



**University of
Reading**

**SYSTEMATICS AND EVOLUTION OF
THE RATTAN GENUS *KORTHALSIA*
BL. (ARECACEAE) WITH SPECIAL
REFERENCE TO DOMATIA**

A thesis submitted by

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For the Degree of Doctor of Philosophy

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February 2018

Declaration

I can confirm that is my own work and the use of all material from other sources have been properly and fully acknowledged.

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ABSTRACT

Korthalsia is a genus of palms endemic to Malesian region and known for the several species that have close associations with ants. In this study, 101 new sequences were generated to add 18 *Korthalsia* species from Malaysia, Singapore, Myanmar and Vietnam to an existing but unpublished data set for calamoid palms. Three nuclear (*prk*, *rpb2*, and ITS) and three chloroplast (*rps16*, *trnD-trnT* and *ndhF*) markers were sampled and Bayesian Inference and Maximum Likelihood methods of tree reconstruction used. The new phylogeny of the calamoids was largely congruent with the published studies, though the taxon sampling was more thorough. Each of the three tribes of the Calamoideae appeared to be monophyletic. The Eugeissoneae was consistently resolved as sister to Calameae and Lepidocaryeae, and better resolved, better supported topologies below the tribal level were identified. *Korthalsia* is monophyletic, and novel hypotheses of species level relationships in *Korthalsia* were put forward.

These hypotheses of species level relationships in *Korthalsia* served as a framework for the better understanding of the evolution of ocrea. The morphological and developmental study of ocrea in genus *Korthalsia* included detailed study using Light and Scanning Electron Microscopy for seven samples of 28 species of *Korthalsia*, in order to provide understanding of ocrea morphological traits. The new phylogenetic hypothesis allowed homology of ocrea types to be tested. Ancestral reconstructions revealed that the inflated ocreas are not homologous; based on these reconstructions, it is suggested that ocreas that provide domatia for ants evolved more than once. Despite their non-homology, ocreas are remarkably uniform developmentally. New time calibrated trees estimated the divergence time for the *Korthalsia* crown node to be 19.89 MYR and ocrea to appear 17.06 MYR consistent with the published estimates for other groups.

A taxonomic treatment of the ant *Korthalsia* species was based on material gathered from four herbaria and during the two field trips. Ten species were recognised in the treatment. Descriptions, keys and distribution maps to all species are presented.

ACKNOWLEDGEMENTS

In the name of Allah, Most Gracious, Most Merciful

First of all, I would like to express my sincere gratitude to my both supervisors, Prof. Julie Hawkins (University of Reading) and Dr. William Baker (Royal Botanic Garden, Kew) for all continuous support, patience, motivation, advice and immense knowledge. I am immensely grateful to both of them for their guidance and for always believing in me.

I am grateful to the staff of Royal Botanic Gardens, Kew for the access to their facilities. I would like to thank Dr. Paula Rudall, Chrissie Prychid and Maria Conejero, staff of Jodrell Laboratory for their advice and assistance with anatomical work. In Reading, my thanks goes to Dr. Stephen Ansel for his amazing help in the lab.

Special thanks to Dr. John Dransfield and Palm Group at Kew for the lively discussion and friendship. In particular, I would like to thank Dr. Wolf Eiserhardt for his advice and for helping me analyses the data. My appreciation also extended to my officemates, Mazhani, Yati, Natali, Marco, Estevão, Deniz, Sawai, Paul, Naila, Riyadh, Aseel, Rahmah, and Lina for giving me motivation and helps in numerous ways.

I would like to thank Dr. Asyraf Mansur and Dr. Rahmad Zakaria from University Science Malaysia, Dr. Saw Leng Guan and Dr. Richard Chung from Forest Research Institute Malaysia (FRIM) for providing me an excellent assistant during my field work. My thanks goes to Dr. David Middleton, Ms Serena Lee and Ms Bazilah for their hospitality during my visit to Singapore Herbarium and Singapore Botanic Garden, and Dr. Adrian Loo for helping me collected the specimens.

To my dearest friend Azi Jamaludin, thank you for being you. If not for your advice and encouragement, I wouldn't be here. To Ahmad Naqib and Kak Roziana I am forever be grateful for your help that you give me in many ways. I owed you guys a lot.

To my scholarship sponsor the Ministry of Higher Education Malaysia (MOHE) and also my employer, University Malaysia Terengganu, thank you so much for believing in me and making it possible for me to complete my Ph.D in the University of Reading.

Last but not least, I would like to express my gratitude and dedicate this thesis to my beloved family, who shown their tremendous support, unconditional love and infinite prayers.

Thank you all.

CONTENTS

ABSTRACT.....	iii
ACKNOWLEDGEMENTS.....	iv
CONTENTS.....	v
LIST OF TABLES.....	viii
LIST OF FIGURES	ix
CHAPTER 1	1
GENERAL INTRODUCTION.....	1
1.1 Overview	1
1.2 Malesian region.....	1
1.3 Palms	3
1.3.1 Palm classification and phylogeny	5
1.3.2 Subfamily Calamoideae.....	8
1.3.3 Genus <i>Korthalsia</i>	10
1.4 Ant-plant relationships	12
1.4.1 Ant and rattan associations	13
1.4.2 Ants and <i>Korthalsia</i>	15
1.5 Aims and thesis structure	17
CHAPTER 2	18
A PHYLOGENETIC FRAMEWORK OF THE CALAMOIDEAE.....	18
2.1 Introduction	18
2.1.1 Objectives.....	20
2.2 Material and methods	21
2.2.1 Taxon sampling	21
2.2.2 DNA isolation.....	21
2.2.3 DNA amplification and purification.....	21
2.2.4 DNA sequencing	30
2.2.5 Sequence assembly and alignment	31
2.2.6 Phylogenetic analyses.....	31
2.2.6.1 Maximum Likelihood	31
2.2.6.2 Bayesian Inference.....	31

2.3 Results	33
2.3.1 Monophyly of the tribes and inter-tribal relationships	33
2.3.2 Monophyly of the subtribes	39
2.3.3 Relationship between the subtribes	40
2.3.4 Species relationship with <i>Calamus</i> and <i>Korthalsia</i>	41
2.3.4.1 Delimitation and subdivision of <i>Calamus</i>	41
2.4 Discussion	42
2.4.1 Evaluating higher-level classification	42
2.4.2 New insights into the relationships among subtribes and between genera	44
2.4.3 Species relationships within genera: <i>Calamus</i> and <i>Korthalsia</i>	45
 CHAPTER 3	47
MORPHOLOGICAL CHARACTERISATION, DEVELOPMENT AND EVOLUTION OF THE OCREA IN <i>KORTHALSIA</i>	47
3.1 Introduction	47
3.1.1 Aims of this study.....	50
3.2 Material and methods	51
3.2.1 Developmental anatomy	51
3.2.1.1 Fieldwork	51
3.2.1.2 Ocrea dissection.....	53
3.2.1.3 Light Microscopy (LM)	55
3.2.1.4 Scanning Electron Microscopy (SEM)	55
3.2.2 Evolution of ocrea	55
3.2.2.1 Reconstruction of a time calibrated phylogeny	55
3.2.2.2 Stochastic character mapping	56
3.3 Results	57
3.3.1 Ocrea development.....	57
3.3.2 Ocrea evolution	64
3.4 Discussion	73
 CHAPTER 4	78
A TAXONOMIC REVISIONS OF THE ANT-RATTAN SPECIES <i>KORTHALSIA</i> ...	78
4.1 Introduction	78
4.1.1 Taxonomic history	80

4.1.2 Taxonomic revision	81
4.2 Material and methods	81
4.2.1 Herbarium studies.....	81
4.2.2 Field studies	82
4.3 Results	83
4.3.1 Morphology of <i>Korthalsia</i>	83
4.3.1.1 Habit.....	83
4.3.1.2 The stem.....	84
4.3.1.3 The leaf	85
4.3.1.4 The ocrea.....	86
4.3.1.5 Inflorescence	87
4.3.1.6 Flower	88
4.3.1.7 Fruit.....	89
4.3.2 Keys to ant-rattan species of <i>Korthalsia</i>	90
4.3.3 Taxonomic treatment.....	91
 CHAPTER 5	135
GENERAL CONCLUSIONS AND FUTURE PERSPECTIVES	135
5.1 Reflections on the work	135
5.1.1 The phylogeny	135
5.1.2 Ocrea evolution	137
5.1.3 The taxonomy	138
5.2 Priorities for future work.....	139
5.2.1 Phylogeny and ocrea evolution	139
5.2.2 Taxonomy.....	140
 REFERENCES	141
APPENDICES	159

LIST OF TABLES

Table 1.1 List of all <i>Korthalsia</i> species which have ant associations.. ..	16
Table 2.1 List of specimens used in this study.....	23
Table 2.2 List of primers used for amplification of all samples.....	30
Table 2.3 PCR profiles.....	30
Table 2.4 Models used in this study.	32
Table 2.5 Alignment statistics for the different regions analysed.....	33
Table 2.6 Support for the monophyly of the tribes, and for inter-tribal relationships.....	39
Table 2.7 Support for the monophyly of the subtribes.....	40
Table 2.8 Comparison of taxon and marker sampling between Baker <i>et al.</i> (2009) and this study.....	44
Table 3.1 Specimens used in this study.....	51
Table 3.2 The nine characters describing variation amongst the 28 species of <i>Korthalsia</i>	65
Table 3.3 Scores for the nine characters describing variation amongst 18 species of <i>Korthalsia</i>	66

LIST OF FIGURES

Figure 1.1 Map of Malesian region.....	2
Figure 1.2 The distribution of living palms around the world.. ..	4
Figure 1.3 Summary schematic tree showing the phylogenetic relationships among subfamilies and tribes of the Arecaceae.....	7
Figure 1.4 Distribution of subfamily Calamoideae.....	9
Figure 1.5 Distribution of <i>Korthalsia</i> in highlighted area based on Dransfield <i>et al.</i> (2008)...	11
Figure 1.6 Phylogenetic distribution of myrmecophytic rattans.....	14
Figure 1.7 Example types of ocrea.....	15
Figure 2.1 Majority rule consensus tree resulting from Bayesian Inference of the 6-gene data set.....	35
Figure 2.2 Phylogenetic relationships reconstructed using Maximum Likelihood of the 6-gene data set prescribed as on phylogram.. ..	36
Figure 2.3 Summarises the relationships recovered across the two 6-genes trees and for combined chloroplast and combined nuclear trees.. ..	37
Figure 3.1 Four types of ocrea according to Dransfield (1981).....	48
Figure 3.2 Field photographs of ocrea.. ..	50
Figure 3.3 The ocrea were preserved in 70% ethanol.....	52
Figure 3.4 The distribution of collection site in Peninsular Malaysia and Sarawak.....	52
Figure 3.5 The distribution of collection sites in Singapore.. ..	53
Figure 3.6 The ocrea dissecting process to remove outer stages of the developing leaves.....	54
Figure 3.7 Photomicroscope images of developing ocrea (boxed area).....	58
Figure 3.8 Early stages of development of the ocrea (highlighted area) (SEM).....	60
Figure 3.9 Leaf developmental series (SEM).....	62
Figure 3.10 Leaf developmental series (SEM).....	63
Figure 3.11 Leaf surface of four different species of <i>Korthalsia</i> (SEM).....	64

Figure 3.12 The Bayesian maximum clade credibility chronogram for the Calamoideae.....	67
Figure 3.13 The Bayesian maximum clade credibility chronogram for <i>Korthalsia</i> , with clades with less than 50% posterior probability collapsed, and with 95% highest posterior distributions of dates indicated by grey bars.....	68
Figure 3.14a The morphological traits of possible importance in the evolution of domatia and the ant-rattan relationship, and the evolution of characters that describe the variation amongst species of <i>Korthalsia</i>	69
Figure 3.14b The morphological traits of possible importance in the evolution of domatia and the ant-rattan relationship, and the evolution of characters that describe the variation amongst species of <i>Korthalsia</i>	70
Figure 3.14c The morphological traits of possible importance in the evolution of domatia and the ant-rattan relationship, and the evolution of characters that describe the variation amongst species of <i>Korthalsia</i>	71
Figure 3.15 The summarize evolution of all characters.. ..	72
Figure 4.1 Distribution map of <i>Korthalsia</i> spp in Malesia region (full line).. ..	79

CHAPTER 1

GENERAL INTRODUCTION

1.1 Overview

This thesis is a systematic study of *Korthalsia* Blume, a genus of palms endemic to the Malesian region. The genus includes several species that have close associations with ants. This introductory chapter has four sections. The first (Section 1.2) describes the Malesian region, its plant species richness and the origins of the region's plant biodiversity. Next (Section 1.3), I present an overview of the palms and their classification, also providing an introduction to the genus *Korthalsia*. Ant-plant relationships are then reviewed, with an introduction to what is known about ant-rattans, and ant relationships in *Korthalsia*. This section includes a brief introduction to the ocrea, a structure that forms domatia (chambers or hollow structures occupied by ants) in some rattan palms, including some species of *Korthalsia*. Finally, the objectives and structure of the thesis is outlined.

1.2 Malesian region

Malesia comprises the Malay Peninsula, Borneo, the Philippines, and the archipelago of islands stretching from Sumatra to New Guinea (Figure 1.1), and is a centre of plant megadiversity (Wallace, 1869; Myers, 1988; Marsh *et al.*, 2009; Raes and van Welzen 2009). Conservative estimates of the diversity of vascular plants within Malesia indicate that at least 45,000 species may be present (Thomas and Roos, 2016). New Guinea and Borneo are two major centres of plant diversity in Malesia. New Guinea, the largest of tropical islands, has at least 14,000 species of plants and Borneo at least 14,500 species (Roos *et al.*, 2004). Phylogenetic studies are contributing to understanding of centres of plant diversity. For example, de Bruyn *et al.* (2014) recently identified Borneo and Indochina as ‘evolutionary hotspots’ in a phylogenetic meta-analysis of both flora and

fauna, and several studies cite Borneo as the centre of diversification for multiple taxa (Nauheimer *et al.*, 2012; Webb and Ree, 2012).

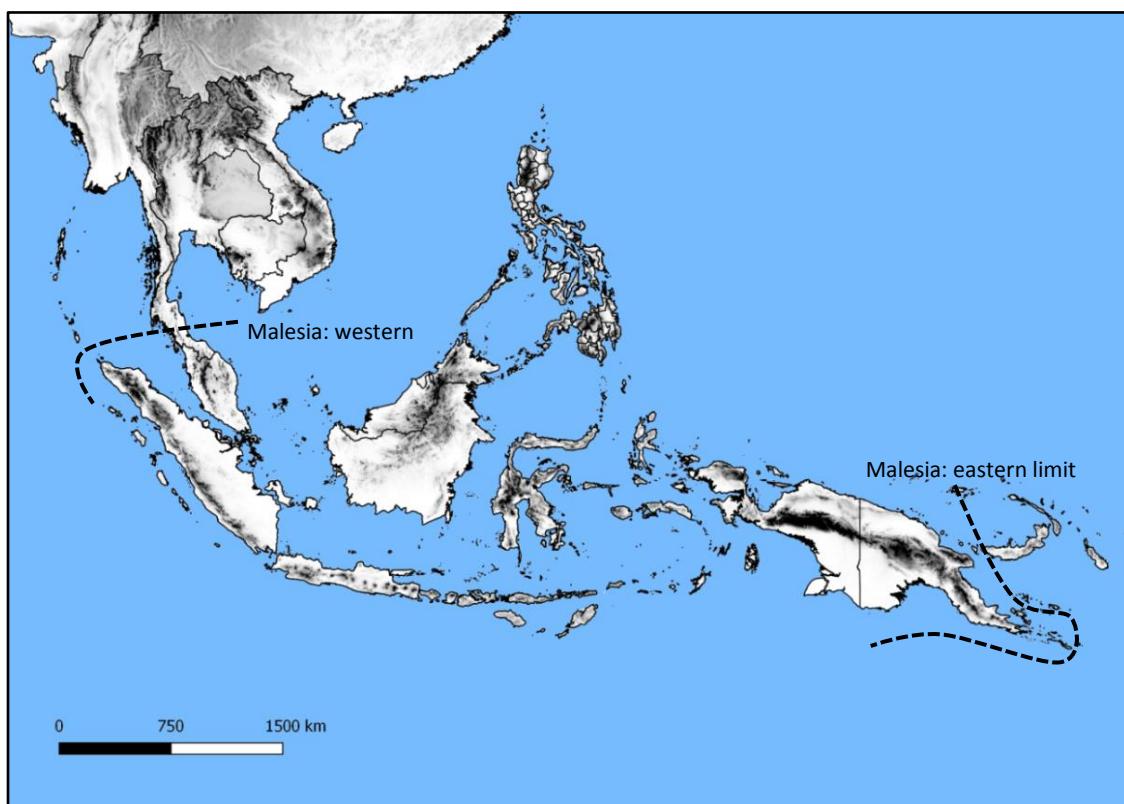


Figure 1.1 Map of Malesian region. Map prepared by Salwa Shahimi, using QGIS.

The Malesian region has for quite some time been acknowledged as a place of extraordinary biogeographic intrigue (Wallace, 1869), providing numerous literature in this area (e.g. Croizat, 1958; MacArthur and Wilson, 1967; Michaux, 1991). The ancestors of the Malesia biota have arrived by three major routes (Webb and Ree, 2012). The first route is Laurasian (including Boreotropical) clades arriving from the west (e.g. for plants, *Trigonobalanus* Forman and *Lithocarpus* Blume, Morley, 2000). The second route is that of the Gondwanan clades arriving from the west, via Africa or the Indian raft and Sundaland (e.g. Dipterocarpaceae; many Annonaceae, Richardson *et al.*, 2004, Crypteroniaceae, Moyle, 2004). The last route is Gondwanan clades arriving from the east, via the Australian raft (e.g. Proteaceae, Barker *et al.*, 2007; Cunoniaceae; Monimiaceae; *Phyllocladus* Rich. ex Mirb., *Nothofagus* Blume, Morley, 2000; *Eucalyptus* L'Hér., Ladiges *et al.*, 2003).

The archipelagic nature of the region and its complex landscapes imply that allopatric speciation has played a major role in generating this diversity, but this may not account for the extremes of species richness observed in the region (Dransfield, 1987; Baker *et al.*, 1998; Baker and Couvreur, 2012). Among other key drivers, the role of biotic interactions, such as pollinator relationships and other mutualisms, remain poorly explored in Malesia. Studies of phylogenetic relationships of Malesian lineages are beginning to shed light on the patterns and drivers of diversification within the region (Baker *et al.*, 1998; Schneider *et al.*, 2004; Nauheimer *et al.*, 2012).

The Malesian and West Pacific region represents one of the richest areas of palm diversity in the world. An estimated 1,200 species in 50 genera are found around in that area (Dransfield *et al.*, 2008; Baker and Couvreur, 2012). There is significant differentiation in species richness patterns throughout the Malesian region even though Malesia is regarded as palm diversity hotspot (Baker and Couvreur, 2013). In the islands of the Malesian region, majority group of palms are still neglected by the general collector and seem to display a high rate of endemism (especially the rattans).

1.3 Palms

It is estimated that there are approximately 2,600 species (Baker and Dransfield, 2016) of palms (palms belonging to family Arecaceae) spread throughout the tropical and subtropical regions of the world (Figure 1.2) (Dransfield *et al.*, 2005; Govaerts and Dransfield, 2005). They are among the most distinctive of all flowering plants, and yet their diversity of form and ecology is remarkable.

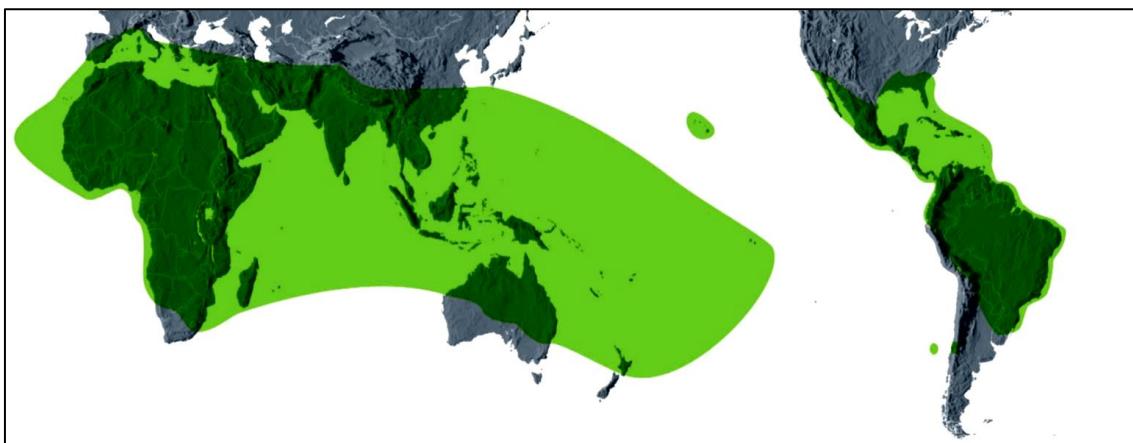


Figure 1.2 The distribution of living palms around the world. (Source: Dransfield *et al.*, 2008).

Currently, the palm family consists of five subfamilies, 28 tribes, 27 subtribes, 181 genera (Baker and Dransfield, 2016). Malesia is home to the largest palm flora, estimated to consist of about 50 genera and almost 1,200 species (Dransfield, *et al.*, 2008). The Sunda Shelf and New Guinea are hotspots for distribution of palm species richness, in comparison to the Americas which is populated by only 730 species, and Africa with only 65 species (Dransfield *et al.*, 2008).

The palms have undergone significant diversification of growth forms to include palms with clustered stems, acaulescent palms and climbing palms (Dransfield, 1978; Dransfield *et al.*, 2008; Balslev *et al.*, 2011). According to Baker *et al.* (2000), within the subfamily Calamoideae, the climbing habit has evolved several times. In climbing palms, spines are present on almost all organs (Tomlinson and Fisher, 2000). There are two unique climbing organs in climbing palms which are the flagellum (a modified, sterile inflorescence, armed with reflexed grapnel spines, which is only found in genus *Calamus* L.) and the cirrus (an extension of the leaf rachis, is whip-like, armed with reflexed grapnel spines). These organs are highly effective attachment structures for climbing (Dransfield *et al.*, 2008; Isnard and Rowe, 2008).

Most palm stems are erect and solitary or clustered. The stem is composed of three discrete regions, the epidermis, cortex and central cylinder (Tomlinson, 1990). Palm stems are characterized by having a single apical meristem or growing point, which is

also referred to as the bud or heart. There are no lateral meristems or vascular cambium in palms. In climbing palms, the stems are narrow, elongated, flexible and internodes are long (Tomlinson *et al.*, 2011). The stem can reach up to 150 m in length (Burkill, 1966).

Many palms are recognisable by the large, evergreen leaves that emerge from the top of the trunk. The leaves are either fan-shaped (palmate) or feather-shaped (pinnate), and usually arranged in a spiral at the top of the stem (Dransfield *et al.*, 2008). The leaves are usually pinnate in climbing palms and they grow along the stem instead of forming a dense crown. Sometimes the leaves are equipped with spines or marginal teeth on sheath, petiole, or blade, or on all parts (Tomlinson, 1990). The sheaths of palm leaves are sometimes elongated or tubular and are referred to as crown shafts when they appear to form a continuation from the stem.

Due to their diversity, abundance and interactions, many palms play notable ecological roles. They provide many ecosystem services and have great economic importance (Johnson, 2011; Cámara-Leret *et al.*, 2017), providing food, construction materials, and medicines, especially in tropical, rural, and poor communities (Jones, 1995; Cámara-Leret *et al.*, 2017). Coconut, date, betel nut and oil palm are some examples of major crops, but the majority of resources from palms are extracted from the wild. Besides that, rattans also have commercial value in economic importance for the cane-furniture industry (Corner, 1966; Wickens, 2001; Barfod *et al.*, 2015).

1.3.1 Palm classification and phylogeny

Genera Palmarum is a standard reference for the palm family. The work includes a classification and generic-level treatments, in addition to essays providing contextual information. In the 12 years since the first publication of *Genera Palmarum* in 1987, data, information and research on palms increased tremendously, thus even before the second edition Uhl and Dransfield (1999) made some changes to the classification,

reducing the number of genera from 200 to 189, changing the composition of tribes and subtribes, but maintaining the number of subfamilies. Six years after that, Dransfield *et al.* (2005) published a paper with another new classification of the palm family. This classification was based on phylogenetic studies. They recognised five rather than six subfamilies. Phytelephantoideae (Seemann) Drude *sensu* Uhl and Dransfield (1987) was included within Ceroxyloideae Drude (as tribe Phytelepheae Horan.). There were further significant changes: removing the tribe Caryoteae Drude from Arecoideae Brunett and placement within Coryphoideae Brunett; and removing Hyophorbeae Luerss. from Ceroxyloideae Drude and placement within Arecoideae Brunett. There were several subtribes which were recognized for the first time, such as Rhapidinae J. Dransf., N. W. Uhl, C. Asmussen, W. J. Baker, M. M. Harley & C. Lewis, Basseliinae J. Dransf., N. W. Uhl, C. Asmussen, W. J. Baker, M. M. Harley & C. Lewis, Carpoxylinae J. Dransf., N. W. Uhl, C. Asmussen, W. J. Baker, M. M. Harley & C. Lewis, Clinospermatinae J. Dransf., N. W. Uhl, C. Asmussen, W. J. Baker, M. M. Harley & C. Lewis, Rhopalostylidinae J. Dransf., N. W. Uhl, C. Asmussen, W. J. Baker, M. M. Harley & C. Lewis and Verschaffeltiinae J. Dransf., N. W. Uhl, C. Asmussen, W. J. Baker, M. M. Harley & C. Lewis. In 2008, a new *Genera Palmarum* (GP2) was published (Dransfield *et al.*, 2008). In this new classification, Arecaceae was divided into five subfamilies, 28 tribes and 27 subtribes with 183 genera. This classification was based on significant molecular phylogenetic research. Eight years after *Genera Palmarum* (GP2) was published, Baker and Dransfield (2016) updated the classification, presenting a schematic phylogenetic tree (Figure 1.3) and making some changes at genus level. Currently, 181 genera are accepted, two fewer than recognised by Dranfield *et al.* (2008).

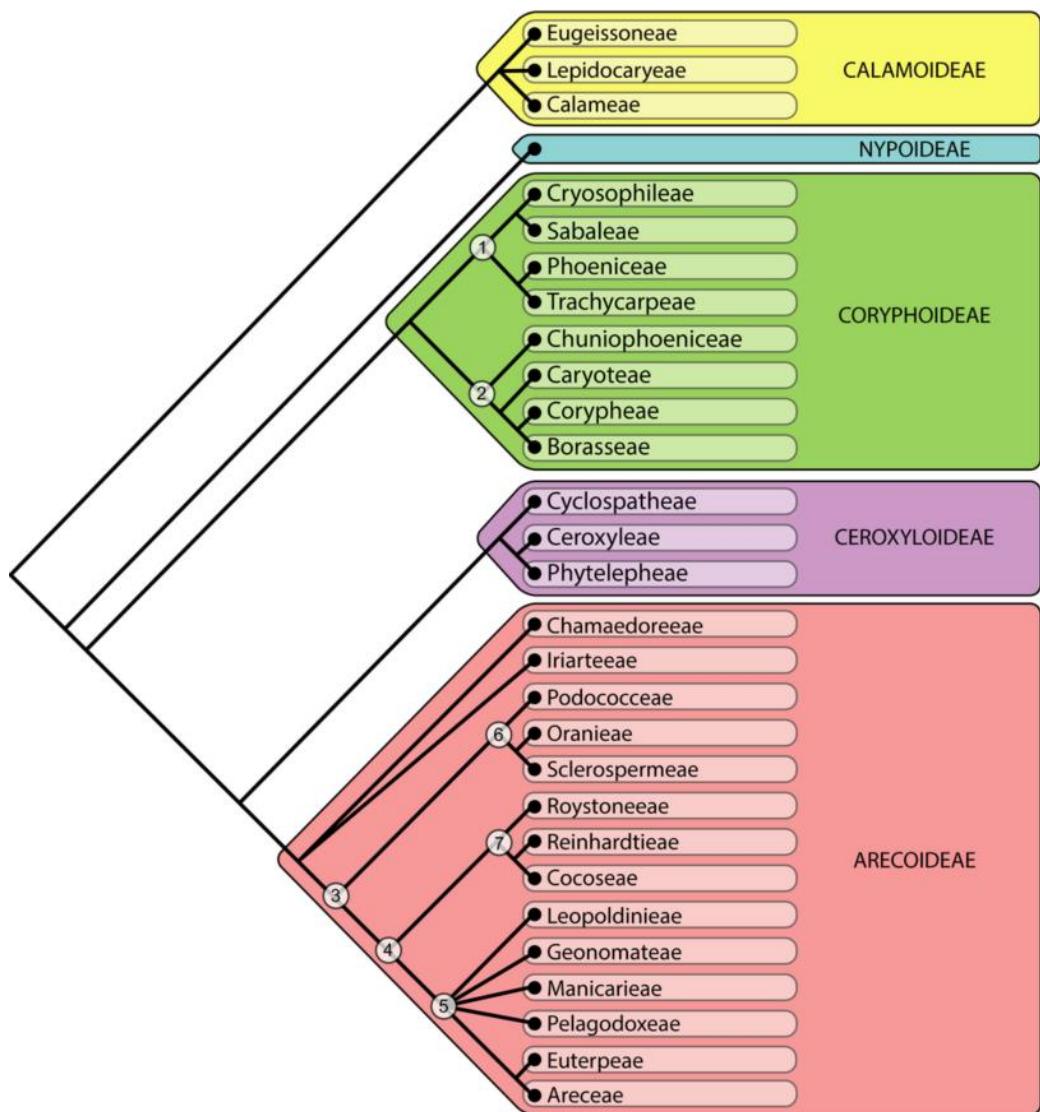


Figure 1.3 Summary schematic tree showing the phylogenetic relationships among subfamilies and tribes of the Arecaceae. (Source: Baker and Dransfield, 2016).

In 2009, Baker *et al.* published the first complete generic level phylogenetic analysis of palms and brought all major phylogenetic datasets together using a supermatrix and supertree approach. This work was considered the most prominent contribution to family-wide phylogenetics of palms since GP2 (Baker and Dransfield, 2016). The outcomes from Baker *et al.* (2009) were utilized greatly as evidence to support the GP2 classification, although they have been published after GP2. Faurby *et al.* (2016) attempted to reconstruct a species level phylogeny of all palms using model-based methods and building on the Baker *et al.* (2009) supermatrix. Fortunately, the result did

not challenge the classification in GP2 and the issues highlighted by Faurby *et al.* (2016) were already discussed by Baker *et al.* (2009).

Aside from the family-wide studies, there are a few phylogenetic studies that only focus on genera within the family including studies of *Sabal* Adans. (Zona, 1990), *Phoenix* L. (Barrow, 1998; Pintaud *et al.*, 2010), *Caryota* L. (Hahn and Sytsma, 1999), *Hyophorbe* Gaertn. (Lewis and Martinez, 2000), *Calamus* L. (Baker *et al.*, 2000a), *Asteroxyne*H.Wendl. ex Hook.f. (Stauffer *et al.*, 2003), *Chamaedorea* Willd. (Thomas *et al.*, 2006), *Lanonia* A.J. Hend. & C.D. Bacon (Henderson and Bacon, 2011), *Livistona* R.Br. (Crisp *et al.*, 2010), *Phytelepheae* Horan. (Barfod *et al.*, 2010), *Geonoma* Willd. (Henderson, 2011; Roncal *et al.*, 2011), *Astrocaryum* G.Mey. (Roncal *et al.*, 2012), *Pritchardia* Seem. & H.Wendl. (Bacon, 2012), *Ceroxylon* Bonpl. ex DC. (Sanín *et al.*, 2016) and *Johannesteijsmannia* H.E.Moore (Bacon *et al.*, 2016).

1.3.2 Subfamily Calamoideae

The Calamoideae consist of 17 genera and about 645 species, making it the second largest palm subfamily. Calamoideae are distributed throughout the wet tropical regions of the world (Figure 1.4). The group is ecologically diverse, but usually found within forest communities. *Calamus* is the largest genus in the palm family consisting of about 520 species (Baker, 2015). *Calamus* is also the most widespread genus in the subfamily, distributed throughout the Western Ghats of India to the west and Fiji to the east, but represented by single species *C. deerratus* endemic to Africa (Baker and Dransfield, 2000). Of the 17 genera, there are three endemic to Africa (*Laccosperma* (G. Mann & H. Wendl.) Drude, *Eremospatha* (G.Mann & H.Wendl.) Schaadtler and *Oncocalamus* (G. Mann & H. Wendl.) H.Wendl.) and three more to South America (*Lepidocaryum* Mart., *Mauritia* L.f. and *Mauritiella* Burret). *Raphia* P.Beauv. is mostly in Africa. In both Madagascar and South America, *Raphia* is represented by single species, *R. farinifera* in Madagascar and *R. taedigerrra* in South America. In Madagascar, there are no other genera of Calamoideae found. The remaining genera occur in Asia.

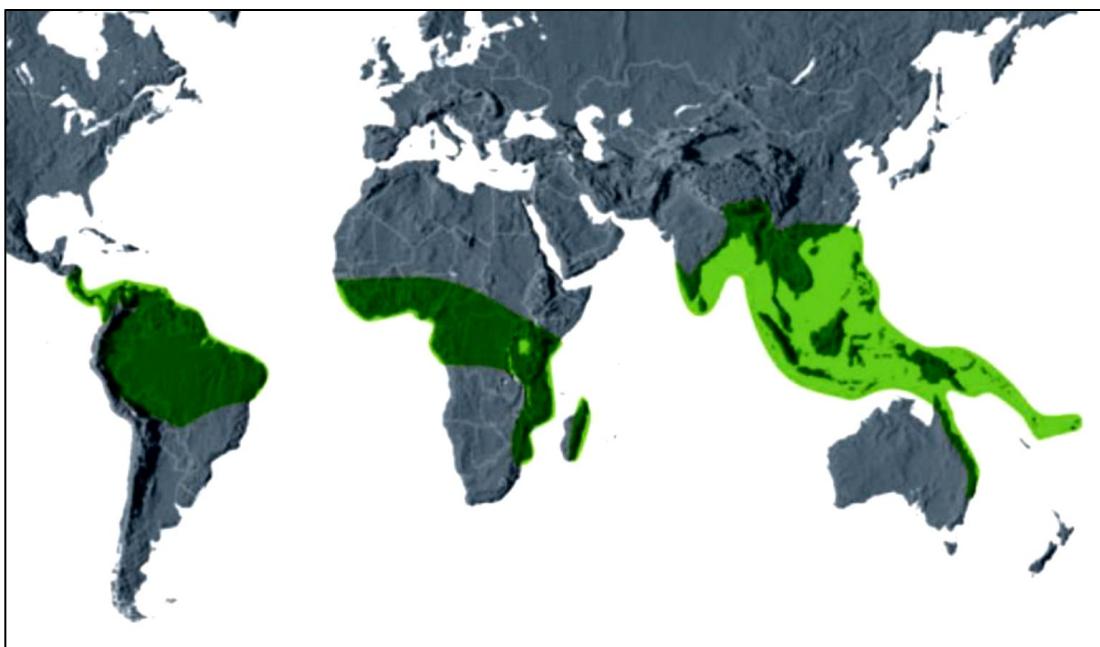


Figure 1.4 Distribution of subfamily Calamoideae. (Source: Dransfield *et al.*, 2008).

The Calamoideae includes massive tree palms, undergrowth palms and acaulescent palms but is best known for its climbing members, the rattans (Dransfield, 1978). All rattans belong to the subfamily Calamoideae and come from the Old World (Dransfield and Manokaran, 1994; Vorontsova *et al.*, 2017). The rattans (derived from the Malay word rotan) are spiny climbing palms. Climbing palms show a strong peak of species richness in Southeast Asia, unlike other palms, and this is explained by climate (present and past) and forest canopy height (Couvreur *et al.*, 2015). According to Baker *et al.* (1999b), some rattan genera include species that are not climbers, and these species are usefully called non-climbing or acaulescent rattans given their close relationship with true rattans.

The Calamoideae contains both pinnate and palmate leaved genera and includes a remarkable range of inflorescence morphology. Despite the breadth of variation observed in the Calamoideae, some unusual character states define the group, many associated with gynoecium structure (Baker *et al.* 1999b). All the species within the Calamoideae are conspicuously spiny. In some species, spines are an adaptation for climbing, but in some species especially in subtribe Mauritiinae, the spines are very inconspicuous. All the species within the Calamoideae are presence of overlapping

reflexed scales on the fruit, which make the fruit its distinctive and unique appearance (Guevara *et al.*, 2010).

All phylogenies of the palm family shows Calamoideae is resolved as a well-supported monophyletic group (e.g. Uhl *et al.*, 1995; Baker *et al.*, 1999a; Asmussen *et al.*, 2000; Asmussen and Chase, 2001, Lewis and Doyle, 2001; Hahn, 2002). Asmussen and Chase (2001) found Calamoideae sister to the rest of the palms. However, according to Uhl *et al.* (1995) and Lewis and Doyle (2001), Calamoideae resolves as sister to all palms except Nypoideae. In recent studies by Asmussen *et al.* (2006), Baker *et al.* (2009) and Faurby *et al.* (2016), Calamoideae is sister to the rest of palms with high support.

1.3.3 Genus *Korthalsia*

Korthalsia Blume is a genus of clustering climbing palms (rattans) which belong to the subfamily Calamoideae. The genus name honours the Dutch botanist and explorer Pieter Willem Korthals (1807–1892), who first collected specimens in Indonesia (Riffle and Craft, 2003). The common name of *Korthalsia* is “ant-rattan” (Uhl and Dransfield, 1987). They are 28 species of *Korthalsia* (WCSP, 2017).

The genus *Korthalsia* is distributed from the Sunda Shelf to the north of Indochina, Burma and the Andaman Islands and southeastward to Celebes and New Guinea (Figure 1.5) with three species being found east of Wallace’s Line (Dransfield, 1981). Among the 28 species, about 18 species are concentrated in the Malay Peninsula, Borneo and Sumatra—a region which appears to be the centre of the genus (Furtado, 1951). All species are limited to lowland and hill tropical rain forests and are absent in montane forest. Most species have a wide ecological range, and although plentiful in primary forest, also seem to be tolerant of disturbance: they are a conspicuous feature of old secondary forest or regenerated logged forest. It has been suggested that the hapaxanthic (individuals flowers only once in their lifetime and die subsequently) habit

may be an adaptation to colonizing secondary habitats (Dransfield, 1978). A system of inflorescences each subtended by a reduced leaves and are not typical of those of the normal vegetative phase.

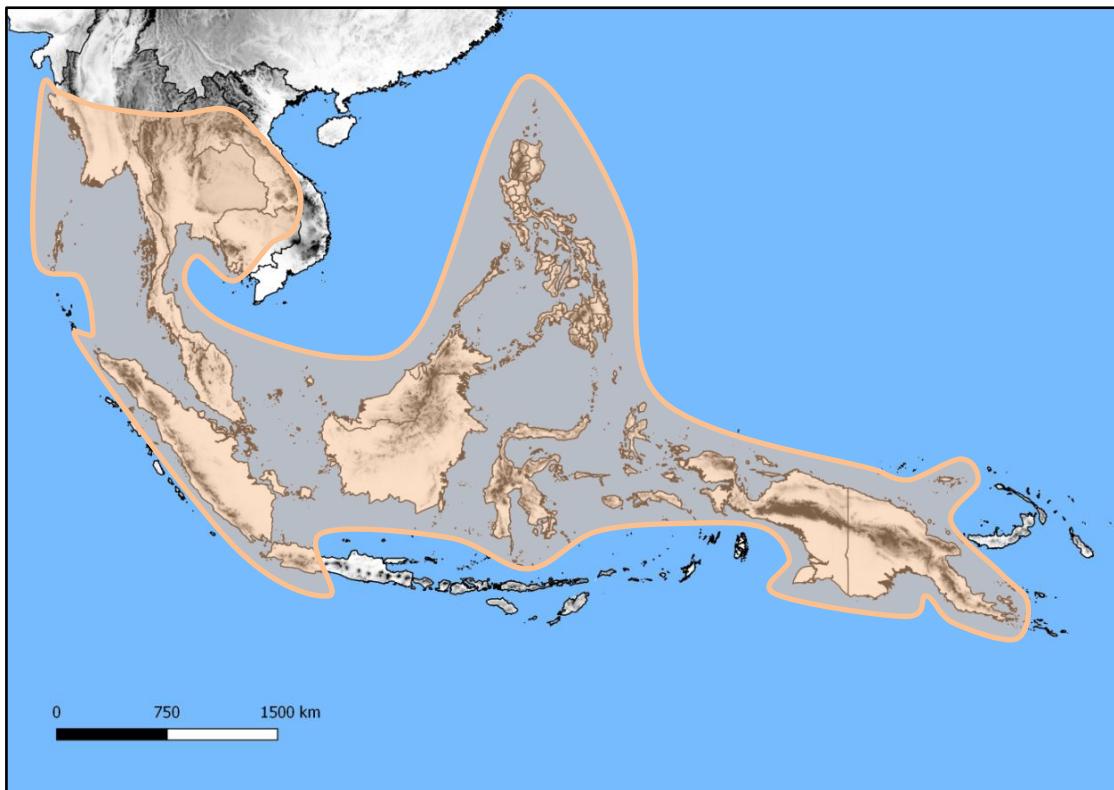


Figure 1.5 Distribution of *Korthalsia* in highlighted area based on Dransfield *et al.* (2008). Map prepared by Salwa Shahimi, using QGIS.

Korthalsia is slender to moderately-stemmed, clustered, spiny, high-climbing and aerially branching, monoecious rattan palm. The stems are hard and durable. *Korthalsia* is the only genus of Asiatic rattans which consistently branches in the canopy. In addition, the nodes are often uneven and marked with scars of branch (Dransfield, 1981). *Korthalsia* leaves are pinnate with a cirrus, variously armed with spines, sometimes splitting longitudinally opposite the petiole. Leaflets are usually rhomboid, but in a few species, are lanceolate. In many species, the leaflets are relatively few, with a single fold and carried on a short laterally flattened stalk. The ocrea is referred to an extension of the leaf sheath beyond the insertion of the petiole (Dransfield, 1981). The ocrea sometimes forms a chamber inhabited by ants (see 1.4.2). The flowering behaviour in all *Korthalsia* species is hapaxanthic. The number of nodes producing inflorescences at the tip of flowering stems rarely exceeds six (Dransfield, 1981).

