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Out of Borneo: biogeography, phylogeny and divergence date estimates of *Artocarpus* (Moraceae)

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• **Background and Aims** The breadfruit genus (*Artocarpus*, Moraceae) includes valuable underutilized fruit tree crops with a centre of diversity in Southeast Asia. It belongs to the monophyletic tribe Artocarpeae, whose only other members include two small neotropical genera. This study aimed to reconstruct the phylogeny, estimate divergence dates and infer ancestral ranges of Artocarpeae, especially *Artocarpus*, to better understand spatial and temporal evolutionary relationships and dispersal patterns in a geologically complex region.
• **Methods** To investigate the phylogeny and biogeography of Artocarpeae, this study used Bayesian and maximum likelihood approaches to analyze DNA sequences from six plastid and two nuclear regions from 75% of *Artocarpus* species, both neotropical Artocarpeae genera, and members of all other Moraceae tribes. Six fossil-based calibrations within the Moraceae family were used to infer divergence times. Ancestral areas and estimated dispersal events were also inferred.
• **Key Results** Artocarpeae, *Artocarpus* and four monophyletic *Artocarpus* subgenera were well supported. A late Cretaceous origin of the Artocarpeae tribe in the Americas is inferred, followed by Eocene radiation of *Artocarpus* in Asia, with the greatest diversification occurring during the Miocene. Borneo is reconstructed as the ancestral range of *Artocarpus*, with dozens of independent *in situ* diversification events inferred there, as well as dispersal events to other regions of Southeast Asia. Dispersal pathways of *Artocarpus* and its ancestors are proposed.
• **Conclusions** Borneo was central in the diversification of the genus *Artocarpus* and probably served as the centre from which species dispersed and diversified in several directions. The greatest amount of diversification is inferred to have occurred during the Miocene, when sea levels fluctuated and land connections frequently existed between Borneo, mainland Asia, Sumatra and Java. Many species found in these areas have extant overlapping ranges, suggesting that sympatric speciation may have occurred. By contrast, *Artocarpus* diversity east of Borneo (where many of the islands have no historical connections to the landmasses of the Sunda and Sahul shelves) is unique and probably the product of over water long-distance dispersal events and subsequent diversification in allopatry. This work represents the most comprehensive *Artocarpus* phylogeny and biogeography study to date and supports Borneo as an evolutionary biodiversity hotspot.

Key words: Ancestral area reconstruction, Artocarpeae, *Artocarpus*, Borneo, dispersal, divergence date estimates, historical biogeography, Moraceae, phylogeny, Southeast Asia.

INTRODUCTION

Artocarpus (Moraceae – mulberry family) is an economically and ecologically important genus of approx. 70 tree species native to South and Southeast Asia and Oceania (Jarrett, (1959*a*; Kochummen, 2000; Berg *et al*., 2006; Zerega *et al*., 2010). All members of the genus have fleshy compound infructescences (syncarps), which develop from inflorescences with up to thousands of tiny flowers tightly packed and condensed on a receptacle. Several species, including breadfruit [*A. altilis* (Parkinson) Fosberg], jackfruit (*A. heterophyllus* Lam.) and cempedak [*A. integer* (Thunb.) Merr.], produce large, edible syncarps and are valuable crops (Fig. 1). Many *Artocarpus* species also serve as important food sources for forest animals, such as elephants and orangutans (Campbell-Smith *et al*., 2011; Sekar *et al*., 2015). Much of the native *Artocarpus* range is in a geologically complex region of the world and encompasses large, biodiverse forests that are under threat due to development and agriculture (Wilcove *et al*., 2013). Some *Artocarpus* species are classified as vulnerable on the IUCN red list, although most species have not been assessed. Understanding the biogeography and evolutionary history of the genus will be important for advancing further research and for informing conservation efforts including of crop wild relatives.

Artocarpus is part of the tribe Artocarpeae, which also includes two small neotropical genera, *Batocarpus* and *Clarisia* (three species each). The tribe has a disjunct distribution, with *Artocarpus* diversity centred in Southeast Asia, and *Batocarpus*

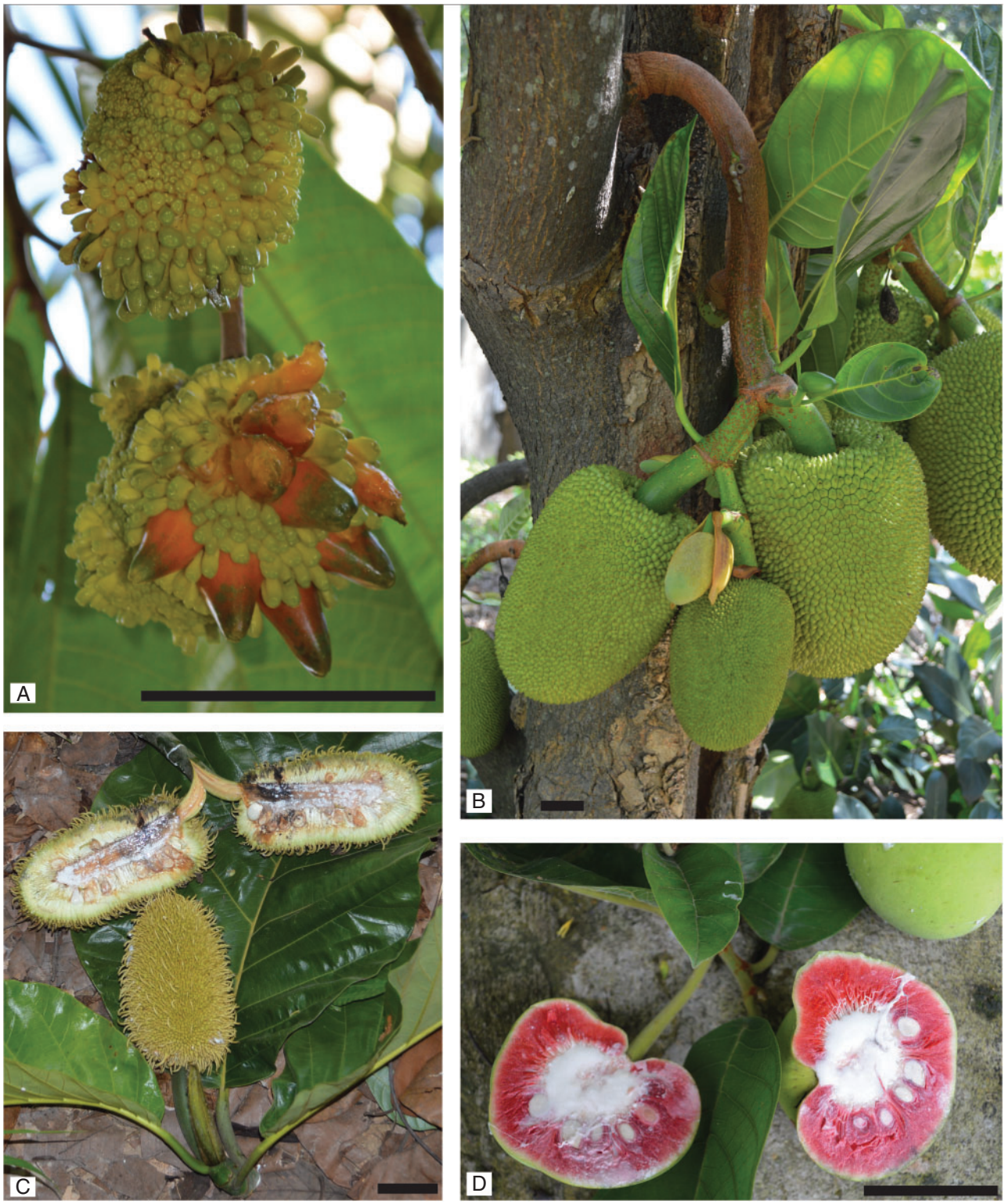


FIG. 1. Representative species from *Artocarpus* subgenera. (A) Subgenus *Prainea*: *A. limpato*. (B) Subgenus *Cauliflori*: *A. heterophyllus*. (C) Subgenus *Artocarpus*: *A. sericicarpus*. (D) Subgenus *Pseudojaca*: *A. dadah*. All scale bars are 5 cm.

