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„Phylogenetic community structure assessment of a mixed Dipterocarp forest using DNA barcoding and molecular phylogeny of the dominant tree family Dipterocarpaceae“

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CONTENT

ABSTRACT

For DNA barcoding, the two markers *ribulose-1,5-bisphosphate carboxylase/oxygenase* (*rbcL*) and *maturase K* (*matK*) are widely used in plants. In contrast to the well conserved *rbcL* barcode region, the *matk* is rapidly evolving and therefore highly variable, approx. three times more variable than that of *rbcL*. PCR amplification of *matK* is often difficult especially when dealing with many different plant families. Earlier DNA barcoding studies have used different primer pair combinations for different plant families which is time consuming and costly. In this project, 14 universal *matk* primers were developed using complete *matk* sequences available from GenBank. These primers, when combined in a multiplex PCR, amplify the target region across a wide range of Angiosperms families. In this study, the *matK*, together with the *rbcL* barcode region, successfully identified trees and shrubs (3237 individuals) mostly at generic level, which helped to assess the phylogenetic community structure in several subplots of a 25 ha mixed Dipterocarp forest in Brunei Darussalam (Borneo, Southeast Asia). The combined matrix of *rbcL* + *matK* barcodes [555 haplotypes which belong to ≥154 genera, 68 families, 25 orders *sensu* Angiosperm Phylogeny Group (APG)] was used to reconstruct phylogenetic relationships, with and without constraining the topology of taxonomic orders to match that proposed by the Angiosperm Phylogeny Group (APG III). A third phylogenetic tree was reconstructed using the program Phylomatic which trims a reference tree to taxa occurring in the community. This program is traditionally used by many ecologists. The different phylogenetic trees obtained were used to calculate community metrics [net relatedness index (NRI) and nearest taxon index (NTI)]. The community indices detected the same patterns of community structure: in most cases it was either random community assembly or phylogenetically clustering, irrespective of the different phylogenetic trees used for calculations. Phylogenetic clustering indicates that habitat filtering plays a role in assembly processes. However, the Phylomatic tree produces greater variation across the plots for NRI and NTI, presumably due to noise introduced by using an unresolved phylogenetic tree.

Dipterocarpaceae (Malvales) are the dominant trees in the study plot, which led to investigation of the molecular phylogeny of the whole family including all subfamilies. Dipterocarpaceae comprises three subfamilies: the largest Asian subfamily Dipterocarpoideae, Monotoideae from Africa, Madagascar and the Colombian Amazon, and Pakaraimaeoideae from Guaianan Highlands and Venezuela. Molecular clock analysis reveals that extant Dipterocarpoideae diverged approx. 55 Mya.

Phylogenetic analysis of plastid regions including all three subfamilies as well as representatives of closely related families Sarcolaenaceae, Cistaceae, and Bixaceae, highlights differences from the previous morphological classifications. *Pakaraimaea* of the monotypic subfamily Pakaraimaeoideae is assigned to Cistaceae. Monotoideae is weakly supported as sister to Dipterocarpoideae. In the subfamily Dipterocarpoideae, *Dipterocarpus* is sister to *Dryobalanops* and tribe Shoreeae which contradicts the morphological concepts of the tribes Dipterocarpeae (*Anisoptera*, *Cotylelobium*, *Dipterocarpus*,

Stemonoporus, *Vatica*, *Vateria*, and *Vateriopsis*) and Shoreeae (*Hopea*, *Parashorea*, *Neobalanocarpus* und *Shorea*) *sensu* Ashton. Further, the genus *Shorea (sensu* Ashton) is not monophyletic.

Genome sizes of the species examined are small (0.3264–0.6724 pg). New chromosome numbers are reported (*Dipterocarpus zeylanicus* Thwaites: 2n = 22; *Shorea megistophylla* P.S.Ashton: 2n = 14; *Hopea jucunda* Thwaites: 2n = 21; *Shorea oblongifolia* Thwaites: 2n = 14; and *Vatica endertii* Slooten: 2n = 22). These correspond to earlier records in the family Dipterocarpaceae.

RADseq (restriction site associated DNA sequencing; next generation sequencing) was successfully used to infer species relationships within the tribe Shoreeae. Analyses of thousands of RAD derived SNPs lead to congruent and much better resolved trees compared to those obtained by sanger sequencing of plastid regions. Regarding the polyphyletic genus *Shorea*, taxonomic grouping based on wood anatomy is supported by RADseq, but it contradicts some (sub-)sectional relationships proposed by Ashton. This was also the case for some (sub-)sections of the genus *Hopea*. Insights into the evolution of floral traits support the hypothesis that flowers with large, oblong anthers with short appendages and more than 15 stamens are plesiomorphic characters in the subfamily Dipterocarpoideae, as hypothesised previously based on pollination biology and biogeography.

ZUSAMMENFASSUNG

Für das Barcoding von Pflanzen werden standardmäßig zwei Marker verwendet: *Ribulose-1,5-bisphosphat-Carboxylase/Oxygenase* (*rbcL*) und *Maturase K* (*matK*). Im Gegensatz zu der weitestgehend konservierten *rbcL*-Barcoding-Region ist die *matK*-Barcoding-Region sehr variable (ca. dreimal so variable wie *rbcL*), was mit Schwierigkeiten der Auswahl der Primer, welche für die Vervielfältigung der DNS während der Polymerase-Ketten-Reaktion (PCR) benötigt werden, einhergeht. Dies ist insbesondere der Fall, wenn ein weites Spektrum an verschiedenen Pflanzenfamilien untersucht wird. Frühere Studien verwendeten jeweils unterschiedliche Primerkombinationen für verschiedene Familien. Dieses Verfahren ist jedoch zeitaufwendig und teuer. In der ersten Studie dieser Arbeit, wurden 14 universelle *matK*-Primer, unter Verwendung von kompletten *matK*-Sequenzen aus GenBank, entwickelt. Mittels Kombination dieser Primer in einer Multiplex-PCR konnte die *matK*-Barcoding-Region aller Angiospermen erfolgreich amplifiziert werden. Die *matK*-Barcoding-Region wurde, zusammen mit der *rbcL*-Barcoding-Region, in einer zweiten Studie verwendet, um Bäume und Sträucher (3237 Individuen) in mehreren Quadraten eines 25 ha Dipterocarpaceaen-Mischwaldes in Brunei Darussalam (Borneo, Südostasien) meist auf Gattungsebene zu identifizieren und die phylogenetische Gemeinschaftsstruktur dieses Waldes zu beurteilen. Die kombinierte Matrix aus *rbcL*- und *matK*-Barcoding-Regionen [555 Haplotypen, die zu ≥154 Gattungen, 68 Familien, 25 Ordnungen nach der Angiosperm Phylogeny Group (APG) gehören], wurde verwendet, um phylogenetische Verwandtschaftsbeziehungen zu erstellen [mit und ohne Verwendung eines APG-Baums auf Ordnungsebene (APGIII)]. Ein dritter phylogenetischer Baum wurde unter Verwendung des Programms Phylomatic rekonstruiert. Dieses Programm wird traditionell von vielen Ökologen verwendet und reduziert einen existierenden Referenzbaum so, dass nur Taxa, welche in der betreffenden Pflanzengesellschaft vorkommen, enthalten sind. Die verschiedenen phylogenetischen Bäume wurden verwendet um Gesellschaftsindizes [Net Relatedness Index (NRI) and Nearest Taxon Index (NTI)] zu berechnen. Die Gesellschaftsindizes deckten jeweils die gleichen Formen der Gesellschaftsstruktur auf: in den meisten Fällen zufällige Verteilung oder phylogenetische Gruppierung. Phylogenetische Gruppierung weist auf den möglichen Einfluss abiotischer Faktoren auf die Gesellschaftsstruktur des Waldes hin. Allerdings wiesen die Gesellschaftsindizes unter Verwendung des Phylomatic-Baumes eine deutlich höhere Variation auf, welche sich mit statistischem Rauschen, einhergehend mit der niedrigen Auflösung dieses phylogenetischen Baumes, erklären lässt.

Weiterhin beschäftigt sich diese Arbeit mit der molekularen Phylogenie der ökologisch und ökonomisch wichtigen Familie der Flügelfluchtgewächse (Dipterocarpaceae, Malvales), welche die dominierenden Bäume im untersuchten Wald darstellen. Die Familie der Flügelfruchtgewächse wird in drei Unterfamilien gegliedert: Dipterocarpoideae, die größte, vorwiegend in Asien vorkommende Unterfamilie, Monotoideae in Afrika, Madagaskar und dem kolumbianischen Amazonas und Pakaraimaeoideae aus den Hochländern Guayanas und Venezuela. Datierungsanalysen, basierend auf

DNA-Sequenzdaten zeigen, dass die Dipterocarpoideae ca. 55 Millionen Jahre alt sind. Phylogenetische Analyse verschiedener Plastidenregionen aller drei Unterfamilien, sowie Vertreter der nah verwandten Familien Sarcolaenaceae, Cistaceae und Bixaceae zeigt verwandtschaftliche Unterschiede zu jenen in bisherigen morphologischen Klassifikationen vorgeschlagenen. *Pakaraimaea* (Unterfamilie Pakaraimaeoideae) bildet eine Gruppe mit Cistaceaea. Monotoideae bildet eine schwach unterstützte Schwestergruppe zu Dipterocarpoideae. In Bezug auf die Unterfamilie Dipterocarpoideae, ist *Dipterocarpus* Schwestergruppe zu *Dryobalanops* und Shoreeae, was dem morphologischen Konzepte der beiden Tribus Dipterocarpeae (*Anisoptera*, *Cotylelobium*, *Dipterocarpus*, *Stemonoporus*, *Vatica*, *Vateria* und *Vateriopsis*) und Shoreeae (*Hopea*, *Parashorea*, *Neobalanocarpus* und *Shorea*) im Sinne von Ashton widerspricht. Weiterhin ist die Gattung *Shorea* (*sensu* Ashton) nicht monophyletisch.

Die untersuchten Genomgrößen sind klein (0,3264-0,6724 pg). Neue Chromosomenzahlen wurden ermittelt (*Dipterocarpus zeylanicus* Thwaites: 2n = 22; *Shorea megistophylla* P.S.Ashton: 2n = 14; *Hopea jucunda* Thwaites: 2n = 21; *Shorea oblongifolia* Thwaites: 2n = 14; and *Vatica endertii* Slooten: 2n = 22). Diese entsprechen früheren Aufzeichnungen in der Familie Dipterocarpaceae.

RAD-Sequenzierung (Restriction Site Associated DNA Sequencing, eine Next-Generation-Sequenziermethode) wurde erfolgreich verwendet, um phylogenetische Verwandtschaftsbeziehungen innerhalb der Shoreeae aufzulösen. Analysen von Tausenden von RAD-abgeleiteten SNPs (Single Nucleotid Polymorphisms) führen zu kongruenten, aber viel besser aufgelösten Bäumen im Vergleich zu jenen, die aus Sanger-Sequenzierung von Plastidenregionen resultieren. Bei der polyphyletischen Gattung *Shorea* wird auch die Monophylie einiger (Unter-)Sektionen nach Ashton nicht gestützt, während Gruppierungen nach Maury unterstützt werden. Dies trifft ebenfalls für einige (Unter-)Sektionen der monophyletischen Gattung *Hopea* zu. Einblicke in die Evolution von Blütenmerkmalen stützen die auf Bestäubungsbiologie und Biogeographie basierende Hypothese, dass Blüten mit großen, länglichen Antheren und mehr als 15 Staubblätternmit kurzen Anhängen einen plesiomorphen Merkmalszustand in der Unterfamilie Dipterocarpoideae darzustellen scheinen.

INTRODUCTION

In this thesis, molecular approaches are used to study phylogenetic community structure as well as plant systematics and evolution. The thesis is divided into four chapters. The first two chapters of the thesis focus on the development of DNA barcode markers and their utility in assessing phylogenetic community structure of a mixed dipterocarp forest in Brunei Darussalam. The third and fourth chapter emphasise on molecular systematics of the dominant tree family Dipterocarpaceae. Using phylogenetic (plastid sequences obtained by traditional sanger sequencing) and phylogenomic (single-nucleotide markers identified by restriction site associated sequencing (RADseq) approaches, this part aims at obtaining a well resolved phylogeny of the whole family with emphasis on the taxonomically difficult tribe Shoreeae and insights into the evolution of floral traits.

Phylogenetic Community Structure Assessment

Tropical forests are being lost at high rates due to global changes such as climate, pollution, agriculture, logging, mining, and infrastructural development (Foley *et al.*, 2005; Chapin *et al.*, 2008; Anderson-Teixiera, 2015). Therefore, a clear understanding of evolutionary processes that drive patterns of species diversity, differentiation, and coexistence in these ecosystems is mandatory with respect to management and restauration of damaged ecosystems (Cavender-Bares *et al.*, 2009). The assessment of communities can provide important information and insights in the structure of forests and mechanisms of community assembly [niche-related (e.g. Weiher & Keddy, 1999; neutral (e.g. Hubell, 2001); and historical processes (e.g. Ricklefs, 1987)]. A set of metrics to determine these mechanisms in a phylogenetic context was introduced by Webb (2000). In his work, phylogenetic trees of species occurring in a community are used to calculate phylogenetic diversity metrics [net relatedness (NRI) and nearest taxon index (NTI] which in turn can be used to infer phylogenetic community structure. The three basic forms of community structure are random assembly, clustering (co-occurring species are more closely related than expected by chance), and overdispersion (co-occurring species are more distantly related than expected by chance; Webb, 2000). While phylogenetic clustering is often used as a proxy for abiotic-driven assembly processes, like habitat filtering, phylogenetically overdispersed communities can hint on biotic interactions, such as interspecific competition (Webb *et al.*, 2002). Traditionally, the online interface "Phylomatic" has been used to achieve a rapid reconstruction of phylogenetic relationships by trimming a reference phylogeny to taxa occurring in the community (Webb & Donoghue, 2006). A disadvantage of this fast approach is that it only produces trees resolved at family or generic level.

DNA barcoding

DNA barcoding (see workflow in Fig. 1) has become a fast and reliable tool to assess and monitor biodiversity (Kress *et al.*, 2015) and the community structure of forests. DNA barcodes are

short gene sequences taken from a standardized portion of the genome (Hebert *et al.*, 2003). For animals, a segment of the mitochondrial gene *cytochrome oxidase I* (*COI*, Hebert *et al.*, 2003) and for fungi, the nuclear ribosomal internal transcribed spacer (ITS) region is used (Schoch *et al.*, 2006). For plants, multiple regions, the portions of two plastid genes, *matK* and *rbcL*, have been recommended by the Consortium for the barcode of Life (CBOL) Plant Working Group (CBOL Plant Working Group: Hollingsworth *et al.*, 2009). While the *rbcL* barcoding region is very universal and therefore exhibits high sequence recovery rates across land plants (Fazekas *et al.*, 2008), *matk*, one of the most rapidly evolving sections of the plastid genome (Hilu & Liang, 1997; Chase *et al.*, 2007) has a much higher discrimination power (Hollingswoth *et al.*, 2011) and can lead to a better resolution of the tips of a phylogenetic tree compared to the ones obtained by the traditionally used program Phylomatic (Kress *et al.*, 2009). DNA barcodes were first used in plant community phylogenetics by reconstruction of the relationships of 281 tree species in the Barro Colorado Island Forest Dynamics Plot in Panama (Kress *et al.*, 2009) and are widely used since then (e.g. Kress *et al.*, 2010; Pei *et al.*, 2011; Baraloto *et al.*, 2012; Whitfeld *et al.*; 2012,Bennet *et al.*, 2013; Yessoufou *et al.*, 2013; Erickson *et al.*, 2014; Muscarella *et al.*, 2014).

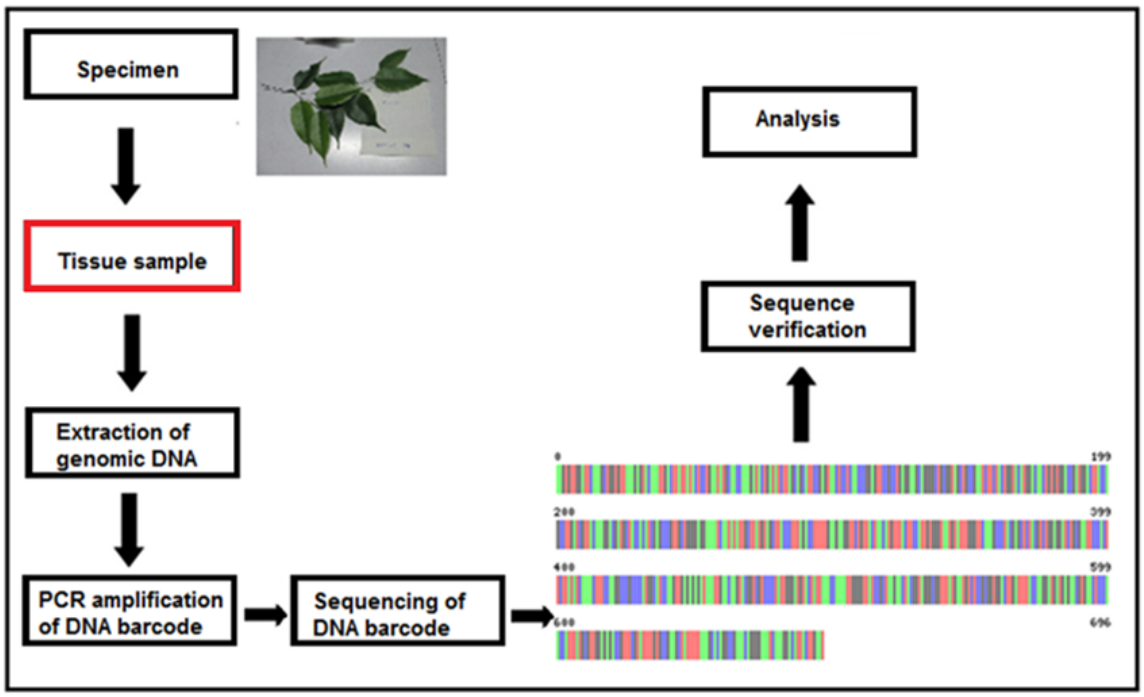

Fig. 1: DNA barcoding workflow: Genomic DNA is extracted from a tissue sample, the DNA barcode region is amplified in a PCR and subsequently sequenced bidirectionally. The sequences are assembled into contigs and edited. Editing and sequencing errors can be verified by frameshifts and the presence of stop codons. Verified sequences are uploaded to a reference database and used for further analyses.

CTFS-ForestGEO: a worldwide network monitoring forests

The research plot which is investigated in this study is part of the Center for Tropical Forest Science - Forest Global Earth Observatory (CTFS-ForestGEO) network which comprises over 60 forest dynamics

plots in 24 countries. In total, it monitors approx. six million trees which can be assigned to 10,000 species (http://www.ctfs.si.edu/group/About/, last accessed 2017-10-10). The focus is on tropical regions, but plots located in temperate regions (i.e. Europe and North America) are also included (http://www.ctfs.si.edu/group/About/, last accessed 2017-10-10). In each plot (median size 25 ha), all stems ≥1 cm diameter will be identified to species, mapped, and regularly censured according to standardized protocols (Anderson-Teixeira *et al.*, 2015). This long-term, large-scale research on forests around the world aims at increasing the scientific understanding of forest ecosystem, monitoring the impacts of global change, and guiding sustainable forest management and natural-resource policies (http://www.ctfs.si.edu/group/About/, last accessed 2017-10-10).

The 25 ha Kuala Belalong plot

The Kuala Belalong plot is located in a lowland mixed dipterocarp forest in the Batu Apoi Forest Reserve in Temburong, Brunei Darussalam on the island of Borneo. It was set up by the Kuala Belalong Field Studies Centre, Universiti Brunei Darussalam. With a total number between 850 and 1050 species of trees and shrubs (Anderson-Teixeira *et al.*, 2015), this plot becomes the second species richest plot in the Paleotropics after the Lambir plot in Malaysia which harbours 1182 species (Anderson-Teixeira *et al.*, 2015). Being the second largest tropical island in the world after New Guinea, Borneo features a high biodiversity, possibly including up to 15,000 different plant species of which approx. 3000 species are trees (MacKinnon *et al.*, 1996). Most of the tree species are found in the lowland rain forests. In Borneo they are usually dominated by species of the Dipterocarpaceae (Whitmore, 1984). The mixed dipterocarp forests occur from sea level to approx. 300 m, on hills up to 750-1200 m elevation (Davies & Kamariah, 1999) and are very species rich and thus ecologically complex (Whitmore, 1984). In contrast to the Malaysian states of Sabah and Sarawak which have become global hotspots of forest degradation due to timber as well as oil palm industries**,** only 15% of the forest is degraded or severely degraded in Brunei (Bryan *et al.*, 2013). Although Brunei's forest cover is the highest on Borneo by percentage, Brunei is a small country with a land area of 5765 km^2 (less than 1% of the whole island of Borneo) of which 3162 km^2 are intact forests (Bryan *et al.*, 2013). Because small areas are extremely vulnerable to environmental and ecological changes, conservation-conscious approaches to land use have to be adopted in order to maintain the high forest cover (Kamariah & Wong, 1999). Moreover, earlier plot surveys studying mainly the tree species in Brunei's mixed dipterocarp forests have shown that rare species build the majority of the diversity (Davies & Kamariah, 1999). Species with a large number of individuals are often outnumbered by species that occur in lower densities (Davies & Kamariah, 1999). Consequently, a clear understanding of processes that are responsible for species interactions and thus the community structure of the forests is important. However, there is a lack of community phylogenetic analyses based on DNA barcoding for the Bruneian and most of the Southeast Asian tropical rainforests.

Dipterocarpaceae

Besides studying the phylogenetic and community structure of the mixed dipterocarp forest, the phylogenetic relationships of the dominant tree family Dipterocarpaceae is investigated in this study. Members of this ecologically important family, are large canopy or emergent, resinous trees (Ashton, 2004) with the tallest known living tropical tree (*Shorea faguetiana* Heim: 89.5 m tall; https://www.newscientist.com/article/2092974-tallest-known-tropical-tree-discovered-in-malaysias-lost-world/, last accessed 2017-10-10). The name Dipterocarpaceae refers to the two-winged fruit of the type genus *Dipterocarpus* C.F.Gaertn. Within the order Malvales, Dipterocarpaceae are placed in a clade with Sarcolaenaceae (trees endemic to Madagascar) and Cistaceae (temperate shrubs, Alverson *et al.*, 1998). The family Dipterocarpaceae was traditionally divided into three subfamilies (Maguire & Ashton, 1977), Pakaraimaeoidea, Monotoideae, and Dipterocarpoideae. Subfamily Pakaraimaeoideae is monotypic with *Pakaraimaea dipterocarpacea* Maguire & P.S. Ashton occurring in the western highlands of Guyana and in Venezuela (Maguire & Ashton, 1980). Subfamily Monotoideae includes three genera: *Marquesia* Gilg with three species, native to Africa, *Monotes* A. DC. having 26 species, distributed across Africa and Madagascar, and monotypic *Pseudomonotes* A.C.Londoño, E.Alvarez & Forero, endemic to the Colombian Amazon. The largest subfamily is Dipterocarpoideae with approx. 470 (Ashton, 2004) to 680 species (http://www.mobot.org/mobot/research/apweb/orders/malvalesweb.htm#Dipterocarpaceae, last accessed: 2017-10-10) belonging to nine to 19 genera depending on the author. Dipterocarpoideae are confined to the Asian tropics between the Seychelles and South Asia to New Guinea (Ashton, 2004). Characteristic for the aseasonal Southeast Asian dipterocarp forests is the supra-annual mass flowering which takes places at irregular intervals of two to ten years (Ashton, Givnish, Appanah, 1988). While dipterocarp seedlings experience little predation, the large, winged, energy-rich seeds are chemically poorly protected and therefore parasited by weevils and eaten by wild pigs, but do not depend on animals for dispersal, as being dispersed by gyration (Ashton, Givnish, Appanah, 1988). The mast fruiting is considered as an adaptation to reduce seed predation through satiation (Janzen, 1974). Dipterocarpoideae were traditionally widely used. Several species are valuable for their nuts, wood oil, resin, and camphor (Shiva & Jantan, 1998). Since the early seventies, Dipterocarpoideae are used as a major source of commercial hardwood (Ashton, 2004).

Taxonomic uncertainties in the family Dipterocarpaceae

Understanding the evolution of Dipterocarpaceae has been compressed by some taxonomic uncertainties. The subfamiliar status of Pakaraimaeoideae is widely discussed (see APG IV, 2016) and existing morphological classifications of the Dipterocarpoideae differ with respect to numbers of genera, sections and subsections. According to Ashton (1979) the subfamily consists of two tribes, Dipterocarpeae with genera *Anisoptera* Korth., *Cotylelobium* Pierre, *Dipterocarpus* C.F.Gaertn.,

Stemonoporus Thwaites, *Upuna* Symington, *Vateria* L., *Vateriopsis* F.Heim, and *Vatica* L. and tribe Shoreeae with the genera *Dryobalanops* C.F.Gaertn., *Hopea* Roxb., *Parashorea* Kurz, and *Shorea*. Roxb. ex C.F.Gaertn. Generic and subgeneric classifications in the tribe Shoreeae *sensu* Ashton are very complex. Ashton's (1982) and Maury's (1978) classifications of the large genus *Shorea* are based on four groups [Balau, Damar Hitam (yellow Meranti), Meranti Pa'ang (white Meranti), and red Meranti] defined by Symington (1943) on the basis of timber characters. While Ashton (1982) kept them in a single genus *Shorea* consisting of eleven sections and eight subsections, Maury (1978), established the following six genera: *Anthoshorea* Pierre (= white meranti), *Rubroshorea* (= red meranti), *Richetia* F.Heim (= yellow meranti), and *Shorea* (= selangang batu), *Doona* Thwaites, and *Pentacme* A. DC. The following issues were considered in this study: (1) the position of subfamilies Pakaraimaeoideae and Monotoideae in Dipterocarpaceae; (2) phylogenetic relationships within *Shorea*; (3) position of *Dipterocarpus*, which has been placed into Dipterocarpeae based on morphology by Ashton; (4) an examination of the position of *Dryobalanops* previously assigned to tribe Shoreeae by Ashton (1979), but placed in an intermediate position between Shoreeae and Dipterocarpeae by Maury-Lechon (1979), and (5) phylogenetic relationships of *Hopea*, *Parashorea* and *Shorea* of the tribe Shoreeae.

Molecular systematics of Dipterocarpaceae using phylogenetic and phylogenomic approaches

Several molecular phylogenetic studies have been conducted on Dipterocarpaceae, including use of restriction fragments (Tsumura *et al.*, 1996; Indrioko, Gailing & Finkeldey, 2006), RAPD (Rath *et al.*, 1998), AFLPs (Cao *et al.*, 2006), other plastid sequences (Kajita *et al.*, 1998; Kamiya *et al.*, 1998; Dayanandan *et al.*, 1999; Gamage *et al.*, 2003, 2006; Yulita, Bayer & West, 2005; Choong *et al.*, 2008; Tsumura *et al.*, 2011; Yulita, 2013), the nuclear gene *Pgi*C (Kamiya *et al.*, 2005; Choong *et al.*, 2008) and internal transcribed spacer regions (Yulita *et al.*, 2005). These studies were either based on one to three plastid or single nuclear markers, have limited number of taxa, or do not included all three subfamilies. In the project, Dipterocarpaceae occuring in the 25 ha plot were barcoded. Barcoding the Dipterocarpaceae species using small fragments of *matk* (approx. 850 bp) and *rbcL* (approx. 700 bp) can lead to an unresolved backbone in the phylogenetic tree (especially in the large genus *Shorea*) because closely related species might have identical sequences. By including additional plastid markers (e.g. *trnK* intron including complete *matK* and *trnT-trnL-trnF*) as well as by including wide range of species from all three subfamilies and related families a more robust framework phylogeny of the whole Dipterocarpaceae can be achieved. Furthermore, phylogenetically informative variation of entire genomes obtained by high-throughput sequencing technologies, can be used to assess relationships more accurately. Short-read technologies like e.g. Illumina for sequencing restriction-site associated DNA (RADseq) allows sampling of genome-wide SNPs at 1000s of loci and has been successfully applied for phylogenetic reconstruction (Rubin *et al.*, 2012; Cariou *et al.*, 2013). Thus, RADseq is promising to resolve positions in taxonomic problematic groups, such as the tribe Shoreeae.

Insights into evolution of floral traits

Dipterocarpoideae show a high floral diversity (Fig. 2) and different pollinators are observed. *Dipterocarpus* (Fig. 2A) is mainly pollinated by nectarivorous Lepidoptera (Ghazoul, 1997; Harrison *et al.*, 2005; Ashton, 2014), and has less than or 15 stamens and large, oblong, yellow anthers with prominent stoutly acicular tapering erect glabrous appendages (Ashton, 2003). *Dyrobalanops*, *Neopbalanocarpus*, *Vateria*, *Vateriopsis*, *Stemonoporus*, *Parashorea,* and *Shorea* sections *Doona* (Fig. 2B) and *Pentacme* generally have large, less abundant flowers, with generally more than 15 stamens and large, oblong, often yellow anthers with mainly short, glabrous appendages (Ashton, 2003). Their anthers carry many pollen grains and may serve as food for their pollinators which are bees (e.g. Dayanandan *et al.*, 1990; Momose *et al.*, 1998). They are most abundant where forest species is low and often flower either sporadically (*Neobalanocarpus*, *Dryobalanops*) or regularly (*Stemonoporus*, some *Shorea* section *Doona*, *Vateria*) outside the simultaneous mass flowering (Ashton, 2003). *Hopea*, *Shorea* sections *Pachycarpa*, *Mutica*, *Brachyptera*, *Richetioides* (except *S. polyandra*) have small, more abundant flowers with mostly 15 stamens and small, (sub-)globose, pale anthers and variable appendages (often slender and ciliate; Ashton, 2003). For *Shorea* section *Mutica*, thrips have been observed as pollinators (Appanah & Chan, 1981). Other pollinators are beetles (Sakai *et al.*, 1999) and flies (Khatua *et al.*, 1998). In contrast to bees, these pollinators are fecund and have short life cycles which enables them to build up the number necessary at the onset of the supra-annual mass flowering. Ashton hypothesises that in the subfamily Dipterocarpoideae, flowers with small, pale, (sub-)globose anthers with long appendage and generally 15 stamens have evolved from flowers with large, oblong anthers, short appendages, and often more than 15 stamens. This is based on the assumption that when the early Dipterocarpacoideae were diversifying into modern genera, forests which were already associated with mass flowering were much less species rich (Ashton personal communication). Thus, large insect-pollinated species were enabled to build up the abundance of resources to attract swarms of such pollinators. The forests became more species rich with the opening of the perhumid Sunda lowlands and competition increasingly favoured species with flowers that attract pollinators who can respond to mass flowering initiation by rapid reproduction (e.g. thrips).

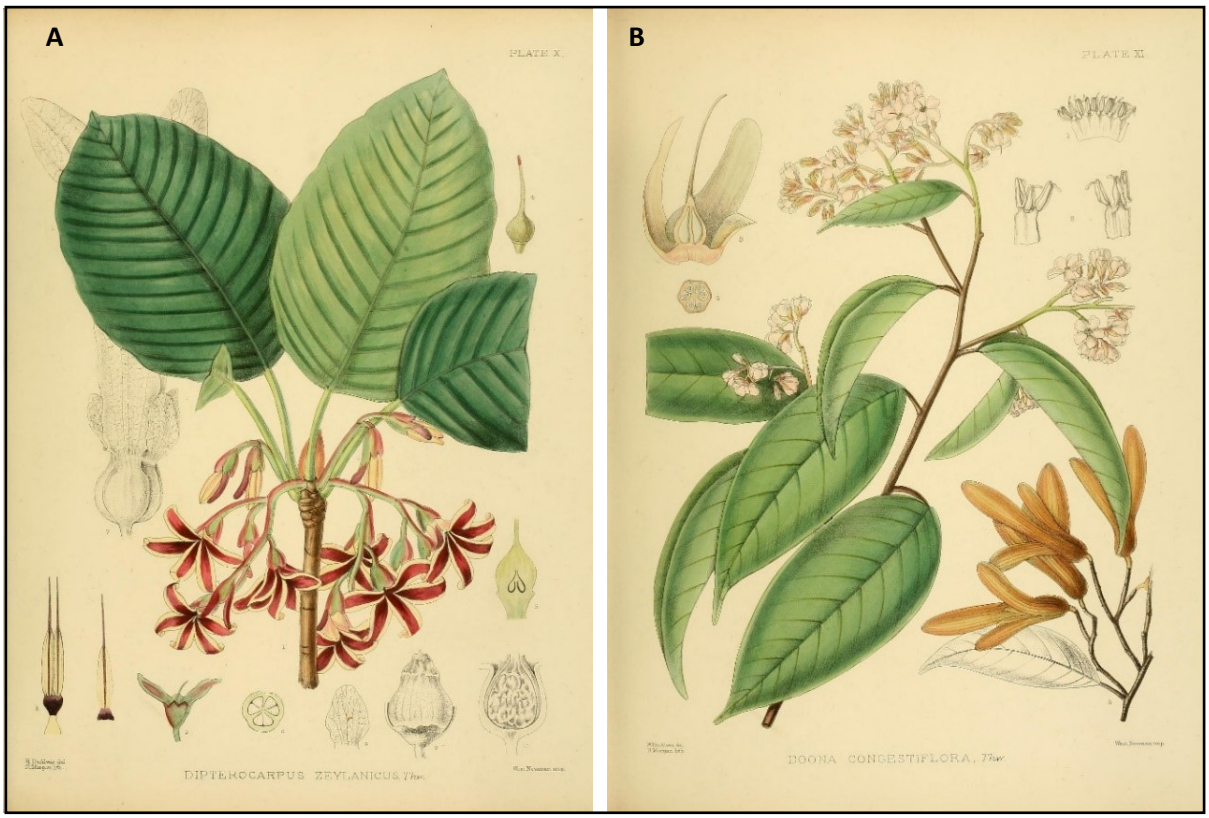

Fig. 2: A: *Dipterocarpus zeylanicus* Thwaites. 1, Twig with flowers; 2, a calyx; 3, stamens, x; 4, pistil x; 5, vertical and 6, transverse section of ovary, x; 7, ripe fruit in persistent calyx; 8, one of the smaller lobes of persistent calyx; 9, ripe fruit with the calyx removed; 10, vertical section of same. B: *Shorea congestiflora* (Thwaites) P.S.Ashton. Branch with flowers; 1, stamens, x; 2, same more enlarged, x; 3, vertical and 4, transverse section of ovary, x; 5, ripe fruit. Images downloaded from Biodiversity Heritage Library http://www.biodiversitylibrary.org/Images; last accessed: 2017-10-10. Images are from Trimen, H (1893): Plates in Illustration of a Handbook to the Flora of Ceylon. Dulau and Co., London.

AIMS

The aims of this Ph.D project were defined by the following major research questions and topics:

1. Development of multiplexable primers which can be used to amply the *matk* barcoding region across a wide range of Angiosperms
2. How useful are the standard DNA barcodes (*rbcL* and *matK*) in identification of morphotaxa occurring in a 70 subplots of the 25-ha forest-dynamics plot?
3. Does a community structure analysis based on *rbcL* and *matK* barcoding sequence data show significant benefits over the traditional approach using the program Phylomatic?
4. What are the patterns of phylogenetic community structure in the forest under investigation and what do they tell us about drivers of community assembly?
5. Investigation of the phylogenetic relationships of the family Dipterocarpaceae including clarification of ambiguous or not well supported positions of the major clades outlined in the introduction
6. Age estimations of major clades in the large subfamily Dipterocarpoideae
7. Examination of genome-size and chromosomal diversity of the Asian subfamily Dipterocarpoideae
8. Comprehensive understanding of the phylogeny and generic limits of the tribe Shoreeae
9. To test the hypothesis that flowers with large, oblong anthers with short appendages, and generally more than 15 stamens represent a plesiomorphic character state in the family Dipterocarpoideae

To address these topics, the thesis is divided in four parts which correspond to the four chapters of the thesis: (1) “Universal multiplexable *matK* primers for DNA barcoding of angiosperms”, pp. 27-35; (2) “Plant DNA barcodes and phylogenetic community structure of a tropical mixed dipterocarp forest in Brunei Darussalam”,pp. 37-99; (3) “Phylogenetic analyses of plastid DNA suggest a different interpretation of morphological evolution than those used as the basis for previous classifications of Dipterocarpaceae (Malvales)”, 101-139; and (4) “Phylogenomics resolve evolutionary relationships and provide first insights into floral evolution in the tribe Shoreeae (Dipterocarpaceae)”, pp. 141-181.

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PART 1
DNA barcodes and phylogenetic community structure

CHAPTER 1

Universal multiplexable *matK* primers for DNA barcoding of angiosperms

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Contribution: primer development, primer testing, data analysis, visualization, writing - original draft preparation, writing - review and editing

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Primer Note

Universal multiplexable *matK* primers for DNA barcoding of angiosperms[1]

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- *Premise of the study:* PCR amplification of the *matK* barcoding region is often difficult when dealing with multiple angiosperm families. We developed a primer cocktail to amplify this region efficiently across angiosperm diversity.
- *Methods and Results:* We developed 14 *matK* primers (seven forward, seven reverse) for multiplex PCR, using sequences available in GenBank for 178 taxa belonging to 123 genera in 41 families and 18 orders. Universality of these new multiplexed primers was tested with 53 specimens from 44 representative angiosperm families in 23 different orders. Our primers showed high PCR amplification and sequencing success.
- *Conclusions:* These results show that our newly developed primers are highly effective for multiplex PCR and can be employed in future barcode projects involving taxonomically diverse samples across angiosperms. Using multiplex primers for barcoding will reduce the cost and time needed for PCR amplification.

Key words: degenerate primers; DNA barcoding; *matK*; multiplex PCR.

The rapidly evolving and highly variable gene maturase K (*matK*; Hilu and Liang, 1997) 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). Amplification and sequencing of the *matK* barcoding region is difficult due to high sequence variability in the primer binding sites (Hollingsworth et al., 2011). Currently, there are three popular *matK* primer pairs available to amplify approximately the same region of the gene: 390F and 1326R (Sun et al., 2001; Cuénoud et al., 2002), XF and 5R (Ford et al., 2009), and 1R_KIM and 3F_KIM (Hollingsworth et al., 2009; Jeanson et al., 2011). Kress et al. (2009) used these three primer pairs to amplify DNA barcodes from 296 shrub and tree species. These primer combinations showed amplification success in 85% and sequencing success in 69% of the species, proving that reliable amplification is possible across a range of plants, using several primer combinations. However, using more than one primer pair can be time consuming as well as costly and is often complex for large-scale projects (e.g., Heckenhauer et al., unpublished data).

Here, we report a set of universal primers that can be multiplexed in one PCR to amplify *matK* successfully in angiosperms and expedite high-throughput, rapid, automated, and cost-effective species identification. We present methods that enable efficient PCR amplification and sequencing of the *matK* barcode region.

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METHODS AND RESULTS

Sequences of the *matK* gene from 178 taxa belonging to 123 genera and 41 families were obtained from GenBank (www.ncbi.nlm.nih.gov/genbank; Appendix S1) and aligned using the MAFFT plugin (Katoh and Standley, 2013) in Geneious (version 8.0.5; Kearse et al., 2012). Because primers were initially developed for a barcoding project dealing primarily with the tree flora of Southeast Asia, *matK* sequences of the most representative genera and families of dicots and monocots were used. The target DNA region was located between positions 383 and 1343 of the *matK* gene (with respect to *Arabidopsis thaliana* (L.) Heynh.) and includes the binding sites of the three commonly used *matK* primer pairs. Primers were designed at the most conserved regions, resulting in a fragment between positions 383 and 1256 (positions 414–1226, excluding the primer sequences). Forward primers are at a similar position to the 390F and XF primers, whereas the reverse primers are located downstream from the above-cited reverse primers to avoid a region of up to 11 adenine bases (e.g., *Sterculia tragacantha* Lindl. AY321178, positions 1257–1267), which could cause PCR and sequencing problems. To minimize primer degeneracy, aligned sequences were clustered into seven groups according to their genetic similarity in the MAFFT alignment, in which sequences are sorted according to their pairwise distances. Thus, for each cluster, primers with no more than five degenerate nucleotide positions were developed. Primers were developed manually considering primer properties (annealing temperature, 3′ and 5′ end stability) and primer secondary structures (cross dimers, dimers, hairpins) with the use of NetPrimer (PREMIER Biosoft International, Palo Alto, California, USA; www.premierbiosoft.com/netprimer/netprlaunch/netprlaunch.html). Primers were designed at the same positions in the *matK* gene for the forward and reverse primers so that they could be multiplexed in a single PCR for each sample. Seven forward and seven reverse primers were developed. Because using more primer combinations in a multiplex PCR reduces the probability of the most appropriate primers binding to the target region, only five forward and five reverse primers for the most frequent sequences in our alignment were multiplexed (Table 1: C_MATK_F/C_MATK_R). Primers were mixed in different ratios depending on their level of degeneration (Table 1). The remaining two forward and two reverse primers serve as spares for amplification of taxa that fail amplification using the previous five-primer combination. Primers were compared against the National Center for Biotechnology Information (NCBI) GenBank nucleotide reference database using the Mega BLAST algorithm (blast.ncbi.nlm.nih.gov/Blast.cgi). Table 2 shows BLAST results with no mismatches in forward or reverse primers at the family level. Thus, in studies where the species are identified to family level, primers can be combined accordingly in a multiplex PCR. To evaluate the universality of the primers, multiplex PCR was conducted on DNA of 54 species from 48 families, representing frequently occurring trees and palms (e.g., Arecaceae, Dipterocarpaceae, Euphorbiaceae) in

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TABLE 1. Primers developed for multiplex PCR used to amplify the *matK* barcoding region. The forward (C_MATK_F) and reverse (C_MATK_R) primer cocktail as well as the four additional primers are given with their proportions in the primer cocktail.

Cocktail name/Primer name (Direction)	Proportion in primer cocktail	Primer sequence (5′–3′)[a]	Primer position[b]
C_MATK_F			383–413
matK-413f-1 (Forward)	2	TAATTTAC**R**ATCAATTCATTCAATATTTCC	
matK-413f-2 (Forward)	2	TAATTTACGATC**Y**ATTCATTCAATATTTCC	
matK-413f-3 (Forward)	1	TAATTTACGATCAATTCATTCAACATTTCC	
matK-413f-4 (Forward)	2	TAATTT**M**C**R**ATCAATTCATTCCATATTTCC	
matK-413f-5 (Forward)	1	TAATTTACGATCAATTCATTCACTATTTCC	
C_MATK_R			1227–1256
matK-1227r-1 (Reverse)	3	GA**R**GA**Y**CC**R**CT**R**T**R**ATAATGAGAAAGATTT	
matK-1227r-2 (Reverse)	1	GAAGA**Y**CCGCTATGATAATGAGAAAGGTTT	
matK-1227r-3 (Reverse)	2	GA**R**GATCC**R**CT**R**T**R**ATAATGAAAAAGATTT	
matK-1227r-4 (Reverse)	2	GA**R**GATCC**R**CT**R**T**R**ATAATGAGAAAAATTT	
matK-1227r-5 (Reverse)	2	GA**R**GATCC**R**CT**R**T**R**ATAATGAGAAATATTT	
Additional primers			
matK-413f-6 (Forward)	2	TAATTTACGATC**W**ATTCATTC**M**ATTTTTCC	383–413
matK-413f-7 (Forward)	1	TAATTTACAATC**M**ATTCATTCAATATTTTC	383–413
matK-1227r-6 (Reverse)	2	GA**R**GATCCGCT**R**TAATAATGCGAAAGATTT	1227–1256
matK-1227r-7 (Reverse)	2	GA**R**GATCCGCTAT**R**ATAATGATAAATATTT	1227–1256

[a]Ambiguous bases are set in boldface.
[b]Primer position is given for *Arabidopsis thaliana* (GenBank accession no. AF144378.1).

Southeast Asia (Table 3), along with other taxa from other parts of the world to improve the coverage of angiosperms (e.g., *Leontodon* [Asteraceae], *Tillandsia* [Bromeliaceae], *Helianthemum* [Cistaceae], *Polystachya* [Orchidaceae]). Approximately 30 mg of silica gel–dried material (bark or leaves) was transferred into a 96-well plate, and genomic DNA was extracted using the DNeasy 96 Plant Kit (QIAGEN, Hilden, Germany). PCRs included 5 µL of 2× ReddyMix PCR Master Mix with 1.5 mM $MgCl_2$ (#AB-0575/DC/LD/A; Thermo Fisher Scientific, Waltham, Massachusetts, USA), 0.1 µL of forward and reverse primer cocktail each at 50 µM (final concentration 0.5 µM), 1 µL of template DNA, and H_2O up to a final volume of 10 µL. Thermocycler conditions were as follows: 95°C for 2 min; five cycles of 95°C for 25 s, 46°C for 35 s, and 70°C for 1 min; 35 cycles of 95°C for 25 s, 48°C for 35 s, and 70°C for 1 min; and a final extension at 72°C for 5 min. For samples that did not amplify using the above-mentioned protocol, the 2× Phusion Green HS II Hi-Fi PCR Master Mix with 1.5 mM $MgCl_2$ (#F-566S, Thermo Fisher Scientific) was used with the following thermocycler conditions: 98°C for 30 s; five cycles of 98°C for 10 s, 53°C for 30 s, and 72°C for 30 s; 35 cycles of 98°C for 10 s, 55°C for 30 s, and 72°C for 30 s; and a final extension at 72°C for 5 min. PCR products were visualized on a 1.5% TAE agarose gel using ethidium bromide staining. After cleaning the PCR products with 1 µL exonuclease I and FastAP thermosensitive alkaline phosphatase mixture (7 units Exo I, 0.7 units FastAP; Thermo Fisher Scientific) at 37°C for 45 min and 85°C for 15 min, barcodes were Sanger sequenced with the BigDye Terminator Kit version 3.1 (Thermo Fisher Scientific) according to the manufacturer's instructions. Sequencing was carried out using an ABI 3730xL DNA Analyzer (Applied Biosystems, Foster City, California, USA) at the Department of Botany and Biodiversity Research, University of Vienna. Bidirectional sequences were assembled in Geneious and edited.

Using 2× ReddyMix PCR Master Mix, all samples could be amplified except for one sample with low-quality DNA (Fig. 1, slot 30). This sample was successfully amplified in a PCR with 2× Phusion Green HS II Hi-Fi PCR Master Mix (Fig. 1, slot 31). Overall, the newly designed degenerate primer cocktails were very effective (100%) in amplifying the target *matK* region, with a product of 813 bp in length in *Arabidopsis thaliana*. By multiplexing the primers in a single PCR, barcodes were recovered from all samples.

CONCLUSIONS

We developed 14 universal, partly degenerate primers suitable for DNA barcoding of angiosperms that may also be suitable for multiplexed amplicon sequencing approaches on next-generation sequencing platforms (e.g., fusion primers on the Illumina system, see Elbrecht and Leese, 2015). We confirmed the effectiveness of our multiplexed primers on 53 species from 44 different plant families. Amplification success for these multiplexed primers in the cross-transferability tests with plant families outside Southeast Asia extends their potential usefulness, especially for large-scale barcoding projects with a diverse composition of plant families. Furthermore, by improving the routine amplification of the *matK* barcode, the establishment of our multiplex PCR approach will reduce laboratory costs as well as potential laboratory errors.

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TABLE 2. Recommended use of primers for different families, based on BLAST matches with no mismatches.[a]

Order	Family	Appropriate forward primer	Appropriate reverse primer
Alismanthales	Alismataceae	matK-413f-2	matK-1227r-1, matK-1227r-3
	Araceae	matK-413f-2, matK-413f-5	matK-1227r-1
Apiales	Araliaceae	matK-413f-2, matK-413f-5	matK-1227r-1, matK-1227r-4
	Apiaceae	matK-413f-7	matK-1227r-1, matK-1227r-5
Aquifoliales	Aquifoliaceae	matK-413f-1	matK-1227r-1, matK-1227r-3
	Cardiopteridaceae (*Gonocaryum minus*)	matK-413f-1	matK-1227r-1, matK-1227r-3
	Stemonuraceae	matK-413f-1	matK-1227r-1, matK-1227r-3
Arecales	Arecaceae (Arecaceae sp.)	matK-413f-2	matK-1227r-1, matK-1227r-3
Asparagales	Amaryllidaceae	matK-413f-6	matK-1227r-1, matK-1227r-3
	Asparagaceae	matK-413f-6	matK-1227r-1, matK-1227r-4, matK-1227r-5
	Hyacinthaceae	matK-413f-6	matK-1227r-1, matK-1227r-3
	Iridaceae	matK-413f-6	matK-1227r-1, matK-1227r-3, matK-1227r-5
	Orchidaceae (*Polystachya humbertii*)	matK-413f-1, matK-413f-2, matK-413f-3, matK-413f-6	matK-1227r-1, matK-1227r-2, matK-1227r-3
	Tecophilaeaceae	matK-413f-6	matK-1227r-1
	Xanthorrhoeaceae	matK-413f-6	matK-1227r-1, matK-1227r-5
Asterales	Asteraceae (*Leontodon hispidus*)	matK-413f-1	matK-1227r-1, matK-1227r-2, matK-1227r-3, matK-1227r-4, matK-1227r-5
	Campanulaceae	matK-413f-2	matK-1227r-1,matK-1227r-5
	Goodeniaceae	matK-413f-4	matK-1227r-1
Austrobaileyales	Austrobaileyaceae	matK-413f-2	matK-1227r-2
	Schisandraceae	matK-413f-2	matK-1227r-2
	Trimeniaceae	matK-413f-2	matK-1227r-2
Berberidopsidales	Berberidopsidaceae	matK-413f-1	matK-1227r-1
Boraginales	Boraginaceae	matK-413f-1, matK-413f-4	matK-1227r-1, matK-1227r-3, matK-1227r-5
	Ehretiaceae	matK-413f-1	matK-1227r-1
Brassicales	Brassicaceae	matK-413f-1, matK-413f-4, matK-413f-6	matK-1227r-1, matK-1227r-5
	Capparaceae	matK-413f-1	matK-1227r-1
	Caricaeae	matK-413f-1	matK-1227r-1
	Cleomaceae	matK-413f-1, matK-413f-3, matK-413f-4, matK-413f-7	matK-1227r-1, matK-1227r-2, matK-1227r-4, matK-1227r-5
	Moringaceae	matK-413f-1	matK-1227r-1, matK-1227r-5
	Resedaceae	matK-413f-1	matK-1227r-1
Bruniales	Brunelliaceae	matK-413f-1	matK-1227r-1
Buxales	Bucaceae	matK-413f-1	matK-1227r-1
Caryophyllales	Amaranthaceae	matK-413f-1	matK-1227r-1
	Cactaceae	matK-413f-1	matK-1227r-1
	Polygonaceae	matK-413f-1	matK-1227r-1, matK-1227r-2, matK-1227r-5
	Simmondsiaceae	matK-413f-1	matK-1227r-3
	Tamaricaceae	matK-413f-1	matK-1227r-1
Celastrales	Celastraceae	matK-413f-1, matK-413f-4, matK-413f-6	matK-1227r-1, matK-1227r-2, matK-1227r-3, matK-1227r-4, matK-1227r-5
	Lepidobotryaceae	matK-413f-1	matK-1227r-5
Chloranthales	Chloranthaceae	matK-413f-2	matK-1227r-1, matK-1227r-5
Commelinales	Commelinaceae	matK-413f-2	matK-1227r-1
	Haemodoraceae	matK-413f-2	matK-1227r-1, matK-1227r-2, matK-1227r-5
Cornales	Cornaceae (*Alangium* cf. *javanicum, Mastixia* sp.)	matK-413f-1, matK-413f-3	matK-1227r-1, matK-1227r-3, matK-1227r-4, matK-1227r-5
	Grubbiaceae	matK-413f-1	matK-1227r-1
	Hydrangeaceae	matK-413f-1	matK-1227r-1, matK-1227r-4
	Loasaceae	matK-413f-1, matK-413f-7	matK-1227r-1, matK-1227r-4
Crossosomatales	Stachyuraceae	matK-413f-1	matK-1227r-1
	Staphyleaceae	matK-413f-1	matK-1227r-1, matK-1227r-5
	Strasburgeriaceae	matK-413f-1	matK-1227r-1
Cucurbitales	Anisophylleaceae (*Anisophyllea* sp.)	matK-413f-1, matK-413f-6	matK-1227r-1
	Begoniaceae	matK-413f-1, matK-413f-6	matK-1227r-1
	Coriariaceae	matK-413f-2	matK-1227r-1
	Cucurbitaceae	matK-413f-2	matK-1227r-1, matK-1227r-3, matK-1227r-4, matK-1227r-5
	Datiscaceae	matK-413f-1	matK-1227r-1
	Tetramelaceae	matK-413f-1	matK-1227r-3, matK-1227r-5
Dipsacales	Adoxaceae	matK-413f-4	matK-1227r-1
	Caprifoliaceae	matK-413f-1, matK-413f-5	matK-1227r-1

Table 2. Continued.

Order	Family	Appropriate forward primer	Appropriate reverse primer
Ericales	Ebenaceae (*Diospyros* sp.)	matK-413f-1	matK-1227r-1, matK-1227r-3, matK-1227r-6
	Ericaceae	matK-413f-1, matK-413f-4	matK-1227r-1, matK-1227r-5
	Lecythidaceae (*Barringtonia curranii*)	matK-413f-5	matK-1227r-1
	Pentaphylacaceae	matK-413f-1	matK-1227r-1
	Primulaceae (*Ardisia* sp.)	matK-413f-1, matK-413f-2	matK-1227r-3, matK-1227r-1, matK-1227r-5, matK-1227r-7
	Styracaceae	matK-413f-1	matK-1227r-1
	Symplocaceae (*Symplocos crassipes*)	matK-413f-1	matK-1227r-1
	Theaceae	matK-413f-1	matK-1227r-4
Escalloniales	Escalloniaceae	matK-413f-1	matK-1227r-1
Fabales	Fabaceae (*Fordia splendidissima*)	matK-413f-1, matK-413f-2, matK-413f-4, matK-413f-6, matK-413f-7	matK-1227r-1, matK-1227r-3, matK-1227r-5
	Polygalaceae (*Xanthophyllum beccarianum*)	matK-413f-1, matK-413f-2	matK-1227r-1
Fagales	Betulaceae	matK-413f-2	matK-1227r-1
	Casuarinaceae	matK-413f-2	matK-1227r-1
	Fagaceae (*Lithocarpus* sp.)	matK-413f-2	matK-1227r-1, matK-1227r-3, matK-1227r-5
	Juglandaceae	matK-413f-1	matK-1227r-1, matK-1227r-6
Garryales	Garryaceae	matK-413f-1	matK-1227r-1, matK-1227r-4, matK-1227r-6
Gentianales	Apocynaceae (*Tabernaemontana* sp.)	matK-413f-1, matK-413f-3, matK-413f-4, matK-413f-5, matK-413f-6	matK-1227r-1, matK-1227r-2, matK-1227r-6
	Loganiaceae	matK-413f-1	matK-1227r-1, matK-1227r-5
	Rubiaceae (*Urophyllum* sp., *Psychotria* sp.)	matK-413f-1, matK-413f-5	matK-1227r-1, matK-1227r-2
Geraniales	Geraniaceae	matK-413f-1, matK-413f-6	matK-1227r-1
	Melianthaceae	matK-413f-1, matK-413f-6	matK-1227r-1
Gunnerales	Gunneraceae	matK-413f-1, matK-413f-2	matK-1227r-1
Huerteales	Dipentodontaceae	matK-413f-1	matK-1227r-1
	Gerrardinaceae	matK-413f-1	matK-1227r-1
	Tapisciaceae	matK-413f-1	matK-1227r-1, matK-1227r-5
Icacinales	Icacinaceae	matK-413f-1	matK-1227r-1, matK-1227r-3
Lamiales	Acanthaceae	matK-413f-1	matK-1227r-1, matK-1227r-2, matK-1227r-4, matK-1227r-5
	Gesneriaceae	matK-413f-1	matK-1227r-1, matK-1227r-2, matK-1227r-5
	Lamiaceae (*Teijsmanniodendron* sp.)	matK-413f-1	matK-1227r-1, matK-1227r-2, matK-1227r-5
	Lentibulariaceae	matK-413f-1	matK-1227r-1
	Myrsinaceae	matK-413f-1	matK-1227r-1
	Oleaceae	matK-413f-1	matK-1227r-1, matK-1227r-2, matK-1227r-3, matK-1227r-4
	Orobanchaceae	matK-413f-1	matK-1227r-1, matK-1227r-3, matK-1227r-4
Laurales	Hernandiaceae	matK-413f-2	matK-1227r-1
	Lauraceae (*Litsea sarawacensis*)	matK-413f-2	matK-1227r-1, matK-1227r-3
	Siparunaceae	matK-413f-2	matK-1227r-3
Liliales	Smilacaceae	matK-413f-2	matK-1227r-1, matK-1227r-5
Magnoliales	Annonaceae	matK-413f-2	matK-1227r-1, matK-1227r-4, matK-1227r-5
	Degeneriaceae	matK-413f-2	matK-1227r-1
	Eupomatiaceae	matK-413f-2	matK-1227r-1
	Himantandraceae	matK-413f-2	matK-1227r-1
	Magnoliaceae (*Magnolia* sp.)	matK-413f-2, matK-413f-6	matK-1227r-1
	Myristicaceae	matK-413f-2, matK-413f-4	matK-1227r-1, matK-1227r-5
Malpighiales	Clusiaceae (*Garcinia* sp.)	matK-413f-1	matK-1227r-1, matK-1227r-3, matK-1227r-4, matK-1227r-5
	Euphorbiaceae (*Antidesma* sp., *Drypetes* sp., *Koilodepas* sp., *Macaranga hosei*, *Mallotus* sp.)	matK-413f-1	matK-1227r-1, matK-1227r-3, matK-1227r-4, matK-1227r-5
	Linaceae	matK-413f-1	matK-1227r-1
	Passifloraceae	matK-413f-1	matK-1227r-1
	Phyllanthaceae	matK-413f-1, matK-413f-2, matK-413f-7	matK-1227r-1
	Putranjivaceae	matK-413f-1	matK-1227r-5
	Rhizophoraceae	matK-413f-5	matK-1227r-1, matK-1227r-3
	Salicaceae	matK-413f-1	matK-1227r-1, matK-1227r-5
	Violaceae (*Rinorea* sp.)	matK-413f-1	matK-1227r-1, matK-1227r-6

TABLE 2. Continued.

Order	Family	Appropriate forward primer	Appropriate reverse primer
Malvales	Elaeocarpaceae	matK-413f-1	matK-1227r-1
	Malvaceae (*Durio griffithii*, *Leptonychia* sp., *Sterculia* sp.)	matK-413f-1	matK-1227r-1
Myrtales	Lythraceae	matK-413f-1, matK-413f-5	matK-1227r-1, matK-1227r-3
	Melastomataceae	matK-413f-7	matK-1227r-1, matK-1227r-4
	Myrtaceae (*Syzygium* sp.)	matK-413f-1, matL-413f-4, matK-413f-6	matK-1227r-1, matK-1227r-3, matK-1227r-4, matK-1227r-5
	Onagraceae	matK-413f-3	matK-1227r-1
Oxalidales	Brunelliaceae	matK-413f-1	
	Cunoniaceae	matK-413f-1	matK-1227r-1
	Huaceae	matK-413f-6	matK-1227r-1
Pandanales	Cyclanthaceae	matK-413f-2	matK-1227r-1
	Pandanaceae	matK-413f-2	matK-1227r-1
Paracryphiales	Paracryphiaceae	matK-413f-1	matK-1227r-1
Piperales	Aristolochiaceae	matK-413f-2	matK-1227r-1, matK-1227r-5
	Piperaceae	matK-413f-2	matK-1227r-3
	Saururaceae	matK-413f-2	matK-1227r-1
Poales	Bromeliaceae (*Tillandsia* cf. *caloura*)	matK-413f-2, matK-413f-6	matK-1227r-1, matK-1227r-3
	Typhaceae	matK-413f-2	matK-1227r-1, matK-1227r-3
Proteales	Nelumbonaceae	matK-413f-1	matK-1227r-1
	Platanaceae	matK-413f-1	matK-1227r-1
	Proteaceae	matK-413f-1, matK-413f-2, matK-413f-3	matK-1227r-1, matK-1227r-3, matK-1227r-4, matK-1227r-5
Ranunculales	Berberidaceae	matK-413f-3	matK-1227r-1
	Eupteleaceae	matK-413f-1, matK-413f-2	matK-1227r-1
	Lardizabalaceae	matK-413f-1	matK-1227r-1, matK-1227r-5
	Papaveraceae	matK-413f-1, matK-413f-2, matK-413f-3, matK-413f-5	matK-1227r-1, matK-1227r-3, matK-1227r-5
	Ranunculaceae	matK-413f-4	matK-1227r-1, matK-1227r-6, matK-1227r-4, matK-1227r-5
Rosales	Cannabaceae (*Gironniera nervosa*)	matK-413f-1, matK-413f-3	matK-1227r-1, matK-1227r-3
	Moraceae (*Artocarpus elasticus*)	matK-413f-1	matK-1227r-3
	Rhamnaceae (*Ziziphus angustifolius*)	matK-413f-1, matK-413f-7	matK-1227r-1, matK-1227r-3
	Rosaceae	matK-413f-1, matK-413f-2, matK-413f-6	matK-1227r-1, matK-1227r-3, matK-1227r-4, matK-1227r-5
	Ulmaceae	matK-413f-1	matK-1227r-3
	Urticaceae	matK-413f-1	matK-1227r-3
Sabilales	Sabiaceae (*Meliosma sumatrana*)	matK-413f-1, matK-413f-2	matK-1227r-1, matK-1227r-4
Santalales	Loranthaceae	matK-413f-4	matK-1227r-1, matK-1227r-4
	Opiliaceae	matK-413f-1, matK-413f-2	matK-1227r-1
	Santalaceae	matK-413f-1, matK-413f-2	matK-1227r-1, matK-1227r-5
	Schoepfiaceae	matK-413f-1	matK-1227r-1, matK-1227r-4
Sapindales	Meliaceae (*Aglaia* sp.)	matK-413f-1, matK-413f-7	matK-1227r-1, matK-1227r-5
	Rutaceae (*Glycosmis macrantha*)	matK-413f-1	matK-1227r-1, matK-1227r-6, matK-1227r-5
	Sapindaceae (*Lepisanthes* sp.)	matK-413f-4	matK-1227r-1, matK-1227r-3, matK-1227r-5
Saxifragales	Cercidiphyllaceae	matK-413f-1, matK-413f-7	matK-1227r-1
	Haloragaceae	matK-413f-1	matK-1227r-1
	Hamamelidaceae	matK-413f-1, matK-413f-5	matK-1227r-1
	Paeoniaceae	matK-413f-1	matK-1227r-1
	Saxifragaceae	matK-413f-1, matK-413f-4, matK-413f-5	matK-1227r-1
Solanales	Montiniaceae	matK-413f-1	matK-1227r-1
	Solanaceae	matK-413f-1, matK-413f-3	matK-1227r-3
Trochodendrales	Trochodendraceae	matK-413f-1, matK-413f-6	matK-1227r-1
Vitales	Vitaceae	matK-413f-1	matK-1227r-1, matk-1227r-2, matK-1227r-5

[a] Species/genera in parentheses were successfully amplified in the family using the primer cocktail C_MATK_F/C_MATK_R.

Table 3. Taxa used for primer testing.

No.[a]	Order: Family	Species	GenBank accession no.
1	Laurales: Lauraceae	*Litsea sarawacensis* Gamble	KU519656
2	Malphigiales: Euphorbiaceae	*Antidesma* L.	KU519677
3	Magnoliales: Myristicaceae	*Knema* Lour.	KU519655
4	Asparagales: Orchidaceae	*Polystachya humbertii* H. Perrier*	KU519659
5	Arecales: Arecaceae	Arecaceae Bercht. & J. Presl	KU519652
6	Poales: Bromeliaceae	*Tillandsia* cf. *caloura* Harms*	KU519653
7	Dilleniales: Dilleniaceae	*Dillenia suffruticosa* Martelli	KU519692
8	Malpighiales: Achariaceae	*Hydnocarpus borneensis* Sleumer	KU519671
9	Malpighiales: Calophyllaceae	*Kayea oblongifolia* Ridl.	KU519679
10	Malpighiales: Euphorbiaceae	*Macaranga hosei* King ex Hook. f.	KU519674
11	Malpighiales: Euphorbiaceae	*Koilodepas* Hassk.	KU519675
12	Malpighiales: Pandaceae	*Galearia fulva* Miq.	KU519670
13	Gentianales: Apocynaceae	*Tabernaemontana* L.	KU519697
14	Malpighiales: Violaceae	*Rinorea* Aubl.	KU519676
15	Malpighiales: Clusiaceae	*Garcinia* L.	KU519698
16	Malpighiales: Euphorbiaceae	*Drypetes* Vahl	KU519669
17	Malpighiales: Ctenolophonaceae	*Ctenolophon parvifolius* Oliv.	KU519672
18	Fabales: Fabaceae	*Fordia splendidissima* (Blume ex Miq.) Buijsen	KU519701
19	Fabales: Polygalaceae	*Xanthophyllum beccarianum* Chodat	KU519700
20	Rosales: Cannabaceae	*Gironniera nervosa* Planch.	KU519681
21	Rosales: Moraceae	*Artocarpus elasticus* Reinw.	KU519682
22	Rosales: Chrysobalanaceae	*Atuna racemosa* Raf.	KU519699
23	Rosales: Rhamnaceae	*Ziziphus angustifolia* (Miq.) Hatus. ex Steenis	KU519680
24	Curcurbitales: Anisophyllaceae	*Anisophyllea* R. Br. ex Sabine	KU519651
25	Fagales: Fagaceae	*Lithocarpus* Blume	KU519693
26	Sapindales: Anacardiaceae	*Gluta laxiflora* Ridl.	KU519684
27	Sapindales: Meliaceae	*Aglaia* F. Allam.	KU519686
28	Sapindales: Sapindaceae	*Lepisanthes* Blume	KU519685
29	Sapindales: Rutaceae	*Glycosmis* Corrêa	KU519687
30, 31	Malvales: Dipterocarpaceae	*Dipterocarpus palembanicus* Slooten	KU519691
32	Malvales: Cistaceae	*Helianthemum obscurum* Pers.*	KU519702
33	Malvales: Malvaceae	*Leptonychia* Turcz.	KU519688
34	Malvales: Malvaceae	*Durio griffithii* Bakh.	KU519689
35	Malvales: Malvaceae	*Sterculia* L.	KU519690
36	Cornales: Cornaceae	*Alangium* cf. *javanicum* (Blume) Wangerin	KU519664
37	Cornales: Cornaceae	*Mastixia* Blume	KU519663
38	Sapindales: Anacardiaceae	*Saurauia* Willd.	KU519661
39	Ericales: Ebenaceae	*Diospyros* L.	KU519660
40	Ericales: Lecythidaceae	*Barringtonia curranii* Merr.	KU519662
41	Ericales: Primulaceae	*Ardisia* Sw.	KU519667
42	Ericales: Symplocaceae	*Symplocos crassipes* C. B. Clarke	KU519658
43	Gentianales: Rubiaceae	*Urophyllum* Jack ex Wall.	KU519696
44	Solanales: Convolvulaceae	*Erycibe* cf. *glomerata* Blume	KU519694
45	Gentianales: Rubiaceae	*Psychotria* L.	KU519695
46	Magnoliales: Magnoliaceae	*Magnolia* L.	KU519654
47	Myrtales: Myrtaceae	*Syzygium* P. Browne ex Gaertn.	KU519678
48	Sabiales: Sabiaceae	*Meliosma sumatrana* (Jack) Walp.	KU519657
49	Malpighiales: Euphorbiaceae	*Mallotus* Lour.	KU519673
50	Lamiales: Lamiaceae	*Teijsmanniodendron* Koord.	KU519668
51	Santalales: Olacaceae	*Strombosia ceylanica* Gardner	KU519665
52	Aquifoliales: Cardiopteridaceae	*Gonocaryum minus* Sleumer	KU519666
53	Sapindales: Burseraceae	*Dacryodes excelsa* Vahl	KU519683
54	Asterales: Asteraceae	*Leontodon hispidus* L.*	KU519703

* Species not found in Southeast Asia.
[a] Number according to Fig. 1.