Inflorescences are produced simultaneously in the axils of the uppermost reduced leaves, branching to once or twice and producing pendulous flower. The peduncle is adnate to the internode above the subtending leaf (Dransfield *et al.*, 2008). The flower of *Korthalsia* is hermaphrodite (having both male and female reproductive organs). *Korthalsia* is the only Asiatic rattan genus with a hermaphrodite flower, and in this aspect, it is similar to the hermaphrodite endemic African rattan genera, *Eremospatha* (, *Laccosperma* and *Oncocalamus*. (Dransfield, 1981). The calyx consists of three sepals while the corolla consists of three valvate petals. The calyx is usually shorter than the corolla. The flower contains 6–9 stamens. The fruits are globose to ovoid; scales arranged in regular vertical rows (Dransfield *et al.*, 2008). The fruits of most species appear to be attractive to animals because of sweet thin-fleshy layer surrounding the seed (Dransfield, 1981).

To date, there is no phylogenetic study of *Korthalsia*. Most higher-level studies use one species to represent the genus (Asmussen *et al.*, 2006; Baker *et al.*, 2009; Faurby *et al.*, 2016). Furthermore, the recent taxonomic account of *Korthalsia* was made by Dransfield (1981). Although in this account he updated descriptions with new information for the established species, it lacks distribution maps, some quantitative data and list of specimens examined. After 1981, there are several species that have been covered in different taxonomic account; The rattan of Sabah (Dransfield, 1984), The rattan of Sarawak (Dransfield, 1992), The rattan of Brunei Darussalam (Dransfield, 1997), A field guide to Philippine rattan (Lapis, 2010) and Arecaceae (Palmae) (Barfod and Dransfield, 2013).

1.4 Ant-plant relationships

In a tropical forest, plants known as ant-plants, or myrmecophytes, are involved with ants in tight, often obligatory relationships. The symbioses of ant-plants have figured prominently in ecology as classic examples of mutualism that coevolved for over 100 million years (Mayer *et al.*, 2014). Ant-plant interactions are geographically widespread and vary in specificity, ranging from mutualistically symbiotic relationships that

provide benefit to both partners to purely antagonistic ones, where ants exploit plants (extensively reviewed by Rico-Gray and Oliveira, 2007; Beattie, 2010). Some of these plants have structures that host the ant colonies, called domatia. These are specialised hollow structures (Bailey, 1924; Brouat and McKey, 2000; Heil and McKey, 2003; Edwards *et al.*, 2006). Stems, thorns, stipules, petioles or leaves are the structures that are involved, depending on the plant species. These plants may produce extrafloral nectar (honeydew) and/or food bodies to sustain their ant colony and to stabilise these obligate mutualisms (Chomicki *et al.*, 2016). The ants get benefit from the food source, increase their colony size and often farming of fungi, scale insects and/or aphids occurs in the domatia (Janzen, 1966; Miler *et al.*, 2016). In these mutualistic interactions, ants contribute to protecting the plant against herbivores, fungal pathogens and competing plants (Miler *et al.*, 2016). The plant also gets nutrients from decomposed matter left behind by the ants in the chamber within the stem (Plumber, 2000). The common ant genera that inhabit domatia include *Camponotus* Mayr (Formicinae), *Iridomyrmex* Mayr and *Azteca* Forel (Dolichoderinae), *Pheidole* Westwood and *Crematogaster* Lund (Myrmicinae), *Pachycondyla* Smith and *Odontomachus* Latreille (Ponerinae) and *Pseudomyrmex* Lund (Pseudomyrmecinae) (Beattie, 1989).

The study of ant-plant interactions offers an excellent opportunity to analyze the effects of both historical and ecological factors on the evolution of mutualisms. Mutualisms have been of interest to researchers for centuries because they are good examples to provide a better understanding of co-evolution (Darwin, 1862, 1872; Janzen, 1966; Rico-Gray and Oliveira, 2007; Blatrix *et al.*, 2009). Questions of general interest in ecology and evolutionary biology can be addressed using ant-plant mutualism as a model system (Feldhaar *et al.*, 2000; Mayer *et al.*, 2014).

1.4.1 Ant-rattan relationships

Interactions between ants and rattans have frequently been described (Holtum, 1969; Dransfield, 1984; Höldobler and Wilson, 1990; Dransfield and Manokaran, 1994; Sunderland, 2004; Miler *et al.*, 2016). There are several morphological adaptations

providing nesting places for ants within several species of rattans such as inflated leaf sheath extensions, interlocking spine combs that form galleries, reflexed leaflets that tightly clasp the stem, or hollowed out acanthophylls (thorn-like organs) (Dransfield, 2001). Figure 1.6 shows the distribution of rattans with the domatia occupied by ants. The extension of leaf sheath beyond petiole insertion is called the ocrea. The ocrea are morphologically diverse, ranging from a short, collar-like rim (e.g. *Calamus australis*), inflated chamber (e.g. *Korthalsia echinometra*) or to an elongate papery structure of up to 1.5 meters in length (e.g., *Calamus paspalanthus*) (Merklinger *et al.*, 2014).

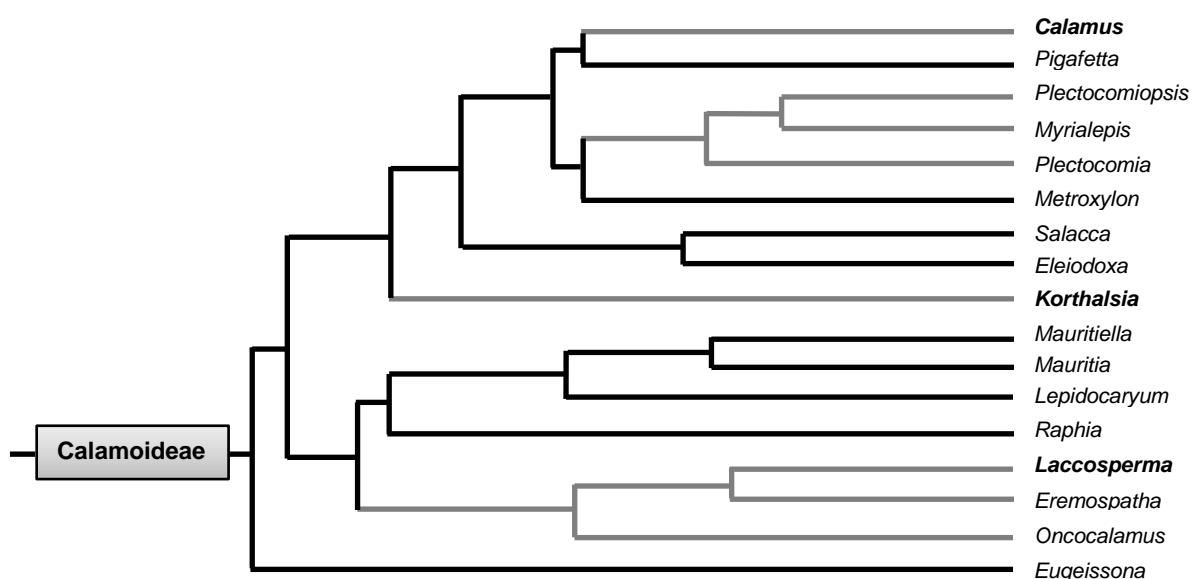


Figure 1.6 Phylogenetic distribution of myrmecophytic rattans. Grey branches indicate rattans. The distribution of genera with well-developed ocrea and inhabited by ants are indicated with bold. Image is redrawn from Merklinger *et al.* (2014).

Many species of rattans in the Malesian region are associated with ants (Beccari, 1884; Dransfield, 1979, 1984; Ridley, 1910; Zizka, 1990). Perhaps the most conspicuous or best known belong to genus *Korthalsia*. Indeed the commonly used name for the genus is “ant-rattan”. There is a symbiotic relationship between ants and aphids within the ocrea.

1.4.2 Ants and *Korthalsia*

Among the 28 species of *Korthalsia* (WCSP, 2017), there are 10 species of *Korthalsia* which have an association with ants (Dransfield, 1981; Mattes *et al.*, 1998; Chan *et al.*, 2012). All of the species with ant associations have modified ocrea serving as domatia. In *Korthalsia*, there is a diversity of ocrea forms. Dransfield (1981) classified the ocreas of *Korthalsia* into four types: inflated, divergent, tightly sheathing and fibrous net-like types (Figure 1.7). The first two have ant associations, whereas the latter two have no ant relationships.

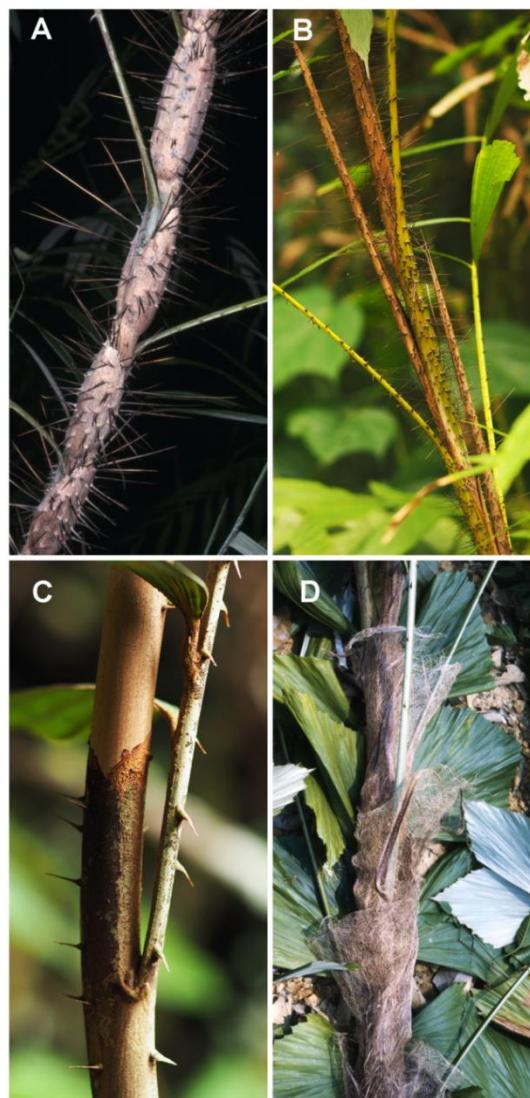


Figure 1.7 Example types of ocrea. A) *Korthalsia echinometra* Becc., inflated ocrea (ants); B) *Korthalsia hispida* Blume, divergent ocrea (ants); C) *Korthalsia debilis* Blume, tightly sheathing ocrea; D) *Korthalsia jala* J.Dransf., conspicuous net-like ocrea. Photographs© Salwa Shahimi, John Dransfield.

There have been several studies of the ant association of *Korthalsia* and the biology and the ecology. The rattan receives protection against predators from the ants (e.g., Ridley, 1910; Dransfield, 1979, 1984, 1992; Mattes *et al.*, 1998; Edwards *et al.*, 2010; Chan *et al.*, 2012). Edwards *et al.* (2010) observed levels of cooperativeness between two different genera of ants that associated with *Korthalsia furtadoana*, *Camponotus* sp. and *Crematogaster* sp., and they conclude that despite some species being mutualistic, other ants can also be parasitic, providing no benefit to the rattan. Table 1.1 shows the *Korthalsia* species which have ant associations.

Table 1.1 List of all *Korthalsia* species which have ant associations. The references identifying ant species are cited.

Species	Notes
<i>Korthalsia angustifolia</i> Blume	Observation by Dransfield, 1974
<i>Korthalsia cheb</i> Becc.	Observation by Dransfield, 1992
<i>Korthalsia echinometra</i> Becc.	<i>Camponotus</i> sp. and <i>Iridomyrmex</i> sp. (Chan <i>et al.</i> , 2012); Observation by Dransfield, 1997
<i>Korthalsia furcata</i> Becc.	Observation by Dransfield, 1992
<i>Korthalsia furtadoana</i> J.Dransf.	<i>Camponotus</i> sp. and <i>Polycharchis</i> sp. (Chan <i>et al.</i> , 2012); <i>Camponotus</i> sp. and <i>Crematogaster</i> sp. (Edwards <i>et al.</i> , 2010); Observation by Dransfield, 1997
<i>Korthalsia hispida</i> Becc.	<i>Camponotus</i> sp. (Mattes <i>et al.</i> , 1998); Observation by Dransfield, 1997
<i>Korthalsia robusta</i> Blume	<i>Camponotus</i> sp. (Mattes <i>et al.</i> , 1998); <i>Camponotus</i> sp. and <i>Dolichoderus</i> sp. (Chan <i>et al.</i> , 2012); Observation by Dransfield, 1992
<i>Korthalsia rostrata</i> Blume	<i>Dolichoderus</i> sp. and <i>Philidris</i> sp. (Chan <i>et al.</i> , 2012); Observation by Dransfield, 1997
<i>Korthalsia scaphigeroides</i> Becc.	Observation by de Guzman and Fernando, 1986
<i>Korthalsia scorchedinii</i> Becc.	Observation by Dransfield, 1979

The farming scale of aphids by the ants in *Korthalsia* domatia has also been studied. Mattes *et al.* (1998) studied the myrmecophytic association between *Korthalsia robusta* and its ant and aphid partners, and found that nutrition from the honeydew that the ants receive is dependent on their association with aphids. *Cerataphis* aphids responded to

Campponotus ants by increasing the amount of honeydew and maximize excretion role significantly (Mattes *et al.*, 1998). According to Chan *et al.* (2012), the ant-hemipteran association in *Korthalsia echinometra* and *K. rostrata* is between species of *Iridomyrmex* ants and the aphid *Cerataphis orchidearum aptera*. The aphids produce the honeydew from rattan phloem cells for the ants can to feed on.

1.5 Aims and thesis structure

This thesis takes three different approaches to better understanding genus *Korthalsia*, molecular phylogeny, anatomy and morphology and alpha taxonomy. Chapter 2 consists of a molecular study of the subfamily Calamoideae based on DNA sequences data from three chloroplast regions (*rps16*, *trnD-trnT* and *ndhF*) and nuclear regions (*prk*, *rpb2* and ITS). Both Maximum Likelihood and Bayesian Inference analyses are presented. Chapter 3 explores homologies of the *Korthalsia* ocrea, using complementary developmental and phylogenetic approaches. Chapter 4 focuses on 10 species *Korthalsia* which have ant-association, using data from herbaria and the field to produce a full taxonomic revision.

CHAPTER 2

A PHYLOGENETIC FRAMEWORK OF THE CALAMOIDEAE

2.1 Introduction

An increasing number of taxonomies are being produced based upon a backbone of molecular data and molecular phylogenies are also informing our understanding of evolutionary change. Molecular phylogenetic methodologies are being used to investigate taxonomic problems and address evolutionary questions at all levels, from the origin and higher level systematics of the angiosperms (Zeng *et al.*, 2014; Magallón *et al.*, 2015) to processes in populations (Sukumaran and Knowles, 2017). These methodologies are now treated as important sources of novel hypotheses within many biological disciplines. Furthermore, it is well established that molecular phylogenies are a generally reliable source upon which to build classifications.

Most classical taxonomists are also morphologists, identifying plants by their external, physical attributes and grouping them based on inferred similarities and hypothesized developmental series. The majority of morphologists acknowledge the limitations of such work; intuitively such researchers often ‘know’ there is more to a taxonomic problem than meets the eye (literally), but cannot provide a definitive answer due to limitations of morphology and its interpretation. As decisions about classification are now made based on phylogenies, there are sometimes conflicts between morphological characters and molecular phylogenies regarding the decisions on classification (Schmidt-Lebuhn, 2012). Increasingly, classifications are based on molecular phylogenies, and new characters are sought to support novel taxa (Pennington and Gemeinholzer, 2000).

The classification of palms, like many plant families have been significantly revised in the light of molecular phylogeny. In 1995, Uhl *et al.* conduct the first molecular study of palms to examine representatives of 67 taxa from all tribes (*sensu* Dransfield and

Uhl, 1986), including 10 from subfamily Arecoideae by using morphological data and chloroplast restriction fragment length polymorphisms (RFLP). Molecular systematics research of the Arecaceae is affected by one general quality of palm molecular evolution: palm DNA evolves slowly. There is extreme heterogeneity in the plastid substitution rates across the commelinid orders (Barrett *et al.*, 2016). Slow rates for palms were identified as early as 1990, according to Wilson *et al.* (1990), in chloroplast DNA, the substitution rate estimate from restriction site variation was found to be 5- to 13-fold slower than the rate estimate for grasses. This finding was confirmed by Gaut *et al.* (1992), who found that the rate in the chloroplast-encoded gene *rbcL* in grasses was five times faster than that in palms. The average for palms was 0.009 substitutions per site per year. The estimated substitution rate between *Calamus* (Calamoideae) is 1.3×10^{-10} substitutions per site per year compared to the other palms and 5.2×10^{-11} for *Ceroxylon* (Ceroxyloideae) (Comer *et al.*, 2015). The estimates were calculated based on fossil data (Daghlian, 1981; Muller, 1981). Clegg *et al.* (1994) found that the grass family (Poaceae), Bromeliales, Liliales, and Orchidales have higher substitution rates compared to palms. Barrett *et al.* (2016) surveyed whole plastomes to identify rate heterogeneity, noting decelerations in the palms. These studies confirmed the suspicions of palm systematists, whose phylogenetic trees suffered from a lack of resolution and/or support. One partial solution to the problem has been to combine several data sets (Asmussen *et al.*, 2000; Baker *et al.*, 2000, 2000a, Asmussen and Chase, 2001; Hahn, 2002a). The first studies combined chloroplast DNA genes and nuclear ribosomal genes to provide insight into higher-level palm systematic studies.

Another area of emphasis has been finding new genes, particularly nuclear genes, for use in phylogeny reconstruction. The nuclear genome is many times larger and potentially contains many genes of phylogenetic utility than the chloroplast, at all taxonomic levels. Studies by Sang (2002), Mort and Crawford (2004) and Small *et al.* (2004), suggest that low-copy nuclear DNA regions hold valuable phylogenetic information and have several advantages over chloroplast DNA; particularly, when applied to low-level systematic studies of plants. According to Thomas *et al.* (2006), the low-copy nuclear DNA regions are sufficiently variable and informative within genus *Chamaedorea* compare to Hahn (2000a) which he used plastid marker in his

study on genera of Arecoid Line. Based on these studies, it suggests that nuclear DNA is valuable tools for investigating phylogenetic signal at low taxonomic levels in palms.

All phylogenetic studies for subfamily Calamoideae based on morphological and molecular data for the entire palm family strongly support Calamoideae as monophyletic (Uhl *et al.*, 1995; Baker *et al.*, 1999a;asmussen *et al.*, 2000; Asmussen and Chase, 2001, Lewis and Doyle, 2001; Hahn, 2002; Asmussen *et al.*, 2006; Baker *et al.*, 2009; Faurby *et al.*, 2016). The calamoid palms have been investigated in depth, and some realignment of the internal structure of the subfamily has been achieved (Baker *et al.*, 1999b, 2000, 2000a, 2000b). Nevertheless, there are outstanding questions regarding relationships within the subfamily.

2.1.1 Objectives

The first objective of this study is to use DNA sequence from three plastid regions (*rps16*, *trnD-trnT* and *ndhF*) and three nuclear regions (*prk*, *rpb2* and ITS) to generate phylogenetic relationships for the Calamoideae with an emphasis on the genus *Korthalsia*. This new phylogeny will allow some question regarding the relationships within the subfamily of Calamoideae to be answered. Specifically I ask whether *Korthalsia* is monophyletic and what its relationships within tribe Calameae are. In subsequent chapters, this phylogeny will be used to investigate morphological ocrea evolution of *Korthalsia*.

At the outset of this study Dr. W. Baker provided sequences that had not been included in published studies nor uploaded onto GenBank. This chapter includes published data from GenBank for six regions (*rps16*, *trnD-trnT*, *ndhF*, *prk*, *rpb2* and ITS), as well as unpublished data for two regions (*prk* and *rpb2*) and data I have generated myself for this study for four regions (*rps16*, *trnD-trnT*, *prk*, *rpb2*).

2.2 Material and methods

2.2.1 Taxon sampling

Representatives of all three tribes and nine subtribes of subfamily Calamoideae were included in this study. Nine outgroups were selected from the four remaining subfamilies, Nypoideae, Coryphoideae, Ceroxyloideae and Arecoideae. All samples are vouchered in herbarium collections. Table 2.1 shows the list of specimens included in the molecular study.

2.2.2 DNA isolation

Silica gel-dried leaf materials were used for DNA extraction (Chase and Hills, 1991). Approximately 0.020–0.025 gram of silica-gel dried material was ground into a thick slurry using pestle and mortal. All DNA extractions used a modified Cetyltrimethylammonium bromide (CTAB) protocol from Doyle and Dickson (1987) (Appendix 2.1). The extracted DNA was then stored in 40 µl of TE buffer at -20°C for subsequent use.

2.2.3 DNA amplification and purification

Templates for Polymerase Chain Reaction (PCR) amplification consist of total genomic DNA. Standard PCR protocols were used to amplify four regions (*prk*, *rpb2*, *rps16* and *trnD-trnT*) and was performed in 20 µl reaction mixtures containing 10 µl BioMix™ of from Bioline, 0.50 µl of Bovine Serum Albumin (BSA), 0.75 µl of 10 µM of each primer: forward and reverse, 6 µl of Milli-Q dH₂O and 2 µl of 50 to 100ng/µl template DNA. Bovine Serum Albumin (BSA) was added to remove phenolics which can have an inhibiting effect on the reaction. The primers used are listed in Table 2.2. All PCR reactions were carried out in Biometra Tgradient thermal cycler. The thermal profiles were unique for each region and are listed in Table 2.3. On completion, a 2 µl of PCR

product was run on a 1% agarose gel made up in 1 x TAE buffer with a molecular weight marker to find out whether or not reaction had been successful and to ascertain the size of PCR products. DNA bands were visualized using UV transilluminator. Reactions were cleaned using QIAquick PCR Purification kit from QIAGEN according to the manufacturer's instruction with the final elution of 30 μ l EB buffer.

Table 2.1 List of specimens used in this study. Voucher specimens indicate the collector and collector number of specimens, and the herbarium where the specimen is deposited (abbreviation following Index Herbariorum). GenBank Accession Numbers are provided where sequences were deposited at the outset of this study. WJB in this field indicates that sequences not yet deposited were provided by Dr. W. Baker; SS indicates sequences generated for this study by Salwa Shahimi.

Species	Voucher specimens	Collection locality	GenBank Accession No.					
			<i>prk</i>	<i>rpb2</i>	ITS	<i>rps16</i>	<i>trnD-trnT</i>	<i>ndhF</i>
ingroup								
<i>Calamus aruensis</i> Becc.	Dransfield, JD7571 (K)	Papua, Indonesia	AM900751	AM903105	AJ242053	AJ241279	EU117438	EU186191
<i>Calamus castaneus</i> Griff.	Baker, WJB507 (KEP)	Malaysia	-	WJB	AJ242047	AJ242155	EU117439	EU186192
<i>Calamus calospathus</i> (Ridl.) W.J.Baker & J.dransf	1990-2783 (K)	Not mention	WJB	WJB	AJ242066	AJ242161	EU117444	EU186197
<i>Calamus concolor</i> (Blume) W.J.Baker	Baker, WJB559 (K)	Sabah, Malaysia	-	WJB	AJ242068	AJ242162	EU117446	EU186199
<i>Calamus conirostris</i> Becc.	Baker, WJB516 (K)	Sarawak, Malaysia	WJB	WJB	AJ242048	AJ242156	EU117440	EU186193
<i>Calamus deerratus</i> G.Mann & H.Wendl.	Tsiforkor, s.n. (K)	Ghana	-	WJB	AJ242051	AJ242157	EU117441	EU186194
<i>Calamus didymophyllus</i> (Becc.) Ridl.	Baker, WJB692 (K)	Sarawak Malaysia	WJB	WJB	AJ242070	AJ242165	EU117436	EU186190
<i>Calamus dumetosus</i> (Dransfield) Henderson & Fonda	Baker, WJB530 (K)	Temburong, Brunei	WJB	WJB	AJ242081	AJ242166	EU117447	EU186200
<i>Calamus fissus</i> (Blume) Miq.	Baker, WJB546 (K)	Sabah, Malaysia	WJB	WJB	AJ242074	AJ242164	EU117437	EU186189

Table 2.1 continued

Species	Voucher specimens	Collection locality	GenBank Accession No.					
			prk	rpb2	ITS	rps16	trnD-trnT	ndhF
<i>Calamus nanodendron</i> J.Dransf.	Baker, WJB720 (K)	Sarawak, Malaysia	WJB	WJB	AJ242062	AJ242154	EU117442	EU186195
<i>Calamus thysanolepis</i> Hance	Baker & Utteridge 13 (K)	Hong Kong, China	WJB	WJB	AJ242064	AJ242158	EU117443	EU186196
<i>Calamus ursinus</i> (Becc.) W.J.Baker	Baker, WJB517 (K)	Sarawak, Malaysia	-	WJB	AJ242076	AJ242163	EU117445	EU186198
<i>Eleiodoxa conferta</i> (Griff.) Burret	Dransfield, JD6514 (K)	Sarawak, Malaysia	WJB	WJB	AJ242092	AJ242179	EU117432	EU186185
<i>Eremospatha wendlandiana</i> Becc.	Dransfield, JD7004 (K)	Not mention	FR729730	FR729729	AJ242129	AJ240868	EU117426	EU186180
<i>Eugeissona tristis</i> Griff.	Baker, WJB501 (K)	Malaysia	WJB	WJB	AJ242116	AJ240869	EU117427	-
<i>Eugeissona utilis</i> Becc.	Baker, WJB712 (SAR)	Sarawak, Malaysia	WJB	WJB	AJ242119	AJ242180	EU117428	EU186181
<i>Korthalsia cheb</i> Becc.	Baker, WJB513 (K)	Sarawak, Malaysia	WJB	WJB	AJ242101	AJ242175	EU117431	EU186184
<i>Korthalsia concolor</i> Burret	Baker, WJB562 (K)	Sabah, Malaysia	SS	SS	-	SS	SS	-
<i>Korthalsia debilis</i> Blume	Shahimi, SS28 (K)	Sarawak Malaysia	SS	SS	-	SS	SS	-
<i>Korthalsia debilis</i> Blume	Baker, WJB526 (K)	Temburong, Brunei	SS	SS	-	-	SS	-

Table 2.1 continued

Species	Voucher specimens	Collection locality	GenBank Accession No.					
			prk	rpb2	ITS	rps16	trnD-trnT	ndhF
<i>Korthalsia echinometra</i> Becc.	Shahimi, SS9 (K)	Terengganu, Malaysia	SS	SS	-	SS	SS	-
<i>Korthalsia echinometra</i> Becc.	Shahimi, SS10 (K)	Terengganu, Malaysia	SS	SS	-	SS	SS	-
<i>Korthalsia ferox</i> Becc.	Baker, WJB515 (K)	Sarawak, Malaysia	SS	-	-	SS	SS	-
<i>Korthalsia flagellaris</i> Miq.	Shahimi, SS3 (K)	Negeri Sembilan, Malaysia	SS	-	-	SS	SS	-
<i>Korthalsia flagellaris</i> Miq.	Shahimi, SS20 (K)	Nee Soon Swamp Forest, Singapore	SS	SS	-	SS	SS	-
<i>Korthalsia furtadoana</i> J.Dransf.	Baker, WJB553 (K)	Sabah, Malaysia	SS	SS	-	SS	SS	-
<i>Korthalsia hispida</i> Becc.	Shahimi, SS11 (K)	Johor, Malaysia	SS	SS	-	-	SS	-
<i>Korthalsia hispida</i> Becc.	Shahimi, SS12 (K)	Johor, Malaysia	SS	SS	-	-	SS	-
<i>Korthalsia jala</i> J.Dransf.	Baker, WJB558 (K)	Sabah, Malaysia	SS	SS	AJ242104	SS	SS	-
<i>Korthalsia laciniosa</i> (Griff.) Mart	Henderson, H3610 (NY)	Binh Thuan Province, Vietnam	SS	SS	-	SS	SS	-
<i>Korthalsia laciniosa</i> (Griff.) Mart	Henderson, H3955 (NY)	Taninthayi, Myanmar	SS	SS	-	SS	SS	-
<i>Korthalsia lanceolata</i> J.Dransf.	Baker, WJB504 (K)	Selangor, Malaysia	SS	SS	-	-	SS	-

Table 2.1 continued

Species	Voucher specimens	Collection locality	GenBank Accession No.					
			prk	rpb2	ITS	rps16	trnD-trnT	ndhF
<i>Korthalsia minor</i> A.J.Hend. & N.Q.Dung	Henderson, H3390 (NY)	Dong Nai Province, Vietnam	SS	SS	-	SS	SS	-
<i>Korthalsia minor</i> A.J.Hend. & N.Q.Dung	Henderson, H3632 (NY)	Binh Thuan Province, Vietnam	SS	-	-	SS	SS	-
<i>Korthalsia rigida</i> Blume	Shahimi, SS2 (K)	Negeri Sembilan, Malaysia	SS	SS	-	SS	SS	-
<i>Korthalsia rigida</i> Blume	Shahimi, SS18 (K)	MacRitchie Reservoir, Singapore	SS	SS	-	SS	SS	-
<i>Korthalsia robusta</i> Blume	Shahimi, SS26 (K)	Sarawak, Malaysia	SS	SS	-	SS	SS	-
<i>Korthalsia rostrata</i> Blume	Shahimi, SS25 (K)	MacRitchie Reservoir, Singapore	SS	SS	-	SS	SS	-
<i>Korthalsia rostrata</i> Blume	Shahimi, SS27 (K)	Sarawak, Malaysia	SS	SS	-	SS	SS	-
<i>Korthalsia scortechinii</i> Becc.	Shahimi, SS15 (K)	Penang, Malaysia	SS	SS	-	SS	SS	-
<i>Korthalsia</i> sp.	Henderson, H3990	Andaman Islands	SS	SS	-	SS	SS	-
<i>Korthalsia tenuissima</i> Becc.	Shahimi, SS14 (K)	Penang, Malaysia	SS	SS	-	SS	SS	-
<i>Korthalsia zippelii</i> Blume	Utteridge, TU20 (K)	Madang, Papua New Guinea	SS	SS	-	SS	SS	-
<i>Korthalsia zippelii</i> Blume	Maturbongs, RAM98 (K)	Papua, Indonesia	SS	SS	-	SS	SS	-

Table 2.1 continued

Species	Voucher specimens	Collection locality	GenBank Accession No.					
			<i>prk</i>	<i>rpb2</i>	<i>ITS</i>	<i>rps16</i>	<i>trnD-trnT</i>	<i>ndhF</i>
<i>Laccosperma acutiflorum</i> (Becc.) J.Dransf.	Dransfield, JD7006 (K)	Not mention	WJB	WJB	AJ242122	AJ240867	EU117424	EU186178
<i>Laccosperma opacum</i> (G. Mann & H. Wendl.) Drude	Sunderland 1750 (K)	SW Province, Cameroon	WJB	WJB	AJ242125	AJ242181	EU117425	EU186179
<i>Lepidocaryum tenue</i> Mart.	Dransfield, JD7012 (K)	Ucayali River, Peru	WJB	WJB	AJ242140	AJ242182	EU117457	EU186210
<i>Mauritia flexuosa</i> L.f.	Ely et al. 17 (K)	Nicolas Suarez Province, U.S.A	WJB	WJB	AJ242141	AJ240872	EU117456	EU186209
<i>Mauritiella armata</i> (Mart.) Burret	Henderson s.n. (K)	Brazil	WJB	WJB	AJ242146	AJ242183	-	-
<i>Metroxylon sagu</i> Rottb.	Baker, WJB550 (SAN)	Sabah, Malaysia	WJB	WJB	AJ242105	AJ242174	EU117429	EU186182
<i>Metroxylon salomonense</i> (Warb.) Becc.	Zona 651 (FTG)	Western Province, Solomon Islands	-	WJB	AJ242107	AJ242173	EU117430	EU186183
<i>Myrialepis paradoxa</i> (Kurz) J.Dransf.	Baker, WJB491 (KEP)	Selangor, Malaysia	WJB	WJB	AJ242083	AJ242169	EU117448	EU186201
<i>Oncocalamus tuleyi</i> Sunderl.	Sunderland 1759 (K)	SW Province, Cameroon	WJB	WJB	WJB	WJB	EU117455	EU186208
<i>Pigafetta elata</i> (Mart.) H.Wendl.	Baker, WJB508 (K)	Kuala Lumpur, Malaysia	WJB	WJB	AJ242112	AJ242171	EU117452	EU186205
<i>Pigafetta filaris</i> (Giseke) Becc.	Dransfield, JD7610 (K)	Papua, Indonesia	-	WJB	AJ242114	AJ242172	EU117453	EU186206

Table 2.1 continued

Species	Voucher specimens	Collection locality	GenBank Accession No.					
			prk	rpb2	ITS	rps16	trnD-trnT	ndhF
<i>Plectocomia elongata</i> Mart. ex Blume	1984-4821 (K)	Royal Botanic Gardens Kew, U.K	WJB	WJB	AJ242085	AJ242167	EU117450	EU186203
<i>Plectocomia mulleri</i> Blume	Baker, WJB563 (K)	Sabah, Malaysia	WJB	WJB	AJ242087	AJ242168	EU117451	EU186204
<i>Plectocomiopsis geminiflora</i> (Griff.) Becc.	Baker, WJB492 (K)	Pahang, Malaysia	WJB	WJB	AJ242089	AJ242170	EU117449	EU186202
<i>Raphia farinifera</i> (Gaertn.) Hyl.	Rutherford 156 (K)	Cultivated	WJB	WJB	AJ242131	AJ242184	EU117454	EU186207
<i>Salacca glabrescens</i> Griff.	1984-3791 (K)	Royal Botanic Gardens Kew, U.K	WJB	WJB	AJ242095	AJ242177	EU117433	EU186186
<i>Salacca ramosiana</i> J.P.Mogea	1979-4409 (K)	Royal Botanic Gardens Kew, U.K	WJB	WJB	AJ242097	AJ242176	EU117434	EU186187
<i>Salacca rupicola</i> J.Dransf.	Baker, WJB710 (K)	Sarawak, Malaysia	WJB	WJB	AJ242099	AJ242178	EU117435	EU186188
Outgroups								
<i>Areca triandra</i> Roxb. ex Buch.-Ham.	1984-2295	Not mention	AY348912	AY543115	AB271413	AJ404945	EU117464	AY044535
<i>Asteroxyne martiana</i> (H.Wendl.) H.Wendl. ex Hemsl	L-81.0284 (BH)	Not mention	AF453334	AJ830154	AJ242152	AJ241314	-	-

Table 2.1 continued

Species	Voucher specimens	Collection locality	GenBank Accession No.					
			prk	rpb2	ITS	rps16	trnD-trnT	ndhF
<i>Caryota mitis</i> Lour.	464-85.05037	Royal Botanic Gardens Kew, U.K	AF453338	-	-	AJ240883	EU117461	AY044531
<i>Ceroxylon quindiuense</i> (H. Karst) H.Wendl.	1976-1160 (K)	Royal Botanic Gardens Kew, U.K	AJ831349	AJ830157	AJ242150	AJ241284	EU117459	EU186212
<i>Chamaerops humilis</i> L.	CEL99_012	Not mention	AF453339	AY543097	-	AM116777	EU117460	DQ273117
<i>Kentiopsis oliviformis</i> (Brongn. & Gris) Brongn.	Pintaud358	Not mention	AF453353	AY543100	-	AJ240892	-	-
<i>Kerriodoxa elegans</i> J.Dransf.	1987-2685 (K)	Royal Botanic Gardens Kew, U.K	AJ831355	AJ830170	AJ242148	AJ241270	EU117458	EU186211
<i>Lodoicea maldivica</i> (J.F.Gmel.) Pers. ex H.Wendl.	1994-3231	Royal Botanic Gardens Kew, U.K	AF453357	-	-	AJ240864	EU117462	EU186215
<i>Nypa fruticans</i> Wurmb.	Baker, WJB512 (SAR)	Sarawak, Malaysia	-	AJ830174	-	AJ242185	EU117463	AY044525

Table 2.2 List of primers used for amplification of all samples.

Region	Primer Name	Primer Sequence (5' to 3')	References
<i>prk</i>	PRKF	GTGATATGGAAGAACGTGG	Lewis and Doyle (2002); Roncal <i>et al.</i> (2005)
	PRKR	ATTCCAGGGTATGAGCAGC	
<i>rpb2</i>	RPB2F	CAACTTATTGAGTCATCATGG	Lewis and Doyle (2002); Roncal <i>et al.</i> (2005)
	RPB2R	CCACGCATCTGATATCCAC	
<i>rps16</i>	RPS16F	GTGGTAGAAAGCAACGTGCGACTT	Oxelman <i>et al.</i>
	RPS16R	TCGGGATCGAACATCAATTGCAAC	(1997)
<i>trnD-</i>	trnD	ACCAATTGAAC TACAATCCC	Demesure <i>et</i>
<i>trnT</i>	trnT	CTACC ACTGAGTTAAAAGGG	<i>al.</i> (1995)

Table 2.3 PCR profiles.

	<i>prk</i>	<i>rpb2</i>	<i>rps16</i>	<i>trnD-trnT</i>
Initial denaturing temperature/time	94°C/4:00	94°C/4:00	97°C/1:00	94°C/5:00
Denaturation temperature/time	94°C/0:30	94°C/0:30	97°C/1:00	92°C/0:45
Annealing temperature/time	53°C/0:30	53°C/0:30	54°C/1:00	57.5°C/0:45
Extension temperature/time	72°C/1:00	72°C/1:00	72°C/2:00	72°C/4:00
Final extension temperature/time	72°C/7:00	72°C/7:00	72°C/7:00	72°C/10:00
No. of cycles	31	31	28	30

2.2.4 DNA sequencing

The PCR products were sent to Source Bioscience Sequencing in Oxford to be sequenced. Purified PCR products are sequenced in both directions (5' and 3') using a modification of the method of Sanger *et al.* (1977). Amplification primers were also used as sequencing primers. Contiguous sequences were assembled and edited using the SeqMan program (DNASTAR, Lasergene).

2.2.5 Sequence assembly and alignment

All sequence data were uploaded in BLAST search on GenBank to ensure that none of the sequences acquired were contaminants. The resulting sequences together with the sequences from GenBank were then aligned using the multiple alignment Clustal W algorithm as implemented in BioEdit version 7·2·5 (Hall, 1999) with further visual and manual adjustments, including misaligned regions. Sequences that could not be aligned were excluded and indels were treated as missing data. A sequence alignment was prepared for each of the regions: *prk*, *rpb2*, *rps16* and *trnD-trnT*, as well as a combined nuclear matrix, a combined chloroplast matrix and a total evidence 6-gene of all of the six regions. In combined matrices all taxa were included and missing sequences from each region were treated as missing data. Areas of ambiguous alignment were excluded prior to analysis.

2.2.6 Phylogenetic analyses

2.2.6.1 Maximum Likelihood

Maximum likelihood analyses were conducted using GTRGAMMA Model in RAxML version 8.2.9 (Stamatakis, 2014) on the CIPRES web-portal. Maximum Likelihood bootstrap analyses and the inference of the optimal tree were conducted simultaneously. Branch support was assessed using 100 replicates of non-parametric bootstrap analysis. The best scoring trees with bootstrap values were saved and the result was viewed using FigTree version v1.4.2 (Rambaut, 2014). Trees figures were prepared using iTOL online (Letunic and Bork, 2006).

2.2.6.2 Bayesian Inference

Bayesian inference was conducted in MrBayes version 3.2.6 on the CIPRES portal by first determining the optimal substitution model using jModelTest (Guindon and Gascuel, 2003; Darriba *et al.*, 2012). The best-fitting model of evolution for each region

was selected using the Akaike Information Criterion (AIC) as a measure of optimality. The models for each gene are shows in Table 2.4. Two independent runs each with four Markov Chain Monte Carlo replicates (MCMC) (one cold and three heated) were run for 1,000,000 generations for all of the regions except for *rps16* and the combined matrices which were run for 2,000,000 generations. Analyses were run until the average standard deviation of split frequencies reaches a value below 0.01.

Table 2.4 Models used in this study.

	Model	Partitions	-lnL	K
<i>prk</i>	GTR+G	012345	26551838	135
<i>rpb2</i>	GTR+G	012345	5866.4871	139
ITS	SYM+G	012345	96339916	80
<i>rps16</i>	GTR+I	012345	2286.8744	139
<i>trnD-trnT</i>	HKY+I+G	010010	2682.7518	140
<i>ndhF</i>	GTR+I+G	012345	2752.1478	88

A plot of negative log likelihoods (LnL) against tree likelihood (TL) was made to measure the burn-in. The output log files of the two independent runs for both individual regions and combined matrix were assessed using Tracer v1.6 (Rambaut *et al.*, 2014) to check for the convergence as well as the suitable burn-in. The first 25,000 trees (25%) of the sampled trees were discarded as ‘burn-in’ and the phylogenetic inference was based on the remaining 75,000 trees for which 50% majority rule consensus tree with posterior probability were calculated. The consensus tree was exported and viewed using FigTree version v1.4.2 software (Rambaut, 2014). Trees were prepared using iTOL online (Letunic and Bork, 2006).

2.3 Results

Statistics for each partitions are details in Table 2.5.

Table 2.5 Alignment statistics for the different regions analysed. PICs and percentage of the parsimony informative characters calculated using PAUP (Swofford, 2001). PIC: Parsimony Informative Characters.

	Nuclear			Chloroplast		
	<i>prk</i>	<i>rpb2</i>	ITS	<i>rps16</i>	<i>trnD-</i> <i>trnT</i>	<i>ndhF</i>
Number of terminals	64	66	38	66	68	40
Aligned length (bp)	430	923	704	765	922	921
PIC/percent variable	114/26.5	207/22.4	367/52.1	54/7.1	83/9.0	83/9.0

Phylogenetic trees resulting from Bayesian Inference and Maximum Likelihood analysis of each partition, the 6-gene alignment and the combined chloroplast and combined nuclear alignments are presented in Appendix 2.2. Figure 2.1 shows the majority rule consensus tree resulting from Bayesian Inference applied to all 6 gene regions, and Figure 2.2 the phylogram reconstructed under Maximum Likelihood. Figure 2.3 show the summarises the relationships recovered across the two 6-gene trees, and for combined chloroplast and combined nuclear trees.

