Biogeography and evolution of the screw-pine genus Benstonea Callm. & Buerki (Pandanaceae)

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Abstract

BUERKI, S., T. GALLAHER, T. BOOTH, G. BREWER, F. FOREST, J.T. PEREIRA & M.W. CALLMANDER (2016). Biogeography and evolution of the screw-pine genus Benstonea Callm. & Buerki (Pandanaceae). *Candollea* 71 : 217-229. In English, English abstract. DOI : http://dx.doi.org/10.15553/c2016v712a8

This study investigates the biogeography, evolution and systematics of *Benstonea* Callm. & Buerki *(Pandanaceae)* based on six plastid DNA regions and 54 specimens representing 36 species (60 % of species generic diversity). Our maximum likelihood and Bayesian phylogenetic inferences support the monophyly of *Benstonea* and its close relationship with the speciose *Pandanus* Parkinson. *Benstonea* is subdivided into three clades exhibiting contrasting species diversities. Clades I and II have seven species each, whereas most of the species diversity occurs in clade III with 21 species. None of the sections defined by Stone in *Pandanus* subgenus *Acrostigma* (Kurz) B.C. Stone (now *Benstonea*) are retrieved monophyletic by our analyses. Biogeographical inference supports the origin of *Benstonea* on the Sunda shelf during the Miocene and shows several subsequent exchanges between Peninsular Malaysia and Borneo. Species in Indochina and the Indian continent originated in Peninsular Malaysia and all belong to clade I. Wallacea was colonized at least twice from Borneo sometimes during the Miocene and no back-dispersals were inferred. The Sunda shelf was colonized once, most likely from Halmahera. Finally, our analyses suggest that the Fijian endemic *Benstonea thurstonii* (C.H. Wright) Callm. & Buerki dispersed from either Australia or New Guinea during the Pleistocene.

Keywords

PANDANACEAE – *Benstonea* – Biogeography – South-East Asia – Systematics – Wallacea

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Submitted on May 30, 2016. Accepted on July 7, 2016.

ISSN : 0373-2967 – Online ISSN : 2235-3658 – *Candollea* 71(2) : 217-229 (2016)

Introduction

Pandanaceae is a small paleotropical family divided into five well-circumscribed dioecious genera: *Pandanus* Parkinson, with ca. 450 spp. of trees and shrubs confined to the paleotropics; *Freycinetia* Gaudich. with ca. 200 spp. of lianas from Sri-Lanka to the South-Pacific; *Martellidendron* (Pic. Serm.) Callm. & Chassot with seven spp. of trees from Madagascar and the granitic Seychelles and *Sararanga* Hemsl. with two species of large trees from the Philippines to New Guinea and the Solomon Islands. The fifth genus, *Benstonea* Callm. & Buerki *(Pandanaceae)* is the last one described in the family and has been segregated from *Pandanus* based on phylogenetic and morphological evidence (Buerki et al., 2012; Callmander et al., 2012). *Benstonea* currently includes 60 species (two new species from Borneo are described in this issue; Callmander & Buerki, 2016) and is morphologically defined by its sharp spiniform styles with stigmatic grooves consistently placed on the abaxial side of the style, staminate flowers reduced to a few stamens and an epiphytic (e.g. *B. epiphytica* (Martelli) Callm. & Buerki) to mainly acaulescent shrub habit (rarely large trees, e.g. *B. atrocarpa* (Griff.) Callm. & Buerki). *Benstonea* includes species previously placed in *Pandanus* subg. *Acrostigma* (Kurz) B.C. Stone. This latter subgenus was further divided into four sections based on morphology: *P.* sect. *Acrostigma* Kurz, *P.* sect. *Epiphytica* Martelli *P.* sect. *Fusiforma* H. St. John and *P.* sect. *Pseudoacrostigma* B.C. Stone (Stone, 1968, 1971, 1974, 1978, 1983). This infra-generic classification has not been recognized in *Benstonea* (Callmander et al., 201 2). *Benstonea* exhibits an interesting distribution range across the old world from tropical India to the South Pacific with centres of diversity in Peninsular Malaysia, Borneo and New Guinea (Callmander et al., 2012, 2013, 2014). Although species of *Freycinetia* and *Pandanus* have successfully colonized New Caledonia, *Benstonea* species occur in the Solomon Islands and Fiji as well as Australia, but did not colonize the New Caledonia archipelago (Callmander et al., 2012).

Although several taxonomic studies have been recently published assessing species circumscriptions within *Benstonea* (Callmander et al., 2012, 2013, 2014), the understanding of phylogenetic relationships within this genus is still very limited. Buerki et al. (2012) included nine species of *Benstonea* in their phylogenetic analysis of *Pandanaceae* based on three plastid DNA regions *(matK, trnQ-rps16, trnL-trnF)*. A lack of DNA polymorphism and taxon sampling in the latter study prevented the inference of the phylogenetic position of *Benstonea* and the confirmation of its monophyly (all *Benstonea* species formed a clade that was embedded in a polytomy with species of *Pandanus* and *Martellidendron*). Later, Gallaher et al. (2015) published a new phylogenetic framework of *Pandanaceae* (focusing on the *Pandanus tectorius* complex) including only three species of *Benstonea* and based on three plastid (*trnL-trnF*, *ndhF-rpl32*, *trnQ-rps16*) and two nuclear (*LFY*, *PHYC*) DNA regions. The addition of DNA regions improved the inference of the phylogenetic position of *Benstonea*, which was recovered sister to *Pandanus*, but the limited sampling did not allow a comprehensive evaluation of its monophyly.

In this study, we expanded the taxon sampling of *Benstonea* to 36 species and sequenced six plastid DNA regions to: i) assess the monophyly of the genus and its phylogenetic position within the family; ii) infer phylogenetic relationships within the genus and discuss this result in light of Stone's infra-generic classification (1974) and iii) infer the biogeographical history of *Benstonea* as well as unravel its major evolutionary trends.

Material and Methods

Sampling

Previous phylogenetic studies on *Pandanaceae* included a limited sampling of *Benstonea*: nine spp. in Buerki et al. (2012) and three spp. in Gallaher et al. (2015). We greatly expanded the sampling of *Benstonea* by including 54 samples representing 36 species (60% of the species generic diversity). To assess the monophyly of *Benstonea*, representatives of all the other genera of *Pandanaceae* were included following results of Buerki et al. (2012) and Gallaher et al. (2015). *Barbacenia elegans* Pax *(Velloziaceae)* was used as the most external outgroup taxon to root the phylogenetic analyses (Buerki et al., 2012). Two other representatives of *Velloziaceae* were also included to serve as additional outgroup taxa. The full list of the species sampled, voucher information and DNA GenBank accession numbers is provided in Appendix 1.

DNA extraction, amplification and sequencing

Genomic DNA was extracted from both silica-gel dried and herbarium leaf material. Extractions of total DNA were performed using the same modified 2 × CTAB method as in our previous study on *Pandanaceae* (Buerki et al., 2012) and samples were stored at the Royal Botanic Gardens, Kew's DNA bank [http://apps.kew.org/dnabank/homepage.html].