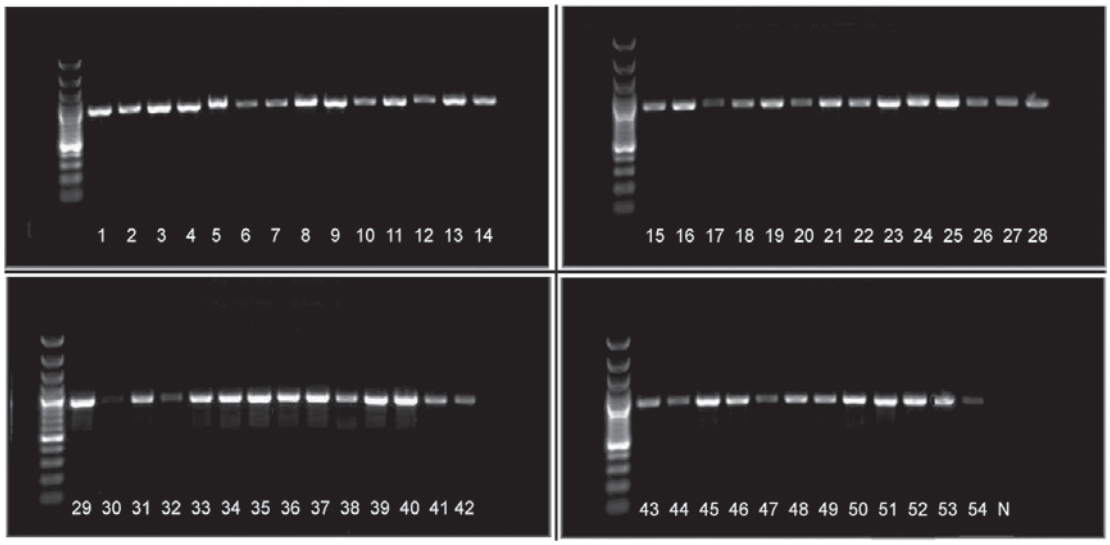

Fig. 1. Images of PCR amplicons for representatives of 53 angiosperm families using multiplex PCR with the newly developed degenerate primers (matK-413f-1 to matK-413f-5, matK-1227r-1 to matK-1227r-5). Bands are approximately 900 bp. Most of the samples were amplified using 2× ReddyMix. Low-quality DNA samples (slot 30) that failed PCR could be amplified using 2× Phusion Green HS II Hi-Fi PCR Master Mix (slot 31). For detailed sample description, see Table 3. Ladder: GeneRuler 100 bp Plus DNA Ladder (#SM0321; Thermo Fisher Scientific, Waltham, Massachusetts, USA). N = negative control.

PART 1
DNA barcodes and phylogenetic community structure

CHAPTER 2

Plant DNA barcodes and assessment of phylogenetic community structure of a tropical mixed dipterocarp forest in Brunei Darussalam (Borneo)

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Contribution: collection of material, collection of data, data curation, formal analyses, visualization, writing- original draft preparation, writing – review and editing

Abstract

DNA barcoding is a fast and reliable tool to assess and monitor biodiversity and, via community phylogenetics, to investigate ecological and evolutionary processes that may be responsible for the community structure of forests. In this study, DNA barcodes for the two widely used plastid coding regions *rbcL* and *matK* are used to contribute to identification of morphologically undetermined individuals, as well as to investigate phylogenetic structure of tree communities in 70 subplots (10 × 10m) of a 25-ha forest-dynamics plot in Brunei (Borneo, Southeast Asia). The combined matrix (*rbcL* + *matK*) comprised 555 haplotypes (from ≥154 genera, 68 families and 25 orders sensu APG, Angiosperm Phylogeny Group, 2016), making a substantial contribution to tree barcode sequences from Southeast Asia. Barcode sequences were used to reconstruct phylogenetic relationships using maximum likelihood, both with and without constraining the topology of taxonomic orders to match that proposed by the Angiosperm Phylogeny Group. A third phylogenetic tree was reconstructed using the program Phylomatic to investigate the influence of phylogenetic resolution on results. Detection of non-random patterns of community assembly was determined by net relatedness index (NRI) and nearest taxon index (NTI). In most cases, community assembly was either random or phylogenetically clustered, which likely indicates the importance to community structure of habitat filtering based on phylogenetically correlated traits in determining community structure. Different phylogenetic trees gave similar overall results, but the Phylomatic tree produced greater variation across plots for NRI and NTI values, presumably due to noise introduced by using an unresolved phylogenetic tree. Our results suggest that using a DNA barcode tree has benefits over the traditionally used Phylomatic approach by increasing precision and accuracy and allowing the incorporation of taxonomically unidentified individuals into analyses.

Introduction

Understanding community assembly and processes that are responsible for community diversity, species differentiation, and coexistence are important in the face of rapid global ecosystem change [1]. Three mechanisms are often put forward as drivers of community assembly [1]: (1) niche-related processes, in which community assembly is influenced by competition [2] and/or abiotic filters [3], (2) neutral processes, in which species are ecologically equivalent [4, 5, 6], and (3) historical processes, which bring an evolutionary perspective into community ecology [7, 8]. The relative importance of these processes for the assembly of communities and coexistence of species has been often debated [1, 5, 6, 9, 10, 11, 12]. Quantification of the phylogenetic component of biodiversity has become important in studying community assembly [13, 14] and holds promise to resolve the controversy over the relative importance of neutral vs. niche-related processes [1]. Phylogenetic information permits an understanding of how communities have evolved through time [15] and is being used increasingly to answer questions of community assembly e.g. [1, 13, 14, 16, 17, 18]. Community phylogenetic structure can exhibit three basic forms, random, clustered and overdispersed [13], although these should be viewed as part of a continuum. In a phylogenetically clustered community, co-occurring

species are more closely related than expected by chance. Conversely, a phylogenetically overdispersed community contains species that are more distantly related than expected by chance. In turn, these forms are used as a proxy to suggest underlying mechanisms of community assembly [14]. Phylogenetic clustering can hint at abiotic-driven assembly processes (habitat filtering), which is based on the fact that under a given set of environmental conditions, closely related species are more likely to be similar in abiotically adaptive traits (trait conservatism). In contrast, in phylogenetically overdispersed communities, biotic interactions (e.g. interspecific competition) may be important in structuring the local community e.g. [19, 20]. These biotic factors can include herbivores and pathogens [21, 22, 23] because they are often specialized for the chemistry of related plants and therefore host shifts in general tend to occur among plants of similar chemistry [24]. Consequently, sharing of herbivores and pathogens could limit the coexistence of closely related plants that are similar in morphology and chemistry but facilitate coexistence of more distantly related plants with different traits.

Community phylogenetics uses phylogenetic trees of co-occurring species within a community to calculate phylogenetic diversity statistics (e.g. phylogenetic diversity [25]), net relatedness index (NRI; [13]), and nearest taxon index (NTI; [13]). Rapid construction of a community phylogenetic tree is often achieved using the online interface Phylomatic [26], which trims a reference tree for plants (Angiosperm Phylogeny Group, APG) to taxa occurring in the community. However, the Phylomatic procedure often provides little or no resolution of relationships among closely related species or even genera [27]. Moreover, for analyses using Phylomatic, the correct identification of individuals is mandatory, and this is often lacking in species-rich tropical forests. DNA barcoding has a high potential to reduce the number of unidentified individuals. DNA barcoding, besides its application in species identification and discovery of cryptic species e.g. [28, 29, 30], has a potential role to play in community phylogenetics [31]. For example, by using DNA barcode sequences to generate a phylogenetic hypothesis for a local species assemblage of woody plants of a forest-dynamics plot, Kress *et al.* [32] investigated community assembly on Barro Colorado Island, Panama. Since then, DNA barcode sequences have been successfully applied in studying the phylogenetic community structure of forests and other ecosystems e.g. [32, 33, 34, 35, 36, 37, 38, 39, 40, 41]. For plants, portions of two plastid genes, *matK* and *rbcL*, have been recommended by the Consortium for the Barcode of Life (CBOL) Plant Working Group [42]. In addition, a third marker, the plastid intergenic spacer *trnH-psbA* was proposed [43, 44] and has been used in phylogenetic community structure analyses [32, 33, 34, 40]. A disadvantage of DNA barcode phylogenetic trees of a single community is that due to sparse taxon sampling across the whole angiosperm tree (missing many families, genera, and species), they can be incongruent in topology with the accepted Angiosperm Phylogeny Group (APG) classification [45, 46]. Therefore, recently published studies e.g. [33, 39, 40] used the ordinal-level topologies of the Phylomatic tree as constraints in phylogenetic analyses of the barcoding data. This allows resolution of the tips of the Phylomatic tree while the deeper APG relationships are retained.

In contrast to Neotropical forests, where phylogenetic clustering is consistently reported as the predominant pattern [35, 47], most of the Southeast Asian forests are dominated by one particular

angiosperm family, Dipterocarpaceae [48]. Therefore, interactions between close relatives that might promote overdispersion may be more important in structuring Southeast Asian forests. Patterns of phylogenetic community structure and phylodiversity have been investigated in a Southeast Asian forest before [13, 49] but using phylogenetic trees generated via the Phylomatic procedure that does not resolve relationships among genera or among species within genera, which is particularly important for detecting overdispersion. To date, no studies have been conducted on the phylogenetic structure of tree communities in Southeast Asia using DNA barcode sequences. Thus, such an analysis is imperative because the pattern of community structure may contrast with the existing view that phylogenetic clustering is paramount in tropical rain forests.

In this study, we assessed the phylogenetic structure for 70 subplots (10 × 10 m) within a 25-ha (500 × 500 m) of mixed dipterocarp forest in Kuala Belalong, Temburong, Brunei Darussalam, on the island of Borneo. An earlier study of a 1 ha plot in the same area as the research plot revealed the presence of 231 tree species [50]. As identification is ongoing, the exact number of species is still unknown, but estimates range between 850–1050 species across the 25-ha, making it among the most species-rich plots in Indomalayasia [51]. This high species-richness, much of which is contributed by species from species-rich genera (i.e. *Shorea*, *Syzygium*, and *Diospyros*) makes the Kuala Belalong plot an ideal location to assess the utility of DNA barcode sequences in a community phylogenetic study.
In this paper, we address the following questions:

1. Do the standard DNA barcodes (*rbcL* and *matK*) contribute to identification of morphotaxa occurring in the 70 subplots of the 25-ha forest-dynamics plot? We predict that the combination of conserved (*rbcL*) and a rapidly evolving (*matK*) barcoding regions allows identification of morphotaxa at least to genus-level if their sequences are already available in reference databases [52, 53], including the contributions to these from this study.

2. Does a community analysis based solely upon *rbcL* and *matK* barcoding sequence data offer significant benefits over one based on a phylogenetic tree constructed using Phylomatic? We expect that the high resolution predicted in the barcode tree decreases the bias and noise in NTI and NRI values, which have been commonly observed with Phylomatic trees due to a decrease in phylogenetic resolution [32].

3. What are the patterns of phylogenetic community structure in this forest and what do they tell us about drivers of community assembly? We suggest that Southeast Asian forests may show greater phylogenetic overdispersion than Neotropical forests because they are often disproportionately dominated by one clade of trees (in most cases, Dipterocarpaceae), thus increasing the general intensity of interspecific competition [14, 54]. In addition, the Bruneian research plot receives a high mean annual precipitation (5203 mm per year), which could allow for more natural enemies (pathogens) such as bacteria, fungi, and viruses that can promote phylogenetic overdispersion [21, 22].

Material and Methods

Study site and sampling

All necessary permissions for this study were obtained in agreement with all relevant guidelines and policies as outlined in the collaboration agreement between Institute for biodiversity and environmental research (IBER), Universiti Brunei Darussalam and University of Vienna, Austria. The Biodiversity Research and Innovation Centre (BIORIC), Ministry of Industry and Primary Resources Brunei Darussalam granted export of biological specimens for research purposes under reference number BioRIC/HOB/TAD/51 - 30 and BioRIC/HOB/TAD/51 - 46.

The study was conducted in a long-term forest-dynamics plot (latitude: 4.634, longitude: 115.228, http://www.ctfs.si.edu/site/Kuala+Belalong, last accessed: 2017-08-19) that was established at the Kuala Belalong Field Studies Centre (KBFSC) of Universiti Brunei Darussalam in 2009 following the protocols of Condit [55]. It is part of the Center for Tropical Forest Science – Forest Global Earth Observatory (CTFS-ForestGEO; [50]) that includes 63 large-scale demographic tree plots across the Americas, Africa, Asia, and Europe, focusing mainly on the tropics [56]. The Bruneian plot is located in a primary, mixed dipterocarp forest in the Batu Apoi Forest Reserve at Temburong. This region is characterized by a tropical climate with significant year-round mean annual precipitation of 5203 mm and a mean annual temperature of 26.5 °C [51]. It has a steep topography and elevation ranging from 160 to 320 m. The dominant soils are silty clay dominated by quartz and kaolinite (ultisoils). Besides being high in iron and aluminium oxides, they are extremely low in basic plant nutrients [50]. The natural disturbance regime is characterized by landslides [57]. The plot is dominated by broadleaf evergreen vegetation. The 25-ha plot is divided into 2500 subplots of 100 m^2. All free-standing woody stems ≥ 1 cm diameter at breast height are tagged with individual numbers, measured, and mapped spatially. Reference vouchers are deposited at the University of Brunei Darussalam Herbarium (UBDH), and the tagged stem itself serves as an additional living voucher for the individuals sampled. Morphological identifications of the individuals are on-going. Following the CTFS standard protocol, specimens have been sorted to families, genera, and "morphospecies" and in this case have been identified by author the S. Tan. However, these "morphotaxa" have not yet been verified by comparison with vouchers at all pertinent herbaria, and a large number remain unidentified to species level. Across the 25-ha plot, 70 subplots (100 m^2 each) were selected in a stratified random pattern including different topographical attributes. According to the list of individuals provided by UBD-CTFS, there are 4348 tagged trees in the 70 subplots. However, several tree tags were not found during sampling (presumably fallen off or removed by people). Leaf or bark material was sampled only from tagged individuals, leading to 3930 samples, which were dried in silica gel [58].

Topographical analyses

Topographical raw data were provided by the UBD-CTFS and generated following standard protocols described by Condit [55]. Using the CTFS R package [59] a contour map was constructed (Fig 1). Three topographical parameters were calculated for each subplot ("S2 Table"): elevation (E), slope

(S), and convexity (C). Elevation was defined as the mean elevations at the four corners of each quadrant [60]. Following Yamakura *et al.* [61], the convexity of each subplot was determined by calculating the difference of the mean elevation of the focal quadrat and mean elevation of 12 points along a grid of eight subplots surrounding the focal quadrat. For subplots located at the edge of the 25-ha plot, convexity was the elevation of the center point minus the mean of the four corners. Convex surfaces are indicated by positive values, whereas negative values indicate concave surfaces. Slope was calculated for each subplot using the quadslope function of the CTFS R package [62]. The three topographical variables were used to assign each of the 70 subplots to one of five habitats according to earlier studies e.g. [63]. These habitat types are (Fig 1, Table 1): valley (S < S*mean*, E < E*mean*); low slope (S ≥S*mean*, E < E*mean*); high slope (S ≥ S*mean*, E ≥ E*mean*, convexity > 0); high gully (S ≥ S*mean*, E ≥ E*mean*, convexity < 0); ridge top (S≤ S*mean*, E ≥ E*mean*).

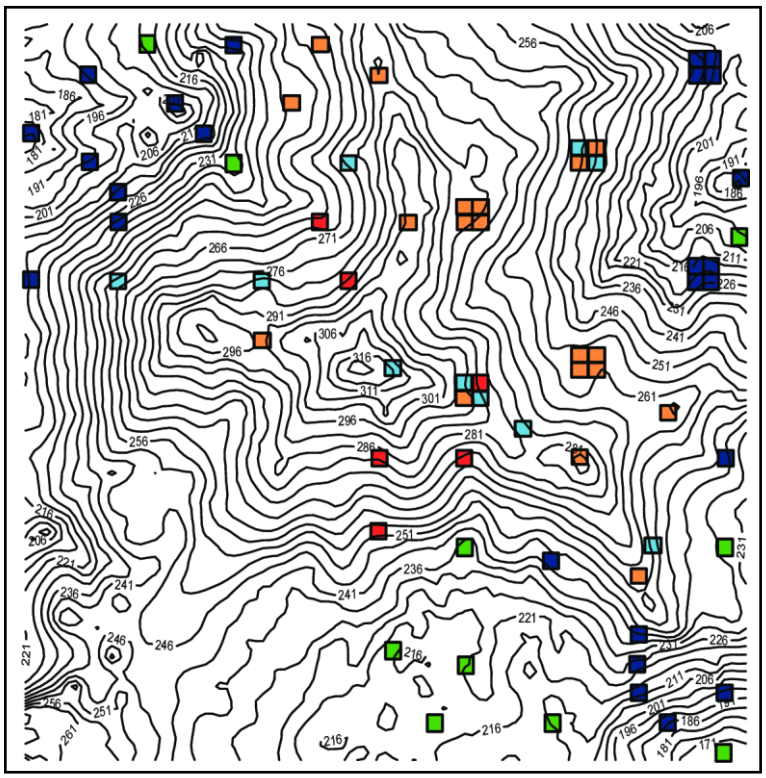

Fig 1. Contour map of the 25 ha plot in Kuala Belalong-Brunei Darussalam and location of the 70 subplots sampled in this study. Habitat types are given for each subplot: valley (green), low slope (dark blue); high slope (light blue); high gully (red); ridge top (orange).

Table 1. Habitat classification.

	Habitat				
	High gully (hg)	**High slope (hs)**	**Low slope (ls)**	**Ridge top (rt)**	**Valley (v)**
Number of plots	6	10	25	19	10
Slope (°)	≥ 27.5	≥ 27.5	≥ 27.5	≤ 27.5	< 27.5
Elevation (m)	≥ 243.5	≥ 243.5	< 243.5	≥ 243.5	< 243.5
Convexity (°)	< 0	> 0	All	all	all

Criteria (slope, elevation, and convexity) used for habitat classification are given.

DNA barcode reference database and identification of morphologically unidentified individuals

DNA extraction, PCR amplification and sequencing

Prior to DNA extraction, samples were frozen in liquid nitrogen and ground into fine powder. Subsequently, genomic DNA was extracted from approximately 20 mg of material using the DNeasy 96 Plant Kit (QIAGEN, Hilden, Germany) following the manufacturer's protocol. Working stocks of 10× diluted DNA were prepared. In total 3300 individuals were included. Two coding plastid regions, *rbcL* and *matK*, were amplified. For amplification of the *rbcL* region primers rbcLa_f [64] and rbcL 724R [65] were used. PCR reactions included 5 µL of 2× ReddyMix PCR Master Mix with 1.5 mM $MgCl_2$ (#AB-0575/DC/LD/A; Thermo Fisher Scientific, Vienna, Austria), 0.1 µl 4.0% bovine serum albumin, 0.1 µl each primer (0.32 µM), 1 µl template DNA and H_20 up to a final volume of 10 µl. Thermal cycle conditions were as follows: initial denaturation at 98°C for 30 sec, 35 cycles of denaturation at 98°C for 10 sec, annealing at 63°C for 30 sec and extension at 72°C for 30 sec, followed by final extension of 5 min at 72 °C. At the beginning of the study, there were three frequently used *matK* primer pairs available to amplify approximately the same region of the gene: 390F and 1326R [66, 67], XF and 5R [68], and 1R_KIM and 3F_KIM [42, 69]. Initially, all three primer pairs were used in this study following the authors' protocols. In the course of generating *matK* sequences, a universal set of primers that can be multiplexed in one PCR reaction was developed (C_MATK_F and C_MATK_R, [70]). This set of primers was then used as follows: 5 µL of 2× ReddyMix PCR Master Mix with 1.5 mM $MgCl_2$ (#AB-0575/DC/LD/A; Thermo Fisher Scientific, Vienna, Austria), 0.1 µL of forward and reverse primer cocktail each at 50 µM (final concentration 0.5 µM), 1 µL of template DNA, and H_2O up to a final volume of 10 µL. Thermocycler conditions were as follows: 95°C for 2 min: five cycles of 95°C for 25 s, 46°C for 35 s, and 70°C for 1 min; 35 cycles of 95°C for 25 s, 48°C for 35 s, and 70°C for 1 min; and a final extension at 72°C for 5 min. For samples that did not amplify using the above-mentioned protocol, the 2× Phusion Green HS II Hi-Fi PCR Master Mix with 1.5 mM $MgCl_2$ (#F-566S, Thermo Fisher Scientific, Vienna, Austria) was used with the following thermocycler conditions: 98°C for 30 s; five cycles of 98°C for 10 s, 53°C for 30 s, and 72°C for 30 s; 35 cycles of 98°C for 10 s, 55°C for 30 s, and 72°C for 30 s; and a final extension at 72°C for 5 min. PCR products were cleaned with 1.5 µL exonuclease I and FastAP thermosensitive alkaline phosphatase mixture (7 U Exo I, 0.7 U FastAP, Thermo Fisher Scientific, Vienna, Austria) at 37°C for 45 min and 85°C for 15 minutes. Sequencing reactions were performed with the BigDye Terminator Kit v3.1 (Thermo Fisher Scientific, Vienna, Austria) using the amplification primers according to the manufacturer's instructions. Sanger sequencing was carried out using a 3730 DNA analyzer (Thermo Fisher Scientific, Vienna, Austria) at the Department of Botany and Biodiversity Research, University of Vienna.

Sequence assembly, editing, and alignment

Bidirectional sequences were trimmed, assembled into contigs, and edited in Geneious (version 8.0.5, [71]). Edited sequences were checked for contamination using BLAST [72]. Contaminated sequences, as well as samples that failed to produce quality reads for *matK* and *rbcL* were removed from

the dataset, leading to a total of 3118 sequences for *rbcL* and 2598 sequences of *matK*. A local reference database for taxa occurring in the 70 subplots of the 25-ha plot was built by uploading all sequences to the Barcode of Life Datasystem [53] under code DS-PCSBRU1. Sequences were sorted according to their haplotypes by aligning them with MAFFT version 7.017 implemented in Geneious version 8.0.5 [71]. A representative for each haplotype was blasted against the Barcode of Life reference (BOLD) database [53] as well as to the National Center for Biotechnology Information (NCBI) reference database Genbank [52]. The resulting identifications were compared with the preliminary morphological identifications. Morphologically unidentified individuals were identified to family or generic level according to their DNA sequence. To decrease computation time in subsequent analyses, a pruned data matrix using one representative per haplotype and morphotaxon was used. If the same morphotaxon exhibited different haplotypes, a representative for each haplotype was included ("S1 Table"). Due to the absence of indel variation, *rbcL* sequences were aligned directly in BioEdit v.7.0.4 [73]. Following translation into amino acids, *matK* sequences were aligned in BioEdit. The translated *matK* matrix was then edited manually. Both alignment files for each marker were combined. For analysis, unsequenced regions and gaps were coded as missing data.

Reconstruction of phylogenetic community trees

To compare resolution and node support of different phylogenetic approaches, three trees were constructed in this study. A tree based on the most recent reference tree R20120829 (APG III, [45]) was built using the online version of Phylomatic [26]. For this, a list of taxa occurring in the barcode matrix was submitted to the program, which tries to match the taxa to the most resolved position in a stored tree. This rapid phylogenetic reconstruction represents a classic and widely used approach in community phylogenetics [74, 75, 76]. Trees were also inferred from the barcode data. Substitution rates were estimated independently for each gene. Here, the rapid bootstrapping algorithm (1000 replicates), which does a complete analysis (ML search and bootstrapping) in one step was conducted using RaxML v8.2.0 [77]. The general time reversible model with six substitution rates (one for each pair of nucleotides) and gamma-distributed rate variation across sites (GTRGAMMA) was chosen for the analysis based on jModeltest2 [78]. The tree constructed by Phylomatic mostly resolves relationships at family level, whereas the barcode data helps to resolve relationships at generic or even species-level. An additional analysis was conducted here because deep nodes in a community phylogenetic tree based on barcodes may not resolve relationships correctly because of taxon-sampling issues. To correct this, deep-level phylogenetic relationships were fixed using a constraint tree based on the APG classification e.g. [33, 39, 40] and the terminal tips were resolved using the barcode sequences. This constraint tree was built using the package "ape" [79] with the R programming language. All taxa were present in the constraint tree, but within each order species were arrayed as polytomies. The constraint tree was implemented in a RaxML analysis as described above, and only trees concordant with ordinal relationships of the APG tree were retained ("S4 Text").

For phylogenetic community structure analyses, ultrametric trees are normally used. For the Phylomatic tree, this is typically done using the command "bladj" in Phylocom [80]. This command was used to obtain a pseudo-chronogram with adjusted branch lengths based on the node calibrations of Wikström *et al.* [81]. Both the unconstrained as well as the constrained trees obtained from the maximum likelihood analyses were transformed into ultrametric chronograms with the mean-path-length method (MPL, [82]) in PATHd8 [83] using age constraints of Magallón & Castillo [84]. They included one fixed age for the angiosperm crown group and 28 (unconstrained tree, "S5 Text") or 29 (constrained tree, "S6 Text") minimal age estimates.

Phylogenetic community structure analyses

To enable a direct comparison among the phylogenetic approaches (Phylomatic and the two ML analyses with barcode sequences, unconstrained and constrained), all three chronograms were used to quantify the phylogenetic structure of 70 communities in the 25-ha forest research plot. If species showed more than one haplotype, we aimed at sequencing all individuals of those species in the plot to assign them to a single tip in the phylogenetic tree. Representatives (3241 individuals in total) for most of the morphotaxa were sequenced. Based on the assumption that the individuals of the same morphotaxon will have identical sequences for *matK* and *rbcL*, unsequenced individuals with a morphological identification were assigned to the haplotype (i.e. tip in the tree) corresponding to sequenced individuals with the same morphological identification. Thus, only taxa that lacked either morphological or sequence information (only 3.3 % of the total number of individuals) were excluded from the community data matrix ("S7 Text"). In order to determine if our results were consistent without making this assumption, we repeated phylogenetic community structure analyses using only sequenced individuals (75% of individuals in the 70 communities). Results of this analysis are referred to as "Barcode only" in the text. In this sensitivity analysis, only 68 subplots were included, as in two subplots, most individuals lacked sequences. The reduced community data matrix with only sequenced individuals is given in "S8 Text". Common phylogenetic diversity metrics were estimated with the remaining data using the package "picante" [85] in R. The widely used quantitative measure of phylogenetic diversity, PD, [25] was calculated on the basis of a chronogram using the "pd" function. In this approach, the branch lengths of a phylogenetic tree, in units of time, are measured and summed. To compare each of the three trees, PDs were compared for subplots using a paired t-test. The phylogenetic trees were then converted into an interspecific phylogenetic distance matrix using the "cophenetic" function in "picante". Based on this distance matrix, mean pairwise distance (MPD; [14]) and mean nearest taxon distance (MNTD; [86]) were calculated. The function "mpd" calculates the mean pairwise distance between all species or individuals in each community, and "mntd" calculates the mean nearest taxon distance, the average distance separating each species or individual in the community from its closest heterospecific relative. MPD and MNTD were weighted by species abundance. Using the functions "ses.mpd" and "ses.mntd", a standardized effect size (SES) of the metric within each local community was calculated based on a comparison of observed MPD/MNTD (obs)

values with the distribution of MPD/MNTD expected under a null model of community assembly where subplots have the same species richness, but species identities are randomised by randomly shuffling tip labels across the entire tree (rand; number of randomizations: 1000). To test for phylogenetic clustering and overdispersion, the net relatedness index (NRI) and the nearest taxon index (NTI) were calculated [13]. NRI and NTI are defined as $[-(\text{metric}_{obs} - \text{mean}(\text{metric}_{rand}))/\text{sd}(\text{metric})_{rand}]$, where the metric is either MPD (for NRI) or MNTD (for NTI). Thus, they are equivalent to the inverse of ses.MPD and ses.MNTD. Positive indices indicate that co-occurring species are more closely related than expect by chance (phylogenetically clustered), whereas negative indices indicate that co-occurring species are more distantly related than expected by chance (phylogenetically overdispersed). NRI and NTI were compared between the different habitats. To investigate if there is a correlation between the environmental variables (mean elevation, slope, convexity) and community structure metrics (PD, NRI, NTI), Pearson product-moment correlation tests were conducted.

Results

DNA barcode reference database and identification of morphologically unidentified individuals

DNA barcode sequence recovery and abundance of families

A DNA barcode reference database was successfully built for individuals occurring in the studied subplots of the 25-ha forest-dynamics plot. In total, DNA barcode sequence data was successfully recovered from 95.5% (*rbcL*), 78.7% (*matK*), and 71.6% (*rbcL* + *matK*) of sequenced individuals. The combined data matrix represented 555 haplotypes (from ≥ 154 genera, 68 families, 25 orders). The DNA barcode sequences were useful for determinations of taxa morphologically unidentified to family or genus, which is necessary for inclusion in phylogenetic reconstruction using Phylomatic. For 500 morphological unidentified individuals, DNA barcodes gave clear identification at genus or family level. Among the 69 families detected by both morphology and molecular identification, Dipterocarpaceae and Euphorbiaceae are dominant, with 16% and 9% of stems, respectively in the study plot. Other frequent families were Rubiaceae (7%) and Achariaceae (6%). The most abundant families are shown in Fig 2.

Characteristics of the alignments

The two-gene alignment included a total of 1820 base pairs (bp), 697 bp from *rbcL* and 1123 from *matK*. The number of variable characters of the combined data matrix was 1087, and the proportion of gaps and completely undetermined characters was 21.17%. Variable characters observed for each marker were 304 (*rbcL*) and 783 (*matK*). The number of gaps and undetermined characters were 0.54% for *rbcL* and 26.68% for *matK*. Population-level variation was detected in one or both loci for only 15 "morphospecies". Additionally, six taxa (*Koompassia excelsa* and five species of *Xanthophyllum*) exhibited stop codons in the *matK* barcode region and were therefore classified as pseudogenes, but they were included in the analysis because these taxa fell in phylogenetic positions reflecting their taxonomy.

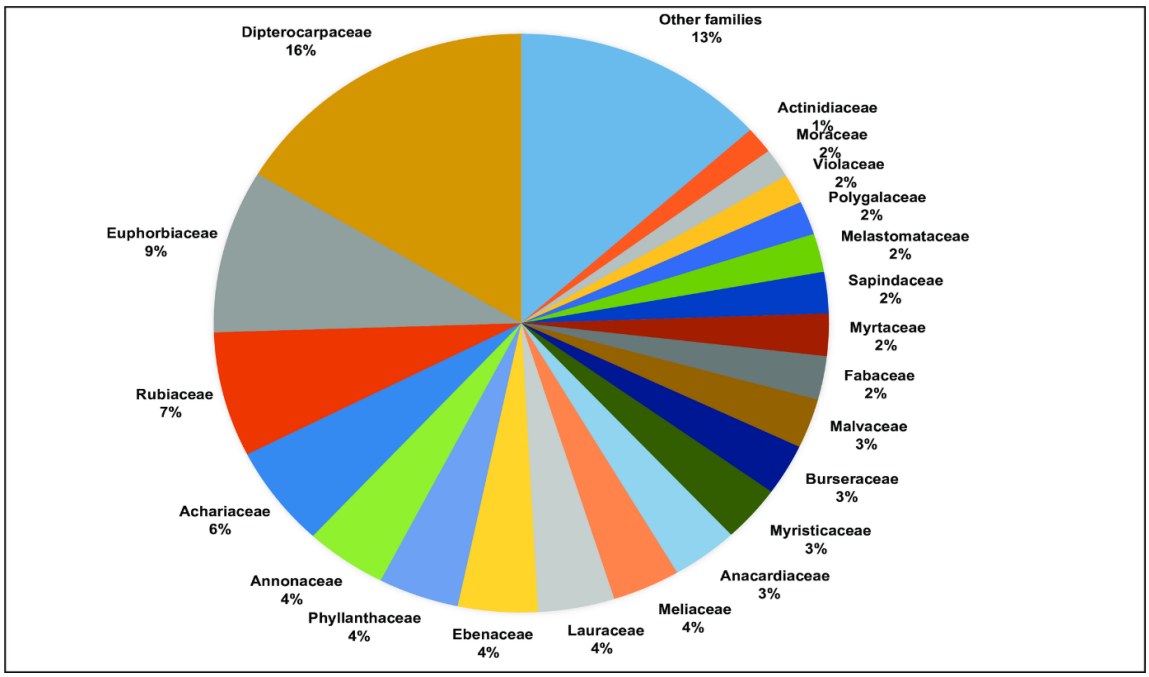

Fig 2: Abundance of plant families. Abundance of frequent plant families in the 70 subplots of the 25 ha forest dynamics plot in Kuala Belalong.

Reconstruction of phylogenetic community trees

All trees produced in this study are given in "S4 Text". The trimmed APG reference tree (R20120829) obtained by Phlylomatic includes 186 resolved nodes, mainly at ordinal and family level, but in some cases resolving relationships among genera within families. Other than Proteales, all other orders were monophyletic. The two families of Proteales were unresolved, a result in common with many other analyses e.g. [87]. Bootstrap support (BS) for this placement was not strong in earlier studies ([88]: BS: 59; [89]: BS: 63), even with complete plastid genomes. In the ML tree constructed using the barcode data, 42.7% of the nodes exhibited high bootstrap support (BS > 85) and a majority (52.4%) showed at least moderate support (BS ≥ 70). Contrary to the Phylomatic tree, the DNA barcode markers were able to resolve relationships at all taxonomic levels, with better resolution at generic and especially species level. Examples of these fine-scale relationships are the genera *Diospyros* and *Shorea* for which species relationships remain completely unresolved in the Phylomatic tree ("S4 Text"). Furthermore, all families were grouped into the same orders as in APG III [45] and APG IV [46], Sabiaceae and Proteaceae (Proteales). However, the topology of the tree differed from the accepted APG classification at the ordinal level (Fig 3). The constrained tree successfully resolved relationships at all taxonomic levels. Compared to the Phylomatic and the unconstraint barcoding trees, the constrained tree showed the highest percentage of highly supported nodes (BS > 85: 43.6% and BS ≥ 70, 53.8%). Proteales were paraphyletic in the constrained analysis, and all other families clustered in the APG IV orders [46]. Three families, Olacaceae (Santalales), Anacardiaceae (Sapindales) and Loganiaceae (Gentianales) were not monophyletic, but monophyly of the remaining families was highly supported (BS ≥ 85), except for Euphorbiaceae (BS 46; Fig 4).

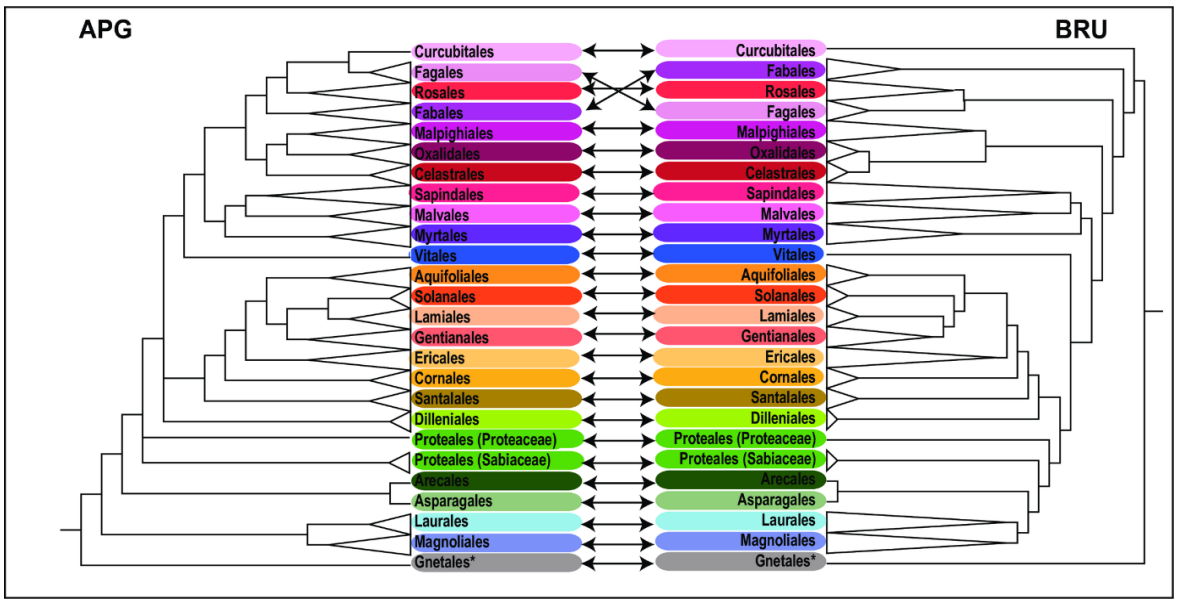

Fig 3. Comparison of ordinal-level topologies of the trimmed APG tree obtained by Phylomatic (APG) and the barcode tree (*rbcL* + *matK*; BRU) obtained from maximum likelihood analysis. Orders are connected by arrows. *: The order Gnetales represents the gymnosperms, whereas all other orders are angiosperms.

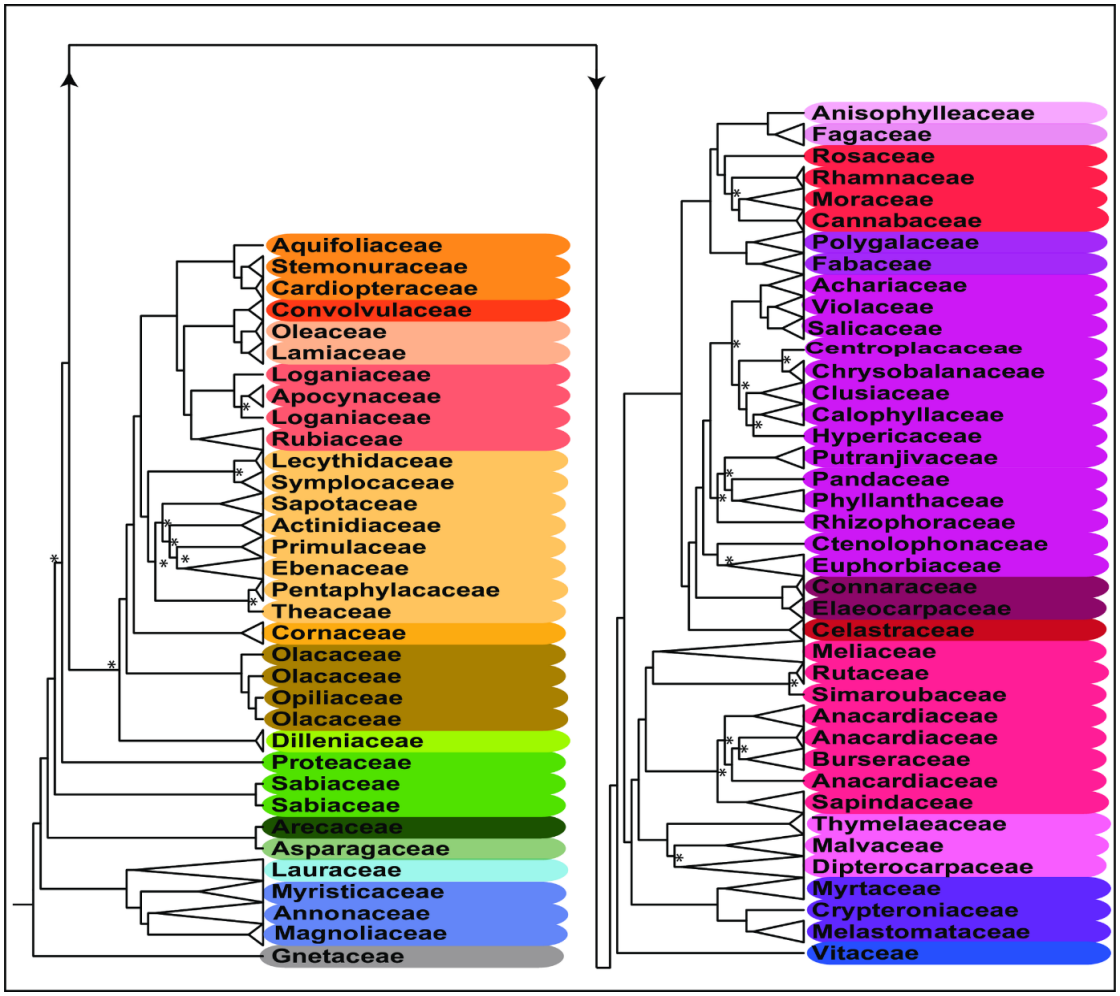

Fig 4. Cladogram of phylogenetic relationships of the woody plant taxa in the Kuala Belalong Forest Dynamics Plot, Brunei Darussalam (BRU). Best-scoring tree obtained from maximum likelihood analysis of the barcode data (*rbcL+matk*) with application of an APG III-based ordinal-level constraint tree. The tree is collapsed to family level. For presentation purposes a cladogram is given. An uncollapsed tree including branch lengths is given in "S4 Text". Nodes with an * have bootstrap support < 70.

Assessment of phylogenetic community structure

Phylogenetic diversity (PD)

Mean PD of the subplots varied among the different trees (Fig 5, "S3 Table"). It was highest when calculated based on a constrained ML tree (4980.46 myrs; Barcode only: 4479.02 myrs), followed by the unconstrained ML tree (4891.62 myrs; Barcode only: 4401.81 myrs) and the tree constructed using Phylomatic (4519.63 myrs: Barcode only: 3956.84 myrs). Using paired t-tests, differences in PD between the calculations based on the unconstrained and constrained ML analyses were significant (t = 8.7228, df = 69, p-value = 9.544e-13; Barcode only: t = 8.5322, df = 67, p-value: 2.646e-12). Highly significant differences were detected between calculations using Phylomatic and the unconstrained (t = -9.7575, df = 69, p-value = 1.271e-14; t = -14.686, Barocode only: df = 67, p-value < 2.2e-16), as wellas the constrained (t = -12.365, df = 69, p-value < 2.2e-16; Barcode only: t = -17.857, df = 67, p-value < 2.2e-16) barcoding ML analyses (Fig 5).

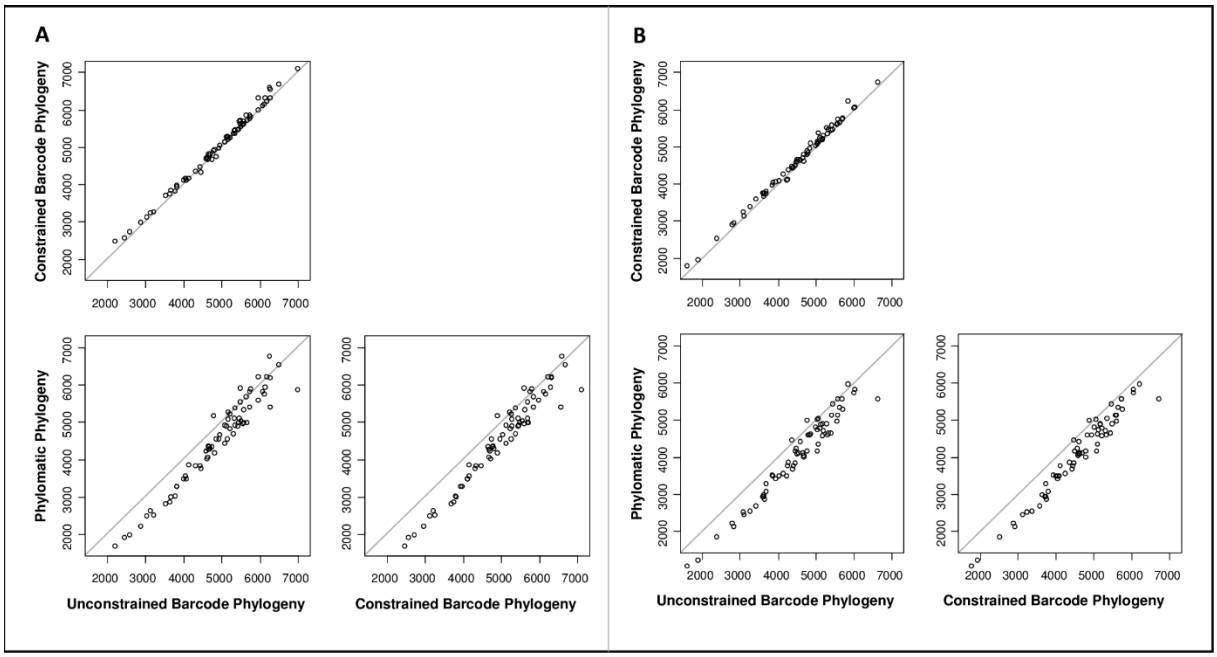

Fig 5. Pairwise comparison of phylogenetic diversity (PD). PD was calculated based on three different phylogenetic hypotheses (Phylomatic: APG classification, Barcode.con: constrained barcode tree, Barcode.uncon: unconstrained barcode tree). A: Calculations based on sequenced and morphologically identified individuals. B: Calculations based on sequenced individuals only.

Phylogenetic community structure

Comparing the NRI and NTI metrics, similar patterns of phylogenetic community structure were observed. In some cases, patterns of phylogenetic community structure varied with respect to the tree used for calculation (Fig 6, "S3 Table"). Looking at the NRI metric of each subplot, the Phylomatic tree detected significant phylogenetic clustering in 16 subplots (Barcode only: nine) and significant phylogenetic overdispersion in two subplots (Barcode only: two). Using the barcode tree, significant clustering was detected in 13 (constrained, Barcode only: eight) and 12 (unconstrained, Barcode only: eight) subplots. Overdispersion was detected in one subplot using the unconstrained barcode tree. For

the NTI metric, phylogenetic clustering was detected in 14 subplots (Barcode only: seven) using the Phylomatic tree, whereas the barcoding trees detected clustering in nine (constrained, Barcode only: nine) or eight (unconstrained, Barcode only: six) subplots. The unconstrained barcode tree revealed phylogenetic overdispersion in one subplot. Overall, the Phylomatic tree not only exhibited a higher mean for NTI and NRI, but also a much greater variance (variance of NRI: Phylomatic: 1.19 (Barcode only: 0.88), Barcode.con: 0.56 (Barcode only: 0.41), Barcode.uncon: 0.42 (Barcode only: 0.46); variance of NTI; Phylomatic 0.96 (Barcode only: 0.83), Barcode.con: 0.71 (Barcode only: 0.66), Barcode.uncon: 0.76 (Barcode only: 0.67)). A summary of subplots exhibiting significantly phylogenetic structuring with respect to different trees is given in "S3 Table". Although there were differences in detecting phylogenetic structure with different phylogenetic trees and community data matrices, no reversed inferences for NRI and NTI were observed. Furthermore, phylogenetic clustering was detected in all habitats (Fig 7). To conclude, other than a few cases of significant phylogenetic overdispersion, the general pattern of either random structuring or phylogenetic clustering did not differ with respect to phylogenetic tree, habitat or community matrix (Fig 6, Fig 7).

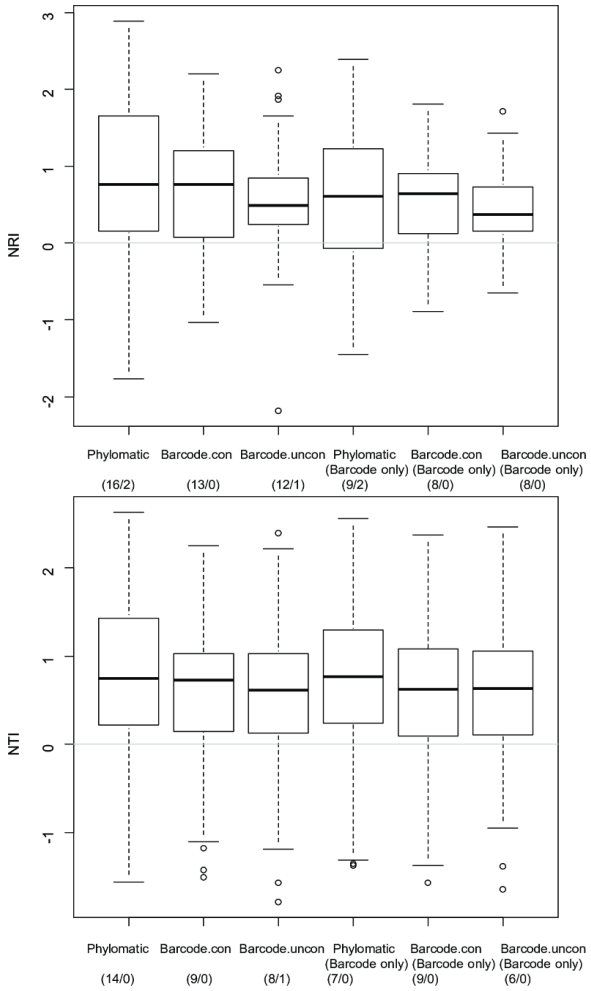

Fig 6. Net relatedness index (NRI) and nearest taxon index (NTI). NRI and NTI were calculated based on three different phylogenetic hypotheses (Phylomatic: APG classification, Barcode.con: constrained barcode tree, Barcode.uncon: unconstrained barcode tree). The number of subplots showing significant phylogenetic structuring (clustering/overdispersion) is given in brackets. A: Calculation based on sequenced and morphologically identified individuals. B: Calculations based on sequenced individuals only.

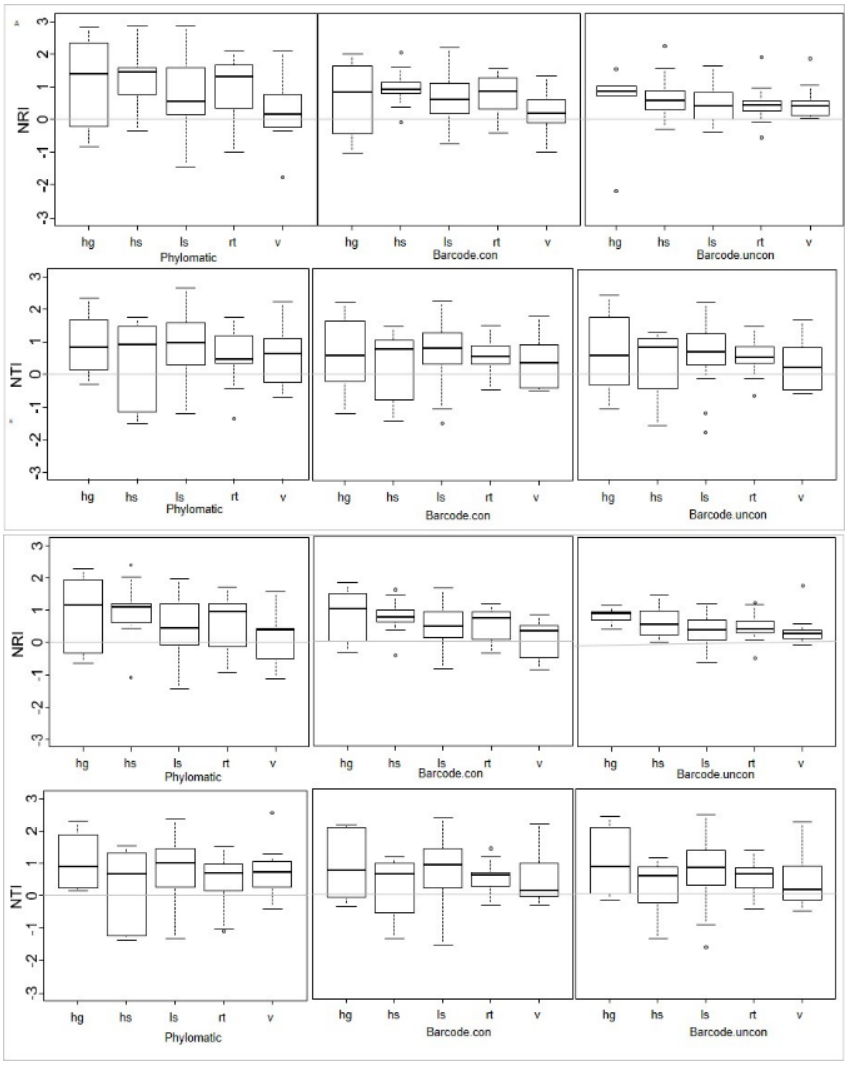

Fig 7. Comparison of net relatedness index (NRI) and nearest taxon index (NTI) in different habitats. NRI and NTI were calculated based on three different phylogenetic hypotheses (Phylomatic: APG classification, Barcode.con: constrained barcode tree, Barcode.uncon: unconstrained barcode tree) in different habitats (hg: high gully, hs: high slope, ls: low slope, rt: ridge top, v: valley). A: Calculation based on sequenced and morphologically identified individuals. B: Calculations based on sequenced individuals only.

Processes responsible for community assembly

Patterns of phylogenetic structuring are often used as a proxy for the mechanism responsible for community assembly. Phylogenetic clustering can suggest the influence of abiotic factors on community assembly [14]. Consequently, three environmental variables (mean elevation, slope, and convexity) were tested for correlations between PD, NRI, and NTI. Pearson product-moment correlations detected a weak positive correlation between the PD and mean elevation when calculated on basis of the unconstrained barcoding tree (Table 2). A moderate positive correlation between mean elevation and NRI was observed when calculated using the Phylomatic tree and the constrained barcoding tree. Significant, albeit generally weak, positive correlations between convexity and PD, as well as between convexity and NRI, were observed in analyses of all three phylogenetic trees. Analysis based on the barcode data only revealed a positive correlation between NRI and elevation, as well as between NRI and convexity when the Phylomatic and the constrained barcode tree were used, whereas the unconstrained barcode tree showed positive correlation between NRI and convexity only (Table 2).

Table 2. Results of Pearson moment-product correlation test. EV: Environmental variable, ME: mean elevation, S: slope, C: convexity

EV	Phylomatic			Barcode.con			Barcode.uncon		
	PD	**NRI**	**NTI**	**PD**	**NRI**	**NTI**	**PD**	**NRI**	**NTI**
ME	r = 0.18	**r = 0.39**	r = 0.1	r = 0.23	**r = 0.35**	r = -0.09	**r = 0.23**	r = 0.11	r = - 0.07
	p = 0.13	**p < 0.05**	p = 0.41	p = 0.05	**p < 0.05**	p = 0.47	**p = 0.049**	p = 0.34	p = 0.54
S	r = 0.04	r = 0.11	r = 0.03	r = 0.04	r = 0.11	r = -0.02	r = 0.02	r = 0.05	r = 0.005
	p = 0.72	p = 0.35	p = 0.81	p = 0.77	p = 0.38	p = 0.87	p = 0.84	p = 0.66	p = 0.97
C	**r = 0.38**	**r = 0.44**	r = 0.08	**r = 0.36**	**r = 0.46**	r = 0.13	**r = 0.37**	**r = 0.27**	r = 0.14
	p < 0.05	**p < 0.05**	p = 0.51	**p < 0.05**	**p < 0.05**	p = 0.29	**p < 0.05**	**p < 0.05**	p = 0.25
EV	**Phylomatic Barcode only**			**Barcode.con Barcode only**			**Barcode.uncon Barcode only**		
	PD	**NRI**	**NTI**	**PD**	**NRI**	**NTI**	**PD**	**NRI**	**NTI**
ME	r = 0.15	**r = 0.36**	r = -0.11	r = 0.19	**r = 0.38**	r = -0.12	r = 0.19	r = 0.23	r = -0.1
	p = 0.21	**p < 0.05**	p 0.38	p = 0.1186	**p < 0.05**	p = 0.32	p = 0.13	p = 0.06	p = 0.4
S	r =0.05	r = 0.11	r = 0.02	r = 0.05	r = 0.10	r = -0.002	r = 0.04	r = 0.05	r = 0.01
	p = 0.67	p = 0.39	p = 0.88	p = 0.68	p = 0.4	p = 0.99	p = 0.7	p = 0.67	p = 0.9
C	**r = 0.33**	**r = 0.44**	r = 0.11	**r = 0.32**	**r = 0.4**	r = 0.1	**r = 0.32**	**r = 0.27**	r = 0.11
	p = 0.006	**p < 0.05**	p = 0.38	**p = 0.006**	**p < 0.05**	p = 0.4	**p = 0.006**	**p < 0.05**	p = 0.37

Bold values show significant positive correlations between environmental variables and phylogenetic diversity metrics with respect to different trees (Phylomatic: APG tree, Barcode.con: constrained barcode tree, Barcode.uncon: unconstrained barcode tree) and community data matrices.

Discussion

DNA barcode reference database and identification of morphologically undetermined individuals

The first step in DNA barcoding and DNA-based community structure analyses is development of a comprehensive barcode sequence library. In this study, a regional plant *rbcL* and *matK* barcode reference database was successfully generated 3241 individuals reported in the studied subplots of the 25-ha forest research plot. DNA barcode recovery rates were higher for *rbcL* (95.1% of individuals sequenced) than for *matK* (88.5% of individuals sequenced). One reason for this is that the *rbcL* primers worked well across all angiosperms, whereas *matK* is much more difficult to amplify across a wide range of species. However, the use of recently published primer cocktails [70] increased amplification and sequencing of the *matK* barcode compared to earlier studies (e.g. [32]: 69% of species, [33]: 70.4% of species). In total, 69 families were detected by morphology and molecular (barcode) identification. The abundance of these families represents the typical composition of tropical rain forests in Southeast Asia [48, 90, 91]. As expected, the dominant tree family in the examined plots was Dipterocarpaceae with 16% of individuals followed by Euphorbiaceae with 9% (Fig 2). DNA barcodes are especially important when some individuals have not been identified, which is often the case in species-rich tropical forests where it is difficult to obtain flowers and or fruits, which are critical for morphological identification yet often not present when sampling takes place. Using DNA barcodes, phylogenetic trees can be constructed that include morphologically unidentified individuals as long as sequences have been

obtained. In addition, families and genera of unidentified individuals can be extracted from BOLD and/or GenBank based on sequence similarity. Here, accessions were successfully assigned to generic or family level using the BOLD Identification System for *rbcL* and *matK* as well as GenBank. However, identification to species level was not achieved because of two issues. Firstly, DNA barcodes, especially *rbcL*, could not distinguish closely related species, leading to more than one high match with the sequences in reference databases. Similar results were observed by Gonzales *et al*. [92] in their study on Amazonian trees, in which neither of the plastid markers tested (including *rbcL* and *matK*), alone or combined, achieved a rate of correct identification greater than 70%. This was especially true for a few species-rich clades that showed little or no variation in these markers. Secondly, some sequences had poor matches in reference databases, reflecting lack of sequences from some species in clades included in our study. Such lack of sequence availability in the reference databases, such as BOLD and GenBank, demonstrates the need for more exhaustive and accurate databases including more species and intra-specific haplotype diversity [93]. Our newly generated sequences make a good contribution to the expansion of these databases.

Comparison of Phylomatic versus barcode trees

Previous studies have shown that the degree of resolution in community phylogenetic trees plays an important role in detecting non-random patterns of phylogenetic community structure [32, 33, 34, 40, 94]. A high degree of phylogenetic resolution is necessary in phylogenetic community structure analysis because poorly resolved trees can reduce statistical power for detecting non-random forms of community structure, especially when deeper nodes are unresolved [94]. In this study, several approaches were used for phylogenetic reconstruction and compared with respect to resolution and topology: (1) Phylomatic, (2) ML analysis of DNA barcode sequences, and (3) ML analysis with application of a constraint tree (ordinal-level APG topologies). Although time and cost efficient, the Phylomatic approach has disadvantages, for example requiring accurate morphological species identifications at least to family/genus level, because the online phylogenetic query tool requires a list of identified individuals. Furthermore, Phylomatic often provides little or no resolution of phylogenetic relationship among closely related taxa [27]. Compared to the tree obtained by Phylomatic, the barcoding trees yielded better resolution at generic and species levels. An earlier study in a Panamanian forest plot by Kress *et al.* [32] has shown that DNA barcode data alone are sufficient to build phylogenetic trees that closely agree with the APG classification. However, a follow up study in a Puerto Rican forest-dynamics plot showed significantly less concordance with APG [33]. Phylogenetic trees constructed by the use of DNA barcodes often represent single geographic areas, with limited taxon sampling, and therefore lack representatives of many angiosperm families. One could expect such analyses with limited taxon-sampling to differ in topology [95, 96] from the APG classification.

Parallel to the observations in Puerto Rican forest [33], our study showed ordinal-level discrepancies between the tree constructed by Phylomatic and that resulting from ML analysis (Fig 3). In order to build a community tree resolved both at deep and shallow nodes, the ordinal-level APG tree was

incorporated as a constraint tree for ML analysis. Many polytomies in the Phylomatic tree were resolved with the barcode data (Fig 4). Proteales were non-monophyletic in the constrained barcode analysis. Although monophyly of the most families was highly supported in our results, three families, Olacaceae, Loganiaceae, and Anacardiaceae (Fig 4), and some genera (e.g. *Shorea*, Dipterocarpaceae; "S4 Text") were not monophyletic. This does not mean that the phylogenetic tree reconstructed with the DNA barcodes is wrong, but in many cases reflects non-monophyly of some taxonomic groups (e.g. paraphyly of Olacaceae [97]; and *Shorea* [98]).

Assessment of phylogenetic community structure and implications

Substantially different results in detection of non-random community structure have been inferred with different phylogenetic approaches (e.g. [32, 34, 40, 94]). For example, in a study of a Chinese subtropical forest, analyses based on a more resolved molecular tree showed more phylogenetic clustering than analyses using a Phylomatic tree [34]. Furthermore, a simulation-based study has shown that measures of phylogenetic diversity and community structure are more sensitive to loss of resolution basally in the tree and less sensitive to loss of resolution terminally [94]. In our study, mean PD was underestimated when calculated based on the Phylomatic tree, which corresponds to Swenson's observation [94]. Regarding NRI and NTI, different phylogenetic trees gave the same overall result, but the Phylomatic tree detected greater phylogenetic clustering (Fig 6). The well-resolved barcode trees are more likely to influence the inference of patterns of community structure at low taxonomic levels. If competition or interaction with natural pests and diseases is influencing the assembly of co-occurring species, the DNA sequence trees are expected to exhibit lower values of NRI and NTI. The Phylomatic tree not only revealed bias (an upwardly shifted mean in values for NRI and NTI), but also a much greater variance due to noise introduced with the decreased resolution. This corresponds with the results of Kress *et al.* [32], where only two of the five cases of significant phylogenetic structuring detected with analysis using a Phylomatic tree were supported by a barcode tree. On the other hand, analyses based on their barcode tree identified significant phylogenetic structure in five cases for which the Phylomatic approach did not.

In this study, analyses were conducted applying two different community data matrices. To obtain information of the relatedness measures of most individuals, a community data matrix was created based on the assumption that the same morphospecies have identical sequences. This standard approach allows inclusion of individuals for which molecular information is not available. Morphological species identifications in the 25-ha forest dynamics plot are not yet complete, and a large number of species (especially species-rich genera, such as *Aglaia*, *Syzygium*) remain unidentified at the species level (Stuart Davies, personal comm.). However, species-level identification is not an essential aspect of this study, because it is not needed for community structure analysis if DNA sequences are available for a large number of individuals. Therefore, a second analysis was conducted without any morphological identifications, i.e. including only individuals that were sequenced in the community matrix data file. Regardless of which community data matrix was used, having all the individuals from

the plot (identified or not at species level) or taking only the sequenced individuals, the general pattern inferred for forest community phylogenetic structure was clustering.

Studies on scale dependence in community phylogenetic analysis of plant communities have shown that phylogenetic clustering increases with spatial scale [47, 99, 100] because this usually includes greater environmental heterogeneity. This leads closely related species sharing environmental factors to sort across contrasting environments [1]. Although there is no standard plot size, we acknowledge that the size of the subplots under investigation (10 × 10 m) is small compared to other studies on phylogenetic community assembly. In our study, six sets of four of the examined 10 × 10 m subplots are adjacent, forming six plots of 20 × 20 m (Fig 1). We compared NRI and NTI of each of the six 20 × 20 m plots with the metrics of the corresponding 10 × 10 m plots and found equivalent results (not shown). Furthermore, as phylogenetic clustering was the general pattern observed in this study, we conclude that the small size of the plots did not negatively bias the detection of phylogenetic clustering.

A central focus in community ecology is the investigation of processes responsible for community assembly, and much research has focused on the phylogenetic consequences of competitive interaction and environmental filtering [101]. We observed phylogenetic clustering in many subplots ("S3 Table"), contrary to our prediction of phylogenetic overdispersion, which was based upon the dominance of Dipterocarpaceae in Southeast Asian rain forests. Our results of phylogenetic clustering may reflect that Dipterocarpaceae actually account only for 16% (Fig 2) of all trees with ≥ 1 cm diameter in breast height occurring in the studied subplots. Although this is the first study on phylogenetic community assembly in a Southeast Asian forest based on DNA barcode sequences, traditional approaches (i.e. Phylomatic) have been used to explore the phylogenetic structure of tree communities on Indonesian Borneo. Webb [13] found evidence that co-occurring species were more closely related than expected by chance (phylogenetically clustered). Moreover, Webb *et al.* [21], detected overdispersion at seedling level in the same forest, suggesting that sharing of herbivores is important at that life stage but maybe not for adult trees.

