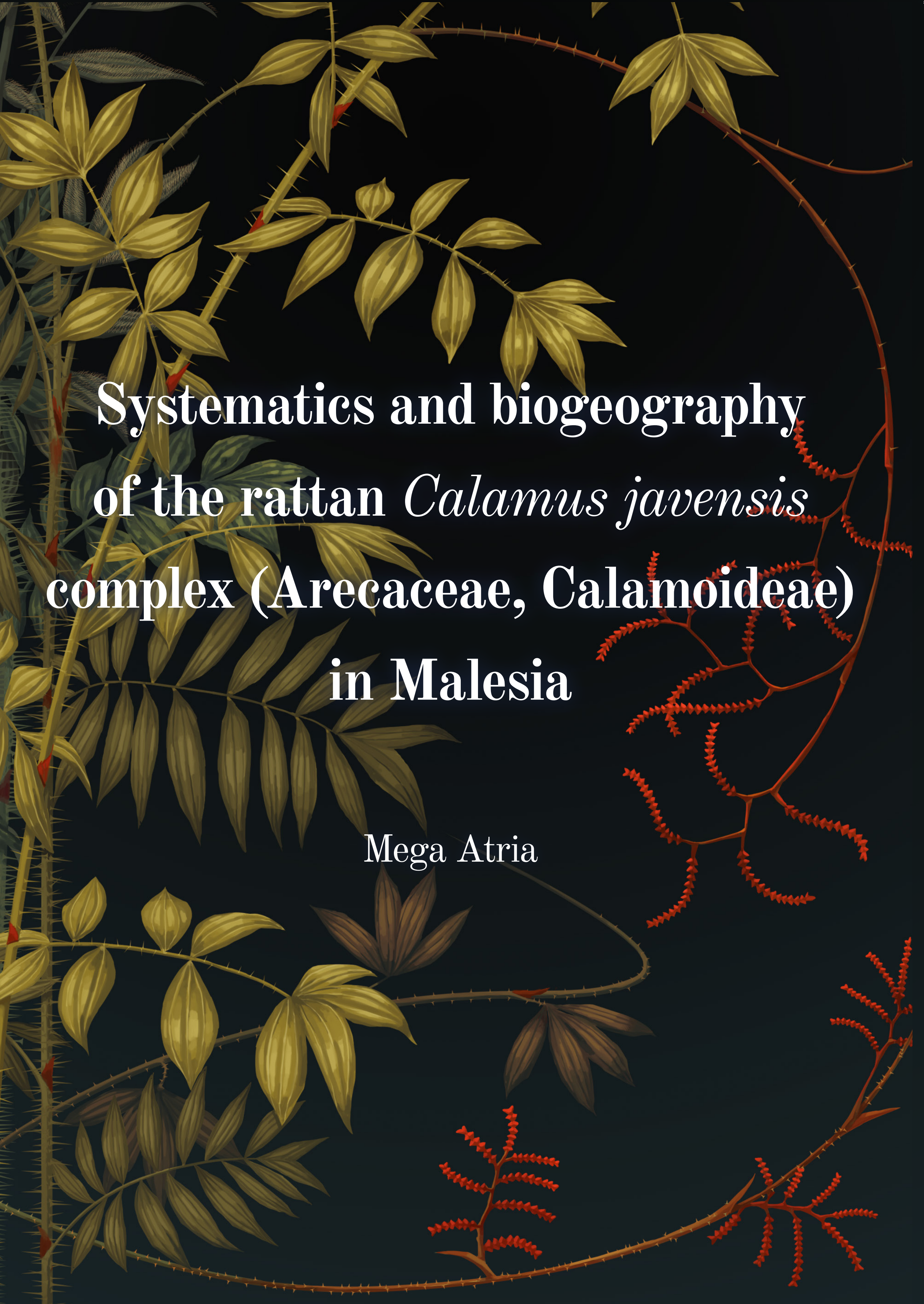

Systematics and biogeography of the rattan *Calamus javensis* complex (Arecaceae, Calamoideae) in Malesia

Mega Atria

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Systematics and biogeography of the rattan *Calamus javensis* complex (Arecaceae, Calamoideae) in Malesia

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This thesis is dedicated to my family, Singgih, Anisa and Bayuaji

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Chapter 1

Introduction

1.1. Polymorphism in Plants

Polymorphism is the presence of two or more, different, continuous or discontinuous morphological forms within populations existing in the same or different habitats. It is the result of a discontinuous genetic variation. Polymorphism in plants is very common and is related to variation on the gene level, adaptation and diversity (Schlichting, 1986; Dudley & Schmitt, 1996; Franks et al., 2014; Merila & Hendry, 2014). At the genetic level, polymorphism is a condition of two or more possible alleles in one gene (alternative genotypes), which is a genetic change starting in an individual that allows the emergence of various forms. The different genotypes result in different phenotypes (polymorphism). The different phenotypes can also develop when in different individuals different genes are activated (morphogenetic polymorphism). Polymorphisms are maintained in nature as there is a balance between morphological forms or variations formed as a result of mutations and/or natural selection (Ford, 1966; Ford, 1977; Alonso-Blanco et al., 2005).

Plant reactions to various environmental conditions are expressed through variation and differentiation based on phenotypic plasticity. Populations from different environmental conditions will exhibit different morphological expressions. Not only morphology, but changes also occur in chemical conditions, physiology, and genetics due to environmental instability (Brown, 1979; Hamrick, 1983; Palacio-Lopez et al., 2015). Natural selection always favours characters that maximize the fitness of the plants in their local environment (Linhart & Grant, 1996; Anderson, 2016), but between environments, there are two possible evolutionary responses. Populations can differentiate genetically to become locally adapted (Futuyma & Moreno, 1988; Kawecki & Ebert, 2004; Gould et al., 2014) or individuals may be phenotypically plastic, expressing the optimal phenotype in both environments without genetic differentiation (Bradshaw, 1965;

Schlichting, 1986; Schlichting & Smith, 2002).

Plants are generally highly plastic, and, during their development, plasticity is an adaptation mechanism that allows plants to respond optimally to environmental variations (Alpert & Simms, 2002; Callahan et al., 2005). Phenotypic plasticity is the ability or capacity of variation of a phenotype/expression of a phenotype due to environmental influences on genotypes during development. Plant populations exhibit various variations (intraspecific), which is a reflection or adaptation to differences in natural environmental conditions, this phenomenon or intraspecific variation is considered as the source of speciation (Linhart & Grant, 1996). An individual's plastic ability is known to develop or evolve in disturbed and dynamic habitats, where it reduces the detrimental effects of selection (http://agron-www.agron.iastate.edu/~weeds/AG517/Content/LifeHistory/PhenoT/LHPlastic.html; Lande & Shannon 1996; Barrett & Schluter 2008). In spatially different environmental conditions, local genetic adaptation occurs if gene flow is limited and may result in speciation. If gene flow can still be extensive and environmental conditions support it, then phenotypic plasticity becomes important (Emery, 2009; Scheiner, 2013; Palacio-Lopez et al., 2015).

The expression of developmental characteristics in plants is highly dependent on environmental conditions. Because of their inability to move, plants have a unique plasticity in the development of characters. An example of plasticity is the capacity of a genotype to express different phenotypes adapted to environmental conditions. Environmental factors that affect plant development include all abiotic factors such as light, temperature, wind, humidity, water availability or nutrient sources, in addition biotic components also play a role such as pathogens and competitors (Tonsor et al., 2005). The concept of "environmental context-dependency" has been mentioned to play a role in many phenomena of developmental character variation and variations in phenological entities during time (Sultan, 2000; Casal et al., 2004).

1.2. The effect of polymorphism

The condition of polymorphism can create complex species, which consists of groups of organisms that share morphological similarities, but the differences are not constant enough to distinguish them as separate species. Complex species are often also associated with cryptic species, which are conditions in which the morphology is (almost) similar, but the genetic makeup is such that different species can be distinguished. If not recognized as two or more species, they will all be united under the same name. Complex plant species show a condition in which the entities or species have similarities, but also a complex pattern of disjunctions in the ranges of variations in characters, some of which can be linked to ecological factors, geography, others being apparently random (White, 1962; Zheng, 2017). Complex species usually form monophyletic groups that have diversified recently and/or are still diversifying.

Polymorphism often raises problems and complexities, one of which is in the field of taxonomy, as polymorphic species generally occur as complex species. Polymorphic plant species typically exhibit large intraspecific variations with complex evolutionary histories, and thus pose a major challenge to taxonomic research (Slovak et al., 2012). Polymorphic conditions can make it difficult to recognize the species delimitations and the resulting nomenclature can be complex or confusing too as different researchers have different delimitations. Identifying discontinuous entities within species complexes is a major topic in systematic and evolutionary biology. Multidisciplinary approaches are crucial to understand the species integrity and boundaries within species complexes. Morphometrics, cytogenetics, anatomy, crossing experiments, and molecular markers have been combined in different ways to investigate species complexes (Dransfield, 1999; Pinheiro et al., 2018).

Species delimitation is not always difficult. In cases where speciation occurred a long time ago, newly discovered species will fit into most species' concepts. The major difficulty in delimiting species is when speciation starts. It is particularly complicated in the following situations: (1) when diversification (or lineage formation) arises with little morphological change, known as cryptic or sibling species (Bickford et al., 2007); (2) when species exhibit extensive morphological diversity and little genetic divergence, commonly occurring in evolutionary radiations (Shaffer & Thomson, 2007; Barley et al., 2013). In species complexes, incomplete knowledge of the biodiversity status of the whole group may lead to erroneous taxon sampling and flawed assessments of biodiversity, biogeography, and speciation processes (Heath et al., 2008; Pinheiro et al., 2018). Therefore, it is very important to determine the situation in a complex species group, whether they are a group of different species but have a similar morphological appearance or are actually one species with a very wide morphological variation.

1.3. Species Complex in Palms

Most palms display a wide variability in morphological characters, variability within individuals, for example, between the seedling and sucker shoot leaf form and that of the adult and also variability within populations. Some of this variation cuts across morphological differences that have been used to separate species. "We should not be surprised that morphological characters that can be used reliably to distinguish species in one group of palms may prove to be unreliable in other groups" (Dransfield, 1999). The great diversity of palms in the Malesian region is in part due to the complex geological and climatic history of the region and the fact that the region comprises one huge archipelago with numerous barriers that will have provided opportunities for the development of species in isolation. Examples of complex species in palm are the *Chamaedorea pinnatifrons* (Jacq.) Oerst. complex (subfam. Arecoideae), a very widespread and variable species in the genus, distributed from Bolivia to Mexico and the *Calamus javensis* complex (subfam. Calamoideae) in Southeast Asia. Other species complexes in new world palms are in the genera *Aiphanes* Willd., *Bactris* Jacq. ex Scop., and *Geonoma* Willd., where as many as three morphologically distinct forms may grow sympatrically with no apparent intermediates (Henderson et. al, 1995; Borchsenius, 1999; Dransfield, 1999)).

The most problematic cases in the classification of species complexes occur when in one geographical area variation may be great but continuous, but in another area similar variation may be discontinuous. In the latter area one can distinguish different taxa, but their distinctness disappears in the first area. Yet, at the local level distinctive elements remain. Subsuming all under one name results in a loss of information, because in line with adaptation to their environment, each local population undergoes morphological and ecological changes that show its evolutionary stages (Wilson, 1959, 1961; Dransfield, 1999). The high levels of variability in a complex species may be based on different ecological circumstances, and these may represent an intermediate step in sympatric speciation (Henderson, 2011).

1.4. Calamus javensis Blume complex

The rattan *Calamus javensis* Blume is known as a polymorphic species, a taxon with a high phenotypic variation and infraspecific complex. The species has a very high morphological diversity and intermediate forms have also been found (Dransfield, 1999; Dransfield et al., 2008). To date, many forms within the complex have been described and named, many new collections do not fit the existing taxa. Therefore, two options exist: Dransfield (1979) suggests that one option is to continue to capture the variability by creating new varieties, while the other option is to unite all taxa and recognize them as a single variable taxon.

Calamus javensis is widely distributed in Southeast Asia. It is a liana common in the understorey of the humid forests in Myanmar, Southern Thailand, Malay Peninsula, throughout Borneo, Sumatra, Java and the Philippines (Palawan and Luzon). The centre of diversity is in northern Borneo where the greatest morphological variation can be found. Meanwhile, on Java, where the syntypes are collected, the variation is not very significant. This species can be found in the lowlands to an altitude of 1200 m above sea level, it can grow in various types of soil and forest types except for mangroves (Dransfield, 1976; Mogea, 1996; Barfod & Dransfield, 2013). The very high morphological diversity within the species *C. javensis* is likely due to the wide range of habitats in which it lives (Dransfield, 1979, 1984, 1999; Uhl & Dransfield, 2008; Watanabe et al., 2006).

The rattans (derived from the Malay word *rotan*) are spiny climbing palms from the old world, all of which belong to subfamily Calamoideae (Dransfield, 1999). They cover 600 species under 13 genera (Dransfield, 1992b; Sunderland, 1999). The largest rattan genus is *Calamus* (Uhl & Dransfield, 1987; Henderson, 2020) with about 385 species (Sheng, 2010; Henderson, 2020). The rattan *C. javensis* is widely used by local people in Southeast Asia. The excellent cane (also called rotan or rattan) is used for bindings, house construction, to manufacture baskets and snares, and the leaf midrib is a tool for grating coconut (Ave, 1988;

Mogea, 1996; Barfod & Dransfield, 2013). Due to its strong and flexible trunk, the cane is considered excellent in Sarawak, and is often used to make baskets, cordage and handicrafts. In Palawan (The Philippines), *C. javensis* cane is considered an excellent rattan and is the second choice to *C. caesius* Blume (a well-known species in the rattan trade; Mogea, 1994; Shim & Tan, 1994). The FAO includes *C. javensis* in the major commercial species of rattan as identified for Asia by Dransfield & Manokaran (1993). Thus *C. javensis* can be a new alternative rattan source for industrial needs. However, the fact that *C. javensis* is a polymorphic species complex with variable quality of the cane makes it less valuable unless the complex is taxonomically resolved.

Typical for all forms of *C. javensis* is that they are small rattans with a stem diameter of 2–6 mm without leaf sheaths and to 10 mm with sheaths, with sparsely to robust spines, long and flexible internodes up to 30 cm long (usually shorter), a distinct ocrea, deep crimson when young; pinnate, ecirrate leaves to 40 cm long, flabellate terminal leaflets and the lowermost pair often swept back across the stem; a flagellum to 75 cm long, long inflorescences with red rachilla and ripe fruits ovoid in shape.

Calamus javensis is recognized as a phylogenetic species and considered as an ochlospecies (Henderson, 2020). Cronk (1998) considers species that have strong morphological similarities and that are taxonomically difficult as an ochlospecies, which is a polymorphic species with a very complicated infraspecific variation, that is ultimately difficult to represent in a formal taxonomy. Several general traits of ochlospecies are that they are usually geographically and ecologically widespread, different and distinct forms might be recognizable and present in one area, but in other localities different, related forms occur, which are likely closely related but morphologically distinct 'satellite' species/forms, but also there might be similar variants in widely separate localities with a similar ecology, and these might appear to be polytypic in origin. Generally, many synonymous names are the result (Barbosa, 2012; Henderson, 2020). Morphotypes are not named, not only because of their high number, but also because of intermediate forms and because it seems that several of these morphotypes may be polytypic in origin (Henderson, 2020). Species included in the species complex show a huge diversity of forms, but intermediate forms are also seen. The complex contains:

C. acuminatus Becc., *C. amplijugus* J.Dransf., *C. congestiflorus* J.Dransf., *C. corrugatus* Becc., *C. elopurensis* J.Dransf., *C. hypertrichosus* Becc., *C. impar* Becc., and *C. tenompokensis* Furtado.

Calamus javensis was described by Blume (1847) based on specimens collected by Blume himself from West Java, he already added his *C. equestris* Blume to the synonymy of *C. javensis*. Based on the shape of the leaflets and the state of the spines on the leaf midrib, Blume (1847) divided *C. javensis* into 2 varieties, namely var. *firmus* and var. *tetrasticus*. Since then, many varieties and subvarieties have been described and recognized by Beccari (1908, 1913), Ridley (1925), Furtado (1956), Dransfield (1999) and Mogea (1996). An overview of all recognized taxa can be found in Chapter 2.

1.5. Approaches to understand the complex species

Problems of infraspecific variation in plants have been commonly addressed by multivariate statistical techniques (Chandler & Crisp, 1998; Loo et al., 2001; Gengler-Nowak, 2002; Henderson & Martins, 2002; Knudsen, 2002; Bacon & Bailey, 2006; Henderson, 2006). The morphological approach is usually the initial attempt to solve the problem of polymorphism, whereby generally morphometric analysis in combination with extensive traditional morphological examinations is thought to be able to solve the circumscription of taxa. Some of the commonly used methods in morphometric analysis are multivariate analyses, such as Cluster Analysis (CA) and Principal Component Analysis (PCA). In some cases, these methods have been proven to help solve problems in systematic research based on herbarium specimens (Henderson, 2006). Tasrif et al. (2004) used CA to describe the variation patterns in the grass *Echinochloa crus-galli* (L.) P.Beauv. var. *crus-galli* in different ecotypes in Malaysia and Indonesia, and they concluded that in addition to genetic factors, the environment plays a role in the expression of the grass phenotype. Within the palms, morphometric analyses have also been frequently carried out. In the palm subfamily Calamoideae, McClatchey (1996) conducted some preliminary morphological analyses in *Metroxylon* Rottb. sect *Coelococcus* Becc.; the resulting taxa were then used for a cladistic analysis in a further study (McClatchey, 1998). The first to carry out a multivariate analysis in *C. javensis* was Madulid (1981), using CA and PCA to classify the species using 35 leaf characters from 125 herbarium specimens. The results of the grouping did not show clear boundaries between groups of specimens. This is caused by an error in the weighting factor (scoring) of the type of variable used. A CA and PCA analysis of 39 morphological characters of 59 herbarium specimens were used by Rustiami (1999) to study a unique 'dragon blood' group in the genus *Daemonorops* Blume section *Piptospatha* Becc. Furthermore, Mogea (1999) managed to identify 4 groups within *Arenga* Labill. ex DC. based on 51 qualitative morphological characters from 22 *Arenga* species using CA. A PCA on 23 morphological characters was used to see the distribution of variation in *C. javensis* and it showed ecotypes on Java, Sumatra and Borneo (Atria, 2009). In his revision work on *Calamus*, Henderson (2020) treated the 11 preliminary species in the *C. javensis* complex as an ochlospecies, with 17 variables treated as traits.

Morphological features alone are inadequate for studying genetic polymorphism, owing to their limited representation of the total genome and the high degree of environmental modification to which they are susceptible (Forrest et al., 2000). In his treatment in *Geonoma* Willd., Henderson (2011) concluded that the infraspecific circumscriptions in *Geonoma* are taxonomically difficult and cannot easily be understood with only morphological data based on herbarium specimens. In his monograph on *Geonoma* Henderson (2011) combined a multivariate approach with a phylogenetic analysis to revise the genus. *Geonoma* is widely distributed from southern Mexico to Bolivia and Paraguay to the Lesser Antilles and Hispaniola. Th phylogenetic study resulted in the recognition of 68 species, a further analysis on traits, geographic distribution, and quantitative variables resulted in the recognition of 90 subspecies in 18 of the species (giving a total of 148 taxa).

Studies on the taxonomy and systematics of palms have progressed tremendously in recent years with a notable increase in the diversity of approaches taken. From earlier advances in anatomy, palynology, and phytochemistry, the past 20 years have witnessed the inclusion of molecular (i.e., DNA) data in evolutionary studies of palms and an increase in our understanding of relationships at all taxonomic levels in the family (Uhl et al., 1995). Molecular phylogenetics at the species level have been conducted in all subfamilies of palms using a wide range of techniques The first of these employed chloroplast restriction site surveys in the southeast Asian genus *Caryota* L. (Hahn & Systsma, 1993).

Different nuclear DNA and Plastid DNA regions have been used in palm phylogenetics. Research using multicopy and low-copy nuclear ribosomal DNA include 18S gene (Hahn, 2002a), ITS between 18S and 26S including 5.8S gene (Baker et al., 2000a, 2000b) and the 5S gene (Barrow, 1998, 1999; Baker, 2000c). The use of plastid DNA has a limitation in resolving the relationships between closely related species (Asmussen, 1999a, 1999b, Asmussen et al., 2000; Baker et al., 1999a; Hahn, 2002b; Lewis & Doyle, 2002; Gunn, 2004; Roncal et al., 2005; Baker et al., 2000b; Thomas et al., 2006; Dransfield et al., 2008).

The 5S nuclear DNA gene was used to resolve polymorphism issues on tribe, generic and species level, and speciation can be detected with 5S nrDNA due to the high and diverse sequence variability (Scoles et al., 1988; Vakhitov & Nikonorov, 1989; McIntyre et al., 1992; Crisp et al., 1999; Poczai & Hyvönen, 2010; Baker et al. 2000b). The 5S unit of the rDNA is organized in repetitive tandem arrays, each separated by a non- transcribed region (NTS). Each 5S DNA unit is composed of a gene region of 120 bp long and a spacer of 100–700 bp long. In plants, the total number of repeats (gene + spacer) per genome varies from less than 1,000 to over 100,000 (Schneeberger et al., 1989; Sastri et al., 1992; Baum & Apples, 1992; Cronn et al., 1996; Crisp et al., 1999; Baker et al., 2000b; Rebordinos et al., 2013; Baum et al., 2015). The intergenic spacer evolves rapidly, and so has the potential to resolve phylogenetic relationships at low taxonomic levels, also among populations and species. The 5S spacer sequences have proven to be informative in groups like *Gossypium* L. (Malvaceae; Cronn et al., 1996), *Acacia* Mill. (Fabaceae; Playford et al., 1992), *Pinus* L. (Pinaceae; Moran et al., 1992), tribe Triticeae (Poaceae; Baum & Appels, 1992; Kellogg & Appels, 1995) and *Eucalyptus* L'Hér. (Myrtaceae; Udovicic et al., 1995). In palms, analyses using nuclear 5S nontranscribed regions were performed on the species level in *Phoenix* L. (Coryphoideae; Barrow et al., 1999) but with limited success. Complicated stretches of repetitive DNA are often found in these spacers (Udovicic et al. 1995; Kellog & Apples, 1995; Hahn, 1999). Ehara et al. (2019) studied phylogenetic relationship of *Metroxylon* Rottb. palms in Southeast Asia and Oceania based on 5S nrDNA spacer sequence data. Baker et al. (2000b) reported the molecular phylogenetics of *Calamus* and related rattan genera based on 5S nrDNA spacer sequence data.

Plastid DNA sequences were useful in resolving relationship at low taxonomic level. The chloroplast DNA (cpDNA) restriction-site variation appeared to be well-suited for studies of genetic relationships at or below the family level (Asmussen & Chase, 2001; Hahn, 2002; Guzman & Vargas, 2005; Roncal et al., 2009; Abbas et al., 2009; Bieniek et al., 2015; Pastore et al., 2017). The cpDNA has already been used often for reconstructing the phylogeny of palm groups. Asmussen et al. (2006) used the plastid DNA regions trnL-trnF, rps16 intron, rbcL and matK to resolve subfamilies within the palms. The phylogenetic analysis of tribe Chamaedoreeae (Arecaceae) has been studied using parsimony and Bayesian analyses of plastid DNA sequences (matK, rps16 intron, 3′ region of ndhF, and trnD-trnT; Cuenca & Asmussen, 2007). Baker et al. (2000a) investigated the relationship among 22 genera in the subfamily Calamoideae using nrDNA ITS and cpDNA rps16 intron sequence data.

1.6. Aims and scope of the thesis

This thesis discusses and looks at problems existing in *Calamus javensis*, which is a complex species, via various approaches.

The major research question is whether *C. javensis* is a 'big' single but variable species that has a very diverse morphological variation, or that the *C. javensis* complex consisting of cryptic species. This research question will be answered through several research objectives, namely:

1. To analyse the character distributions within the species complex using overall morphological characters that can seemingly be used to identify smaller units. A morphometric approach is used to obtain the best results.
2. To see if the *Calamus javensis* complex can be subdivided in monophyletic, recognisable units, which may corroborate the results of the morphometric study.
3. To see if recognisable forms are the result of historical geographic patterns.
4. To present identification keys, descriptions and notes of all recognisable forms, varieties and species included in the *Calamus javensis* complex.

The first approach was morphological, as well alpha-taxonomical as numerical/morphometric, to see if discontinuous character states existed that could break up the complex species into separate taxonomic units. Independantly, Henderson (Henderson, 2020) revised the genus *Calamus* and his results already made major changes to the position of *C. javensis*. However, Henderson included the results of the morphometric analysis (Atria et al., 2017) presented in this thesis by recognising *C. tenompokensis* as a separate species, next to *C. javensis*.

The various approaches to tackle the morphological and molecular variation in *C. javensis* are divided over the various chapters.

In Chapter 2 a multivariate morphological analysis examines the possible existence of gaps in morphology that can be used to distinguish forms as separate entities. The complex comprises much morphological variation, which is difficult to comprehend with a traditional descriptive approach. Two different machine learning algorithms were used, unsupervised and supervised learning, to allow for two different perspectives on the problem. Classification and clustering of the datasets was obtained with Multidimensional Scaling (MDS) to overview the distribution of the variation within the *C. javensis* complex, and Hierarchical Cluster Analysis (HCA) using an unweighted cluster method (UPGMA = Unweighted Pair Group Method with Arithmetic Mean).

In Chapter 3, a molecular approach is used to see if the various forms are phylogenetically distinct, i.e, form monophyletic groups. The phylogeny is compared with the results of the morphometric analyses, to see if they corroborate each other. Two markers were used, namely the 5S spacer (5S nrDNA) and the chloroplast Maturase K (matK). The sequences, after alignment, were analysed via a Bayesian algorithm to obtain as much resolution as possible.

In Chapter 4 the historical geographic situation is analysed via three methods: parsimony-based S-DIVA (Statistical, DIspersal-VIcariance analysis), maximum likelihood-based DEC (Dispersal Extinction Cladogenesis), and S-DEC (Statistical DEC). The results show that several morphological forms are geographically coherent, other forms are likely the result of similar ecological conditions in non-related populations.

In Chapter 5 a summing up is provided of the various forms that are morphologically recognisable, whether they are species, subspecies or non-related ecological variants. Detailed descriptions of all species and forms are provided together with an identification key.

In conclusion the complex can be divided into two species, *C. tenompokensis* and still a very variable *C. javensis* with one recognisable variety *C. javensis* var. *polyphyllus*.

Chapter 2

Morphometric Analysis of the rattan *Calamus javensis* Complex (Arecaceae, Calamoidae)

Mega Atria, Harald van Mil, William J. Baker, John Dransfield, Peter C. van Welzen

Systematic Botany (2017), 42(3): pp. 494–506.

Abstract -- *Calamus javensis* (Calamoideae) is a slender rattan common in tropical rainforests. The species is very polymorphic and forms a species complex together with some taxonomically still non-recognized forms and the species *C. acuminatus*, *C. amplijugus*, *C. congestiflorus*, *C. corrugatus*, *C. elopurensis*, *C. hypertrichosus*, *C. impar* and *C. tenompokensis*. Within the complex the morphological variation and similarities among the entities are too difficult to solve with traditional morphological observation. Therefore, two multivariate analyses, MDS (Multidimensional scaling) and Hierarchical Cluster Analysis (HCA) with UPGMA (Unweighted Pair Group Method with Arithmetic Mean) were used for two datasets. The inclusion of categorical characters did not make significant differences in the cluster results. Of all 9 taxa included in the complex, only 2 clusters resulted from the analysis next to two forms based on few specimens. Only *C. tenompokensis* was recognized as a separate cluster, while all other taxa were combined into one typical *C. javensis* cluster. More material may result in the recognition of the two forms as distinct species.

2.1. Introduction

Calamus javensis Blume is an understorey rattan belonging to the subtribe Calaminae, tribe Calameae of the palm subfamily Calamoideae (Dransfield et al.

2008). This slender, generally sparsely spiny, climbing palm is widely distributed in South-East Asia, ranging from Southern Thailand and Peninsular Malaysia, to Sumatra, Java, Borneo and Palawan. Within the genus *Calamus*, *C. javensis* is known as an extremely polymorphic species (Dransfield, 1992). Characters are (most typical ones in italics): stem without leaf sheath 2–6 mm in diameter; with sheaths to 10 mm in diameter; internodes up to 30 cm long but usually shorter; *ocrea conspicuously deep crimson when young*; leaves pinnate, ecirrate, up to 40 cm long; *terminal leaflets flabellate, lowermost pair often swept back across the stem*; flagellum up to 75 cm long; *inflorescence long, with red crimson rachilla*; ripe fruit ovoid (Dransfield, 1992; Dransfield and Manokaran 1993).

People make bindings, rope and baskets from the cane. The fruits are edible for small mammals and a few birds. The raw cabbage can be eaten and is sometimes used to cure coughs. In Sarawak the cane is considered excellent because of its length and strength, but it is only sold locally for baskets, cordage and handicrafts. In Palawan, the cane is deemed only second in quality to *Calamus caesius* Blume (a well-known species in the rattan trade) (Mogea, 1994; Shim & Tan 1994). According to the FAO, *C. javensis* is included in the major commercial species of rattan as identified for Asia by Dransfield and Manokaran (1993). Then *C. javensis* may become a new alternative rattan source for industrial needs. However, the fact that *C. javensis* is a polymorphic species complex with variable quality of the cane makes it less valuable unless the complex is taxonomically resolved.

Calamus javensis was first described by Blume (1847). He transferred his own *Calamus equestris* into the synonymy of *C. javensis*. He also noted morphological variation within *C. javensis* and distinguished 3 varieties based mainly on the variation in the leaves and spines on the leaf sheaths (Table 2-1; var. α is considered to be the typical variety and should according to modern nomenclature be called var. *javensis*). Beccari (1884, 1908) recognized Blume's varieties and made modifications, creating his own infraspecific taxonomy. Since then (Table 2-1), many changes in the infraspecific taxa occurred, forms were united, separated, etc. Also, on the species level many exceptional forms were described as new species and synonimized again, the latter especially by Dransfield (1992, 1999), who mainly recognized one species without infraspecific taxa. As a result, *C._javensis* (Dransfield, 1999) is taxonomically the most difficult rattan species complex in the Southeast Asian region. Table 2-1 shows that no subdivision of the complex provided a satisfactory solution, forms were described at various levels or moved to different levels, which also shows that the characters used for recognition were doubtful too. Madulid (1981) treated *C. javensis* as a species complex because of the extreme variation within the species and the relatively subtle differences among morphologically similar groups of specimens. To solve the problem, Dransfield (1999) suggested 2 options, either maintaining several varieties or blend them all into one species. Alternatively, add comprehensive studies in the field next to phenetic and molecular-phylogenetic techniques to examine the circumscription of *C. javensis*.

In species complexes variation is great often showing complex patterns of disjunctions in the ranges of variation (often recognised by separate names), some of which can be linked to ecological factors or geography, others being apparently random. Subsuming all variation under one name results in loss of information. In Java, where the type was originally collected, the species is not very variable (Dransfield, 1999). In West Java, *C. javensis* exhibits a more homogeneous form, though forms with smaller leaflets and shorter rachillae are among the specimens collection from Ujung Kulon (Atria, 2008, unpublished). Variation in Sumatra is similar to that in Peninsular Malaysia (Dransfield, 1999). In the Malay Peninsula, many intermediates are named as varieties. Dransfield (1979) included all names in *C. javensis*. In Sabah (N Borneo) variation is more discontinuous, certain populations are rather distinct, but there appear to be intermediates forms. One particular distinct form is a non-climbing plant on ridge tops above Tenom (Sabah), it has narrow, lanceolate leaflets, like *C. acuminatus* Becc., but the fruit is ovoid just like the other populations of *C. javensis* (Dransfield, 1994). In Sarawak (NW Borneo), on Mount Mulu, a plant was found to resemble *C. tenompokensis* Furtado, but the leaflets are much smaller and have a different texture and the inflorescence is much smaller (Dransfield, 1992).

Problems of intraspecific and infraspecific variation in plants have been commonly addressed by multivariate statistical techniques (Chandler & Crisp, 1998; Loo et al., 2001; Gengler-Nowak, 2002; Henderson & Martins, 2002; Knudsen, 2002; Bacon & Bailey, 2006; Henderson, 2006). In the *C. javensis* complex, Madulid (1981) analysed the leaf variation using Principal Components Analysis (PCA), which resulted in a few discernible groupings. However, Madulid also showed a continuous range in leaf variation. A re-appraisal of *C. javensis* is necessary to clear and put into order its present chaotic taxonomic status. "Morphological characters of plant parts other than the leaves need to be thoroughly examined for more taxonomic evidence that could lead to a better understanding of the true nature of the *C. javensis* complex" (Madulid, 1981). A preliminary study of *C. javensis*' morphological variation over its distribution was done by using PCA; the results showed that geographic variation in morphology exists, which probably reflects phenotypic responses to environmental gradients (Atria, 2008, unpublished), but this study did not cover all forms in the distribution and all named taxa within the *C. javensis* complex.

In the present study, the selected morphological characters will be analysed using multivariate analyses to see the character distribution in the *C. javensis* complex and to investigate the distinctiveness of some of the character combinations to recognize (some) forms as distinct taxa.

The objectives of this study are: (1) to analyse the character distribution within the species complex using overall morphological characters; (2) to characterize probable characters that significantly influence the species complex. With the right selection of characters and the availability of adequate material throughout the distribution area, it is expected that the distribution of morphological variation in the *C. javensis* complex in West Malesia becomes clearer. The result of this study will be used as a preliminary for the molecular examination of the species complex in order to construct a comprehensive classification of the *C. javensis* complex.

2.2. Material and Methods

Herbarium Specimens

From a total of 226 herbarium specimens, we selected 177 as a representative subset for multivariate statistical analyses; 86 of these were from northern Borneo, where the greatest variation occurs. The specimens of *C. javensis* and related taxa were selected from three herbaria, which store the best and most variable collections: Herbarium Bogoriense (BO), Leiden (L) and Kew (K). Photo material of types of varieties was received from Herbarium Firenze (FI), and particular forms were examined in two Bornean herbaria, Sandakan (SAN) and Kuching (SAR). Type

Table 2-1. The chronological history of names and taxa involved in the Calamus javensis Blume species complex (adapted from Madulid, 1981).