2.3.1 Monophyly of the tribes and inter-tribal relationships

Each of the three tribes of the Calamoideae are recovered as monophyletic in the 6-gene, combined chloroplast and combined nuclear analyses (Table 2.6). In the Bayesian analysis of all gene regions (Figure 2.1), support for each tribe is high (1pp). Maximum Likelihood analysis of all six regions finds 100% support for tribes Calameae and *Eugeissona*, and 96% for Lepidocaryeae (Figure 2.2). Both combined chloroplast and

combined nuclear partitions under Bayesian Inference and Maximum Likelihood reconstructions also find moderate to high support for the monophyly of each of the tribes. Considering the separate partitions, the monophyly of Lepidocaryeae is not recovered in all nuclear partitions (Table 2.6), and none of the tribes are monophyletic in analyses of single chloroplast regions. The failure to recover monophyletic tribes by individual partitions is attributed to the low variation. Instability in the placement of *Raphia farinifera*, and the anomalous placement of *Eugeissona utilis* as sister to *Chamaerops humilis* in *ndhF* tree is noted.

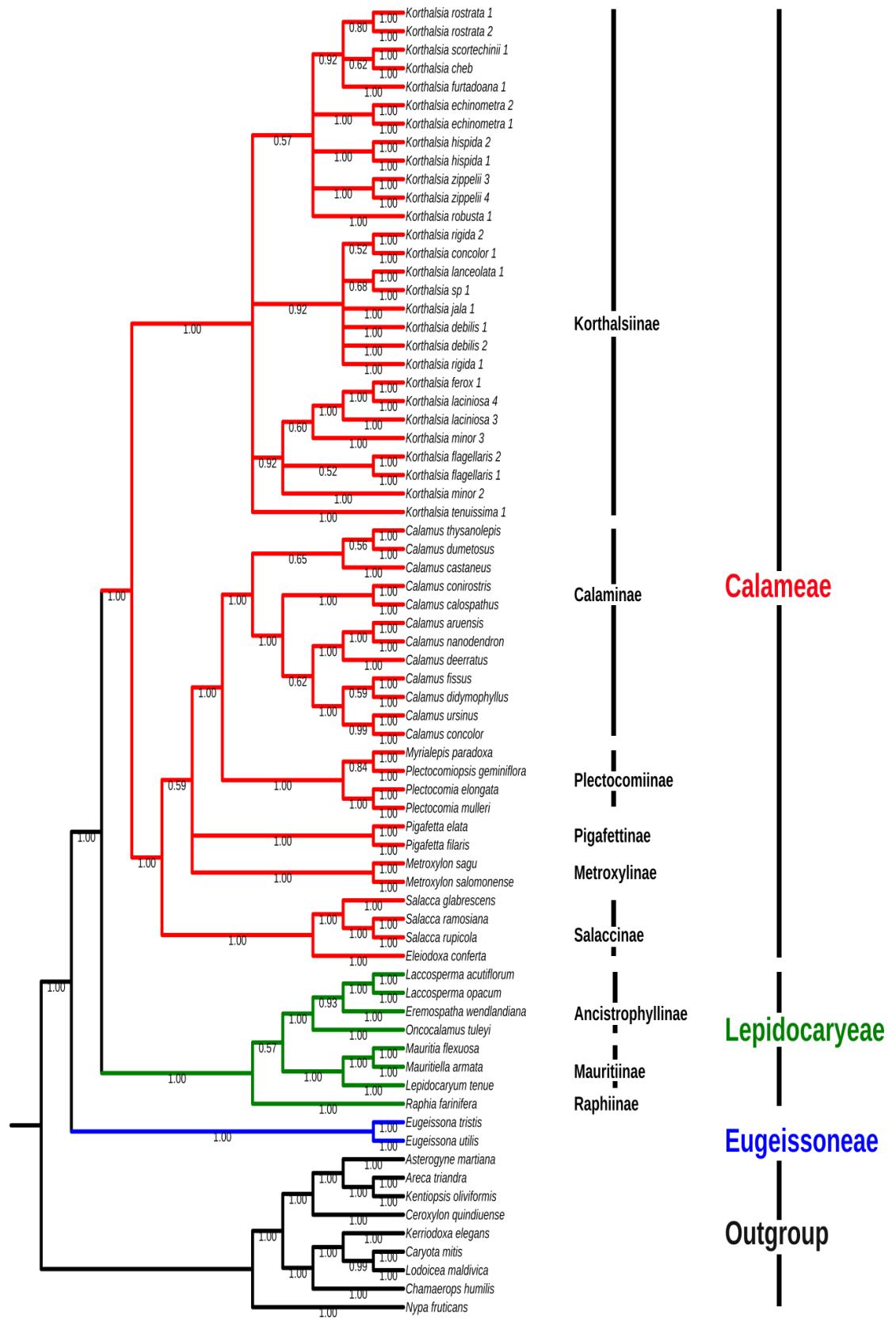


Figure 2.1 Majority rule consensus tree resulting from Bayesian Inference of the 6-gene data set. Tribes and subtribes are indicated, with tribes shown in colour. Values below the branches are posterior probabilities. The posterior probability threshold chosen for the consensus tree (0.5).

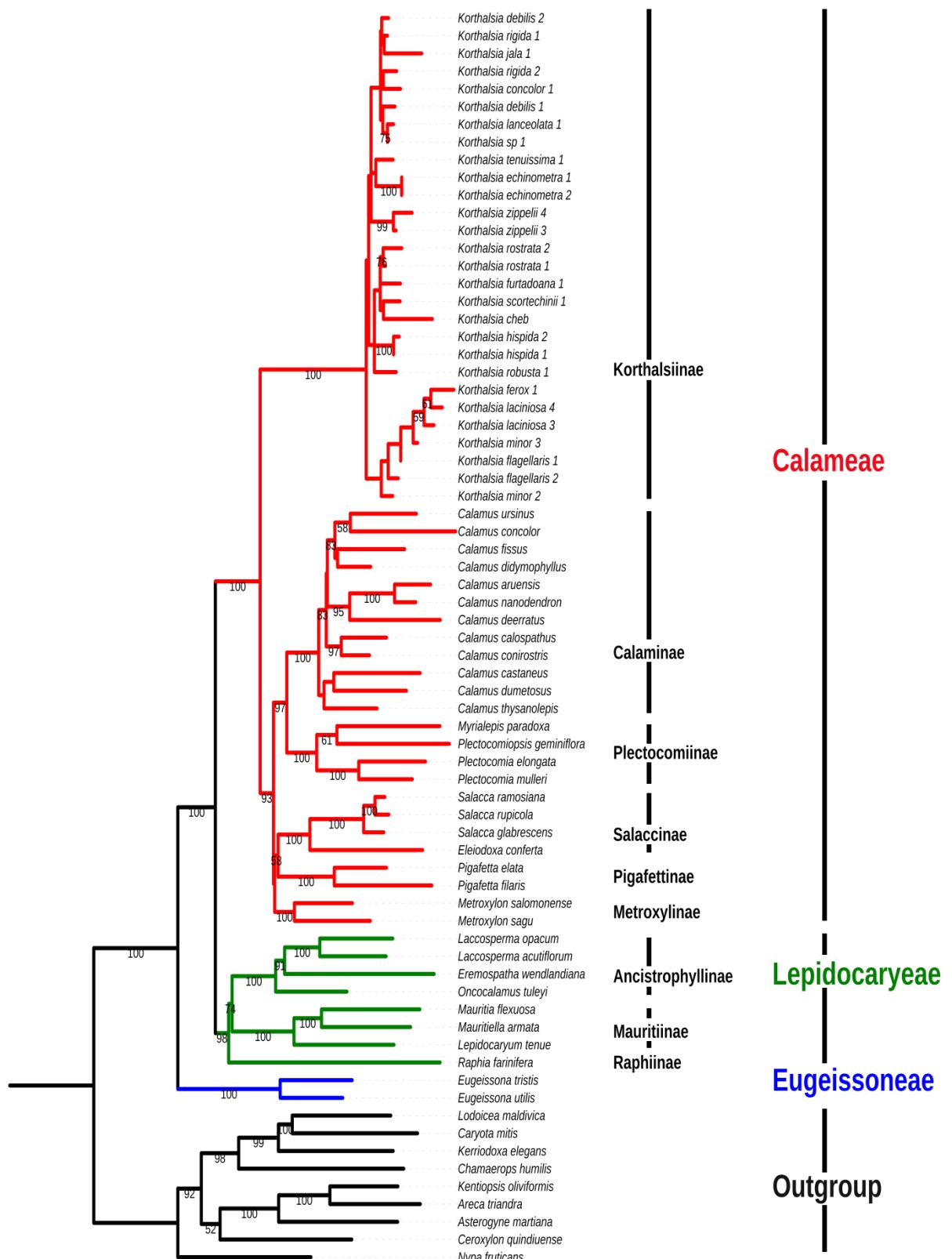


Figure 2.2 Phylogenetic relationships reconstructed using Maximum Likelihood of the 6-gene data set prescribed as on phylogram. Tribes and subtribes are indicated, with tribes shown in colour. Values below the branches are bootstrap value.

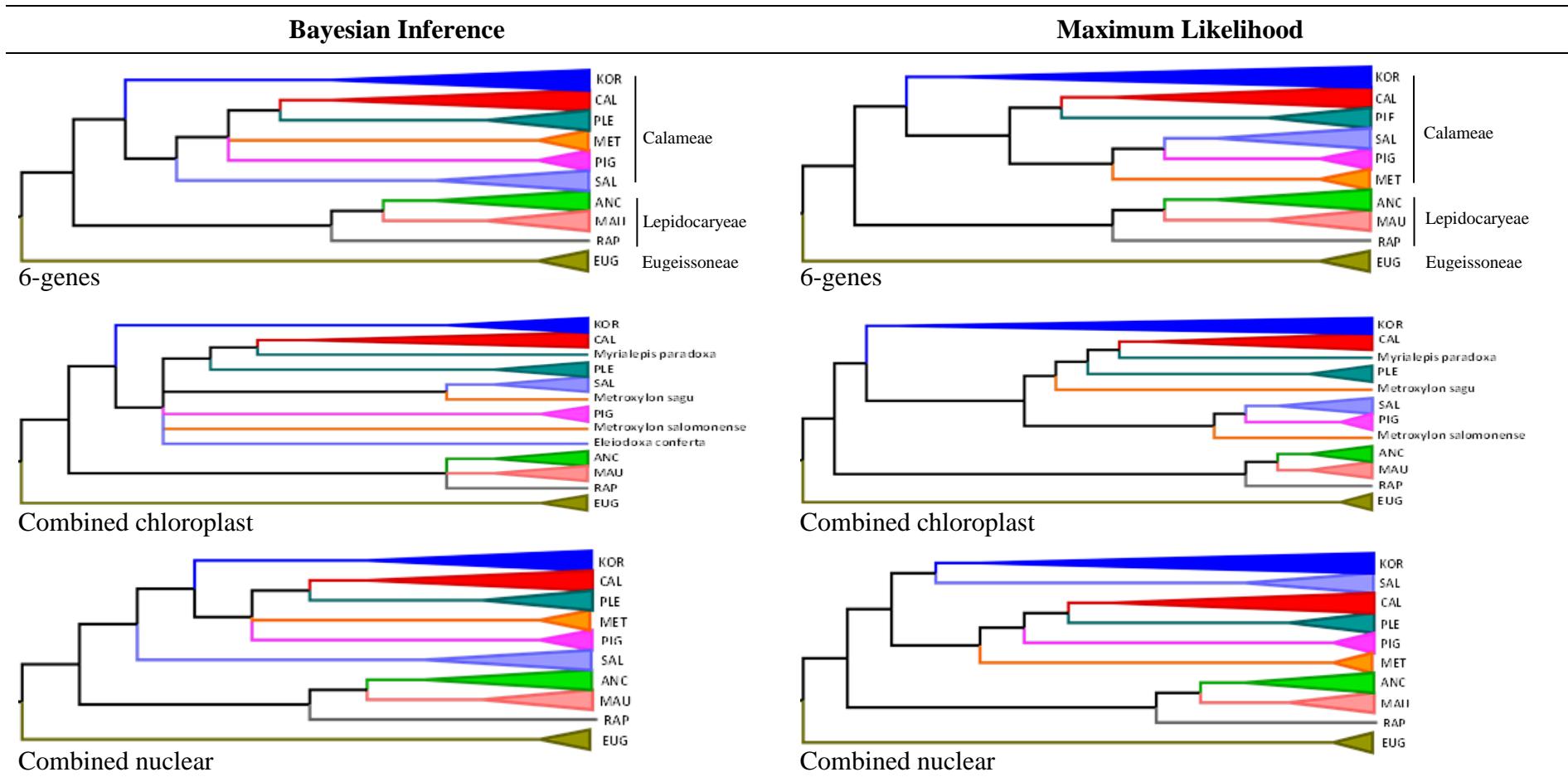


Figure 2.3 Summarises the relationships recovered across the two 6-genes trees and for combined chloroplast and combined nuclear trees. Tribal and subtribal name abbreviation, KOR: Korthalsiinae; CAL: Calaminae; PLE: Plectocomiinae; MET: Metroxylinae; PIG: Pigafettinae; SAL: Salaccinae; ANC: Ancistrophyllinae; MAU: Mauritiinae; RAP: Raphiinae; EUG: Eugeissoneae.

Figures 2.1 and 2.2 show a sister relationship between Calameae and Lepidocaryeae (1pp; 100% BS), with tribe Eugeissoneae recovered as sister to this sister pair (1pp; 100% BS). The same inter-tribal relationships are recovered in the combined nuclear and combined chloroplast analyses, with very strong support from combined chloroplast analyses, but weak to moderate support from the combined nuclear analysis. To consider how individual partitions contribute to these topologies, the trees reconstructed using each partition were examined. None of the individual partitions recovered the sister relationships between tribes, mainly because the tribes themselves were unresolved, and not monophyletic. However, there is notable well-supported conflict in inter-tribal relationships when comparing the topologies from individual nuclear partitions. Specifically, *prk* finds Eugeissoneae sister to Calameae (0.95pp; 88% BS), and *rpb2* finds Lepidocaryeae sister to Calameae (0.95pp; 65% BS).

Table 2.6 Support for the monophyly of the tribes, and for inter-tribal relationships. Superscripts indicate individual partitions not supporting the relationships shown by the combined nuclear or combined chloroplast phylogenies. ^{*1} not recovered by ITS (core Lepidocaryeae recovered, excluding *Raphia farinifera*). ^{*2} not recovered by *trnD-trnT* (part of polytomy). ^{*3} not recovered by *ndhF* because one species found outside of the Calamoideae, sister to *Chamaerops humilis* with 1pp and 98% BS. ^{*4} not recovered by *ndhF* (core Lepidocaryeae recovered, excluding *R. farinifera*), by *trnD-trnT* (part of a polytomy). ^{*5} not recovered by *trnD-trnT* (paraphyletic Calameae). ^{*6} not recovered by *ndhF* or *trnD-trnT* (polyphyletic, but groupings not supported). ^{*7} not recovered by ITS (Lepidocaryeae not monophyletic), nor *prk* (Eugeissoneae sister to Calameae). ^{*8} not recovered by any single partition. “Tribal” grouping describes groups and whether they were recovered, whereas “Inter-tribal” grouping describe relationships, e.g. (Lepidocaryeae Calameae) is Lepidocaryeae as sister to Calameae and (Eugeissoneae (Lepidocaryeae Calameae)) is Eugeissonea as sister to Lepidocaryeae and Calameae. The group Calamoideae was always monophyletic.

Grouping	Bayesian Inference			Maximum Likelihood		
	6-genes	Nuclear	Chloroplast	6-genes	Nuclear	Chloroplast
Tribal						
Calameae	1	1	0.99 ^{*2}	100	100	95 ^{*5}
Eugeissoneae	1	1	1 ^{*3}	100	100	100 ^{*3}
Lepidocaryeae	1	0.94 ^{*1}	0.71 ^{*4}	98	95 ^{*1}	90 ^{*6}
Inter-tribal						
(Lepidocaryeae Calameae)	1	0.77 ^{*7}	1 ^{*8}	100	53 ^{*7}	100 ^{*8}
(Eugeissoneae (Lepidocaryeae Calameae))	1	1 ^{*7}	1 ^{*8}	100	100 ^{*7}	100 ^{*8}

2.3.2 Monophyly of the subtribes

Both 6-gene analyses, under Bayesian Inference and Maximum Likelihood, provide strong support for the monophyly of the subtribes. The combined nuclear phylogenies also provide strong support for the subtribes, but support is weak, and in some cases the subtribes are not monophyletic, in analyses of the combined chloroplast regions (Table 2.7).

Table 2.7 Support for the monophyly of the subtribes. Subtribes described as unresolved have species placed in a polytomy at the base of the Calameae. The paraphyletic Plectocomiinae in the Bayesian Inference and Maximum Likelihood analyses of the combined chloroplast data is part of a monophyletic group that includes the Calaminae.

Subtribe	Bayesian Inference			Maximum Likelihood		
	6-genes	Nuclear	Chloroplast	6-genes	Nuclear	Chloroplast
Korthalsiinae	1	1	1	100	100	100
Salaccinae	1	1	Unresolved	100	99	43
Metroxylinae	1	1	Unresolved	100	99	Unresolved
Pigafettinae	1	1	1	100	100	98
Plectocomiinae	1	1	Paraphyletic*	100	100	Paraphyletic*
Calaminae	1	1	0.99	93	100	86
Ancistrophyllinae	1	1	0.99	100	100	100
Raphiinae	1	0.94	Unresolved	100	95	90
Mauritiinae	1	1	0.86	100	100	94

2.3.3 Relationship between the subtribes

The relationships of the subtribes of the Calameae for the 6-gene alignment differ under Bayesian Inference and Maximum Likelihood. The Bayesian topology (Figure 2.1) finds Korthalsiinae is sister to the remainder the Calameae with high support (1pp). The monophyletic group comprising the remainder of the tribes also finds high support (1pp), with Salaccinae sister to the rest (1pp). Calaminae is sister to Plectocomiinae (1pp), but the relationships of Metroxylinae and Pigafettinae are unresolved. The Maximum Likelihood topology (Figure 2.2) also finds Korthalsiinae is sister to the remainder of the Calameae (100% BS). However, the reminder of the Calameae comprise two clades, one comprising Calaminae sister to Plectocomiinae (97% BS), and the other Metroxylinae sister (39% BS) to a sister pair comprising Salaccinae and Pigafettinae (58% BS). The notable difference between the two topologies therefore is the position of the Salaccinae, either as sister to all the remainder of the Calameae except Korthalsiinae, or nested in the Calameae as sister to Pigafettinae.

Relationships between subtribes of the Calameae are inconsistent also between the combined chloroplast and combined nuclear topologies, with the position of Salaccinae being unstable. Although overall the combined chloroplast topologies are poorly supported, Korthalsiinae is sister to the remainder in both combined chloroplast topologies and the Salaccinae groups with the remaining poorly supported tribes. However, in the combined nuclear topologies, the Salaccinae has a more basal position, being sister to the remainder of the tribe in the Bayesian Inference analysis, and sister to Korthalsiinae in the Maximum Likelihood analysis.

In contrast to relationships within the Calameae, relationships of subtribes within the Lepidocaryeae are stable, with Ancistrophyllinae sister to Mauritiinae, and Raphiinae sister to that sister pair. Low support for the relationships might be attributed to instability of the placement of *Raphia farinifera* in the analyses of separate chloroplast partitions. The 6-gene topologies show weak support for the sister relationship between Ancistrophyllinae and Mauritiinae (0.57pp; 74% BS); this is more support than the chloroplast partitions (not recovered; 50% BS), but less than the nuclear partitions (0.64pp; 74% BS).

2.3.4 Species relationship with *Calamus* and *Korthalsia*

2.3.4.1 Delimitation and subdivision of *Calamus*

The result for both of the 6-gene analyses, under Bayesian Inference (Figure 2.1) and Maximum Likelihood (Figure 2.2), shows genus *Korthalsia* resolved as monophyletic with strong support (1pp; 100% BS). Despite overall a poorly resolved backbone for the *Korthalsia* clade, three groups are emerging. *Korthalsia cheb*, *K. echinometra*, *K. furtadoana*, *K. hispida*, *K. robusta*, *K. rostrata*, *K. scortechinii* and *K. zippelii* (0.57pp) are in one group. *K. ferox*, *K. flagellaris*, *K. laciniosa* and *K. minor* (0.92pp) are in other group while *K. concolor*, *K. debilis*, *K. jala*, *K. lanceolata*, *K. rigida* and *K. sp.* (0.92pp) are in another. There have been conflict between nuclear and chloroplast partitions for species relationships in both analyses. Comparing the partitions, there are no groups

emerging from analysis of the chloroplast partitions. Mostly the relationships between species in chloroplast partitions are unresolved compare to nuclear partition, so that phylogenetic structure in the combined analysis is congruence with the analysis of combined nuclear partitions.

2.4 Discussion

2.4.1 Evaluating higher-level classification

Reviewing the status of palm systematics in 2016, Baker and Dransfield considered the deep relationships in the Calamoideae an open question (Baker and Dransfield, 2016). They highlighted conflict in the placement of Eugeissoneae, and a sometimes paraphyletic Lepidocaryeae as areas of uncertainty. This study finds strong support for a monophyletic Lepidocaryeae, and for the intertribal relationships (Eugeissoneae (Calameae, Lepidocaryeae)). Ambiguity in the placement of Eugeissoneae and the status of Lepidocaryeae is marked in previous phylogenetic studies. The tribe Eugeissoneae was described based on Baker *et al.*'s earliest molecular phylogenetic studies of the subfamily (Baker *et al.*, 2000), although the position of the Eugeissoneae was labile. Sometimes it was recovered as sister to the sister pair (Calameae, Lepidocaryeae), in other analyses Eugeissoneae was nested in Lepidocaryeae, as sister to *Raphia* (Baker *et al.*, 2000). These topologies resulted from analyses of two gene regions, ITS and the *rps16* intron, and 25, eight and two taxa were sampled to represent the tribes Calameae, Lepidocaryeae and Eugeissoneae respectively. Later studies increased gene region sampling.

A study of the family by Asmussen *et al.* (2006) sampled 12 Calamoideae for four plastid regions, *trnL-F*, *rps16*, *rbcL* and *matK*, finding (Eugeissoneae (Calameae, Lepidocaryeae)) but their analysis, which used parsimony, only weakly supported the (Calameae, Lepidocaryeae) sister relationship (59% BS). Baker *et al.* (2009) presented a supermatrix phylogeny and a supertree phylogeny for all palms, focussing sampling at the generic level and using available data for 14 gene regions plus restriction fragment

and morphological data. In total 205 taxa were sampled, of which 14 represented the Calameae, seven Lepidocaryeae and one Eugeissoneae. The supermatrix recovered a paraphyletic Lepidocaryeae with Eugeissoneae nested in it, whereas the supertree analysis based on Matrix Representation with Parsimony of trees from 26 analyses of subsets of taxa and partitions, recovered (Eugeissoneae (Calameae, Lepidocaryeae)). One source of conflicting relationships between the supermatrix and supertree topologies in this study could be the amount of missing data in the supermatrix analysis. Overall, the supermatrix lacked data for around half the taxa for each partition. For the Calamoideae, only three of the nine gene partitions included all the 14 Calameae, three included all seven Lepidocaryeae, and the single Eugeissoneae was represented by five of the partitions (Table 2.8). It is possible that using subsets of partitions to reconstruct subtrees reduced the impact of missing data in their study. My study limits missing data by focussing on a subset of 6-gene partitions and by generating new data for the complete or almost complete set regions for of 62 Calamoideae, almost three times as many as were included in the Baker *et al.* (2009) study (22). The Baker *et al.* (2009) study relied on parsimony methods for tree reconstruction. Model based methods have been used to infer relationships within the Calamoideae since 2009, but have employed very different gene and taxon sampling strategies. For example, Faurby *et al.* (2016) attempted to reconstruct a species level phylogeny of all palms using model-based methods and building on the Baker *et al.* (2009) supermatrix. Their topology revealed a paraphyletic Lepidocaryeae, and Eugeissoneae sister to Calameae.

Barret *et al.* (2016) sequenced whole plastid genomes using next-generation sequencing (NGS) in a study of the commelinid monocots. They sampled seven Calamoid palms, and although the tribes were monophyletic, their topology (Eugeissoneae (Calameae, Lepidocaryeae)) was unsupported. The present study could be considered the most robust estimate of relationships within the Calamoideae to date, employing model-based methods, dense taxon sampling and with minimal missing data. The next step is to generate more fully sampled NGS datasets and that the palm community is doing just that in its collaborative ambition to produce a NGS phylogeny of all palm species.

Table 2.8 Comparison of taxon and marker sampling between Baker *et al.* (2009) and this study. This study employs dense sampling of taxa and even represents action of markers compared to the study of Baker *et al.* (2009). Tribal name abbreviation, CAL: Calameae, LEP: Lepidocaryeae, E: Eugeissoneae.

	Baker <i>et al.</i> (2009) supermatrix			This study		
	CAL	LEP	E	CAL	LEP	E
<i>prk</i>	-	1	-	47	7	2
<i>rpb2</i>	-	1	-	50	7	2
ITS	14	7	1	26	7	2
<i>rps16</i>	14	7	1	48	7	2
<i>trnD-trnT</i>	1	1	-	53	6	2
<i>ndhF</i>	1	1	-	26	6	1
18S	3	1	-	-	-	-
<i>atpB</i>	3	1	-	-	-	-
<i>matK</i>	6	5	1	-	-	-
<i>ms</i>	3	1	-	-	-	-
<i>rbcL</i>	14	7	1	-	-	-
<i>trnL-trnF</i>	6	5	1	-	-	-
<i>trnQ-rps16</i>	1	1	-	-	-	-

2.4.2 New insights into the relationships among subtribes and between genera

The topologies presented here, being the most densely sampled and data rich to date for the Calamoideae, provide insights into relationships within the tribes Calameae and Lepidocaryeae. Tribe Calameae resolved in previous studies as monophyletic with moderate to high support (Baker *et al.*, 2000, 2000b, 2009; Asmussen *et al.*, 2006; Faurby *et al.*, 2016). The topology from the combined 6-gene analysis in this study supports view that tribe Calameae is monophyletic with strong support. The relationships among subtribes from this study are very similar to those found by Faurby *et al.* (2016). Among subtribes of the Calameae, Korthalsiinae is sister to all remaining Calameae with high support, with Salaccinae sister to the rest also with strong support (Figure 2.1) except in Maximum Likelihood and combined nuclear and chloroplast for both analyses. This study and previous ones find conflict in the placement of Salaccinae. Baker *et al.* (2009) found Salaccinae is sister to Korthalsiinae with moderate support, while in Faurby *et al.*'s (2016), shows Salaccinae is sister to the remainder of Calameae expect Korthalsiinae. In this study, the position of Salaccinae is

unstable with different placement in the combined chloroplast and combined nuclear topologies. The conflicting position of the Salaccinae in this study is not strongly supported. More data are needed before hypotheses to explain the conflicting placement of Salaccinae are put forward.

There are three subtribes recognised in tribe Lepidocaryeae. This study resolved the relationship between subtribes as (Raphiinae (Ancistrophyllinae, Mauritiinae)) with strong support in both Bayesian Inference and Maximum Likelihood analyses. This result is contrast to the previous study by Faurby *et al.* (2016), which found Raphiinae is sister to Mauritiinae and this sister pair as sister to Ancistrophyllinae. Within subtribe Ancistrophyllinae, genus *Laccosperma* is sister to *Eremospatha* with strong support, in agreement with the study made by Faye *et al.* (2014) and Faye *et al.* (2016).

2.4.3 Species relationships within genera: *Calamus* and *Korthalsia*

Although the data presented here are adequate to resolve higher-level relationships, overall species-level relationships are poorly resolved in two genera that are represented by several exemplars in this study, *Calamus* and *Korthalsia*.

Previous studies have found conflict between nuclear and chloroplast regions at the species level for *Calamus*. However, following previous phylogenetic studies (Baker *et al.*, 2000, 2000a, 2000b), the genus *Calamus* was re-circumscribed to include five genera of Calaminae nested within it (Baker, 2015). The currently accepted classification for expanded *Calamus* includes *Calospatha* Becc., *Ceratolobus* Blume ex Schult. & Schult.f., *Daemonorops* Blume, *Pogonotium* J.Dransf. and *Retispatha* J.Dransf. This study places species previously placed in these five genera within a strongly supported *Calamus sensu lato*, supporting the re-circumscription of the genus.

For the first time, this study sampled enough exemplars of *Korthalsia* to make a meaningful test of monophyly. The recovery of the genus *Korthalsia* as monophyletic with strong support (1pp; 100% BS) (Figure 2.1 and Figure 2.2) is therefore a significant finding for this study. The three subgeneric groups emerging can be interpreted in terms of ocrea morphology (Chapter 4).

CHAPTER 3

MORPHOLOGICAL CHARACTERISATION, DEVELOPMENT AND EVOLUTION OF THE OCREA IN *KORTHALSI*A

3.1 Introduction

Rattans the spiny, climbing palms of the Old World, belong to subfamily Calamoideae (Uhl and Dransfield, 1987). Although the subfamily includes tree-like, shrubby and acaulescent palms, the majority of species are lianas exhibiting morphological adaptations to the climbing habit, such as elongated stems, and climbing organs on the leaf (the cirrus). Leaf sheaths of rattans are thickened, elongated and surround the stems, conferring mechanical properties on the stem thus aiding climbing (Rowe *et al.*, 2004; Isnard and Rowe, 2007). A further modification of the leaf sheath is the extension beyond the point of insertion of the petiole, known as the ocrea (Figure 3.1). The ocrea may be more or less elongate or appressed to the stem, and may form domatia (small chamber or hollow structure) occupied by ants in some species. The ant domatia have rendered the rattans important species in studies of the evolution of ant-plant mutualisms (Mattes *et al.*, 1998; Moog *et al.*, 2003; Edwards *et al.*, 2010; Merklinger *et al.*, 2014).

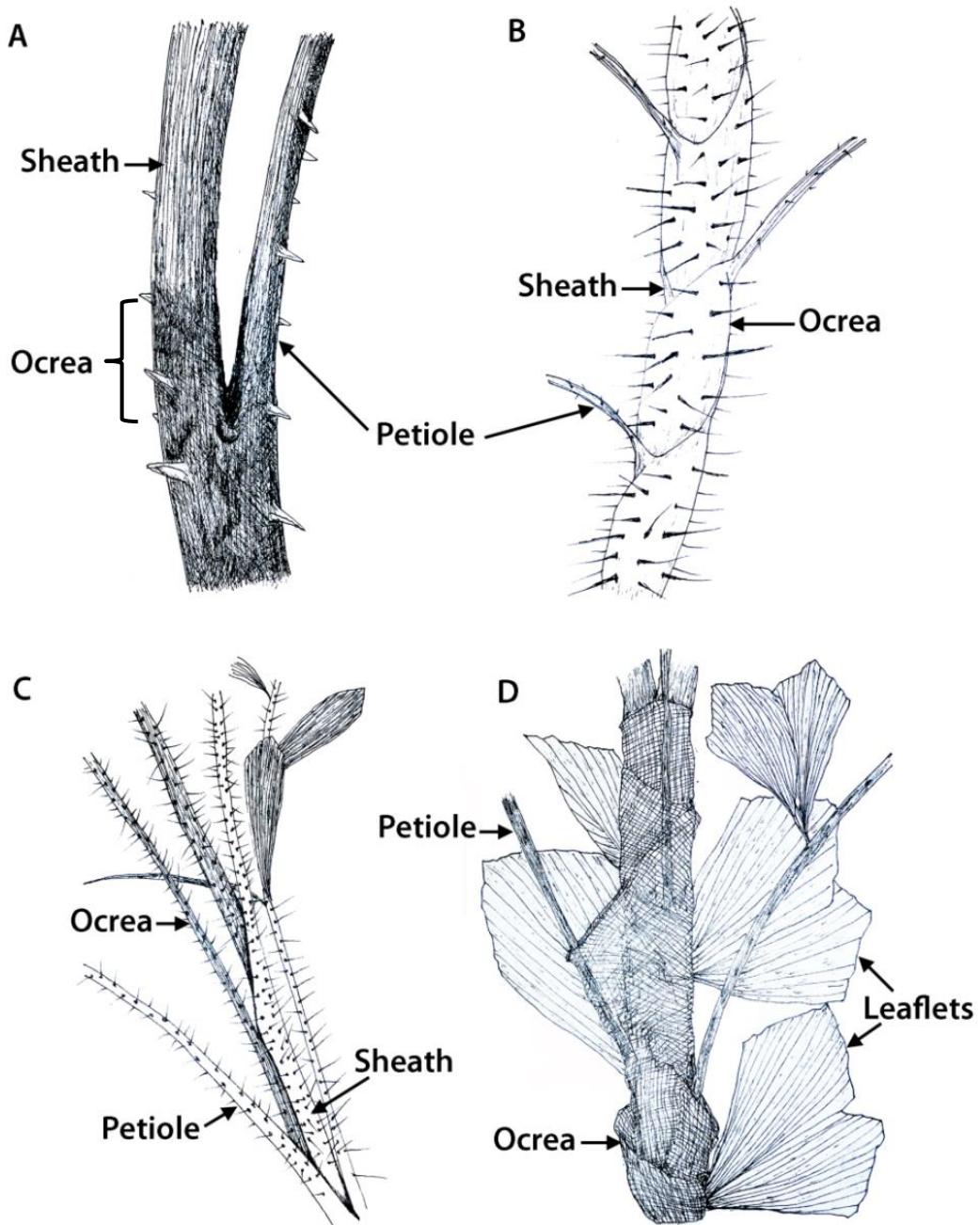


Figure 3.1 Four types of ocrea according to Dransfield (1981). A) ocrea tightly sheathing; B) ocrea inflated; C) ocrea diverging; D) ocrea fibrous net-like. Drawn by Mohd Fadzil.

To date ocreas have been characterised in detail for only three species of palms, all members of genus *Calamus* (Merklinger *et al.*, 2014). Merklinger *et al.*'s (2014) study was the first and remains the only study of the development of the palm ocrea. The study confirmed the origin of the ocrea as an extension of the leaf sheath initiated shortly after differentiation of the lamina and sheath. It also described the

developmental trajectory apparently unique to the inflated, sac-like ocrea of *Calamus longipinna*. In this species plications develop on the surface of the ocrea, and persist as folds on the ocrea surface that allow the ocrea to expand. The relationship of the ocrea to the ligule (a distal projection of the leaf sheath) or to stipules (small, paired leaf-like appendages to a leaf petiole in Angiosperms) is uncertain (Merklinger, et al., 2014), and whether the developmental features of domatia in *C. longipinna* are shared with other ant-domatia forming rattan species has not been determined.

Modifications to ocreas so that they serve as domatia for ants have apparently evolved multiple times in the Calamoideae especially in the two largest and widespread rattan genera, *Calamus* and *Korthalsia*. Ocreas that form domatia are present in four rattans according Merklinger *et al.*, (2014), of which three are still recognised: *Calamus*, *Korthalsia* and *Laccosperma*, since *Pogonotium* has been placed in *Calamus* (Baker, 2015). This study is focussed on the genus *Korthalsia*. Currently, 28 species are accepted (WCSP, 2017), distributed from the north of Indochina, Burma and the Andaman Islands and south-eastward to Celebes and New Guinea (Dransfield *et al.*, 2008). In *Korthalsia*, the ocrea is extremely diverse in form in different species (Figure 3.2). In the species with ocreas tightly sheathing the stem, the ocrea does not serve as a domatium; species which have fibrous net-like ocreas do not have ant relationships either (Dransfield *et al.*, 2008). However, Dransfield *et al.*, (2008) recognised two types of ocrea which serve as domatia which are inflated ocreas and divergent ocreas. The ocrea can be unarmed or variously spiny. The inflated ocrea is a notable adaptation, compared to other rattan structures that are utilized by ants, since it is apparently highly specialized to facilitate work by ants (Ridley, 1910; Moog *et al.*, 2003; Sunderland, 2004; Edwards *et al.*, 2010; Chan *et al.*, 2012). However, divergent ocreas are also occupied by ants (Mattes *et al.*, 1998; Chan *et al.*, 2012).



Figure 3.2 Field photographs of ocrea. A–C: inflated ocrea (A) *Korthalsia echinometra*; B) *K. rostrata*; C) *K. scortechinii*); D–E: diverging ocrea (D) *K. robusta*; E) *K. hispida*); F–G: tightly sheathing ocrea (F) *K. debilis*; G) *K. rigida*); H: fibrous, net-like ocrea (H) *K. jala*). Photographs© Salwa Shahimi, William Baker, John Dransfield.

3.1.1 Aims of this study

Korthalsia, a genus with a diversity of ocrea types is an ideal model for exploring the evolution of ocrea types, yet the variation in ocrea morphology in the genus has not been the focus of a thorough descriptive and comparative study. Developmental studies inform interpretation of morphological traits, but despite recent studies characterising the development of *Calamus longipinna* (Merklinger *et al.*, 2014), comparative developmental studies that could provide insights into elaboration of the ant-ocreas are lacking. Here I will identify the homologies of the *Korthalsia* ocrea, using complementary developmental and phylogenetic approaches. Specifically, I will use ancestral reconstruction to examine the evolution of the ant-ocreas, optimizing traits which explain ocrea diversity onto a chronogram representing species with and without ant domatia. Interpretation of the history of the ocrea traits is expected to shed light on the evolution of the ant-plant mutualism.

3.2 Material and methods

3.2.1 Developmental anatomy

3.2.1.1 Fieldwork

Fresh specimens of nine species used in this study were obtained from eight localities in Malaysia and Singapore during field trips undertaken in November 2014 and September 2015. Specimens of nine species had been successfully collected from eight different locations (Table 3.1). Voucher specimens including stem, whole leaf and ocrea were collected (Appendix 3.1). The ocrea was preserved in 70% ethanol for developmental studies (Figure 3.3). Figure 3.4 and Figure 3.5 show map localities where the specimens were sampled.

Table 3.1 Specimens used in this study. Vouchers were collected.

Species	Voucher specimens	Locality
<i>Korthalsia debilis</i> Blume	Shahimi, SS28 (K)	Sarawak, Malaysia
<i>Korthalsia echinometra</i> Becc.	Shahimi, SS9 (K)	Terengganu, Malaysia
<i>Korthalsia echinometra</i> Becc.	Shahimi, SS10 (K)	Terengganu, Malaysia
<i>Korthalsia echinometra</i> Becc.	Shahimi, SS13 (K)	Johor, Malaysia
<i>Korthalsia echinometra</i> Becc.	Shahimi, SS23 (K)	MacRitchie Reservoir, Singapore
<i>Korthalsia flagellaris</i> Miq.	Shahimi, SS3 (K)	Negeri Sembilan, Malaysia
<i>Korthalsia flagellaris</i> Miq.	Shahimi, SS20 (K)	Nee Soon Swamp Forest, Singapore
<i>Korthalsia hispida</i> Becc.	Shahimi, SS11 (K)	Johor, Malaysia
<i>Korthalsia hispida</i> Becc.	Shahimi, SS12 (K)	Johor, Malaysia
<i>Korthalsia rigida</i> Blume	Shahimi, SS2 (K)	Negeri Sembilan, Malaysia
<i>Korthalsia rigida</i> Blume	Shahimi, SS8 (K)	Selangor, Malaysia
<i>Korthalsia rigida</i> Blume	Shahimi, SS18 (K)	MacRitchie Reservoir, Singapore
<i>Korthalsia robusta</i> Blume	Shahimi, SS26 (K)	Sarawak, Malaysia
<i>Korthalsia rostrata</i> Blume	Shahimi, SS27 (K)	Sarawak, Malaysia
<i>Korthalsia rostrata</i> Blume	Shahimi, SS25 (K)	MacRitchie Reservoir, Singapore
<i>Korthalsia scortechinii</i> Becc.	Shahimi, SS15 (K)	Penang, Malaysia
<i>Korthalsia tenuissima</i> Becc.	Shahimi, SS14 (K)	Penang, Malaysia



Figure 3.3 The ocrea were preserved in 70% ethanol. Photographs© Salwa Shahimi.

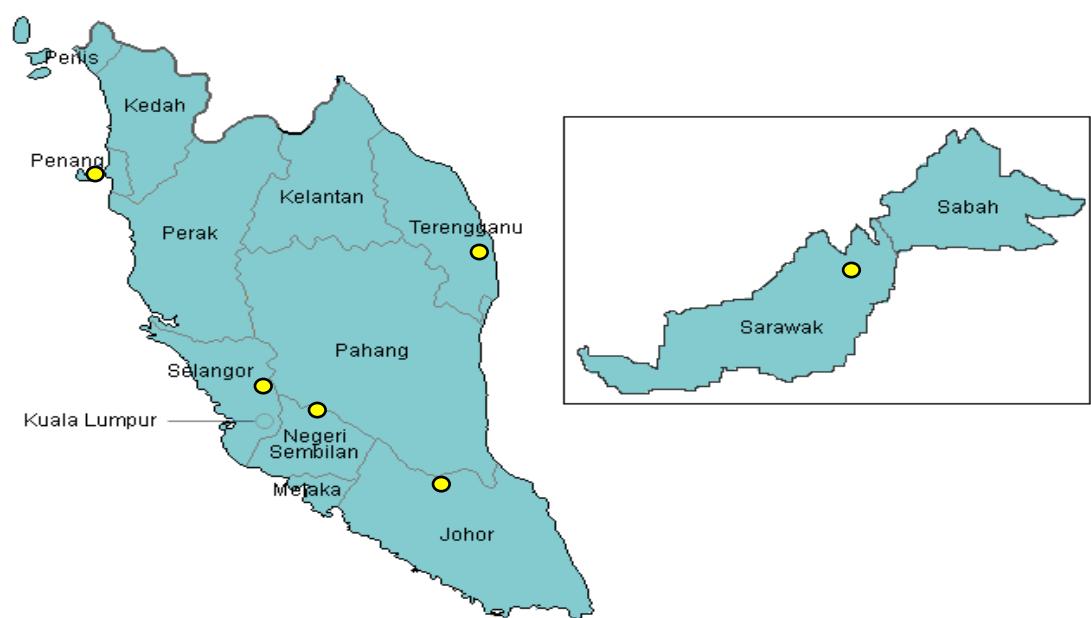


Figure 3.4 The distribution of collection site in Peninsular Malaysia and Sarawak. Peninsular Malaysia (Left), Sabah and Sarawak (Right). Dots are point localities of specimens collected.



Figure 3.5 The distribution of collection sites in Singapore. Dots are point localities of specimens collected.

3.2.1.2 Ocrea dissection

The preserved ocrea were dissected. Each sheath was removed until the outermost sheath was approximately the diameter of a pencil. In the case of the dissection shown in Figure 3.6 B, five sheaths were removed. The samples were then fixed in 70% ethanol (Figure 3.6).



Figure 3.6 The ocrea dissecting process to remove outer stages of the developing leaves. (A) the undissected stem as collected and preserved; (B) the stem shown in (A), with successive sheaths removed down to reveal innermost sheath (bottom); (C) dissected sheaths labelled and stored for further dissection. Photographs© Salwa Shahimi.

