Blumea 65, 2020: 205–211
www.ingentaconnect.com/content/nhn/blumea

RESEARCH ARTICLE

https://doi.org/10.3767/blumea.2020.65.03.04

Phylogenetic analysis of the *Calamus javensis* complex (*Arecaceae*: *Calamoideae*) in Malesia

M. Atria[1,2,3,*], M. Eurlings[1], W.J. Baker[4], J. Dransfield[4], P.C. van Welzen[1,3]

Key words

Calamoideae
Calamus javensis
chloroplast DNA
intraspecific variation
*mat*K
molecular phylogeny
nuclear DNA
paraphyletic
rattan

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.

Citation: Atria M, Eurlings M, Baker WJ, et al. 2020. Phylogenetic analysis of the Calamus javensis complex (Arecaceae: Calamoideae) in Malesia. Blumea 65 (3): 205–211. https://doi.org/10.3767/blumea.2020.65.03.04.
Effectively published online: 22 December 2020.

INTRODUCTION

Calamus L. is the largest genus among the climbing palms, also called rattans (http://www.fao.org/3/Y2783E/y2783e05.htm; Sreekumar & Renuka 2008), and is classified in subtribe *Calaminae*. The generic subdivision of the subtribe appeared to be as difficult as the *C. javensis* complex. The phylogeny of the rattans by Kramadibrata (1992) suggested *Calamus* to be 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 [the genera] *Ceratolobus* [Blume ex Schult. & Schult.f.], *Daemonorops* [Blume], *Pogonotium* [J.Dransf.] and *Retispatha* [J.Dransf.] are placed in synonymy with *Calamus*. This is presented as a stable, alternative and pragmatic taxonomic solution for this problematic group".

Calamus javensis Blume (*Arecaceae*: *Calamoideae*) is a slender rattan common in southeast Asian tropical rainforests. The species is very polymorphic and includes some taxonomically non-recognized, but morphologically distinct forms, next to a range of species that were ever split off: *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 (synonyms can be found in Barfod & Dransfield 2013). The distribution of the species complex ranges from Southern Thailand and Peninsular Malaysia to Sumatra, Java, Borneo and Palawan (Dransfield 1992, Barfod & Dransfield 2013). The greatest morphological diversity is in north Borneo. Typical for all forms 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 the stem; a flagellum to 75 cm long, long inflorescences with red rachillae and ripe fruits ovoid in shape.

The morphological variation within *C. javensis* is complex and large and there are hardly constant differences among the entities. As a result, traditional morphological observations did not provide a satisfactory solution (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, *C. 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 (vs not swollen in *C. javensis*). The pistillate inflorescences have rachilla bracts that are different in the broadly cupuliform limb (vs bracts tightly sheathing in *C. javensis*).

In this study we conduct a phylogenetic analysis of the *C. javensis* complex based on two different regions, 5S nrDNA and *mat*K (formerly used by Baker et al. 2000a, b). Aim is to see if

[1] Naturalis Biodiversity Center, Research Group Tropical Botany, P.O. Box 9517, 2300 RA Leiden, The Netherlands; corresponding author e-mail: mega.atria@sci.ui.ac.id.
[2] Departemen Biologi, Fakultas Matematika dan Ilmu Pengetahuan Alam, Universitas Indonesia (UI), Depok 16424, Indonesia.
[3] Institute of Biology Leiden, University of Leiden, P.O. Box 9505, 2300 RA Leiden, The Netherlands.
[4] Royal Botanic Gardens, Kew, Richmond, TW9 3AB, Surrey, United Kingdom.

the complex can be subdivided in monophyletic, recognisable units, which may corroborate the morphometric study (Atria et al. 2017).

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 *C. 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 Appendix 1.

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).

Two DNA regions were amplified, the nuclear 5S spacer and the chloroplast marker *mat*K. Primers (Table 1) for this study were designed specifically for the group based on the work of Baker et al. (2000b) using Geneious v. 10.1.3 (http://www.geneious.com). Primers for the 5S spacer (Table 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 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 25 µl containing 5 µl of 5× 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 10 mM dNTP, and 1 µl 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 Appendix 1).

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 datasets and combined.

A parsimony analysis was performed with PAUP* v. 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 1 000th generation whereby the first 25 % of the samples were discarded as burnin. We ran 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 of the chains. The two tree files of the combined markers were then combined using LogCombiner v. 1.10.3 (in BEAST v. 1.10.3; 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 v. 1.10.3 (in BEAST v. 1.10.3) to create the Maximum Clade Credibility (MCC) tree, which was visualised with FigTree v. 1.4.3 (Rambaut 2010).

