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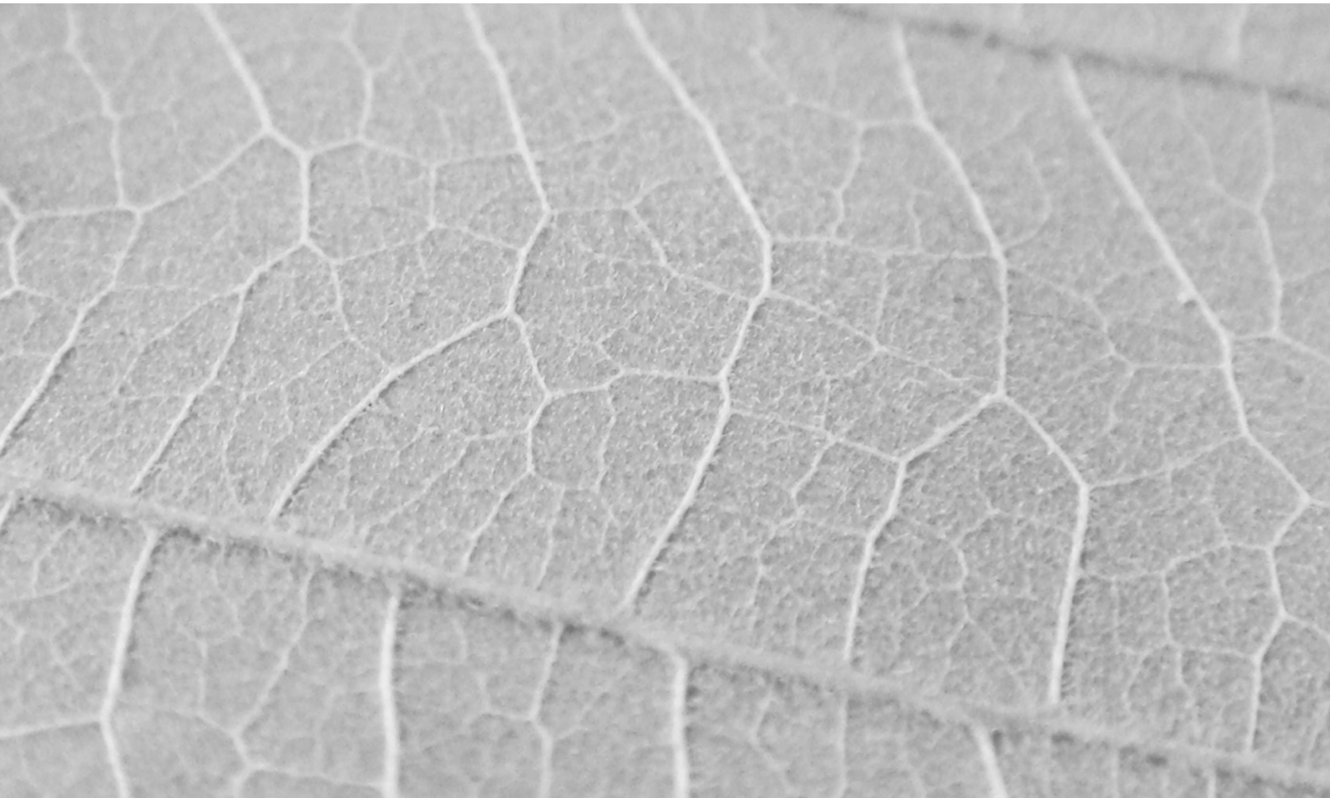
Chapter 6

Historical biogeography of *Aganope*, *Brachypterum* and *Derris* (Fabaceae, tribe Millettieae): Insights into the origins of palaeotropical intercontinental disjunctions and cross-taxon biogeographic patterns in Southeast Asia



Historical biogeography of *Aganope*, *Brachypterum* and *Derris* (Fabaceae, tribe Millettieae): Insights into the origins of palaeotropical intercontinental disjunctions and cross-taxon biogeographic patterns in Southeast Asia

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Abstract

Aim The historical biogeography of three palaeotropical legume genera, *Aganope*, *Brachypterum* and *Derris*, was investigated with the aims to i) discuss the selection between competing hypotheses on the origins of palaeotropical intercontinental disjunctions (PIDs), and ii) to infer spatio-temporal diversification patterns in tropical Southeast Asia.

Location African and Asian Palaeotropics.

Methods Plastid (*trnL-F IGS*, *psbA-trnH IGS* and *trnK-matK* ORF) and nuclear ribosomal (ITS/5.8S) DNA sequence data of endemic and widespread species, covering the complete geographic distribution of all three genera, were analysed using an uncorrelated-rates relaxed molecular clock model. Ancestral areas were reconstructed using a likelihood approach implementing the dispersal-extinction-cladogenesis model and a Bayesian approach to dispersal-vicariance analysis.

Results A wide ancestral distribution in Africa and Asia was inferred for the *Aganope* stem and crown groups, and vicariance between Africa and Asia in the early Miocene was supported. The SE Asian mainland was inferred as ancestral area for both the *Brachypterum* and the *Derris* crown groups. The reconstructions indicated numerous dispersal events westward to India, and eastward across Wallace's Line to New Guinea from the middle Miocene onwards. Two dispersal events from Asia to Africa, in the Miocene-Pliocene in *Brachypterum* and in the Pliocene-Pleistocene in *Derris*, were inferred.

Main conclusions The PID in *Aganope* is likely the result of vicariance, possibly caused by climate deterioration subsequent to the Middle Miocene Climatic Optimum. The inferred temporal origins of PIDs in *Brachypetrum* and *Derris* in the Middle Miocene to Pliocene-Pleistocene are consistent with long-distance dispersal. The biogeographical patterns of *Brachypterum* and *Derris* are similar to patterns identified in other SE Asian plant taxa, and highly congruent with geological events in SE Asia facilitating dispersal from the early Miocene until present, i.e. convergence and collision of the Australian and the Eurasian plates and associated emergence of substantial land and island arcs in central Malesia from the middle Miocene onwards. Preadaptation to a spectrum of environmental conditions and habitats including mangrove swamps, and high dispersal capabilities by hydrochory may explain wide distributions of some species, and frequent dispersal across oceanic water bodies separating western and eastern Malesia.

Keywords

Aganope, Ancestral area reconstruction; *Brachypterum*; *Derris*; Paleotropical disjunction

Introduction

Derris-like legume genera of the old world (sensu Sirichamorn et al., 2012b, see chapter 3 and 4) include *Aganope* Miq., *Brachypterum* (Wight & Arn.) Benth., *Derris* Lour. [including *Paraderris* (Miq.) Geesink], *Leptoderris* Dunn, *Philenoptera* Fenzl ex A. Rich. and *Ostryocarpus* Dunn. These genera were long considered to constitute a highly problematic, complex genus group (Geesink, 1984; Hu et al., 2000; 2002; Schrire, 2005), whose poorly understood generic delimitations and infrageneric relationships were major impediments to understanding their biogeography. Recent molecular phylogenetic studies (Sirichamorn et al., 2012b) have clarified generic delimitations and relationships among the *Derris*-like legume genera, moreover, providing a phylogenetic framework with the power to facilitate biogeographic analyses.

While *Leptoderris*, *Philenoptera* and *Ostryocarpus* are exclusively distributed in Africa, *Aganope*, *Brachypterum* and *Derris* occur in both the Asian and African tropics. This study investigates the historical biogeography of the latter three palaeotropical genera focusing on the selection between competitive hypotheses explaining their palaeotropical disjunctions, and inferring spatio-temporal diversification patterns in the Asian tropics.

Aganope includes c. 8 species of mostly woody climbers (Schrire, 2005). Most species grow in water-associated vegetation such as forests along rivers and mangrove swamps, however, occurrence in agricultural areas, degraded peat swamps and other disturbed vegetation types were also reported (Giesen et al., 2007; Lebrun and Stork, 2008a; Sirichamorn et al., 2012a, chapter 2). In contrast to other species of *Aganope*, *A. stuhlmannii* (Taub.) Adema grows in semi-arid areas in Africa (Lebrun and Stork, 2008b).

Brachypterum comprises c. 11 species of woody climbers and trees (Geesink, 1984; Sirichamorn et al., 2013, chapter 5). Most species range from continental SE Asia across Malesia to Australia with several species endemic to Australia, New Guinea and the Philippines. Other species show wide distributions, e.g., *B. scandens* (Roxb.) Miq., is widespread in Asia and Australia, but also occurs on Reunion Island. Two other widespread Southeast Asian tree species, *B. microphyllum* Miq. and *B. robustum* (Roxb. ex DC.) Dalzell & A. Gibson, also occur in Africa, but probably due to human introduction and cultivation of Asian-origin material (Polhill, 1990; Du Puy and Labat, 2002). The genus includes several drought tolerant species, which occur in dry habitats such as fire-prone areas of deciduous forest, grassland, cliffs and rocky slopes, and sometimes heavily disturbed areas and roadsides.

Derris (sensu Sirichamorn et al., 2012b) includes c. 55–60 species (Schrire, 2005) of all woody climbers. The genus shows a centre of diversity in Southeast Asia. Several species show wide distributions in tropical Asia, and the widespread type species, *D. trifoliata* Lour. also occurs along the southern and eastern coasts of Africa, in Madagascar, throughout Malesia and Pacific. Similar to *Aganope* species, most *Derris* species grow in fully exposed areas near or along waterways and only a few species are found in habitats, where water is limited, such as limestone karsts (e.g., *D. tonkinensis* Gagnep.).

Most species of *Aganope*, *Brachypterum* and *Derris* show adaptations to water and/or wind dispersal including indehiscent, winged pods, which are light weight and buoyant, and show a tough, water impermeable lignified pericarp. Endozoochory by herbivores is less likely, because the seeds are usually poisonous (Gomes et al., 1981; Geesink, 1984; Evans et al., 1985).

Because of their wide distribution in the Palaeotropics, the presence of palaeotropical intercontinental disjunctions (PIDs) at infrageneric (*Aganope*) and infraspecific (*Brachypterum scandens*, *Derris trifoliata*) levels, and a good representation with regards to species numbers in Southeast Asia, detailed investigations of the historical biogeography of these genera can address questions related to two complexes: (i) the origin of PIDs, and (ii) the identification of cross-taxon biogeographic patterns in SE Asia.

The origin of PIDs: Wide disjunctions between the African and Asian tropics pose an explanatory challenge and four main competitive hypothesizes have been advanced to explain this major biogeographic pattern: i) Rafting of biota of Gondwanan origin on the Indian plate and dispersal to Asia subsequent or slightly prior to collision with the Eurasian plate in the Cenozic (the "out-of-India" hypothesis; McKenna, 1973; Morley, 1998; Conti et al., 2002), ii) dispersal through extensive boreotropical forests of the Palaeocene and Eocene (the "boreotropical migration hypothesis"; Wolfe, 1975; Morley, 2000, 2007), iii) transoceanic long-distance dispersal (e.g., Li et al., 2009; Warren et al., 2010) and iv) overland migration between Africa and Asia across Arabia during a warm phase in the early to middle Miocene (e.g. Zhou et al., 2012). Molecular divergence age estimates and ancestral area reconstructions of *Aganope*, *Brachypterum*, and *Derris* may help to select between these hypotheses and provide insights into the historical assembly of Palaeotropical legume floras.

Cross-taxon biogeographic patterns in SE Asia: Biogeographic studies of palaeotropic legumes are sparse, and studies integrating a robust taxon sample and molecular divergence time estimation have not been published to-date. The Millettiod-Phaseoloid genera *Fordia* Hemsley (Schot, 1991) and *Spatholobus* Hassk. (Ridder-Numan, 1998) have been analysed based on phylogenies derived from morphological and anatomical data. Correlations between historical events such as eustatic sea-level changes repeatedly separating and uniting parts of western Malesia (the Sunda Shelf) and diversification patterns have been hypothesized (Schot, 1991; Ridder-Numan, 1998). Molecular divergence time estimation and ancestral area reconstructions of several other Southeast Asian angiosperm taxa have indicated that the oceanic water bodies separating western and eastern Malesia constitute limited dispersal barriers, and these studies inferred major diversification in a Pliocene or Pliocene-Pleistocene timeframe (Muellner et al., 2008; Su and Saunders, 2009; Nauheimer et al., 2012; Thomas et al., 2012). Frequent eustatic sea-level changes and orogeny in the Pliocene and Pleistocene and associated isolation and vicariance dynamics have been hypothesized as major drivers of diversification (Muellner et al., 2008; Su and Saunders, 2009; Nauheimer et al., 2012; Thomas et al., 2012). Molecular divergence time estimates and ancestral area reconstructions of *Aganope*, *Brachypterum* and *Derris* can be used to test apparent generalisation derived from this very limited number of studies and contribute to the identification of cross-taxon biogeographic patterns in the region.

To achieve these aims, we used molecular divergence time estimation based on nuclear ribosomal and plastid DNA sequence data in combination with ancestral area reconstructions.

Materials and Methods

Taxon sampling, DNA sequencing and sequence alignment—The taxon sampling used in this study is the same as that used to reconstruct the phylogeny of Asian *Derris*-like taxa by Sirichamorn et al. (2012b, chapter 3), i.e. over 90% of the known species of *Aganope* and *Brachypterum* (Geesink, 1984; Schrire, 2005), and about 50% of the *Derris* species (Adema, 2003a and 2003b; Schrire, 2005) were included. Voucher information and GenBank accession numbers are shown in Appendix 4-1 (see chapter 4). The chloroplast *trnL-F IGS*, *psbA-trnH IGS* and *trnK-matK* ORF and nuclear ribosomal ITS/5.8S were sampled. See Sirichamorn et al. (2012b, chapter 3) for details about DNA extraction, sequencing, and alignment.

Fossil selection and age constraints—Two fossil calibrations were used. The first calibration is based on *Derris prakashii* Prasad, Ghosh & Tripathi (Prasad et al., 2004), *Derrisocarpon miocenicum* Mitra & Banerjee (2004) and *Derrisophyllum siwalicum* Mitra & Banerjee (2004), described from middle-Miocene strata (c. 16.4–11.2 Ma) from India. The leaflet "*Derrisophyllum*" fossil was collected in close association with the fruit "*Derrisocarpon*" fossil (Mitra and Banerjee, 2004). These three fossils are similar to extant *Derris trifoliata* because of the higher order vein architecture (Mitra and Banerjee, 2004) and the broadly ovate-elliptic shape of the leaflets with a gradually attenuate apex and the obliquely orbicular, single-seeded pods with a winged upper suture. The characteristic shape of the leaflet found in *Derrisophyllum siwalicum* and pods of *Derrisocarpon miocenicum* and *Derris prakashii* are synapomorphies of *Derris elegans* Graham ex Benth., *D. glabra* Sirich., *D.* "*laotica*", *D. spanogheana* Blume ex Miq. and *D. trifoliata*, and allowed a clear clade assignment.

The second calibration was based on an leaflet-impression fossil of *Brachypterum*. This fossil, *Derris champarensis* Awasthi & Lakhanpal (1990), dates from the middle Pliocene of India (c. 3.5 Ma) and shows clear affinity with the extant type species, *B. scandens*. Its size, texture and venation pattern are diagnostic for *B. scandens* and *B.* "*philippinensis*", and the fossil was used to define a calibration prior for the crown node of the clade including these two species.