Phylogenetic relationships within *Benstonea* were reconstructed using six plastid DNA regions, of which one is coding *(matK)* and five are intergenic spacers *(atpB-rbcL*, *trnQ-5'-rps16*, *trnL-trnF*, *trnV-ndhC, ndhF-rpl32)*. Information on primers and PCR protocols for *matK*, *trnQ-5'-rps16* and *trnL-trnF* are described in Buerki et al. (2012). This latter information for *atpB-rbcL, trnV-ndhC* and *ndhF-rpl32* is available respectively in Shaw et al. (2007), Schnitzler et al. (2011) and Forest et al. (2014). All PCR products were purified using DNA purification columns according to the manufacturers' protocols (QIAquick; Qiagen Ltd, Crawley, UK.). Dideoxy cycle sequencing was then performed using the chain termination method and ABI Prism Big Dye v. 3.1 reaction kit, following the manufacturer's protocols, but using

0.5µl of reaction mix (Applied Biosystems Inc., Warrington, UK). The products were prepared for sequencing using the ethanol-precipitation method and visualised on an ABI 3730 DNA Analyzer, also according to the manufacturer's protocols.

Alignment and phylogenetic analyses

The program Geneious v. 8.1.3 (Biomatters, Aukland, New Zealand) was used to assemble complementary strands, verify software base-calling and produce the alignments (using MUSCLE; Edgar, 2004). Single-gene and partitioned phylogenetic inferences were carried out employing both maximum likelihood (ML) and Bayesian Markov chain Monte Carlo (MCMC) analyses. In the case of the partitioned analyses, the dataset was divided into six partitions and each locus was allowed to have partition-specific model parameters. The phylogenetic analyses were done using the facilities offered by the CIPRES portal in San-Diego, USA [http://www.phylo.org].

The ML analyses were performed using RAxML v. 8.1.11 (Stamatakis, 2006; Stamatakis et al., 2008) with a 1,000 rapid bootstrap analysis followed by the search of the best-scoring ML tree in one single run. The default model, GTRCAT, was used to perform the ML analyses. The Bayesian MCMC analyses were performed in MrBayes v. 3.2 (Ronquist et al., 2012) and the best-fit model for each DNA region was estimated using MrModeltest v. 2.3 (Nylander, 2004) and the Akaike Information criterion (see Table 1 for best-fit models). Three Metropolis-coupled Markov chains with an incremental heating temperature of 0.2 were run for 10 hours on the CIPRES portal (yielding 14,821,000 generations for the partitioned analysis) and sampled every 1,000th generation. Each analysis was repeated twice starting with random trees. The MCMC sampling was considered sufficient when the effective sampling size (ESS) was higher than 200, as verified in Tracer v. 1.4 (Rambaut & Drummond, 2007). After a burn-in period of 25% per run, the remaining trees were used to construct a majority-rule consensus from MrBayes (half-compatible maximum credibility tree in BEAST) and its associated Bayesian posterior probabilities (BPP).

Divergence time estimations

Divergence time estimates were obtained using the Bayesian inference approach implemented in the package BEAST v. 1.8.0 (Drummond & Rambaut, 2007), applying the same partition delimitation and evolutionary models as those used for the MrBayes analysis. We used an uncorrelated relaxed molecular clock with a lognormal distribution of rates and a Yule speciation model. The analysis was run twice on the CIPRES portal for 10 million generations, sampling one tree every 1,000th generation. Parameter convergence was confirmed following the same approach as in the MrBayes analysis (see above). Following a burn-in period of one million generations, a maximum clade credibility tree with median branch lengths and 95% highest posterior density (HPD) interval on nodes was reconstructed using TreeAnnotator v. 1.8.0 (Drummond & Rambaut, 2007). HPD was only inferred for nodes with BPPs ≥ 0.5.

The calibration of the phylogenetic tree to obtained absolute age estimates was performed using four secondary calibration points obtained by Gallaher et al. (2015; see Table 1 in reference and values of 95% HPD obtained from *Cyclanthus* and *Pandanaceae* fossils). Uniform priors were set as follows (in million years): a) Crown of *Pandanaceae* (upper: 97.5, lower: 41.9) and splits between b) *Freycinetia* and clade comprising *Martellidendron*, *Pandanus* and *Benstonea* (upper: 60.7, lower: 24.6), c) *Martellidendron* and clade comprising *Pandanus* and *Benstonea* (upper: 38.1, lower: 15.1) and d) *Pandanus* and *Benstonea* (upper: 33.0, lower: 12.5).

Biogeographical inference

Geographical areas were defined based on the current distribution of *Benstonea* species and paleogeological data (Hall, 2009). We recognized five areas: A. India and Indochina (the limit of this area is set at the Isthmus of Kra; Parnell, 2013), B. Sunda shelf (including Peninsular Malaysia, Sumatra and Java, but excluding the Philippines, which are here included in the Wallacea region), C. Borneo, D. Wallacea (including Sulawesi, the Philippines and the Moluccas Islands) and E. Sahul shelf (including New Guinea and northern Australia) and Pacific islands (here the Solomon Islands and Fiji).

Table 1. – Characteristics of the six DNA plastid regions used in the phylogenetic analyses of *Pandanaceae.*

	atpB-rbcL	***matK***	***ndhF-rpl32***	***trnL-trnF***	***trnQ-rps16***	***trnV-ndhC***	**Supermatrix**
N sequences	50	49	62	75	80	46	80
Alignment length (bp)	950	673	1105	497	1385	842	5452
Best-fit model	GTR+G+I	GTR+G	GTR+G+I	GTR+G	GTR+G	GTR+G	GTR+G
N constant char. (%)	835 (87.9)	551 (81.9)	875 (79.2)	402 (80.9)	1026 (74.1)	691 (82.1)	4380 (80.3)
N variable char. (%)	115 (12.1)	122 (18.1)	230 (20.8)	95 (19.1)	359 (25.9)	151 (17.9)	1072 (19.6)
N Parsimony informative char. (%)	51 (5.4)	46 (6.8)	114 (10.3)	56 (11.3)	206 (14.9)	45 (5.3)	518 (9.5)

The dispersal–extinction–cladogenesis (DEC) likelihood model implemented in Lagrange v. 2.0.1 (Ree et al., 2005; Ree & Smith, 2008) was used to investigate the biogeographical history of *Benstonea* (further details on this method are presented in Buerki et al., 2011). We followed the same approach as in Forest et al. (2014) and performed the Lagrange analysis on the BEAST maximum clade credibility tree excluding all taxa expect those belonging to *Benstonea* and pruning the dated tree at the species level (i.e. only one accession per species was kept). The maximum number of areas at nodes was constrained to two; however additional areas were included in the Lagrange analysis to account for the widespread species, *B. affinis* (Kurz) Callm. & Buerki, occurring in areas B, C and D (only species occurring in more than two areas). Ancestral area reconstructions for each node were plotted on the BEAST tree using pie charts and the biogeographical scenario was produced using a collection of R scripts following Buerki et al. (2013). This latter procedure (i.e. the type and frequency of transition events between ancestral and descendant nodes along the dated phylogenetic tree) was inferred according to the *Q matrix* implemented in the DEC model (Ree et al., 2005; Ree & Smith, 2008).