Blume (1847)	**Beccari & Hook.f. (1892)**	**Ridley (1907)**	**Beccari (1908, 1913)**	**Ridley (1925)**	**Furtado (1956)**	**Dransfield (1984, 1992)**
C. javensis	*C. javensis*	*C. penicillatus*	*C. javensis*	*C. javensis*	*C. javensis*	*C. javensis*
var. α	'forma *typica*'					(Dransfield & Manokaran, 1993; Govaerts & Dransfield, 2005)
var. β (*firmus*)						
		var. inermis		var. *inermis*	var. *inermis*	
					var. *laevis*	
	var. *peninsularis* subvar *purpurascens*	var. *purpurascens*	var. *peninsularis* subvar *purpurascens*	var. *purpurascens*	var. *peninsularis*	
					var. *purpurascens*	
	subvar. *tenuissimus*		var. *tenuissimus*		var. *tenuissimus*	
	subvar. *pinangianus*	var. *pinangianus*	var. peninsularis	var. *pinangianus*	var. *pinangianus*	
			var. pinangianus			
	subvar. polyphyllus		var. *polyphyllus*		var. *polyphyllus*	
			var. *intermedius*			
var. γ (*tetrastichus*)			var. *tetrastichus*			
			subvar. *mollispinus*			
			var. *sublaevis*			
			var. *acicularis*			
			var. *exilis*			
					C. kemamanensis	
			C. corrugatus			*C. corrugatus*
			C. filiformis			

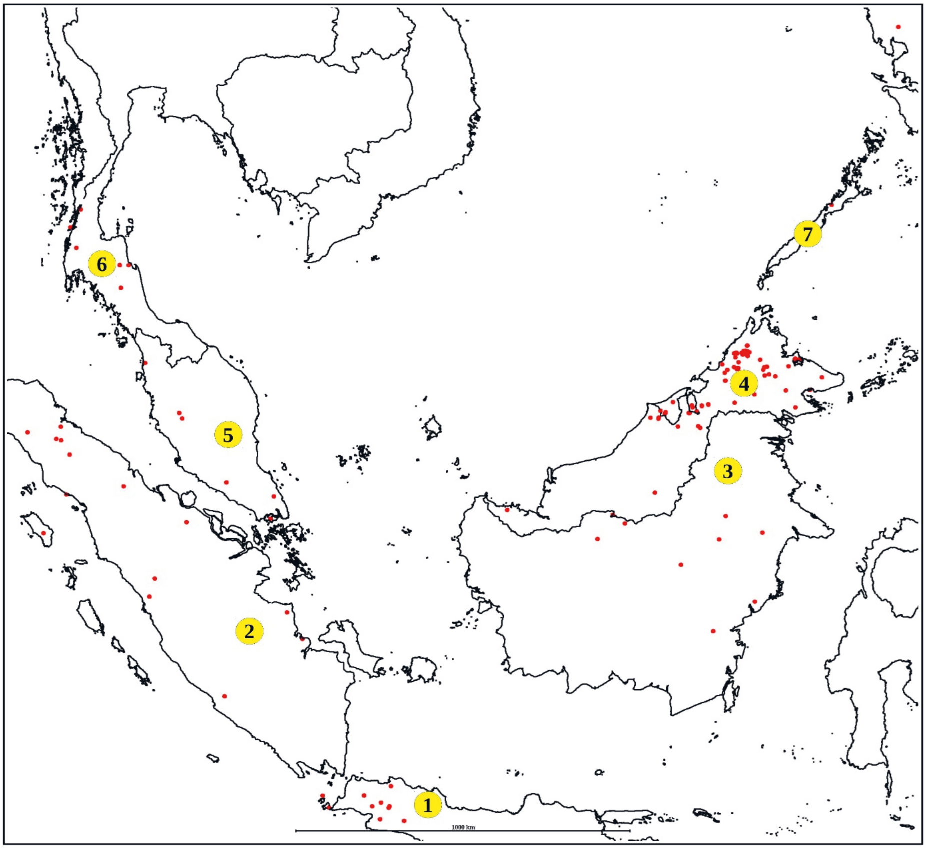

Figure 2-1. Samples locality and collection sites. 1=West Java, 2=Sumatra, 3=Kalimantan, 4=Northern Borneo, 5=Malay Peninsula, 6=Thailand, 7=the Philippines.

specimens were included except for Beccari's varieties as the photo material was unsuitable for measurements. Representative herbarium specimens were also collected during fieldwork in West Java and several parts of Sabah and Brunei. Collection and preparation followed the standard procedure by Dransfield (1986). Fertile and sterile specimens were both collected to represent the distributions of all forms. Sample localities and collection sites are shown in Figure 2-1.

Representatives of as many as 9 taxa that are generally accepted (Blume, 1847; Beccari, 1908; Furtado, 1956; Dransfield, 1984; Dransfield, 1992; Govaerts & Dransfield, 2005) were included in this study: *Calamus acuminatus* Becc., *C. amplijugus* J.Dransf., *C. congestiflorus* J.Dransf., *C. corrugatus* Becc., *C. elopurensis* J.Dransf., C. *hypertrichorus* Becc., *C. impar* Becc., *C. javensis* Blume and *C. tenompokensis* Furtado (see also Table 2-1).

Morphometry was conducted according to Rohlf (1990), Chandler & Crisp (1997), Loo et al. (2001) and Henderson (2006). Specimens were not assigned to groups a priori and each herbarium collection was considered as an individual Operational Taxonomic Unit (OTU) in tabulating the data matrix (Loo et al., 2001). Multiple herbarium sheets from a single collection were grouped into a single OTU. The characters were chosen based on the differences among taxa. Thirty-two quantitative and qualitative characters, like sizes, shapes and textures of vegetative and floral parts, were measured using a ruler, measuring tape and calliper (Table 2-2) (Gengler-Nowak, 2002; Henderson & Martins, 2002; Sreekumar et al., 2006). Missing values were left blank in the matrix. The measurements derived from observations on herbarium specimens were assigned to a matrix. The 32 characters by 177 Operational Taxonomic Units (OTUs) data matrix was then analysed using multivariate analyses.

Multivariate Analyses

All statistical analyses were performed in the statistical computing environment R (R Core Team, 2016). Missing values were imputed using Multiple Imputation by Chained Equation (MICE) algorithms provided by the MICE package (van Buuren & Groothuis-Oudshoorn, 2011). The default in the MICE package for R is k=5, “k” is how many cases should be in each match set. That is, each case with missing data on x is matched to 5 cases (with data present) that have the closest predicted values (Allison, 2015). To investigate the effect of categorical variables on the classification problem, we made a second dataset without categorical data (qualitative characters).

We used two types of statistical learning algorithms, unsupervised and supervised to allow for two different perspectives on the problem. Multidimensional Scaling (MDS) was applied to overview the distribution of the variation within the *C. javensis* complex. MDS has become a popular technique for both multivariate and exploratory data analysis (Krauss, 1996; Chandler & Crisps, 1998; Streiber et al., 1999; Wicklermaier, 2003). Additionally, we used Hierarchical Cluster Analysis (HCA) using an unweighted cluster method (UPGMA = Unweighted

Pair Group Method with Arithmetic Mean)).

The results presented in this paper were obtained using an Euclidean similarity measure for the numerical dataset and the Gower measure for the dataset with categorical data (Gower, 1971; Struyf et al., 1997). In the Hierarchical Cluster Analysis (HCA) this was combined with the average agglomeration measure for UPGMA. Other distance and agglomeration measures were applied to check their effect on the cluster analysis. To assess the uncertainty of the HCA we used a bootstrap method as implemented in the pvclust R package (Suzuki & Shimodaira, 2006).

To get another perspective on the data supervised methods of Classification Trees (CT; Breiman et al., 1984; De'ath, 2002; Hothorn et al., 2006) and Random Forest learning algorithm (RF; Breiman, 2001; Liaw & Wiener, 2002; Cutler et al., 2007) were used to see how well these algorithms predicted the classifications done by experts. Here we draw on a much used metaphor used in the context of tree methods like CT and RF; the resulting decision trees and community of decision trees are seen as a virtual expert (CT) or community of experts (RF). Confusion matrices and cross validation error were used to evaluate quality of the classification procedure where confusion is equated as the lack of consensus between experts.

Table 2-2. List of morphological characters, states and units of measurement used in the study.

No	Character (names and abbreviation)	States / Measurem/ents / Type of data	Vegetative/ Generative	remarks
1	Stem diameter with sheath (LS diam)	Cm / numeric / Continuous	Vegetative	
2	Stem diameter without sheath	Cm / numeric / Continuous	Vegetative	
3	Leaf sheath spine density (spinedens)	(0) no spine; (1) sparse; (2) moderate; (3) dense; (4) very dense / discrete / semi-continuous	Vegetative	used in the analysis
4	Spine shape (Spinshap)	(0) tape; (1) swollen-based; (2) broad swollen-based short; (3) swollen-based pointing; (4) clawed; (5) no spines / discrete	Vegetative	used in the analysis
5	Ocrea (Ocrea)	(0) none, (1) obvious (<2 cm), (2) conspicuous (2 cm) / categorical	Vegetative	used in the analysis
6	Knee (Knee)	(0) none, (1) obvious, (2) conspicuous	Vegetative	used in the analysis
7	Leaf length	Cm / numeric / Continuous	Vegetative	used in the analysis

No	Character (names and abbreviation)	States / Measurem/ents / Type of data	Vegetative/ Generative	remarks
8	Petiole length (Petiolength)	Cm / numeric / Continuous	Vegetative	used in the analysis
9	Leaflet pair number including flabellate pair (Leafpair)	Count / numeric / Discrete	Vegetative	used in the analysis
10	Leaflets uppermost length (Upperlength)	Cm / numeric / Continuous	Vegetative	used in the analysis
11	Leaflets uppermost width (Upperwidth)	Cm / numeric / Continuous	Vegetative	used in the analysis
12	Leaflets penultimate length (Penlength)	Cm / numeric / Continuous	Vegetative	used in the analysis
13	Leaflets penultimate width (Penwidth)	Cm /numeric / Continuous	Vegetative	used in the analysis
14	Leaflets median length (Medlength)	Cm / numeric / Continuous	Vegetative	used in the analysis
15	Leaflets median width (Medwidth)	Cm / numeric / Continuous	Vegetative	used in the analysis
16	Leaflets lowermost length (Lowlength)	Cm / numeric / Continuous	Vegetative	used in the analysis
17	Leaflets lowermost width (Lowwidth)	Cm / numeric / Continuous	Vegetative	used in the analysis
18	Prophyll armature (Propharm)	(0) smooth; (1) prickly; (2) spiny; (3) with indument / Categorical	Generative	used in the analysis
19	Peduncular bracts shape (Pedbrashape)	(0) loose; (1) tightly sheathing; (2) tightly sheathing with limb; (3) tightly sheathing with very long limb/ Categorical	Generative	used in the analysis
20	Rachis bracts armature (Rachbracharm)	(0) smooth; (1) prickly; (2) spiny; (3) with indument / Categorical	Generative	used in the analysis
21	Number of partial inflorescences (cluster)	Count / numeric / Discrete	Generative	
22	Cluster length (the length of the partial inflorescence)	Cm / numeric / Continuous	Generative	
23	Number of rachillae	Count / numeric / Discrete	Generative	used in the analysis
24	Rachilla length (Rachilleng)	Cm/ numeric/ Continuous	Generative	
25	Inflorescence length	(0) short (<1 m); (1) long (>1 m) / Categorical	Generative	
26	Prophyll length	(0) short (<10 cm); (1) long (>10 cm) / Categorical	Generative	
27	Leaflet blade texture	(0) glabrous; (1) hairy / Categorical	Vegetative	
28	Stem habit (Stem)	(0) climbing; (1) short erect / Categorical	Vegetative	
29	Pistillate flower bract texture	(0) smooth; (1) striate; (2) prickly / Categorical	Generative	
30	Pistillate flower bract shape	(1) loose; (2) tight / Categorical	Generative	
31	Staminate flower bract texture	(0) smooth; (1) striate; (2) prickly / Categorical	Generative	
32	Staminate flower bract shape	(1) loose; (2) tight / Categorical	Generative	used in the analysis

2.3. Results

The results of different types of analysis are shown, starting with the results of (alpha-) taxonomic comparisons of the specimens, field observations and finally continued by multivariate results.

Morphological Observations

The observations and examination of the *C. javensis* complex resulted in several tentative form groups, form 1–4 next to various forms recognized as species (Table 2-3), whereby several groups are represented by a very limited number of specimens. Two of Beccari's species, *C. corrugatus* and *C. hypertrichosus*, were grouped with the typical *C. javensis*. The two species have a close resemblance with typical *C. javensis*. Of *C. hypertrichosus* only a single specimen was observed, which had one very distinctive character, white hairy leaflets and leaf sheaths. In *C. corrugatus* intermediate specimens showed that the ring-like corrugated/wrinkled leaf sheath is variable and not distinctive, it ranges between a half circle to a full ring-like, corrugated sheath.

Detailed observations from field and herbarium examinations showed that members of the *C. javensis* complex exhibit a wide range of morphological variation. Intermediates were identified, especially among the very diverse specimens from Northern Borneo. Specimens were collected in an altitude range of 20–2,000 m above sea level. Fieldwork showed the overlapping occurrence of two taxa, *C. javensis* var. *polyphyllus* and *C. acuminatus*, and other growth forms together in the same place with typical *C. javensis*.

Variable characters observed in the *C. javensis* complex are:

◊ Stem and leaf sheath. All members of the *C. javensis* complex are clustering rattans, which climb with the flagella except for *Form 3* and *C. tenompokensis*, which are short-stemmed, erect rattans (acaulescent). The stem diameter, including the sheaths, varies from 2–30(–35) mm in diam. with 5–10 mm as common diameter found. The smallest stem diameter belongs to *C. javensis* and sturdier stems belong to *C. tenompokensis.*

◊ The spines on the leaf sheaths differ in size, shape and density. The shape varied from small, clawed to triangular flat, short to long pointed, with mostly a swollen base. Almost all specimens are spiny, with scattered to many, slender

Table 2-3. Groups distinguished in the Calamus javensis complex based on morphological observations.

No	Taxa	No of Specimens	Remarks	Distribution
1	*Calamus amplijugus*	10	Broadly elliptic leaflets, rachilla very long up to 25 cm	Brunei, Malaysia: Sabah
2	*Calamus congestiflorus*	6	Short and congested rachilla	Indonesia: East Kalimantan, West Java; Malaysia: Sabah
3	*Calamus elopurensis*	4	Leaflets in 2 or 3 pair rosette to the terminal, Ocrea conspicuous, tubular triangle limb, reddish, up to 6 cm, petiole long, c. 9-15 cm, floccose, sometimes abundant.	Malaysia: Sabah
4	*Calamus impar*	9	Leaflets 2 pairs, deeply bilobed flabellate pair, ocrea short-truncated	Indonesia: East Kalimantan, West Java; Malaysia: Sabah; Thailand: Phang Nga
5	*Calamus javensis* (typical)	89	ocrea conspicuously deep crimson when young; leaflets 4-7 pairs, terminal leaflets flabellate, lowermost pair often swept back across the stem; inflorescence long, with red crimson rachilla; ripe fruit ovoid	Indonesia: West Java, Sumatra, Kalimantan; Southern Thailand, Malaysia: Malay Peninsula, Sabah, Sarawak.
	Calamus corrugatus	6	Leaf sheath glabrous with corrugated horizontal lines.	Indonesia: Central Kalimantan Sarawak
	Calamus hypertrichosus	1	Leaf sheath glabrous, leaflets with abundant hairs on both surfaces.	Sarawak
6	*Calamus javensis* var. *polyphyllus* + *Calamus acuminatus*	15 + 13	Leaflets 8-12 pairs, apetiolate to short petiolate	Brunei, Indonesia: Sumatra, Malaysia: Peninsular Malaysia, Sabah, Sarawak. Sabah: Tenom, Keningau
7	*Calamus tenompokensis*	10	Stem short, moderate stem with robust spines, flagellum absent, inflorescence short,	Malaysia: Sabah, Sarawak
8	*Form 1*	2	Lack of flagellum and cirrus, short staminate inflorescence, warty rachilla bracts	Sarawak (Mt. Mulu), 1200-1500 m alt.
9	*Form 2*	1	Short setae along the veins and one long black setae abaxial main veins	The Philippines, Pampanga, Mount Arayat
10	*Form 3*	2	Leaflets only 2 or 3 pairs, tomentose	Malaysia: Sabah, Sarawak
11	*Form 4*	9	Ocrea rough and hirsute, pistillate inflorescence short	Indonesia: Sumatra, Malaysia: Sabah

to robust spines, but *C. acuminatus* specimens have an almost unarmed leaf sheath with only very few, scattered spines.

◊ Flagella are always present except in *Form 3* and *C. tenompokensis*, where, if present the flagellum is short, less than 1 m long. The flagella are armed with clawed spines, 2–5 arranged in a group. The tip of the spines is almost always black (black-tipped spine).

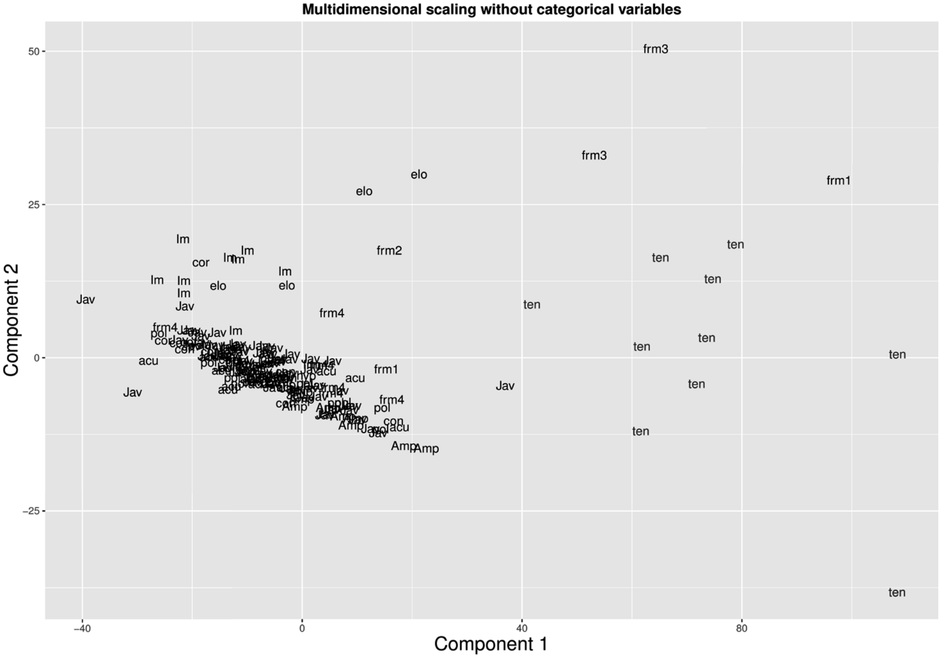

Figure 2-2. Clustering result of unsupervised method (MDS without categorical variables). acu = C. acuminatus, amp = C. amplijugus, con = C. congestiflorus, cor = C. corrugatus, elo = C. elopurensis, frm1 = Form 1, frm2 = Form 2, frm3 = Form 3, frm4 = Form 4, hyp = C. hypertrichosus, im = C. impar, jav = C. javensis, pol = C. javensis var. polyphyllus, ten = C. tenompokensis.

◊ The ocrea is tubular, dark red (maroon) and sometimes torn apart in older plant parts. Ocrea length varies from 0.5 to more than 2 cm. The ocrea is usually glabrous, but in *Form 4* it is hirsute with stiff hairs. In *C. elopurensis* it is prolonged on the far side to the petiole whereas in other forms the ocrea is prolonged next to the petiole.

◊ The leaves and leaflets show most variation. The **leaf** is pinnately compound and ecirrate with a length of 20 cm to more than 140 cm (*C. tenompokensis*). Mature plants usually have a very short petiole, but *C. javensis* var. *polyphyllus* and *C. acuminatus* have sessile leaves. Juvenile or young plants usually have a longer petiole than the older plants. In *C. elopurensis*, the petiole is quite distinctive from c. 9-15 cm long. *Calamus tenompokensis* has the longest petioles, 22–27(–60) cm long.

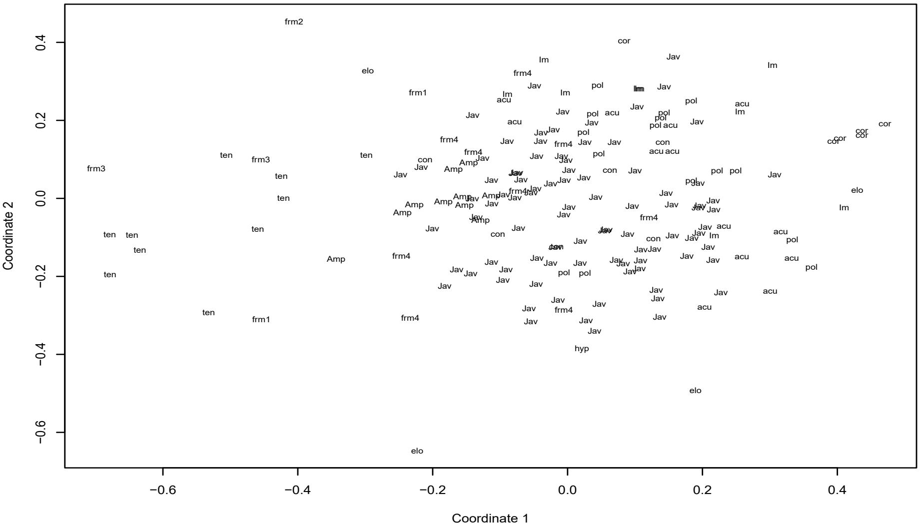

Figure 2-3. Clustering result of unsupervised method (MDS with categorical variables). acu = C. acuminatus, amp = C. amplijugus, con = C. congestiflorus, cor = C. corrugatus, elo = C. elopurensis, frm1 = Form 1, frm2 = Form 2, frm3 = Form 3, frm4 = Form 4, hyp = C. hypertrichosus, im = C. impar, jav = C. javensis, pol = C. javensis var. polyphyllus, ten = C. tenompokensis.

- ◊ Four distinct leaflet shapes are observed, elliptic, oblong, oblanceolate and lanceolate. The apices are usually acute, but can also be acuminate. The leaflets have black bristle apically and along the margins.
- ◊ Leaflets are in 2 (*C. impar*)–8(–12: *C. tenompokensis*, *C. acuminatus*, *C. javensis* var. *polyphyllus* and *Form 1*) pairs; most *C. javensis* specimens have 5–7 pairs.
- ◊ The uppermost leaflets are partially bifid and joined for 1/3–2/3 of their length. The basal or the lowest pair usually are the smallest leaflets, which vary from swept back across the stem (enclosing the stem) to spreading.
- ◊ The staminate and pistillate inflorescences are quite similar. The inflorescences are long and mainly pendulous, whereby the first part is erect followed by a slightly curved part and the longest, terminal part is hanging. The inflorescence

Table 2-4. Tree model confusion table. horizontal = actual taxon; vertical = predicted taxon. Abbreviation of taxa: acu = C. acuminatus, amp = C. amplijugus, con = C. congestiflorus, cor = C. corrugatus, elo = C. elopurensis, frm1 = Form 1, frm2 = Form 2, frm3 = Form 3, frm4 = Form 4, hyp = C. hypertrichosus, im = C.impar, jav = C. javensis, pol = C. javensis var. polyphyllus, ten = C. tenompokensis. Number indicates the number of OTUs (or collection specimens).

PREDICTED

ACTUAL	acu	amp	con	cor	elo	frm1	frm2	frm3	frm4	hyp	im	jav	pol	ten
Acu	4	0	0	0	0	0	0	0	0	0	0	1	8	0
amp	0	5	0	0	0	0	0	0	0	0	0	5	0	0
Con	0	0	0	0	0	0	0	0	0	0	0	6	0	0
Cor	1	0	0	0	0	0	0	0	0	0	1	4	0	0
Elo	0	0	0	0	0	0	0	0	0	0	2	0	0	2
frm1	0	0	0	0	0	0	0	0	0	0	0	0	1	1
frm2	0	0	0	0	0	0	0	0	0	0	0	0	0	1
frm3	0	0	0	0	0	0	0	0	0	0	0	0	0	2
frm4	0	0	0	0	0	0	0	0	6	0	0	3	0	0
Hyp	0	0	0	0	0	0	0	0	0	0	0	1	0	0
Im	0	0	0	0	0	0	0	0	0	0	9	0	0	0
Jav	1	4	0	0	0	0	0	0	1	0	2	81	0	0
Pol	3	1	0	0	0	0	0	0	0	0	0	2	9	0
Ten	0	0	0	0	0	0	0	0	0	0	0	1	0	9

length varies from 50 cm to more than 1m. Short and erect inflorescences are present in *C. tenompokensis* (less than 1 m long), *Form 1* and *Form 3*. Typical *C. javensis* has (2–5)–8 partial inflorescences; inflorescences bearing > 8 partial inflorescences can be found in *C. javensis* var. *polyphyllus*, *C. elopurensis*, and *C. amplijugus*.

◊ The prophyll is 7–30 cm long, closely sheathing, with spines, but some forms have an unarmed prophyll, or the prophyll is glabrous except for a spiny tip. Rachis bracts are mostly spiny or prickly, some are glabrous, others have hairs.

◊ The pistillate rachillae are borne on the 2nd order branches and staminate rachillae are on the 3rd order branches. Staminate and pistillate rachillae

Table 2-5. Confusion matrix of Random Forest analysis. acu = C. acuminatus, amp = C. amplijugus, con = C. congestiflorus, cor = C. corrugatus, elo = C. elopurensis, frm1 = Form 1, frm2 = Form 2, frm3 = Form 3, frm4 = Form 4, hyp = C. hypertrichosus, im = C. impar, jav = C. javensis, pol = C. javensis var. polyphyllus, ten = C. tenompokensis. Number indicates the number of OTUs (or collection specimens).

	PREDICTED													
ACTUAL	acu	amp	con	cor	elo	frm1	frm2	frm3	frm4	hyp	im	jav	pol	ten
Acu	4	0	0	0	0	0	0	0	0	0	0	6	3	0
amp	0	4	0	0	0	0	0	0	0	0	0	6	0	0
con	0	0	0	0	0	0	0	0	0	0	0	6	0	0
cor	0	0	0	4	0	0	0	0	0	0	0	1	1	0
Elo	0	0	0	0	3	0	0	0	0	0	0	1	0	0
frm1	0	0	0	0	0	0	0	0	0	0	0	1	0	1
frm2	1	0	0	0	0	0	0	0	0	0	0	0	0	0
frm3	0	0	0	0	0	0	0	2	0	0	0	0	0	0
frm4	0	0	0	0	0	0	0	0	5	0	0	4	0	0
hyp	0	0	0	0	0	0	0	0	0	0	0	1	0	0
Im	0	0	0	0	0	0	0	0	0	0	7	2	0	0
Jav	1	3	0	0	0	0	0	0	1	0	0	80	4	0
Pol	4	0	0	0	0	0	0	0	0	0	0	9	2	0
Ten	0	0	0	0	0	1	0	0	0	0	0	0	0	9

have the same color, red when mature and green in younger stages. Staminate rachillae have a more simple structure, one bracteole subtending one staminate flower along each side of the rachilla; while pistillate rachillae are more complex, with an arrangement in ’dyads’, with one rachilla bract subtending 2 flowers, each with its own bracteole, one fertile pistillate flower and one sterile staminate (which is usually already caducous when collected and leaving a scar next to the pistillate flower). The rachilla length in *C. javensis* varies from 4–8 cm in staminate inflorescences and is up to 12 cm long in pistillate inflorescences. The average length of the staminate rachilla is 5.5 cm and that of the pistillate rachillae 6.5 cm. In other forms the rachillae are 2–12 cm long; short and congested in *C. congestiflorus* with the rachillae only 2–4

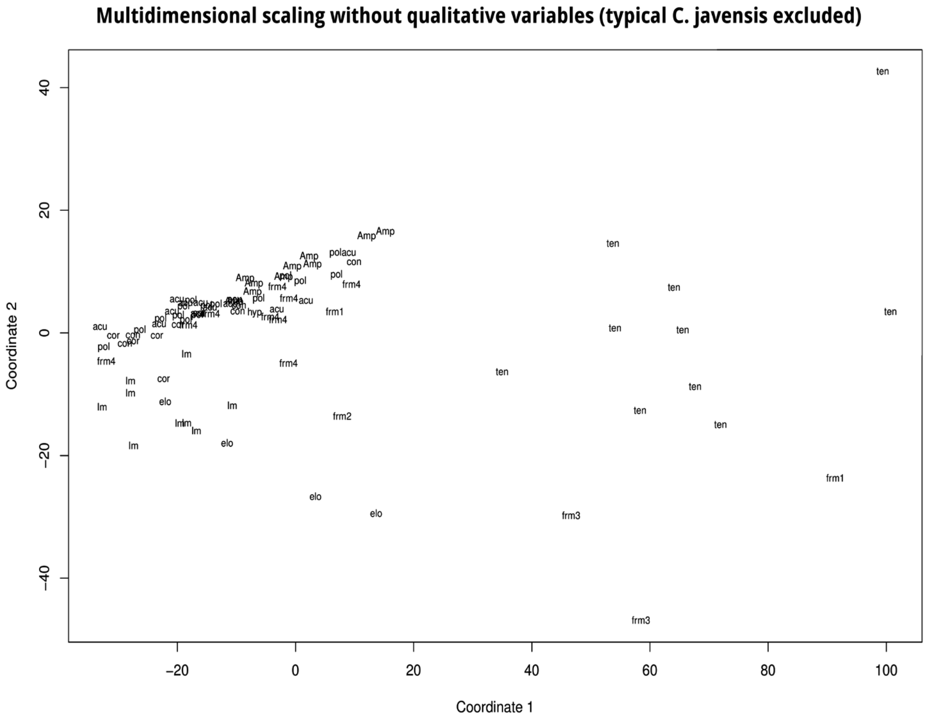

Figure 2-4. MDS clustering analysis without categorical variables and typical C. javensis excluded from the data matrix. Abbreviation of taxa: acu = C. acuminatus, amp = C. amplijugus, con = C. congestiflorus, cor = C. corrugatus, elo = C. elopurensis, frm1 = Form 1, frm2 = Form 2, frm3 = Form 3, frm4 = Form 4, hyp = C. hypertrichosus, im = C. impar, pol = C. javensis var. polyphyllus, ten = C. tenompokensis.

cm long, but up to 25 cm long in *C. amplijugus*.

Multivariate analysis

The dataset consisted of 177 OTUs with 32 characters. The dataset suffered from missing data, mostly in the inflorescence characters. Missing data were due to incomplete parts of the specimens, as can be expected, fruiting specimens have no floral characters, pistillate specimens no fruits or staminate floral characters, etc. To minimize the missing values, there was a pre-selection of variables based on the availability of observed characters. Twenty characters were selected, and the

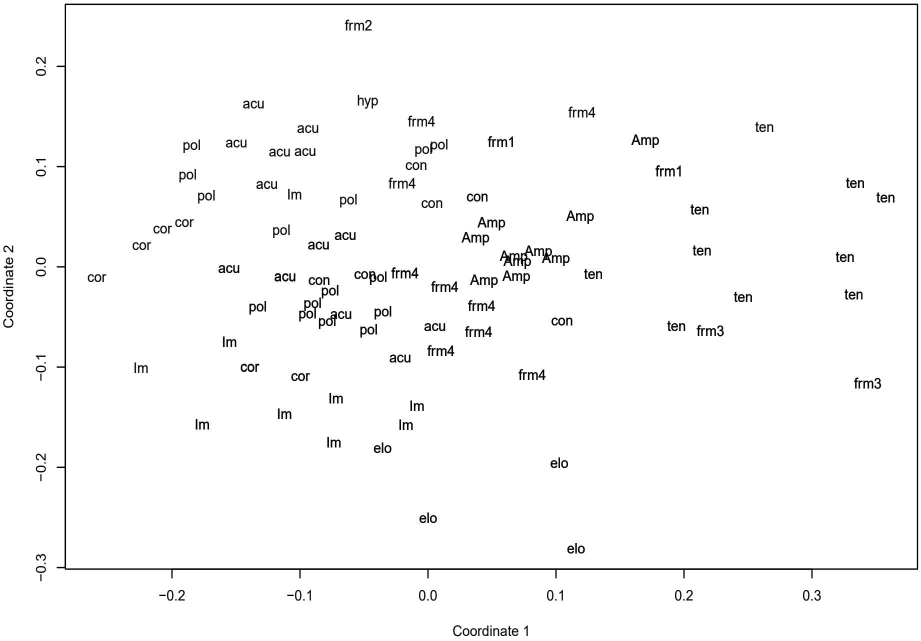

Figure 2-5. MDS clustering analysis with categorical variables, with typical C. javensis excluded from the data matrix. Abbreviation of taxa: acu = C. acuminatus, amp = C. amplijugus, con = C. congestiflorus, cor = C. corrugatus, elo = C. elopurensis, frm1 = Form 1, frm2 = Form 2, frm3 = Form 3, frm4 = Form 4, hyp = C. hypertrichosus, im = C. impar, pol = C. javensis var. polyphyllus, ten = C. tenompokensis.

remaining missing data were imputed using MICE (Table 2-2).

We made a comparison between two datasets: a dataset consisting only of numerical data (MICE + numerical data) and a dataset with numerical and categorical data (MICE + Numerical and Categorical data). Categorical data were included in order to show their influence on ordination and clustering.

Unsupervised methods were applied to both datasets, with and without categorical variables. Distance matrices were computed, using a Euclidean measure, and visualized using a heat map representation. Only one small cluster, consisting of *C. tenompokensis*, is observed in an otherwise homogenous heat map; using different distance measures did not affect this result. MDS produces

Table 2-6. Tree model confusion table for matrix without typical C. javensis. Abbreviation of taxon: acu = C. acuminatus, amp = C. amplijugus, con = C. congestiflorus, cor = C. corrugatus, elo = C. elopurensis, frm1 = Form 1, frm2 = Form 2, frm3 = Form 3, frm4 = Form 4, hyp = C. hypertrichosus, im = C. impar, jav = typical C. javensis, pol = C. J*avensis var. polyphyllus, ten = C. tenompokensis. Number indicates the number of OTUs (or collection specimens).*

PREDICTED

ACTUAL	acu	amp	con	cor	elo	frm1	frm2	frm3	frm4	hyp	im	jav	pol	Ten
Acu	4	0	0	1	0	0	0	0	0	0	0	0	8	0
Amp	0	10	0	0	0	0	0	0	0	0	0	0	0	0
Con	0	0	0	0	0	0	0	0	6	0	0	0	0	0
Cor	1	0	0	5	0	0	0	0	0	0	0	0	0	0
Elo	0	0	0	0	0	0	0	0	0	0	2	0	0	2
frm1	0	0	0	0	0	0	0	0	0	0	0	0	1	1
frm2	0	0	0	0	0	0	0	0	0	0	0	0	0	1
frm3	0	0	0	0	0	0	0	0	0	0	0	0	0	2
frm4	0	0	0	0	0	0	0	0	8	0	0	0	0	1
Hyp	0	0	0	1	0	0	0	0	0	0	0	0	0	0
Im	0	0	0	0	0	0	0	0	2	0	7	0	0	0
Jav	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pol	3	1	0	0	0	0	0	0	0	0	0	0	11	0
Ten	0	0	0	0	0	0	0	0	0	0	0	0	0	10

analogous results with a compact cluster for all OTUs except *C. tenompokensis* with goodness of fit measures of 0.876 (Figure 2-2).

When categorical variables are included, members of *C. tenompokensis* remain separate away from the main cluster; the main cluster included all other species/specimens but is less condensed; the goodness of fit of the non-metric fit is again high (0.928). We note, however, that the main cluster is partitioned into two regions that are related to the character ‘bract shape’ but OTUs representing the same species were present in both partitions (Figure 2-3).

Hierarchical clustering of both datasets gave similar results independent of the agglomeration method used: *C. tenompokensis* forming a separate cluster in the presence of *Form 1* and *3*. Bootstrapping the cluster tree indicated the *C. tenompokensis* cluster to be very robust with approximately unbiased p-values

Table 2-7. Confusion matrix of Random forest analysis for matrix data without typical C. javensis. Abreviation of taxon: acu = C. acuminatus, amp = C. amplijugus, con = C. congestiflorus, cor = C. corrugatus, elo = C. elopurensis, frm1 = Form 1, frm2 = Form 2, frm3 = Form 3, frm4 = Form 4, hyp = C. hypertrichosus, im = C. impar, pol = C. J*avensis var. polyphyllus, ten = C. tenompokensis. Number indicates the number of OTUs (or collection specimens).*

PREDICTED

ACTUAL	acu	amp	con	cor	elo	frm1	frm2	frm3	frm4	hyp	im	pol	ten
Acu	8	0	0	0	0	0	0	0	0	0	0	5	0
Amp	0	10	0	0	0	0	0	0	0	0	0	0	0
Con	0	3	2	0	0	0	0	0	0	0	0	1	0
cor	0	0	0	4	0	0	0	0	0	0	1	1	0
elo	0	0	0	0	3	0	0	0	0	0	1	0	0
frm1	0	0	1	0	0	0	0	0	0	0	0	0	1
frm2	1	0	0	0	0	0	0	0	0	0	0	0	0
frm3	0	0	0	0	0	0	0	1	0	0	0	0	1
frm4	0	0	1	0	0	0	0	0	8	0	0	0	0
hyp	0	0	0	0	0	0	0	0	0	0	0	1	0
im	1	0	0	1	0	0	0	0	0	0	7	0	0
pol	2	2	0	1	0	0	0	0	0	0	0	10	0
ten	0	0	0	0	0	1	0	0	0	0	0	0	9

greater than 0.95 (Suzuki & Shimodaira, 2006). The other clusters consisted of a mixture of all OTUs representing a mix of the species but without a particular distribution in distinct clusters as observed earlier in the MDS with categorical data for the bract shape.

When using the above unsupervised methods, we did not include the information of the species. When using supervised methods, species are used as a supervisory signal for classification problems. From the confusion matrix of the Confusion Table (CT) analysis (Table 2-4), we infer that most species are classified as *C. javensis*, including one member of *C. tenompokensis*. Nine *C. tenompokensis* are classified correctly as is the complete sample of *C. impar*. Some *C. elopurensis*, *Form 1* and *Form 2* are misclassified as *C. tenompokensis* and some *C. javensis, C. elopurensis and C. corrugatus* were misclassified as *C. impar*.