3.2.1.3 Light Microscopy (LM)

The early stage of development of the ocrea of each specimen, following removal of several, outer sheath layers as shown in Figure 3.6 B, was imaged using a Leica Photo Microscope M400.

3.2.1.4 Scanning Electron Microscopy (SEM)

For examination of early leaf development, samples with outer sheaths removed for LM work were dissected further and then dehydrated through an alcohol series (Appendix 3.2). The samples were transferred to Autosamdry 815B CPD for critical-point drying. After that, samples were mounted onto SEM stubs, and coated with platinum in an Emitech K550 sputter coater. Samples were examined using a Hitachi S-4700 cold-field emission SEM at RBG Kew.

The mature ocrea surface of four species with different ocrea morphologies was examined using Scanning Electron Microscopy (SEM). They were *Korthalsia debilis* (Shahimi, SS28), *K. hispida* (Shahimi, SS11), *K. scorchedinii* (Shahimi, SS15) and *K. jala* (Dransfield et al. 4652).

3.2.2 Evolution of ocrea

3.2.2.1 Reconstruction of a time calibrated phylogeny

Using an existing matrix of DNA sequence data for calamoid palms, with emphasis on *Korthalsia* (Chapter 2), a set of time-calibrated trees was reconstructed using BEAST v2.4.7 (Bouckaert et al., 2014). The dataset comprises a total of 71 taxa including 62 taxa from subfamily Calamoideae and nine outgroup taxa to represent all subfamilies in the Arecaceae. One of the calibration points previously identified and used by Couvreur et al. (2011) to reconstruct their time-calibrated genus-level phylogeny of palms was

used here. Couvreur *et al.* (2011) noted that pollen of subtribe Mauritiinae corresponds closely to the fossil pollen in the genus *Mauritiidites*, a genus recorded from the Maastrichtian of Africa, and with numerous subsequent records from South America which motivates the use of a calibration for the stem node of the Mauritiinae of 65 Ma. A second calibration point was used in this study: the root node was constrained to 100.1 Ma in accordance with the Couvreur *et al.* (2011) study.

To implement BEAST v2.4.7 the alignment was imported into BEAUTi v.2.4.7 (Bouckaert *et al.*, 2014) to generate a BEAST input file. A relaxed clock log normal prior (Drummond *et al.*, 2006) was used, and the model of molecular evolution was set to be GTR with gamma-distributed rate variation (G). The tree prior was a birth-death tree, with MCMC chain lengths 50 million generations, sampling every 5000 generation. The convergence statistics were checked using Tracer v1.6 (Rambaut *et al.*, 2014). TreeAnnotator v2.4.7 (Bouckaert *et al.*, 2014) was used to produce the maximum clade credibility tree. Tree files were viewed using FigTree version v1.4.2 (Rambaut, 2014). The tree with minimum 50% clade frequency was produced using the sumtree.py program in DendroPy (Sukumaran and Holder, 2010). Tree figures were prepared using iTOL online (Letunic and Bork, 2006).

3.2.2.2 Stochastic character mapping

Specimens with representative mature ocrea were examined in the field (see 3.2.1.1) and from herbarium specimens (see chapter 4). Based on these observations, characters were described and scored for all 28 species recognized in the genus. An additional character was scored to describe whether the species had an ant association. All characters were scored as binary characters.

The evolution of ocrea characters and ant symbioses was studied by applying stochastic character mapping using the R package phytools (Revell, 2012). The phytools character mapped fitted a continuous-time reversible Markov model for the evolution of each

character and then simulated stochastic character histories using that model and the tip states on the tree. This is the same procedure that is described in Bollback (2006). A maximum-clade credibility tree (mcct) from the BEAST analysis, without collapsed branches, was used to reconstruct ancestral states for each character using the commands `make.simmap()` and `densityMap()` (Revell, 2013). Prior to the reconstructions, the mcct was pruned to include only members of genus *Korthalsia* using the `drop.tip()` function in ape (Paradis *et al.*, 2004). For each character trait, 500 stochastic character maps were simulated (`nsim=500`).

3.3 Results

3.3.1 Ocrea development

Figure 3.7 shows the results of a survey using Light Microscopy (LM), illustrating the comparative morphology of six species of *Korthalsia*, following dissection down to the innermost sheath of the youngest leaf. The six species represent three of the four ocrea types identified by Dransfield (1981). In Figure 3.7 A–C, *K. echinometra*, *K. scorchedinii* and *K. rostrata* represent the inflated type *sensu* Dransfield (1981); in Figure 3.7 D, *K. robusta* represents the diverging type and in Figure 3.7, E–F *K. debilis* and *K. rigida* represent the tightly sheathing type. It is notable that the three inflated species, Figure 3.7, A–C, differ in the origin of the inflation. *K. echinometra* shows inflation from the point of attachment, whereas the other two species with inflated ocrea show several millimetres of tight clasping before the origin of inflation. At the stage recorded here, the tightly clasping part is almost as long as the inflated part, though in the mature ocrea the clasping part is much less. *K. echinometra* also differs from *K. scorchedinii* and *K. rostrata* in that it lacks notching of the ocrea apex that results in a bifid shape in *K. scorchedinii* and *K. rostrata*. In contrast the apex is truncate in the species with tightly sheathings ocrea (*K. debilis* and *K. rigida*; E–F). Figure 3.7 A–D shows ocreas with spines at this early stage while in E–F the spines are absent. Thus, at a relatively early stage of development of the ant ocrea, both inflated and divergent types have spines. They also differ from the tightly-sheathing species in that the ocrea apex is cleft (bifid) or angled. The tightly sheathing ocrea species investigated here lack

spines when visualised by LM, and have a truncate ocrea apex that is perpendicular to the stem.

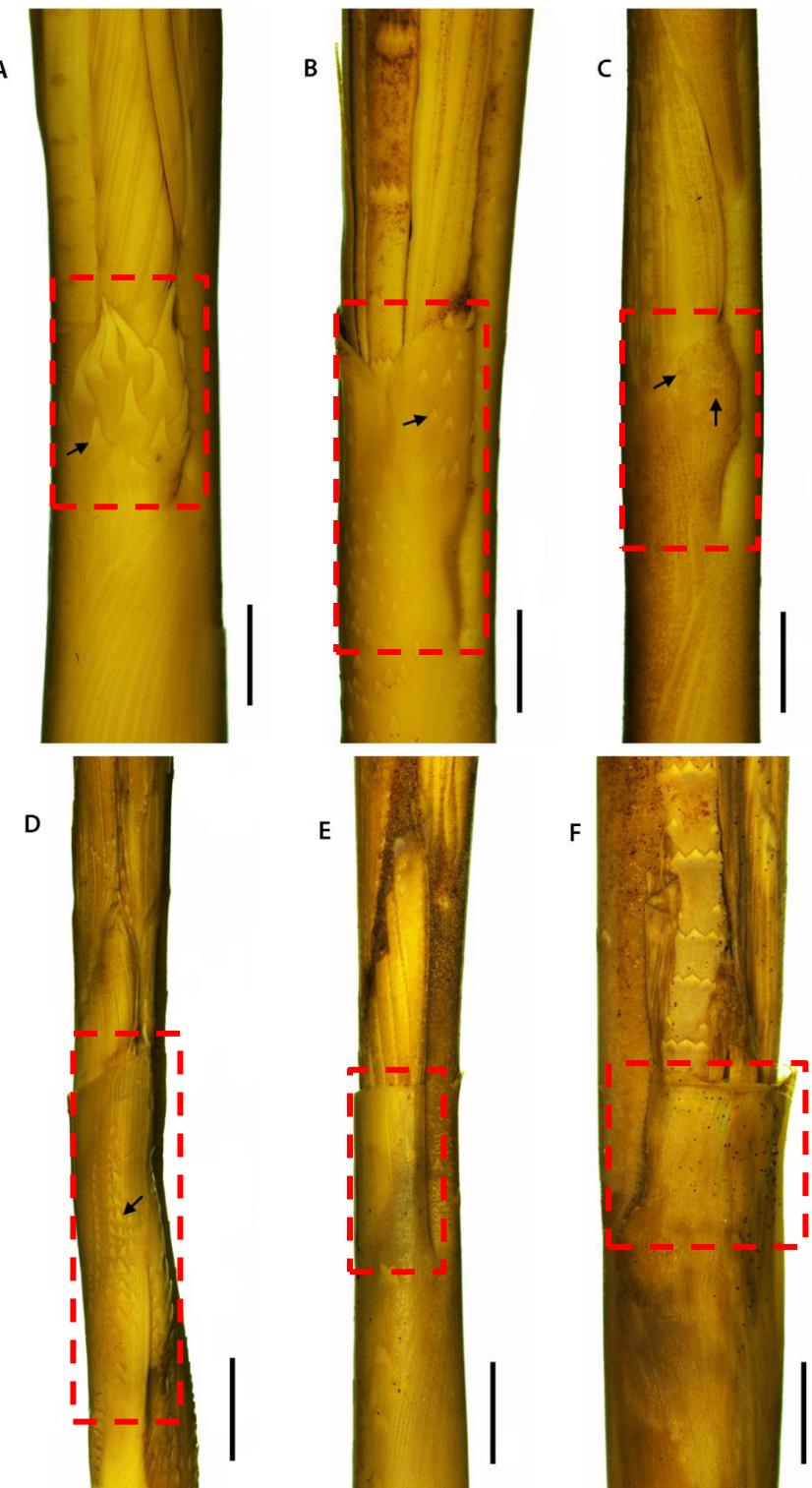


Figure 3.7 Photomicroscope images of developing ocrea (boxed area). A) *Korthalsia echinometra*; B) *K. scorchedii*; C) *K. rostrata* D) *K. robusta*; E) *K. debilis*; F) *K. rigida*. The spines are shown by arrows. Bar 10 mm.

Many of these differences are more clearly seen in the SEMs than in the LM images, although the SEMs are prepared following removal of another leaf sheath to reveal the ocrea at an even earlier stage. Figure 3.8 shows three inflated ocrea species *K. echinometra*, *K. scortechinii* and *K. rostrata* (Fig. 3.8 A–C), *K. robusta* (Fig. 3.8 D), a species with divergent ocreas and *K. debilis* and *K. tenuissima* (Fig. 3.8 E–F), representing species with tightly sheathing ocreas. The degree of inflation differs between *K. echinometra*, *K. scortechinii* and *K. rostrata*. At this early stage there is very slight inflation of the ocrea of *K. echinometra*, no inflation of *K. scortechinii* but the inflation is clearly apparent in *K. rostrata* as a swelling. Whether the ocrea apex is bifid or not is much clearer, at least in *K. rostrata*: Figure 3.8 C shows the strongly bifid ocrea of *K. rostrata*; *K. scortechinii* also appears bifid, as in the LM image Figure 3.7 B, but this is less apparent at the angle at which the ocrea is orientated. Although spines were not apparent on the ocrea on *K. debilis* in the LM image (Fig. 3.7 E), they are apparent at much higher magnification in the earlier developmental stage (Fig. 3.8 D).

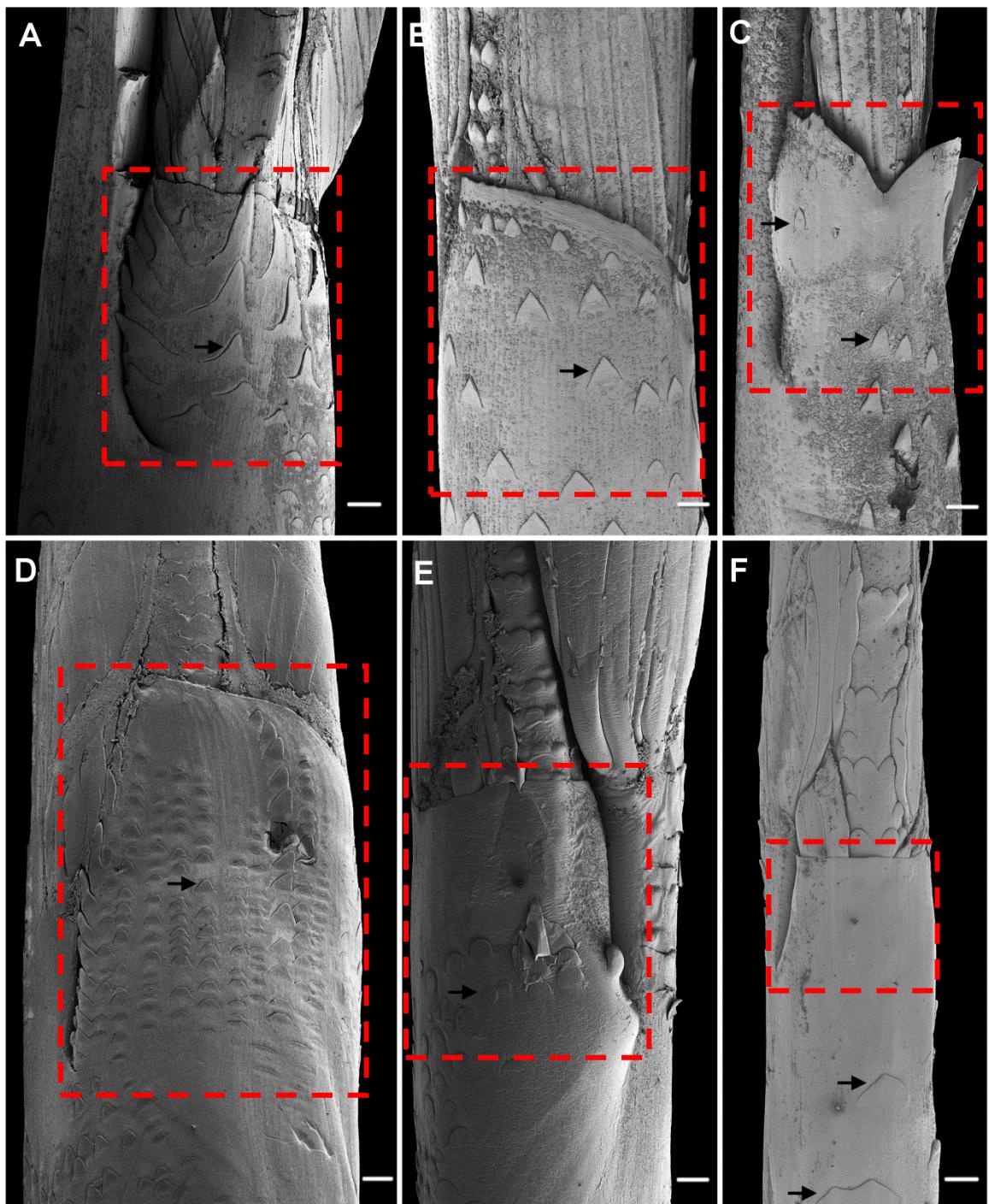


Figure 3.8 Early stages of development of the ocrea (highlighted area) (SEM). A) *Korthalsia echinometra*; B) *K. scortechinii*; C) *K. rostrata*; D) *K. robusta*; E) *K. debilis*; F) *K. tenuissima*. The spines are show by arrows. Bars 100 µm.

Leaf developmental series were examined for *K. echinometra* (Fig. 3.9) and *K. debilis* (Fig. 3.10), inflated and tightly sheathing species respectively, to observe similarities and differences in development between these species. Figures 3.9 A and 3.10 A both illustrate the smallest leaf it was possible to dissect down to, referred to here as the leaf primordium. Figures 3.9 B–D and 3.10 B–D show successively older stages, revealed when fewer layers were removed. The stage illustrated Figures 3.9 B and 3.10 B is one or two layers older, and the stage illustrated in Figures 3.9 C and 3.10 C is another one or two layers older. Although the ocreas differ in these species, these early developmental stages are remarkably similar. Both species have a plicate leaf surface either side of the lamina visible at the stage shown in Figures 3.9 B and 3.10 A. There are no plications apparent on the ocrea itself in either species at any stage. Figures 3.9 D and 3.10 D show that in both species the ocrea only becomes visible and begins to show differences at a late stage, at a layer approximately 3–6 layers later than the first leaf primordium shown in Figures 3.9 A and 3.10 A.

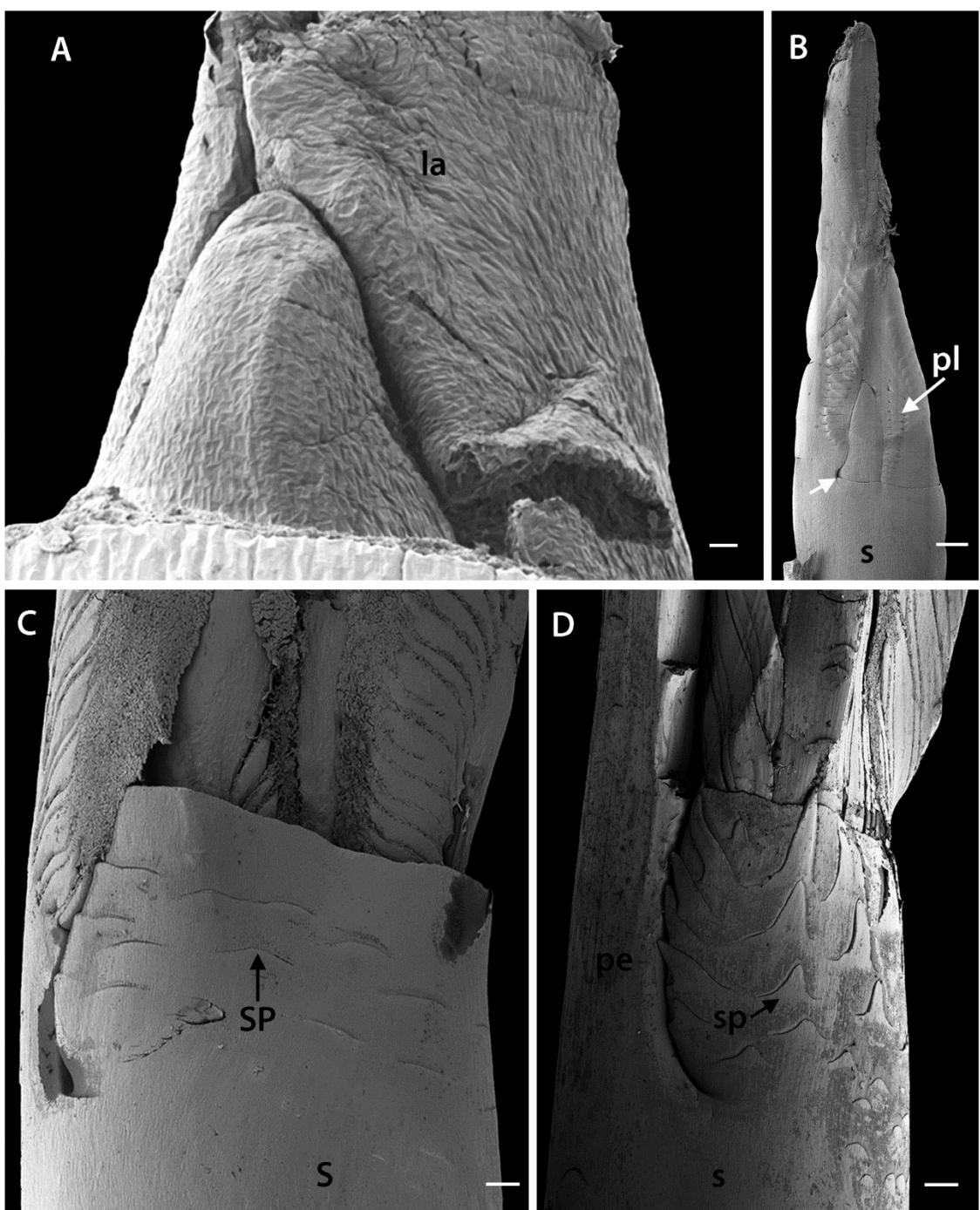


Figure 3.9 Leaf developmental series (SEM). A–D developmental stages for *K. echinometra*. A) Leaf primordium differentiated into a distal lamina but lacking plications at this stage. B) Successive stages of leaf elongation, with leaf plication becoming more pronounced on both sides of the lamina and small lobes present at the top of the leaf sheath (arrow) indicating the first stage of ocrea development. C) At this stage, the petiole has begun to elongate, the spines have begun to develop and the ocrea is more visible. D) The ocrea is a clearly visible and well-delimited structure that will persist into the adult organ. Bars 100 µm. la lamina, s sheath, sp spines, pe petiole, pl leaf plications.

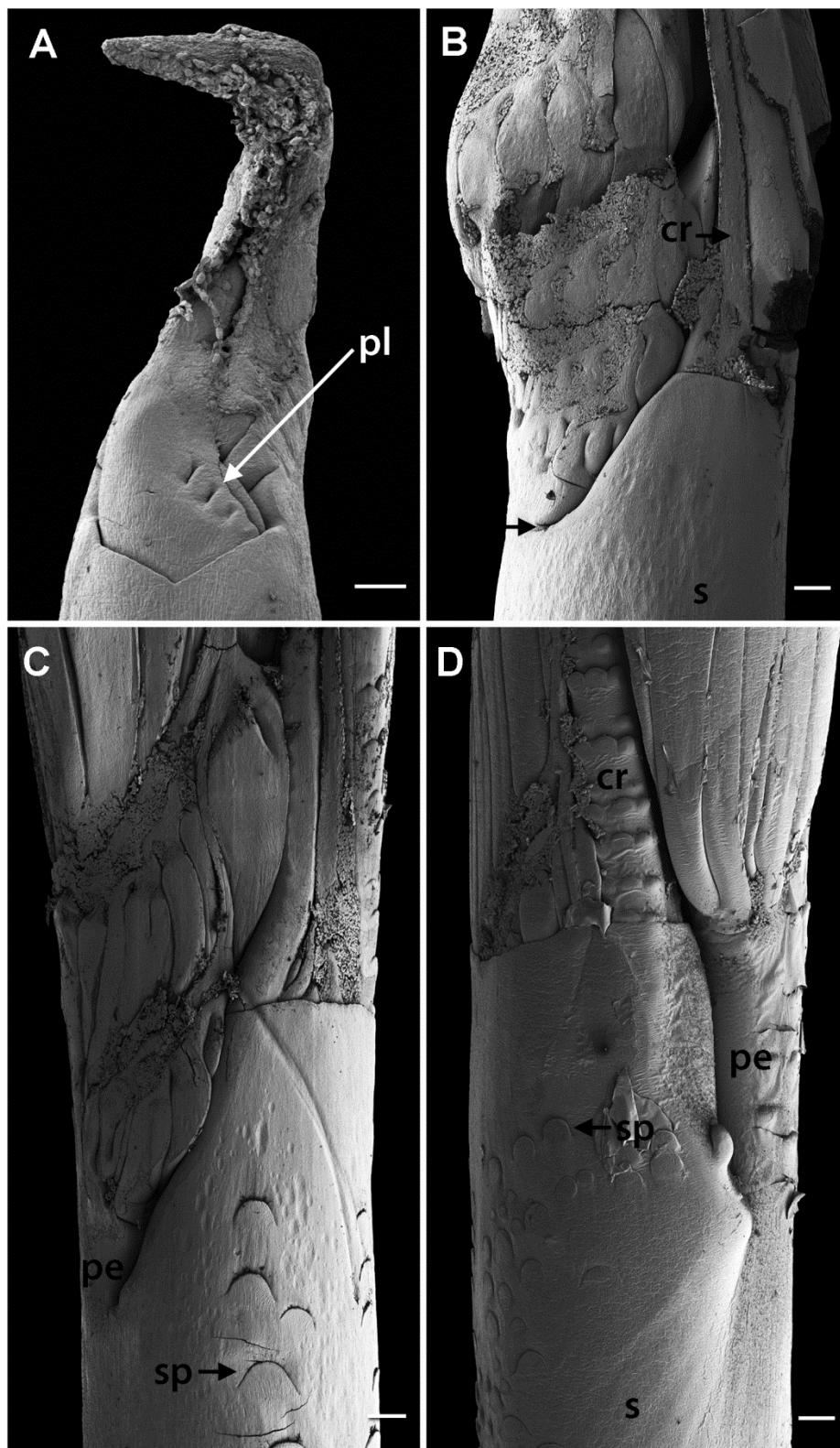


Figure 3.10 Leaf developmental series (SEM). A–D developmental stages for *K. debilis*. A) Plication inception is visible on the leaf and start to develop. B) The ocrea emerge above the petiole (arrow). C) At this stage, the petiole begun to elongate, the spines have begun to develop and the ocrea become more visible. D) The ocrea is a clearly visible and well-delimited structure that will persist into the adult organ. Bars 100 µm. s sheath, sp spines, cr cirrus, pe petiole, pl leaf plications.

The mature surface ocrea of four different species examined using SEM is illustrated in Figure 3.11. Samples include a divergent species (Fig. 3.11 A), an inflated species (Fig. 3.11 B), a tightly sheathing species (Fig. 3.11 C) and a fibrous net-like species (Fig. 3.11 D).

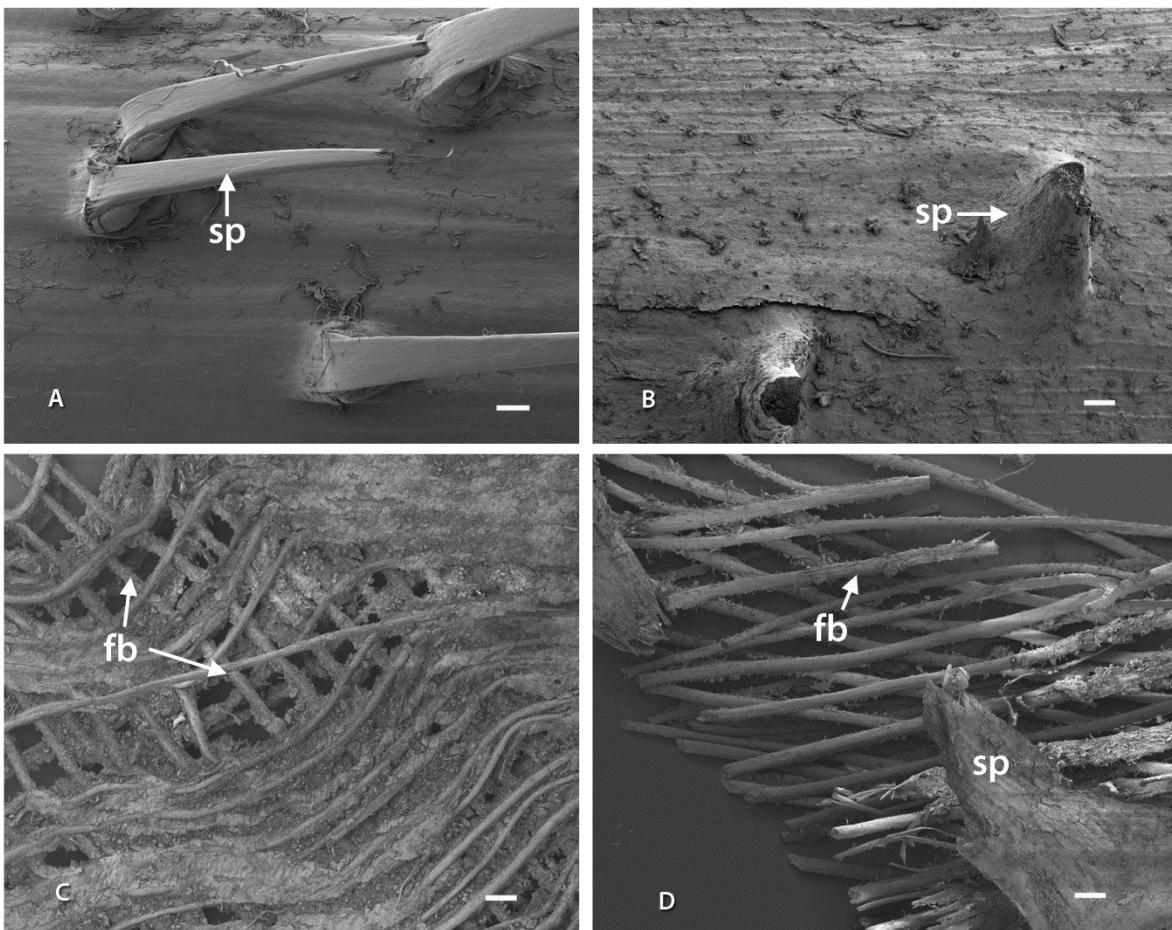


Figure 3.11 Leaf surface of four different species of *Korthalsia* (SEM). a) *K. robusta*; b) *K. scortechinii*; c) *K. debilis*; d) *K. jala*. Bars 100 µm. The specimen of *K. jala* used here was Dransfield et al. 4652. sp spines, fb fibrous.

3.3.2 Ocrea evolution

Nine ocrea characters were used to describe variation in ocrea morphology amongst the 28 species (Table 3.2). All of the ocrea characters were scored from the herbarium and field material. The data matrix for these nine characters and 18 species is presented in Table 3.3. Table 3.3 also indicates the ocrea types *sensu* Dransfield (1981). According

to my scoring, Dransfield's ocrea types that are associated with ants are scored for key domatia characters as follows:

- Divergent type: separated but not clasping
- Inflated type: separated and clasping

The rationale is that the term “separated” describes the separation of the ocrea from the stem. In inflated types, the ocrea comes into contact at a more distal point further up the stem, creating a chambered domatium. The divergent types are also considered separated because the ocrea is separated from the stem. However, in contrast, they are not considered clasping because there is not a more distal point of close contact, and therefore no chambered domatia. Dransfield's two types that are not associated with ants are scored as not separated, nor clasping, but they are distinguished by the funnelling character because only the fibrous and net-like species are scored as funnelling. The sheathing character is different from the separation character because in some separating species there is a basal part of the ocrea that is tightly sheathing, before the ocrea separates from the stem.

Table 3.2 The nine characters describing variation amongst the 28 species of *Korthalsia*.

Character	Characters states
1. Ocrea integrity	0: not disintegrating; 1: disintegrating into a fibrous net
2. Ocrea sheathing	0: no part of ocrea tightly sheathing the stem; 1: the basal part or all of the ocrea tightly sheathing
3. Ocrea separation	0: not separating from the stem at all; 1: separating at least in part from the stem
4. Ocrea clasping	0: absent; 1: in the case of separated ocrea, part of the ocrea has a more distal point of close contact to form a chamber
5. Ocrea inrolling	0: absent; 1: in the case of separated ocrea, there is no point of close contact more distally, and instead the elongate ocrea continues to diverge from the stem and rolls into a tube
6. Funnelling of ocrea	0: absent; 1: present
7. Ocrea size	0: 0–5 cm long; 1: > 5 cm long
8. Ocrea apex	0: truncate; 1: pointed and more or less bifid
9. Spines	0: absent; 1: present

Table 3.3 Scores for the nine characters describing variation amongst 18 species of *Korthalsia*. Whether or not the species have an ant association and the type of ocrea sensu Dransfield (1981) is also indicated. I = inflated ocrea type; D = divergent ocrea type; F = fibrous, net-like ocrea type; T = tightly sheathing ocrea type; Y = yes; N = no.

Species	Characters									Ants	<i>sensu</i> Dransfield (1981)
	1	2	3	4	5	6	7	8	9		
<i>K. cheb</i> Becc.	0	1	1	1	0	0	1	1	1	Y	I
<i>K. echinometra</i> Becc.	0	1	1	1	0	0	1	1	1	Y	I
<i>K. furtadoana</i> J.Dransf.	0	1	1	1	0	0	1	1	1	Y	I
<i>K. rostrata</i> Blume	0	1	1	1	0	0	0	1	1	Y	I
<i>K. scortechinii</i> Becc.	0	1	1	1	0	0	1	1	1	Y	I
<i>K. hispida</i> Becc.	0	0	1	0	1	0	1	1	1	Y	D
<i>K. robusta</i> Blume	0	0	1	0	1	0	1	1	1	Y	D
<i>K. jala</i> J.Dransf.	1	1	0	0	0	1	1	0	1	N	F
<i>K. concolor</i> Burret	0	1	0	0	0	0	1	0	1	N	T
<i>K. debilis</i> Blume	0	1	0	0	0	0	0	0	1	N	T
<i>K. ferox</i> Becc.	0	1	0	0	0	0	1	0	1	N	T
<i>K. flagellaris</i> Miq.	0	1	0	0	0	0	1	0	0	N	T
<i>K. laciniosa</i> (Griff.) Mart.	0	1	0	0	0	0	1	0	1	N	T
<i>K. lanceolata</i> J.Dransf.	0	1	0	0	0	0	0	0	1	N	T
<i>K. minor</i> A.J.Hend. & N.Q.Dung	0	1	0	0	0	0	1	0	1	N	T
<i>K. rigida</i> Blume	0	1	0	0	0	0	0	0	1	N	T
<i>K. tenuissima</i> Becc.	0	1	0	0	0	0	0	0	0	N	T
<i>K. zippelii</i> Blume	0	1	0	0	0	0	1	0	1	N	T

The full time calibrated phylogeny for the Calamoideae showing error bars on ages is shown in Appendix 3.3. Figure 3.12 shows the Bayesian maximum clade credibility chronogram for the Calamoideae and Figure 3.13 shows the Bayesian maximum clade credibility chronogram for *Korthalsia*, clades with less than 50% posterior probability collapsed, and with 95% highest posterior distributions of dates indicated by blue bars. The evolutionary reconstructions for each of the nine characters plus the relationship with ants are shown in Figure 3.14a–c and all characters are summarised shown in Figure 3.15.

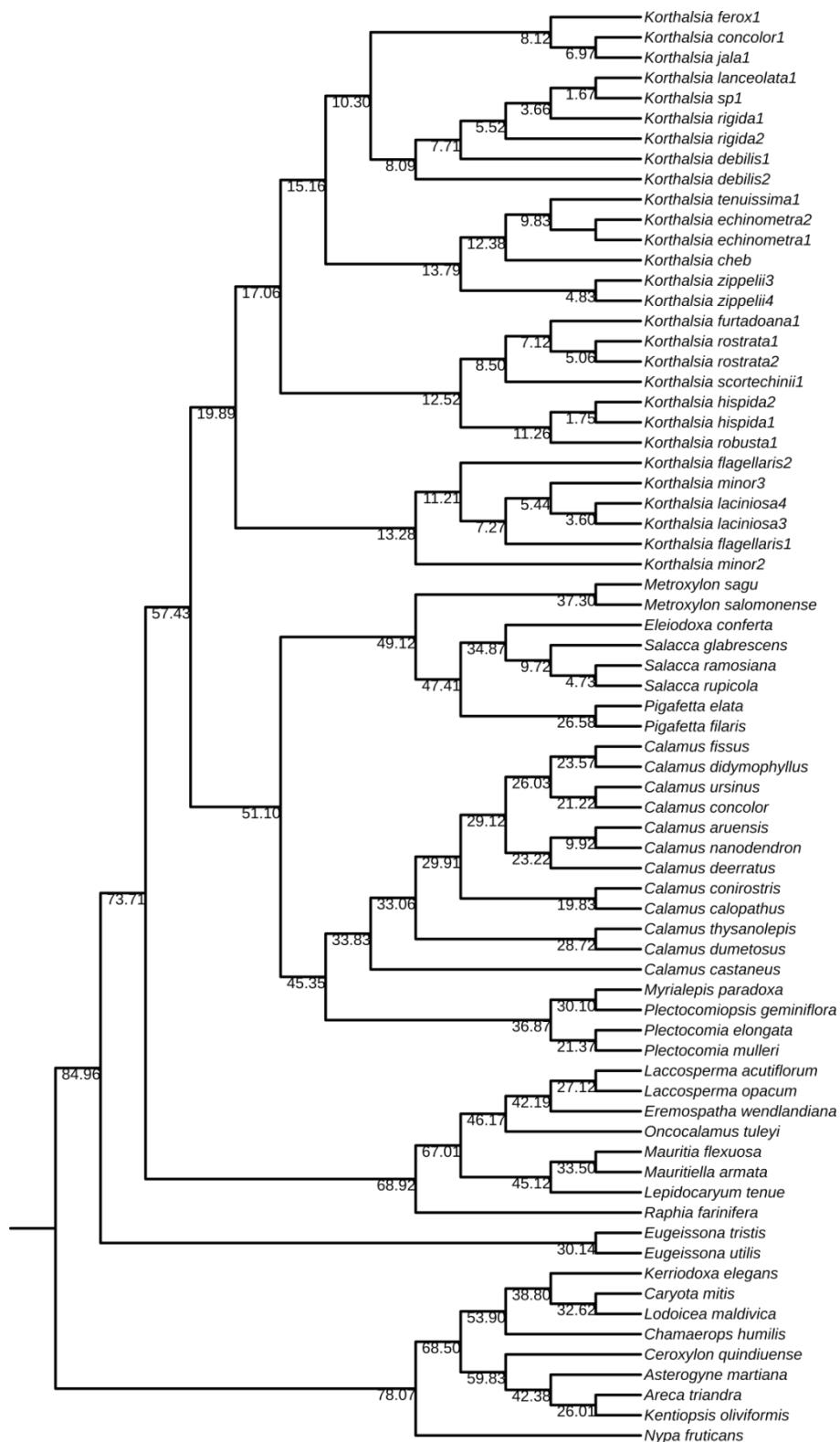


Figure 3.12 The Bayesian maximum clade credibility chronogram for the Calamoideae. Values associated with the nodes are ages.

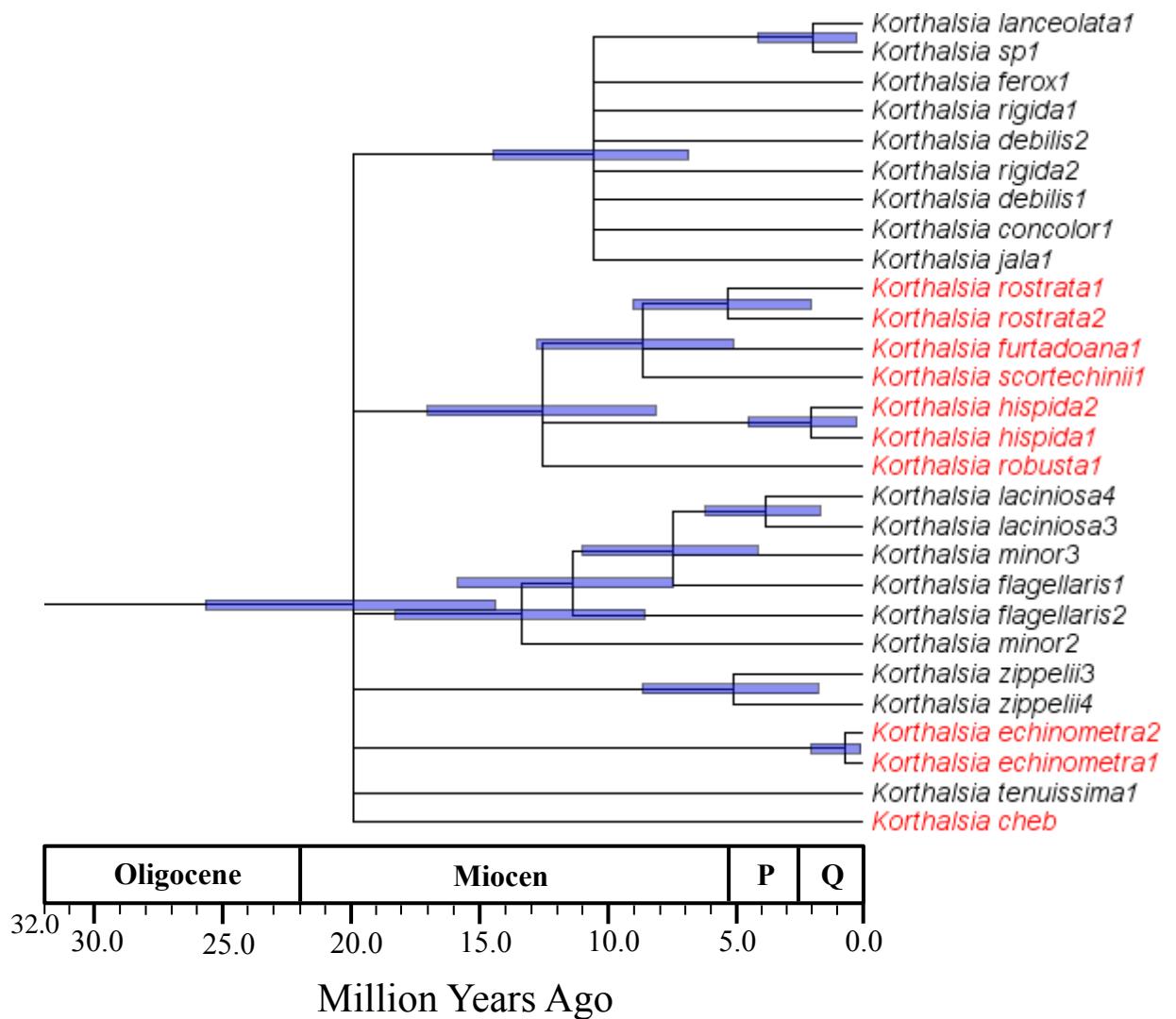
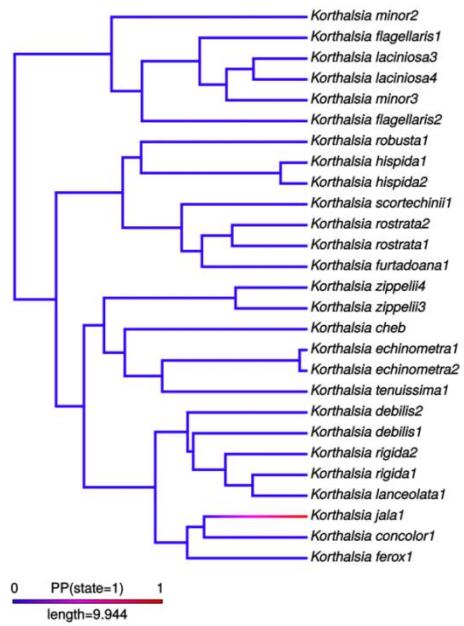
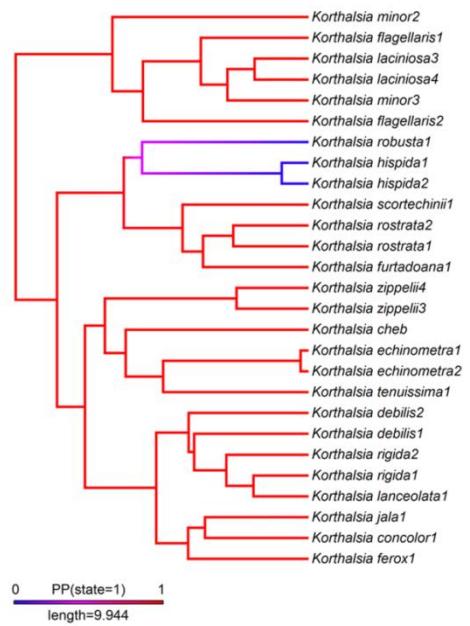


Figure 3.13 The Bayesian maximum clade credibility chronogram for *Korthalsia*, with clades with less than 50% posterior probability collapsed, and with 95% highest posterior distributions of dates indicated by grey bars. Whether species have ant association is indicated using red font for species with ant associations, and black font for species without.