Results

Phylogenetic analyses

The numbers of accessions per DNA region are provided in Table 1 together with various statistics. The ML and Bayesian MCMC single-partition analyses yielded congruent topologies, i.e. no incongruence with BPP > 0.95 or bootstrap support (BS) > 75 % was found between the plastid regions, thus allowing performing a combined partitioned analysis. An identical situation was observed between the combined ML and Bayesian MCMC trees, where no supported incongruence was recognized. Only the half-compatible maximum credibility tree from the combined Bayesian analysis is presented here (including BPP and BS values on nodes; Fig. 1).

Phylogenetic analyses support the monophyly of all the genera of *Pandanaceae* (Fig. 1). Within this framework, *Sararanga* (BPP : 1, BS : 100 %) is inferred as the sister lineage to all other *Pandanaceae* with *Freycinetia* (BPP : 1, BS : 100 %) and *Martellidendron* (BPP : 1, BS : 94 %) as subsequent sister lineages to *Benstonea* + *Pandanus* (Fig. 1). Although moderately supported by the combined partitioned analyses (BPP : 0.95, BS : 74 %), our phylogenetic inferences infer *Pandanus* (BPP : 1, BS : 100 %) as sister clade to *Benstonea* (BPP : 1, BS : 85 %) (Fig. 1). Three clades are retrieved within *Benstonea* : clade I (BPP : 1, BS : 85 %), clade II (BPP : 0.98, BS : 88 %) and clade III (BPP : 1, BS : 84 %) (Fig. 1). Clade III is further divided into three moderately to strongly supported subclades to facilitate the discussion on the evolution and biogeography of the genus : clade IIIa (BPP : 1, BS : 99 %), clade IIIb (BPP : 1, BS : 65 %) and clade IIIc (BPP : 1, BS : 94 %) (Fig. 1). Finally, the phylogenetic relationship between these subclades is only moderately supported (BPP : 0.97, BS : 79 %).

Divergence time estimations and biogeographical inference

The BEAST maximum clade credibility tree of *Pandanaceae* is displayed on Fig. 2. The topology and node support are highly congruent with the MrBayes and RAxML analyses (Fig. 1). The temporal framework is in agreement with Gallaher et al. (2015). The biogeographical analysis suggested an origin of *Benstonea* on the Sunda shelf (areas B and C) sometime during the Miocene (Fig. 3, 4). Clade I remained on the Sunda shelf and two independent dispersals to area A (more specifically India, Burma and Sri Lanka) took place at the end of the Miocene (c. 10 million years ago) (Fig. 3, 4). A vicariance event is inferred at the most recent common ancestor of clades II and III between Peninsular Malaysia and Borneo (Fig. 3). Clade II most likely originated on the Peninsular Malaysia (area B), with two subsequent dispersals to Borneo (area C) and another dispersal from Borneo back to Peninsular Malaysia (Fig. 3, 4). Clade III originated in Borneo and a dispersal event is inferred from this area to Wallacea (area D) at the most recent common ancestor of subclades IIIb and IIIc (Fig. 3, 4). A dispersal event into the Sahul shelf (New Guinea and Australia) from Wallacea (including the Philippines) is inferred at the origin of subclade IIIb. Within area E, the spacial origin of Fijian *Benstonea* remains unclear, but the most likely hypothesis suggests a dispersal during the Pleistocene from either Australia or New Guinea as shown by the close relationships between the Fijian endemic *B. thurstonii* (C.H. Wright) Callm. & Buerki and the Australia and New Guinean *B. lauterbachii* (K. Schum. & Warb.) Callm. & Buerki (Fig. 3). However this latter phylogenetic relationship is poorly supported (BPP<0.5) and has to be taken with caution. Subclade IIIc originated in Borneo and the two lineages underwent contrasting biogeographical histories (Fig. 3). The lower lineage (including the type species, *B. affinis*) dispersed three times towards Peninsular Malaysia and another time towards Wallacea (more precisely the Philippines in the case of *B. affinis*) from Borneo, whereas the upper lineage underwent sympatric speciation in Borneo (Fig. 3).

Discussion

Systematics and evolution of Benstonea

Our phylogenetic analyses support the monophyly of *Benstonea* and suggest its close relationship with the largest genus of the family, *Pandanus* (Fig. 1). These results are in agreement with previous findings from Gallaher et al. (2015) based on very limited taxon sampling. *Benstonea* is subdivided into three clades