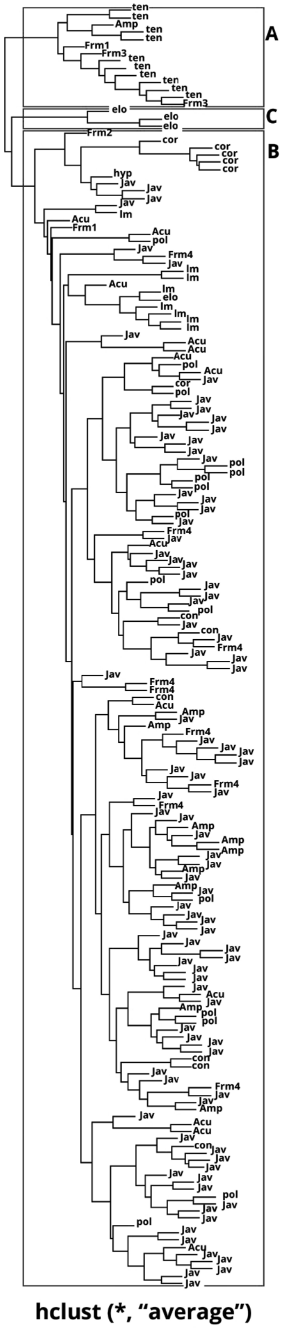

Figure 2-6. Dendogram based on all characters for all specimens using Hclust (‘mcquitty’) analysis.

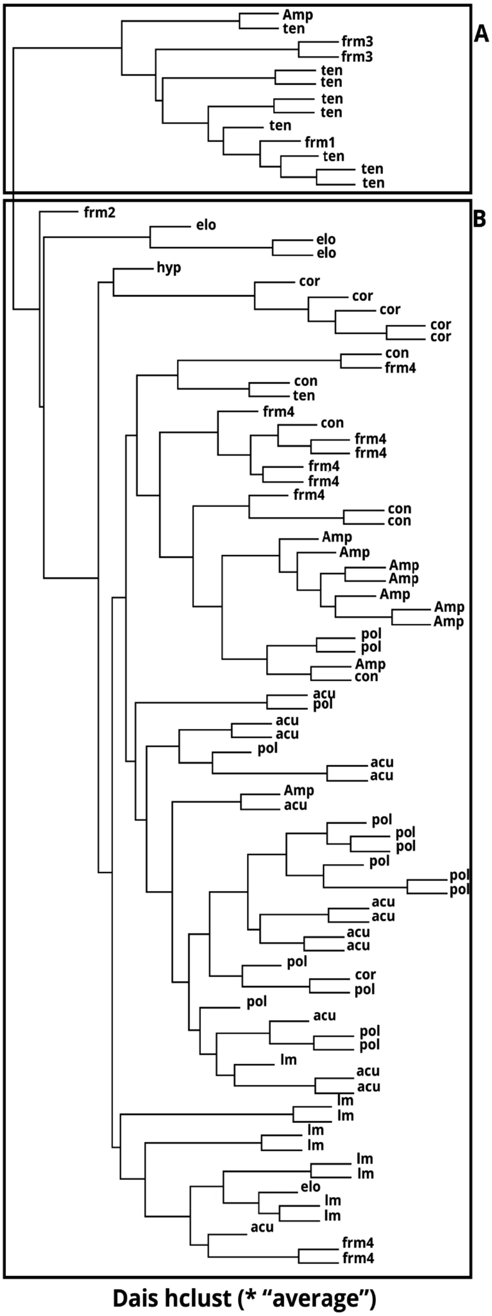

Figure 2-7. Dendogram based on all characters for all specimens excluding typical C. javensis using Hclust (‘average’) analysis.

In Table 2-5 we tabulate the confusion matrix computed using the Random Forest algorithm. Here the confusion matrix is based on a consensus tree, the decision tree with the highest occurrence in the forest.

The evaluation over the correct or incorrect prediction made by the model can be shown by the error rate value. Here the error rate of 28.25% is misleading due to the number of correctly classified members of *C. javensis*. If we disregard *C. javensis* from this table, 57 % species are misclassified and thus in disagreement with the experts. *Calamus tenompokensis* is, however, for 90 % correctly classified. These results point again to the problematic classification of the *C. javensis* complex based on the morphological features.

The Exclusion of typical C. javensis from the data matrix

Most extreme forms (often recognized as separate species) are only represented by a low number of OTUs, the majority of specimens represent typical *C. javensis*. It may well be that the bulk of typical *C. javensis* OTUs obscures groupings of the extreme forms, therefore we excluded all typical *C. javensis* OTUs from the data matrix. The unsupervised MDS analysis without categorial variables showed no significant differences (Figure 2-4) with when we included typical *C. javensis* in the analysis (Figure 2-2). Two clusters were observed: one big cluster consisting of all forms except members of *C. tenompokensis* (ten in Figure 2-4) and one member of *Form 1* and 2 members of *Form 3*. Members of *C. impar* (im) and *C. elopurensis* (elo) are at the periphery of the big cluster. Form 1 and Form 4 were mingled- with the big cluster (Figure 2-4). The same result was obtained when the categorical characters were included (Figure 2-5), but the clustering is less compact, but the group of *C. tenompokensis*, *Form 1* and *Form 3* still show a tendency to form a separate cluster. Typical here is the grouping of *C. acuminatus* (acu) and *C. javensis* var. *polyphyllus* (pol) (Figure 2-5).

The exclusion of typical *C. javensis* from the data matrix, gave a slightly different result for the data prediction in the supervised methods as shown in Table 2-6 and Table 2-7. All Forms (frm1–frm4) were misclassified as *C. tenompokensis*. Members of *C. acuminatus* and *C. javensis* var. *polyphyllus* were alternately misclassified. Species *C. corrugatus* and *C. impar* were almost correctly classified and the only *C. hypertrichosus* was misclassified as *C. corrugatus* or *C. javensis* var. *polyphyllus*. Almost all members of *C. amplijugus* were perfectly classified.

Hierarchical Custer Analysis produced dendograms for 2 sets of data, one including *C. javensis* (Figure 2-6) and one excluding *C. javensis* (Figure 2-7). The two dendograms support the distribution of the characters from the MDS analysis and the prediction made by the TREE and rF analysis. There are two groups, one small group (group A) consisting of *C. tenompokensis*, Form 1 and Form 3 (Figure 2-6 and Figure 2-7); and the rest formed a large group (group B) consisting of all typical *C. javensis* and the rest of the species. Noted here a small group within group B, indicated as C, contains members of *C. elopurensis* only (Figure 2-6). The exclusion of typical *C. javensis* yielded a similar result, there are also 2 main groups but here, in group B, all the species with peculiar characters are grouped together (Figure 2-7).

2.4. Discussion

Herbarium studies and characters

The characters used in the analyses did not lead to a clear distinction between species except for the recognition of *C. tenompokensis* separate from *C. javensis*. There are two reasons why *C. javensis* appears to be a good, but variable species. 1. The variation in characters is well spread among all OTUs, thus extreme forms do not show a constant set of different character states. 2. Specimens with intermediate forms between the distinguished taxa were observed during the fieldwork and in the herbarium material.

Not all characters and characters states were used in the multivariate analyses (often present in a single taxonomic unit or too many missing data). These and the characters used are discussed here.

Leaves and Leaf sheath are the two most variable characters. Beccari (1913) described *C. hypertrichosus* based on a sterile specimen from Borneo; the type has leaflets in 4 pairs and a very long petiole (c. 30 cm long). Seemingly the type was a young plant. Other specimens examined were full-grown (flowers, fruits) and showed leaves with 7 pairs of leaflets in an arrangement that resembles typical *C. javensis*. The type specimen of *C. hypertrichosus* only differs from typical *C. javensis* in having the leaflets covered with whitish hairs. It was the only specimen with this type of indumentum, and, therefore, the character state was not used in the multivariate analyses. On the other hand, because the white hairs are only shown by a single, immature specimen, no taxonomic value is given to this character state.

Smooth or almost smooth leaf sheaths were found among the OTUs called *C. corrugatus*. Instead of having spines, members of *C. corrugatus* have corrugated ring-like lines on the leaf sheath. However, the arrangement of the leaflets is similar to that of typical *C. javensis*. The corrugate lines are not completely circular in all specimens and a specimen from Central Kalimantan (*Mogea 3615*) has the typical *Calamus javensis* feature of an incompletely corrugated leaf sheath.

Leaflet shape is another character that is variable and apparently distributed over all samples. Already in typical *C. javensis* there is a broad variety in shapes.

Calamus amplijugus can easily be recognized by its many broadly elliptic leaflets, a long pistillate rachilla and narrow fruit scales. In the confusion matrix (Table 2-6 and Table 2-7) *C. amplijugus* was well recognized and correctly classified. Intermediate specimens with *C. javensis* were found among the herbarium specimens and several were observed during field work. All these collections are from Sabah (N. Borneo) only.

The number of leaflet pairs varies with age (less when young, more when older), but several forms were distinguished based on the number of leaflet pairs. *Calamus javensis* var. *polyphyllus* and *C. acuminatus* are synonymous. Beccari (1908) described *C. javensis* var. *polyphyllus* based on the presence of 9–12 pairs of leaflets; and later he (1913) described *C. acuminatus* as a species with 10–11 pairs, but with an acuminate leaf tip (see also below).

A low number (2 or 3) of leaflet pairs is observed among the members of *C. elopurensis*, *C. impar* and *Form 4*. The arrangement of these leaflets (not used as character in the multivariate analyses) can differ from typical *C. javensis*. The penultimate pair in *C. javensis* is almost always opposite and close to the flabellum; whereas *Form 4* has subopposite or alternate penultimate leaflets of which one is very close to the terminal pair. *Form 4* resembles *C. elopurensis* (3 pairs of leaflets) and *C. impar* (2 pairs of leaflets). However, the size of the leaflets of *C. impar* is smaller than those of *C. elopurensis* (up to 20 cm and up to 35 cm, respectively). Moreover, in *C. elopurensis* the staminate inflorescence has conspicuous bracts along the peduncle with a long limb of up to 10 cm long; in *C. impar* the bract of peduncle has tubular, c. 13 cm long, closely sheathing, distally with an extended limb to 2.5 cm long; whereas the peduncle bracts of *Form 4* are tubular, closely sheathing, later with extended elliptic limb to 5 mm long, c. 35 cm long.

Imputation (MICE) eliminated several generative characters, because these were not present in all OTUs and did not contribute significantly to the results of the multivariate analyses. One character remaining was the rachilla length. Rachilla length was expected to differentiate *C. congestiflorus* from the other taxa in the complex. Dransfield (1982) described this taxon because of a congested, short rachilla (2.5–5 cm long) and a lanceolate rachis limb. However, specimens were found with a variable short rachilla length and various degrees of congestion.

Multivariate analyses and taxon recognition

The conclusion from all multivariate analyses is that the *C. javensis* complex with its 9 described species, several infraspecific taxa and 4 tentative forms can be reduced to only 2 species. The majority of specimens constitutes *C. javensis* and a small group can be recognized as *C. tenompokensis*, retained as a different species. The tentative forms still need further attention, but extra material is needed to correctly establish their status. This division is supported by all analyses used.

The unsupervised analysis showed generally that two clusters can be distinguished: All OTUs forming one group (*C. javensis*), except those forming *C. tenompokensis*. The big cluster of typical *C. javensis* indicates an even distribution of all variation among the OTUs. There were no significant groups within this big cluster, only an indication that several forms could be distinguished. In fact, the analyses without typical *C. javensis* gave a similar, but somewhat less compact clustering, which means that all extreme forms, recognized as separate species, do not differ in distinct character states from each other, intermediates are always present. The two confusion tables of the data set including typical *C. javensis* (Table 2-4 and Table 2-5) support the conclusion of a less restricted definition of *C. javensis*, because many to most to all members of special forms appeared to be part of typical *C. javensis*.

Members of *C. tenompokensis* formed a separate group. The clustering made with MDS, when tested, showed in the confusion tables (Table 2-4 and Table 2-5) that only one OTU of *C. tenompokensis* was misclassified. The members of the *C. tenompokensis* group share the presence of flabellate leaflets with *C.*

javensis. However, *C. tenompokensis* can easily be distinguished from other taxa within the *C. javensis* complex by the vegetative parts: the angular petiole and rachis, a very different leaf sheath appearance, the sheaths being massive and robust, and the number and arrangement of the leaflets, *C. tenompokensis* has 9 pairs of large, lanceolate leaflets, which are almost always regularly arranged. The staminate inflorescence resembles those of *C. javensis*, but the base of the calyx is swollen. The pistillate inflorescences have rachilla bracts that are different in the broadly cupuliform limb (specimen *Chew & Corner RSNB 1892*).

Several peculiar forms, *Form 1–4* cannot be treated well for now, because of a lack of sufficient samples. *Form 4* is already discussed above.

Form 2 is only represented by a single sample collected from the Philippines, and the leaflets have scattered setae along veins and one black long seta on the main vein on the abaxial surfaces. The leaflets resemble the arrangement of typical *C. javensis*, but with quite long petioles and peculiar rachis spines.

Morphological examination of *Form 1*, consisting of two specimens, shows that one is quite distinct, grouping with *C. tenompokensis* (Figure 2-2, Figure 2-3, Figure 2-4 and Figure 2-5), the other is part of the typical *C. javensis* group, a result confirmed by the confusion tables (Table 2-4, Table 2-5, Table 2-6, and Table 2-7). This second specimen appears to be intermediate. *Form 1* is, like *C. tenompokensis* and *Form 3*, only known from higher altitudes (1200–1500 m). One member of *Form 1* is clearly different from *C. javensis* in the leaves and arrangement of leaflets, leaf sheath features, the short staminate inflorescences, tubular, warty rachilla bracts and the lack of a flagellum and a cirrus. *Calamus tenompokensis* resembles this taxon, also lacks the flagellum, but *Form 1* has smaller leaflets (c. 15 cm long, *C. tenompokensis* up to 35 cm long), the tubular and warty rachilla bracts (*C. tenompokensis*: explanate and smooth) and the staminate inflorescence borne on the 2nd branch (3rd branch in *C. tenompokensis*). *Form 3*, collected from Sarawak (*S 44664*) and Sabah (*SAN 144483*), remains separate from the typical *C. javensis* cluster and is closer to *C. tenompokensis* in all analyses (Figure 2-2, Figure 2-3, group A in Figure 2-6). Specimen *SAN 144483* has leaflets without hairs, but there are remnants of hairs. This entity differs from other specimens in the *C. javensis* complex, because it is stemless, has a peculiar arrangement of the tomentose leaflets, and the stigmas show a curled tip. Tomentose leaflets are also present in *C. hypertrichosus*, but the latter is climbing, has 7 leaflets pair (compare to 3 pairs in this taxon) with the leaflets arranged as in typical *C. javensis*.

The resemblance between *C. acuminatus* and *C. javensis* var. *polyphyllus* was shown to be high. The confusion tables (Table 2-4, Table 2-5, Table 2-6, and Table 2-7) also clearly indicate this as both species are interchangeable as they alternately show up as misclassified. Some members of these two taxa are also misclassified as typical *C. javensis*, which also indicates that they are part of this cluster, because they share many character states. This is also clearly shown in Figure 2-7, where members of *C. acuminatus* and *C. javensis* var. *polyphyllus* are in the same cluster. Beccari (1908) stated that *C. acuminatus* is indistinguishable from some *C. javensis* varieties. Based on the observations of the first author, *C. acuminatus* is similar to *C. javensis* var. *polyphyllus* (Beccari, 1908), but *C. acuminatus* has an almost smooth leaf sheath, smaller flowers and fruits and the bracts of the peduncle are more cupuliform. Beccari (1913) also suspected that *C. acuminatus* may be considered as subspecies. However, there are specimens observed with intermediate character states between *C. acuminatus* and *C. javensis*, e.g., specimens with leaflets varying between lanceolate and narrowly elliptic and leaf sheaths ranging from smooth to moderately spiny; the inflorescence can range from fine to the size of typical *C. javensis*. In conclusion, both *C. acuminatus* and *C. javensis* var. *polyphyllus* are part of the typical *C. javensis* complex and no separate entities.

Calamus impar and *C. elopurensis* also show a tendency to group together, but always in close proximity of the *C. javensis* cluster. When the categorical data were added the clustering became less distinct. The differences with *C. javensis* are discussed above. The confusion matrix of both analysis TREE and Random Forest (Table 2-4, Table 2-5, Table 2-6, and Table 2-7) resulted in a clear indication that each form is distinct, but they are not significantly different enough to separate them from typical *C. javensis*.

All other species are part of the typical *C. javensis* complex as indicated by Figure 2-2, Figure 2-3, Figure 2-4 and Figure 2-5.

The dendogram of all specimens (Figure 2-6) shows that *C. javensis* specimens are among almost all members of the complex, except group A of *C. tenompokensis*, *Form 1* and *Form 3*. *Calamus javensis* is a polymorphic species whereby many intermediate characters were found among the members of the complex (Group B, Figure 2-6). A number of *C. corrugatus* and *C. impar* specimens form subgroups but other members of both species are mingled with the rest of the specimens. This can best be seen in Figure 2-7, where the distinctive variation among extreme forms is shown.

Calamus javensis was recently discussed by Syam et al. (2016) in their work on the *Calamus flabellatus* complex in the Malesian region. They included *C. javensis* and other species within the complex, like *C. acuminatus, C. amplijugus, C. congestiflorus, C. corrugatus, C. hypertrichosus* and *C. ruvidus*. Their use of *C. flabellatus* to indicate the complex is unfortunate as *C. javensi*s is the oldest name and the most widespread species. The results of Syam et al. (2016) differ from our study. Two species, *C. corrugatus* and *C. hypertrichosus*, were separate from the *C. javensis* cluster in their cluster analysis. Our analysis showed that *C. corrugatus* was among typical *C. javensis*, which is corroborated by the presence of intermediate specimens; whereas *C. hypertrichosus*, with only one observed specimen, was also placed among typical *C. javensis*, though slightly separated in Figure 2-5 due to the distinctive hairs. Like with us, Syam et al. (2016) found a major cluster consisting of *C. javensis* and satellite species. Strangely enough, they named all groups of *C. flabellatus*, but did not do the same with those of *C. javensis*. We do not consider their study to be very representive, as the samples are few (seemingly 20 OTUs in the cluster analysis made with an unknown program) and not well-spread over the variation of the species, mainly N. Borneo is lacking. Unfortunately, as no data matrices are provided their work cannot be reproduced.

2.5. Conclusion

The supervised classification results in a set of rules that can be represented as a decision tree that experts would use to classify a species. The resampling methods of Random Forest produces many decision trees and thus creates, as its name indicates, even a decision tree forest. In the current study we interpret the miss-classifications observed, based on the morphological variables, as disagreement between the virtual experts and the real experts. The observation that most of the samples are classified as *C. javensis* points in the direction of a variable species or cryptic species rather than distinct species with the exception of *C. tenompokensis*. The multivariate analysis clearly indicates that *C. javensis* is a distinct species in its very broad definition, with most other entities, ever separated from it as species, as synonyms. The only exception is *C. tenompokensis*, which can still be recognized as distinct. If more material becomes available, some of the four forms might also be recognized as species.

A future molecular analysis will be used to test the two species hypothesis as put forward by the multivariate analyses. Once agreement exists a new classification and description of *C. javensis* and *C. tenompokensis* will be provided.

Chapter 3

Phylogenetic Analysis of the *Calamus javensis* complex (*Arecaceae, Calamoideae*) in Malesia

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Abstract -- A phylogenetic analysis on specimen level was made in possible support of a multivariate analysis of the *Calamus javensis* complex. Nine species, at some time recognized within the complex, and several recognisable forms were included. The phylogenetic markers used were the nuclear 5S spacer (5S nrDNA) and the chloroplast Maturase K (*mat*K). The Bayesian analysis showed that only 5S provided some resolution. The 50% majority rule consensus showed one major polytomy with a few supported groups, which were mainly morphologically unsupported pairs of specimens. However, one group, the form *C. tenompokensis* (the only distinct group in a multivariate analysis) is morphologically distinct and phylogenetically monophyletic and can be recognized as a species. Of all other recognizable forms, we only consider *C. acuminatus* to be regarded as a variety as it was not supported in the morphometric analysis.

3.1. Introduction

Calamus javensis Blume (*Arecaceae*: *Calamoideae*) is a slender rattan common in southeast Asian tropical rainforests. The species is very polymorphic and forms a species complex, including some taxonomically non-recognized, but morphologically distinct forms, and the species *C. acuminatus* Becc., *C. amplijugus* J.Dransf., *C. congestiflorus* J.Dransf., *C. corrugatus* Becc., *C. elopurensis* J.Dransf., *C. hypertrichosus* Becc., *C. impar* Becc. and *C. tenompokensis* Furtado. The distribution of the complex ranges from Southern Thailand and Peninsular

Malaysia to Sumatra, Java, Borneo and Palawan (Dransfield, 1992). The greatest morphological diversity is in Northern Borneo. Typical for all forms is that the stem without leaf sheaths is 2–6 mm in diameter; with sheaths to 10 mm in diameter; the internodes are up to 30 cm long but are usually shorter; the ocrea is distinct and deep crimson when young; leaves are pinnate, ecirrate and up to 40 cm long, the terminal leaflets are flabellate, and the lowermost pair is often swept back across the stem; the flagellum is up to 75 cm long; the inflorescences are long, and have red rachillae; the ripe fruits are ovoid.

The cane is useful and forms one of the minor forest products. According to the FAO, *C. javensis* is included in the major commercial species of rattan as identified for Asia by Dransfield and Manokaran (1993). Local people make bindings, baskets, cordages and ropes from the cane (Dransfield, 1992; Dransfield and Manokaran, 1994; Mogea, 1994; Watanabe et al., 2006).

Polymorphic plant species with large intraspecific variation and complicated evolutionary histories are undoubtedly a challenge for taxonomic research (Slovak et al., 2012). Because of the complex morphological variation without any constant differences among the entities, the species complex is too difficult to solve via traditional morphological observation (Dransfield, 1992; Dransfield et al. 2008; Atria et al. 2017). A recent morphometric study by Atria et al. (2017) showed that within the complex only two clearly defined taxa could be circumscribed, *Calamus javensis* with a broad range of variation and a far less variable *C. tenompokensis*. *Calamus tenompokensis* can easily be distinguished from other taxa within the *C. javensis* complex by its short stem, angular petiole and rachis, a very different leaf sheath appearance (the sheaths being massive and robust), and the number and arrangement of the leaflets. *Calamus tenompokensis* has 9 pairs of large, lanceolate leaflets, which are almost always regularly arranged, while in the rest of the complex the leaflets are smaller, 6 or 7, and ovate to broadly ovate. The staminate flowers resemble those of *C. javensis*, but the base of the calyx is swollen (versus not swollen in *C. javensis*). The pistillate inflorescences have rachilla bracts that are different in the broadly cupuliform limb (versus bracts tightly sheathing in *C. javensis*).

Calamus is the largest genus among the climbing palms, also called rattans (http://www.fao.org/3/Y2783E/y2783e05.htm; Sreekumar & Renuka 2008) and forms subtribe *Calaminae*, together with *Ceratolobus* Blume ex Schult.

& Schult.f., *Daemonorops* Blume, *Pogonotium* J.Dransf. and *Retispatha* J.Dransf. The phylogeny of the rattans by Kramadibrata (1992) suggested that *Calamus* is paraphyletic. This was confirmed by Baker et al. (2000b) in their phylogenetic study based on the 5S nrDNA spacer. They found four major lineages within the genus (Baker et al. 2000b), whereby the other genera of the Calaminae are nested within *Calamus*. Baker (2015): "A revised classification is proposed in which *Ceratolobus*, *Daemonorops*, *Pogonotium* and *Retispatha* are placed in synonymy with *Calamus*. This is presented as a stable, alternative and pragmatic taxonomic solution for this problematic group".

Cronn et al. (1996) were the first to study polymorphism in *Gossypium* L. (Malvaceae) using 5S nrDNA. Polymorphism is always detected in 5S nrDNA, but less often in 18S-26S nrDNA (Baker et al., 2000b). The spacer regions of the nrDNA possess information useful for plant systematics from species to generic level. They have also been used in studies of speciation and biogeography, due to their high sequence variability and divergence (Poczai & Hyvönen, 2010). The 5S nrDNA unit provides a useful marker for studying the evolutionary relationships between species on tribal level (Scoles et al., 1988; Vakhitov & Nikonorov,1989; McIntyre et al., 1992; Crisp et al., 1999). The 5S unit of the rDNA is organized in repetitive tandem arrays, each separated by a non- transcribed region (NTS). Each 5S DNA unit is composed of a gene region of 120 bp long and a spacer of 100–700 bp long. In plants, the total number of repeats (gene + spacer) per genome varies by over two orders of magnitude, from less than 1,000 to over 100,000 (Schneeberger et al., 1989; Sastri et al.,1992; Baum & Apples, 1992; Cronn et al. 1996, Crisp et al. 1999, Baker et al. 2000b, Baum et al. 2015). It is easy to amplify and to sequence the intergenic spacer, which evolves rapidly, and so has the potential to resolve phylogenetic relationships at low taxonomic levels, also among populations and species. The 5S spacer sequences have proven to be informative in such groups as *Acacia* Mill. (Fabaceae; Playford et al., 1992), *Pinus* L. (Pinaceae; Moran et al., 1992), tribe Triticeae (Poaceae; Baum & Appels, 1992; Kellogg & Appels, 1995) and *Eucalyptus* L'Hér. (Myrtaceae; Udovicic et al. 1995).

The maturase K (*mat*K) is a rapidly evolving and highly variable gene, and has been recommended as a locus for DNA barcoding by the Consortium for the Barcode of Life (CBOL) Plant Working Group (Hollingsworth et al., 2009;

Yu et al., 2011; Bieniek, 2015). The chloroplast DNA (cpDNA) restriction-site variation appeared to be well-suited for studies of genetic relationships at or below the family level (Asmussen & Chase, 2001; Hahn, 2002; Guzman & Vargas 2005; Roncal et al., 2009; Abbas et al., 2009; Bieniek, 2015; Pastore et al., 2017). The cpDNA has already been used often for reconstructing the phylogeny of palm groups. Asmussen et al. (2006) used the plastid DNA regions *trn*L-*trn*F, rps16 intron, rbcL and *mat*K to resolve subfamilies within the palms. The phylogenetic analysis of tribe *Chamaedoreeae* (*Arecaceae*) has been studied using parsimony and Bayesian analyses of plastid DNA sequences (*mat*K, rps16 intron, 3′ region of ndhF, and trnD-trnT) (Cuenca & Asmussen-Lange, 2007). Baker et al. (2000a) investigated the relationship among 22 genera in the subfamily *Calamoideae* using nrDNA ITS and cpDNA *rps*16 intron sequence data.

In this study we conduct a phylogenetic analysis of the *C. javensis* complex based on two different regions, 5S nrDNA and *mat*K. Aim is to see if the complex can be subdivided in monophyletic, recognisable units, which may corroborate the morphometric study (Atria et al., 2017).

3.2. Materials and Methods

Taxon sampling

About 52 samples of silica gel-dried leaf fragments and herbarium specimens were used for DNA sequencing. One silica sample of *Calamus flabellatus* was included as outgroup. One of the syntypes, from west Java (*Blume* s.n. (L, sheet 900.182-94) and silica gel-dried material from the type locality were included. All samples cover the distribution area of the *C. javensis* complex. A list of voucher specimens can be found in Table 3-2.

DNA extraction, amplification and sequencing

DNA extraction from the silica gel-dried leaves and herbarium vouchers was done using the magnetic bead-based isolation procedure (NucleoMag® 96 Plant kit, Macherey-Nagel; https://www.mn-net.com), carried out on an automated KingFisher extractor (https://www.thermofisher.com/nl/en/home.html). Additional sequences of the outgroups were obtained from the NCBI GenBank sequence database (https://www.ncbi.nlm.nih.gov/Genbank, see Table 3-2).

Two DNA regions were amplified, the nuclear 5S spacer and the chloroplast marker *mat*K. Primers (Table 3-1) for this study were designed specifically for the group based on the work of Baker et al. (2000b) using Geneious ver. 10.1.3 (http://www.geneious.com). Primers for the 5S spacer (Table 3-1) were M13-R435R (R2) and M13F-ITS103F (F2). The *mat*K sequences were amplified from total genomic DNA using the designed primers M13R-831R*mat*K3 (M3) and M13F-578F*mat*K3 (F3). M13 tails (M13F TGTAAAACGACGGCCAGT, M13R CAGGAAACAGCTATGAC) used are from Messing (1983).

Table 3-1. Primers designed for this study oriented 5'–3', m13 tails not included.

DNA region	Primer name	Primer sequence
*mat*K	F3	CAGGAAACAGCTATGACGCTGGTCC
	M3	TGTAAAACGACGGCCATTTTTTCATA
5S spacer	F2	TGTAAAACGGCCACTTCCTTGTGT
	R2	CAGGAAACAGCTATGACCATCGTGGG

The Polymerase Chain Reactions (PCRs) were carried out with an end volume of 25µl containing 5 µl of 5x Phire green reaction Buffer (F-527, Thermo Fischer Scientific), ultrapure water 10.5 µl, 1 µl of each 10 µM forward and reverse primer, 1 µl of 100 mg/ml Polyvinylpyrrolidone (PVP), 0.5 µl of 2 U/µl Phire™ Hot Start II DNA Polymerase (F-122S, Thermo Fischer Scientific), 1 µl of 10mM dNTP, and 1µl of DNA template.

The amplifications were conducted in a 96+ Grad 1000S thermocycle, programmed as follows: initial denaturation step at 98°C for 30 sec, followed by 35 cycles of denaturation steps at 98°C for 5 sec, an annealing step at 55°C for 5 sec, extension step at 72°C for 15 sec; final extension at 72°C for 1 min.

PCR results were checked in standard 1% agarose gel electrophoresis. Gels were stained and immersed in 0.5 µg/ml ethidium bromide solution for 30 min, visualized and recorded on a Gel Doc Systems (Bio-Rad, Barcelona, Spain; https://www.bio-rad.com/). All selected PCR amplification products were sent to BaseClear (https://www.baseclear.com). The resulting chromatograms were then assembled and edited using Sequencher™ 4.1.4 (Gene Codes Corp., Ann Arbor, Michigan, USA; https://genecodes.com/). To ensure that the DNA isolated was not contaminated, all sequences were BLAST-searched in GenBank. The sequence results of all markers were submitted to the NCBI GenBank sequence database (see Table 3-2).

Table 3-2. Species and samples of Calamus javensis complex and outgroup taxon sequenced for 5S Spacer with reference to collecting location and GenBank number. The Group abbreviation, where present, refers to the supported clades in Fig. 2 (groups E and F nested in G, G nested in H); the numbers between brackets after the species names correspond with the numbers in Figs. 1 & 2, the abbreviations after the collector name indicate the herbarium where the voucher is stored, including the barcode number when available, K = Royal Botanic Gardens Kew, UK; L = Naturalis Biodiversity Center, Leiden, Netherlands).

Group	Species or Form (voucher code)	Voucher and source (herbarium)	Location	GenBank number 5SnrDNA
F	*C. acuminatus* (10A)	*Mega MAT 037* (L)	Kabili-Sepilok FR, Sabah, North Borneo	MT273960
F	*C. acuminatus* (10B)	*Mega MAT 028* (L)	Kabili-Sepilok FR, Sabah, North Borneo	MT273958
F	*C. acuminatus* (10C)	*SAN (Nordin Abas) 85869* (L 0617865)	Crocker range, Keningau, Sabah, North Borneo	MT273963
F	*C. acuminatus* (10D)	*SAN (Amin & Jarius) 116575* (L 0617894)	Ranau, Sabah, North Borneo	MT273962
F	*C. acuminatus* (10E)	*Mega MAT 033* (L)	Kabili-Sepilok FR, Sabah, North Borneo	MT273959
B	*C. acuminatus* (6G)	*Dransfield JD 5584* (L 0618191)	Tenom, Sabah, North Borneo	MT273961
	C. amplijugus (1E)	*Mega MAT 109* (L)	Teraja, Mendaram, Brunei	MT273965
	C. amplijugus (3D)	*Mega MAT 045* (L)	Tawai FR, Telupid, Sabah, North Borneo	MT273964
	C. amplijugus (3F)	*Mega MAT 109C* (L)	Teraja, Mendaram, Brunei	MT273966
	C. congestiflorus (12E)	*Mega MAT 079* (L)	Mesilau nature center, Ranau, Sabah, North Borneo	MT273967
	C. corrugatus (6C)	*Dransfield JD 6080* (L 0618038)	Sabal Tapang FR, Sarawak, North Borneo	MT273970
A	*C. corrugatus* (6E)	*Mogea 3615* (L 0618040)	Central Kalimantan, South Borneo	MT273969
	C. corrugatus (6F)	*Dransfield JD 5868* (L 0618039)	Mt. Matang, Sarawak, North Borneo	MT273968
H	*C. elopurensis* (10F)	*Dransfield JD 6265* (L 0618008)	Danum Valley, Sabah, North Borneo	MT273971
G	*C. impar* (11A)	*Ave 136* (L 3928259)	Perak, West Malaysia	MT273974
G	*C. impar* (11D)	*SAN (Meijer) 21064* (K 000113394)	Bukit Ampuan, Ranau, Sabah, North Borneo	MT273973
G	*C. javensis* (11B)	*Niyomdham 1254* (L 3933498)	Klong Seang, Pangnga, Thailand	MT273975
	C. javensis (1A)	*Mega MAT 001* (L)	West Java, Indonesia	MT274005
	C. javensis (1B)	*Mega MAT 093* (L)	Ulu Temburong, Brunei	MT274006

Group	Species or Form (voucher code)	Voucher and source (herbarium)	Location	GenBank number 5SnrDNA
	C. javensis (1C)	*Mega MAT 022* (L)	West Java, Indonesia	MT273992
D	*C. javensis* (1D)	*Mega MAT 008* (L)	West Java, Indonesia	MT274004
C	*C. javensis* (1F)	*Mega MAT 011* (L)	West java, Indonesia	MT273991
	C. javensis (1G)	*Mega MAT 005* (L)	West Java, Indonesia	MT273989
	C. javensis (2C)	*Mega MAT 100B* (L)	Kuala Belalong, Brunei	MT273996
	C. javensis (2E)	*Mega MAT 024* (L)	West Java, Indonesia	MT273993
	C. javensis (3G)	*Mega MAT 007* (L)	West Java, Indonesia	MT273990
	C. javensis (3H)	*Mega MAT 002* (L)	West java, Indonesia	MT274007
	C. javensis (4A)	*Ave 114* (L)	Malaysia-Peninsula	MT273983
	C. javensis (4B)	*Dransfield JD 4728* (L 0617876)	Gunung Matang, Sarawak, North Borneo	MT273986
	C. javensis (4C)	*Ambri & Arifin W 915* (L 0617975)	Wanariset, Kalimantan Timur, Indonesia	MT273987
	C. javensis (4D)	*Kato & Wiriadinata B 4943* (L 0617908)	East Kalimantan, Indonesia	MT273988
	C. javensis (4H)	*Dransfield JD 4650* (L 0617884)	Sabal Tapang FR, Sarawak, North Borneo	MT273977
	C. javensis (5A)	*Dransfield JD 4519* (L 3928253)	Tapah Hill, Perak, Malay Peninsula	MT273982
	C. javensis (5B)	*S (Awa & Lee) 50593* (L 0617892)	Bukit Lawi, Sarawak, North Borneo	MT273978
B	*C. javensis* (5E)	*Dransfield JD 3613* (L 3928265)	Bengkulu, Sumatra, Indonesia	MT273984
	C. javensis (6A)	*S (Lee Meng Hock) 54137* (L 0617893)	Gn. Bawang, Matang, Sarawak, North Borneo	MT273979
C	*C. javensis* (6B)	*Van Valkenburg 1320* (L 0372224)	Kutai, East Kalimantan	MT273976
	C. javensis (7A)	*Mega MAT 097* (L)	Sungai Belalong, Brunei	MT273995
	C. javensis (7C)	*S (Lee) 52424* (L 0617902)	Bukit Tebunan, Sarawak, North Borneo	MT273980
	C. javensis (7F)	*Dransfield JD 2553* (L 3928344)	Sungai Air Hitam, Berbak, Jambi, Sumatra, Indonesia	MT273985
	C. javensis (8B)	*Mega MAT 057* (L)	Tenompok Forest Reserve, Sabah, North Borneo	MT273994
A	*C. javensis* (8D)	*Ave 216* (L 3928252)	Perak, Malay Peninsula	MT273981
	C. javensis var. *polyphyllus* (2B)	*Mega MAT 103A* (L)	Kuala Belalong, Brunei	MT274001
H	*C. javensis* var. *polyphyllus* (10G)	*Mega MAT 027* (L)	Kabili-Sepilok FR, Sabah, North Borneo	MT273997

Group	Species or Form (voucher code)	Voucher and source (herbarium)	Location	GenBank number 5SnrDNA
	C. javensis var. *polyphyllus* (12B)	*Mega MAT 095* (L)	Ashton Trail, Ulu Temburong, Brunei	MT274000
	C. javensis var. *polyphyllus* (12C)	*Mega MAT 080* (L)	Crocker Range Nature Center, Keningau, Sabah, North Borneo	MT273999
	C. javensis var. *polyphyllus* (3C)	*Mega MAT 058* (L)	Bukit Hampuan, Sabah, North Borneo	MT273998
E	*C. tenompokensis* (10H)	*Mega MAT 055* (L)	Tenom, Sabah, North Borneo	MT274003
E	*C. tenompokensis* (11E)	*Mega MAT 054* (L)	Tenom, Sabah, North Borneo	MT274002
	Form 2 (8E)	*Madulid et al. 7172* (L 3928434)	Mt. Arayat, Pampanga, Luzon, the Philippines	MT273955
D	Form 4 (11G)	*Chew & Corner RSNB 4835* (L 0617867)	Mesilau Cave, Mt. Kinabalu, Sabah, North Borneo	MT273956
G	Form 4 (12D)	*Mega MAT 065* (L)	Bukit Hampuan, Sabah, North Borneo	MT273957
Sister taxon	*Calamus flabellatus*	*Mega MAT 102* (L)	Kuala Belalong, Brunei, North Borneo	MT273972

Alignment of Sequences and Phylogenetic Analysis

The alignment of the forward and reverse sequences were checked manually with Sequencher 4.1.4. The 5S nrDNA sequences showed in most cases little ambiguity in the alignments. Refined sequences or multiple sequence alignments were made using CLUSTAL W option of the program Bioedit v.7.0.9 (Hall 1999), which was also used for the *mat*K sequences.