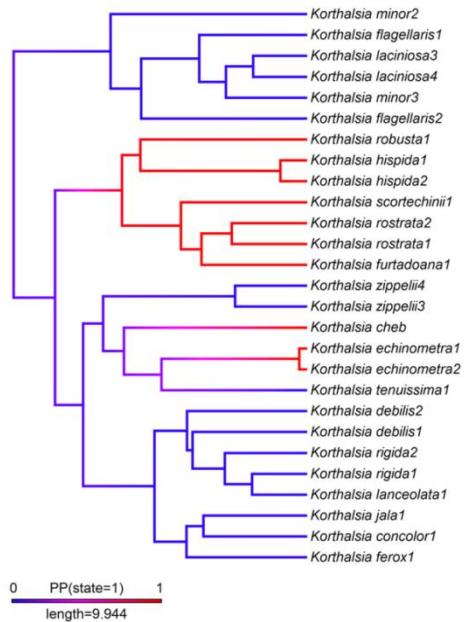
Character 1



Character 2



Character 3



Character 4

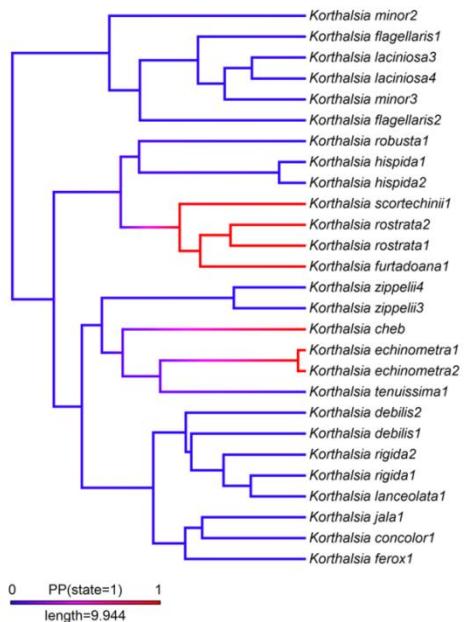
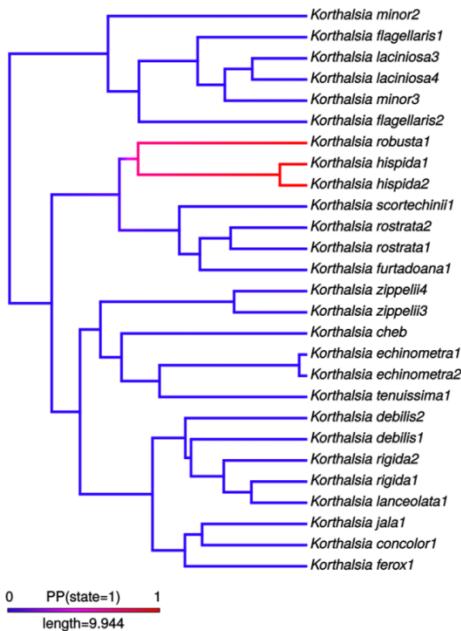
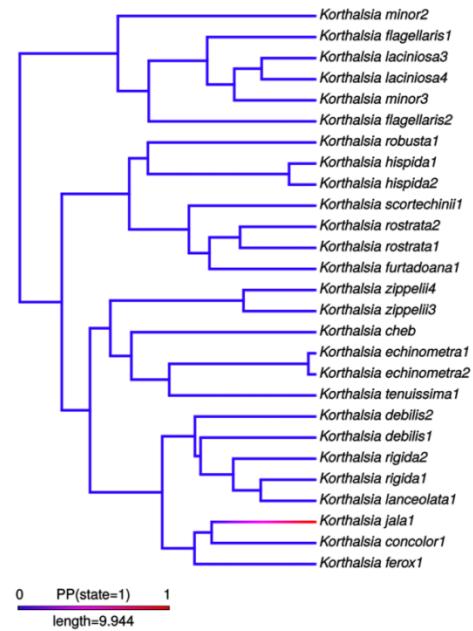


Figure 3.14a The morphological traits of possible importance in the evolution of domatia and the ant-rattan relationship, and the evolution of characters that describe the variation amongst species of *Korthalsia*. Character 1 is ocrea integrity. Character 2 is ocrea sheathing. Character 3 is ocrea separation. Character 4 is clasping ocrea.

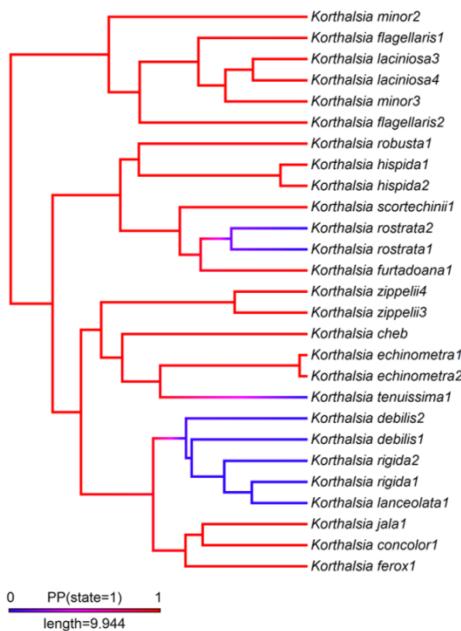
Character 5



Character 6



Character 7



Character 8

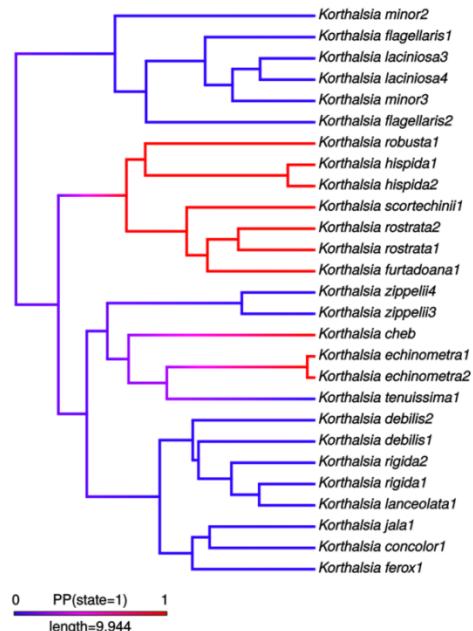


Figure 3.14b The morphological traits of possible importance in the evolution of domatia and the ant-rattan relationship, and the evolution of characters that describe the variation amongst species of *Korthalsia*. Character 5 is ocrea inrolling. Character 6 is funnelling of ocrea. Character 7 is ocrea size.

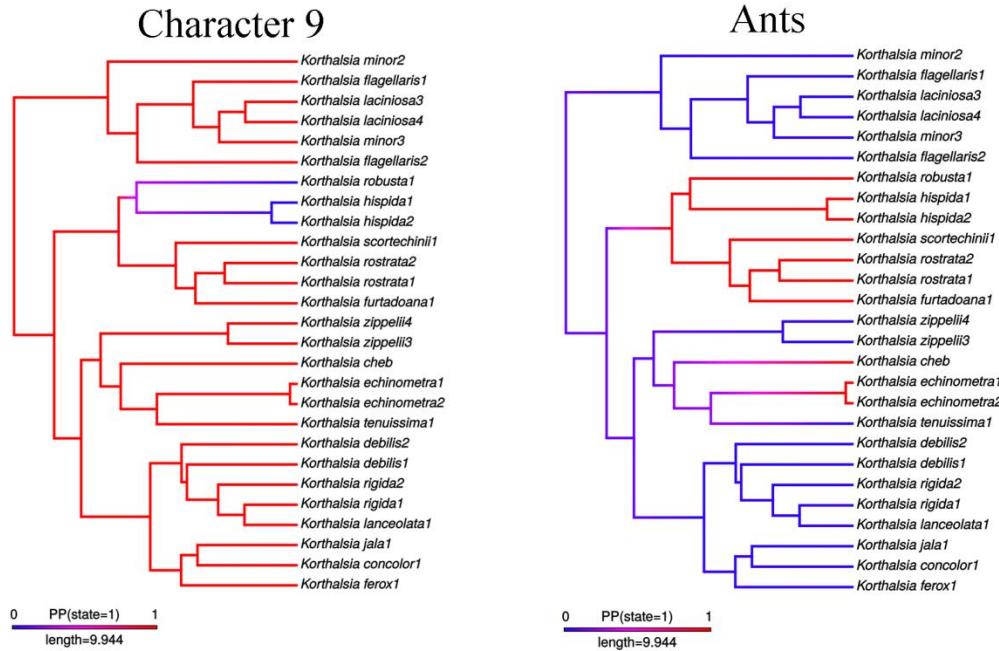


Figure 3.14c The morphological traits of possible importance in the evolution of domatia and the ant-rattan relationship, and the evolution of characters that describe the variation amongst species of *Korthalsia*. Character 9 is spines.

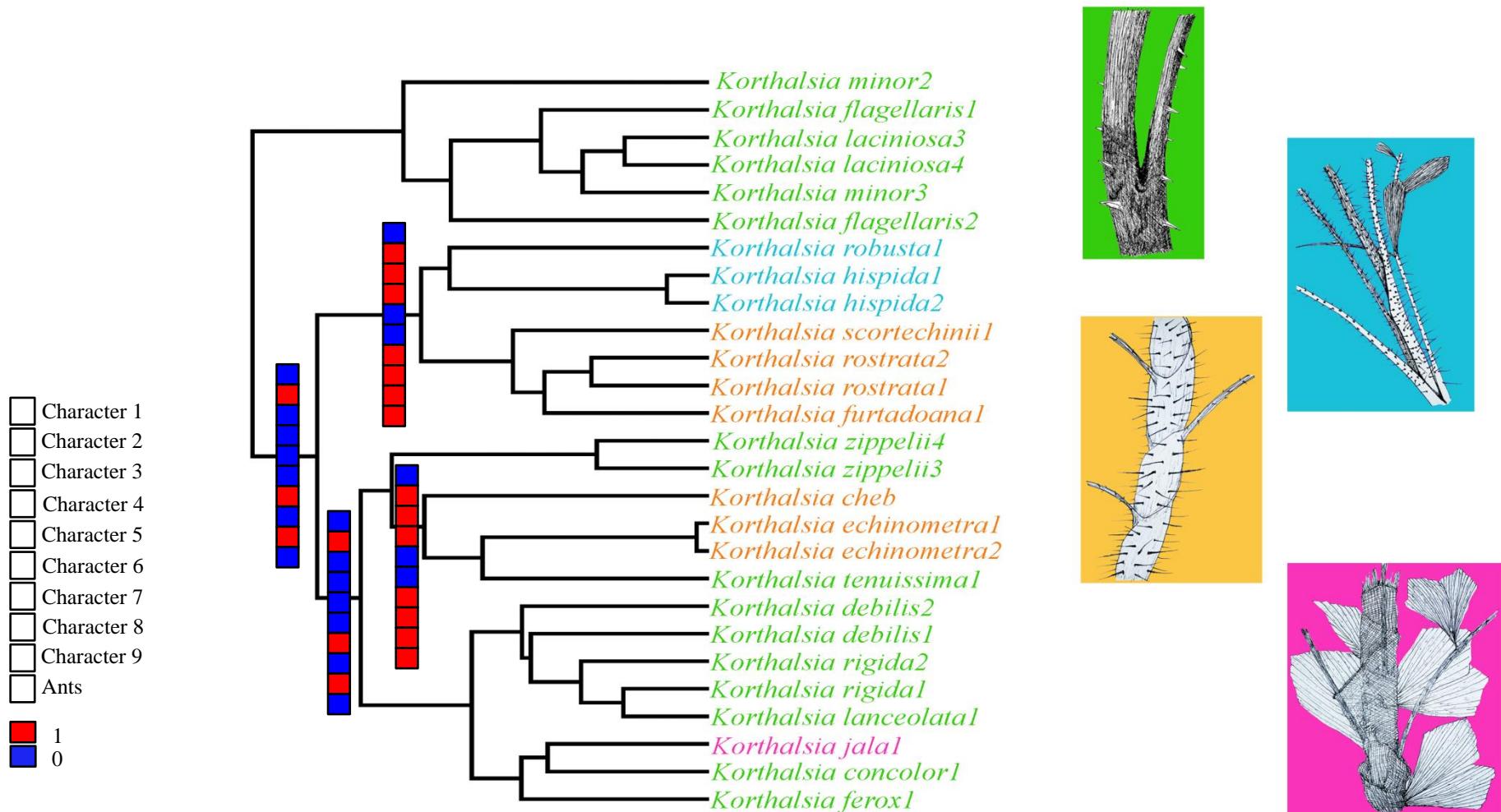


Figure 3.15 The summarize evolution of all characters. Character 1 is ocrea integrity. Character 2 is ocrea sheathing. Character 3 is ocrea separation. Character 4 is clasping ocrea. Character 5 is ocrea inrolling. Character 6 is funnelling of ocrea. Character 7 is ocrea size. Character 8 is ocrea apex and Character 9 is spines. The colours in the species name and the pictures represent types of ocrea. Green: tightly sheathing ocrea. Turquoise: divergent ocrea. Orange: inflated ocrea. Pink: fibrous net-like ocrea.

3.4 Discussion

Dransfield (1981) described four ocrea types. This chapter characterises those ocrea types in detail, and I now have better understanding of the constituent characters that underpin ocrea diversity in the mature ocrea. Amongst the 28 species there were nine unique character combinations. These represent the unique character combinations that remain after grouping types that differed only in ocrea sheathing, ocrea separation, ocrea clasping, ocrea inrolling and funnelling of ocrea. Both the developmental and evolutionary origins of these characters have been identified, and this gives us preliminary assessments of the homologies of the *Korthalsia* ocrea. The analyses show that ocreas that provide domatia for ants evolved more than once, and also that there were shifts between domatia types. As I go on to discuss below, at least the inflated ocreas are not homologous in that this ocrea type does not have a single origin (following Patterson's definition of homology, any structure that is not a synapomorphy is not homologous, Patterson, 1988). However, despite their non-homology, developmentally ocreas are remarkably uniform. Here these main findings are elaborated in more detail, and the significance in terms of the wider literature is discussed. The consideration of ocrea types and their delimitation is also pertinent to the new taxonomic treatment for ant-associated species of *Korthalsia* presented in Chapter 4.

Our time calibrated phylogenies suggest that the relationship with ants evolved at least twice. Throughout the discussion of the evolution of ocrea types and ant relationships, it is important to remember that the phylogeny on which the traits were reconstructed was generally weakly supported. The phylogeny used for the character mapping was the Bayesian maximum clade credibility chronogram; several of the branches in this topology find less than 0.55pp. One clade includes the ant-associated species *K. robusta*, *K. hispida*, *K. scortechinii*, *K. rostrata* and *K. furtadoana*. I refer here to this clade as the main ant clade, and this clade finds 0.73 posterior probability. It comprises species that have divergent ocreas according to Dransfield's (1981) types, as well as species with inflated ocreas. Inspection of the characters that are found amongst the

species in this clade suggests that the separation of the ocrea was the character first associated with the relationship with the ants. The differentiation into the two main types of ant structures evolved within the clade. This presents an interesting evolutionary scenario, whereby the loss of tight sheathing around the stem gave rise to these two specialisations, these being the fully chambered inflated ocrea type and the divergent ocrea type where the ants inhabit an inrolled tube divergent from the main stem. It is interesting therefore to consider the character describing whether any part of the ocrea is tightly sheathing in these species. The two divergent species of this clade have no clasping at all, whereas the inflated species have at least the basal part of the ocrea tightly clasping the stem. The anatomical study of the inflated species in this clade shows that a clasping basal part of the ocrea is evident at early stages of development, though its relative size decreases as the ocrea matures. The anatomy of the divergent species shows no such clasping part at any stage. The character reconstructions suggest separation pre-dated evolution of the divergent and inflated ocrea, but anatomical studies show this separation is more complete in the species with divergent ocrea types.

The timing of the origin of this main ant clade is of interest, since it is the first time that analyses have been carried out to explicitly put dates on the evolution of ant-ocrea associations in rattans. This main ant clade originated 12.52MYR (95% HPD: 8–17). Chomicki and Renner (2015) compiled an up-to-date list of ant domatium-bearing plants, and used local phylogenies for the main ant-associated groups, that represented nearly half of the myrmecophyte-containing lineages. They found 681 vascular plants with domatia distributed in 159 genera in 50 families, mostly from tropical regions of Australasia, Africa and the New World. Their survey revealed domatium-bearing plants in one family of ferns, but not in gymnosperms, absent in basal eudicots but otherwise widespread in angiosperms. Rubiaceae had the highest number, then Melastomataceae. They showed that there had been more than 150 independent origins of domatia and more than 40 losses in the last the last 19MYR. The lineages they performed reconstructions for did not include palms. The time of origin in main ant clade for *Korthalsia* falls within the 19MYR window in which Chomicki and Renner (2015) identified gains and losses of domatia.

The second clade includes two ant associated species, *K. cheb* and *K. echinometra*, both with the inflated type of ocrea. However, *K. tenuissima* is part of the clade that includes the most recent common ancestor of *K. cheb* and *K. echinometra*, though this species does not have any ant association. In fact it has the tightly sheathing ocrea type *sensu* Dransfield (1981). This represents a possible reversal to tight sheathing with loss of clasping. Chomicki and Renner's (2015) study identified gains to losses of 3.75:1; my study identifies two gains and one loss, a 2:1 ratio. *Korthalsia* does not appear to be an outlier in terms of gains and losses of domatia. The separation of the sheath and clasping associated with tightly sheathing ocrea occurred 12.38MYR (95% HPD: 8–16). This is broadly consistent with the origin of the relationship with ants in the main ant clade.

In the light of the phylogenetic hypothesis, I asked whether there were differences between the inflated ocreas of species in the two ant clades, since inflated ocreas have evolved at least twice. There are size differences amongst the inflated ocreas, with the main clade including small and large inflated ocreas, but the second ant clade only including species with large inflated ocrea. This seems to be attributed to the reduction in size of the ocrea in *Korthalsia rostrata*. In the case of *K. rostrata*, the whole plant is small, with scaling maintained as both ocrea and plant reduce in size. It is interesting that there does not seem to be evolutionary pressure to maintain ocrea size, and it would be biologically interesting to determine what impact this has on the ants recruited to defend the plant, and the ants' behaviour. In other ant-plants, the number of domatia rather than size of the domatia determines total nesting space and therefore may be limiting the population size of ants (Fonseca, 1993; 1999).

I also inspected the developmental data for *K. cheb* and *K. echinometra* to see if these inflated ocreas showed any developmental difference to the species with inflated ocrea in the main ant clade, *K. rostrata* and *K. scorchedinii*. There are differences between *K. cheb* and *K. echinometra* to take into account when comparing the inflated ocreas from the two clades. I found *K. echinometra* lacks any basal part of the ocrea in close contact with the stem, even in early stages, whereas *K. cheb* has a short part of the ocrea tightly sheathing the stem. These small developmental differences are apparent at an early

stage. Aside from these differences within the main clade inflated ocrea species, there are no developmental differences between the different species that Dransfield (1981) recognised as having inflated ocreas. These very minor developmental differences are unlikely to have ecological or evolutionary implications. They are highlighted here to illustrate that the early stage developmental differences. Apparently, convergent ocrea in different clades is so subtle.

In 1984, Shirley Tucker, the floral developmental anatomist, building on a rich history of comparative anatomy to investigate evolutionary relationships (Hall, 1992) suggested that at least in flowers, generalized character states are expressed in early stages of ontogeny and that specialized character states are expressed late in ontogeny (Tucker, 1997). She defined characters as early stage, mid-stage and late stage. She found the early stages differed in different lineages, and late stages differed between more closely related species. My findings of relatively few early stage differences in *Korthalsia* ocrea development would be expected under her Hierarchical-Significance Hypothesis, since I am comparing closely related species. It is interesting in this context to compare the development of *Korthalsia* to the development of *Calamus longipinna* described by Merklinger *et al.* (2014). They found that the result of plications which develop on the ocrea surface make the ocrea subsequently become sac-like and inflated. These plications are similar to the lamina at the early stage but the orientation is different compared to lamina. In other words, at deeper hierarchical levels there are earlier stage differences in ocrea development.

The costs and benefits to host plants of ant-plant protective mutualisms have been the focus for field research and reviews (Mayer, 2014; Trager, 2010). These studies highlight cost benefit analyses as valuable approaches to examining ant-plant mutualism. Whether plants experience fitness benefits (Trager, 2010) is a question of great importance if we seek to understand the maintenance of ocrea and ant-plant mutualisms through evolutionary time. Our findings, suggesting loss of the mutualistic relationship in at least one lineage (*K. tenuissima*) might be interpreted in the light of cost-benefit analysis. The stability of mutualism is of great interest (Mayer, 2014), and our study highlights the loss of the mutualism in *K. tenuissima* as a study system to

better understand cost-benefits and stability. Field experiments comparing reproductive success in the lineage including *K. tenuissma* might be very informative in this context. Evolutionarily-informed manipulations of the ants, swapping ants between close and distantly related plants, might be of interest.

It would be exciting to integrate what is known about the field biology of the ant mutualism in *Korthalsia* with what is found here in terms of phylogeny. Table 1.1 lists all the existing papers and reports of ants in association with *Korthalsia* species. There is very scanty information available, with identifications of ants made from only five species, of which five are sampled and included in the phylogeny. There are seven genera of ants in association, but only one of them is found in more than one *Korthalsia* species, *Camponotus* sp. Considering the distribution of species of this genus in the evolutionary lineages of *Korthalsia*, we can see little evidence of phylogenetic pattern in occupation of related species. *Camponotus* sp. is found in four species, but these are not in one clade, suggesting independent recruitment of these ants to these plant species, and no preferences amongst ants for phylogenetically close relatives. Considering the distribution of the plants that are found with ocrea occupied by *Camponotus* sp., we find that these species are found in Borneo and Peninsular Malaysia, suggesting little geographical structuring in the distribution of *Camponotus* sp. on *Korthalsia*. Ultimately considerably more field work is needed to characterise the ants in association with *Korthalsia*. With more data available, biogeographic patterns as well as phylogenetic ones might emerge.

Camponotus is a hyperdiverse genus (> 1,000 species, > 400 subspecies) of carpenter ants; in the Neotropics, *Camponotus* species may be ant-gardeners (Youngsteadt *et al.*, 2009), nectar feeders (Josens *et al.*, 1998) and also occupy the domatia of *Cecropia* species (Bonato *et al.*, 2003). In South-East Asia, the ant-plant *Macaranga puncticulata* (Federle *et al.*, 1998) and *Clerodendrum fistulosum* (Maschwitz *et al.*, 1994) are also associated with *Camponotus*. Clearly *Camponotus* species are not uniquely recruited to *Korthalsia* domatia.

CHAPTER 4

A TAXONOMIC REVISIONS OF THE ANT-RATTAN SPECIES *KORTHALSIAS*

4.1 Introduction

The palms (Arecaceae) are among the most prominent and diverse components of tropical ecosystems worldwide. Almost 40% (>1,000 species) of global palm diversity (ca. 2,600 species) occurs in the Malesian region (Dransfield, *et al.*, 2008).

Korthalsia is endemic to this region. It is one of the eight genera of rattans, which are spiny climbing palms in the subfamily Calamoideae (Vorontsova *et al.*, 2016). The genus has distinctive vegetative morphology with jagged, diamond-shaped leaflets and unusual aerially branching stems. In common with a few other palm genera, *Korthalsia* species flower in a single, terminal events that result in the death of the stem. The tubular sheathing base of the leaf is unusual in forming a conspicuous extension above the point of attachment of the leaf petiole (stalk). This structure, known as an ocrea, is extremely diverse in form, sometimes tightly clasping the stem or forming a fibrous expanded network or inflated to form an ant nest-chamber, or diverging from the stem with inrolled margins, also forming a chamber for ants (Dransfield *et al.*, 2008). Several species from genus *Korthalsia* have an association with ants from genus *Camponotus*, *Crematogaster*, *Dolichoderus*, *Iridomyrmex* or *Polycharchis* sp. (Dransfield, 1981; Mattes *et al.*, 1998; Edwards *et al.*, 2010; Chan *et al.*, 2012). The ants live in the chambers of inflated and diverging ocrea at the leaf base. The ants make a rustling noise by banging their abdomens against the ocrea to create a precautionary alarm when the plant is disturbed. *Korthalsia* is an isolated group and the sole member of its tribe Korthalsiinae in tribe Calameae of subfamily Calamoideae.

In phylogenetic study (Chapter 2), total evidence analyses strongly support the monophyly of *Korthalsia* and its placement as sister to the remaining members of

subtribe Calameae. These findings consistent with some (Baker *et al.* 2000a, 2000b), but not all previously published results (Baker *et al.* 2000, 2006, 2009), some of which found relationships with genera such as *Eleiodoxa* (Becc.) Burret, *Metroxylon* Rottb., *Pigafetta* (Blume) Becc. and *Salacca* Reinw.

Korthalsia is distributed from the north of Indochina, Burma and the Andaman Islands and south-eastward to Celebes and New Guinea (Dransfield *et al.*, 2008). Currently, 28 species are accepted (WCSP, 2017). The highest diversity of *Korthalsia* is found in Borneo, Malay Peninsula and Sumatra. The focus of this revision is on the ant-species of *Korthalsia*. Currently, 10 ant-species of *Korthalsia* are accepted. The ant species do not occur throughout the entire range of the genus and are restricted to Borneo, Malay Peninsula, Philippines (Palawan and Mindanao), Singapore and Thailand (Figure 4.1).

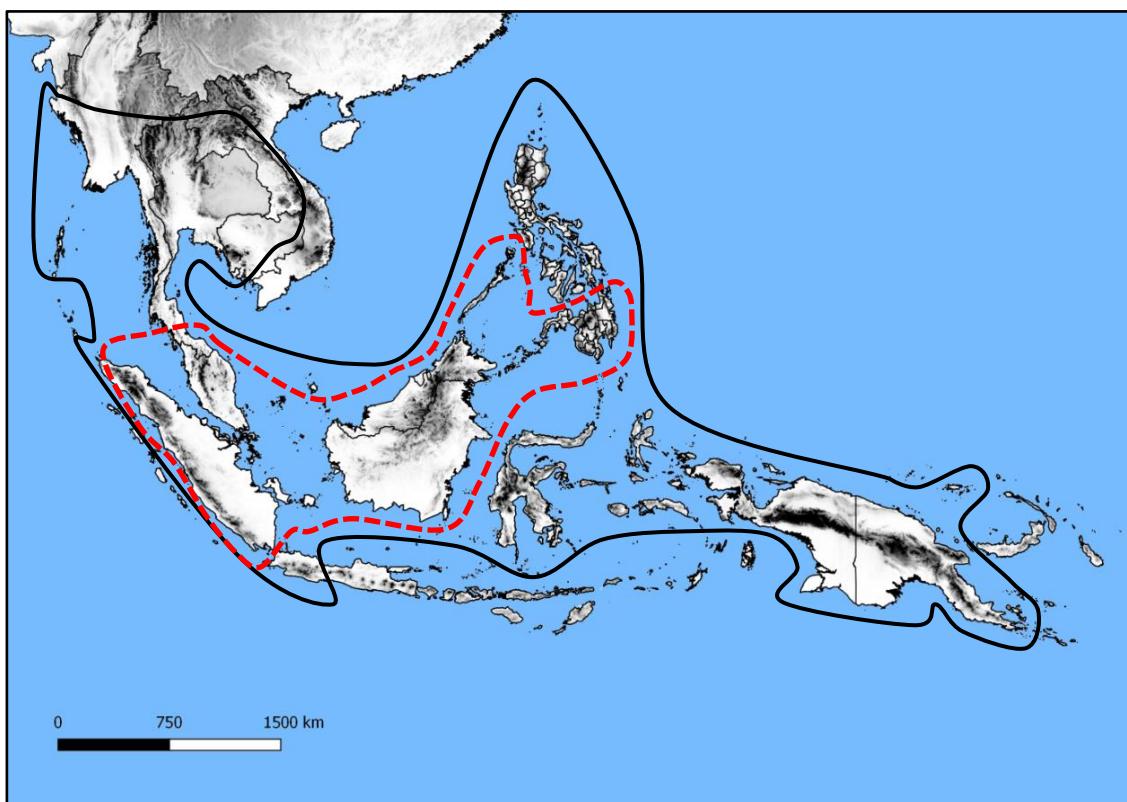


Figure 4.1 Distribution map of *Korthalsia* spp in Malesia region (full line). Distribution of ant-species *Korthalsia* (broken red line). Map prepared by Salwa Shahimi, using QGIS.

4.1.1 Taxonomic history

The genus *Korthalsia* was first described in 1843 by Carl Ludwig Blume, based on type specimen of *K. rigida* Blume. In the same year, Blume also described six more species of *Korthalsia*. In 1845, William Griffith described a new plant and named as *Calamosagus* Griff. It appeared that the genus has very similar morphology characters to *Korthalsia*. Because of that similarity, five species *Calamosagus* recognized by Griffiths are now included in *Korthalsia*.

Odoardo Beccari made a tremendous contribution by describing the Calamoid palms. Between years 1884–1918, Beccari described almost all species of *Korthalsia*. In 1884, Beccari wrote a monograph of *Korthalsia* species for *Malesia, raccolta d'osservazioni lese e papuano*. In 1951, Furtado published the Malayan species of *Korthalsia* focusing with only eight recorded species of *Korthalsia*.

In 1981, Dransfield published a synopsis of *Korthalsia*. In this account, Dransfield organized nomenclature and made new synonymies. He also updated descriptions with new information for the established species and described new species in full. *Korthalsia furtadoana* J.Dransf. and *Korthalsia jala* J.Dransf. were the two new species described by Dransfield.

Since Dransfield's synopsis, *K. minor* A.J.Hend. & N.Q.Dung was the only new species added to the genus (Henderson and Nguyen, 2013). The species was described based on a herbarium specimen of *K. laciniosa* (Griff.) Mart. which has significant variation in the size of pinnae. There are two groups of the specimen in herbaria, one with larger-sized and the other with smaller-sized of pinnae. Henderson and Nguyen decided that the larger-sized specimens from Laos and Vietnam represented *K. laciniosa* (Griff.) Mart. and the smaller-sized one represented a new species (*K. minor* A.J.Hend. & N.Q.Dung) which differs from *K. laciniosa* (Griff.) Mart. in smaller pinnae, size, scale and colour of fruit and seed.

4.1.2 Taxonomic revision

Taxonomic revisions may be mainly based on the study of herbarium collections. The study on early publication and field observations also are the primary component of taxonomic revisions. The elements of treatment are the names accepted and names put into synonymy, the citation of protogues, type, and other specimens examined, description of the species, distribution maps, and notes on habitat. Other notes might include ethnobotanical uses, local names, phenology, and biotic or edaphic associations.

A taxonomic revision reconsiders the boundaries of the species within the study group. This is testing hypotheses of species (Wheeler, 2004). As part of the reviewing process, new species hypotheses may be put forward. Either completely new species might be described, or the variation and/or distribution of existing species might be redrawn. A revision may, therefore, present different descriptions, maps, and species to the previous treatment.

For genus *Korthalsia*, a revision of 10 species of *Korthalsia* which has ant-association as the main focus here. Although the species with ant associations do not appear to comprise a monophyletic group (Chapter 3) they are a distinctive subcomponent of the genus. By treating the species with ant-associations here I provide a tool of use to the ecologist making field studies of these plants. A new treatment for the genus is needed because these species have not been the subject of a modern treatment across their ranges. Only regional treatments have been published.

4.2 Material and methods

4.2.1 Herbarium studies

An extensive study of specimens at Kew herbarium and three international herbaria, namely E, SING and KEP (herbarium acronyms follow Holmgren *et al.*, 1990)

underpins this study. Every specimen with information relating to collector name and number, collection date and locality, vernacular name(s), habitat and uses was collected and compiled in a data sheet.

Living rattans and dried herbarium were fully measured and the information obtained was recorded in data sheets. The information on morphological features such as habit, stems, leaves, inflorescences, fruits and seeds observed in living rattan and dried herbarium specimens were used to describe each taxon and taxa. The key species was constructed using the information on diagnostic characters.

In total, almost 230 distinct herbarium collections were studied and observed. Appendix 4.1 shows the data sheet that has been used for data collection. The exclamation mark (!) indicate that where I have seen the specimens directly myself or have studied on online digital image.

4.2.2 Field studies

To gain more information and knowledge on *Korthalsia*, two field trips were conducted: Peninsular Malaysia and Singapore (November, 2014) and Borneo and Singapore (September, 2015). For each population, herbarium material was collected according to the method of Dransfield (1986). Geo referencing was carried out using a global positioning system (GPS) receiver to record the latitude and longitude for each specimen. The targeted parts of the plant such as stem, leaves were collected. The specimens were collected in up to three duplicates. Duplicate specimens of all collections made were deposited in herbaria K, KEP and SING. The ocrea was preserved in 70% ethanol for anatomical studies. The sample extracted from leaves for each specimen was dried and preserved in silica gel for molecular works. Extensive notes were taken for each specimen. The general morphology of the plant was recorded, and several photographs were taken of each rattan including images of the rattan in habitat.

4.3 Results

4.3.1 Morphology of ant-associated *Korthalsia*

4.3.1.1 Habit

These species of *Korthalsia* are moderate to robust, clustering high-climbing rattans up to 60 m or more. Some species can reach the forest canopy. They are found only in lowland and hill tropical forest, being absent in montane forest. Most of the species have a wide ecological range and are abundant in primary forest. Some species (e.g. *K. echinometra*) are quite variable in habit, but others (e.g. *K. furcata*) appear rather uniform.



Left: Habit of *K. scorutchinii*. Right: Habit of *K. echinometra*.

4.3.1.2 The stem

The stem size of the ant-associated *Korthalsia* varies from slender (0.2–0.8 cm in diameter without sheath) to moderately large (1.0–4.0 cm in diameter without sheath). The internodes are elongate and variable in length. Nodal scars of *Korthalsia* species are often uneven. Aerial branching sometimes occurs due to parallel forking (dichotomy).



Left: Stem slender (e.g. *K. rostrata*). Right: Stem moderate large (e.g. *K. rigida*)

4.3.1.3 The leaf

All ant-associated species of *Korthalsia* in this study have pinnate leaves with a cirrus. The sheath is tubular, bright green to green in colour and turn into light brown as they age, usually with caducous indumentum. The sheath is sometimes unarmed or variously armed with spines. The petiole ranges from absent/short (up to 1.5 cm) to long (up to 40 cm). The leaflets are regularly arranged and usually rhomboid with distal margins praemorse, but in few species, they are lanceolate, still with praemorse margins. The adaxial surface of the leaflets is usually bright or dark green, and the abaxial surface was covered in white or grey indumentum, or sometimes with caducous, orange and/or brown to dark brown indumentum. The number of leaflets on each side of the rachis varies from one to 25; with the smallest number belong to *K. furcata* and the highest in *K. echinometra*. The main veins diverge from the leaflet base. The transverse veinlets are conspicuous or unclear.



Left: Leaflets rhomboid (e.g. *K. robusta*). Right: Leaflets lanceolate (e.g. *K. echinometra*).

4.3.1.4 The ocrea

The term ocrea refers to an extension of leaf sheaths above the petiole insertion. The ocrea is usually well-developed, unarmed or variously spiny. It is morphologically diverse, ranging from net-like, collar-like rim to elongate papery. In *Korthalsia*, it has four broad type of ocrea. First, the simple ocrea is truncate, tightly sheathing and regularly spiny (e.g. *K. bejaudii*, *K. brassii*, *K. celebica*, *K. concolor*, *K. debilis*, *K. ferox*, *K. flagellaris*, *K. junghuhnii*, *K. laciniosa*, *K. lanceolata*, *K. merrillii*, *K. minor*, *K. paucijuga*, *K. rigida*, *K. rogersii*, *K. tenuissima* and *K. zippelii*). Secondly, only belonging to *K. jala* which the ocrea is expended and forming a funnel-shape fibrous net-like. The third ocrea type which the ocrea clasping the stem and inflated (e.g. *K. angustifolia*, *K. cheb*, *K. echinometra*, *K. furcata*, *K. furtadoana*, *K. rostrata*, *K. scaphigeroides* and *K. scortechinii*). This ocrea type associated with ants because the ocrea form like a chamber for the ants nested within. The fourth type is where the ocrea is not clasping the stem but diverging at an acute angle and margins tending to inroll (e.g. *K. hispida* and *K. robusta*). This ocrea is variously armed with long spines and is also inhabited by fierce ants. The ants are capable of making noise.



Left: Ocrea divergence (e.g. *K. robusta*). Right: Ocrea inflated (e.g. *K. echinometra*).

4.3.1.5 Inflorescence

The flowering behavior in the species surveyed is hapaxanthic (individual stems flower only once in their lifetime and die subsequently). The inflorescences are borne at the apex of the stem and are lax to congested, with one to two orders of branching. The peduncle is adnate to the internode above the subtending leaf (Dransfield *et al.*, 2008). The prophylls have 2-keels and are tightly sheathing. Rachis bracts can be tubular, tightly sheathing and for some species somewhat inflated. The bracts can be unarmed or sparsely armed and densely covered with caducous indumentum. The rachillae are cylindrical and catkin-like, with densely arranged rachilla bracts, sometimes with hairs in between. The rachillae can be slender or congested.



Left: Inflorescence congested (e.g. *K. robusta*). Right: Inflorescence slender (e.g. *K. cheb*).

4.3.1.6 Flower

In all *Korthalsia* species examined here, the flower is hermaphrodite and borne in pits in the catkin-like rachillae. According to Dransfield (1981), *Korthalsia* is the only Asiatic rattan genus with a hermaphrodite flower. The calyx is tubular at the base, with three sepals and usually shorter than corolla. The corolla consists of three valvate petals. The flower contains 6–9 stamens, borne on the petal.



Flower of *K. laciniosa*.

4.3.1.7 Fruit

The fruit of *Korthalsia* species examined here is globose to ovoid, with one seed. The epicarp is thin and covered with vertical rows of imbricate scales. The scales are usually brown in colour, rarely straw-coloured except for *K. robusta*. The mesocarp develops as a thin sweet-fleshy layer surrounding the seed, and the endocarp is not differentiated. The seed is attached basally. The lack of sarcotesta (fleshy layer developed from the outer seed coat) makes this genus unique amongst the rattans. In most of the species, the endosperm is ruminant or sometimes homogeneous in a few species (e.g. *K. hispida*). The fruits of most species appear to be attractive to animals (Dransfield, 1981).



Left: Fruit of *K. echinometra*. Right: Fruit of *K. robusta*.

4.3.2 Keys to ant-rattan species of *Korthalsia*

1. Ocrea not clasping, diverging at an angle from the stem, margins tending to inroll (Borneo, Peninsular Malaysia, Philippines)..... 2
1. Ocrea clasping the stem and inflated (Borneo, Malay Peninsula, Sumatra, Singapore, Thailand, Philippines)..... 3
 2. Ocrea 18.5–30.0 × 3.0–4.5 cm long, ocrea, sheath and inflorescences bracts armed with scattered black spines, with abundant black spicules (a very slender brittle, needle-like structure) (Peninsular Malaysia, Borneo)..... *K. hispida*
 2. Ocrea 16.0–40.0 × 3.0–7.0 cm long, ocrea, sheath and inflorescences bracts armed with scattered black spines, with black spicules absent (Borneo, Philippines) *K. robusta*
3. Leaflets rhomboid (rarely narrowly rhomboid) 4
3. Leaflets narrowly lanceolate (Peninsular Malaysia, Borneo, Singapore) *K. echinometra*
 4. Leaflets only one on each side of the rachis (Sarawak) *K. furcata*
 4. Leaflets usually more than three on one each side of rachis 5
 5. Stem slender, with sheath less than 1.5 cm diameter 6
 5. Stem robust, with sheath at least 2.0 cm diameter 8
 6. Leaflets intensely indumentose on undersurface (Philippines). *K. scaphigeroides*
 6. Leaflets sparsely indumentose on undersurface 7

7. Ocrea 2.5–5.0 × 1.0–3.0 cm, transverse veinlets space moderately close, rachillae slender (Peninsular Malaysia, Borneo, Singapore, Sumatra) *K. rostrata*
7. Ocrea 2.5–8.3 × 1.0–1.5 cm, transverse veinlets fine and closely spaced, rachillae robust and congested (Borneo) *K. furtadoana*
8. Ocrea armed with scattered triangular spines up to 2.0 cm long and ocrea about 20–26 cm long 9
8. Ocrea armed with scattered very short triangular spines about 3 mm long and ocrea about 10–15 cm long (Peninsular Malaysia, Thailand) *K. scortechinii*
9. Leaflets rhomboid to broadly rhomboid, 6–8 leaflets each side of rachis and petiole 10–25 cm long *K. cheb*
9. Leaflets rhomboid, 7–12 leaflets each side of rachis and very long petiole ca. 40 cm long *K. angustifolia*

4.3.3 Taxonomic treatment

Korthalsia Blume (1843: 166). Lectotype: *K. rigida* Blume.

Calamosagus Griff. (1844: 22). Lectotype: *C. laciniosus* Griff. (= *Korthalsia laciniosa* (Griff.) Mart.)

1. ***Korthalsia angustifolia*** Blume (1843: 172). Type:—INDONESIA. South Kalimantan: Sungai Dusun, *Korthals & Muller s.n.* (holotype L!; isotype FI!). MAP 1. PLATE 1.

