eastern States
15	<i>C. lacciferus</i> Lakshmana & Renuka	Western Ghats
16	<i>C. lakshmanae</i> Renuka.	Western Ghats
17	<i>C. longisetus</i> Griff.	Andaman and Nicobar Islands
18	<i>C. metzianus</i> Schlecht	Western Ghats
19	<i>C. nagbettai</i> Fernandez & Dey	Western Ghats
20	<i>C. neelagiricus</i> Renuka	Western Ghats
21	<i>C. palustris</i> Griff.	Andaman and Nicobar Islands
22	<i>C. prasinus</i> Lakshmana & Renuka	Western Ghats
23	<i>C. pseudotenuis</i> Becc.	Western Ghats
24	<i>C. rotang</i> L.	Western Ghats
25	<i>C. rivalis</i> Thw. ex Trim.	Western Ghats
26	<i>C. shendurunii</i> Anto, Renuka et Sreekumar	Western Ghats
27	<i>C. stoloniferus</i> Renuka	Western Ghats
28	<i>C. travancoricus</i> Bedd.	Western Ghats
29	<i>C. thwaitesii</i> Becc.	Western Ghats
30	<i>C. vattayila</i> Renuka	Western Ghats
31	<i>C. viminalis</i> Willd.	Andaman and Nicobar Islands

**Table 3. 2. Beccari's Grouping (1908) and the species reported from the Western Ghats after Beccari**

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***Beccari's Grouping***

Group 1 A	<i>C. erectus</i> Roxb.
Group 1 B	<i>C. flagellum</i> Griff.
Group 2 A	<i>C. longisetus</i> Griff., <i>C. thwaitesii</i> Becc.
Group 2 B	<i>C. dilaceratus</i> Becc.
Group 5	<i>C. viminalis</i> Willd., <i>C. metzianus</i> Schlecht, <i>C. rivalis</i> Thw. ex Trim. <i>C. pseudotenuis</i> Becc., <i>C. hookerianus</i> Becc., <i>C. rotang</i> L., <i>C. brandisii</i> Becc., <i>C. delessertianus</i> Becc. and <i>C. acanthospathus</i> Griff.
Group 7	<i>C. travancoricus</i> Bedd.
Group 8	<i>C. huegelianus</i> Mart. and <i>C. gamblei</i> Becc.
Group 9	<i>C. gracilis</i> Roxb.
Group 14	<i>C. andamanicus</i> Kurz.
Group 15	<i>C. palustris</i> Griff. and <i>C. khasianus</i> Becc.

---

***Species reported after Beccari***

1. *C. vattayila* Renuka
  2. *C. prasinus* Lakshmana et Renuka
  3. *C. dransfieldii* Renuka
  4. *C. lacciferus* Lakshmana et Renuka
  5. *C. shendurunii* Anto, Renuka et Sreekumar
  6. *C. nagbettai* Fernandez et Dey
  7. *C. lakshmanae* Renuka
  8. *C. karnatakensis* Renuka et Lakshmana
  9. *C. stoloniferus* Renuka
  10. *C. neelagiricus* Renuka
-

**Table 3.3. List of characters selected for phenetic and phylogenetic analysis**

No.	Characters	Character states
1	Habit	Clustering (0); Solitary (1)
2	Stem diameter	≤ 1.5 cm (0); > 1.5 cm (1)
3	Stem with aerial branches	Absent (0); Present (1)
4	Leaves	Ecirrate (0); Cirrate (1)
5	Mouth of leaves sheath	Not spiny (0); Spiny (1)
6	Leafsheath Indumentum	Absent (0); Present (1)
7	Leafsheath spines	Not organized (0); Organised (1)
8	Leafsheath spine nature	Sparse (0); Dense (1)
9	Leafsheath spine base	Bulbous based (0); Not Bulbous based (1)
10	Leafsheath surface horizontal ridges	Absent (0); Present (1)
11	Knee	Absent (0); Present (1)
12	Knee spine	Armed (0); Unarmed (1)
13	Flagellum	Absent (0); Present (1)
14	Ochrea	Conspicuous (0); Inconspicuous (1)
15	Ochrea	Not Papery (0); Papery (1)
16	Petiole	Inconspicuous (0); Conspicuous (1)
17	Petiole length	≤ 25 cm (0); > 25 cm (1)
18	Petiole width	≤ 2 cm (0); > 2 cm (1)
19	Rachis claws	Not grouped (0); Grouped (1)
20	Leaflets	Not grouped (0); Grouped (1)
21	Leaflet shape	Broad lanceolate (0); Narrow linear lanceolate (1)
22	Leaflet length	≤ 45 cm (0); > 45 cm (1)
23	Leaflet apex	Not bristly (0); Bristly (1)
24	Leaflet upper midvein	Not bristly (0); Bristly (1)
25	Leaflet lower midvein	Not bristly (0); Bristly (1)
26	Leaflet margin	Not spiny (0); Spiny (1)
27	Leaflet terminal pair	Fused (1); Free (0)
28	Rachilla form	Stout, stiff (1); Slender (0)
29	Male rachillae length	≤ 5 cm (0); > 5 cm (1)
30	Female rachillae length	≤ 10 cm (0); > 10 cm (1)
31	Primary spathe	Armed (0); Unarmed (1)
32	Primary spathe	Tightly sheathing (0); Loosely sheathing (1)
33	Primary spathe mouth	Not lacerated (0); Lacerated (1)
34	Secondary spathe	Armed (0); Unarmed (1)
35	Secondary spathe	Tightly sheathing (0); Loosely sheathing (1)

36	Male flower	≤ 5 cm (0); > 5 cm (1)
37	Female flower	≤ 5 cm (0); > 5 cm (1)
38	Involucrophorum	Sessile (0); Pedicelliform (1)
39	Fruit stigmatic remains	Cauduous (0); Persistent (1)
40	Fruit shape	Globose/Ovoid (0); Ellipsoidal/Oblong (1)
41	Fruit diameter	≤ 1.5 cm (0); > 1.5 cm (1)
42	Fruit scales count	≤ 15 cm (0); > 15 cm (1)
43	Fruit scales channel	Present (0); Absent (1)
44	Fruiting perianth	Not explanate (1); Explanate (0)
45	Embryo position	Basal (0); Lateral (1)
46	Seed surface	Smooth (0); Rough (1)
47	Endosperm	Homogenous (0); Ruminant (1)

### 3.3. Results

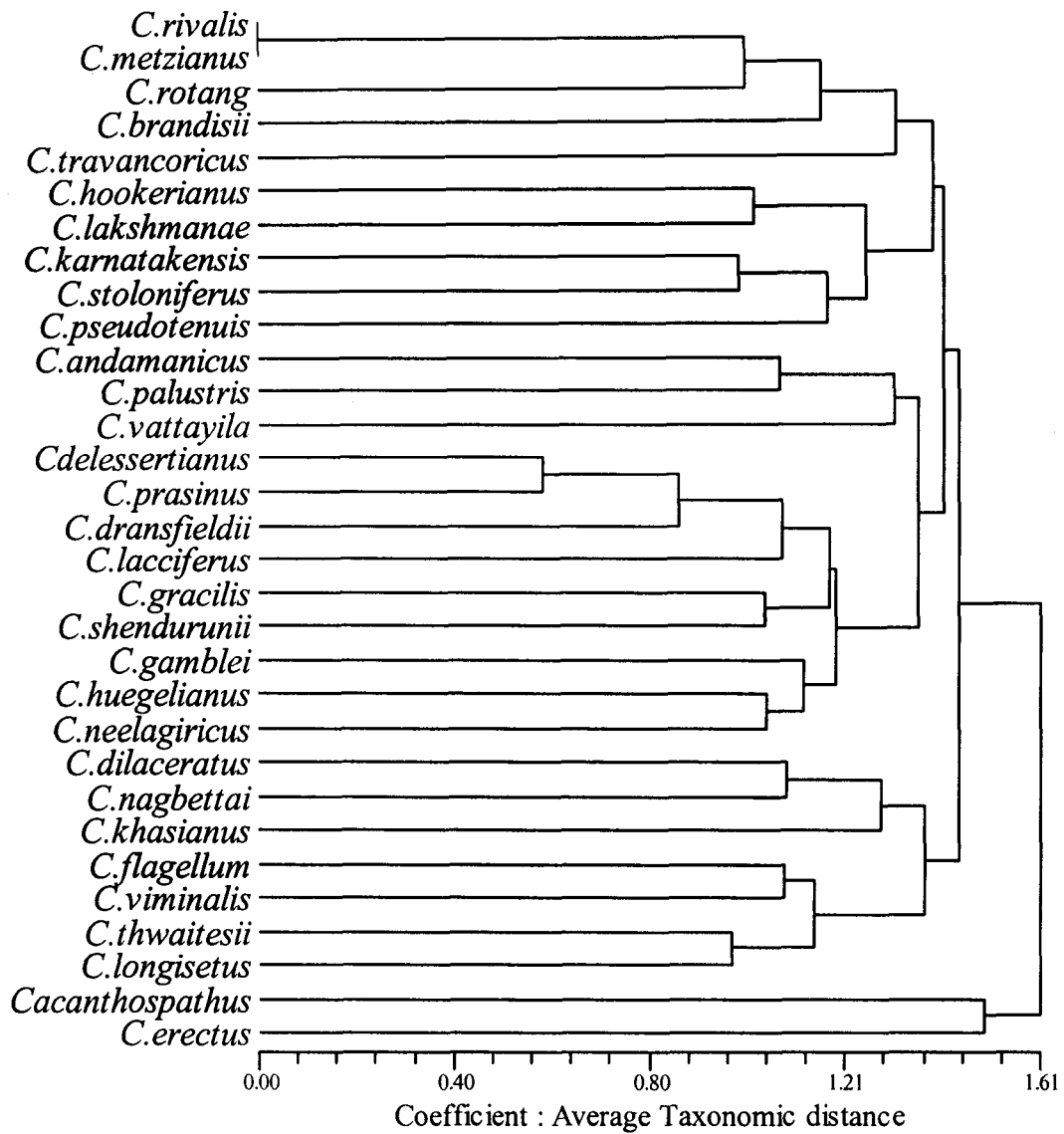
#### 3.3.1. Phenetic analysis

The cluster analysis showed a good clustering pattern in the dendrogram (Figure 3.1) reflecting the natural relationship among species, which is partially in agreement with the grouping proposed by Beccari (1908). The dendrogram consisted of eight major clusters with a cophenetic correlation ( $r$ ) of 0.81.

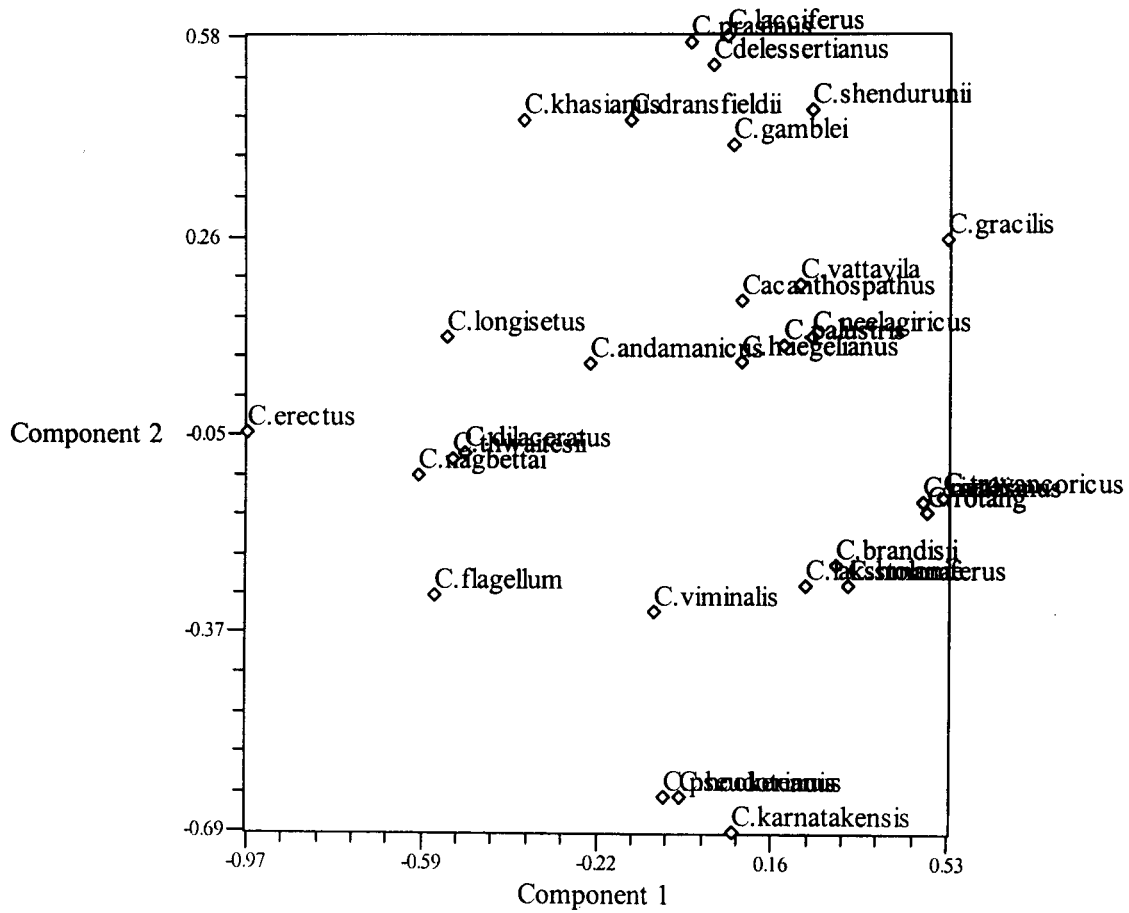
The major eight clusters are the following:

- Cluster 1 - *C. erectus* and *C. acanthospathus*.
- Cluster 2 - *C. longesetus*, *C. thwaitesii*, *C. viminalis* and *C. flagellum*.
- Cluster 3 - *C. khasianus*, *C. nagbettai* and *C. dilaceratus*.
- Cluster 4 - *C. neelagiricus*, *C. huegelianus* and *C. gamblei*.
- Cluster 5 - *C. shendurunii*, *C. graclis*, *C. lacciferus*, *C. dransfieldii*, *C. prasinus* and *C. delessertianus*.
- Cluster 6 - *C. vattayila*, *C. palustris* and *C. andamanicus*.
- Cluster 7 - *C. pseudotenuis*, *C. stoloniferus*, *C. karnatakensis*, *C. lakshmanae* and *C. hookerianus*.
- Cluster 8 - *C. metzianus*, *C. rivalis*, *C. rotang*, *C. travancoricus* and *C. brandisii*.

The first cluster included *C. erectus* and *C. acanthospathus*, which were grouped at a dissimilarity coefficient of 1.46 and linked to the remaining species at 1.39 level. The second cluster consisted of *C. flagellum*, *C. viminalis*, *C. thwaitesii* and *C. longesetus*. In this cluster, *C. thwaitesii* was closely related to *C. longesetus* found in Andaman Islands, which was linked to *C. flagellum* and *C. viminalis* at a dissimilarity coefficient of 1.17. Third cluster included *C. dilaceratus* (Nicobar Islands), *C. nagbettai* (Western Ghats) and *C. khasianus* (North Eastern states). *C. dilaceratus* and *C. nagbettai* are separated from *C. khasianus* at a distance of 1.24. The fourth cluster consisted of three species from the Western Ghats viz. *C. gamblei*, *C. huegelianus* and *C. neelagiricus*. *C. gamblei* was grouped to *C. huegelianus* and *C. neelagiricus* at a distance of 1.10. In the fifth cluster, *C. shendurunii* and *C. gracilis* are separated from *C. lacciferus*, *C. dransfieldii*, *C. prasinus* and *C. delessertianus* at a distance of 1.17. *C. delessertianus* is closely allied to *C. prasinus*. Sixth cluster included *C. palustris* and *C. andamanicus* from Andaman and Nicobar Islands and *C. vattayila* from the Western Ghats. *C. vattayila* was separated from *C. palustris* and *C. andamanicus* at a distance of 1.32 level. In the seventh cluster, *C. hookerianus* and *C. lakshmanae* are linked to *C. karnatakensis*, *C. stoloniferus* and *C. pseudotenuis* at a dissimilarity coefficient of 1.24. All the members of this cluster are found in the Western Ghats. *C. rivalis* and *C. metzianus* shows 100 per cent similarity in the eighth cluster. This cluster also included *C. rotang*, *C. brandisii* and *C. travancoricus*. This cluster was separated from *C. hookerianus* – *C. pseudotenuis* cluster at a distance of 1.39.



**Figure 3.1. UPGMA phenogram based on morphological characters from 31 species of *Calamus*. Cophenetic correlation coefficient ( $r$ ) = 0.81**



**Figure 3.2. Two-dimensional scattergram of 31 species of *Calamus* from the principal component analysis showing species grouping**

**Table 3.4. Percentage of the variance explained by first two axis**

	Eigen value	Percent	Cumulative
1	8.77845914	34.4223	34.4223
2	6.94501773	22.6490	57.0713

**Table 3. 5. Character loading values for the first two Principal Components (PC) axes**

	1	2
Rachilla form	0.6091	-0.3455
Fruit scales count	0.5022	0.0436
Ochrea form	0.4894	0.4671
Flagellum	0.4666	-0.0626
Embryo position	0.4221	-0.2801
Leafsheath spine base	0.2655	0.6043
Knee	0.2228	0.1754
Petiole length	0.2042	-0.1047
Habit	0.1129	0.3219
Mouth of the leafsheath	0.1021	-0.5707
Stem with aerial branches	0.0940	0.3625
Leaflet length	-0.6044	0.4129
Endosperm	0.0688	0.4761
Male flower length	-0.6980	0.3191
Involucrophorum	0.2435	0.4488
Primary spathe mouth	-0.2993	0.2696
Fruit diameter	-0.1744	0.6386
Fruit scales channel	-0.2887	0.3758

Principal Component analysis summarised the phenetic relationships between taxa. (Figure 3.2) The first principal component accounted for the 34.42 per cent of the total variation with rachilla form, embryo position, ochrea form and flagellum having highest loadings (Table 3.4). The second principal component accounted for 22.64 per cent of the total variation. The fruit diameter, leafsheath spine, ochrea form and leaflength had highest loadings (Table 3.5). Ordination allowed almost the same pattern of

relationships corresponding to cluster analysis. *C. longesetus*, *C. thwaitesii*, *C. flagellum*, *C. khasianus*, *C. nagbettaii* and *C. viminalis* were seen slightly apart towards the lower part. The lower and right part of the phenetic space consisted of members of the Western Ghats which were arranged along a gradient of increasing values on C1, and decreasing values on C2. This group consisted of more closely related members such as *C. rivalis*, *C. metzianus*, *C. rotang*, *C. brandisii* and *C. travancoricus*, *C. hookerianus* – *C. lakshmanaee*, *C. karnatakensis*- *C. pseudotenuis*- *C. stoloniferus*. The right and upper part consisted of members of a large group with the following species, *C. andamanicus*, *C. acanthospathus*, *C. neelagiricus*, *C. huegelianus* and *C. palustris*. They were arranged along a gradient of increasing values on both C1 and C2.

### 3.3.2. Phylogenetic analysis

The phylogenetic analysis resulted in a strict consensus tree. (Figure 3.3). This was almost consistent with the phenetic dendrogram. *C. erectus* and *C. acanthospathus* form primitive taxa and formed sister species to the remaining members. These two species are distributed in north eastern states. The clade *C. vattayila* – *C. andamanicus* was distantly placed from other species. In this clade *C. palustris* and *C. andamanicus* were closely related; both species are solitary, cirrate and large diameter rattans. However, *C. vattayila* is solitary, ecirrate medium diameter cane found in the Western Ghats. The clade *C. dransfieldii* – *C. delessertianus* consisted of four species, three species (*C. dransfieldii*, *C. delessertianus* and *C. prasinus*) from the Western Ghats and the fourth one *C. khasianus* from North eastern states. In this clade *C. dransfieldii* was found to be sister species for the remaining taxa and *C. khasianus* and *C. delessertianus* were found to be more advanced species. This clade was followed by *C. neelagiricus*, a solitary moderate sized rattan, seen as isolated and formed sister species for all the remaining

species. This species lack cilia on the veins of leaflets and possess inconspicuous ochrea. *C. gracilis* – *C. shendurunii* clade includes three species viz. *C. gracilis*, *C. lacciferus* and *C. shendurunii*; all are cluster forming rattans. *C. gracilis* is characterized by the absence of cilia on the lower veins of leaflets and ruminant endosperm with lateral embryo. *C. lacciferus* having aerial branches on the stem, this species is closely allied to *C. shendurunii*. The position of *C. gamblei* and *C. huegelianus* were found to be isolated from the remaining species. The former possess bulbous based spines on the leaf sheath with deeply channeled pale yellow shiny fruits. *C. huegelianus* is a cluster forming moderate sized rattan with closely sheathing, spiny primary spathe and jet black fruits. *C. rivalis* and *C. metzianus* showed close relationship suggesting the possibility of a single species and they were linked to *C. rotang* and this clade shared the characters of absence of spines on the knee, bristly leaf apex, persistent fruit stigmatic remains. *C. brandisii* and *C. travancoricus* are clustering, slender diameter rattans with ecirrate leaves and distinctly grouped leaflets. *C. stoloniferus*, *C. karnatakensis* and *C. pseudotenius* formed a distinct clade, characterized by cluster forming habit with homogenous endosperm. *C. lakshmanae* was found to be closely allied to *C. hookerianus* whereas *C. vimialis* is closely related to *C. flagellum*. *C. nagbettai*, formed sister to *C. dilaceratus*, *C. longesetus* and *C. thwaitesii*. In this clade *C. dilaceratus* and *C. nagbettai* possess cirrate leaf which is absent in the later two taxa. *C. thwaitesii* and *C. longesetus* are characterized by flagellate spadix, with lacerated mouth of the primary spathe and ellipsoid fruits.

### 3.4. Discussion

The results of phenetic and cladistic analysis show systematic relationships among members of the Western Ghats and their affinities with taxa from Northeastern states and Andaman and Nicobar Islands. Beccari (1908) proposed 16 groups and included 11 species from Western Ghats in these groups. In the classification he had proposed groups in accordance with the natural affinities of the species, but in some cases the species description were partial due to lack of enough materials representing both vegetative and reproductive parts and hence he pointed out that this arrangement is far from satisfactory. Beccari placed *C. erectus* and *C. flagellum* in the Group 1. But in the present study *C. erectus* is found to be more related to *C. acanthospathus*, both phenetically and phylogenetically. Hence the position of *C. acanthospathus* is in Group V. Beccari divided the second group in two sections in which in the first section he placed *C. longesetus* and *C. thwaitesii*. In this study also *C. thwaitesii* is found to be closely associated with *C. longesetus* in both the analysis. *C. dilaceratus* is included in the second section of group II of Beccari; the results of both analyse supported the close relationship of *C. nagbettai* to *C. dilaceratus*. *C. nagbettai* is a cirrate species reported by Fernandez and Dey in 1970 where as *C. dilaceratus* is endemic to Nicobar Islands. Beccari placed *C. viminalis*, *C. metzianus*, *C. rivalis*, *C. pseudotenuis*, *C. hookerianus*, *C. rotang*, *C. delessertianus*, *C. brandisii*, and *C. acanthospathus* in the fifth group. The members of the fifth group showed different clustering pattern in the dendrogram. *C. acanthospathus* is clustered to *C. erectus* and *C. delessertianus* is clustered with *C. prasinus*. Similarly the overall morphological similarity between *C. brandisii* and *C. travancoricus* suggests that the separation of two species into two groups as done by Beccari is not justifiable. In the cladogram all these species does not from monophyletic

group but supported close relationship of *C. metzianus*, *C. rivalis*, *C. rotang*, *C. hookerianus* and *C. pseudotenius*. This clade also consists of the other newly reported species such as *C. lakshmanae*, *C. karnatakensis* and *C. stoloniferus*. The position of *C. viminalis* in the fifth group of Beccari is not supported in the present study. But it is closely allied to *C. flagellum*. Similarly *C. brandisii* is found to be allied to *C. travancoricus*, but these two species were placed in two different groups by Beccari. The eighth group consists of *C. huegelianus* and *C. gamblei*. Phylogenetically these species are closely similar and form sister group for the remaining members. *C. gracilis* is placed in the eighth group. This species is closely related to *C. lacciferus* and *C. shendurunii*. The fourteenth group consists of cirrate, explanate fruiting perianth with ruminant endosperm. Beccari placed *C. andamanicus* in this group and *C. palustris* in the fifteenth group. Both species are closely related both phylogenetically and phenetically showing that members of these groups are closely related. Moreover, *C. vattayila* is also seen in this clade indicating its close relationship with the members of these two groups.

On the basis of the phylogenetic analysis based on the forty-seven morphological characters the members of the Western Ghats are found in nine major lineages. The first clade consists of *C. vattayila* and second includes *C. dransfieldii*, *C. prasinus* and *C. delessertianus*. Only one species, *C. neelagiricus* is represented in the third lineage. The fourth contained *C. lacciferus* and *C. shendurunii*. The fifth and sixth includes *C. gamblei* and *C. huegelianus* while the seventh consists of *C. rotang*, *C. rivalis*, *C. metzianus*, *C. travancoricus* and *C. brandisii*. This is followed by *C. stoloniferus*, *C. karnatakensis*, *C. pseudotenius*, *C. lakshmanae* and *C. hookerianus* in the eighth and *C. nagbettai* and *C. thwaitesii* in the ninth.

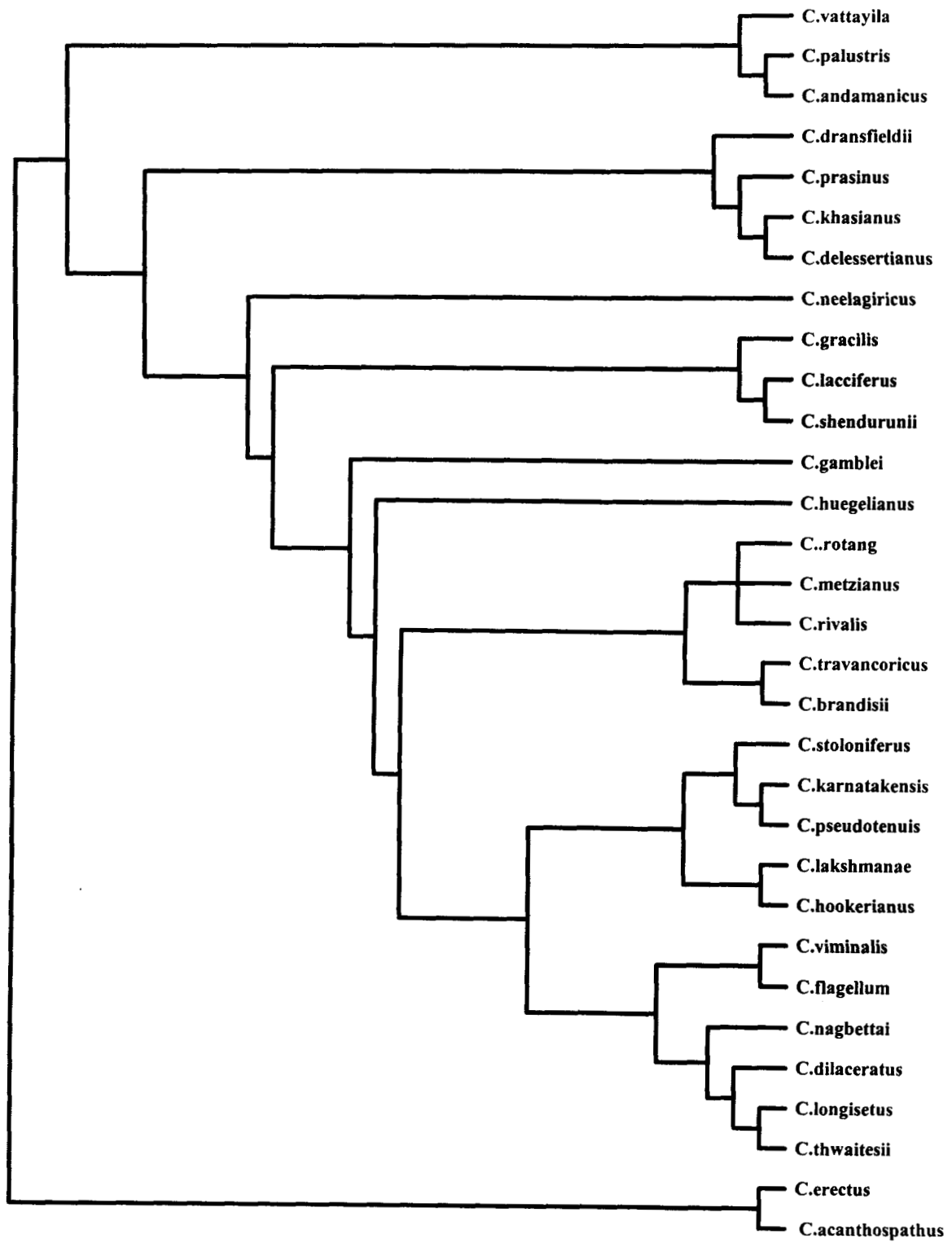


Figure 3.3. Strict consensus tree derived from the analysis of the 31 species of *Calamus*

The genus *Calamus* is placed in the subtribe Calaminae which includes six genera. The cladistic study revealed that the subtribe Calaminae is not monophyletic and suggested that several genera including *Calamus* and *Daemonorops* need to expect change in their generic delimitation and require modern treatments (Baker *et al.*, 2000). The main characters which separate the remaining related genera are the presence of persistent tubular bracts. In the present study the position of species of the Western Ghats, nested among other members of North eastern states and Andaman and Nicobar Islands, clearly indicates the non-monophyletic nature of members of the Western Ghats. Moreover highly complex pattern of morphological variation has been found within the group. Many characters, especially the habit and presence of cirrus have been found to arise in more than one occasion.

In the phenetic analysis it was observed that there is a hundred per cent similarity between *C. rivalis* and *C. metzianus*. Hence detailed morphometric and genetic variation studies were undertaken for these two species to understand the pattern of phenetic structure among population.

# Morphometric studies

Sreekumar V.B “Systematics and phylogeny of the genus *calamus* linn. (arecaceae) in the western ghats ” Thesis. Kerala Forest Research Institute, University of Calicut, 2004

# *Morphometric studies*

on *Calamus rivalis* Thw. ex Trim. and *C. metzianus* Schlecht

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## 4. Morphometric studies on *Calamus rivalis* Thw. ex Trim. and *C. metzianus* Schlecht

### 4.1. Introduction

The present chapter deals with the taxonomic identity of two species of *Calamus*, viz. *C. metzianus* and *C. rivalis*. The species *C. metzianus* is collected by Rev. Metz from Canara district of Karnataka and published by Schlecht in *Linnaea* in 1853. Renuka and Bhat (1987) reported the same species from Nilambur, Kerala. *C. rivalis* is originally described from Sri Lanka by Thwaites and later validly published by Trimen in *Journal of Botany* in 1885 which has been recently collected from Ashramam (Kollam) and Chertala (Alapuzha) of India (Renuka and Sreekumar, in press).

A critical study of these specimens revealed that these two specimens are very similar phenotypically as supported by both the phenetic and phylogenetic analysis. According to Beccari (1908) *C. metzianus* is only the continental form of *C. rivalis*. The main difference of *C. rivalis* from *C. metzianus* is that the latter is characterised in having larger fruits with distinctly channeled scales. In the present investigation, detailed morphometric studies were carried out for both these species to understand the pattern of phenetic structure among populations and to demarcate the species boundaries.

Morphometric studies have been widely recognized as a suitable tool in systematic studies in making decisions on species delimitations. Of the various multivariate methods, cluster analysis and ordination techniques are commonly used to quantify the morphological variability within species. The cluster analysis is better for representing distances among similar specimens. This generates a hierarchic dendrogram, produced by a sequence of fusion between Operational taxonomic Units (OTUs) or groups of

OTUs. Principal component analysis (PCA) is one of the ordination techniques, which is better in representing distances among groups of specimens (Sneath and Sokal, 1963). The taxonomic problems regarding species identity based on morphological variability have been addressed using morphometric analysis on various groups of plants. (Naczi *et al.*, 1998; Levin, 1999; Fritsch and Lucas, 2000; Spooner, *et al.*, 2001). In the case of palms similar studies were carried out on *Licuala* (Loo, 2001) in Peninsular Malaysia, *Archontophoenix* (Dowe and Hodel, 1994) *Livistona* (Dransfield and Beentje, 1995) *Genoma cuneata* (Borchsenius, 1999) and *Hyphaene* (Dransfield, 1999).

## 4.2. Materials and Methods

Two populations of *C. rivalis* from the Western Ghats of India and five from Sri Lanka and two populations of *C. metzianus* from the Western Ghats are included in this study (Table 4.1). The specimens were collected and herbarium were prepared from all these populations. The voucher specimens are deposited in KFRI herbarium.

The Operational Taxonomic Units (OTUs) included for the morphometric analysis are given in Appendix 1. The characters were chosen on the basis of differences among taxa in the vegetative and reproductive parts. Only the mature plants were considered and a total of 32 characters, both quantitative and qualitative were selected for the study (Table 4.2). The characters were scored as 0 for absence and 1 for the presence. Multiple herbarium sheets from a single collection were grouped into a single OTU and 107 such OTUs were used for the analysis.

Cluster analysis (CA) and Principal Component Analysis (PCA) were performed using NTSYS pc (Rolf, 2000). The standardised data were used to compute a distance matrix based on average taxonomic distances, manhattan distances and euclidian distances and

was then subjected to the Unweighted pair-group method with arithmetic averages (UPGMA) clustering algorithm. To test the consistency between the resulting phenogram and the original data, co-phenetic correlation coefficient was calculated for each phenogram - data matrix pair. The phenogram with highest co-phenetic correlation coefficient ( $r$ ) is reported here.

The principal component analysis was done to summarize the correlational structure among variables and to display the phenetic relationship among OTUs in a non-hierarchical way. The standardised data were used to compute a character by character correlation matrix, from which eigen vectors were extracted. OTUs were plotted in the three dimensional space defined by the first three eigen vectors.