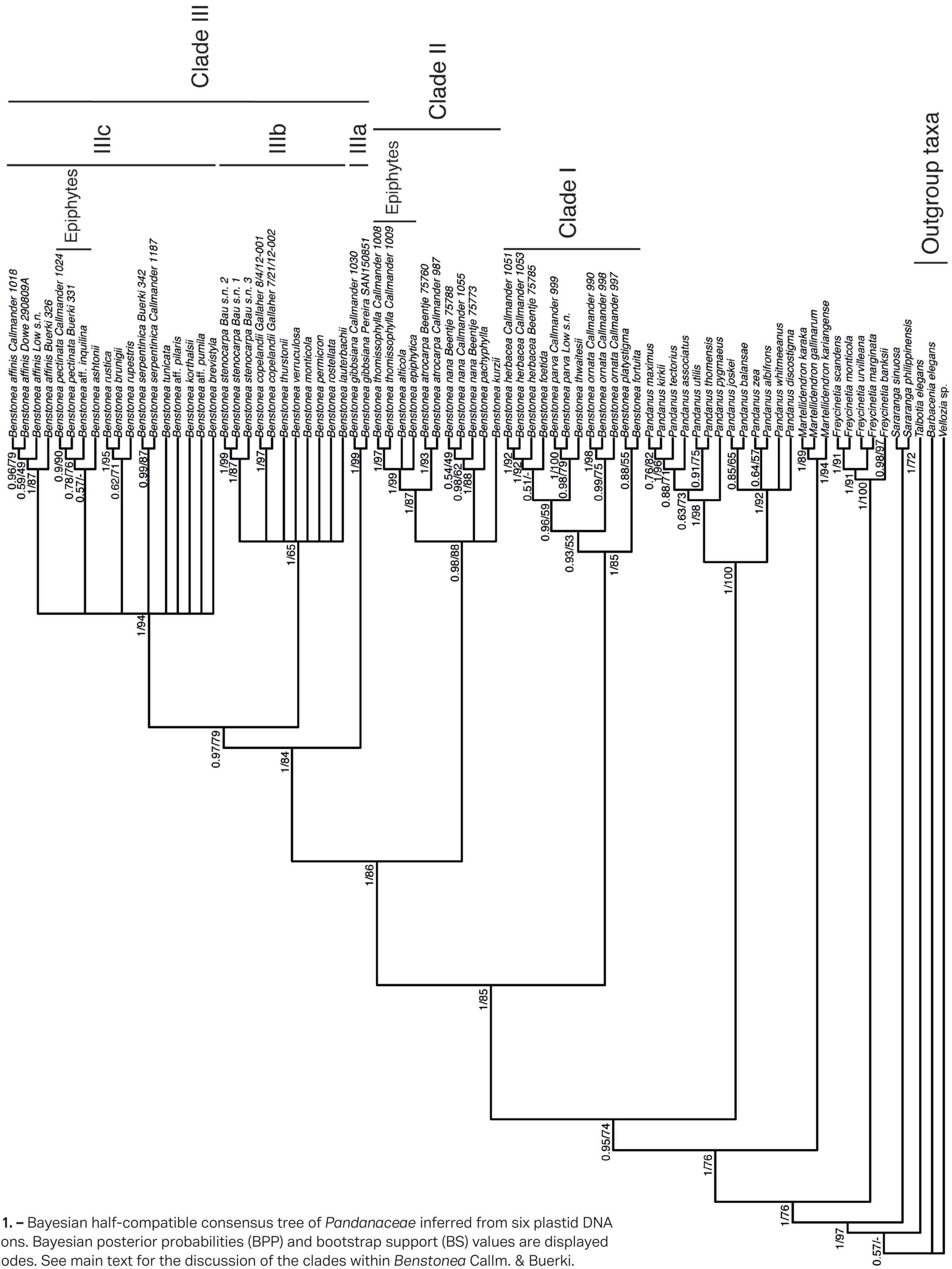

Fig. 1. – Bayesian half-compatible consensus tree of *Pandanaceae* inferred from six plastid DNA regions. Bayesian posterior probabilities (BPP) and bootstrap support (BS) values are displayed at nodes. See main text for the discussion of the clades within *Benstonea* Callm. & Buerki.

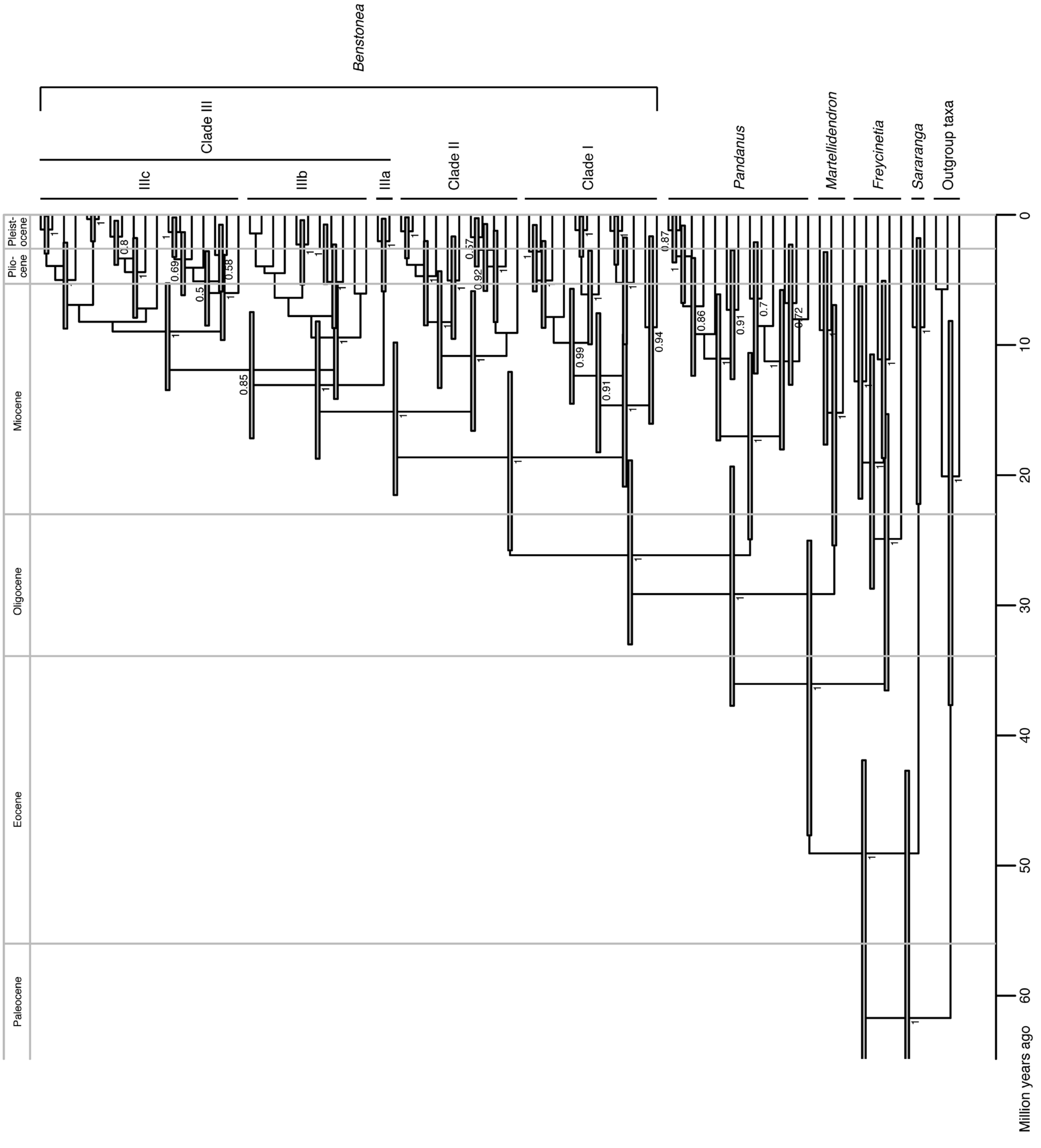

Fig. 2. – BEAST maximum clade credibility tree of *Pandanaceae*. Bayesian posterior probabilities (BPP) and 95% highest posterior density (HPD) interval on nodes are displayed. See main text for more details on clades.

exhibiting contrasting species diversities. Clades I and II have seven species each, whereas most of the species diversity occurs in clade III with 21 species (Fig. 1). Such uneven species diversity pattern is most likely not due to a bias in our taxon sampling since a taxonomic study on New Guinean species of *Benstonea* (all belonging to clade IIIb; Fig.1) indicated that this area is more speciose than previously expected (e.g. Callmander et al., 2014). None of the clades within *Benstonea* reflect the infrageneric classification proposed by Stone (1974, 1978, 1983) in *Pandanus* subg. *Acrostigma*. Species of the largest *P.* sect. *Acrostigma* (further subdivided into 15 subsections; see Stone, 1978) are scattered across the clades. Sampled species of *P.* sect. *Pseudoacrostigma* (two species, only *B. platystigma* was sampled) and *P.* sect. *Epiphytica* (one species, *B. epiphytica* sampled here) are nested within species of *P.* sect. *Acrostigma* in respectively clades I and II (Fig. 1). *Pandanus* sect. *Fusiforma* as defined by Stone (1968, 1974) included six species. The same author later in his revision of the subgenus *Acrostigma* (Stone, 1978) only accepted four species, but stated difficulties in identifying clear morphological characters discriminating section *Fusiforma* from section *Acrostigma* subsect. *Dismissistily* (where Stone moved in 1978 other species previously placed in *Fusiforma*). *Benstonea nana* (Martelli) Callm. & Buerki, the accepted name of *Pandanus magnifibrosus* H. St. John (see Stone, 1978; Callmander et al., 2012), should be placed in sect. *Fusiforma*, a species sampled in our studies. Furthermore, another species accepted in this section by Stone (1978), *Pandanus sobolifer* B.C. Stone (currently accepted as *Benstonea sobolifera* (B.C. Stone) Callm. & Buerki) will be treated as a synonym of *B. nana* in the upcoming treatment of the family *Pandanaceae* for the "Flora of Peninsular Malaysia" (Beentje & Callmander, unpubl. data). The only species of section *Fusiforma* sampled here is also nested in section *Acrostigma* in clades II (Fig. 1).

The polyphyly of the sections suggest that key morphological characters used by Stone for his infra-generic classification evolved multiple times independently. For instance, species with scaly surface pileus previously gathered in *Pandanus* subsect. *Scabridi* B.C. Stone belong to clade II (*Benstonea kurzii* (Merr.) Callm. & Buerki, *B. atrocarpa*) and clade III (*B. gibbsiana* (Martelli) Callm. & Buerki). Species of *Pandanus* subsection *Ornati* B.C. Stone, a group defined by its narrow and linear leaves with ventral pleats gradually attenuated with very small teeth and oblong or cylindrical syncarps (Stone, 1974: 525) are shared between clade I (*B. ornata* (Kurz) Callm. & Buerki) and clade IIIc (*B. rustica* (B.C. Stone) Callm. & Buerki).

On the other hand, it is interesting to note that several small understorey species distributed in lowland Bornean evergreen forests are restricted to clade IIIc, i.e. *B. brevistylis* (B.C. Stone) Callm. & Buerki, *B. brunigii* (B.C. Stone) Callm. & Buerki or *B. rustica*. The same is also true for another group with the same ecology in New Guinea, i.e. *B. permicron* (Kaneh.) Callm. & Buerki and *B. rostellata* (Merr. & L.M. Perry) comb. ined. in clade IIIb. This pattern suggests that species occurring in the same ecological conditions in Borneo and New Guinea radiated following similar processes and that most of the morphological characters used by Stone are homologous. Finally, the epiphyte habit evolved at least twice during the evolution of *Benstonea* in clades II and IIIc (Fig. 1). Phylogenetic evidence suggests that at least three facultative epiphytic species (clade II) are derived from acaulescent species (Fig. 1). This shift of habit might have been key in enabling *Benstonea* species (especially *B. thomissophylla* (B.C. Stone) Callm. & Buerki) to colonize drier habitats on the Sunda shelf on limestone at low elevations (Callmander et al., 2012). Water supply on limestone is very limited due to high rates of drainage; however the epiphytic habit allows *Benstonea* species to trap water and debris into their leaves for long periods of time therefore maintaining a suitable growing environment (see Zona & Christenhusz, 2015 for a review).

Biogeographical history of Benstonea

Most islands in the Wallacea region were created from the Eocene-Oligocene boundary onwards (as a result of the collision between the Australian and Eurasian plates; Hall, 2009) with a peak of tectonic activities during the Miocene. A previous study on *Sapindaceae* showed that Wallacea acted as a hub connecting the Sunda and Sahul shelves and triggered the diversification of that group of plants (Buerki et al., 2013). A review published by Crayn et al. (2015) concluded that most plant lineages exhibiting the same distribution as *Benstonea* originated on the Sunda shelf during the Miocene and subsequently dispersed towards the Sahul shelf by using the islands in the Wallacea region as stepping-stones.

Our biogeographical analysis inferred an origin of *Benstonea* on the Sunda shelf during the Miocene followed by several (almost symmetrical) exchanges between Peninsular Malaysia and Borneo (Fig. 3, 4). The dispersals are not restricted to a specific clade and occurred throughout the phylogenetic tree of the genus (Fig. 3, 4). Only one lineage within subclade IIIc is restricted to Borneo and it apparently underwent significant diversification (seven species; Fig. 4). This endemic Bornean radiation occurred within the last five million years and might result from the isolation of Borneo from Peninsular Malaysia due to Quaternary sea level rise and/or orographic effects (see Cannon et al., 2009; Hall, 2009). Our analysis also inferred at least two northern dispersals in clade I from Peninsular Malaysia to Indochina and the Indian continent (Fig. 3, 4).

With the exception of *B. affinis* (belonging to clade IIIc, which colonized the Philippines from Borneo, most likely by taking advantage of low sea levels enabling species adapted to swamps to disperse across the Sunda shelf and parts of the Philippines; Cannon et al., 2009), all the species occurring in Wallacea, the Sahul and the Pacific islands are restricted to clade IIIb (Fig. 2, 3). Species of clade IIIb invaded the Wallacea region (including the Philippines) from Borneo during