Clustering rattan climbing to 50 m. **Stem** with sheaths 35–50 mm diam., without sheaths 20–30 mm diam.; internodes 10–25 cm. **Leaf** 2–3 m long including cirrus and

petiole; sheath green, with grey indumentum, sheath sparsely armed with brown to dark brown triangular spines, spines more abundant near sheath mouth, spines 3–13 mm long, ca. 1 mm wide; ocrea 24.2–25.5 × 2.5–3.5 cm, very large and conspicuous, elongate inflated, clasping the stem, tough, brown (when dry), armed with 1.0–2.0 cm long dark brown, planar spines, ocrea occupied by ants; cirrus ca. 1 m; petiole ca. 40 cm, 10–15 mm wide and 3–4 mm thick at base, flattened adaxially, abaxially rounded, with grey indumentum as sheath, sparsely armed with spines; rachis ca. 0.9 m, armed with recurved grapple spines organised in whorls; 7–12 leaflets each side of rachis, regularly arranged, rhomboid, mid-leaf leaflets 18.0–23.0 × 4.7–5.9 cm, leaflet dark green above, glabrous, with caducous, grey-brown indumentum on undersurface, distal margin conspicuously praemorse, transverse veinlets moderately conspicuous, moderately spaced ca. 1.0 mm. **Inflorescence** lax, 47.0–61.7 cm long, branched to 2 orders; rachis bracts up to 6.2–7.0 × 3.5–3.7 cm, tightly sheathing and splitting, with caducous, dark brown indumentum; primary branches 1–2, 4.0–8.0 cm apart, proximal primary branch 20.2–22.1 cm long, with up to 2 rachillae; rachillae 17.8–24.8 cm long, including 1.5–3.7 cm visible stalk, 0.7–1.0 cm wide, densely hairy between rachilla bracts. **Flower** not seen. **Fruit** mature fruit not seen. **Seed** not seen.

Distribution:—Borneo (Central and South Kalimantan).

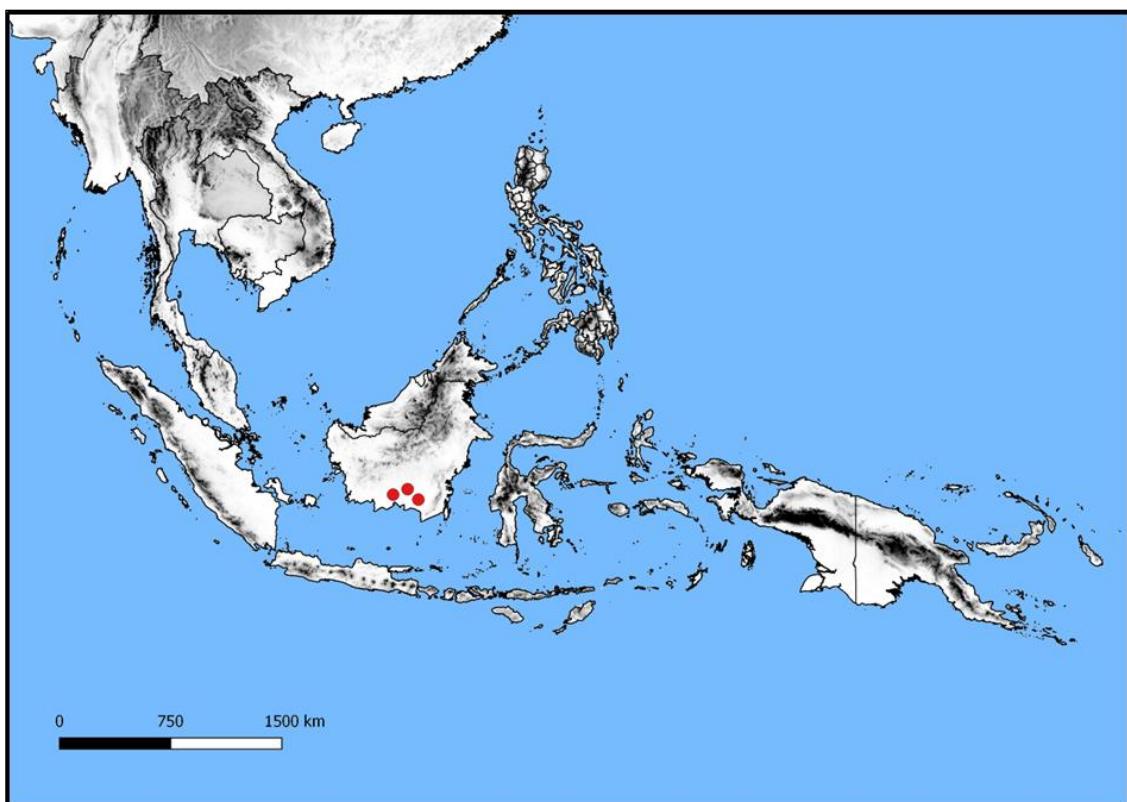
Habitat:—Along river banks and in freshwater swamp on alluvial soils.

Uses:—Not recorded.

Vernacular name:—*Ahas, Rotan ahas, Rotan patung* (Indonesia).

Specimens examined:—INDONESIA. Kalimantan Tengah: Pelangka Jaya, 2°12' S, 113°50' E, 25 January 1974, Dransfield 3924 (K!, L). Kalimantan Selatan: Barito River, 12 km upstream of Marabahan, 2°50' S, 114°29' E, 03 January 1989, Geisen 89 (K!); Sampit, Sei Pemalian, 2°32' S, 112°57' E, July 1986, Sutisna 70 (K!).

Notes:—Very little herbarium material of *K. angustifolia* is available. Although *K. angustifolia* is vegetatively very similar to *K. cheb* and *K. scortechinii* but it has very long petiole compare to *K. cheb* and *K. scortechinii*.



MAP 1 Distribution of *Korthalsia angustifolia* Blume. Dots are point localities of specimens examined. Map prepared by Salwa Shahimi, using QGIS.

PLATE 1



Korthalsia angustifolia Blume. Top left: ocrea (Sutisna 070). Top right: inflorescence (Giesen 89). Bottom: leaf arrangement (Sutisna 070).

2. *Korthalsia cheb* Becc. (1884a: 67). Type:—MALAYSIA. Sarawak: Gunung Matang, 1 June 1866, Beccari PB 1936 (holotype FI!). MAP 2. PLATE 2.

Robust, clustering rattan climbing to 30 m. **Stem** with sheaths 20–40 mm diam., without sheaths 10–25 mm diam.; internodes 25–30 cm. **Leaf** 2.2–3.5 m long including cirrus and petiole; sheath bright green, almost completely obscured by ocrea, with caducous, black indumentum, sheath sparsely armed with brown, triangular spines, spines 7–12 mm long, ca. 2 mm wide; ocrea 20.0–26.0 × 5.0–8.0 cm, very large and conspicuous, tightly sheathing above the petiole then inflated, clasping the stem, tough, brown, armed with 6–26 mm long, dark brown, planar spines, ocrea occupied by numerous ants, entry hole made by ants often visible; cirrus 0.7–1.0 m, armed with recurved grapnel spines organised in whorls; petiole 10–25 cm, 14–18 mm wide and 4–5 mm thick at base, flattened adaxially, abaxially rounded, with brown indumentum, armed with spines as sheath; rachis 0.80–2.03 m, armed with recurved grapnel spines organised in whorls; 6–8 leaflets each side of rachis, regularly arranged, rhomboid to broadly rhomboid, mid-leaf leaflets 20.8–27.3 × 4.3–7.7 cm, leaflet light to dark green above, brownish undersurface covered with caducous, orange brown indumentum when young, distal margin conspicuously praemorse, transverse veinlets conspicuous, moderate closely spaced ca. 1.5 mm. **Inflorescence** lax, 52.9–54.0 cm long including 11.5 cm peduncle, branched to 2 orders; prophyll 4.5–6.5 × 0.8–1.8 cm; rachis bract 2.0–8.7 × 1.9–2.3 cm, tightly sheathing, with caducous, dark brown indumentum; primary branches 1–2, 0.5–1.0 cm apart proximal primary branch 16.8–21.0 cm long, with 1–4 rachillae; rachillae 13.5–23.6 cm long and slender including 0.5–1.5 cm visible stalk, 0.5–1.0 cm wide, densely hairy between rachilla bracts. **Flower** 4 × 2 mm in the bud. **Fruit** somewhat oblong, 1.4–2.2 × 0.9–1.8 cm covered in 16–18 vertical rows of yellow-brown scales. **Seed** 1.2 x 0.1 cm; endosperm ruminate.

Distribution:—Borneo (in the 1st Division of Sarawak, Sabah, East and South Kalimantan).

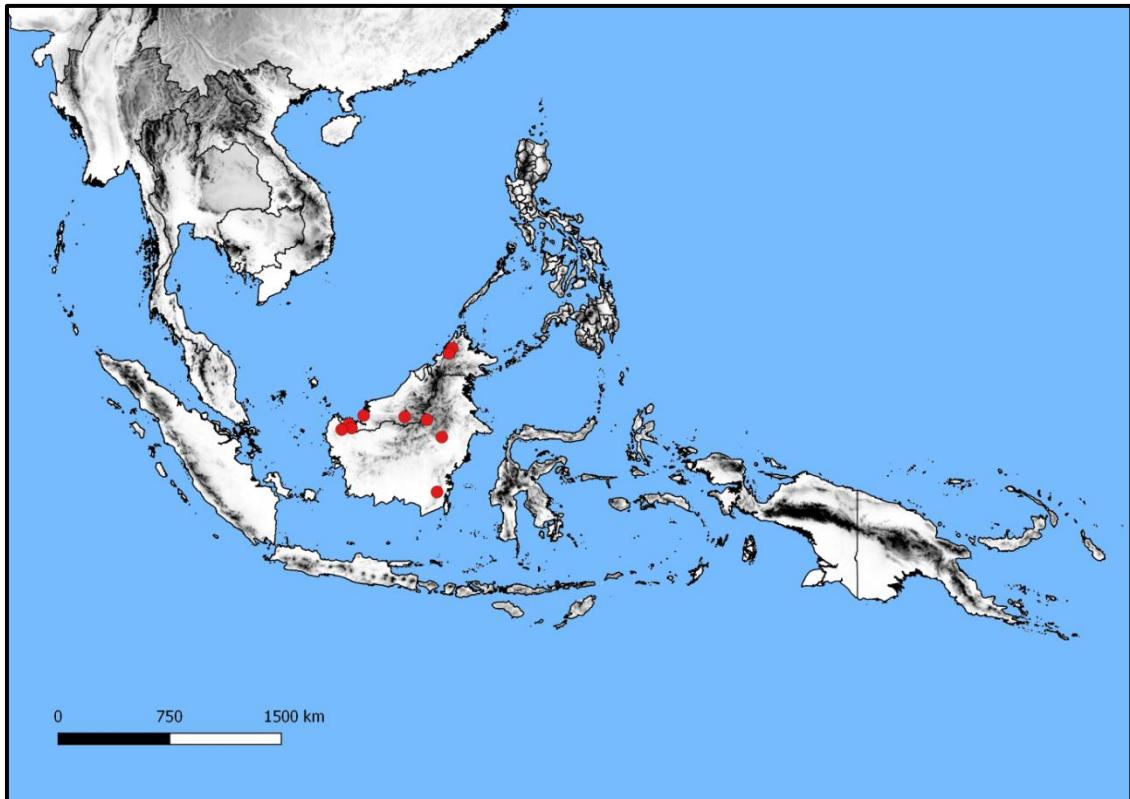
Habitat:—Hill Dipterocarp forest at altitudes up to 800 m, usually in humid valleys.

Uses:—Making a basket and as a binding material for constructing pig-sties.

Vernacular name:—*Wee jematang tengah* (Kayan), *Lasas* (Petung), *Wae dura* (Penan), *Uei sanam* (Kenyah), *Rua* (Bidayuh).

Specimens examined:—INDONESIA. Kalimantan Timur: Gunung Mendam at foot north of Tabang, 100 m, 0°37' N, 115°54' E, 16 January 1979, *Mogea et al.* 1630 (K!); Surroundings of Long Sungai Barang, 800 m, 1°40' N, 115°0' E, 30 December 1992, *van Valkenburg* 1206 (K!). Kalimantan Selatan: Gunung Besar, 800 m, 2°43' S, 115°37' E, 19 February 1979, *Mogea et al.* 1686 (K!). MALAYSIA. Sabah: Tambunan, Crocker Range National Park NW of Kampung Kuyongon close to Tundulu river, 950 m, 5°40' N, 116°20' E, 13 September 2000, *Andersen* 175 (K!, SAN, KEP, C); Tambunan, Mile 8, road to Kampung Kaingaran, 1150 m, 26 March 1999, *Diwol SAN* 141821 (K!, SAN); Kota Kinabalu, Mile 24, Sinsuron Road, Crocker Range, 800 m, 21 August 1979, *Dransfield et al.* 5535 (K!, L, SAR, SAF); Ranau, Kampung Bundu Tuhan, 5°59' N, 116°32' E, 08 January 1994, *Soibeh* 683 (K!). Sarawak: Kapit, Batang Balleh, 1°50' N, 113°40', 16 July 1987, *Lee S* 54592 (K!, L); Kapit, Belaga, 200 m, 1°55' N, 111°11' E, 09 August 1975, *Dransfield et al.* 4671(K!, KEP); Kuching, 1st Division, Gunung Matang, 750 m, 09 April 1981, *Dransfield et al.* 5882 (K!, NY, L, B, PNH); Kuching, 1st Division, Kampung Kakeng, 200 m, 1°9' N, 110°27' E, 20 July 1993, *Bunker* 51 (K!); Kuching, Lundu, Waterfall trail Gunung Gading, 300 m, 25 April 1996, *Baker* 742 (SING!, K, KEP, SAN); Kuching, Siburan, Semenggoh Arboretum, 1°24' N, 110°18' E, 09 February 1995, *Baker* 513 (K!, KEP).

Notes:—*Korthalsia cheb* is a very distinctive species with its very large inflated ocrea and broad diamond-shape (rhomboid) leaflets sometimes it can be confused with *K. angustifolia*. Based on the phylogenetic results of this study, *K. cheb* is sister to *K. scorTechinii* with moderate support.



MAP 2 Distribution of *Korthalsia cheb* Becc. Dots are point localities of specimens examined. Map prepared by Salwa Shahimi, using QGIS.

PLATE 2



Korthalsia cheb Becc. Top left: inflorescence (Lee S.54592). Top right: ocrea (Van Valkenburg 1206). Bottom left: leaflets (Van Valkenburg 1206). Bottom right: fruit (Dransfield et al. 5882).

3. *Korthalsia echinometra* Becc. (1884a: 66). Type:—MALAYSIA. Sarawak: Gunung Matang, 1 June 1866, Beccari PB 1935 (holotype FI!). MAP 3. PLATE 3.

Korthalsia angustifolia var. *gracilis* Miq. (1868: 16). Type:—INDONESIA. Sumatra, Palembang, De Vriese (holotype L).

Korthalsia horrida Becc. (1884a: 66). Type:—MALAYSIA. Sarawak: Gunung Matang, 1 June 1866, Beccari PB 1918 (holotype FI!).

Daemonorops ochreata Teijsm. & Binn. (1866: 74), nom. inval.

Calamus ochreatus Miq. (1868: 29), nom. inval.

Moderately robust, clustering rattan climbing to 50 m. **Stem** with sheaths 12–35 mm diam., without sheaths 9–20 mm diam.; internodes 12–25 cm. **Leaf** 1–2.5 m long including cirrus and petiole; sheath green, almost entirely obscured by ocrea, with indumentum grey in colour, sheath sparsely armed with scattered, brown triangular spines, spines more abundant near sheath mouth, spines 2–20 mm long, 1–3 mm wide; ocrea 6.0–15.5 x 3.5–5.0 cm, conspicuous, inflated and elongate, clasping the stem, tough, brown, armed with 1.0–7.7 cm long, dark brown to black, planar spines, numerous ants occupied within the ocrea, entry hole made by ants often visible; cirrus 0.75–1.80 m, armed with recurved grapnel spines organised in whorls; petiole 5.0–40.0 cm, 7–20 mm wide and 3–5 mm thick at base, flattened adaxially, abaxially rounded, with caducous, brown indumentum, sparsely armed with spines; rachis 0.49–1.50 m, armed with recurved grapnel spines at base and then the spines organised in whorls before the cirrus; 8–25 leaflets each side of rachis, regularly arranged, narrowly lanceolate, mid-leaf leaflets 24.6–31.4 x 1–2 cm, leaflets dull to dark green above, glabrous, with caducous, whitish indumentum on undersurface, distal margin conspicuously praemorse, transverse veinlets conspicuous, moderate closely spaced, 1.0–1.5 mm. **Inflorescence** lax, 64.3–150 cm long including ca. 19.2 cm peduncle, branched to (1–)2 orders; prophyll 13.2–15.2 x 1.2–2.0 cm; rachis bracts up to 5.7–7.0 x 2.0–2.7 cm, tightly sheathing, with caducous, dark brown indumentum; primary

branches 1–4, 6.5–8.5 cm apart; proximal primary branch 18.0–29.5 cm long, with up to 1–4 rachillae; rachillae 14.5–26.2 cm long and slender including 0.7–4.2 cm visible stalk, 0.7–1.0 cm wide, densely hairy between rachilla bracts, orange-brown tomentose. **Flower** not seen. **Fruit** ovoid, 1.9–2.7 × 1.2–1.9 cm covered in 16–21 vertical rows of reddish-brown scales. **Seed** 1.7 x 1.0 cm; endosperm ruminant.

Distribution:—Peninsular Malaysia, Borneo (Central and East Kalimantan, Brunei), Singapore.

Habitat:—Lowland and hill Dipterocarp forest up to 1000 m above sea level.

Uses:—Cane used to make basket frame, weaving handicraft, and also to tie planks on dugouts to raise the side wall of canoes, sap can be drunk for fever.

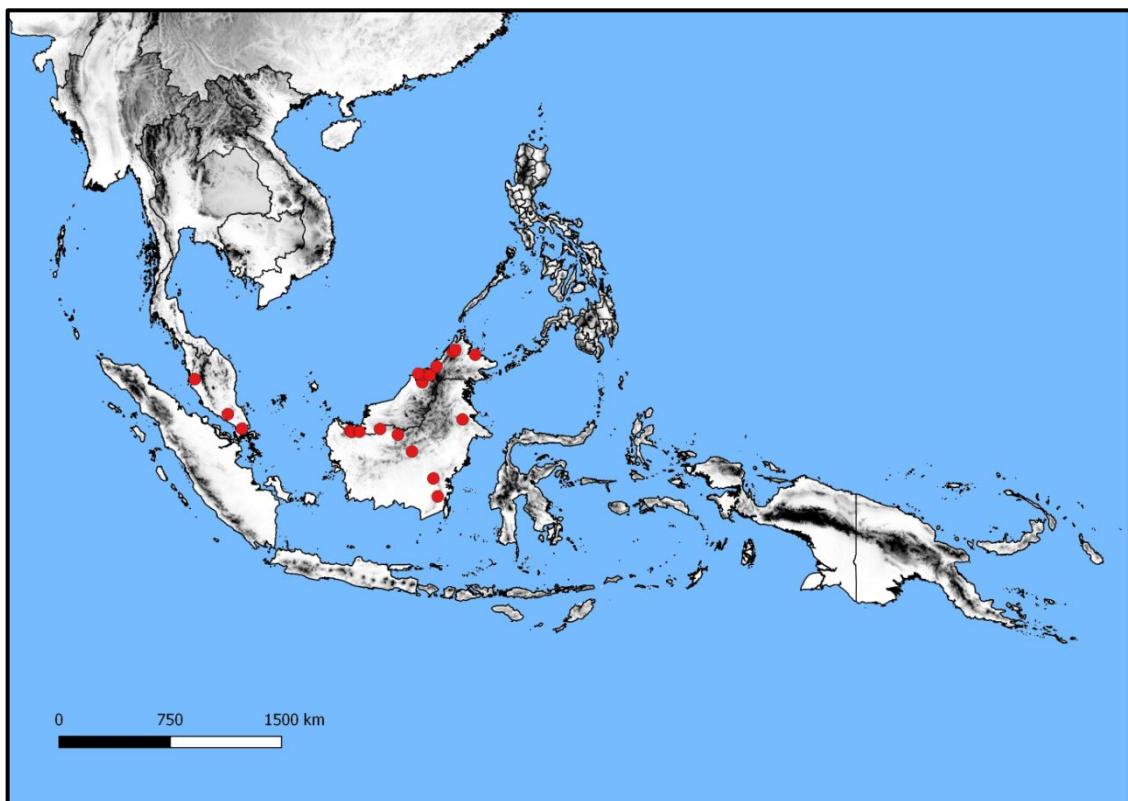
Vernacular name:—*Wi wisro* (Iban), *Wee tujah* (Kayan), *Owei lia* (Murut), *Uwai kapot alot* (Kenyah), *Paikat talusuk* (Dayak lawangan), *Rotan keruk* (Iban), *Rotan udang* (Jakun), *Rotan seru* (Iban), *Rotan lia* (Dusun), *Pipit gading* (Dusun), *Wi serok* (Iban), *Rotan semut udang* (Dayak), *Semeii* (Bidayuh).

Specimens examined:—BRUNEI. Belait: along Sungai Ingei, 4°10' N, 114°43' E, 05 January 1989, Wong s.n. (K!). Tutong: Bukit Ladan Forest Reserve, Compartment 69, 4°38' N, 114°48' E, 13 April 1993, Ibrahim 15126 (K!); Sungai Liang, Sungai Liang Arboretum Reserve, 4°41' N, 114°29' E, Wong 143 (K!). Temburong: Batu Apoi, Ridge running W of landing place, 250 m, 4°36' N, 115°11' E, Simpson 2365 (K); Amo, Sungai Temburong at Kampung Belalong, ridge west of river, 150 m, 4°36' N, 115°11' E, 25 June 1989, Dransfield 6721 (K!). INDONESIA. Kalimantan Barat: Mendalam River, Betung Kerihun National Park, 200 m, 0°59' N, 113°15' E, 01 August 2001, Watanabe 14 (K!, BO); Sanggau, near village of Bedigong, 02 February 1996, Graefen 2 (K!). Kalimantan Selatan: District Tabalong, Upper Tabalong Area, PT Aya Yayang Indonesia Concession, Misim, 50 m, 1°40' S, 115°24' E, 05 August 2000, Moga 7403 (BO, K!, L, NY, WAN); P.T. Yayang Tanjung, 200 m, 22 August 1985, Auggana 047 (K); Barabai, Pergunungan Moratus, foothills of Gunung Bosar, 500 m, 2°45' S, 115°40' E, 18 October 1972, Dransfield 2828 (K!, L, BH). Kalimantan Tengah: near Rekut base camp, 250 m, 0°2' S, 114°6' E, 11 April 1992, Awmack 296 (K!). Kalimantan Timur: Berau, RKT 92-93, Inhutani I concession, 250 m, 08 October 1991,

Stockdale 143 (K!); Berau, near km 37 Camp, Inhutani I, 80 m, 1°55' N, 117°10' E, 09 August 2003, *Watanabe* 60 (K!); between Sinara and Entang Rivers on [floodplain] of Sangatta River, Kutai Reserve, 200 m, 31 October 1991, *Stockdale* 87 (K!). Sumatra: Bengkulu, Muara Aman, Ketenong, 675 m, 18 December 1984, *Uhaedi* 009 (K!); Silo Maradja, Asahan, in the vicinity of Taloen Djoring, December 1927, *Toroes* 64 (K!); Batang Tebopandak, Jambi, nearest city muarabungo, 300 m, September 1993, *Trichon* 796 (K!); Riau, Bukit Suligi, 200 m, August 1986, *Sutisna* 081 (K!). Northern part of island Nunukan, N.E. Borneo (Kalimantan), km 3 from the coast, 06 November 1953, *Meijer* 2054 (K!). MALAYSIA. Johor: Muar, Ma' Okil Forest Reserve, Cpt. 218, valley bottom, 50 m, 4°09' N, 103°19' E, 7 June 1977, *Dransfield* 5019 (K!, KEP!). Pahang: Maran, Cehabu, near Gun Tung Kat, north of Sori Jaya, 17 July 1987, *Gianno* 119 (KLU, A, BISH, BO, K!, KEP!, L, LAE, P, PNH, SAN, SAR, SING, TI, TNS, UC, US); Pekan, near kmomol, Ulu Jeram, 76.2 m, 02 July 1987, *Gianno* 79 (KLU, A, BISH, BO, K!, KEP!, L, LAE, P, PNH, SAN, SAR, SING, TI, TNS, UC, US); Kuantan, Bukit Ibam, 30 May 1987, *Gianno* 36 (KLU, A, BISH, BO, K!, KEP!, L, LAE, P, PNH, SAN, SAR, SING, TI, TNS, UC, US). Perak: Taiping, Maxwell's hill, 975.36 m, 09 March 1924, *Burkill* 12787 (K); Bota, Universiti Teknologi Mara, Bandar Seri Iskandar, 4°21' N, 100°57' E, 09 January 2007, *Chan FRI* 49263 (K!, KEP!). Sabah: Sandakan, Kabili-Sepilok Forest Reserve, 5°50' N, 117°56' E, 12 June 1937, *Enggoh* 7432 (K!); Semporna, Mapat Reserve, Timbun Mata Forest Reserve, 01 August 1937, *Puasa* 7404 (K!); Kampung Kauluan, 6°0' N, 116°35' E, 09 May 1994, *Sambuling* 169 (K!); Ranau, Kampung Takutan, 6°7' N, 116°43' E, 29 March 1995, *Tadong* 179 (K!); Ranau, Kampung Poring. Minintaiku Forest Reserve, 04 May 1994, *Sambuling* 144 (K!); Kampung Poring, on the hill top, 04 January 1994, *Sambuling* 45 (K!); Kampung Poring, 09 September 1992, *Giking* 22 (K!); Kampung Poring, 10 February 1994, *Sambuling* 82 (K!); Kampung Tensungoi, 5°7' N, 115°35' E, 02 June 1994, *Sambuling* 202 (K!); Mile 55, Sandakan-Telupid, 100 m, 26 October 1979, *Dransfield et al.* 5818 (K!, SAR, SAF). Sarawak: Kuching, Gunung Serapi, Kubah National Park, forest trail, 274.32 m, 07 February 1995, *Baker* 509 (K!, KEP!); Kuching, 1st Division, Kampung Semedang, 24th Mile Penrissen Road, 11 August 1985, *Saigol* 15 (K!); 1st Division, Kampung Gayu, 250 m, 1°13' N, 110°21' E, 15 July 1993, *Bunker* 27 (K!); 1st Division, Kampung Kakeng, 200 m, 1°9' N, 110°27' E, 20 July 1993, *Bunker* OX39 (K!); Kapit, 7th Division, 8 km Logging Camp, Nanga Mujong, 28 August 1985, *Saigol* 25 (K!); Lubok Antu, 2nd Division, Delok River, on slope in open secondary forest near Nanga

Sumpa, 250 m, 1°20' N, 112°10' E, 05 March 1993, *Christensen* 1211 (K!); Simunjan, 1st/2nd Division Border Gunung Buri, near summit, 700 m, 1°10' N, 110°55' E, 22 May 1981, *Dransfield et al.* 6115 (K!, NY, L); Baram, on hill behind house, Long Selatong Ulu, 228.6 m, 15 July 1977, *Chung* 2768 (K!); Belaga, 3rd Division, Sungai Masoh, rumah Nyaving, Kuala Linau, Ulu Belaga, 200 m, 09 August 1975, *Dransfield* 4688 (K!, KEP!, L). Terengganu: Kemaman, Bukit Kajang, 14 November 1935, *Corner* 30467 (K, SING); Dungun, Jerangau Dalam, 18 November 2014, *Shahimi* 9 (K!, KEP!). Singapore: Herbarium Botanic Gardens, 15 June 1949, *Furtado* 37946 (SING!, BH, K!, L, BO, AA, PNH, LAE, KEP, SAR, PAR); Chau Chu Raug, 10 December 1892, *Ridley* 3521 (K!); Nee Soon jungle, Thomson Road, *bin Rani* 27 (SING, K); Nee Soon Swamp Forest, *Rajasegar* 18 (K!); MacRitchie Forest, 39 m, 1°21' N, 103°48' E, 09 December 2014, *Shahimi* 23 (K!, SING!).

Notes:—*Korthalsia echinometra* is the commonest ant species in the genus *Korthalsia*. It is a very distinctive species, yet from a distance, it can be mistaken for as a species of a rattan genus other than *Korthalsia* because of its narrow lanceolate leaflets. Its large inflated ocrea with long slender spines and narrowly lanceolate leaflets are diagnostic. The ocreas of *K. echinometra* are inhabited by ants of *Camponotus* sp. and *Iridomyrmex* sp. (Chan *et al.*, 2012). *Daemonorops ochreata* and *Calamus ochreatus* are invalid name because the names have not been published properly according to Art. 6.2 in International Code of Nomenclature (ICN).



MAP 3 Distribution of *Korthalsia echinometra* Becc. Dots are point localities of specimens examined. Map prepared by Salwa Shahimi, using QGIS.

PLATE 3



Korthalsia echinometra Becc. Top left: ocrea. Top right: leaflets arrangement (Bunker 27). Bottom left: inflorescence (Dransfield 2828). Bottom right: fruit (Dransfield 2828).

4. *Korthalsia furcata* Becc. (1918: 120). Type:—INDONESIA. West Kalimantan: Sungai Kenepai, *Hallier 2034* (holotype BO; isotype FI!). MAP 4. PLATE 4.

Slender, clustering rattan climbing to 20 m. **Stem** with sheaths 4–6 mm diam., without sheaths 2–3 mm diam.; internodes ca. 9 cm. **Leaf** ca. 0.6 m long including cirrus and petiole; sheath brown, with caducous, brown coloured indumentum, sheath armed with scattered abundant brown triangular spines, spines 1–3 mm long, ca. 1 mm wide; ocrea 20–25 × 10–11 mm, small, almost rounded and start inflated above the petiole, clasping the stem, tough, brownish, armed with ca. 2 mm long tiny triangular spines, ant present; cirrus ca. 0.28 m, armed with recurved grapnel spines organised in whorls; petiole ca. 2 cm, ca. 4 mm wide and ca. 2 mm thick at base, flattened adaxially, abaxially rounded, with caducous, indumentum as sheath, sparsely armed with spines; rachis 3.5–4.0 cm in length, armed with recurved grapnel spines; usually only one leaflets each side of rachis, regularly arranged, furcate (bilobed), mid-leaf leaflets 33.8–36.7 × 4.2–4.6 cm, leaflet dull green above, glabrous, caducous, grey-whitish indumentum on undersurface, with inconspicuously praemorse margin, transverse veinlets moderately conspicuous, fine and closely spaced, ca 0.5 mm. **Inflorescence** lax, ca. 35 cm long, branched to 2 orders; rachis bracts up to 3.0–7.7 × 0.7–1.5 cm, splitting, with caducous, dark brown indumentum; primary branches 2, 3.5–8.3 cm apart; proximal primary branch 14.3 cm long, with up to 2 rachillae; rachillae 4.7–17.2 cm long and slender including 0.6–1.2 cm visible stalk, 0.6–0.7 cm wide, densely hairy between rachilla bracts. **Flower** not seen. **Fruit** mature fruit not seen. **Seed** not seen.

Distribution:—Borneo (1st Division at Sarawak, Malaysia).

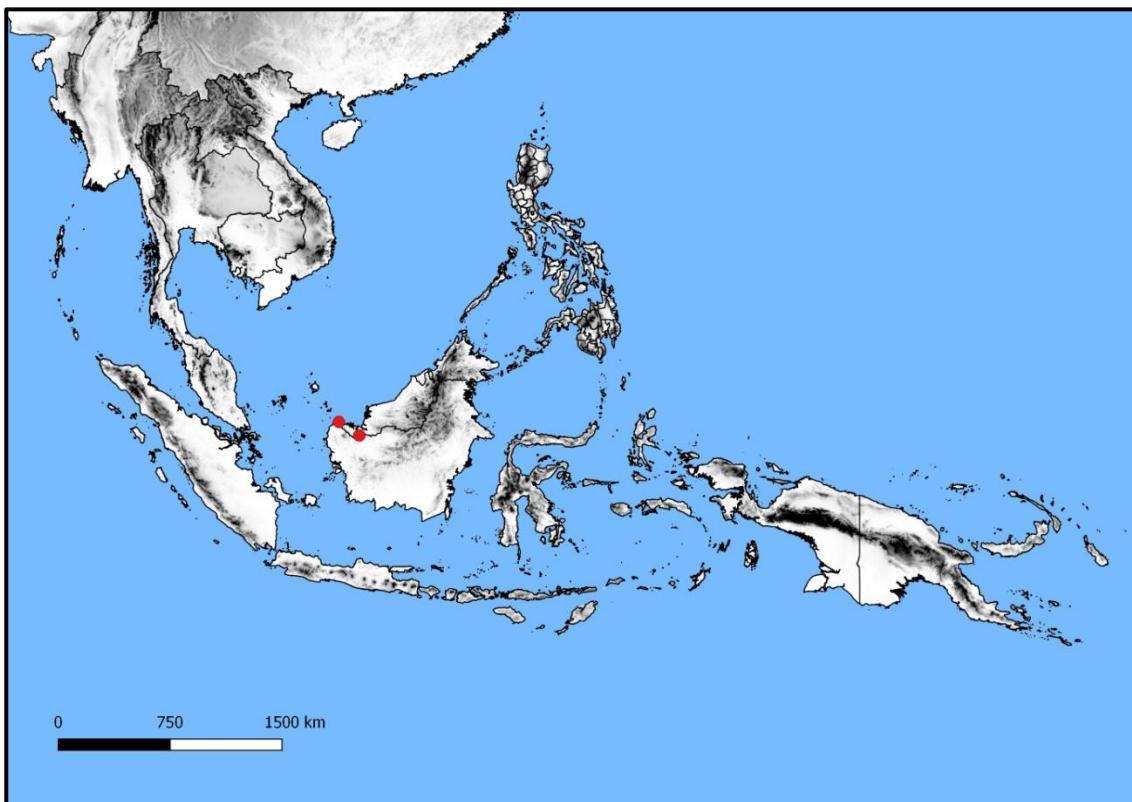
Habitat:—In valley bottom in transitional between hill Dipterocarp forest and Kerangas at 350 m altitude.

Uses:—Not recorded.

Vernacular name:—Not recorded.

Specimens examined:—MALAYSIA. Sarawak: Serian, 1st Division, Sabal Tapang Forest Reserve, Block 14, 350 m, 18 May 1981, Dransfield et al. 6074 (K!, NY, L, B); Kuching, Sungai Kopak, Ulu Samunsam, 200 m, 21 August 1998, Rantai & Lai 74544 (K!, KEP, L).

Notes:—*Korthalsia furcata* is very similar to *K. rostrata* but can be readily distinguished by the leaf which has only two leaflets which appear forked.



MAP 4 Distribution of *Korthalsia furcata* Becc. Dots are point localities of specimens examined. Map prepared by Salwa Shahimi, using QGIS.

PLATE 4



Korthalsia furcata Becc. Top left: leaflets (Dransfield 6075). Top right: inflorescence (Dransfield 6075). Bottom: ocrea (Dransfield 6075).

5. *Korthalsia furtadoana* J.Dransf. (1981: 185). Type:—MALAYSIA. Sabah, 16 October 1979, Dransfield et al. JD 5763 (holotype K!; isotype SAN, SAR). MAP 5. PLATE 5.

Slender, clustering rattan climbing to 20 m. **Stem** with sheaths 8–11 mm diam., without sheaths 5–8 mm diam.; internodes 7–12 cm. **Leaf** 0.60–0.88 m long including cirrus and petiole; sheath green, with brown indumentum in colour, sheath armed with scattered abundant black triangular spines, spines 1–6 mm long, 1–2 mm wide; ocrea 25–83 × 10–15 mm, conspicuous, tightly sheathing above the petiole and then elongate inflated, clasping the stem, tough, brown, armed with 1–6 mm long, triangular black spines, numerous ants within the ocrea, entry hole made by ants visible; cirrus 0.3–0.7 m, armed with recurved grapnel spines organised in whorls; petiole 25–70 mm, 5–7 mm wide and 2–3 mm thick at base, flattened adaxially, abaxially rounded, with few spot of brown indumentum, armed with spines as sheath and reflexed grapnel spines; rachis 0.27–0.43 m, armed with recurved grapnel spines; 5–6 leaflets each side of rachis, regularly arranged, narrowly rhomboid, mid-leaf leaflets 9.0–19.7 × 4.4–6.6 cm, leaflet dull to dark green above, glabrous, with lacking indumentum on undersurface, with inconspicuously praemorse margin, transverse veinlets conspicuous, fine and closely spaced, ca. 0.5 mm. **Inflorescence** lax, 26.5–100 cm long including ca. 10 cm peduncle, branched to 2 order; prophyll 1.5–6.5 × 0.5–0.7 cm; rachis bract up to 3.5–5.9 × 1.3–3.3 cm, tightly sheathing and splitting, caducous, brown indumentum with several tiny spines; primary branches 1–2, 9.5–13.5 cm apart; proximal primary branch 13.5–19.5 cm long, with up to 1–3 rachillae; rachillae 12.0–19.8 cm long and congested, stalk not visible, 0.7–1.4 cm wide, densely hairy between rachilla bracts. **Flower** 8 × 4 mm in the bud. **Fruit** ovoid, 1.3–2.2 × 0.8–1.2 cm covered in 15–23 vertical rows of yellow-brownish scales. **Seed** 1.2 x 0.8 cm; endosperm ruminate.

Distribution:—Borneo (Brunei, Sabah, East and South Kalimantan).

Habitat:—Occasionally occurs at lowland Dipterocarp forest.

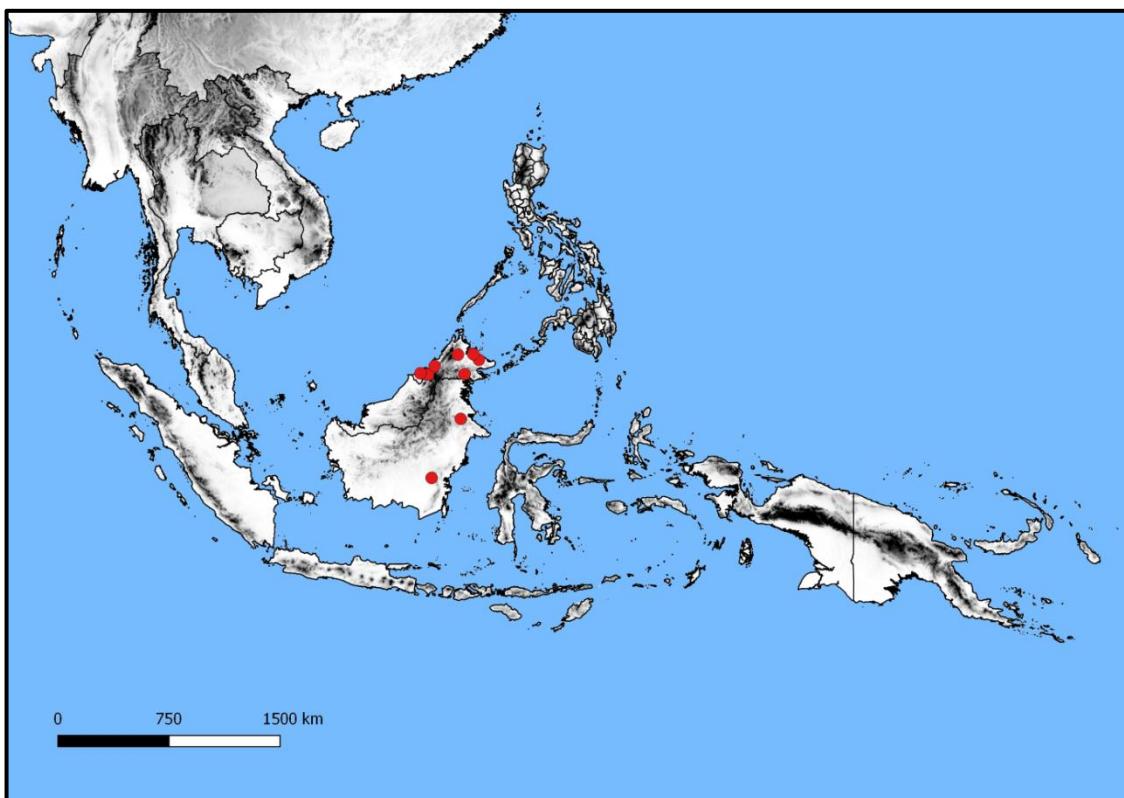
Uses:—Not recorded.

Vernacular name:—Not recorded.

Specimens examined:—BRUNEI. Temburong: Amo, beside the Belalong River, 4°37' N, 115°6' E, *Wong* 1326 (K!); Batu Apoi, Bukit Belitun, Sungai Sinupoi, south of village, 40 m, 4°36' N, 115°11' E, 21 January 1994, *Dransfield* 7477 (K!). Tutong: Lamunin, Compartment 1, 30 m, 4°41' N, 114°43' E, November 1990, *Dransfield et al.* 6875 (K!). INDONESIA. Kalimantan Selatan: Tabaling, Upper Tabalong Area, PT Aya Yayang Indonesia Concession, Kebun Bibit, km 54, Sungai Papun, 200 m, 06 August 2000, *Mogea* 7431 (BO, K!, L, NY, WAN); Tabalong, Upper Tabalong Area, PT Aya Yayang Indonesia Concession, cutting block 1999/2000 near km 54 Kebun Bibit, 400 m, 12 August 2000, *Mogea* 7436 (BO, K!, L, NY, WAN); Upper Taba-ong Area, PT Aya Yayang Indonesia Concession, Misim, 50 m, 1°40' S, 115°24' E, 05 August 2000, *Mogea* 7408 (BO, K!, L, NY, WAN); PT Yayang Tanjung, 20 August 1985, *Anggana* 039 (K). Kalimantan Tengah: Bukit Raya, *Leighton* 376 (K). Kalimantan Timur: Berau, near km 37 Camp, Inhutani I, 80 m, 1°55' N, 117°10' E, 09 August 2003, *Watanabe* 59 (K!, BO); 7 km from Eheng (village), Barong Tongkok (district) near Kelian, upper Mahakan, 10 November 1991, *Stockdale* 101 (K!); Lingau, 2 km from Prampus, P.T. KEM concession near Kelian, upper Mahakan River, 11 November 1991, *Stockdale* 104 (K!); near Sangatta River, Kutai Reserve, halfway between "Prevab" camp and "Mentoko" camp, 20 m, 30 October 1991, *Stockdale* 78 (K!); Mentoko Camp, Kutai Reserve, 50 m, 31 October 1991, *Stockdale* 85 (K!). MALAYSIA. Sabah: Kalabakan, Hap Seng logged area, km 12, 12 June 1982, *Krispinus* 94884 (K); Kota Kinabatangan, Hutan Simpan Pin-Supu, 80 m, 2 April 1992, *Dewol* SAN 132477 (K!, SAN); Lahad Datu, Forest District Lahad Datu, 05 November 1949, *Cuadra* 2498 (K!); Lahad Datu, Block 43 Bagahak, 04 April 1962, *Howroyd* SAN 29366 (K!); Lahad Datu, Cpt. 54 Bakapit, Kennedy Bay, 457.2 m, June 1962, *Burgess* SAN 30782 (K!); Nabawan, Mile 2½ Rahsna Road, 19 January 1978, *Abas* SAN 85854 (K!, L, SAR); Pinangah, Ulu Sg.Melikop, 30 August 1984, *Leopold* SAN 64584 (K!); Ranau, Ulu Tungud Forest Reserve, Gunung Monkobo Expedition, 471 m, 5°49' N, 117°0' E, 25 July 2005, *Saw* SAN 146691 (SAN, KEP!, SAR, K!, L); Ranau, Kampung Poring, 11 February 1994, *Sambuling* 93 (K!); Sandakan, Sungai Menenggul, 5°30' N, 118°16' E, 27 February 1985, *Amin* SAN 68050 (K!, KEP!); Sandakan, Sepilok Forest Reserve, Forest District Elopura, 5°52' N, 117°55' E, 24 September 1949, *Kadir* 2643 (K!, KEP!); Sandakan, Sepilok Forest Reserve, 50 m, 28 February 1995, *Baker* 553 (K!); Sandakan, Kabil-

Sepilok Forest Reserve, 5°50' N, 117°56' E, 25 07 1937, *Matusop* 7427 (K!); Sandakan, Ulu Dusun Agriculture station, 7 m, 16 September 1979, *Dransfield et al.* 5763 (K!, L, SAR, SAF); Sandakan, Subak camp, Jesselton District, 19 December 1962, *Madani SAN* 33215 (K!); Tawau, St Lucia, Ranggu Mile 10 3/4, state forest, 16 September 1949, *Cuadra* 2377 (KEP); Tawau, Luasong Forest Reserve, logging area, 4°38' N, 117°24' E, 16 August 1977, *Fedilis SAN* 87333 (K!, L, SAR, KEP!, SING!); Tawau, mile 10 6 State Forest, Forest District St. Lucia, 16 September 1949, *Lenedia* 2377 (K!); Tawau, Elphinstone Province, October 1922, *Elmer* 20476 (K!); Tenom, Paling-paling Hills, Lagud, 300 m, 04 September 1979, *Dransfield et al.* 5591 (K!, L, SAR, SAF); Danum Valley, Ulu Segama, plot 2, west trail from field station, 150 m, 09 March 1987, *Argent* 291987 (E!, K!); BetoTan, 15.24 m, 16 May 1933, *Castro* 3201 (K); Kampung Wolit, ½ km from house to east, 29 August 1997, *Jinuan* 24 (K); Ulu Segama, west trail, 200 m, 16 February 1986, *Dransfield* 6260 (K); Kampung Tensungoi, 5°7' N, 115°35' E, 02 June 1994, *Sambuling* 198 (K!).