The ages of the used *Brachypterum* and *Derris* fossils were reported as "middle Pliocene" and "middle Miocene", respectively (Prasad et al., 2004; Mitra and Banerjee, 2004). Thus intervals, 4–3 Ma for the middle Pliocene fossil of *Brachypterum* and 16.4–11.2 Ma for the middle Miocene fossils of *Derris*, were assigned using the time scale of Berggren et al. (1995). In order to avoid subjective drawbacks of parametric calibration prior distributions (Ho, 2007), uniform distributions were assigned to the calibration points. The middle Pliocene and middle Miocene intervals were used as boundaries to account for the uncertainty of fossil ages. Divergence date estimates accordingly represent minimum ages. In addition, a secondary calibration was employed by assigning a normally distributed prior with a mean of 45.2 Ma and a standard deviation (SD) of 1.7 to the millettioid crown node ("MC" in Fig. 6-1) based on divergence age estimates of 48.4–40.2 Ma by Lavin et al. (2005).

Molecular dating analysis—The molecular dating analyses were performed in a Bayesian framework using the program BEAST 1.7.5 (Drummond and Rambaut, 2007) using the XSEDE package available online through the Cipres Science Gateway v.3.3 (Miller et al., 2010). The input files were created using BEAUti 1.7.5, in which four molecular partitions, were specified. The best performing evolutionary model for each molecular marker was identified using MrModelTest v.2.2 (Nylander, 2004) under the Akaike information criterion. The model GTR+G was selected for ITS/5.8S, *trnK/matK* and *trnL-F* IGS, GTR+I+G for ITS/5.8S and HKY+G for *psbA-trnH*. An uncorrelated relaxed clock model assuming a lognormal distribution of rates was used, and the Yule process was selected as tree prior (Drummond et al., 2006). A starting tree was generated by taking a 50% majority rule consensus tree from a Bayesian analysis in MrBayes v.3.1.2 (Ronquist and Huelsenbeck, 2003), anchoring the calibration nodes at ages concordant with hard calibration priors and transforming it into an otherwise random ultrametric tree in Mesquite (Maddison and Maddison, 2011). Two independent Markov Chain Monte Carlo runs were performed, each for 80,000,000 generations, with sampling every 1,000th generation. Time-series plots of all parameters were checked with Tracer v.1.5 (Rambaut and Drummond, 2009) for adequate effective sampling sizes (ESS > 200) and convergence of parameters between runs. The two runs were combined with LogCombiner v.1.7.4 (Drummond and Rambaut, 2007), setting the initial burn-in to 25%. The results were summarized using the maximum clade credibility (MCC) tree option in TreeAnnotator v.1.7.4. FigTree v1.4 (Rambaut, 2009) was used to visualize the BEAST topology.

Ancestral Area Reconstruction—Six biogeographic areas were delimited based on the distribution of extant species of *Aganope*, *Brachypterum* and *Derris* (Fig. 6-2): (A) Continental Africa, Madagascar and Mascarene Islands; (B) India and adjacent areas; (C) Continental SE Asia including S China, Taiwan and Hainan; (D) the Sunda Shelf and Wallacea, extending from Malay Peninsula through Indonesia; (E) the Philippines; (F) the Sahul Shelf (New Guinea and NE Australia). Wallacea does not harbour any endemic species and the majority of species occurring in this transitional region between the Sunda Shelf in the west and the Sahul Shelf in the east, also occur on the Sunda Shelf. Wallacea was, therefore, combined with the Sunda Shelf region.

Parsimony-based S-DIVA (Statistical Dispersal-Vicariance-Analysis), modified from DIVA (Ronquist, 1997) to account for uncertainty of both phylogenetic and ancestral area reconstructions (Nylander et al., 2008, Harris and Xiang, 2009; Yu et al., 2010; 2013), implemented in RASP v2.0b (Yu et al., 2010; 2013), and likelihood-based analyses under the dispersal-extinction-cladogenesis model implemented in Lagrange (Ree et al., 2005; Ree and Smith, 2008) were used to reconstruct ancestral areas at internal nodes. As several extant species show wide distributions, the maximum number of areas in ancestral ranges was unconstrained (6). Nine area combinations (ABE, ABF, ACF, AE, BCF, BE, BEF, BF, CF) representing biologically implausible ancestral area configurations were excluded prior to analysis.

Two sets of input trees were generated by pruning the MCC tree and the posterior post burn-in trees of the Beast analyses using Mesquite (Maddison and Maddison, 2011). The first set consisted of trees including *Aganope* and its sister *Ostryocarpus*, the second set consisted of trees including *Brachypterum*, *Derris*, *Fordia* and *Millettia pinnata*. The other taxa, all poorly sampled and serving merely as phylogenetic context, were pruned from these input trees. For species represented by multiple accessions all but one accession were pruned. The only exception was *Derris elliptica* (Wall.) Benth. for which multiple accessions representing different morphological forms and possibly cryptic species, were included. Cultivated material, "*elliptica* C", was also pruned as its origin and distribution is unknown.

For the S-DIVA analyses, 8000 input trees were selected by resampling from the post-burn-in sample of the BEAST analysis at lower frequency using LogCombiner. Relative frequencies of ancestral areas reconstructed for each node were recorded and plotted onto the MCC tree from the BEAST analysis.

The Lagrange online configurator (http://www.reelab.net/lagrange/ configurator/ index) was used to create input files. The two versions of the MCC tree from the BEAST analysis were used as input trees. Dispersal probability scaling matrixes for three time slices were specified (see Fig. 6-2) based on changes in the proximity and connectivity of areas during the early to middle Miocene (23.7–16.4 Ma; Time slice 3); the middle to late Miocene (16.4–11.2 Ma; Time slice 2), and the late Miocene to Quaternary (11.2–0 Ma; Time slice 1) (Rögl, 1998, 1999; Hall, 2009).

Results

Molecular divergence time estimation—The maximum clade credibility tree from the BEAST analysis is shown in Fig. 6-1. The inferred mean age of the Millettieae crown group is 48.5 Ma (95% highest posterior density interval: 51.5–42.3 Ma), suggesting an origin of the tribe in the middle Eocene (indicated by "MC" in Fig. 6-1). The divergence times of the crown groups of *Aganope* ("A" in Fig. 6-1), *Brachypterum* ("B" in Fig. 6-1) and *Derris* ("D" in Fig. 6-1) fall into an Miocene timeframe with estimated divergence times of 18.8 (29.3–14.8) Ma, 16.5 (20.7–11.3) Ma, and 19.4 (25.0–16.1) Ma, respectively. The age of the African crown group of *Aganope* ("AF" in Fig. 6-1) was estimated at 14.9 Ma, while the age of the Asian crown group ("AA" in Fig. 6-1) was inferred at 10.3 Ma. The split between the New Guinea-Australian subclade of *Brachypterum* ("BNA" in Fig. 6-1) from the remaining SE Asian species is inferred in the middle Miocene (11.8, 15.5–7.0 Ma), and the divergence of the "tree habit" subclade of *Brachypterum* ("BT" in Fig. 6-1) occurred at 6.1 (14.5–5.5) Ma. The split of the *Paraderris* subclade ("P" in Fig. 6-1) from the other *Derris* species was dated to the late Miocene, 6.3 (9.0–4.0) Ma.

Ancestral Area Reconstruction—Ancestral area reconstructions are illustrated in Figs 6-3 and 6-4, and relative and marginal probability values of some important nodes are summarised in Table 6-1. S-DIVA and Lagrange analyses yielded similar results (differences shown in Table 1).

For *Aganope*, the optimisations for the stem (node 15, Fig. 6-3A) and crown nodes (node 14) indicated wide ancestral areas in Africa and Asia (African Palaeotropics and Southeast Asian mainland in the likelihood reconstructions; all six areas in the S-DIVA analyses), and vicariance into an Asian and an African clade at the crown node.

The SE Asian mainland (area C) was inferred as the most probable ancestral area at the *Brachypterum* crown node (node 77, Fig. 6-3B). Several nodes along the backbone show highly ambiguous reconstructions (Table 1), but both dispersal eastwards to western Malesia (probably between nodes 77 and 76, and 70 and 69), eastern Malesia and Australia (probably between nodes 77 and 76) and eastwards to India can be identified (between nodes 70 and 69). Most likely reconstructions indicate a single dispersal event from Asia to Africa between nodes 72 and 71, or just along the branch leading to the terminal.