Phylogenetic analysis

Sequences with indels are included to provide as much phylogenetic information as possible (Baum et al., 1994). 5S and *mat*K were analysed both as separate data sets and combined.

A parsimony analysis was performed with PAUP* 4.0a157 (Swofford, 2002), but because of the low variability many cladograms were possible and the program had to be terminated as the swapping ran too long. An analysis of the two markers via Bayesian inference using Markov Chain Monte Carlo algorithms (MCMC) as implemented in MrBayes v.3.2.6 (Ronquist et al., 2012) with 10,000,000 generations gave better results. Most default values were used: 4 chains of which 1 cold and 3 heated in two simultaneous runs, the markov chain was sampled every 1000th generation whereby the first 25% of the samples were discarded as burnin. We run MrBayes in XSEDE via the CIPRES science gateway (http://www.phylo.org/; Miller et al. 2015). The substitution model used is GTR (generalized time-reversible) (Ronquist et al., 2012, De Salle & Rosenfeld 2013). We checked the Potential Scale Reduction Factors (PSRF) in the MrBayes SUMP output, the values were 1 or close to 1, which also indicates correct convergence. The two tree files per marker were then combined using LogCombiner v1.10.3 (in BEAST v1.10.3; http://tree.bio.ed.ac.uk; Drummond et al. 2002. Drummond & Rambaut 2007. Suchard et al. 2018), whereby the dot in the names of the trees in the MrBayes output files were replaced by dashes to make the MrBayes files readable. The output file was used via TreeAnnotator v1.10.3 (in BEAST v1.10.3) to create the Maximum Clade Credibility (MCC) tree, which was visualised with FigTree v1.4.3 (Rambaut 2010).

3.3. Results

DNA extraction and amplification

All 53 samples were successfully amplified for 5S and 57 amplified for *mat*K. The designed primers resulted for *mat*K in sequences of 253 bp long and for 5S of 332 bp long. In the combined matrix there were 52 entities and 561 characters, of which 484 characters were constant, 53 were variable characters but parsimony-uninformative, and 24 characters were parsimony informative.

Phylogenetic analysis

For all analyses (both markers separately and combined) 50% majority consensus cladograms were produced by MrBayes and via the BEAST software MCC trees were created. The data that resulted from the analysis did not provide the desired resolution. The 50% majority consensus tree for *mat*K showed no support for any clade except for the complete *C. javensis* complex and a subclade formed by Form 4 (the latter is not supported by any other analysis; results not shown here). The 5S 50% majority rule consensus and the MCC tree of the combined markers (Figure 3-1 and Figure 3-2, respectively) are compared. The topologies in both trees are similar with also a similar (lack of) support for the various clades. The 5S 50% consensus tree, with its large polytomy, shows lack of support for the majority of

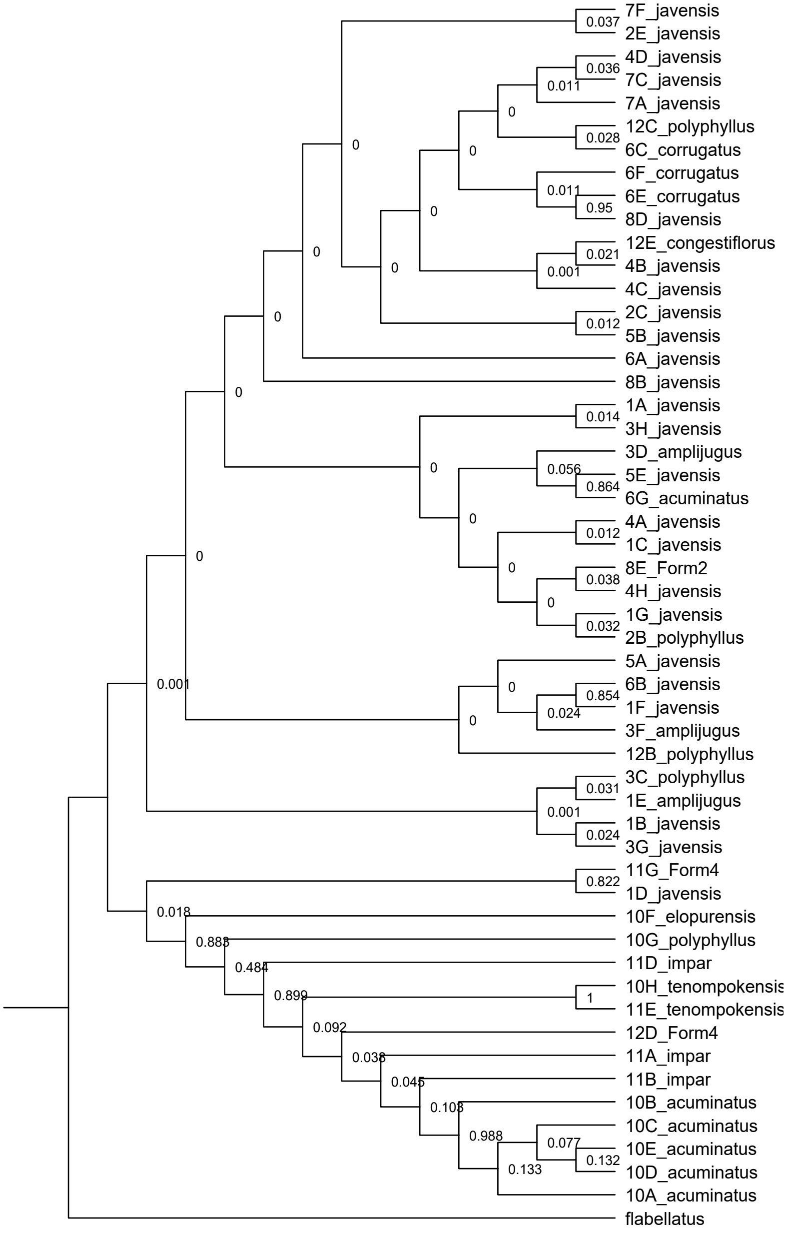

Figure 3-1. Cladogram of 5S 50% majority rule consensus tree from MrBayes analysis using MCMC algorithm. Cladogram shows lack of support for the majority of samples. There is only full support (posterior probability (PP) = 1) for the complete C. javensis complex as the outgroup is very different.

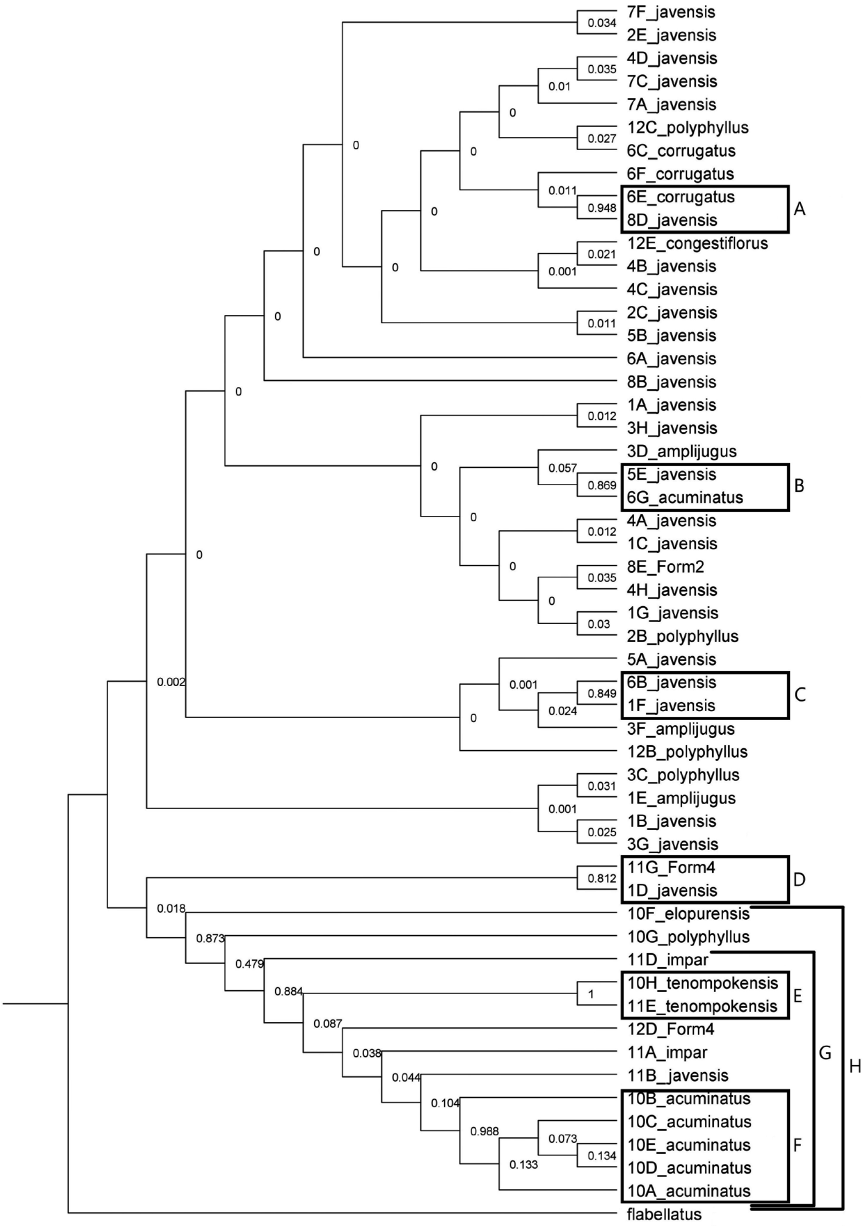

Figure 3-2. The MCC tree of the combined markers (matK and 5S). Several subclades with reasonable support (PP > 0.8) are present, all other branches lack support (PP usually < 0.1).

samples (Figure 3-1); there is only full support (posterior probability (PP) = 1) for the complete *C. javensis* complex as the outgroup is very different. Several subclades (framed in Figure 3-2) with reasonable support (PP > 0.8) are present, all other branches lack support (PP usually < 0.1).

Both cladograms, the 5S 50% majority and the MCC tree of the combined markers, show higher support (PP > 0.8) for 4 pairs of specimens (Groups A-D in Figure 3-2). One larger clade (Group H, Figure 3-2) has a PP of almost 0.9, within this clade Group G forms another well-supported group (PP = 0.9) and it contains two subclades, *C. tenompokensis* (Group E; PP = 1) and *C. acuminatus* (Group F; PP = 0.99). Group H comprises mainly entities from northern Borneo with slightly different morphological characters, it includes *C. tenompokensis* and *C. acuminatus*, *C. impar*, *C. elopurensis*, Form 4 (incomplete) and only one specimen of *C. javensis* var. *polyphyllus* (10G). In Group F (Figure 3-2), *C. acuminatus*, whereby not all forms identified as *C. acuminatus* are included, Group B also comprises one. All other pairs of supported entities form strange combinations, morphologically and geographically: Group A (Figure 3-2; *C. corrugatus* from Kalimantan and *C. javensis* from the Malay Peninsula), Group B (*C. javensis* from Sumatra and *C. acuminatus* from Sabah), Group C (*C. javensis* from Kalimantan and Java), and Group D (*C. javensis* from Java and Form 4 from Sabah).

3.4. Discussion

The topologies of the supported clades in the 5S 50% majority rule consensus tree and the MCC tree of the combined markers are similar, which indicates that the 5S sequences determine the phylogeny found and that *mat*K did not contribute at all. The variable and informative characters (24) are only in the 5S sequences and their number is insufficient to solve the cladogram. This explains why so little resolution was reached and why the parsimony analysis failed. Unfortunately, no other markers could be sequenced in the time and financial means alloted to this project.

The multivariate analysis (Atria et al., 2017) of the same taxa showed two groups, *C. javensis* and *C. tenompokensis*. The phylogenetic results show that, based on the two markers used, the *C. javensis* complex cannot easily be split in various taxa as supported main clades are absent. *Calamus tenompokensis*

(Group E, Figure 3-2) forms a distinct subclade. Recognizing it as a species would render the remaining part paraphyletic, which is not acceptable for phylogenies of species. However, species do not always have to split up (as it is always simplified in cladograms), but small populations may split off and form new species, thus leaving a 'paraphyletic' species behind. Ferris et al. (2014) stated that local speciation occurring on a small geographic scale can be the dominant mode of speciation in plants, where small populations split off from a broad-ranged progenitor species; but there are still limited examples to verify the case (see also Crisp & Chandler 1996, De Queiroz 1998). The characters typical for group E are already mentioned in the introduction, one can add some details: lack of an ocrea, no flagella and cirri and inflorescences generally shorter than the leaves; pistillate inflorescences to 80 cm long; staminate inflorescences robust, up to 1 m long, but some short only c. 25 cm long. *Calamus tenompokensis* is a Bornean mountain endemic of Gunung Mulu (Sarawak) and Gunung Kinabalu and the Crocker Range (Sabah) at altitudes from 1200 to c. 1800 m. (Dransfield, 1992). This narrow distribution and ecological specialization supports the view to regard it as a distinct species.

The other well-supported group, Group F (PP = 0.9), consists of samples of *C. acuminatus* (10A-10E). *Calamus acuminatus* is less recognizable than *C. tenompokensis*, but is still distinct by a set of characters. Typical are the many (9-12) narrow leaflets that are regularly arranged in a subopposite way and the leaf sheath is smooth or almost smooth. There is variation in the appearance of the leaf sheath and leaflet arrangement, but the most frequent form bears 10 or 11 sub-equidistant linear leaflets. The inflorescences resemble those of *C. javensis*, but they are mostly smaller or finer. The size of the fruit varies. Beccari (1908) stated that *C. acuminatus* is indistinguishable from some *C. javensis* varieties. Based on observations of the first author, *C. acuminatus* resembles Beccari's form *C. javensis* var. *polyphyllus* Becc. (Beccari, 1908), but the leaf sheath of *C. acuminatus* is smoother, the flowers and fruits are smaller and the bracts of the peduncle are more cupuliform. However, several specimens with characters partly intermediate can still be recognized. Specimen 10G (*Mega MAT 027*, Sabah) is identified as *C. javensis* var. *polyphyllus*, it exhibits the characters of var. *polyphyllus*, but the leaf sheath is almost smooth, like the leaf sheath of *C. acuminatus*. Based on the phylogeny and sheath character we consider this specimen to be *C. acuminatus*.

One sample, identified as *C. acuminatus* (6G, *Dransfield JD 5584*), was placed in group B within the typical *C. javensis* group; this sample agrees with *C. acuminatus* in the almost smooth leaf sheath, numerous (9) leaflets and prickly peduncular bracts (in *C. javensis* a robustly spiny leaf sheath, 8 leaflets and spiny peduncular bracts), but the fruits are more ovoid, like *C. javensis*, and not spherical as typical *C. acuminatus* (Dransfield, 1984). Specimen 6G is an intermediate form between *C. javensis* var *polyphyllus* and *C. acuminatus*. Because of this specimen, and its placement outside the rest of the *C. acuminatus* specimens, *C. acuminatus* can, at most, be recognized at variety level. *Calamus acuminatus* is endemic to and widespread throughout Sabah. It is particularly abundant in the Tenom and Keningau districts, where it is very much a feature of the secondary forests and roadside belukar (Dransfield, 1984). We found *C. acuminatus* abundantly growing in an open area in Mamut Copper Mine (MCM) in Bukit Hampuan, Ranau, Sabah; which is a no longer operational. "The MCM site has several unusual geochemical features on account of the concomitant occurrence of acid forming sulphide porphyry rocks and alkaline serpentinite minerals, and unique biological features because of the very high plant diversity in its immediate surroundings. The area is reported to contain acid mine drainage (AMD) pollution as is gradually becoming evident at the abandoned mine area" (Jopony & Tongkul 2009; Van der Ent & Edraki 2016). The narrow distribution and ecological preferences support the idea to recognise *C. acuminatus* as a distinct entity.

Group G (Figure 3-2) contains all forms identified as *C. impar* (11A from Malay Peninsula and 11D from Sabah). It does not form a supported group, but morphologically it is distinct from the rest of *C. javensis*, as it differs in the number of leaflets and their arrangement. *Calamus impar* always has 2 pairs of leaflets; the penultimate pair in *C. javensis* is almost always opposite and close to the flabellum, whereas in *C. impar* the pair is subopposite or alternate with one leaflet very close to the terminal pair. The ocrea of *C. impar* is cylindrical and truncated, while in typical *C. javensis* the ocrea is always quite conspicuous, quickly tattering, rarely persistent. *Calamus elopurensis* (10F), also in groups G and H (Figure 3-2), is only represented by a single specimen. This taxon is easily distinguished by the rosette of the large leaves and the peculiar long rachis bracts. It should not be confused with *C. impar* (Group G, included in Group H), which also has few leaflets, but a much smaller stem (5 mm vs up to 8 mm in *C. elopurensis*), shorter staminate rachilla (5 cm vs up to 12 cm in *C. elopurensis*) and broadly elliptic leaflets (vs narrowly elliptic in *C. elopurensis*). However, the size of the leaflets of *C. impar* is smaller than those of *C. elopurensis* (to 20 cm long vs to 35 cm long), moreover, in *C. elopurensis*, the staminate inflorescence has conspicuous peduncle bracts with a long limb of up to 10 cm long. Because of significant morphological differences *C. impar* appears to be recognizable as a distinct form. However, *C. impar* is regarded as probably merely a form of *Calamus javensis* by Dransfield & Patel (2005). Specimens studied cover a wide distribution Berau (East Kalimantan), Perak (Malay Peninsula), Ranau, Mt. Kinabalu (Sabah) and Klong Seang (Thailand). At least one specimen was collected from ultrabasic soil at the altitude of 900 m. Ultrabasic rock often triggers speciation, but in most areas of the specimens studied it is absent. Because of the wide distribution, and unspecialized ecology we consider the morphological differences to have appeared multiple times, and we adhere to the conclusion to consider *C. impar* as a mere form of *C. javansis*, not to be recognized in a formal classification.

Within group H a few other specimens are included. The specimen representing var. *polyphyllus*, does not form a distinct group as two other specimens are in the main *C. javensis* clade, therefore no status will be given to this form. Form 4 is partly in group H (12D, *Mega MAT 065*, Sabah) and partly in group D (11G, *Chew & Corner RSNB 4835*, Sabah). Morphologically this form is different from typical *javensis*, as it has robust leaf sheaths with a triangular, flat, hairy margin, the ocrea is rough and hirsute or with spines, the young staminate inflorescence bracts are reddish, and the pistillate inflorescences are short, 16-18 cm long. However, as this form is split over two supported clades, no taxonomic value is given. Also, because specimen 1D, also grouped in group D, has a typical *javensis* form.

Group A comprises specimen 8D from the Malay Peninsula, typical *javensis* (except for the wider leaf sheaths with quite robust spines) and 6E from Kalimantan. The latter represents *C. corrugatus* with typical ring-like wrinkled sheaths without spines. Two others specimens with the same characters are in the unsupported sister clade. Specimen 6E (*Mogea 3615*, Central Kalimantan) is in fact intermediate, as the wrinkles are only in half of the sheath. Because of the union with specimen 8D, which is morphologically nor geographically supported, and the presence of an intermediate specimen, no taxonomic status will be given anymore to the *corrugatus* form.

Group B contains specimen 5E (typical *C. javensis* from Sumatra) and 6G (*C. acuminatus* from Sabah). The latter specimen is discussed above. Both do not share any typical morphological characters and are not recognised as a taxon. Group C combines specimen 6B and 1F. Specimen 6B (*van Valkenbug JVV 1320*) is typical in its leaf sheath with robust spines and the broad leaflets. Specimen 1F (*Mega MAT 011*, from West Java) is typical *C. javensis*. As a group they are not morphologically distinct and do not deserve any taxonomic status.

The concept of genetic assimilation (Pigliucci et al., 2006) can be applied here to explain what is present. 'A population will produce novel phenotypes when pressured by environmental conditions via pre-existing reaction mechanisms for which no initial genetic change is necessary. A genetic fixation happens when natural selection continues to work under the new environmental conditions when the new phenotype prevails' (Pigliucci et al., 2006). Seemingly the *C. javensis* complex is adapting to locally different environments, resulting now in a few morphologically, but not yet genetically distinct forms. The multivariate analysis (Atria et al., 2017) showed one (multi) group indicated as *C. javensis*, with at the outside of the big cluster *C. tenompokensis* and *C. acuminatus* as more or less distinct groups. Genetically, they are seemingly also differentiating, but not yet enough to recognize them as distinct species in well-supported clades.

3.5. Conclusions

The markers used gave little resolution, but a few groups in the *C. javensis* complex are phylogenetically and morphologically supported to some degree. More sequences of other markers should be added before a clear picture of the complex can be given.

Calamus tenompokensis, distinctive in the multivariate analysis, and in this phylogenetic analysis, should be recognized on species level. The form *C. acuminatus* may have a status as variety.

Chapter 4

The *Calamus javensis* (Arecaceae, Calamoideae) complex in historical biogeographic context

Mega Atria and Peter C. van Welzen

Reinwardtia 20(1): 1-7. (2021)

Abstract -- The *Calamus javensis* (Arecaceae: Calamoideae) complex in historical biogeographic context. Reinwardtia xx: xx−xx. — *Calamus javensis* is a very polymorphic species with a number of recognisable forms (of which several were recognized at species level). A historical biogeographic analysis showed no historical distribution pattern in the diversification of these various forms. The forms are very likely the result of adaptation to local circumstances, whereby more or less identical forms can develop under similar niche circumstances in disjunct areas. Exceptions are the '*acuminatus-polyphyllus*' form and *C. tenompokensis*.

4.1. Introduction

Presently, the largest genus in the rattans, climbing palms, is *Calamus* L. Especially since phylogenetic analyses (Kramadibrata, 1991; Baker *et al.* 2000a, 2000b) proved it to be paraphyletic with the genera *Ceratolobus* Blume ex Schult. & Schult.f., *Daemonorops* Blume, *Pogonotium* J.Dransf. and *Retispatha* J.Dransf. nested within it, which Baker (2015) synonymized with *Calamus*.

Within *Calamus*, *C. javensis* Blume is a slender, very polymorphic rattan commonly present in the everwet rainforests of southeast Asian. Many taxa have been split from it and various forms are recognizable (see names in Table 4-1 and Figure 4-2). Most names have been synonymized with *C. javensis* (Barfod & Dransfield, 2013; Henderson, 2020). Within the complex only *C. tenompokensis*

Furtado proved to be recognizable, in a morphometric, phenetic analysis (Atria *et al.*, 2017) as well as in a specimen-level phylogenetic analysis (Atria *et al.*, 2020). This species is generally an accepted taxon. Unfortunately, the phylogenetic analysis (Atria *et al.*, 2020), based on two markers (the chloroplast *mat*K and the nuclear 5SnrDNA), gave no real satisfying resolution for most specimens in the analysis. Only the 5S marker provided some structure in the cladogram in which most branches were not supported (in Figure 4-2 the highly supported groups are indicated as groups A–H).

Typical for all forms of *C. javensis* is a stem diameter of 2–6 mm without leaf sheaths and to 10 mm with sheaths; internodes up to 30 cm long (usually shorter); a distinct ocrea, deep crimson when young; pinnate, ecirrate leaves to 40 cm long, flabellate terminal leaflets and the lowermost pair often swept back across

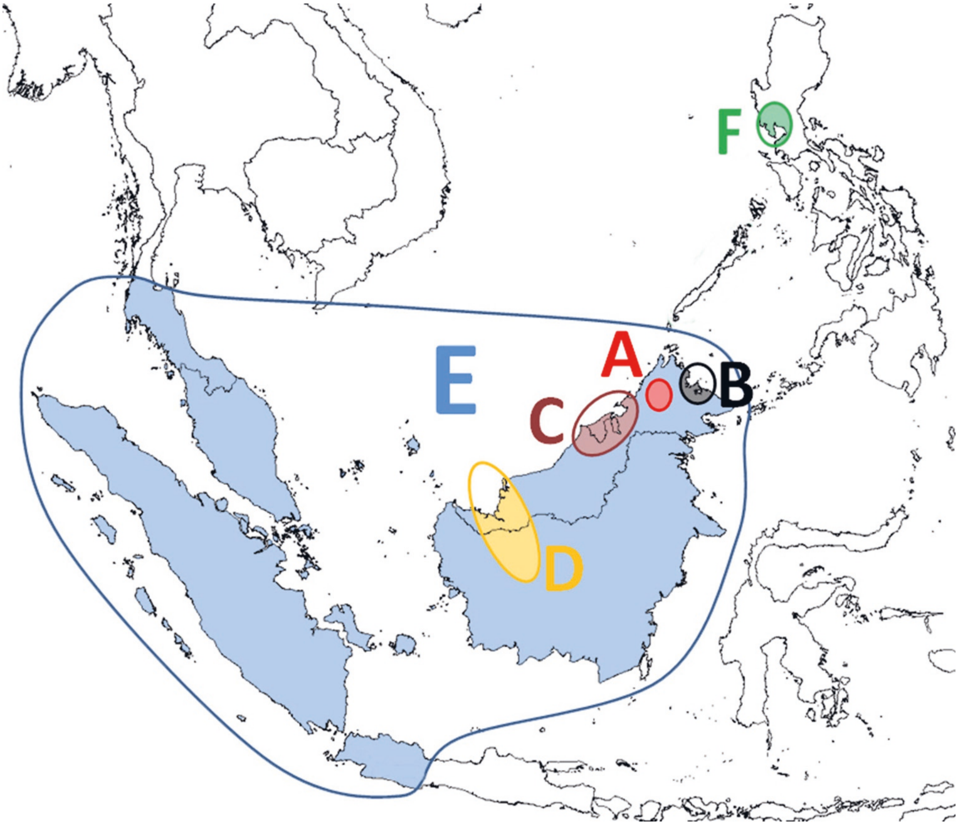

Figure 4-1. Areas as used in the historical biogeographic analysis.

the stem; a flagellum to 75 cm long, long inflorescences with red rachillae and ripe fruits ovoid in shape. *Calamus tenompokensis* can be distinguished from the *C. javensis* complex by its short stem, angular petiole and rachis, a very different leaf sheath appearance (the sheaths being massive and robust), number (9 pairs) and almost always regularly arrangement of the leaflets, staminate calyx swollen (versus not swollen in *C. javensis*), pistillate inflorescences with rachilla bracts with a broadly cupuliform limb (versus bracts tightly sheathing in *C. javensis*).

One other form is phenetically and phylogenetically rather distinct, '*acuminatus*' (to be united with form '*polyphyllus*'; will become a variety of *C. javensis* (Atria *et al.*, ms., to be submitted). This taxon always has many (9–12) narrow or linear leaflets, subequidistant to regularly arranged, subopposite and with a smooth or almost smooth leaf sheath. The inflorescences resemble those of form *javensis*, but those are mostly smaller or finer. The size of flowers and fruits is smaller, and the bracts of the peduncle are more cupuliform than form *javensis*.

The biogeographical range of the *C. javensis* complex includes southern Thailand, the Malay Peninsula, Sumatra, west Java, Borneo and Luzon in the Philippines (Figure 4-1). The greatest morphological diversity is found in north Borneo. The aim of this study is to see if historical geographic patterns developed in the various forms.

Table 4-1. Distribution area (Figure 4-1) of the samples used in the phylogenetic analysis (Atria et al. 2021). The group names (column 1) refer to mainly informal groups within C. javensis except for C. tenompokensis (recognized as species) and acuminatus (to be ***recognized as variety polyphyllus within C. javensis). All vouchers are in L (Naturalis Biodiversity Center, Leiden, The Netherlands) except SAN 21064 in K (Royal Botanic Gardens Kew, UK)***

Group name	Reference to molecular sample	Area	Voucher	Location
acuminatus	10A	B	Mega MAT 037	Sabah
acuminatus	10B	B	Mega MAT 028	Sabah
acuminatus	10C	C	SAN 85869	Sabah
acuminatus	10D	C	SAN 126575	Sabah
acuminatus	10E	B	Mega MAT 033	Sabah

Group name	Reference to molecular sample	Area	Voucher	Location
acuminatus	6G	C	Dransfield JD 5584	Sabah
amplijugus	1E	C	Mega MAT 109	Brunei
amplijugus	3D	B	Mega MAT 045	Sabah
amplijugus	3F	C	Mega MAT 109C	Brunei
congestiflorus	12E	C	Mega MAT 079	Sabah
corrugatus	6C	D	Dransfield JD 6080	Sarawak
corrugatus	6E	D	Mogea 3615	Central Kalimantan
corrugatus	6F	D	Dransfield JD 5868	Sarawak
elopurensis	10F	B	Dransfield JD 6265	Sabah
Form2	8E	F	Madulid et al. 7172	Luzon
Form4	11G	B	Chew & Corner RSNB 4835	Sabah
Form4	12D	A	Mega MAT 065	Sabah
impar	11A	E	Ave 136	Malay Peninsula
impar	11B	E	Niyomdham 1254	S. Thailand
impar	11D	A	SAN 21064	Sabah
javensis	1A	E	Mega MAT 001	W Java
javensis	1B	C	Mega MAT 093	Brunei
javensis	1C	E	Mega MAT 022	W. Java
javensis	1D	E	Mega MAT 008	W. Java
javensis	1F	E	Mega MAT 011	W. Java
javensis	1G	E	Mega MAT 005	W. Java
javensis	2C	C	Mega MAT 100B	Brunei
javensis	2E	E	Mega MAT 024	W. Java
javensis	3G	E	Mega MAT 007	W. Java
javensis	3H	E	Mega MAT 002	W. Java
javensis	4A	E	Ave 114	Malay Peninsula
javensis	4B	D	Dransfield JD 4728	Sarawak
javensis	4C	E	Ambri & Arifin W 915	E. Kalimantan
javensis	4D	E	Kato & Wiriadinata B 4943	E. Kalimantan
javensis	4H	D	Dransfield JD 4650	Sarawak

Group name	Reference to molecular sample	Area	Voucher	Location
javensis	5A	E	Dransfield JD 4519	Malay Peninsula
javensis	5B	C	S 50593	Sarawak
javensis	5E	E	Dransfield JD 3613	Sumatra
javensis	6A	D	S 54137	Sarawak
javensis	6B	E	van Valkenbrg 1320	E. Kalimantan
javensis	7A	E	Mega MAT 097	Brunei
javensis	7C	C	S 52424	Sarawak
javensis	7F	E	Dransfield JD 2553	Sumatra
javensis	8B	E	Mega MAT 057	Sabah
javensis	8D	E	Ave 216	Malay Peninsula
polyphyllus	2B	C	Mega MAT 103A	Brunei
polyphyllus	3C	A	Mega MAT 058	Sabah
polyphyllus	10G	B	Mega MAT 027	Sabah
polyphyllus	12B	C	Mega MAT 095	Brunei
polyphyllus	12C	E	Mega MAT 080	Sabah
tenompokensis	10H	A	Mega MAT 055	Sabah
tenompokensis	11E	A	Mega MAT 054	Sabah

4.2. Materials and Methods

The results of the phylogenetic analysis (Figure 2; Atria *et al.*, 2020 or Figure 3-2) formed the basis for the historical biogeographic analysis. A total of 52 samples was used to sequence the nuclear 5S spacer and the chloroplast *mat*K regions. All samples, molecular methodology and phylogenetic methods are explained in Atria *et al.* (2020). *Calamus flabellatus* Becc. (silica sample) acted as outgroup in the phylogenetic parsimony and Bayesian phylogenetic analyses. The outgroup was removed again in the historical biogeography as it was unknown how closely related the *C. flabellatus* is to *C. javensis*.

Taxon sampling

The 52 samples of silica gel-dried leaf fragments and herbarium specimens comprised one of the syntypes, from west Java (*Blume* s.n. (L, sheet 900.182-94), while also silica gel-dried material from the type locality was included. All samples cover the distribution area of the *C. javensis* complex. A list of voucher specimens can be found in Appendix 1 of Atria *et al.* (2020) .

Areas

Areas are recognized via a combination of the distribution of informal groups (names in the phylogeny, Figure 2; Atria *et al.*, 2020 or Figure 3-2) and monophyletic groups. This resulted in the discrimination of the following areas (Figure 4-1): A = NW Sabah (Borneo), B = NE Sabah (Borneo), C = Brunei with small parts of N Sarawak and SE Sabah (Borneo), D = central part of Sarawak and part of Kalimantan Barat (Borneo), E = remaining area (S Thailand up to Java and rest of Borneo), F = W Luzon (Philippines). Table 4-1 shows the areas as per specimen.

Historical Biogeographic Analysis

All analyses were carried out in RASP v.4.0 (Reconstruct Ancestral State in Phylogenies; Yu *et al.*, 2015). Three modules in RASP were used: parsimony-based S-DIVA (Statistical, DIspersal-VIcariance analysis, based on the DIVA method of Ronquist, 1997), maximum likelihood based DEC (Dispersal Extinction Cladogenesis; Ree *et al.*, 2005; Ree & Smith, 2008) and S-DEC (Statistical DEC). As input all cladograms (18,000, burn-in removed) found with a bayesian phylogeny analysis (Atria *et al.*, 2020) were used and the Maximum Clade Credibility (MCC) tree derived from those cladograms. DEC only uses the MCC tree for the analysis, the Statistical modules reconcile all trees against the MCC tree, thus introducing a kind of consensus per branch in the MCC tree (and adding a more Bayesian approach to DIVA).

In all modules the number of areas allowed per node was increased from 2 to 6 (maximum possible) per analysis. All other settings were default, no additional time frames were added in DEC as the MCC tree (Atria *et al.*, 2020) was not dated.

4.3. Results

The results of all analyses are summarized in Figure 4-2 for the maximum number of areas (6) per node. Even though more areas were allowed, at most only 3 areas resulted as best optimisation per node, but usually 2 or a single area proved to be the best results. As could be expected, these optimisations, independent of the method, generally include area E as this is by far the largest surface and most specimens have this distribution. The areas mentioned per node (Figure 4-2) are the ones with the highest probabilities, but as most node support was very low, therefore, these highest probabilities are also low and often many possible optimisations per node exist (not shown).