Notes:—*Korthalsia furtadoana* is distinctive when fertile, but is much harder to determine when sterile. It is similar and closely related to *K. rostrata* but can be distinguished from *K. rostrata* by its very robust rachilla and conspicuous fine closely spaced transverse veinlets in leaf. *K. furtadoana* inhabited by ants of *Crematogaster* sp. (Edwards *et al.*, 2010), *Camponotus* sp. and *Polycharchis* sp. (Chan *et al.*, 2012). Based on the phylogenetic results of this study, *K. furtadoana* has a close relationship with *K. rostrata*.



MAP 5 Distribution of *Korthalsia furtadoana* J.Dranf. Dots are point localities of specimens examined. Map prepared by Salwa Shahimi, using QGIS.

PLATE 5



Korthalsia furtadoana J.Dranf. Top left: leaflets arrangement (Howrayd SAN 29366). Top right: ocrea (Howrayd SAN 29366). Bottom left: inflorescence (Dransfield 5590). Bottom right: fruits (Amin et al. 68050).

6. *Korthalsia hispida* Becc. (1884a: 71). Type:—INDONESIA. Sumatra, 1 August 1878, Beccari PS 643 (holotype FI!: isotype K!). MAP 6. PLATE 6.

Moderately robust, clustering rattan climbing to 20 m. **Stem** with sheaths 11–25 mm diam., without sheaths 8–10 mm diam.; internodes 10–20 cm. **Leaf** 1.15–2 m long including cirrus and petiole; sheath bright green, with caducous, brown coloured indumentum, sheath armed with scattered abundant, black, needle-like spines, spine 2–30 mm long, ca. 1 mm wide; ocrea 18.5–30.0 × 3.0–4.5 cm, conspicuous, diverging from the angle at stem just above the petiole, the margins tending to enroll, papery, brown, armed with 3–30 mm long black spines and abundant chocolate spicules, abundant black fierce and very noise ants nested within the ocrea; cirrus 0.5–1.0 m, armed with recurved grapnel spines organised in whorls; petiole 10.0–35.0 cm, 10–20 mm wide and 3–5 mm thick at base, flattened adaxially, abaxially rounded, with brown indumentum, sparsely armed with fine spines as sheath; rachis 0.30–1.07 m, armed with recurved grapnel spines; 5–8 leaflets each side of rachis, regularly arranged, rhomboid, mid-leaf leaflets 15.0–19.6 × 5.0–8.6 cm, leaflet dull to light green above, glabrous, with caducous, whitish indumentum on undersurface, distal margin conspicuously praemorse, transverse veinlets moderate conspicuous and moderately spaced, 1.0–1.5 mm. **Inflorescence** lax to congested, 30.0–50.2 cm long including ca. 45 mm peduncle, branched to (1–)2 orders; prophyll 4.5 × 1.0 cm, with caducous, dark brown indumentum with scattered tiny spines; rachis bracts up to 8.3–9.5 × 2.6–3.4 cm, conspicuous, inflated and splitting, with caducous, dark brown indumentum and densely covered with chocolate scales and black spicules; primary branches 1–4, 4.0–6.6 cm apart; proximal primary branch 17.0–27.5 cm long, with up to 1–3 rachillae; rachillae 11.5–22.3 cm long and slender, stalk not visible, 1.0–1.8 cm wide, lacking hairy between rachilla bracts. **Flower** 8–8.1 × 3.3–3.7 mm in the bud. **Fruit** round to oblong, 1.8–2.3 × 0.9–1.6 cm covered in 15–18 vertical rows of reddish-brown scales. **Seed** 1.1 x 0.8 cm; endosperm homogenous.

Distribution:—Peninsular Malaysia, Borneo (Brunei, Sarawak, East and South Kalimantan).

Habitat:—Along steep-sides river valleys in lowland and hill Dipterocarp forest at altitudes up to 900 m above sea level.

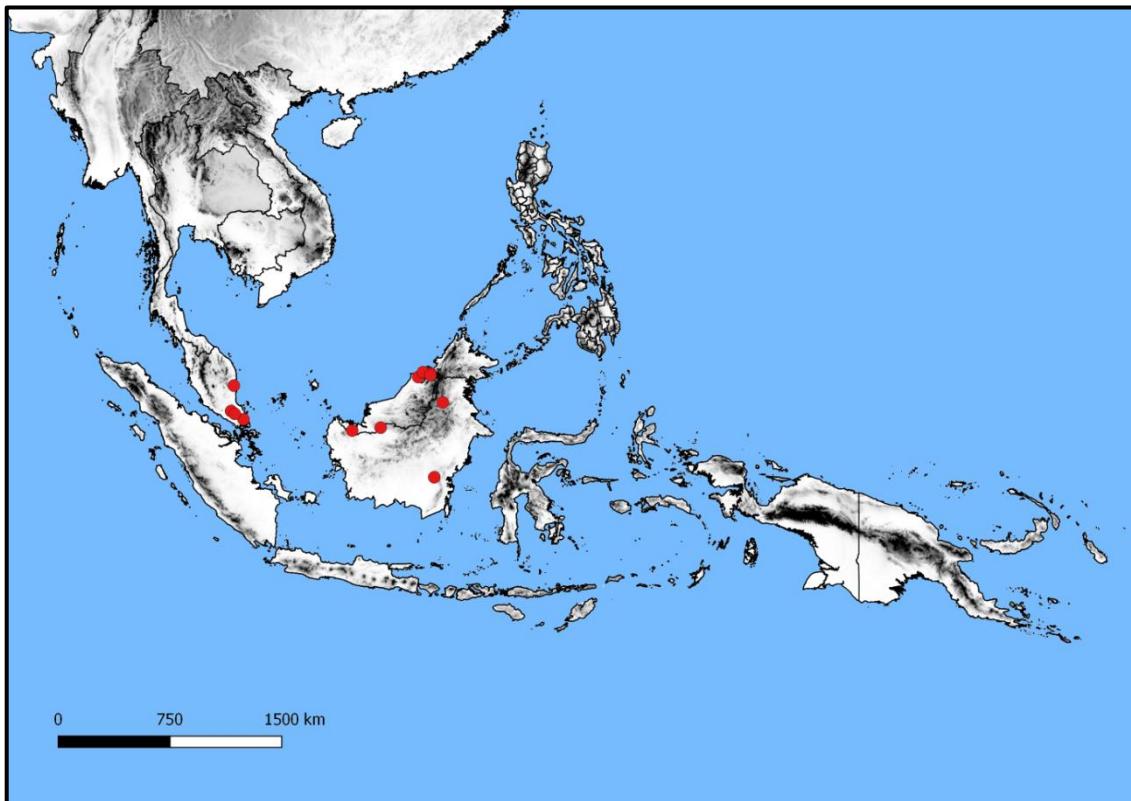
Uses:—Fibre for the plaiting of baskets, craft and binding constructions.

Vernacular name:—*Rotan semut* (Iban), *Paikat ayah pipit* (Dayak Lawangan), *Wi asas* (Iban), *Wi semut* (Iban), *Lesat* (Lundaya), *Uwai saar* (Kenyah), *Uei sanam* (Kenyah), *Laku sepuru* (Punan), *Ohot* (Dayak Tunjung).

Specimens examined:—BRUNEI. Belait: Labi, Teraja Longhouse, 4°23' N, 114°26' E, *Wong & Dransfield 540* (K!, SING!). Temburong: Amo, Belalong River bank near Field Studies Centre, east side, 300 m, 4°33' N, 115°9' E, 06 September 1991, *Stockdale 31* (K!); Batu Apoi, Kerangan Batu Semawat, west-north-west of village, 30 m, 4°36' N, 115°11' E, *Dransfield et al. 7494* (K!); Batu Apoi National Park, Kuala Belalong Field Centre Temburong river, 50 m, 15 February 1995, *Baker 531* (K!, BRUN, UBD); South Temburong, above Kuala Belalong, 75 m, 4°30' N, 15°10' E, 21 February 1991, *Argent et al. 9168* (K!). Tutong: Lamunin, between the rivers Batas and Tutong, 4°41' N, 114°43' E, *Wong 1663* (K!). INDONESIA. Kalimantan Selatan: Tabalong, upper Tabalong area, PT Aya Yayang Indonesia Concession, km 69, 250 m, 07 August 2000, *Mogea 7412* (BO, K!, L, NY, WAN); Tabalong, Upper Tabalong Area, PY Aya Yayang Indonesia Concession, Misim, 50 m, 1°40' S, 115°24' E, 06 August 2000, *Mogea 7410* (BO, K!, L, NY, WAN); P.T. Yayang Tanjung, 100 m, 22 August 1985, *Anggana 048* (K). Kalimantan Timur: Bordering Talib's Farm, Lingon, 2 km from Prampus, PT KEM concession, Kelian, upper Mohakan River, 11 November 1991, *Stockdale 106* (K!); Surroundings WWF basecamp, Kayan Mentarang Reserve, 400 m, 2°51' N, 115°55' E, 24 November 1991, *van Valkenburg 1063* (K!); Berau, RKT 92-93, Inhutani I Concession, 250 m, 08 December 1991, *Stockdale 142* (K!). Sumatra: Padang, Ayer maniciar (Ajer mantjoer), August 1878, *Beccari 643* (K!). MALAYSIA. Johor: Bekuk, Hutan Lipur Batang, 90 m, 2°20' N, 103°09' E, 24 November 2014, *Shahimi 11* (K!, KEP!); Kota Tinggi, Panti Forest Reserve, 10 m, 10 June 1977, *Dransfield 5035* (K!); Kota Tinggi, Gunong Panti, East Forest Reserve, 20 m, 1°50' N, 103°54' E, 07 January 1973, *Dransfield 3037* (L, K!, SING!, MAN, PAR, LAE, G, SAR, BH, NSW, NY, CAL, KEP, BRI, CANB, SAN, US, BO); Labis, Labis Forest Reserve, Ulu Endau, Gunung Janung, valley bottom, 100 m, 2°10' N, 103°22' E, 16 June 1977, *Dransfield 5090* (K!). Pahang: Kuantan, Galing Forest Reserve, 20 m, 3°52'

N, 103°19' E, 11 August 1977, *Dransfield* 5227 (K!). Sabah: Telupid, c. 8 miles, Entelebun-Menanam, 150 m, 24 September 1979, *Dransfield et al.* 5803 (K!, SAR, SAF); Tenom, hills above Kampung Baru Jumpa, 700 m, 05 September 1979, *Dransfield et al.* 5598 (K!, SAR, KEP!, SAF); Kampung Kiau Nuluh, 19 September 1992, *Duaneh* 118 (K); Kampung Sorinsim, 17 March 1993, *Sibil* 162 (K!). Sarawak: Baram, 4th Division Around Long Selatong Lepo Ga', on hill behind house, banks of Benuon river, 400 m, 19 April 1980, *Chin* 3008 (K!); Belaga, 3rd Division, Sungai Masoh, rumah Nyaving, Kuala Linau, Ulu Belaga, 200 m, 10 August 1975, *Dransfield et al.* 4705 (K!, KEP!, L, BH, SING!); Kapit, upper Rejang River, *Clemens* 22085 (K!); Kuching, 1st Division, Kampung Kakeng, 200 m, 1°9' N, 110°27' E, 20 July 1993, *Bunker* 36 (K!); Tawau, Elphinstone Province, British North Borneo, October 1922, *Elmer* 21553 (K!); Lubok Antu, 2nd Division, Delok River, on steep slope in secondary forest near Nanga Sumpa, 200 m, 1°20' N, 112°10' E, 31 May 1993, *Christensen* 1188 (K!).

Notes:—*Korthalsia hispida* and *K. robusta* are the only two species in *Korthalsia* with ocreas that do not clasp the stem but diverge at an acute angle just above the petiole. These two species are the noisy rattans. The ocreas of *K. hispida* are inhabited by ants of *Camponotus* sp. (Mattes *et al.*, 1998). The ants make a rustling noise by banging their abdomens against the ocrea when the plant is disturbed. *K. hispida* can be distinguished from *K. robusta* by the presence of black spicules on the ocrea and sheaths. Based on the phylogenetic results of this study, *K. hispida* is closely related to *K. robusta*.



MAP 6 Distribution of *Korthalsia hispida* Becc. Dots are point localities of specimens examined. Map prepared by Salwa Shahimi, using QGIS.

PLATE 6



Korthalsia hispida Becc. Top left: habit. Top right: ocrea. Bottom left: inflorescence (Dransfield et al. 7494). Bottom right: fruit (Argent and Mitchell 9168).

7. *Korthalsia robusta* Blume (1843: 170). Type:—INDONESIA. Sumatra, *Praetorius s.n.* (holotype L: isotype K!, L!). MAP 7. PLATE 7.

Korthalsia squarrosa Becc. (1909: 620). Type:—PHILIPPINES. Palawan, Curran 4155 (holotype FI; isotype K!).

Korthalsia macrocarpa Becc. (1918: 149). Type:—INDONESIA. Borneo, H. Winkler 2777 (holotype FI).

Robust, clustering rattan climbing to 40 m. **Stem** with sheaths 20–35 mm diam., without sheaths 14–20 mm diam.; internodes 24–25 cm. **Leaf** 1.5–3 m long including cirrus and petiole; sheath bright green to green, with abundant dark brown to brown-black floccose, sparsely armed with scattered black, triangular spine, spines 2–30 mm long, 1–2 mm wide; ocrea 16.0–41.0 × 3.0–7.0 cm, conspicuous, diverging from the angle at stem just above the petiole, the margins tending to inroll, papery, brown in colour, armed with abundant 2–30 mm long dark brown spines, , black ants usually abundant and very noisy; cirrus 0.50–1.75 m, armed with recurved grapnel spines organised in whorls; petiole 10–35 cm, 12–30 mm wide and 4–6 mm thick at base, flattened adaxially, abaxially rounded, with slightly grey indumentum, sparsely armed with spines as sheath; rachis 0.65–1.35 m, armed with recurved grapnel spines; 6–10 leaflets each side of rachis, regularly arranged, rhomboid to broadly rhomboid, mid-leaf leaflets 20.2–27.8 × 4.2–13.8 cm, leaflet dull green to dark green above, glabrous, with grey-whitish indumentum under surface, distal margin conspicuously praemorse, transverse veinlets conspicuous, fine and closely spaced ca. 0.5 mm. **Inflorescence** congested, 34.9–51.0 cm long including 3.5–15.0 cm peduncle, branched to 2 orders; prophyll 2.5–8.0 × 2.2–3.7 cm, with caducous, brown indumentum; rachis bracts up to 12.3–16.2 × 4.7–8.0 cm, conspicuous, inflated, with caducous, dark brown indumentum and black spicules; primary branches 3, 3.0–4.5 cm apart; proximal primary branch 16.0–17.0 cm long, with up to 2 rachillae; rachillae 13.0–16.0 cm long and slender, stalk not visible, 1.2–2.0 cm wide, lacking hairy between rachilla bracts. **Flower** 8.4–11.0 × 3.4–5.0 mm in the bud. **Fruit** round to oblong, 1.8–2.3 × 0.9–1.6 cm covered in

16–18 vertical rows of mid-brown scales with darker intermarginal and straw-coloured margins. **Seed** 1.8 x 1.0 cm; endosperm homogenous.

Distribution:—Borneo (Sabah, Sarawak, East and South Kalimantan), Palawan (Philippines).

Habitat:—Lowland Dipterocarp forest.

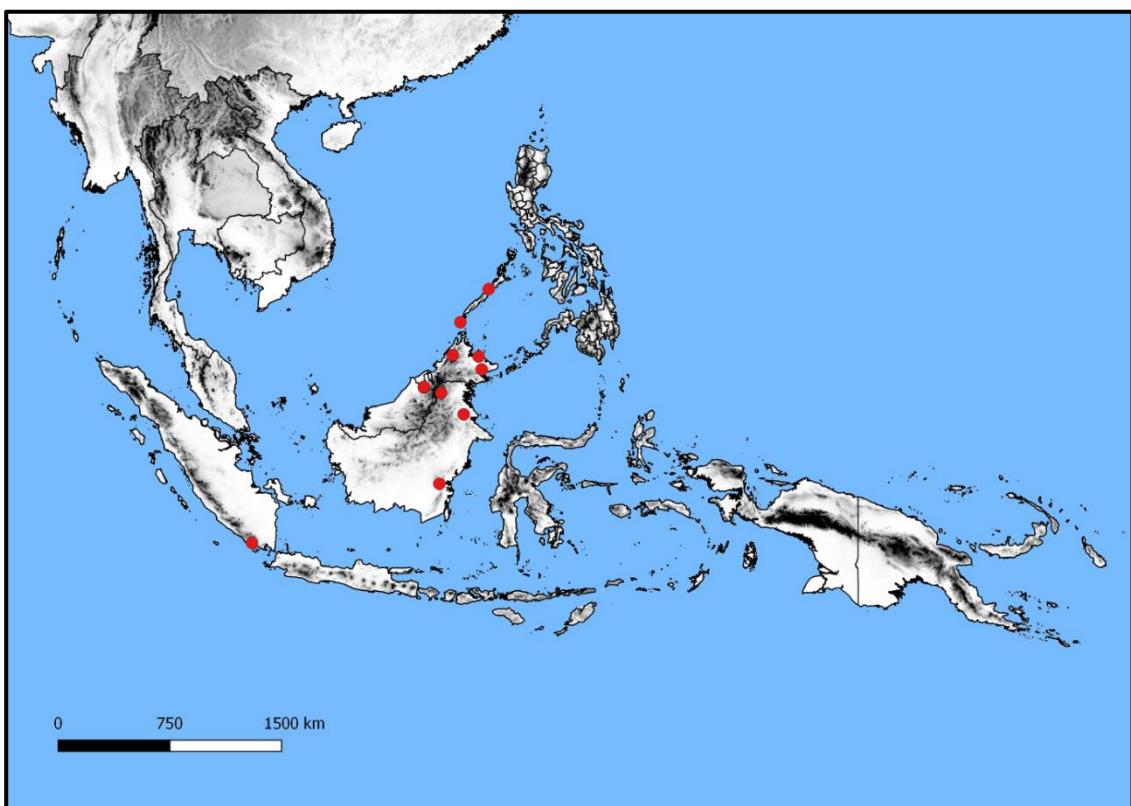
Uses:—Stem used for tying and weaving of baskets, shoots edible, handicraft and construction.

Vernacular name:—*Oros* (Kutai dialect), *Rotan lasas* (Kadazan), *Wi semut* (Iban), *Wae asa* (Kelabit language), *Wae perah* (Penan), *Ayas* (Pasir language).

Specimens examined:—BRUNEI. Temburong: Batu Apoi National Park, Kuala Belalong Field Centre Jalan Tengah, path to Bukit Belalong, 1 km from field centre, 300 m, 16 February 1995, *Baker* 534 (K!, BRUN, UBD). INDONESIA. Kalimantan Tengah: Bukit Raya, *Leighton* 947 (K!). Kalimantan Timur: Berau, near km 37 Camp, Inhutani I, 30 m, 2°22' N, 117°12' E, 18 August 2002, *Watanabe* 44 (K!, BO); Halfway between Prevab and Mentoko Camps, Kutai Reserve, floodplain of Sangatta River, 20 m, 30 October 1991, *Stockdale et al.* 80 (K); Tabang, Gunung Mendam, KTI, 400 m, 16 January 1979, *Mogea* 1629 (K!, KYO); Ma Ancalong, Ma Lun, Sungai Kelinjau, 04 October 1980, *Mogea* 2708 (K!). Kalimantan Selatan: Sungai Rusi near Mount Sarempaka, 450 m, 1°49' S, 115°44' E, 04 November 1972, *de Vogel* 2073 (K!); Datar Alai, Pegunungan Meratus Barabai, 350 m, 26 October 1972, *Dransfield* 2967 (K!, L, BH). Sumatra: Lampung, NW of Kota Agung, 350 m, 5°23' S, 104°25' E, 09 May 1968, *Jacobs* 8295 (K!). MALAYSIA. Sabah: Lahad Datu, Masuri, Ulu Segama, 5°5' N, 118°17' E, 20 November 1949, *Cuadra* 248 (K!, KEP!); Ranau, Kampung Bundu Tuhan, Kinatongan, 5°57' N, 116°32' E, 07 September 1994, *Soibeh* 828 (K!); Ranau, Poring, near hot springs, 700 m, 29 August 1979, *Dransfield et al.* 5563 (K!, SAR, KEP!, SAP); Ranau, Kampung Poring, next to Mamut Copper Mine, 03 May 1994, *Sambuling* 133 (K!); Sandakan, Elopura, Mile 15 state forest, 5°41' N, 118°6' E, 24 March 1949, *Cuadra* 2226 (K!, KEP!); Sandakan, Elopura, Sapagaya River, 21 May 1949, *Cuadra* 2275 (K!, KEP!); Sandakan, Sepilok Forest Reserve, 50 m, 28 February 1995, *Baker et al.* 552 (K!); Sandakan, Virgin Jungle Reserve, Mile 42 Segaliud-Lokan

Forest Reserve, 50 m, 18 October 1979, *Dransfield et al.* 5772 (K!, SAR, SAF); Tambunan, Kampung Mansaralong mile 56 ½, 11 July 1978, *Abas SAN* 85923 (K!); Kinabatangan Besar, Kori, timber camp, 5°38' N, 118°36' E, 02 November 1948, *Cuadra* 2158 (K!, KEP!); Kota Marudu District, Kampung Serinsim, 27 April 1995, *Bakia* 339 (K!). Sarawak: Baram, Sungai Tutoh, 45.72 m, 4°0' N, 114°48' E, 11 February 1966, *Wee-Lek* 1086 (K!); Kapit, 7th Division, 8 km Logging Camp, Nanga Mujong, 28 August 1985, *Saigol* 33 (K!); Miri, Gunung Mulu National Park, 4th Division, on banks of Sungai Melinau near base camp, 30 m, 03 October 1977, *Dransfield* 5262 (K!, NY); Miri, Gunung Mulu National Park, 35 m, 4°2' N, 114°48' E, 14 September 2015, *Shahimi* 26 (K!, KEP!); 4th Division, Kelabit Highlands on slope in secondary forest near Ramudu, 900 m, 3°40' N, 115°50' E, 06 October 1993, *Christensen* 185 (K!). PHILIPPINES. Palawan: Balabac Island, 7°56' N, 117°0' E, 16 October 1906, *Merrill* 5384 (K!); Kampo 1, 05 March 1984, *Madulid* 1010 (K); St. Paul's Bay National Park, Lion Cave, 15 m, 07 May 1984, *Dransfield* 6177 (K!); Pulot III, North of Brooke's Point, 100 m, 22 May 1984, *Dransfield* 6206 (K!); Puerto Princesa, Bagumbayan, 22.86 m, 18 November 1983, *Baja-Lapis* 072 (K!); Puerto Princesa, on road between Puerto Princesa and Palawan Consolidated Mining Corporation's Concession, 100 m, 9°56' N, 118°42' E, 11 April 1979, *Dransfield* 5492 (K!).

Notes:—*Korthalsia robusta* is similar to *K. hispida* but can be easily distinguished from *K. hispida* by the absence of black spicules on the sheaths, ocreas, and bracts, and by the very congested inflorescences. *K. robusta* is a noisy rattan and inhabited by ants of *Camponotus* sp. and *Dolichoderus* sp. (Chan *et al.*, 2012). These ants are big, black and very fierce and make this rattan difficult to collect. Based on the phylogenetic results of this study, *K. robusta* is closely related to *K. hispida*.



MAP 7 Distribution of *Korthalsia robusta* Blume. Dots are point localities of specimens examined. Map prepared by Salwa Shahimi, using QGIS.

PLATE 7



Korthalsia robusta Blume. Top left: leaflets arrangement. Top right: ocrea. Bottom left: inflorescence (Dransfield 5262). Bottom right: fruit (Dransfield 5262).

8. *Korthalsia rostrata* Blume (1843: 168). Type:—INDONESIA. Borneo: Sungai Dusun, *Muller s.n.* (holotype L!). MAP 8. PLAT 8.

Ceratolobus rostratus (Blume) Becc. (1919: 11).

Korthalsia scaphigera Mart. (1845: 211). *Calamosagus scaphiger* (Mart.) Griff. (1850: 30). Type:—MALAYSIA. Malacca, *Griffith s.n.* (holotype BR; isotype K!).

Korthalsia lobbiana H.Wendl. (1859: 174). Type:—MALAYSIA. Sarawak, *Lobb 106* (holotype K!).

Korthalsia machadonis Ridl. (1907: 216). Type:—MALAYSIA. Perak: K amuning, *Machado s.n.*

Slender, clustering rattan climbing to 20 m. **Stem** with sheaths 5–15 mm diam., without sheaths 4–6 mm diam.; internodes 10–12 cm. **Leaf** 0.4–1.1 m long including cirrus and petiole; sheath green, with dark brown to black indumentum, sheath armed with scattered tiny abundant yellow-brown to black triangular spines, spines 1–4 mm long, 1–2 mm wide; ocrea 25–50 × 10–30 mm, conspicuous, almost rounded and start inflated above the petiole, tough, pale brown to light brown in colour, armed with 2–5 mm long, tiny triangular brown to dark brown spines, ants abundant within the ocrea; cirrus 0.30–1.25 m, armed with recurved grapnel spines organised in whorls; petiole 2–18 cm, 5–19 mm wide and 2–3 mm thick at base, flattened adaxially, abaxially rounded, with caducous brown indumentum, sparsely armed with spines as sheath; rachis 0.25–1.45 m, armed with recurved grapnel spines; 4–6 leaflets each side of rachis, regularly arranged, broadly rhomboid, mid-leaf leaflets 13.5–20.5 × 5.0–6.5 cm, sometime (rarely) the leaflet narrowly lanceolate, to 21.5–30.8 × 1.5–2.8 cm, leaflet dark green above, glabrous, with caducous, chalky-white indumentum on undersurface, distal margin conspicuously praemorse, transverse veinlets conspicuous, moderate closely spaced, 1.0–1.5 mm. **Inflorescence** lax, 35.2–77.5 cm long including 8.7–9.0 cm peduncle, branched to 2 orders; prophyll 3.5–6.0 × 0.7–0.8 cm, with caducous, brown indumentum; rachis bracts up to 4.5–8.7 × 0.9–3.3 cm, tightly sheathing, splitting, with

caducous, brown indumentum with several tiny spines; primary branches 2–4, 4.1–11.2 cm apart; proximal primary branch 14.7–19.0 cm long, with up to 2–4 rachillae; rachillae 12.7–18.6 cm long and slender, including 0.7–1.7 cm visible stalk, 0.5–0.7 cm wide, lack to hairy between rachilla bracts. **Flower** 5.0–5.1 × 2.0–2.2 mm in the bud. **Fruit** 2.4–2.5 × 1.6–1.7 cm covered in 15–19 vertical rows of orange-brown scales. **Seed** 1.1–1.6 × 0.7–1.0 cm, endosperm ruminate.

Distribution:—Peninsular Malaysia, Borneo (Brunei, Sarawak), Singapore, Sumatra.

Habitat:—Occurs in a variety habitats including lowland and hill Dipterocarp forest and Kerangas.

Uses:—Stems used for weaving basket and mats.

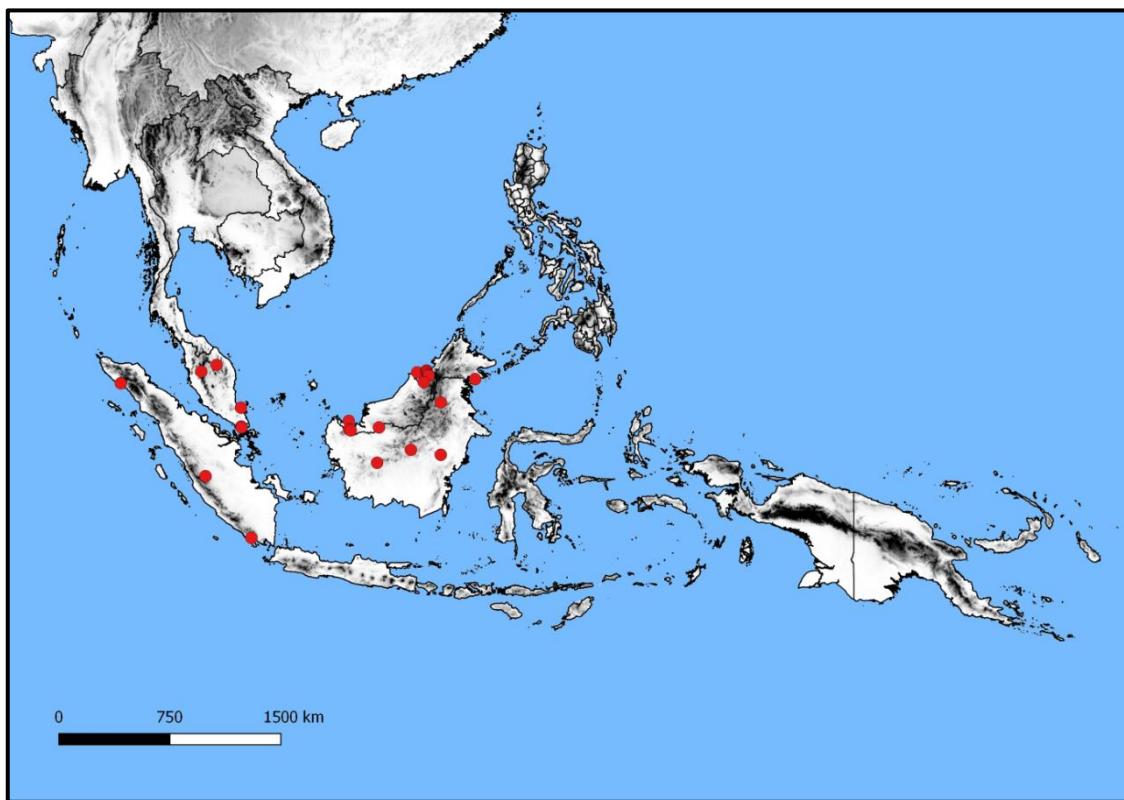
Vernacular name:—*Wi cit* (Iban), *Wi Semut* (Iban), *Uwai merah* (Dusun), *Rotan kawan* (Aceh), *Wee pak lavo* (Kayan), *Laku sepuru* (Punan), *Uei saman* (Kenyan), *Lempinit ralun* (Murut), *Wi nang* (Iban), *Rotan seru* (Iban), *Rotan semut* (Iban).

Specimens examined:—BRUNEI. Belait: Sungai Liang, Sungai Liang Arboretum Reserve, 4°41' N, 114°29' E, Wong 137 (K!). Temburong: Temburong River Valley, in area of river bed near helicopter pad, 50 m, 4°48' N, 115°3' E, Johns 7282 (K!); Jalan Tengah 1850 m from north end, 100 m, 4°43' N, 115°4' E, 01 August 1991, Stockdale 14 (K); Amo, Temburong River upstream from the Wong Nguan gorge, 500 m, 4°37' N, 115°6' E, 10 April 1990, Wong 1738 (K!); Amo, Kuala Belalong, 300 m, 4°32' N, 115°9' E, 14 February 1992, Dransfield 7064 (K!); Bukit Belalong, north ridge, 4°29' N, 115°11' E, 20 July 1989, Wong s.n. (K!). INDONESIA. Kalimantan Tengah: near Rekut base camp, 250 m, 0°2' S, 114°6' E, 11 April 1992, Awmack 299 (K!). Kalimantan Timur: Berau, RKT 92-93, Inhutani I Concession, 250 m, 08 December 1991, Stockdale 137 (K!); near Sedulan, ca 50 km north of Sebulu, 27 December 1978, Mogaia et al. 1535 (K!); surrounding WWF basecamp Kayan Mentarang Reserve, 350 m, 2°51' N, 115°55' E, 23 November 1991, van Valkenburg 1055 (K!); about 30 km north of Sebulu, 30 December 1978, Mogaia et al. 1548 (K!); Kalimantan, Sintang, HPH km 86 - 85, along subsidiary road south towards Riam Batang, 80 m, 0°49' S, 112°3' E, 04 May 1994, Church 1302 (K!); Kelian, K. Equatorial Mining area, 250 m, 0°20' S, 115°55' E, 17 March 1997, Kessler 2080 (K!, L). Sumatra: Aceh Selatan, collected on

Gunung Simpang Kiri, 23 October 1997, *Sangaji & Barrow* 11 (K!); Aceh Selatan, Kampong Lamee, 72.85 m, 4°1' N, 96°29' E, 21 October 1997, *Sangaji & Barrow* 6 (K!); East Coast, Silo Maradja, Asahan, May 1927, *Bartlett* 8132 (K!); East Coast, General region of Marbau, Bilah, near Bilah Pertama (Parbasiran), February 1928, *Toroes* 200 (K!); Jambi, Batang Tebopandak, 300 m, 1°38' S, 101°38' E, September 1993, *Trichon* 801 (K!); Lampung, NW of Kota Agung, 350 m, 5°23' S, 104°25' E, 18 May 1968, *Jacobs* 8482 (K!); Pajakumbuh, Taram, sandstone region of River Tjampo, 500 m, 23 August 1957, *Meijer* 6881 (K!); Sumatra Barat, Sijunjung, Muro Kulampi, 200 m, 26 February 1974, *Dransfield* 3957 (K!); Bengkulu, Ketahun, 40 m, 26 December 1984, *Uhaedi* 019 (K!). MALAYSIA. Sabah: Tawau, St Lucia, Ranggu, Mile 10, State forest, 18 September 1949, *Cuadra* 2383 (K!, KEP!); Tawau, Membalua Forest Reserve, 50 m, 4°16' N, 118°0' E, 03 November 1979, *Dransfield et al.* 5855 (K!, SAR, SAF); Mile 46, Nabawan, 400 m, 11 September 1979, *Dransfield et al.* 5636 (K!, L, SAR, KEP, SAF); Telupid, c 5 miles on road Telupid-Karamuak, 24 October 1979, *Dransfield et al.* 5798 (K!, SAR, SAF). Sarawak: Kapit, 7th Division, 8 km Logging Camp Nanga Mujong, 28 August 1985, *Saigol* 35 (K!); Kuching, 1st Division, Sungai Sendok, Proposed Matang National Park, Matang, 10 May 1987, *Hock* 53881 (K!, L, NY); 1st Division, Wildlife Observation Path, Bako National Park, 19 August 1975, *Jawa* 36609 (K!, NY, L, SAN); Gunung Gading, Lundu District, 17 April 1996, *Ara* 73906 (K!, SING!); Gunung Santubong, 100 m, 1°44' N, 110°20' E, 06 June 1964, *Ashton* 21471 (K!, NY); Lubok Antu, 2nd Division, Delok River, on steep slope in secondary forest near Nanga Sumpa, 150 m, 1°20' N, 112°10' E, 04 June 1993, *Christensen* 1183 (K!); Miri, Gunung Mulu National Park, 4th Division, proposed extension, hills near Gunung Buda, 250 m, 14 October 1977, *Dransfield* 5322 (K!); Miri, Gunung Mulu National Park, 4th Division, lower slopes of Gunung Mulu near Camp 1, 250 m, 4°5' N, 114°53' E, 05 October 1977, *Dransfield* 5272 (K!, NY, L); Serian, 1st Division, Sabal Tapang Forest Reserve Mile 70, 250 m, 19 May 1981, *Dransfield et al.* 6085 (K!, NY, L); 4th Division, Dulit Range, 350 m, 09 October 1983, *Awa* 46688 (K!, L); Wooded slopes of Mount Mattang above water works, 31 December 1963, *Moore* 9084 (K); 5th Division, Path to Bukit Pagon, Limbang, 29 July 1984, *Awa* 47554 (K!, KEP!); 3rd Division, Sungai Masoh, rumah Nyaving, Kuala Linau, Ulu Belaga, 200 m, 09 August 1975, *Dransfield et al.* 4680 (K!, KEP, L, BH, SING!); Semenggoh Forest Reserve, 12 miles from Kuching, 20 December 1955, *Tomlinson* 6 (K); Semenggoh arboretum, 09 February 1995, *Baker* 514 (K!, KEP!); 1st

Division, Kampung Gayu, 250 m, 1°13' N, 110°21' E, 15 July 1993, *Bunker* 30 (K!); 1st Division, Kampung Kakeng, 200 m, 1°9' N, 110°27' E, 20 July 1993, *Bunker* 33 (K). Johor: Endau, between Bukit Petar and Sungai, 07 September 1987, *Gianno* 151 (A, BISH, BO, K!, KEP!, L, LAE, P PNH, SAN, SAR, SING!, TI, TNS, UC, US). Mersing, Gunung Arong Forest Reserve Cpt. 9, 20 m, 2°32' N, 103°47' E, 13 June 1977, *Dransfield* 5064 (K); Ulu Kahang, 106.68 m, 02 June 1923, *Holttum* 10916 (K!). Pahang: Aur Forest Reserve, 38 miles inland from Kuala Rompin, 50 m, 24 July 1975, *Dransfield* 4583 (K!). Perak: Ipoh, Kinta Hills Forest Reserve, Compt. 78, near Ampang valley bottom, 350 m, 08 July 1975, *Dransfield* 4477 (K!); Kuala Kangsar, Gunung Bubu Forest Reserve Cpt. 77 Ulu Trong, 250 m, 4°44' N, 101°22' E, 18 November 1977, *Dransfield* 5381 (K!). Larut, 152.4 m, October 1883, *Dr King's collector* 5047 (K!); Ridge of main range near Kampung Temakah, downstream from Pos Legap, Sungai Plus, 10 January 1988, *Gianno* 320 (A, BISH, BO, K!, KEP!, L, LAE, P PNH, SAN, SAR, SING!, TI, TNS, UC, US). Selangor: Semenyih, Sungai Lalang Forest Reserve, 200 m, 3°4' N, 101°52' E, 22 April 1977, *Dransfield* 4950 (K!). Terengganu: Besut, Ulu Setiu Forest Reserve foothills of Gunung Lawit, 100 m, 5°8' N, 102°18' E, 03 August 1977, *Dransfield* 5142 (K!). SINGAPORE. Nee Soon Swamp Forest, 1°23' N, 103°48' E, *Rajasegar & Loo* 24 (K!); MacRitchie Forest, 17 m, 1°20' N, 103°49' E, 11 December 2014, *Shahimi* 25 (K!, SING!).

Notes:—*Korthalsia rostrata* is almost distinguishable vegetatively to *K. furtadoana*. *K. rostrata* tend to have small, almost rounded ocrea compare to *K. furtadoana*. When it is fertile, it can easily be distinguished from *K. furtadoana* by its slender rachillae. In Brunei, *K. rostrata* occur to have lanceolate leaflets form rather than rhomboid. There is an only little collection in the herbarium. Although the shape of leaflets seems to be distinctive, other than that it appears to be identical to *K. rostrata*.



MAP 8 Distribution of *Korthalsia rostrata* Blume. Dots are point localities of specimens examined. Map prepared by Salwa Shahimi, using QGIS.

PLATE 8



Korthalsia rostrata Blume. Top left: Habit. Top right: inflorescence (Church et al. 1302). Bottom left: ocrea (Dransfield et al. 6085). Bottom right: fruit (Laumonier TFB 1155).

9. *Korthalsia scaphigeroides* Becc. (1909: 619). Type:—PHILIPPINES.
Mindanao: Zamboanga, 1 July 1906, Hutchinson 4816 (holotype FI: isotype NY!, US!). MAP 9. PLATE 9.

Slender, clustering rattan climbing to ca. 5 m. **Stem** with sheaths ca. 10 mm diam., without sheaths ca. 8 mm diam.; internodes 9–23 cm. **Leaf** ca. 1.5 m long including cirrus and petiole; sheath brown, with caducous, grey-black indumentum, sheath armed with scattered abundant, brown, triangular spines, spines 2–7 mm long, ca. 2 mm wide; ocrea 4.5–10.0 × 1.5–2.5 cm, conspicuous, elongate and inflated, clasping the stem, tough, brown, armed with 1–5 mm long tiny triangular spines, abundant black ants nested within the ocrea, entry hole made by ants visible; cirrus ca. 45 cm, armed with recurved grapnel spines organised in whorls; petiole 9.7–13.0 cm, 5–6 mm wide and ca. 3 mm thick at base, flattened adaxially, abaxially rounded, with brown indumentum as sheath, sparsely armed with spines as sheath; rachis 35–38 cm, armed with recurved grapnel spines; 4 leaflets each side of rachis, regularly arranged, rhomboid, mid-leaf leaflets 17.0–27.0 × 6.0–7.6 cm, leaflet dark green above, glabrous, with caducous, chalky-white indumentum on undersurface, distal margin conspicuously praemorse, transverse veinlets conspicuous, moderate closely spaced, 1.0–1.5 mm. **Inflorescence** not seen. **Flower** not seen. **Fruit** not seen. **Seed** not seen.