**Table 4.1. Details of selected populations of *C. metzianus* and *C. rivalis* included in the analysis**

Population	No of plants	Country	Latitude ( $^{\circ}$ N)	Longitude ( $^{\circ}$ L)
<b><i>C. metzianus</i></b>				
Honaver, Western Ghats	15	India	14 $^{\circ}$ 08'	74 $^{\circ}$ 30'
Nilambur, Western Ghats	20	India	11 $^{\circ}$ 17'	76 $^{\circ}$ 28'
<b><i>C. rivalis</i></b>				
Ashramam, Western Ghats	18	India	08 $^{\circ}$ 53'	76 $^{\circ}$ 35'
Chertala, Western Ghats	15	India	09 $^{\circ}$ 42'	76 $^{\circ}$ 19'
Watareke, Sri Lanka	9	Sri Lanka	06 $^{\circ}$ 51'	80 $^{\circ}$ 03'
Yagirala, Sri Lanka	13	Sri Lanka	06 $^{\circ}$ 28'	80 $^{\circ}$ 11'
Matugama, Sri Lanka	10	Sri Lanka	06 $^{\circ}$ 31'	80 $^{\circ}$ 07'
Ratnapura, Sri Lanka	3	Sri Lanka	06 $^{\circ}$ 42'	80 $^{\circ}$ 23'
Walakanda, Sri Lanka	4	Sri Lanka	06 $^{\circ}$ 21'	80 $^{\circ}$ 42'

**Table 4. 2. Characters and character states evaluated in *C. rivalis* Thw. ex Trim. and *C. metzianus* Schlecht**

No.	Character	Character states
1	Stem diameter	cm
2	Internodal length	cm
3	Leafsheath spine length	cm
4	Leafsheath spine	Sparse (0); Dense (1)
5	Leafsheath spine hairs	Absent (0); Present (1)
6	Petiole	Absent (0); Present (1)
7	Leaf length	m
8	Leaflet length	cm
9	Leaflet width	cm
10	Leaflet upper midvein cilia length	cm
11	Leaflet lower midvein cilia length	cm
12	Terminal leaflet length	cm
13	Terminal leaflet width	cm
14	Partial Inflorescence length	cm
15	Number of male rachilla/partial inflorescence	number
16	Number of female rachilla/partial inflorescence	number
17	Number of fruits per rachillae	number
18	Male rachillae length	cm
19	Female rachillae length	cm
20	Inflorescence caudex length	cm
21	Rachillae prophyll length	cm
22	Rachillae prophyll width	cm
23	Fruit apical beak length	cm
24	Fruit diameter	cm
25	Fruit length	cm
26	Fruit scales length	cm
27	Fruit scales width	cm
28	Fruit scales margin	Reddish-Brown (0); White (1)
29	Number of vertical rows of scales in fruit	Number
30	Fruit scales channel	Absent (0); Present (1)
31	Seed length	cm
32	Seed width	cm

## **4.3. Results**

### **4.3.1. Cluster analysis**

The phenogram constructed using Manhattan distances showed least distortion with co-phenetic correlation coefficient  $r = 0.8713$  where as in euclidian distances,  $r = 0.86829$  and average taxonomic distances,  $r = 0.8682$  (Figure 4.1). The OTUs fell into three major and two minor clusters. The first and second cluster comprised of complex series of closely nested clusters of majority of the plants of the Western Ghats (Honaver, Nilambur, Chertala and Ashramam) and all OTUs from Matugama of Sri Lanka. The third cluster included all remaining Sri Lankan OTUs and some OTUs from Chertala. The first cluster (Hon 1 to Nil 11) mainly consists of OTUs from Chertala and Ashramam to which the OTUs from Honaver and Nilambur were overlapped. The second cluster (Nil 10 to Hon 13) included the OTUs from Nilambur, Ashramam, Honaver, Chertala and Matugama. The OTUs from Nilambur, Honaver and Chertala were found to be scattered among different clusters. The majority of OTUs from Ashramam were found to be clustered together with OTUs from Nilambur. Among, Sri Lankan specimens OTUs from Matugama formed a distinct group linked with some OTUs of Chertala, Honaver and Watareke, Yagirala and Walakanda. The remaining OTUs, ie., from Watareke, Yagirala, Walakanda and Ratnapura were scattered together and intermingled with OTUs from Chertala.

### **4.3.2. Principal Component analysis**

In ordination, the first principal component explained 38.58% (Table. 4.3) of the total variation with leaf sheath spine length, number of male rachilla/partial inflorescence, number of female rachilla/ partial inflorescence, fruit apical beak length, number of

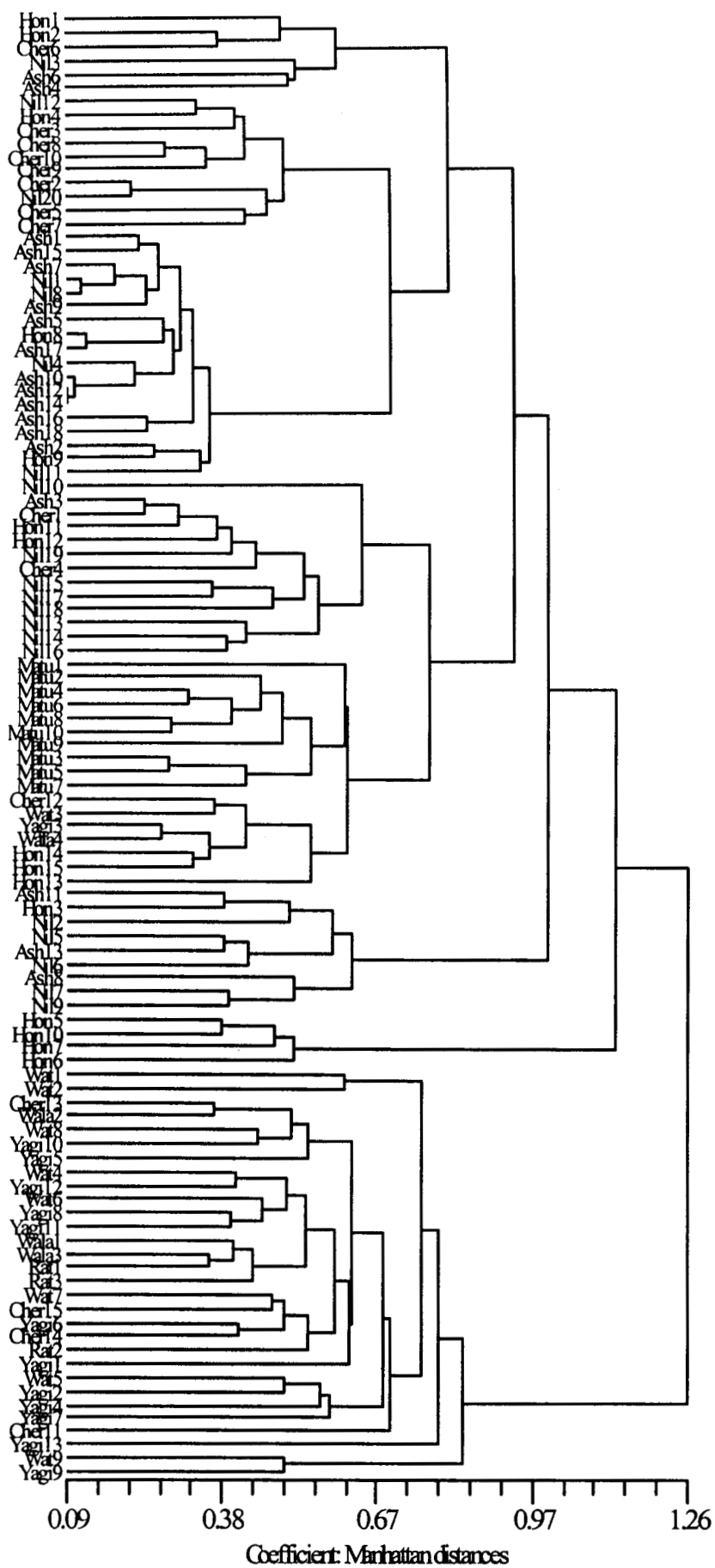
fruits per rachillae, inflorescence caudex length and fruit diameter having the highest loading (Table. 4.4).

The second principal component explained 22.25% of the total variation with stem diameter, nature of leafsheath spine and seed length with highest loading. Only 9.17% of the total variation was accounted for by the third principal component with leaflet length and inflorescence caudex length but the correlation was found to be less than 0.5.

The result of Principal Component Analysis also failed to disclose any particular major gaps in the pattern of morphological variation, clearly indicating lack of distinct phenetic structure (Figure 4.2); however, there is some tendency for OTUs from geographic regions to fall together in a given proportion of the three dimensional factor space as similar to cluster analysis.

#### **4.4. Discussion**

The phenetic species concept implies that the overall similarity of members of a species or population of species should be clearly separated from other species by a gap in variation (Davis and Heywood, 1963). The variation within species involves morphological, physiological and biochemical attributes and these exist at three different levels such as between populations, within populations and within an individual (Snaydon, 1984). Bradshaw (1965) pointed out that the taxonomic confusion is often due to the plastic response of the different individuals of a species in relation to a particular environmental influence.



**Figure 4.1. UPGMA dendrogram of 107 individuals of nine populations based on Manhattan distance.**

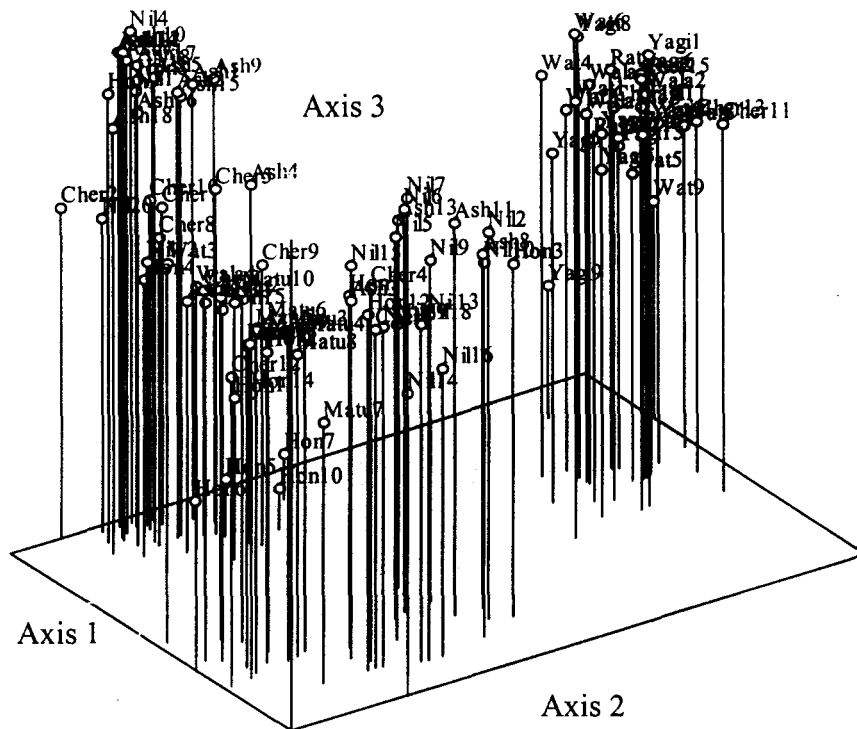


Figure 4. 2. Principal component analysis (PCA) of 32 morphological characters

Table 4. 3. Variation explained by the first three principal components

i	Eigen value	Percent of variance	Percent Cumulative
1	9.14708470	38.5846	38.5846
2	4.92040961	22.2513	60.8359
3	2.93576523	9.1743	69.0102

**Table 4. 4. Eigen vector coefficient for Principal Component Analysis. Character loading values are for the first three Principal Component (PC) axes**

No.	Characters	PC 1	PC 2	PC 3
1	Stem diameter	0.3242	0.5600	0.0715
2	Internodal length	0.5943	0.4375	-0.0841
3	Leafsheath spine length	0.8674	0.2061	0.1302
4	Leafsheath spine	-0.7341	0.5175	-0.1292
5	Leafsheath spine hairs	-0.7390	-0.3098	-0.0563
6	Petiole	0.3039	-0.4999	-0.1819
7	Leaf length	0.3616	-0.0221	-0.2955
8	Leaflet length	0.5021	0.1985	0.3648
9	Leaflet width	0.3847	0.0304	-0.0887
10	Leaflet upper midvein cilia length	0.5141	-0.5465	-0.0791
11	Leaflet lower midvein cilia length	0.4813	-0.5847	-0.0740
12	Terminal leaflet length	0.1412	-0.4591	-0.1395
13	Terminal leaflet width	-0.6926	-0.5647	-0.2530
14	Partial Inflorescence length	-0.1342	0.3417	-0.6384
15	Number of male rachilla/partial inflorescence	0.6809	-0.0919	-0.3409
16	Number of female rachilla/partial inflorescence	0.6809	0.1761	-0.2767
17	Number of fruits per rachillae	0.7012	-0.0399	-0.0737
18	Male rachillae length	0.0312	-0.2876	-0.5300
19	Female rachillae length	0.8515	-0.0476	0.1849
20	Inflorescence caudex length	0.8120	-0.1423	0.3063
21	Rachillae prophyll length	0.5345	0.1184	0.1657
22	Rachillae prophyll width	-0.4205	-0.7203	-0.2162
23	Fruit apical beak length	0.6522	-0.2468	-0.2856
24	Fruit diameter	0.7826	0.0027	-0.2615
25	Fruit length	0.1588	-0.1762	-0.1572
26	Fruit scales length	0.1184	0.2062	-0.6231
27	Fruit scales width	0.0393	0.2376	-0.6951
28	Fruit scales margin	-0.9415	-0.0061	-0.0882
29	Number of vertical rows of scales in fruit	-0.4868	0.3799	-0.3524
30	Fruit scales channel	0.0145	-0.3941	0.0357
31	Seed length	0.1669	0.5111	-0.2912
32	Seed width	0.3282	-0.0201	-0.4209

Palms are considered as taxonomically one of the most difficult group (Tomlinson, 1990) due to wide range of morphological variability, even within individuals (Dransfield, 1999). Henderson *et al.* (1995) reported that at least 10 per cent of the palm species were considered as species complexes, which contain more or less distinct forms joined by several intermediates.

Morphometric studies revealed high degree of morphological similarity among populations and there is no distinct phenetic gap between two species, rather the morphological characters are found to be overlapping. The OTUs from Nilambur and Honaver corresponding to two different populations of *C. metzianus* is found to be intermixed with the remaining OTUs representing different populations of *C. rivalis*. The lack of distinct phenetic structure in the principal component analysis also indicates close similarity of these two species. So the result of both cluster and principal component analysis failed to separate *C. rivalis* and *C. metzianus* as distinct groups corresponding to two species. The studies of Karla (2002) on *Malesherbia* complex and Delgadillo and Villasenor (2002) on *Grimmia* resulted in distinct clustering and separation of OTUs in the morphometric analysis where as in the studies on *Peracarpa*, Barnesky and Lammers (1997) failed to disclose groups of population similar to the present study. So the study supports the high rate of morphological variation existing in different species of *Calamus* as reported by Renuka (1994) and Dransfield (1979). Dransfield (2000) pointed out the proliferation of taxon names within *Calamus* and in many cases narrow species concept is used and the studies on other palm genera also had similar conclusion as in *Bactris* (Trail, 1877), *Pinanga* in Java (Dransfield, 2000) and *Dypsis* in Madagascar (Dransfield and Beentje, 1995).

Wessels Boer (1968) noticed that most of the palms are phenotypically variable according to the habitat and elevation. In the case of phenotypically variable species it is very difficult to differentiate stable and non-overlapping characters. A real species should possess “good characters”, which is phenotypically least variable in contrast to the bad characters (Cook, 1968).

The distinction of *C. metzianus* from *C. rivalis* is based on its larger fruit size and fruit scale channel in the middle and the rest of the morphological features are almost same for both species. But it is found that these characters, fruit size and fruit scale channel shows considerable amount of variation within the individuals of all the studied populations and these features are found to be unreliable for species distinction.

The morphometric analysis based on 32 characters on all the available populations is unable to demarcate *C. rivalis* from *C. metzianus*. Clear zones of overlapping revealed both by phenogram and scatter plot might be indicative of species similarity and possible merging.

# Genetic variation studies

Sreekumar V.B “Systematics and phylogeny of the genus *calamus* linn. (arecaceae) in the western ghats ” Thesis. Kerala Forest Research Institute, University of Calicut, 2004

*Genetic variation studies*  
on *Calamus rivalis* Thw. ex Trim. and *C. metzianus* Schlecht

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## **5. Genetic variation studies on *Calamus rivalis* Thw. ex Trim. and *C. metzianus* Schlecht**

### **5.1. Introduction**

The continuous loss of floral and faunal wealth and decline of the population size of many species are most significant problems of global concern. Sound knowledge of morphological features, ecological adaptations and genetic structure in natural populations are essential to understand the species identity and for undertaking judicious decisions regarding future plant improvements and conservation activities (Young *et al.*, 2000).

In recent years, there has been a radical change in the way the taxonomic groups are defined. The modern taxonomic methods using morphological and DNA sequences are widely applied in solving taxonomic confusions.

Genetic variation of a species is closely related to geographic distribution of genotypes that constitute subspecies, races or ecotypes. The DNA based variation detected by molecular markers can be defined as “genetic variation” to differentiate it from the phenotypic variation evolved through the adaptive potential of populations. The study on distribution of genetic diversity among populations provides critical information for the formulation of management and conservation strategies (Ledig, 1998; Hamrick *et al.*, 1991).

Molecular markers are fundamental tools for plant biologists. Molecular markers such as Restriction Fragment Length Polymorphisms (RFLPs), Randomly Amplified Polymorphic DNAs (RAPDs), Amplified Fragment Length Polymorphism (AFLP), Single Sequence Repeats (SSR) and Single Strand Conformation Polymorphism (SSCP) are widely applied to study genetic variation, varietal or species identification or to establish phylogenetic relationships and genetic mapping. RAPD technique gained

importance due to its simplicity, efficiency, relative ease to perform and non-requirement of sequence information (Williams *et al.*, 1990; Welsh and McClelland, 1990). Polymorphism detected by RAPD was determined by the different DNA sequence of the sites, which the primer bound, or by the insertions and deletions occurring between these sites. RAPD technique has been widely applied in phylogenetic (Rath *et al.*, 1998), population genetic (Smith and Pham, 1996; Kwon and Morden, 2002) and species differentiation studies (Luo *et al.*, 2000; Lihova *et al.*, 2000).

This chapter discusses the taxonomic identity and genetic variation of *C. metzianus* and *C. rivalis*. The morphometric analysis based on 32 morphological characters on all the available populations failed to separate distinct phenetic groups between these two species and in this context the main aspects of the present chapter is to confirm the taxonomic identity of *C. rivalis* and *C. metzianus* genetically and to identify the extent of genetic grouping and differentiation among population.

## **5.2. Materials and Methods**

### **5.2.1. Sample collection**

Juvenile leaf tissues were collected from all the available four populations from India and three populations from Sri Lanka. A total of 60 individual samples were selected for the analysis (Table 5.1).

### **5.2.2. DNA Extraction**

The leaf specimens were collected in plastic bags and kept chilled at  $-20^{\circ}\text{C}$  until DNA was extracted. For Sri Lankan samples dried leaves were used for DNA extraction. Total DNA was extracted from 1 g of the fresh leaf tissues using the modified CTAB protocol of Doyle and Doyle (1990) containing 2 % CTAB, 100mM Tris pH 8.0, 20mM EDTA pH 8.0 and 1.4 M NaCl. The homogenate was incubated on a water bath at  $60^{\circ}$

C for half an hour with occasional shaking. Organic extractions were carried out twice at room temperature, first with chloroform/isoamyl alcohol (24:1) and then with chloroform. After centrifugation at 10000 rpm for 10 minutes, the aqueous phase was eluted out. To the aqueous layer, double the volume of ice cold (-20° C) absolute alcohol was added. After incubation at -20° for 12 hours, the precipitated DNA was pelleted at low speed (1000rpm). The DNA pellets were washed with 95% ethanol, centrifuged at 1000rpm and the samples were air dried. Finally the pellets were dissolved in 100 µl double distilled water and stored at -80° until use. Total DNA was estimated at A<sub>260</sub> (1OD = 50µg ml<sup>-1</sup>) using UV spectrophotometer (Gallagher, 1996).

### **5.2.3. Polymerase Chain Reaction**

PCR-RAPD analysis was carried out according to Williams *et al.* (1990) protocol using ten primers *viz.* OPAU02, OPA04, OPA18, OPAW07, OPAW09, OPAW10, OPAW20, OPE02, OPE18 and OPB15 (Operon Technologies, Alameda, CA) selected out of the 65 primers based on repeatability of DNA band profile. Amplifications were performed on PTC-100 Thermal cycler (MJ Research Inc., USA) with 25µl reaction mixtures containing 50-100 ng of template DNA, 200 µM each of dATP, dTTP, dCTP and dGTP, 3 units of Taq DNA polymerase, 1µl (20pm) of each primer and 5µl Taq buffer with 1.5 mM MgCl<sub>2</sub> (Genei, Bangalore, India). The amplification regime was performed with 45 cycles, each of 60 Seconds denaturation (94° C), 60 Seconds annealing (36° C) and 120 Seconds extension (72° C). The last cycle was followed by incubation for 10 minutes at 72° C.

### **5.2.4. Separation and visualisation of the amplified products**

The RAPD products were electrophoresed on 1.5 % horizontal agarose gel (Sigma, USA) in TBE buffer (40 mM Tris-borate, 1 mM EDTA, pH 8.0). The gel, after the completion of electrophoresis was stained with ethidium bromide and bands were

compared with DNA markers. [100-bp DNA ladder and Low range DNA ruler (Genei)]. The gels were documented using Kodak Digital Science Electrophoresis Documentation and Analysis System 120 (Kodak, USA).

#### **5.2.5. Data analysis**

RAPD products were scored for presence (1) and absence (0) of bands. The data matrices were entered into the PopGene Version 1.31 package and pairwise comparison of populations were made (Yeh *et al.*, 1999). The genetic diversity parameters within population *viz.* number of polymorphic loci and gene diversity were determined. Genetic differentiation between the analysed populations were calculated according to Nei (1973).

Genetic distance (Nei, 1978) between all populations were obtained from PopGene Version 1.31 and the resulting distance matrices were then used to construct an unrooted phenetic tree of the different populations using Fitch Program of PHYLIP (Phylogeny Inference Software Package) Version 3.5 (Felsenstein, 1993).

Cluster analysis and principal coordinate analysis were carried out in NTSys version 2.1 (Rolf, 2000). Genetic similarities based on Jaccard's coefficient (Jaccard, 1908) were calculated among all possible pairs using the SIMQUAL option and ordered in a similarity matrix. The similarity matrix was run on sequential, agglomerative, hierarchical and nested clustering SAHN (Sneath and Sokal, 1963) using UPGMA (unweighted Pair group method with arithmetic average) as an option (Sokal and Michener, 1958). Cophenetic correlation was calculated to measure goodness of fit.

Principal co-ordinate analysis (PCO) was performed using the following modules of the NTSys program: STAND, SIMINT, DECENTER and EIGEN to identify the number of

groups based on eigen vectors. Three dimensional ordination provided an additional representation of genetic relationships among the individuals of populations.

**Table 5.1. Details of selected populations of *C. metzianus* and *C. rivalis* included in the analysis**

Population	No of plants	Country	Latitude ( <sup>o</sup> N)	Longitude ( <sup>o</sup> L)
<b><i>C. metzianus</i></b>				
Honaver, Karnataka	10	India	14° 08'	74° 30'
Nilambur, Kerala	10	India	11° 17'	76° 28'
<b><i>C. rivalis</i></b>				
Ashramam, Kerala	10	India	08° 53'	76° 35'
Chertala, Kerala	10	India	09° 42'	76° 19'
Watareke, Sri Lanka	10	Sri Lanka	06° 51'	80° 03'
Yagirala, Sri Lanka	5	Sri Lanka	06° 28'	80° 11'
Matugama, Sri Lanka	5	Sri Lanka	06° 31'	80° 07'

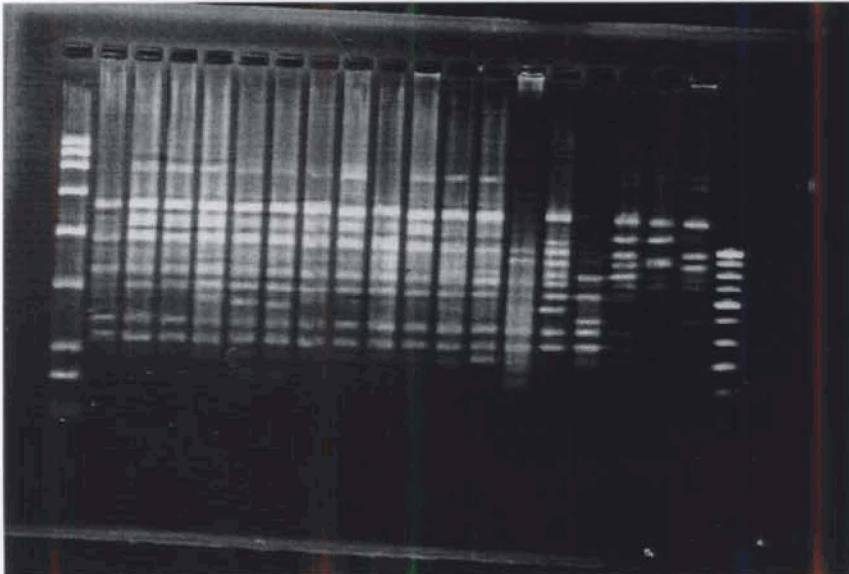
## 5.3. Results

### 5.3.1. Primer utility

Sixty five primers from Kit OPA, OPB, OPE, OPAU and OPAW (Operon Technology, USA) were initially screened. Out of these, ten (Table 5.2) were chosen for further analysis based on the number and reproducibility of amplified products (Plate 5 a – e). A total of 117 markers were obtained with molecular size ranging from 100bp to 2500bp. The number of scorable bands amplified by each primer varied (Table 5.2) from 7 (OPAW 20) to 15 (OPA 4 and OPA 18). Polymorphism was very pronounced, with 95% polymorphic markers across all primers.

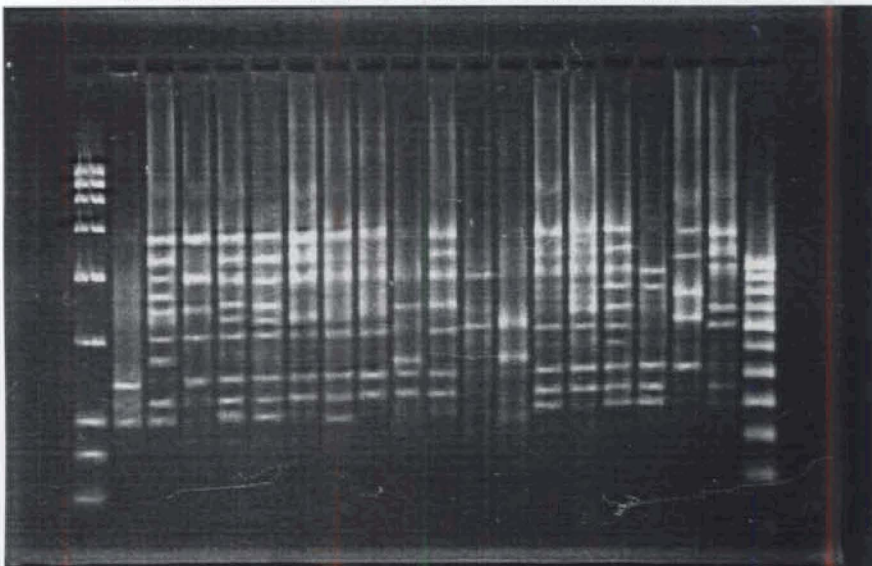
The percentage of polymorphic loci (ppl) vary from 26.5 in Matugama population to 68.38 in Chertala population. The Chertala population had highest genetic diversity

L 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 M



RAPD profiles of eighteen individuals of two Western Ghats populations by OPA 18 primer: L: Low range DNA ruler and M: DNA Marker (100bp ladder) Lane 1-10: Samples from Honaver and 11 – 18: Samples from Nilambur.

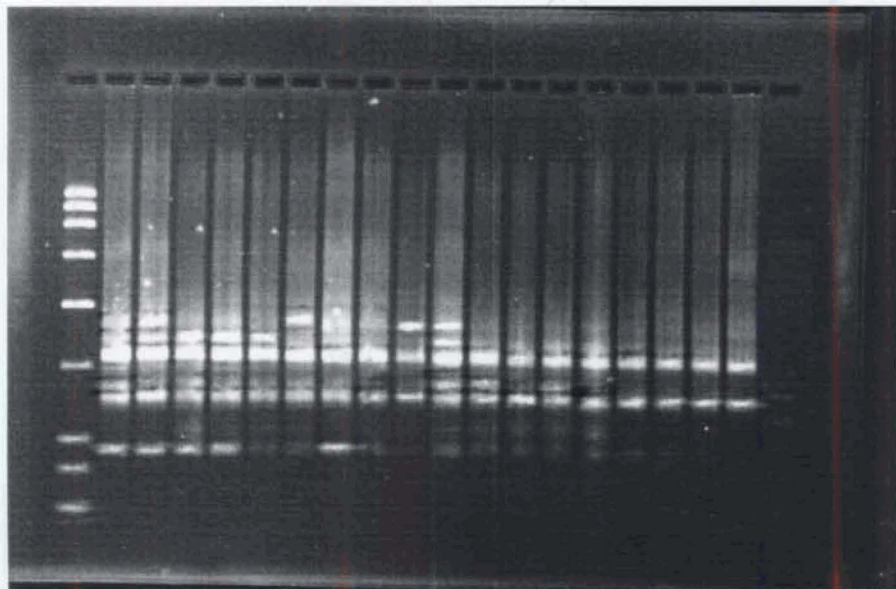
L 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 M



RAPD profiles of eighteen individuals of two Western Ghats populations by OPA 4 primer: L: Low range DNA ruler and M: DNA Marker (100bp ladder). Lane 1-10: Samples from Honaver and 11 – 18: Samples from Nilambur.

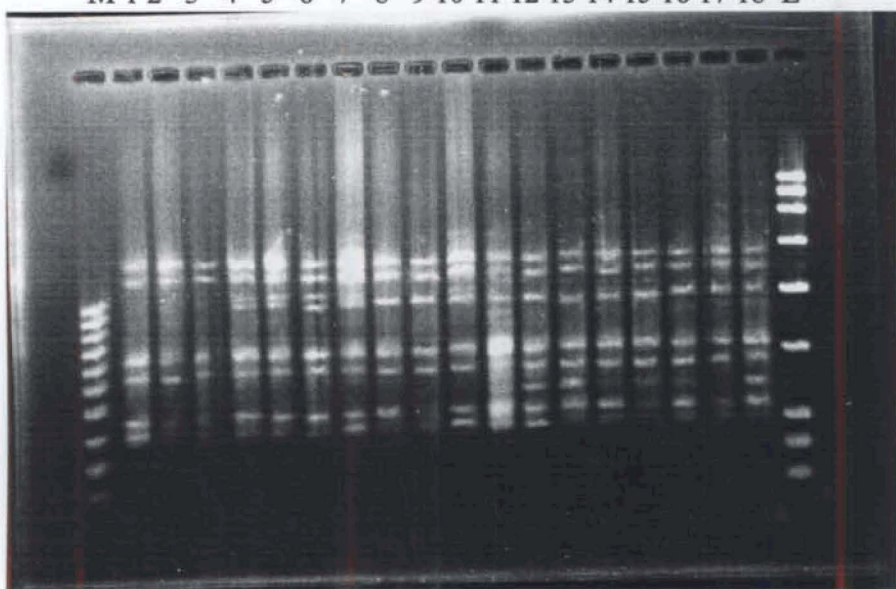
53B

L 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 M



RAPD profiles of eighteen individuals of two Western Ghats populations by OPAU 2 primer: L: Low range DNA ruler and M: DNA Marker (100bp ladder). Lane 1-10: Samples from Ashramam and 11 – 18: Samples from Chertala.

M 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 L



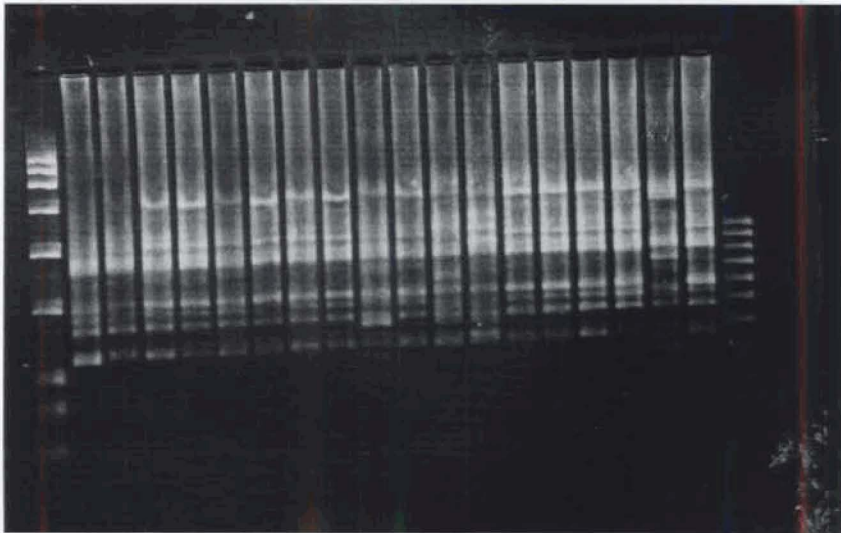
RAPD profiles of eighteen individuals of two Western Ghats populations by OPAW 10 primer: M: DNA Marker (100bp ladder) and L: Low range DNA ruler. Lane 1-10: Samples from Honaver and 11 – 18: Samples from Nilambur.

**Plate – 5b**

19

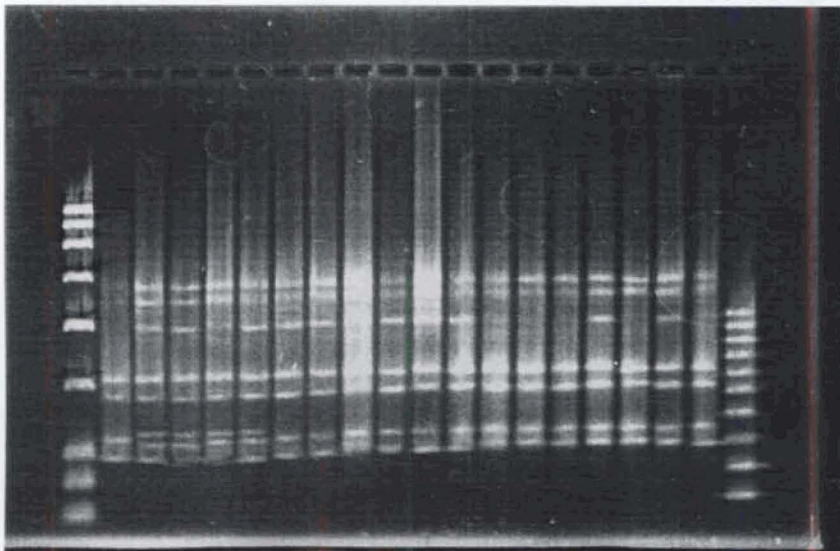
53c

L 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 M



RAPD profiles of eighteen individuals of two Western Ghats populations by OPAW 13 primer: L: Low range DNA ruler and M: DNA Marker (100bp ladder). Lane 1-10: Samples from Honaver and 11 - 18: Samples from Nilambur.