TABLE 1. *Taxa included in this study*

Genus/subgenus (taxa included/total no. of taxa in group)	Taxon	Geographical region(s) assigned for biogeographical analyses	No. of individuals in 'Full' dataset	No. of individuals in 'Reduced' dataset	No. of individuals in Exemplar dataset
Artocarpus/Artocarpus (24/31)	*Artocarpus altilis*	ES	6	2	1
Artocarpus/Artocarpus (24/31)	*Artocarpus anisophyllus*	B, Sum, TM	7	6	1
Artocarpus/Artocarpus (24/31)	*Artocarpus blancoi*	P	1	1	1
Artocarpus/Artocarpus (24/31)	*Artocarpus brevipedunculatus*	B	2	2	1
Artocarpus/Artocarpus (24/31)	*Artocarpus camansi*	ES	2	2	1
Artocarpus/Artocarpus (24/31)	*Artocarpus chama*	TM, IB, SC	2	2	1
Artocarpus/Artocarpus (24/31)	*Artocarpus elasticus*	B, Sum, J, TM, IB	3	2	1
Artocarpus/Artocarpus (24/31)	*Artocarpus excelsus*	B	2	2	1
Artocarpus/Artocarpus (24/31)	*Artocarpus hirsutus*	WG	1	1	1
Artocarpus/Artocarpus (24/31)	*Artocarpus hispidus*	TM	1	1	1
Artocarpus/Artocarpus (24/31)	*Artocarpus kemando*	B, Sum, TM, IB	2	2	1
Artocarpus/Artocarpus (24/31)	*Artocarpus lanceifolius*	B, Sum, TM, IB	4	2	1
Artocarpus/Artocarpus (24/31)	*Artocarpus lowii*	Sum, TM	3	2	1
Artocarpus/Artocarpus (24/31)	*Artocarpus maingayi*	Sum, TM	2	2	1
Artocarpus/Artocarpus (24/31)	*Artocarpus mariannensis*	ES	1	1	1
Artocarpus/Artocarpus (24/31)	*Artocarpus obtusus*	B	1	1	1
Artocarpus/Artocarpus (24/31)	*Artocarpus odoratissimus*	B, P	3	2	1
Artocarpus/Artocarpus (24/31)	*Artocarpus rigidus*	B, Sum, J, TM, IB	3	3	1
Artocarpus/Artocarpus (24/31)	*Artocarpus scortechinii*	Sum, TM	2	2	1
Artocarpus/Artocarpus (24/31)	*Artocarpus sepicanus*	ES	1	1	1
Artocarpus/Artocarpus (24/31)	*Artocarpus sericicarpus*	B, P, ES, Sul	2	2	1
Artocarpus/Artocarpus (24/31)	*Artocarpus tamaran*	B	2	2	1
Artocarpus/Artocarpus (24/31)	*Artocarpus teijsmannii*	B, Sum, TM, ES, Sul	3	2	1
Artocarpus/Artocarpus (24/31)	*Artocarpus treculianus*	P	1	1	1
Artocarpus/Cauliflori (3/3)	*Artocarpus annulatus*	B	1	1	1
Artocarpus/Cauliflori (3/3)	*Artocarpus heterophyllus*	WG	6	2	1
Artocarpus/Cauliflori (3/3)	*Artocarpus integer*	B, Sum, TM, IB, ES, Sul	4	2	1
Artocarpus/Prainea (2/4)	*Artocarpus limpato*	B, Sum, TM	2	2	1
Artocarpus/Prainea (2/4)	*Artocarpus papuanus*	ES	1	1	1
Artocarpus/Pseudojaca (20/24)	*Artocarpus altissimus*	B, Sum, TM, IB	1	1	1
Artocarpus/Pseudojaca (20/24)	*Artocarpus dadah***	B, Sum, TM, IB	10	8	2
Artocarpus/Pseudojaca (20/24)	*Artocarpus fretessii***	B, ES, Sul	3	3	1
Artocarpus/Pseudojaca (20/24)	*Artocarpus fulvicortex*	Sum, TM	2	2	1
Artocarpus/Pseudojaca (20/24)	*Artocarpus glaucus*	B, Sum, J, TM	1	1	1
Artocarpus/Pseudojaca (20/24)	*Artocarpus gomezianus* subsp. *gomezianus*	Sum, J, TM, IB	3	2	1
Artocarpus/Pseudojaca (20/24)	*Artocarpus lacucha***	TM, IB, SC	7	6	2
Artocarpus/Pseudojaca (20/24)	*Artocarpus nitidus cf.* subsp. *humilis**	B	2	2	1
Artocarpus/Pseudojaca (20/24)	*Artocarpus nitidus* subsp. *borneensis**	B	3	2	1
Artocarpus/Pseudojaca (20/24)	*Artocarpus nitidus* subsp. *griffithii**	TM, IB, SC	3	2	1
Artocarpus/Pseudojaca (20/24)	*Artocarpus nitidus* subsp. *lingnanensis**	IB, SC	5	2	1
Artocarpus/Pseudojaca (20/24)	*Artocarpus ovatus***	P	1	1	1
Artocarpus/Pseudojaca (20/24)	*Artocarpus primackii*	B	4	2	1
Artocarpus/Pseudojaca (20/24)	*Artocarpus rubrovenius*	P	1	1	1
Artocarpus/Pseudojaca (20/24)	*Artocarpus styracifolius*	IB, SC	1	1	1
Artocarpus/Pseudojaca (20/24)	*Artocarpus thailandicus*	IB	3	3	1
Artocarpus/Pseudojaca (20/24)	*Artocarpus tomentosulus*	B	2	2	1
Artocarpus/Pseudojaca (20/24)	*Artocarpus tonkinensis*	IB, SC	2	2	1
Artocarpus/Pseudojaca (20/24)	*Artocarpus vrieseanus* var. *vrieseanus*	ES, Sul	1	1	1
Artocarpus/Pseudojaca (20/24)	*Artocarpus xanthocarpus**	P, SC	1	1	1
Batocarpus	*Batocarpus costaricensis*	NCA, SA	1	1	1
Batocarpus	*Batocarpus* sp.		1	1	
Clarisia	*Clarisia biflora*	NCA, SA	1	1	1
Outgroup (Moraceae)	*Antiaris toxicaria* subsp. *madagascariensis*	A	1	1	1
Outgroup (Moraceae)	*Antiaropsis decipiens*	ES	1	1	1
Outgroup (Moraceae)	*Bagassa guiannensis*	SA	1	1	1
Outgroup (Moraceae)	*Brosimum lactescens*	NCA, SA	1	1	1
Outgroup (Moraceae)	*Broussonetia cf. kurzii*	Sum, IB, SC	1	1	1
Outgroup (Moraceae)	*Broussonetia greveana*	A	1	1	1
Outgroup (Cannabaceae)	*Cannabis sativa*	E	1	1	1
Outgroup (Moraceae)	*Castilla elastica*	NCA, SA	1	1	1
Outgroup (Moraceae)	*Dorstenia choconiana*	NCA	1	1	1
Outgroup (Moraceae)	*Fatoua villosa*	E	1	1	1

(continued)

TABLE 1. *Continued*

Genus/subgenus (taxa included/total no. of taxa in group)	Taxon	Geographical region(s) assigned for biogeographical analyses	No. of individuals in 'Full' dataset	No. of individuals in 'Reduced' dataset	No. of individuals in Exemplar dataset
Outgroup (Moraceae)	*Ficus carica*	E	1	1	1
Outgroup (Moraceae)	*Ficus insipida*	NCA, SA	1	1	1
Outgroup (Moraceae)	*Ficus pachyclada*	A	1		1
Outgroup (Rosaceae)	*Fragaria vesca*	NCA			1
Outgroup (Moraceae)	*Hullettia dumosa*	Sum, TM	1	1	1
Outgroup (Moraceae)	*Hullettia griffithiana*	TM	1	1	1
Outgroup (Cannabaceae)	*Humulus lupulus*	E	1	1	1
Outgroup (Moraceae)	*Maclura africana*	A	1		1
Outgroup (Moraceae)	*Maclura amboinensis*	TM, IB, ES, SC			1
Outgroup (Moraceae)	*Maclura andamanica*	IB			1
Outgroup (Moraceae)	*Maclura brasiliensis*	NCA, SA			1
Outgroup (Moraceae)	*Maclura cochinchinensis Asia*	B, P, Sum, J, TM, IB, ES, Sul, SC, E			1
Outgroup (Moraceae)	*Maclura cochinchinensis Borneo*	B, P, Sum, J, TM, IB, ES, Sul, SC, E			1
Outgroup (Moraceae)	*Maclura fruticosa*	TM, IB, SC			1
Outgroup (Moraceae)	*Maclura pomifera*	NCA	1	1	1
Outgroup (Moraceae)	*Maclura spinosa*	WG			1
Outgroup (Moraceae)	*Maclura thorelii*	IB			1
Outgroup (Moraceae)	*Maclura tinctoria* subsp. *mora*	SA			1
Outgroup (Moraceae)	*Maclura tinctoria* subsp. *tinctoria*	NCA, SA	1	1	1
Outgroup (Moraceae)	*Maclura tricuspidata*	SC	1	1	1
Outgroup (Moraceae)	*Melicia excelsa*	A			1
Outgroup (Moraceae)	*Morus alba*	E	1	1	1
Outgroup (Moraceae)	*Morus notabilis*	SC			1
Outgroup (Moraceae)	*Parartocarpus bracteatus*	B, Sum, P	1	1	1
Outgroup (Moraceae)	*Parartocarpus venenosus*	B	1	1	1
Outgroup (Urticaceae)	*Pilea microphylla*	NCA, SA	1	1	1
Outgroup (Moraceae)	*Sorocea briquetii*	SA	1	1	1
Outgroup (Moraceae)	*Sorocea steinbachii*		1	1	
Outgroup (Moraceae)	*Sparattosyce dioica*	ES	1	1	1
Outgroup (Moraceae)	*Treculia africana*	A	1	1	1
Outgroup (Moraceae)	*Treculia obovoidea*		1	1	

Taxa are organized alphabetically, by genus and species. For ingroup Artocarpeae taxa, the genus, and within *Artocarpus* the subgenus, are indicated (number of species sampled in taxon group/total number of species in specified taxon group based on Zerega *et al*., 2010). Outgroup taxa are also listed and are shown in the phylogeny of the full dataset in Fig. S1. Range distributions used in biogeography analyses are shown for each taxon as follows: B = Borneo and Palawan, Sum = Sumatra, TM = Thai-Malay peninsula, IB = IndoBurma, P = Philippines, J = Java and the lesser Sunda Islands, WG = Western Ghats, Sul = Sulawesi, ES = East of Sulawesi, SC = southern China, A = Africa, NCA = North and Central America, SA = South America, E = Eurasia. The number of individuals included in the full, reduced and exemplar datasets for each taxon is indicated.

**A. nitidus sensu* Berg *et al*. (2006) and Jarrett (1960); ***A. lacucha sensu* Berg *et al*. (2006).

and *Clarisia* restricted to Central and South America (Zerega *et al*., 2010; Berg, 2001). It is unclear when, where and how the tribe diversified and dispersed into its current disjunct range. In a family-level study, Zerega *et al*. (2005) estimated the crown age of the Artocarpeae tribe at 65·1 (52·2–80·6) Mya. Based on locations of fossils, paleoclimate, and geological evidence, they proposed that a Eurasian origin of Moreaceae, followed by migration across the boreotropical North Atlantic Land Bridge during the Eocene, was at least as likely as a previously proposed Gondwana origin for the family. However, only five species of *Artocarpus* were included in the study and no ancestral range reconstructions were conducted, leaving the ancestral range and the influence of the complex biogeography of Southeast Asia on the spatial and temporal evolution of *Artocarpus* unexplored.

The geological (Hall, 2002, 2009; http://searg.rhul.ac.uk/) and floristic history (Morley, 2000, 2012; de Bruyn *et al*., 2014) of Southeast Asia have been described and summarized by several authors. Geologically, the region includes Sundaland (Sunda shelf including southern Indochina, peninsular Thailand and Malaysia, Sumatra, and parts of Borneo and Java), the Sahul shelf (including Australia and the island of New Guinea), the eastern Pacific Ocean and Philippine Sea plates, and Wallacea (the area of collision between the Sahul and Sunda shelves, including numerous islands such as Sulawesi, the Moluccas and the Lesser Sunda Islands). The Wallacean Islands have various origins in the West Pacific and Australia and have never been connected with Sundaland nor with the Sahul shelf. The landmasses of the Sahul shelf have never been connected to Sundaland nor to the Eurasian continent. In contrast, Sundaland has been part of the Eurasian continent since the Mesozoic and the islands of Sundaland are largely of continental origin. They formed a contiguous landmass with Eurasia during times of low sea levels, and during the middle Eocene the Sunda Shelf is thought to have experienced its greatest land area (de Bruyn *et al*., 2014). From the Oligocene

into the early Quaternary, sea levels fluctuated frequently, with landmasses of Sundaland variously submerged and emergent. Throughout this time, central and north-western Borneo remained emergent and connected to the mainland (Hall, 2009; de Bruyn *et al*., 2014). It is only in relatively recent times that the continuous landscape disappeared and was replaced by island chains (Bendiksby *et al*., 2010), and the present-day geography is atypical of what it has looked like during most of the past tens of thousands of years.

The geological history of Southeast Asia has been described as being the result of more than 300 million years of 'Colliding Worlds' (van Oosterzee, 1997) due to its position at the interface of the Sunda and Sahul plates. Dramatic sea-level fluctuations throughout the past hundreds of millions of years have led to large variations in the amount of exposed land area and terrestrial connections among the islands and has had an impact on the evolution of the biota in the region (Hall, 2009, 2012; de Bruyn *et al*., 2014). Studying taxa that are centred in this region can contribute to a more complete understanding of how, when and where taxa diversified and dispersed, and may reveal common patterns. Within Southeast Asia, certain regions may have higher rates of endemism and diversification. For example, de Bruyn *et al*. (2014) recently identified Borneo and Indochina as 'evolutionary hotspots' in a phylogenetic meta-analysis of both flora and fauna, and several studies cite Borneo as the centre of diversification for multiple taxa (Nauheimer *et al*., 2012; Webb and Ree, 2012).

The most recent phylogeny of *Artocarpus* (Zerega *et al*., 2010) supports its monophyly and recognizes four subgenera: *Artocarpus*, *Pseudojaca*, *Cauliflori* and *Prainea* (Table 1; Zerega *et al*., 2010), but not all subgeneric sections and series (*sensu* Jarrett, 1959*c*, 1960) were included in that analysis. The subgenera are not geographically restricted, and taxa from all subgenera are found throughout the Sunda and Sahul shelves as well as in Wallacea and the Philippines. A well-resolved phylogeny will help understand the complicated biogeography of *Artocarpus* as well as help address species delineation for several species with broad geographical ranges. For example, the range of *Artocarpus nitidus* Trécul (*sensu* Jarrett, 1960) includes the Philippines, Borneo, Sumatra, mainland Southeast Asia and China, but it has been variously sunken or segregated into separate species, subspecies or varieties based on size and indumentum of the syncarp, and slight differences in the shape and venation of the leaves, as well as variations in geographical range. In a recent taxonomic treatment, Berg *et al*. (2006) treated *A. nitidus* as having several 'informal entities' that aligned to some degree with formerly treated subspecies, while previous authors treated them as five separate species as detailed in Jarrett (1960). Another example is *A. lacucha* Buch.-Ham. Jarrett (1960) recognized a well-defined *A. lacucha* restricted in its range from India into Indo-Burma and southern China, while Berg *et al*. (2006) sunk several morphologically diverse species into *A. lacucha*, extending its range into the Philippines, the Indo-Pacific Islands and New Guinea. Berg justified this approach based on shared features that indicate intermittent growth in combination with deciduousness; however, he also recognized informal 'forms' within *A. lacucha* based on variations in leaf shape and inflorescence morphology.

The aims of the present study were to employ data from eight loci (two nuclear and six chloroplast) and extensive taxon sampling to reconstruct the evolutionary history of Artocarpeae, especially *Artocarpus*, in order to test the monophyly of and relationships among *Artocarpus* subgenera, and to help inform the species boundaries of difficult to delineate *Artocarpus* species. In addition, we estimated divergence dates and inferred ancestral ranges within Artocarpeae to understand dispersal patterns in a highly complex biogeographical region. Specifically, we aimed to identify the ancestral range of tribe Artocarpeae and test if Borneo is an evolutionary hotspot for the genus. Investigating the evolutionary and biogeographical history of *Artocarpus* species is also of interest due to the economic importance of the genus. Understanding its origins, diversification and crop wild relatives will be important for conservation efforts.

MATERIALS AND METHODS

Taxon sampling

Outgroup sampling included taxa in the Cannabaceae, Urticaceae and Rosaceae as well as 26 taxa from 15 genera in Moraceae, encompassing all Moraceae tribes recognized by Clement and Weiblen (2009). Ingroup sampling represented all Artocarpeae genera, all four *Artocarpus* subgenera (*Artocarpus*, *Prainea*, *Cauliflori*, *Pseudojaca*; Zerega *et al*., 2010), as well as all of Jarrett's (1959*a*, *b*, *c*, 1960) named sections and series (Table 1). In most cases, there were at least two exemplars for each taxon. For taxa with difficult to delineate species boundaries (*A. nitidus* and *A. lacucha*), we included multiple exemplars of the taxa that have been placed in these species (14 and 19, respectively, Table 1). We included a total of two *Batocarpus*, one *Clarisia* and 52 *Artocarpus* taxa (Table 1; Table S1). We used three different datasets for phylogenetic inference, dating and dispersal approximations: the full dataset used all accessions, the reduced dataset used up to two accessions per taxon, and the exemplar dataset used a single accession per taxon (Table 1, see Results for an explanation of the criteria for each dataset).

DNA extraction and sequencing

While some DNA sequences used in this study came from Zerega *et al*. (2010), most of the samples were generated for the present study as follows. We extracted DNA using a CTAB method (Zerega *et al*., 2002) or Qiagen DNeasy Mini Plant Kit (cat. no. 69104, Qiagen, Valencia, CA, USA). For recalcitrant herbarium samples we modified the Qiagen protocol and added 35 μL of proteinase K and 75 μL β-mercaptoethanol to each sample with the lysis buffer and incubated at 45 °C overnight. An additional 20 μL of proteinase K was added before incubation for 12 h at 45°C. After the addition of buffer AP2 (Qiagen), samples were incubated in a −20 °C freezer overnight, and then the recommended kit protocol was followed. CTAB DNA extractions from herbarium samples were cleaned using a QIAquick PCR Purification Kit (cat. no. 28104, Qiagen). We quantified DNA using a NanoDrop 2000 device

(Thermo Scientific, Waltham, MA, USA) and visualized DNA by running samples out on a 1 % agarose gel.

We used the same PCR recipe for each region [5 μL of 2× MyTaq Mix (cat. no. BIO-25041, Bioline, London, UK), 3 μL of water, 0·5 μL of each 10 mM primer and 1 μL of DNA template] with the exception of *G3pdh* which included the addition of 0·4 % bovine serum albumin to each PCR. We developed internal primers for *rbcL* and *matK* (Supplementary Data Table S2). PCR conditions were as follows: *ITS*: 94 °C/5 min, then 30 cycles of [94 °C/30 s, 53 °C/30 s, 72 °C/2 min], then a final extension of 72 °C/10 min; *G3pdh*: 94 °C/3·5 min, then 36 cycles of [95 °C/1 min, 55 °C/1 min and 72 °C/min], then a final extension at 72 °C/7 min; *matK*: 94 °C/5 min, then 35 cycles of [94 °C/30 s, 52 °C/20 s and 72 °C/50 s], then a final extension at 72 °C/5 min; *rbcL*: 95 °C/4 min, then five cycles of [94 °C/30 s, 55 °C/1 min and 72 °C/1 min], then 30 cycles of [94 °C/30 s and 54 °C/1 min]; *trnL-trnF*: 94 °C/3 min, then 32 cycles of [94 °C/45 s, 52 °C/30 s and 72 °C/90 s], then a final extension of 74 °C/7 min; *trnH-psbA*: 80 °C/5 min, then 35 cycles of [94 °C/30 s, 58–48 °C (touchdown)/30 s, 72 °C/1 min], then a final extension at 72 °C/10 min; *trnS-G*: 80 °C/5 min, then 30 cycles of [95 °C/1 min, 66 °C/1 min], then a final extension at 66 °C/10 min; *trnV-ndhC*: 80 °C/5 min, then 35 cycles of [94 °C/30 s, 55 °C/30 s, 72 °C/2 min], then a final extension at 65 °C/5 min. We used gel electrophoresis to confirm that PCR was successful. We cleaned PCR products using the QIAquick PCR Purification Kit or an ethanol cleaning using a centrifuge spin-down in 100 % ethanol for 30 min at 4 °C, followed by a wash in 70 % ethanol for 15 min at 4 °C.

To cycle sequence PCR products we used: 3 μL of water, 1 μL of ABI Big Dye (Applied Biosystems, Foster City, CA, USA), 3 μL of 100× Big Dye buffer, 1 μL of either the forward or reverse 10 mM primer and 2 μL of PCR product. Conditions for cycle sequencing were 95 °C/1 min, then 32 cycles of [96 °C/10 s, 50 °C/5 s and 60 °C/30 s]. We cleaned the product using the ethanol protocol described above with a 7 % addition of 125 mM EDTA to the preliminary 100 % ethanol. We added 10 μL of HiDi formamide before running plates on an Applied Biosystems 3730 sequencer.

We trimmed traces and edited contigs manually using CodonCode v.5.1. We checked each sequence against the NCBI Nucleotide database using BLAST (Altschul *et al*., 1990, 1997) to identify contamination. Sequences were aligned using MAFFT (Katoh and Standley, 2013) and we manually checked alignments in Mesquite (Maddison and Maddison, 2011).

Phylogenetic analyses

To determine the best model of evolution, we analysed each region in jmodeltest2 (Guindon and Gascuel, 2003; Darriba *et al*., 2012) with five substitution schemes, +F, +I and +G, ML optimized, and NNI tree search. We chose the best model based on likelihood scores (Table S2). We used Bayesian inference (BI) as implemented in MrBayes (Ronquist *et al*., 2012) (5000000 generations, 25 % burn-in, temperature set to allow >50 swapping frequency among chains, 4 chains, 2 runs) and maximum-likelihood (ML) in RAxML using default settings and data partitioned by locus. The full dataset was analysed using RAxML, while the reduced and exemplar datasets were analysed in RAxML and MrBayes. All analyses were performed on the CIPRES computing core (Miller *et al*., 2010). To investigate discordance between the two nuclear regions and the chloroplast, three ML trees were estimated in RAxML using *ITS*, *G3pdh* and the combined chloroplast regions, respectively. We then used these trees as input for ASTRAL, which estimates species trees by decomposing input trees into quartets and calculates the proportion of these quartets represented in the final species tree (Mirarab *et al*., 2014).

Dating

We used Beast v.1.8.1 (Heled and Drummond, 2010) to date species divergence using the exemplar (single accession per taxon) dataset, which also included outgroup sampling. We formatted datasets in Beauti v.1.8.1 with separate rate models for each locus, an uncorrelated relaxed clock (priors: ucld.stdev with exponential distribution, mean = 0·33; ucld.mean with gamma distribution shape and scale = 1), Yule process speciation (prior yule.birthRate with gamma distribution shape = 0·001, scale = 1000), and six lognormal fossil-based calibrations (see below) within Moraceae using the option 'real space'. The mean was set to 20 and the log (Stdev) was set to 0·75 for all fossil priors. The root of the tree was *Fragraria vesca* (Rosaceae, Rosales) and was constrained to 110 Mya with a uniform prior based on estimates of the Rosales (Wang *et al*., 2009), the order to which Moraceae belongs.