Distribution:—Philippines (Mindanao).

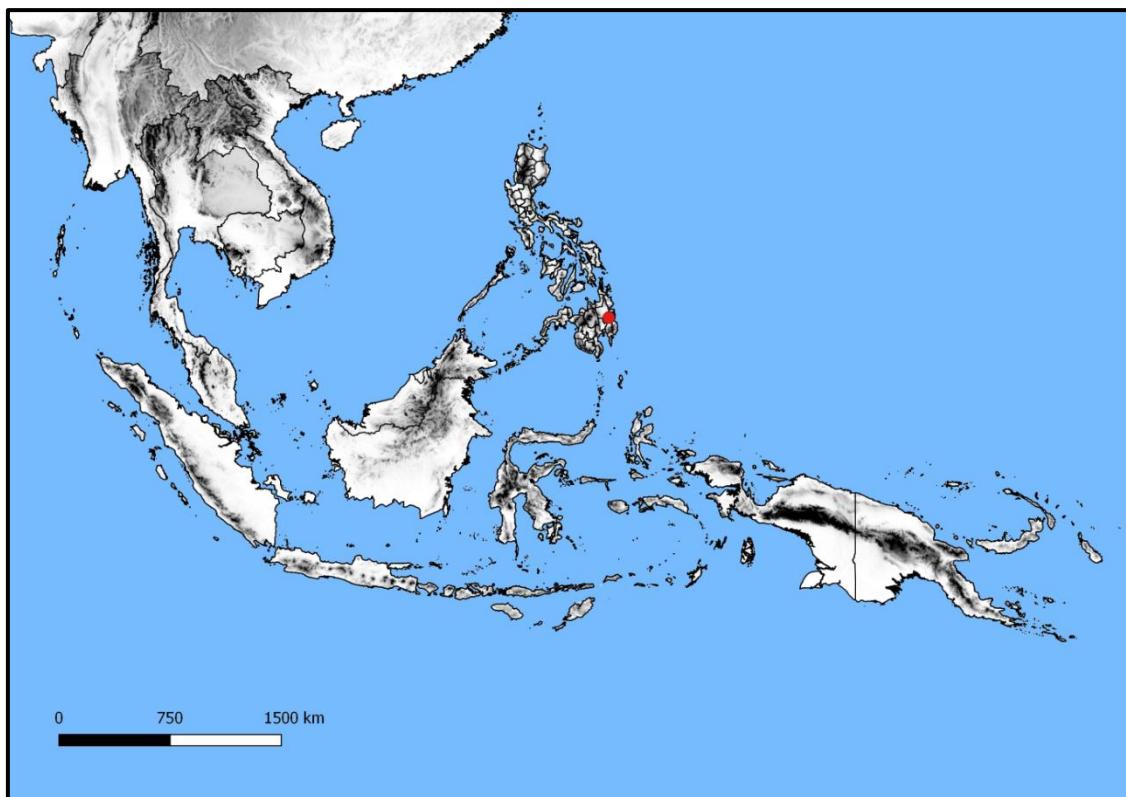
Habitat:—Growing in Dipterocarp forest at low altitude.

Uses:—Furniture and basket making.

Vernacular name:—*Kaporigid* (Philippines).

Specimens examined:—PHILIPPINES. Mindanao: Agusan del Sur, Trento near Simulao, 100 m, 8°3' N, 126°4' E, 18 July 1984, Fernando 411 (K!); Agusan del Sur, Experimental Forest, FORI, 8°02' N, 126°03' E, Baja-Lapis 112 (K!); Surigan, April 1915, Ponce 23913 (K!).

Notes:—This species is still known only from the sterile material. There is very little herbarium material is available. Although *K. scaphigeroides* is quite similar to *K. rostrata*, *K. scaphigeroides* has ocreas more than 5 cm longer than those of *K. rostrata* and the abaxial of leaflets have very dense chalky white indumentum.



MAP 9 Distribution of *Korthalsia scaphigeroides* Becc. Dots are point localities of specimens examined. Map prepared by Salwa Shahimi, using QGIS.

PLATE 9



Korthalsia scaphigeroides Becc. Top left: leaf arrangement (Baja-Lapis 112). Top right: ocrea (Baja-Lapis 112).

10. *Korthalsia scortechinii* Becc. in J.D.Hooker (1893: 475). Type:—
MALAYSIA. Perak, *Scortechini s.n.* (holotype FI!). MAP 10. PLATE 10.

Clustering rattan climbing to 20 m. **Stem** with sheaths 20–25 mm diam., without sheaths 10–15 mm diam.; internodes 25–30 cm. **Leaf** 1–2.5 m long including cirrus and petiole; sheath green, almost entirely obscured by ocrea, with caducous, grey-black coloured indumentum, sheath sparsely armed with scattered short brown triangular spines, spines 1–4 mm long, 1.0–1.5 mm wide; ocrea 8–15 × 4–5 cm, conspicuous, swollen and elongate, base of ocrea tightly sheathing, margin tattering (net-like) (for some species), clasping the stem, tough, dull pale brown with caducous, chocolate indumentum, armed with 1–3 mm long, scattered short tiny triangular caducous, chocolate spines, numerous black ants within the ocrea; cirrus 0.30–1.25 m, armed with recurved grapnel spines organised in whorls; petiole 5–10 cm, 10–18 mm wide and ca. 4–5 mm thick at base, flattened adaxially, abaxially rounded, indumentum, sparsely armed with spines; rachis 0.45–1.15 m, armed with recurved grapnel spines organised in whorls; 8–13 leaflets each side of rachis, regularly arranged, narrowly rhomboid, mid-leaf leaflets 21.5–30.1 × 2.5–5.2 cm, leaflet dull to dark green above, glabrous, with whitish indumentum on undersurface, distal margin conspicuously praemorse usually the upper V*, transverse veinlets moderate conspicuous, moderate closely spaced, 1.0–1.5 mm. **Inflorescence** lax, 40.5–72.7 cm long including ca. 6 mm peduncle, branched to 2 orders; prophyll ca. 4.0 × 0.7 cm, with caducous, dark brown indumentum; rachis bracts up to 4.1–9.0 × 1.7–3.7 cm, splitting, with caducous, dark brown indumentum with several tiny spines; primary branches 2–4, 5.7–10.6 cm apart; proximal primary branch 26.2–30.7 cm long, with up to 2–3 rachillae; rachillae 14.0–28.2 cm long and slender, including 0.5–1.0 cm visible stalk, ca. 0.7 cm wide, lacking hairy between rachilla bracts. **Flower** not seen. **Fruit** 1.6–1.7 × 1.1–1.5 cm covered in 16–19 vertical rows of orange-brown scales. **Seed** 1.3 × 0.7 cm, endosperm ruminate.

Distribution:—Peninsular Malaysia, Thailand.

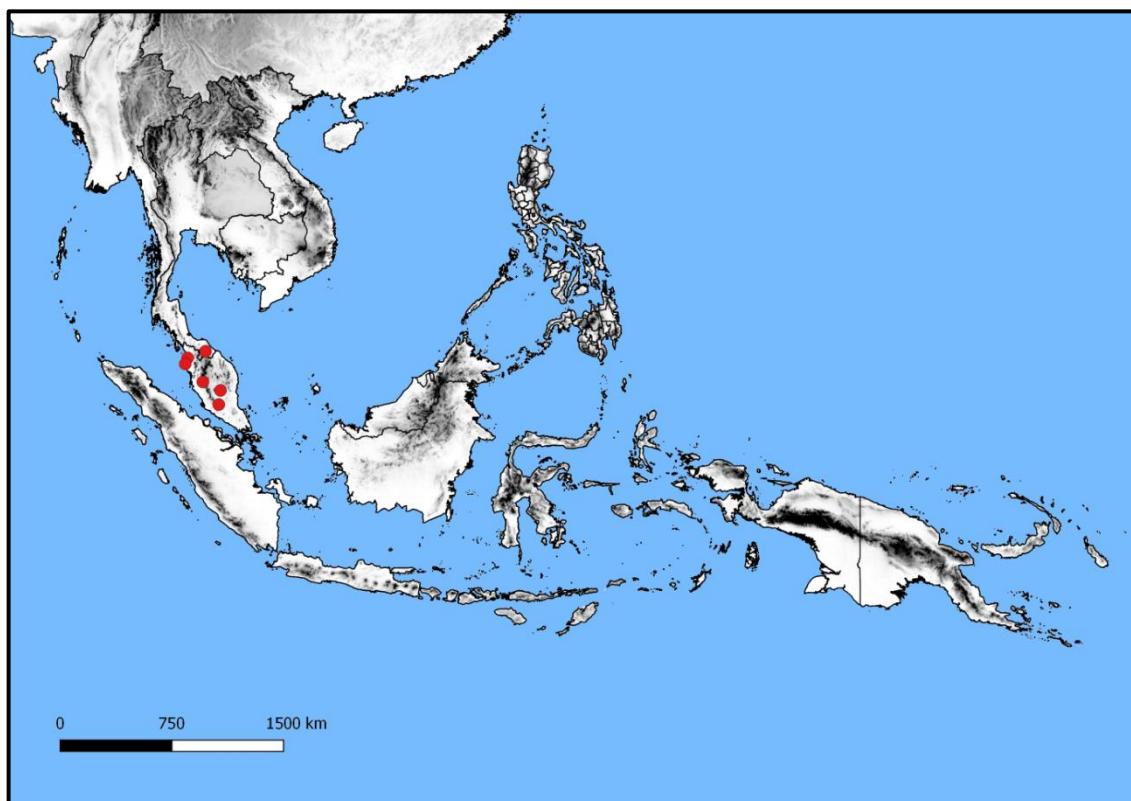
Habitat:—Lowland and hill dipterocarp forest up to 900 m altitude.

Uses:—Cane used to make baskets, also as binding material.

Vernacular name:—*Rotan dahan* (Iban), *Rotan semut*, *Wai doa* (Thailand).

Specimens examined:—MALAYSIA. Kedah: Sungai Patani, Gunung Jerai, 600 m, 5°47' N, 100°25' E, 06 July 1977, Dransfield 5106 (K!). Negeri Sembilan: Pasoh Forest Reserve, 100 m, 2°58' N, 102°18' E, 09 May 1977, Dransfield 4976 (K!). Pahang: Genting Highlands, 30 January 1995, Baker 497 (KEP!); Kuala Lipis, 18 August 1925, Somerville 10484 (K!). Tasak Bera, 3°49' N, 102°24' E, 03 September 1982, Gianno 512 (K!). Penang: Penang Hill, 423 m, 5°25' N, 100°16' E, 03 March 2014, Shahimi 15 (K!, KEP!). Perak: Tapah, 700 m, 4°20' N, 101°20' E, 23 October 1982, Ave 178 (K). THAILAND. Narathiwat: Si Sakhon, 550 m, 6°11' N, 101°30' E, 07 March 2001, Niyomdham & Puudja 6446 (K!).

Notes:—*Korthalsia scortechinii* sometimes has been confused with *K. echinometra* because large swollen inflated ocrea but can be distinguished by ocrea bearing numerous short triangular spines and the narrowly rhomboid leaflets. Based on the phylogenetic results of this study, *K. scortechinii* has a close relationship with *K. cheb.*



MAP 10 Distribution of *Korthalsia scortechinii* Becc. Dots are point localities of specimens examined. Map prepared by Salwa Shahimi, using QGIS.

PLATE 10



Korthalsia scortechinii Becc. Top left: Habit. Top right: ocrea. Bottom left: leaf arrangement (Shahimi 15). Bottom right: inflorescence (Dransfield 4976).

CHAPTER 5

GENERAL CONCLUSIONS AND FUTURE PERSPECTIVES

This thesis presents new taxonomic treatments for 10 species of *Korthalsia* which have ant-associations, including new keys and distribution maps. I have also reconstructed a new phylogenetic hypothesis for calamoid palms. This allows an exploration of the relationships between species in the calamoid tribe Calameae, with emphasis on *Korthalsia*. In terms of understanding the origins of biological diversity I have used ancestral reconstruction in the context of my new phylogeny to understand the evolution of ant-domatia in genus *Korthalsia*. These studies have advanced our knowledge of the genus *Korthalsia* and particularly the species of *Korthalsia* with domatia.

5.1 Reflections on the work

5.1.1 The phylogeny

This study uses published data, and unpublished data that were made available to me. As such, the work depends on the available data being of high quality. Unlike many studies using data that have not been directly generated for the present work, I was lucky because the unpublished data I was provided with was all from specimens that had vouchers and that were determined by my supervisor, Dr. W. Baker. I am also confident of the identifications of the plants I sampled in the field, because I worked intensively on the morphology of the plants, because I included them in the taxonomic treatment.

The species I sampled in the field for inclusion in this study were species of *Korthalsia*, and I designed my fieldwork to sample as many species of this genus as possible. Ultimately, my phylogeny sampled 19 of the 28 species. It would have been good to

have included all 28 species, but the nine species I didn't sample are from the Cambodia, New Guinea, Philippines, the Andaman Islands, and Indonesia, places difficult for me to visit. Since previous studies had never sampled more than one or two species the effort in adding species has made a considerable contribution. It is common to increase sampling for DNA work by sampling leaf material from herbarium specimens, particularly as new sequencing methods make it possible to get sequence data from degraded DNA (Särkinen *et al.*, 2012). I was using Sanger sequencing, and it seemed unlikely that PCR-Sanger methods would work for the specimens in the herbarium I had access to. One species without a name, provided by Dr. A. Henderson from his fieldwork in the Andaman Islands and included in the phylogeny here as *Korthalsia* sp. is probably *K. rogersii*, a species endemic to the Andaman Islands. The identification was provisional because there was no voucher to determine, only silica-dried leaf material was sent. Three of the species not included in this study had ant ocrea. These are *K. angustifolia*, *K. furcata* and *K. scaphigeroides*. It would be particularly good to include these species, and their inclusion could contribute to the understanding of ocrea evolution.

There were some problems with collecting sequence data for some regions. I spent a lot of time trying to optimise the *rps16* region. One advantage of using existing unpublished data was that I had a lot of data to work with. But if I was to design a study from scratch I probably would not have included the *rps16* region in the set of regions I sequenced. Most molecular phylogenetic studies using Sanger sequencing would usually carry out a pilot study and select the most variable and most tractable (easy to work with) regions.

One aspect of my work that should be noted here relates to the outcome of the phylogenetic analysis: not all nodes were well supported and some relationships relatively poorly resolved. This is attributed to the slow evolution of palm DNA and the recent origin of *Korthalsia*. Despite the lack of resolution and support, at higher levels our new phylogeny of the calamoids supports revised taxonomy. Also, since the tree in this study has dense taxonomic sampling at species level especially in genus *Korthalsia*,

and can still serve as a valuable framework for the comparative research described in Chapter 3 (Heath *et al.*, 2008).

5.1.2 Ocrea evolution

One thing I benefited from in the design of this Ph.D project was the ample opportunity for fieldwork. To see and understand ocrea in the field was essential for me to make sense of the palm ocrea. In the field I was able to use a machete to cut up *Korthalsia* palms, and this gave me the insights I needed to develop the comparative morphology and anatomy work. Fieldwork also gave me the opportunity to experience how well defended the rattans are by the ants that live in the domatia, as well as by the spines that most species have.

Dissection of ocrea and the interpretation of the ocrea structures was challenging. I found it a challenge to interpret the ocrea morphologies because the only published comparative developmental data was for a species of *Calamus* (Merklinger *et al.*, 2014). The development of the *Korthalsia* ocrea is different. It was a significant amount of work to get all the stages of all the seven species I included in comparative study, from the field to the final images. In this light it was disappointing that the developmental stages were so similar. If I was starting this work again, I might focus on species that from the phylogeny I expected to be different, rather than trying to make a complete set of images for all the species I had material for. Another challenge relates to the description of stages in these plants. Whereas in flowering plants generally it is fairly straightforward to describe the ages of stages, by numbering the leaf primordia starting with the youngest (Bar and Ori, 2014), in the plants I worked with there is no clear way of identifying relative ages. This is because when I remove layers and chose an inner layer to image, I don't know how many primordia there are that are younger than the one I have imaged.

The comparative morphology is a contribution to the understanding of ocrea in its own right, but considering ocrea types in the light of the phylogeny allows deeper

understanding of ocrea. It was unfortunate that the phylogenies I had to work with were not very resolved or supported. Nevertheless, I do have a better understanding now of when ocreas evolved, and I know that different ocrea types have evolved more than once.

5.1.3 The taxonomy

As for the taxonomic treatment for the *Korthalsia* species, it is important that the differences between species are clearly understood and how they can be distinguished from other species. This knowledge is necessary in order to undertake important records of all species and to be able to consider the potential of each species for cultivation and sustainable management. To ensure any experimental or development work undertaken is replicable, a structured framework is essential. In a nutshell, conservation and sustainability of world remaining resource can be done by taxonomy. In order to face global biodiversity challenge, it is better to integrate the science of taxonomy back into the conservation world (Sunderland, 2012).

During the fieldwork, I saw nine of the species of *Korthalsia* that I went on to describe. This is particularly important for palms because the plants are so big, and getting an impression of the plant from the herbarium specimen is hard. It was harder to prepare the descriptions of the fruit and inflorescence characters because I didn't see them in the field.

At the outset of this project I made a case that it was timely to make a new treatment for *Korthalsia*, since the last treatment was made in 1981 (Dransfield, 1981). Having completed the treatment it is possible to assess how many specimens of *Korthalsia* were available to me that were not available to Dransfield when he wrote his treatment. I studied 117 specimens collected in 1982 or later, including five specimens that I collected myself. This significant increase in specimens available to study indicates that as previously suggested, a revision at this time would add new information.

5.2 Priorities for future work

5.2.1 Phylogeny and ocrea evolution

With the advent of next-generation sequencing (NGS) technologies in recent years, improvement in high-throughput, time-saving and affordability seems poised to resolve many of the problematic relationships in flowering plants, thus, clarifying remaining problematic deep level questions in palms in the near future would much easier (Schuster, 2007; Soltis *et al.*, 2010; McCormack *et al.*, 2013). An NGS-based hypothesis of species relationships of *Korthalsia* would significantly improve the estimation of relationships and therefore also our hypotheses about ocrea evolution.

I could also improve the developmental work I carried out. Because of the limitation of material available, I characterised only diverging, inflated and tightly sheathing ocrea using developmental series under light and electron microscopy. Future work should include characterisation of the fibrous net-like type of ocrea.

The work I present here does not include any biogeography, such as ancestral area analysis (Bremer, 1992). It would be interesting to look at where the ant ocrea types evolved, now I know more about when they evolved. For example, it appears that all the ant-associated *Korthalsia* are on west side of the Wallace's line and there are only two species on east of Wallace's line which are not ant associations; one species in New Guinea and another one in Sulawesi. There are other biological questions that could be interesting to consider, that are suggested by the phylogeny. It is interesting that divergent ocreas may have later in evolutionary time become inflated. The relative advantages to the plants of entirely enclosed domatia could be investigated experimentally. All of the experimental work on *Korthalsia* ocrea to date (Edwards *et al.*, 2010; Chan *et al.*, 2012; Miler *et al.*, 2016) have been done on species with inflated ocreas; it would be interesting to compare the biology and ecology of ants in inflated and divergent ocreas.

5.2.2 Taxonomy

The taxonomic revision in this thesis focuses only on 10 species of *Korthalsia*, the species which have ant-associations, but the other 18 species also need a new treatment as only a regional treatment has been published (Blume, 1843; Beccari, 1884; Martius, 1845; Beccari, 1909; Dransfield, 1981). It is important to make a new treatment for the remaining species as these species have not been the subject of any integrative treatment across their range.

Apart from that, there are no conservation status assessments for *Korthalsia*. None have ever been made. Although I think it is unlikely that any species in my treatment would be considered as threatened, as many of this species can be commonly found throughout the region with multiple individual in each population, it is important to document the status of each species. Conservation measures may be needed if the status of any species may change due to habitat loss and land use changes.

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APPENDICES

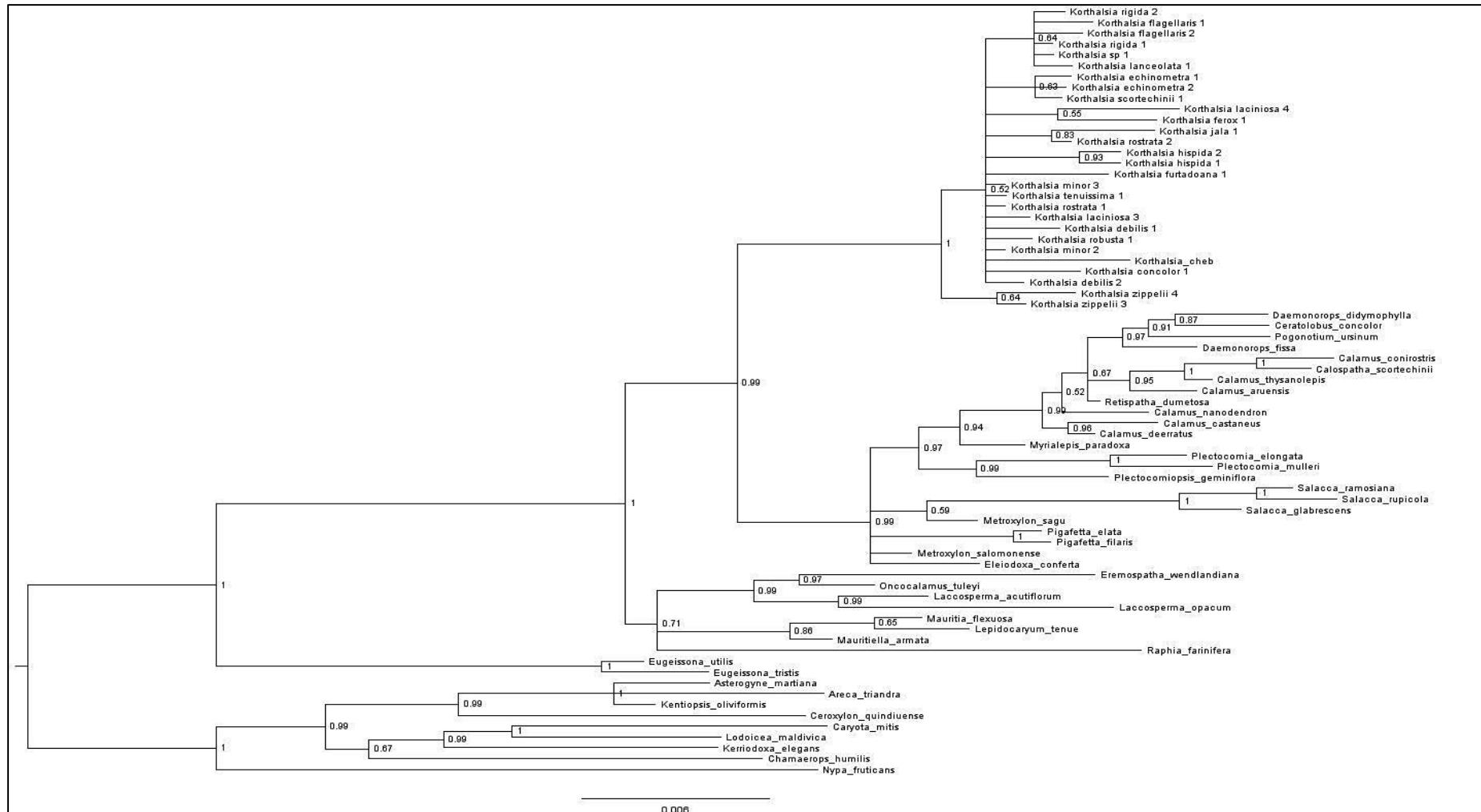
Appendix 2.1

Modified Doyle and Dickson (1987) of DNA extraction procedure for silica-gel dried material:

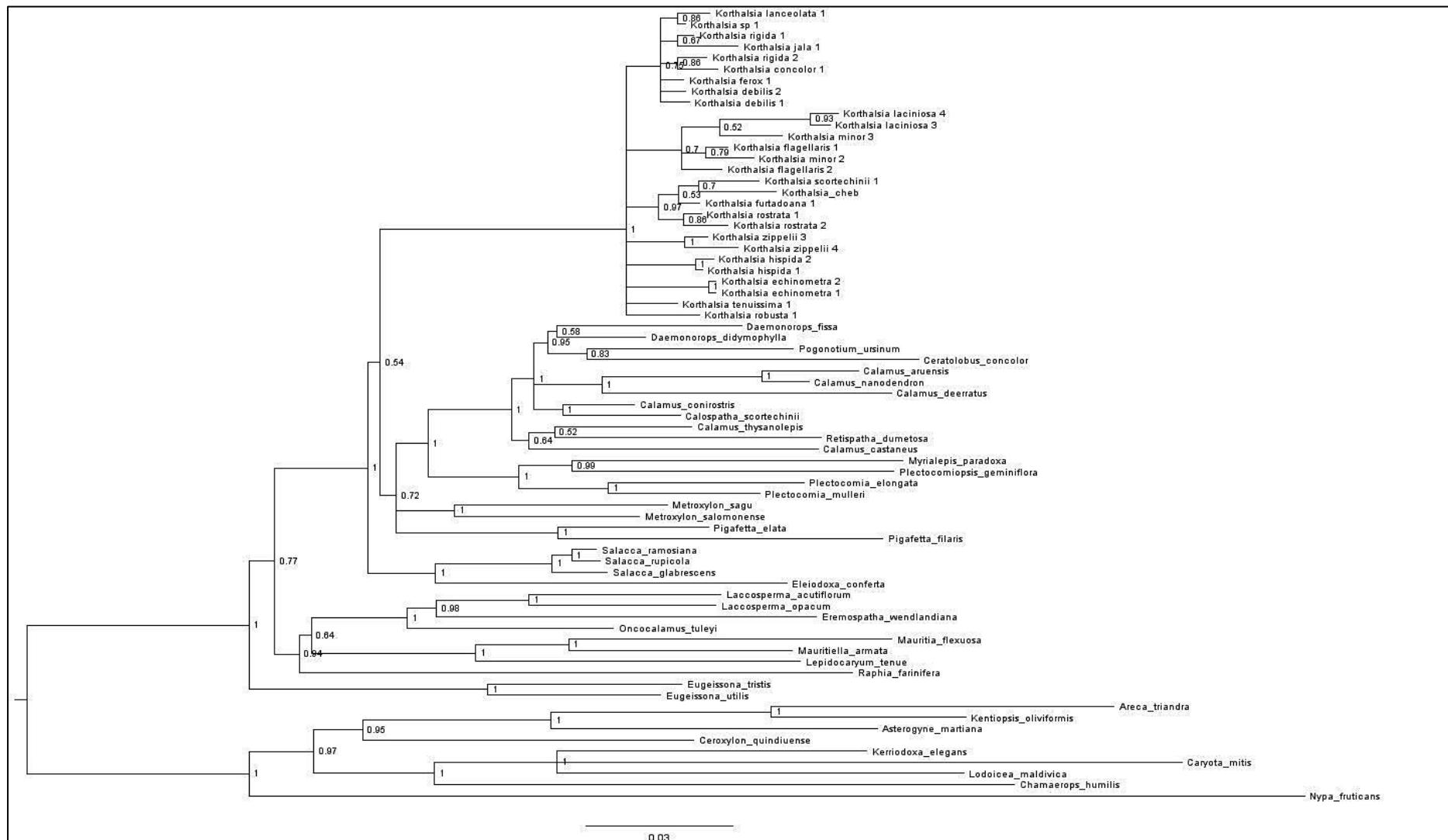
1. 0.02–0.03 g of leaf material were ground to a fine powder using pestle and mortar with acid washed sand.
2. The powders was placed in 1.5 ml eppendorf and were added with 500 µl CTAB buffer, 50 µl sarkosyl and 5 µl B-mercaptoethanol. These eppendorf were then vortexed for 10s and incubated at 60°C for 1 hour with occasional mixing.
3. An equal volume of 24:1 chloroform/isoamyl alcohol was added to the solution, vortexed for 10s and centrifuged at 13,000 rpm for 3 min to produce a clear supernatant. The upper aqueous layer in the tube was removed carefully to a clean tube. Re-extraction was repeated and was performed the same way for a second time.
4. 2/3 volume of ice-cold 100% isopropanol was added to the supernatant and the samples were mixed and placed on ice for 60 min or overnight in the -20 °C freezer. The DNA precipitate was collected by centrifuging at 10,000 rpm for 3 min.
5. The alcohol supernatant was discarded. The DNA pellet was washed with 500 µl of 70% ethanol and centrifuged at 13, 000 rpm for 1 min. The liquid phase was then released and the pellet was added with 0.1M TE buffer and 3M sodium acetate. This solution were mixed until the DNA was suspended followed by adding ice-old 100% ethanol. The solution with pellet DNA was incubated on ice for 60 min.
6. The pellet then was centrifuged again at 10,000 rpm for 3 min. The ethanol was removed and the pellet was set to dry by overnight drying at room temperature.
7. The pellet was re-suspended in 50 µl of 0.1M TE buffer and stored at -20 °C.

Appendix 2.2

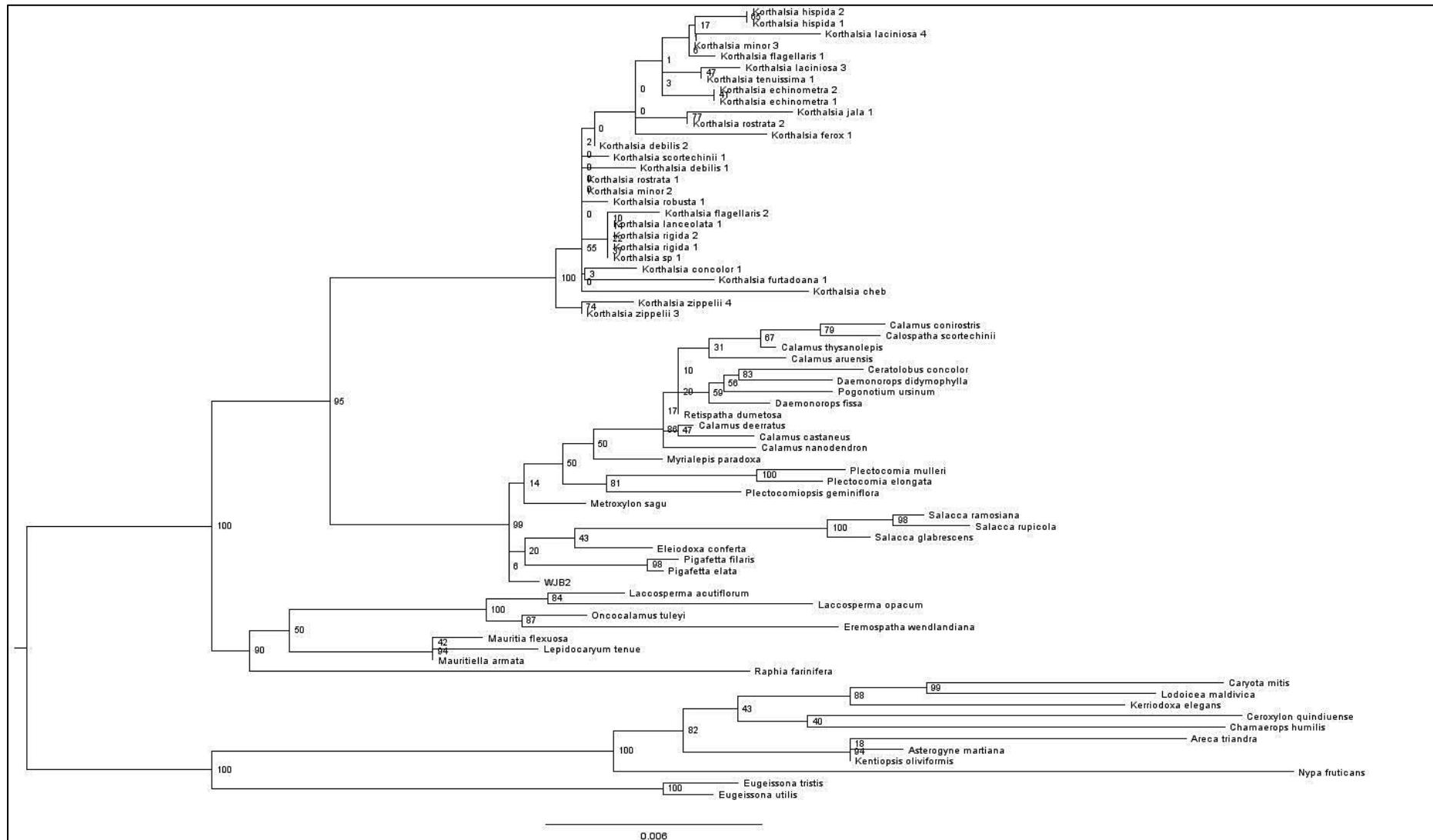
2.2.1 Bayesian analysis, combined chloroplast



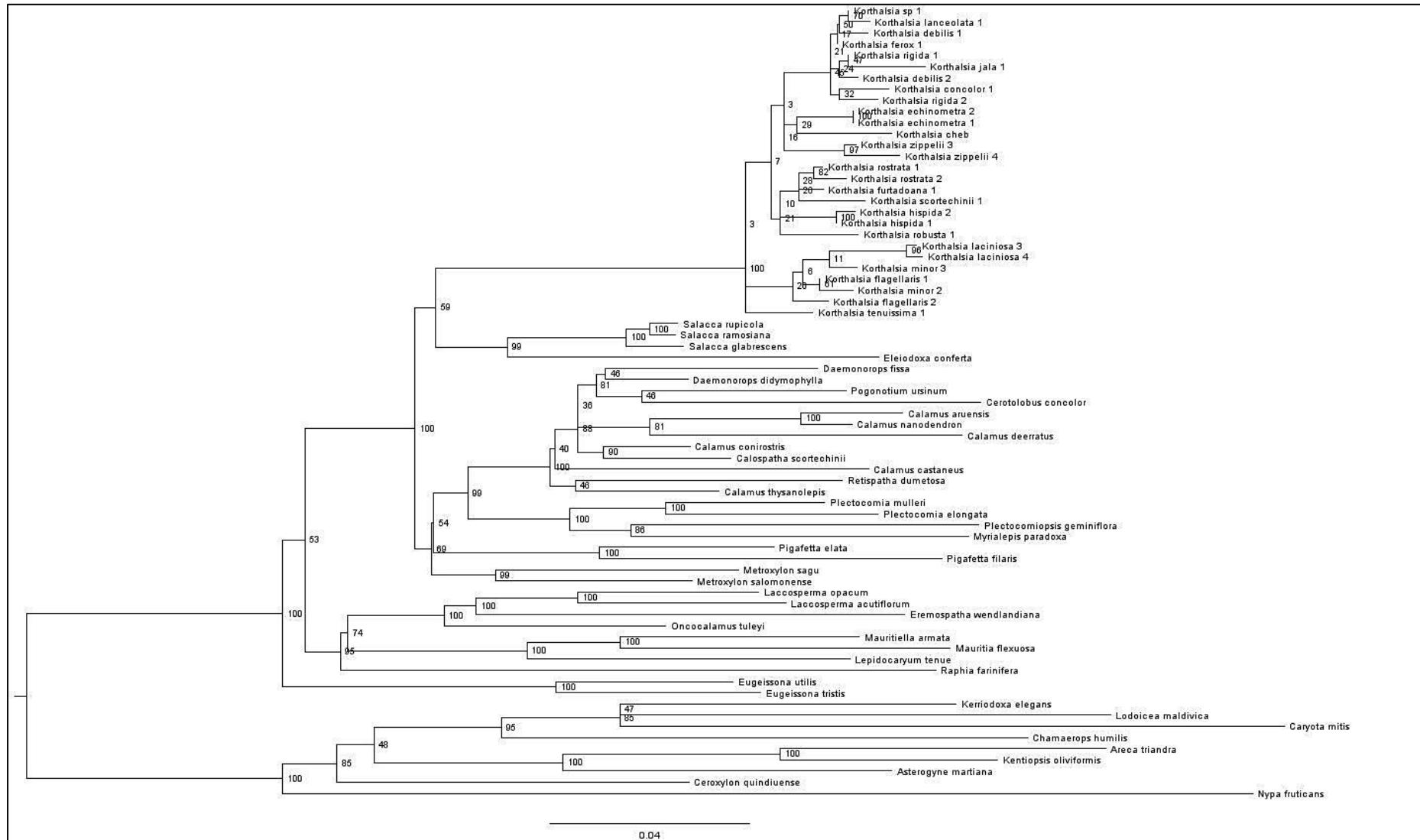
2.2.2 Bayesian analysis, combined nuclear



2.2.3 Maximum Likelihood, combined chloroplast



2.2.4 Maximum Likelihood, combined nuclear



Appendix 3.1

Voucher specimens including stem, whole leaf and ocrea



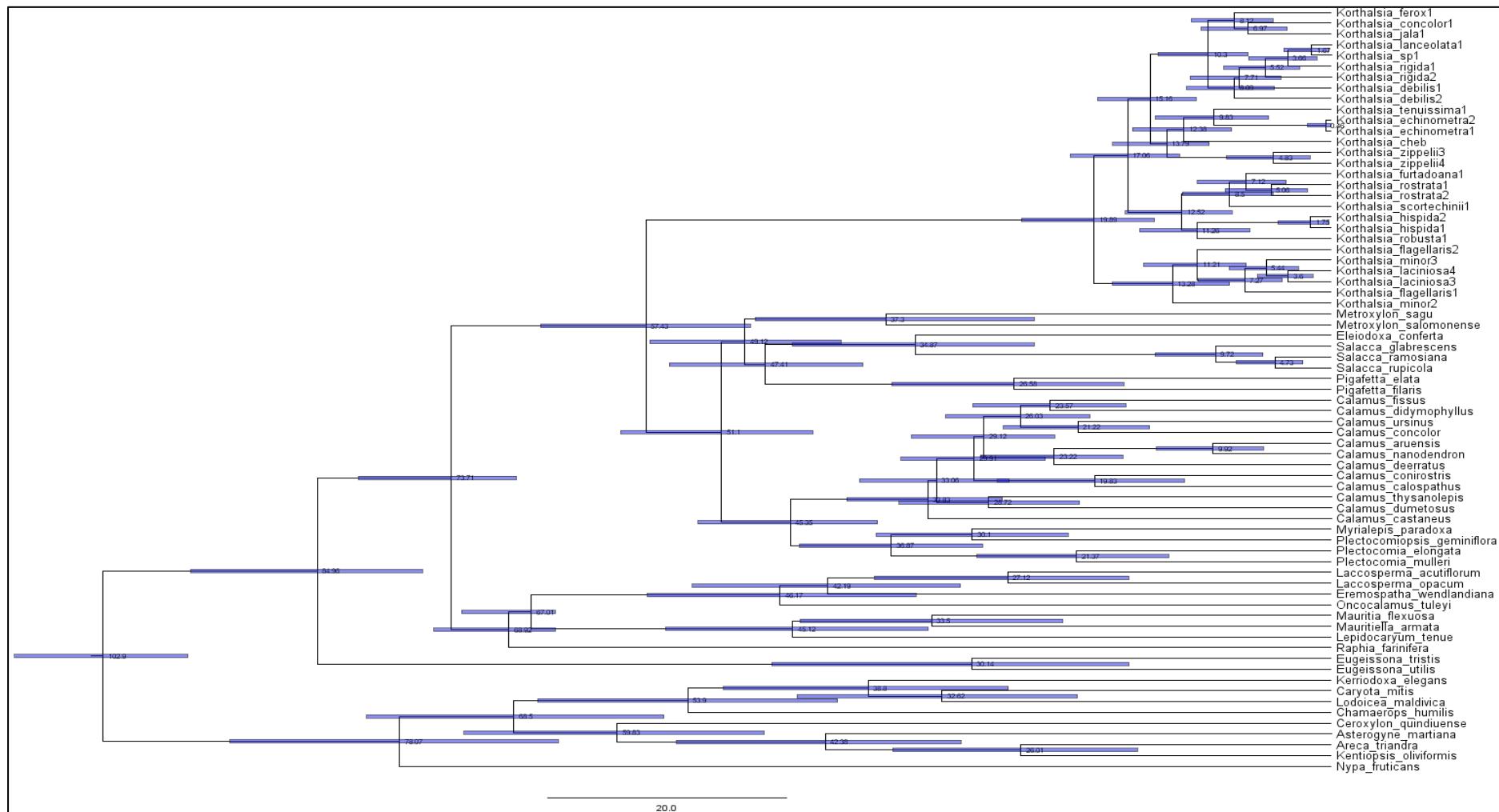
Appendix 3.2

Ethanol series:

1. The samples transfer through an ethanol series of 70% - 90% - 100% - 100%.
Approximately 1 hour in each solution.
2. The samples were leaves in 100% ethanol for overnight.

Appendix 3.3

Bayesian maximum clade credibility chronogram for the Calamoideae



Appendix 4.1

DATA SHEET		DATE:			
Collector & number:	Herb:				
Name:	Det by:				
Vernacular:	Ethnobotany:				
Locality:	Habitat:				
Stem					
STEM	Solitary / Clustering	Max height:	Diam. with sheath:	Diam. without sheaths:	Internode length:
Stem notes:					
Sheath					
Sheath spines:					
Sheath notes (indument, color):					
Ocrea:					
Leaf Cirrus: yes/no					
Leaf length (inc. cir, pet)	Petiole length	Rachis length	Cirrus length		
Leaf notes (indumenta, hairs, spines, etc.):					
Leaflet Leaflet shape: Leaflet arrangement:					
Largest dimensions (L x W)	Smallest dimension (L x W)	Mid-rachis dimension (L x W)	Leaflet number (on 1 side)		
Leaflet notes (indumenta, hairs, spines, venation, transverse veinlets, etc.):					

Inflorescence

lax/congested/erect

Inflorescence length	Peduncle length	Prophyll length	No. of orders of branching

Primary branches notes (how many, Z cm apart, proximal, X-Y cm long, etc.):

Rachilla

Largest rachilla length	Smallest rachilla length

Rachilla bract notes:

Rachilla notes (shape, the arrangement of flower, etc.):

Flower

(X x Y) mm in bud:

Fruit Mature / Immature

Fruit dimension inc. beak (H x W)	No. of vertical rows of scales

Fruit notes (shape, beak, color, arrangement, etc.):

Seed Seed dimensions (H x W x D):

Seed notes (shape, pit ornamentation):

Endosperm and embryo: