A dated phylogeny of the palm tribe Chamaedoreeae supports Eocene dispersal between Africa, North and South America

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Received 9 July 2007; revised 14 September 2007; accepted 13 October 2007
Available online 22 October 2007

Abstract

The palm tribe Chamaedoreeae reaches its higher diversity in Central America, however, its distribution ranges from the north eastern part of Mexico to Bolivia with a disjunction to the Mascarene Islands in the Indian Ocean. The disjunct distribution of Chamaedoreeae is generally considered a result of Gondwana vicariance and extinction from Africa and/or Madagascar. However, latitudinal migrations and their role in shaping the distribution of this tribe in the Americas have been largely overlooked. In this study we used seven plastid and two nuclear DNA regions to investigate the phylogenetic relationships and biogeography of the Chamaedoreeae. The resulting phylogeny fully resolved the generic relationships within the tribe. The exact area of origin of the tribe remains uncertain, but dating analyses indicated an initial diversification of the Chamaedoreeae during the Early Eocene, followed by long distance dispersion to the Mascarene Islands in the late Miocene. The radiation of Hyophorbe could have taking place on islands in the Indian Ocean now submerged, but its former presence in Africa or Madagascar cannot be ruled out. At least two independent migrations between North and South America predating the rise of the Panama isthmus need to be postulated to explain the distribution of Chamaedoreeae, one during the Middle Eocene and a second during the Miocene. Whereas the traditional interpretation of distribution of Chamaedoreeae species assumes a west Gondwana origin of the group, the findings presented in this paper make it equally possible to interpret the group as a primarily boreotropical element.

Keywords: Antilles; Arecaceae; Boreotropics; Caribbean geology; Central America; Chamaedorea; Dispersal; Gaussia; Gondwana break-up; Hyophorbe; Eocene land bridges; Latitudinal migrations; Mascarene Islands; Neotropics; Panama isthmus; Synechanthus; Wendlandiella; ndhF; matK; trnL-trnF; trnD-trnT; rps16 intron; PRK; RPB2

1. Introduction

Explaining disjunct distribution is an important aim of biogeography. Following the 1960s general acceptance of the theory of continental drift, vicariance became the predominant driver in historical biogeography. However, an increasing number of studies using dated phylogenies indicate that dispersal and not Gondwana vicariance often has been the driving force shaping the distribution of plant groups in the southern hemisphere (Davis et al., 2002; Muellner et al., 2006; Renner, 2004a; Sanmartín and Ronquist, 2004; Zerega et al., 2005), although Gondwana vicariance still holds in a number of cases (Sytsma et al., 2004). Proposed explanations for these findings include interplate dispersal routes across now submerged land bridges or island chains (Morley and Dick, 2003) or dispersal in sea water or on floating mats of vegetation following ocean currents and predominant wind directions (Renner, 2004a; Zhang et al., 2007). In the palm family, dated phylogenies of the Cocoseae (Gunn, 2004) and the Ceroxylloideae (Trénel et al., 2007) both indicate Eocene trans-oceanic dispersal between America and Africa. These results are also corroborated in a dated phylogeny of the palm subfamilies Ceroxylloideae and Arcoideae presented
by Savolainen et al. (2006). The dismissal of the Gondwana vicariance hypothesis for these palm groups raises the question of whether Eocene interplate dispersal is a general explanation for amphicontinental disjunctions in the palm family and which dispersal routes are most likely to explain such events.

One of the most extreme examples of disjunct distribution within the palm family is presented by the tribe Chamaedoreeae consisting of four genera occurring in Central and South America and one endemic to the Mascarene Islands in the Indian Ocean. Included in the tribe is the largest Neotropical palm genus Chamaedorea, with 107 species ranging from Mexico to Bolivia (Govaerts et al., 2006). The genus has two pronounced centres of diversity in Central America, the first in the southern part of Mexico and Guatemala, and the second in the mountains of Costa Rica and Panama (Hodel, 1992, 1999). The other Neotropical genera are less diverse and have more restricted ranges. Gaussia contains five species distributed in Mexico and the Caribbean region (Greater Antilles). Synchactus includes two species ranging from Mexico to Ecuador along the Pacific Ocean. Wendlandiella with a single species is endemic to western Amazon region (Henderson et al., 1995).

The last genus of the tribe, Hyophorbe, contains five species found on different islands of the Mascarene Archipelago (Uhl and Dransfield, 1987). All are listed as critically endangered, and one of them, H. amaricaulis is recognized as the most endangered plant species in the world, having only one remaining living individual in the wild (IUCN, 2006). Hyophorbe species are popular ornamental palms owing to their bottle shaped trunks and are widely cultivated in tropical gardens throughout the world. Also Gaussia and Chamaedorea include popular ornamental palms. Species of the latter genus are also used for cutting leaves for floral arrangements and dietary uses, a practice which has led to a poor conservation status of some species.