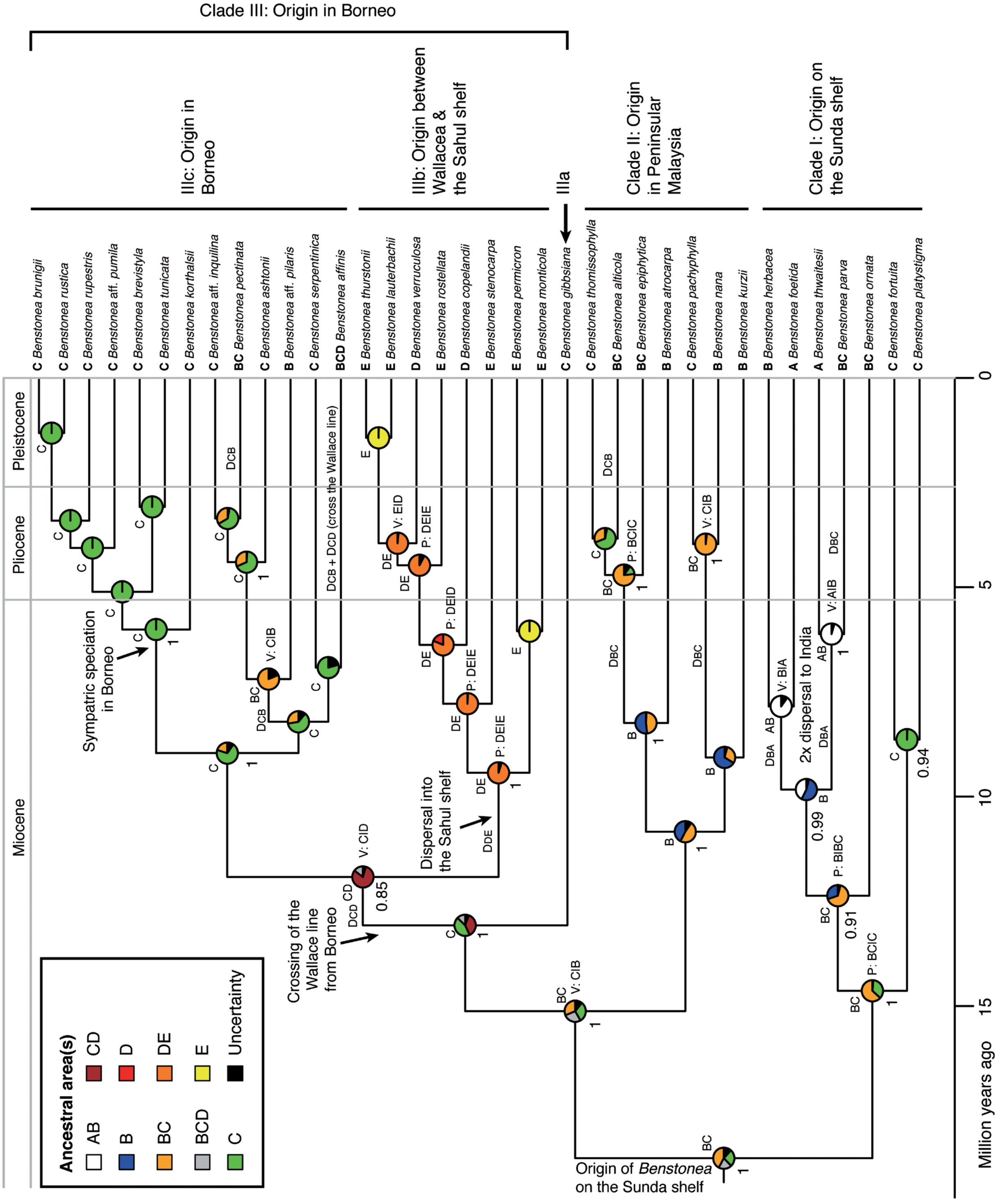

Fig. 3. – Biogeographical scenario of *Benstonea* Callm. & Buerki inferred using the DEC model implemented in Lagrange and displayed on the BEAST maximum credibility clade tree. Abbreviations for the biogeographical areas: A. India and Indochina (the limit of this area is set at the Isthmus of Kra; Parnell, 2013), B. Sunda shelf (including Peninsular Malaysia, Sumatra and Java, but excluding the Philippines, which are here included in the Wallacea region), C. Borneo, D. Wallacea (including Sulawesi, the Philippines and the Mollucas Islands) and E. the Sahul shelf (including New Guinea and Northern Australia) and the Pacific islands (here the Solomon islands and Fiji). Please see figure 4 for more details on the geographical boundaries of the biogeographical areas. Other abbreviations: P: peripheral isolate; V: vicariance; D: dispersal event.

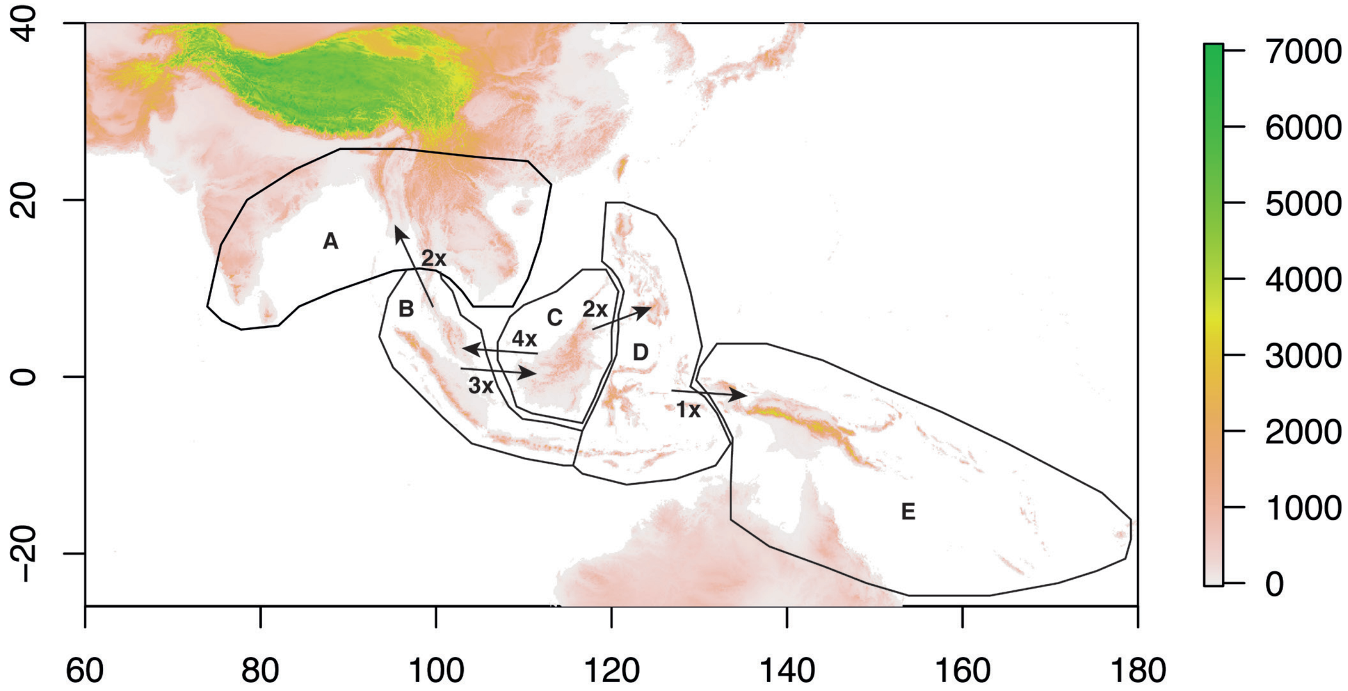

Fig. 4. – Biogeographical areas used for the Lagrange analysis of *Benstonea* Callm. & Buerki displayed on an elevation map retrieved from the WorldClim database [http://www.worldclim.org/current]. Dispersal events inferred by the Lagrange analysis are also indicated. See legend of Fig. 3 for more details on biogeographical areas.

the Miocene (c. 10 million years ago) and no back-dispersals were inferred (Fig. 3, 4). A study comparing the dispersal modes of *Benstonea* species would be required to test if the absence of back dispersals observed here is associated with adaptations to local dispersers. The fauna on each side of the Wallace line are significantly different (Huxley, 1868) and we could hypothesize that such difference might have played a role in shaping distribution patterns of *Benstonea* species.