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 53 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 (Fig. 1 & 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 samples (Fig. 1); there is only full support (posterior probability

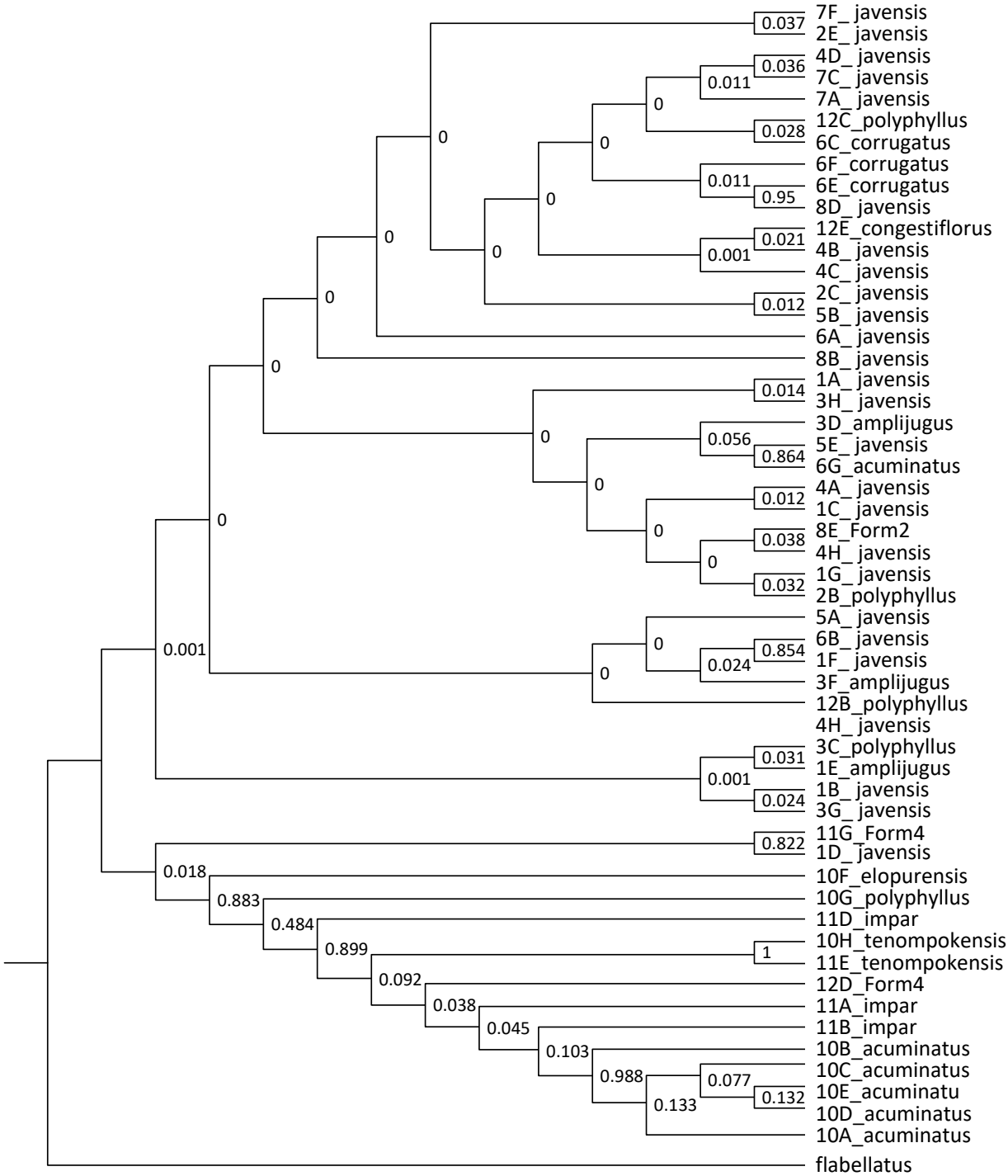

Fig. 1 Cladogram of 5S 50 % majority rule consensus tree from MrBayes analysis using MCMC algorithm. Cladogram shows lack of support for the majority of samples. There is only full support (posterior probability (PP) = 1) for the complete *C. javensis* complex as the outgroup is very different.