Reconstructions at the *Derris* crown group (node 65, Fig. 6-3B) indicate continental SE Asia as most likely ancestral area. The genus splits into two lineages before the

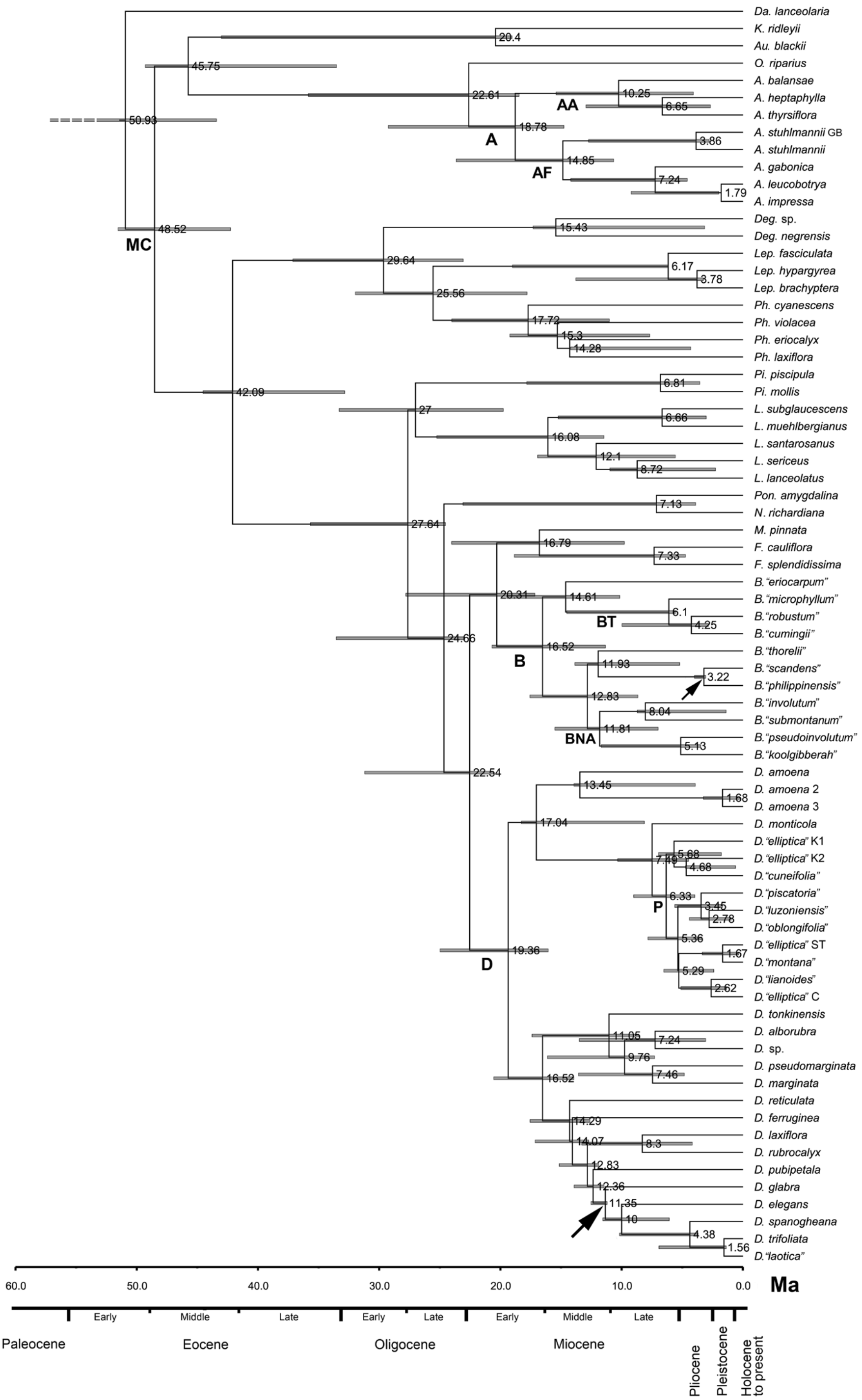

Da. lanceolaria
K. ridleyii
Au. blackii
O. riparius
A. balansae
A. heptaphylla
A. thyrsiflora
A. stuhlmannii GB
A. stuhlmannii
A. gabonica
A. leucobotrya
A. impressa
Deg. sp.
Deg. negrensis
Lep. fasciculata
Lep. hypargyrea
Lep. brachyptera
Ph. cyanescens
Ph. violacea
Ph. eriocalyx
Ph. laxiflora
Pi. piscipula
Pi. mollis
L. subglaucescens
L. muehlbergianus
L. santarosanus
L. sericeus
L. lanceolatus
Pon. amygdalina
N. richardiana
M. pinnata
F. cauliflora
F. splendidissima
B."eriocarpum"
B."microphyllum"
B."robustum"
B."cumingii"
B."thorelii"
B."scandens"
B."philippinensis"
B."involutum"
B."submontanum"
B."pseudoinvolutum"
B."koolgibberah"
D. amoena
D. amoena 2
D. amoena 3
D. monticola
D."elliptica" K1
D."elliptica" K2
D."cuneifolia"
D."piscatoria"
D."luzoniensis"
D."oblongifolia"
D."elliptica" ST
D."montana"
D."lianoides"
D."elliptica" C
D. tonkinensis
D. alborubra
D. sp.
D. pseudomarginata
D. marginata
D. reticulata
D. ferruginea
D. laxiflora
D. rubrocalyx
D. pubipetala
D. glabra
D. elegans
D. spanogheana
D. trifoliata
D."laotica"
20.4
45.75
50.93
22.61
10.25
AA
6.65
18.78
A
3.86
14.85
AF
7.24
1.79
48.52
MC
15.43
29.64
6.17
3.78
25.56
17.72
15.3
14.28
42.09
6.81
27
6.66
16.08
12.1
8.72
7.13
27.64
16.79
7.33
20.31
14.61
6.1
BT
4.25
24.66
16.52
B
11.93
3.22
12.83
8.04
11.81
BNA
5.13
22.54
13.45
1.68
17.04
7.49
5.68
4.68
6.33
P
3.45
2.78
5.36
1.67
19.36
D
5.29
2.62
11.05
7.24
9.76
16.52
7.46
14.29
14.07
8.3
12.83
12.36
11.35
10
4.38
1.56
60.0
50.0
40.0
30.0
20.0
10.0
0.0
Ma
Early
Middle
Late
Early
Late
Early
Middle
Late
Paleocene
Eocene
Oligocene
Miocene
Pliocene
Pleistocene
Holocene to present

Fig. 6-1. . Maximum clade credibility (MCC) tree derived with BEAST. The fossil calibration points are indicated by arrows. "MC" indicates "Millettiod Crown node" according to Lavin et al. (2005) to which the age 45.2 Ma was assigned. "A" = *Aganope* clade, "AA" = Asian *Aganope* clade, "AF" = African *Aganope* clade, "B" = *Brachypterum* clade, "BT" = tree-habit *Brachypterum* clade, "D" = *Derris* major clade (including Paraderris), "P" = *Paraderris* clade within *Derris* major clade. The bars indicate age intervals (95% highest posterior density interval, HPD). As the bar at the root node is too long (credibility intervals: 82.7–43.4 Ma), it is shown as a dashed bar.