Phylogenetic clustering is often used as a proxy for habitat filtering [14]. It has been reported that the floristic composition of mixed dipterocarp forests varies with precipitation, soil nutrients and topography [102, 103]. Moreover, in a study of a species-rich mixed dipterocarp rain forest in Indonesian Borneo, Webb and Peart [104] have shown that distribution and abundance of many species are influenced by local heterogeneity in physical habitat variables. Considering our observations, all three phylogenetic trees revealed significant phylogenetic clustering in most habitats (Fig 7, "S3 Table"). Furthermore, PD and NRI showed significantly positive correlations with convexity (Table 2), indicating that dynamics of Bruneian forest are, at least partly, shaped by environmental filtering at the community scale. This supports the hypothesis that habitat filtering is an important mechanism responsible for phylogenetic clustering in tropical forests, in accordance with results from most tropical tree communities [13, 35, 41, 47]. On the other hand, those predictions have to be taken with caution because competition might promote phylogenetic clustering [105]. Phylogenetically conserved traits might determine whether a species is a good competitor, which possibly leads to overrepresentation of

a clade of good competitors, resulting in phylogenetic clustering [18]. However, further investigations including data on functional niche-associated traits and additional environmental factors (e.g. soil composition) are needed for solid conclusions.

Conclusion

Although DNA barcodes cannot always be used for species-level identification because reference databases often lack species and haplotype diversity, they can still be useful in species-diverse communities such as tropical rain forests where morphological identification is challenging. In this study, phylogenetic information from two DNA barcoding plastid regions was successfully combined with the APG tree by incorporating ordinal-level constraints on topology. This approach led to a highly resolved tree, which when used in community structure analyses, decreased false positive and false negative observations. The pattern of phylogenetic clustering observed in this study, one of the first using a barcode phylogenetic trees in a Southeast Asian tropical rain forest, gives insights into phylogenetic community structure and corresponds to earlier findings in other tropical forests. Once morphological identification is completed and names of the taxa are validated, the phylogenetic trees constructed here can be used in further studies, and mechanisms responsible for the observed phylogenetic structuring can be identified once niche-associated plant functional traits are integrated.

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Supporting information

S1 Table. List of haplotypes included in the study.

S2 Table. Mean elevation, slope, and convexity for all subplots.

S3 Table. Calculations of PD, MPD, MNTD, NRI, and NTI for each subplot using different trees (Phylomatic, un-, constrained ML trees) and community data matrices

S4-S8 are not given here because these are nexus/input files.

S4 Nexus file containing different phylogenies reconstructed in this study

S5 Input file used for dating of the unconstrained ML tree in PATHD8.

S6 Input file used for dating of the constrained ML tree in PATHD8.

S7 Input file. Community data matrix including sequenced and morphological identified individuals.

S8 Input file. Community data matrix including sequenced individuals only

S1 Table. List of haplotypes (H), BLAST ID, GenBank accession numbers for *rbcL* and *matK*, and voucher/tree tag numbers. [s]: sequences with stop codons; n/a: sequence not available. Vouchers of the trees are stored under the tree tag number at the Universiti Brunei Darussalam.

Haplotype	BLAST ID	BOLD-ID	*rbcL*	*matK*	Vocher/Tree Tag Number
H1_ILEXCY	*Ilex sp.*	BABRU2813-15	MF435396	MF418661	16-2912
H2_GONOMI	*Gonocaryum sp.*	BABRU876-14	MF435522	MF418662	04-4020
H3_GONOSP	*Gonocaryum sp.*	BABRU2405-15	MF435521	n/a	25-0036
H4_GROMQU	*Gomphandra sp.*	BABRU4143-15	MF435660	MF418716	24-5844
H5_STEMMA	Stemonuraceae	BABRU2535-15	MF435792	MF418717	22-1041
H6_STEMUM	Stemonuraceae	BABRU2922-15	MF435823	MF418718	24-3882
H7_PINAAU	Arecaceae	BABRU1487-15	MF435835	MF419145	16-2462
H8_DRACSP	*Dracaena sp.*	BABRU2080-15	n/a	MF419146	22-0372
H9_SALACA	*Salacia sp.*	BABRU3098-15	MF435859	MF419152	24-4063
H10_LOPHBE	*Lophopetalum sp.*	BABRU1810-15	MF435870	MF419153	23-3267
H11_LOPHSU	*Lophopetalum sp.*	BABRU400-14	MF435291	MF419154	09-4159
H12_ALANSP1	*Alangium sp.*	BABRU1753-15	MF43530	MF418665	25-1862
H13_ALANJAE	*Alangium sp.*	BABRU2907-15	MF435339	MF418668	24-3867
H14_ALANJAJ1	*Alangium sp.*	BABRU2800-15	MF435325	MF418667	16-2899
H15_ALANJAJ2	*Alangium sp.*	BABRU398-14	MF435314	MF418666	09-4157
H16_MASTRO	*Mastixia sp.*	BABRU3317-15	MF435351	MF418664	16-4625
H17_ANISBE	*Anisophyllea sp.*	BABRU2252-15	MF435362	MF419147	24-0498
H18_DILLEX	*Dillenia sp.*	BABRU3907-15	MF435373	MF418758	24-5580
H19_DILLSU	*Dillenia sp.*	BABRU3585-15	MF435383	MF418759	20-3821
H20_SAURSP1	*Saurauia sp.*	BABRU2382-15	MF435395	MF418703	25-0013
H21_SAURSP2	*Saurauia sp.*	BABRU1240-14	MF435407	MF418706	11-4097
H22_SAURLO	*Saurauia sp.*	BABRU2487-15	MF435424	MF418704	23-0210
H23_SAURSU	*Saurauia sp.*	BABRU2271-15	MF435435	MF418705	24-0519
H24_DIOSSP2	*Diospyros sp.*	BABRU1449-15	MF435458	MF418682	13-2673
H25_DIOSSP1	*Diospyros sp.*	BABRU3444-15	MF435446	MF418679	20-3676
H26_DIOSSP3	*Diospyros sp.*	BABRU2950-15	MF435475	MF418684	24-3910
H27_DIOSSP4	*Diospyros sp.*	BABRU2222-15	MF435487	MF418692	16-0891
H28_DIOSSP5	*Diospyros sp.*	BABRU2945-15	MF435486	n/a	24-3905
H29_DIOSAR	*Diospyros sp.*	BABRU473-14	MF435499	MF418688	11-5698
H30_DIOSBO	*Diospyros sp.*	BABRU3744-15	MF435512	MF418683	20-5422
H31_DIOSBU	*Diospyros sp.*	BABRU1160-14	MF435520	MF418695	04-4864

H32_DIOSCL	*Diospyros sp.*	BABRU2221-15	MF435534	MF418694	16-0890
H33_DIOSEL	*Diospyros sp.*	BABRU961-14	MF435550	MF418696	04-4645
H34_DIOSFE	*Diospyros sp.*	BABRU288-14	MF435561	MF418690	12-4188
H35_DIOKO	*Diospyros sp.*	BABRU1963-15	MF435581	MF418689	16-1786
H36_DIOSLA	*Diospyros sp.*	BABRU1605-15	MF435592	MF418680	20-2941
H37_DIOSOL	*Diospyros sp.*	BABRU3715-15	MF435606	MF418681	20-5384
H38_DIOSPE	*Diospyros sp.*	BABRU3371-15	MF435618	MF418687	20-3602
H39_DIOSSB	*Diospyros sp.*	BABRU4107-15	MF435632	MF418685	24-5803
H40_DIOSSU	*Diospyros sp.*	BABRU1200-14	MF435648	MF418693	04-4986
H41_DIOSTO	*Diospyros sp.*	BABRU837-14	MF435659	MF418691	07-5771
H42_DIOSWA	*Diospyros sp.*	BABRU2100-15	MF435672	MF418686	15-0347
H43a_BARRCU	*Barringtonia sp.*	BABRU4005-15	MF435684	MF418707	24-5693
H43b_BARRLA	*Barringtonia sp.*	BABRU3337-15	MF435685	MF418708	16-4645
H44_ADINS1	*Adinandra sp.*	BABRU1232-14	MF435709	MF418698	11-4089
H45_ADINAC	*Adinandra sp.*	BABRU1856-15	MF435697	MF418697	13-1859
H46_ARDIS1	*Ardisia sp.*	BABRU2356-15	MF435740	MF418754	19-0275
H47a_ARDIS3	*Ardisia sp.*	BABRU3598-15	MF435729	MF418753	20-3835
H47b_ARDIFE	*Ardisia sp.*	BABRU4167-15	MF435728	MF418752	24-5870
H48_ARDIS4	*Ardisia sp.*	BABRU2227-15	MF435752	MF418756	16-0896
H49_ARDIS2	*Ardisia sp.*	BABRU864-14	MF435768	MF418755	04-4003
H50_MADHKI	*Madhuca sp.*	BABRU1855-15	MF435780	MF418669	13-1858
H51_PALAS1	*Palaquium sp.*	BABRU2674-15	MF435817	MF418677	16-2990
H52_PALAS2	*Palaquium sp.*	BABRU150-14	MF435818	MF418672	13-6047
H53_PALACA	*Palaquium sp.*	BABRU168-14	MF435791	MF418674	13-6065
H54_PALAEL	*Palaquium sp.*	BABRU2391-15	MF435813	MF418676	25-0022
H55_PALAGU	*Palaquium sp.*	BABRU1846-15	MF435816	MF418671	13-1848
H56_PALASE	*Palaquium sp.*	BABRU284-14	MF435802	MF418673	12-4184
H57_SAPOXS1	*Madhuca sp.*	BABRU926-14	MF435819	MF418678	04-4708
H58_SAPOXS2	*Madhuca sp.*	BABRU2693-15	MF435820	MF418670	16-3065
H59_SAPOXS3	*Manilkara sp.*	BABRU4013-15	MF435821	MF418675	24-5701
H60_SYMPSP1	*Symplocos sp.*	BABRU827-14	MF435822	MF418699	03-5562
H61_SYMPSP2	*Symplocos sp.*	BABRU4002-15	n/a	MF418700	24-5690
H62_SYMPCO	*Symplocos sp.*	BABRU1143-14	MF435824	MF418701	03-5122
H63_SYMPCR	*Symplocos sp.*	BABRU095-14	MF435825	MF418702	09-3687
H64_THEAX	*Tutcheria sp.*	BABRU1462-15	MF435826	MF418709	16-2436
H65_ADENX	*Adenanthera sp.*	BABRU3185-15	MF435827	MF419047	16-4488
H66_ARCHJI	Fabaceae	BABRU3537-15	MF435828	MF419048	20-3772
H67_ARCHTR	Fabaceae	BABRU2545-15	MF435829	MF435873	22-1051
H68_CRUDRE	Fabaceae	BABRU3714-15	MF435830	MF419050	20-5383
H69_DIALKUKU	*Dialium sp.*	BABRU1595-15	MF435832	n/a	20-2931
H70_DIALKU	*Dialium sp.*	BABRU4228-17	MF435831	MF419049	24-3849
H71_FABABR	*Fordia sp.*	BABRU2304-15	MF435693	MF419064	19-0223
H72_FORDSP	*Fordia splendidissima*	BABRU1608-15	MF435692	MF419063	20-2944
H73_KOOMEX[s]	*Koompassia sp.*	BABRU2455-15	MF435833	MF435890[s]	25-0349
H74_SARADE	*Saraca sp.*	BABRU794-14	MF435834	MF419055	01-5515
H75a_SINDSP	*Sindora sp.*	BABRU2734-15	MF435836	MF419051	16-3107
H75b_SINDBE	*Sindora sp.*	BABRU2188-15	MF435837	MF419054	16-0856
H75c_SINDCO	*Sindora sp.*	BABRU2403-15	MF435838	MF419052	25-0034
H76_SINDLE	*Sindora sp.*	BABRU2822-15	MF435839	MF419053	16-2921
H77_XANTSP1[s]	*Xanthophyllum sp.*	BABRU2170-15	MF435840	MF435891[s]	15-0418

H78_XANTSP2	*Xanthophyllum sp.*	BABRU1978-15	MF435841	MF419056	19-1899
H79_XANTSP3	*Xanthophyllum sp.*	BABRU4026-15	n/a	MF419061	24-5719
H80_XANTBE	*Xanthophyllum sp.*	BABRU1192-14	MF435843	MF419059[s]	04-4951
H81_XANTCL	*Xanthophyllum sp.*	BABRU1690-15	MF435844	MF419058	22-1841
H82_XANTFE[s]	*Xanthophyllum sp.*	BABRU420-14	MF435845	MF435892[s]	09-4182
H83_XANTGR[s]	*Xanthophyllum sp.*	BABRU1615-15	MF435847	MF435893[s]	20-2952
H84_XANTMA[s]	*Xanthophyllum sp.*	BABRU1923-15	MF435846	MF435894[s]	16-1741
H85_XANTPA	*Xanthophyllum sp.*	BABRU3748-15	MF435848	MF419057	20-5427
H86_XANTPE	*Xanthophyllum sp.*	BABRU2569-15	MF435842	MF419060	22-1076
H87_XANTRU[s]	*Xanthophyllum sp.*	BABRU3582-15	MF435849	MF435895	20-3818
H88_XANTST	*Xanthophyllum sp.*	BABRU1723-15	MF435850	MF419062	25-1831
H89_CASTSP	*Castanopsis sp.*	BABRU2352-15	MF435851	n/a	19-0271
H90_CASTMO	*Castanopsis sp.*	BABRU2947-15	MF435852	MF419148	24-3907
H91a_LITHCL	*Lithocarpus sp.*	BABRU1365-15	MF435855	n/a	13-3482
H91b_LITHNI	*Lithocarpus sp.*	BABRU1307-15	MF435854	n/a	18-3132
H92_LITHTE1	*Lithocarpus sp.*	BABRU361-14	MF435856	MF419150	12-5331
H93_LITHTE2	*Lithocarpus sp.*	BABRU1770-15	MF435857	MF419149	25-1879
H94_LITHLE	*Lithocarpus sp.*	BABRU1071-14	MF435853	MF419151	01-3878
H95_DYERCO	*Dyera costulata*	BABRU3566-15	MF435858	MF418719	20-3801
H96_TABEAN	*Tabernaemontana sp.*	BABRU077-14	MF435860	MF418720	09-3667
H97_TABEPA	*Tabernaemontana sp.*	BABRU1610-15	MF435861	MF418721	20-2947
H98_NORRMA	*Norrisia sp.*	BABRU1246-14	MF435862	MF418757	11-4109
H99_UTANSP	Loganiaceae	BABRU856-14	MF435863	MF419174	06-5362
H100_RUBIX2	*Rubiaceae sp.*	BABRU898-14	MF435283	MF418728	04-4078
H101_RUBIX1	*Rubiaceae sp.*	BABRU2058-15	MF435864	MF418722	22-0348
H102_AIDISP1	*Rubiaceae sp.*	BABRU881-14	MF435865	MF418723	04-4027
H103_AIDISP2	*Rubiaceae sp.*	BABRU1395-15	MF435866	MF418724	13-3513
H104_CANTCO	*Rubiaceae sp.*	BABRU079-14	MF435867	MF418730	09-3669
H105_DISCSP	*Rubiaceae sp.*	BABRU1095-14	MF435868	MF418725	01-3921
H106_GAERVA	*Gaertnera sp.*	BABRU2334-15	MF435869	MF418749	19-0253
H107_GARDEL	*Gardenia sp.*	BABRU2143-15	MF435281	MF418726	15-0391
H108_GARDLO	*Gardeniopsis longifolia*	BABRU3392-15	MF435282	MF418743	20-3623
H109_IXORSP3	*Ixora sp.*	BABRU2433-15	MF435288	MF418735	25-0326
H110_IXORSP1	*Ixora sp.*	BABRU1562-15	MF435284	MF418731	20-2897
H111_IXORSP2	*Ixora sp.*	BABRU085-14	MF435285	MF418734	09-3675
H112_IXORGL	*Ixora sp.*	BABRU1072-14	MF435286	MF418733	01-3879
H113_IXORPU	*Ixora sp.*	BABRU090-14	MF435287	MF418732	09-3681
H114_LASIRO	*Lasianthus sp.*	BABRU4076-15	MF435810	MF435877	24-5772
H115_NEONSP	*Neonauclea sp.*	BABRU1229-14	MF435289	MF418736	11-4085
H116_NEONSU	*Neonauclea sp.*	BABRU3578-15	MF435290	MF418737	20-3814
H117_PLEISP	Rubiaceae	BABRU1265-14	MF435811	MF435878	11-4132
H118_PARVSP	Rubiaceae	BABRU3756-15	MF435292	MF418738	20-5440
H119_PORTCA	*Porterandia sp.*	BABRU221-14	MF435293	MF418727	14-4342
H120a_PRISSP	*Prismatomeris sp.*	BABRU4200-15	MF435294	MF418744	24-5905
H120b_PRISBE	*Prismatomeris sp.*	BABRU4198-15	MF435295	MF418745	24-5903
H121_PSYCSP	*Psychotria sp.*	BABRU3315-15	MF435296	MF418747	16-4623
H122_RENNEL	Rubiaceae	BABRU3579-15	MF435297	MF418746	20-3815
H123_SAPRSP	*Saprosma sp.*	BABRU1494-15	MF435298	MF418748	16-2471
H124_TARESP1	*Tarenna sp.*	BABRU1589-15	MF435299	MF418729	20-2925
H125_TARESP2	*Tarenna sp.*	BABRU3913-15	MF435300	n/a	24-5586
H126_UROPS2	*Urophyllum sp.*	BABRU4125-15	MF435304	MF418742	24-5821
H127_UROPS1	*Urophyllum sp.*	BABRU4097-15	MF435302	MF418741	24-5793
H128_UROPSP3	*Urophyllum sp.*	BABRU2572-15	MF435305	MF418740	22-1079
H129_UROPSP4	*Urophyllum sp.*	BABRU838-14	MF435306	n/a	07-5773
H130_UROPSP5	*Urophyllum sp.*	BABRU2810-15	MF435307	n/a	16-2909
H131_UROPCO	*Urophyllum sp.*	BABRU202-14	MF435301	MF418739	14-4323
H132_UROPWO	*Urophyllum sp.*	BABRU4041-15	MF435308	n/a	24-5736
H133_GNETGN	*Gnetum sp.*	BABRU2567-15	MF435309	n/a	22-1074
H134_TEIJCO	Lamiaceae	BABRU440-14	MF435310	MF418711	09-4203
H135_TEIJSI	Lamiaceae	BABRU1683-15	MF435311	MF418712	22-1834
H136_VITEVI	*Vitex sp.*	BABRU1930-15	MF435312	MF418713	16-1749
H137_CHIOSP4	*Oleaceae sp.*	BABRU2331-15	MF435313	MF418714	19-0250
H138_OLEASP	*Oleaceae sp.*	BABRU2992-15	MF435315	MF418715	24-3952
H139_ALSEBA	Lauracee	BABRU3775-15	MF435319	MF419083	20-5461
H140_ACTISP	*Actinodaphne sp.*	BABRU2765-15	MF435318	MF419066	16-3019

H141_ACTIBO	*Actinodaphne sp*	BABRU2024-15	MF435316	MF419065	19-1945
H142_ACTIPR	*Actinodaphne sp*	BABRU3546-15	MF435317	MF419082	20-3781
H143_BEILGL	*Beilschmiedia sp.*	BABRU1036-14	MF435321	n/a	01-3840
H144_BEILTA	*Beilschmiedia sp.*	BABRU888-14	MF435320	MF419086	04-4061
H145_CINNJA	Lauraceae	BABRU3728-15	MF435322	MF419081	20-5404
H146_CRYPER	*Cryptocarya sp.*	BABRU1967-15	MF435324	n/a	16-1790
H147_CRYPTO	*Cryptocarya sp.*	BABRU356-14	MF435323	MF419088	12-5325
H148_ENDICO	*Endiandra sp.*	BABRU3845-15	MF435326	MF419087	20-5554
H149_LITSSP6	*Litsea sp.*	BABRU3299-15	MF435331	n/a	16-4606
H150a_LITSSP9	*Litsea sp.*	BABRU3161-15	MF435334	MF419070	16-4462
H150b_LITSSP10	*Litsea sp.*	BABRU1824-15	MF435335	MF419076	23-3282
H150c_LITSFU	*Litsea sp.*	BABRU1322-15	MF435336	MF419071	18-3149
H150d_LITSLA	*Litsea sp.*	BABRU1510-15	MF435337	MF419072	16-2488
H151_LITSSP8	*Litsea sp.*	BABRU2007-15	MF435333	MF419069	19-1928
H152_LITSSP7	*Litsea sp.*	BABRU1926-15	MF435332	MF419085	16-1745
H153_LITSAC1	*Litsea sp.*	BABRU1188-14	MF435340	MF419077	04-4944
H154_LITSAC2	*Litsea sp.*	BABRU2353-15	MF435341	MF419079	19-0272
H155_LITSCA	*Litsea sp.*	BABRU2615-15	MF435342	MF419074	22-1125
H156_LITSCO	*Litsea sp.*	BABRU1839-15	MF435343	MF419075	13-1841
H157_LITSFE	*Litsea sp.*	BABRU4004-15	MF435344	MF435874	24-5692
H158a_LITSME	*Litsea sp.*	BABRU3842-15	MF435812	MF435880	20-5551
H158b_LITSAN	*Litsea sp.*	BABRU371-14	MF435338	MF435879	08-5660
H159_LITSRU	*Litsea rubicunda*	BABRU4161-15	MF435345	MF419080	24-5864
H160_LITSSP1	*Litsea sp.*	BABRU3859-15	MF435327	MF419067	20-5568
H161_LITSSP2	*Litsea sp.*	BABRU3472-15	MF435328	MF419073	20-3704
H162_LITSSP3	*Litsea sp.*	BABRU3722-15	MF435329	MF419068	20-5393
H163_LITSSP4	*Litsea sp.*	BABRU3944-15	n/a	MF419078	24-5621
H164_LITSSP5	*Litsea sp.*	BABRU941-14	MF435330	n/a	04-4619
H165_PHOEGR	Lauraceae	BABRU457-14	MF435346	MF419084	08-6445
H167_PROTME	*Potoxylon melagangai*	BABRU830-14	MF435347	MF419089	03-5567
H168_ANNOX1	*Annonaceae sp.*	BABRU820-14	MF435348	MF419093	01-5545
H169_ANNOX2	*Annonaceae sp.*	BABRU824-14	MF435349	MF419117	01-5549
H170_ANNOX3	*Annonaceae sp.*	BABRU805-14	MF435350	MF419094	01-5526
H171_ANNOX4	*Annonaceae sp.*	BABRU3017-15	MF435352	MF419097	24-3979
H172_ANNOX5	Annonaceae	BABRU1913-15	MF435388	MF419101	25-2695
H173_ANNOX6	Annonaceae	BABRU064-14	MF435389	MF419102	09-3654
H174_XYLOMA	*Xylopia sp.*	BABRU2532-15	MF435387	MF419123	13-0920
H175_DREPHA	Annonaceae	BABRU214-14	MF435353	MF419121	14-4335
H176_GONISP	*Goniothalamus sp.*	BABRU309-14	MF435356	MF419125	12-5725
H177_GONIME	*Goniothalamus sp.*	BABRU2627-15	MF435354	MF419124	22-1137
H178_GONIPA	*Goniothalamus sp.*	BABRU2204-15	MF435355	MF419126	16-0872
H179_GONITA	*Goniothalamus sp.*	BABRU025-14	MF435357	MF419128	05-5376
H180_GONIVE	*Goniothalamus sp.*	BABRU2543-15	MF435358	MF419127	22-1049
H181_HUBERU	Annonaceae	BABRU3448-15	MF435359	MF419118	20-3680
H182_MAASGL	*Maasia sp.*	BABRU172-14	MF435360	MF419120	13-6069
H183_MAASSU	*Maasia sp.*	BABRU1334-15	MF435361	MF419119	13-3449
H184_MEIOSP	Annonaceae	BABRU2417-15	MF435363	MF419099	25-0309
H185_MEZZMA	*Mezzettia sp.*	BABRU1381-15	MF435364	MF419122	13-3498

H186_MITRLO	Annonaceae	BABRU3840-15	MF435365	MF419106	20-5549
H187_MITRMA	Annonaceae	BABRU1106-14	MF435366	MF419100	03-5083
H188_MONOEU	*Monocarpia sp.*	BABRU2441-15	MF435367	MF419105	25-0335
H189_MONOLA	Annonaceae	BABRU4169-15	MF435368	MF419096	24-5872
H190_NEOUPR	*Neo-uvaria sp.*	BABRU3822-15	MF435369	MF419098	20-5521
H191_PHAESP	Annonaceae	BABRU3480-15	MF435370	MF419095	20-3712
H192_PHAEOP	*Phaeanthus sp.*	BABRU2265-15	MF435371	MF419116	24-0512
H193_POLYSP1	*Polyalthia sp.*	BABRU3347-15	MF435372	MF419109	16-4655
H194_POLYSP3	*Polyalthia sp.*	BABRU4148-15	MF435378	MF419115	24-5851
H195_POLYSP5	*Polyalthia sp.*	BABRU3397-15	MF435379	MF419112	20-3628
H196_POLYBO	*Polyalthia sp.*	BABRU840-14	MF435374	MF419111	06-5345
H197_POLYBU	*Polyalthia sp.*	BABRU1065-14	MF435375	MF419110	01-3871
H198_POLYCA1	*Polyalthia sp.*	BABRU2220-15	MF435376	MF419114	16-0889
H199_POLYCA2	*Polyalthia sp.*	BABRU148-14	MF435377	MF419113	13-6045
H200a_POPOHI	*Popowia*	BABRU1800-15	MF435381	MF419107	23-3256
H200b_POPOPI	*Popowia*	BABRU1830-15	MF435382	MF419108	23-3288
H201_POPOSP1	*Popowia sp.*	BABRU2654-15	MF435380	MF435875	16-2970
H202_SAGELA	*Sageraea*	BABRU3419-15	MF435384	MF419103	20-3650
H203_SAGESA	*Sageraea*	BABRU2628-15	MF435385	MF419104	22-1138
H204_UVARSXI	*Uvaria sp.*	BABRU313-14	MF435386	MF419129	12-5280
H205_MAGNS1	*Magnolia sp.*	BABRU432-14	MF435390	MF419090	09-4194
H206_MAGNAS	*Magnolia sp.*	BABRU424-14	MF435391	MF419092	09-4186
H207_MAGNGI	*Magnolia sp.*	BABRU345-14	MF435392	MF419091	12-5313
H208_GYMNFA	*Gymnacranthera sp.*	BABRU4084-15	MF435400	n/a	24-5780
H209_GYMNFO	*Gymnacranthera sp.*	BABRU4205-15	MF435401	MF419131	24-5910
H210_HORSGR	*Horsfieldia sp.*	BABRU239-14	MF435402	MF419142	12-4139
H211_HORSFL	*Horsfieldia sp.*	BABRU3696-15	MF435403	MF419143	20-5358
H212_KNEMS1	*Knema sp.*	BABRU2187-15	MF435404	MF419134	16-0855
H213_KNEMS2	*Knema sp.*	BABRU3072-15	MF435405	MF419138	24-4037
H214_KNEMS3	*Knema sp.*	BABRU3360-15	MF435406	MF419135	16-4669
H215_KNEMS6	*Knema sp.*	BABRU949-14	MF435410	n/a	04-4633
H216_KNEMS7	*Knema sp.*	BABRU819-14	MF435399	MF419133	01-5544
H217_KNEMS4	*Knema sp.*	BABRU2516-15	MF435408	MF419136	13-0899
H218_KNEMS5	*Knema sp.*	BABRU4191-15	MF435409	MF419141	24-5896
H219_KNEMSE	*Knema sp.*	BABRU842-14	MF435411	MF419137	06-5347
H220_MYRXS1	*Myristicaceae sp.*	BABRU1672-15	MF435393	MF419130	22-1821
H221_MYRXS2	*Myristicaceae sp.*	BABRU2228-15	MF435394	MF419132	16-0898
H222_MYRXSIII	*Myristica sp.*	BABRU155-14	MF435398	MF419140	13-6052
H223_MYRIS4	*Myristica sp.*	BABRU3697-15	MF435397	MF419139	20-5360
H224_HYDNS1	*Hydnocarpus sp.*	BABRU4109-15	MF435412	MF418945	24-5805
H225a_HYDNS2	*Hydnocarpus sp.*	BABRU872-14	MF435414	MF435881	04-4011
H225b_HYDNBO	*Hydnocarpus sp.*	BABRU3110-15	MF435415	MF418949	24-4075
H225c_HYDNSB	*Hydnocarpus sp.*	BABRU2726-15	MF435416	MF418950	16-3099
H225d_HYDNSM	*Hydnocarpus sp.*	BABRU1722-15	MF435417	MF418951	22-1874
H226_HYDNKU	*Hydnocarpus sp.*	BABRU3189-15	MF435413	MF418946	16-4492
H227_HYDNPI	*Hydnocarpus sp.*	BABRU3119-15	MF435418	MF418948	16-4419
H228_HYDNWO	*Hydnocarpus sp.*	BABRU896-14	MF435419	MF418947	04-4070
H229a_RYPAS1	Achariaceae	BABRU1018-14	MF435420	MF418952	01-3822

H229b_RYPAQU	Achariaceae	BABRU1498-15	MF435421	MF418954	16-2475
H229c_RYPAHU	Achariaceae	BABRU125-14	MF435422	MF418953	10-5215
H230d_RYPAHI	Achariaceae	BABRU427-14	MF435423	MF435882	09-4189
H231_CALLS3	*Calophyllum sp.*	BABRU2431-15	MF435427	MF419157	25-0324
H232_CALLS1	*Calophyllum sp.*	BABRU2574-15	MF435425	MF419155	22-1081
H233_CALLS2	*Calophyllum sp.*	BABRU807-14	MF435426	MF419158	01-5528
H234_CALLDE	*Calophyllum sp.*	BABRU2691-15	MF435428	MF419160	16-3063
H235_CALLFE	*Calophyllum sp.*	BABRU2378-15	MF435429	MF419156	25-0009
H236_CALLWO	*Calophyllum sp.*	BABRU2930-15	MF435430	MF419159	24-3890
H237_KAYES1	*Kayea sp.*	BABRU3178-15	MF435431	MF419161	16-4479
H238_KAYEFE	*Kayea sp.*	BABRU2633-15	MF435432	MF419162	16-2949
H239_KAYEMA	*Kayea sp.*	BABRU3420-15	MF435433	MF419163	20-3651
H240_KAYEOB	*Kayea sp.*	BABRU3824-15	MF435434	MF419164	20-5527
H241_BHESPA	*Bhesa paniculata*	BABRU4163-15	MF435436	MF419020	24-5866
H242_ATUNRA	*Atuna sp.*	BABRU1050-14	MF435803	MF435883	01-3854
H243_PARIEL	*Parinari sp.*	BABRU1225-14	MF435805	MF435884	04-5020
H244_KOSTHE	*Kostermanthus sp.*	BABRU3399-15	MF435814	n/a	20-3630
H245_GARCSPI	*Garcinia sp.*	BABRU2795-15	MF435437	MF419166	16-2894
H246_GARCSP2	*Garcinia sp.*	BABRU1517-15	MF435438	MF419172	20-2851
H247_GARCS7	*Garcinia sp.*	BABRU1968-15	MF435443	MF419170	19-1887
H248_GARCS4	*Garcinia sp.*	BABRU1114-14	MF435440	MF419167	03-5091
H249_GARCS5	*Garcinia sp.*	BABRU2181-15	MF435441	MF435876	15-0431
H250_GARCS6	*Garcinia sp.*	BABRU2814-15	MF435442	MF419168	16-2913
H251_GARCSP3	*Garcinia sp.*	BABRU3492-15	MF435439	MF419169	20-3725
H252_GARCHA	*Garcinia sp.*	BABRU308-14	MF435444	MF419173	12-5274
H253_GARCLA	*Garcinia sp.*	BABRU1569-15	MF435445	n/a	20-2904
H254_GARCMA	*Garcinia sp.*	BABRU2236-15	MF435808	n/a	16-0908
H255_GARCSA	*Garcinia sp.*	BABRU1565-15	MF435447	MF419171	20-2900
H256_CTENPA	*Ctenolophon sp.*	BABRU1463-15	MF435448	MF418992	16-2437
H257a_BLUMBO	*Blumeodendron sp.*	BABRU2138-15	MF435449	MF418958	15-0385
H257b_BLUMCA	*Blumeodendron sp.*	BABRU2474-15	MF435450	MF418959	23-0184
H258_BLUMKU	*Blumeodendron sp.*	BABRU2194-15	MF435451	MF418961	16-0862
H259_BLUMSU	*Blumeodendron sp.*	BABRU3523-15	MF435452	MF418960	20-3757
H260_BLUMTO	*Blumeodendron sp.*	BABRU1404-15	MF435453	MF418962	13-3522
H261_CEPHMA	*Cephalomappa sp.*	BABRU2458-15	MF435815	MF418982	25-0352
H262_CHAECA	*Chaetocarpus castanocarpus*	BABRU2929-15	MF435454	MF418984	24-3889
H263_CHEIMO	*Cheilosa montana*	BABRU1055-14	MF435455	MF418955	01-3859
H264_ELATTA	*Elateriospermum tapos*	BABRU2013-15	MF435456	MF418977	19-1934
H265_ERISLE	*Erismanthus leembruggianus*	BABRU1123-14	MF435457	MF418978	03-5100
H266_HANCEU	*Hancea eucaustus*	BABRU408-14	MF435459	MF418976	09-4167
H267_KOILLO	*Koilodepas sp.*	BABRU453-14	MF435460	MF418983	08-6441
H268a_MACASP	*Macaranga sp.*	BABRU3916-15	MF435461	MF418964	24-5589
H268b_MACAHU	*Macaranga sp.*	BABRU3699-15	MF435462	MF418965	20-5363
H268c_MACALA	*Macaranga sp.*	BABRU2771-15	MF435463	MF418966	16-3025

H268_MACATR	*Macaranga sp.*	BABRU1864-15	MF435464	MF418967	13-1868
H269_MACABE	*Macaranga sp.*	BABRU1917-15	MF435465	MF418968	25-2701
H270a_MACABR	*Macaranga sp.*	BABRU892-14	MF435466	MF418969	04-4066
H270b_MACAHO	*Macaranga sp.*	BABRU3431-15	MF435467	MF418971	20-3663
H270c_MACALO	*Macaranga sp.*	BABRU3837-15	MF435468	MF418970	20-5546
H271_MALLS1	*Mallotus sp.*	BABRU2241-15	MF435469	MF418972	16-0914
H272_MALLS2	*Mallotus sp.*	BABRU1651-15	MF435470	MF418974	22-2146
H273_MALLLE	*Mallotus sp.*	BABRU1166-14	MF435471	MF418975	04-4870
H274_MALLWR	*Mallotus sp.*	BABRU431-14	MF435472	MF418973	09-4193
H275_NEOSKI	*Neoscortechinia sp.*	BABRU1885-15	MF435473	MF418956	13-1890
H276_NEOSSU	*Neoscortechinia sp.*	BABRU3411-15	MF435474	MF418957	20-3642
H277_PIMEGR1	*Pimelodendron sp.*	BABRU3569-15	MF435476	MF418979	20-3804
H278_PIMEGR2	*Pimelodendron sp.*	BABRU141-14	MF435477	MF418980	10-5232
H279_PIMEGR3	*Pimelodendron sp.*	BABRU415-14	MF435478	MF418981	09-4177
H280_PTYCAR	Euphorbiaceae	BABRU2057-15	MF435479	MF418963	22-0347
H281_TRIGMA	*Trigonopleura malayana*	BABRU3275-15	MF435480	MF418985	16-4582
H282_CRATFO	*Cratoxylum sp.*	BABRU3368-15	MF435481	MF419165	16-4679
H283_GALEFU	*Galearia sp.*	BABRU3706-15	MF435482	MF418986	20-5373
H284_CARRSP	*Carralia sp.*	BABRU1425-15	MF435483	MF419002	13-2647
H285_ANTISP1	*Antidesma sp.*	BABRU920-14	MF435484	n/a	04-4494
H286_ANTILI	*Antidesma sp.*	BABRU1593-15	MF435489	MF419005	20-2929
H287_ANTISP2	*Antidesma sp.*	BABRU379-14	MF435485	MF419003	08-5671
H288_ANTILE	*Antidesma sp.*	BABRU446-14	MF435488	MF419004	08-6434
H289_ANTIST	*Antidesma sp.*	BABRU3801-15	MF435490	n/a	20-5493
H290_APORS3	*Aporosa sp.*	BABRU2348-15	MF435494	MF419009	19-0267
H291_APORS1	*Aporosa sp.*	BABRU4065-15	MF435491	n/a	24-5761
H292_APORS2	*Aporosa sp.*	BABRU3694-15	MF435493	MF419012	20-5356
H293_APORS4	*Aporosa sp.*	BABRU4185-15	MF435495	MF419007	24-5890
H294_APORAC	*Aporosa sp.*	BABRU3220-15	MF435492	MF419006	16-4523
H295_APORBE	*Aporosa sp.*	BABRU2537-15	MF435498	MF419010	22-1043
H296_APORBU	*Aporosa sp.*	BABRU3663-15	MF435496	n/a	20-5323
H297_APORSA	*Aporosa sp.*	BABRU2060-15	MF435496	MF419008	22-0350
H298_APORSU1	*Aporosa sp.*	BABRU1485-15	MF435500	MF419011	16-2460
H299_APORSU2	*Aporosa sp.*	BABRU1308-15	MF435501	n/a	18-3133
H300_BACCS1	*Baccaurea sp.*	BABRU4003-15	MF435502	MF419013	24-5691
H301_BACCS2	*Baccaurea sp.*	BABRU2358-15	MF435507	MF419017	19-0277
H302a_BACCS3	*Baccaurea sp.*	BABRU2501-15	MF435503	MF419014	13-0884
H302b_BACCRA	*Baccaurea sp.*	BABRU2439-15	MF435505	MF419016	25-0333
H303_BACCCO	*Baccaurea sp.*	BABRU024-14	MF435506	MF419019	05-5375
H304_BACCLA	*Baccaurea sp.*	BABRU3530-15	MF435508	MF419015	20-3764
H305_BACCPY	*Baccaurea sp.*	BABRU4017-15	MF435509	MF419018	24-5706
H306_BACCSA	*Baccaurea sp.*	BABRU1281-15	MF435504	n/a	18-3104
H307_CLEIHI	*Cleisthanthus sp.*	BABRU2493-15	MF435510	MF419021	13-0876
H308_GLOCSP	*Glochidion sp.*	BABRU2273-15	MF435513	MF419023	24-0521
H309_PHYLSP	*Cleisthanthus sp.*	BABRU1754-15	MF435511	MF419022	25-1863

H310_DRYSP1	*Drypetes sp.*	BABRU1637-15	MF435515	MF418991	22-2130
H311_DRYPER	*Drypetes sp.*	BABRU901-14	MF435514	MF418987	04-4081
H312_DRYPFA	*Drypetes sp.*	BABRU050-14	MF435516	MF435872	05-5404
H313_DRYPLO	*Drypetes sp.*	BABRU1874-15	n/a	MF418990	13-1878
H314_DRYPMY	*Drypetes sp.*	BABRU2381-15	n/a	MF418989	25-0012
H315_DRYPPO	*Drypetes sp.*	BABRU868-14	MF435517	MF418988	04-4007
H316_CASEGR	*Casearia sp.*	BABRU4216-15	MF435518	MF419001	24-5921
H317_FLACRU	*Flacourtia sp.*	BABRU854-14	MF435519	MF418998	06-5360
H318_HOMAIN	*Homalium sp.*	BABRU2404-15	MF435523	MF419000	25-0035
H319_SCOLSP	*Scolopia spinosa*	BABRU134-14	MF435524	MF418999	10-5225
H320_RINOS1	*Rinorea sp.*	BABRU2949-15	MF435525	MF418993	24-3909
H321_RINOS2	*Rinorea sp.*	BABRU2166-15	MF435526	MF418994	15-0414
H322_RINOS3	*Rinorea sp.*	BABRU1738-15	MF435529	MF418995	25-1847
H323_RINOHO	*Rinorea sp.*	BABRU815-14	MF435527	MF418997	01-5540
H324_RINOLO	*Rinorea sp.*	BABRU3086-15	MF435528	MF418996	24-4051
H325_ANISLA	*Anisoptera sp.*	BABRU1400-15	MF435530	MF418805	13-3518
H326_DIPTCASP	*Dipterocarpus sp.*	BABRU1378-15	MF435531	MF418855	13-3495
H327a_DIPTPASB	*Dipterocarpus sp.*	BABRU1571-15	MF435532	MF418856	20-2907
H327b_DIPTCA	*Dipterocarpus sp.*	BABRU2178-15	MF435533	MF418857	15-0427
H328_DRYOBE	*Dryobalanops sp.*	BABRU229-14	MF435535	MF418853	14-4350
H329_DRYOLA	*Dryobalanops sp.*	BABRU810-14	MF435536	MF418854	01-5535
H330_HOPEBR	*Hopea sp.*	BABRU1357-15	MF435537	MF418847	13-3473
H331_HOPDR	*Hopea sp.*	BABRU2282-15	MF435538	MF418849	24-0530
H332_HOPDR1	*Hopea sp.*	BABRU300-14	MF435539	MF418850	12-5263
H333_HOPENE	*Hopea sp.*	BABRU1161-14	MF435540	MF418848	04-4865
H334_PARAMAC	*Parashorea*	BABRU4229-17	MF435809	MF435889	24-4723
H335_PARAMA	*Parashorea*	BABRU2401-15	MF435541	MF418815	25-0032
H336_PARATO	*Parashorea*	BABRU3603-15	MF435542	MF418814	20-3841
H337a_SHORSP1	*Shorea sp.*	BABRU3446-15	MF435545	MF418845	20-3678
H337b_SHORFAGUE_2	*Shorea sp.*	BABRU3720-15	MF435549	MF418841	20-5391
H338_SHORSP2	*Shorea sp.*	BABRU304-14	MF435551	MF418843	12-5270
H339a_SHORPAL	*Shorea sp.*	BABRU3870-15	MF435578	n/a	20-5582
H339b_SHORPAU	*Shorea sp.*	BABRU2573-15	MF435579	n/a	22-1080
H340_SHORSP2	*Shorea sp.*	BABRU967-14	MF435548	MF418840	04-4651
H341_SHORSP4	*Shorea sp.*	BABRU1609-15	MF435565	MF418844	20-2946
H342_SHORSP5	*Shorea sp.*	BABRU1096-14	MF435570	MF418838	01-3922
H343_SHORSP9	*Shorea sp.*	BABRU2833-15	MF435580	MF418835	16-2932
H344a_SHOAG	*Shorea sp.*	BABRU4230-17	MF435801	MF435888	05-4419
H344b_SHORCO	*Shorea sp.*	BABRU4231-17	MF435557	MF418851	25-4813
H345_SHORAM	*Shorea sp.*	BABRU2097-15	MF435553	MF418837	15-0344
H346_SHORBA	*Shorea sp.*	BABRU053-14	MF435544	MF418839	05-5407

H347_SHORBECC	*Shorea sp.*	BABRU3151-15	MF435543	MF418825	16-4452
H348_SHORBI	*Shorea sp.*	BABRU3881-15	MF435555	MF418823	20-5597
H349_SHOREX	*Shorea sp.*	BABRU376-14	MF435554	MF418821	08-5668
H350_SHORASA	*Shorea sp.*	BABRU1414-15	MF435552	MF418816	13-2636
H351_SHORFAGT	*Shorea sp.*	BABRU2601-15	n/a	MF435887	22-1109
H352_SHORFAGU_1	*Shorea sp.*	BABRU3883-15	MF435556	MF418842	20-5601
H353_SHORDO	*Shorea sp.*	BABRU355-14	MF435558	MF418822	12-5324
H354_SHORFA	*Shorea sp.*	BABRU2962-15	MF435559	MF418834	24-3922
H355_SHORHA1	*Shorea sp.*	BABRU2753-15	MF435568	MF418818	16-3007
H356_SHORHA2	*Shorea sp.*	BABRU2783-15	MF435569	MF418819	16-3040
H357_SHORAT	*Shorea sp.*	BABRU966-14	MF435563	MF418824	04-4650
H358a_SHORLE	*Shorea sp.*	BABRU4048-15	MF435571	MF418829	24-5744
H358b_SHORSP6	*Shorea sp.*	BABRU4232-17	MF435573	MF418831	19-1085
H358c_SHORSC	*Shorea sp.*	BABRU4043-15	MF435572	MF418828	24-5738
H358d_SHORFE	*Shorea sp.*	BABRU1478-15	MF435574	MF418832	16-2452
H358e_SHORMASB	*Shorea sp.*	BABRU2933-15	MF435575	MF418827	24-3893
H359_SHORMASB2	*Shorea sp.*	BABRU004-14	MF435576	MF418833	05-5346
H360_SHORMASM	*Shorea sp.*	BABRU3358-15	MF435577	MF418830	16-4666
H361_SHORMA	*Shorea sp.*	BABRU1545-15	MF435560	MF418817	20-2879
H362_SHORGI	*Shorea sp.*	BABRU2774-15	MF435560	MF418846	16-3031
H363_SHOROB	*Shorea sp.*	BABRU4035-15	MF435562	MF418820	24-5730
H364_SHOROC	*Shorea sp.*	BABRU1627-15	MF435564	MF418852	22-2120
H365_SHORPARSVE	*Shorea sp.*	BABRU1916-15	MF435566	MF418826	25-2700
H366_SHORPAT	*Shorea sp.*	BABRU984-14	MF435547	n/a	04-4670
H367_SHORPI	*Shorea sp.*	BABRU327-14	MF435567	MF418836	12-5295
H568_SHORLA	*Shorea sp.*	BABRU4233-17	MF435807	MF435886	05-3814
H369_VATISP2	*Vatica sp.*	BABRU4196-15	MF435587	MF418812	24-5901
H370_VATIDU	*Vatica sp.*	BABRU1355-15	MF435583	MF418813	13-3471
H371_VATIEN	*Vatica sp.*	BABRU1275-14	MF435582	MF418806	04-4496
H372_VATIMI	*Vatica sp.*	BABRU1629-15	MF435584	MF418807	22-2122
H373_VATINI	*Vatica sp.*	BABRU1695-15	MF435589	MF418809	22-1846
H374_VATIOBOB	*Vatica sp.*	BABRU2114-15	MF435585	MF418810	15-0361
H375_VATIOD	*Vatica sp.*	BABRU4179-15	MF435586	MF418811	24-5883
H376_VATISA	*Vatica sp.*	BABRU3619-15	MF435588	MF418808	20-5275
H377_DURIS	*Durio sp.*	BABRU2626-15	MF435594	MF418785	22-1136
H378_DURIGRA	*Durio sp.*	BABRU1783-15	MF435590	n/a	25-1893
H379_DURIGR	*Durio sp.*	BABRU1581-15	MF435591	MF418784	20-2917
H380_DURIGRI	*Durio sp.*	BABRU1590-15	MF435593	KU519689	20-2926
H381_HERISI	*Heritiera sp.*	BABRU2793-15	MF435595	MF418791	16-2892
H382_LEPTCA	*Leptonychia sp.*	BABRU3323-15	MF435596	MF418796	16-4631
H383_MICRBL	*Microcos sp.*	BABRU3416-15	MF435597	MF418797	20-3647

H84_MICRFI	*Microcos sp.*	BABRU870-14	MF435601	MF418800	04-4009
H385a_MICRHI	*Microcos sp.*	BABRU153-14	MF435598	MF418799	13-6050
H385b_MICRLA	*Microcos sp.*	BABRU1907-15	MF435600	MF418802	25-2685
H385c_MICRCI	*Microcos sp.*	BABRU1299-15	MF435599	MF418803	18-3123
H386a_MICROS	*Microcos sp.*	BABRU3680-15	MF435602	MF418798	20-5342
H386b_MICRST	*Microcos sp.*	BABRU260-14	MF435603	MF418801	12-4160
H387_PENTAD	*Pentace sp.*	BABRU1058-14	MF435626	MF418794	01-3862
H388_PTERSU	*Pterospermum sp.*	BABRU2480-15	MF435604	MF418795	23-0192
H389_SCAPAL	*Scaphium sp.*	BABRU1479-15	MF435605	MF418793	16-2453
H390_SCAPMA	*Scaphium sp.*	BABRU2362-15	MF435607	MF418792	19-0281
H391_STERS	*Sterculia* sp.	BABRU381-14	MF435609	MF435885	09-4140
H392_STERCO	*Sterculia* sp.	BABRU341-14	MF435611	MF418790	12-5309
H393a_STERRY	*Sterculia* sp.	BABRU1443-15	MF435608	MF41878	13-2666
H393b_STERS1	*Sterculia* sp.	BABRU130-14	MF435610	MF418789	10-5220
H394_STERRU	*Sterculia* sp.	BABRU1411-15	MF435613	MF418787	13-2633
H395_STERST	*Sterculia* sp.	BABRU2118-15	MF435612	MF418788	15-0365
H396_AQUILBE	*Aquilaria sp.*	BABRU2425-15	MF435614	MF418804	25-0317
H397_AQILMI	*Aquilaria sp.*	BABRU3463-15	MF435615	n/a	20-3695
H398_GONYSP	*Gonystylus sp.*	BABRU430-14	MF435616	n/a	09-4192
H399_CRYPMA	*Crypteronia sp.*	BABRU1619-15	MF435617	n/a	22-2112
H400_LIJNLA	Melastomataceae	BABRU3303-15	MF435619	MF419175	16-4611
H401_MEMEAR	*Memecylon sp.*	BABRU413-14	MF435620	MF419176	09-4172
H402_MEMECA	*Memecylon sp.*	BABRU1104-14	MF435804	n/a	03-5081
H403_MEMEFL	*Memecylon sp.*	BABRU1827-15	MF435621	n/a	23-3285
H404a_MEMELO	*Memecylon sp.*	BABRU173-14	MF435623	n/a	13-6070
H404b_MEMEPA	*Memecylon sp.*	BABRU3865-15	MF435624	n/a	20-5576
H405_MEMEME	*Memecylon sp.*	BABRU3648-15	MF435622	n/a	20-5306
H406_OXYSBE	Melastomataceae	BABRU1511-15	MF435625	MF419180	16-2489
H407a_PTERS1	*Pternandra sp.*	BABRU971-14	MF435627	n/a	04-4656
H407b_PTERCR	*Pternandra sp.*	BABRU2941-15	MF435628	n/a	24-3901
H408_PTERAZ	*Pternandra sp.*	BABRU2926-15	MF435631	MF419178	24-3886
H409_PTEREC	*Pternandra echinata*	BABRU456-14	MF435630	MF419179	08-6444
H410_PTERMU	*Pternandra sp.*	BABRU2457-15	MF435629	MF419177	25-0351
H411_SYZYSP	*Syzygium sp.*	BABRU480-14	MF435633	MF418783	11-5705
H412_SYZYS1	*Syzygium sp.*	BABRU2533-15	MF435653	MF418782	13-0921
H413_SYZYS3	*Syzygium sp.*	BABRU2454-15	MF435646	MF418767	25-0348
H414_SYZYS4	*Syzygium sp.*	BABRU1812-15	MF435654	MF418775	23-3269
H415_SYZYS8	*Syzygium sp.*	BABRU3595-15	MF435656	MF418774	20-3832
H416_SYZYS14	*Syzygium sp.*	BABRU2966-15	MF435634	MF418777	24-3926
H417_SYZYS17	*Syzygium sp.*	BABRU2012-15	MF435635	MF418773	19-1933

H418_SYZYS19	*Syzygium sp.*	BABRU017-14	MF435636	MF418766	05-5362
H419_SYZYS22	*Syzygium sp.*	BABRU3954-15	MF435637	MF418771	24-5636
H420_SYZYS23	*Syzygium sp.*	BABRU414-14	MF435638	MF418772	09-4173
H421_SYZYS25	*Syzygium sp.*	BABRU4213-15	MF435639	MF418768	24-5918
H422_SYZYS28	*Syzygium sp.*	BABRU3554-15	MF435640	n/a	20-3789
H423_SYZYS29	*Syzygium sp.*	BABRU2828-15	MF435645	MF418769	16-2927
H424_SYZYS30	*Syzygium sp.*	BABRU1211-14	MF435647	n/a	04-4997
H425_SYZYS32	*Syzygium sp.*	BABRU2217-15	MF435649	MF418770	16-0886
H426_SYZYS33	*Syzygium sp.*	BABRU3608-15	MF435650	MF418778	20-3846
H427_SYZYS34	*Syzygium sp.*	BABRU3487-15	MF435651	MF418780	20-3719
H428_SYZYS38	*Syzygium sp.*	BABRU2712-15	MF435652	MF418781	16-3085
H429a_SYZYS39	*Syzygium sp.*	BABRU131-14	MF435641	MF418776	10-5222
H429b_SYZYS40	*Syzygium sp.*	BABRU3784-15	MF435642	MF418764	20-5473
H429c_SYZYS41	*Syzygium sp.*	BABRU3650-15	MF435643	MF418763	20-5308
H429d_SYZYS15	*Syzygium sp.*	BABRU2308-15	MF435644	MF418765	19-0227
H430SYZYS42	*Syzygium sp.*	BABRU2716-15	MF435655	MF418779	16-3089
H431_CONAS1	*Connaraceae sp.*	BABRU3788-15	MF435657	MF418942	20-5477
H232_CONAS2	*Connaraceae sp.*	BABRU3233-15	MF435658	MF418943	16-4536
H433_ELAECL	*Elaeocarpus sp.*	BABRU1868-15	MF435661	n/a	13-1872
H434_ELAENI	*Elaeocarpus sp.*	BABRU1388-15	MF435662	MF418944	13-3506
H435_SLOAJA	*Sloanea sp.*	BABRU094-14	MF435663	n/a	09-3686
H436_HELIPE	*Helicia sp.*	BABRU3754-15	MF435664	n/a	20-5434
H437_MELISP	*Meliosma sp.*	BABRU946-14	MF435665	n/a	04-4629
H438_MELISU	*Meliosma sp.*	BABRU3379-15	MF435666	MF419144	20-3610
H439_GIRONE	*Gironniera sp.*	BABRU3210-15	MF435667	MF419043	16-4513
H440_GIROSU	*Gironniera sp.*	BABRU3725-15	MF435668	MF419044	20-5397
H441_ARTOS1	*Artocarpus sp.*	BABRU1980-15	MF435677	MF419030	19-1901
H442_ARTOS2	*Artocarpus sp.*	BABRU3987-15	MF435678	MF419031	24-5672
H443_ARTOAN	*Artocarpus sp.*	BABRU3737-15	MF435669	MF419025	20-5415
H444_ARTOIN	*Artocarpus sp.*	BABRU1563-15	MF435670	MF419032	20-2898
H445_ARTONI	*Artocarpus sp.*	BABRU2036-15	MF435671	MF419024	19-1962
H446_ARTOKE	*Artocarpus sp.*	BABRU1139-14	MF435673	MF419029	03-5117
H447_ARTOME	*Artocarpus sp.*	BABRU3868-15	MF435674	MF419026	20-5579
H448_ARTOOD1	*Artocarpus sp.*	BABRU2196-15	MF435675	MF419028	16-0864
H449_ARTOOD2	*Artocarpus sp.*	BABRU3869-15	MF435676	MF419027	20-5580
H450_ARTOTA	*Artocarpus sp.*	BABRU3925-15	MF435679	n/a	24-5599
H451_FICUSSP1	*Ficus sp.*	BABRU1228-14	MF435686	MF419037	11-4084
H452_FICUS2	*Ficus sp.*	BABRU4121-15	MF435687	MF419040	24-5817
H453_FICUS3	*Ficus sp.*	BABRU2284-15	MF435688	MF419038	24-0532
H454_FICUS4	*Ficus sp.*	BABRU853-14	MF435689	MF419035	06-5359
H455_FICUS5	*Ficus sp.*	BABRU1663-15	MF435690	MF419041	22-2159
H456_FICUS6	*Ficus sp.*	BABRU3981-15	MF435691	MF419042	24-5665
H457_FICUAU	*Ficus sp.*	BABRU1179-14	MF435680	MF419033	04-4931
H458a_FICUBE	*Ficus sp.*	BABRU373-14	MF435681	MF419036	08-5664
H458b_FICUME	*Ficus sp.*	BABRU448-14	MF435682	MF419036	08-6436
H459_FICUSE	*Ficus sp.*	BABRU1239-14	MF435683	MF419034	11-4096

H460_RHAMSP	*Ziziphus angustifolius*	BABRU788-14	MF435694	MF419046	01-5417
H461_ZIZIS1	*Ziziphus sp.*	BABRU1300-15	MF435695	MF419045	18-3124
H462_PRUNGR	*Prunus sp.*	BABRU3601-15	MF435806	n/a	20-3839
H463_ANACFR	*Anacolosa sp.*	BABRU883-14	MF435696	MF418750	04-4029
H464_OCHAAM	*Ochanostachys amentacea*	BABRU1108-14	MF435699	MF418663	03-5085
H465_STROCE	*Strombosia sp.*	BABRU1656-15	MF435698	KU519665	22-2151
H466_OIPLX	*Opiliaceae sp.*	BABRU3310-15	MF435700	MF418751	16-4618
H467_ANACSP	*Anacardiaceae sp.*	BABRU3921-15	MF435701	MF418858	24-5594
H468_BUCHSE	Anarcadiaceae	BABRU2451-15	MF435702	MF418860	25-0345
H469_CAMPAU	*Campnosperma sp.*	BABRU1230-14	MF435703	MF418859	11-4086
H470_DRYMLU	Anarcadiaceae	BABRU2563-15	MF435704	MF418880	22-1070
H471_GLUTLA	*Gluta sp.*	BABRU212-14	MF435705	MF418890	14-4333
H472_GLUTMA	Anarcadiaceae	BABRU4025-15	MF435707	n/a	24-5718
H473_GLUTRU	*Gluta sp.*	BABRU2359-15	MF435706	MF418891	19-0278
H474_GLUTWA	*Gluta sp.*	BABRU322-14	MF435708	MF418892	12-5289
H475a_MELASP	Anarcadiaceae	BABRU2629-15	MF435713	MF418883	16-2944
H475b_MELAS1	Anarcadiaceae	BABRU795-14	MF435714	MF418884	01-5516
H475c_MELAS2	Anarcadiaceae	BABRU3831-15	MF435715	MF418885	20-5539
H475d_MELABE	Anarcadiaceae	BABRU1811-15	MF435710	MF418881	23-3268
H475e_MELABU	Anarcadiaceae	BABRU3984-15	MF435711	MF418882	24-5669
H476_MELATO	Anarcadiaceae	BABRU119-14	MF435712	MF418889	10-5209
H477_PARISP	*Parishia sp.*	BABRU2736-15	MF435716	MF418888	16-3109
H478_SEMEEU	*Semecarpus sp.*	BABRU1943-15	MF435718	MF418887	16-1764
H479_SEMERU	*Semecarpus sp.*	BABRU3993-15	MF435717	MF418886	24-5680
H480_SWINFO	Anarcadiaceae	BABRU1311-15	MF435719	MF418893	18-3136
H481_SWINGL	Anarcadiaceae	BABRU3894-15	MF435720	n/a	20-5613
H482_BURSXXI	*Burseracea sp.*	BABRU2661-15	MF435721	MF418861	16-2977
H483_CANASP	Burseraceae	BABRU3862-15	MF435723	MF418878	20-5571
H484_CANASP2	Burseraceae	BABRU4212-15	MF435724	MF418876	24-5917
H485_CANALI	Burseraceae	BABRU1900-15	MF435722	MF418875	25-2677
H486_DACRCO	*Dacryodes sp.*	BABRU2127-15	MF435725	MF418863	15-0374
H487_DACRIN	*Dacryodes sp.*	BABRU3799-15	MF435727	MF418877	20-5491
H488_DACRLA	*Dacryodes sp.*	BABRU2598-15	MF435730	MF418864	22-1106
H489_DACRYRO1	*Dacryodes sp.*	BABRU1005-14	MF435732	MF418867	04-5263
H490_DACRYRO2	*Dacryodes sp.*	BABRU2595-15	MF435733	MF418868	22-1103
H491_DACRRURU	*Dacryodes sp.*	BABRU1823-15	MF435731	MF418865	23-3281
H492_DACRRU	*Dacryodes sp.*	BABRU3691-15	MF435726	MF418874	20-5353
H493_DACRSP	*Dacryodes sp.*	BABRU2519-15	MF435734	MF418866	13-0904
H494_SANTSP	Burseraceae	BABRU421-14	MF435737	MF418873	09-4183
H495_SANTAP	Burseraceae	BABRU179-14	MF435735	MF418872	13-6076
H496_SANTAPVA	Burseraceae	BABRU4083-15	MF435736	MF418862	24-5779
H497_SANTGR	Burseraceae	BABRU877-14	MF435738	MF418869	04-4023
H498_SANTOB	Burseraceae	BABRU1898-15	MF435739	MF418871	25-2675
H499_SANTTO	Burseraceae	BABRU1417-15	n/a	MF418870	13-2639
H500_TRIOMA	Burseraceae	BABRU2565-15	MF435741	MF418879	22-1072
H501_AGLASXI	*Aglaia sp.*	BABRU3900-15	MF435742	MF418894	24-5572
H502_AGLASP	*Aglaia sp.*	BABRU3442-15	MF435743	MF418911	20-3674
H503_AGLAS2	*Aglaia sp.*	BABRU848-14	MF435751	MF418915	06-5353
H504_AGLAS1	*Aglaia sp.*	BABRU070-14	MF435756	MF418920	09-3660
H505_AGLAS13	*Aglaia sp.*	BABRU3136-15	MF435757	MF418898	16-4437
H506_AGLAS7	*Aglaia sp.*	BABRU1995-15	MF435749	MF418909	19-1916

H507_AGLAS9	*Aglaia sp.*	BABRU817-14	MF435750	MF418913	01-5542
H508_AGLAS10	*Aglaia sp.*	BABRU2386-15	MF435745	MF418908	25-0017
H509_AGLAS12	*Aglaia sp.*	BABRU1850-15	MF435746	MF418899	13-1853
H510_AGLAS11	*Aglaia sp.*	BABRU2422-15	MF435747	MF418896	25-0314
H511_AGLAS15	*Aglaia sp.*	BABRU2347-15	MF435748	MF435871	19-0266
H512_AGLAS24	*Aglaia sp.*	BABRU3848-15	MF435753	MF418897	20-5557
H513_AGLAS4	*Aglaia sp.*	BABRU4098-15	MF435754	MF418895	24-5794
H514_AGLAS3	*Aglaia sp.*	BABRU2290-15	MF435755	MF418906	24-0538
H515_AGLASRU	*Aglaia sp.*	BABRU3684-15	MF435758	MF418905	20-5346
H516_AGLASI	*Aglaia sp.*	BABRU4064-15	MF435744	MF418912	24-5760
H517_CHISSP2	Meliaceae	BABRU4066-15	MF435765	n/a	24-5762
H518_CHISSP4	Meliaceae	BABRU3983-15	MF435760	MF418921	24-5668
H519_CHISS5	Meliaceae	BABRU1178-14	MF435759	MF418917	04-4930
H520_DYSOSP	Meliaceae	BABRU1441-15	MF435761	MF418922	13-2664
H521_DYSOS1	Meliaceae	BABRU3851-15	MF435762	MF418910	20-5560
H522_DYSOS3	Meliaceae	BABRU2324-15	MF435767	MF418902	19-0243
H523_DYSOS4	Meliaceae	BABRU4032-15	MF435771	MF418900	24-5727
H524_DYSOS5	Meliaceae	BABRU3952-15	MF435772	MF418901	24-5634
H525_DYSOS6	Meliaceae	BABRU4234-17	MF435770	MF418903	24-3851
H526_DYSOS8	Meliaceae	BABRU1521-15	MF435769	MF418904	20-2855
H527_DYSOS10	Meliaceae	BABRU283-14	MF435764	MF418919	12-4183
H528_DYSOS13	Meliaceae	BABRU1003-14	MF435763	n/a	04-5261
H529_DYSOS14	Meliaceae	BABRU2219-15	MF435766	MF418916	16-0888
H530_LANSDO	*Lansium sp.*	BABRU1112-14	MF435773	MF418907	03-5089
H531_MELIXXI	*Meliaceae*	BABRU022-14	MF435774	MF418914	05-5372
H532_REINHU	*Meliaceae*	BABRU2760-15	MF435775	MF418918	16-3014
H533_WALSSXI	*Walsura sp.*	BABRU3479-15	MF435776	MF418923	20-3711
H534_WALSSXII	*Walsura sp.*	BABRU1469-15	MF435777	MF418925	16-2443
H535_WALSSXIII	*Walsura sp.*	BABRU3339-15	MF435778	MF418924	16-4647
H536_GLYCSP	*Glycosmis sp.*	BABRU2438-15	MF435781	MF418941	25-0332
H537_GLYCMA	*Glycosmis sp.*	BABRU1763-15	MF435779	MF418940	25-1872
H538_LEPIS3	Sapindaceae	BABRU2108-15	MF435782	MF418927	15-0355
H539_LEPIS4	Sapindaceae	BABRU2061-15	MF435783	MF418939	22-0351
H540_LEPIFR	Sapindaceae	BABRU1613-15	MF435784	MF418938	20-2950
H541_LEPITE	Sapindaceae	BABRU879-14	MF435785	MF418928	04-4025
H542_NEPHCU	*Nephelium sp.*	BABRU3276-15	MF435786	MF418932	16-4583
H543_NEPHSP	*Nephelium sp.*	BABRU1982-15	MF435787	MF418933	19-1903
H544_NEPHS3	*Nephelium sp.*	BABRU3818-15	MF435789	MF418929	20-5515
H545_NEPHS4	*Nephelium sp.*	BABRU1103-14	MF435788	MF418936	03-5080
H546_NEPHME	*Nephelium sp.*	BABRU3604-15	MF435794	MF418934	20-3842
H547_NEPHSU	*Nephelium sp.*	BABRU3050-15	MF435790	MF418937	24-4015
H548_NEPHUN	*Nephelium sp.*	BABRU1987-15	MF435793	MF418935	19-1908
H549_POMEPI	Sapindaceae	BABRU818-14	MF435795	MF418931	01-5543
H550_SAPISP2	Sapindaceae	BABRU897-14	MF435796	MF418930	04-4071

H551_SIMASP	*Eurycoma*	BABRU2832-15	MF435797	MF418926	16-2931
H552_ERYCCR	*Erycibe sp.*	BABRU2396-15	n/a	MF418761	25-0027
H553_ERYCGL	*Erycibe sp.*	BABRU3053-15	MF435798	MF418760	24-4018
H554_ERYCST	*Erycibe sp.*	BABRU144-14	MF435799	MF418762	10-5236
H555_LEEAIN	*Leea sp.*	BABRU2074-15	MF435800	MF418710	22-0365

Morphological identities are available on request from Dr. Kamariah Abu Salim (Environmental and Life Sciences, Faculty of Science, University of Brunei Darussalam, Brunei Darussalam).

S2 Table. Mean elevation, slope, and convexity for all subplots.

Subplot	mean elevation	slope	convexity	habitat
01_22a	178,4576516	31,5032799	-2,94267268	ls
03_24a	196,2842815	30,0856532	0,62594553	ls
04_19a	232,7530285	43,0867831	1,66517425	ls
05_25a	212,471505	27,0565105	-0,13658501	valley
07_22a	214,9757554	30,2570571	-3,94526171	ls
09_15a	295,8817382	9,83767983	0,81398377	rt
10_23a	252,529537	12,9260782	0,26558772	rt
13_24a	283,6371823	11,775248	3,59668129	rt
14_19a	296,4899417	24,256739	3,16181038	rt
12_17a	288,0741697	38,9889787	-1,2903252	hg
12_21a	276,6725438	31,4367849	2,44494731	hs
06_23a	198,6624924	29,8426512	-8,15999413	ls
01_17a	218,1053213	35,4814063	-0,3784801	ls
08_21a	235,5897581	22,2436987	-2,79023946	valley
04_17a	258,118551	39,76937	2,30538922	hs
09_17a	281,9405234	36,5114584	3,04719965	hs
03_21a	198,168061	31,1671716	-1,61519948	ls
04_20a	217,5154344	38,633925	-0,0333544	ls
08_25a	237,4883163	41,025268	-3,08532074	ls
11_25a	263,5684532	20,3026443	-1,77818584	rt
11_19a	263,6924432	31,8302265	-2,11927643	hg
22_03a	212,2330625	40,3716892	0,90964324	ls
15_02a	218,3099557	6,64881193	1,00985041	valley
16_04a	221,0776884	12,7238584	1,35341143	valley
24_04a	214,0093566	35,9376441	-1,79824283	ls
19_02a	211,5508547	15,1894523	-1,12167875	valley
25_01a	168,0665157	18,2382694	-1,08221133	valley
25_03a	195,3323768	41,7407812	-0,80547051	ls
23_02a	187,7208723	38,8073314	-2,89800547	ls
13_04c	218,1038375	26,0921979	-2,43060061	valley
16_08a	235,4744345	20,925626	-5,12084993	valley
22_05a	240,2509411	38,9355357	1,97679112	ls
25_08a	234,3266504	17,9906485	0,18443744	valley
22_08b	254,8102015	43,063407	1,78972334	hs
20_11a	284,2155631	24,9160683	5,78803239	rt
13_08d	253,083767	33,0378432	-1,35876054	hg
19_07d	242,8274439	28,5213926	0,27070838	ls
23_12d	264,9302957	19,0099288	3,94095786	rt

22_07a	258,7057741	14,9759648	4,3929814	rt
13_11a	282,2842427	31,4416842	-1,65352933	hg
13_14b	314,1090403	34,7276648	5,69961019	hs
16_11a	269,9460682	30,5191965	-2,63258682	hg
18_12a	278,2592676	30,4068584	0,33138525	hs
25_11a	242,5889064	30,1767004	1,46259818	ls
16_13b	287,7355876	31,6886758	1,45480036	hs
16_13d	291,6528584	29,6117316	0,28358218	hs
25_18c	202,8897261	21,8013887	0,0747914	valley
25_20c	185,9237815	32,1230488	-4,24047213	ls
16_13c	285,7035377	32,9996047	-0,81893158	hg
16_13a	292,266023	20,6505471	2,10552861	rt
24_17a	222,2607122	38,5344067	0,19563778	ls
24_17b	225,0011896	34,2995959	1,79858419	ls
24_17c	218,6542312	31,8334342	1,55296713	ls
24_17d	216,9758455	36,2666077	0,50404433	ls
16_19a	281,6701319	17,5537974	0,37726078	rt
16_19b	279,0469529	27,0309891	1,39786689	rt
16_19c	281,6543205	18,735866	2,65392435	rt
16_19d	283,393824	6,21792707	0,62149278	rt
20_14a	258,5917461	9,554597	-0,98708557	rt
20_14b	259,5777082	16,8760922	0,33925527	rt
20_14c	257,8032303	12,3817182	0,54081893	rt
20_14d	257,1991809	8,88190035	-0,10009444	rt
20_21a	251,139303	26,726469	1,32260204	rt
20_21b	245,5989398	32,5440076	1,69896763	hs
20_21c	246,5797346	33,317699	1,13246669	hs
20_21d	252,1078425	24,5099767	0,98565273	rt
24_24a	218,5365666	34,4045056	2,62327394	ls
24_24b	212,2308002	33,198608	0,74867202	ls
24_24c	212,8977412	30,1847214	1,42091101	ls
24_24d	217,9678983	31,1393467	3,07388103	ls

S3 Tables. Calculations of PD, MPD, MNTD, NRI, and NTI for each subplot using different trees (Phylomatic, un-, constrained ML trees) and community data matrices

PD

Subplot	Phylomatic	Barcode.con	Barcode.uncon	Phylomatic.Barcode.only	Barcodeonyl.con	Barcodeonly.uncon
01_22a	3817,693223	4347,814446	4314,973721	3471,330013	4061,687309	4033,930763
03_24a	1900,623564	2563,703731	2465,754505	1064,761884	1775,115033	1611,012442
04_19a	5927,134097	6305,139732	6132,020843	5113,459788	5581,759296	5415,391865
05_25a	4223,705828	4699,607606	4595,963518	3490,916728	4017,666103	3871,172563
07_22a	1689,299874	2471,812526	2201,347383	1224,092958	1942,284827	1901,218554
09_15a	4653,937546	5044,809076	4960,668421	4218,551129	4570,577852	4468,238719
10_23a	3008,289042	3829,714215	3681,272672	2931,52714	3747,665435	3599,443404
13_24a	3845,586199	4162,544867	4134,527309	3506,192331	3946,579569	3849,549217
14_19a	3031,090338	3816,629635	3785,570715	2538,765415	3373,424213	3274,941797
12_17a	4349,411184	4802,679016	4655,637569	4084,230784	4646,002803	4498,458639

12_21a	4961,602601	5598,679139	5565,585271	4595,089992	5341,404004	5308,859711
06_23a	2512,395855	3261,273853	3229,13062	2445,332363	3129,850776	3100,921829
01_17a	6529,9922	6672,094765	6497,811744	5719,173722	6043,985186	6006,293873
08_21a	2201,837046	2986,108109	2885,188927	2109,49578	2936,818635	2836,083664
04_17a	6206,303437	6321,373236	5969,467041	5953,40428	6209,098595	5858,534505
09_17a	4983,842057	5698,505545	5588,71956	4648,769966	5451,067136	5341,785576
03_21a	4545,212778	4968,6809	4933,893266	4110,636129	4693,872037	4659,581595
04_20a	5740,15316	6159,355155	6121,365225	4966,142754	5590,454605	5550,180823
08_25a	2852,32286	3751,946078	3645,681394	2852,32286	3751,946078	3645,681394
11_25a	3541,229975	4155,857694	4053,526375	3413,778995	4042,318368	3940,133116
11_19a	2496,576102	3134,801917	3033,428338	2214,120449	2889,781687	2789,373823
22_03a	2810,740602	3694,587187	3536,20676	2667,601012	3587,510408	3430,472086
15_02a	5593,647987	5990,530586	5956,915515	5122,418227	5609,645145	5573,671257
16_04a	4912,165325	5124,338105	5094,224627	4399,185246	4623,285209	4590,548299
24_04a	3764,564641	4317,755612	4449,192374	3488,719979	4096,417927	4228,64368
19_02a	5893,241437	5797,492552	5756,475453	5557,902909	5732,676873	5691,659774
25_01a	3266,975789	3932,065204	3831,60593	3061,142456	3797,662728	3697,606963
25_03a	4346,315571	4776,327994	4671,821862	4104,876884	4645,543918	4542,287786
23_02a	1986,462251	2729,351304	2583,956789	1848,390353	2531,032027	2385,980886
13_04c	3268,852883	3986,946956	3836,704918	2511,454498	3234,323746	3087,043902
16_08a	4984,154352	5488,528503	5452,070855	4336,519869	5103,694161	5064,514299
22_05a	5869,654405	7098,758424	7002,803577	5570,340839	6732,706074	6638,06518
25_08a	4051,28021	4670,470071	4636,478221	3821,079464	4480,501583	4447,014158
22_08b	4989,18309	5714,153027	5671,383319	4631,167218	5440,637118	5401,262783
20_11a	4341,811285	4666,223476	4739,727593	4038,235183	4597,377642	4670,881759
13_08d	5529,979376	5700,83862	5478,565662	5024,005067	5355,568896	5061,190728
19_07d	5680,301985	5841,825798	5644,438268	4889,214229	5181,037237	5165,399857
23_12d	4293,080147	4814,546305	4710,939693	3841,508714	4367,578747	4267,362042
22_07a	4685,745995	5366,788765	5315,088841	4145,920645	5092,57989	5040,946462
13_11a	4551,944133	5264,158598	5155,463852	4000,40508	4794,603331	4686,791629
13_14b	3471,268594	4114,812845	4084,928525	2966,649412	3652,10415	3625,09131
16_11a	5409,940595	6555,220223	6278,660899	4893,186704	5498,115048	5282,589947
18_12a	3826,024564	4475,586912	4439,386663	3770,795804	4449,475801	4413,942219
25_11a	3483,883799	4111,851971	4006,534617	2935,389395	3726,39166	3622,350454
16_13b	5401,968701	5839,98876	5728,323796	4722,917965	5136,916299	5030,739896
16_13d	5258,318915	5209,183371	5180,972478	5001,851035	5062,928858	5033,448734
25_18c	4020,309578	4725,748053	4623,25518			
25_20c	2619,2893	3229,089143	3130,332362			
16_13c	4276,179576	4690,28248	4653,949135	3263,437995	3727,714595	3698,562731
16_13a	4906,11005	5456,746657	5353,065977	4703,604599	5304,48382	5201,423072
24_17a	5334,45418	5617,64764	5576,967338	4874,360353	5170,041442	5136,037104
24_17b	6174,623843	6308,924861	6271,441542	5806,63154	6058,601117	6020,687875
24_17c	4539,990987	4741,879262	4857,894237	3752,910123	4127,532116	4247,198303
24_17d	5812,021045	5762,034359	5746,511316	5415,229549	5467,989685	5454,090409
16_19a	4813,308594	5245,959274	5214,015201	4142,199704	4790,059031	4759,420031
16_19b	5808,777193	6112,849688	6078,94638	5278,433243	5750,57917	5717,890976
16_19c	4176,455714	4915,562553	4815,188702	3549,872294	4250,501997	4152,658647
16_19d	5096,024353	5439,47256	5335,318811	4786,999552	5220,399629	5116,245879

20_14a	5110,017203	5694,281564	5459,159677	4615,83108	5098,145515	4864,741469
20_14b	4886,205964	5454,908735	5416,55186	4083,588463	4599,766246	4494,340246
20_14c	5020,385009	5546,594918	5513,338658	4564,262057	5211,977266	5179,269467
20_14d	4429,95183	5123,525551	5087,7443	3666,192807	4429,812915	4399,454284
20_21a	6217,434028	6225,703195	6183,763579	5326,359488	5653,410236	5619,023662
20_21b	5388,414135	5374,655483	5345,000972	4791,788204	5026,392215	4996,991534
20_21c	6770,73363	6598,840765	6249,492467	5551,173917	5734,973232	5583,401045
20_21d	4893,653638	5283,277471	5130,953316	4143,25867	4494,236716	4461,096936
24_24a	5904,745761	5610,910427	5490,940386	4995,523619	4890,692952	4778,802484
24_24b	5218,357949	5260,078406	5212,20637	4592,727167	4837,319899	4793,057476
24_24c	5084,93546	5279,879167	5174,975978	4595,597033	4943,714628	4841,682576
24_24d	5180,763972	4897,977943	4797,766214	4455,825695	4469,725437	4370,134402

MPD: Phylomatic

Subplot	mpd,obs	mpd,rand,mean	mpd,rand,sd	mpd,obs,rank	mpd,obs,z	mpd,obs,p	runs	NRI
01_22a	442,976597	429,156595	19,9926707	745	0,69125338	0,745	999	-0,69125338
03_24a	443,12017	398,63437	31,1569781	956	1,42779571	0,956	999	-1,42779571
04_19a	401,933863	436,698678	16,2864513	29	-2,13458504	0,029	999	2,13458504
05_25a	393,529277	432,86327	18,7558993	26	-2,09715313	0,026	999	2,09715313
07_22a	361,473048	395,229306	31,4855088	163	-1,07212043	0,163	999	1,07212043
09_15a	384,632711	425,063788	21,7371349	33	-1,86000026	0,033	999	1,86000026
10_23a	437,478114	428,054466	21,2242518	639	0,44400378	0,639	999	-0,44400378
13_24a	439,237037	414,918127	24,3401674	821	0,99912667	0,821	999	-0,99912667
14_19a	380,528649	405,707715	27,5368607	187	-0,91437675	0,187	999	0,91437675
12_17a	449,56927	436,100098	16,621594	791	0,81034178	0,791	999	-0,81034178
12_21a	381,816605	427,915879	19,0715132	8	-2,4171797	0,008	999	2,4171797
06_23a	452,973199	420,909068	23,5948491	941	1,35894622	0,941	999	-1,35894622
01_17a	414,237406	439,579927	14,8764006	46	-1,70353848	0,046	999	1,70353848
08_21a	419,524368	410,130133	27,6835574	585	0,3393435	0,585	999	-0,3393435
04_17a	432,014412	437,875029	15,3662207	350	-0,3813961	0,35	999	0,3813961
09_17a	415,860695	438,348097	14,9262412	78	-1,50656835	0,078	999	1,50656835
03_21a	431,265392	428,793109	18,8560325	555	0,13111368	0,555	999	-0,13111368
04_20a	394,366526	407,593662	21,5478401	282	-0,61384976	0,282	999	0,61384976
08_25a	400,378825	398,456389	30,0350071	506	0,06400651	0,506	999	-0,06400651
11_25a	438,378083	429,894612	20,7508473	625	0,40882525	0,625	999	-0,40882525
11_19a	344,770629	408,562783	27,0255295	14	-2,36044049	0,014	999	2,36044049
22_03a	353,399467	361,480417	34,6778158	409	-0,23302939	0,409	999	0,23302939
15_02a	426,13875	433,843609	16,9204032	327	-0,45535907	0,327	999	0,45535907
16_04a	425,220086	437,460181	15,935309	236	-0,76811154	0,236	999	0,76811154
24_04a	409,599618	419,492411	24,1988358	328	-0,40881276	0,328	999	0,40881276
19_02a	440,147845	442,280739	13,0891286	405	-0,16295155	0,405	999	0,16295155
25_01a	462,001356	420,98183	23,1345616	985	1,77308422	0,985	999	-1,77308422
25_03a	434,226324	438,502073	15,6041625	373	-0,27401335	0,373	999	0,27401335
23_02a	346,542986	353,853488	38,1910291	415	-0,19141934	0,415	999	0,19141934
13_04c	320,990734	318,645511	28,2935782	588	0,08288889	0,588	999	-0,08288889
16_08a	437,181895	440,153987	15,1555461	404	-0,19610588	0,404	999	0,19610588
22_05a	404,592498	436,955637	15,830472	20	-2,04435717	0,02	999	2,04435717

25_08a	413,414147	436,299117	17,5466232	102	-1,30423787	0,102	999	1,30423787
22_08b	420,034519	436,165215	16,8616431	173	-0,95665029	0,173	999	0,95665029
20_11a	363,247458	406,441466	23,6259559	23	-1,82824385	0,023	999	1,82824385
13_08d	444,711999	442,065693	13,349626	554	0,19823073	0,554	999	-0,19823073
19_07d	431,413026	439,258227	15,118517	305	-0,51891339	0,305	999	0,51891339
23_12d	430,282838	436,667154	16,3104308	332	-0,3914253	0,332	999	0,3914253
22_07a	414,146369	435,978804	16,8344826	109	-1,29688781	0,109	999	1,29688781
13_11a	397,894151	439,731391	14,8666598	5	-2,81416541	0,005	999	2,81416541
13_14b	331,131004	409,398946	27,2353302	4	-2,87376515	0,004	999	2,87376515
16_11a	429,577404	437,811617	15,4260453	284	-0,53378635	0,284	999	0,53378635
18_12a	400,305474	431,86678	19,554788	60	-1,61399376	0,06	999	1,61399376
25_11a	397,573536	427,410408	20,6748777	83	-1,4431462	0,083	999	1,4431462
16_13b	444,233257	439,162211	14,6906415	616	0,34518882	0,616	999	-0,34518882
16_13d	428,967375	440,189836	14,7504637	222	-0,76082089	0,222	999	0,76082089
25_18c	437,018337	432,949148	18,7076181	559	0,2175151	0,559	999	-0,2175151
25_20c	435,692469	424,068555	23,3993877	664	0,49676146	0,664	999	-0,49676146
16_13c	394,269513	434,105666	17,7070719	15	-2,24973125	0,015	999	2,24973125
16_13a	414,907651	440,265726	14,4322982	47	-1,75703655	0,047	999	1,75703655
24_17a	438,836611	441,141005	14,1931962	417	-0,16235903	0,417	999	0,16235903
24_17b	433,356269	440,711419	13,6713285	290	-0,5379982	0,29	999	0,5379982
24_17c	423,837181	434,240725	17,8214434	277	-0,58376551	0,277	999	0,58376551
24_17d	427,622442	442,33809	13,2477492	135	-1,11080366	0,135	999	1,11080366
16_19a	418,872206	437,432296	15,8837396	122	-1,16849623	0,122	999	1,16849623
16_19b	420,393121	442,53457	12,9768003	57	-1,7062333	0,057	999	1,7062333
16_19c	401,450451	436,473111	16,7823745	23	-2,08687157	0,023	999	2,08687157
16_19d	441,641714	440,153043	14,8335354	527	0,10035847	0,527	999	-0,10035847
20_14a	410,078168	436,273894	15,7640574	51	-1,6617375	0,051	999	1,6617375
20_14b	415,345485	437,35143	16,5485515	97	-1,32978076	0,097	999	1,32978076
20_14c	414,479782	437,279407	16,3643128	89	-1,39325286	0,089	999	1,39325286
20_14d	411,66829	424,914161	21,5451929	268	-0,61479474	0,268	999	0,61479474
20_21a	422,488436	442,933112	12,5302837	66	-1,63162117	0,066	999	1,63162117
20_21b	419,031181	441,390695	13,9922132	70	-1,5979969	0,07	999	1,5979969
20_21c	424,216741	442,443171	13,1514989	97	-1,38588231	0,097	999	1,38588231
20_21d	430,526973	435,748679	16,9442094	352	-0,3081705	0,352	999	0,3081705
24_24a	413,415618	442,491534	12,8253986	16	-2,26705747	0,016	999	2,26705747
24_24b	398,394604	440,337709	14,4921357	6	-2,89419768	0,006	999	2,89419768
24_24c	411,24073	441,92254	13,2047873	21	-2,32353685	0,021	999	2,32353685
24_24d	412,87664	438,105242	15,8646481	61	-1,59024027	0,061	999	1,59024027

MNTD: Phylomatic

Subplot	**mntd,obs**	**mntd,rand,mean**	**mntd,rand,sd**	**mntd,obs,rank**	**mntd,obs,z**	**mntd,obs,p**	**runs**	**NTI**
01_22a	168,955222	185,124033	28,8612603	305	-0,56022538	0,305	999	0,56022538
03_24a	308,654192	284,709102	54,2311705	652	0,44153739	0,652	999	-0,44153739
04_19a	137,647548	142,510531	19,215368	419	-0,25307781	0,419	999	0,25307781
05_25a	106,779863	161,900856	24,9860882	10	-2,20606734	0,01	999	2,20606734
07_22a	262,358274	274,272156	53,0306783	386	-0,22466018	0,386	999	0,22466018
09_15a	111,008914	155,443114	28,3546869	36	-1,56708483	0,036	999	1,56708483

10_23a	167,299532	202,829394	32,4235876	142	-1,09580292	0,142	999	1,09580292
13_24a	236,459146	186,689404	35,8394536	917	1,38868585	0,917	999	-1,38868585
14_19a	189,330651	204,463085	42,4330054	381	-0,35661944	0,381	999	0,35661944
12_17a	134,564991	164,96826	22,8220077	88	-1,33219085	0,088	999	1,33219085
12_21a	112,665177	154,563927	26,2836709	37	-1,59409808	0,037	999	1,59409808
06_23a	193,358211	230,902307	38,1924883	162	-0,98302304	0,162	999	0,98302304
01_17a	139,426898	142,39275	18,0729784	455	-0,16410418	0,455	999	0,16410418
08_21a	196,105212	241,821671	42,7163067	152	-1,07023435	0,152	999	1,07023435
04_17a	182,153306	151,56567	19,6027886	926	1,56037165	0,926	999	-1,56037165
09_17a	137,804603	154,36035	18,6406165	192	-0,88815444	0,192	999	0,88815444
03_21a	141,193624	168,577651	26,3921747	148	-1,03758131	0,148	999	1,03758131
04_20a	126,41856	151,987461	35,3030314	265	-0,72426926	0,265	999	0,72426926
08_25a	294,162153	234,022872	48,5989806	890	1,23745971	0,89	999	-1,23745971
11_25a	182,583432	192,41714	27,6338294	383	-0,35585757	0,383	999	0,35585757
11_19a	107,362227	202,664967	41,240714	7	-2,31088967	0,007	999	2,31088967
22_03a	220,257768	212,650911	58,696188	561	0,12959713	0,561	999	-0,12959713
15_02a	132,079476	146,723366	21,1005865	261	-0,69400391	0,261	999	0,69400391
16_04a	143,849535	154,469867	20,485233	296	-0,51843841	0,296	999	0,51843841
24_04a	136,74516	187,073712	33,5438032	62	-1,500383	0,062	999	1,500383
19_02a	127,965206	141,952065	15,1497947	175	-0,92323754	0,175	999	0,92323754
25_01a	226,189957	203,483924	35,67092	744	0,63654182	0,744	999	-0,63654182
25_03a	129,532287	162,906456	20,4020841	44	-1,63582155	0,044	999	1,63582155
23_02a	178,700149	237,971366	67,4794996	203	-0,87835886	0,203	999	0,87835886
13_04c	177,671737	204,045833	70,356508	423	-0,37486363	0,423	999	0,37486363
16_08a	164,546636	159,525144	18,8221139	609	0,26678681	0,609	999	-0,26678681
22_05a	150,611962	144,770921	19,8269681	636	0,29460083	0,636	999	-0,29460083
25_08a	140,223295	169,589319	23,7452791	108	-1,23670997	0,108	999	1,23670997
22_08b	194,557166	167,546202	23,2570561	881	1,16140941	0,881	999	-1,16140941
20_11a	140,369276	154,356149	35,2672711	416	-0,3965964	0,416	999	0,3965964
13_08d	150,845705	152,921996	17,6039239	471	-0,11794478	0,471	999	0,11794478
19_07d	117,428654	146,899918	18,2602143	44	-1,61396046	0,044	999	1,61396046
23_12d	124,077221	161,104744	21,420779	44	-1,72857968	0,044	999	1,72857968
22_07a	126,749952	161,2526	21,9917358	48	-1,56889154	0,048	999	1,56889154
13_11a	155,477742	160,185398	19,1316915	421	-0,2460659	0,421	999	0,2460659
13_14b	135,000585	181,891591	38,540277	114	-1,21667537	0,114	999	1,21667537
16_11a	163,836188	157,372407	19,1271974	637	0,33793668	0,637	999	-0,33793668
18_12a	153,133005	175,719823	26,2160773	202	-0,86156357	0,202	999	0,86156357
25_11a	139,80072	185,623721	29,5946763	64	-1,54835284	0,064	999	1,54835284
16_13b	182,247556	154,312028	18,4090433	931	1,51748941	0,931	999	-1,51748941
16_13d	144,727951	152,676009	17,8173972	332	-0,44608411	0,332	999	0,44608411
25_18c	200,800887	182,566254	24,5975949	756	0,74131773	0,756	999	-0,74131773
25_20c	188,045468	214,358099	34,1941418	220	-0,76950699	0,22	999	0,76950699
16_13c	126,171599	163,918041	22,9727077	43	-1,64309939	0,043	999	1,64309939
16_13a	151,633398	157,220348	17,9332691	374	-0,3115411	0,374	999	0,3115411
24_17a	139,194407	155,558933	17,6497967	176	-0,92717925	0,176	999	0,92717925
24_17b	136,304542	144,211425	17,5531529	339	-0,45045368	0,339	999	0,45045368
24_17c	141,654068	163,642006	22,5166768	158	-0,97651792	0,158	999	0,97651792
24_17d	120,155714	145,05417	16,3872642	73	-1,51937843	0,073	999	1,51937843

16_19a	136,13936	156,104715	20,0603551	167	-0,99526428	0,167	999	0,99526428
16_19b	144,473013	149,683346	16,8095072	378	-0,30996348	0,378	999	0,30996348
16_19c	156,09585	168,886795	21,9017558	290	-0,58401459	0,29	999	0,58401459
16_19d	153,851142	155,125441	19,1315851	477	-0,06660705	0,477	999	0,06660705
20_14a	145,599667	154,493334	20,8739026	357	-0,42606629	0,357	999	0,42606629
20_14b	145,763812	159,396827	21,312984	268	-0,63965769	0,268	999	0,63965769
20_14c	127,381174	151,963925	19,6825993	109	-1,24895854	0,109	999	1,24895854
20_14d	194,982929	181,051183	30,4548921	683	0,45745511	0,683	999	-0,45745511
20_21a	144,380424	142,873469	14,3824162	572	0,10477759	0,572	999	-0,10477759
20_21b	117,335978	147,012161	17,2520005	41	-1,72015894	0,041	999	1,72015894
20_21c	111,690929	133,394101	15,1250898	75	-1,43491197	0,075	999	1,43491197
20_21d	131,108946	158,777433	20,1567132	79	-1,37266863	0,079	999	1,37266863
24_24a	98,8083787	137,151634	14,7232455	3	-2,60426654	0,003	999	2,60426654
24_24b	104,150605	146,643753	17,1435692	4	-2,47866404	0,004	999	2,47866404
24_24c	101,535847	144,212226	16,2197143	4	-2,63114248	0,004	999	2,63114248
24_24d	99,3128048	145,896765	18,991542	3	-2,45287932	0,003	999	2,45287932

MPD: Barcode.con

Subplot	mpd,obs	mpd,rand,mean	mpd,rand,sd	mpd,obs,rank	mpd,obs,z	mpd,obs,p	runs	NRI
01_22a	500,24077	482,990623	36,5620695	755	0,47180445	0,755	999	-0,47180445
03_24a	485,441283	446,839879	55,9896349	859	0,68943839	0,859	999	-0,68943839
04_19a	452,230918	492,902225	25,9734836	34	-1,56587801	0,034	999	1,56587801
05_25a	448,019258	488,275611	29,8859543	53	-1,34699908	0,053	999	1,34699908
07_22a	392,835854	448,977112	60,9347047	111	-0,9213347	0,111	999	0,9213347
09_15a	417,785541	480,51507	39,7016208	7	-1,58002437	0,007	999	1,58002437
10_23a	494,200659	482,712249	38,6368223	685	0,29734355	0,685	999	-0,29734355
13_24a	487,742458	468,499761	45,448678	756	0,423394	0,756	999	-0,423394
14_19a	417,713178	458,52323	52,5565906	160	-0,77649732	0,16	999	0,77649732
12_17a	505,847564	492,581191	29,3845687	757	0,45147416	0,757	999	-0,45147416
12_21a	431,845636	482,398706	31,2558653	17	-1,61739465	0,017	999	1,61739465
06_23a	510,205517	477,05108	43,6244523	918	0,75999663	0,918	999	-0,75999663
01_17a	461,70917	497,464043	27,2777676	41	-1,31076976	0,041	999	1,31076976
08_21a	473,916054	464,705452	46,7708855	615	0,19693024	0,615	999	-0,19693024
04_17a	484,774673	494,617956	24,2286533	329	-0,40626619	0,329	999	0,40626619
09_17a	466,013895	496,236086	26,1491482	84	-1,15576194	0,084	999	1,15576194
03_21a	482,708315	485,303123	33,3124382	514	-0,07789309	0,514	999	0,07789309
04_20a	436,85175	459,754983	36,9918337	223	-0,61914294	0,223	999	0,61914294
08_25a	445,63154	449,419205	55,7642283	504	-0,06792284	0,504	999	0,06792284
11_25a	487,680179	486,533888	32,7281931	533	0,03502459	0,533	999	-0,03502459
11_19a	370,052573	463,278741	55,9296739	5	-1,66684626	0,005	999	1,66684626
22_03a	387,996769	405,876953	57,9267497	393	-0,30866886	0,393	999	0,30866886
15_02a	480,723974	491,648919	41,0644947	412	-0,26604358	0,412	999	0,26604358
16_04a	474,527354	494,46403	31,6064792	227	-0,63077813	0,227	999	0,63077813
24_04a	447,153061	472,946716	38,7288278	227	-0,66600659	0,227	999	0,66600659
19_02a	494,127482	500,111372	26,2629471	425	-0,22784533	0,425	999	0,22784533
25_01a	514,216877	474,148892	40,8909817	933	0,97987338	0,933	999	-0,97987338
25_03a	489,524364	495,220959	28,7921955	437	-0,19785208	0,437	999	0,19785208

23_02a	386,92353	400,728678	55,6344505	452	-0,24814029	0,452	999	0,24814029
13_04c	365,721899	360,73235	54,9864849	674	0,09074136	0,674	999	-0,09074136
16_08a	491,476943	496,181718	26,1032818	459	-0,18023693	0,459	999	0,18023693
22_05a	481,276883	492,273455	27,70028	337	-0,39698417	0,337	999	0,39698417
25_08a	466,917903	491,43656	31,1880798	167	-0,78615476	0,167	999	0,78615476
22_08b	467,377974	491,711655	28,5284926	161	-0,85296065	0,161	999	0,85296065
20_11a	398,752191	458,207298	47,170807	9	-1,26042165	0,009	999	1,26042165
13_08d	498,964421	499,227655	23,5317732	541	-0,01118632	0,541	999	0,01118632
19_07d	482,09318	494,923779	25,2076237	311	-0,50899674	0,311	999	0,50899674
23_12d	480,925034	491,561384	30,045445	350	-0,35400874	0,35	999	0,35400874
22_07a	464,404312	493,749843	33,3913752	130	-0,87883564	0,13	999	0,87883564
13_11a	442,821445	496,040087	26,5305265	10	-2,0059399	0,01	999	2,0059399
13_14b	367,498045	463,937032	47,161053	4	-2,04488621	0,004	999	2,04488621
16_11a	522,362362	495,265028	26,3976878	912	1,02650408	0,912	999	-1,02650408
18_12a	451,829142	486,807932	35,888553	121	-0,97465033	0,121	999	0,97465033
25_11a	440,743258	480,378513	35,7792915	82	-1,1077708	0,082	999	1,1077708
16_13b	499,324668	496,906338	29,5285014	616	0,08189814	0,616	999	-0,08189814
16_13d	475,669785	496,559832	24,9485319	172	-0,8373257	0,172	999	0,8373257
25_18c	488,709606	488,823876	32,0911283	517	-0,0035608	0,517	999	0,0035608
25_20c	489,915262	478,841362	38,2304812	675	0,28966154	0,675	999	-0,28966154
16_13c	441,418856	490,82236	29,8002747	19	-1,65782041	0,019	999	1,65782041
16_13a	459,707893	496,469701	24,7539405	36	-1,48508913	0,036	999	1,48508913
24_17a	488,470346	497,05702	23,9361573	345	-0,35873233	0,345	999	0,35873233
24_17b	479,094585	497,521264	23,5302582	169	-0,78310571	0,169	999	0,78310571
24_17c	468,697275	489,550775	27,6159288	199	-0,75512579	0,199	999	0,75512579
24_17d	476,276839	498,362082	24,5565456	136	-0,89936279	0,136	999	0,89936279
16_19a	469,829441	492,395476	27,4388838	163	-0,8224108	0,163	999	0,8224108
16_19b	468,462756	499,134699	23,4540053	61	-1,30774863	0,061	999	1,30774863
16_19c	452,840333	491,491699	27,1524981	58	-1,42349209	0,058	999	1,42349209
16_19d	496,461026	497,153414	26,5931956	538	-0,02603628	0,538	999	0,02603628
20_14a	461,986394	493,366762	29,4751058	83	-1,06463969	0,083	999	1,06463969
20_14b	464,220126	491,237083	28,1303663	122	-0,9604197	0,122	999	0,9604197
20_14c	465,9026	493,104646	27,1951019	114	-1,00025534	0,114	999	1,00025534
20_14d	455,230433	477,459677	36,8685668	252	-0,60293215	0,252	999	0,60293215
20_21a	471,569987	500,650761	21,8876389	54	-1,32863917	0,054	999	1,32863917
20_21b	471,341755	497,887293	24,521975	94	-1,08252037	0,094	999	1,08252037
20_21c	480,288477	498,287591	22,0779896	170	-0,81525151	0,17	999	0,81525151
20_21d	484,470451	491,901911	27,5030159	384	-0,27020529	0,384	999	0,27020529
24_24a	466,960283	501,226373	20,6275623	24	-1,66117983	0,024	999	1,66117983
24_24b	443,119036	496,539689	24,1717242	4	-2,21004728	0,004	999	2,21004728
24_24c	457,097634	498,267578	23,3094044	13	-1,76623751	0,013	999	1,76623751
24_24d	461,571158	494,757282	27,5915576	76	-1,20276371	0,076	999	1,20276371

MNTD: Barcode.con

Subplot	mntd,obs	mntd,rand,mean	mntd,rand,sd	mntd,obs,rank	mntd,obs,z	mntd,obs,p	runs	NTI
01_22a	125,363395	164,98606	44,9150113	168	-0,88216976	0,168	999	0,88216976
03_24a	272,229951	296,264531	75,4547516	370	-0,31852971	0,37	999	0,31852971

04_19a	104,316411	103,909437	26,1847503	568	0,01554239	0,568	999	-0,01554239
05_25a	75,5777798	132,109727	31,8789631	20	-1,77333079	0,02	999	1,77333079
07_22a	279,868435	282,226604	71,8450094	482	-0,03282301	0,482	999	0,03282301
09_15a	73,1779786	122,883155	37,4400164	68	-1,32759494	0,068	999	1,32759494
10_23a	148,932653	187,73259	43,0073062	172	-0,90217083	0,172	999	0,90217083
13_24a	191,985709	168,856319	48,7893317	714	0,47406654	0,714	999	-0,47406654
14_19a	148,713896	184,959753	55,6631914	269	-0,65116384	0,269	999	0,65116384
12_17a	106,377365	136,442622	30,5515047	146	-0,98408435	0,146	999	0,98408435
12_21a	93,8998662	120,111164	33,2214807	205	-0,78898643	0,205	999	0,78898643
06_23a	183,468952	222,828928	51,6188065	223	-0,76251232	0,223	999	0,76251232
01_17a	101,626373	103,793664	26,9444037	538	-0,08043568	0,538	999	0,08043568
08_21a	198,985015	240,911982	60,7342229	232	-0,69033512	0,232	999	0,69033512
04_17a	137,28533	116,492219	27,121964	813	0,76665212	0,813	999	-0,76665212
09_17a	111,335927	124,266222	25,8562166	322	-0,50008457	0,322	999	0,50008457
03_21a	107,107218	140,948076	36,9817005	169	-0,91507036	0,169	999	0,91507036
04_20a	103,828983	115,358808	48,8270405	523	-0,23613606	0,523	999	0,23613606
08_25a	332,294487	229,721035	68,049339	944	1,50733943	0,944	999	-1,50733943
11_25a	157,439607	174,434918	37,406647	346	-0,45433933	0,346	999	0,45433933
11_19a	64,7758261	186,173294	54,8455415	2	-2,21344278	0,002	999	2,21344278
22_03a	175,085625	201,941491	86,7210994	408	-0,30968088	0,408	999	0,30968088
15_02a	114,142884	112,510908	29,4601811	581	0,05539599	0,581	999	-0,05539599
16_04a	98,536295	123,596772	27,733353	175	-0,90362232	0,175	999	0,90362232
24_04a	129,18366	166,945351	47,6996673	217	-0,79165522	0,217	999	0,79165522
19_02a	86,6615937	103,343947	20,3652386	204	-0,81915825	0,204	999	0,81915825
25_01a	208,342835	188,66343	49,0250644	700	0,40141517	0,7	999	-0,40141517
25_03a	97,5246951	135,058354	28,7535158	84	-1,30535893	0,084	999	1,30535893
23_02a	187,828573	239,628027	88,8771388	282	-0,5828209	0,282	999	0,5828209
13_04c	235,805872	192,428598	91,8232571	673	0,47239965	0,673	999	-0,47239965
16_08a	130,876366	131,042997	27,898585	538	-0,00597276	0,538	999	0,00597276
22_05a	138,970411	109,978104	27,4419692	872	1,05649513	0,872	999	-1,05649513
25_08a	113,232395	141,838774	32,1991607	182	-0,88842002	0,182	999	0,88842002
22_08b	184,068892	139,810032	31,0218428	925	1,42669988	0,925	999	-1,42669988
20_11a	93,4024881	124,759694	50,4423491	294	-0,62164444	0,294	999	0,62164444
13_08d	112,780588	117,32747	22,7160468	439	-0,20016166	0,439	999	0,20016166
19_07d	83,8108609	110,241237	25,7861285	133	-1,02498425	0,133	999	1,02498425
23_12d	88,0179204	129,160931	27,4595387	50	-1,49831397	0,05	999	1,49831397
22_07a	105,616284	131,217355	29,2467183	195	-0,8753485	0,195	999	0,8753485
13_11a	136,32173	130,438668	26,8087783	617	0,21944534	0,617	999	-0,21944534
13_14b	107,65045	160,795087	50,7300448	140	-1,04759689	0,14	999	1,04759689
16_11a	160,889825	127,07186	28,6640137	890	1,17980563	0,89	999	-1,17980563
18_12a	118,300199	149,571987	35,9239377	176	-0,87050001	0,176	999	0,87050001
25_11a	113,674539	165,071571	40,4791229	92	-1,26971704	0,092	999	1,26971704
16_13b	151,266608	122,87634	25,6248513	877	1,10791936	0,877	999	-1,10791936
16_13d	89,6126549	118,71794	24,378566	109	-1,19388833	0,109	999	1,19388833
25_18c	179,824782	161,518689	36,2697706	749	0,50472039	0,749	999	-0,50472039
25_20c	163,208444	202,466699	48,1844928	204	-0,81474876	0,204	999	0,81474876
16_13c	79,6067659	134,821848	33,7724378	23	-1,63491551	0,023	999	1,63491551
16_13a	119,227491	126,907618	26,4458279	406	-0,2904098	0,406	999	0,2904098

24_17a	98,2711962	123,751905	24,1552078	138	-1,05487432	0,138	999	1,05487432
24_17b	94,4108188	106,839694	24,3212613	320	-0,51102924	0,32	999	0,51102924
24_17c	94,2127612	134,74356	32,1442668	83	-1,2609029	0,083	999	1,2609029
24_17d	62,7569727	109,565208	23,1711805	8	-2,02010578	0,008	999	2,02010578
16_19a	98,7647475	121,436701	28,7957569	207	-0,7873366	0,207	999	0,7873366
16_19b	101,977588	114,849668	23,2875173	294	-0,55274591	0,294	999	0,55274591
16_19c	138,112188	142,696681	30,6177519	475	-0,14973316	0,475	999	0,14973316
16_19d	117,556697	125,602946	26,7321404	411	-0,30099529	0,411	999	0,30099529
20_14a	112,437927	121,69402	28,4185091	409	-0,32570649	0,409	999	0,32570649
20_14b	113,247201	129,766899	29,7498773	290	-0,55528624	0,29	999	0,55528624
20_14c	93,2541485	119,21054	28,4491819	171	-0,91237745	0,171	999	0,91237745
20_14d	161,420428	161,395848	43,4663777	543	0,00056551	0,543	999	-0,00056551
20_21a	95,4123098	104,975109	19,8796621	334	-0,48103431	0,334	999	0,48103431
20_21b	75,9826307	111,82067	24,0719576	49	-1,48878791	0,049	999	1,48878791
20_21c	73,3532974	91,0248513	22,6839766	203	-0,77903245	0,203	999	0,77903245
20_21d	101,383384	126,63105	28,4133068	191	-0,88858598	0,191	999	0,88858598
24_24a	59,307247	98,0829103	21,1098933	10	-1,83684791	0,01	999	1,83684791
24_24b	64,4715972	112,11477	23,3271359	7	-2,04239272	0,007	999	2,04239272
24_24c	62,4423689	108,481448	22,092805	4	-2,0838947	0,004	999	2,0838947
24_24d	50,2645314	111,045139	26,9567397	3	-2,25474623	0,003	999	2,25474623

MPD: Barcode.uncon

Subplot	**mpd,obs**	**mpd,rand,mean**	**mpd,rand,sd**	**mpd,obs,rank**	**mpd,obs,z**	**mpd,obs,p**	**runs**	**NRI**
01_22a	439,068606	453,743026	26,1077431	183	-0,56207155	0,183	999	0,56207155
03_24a	397,932965	423,287632	46,2092013	184	-0,54869305	0,184	999	0,54869305
04_19a	434,862817	464,172317	22,9265021	6	-1,27841131	0,006	999	1,27841131
05_25a	419,387946	456,845707	20,014822	7	-1,87150105	0,007	999	1,87150105
07_22a	404,704712	421,076135	42,2905106	262	-0,38711813	0,262	999	0,38711813
09_15a	398,939085	451,191371	27,2456174	8	-1,91782353	0,008	999	1,91782353
10_23a	438,083875	452,484182	27,4088601	196	-0,52538878	0,196	999	0,52538878
13_24a	457,487244	440,263893	31,9155056	895	0,53965465	0,895	999	-0,53965465
14_19a	396,398764	431,977641	36,3815665	56	-0,97793691	0,056	999	0,97793691
12_17a	441,418233	462,623265	23,84333	55	-0,88934859	0,055	999	0,88934859
12_21a	406,416895	454,09926	30,1181457	3	-1,58317729	0,003	999	1,58317729
06_23a	445,645363	446,953945	33,4423331	580	-0,03912951	0,58	999	0,03912951
01_17a	450,056351	463,893137	16,4587748	112	-0,84069357	0,112	999	0,84069357
08_21a	413,421152	433,633852	34,821099	168	-0,58047279	0,168	999	0,58047279
04_17a	453,665333	464,438924	20,9488622	209	-0,51428048	0,209	999	0,51428048
09_17a	446,971229	466,852448	22,0355356	51	-0,90223445	0,051	999	0,90223445
03_21a	459,896431	455,504245	28,3535188	741	0,15490798	0,741	999	-0,15490798
04_20a	433,537611	432,037601	31,7412822	655	0,0472574	0,655	999	-0,0472574
08_25a	434,223439	422,849263	43,4125452	776	0,26200206	0,776	999	-0,26200206
11_25a	448,361046	457,092725	27,1229545	344	-0,32192949	0,344	999	0,32192949
11_19a	384,969946	430,388106	29,2295878	28	-1,55384195	0,028	999	1,55384195
22_03a	380,619244	380,404705	38,4643664	439	0,00557758	0,439	999	-0,00557758
15_02a	456,823791	460,026564	21,8036443	486	-0,14689162	0,486	999	0,14689162
16_04a	457,069619	463,152797	19,891957	377	-0,3058109	0,377	999	0,3058109

24_04a	435,855938	444,538456	35,5048209	385	-0,24454475	0,385	999	0,24454475
19_02a	464,046584	469,234985	17,4925003	422	-0,29660719	0,422	999	0,29660719
25_01a	431,050217	445,067278	26,6539647	211	-0,52589027	0,211	999	0,52589027
25_03a	447,171142	463,974918	17,9289103	74	-0,93724466	0,074	999	0,93724466
23_02a	358,799726	374,229052	34,363995	209	-0,44899686	0,209	999	0,44899686
13_04c	334,285801	338,472631	41,1384105	461	-0,10177425	0,461	999	0,10177425
16_08a	455,127931	466,610798	21,7742273	215	-0,52736049	0,215	999	0,52736049
22_05a	471,276201	462,779908	23,100243	838	0,36780101	0,838	999	-0,36780101
25_08a	435,19159	461,184403	24,4386469	22	-1,0635946	0,022	999	1,0635946
22_08b	469,36334	462,453714	25,5858388	839	0,27005667	0,839	999	-0,27005667
20_11a	414,091365	432,502812	29,2871139	150	-0,62865349	0,15	999	0,62865349
13_08d	453,929683	467,082826	18,1197198	111	-0,72590209	0,111	999	0,72590209
19_07d	456,719628	463,575559	19,6211725	338	-0,34941496	0,338	999	0,34941496
23_12d	453,145228	462,944765	20,874432	267	-0,46945167	0,267	999	0,46945167
22_07a	446,128861	462,790924	28,405377	125	-0,58658131	0,125	999	0,58658131
13_11a	444,113069	465,977188	21,2000972	31	-1,03132166	0,031	999	1,03132166
13_14b	362,104069	436,19564	32,9266365	1	-2,2502016	0,001	999	2,2502016
16_11a	507,79115	463,495378	20,3324576	962	2,17857441	0,962	999	-2,17857441
18_12a	449,494615	456,899424	25,1081559	363	-0,29491648	0,363	999	0,29491648
25_11a	437,503015	450,385119	20,9443607	199	-0,61506313	0,199	999	0,61506313
16_13b	454,183391	465,257241	17,8361278	204	-0,62086625	0,204	999	0,62086625
16_13d	461,413394	467,277988	21,0487879	425	-0,27861909	0,425	999	0,27861909
25_18c	459,705641	461,028132	26,3914284	620	-0,0501106	0,62	999	0,0501106
25_20c	436,419099	448,421172	28,9749841	290	-0,41422189	0,29	999	0,41422189
16_13c	440,608892	459,739579	22,5302863	77	-0,84910981	0,077	999	0,84910981
16_13a	457,703958	466,477229	20,0975855	284	-0,43653358	0,284	999	0,43653358
24_17a	454,847131	467,64437	21,0409857	175	-0,60820529	0,175	999	0,60820529
24_17b	459,25151	467,370921	21,0591016	340	-0,38555356	0,34	999	0,38555356
24_17c	466,271791	459,642603	21,583574	799	0,30714042	0,799	999	-0,30714042
24_17d	463,178417	467,751715	17,1566833	427	-0,26656074	0,427	999	0,26656074
16_19a	458,54183	463,495472	22,2393303	434	-0,2227424	0,434	999	0,2227424
16_19b	467,302853	469,009817	19,9998713	611	-0,08534872	0,611	999	0,08534872
16_19c	442,376623	462,963484	24,3510128	81	-0,84542115	0,081	999	0,84542115
16_19d	459,339061	465,651172	20,2540962	388	-0,31164611	0,388	999	0,31164611
20_14a	449,304496	462,802892	23,1626193	156	-0,58276635	0,156	999	0,58276635
20_14b	452,117058	461,966112	21,9430667	252	-0,44884583	0,252	999	0,44884583
20_14c	450,512864	464,056658	23,3729577	178	-0,5794643	0,178	999	0,5794643
20_14d	452,071458	449,894065	31,1652378	669	0,06986608	0,669	999	-0,06986608
20_21a	462,231418	470,024195	16,9141936	301	-0,46072416	0,301	999	0,46072416
20_21b	458,614543	465,493991	17,1486164	321	-0,40116636	0,321	999	0,40116636
20_21c	452,266407	468,964675	18,4067465	57	-0,90718194	0,057	999	0,90718194
20_21d	458,181736	463,666972	24,3651029	488	-0,22512672	0,488	999	0,22512672
24_24a	443,704264	469,251933	16,3853314	4	-1,5591793	0,004	999	1,5591793
24_24b	442,804709	465,999982	18,2883172	19	-1,26831095	0,019	999	1,26831095
24_24c	445,613302	468,825192	19,5170056	9	-1,18931614	0,009	999	1,18931614
24_24d	430,826643	463,735086	19,8502904	2	-1,65783183	0,002	999	1,65783183

MNTD: Barcode.uncon

Subplot	mntd,obs	mntd,rand,mean	mntd,rand,sd	mntd,obs,rank	mntd,obs,z	mntd,obs,p	runs	NTI
01_22a	128,04838	162,46795	38,4119165	191	-0,89606491	0,191	999	0,89606491
03_24a	292,881488	294,724065	67,7194976	502	-0,02720896	0,502	999	0,02720896
04_19a	99,4927908	102,90513	26,8425632	498	-0,12712419	0,498	999	0,12712419
05_25a	76,762739	131,25113	32,5588651	17	-1,6735347	0,017	999	1,6735347
07_22a	289,206294	280,355655	68,8884296	537	0,12847788	0,537	999	-0,12847788
09_15a	76,9609162	122,913061	39,560434	77	-1,16156826	0,077	999	1,16156826
10_23a	148,483151	186,94292	40,2233101	170	-0,95615624	0,17	999	0,95615624
13_24a	194,930602	165,364655	45,1150749	758	0,65534518	0,758	999	-0,65534518
14_19a	154,351234	184,961055	57,008405	303	-0,53693523	0,303	999	0,53693523
12_17a	106,279817	133,566647	27,8919571	151	-0,9783046	0,151	999	0,9783046
12_21a	99,0122832	120,318839	32,5189646	269	-0,65520399	0,269	999	0,65520399
06_23a	190,92403	220,940606	49,1039139	279	-0,61128684	0,279	999	0,61128684
01_17a	98,2863112	101,534597	23,2810262	487	-0,13952504	0,487	999	0,13952504
08_21a	206,222749	237,035265	59,0094803	302	-0,52216213	0,302	999	0,52216213
04_17a	126,76281	115,937019	24,9098727	731	0,43459842	0,731	999	-0,43459842
09_17a	112,340924	121,1476	24,0376068	376	-0,36637075	0,376	999	0,36637075
03_21a	106,568237	139,975071	34,2960354	155	-0,97407275	0,155	999	0,97407275
04_20a	103,586973	115,809565	43,6006283	491	-0,28033064	0,491	999	0,28033064
08_25a	334,365265	226,472386	60,5468132	962	1,78197453	0,962	999	-1,78197453
11_25a	156,88354	173,32542	36,4506882	332	-0,45107187	0,332	999	0,45107187
11_19a	60,0165155	187,001312	52,912106	2	-2,39991954	0,002	999	2,39991954
22_03a	170,184529	206,865159	82,7781676	339	-0,44311961	0,339	999	0,44311961
15_02a	113,766881	110,230953	28,0165689	590	0,12620849	0,59	999	-0,12620849
16_04a	98,4923958	121,319251	26,4008481	193	-0,86462583	0,193	999	0,86462583
24_04a	138,251238	167,362369	46,6905453	272	-0,62349093	0,272	999	0,62349093
19_02a	86,5244001	101,677128	20,0673325	231	-0,75509427	0,231	999	0,75509427
25_01a	210,438461	186,311681	45,3820098	717	0,53163753	0,717	999	-0,53163753
25_03a	99,1271866	132,059581	26,8799519	94	-1,22516568	0,094	999	1,22516568
23_02a	186,994292	240,942236	84,5255958	279	-0,63824388	0,279	999	0,63824388
13_04c	234,964344	191,572583	94,5862778	678	0,45875324	0,678	999	-0,45875324
16_08a	136,043392	128,90194	25,5041879	638	0,28001097	0,638	999	-0,28001097
22_05a	138,848947	108,370934	26,0232697	886	1,17118308	0,886	999	-1,17118308
25_08a	117,495194	140,914517	28,8876377	220	-0,810704	0,22	999	0,810704
22_08b	183,29567	137,775566	29,0273632	941	1,56817909	0,941	999	-1,56817909
20_11a	93,4065761	123,157316	52,1945563	298	-0,56999698	0,298	999	0,56999698
13_08d	113,38703	117,48898	21,4779811	434	-0,19098398	0,434	999	0,19098398
19_07d	82,0122701	108,115655	24,3161781	118	-1,07349869	0,118	999	1,07349869
23_12d	87,8421872	128,762345	27,8899829	59	-1,46719908	0,059	999	1,46719908
22_07a	106,8911	132,48194	30,2177896	196	-0,84687992	0,196	999	0,84687992
13_11a	135,710285	127,803076	24,0246444	641	0,32912908	0,641	999	-0,32912908
13_14b	109,357733	161,050341	52,0566662	140	-0,9930065	0,14	999	0,9930065
16_11a	154,699539	125,725077	27,60004	871	1,04979781	0,871	999	-1,04979781
18_12a	117,059338	151,097673	33,9107225	154	-1,00376318	0,154	999	1,00376318
25_11a	115,778454	165,284011	39,4838387	89	-1,25381824	0,089	999	1,25381824
16_13b	152,07456	120,951854	26,1254808	894	1,19127785	0,894	999	-1,19127785

16_13d	92,3275697	118,734161	23,5246092	119	-1,12250923	0,119	999	1,12250923
25_18c	179,346046	159,3258	33,9844474	751	0,58910024	0,751	999	-0,58910024
25_20c	170,745458	201,97067	45,4774938	252	-0,68660801	0,252	999	0,68660801
16_13c	79,1811813	133,553823	31,0912056	17	-1,74881097	0,017	999	1,74881097
16_13a	118,91539	125,034874	25,3121074	443	-0,24176115	0,443	999	0,24176115
24_17a	97,709438	121,795771	23,3562288	146	-1,03125949	0,146	999	1,03125949
24_17b	96,0609688	105,938608	23,9888311	353	-0,41175992	0,353	999	0,41175992
24_17c	102,151811	134,491951	31,6521392	125	-1,02173632	0,125	999	1,02173632
24_17d	64,9857361	108,04678	22,1198247	10	-1,94671723	0,01	999	1,94671723
16_19a	100,600477	120,657237	26,7869531	230	-0,74875108	0,23	999	0,74875108
16_19b	101,829291	112,652618	22,6812502	346	-0,47719274	0,346	999	0,47719274
16_19c	137,957417	140,962449	28,9509446	491	-0,10379736	0,491	999	0,10379736
16_19d	117,241607	123,265418	24,7108685	424	-0,24377175	0,424	999	0,24377175
20_14a	108,324417	120,670355	26,9772091	343	-0,45764324	0,343	999	0,45764324
20_14b	115,292673	129,144371	29,2613765	325	-0,47337821	0,325	999	0,47337821
20_14c	95,0762206	118,680317	27,8323275	177	-0,84808202	0,177	999	0,84808202
20_14d	164,588439	159,806966	39,1057646	565	0,12227028	0,565	999	-0,12227028
20_21a	94,7564992	104,962757	19,4221311	306	-0,5254963	0,306	999	0,5254963
20_21b	83,0578788	111,829167	23,9337881	84	-1,20212012	0,084	999	1,20212012
20_21c	63,7606341	89,5714859	19,5710799	62	-1,31882614	0,062	999	1,31882614
20_21d	97,6963199	128,640654	28,8514737	137	-1,07253913	0,137	999	1,07253913
24_24a	58,5972544	95,4380921	19,8930418	16	-1,85194592	0,016	999	1,85194592
24_24b	64,0045608	109,180525	21,9016378	6	-2,06267517	0,006	999	2,06267517
24_24c	62,5624429	104,72401	20,0622884	11	-2,10153332	0,011	999	2,10153332
24_24d	50,0919374	110,230182	27,1346659	3	-2,21628836	0,003	999	2,21628836

MPD:Phylomatic.Barcode.only

Subplot	mpd.obs	mpd.rand.mean	mpd.rand.sd	mpd.obs.rank	mpd.obs.z	mpd.obs.p	runs	NRI
01_22a	446,631938	422,734715	22,0488313	854	1,08383175	0,854	999	-1,08383175
03_24a	405,476178	339,140726	48,25979	989	1,37454911	0,989	999	-1,37454911
04_19a	410,124446	438,928334	14,7828293	37	-1,94846921	0,037	999	1,94846921
05_25a	402,353168	432,159805	18,8643759	58	-1,58004891	0,058	999	1,58004891
07_22a	351,244848	367,191188	37,5408317	336	-0,42477322	0,336	999	0,42477322
09_15a	416,732149	435,296488	17,2636268	150	-1,07534408	0,15	999	1,07534408
10_23a	434,490404	426,261403	21,0976196	624	0,39004407	0,624	999	-0,39004407
13_24a	439,463014	417,374558	23,9649254	789	0,92169932	0,789	999	-0,92169932
14_19a	372,838267	400,914788	28,9989879	171	-0,96818969	0,171	999	0,96818969
12_17a	446,406015	434,666678	18,117953	717	0,64793947	0,717	999	-0,64793947
12_21a	386,171235	430,737586	18,5597031	11	-2,40124268	0,011	999	2,40124268
06_23a	454,320994	419,873509	23,7656761	952	1,44946372	0,952	999	-1,44946372
01_17a	410,26645	437,895741	16,1445962	48	-1,71136465	0,048	999	1,71136465
08_21a	430,436881	406,06381	28,6798293	773	0,84983321	0,773	999	-0,84983321
04_17a	426,150447	437,879469	15,45807	230	-0,75876369	0,23	999	0,75876369
09_17a	418,729802	437,535459	16,254771	125	-1,15693156	0,125	999	1,15693156
03_21a	425,403947	424,827638	21,3946738	516	0,02693704	0,516	999	-0,02693704
04_20a	399,017734	404,593586	23,7917341	447	-0,23436088	0,447	999	0,23436088
08_25a	408,307399	405,253122	29,2384423	523	0,10446101	0,523	999	-0,10446101

11_25a	436,242565	429,74294	19,5712997	587	0,33209984	0,587	999	-0,33209984
11_19a	352,774572	399,399736	28,8795134	54	-1,61447195	0,054	999	1,61447195
22_03a	371,0253	379,557007	34,2238566	407	-0,24929122	0,407	999	0,24929122
15_02a	413,987354	431,684969	17,9596565	173	-0,98540942	0,173	999	0,98540942
16_04a	425,479861	433,312958	17,1900791	327	-0,45567544	0,327	999	0,45567544
24_04a	411,355777	425,029732	21,9268021	256	-0,6236183	0,256	999	0,6236183
19_02a	436,809346	441,862243	13,7383835	328	-0,3677941	0,328	999	0,3677941
25_01a	449,597034	423,27517	23,5561693	877	1,11740853	0,877	999	-1,11740853
25_03a	435,688006	436,169643	16,8412669	466	-0,02859864	0,466	999	0,02859864
23_02a	397,39741	378,179931	33,4381327	707	0,57471748	0,707	999	-0,57471748
13_04c	324,690903	309,839797	29,8652786	739	0,49726995	0,739	999	-0,49726995
16_08a	433,070907	435,440168	16,8605708	411	-0,14052081	0,411	999	0,14052081
22_05a	403,982715	434,44749	17,2447088	38	-1,76661579	0,038	999	1,76661579
25_08a	425,468238	433,265194	18,5598674	302	-0,4200976	0,302	999	0,4200976
22_08b	412,398947	433,88736	17,6129435	120	-1,2200353	0,12	999	1,2200353
20_11a	366,785742	407,207268	23,7335964	40	-1,70313531	0,04	999	1,70313531
13_08d	444,07081	439,320508	14,7845016	605	0,32130285	0,605	999	-0,32130285
19_07d	431,21532	435,964157	16,9525091	367	-0,2801259	0,367	999	0,2801259
23_12d	434,755306	435,517806	17,3497741	453	-0,04394868	0,453	999	0,04394868
22_07a	404,432859	429,241572	19,5559667	103	-1,26860067	0,103	999	1,26860067
13_11a	401,005273	437,664878	15,9966526	20	-2,29170475	0,02	999	2,29170475
13_14b	339,087708	399,24757	29,5026909	26	-2,03913134	0,026	999	2,03913134
16_11a	427,130599	437,635239	16,1779931	251	-0,64931659	0,251	999	0,64931659
18_12a	411,615871	432,140079	18,8007466	134	-1,09166983	0,134	999	1,09166983
25_11a	398,764365	425,859274	21,7217361	120	-1,2473639	0,12	999	1,2473639
16_13b	454,827862	437,483797	16,138181	856	1,07472239	0,856	999	-1,07472239
16_13d	429,695455	438,617427	15,01798	277	-0,59408605	0,277	999	0,59408605
16_13c	396,045313	431,929495	18,7138233	35	-1,91752274	0,035	999	1,91752274
16_13a	418,345791	438,485787	15,3295908	102	-1,31379864	0,102	999	1,31379864
24_17a	441,084452	438,536965	15,0356391	537	0,1694299	0,537	999	-0,1694299
24_17b	432,490386	438,541281	15,025852	333	-0,40269894	0,333	999	0,40269894
24_17c	424,046673	432,41856	18,5967438	319	-0,45018025	0,319	999	0,45018025
24_17d	428,763405	439,488404	14,4665755	230	-0,74136405	0,23	999	0,74136405
16_19a	419,19948	433,662075	17,4582629	200	-0,82840971	0,2	999	0,82840971
16_19b	419,83029	439,499969	14,7357166	92	-1,33483021	0,092	999	1,33483021
16_19c	414,55618	434,231378	17,1339602	126	-1,14831583	0,126	999	1,14831583
16_19d	451,787991	436,736923	16,1815477	807	0,93013769	0,807	999	-0,93013769
20_14a	409,058388	433,150096	18,2291614	102	-1,32160264	0,102	999	1,32160264
20_14b	417,610917	433,978153	17,8635222	178	-0,9162379	0,178	999	0,9162379
20_14c	417,822984	434,658953	17,6158374	187	-0,95572909	0,187	999	0,95572909
20_14d	391,25618	416,017514	24,8879518	157	-0,99491249	0,157	999	0,99491249
20_21a	433,37321	441,335412	13,8188804	261	-0,57618289	0,261	999	0,57618289
20_21b	431,337852	438,148713	15,3233736	324	-0,44447532	0,324	999	0,44447532
20_21c	423,246863	439,648326	15,1017423	152	-1,08606427	0,152	999	1,08606427
20_21d	442,772958	437,279607	17,0802932	615	0,32161927	0,615	999	-0,32161927
24_24a	432,349747	440,001322	14,1520222	296	-0,54067006	0,296	999	0,54067006
24_24b	415,501769	438,018857	15,8619482	91	-1,41956637	0,091	999	1,41956637
24_24c	412,066935	439,915772	14,5498899	35	-1,91402387	0,035	999	1,91402387

24_24d	413,614182	434,027195	17,1425048	123	-1,19078358	0,123	999	1,19078358

MNTD:Phylocom.Barcode.only

Subplot	mpd,obs	mpd,rand,mean	mpd,rand,sd	mpd,obs,rank	mpd,obs,z	mpd,obs,p	runs	NTI
01_22a	183,971718	195,446321	33,4794506	383	-0,34273571	0,383	999	0,34273571
03_24a	445,71428	370,871924	83,4110528	787	0,89727144	0,787	999	-0,89727144
04_19a	131,508718	151,963059	17,7803281	122	-1,15039166	0,122	999	1,15039166
05_25a	109,675522	176,65828	26,1012551	1	-2,56626578	0,001	999	2,56626578
07_22a	314,787815	328,747824	71,9399139	412	-0,19405095	0,412	999	0,19405095
09_15a	134,124148	168,14861	22,6082527	64	-1,50495762	0,064	999	1,50495762
10_23a	176,330924	208,018602	32,1619245	162	-0,98525442	0,162	999	0,98525442
13_24a	235,390753	195,730305	37,7130646	848	1,05163682	0,848	999	-1,05163682
14_19a	203,305541	219,198951	46,3795113	374	-0,34268171	0,374	999	0,34268171
12_17a	138,531787	170,263208	22,651665	79	-1,40084276	0,079	999	1,40084276
12_21a	123,287213	159,549988	23,6057866	61	-1,53618158	0,061	999	1,53618158
06_23a	213,852392	234,145025	39,9032083	309	-0,5085464	0,309	999	0,5085464
01_17a	146,969095	151,866021	20,1547317	424	-0,24296656	0,424	999	0,24296656
08_21a	251,220041	255,27653	48,206289	485	-0,08414854	0,485	999	0,08414854
04_17a	179,121409	155,051483	19,7838791	879	1,2166434	0,879	999	-1,2166434
09_17a	137,712065	161,551829	19,8356232	121	-1,20186619	0,121	999	1,20186619
03_21a	169,687781	184,406237	30,5419933	343	-0,48190882	0,343	999	0,48190882
04_20a	134,04201	162,255001	36,2402665	252	-0,77849843	0,252	999	0,77849843
08_25a	293,691227	233,663278	45,8372149	899	1,30958979	0,899	999	-1,30958979
11_25a	175,38189	195,952082	29,0691876	227	-0,70762871	0,227	999	0,70762871
11_19a	123,643041	231,85795	46,9652749	5	-2,30414725	0,005	999	2,30414725
22_03a	231,664264	230,693919	54,0234413	502	0,01796155	0,502	999	-0,01796155
15_02a	135,453979	153,916245	24,3835606	231	-0,75716039	0,231	999	0,75716039
16_04a	149,304199	166,336182	23,3666013	242	-0,7289029	0,242	999	0,7289029
24_04a	146,162423	193,674799	30,9260478	62	-1,53632226	0,062	999	1,53632226
19_02a	129,315977	147,148756	16,4983245	134	-1,08088425	0,134	999	1,08088425
25_01a	201,583669	211,311456	34,1349033	379	-0,28498066	0,379	999	0,28498066
25_03a	142,264197	172,612229	22,6970124	97	-1,33709368	0,097	999	1,33709368
23_02a	199,126199	254,916233	56,590095	192	-0,98586217	0,192	999	0,98586217
13_04c	198,08914	242,691111	79,7008748	340	-0,55961709	0,34	999	0,55961709
16_08a	182,89074	173,845798	22,3807644	651	0,40413911	0,651	999	-0,40413911
22_05a	151,636137	151,507195	21,2051316	514	0,00608068	0,514	999	-0,00608068
25_08a	144,51812	176,264817	24,9116821	98	-1,27436988	0,098	999	1,27436988
22_08b	208,817952	174,965298	24,6243045	905	1,3747659	0,905	999	-1,3747659
20_11a	144,037806	162,415957	37,8809691	379	-0,48515525	0,379	999	0,48515525
13_08d	153,593859	161,266512	18,4547913	336	-0,41575399	0,336	999	0,41575399
19_07d	122,00497	158,921165	21,5757227	40	-1,71100615	0,04	999	1,71100615
23_12d	141,74371	171,063221	23,087685	97	-1,2699199	0,097	999	1,2699199
22_07a	144,533401	175,865832	26,5452782	123	-1,18033916	0,123	999	1,18033916
13_11a	166,099073	171,55984	22,1656374	420	-0,24636184	0,42	999	0,24636184
13_14b	135,505001	197,555976	44,4541233	77	-1,39584294	0,077	999	1,39584294
16_11a	161,783783	164,833636	20,6019571	468	-0,14803702	0,468	999	0,14803702
18_12a	164,424896	178,430195	26,1653292	302	-0,53526172	0,302	999	0,53526172