Acknowledgements

A preliminary study was presented at the IX Flora Malesiana symposium in Bogor in 2013 and we are thankful to the organisers of the meeting and participants of the *Pandanaceae* session for their enthusiasm and for providing DNA material, especially Henk Beentje, Ary Keim and Altafhusain Nadaf. The authors are also deeply grateful to the following people for providing material and/or helping with material collection and/or interests in our work, without whom this study would not have been possible: Sharon Christopher and Chris Davidson (Boise, Idaho, U.S.A.); Khoon-Meng Wong and Yee Wen Low (SING); Yong Kien-Thai (KLU); Jérôme Munzinger (MPU); Laure Barrabé, Philippe Birnbaum, Jacqueline Fambart-Tinel, Céline Grignon and Vanessa Hequet (NOU); Voradol Chamchumroon, Nannapat Pattharahirantricin, Rachun Pooma Sommanussa Saengrit and Sukontip Sirimongkol (BKF); John B. Sugau, Postar Miun, Jeisin Jumian, and Nur Adillah binti Mohd Yusof (SAN); Mohizah Mohamad and Yahud Hj. Wat (SAR); John Leslie Dowe (JCT); Marika Tuiwawa and Alifereti Naikatini (SUVA); William Baker and Timothy Utteridge (K); Chiara Nepi (Museo di Storia Naturale dell'Università degli Studi di Firenze, Italia); Mary Merello, Pete Lowry and Pete Phillipson (MO); Rahul Zanan (BSI); Billy Bau, Kipiro Damas and Peter Homot (LAE); Chrissen Gemmill (WAIK); Thomas

Haevermans and Odile Poncy (P); Susana Arias Guerrero and Nicolien Sol (L), and Nicolas Fumeaux, Laurent Gautier and Pierre-André Loizeau (G). MC thanks PT Weda Bay Nickel for the opportunity to participate in their Flora Inventory Programme on Halmahera, with special thanks to Tjut Fatisa Bangun, Bahar Fabanyo, Iska Gushilman, Idris Haris, Samsul Lasut, Gavin Lee, Roji Mahroji, Edi Permana and Bilal Sau. SB and MC are very grateful to the Idaho Botanical Research Foundation for generous support for fieldwork. GB and TB are grateful to Dion Devey (K) for his support in the laboratory. Finally, we would like to thank Nadir Alvarez and Mathieu Perret for their careful review of this manuscript.

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Appendix 1. – List of sequences produced in this study. Taxa, voucher information, location and GenBank accession numbers for the plastid DNA regions.

Taxon	Voucher	Location	*atpB-rbcL*	*matK*	*ndhF-rpl32*	*trnL-trnF*	*trnQ-rps16*	*trnV-ndhC*
Barbacenia elegans	–	–	JX286710	–	–	JX287059	–	
Benstonea aff. *inquilina*	*Callmander 1192*, G	Malaysia [Borneo]	KX544860	–	KX544957	KX545020	KX545095	–
Benstonea aff. *pilaris*	*Low s.n.*, SING	Malaysia [Peninsular]	–	KX544923	KX544989	KX545053	KX545128	–
Benstonea aff. *pumila*	*Callmander 1036*, G	Malaysia [Borneo]	–	KX544910	KX544966	KX545030	KX545105	KX545170
Benstonea affinis	*Buerki 326*, K	Malaysia [Borneo]	–	KX544907	KX544964	KX545027	KX545102	KX545168
Benstonea affinis	*Callmander 1018*, G	Malaysia [Borneo]	–	KX544906	KX544963	KX545026	KX545101	KX545167
Benstonea affinis	*Dowe 290809A*, JCT	Australia [cultivated]	–	–	KX544961	KX545024	KX545099	–
Benstonea affinis	*Low s.n.*, SING	Malaysia [Peninsular]	–	KX544905	KX544962	KX545025	KX545100	–
Benstonea alticola	*Callmander 1010*, G	Malaysia [Borneo]	KX544889	KX544927	KX544998	K X545062	KX545138	KX545199
Benstonea ashtonii	*Low 217*, SING	Malaysia [Borneo]	–	KX544909	–	KX545029	KX545104	–
Benstonea atrocarpa	*Beentje 75760*, K	Malaysia [Peninsular]	KX544884	–	–	–	KX545132	KX545193
Benstonea atrocarpa	*Callmander 987*, G	Singapore	KX544865	–	KX544967	KX545031	KX545106	–
Benstonea brevistyla	*Callmander 1199*, G	Malaysia [Borneo]	KX544861	–	KX544958	KX545021	KX545096	–
Benstonea brunigii	*Callmander 1012*, G	Malaysia [Borneo]	KX544867	–	KX544969	KX545033	KX545108	KX545172
Benstonea copelandii	*Gallaher 7/21/12–002*, BISH	Philippines	KX544868	–	KX544970	KX545034	KX545109	KX545173
Benstonea copelandii	*Gallaher 8/4/12–001*, BISH	Philippines	KX544869	–	KX544971	KX545035	KX545110	KX545174
Benstonea epiphytica	*Callmander 1193*, G	Malaysia [Borneo]	KX544859	–	KX544956	KX545019	KX545094	–
Benstonea foetida	*Zanan 4*, BSI	India	–	KX544911	KX544972	KX545036	KX545111	KX545175
Benstonea fortuita	*Callmander 1197*, G	Malaysia [Borneo]	KX544858	–	KX544955	KX545018	KX545093	–
Benstonea gibbsiana	*Callmander 1030*, G	Malaysia [Borneo]	KX544871	KX544913	KX544974	KX545038	KX545113	KX545177
Benstonea gibbsiana	*Perreira SAN150851*, SAN	Malaysia [Borneo]	KX544870	KX544912	KX544973	KX545037	KX545112	KX545176
Benstonea herbacea	*Beentje 75785*, K	Malaysia [Peninsular]	KX544872	KX544914	KX544975	KX545039	KX545114	KX545178
Benstonea herbacea	*Callmander 1051*, G	Thailand	KX544874	KX544916	KX544977	KX545041	KX545116	–
Benstonea herbacea	*Callmander 1053*, G	Thailand	KX544873	KX544915	KX544976	KX545040	KX545115	KX545179
Benstonea korthalsii	*Callmander 1002*, G	Malaysia [Borneo]	KX544875	–	KX544978	KX545042	KX545117	KX545180
Benstonea kurzii	*Callmander s.n.*, G	Indonesia [Java]	KX544876	KX544917	KX544979	KX545043	KX545118	KX545181
Benstonea lauterbachii	*Sands 7218*, K	Indonesia [New Guinea]	KX544855	–	KX544953	KX545015	KX545090	–
Benstonea monticola	*Dowe 120709D*, JCT	Australia	–	KX544918	–	KX545044	KX545119	–
Benstonea nana	*Beentje 75773*, K	Malaysia [Peninsular]	–	–	KX544980	KX545045	–	KX545182
Benstonea nana	*Beentje 75788*, K	Malaysia [Peninsular]	KX544885	–	KX544993	KX545057	KX545133	KX545194
Benstonea nana	*Callmander 1055*, G	Thailand	KX544866	–	KX544968	KX545032	KX545107	KX545171
Benstonea ornata	*Callmander 990*, G	Singapore	KX544878	KX544919	KX544982	KX545047	KX545121	KX545184
Benstonea ornata	*Callmander 997*, G	Singapore	KX544879	KX544920	KX544983	KX545048	KX545122	KX545185
Benstonea ornata	*Callmander 998*, G	Singapore	KX544877	–	KX544981	KX545046	KX545120	KX545183
Benstonea pachyphylla	*Callmander 1020*, G	Malaysia [Borneo]	–	–	KX544984	KX545049	KX545123	KX545186
Benstonea parva	*Callmander 999*, G	Singapore	–	KX544922	KX544986	KX545050	KX545125	KX545187
Benstonea parva	*Low s.n.*, SING	Malaysia [Peninsular]	–	KX544921	KX544985	–	KX545124	–
Benstonea pectinata	*Buerki 331*, K	Malaysia [Borneo]	KX544880	–	KX544987	KX545051	KX545126	KX545188
Benstonea pectinata	*Callmander 1024*, G	Malaysia [Borneo]	KX544881	–	KX544988	KX545052	KX545127	KX545189
Benstonea permicron	*Wiriadinata HW13274* , BO	Indonesia [New Guinea]	KX544863	–	KX544960	KX545023	KX545098	–
Benstonea platystigma	*Callmander 1001*, G	Malaysia [Borneo]	–	–	KX544990	KX545054	KX545129	KX545190
Benstonea rostellata	*Willis 118*, K	Indonesia [New Guinea]	KX544856	–	–	KX545016	KX545091	–
Benstonea rupestris	Callmander 1028, G	Malaysia [Borneo]	KX544883	–	KX544992	KX545056	KX545131	KX545192
Benstonea rustica	*Callmander 1011*, G	Malaysia [Borneo]	KX544882	–	KX544991	KX545055	KX545130	KX545191
Benstonea serpentinica	*Buerki 342*, K	Malaysia [Borneo]	KX544862	–	KX544959	KX545022	KX545097	–
Benstonea serpentinica	*Callmander 1187*, G	Malaysia [Borneo]	KX544857	–	KX544954	KX545017	KX545092	–
Benstonea stenocarpa	*Bau s.n. 1*, LAE	Papua New Guinea	–	KX544924	KX544994	KX545058	KX545134	KX545195
Benstonea stenocarpa	*Bau s.n. 2*, LAE	Papua New Guinea	KX544886	KX544925	KX544995	KX545059	KX545135	KX545196
Benstonea stenocarpa	*Bau s.n. 3*, LAE	Papua New Guinea	KX544887	–	KX544996	KX545060	KX545136	KX545197