Even though most specimens occurred in area E, Borneo (areas A–D) is very important. In the upper clade (Figure 4-2, nodes 54–67, containing the groups D–H) especially area B (NE Sabah), is important, followed by areas A (Kinabalu and adjacent areas) and C ("Brunei"). In the other clade (Figure 4-2, nodes 68–103) it are especially areas C and D (central Sarawak, part Kalimantan Barat) on Borneo. Of course, area E comprises, among other areas, the rest of Borneo. No other obvious patterns were visible per form name or in most clades.

Of the groups that can be recognized it is obvious that *C. tenompokensis* (Figure 4-2, group E) originated in area A. The only other taxon is *C. javensis* var. *acuminatus* (Figure 4-2, mainly group F), which occurs in the Bornean areas B and C. Unfortunately, only represented once in the phylogeny, is 'Form 2', the only one found in the Philippines on Palawan (Figure 4-2, halfway, blue).

4.4. Discussion

The basal node (Figure 4-2, node 104) indicates as best optimisation area BCE or BE, but these areas cannot be regarded as the area of origin of *C. javensis*. For that purpose, this phylogeny should be embedded in a much larger phylogeny, preferably the whole of *Calamus* (which has to be based on more markers than the phylogeny used here). Borneo is one of the oldest areas in W Malesia that was already (partly) above water more than 60 Ma (Hall, 2013), especially the southern part. This in combination with the high variability on Borneo may indicate that perhaps the species originated on Borneo, but at least it was already present on the island in an early phase of its evolution.

Malesia, as a tropical rainforest area, differs from Amazonia and the Congo rainforest. During interglacial periods, like the present, the latter two occupy their largest surface area, while Malesia is then at its smallest (e.g., Morley & Flenley, 1987; Cannon *et al.*, 2009). During glacial periods these reverses,

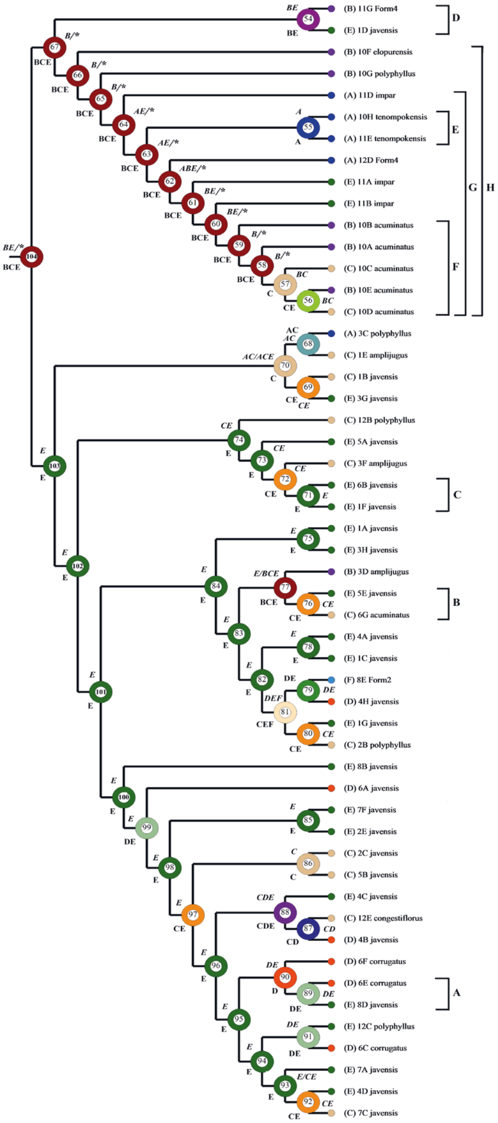

Figure 4-2. Areacladogram for the Calamus javensis complex. The groups indicated are the ones with a high (0.85 posterior probability) in the phylogenetic analysis (Atria et al. 2020). The terminal taxa have their distribution between brackets in front of the name. The various areas can be found in Figure 4-1. On the internal nodes the optimisations according to S-DIVA (bold), DEC (bold italics) and S-DEC (if similar to DEC then only one value, otherwise behind the /).

drought reduces the rainforest areas in Amazonia and the Congo to refuge areas, while the forest in Malesia extends to its maximum due to the emergence of a dry Sunda Shelf (connecting the Malay Peninsula, Sumatra, Borneo and Java) and Sahul Shelf (connecting New Guinea to Australis). However, not the whole Sunda Shelf becomes an everwet area during glacial periods, a large savannah corridor (complete extend unknown: Cannon *et al.*, 2009) develops from the Malay Peninsula via the lowest areas towards Java and involves the southern part of Borneo. NE Borneo (area B) often acts as a refuge area for species, as shown for the Dipterocarpaceae by Raes et al. (2014) via species distribution modelling for the last glacial maximum (21 kya). This might also have been an important refuge area for *Calamus javensis*, as area B is important in the upper clade (Figure 4-2, nodes 54–67).

Calamus tenompokensis (group E in Figure 4-2) is typically a local endemic that originated at the higher altitudes of Mount Kinabalu and the Crocker Range in NE Sabah (area A). Something similar applies to the '*acuminatus*' form (group F in Figure 4-2), but the ecological conditions under which the form develops are less obvious. It occurs in areas B (NE Sabah) and C ("Brunei") and if the strongly resembling form '*polyphyllus*' is also included, then also once in area A (NW Sabah). All other forms (see names in Figure 4-2) occur mixed throughout the phylogeny. This likely indicates that *C. javensis* is genetically quite plastid, adapting (relatively?) easily to varying environmental circumstances as long as these are in the more everwet areas, whereby similar forms can occur in very disjunct areas, that probably have a more or less similar habitat. This can be seen in Table 4-1 as various recognisable forms occur in different areas.

During glacial periods land bridges occurred, one ranges from NE Borneo via Mindanao to Luzon (*e.g.*, Morley & Flenley, 1987: fig. 5.5). *Calamus javensis* may have dispersed to Luzon via this bridge, but as the climate and soils differed the species adapted, which resulted in 'Form 2'. can for instance also be seen in *Guioa pleuropteris* (Blume) Radlk. (Sapindaceae), where the leaflets became much smaller with a different hair type than found on Borneo (van Welzen, 1989: fig. 110). Similar geoclines, indicative of the landbridge, are also present in two other Sapindaceae: *Gloeocarpus patentivalvis* (Radlk.) Radlk. (van Welzen, 1991) and *Lepidopetalum perrottetii* (Cambess.) Blume (van Welzen *et al.*, 1992).

In conclusion, partly due to the lack of phylogenetic signal, it is obvious that there is no historical biogeographic pattern behind the various recognisable forms in the *C. javensis* complex. Forms are very likely the result of adaptation to local circumstances, whereby more or less identical forms can develop under similar niche circumstances in disjunct areas. Exceptions are the ‘*acuminatus-polyphyllus*’ form and *C. tenompokensis*.

Chapter 5

Revision of the rattan *Calamus javensis* Blume Complex (Arecaceae, Calamoideae) in Malesia with notes on all recognisable taxa and forms

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Abstract -- The problem of polymorphism in the *Calamus javensis* complex has been examined by various techniques in several publications. A wide range of morphological variation was identified during these studies. Characters showing high variation were leaf sheath and ocrea morphology, number and arrangement of leaflets, length, and arrangement of the rachillas. All names used in the complex are regarded as synonyms of *C. javensis* except *C. tenompokensis*, which is retained as a distinct species. One variety is here recognized, *C. javensis* var. *polyphyllus* (including *C. acuminatus*). Using morphological characters, we recognized 11 informal (often not monophyletic) forms within *C. javensis* var. *javensis*. Here we present identification keys, descriptions and notes of all recognisable forms, varieties and species included in the *Calamus javensis* complex.

5.1. Introduction

As a large genus, *Calamus* has long been of interest in the world of palm taxonomy. Recently, Henderson (2000) has revised the entire genus, which resulted in 411 phylogenetic species; of these, 38 are recognized as new and eight species as ochlospecies. *Calamus javensis* has been identified as an ochlospecies with 11 species names subsumed in it, namely *C. acuminatus Becc., C. amplectens Becc., C. amplijugus J.Dransf., C. congestiflorus J.Dransf., C. corrugatus Becc., C. elopurensis J.Dransf., C. filiformis Becc., C. hypertrichosus Becc., C. impar*

Becc., C. javensis Blume, and *C. kemamanensis Furtado*. Henderson (2020) treated morphotypes as recognized but not named.

Calamus javensis Blume is a polymorphic species that forms one of the most difficult complex species in Southeast Asian palms (Dransfield,1999; Henderson, 2020). The species has a wide distribution, ranging from Southern Myanmar, Southern Thailand, and Peninsular Malaysia, throughout Sumatra, Java, and Borneo to Palawan and Luzon in the Philippines. *Calamus javensis* occurs in all major forest types except mangrove, from sea level to about 2000 m altitude and is found on a wide range of soils in tropical rainforest (Dransfield and Manokaran, 1993 Dransfield, 1999; Watanabe and Suzuki, 2007). Rattans prefer soils rich in organic carbon that are not subjected to severe dry conditions and water logging (Pantanella, 2005). However, we observed that some forms of *C. javensis* grew abundantly outside the dense forest in a soil mixed with very fine gravels in Bukit Hampuan and Telupid, Sabah.

All forms in the *C. javensis* complex are defined by the presence of a flagellum, papyraceous leaves, the terminal leaflet pair conspicuously connate, distant partial inflorescences, with rachillae generally diverging at 90 degrees with rather distinctive, closely set rachilla bracts, the whole inflorescence cherry-red at anthesis, and fruits with rather flat, white scales (Dransfield, 1992). Because of a high variation in width of stem, leaf sheath profile and spines, ocrea, leaflet number, shapes, and arrangement, inflorescence structure, and fruit shape and scales, a description is long and with few non-variable characters (see below).

The high variation resulted in the description of various forms as species or infraspecific taxa. A chronological history of names and taxa involved in the *Calamus javensis* complex was presented in Atria et al. (2017). The status of these names is variable and inconsistent as some forms are more obvious and without transitional forms than others. However, a taxonomic revision of the *C. javensis* complex was never completed. Interpretations of species boundaries based on morphological characters alone frequently generated misleading results when only a limited number of samples were used, because discontinuity in a character can disappear once the sampling intensity is increased (Dransfield, 1999).

A series of studies revealed the polymorphic problem in the *C. javensis* species complex. The two most distinctive taxa in Sabah related to *C. javensis* were separated at the specific level, *C. tenompokensis* Furtado and *C. amplijugus*

J.Dransf. The other taxa in this complex appear to intergrade (Dransfield, 1997). But, based on recent work with multivariate (Atria et al., 2017) and molecular analysis (Atria et al., 2020), *C. amplijugus* is part of the *C. javensis* group/clade and not distinctive. Both studies showed that in the complex only two groups of specimens can be distinguished, one is the robust short-stemmed rattan *C. tenompokensis* and the other is the large group of *C. javensis*, which consists of all the other forms and taxa formerly described in the complex. The markers used (*mat*K and 5SnrDNA) gave little resolution in the phylogeny reconstruction (Atria et al., 2020), but a few groups in the *C. javensis* complex are phylogenetically and morphologically supported to some degree. More sequences of other markers or complete genomes should be added before a clear picture of the complex can be given.

The multivariate analysis (Atria et al., 2017) also showed *C. acuminatus* Becc. as more or less distinct. Phylogenetically (Atria et al., 2020), this form is less apparent, but the narrow distribution and ecological preferences support the idea to recognise *C. acuminatus* as a distinct entity at the varietal level. Morphologically it cannot be separated from *C. javensis* Blume var. *polyphyllus* (Becc.) Becc. with which it is synonymized here. Notes about *C. acuminatus* will be presented under the description of the latter variety.

Calamus javensis seemingly adapts morphologically very well to local circumstances. This probably also means that local, recognizable forms may arise independently in parallel as several forms have disjunct distributions and/or are present in different clades (Atria et al., 2020). Recognition of the forms in informal groups, as we will do here, likely indicates that the specimens in these groups live under comparable conditions.

The polymorphism may also occur as a result of hybridization. In the Arecaceae, hybridization is common in date palm species, *Phoenix* spp. (Coryphoideae) (Pintaud et al., 2010; Gros-Balthazard, 2013; Flowers et al., 2019). The possibility of hybridization may occur in *Calamus javensis* but in our series of studies we did not focus on this nor did the material or results give rise to suspect this. There is no report on hybridization in ratan species so far.

The aim of this paper is to describe the various taxa and informal groups that are morphologically recognisable.

5.2. Materials and Methods

In this study we use the groups described by Atria et al. (2017, 2021) and the recent revision of *Calamus* by Henderson (2020) and the revision of the Aceraceae for Thailand by Barfod and Dransfield (2013). Morphological examinations were conducted on fresh material collected during fieldwork in Brunei, Java, and Sabah, and on herbarium specimens made available by several herbaria: Herbarium Bogoriense (BO), Herbarium Firenze (FI), Royal Botanic Gardens Kew (K), Naturalis Biodiversity Center (L, U), the Sandakan Herbarium (SAN), and the Sarawak Herbarium (SAR) (see Thiers, continuously updated, for herbarium abbreviations).

5.3. Results

Key to the species and varieties in the *Calamus javensis* complex

1a. Leaf sheath abundantly covered with horizontal, hairy, black-tipped spines, 1–5 cm long. Flagellum absent or short (rarely longer than 1 m). Petiole and rachis somewhat angular. Leaflets 8 or 9 pairs, opposite, narrowly to broadly elliptic. Inflorescences to c. 1 m long, but some only c. 25 cm long. Staminate calyx tubular, lobes swollen. Pistillate rachillae bracts with a broadly cupuliform limb **2. C. tenompokensis**

b. Leaf sheath (smooth to) mostly sparsely (to densely) armed with various spines, spines mostly glabrous (some with hairs along the margin), straight and patent, reflexed upward or downward or mixed in orientation, stout, clawed to solid pointed, mostly with a black tip, 2–7(–15) cm long. Flagellum to c. 150 cm long. Petiole and rachis rounded. Leaflets 2–12 pairs, usually opposite to subopposite (to alternate), mostly lanceolate to ovate, very rarely broad-ovate. Inflorescences up to 1.5 m long. Staminate calyx basally tubular, lobes not swollen. Pistillate rachilla with tightly sheathing bracts **2 (1. C. javensis**)

2a. Leaflets in 1–6 pairs, rarely to 12 pairs, grouped or more rarely subregular, usually opposite to subopposite, some alternate, mostly lanceolate to ovate, very rarely broadly ovate. Inflorescences up to 1.5 m long, staminate and pistillate ones almost similar. Fruits oblong to some ellipsoid to globose, up to 10 by 8 mm; pericarp covered with 15–19 vertical rows of scales, yellow to light brown scales with reddish-brown lines, apex mucronate **1a. C. javensis** var. **javensis**

b. Leaflets in 9–12 pairs, regularly arranged or sub-equidistant, subopposite to alternate, lanceolate to narrowly elliptic, some elliptic. Staminate inflorescences c. 50 cm long; pistillate ones to c. 140 cm long. Fruits broadly ovoid to globose, 4–6 by 3–4 mm; pericarp covered with 8 vertical rows of scales, yellowish with red tinged acuminate apex **1b. C. javensis** var. **polyphyllus**

1. Calamus javensis Blume

Calamus javensis **Blume (1847, p. 62)**

Based on the same type: *Palmijuncus javensis* (Blume) Kuntze (1891, p. 733).

Type: Indonesia. Java, *C.L. Blume s.n.* (lectotype: L-0699971!, selected by Henderson (2020, p. 217); isolectotype: P-02147162! [photo image]).

Taxonomic synonyms: *Calamus javensis* var. *firmus* Blume (1847, p. 62). **Type:** not indicated by Henderson (2020, p. 217).

– *Calamus javensis* var. *tetrastichus* Blume (1847, p. 62), basionym of *Calamus tetrastichus* (Blume) Blume (1847, p. 62, tab. 153), based on the same type as *Palmijuncus tetrastichus* (Blume) Kuntze (1891, p. 733). Type: Blume, 1847, t. 153 (lectotype, designated by Henderson (2020, 217)).

– *Calamus borneensis* Miq. (1850, p. 4). Based on the same type as *Palmijuncus borneensis* (Miq.) Kuntze (1891, p. 733). **Type:** not indicated.

– *Calamus amplectens* Becc. (1884, p. 78), based on the same type as *Palmijuncus amplectens* (Becc.) Kuntze (1891, p. 733). **Type:** Malaysia. Sarawak, *O. Beccari s.n.* (lectotype: FI- 013529 selected by Henderson (2020, p. 217)).

– *Calamus javensis* var. *peninsularis* Becc. (1892, p. 442). **Type**: Malaysia. Perak, *King's collector 1996* (female) (lectotype: K-000207740!, designated here). Henderson (2020) indicated *Scortechini 236b* (FI) as lectotype, but this collection is not mentioned by Beccari (1892) and cannot serve as lectotype.

– *Calamus javensis* var. *peninsularis* subvar. *intermedius* Becc. (1892, p. 443), basionym for *Calamus javensis* var. *intermedius* (Becc.) Becc. (1902, p. 201). **Type:** Malaysia. Perak, *B. Scortechini 236b* (lectotype: FI-018943*), indirectly selected by Henderson (2020, p. 217)). Henderson (2020) regarded the specimen in FI as holotype, but Beccari (1892) did not indicate a holotype, which makes all duplicates syntypes, therefore we consider Henderson's choice as a lectotypification.

- *Calamus javensis* var. *peninsularis* subvar. *penangiana* Becc. (1892, p. 443). *Calamus penicillatus* var. *penangiana* (Becc.) Ridl. (1907, p. 192). Based on the same type of *Calamus javensis* var. *penangiana* (Becc.) Ridl. (1925, p. 51). **Type:** Malaysia. Pulau Penang: Mount Elvira, *C. Curtis s.n.* (lectotype: SING-0186878 (photo image), selected by Henderson (2020, p. 217)).
- *Calamus javensis* var. *peninsularis* subvar. *purpurascens* Becc. (1892, p. 443). **Type:** Malaysia. Perak, *King's collector 7932* (lectotype CAL, *n.v.*; isolectotypes: BM, *n.v.*, FI-018947*(photo image), K-000207739!, selected by Henderson (2020, p. 217).
- *Calamus javensis* var. *peninsularis* subvar. *tenuissimus* Becc. (1892, p. 443). Basionym of *Calamus javensis* var. *tenuissimus* (Becc.) Becc. (1902, p. 201). **Type:** Malaysia. Perak: summit of Gunong Tambang Batok, *Scortechini 648b* (lectotype FI-018949*(photo image), indirectly selected by Henderson (2020, p. 217)). Henderson (2020) regarded the specimen in FI as holotype, but Beccari (1892) did not indicate a holotype, which makes all duplicates syntypes, therefore we consider Henderson's choice as a lectotypification.
- *Calamus filiformis* Becc. (1902, p. 609). **Type:** Malaysia. Sarawak: Mount Mattang, prop. Kutcing [Kuching], *O. Beccari PB 1909* (holotype: FI- 008448).
- *Calamus corrugatus* Beccari (1902, p. 201). **Type:** Malaysia: Sarawak, *O. Beccari PB 1910* (holotype: FI- 008446).
- *Calamus penicillatus* var. *purpurascens* Ridl. (1907, p. 192). Basionym of *Calamus javensis* var. *purpurascens* (Ridl.) Ridl. (1925, p. 49). **Type:** Malaysia. Perak: Gunong Batu Putih, *L. Wray 981* (lectotype: CAL, *n.v.*, selected by Henderson 2020, p. 217).
- *Calamus penicillatus* var. *inermis* Ridl. (1907, p. 192). Basionym of *Calamus javensis* var. *inermis* (Ridl.) Ridl. (1925, p. 51). **Type:** Malaysia. Negeri Sembilan: Gunong Angsi, *H.N. Ridley s.n.* (lectotype: SING- 0056674, selected by Furtado (1956, p. 176).
- *Calamus javensis* var. *javensis* subvar. *exilis* Becc. (1908, p. 178). **Type:** Indonesia. Java, *C.G.C. Reinwardt s.n.*, (holotype: M-0199069*[photo image]).

– *Calamus javensis* var. *sublaevis* Becc. (1908, p. 184). **Type:** Malaysia. Sarawak: Kutcing [Kuching], Mt. Mattang, *O. Beccari PB 1694* (holotype: FI-013528).

– *Calamus javensis* var. *acicularis* Becc. (1908, p. 185). **Type:** Malaysia. Sarawak: Mt. Mattang [Matang], *R.W. Hullett 3471* (lectotype: FI-018945* (photo image), indirectly selected by Henderson (2020, p. 217); isolectotype: SING-0192571). Henderson (2020) regarded the specimen in FI as holotype, but Beccari (1908) did not indicate a holotype, which makes all duplicates syntypes, therefore we consider Henderson's choice as a lectotypification.

– *Calamus javensis* var. *tetrastichus* subvar. *mollispinus* Becc. (1913, p. 15). **Type:** Indonesia. Borneo: Sungei Mandai, *J.G.(H.) Hallier 2569* (holotype: BO-0036501; isotype: FI-018948* (photo image)).

– *Calamus hypertrichosus* Becc. (1913, p. 17). **Type**: Indonesia, Borneo: *J.E. Teijsmann HB 16330* ((holotype: BO-0039113!; isotype: iso FI-018946* (photo image)).

– *Calamus impar* Becc. (1913, p. 19). **Type**: Indonesia, Borneo: Sungei Kenepai, *J.G. (H.) Hallier 2033* (holotype: BO-0063384!; isotype: FI, *n.v.*)).

– *Calamus fissijugatus* Burret, (1943, p. 804). **Type:** Indonesia. North Sumatra: Sabang, *B. Krug 145* (holotype: B, destroyed).

– *Calamus kemamanensis* Furtado (1956, p. 170). **Type:** Malaysia. Terengganu: Kemaman, Sungai Nipa, *E.J.H. Corner SFN 30535* (holotype: SING-0056675; isotypes: BH, *n.v.*, K-000207734!).

– *Calamus javensis* var. *laevis* Furtado (1956, p. 177). **Type:** Malaysia. Johore: Sedinak 8, *H.N. Ridley 13518* (holotype: SING-0056830; isotype: BM, *n.v.*).

– *Calamus congestiflorus* J.Dransf. (1982, p. 785). **Type:** Malaysia. Sabah: Nabawan District, mile 46, Nabawan, *J. Dransfield et al. 5635* (holotype: K-000113076!; isotypes: KEP, *n.v.*, L!, SAN, *n.v.*, SAR, *n.v.*).

– *Calamus amplijugus* J.Dransf. (1982, p. 787). **Type:** Malaysia. Sabah: Sandakan District, Lungamis Experimental Plots, *J. Dransfield 5775* (holotype: K-K000112913!; isotypes: L, *n.v.*, SAN, *n.v.*, SAR, *n.v.*).

– *Calamus elopurensis* J.Dransf. (1982, p. 787). **Type:** Malaysia. Sabah: Sandakan, Elopura, Sepilok Forest Reserved, *B. Kadir A 2651* (holotype: K-000113249!; isotypes: KEP, *n.v.*, SAN, *n.v.*, SING, *n.v.*).

– *Calamus equestris* auct. non Willd.: Blume (1830, p. 1330).

(Shrub to) liana, small, clustering, dioecious; stem with leaf sheaths 5–20(–35) mm in diameter, without sheaths to about 8(–10) mm in diameter; internodes to 30 cm long. *Sheaths* light green to dark brown when dry; smooth to distinctly striate; (smooth to) mostly sparsely (to densely) armed with various spines, spines straight and patent, pointing upward or downward or mixed in orientation, flattened triangular, stout, clawed to solid pointed, mostly with a black tip, 2–7(–15) cm long, base almost always swollen, apex usually with a black tip; knee present, sometimes conspicuous; ocrea reddish, quite conspicuous, quickly tattering, rarely persistent. *Flagellum* up to 150 cm long, with groups of 2- or 3-clawed spines. *Leaves* alternate, ecirrate, 35–40(–60) cm long, petiole usually very short, only 2–5 cm long, but occasionally longer, with abundant dark hairs in young plants; rachis sparsely armed with small, curved or clawed spines, sometimes in groups of 2 or 3, rarely smooth, sometimes with some indumentum. *Leaflets* 2–6(–12) on each side of the rachis, grouped or more rarely subregular, usually opposite to subopposite (to alternate), mostly lanceolate to ovate, very rarely broad-ovate, the largest ones up to 29 by 4 cm, with 3 conspicuous parallel main veins; apex acuminate, mostly with spinules, latter sometimes also along the margin; lowermost pair always swept back across the stem, in some forms clasping the stem; median leaflet pairs usually smaller than the other two above, but sometimes the longest pair; adult leaves always with a terminal flabellum formed by 2 apical leaflets joined for ¼–¾ of their length, the latter do not always form the largest leaflets; the pair of leaflets below the flabellum (penultimate) usually very close (0–1 cm distant) to the flabellum, rarely far apart (more than 3 cm), opposite to subopposite, rarely regularly arranged, mostly not grouped. *Inflorescences* up to 1.5 m long, pendulous, staminate and pistillate ones almost similar, staminate ones finer/more slender than pistillate ones, each with 4–8 partial inflorescences, rachillae 4–8 cm long in staminate inflorescences and up to 12 cm long in pistillate inflorescences. *Prophyll* tubular at base, tightly enclosing the rachis, some loosely enclosing with a long distal opening, smooth to more spiny with sparse, small, black tipped spines, curved or tending to be so in the end; peduncular bracts with tubular base, enclosing the rachis but free at the end, with sparse, small, curved spines; rachis bracts enclosing the rachis tightly, some loose and keeled, mostly smooth or prickly with sparse, small, curved spines, rarely densely so. *Staminate rachillae* with bracts, explanate, 1.5–2 mm by c. 1 mm; 1 striated tubular prophyllar bracteole subtending one staminate flower within the axil of the rachilla bract. *Pistillate rachillae* bigger than staminate one, with tightly sheathing rachilla bracts; prophyllar bracteoles tubular with two opposite acute tips, striate, subtending a dyad of a pistillate and a sterile staminate flower, each flower with only c. 1 mm interval on each side and thus appearing crowded. *Staminate flowers*: calyx basally tubular, deeply divided into 3 lobes (lobes not swollen), valvate, free on the top with acute tip; lobes elliptic, c. 1.5 by 1 mm; corolla usually quite far exceeding the calyx, petals oblong, 3–3.5 by 8–10 mm; stamens six, filaments narrow, tapering to the top, 1.5–2 mm long, tip hooked, anthers c. 1.5 mm long, dorsifixed, hanging freely, versatile, opening latrorse with longitudinal slit. *Pistillate flowers*: calyx tubular, basally connate, shallowly divided into 3 valvate lobes, latter broadly elliptic, 1–1.8(–2) by 1.3–1.5(–1.8) mm; corolla rarely or slightly exceeding the calyx, petals ovate to elliptic, 2–2.5 by 0.8–1.5 mm, apex acute to acuminate; staminodes 6, epipetalous, the filaments united into a membranous, toothed-ring with empty anthers attached on top; pistil about 2–2.5 mm long, style short, together with stigma only 1–1.5 mm long, with 3 reflexed fleshy stigmas. *Fruits* one-seeded, oblong to somewhat ellipsoid to globose, up to 12–15 by c. 8 mm, mucronate, pericarp covered with 8–15(–19) scales, c. 3 by 1–1.5 mm, yellow to brown, with reddish brown margins. *Seeds* rounded, somewhat angular, 8–11 by 7–10 mm, endosperm homogenous.

Distribution

Southern Myanmar, Peninsular Thailand, Peninsular Malaysia, Singapore, Sumatra, western Java, Borneo, and the Philippines (Palawan & central Luzon).

Habitat & ecology

Calamus javensis ranges from the lowlands to up to 2000(–2600) m altitude and occurs on a wide variety of soils. Flowering: whole year through, but mainly April to August; fruiting: June to November.

Vernacular names

Thailand: Rote batu, Wai ukan, Wai tek (S part). Malay Peninsula: rotan lilin (throughout the peninsula); coonk stook (Perak). Sumatra: Rotan opot (Bengkulu). Java: Howe cacing (Western Java). Borneo: Sabah: Lempinit ular-ular; Kalimantan: Rotan lilin. Philippines: Arorog, Arurug (Palawan).

Uses

People make baskets and musical instruments from the cane, and they use it as binding material, for traps and carrying baskets. The length and strength of the cane itself is excellent and good in quality second to *C. caesius* Blume. The raw cabbage is eaten to cure coughs (Dransfield J. and Manokaran, 1993; Watanabe et al., 2006; Barfod and Dransfield, 2013).

Notes

The type of *Calamus javensis* Blume (1847) was collected on the Halimun-Salak mountain in West Java. However, already at this location, individuals showed variation in leaf blades and leaf sheaths, linked by intermediates. *Calamus javensis* populations found in northern Borneo show most variation, perhaps dependent on the various ecological niches, not only in the leaf sheath spines, leaflets number and arrangement, but also the ocrea, inflorescence rachillae, and some exhibit an erect habit; but individuals with intermediate characters can also be found. The previous studies on morphometry and phylogeny of *C. javensis* (Madulid 1981, Atria et al. 2017, Atria et al. 2020, Henderson 2020) showed that almost all variation (*C. tenompokensis* excepted) is part of one big cluster of specimens, *C. javensis*. The complex species of *C. javensis* have also been discussed in a work of Syam et al. (2016). Their treatment of *C. javensis* differed from our (Atria, 2017), as they regarded all names as part of the *C. flabellatus* Furtado complex (including not only *C. javensis*, but also *C. acuminatus, C. amplijugus, C. congestiflorus, C. corrugatus, C. hypertrichosus,* and *C. ruvidus*). The name is incorrect as *C. javensis* is the oldest name. *Calamus javensis* is a polymorphic taxon and consists of several small groups of specimens that show recognizable characters next to the more amorphous mass of most specimens. These recognizable groups are here described as informal taxa (group). As most specimens group under Form *Javensis*, this group will not be keyed out, due to too much overlap; therefore, all specimens that cannot be assigned to any of the Forms are part of Form *Javensis*. Specimens examined for Form *Javensis* are listed in Table 5-1.

Table 5-1. Specimens examined for Calamus javensis var javensis

Brunei, Mendaram: Atria MAT 108 (BRUN, L); Mendaram, Atria MAT 108 B (BRUN, L); Mendaram, Atria MAT 108 C (BRUN, L); Mendaram, Atria MAT 110 (BRUN, L); Kuala Belalong, Sg. Belalong riverside, Atria MAT 097 (BRUN, L); Sg. Belalong riverside, Atria MAT 100 B (BRUN, L); Sg. Belalong riverside, Atria MAT 100 C (BRUN, L); Temburong, North ridge of Bukit Retak, K.M. Wong WKM 745 (K); Bukit Belalong, south ridge, K.M. Wong WKM 1386 (K); Kuala Belalong, Canopy Trail, Atria MAT 103 A (BRUN, L); Sg. Belalong, Atria MAT 097 (BRUN, L); Kuala Belalong, Ashton trail, Atria MAT 092 (BRUN, L); Kuala Belalong, Ashton trail, Atria MAT 093 (BRUN, L); Kuala Belalong, Ashton trail, Atria MAT 095 (BRUN, L); Kuala Belalong, Ashton trail, M.J.E. Coode 7022 (K). Indonesia, Java: Lotzy s.n. (L); Batavia, Tjimanglit, Buitenzorg, Backhuizen vd brink 6273 (L); Buitenzorg, cultivated, Furtado SFN 30842 (L); West Java, Jasinga, Mt. Gede, Backer 9996 (L); West Java: Mt. Sanggabuana, Backer 23821 (L); West Java, Cibodas, forest fragmen in garden, Dransfield JD 1106 (L); West Java, Sukabumi, Jampang Tengah, Lengkong, Kosterman 23841 (L); West Java, Sukabumi, Jampang Tengah, Lengkong, Dransfield JD 1033 (L); West Java, Mt. Patuha, Junghun 43 (L); West Java, Sukabumi, Ciayunan, Dransfield JD 3086 (L); West Java, Junghun sn (L); West Java, Preanger, Cibodas, Sapiin W 2374 (L); Mt. Halimun NP, Atria MAT 001 (BO, L); Mt. Halimun NP, Atria MAT 002 (BO, L); Mt. Halimun NP, Atria MAT 004 (BO, L); Mt. Halimun NP, Atria MAT 005 (BO, L); Mt. Halimun NP, Atria MAT 007 (BO, L); Mt. Halimun NP, Atria MAT 008 (BO, L); Mt. Halimun NP, Atria MAT 010 (BO, L); Mt. Halimun NP, Atria MAT 011 (BO, L); Mt. Halimun NP, Atria MAT 013 (BO, L); Mt. Halimun NP, Atria MAT 018 (BO, L); Mt. Halimun NP, Atria MAT 021 (BO, L); Mt. Halimun NP, Atria MAT 022 (BO, L); Mt. Halimun NP, Atria MAT 024 (BO, L); South west Java, Ujung Kulon, Mt. Payung, Kostermans s.n. (L); Ujung Kulon, Cijaga watershed, Cijaga Besar Forest, 5 km SE Taman Jaya, Mc Donald & Afriastini 3443 (K); Kalimantan, East Kalimantan, Wanariset Samboja, Ambi & Arifin AA 453 (L); Tabang, Mt. Mendam, Gen Murata, Kunio Iwatsaki, Masahiro Kato, JP Mogea B 2612 (L); Kutai, Mt. Mendam, Mogea JPM 1620 (L); Balikpapan, Kenayan, Dransfield JD 4375 (L); Berau, Clemens MCS 140 (L); North west Tabang, Mt. Batukenye, J.P. Mogea, Kunio Iwatsuki, Gen Murata, Masahiro Kato B 1590 (L); Mt. Batukenye, J.P. Mogea JPM 1591 (L); Kutai, Bantian Besar, Dilang Putih, Van Valkenburg JVV 1320 (L); Wanariset Rintis Wartono Kadri area, Ambri & Arifin W 915 (L); Mt. Kongkat, Masahiro Kato & Harry Wiriadinata B 4943 (L); West Kalimantan, Betung Kerihun NP, Putussibau, Mendalam river bank, Sidiyasa et al. 1953 (L); South Kalimantan, Kab. Tanjung, Muara Uja, Near Mt. Sarempaka, EF de Vogel 1769 (L); Mt. Sarempaka, EF de Vogel 1701 (L); Kalimantan Selatan, J.P. Mogea, Gen Murata, Masahiro Kato B 1692 (L); South Kalimantan, Banjarmasin, Batang Sengaleng, Mueller s.n. (L); South Kalimantan, Banjarmasin, Batang Sengaleng, Korthals s.n. (L); Borneo, Korthals no 9 (L). Central Kalimantan, Katingan, Bukit Raya, Mendawai area, J.P. Mogea 3988 (L); Bukit Raya, Tumbang Tapi, Hary Wiriadinata HW 3299 (L); Sumatra, Dransfield JD 3355 (L); East Sumatra, Parbasiran Merbau, Rahmat Si Toroes No 331 (L); East coast Sumatera, Mt. Pangaribuan, Labuan Batu, Rahmat Si Toroes No 4350 (L); Karo, Carel Hamel & Rahmat Si Toroes No 765 (L); Silo Maradja, Asahan, Bartlett 8095 (L); North Sumatera, Sibolangit, Karo Plateu, Kabanjahe, Lorzing 8744 (L); Karo, Kabanjahe, Lorzing 13718 (L); Bohorok, Bkt lawang, Mt. Sinar Alas, Harry Wiriadinata & Mashuri 669 (L); Masihi Forest Reserves, Asahan east coast, BA Krukoff 4181 (L); West coast, Batoe Island, Haap 556 (L); Tanjung Pura, Ulu Besitang, Dransfield JD & D. Saerudin 1830 (L); Southwest Sumatra, Enggano Island, WJ Lutjeharms WJL 4253 (L); West Sumatera, Muro Kulampi, Gunung Putih, Dransfield & J.P. Mogea JD JPM 3967 (L); Payakumbuh, Mt. Sago, Ladang lawas, W. Meijer No 4038 (L). Aceh Tenggara, Mt Kemiri, J.P. Mogea 626 (L); Jambi, Sungai Penuh, Tapan km 19, Dransfield & J.P. Mogea JD & JPM 4075 (L); Sungai Air Hitam, Berbak, peat swamp forest, Dransfield JD 2553 (L); Bengkulu, Kerinci Sebelat, Timothy J Forsyth TJF 29 (L); Bengkulu, Kerinci Sebelat, Kathrin Shreckenberg KS 19 (L); Bengkulu, Kepahiang km 20, Dransfield JD 3595 (L); Bengkulu, Kepahiang, Pagar Gunung, Dransfield JD 3613 (L); South Sumatra, Palembang, Kebau, Pematang Ulu, Lambach 1328 (L); Palembang, Furtado 30829 (L). Malaysia: Sabah, Tawau, Elmer 21331 (L); Sandakan, Pulau Sapi, Bukit Marango, Dransfield JD 5737 (L); Sandakan, Lungmanis, GHS Wood San A 3990 (L); Sandakan, Fidilis Krispinus SAN 95821 (L); Sandakan, Moore (L); Sandakan, Kabili – Sepilok FR, Atria MAT 030 (SAN, L); Kinabalu, Dransfield JD 5672 (L); Crocker range, Nordin Abas SAN 85981 (L); Kinabalu, Ranau, Dransfield JD 5699 (L);

Kinabalu, Dransfield JD 5673 (L); Kinabalu, Dransfield JD 5672 (L); Kinabalu National Park, Pandanus Trail, Atria MAT 066 (SAN, L); Kinabalu National Park, Pandanus Trail, Atria MAT 067 (SAN, L); Kinabalu National Park, Pandanus Trail, Atria MAT 068 (SAN, L); Kinabalu National Park, Pandanus Trail, Atria MAT 069 (SAN, L); Kinabalu National Park, Pandanus Trail, Atria MAT 070 (SAN, L); Mesilau Nature Center, pathway in front of the HQ, Atria MAT 071 (SAN, L); Mesilau Nature Center, pathway in front of the HQ, Atria MAT 072 (SAN, L); Mesilau Nature Center, pathway in front of the HQ, Atria MAT 073 (SAN, L); Mesilau Nature Center, Nepenthes trail gate, Atria MAT 074 (SAN, L); Mesilau Nature Center, Nepenthes trail gate, Atria MAT 075 (SAN, L); Mesilau Nature Center, Nepenthes trail gate, Atria MAT 076 (SAN, L); Beaufort, Halojilat, Padas river, Cuadra A 3023 (L); Ranau, Sagindai, Dewol S et al. SAN 100151 (L). Keningau, Sepulut FR, Amin & Sigin SAN 69127 (L); Keningau, Crocker Range Nature Center, Atria MAT 078 (SAN, L). Telupid, Sapa Payau FR, Dransfield JD 5811 (L); Telupid, slope of Bukit Tangkunan, Dransfield JD 5783 (L); Mount Tawai, Vermeulen No 869 (L); Tawai FR, Atria MAT 045 (SAN, L); Tawai FR, Atria MAT 047 (SAN, L); Tawai FR, Atria MAT 048 (SAN, L); Tawai FR, Atria MAT 049 (SAN, L); Tawai FR, Atria MAT 050 (SAN, L). Lahad Datu, Pulau Sakar, Aban Sibot SAN 54826 (K); Dumpinit, Balajadia 3755 (K); Ranau, Poring, Bukit hampuan FR, Atria MAT 052 (SAN, L); Ranau, near Mamut Copper Mine, Atria MAT 063 (SAN, L); Ranau, near Mamut Copper Mine, Atria MAT 064 (SAN, L); Ranau, near Mamut Copper Mine, Atria MAT 065 (SAN, L). Sipitang, Mandulang-Maligan, Y.F. Lee & Dewol SAN 69853 (L). Sarawak: Sabal Tapang FR, Dransfield JD 4650 (L); Belaga, Ulu Belaga, Kuala Linau, Rumah Nyauing, Sg. Masoh, Dransfield JD 4706 (L). Kuching, Mt. Matang, Dransfield JD 4728 (L); Mt. Matang, Bernard Lee Meng Hock S 54137 (L); Mt. Matang, Ridley s.n. 2 (K); Lawas, Ulu Trusan, Bukit Tebunan, Bernard Lee S 52424 (L); Kelabit, Bukit Batu Lawi, Bario, Dyg. Awa & Bernard Lee S 50593 (L); Kapit, Balleh, Ulu mengiong, Sg. Entejum, Hj. Othman, Rantai & Jugah S 56410 (K). Malay Peninsula: Ipoh, Kledang Saiong FR, Dransfield JD & N. Manokaran JD 4485 (L); Kedah, Furtado SFN 33053 (L); Johor, NW Johore, Kepong, Mt. Ledang (Mt. Ophir), Whitmore FRI 12330 (L); Perak, Tapah, Kp. Sekam, Ave 218 (L); Tapah, Kp. Sekam, Ave 229 (L); Tapah, Kp. Sekam, Ave No 114 (L); Tapah, Kp. Sekam, Ave 4 (L); Perak, Tapah Hill, Dransfield JD 4519 (L); Northeast Tapah, Kp.

Sekam, Ave 145 (L); Northeast Tapah, Kp. Sekam, Ave 55 (L); Kp. Sekam, Ave 298 (L); Tapah, Kp. Sekam, Ave 216 (L); Tapah, Kp. Sekam, Ave No 218 (L). Pahang, Bentong-Raub boundary, Furtado SFN 33102 (L); Tasek Bera, Rosemarry Gianno A 668 (L). Terengganu, Kuala Trengganu, Ulu Nerus Forest Reserves, Dransfield JD 6506 (L). Singapore: Nee Soon swamp forest, Rajasegar & Adrian Loo 19 (K). Thailand: Nackhon Si Thammarat, T. Smitinand No 637 (L); Nackhon Si Thammarat, T. Smitinand No 841 (L); Pang Nga, Klong Saeng, C. Niyomdham 1254 (L); Pang Nga, Klong Saeng, non-hunting area, N. Niyomdhan 1253 (L); Pattani, Bachao district, AFG Kerr 7186 (L); Kao Kalakari, AFG Kerr 7785 (K); Province Ranong, Laemson NP, Ban Hin substation, A.S. Barford 45253 (K); Trang, Vongkaluang 210 (K); Ranawang (Ranong), AFG Kerr 11753 (L). The Philippines: Palawan, St. Paul's Bay NP, Dransfield, Madulid, Reynoso JD 6176 (L); Lion cave, Madulid F1008 (L).

1b. *Calamus javensis* Blume var. **polyphyllus** (Becc.) Becc.

Calamus javensis Blume var. **polyphyllus** (Becc.) Becc. (1902, 201)

Calamus javensis Blume var. *polyphyllus* (Becc.) Becc. (1902, 201); 1908, 184). Basionym for *Calamus javensis* Blume var. *peninsularis* Becc. subvar. *polyphyllus* Becc. (1892, 443). **Type**: Malaysia, Perak, summit of Gunong Tambang Batak, *B. Scortechini 657b* (lectotype: FI-1018944* [photo image], indirectly indicated by Henderson 2020)). Henderson (2020) indicates that *Scortechini 657b* (FI) is the holotype, but Beccari (1892) mentions two collections that are syntypes, therefore we consider Henderson's choice as a lectotypification.

Calamus acuminatus (1913, 16). **Type**: Malaysia, British North Borneo (= Sabah), Tenom, 700 ft., *L.S. Gibbs 4349* (lecto FI-1014285* [photo image] (indirectly selected by Henderson 2020), iso BM *n.v.*)). Beccari (1913) did not indicate a holotype, therefore all duplicates are syntypes and Henderson's (2020) selection of the FI specimen as holotype is regarded here as a lectotypification.

Clustering rattan. *Stem* to 10 m long, c. 8 mm in diameter with leaf sheath, c. 5 mm in diameter without leaf sheath. *Leaf sheath* striate, mostly smooth or almost smooth or with few small, clawed spines, or pointing, swollen-based spines, 3–5 mm long. *Ocrea* tubular, persistent, 20–30(–40) mm long. *Knee* conspicuous. *Flagellum* c. 1 m long. *Leaves* ecirrate, 35–40(–58) cm long; petiole very short, less than 2 (sometimes to 16) cm long; rachis with small, clawed, some black-tipped, swollen-based spines. *Leaflets* 9–12 pairs, regularly arranged or subequidistant, subopposite to alternate, lanceolate to narrowly elliptic, some elliptic, 9–15(–24) by 1.5–4 cm, conspicuously 3-nerved, several black-tipped spines along the nerves, transverse veinlets conspicuous, margin with small, soft spines, apex acuminate (to cuspidate), abaxial surface with red soft spines along main vein; uppermost pair joined for 1/3–1/2, forming a flabellum, c. 8(–13–19) by 1–3.5 cm; penultimate pair very close; basal pair smallest, spreading or swept back, 8–10 by 1–1.5 cm. *Staminate inflorescences* c. 50 cm long; prophyll tubular, closely sheathing, up to 27 cm long, prickly or with small, clawed spines; peduncular bracts tubular, sparsely armed with small black, clawed spines; rachis bracts tubular, smooth, 11–16 cm long, closely sheathing, with short longitudinal opening and asymmetrically extended into narrowly elliptic limb, c. 3 mm long, with several small, brown, swollen-based, downward pointing, c. 1 mm long spines, subtending 2 partial inflorescences each with up to 10 rachillae; rachillae 4–8 cm long; rachilla bracts strongly striate, tubular, later slightly cupuliform, with small extended limb; rachilla bracts strongly striate, shortly tubular, later cupuliform or explanate with broadly ovate limb asymmetrically extended into acuminate tip; floral bracteole explanate, shortly tubular with 2 acute tips. *Staminate flowers* c. 2 mm long; inserted at an angle of 45° with the axis; calyx valvate, deeply divided into 3 lobes, lobes broadly elliptic, c. 1.5 by 1 mm, apex acuminate; petals 3, oblong, 1–2.5 by c. 0.5 mm; stamens 6, filaments triangular, flat, tapering, c. 1.5 mm long, anthers hastate, 1–1.5 mm long, versatile to the filament; pistillode with 3 reddish, linear stigmas, c. 1 mm long. *Pistillate inflorescences* to 140 cm long, main inflorescence axis inserted in a conspicuous, swollen-based prophyll; prophyll tubular, c. 19 cm long, closely sheathed, with few small, clawed spines; peduncular bract tubular, closely sheathed, c. 21 cm long, with short, broad limb, sparsely armed with small, clawed spines; rachis bract tubular, c. 17 cm long, with limb expanded to 4 cm long, armed with clawed spines, subtending 3 partial inflorescences; subsequent bracts first tubular, closely sheathing, 12–20 cm, moderately armed with small, brown, downward pointing spines, later with expanded limb to 6 cm long; rachillae 4–8, 4–8.5 cm long; rachilla bracts striate, tubular, smooth or almost smooth, reddish, closely sheathing, later cupuliform, with one-sided extension into an acuminate tip, 10–20 mm long; rachilla bracts strongly striate, tubular, later cupuliform with extension on one side ending into an acuminate tip, subtending 2 floral bracteoles; cupuliform, subtending a fertile pistillate flower. *Pistillate flowers* seen young, c. 2 mm long; calyx valvate, with 3 ovate lobes, tip acuminate, c. 1.5 by 1 mm; petals three, small, ovate, c. 2 by 1.8 mm long; staminodes thin, connected at the base, filaments short, less than 0.5 mm long, anther empty, sagittate, dorsifixed, yellow, short, c. 0.5 mm long; stigmas 3, reddish, ovate, short, c. 0.5 mm long. *Fruits* broadly ovoid to globose, 4–6 by 3–4 mm, beak c. 1 mm long; scales narrowly elliptic to elliptic, in 8 vertical rows, yellowish with red tinged acuminate tip. *Seed* oblong, c. 30 by 22 mm, to globose, c. 50 by 48 mm; endosperm ruminate.

Distribution

Peninsular Malaysia, Sumatra, Borneo (Brunei, Sarawak, and Sabah).

Habitat & Ecology

Lowland mixed dipterocarp primary forest to rocky hill slopes and ridges, in Sabah often in secondary forests and roadside ‘belukar’ (weedy vegetation of small woody plants) (Dransfield, 1984), up to 1350 m altitude. Flowering: February, May to August, November, December; fruiting: January, February, April, September, November. Seemingly, this taxon can withstand areas affected by pollution as it was abundant in an open area in Mamut Copper Mine (Sabah) (Jopony and Tongkul, 2009; Van der Ent and Edraki, 2016).

Specimens examined

Brunei: Kuala Belalong, *Atria MAT 103A* (L); Temburong, Gunong Retak, *Johns 6509* (L). Indonesia: Sumatra, Aceh, Gunung Leuser Nature Reserve, *de Wilde & de Wilde-Duyfjes 12847* (L). Malaysia: Kedah, Bukit Seblak-Weng, *Furtado SFN 33068* (L); Sabah: Kabili-Sepilok, *Atria MAT 025* (L); Kabili-Sepilok, *Atria MAT 026* (L); Kabili-Sepilok, *Atria MAT 027* (L); Kabili-Sepilok, *Atria MAT 028* (L); Kabili-Sepilok, *Atria MAT 029* (L); Kabili-Sepilok, *Atria MAT 030* (L); Kabili-Sepilok, *Atria MAT 031* (L); Kabili-Sepilok, *Atria MAT 033* (L); Kabili-Sepilok, *Atria MAT 034* (L); Kabili-Sepilok, *Atria MAT 036* (L); Kabili-Sepilok, Atria

MAT 037 (L); Kabili-Sepilok, Atria MAT 038 (L); Kabili-Sepilok, *Atria MAT 039* (L); Kabili-Sepilok, *Atria MAT 056* (L); Kabili-Sepilok, *Atria MAT 057* (L); Kabili-Sepilok, *Atria MAT 058* (L); Kabili-Sepilok, *Atria MAT 059* (L); Kabili-Sepilok, *Atria MAT 060* (L); Kabili-Sepilok, *Atria MAT 061* (L); Kabili-Sepilok, *Atria MAT 062* (L); Ranau, Asin & Jarius SAN 121188 (L); Ranau, *Amin & Jarius SAN 116575* (L); Keningau, *Krispinus SAN 121886* (K, L); Keningau, *Ag. Abas SAN 85869* (L); Ulu Tungud FR, *Saw et al. SAN 146090* (K); Bukit Tenom, *Moore & Meijer 9208* (L); Tambunan, *Maikin & Joseph SAN 125852* (K). Keningau, along road Keningau-Kimanis, *Vermeulen & Duistermaat 679* (L); Keningau, Kg. Bandukan, *Clemens 5982* (K); Mt. Kinabalu, *J. & M.S. Clemens 27320* (L); Pensiangan, *SAN (Jimpin) 139385* (K); Pinangah Tongod, Dewol & Mansur SAN107646 (K, L); Sandakan: *Dransfield JD 5584* (L); Kinabatangan, *Sundaling SAN 97700* (L); Sarawak: Sematan, Gn. Pueh, *Dransfield et al. JD 5992* (L).

Notes

This taxon always has many (9–12) narrow leaflets, regularly arranged, subopposite and with a smooth or almost smooth leaf sheath. The appearance of the leaf sheath and leaflet arrangement is variable, but the most frequent form bears 10 or 11 subequidistant linear leaflets. The inflorescences resemble those of form *javensis* in the var. *javensis*, but those are mostly smaller or finer. The size of the fruit varies. Beccari (1908) stated that *C. acuminatus* is indistinguishable from some *C. javensis* varieties. The first author observed that *C. acuminatus* is similar to Beccari's *C. javensis* var. *polyphyllus* (Beccari, 1908: pl. 40), but *C. acuminatus* has an almost smooth leaf sheath, smaller flowers and fruits and the bracts of the peduncle are more cupuliform. Beccari (1913) considered to treat this entity as a subspecies. However, several specimens with characters partly intermediate can still be recognized. *Mega MAT 027* (Sabah) was identified as *C. javensis* var. *polyphyllus* as it exhibits the characters of var. *polyphyllus*, but the leaf sheath is almost smooth, like the leaf sheath of *C. acuminatus*. One sample, identified as *C. acuminatus* (*Dransfield JD 5584*), agrees with *C. acuminatus* in the almost smooth leaf sheath, numerous (9) leaflets and prickly peduncular bracts (in *C. javensis* var. *javensis* a robustly spiny leaf sheath, eight leaflets and spiny peduncular bracts), but the fruits are more ovoid, like *C. javensis* var. *javensis*, and not spherical as typical *C. acuminatus* (Dransfield 1984). In the phylogenic analysis (Atria et al., 2020) it groups in group B, not even closely related to the rest of the *C. acuminatus* specimens. Other intermediate specimens have leaflets varying between lanceolate and narrowly elliptic and leaf sheaths ranging from smooth to moderately spiny; the inflorescence can range from fine to the size of typical *C. javensis* var. *javensis*. *J & M.S. Clemens 27320* resembles typical *C. acuminatus*, but with a petiole to 17 cm long (absent in *C. acuminatus*). *Moore & Meijer 9208* has characters like *C. acuminatus*: a smooth leaf sheath, 11 subopposite leaflets, lowest pair swept back, no petiole, but the pistillate inflorescences resemble those of *C. javensis* var. *javensis*. In his description, Beccari (1908) mentioned that the female prophyll of *C. acuminatus* has a very conspicuous auxillary callus at the insertion with the stem. However, this character is not typical for *C. acuminatus* as a typical *C. javensis* var. *javensis* specimen from Java (*Kostermans 136*) shows the callus too. As there are no constant differences between both taxa, *Calamus acuminatus* is synonymized with *C. javensis* var. *polyphyllus* and recognized at the variety level. This variety is polyphyletic in the phylogeny (Atria et al. 2020).

Key to Groups

(non-fitting specimens are Form *Javensis*)

1. Stem slender, c. 5–6 mm in diameter with leaf sheath, 2–3 mm in diameter without leaf sheath. Leaf sheath smooth, without spines ……………...... 2
   —Stem slender to robust, 5–25 mm in diameter with leaf sheath, 2.5–10 mm in diameter without leaf sheath. Leaf sheath with spines …..........….….. 5
2. Leaflets only 1 pair (flabellate) or 2 pairs (upper most pair biggest and flabellate).. 3
   — Leaflets in 3–9 pairs, uppermost pair flabellate, not the biggest ……...... 4
3. Leaflets only 1 pair (2 pairs when juvenile), flabellate, joined for 1/2 of their length, abaxial midrib with few clawed spines ……….. group *Kemamanensis*
   — Leaflets in 2 pairs, the uppermost pair largest, forming a flabellum, joined for ¾ of their length, penultimate pair always sub opposite to alternate, abaxial midrib smooth ……………………………….....…...……..... group *Impar*
4. Leaf sheath with corrugated horizontal lines, mostly connecting and forming a circle. Flagellum thick, 20–30 cm long. Leaf 25–37 cm long, petiole 2–10 cm long, dark indumentum presents on the long petioles; rachis pilose, with

abundant red hairs. Leaflets in 3–5(–9) pairs, opposite (some subopposite), glabrous; terminal pair joined for 1/4–3/4 of their length, not the biggest pair .... group *Corrugatus*

— Leaf sheath smooth. Flagellum very fine, c. 50 cm long. Leaf up to 40 cm long; petiole very short, less than 2 cm long, floccose; rachis floccose. Leaflets in 7 pairs, middle pair alternate, both margin and surface tomentose, hairs whitish yellow, swollen-based; terminal pair joined for 3/4 of their length, biggest of all pairs .... group *Hypertrichosus*

5. Leaflets in 2 or 3 pairs, terminal pair (flabellum) usually very big and the biggest pair 6

— Leaflets in 4–13 pairs, terminal pair (flabellum) may not the biggest pair 8

6. Stem very short, to 1.5 m tall. Flagellum absent. Leaflets in 3 pairs, surfaces floccose with yellow hairs, terminal pair much bigger than the other pairs; abaxial main vein floccose, and with small spines till halfway the vein. Pistillate inflorescence c. 20 cm long, all bracts abundantly floccose... group *Acaulis*

— Stem 2–10 m tall. Flagellum to 1 m long. Leaflets in 2 or 3 pairs, glabrous, terminal pair not always the biggest; abaxial main vein glabrous. Pistillate inflorescences c. 40 cm long, all bracts glabrous (unknown for group *Elopurensis*) 7

7. Stem to 10 m tall. Flagellum up to 1 m long. Ocrea with triangular limb, up to 6 cm long. Leaves 27–50 cm long, petiole 9–15 cm long; Leaflets in 2 or 3 pairs, if 2 pairs then all leaflets in rosette; if in 3 pairs, then penultimate usually opposite; narrowly elliptic to broadly elliptic. Staminate inflorescences more than 1 m long; prophyll c. 28 cm long, closely sheathing, smooth .... group *Elopurensis*

— Stem 2–3 m tall. Flagellum c. 60 cm long. Ocrea apically truncate, short. Leaves c. 32 cm long, petiole 4–5 cm long. Leaflets in 2 pairs, penultimate pair always subopposite to alternate, narrowly elliptic. Staminate inflorescences very fine or slender, c. 56 cm long; prophyll c. 8 cm long, closely sheathing, but later with extended limb, sparsely armed with small, clawed spines .... group *Impar*

8. Petiole very short or absent. Leaflets in 6 pairs, narrowly obovate to elliptic.

Pistillate rachillae short, congested, to only 2.5–5 cm long..form *Congestiflorus* — Petiole usually longer, to 28 cm long, if absent or very short then leaflets in 8–13 pairs. Leaflets 4–13 pairs, narrowly elliptic to broadly elliptic. Pistillate rachillae long (more than 5 cm) or not congested (unknown for form *Rubro-ochreatus* and form *Setosus*) …….. 9

9. Leaflets abaxially with several setae along the veins, one black long setae on the main vein, c. 9 mm long .... group *Setosus* — Leaflets abaxially smooth ……………………………................... 10
10. Flagellum and cirrus absent ……............................... group *Rubro-ochreatus* — Flagellum present and cirrus absent or present …………………..... 11
11. Ochrea absent of poorly developed, glabrous. Pistillate inflorescence long, up to 1.8 m long; prophyll to 25 cm long, later extended into an ovate limb, c. 4 cm long ………….................……. group *Amplijugus* — Ochrea conspicuous, hirsute with stiff hairs or spines, partly torn apart. Pistillate inflorescence short, only 16–18 cm long; prophyll to c. 17 cm long, truncate, without limb …...………………………… group *Brevipaniculatus*

Informal groups in var. *javensis*

The synonyms pertaining to a group are only mentioned, the full nomenclature is provided under the species.

Group Acaulis

Representative specimen: Malaysia. North Borneo: Sarawak, Gunung Murud National Park, 15 September 1982, *Yii Puan Ching S 44664* (K),

Non-climbing rattan. *Stem* very short, to 1.5 m tall; with leaf sheath c. 15 mm in diameter, without leaf sheath c. 10 mm in diameter. *Leaf sheath* abundantly floccose, moderately armed with flat, pointing, horizontal or upward pointing spines, to 15 mm long. *Ocrea* absent. *Knee* absent. *Flagellum* absent. *Leaves* ecirrate, c. 80 cm long; petiole long, c. 35 cm long, abundantly floccose, proximally armed with several flat, pointing spines of up to 5 cm long, then with several small spines; rachis floccose, glabrous or with few horizontal, pointing black-tipped spines, 5–9 mm long. *Leaflets* in 3 pairs, elliptic to broadly elliptic, tip acuminate, hairy, surfaces and margin tomentose with bulbous-based yellow hairs; conspicuously 3-nerved, transverse veins obvious; terminal pair very big, forming a flabellum, bifid, joined for 2/3, c. 33 by 4 cm, main vein floccose on abaxial part, and with small horizontal, swollen-based spines, only to halfway the vein, c. 1 mm long; penultimate pair close to flabellum, c. 29 by 5.5 cm; basal pair spread, c. 25 by 5 cm, c. 15 cm below penultimate pair. *Staminate inflorescences* and *flowers* unknown. *Pistillate inflorescences* short, c. 20 cm long; all bracts abundantly floccose; prophyll tubular, glabrous, closely sheathing, extended into an elliptic limb of c. 1.5 cm long; peduncular bracts tubular, glabrous or with few short spines, closely sheathing, later with an extended, split limb, c. 2 cm long; rachis bracts tubular, smooth, closely sheathing, later with an auriculiform limb to 6 mm long; rachillae 4–6 cm long; rachilla bracts very shortly tubular, later infundibuliform, with an ovate extension at one side, tip acuminate, c. 2 by 1 mm.; prophyllar bracteoles striate, short tubular, explanate, subtending 2 flowers. *Pistillate flowers* c. 4.5 mm long; calyx valvate, deeply divided into 3 elliptic lobes, lobes c. 2 by 1.5 mm, apex acuminate; petals 3, ovate, c. 4.5 by 1 mm; staminodes 6, thin, filaments triangle, 3 filaments attached to the petals, c. 3.5 mm long, 3 others alternate to the petals, c. 2.5 mm long, connected at the base or half of their length, reddish; anther empty, sagittate, dorsifixed, yellow, c. 1.5 mm long; stigmas 3, curled. *Fruits* seen in young stage only, elliptic, c. 7 by 4 mm, beak to 1.8 mm long, scales c. 1.5 by 1 mm, in 10 vertical rows, light brown, with red line margin and tip. *Seed* unknown.

Distribution

Borneo (Sabah & Sarawak).

Habitat & Ecology

Mixed dipterocarp hill forest and lower montane forest; altitude up to 1300 m.

Specimen examined

Malaysia: Sabah, Sipitang, *Diwol & John et al. SAN 144483* (K, L).

Notes

‘Group *Acaulis*’ was called ‘Form 3’ in Atria et al. (2017). Unfortunately, the specimens did not yield usable DNA sequences and are absent in the phylogeny (Atria et al., 2020).

This entity differs from other specimens in the *C. javensis* complex, because it is stemless, has a peculiar arrangement of the floccose leaflets, and stigmas with a curled tip. Tomentose leaflets are also present in ‘group *Hypertrichosus*’, but the latter is climbing, has 7 leaflet pairs (3 pairs in this form) with a leaflet arrangement as in typical *C. javensis*.

The two specimens are both pistillate. *SAN 144483* has leaflets without hairs, but there are remnants of hairs.

Group Amplijugus

Based on *Calamus amplijugus* J. Dransf., see nomenclature under species.

Clustering rattan. *Stem* robust, up to 15 m high; 1.5–2.5 cm in diameter with leaf sheath, to 1 cm in diameter without leaf sheath. *Leaf sheath* moderate to densely armed with flat horizontal spines, up to 2.5 cm long. *Ocrea* absent or poorly developed, glabrous. *Knee* conspicuous. *Flagellum* c. 1.5 m long, armed with small, downward pointing, black spines, solitary, not in groups. *Leaves* ecirrate, up to 65 cm long; petiole absent or very short; rachis moderately armed with small but stout, swollen based spines, c. 3 mm long. *Leaflets* in 8–13 pairs, elliptic to broadly elliptic, conspicuously 3-nerved, transverse veins not so obvious, tip acuminate, regularly arranged, opposite to subopposite, abaxially smooth; the terminal pair forming a flabellum, joined for 2/3, 16–25 by 4.5–6 cm, median leaflets usually bigger than other pairs, 17–26 by 5–8 cm, the last 2 pairs amplexicaul, smaller, c. 8 by 2.5 cm. *Staminate inflorescences* and *staminate flowers* unknown. *Pistillate inflorescences* robust, up to 1.8 m long; prophyll tubular to 25 cm long, closely sheathing, later extended into an ovate limb of c. 4 cm, lower part with small clawed spines, upper part glabrous; peduncular bract tubular, closely sheathing, c. 60 cm long, sparsely armed with small clawed spines, upper part with longitudinal opening of 2.5–10 cm long; rachis bracts tubular, armed with small, clawed, black-tipped spines, upper part asymmetrically extended into an acute tip, c. 4 cm long; rachilla very long, up to 25 cm; rachilla bracts first tubular then cupuliform, extended on one side into an acuminate tip, striate, c. 2–3 mm long; floral bracteoles short-cupuliform, extended into 2 acute tips, striate. *Pistillate flowers*: pedicel short, 3–4 mm long; calyx valvate, shallowly divided into 3 broadly ovate lobes of c. 2 by 1.5 mm; petals 3, ovate, c. 2.5 by 1 mm; staminodes thin, 6, filaments valvate, connected at the base, narrowly elliptic lobes; anthers empty; stigmas 3, curled. *Fruit* globose, 5–6 x 5–6 mm, or oblong, c. 5 by 3.5 mm, with beak to 1.5 mm long; scales narrow, c. 3 by 1 mm, tip acute to acuminate, yellow, red or dark red lines along the margin and on the tip. *Seeds* broad elliptic to globose, c. 4–5 by 3 mm; endosperm ruminate.

Distribution

Borneo (Brunei and Sabah, in latter especially abundant in Tawai Forest Reserve in Telupid district).

Habitat & Ecology

In disturbed and primary mixed dipterocarp forest, areas with high relief, on ultrabasic soil and sediments; altitude up to 300 m. Flowering: January and May; fruiting: June to July.

Specimens examined

Brunei: Belait, Teraja FR, *Atria MAT 109* (L); Teraja FR, *Atria MAT 109C* (L); Labi Mendaram, *Dransfield JD 6548* (K). Malaysia: Sabah, Telupid, Tawai FR, *Atria MAT 043* (L); Tawai FR, *Atria MAT 044* (L); Tawai FR, *Atria MAT 045* (L); Tawai FR, *Atria MAT 046* (L); Tawai FR, *Atria MAT 048* (L); Tawai FR, *Atria MAT 049* (L); Tawai FR, *Atria MAT 050* (L); Dengiranuk, *Lugas 1600* (K); Sandakan, Bukit Silam, *Perumal & Diwol S. SAN 134344* (K); Kabili-Sepilok, *Puasa 6712* (K); Kampung Takutan, *Tadong 320* (K).

Notes

This group can easily be recognized by its many (8–13 pairs) of shiny, broadly elliptic leaflets; a long pistillate rachilla; and narrow fruit scales.

Group Brevipaniculatus

Representative specimen: North Borneo, Sabah, Mt. Kinabalu, Mesilau Cave, *Chew & Corner RSNB 4835* (L).

Clustering rattan. *Stem* to 10 m tall; c. 8 mm in diameter with leaf sheath, up to 6 mm in diameter without leaf sheath. *Leaf sheath* robust with a triangular, flat, hairy margin, covered with spines, the latter swollen-based, mostly pointing but several bent upward, the longest up to 3 cm long. *Ocrea* conspicuous, partly torn apart, hirsute (covered with long stiff hairs) or spines. *Knee* conspicuous. *Flagellum* 35–60(–100) cm long. *Leaves* ecirrate, c. 56 cm long; petiole c. 5 cm long, armed with a few triangular, c. 11 mm long spines; rachis floccose, sparsely armed with horizontal, flattened spines, 8–10 mm long or clawed spines. *Leaflets* in 4–6 pairs, narrowly elliptic, conspicuously 3-nerved, transverse veins conspicuous, margin with soft spines, apex acuminate with ciliate tip, abaxially smooth; upper most pair forming a flabellum, joined for 1/2, c. 21 by 2.5 cm; penultimate pair largest, c. 25 by 3.5 cm, very close to the flabellum; the lowest pair when 4 pairs of leaflets not swept back across stem or spreading, c. 19 by 3.5 cm, when 6 pairs of leaflets then lowest pair swept back. *Staminate inflorescences* quite long, c. 25 cm long, in young inflorescences all bracts reddish; prophyll tubular, first with few small, clawed spines, later smooth, c. 12.5 cm long, with a narrowly elliptic limb to 4.5 cm long; peduncular bract tubular, closely sheathing, c. 35 cm long, armed with several clawed, swollen-based spines, later with extended elliptic limb to 5 mm long, subtending 5–7 partial inflorescences; rachis bracts tubular, closely sheathing, c. 6 cm long, later with c. 5 cm long limb, armed with dull brownish yellow, downward pointing, swollen-based spines, up to 2 mm long; rachilla to 8 cm long; prophyll bracts smooth, tubular, up to 3 cm long, first closely sheathing then loose, opening longitudinally with acuminate tip; rachilla bracts strongly striate, cupuliform, one side extended into acuminate tip; prophyllar bracteole strongly striate, cupuliform with 2 acute tips, subtending staminate flower. *Staminate flowers* young, inserted at c. 45^0, c. 4 mm diameter; calyx valvate, shallowly divided into 2 elliptic lobes of c. 3 by 1.5 mm; petals three, narrowly elliptic, c. 3 by 1 mm; stamens 6, filaments ensiform, brown, c. 2 mm long, anther sagittate, dorsi-versatile, yellow, c. 2 mm long; pistillode with 3 linear, reddish stigmas, c. 1.3 mm long. *Pistillate inflorescences* short, 16–18 cm long; prophyll tubular, c. 17 cm long, closely sheathing, truncate, armed with several small, clawed spines, gradually glabrous distally; peduncular bract c. 22 cm long; rachis bract c. 21 cm long, with an extended limb of 2–3 cm long, narrowly elliptic, apex acute, subtending 2 partial inflorescences, each with 4 or 5 rachillae, all bracts sparsely armed with small, clawed spines; subsequent rachis bracts tubular, closely sheathing, 10–18 cm long, later with c. 2.5 cm long narrowly elliptic limb, armed with small, brown, stout, clawed, swollen-based spines; rachillae up to 9 cm long, in one specimen twisted (*Chew et al. 168)*; prophyll bracts smooth, tubular, closely sheathing, with several small, stout, black-tipped, swollen-based spines; rachilla bracts tubular, later with one side extending into an acuminate tip of c. 2 mm long; floral bracteoles 2, strongly striate. *Pistillate flowers* c. 3 mm long, rather distantly arranged, c. 8 mm apart; calyx shallowly divided into 3 lobes, latter broad-elliptic, c. 2 by 1.5 mm, apex obtuse-acuminate; petals 3, slightly longer than calyx, elliptic, c. 2.5 mm long, apex acute; staminodes 6, united into a ring at the base of the ovary, each with a free, empty anther; stigmas 3, reddish, fleshy; some pistillate flowers longer, up to 8 mm long; calyx like other flowers, but base swollen; petals oblong, c. 6 by 1.5 mm, apex acuminate; staminodes like in smaller flowers, anther hastate, c. 0.5 mm high; stigmas 3, c. 1 mm long, dark spotted, fleshy. *Fruit* ellipsoid, c. 15 by 10 mm, yellowish brown, beak to 2 mm long, scales c. 2 by 1 mm, in 10 vertical rows.

Distribution

Sumatra and Borneo (Sabah).

Habitat & Ecology

In mossy montane heath forest, altitude 1300–1800 m. Fruiting specimens collected in February, April and July.

Specimens examined

Indonesia: West Sumatra, Payakumbuh, Gunung Sago, *Mejia 2019* (K); Central Sumatra, Kerinci, *Aumeeruddy YA 308* (K). Malaysia, Sabah: Mt. Kinabalu, Mesilau Cave, *Chew & Corner RSNB 4835* (L); Mt. Kinabalu, Mesilau river, *Chew & Corner RSNB 7025* (K); Mt. Kinabalu (eastern shoulder), *Chew et al. 168* (K); Ulu Meligan, *Diwol et al. SAN 144475* (K).

Notes

This form was called ‘Form 4’ in Atria et al (2017, 2020). This group is easily recognized by its peculiar rough ocrea texture, which is typically covered either with white setae or hirsute hairs or spines. Typical *C. javensis* almost always has a tubular, smooth, reddish, and glabrous ocrea. Other characters that distinguish this form from typical *C. javensis* are the length of the pistillate inflorescences; which are shorter, up to only 18 cm and which have a twisted rachilla (especially specimen *Chew et al. 168*) and the flowers are rather far apart; while typical *C. javensis* usually has a longer pistillate inflorescences, up to 1.5 m long, with a straight rachilla and closely arranged flowers. *Chew & Corner RSNB 4835*, selected as representative specimen, has only 4 pairs of leaflets, but other specimens resemble the arrangement of *C. javensis* with 5 or 6 pairs of leaflets. The label of *Chew & Corner RSNB 4835* states that the flowers are purple instead of the typical red of *C. javensis*.

This form differs from 'group *elopurensis*', which also has 4 pairs of leaflets, in the staminate inflorescence: the rachilla is longer in 'group *elopurensis*' (up to 14 cm versus c. 9 cm), the bracts are much longer in 'group *elopurensis*' (up to 16 cm vs c. 3 cm).