25_11a	171,513631	203,793746	31,03891	148	-1,03998869	0,148	999	1,03998869
16_13b	195,513259	166,487729	21,5060451	907	1,34964519	0,907	999	-1,34964519
16_13d	150,524973	156,622337	19,5806914	390	-0,31139673	0,39	999	0,31139673
16_13c	132,365464	182,586465	26,2714685	16	-1,91161758	0,016	999	1,91161758
16_13a	163,106087	162,717795	19,2918349	516	0,02012725	0,516	999	-0,02012725
24_17a	140,854567	162,09461	19,701405	147	-1,07809788	0,147	999	1,07809788
24_17b	134,940734	148,902309	18,3223358	222	-0,76199754	0,222	999	0,76199754
24_17c	136,892378	175,96296	24,7560152	49	-1,57822579	0,049	999	1,57822579
24_17d	129,600959	152,330082	18,3379807	101	-1,23945615	0,101	999	1,23945615
16_19a	147,346066	166,605196	22,9981534	190	-0,83742072	0,19	999	0,83742072
16_19b	145,401926	156,116354	18,0783248	287	-0,59266713	0,287	999	0,59266713
16_19c	158,944533	180,613959	24,8343901	199	-0,8725572	0,199	999	0,8725572
16_19d	169,960405	166,605116	21,9056504	566	0,15317002	0,566	999	-0,15317002
20_14a	154,425739	164,578061	23,2159009	345	-0,43730039	0,345	999	0,43730039
20_14b	137,609876	170,56462	24,311158	79	-1,35553986	0,079	999	1,35553986
20_14c	142,815907	164,780099	22,6914805	161	-0,96794883	0,161	999	0,96794883
20_14d	208,461474	202,364584	38,0305273	578	0,1603157	0,578	999	-0,1603157
20_21a	177,176292	157,00719	18,4808047	863	1,09135413	0,863	999	-1,09135413
20_21b	144,430806	161,128758	20,1203545	197	-0,82990346	0,197	999	0,82990346
20_21c	122,617968	145,282304	17,2447449	95	-1,31427493	0,095	999	1,31427493
20_21d	150,444591	167,952535	21,2205657	204	-0,82504602	0,204	999	0,82504602
24_24a	111,036629	151,253175	17,9007853	11	-2,24663583	0,011	999	2,24663583
24_24b	131,602945	160,992968	21,2331204	78	-1,38415939	0,078	999	1,38415939
24_24c	110,54438	152,186009	17,5438681	8	-2,37357171	0,008	999	2,37357171
24_24d	112,552263	161,792503	22,3071524	8	-2,20737454	0,008	999	2,20737454

MPD: Barcodeonly.con

Sublot	mpd.obs	mpd.rand.mean	mpd.rand.sd	mpd.obs.rank	mpd.obs.z	mpd.obs.p	runs	NRI
01_22a	503,636476	478,31444	37,7803769	822	0,67024307	0,822	999	-0,67024307
03_24a	448,267933	382,437749	75,3868312	895,5	0,87323187	0,8955	999	-0,87323187
04_19a	465,027963	494,371574	25,1291243	100	-1,16771323	0,1	999	1,16771323
05_25a	460,286951	487,978385	33,5320167	157	-0,82582071	0,157	999	0,82582071
07_22a	373,063243	414,870465	59,3382309	217	-0,70455794	0,217	999	0,70455794
09_15a	455,995719	491,472069	31,1038736	92	-1,14057658	0,092	999	1,14057658
10_23a	491,477125	481,473068	34,8486623	620	0,28707148	0,62	999	-0,28707148
13_24a	487,929013	470,675519	46,662002	712	0,36975469	0,712	999	-0,36975469
14_19a	406,238344	452,169625	58,3064127	147	-0,78775693	0,147	999	0,78775693
12_17a	502,021076	491,74612	31,6199402	690	0,32495179	0,69	999	-0,32495179
12_21a	434,811144	486,643405	35,9488594	19	-1,44183326	0,019	999	1,44183326
06_23a	510,155674	473,818972	41,9646131	928	0,86588912	0,928	999	-0,86588912
01_17a	455,637615	493,685661	27,3124454	43	-1,39306627	0,043	999	1,39306627
08_21a	489,862479	459,976413	51,0203368	803	0,58576771	0,803	999	-0,58576771
04_17a	477,925928	494,642474	27,9053468	253	-0,59904455	0,253	999	0,59904455
09_17a	470,230102	496,653183	30,1737477	143	-0,8756977	0,143	999	0,8756977
03_21a	476,048774	479,778245	35,477522	491	-0,10512208	0,491	999	0,10512208
04_20a	442,711154	455,984831	37,750436	383	-0,35161652	0,383	999	0,35161652
08_25a	454,59062	457,725169	48,4380179	490	-0,06471258	0,49	999	0,06471258

11_25a	486,73456	486,122366	35,6321014	527	0,01718096	0,527	999	-0,01718096
11_19a	381,262779	450,124388	46,9884331	44	-1,46550128	0,044	999	1,46550128
22_03a	408,403255	428,644747	50,5541086	355	-0,40039261	0,355	999	0,40039261
15_02a	465,882201	486,250411	29,3182136	215	-0,69472887	0,215	999	0,69472887
16_04a	476,458838	492,528957	33,5930383	294	-0,47837648	0,294	999	0,47837648
24_04a	446,996871	479,742466	36,2796433	133	-0,90258867	0,133	999	0,90258867
19_02a	490,119593	497,111386	22,68508	385	-0,30821111	0,385	999	0,30821111
25_01a	506,652616	477,396427	33,2045428	886	0,88108994	0,886	999	-0,88108994
25_03a	489,024786	495,438536	31,501233	419	-0,20360313	0,419	999	0,20360313
23_02a	447,297935	429,007575	56,0577155	666	0,3262773	0,666	999	-0,3262773
13_04c	370,181523	346,973041	45,530659	788	0,50973305	0,788	999	-0,50973305
16_08a	486,815109	492,204548	28,7746725	442	-0,187298	0,442	999	0,187298
22_05a	486,284521	490,581348	30,5556849	469	-0,14062284	0,469	999	0,14062284
25_08a	480,316321	489,66177	30,1746198	362	-0,30971224	0,362	999	0,30971224
22_08b	458,538503	489,769272	32,6348254	120	-0,95697673	0,12	999	0,95697673
20_11a	402,576711	458,356548	48,5932744	33	-1,14789213	0,033	999	1,14789213
13_08d	498,633673	498,383344	27,080106	556	0,00924399	0,556	999	-0,00924399
19_07d	481,072613	493,308792	27,1000226	330	-0,45151914	0,33	999	0,45151914
23_12d	486,70603	492,296314	30,761806	439	-0,1817281	0,439	999	0,1817281
22_07a	452,447281	483,18809	33,6989794	143	-0,91221781	0,143	999	0,91221781
13_11a	443,472774	494,211339	28,0432934	19	-1,80929411	0,019	999	1,80929411
13_14b	375,788023	450,704175	46,8059442	29	-1,60056917	0,029	999	1,60056917
16_11a	474,897861	493,891158	27,8131787	212	-0,68288838	0,212	999	0,68288838
18_12a	462,194219	487,607328	32,7788613	166	-0,77528955	0,166	999	0,77528955
25_11a	438,503878	482,187965	41,4965361	92	-1,05271646	0,092	999	1,05271646
16_13b	507,534902	493,884137	30,8155796	757	0,44298259	0,757	999	-0,44298259
16_13d	476,998748	494,647669	25,7128741	232	-0,68638459	0,232	999	0,68638459
16_13c	445,452097	489,487485	34,0560817	48	-1,29302568	0,048	999	1,29302568
16_13a	464,408597	496,390856	27,7086047	72	-1,15423563	0,072	999	1,15423563
24_17a	490,298622	496,184557	27,9031346	430	-0,21094173	0,43	999	0,21094173
24_17b	479,062688	495,58419	24,6415786	234	-0,67047255	0,234	999	0,67047255
24_17c	469,988926	489,186033	29,7340172	235	-0,64562776	0,235	999	0,64562776
24_17d	477,573164	496,574731	24,8376244	190	-0,76503156	0,19	999	0,76503156
16_19a	471,781777	491,894341	34,5906627	230	-0,58144489	0,23	999	0,58144489
16_19b	469,385194	497,931557	26,2390954	105	-1,08793245	0,105	999	1,08793245
16_19c	469,595924	490,453214	32,5061847	214	-0,64164069	0,214	999	0,64164069
16_19d	506,640134	495,705406	31,2241449	739	0,35020104	0,739	999	-0,35020104
20_14a	460,157505	488,873778	32,299003	125	-0,88907614	0,125	999	0,88907614
20_14b	465,823632	490,52089	32,1418762	167	-0,76838259	0,167	999	0,76838259
20_14c	468,714265	491,298927	31,2698272	184	-0,72225093	0,184	999	0,72225093
20_14d	429,378967	469,404715	44,3749692	123	-0,90198931	0,123	999	0,90198931
20_21a	483,886264	498,383947	24,0421028	244	-0,60301227	0,244	999	0,60301227
20_21b	485,421917	494,608759	26,8949939	355	-0,34158186	0,355	999	0,34158186
20_21c	477,501915	497,144537	27,5121589	199	-0,71396147	0,199	999	0,71396147
20_21d	497,830524	493,581897	32,299754	625	0,13153744	0,625	999	-0,13153744
24_24a	486,608744	498,374344	25,9992643	302	-0,45253586	0,302	999	0,45253586
24_24b	459,69211	494,587691	28,0141236	62	-1,24564244	0,062	999	1,24564244
24_24c	455,34208	498,824563	26,4885805	17	-1,64155582	0,017	999	1,64155582

24_24d	462,76563	492,942707	32,3329652	124	-0,93332231	0,124	999	0,93332231

MNTD: Barcodeonly.con

Subplot	mntd.obs	mntd.rand.mean	mntd.rand.sd	mntd.obs.rank	mntd.obs.z	mntd.obs.p	runs	NTI
01_22a	139,389448	183,432078	47,0206986	143	-0,93666474	0,143	999	0,93666474
03_24a	398,322566	398,99738	105,760488	439,5	-0,00638058	0,4395	999	0,00638058
04_19a	91,0628137	118,475764	26,4719915	130	-1,03554544	0,13	999	1,03554544
05_25a	74,4380113	147,978983	33,6492362	3	-2,18551683	0,003	999	2,18551683
07_22a	305,870393	352,576199	93,5331596	306	-0,49935025	0,306	999	0,49935025
09_15a	97,3473408	141,858608	31,5138818	58	-1,41243365	0,058	999	1,41243365
10_23a	156,153012	191,204429	41,1082695	185	-0,85266098	0,185	999	0,85266098
13_24a	196,326811	178,828248	50,5122613	681	0,34642208	0,681	999	-0,34642208
14_19a	168,101837	207,659918	60,3807249	287	-0,65514419	0,287	999	0,65514419
12_17a	115,98839	144,316911	32,1511166	190	-0,88110536	0,19	999	0,88110536
12_21a	109,977941	127,820467	33,7569333	305	-0,52855885	0,305	999	0,52855885
06_23a	192,166824	229,243145	51,5444367	228	-0,71930791	0,228	999	0,71930791
01_17a	112,621904	116,932034	28,619855	481	-0,15059929	0,481	999	0,15059929
08_21a	263,531755	259,557774	65,0166633	535	0,06112249	0,535	999	-0,06112249
04_17a	136,990045	121,575831	26,9531511	730	0,57188914	0,73	999	-0,57188914
09_17a	119,25659	129,88184	26,6543463	363	-0,39863108	0,363	999	0,39863108
03_21a	148,511466	159,089159	43,0251189	431	-0,24584925	0,431	999	0,24584925
04_20a	116,284392	127,438411	50,1587784	506	-0,22237422	0,506	999	0,22237422
08_25a	329,208299	230,687305	62,6792348	951	1,57182828	0,951	999	-1,57182828
11_25a	154,821308	178,352249	39,8264862	272	-0,59083648	0,272	999	0,59083648
11_19a	83,8199125	222,636675	64,5025949	2	-2,15211128	0,002	999	2,15211128
22_03a	210,306421	220,517238	76,2377808	457	-0,13393381	0,457	999	0,13393381
15_02a	117,662759	120,844371	33,5332081	506	-0,09487944	0,506	999	0,09487944
16_04a	96,0991973	136,961076	30,7738854	71	-1,3278102	0,071	999	1,3278102
24_04a	122,610008	174,612097	44,899371	105	-1,15819192	0,105	999	1,15819192
19_02a	90,9101334	110,987146	22,6786459	181	-0,88528271	0,181	999	0,88528271
25_01a	201,064063	199,646192	48,9599549	525	0,0289598	0,525	999	-0,0289598
25_03a	113,436906	146,707332	31,1096292	127	-1,06945748	0,127	999	1,06945748
23_02a	195,422611	258,75735	73,5581807	211	-0,86101557	0,211	999	0,86101557
13_04c	253,472343	235,050496	100,700513	545	0,18293698	0,545	999	-0,18293698
16_08a	158,034536	147,119028	29,4525555	668	0,37061329	0,668	999	-0,37061329
22_05a	143,359982	116,991579	30,5587952	827	0,86287441	0,827	999	-0,86287441
25_08a	118,882166	149,866416	32,2320433	162	-0,9612872	0,162	999	0,9612872
22_08b	196,583582	148,913602	34,7124813	925	1,37328069	0,925	999	-1,37328069
20_11a	102,047414	133,035603	52,4418684	294	-0,5909055	0,294	999	0,5909055
13_08d	114,041858	129,286041	25,39856	264	-0,60019871	0,264	999	0,60019871
19_07d	85,9237958	128,111331	30,0236607	46	-1,40514295	0,046	999	1,40514295
23_12d	110,732686	143,997257	30,8960804	135	-1,07665991	0,135	999	1,07665991
22_07a	137,135477	151,409602	36,6561115	375	-0,3894064	0,375	999	0,3894064
13_11a	155,521657	143,989497	30,1078954	678	0,38302775	0,678	999	-0,38302775
13_14b	110,2772	180,77522	60,999685	106	-1,15571122	0,106	999	1,15571122
16_11a	138,235243	135,386267	27,8777617	560	0,10219529	0,56	999	-0,10219529
18_12a	130,469102	152,94586	32,6112066	246	-0,68923417	0,246	999	0,68923417

25_11a	181,222702	184,986512	41,937978	485	-0,08974708	0,485	999	0,08974708
16_13b	159,377791	138,412577	28,8381365	785	0,72699615	0,785	999	-0,72699615
16_13d	100,113845	125,925548	27,1734641	156	-0,94988637	0,156	999	0,94988637
16_13c	84,4644271	160,35566	36,9956602	5	-2,05135502	0,005	999	2,05135502
16_13a	129,557967	133,147671	27,3602712	464	-0,13120131	0,464	999	0,13120131
24_17a	95,1865648	131,32001	25,5515069	76	-1,41414147	0,076	999	1,41414147
24_17b	98,4425275	113,600913	24,55816	302	-0,61724434	0,302	999	0,61724434
24_17c	92,971991	150,862345	32,3543184	21	-1,78926206	0,021	999	1,78926206
24_17d	78,8128256	120,085587	25,1604409	35	-1,64038309	0,035	999	1,64038309
16_19a	120,116152	138,540794	33,6929857	328	-0,54683911	0,328	999	0,54683911
16_19b	110,360076	123,689199	25,869867	319	-0,51523737	0,319	999	0,51523737
16_19c	137,336994	158,93296	35,2219863	274	-0,61313878	0,274	999	0,61313878
16_19d	137,676584	136,684462	28,7529201	547	0,03450509	0,547	999	-0,03450509
20_14a	112,292304	134,866349	33,2089046	247	-0,6797588	0,247	999	0,6797588
20_14b	104,643305	145,30614	35,2311963	95	-1,15417128	0,095	999	1,15417128
20_14c	112,975262	135,726982	32,836215	246	-0,69288498	0,246	999	0,69288498
20_14d	185,716442	185,210928	52,604613	518	0,00960967	0,518	999	-0,00960967
20_21a	132,07372	125,424093	23,2734162	658	0,28571769	0,658	999	-0,28571769
20_21b	97,124569	128,098639	28,3596584	118	-1,0921877	0,118	999	1,0921877
20_21c	90,6125541	107,710172	22,8724449	234	-0,74752035	0,234	999	0,74752035
20_21d	118,538741	138,176326	31,1353945	270	-0,63071579	0,27	999	0,63071579
24_24a	63,1894314	116,153063	22,2660837	4	-2,37866848	0,004	999	2,37866848
24_24b	96,8732544	127,616719	26,8708134	118	-1,14412109	0,118	999	1,14412109
24_24c	77,8648438	117,447722	24,7085756	36	-1,6019895	0,036	999	1,6019895
24_24d	62,4307663	132,107057	31,0155459	1	-2,2464957	0,001	999	2,2464957

MPD: Barcodeonly.uncon

Subplot	mpd.obs	mpd.rand.mean	mpd.rand.sd	mpd.obs.rank	mpd.obs.z	mpd.obs.p	runs	NRI
01_22a	435,900976	447,920047	30,2132258	257	-0,39780824	0,257	999	0,39780824
03_24a	353,8728	361,192543	58,5892455	384,5	-0,1249332	0,3845	999	0,1249332
04_19a	446,727213	465,280206	18,7314845	60	-0,99047103	0,06	999	0,99047103
05_25a	416,831918	457,847805	23,8493046	3	-1,71979385	0,003	999	1,71979385
07_22a	375,557121	390,70443	48,314543	297	-0,3135145	0,297	999	0,3135145
09_15a	429,240822	461,015825	26,3844989	14	-1,2043057	0,014	999	1,2043057
10_23a	435,07594	451,558077	28,1457775	164	-0,58559893	0,164	999	0,58559893
13_24a	454,324456	440,441676	26,7003313	845	0,51994787	0,845	999	-0,51994787
14_19a	387,831625	422,835431	31,0351053	58	-1,1278778	0,058	999	1,1278778
12_17a	439,692474	459,957545	22,5405716	78	-0,89904867	0,078	999	0,89904867
12_21a	421,440957	457,881547	25,4662795	9	-1,430935	0,009	999	1,430935
06_23a	448,460257	445,930942	35,1255921	662	0,07200777	0,662	999	-0,07200777
01_17a	449,029986	462,985334	18,6455112	143	-0,74845619	0,143	999	0,74845619
08_21a	428,106797	428,198955	33,5419366	537	-0,00274754	0,537	999	0,00274754
04_17a	452,383661	464,783721	25,3692126	219	-0,48878382	0,219	999	0,48878382
09_17a	448,678969	465,644551	23,9851487	120	-0,70733695	0,12	999	0,70733695
03_21a	457,553817	448,589718	25,3191809	815	0,35404377	0,815	999	-0,35404377
04_20a	432,276636	428,026041	28,1496965	705	0,15099968	0,705	999	-0,15099968
08_25a	440,211351	427,784417	33,855188	790	0,36706145	0,79	999	-0,36706145

11_25a	445,065257	455,2803	27,4713758	277	-0,37184318	0,277	999	0,37184318
11_19a	391,65161	424,106873	35,1984605	70	-0,92206485	0,07	999	0,92206485
22_03a	401,611637	402,138764	42,6171096	486	-0,01236892	0,486	999	0,01236892
15_02a	454,412991	457,437002	30,8599162	547	-0,09799154	0,547	999	0,09799154
16_04a	451,912165	462,264266	29,8603315	321	-0,34668404	0,321	999	0,34668404
24_04a	438,581707	450,756457	34,7103	279	-0,35075322	0,279	999	0,35075322
19_02a	463,178959	468,475068	21,422193	469	-0,24722533	0,469	999	0,24722533
25_01a	443,742026	448,599007	30,8217619	477	-0,15758285	0,477	999	0,15758285
25_03a	448,419619	462,21499	23,0178192	186	-0,59933437	0,186	999	0,59933437
23_02a	395,602594	402,420807	43,8301052	378	-0,15556004	0,378	999	0,15556004
13_04c	330,756829	326,458382	41,9708519	607	0,10241505	0,607	999	-0,10241505
16_08a	455,972774	462,452796	25,7064897	421	-0,25207726	0,421	999	0,25207726
22_05a	473,453744	459,617899	21,4969216	898	0,64361983	0,898	999	-0,64361983
25_08a	445,835548	459,413537	24,2469039	199	-0,55998856	0,199	999	0,55998856
22_08b	460,147169	459,115047	24,1655386	656	0,04271049	0,656	999	-0,04271049
20_11a	418,0496	429,439828	22,8162051	270	-0,49921658	0,27	999	0,49921658
13_08d	449,459578	466,751552	21,4574739	71	-0,80587184	0,071	999	0,80587184
19_07d	456,179755	462,015979	19,8074349	385	-0,29464819	0,385	999	0,29464819
23_12d	453,060266	461,811153	22,9775198	303	-0,38084562	0,303	999	0,38084562
22_07a	440,266256	455,608155	23,1626318	149	-0,66235561	0,149	999	0,66235561
13_11a	448,819744	462,56511	21,1846922	152	-0,64883485	0,152	999	0,64883485
13_14b	365,745917	422,619428	46,0899576	23	-1,23396754	0,023	999	1,23396754
16_11a	456,530325	464,281855	21,2169744	334	-0,3653457	0,334	999	0,3653457
18_12a	456,302751	457,113819	24,527174	558	-0,03306816	0,558	999	0,03306816
25_11a	438,473406	450,72833	24,1492334	236	-0,50746636	0,236	999	0,50746636
16_13b	449,51923	461,953901	21,1834088	164	-0,58700046	0,164	999	0,58700046
16_13d	459,168077	465,343464	21,0550577	396	-0,29329708	0,396	999	0,29329708
16_13c	430,113316	458,163791	25,5026303	23	-1,09990518	0,023	999	1,09990518
16_13a	460,041699	465,228815	20,5654175	456	-0,25222517	0,456	999	0,25222517
24_17a	448,4694	465,609328	19,7144962	95	-0,86940735	0,095	999	0,86940735
24_17b	454,541034	465,608261	22,4812298	194	-0,4922874	0,194	999	0,4922874
24_17c	457,936153	458,859989	24,9676451	609	-0,03700133	0,609	999	0,03700133
24_17d	458,877523	465,773758	19,9256164	368	-0,34609896	0,368	999	0,34609896
16_19a	454,691344	460,251934	21,2843894	430	-0,26125202	0,43	999	0,26125202
16_19b	466,305149	467,115469	20,8694576	643	-0,03882803	0,643	999	0,03882803
16_19c	441,177827	460,240077	24,409357	100	-0,78094026	0,1	999	0,78094026
16_19d	456,043078	463,143609	21,5894189	347	-0,32888939	0,347	999	0,32888939
20_14a	445,863672	458,614998	24,7471111	197	-0,51526523	0,197	999	0,51526523
20_14b	443,336799	458,916335	22,6426455	150	-0,68806166	0,15	999	0,68806166
20_14c	449,712472	460,434927	21,6234317	246	-0,49587203	0,246	999	0,49587203
20_14d	437,039809	441,358044	34,9441577	471	-0,12357528	0,471	999	0,12357528
20_21a	462,041337	467,428576	19,1514005	436	-0,28129738	0,436	999	0,28129738
20_21b	460,288288	464,920851	21,2908063	461	-0,21758513	0,461	999	0,21758513
20_21c	448,735229	466,144326	18,2806213	63	-0,95232526	0,063	999	0,95232526
20_21d	456,775285	463,013675	22,282192	415	-0,27997198	0,415	999	0,27997198
24_24a	446,601608	467,213132	18,3053287	33	-1,12598492	0,033	999	1,12598492
24_24b	454,778063	463,774458	20,2516133	281	-0,44423101	0,281	999	0,44423101
24_24c	443,967062	467,120602	19,5776279	24	-1,18265296	0,024	999	1,18265296

24_24d	435,844091	461,67819	22,1577624	24	-1,16591642	0,024	999	1,16591642

MNTD: Barcode.only.uncon

Subplot	mntd.obs	mntd.rand.mean	mntd.rand.sd	mntd.obs.rank	mntd.obs.z	mntd.obs.p	runs	NTI
01_22a	143,431163	178,353824	43,0070836	209	-0,81202115	0,209	999	0,81202115
03_24a	390,237598	399,530922	97,6412723	392	-0,09517823	0,392	999	0,09517823
04_19a	85,1431356	117,420072	23,207337	72	-1,3908074	0,072	999	1,3908074
05_25a	74,1452672	148,150211	33,0255924	2	-2,24083622	0,002	999	2,24083622
07_22a	311,150883	344,211466	86,8889833	344	-0,38049223	0,344	999	0,38049223
09_15a	100,386315	138,715485	28,1039287	77	-1,36383672	0,077	999	1,36383672
10_23a	155,655127	190,457356	40,4118741	193	-0,8611882	0,193	999	0,8611882
13_24a	195,891026	178,317346	49,9862691	670	0,35157015	0,67	999	-0,35157015
14_19a	170,225903	207,1877	60,2099012	275	-0,61388237	0,275	999	0,61388237
12_17a	115,856543	143,429773	32,6173206	201	-0,84535548	0,201	999	0,84535548
12_21a	116,149162	127,802773	33,4190083	397	-0,34871207	0,397	999	0,34871207
06_23a	200,615733	231,952149	49,3488066	280	-0,63499844	0,28	999	0,63499844
01_17a	112,251972	115,848867	27,3762	487	-0,13138765	0,487	999	0,13138765
08_21a	272,715716	259,33347	66,4424807	587	0,20141099	0,587	999	-0,20141099
04_17a	125,303939	118,561641	26,3031335	636	0,2563306	0,636	999	-0,2563306
09_17a	120,468496	130,049846	26,5899766	367	-0,36033689	0,367	999	0,36033689
03_21a	147,905385	159,356145	39,2299971	395	-0,29188788	0,395	999	0,29188788
04_20a	115,964049	129,842687	49,979762	487	-0,27768515	0,487	999	0,27768515
08_25a	331,574153	232,364021	60,2501151	956	1,64663806	0,956	999	-1,64663806
11_25a	154,235912	178,14413	37,2450102	270	-0,64191733	0,27	999	0,64191733
11_19a	77,078924	227,24279	62,7838355	2	-2,39175999	0,002	999	2,39175999
22_03a	204,251298	226,247544	73,3041361	384	-0,30006827	0,384	999	0,30006827
15_02a	117,245646	121,43103	33,6012907	482	-0,12456023	0,482	999	0,12456023
16_04a	95,9354148	136,760435	30,1453108	71	-1,3542743	0,071	999	1,3542743
24_04a	134,093579	173,898391	41,0914898	159	-0,96868748	0,159	999	0,96868748
19_02a	90,6790025	110,197015	22,5378699	191	-0,86600963	0,191	999	0,86600963
25_01a	204,091194	195,205695	45,359042	588	0,19589256	0,588	999	-0,19589256
25_03a	115,491427	146,170658	28,3767667	127	-1,08113907	0,127	999	1,08113907
23_02a	194,578908	257,143649	76,0453866	218	-0,82272894	0,218	999	0,82272894
13_04c	252,582609	233,313632	101,558047	544	0,18973364	0,544	999	-0,18973364
16_08a	164,372567	148,859294	29,9943231	736	0,51720696	0,736	999	-0,51720696
22_05a	143,28319	114,975255	29,9528267	857	0,94508392	0,857	999	-0,94508392
25_08a	124,006513	152,592407	34,1873341	186	-0,83615453	0,186	999	0,83615453
22_08b	195,745734	148,603433	33,9827499	925	1,38724208	0,925	999	-1,38724208
20_11a	102,044694	132,429783	51,0641134	304	-0,59503802	0,304	999	0,59503802
13_08d	107,489341	127,905636	24,1533524	193	-0,8452779	0,193	999	0,8452779
19_07d	89,4607329	125,826171	26,9797485	78	-1,34787907	0,078	999	1,34787907
23_12d	110,576788	143,693427	31,5753252	138	-1,04881387	0,138	999	1,04881387
22_07a	141,922717	152,274887	34,404873	397	-0,30089256	0,397	999	0,30089256
13_11a	149,815791	145,193143	26,7173238	590	0,17302061	0,59	999	-0,17302061
13_14b	114,765703	178,559339	56,535404	132	-1,12838384	0,132	999	1,12838384
16_11a	133,530731	134,039737	27,0801026	521	-0,01879631	0,521	999	0,01879631
18_12a	129,392128	155,237652	34,2755066	243	-0,75405229	0,243	999	0,75405229

25_11a	176,652162	186,697241	41,4774395	430	-0,24218176	0,43	999	0,24218176
16_13b	160,820717	139,111001	28,9028257	802	0,7511278	0,802	999	-0,7511278
16_13d	102,931694	124,528189	25,6259041	199	-0,84276033	0,199	999	0,84276033
16_13c	92,5482666	162,009703	34,033527	12	-2,04097085	0,012	999	2,04097085
16_13a	129,176771	131,507244	25,8410292	492	-0,090185	0,492	999	0,090185
24_17a	97,8631986	132,052494	25,3571635	68	-1,34830915	0,068	999	1,34830915
24_17b	100,301536	112,1939	25,0280059	336	-0,47516227	0,336	999	0,47516227
24_17c	103,176426	152,084724	33,2374904	62	-1,47147985	0,062	999	1,47147985
24_17d	81,4276781	118,941825	23,370496	50	-1,60519257	0,05	999	1,60519257
16_19a	122,384417	136,902619	30,4031037	334	-0,47752368	0,334	999	0,47752368
16_19b	110,302254	123,836539	23,9301783	300	-0,56557394	0,3	999	0,56557394
16_19c	137,298702	157,578037	32,7863941	268	-0,61852899	0,268	999	0,61852899
16_19d	137,252604	136,744686	27,4923756	541	0,01847489	0,541	999	-0,01847489
20_14a	107,567448	134,029286	29,9947798	195	-0,88221475	0,195	999	0,88221475
20_14b	108,249964	144,365486	31,9989181	108	-1,12864822	0,108	999	1,12864822
20_14c	115,172592	135,053581	29,2951345	246	-0,67864474	0,246	999	0,67864474
20_14d	179,603133	183,836535	47,1015441	492	-0,08987822	0,492	999	0,08987822
20_21a	134,673735	124,770022	22,5572763	702	0,43904738	0,702	999	-0,43904738
20_21b	104,905618	128,973446	28,3076531	187	-0,85022338	0,187	999	0,85022338
20_21c	89,1112559	108,343519	23,6265362	183	-0,81401112	0,183	999	0,81401112
20_21d	118,20425	141,02766	28,8985638	211	-0,78977662	0,211	999	0,78977662
24_24a	62,6577491	116,298543	23,0384471	4	-2,3283164	0,004	999	2,3283164
24_24b	96,273272	126,124985	26,0249198	103	-1,14704343	0,103	999	1,14704343
24_24c	79,3151878	117,233584	22,5274191	32	-1,68321085	0,032	999	1,68321085
24_24d	62,2953958	131,956046	28,2068495	1	-2,46963598	0,001	999	2,46963598

PART 2

Molecular phylogeny and phylogenomics of Dipterocarpaceae

CHAPTER 3

Phylogenetic analyses of plastid DNA suggest a different interpretation of morphological evolution than those used as the basis for previous classifications of Dipterocarpaceae (Malvales)

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Phylogenetic analyses of plastid DNA suggest a different interpretation of morphological evolution than those used as the basis for previous classifications of Dipterocarpaceae (Malvales)

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Phylogenetic and molecular clock analyses were performed including all genera except one (*Pseudomonotes*) for the three subfamilies of Dipterocarpaceae. We also included representatives of Sarcolaenaceae and Cistaceae with Bixaceae as the ultimate outgroup. Three plastid regions (six markers), partial *rbcL*, *trnK-matK-trnK* (partial *trnK* intron including complete *matK*) and *trnT-trnL-trnF* (partial *trnT*, complete *trnT-trnL* intergenic spacer, complete *trnL*, complete *trnL-trnF* intergenic spacer and partial *trnF*), were analysed. We also investigated additional accessions for genome size and chromosome numbers. Our phylogenetic results differ in three important respects from previous interpretations of morphological characters, as reflected in recent classifications. First, our analyses strongly support assignment of *Pakaraimaea* (subfamily Pakaraimaeoideae) to Cistaceae. Second, the morphological concepts of Dipterocarpeae and Shoreeae in subfamily Dipterocarpoideae are not supported because *Dipterocarpus* is sister to *Dryobalanops* plus tribe Shoreeae. Our analysis revealed four clades: (1) *Dipterocarpus*; (2) *Dryobalanops*, for which tribal assignment has been contentious; (3) genera of Shoreeae; and (4) the remaining genera of Dipterocarpeae. Third, *Shorea* is not monophyletic. Monotoideae are weakly supported as sister to Dipterocarpoideae; Sarcolaenaceae (endemic to Madagascar) are sister to this pair. Divergence in extant Dipterocarpoideae occurred *c.* 55 Mya. Genome sizes for all accessions examined are small (0.3264–0.6724 pg), and the additional chromosome numbers we collected fit into the patterns previously observed for Dipterocarpaceae.

ADDITIONAL KEYWORDS: Cistaceae – chromosome numbers – Dipterocarpoideae – genome size – Monotoideae – Pakaraimaeoideae – Sarcolaenaceae.

INTRODUCTION

Dipterocarpaceae comprise > 500 species and have usually been considered to include three subfamilies (Maury-Lechon & Curtet, 1998), Monotoideae with three genera (30 species), monospecific Pakaraimaeoideae and Dipterocarpoideae (470 species), with nine to 19 genera depending on the author (Table 1). Their distribution is pantropical, with Monotoideae (Gilg, 1925) in Africa, Madagascar and the Colombian Amazon, Pakaraimaeoideae (Maguire &

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Table 1. Comparative classifications of Dipterocarpaceae according to different authors after Maury-Lechon & Curtet (1998)

Authors	Genera	Section (s.)/subgenus (s.g.)	Subsection (s.s)/subgroup (s.gr.)
Ashton (1964, 1968, 1980, 1982)	*Hopea**	s. *Hopea*	s.s. *Hopea*
			s.s *Pierra*
		s. *Dryobalanoides*	s.s. *Dryobalanoides*
			s.s. *Sphaerocarpae*
	*Neobalanocarpus**	–	
	*Shorea**	s. *Shorea*	s.s. *Shorea*
			s.s. *Barbata*
		s. *Richetioides*	s.s. *Richetioides*
			s.s. *Polyandrae*
		s. *Anthoshorea*	
		s. *Mutica*	s.s. *Mutica*
			s.s. *Auriculatae*
		s. *Ovalis*	–
		s. *Neohopea*	–
		s. *Rubella*	–
		s. *Brachypterae*	s.s. *Brachypterae*
			s.s. *Smithiana*
		s. *Pachycarpae*	–
		s. *Doona*	–
		s. *Pentacme*	–
	*Parashorea**	–	
	*Dryobalanops**	–	
	*Dipterocarpus**	–	
	*Anisoptera**	s. *Anisoptera*	–
		s. *Glabrae*	–
	*Upuna**	–	
	*Cotylelobium**	–	
	*Vatica**	s. *Sunaptea*	–
		s. *Vatica*	–
		(s. *Pachynocarpus*, 1964)	
	*Stemonoporus**	–	
	*Vateria**	–	
	*Vateriopsis**	–	
	*Marquesia***	–	
	*Monotes***	–	
	*Pakaraimaea****	–	
Meijer & Wood (1964, 1976), Meijer (1979)	*Hopea*	–	
	Shorea	s.g. *Euchorea* = *Shorea*	–
		s.g. *Richetia*	–
		s.g. *Anthoshorea*	–
		s.g. *Rubroshorea*	s.gr. *Parvifolia*
			s.gr. *Ovalis*
			s.gr. *Pauciflora*
			s.gr. *Smithiana*
			s.gr. *Pinanga*
	Parashorea	–	
	Dryobalanops	–	
	Dipterocarpus	–	
	Anisoptera	s. *Pilosa*	–

Table 1. *Continued*

Authors	Genera	Section (s.)/subgenus (s.g.)	Subsection (s.s)/subgroup (s.gr.)
		s. *Glabrae*	–
	Upuna	–	
	Cotylelobium	–	
	Vatica	s.g. *Synaptea*	–
		s.g. *Isauxis*	–
		s.g. *Pachynocarpus*	–
Maury (1978), Maury-Lechon (1979a, b)	*Hopea*	s. *Hopea*	s.s. *Hopea*
			s.s. *Pierra*
		s. *Dryobalanoides*	s.s. *Dryobalanoides*
			s.s. *Sphaerocarpae*
	Balanocarpus heimii	–	
	Shorea	s. *Shoreae*	–
		s. *Barbatae*	–
	Richetia	s. *Richetioides*	–
		s. *Maximae*	–
	Anthoshorea	–	
	Rubroshorea	s. *Mutica*	s.s. *Mutica*
			s.s. *Auriculatae*
		s. *Ovalis*	–
		s. *Neohopea*	–
		s. *Rubella*	–
		s. *Brachypterae*	s.s. *Brachypterae*
			s.s. *Smithianeae*
		s. *Pachycarpa*	–
	Doona	–	
	Pentacme	–	
	Parashorea	–	
	Dryobalanops	–	
	Dipterocarpus	–	
	Anisoptera	s. *Anisoptera*	–
		s. *Glabrae*	–
	Upuna	–	
	Cotylelobium	–	
	Sunaptea	–	
	Vatica	s. *Vatica*	–
		s. *Pachynocarpus*	–
	Stemonoporus	–	
	Vateria	–	
	Vateriopsis	–	

–, no further classification; *, subfamily Dipterocarpoideae; **, subfamily Monotoideae; ***, subfamily Pakaraimaeoideae.

Ashton, 1977) in the Guianan Highlands of South America and Dipterocarpoideae in the Seychelles, Sri Lanka, India and Southeast Asia to New Guinea. The last have their greatest diversity in Borneo, where they dominate the canopy of lowland forests (Ashton, 1988).

Ashton (2003) defined Dipterocarpaceae by their diversity of epidermal hairs, especially fascicled hair tufts (a malvalean character), spiral or alternate geniculate entire penninerved leaves with paired stipules and mainly paniculate or racemose inflorescences with paired bracteoles. The bisexual actinomorphic scented flowers are pentamerous with an imbricate perianth and have a persistent calyx with the sepals becoming aliform in fruit. The petals have unicellular hairs outside. The stamens are centrifugally arranged with basifixed (Dipterocarpoideae) or versatile (Monotoideae, Pakaraimaeoideae) anthers that are two-celled and generally latrorse. The anthers have (two–) four pollen sacs with more or less prominent connectival appendages. The superior ovary has three (–five) locules, each locule with two (–four) axile anatropous ovules. Ovules

are bitegmatic, with a ventral raphe and a superior micropyle, and only one survives as a viable seed. The indehiscent fruit has a woody pericarp splitting irregularly or along three sutures with persistent sepals. The embryo sac development is of the *Polygonum* type, and endosperm is of the nuclear type. The ripe seeds generally lack endosperm. The cotyledons are generally unequal, one more or less enclosing the other, laminar or fleshy, entire or lobed enclosing the radical. Ashton regarded the presence of many stamens and ovules, the pentaloculate ovary and loculicidally dehiscent pericarp in some taxa to be primitive generalized traits in the family. Dipterocarpaceae are ectotrophic and mycorrhizal (Malloch, Pirozynski & Raven, 1980; Smits, 1994; Tedersoo *et al.*, 2007; Brearley, 2012; Phosri *et al.*, 2012; Sato, Tanabe & Toju, 2015); their seeds lack dormancy.

Although the phylogenetic assignment of Dipterocarpaceae among angiosperms has previously been problematic, Ashton (1982) supported their placement in the order Malvales, a position formally accepted by the Angiosperm Phylogeny Group (APG) (1998, 2003, 2009, 2016). Ashton recognized similarities with Tiliaceae and also cited Sarcolaenaceae as tropical evergreen canopy trees with compatible biogeography. Fascicled hairs, stipules, floral characters and loculicidal capsules of Dipterocarpaceae are shared with many Malvales (Kubitzki & Chase, 2003). More morphological characters are given in Table 2; a review of these and further characters is provided in Maury-Lechon & Curtet (1998). Vestured pits are shared by some Dipterocarpaceae and Cistaceae (Arrington & Kubitzki, 2003). A distinct 'bixoid' chalazal region of the seed coat is shared by Monotoideae and Pakaraimaeoideae with Cistaceae and Bixaceae (including *Cochlospermum* Kunth; Nandi, 1998).

Dayanandan *et al.* (1999) concluded based on molecular evidence that Dipterocarpaceae, including *Monotes* A.DC. (Monotoideae) and *Pakaraimaea* Maguire & P.S.Ashton (Pakaraimaeoideae), form a clade closely related to Sarcolaenaceae, but they did not include enough outgroup genera (e.g. Cistaceae) to make any conclusive assessment of interfamilial relationships, leaving the positions of Monotoideae and Pakaraimaeoideae under discussion. According to the recent APG IV classification (2016), *Pakaraimaea* should be considered a member of an expanded Cistaceae based on the plastid *rbcL* analysis of Ducousso *et al.* (2004), in which *Pakaraimaea* was sister (with 88% bootstrap) to the two genera of Cistaceae included in that study. *Monotes* and *Pseudomonotes* A.C.Londoño, E.Alvarez & Forero (Monotoideae) were moderately supported (88%) as sister to Sarcolaenaceae plus Dipterocarpoideae, with Sarcolaenaceae weakly supported (62%) as sister to Dipterocarpoideae. A recent molecular phylogenetic study of Sarcolaenaceae that included several genera of Cistaceae and Dipterocarpaceae raised questions about the monophyly of Dipterocarpaceae with respect to Sarcolaenaceae (Aubriot *et al.*, 2016). Several other molecular phylogenetic studies have been conducted on Dipterocarpaceae, including use of PCR-RFLP (Tsumura *et al.*, 1996; Indrioko, Gailing & Finkeldey, 2006), RAPD (Rath *et al.*, 1998), AFLPs (Cao *et al.*, 2006), other plastid sequences (Kajita *et al.*, 1998; Kamiya *et al.*, 1998; Dayanandan *et al.*, 1999; Gamage *et al.*, 2003, 2006; Yulita, Bayer & West, 2005; Choong *et al.*, 2008; Tsumura *et al.*, 2011; Yulita, 2013), the nuclear gene *Pgi*C (Kamiya *et al.*, 2005; Choong *et al.*, 2008) and internal transcribed spacer regions (Yulita *et al.*, 2005). These studies have used only one to three plastid or nuclear markers (e.g. Kamiya *et al.*, 1998: *trnL* intron and intergenic spacer between *trnL* and *trnF*; Dayanandan *et al.*, 1999: *rbcL*; Gamage *et al.*, 2003: *trnL-trnF* spacer and *trnL* intron region; Gamage *et al.*, 2006: *matK*, *trnL* intron and *trnL-trnF* intergenic spacer region), only included a limited number of taxa (e.g. Kajita *et al.*, 1998: 17 species; Rath *et al.*, 1998: 12 species; Tsumura *et al.*, 1996: 30 species; Dayanandan *et al.*, 1999: 35 species, Choong *et al.*, 2008: 30 species) or did not include all three subfamilies.

Reconciliation of discordant intuitively constructed morphological classifications and molecular phylogenetics in some cases has presented problems (e.g. sectional classifications in *Leontodon* L., Asteraceae, Samuel *et al.*, 2003; *Diospyros* L., Ebenaceae, Duangjai *et al.*, 2009; *Polystachya* Hook., Orchidaceae, Russell *et al.*, 2010). Molecular phylogenetic studies have paved the way to reclassifications at tribal level in Rubioideae (Bremer & Manen, 2000) and Orchidaceae (Chase *et al.*, 2015). A taxonomic revision of Bromeliaceae subfamily Tillandsioideae was based on molecular phylogenetics of plastid and nuclear markers and new or re-evaluated morphology, which enabled circumscription of monophyletic units using synapomorphic combination of diagnostic morphological characters (Barfuss *et al.*, 2016). In general, traditional classifications have been based on a few characters intuitively selected by a well-informed specialist, and these classifications have typically excluded other generally conflicting characters; these classifications generally cannot be reproduced with a formal cladistic analysis of these data for the same group of organisms. For example, molecular phylogenetic results for the angiosperms (e.g. Chase *et al.*, 1993) appeared to be in conflict with previous 'morphological' systems (e.g. Cronquist, 1981). However, it became clear that when a formal non-molecular cladistic analysis was performed (Nandi, Chase & Endress, 1998), the conflict was not between morphology and molecules, but rather between an intuitive interpretation of a few characters and a formal objective analysis of a broader set of data. In general, such intuitive

Table 2. Distinctive morphological characters of Cistaceae, Sarcolaenaceae and Dipterocarpaceae according to Ashton (2003), Maury-Lechon & Curtet (1998) and Watson & Dallwitz (http://delta-intkey.com/angio/www/cistacea.htm, accessed 14 July 2017)

Character	Cistaceae	Sarcolaenaceae	Dipterocarpoideae	Monotoideae	Pakaraimaeoideae
Inflorescence					
paniculate			+	+	
racemi-paniculate		+	(+)	+	+
cyme	+		(+)		
Perianth pentamerous	x	x	+	+	+
Flower bud sepals					
imbricate	+	+	+		+
valvate			+	+	
Leaves					
alternate	x	+	+	+	+
opposite	x				
Stipules	+	+	x	x	x
One- or two-layered hypodermis		+	x		+
Contorted corolla	x	+	x	+	+
Two-celled anthers generally dehiscing longitudinally	+		x	+	+
Subversatile anthers			+	+	+
Imbricate perianth with unequal persistent sepals					
two smaller sepals: outer	+	+			
two smaller sepals: inner			+	+	+
Mucilage canals and cells in epidermis	+	+	+		+
Fruit					
capsular	+	+	+	+	+
nut		+	+	+	
dehiscent	+	+		+	+
indehiscent		+	+	+	

+, present; x, present and other possibilities; in parentheses, exceptions.

classifications have been re-interpreted in the face of consistent, well-supported, 'conflicting' results of molecular analyses (e.g. the intuitive interpretation of morphological data upon which these classifications have been based is discarded), generally leading to the conclusion that morphological evolution has been more complicated than previously assumed. Our intention in this study was to compare our molecular results with the previous classifications (Ashton, 1964; Meijer & Wood, 1964, 1976; Ashton, 1968, 1980, 1982; Maury, 1978; Maury-Lechon 1979a, b; Meijer, 1979) to determine to what extent they were mutally corroborative. We do not here undertake a formal analysis of morphological data, which is beyond the scope of this study.

Beside phylogenetic relationships, the age of clades is of interest so that an appropriate geographical interpretation of the evolutionary history of a group can be developed. The three subfamilies occupy different phytogeographical zones along the tropical belt of three continents with Wallace's Line as a major phytogeographical boundary in Southeast Asia (Maury-Lechon & Curtet, 1998). A Gondwanan origin, with subsequent migration to Indomalesia, was proposed by Croizat (1952, 1964) and Ashton (1982). This is supported by the significant decline in the number of species to the east of Wallace's Line. Based on an assumption that high species diversity of Dipterocarpaceae in Southeast Asia is associated with their origin, another hypothesis suggested that Dipterocarpaceae originated on the Eurasian plate with subsequent migration to South Asia, Africa and South America (Merrill, 1923; Prakash, 1972; Meher-Homji, 1979). Both hypotheses involve overland seed dispersal, which was suggested by Ashton (1982) on the basis of the limited seed dispersal capacity of these species, ectomycorrhizal symbiosis, lack of seed dormancy and salt-intolerant seeds. Morley (2000)

inferred the likely migration of Dipterocarpoideae to India/Seychelles directly from Africa, which is consistent with the presence of fossil wood identified as *Dipterocarpus* C.F.Gaertn. in East Africa in the Tertiary (Bancroft, 1935; Ashton & Gunatilleke, 1987). A phylogenetic and ectomycorrhizal study revealed that Sarcolaenaceae (endemic to Madagascar) and Dipteocarpoideae share an ectomycorrhizal common ancestor (Ducousso *et al.*, 2004). Ducousso *et al.* (2004) further suggested that the last common ancestor was located on the India–Madagascar landmass and produced the current Sarcolaenceae in southeastern Madagascar, whereas the Asian Dipterocarpaceae drifted away with the India–Seychelles landmass and then dispersed throughout Asia. Ducousso *et al.* (2004) cited Bossuyt & Milinkovitch (2001), who proposed a similar scenario for amphibians. The separation of Madagascar from the India–Seychelles block occurred 87.6 ± 0.6 Mya.

Chromosome counts are available for seven genera of Dipterocarpoideae (Rice *et al.*, 2015), which indicated the basic chromosome number in Dipterocarpeae is $x = 11$, but $x = 7$ for Shoreeae (Jong & Kaur, 1979). Most species appear to be diploid, but there are a few reports of polyploidy in *Shorea* Roxb. ex C.F.Gaertn. and *Hopea* Roxb. ranging from triploid and near triploid to tetraploid: e.g. *Hopea beccariana* Burck.: $2n = 20–22$ (Ashton, 1982) and *Shorea ovalis* (Korth.) Blume: $2n = 28$ (Kaur *et al.*, 1986). Based on published genome size measurements, most species of Dipterocarpaceae are characterized by small genomes (Ohri & Kumar, 1986; Ng *et al.*, 2016). Recently published genome size values showed a 2.64-fold difference, ranging from 0.267 pg in *Shorea hemsleyana* King ex Foxw. to 0.705 pg in *Shorea ovalis* (Ng *et al.*, 2016).

There have been morphological classifications of Dipterocarpaceae that differ with respect to numbers of genera, sections and subsections (Table 1), and the molecular studies cited above also exhibited some consistent differences in topology from those classifications. This has compromised understanding of the evolution of Dipterocarpaceae. However, the intention of this study is not to reclassify Dipterocarpaceae or to attempt a formal analysis of character evolution, but to obtain information that could help to solve some uncertainities in the current classification of this ecologically and economically important family. We address here the following topics: (1) clarification of the position of subfamilies Pakaraimaeoideae and Monotoideae; (2) phylogenetic placement of *Hopea*, *Parashorea* Kurz and *Shorea* (tribe Shoreeae) and phylogenetic relationships within *Shorea*, which comprises > 190 species; (3) placement of *Dipterocarpus*, which has been placed in Dipterocarpeae with other genera based on morphology, but showed a closer relationship to members of Shoreeae than to other members of Dipterocarpeae in previous molecular studies; (4) an examination of the position of *Dryobalanops* C.F.Gaertn. previously assigned to tribe Shoreeae by Ashton (1979) and placed in an intermediate position between Shoreeae and Dipterocarpeae by Maury-Lechon (1979a); (5) estimation of divergence times of the major clades in Dipterocarpaceae; and (6) investigation of genome size and chromosomal diversity using published as well as newly collected data.

MATERIAL AND METHODS

Plant material

Here, 238 accessions of Dipterocarpoideae representing 143 species were included. Of the 11 sections and eight subsections in the species-rich genus *Shorea* reported by Ashton (1964, 1968, 1980, 1982), nine sections and seven subsections were represented in this study. Samples were mainly collected in Brunei, Sri Lanka and Thailand. Detailed sampling locations can be found in the appendix (Supporting Information, Table S1). The sampling further comprised two accessions of the single species of *Pakaraimaea* and four species of *Marquesia* Gilg and *Monotes* in Monotoideae. This covers all described genera except for *Pseudomonotes* (Monotoideae), a notable increase in generic coverage over previous studies. Even though included in Ducousso *et al.* (2004), *Pseudomonotes*, which paired with *Monotes* (98% bootstrap), was omitted because the *rbcL* sequence used by Ducousso *et al.* (2004) was not available in GenBank. Furthermore, only sequences for which at least sequences of two of the three matrices (1) *rbcL*, (2) *trnK-matK-trnK* and (3) *trnT-trnL-trnF* were available were included in the combined analysis. Additionally, four species belonging to three genera of the closely related families Sarcolaenaceae (three genera) and three genera of Cistaceae (three species) were included. Outgroup sampling included members of Bixaceae, *Bixa orellana* L. and *Cochlospermum vitifolium* Spreng. (Supporting Information, Table S1).

DNA extraction and PCR amplification

For some accessions, sequence data were obtained from previous studies (Kajita *et al.*, 1998; Kamiya *et al.*, 1998; Gamage *et al.*, 2003, 2006: see Supporting Information, Table S1). For new accessions, DNA from the Royal Botanic Gardens, Kew, DNA Bank (apps.kew.org/dnabank/, accessed 14 July 2017) was used or genomic DNA was extracted from *c.* 20 mg of silica gel-dried (Chase & Hills, 1991) material (bark or leaves) using the DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) following the manufacturer's protocol. To

avoid degradation, material was frozen in liquid nitrogen and then ground to a fine powder using glassbeads. To remove mucilaginous polysaccharides, which are a problem for many members of Malvales due to the mucilaginous epidermal cells, the ground material was initially washed with sorbitol buffer (Russell *et al.*, 2010; Souza *et al.*, 2012) until there was no visible mucilage in the sample.

Three plastid regions (including six markers) were amplified: partial *rbcL*, *trnK-matK-trnK* (partial *trnK* intron including complete *matK*) and *trnT-trnL-trnF* (partial *trnT*, complete *trnT-trnL* intergenic spacer, complete *trnL*, complete *trnL-trnF* intergenic spacer and partial *trnF*), resulting in a *c.* 5.9 kb alignment. PCRs included 7.5 µL 2× Phusion Green HF HS PCR Master Mix with 1.5 mM $MgCl_2$ (Life Technologies, LT, Vienna, Austria), 0.15 µL bovine serum albumin (0.2 g/L), 1.5 µL each primer (3.2 µM), 1 µL template DNA and H_2O up to a final volume of 15 µL. The primers used in this study are provided in Table 3. Thermal cycle conditions were as follows: initial denaturation at 98 °C for 30 s, 35 cycles of denaturation at 98 °C for 10 s, annealing at 63–68 °C (depending on the primers, Table 3) for 30 s and extension at 72 °C for 30 s (*rbcL*) to 1 min (*trnK-matK-trnK, trnT-trnL-trnF*), followed by final extension of 5 min at 72 °C. PCR products were cleaned with 1.5 µL exonuclease I and FastAP thermosensitive alkaline phosphatase mixture (7 U Exo I, 0.7 U FastAP) at 37 °C for 45 min and 85 °C for 15 min. Sequencing reactions were performed with the BigDye Terminator Kit v3.1 (LT) using the same primers that were used for amplification or with internal primers (Table 3) according to the manufacturer's instructions. Sanger sequencing was carried out using a 3730 DNA analyser (LT).

Sequence alignment and phylogenetic analyses

Sequences were assembled and edited using Geneious (version 8.0.5, http://www.geneious.com; accessed 14 July 2017; Kearse *et al.*, 2012). To generate the *trnT-trnL-trnF* alignment, the partial *trnL* intron and the *trnL-trnF* accessions obtained from GenBank were combined in BioEdit v7.0.4 (Hall, 1999). The final alignment was performed online using MAFFT version 7 (http://mafft.cbrc.jp/alignment/server/, accessed 14 July 2017) and inspected manually with BioEdit v7.0.4. Unsequenced regions were coded as missing data in the combined matrix. To infer phylogenetic relationships, maximum parsimony (MP), maximum likelihood (ML) and Bayesian inference (BI) analyses were performed. MP analyses were conducted in PAUP version 4.0a149 (Swofford, 2016). For each data set, heuristic searches were conducted using 1000 replicates of random addition sequence, tree-bisection–reconnection (TBR) branch-swapping and 'keeping multiple trees' (MulTrees), but saving only 20 trees per replicate. Clade support was estimated by the bootstrap (Felsenstein, 1985) with 1000 replicates, TBR branch swapping and simple addition sequence. To explore the variability of each marker, four matrices were analysed with MP: (1) *rbcL*, (2) *trnK-matK-trnK*, (3) *trnT-trnL-trnF* and (4) all regions combined. Information about the alignment characteristics and number of variable and potentially parsimony informative sites is presented in Table 4. ML and BI analyses were conducted using the combined data only. An ML rapid bootstrap analysis (1000 replicates) with search for best-scoring ML tree in one run was conducted in RAxML v8.2.0 (Stamatakis, 2014). The best fitting substitution model was determined with jModeltest v2.1.7 (Darriba *et al.*, 2012; Guindon & Gascuel, 2003) using the Akaike information criterion. Evolutionary substitution models for each marker were calculated. The most complex substitution model, general time reversible (GTR+I+GAMMA) model with six substitution types (one for each pair of nucleotides) and gamma-distributed rate variation across sites and a proportion of invariable sites was finally chosen for the analysis. BI was performed using MrBayes v3.2.6 (Huelsenbeck & Ronquist, 2001; Ronquist & Huelsenbeck, 2003). A partition scheme was set up by creating character sets for each of the three combined parts of the alignment: (1) *rbcL*, (2) *trnK-matK-trnK* and (3) *trnT-trnL-trnF*. Parameters were unlinked so that each partition has its own parameters. Overall rate variation was allowed to be different across partitions. By changing it to variable, the rates are allowed to vary under a flat Dirichlet prior. Two independent Metropolis-coupled Markov chain Monte Carlo (MCMC) analyses each with 10 million generations, sampling each 1000th generation, were run. The initial 25% of trees obtained from each MCMC run was removed as the burn-in. Each run consisted of three heated and one cold chain. A 50% majority rule consensus tree was calculated using the remaining trees to obtain posterior probabilities for each node. Outgroup taxa were specified to be Bixaceae. Trees were visualized and edited in FigTree v1.4.1 (http://tree.bio.ed.ac.uk/software/figtree/, accessed 14 July 2017).

Molecular clock analysis

To obtain age estimates for the major clades of the groups of interest, a molecular clock analysis was performed in BEAST v2.4.4. (Drummond *et al.*, 2012) with an uncorrelated log-normal relaxed clock excluding the proportion of invariant sites parameter under the TVM+G4 model. This model was obtained by the model test implemented in IQ-TREE software (http://www.iqtree.org/, accessed 14 July 2017) under the

Table 3. Details of primers used in this study

Region	Primer	Sequence (5′–3′)	Usage	T_A (°C)	Reference
rbcL	rbcLa_f	ATGTCACCACAAACAGAGACTAAAGC	PCR and sequencing	63	Levin *et al.* (2003)
	rbcL_724R	TCGCATGTACCTGCAGTAGC	PCR and sequencing		Fay, Swensen & Chase (1997)
trnK-matK-trnK	trnK-799f	CCYTGTTYTRACYRTATYGCACTATGTAT	PCR and sequencing	65	Barfuss *et al.* (2016)
	trnK-2662r	CTCGAACCCGGAACTAGTCGG	PCR and sequencing		Castello *et al.* (2016)
	matK-DipF* (ratio 1:2):		Sequencing		Heckenhauer, Barfuss & Samuel (2016)
	matK-413f-1	TAATTTACRATCAATTCATTCAATATTTCC			
	matK-413f-4	TAATTTMCRATCAATTCATTCCATATTTCC			
	matK-DipR* (ratio 1:1:1):		Sequencing		
	matK-1227r-4	GARGATCCRCTRTRATAATGAGAAAAATTT			Heckenhauer, Barfuss & Samuel (2016)
	matK-1227r-5	GARGATCCRCTRTRATAATGAGAAATATTT			
	matK-1227r-7	GARGATCCGCTATRATAATGATAAATATTT			
trnT-trnL-trnF	a†	CATTACAAATGCGATGCTCT	PCR and sequencing	60	Taberlet *et al.* (1991)
	f†	ATTTGAACTGGTGACACGAG	PCR and sequencing		Taberlet *et al.* (1991)
	a_mod†	CATTACAAATGCGATGCTCTAAC	PCR and sequencing	68	This study
	f_mod†	ATTTGAACTGGTGACACGAGGAT	PCR and sequencing		This study
	c	CGAAATCGGTAGACGCTACG	Sequencing		Taberlet *et al.* (1991)
	h	CCATTGAGTCTCTGCACCTATC	Sequencing		Taberlet *et al.* (2007)

*Primers *matK*-DipF and *matK*-DipR were obtained by multiplexing several degenerate primers in different ratios according to Heckenhauer *et al.* (2016).
†Because of a higher annealing temperature (T_A), predominantly modified primers (a_mod and f_mod) of Taberlet *et al.* (1991) were used for amplification of *trnT-trnL-trnF*.

Bayesian information criterion (BIC). The input file for BEAST was first generated using Beauti implemented in BEAST and edited manually. The dating analysis was based on the study of Ducousso *et al.* (2004), which revealed that the last common ancestor of Sarcolaenaceae and Asian dipterocarps was ectomycorrhizal before the India–Madagascar separation, *c.* 87.6 ± 0.6 Mya.

There are fossils attributed to Dipterocarpaceae (e.g. Maury-Lechon & Curtet, 1998; Dutta *et al.*, 2011; Feng *et al.*, 2013), but they are not clearly assignable to any extant clade of the family, making them unusable as calibration points. Without expanding our analysis to include a much greater set of Malvales, we were unable to use fossils as calibration points. Here, a log-normal distribution with a mean of 87.5 My was used as calibration point to the most recent common ancestor of Sarcolaenaceae and Dipterocarpoideae. The following time of most recent common ancestor settings were log normal prior distribution with a mean of 87.5 and log standard deviation of 0.015 (real space). A log-normal prior with mean of 0.005 and standard deviation of 0.5 was placed on the mean of the log-normal relaxed clock rate. In our analyses, Monotoideae were sister to Dipterocarpoideae (Figs 1, 2), but this is not well supported. Thus, the correct position of Monotoideae remains unclear, and two alternative dating analyses were therefore run. In the first, a constraint consisting of Sarcolaenaceae, Monotoideae and Dipterocarpoideae was defined. In the second analysis, Sarcolaenaceae and Dipterocarpoideae were considered a clade. For each of our two analyses, we ran two separate chains for 300 million generations to achieve

Table 4. Parsimony characteristics and molecular evolutionary model for each locus and combined data set including Bixaceae, Cistaceae, Sarcolaenaceae and Dipterocarpaceae

	rbcL	*trnK-matK-trnK*	*trnT-trnL-trnF*	Combined data
Total number of accessions	192	252	250	254
Length of alignment	697	1908	3306	5911
Number of variable characters (%)	125 (17.9)	765 (40.1)	961 (29.1)	1851 (31.3)
Number of potentially parsimony-informative characters (%)	98 (14.1)	546 (28.6)	648 (19.6)	1292 (21.9)
Tree length of best parsimony tree (steps)	254	1280	1588	3185
Trees saved (parsimony analysis)	11460	20000	3600	14000
Consistency index	0.58	0.76	0.75	0.73
Retention index	0.93	0.95	0.71	0.68
Molecular evolutionary model	TVM+I+G	TVM+G	TVM+G	TVM+G
Number of substitution types (Nst)	6	6	6	6
Rates	Gamma shape	Gamma shape	Gamma shape	Gamma shape
Number of rate categories (Ncat)	4	4	4	4

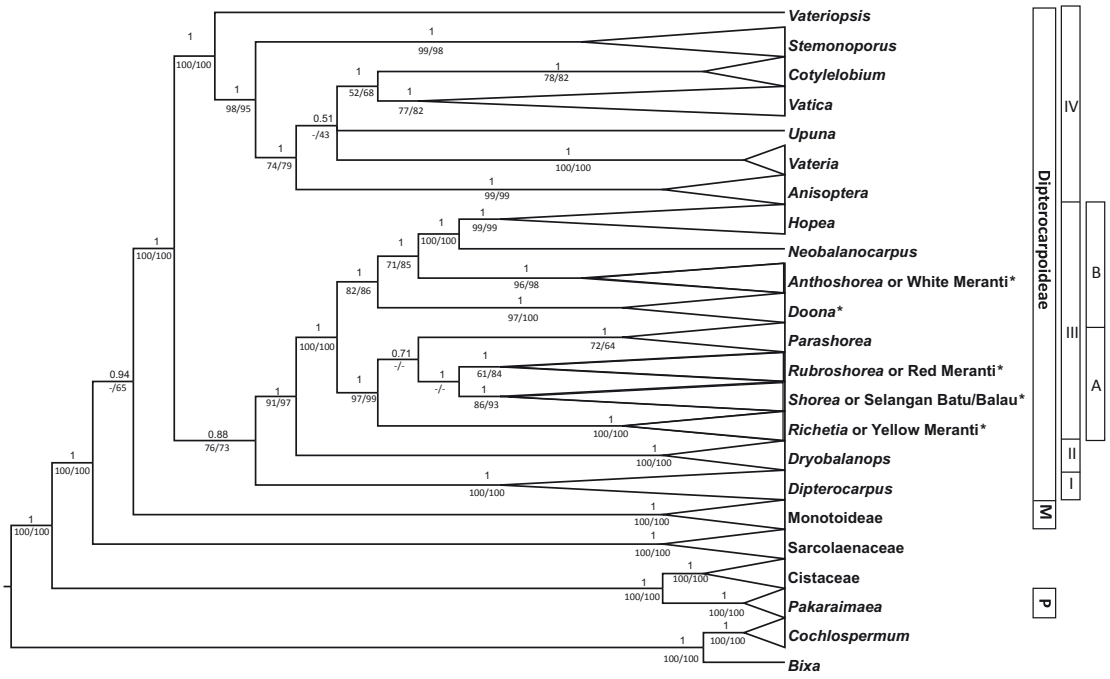
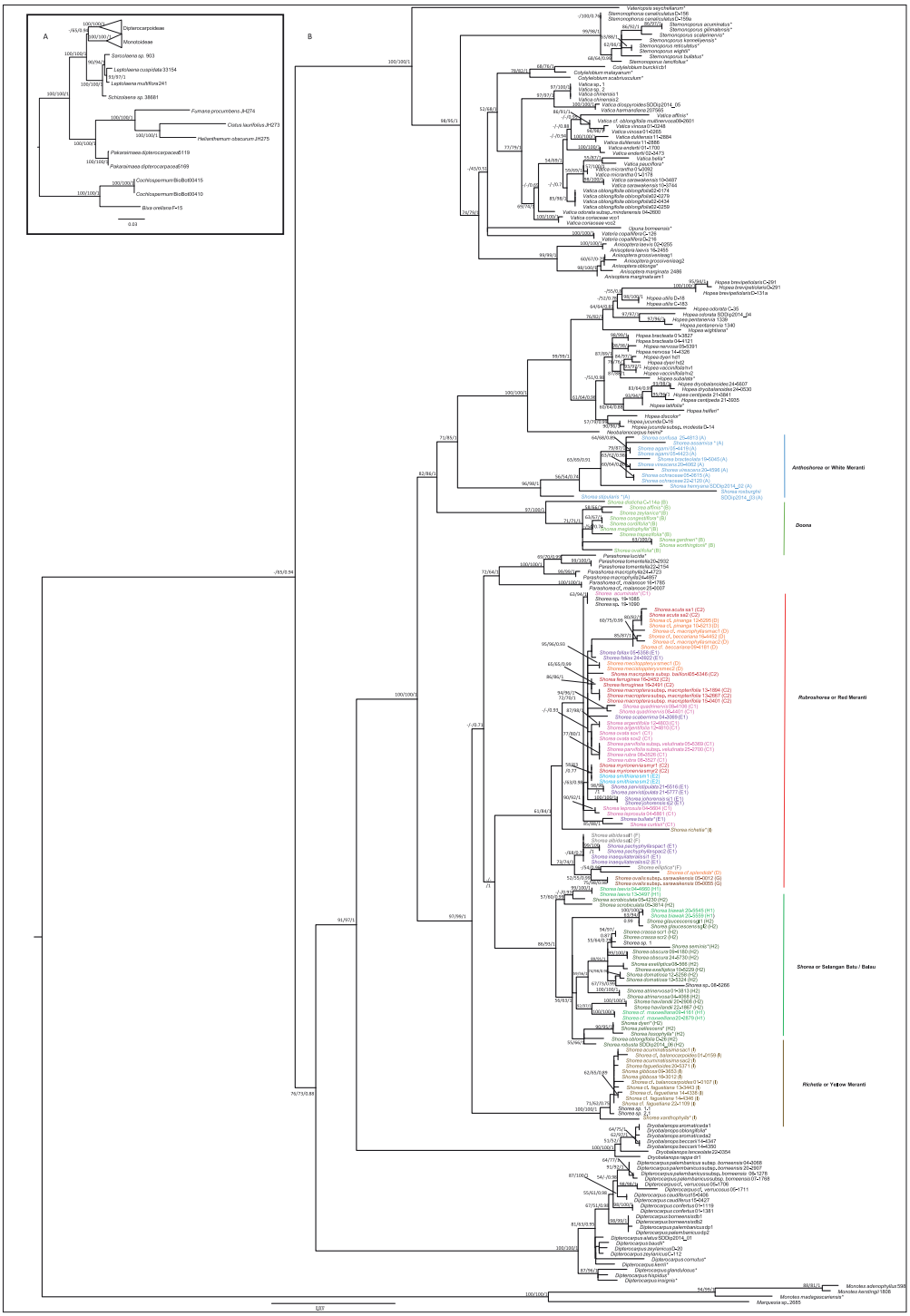


Figure 1. Bayesian 50% majority rule consensus tree from analyses of the combined plastid loci. Taxa are collapsed to major clades. Posterior probabilities ($BI_{PP} \geq 0.7$) are given above the nodes and bootstrap percentages (≥ 50%) from maximum parsimony and maximum likelihood analyses are shown below the nodes in this order. A hyphen indicates bootstrap support < 50%. The current classification of Dipterocarpaceae (Dipterocarpoideae, Monotoideae = M, Pakaraimaeoideae = P) is shown. The four major clades (I, II, III, IV) of Dipterocarpoideae and subclades (A, B) of the tribe Shoreeae are indicated. Different groups of *Shorea* are marked with an asterisk.

a reasonable effective sample size (ESS) of at least 200. Convergence and mixing of each run were assessed with Tracer v1.5.0 (http://tree.bio.ed.ac.uk/software/tracer/, accessed 14 July 2017). Both log and tree files were then trimmed to 250 million generations. The two log files were combined using LogCombiner using 5000


A
B
Dipterocarpoideae
Monotoideae
Sarcolaena sp. 903
Leptolaena cuspidata 33154
Leptolaena multiflora 241
Schizolaena sp. 38681
Fumaria procumbens JH274
Cistus laurifolius JH273
Helianthemum obscurum JH275
Pakaraimaea dipterocarpacea5119
Pakaraimaea dipterocarpacea5169
Cochlospermum BioBot00415
Cochlospermum BioBot00410
Bixa orellana F-15
0.03
Anthoshorea or White Meranti
Doona
Rubroshorea or Red Meranti
Shorea or Selangan Batu / Balau
Richetia or Yellow Meranti
0.01
Monotes adenophyllus 598
Monotes kerstingii 1808
Marquesia sp. 2695

state samples each. For each chain, 60% of generations were discarded as burn-in. We combined the post burn-in trees in TreeAnnotator to construct a maximum clade credibility tree, which was displayed with Figtree v1.4.1. Since we are interested in the ages of the major clades, the maximum clade credibility tree was collapsed. To explore which of our two hypotheses [monophyly of (Sarcolaenaceae+Monotoideae+Dipterocarpoideae) or monophyly of only (Sarcolaenaceae+Dipterocarpoideae)] is better supported, we estimated marginal likelihoods for the two models using the path sampling (PS) method implemented in BEAST. PS analyses were conducted with 112 path steps, each run until the ESS reached 200. Marginal likelihood estimates where then used for calculation of the Bayes factor. We included the whole dataset used in the combined analysis, but the node used for the calibration thus becomes the root of the analysis, arranging the outgroups as sister to Sarcolaenaceae/Dipterocarpoideae. Their age assignments were thus not correctly estimated and are therefore not discussed here.