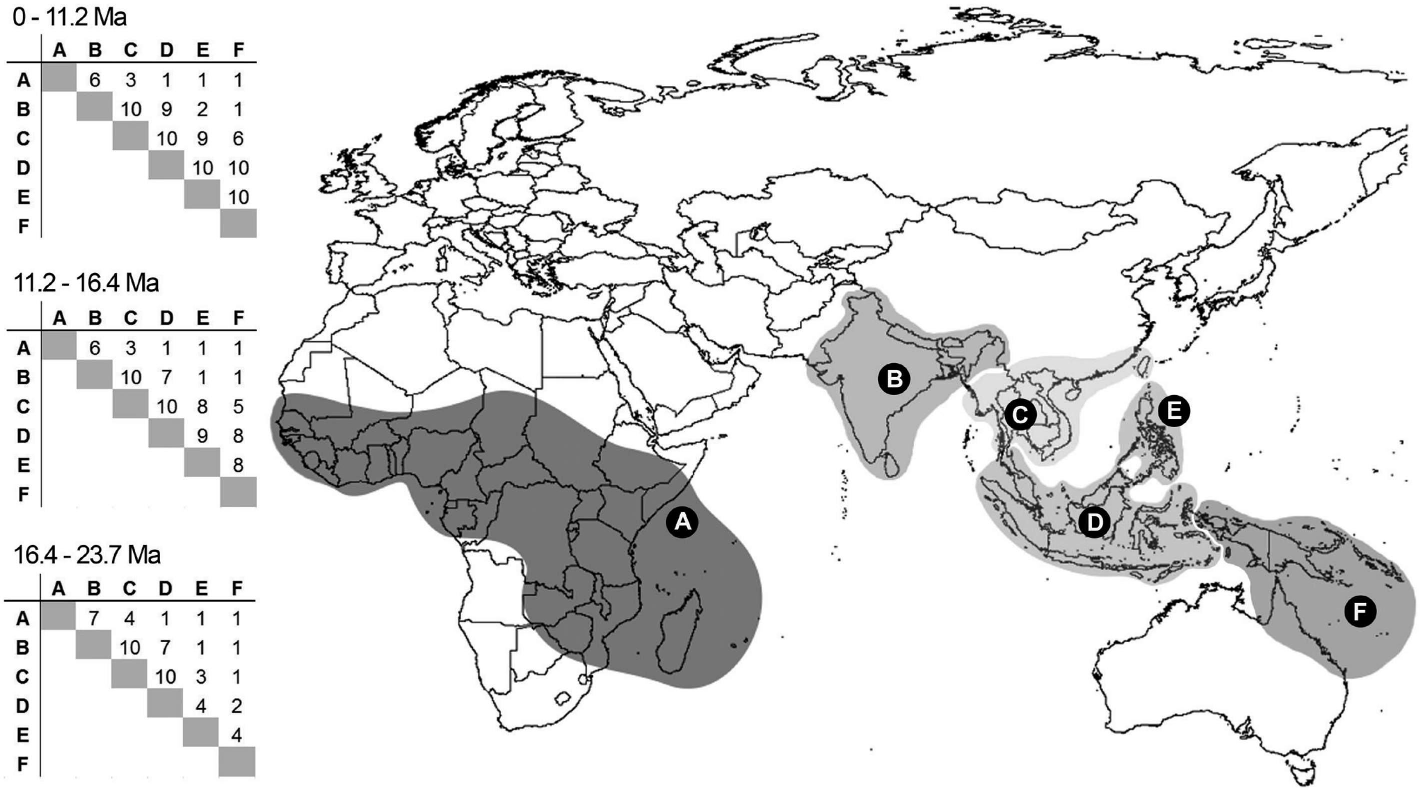
middle Miocene: one ("DI" in Fig. 6-3B, node 50) leading to *Paraderris* and the other ("DII" in Fig. 6-3B, node 64) consisting of *Derris* sensu Geesink (1984). For the first lineage (node 50) S-DIVA inferred an ancestral area in continental SE Asia (C) and Sunda shelf + Wallacea (D), and subsequent dispersal westward to India (B) and eastward to the Philippines (E) and to the Sahul Shelf (F) was inferred. For the crown group of the second lineage (DII: node 64) continental SE Asia was inferred as ancestral area. Reconstruction on the subsequent nodes indicate several dispersal events including dispersal westwards to India and eastwards to west and central Malesia, the Philippines (E) and the Sahul Shelf (F). Most likely reconstructions indicate a single dispersal event from Asia to Africa (between nodes 56 and 55, or along the branch leading to the terminal *D. trifoliata*).

Discussion

The origin of palaeotropical intercontinental disjunctions in *Aganope*, *Brachypterum* and *Derris*—The inferred timing of vicariance between African and Asian clades in *Aganope* in the Miocene, and dispersal from Asia to Africa in *Brachypterum* and *Derris* in the Middle Miocene to Plio-Pleistocene indicate that neither Gondwanan vicariance nor migration through the Eocene boreotropics are underlying PIDs in these genera.

Reconstructions for the *Aganope* crown node (dated 18.8 Ma) indicate a wide distribution including a combination of Africa, India and continental SE Asia, and subsequent vicariance into an African and an Asian lineage. Separation of the Indian subcontinent from the Madagascan fragment in the Late Cretaceous, 90 to 85 Ma, and the collision of the Indian fragment with the Eurasian continent, reconstructed at c. 50 Ma (van Hinsbergen et al., 2012) to 35 Ma (Ali and Aitchison, 2008), are preceding the inferred mean divergence time estimates of the *Aganope* stem lineage during the early Miocene by a considerable margin. Thus, it is unlikely that the ancestral species dispersed from Africa to Asia via the Indian raft ("out-of-India" hypothesis; McKennan, 1973; Morley, 1998; Conti et al., 2002). Migration through a northern mid-latitude corridor of frost-free humid climates in the Eocene (the

Fig. 6-2. Delimitation of the eight areas used for the ancestral area reconstruction by S-DIVA and Lagrange and three time slices showing the dispersal probability throughout three defined time periods (0-11.2 Ma: present to late Miocene, 11.2-16.4 Ma: late to middle Miocene, 16.4-23.7 Ma: middle to early Miocene). Area designations are as follows: A = Continental Africa including Madagascar and Mascarene Islands; B = India including adjacent areas; C = Continental SE Asia (northern region of Kra Isthmus) including South and SE China, Taiwan and Hainan; D = the Sunda Shelf and Wallacea, extending from Malay Peninsula through Indonesia; E = the Philippines; F = the Sahul Shelf (New Guinea and NE Australia).



0 - 11.2 Ma

	A	B	C	D	E	F
A	▨	6	3	1	1	1
B		▨	10	9	2	1
C			▨	10	9	6
D				▨	10	10
E					▨	10
F						▨

11.2 - 16.4 Ma

	A	B	C	D	E	F
A	▨	6	3	1	1	1
B		▨	10	7	1	1
C			▨	10	8	5
D				▨	9	8
E					▨	8
F						▨

16.4 - 23.7 Ma

	A	B	C	D	E	F
A	▨	7	4	1	1	1
B		▨	10	7	1	1
C			▨	10	3	1
D				▨	4	2
E					▨	4
F						▨

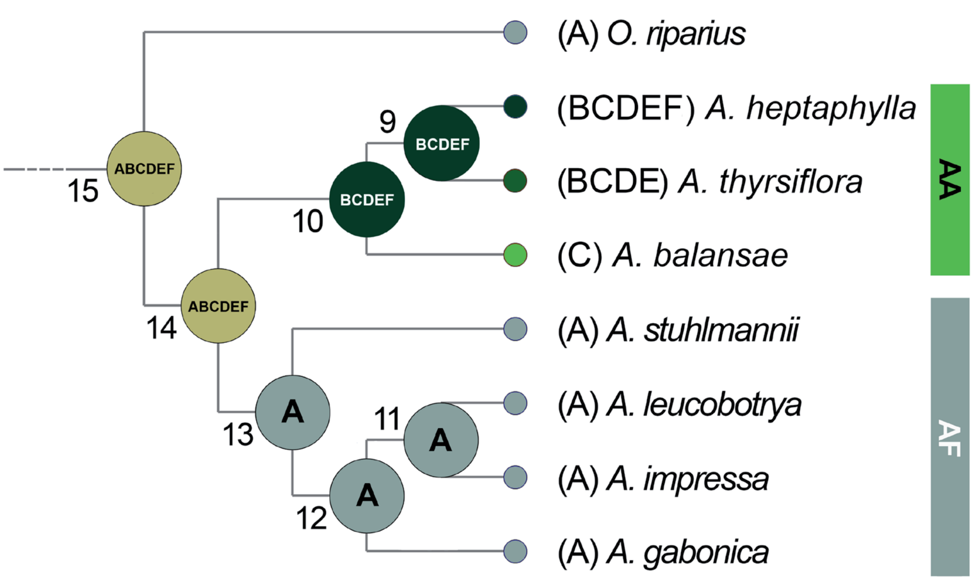


Fig. 6-3. Ancestral area reconstruction with S-DIVA showing some parts of the MCC tree. **6-3A**: clade of *Aganope* and its close relative, *Ostryocarpus*. AA = Asian *Aganope* clade, AF = *African* Aganope clade. For area abbreviations see the legend of Fig. 2. The distrubutions of the (terminal) species are in front of the species names. Numbers at nodes represent node names.