A short rachilla in the *C. javensis* complex is also shown by 'group *congestiflorus*', but the latter has a short rachilla bearing very congested flowers. The leaflets in 'group *congestiflorus*' are obovate to elliptic and not narrowly elliptic as in 'group *brevipaniculatus*'. Beccari (1908, plate 41) described *C. javensis* var. *acicularis* (belonging to typical *C. javensis*) based on a slender specimen (SING 0192571*, collected by R.H. Hullet in 1890) which also has a short pistillate inflorescence with short rachillae, but the features of the leaf sheath, ocrea and leaflets are different from 'group *brevipaniculatus*'. In the *acicularis* group of *C. javensis* the leaf sheath is armed with scattered short horizontal spines, ochrea tubular and glabrous, leaflets in two pairs and there is a pair of acicular spines (3–3.5 cm long) inserted between petiole and leaf sheath.

'Group *brevipaniculatus*' is apparently confined to higher altitudes between 1300–1800 m and '*acicularis* form of Form *javensis*' was found in lowland of c. 240 m a.s.l.

Group Congestiflorus

Based on *Calamus congestiflorus* J.Dransf., see nomenclature under species.

Clustering rattan. *Stem* to 33 m high; c. 6 mm diameter with sheath, c. 4 mm diameter without sheath. *Leaf sheath* slightly striate, with scattered, small, flat, bulbous spines, facing downward. *Ocrea* obvious, first tubular later torn apart, up to 8 mm long. *Knee* conspicuous. *Flagellum* 28–60 cm long. *Leaves* ecirrate, 24–46 cm long; petiole very short or absent; rachis floccose, with several small, black tipped, clawed spines. *Leaflets* in 6 pairs, narrowly obovate to elliptic, acuminate, tip with soft spines, 3 main nerves and transverse veinlets conspicuous, margin glabrous; uppermost pair joined for c. 1/3, 12–17 by 2–3.5 cm; penultimate pair opposite, c. 1 cm below the terminal one, c. 15 by 3.5 cm; median pair subopposite to alternate, c. 7 cm below the penultimate one, c. 15 by 3 cm; lowest pair swept back across stem, 7–10 by 1.5–2.5 cm. *Staminate inflorescence* and *flowers* not known. *Pistillate inflorescences* short, 27–40 cm long; prophyll tubular, mainly on the proximal part of 10–28 cm long, closely sheathing, with several small, clawed, black tipped spines, limb c. 5 cm long; peduncular bract tubular, closely sheathing, c. 14 cm long, armed with scattered small black-tipped spines, subtending 2 partial inflorescences; bracts much longer or the same length as the partial inflorescence; rachis bracts tubular, closely sheathing, 10–15 cm long, distally armed with a few very small, black, clawed spines, either with a prickly lanceolate limb, up to 13 by 0.5 cm or an oblong limb of c. 3.5 by 1.5 cm; prophyll bracts tubular, closely sheathing, floccose, c. 1.5 mm long or less, some with several short black spines, with c. 4 mm extension limb enclosing the rachilla; rachillae short, congested, 2.5–5 cm long; rachilla bracts very short, only c. 1 mm long, with conspicuous acuminate tip limb; floral bracteoles strongly striate, explanate. *Pistillate flowers* very close together (crowded); calyx valvate, deeply divided into 3 broad elliptic lobes, tip acuminate, sepals c. 2 by 1 mm; petals 3, elliptic, c. 3 by 1.5 mm. staminodes thin, valvate, with 6 small elliptic lobes, c. 0.5 mm long; stigmas 3, curled. *Fruit* in young stage, small, c. 4 mm long; with c. 8 diamond-shaped scales, of c. 2 by 2 mm, yellow, margin white; beak c. 1.5 mm. *Seed* broadly ellipsoid, c. 7 by 5 mm to globose and up to 10 by 8 mm; endosperm ruminate.

Distribution

West Java, Borneo (Sabah and East Kalimantan).

Habitat & Ecology

Flowering: February; fruiting: February, March and November.

Specimens examined

Indonesia: East Kalimantan, Maruwai, *Kessler et al. PK 2688* (L); West Java: Ujung Kulon Reserves, Mt. Pajung, *Kostermans et al. 136* (L). Malaysia, Sabah: Telupid, Sapi Nangoh FR, *Atria MAT 042* (SAN, L); Keningau, Crocker Range Nature Center, *Atria MAT 079* (SAN, L); Crocker Range Nature Center, *Atria*

MAT 080 (SAN, L); Nabawan, *Dransfield JD 5635* (K); Pandewan, Mesopo river, *SAN (Krispinus) 114061* (L); Sandakan, Tawau, *SAN (Sundaling) 105576* (K).

Notes

This group, only represented by a single entry in the phylogeny (Atria et al. 2020), has a disjunct distribution and probably represents parallel, locally developed forms.

The form is easily recognized by its crowded and congested flowers due to the very short rachillae. A few specimens consigned to this group are somewhat intermediate with more typical *javensis*. *SAN 105576* from Tawau (Sabah) has the closest resemblance with the type of *C. congestiflorus*, but it slightly differs in the smaller rachis limb that is oblong instead of lanceolate as in more typical 'group *congestiflorus*'. *SAN 114061* (Pandewan, Sabah) has a similar rachis limb as in typical 'group *congestiflorus*', but the prophyll is longer (c. 40 cm long) versus c. 15 cm in 'group *congestiflorus*'; and the shape of the leaflets and their arrangement is closer to typical *Calamus javensis* than to 'group *congestiflorus*'. Included is a specimen from West Java (*Kostermans et al. 136*), because it also has a subtending oblong bract, a short pistillate inflorescence and a congested rachilla of c. 4.5 cm long (versus up to 11 cm in typical *C. javensis*), but that is still longer than the rachilla in the Borneo specimens (only c. 2–2.5 cm).

Group Corrugatus

Based on *Calamus corrugatus* Becc., see nomenclature under species.

Slender stem, c. 6 mm in diameter with leaf sheath, c. 2 mm in diameter without leaf sheath. *Leaf sheath* striate, glabrous (without spines), with corrugated horizontal lines, mostly connecting and forming a circle. *Ocrea* conspicuous, tubular, c. 2 cm long, floccose. *Knee* prominent. *Flagellum* 20–30 cm long. *Leaves* ecirrate, about 25–37 cm long; petiole 2–10 cm long, with few, small, clawed spines, dark indumentum present on the long petioles; rachis pilose, with abundant reddish hairs, sparsely armed with small, swollen-based, black-tipped, clawed spines, some in groups of 3. *Flagellum* thick, 20–30 cm long. *Leaflets* in 3–5(–9) pairs, opposite, some subopposite, narrowly elliptic, apex acuminate to caudate, glabrous; terminal pair forming a flabellum, joined for 1/4–3/4, not the biggest pair, 9–13 by 2–3 cm; penultimate pair 2–4 cm below flabellum, 11–15 by 2–3.5 cm; 2 lower pairs swept back across stem, 7–11 by 2–2.5 cm and the lowest pair 5–7.5 by 0.5–2 cm. *Pistillate flowers* small, c. 3 mm high; calyx valvate, shallowly divided into 3 elliptic lobes, c. 1.5 by 0.8 mm; petals 3, narrowly elliptic, c. 3 by 1 mm; staminodes thin, 6, c. 2.5 mm long, filaments valvate, triangular, anther empty, sagittate, c. 1 mm long; pistil c. 3 mm long, with 3 stigmas, not curved.

Distribution

Borneo (Sarawak, Central Kalimantan).

Habitat & Ecology

In primary mixed, dipterocarp forest, secondary forest and heath forest, on (steep) slopes, soil with clay or sandstone, at low altitudes up to 900 m. Flowering: March.

Specimens examined

Indonesia: Central Kalimantan, Bukit Raya, 24 Nov 1982, *Mogea 3615* (L). Malaysia, Sarawak: Mt. Mattang, 8 Apr 1981, *Dransfield JD 5868* (L); Sabal Tapang, 19 May1981, *Dransfield JD 6080* (L); Semongoh FR, 9 Jul 1976, *Dransfield JD 4930* (L). Lubuk Antu, 5 Jun1993, *Dransfield JD 1428* (K); Ulu Temalad, Hose mountain, 27 Mar 1964, *S (Ashton) 19775* (K).

Notes

This group has two unique characters, the corrugated ring-like lines on the sheaths and the complete absence of the spines. However, the arrangement of the leaflets is similar to that of typical *C. javensis*. The corrugate lines are not completely circular in some specimens. A specimen from Central Kalimantan (*Mogea 3615*) has the typical *Calamus javensis* feature of an incomplete corrugate leaf sheath. In the phylogeny (Atria et al. 2020) the three included specimens are para/polyphyletic in not supported clades.

Group Elopurensis

Based on *C. elopurensis* J. Dransf., see nomenclature under species.

Clustering rattan. *Stem* to 10 m tall, c. 5–8(–10) mm in diameter with leaf sheath, up to 2.5–5 mm in diameter without leaf sheath. *Leaf sheath* smooth or striated, moderately armed with small clawed-spines, or spines short triangular bulbous, or tubular and pointing horizontally, to 5 mm long. *Ocrea* conspicuous, tubular with triangular limb, reddish, up to 6 cm long. *Knee* conspicuous. *Flagellum* to 1 m long. *Leaves* ecirrate, about 27–50 cm long; petiole floccose, sometimes abundantly hairy, c. 9–15 cm long, with short, clawed, swollen based spines, some in groups or with triangular flat spines, c. 3 cm long; rachis abundantly floccose, very short in specimens with only two pairs of leaflets and c. 13 cm long in specimens with three pairs, armed with a few small, stout, clawed, bulbous-based spines. *Leaflets* in 2 or 3 pairs, narrowly elliptic to broadly elliptic, glabrous (also abaxial main veins), conspicuously 3-nerved, transverse veins obvious, margin smooth, apex acuminate with ciliate tip; when in 2 pairs, then penultimate pair forming a rosette with the terminal one, upper most pair forming a big flabellum, joined for 3/4, c. 20–35 by 3–6 cm; penultimate pair c. 13–33 by 3–8 cm, very close to the flabellum; if in 3 pairs, then the flabellum 25–36 by 3–6 cm, penultimate pair usually opposite, narrowly elliptic to broadly elliptic, c. 33 by 6.5 cm, basal pair 8–24 by 2.5–6 cm. *Staminate inflorescences* long, more than 1 m; prophyll tubular, closely sheathing, smooth, c. 28 cm long; peduncular bract tubular, closely sheathing, c. 30 cm long, armed with several clawed, black, bulbous-based spines; rachis bract tubular, c. 20 cm long, with an extended limb of c. 9 cm long, armed with small black, clawed, bulbous based spines, subtending six partial inflorescences; subsequent rachis bracts floccose, tubular, closely sheathing, 13–15 cm long, older ones with a 3–5 cm long limb, armed with dark, downward, swollen based spines, up to 2 mm long; rachilla 8–12 cm long; rachilla bracts strongly striate, infundibuliform, with a 1-sided extension into an acuminate tip; floral bracteole strongly striate, cupuliform with 2 acute tips, subtending staminate flower. *Staminate flowers*, *Pistillate inflorescences* and *flowers* and *fruits unknown*.

Distribution

North Borneo (Sabah).

Habitat & Ecology

Disturbed mixed, lowland dipterocarp forest, recorded from swampy hollows, altitude 50–200 m. Flowering: October.

Specimens examined

Malaysia, Sabah: Kinabatangan, *Andersen & Rasit bin Abdullah 224* (K);

Sandakan, Segaliud Lokan mile 42, Virgin Jungle Reserves, *Dransfield JD 5774* (K); Danum valley, *Dransfield 6265* (L); Ranau, *Sani Sambuling 130* (K).

Notes

This group can easily be distinguished by the rosette of large leaflets and the peculiar rachis bracts with a long-extended limb to 9 cm. It should not be confused with 'group *Impar*', which also has few leaflets, but with a much narrower stem (c. 5 mm vs. to 10 mm), and a different staminate rachilla (up to 12 cm in 'group *Elopurensis*' vs c. 5 cm in 'group *Impar*').

Group Hypertrichosus

Based on *C. hypertrichosus* Becc., see nomenclature under species.

Stem slender, c. 6 mm in diameter with leaf sheath. *Leaf sheath* smooth, striate, glabrous. *Ocrea* tubular, conspicuous, truncate. *Flagellum* very fine, c. 50 cm long. *Leaves* up to 40 cm long; petiole very short, less than 2 cm long, floccose (densely covered with dark woolly tufts of hairs); rachis floccose. *Leaflets* in 7 pairs, narrowly elliptic, apex acuminate, both margins and surfaces tomentose with swollen-based, whitish yellow hairs, conspicuously 3-nerved, transverse veinlets not obvious; terminal pair forming a flabellum, joined for 3/4, c. 16 by 3 cm, bigger than all other pairs; penultimate pair c. 2 cm below the flabellum, subopposite to alternate; middle pairs alternate, 11–14 by 2–3.5 cm; 2 lowest pairs swept back across stem, 5–7.5 by 1–3 cm. *Inflorescences*, *flowers* and *fruits* unknown.

Distribution

Endemic in Borneo (Sarawak).

Habitat & Ecology

Hill slope in lowland mixed dipterocarp, altitude: c. 25 m.

Specimen examined

Malaysia, Sarawak, Semongoh FR, near Kuching, *Dransfield JD 4637* (K!, L!).

Notes

Beccari (1913) described *C. hypertrichosus* based on a sterile specimen from

Borneo; the type has leaflets in four pairs and a very long petiole (c. 30 cm long). Seemingly the type was a young plant, while the specimen examined is fully grown with leaves with seven pairs of leaflets in an arrangement that resembles typical *C. javensis*. The specimen studied only differs from typical *Calamus javensis* in having the leaflets covered with whitish yellow hairs.

This group was not represented in the phylogenetic analysis (Atria et al., 2020) as the DNA extraction failed.

Group Impar

Based on *C. impar* Becc., see nomenclature under species.

Stem 2–3 m high; c. 5 mm in diameter with leaf sheath, c. 3 mm in diameter without leaf sheath. *Leaf sheath* striate, some floccose, unarmed or sparsely armed with small, black-tipped, swollen-based spines. *Ocrea* conspicuous, closely sheathing, truncate, short. *Flagellum* c. 60 cm long. *Leaves* c. 32 cm long; petiole floccose, 4–5 cm long; smooth or with spines. *Leaflets* in 2 pairs, narrowly elliptic, glabrous (including abaxial vein), the uppermost pair largest, forming a flabellum, joined for c. 3/4, 23–26 by 2–3 cm; penultimate pair always subopposite to alternate, one leaflet 0.5–4 cm below the flabellum, c. 17 by 1.5–2.5 cm. *Staminate inflorescences* very fine or slender, c. 56 cm long; prophyll tubular c. 8 cm long, closely sheathing, later with extended limb, sparsely armed with small, clawed spines; rachis bracts subtending inflorescences 2, 10–12.5 cm long, tubular, distally with an extended limb of 1–1.5 cm long and longitudinally opening, armed with solitary, clawed, black-tipped spines; subtending 3 partial inflorescences; subsequent rachis bracts tubular, closely sheathing, up to 11 cm long, extended limb up to 7 mm long, almost smooth or with few small clawed spines; rachillae c. 5 cm long; rachilla bracts striate, infundibuliform, c. 1 mm long, shorter in mature inflorescences, slightly extended on one side into a conspicuous acuminate tip, bent in some specimens, subtending one prophyllar bracteole, strongly striate, short-cupuliform, with two acute tips. *Staminate flowers* small, attached at 90° to the axis; 4–5 mm long; calyx valvate, tubular, deeply divided into 3 lobes, lobes elliptic, c. 1 by 0.8 mm, apex acute to acuminate; petals slightly longer than calyx, oblong-elliptic, c. 3 by 1 mm, apex acute; stamens 6, dorsi-versatile, filament triangular, tapering, c. 1.5 mm long, anther sagittate, yellow, c. 1.5 mm long; pistillode with 3 red, slightly falcate stigmas, c. 1.5 mm long. *Pistillate inflorescences* slender, c. 40 cm long; prophyll tubular, c. 17 cm long, smooth, with an extended lanceolate limb of c. 10 mm long; peduncular bracts tubular, closely sheathing, c. 23 cm long, with few small clawed spines on swollen bases; rachis bracts tubular, closely sheathing, c. 20 cm long, with a small limb of c. 8 mm long, subtending 2 rachillae; each rachilla 7–8 cm long, subsequent bracts tubular, more than 9 cm long, with a few brown, downward pointing, swollen-based spines; prophyll bracts tubular, closely sheathing, 2–3 cm long, smooth or with one or two small bulbous-based spines; prophyllar bracteoles striate, first tubular then cupuliform, reddish, 2–3 mm long. *Pistillate flower* unknown. *Fruits* broadly elliptic, c. 8 by 6 mm, with yellow scales, latter in 10 rows, margin, and beak reddish, beak to 7 mm long.

Distribution

Peninsular Thailand, Malay Peninsula (Perak), Borneo (Sabah, East Kalimantan).

Habitat & Ecology

In primary forest, often on steep hills or ridges, locally on ultrabasic soil with loose stones and boulders and thick layer of litter, or on bare rock in full sun; altitude c. 900 m. Flowering: February, April.

Specimens examined

Indonesia: East Kalimantan, Berau, *Watanabe NMW 41* (K); West Java, G. Kendeng, *Dransfield JD 1434* (L). Malaysia, Sabah: Ranau, Mt. Kinabalu, *de Vogel 8695* (L); Kinabalu, *Lugas 14* (K); Ranau, *Meijer SAN 21064* (K, L); Kampung Poring, *Sambuling 115* (K); Kampung Poring, *Sambuling 130* (K); Ranau, *SAN (Amin et al.) 114102* (L); Kinabalu, *Lugas 1626* (K); Perak: Kp. Tapah, *Ave 136* (L). Thailand: Klong Seang, Pangnga, *Niyomdham 1253* (L).

Notes

The description of Beccari (1913) is broadened by adding more variability. Beccari (1913) described *C. impar* as a taxon with a deeply bilobed flabellum, but also observed were specimens with the flabellate pair joined for 2/3rd. The type has the penultimate pair of leaflets alternate, one leaflet growing directly below the flabellum and the other leaflet 4–5 cm further below. In a specimen from Sabah (*SAN 21064*), two forms of penultimate arrangement can be observed, one alternate with one leaflet closely attached to the flabellum and the other rather distant, but also opposite penultimate pairs in other leaves.

From typical *C. javensis* it differs in the number of leaflets and the arrangement. The penultimate pair in *C. javensis* is almost always opposite and close to the flabellum. Among other forms within the *C. javensis* complex, this taxon resembles 'group *elopurensis*' as both have a few pairs of leaflets (two pairs in 'form *impar*' and up to three pairs in 'form *elopurensis*'). The ocrea of 'group *impar*' is said to be cylindrical and truncate, however, one specimen from Ranau, Sabah (*Sambuling 130*), has the typical 'group *impar*'s conspicuous ocrea, closely sheathing, truncate, and short, but the leaflet arrangement resembles 'group *elopurensis*'. Another specimen from Ranau, Sabah (*SAN 114102*), has two pairs of leaflets, one pair flabellate and the penultimate pair alternate but the upper leaflet not very close to the terminal pair, and it has an ocrea that resembles 'group *elopurensis*'. However, the size of the leaflets in 'groupm *impar*' is smaller than in 'group *elopurensis*' (up to 20 cm versus to up to 35 cm long, respectively), moreover, in 'group *elopurensis*', the staminate inflorescence, has a conspicuous peduncle bract with a long limb up to 10 cm long, while in 'group *impar*' the peduncle bract is tubular, c. 13 cm long, closely sheathing, and with an extended limb to 2.5 cm long. These differences clearly segregate both forms.

Group Kemamanensis

Based on *C. kemamanensis* Furtado, see nomenclature under species.

Slender clustering rattan. *Stem* c. 4 m long; c. 6 mm in diameter with leaf sheath, c. 3 mm in diameter without leaf sheath. *Leaf sheath* striate, glabrous. *Ocrea* obvious, tubular, to 1 cm long. *Knee* conspicuous. *Flagellum* c. 75 cm long, with small, clawed spines. *Leaves* ecirrate, 27–35 cm long; petiole floccose, 5–7 cm long, longer in juvenile plant, armed with clawed spines. *Leaflets* narrowly elliptic, in adult plant only 1 pair, flabellate, joined for ½, 26–29 by 4.5–5 cm, apex acute, with setae, conspicuously 3-nerved, transverse veins obvious, abaxial midrib with few clawed spines. *Staminate* and *Pistillate inflorescence, Flowers* and *Fruits* unknown.

Distribution

Endemic in Peninsular Malaysia (Trengganu).

Habitat & Ecology

Hill dipterocarp forest, altitude: c. 350 m.

Specimens examined

Malaysia: Kuala Trengganu, Ulu Nerus Forest Reserves, *Dransfield et al. JD 6506* (K); Kemaman, Sungai Nipa, *SFN (Corner) 30535* (K).

Notes

This is the only form with one pair of leaflets in adult plants; Corner's specimen is a juvenile specimen with two pairs of leaflets in a rossette. It is not present in the phylogenetic analysis (Atria et al., 2020) due to a DNA isolation failure.

Group Rubro-ochreatus

Representative specimen: North Borneo: Sarawak, Mt. Mulu National Park, *Dransfield JD 5301* (L).

Clustering rattan. *Stem* up to 3 m tall; c. 15 mm in diameter with sheath, c. 8 mm in diameter without sheath. *Leaf sheath* striate, robustly armed with flat spines, pointing horizontally or upward, some reaching up to 15 mm long. *Ocrea* conspicuous, persistent, to 3.5(–10) cm long, covered with abundant red hairs, torn apart. *Knee* not conspicuous. *Flagellum* absent. *Cirrus* absent. *Leaves* to 60 cm long; petiole up to 25 cm long, with a few, flat, horizontally pointing spines, 6–8 mm long; rachis striate, floccose, with a few pointing, black-tipped spines with swollen base, up to 17 mm long. *Leaflets* in 10 or 11 pairs, very regularly arranged, more or less the same size except for the lowest pair, narrowly elliptic to elliptic, conspicuously 3-nerved, transverse veins distinct, margin smooth, apex acuminate, tip hairy, abaxially smooth; terminal pair joined for 1/2, c. 15 by 2.5 cm, lowest pair swept back across stem, c. 9 by 1.5 cm. *Staminate inflorescences* short, c. 20 cm long; bracts of inflorescence red floccose; prophyll smooth, tubular, closely sheathing, base swollen, c. 7 cm long, with a small elliptic limb; rachis bract tubular, closely sheathing, glabrous, c. 4 cm long, later with an elliptic, c. 3 mm long limb, bearing 2 partial inflorescences; rachilla 3–4 cm long; prophyll bracts tubular, smooth, with 2 or 3 short swollen spines in the first part then smooth, c. 2.5 cm long, with extended broadly ovate limb, c. 3 mm high, enclosing rachilla and the subsequent bracts; floral bracteole warty, striate, shortly tubular, with one-sided extension into a small acuminate tip, the tip usually bent. *Staminate rachilla* borne on the 2nd branch of the inflorescence. *Staminate flowers* unknown. *Pistillate inflorescences*, *flowers*, and *fruits* unknown.

Distribution

Borneo (Sarawak: Mount Mulu).

Habitat & Ecology

Lower montane forest; altitude 1200–1500 m.

Specimen examined

North Borneo: Sarawak, Mt. Mulu National Park, *Dransfield JD 5353* (L).

Notes

This group was called 'Form 1'in Atria et al. (2017). Unfortunately, the two specimens did not provide useful DNA and are lacking in the phylogenetic analysis (Atria et al., 2020).

Typical for this taxon are the short staminate inflorescences, the tubular, warty rachilla bracts, the lack of a flagellum and a cirrus. This taxon is clearly different from typical *C. javensis* in the leaves and arrangement of leaflets, leaf sheath features, and all inflorescence bracts. *Calamus tenompokensis* resembles this taxon, but 'group *rubro-ochreatus*' differs in the smaller leaflets (c. 15 cm long, *C. tenompokensis* up to 35 cm long), the tubular and warty rachilla bracts (*C. tenompokensis*: explanate and smooth) and the staminate inflorescence borne on the 2nd branch (3rd branch in *C. tenompokensis*). The multivariate analysis by Atria et al (2017) showed that the two specimens of 'group *rubro-ochreatus*' are separated far apart, one near the typical *javensis* cluster and the other one is outside this cluster; this indicates that the resemblance that unites them is only in the key characters mentioned.

Group Setosus

Representative specimen: Philippines: Luzon, Pampanga, Mt. Arayat, *Madulid et al. 7172* (L).

Slender rattan. *Stem* c. 8 mm in diameter with leaf sheath, c. 5 mm without sheath. *Leaf sheath* striate, moderately armed with cylindrical, pointing and flat spines, horizontal or some slightly directed upward. *Ocrea* not so obvious, torn apart, the remnant attached to the petiole. *Knee* conspicuous. *Flagellum* absent. *Leaves* ecirrate, c. 50 cm long; petiole smooth, c. 28 cm long, with horizontal stiff pointing spines of c. 8 mm long; rachis smooth, with several scattered horizontal stiff pointing, black-tipped spines, up to 20 mm long. *Leaflets* in 6 pairs, opposite, grouped, narrowly elliptic, conspicuously 3-nerved, transverse veins distinct, margin with short setae up to the apex, apex acuminate, abaxial parts with few setae along veins and one black long seta on the main vein, to 9 mm long; the terminal pair could not be examined thoroughly, penultimate pair very close to the terminal one, opposite; the 3rd pair c. 7 cm below the penultimate one, close to the 4th pair of 12–14 by 2–2.5 cm; the basal two pairs close to each other, c. 7 cm below the middle pairs; the 5th pair c. 12 by 1 cm, the basal or 6th pair, c. 12 by 2 cm. *Inflorescences*, *flowers*, *fruits* unknown.

Distribution

Philippines (Luzon: Pampanga.

Habitat & Ecology

In primary forest; altitude c. 500 m.

Notes

This group was formerly called ‘form 2’ in Atria et al. (2017, 2020).

Typical for this group are the long black setae on the abaxial side of the main veins of the leaf blades, and one long seta on each leaflet, a character not present in other forms within the *C. javensis* complex. The leaflets resemble the arrangement of typical *C. javensis*, but with quite long petioles and peculiar rachis spines. The ocrea of this group is not tubular as it is in typical *javensis*. In Atria et al. (2017) ‘group *setosus*’ was separated from the cluster of typical *javensis*, but as it is only represented by a single specimen with incomplete flower and fruit data, it cannot be described as taxon.

2. *Calamus tenompokensis* Furtado

***Calamus tenompokensis* Furtado (1935, p. 260)**

Calamus tenompokensis Furtado (1935, 260). **Type**: British North Borneo (= Sabah), Mt. Kinabalu, Tenompok, towards Lumu, 5,500 ft., *C. Furtado (M.S.*

Clemens 28408) (holotype: SING-0056713* [photo image] over 2 sheets; iso A n.v, BM *n.v.*, BO *n.v.*, G-00039991* [photo image], K-000521383!, NY n.v.).

Calamus nanus Burret, (1943) 818. **Type**: Malaysia, Borneo, Sabah, Mount Kinabalu, Gurulau Spur, *J. & M.S. Clemens 50397* (lectotype: BM-000517233* [photo image], designated by Henderson 2020, the holotype at B destroyed).

Clustering and moderate rattan. *Stem* erect, 2–4 m long; c. 3 cm in diameter with leaf sheath, c. 2 cm in diameter without sheath. *Internodes* short, less than 10 cm long. *Leaf sheath* abundantly covered with triangular, flat, horizontal, margin hairy, black-tipped spines, 1–5 cm long. *Ocrea* conspicuous, to 6 cm long, spiny. *Knee* poorly developed. *Flagellum* absent or short (rarely longer than 1 m). *Leaves* ecirrate, c. 1 m long; petiole angular, 22–37 cm long, with several flat, triangular spines, 1–5 cm long, short-clawed spines towards the distal part; rachis angular, floccose, with a few small, floccose, clawed spines. *Leaflets* in 8 or 9 pairs, regularly arranged, opposite, narrowly to broadly elliptic, conspicuously 3-nerved, transverse veins conspicuous, margin with small, black soft spines (setae), apex acuminate; terminal pair forming a flabellum, joined for 1/2–3/4 , 18–23 by 2–6 cm; middle pairs 16–35 by 3–8 cm; lowest pair swept back across stem, 19–28 by 3–6 cm. *Staminate inflorescences* robust, up to 1 m long, but some short, only c. 25 cm long; prophyll short, c. 8 cm long, with longitudinal opening c. 5 cm long, indumentum present, with one or two short spines; peduncular bracts tubular, more than 16 cm long, extended on one side into an elliptic, acute point, c. 6 cm long, with small, black-tipped spines and swollen bases; rachis bracts smooth, tubular, closely sheathing, 15–30 cm long, including the upper part with long splitting limb to c. 5 cm long, subtending 3–5 partial inflorescences; rachillae 6–9, each rachilla 5–12 cm long; rachilla bracts short, (slightly) swollen, cupuliform, some explanate, with a one-sided extension into an acuminate tip, subtending one flower bract; floral bracteole not tubular. *Staminate flowers* alternate, 4–6 mm long; calyx valvate, divided into 3 lobes, tubular, base swollen, elliptic, c. 2 by 1 mm, apex acute; petals 3, oblong, 4–6 by 0.5–1 mm; stamens 6, dorsifixed, c. 5 mm long; filament ensiform, yellowish brown, c. 3.5 mm long; anther sagittate, light yellow, 2–3 mm long; pistillode with 3 reddish, apically slightly falcate, c. 1.5 mm long stigmas. *Pistillate inflorescences* to 80 cm long, bearing 2 partial inflorescences; prophyll short, c. 7 cm long, smooth or almost smooth, with a longitudinal opening of c. 9 cm; peduncular bracts tubular, at first closely sheathing, later splitting longitudinally into 2 limbs, c. 12 cm long, armed with stout, black-tipped, downward pointed spines on very swollen bases, latter 3–4 mm long, some in groups of 3; secondary bracts tubular, c. 10 cm long, closely sheathing, later split longitudinally, c. 18 cm long, subtending the inflorescence, with sparse, black-tipped spines on swollen bases, with a tendency to become clawed; rachis bracts tubular, closely sheathing, floccose, 6–9 cm long, armed with downward pointing, yellowish spines, these on swollen bases, some in groups of 3, the upper part of the bract with long splitting limb to c. 10 cm long, subtending 2 partial inflorescences; with 5 or 6 rachillae, each rachilla 4–6 cm long; prophyll bracts smooth, 5–6 mm long, at first short-tubular later split into 2 or 3 broadly ovate limbs, 4–8 mm long; rachilla bracts smooth, short, tubular, only c. 1 mm long, cupuliform, with a broad limb enclosing the young flower, two-keeled with acuminate tip, c. 4 mm long, subtending 2 floral bracteoles. *Pistillate flowers* 5–11 mm long; calyx valvate, shallowly divided into 3 lobes, lobes elliptic to broadly elliptic, 2–4 by 1.5–1.8 mm, apex acute to acuminate; petals 3, elliptic, 2–5 by 0.5–3 mm; staminodes 6, thin, adnate to the base of the ovary, with a free, empty anther at the top; pistil with 3 stigmas, c. 1.5 mm long, reddish. *Fruit* unknown.

Distribution

Endemic to Borneo (Brunei, Sabah, Sarawak).

Habitat & Ecology

In swampy places and steep slopes in lower montane forest and on ridges, altitude: 1300–1700 m. Observations in the field showed that the rattan lives in a moist and open areas on hillsides. Flowering: January, August, September.

Specimen examined

Brunei: Temburong, north ridge of Bukit Retak, *Wong WKM 792* (K). Malaysia: Sabah: Tenompok FR, *Atria MAT 053* (L); Tenompok FR, *Atria MAT 054* (L), Tenompok FR, *Atria MAT 055* (L); Mt. Kinabalu, Ulu langanani, *Chew et al. RSNB 1653* (K, L); Mt. Kinabalu, *Chew & Corner RSNB 1892* (L); Mt. Kinabalu, Mesilau river, *Chew & Corner RSNB 4056* (K); Mt. Kinabalu, *Dransfield et al. JD 5707* (L); Mt. Kinabalu, Pinosok, *Dransfield et al. JD 5554* (K). Malaysia:

Sarawak: Lawas, *S (Lee) 52573* (L).

Notes

Calamus tenompokensis can easily be distinguished from other forms within the *C. javensis* complex by its vegetative parts: its angular petiole and rachis, a very different leaf sheath appearance, because of the massive and robust sheath, and the number and arrangement of the leaflets. *Calamus tenompokensis* has eight or nine pairs of large, elliptic leaflets, which are almost always regularly arranged. The staminate inflorescence resembles that of *C. javensis*, but the base of the calyx lobes is swollen. The pistillate inflorescences have rachilla bracts that are different in the broadly cupuliform limb (*Chew & Corner RSNB 1892*).

Chapter 6

Summary and Conclusions

Polymorphism in plants is and will remain the source of a long-lasting problem in taxonomy, especially in terms of morphological species recognition, nomenclature and classification. Phenotypic variations in plants are a result of adaptations to the environment in which they live. The rattan *Calamus javensis* Blume is known as a polymorphic species and it forms the most complex species among Southeast Asian palms (Dransfield, 1999; Henderson, 2020).

This thesis describes and analyses the intricacies of *C. javensis*. It, at least, reveals the variation in morphological and spatial distribution patterns and shows how certain forms can be delimited as separate taxa or as local variants. The complexity of the species necessitated a thorough examination of all existing *C. javensis* samples, both fresh and herbarium material. The specimens were first analysed morphologically and classified in forms, which were then tested with multivariate statistics (chapter 2). The resulting groups were then independently tested via a phylogenetic approach based on molecular DNA data (chapter 3). The latter data were also used for a phytogeographic analysis to see if forms were spatially coherent (chapter 4). Finally, all recognizable forms, representing informal or formal taxa, were described (chapter 5).

The polymorphism in *C. javensis* is, among others, shown by the many taxa that were once described based on morphological characters. Most of them are linked by intermediate forms. ‘Species’ included in the species complex are *C. acuminatus* Becc., *C. amplijugus* J.Dransf., *C. congestiflorus* J.Dransf., *C. corrugatus* Becc., *C. elopurensis* J.Dransf., *C. hypertrichorus* Becc., *C. impar* Becc., *C. javensis* Blume and *C. tenompokensis* Furtado. The typical characters of *C. javensis* are a stem diameter of 2–6 mm without leaf sheaths and to 10 mm with sheaths; internodes up to 30 cm long (usually shorter); a distinct ocrea, deep crimson when young; pinnate, ecirrate leaves to 40 cm long, flabellate terminal leaflets and the lowermost pair often swept back across the stem; a flagellum to

75 cm long, long inflorescences with red rachillae and ripe fruits ovoid in shape. Specimens were collected in an altitude range of 20–2,000 m above sea level. The species occurs from SE Asia mainland to Java and Borneo and a few specimens from the Philippines are known. Detailed observations from field and herbarium examinations showed that specimens often showed exceptions, and this made the morphological variation very wide. Due to intermediate forms an unambiguous species delimitation was not possible. Most diverse forms and intermediates were found in Northern Borneo with especially variation in leaves, leaf sheaths, spines, and rachillas. A few examples, there is a population or form with longer and denser (robust) spines (Form *amplijugus*). There is also a form that has no spines at all on its leaf sheath but possesses white hairs (Form *hypertrichosus*) and corrugate stripes that seem to replace the spines (Form *corrugatus*). Most deviating from the typical *C. javensis* is *C. tenompokensis*, which has as typical characters an erect stem, massive and robust spiny sheaths, angular petioles and rachises, 8 or 9 pairs of large elliptic leaflets, swollen calyx lobes in staminate flowers and broadly cupuliform limbs in pistillate flowers (specimen *Chew & Corner RSNB 1892*).