Many of the fossils attributed to the family Moraceae are leaf impressions that are often poorly preserved and lack truly diagnostic characters, leaving limited numbers of definitive fossils that could be used for calibrations. Collinson (1989) reviewed fossils of Moraceae and related families and confirmed the identification of several fossils from reproductive and wood structures. The oldest fossil fruits with diagnostic features of modern *Ficus* are known from early Eocene formations in southern England (Chandler, 1962, 1963*a*, *b*). *Broussonetia* fossil fruits with diagnostic characters are recorded from the upper Eocene of southern England (Chandler, 1961; Collinson, 1989). *Morus* fruits have been recorded from the early Eocene or later in southern England (Chandler, 1963*a*), the USSR (Takhtajan, 1982) and Germany (Gregor, 1978). Fossil fruit from *Chlorophora bicarinata* [resembling the extant *Milicia excelsa* (Welw.) C.C.Berg] have been recorded from the middle Eocene of southern England (Chandler, 1961). The earliest reliable fossil with affinities to modern *Artocarpus* comes from fossil wood described as *Artocarpoxylon deccanensis* from the Deccan Intertrappean beds of the Mandla district, Madhya Pradesh, India. This formation has been dated to 54·4 ± 8·1 Mya (Srivastava *et al*., 1986). Fossil wood described as *Cudrianoxylon engolismense* from the Eocene of France is assigned to *Maclura* section *Cudrania*, and the formation from which it was reported is broadly dated to the Eocene (Dupéron-Laudoueneix, 1980). We used the above fossils for calibrations, and if they were assigned to a broad time range (i.e. Eocene), fossil offsets were selected at the youngest age within the range. We used this broad approach for *Broussonetia* (youngest date within the upper Eocene), *Ficus* (youngest date within the lower Eocene), *Morus* (youngest date in the lower Eocene), *Milicia* (youngest date within the mid-Eocene) and *Maclura*

(Eocene). Based on the above information and using this approach, we placed the following fossil offsets at the stem node for the following clades: *Artocarpus* 54 Mya, *Broussonetia* 34 Mya, *Ficus* 48 Mya, *Morus* 48 Mya, *Maclura* section *Cudrania* 34 Mya and *Milicia* 38 Mya.

Biogeography

We used *Artocarpus* species distribution information from Jarrett (1959*a*, *b*, *c*, 1960, 1975), Kochummen (2000), Soepadmo and Saw (2000), Berg (2001, 2005), Zhekun and Gilbert (2003) and Berg *et al.* (2006, 2011) to assign *Artocarpus* taxa to the following areas, modified from Turner *et al.* (2001): Southern China, Western Ghats of India, Indo-Burma *sensu* Myers *et al.* (2000), Thai-Malay Peninsula, Borneo, Sumatra, Java, Philippines, Sulawesi and east of Sulawesi (Table 1). For other Artocarpeae and outgroup taxa we included Africa, Eurasia, North/Central America, and South America. For taxa found in more than one area, multiple assignments were allowed, with six areas being the highest for a single taxon. To reconstruct ancestral ranges and estimate dispersal events we used S-DIVA (Yu *et al.*, 2010) and Lagrange (Ree and Smith, 2008) as implemented in RASP v.3.02 (Yu *et al.*, 2015) using the single-accession exemplar dataset. In S-DIVA, we used 201 Beast output trees to test two to four areas per node with and without extinction, and no constraints on dispersal. In Lagrange we tested two to three areas per node, with and without dispersal constraints. Dispersal constraints followed those in Webb and Ree (2012), which we coded with the probability of 0·5 to allow dispersals between all areas. To visualize dispersal, the number and direction of dispersal events were calculated based on the most likely (>50 %) ancestral range reconstructions at each node. In cases where the most likely range reconstruction consisted of two areas, all permutations of dispersal were assigned with a value that was proportional such that each node was only counted once. For example, if a dispersal event was reconstructed from a node with one area (X) to a node with two areas (YZ), then X to Y was assigned a value of 0·5, and X to Z was assigned a value of 0·5.

RESULTS

The three datasets (exemplar, reduced, full) each had approx. 18 % missing data and 40·2–44·7 % variable and 20·9–25·1 % parsimony-informative characters. The regions *G3pdh* (27–36·3 % missing samples) and *trnH-psbA* (15–18·6 % missing samples) had the most missing taxa for each dataset. Only three samples did not have any nuclear regions: *A. styracifolius* Pierre and two accessions of *A. kemando* Miq. All taxa had at least one chloroplast region.

Phylogenetic analyses

After analysis of the full dataset (Fig. S1) we reduced the number of individuals per species for the reduced and exemplar datasets based on well-supported (ML bootstrap >95 %) species clades. Within these clades, we randomly chose accession(s) that had sequences from all regions to form the reduced and exemplar datasets. In the reduced dataset, most species (or subspecies) with multiple samples were reduced to two samples, except if the species was paraphyletic in the full dataset. In the exemplar dataset, most species were reduced to a single individual for use in the dating analysis because the Yule prior used assumes each sequence represents a distinct species. Separate ML analyses of each nuclear region (*ITS* and *G3pdh*) were compared with the chloroplast phylogeny. No conflicts with bootstrap values above 70 % were found between the nuclear and chloroplast datasets. Similarly, the proportion of input quartet trees satisfied by the final ASTRAL species tree was 0·92. All regions were subsequently combined and analysed using ML and BI (Fig. 2).

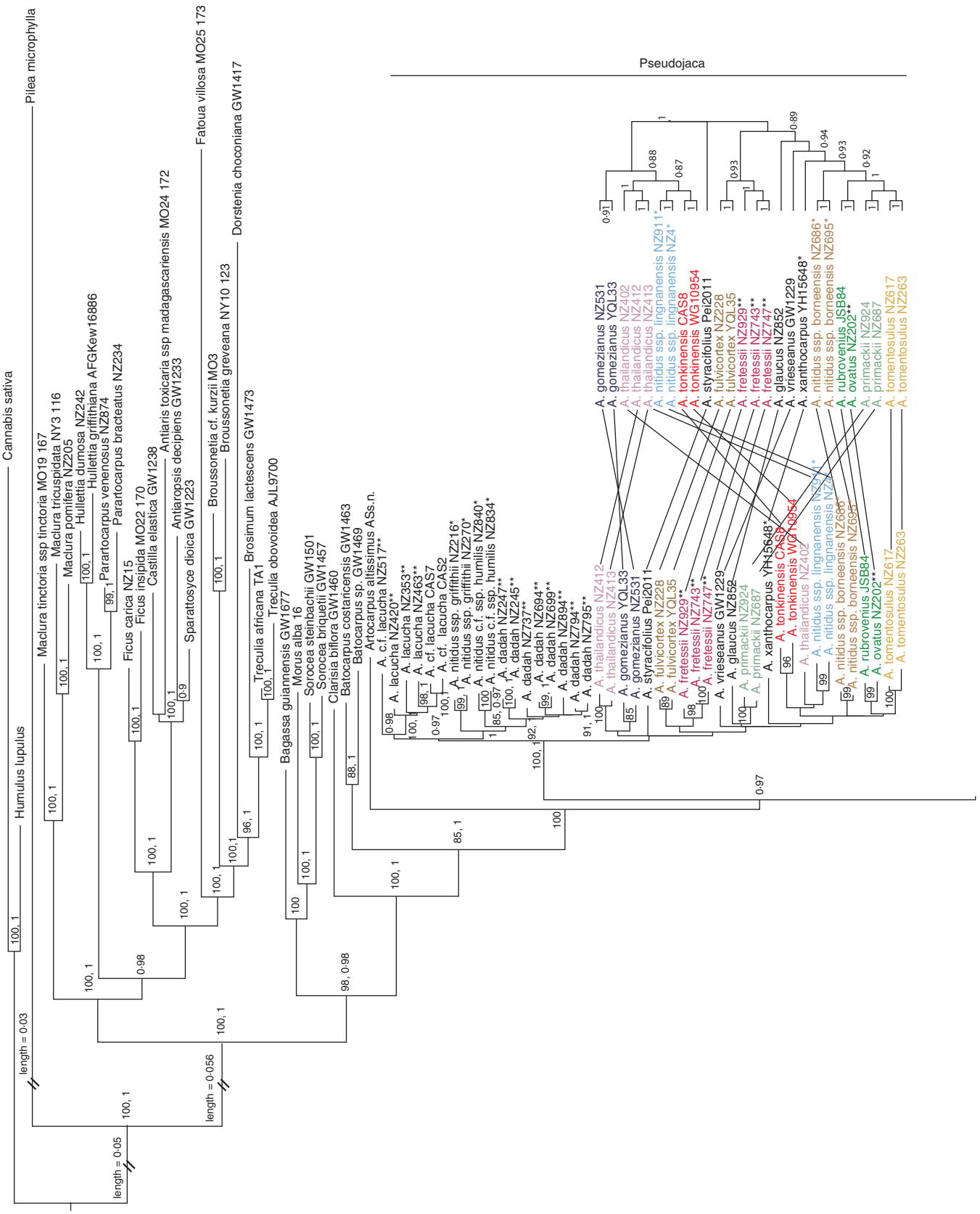
The genus *Artocarpus* as well as three (subgen. *Artocarpus*, *Cauliflori* and *Prainea*) of the four subgenera proposed by Zerega *et al.* (2010) had strong support (Fig. 2) as monophyletic. The fourth subgenus (*Pseudojaca*) was monophyletic if the anomalous *A. altissimus* (Miq.) J. J. Smith, which is sister to the entire genus, was excluded from it.

Within subgenus *Artocarpus*, Jarrett (1959) recognized two sections (section *Artocarpus* with four series, and section *Duricarpus* with two series, Table 1). Neither section was supported as monophyletic, nor were any of the series within them. The placement of a few species differed in the full vs. reduced dataset. In the analysis of the reduced dataset, exemplars of *A. anisophyllus* Miq. formed a clade, but in analysis of the full dataset, *A. brevipedunculatus* (F. M. Jarrett) C. C. Berg was nested within it. Also, exemplars of *A. lanceifolius* Roxb. formed a clade in the full dataset, but they formed a basal grade to *A. anisophyllus* and *A. brevipedunculatus* in the reduced dataset.