L 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 M

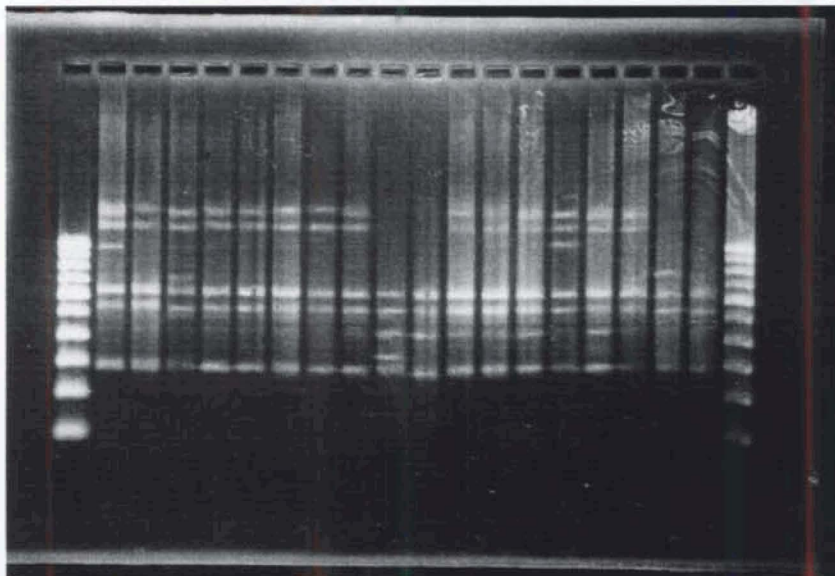


RAPD profiles of eighteen individuals of two Western Ghats populations by OPA 18 primer: L: Low range DNA ruler and M: DNA Marker (100bp ladder). Lane 1-10: Samples from Honaver and 11 - 18: Samples from Nilambur.

5

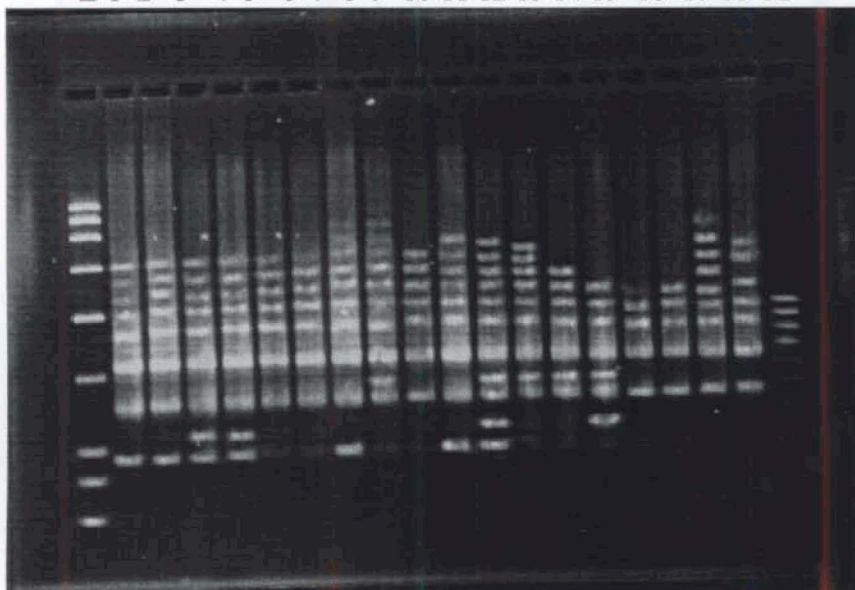
53D

L 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 M



RAPD profiles of eighteen individuals of two Western Ghats populations by OPAW 9 primer: L: Low range DNA ruler and M: DNA Marker (100bp ladder). Lane 1-10: Samples from Honaver and 11 - 18: Samples from Nilambur.

L 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 M

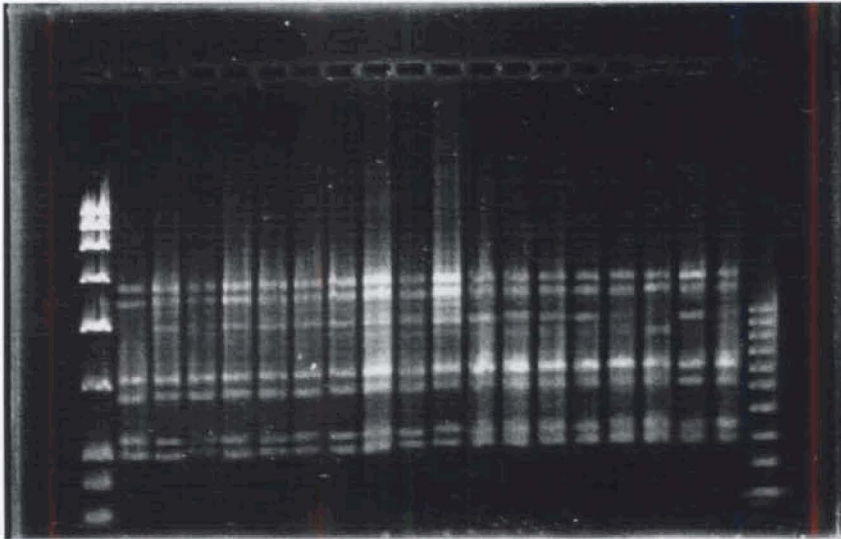


RAPD profiles of eighteen individuals of two Western Ghats populations by OPAW 7 primer: L: Low range DNA ruler and M: DNA Marker (100bp ladder). Lane 1-10: Individuals of Chertala and 11 - 18: Samples from Ashramam.

16

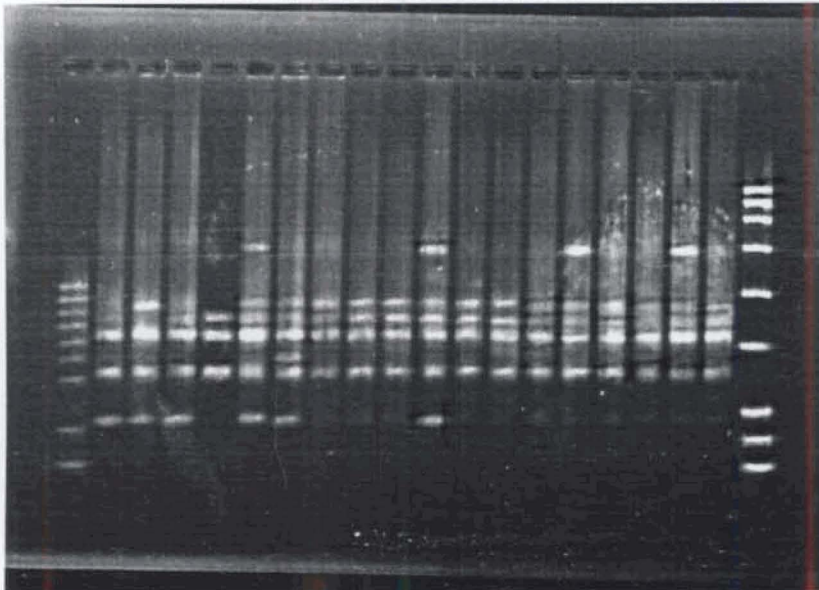
03K1

L 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 M



RAPD profiles of eighteen individuals of 3 Sri Lankan populations by OPE 2 primer: L: Low range DNA ruler and M: DNA Marker (100bp ladder). Lane 1-10: Samples from Matugama, Lane 11 – 15: Samples from Yagirala, Lane 16 –18: Samples from Watareke.

M 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 L



RAPD profiles of eighteen individuals of 3 Sri Lankan populations by OPE 18 primer: M: DNA Marker (100bp ladder) and L: Low range DNA ruler. Lane 1-10: Samples from Matugama, Lane 11 – 15: Samples from Yagirala, Lane 16 –18: Samples from Watareke.

index (Nei, 1973)  $h = 0.2458$  and Matugama population of Sri Lanka with lowest (0.1085) (Table 5. 3).

The estimated average genetic distances between populations (Table 5.4) indicated that the genetic distances between population pairs were ranged from 0.0500 (between Yagirala and Watারেके) to 0.2777 (between Yagirala and Honaver). The relative magnitude of genetic differentiation (Table 5.5) among subpopulation ( $G_{st}$ ) was 0.3820. The mean total genetic diversity ( $H_t$ ) was 0.2903.

The unrooted phenetic tree constructed based on the genetic distance showed a (Figure 5.1) clear distinction between Indian and Sri Lankan populations. Two single clusters were observed among the Indian populations (between Honaver/Chertala and between Nilambur/Ashramam). Among the Sri Lankan populations Yagirala and Watারেके formed a single cluster while Matugama population joined separately to this single cluster.

### **5.3.2. Cluster analysis**

The UPGMA dendrogram based on Jaccard's similarity coefficient depicted the genetic clustering of all the sixty genotypes analysed (Figure 5.2). The cophenetic correlation coefficient between the dendrogram and the original distance matrix was 0.84021 (Figure 5.3). Three major clusters were obtained in the dendrogram. The first cluster consisted of the individuals Hon1 to Cher 8, which mainly consisted of the populations of Honaver and Chertala with similarity of 0.57. The second cluster associated the individuals of Ashramam and Nilambur with similarity of 0.56. Some individuals of Chertala and major individuals of Ashramam were merged together at the level of 0.62 and then joined by individuals of Nilambur at 0.58 level. The third cluster consisted of entire Sri Lankan populations which with similarity of 0.61. The individuals of Watারেके and Matugama were clustered first (level 0.72) and then joined by Yagirala at level of 0.66.

### 5.3.3. Principal Co-ordinate Analysis (PCOA)

The PCOA chart (Figure 5.4) clearly discriminated Sri Lankan populations from Indian populations. The first principal coordinate axis represented the entire Sri Lankan populations where as the second coordinate axis included Nilambur and Chertala populations and the populations of Honaver and Ashramam concentrated in the third axis. The results of principal coordinate analysis were similar to cluster analysis. The first coordinate accounted for the 13.6% of total variance, while the second and third accounted for 11.2% and 6.4% respectively (Table 5.6). The low percentage of the variation (31.1%) explained by the first three axis could be attributed to high extent of genetic variability observed.

**Table 5.2. Nucleotide sequence of selected primers with the number of amplified products and fragment size range (bp)**

Code	Sequence 5' to 3'	No. of amplified products	Fragment size range (bp)
OPAU 02	CCA ACC CGC A	7	250 – 1200
OPA 04	AAT CGG GCT G	15	200 – 1000
OPA 18	AGG TGA CCG T	15	200 – 2500
OPAW 07	AGC CCC CAA G	13	250 – 1600
OPAW 09	ACT GGG TCG G	13	280 – 1500
OPAW 10	GGT GTT TGC C	13	280 – 1500
OPAW 20	TGT CCT AGC C	8	280 – 800
OPE 02	GGT GCG GGA A	10	100 – 1000
OPE 18	GGA CTG CAG A	12	100 – 600
OPB 15	GGA GGG TGT T	11	200 – 1600

**Table 5. 3. Comparison of populations for various genetic diversity measures**

Populations	h	npl	ppl
Honaver	0.1637	47	40.17%
Chertala	0.2458	80	68.38%
Ashramam	0.2086	66	56.41%
Nilambur	0.2326	69	58.97%
Watareke	0.1633	54	46.15%
Matugama	0.1085	31	26.50%
Yagirala	0.1331	38	32.48%
Mean	0.1793	55	47.00%

h = Nei's (1973) gene diversity

npl = Number of polymorphic loci

ppl = Percentage of polymorphic loci

**Table 5. 4. Nei (1978) genetic distance between populations**

Pop1- Honaver, Pop2- Chertala, Pop3-Ashramam, Pop4- Nilambur, Pop5-Watareke, Pop6-Matugama, Pop7- Yagirala.

pop ID	1	2	3	4	5	6	7
1	****						
2	0.1263	****					
3	0.2166	0.1204	****				
4	0.1816	0.1026	0.0526	****			
5	0.2372	0.1702	0.1586	0.1249	****		
6	0.2469	0.1751	0.1988	0.1478	0.0828	****	
7	0.2777	0.1962	0.1866	0.1454	0.0500	0.1132	****

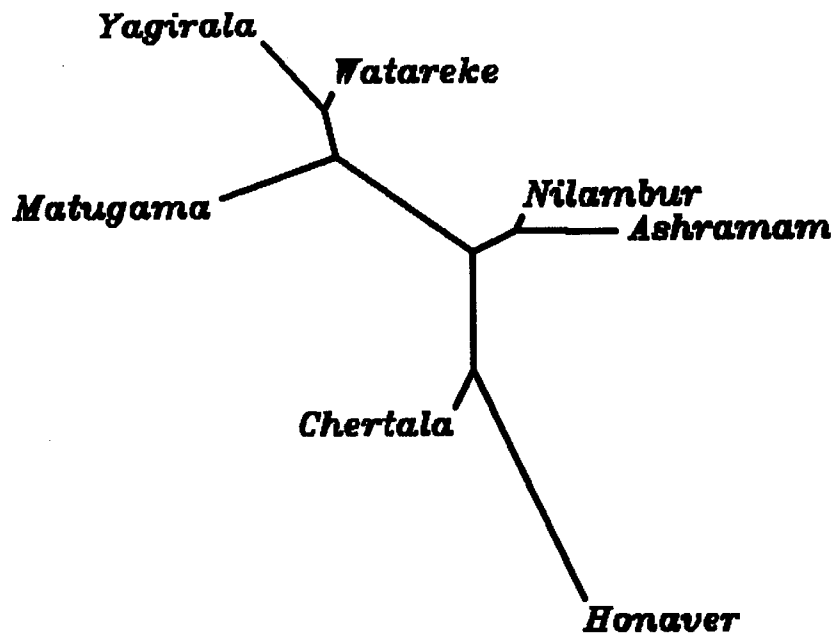
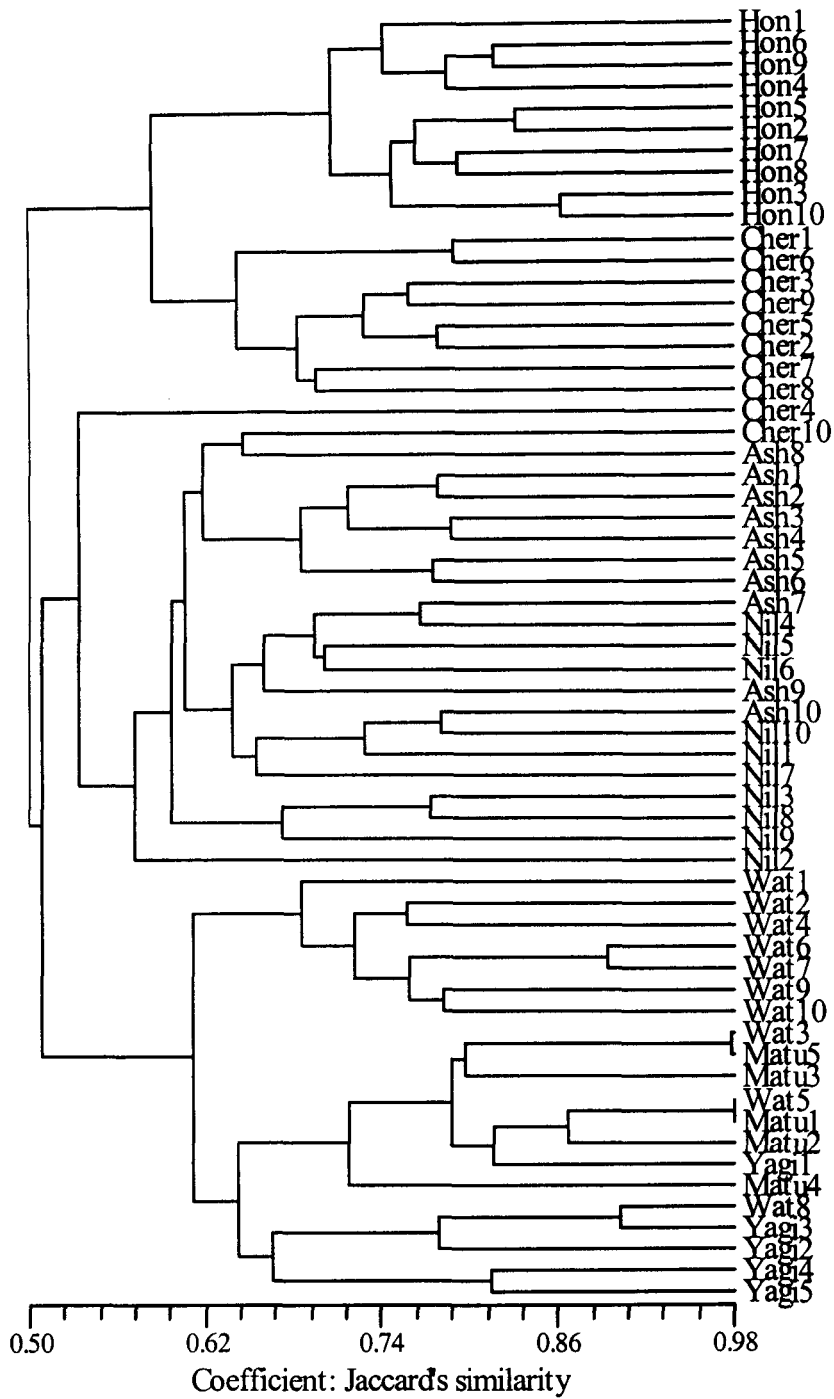
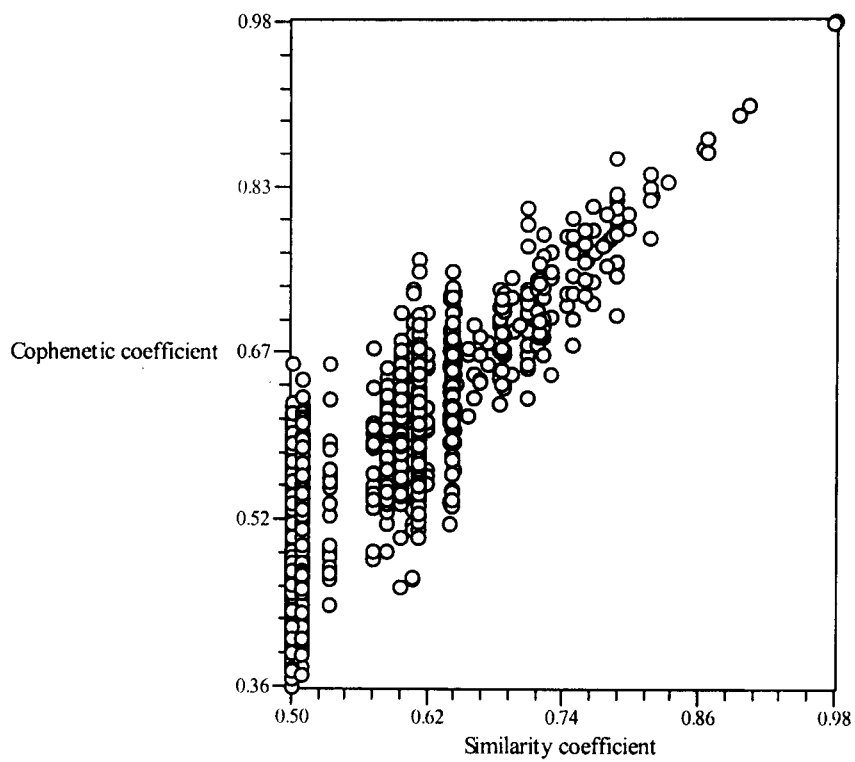


Figure 5.1. Unrooted phenetic tree for all populations based on genetic distance values produced from FITCH method (Fitch-Margoliash method) of PHYLIP (Felsenstein, 1993)

Between	And	Length
1	Chertala	0.01988
1	3	0.05567
3	4	0.07900
4	Matugama	0.05808
4	5	0.02295
5	Yagirala	0.04339
5	Watareke	0.00909
3	2	0.02340
2	Nilambur	0.00762
2	Ashramam	0.04772
1	Honaver	0.12161



**Figure 5.2. UPGMA dendrogram of 60 individuals of seven populations based on Jaccard's similarity coefficient (1908)**



**Figure 5.3. Plot analysis of cophenetic correlation and similarity coefficient represented as a measure of goodness of fit of the similarity indices,  $r = 0.84021$  = normalized Mantel statistic**

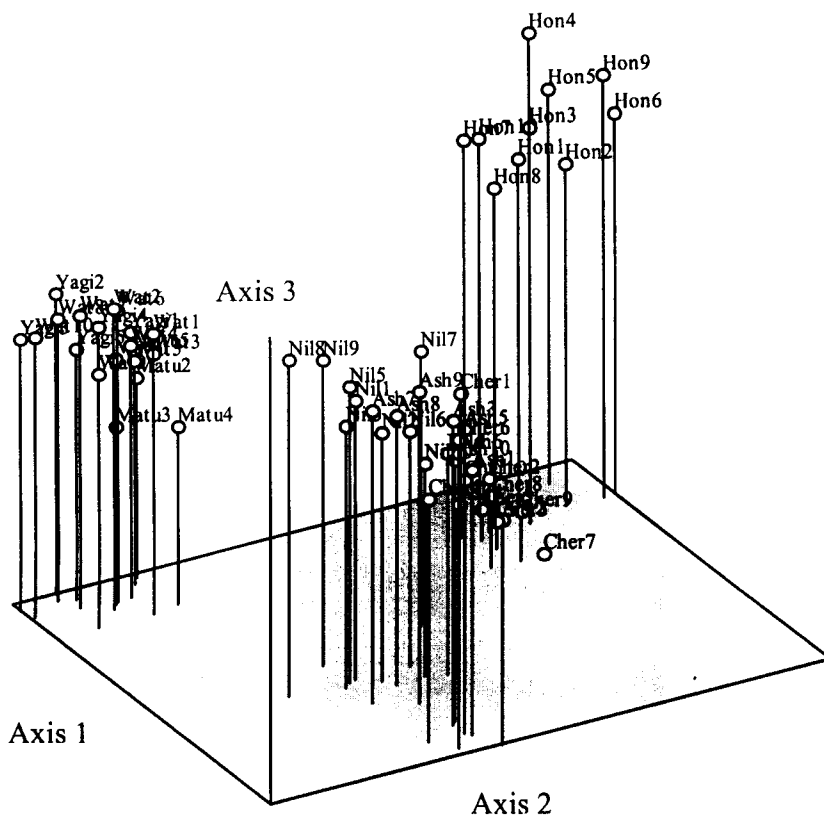
Table 5.5. Nei's (1973) genetic diversity statistics among seven populations used in this study

Locus	Sample Size	Ht	Hs	Gst
OPAU02-1	60	0.0000	0.0000	****
OPAU02-2	60	0.2822	0.2029	0.2810
OPAU02-3	60	0.0000	0.0000	****
OPAU02-4	60	0.0000	0.0000	****
OPAU02-5	60	0.4867	0.3059	0.3715
OPAU02-6	60	0.4518	0.2535	0.4389
OPAU02-7	60	0.4546	0.2444	0.4624
OPA04-1	60	0.0623	0.0499	0.1996
OPA04-2	60	0.2284	0.1370	0.3999
OPA04-3	60	0.0000	0.0000	****
OPA04-4	60	0.2250	0.1416	0.3710
OPA04-5	60	0.1644	0.0664	0.5959
OPA04-6	60	0.1336	0.1131	0.1535
OPA04-7	60	0.2861	0.2030	0.2902
OPA04-8	60	0.0297	0.0270	0.0919
OPA04-9	60	0.2496	0.1649	0.3394
OPA04-10	60	0.1943	0.1324	0.3186
OPA04-11	60	0.2961	0.1900	0.3584
OPA04-12	60	0.1534	0.1183	0.2283
OPA04-13	60	0.4804	0.3450	0.2819
OPA04-14	60	0.2343	0.1282	0.4529
OPA04-15	60	0.2553	0.0139	0.9455
OPA18-1	60	0.0731	0.0669	0.0854
OPA18-2	60	0.4754	0.2354	0.5048
OPA18-3	60	0.4581	0.3105	0.3222
OPA18-4	60	0.3569	0.2650	0.2575
OPA18-5	60	0.2183	0.1506	0.3104
OPA18-6	60	0.4611	0.2505	0.4567
OPA18-7	60	0.4983	0.3449	0.3078
OPA18-8	60	0.3193	0.1988	0.3773
OPA18-9	60	0.2195	0.1775	0.1914
OPA18-10	60	0.4546	0.3272	0.2803
OPA18-11	60	0.4759	0.1803	0.6211
OPA18-12	60	0.4895	0.3979	0.1870
OPA18-13	60	0.4354	0.2738	0.3711
OPA18-14	60	0.2225	0.1736	0.2195
OPA18-15	60	0.0438	0.0409	0.0669
OPAW07-1	60	0.4901	0.4264	0.1300
OPAW07-2	60	0.2164	0.1326	0.3874
OPAW07-3	60	0.4532	0.1917	0.5769
OPAW07-4	60	0.2670	0.1684	0.3693
OPAW07-5	60	0.4836	0.3904	0.1927
OPAW07-6	60	0.4050	0.3355	0.1716
OPAW07-7	60	0.4626	0.2122	0.5413
OPAW07-8	60	0.3876	0.1027	0.7351
OPAW07-9	60	0.3712	0.1781	0.5202

OPAW07-10	60	0.3481	0.1917	0.4493
OPAW07-11	60	0.3481	0.1917	0.4493
OPAW07-12	60	0.1336	0.1131	0.1535
OPAW07-13	60	0.0456	0.0390	0.1434
OPAW09-1	60	0.2631	0.1117	0.5756
OPAW09-2	60	0.4923	0.1526	0.6901
OPAW09-3	60	0.4417	0.2558	0.4209
OPAW09-4	60	0.2183	0.1506	0.3104
OPAW09-5	60	0.3411	0.2077	0.3911
OPAW09-6	60	0.0863	0.0618	0.2839
OPAW09-7	60	0.2614	0.1479	0.4341
OPAW09-8	60	0.1706	0.1546	0.0938
OPAW09-9	60	0.4858	0.3115	0.3587
OPAW09-10	60	0.2888	0.0499	0.8273
OPAW09-11	60	0.2660	0.0270	0.8986
OPAW09-12	60	0.3838	0.2036	0.4695
OPAW09-13	60	0.3411	0.2077	0.3911
OPAW10-1	60	0.1253	0.0942	0.2480
OPAW10-2	60	0.3855	0.2786	0.2772
OPAW10-3	60	0.5000	0.3486	0.3029
OPAW10-4	60	0.1260	0.0934	0.2590
OPAW10-5	60	0.4862	0.2622	0.4606
OPAW10-6	60	0.2343	0.1282	0.4529
OPAW10-7	60	0.1952	0.1236	0.3670
OPAW10-8	60	0.4210	0.1974	0.5312
OPAW10-9	60	0.4284	0.2786	0.3496
OPAW10-10	60	0.3397	0.1091	0.6790
OPAW10-11	60	0.0297	0.0270	0.0919
OPAW10-12	60	0.5000	0.3919	0.2162
OPAW10-13	60	0.0289	0.0278	0.0372
OPAW20-1	60	0.0935	0.0731	0.2184
OPAW20-2	60	0.0000	0.0000	****
OPAW20-3	60	0.1704	0.0976	0.4272
OPAW20-4	60	0.0759	0.0638	0.1599
OPAW20-5	60	0.0863	0.0618	0.2839
OPAW20-6	60	0.4477	0.0664	0.8516
OPAW20-8	60	0.1260	0.0934	0.2590
OPE02-1	60	0.3193	0.1988	0.3773
OPE02-2	60	0.4236	0.3053	0.2792
OPE02-3	60	0.3467	0.2395	0.3092
OPE02-4	60	0.4563	0.3330	0.2703
OPE02-5	60	0.1196	0.0706	0.4095
OPE02-6	60	0.3476	0.2093	0.3979
OPE02-7	60	0.4827	0.3283	0.3198
OPE02-8	60	0.1125	0.0803	0.2861
OPE02-9	60	0.3780	0.2940	0.2222
OPE02-10	60	0.4140	0.1568	0.6213
OPE18-1	60	0.3892	0.2422	0.3776
OPE18-2	60	0.4538	0.2181	0.5194
OPE18-3	60	0.4805	0.3117	0.3514
OPE18-4	60	0.1209	0.0708	0.4144
OPE18-5	60	0.4249	0.2161	0.4914
OPE18-6	60	0.3879	0.2695	0.3054
OPE18-7	60	0.4995	0.2824	0.4347

OPE18-8	60	0.1551	0.1163	0.2500
OPE18-9	60	0.2395	0.1789	0.2530
OPE18-10	60	0.1278	0.1200	0.0608
OPE18-11	60	0.0901	0.0769	0.1468
OPE18-12	60	0.0722	0.0679	0.0597
OPB15-1	60	0.0623	0.0499	0.1996
OPB15-2	60	0.1313	0.1159	0.1169
OPB15-3	60	0.4609	0.2796	0.3934
OPB15-4	60	0.2089	0.1344	0.3566
OPB15-5	60	0.1517	0.1204	0.2063
OPB15-6	60	0.4999	0.3817	0.2365
OPB15-7	60	0.4324	0.3105	0.2820
OPB15-8	60	0.4995	0.2549	0.4896
OPB15-9	60	0.4666	0.3129	0.3294
OPB15-10	60	0.4405	0.4021	0.0871
OPB15-11	60	0.4000	0.3330	0.1673
Mean	60	0.2903	0.1794	0.3820

Note:  $H_t$  = total genetic diversity;  
 $H_s$  = expected heterozygosity;  
 $G_{st}$  = coefficient of gene differentiation.



**Figure 5.4. Principal coordinate analysis of RAPD data, based on RAPD profiles of the 60 individuals**

**Table 5.6. Percentage of variance explained by each Axis**

	Eigen value	Percent	Cumulative
1	8.01570788	13.5859	13.5859
2	6.62523162	11.2292	24.8152
3	3.75374212	6.3623	31.1774

## 5.4. Discussion

### 5.4.1. Species characterisation of *C. metzianus* and *C. rivalis*

RAPD analysis has been efficiently used in characterizing and discriminating different species of *Brassica* (Demeke *et al.*, 1996), *Asphodelus* (Lifante and Agunagalde, 1996), *Eucalyptus* species (Sale *et al.*, 1996) *Astragalus* L. (Luo *et al.* 2000) and *Cardamine* L. (Lihova *et al.*, 2000). In the present study, RAPD was used to check the identity of two morphologically similar species of *Calamus* viz. *C. metzianus* and *C. rivalis*. The ten random primers used in the study produced a total of 117 bands of which 112 bands were scored polymorphic. The lack of any species-specific marker might be due to the identical genetic make up of the species or due to low coverage of genome. In many studies, species-specific RAPD markers has been observed in Cyprus (Banilas *et al.*, 2003) and *Calamus* (Changtragoon *et al.*, 1995). But no striking variations were observed when *C. metzianus* and *C. rivalis* populations were compared for various genetic diversity measures. Further, the result of UPGMA dendrogram based on Jaccard's similarity coefficient, the unrooted phenetic tree based on genetic distance and the principal coordinate analysis failed to separate the populations of *C. metzianus* (Nilambur and Honaver populations) from *C. rivalis* (Ashramam, Chertala and Sri Lanka). However, a clear geographic pattern was portrayed in the dendrogram. In the unrooted phenetic tree, the populations of Honaver (*C. metzianus*) and Chertala (*C. rivalis*) was found to be grouped together. The same pattern was again repeated for Nilambur and Ashramam populations. The similar results indicating genetic relatedness among populations from cluster and principal coordinate analysis shows

that *C. rivalis* cannot be separated from *C. metzianus* and hence *C. rivalis* can be synonymised to *C. metzianus*.

#### 5.4.2. Population based Genetic diversity

Genetic richness can be assessed by estimating the genetic diversity parameters viz. percentage of polymorphic loci and gene diversity index (Yeh, 2000). The percentage of polymorphic loci was 95% in the present study. The high percentage of polymorphism has been reported in tropical plant species such as *Taiwania cryptomerioides* (Lin *et al.*, 1993) *Populus tremuloides* (Yeh *et al.*, 1995) *Faidherbia albida* (Ibrahim, 1996) *Caesalpinia echinata* Lam. (Cardoso *et al.*, 1998) and in *Euterpe edulis* (Cardoso *et al.*, 2000). In contrast to these studies, relatively lower percentage of polymorphic loci was observed in *Gliricidia sepium* (Chalmers *et al.*, 1992) and *Theobroma cacao* (Russell *et al.*, 1993) using RAPDs and in *Moringa oleifera* using AFLPs (Muluvi *et al.*, 1999).

Nei's gene diversity estimate was found to be 0.1793 in the present study. This value is low when compared to other wind pollinated, woody, long lived tree species in which comparatively much variability within population is reported (Hamrick and Godt, 1990) as in *Populus tremuloides* (0.30), *P. grandidentata* (0.35) and *Quercus petraea* (0.298) (Greef *et al.*, 1998). When compared to outcrossing, herbaceous, insect pollinated species, the gene diversity estimate value for the present study is similar to that in *Lilium martagon* (between 0.15 – 0.26) (Persson *et al.*, 1998) and *Argyroxiphium sandwicense* (0.192) (Frier *et al.*, 1996) but higher than those of short lived, annual perennial species of *Oryza glumaepatula* (Buso *et al.*, 1998) in which the Nei's gene diversity estimate was 0.089.

Among the seven analysed populations, the genetic diversity measures were highest in Chertala population (0.2458) followed by Nilambur (0.2326) and Ashramam (0.2086). The higher gene diversity of Chertala population compared to those of other populations might be due to high population density compared to those of other populations. Evolutionary forces can operate on a large number of genotypes resulting in increased amount of gene diversity. This population can be considered as 'hot spot' of genetic variation and important reservoir of potentially useful genes and hence required to provide high priority for management strategies and conservation. Hamrick and Loveless (1989) reported that the species with high fecundities, high rates of out crossing, wind pollination and long generation time often maintain high level of diversity.

Hamrick *et al.* (1991) reported that the key factors affecting genetic diversity within populations are the geographic distribution and mode of seed dispersal. Renuka (1995a) reported that birds and small mammals are the main agents for the dispersal of *Calamus* seeds. The studies on pollination mechanism of three species of *Calamus* (Renuka, 1995a) revealed that they were mostly wind pollinated and pollen grains were found to be shed from the anthers within two hours after anthesis.