Next to the nine taxa included in the complex, there are four unique forms (*Forms 1–4*) recognized during the first morphological examination, and their uniqueness is quite clear, but as they are only represented by a single or two specimens no conclusion about their status could be made. However, it seems likely with more specimens found they constitute distinct taxa. *Form 1* and *Form 3* are like *C. tenompokensis*, only known from higher altitudes (1200–1500 m). *Form 1* differs from *C. javensis* in the short staminate, tubular, warty rachilla bracts and the lack of a flagellum and a cirrus; this is more like *C. tenompokensis* that also lacks a flagellum and cirrus, but *Form 1* (2 specimens, *Dransfield JD 5353* and *Dransfield JD 5301*) is different in the size of the leaflets (smaller in *Form 1*), the rachilla bracts (smooth in *C. tenompokensis*), and the staminate inflorescence position (the staminate inflorescence borne on the 2nd branch, 3rd branch in *C. tenompokensis*). *Form 2* is only represented by a single collection from the Philippines; the prominent differences with *C. javensis* are the presence of scattered setae along the leaflet veins and one long black seta on the main vein on the abaxial surfaces; the leaflets resemble the arrangement of typical *C. javensis*, but with quite long petioles and peculiar rachis spines. The two samples called *Form 3*, from Sarawak and Sabah, remain separate from the typical *C. javensis* cluster in all analyses and are grouped closer to *C. tenompokensis*. This entity differs from other specimens in the *C. javensis* complex, because it is stemless, has a peculiar arrangement of the tomentose leaflets, and the stigmas show a curled tip. The tomentose leaflets resemble the form *C. hypertrichosus*, but the latter is climbing, has 7 leaflet pairs (3 pairs in *Form 3*) with the leaflets arranged as in typical *C. javensis*. *Form 4* (7 specimens) is easily recognized by its peculiar rough ocrea texture, which is typically covered either with white setae or hirsute hairs or spines. Typical *C. javensis* almost always has a tubular, smooth, reddish, and glabrous ocrea. Other characters that distinguish this form from typical *C. javensis* are the length of the pistillate inflorescences, which are shorter, up to only 18 cm long and which have a twisted rachilla (especially specimen *Chew et al. 168*) and the flowers are rather far apart, while typical *C. javensis* usually has a longer pistillate inflorescences, up to 1.5 m long, with a straight rachilla and closely set flowers.

A multivariate analysis was conducted using a supervised and unsupervised approach as two different perspectives on the problem. Supervised learning is used to classify or predict data and unsupervised to understand and see a relationship between datasets. Supervised and unsupervised analyses provide additional assurance that it is very difficult to clearly distinguish between forms within the *C. javensis* species complex. When using supervised methods, entity names are used as a supervisory signal for classification problems (primary grouping via the names). When using unsupervised methods, the name of the entities was not included. From the confusion matrix of the Confusion Table (CT) analysis (supervised), we infer that most species are classified as *C. javensis*, including one member of *C. tenompokensis* (most others are not misclassified). The noteworthy result is the position of *C. javensis* var. polyphyllus and *C. acuminatus*, the confusion tables clearly indicated that both forms are interchangeable as they alternately show up as misclassified. This is not surprising as there were intermediate specimens observed. The Multidimensional scaling (MDS) analysis (unsupervised) is used to visualize relationships in a dataset. Two main groups were formed, one large group that included almost all the variation, this is called 'typical *C. javensis*', and a smaller group consisting of members of *C. tenompokensis* (Figure 2-2). Due to the overlapping characters and the presence of intermediate individuals, the taxa previously described as separate species are hereby morphologically part of the

C. javensis species which consists of individuals with continuous variability, the exception being *Calamus tenompokensis*, which forms a distinct, recognizable and morphological coherent taxon. The multivariate analysis clearly indicates that *C. javensis* is a distinct species in its very broad definition, with most other entities, ever separated from it as species, as synonyms. The observation that most of the samples are classified as *C. javensis* points in the direction of a variable species or cryptic species rather than distinct species.

Another way of testing the outcome of two distinct species is by using a molecular analysis. The results of the molecular analysis do not show results as clear as the multivariate analysis. The markers used in the molecular analysis (*mat*K and 5S spacer) gave little resolution, but a few groups in the *C. javensis* complex are phylogenetically and morphologically supported to some degree. More sequences of other markers should be added before a clear picture of the complex can be given. The phylogenetic results show that the *C. javensis* complex cannot easily be split in various taxa as main clades with statistical support are absent. *Calamus tenompokensis* forms a distinct subclade. Recognizing it as a species would render the remaining part of *C. javensis* paraphyletic (= all specimens derived from the same ancestor, but not all descendants included), which is generally not accepted in phylogenies of species. However, species do not always have to split up (as it is always simplified in cladograms), but small populations may split off and form new species, thus leaving a 'paraphyletic' species behind. Ferris et al. (2014) stated that local speciation occurring on a small geographic scale can be the dominant mode of speciation in plants, whereby small populations split off from a broad-ranged progenitor species; but there are still limited examples to support this mode of speciation (see also Crisp & Chandler, 1996; De Queiroz, 1998). The multivariate analysis (Atria et al., 2017) showed one (multi)group indicated as *C. javensis*, with at the outside of the big cluster *C. tenompokensis* and *C. acuminatus* as more or less distinct groups. Seemingly the *C. javensis* complex is adapting to locally different environments, resulting now in a few morphologically, but not yet genetically distinct forms. Genetically, they are presumably also differentiating, but not yet enough to recognize them as distinct species in well-supported clades. *Calamus tenompokensis*, distinctive in the multivariate analysis, and in this phylogenetic analysis, should be recognized on species level. The form *C. acuminatus* (including var. *polyphyllus*) may have a status as variety.

Unfortunately, the two molecular markers (*mat*K and 5S nrDNA) were not able to unravel the relationships between forms in the *C. javensis* species complex. As the historical biogeographic analysis uses the same data, no historical biogeographic patterns were found for the various forms. The biogeographical range of the *C. javensis* complex includes southern Thailand, the Malay Peninsula, Sumatra, West Java, Borneo and Palawan and Luzon in the Philippines. The greatest morphological diversity is found in north Borneo, where only *C. tenompokensis* displayed a distinct pattern in Sabah, because this species is a local endemic on Mount Kinabalu and the Crocker Range. It likely adapted to higher altitudes and that is also the reason why *Form 1* and *3* resemble *C. tenompokensis*, they also occur on higher altitudes. Borneo is an important area for most of the forms in the *C. javensis* complex. The NE of Sabah comprises most forms, followed by Mt. Kinabalu and adjacent areas, and "Brunei" (see Figure 4-1). Other important areas are central Sarawak and parts of West Kalimantan. The results of the historical biogeographical analysis show that the distribution area for most specimens in "*acuminatus-polyphyllus*" is in Northeast Sabah (Borneo) and "Brunei" with a small portion in North Sarawak and Southeast Sabah. A clear pattern is also seen in the Luzon area (Philippines) where '*Form 2*' was collected, but unfortunately this form only appears once in phylogeny.

The Malesian region has experienced several periods of land emergence and disappearance during glaciation cycles. During the peak of an ice age, with a sea level of minus 120 m, the Malesia Region reaches its maximum extent of land, because the Sunda Shelf becomes a landmass connecting the Malay Peninsula, Sumatra, Borneo and Java. The species *C. javensis* may have originated on Borneo, at least it is likely that this species already existed in this area in the early stages of its evolution. But the evidence is circumstantial at most: Borneo is one of the oldest West Malesian regions, at least partially above sea level for more than 60 Ma (Hall, 2013), and the highest morphological variability is found on Borneo. The subfamily Calamoideae diversified from other palm groups in Eurasia at around 80 Ma, while the divergence of the Calameae tribe is from Eurasia and dated later than 75 Ma to the early Palaeocene 66 Ma (Dransfield, 2008; Baker & Couvreur, 2012). Through his research Raes et al. (2014) explained that NE Borneo was a refuge area for members of the tree family Dipterocarpaceae, likely because not all parts of the Sunda Shelf area were everwet during an ice age as a savannah corridor was formed from the Malay Peninsula to Java and parts of southern Borneo. The NE area of Borneo remained everwet and this may also have been a refuge area for *C. javensis* because the species likes a wet and humid climate, and the analysis shows that Borneo is an important area with most variation. Islands of the Philippines became close with or connected to Borneo during the glaciations, a landbridge ran from NE Borneo to Luzon via Mindanao (e.g., Morley & Flenley, 1987). The *Form 2* phenotype is likely a result of dispersal along the land bridge with adaptation to the new environment. Most forms not only occur in various branches of the phylogeny, but also in non-adjacent, disjunct areas, likely the result of adaptation to local circumstances, whereby more or less identical forms can develop under similar niche circumstances in different areas. Exceptions are the '*acuminatus-polyphyllus*' form and *C. tenompokensis*, which are present in non-disjunct areas.

The last chapter of this thesis was a revision of *C. javensis* to describe the forms as informal groups, to make their identification possible via a key, and to present detailed descriptions of each of them. Henderson (2020) has described *C. javensis* as an ochlospecies (he treated morphotypes/forms informally, he did not name them) of several phylogenetic species. White's (1998) concept of ochlospecies is a species that is problematic but is not the result of well-known processes such as autogamy, hybridization, or apomixis. Cronk (1998) defined ochlospecies as a polymorphic species that causes problems in formal taxonomic treatment. Meanwhile, Barbosa (2012) states that ochlospecies is a species that is highly polymorphic (but weakly polytypic) with morphological characters that vary independently. Often this variation can be partly correlated with geological and ecological conditions. According to Barbosa, ochlospecies usually have a wide geographic and ecological distribution and many synonyms. Two species are recognized, *C. javensis* and *C. tenompokensis*. All formerly described species, *C. acuminatus, C. amplijugus, C. congestiflorus, C. corrugatus, C. elopurensis, C. hypertrichosus, C. impar,* and *C. kemamanensis*, are treated as synonyms of *C. javensis*. There is somewhat an exception for the form *C. acuminatus/polyophyllus*. The multivariate analysis placed *C. acuminatus/polyphyllus* as group in the big cluster of *C. javensis*. However, the phylogenetic results showed that although the clade *C. acuminatus/polyphyllus* was partially well supported (it is among the few groups with statistical support), and as it has a narrow distribution and ecological preference, it is recognized at the varietal level, combined with the inseparable *C. javensis* var. *polyphyllus* (Becc.) Becc., under the latter name (oldest on the level of variety).

The overall summary of this thesis is that *C. javensis* is a rattan that easily adapts to the environment in which it lives, which results in a mosaic of local morphological forms, that are not yet genetically distinct and can thus interbreed and form intermediates. Independent and parallel adaptation processes are occurring in different parts of the distribution when the environment is similar as the same forms are found in disjunct areas, and this is also seen from the presence of the same forms in different clades. The recognition of various forms within an informal group, as we do here, illustrates that the specimens in this group live in similar and comparable environmental conditions. The concept of genetic assimilation (Pigliucci et al., 2006) nicely explains what is happening: 'A population will produce novel phenotypes when pressured by environmental conditions via pre-existing reaction mechanisms for which no initial genetic change is necessary. A genetic fixation happens when natural selection continues to work under the new environmental conditions when the new phenotype prevails'.

Samenvatting en Conclusies

Polymorfisme in planten is en blijft de bron van een langdurig probleem in de taxonomie, vooral wat betreft morfologische soortherkenning, nomenclatuur en classificatie. Fenotypische variaties in planten zijn het resultaat van aanpassingen aan de omgeving waarin ze leven. De rotan *Calamus javensis* Blume staat bekend als een polymorfe soort en vormt de meest complexe soort binnen de Zuidoost-Aziatische palmen (Dransfield, 1999; Henderson, 2020).

Dit proefschrift beschrijft en analyseert de complexiteit van *C. javensis*. Het onthult in ieder geval de variatie in morfologische en ruimtelijke verspreidingspatronen en laat zien hoe bepaalde vormen kunnen worden afgebakend als afzonderlijke taxa of als lokale varianten. De complexiteit van de soort maakte een grondig onderzoek van alle bestaande *C. javensis*-monsters noodzakelijk, zowel vers als herbariummateriaal. De exemplaren werden eerst morfologisch geanalyseerd en geclassificeerd in vormen, die vervolgens werden getest met multivariate statistiek (hoofdstuk 2). De resulterende groepen werden vervolgens onafhankelijk getest via een fylogenetische benadering op basis van moleculaire DNA-gegevens (hoofdstuk 3). Deze laatste gegevens werden ook gebruikt voor een fytogeografische analyse om te zien of vormen ruimtelijk samenhangend waren (hoofdstuk 4). Ten slotte werden alle herkenbare vormen, die informele of formele taxa vertegenwoordigen, beschreven (hoofdstuk 5).

Het polymorfisme bij *C. javensis* blijkt onder andere uit de vele taxa die ooit werden beschreven op basis van morfologische kenmerken. De meeste zijn verbonden door tussenvormen. ‘Soorten’ die deel uitmaken van het soortencomplex zijn *C. acuminatus* Becc., *C. amplijugus* J.Dransf., C. *congestiflorus* J. Dransf., *C. corrugatus* Becc., *C. elopurensis* J.Dransf., *C. hypertrichosus* Becc. , *C. impar* Becc., *C. javensis* Blume en *C. tenompokensis* Furtado. De typische kenmerken van *C. javensis* zijn een stengeldiameter van 2–6 mm zonder bladscheden en tot 10 mm met scheden; internodiën tot 30 cm lang (meestal korter); een duidelijke ocrea, diep karmozijnrood als ze jong zijn; ecirrate bladeren tot 40 cm lang, flabellate eindblaadjes en het onderste paar vaak teruggeslagen over de stengel; een flagellum tot 75 cm lang, lange bloeiwijzen met rode rachillae en rijpe vruchten eivormig. Planten werden verzameld op een hoogte van 20 tot 2000 m boven zeeniveau. De soort komt voor van het vasteland van Zuidoost-Azië tot Java en Borneo en er zijn enkele exemplaren bekend uit de Filippijnen. Gedetailleerde observaties van veld- en herbariumonderzoek toonden aan dat exemplaren vaak uitzonderingen vertoonden, waardoor de morfologische variatie erg groot was. Door tussenvormen was een eenduidige soortafbakening niet mogelijk. De meest uiteenlopende vormen en tussenproducten werden gevonden in Noord-Borneo met vooral variatie in bladeren, bladscheden, stekels en rachillae. Een paar voorbeelden, er is een 'populatie' of 'vorm' met langere en dichtere (robuuste) stekels (Vorm *amplijugus*). Er is ook een vorm die helemaal geen stekels op de bladschede heeft maar witte haren heeft (Vorm *hypertrichosus*) en gegolfde strepen die de stekels lijken te vervangen (Vorm *corrugatus*). Het meest afwijkend van de typische *C. javensis* is *C. tenompokensis*, die heeft als typische kenmerken een rechtopstaande stengel, massieve en robuuste stekelige schedes, hoekige bladstelen en rachises, 8 of 9 paar grote elliptische blaadjes, gezwollen kelklobben in de mannelijke bloemen en breed bekervormige kelklobben in de vrouwelijke bloemen (b.v. exemplaar *Chew & Corner RSNB 189*2).

Naast de negen taxa die in het complex zijn opgenomen, zijn er vier unieke vormen (*Vormen 1-4*) herkend tijdens het eerste morfologische onderzoek, en hun uniekheid is vrij duidelijk, maar aangezien ze slechts worden vertegenwoordigd door een enkel of twee exemplaren, kan er geen conclusie over hun status worden getrokken. Het lijkt echter waarschijnlijk dat als er meer exemplaren zijn gevonden, ze verschillende taxa vormen. *Vorm 1* en *Vorm 3* zijn, als *C. tenompokensis*, alleen bekend van grotere hoogten (1200–1500 m). *Vorm 1* verschilt van *C. javensis* door de korte meeldraden, buisvormige, wrattenachtige rachilla schutbladen en het ontbreken van een flagellum en een cirrus; dit lijkt meer op *C. tenompokensis* die ook geen flagellum en cirrus heeft, maar *Vorm 1* (2 exemplaren, *Dransfield JD 5353* en *Dransfield JD 5301*) verschilt in de grootte van de blaadjes (kleiner in *Vorm 1*), de rachilla schutbladen (gladde in *C. tenompokensis*), en de positie van de mannelijke bloeiwijze (de mannelijke bloeiwijze beginnend op de 2e tak, versus op de 3e tak in *C. tenompokensis*). *Vorm 2* wordt alleen vertegenwoordigd door een enkele collectie uit de Filippijnen; de opvallende verschillen met *C. javensis* zijn de aanwezigheid van verspreide setae langs de bladnerven en één lange zwarte seta op de middennerf abaxiaal; de blaadjes lijken in compositie op die van de typische *C. javensis*, maar met vrij lange bladstelen en eigenaardige rachis stekels. De twee monsters die *Vorm 3* vormen, uit Sarawak en Sabah, blijven in alle analyses gescheiden van het typische *C. javensis*-cluster en zijn dichter bij *C. tenompokensis* gegroepeerd. Deze entiteit verschilt van andere exemplaren in het *C. javensis*-complex, omdat het stengelloos is, een eigenaardige opstelling heeft van de tomentose-blaadjes en de stigma's een gekrulde punt vertonen. De tomentose-blaadjes lijken op de vorm *C. hypertrichosus*, maar de laatste klimt, heeft 7 paar blaadjes (3 paar in *Vorm 3*) met de blaadjes gerangschikt zoals bij typische *C. javensis*. *Vorm 4* (7 exemplaren) is gemakkelijk te herkennen aan zijn eigenaardige ruwe ocrea-textuur, die meestal bedekt is met witte borstelharen of behaarde haren of stekels. Typische *C. javensis* heeft bijna altijd een buisvormig, glad, roodachtige en kale ocrea. Andere kenmerken die deze vorm onderscheiden van de typische *C. javensis* zijn de lengte van de vrouwelijke bloeiwijzen, die korter zijn, tot slechts 18 cm lang en die een gedraaide rachilla hebben (vooral exemplaar *Chew et al. 168*) en de bloemen staan tamelijk uit elkaar, terwijl de typische C. *javensis* meestal langere vrouwelijke bloeiwijzen heeft, tot 1,5 m lang, met een rechte rachilla en dicht op elkaar staande bloemen.

Er werd een multivariate analyse uitgevoerd, waarbij het probleem op twee manieren werd aangepakt, via begeleid en niet-begeleid leren. Begeleid leren wordt gebruikt om gegevens te classificeren of te voorspellen en niet-begeleid leren om een relatie tussen datasets te begrijpen en te zien. Begeleid en niet-begeleide analyses bieden extra ondersteuning bij de conclusie dat het erg moeilijk is om duidelijk onderscheid te maken tussen vormen binnen het *C. javensis* soortencomplex. Bij het gebruik van begeleide methoden worden entiteitsnamen gebruikt als groeperingssignaal voor classificatieproblemen (primaire groepering via de namen). Bij het gebruik van methoden zonder begeleiding werd de naam van de entiteiten niet opgenomen. Uit de verwarringsmatrix van de VerwarringsTabel (VT)-analyse (begeleid) leiden we af dat de meeste exemplaren zijn geclassificeerd als *C. javensis*, waaronder één lid van *C. tenompokensis* (de meeste andere zijn niet verkeerd geclassificeerd). Het opmerkelijke resultaat is de positie van *C. javensis* var. *polyphyllus* en C. *acuminatus*, hierbij gaven de verwarringstabellen duidelijk aan dat beide vormen uitwisselbaar zijn, aangezien ze afwisselend verschijnen als verkeerd geclassificeerd. Dit is niet verwonderlijk aangezien er tussenvormen zijn waargenomen. De ‘Multidimensional Scaling’ (MDS)-analyse (niet-begeleid) wordt gebruikt om relaties in een dataset te visualiseren. Er werden twee hoofdgroepen gevormd, een grote groep die bijna alle variatie omvatte, dit wordt ‘typische *C. javensis*’ genoemd, en een kleinere groep bestaande uit leden van *C. tenompokensis* (Fig. 2, hoofdstuk 2). Vanwege de overlappende kenmerken en de aanwezigheid van intermediaire individuen, maken de taxa die eerder als afzonderlijke soorten werden beschreven, nu morfologisch deel uit van de *C. javensis*-soort die bestaat uit individuen met continue variabiliteit, met uitzondering van *Calamus tenompokensis*, die een apart, herkenbaar en morfologisch coherent taxon vormt. De multivariate analyse geeft duidelijk aan dat *C. javensis* een afzonderlijke soort is in zijn zeer brede definitie, met de meeste andere entiteiten, die er ooit van gescheiden zijn als soort, als synoniemen. De waarneming dat de meeste exemplaren zijn geclassificeerd als *C. javensis* wijst in de richting van een variabele soort of cryptische soorten in plaats van afzonderlijke soorten.

Een andere manier om de uitkomst van twee verschillende soorten te testen, is door een moleculaire analyse te gebruiken. De resultaten van de moleculaire analyse laten niet zulke duidelijke resultaten zien als de multivariate analyse. De markers die in de moleculaire analyse werden gebruikt (*mat*K en de 5S spacer) gaven weinig resolutie, maar een paar groepen in het *C. javensis*-complex worden tot op zekere hoogte fylogenetisch en morfologisch ondersteund. Er moeten meer sequenties van andere markers worden toegevoegd voordat een duidelijk beeld van het complex kan worden gegeven. De fylogenetische resultaten laten zien dat het *C. javensis*-complex niet gemakkelijk in verschillende taxa kan worden opgesplitst, aangezien hoofdgroepen met statistische ondersteuning ontbreken. *Calamus tenompokensis* vormt een aparte tak. Herkenning als soort zou het resterende deel van *C. javensis* parafyletisch maken (= alle exemplaren zijn afgeleid van dezelfde voorouder, maar niet alle nakomelingen inbegrepen), wat over het algemeen niet wordt geaccepteerd in de fylogenieën van soorten. Soorten hoeven echter niet altijd op te splitsen (zoals altijd vereenvoudigd in cladogrammen), maar kleine populaties kunnen zich afsplitsen en nieuwe soorten vormen, waardoor een ‘parafyletische’ soort achterblijft. Ferris et al. (2014) stelde dat lokale soortvorming, die op kleine geografische schaal plaatsvindt, de dominante vorm van soortvorming in planten kan zijn, waarbij kleine populaties zich afsplitsen van een breed verspreide voorlopersoort; maar er zijn nog steeds beperkte voorbeelden die deze vorm van soortvorming ondersteunen (zie ook Crisp & Chandler 1996, De Queiroz 1998). Uit de multivariate analyse (Atria et al., 2017) duidde één (multi)groep aan als *C. javensis*, met aan de buitenkant van het grote cluster *C. tenompokensis* en *C. acuminatus* als min of meer aparte groepen. Het lijkt erop dat het *C. javensis*-complex zich aanpast aan verschillende lokale omgevingen, wat nu resulteert in een paar morfologische, maar nog niet genetisch verschillende vormen. Genetisch differentiëren ze vermoedelijk ook, maar nog niet genoeg om ze te herkennen als afzonderlijke soorten in goed ondersteunde takken. *Calamus tenompokensis*, apart in de multivariate analyse en in deze fylogenetische analyse, dient op soortniveau herkend te worden. De vorm *C. acuminatus* (inclusief var. *polyphyllus*) kan een status als variëteit hebben.

Helaas konden de twee moleculaire markers (*mat*K en 5S nrDNA) de relaties tussen vormen in het *C. javensis*-soortencomplex niet ontrafelen. Omdat de historische biogeografische analyse dezelfde gegevens gebruikt, werden er geen historische biogeografische patronen gevonden voor de verschillende vormen. Het biogeografische verspreidingsgebied van het *C. javensis*-complex omvat Zuid-Thailand, het Maleisische schiereiland, Sumatra, West-Java, Borneo en Palawan en Luzon in de Filippijnen. De grootste morfologische diversiteit wordt gevonden in Noord-Borneo, waar alleen *C. tenompokensis* een duidelijk patroon vertoonde in Sabah, omdat deze soort lokaal endemisch is op Mt. Kinabalu en de Crocker Range. Het heeft zich waarschijnlijk aangepast aan grotere hoogten en dat is ook de reden waarom *Vorm 1* en *3* op *C. tenompokensis* lijken, ze komen ook op grotere hoogten voor. Borneo is een belangrijk gebied voor de meeste vormen in het *C. javensis*-complex. Het NO van Sabah omvat de meeste vormen, gevolgd door Mt. Kinabalu en aangrenzende gebieden, en 'Brunei' (zie figuur 1, hoofdstuk 4). Andere belangrijke gebieden zijn centraal Sarawak en delen van West-Kalimantan. De resultaten van de historische biogeografische analyse laten zien dat het verspreidingsgebied van de meeste exemplaren in '*acuminatus-polyphyllus*' zich in Noordoost-Sabah (Borneo) en 'Brunei' bevindt, met een klein deel in Noord-Sarawak en Zuidoost-Sabah. Een duidelijk patroon is ook te zien in het Luzon-gebied (Filippijnen) waar '*Form 2*' werd verzameld, maar helaas komt deze vorm maar één keer voor in de fylogenie.

De Maleisische regio heeft tijdens ijstijden verschillende perioden van opkomst en verdwijning van land meegemaakt. Tijdens de piek van een ijstijd, met een zeespiegel van min 120 m, bereikt de regio Maleisië zijn maximale landoppervlak, omdat het Sunda-plateau een landmassa wordt die het Maleise schiereiland, Sumatra, Borneo en Java met elkaar verbindt. De soort *C. javensis* is mogelijk ontstaan op Borneo, het is in ieder geval waarschijnlijk dat deze soort al in de vroege stadia van zijn evolutie in dit gebied voorkwam. Maar het bewijs is hooguit indirect: Borneo is een van de oudste regio's van West-Maleisië, tenminste gedeeltelijk boven zeeniveau gedurende meer dan 60 miljoen jaar (Hall, 2013), en de hoogste morfologische variabiliteit wordt gevonden op Borneo. De onderfamilie Calamoideae diversifieerde van andere palmgroepen in Eurazië rond 80 Ma, terwijl de divergentie van de Calameae-stam uit Eurazië komt en dateert tussen 75 Ma en het vroege Paleoceen van 66 Ma (Dransfield, 2008; Baker & Couvreur, 2012). Via hun onderzoek legden Raes et al. (2014) uit dat NO Borneo een toevluchtsoord was voor leden van de boomfamilie Dipterocarpaceae, waarschijnlijk omdat niet alle delen van het Sunda Plateau-gebied altijd nat waren tijdens een ijstijd, aangezien er een savannecorridor werd gevormd van het Maleise schiereiland naar Java en delen van Zuid-Borneo. Het NO-gebied van Borneo bleef altijd nat en dit kan ook een toevluchtsoord zijn geweest voor *C. javensis* omdat de soort van een nat en vochtig klimaat houdt, en de analyse laat zien dat Borneo een belangrijk gebied is met de meeste variatie. Eilanden van de Filipijnen kwamen in de buurt van of waren verbonden met Borneo tijdens de ijstijden, een landbrug liep van NO Borneo naar Luzon via Mindanao (bijv. Morley & Flenley, 1987). Het *Vorm 2*-fenotype is waarschijnlijk het resultaat van verspreiding langs de landbrug met aanpassing aan de nieuwe omgeving. De meeste vormen komen niet alleen voor in verschillende takken van de fylogenie, maar ook in niet-aangrenzende, gescheiden gebieden, waarschijnlijk het resultaat van aanpassing aan lokale omstandigheden, waardoor min of meer identieke vormen zich konden ontwikkelen onder vergelijkbare niche-omstandigheden in verschillende gebieden. Uitzonderingen zijn de vorm '*acuminatus-polyphyllus*' en *C. tenompokensis*, die aanwezig zijn in continue gebieden.

Het laatste hoofdstuk van dit proefschrift was een revisie van *C. javensis* om de vormen als informele groepen te beschrijven, hun identificatie via een sleutel mogelijk te maken, en om gedetailleerde beschrijvingen van elk van hen te geven. Henderson (2020) heeft *C. javensis* beschreven als een ochlospecies (hij behandelde morfotypes/vormen informeel, hij noemde ze niet) van verschillende fylogenetische soorten. White's (1998) concept van ochlospecies is een soort die problematisch is, maar niet het resultaat is van bekende processen zoals autogamie, hybridisatie of apomixis. Cronk (1998) definieerde ochlospecies als een polymorfe soort die problemen veroorzaakt bij de formele taxonomische behandeling. Ondertussen stelt Barbosa (2012) dat de ochlospecies een soort is die zeer polymorf (maar zwak polytypisch) is, met morfologische kenmerken, die onafhankelijk van elkaar variëren. Vaak kan deze variatie gedeeltelijk worden gecorreleerd met geologische en ecologische omstandigheden. Volgens Barbosa hebben ochlospecies meestal een brede geografische en ecologische verspreiding en veel synoniemen. Er worden twee soorten herkend, *C. javensis* en *C. tenompokensis*. Alle eerder beschreven soorten, *C. acuminatus*, *C. amplijugus*, *C. congestiflorus*, *C. corrugatus*, *C. elopurensis*, *C. hypertrichosus*, *C. impar* en *C. kemamanensis*, worden behandeld als synoniemen van *C. javensis*. Er is een uitzondering voor de vorm *C. acuminatus/polyphyllus*. De multivariate analyse plaatste *C. acuminatus/polyphyllus* als groep in het grote *C. javensis* cluster. Echter de fylogenetische resultaten toonden aan dat, hoewel de tak *C. acuminatus/polyphyllus* gedeeltelijk goed werd ondersteund (het is een van de weinige groepen met statistische ondersteuning), en aangezien het een smalle verspreiding en ecologische voorkeur heeft, het wordt erkend op het niveau van de variëteit als *C. javensis* var. *polyphyllus* (Becc.) Becc., de oudste naam op het niveau van variëteit.

De algemene samenvatting van dit proefschrift is dat *C. javensis* een rotan is die zich gemakkelijk aanpast aan de omgeving waarin hij leeft, wat resulteert in een mozaïek van lokale morfologische vormen, die nog niet genetisch onderscheiden zijn en dus onderling kunnen kruisen en tussenvormen kunnen genereren. Onafhankelijke en parallelle aanpassingsprocessen vinden plaats in verschillende delen van de verspreiding wanneer de omgeving vergelijkbaar is, aangezien dezelfde vormen worden aangetroffen in gescheiden gebieden, en dit blijkt ook uit de aanwezigheid van dezelfde vormen in verschillende takken. De herkenning van verschillende vormen binnen een informele groep, zoals we hier doen, illustreert dat de exemplaren in deze groepen in gelijke en vergelijkbare omgevingsomstandigheden leven. Het concept van genetische assimilatie (Pigliucci et al., 2006) legt mooi uit wat er gebeurt: ‘Een populatie zal nieuwe fenotypes produceren wanneer ze onder druk wordt gezet door omgevingsfactoren via reeds bestaande reactiemechanismen waarvoor geen initiële genetische verandering nodig is. Een genetische fixatie vindt plaats wanneer natuurlijke selectie blijft werken onder de nieuwe omgevingsomstandigheden wanneer het nieuwe fenotype de overhand heeft.

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Curriculum Vitae

Mega Atria was born on 26th of August 1969, in Surabaya, Indonesia. When she was 8, she moved with her parents to Den Haag, the Netherlands, where she attended an Indonesian primary school in Wassenaar. At the age of 12 she moved back to Indonesia to attend junior and senior high school in Surabaya and Jakarta. In 1988 she started to study Biology at the University of Indonesia (UI) in Depok and finished in 1995.

She was appointed as a junior lecturer at the Department of Biology, University of Indonesia (UI) in 1997, and to assist with all lectures and practicals. In 2000 she became a permanent lecturer in the Taxonomy Lab. A senior colleague from her lab, Dr. Padmi Kramadibrata introduced her to the rattan genus *Calamus*. During fieldwork with students in Java she deepened her interest in rattan.

In 2007 she participated in the “Ecosystem Approach on biodiversity management” course, a collaboration between the KEHATI Foundation and Utrecht University, The Netherlands. This enabled her to introduce the concept of nature conservation when teaching plant biodiversity, using role play methods and Problem Based Learning discussions.

In 2009, under the supervision of Dr. Johanis P. Mogea, and Dr. Ary P. Keim from Herbarium Bogoriense (BO) and Dr. Noviar Andayani from UI, she obtained her Master degree in biodiversity and conservation from University of Indonesia (UI) with a thesis entitled “the Rattan *Calamus* section *Coleospathus* in West Malesia”.

In 2012, the DIKTI-LEIDEN Cooperation scholarship fund (Directorate General of Higher Education, Ministry of National Education, Indonesia, and Leiden University) provide her with funds for a Ph.D. study entitled “The Biogeography and Evolution of the Rattan *Calamus javensis* (Blume) complex in Malesia” in the Netherlands, which she started in January 2013 at Naturalis Biodiversity Center. Prof.dr. Peter C. van Welzen was her promotor. The study was supported by two rattan specialists, Prof.dr. John Dransfield and Dr. William J. Baker, of the Royal Botanic Gardens of Kew, Richmond, UK, who she visited for 3 weeks.

In 2017, she returned to UI, where she finished the thesis besides her regular teaching activities. She took the initiative to manage the plant collection stored in the Department of Biology. In 2018 she was assigned as head of the Biology Department’s Collection Room.

Publications

Atria, M., Eurlings, M., Baker, W.J., Dransfield, J. and Van Welzen, P.C. 2020. Phylogenetic analysis of the *Calamus javensis* complex (Arecaceae: Calamoideae) in Malesia. Blumea 65: 205–211.

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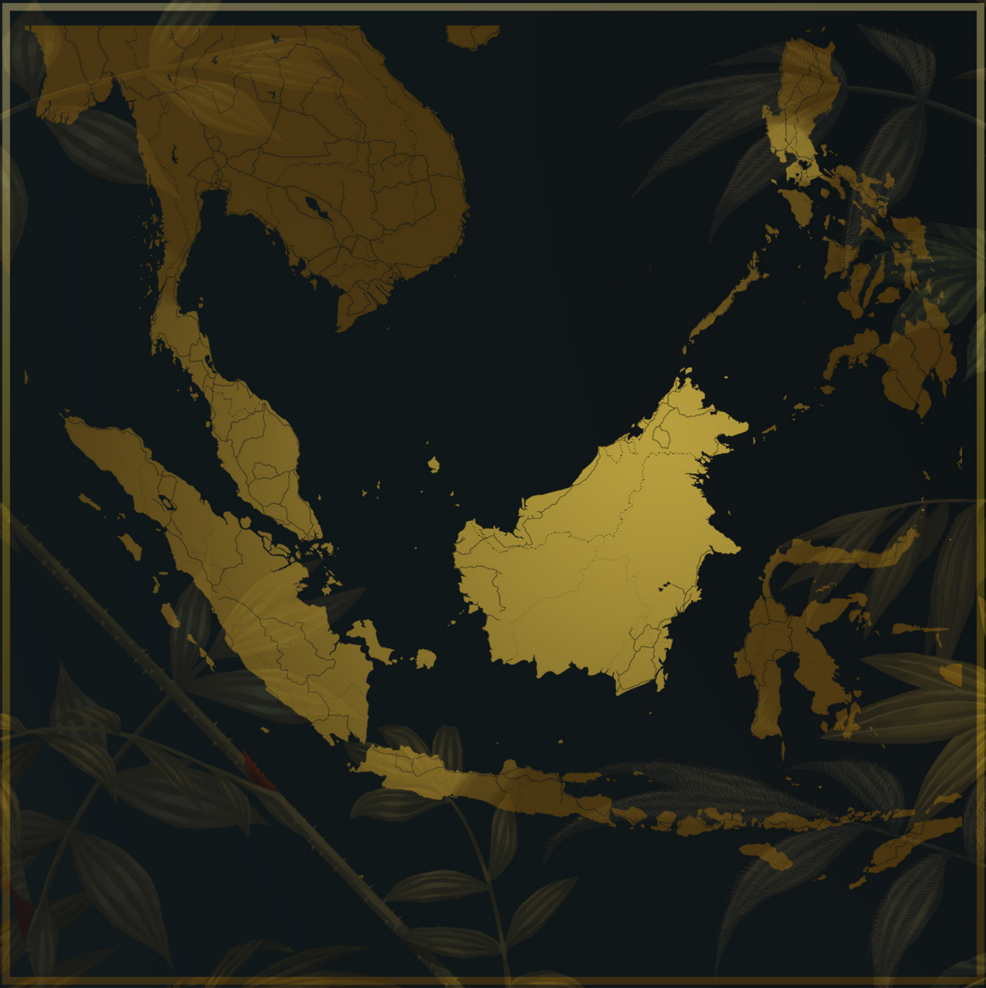

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