Within subgenus *Pseudojaca*, Jarrett (1960) delineated two sections: the monotypic section *Glandulifolium* (*A. altissimus*) and section *Pseudojaca*, which in the present analysis is monophyletic. While there was no strong support for many of the relationships among taxa in section *Pseudojaca*, there was strong support at the tips for nearly all of the species (excluding the difficult to delineate *A. lacucha* and *A. nitidus*) represented by more than one accession. With three accessions, the only species lacking support in the ML analysis was *A. thailandicus* C. C. Berg, although it was well supported in the BI analysis. Within section *Pseudojaca*, Jarrett (1960) recognized two series: *Clavati* (defined by the presence of interfloral bracts with clavate heads) and *Peltati* (defined by the presence of interfloral bracts with peltate heads). Series *Clavati* comprises three species, but only *A. styracifolius* was included in the present study, and it was nested within series *Peltati*. Within series *Peltati*, both *A. lacucha* (*sensu* Berg *et al.*, 2006) and *A. nitidus* (*sensu* Berg *et al.*, 2006; *sensu* Jarrett, 1960) were polyphyletic.

Dating

The tree topology resulting from the BEAST analysis of the single accession per taxon exemplar dataset was the same as the ML and BI topologies with one exception (Fig. 3). In the BEAST analysis *A. altissimus* was sister to subgenus *Prainea* with very low support [(posterior probability (PP) = 0·4), whereas in the ML and BI analyses *A. altissimus* was sister to all other *Artocarpus* taxa. Using the exemplar dataset in


Cannabis sativa
Humulus lupulus
Pilea microphylla
Maclura tinctoria ssp tinctoria MO19 167
Maclura tricuspidata NY3 116
Maclura pomifera NZ205
Hullettia dumosa NZ242
Hullettia griffithiana AFGKew16886
Parartocarpus venenosus NZ874
Parartocarpus bracteatus NZ234
Ficus carica NZ15
Ficus insipida MO22 170
Castilla elastica GW1238
Antiaris toxicaria ssp madagascariensis MO24 172
Antiaropsis decipiens GW1233
Sparattosyce dioica GW1223
Fatoua villosa MO25 173
Broussonetia cf. kurzii MO3
Broussonetia greveana NY10 123
Dorstenia choconiana GW1417
Brosimum lactescens GW1473
Treculia africana TA1
Treculia obovoidea AJL9700
Bagassa guianensis GW1677
Morus alba 16
Sorocea steinbachii GW1501
Sorocea briquetii GW1457
Clarisia biflora GW1460
Batocarpus costaricensis GW1463
Batocarpus sp. GW1469
Artocarpus altissimus ASs.n.
A. c.f. lacucha NZ517**
A. lacucha NZ420**
A. lacucha NZ353**
A. lacucha NZ463**
A. cf. lacucha CAS7
A. cf. lacucha CAS2
A. nitidus ssp. griffithii NZ216*
A. nitidus ssp. griffithii NZ270*
A. nitidus c.f. ssp. humilis NZ840*
A. nitidus c.f. ssp. humilis NZ834*
A. dadah NZ247**
A. dadah NZ245**
A. dadah NZ737**
A. dadah NZ694**
A. dadah NZ699**
A. dadah NZ894**
A. dadah NZ794**
A. dadah NZ795**
A. thailandicus NZ412
A. thailandicus NZ413
A. gomezianus YQL33
A. gomezianus NZ531
A. styracifolius Pei2011
A. fulvicortex NZ228
A. fulvicortex YQL35
A. fretessii NZ929**
A. fretessii NZ743**
A. fretessii NZ747**
A. vrieseanus GW1229
A. glaucus NZ852
A. primackii NZ924
A. primackii NZ687
A. xanthocarpus YH15648*
A. tonkinensis CAS8
A. tonkinensis WG10954
A. thailandicus NZ402
A. nitidus ssp. lingnanensis NZ911*
A. nitidus ssp. lingnanensis NZ4*
A. rubrovenius JSB84
A. nitidus ssp. borneensis NZ686*
A. nitidus ssp. borneensis NZ695*
A. ovatus NZ202**
A. tomentosulus NZ617
A. tomentosulus NZ263
Pseudojaca
length = 0·03
length = 0·056
length = 0·05

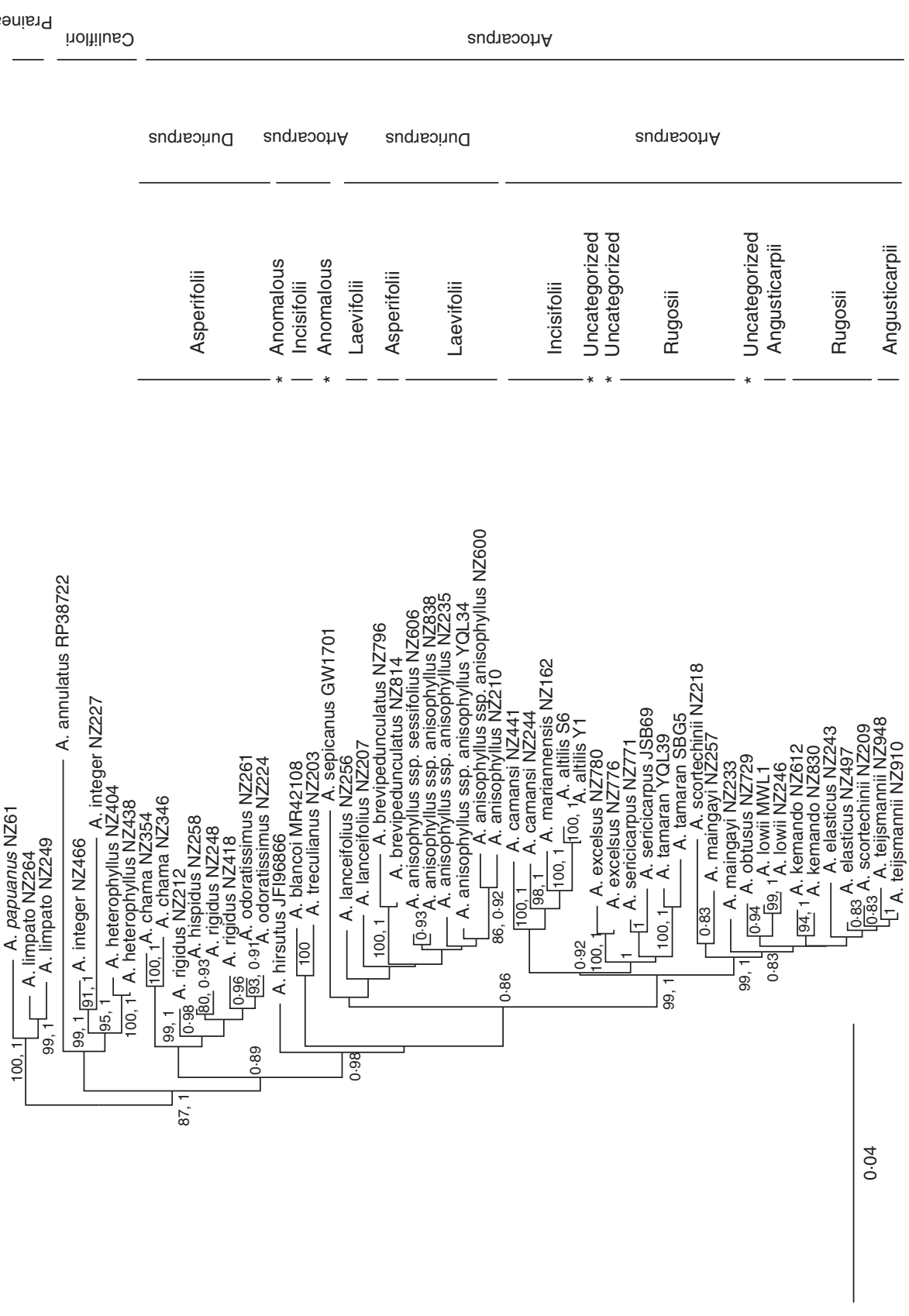


FIG. 2. Maximum likelihood tree of the genus *Artocarpus* based on eight loci (six plastid, two nuclear). Numbers after taxon names refer to specific collections (see Table S1 for details). Whole integer bootstrap values (80–100) are from ML analysis, while BI PP ranges from 0·8 to 1. Highly supported portions (>80, 0·8) of the topology agreed in both ML and BI analyses except in one part, where the corresponding BI tree clade is shown for comparison. Names and vertical lines to the right of the tree represent from left to right: series, sections, and subgenera (sensu Jarrett). **A. nititdus* sense Berg *et al.* (2006) and Jarrett (1960). ***A. lacucha* sense Berg *et al.* (2006).

BEAST, the crown of Artocarpeae was estimated to be 69·61 Mya (61·39–78·47 Mya) and the crown of *Artocarpus* estimated at 40·07 Mya (29·8–50·81 Mya) (Fig. 3). The crown of *Prainea* + *A. altissimus* was estimated at 32·47 Mya (21·6–44·09 Mya). The crown of subgenus *Cauliflori* was estimated at 22·83 Mya (13·43–31·85 Mya). The crown of subgenus *Artocarpus* was estimated at 29·61 Mya (22·33–37·49 Mya) (Fig. 3). Subgenus *Pseudojaca* had the youngest crown estimate at 18·31 Mya (12·89–24·45 Mya). The ages of the outgroups will be further discussed in a forthcoming article.

Biogeography

Analyses using Lagrange and SDIVA produced similar results (Supplementary Data Table S3, Fig. S2), with strong support for Borneo as the ancestral range for *Artocarpus* (Fig. 3). The results presented here are based on allowing two ancestral regions per node and no dispersal constraints. The root of the tribe Artocarpeae is reconstructed as North/Central America and South America. Range reconstruction of *Artocarpus* inferred the most cases of *in situ* diversification in Borneo (42 events) followed by the Thai Malay peninsula (eight events), the Philippines (six events) and East of Sulawesi (five events) (Fig. 4, Supplementary Data Table S4). Borneo was inferred as the source of the most dispersal events (32 events), followed by the Thai-Malay Peninsula (12 events) (Fig. 4, Table S4). Dispersal events from Borneo include movement eastward across Wallace's line (i.e. *A. fretessii*), westward into the Thai-Malay Peninsula (i.e. *A. lanceifolius*), northward into Indo-Burma (*A. dadah* Miq.) and north-east into the Philippines (*A. odoratissimus* Blanco).

DISCUSSION

Phylogenetic analyses

This study comprises more than 75 % of the *Artocarpus* species recognized by the most recent treatments (Jarrett, 1959*a*, *b*, *c*, 1960; Kochummen, 2000; Zhekun and Gilbert, 2003; Berg *et al*., 2006), provides 50–100 % coverage of all subgeneric ranks, includes coverage of all previously defined sections and series (*sensu* Jarrett 1959*a*, *b*, *c*, 1960), and includes more than a dozen taxa that have not been included in previous phylogenetic analyses (Zerega *et al*., 2010). While it is not our aim here to redefine taxonomic divisions, this comprehensive analysis of a large and economically important genus will be important for future revisionary work and some taxonomic implications are briefly discussed.