Moore (1973a,b, 1978) suggested a West Gondwana origin of the Chamaedoreeae, with a subsequent extinction of the African members of the tribe, possible caused by climatic changes on the continent, and with an arrival to the Mascarene Islands either directly from Africa or via Madagascar. Bjorholm et al. (2006) proposed that initial diversification of the subfamily Arecoideae, to which Chamaedoreeae belongs, occurred in the Neotropics where the subfamily has a long history. New insight into the phylogeny of the palm family based on addition of the plastid region ndhF to the dataset of Asmussen et al. (2006) indeed indicates that Chamaedoreeae diverged early in the history of the areoids (Conny Asmussen-Lange, unpublished data).

The radiation of Hyophorbe is assumed to have happened in situ on the Mascarene Islands (Savolainen et al., 2006). Strong evidence indicates that taxa, which colonize ocean archipelagos often speciate by adaptive radiation (Emerson, 2002; Francisco-Ortega et al., 1996; Gillespie, 2004). Our knowledge about the actual historical process of colonization in most archipelagos is, however, still sparse. In the case of the Mascarenes few studies exist. For reptiles such as geckos, tortoises, and skinks, a single colonization of the Mascarenes has been proposed, followed by an in situ radiation (Austin and Arnold, 2001; Austin et al., 2004). Australia and Madagascar have been proposed as the two areas of origin of reptile lineages represented in the Mascarenes. Whereas, long distance dispersal from Africa to the Mascarenes has been shown for the family Psiloxylaceae, part of the Myrtales (Sytsma et al., 2004).

Within the Neotropical Chamaedoreeae it is clear that latitudinal migrations must have occurred. Understanding ancient events of dispersal between North and South America is a complex issue that does not only involve the existence of possible bridges between the land masses but also the past climatic conditions of the region and its orogenetic history. Palm groups occurring in Central America and the Caribbean have complex origins, with a mixture of boreotropical clades and Gondwana/south American groups (Bjorholm et al., 2006). A close relationship exists between certain Asian/Eurasian taxa and boreotropical clades present in Mexico, Central America, and the Caribbean, e.g., in Coryphoideae (e.g., Zona, 1990; Dransfield et al., 2005). Some Coryphoid lineages also managed to disperse into South America. Repeated migration between North and South America has likewise been proposed for subfamily Arecoideae, with events dating back to at least the early Tertiary (Bjorholm et al., 2006). To explain the ancient exchange of biota between North and South America it has been proposed that various land bridges existed for shorter or longer periods prior to the formation of the Panamanian isthmus ca. 3 My ago. These include a very old bridge in the Cretaceous-Palaeocene (Iturralde-Vincent and MacPhee, 1999); a more recent fragmented bridge involving the proto Antilles during the Middle Eocene (Graham, 2003; Pennington and Dick, 2004); and a brief connection involving the submerged Aves ridge in the Eocene-Oligocene boundary (Iturralde-Vincent and MacPhee, 1999).

The tribe Chamaedoreeae has been demonstrated convincingly to be monophyletic (Asmussen and Chase, 2001; Asmussen et al., 2006) but inter-generic relationships within the tribe remain incompletely understood. So does species level phylogeny within its major genera in spite of recent attempts at analysing this using plastid or nuclear DNA markers. Thomas et al. (2006) used two nuclear DNA regions (PRK and RPB2) to reconstruct the phylogenetic relationships within the largest genus Chamaedorea. They found that most the proposed Chamaedorea subgenera (Hodel, 1992) are non-monophyletic. This result has since been corroborated by plastid DNA data (matK, rps16 intron, trnl-trnF, and ndhF; Cuenca and Asmussen-Lange, 2007). In both analyses, species groups not proposed by morphological studies have been identified. In addition, a few clades with well delimited geographic distributions have been recovered, e.g., the “South American”
clade constituted by *C. linearis* and *C. fragrans* (Thomas et al., 2006). So far, no attempt to analyse the biogeography and diversification pattern of the tribe using molecular dating techniques has been undertaken.

Here we use plastid and nuclear DNA sequence data to reconstruct the phylogenetic relationships within Chamaedoreeae and to investigate divergence times within the tribe. The information is used to evaluate existing biogeographic hypotheses for the diversification of Chamaedoreeae regarding to (a) the disjunction between the Neotropics and the Indian Ocean, and possible migratory routes involved with the arrival of *Hyophorbe* to the Mascarene Islands, (b) the time and place of the radiation of extant *Hyophorbe* species, (c) latitudinal migration of the Neotropical members of Chamaedoreeae in relation to proposed land bridges between North and South America, and (d) the diversification of *Gaussia* in the Caribbean regions, particularly the relationship between Central American and Antillean species.