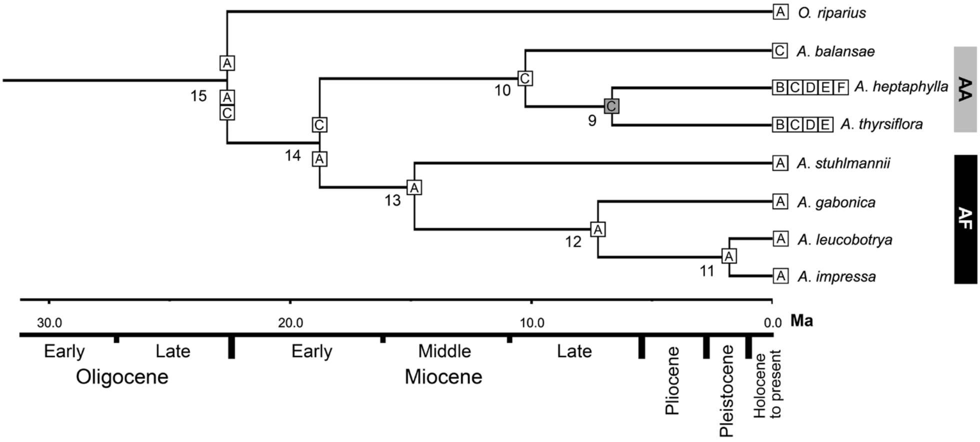


Fig. 6-4. Ancestral area reconstruction with Lagrange. **6-4A**: clade of *Aganope* and its close relative, *Ostryocarpus*. A = *Aganope* clade, AA = Asian *Aganope* clade, AF = African *Aganope* clade. The grey boxes indicate very low (≤ 0.1) relative probability (RP)

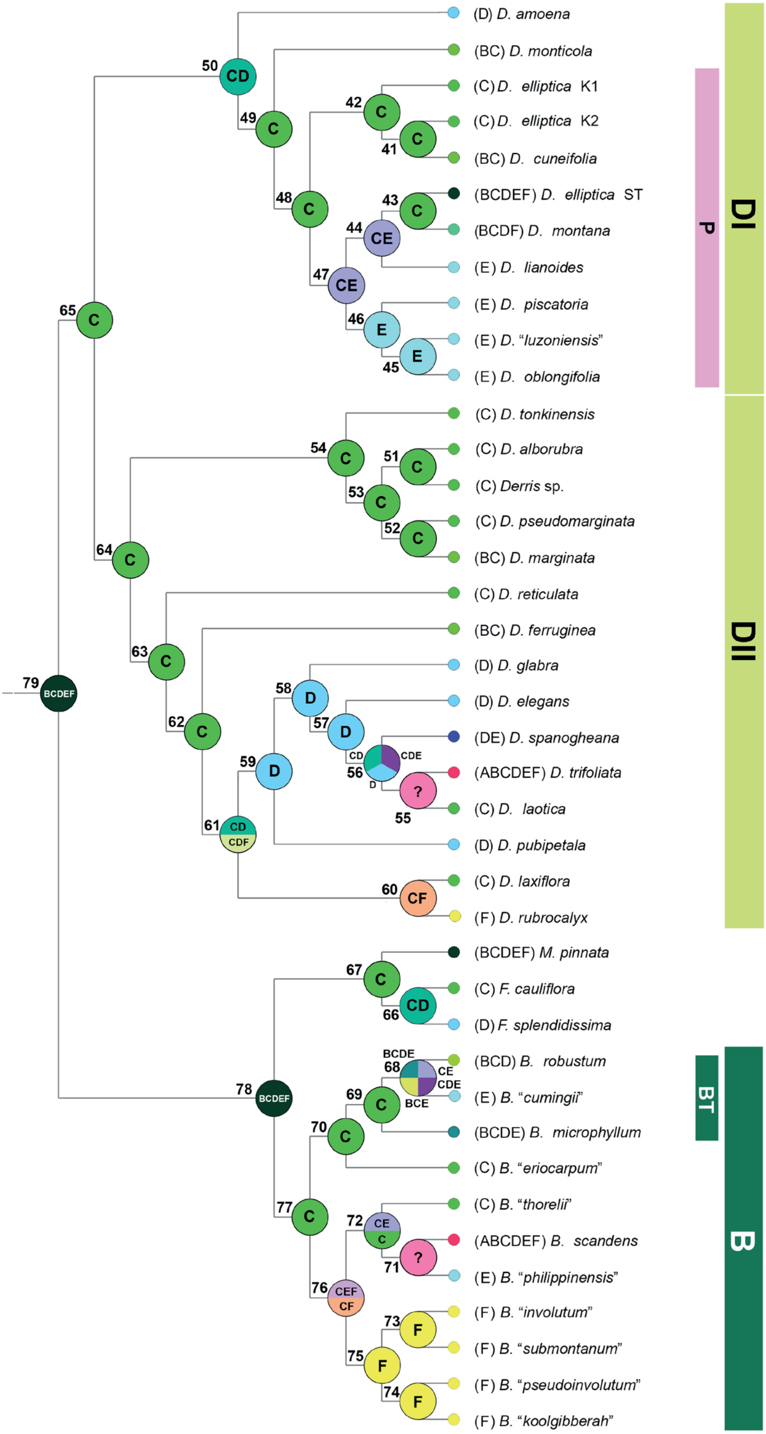


Fig. 6-3. Ancestral area reconstruction with S-DIVA showing some parts of the MCC tree. **6-2B**: *Brachypterum* clade and *Derris* (sensu Sirichamorn et al., 2012b and unpublished) clade, including the clade of *Millettia pinnata* + *Fordia* as local outgroups. Abbreviation for clades: B = *Brachypterum clade*, BT = "Tree habit" *Brachypterum*, DI = the 1st lineage of *Derris* leading to *Paraderris*, DII = the 2nd lineage of *Derris* consisting majority of *Derris* sensu Geesink (1984), P = *Paraderris* clade nested within DI. For area abbreviations see the legend of Fig. 6-2. The distributions of the (terminal) species are in front of the species names. Numbers at nodes represent node names. Reconstruction at node 55 and 71 was ambiguous; resulted in several area combination with low and equal marginal probability and thus were shown by question mark "?". Reconstruction at Node 60 was possibly only when the area combination "CF" was allowed in the analysis.