The partitioning of genetic diversity show high amount of diversity within population ( $G_{st} = 0.3820$ ) which indicated that 38.20 % of the total diversity was among populations while the rest (61.80%) of the total variations occurred within populations. The same pattern was observed in the studies of *Eucalyptus urophylla* (House and Bell, 1994), *Eucalyptus grandis* (Grattapaglia *et al.*, 1997) and *Azadirachta indica* (Kundu, 1999). But studies on pines (Hamrick and Godt, 1990) and *Santalum album* (Suma and Balasundaran, 2003)

indicated a reverse trend in which most of the genetic diversity was found to be among populations than within populations. The mean expected heterozygosity (0.1794) was higher than that of out crossing woody plants (0.154) reported by Moran, (1992); House and Bell (1994) and Martins – Corder and Lopes (1997).

The seven populations in this study cover wide geographic range in latitudes and geographical distances. The most genetically similar populations (Yagirala and Watারেke) are geographically separated by a distance of 25 kms only while, the genetically distant populations (Honaver and Yagirala) were separated by about 900 kms. The genetic separations of the populations in the phenetic tree were in partial agreement with geographical isolation. The reasons for partial or low correlation between geographic and genetic distance could be the high gene flow, mutation, migration, natural selection and the geographical isolation in a small area (Huang and Zhang, 2000). Similar results have been reported in the case of other tropical tree species in the previous studies (Belletti and Lanteri, 1995; Suoheimo *et al.*, 1999).

The populations analysed in the present study is under continuous threat of degradation and in most cases the fragmentation of populations is due to habitat alteration and irregular harvesting of stems for the furniture and handicraft industry. Recently, the population in Ashramam is seriously disturbed due to the promotion of tourism activities. As a prior step for the *ex-situ* conservation for rattans, germplasm collections and seed stands representing all the populations are maintained by Kerala Forest Research Institute.

# Systematic treatment

Sreekumar V.B “Systematics and phylogeny of the genus *calamus* linn. (arecaceae) in the western ghats ” Thesis. Kerala Forest Research Institute, University of Calicut, 2004

*Systematic treatment*

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## 6. Systematic treatment

### CALAMUS Linn.

Linneaus, Sp. Pl. 325. 1753 & in Gen. Pl. (ed.5) 152. 1754; Becc. in Hook. f., Fl. Brit. India 6 : 436. 1892 & in Ann. Roy. Bot. Gard. Calcutta 11: 61. 1908; Furtado in Gard. Bull, Singapore 15: 32. 1956; Dransfield, Man. Ratt. Malay Pen. 122. 1979 & in Ratt. Sabah 89 1984.

**Type species:** *Calamus rotang* L.

Solitary or clustering, acaulescent to high climbing, pleoanthic, dioecious rattans. Leaves cirrate or ecirrate. Sheaths usually heavily armed with spines, spines frequently highly organized, sheath often bearing flagellum, sometimes absent and a cirrus present; knee often present; ochrea sometimes well developed, usually inconspicuous; petiole conspicuous or inconspicuous; leaflets variously arranged. Male and female inflorescences superficially similar, often ending a long flagellum, bracts tubular at base, armed; partial inflorescence longer than subtending bract, very rarely slender; male flower with a short cup shaped calyx, usually with three well defined lobes, corolla split into three petals, stamens 6, shortly epipetalous, pistillode minute; female flower sometimes longer than male, calyx shallowly three lobed, corolla with 3 petals, staminodes six, joined basally to form a cup shaped ring; ovary tipped with 3 stigmas, covered with reflexed scales, locules 3 with one ovule in each. Fruit spherical, ellipsoid to conical – ovoid, covered with several rows of reflexed scales; seed usually one, frequently deeply pitted or grooved; endosperm homogenous or ruminant. Embryo basal or lateral.

Distribution: The genus consists of about 370 species distributed throughout Africa, India, South China, Malay Archipelago, Queensland, Fiji, Sunda shelf and New Guinea.

Based on the phylogenetic analysis, the following groups were recognized for the members of the Western Ghats. The conservation status assigned to each species were based on IUCN (2000).

**Group 1- *Calamus thwaitesii* clade**

Rachilla not pedicellate and partial inflorescence clawed on the axial part.

*C. thwaitesii* and *C. nagbettai*

**Group 2- *C. hookerianus* clade**

Leafsheath tomentose, mouth of the leafsheath spiny and embryo lateral.

*C. hookerianus*, *C. lakshmana*, *C. pseudotenuis*, *C. karnatakensis*  
and *C. stoloniferus*

**Group 3- *C. brandisii* clade**

Slender diameter canes, primary spathe elongate and split longitudinally,  
entirely opened or laminar.

*C. brandisii*, *C. travancoricus*, *C. metzianus* and *C. rotang*

**Group 4 - *C. huegelianus* clade**

Primary spathe tightly sheathing, not lacerate and rachilla arched.

*C. huegelianus*

**Group 5 - *C. gamblei* clade**

Mouth of the leafsheath spines absent, leafsheath spines bulbous based,  
rachillae stout and stiff and secondary spathe armed.

*C. gamblei*

**Group 6 - *C. shendurunii* clade**

Leafsheath sparsely armed and knee unarmed.

*C. shendurunii* and *C. lacciferus*

**Group 7- *C. neelagiricus* clade**

Cilia absent on both surfaces of the leaflets and ochrea inconspicuous,

*C. neelagiricus*

**Group 8- *C. delessertianus* clade**

Leaflets margins bristly on both surfaces and partial inflorescence base bulbous.

*C. delessertianus*, *C. prasinus* and *C. dransfieldii*

**Group 9- *C. vattayila* clade**

Leaflets oblong and partial inflorescence bracts closely sheathing.

*C. vattayila*

*Key to the Species*

- 1a. Leaf tip ending in a cirrus.....*C. nagbetta*  
 1b. Leaf tip not ending in a cirrus .....2  
 2a. Leaf sheath basal flagella prolonged to shoots.....*C. lacciferus*  
 2b. Leaf sheath basal flagella not prolonged to shoots.....3  
 3a. Leaflets and fruits oblong.....*C. vattayila*  
 3b. Leaflets and fruits not oblong .....4  
 4a. Leaflets grouped.....5  
 4b. Leaflets not grouped.....7  
 5a. Mouth of the leafsheath with minute bristles like spines .....*C. brandisii*  
 5b. Mouth of the leafsheath without bristles like spines .....6  
 6a. Primary sheath partly laminar, stem less than 1 cm diameter ..... *C. travancoricus*  
 6b. Primary sheath not laminar, stem above 1 cm in diameter..... *C. thwaitesii*  
 7a. Leafsheath spines numerous and heavily armed .....8  
 7b. Leafsheath spines few, one to three .....*C. shendurunii*  
 8a. Stem with sheath to 1 cm in diameter .....9  
 8b. Stem with sheath more than 1 cm in diameter .....10  
 9a. Leaf sheath spines black tipped, rachis of younger parts  
     covered with greyish pubescence.....*C. metzianus*  
 9b. Leaf sheath, rachis and inflorescence axis glabrous.....*C. rotang*  
 10a. Leaf sheath with tomentum, mouth of the leaf sheath  
     with longer flat like spines.....11  
 10b. Leaf sheath without tomentum, mouth of the leaf sheath  
     without longer flat like spines.....14  
 11a. Fruit black colour, scales flattened.....*C. huegelianus*  
 11b. Fruit not black, scales not flattened.....12  
 12a. Mouth of the leaf sheath with longer 16 cm flat spines.....*C. hookerianus*  
 12b. Mouth of the leaf sheath without long, flat spines .....13  
 13a. Petiole and rachis with needle like spines,  
     fruit yellow.....*C. pseudotenius*  
 13b. Petiole and rachis with slender spines, fruit violet .....*C. karnatakensis*  
 14a. Leaf sheath inner part red colour, seed surface irregularly  
     pitted .....*C. lakshmanae*  
 14b. Leaf sheath inner part not red colour, seed surface smooth .....15  
 15a. Leaflet cilia 2 cm long, sheath light green.....*C. dransfieldii*

- 15b. Leaflet cilia 1 cm long, sheath not light green .....16
- 16a. Leaf sheath when cut, exudate milky latex .....*C. prasinus*
- 16b. Leaf sheath when cut does not exudates milky latex.....17
- 17a. Leaflet margins closely spinulose, spinules more spreading.....*C. delessertianus*
- 17b. Leaflet margin remotely spinulose, cilia not spreading.....18
- 18a. Fruit ovoid, deeply channeled in the middle.....*C. gamblei*
- 18b. Fruit oblate, faintly channeled in the middle .....*C. neelagiricus*

**Group 1- *Calamus thwaitesii* clade**

***Calamus thwaitesii*** Becc. *et* Hook. f. in Hook. f., Fl. Brit. India 6: 452. 1892; Becc., Ann. Roy. Bot. Gard. Calcutta 11: 137. 1908 t. 10 & 11, 1913; Fischer in Gamble, Fl. Pres. Madras 3: 1567. 1931; Basu, Rattans in India 104. 1992; Renuka, Rattans of Western Ghats 33. t. 22. 1992. [Figure 6.1; Plate 6.1]

*Type:* Sri Lanka, S. loc. Thwaites 2873 (isotype K. cibachrome!).

*C. longesetus* Thw. (*non* Griff.) Enum. Plant. Zeylan. 330. 1864.

*Vernacular names:* Pannichural, Thadiyan chural, Vandichural (Malayalam)

Robust, cluster forming, high climbing rattan. Stem with sheaths 5 to 6 cm in diameter and without sheaths 3 to 4 cm; internodes 20 to 25 cm in length. Leaves about 3m long, ecirrate; leaf sheath yellow, densely armed with black spines, arising from a raised rim-like surface, the largest 3 x 0.7 cm, flat, smaller spines scattered in between; knee absent; ochrea inconspicuous; petiole 30 to 40 cm long, trigonous, yellowish, armed with straight, black laminar spines arranged into oblique whorls; rachis yellowish, channeled above, bifaced above, flat beneath, armed with distant claws; leaflets 70 x 4 cm fascicled irregularly, plicate, narrowly lanceolate, elongate-ensiform, narrow at the the base, sharply

spinulose along the margins, midrib prominent, bristly on both sides. Inflorescence to 6 m long, male and female inflorescence superficially similar, elongate, flagelliform, primary sheath long, tubular, tightly sheathing, thin, coriaceous, armed with black hooked spines, mouth lacerated; partial inflorescence 60 to 70 cm long, with 6 - 8 rachillae on each side, secondary sheath tubular, infundibuliform, 4 to 5 cm long, compactly sheathing, obliquely truncate, acuminate at one side, lacerated, armed with short spines, male rachillae 7-8 cm long, large and flattened with distichous flowers on each side; flowers ovate, calyx three toothed, finely striately veined, female rachillae 10-15 cm long, involucrephorum sessile; involucre irregularly cupular, truncate, acutely two keeled; female flowers ovate, 7-8 mm long, calyx with 3 ovate acute tooth; fruiting perianth explanate. Fruit 2 x 1.3 cm, ellipsoid to ovoid; scales arranged in 12 vertical rows with median grooves, yellow with deep brown margins; seed ovoid, compressed, 1.5 x 1 cm, apex obtuse, irregularly pitted on back side; endosperm homogenous; embryo basal.

*Distribution:* In evergreen, semi-evergreen and moist deciduous forests between 75 to 900 m in the Southern Western Ghats.

*Phenology:* Flowering: June-July; Fruiting: April-May.

*Uses:* One of the best quality canes used extensively in furniture industry.

*Conservation status:* Lower risk.

*Specimens examined:* KERALA: Palakkad Dist., Silent Valley, 1980, *Nair N C 129895* (MH); Dhoni hills, 1982 *Nambiar & Renuka 2770* (KFRI); Thrissur Dist., Vazhachal, 1982, *Renuka 2767* (KFRI); Vazhachal, 2001, *Sreekumar 22465* (KFRI); Trivandrum Dist., Palamkanam, 1986 *Mukundakumar 05261* (TBGT); Kannur Dist., Kottiyoor, 2001

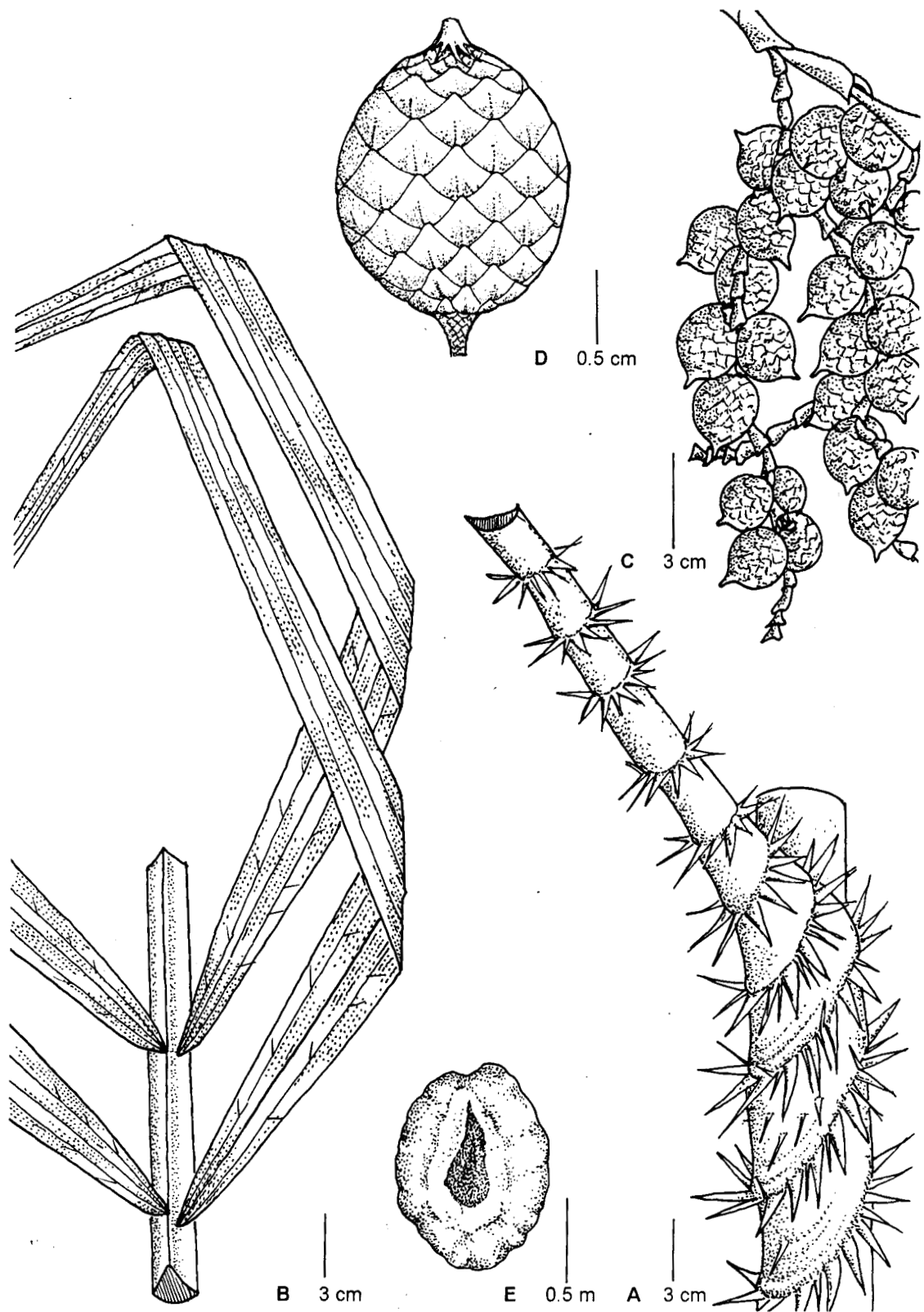


Figure 6.1. *Calamus thwaitesii* Becc. A. leaf sheath; B. a portion of leaf; C. a partial infructescence; D. fruit; E. seed

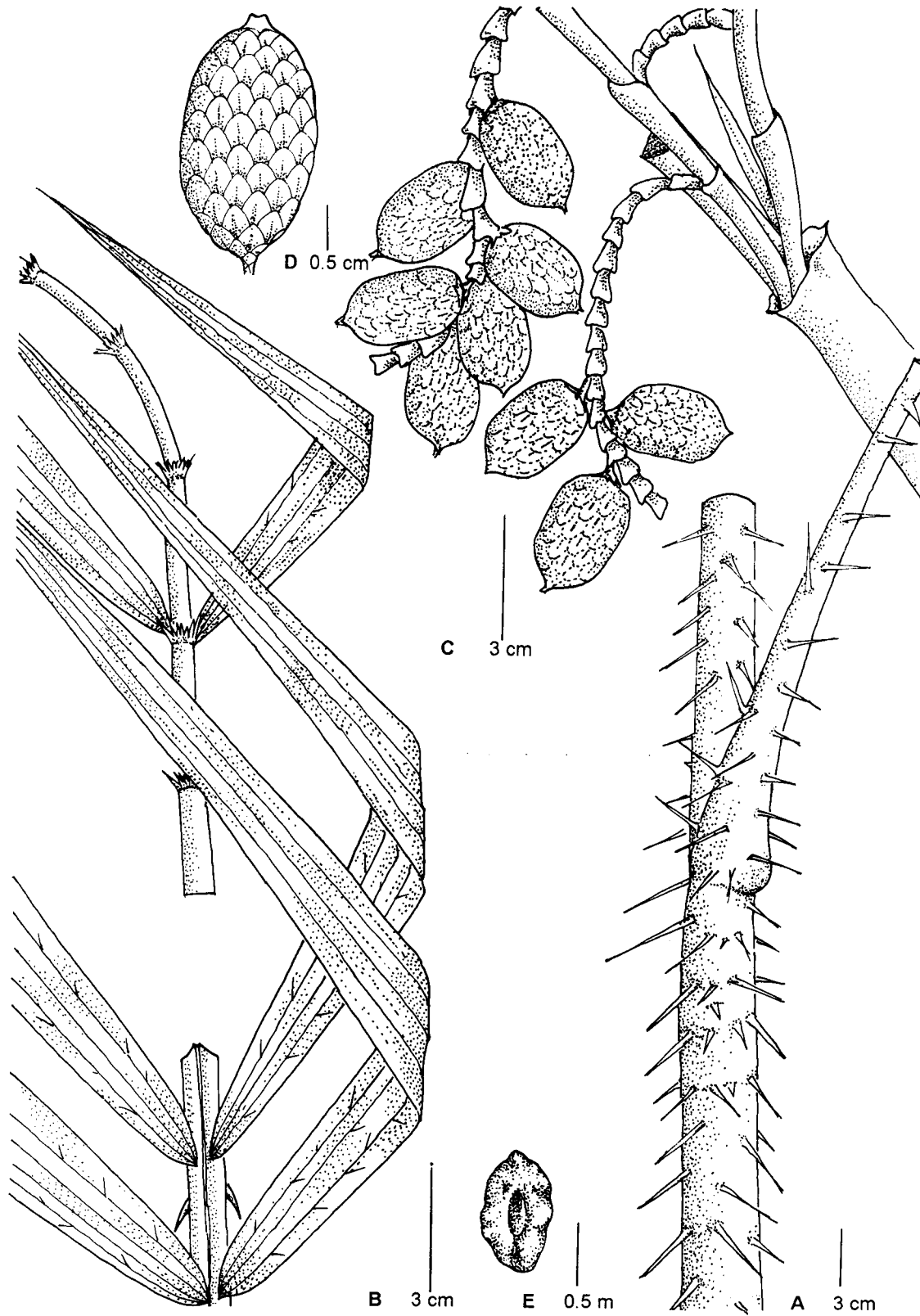


Figure 6.2. *Calamus nagbettai* Fernandez & Dey A. leaf sheath; B. a portion of leaf; C. a partial inflorescence; D. fruit; E. seed

*Sreekumar 22421* (KFRI); Aralam, 2001, *Sreekumar 22438* (KFRI); Kollam Dist., Achenkovil, 2001, *Rangan & Sreekumar 22423* (KFRI); Kulathupuzha, 2001, *Rangan & Sreekumar 22424* (KFRI); Kulathupuzha, 2001, *Rangan & Sreekumar 22425* (KFRI); Arenkavu, 2001, *Rangan & Sreekumar 22426* (KFRI); Rosemala, 2002, *Sreekumar 22465* (KFRI). KARNATAKA: Uttara Kannada Dist., Kanara, 1873, *Beddome 52758* (MH); Kargal, 1988 *Renuka 4070* (KFRI); Honaver, 2001, *Sreekumar & Rangan 21333* (KFRI).

***Calamus nagbetta*** Fernandez *et* Dey, Indian For. 96: 223.1970. *Renuka*, Rattans of Western Ghats 41. t. 17. 1992. [Figure 6.2; Plate 6.1]

*Type*: India, Karnataka, Coorg. *Dey 64* (holotype DD!).

*Vernacular names*: Nagabetha (Kannada).

Robust, clustering, large diameter rattans. Stems 20 to 25 m long or more, with sheaths 4 to 4.5 cm in diameter, without sheaths to 3 cm, with black patches at the basal portion. Leaves green, juvenile ones brown, ecirrate, mature leaves cirrate, 3.5 m long, cirrus to 1.5 m long, armed with stout claws; sheath yellowish green to green, lower half densely armed with spines; spines dimorphic, in groups of two, 3 x 0.5 cm, narrow, triangular, black, flat, intermingled with bristle-like spines, in the upper portion of the sheath few in number, mouth with long spines to 4 cm long, intermingled with narrow shorter spines, young sheaths with brown tomentum; knee not prominent; petiole 40 to 45 cm long, spiny; spines 1.5 to 2 cm long; rachis spiny on the basal portion, spines 1.5 to 2 cm long, upper portion armed with claws; leaflets 50 x 2 cm, regular, linear-lanceolate, ciliate veins on both surfaces. Female inflorescence branching to two orders; primary sheath tightly sheathing,

74B

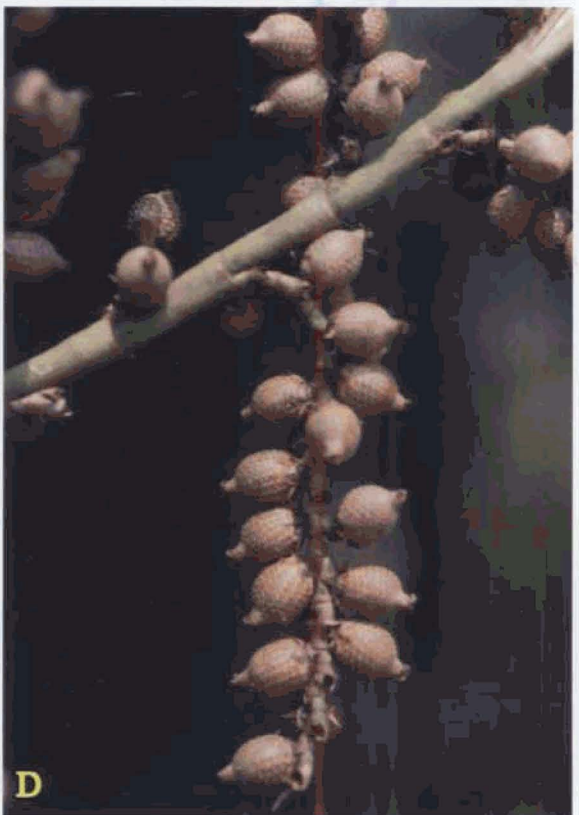


Plate 6.1. *Calamus thwaitesii* Becc.: **A.** Leaf sheath; **B.** Infructescence  
*Calamus nagbettai* Fernandez & Dey: **C.** Leaf sheath; **D.** Infructescence

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prolonged at the distal end into a lanceolate point, with small spines; partial inflorescence to 60 to 70 cm long; secondary sheath tightly sheathing, unarmed; involucre slightly stalked; involucre cup-shaped; fruiting perianth explanate. Fruit ovoid, 1.6 x 0.9 cm; scales in 15 vertical rows, brown with a thin, dark brown margin, deeply channeled in the middle; endosperm ruminant.

*Distribution:* Evergreen forests at about 450 m in Uttara Kannada district of Karnataka.

*Phenology:* Flowering: December – January; Fruiting: May-June.

*Uses:* A very robust cane, suitable for furniture and handicrafts. This cane is considered to be very sacred and is worshiped in many households.

*Conservation status:* Vulnerable.

*Specimens examined:* KARNATAKA: Uttara Kannada Dist., Kanara, 1966 Dey 64 (DD); Subramanya, 1988, *Renuka 4077* (KFRI); Subramanya, 2000, *Anto & Sreekumar 22408* (KFRI); Subramanya, 2001, *Sreekumar 22609* (KFRI); Karike, 2001, *Sreekumar 22427* (KFRI); Subramanya, 2002, *Sreekumar 22480* (KFRI).

#### **Group 2- *C. hookerianus* clade**

***Calamus hookerianus*** Becc., Ann. Roy. Bot. Gard. Calcutta 11: 83, 226. t. 70. 1908;

Fischer in Gamble, Fl. Pres. Madras 3: 1568. 1931; Basu, Rattans in India 82. 1992;

Renuka, Rattans of Western Ghats 30, t. 12. 1992. [Figure 6.3; Plate 6.2]

*Type:* India, Tamil Nadu, Courtallum, *Wight 1142* (holotype K. Cibachrome!)

*C. borneensis* Becc., Rec. Bot. Surv. India. 2: 205. 1926.

*Vernacular names:* Velichural, Kakkachural, Vanthal and Kallan (Malayalam).

Clustering, moderate sized rattans. Stems 8 to 10 m or more long, with sheaths to 4 cm in diameter, without sheaths 2 to 2.5 cm; internodes 20 to 26 cm. Leaves 1.5 to 2 m cirrate; sheath brownish green, densely armed with spines, spines triangular, the largest 2 to 2.5 long, 0.5 cm wide at the base, interspersed with numerous smaller spines and abundant brown tomentum, mouth of the sheath provided with long papery spines 11 to 12 cm long; knee sometimes present, not conspicuous; ochrea 6 to 8 cm, falling off easily; flagellum 4 to 5 m long; petiole 65 to 75 cm; armed with spines; spines 2 to 6 cm long, brown or black, triangular; rachis acutely trigonous, bifaced, smooth above, flat below, with a single row of spines, 1 to 1.5 cm long with reflexed base; leaflets regular, the largest to 55 x 2.5 cm, long, acuminate, tips armed with short bristles, narrowly lanceolate, linear ensiform, midvein ciliate beneath from centre upwards, lateral veins ciliate above. Inflorescence 4 to 5 m long; primary sheath long, narrow, closely sheathing, armed with small spines, split longitudinally upwards; partial inflorescence 60 to 75 cm long; male branching to three orders; secondary sheath 8 to 9 cm long, closely sheathing, narrow at base, expanded into a cup at the upper region, armed with one or two rows of small spines at the region where expansion begins; rachillae 1 to 3 cm long, flowers large, 3 mm long, ovate; female branch to two orders; rachillae 10 to 15 cm long; involucrophorum sessile; involucre cup shaped. Fruiting perianth not distinctly pedicelliform, callous at the base. Fruits subglobose to ovate, 1 x 0.8 cm, scales in 18 rows, not channeled along the middle, yellowish brown with a dark brown border, margin erosely toothed; seed subglobose, irregularly pitted; endosperm homogenous.

*Distribution:* Evergreen forests upto 1000 m in Southern Western Ghats

*Phenology:* Flowering: January – July; Fruiting: July – December.

*Uses:* A good medium diameter cane, used in furniture industry and basket making.

*Conservation status:* Lower risk.

*Specimens examined:* KERALA: Kollam Dist., Arienkavu, 1982, *Sasidharan 1732* (KFRI); Chokkanpatti, 1982, *Mohanan 153674* (MH); Valayam, Achenkovil, 1983, *Nambiar & Renuka 2902* (KFRI); Rosemala, Shenduruni Wild Life Sanctuary, 2000, *Sreekumar & Rangan 22416* (KFRI); Rosemala, Shenduruni Wild Life Sanctuary, 2001, *Sreekumar 22445* (KFRI); Rosemala, Shenduruni Wild Life Sanctuary, 1999, *Sreekumar 22604* (KFRI); Palakkad Dist., Pukuthipalam, Nelliampathy, 1983, *Renuka & Muktesh 2727* (KFRI); Karianchola, Parambikulam, 1983, *Muktesh 2503* (KFRI); Pothupara, 1983, *Renuka & Nambiar 2904* (KFRI); Ernakulam Dist., Kodanad, 1983, *Nambiar & Renuka 2948* (KFRI); Thrissur Dist., Peechi 1984, *Renuka 3009* (KFRI); Wayanad Dist., Chandanathodu, 1984, *Renuka 3030* (KFRI); Pathanamthitta Dist., Moozhiyar, Ranni, 1984, *Renuka 3155* (KFRI); Trivandrum Dist., Mylamoodu, 1986, *S M Kumar & Seeni 05256* (TBGT); TAMILNADU: Kattabomman Dist., Courtallum, 1988, *Renuka 4041* (KFRI); Mundandurai Wildlife Sanctuary, 1988, *Renuka 4045* (KFRI); Shencottah, 1988, *Renuka 4046* (KFRI); Trichy Dist., Kaduvaiperumukku, 1990, *Renuka 6619* (KFRI).

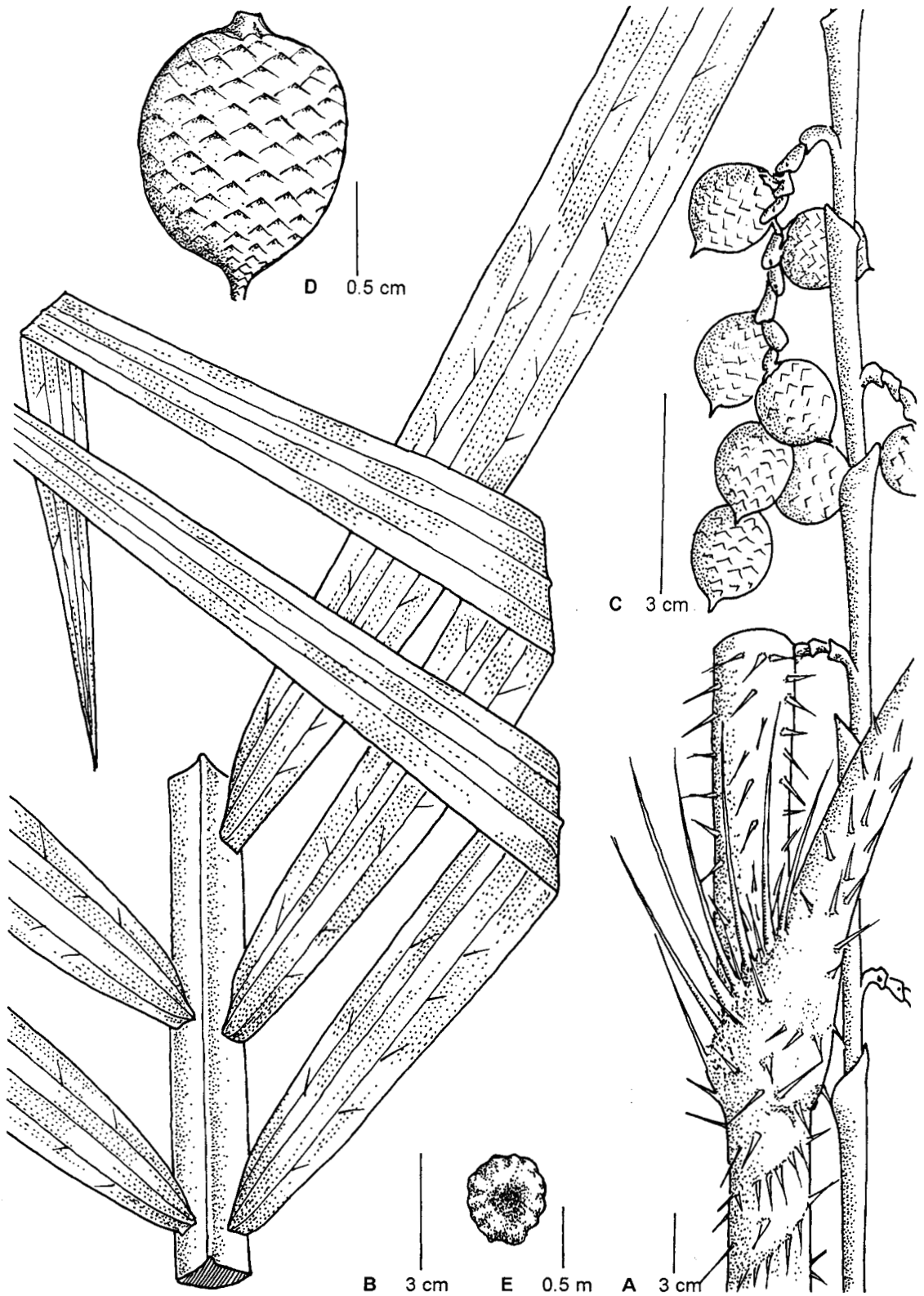


Figure 6.3. *Calamus hookerianus* Becc. A. leaf sheath; B. a portion of leaf; C. a partial infructescence; D. fruit; E. seed

719

**Calamus lakshmanae** Renuka, J. Econ. Tax. Bot., 14: 701. 1990 & in Rattans of Western Ghats 37. t. 15. 1992. [Figure 6.4; Plate 6.2]

*Type:* India, Karnataka, Makkut, *Renuka 4086* (holotype KFRI!)

Clustering, medium diameter rattan. Stem 15 to 20 m long or more, with sheaths 1 to 2.5 cm in diameter, without 1 cm. Leaf 1 to 1.5 m long, ecirrate, leafsheath yellowish green, densely armed with spines, spines 0.5 to 1 cm long, yellowish, bulbous based, pointing horizontally or upwards, distal younger sheaths less spiny and with brown markings; knee present; ochrea not seen. Leaf 1 to 1.60 m long; petiole 20 to 22 cm, armed with small sometimes curved spines; rachis biconvex towards the basal portion, triangular in the upper portion, with many small recurved spines; leaflets to 45 x 2 cm, regular, linear, lanceolate, tip not ciliate, terminal pair 17 x 0.8 cm, confluent to more than half of its length, 3 veined, lateral veins ciliate on the dorsal surface, midvein ciliate below, spinulose at margin. Inflorescence long, pendulous; primary sheath tightly sheathing with small spines; partial inflorescence 55 to 60 cm long, attached well above the mouth of sheath; secondary sheaths 3 to 3.5 cm long, tubular infundibuliform, lightly sheathing, narrow at base, inner side flat, distal region with 3 or 4 small prickles; rachillae 8 to 10 cm long, attached above the mouth of secondary sheath; male flowers glabrous, narrowly oblong, trigonous, obtuse, 4 mm long; female flowers 2 mm long; involucre not stalked; involucre cup-shaped; fruiting perianth slightly callous at base. Fruit ovate, 1 x 0.7 cm, stigma 3 mm long, scales in 26 rows, when young bright green, channeled along the middle; endosperm ruminant.