Chromosome counts and genome size measurements in Dipterocarpoideae

Actively growing root tips were pretreated with 0.002 M 8-hydroxyquinoline for 2.5 h at room temperature and 4 °C for 2.5 h, fixed in 3:1 ethanol/acetic acid and stored at –20 °C until use. Chromosome numbers were initially assessed by standard Feulgen staining of meristematic root cells (Jang *et al.*, 2013). Due to the small size of these chromosomes, additional preparations were also made using enzymatic digestion of cell walls to improve resolution of karyotypes. Preparations were made in a drop of 60% acetic acid with the cover slips off and the material was stained with 2 ng/µL DAPI (4′, 6-diamidino-2-2phenylindole) dissolved in the mounting antifade medium Vectashield (Vector Laboratories, Burlingame, CA, USA). Chromosomes were examined with an AxioImager M2 epifluorescence microscope with a high-resolution microscopy camera (Carl Zeiss, Vienna, Austria), and files were processed using AxioVision 4.8 (Carl Zeiss). At least three well-spread metaphases were analysed for each species.

Genome size was measured with flow cytometry performed on leaf material. Fresh tissue from plants growing in the Hortus Botanicus Vindobonensis (HBV) and recently collected silica-gel dried material from Sri Lanka were used. Together with leaves of the internal standard species, samples were chopped in Otto I buffer (Otto *et al.*, 1981) according to Galbraith *et al.* (1983). Standards were *Solanum pseudocapsicum* L., 1C = 1.30 pg (Temsch, Greilhuber & Krisai, 2010) or *Pisum sativum* L. 'Kleine Rheinländerin', 1C = 4.42 pg (Greilhuber & Ebert, 1994). After filtering of the isolate through a 30-µm nylon mesh, RNA was digested with 15 mg/L RNase A for 30 min at 37 °C. Afterward, DNA was stained in propidium iodide (50 mg/L) complemented with Otto II buffer (Otto *et al.*, 1981). Mean fluorescence intensity of at least 10 000 particles was measured with a CyFlow cytometer (Partec, Münster, Germany) equipped with a green laser (Cobolt Samba, Cobolt AB, Stockholm, Sweden); the 1C-value was calculated according to the formula: (MFIobject/MFIStandard) × 1C-value standard, where MFI is the mean fluorescence intensity of the G1 nuclei population. All measurements were carried out three times.

RESULTS

Sequence and alignment characteristics

There was no length variation in *rbcL* (697 bp), whereas the *trnK-matK-trnK* and *trnT-trnL-trnF* regions were variable among taxa. The aligned sequence length of the partial *trnK* intron region (including complete *matK*) was 1908 bp and that of the *trnT-trnL-trnF* region was 3306 bp. The *trnK-matK-trnK* region was the most informative region with 546 (28.61%) potentially parsimony-informative sites. The number of potentially parsimony-informative sites was 98 (14.06%) and 648 (19.6%) for *rbcL* and *trnT-trnL-trnF*, respectively (Table 4).

Phylogenetic analysis of the plastid loci

All three methods of phylogenetic inference (MP, ML, BI) for the combined data set revealed congruent results for the main clades, but there was some

Figure 2. Best-scoring maximum likelihood tree of a rapid bootstrap analysis with 1000 replicates of the combined data set. Bootstrap values (≥ 50%) obtained from maximum parsimony and maximum likelihood analyses and posterior probabilities ($BI_{PP} \geq 0.7$) obtained from Bayesian interference are given in this order. A hyphen indicates bootstrap support < 50% or $BI_{PP} < 0.7$. The relationships between different (sub-)families used in this study (A) and within Dipterocarpoideae (B) are shown. Sequences obtained from GenBank are indicated with an asterisk (*). Different groups of *Shorea* according to Maury (*Anthoshorea*, *Doona*, *Rubroshorea*, *Shorea*, *Richetia*) are indicated. Sections and subsections according to Ashton are given for each of the *Shorea* accessions: A, section *Anthoshorea*; B, section *Doona*; C, section *Mutica*; C1, section Mutica; C2, section Auriculatae; D, section *Pachycarpae*; E, section *Brachypterae*; E1, subsection *Brachypterae*; E2, subsection *Smithiana*; F, section *Rubella*; G, section *Ovalis*;, H, section *Shorea*; H1, subsection *Barbata*; H2, subsection *Shorea*, I, section *Richetioides*, subsection *Richetioides*.

variation in topologies in the terminal clades. The Bayesian (Fig. 1) and the maximum likelihood (Fig. 2) trees with bootstrap percentages from the MP (BS_{MP}) and ML (BS_{ML}) analyses and posterior probabilities from the BI (PP_{BI}) are shown.

Phylogenetic relationships in Dipterocarpaceae

Our main aim in this study was the clarification of the position of the three subfamilies of Dipterocarpaceae relative to Sarcolaenaceae and Cistaceae. Besides *Bixa* and *Cochlospermum* (Bixaceae), which were used as the outgroup and arranged as a clade sister to all other taxa, our analyses revealed four groups (Figs 1, 2): (1) Cistaceae including *Pakaraimaea* (the sole member of Pakaraimaeoideae; Fig. 1: P; BS_{MP} 100, BS_{ML} 100, PP_{BI} 1.00; this order will be used throughout; a hyphen indicates support < 50; Figs 1, 2), (2) Sarcolaenaceae (100, 100, 1.00), which were strongly supported (100, 100, 1.00) as sister to the clade containing Dipterocarpoideae (100, 100, 1.00) plus Monotoideae (100, 100, 1.00), (3) Monotoideae (consisting of *Monotes* and *Marquesia*, Fig. 1: M) and (4) all taxa belonging to the Asian subfamily Dipterocarpoideae (Fig. 1). The sister relationship between Monotoideae and Dipterocarpoideae was only weakly supported (-, 65, 0.94).

Phylogenetic relationships in subfamily Dipterocarpoideae

Dipterocarpoideae were divided in four clades (Fig. 1: I, II, III, IV), which are almost in accordance to the tribal division *sensu* Ashton except that *Dipterocarpus* (Fig. 1, clade I, 100, 100, 1.00) was weakly supported (76, 73, 0.88) as sister to clades II and III and thus separated from the remaining genera of Dipterocarpeae (clade IV). The sister relationship of *Dryobalanops* (Fig. 1, clade II, 100, 100, 1) to tribe Shoreeae (Fig. 1, clade III) was strongly supported (91, 97, 1.00). This third major clade (Fig. 1, clade III, 100, 100, 1.00) can be further divided into two main subclades (designated as A and B in Fig. 1). Subclade A (97, 99, 1.00) consisted of *Parashorea* (71, 64, 1.00), *Rubroshorea* (ined., 61, 84, 1.00), *Richetia* F.Heim or the yellow meranti group (100, 100, 1.00) and *Shorea* or selangang batu/balau group (86, 93, 1.00). Subclade B (82, 86, 1.00) contained three groups with the following taxa: (1) *Hopea* and *Neobalanocarpus* P.S.Ashton (100, 100, 1.00); (2) *Anthoshorea* Pierre or white meranti wood group (96, 98, 1.00); and (3) *Doona* Thwaites (97, 100, 1.00). It is notable that *Shorea richetia* Symington (obtained from GenBank), which has been assigned to *Richetia*, clustered with *Rubroshorea* (Fig. 2). This is possibly due to a missidentification. Species of *Anisoptera* Korth., *Cotylelobium* Pierre, *Stemonoporus* Thwaites, *Upuna* Symington, *Vateria* L., *Vateriopsis* F.Heim and *Vatica* L. formed a fourth major clade (Fig. 1, IV; 100, 100, 1.00). Monophyly of *Anisoptera* and *Stemonoporus* was strongly supported (99, 99, 1.00 and 99, 98, 1.00, respectively). In *Anisoptera*, *A. laevis* Ridl. was sister to the other three species, *A. grossivenia* Slooten, *A. marginata* Korth. and *A. oblonga* Dyer (Fig. 2). Species of *Vatica* and *Cotylelobium* each formed sister clades with weak to moderate support (77, 79, 1.00 and 78, 82, 1.00, respectively). *Vateriopsis seychellarum* F.Heim was sister to the other genera in that clade (100, 100, 1.00). Positions of *Upuna* and *Vateria* in this major clade were not well supported (Figs 1, 2).

Molecular dating analysis

The Bayes factor tests using the marginal likelihoods from the BEAST analyses found a clear preference (Bayes factor: 5.6) for the model with monophyletic constraint consisting of only Sarcolaenaceae and Dipterocarpoideae (marginal likelihood estimate: −29 614) over the model with the monophyletic constraint consisting of Monotoideae, Sarcolaenaceae and Dipterocarpoideae (marginal likelihood estimate: −29 619.6). Therefore, results from the analysis using the first model are presented. The age estimates obtained for the major clades showed a wide range (e.g. age estimate for Dipterocarpoideae: 39.3–71.6 Mya). The median crown age estimate for Dipterocarpoideae was 54.9 Mya. Further age estimates for the major clades can be found in Figure 5, but because of the way BEAST works (and our decision not to use a fossil to set the age of the deeper nodes because we judged none of them to be specific enough to be of use in our study) the divergences for the outgroup taxa are not relevant and will not be discussed.

Chromosomes and genome sizes in Dipterocarpoideae

The chromosome numbers determined in this study are given in Table 5 with those from earlier reports on Dipterocarpaceae. Chromosome numbers for five species (*Dipterocarpus zeylanicus* Thwaites: $2n = 22$; *Shorea megistophylla* P.S.Ashton: $2n = 14$; *Hopea jucunda* Thwaites: $2n = 21$; *Shorea oblongifolia* Thwaites: $2n = 14$; and *Vatica endertii* Slooten: $2n = 22$) are reported here for the first time (Fig. 3). Most of the newly counted species were diploid (Fig. 3A–B, D–F), but our chromosome counts of *Hopea jucunda* reveal triploidy (Fig. 3C). Karyotypes were similar and symmetrical for all with small metacentric, submetacentric or subtelocetric chromosomes in all analysed species in Dipterocarpaceae, which makes

Table 5. Chromosome numbers for Dipterocarpaceae

Taxon	Chromosome number	Putative ploidy	Reference(s)
Anisoptera costata Korth.	$2n = 20$	$2x$	Tixier (1953)
Anisoptera laevis Ridl.	$2n = 22$	$2x$	Jong & Lethbridge (1967)
Anisoptera scaphula Pierre	$2n = 20$	$2x$	Tixier (1960)
Anisoptera thurifera Blume	$2n = 22$	$2x$	Oginuma *et al.* (1998)
Dipterocarpus alatus Roxb. & G.Don	$2n = 20$	$2x$	Tixier (1953)
	$2n = 22$	$2x$	Roy & Jha (1965)
Dipterocarpus baudii Korth.	$2n = 22$	$2x$	Jong & Lethbridge (1967)
Dipterocarpus costatus C.F.Gaertn.	$2n = 20$	$2x$	Tixier (1960)
Dipterocarpus intricatus Dyer	$2n = 20$	$2x$	Tixier (1953)
Dipterocarpus kunstleri King	$2n = 20$	$2x$	Pancho (1971)
Dipterocarpus oblongifolius Blume	$2n = 22$	$2x$	Kaur *et al.* (1986)
Dipterocarpus sarawakensis Slooten	$2n = 22$	$2x$	Jong & Lethbridge (1967)
Dipterocarpus tuberculatus Roxb.	$2n = 20$ and 30	$2x$ and $3x$	Tixier (1960)
Dipterocarpus turbinatus C.F.Gaertn.	$2n = 20$	$2x$	Tixier (1960)
Dipterocarpus validus Blume	$2n = 20$	$2x$	Pancho (1971)
Dipterocarpus zeylanicus Thwaites	$2n = 22$	$2x$	*(PDA: D-20)
Dryobalanops oblongifolia Dyer	$2n = 14$	$2x$	Jong & Lethbridge (1967), Kaur *et al.* (1986)
Dryobalanops sumatrensis (J.F.Gmel.) Kosterm.	$2n = 14$	$2x$	Jong & Lethbridge (1967), Kaur *et al.* (1986)
Hopea beccariana Burck	$2n = 20, 21, 22$	$2x, 3x$	Ashton (1982)
Hopea jucunda Thwaites	$2n = 21$	$3x$	*(PDA: D-16)
Hopea latifolia Symington	$2n = 21$	$3x$	Jong & Kaur (1979)
Hopea odorata Roxb.	$2n = 20–22$	$3x$	Kaur *et al.* (1986)
	$n = 7$	–	Sarkar *et al.* (1982)
	$2n = 14$	$2x$	Jong & Lethbridge (1967), Roy & Jha (1965)
	$2n = 20$	$2x$	Tixier (1960)
Hopea subalata Symington	$2n = 21$	$3x$	Kaur *et al.* (1986)
	$2n = 21$	$3x$	Jong & Kaur (1979)
Neobalanocarpus heimii (King) P.S.Ashton	$2n = 14$	$2x$	Jong & Lethbridge (1967)
Shorea acuminata Dyer	$2n = 14$	$2x$	Kaur *et al.* (1986)
Shorea agami P.S.Ashton	$2n = 14$	$2x$	Kaur *et al.* (1986)
Shorea argentifolia Symington	$2n = 14$	$2x$	Kaur *et al.* (1986)
Shorea gardneri (Thwaites) P.S.Ashton	$2n = 14$	$2x$	Jong & Kaur (1979)
Shorea leprosula Miq.	$2n = 14$	$2x$	Kaur *et al.* (1986)
Shorea macrophylla (de Vriese) P.S.Ashton	$2n = 14$	$2x$	Jong & Kaur (1979), Kaur *et al.* (1986)
Shorea macroptera Dyer	$2n = 14$	$2x$	Kaur *et al.* (1986)
Shorea megistophylla P.S.Ashton	$2n = 14$	$2x$	*(PDA: D-24)
Shorea multiflora (Burck) Symington	$2n = 14$	$2x$	Kaur *et al.* (1986)
Shorea oblongifolia Thwaites	$2n = 14$	$2x$	*(PDA: D-26)
Shorea ovalis (Korth.) Blume subsp. *ovalis*	$2n = 28$	$4x$	Kaur *et al.* (1986)
Shorea ovalis (Korth.) Blume subsp. *sericea* (Dyer) P.S.Ashton	$2n = 21, 27, 28$	$3x$ and $4x$	Jong & Kaur (1979)
Shorea pauciflora King	$2n = 14$	$2x$	Kaur *et al.* (1986)
Shorea pinanga Scheff.	$2n = 14$	$2x$	Jong & Kaur (1979), Kaur *et al.* (1986)
Shorea platyclados Slooten ex Endert	$2n = 14$	$2x$	Kaur *et al.* (1986)

Table 5. *Continued*

Taxon	Chromosome number	Putative ploidy	Reference(s)
Shorea resinosa Foxw.	2*n* = 21	3*x*	Jong & Kaur (1979), Kaur *et al.* (1986)
Shorea robusta C.F.Gaertn.	2*n* = 14	2*x*	Roy & Jha (1965), Pal *et al.* (1993)
Shorea roxburghii G.Don	2*n* = 14	2*x*	Roy & Jha (1965), *(S. Duangjai_Dip2014_03)
Shorea splendida (de Vriese) P.S.Ashton	2*n* = 14	2*x*	Jong & Kaur (1979), Kaur *et al.* (1986)
Shorea stenoptera Burck	2*n* = 14	2*x*	Jong & Kaur (1979), Kaur *et al.* (1986)
Shorea trapezifolia (Thwaites) P.S.Ashton	2*n* = 14	2*x*	Jong & Kaur (1979)
Vateria indica L.	*n* = 10	–	Mehra (1976)
Vatica endertii Slooten	2*n* = 22	2*x*	*(UBDH: UBD-CTFS: 01-1700)
Vatica odorata (Griff.) Symington	2*n* = 22	2*x*	Roy & Jha (1965)

Previously published chromosome counts and its references were obtained from http://ccdb.tau.ac.il/ (Rice *et al.*, 2015, accessed 14 July 2017). Counts from the present study are indicated with an asterisk (*). Herbarium voucher of mother plant is given in parentheses. –, not indicated.

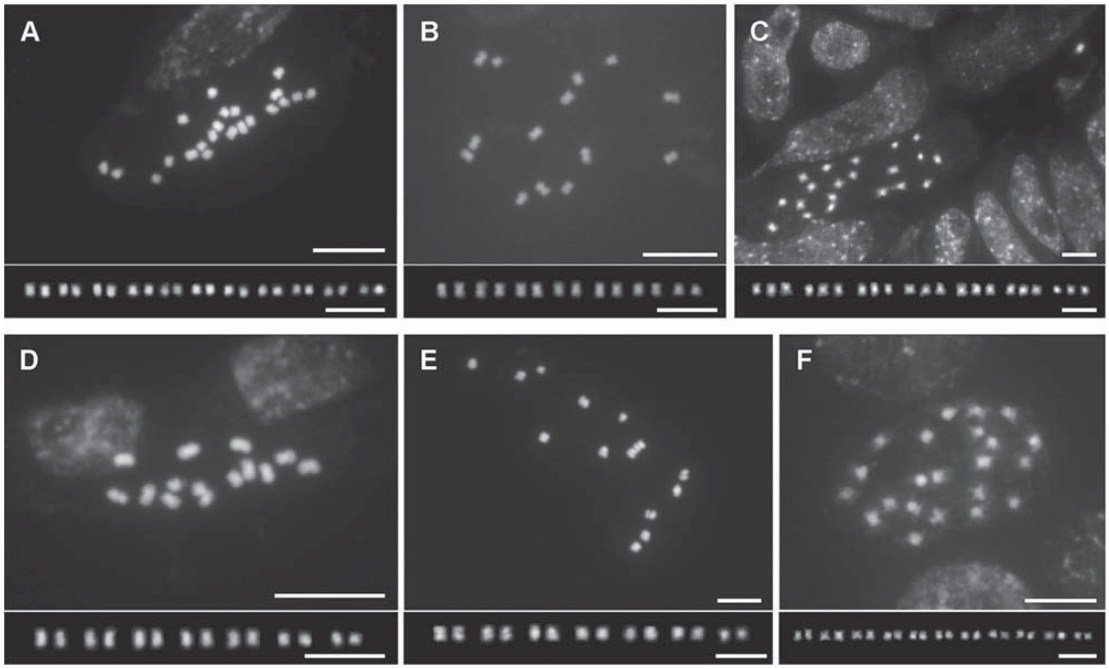


Figure 3. Mitotic chromosomes of some species of Dipterocarpaceae. A, *Dipterocarpus zeylanicus* (2*n* = 2*x* = 22). B, *Shorea megistophylla* (2*n* = 2*x* = 14). C, *Hopea jucunda* (2*n* = 2*x* = 21). D, *Shorea oblongifolia* (2*n* = 2*x* = 14). E, *Shorea roxburghii* (2*n* = 2*x* = 14). F, *Vatica endertii* (2*n* = 2*x* = 22). Scale bars = 5 μm.

identification of individual chromosome pairs difficult (Fig. 3). Similar to a recent study of genome sizes in Dipterocarpaceae (Ng *et al.*, 2016), our measurements of genome size showed differences among and within genera (Table 6, Fig. 4) and range from 1C = 0.3264 pg in *Shorea roxburghii* G.Don to 0.6724 pg in *Vatica*

Table 6. Genome size measurements in Dipterocarpaceae

Taxon	Taxon-ID	C-value	SD
Dipterocarpus hispidus Thwaites	D-7a	0.3287	0.0017
Dipterocarpus zeylanicus Thwaites	D-20	0.3933	0.0178
Hopea brevipetiolaris (Thwaites) P.S.Ashton	D-291	0.3931	0.0016
Hopea brevipetiolaris (Thwaites) P.S.Ashton	C-291	0.3955	0.0028
Hopea brevipetiolaris (Thwaites) P.S.Ashton	D-131a	0.3899	0.0039
Hopea jucunda Thwaites	D-16	0.5949	0.0035
Hopea jucunda subsp. *modesta* (A.DC.) Kosterm.	D-14	0.3277	0.0001
Hopea odorata Roxb.	C-35	0.4216	0.002
Hopea odorata Roxb.	C-36	0.6051	0.0042
Hopea odorata Roxb.	C-37	0.6094	0.0036
Hopea utilis (Bedd.) Bole	D-18	0.3663	0.0013
Shorea lissophylla Thwaites	D-27	0.3586	0.0047
Shorea oblongifolia Thwaites	D-26	0.3669	0.0024
Shorea roxburghii G.Don	S.Duangjai_Dip2014_03	0.3264	0.0033
Shorea zeylanica (Thwaites) P.S.Ashton	C-160	0.4537	0.0012
Stemonoporus canaliculatus Thwaites	D-156	0.4005	0.0011
Vateria copallifera (Retz.) Alston	D-216	0.4581	0.0011
Vatica endertii P.S.Ashton	01-1700	0.5505	0.0006
Vatica diospyroides Symington	S.Duangjai_Dip2014_05	0.6724	0.0079
Vatica sp.	D-198a	0.448	0.0012

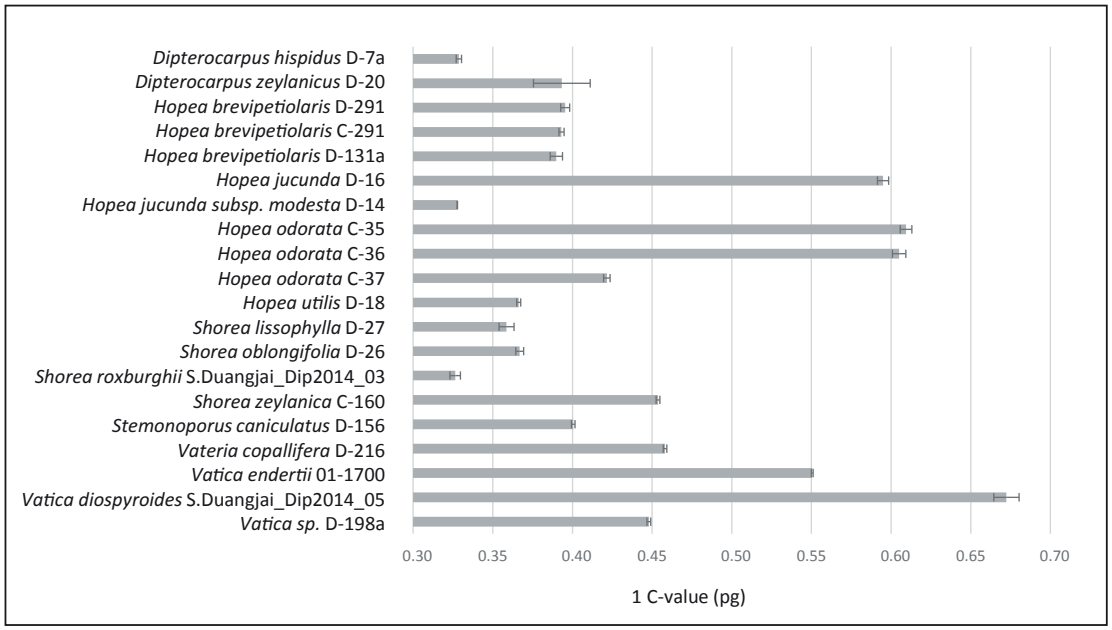


Figure 4. Genome size in several species of Dipterocarpaceae with standard deviation based on three measurements of each individual.

diospyroides Symington. Although most species show uniform genome size, intraspecific variation was detected in *Hopea odorata* Roxb. (1C = 0.4216, 0.6051 and 0.6094 pg).

DISCUSSION

This study provides a comprehensive molecular phylogenetic tree of the ecologically and economically important family Dipterocarpaceae including all three subfamilies, Cistaceae and Sarcolaenaceae, the largest Madagascan endemic family. Taxonomic issues among the three subfamilies, especially in Asian Dipterocarpoideae, could be refined. Molecular phylogenetic analyses have assigned Dipterocarpaceae to Malvales (APG IV, 2016), and recent genetic studies have shown that at least Dipterocarpoideae share a unique common ancestor with Sarcolaenaceae, a family of trees endemic to Madagascar (Dayanandan *et al.*, 1999; Ducousso *et al.*, 2004). The close relationship between Dipterocarpaceae and Sarcolaenaceae has been emphasized by Maguire & Ashton (1977) and Ashton (1982) on morphological evidence and was supported by anatomical features (Capuron, 1970; de Zeeuw, 1977). In addition, results of numerous molecular studies employing plastid and nuclear genes have indicated Cistaceae to be the closest relatives of Dipterocarpaceae in the broadly circumscribed order Malvales (APG, 1998; Savolainen *et al.*, 2000; Soltis *et al.*, 2000). This is supported by the similarity in the structure of the chalazal region of the mature seed (Nandi, 1998) and strongly suggests a common ancestry of at least Monotoideae, Pakaraimaeoideae, Sarcolaenaceae and possibly Dipterocarpoideae, Bixaceae and Cistaceae. All three subfamilies of Dipterocarpaceae (Högberg, 1982; Alexander & Högberg, 1986; Högberg & Piearce, 1986; Lee, 1990; Moyersoen, 2006), Sarcolaenaceae (Ducousso *et al.*, 2004) and Cistaceae (Smith & Read, 1997) are ectomycorrhizal. Our results differed from the widely used subfamily concept for Dipterocarpaceae based on morphological and anatomical evidence consisting of three subfamilies, Dipterocarpoideae, Monotoideae and Pakaraimaeoideae. *Pakaraimaea* is more closely related to Cistaceae, but their exact relationship could not be determined from our limited sampling of the latter. The close relationship between Cistaceae and *Pakariamaea* has been already suggested by Alverson *et al.* (1998), Kubitzki & Chase (2003), Ducousso *et al.* (2004) and Horn, Wurdack & Dorr (2016). *Pakaraimaea* was recently included in Cistaceae in APG (2016). This was an unexpected result from an ecological point of view. Cistaceae are also woody with a few herbaceous members (Proctor, 1978). Dipterocarpaceae (including *Pakaraimaea*) and Sarcolaenaceae are exclusively tropical, but Cistaceae are distributed primarily in the temperate areas of Europe, principally in the Mediterranean Basin and, to a much more limited extent, in North and South America (http://www.mobot.org/mobot/research/apweb/orders/malvalesweb.htm#Cistaceae, accessed 14 July 2017). *Pakaraimaea* are relatively small trees (Maury-Lechon & Curtet, 1998), recalling *Stemonoporus* in architecture. Leaf venation of *Pakaraimaea* shows similarities to those of *Cotylelobium* and *Anisoptera* (Ashton, 2003). On the other hand, there are features not shared by *Pakaraimaea* and Asian Dipterocarpaceae, which supports removing *Pakaraimaea* from Dipterocarpaceae. Contrary to the thick-walled intricately structured pericarp wall of Dipterocarpaceae, the thin fruit pericarp of *Pakaraimaea* has a simple structure. The five-celled fruit dehisces loculidally. There is continuing growth of the cotyledons following germination, and albumen occurs in the ripe embryo, all as in *Monotes*. *Pakaraimaea* petals are shorter than the sepals, and the anthers appear versatile as in Monotoideae (Maury-Lechon & Curtet, 1998). Wood rays are biseriate (Maury-Lechon & Curtet, 1998). The ovary of Dipterocarpoideae and Monotoideae is three-celled, each bearing two seeds (four in *Monotes*). The five-celled ovary of *Pakaraimaea*, each cell bearing two (rarely four) ovules per loculus (Maguire & Ashton, 1977), is unique in Dipterocarpeaceae but typically malvalean and could therefore be primitive within the family. Locules with two to > 30 ovules and two to many have been observed in Sarcolaenaceae (Bayer, 2003) and Cistaceae (Arrington & Kubitzki, 2003), respectively. Ripe fruits of Dipterocarpoideae are one-seeded nuts, generally woody, sometimes corky (Ashton, 2003), and *Pakaraimaea* fruits contain at most one fertile seed although other aborted seeds persist. In *Pakaraimaea* and Monotoideae pollen is tricolporate, with well-developed endexine and a distinct foot layer, whereas in Dipterocarpoideae pollen grains are tricolpate and lack endexine. Anthers are basifixed in Dipterocarpoideae and basi-versatile in Monotoideae and Pakaraimaeoideae. In *Pakaraimaea* and Monotoideae, wood, leaves and ovary are devoid of resin (Maury-Lechon & Curtet, 1998), whereas Dipterocarpoideae are distinguished by the universal presence of intercellular resin canals. Our analyses showed that Monotoideae are probably sister to the Asian dipterocarps, but this was not well supported (Figs 1, 2). The position of Monotoideae needs to be further investigated with a broader taxon sampling and more data. A more detailed analysis is required to obtain further insights into the relationships of Sarcolaenaceae–Dipterocarpaceae–Cistaceae, which could be combined in an expanded family concept as discussed in APG (2009, 2016). Cistaceae is the

oldest name of these three, but conservation of Dipterocarpaceae may be considered as an option to preserve the name of this economically important group of forest trees, if these are to be combined in a single family.

With respect to the large clade of Asian Dipterocarpoideae, we discuss the four clades obtained in our molecular analysis (Fig. 1) and some morphological features. The concept of two tribes, Dipterocarpeae and Shoreeae, was not supported in our analyses. Our results separated *Dipterocarpus* from the remaining genera of Dipterocarpeae (Fig. 1: clades I and IV) and it was weakly supported (76, 73, 0.88) as sister to *Dryobalanops* (Fig. 1: clade II) and Shoreeae (Fig. 1: clade III). This has also been observed in earlier molecular studies (e.g. Kajita *et al.*, 1998; Yulita *et al.*, 2005; Gamage *et al.*, 2006). In the study of Indrioko *et al.* (2006), depending on the outgroup, *Dipterocarpus* was sister either to the remaining Dipterocarpeae (bootstrap support: 80%) or to Shoreeae (bootstrap support: 83%). We acknowledge that the weak support obtained from our analysis limits our ability to interpret this relationship. *Dipterocarpus* could perhaps be sister to other Dipterocarpeae or the latter form a separate tribe. *Dipterocarpus* makes trees that are columnar but hardly buttressed with untidy globose crowns and prominently lenticellate orange–brown massively flaky bark, which at once makes these recognizable as distinct from other large forest dipterocarps. They have the chromosome number, $2n$ = 20–22, as in other Dipterocarpeae (e.g. Tixier, 1953; Tixier, 1960; Table 5) but differ from other Dipterocarpoideae further in their dispersed resin canals in the wood (Meijer, 1979; Ashton, 1982). Other typical characters are large leaf buds, amplexicaul bud scales and stipules furnished with diverse species-defining indumenta, plicate venation resulting in corrugation of their coriaceous leaves, thickly geniculate and often long petioles with often complex rings of vascular bundles and resin canals, large flowers bearing a tubular calyx united at base into a smooth, angled, tuberculate or flanged tube enclosing but free from the ovary, two aliform, valvate sepals, and 15–40 stamens that are larger than in all other dipterocarp taxa and have elongate orange anthers and stout tapering connectival appendages. First-branching Dipterocarpoideae exhibit relatively large orange anthers, whereas those are reduced in size and white in most derived clades. Dipterocarpaceae are pollinated by pollenivores (Thysanoptera, Ashton, Givnish & Appanah, 1988; Kondo *et al.*, 2016; multiple species of Coleoptera, Appanah & Chan, 1981; Momose *et al.*, 1998; Nagamitsu, Harrison & Inoue, 1999; Sakai *et al.*, 1999; flies, Khatua, Chakrabarti & Mallick, 1998; and bees, Khatua, Chakrabarti & Mallick, 1998; Momose, Nagamitsu & Inoue, 1996; see also Corlett, 2004). *Shorea acuminata* Dyer is also pollinated by a species of *Geocoris*, a major predator of thrips (Kondo *et al.*, 2016). *Dipterocarpus* is mainly pollinated by nectarivorous Lepidoptera (Ghazoul, 1997; Harrison *et al.*, 2005; Ashton, 2014), but also by Hymenoptera (Apis; Harrison *et al.*, 2005) and, to a small extent, by Coleoptera (Harrison *et al.*, 2005) and birds (Ghazoul, 1997).

Furthermore, our results revealed a sister relationship of *Dryobalanops* (II) to Shoreeae (III) (91, 97, 1.00; Figs 1, 2), which is in agreement with earlier molecular studies (Tsumura *et al.*, 1996; Kajita *et al.*, 1998, Kamiya *et al.*, 1998, Gamage *et al.*, 2003, 2006; Yulita, 2013). However, in the study of Indrioko *et al.* (2006), depending on the outgroup selection, *Dryobalanops* clustered with either Dipterocarpeae or Shoreeae. This ambiguity over the placement of *Dryobalanops* with either Shoreeae or Dipterocarpeae is reflected in its morphology and chromosome number. It shares wood anatomical characters (fibres with bordered pits, scattered resin canals and solitary vessels) with Dipterocarpeae, whereas its chromosome number, n = 7, and a thickened fruit sepal base are similar to those of Shoreeae (Gottwald & Parameswaran, 1966; Ashton, 1982). Moreover, being subvalvate, the sepals in fruit are intermediate between these tribes (Dipterocarpeae, valvate; Shoreeae, imbricate). Besides the strong bootstrap support, it is not clear from morphological characters if *Dryobalanops* could be included in the tribe Shoreeae or kept as an independent tribe.

Regarding the third clade (Fig. 1, clade III), our analyses clearly showed that *Hopea*, *Parashorea*, *Neobalanocarpus* and paraphyletic *Shorea* (tribe Shoreeae) should probably not be separated into distinct genera without additional evidence. This also has been reported in earlier molecular analyses (e.g. Yulita *et al.*, 2005; Gamage *et al.*, 2006). Pollen morphology of *Shorea*, *Hopea*, *Parashorea* and *Neobalanocarpus* is fairly uniform (Talip, 2008) and there are no obvious morphological characters to separate these four genera. *Anthoshorea* and *Doona* (endemic to Sri Lanka) form distinct groups, sister to *Hopea* and *Neobalanocarpus* (Fig. 1: clade III, subclade B), an observation also reported by Gamage *et al.* (2006). For species-rich *Shorea*, 11 sections have been proposed by Ashton (1982), based on the independent characters of androecium and bark morphology proposed by Symington (1943) and amplified by Whitmore (1963). Sections and subsections for each species of *Shorea* included in this study are given in Figure 2. However, our molecular analyses could not clearly separate these sections, but five groups of *Shorea* were observed. These groups were also recovered by Gamage *et al.* (2006) and correspond to the classification of Maury (1978; Table 1; Fig. 1, clade III; Fig. 2). According to Maury (1978), *Shorea* consists of six genera, *Anthoshorea*, *Rubroshorea*,

Richetia, *Shorea*, *Doona* and *Pentacme* A.DC. (the last was not included in our study), generic limits which correlate with the field characters of bark and wood anatomy proposed by Symington (1943); *Anthoshorea* = white meranti, *Rubroshorea* = red meranti, *Richetia* = yellow meranti and *Shorea* = selangang batu/balau). *Rubroshorea* is held together solely by the red colour of their wood, a character also found in some other *Shorea* spp. All species for which characteristics have been observed (two thereby excepted) are unambiguously attributable to the five sections in *Rubroshorea* (*Brachypterae* F.Heim, *Mutica* P.S.Ashton, *Ovalis* Symington ex P.S.Ashton, *Pachycarpae* P.S.Ashton and *Rubella* P.S.Ashton) recognized by Ashton. To evaluate whether the classifications proposed by Ashton (1964, 1968, 1980, 1982), Maury (1978) and Maury-Lechon (1979a, b) can be supported, *Pentacme* needs to be included in subsequent analyses. Furthermore, in our results, it is obvious that *Shorea* should include *Hopea*, *Parashorea* and *Neobalanocarpus*. Here, *Parashorea* clustered with *Rubroshorea* (red meranti) and *Shorea* (selangan batu/balau). A close relationship between *Shorea* and *Parashorea* was also confirmed in earlier molecular studies (Tsumura *et al.*, 1996; Kajita *et al.*, 1998; Kamiya *et al.*, 2005; Gamage *et al.*, 2003, 2006; Indrioko *et al.*, 2006). In an AFLP analysis, *Parashorea* clustered with *Hopea*, which could be explained by interspecific hybridization or ancestral polymorphisms as suggested by Cao *et al.* (2006). *Neobalanocarpus* was sister to *Hopea* (Figs 1, 2), which contradicts the nuclear *PgiC* analysis of Kamiya *et al.* (2005), in which *Neobalanocarpus* is nested in the white meranti group of *Shorea*. This could indicate hybridization between a species of white meranti and one of *Hopea*, as suggested by Kamiya *et al.* (2005). Evidence for hybridization comes from irregular meiosis (*Neobalanocarpus*) and existence of morphologically intermediate individuals between other species in Shoreeae (Ashton, 2003).

The fourth clade comprised *Anisoptera*, *Cotylelobium*, *Stemonoporus*, *Upuna*, *Vateria*, *Vateriopsis* and *Vatica* (Fig. 1, clade IV). *Anisoptera laevis* was highly divergent from the other three species, *A. grossivenia*, *A. marginata* and *A. oblonga* (Fig. 2) to which it was sister. This fits the classifications of Ashton (1964, 1968, 1980, 1982), Maury (1978) and Maury-Lechon (1979a, b), in which *Anisoptera* is divided into two sections, *Glabrae* (ined.), to which *A. laevis* is assigned, and *Anisoptera* Korth. containing the other three species included in this study. This is well supported by the morphological features of the flower buds, number of stamens, style and stigma (Ashton, 2003). Monophyly of *Stemonoporus*, which is endemic to Sri Lanka, was strongly supported (99, 100, 1.00) in all analyses, consistent with previous molecular studies (Dayanandan *et al.*, 1999; Gamage *et al.*, 2003, 2006) and its distinctive morphological features, including peculiar anthers with apical dehiscence, leaf traces that separate from the central vascular cylinder well before the node and the absence of wing-like sepals (Ashton, 1982). *Cotylelobium* was weakly (MP, ML) to highly supported (BI) as sister to *Vatica* (Figs 1, 2). Similar results have occurred in previous studies (Kajita *et al.*, 1998; Kamiya *et al.*, 1998; Gamage *et al.*, 2006). In our results, the positions of *Vateria* and *Upuna* remained unresolved or weakly supported, although a sister relationship of *Upuna* to *Anisoptera* has been suggested by one of the co-authors (P. S. Ashton, pers. comm.). *Vateriopsis seychellarum*, which is endemic to the Seychelles, has unique anatomical features: many stamens, implying a primitive condition (Ashton, 1982), with anthers of a type attracting bees, although no native bees currently survive on the islands. It is sister to the remaining genera in clade IV (Figs 1, 2). *Stemonoporus* and *Vateria*, the endemic genera of Gondwanan peninsular India, also have wingless fruits.

Besides clarification of phylogenetic relationships in Dipterocarpaceae and allied families, one of the aims in this study was to obtain estimation of divergence times of Dipterocarpaceae and infer ages of major clades in Dipterocarpoideae. The biogeography and origin of Cistaceae, Sarcolaenaceae and Dipterocarpaceae have been widely discussed. The age of crown-group Cistaceae is *c.* (18.5–) 14.2 (–10.2) Mya (Guzmán & Vargas, 2009). Diversification in Sarcolaenaceae possibly began only 4.5 Mya (http://www.mobot.org/mobot/research/apweb/orders/malvalesweb.htm#Sarcolaenacea, accessed 14 July 2017). Wikström, Savolainen & Chase (2001) estimated the origin of Dipterocarpaceae as 14–28 Mya, but these dates are based on an analysis that included only one dipterocarp. Such limited sampling was stated by those authors to underestimate ages in terminal clades. On the other hand, based on the ectomycorrhizal status of *Pakaraimaea*, Moyersoen (2006) suggested that Dipterocarpaceae occurred on Gondwana *c.* 135 Mya. Fossil resin and pollen grains from the early Eocene of western India (Dutta *et al.*, 2009, 2011; Rust *et al.*, 2010) suggested an origin or early occurrence of Dipterocarpaceae in India and later dispersal to Southeast Asia–Malesia and southern China after contact of the two was established *c.* 50 Mya (Feng *et al.*, 2013; Shukla, Mehrotra & Guleria, 2013). In addition, Tertiary fossils of East Africa have been attributed to *Dipterocarpus* (Bancroft, 1935). Thus, *Dipterocarpus* is the only genus of Dipterocarpoideae known from Africa (Bancroft, 1935; Ashton & Gunatilleke, 1987). Although it would seem unlikely that the diversity of other dipterocarpoid genera could all have originated on the Indian Noah's Ark, some may have later dispersed there and gone extinct in Africa. During the late Oligocene and early Miocene

(20–23 Mya), Dipterocarpoideae occurred in the monsoon forests of the Sunda region and were therefore already distributed across Southeast Asia at the time of widespread expansion of evergreen rainforest in the later part of the early Miocene and probably have become a major part of the Southeast Asian rainforest only since then (Morley, 2000). The irregular flowering pattern followed by the distinctive masting behaviour of Dipterocarpaceae, which depends on sudden cool spells resulting from El Niño oscillations, supports their origin in a seasonal climate (Ashton, 1988). For molecular clock analyses, fossils are often used as calibration points for defined clades. Several dipterocarp fossils are reported in the literature (e.g. Dutta *et al.*, 2011; Feng *et al.*, 2013). However, placing fossils in the correct position on the phylogenetic tree is crucial for correct interpretation (Forest, 2009), and we faced several problems in assigning the described fossils to clades in our trees. For example, winged fruits and associated leaves of *Shorea* are reported from the late Eocene of South China by Feng *et al.* (2013) and are described as *Shorea maomingensis* Feng, Kodrul & Jin. According to Feng *et al.* (2013), this fossil can be attributed to *Shorea ovalis* (Korth.)Bl. subsp. *sericea* (Dyer) P.S.Ashton. Feng *et al.* (2013) suggested that the fossil leaves show the greatest similarities to *Shorea*. According to P. S. Ashton (pers. comm.), these leaves differ from Dipterocarpaceae in the nature of their reticulate tertiary venation, whereas the fruit is not that of the tetraploid *S. ovalis*, but almost certainly a species with subauriculate sepal bases in *Shorea* section *Anthoshoreae.* Another problem of using fossils for calibration is that they represent minimum ages. Coetzee & Muller (1984) reported intricate pollen tetrads of extant taxa of Sarcolaenaceae from South Africa in the Miocene, but these probably do not represent the oldest occurrence of this family (Nilsson, Coetzee & Grafström, 1996). It may have been an ancient endemic African taxon that migrated to Madagascar where it became restricted (Raven & Axelrod, 1974). As the Sarcolaneaceae pollen fossils are young (Miocene), we decided not to use them as a calibration point. To avoid the problem of incorrect placement, we did not use any fossils and instead applied the time of separation of Madagascar from the India–Seychelles block (87.6 ± 0.6 Mya) as a calibration point for Sarcolaenaceae plus Dipterocarpoideae. Potentially due to differences between phylogenetic models or implementation in BEAST used for the age estimation, the topology of the dated maximum clade credibility tree (Fig. 5) differed slightly from the trees obtained in our other analyses (Figs 1, 2). However, differences in topologies were not well supported in either result (see posterior probabilities; Fig. 5). Our dating study gives a general time frame for the major clades in Dipterocarpoideae and shows that they had already diverged into the extant genera by the end of the Miocene. Our median crown age estimate for Dipterocarpoideae was 54.9 Mya. The emergence of Dipterocarpeae was dated to 47.7 Mya (crown age). The dating analysis revealed 43.3 Mya as the median age of Shoreeae and *Dryobalanops*. Monotypic *Vateriopsis* is endemic to the Seychelles and has wingless fruits and seeds that are inviable in salt water, implying early separation from other Dipterocarpoideae (63 Mya; Ashton, 2014). Our results here indicate 34.9 Mya as the median age of *Vateriopsis*. The separation of the Seychelles from India began *c.* 63.4 Mya (Collier *et al.*, 2008). Therefore, our age estimates imply that *Vateriopsis* reached its current position not by continental drift, but rather by long-distance dispersal. Our dating estimate of 15.4 Mya for *Vateria* corresponds to the occurrence of the fossil *Vaterioxylon* in northern India in the Miocene (Maury-Lechon & Curtet, 1998) and suggest parallel evolution of *Vateria* and *Upuna*. An expanded analysis including a much larger set of Malvales would permit the use of multiple calibrations points and would be suitable to obtain further insights into the ages of clades in the larger set of taxa included here.

Earlier reports of chromosome numbers for Dipterocarpoideae indicated a high level of uniformity in the species and genera with *Anisoptera*, *Dipterocarpus*, *Upuna* and *Vatica* having $x = 11$ and *Dryobalanops*, *Hopea*, *Neobalanocarpus*, *Parashorea* and *Shorea* having $x = 7$ as the basic chromosome numbers. Some species of the last exhibit a chromosome number of 20, 21 and 22, assuming that $x = 11$ might have been derived from $x = 7$ through hybridization and polyploidization (Bawa, 1998). Our additional chromosome counts confirm those of earlier studies and demonstrate further evidence of polyploidy in Dipterocarpaceae (*Hopea jucunda*: $2n = 21$), which has been reported in *Shorea* [e.g. *S. ovalis* (Korth.) Blume with $2n = 28$ and *S. resinosa* Foxw. with $2n = 21$; Kaur *et al.*, 1986)] and *Hopea* (e.g. *H. odorata*: $2n = 20–22$ and *H. subalata* Symington: $2n = 21$, Kaur *et al.*, 1986). Furthermore, intraspecific variation in chromosome numbers has been observed (e.g. *H. odorata*: $2n = 14, 20, 21, 22$; Jong & Lethbridge, 1967; Kaur *et al.*, 1986). However, variation in chromosome numbers has to be interpreted with caution due to the often small sample size (Bawa, 1998). For example, the form of intraspecific variation in *H. odorata* is dysploid or polyploid, but it remains unclear if it is in the form of occasional dysploid individuals or polyploid populations (Ashton, 1982). Sampling of several individuals in the same population and of the same species from different populations would be helpful in evaluating variation and its significance (Bawa, 1998). Genome

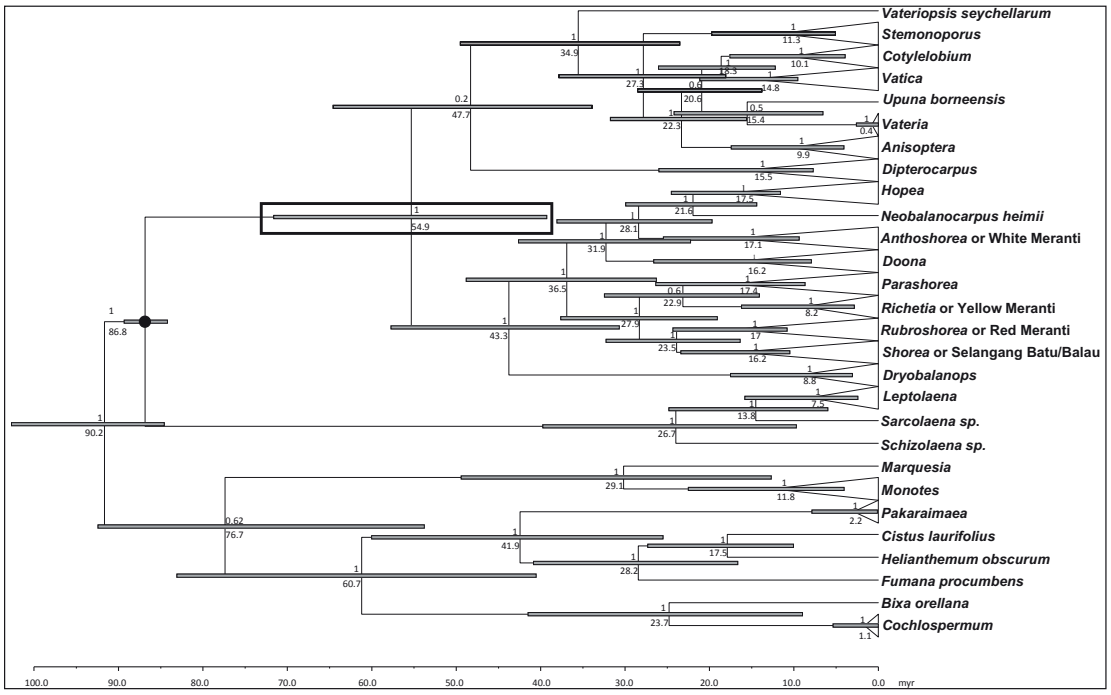


Figure 5. Dated maximum clade credibility tree obtained from BEAST analysis. Taxa are collapsed to major clades. The node that was calibrated is marked with a black dot. Grey node bars represent the 95% highest posterior density interval. Posterior probabilities are given above each node and the mean age estimates are shown below each node. The node in the black box shows the age estimates for Dipterocarpoideae. Geological time scale is given in millions of years.

size of Dipterocarpaceae was first reported from a diploid *Shorea robusta* C.F.Gaertn. (2C = 1.15 pg; Ohri & Kumar, 1986), and insights into evolution of genome size in Dipterocarpaceae were recently reported by Ng *et al.* (2016). Genome sizes of 20 individuals representing 15 species in six genera were obtained in this study (Table 6, Fig. 4) and ranged from 1C = 0.3264 pg in *Shorea roxburghii* to 0.6724 pg in *Vatica diospyroides*. Genome size variation was observed between and within genera, corresponding well to the results of Ng *et al.* (2016; Table 6). Moreover, genome size variation was observed within species, e.g. in *Hopea odorata* (1C = 0.4216, 0.6051 and 0.6094 pg). Dipterocarpaceae have relative small genome sizes, corresponding to previous observations of small genome sizes in woody angiosperms that are hypothesized to rarely experience polyploidization (Ohri, 2005; Chen *et al.*, 2014). Compared to closely related families (Bennett & Leitch, 2012), genome size in dipterocarps was smaller than those in Cistaceae (median 1C = 2.53 and 0.88–4.50 pg, respectively), but larger than those in Bixaceae (1C = 0.20 pg). Although negative correlations have been observed between genome size and species richness (e.g. Vinogradov, 2004; Knight *et al.*, 2005), in their study Ng *et al.* (2016) argued that excluding any correlation between the high species diversity of Dipterocarpoideae and their small genome size is premature, and further studies are needed.

CONCLUSIONS

Several molecular and many morphological studies on Dipterocarpaceae have been conducted in the past. Here, we present the first molecular phylogenetic study including all three subfamilies of Dipterocarpaceae and closely related families. In our study, there are conflicts between molecular results and the distribution of some of the intuitively selected morphological characters that in the past have been the basis of previous classifications. Broad and critical observations on well-defined morphological characters are important for classical taxonomy, but ultimately such decisions should be taken on the bases of all data, not just a set of intuitively selected characters that are thought to be more reliable than others. For example, Ashton's

circumscription of *Shorea* was based on a single character, the number of long versus short fruit sepals. However, many *Shorea* spp. only have short subequal fruit sepals. This concept is further complicated by the fact that *Parashorea* also has unequal fruit sepals, which could be interpreted as three long and two short as in *Shorea*. Our molecular results were not supported well enough to resolve the 11 sections in *Shorea* proposed by Ashton on the basis of morphological characters. We therefore assume that next-generation techniques, such as restriction-site associated sequencing (RADseq), which allows sampling of genome-wide single nucleotide polymorphisms, could give better resolution at the species level in *Shorea* and be able to detect instances of hybridization, which have been suggested in some previous studies (e.g. AFLP; Cao *et al.*, 2006). To conclude, our study strengthens the phylogenetic hypotheses for the larger clade to which Dipterocarpaceae are related (*Pakaraimaea* + Cistaceae) (Sarcolaenaceae + Monotoideae + Dipterocarpoideae). Nevertheless, there are still some relationships between (Sarcolaenaceae + Monotoideae + Dipterocarpoideae) that still need to be clarified. This paper clearly demonstrates that morphological and molecular evidence are both important, although there are still some discrepancies between them that need to be better addressed in future research.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Table S1. Specimens used in this study. The collection number, herbarium voucher, and location is given. GenBank accession numbers of species used for phylogenetic analysis is stated.

Table S1: Specimens used in this study. The collection number, herbarium voucher, and location is given. GenBank accession numbers of species used for phylogenetic analysis is stated.
Field collectors: JH: Jacqueline Heckenhauer, KAS: Kamariah Abu Salim, R.S.: Rosabella Samuel, SD: Sutee Duangjai;
Herbarium codes: BRUN: Brunei Forestry Centre, Brunei Darussalam, Belait; K: Royal Botanical Gardens, U.K. England, Kew; KUFF: Faculty of Forestry, Kasetsart University Herbarium, Thailand, Nakhon Pathom; PDA: Royal Botanical Gardens, Sri Lanka, Peradeniya; UBDH: Universiti Brunei Darussalam Herbarium, Gadong; WU: Herbarium of Institute of Botany, University of Vienna
n.a.: not available
GB: Sequences obtained from GenBank; Location: BD: Brunei Darussalam, UBD-CTFS 25 ha plot: Kuala Belalong, Temburong, Brunei Darussalam;
[1]DNA obtained from Kew (apps.kew.org/dnabank); [2]Material obtained from MO (Missouri Botanical Garden, Saint Louis, USA); [3]Material obtained from Biological Diversity of the Guiana Shield Program, US National Herbarium, Smithsonian Institution; [#]sequences obtained from GenBank consist of *trnL-F* intergenic spacer and *trnL* intron
[gs]: sample used for genome size
[cs]: sample used for chromosom count

Taxon	Collection	Voucher	Location	*rbcL*	*matK*	*trnT-trnL-trnF*[#]
Dipterocarpoideae						
Anisoptera grossivenia Slooten	KASag1	BRUN: KEP 80080/ B 000 014	Sg.Liang. Andulau Forest Reserve, Kuala Belait, BD	KY973095	KY972898	KY972701
	KASag2	BRUN: KEP 80080/ B 000 014	Sg.Liang. Andulau Forest Reserve, Kuala Belait, BD	KY973096	KY972899	KY972702
Anisoptera laevis Ridl.	JH02-0255	UBDH: 02-0255	UBD-CTFS 25 ha plot	KY973097	KY972900	KY972703
	JH16-2455	UBDH: 16-2455	UBD-CTFS 25 ha plot	KY973098	KY972901	KY972704
Anisoptera marginata Korth.	Chase, M.W. 2486[1]	K: n.a.	Java, Kebun Raya, Bogor, Indonesia	KY973100	KY972903	KY972706
	KASam1	BRUN: KEP 30418	UBD plot, Km 13 Bt.Sawat, Kuala Belait, BD	KY973099	KY972902	KY972705
Anisoptera oblonga Dyer	GB		Frim, Kepong, Malayasia		AB006371	AB006405 AB006388
Cotylelobium burckii F.Heim	KAScb1	BRUN: WKM 935/ B 000 033	UBD plot, Km 13, Jln Labi, Bt.Sawat, Kuala Belait, BD	KY973108	KY972911	KY972713
Cotylelobium malayanum Slooten	GB		Frim Arboretum, Malayasia		AB246414	AB246479 AB246544
Cotylelobium scabriusculum Brandis	GB		Kottawa Forest, Sri Lanka		AB246415	AB246480 AB246545
Dipterocarpus alatus Roxb. & G.Don	SDDip2014_01	KUFF: S.Duangjai_Dip2014_01	n.a.	KY973111	KY972914	KY972716
Dipterocarpus baudii Korth.	GB		Frim, Kepong, Malaysia		AB006376	AB006410 AB006393
Dipterocarpus borneensis Slooten	KASdb1	n.a.	n.a	KY973112	KY972915	KY972717
	KASdb2	n.a.	n.a	KY973113	KY972916	KY972718
Dipterocarpus caudiferus Merr.	JH15-0406	UBDH: 15-0406	UBD-CTFS 25 ha plot	KY973120	KY972923	KY972725
	JH15-0427	UBDH: 15-0427	UBD-CTFS 25 ha plot	KY973121	KY972924	KY972726

Dipterocarpus confertus Slooten	JH01-1119	UBDH: 01-1119	UBD-CTFS 25 ha plot	KY973116	KY972919	KY972721
	JH01-1381	UBDH: 01-1381	UBD-CTFS 25 ha plot	KY973117	KY972920	KY972722
Dipterocarpus cornutus Dyer	GB		Frim Arboretum, Malayasia		AB246472	AB246537 AB246602
Dipterocarpus glandulosus Thwaites	GB		Kanneliya Forest Reserve, Sri Lanka		AB246477	AB246542 AB246607
Dipterocarpus hispidus Thwaites	GB		Wilpita Forest, Sri Lanka		AB246476	AB246541 AB246606
	R.S. D-7a[gs]	PDA: D-7a	Arboretum, The Royal Botanical Gardens, Peradeniya, Sri Lanka			
Dipterocarpus insignis Thwaites	GB		Kanneliya Forest Reserve, Sri Lanka		AB246475	AB246540 AB246605
Dipterocarpus kerrii King	GB		Frim, Kepong, Malayasia		AB006375	AB006409 AB006392
Dipterocarpus palembanicus Slooten	KASdp1	n.a.	n.a	KY973122	KY972925	KY972727
	KASdp2	n.a.	n.a	KY973123	KY972926	KY972728
Dipterocarpus palembanicus subsp. *borneensis* P.S.Ashton	JH04-3068	UBDH: 04-3068	UBD-CTFS 25 ha plot	KY973109	KY972912	KY972714
	JH20-2907	UBDH: 20-2907	UBD-CTFS 25 ha plot	KY973110	KY972913	KY972715
	JH06-1278	UBDH: 06-1278	UBD-CTFS 25 ha plot	KY973118	KY972921	KY972723
	JH07-1768	UBDH: 07-1768	UBD-CTFS 25 ha plot	KY973119	KY972922	KY972724
Dipterocarpus cf. verrucosus Foxw. ex Slooten	JH05-1706	UBDH: 05-1706	UBD-CTFS 25 ha plot	KY973114	KY972917	KY972719
	JH05-1711	UBDH: 05-1711	UBD-CTFS 25 ha plot	KY973115	KY972918	KY972720
Dipterocarpus zeylanicus Thwaites	R.S. D-20[cs, gs]	PDA: D-20	Arboretum, The Royal Botanical Gardens, Peradeniya, Sri Lanka	KY973124	KY972927	KY972729
	R.S. C-112	PDA: C-112	Arboretum, The Royal Botanical Gardens, Peradeniya, Sri Lanka	KY973125	KY972928	KY972730
Dryobalanops aromatica C.F.Gaertn.	KASda1	BRUN: BRUN 17436/ B 000 325	UBD plot, Andulau Forest Reserve, Kuala Belait, BD	KY973126	KY972930	KY972732
	KASda2	BRUN: BRUN 17436/ B 000 325	UBD plot, Andulau Forest Reserve, Kuala Belait, BD	KY973127	KY972931	KY972733
Dryobalanops beccarii Dyer	14-4347	UBDH: 14-4347	UBD-CTFS 25 ha plot	KY973128	KY972932	KY972734
	14-4350	UBDH: 14-4350	UBD-CTFS 25 ha plot	KY973129	KY972933	KY972735
Dryobalanops lanceolata Burck	22-0354	UBDH: 22-0354	UBD-CTFS 25 ha plot	KY973130	KY972934	KY972736
Dryobalanops rappa Becc.	KASdr1	BRUN: WKM 917/ B 000 393	UBD plot, Andulau Forest Reserve, Kuala Belait, BD	KY973131	KY972935	KY972737
Dryobalanops oblongifolia Dyer	GB		Frim, Kepong, Malayasia		AB006378	AB006412 AB006395
Hopea bracteata Burck	JH01-3827	UBDH: 01-3827	UBD-CTFS 25 ha plot	KY973134	KY972938	KY972740
	JH04-4121	UBDH: 04-4121	UBD-CTFS 25 ha plot	KY973135	KY972939	KY972741

Hopea brevipetiolaris (Thwaites) P.S.Ashton	R.S. D-131a[gs]	PDA: D-131a	Arboretum, The Royal Botanical Gardens, Peradeniya, Sri Lanka	KY973103	KY972906	KY972709
	R.S. C-291[gs]	PDA: C-291	Arboretum, The Royal Botanical Gardens, Peradeniya, Sri Lanka	KY973101	KY972904	KY972707
	R.S. D-291[gs]	PDA: D-291	Arboretum, The Royal Botanical Gardens, Peradeniya, Sri Lanka	KY973102	KY972905	KY972708
Hopea centipeda P.S.Ashton	JH21-3841	UBDH: 21-3841	UBD-CTFS 25 ha plot	KY973140	KY972944	KY972746
	JH21-3935	UBDH: 21-3935	UBD-CTFS 25 ha plot	KY973141	KY972945	KY972747
Hopea discolor Thwaites	GB		Kanneliya Forest Reserve, Sri Lanka		AB246458	AB246523 AB246588
Hopea dryobalanoides Miq.	JH24-5607	UBDH: 24-5607	UBD-CTFS 25 ha plot	KY973136	KY972940	KY972742
	JH24-0530	UBDH: 24-0530	UBD-CTFS 25 ha plot	KY973137	KY972941	KY972743
Hopea dyeri F.Heim	KAShd1	BRUN: BRUN 3362/ B 000 470	KN Nursery Km 13 Bt Sawat, Kuala Belait, BD	KY973138	KY972942	KY972744
	KAShd2	BRUN: BRUN 3362/ B 000 470	KN Nursery Km 13 Bt Sawat, Kuala Belait, BD	KY973139	KY972943	KY972745
Hopea helferi Brandis	GB		Frim Aboretum, Malayasia		AB246457	AB246522 AB246587
Hopea jucunda Thwaites	R.S. D-16[cs,gs]	PDA: D-16	Arboretum, The Royal Botanical Gardens, Peradeniya, Sri Lanka	KY973142	KY972946	KY972748
Hopea jucunda subsp. *modesta* (A.DC.) Kosterm.	R.S. D-14[gs]	PDA: D-14	Arboretum, The Royal Botanical Gardens, Peradeniya, Sri Lanka	KY973143	KY972947	KY972749
Hopea latifolia Symington	GB		Frim Arboretum, Malayasia		AB246456	AB246521 AB246586
Hopea nervosa King	JH05-5391	UBDH: 05-5391	UBD-CTFS 25 ha plot	KY973144	KY972948	KY972750
	JH14-4326	UBDH: 14-4326	UBD-CTFS 25 ha plot	KY973145	KY972949	KY972751
Hopea odorata Roxb.	R.S. C-35[gs]	PDA: C-35	Arboretum, The Royal Botanical Gardens, Peradeniya, Sri Lanka	KY973146	KY972950	KY972752
	R.S. C-36[gs]	PDA: C-36	Arboretum, The Royal Botanical Gardens, Peradeniya, Sri Lanka			
	R.S. C-37[gs]	PDA: C-37	Arboretum, The Royal Botanical Gardens, Peradeniya, Sri Lanka			
	SDDip2014_04	KUFF: S.Duangjai_Dip2014_04	Botanical Garden, Kasetsart University, Nakhon Pathom, Thailand	KY973147	KY972951	KY972753
Hopea pentanervia Symington ex G.H.S.Wood	KAS1339	BRUN: BRUN 17424/ B 000 591	UBD plot, Km 13 Bt.Sawat, Kuala Belait, BD	KY973148	KY972952	KY972754
	KAS1340	BRUN: BRUN 17424/ B 000 591	UBD plot, Km 13 Bt.Sawat, Kuala Belait, BD	KY973149	KY972953	KY972755
Hopea subalata Symington	GB		Frim Arboretum, Malayasia		AB246455	AB246520 AB246585