ML and BI analysis support the division of the genus *Artocarpus* into the four subgenera proposed by Zerega *et al.* (2010), if *A. altissimus* is segregated into a new monotypic subgenus. Previous phylogenetic analyses did not include *A. altissimus*, but in the present study it was found to be sister to the rest of the genus (Figs S1 and S2) or sister to subgenus *Prainea* (Fig. 3). Jarrett (1960) placed *A. altissimus* in subgenus *Pseudojaca* as they share alternate, distichous leaves with non-amplexicaul stipule scars (compared to spirally alternate leaves with amplexicaul stipule scars found in subgenera *Artocarpus* and *Cauliflori*) and they also share apically fused adjacent carpellate perianths (compared to lack of apical fusion between adjacent carpellate perianths in subgenera *Artocarpus*, *Cauliflori* and *Prainea*). However, *A. altissimus* has long bifid styles, which is uncharacteristic of the subgenus *Pseudojaca* (but present in some members of subgenera *Artocarpus* and *Prainea*), and it also has several vegetative characters that are anomalous for the genus as a whole. These include palmately tri-nerved leaves, geniculate petioles and crenate-dentate leaf margins with glandular tissue evenly spaced along the edge. These anomalies led Jarrett (1960) to place *A. altissimus* in a monotypic section (*Glandulifolium*) within subgenus *Pseudojaca*. The affinities of *A. altissimus* have been difficult to determine ever since it was first described, having been previously placed in *Grewia* (Malvaceae) and *Morus* (Moraceae) (Jarrett, 1960). *Morus* belongs to the sister tribe (Moreae) of Artocarpeae, and *A. altissimus* shares the tri-nerved leaf base and crenate leaf margin present in some *Morus* species. These characters are absent in all other *Artocarpus* taxa. Further analyses will be necessary to determine the precise affinities of *A. altissimus*, but it does not appear to be part of a monophyletic subgenus *Pseudojaca*.

The defining characters of subgenus *Artocarpus* are described in detail in Zerega *et al*. (2010). Some of the most recognizable traits of this subgenus include fleshy perianths of individual flowers fused only medially (apices and proximal portions are typically free) to adjacent perianths on the syncarp, and inflorescences are never rami- or cauliflorous. Subgenus *Artocarpus* is a well-supported clade (ML, 87 %; BI, 1·0 PP, Fig. 2), but there is no support for previous classifications below the subgeneric level, and we make no recommendations for further divisions at that level. Several species in this subgenus were not included in previous phylogenetic analyses. Among these, *A. brevipedunculatus* has indurated perianth apices and falls within a clade that shares this character. *Artocarpus obtusus* Jarrett, with rugose male inflorescences, falls within a clade that largely shares this character. Found at much higher elevations than other species in the subgenus, *A. excelsus* Jarrett grows on Mt Kinabalu in Sabah, Malaysia. Jarrett (1959) discussed the yet to be described *A. excelsus* in her treatment of *A. lowii* King, a lowland species found in the Thai-Malay Peninsula. She noted several morphological affinities between the two species, although they are not sister species in the present analysis. *Artocarpus teysmannii* Miq. has also not been included in previous analyses and is placed in a clade with *A. elasticus* Reinw. ex Blume and *A. scortechinii* King. All three species share the character of having perianth apices of varying lengths, with *A. elasticus* and *A. teijsmannii* also having sterile perianth apices and quite pronounced length variation between sterile and fertile perianths. The two subspecies of *A. anisophyllus* (the only *Artocarpus* species with adult leaves incised all the way to the midrib, appearing compound) are included for the first time in a phylogenetic analysis here. They are differentiated based on the sessile (subsp. *sessifolius*) or petiolate (subsp. *anisophyllus*) nature of the leaflets (lobes). However, the evidence does not support these divisions. In this analysis, one subspecies was nested within the other. In addition, the character itself appears quite labile, and both sessile and petiolate leaflets can be found on the same individual (N. J. C. Zerega, pers. observ.). Finally, within subgenus *Artocarpus* there were three species that were not resolved as monophyletic. *Artocarpus hispidus* is nested within the morphologically

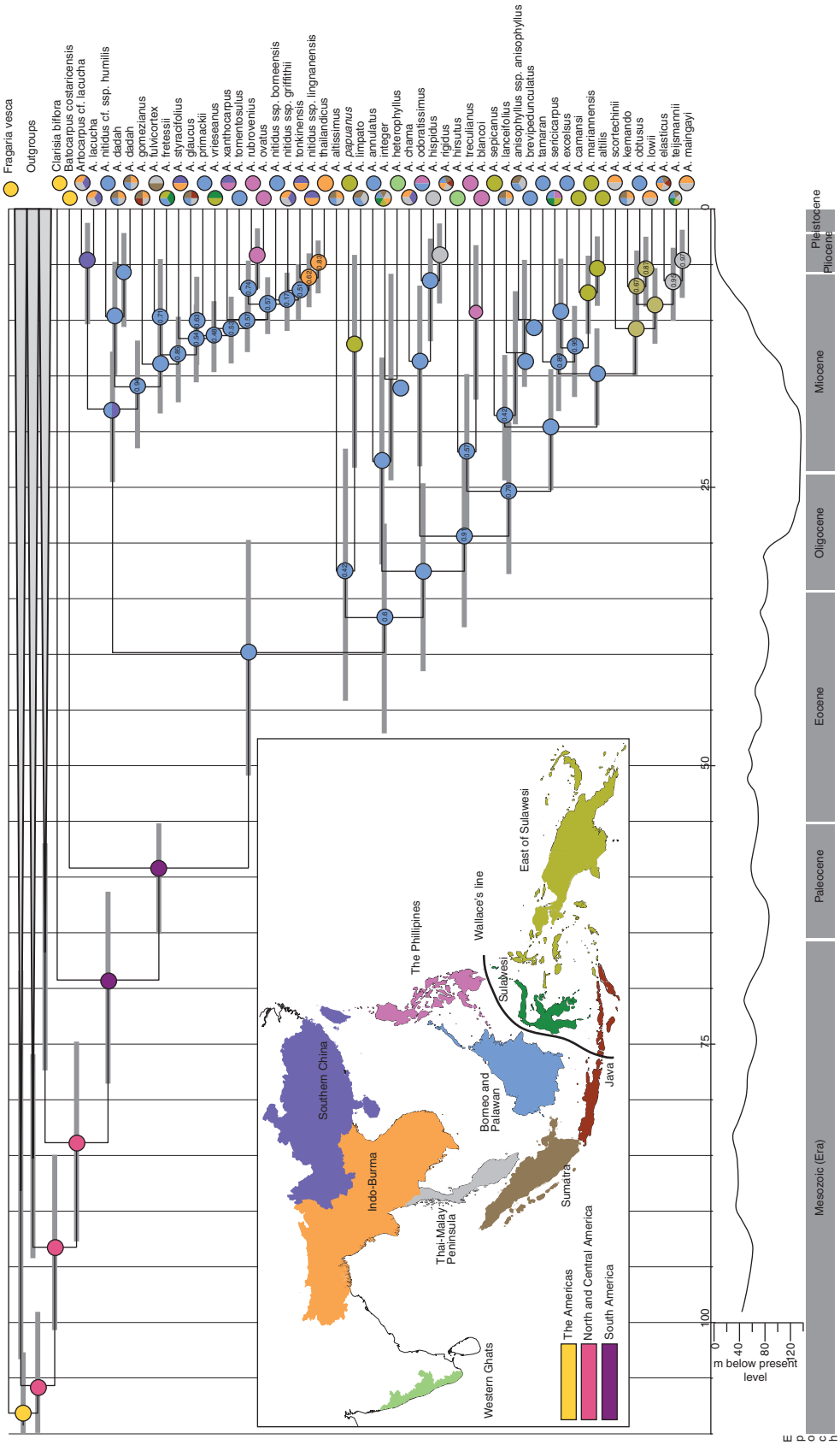


FIG. 3. Divergence date estimates and ancestral range reconstruction for *Artocarpus*. Dating, topology and node posterior support are from a BEAST analysis using six fossil calibrations and a constrained root. Error bars are 95 % confidence intervals. All nodes have posterior probability (PP) of 1·0 except where noted on the node. Coloured circles are the most likely state of a Lagrange ancestral area reconstruction allowing two areas per node. Pie charts at tips indicate the extent of the range for the indicated taxon. Sea level graph from Lambeck and Chappell (2001).

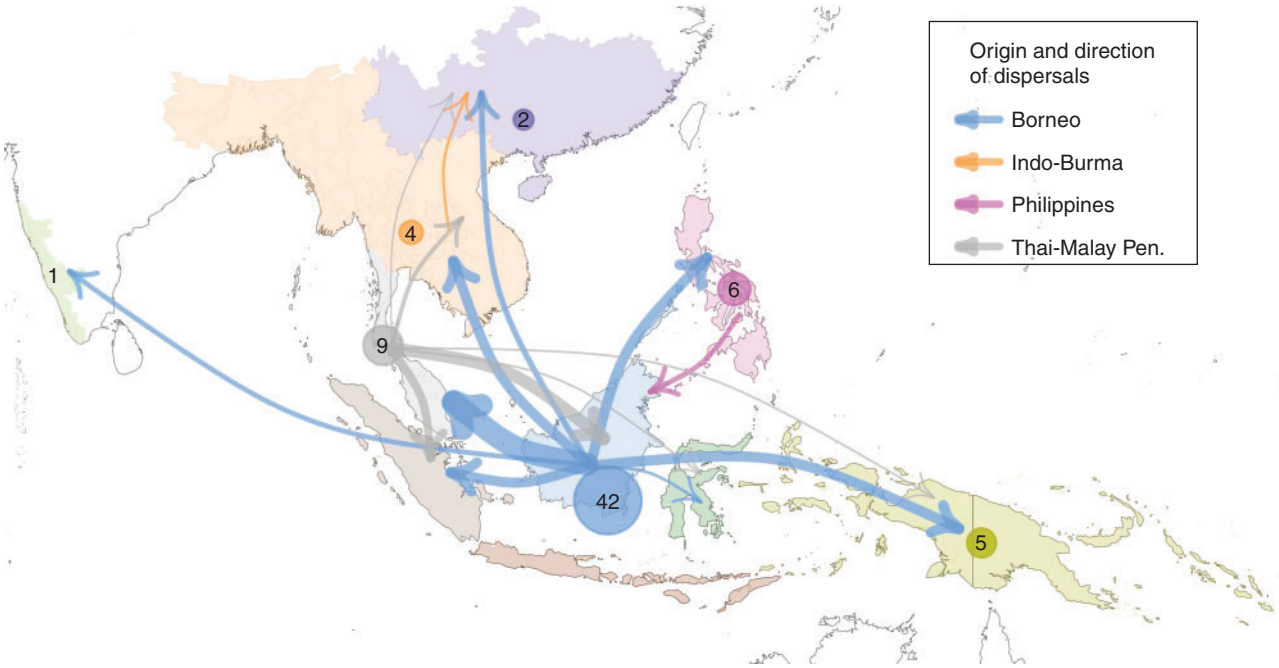


FIG. 4. Dispersal and *in situ* speciation events in *Artocarpus*. Arrows indicate dispersal events, and are colour-coded to match the region from which the event originated. Line thickness is proportional to the number of events. The circled numbers in each area indicate the number of *in situ* diversification events in that region. Events with an occurrence of 1·0 or higher are displayed. See Table S4 for all events.

similar *A. rigidus*, the main difference being the dense hispid pubescence found on the twigs of *A. hispidus* Jarrett compared to the sparser pubescence in *A. rigidus* Blume. With a much more restricted range (Malay Peninsula), *A. hispidus* may be better considered a subspecies of the widespread *A. rigidus*, which is found in Indo-Burma, the Thai-Malay Peninsula, Borneo, Sumatra and Java. Finally, neither of the two exemplars of *A. scortechinii* nor the two exemplars of *A. maingayi* King is monophyletic, and this will require further sampling and investigation.