*Distribution:* Evergreen forests between 100 – 1000 m above MSL. Rosemala in Kerala and Makkut and Honaver in Karnataka.

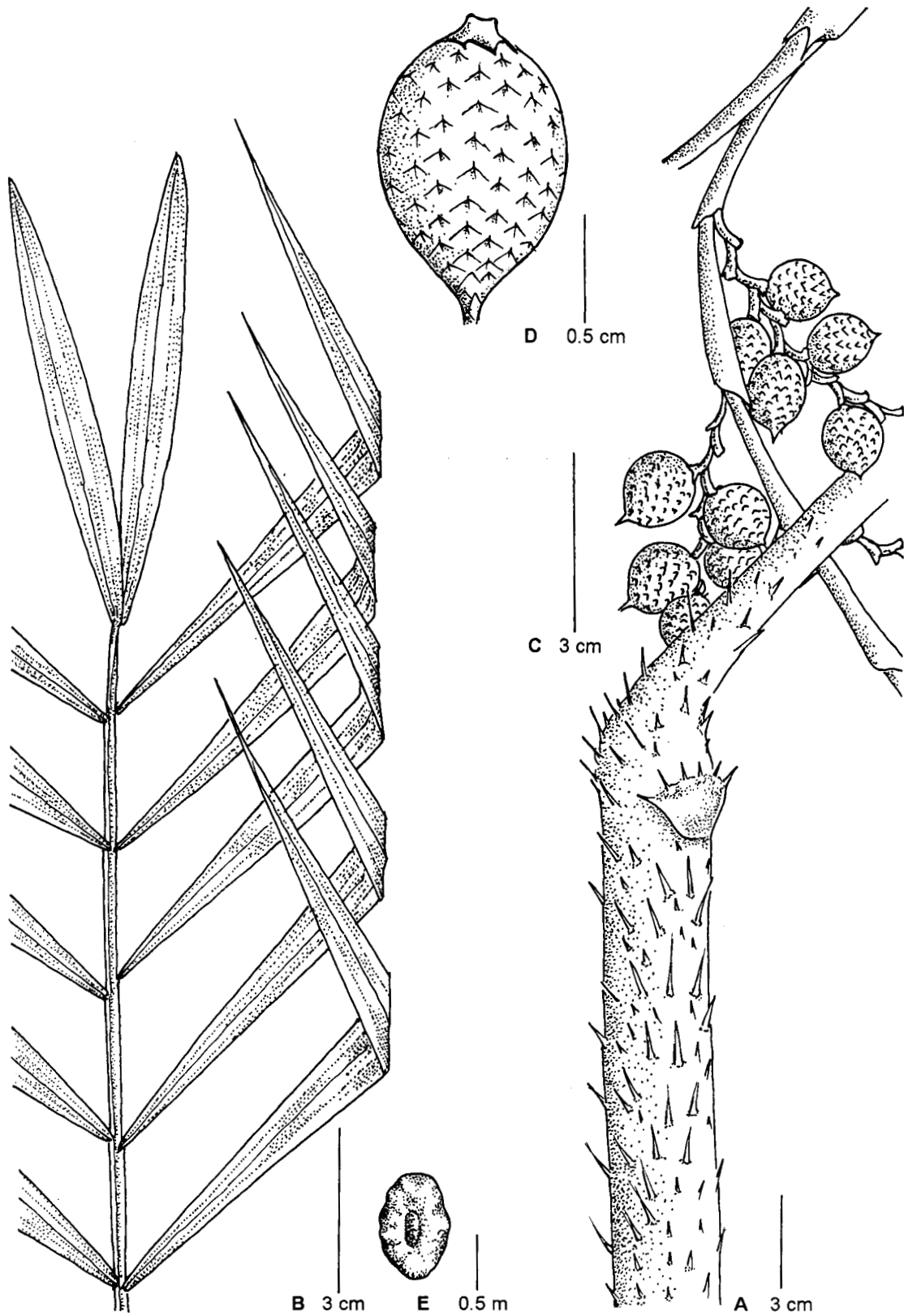


Figure 6.4. *Calamus lakshmanae* Renuka A. leaf sheath; B. a portion of leaf; C. a partial infructescence; D. fruit; E. seed

78B

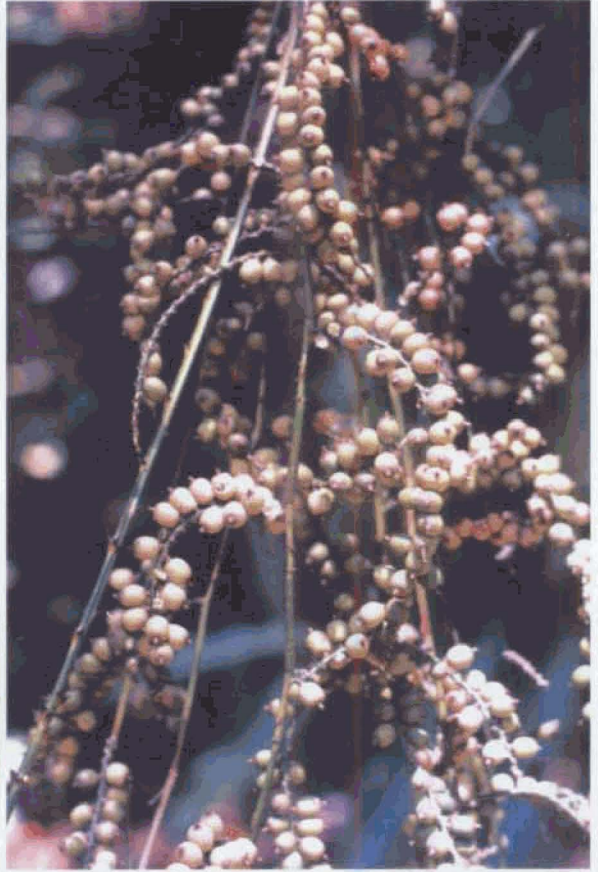


Plate 6.2. *Calamus hookerianus* Becc.: **A.** Leaf sheath; **B.** Inflorescence  
*Calamus lakshmanae* Renuka: **C.** Leaf sheath; **D.** Inflorescence

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*Phenology*: Flowering: October – December; Fruiting: April – May.

*Uses*: A good cane used in furniture industry.

*Conservation status*: Vulnerable.

*Specimens examined*: KERALA: Kollam Dist., Rosemala, 2002, *Sreekumar* 22470 (KFRI); Malappuram Dist., Kidakkamala, Amarambalam, 2002, *Sreekumar & Rangan* 22664 (KFRI); KARNATAKA: Kodagu Dist., Makkut, 1989, *Renuka* 4086 (CAL); 1989, *Renuka* 4086 (KFRI).

***Calamus pseudotenuis* Becc. ex Becc. et Hook. f. in Hook. f., Fl. Brit. India 6: 452. 1892;**  
Becc., Ann. Roy. Bot. Gard. Calcutta 11: 314. t. 69.1908; Fischer in Gamble, Fl. Pres.  
Madras 3: 1567. 1931; Basu, Rattans in India 96. 1992; Renuka, Rattans of Western  
Ghats 33. t. 19. 1992. [Figure 6.5; Plate 6.3]

*Type*: Sri Lanka, Balangodde, Matette, *Thwaites* 2335 (holotype: K. Cibachrome!).

Clustering, moderate sized rattan. Stem with sheaths measures 3 to 3.5 cm in diameter, without sheaths 2 to 2.5 cm. Leaf to 1.6 m long; ecirrate; leafsheath flagelliferous, yellowish green and armed with spines of 3 to 3.5 cm length; spines needle like, yellow in colour and pointing different directions. Brown tomentum seen in between the spines; mouth of the sheath provided with 3 or 4 longer spines of 6 cm length; ochrea large, 8 to 10 cm in length, brown, not spiny, entire; petiole 8 to 10 cm long, armed with straight, yellowish spines, 3 to 4 cm in length; rachis bifaced above, rounded beneath, armed with scattered, needle like flat, solitary, horizontal deflexed spines with 2.5 to 3 cm long,

pointing different directions; leaflets arranged regularly, linear lanceolate, gradually acuminate at the apex; terminal pair free at base. Inflorescence 2 to 3 m long, partial inflorescence 65 to 70 cm long; primary spathe tubular, very long, closely sheathing, acutely two keeled, armed with scattered, straight, horizontal spines; secondary spathe with very narrowly tubular infundibuliform, elongate, unarmed, prolonged into a triangular tip; involucrophorum very short, subcupular; involucre small, shallowly cupular, projecting from the involucrophorum; fruiting perianth pedicelliform, slightly callose at the base. Fruit sub ovoid, 1.5 x 0.8 cm, scales arranged in 18 rows, not channeled along the middle, straw yellow at their base, margin finely toothed; seed subglobose; endosperm homogenous.

*Distribution:* This species is found in evergreen forests generally above 750 m in Southern Western Ghats and Eastern Ghats.

*Phenology:* Flowering: July-August; Fruiting: April-May.

*Uses:* Used in furniture industry and in basket making.

*Conservation status:* Threatened.

*Specimens examined:* KERALA: Idukki Dist., Kozhikkanam, Peermedu, 1982, *Nambiar & Renuka 2620* (KFRI); Valanchankanam, Peermedu, 1982, *Nambiar & Renuka 2625* (KFRI); Mankulam, 1988, *Renuka 4053* (KFRI); Palakkad Dist., Attappady, 1985, *Renuka 3471* (KFRI); Nelliampathy, 2001, *Sreekumar & Rangan 22437* (KFRI); Pathanamthitta Dist., Pamba, 2001, *Sreekumar 22434* (KFRI); TAMILNADU: Dindigul Dist., Pulney hills, 1873, *Beddome 73173 & 73169* (MH); KARNATAKA: Kodagu Dist., Makkut,

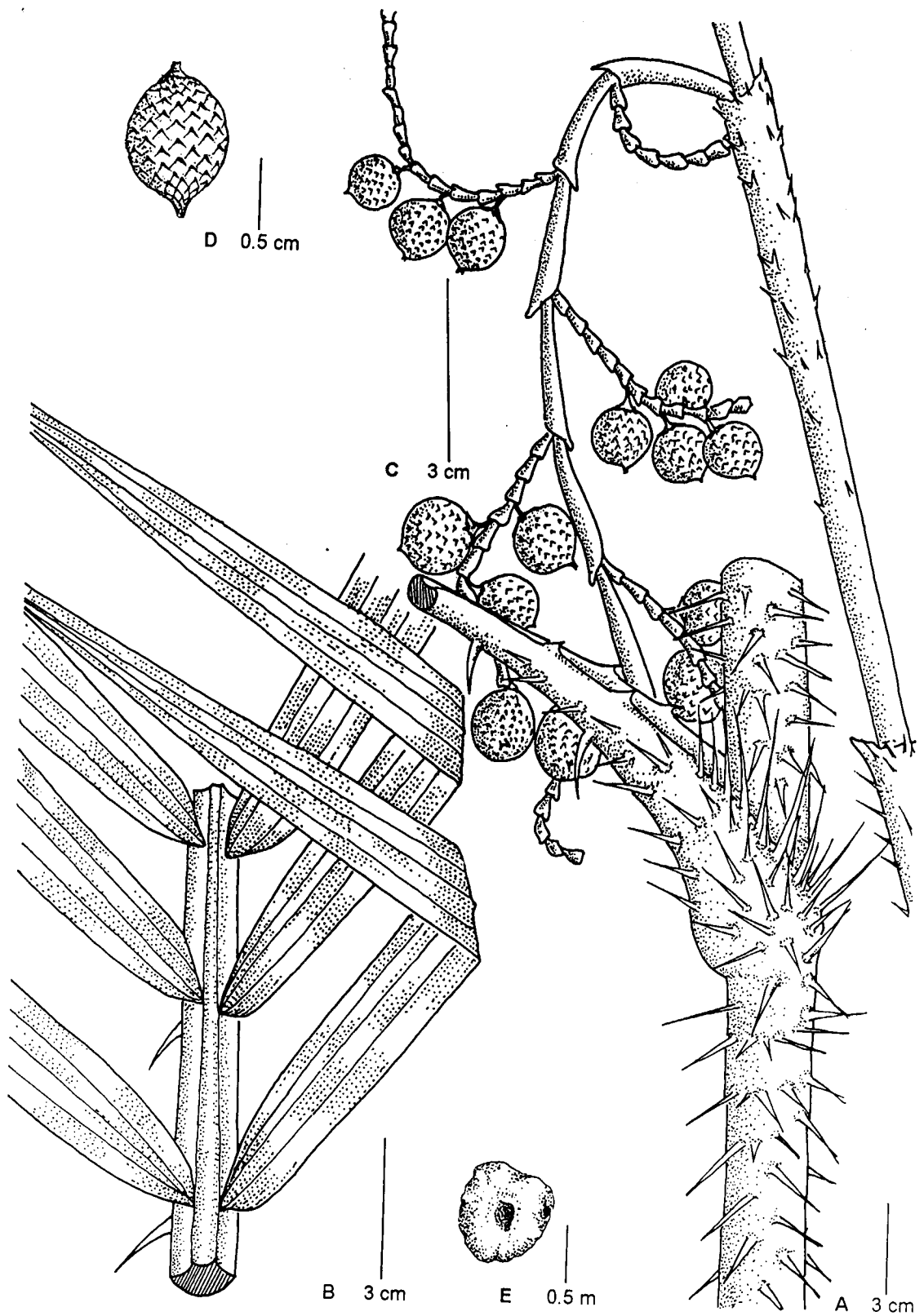


Figure 6. 5. *Calamus pseudotenius* Becc. ex Becc. & Hook. f. A. leaf sheath; B. a portion of leaf; C. a partial inflorescence; D. fruit; E. seed

1984, *Kesava Murthy & Simhan 4715* (RRCBI), Shimoga Dist., Arabithittu, 1984, *Vajravelu 140554* (MH).

***Calamus karnatakensis*** Renuka *et* Lakshmana, RIC Bull. 9: 10. 1990; Renuka, Rattans of Western Ghats 33. 13. 1992. [Figure 6.6; Plate 6.3]

*Type*: India, Karnataka, *Renuka 4068* (holotype: KFRI!).

Clustering, medium diameter rattan. Stem 20 to 25 m long more, with sheaths 2 to 3 cm in diameter, without sheaths 1 to 1.5 cm. Leaves ecirrate, 1 to 1.5 m long; sheath when mature yellow, lower part greenish brown or greenish yellow, young sheaths with brown tomentum, densely armed with spines, spines stout, 2.5 cm long, sometimes forming half spiral, mouth of the sheath provided with long spines 3 to 4 cm; knee prominent, wrinkled; ochrea 5 to 6 cm long, with small spines; petiole 25 to 30 cm long; rachis 0.5 to 1 cm wide; leaflets, 50 x 2.5 cm, regular, 3-veined, veins and margins spinulose, terminal pair united basally. Inflorescences long, flagellate; partial inflorescences 25 to 30 cm, many; primary sheath tubular, very long, closely sheathing, acutely two keeled, armed, spines scattered, secondary sheaths tightly sheathing at first, later split open, sometimes becoming laminar; basal part of secondary sheath narrow, upper part funnel-shaped; rachillae 10 to 15 cm long, spinulose; male flowers small, 3.5 to 4 mm long, ovate; female flowers small, 3 mm; involucre sessile, arising from inside the sheath; involucre sessile, cup-shaped. Fruit globose, light violet when ripe, 7 to 8 mm across; scales in 19 rows, yellow with chestnut brown border; endosperm homogenous, seedling leaf fan-shaped.

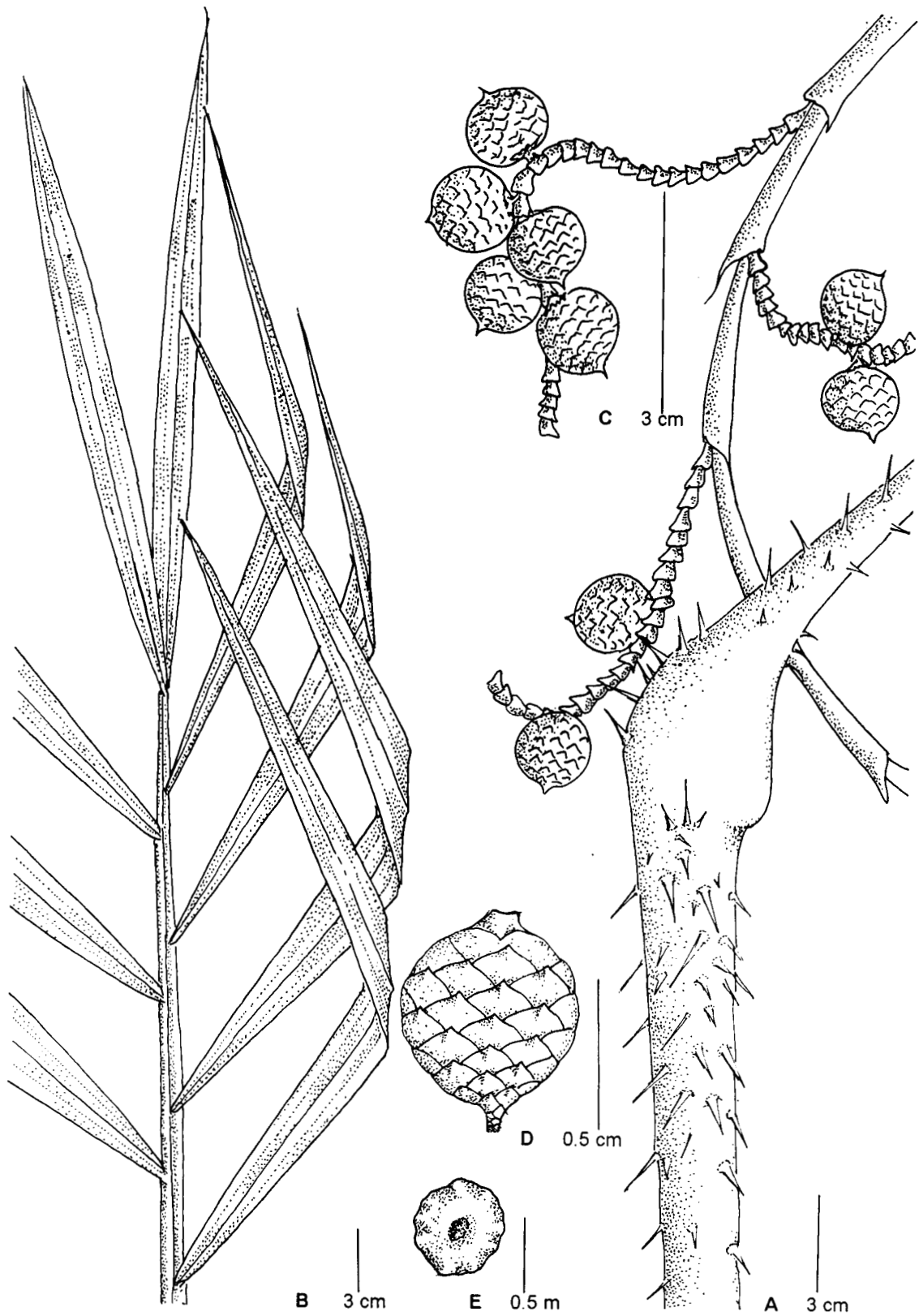


Figure 6.6. *Calamus karnatakensis* Renuka & Lakshmana. A. leaf sheath; B. a portion of leaf; C. a partial infructescence; D. fruit; E. seed

2/2



Plate 6.3. *Calamus pseudotenius* Becc. ex Becc. & Hook. f.: **A.** Leaf sheath; **B.** Inflorescence  
*Calamus karnatakensis* Renuka & Lakshmana: **C.** Leaf sheath; **D.** Inflorescence

*Distribution:* Evergreen forests between 530-1200 m above MSL in Southern Western Ghats of India.

*Phenology:* Flowering: December – January; Fruiting: April – May.

*Uses:* A good medium diameter cane, used in the furniture industry.

*Conservation status:* Vulnerable.

*Specimens examined:* KERALA: Malappuram Dist., New Amarambalam, 2002, *Sreekumar & Rangan* 22666 (KFRI). KARNATAKA: Kodagu Dist., Kargal range, 1988, *Renuka* 4068 & 4069 (KFRI); Shimoga Dist., Agumbe, 1988, *Renuka* 4076 (KFRI); 1989, *Renuka* 4094 & 4095 (KFRI); Kodagu Dist., Thalakkaveri, 1989, *Renuka* 4088 (KFRI); Thalakkaveri, 2000, *Anto & Sreekumar* 22404 (KFRI).

***Calamus stoloniferus*** Renuka, J. Econ. Taxon. Bot. 14: 803. 1990 & in Rattans of Western Ghats 49 t. 21. 1992. [Figure 6.7; Plate 6.4]

*Type:* India, Karnataka, Makkut, *Renuka* 4085 (holotype: KFRI!)

*Vernacular name:* Jedubetha (Kannada).

Clustering, stoloniferous, medium diameter rattans. Stem with sheaths 2 to 3 cm in diameter, without sheaths 1.2 cm. Leaf 1.75 m long, ecirrate; leafsheath green, sparingly spiny, spines 1.5 to 2 cm long; knee present, devoid of spines; ochrea present in young stages; petiole 15 cm long; rachis spiny, spines 2 to 2.5 cm, distally with recurved spines below; leaflets 45 x 3.5 cm, seedling leaflets 5 to 5.5 cm wide, linear lanceolate, the terminal pair confluent basally, apical 5 to 6 leaflets crowded together, more obtuse, margin

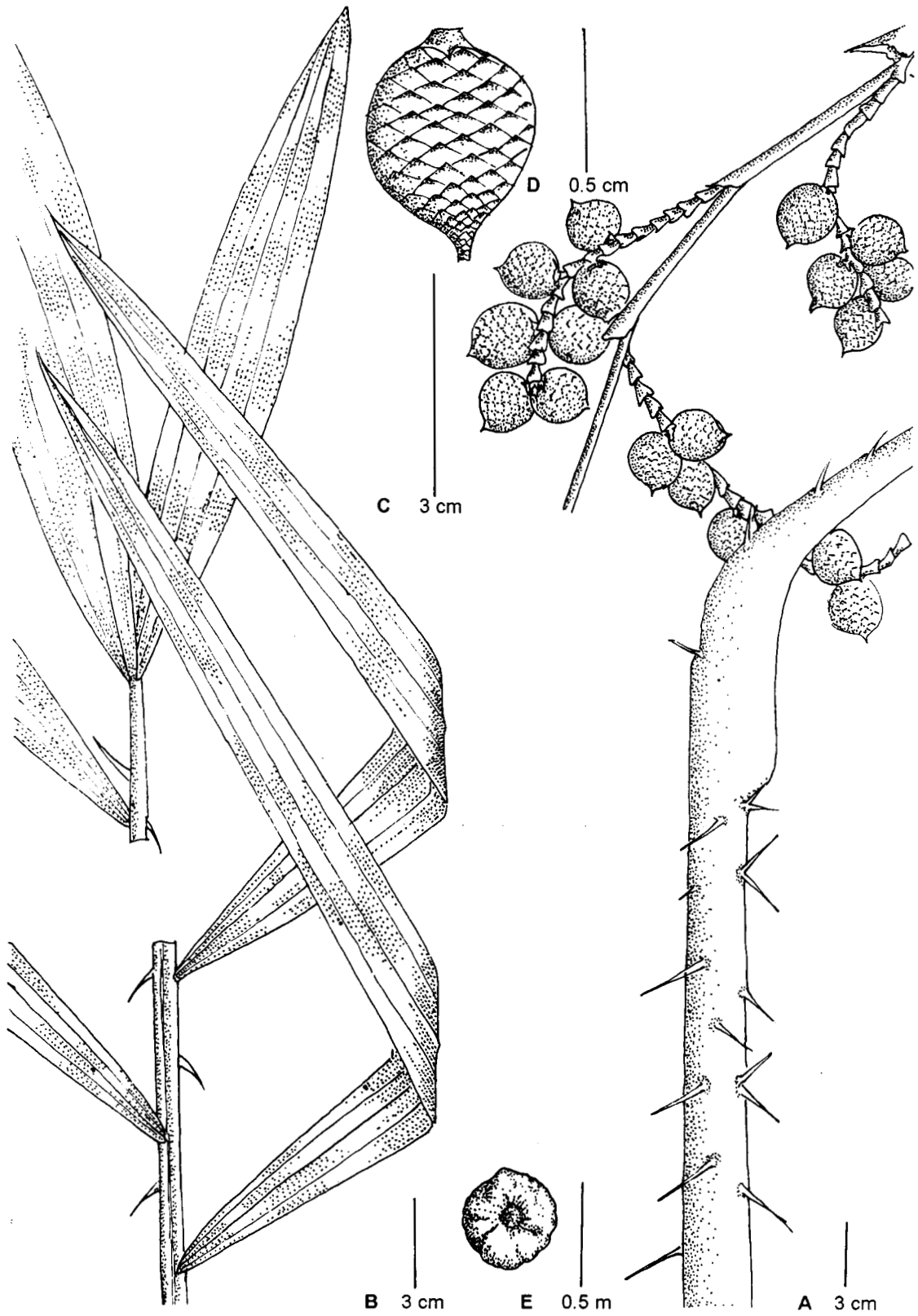


Figure 6.7. *Calamus stoloniferus* Renuka A. leaf sheath; B. a portion of leaf; C. a partial infructescence; D. fruit; E. seed

spinescent, 3 to 5 veined, midvein sparsely ciliate. Inflorescence long, flagellate; primary sheath in male inflorescence expanded into a laminar structure at the mouth, in female inflorescence tapering at the mouth; secondary sheath tightly sheathing, in male produced into a point at one side at the mouth, not spiny; male and female rachillae 8 to 9 cm long, attached at the mouth of the sheath; involucre not stalked; involucre cup-shaped. Fruit, 1 cm across, spherical, scales in 16 rows, yellow with brown border; not channeled; endosperm not ruminant.

*Distribution:* Evergreen forests at 85 m. Found in Makkut of Karnataka State.

*Phenology:* Flowering: November – December; Fruiting: March – April.

*Uses:* A good quality cane, but not available in sufficient quantities.

*Conservation status:* Vulnerable.

*Specimens examined:* KARNATAKA: Kodagu Dist., Makkut, 1989. *Renuka 4085* (KFRI); 1984, Makkut, 2000, *Anto & Sreekumar 22407* (KFRI).

### **Group 3- *C. brandisii* clade**

***Calamus brandisii*** Becc. ex Becc. et Hook. f., in Hook. f., Fl. Brit. India. 6: 448.1892; Becc., Ann. Roy. Bot. Gard. Calcutta 11: 278 t. 102.1908; Fischer in Gamble, Fl. Pres. Madras. 3: 1091.1931; Renuka, Rattans of Western Ghats, 23 t. 9.1992. Basu, Rattans in India 109. 1992. [Figure 6.8; Plate 6.4]

*Type:* India, Tamil Nadu, Courtallum, *Brandis 2340*. (holotype: CAL!).

Clustering, slender, small diameter rattan. Stem 8 to 10 m long, with sheaths 1 to 1.5 cm in diameter, without sheaths 0.8 to 1 cm. Leaf sheath green, flagelliferous, gibbous above with scattered, deflexed, minute slender needle like spines, mouth of the sheath with longer spines to 4 cm long; knee present; ochrea inconspicuous. Leaf 1 m long, ecirrate, petiole 22 cm long, smooth above, convex beneath, margin acute, armed 2 to 3 cm long, often interspersed with a few smaller ones; petiole 14 cm long, flat and smooth above and beneath convex, with acute margins, armed with needle like spines; rachis acutely trigonous, with solitary deflexed spines 2 to 2.5 cm long; leaflets few, distinctly fascicled, linear-lanceolate, acuminate, 25 cm in length, 2 cm in width, midvein prominent, sparingly spiny, apex bristly, terminal pair united at the base. Male and female inflorescences long, slender, flagelliform; primary sheath tightly sheathing basally, tubular, splits open distally, with small spines; partial inflorescence 55 cm long, attached at the mouth of the sheath; secondary sheaths narrow at base, tubular infundibuliform, with small prickles; male rachillae alternate, attached at the mouth of the sheath, 2 to 3 cm long; male flowers greenish yellow, distichous, 3mm; female rachilla 8 cm long, flowers conical, ovoid and acute, 3 mm long; involucre not stalked; involucre cup shaped, veined; fruiting perianth explanate. Fruit 1.8 cm, ovoid; scales in 17 vertical rows, brown with dark brown border, slightly channeled along the middle; endosperm homogenous.

*Distribution:* In evergreen forests of the Western Ghats between 1000 - 1500 m altitude.

*Phenology:* Flowering: October – December; Fruiting: March - May.

*Uses:* Excellent small diameter cane, extensively used in furniture industry.

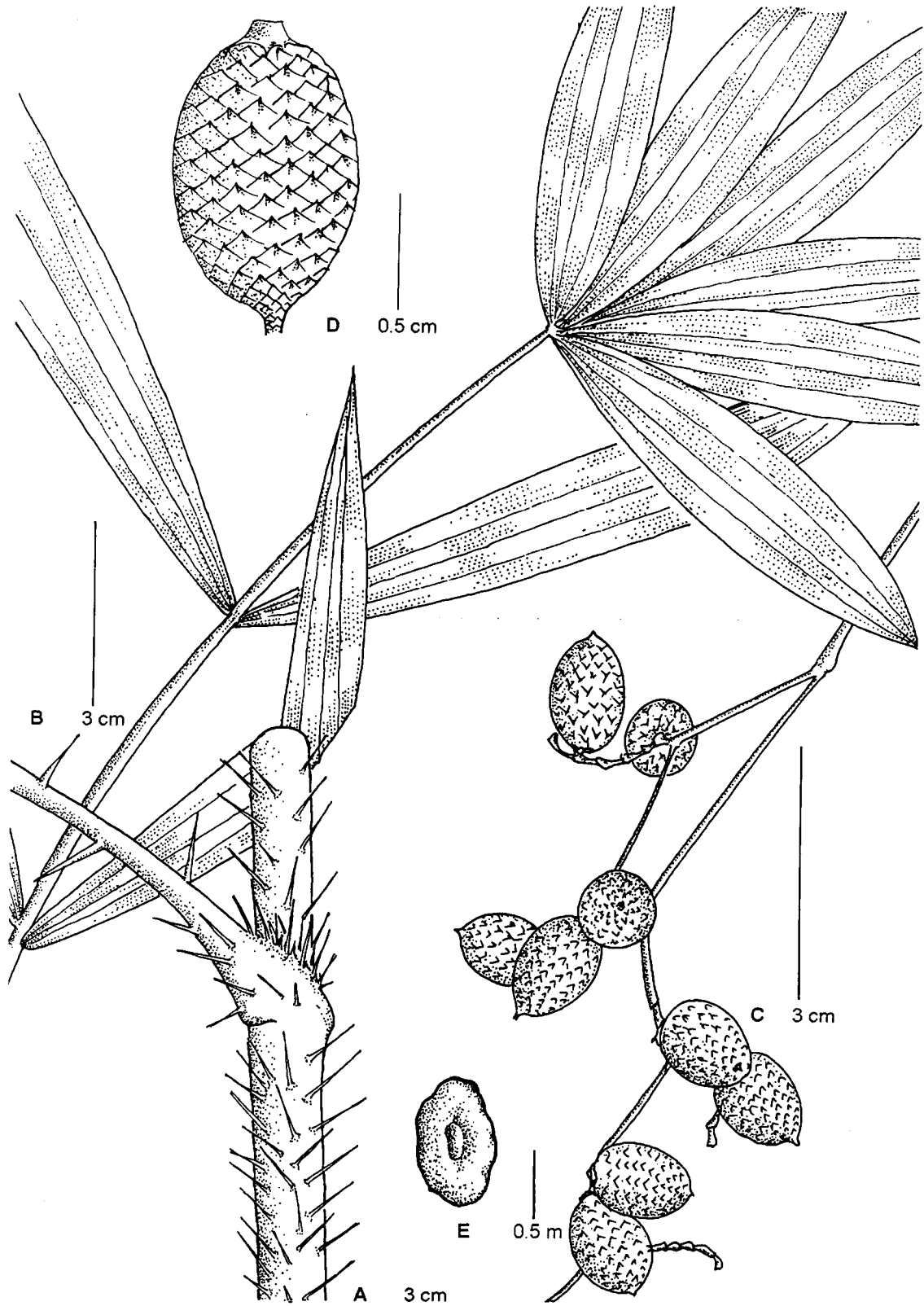


Figure 6.8. *Calamus brandisii* Becc. A. leaf sheath; B. a portion of leaf; C. a partial inflorescence; D. fruit; E. seed



A



B



C



D

Plate 6.4. *Calamus stoloniferus* Renuka: **A.** Leaf sheath; **B.** Inflorescence  
*Calamus brandisii* Becc.: **C.** Leaf sheath; **D.** Inflorescence

29

64 B

*Conservation status:* Vulnerable.

*Specimens examined:* KERALA: Trivandrum Dist., Bonnacaud, 1987, *Nair 6144* (KFRI); Bonnacaud, 1990, *S. M. Kumar 10396* (TBGT); Palakkad Dist., Kalakkadu, 1990, *Renuka 6616* (KFRI); Idukki Dist., Mankulam, 2003, *Sreekumar 24260* (KFRI). TAMILNADU: Kattabomman Dist., Courtallum, 1882, *Brandis 2340* (CAL); Courtallum, 1988, *Renuka 5870* (KFRI); Sengaltheri, 1983, *Vajravelu 80650* (MH); Courtallum, 1988, *Renuka 3817* (KFRI).

***Calamus travancoricus*** Bedd. ex Becc. et Hook. f. in Fl. Brit. India 6: 452. 1892; Becc., in Ann. Roy. Bot. Gard. Calcutta 11: 95, 310. 1908; Fischer in Gamble, Fl. Pres. Madras 3: 1567. 1931; Renuka, Rattans of Western Ghats 53, t. 23. 1992; Basu, Rattans in India 122. 1992. [Figure 6.9; Plate 6.5]

*Type:* India, Kerala, Travancore, *Beddome 291* (holotype: K. Cibachrome!)

*C. gracilis* (non. Roxb) Griff., Palms. Brit. India. 64: 1844.

*Vernacular names:* Arichural (Malayalam); Kiribetha (Kannada).