(PP) = 1) for the complete *C. javensis* complex as the outgroup is very different. Several subclades (framed in Fig. 2) with reasonable support (PP > 0.8) are present, all other branches lack support (PP usually < 0.1).

Both cladograms show higher support (PP > 0.8) for 4 pairs of specimens (Groups A–D in Fig. 2). One larger clade (Group H, Fig. 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 (Fig. 2), *C. acuminatus*, whereby not all forms identified as *C. acuminatus* are included, Group B also contains one. All other pairs of supported entities form strange combinations, morphologically and geographically (see Discussion).

DISCUSSION

The topologies of Fig. 1 and 2 are similar, which means that only the 5S sequences determined the phylogeny, *mat*K did not contribute at all. The variable and informative characters (24) in the 5S sequences are insufficient to solve the cladogram, with a major polytomy as result. No other markers could be sequenced in the time and financial means allotted to this project.

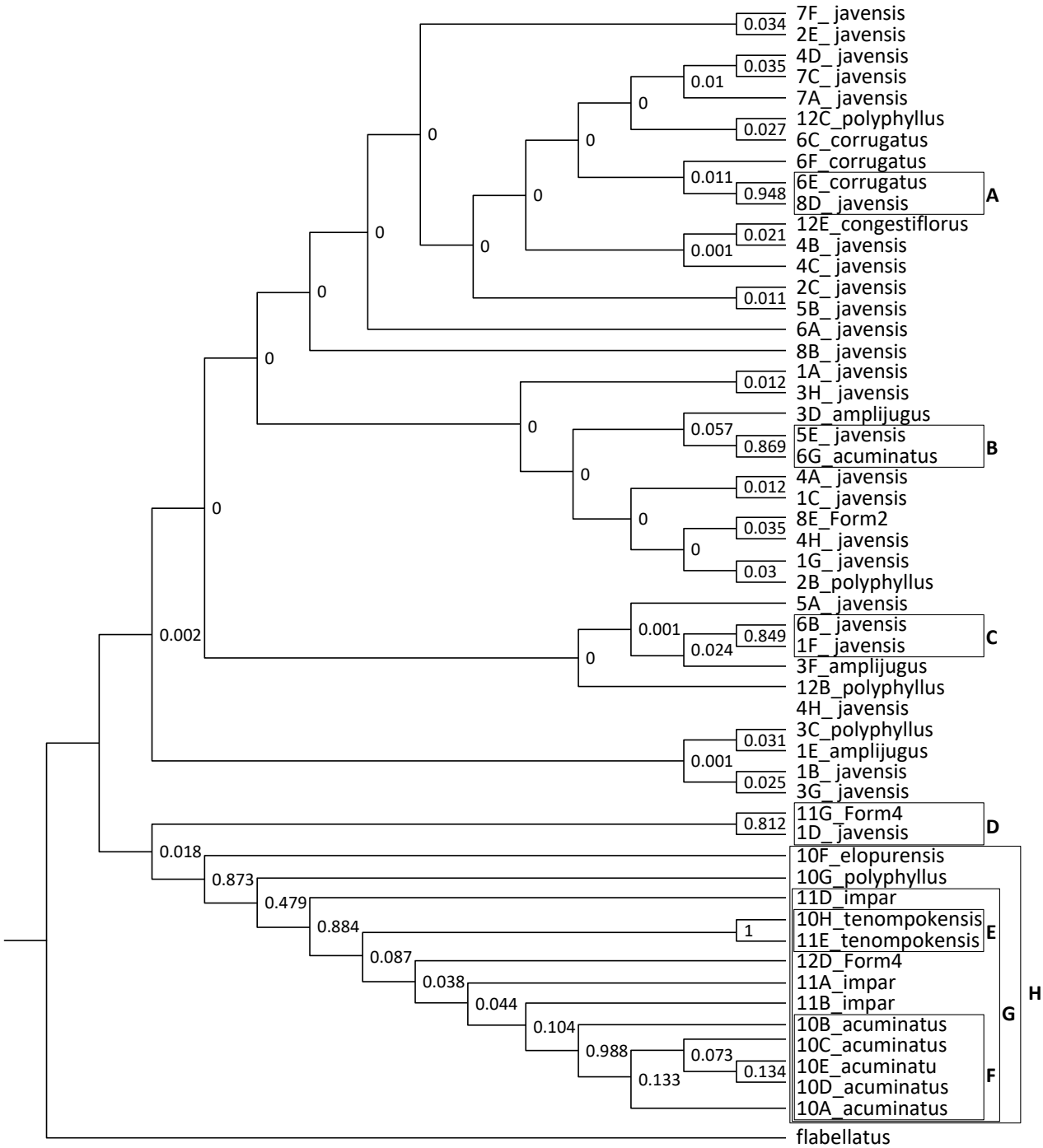

Fig. 2 The MCC tree of the combined markers (*mat*K and 5S). Several subclades with reasonable support (posterior probability PP > 0.8) are present and indicated as Groups A–H, all other branches lack support (PP usually < 0.1).