2. Materials and methods

2.1. Taxon sampling

For the phylogenetic analysis 40 species of tribe Chamaedoreeae were sampled (Appendix 1 in supplementary material), including all species of *Hyophorbe, Gaussia, Synechanthus,* and *Wendlandiella,* and 27 of the 107 species of *Chamaedorea* recognized by Govaerts et al. (2006). The latter included representatives of seven of Hodel’s (1992, 1999) eight subgenera of *Chamaedorea.* In some few cases, problems with amplification of lack of plant material made it necessary to use more than a single accession for some species. Tribe Chamaedoreeae is nested convincingly within subfamily Arecoideae (Asmussen et al., 2006). Therefore, representatives of six other tribes of the Arecoideae were selected as outgroup (Appendix 1). The final taxon sample for dating analyses thus consisted of 46 species including six in the outgroup. That dataset will be referred to as M1 in the rest of this paper.

No reliable fossil record exists for tribe Chamaedoreeae. For dating analyses the M1 sample was therefore expanded to include a number of taxa belonging to related tribes within the Arecoideae, particularly the Cocoseae, where fossil records are more abundant and reliable. Dating methods are sensitive to uneven sampling across groups (Linder et al., 2005). We therefore reduced the number of *Chamaedorea* species in the dataset from 27 to 15, leaving only species where all the sequences were made from a single accession and taking care to include at least two species from each well supported clade. We also removed *H. amaricaulis* from the matrix because the RPB2 and half of the PRK sequence were unavailable for this taxon. The final taxon sample for dating analyses thus consisted of three species from subfamily Coryphoideae, *Nypa fruticans* from subfamily Nypoideae, six members of subfamily Ceroxyloideae, and 62 members of subfamily Arecoideae of which 27 were Chamaedoreae. Representatives of all tribes in the Arecoideae were included. Among the non-chamaedoroid arecoid taxa there were one species from each of the four monogenic tribes Oranieae, Sclerospermeae, Roystonae, and Podococceae, three species from tribe Iriarteeae, 19 species from tribe Cocoseae representing all of its three subtribes, one species from each of the tribes Manicarieae, Geonomateae, Leopoldinieae, and Pelagodoxaeae, and three species from tribe Areceae (Appendix 1 in the supplementary material). This dataset will be referred to as M2 in the rest of this paper.

2.2. DNA extraction, PCR amplification, and sequencing

We sequenced the plastid region *trnL-trnF* and two nuclear DNA regions: the one between the second and third intron of the phosphoribulokinase gene (PRK) and the intron 23 of the second largest subunit of the RNA polymerase II (RPB2). In addition, sequences from four plastid DNA regions (*matK, ndhF, trnD-trnT,* and *rps16* intron) generated by Cuenca and Asmussen-Lange (2007) were included in the analysis. Total genomic DNA was extracted using either the DNeasy Plant Mini Kit (Qiagen, Crawley, West Sussex, UK) or a modification of the CTAB protocol of Doyle and Doyle (1987). The *trnL-trnF* region was amplified and sequenced following the protocol of Baker et al. (1999) and Asmussen et al. (2000). The PRK region was amplified using a two-step PCR procedure. In the first amplification, primers prk488f and prk1167r were used (Lewis and Doyle, 2002). The reaction was performed in 15 µl total volume using 13.5 µl of PCR ready mix (Ampliqon, Denmark), 2.0 mM MgCl₂, 5 pmol of each primer and approximately 25 ng of template DNA. The amplification conditions were: initial denaturalization at 94 °C for 5 min, 32 cycles of 1 min at 94 °C, 1 min at 55 °C, and 1 min at 72 °C, with a final extension step of 72 °C for 5 min. The result of this amplification was used as template for the second amplification. The second amplification was performed using the internal primers prk717f and prk969r (Lewis and Doyle, 2002) in a 50 µl total volume using 45 µl of PCR ready mix, 2.0 mM MgCl₂ (Ampliqon, Denmark), 10 pmol of each primer, and 2 µl of template. The PCR conditions were the same as in the first amplification and the reactions were carried out in an MJ Research thermocycler. The RPB2 region was amplified using the protocols of Thomas et al. (2006) and Loo et al. (2006).

2.3. Phylogenetic analyses of the tribe Chamaedoreeae

Consensus sequences from all taxa in the M1 sample were aligned visually in BioEdit 7.0.1 (Hall, 1999). Regions where the alignment could not be assessed unambiguously were excluded from the analysis, as suggested by Asmussen and Chase (2001) and Hahn (2002). Within PRK we excluded from the analyses a poly-A region at position 205–228 in the alignment and a poly-G region at position
365–387, because both regions could not be aligned unambiguously. The same occurred with a poly-C region positioned from nucleotide 378–442 in the alignment of RPB2. In the plastid DNA regions we excluded a total of 127 characters, most of them belonging to the intergenic spacers heavily populated by indels and repetitive sequences.