Hopea utilis (Bedd.) Bole	R.S. D-18[gs]	PDA: D-18	Arboretum, The Royal Botanical Gardens, Peradeniya, Sri Lanka	KY973104	KY972907	KY972710
	R.S. C-183	PDA: C-183	Arboretum, The Royal Botanical Gardens, Peradeniya, Sri Lanka	KY973105	KY972908	KY972711
Hopea vaccinifolia Ridl. ex P.S.Ashton	KAShv1	BRUN: BRUN 18998/ B 000 822	UBD plot, Km 13 Bt.Sawat, Kuala Belait, BD	KY973150	KY972954	KY972756
	KAShv2	BRUN: BRUN 18998/ B 000 822	Bt. Puan, Labi, Kuala Belait, BD	KY973151	KY972955	KY972757
Hopea wightiana Wall.	GB		Frim Arboretum, Malyasia		AB246461	AB246526 AB246591
Neobalanocarpus heimii (King) P.S.Ashton	GB		Frim, Kepong, Malayasia		AB006383	AB006417 AB006400
Parashorea lucida Kurz	GB		Frim Kepong, Malayasia		AB006382	AB006416 AB006399
Parashorea macrophylla Wyatt-Sm. ex P.S.Ashton	JH24-4723	UBDH: 24-4723	UBD-CTFS 25 ha plot	KY973159	KY972962	KY972764
	JH24-4857	UBDH: 25-4857	UBD-CTFS 25 ha plot	KY973160	KY972963	KY972765
Parashorea malanoon Merr.	JH16-1785	UBDH: 16-1785	UBD-CTFS 25 ha plot	KY973161	KY972964	KY972766
	JH25-0007	UBDH: 25-0007	UBD-CTFS 25 ha plot	KY973162	KY972965	KY972767
Parashorea cf. tomentella (Symington) Meijer	JH20-2932	UBDH: 20-2932	UBD-CTFS 25 ha plot	KY973163	KY972966	KY972768
	JH22-2154	UBDH: 22-2154	UBD-CTFS 25 ha plot	KY973164	KY972967	KY972769
Shorea sp.	KASsp1.1	n.a.	UBD plot, Km 13 Bt.Sawat, Kuala Belait UBD-CTFS 25 ha plot	KY973271	KY973081	KY972884
	KASsp2.1	n.a.	UBD plot, Km 13 Bt.Sawat, Kuala Belait UBD-CTFS 25 ha plot	KY973272	KY973082	KY972885
	JH19-1085	UBDH: 19-1085	UBD-CTFS 25 ha plot	KY973189	KY972997	KY972800
	JH19-1090	UBDH: 19-1090	UBD-CTFS 25 ha plot	KY973190	KY972998	KY972801
	JH08-5266	UBDH: 08-5266	CTFS 25 ha plot	KY973202	KY973012	KY972815
	KASsp1	n.a.	Sg.Liang, Andulau FR, Kuala Belait, BD	KY973251	KY973061	KY972864
Shorea acuminatissima Symington	KASsac1	BRUN: KEP 80128/ B 000 893	KN Nursery Km 13 Bt Sawat, Kuala Belait, BD	KY973167	KY972969	KY972772
	KASsac2	BRUN: KEP 80128/ B 000 893	KN Nursery Km 13 Bt Sawat, Kuala Belait, BD	KY973168	KY972970	KY972773
Shorea acuminata Dyer	GB		Mersing Johor, Malayasia		AB246440	AB246505 AB246570
Shorea acuta P.S.Ashton	KASsa1	BRUN: KEP 80088/ B 000 918	Sg. Liang Arboretum Forest Reserve, Kuala Belait or Andulau FR, Kuala Belait, BD		KY972971	KY972774
	KASsa2	BRUN: KEP 80088/ B 000 918	Sg. Liang Arboretum Forest Reserve, Kuala Belait or Andulau Forest Reserve, Kuala Belait, BD		KY972972	KY972775

Shorea affinis (Thwaites) P.S.Ashton	GB		Kottawa Arboretum, Sri Lanka		AB246471	AB246536 AB246601
Shorea albida Symington ex A.V.Thomas	KASsal1	BRUN: BRUN 26298/ B 000 940	Sg. Mau, Bt.Sawat, Kuala Belait	KY973170	KY972974	KY972777
	KASsal2	BRUN: BRUN 26298/ B 000 940	Sg. Mau, Bt.Sawat, Kuala Belait	KY973171	KY972975	KY972778
Shorea agami P.S.Ashton	JH05-4419	UBDH: 05-4419	UBD-CTFS 25 ha plot	KY973183	KY972989	KY972792
	JH05-4423	UBDH: 05-4423	UBD-CTFS 25 ha plot	KY973184	KY972990	KY972793
Shorea argentifolia Symington	JH12-4803	UBDH: 12-4803	UBD-CTFS 25 ha plot	KY973176	KY972980	KY972783
	JH12-4810	UBDH: 12-4810	UBD-CTFS 25 ha plot	KY973177	KY972981	KY972784
Shorea atrinervosa Symington	JH01-3813	UBDH: 01-3813	UBD-CTFS 25 ha plot	KY973203	KY973013	KY972816
	JH04-4068	UBDH: 04-4068	UBD-CTFS 25 ha plot	KY973204	KY973014	KY972817
Shorea assamica Dyer.	GB		Frim Arboretum, Malayasia		AB246453	AB246518 AB246583
Shorea cf. *balanocarpoides* Symington	JH01-0107	UBDH: 01-0107	UBD-CTFS 25 ha plot		KY972984	KY972787
	JH01-0159	UBDH: 01-0159	UBD-CTFS 25 ha plot		KY972985	KY972788
Shorea cf. *beccariana* Burck	JH09-4181	UBDH: 09-4181	UBD-CTFS 25 ha plot	KY973172	KY972976	KY972779
	JH16-4452	UBDH: 16-4452	UBD-CTFS 25 ha plot	KY973173	KY972977	KY972780
Shorea biawak P.S.Ashton	JH20-5545	UBDH: 20-5545	UBD-CTFS 25 ha plot	KY973180	KY972986	KY972789
	JH20-5559	UBDH: 20-5549	UBD-CTFS 25 ha plot	KY973181	KY972987	KY972790
Shorea bracteolata Dyer	JH19-5045	UBDH: 19-5405	UBD-CTFS 25 ha plot	KY973182	KY972988	KY972791
Shorea bullata P.S.Ashton	GB		Engkabang, Semengoh, Malayasia		AB246435	AB246500 AB246565
Shorea confusa P.S.Ashton	JH25-4813	UBDH: 25-4813	UBD-CTFS 25 ha plot	KY973169	KY972973	KY972776
Shorea congestiflora (Thwaites) P.S.Ashton	GB		Kanneliya Forest Reservere, Sri Lanka		AB246463	AB246528 AB246593
Shorea cordifolia (Thwaites) P.S.Ashton	GB		Kanneliya Forest Reserve, Sri Lanka		AB246462	AB246527 AB246592
Shorea crassa P.S.Ashton	KASscr1	BRUN: BRUN 3080/B 001 134	Sg.Liang Andulau FR, Kuala Belait, BD	KY973185	KY972991	KY972794
	KASscr2	BRUN: BRUN 3080/B 001 134	Berakas FR, Berakas, Muara, BD	KY973186	KY972992	KY972795
Shorea curtisii Dyer ex King	GB		Mersing Johor, Malayasia		AB246433	AB246498 AB246563
Shorea disticha (Thwaites) P.S.Ashton	R.S. C-114a	PDA: C-114a	Arboretum, The Royal Botanical Gardens, Peradeniya, Sri Lanka		KY972929	KY972731
Shorea domatiosa P.S.Ashton	JH12-5258	UBDH: 12-5258	UBD-CTFS 25 ha plot	KY973229	KY973039	KY972842
	JH12-5324	UBDH: 12-5324	UBD-CTFS 25 ha plot	KY973230	KY973040	KY972843
Shorea dyeri F.Heim	GB		Bambarabotuwa Forest Reserve,		AB246446	AB246511

			Malyasia			AB246576
Shorea elliptica Burck	GB		Engkabang, Semengoh, Malayasia		AB246444	AB246509 AB246574
Shore exelliptica Meijer	JH08-5668	UBDH: 08-5668	UBD-CTFS 25 ha plot	KY973187	KY972993	KY972796
	JH10-5229	UBDH: 10-5229	UBD-CTFS 25 ha plot	KY973188	KY972994	KY972797
Shorea cf. *faguetiana* F.Heim	JH13-3443	UBDH: 13-3443	UBD-CTFS 25 ha plot		KY972995	KY972798
	JH22-1109	UBDH: 22-1109	UBD-CTFS 25 ha plot		KY972996	KY972799
	JH14-4338	UBDH: 14-4338	UBD-CTFS 25 ha plot	KY973174	KY972978	KY972781
	JH14-4346	UBDH: 14-4346	UBD-CTFS 25 ha plot	KY973175	KY972979	KY972782
Shorea faguetioides P.S.Ashton	JH20-5371	UBDH: 20-5371	UBD-CTFS 25 ha plot	KY973209	KY973019	KY972822
Shorea fallax Meijer	JH05-5358	UBDH: 05-5358	UBD-CTFS 25 ha plot	KY973241	KY973051	KY972854
	JH24-3922	UBDH: 24-3922	UBD-CTFS 25 ha plot	KY973242	KY973052	KY972855
Shorea ferruginea Dyer ex Brandis	JH16-2452	UBDH: 16-2452	UBD-CTFS 25 ha plot	KY973191	KY972999	KY972802
	JH16-2491	UBDH: 16-2491	UBD-CTFS 25 ha plot	KY973192	KY973000	KY972803
Shore gardneri (Thwaites) P.S.Ashton	GB		Bambarabotuwa Forest Reserve, Sri Lanka		AB246468	AB246533 AB246598
Shorea gibbosa Brandis	JH09-3653	UBDH: 09-3653	UBD-CTFS 25 ha plot	KY973224	KY973034	KY972837
	JH16-3012	UBDH: 16-3012	UBD-CTFS 25 ha plot	KY973225	KY973035	KY972838
Shorea glaucenscens Meijer	KASsgl1	n.a.	KN Nursery Km 13 Bt Sawat, Kuala Belait, BD	KY973195	KY973003	KY972806
	KASsgl2	n.a.	KN Nursery Km 13 Bt Sawat, Kuala Belait, BD	KY973196	KY973004	KY972807
Shorea havilandii Brandis	JH20-2908	UBDH: 20-2908	UBD-CTFS 25 ha plot	KY973197	KY973005	KY972808
	JH22-1867	UBDH: 22-1867	UBD-CTFS 25 ha plot	KY973198	KY973006	KY972809
Shorea henryana Pierre	SDDip2014_02	KUFF: S.Duangjai_Dip2014_02	Botanical Garden, Kasetsart University, Nakhon Pathom, Thailand	KY973199	KY973007	KY972810
Shorea inaequilateralis Symington	KASsi1	BRUN: WKM 3238/ B 040 939	KN Nursery Km 13 Bt Sawat, Kuala Belait, BD		KY973008	KY972811
	KASsi2	BRUN: WKM 3238/ B 040 939	KN Nursery Km 13 Bt Sawat, Kuala Belait, BD		KY973009	KY972812
Shorea johorensis Foxw.	KASsj1	BRUN: BRUN 355/ B 001 436	KN Nursery Km 13 Bt Sawat, Kuala Belait, BD	KY973212	KY973022	KY972825
	KASsj2	BRUN: BRUN 355/ B 001 436	KN Nursery Km 13 Bt Sawat, Kuala Belait, BD	KY973213	KY973023	KY972826
Shorea laevis Ridl.	JH04-4660	UBDH: 04-4660	UBD-CTFS 25 ha plot	KY973178	KY972982	KY972785
	JH13-3497	UBDH: 13-3497	UBD-CTFS 25 ha plot	KY973179	KY972983	KY972786
Shorea leprosula Miq.	JH04-5604	UBDH: 04-5604	UBD-CTFS 25 ha plot	KY973210	KY973020	KY972823
	JH04-5861	UBDH: 04-5861	UBD-CTFS 25 ha plot	KY973211	KY973021	KY972824
Shore lissophylla Thwaites	GB		Kanneliya Forest Reserve, Sri Lanka		AB246447	AB246512 AB246577

	R.S. D-27[gs]	PDA: D-27	Arboretum, The Royal Botanical Gardens, Peradeniya, Sri Lanka			
Shorea cf. *macrophylla* (de Vriese) P.S.Ashton	KASsmac1	BRUN: WKM 1570/ B 001 526	Palm Garden, Sg.Liang, Kuala Belait, BD	KY973214	KY973024	KY972827
	KASsmac2	BRUN: WKM 1570/ B 001 526	Palm Garden, Sg.Liang, Kuala Belait, BD	KY973215	KY973025	KY972828
Shorea macroptera subsp. *baillonii* (F.Heim) P.S.Ashton	JH05-5346	UBDH: 05-5346	UBD-CTFS 25 ha plot	KY973216	KY973026	KY972829
Shorea macroptera subsp. *macropterifolia* P.S.Ashton	JH13-1894	UBDH: 13-1894	UBD-CTFS 25 ha plot	KY973217	KY973027	KY972830
	JH13-2667	UBDH: 13-2667	UBD-CTFS 25 ha plot	KY973218	KY973028	KY972831
	JH15-0401	UBDH: 15-0401	UBD-CTFS 25 ha plot	KY973219	KY973029	KY972832
Shorea cf. *maxwelliana* King	JH09-4161	UBDH: 09-4161	UBD-CTFS 25 ha plot	KY973220	KY973030	KY972833
	JH20-2879	UBDH: 20-2879	UBD-CTFS 25 ha plot	KY973221	KY973031	KY972834
Shorea mecistopteryx Ridl.	KASsmec1	BRUN: BRUN 3280/ B 001 563	Sg.Liang, Andulau Forest Reseve, Kuala Belait, BD	KY973222	KY973032	KY972835
	KASsmec2	BRUN: BRUN 3280/ B 001 563	Sg.Liang, Andulau Forest Reserve, Kuala Belait, BD	KY973223	KY973033	KY972836
Shorea megistophylla P.S.Ashton	GB		Royal Botanical Gardens, Sri Lanka		AB246464	AB246529 AB246594
	R.S. D-24[es]	PDA: D-24	Arboretum, The Royal Botanical Gardens, Peradeniya, Sri Lanka			
Shorea myrionervia Symington ex P.S.Ashton	KASsmyr1	BRUN: KEP 30480/ B 001 633	Sg.Liang Arboretum, Kuala Belait, BD	KY973226	KY973036	KY972839
	KASsmyr2	BRUN: KEP 30480/ B 001 633	Sg.Liang Arboretum, Kuala Belait, BD	KY973227	KY973037	KY972840
Shorea oblongifolia Thwaites	R.S. D-26[es,gs]	PDA: D-26	Arboretum, The Royal Botanical Gardens, Peradeniya, Sri Lanka	KY973228	KY973038	KY972841
Shorea obscura Meijer	JH09-4180	UBDH: 09-4180	UBD-CTFS 25 ha plot	KY973200	KY973010	KY972813
	JH24-5730	UBDH: 24-5730	UBD-CTFS 25 ha plot	KY973201	KY973011	KY972814
Shorea ochracea Symington	JH05-0615	UBDH: 05-0615	UBD-CTFS 25 ha plot	KY973231	KY973041	KY972844
	JH22-2120	UBDH: 22-2120	UBD-CTFS 25 ha plot	KY973232	KY973042	KY972845
Shorea ovalifolia (Thwaites) P.S.Ashton	GB		Gilimale Forest, Sri Lanka		AB246467	AB246532 AB246597
Shorea ovalis (Korth.) Blume subsp. *sarawakensis* P.S.Ashton	JH05-0012	UBDH: 05-0012	UBD-CTFS 25 ha plot	KY973233	KY973043	KY972846
	JH05-0055	UBDH: 05-0055	UBD-CTFS 25 ha plot	KY973234	KY973044	KY972847
Shorea ovata Dyer ex Brandis	KASsov1	n.a.	n.a.	KY973235	KY973045	KY972848
	KASsov2	n.a.	n.a.	KY973236	KY973046	KY972849
Shorea pachyphylla Ridl. ex Symington	KASspac1	n.a.	n.a.	KY973237	KY973047	KY972850
	KASspac2	n.a.	n.a.	KY973238	KY973048	KY972851
Shorea pallescens P.S.Ashton	GB		Kanneliya Forest Reserve, Sri Lanka		AB246448	AB246513 AB246578

Shorea parvifolia Dyer subsp. *velutinata* P.S.Ashton	JH05-5369	UBDH: 05-5369	UBD-CTFS 25 ha plot	KY973239	KY973049	KY972852
	JH25-2700	UBDH: 25-2700	UBD-CTFS 25 ha plot	KY973240	KY973050	KY972853
Shorea parvistupulata F.Heim	JH21-5516	UBDH: 21-5516	UBD-CTFS 25 ha plot	KY973193	KY973001	KY972804
	JH21-5777	UBDH: 21-5777	UBD-CTFS 25 ha plot	KY973194	KY973002	KY972805
Shorea cf. *pinanga* Scheff.	JH10-5213	UBDH: 10-5213	UBD-CTFS 25 ha plot	KY973245	KY973055	KY972858
	JH12-5295	UBDH: 12-5295	UBD-CTFS 25 ha plot	KY973246	KY973056	KY972859
Shorea quadrinervis Slooten	JH08-4106	UBDH: 08-4106	UBD-CTFS 25 ha plot	KY973247	KY973057	KY972860
	JH08-4401	UBDH: 08-4401	UBD-CTFS 25 ha plot	KY973248	KY973058	KY972861
Shorea richetia Symington	GB		Kubah, National Park, Malayasia		AB246442	AB246507 AB246572
Shorea robusta C.F.Gaertn.	SDDip2014_06	KUFF: S.Duangjai_Dip2014_06	Botanical Garden, Kasetsart University, Nakhon Pathom, Thailand	KY973249	KY973059	KY972862
Shorea roxburghii G.Don	SDDip2014_03[cs,gs]	KUFF: S.Duangjai_Dip2014_03	Botanical Garden, Kasetsart University, Nakhon Pathom, Thailand	KY973250	KY973060	KY972863
Shorea rubra P.S.Ashton	JH08-3526	UBDH: 08-3526	UBD-CTFS 25 ha plot	KY973243	KY973053	KY972856
	JH08-3527	UBDH: 08-3527	UBD-CTFS 25 ha plot	KY973244	KY973054	KY972857
Shorea scaberrima Burck	JH04-3069	UBDH: 04-3069	UBD-CTFS 25 ha plot	KY973252	KY973062	KY972865
Shorea scrobiculata Burck	JH05-3814	UBDH: 05-3814	UBD-CTFS 25 ha plot	KY973205	KY973015	KY972818
	JH05-4230	UBDH: 05-4230	UBD-CTFS 25 ha plot	KY973206	KY973016	KY972819
Shorea seminis Slooten	GB		Frim Arboretum, Malayasia		AB246450	AB246515 AB246580
Shorea smithiana Symington	KASsm1	BRUN: BRUN 661/ B 002 013	KN Nursery Km 13 Bt Sawat, Kuala Belait, BD	KY973253	KY973063	KY972866
	KASsm2	BRUN: BRUN 661/ B 002 013	KN Nursery Km 13 Bt Sawat, Kuala Belait, BD	KY973254	KY973064	KY972867
Shorea cf. *splendida* (de Vriese) P.S.Ashton (GenBank: *S. splendens*)	GB		Engkaban, Semengoh, Malayasia		AB246443	AB246508 AB246573
Shorea stipularis Thwaites	GB		Kanneliya Forest Reserve, Sri Lanka		AB246454	AB246519 AB246584
Shorea trapezifloia (Thwaites) P.S.Ashton	GB		Kanneliya Forest Reserve, Sri Lanka		AB246466	AB246531 AB246596
Shorea virescens Parijs	JH20-4062	UBDH: 20-4062	UBD-CTFS 25 ha plot	KY973207	KY973017	KY972820
	JH20-4596	UBDH: 20-4596	UBD-CTFS 25 ha plot	KY973208	KY973018	KY972821
Shorea worthingtonii P.S.Ashton	GB		Kanneliya Forest Reserve, Sri Lanka		AB246469	AB246534 AB246599
Shorea xanthophylla Symington	GB		Frim Arboretum, Malayasia		AB246452	AB246517 AB246582
Shorea zeylanica (Thwaites) P.S.Ashton	GB		Royal Botanical Gardens, Sri Lanka		AB246470	AB246535 AB246600

	R.S. C-160[gs]	PDA: C.160	Arboretum, The Royal Botanical Gardens, Peradeniya, Sri Lanka			
Stemonoporus acuminatus Bedd.	GB		Kanneliya Forest Reserve, Sri Lanka		AB246422	AB246487 AB246552
Stemonoporus bullatus Kosterm.	GB		Kanneliya Forest Reserve, Sri Lanka		AB246426	AB246491 AB246556
Stemonoporus canaliculatus Thwaites	R.S. D-156[gs]	PDA: D-156	Arboretum, The Royal Botanical Gardens, Peradeniya, Sri Lanka	KY973257	KY973067	KY972870
	R.S. D-159a	PDA: D-159a	Arboretum, The Royal Botanical Gardens, Peradeniya, Sri Lanka	KY973258	KY973068	KY972871
Stemonoporus gilimalensis Kosterm.	GB		Gilimale Forest, Sri Lanka		AB246423	AB246488 AB246553
Stemonoporus kanneliyensis Kosterm.	GB		Kanneliya Forest Reserve, Sri Lanka		AB246429	AB246494 AB246559
Stemonoporus lancifolius (Thwaites) P.S.Ashton	GB		Kanneliya Forest Reserve, Sri Lanka		AB246430	AB246495 AB246560
Stemonoporus reticulatus Thwaites	GB		Kanneliya Forest Reserve, Sri Lanka		AB246427	AB246492 AB246557
Stemonoporus scalarinervis Kosterm.	GB		Gilimale Forest, Sri Lanka		AB246424	AB246489 AB246554
Stemonoporus wightii Thwaites	GB		Gilimale Forest, Sri Lanka		AB246428	AB246493 AB246558
Upuna borneensis Symington	GB		Frim, Kepong, Malayasia		AB006374	AB006408 AB006391
Vateria copallifera (Retz.) Alston	R.S. D-216[gs]	PDA: D-216	Arboretum, The Royal Botanical Gardens, Peradeniya, Sri Lanka	KY973260	KY973070	KY972873
	R.S. C-126	PDA: C-126	Arboretum, The Royal Botanical Gardens, Peradeniya, Sri Lanka	KY973259	KY973069	KY972872
Vateriopsis seychellanum F.Heim	GB		Seychelles		AB246432	AB246497 AB246562
Vatica sp.	R.S. 1	PDA: D-198a	Arboretum, The Royal Botanical Gardens, Peradeniya, Sri Lanka	KY973255	KY973065	KY972868
	R.S. 2[gs]	PDA: D-198a	Arboretum, The Royal Botanical Gardens, Peradeniya, Sri Lanka	KY973256	KY973066	KY972869
Vatica affinis Thwaites	GB		Kottawa, Sri Lanka		AB246421	AB246486 AB246551
Vatica bella Slooten	GB		Frim Arboretum, Malayasia		AB246416	AB246481 AB246546
Vatica chinensis L.	R.S. 1	PDA: D-28	Arboretum, The Royal Botanical Gardens, Peradeniya, Sri Lanka	KY973263	KY973073	KY972876
	R.S. 2	PDA: D-28	Arboretum, The Royal Botanical	KY973264	KY973074	KY972877

			Gardens, Peradeniya, Sri Lanka			
Vatica coriaceae P.S.Ashton	KASvco1	n.a.	n.a.	KY973265	KY973075	KY972878
	KASvco2	n.a.	n.a.	KY973266	KY973076	KY972879
Vatica diospyroides Symington	SDDip2014_05	KUFF: S.Duangjai_Dip2014_05	Botanical Garden, Kasetsart University, Nakhon Pathom, Thailand	KY973267	KY973077	KY972880
Vatica dulitensis Symington	JH11-2884	UBDH: 11-2884	UBD-CTFS 25 ha plot	KY973269	KY973079	KY972882
	JH11-2886	UBDH: 11-2886	UBD-CTFS 25 ha plot	KY973270	KY973080	KY972883
Vatica endertii Slooten	JH01-1700[cs,gs]	UBDH: 01-1700	UBD-CTFS 25 ha plot	KY973261	KY973071	KY972874
	JH02-3473	UBDH: 02-3473	UBD-CTFS 25 ha plot	KY973262	KY973072	KY972875
Vatica harmandiana F.Heim	207565[1]	K, 207565	n.a., Thailand	KY973268	KY973078	KY972881
Vatica micrantha Slooten	JH01-0092	UBDH: 01-0092	UBD-CTFS 25 ha plot	KY973273	KY973083	KY972886
	JH01-0178	UBDH: 01-0178	UBD-CTFS 25 ha plot	KY973274	KY973084	KY972887
Vatica cf. oblongifolia subsp. *multinervosa* P.S.Ashton	JH08-2601	UBDH: 08-2601	UBD-CTFS 25 ha plot	KY973277	KY973087	KY972890
Vatica oblongifolia subsp. *oblongifolia*	JH02-0174	UBDH: 02-0174	UBD-CTFS 25 ha plot	KY973275	KY973085	KY972888
	JH02-0259	UBDH: 02-0259	UBD-CTFS 25 ha plot	KY973276	KY973086	KY972889
	JH02-0279	UBDH: 02-0279	UBD-CTFS 25 ha plot	KY973278	KY973088	KY972891
	JH02-0434	UBDH: 02-0434	UBD-CTFS 25 ha plot	KY973279	KY973089	KY972892
Vatica odorata (Griff.) Symington subsp. *mindanensis* (Fowx.) P.S.Ashton	JH04-2600	UBDH: 04-2600	UBD-CTFS 25 ha plot	KY973280	KY973090	KY972893
Vatica pauciflora (Korth.) BI.	GB		Frim Aboretum, Malayasia		AB246417	AB246482 AB246547
Vatica sarawakensis F.Heim	JH10-3487	UBDH: 10-3487	UBD-CTFS 25 ha plot	KY973281	KY973091	KY972894
	JH10-3744	UBDH: 10-3744	UBD-CTFS 25 ha plot	KY973282	KY973092	KY972895
Vatica vinosa P.S.Ashton	JH01-0248	UBDH: 01-0248	UBD-CTFS 25 ha plot	KY973283	KY973093	KY972896
	JH01-0265	UBDH: 01-0265	UBD-CTFS 25 ha plot	KY973284	KY973094	KY972897
Monotoideae						
Marquesia	Daniel K. Harder *et al.*, 2685[2]	MO: 2685	Northwestern Solwezi District; Acres National Forest/ Jirunda Botanical Reserve	KY973154	KY972957	
Monotes adenophylla Gilg	Noah B. Zimba *et al.*, 598[2]	MO: 598	Northwestern Mwinilunga District: Lwawu Mission	KY973155	KY972958	KY972760
Monotes kerstingii Gilg	Heidi H. Schmidt *et al.*, 1808[2]	MO: 1808	Nothern Mole National Park	KY973156	KY972959	KY972761
Monotes madagascariensis Humbert	GB		Fenetrede I'Isalo, Madagascar		AB246478	AB246543 AB246608
Pakaraimaeoidea						
Pakaraimaea dipterocarpacea Maguire & P.S.Ashton	Kelloff 5169[3]	US National Herbarium, Smithsonian Institution: 5169	Guyana, Cuyuni-Mazaruni, Imbamadai	KY973157	KY972960	KY972762
	Wurdack 5119[3]	US National Herbarium, Smithsonian Institution: 5119	Guyana, Cuyuni-Mazaruni, Imbamadai	KY973158	KY972961	KY972763

Leptolaena cuspidata Baker	Chase, M.W. 33154[1]	K: 1000 Plant Genomes Project: 33154	Antsiranana, Madagascar	KY973152		KY972758
Leptolaena multiflora Thou.	Randrianasdo 241[1]	MO: 241	n.a.	KY973153	KY972956	KY972759
Sarcolaena sp.	Chase, M.W. 903[1]	K: 903	n.a., Madagascar	KY973165	KY972968	KY972770
Schizolaena sp.	Chase, M.W. 38681[1]	K: 1000 Plant Genomes Project: 38681	n.a., Madagascar	KY973166		KY972771
Cistaceae						
Cistus laurifolius L.	JH273	WU: JH 273 (photo)	Hortus Botanicus Vindobonensis, Austria, Vienna	KY973107	KY972910	KY972712
Fumana procumbens Gren. & Godr.	JH274	WU: JH 274 (photo)	Hortus Botanicus Vindobonensis, Austria, Vienna	KY973132	KY972936	KY972738
Helianthemum obscurum Pers.	JH275	WU: JH 275 (photo)	Hortus Botanicus Vindobonensis, Austria, Vienna	KY973133	KY972937	KY972739
Bixaceae						
Bixa orellana L.	RSF-15	PDA: F-15	Arboretum, The Royal Botanical Gardens, Peradeniya, Sri Lanka	KY973106	KY972909	
Cochlospermum vitifolium Spreng.	GB	BioBot00410	Area de Conservacion Guanacaste, Sector Santa Rosa, Camino los Borrachos, Costa Rica	JQ591113	JQ587261	
Cochlospermum vitifolium Spreng.	GB	BioBot00415	Area de Conservacion Guanacaste, Sector Santa Rosa, Camino los Borrachos, Costa Rica	JQ591114	JQ587263	

PART 2
Molecular phylogeny and phylogenomics of Dipterocarpaceae

CHAPTER 4

Phylogenomics resolves evolutionary relationships and provides first insights into floral evolution in the tribe Shoreeae (Dipterocarpaceae)

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Status: to be submitted to New Phytologist

Contribution: data curation, formal analysis, visualization, writing- original draft preparation, writing – review and editing

Abstract

The supra-annual, community-level synchronous mass flowering is an ecologically-interesting phenomenon in the aseasonal tropical forests of Southeast Asia. The evolution of mass flowering has been hypothesized to be linked to pollinator shifts, that is from larger, longer-generation to smaller, shorter-generation insects, which are capable of building up the necessary population sizes within a short period. The main component of Southeast Asian forests is the subfamily Dipterocarpoideae (Dipterocarpaceae), which exhibits great floral diversity and a range of pollination syndromes. Phylogenetic relationships within this subfamily, especially in the tribe Shoreea have not been clearly understood up to now. In this study, we use a new pipeline to extract more than one hundred thousand genome-wide SNP markers from restriction site associated DNA sequencing (RADseq) in non-models, and we successfully use this dataset to infer phylogenomic relationships in the tribe Shoreeae of the subfamily Dipterocarpoideae. The phylogenetic trees obtained here with traditional (maximum parsimony, maximum likelihood) and bayesian coalescent-based analyses are largely congruent to phylogenetic trees previously obtained from single loci. The high resolution we obtain from the SNPs data helped to resolve taxonomic uncertainties within the tribe Shoreeae. RADseq data supports taxonomic grouping of the genus *Shorea* based on wood anatomy, but contradicts some of the previously reported (sub-)sectional relationships within genus *Hopea*, as well as *Shorea* groups *Rubroshorea* and *Shorea* (selangang batu). Insights into the evolution of floral traits supports the hypothesis that species with small, (sub-)globose anthers, long appendages and generally fewer stamens per flower have evolved from species having large, oblong anthers, short appendages, and often more than 15 stamens, and this parallel the change in pollinators expected to be linked to switches to mass-flowering.

Key words: Dipterocarpoideae – floral evolution – mass flowering – phylogenomics –Shoreeae – RADseq – species trees

Introduction

The supra-annual, community-level synchronous flowering (referred to as mass flowering or general flowering) in the aseasonal tropical forests of Southeast Asia is an ecologically-fascinating phenomenon which drives not only plant phenology, but also the demography of local insect populations. Drought (Medway, 1972; Appanah, 1985, Sakai *et al.*, 2002; Sakai *et al.*, 2006; Brearley *et al.*, 2007; Kobayashi *et al.*, 2013), increased sunshine hours (Wright & Vanschaik, 1994), and/or a change in temperature (Appanah 1985; Ashton, Givnish, Appanah, 1988; Sakai *et al.*, 2002) as a result of El Nino - Southern Oscillations have been proposed to induce the irregular gregarious flowering in the canopy members of forests dominated by Dipterocarpaceae, but also affecting members of Burseraceae, Fabaceae, Myristicaceae, Polygalaceae, and Sapotaceae (Ashton, Givnish, Appanah, 1988). The evolution of mass flowering has been hypothesized to be linked to a shift in pollinators from larger, longer-generation pollinators to smaller insects that can build up the necessary population sizes within the approx. two months period between the signal to primordial and a mass flowering (Heinrich

& Raven, 1972; Frankie *et al.*, 1974). Janzen (1974) considered the evolution of mass flowering and the subsequent mast fruiting as adaptations to reduce flower and seed predation through satiation, this idea was supported by the observation that individuals that fruit outside mass flowering have higher rates of seed mortality (Burgess, 1972).

Dipterocarpoideae, a main element of Southeast Asian forests, exhibit great floral diversity and a range of pollination syndromes, which could be largely circumscribed to three main groups. The first group is largely represented by *Dipterocarpus* C.F.Gaertn. which is mainly pollinated by nectarivorous Lepidoptera (Ghazoul, 1997; Harrison *et al.*, 2005; Ashton, 2013) and has numerous stamens (i.e. 15-50), and large, oblong, yellow anthers with prominent stoutly acicular tapering erect glabrous appendages (Ashton, 2013). The second group has large, less abundant flowers, with mostly more than 15 stamens and large, oblong, often yellow anthers with mainly glabrous appendages which are generally short (Ashton, 2013). Taxa of this group are *Dyrobalanops* C.F.Gaertn., *Neopbalanocarpus* P.S.Ashton, *Vateria* L., *Vateriopsis* F.Heim, *Stemonoporus* Thwaites, *Parashorea* Kurz, and *Shorea* Roxb. ex. C.F.Gaertn. sections *Doona* Thwaites and *Pentacme* A.DC. Their anthers produce large pollen grains which may serve as food for large insects with relatively long lifecycles, too long to build numbers in the short period between signal to primordial and a mass flowering event. Thus, the pollinator must be able to migrate, by swarming. Observed pollinators in this group are bees, mainly *Apis* L., but also by *Trigona* Jurine (Appanah, 1985; Appanah 1987; Dayanandan, 1990; Momose *et al.*, 1996; Ghazoul *et al.*, 1998; Momose *et al.*, 1998). Taxa in this group are most abundant in forests with low diversity (i.e. on peat swamps or basic volcanic soils, Ashton personal communication) and flower either sporadically (*Neobalanocarpus*, *Dryobalanops*) or regularly (*Stemonoporus*, some *Shorea* section *Doona*, *Vateria*) outside mass flowering (Ashton, 2013). The third group has relatively small, but more abundant flowers with less than or 15 stamens and small, (sub-)globose, often white anthers and variable appendages (in general slender and ciliate; Ashton, 2013). Members of this group are *Hopea* Roxb. and *Shorea* sections *Pachycarpa*, *Mutica*, *Brachyptera* and *Richetioides* (except *S. polyandra* P.S.Ashton). Here, thrips (*Thrips* L. and *Megalurothrips* Bagnall spp.; *Thysanoptera* Haliday) have been mainly observed as pollinators of species of *Shorea* section *Mutica* (Appanah & Chan, 1981). However, beetles (Sakai *et al.*, 1999) and flies (Khatua *et al.*, 1998) are also involved in pollination of this group. All these insects are fecund and have short life cycles which enables them to build up the numbers necessary at the onset of the supra-annual mass flowering making them effective pollinators (Appanah & Chan, 1981).

Based on the assumptions that when the early Dipterocarpoideae were diversifying into modern genera, forests were already associated with mass flowering and they were much less species rich, large insect-pollinated tree species were enabled to build up the abundance of resources to attract swarms of pollinators. In the early Mioecene (about 20 mya) tropical lowland evergreen rain forests became extensive in Southeast Asia having diverse Dipterocarpaceae as their prominent members (Morley, 2000) and forests thus became more species rich. The subsequent competition might have increasingly favoured species that could maintain fecundity and pollinators who could respond to mass flowering

initiation by rapid reproduction. *Parashorea* which probably originated in seasonal region with annual flowering subsequently migrated to Sunda, where *Rubroshorea* and *Richetia* F.Heim might have started to diversify from it. The first of these *Rubroshorea* to evolve is hypothesized to be section *Rubella* which retained the large and many oblong anthers and thus large insect pollinators of *Parashorea*. We therefore hypothesize that in the subfamily Dipterocarpoideae, in parallel to changing pollinators, species with small, pale, (sub-)globose anthers, long appendages and generally fewer stamens per flower have evolved from species with flowers having large, oblong anthers, short appendages, and often more than 15 stamens.

To test this hypothesis a well resolved phylogeny is required. Recently, a published molecular phylogenetic study utilizing six plastid markers and including a broad taxon sampling provided a provisional framework phylogeny of the whole family Dipterocarpaceae (Heckenhauer *et al.*, 2017). However, some relationships, especially in the tribe Shoreeae still remain unresolved which does not allow for in depth testing of the hypothesis of floral trait evolution outlined above. Even higher level taxonomic classifications within the species-rich Dipterocarpoideae have been a long-standing problem. According to Ashton (1979), Dipterocarpoideae consists of two tribes, Dipterocarpeae and Shoreeae, whereas Maury-Lechon (1979), divided the subfamily into two groups, "Valvate" and "Imbricate", based on fruit-sepal base arrangement in the ripe fruit. Generic and subgeneric classifications in the tribe Shoreeae *sensu* Ashton are very complex which leads to difficulties in defining morphological boundaries for the taxonomic status. Different classifications systems proposed by different authors are given in Table 1.

According to Ashton (1979) the tribe Shoreeae comprises five genera: *Dryobalanops*, *Hopea*, monotypic *Neobalanocarpus*, *Parashorea*, and the large genus *Shorea* with approximately 360 species. Generic limits in this tribe, especially that of the closely related genera *Hopea* and *Shorea* are obscure. In addition, species relationships within the genera *Hopea* and *Shorea* are uncertain. *Hopea* exhibits a great variability in ovary, leaf, and bark, making it one of the most diverse dipterocarp genera (Ashton, 2004). Ashton (1979) and Maury (1978) recognized two sections having two subsections each (Table 1). However, these entities have a great floral diversity and several species reveal intermediate characters (Ashton, 2004). Regarding genus *Shorea*, Heim (1892) and Symington (1943) proposed groups of species having floral characters correlating with bark morphology and wood anatomy. Classifications of Ashton (1982) and Maury (1978) are based on four *Shorea* groups (selangan batu, damar hitam (yellow meranti), meranti Pa'ang (white meranti), and red meranti) defined based on timber characters by Symington (1943). According to Maury (1978), *Shorea* consists of the following six genera: *Anthoshorea* (= white meranti), *Rubroshorea* (= red meranti), *Richetia* (= yellow meranti), and *Shorea* (= selangang batu), *Doona*, and *Pentacme*. In contrast to this, Ashton (1982) kept them in a single genus *Shorea* with eleven sections and eight subsections (Table 1).

Utilizing the enormous throughput of next-generation sequencing restriction-site associated DNA (RADseq) is able to detect thousands of polymorphic genetic markers across the investigated genomes. RADseq data has proven to be an efficient and cost-effective approach for interspecific

phylogenetic reconstruction (e.g., Rubin *et al.*, 2012; Cariou *et al.*, 2013) even for groups which radiated rapidly or recently (e.g. Paun *et al.*, 2016; Bateman *et al.*, 2017). Contrary to traditional sanger sequencing approaches, RADseq samples many short loci across the entire genome (Miller *et al.*, 2007; Baird *et al.*, 2008; Rowe *et al.*, 2011), making it useful in families like Dipterocarpaceae where a few plastid DNA regions do not provide enough variability to resolve phylogenetic relationships at lower taxonomic levels.

Using a taxonomic diverse sampling of the tribe Shoreeae in this study, we extract SNP markers from RADseq to built traditional and coalescent-based species trees of the group in order to (1) test if RADseq can help to resolve taxonomic uncertainties that exist with the tribe Shoreeae, (2) clarify if RADseq data leads to the same taxonomic grouping of *Shorea* as the one based on wood anatomy established by Symington (1943) and confirmed by Maury (1978) and detected by analysing plastid regions (i.e. Heckenhauer *et al.*, 2017), and (3) to examine if (sub-)sectional relationships within the genera *Hopea* and *Shorea* proposed by Ashton are supported. Further, we test the hypothesis that flowers with large, oblong anthers with short appendages, and generally more than 15 stamens represent a plesiomorphic character state in the family Dipterocarpoideae with regard to pollination biology and biogeography.

Table 1: Comparative classifications of genera *Hopea, Neobalanocarpus, Parashorea, Shorea* after Maury-Lechon (1998).

Authors	**Tribe**	**Subtribe**	**Genera**	**Section (s.) / Subgroup (s.g.)**	**Subsection (s.s) / Subgroup (s.gr.)**
Meijer and Wood (1964, 1976)			*Hopea* *Shorea* *Parashorea*	 s.g. *Euchorea* = *Shorea* s.g. *Richetia* s.g. *Rubroshorea* s.g. *Anthoshorea*	 s.gr. *Ovalis* s.gr. *Pauciflora* s.gr. *Smithiana* s.gr. *Pinanga* s.gr. *Parvifolia*
Maury (1978)	*Shoreeae*	*Shorineae*	*Hopea* *Balanocarpus heimii* *Shorea* *Richetia* *Rubroshorea*	s. *Hopea* s. *Dryobalanoides* s. *Shoreae* s. *Barbatae* s. *Richetioides* s. *Maximae* s. *Mutica* s. *Ovalis* s. *Neohopea* s. *Rubella*	s.s. *Hopea* s.s. *Pierra* s.s. *Dryobalanoides* s.s. *Sphaerocarpae* s.s. *Mutica* s.s. *Auriculatae*

		Anthoshorinae *Parashorinae*	*Anthoshorea* *Doona* *Pentacme* *Parashorea*	s. *Brachypterae* s. *Pachycarpa* s. *Anthoshoreae*	s.s. *Brachypterae* s.s. *Smithianeae*
Ashton (1982)	*Shoreeae*		*Hopea* *Neobalanocarpus* *Shorea* *Parashorea*	s. *Hopea* s. *Dryobalanoides* s. *Shorea* s. *Richetioides* s. *Mutica* s. *Ovalis* s. *Neohopea* s. *Rubella* s. *Brachypterae* s. *Pachycarpae* s. *Anthoshorea* s. *Doona* s. *Pentacme*	s.s. *Hopea* s.s *Pierra* s.s. *Dryobalanoides* s.s. *Sphaerocarpae* s.s. *Shorea* s.s. *Barbata* s.s. *Richetioides* s.s. *Polyandrae* s.s. *Mutica* s.s. *Auriculatae* s.s. *Brachypterae* s.s. *Smithiana*
Kostermans (1983, 1984, 1992)			*Hopea* *Neobalanocarpus* *Balanocarpus* *Shorea* *Doona* *Parashorea*		

Material and Methods

Plant material and DNA isolation

Leaf or bark material was collected and stored in silica gel in Kuala Belalong (Brunei Darussalam), with additional samples originating from other parts of Brunei Darussalam as well as from Sri Lanka (Table S1). Initially, 137 Dipterocarpoideae samples were extracted and sequenced, but 11 were excluded from further analysis due high frequency of missing data. The final dataset represents 71 species mainly belonging to the genera *Parashorea* (three species), *Hopea* (11 species), and *Shorea sensu* Ashton (51 species). Of the eleven sections and eight subsections in the species-rich genus *Shorea* reported by Ashton (1982), nine sections and seven subsections are represented in this study. These accessions represent five of the six genera (*Doona*, *Anthoshorea*, *Shorea*, *Richetia*, *Rubroshorea*)

introduced by Maury (1978). The sampling further comprised accessions of *Dryobalanops* (two species) and *Dipterocarpus* (one species). Outgroup sampling included members of *Vatica* (two species).
Total genomic DNA was extracted from approximately 40 mg of silica gel-dried (Chase & Hills, 1991) tissue. To avoid degradation, the material was frozen in liquid nitrogen and then ground to a fine powder using glass-beads. Malvales often harbour a high amount of secondary compounds (e.g. polyphenol or polysaccharides due to mucilaginous epidermal cells), which can lead to difficulties in DNA extraction. To qualitatively and quantitatively maximize the DNA obtained, a modified sorbitol/ high-salt CTAB (cetyltrimethylammonium bromide) protocol for difficult plant tissues (Tel-Zur *et al.* 1999, Russell *et al.* 2010) was combined with the silica-membrane-binding DNeasy Plant Mini Kit (QIAGEN) according to Barfuss *et al.* (2016). The DNA content was quantified using the Qubit 3.0 Fluorometer with dsDNA HS Assay Kit (Thermofisher).

RADseq Library Preparation

With a range of 0.267 to 0.705 pg Dipterocarpaceae have relatively small genomes (Ohri & Kumar, 1986; Ng *et al.*, 2016; Heckenhauer *et al.*, 2017). Single-digest RADseq libraries were prepared using the *PstI* restriction enzyme (New England Biolabs) following a modified protocol adapted from Baird *et al.* (2008) which has been successfully used in previous studies (Paun *et al.*, 2016; Trucchi *et al.*, 2016; Brandrud *et al.*, 2017). Altogether, four libraries were prepared. In the first library 120 ng DNA was used per individual. As the available DNA quantity was not a limitation, the DNA amount was increased to 200 ng in subsequent libraries. The fourth library was prepared to add few additional individuals as well as to increase the number of reads of selected samples which were underrepresented in the first libraries. After restriction digest with 15 U *PstI* for two hours at 37°C and deactivation for 20 minutes at 80°C, depending on the DNA amount 130 nM (for first library only), respectively 300 nM P1 adapters, were ligated to the restricted samples overnight at 16°C. Groups of samples barcoded with different P1 barcodes were pooled together. To obtain an average size of 400 bp, DNA shearing by sonication was performed using a Bioruptor Pico (Diagenode) with three cycles of 30 s "on" and 60 s "off" at 6°C. Samples were purified with the Qiagen Reaction clean up columns and a size selection (left side: 0.7x, right side: 0.55x) was carried out using the SPRIselect Reagent Kit (Beckman Coulter). To polish the ends of the sonicated DNA fragments, blunting was performed using the Quick Blunting Kit (New England Biolabs). After another purification step with the Qiagen MinElute PCR Cleanup Kit, dATP was added. Following an additional purification with the Qiagen PCR Cleanup Kit, ligation of P2 adapters was carried out. After quantification all samples were pooled together, with the aim that each sample is equally represented. To remove unwanted primer dimers, SPRI size selections (left side: 0.7x) were performed before and after 18 cycles of PCR amplification with the Phusion Master Mix (Thermo Fischer Scientific). An additional size selection targeting a fragment range of 220 to 850 bp was achieved using Pippin Prep (Sage Science) and a 1.5% dye-free cassette. The quality of libraries was checked on Bioanalyzer with the High-Sensitivity DNA Kit (Agilent Genomics) before sequencing them

on an Illumina HiSeq 2500 at VBCF Vienna (http://vbcf.ac.at/ngs/, last accessed: 2017-10-05) as 100 bp single-end reads.

Identification of RAD loci and SNPs filtering

Quality filtering and demultiplexing of the raw reads from all sequenced samples was conducted according to individual barcodes with the script process_radtags.pl implemented in the Stacks package v1.46 (Catchen *et al.*, 2011) using the following settings: *PstI* as restriction enzyme, removal of any read with an uncalled base, discarding reads with low quality scores, and rescuing barcodes and RAD-Tags with 1 nt mismatch to retain only full-length reads (94 bp after barcode trimming) free from adapter sequences. The files of samples which were sequenced twice in different libraries were concatenated. The RAD loci were assembled and SNPs were called using the denovo_map.pl pipeline in STACKS. Following Paun *et al.* (2016), in order to find the best settings for catalogue building, i.e. settings that maximised the number of reliable loci identified from the reads, the minimum number of identical reads required to create a stack ("m"), the maximum mismatch between loci when processing a single individual ("M") and when building the catalogue ("n") were varied, starting from initial values m = 5, M = 1 and n = 1 (Table S2). The settings that were used to produce the final catalogue were m = 5, M = 1, and n = 2, because they resulted in the highest number of polymorphic stacks with 1-10 SNPs and 1-15 SNPs that were covered in at least 100 individuals.

To optimize the recovery of loci across the phylogenetic and coverage depth in the dataset, a reference was further prepared from the previously created catalogue by extracting haplotype consensuses of loci that were present in at least 37 samples and contained 1-20 SNPs using the function export_sq.pl of Stacks. The demultiplexed reads were mapped to this reference with BWA-MEM (Li & Durbin, 2010) which aligns query sequences by seeding alignments with maximal exact matches (MEMs). The option -M was applied to flag shorter split hits as secondary. The resulting aligned sam file was sorted by coordinate and read groups were added in an outputted bam file using picard tools (Wysoker *et al.*, 2013; http://broadinstitute.github.io/picard/; last accessed 2017-10-05). A realignment around indels was performed with the Genome Analysis Toolkit v3.7.0 (McKenna *et al.*, 2010). The realignment process consists of two steps. First, suspicious (small) intervals which are likely in need of realignment are determined with the Realigner Target Creator tool. Secondly, a realignment is attempted over those intervals using the Indel Realigner tool, thinning the data to a maximum of 100,000 reads per interval. Variants were called from the realigned bam files using the ref_map.pl pipeline in STACKS with the default requirement of a minimum number of three identical, raw reads required to create a stack. The program populations from Stacks was used to output SNPs, by retaining only those positions with a maximum observed heterozygosity 0.65 to avoid further use of any pooled paralogs. The program vcftools v0.1.13 (Danecek, 2011) was finally used to further filter data, by removing indels and retaining only polymorphic sites with a minor allele frequency ≥ 0.014 (i.e., four haplotype copies).

Phylogenomic analyses using MP and ML

To investigate phylogenetic relationships between, as well as within genera or groups of the tribe Shoreeae *sensu* Ashton phylogenomic analyses were conducted. Missingness per individual was estimated with vcf tools. Individuals with more than 35% missing data (11 samples) were excluded from the analysis using the remove-indv function in vcftools, leading to a final dataset of 126 accessions, representing 71 species. The minor allele frequency (≥ 0.015) was adjusted according to the new number of individuals included in the analysis. Invariable sites were filtered and excluded. The filtered vcf files were converted into phylip and nexus files with PGDSpider v2.1.1.0 (Lischer & Excoffier, 2012).
Maximum likelihood analyses were conducted using RAxML v8.2.4 (Stamatakis, 2014), using the correction type of the likelihood for ascertainment bias by Lewis (2001), as recommended for concatenated SNP datasets. Rapid Bootstrap analyses (1,000 replicates), searching for the best-scoring ML tree were conducted in a single program run. An inference under the General Time Reversible model of nucleotide substitution under the Gamma model of rate heterogeneity (i.e., the GTRCAT model) was executed. The substitution rates and site-specific evolutionary rates, categorized into distinct categories, were optimized. Several ML analyses were conducted with different amounts of missing data allowed (filtered with the max-missing function in vcftools) ranging from 0 to 30%. As results were largely congruent, the final dataset (20% missing data, hereafter dataset DF) was chosen according to the maximum percentage of nodes with high bootstrap support in the resulting trees. Trees were also estimated with maximum parsimony using dataset DF in PAUP version 4.0a149 (Swofford, 2016) via heuristic search with stepwise addition, 1,000 replicates of random addition sequence and tree-bisection–reconnection (TBR) branch-swapping. Clade support was estimated by bootstrapping (Felsenstein, 1985) with 1,000 replicates. The resulting trees were rooted with *Vatica* (two species) and visualized with FigTree v1.4 (available from http://tree.bio.ed.ac.uk/software/figtree/, last accessed: 2017-10-05).

Detection of patterns of reticulation

Dataset DF was used to produce phylogenetic networks for two different clades (1) *Hopea-Anthoshorea-Doona*, and (2) *Rubroshorea* in SPLITSTREE 4.10 (Huson & Bryant, 2006) to detect patterns of reticulation which might hint on hybridisation or incomplete lineage sorting. Splits were drawn using the uncorrected p method that is identical to the Hamming method (Hamming 1950), because the final data was lacking indels. Trees were visualised as neighbour nets with each end node representing an individual.

Species tree estimation with SNAPP

Species trees were inferred with the Bayesian coalescent method implemented in SNAPP (Bryant *et al.*, 2012). To lower computational cost, we built separate species trees for large monophyletic clades, visualized from RAxML trees, containing (1) *Rubroshorea-Shorea-Richetia-Parashorea* and (2) genus *Hopea*. Here, no missing data was allowed. SNAPP is designed to deal with biallelic unlinked

data. Thus one random SNP per RADseq locus was extracted. For the SNAPP analyses, individuals were a priori grouped to species, apart from those species that were non-monophyletic in the ML tree. *H. jucunda* and *H. juncunda* subsp. *modesta* were excluded because they are different subspecies. These were excluded from the analysis. Further, only species which were represented by at least two individuals were included in the SNAPP analyses. MCMC chain was run for 10^7 (*Hopea*), respectively $9x10^6$ *(Rubroshorea-Shorea-Richetia-Parashorea)*, sampling every 100^{th} generation. Burn-in was checked visually and convergence was determined in Tracer v. 1.6. All of the Effective Sample Site (ESS) values were over 200 for the analysis of the *Hopea* clade, while few ESS values remained below 200, but over 120 in the analysis of *Rubroshorea-Shorea-Richetia-Parashorea* after removing 4,500,000 (i.e., 50%) generations as burn-in. Resulting trees were visualized in DensiTree (Bouckaert, 2010) and summarized in a set of consensus trees.

Ancestral state reconstruction of floral traits

To test the hypothesis that a high number of stamens (> 15) and large, oblong anthers with short appendages are plesiomorphic characters we attempted to reconstruct ancestral states of floral traits in the tribe Shoreeae *sensu* Ashton along the inferred phylogeny with the Lagrange dispersal, extinction, and cladogenesis model (Ree & Smith, 2008) implemented in RASP v.3.02 (Yu *et al.*, 2015). As species trees built with SNAPP were largely congruent with the RAxML phylogeny, we used the later tree as a backbone to perform floral trait evolution analyses. These were carried out for (1) the evolution of the anther/appendage size (large anthers with short appendages, small anthers with long appendages, appendages as long as anthers), (2) anther structure [oblong vs. (sub-)globose], and (3) number of stamens (less than or equal 15 vs. more than 15). We allowed for ancestral ranges combining a maximum of two states. Information on traits were extracted from Ashton (1964, 2004) and are given in Table S1. Species, for which trait information was missing were pruned from the ML phylogeny in R with the command "drop.tip".

Results

Phylogenomic inference

Demultiplexing, trimming and filtering the raw reads from four RADseq libraries resulted in an average of 2.98 million high-quality pairs of reads per individual. After parameter optimization as described above, the assembly pipeline of STACKS produced 16,238 loci. The filtered data (dataset DF) used for phylogenetic reconstructions with MP and ML included 101,066 SNPs. Of the characters, 91,440 characters were parsimony informative. The MP analysis resulted in a single best parsimonious tree with a length of 158,467 steps. Further statistics were: retention index: 0.907, consistency index 0.638, rescaled consistency index 0.578, and homoplasy index 0.362.

The topologies of the trees resulting from the MP and ML analyses were generally congruent. Bootstrap analysis revealed high support (BS: 91-100) for 82.3% (MP) / 91.9 % (ML) of the nodes and moderate support (BS: 71-90) for 8.1% (MP) / 2.4% (ML) of the nodes. Only 7.3% (MP) / 4.9% (ML)

of the nodes had a low support value (50-70) and 2.4% (MP) / 0.8% (ML) of the nodes remained unresolved (BS: < 50). Analyses resulted in four highly supported major clades (I-IV) with respect to the ingroup (Fig. 1, Fig. S1). In the ML tree, clade I contains the *Dipterocarpus* individual included in the analyses and clade II consists of *Dryobalanops*. These positions were reversed in the MP tree (Fig. S1). According to Ashton (1979), *Dryobalanops* is assigned to tribe Shoreeae, whereas Maury-Lechon (1979) placed in an intermediate position between tribes Shoreeae and Dipterocarpeae.

However, the main interest of the study are relationships of the genera *Shorea*, *Parashorea*, and *Hopea* which are represented in clade III and IV. While genera *Hopea* and *Parashorea* are monophyletic, genus *Shorea sensu* Ashton is divided into five separate groups which correspond to classifications of Maury (1979) based on morphology and anatomy of the bark: *Anthoshorea*, *Doona*, *Richetia*, *Shorea* (selangang batu), and *Rubroshorea*.

Specifically, clade III consists of three subclades: *Doona* (SD), *Anthoshorea* (SA), and *Hopea* (HO). Subclade HO includes members of the sections *Dryobalanoides* (subsections *Dryobalanoides* and *Sphaerocarpae*) and *Hopea* (subsection *Hopea*). However, neither the sections nor subsections in this subclade are monophyletic. In detail, subclade HO can be further divided into four smaller clades consisting of: (1) *H. pentanerviana* Symington ex G.H.S.Wood, *H. odorata* Roxb. (section *Hopea*, subsection *Hopea*), *H. utilis* (Bedd.) Bole and *H. brevipetiolaris* (Thwaites) P.S.Ashton (endemic to South India and Sri Lanka); (2) *H. jucunda* Thwaites and *H. jucunda* Thwaites subsp. *modesta*, endemic to Sri Lanka; (3) *H. centipeda* P.S.Ashton (section *Hopea*, subsection *Hopea*) and non-monophyletic *H. dryobalanocarpoides* Miq. (section *Dryobalanoides*, subsections *Dryobalanoides*); and (4) *H. bracteata* Burck, *H. nervosa* King, *H. vaccinifolia* Ridl. ex P.S.Ashton (all section *Dryobalanoides*, subsection *Sphaerocarpae),* but also *H. dyeri* F.Heim(section *Dryobalanoides*, subsections *Dryobalanoides*). Clade IV consisted of four monophyletic subclades: subclade PA is formed by the genus *Parashorea* and is sister to subclade SRI consisting of *Richetia.* Subclade SS contains members of *Shorea* (selangang batu). Here, subsections *Shorea* and *Barbata* of section *Shorea* are not monophyletic. Subclade SS is sister to subclade SRU and includes members of *Rubroshorea*. Within *Rubroshorea*, some sectional and subsectional classifications were monophyletic and some relationships remained poorly supported (Fig. 1).

The SplitsTree network of the *Hopea-Anthoshorea-Doona* clade (Fig. 2a) reflects the general pattern revealed in the phylogenetic analyses. Whereas genus *Hopea* (Fig. 2a) shows some reticulation, *Doona* (Fig. 2a, green) and *Antoshorea* (Fig. 2a, violet) are less reticulated. Because we were interested in *Rubroshorea* which contains some low supported nodes, we also reconstructed a SplitsTree network of this subclade (Fig. 2b). The groups detected correspond to those in the MP and ML trees. Most of the groups in the network outline reticulation, possibly indicating incomplete lineage sorting.

The filtered matrices used for the SNAPP analyses contained 3,861 SNPS for the clade consisting of *Rubroshorea*, *Shorea*, *Richetia*, and *Parashorea* (Fig. 1: IV) and 6,919 SNPS for the *Hopea* clade (Fig. 1: III HO). The relationships between the four subclades *Rubroshorea-Shorea-Richetia-Parashorea* (Fig. 3a) were constant between the SNAPP and MP/ML analyses. Terminal relationships

within the group *Shorea* (selangang batu; Fig. 3a: green) reflect the relationships observed in the MP and ML trees, indicating rapid divergence in the clade formed by *S. exelliptica* Meijer, *S. maxwelliana* King, *S. obscura* Meijer and *S. crassa* P.S.Ashton. Regarding the group *Rubroshorea*, the SNAPP analysis detected *Shorea johorensis* Foxw. as the sister species to the rest of *Rubroshorea*. This is in contrast to the MP/ML analyses, in which a monophyletic group of *S. albida* Ridl., *S. pachyphylla* Ridl. ex Symington, and *S. inaequilateralis* Symington is sister to the remaining members of *Rubroshorea*. Further, it also reveals close relationship between *S. macroptera* subsp. *macropterifolia* P.S.Ashton, *S. smithiana* Symington, *S. fallax* Meijer, *S. sp*., and *S. parvistipulata* F.Heim. However, in the ML analysis *S. macroptera* subsp. *macropterifolia* is clearly separated from these taxa. The topologies of the *Hopea* species trees (Fig. 3b) corresponds to the clades detected with the MP and ML analyses (Fig. 1 III HO), but there are some differences at lower taxonomic levels regarding subclade III HO 1. Specifically, *H. pentanervia* is sister to *H. brevipetiolaris* and *H. utilis* is sister to both.

Fig. 1: Best-scoring maximum likelihood tree of a rapid bootstrap analysis with 1,000 replicates. Bootstrap values (> 70%) are given. I: *Dryobalanops*, II: *Dipterocarpus*, III: *Doona* (SD) – *Anthoshorea* (SA) – *Hopea* (HO), IV: *Parashorea* (PA) – *Richetia* (SRI) – *Shorea* (selangan batu, SS) – *Rubroshorea* (SRU). 1-4 indicate different clades within HO. Sections and subsections according to Ashton are given in brackets for each of the *Shorea* and *Hopea* accessions: (A), section *Anthoshorea*; (B), section *Doona*; (C), section *Mutica*; (C1), subsection Mutica; (C2), subsection Auriculatae; (D), section *Pachycarpae*; (E), section *Brachypterae*; (E1), subsection *Brachypterae*; (E2), subsection *Smithiana*; (F), section *Rubella*; (G), section *Ovalis;* (H), section *Shorea*; (H1), subsection *Barbata*; (H2), subsection *Shorea*; (I), section *Richetioides*; subsection *Richetioides*; (J), section *Dryobalanoides*; (J1), subsection *Sphaerocarpae;* (J2), subsection *Dryobalanoides*; (K), section *Hopea;* subsection *Hopea.*

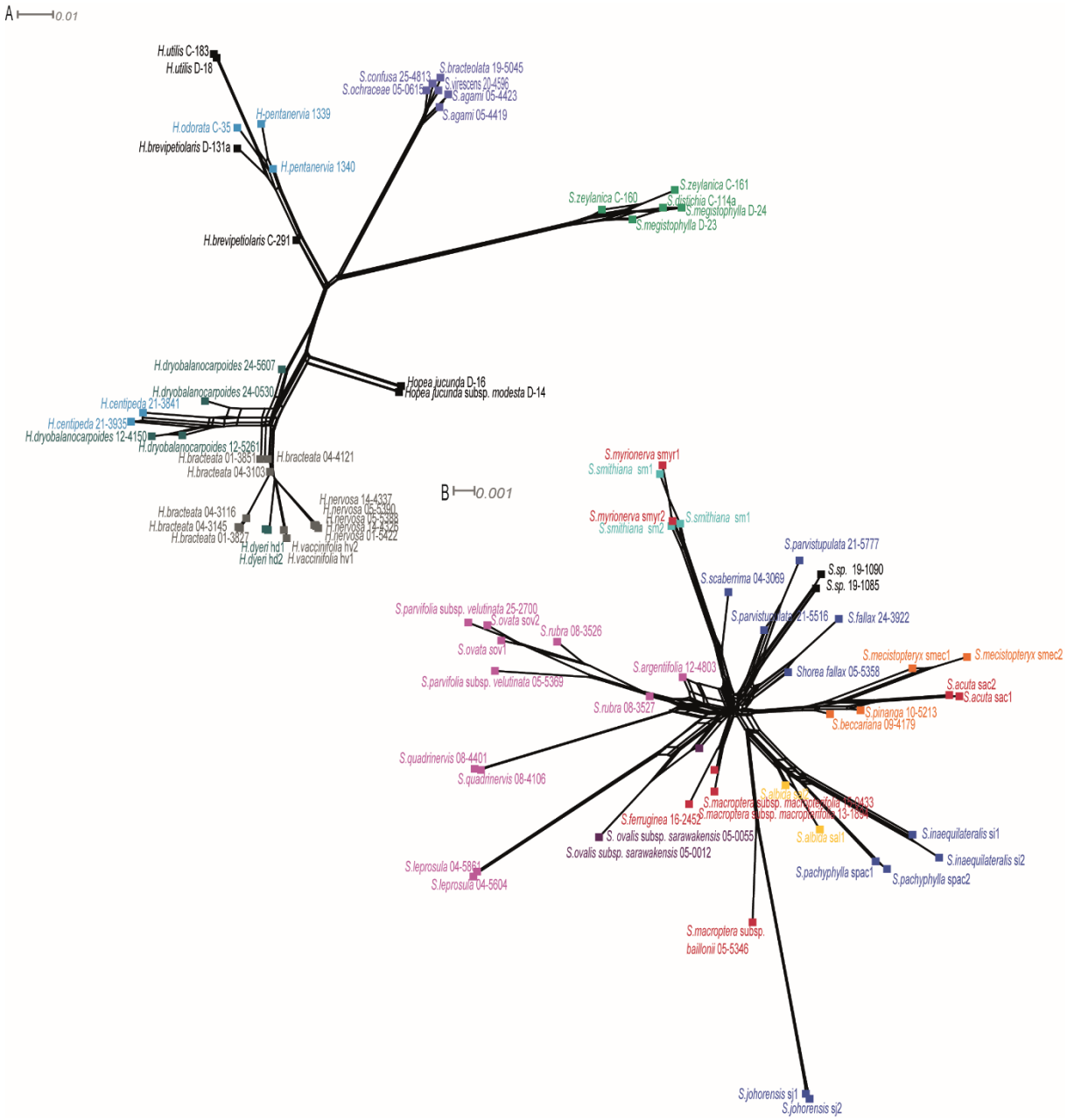

Fig. 2: NeighbourNets based on uncorrected p distance for a) *Hopea-Anthoshorea-Doona* and b) *Rubroshorea*.