Clustering, small diameter rattan. Stem to 15 m long or more, with sheaths to 0.8 cm in diameter, without sheaths to 0.4 cm in diameter; internodes to 22 cm long. Leaf to 45 cm long, ecirrate; sheath green, spiny, spines to 0.5 cm long, mouth of the sheath with longer spines to 1 cm long; knee small; ochrea short, papery, obliquely truncate, 1.5 cm long; flagellum to 1.3 m long, slender, filiform; petiole to 11 cm, armed with small spines; rachis armed with claw-like spines; leaflets in groups of varying numbers, the longest to 25 x 2 cm, long acuminate, narrowly oblanceolate, attenuate at base, trinerved, margin bristly.

Male inflorescences to 1 m long; primary sheath elongate, basal one to 25 cm long, yellowish brown, edge spinulose, gradually becoming shorter upwards, bursting longitudinally to form a laminar structure, membranous, glabrous, only the lowest sheath provided with spinules; partial inflorescences 8 to 10 cm long; secondary sheaths to 15 cm long, tightly sheathing, smooth, subtending branches very thin; rachillae very small, to 1 cm long, with 4 to 5 flowers, upper rachillae smaller; male flowers oblong, cylindraceous, obsoletely trigonous, obtuse, 3.5 mm long. Female inflorescence to 40 cm long; primary sheath elongate, tubular, to 16 cm long; tightly sheathing basally, splitting open distally to a laminal structure, glabrous; partial inflorescences 3 to 4, 12 cm long; secondary sheaths elongate, infundibuliform, tightly sheathing, acute or acuminate, slender upwards; rachillae to 2.5 cm long; female flowers ovoid, 2.5 mm, involucre slightly concave; calyciform, fruiting perianth pedicelliform. Fruit 1 cm across, globose, scales in 24 rows, straw yellow with a dark brown border; endosperm ruminant.

*Distribution:* Evergreen forests from 200 to 500 m in Western Ghats and Eastern Ghats.

*Phenology:* Flowering: October – November; Fruiting: May- June.

*Uses:* A very good small diameter cane; used in handicrafts and furniture industry.

*Conservation status:* Endangered.

*Specimens examined:* KERALA: Kollam Dist., Arienkavu, 1982, *Sasidharan 1731* (KFRI); 1983, *Nambiar & Renuka 2925* (KFRI); Palaruvi, 1998, *Kiranraj, 38562* (TBGT); Vilakkumaram, Rosemala, 1999, *Sreekumar 22606* (KFRI); Rosemala, 2001, *Sreekumar 22440* (KFRI); Ernakulam Dist., Malayattoor, 1983, *Nambiar & Renuka 2949* (KFRI); Pathanamthitta Dist., Moozhiyar, Ranni, 1984, *Renuka 3154* (KFRI); Thrissur Dist.,

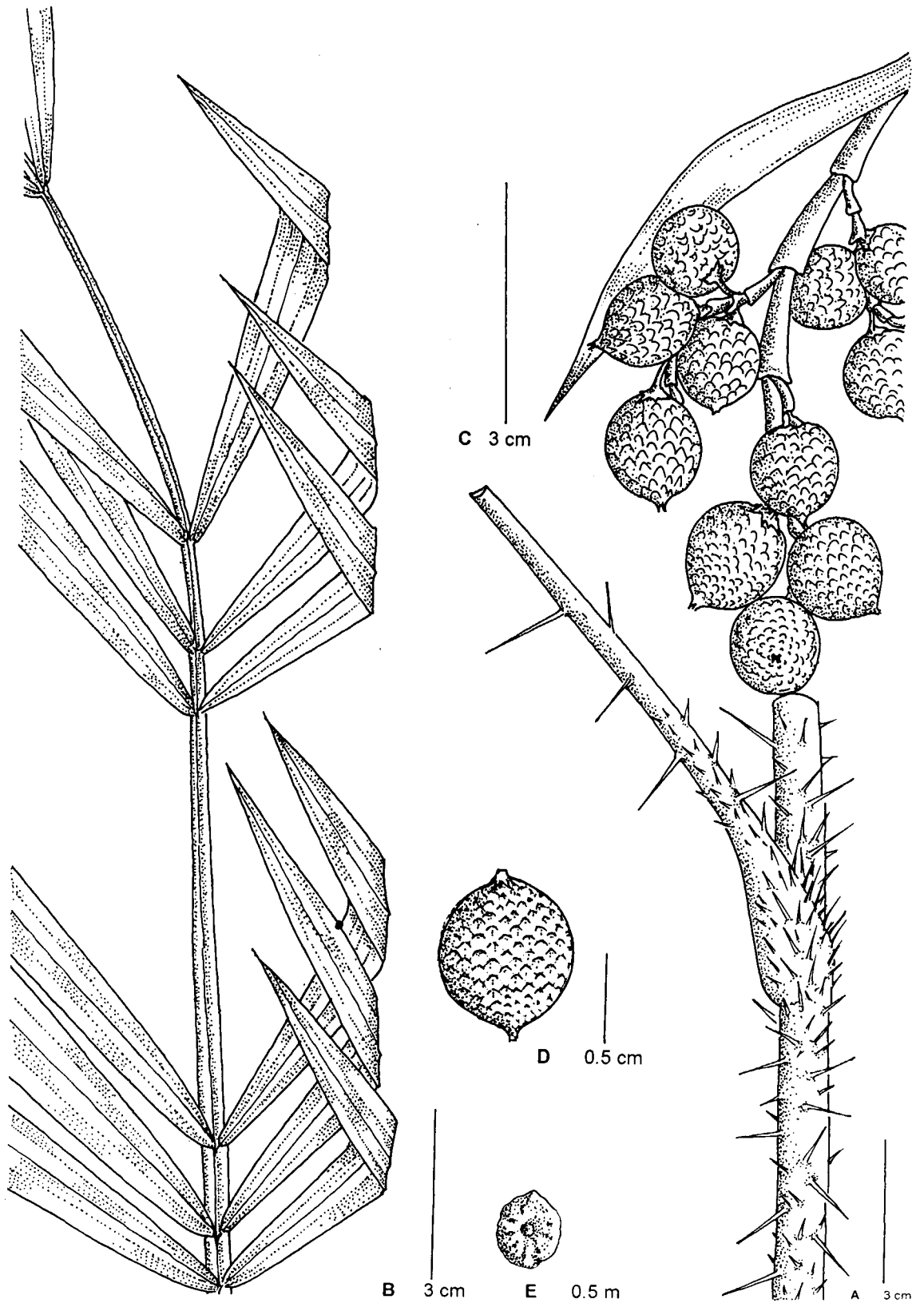


Figure 6.9. *Calamus travancoricus* Bedd. A. leaf sheath; B. a portion of leaf; C. a partial infructescence; D. fruit; E. seed

Vazhachal, 2002, *Sreekumar & Rangan 22472* (KFRI); Malappuram Dist., Nadukani, 2001, *Sreekumar & Rangan 22648* (KFRI). KARNATAKA: Kodagu Dist., Coorg, 1825, *Balwasar 52725* (MH).

**Calamus metzianus** Schlecht in *Linnaea* 26: 727. 1853; Becc. in Hook.f., *Fl. Brit. India* 6: 462. 1892; Becc., in *Ann. Roy. Bot. Gard. Calcutta* 11: 82, 221. t. 67. 1908. Renuka, *Rattans of Western Ghats* 53, t. 16. 1992 [Figure 6.10; Plate 6.5]

*Type*: India, Karnataka, Honor, *R. T. Hokanaker 4906* (holotype, FI. Cibachrome!)

*C. rudentum* Mart., *Hist. Nat. Palm.* 3: 340. 1823 – 1853.

*C. rivalis* Thw. *ex Trim. J. Bot.* 23: 268, 1885, **Syn. nov.**

Clustering, slender cane, climbing to a height of 10 to 15 m. Stem diameter with sheath 1 to 2 cm, without sheath to 1 cm. Leaf sheath green, armed with solitary, yellowish, horizontal or slightly deflexed spines up to 10 mm long; knee present; ochrea short, deciduous. Leaves 1.5 m long; petiole short or absent; rachis acutely trigonous, bifaced and naked above; leaflets 40 x 2 cm, regular, linear, acuminate to a slender apex, lanceolate, on the upper surface mid vein without spines or spinulose at the apex, ciliate beneath from centre upward, margin spinulose, upper leaflets small, bristly, terminal pair free at base, Inflorescences flagellate; male inflorescence 3 m. long; partial inflorescence 20–30 cm long, rachilla 3 cm long; female inflorescence 3 m long, partial inflorescence 70 cm long, rachillae 5 to 10 on each side, terminating in a filiform caudate apex, 8 cm long, primary spathe very long; narrow, closely sheathing, armed with numerous reflexed scattered spines, obliquely truncate at the mouth, prolonged in to a short triangular point; secondary

spathe very narrow tubular infundibuliform; 2.5 cm length, spiny and narrow at base; male flowers 4 mm long, acute, calyx veined; female flowers 3 mm long, ovate; involucre cupular, unequal margin; fruiting perianth not pedicelliform. Fruit very broadly ovoid, 7 to 8 mm broad and 11 mm long; scales on 21 vertical rows, very faintly channeled, straw yellow, violet when ripe, margin erosely toothed; seed ovate, 9 mm broad and 5 mm thick. Endosperm not ruminant; embryo basal.

*Distribution:* Restricted to plains along the backwaters and coasts and in sacred groves of Kerala and Karnataka in India and Sri Lanka.

*Phenology:* Flowering: September – October; Fruiting: March – April.

*Uses:* Mainly used in handicraft industry and for basket making.

*Conservation status:* Near threatened.

*Specimens examined:* INDIA, KERALA: Malappuram Dist., Pattakkarimbu, Nilambur, 1988, Babu 4081, 4082, 4083 & 4084 (KFRI); Nilambur, Perrie 49362, 49363, 49364, 49365 & 49366 (CAL); Nilambur, Thalaumkolli, 1984, Renuka 3061, 4031, 4032 & 4033 (KFRI); Nilambur, Thalaumkolli, 1999, Sreekumar, 8448 (KFRI); Nilambur, Pattakarimba, 2002, Sreekumar 8450, 8451 & 8452 (KFRI). Kollam Dist., Ashramam, 1997, Renuka & Sasidharan 3443 (KFRI), Ashramam, 1999, Renuka 4004, 6221 & 6799 (KFRI), Ashramam, 2001 Sreekumar 8441 & 8447 (KFRI), Alapuzha Dist., Chertala, 1992, Renuka 5884 (KFRI); Sreekumar 8469 (KFRI); KARNATAKA: Uttara Kannada Dist., Honaver, Renuka 6013 (KFRI); Honaver, Renuka 5884 (KFRI); Honaver, Renuka 7555 (KFRI); Honaver, 2000, Anto & Sreekumar 22401 (KFRI); Honaver, 2002, Sreekumar 8444 (KFRI); SRI LANKA: Watারেක, Neela De Zoysa 6814 (KFRI); Ediriweera 8445

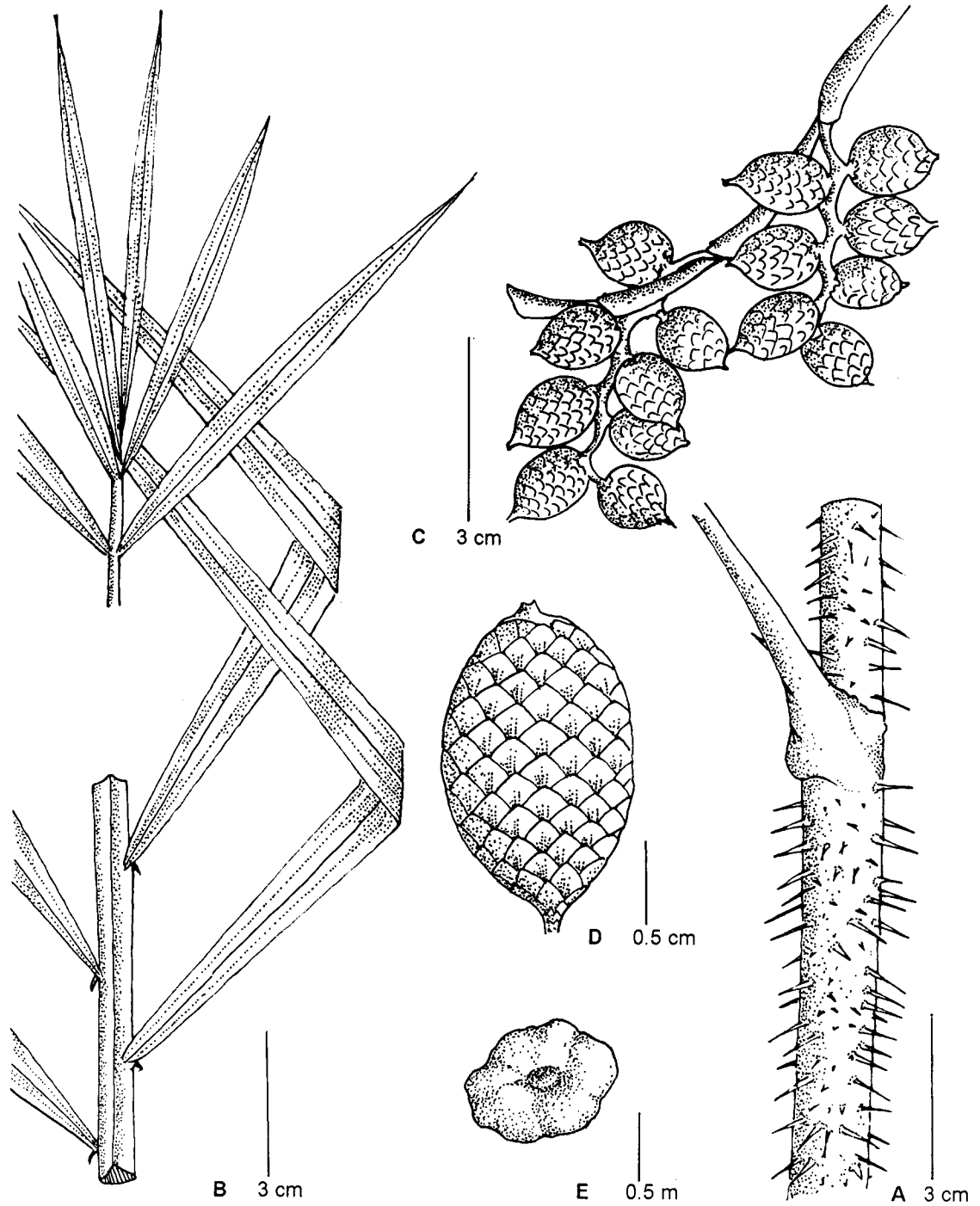


Figure 6.10. *Calamus metzianus* Schlecht A. leaf sheath, B. a portion of leaf, C. a partial infructescence; D. fruit; E. seed



Plate 6.5. *Calamus travancoricus* Bedd.: **A.** Leaf sheath; **B.** Infructescence  
*Calamus metzianus* Schlecht: **C.** Leaf sheath; **D.** Infructescence

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8813

(KFRI); Matugama, *Ediriweera 8444* (KFRI); Yagirala, *Ediriweera 8442 & 8443* (KFRI); Walakanda, *Neela De Zoysa 6814* (KFRI); Ratnapura, *Neela De Zoysa 6816* (KFRI).

**Calamus rotang** Linn., Sp. Pl. 1: 323. 1753. Mart., Hist. Nat. Palm. 3: 334. t. 122, f. 12. 1823-1853; Bl. in Rumphia 3: 33. 1849; Becc. in Hook. f., Fl. Brit. India 6: 447. 1892; Becc. Ann. Roy Bot. Gard. Calcutta 11: 98, 269. 1908; Fischer in Gamble, Fl. Pres. Madras 3: 1568. 1931. [Figure 6.11; Plate 6.6]

*Type*: Sri Lanka, Yala, *Thawites 3388* (holotype: K. Cibachrome!).

*C. roxburghii* Griff. in Cal. J. Nat. Hist. 43. 1844.

*C. monoecus* Roxb. Fl. Ind. 783. 1832; Mart. Hist. Nat. Palm. 209. 1837-1853.

*Vernacular name*: Sothu perambu (Tamil)

Slender clustering rattan, climbing to a height of 15 m. Stem diameter with sheath 0.8 to 1.6 cm. Leaf sheath green, flagelliferous, gibbous above, glabrous, armed with spines, scattered spines; spines solitary, horizontal or slightly ascendent, with a broad base and a blackish point, some 10 to 15 mm long, others small and tuberculiform; knee present; ochrea short, truncate, brown, glabrous, unarmed. Leaves ecirrate, 40 to 80 cm long; petiole very short, obsolete; leaflets 30 x 2 cm; leaflets numerous, equidistant, alternate, narrowly lanceolate, papyraceous, regular equidistant, alternate or subopposite, narrowly lanceolate, base attenuate, acuminate at the apex, margin spinulose, median vein with robust spinules at the base. Inflorescence flagellate, 1 to 3 m. long; partial inflorescence arising from inside their own sheath, larger ones 15 to 20 cm long; primary sheath cylindraceous, closely sheathing, truncate at mouth; secondary sheath narrowly tubular;

infundibuliform, glabrous, finely straitely veined, obliquely truncate at mouth, apiculate at one side, involucrophorum sub cupular, sessile; involucre cupular, irregular and obseletely toothed margin; rachilla 3 to 5 cm long, 5 to 8 on either side with a terminal one; fruiting perianth distinctly pedicelled. Male flowers distichous, subtrigonus, ovate, acute, 3.5 mm long; female flowers small, ovoid, 3 mm long; Fruit globose or slightly longer than broad, minutely apiculate, 13-15 x 12-13 mm; scales on 21 vertical rows, faintly channeled in the middle, straw yellow with pale margins, erosely toothed; seed compressed, irregularly pitted; endosperm homogenous; embryo basal.

*Distribution:* Restricted to plains and coastal areas in Tamil Nadu and Andhra Pradesh in India and Sri Lanka.

*Phenology:* Flowering: September – October; Fruiting: March – April

*Conservation status:* Near threatened

*Uses:* Used for making handicrafts and furniture. Fruits are edible.

*Specimens examined:* INDIA, TAMILNADU: Trichy Dist., Mukkombu, 1999, *Sreekumar* 22432 (KFRI); Trichy, 2000, *Sreekumar* 22409 (KFRI); ANDHRA PRADESH: Sriharikotta, undated, *Gamble* 12821 (MH); *Gamble* 73178 (MH); Kurnool Dist., Krishna, undated, *Ellis* 25527 (MH); Thirupathi Dist., Kambakkam Hills, undated, *Ellis* 1374 (MH); SRI LANKA: Kegalle Dist., Matugama, 1992, *Neela De Zoysa* 82 (KFRI); Matugama, 1997, *Hellige & Pinnapola* 6815 & 6816 (KFRI).

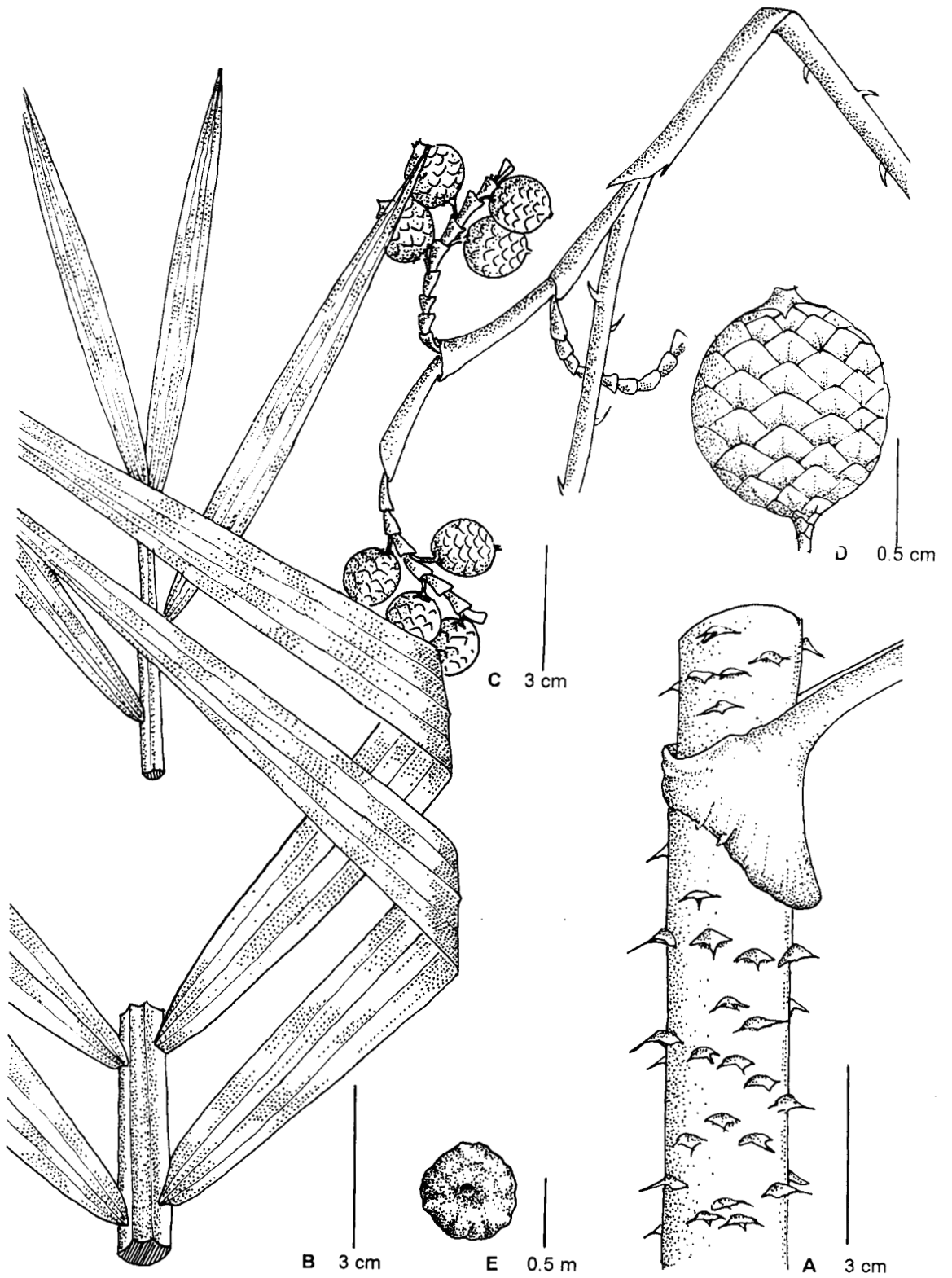


Figure 6.11. *Calamus rotang* L. A. leaf sheath; B. a portion of leaf; C. a partial inflorescence; D. fruit; E. seed

4/0/15

#### Group 4 - *C. huegelianus* clade

*Calamus huegelianus* Mart., Hist. Nat. Palm. 3: 330. t. 112: F. 3. 1853; Becc. in Hook. f., Fl. Brit. India 6: 452. 1892; Becc., Ann. Roy. Bot. Gard. Calcutta 11: 314. t. 122. 1908; Fischer in Gamble, Fl. Pres. Madras 3: 1568.1931; Basu, Rattans in India 96. 1992. Renuka, Rattans of Western Ghats 33. 1992. [Figure 6.12; Plate 6.6]

*Type:* India, Kerala, Sispara, Silent Valley National Park, 1850, *Martius 2760* (isotype: K. Cibachrome!).

Clustering, medium diameter rattan. Stem to 30 m long or more, with sheaths to 3 cm in diameter, without sheaths to 2 cm. Leaf to 2 m long, ecirrate; sheath brownish green, flagelliform, densely covered with grayish brown tomentum, abundant in younger parts, gibbous above, densely armed with spines, spines solitary straight or subseriate, the largest 10 to 15 mm long interspersed with abundant brown tomentum; knee present; ochrea very short, coriaceous, truncate. Leaf to 2 m long, ecirrate; petiole to 30 cm long, green when dry, convex on both sides, underneath armed, margin acute, armed with spines, spines similar to those of the sheaths, gradually transformed into hooked spines upwards; rachis armed below with hooked spines; leaflets ca 50 x 2.5 cm, upper leaflets smaller, regular, long acuminate, narrowly lanceolate, equidistant, ensiform, paler beneath, tri-nerved, midvein acute, prominent, the terminal pair less acuminate, free at the base. Inflorescences long, flagellate; primary sheath closely sheathing, armed with small straight spines; partial inflorescences erect, the largest 30 to 40 cm long, rather densely paniced; secondary sheaths tubular infundibuliform, obliquely truncate, ciliate at the mouth, prolonged in to a triangular point; rachillae 5 to 7 pairs, distichous, rigid, arched, inserted just at the mouth of

the spathes, with a distinct axillary callus; male flowers large, oblong, 5 mm; female flowers 6 mm, ovoid; involucrophorum more or less distinctly pedicelliform; involucre slightly concave. Fruit 15 to 18 mm across, almost spherical, quite black, contracted in to a short beak, scales in 21 rows shiny, not channeled in the middle. Seed globular; endosperm ruminant; embryo basal.

*Distribution:* Evergreen forests between 1300-2000 m. Sispara in the Nilgiri mountains, Conoor, Marapalem and Naduvettum in Tamilnadu and Kodagu and Chikmagalur in Karnataka States.

*Phenology:* March - April; Fruiting: October – November.

*Uses:* Reported to be a good quality cane.

*Conservation status:* Critically endangered.

*Specimens examined:* KERALA: Palakkad Dist., Sispara, Silent Valley National Park, 1997, *Anto 7092* (KFRI); Sispara, Silent Valley National Park, 2001, *Sreekumar and Rangan 22442* (KFRI); Kottayam Dist., Kottayam, 1966, *Shetty 61259* (MH); TAMIL NADU: Conoor Dist., conoor, 1883, *Gamble 12355* (CAL); Nilgiri Dist., Marapalem, 1972, *Sharma 40352* (MH); Naduvettam, 1886, *Gamble 73184* (MH); KARNATAKA: Kodagu Dist., Solle Kolli, 1983, *Keshava Murthy & Simhan 3337* (RRCBI); Chikmagalur Dist., Mallam 1984, *Vajravelu 140644* (MH).

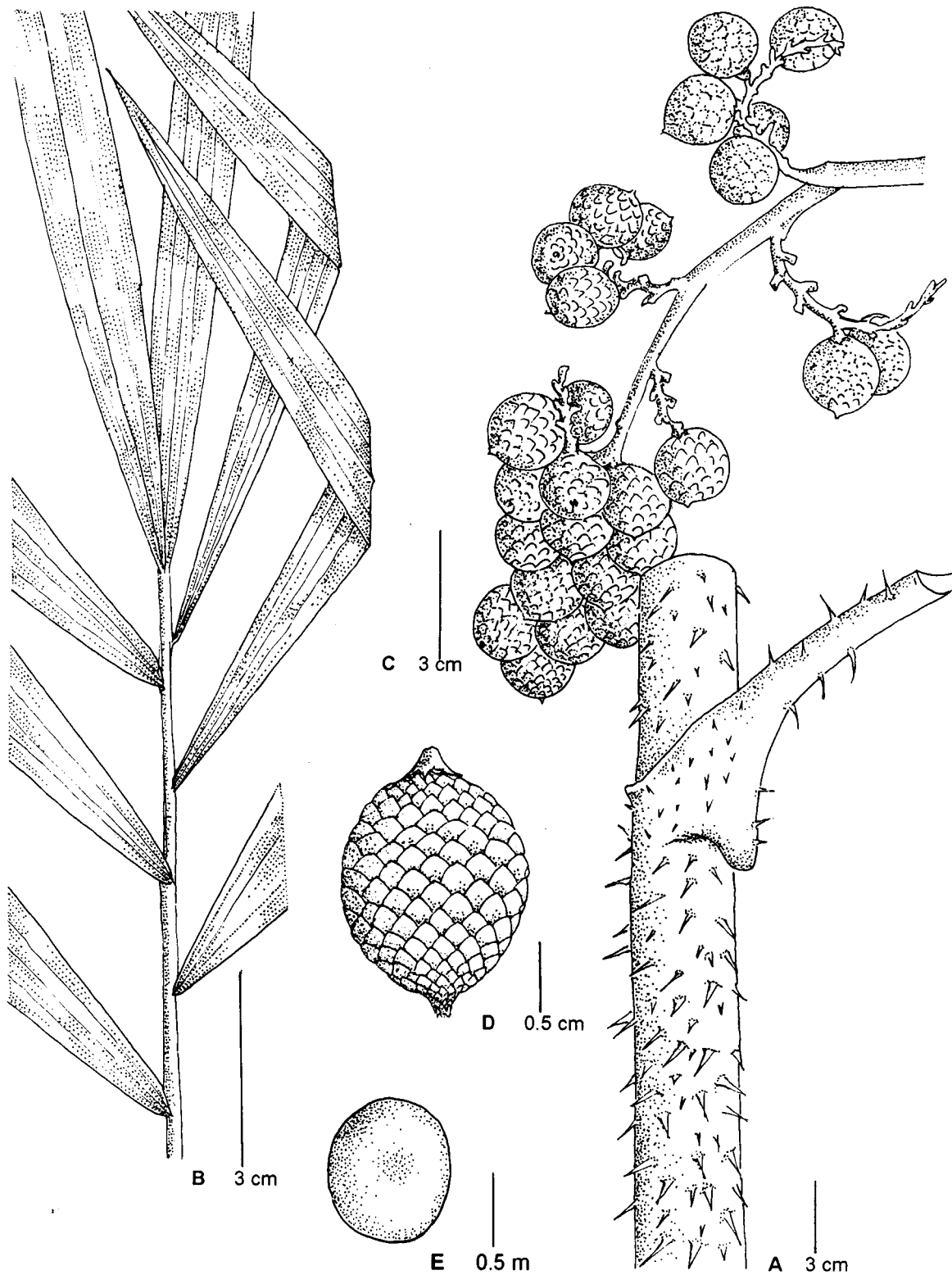


Figure 6.12. *Calamus huegelianus* Mart. A. leaf sheath; B. a portion of leaf; C. a partial infructescence; D. fruit; E. seed



Plate 6.6. *Calamus rotang* L.: **A.** Leaf sheath; **B.** Infructescence  
*Calamus huegelianus* Mart.: **C.** Leaf sheath; **D.** Infructescence

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### Group 5 - *C. gamblei* clade

*Calamus gamblei* Becc. in Hook. f., Fl. Brit. India 6: 493.1892; Becc., Ann. Roy. Bot. Gard. Calcutta 11: 316. t. 123. 1908; Fischer in Gamble, Fl. Pres. Madras 3: 1093. 1931; Basu, Rattans in India 98.1992; Renuka, Rattans of Western Ghats 26, t. 10.1992. [Figure 6.13; Plate 6.7]

*Type:* India, Karnataka, Makurti forests, *Gamble 14405* (isotype K. Cibachrome!)

*Vernacular names:* Pachachural, Ottamoodan (Malayalam); Hasirubetha (Kannada); Othaiyadi perambu (Tamil).

Clustering, medium diameter rattan, Stem 25 to 30 m long, with sheaths 2.5 cm in diameter, without sheaths 1.5 cm; internodes 30 to 35 cm long. Sheath green, armed with spines, spines 0.5 to 1.5 cm long, horizontally held, bulbous based, tip slightly pointing upwards; knee present; ochrea not well developed; flagellum 3 to 4 m long. Leaves ecirrate, to 1.2 m long; petioles to 25 cm long, armed with spines, spines 1.5 to 2 cm long; rachis bifaced, above, rounded beneath, armed with hooked spines; leaflets regular, 40 x 2.5 cm, long narrowly lanceolate, ensiform, tips armed with short bristles, three veined, veins ciliate on both surfaces. Inflorescence flagellate, 2.5 to 3 m long; partial inflorescences 5-6, 90 cm long, arising well above the mouth of the sheath; male inflorescence with branches of 3 orders; primary sheath tightly sheathing, distally produced into a lanceolate point, armed with small spines, spines 0.5 cm long; secondary sheaths tight, narrow at base, expanded distally; male rachillae 1.5 to 2 cm long; male flowers ovoid, 4 mm long; female inflorescence with branches of 2 orders; primary sheath tight, provided with prickles; partial inflorescence 80 to 90 cm long; secondary sheaths 2 cm long, unarmed, cylindrical

at the base and slightly expanded distally, closely sheathing; rachillae 6 to 8 cm long, the upper ones shorter, arched and recurved, base ascendent distinctly callous at their insertion; female flowers 5 mm long, ovate, arranged in two series, pointing upwards; involucre distinctly pedicelliform; involucre disc-shaped. Fruit, 2 cm long, spherical or slightly tapering at the base, short stalked, scales in 23 rows, pale yellow, shiny, deeply channeled; seed globose, smooth; endosperm ruminant; basal embryo.

*Distribution:* Evergreen forests above 700 m in Southern Western Ghats and Eastern Ghats and rarely seen at lower altitudes.

*Phenology:* Flowering: December – January; Fruiting: May - June.

*Uses:* Used in handicraft industry and for basket making.

*Conservation status:* Lower risk.

*Specimens examined:* KERALA: Kollam Dist., Arienkavu, 1962, *Henry A N 93049* (MH); Wayanad Dist., Chandanathodu, 1984, *Renuka & Nambiar 3028* (KFRI); Thirunelli, 2001, *Sreekumar 22611* (KFRI). Pathanamthitta Dist., Ranni, 1984, *Renuka 3173* (KFRI); Pampa, 2001, *Sreekumar 22439* (KFRI); Kudremukh, 1984, *Vajravelu 77850* (MH); Idukki Dist., Pannithavarnachola, 1984, *Renuka 3401* (KFRI); Palakkad Dist., Attappady, 1985, *Renuka 3473* (KFRI); Silent Valley National Park, 1985, *Renuka 3474* (KFRI); Trivandrum Dist., Arippa, 1985, *Renuka 3433* (KFRI); Kadamankunnu, 1986, *Mukundakumar 0518* (TBGT); TAMIL NADU: Madura Dist., Madurai, 1975, *Jacob 17684* (MH); Kanyakumari Dist., Kilaviarumalai, 1976, *Henry 48117* (MH). KARNATAKA: Chickmangalur Dist., Mallam, 1984, *Vajravelu 140643* (MH).