The defining characters of subgenus *Cauliflori* are described in detail in Zerega *et al.* (2010). The most striking synapomorphy of the clade is the presence of rami- or cauliflorous istillate inflorescences. With only three species, subgenus *Cauliflori* included 100% taxon sampling and is well supported.

The defining characters of subgenus *Pseudojaca* are described in detail in Jarrett (1959*a*, *b*, *c*, 1960) and Zerega *et al.* (2010). The most recognizable traits of this subgenus are the alternate distichous leaf arrangement with non-amplexicaul stipules, coupled with the medial and apical (and sometimes basal) fusion of fleshy perianth tissue of individual flowers to adjacent perianth tissue on the syncarp. Subgenus *Pseudojaca* (excluding *A. altissimus*) is well supported, but many of the shallower nodes are poorly supported. The much shorter branch lengths in this subgenus compared to the other subgenera could indicate a more recent radiation, supported in the dating analysis (Fig. 3). Alternatively, variation in the rates of evolution could lead to short branch lengths, which we did not test here. There is much less character variation in subgenus *Pseudojaca* compared to the other subgenera, and there is a great deal of character overlap among the leaves and inflorescences across the subgenus (Jarrett, 1960; Berg *et al.*, 2006). These apparently reduced levels of variation could be indicative of a more recent radiation of subgenus *Pseudojaca*.

Several species in subgenus *Pseudojaca* have been variously reduced and expanded by different authors due to a paucity of variable characters and difficulties with species delineation. Several exemplars of two such species are included in the analysis: *A. nitidus* and *A. lacucha*. *Artocarpus nitidus* has been considered as five different species, a single species with five subspecies, and a single species with no intraspecific taxa but with four informal entities recognized (Jarrett, 1960; Berg *et al.*, 2006). The present analysis includes four of the five putative subspecies *sensu* Jarrett (1960). Phylogenetic reconstruction indicates that they represent four distinct lineages (Fig. 2), and they should be elevated to species rank. Additionally, Berg *et al.* (2006) reduced *A. xanthocarpus* Teysm. and Binnend. into *A. nitidus*, but this is not supported here. *Artocarpus lacucha* has been treated as a species restricted to Indo-Burma (Jarrett, 1960), or as a highly variable species ranging from the western Ghats of India to east of Wallace's line (Berg *et al.*, 2006). Compared to Jarrett's (1960) circumscription, Berg *et al.* (2006) reduced several taxa into *A. lacucha* (Fig. 2). The present analysis supports the treatment of Jarrett (1960) and the recognition of *A. lacucha*, *A. dadah*, *A. fretessii* Teysm. & Binnend. and *A. ovatus* Blanco as distinct taxa at the specific rank. Both the *A. nitidus* and the *A. lacucha* species complexes would benefit from additional phylogeographical study and detailed morphological studies.

Subgenus *Prainea* (2010) was recognized at the sectional level within the genus *Artocarpus* by Renner (1907), and

subsequently raised to the generic level by Jarrett (1959). A readily recognizable synapomorphy of the clade is that the fleshy perianths of individual flowers are not fused at all to the perianths of adjacent flowers on the syncarp. Two of the four species in subgenus *Prainea* are included in the present analysis, and there is strong support for its monophyly. However its placement within the genus is uncertain. In the ML and BI analyses it is sister to subgenus *Artocarpus* + *Cauliflori* with no support for this placement (Fig. 2). In the Beast analysis it is placed sister to the anomalous *A. altissimus*, which are both in turn sister to subgenus *Artocarpus* + *Cauliflori* (Fig. 3). However, this is also not well supported. *Prainea* has historically been difficult to place as it shares leaf phyllotaxy and stipule characters with subgenus *Pseudojaca* but anatomical leaf glandular characters with subgenus *Artocarpus* (Renner, 1907; Zerega *et al*., 2010), while the lack of fusion of adjacent perianths sets it apart from both subgenera, leading Jarrett (1959*a*, *b*, *c*) to treat it as its own genus. However, studies have shown that in young pistillate inflorescences of species from both subgenera *Artocarpus* and *Pseudojaca*, the perianths are unfused and only fuse later due to rapid divisions and subsequent enlargement of the ground tissue (Jarrett, 1959*a*, *b*, *c*; Sharma, 1965; Moncur, 1985). Complete sampling of subgenus *Prainea* and more extensive data sampling from the nuclear and chloroplast genomes, combined with detailed developmental anatomical study, may help to resolve relationships.

Dating and biogeography

In the mid- to late Cretaceous (83·8 Mya, 74·85–92·65 Mya) the stem node of the tribe Artocarpeae diverged from the rest of the family Moraceae. The biogeographical reconstruction infers a likely origin of the tribe in the Americas. The split between American (*Clarisia* and *Batocarpus*) and Asian Artocarpeae (*Artocarpus*) occurred in the Palaeocene (59·67 Mya, 55·24–65·03 Mya) with a radiation of *Artocarpus* from Borneo in the Eocene to Oligocene (40·07 Mya, 29·8–50·81 Mya). The time frame in which *Artocarpus* radiated coincides with boreotropical flora and a North Atlantic Land Bridge that could have allowed for dispersal from the Americas into Eurasia (McLoughlin, 2001). We are unaware of any fossil evidence for *Batocarpus* and *Clarisia*, but there are several records of fossils of *Artocarpus* and related extinct taxa (*Artocarpoxylon*, *Artocarpoxidium* and *Artocarpoides*) in Austria, France, Texas, Colorado, Louisiana, Canada, Kansas and Greenland from the Cretaceous into the Eocene (Collinson, 1989). These fossils support the possible presence of *Artocarpus* ancestors in areas where they might be expected if there had been dispersal across a North Atlantic Land Bridge. However, they must be viewed with some caution, and this is why they were not used in divergence date estimates. The vast majority of the fossils are from deeply cleft fossil leaves, which share gross similarities with some extant *Artocarpus* species, but the fossils lack cuticles and have poorly preserved venation. In her review of Moraceae fossils, Collinson (1989) indicated that these leaf fossils may be correctly identified as *Artocarpus* ancestors, but they are not 100 % diagnostic. In summary, the data presented here suggest an ancestral range for Artocarpeae in the Americas and dispersal across a North Atlantic Land Bridge in the Eocene during a time of boreotropical flora. Further detailed examination of fossil data is warranted to determine how well they support this proposal.

Artocarpus ancestors probably spread throughout Eurasia in the Eocene and began to diversify during this period of higher global temperatures. There are limited fossils of *Artocarpus* and its ancestors in Asia, but there are well-characterized wood fossils from the Intertrappean Deccan Beds in India dated from the Palaeocene to the Miocene periods, suggesting the genus or its ancestors had reached India by then (Mehrotra *et al*., 1984). This is consistent with dispersal across land, as India collided with Asia sometime between 43 and 50 Mya (McLoughlin, 2001; Sanmartín and Ronquist, 2004). Also, the first ever *Artocarpus* fossil from China was recently described from a site that is considered to have strong phytogeographical connections with India (Jacques *et al*., 2015). The fossil is well preserved and comes from the middle Miocene Fotan flora of Zhangpu County, South Fujian, China, an area that has been considered to represent tropical rainforest based on the occurrence of distinctive winged fruit fossils (Jacques *et al*., 2015).

From southern Asia *Artocarpus* could have dispersed across land into what is now the island of Borneo. While *Artocarpus* may ultimately derive from extinct taxa in mainland Asia, Borneo is reconstructed as the greatest incubator of extant diversity and the ancestral range of *Artocarpus* as it exists today. Diversification in Borneo may have been followed by several separate dispersal events (and subsequent radiations) throughout Southeast Asia and Malesia (Figs 3 and 4). Radiation of *Artocarpus* is reconstructed as beginning in the Eocene and continuing through the Oligocene and into the Miocene and Pliocene, with the greatest diversification inferred to occur in the Miocene. Fluctuating sea levels during these periods may have allowed for isolation and diversification during times of high sea levels, followed by radiation and dispersal during periods of lower sea levels (Turner *et al*., 2001). During the Miocene, sea levels were generally lower than they are today, and frequent land connections existed between mainland Asia, the Thai/Malay peninsula and what is now northern and central Borneo, and parts of Sumatra and western Java (Hall, 2009, 2012; de Bruyn *et al*., 2014). However, the Philippines, Sulawesi, the Moluccas and other islands of Wallacea were frequently submerged until the mid-Miocene to Pliocene and did not share land connections to Borneo or to each other.

Considering the extant range for *Artocarpus* species included in this study, Borneo experienced higher levels of *in situ* diversification and emigration than any other area, especially during the Miocene. Borneo, the Thai-Malay Peninsula, mainland Asia, and parts of Sumatra and Java were frequently connected between 60 and 5 Mya (Hall, 2009; de Bruyn *et al*., 2014), and the species found in these areas have largely overlapping ranges today, suggesting that sympatric speciation may have occurred followed by dispersal (Fig. 4). The Thai-Malay Peninsula may have been a gateway for *Artocarpus* from Borneo into mainland Asia, Sumatra and Java. For example, half of the Thai-Malay taxa included in the study diversified in Borneo. All of the *Artocarpus* taxa in Java and Sumatra are a subset of what is found in the Thai-Malay Peninsula. Sumatra was connected to the Thai-Malay Peninsula and parts of Borneo (but closer to the former) from the Palaeocene into the Oligocene, and variously connected and disconnected due to fluctuating water levels in

the Miocene. Several of the species in Sumatra are not found in Borneo and are only otherwise known from the Thai-Malay Peninsula. This, along with ancestral range reconstructions, suggests that dispersal into Sumatra came through the Thai-Malay Peninsula (Fig. 3). Java in turn houses a subset of the Sumatran taxa, suggesting taxa dispersed into Java from Sumatra, probably during the late Miocene and early Pliocene when dispersal would have been more likely. Moving northward, based on the taxa in this study, Indo-Burma houses 73 % of the taxa found in the Thai-Malay Peninsula (but only 40 % of Borneo taxa), and southern China in turn houses 83 % of the taxa found in Indo-Burma (but only 33 % of Thai-Malay Peninsula and 0 % of Borneo taxa) (Table S1). This suggests northward dispersal of taxa out of Borneo into the Thai-Malay Peninsula, Indo-Burma and southern China, with some *in situ* diversification of new species throughout the Miocene and into the Pliocene (Figs 3 and 4). An alternative, and not mutually exclusive, explanation is allopatric speciation on the variously isolated landmasses during times of sea-level fluctuations with secondary contact after dispersal to Borneo. Possible dispersal routes over water are discussed below.