Appendix 1. – *Cont.*

Taxon	**Voucher**	**Location**	***atpB-rbcL***	***matK***	***ndhF-rpl32***	***trnL-trnF***	***trnQ-rps16***	***trnV-ndhC***
Benstonea thomissophylla	*Callmander 1008*, G	Malaysia [Borneo]	KX544890	KX544928	KX544999	KX545063	KX545139	KX545200
Benstonea thomissophylla	*Callmander 1009*, G	Malaysia [Borneo]	KX544864	KX544908	KX544965	KX545028	KX545103	KX545169
Benstonea thurstonii	*Callmander 937*, G	Fiji	–	–	–	KX545064	KX545140	KX545201
Benstonea thwaitesii	*Zanan 58*, BSI	India	–	KX544929	KX545000	KX545065	KX545141	KX545202
Benstonea tunicata	*Callmander 1026*, G	Malaysia [Borneo]	KX544891	KX544930	KX545001	KX545066	KX545142	KX545203
Benstonea verruculosa	*Callmander 1151*, G	Indonesia [Moluccas]	KX544888	KX544926	KX544997	KX545061	KX545137	KX545198
Freycinetia banksii	*Gemmi 734*, WAIK	New Zealand	–	KX544931	–	KX545067	KX545143	–
Freycinetia marginata	*Dowe 290809L*, JCT	Australia	KX544892	KX544932	KX545002	KX545068	KX545144	–
Freycinetia monticola	*Munzinger 3230*, NOU	New Caledonia	KX544893	KX544933	KX545003	KX545069	KX545145	–
Freycinetia scandens	*Dowe 111009A*, JCT	Australia	KX544894	KX544934	KX545004	KX545070	KX545146	KX545204
Freycinetia urvilleana	*Callmander 942*, G	Fiji	KX544895	KX544935	KX545005	KX545071	KX545147	–
Martellidendron gallinarum	*Callmander 114*, G	Madagascar	–	KX544936	KX545006	–	KX545148	KX545205
Martellidendron karaka	*Callmander 70*, G	Madagascar	KX544896	–	KX545007	KX545072	KX545149	KX545206
Martellidendron kariangense	*Callmander 111*, G	Madagascar	KX544897	KX544937	KX545008	–	KX545150	KX545207
Pandanus albifrons	*Callmander 1005*, G	Malaysia [Borneo, cultivated]	KX544898	KX544938	KX545009	KX545073	KX545151	–
Pandanus associatus	*Callmander 52*, G	Comoros [Mayotte]	–	KX544939	–	KX545074	KX545152	–
Pandanus balansae	*Grignon 517*, NOU	New Caledonia	–	KX544940	–	KX545075	KX545153	–
Pandanus discostigma	*Callmander 1034*, G	Malaysia	KX544899	KX544941	KX545010	KX545076	KX545154	KX545208
Pandanus joskei	*Callmander 959*, G	Fiji	–	KX544942	–	KX545077	KX545155	KX545209
Pandanus kirkii	*Temu s.n.*, MO	Tanzania	–	KX544943	–	KX545078	–	–
Pandanus maximus	*Callmander 50*, G	Comoros [Grande Comore]	KX544900	KX544944	KX545011	KX545079	KX545156	–
Pandanus pygmaeus	*Callmander 84*, G	Madagascar	KX544901	KX544945	KX545012	KX545080	KX545157	–
Pandanus tectorius	*Dowe 111009B*, JCT	Australia	–	KX544946	–	KX545081	KX545158	–
Pandanus thomensis	*Callmander 165*, G	Sao Thomé-et-Principe [Sao Thomé]	KX544902	KX544947	KX545013	KX545082	KX545159	KX545210
Pandanus utilis	*Strasberg s.n. 6*, REU	Reunion	KX544903	KX544948	KX545014	KX545083	KX545160	KX545211
Pandanus whitmeeanus	*Callmander 938*, G	Fiji	–	KX544949	–	KX545084	KX545161	–
Pandanus zea	*Dowe 201009C*, JCT	Australia	–	KX544950	–	KX545085	KX545162	–
Sararanga philippinensis	*Callmander 921*, G	Hawaii [Cultivated]	KX544904	KX544951	–	KX545086	KX545163	–
Sararanga sinuosa	*Callmander 919*, G	Hawaii [Cultivated]	–	KX544952	–	KX545087	KX545164	–
Talbotia elegans		–	–	–	–	KX545088	KX545165	–
Vellozia sp.		–	–	–	–	KX545089	KX545166	–