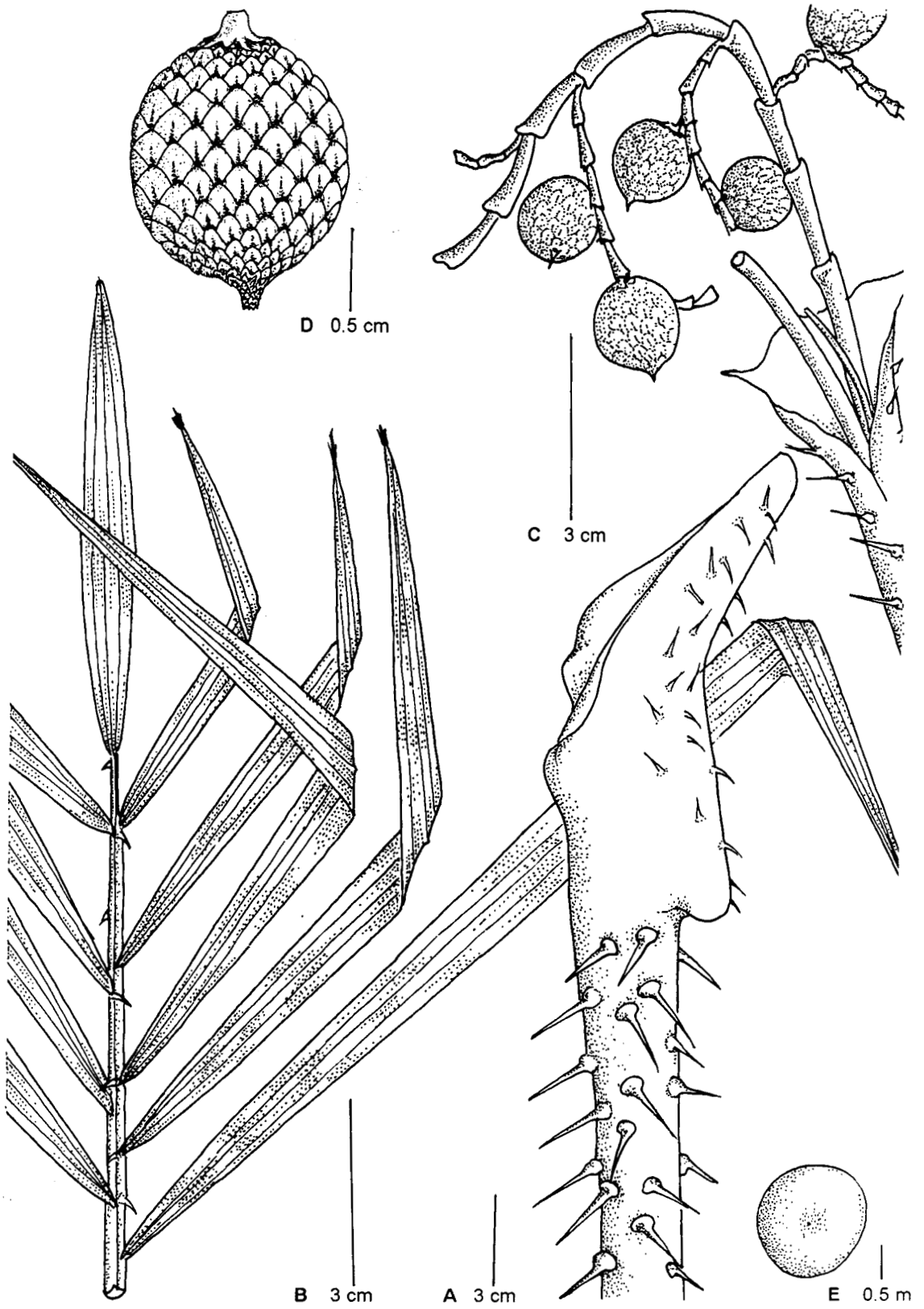


Figure 6.13. *Calamus gamblei* Becc. A. leaf sheath; B. a portion of leaf; C. a partial inflorescence; D. fruit; E. seed

**Group 6 - *C. shendurunii* clade**

***Calamus shendurunii*** Anto, Renuka *et* Sreekumar, *Rheedea* 11: 1, 2001. [Figure 6.14; Plate 6.7]

*Type:* India, Kerala, Kollam, Shenduruni Valley, Evergreen forest, *Anto 006680* (holotype: KFRI!).

Clustering, medium sized rattan, stem reaching to 20 m, with sheath up to 2 cm diameter, without sheath up to 1.5 cm diameter; internodes up to 25 cm long. Sheath dark green, shining, spiny; spines very few, 1-4 scattered with bulbous base, tip black, 1 cm long; knee conspicuous, devoid of spines; flagellum up to 2 m long; leaves ecirrate, to 1 m long; petiole 25 cm long, armed on the dorsal side; rachis channeled on the dorsal side, ridges spiny, armed on the ventral side with recurved spines; spines 1 cm long, sturdy, bulbous based; leaflets long acuminate, regular, 35 x 3 cm; midvein setose ventrally, setae up to 1 cm long, lateral and mid veins setose dorsally towards the tip, setae up to 0.5 cm long, apical leaflets basally united. Inflorescence flagellate, 2 cm long; partial inflorescence 3 to 4, up to 20 cm long, slender; primary sheath closely sheathing, mouth of the leafsheath with bristles; secondary sheath sparingly spiny; rachillae to 5 cm long. Fruit globose or obpyriform, 1 x 1.8 cm, with stalk like involucrophores, stalk to 2 mm long, the pedicel of the neuter flower persistent, represented by a small projecting tuberculae, scales in 25 vertical rows, pale green, without any border, shiny, shallowly channeled along the middle; endosperm ruminant.

*Distribution:* This species is endemic to Shenduruni Wildlife Sanctuary, Kollam.

*Phenology:* Flowering: June-August; Fruiting: March-April.

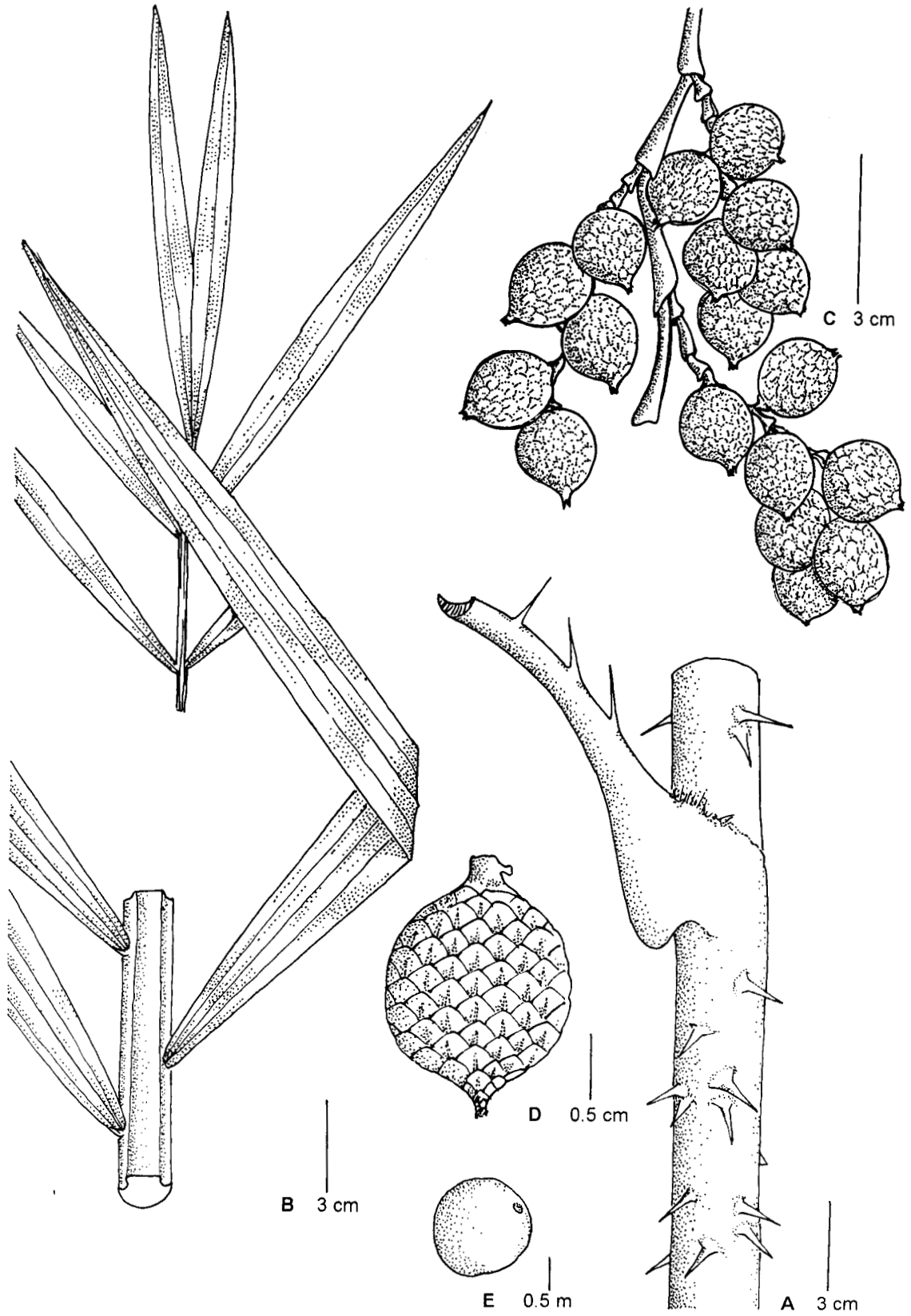


Figure 6.14. *Calamus shendurunii* Anto, Renuka et Sreekumar A. leaf sheath; B. a portion of leaf; C. a partial inflorescence; D. fruit; E. seed



Plate 6.7. *Calamus gamblei* Becc.: **A.** Leaf sheath; **B.** Infructescence  
*Calamus shendurunii* Anto, Renuka et Sreekumar: **C.** Leaf sheath; **D.** Infructescence

*Uses:* Used in furniture industry and in basket making.

*Conservation status:* Endangered.

*Specimens examined:* KERALA: Kollam Dist., Shenduruni Wild Life Sanctuary, 1999, *Anto 006680* (KFRI); Vilakkumaram, Rosemala, Shenduruni Wild Life Sanctuary, 2000, *Sreekumar 20621* (KFRI); Rosemala, Shenduruni Wild Life Sanctuary, 2001, *Sreekumar 22654* (KFRI); Rosemala, Shenduruni Wild Life Sanctuary, 2002, *Sreekumar 22464* (KFRI).

*Calamus lacciferus* Lakshmana *et* Renuka, J. Econ. Tax. Bot. 14: 705. 1990; Renuka, Rattans of Western Ghats 35, t. 14. 1992. [Figure 6.15; Plate 6.8]

*Type:* Karnataka, Uttara Kannada, Kenganahonda, *Renuka 4078* (holotype: KFRI!).

*Vernacular names:* Neerubetha (Kannada)

Clustering, medium diameter rattans. Stem 10 to 15 m or more in length, very thin at the base, 0.5 to 0.8 cm in diameter, getting thicker upwards, distally with sheaths 3 to 3.5 cm in diameter, without sheaths 1 to 1.5 cm, basal nodes producing new shoots. Leaf 1 to 2 m long, ecirrate; sheath green in the basal portions of the plant and greyish green distally, sparingly spiny, spines 0.5 cm long, bulbous at base; knee very conspicuous, unarmed; petiole 30 to 40 cm long, biconvex, spiny; rachis with recurved spines on the under surface, when cut exudes milky latex; leaflets 65 x 2.8 cm gradually becoming smaller towards the tip, the terminal pair 20 x 1.2 cm, slightly united at the base, regular, linear – lanceolate, ciliated at tip, 3 – veined, midvein ciliated abaxially on the upper half of leaflets, lateral veins ciliate abaxially on the upper half. Inflorescences long, flagellate; flagella often

getting modified into suckers; primary sheath tightly sheathing, spiny; partial inflorescence 10 to 15 cm long; secondary sheaths tubular, inflated at the mouth; rachillae 4 to 5 cm long, arched and recurved, the upper ones gradually diminishing 1 cm long; male flowers distichous, 3 mm long; female flowers ovate, obtuse, 3 mm long; involucre stalked, conspicuously callous at its axilla next to the axis; involucre disc shaped; fruiting perianth pedicellate. Fruits 12 x 2 cm, globose, scales in 19 rows, yellow with brown border, shiny, deeply channeled in the middle; endosperm ruminant.

*Distribution:* Evergreen forests at about 950 m., usually seen near water course. Behaves like a runner. Found in Uttara Kannada, Kodagu and Shimoga forest areas of Karnataka. Recently reported from New Amarambalam Forest Reserve in Nilambur, Kerala.

*Phenology:* Flowering : November – December; Fruiting : May-June.

*Uses:* Not much used because of low quality.

*Conservation status:* Endangered.

*Specimens examined:* KERALA: Malappuram Dist., Nilambur, 1988 *Renuka 4078* (KFRI); New Amarambalam, 2000, *Anto & Sreekumar 22405* (KFRI), KARNATAKA: Uttara Kannada Dist., Kenganahonda, 1988, *Renuka 4078* (KFRI); Shimoga Dist., Agumbe, 1989, *Renuka 4096* (KFRI); Kodagu Dist., Thalakkaveri, 2000, *Anto & Padmakumar 22405* (KFRI); Thalakkaveri, 2001, *Sreekumar 22612* (KFRI).

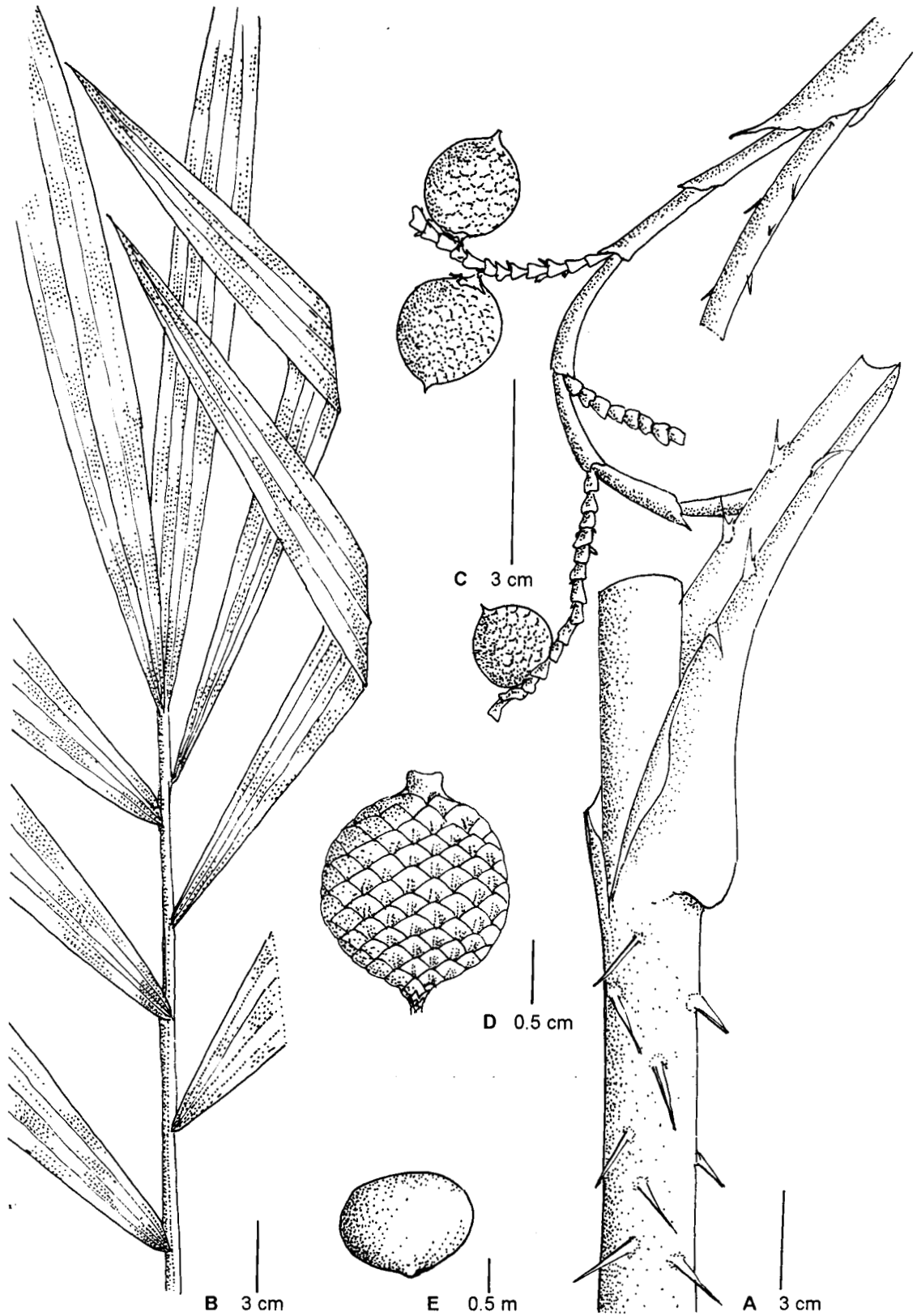


Figure 6.15. *Calamus lacciferus* Lakshmana & Renuka A. leaf sheath; B. a portion of leaf; C. a partial inflorescence; D. fruit; E. seed

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**Group 7- *C. neelagiricus* clade**

***Calamus neelagiricus*** Renuka, *Rheedea* 7: 2, 1997 [Figure 6.16; Plate 6.8].

***Type:*** India, Kerala, Silent valley, *Anto 7100* (holotype: KFRI!).

Solitary, moderate sized rattan, stem reaching up to 25 m, with sheath 2.5 –5 cm diameter, without sheath 3 cm, internodes 21 cm long. Sheath green, spiny; spines 1 cm long, sturdy with a bulbous base; knee present, ochrea inconspicuous; flagellum 3 to 4 m long. Leaf ecirrate, 2 m long; petiole 25 cm, petiole and rachis armed on the dorsal side with spines; spines 0.5 cm long, sturdy, bulbous based, ventral side spiny at the point of attachment of leaflet with the rachis; leaflets long acuminate, regular, 46 x 2 cm, midvein ciliate dorsally, lateral veins ciliate ventrally, cilia 0.3 cm, apical leaflets free. Inflorescence flagellate, 2.5 cm long, 5 to 6 partial inflorescences, heavy with profuse fruiting, pendulous; partial inflorescence 30 cm long; primary sheath closely sheathing, completely armed with sturdy spines; rachillae 10 to 13 cm long; strongly recurved and recoiled; secondary sheath closely sheathing. Fruit oblate, 1 x 0.5 cm; fruiting perianth pedicellate, scales straw yellow when dry, channeled in the middle, in 21 series; endosperm ruminant.

***Distribution:*** Endemic to evergreen forests of Silent Valley National park.

***Phenology:*** Flowering: October – November; Fruiting: May- June.

***Uses:*** A very good medium diameter cane; used in handicrafts and furniture industry.

***Conservation status:*** Critically endangered.

***Specimens examined:*** KERALA: Palakkad Dist., Silent Valley National Park, 1997, *Anto 7100* (KFRI); Silent Valley National Park, 1997, *Sasidharan & Anto 3698* (KFRI); Silent

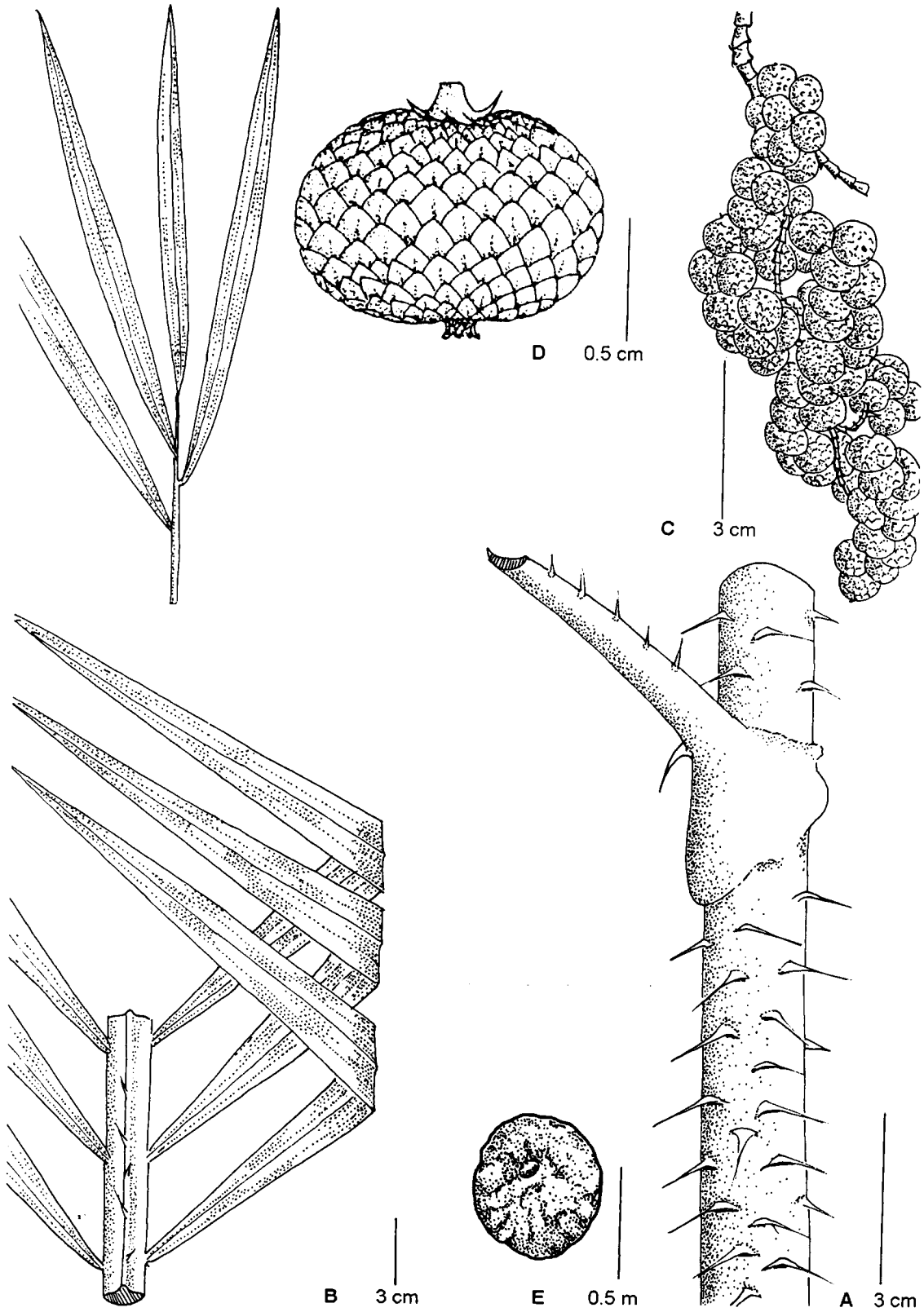


Figure 6.16. *Calamus neelagiricus* Renuka A. leaf sheath; B. a portion of leaf; C. a partial infructescence; D. fruit; E. seed

1997

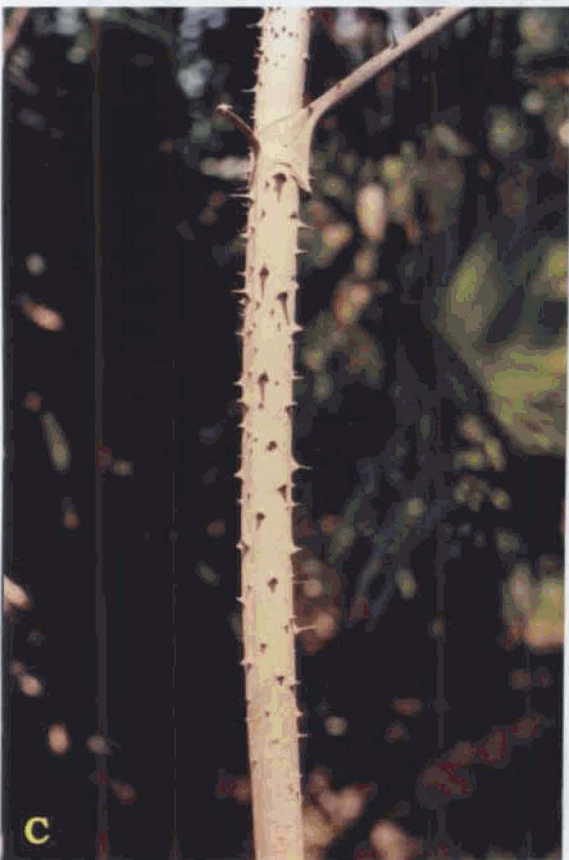


Plate 6.8. *Calamus lacciferus* Lakshmana & Renuka: **A.** Leaf sheath; **B.** Infructescence  
*Calamus neelagiricus* Renuka: **C.** Leaf sheath; **D.** Infructescence

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Valley National Park, 2001, *Sreekumar & Rangan 22441* (KFRI); Silent Valley National Park, 2002, *Rangan 22468* (KFRI).

**Group 8- *C. delessertianus* clade**

***Calamus delessertianus*** Becc., Ann. Roy. Bot. Gard. Calcutta 11: 276. t. 101. 1908; Basu, Rattans in India 92.1992. Renuka, Rattans of Western Ghats: 23.1992. [Figure 6.17; Plate 6.9]

***Type:*** India, South India, S. loc. *Roxburgh 4802* (holotype: K. Cibachrome!)

Solitary, large diameter rattan. Stem to 15 m long or more, with sheaths 4 cm in diameter, without sheaths 2.5 to 3 cm. Leaf 2 m long, ecirrate; sheath green with bulbous based spines; knee present; ochrea inconspicuous; petiole to 50 cm long; rachis upper subtrigonal, convex beneath, armed with numerous spines; spines strong, solitary, bulbous based, black tipped; leaflets about 35 cm length and 2.5 cm width, linear-lanceolate, pale green when dry, veins ciliated on both surfaces, cilia 1.5 cm long, leaf margin closely spinulose, sometimes a small spinule occurs at the base of the midvein on the upper surface. Male and female inflorescences large; primary sheath pale green, coriaceous, tubular, closely sheathing densely armed with small, broad based, straight spines; spines to 1.3 cm long; prolonged at one side to a triangular point; partial inflorescence to 40 cm long, attached inside the sheath, arising erect at first and then spreading; secondary sheath tubular infundibuliform, loosely sheathing, unarmed; rachillae 10 cm long, horizontally truncate, inserted at the mouth of the sheath, with a distinct axillary callus, rigid, arched, spreading, basal ones branched; involucrophorum almost

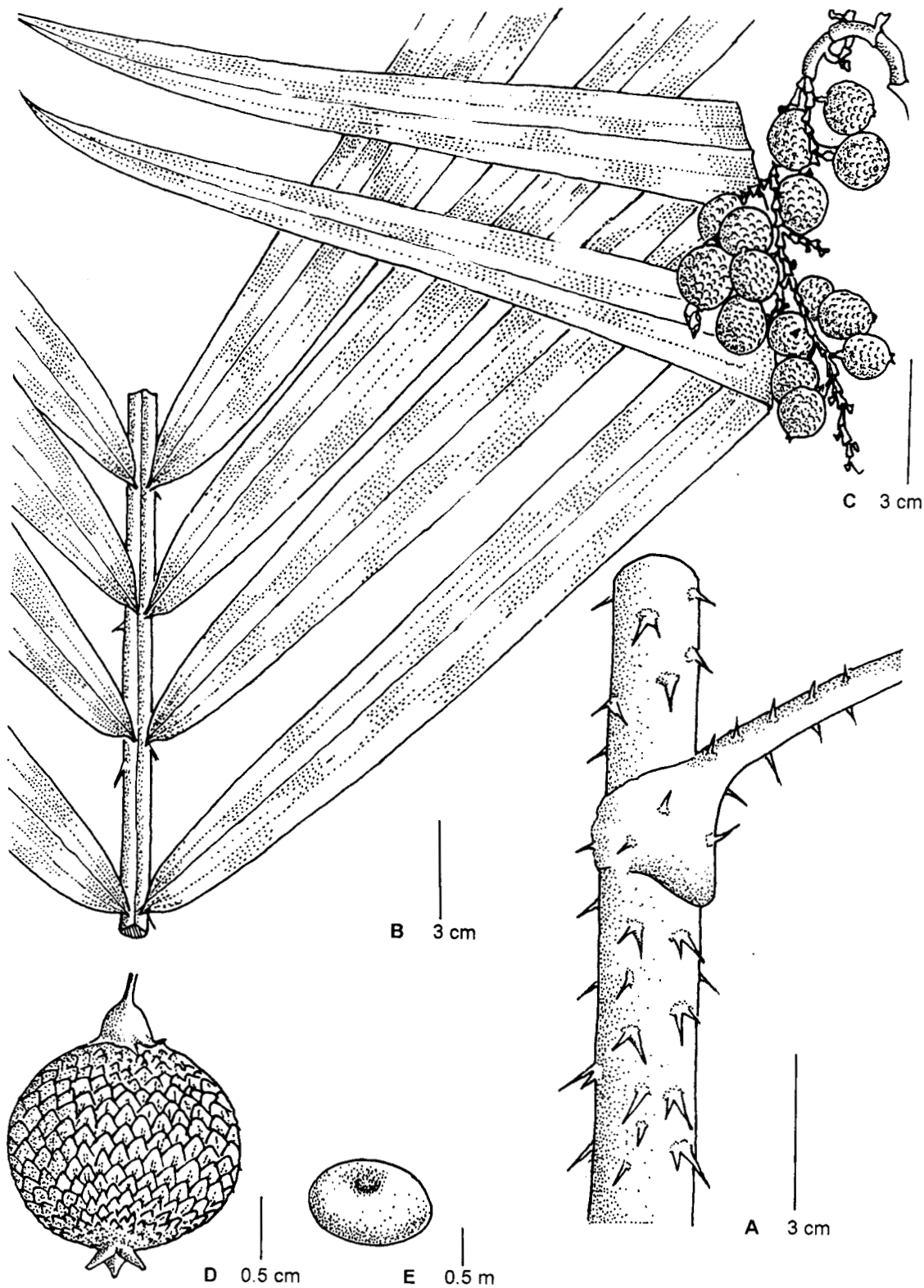


Figure 5. 17. *Calamus delessertianus* Becc. A. leaf sheath; B. a portion of leaf; C. a partial infructescence; D. fruit; E. seed

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exerted from the own sheath, to 0.8 cm long; involucre disc shaped; male flowers ovoid, 2 mm long, female flowers 3 mm long, very regularly bifarious, inserted at an angle of 45 °. Fruit globose to obovate, 1.4 cm across, scales in 28 vertical rows, light green when fresh, straw yellow when dry, deeply channeled; endosperm ruminant.

*Distribution:* In evergreen forests between 700 to 1000 m. Trivandrum and Palakkad forest areas in Kerala, Nagercoil in Tamil Nadu and Uttara Kannada in Karnataka.

*Phenology:* Flowering: August – September; Fruiting: April-May.

*Uses:* Used for making baskets, furniture etc.

*Conservation status:* Lower risk.

*Specimens examined:* KERALA: Trivandrum Dist., Theemparai, 1986, *Mukundakumar*, 0516 (TBGT); Palakkad Dist., Nelliampathy, 2002, *Rangan & Sreekumar* 22436 (KFRI); Nelliampathy, 2001, *Sreekumar* 22433 (KFRI); TAMIL NADU: Nagercoil Dist., Nariakkad estate, 1992, *Renuka* 4079 (KFRI). KARNATAKA: Uttara Kannada Dist., Moodegiri, 1992, *Renuka* 6622 & 7004 (KFRI); Subramanya, 2002, *Sreekumar* 22433 (KFRI).

*Calamus prasinus* Lakshmana *et* Renuka, J. Econ. Tax. Bot., 14: 705. 1990; Renuka, Rattans of Western Ghats 41. t. 43. 1992. [Figure 6.18; Plate 6.9]

*Type:* India, Karnataka, Sampaje Ghats, *Renuka* 4082 (KFRI!).

High climbing rattan, solitary, with sheath 3 to 4 cm, without sheath 1.5 cm; sheaths densely armed with 1 cm long spines; knee conspicuous; Leaves 2 to 2.5 m long, ecirrate; petiole 2 to 30 cm long, biconvex, armed with short spines, spines underneath recurved,

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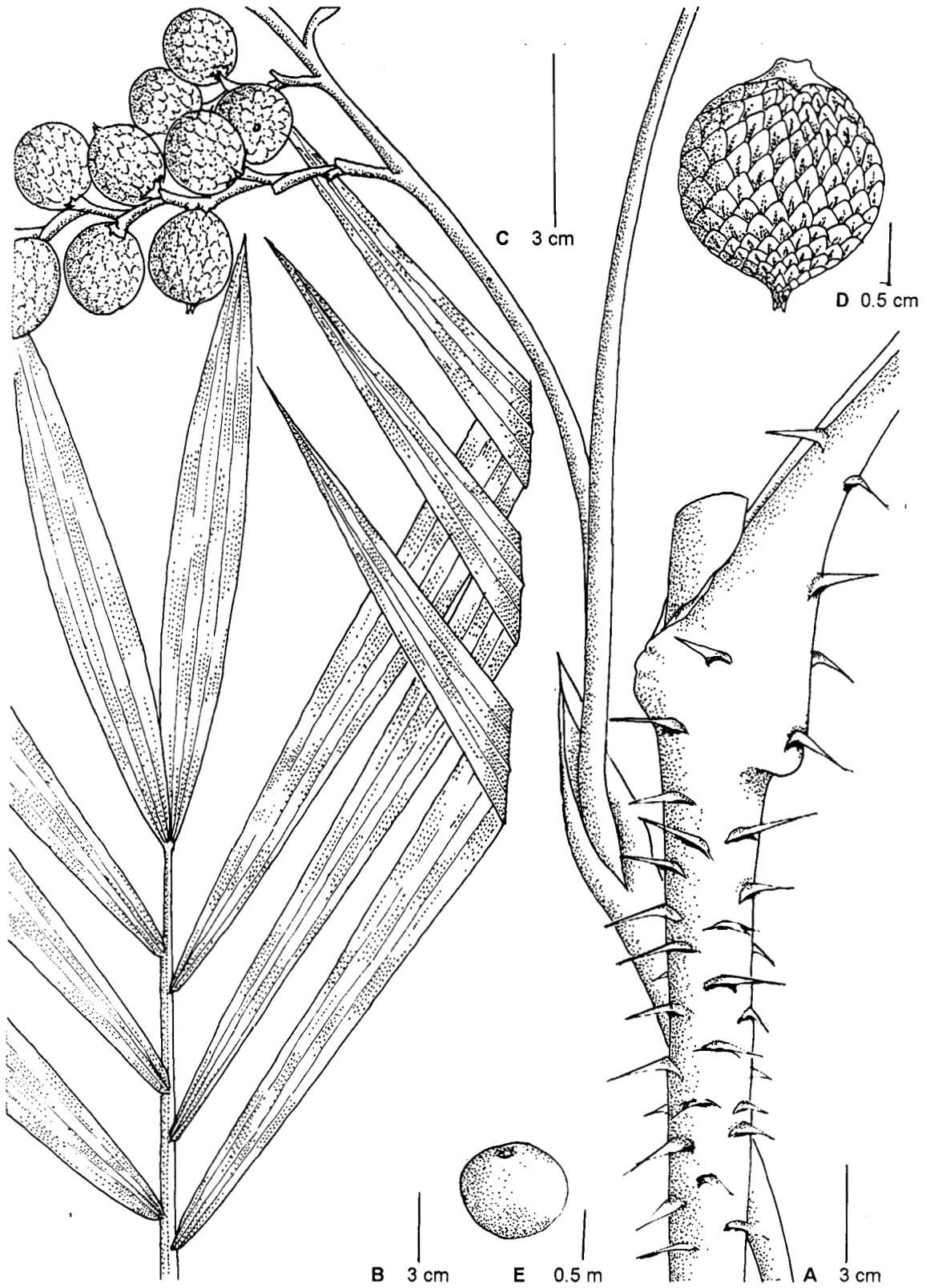


Figure 6.18. *Calamus prasinus* Lakshmana & Renuka A. leaf sheath; B. a portion of leaf; C. a partial infructescence; D. fruit; E. seed

1504



Plate 6.9. *Calamus delessertianus* Becc.: A. Leaf sheath; B. Infructescence  
*Calamus prasinus* Lakshmana & Renuka: C. Leaf sheath; D. Infructescence

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rachis triangular, armed with recurved spines below; petiole and rachis exudates a milky latex; leaflets 50 cm x 2.5 cm, linear, lanceolate, three nerved, veins ciliate in the upper half, lateral veins ciliate above, terminal pair fused basally. Inflorescences elongate, drooping; primary sheath tightly sheathing, two keeled with lacerate spiny tip; partial inflorescence 10 to 18 cm long; secondary sheath unarmed, tightly sheathing, tubular, rachillae 5 cm long; male flowers 4 mm long, bifarious, ovate; female flowers 5 mm long; involucre with stalk, 2 to 3 mm long, borne on above the mouth of the sheath; involucre disciform. Fruits globose, with 26 rows of scales, scales yellow, deeply channeled along the middle; endosperm ruminant.