The higher support for Group A–D (Fig. 2) is likely coincidence, neither geography nor morphology do support the four groups. None of them is therefore recognized as a taxon. Group A contains *C. javensis* (*Avé 216*, 8D) from the Malay Peninsula (typical form except for the wider leaf sheaths with quite robust spines) and '*C. corrugatus*' (*Mogea 3615,* 6E) from Kalimantan (with typical ring-like wrinkled sheaths without spines). Group B comprises *C. javensis* (*Dransfield JD 3613*, 5E) from Sumatra and *C. acuminatus* from Sabah (*Dransfield JD 5584,* 6G; see below). Group C has typical *C. javensis* from Kalimantan (*Van Valkenburg 1320,* 6B) and Java (*Mega MAT 011*, 1F), whereby the Borneo specimen has more robust spines on the leaf sheath and broader leaflets. In Group D are *C. javensis* (*Mega MAT 008*, 1D) from Java and 'Form 4' from Sabah (*Chew & Corner RSNB 4835*, 11G); the latter can be distinguished by robust leaf sheaths with a triangular, flat, hairy margin, a rough ocrea that is either hirsute or with spines, young red staminate inflorescence bracts, and short (16–18 cm long) pistillate inflorescences. However, the other 'Form 4' specimen (*Mega MAT 065*, 12D, Sabah) is included in Clade G, thus no taxonomic value can be given to this form.

Clade H comprises Clade G plus 2 specimens, while Clade G encompasses Clades E and F plus three specimens (Fig. 2).

Clade E (*Mega MAT 055*, 10H, and *Mega MAT 054*, 11E in Fig. 2) is *C. tenompokensis*, the only taxon, besides *C. javensis*, recognized in the multivariate analysis of Atria et al. (2017). The phylogenetic results show that the *C. javensis* complex cannot easily be split in various taxa as supported main clades are absent. *Calamus tenompokensis* forms a distinct subclade. Recognizing it as a species would render the remaining part

paraphyletic, which is not acceptable for higher taxonomic levels like genera, families, etc. 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. *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 support 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–)10–11(–12)) linear, subopposite, regularly arranged leaflets, the (almost) smooth leaf sheath, and the inflorescences that are mostly smaller and finer than those of the resembling *C. javensis*. Beccari (1908) stated that '*C. acuminatus*' is indistinguishable from his *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 are intermediate between both forms. *Mega MAT 027* (10G in Fig. 2, part of Clade H) from Sabah, identified as *C. javensis* var. *polyphyllus*, has almost smooth leaf sheath as '*C. acuminatus*'. One sample, identified as '*C. acuminatus*' (*Dransfield JD 5584*, 6G in Clade B in Fig. 2, also from Sabah), 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). Therefore, '*C. acuminatus*' can, at most, be recognized at variety level. Biogeographically, '*C. 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). The relatively narrow distribution and the ecological preferences support the idea to recognise '*C. acuminatus*' as a distinct entity.

Group G (Fig. 2) contains two specimens identified as '*C. impar*' (*Avé 136*, 11A, from the Malay Peninsula and *SAN 21064*, 11D, from Sabah), morphologically recognisable, but biogeographically not coherent (widely distributed, ranging from S Thailand and Peninsular Malaysia to east and north Borneo). '*Calamus impar*' is regarded as merely a form of *C. javensis* by Dransfield & Patel (2005) and the name can at most be used to indicate specimens with as morphological features the presence of 2 pairs of leaflets of which the penultimate pair subopposite or alternate with one leaflet very close to the terminal pair (opposite and not close to the terminal pari in *C. javensis*) and a cylindrical and truncated, persistent ocrea (in *C. javensis* the ocrea is always quite conspicuous, quickly tattering, rarely persistent).