An outlier in *Artocarpus* distribution is the Western Ghats of southern India. An Indo-Malayan influence in the flora and fauna of southern India has long been recognized (Hora, 1944, 1949), and there are many examples of extant plant taxa present in the wet tropical forests of the Western Ghats and north-eastern India, but absent from the more arid central Indian region (Bahulikar *et al*., 2004; Apte *et al*., 2006; Banu *et al*., 2009; Kuttapetty *et al*., 2014). Recent analysis of fossil flora from the Deccan Intertrappean beds in central India suggests that the wet tropical forests, similar to present-day forests of the Western Ghats and north-east India, were flourishing in central India during the late Cretaceous into the Oligocene (Kapgate, 2013). *Artocarpus hirsutus* is restricted to the Western Ghats and it is reconstructed with an ancestral area of Borneo. Its extant distribution may be the result of long-distance overwater dispersal from Borneo to the Indian peninsula, or it may be the result of overland dispersal of its ancestral lineage through the Thai-Malay Peninsula into the Asian mainland and into India during a period when central India would have been home to wet tropical forests, but it has subsequently gone extinct outside of the Western Ghats (Figs 3 and 4). Given that support for its position in the phylogeny is low and it has been considered a morphologically anomalous species difficult to place (Jarrett, 1959*a*, *b*, *c*; Zerega *et al*., 2010), further work is needed to elucidate the evolutionary and biogeographical history of this species. Another Western Ghats species (*A. heterophyllus*, jackfruit) is a complicated taxon, as it is an economically important crop that is widely cultivated throughout the tropics today. It exhibits high levels of morphological (Azad *et al*., 2007; Khan *et al*., 2010) and genetic diversity (Melhem, 2015) in the Western Ghats, and this area has been proposed as its area of origin. However, high levels of morphological and genetic diversity also exist in Indo-Burma (Bangladesh) (Khan *et al*., 2010; Witherup, 2013; Witherup *et al*., 2013), and its centre of diversity and wild relative(s) remain unclear. Given that it may also be native in Indo-Burma and that its sister species, *A. integer* (cempedak, an important crop in Malaysia), is native to the Thai-Malay Peninsula and Borneo, *A. heterophyllus* may have reached the Western Ghats via overland dispersal through Indo-Burma. Inclusion of the Sri Lankan endemic, *A. nobilis* Thwaites, in future phylogenetic analyses, as well as phylogeographical studies of Western Ghats species such as *A. heterophyllus* and *A. hirsutus*, could help further elucidate biogeographical patterns in *Artocarpus* between Indo-Malaya and India.

Apart from one very widely distributed taxon (*A. teijsmannii*), Sulawesi and islands eastwards harbour very different species diversity than mainland Asia, Sumatra and Java, but they share diversity with Borneo. Sulawesi taxa are largely a subset of Borneo taxa, and our results infer a dispersal event to Sulawesi from Borneo during the Miocene (Table S4). Taxa present eastward, in Wallacea and Oceania, are the same as those in Sulawesi plus three additional lineages that may have diversified in New Guinea and Oceania [*A. papuanus* (Becc.) Renner, *A. sepicanus* Diels, and the lineage containing *A. altilis*, *A. camansi* Blanco and *A. mariannensis* Trécul – breadfruit and its wild progenitors]. Because there were no land connections from Borneo into Sulawesi and Wallacea, this finding suggests overwater dispersal from Borneo, mostly during the Miocene. The *Artocarpus* taxa on the islands of the Philippines are quite distinct. Of the Philippine taxa included in this study, only 29 % of them have distributions overlapping with Borneo and there is no overlap with any other region apart from the widespread *A. sericicarpus* Jarrett. The Philippines is home to a high number of endemic taxa that diversified in the mid-Miocene to Pliocene, when the Philippine islands became emergent (Hall, 1998). There are additional endemic Philippine *Artocarpus* taxa that we were unable to include in the present study. They have morphological affinities to taxa included in the present study, suggesting that once taxa reached the islands, *in situ* species radiation was not uncommon.

With several inferred dispersal events across large expanses of water from Borneo into Sulawesi and eastward (Fig. 4, Table S4), it is important to consider how these may have occurred. Dispersal of syncarps and seeds in *Artocarpus* is not well studied. Large syncarps often drop and germinate near the mother tree (N. J. C. Zerega, pers. observ.) or are consumed by large mammals, such as elephants, orangutans and flying foxes (Campbell-Smith *et al*., 2011; Canale *et al*., 2013; Sekar *et al*., 2015; Sekar and Sukumar, 2015). However, whether the seeds survive passage through mammalian guts or if such passage increases germination rates is largely unknown. Recent studies examined whether Asian tapirs could facilitate long-distance seed dispersal in several species including *A. integer* (Thunb.) Merr. (Campos-Arceiz *et al*., 2012). They found that the tapirs consumed very few seeds, and of those that were consumed only 2·8 % of *A. integer* seeds survived passage through the gut and 0 % were able to germinate (Campos-Arceiz *et al*., 2012). Sekar *et al*. (2015) tested how well domestic bovids (*Bos primigenius* – cattle, and *Bubalus bubalis* – buffalo) and Asian elephants (*Elephas maximus*) in India could disperse *A. chama* seeds. They found that seeds passing through elephants are more likely to survive and germinate compared to seeds passing through bovids, and that elephants can act as dispersers of *A. chama* seeds. The ancestors of modern Asian elephants diverged from mastodons in the Oligocene and diverged from the African elephant (*Loxodonta africana*) in the late Miocene (Kappelman *et al*., 2003; Shoshani *et al*., 2006; Rohland *et al*., 2007). Asian elephants and their ancestors may have been

important dispersers of several *Artocarpus* species (Sekar *et al*., 2015). This mode of dispersal may help to explain the expansion of *Artocarpus* from Borneo throughout parts of mainland Asia. As the range of the modern Asian elephant and other large mammals shrinks, so too may the dispersal of *Artocarpus* species.

With regard to dispersal across long distances of open water to the islands east of Borneo, a possible disperser may have been flying foxes (*Pteropus* ssp.). Much of the diversification within *Pteropus* occurred in the Miocene to Pliocene, coinciding with *Artocarpus* diversification (Almeida *et al*., 2014). Various *Artocarpus* species have been recorded as preferred roosting sites and a food source for several *Pteropus* species in the Caroline Islands, Philippines and elsewhere (Mildenstein *et al*., 2005; Buden *et al*., 2013). *Pteropus* species are predominantly insular species with restricted ranges and are capable of flying up to 50 km in a single night (Mickleburgh *et al*., 1992; Almeida *et al*., 2014). There is a great deal of diversity and endemism of *Pteropus* species, which can be explained by its being a specialized island taxon. Islands provide isolated areas (allopatry) where divergence can proceed relatively quickly by genetic drift without interference from frequent gene flow. Sympatry of *Pteropus* species most often results from multiple colonization events rather than *in situ* speciation (Almeida *et al*., 2014). This same pattern is observed in *Artocarpus* species in the islands east of Borneo; however, *in situ* diversification is also important (Fig. 4, Table S4). Further investigation of the ecological interactions between *Artocarpus* and *Pteropus* is warranted.

CONCLUSION

We present a much expanded *Artocarpus* phylogeny that will be useful for future revisionary work, and we infer the biogeographical history of this important genus. Borneo is reconstructed as being central in the diversification of the genus *Artocarpus*, and it probably served as the centre from which extant species dispersed and diversified in several directions. Much of this probably occurred during the Miocene, a period when sea levels were frequently low, providing land connections between Borneo, present-day mainland Asia, Sumatra and Java. The Thai-Malay Peninsula may have been a gateway for *Artocarpus* into mainland Asia, Sumatra and Java, and some of the species found in these areas have extant overlapping ranges, suggesting that sympatric speciation may have occurred. In contrast, *Artocarpus* diversity east of Borneo, including Sulawesi, Wallacea, Oceania and the Philippines, is markedly different from the Thai-Malay group, with Philippine diversity being particularly unique and home to several endemic taxa. Also in contrast to the Thai-Malay group, reaching these islands probably involved long-distance overwater dispersal as opposed to overland dispersal. While the barrier to crossing Wallace's line has proved to be a hindrance for the dispersal in many faunal groups, it is less so among plants (Van Welzen *et al*., 2011). Other examples of an inferred origin of flora in Borneo and subsequent dispersal across Wallace's line during the Miocene include *Rhododendron*section *Vireya* (Ericaceae) (Brown *et al*., 2006; Webb and Ree, 2012), *Alocasia* (Araceae) (Nauheimer *et al*., 2012) and *Begonia* (Thomas *et al*., 2012). Finally, the dispersal of two taxa native to the Western Ghats of India may be the result of overland or overwater dispersal.

Borneo houses the highest levels of extant *Artocarpus* endemism, and our results support other studies showing Borneo to be a biodiversity and evolutionary hotspot (Myers *et al*., 2000; Mittermeier *et al*., 2005; de Bruyn *et al*., 2014). These findings offer further support for the critical importance of conservation of this area in the face of rapid rates of deforestation and development (Koh and Sodhi, 2010; Wilcove *et al*., 2013).

SUPPLEMENTARY DATA

Supplementary data are available online at www.aob.oxfordjournals.org and consist of the following: Table S1: a list of specimens sampled, their geographical origin and abbreviations used in the study, together with GenBank accession numbers for the resulting DNA sequences (provided as an Excel file). Table S2: primers used, primer sources and the model of evolution used for Bayesian analysis. Table S3: results from LaGrange and S-DIVA biogeography analyses, with nodes that correspond to Fig. S2. Table S4: number of dispersal and *in situ* speciation events in *Artocarpus*. Figure S1: maximum-likelihood tree of the full dataset. Figure S2: consensus tree from BEAST with labelled nodes corresponding to Table S3.

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