*Distribution:* Evergreen forests at about 500 m, frequently found in Sampaje, Karike, Thalakkaveri and Magunde forest areas.

*Phenology:* Flowering: November - December; Fruiting: May - June.

*Uses:* Used for furniture industry.

*Conservation status:* Near threatened.

*Specimens examined:* KARNATAKA: Dakshina Kannada Dist., Sampaje Ghats, 1988, *Renuka 4082* (KFRI); Magunde, 1989, *Renuka 4092* (KFRI); Karike, 2001, *Sreekumar 22430* (KFRI), Kodagu Dist., Thalakkaveri, 2001, *Sreekumar 22661* (KFRI).

*Calamus dransfieldii* Renuka, Kew Bull. 42: 433, 1987; Basu, Rattans in India 109. 1992; Renuka, Rattans of Western Ghats 26. t. 10. 1992. [Figure 6.19; Plate 6.10]

*Type:* Palakkad District, Dhoni hills, *Renuka 2982* (holotype: KFRI!)



Solitary, large diameter rattan. Stem 10 m long, with sheaths 3 to 3.5 cm in diameter, without sheaths 2.5 cm; internodes 25 to 30 cm. Leaves 2 m long, ecirrate; sheath pale green, sparingly spiny; spines 1 cm long, bulbous based; knee conspicuous; ochrea absent; flagellum 6 m long; petiole 55 cm, spiny, elongate, spines 6 mm long; bulbous based; rachis armed with distant row of very small spines; leaflets ca 45 x 2 cm, narrowly lanceolate, regularly arranged, long-acuminate, pale green; veins ciliate on both surfaces, cilia 2 cm long. Inflorescences 3.5 m long, flagellate, axis 1.5 cm in diameter; partial inflorescences 4 to 5; primary sheath cylindrical; basal sheath smaller in younger plants, spines 1 to 1.3 cm long, black, bulbous-based; partial inflorescence of male 9 cm long, very slender; rachillae 2 cm long, smaller upwards; staminate flowers 8 mm long; partial inflorescence of female 25 cm long, attached at the mouth of the sheath; female flowers 6 mm long, ovoid, calyx veined, glabrous; secondary sheaths cylindrical at base, funnel shaped distally; rachillae attached above the mouth of its own sheath; involucrophorum stalked, 7 mm long; involucre disc shaped; fruiting perianth callous at the base. Fruit globose, 1.5 cm diameter, 27 vertical rows of scales, deeply channeled in the middle, shiny yellow; endosperm ruminant.

*Distribution:* Evergreen forests at 300 m and endemic to Dhoni forests in Kerala.

*Phenology:* Flowering: November – December; Fruiting : January – March.

*Uses:* The cane is of good quality, suitable for furniture industry.

*Conservation status:* Critically endangered.

*Specimens examined:* KERALA: Palakkad Dist., Dhoni forests, 1983, *Renuka* 2982, 2983 (KFRI); 1987, *Renuka* 4030, 4031 (KFRI).

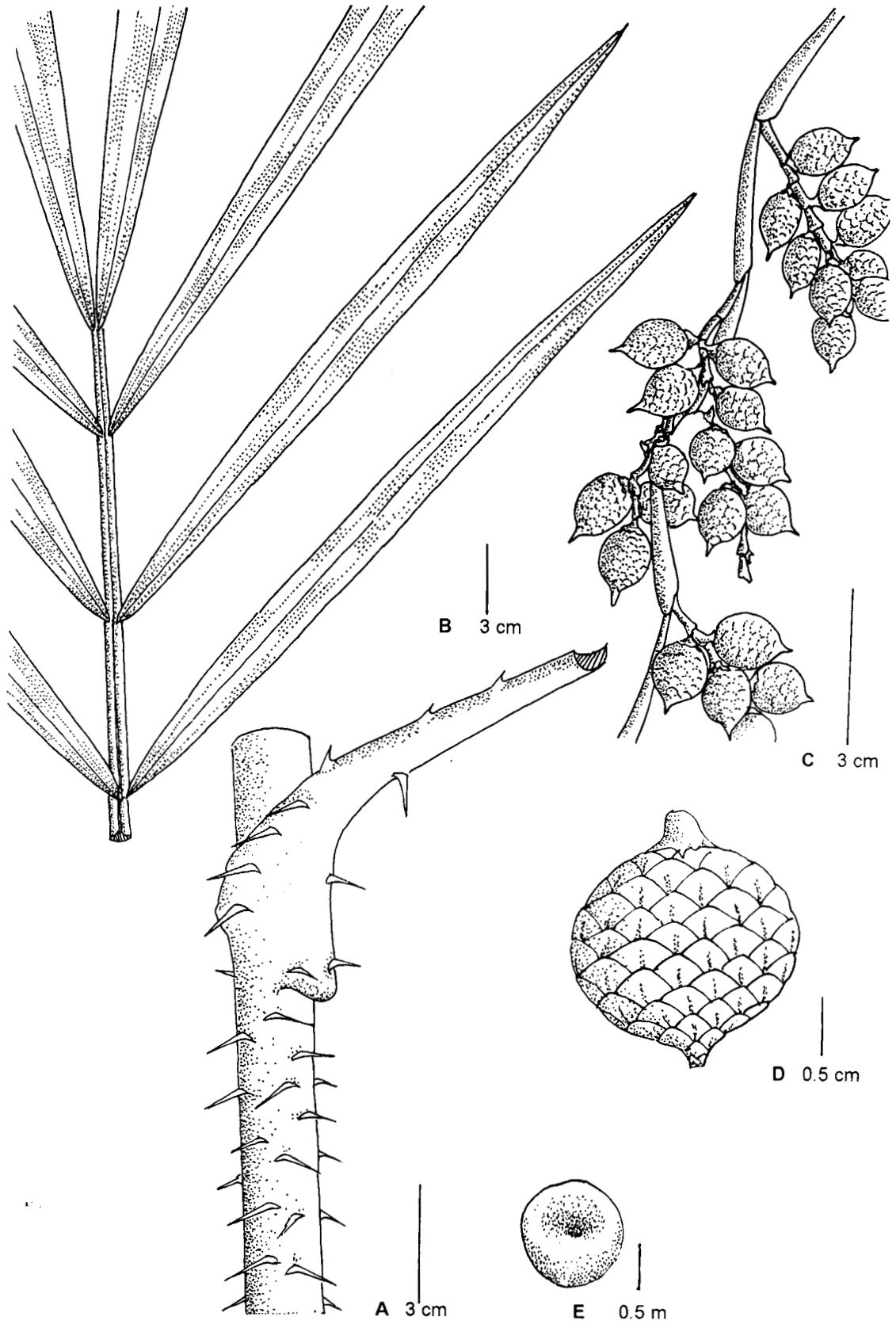


Figure 6.19. *Calamus dransfieldii* Renuka A. leaf sheath; B. a portion of leaf; C. a partial infructescence; D. fruit; E. seed

**Group 9- *C. vattayila* clade**

*Calamus vattayila* Renuka, Curr. Sci. 56: 1012. 1987 & in Rattans of Western Ghats 53. t. 56. 1992. [Figure 6. 20; Plate 6.10]

*Type:* India, Kerala, Wayanad, Chembra, *Renuka 4001* (holotype: KFRI!)

*Vernacular names:* Vattayilayan, Ottaman (Malayalam); Devarubetha (Kannada); Maniperambu (Tamil)

Solitary, small diameter rattan. Stem to 30 m long, with sheaths 2.5 cm in diameter, without sheaths 1.8 cm, internodes 27 cm. Leaf ecirrate, 1 m long; sheath dark green, sparingly spiny, spines 2 cm long, sometimes pointing upwards; knee conspicuous; ochrea small, 0.5 cm long; flagellum dark green, 4 m long; petiole 25 cm, armed with spines 1 cm long; rachis armed with claw-like spines in 3 rows; leaflets alternate, 40 x 10 cm, elliptic, dark green, 6 veined, tips armed with short bristles. Female inflorescence in heavy bunches, 1 m long; primary sheath slightly flattened, biconvex, with short stout spines 0.5 cm long; partial inflorescence 40 cm long, shorter upwards; secondary sheath 2.5 cm long, funnel-shaped, with short stout spines; rachillae 9 cm long, decreasing in length distally; involucre sessile; involucre cup-shaped, fruiting perianth explanate. Fruit ca 2.5 x 0.8 cm, oblong; stigma to 0.4 cm long, scales in 27 rows, longer than broad, not channeled, chestnut brown; endosperm ruminant. Seedling leaf bifid.

*Distribution:* Evergreen forests between 200 to 750 m., very sporadic. *Phenology:* Flowering: September – October; Fruiting: April - May.

*Uses:* A good quality cane used in furniture industry, but not available in required quantities.

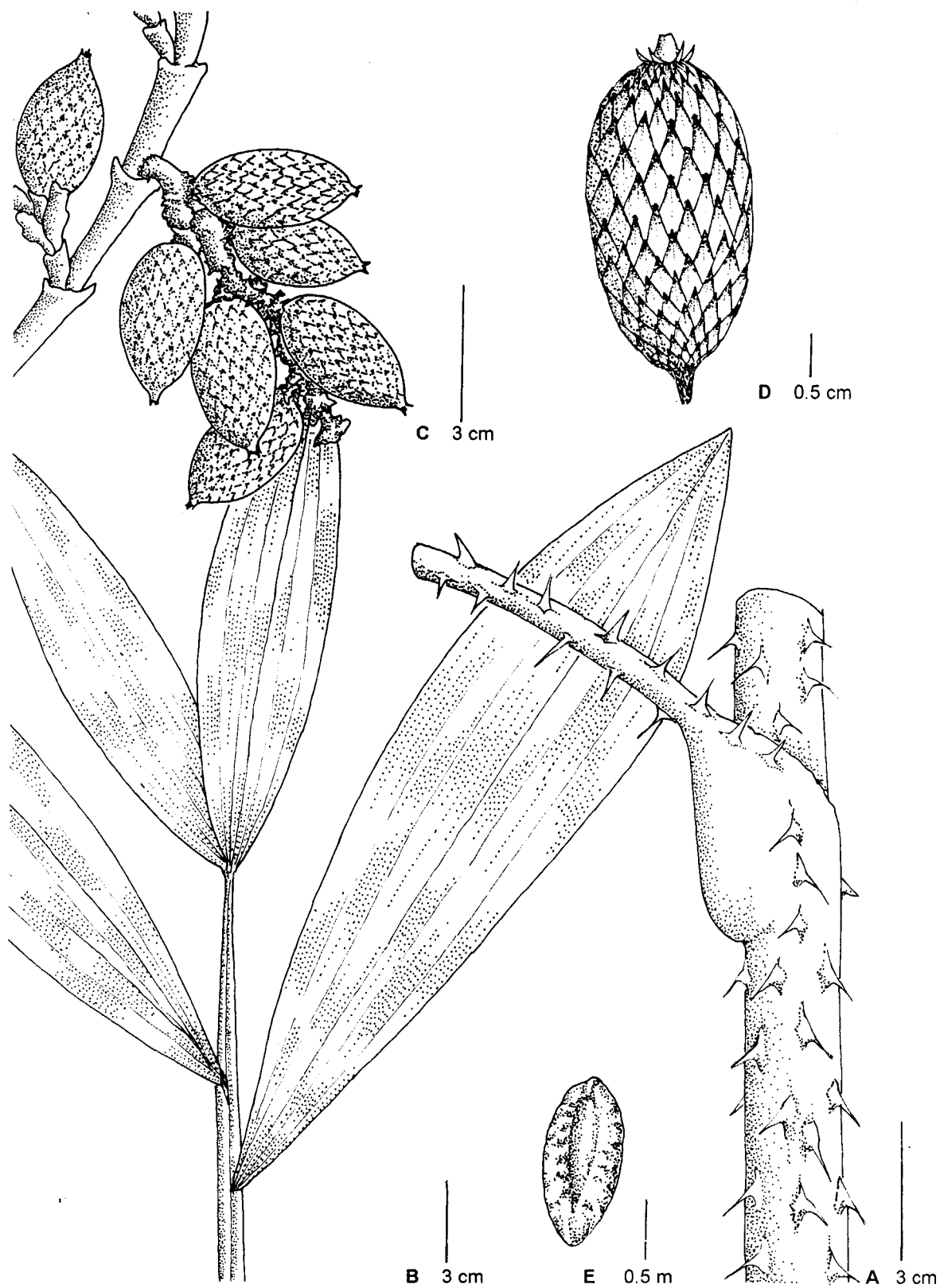


Figure 6.20. *Calamus vattayila* Renuka A. leaf sheath; B. a portion of leaf; C. a partial inflorescence; D. fruit; E. seed



Plate 6.10. *Calamus dransfieldii* Renuka: **A.** Leaf sheath; **B.** Inflorescence  
*Calamus vattayila* Renuka: **C.** Leaf sheath; **D.** Inflorescence

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*Conservation status:* Endangered.

*Specimens examined:* KERALA: Kollam Dist., Chittar valley, Achenkovil, 1983, *Renuka & Nambiar 2907* (KFRI); Thenmala, 1985, *Renuka 4032* (KFRI); Wayanad Dist., Chandanathodu, 1984, *Renuka & Nambiar 3029* (KFRI); Pathanamthitta Dist., Moozhiyar, 1985, *Renuka 4081* (KFRI); Thrissur Dist., Sholayar, 1989, *Sasidharan 5421* (KFRI); Palakkad Dist., Nelliampathy, 2001, *Sreekumar 22435 & 22481* (KFRI).

### SPECIES IMPERFECTLY KNOWN

*Calamus rheedei* Griff., Calc. J. Nat. Hist., 5:73. 1845; Becc. *et* Hook. f. in Hook. f., Brit. India 6:452. 1892. Becc., Ann. Roy. Bot. Gard. Culcutta 11: 95, 313. t. 226. 1908.

N. Burman (1768:84) misidentified Rheede's element as *Calamus rotang* L. In 1814 Roxburgh founded the species *C. latifolius* and he cited Rheede's element also. But his specimen was collected from Chittagong with the local name 'korak bet'. From the description provided (Roxburg, 1814) it is clear that Roxburgh's specimen is entirely different from Rheede's. *C. latifolius* Roxb. is having cirrate leaves whereas Rheede's specimen is ecirrate. *C. latifolius* Roxb. (1832) is also collected from Chittagong with the local name 'korak bet'. Griffith (1844 - 45) treated Rheede's specimen as a separate species, *C. rheedei* Griff. According to Beccari (Ann. Royal Bot Gard. Calcutta 11: 67, 95, 313, 1913) this specimen is allied to *C. travancoricus*, because the fruits are similar to the fruits collected by Campbell in Malabar (illustr. in t. 226). However no one has matched the leaves as illustrated in Rheede's and characterized by Beccari as "not cirriferous, leaflets distinctly grouped in 4 fascicles with a long vacant space interposed".

Nicolson *et al.* (1988) treated t.65 as *C. latifolius* Roxb. But *C. latifolius* is a large diameter rattan, the diameter of the sheathed stem being 3cm. Moreover the leaf is cirrate. In Katu Chural the leaf is ecirrate and hence this species is not *C. latifolius* Roxb. Basu (1992) described a specimen of *C. rheedei* from Coorg (M.H. Acc.No. 52725) stating that this material is perfectly matching with Rheede's plate and description. But on close examination this material was proved to be *C. travancoricus*. In the Western Ghats there are only two species of *Calamus* with slender cane and grouped leaflets, *C. travancoricus* and *C. brandisii*. *C. travancoricus* is slightly slender than *C. brandisii*, the diameter of the sheathed stem being 0.8 cm in the former and 1.5cm in the latter. *C. brandisii* has a tuft of spines at the mouth of the sheath. Rheede's 'Tsjeru-tsjurel' is *C. travancoricus* and he has mentioned that 'Katu Chural' "differs from the former only in that it is with larger leaves and also fruits, which when ripe become black and are eaten". The leaf of Katu Chural is similar to *C. brandisii* but the laminar primary sheath seen in the fruiting specimen of Katu Chural is absent in *C. brandisii*. Here the primary sheath is tightly sheathing basally and splits open distally, but not produced into laminar part. As stated by Beccari, the drawings of *Hortus Malabaricus* is very good for its time, and hence it is evident that Tsjeru-tsjurel and Katu Chural are two distinct species. A specimen similar to Katu Chural was not collected till date. Based on Rheede (1693) and Beccari (1908) the species can be described as follows:

Clustering, slender rattan. Leaves ecirrate; petiole and rachis rather densely clawed; leaflets narrowly lanceolate, the longest to 40 x 2.5cm, arranged in groups of 3 leaflets each, with long vacant spaces interposed; one leaflet of each group on one side of the rachis and two of the other, 5 leaflets terminate the rachis, terminal pair free at base. Inflorescence

long flagellate; primary sheath split open at the distal end to form a laminar portion; partial inflorescence shorter than the sheath. Fruit *ca* 2 x 1.2 cm, ovoid or ellipsoid.

*Note:* In Rheede's plate the fruits are not attached to the leaves (as shown tied together) and there is a possibility that the materials are mixed.

# Summary

Sreekumar V.B “Systematics and phylogeny of the genus *calamus* linn. (arecaceae) in the western ghats ” Thesis. Kerala Forest Research Institute, University of Calicut, 2004

## *Summary*

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## 7. Summary

The genus *Calamus* L., commonly known as 'rattan' are spiny climbing palms belonging to the subfamily Calamoideae. They are mostly distributed in humid tropical forests and show rather high degree of endemism. In the Western Ghats, the genus is represented by 21 species among which 16 are endemic. Extensive field survey was undertaken throughout the Western Ghats, North Eastern States and Andaman and Nicobar Islands and herbarium specimens were prepared and deposited in Kerala Forest Research Institute. The specimens were compared with the type specimens from various National and International Herbaria. To understand the pattern of morphological variations and systematic relationships among various species of *Calamus* in the Western Ghats, phenetic and phylogenetic studies were carried out. Thirty one species of *Calamus*, representing three phyto-geographical areas viz. Western Ghats, Eastern Himalayas and Andaman and Nicobar Islands were included. A total of forty-seven morphological characters from both vegetative and reproductive parts were selected for the analysis. The cluster analysis divided the species into eight groups. Ordination showed the same pattern of species relationships. Phylogenetic analysis confirmed the non-monophyly of members of Western Ghats and complex pattern of variation identified within the study group. The phenetic analysis showed that there was hundred per cent similarity between *C. rivalis* and *C. metzianus*. Morphometric analysis was carried out to understand the pattern of phenetic structure among populations and to demarcate the species boundaries of *C. metzianus* and *C. rivalis*. Total of 107 Operational Taxonomic Units (OTUs) were selected from both the Western

Ghats and Sri Lanka and a total of 32 morphological quantitative and qualitative characters were included in the analysis. The results of both cluster analysis and principal component analysis failed to show a distinct phenetic gap between the two species.

RAPD techniques were used to confirm the taxonomic identity and population level genetic structure of *C. metzianus* and *C. rivalis*. Sixty individuals from seven populations representing both the Western Ghats and Sri Lanka were included in the study. Out of the sixty five primers from KIT OPA, OPB, OPE, OPAU and OPAW screened, ten were chosen based on the number and reproducibility of amplified products. A total of 117 markers were obtained of which 112 (95%) were polymorphic. The analysis could not identify any species specific markers within these two species. The various genetic diversity parameters, viz. the percentage of polymorphism, number of alleles and total gene diversity, were also studied.

The Chertala population had highest genetic diversity index of 0.2458 and Matugama population of Sri Lanka, the lowest (0.1085). The mean total genetic diversity ( $H_t$ ) was 0.2903. The relative magnitude of genetic differentiation among subpopulation ( $G_{st}$ ) was 0.3820 which indicated that 38.20 per cent of the total diversity was among populations while the rest (61.80%) of the total variations occurred within populations. So the partitioning of genetic diversity showed high amount of variability within populations. The estimated average genetic distances between populations indicated that the genetic distances between population pairs ranged from 0.0500 (between Yagirala and Watারেke) to 0.2777 (between Yagirala and Honaver). Hence the maximum genetically similar populations are Yagirala and Watারেke and most genetically distant ones are Honaver and Yagirala. The unrooted phenetic tree based on genetic distance depicts relationships among

populations. The genetic separations of the populations in the phenetic tree were in partial agreement with geographical isolation. The UPGMA dendrogram based on Jaccard's similarity coefficients depicted genetic clustering of all the sixty genotypes analysed. The cophenetic correlation coefficient between the dendrogram and the original distance matrix was 0.840. Three major clusters were obtained in the dendrogram. The result of both cluster and principal coordinate analysis failed to separate the populations of *C. metzianus* from *C. rivalis* into two distinct species, rather a clear geographic pattern was observed. Chertala population can be considered as 'hot spot' of genetic variation and important reservoir of potentially useful genes and hence required to be conserved.

Diminishing forest cover and over exploitation of commercially important species have seriously depleted the wild populations in the rattans of the Western Ghats. The species like *C. dransfieldii*, *C. neelagiricus* and *C. huegelianus* are in the verge of extinction.

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**Appendix 1.** List of OTUs (Operational Taxonomic Units) used in the morphometric analysis.  
 (Nil = Nilambur; Hon = Honaver; Cher = Chertala; Ash = Ashramam; Wata = Watareke; Matu = Matugama; Yagi = Yagirala; Wala = Walakanda; Rat = Ratnapura.

- Nil 1 *Babu, 4081*, Pattakkarimbu, Nilambur, Kerala, India, KFRI.  
 Nil 2 *Babu, 4082*, Pattakkarimbu, Nilambur, Kerala, India, KFRI.  
 Nil 3 *Babu, 4083*, Pattakkarimbu, Nilambur, Kerala, India, KFRI.  
 Nil 4 *Babu, 4084*, Pattakkarimbu, Nilambur, Kerala, India, KFRI.  
 Nil 5 *Renuka, 4031*, Thalaumkolli, Nilambur, Kerala, India, KFRI.  
 Nil 6 *Renuka, 4032*, Thalaumkolli, Nilambur, Kerala, India, KFRI.  
 Nil 7 *Renuka, 4033*, Thalaumkolli, Nilambur, Kerala, India, KFRI.  
 Nil 8 *Sreekumar, 8448*, Thalaumkolli, Nilambur, Kerala, India, KFRI.  
 Nil 9 *Padmakumar, 1001*, Thalaumkolli, Nilambur, Kerala, India, S.V.R.N.S.S.College, Vazhoor.  
 Nil 10 *Padmakumar, 1880*, Thalaumkolli, Nilambur, Kerala, India, S.V.R.N.S.S.College, Vazhoor.  
 Nil 11 *Perrie, 49362*, Nilambur, India, CAL.  
 Nil 12 *Perrie, 49363*, Nilambur, India, CAL.  
 Nil 14 *Perrie, 49364*, Nilambur, India, CAL.  
 Nil 15 *Perrie, 49365*, Nilambur, India, CAL.  
 Nil 16 *Perrie, 49366*, Nilambur, India, CAL.  
 Nil 17 *Renuka, 3061*, Nilambur, Kerala, India, CAL.  
 Nil 18 *Sreekumar, 8450*, Pattakkarimbu, Nilambur, Kerala, India, KFRI.  
 Nil 19 *Sreekumar, 8451*, Pattakkarimbu, Nilambur, Kerala, India, KFRI.  
 Nil 20 *Sreekumar, 8452*, Pattakkarimbu, Nilambur, Kerala, India, KFRI.  
 Hon 1 *Sreekumar, 8444a*, Honaver, Karnataka, India, KFRI.  
 Hon 2 *Sreekumar, 8444b*, Honaver, Karnataka, India, KFRI.  
 Hon 3 *Sreekumar, 8444c*, Honaver, Karnataka, India, KFRI.  
 Hon 4 *Sreekumar, 8444d*, Honaver, Karnataka, India, KFRI.  
 Hon 5 *Sreekumar, 8444e*, Honaver, Karnataka, India, KFRI.  
 Hon 6 *Renuka, 6793*, Honaver, Karnataka, India, KFRI.  
 Hon 7 *Renuka, 6822*, Honaver, Karnataka, India, KFRI.  
 Hon 8 *Padmakumar, 1221*, Honaver, Karnataka, India, S.V.R.N.S.S.College, Vazhoor.  
 Hon 9 *Padmakumar, 1345*, Honaver, Karnataka, India, S.V.R.N.S.S.College, Vazhoor.  
 Hon 10 *Padmakumar, 1190*, Honaver, Karnataka, India, S.V.R.N.S.S.College, Vazhoor.  
 Hon 11 *Renuka, 6013a*, Honaver, Karnataka, India, KFRI.  
 Hon 12 *Renuka, 6013b*, Honaver, Karnataka, India, KFRI.  
 Hon 13 *Renuka, 6013c*, Honaver, Karnataka, India, KFRI.  
 Hon 14 *Renuka, 6013d*, Honaver, Karnataka, India, KFRI.  
 Hon 15 *Renuka, 6013e*, Honaver, Karnataka, India, KFRI.  
 Cher 1 *Renuka, 5884a*, Chertala, Alapuzha, Kerala, India, KFRI.  
 Cher 2 *Renuka, 5884b*, Chertala, Alapuzha, Kerala, India, KFRI.

- Cher 3 *Renuka, 5884c*, Chertala, Alapuzha, Kerala, India, KFRI.
- Cher 4 *Padmakumar, 1880*, Chertala, Kerala, India, S.V.R.N.S.S.College, Vazhoor.
- Cher 6 *Padmakumar, 1881*, Chertala, Kerala, India, S.V.R.N.S.S.College, Vazhoor.
- Cher 7 *Sreekumar, 8469a* Chertala, Alapuzha, Kerala, India, KFRI.
- Cher 8 *Sreekumar, 8469b* Chertala, Alapuzha, Kerala, India, KFRI.
- Cher 9 *Sreekumar, 8469c* Chertala, Alapuzha, Kerala, India, KFRI.
- Cher 10 *Sreekumar, 8469d* Chertala, Alapuzha, Kerala, India, KFRI.
- Cher 11 *Sreekumar, 8469e* Chertala, Alapuzha, Kerala, India, KFRI.
- Cher 12 *Sreekumar, 8469f* Chertala, Alapuzha, Kerala, India, KFRI.
- Cher 13 *Sreekumar, 8469g* Chertala, Alapuzha, Kerala, India, KFRI.
- Cher 14 *Sreekumar, 8469h* Chertala, Alapuzha, Kerala, India, KFRI.
- Cher 15 *Sreekumar, 8469i* Chertala, Alapuzha, Kerala, India, KFRI.
- Ash 1 *Sreekumar, 8447a*, Ashramam, Kollam, Kerala, India, KFRI.
- Ash 2 *Sreekumar, 8447b*, Ashramam, Kollam, Kerala, India, KFRI.
- Ash 3 *Sreekumar, 8441a*, Ashramam, Kollam, Kerala, India, KFRI.
- Ash 4 *Sreekumar, 8441b*, Ashramam, Kollam, Kerala, India, KFRI.
- Ash 5 *Renuka, 7555*, Ashramam, Kollam, Kerala, India, KFRI.
- Ash 6 *Renuka & Sasidharan, 3443a*, Ashramam, Kollam, Kerala, India, KFRI.
- Ash 7 *Renuka & Sasidharan, 3443b*, Ashramam, Kollam, Kerala, India, KFRI.
- Ash 8 *Renuka, 6621*, Ashramam, Kollam, Kerala, India, KFRI.
- Ash 9 *Renuka, 6799*, Ashramam, Kollam, Kerala, India, KFRI.
- Ash 10 *Sreekumar, 7535*, Ashramam, Kollam, Kerala, India, KFRI.
- Ash 11 *Sreekumar, 7536*, Ashramam, Kollam, Kerala, India, KFRI.
- Ash 12 *Sreekumar, 7537*, Ashramam, Kollam, Kerala, India, KFRI.
- Ash 13 *Padmakumar, 1092*, Ashramam, Kerala, India, S.V.R.N.S.S.College, Vazhoor.
- Ash 14 *Padmakumar, 1093*, Ashramam, Kerala, India, S.V.R.N.S.S.College, Vazhoor.
- Ash 15 *Renuka, 4004a*, Ashramam, Kollam, Kerala, India, KFRI.
- Ash 16 *Renuka, 4004b*, Ashramam, Kollam, Kerala, India, KFRI.
- Ash 17 *Renuka, 4004c*, Ashramam, Kollam, Kerala, India, KFRI.
- Ash 18 *Renuka, 4004d*, Ashramam, Kollam, Kerala, India, KFRI.
- Wata 1 *Ediriweera, S. 8445a*, Watareke, Sri Lanka; KFRI.
- Wata 2 *Ediriweera, S. 8445b*, Watareke, Sri Lanka; KFRI.
- Wata 3 *Ediriweera, S. 8445c*, Watareke, Sri Lanka; KFRI.
- Wata 4 *Ediriweera, S. 8445d*, Watareke, Sri Lanka; KFRI.
- Wata 5 *Ediriweera, S. 8445e*, Watareke, Sri Lanka; KFRI.
- Wata 6 *Ediriweera, S. 8445f*, Watareke, Sri Lanka; KFRI.
- Wata 7 *Ediriweera, S. 8445g*, Watareke, Sri Lanka; KFRI.
- Wata 8 *Ediriweera, S. 8445h*, Watareke, Sri Lanka; KFRI.
- Wata 9 *Ediriweera, S. 8445i*, Watareke, Sri Lanka; KFRI.
- Matu 1 *Ediriweera, S. 8444a*, Matugama, Sri Lanka; KFRI.

- Matu 2 *Ediriweera, S. 8444b*, Matugama, Sri Lanka; KFRI.  
 Matu 3 *Ediriweera, S. 8444c*, Matugama, Sri Lanka; KFRI.  
 Matu 4 *Ediriweera, S. 8444d*, Matugama, Sri Lanka; KFRI.  
 Matu 5 *Ediriweera, S. 8444e*, Matugama, Sri Lanka; KFRI.  
 Matu 6 *Ediriweera, S. 8444f*, Matugama, Sri Lanka; KFRI.  
 Matu 7 *Ediriweera, S. 8444g*, Matugama, Sri Lanka; KFRI.  
 Matu 8 *Ediriweera, S. 8444h*, Matugama, Sri Lanka; KFRI.  
 Matu 9 *Ediriweera, S. 8444i*, Matugama, Sri Lanka; KFRI.  
 Matu 10 *Ediriweera, S. 8444j*, Matugama, Sri Lanka; KFRI.  
 Yagi 1 *Ediriweera, S. 8442a*, Yagirala, Sri Lanka; KFRI.  
 Yagi 2 *Ediriweera, S. 8442b*, Yagirala, Sri Lanka; KFRI.  
 Yagi 3 *Ediriweera, S. 8442c*, Yagirala, Sri Lanka; KFRI.  
 Yagi 4 *Ediriweera, S. 8442d*, Yagirala, Sri Lanka; KFRI.  
 Yagi 5 *Ediriweera, S. 8442e*, Yagirala, Sri Lanka; KFRI.  
 Yagi 6 *Ediriweera, S. 8442f*, Yagirala, Sri Lanka; KFRI.  
 Yagi 7 *Ediriweera, S. 8443a*, Yagirala, Sri Lanka; KFRI.  
 Yagi 8 *Ediriweera, S. 8443b*, Yagirala, Sri Lanka; KFRI.  
 Yagi 9 *Ediriweera, S. 8443c*, Yagirala, Sri Lanka; KFRI.  
 Yagi 10 *Ediriweera, S. 8443d*, Yagirala, Sri Lanka; KFRI.  
 Yagi 11 *Ediriweera, S. 8443e*, Yagirala, Sri Lanka; KFRI.  
 Yagi 12 *Ediriweera, S. 8443f*, Yagirala, Sri Lanka; KFRI.  
 Yagi 13 *Ediriweera, S. 8443g*, Yagirala, Sri Lanka; KFRI.  
 Wala 1 *De Zoysa, N.D. 6814a*, Walankanda, Sri Lanka; KFRI.  
 Wala 2 *De Zoysa, N.D. 6814b*, Walankanda, Sri Lanka; KFRI.  
 Wala 3 *De Zoysa, N.D. 6814c*, Walankanda, Sri Lanka; KFRI.  
 Wala 4 *De Zoysa, N.D. 6814d*, Walankanda, Sri Lanka; KFRI.  
 Rat 1 *De Zoysa, N.D. 6816a*, Ratnapura, Sri Lanka; KFRI.  
 Rat 2 *De Zoysa, N.D. 6816b*, Ratnapura, Sri Lanka; KFRI.  
 Rat 3 *De Zoysa, N.D. 6816c*, Ratnapura, Sri Lanka; KFRI.



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