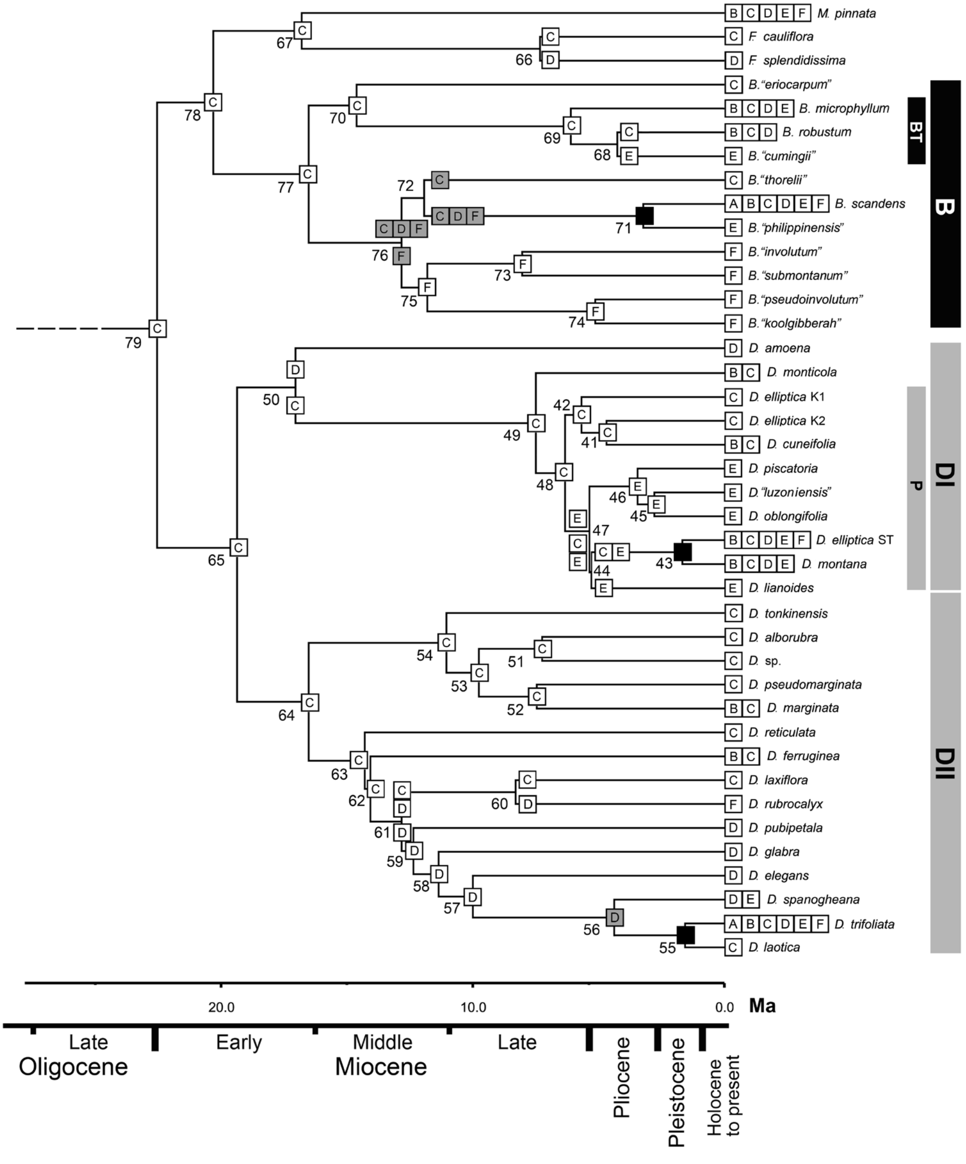


Fig. 6-4. Ancestral area reconstruction with Lagrange. **6-4B**: *Brachypterum* clade and *Derris* (sensu Sirichamorn et al., 2012b and unpublished) clade, including the clade of *Millettia pinnata* + *Fordia* as local outgroups. Abbreviation for clades and numbers of nodes are the same as presented in Fig. 6-3. The grey boxes indicate very low (≤ 0.1) relative probability (RP) and the black ones at node 43, 55 and 71 indicate splits between widespread and uncertain area(s).

"boreotropical migration hypothesis"), is also unlikely, as this corridor got disrupted by climate deterioration in the late Eocene and a drastic temperature drop at the Eocene-Oligocene border, c. 34 Ma (Wolfe, 1975; Zachos et al., 2001; Morley, 2000, 2007). Mean divergence time estimates at 18.8 (29.3–14.8) Ma for the *Aganope* crown node, however, are too recent to be consistent with this hypothesis.

Adaptations to hydrochory (dispersal by water) and habitats associated with both fresh and brackish water may have facilitated wide ancestral distributions along the coasts. The pods of extant *Aganope*, *Brachypterum* and *Derris* species are light, buoyant and show indehiscent, water impermeable pericarps. Some species, such as the exceptionally widespread species *A. heptaphylla*, *B. scandens*, and *D. trifoliata*, can be found in mangrove vegetation (Giesen et al., 2007, Sirichamorn et al., 2012a, chapter 2), strongly indicating that their fruit and seed are adapted to prolonged exposure of brackish water.

Prior to the initial diversification of *Aganope*, the most common recent ancestor might already have had a broad distribution in Africa, along the Arabian coast and eastward to India and SE Asia. Fossil casts of mangrove roots are described from the early Miocene strata of western Arabia (Whybrow and McClure, 1980), and bioconnectivity within the eastern mangrove flora region (eastern Africa, Arabia, Asia, Australia) during the middle Miocene has been hypothesized (Plaziat et al., 2001). Drier, colder climates subsequent to the Middle Miocene Climatic Optimum (Zachos et al., 2001) resulted in receding tropical forests and expansion of deciduous forest, drought adapted savannah, and grassland (Jacobs, 2004; Pound et al., 2012). This climate deterioration may also have resulted in geographic isolation of previously connected populations of a widespread mangrove-associated ancestor of *Aganope* and subsequent vicariance.

PIDs at the infraspecific level can be identified in the two exceptionally widespread species: *Brachypterum scandens*, which is a lowland species found in various habitats such as secondary shrub and monsoon forests, but it is also regularly found in mangroves, and *Derris trifoliata*, which grows on the landward margin of mangrove habitats (Giesen et al., 2006). Both taxa are deeply nested within clades of species showing distributions in Asia or Australia, and the analyses strongly indicate relatively recent dispersal (late Miocene-Pliocene in *Brachypterum*; Pliocene-Pleistocene in *Derris*) from Asia to Africa. Inferred dispersal events between the African and Asian Palaeotropics in late Miocene or Plio-Pleistocene have frequently been explained by long-distance dispersal facilitated by island hopping routes across the western Indian Ocean, rafting of vegetation mats and prevailing wind and oceanic currents (e.g. Renner, 2004; Li et al., 2009; Warren et al., 2010), or by overland dispersal from Africa, across Arabia, and on to India and SE Asia. The latter scenario has been hypothesized for tropical forest plant taxa based on fossil (Prive-Gill et al., 1999), or molecular data (Zhou et al., 2012), as land connections formed during the early to middle Miocene between Africa and Eurasia (Rögl, 1998, 1999) coinciding with a warm phase peaking in the Middle Miocene Climatic Optimum, c.17-15 Ma (Zachos et al., 2001). However, given the inferred timeframe of dispersal events in *Brachypterum* and *Derris* subsequent to the Middle Miocene Climatic Optimum, as well as their apparent high dispersal capabilities, long-distance dispersal by floating in water is the most likely process underlying the current palaeotropical distribution patterns in these two widespread mangrove-associated species. Samonds et al. (2012) indicate that most vertebrate mammals reached Madagascar from Africa main land till 20-15 Ma, after which prevailing sea currents shifted and favoured immigration from Asia, which coincides with the migration dates of *Derris* and *Brachypterum*.

Biogeographic patterns and diversification in tropical Asia—Ancestral areas in continental Southeast Asia and divergence in the early to middle Miocene have been inferred for the crown groups of *Brachypterum* and *Derris*. The inferred crown group age of *Derris* of c. 19.4 Ma (early Miocene) is considerably older than previous estimates (c. 8 Ma, Lavin et al., 2005), which can at least partially be explained by the much more robust taxon sample in the present study. Dispersal from the ancestral area in continental Southeast Asia to western and central Malesia has been inferred before the middle Miocene, and at least three dispersal events further eastwards to the Sahul Shelf (New Guinea and Australia) can be identified from the middle Miocene onwards. Most probable ancestral area reconstructions indicate six to seven dispersal events westwards to India from the middle Miocene onwards.