'*Calamus elopurensis*' is represented by a single specimen (*Dransfield JD 6265*, 10F, Sabah), and present in Clade H (Fig. 2). This form is easily distinguished by the rosette of large leaves and the peculiar long rachis bracts. Vegetatively, it should not be confused with '*C. impar*', 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 (vs narrowly elliptic in '*C. elopurensis*') smaller leaflets of '*C. impar*' (to 20 cm long vs to 35 cm long in '*C. elopurensis*').

Other forms represented in the phylogeny ('*C. congestiflorus*', '*C. corrugatus*', 'var. *polyphyllus*', 'Form 2') are also all only morphologically recognisable forms.

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). Polymorphism in *C. javensis* has been reported in several articles and field observations, but there were no studies on hybridization or whole genome tracing so far. From the results of this study and confirmation from field observations, it may be assumed that the phenotypes shown by *C. javensis* populations were the result of genetic material exchange events, such as hybridization or introgression. Hybridization is very common in plants, and is likely enhanced by habitat disturbance, which often brings formerly geographically separated lineages together (Choler et al. 2004, Naciri & Linder 2015, Schilling et al. 2018). The introduction of new alleles has effects on speciation and adaptation to local surroundings, either slowing or accelerating the isolation between populations through varying gene flow and recombination, which can blur species boundaries (Schilling et al. 2018). Naciri & Linder (2015) mention two studies concerning chloroplast haplotype sharing between species, which was seen among closely related species of *Solidago* subsect. *Humiles* (Rydb.) Semple and *Salix* L. (Percy et al. 2014). This also indicates that molecular and morphological rates of divergence might be uncoupled (Vanderpoorten & Shaw 2010), a phenomenon certainly demonstrated here for the molecular markers used, which showed no or only some variation, while the morphology varies enormously among the specimens.

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. However, ancestral polymorphism may have been present in *C. javensis*, and distant specimens or various forms may share the same haplotype, primitively or by later hybridization. More molecular studies, involving perhaps complete genomes are necessary to understand the situation in *C. javensis*.

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. All other 'taxa' are morphologically recognisable forms only. In a future article the formal taxa will be described and keyed out, just as the morphological forms.

Acknowledgements We especially thank the Ministry of Research, Technology and Higher Education of the Republic of Indonesia (MENRISTEK-DIKTI) for funding the PhD study of the first author through DIKTI-Leiden University joined scholarship, which made this research possible. We would like to acknowledge the Alberta Mennega Stichting, Leiden University Funds (LUF), Maatschappij voor Wetenschappelijk Onderzoek in de Tropen / The Society for the Advancement of Research in the Tropics (Treub-Maatschappij) for funding the field trip of the first author and the latter fund is also acknowledged by the last author for funding the Ornstein chair in Tropical Plant Biogeography. We greatly appreciate the help during fieldwork by Dr. Joan Pereira from SAN; Dr. Ferry Slik, Dr. Rahayu Sukmaria binti Hj Sukri from the University of Brunei (UBD) and Herbarium Brunei (BRUN) and Dr. Mohizah Bt. Mohamad (SAR).

REFERENCES

Atria M, Van Mil H, Baker WJ, et al. 2017. Morphometric analysis of the rattan Calamus javensis complex (Arecaceae: Calamoideae). Systematic Botany 42: 494–506.

Baker WJ. 2015. A revised delimitation of the rattan genus Calamus (Arecaceae). Phytotaxa 197: 139–152.

Baker WJ, Hedderson TA, Dransfield J. 2000a. Molecular phylogenetics of subfamily Calamoideae (Palmae) based on nrDNA ITS and cpDNA rps16 Intron sequence data. Molecular Phylogenetics and Evolution 14: 195–217.

Baker WJ, Hedderson TA, Dransfield J. 2000b. Molecular phylogenetics of Calamus (Palmae) based on 5S nrDNA spacer sequence data. Molecular Phylogenetics and Evolution 14: 218–231.

Barfod AS, Dransfield J. 2013. Arecaceae (Palmae). In: Santisuk T, Larsen K (eds), Flora of Thailand 11, 3. The Forest Herbarium, Bangkok.

Baum DA, Systsma KJ, Hoch PC. 1994. A phylogenetic analysis of Epilobium (Onagraceae) based on nuclear ribosomal DNA sequences. Systematic Botany 19: 363–388.