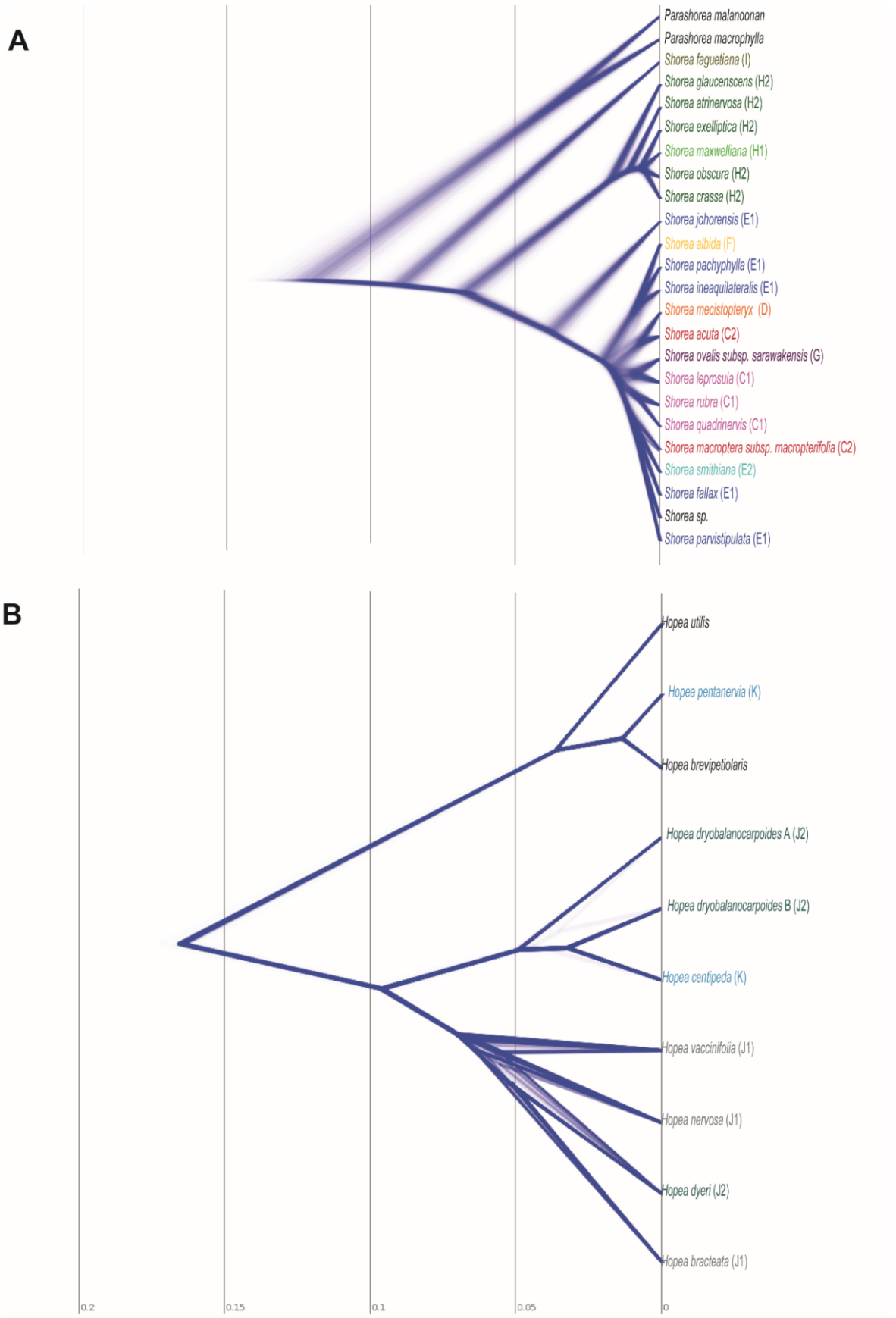

Fig. 3: Consensus trees generated by SNAPP analyses for a) *Rubroshorea-Shorea-Richetia-Parashorea* and b) *Hopea*. The distances on the horizontal axis are relative measures of substitutions per site. For abbreviations see legend Fig. 1.

Ancestral states and floral evolution of Dipterocarpoideaea

Lagrange ancestral state reconstructions of floral characters on the phylogeny of the tribe Shoreeae clearly confirmed the hypothesis that the flowers of the most common ancestor of the Shoreeae had large, oblong anthers with short appendages and probably more than 15 stamens (Fig. 4). Specifically, regarding the anther structure, the reconstructions support at least two independent origins of (sub-)globose flowers (Fig. 4a, orange): one at the base of *Hopea* (Fig. 4a, node 1) and another one at the base of section *Mutica*, subsection *Mutica* (Fig. 4a, node 2). Two more possible gains are observed in *Rubroshorea* (*S. pachyphylla*; Fig 4a, node 3) and *Richetia* (*S. faguetiodes* P.S.Ashton, Fig. 4a, node 4). With respect to the size of anthers and length of appendages, there is a tendency for the gain of small anthers with long appendages all over the phylogenetic tree (Fig. 2b, pink): one at the base of *Anthoshorea* and *Hopea* (Fig. 4b, node 5), one at the base of the clade containing members of *Shorea* (selangang batu; Fig. 4b, node 6) and several in *Rubroshorea*. The hypothesis of having more than 15 stamens being an ancestral character state was represented by 22% in the piechart (Fig. 4c, node *), whereas the rest 78% supported either character state. Few stamens as ancestral character received no clear support by itself.

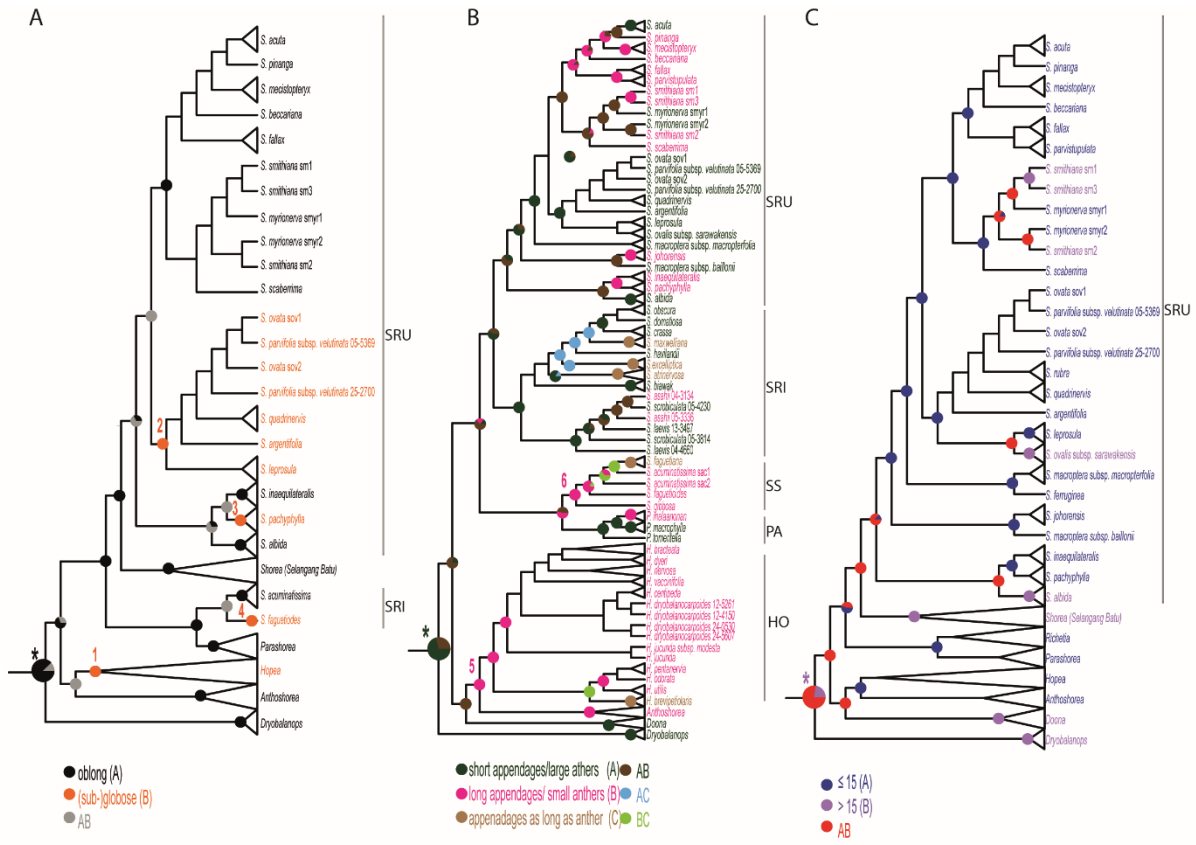

Fig. 4: Lagrange ancestral state reconstructions of floral characters on the phylogeny of the Dipterocarpoideae for a) anther structure, b) anther and appendage size, and c) number of stamens. Ancestral states are shown at the nodes, current states are given at the tips. * indicates the most recent common ancestor of the Shoreeae, HO: *Hopea*, PA: *Parashorea*, SRI: *Richetia*, SS: *Shorea* (selangan batu), SRU: *Rubroshorea*.

Discussion

Phylogenomic relationships within the tribe Shoreeae

The systematics of the subfamily Dipterocarpoideae has been widely debated, which is reflected by the presence of several different classification systems (Table 1) proposed mainly based on morphological characters. Several molecular studies have been also carried out in the past to investigate the phylogenetic relationships within Dipterocarpaceae. These made use of PCR-RFLPs (Tsumura *et al.*, 1996; Indrioko *et al.*, 2006), RAPDs (Rath *et al.*, 1998), AFLPs (Cao *et al.*, 2006), plastid sequences (Kajita *et al.*, 1998; Kamiya *et al.*, 1998; Dayanandan *et al.*, 1999; Gamage *et al.*, 2003; Yulita, Bayer & West, 2005; Gamage *et al.*, 2006; Choong *et al.*, 2008; Tsumura *et al.*, 2011; Yulita, 2013), nuclear *pgiC* gene (Kamiya *et al.*, 2005; Choong *et al.*, 2008) and ITS regions (Yulita *et al.*, 2005). However, these previous studies often used only one or few markers and a limited number of species. The most recently published molecular phylogenetic study (Heckenhauer *et al.*, 2017) provides a comprehensive phylogeny of the whole family Dipterocarpaceae based on plastid markers. However, for some taxonomic groups, especially in the large tribe Shoreeae relationships remained largely unresolved. In addition, as the plastid genome is inherited as a linkage block, the phylogeny obtained represents a gene tree that may not necessarily reflect the true species phylogeny of the family (i.e. Rosenberg, 2002; Degnan & Rosenberg 2006).

In the present investigation, more than a hundred thousand genome-wide SNPs were used for phylogenetic analyses, including coalescent-based species tree inference for the tribe Shoreeae. RADseq has been successfully applied in phylogenomic studies of non-model organisms such as parasitic plants (*Pedicularis*, Orobanchaceae; Eaton & Ree, 2013), Poaceae (Bambusoideae; Wang *et al.*, 2013), subshrubs (*Diapensia*, Diapensiaceae; Hou *et al.*, 2015), shrubs (*Eriobotrya*, Rosaceae; Yang *et al.*, 2017), temperate (*Quercus*, Fagaceae; Hipp *et al.*, 2014) and tropical trees (*Myrica*, Myricaceae; Liu *et al.*, 2015; *Diospyros*, Ebenaceae; Paun *et al.*, 2016). We propose here a new analyses pipeline for non-model RADseq data, i.e., *de novo* catalog building followed by extracting a pseudoreference from the loci obtained, and calling variants based on a mapping approach. This pipeline allows spanning biological and technical variability across a large number of samples in a non-model group at great phylogenetic width, which maximises the amount of usable loci by reducing significantly the amount of missingness present in the data. The average level of missing data in our raw catalog after reference mapping was 0.22, whereas in the original Stacks catalog 60% of the loci were missing on average.

The phylogenetic trees obtained in the present study (MP, ML, SNAPP) proved to be largely congruent to those revealed in a previous study based on plastid markers (Heckenhauer *et al.*, 2017; for comparison see Fig. S2), but improved significantly the resolution of the phylogenetic relationships. Most of the branches in the RADseq-derived phylogenetic trees receive 100% bootstrap support. In addition, the relationships reported here represent the history along the genome, improving the confidence obtained from trees built based on single loci (e.g. the plastid genome). Further, phylogenetic trees from different genes often exhibit conflicting branching patterns (Degnan & Rosenberg, 2009). Whereas gene trees inference was traditionally used in phylogenetics, recent studies focus on multilocus inference of species

trees (Degnan & Rosenberg, 2009). We successfully build coalescent based species trees which avoid potentially misleading histories of single loci. The concatenated SNP datasets analysed with traditional methods (e.g. MP and ML; Fig. 1 and Fig. S1) were largely congruent with the coalescent based species tree (Fig. 3) and therefore constitute good proxies of species trees in our study group.

We report here that the two monophyletic genera *Hopea* and *Parashorea* are clearly nested within the genus *Shorea sensu* Ashton, thus the latter becoming polyphyletic. *Shorea* appears divided into five groups which correspond to earlier morphological classifications of Maury (1978): *Doona*, *Anthoshorea*, *Rubroshorea*, *Shorea* (selangang batu), and *Richetia* (Fig. 1, Fig. S1). The SNPs derived trees provide good resolution for some previously unresolved aspects in the relationships between and within the genera *Hopea*, *Parashorea*, and *Shorea*. While the monophyly of some of the subclades was only moderately supported in the phylogenetic trees resulting from plastid analysis (i.e. *Rubroshorea*: 84%, *Shorea* (selangang batu): 93%, *Richetia*: < 70%, *Parashorea* < 70%, *Anthoshorea* < 70%; Fig. S2, Heckenhauer *et al.*, 2017), they receive highest support in the RADseq-derived dataset. The current study clearly resolves the positions of the subclades in clade IV which comprises *Rubroshorea*, *Shorea* (selangang batu), *Richetia*, and *Parashorea*. It recovers sister relationship between *Rubroshorea* and *Shorea* (selangang batu), as well as *Parashorea* and *Richetia*.

As a direct result of our study, the polyphyletic *Shorea* could be considered to one large monophyletic genus, including *Hopea* and *Parashorea*. However, there are remarkable morphological differences between the genera *Hopea* and *Shorea* which have to be taken in consideration. Members of *Shorea* are mainly tall emergent or canopy trees. *Hopea* is often represented by main canopy but also understorey trees (Maury-Lechon & Curtet, 1998). Morphologically, most *Hopea* species differ from *Shorea* in having stilt roots and flying buttresses which are virtually absent in *Shorea*. Furthermore, *Hopea* has more slender twigs, smaller flowers and two-winged fruits that are usually also smaller and generally glabrous (Ashton, 2004). Both genera, *Shorea* and *Hopea,* differ consistently in the number and morphology of the flower calyx lobes. In *Hopea*, the two outer lobes are thicker and more acuminate than the three inner ones, whereas in *Shorea* the three outer ones are thicker and more acuminate than the three inner ones (Ashton, 2004). An alternative to the above-suggested fusion of the three genera into one larger monophyletic genus, could be to divide the genus *Shorea* into several clades corresponding to the ones proposed by Maury (1979), which are well supported in the present study. For this, well-defined morphological features unique for each of these groups of *Shorea* should be defined.

Interestingly, speciation rates in *Hopea* seem to be lower than in *Shorea* (Fig. 3 a and b). A potential explanation for this finding could be faster generation times in *Shorea*. However, members of *Shorea* are tall, emergent trees whereas members of *Hopea* are main canopy but also sub-canopy trees that, if anything, would thus be expected to have a shorter generation time. An alternative explanation could be that *Shorea* has a much smaller effective population size, leading to faster speciation rates. Finally, *Hopea* could have experienced increased extinction rates as a result of evolutionary or ecological factors,

which would also produce a difference in net diversification rates between the two groups. However, further studies including a denser sampling of these groups are needed for confident conclusions.

Phylogenomic inferences within Hopea and Shorea sensu Ashton

The relationships within *Hopea* and *Shorea* are much better resolved compared to earlier studies and only few relationships still remain unresolved in the group *Rubroshorea* (Fig. 1). According to Ashton, the genus *Shorea* should be divided into eleven sections and eight subsections. Our analysis reveals that within the group *Rubroshorea* the subsections *Auriculatae* (section *Mutica;* Fig. 1: C2), *Brachypterae*, *Smithiana* (both section *Brachypterae;* Fig. 1: E1 and E2), as well as the section *Pachycarpae* (Fig. 1: D) are not forming monophyletic groups. This is also true for subsections *Barbata* and *Shorea* of section *Shorea* (*Shorea* / selangang batu group; Fig. 1: H1 and H2). Thus, Ashton's sectional and subsectional classification is not supported by the present results. Well-defined morphological characters as well as extended molecular studies with many species (in case of genera with large number of species) are important. This, again shows that well-defined morphological characters as well as molecular studies are important in systematics as pointed out in Heckenhauer *et al.* (2017).

Differences from morphological classifications were also detected for genus *Hopea*. *Hopea* is one of the most diverse dipterocarp genera, and exhibits great variability in ovary, leaf, bark, and mature habit (Ashton, 2004). Ashton (1982) and Maury (1978) recognised two sections with two subsections each: (1) section *Dryobalanoides* (Fig.1: J) with subsections *Dryobalanoides* and *Sphaerocarpae*, and (2) section *Hopea* (Fig.1: K), with subsections *Hopea* and *Pierra*. There are several characters used to separate the (sub-)sections. Subsections *Dryobalanoides* can be distinguished by its leaf venation which is dryobalanoid, i.e. with many fine but arched and unequal lateral veins and obscurely reticulate intercostal venation. Leaf venation of the other three subsections is either of hopea-type (i.e. all lateral veins are more or less parallel and reach the leaf margins, without intermediates, and with scalariform intercostal venation) or subdryobalanoid, which is an intermediate between both (Ashton, 1964). However, *Hopea* exhibitsgreat variability in the ovary, leaf, and bark and the floral diversity of the previously proposed (sub-)sections is even greater than in sections of *Shorea* (Ashton, 2004) which might explain why neither sections nor subsections included in our study are monophyletic.

Patterns of reticulation

The relationships revealed by the SplitsTree networks correspond to those in the MP and ML trees. Patterns of reticulation are especially detected in *Hopea*. Although hybridisation is assumed to be rare in tropical trees (Ashton, 1969), it has been reported in Dipterocarpaceae (Bawa, 1998). However, polyploidy has been detected in only few Dipterocarpaceae species [triploidy: *Dipterocarpus tuberculatus* Roxb. (Tixier, 1960), *H. beccariana* Burck (Ashton, 1982), *H. jucunda* (Heckenhauer *et al.*, 2017), *H. latifolia* Symington (Jong & Kaur, 1979), *H. odorata* (Kaur *et al.*, 1986), *H. subalata* Symington (Jong & Kaur, 1979), *Shorea ovalis* (Korth.) Blume subsp. *sericea* (Dyer) P.S.Ashton (Jong & Kaur, 1979); tetraploidy: *Shorea ovalis* (Korth.) Blume subsp. *sericea* (Dyer) P.S.Ashton (Jong &

Kaur, 1979), *Shorea ovalis* (Korth.) Blume subsp. *ovalis* (Kaur et *al.*, 1986)]. According to Ashton (1982) triploid taxa might be of interspecific hybrid origin (e.g. *H. subalata*, *H. odorata*). However, we assume that the patterns of reticulation detected in our analysis indicate incomplete lineage sorting rather than recent or ongoing hybridisation.

Ancestral states and floral evolution of Dipterocarpoideaea

The excellent resolution of the phylogenetic trees in this study makes it possible to investigate floral trait evolution in the tribe Shoreeae and test a hypothesis formulated based on biogeography and pollination biology. Reconstructions of the ancestral flower states indicate that the most recent common ancestor of the members of the tribe Shoreeae had large, oblong anthers with short appendages and more than 15 stamens. The high number of stamens observed in the closely related family Sarcolaenaceae (Bayer, 2003), as well as in the subfamily Monotoideae (Dipterocarpaceae; Ashton, 2013) together with our molecular evidence obtained in this study indicates that this is a an ancestral state. The flowers of species with oblong anthers and shorter appendages, have been observed to be bee pollinated (Appanah, 1985; Appanah 1987; Ghazoul *et al.*, 1998; Dayanandan, 1990; Momose *et al.*, 1996; Momose *et al.*, 1998). They often occur in forests where species richness is low and canopy flowering is annual (Ashton, 2013). Our results support the hypothesis of a shift in pollinators as species richness increased during diversification of the modern Dipterocarpoideae. The subsequent competition favoured species that could maintain pollinators which could respond to mass flowering initiation by rapid reproduction of the pollinators concerned. These are species with small, (sub-)globose anthers and long appendages which are pollinated by small fecund insects, mainly thrips (Appanah & Chan, 1981). However, there are sections/species which retained some of the primitive characters (large anthers (large, oblong anthers with short appendages, and more that 15 stamens), i.e. *Shorea* section *Rubella*. Section *Rubella* species increasingly became restricted due to competition from advanced, small anthered species. They only survive in habitats lacking either competition (i.e. *S. albida*) or by receiving visits by swarms of large pollinators (mainly bees) which are attracted by species whose pollen production act as baits (i.e. *Dryobalanops,* Ashton personal communication). To detect further support for this hypothesis, more species have to be included in subsequent studies. Particular interest would be the inclusion of *Shorea* section *Pentacme* which is not included in our phylogenetic study. Pentacme has 15 stamens and yellow, oblong anthers. It is abundant in seasonal lowland forests, where it flowers annually and it is pollinated by bees. In this study, *Shorea* section *Rubella* is presented by one species only (*S. albida Symington* ex. A.V. Thomas), but ideally more species of this section should be included for solid conclusions. Moreover, there are interesting species, such as *H. plagata* and *S. polyandra* (section *Richetioides,* subsection *Polyandrae*) which differ from other taxa in *Hopea,* as well as *Richetoides* by their high number of stamens (approx. 35 in *H. plagata* (Blanco) S.Vidal and more than 100 in *S. polyandra;* Ashton, 2004). These are not included in this study, but are of value for further investigations. Further, it would be interesting to search for particular genomic regions that have been affected as a result of positive selection for flowers with small, (sub-) globose anthers with long appendages.

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Supplementary

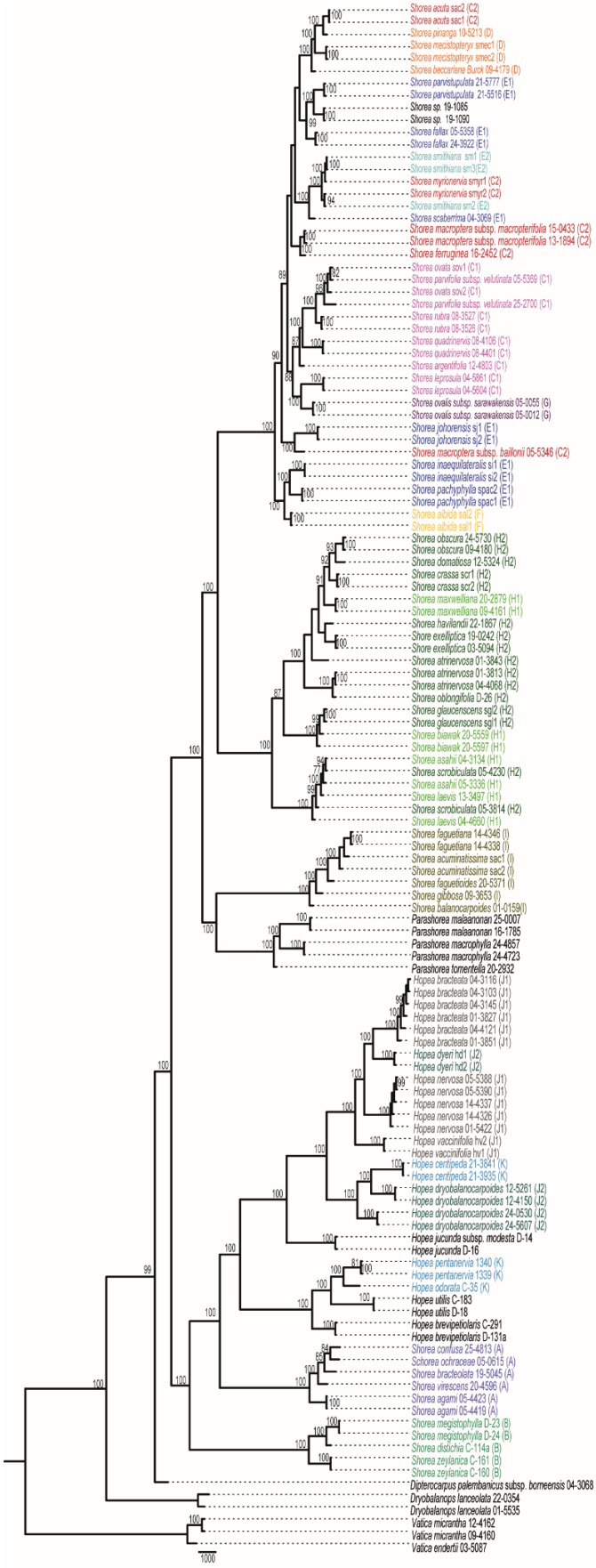

Fig. S1: Most parsimonious tree based on 91,440 parsimony informative SNPS derived from RADseq. Numbers indicate bootstrap support values over 70%. For abbreviations see legend of Fig. 1.

Fig. S2: Best scoring maximum likelihood tree obtained from six plastid DNA regions (5,911 bp) in a previous study (Heckenhauer *et al.*, 2017). For comparative reasons, the tree is pruned to species investigated in this study. Bootstrap values higher than 70% are given. For abbreviations see legend of Fig. 1.

Table S1: List of taxa used for analysis. Collection numbers, herbarium vouchers, and sampling locations are given, as well as information on floral characteracters.

Taxon	Collection	Voucher	Location	Anther structure	Appendage size	No. of stamens
Dipterocarpus palembanicus subsp. *borneensis* P.S.Ashton	JH04-3068	UBDH: 04-3068	UBD-CTFS 25 ha plot			
Dryobalanops lanceolata Burck	JH22-0354	UBDH: 22-0354	UBD-CTFS 25 ha plot	oblong	short	> 15
	JH01-5535	UBDH: 01-5535	UBD-CTFS 25 ha plot			
Hopea bracteata Burck	JH01-3827	UBDH: 01-3827	UBD-CTFS 25 ha plot	(sub-)globose	as long as anther	≤ 15
	JH04-3116	UBDH: 04-3116	UBD-CTFS 25 ha plot			
	JH04-4121	UBDH: 04-4121	UBD-CTFS 25 ha plot			
	JH04-3103	UBDH: 04-3103	UBD-CTFS 25 ha plot			
	JH04-3145	UBDH: 04-3145	UBD-CTFS 25 ha plot			
	JH01-3851	UBDH: 01-3851	UBD-CTFS 25 ha plot			
Hopea brevipetiolaris (Thwaites) P.S.Ashton	R.S. D-131a	PDA: D-131a	Arboretum, The Royal Botanical Gardens, Peradeniya, Sri Lanka	(sub-)globose	long	≤ 15
	R.S. C-291	PDA: C-291	Arboretum, The Royal Botanical Gardens, Peradeniya, Sri Lanka			
Hopea centipeda P.S.Ashton	JH21-3935	UBDH: 21-3935	UBD-CTFS 25 ha plot	(sub-)globose	long	≤ 15
	JH21-3841	UBDH: 21-3841	UBD-CTFS 25 ha plot			
Hopea dryobalanoides Miq.	JH24-5607	UBDH: 24-5607	UBD-CTFS 25 ha plot	(sub-)globose	long	≤ 15
	JH12-4150	UBDH: 12-4150	UBD-CTFS 25 ha plot			
	JH12-5262	UBDH: 12-5262	UBD-CTFS 25 ha plot			
	JH24-0530	UBDH: 24-0530	UBD-CTFS 25 ha plot			
Hopea dyeri F.Heim	KAShd1	BRUN: BRUN 3362/ B 000 470	KN Nursery Km 13 Bt Sawat, Kuala Belait, BD	(sub-)globose	long	≤ 15
	KAShd2	BRUN: BRUN 3362/ B 000 470	KN Nursery Km 13 Bt Sawat, Kuala Belait, BD			
Hopea jucunda Thwaites	R.S. D-16	PDA: D-16	Arboretum, The Royal Botanical Gardens, Peradeniya, Sri Lanka	(sub-)globose	long	≤ 15
Hopea jucunda subsp. *modesta* (A.DC.) Kosterm.	R.S. D-14	PDA: D-14	Arboretum, The Royal Botanical Gardens, Peradeniya, Sri Lanka	(sub-)globose	long	≤ 15

Hopea nervosa King	JH01-5422	UBDH: 01-5422	UBD-CTFS 25 ha plot	(sub-)globose	long	≤ 15
	JH05-5388	UBDH: 05-5388	UBD-CTFS 25 ha plot			
	JH05-5390	UBDH: 05-5390	UBD-CTFS 25 ha plot			
	JH14-4337	UBDH: 14-4337	UBD-CTFS 25 ha plot			
	JH14-4326	UBDH: 14-4326	UBD-CTFS 25 ha plot			
Hopea odorata Roxb.	R.S. C-35	PDA: C-35	Arboretum, The Royal Botanical Gardens, Peradeniya, Sri Lanka	(sub-)globose	long	≤ 15
Hopea pentanervia Symington ex G.H.S.Wood	KAS1339	BRUN: BRUN 17424/ B 000 591	UBD plot, Km 13 Bt.Sawat, Kuala Belait, BD	(sub-)globose	long	≤ 15
	KAS1340	BRUN: BRUN 17424/ B 000 591	UBD plot, Km 13 Bt.Sawat, Kuala Belait, BD			
Hopea utilis (Bedd.) Bole	R.S. D-18	PDA: D-18	Arboretum, The Royal Botanical Gardens, Peradeniya, Sri Lanka	(sub-)globose	long	≤ 15
	R.S. C-183	PDA: C-183	Arboretum, The Royal Botanical Gardens, Peradeniya, Sri Lanka			
Hopea vaccinifolia Ridl. ex P.S.Ashton	KAShv1	BRUN: BRUN 18998/ B 000 822	UBD plot, Km 13 Bt.Sawat, Kuala Belait, BD	(sub-)globose	long	≤ 15
	KAShv2	BRUN: BRUN 18998/ B 000 822	Bt. Puan, Labi, Kuala Belait, BD			
Parashorea macrophylla Wyatt-Sm. ex. P.S.Ashton	JH24-4723	UBDH: 24-4723	UBD-CTFS 25 ha plot	oblong	long	≤ 15
	JH24-4857	UBDH: 25-4857	UBD-CTFS 25 ha plot			
Parashorea malaanonan (Blco) Merr.	JH16-1785	UBDH: 16-1785	UBD-CTFS 25 ha plot	oblong	long	≤ 15
	JH25-0007	UBDH: 25-0007	UBD-CTFS 25 ha plot			
Parashorea tomentella (Symington) Meijer	JH20-2932	UBDH: 20-2932	UBD-CTFS 25 ha plot	oblong	short	≤ 15
Shorea sp.	JH19-1085	UBDH: 19-1085	UBD-CTFS 25 ha plot			
	JH19-1090	UBDH: 19-1090	UBD-CTFS 25 ha plot			
Shorea acuminatissima Symington	KASsac1	BRUN: KEP 80128/ B 000 893	KN Nursery Km 13 Bt Sawat, Kuala Belait, BD	oblong	long	≤ 15
	KASsac2	BRUN: KEP 80128/ B 000 893	KN Nursery Km 13 Bt Sawat, Kuala Belait, BD			
Shorea acuta P.S.Ashton	KASsa1	BRUN: KEP 80088/ B 000 918	Sg. Liang Arboretum Forest Reserve, Kuala Belait or Andulau FR, Kuala Belait, BD	oblong	short	≤ 15

	KASsa2	BRUN: KEP 80088/ B 000 918	Sg. Liang Arboretum Forest Reserve, Kuala Belait or Andulau Forest Reserve, Kuala Belait, BD			
Shorea albida Symington ex A.V.Thomas	KASsal1	BRUN: BRUN 26298/ B 000 940	Sg. Mau, Bt.Sawat, Kuala Belait	oblong	short	> 15
	KASsal2	BRUN: BRUN 26298/ B 000 940	Sg. Mau, Bt.Sawat, Kuala Belait			
Shorea agami P.S.Ashton	JH05-4419	UBDH: 05-4419	UBD-CTFS 25 ha plot	oblong	long	≤ 15
	JH05-4423	UBDH: 05-4423	UBD-CTFS 25 ha plot			
Shorea argentifolia Symington	JH12-4803	UBDH: 12-4803	UBD-CTFS 25 ha plot	(sub-)globose	short	≤ 15
Shorea asahii P.S. Ashton	JH04-3134	UBDH: 04-3134	UBD-CTFS 25 ha plot	oblong	long	> 15
	JH05-3336	UBDH: 05-3336	UBD-CTFS 25 ha plot			
Shorea atrinervosa Symington	JH01-3813	UBDH: 01-3813	UBD-CTFS 25 ha plot	n.a.	as long as anther	> 15
	JH01-3843	UBDH: 01-3843	UBD-CTFS 25 ha plot			
	JH04-4068	UBDH: 04-4068	UBD-CTFS 25 ha plot			
Shorea balanocarpoides Symington	JH01-0159	UBDH: 01-0159	UBD-CTFS 25 ha plot	n.a	n.a	≤ 15
Shorea beccariana Burck	JH09-4179	UBDH: 09-4179	UBD-CTFS 25 ha plot	oblong	long	≤ 15
Shorea biawak P.S.Ashton	JH20-5597	UBDH: 20-5597	UBD-CTFS 25 ha plot	oblong	short	> 15
	JH20-5559	UBDH: 20-5549	UBD-CTFS 25 ha plot			
Shorea bracteolata Dyer	JH19-5045	UBDH: 19-5405	UBD-CTFS 25 ha plot	oblong	long	≤ 15
Shorea confusa P.S.Ashton	JH25-4813	UBDH: 25-4813	UBD-CTFS 25 ha plot	oblong	long	≤ 15
Shorea crassa P.S.Ashton	KASscr1	BRUN: BRUN 3080/B 001 134	Sg.Liang Andulau FR, Kuala Belait, BD	oblong	short	> 15
	KASscr2	BRUN: BRUN 3080/B 001 134	Berakas FR, Berakas, Muara, BD			
Shorea disticha (Thwaites) P.S.Ashton	R.S. C-114a	PDA: C-114a	Arboretum, The Royal Botanical Gardens, Peradeniya, Sri Lanka	n.a	short	≤ 15

Shorea domatiosa P.S.Ashton	JH12-5324	UBDH: 12-5324	UBD-CTFS 25 ha plot	oblong	short	> 15
Shorea exelliptica Meijer	JH03-5094	UBDH: 03-5094	UBD-CTFS 25 ha plot	n.a	as long as anther	> 15
	JH19-0242	UBDH: 19-0242	UBD-CTFS 25 ha plot			
Shorea faguetiana F.Heim	JH14-4338	UBDH: 14-4338	UBD-CTFS 25 ha plot	n.a	as long as anther	≤ 15
	JH14-4346	UBDH: 14-4346	UBD-CTFS 25 ha plot			
Shorea faguetioides P.S.Ashton	JH20-5371	UBDH: 20-5371	UBD-CTFS 25 ha plot	(sub-)globose	long	≤ 15
Shorea fallax Meijer	JH05-5358	UBDH: 05-5358	UBD-CTFS 25 ha plot	oblong	long	≤ 15
	JH24-3922	UBDH: 24-3922	UBD-CTFS 25 ha plot			
Shorea ferruginea Dyer ex Brandis	JH16-2452	UBDH: 16-2452	UBD-CTFS 25 ha plot	n.a	n.a.	≤ 15
Shorea gibbosa Brandis	JH09-3653	UBDH: 09-3653	UBD-CTFS 25 ha plot	n.a	long	≤ 15
Shorea glaucenscens Meijer	KASsgl1	n.a.	KN Nursery Km 13 Bt Sawat, Kuala Belait, BD	n.a	n.a	n.a
	KASsgl2	n.a.	KN Nursery Km 13 Bt Sawat, Kuala Belait, BD			
Shorea havilandii Brandis	JH22-1867	UBDH: 22-1867	UBD-CTFS 25 ha plot	n.a	short	> 15
Shorea inaequilateralis Symington	KASsi1	BRUN: WKM 3238/ B 040 939	KN Nursery Km 13 Bt Sawat, Kuala Belait, BD	oblong	long	≤ 15
	KASsi2	BRUN: WKM 3238/ B 040 939	KN Nursery Km 13 Bt Sawat, Kuala Belait, BD			
Shorea johorensis Foxw.	KASsj1	BRUN: BRUN 355/ B 001 436	KN Nursery Km 13 Bt Sawat, Kuala Belait, BD	n.a	long	≤ 15
	KASsj2	BRUN: BRUN 355/ B 001 436	KN Nursery Km 13 Bt Sawat, Kuala Belait, BD			
Shorea laevis Ridl.	JH04-4660	UBDH: 04-4660	UBD-CTFS 25 ha plot	oblong	short	> 15
	JH13-3497	UBDH: 13-3497	UBD-CTFS 25 ha plot			
Shorea leprosula Miq.	JH04-5604	UBDH: 04-5604	UBD-CTFS 25 ha plot	(sub-)globose	short	≤ 15
	JH04-5861	UBDH: 04-5861	UBD-CTFS 25 ha plot			
Shorea macroptera subsp. *baillonii* (F.Heim) P.S.Ashton	JH05-5346	UBDH: 05-5346	UBD-CTFS 25 ha plot	n.a	short	≤ 15
Shorea macroptera subsp. *macropterifolia* P.S.Ashton	JH13-1894	UBDH: 13-1894	UBD-CTFS 25 ha plot	n.a	short	≤ 15
	JH15-0433	UBDH: 15-0433	UBD-CTFS 25 ha plot			
Shorea maxwelliana King	JH09-4161	UBDH: 09-4161	UBD-CTFS 25 ha plot	n.a	as long as anther	> 15

	JH20-2879	UBDH: 20-2879	UBD-CTFS 25 ha plot			
Shorea mecistopteryx Ridl.	KASsmec1	BRUN: BRUN 3280/ B 001 563	Sg.Liang, Andulau Forest Reseve, Kuala Belait, BD	oblong	long	≤ 15
	KASsmec2	BRUN: BRUN 3280/ B 001 563	Sg.Liang, Andulau Forest Reserve, Kuala Belait, BD			
Shorea megistophylla P.S.Ashton	R.S. D-23	PDA: D-24	Arboretum, The Royal Botanical Gardens, Peradeniya, Sri Lanka	n.a	short	> 15
	R.S. D-24	PDA: D-24	Arboretum, The Royal Botanical Gardens, Peradeniya, Sri Lanka			
Shorea myrionervia Symington ex P.S.Ashton	KASsmyr1	BRUN: KEP 30480/ B 001 633	Sg.Liang Arboretum, Kuala Belait, BD	oblong	short	≤ 15
	KASsmyr2	BRUN: KEP 30480/ B 001 633	Sg.Liang Arboretum, Kuala Belait, BD			
Shorea oblongifolia Thwaites	R.S. D-26	PDA: D-26	Arboretum, The Royal Botanical Gardens, Peradeniya, Sri Lanka	n.a	n.a	n.a
Shorea obscura Meijer	JH09-4180	UBDH: 09-4180	UBD-CTFS 25 ha plot	oblong	short	> 15
	JH24-5730	UBDH: 24-5730	UBD-CTFS 25 ha plot			
Shorea ochracea Symington	JH05-0615	UBDH: 05-0615	UBD-CTFS 25 ha plot	oblong	long	≤ 15
Shorea ovalis (Korth.) Blume subsp. *sarawakensis* P.S.Ashton	JH05-0012	UBDH: 05-0012	UBD-CTFS 25 ha plot	n.a	short	> 15
	JH05-0055	UBDH: 05-0055	UBD-CTFS 25 ha plot			
Shorea ovata Dyer ex Brandis	KASsov1	n.a.	n.a.	(sub-)globose	short	≤ 15
	KASsov2	n.a.	n.a.			
Shorea pachyphylla Ridl. ex Symington	KASspac1	n.a.	n.a.	(sub-)globose	long	≤ 15
	KASspac2	n.a.	n.a.			
Shorea parvifolia Dyer subsp. *velutinata* P.S.Ashton	JH05-5369	UBDH: 05-5369	UBD-CTFS 25 ha plot	(sub-)globose	short	≤ 15
	JH25-2700	UBDH: 25-2700	UBD-CTFS 25 ha plot			
Shorea parvistupulata F. Heim	JH21-5516	UBDH: 21-5516	UBD-CTFS 25 ha plot	n.a	long	≤ 15
	JH21-5777	UBDH: 21-5777	UBD-CTFS 25 ha plot			
Shorea pinanga Scheff.	JH10-5213	UBDH: 10-5213	UBD-CTFS 25 ha plot	oblong	long	≤ 15
Shorea quadrinervis Slooten	JH08-4106	UBDH: 08-4106	UBD-CTFS 25 ha plot	(sub-)globose	short	≤ 15
	JH08-4401	UBDH: 08-4401	UBD-CTFS 25 ha plot			
Shorea rubra P.S.Ashton	JH08-3526	UBDH: 08-3526	UBD-CTFS 25 ha plot	n.a	n.a	≤ 15
	JH08-3527	UBDH: 08-3527	UBD-CTFS 25 ha plot			

Shorea scaberrima Burck	JH04-3069	UBDH: 04-3069	UBD-CTFS 25 ha plot	oblong	long	≤ 15
Shorea scrobiculata Burck	JH05-3814	UBDH: 05-3814	UBD-CTFS 25 ha plot	oblong	short	> 15
	JH05-4230	UBDH: 05-4230	UBD-CTFS 25 ha plot			
Shorea smithiana Symington	KASsm1	BRUN: BRUN 661/ B 002 013	KN Nursery Km 13 Bt Sawat, Kuala Belait, BD	oblong	long	> 15
	KASsm2	BRUN: BRUN 661/ B 002 013	KN Nursery Km 13 Bt Sawat, Kuala Belait, BD			
	KASsm3	BRUN: BRUN 661/ B 002 013	KN Nursery Km 13 Bt Sawat, Kuala Belait, BD			
Shorea virescens Parijs	JH20-4596	UBDH: 20-4596	UBD-CTFS 25 ha plot	oblong	long	≤ 15
Shorea zeylanica (Thwaites) P.S.Ashton	R.S.C-161	PDA: C-160	Arboretum, The Royal Botanical Gardens, Peradeniya, Sri Lanka	n.a.	short	> 15
	R.S. C-160	PDA: C-160	Arboretum, The Royal Botanical Gardens, Peradeniya, Sri Lanka			
Vatica endertii Slooten	JH03-5087	UBDH: 03-5087	UBD-CTFS 25 ha plot			
Vatica micrantha Slooten	JH09-4160	UBDH: 09-4160	UBD-CTFS 25 ha plot			
	JH12-4162	UBDH: 12-4162	UBD-CTFS 25 ha plot			

Table S2. Processing of RADseq reads. Selecting settings for maximizing number of reliable loci**.**
The number of stacks obtained with STACKS differed by varying the settings *m* (minimum number of identical reads required to create a stack, *M* (number of mismatches allowed between loci when processing a single individual), and *n* (number of mismatches allowed between loci when building the catalog). m = 6, M = 1, and n= 1 were used as a starting point and various values were tested for these three parameters. The resulting files were loaded into a MySQL database with load_radtags.pl and the amount of reliable loci obtained from each of the runs was compared using the following filters: (1) only allowing loci with 1-10 SNPs in at least 100 individuals and (2) only allowing loci with 1-20 SNPs in at least 100 individuals. The value that maximized the amount of loci was chosen.

Settings (m, M, n)	**Loci with 1 - 15 SNPs, covered in ≤ 100 individuals**	**Loci with 1 - 10 SNPs, covered in ≤ 100 individuals**
611	801	1424
511	1250	2174
521	948	1907
512	1195	3362
513	998	3347

CONCLUSIONS

The questions and aims (see "Aims" at page 18) of this project are successfully answered in this thesis.

Development of multiplexable primers which can be used to amplify the *matK* barcoding region across a wide range of Angiosperms was achieved. Initially, primers were designed for this study, i.e. targeting amplification and sequencing of Southeast Asian shrubs and trees. However, cross-transferability tests using plant families from other parts of the world (e.g., *Leontodon* [Asteraceae], *Tillandsia* [Bromeliaceae], *Helianthemum* [Cistaceae], *Polystachya* [Orchidaceae]) show that primers are also useful for other large-scale barcoding studies especially if these underlie a diverse composition of angiosperm families. The multiplex PCR approach improved the routine amplification of the *matK* barcode and thereby time in the laboratory.

DNA barcodes helped in identification of individuals in the 25-ha forest dynamics plot where morphological identifications is not yet complete. The combination of two DNA standard barcodes (*rbcL* and *matK*) is useful in identification of individuals at generic or family level. To achieve species-level identification, earlier studies have used a third barcode region such as *psbA-trnH* intergenic spacer. However it is very difficult to align a highly variable spacer region across a wide spectrum of angiosperms. The main reason why it was not possible to identify individuals at species-level in this study was that reference databases (GenBank, BOLD) often lack species and haplotype diversity, especially for Southeast Asian trees. Once the morphological identifications of our study plot are complete, already sequenced barcode regions (*matK* and *rbcL*) will be a great contribution to the existing reference database.

A relatively new methods which might increase species delimitation and extend existing barcodes is low-coverage shotgun sequencing of genomic DNA (genome skimming). This approach does not only recover the standard barcoding regions, but also provides sequence data from many other loci. It is in use for large scale projects, such as the Norwegian initiative for Barcoding of Life (including 3000 specimens of vascular plants of the arctic-boreal flora) and the PhyloAlps project which covers nearly all vascular plant species of the Alpine flora.

Phylogenetic analyses of the barcode sequences lead to reconstruction of highly resolved trees which have benefits over the traditionally used Phylomatic approach as they decrease false positive and negative observations. This is the first study that contributed to community assembly of a Southeast Asian tropical rain forest using barcode phylogenies, thus it is useful to obtain first insights into the forest's community structure. There is a great potential for follow up studies in this forest dynamics plot. Once morphological identification is completed, the DNA barcodes can be used to tests species boundaries and identification of cryptic species. The phylogenetic barcode trees can be applied in studies of functional and phylogenetic diversity. Mechanisms responsible for the observed phylogenetic clustering can be identified once niche-associated plant functional traits are available.

Molecular dating analysis gives a general time frame for the major clades in Dipterocarpoideae and revealed that divergence in extant Dipterocarpoideae occurred *c.* 55 Mya. An expanded analysis including a much larger set of Malvales would allow the use of multiple calibrations points and consequently help to obtain further insights into the biogeography and origin of Dipterocarpaceae and related families.

Results from analysis of plastid markers strengthen the phylogenetic hypotheses for the major clade to which Dipterocarpaceae are related (*Pakaraimaea* + Cistaceae) (Sarcolaenaceae + Monotoideae + Dipterocarpoideae). However, uncertainties remain in the phylogenetic relationships between Sarcolaenaceae, Monotoideae, and Dipterocarpoideae, as well as within the large non-monophyletic genus *Shorea*.

Analyses of RADseq derived SNPs were successfully used to infer species relationships within the tribe Shoreeae. Species delimitations detected with RADseq are congruent to those obtained from plastid markers and relationships in the tribe are better resolved in most cases. To get a full understanding of the relationships of the whole clade (Sarcolaenaceae + Monotoideae + Dipterocarpoideae), the RADseq dataset has to be extended to more species of the genus *Shorea*, especially sections which are missing in our study (e.g. *Pentacme*), but also to the other tribe of Dipterocarpoideae (Dipterocarpeae) and ideally to the closely related (sub-)families Monotoideae (including genus *Pseudomonotes* which is missing in the study of plastid markers) and Sarcolaenaceae. The phylogenetic trees, gave way for testing hypothesis regarding the evolution of floral traits, and this indicates that flowers with large, oblong anthers with short appendages are plesiomorphic characters in the subfamily Dipterocarpoideae. It will be good to get a clear understanding of this phenomena having the family Dipterocarpaceae as a whole.

APPENDIX

Abstracts of conference contributions (oral presentations and posters)

Assessment of Phylogenetic Community Structure for a Mixed Dipterocarp Forest in Brunei Darussalam using DNA barcoding

Jacqueline Heckenhauer[1], Mark W. Chase[2,3], Kamariah Abu Salim[4], Toby R. Pennington[5], David F. R. P. Burslem[6], Maria Ellen Kaye[6], Michael H. J. Barfuss[1], Rosabelle Samuel[1]

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Oral Presentation

DNA barcoding is a fast and reliable tool to assess and monitor biodiversity and has a role to play in investigating processes that are responsible for species interactions and thus the community structure of forests. Here, we use DNA barcoding to assess phylogenetic community structure of a 25 ha forest dynamics plot in a mixed dipterocarp forest in Brunei Darussalam which is part of the Center for Tropical Forest Science - Forest Global Earth Observatory (CTFS-ForestGEO) network of plots. Leaves and bark of shrubs and trees were sampled from 70 subplots (10x10 m) that varied in relative elevation, convexity and slope. We generated DNA sequence data for the two widely used plastid coding regions *rbcL* (3118 sequences) and *matK* (2598 sequences) for 599 morphotaxa identified in the 70 subplots. These represented 24 orders, 69 families and 189 genera with Dipterocarpaceae (16%) and Euphorbiaceae (9%) as the dominant families. Based on the barcode sequences, we reconstructed phylogenetic relationships among the taxa. To compare phylogenetic resolution and to test if it has an influence on phylogenetic diversity (PD) metrics, we generated a second phylogeny using the program Phylomatic which is widely used to infer phylogenetic community structure. It estimates the phylogenetic hypothesis for the taxa in the study plot on the basis of the Angiosperm Phylogeny Group III. The phylogenetic trees were largely congruent, but our community scale phylogeny obtained from barcode sequences showed higher resolution than the Phylomatic tree, which resolved only at family or generic level. Both phylogenetic trees will be used to calculate two widely used metrics of PD, Mean Phylogenetic Distance (MPD) and Mean Nearest Taxon Distance (MNTD). Further, Webb's Net Relatedness index (NRI) and Nearest Taxon Index (NTI) will be calculated. The metrics will be compared to a null model of community assembly to determine whether species are assembled into local communities at random or if they are more closely (phylogenetic clustering) or more distantly (overdispersion) related than expected by chance. Further, besides biotic interactions habitat filtering often plays a role in species interactions. We will investigate the influence of environmental factors on community structure.

10th International Flora Malesiana Symposium, Royal Botanic Garden, Edingburgh, UK, 11-15th July, 2016

DNA Barcoding of a mixed Dipterocarp forest in Brunei Darussalam and phylogenetic analysis of Dipterocarpaceae

Jacqueline Heckenhauer[1], Kamariah Abu Salim[2], Mark Chase[3,4], Rosabelle Samuel[1]

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Oral Presentation

A clear understanding of how forest communities are structured is important for conservation and restoration of ecosystems and thus their contribution to sustain human population. Here, we asses, using DNA barcoding, the biodiversity and phylogenetic community structure of a 25 ha mixed dipterocarps forest at Kuala Belalong (Brunei Darussalam), set up by the Centre of Tropical Forest Science (CTFS). Taking different ecological niches of the 25 Ha forest into consideration, individuals of woody plants (> 1 cm diameter at breast height) were sampled from 70 subplots (10x10 m) leading to a total of 3300 specimens for barcoding. Standard plant barcoding markers *rbcL* and *matK* revealed < 60 plant families in the 25 Ha forest with Dipterocarpaceae being the dominant one. Phylogenetic relationships in the South Asian subfamily Dipterocarpoideae remain poorly resolved, barcoding its species using small fragments of *matk* and *rbcL* leads to an unresolved backbone in the phylogenetic tree since closely related species have identical sequences. In order to address this issue, we conducted phylogenetic analysis using *rbcL*, complete *matk* and *trnT-trnL-trnF* chloroplast DNA sequences. Here we included most of the genera of the subfamily Dipterocarpoideae from different parts of Asia (Sri Lanka, Thailand, Brunei), as well as individuals of the other subfamilies Monotiodeae and Pakaraimeaoideae. Phylogenetic trees were calculated using Maximum Parsimony, Maximum Likelihood and Bayesian interference. The topologies of the trees are largely consistent with the morphological taxonomy as well as previous molecular studies and provide new insights into the large genus *Shorea* of Dipterocarpoideae.

Barcoding of a 25 ha tropical forest in Kuala Belalong (Brunei) and molecular phylogeny of the dominant family Dipterocarpaceae in the plot

Heckenhauer, J.[1], Jang, T[1], Turner, B.[4], Barfuss, M.H.J.[1],Temsch, E. [1], Chase, M.W.[2,3,] Samuel, R.[1]

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Oral presentation

DNA barcoding is a reliable tool to assess biodiversity and can be used to identify species even when morphological characters are not available. Here, we asses biodiversity and phylogenetic community structures of a 25 ha mixed dipterocarps forest at Kuala Belalong (Brunei Darussalam) which is set up by the Centre of Tropical Forest Science (CTFS). Considering different ecological niches, individuals of woody plants (> 1 cm diameter at breast height) were sampled from 70 subplots (10x10 m) giving a total of 3300 specimens for barcoding. Samples were barcoded with the standard plant barcoding markers *rbcL* and *matk*. As amplification of the *matK* barcoding region is often difficult when dealing with multiple angiosperm families, we developed a primer cocktail to amplify this region efficiently across angiosperm diversity. Our barcoding data revealed more than 60 plant families in the 25 ha forest with Dipterocarpaceae being the dominant one. Barcoding the Dipterocarpaceae species using small fragments of *matk* and *rbcL* leads to an unresolved backbone in the phylogenetic tree (especially in large genus *Shorea* (about 196 species)) because closely related species have identical sequences. To obtain a robust phylogeny and to test the monophyly of the genus *Shorea*, we used DNA sequences from three plastid regions (*rbcL*, *trnK-matK-trnK* and *trnT-trnL-trnF*) and included more than 160 species from different parts of Asia (Sri Lanka, Thailand, Brunei). Phylogenetic trees were calculated using maximum parsimony and Bayesian interference. The topologies of the trees provide new insights into the large genus *Shorea*, which is not monophyletic as suggested. Chromosome numbers and genome size play an important role in the phylogeny and evolution of Dipterocarpaceae. The basic chromosome number in Dipterocarpaceae is 2n = 2x = 11 for the tribe Shoreae and 2n=2x=7 for the tribe Dipterocarpeae. Chromosome numbers for five species are reported for the first time. Most of them are diploid (Tribe Dipterocarpeae: *Dipterocarpus zeylanicus*: 2n=22, *Vatica endertii*: 2n=22, Tribe Shoreae: *Shorea megistophylla*: 2n=14, *Shorea oblongifolia*: 2n=14,), but also a triploid species was detected (Tribe

Shoreae: *Hopea secunda*: 2n=21). Genome sizes were measured for 17 species and ranged from 1C DNA content of 0.3043 ± 0.0003 pg in *Shorea roxburghii* to 0.6724 ± 0.0079 pg in *Vatica diospyroides.* Interspecific variation in genome size was observed in *Hopea odorata* (1C = 0.4216 ± 0.002 pg, 0.6051 ± 0.0042 pg and 0.6094 ± 0.00361 pg) indicating presence of polyploidy.

Austrian Barcode of Life (ABOL) Meeting, Linz, Austria, 05-06th November, 2015

DNA barcoding und phylogenetische analyse der Pflanzengesellschaften eines 25 ha Dipterocarpaceaen-mischwaldes in Brunei Darussalam

Jacqueline Heckenhauer[1], Michael H. J. Barfuss[1],Kamariah Abu Salim[2], Ovidiu Paun[1], Mark W. Chase[3,4] ,Toby R. Pennington[5], Rosabelle Samuel[1]

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Poster presentation

Tropische Regenwälder sind eine der größten Kohlenstoff-Speicher und CO_2-Fixierer der Erde, jedoch sind sie in hohem Maße durch unterschiedliche menschliche Aktivitäten (landwirtschaftliche Tätigkeit, Abholzung, Weidelandgewinnung) bedroht. Ein klares Verständnis der Struktur und der genetischen Vielfalt des Waldes ist daher von großer Bedeutung. Mittels genetischer DNA Barcoding-Analysen erfolgt eine Biodiversitätserfassung der im Waldstück vorkommenden Arten. Diese Methode zur Artenbestimmung anhand der DNA-Sequenz eines Markergens bietet eine standartisierte und akkurate Bestimmung selbst kryptischer Arten (morphologisch nicht-unterscheidbare Arten) oder wenn morphologische Charakteristika (z.B. Blüten, Früchte) nicht vorhanden sind. Genetische Diversität ist Grundlage für die Anpassungsfähigkeit und den evolutionären Erfolg von Organismen. Zur Analyse der drei grundlegenden Formen von phylogenetischen Gesellschaftsstrukturen (zufällige Verteilung, Überstreuung (entfernter verwandt als erwartet) und Gruppierung (näher verwandt als erwartet)) werden Gesellschaftsindizes (Netz-Verwandtschafts-Index (NRI) und Nächstes-Taxon-Index (NTI)) in jedem Teilstück berechnet. Um den Einfluss von abiotischen Faktoren auf die Gesellschaftsstruktur der Teilstücke zu quantifizieren, soll ein Zusammenhang zwischen Gesellschaftsindizes und ökologischen Parametern ermittelt werden. Die Ergebnisse sollen Grundlagen für die Bewertung der Arten-, der phylogenetischen und funktionellen Diversität liefern, welche im Hinblick auf das Erstellen von Schutzgebieten sowie für Wiederaufforstung von gestörten Ökosystemen unabdingbar sind.

DNA Barcoding und phylogenetische Analyse der Pflanzengesellschaften eines 25 ha Dipterocarpaceaen-Mischwaldes in Brunei Darussalam

Jacqueline Heckenhauer[1], Michael Barfuss[1], Kamariah Abu Salim[2], Ovidiu Paun[1], Mark Chase[3,4], Toby Pennington[5], Rosabelle Samuel[1]
[1]Department for Botany and Biodiversity Research, University of Vienna, Rennweg 14, Vienna 1030, Austria [2]Universiti Brunei Darussalam, Department of Biology Jalan Tungku Link, Gadong, BE 1410, Negara Brunei Darussalam; [3]Jodrell Laboratory, Royal Botanic Gardens, Kew, Richmond, Surrey TW9 3DS, UK; [4]School of Plant Biology, The University of Western Australia, Crawley WA 6009, Australia; [5]Royal Botanic Garden Edinburgh, Edinburgh, EH3 5LR, Scotland, U.K.

Einleitung

Tropische Regenwälder sind eine der größten Kohlenstoff-Speicher und CO_2-Fixierer der Erde, jedoch sind sie in hohem Maße durch unterschiedliche menschliche Aktivitäten (landwirtschaftliche Tätigkeit, Abholzung, Weidelandgewinnung) bedroht. Ein klares Verständnis der Struktur und der genetischen Vielfalt des Waldes ist daher von großer Bedeutung.

Die Tiefland-Dipterocarpaceen- Mischwälder in Brunei Darussalam beheimaten mehr als 3500 Gefäßpflanzenarten und bilden somit eines der reichsten Ökosysteme der Erde. Seinen Namen bezieht dieser Waldtyp von der dort vorherrschenden, ökonomisch wichtigen Pflanzenfamilie Dipterocarpaceae (Flügelfruchtgewächse, Malvales). Das 25 ha große Waldstück ist Teil des CTFS-Netzwerkes[1] mit mehr als 60 Waldstücken in 24 Ländern.

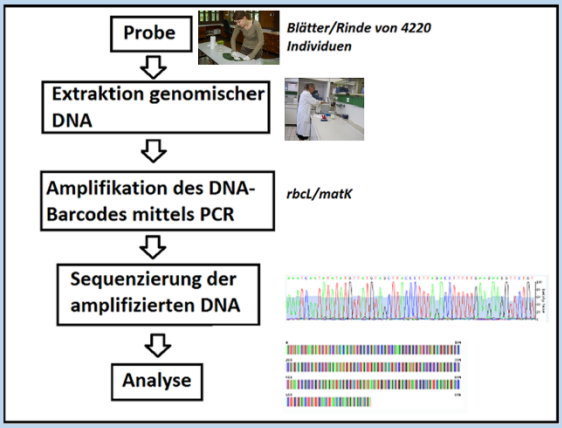
Mittels genetischer DNA Barcoding-Analysen erfolgt eine Biodiversitätserfassung der im Waldstück vorkommenden Arten. Diese taxonomische Methode zur Artenbestimmung anhand der DNA-Sequenz eines Markergens bietet eine standartisierte und akkurate Bestimmung selbst kryptischer Arten (morphologisch nicht-unterscheidbare Arten) oder wenn morphologische Charakteristika (z.B. Blüten, Früchte) nicht vorhanden sind.



Zentrale Forschungsfragen:

1. Biodiversitätserfassung: Welche Arten kommen gemeinsam vor?
   → Verlässliche und schnelle Charakterisierung der Artengemeinschaft in 76 Teilstücken (10x10 m) mittels DNA Barcoding
2. Was ist die phylogenetische Struktur in den Teilstücken des 25 ha großen *Dipterocarpus*-Waldstück in Kuala Belalong?
   → Phylogenetische Anaylse der Pflanzengesellschaften
3. Was sind mögliche Gründe für das gemeinsame Vorkommen von Pflanzen?
   → Untersuchung abiotischer/ biotischer Faktoren im Zusammenhang mit der Zusammensetzung der Pflanzengesellschaften

Methoden – DNA Barcoding Workflow



Erste Ergebnisse

Die rbcL Barcode-Sequenzen wurden mit der Referenzdatenbank verglichen und Familien zugeordnet. Insgesamt ließen sich 56 verschiedene Pflanzenfamilien ermitteln (siehe Abbildung1).

Da *matK* eine schnell evolvierende Region ist, wird mittels *matk* Barcode-Sequenzen eine genauere Bestimmung auf Artniveau erfolgen.

Abbildung 1: Pflanzenfamilien im 25 ha Forschungswaldstück in Brunei Darussalam

Die dominatesten Familien sind Dipterocarpaceae (19,8%), gefolgt von Euphorbiaceae (9,9%) und Rubiaceae (8,8%; siehe Abbildung 2).

Dies ist typisch für die tropischen Wälder Südostasiens und unterscheidet sich von den afrikanischen und neuweltlichen Tropen, die von Fabaceae dominiert werden.

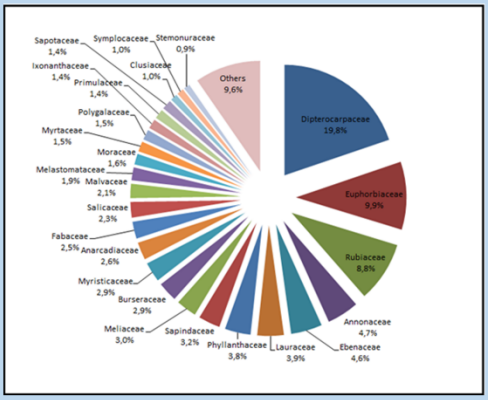


Abbildung 2: Häufigkeit der Pflanzenfamilien

Ausblick

Genetische Diversität ist Grundlage für die Anpassungsfähigkeit und den evolutionären Erfolg von Organismen.

Zur Analyse der drei grundlegenden Formen von phylogenetischen Gesellschaftsstrukturen (zufällige Verteilung, Überstreuung (entfernter verwandt als erwartet) und Gruppierung (näher verwandt als erwartet)) werden mit dem Programm Phylocom[3] Gesellschaftsindizes (Netz-Verwandtschafts-Index (NRI) und nächstes-Taxon-Index (NTI)) in jedem Teilstück berechnet.

Um den Einfluss von abiotischen Faktoren auf die Gesellschaftsstruktur der Teilstücke zu quantifizieren, soll ein Zusammenhang zwischen Gesellschaftsindizes und ökologischen Parametern ermittelt werden.

Die Ergebnisse sollen Grundlagen für die Bewertung der Arten-, der phylogenetischen und funktionellen Diversität liefern, welche im Hinblick auf das Erstellen von Schutzgebieten sowie für Wiederaufforstung von gestörten Ökosystemen unabdingbar sind.

[1]Center for Tropical Forest Science: http://www.ctfs.si.edu/plots/
[2]Barcode of Life: http://boldsystems.org/
[3]Webb *et al.*, 2008: Phylocom: software for the analysis of phylogenetic community structure and trait evolution.

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Projektleiterin: Prof. Samuel

DNA Barcoding and Community Structure Assessment of a mixed Dipterocarp forest in Brunei Darussalam

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Oral Presentation

Maintaining wide genetic diversity is essential for forest health, as it allows for adaption of species and conservation of ecosystem function. A clear understanding of how forest communities are structured is important for protection and restoration of ecosystems and their services that sustain human population. DNA Barcoding is a fast and reliable tool to asses and monitor biodiversity. As a DNA based method it can be used to identify species even when morphological characters (i.e. flowers) are not available, as well as to detect undescribed and cryptic species. Here, we asses biodiversity and phylogenetic community structures of a 25 ha mixed dipterocarps forest at Kuala Belalong (Brunei Darussalam) which is set up by the Centre of Tropical Forest Science (CTFS). Considering different ecological niches, all individuals of woody plants (> 1 cm diameter at breast height) were sampled from 38 subplots (10x10 m) leading to a total number of 2040 specimens. Samples were barcoded with the standard plant barcoding markers *rbcL* and *matK*. Initial investigations with the *rbcL* marker revealed 56 families which belong to 21 different orders. The available *rbcL* sequences were used to reconstruct phylogenetic relationships to get an insight into evolutionary processes responsible for species diversity. Phylogenetic analysis of the sequence data was consistent with the APG III phylogeny. To achieve higher resolution at species level *matK* barcode sequences will be included into the existing matrix of the *rbcL* sequences.

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DNA barcoding and community structure assessment in a tropical forest: A 25 ha mixed dipterocarp forest at Kuala Belalong Brunei Darussalam as a model

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Oral Presentation

Species diversity in tropical rain forests is maintained by various ecological and evolutionary processes. However, there is a lack of community phylogenetic analyses based on DNA barcoding for the Southeast Asian tropical rainforest. Here, we present first insights into the community structure of a 25 hectare forest dynamics plot at Kuala Belalong, which is one of the sites set up by the Center for Tropical Forest Science (CTFS) in collaboration with University of Brunei Darussalam for investigations. Considering different ecological niches, all individuals (> 1 cm dbh) of several subplots were sampled. For barcoding, plastid regions *rbcL* and *matK* were sequenced as recommended by the Consortium for the Barcode of Life (CBOL). These DNA barcode sequences are used to reconstruct evolutionary relationships, which are compared with existing phylogenetic patterns (APG III) to examine the presence or absence of phylogenetic clustering. FWF (Austrian Science Fund) project AP26548-B22 funded to Prof. Rosabelle Samuel.