The crown group of *Brachypterum* originated during the transition between the early and middle Miocene (c. 16.5 Ma), and diversified in the Miocene to the late Pliocene. Similar to the patterns in *Derris*, the earliest dispersal events eastward across Wallace's Line to the Sahul Shelf were inferred in the Middle Miocene. At this time, the main phase of collision between Sundaland and the New Guinea/Australian plate had already taken place (Morley, 1998; Hall, 2002, 2009). The "west to east" dispersal found in *Brachypterum* and *Derris* is a pattern inferred in several other SE Asian plant taxa from various families, e.g. *Alocasia* (Araceae; Nauheimer et al., 2012), *Begonia* (Begoniaceae; Thomas et al., 2012), and *Pseuduvaria* (Annonaceae; Su and Sauders, 2009). The emergence of large parts of Wallacea, New Guinea and island arcs connecting these areas from the late Miocene onward (Hall, 2009), in combination with substantial coinciding eustatic sea-level changes (Miller et al., 2011) may have facilitated dispersal and possibly explains similar dispersal patterns observed in disparate taxa showing different life forms, generation times and dispersal vectors and capabilities (Hall, 2009; Richardson et al., 2012; Thomas et al., 2012).

Most diversification within the *Derris*-like genera, however, falls into a Miocene timeframe, similar to patterns inferred in *Aglaia* (Meliaceae, Muellner et al., 2008), whereas in *Pseuduvaria, Alocasia*, and *Begonia* divergence time estimates indicated that most diversification occurred in the Pliocene-Pleistocene (Su and Saunders, 2009; Nauheimer et al., 2012; Thomas et al., 2012). The latter three genera comprise many narrowly endemic species, and show apparently poor dispersability. In contrast to this, *Aglaia* and the *Derris*-like legume genera comprise many widespread species, and show apparently relatively high dispersability (see Muellner et al., 2008 for *Aglaia*). Water is the crucial dispersal vector of the three *Derris*-like genera, as most species show distinct adaptations to hydrochory (light, buoyant pods with indehiscent, water impermeable pericarps) and some species show adaptations to mangrove habitats (Giesen et al., 2007). Wind dispersal is also possible for some species, which grow in drier areas far from watercourses (mostly found in *Brachypterum*). Dispersability and niche breadth are likely crucial factors determining the effectiveness of colonization of newly available habitats, e.g., subsequent to emergence of new land in Malesia. The evolution of effective hydrochory and adaptations to salt water exposure in *Derris*-like genera explain why the oceanic water bodies separating western and eastern Malesia apparently have not been a strong barrier to dispersal in these taxa. Differences in dispersability may also have had a crucial impact, however, on the potential of hypothesized drivers of diversification such as mountain uplift and associated formation of topological heterogeneity or frequent eustatic sea-level changes in the Pliocene and Pleistocene to geographically isolate populations and cause (micro-) allopatric speciation. These drivers may have played an important role in diversification of poor dispersers such as *Begonia*, but the impact on diversification of effective dispersers similar to several extant *Aganope*, *Brachypterum*, and *Derris* species was likely much less pronounced.

Conclusions

The molecular divergence time estimates and ancestral area reconstructions provide insights into the processes underlying the historical assembly of Palaeotropical legume floras. The *Aganope*, *Derris* and *Brachypterum* crown groups likely originated in the Miocene. The ancestral area of the most recent common ancestor of *Aganope* included both Africa and Asia, and the PID in *Aganope* likely resulted from vicariance in the Miocene. The PIDs in *Brachypterum* and *Derris* in the Plio-Pleistocene and inferred origins of the respective crown groups on the Southeast Asian mainland are consistent with long-distance dispersal from Asia to Africa.

The results also corroborate previously identified cross-taxon biogeographic patterns in the Southeast Asian tropics. *Brachypterum* and *Derris* probably originated on the SE Asian mainland and dispersed multiple times westward to India, and eastward throughout Malesia, across Wallace's Line to New Guinea from the middle Miocene onwards. During this time the New Guinea/Australian plate collided with Sundaland and substantial islands started to emerge in Wallacea providing stepping stones for migration between the two areas. Preadaptation to a spectrum of environmental conditions and habitats as well as effective hydrochory may explain why the oceanic water bodies separating western and eastern Malesia were no major dispersal barrier for the *Derris*-like genera once new land became available in central Malesia (Wallacea).

Acknowledgements

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Table 6-1. The comparative Ancestral Area Reconstructions (AAR) using the Dispersal-Extinction–Cladogenesis (DEC) model implemented in Lagrange and S-DIVA (Statistical Dispersal-Vicariance) implemented in RASP. Numbers between brackets are the relative probability (RP) and the marginal probability (MP) for Lagrange and S-DIVA, respectively. Only some interesting nodes and the probability which is higher than 0.10 (for RP) or 10.00 (for MP) are presented. For Area designations see the legend to Fig. 6-2.

No. of Node	Node description	Lagrange (RP)	S-DIVA (MP)
15	Split between *Aganope*/*Ostryocarpus*	A|AC (0.26) A|A (0.11)	ABCDEF (91.76)
14	*Aganope* ancestral node (Split between Asian / African clade)	A|C (0.32)	ABCDEF (91.76)
10	Ancestral node of Asian *Aganope*	C|C (0.31)	BCDEF (91.76)
13	Ancestral node of African *Aganope* (Split between *Ostryoderris* /*Xeroderris*)	A|A (1.00)	A (100.00)
78	Split between *Brachypterum* calde /*Fordia* + *M. pinnata* clade	C|C (0.27)	BCDEF (52.44)
77	Ancestral node of *Brachypterum*	C|C (0.23)	C (52.45) ABCDEF (23.74) ABCEF (23.74)
70	Split between *B.* "*eriocarpum*" / tree clade	C|C (0.81)	C (100.00)
69	Ancestral node of "tree" *Brachypterum*	C|C (0.18)	C (100.00)
68	Split between widespread *B. robustum* and endemic *B. cumingii*	C|E (0.25) BCD|E (0.20) CD|E (0.20) BC|E (0.15)	CDE (25.00) BCDE (25.00) CE (25.00) BCE (25.00)
75	Ancestral node of species endemic to New Guinea and Australia	F|F (0.99)	F (100)
71	Split between widespread *B. scandens* and endemic *B.* "*philippinensis*"	ABCDEF|E (0.45) ABCDF|E (0.12) BCDEF|E (0.10)	more than 20 possible scenarios MP with less than 10.00
79	Split between *Derris* clade and other close relatives	C|C (0.19)	BCDEF (64.35) ABCDEF (35.64)
65	Ancestral node of *Derris* sensu Sirichamorn et al. (2012b and unpublished)	C|C (0.62) D|DE (0.16)	C (99.97)
50	Ancestral node of the first lineage of *Derris* (DI) leading to *Paraderris* clade	D|C (0.33) C|C (0.32)	CD (98.17)
48	Ancestral node of *Paraderris* sensu Geesink (1984)	C|C (0.32) C|E (0.29) C|CE (0.11)	C (51.85) CE (48.14)
64	Ancestral node of the second lineage of *Derris* (DII), consisting most species of *Derris* sensu Geesink (1984)	C|C (0.93)	C (100.00)

No. of Node	Node description	Lagrange (RP)	S-DIVA (MP)
60	Split betwen *D. laxiflora* and *D. rubrocalyx*	C|D (0.32) CD|F (0.25) C|DF (0.24)	CF* (100.00) (only when CF was allowed)
56	Split betwen *D. spanogheana* and (*D. trifoliata* + *D.* "*laotica*") clade all with RP less than 0.10	more than 60 possible scenarios	D, CD, CDE (all 33.33)
55	Split between widespread *D.trifoliata* and endemic *D.* "*laotica*"	ABCDEF|C (0.3496) BCDEF|C (0.1386)	17 possible scenarios with all MP less than 10