Beccari O. 1908. Asiatic Palms-Lepidocaryeae. Part I. The species of Calamus. Annals of the Royal Botanic Garden, Calcutta 11: 1–518.

Choler P, Erschbamer B, Tribsch A, et al. 2004. Genetic introgression as a potential to widen a species' niche: Insights from alpine Carex curvula. Proceedings of the National Academy of Sciences of the United States of America 101: 171–176. https://doi.org/10.1073/pnas.2237235100.

Crisp MD, Chandler GT. 1996. Paraphyletic species. Telopia 6: 813–844.

De Queiroz K. 1998. The general lineage concept of species, species criteria, and the process of speciation. In: Howard DJ, Berlocher SH (eds), Endless forms: species and speciation: 57–75. Oxford University Press Inc., New York.

De Salle R, Rosenfeld JA. 2013. Phylogenomics: A primer. Garland Science, New York.

Dransfield J. 1984. The rattans of Sabah. Sabah Forest Records 13. Sabah Forest Department, Sabah, Sandakan.

Dransfield J. 1992. The rattans of Sarawak. Royal Botanic Gardens, Kew.

Dransfield J, Patel M. 2005. Rattans of Borneo, an interactive key. Royal Botanic Gardens, Kew.

Dransfield J, Uhl NW, Asmussen CB, et al. 2008. Genera Palmarum: The evolution and classification of palms. Royal Botanic Gardens, Kew.

Drummond AJ, Nicholls GK, Rodrigo AG, et al. 2002. Estimating mutation parameters, population history and genealogy simultaneously from temporally spaced sequence data. Genetics 161: 1307–1320.

Drummond AJ, Rambaut A. 2007. BEAST: Bayesian Evolutionary Analysis by Sampling Trees. Evolutionary Biology 7: 214–221.

Ferris KG, Sexton JP, Willis JH. 2014. Speciation on a local geographic scale: the evolution of a rare rock outcrop specialist in Mimulus. Philosophical Transactions of the Royal Society B 368: 1–12.

Hall TA. 1999. BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symposium Series. 41: 95–98.

Kramadibrata P. 1992. A revision of the genus Calamus (Palmae) section Macropadus sensu Furtado (dissertation). Reading, School of Plant Sciences, University of Reading.

Messing J. 1983. New M13 vectors for cloning. Methods in Enzymology 101: 20–78.

Miller MA, Schwartz T, Pickett BE, et al. 2015. A RESTful API for Access to Phylogenetic Tools via the CIPRES Science Gateway. Evolutionary Bioinformatics 11: 43–48.

Naciri Y, Linder HP. 2015. Species delimitation and relationships: The dance of the seven veils. Taxon 64: 3–16.

Percy DM, Argus GW, Cronk QC, et al. 2014. Understanding the spectacular failure of DNA barcoding in willows (Salix): Does this result from a trans-specific selective sweep? Molecular Ecology 19: 4737–4756.

Pigliucci M, Murren J, Schlichting CD. 2006. Phenotypic plasticity and evolution by genetic assimilation. The Journal of Experimental Biology 209: 2362–2367.

Rambaut A. 2010. FigTree v1.3.1. Institute of Evolutionary Biology, University of Edinburgh, Edinburgh. http://tree.bio.ed.ac.uk/software/figtree/.

Ronquist F, Teslenko M, Van der Mark P, et al. 2012. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Systematic Biology 61: 539–542.

Schilling MP, Gompert Z, Li FW, et al. 2018. Admixture, evolution, and variation in reproductive isolation in the Boechera puberula clade. Evolutionary Biology. 18: 61.

Sreekumar VB, Renuka. 2008. Phenetic and phylogenetic analysis of the genus Calamus L. (Arecaceae) in India. Proceedings of the 20th Kerala Science Congress at Thiruvananthapuram 20.

Suchard MA, Lemey P, Baele G, et al. 2018. Bayesian phylogenetic and phylodynamic data integration using BEAST 1.10. Virus Evolution 4: vey016.

Swofford DL. 2002. PAUP*. Phylogenetic analysis using parsimony (*and other methods), Version 4.0b10. Sinauer Associates, Sunderland, Massachusetts.

Vanderpoorten A, Shaw AJ. 2010. The application of molecular data to the phylogenetic delimitation of species in bryophytes: A note of caution. Phytotaxa 9: 229–237. https://doi.org/10.11646/phytotaxa.9.1.12.

Appendix 1 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 Fig. 1 and 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, The 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
	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
	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