The two nuclear DNA regions PRK and RPB2 were analysed separately. In addition a concatenated matrix including the five plastid regions (trnL-trnF, rps16, ndhF, matK, and trnD-trnT) and the two nuclear DNA regions was constructed and analysed. To test for compatibility among DNA regions a partition homogeneity test (PHT) was performed in PAUP with heuristic search and 1000 replicates with 10 random stepwise addition sequences per replicate, saving maximum five trees per replicate. The PHT was performed for the combined plastid DNA data set tested against each of the nuclear DNA regions, and the two nuclear DNA regions were tested against each other. In all cases the PHT showed incongruence among datasets ($p < 0.05$). To explore the level of incongruence among different partitions we used instead Partition Bremer Support (PBS, Baker and DeSalle, 1997) as calculated in the program TreeRot V.2. (Sorenson, 1999) using a heuristic search with 100 replications, random stepwise addition to construct a starting tree for each replication, and saving maximum 500 trees in each replication. Decay indices (DI) for the combined tree were calculated by the same procedure.

Parsimony analyses were carried on in PAUP* version 4.0b10 (Swofford, 2002), excluding all parsimony-uninformative characters and with equal character weighting. An initial heuristic search was carried out doing 1000 replicates and holding maximum 10 trees per replicate. Random stepwise addition was used to get the starting trees. Branch swapping was performed using the tree-bisection-reconnection (TBR) algorithm with MulTrees in effect. Branches with maximum length equal to zero were collapsed. A second search was performed by TBR swapping the trees saved from the 1000 replicates in the first search. The trees in this second search were swapped until completion. Bootstrap values were calculated based on 1000 bootstrap replicates, using the same search strategy as in the heuristic search, but with only 10 random stepwise addition sequences performed per replicate.

Bayesian analysis was performed using MrBayes v. 3.1 (Huelsnbeck and Ronquist, 2003; Ronquist et al., 2005). The different models of DNA substitution were evaluated using ModelTest (Posada and Crandall, 1998). The GTR+G (General time reversible (Tavaré, 1986) model+ gamma distribution) was used for all partitions, except RPB2 where the HKY+G model was selected. MrBayes was run with two simultaneous analyses with four parallel chains in each, sampling one tree for each 100 generations, starting with a random tree, and using the default priors, except for the temperature value that was set to 0.1. The program was run for 3 million generations at which point the standard deviation of split frequencies between the two simultaneous analyses was less than 0.005. Samples obtained prior to stabilization of the parameter estimates around a likelihood maximum were discarded (burn-in; 2500 samples).

2.4. Construction of the phylogenetic trees used in the molecular dating

Consensus sequences for all taxa in the M2 taxon sample were aligned visually in BioEdit 7.0.1 (Hall, 1999). The same criteria were employed for exclusion of nucleotide characters as for the M1 matrix. This included several repetitive regions within PRK and RPB2 (188 bases) and 289 characters in the plastid dataset. For reasons given above a concatenated matrix including the five plastid DNA regions (trnL-trnF, rps16, ndhF, matK, and trnD-trnT) and the two nuclear DNA regions (PRK and RPB2) was constructed without a previous PHT. We assume that simultaneous analyses based on different loci will detect the week signal in single datasets (Renner, 2004a). A maximum parsimony (MP) analysis of the concatenated matrix was performed following the same strategy described for the M1 sample. A likelihood ratio test of rate constancy was performed applying the evolutionary model and parameter estimates calculated by ModelTest using the Akaike information criterion for model choice. A strict molecular clock was rejected; therefore local clock methods allowing for varying substitution rates in different clades were employed. These included the Bayesian approach to divergence time estimation using the baseml of the PAML 3.14 packages (Yang, 1997) and multidivtime (Kishino et al., 2001; Thorne and Kishino, 2002; Thorne et al., 1998); and the non-parametric rate smoothing approach, penalized likelihood (PL; Sanderson, 1997), as implemented in the program r8s v. 1.7 (Sanderson, 2004).

Bayesian dating requires as input a fixed topology in addition to the nucleotide sequences of each partition. For the topology we used one tree selected at random from the most parsimonious trees obtained from the heuristic search of the M2 matrix. The parameters of the nucleotide substitution model in each dataset were estimated using baseml. To run baseml all characters with a gap or an ambiguity in at least half of the taxa in the analysis were excluded manually from the alignment, following Renner (2004a,b). This left a total of 6498 characters in the concatenated dataset. The programs paml2modelinf and estbranchs, both part of multidivtime (Thorne and Kishino, 2002) were used to estimate the branch lengths for the rooted tree and the variance–covariance matrix for the datasets. This information is needed for multidivtime to calculate the node divergence time under the fixed topology. Multidivtime was run with the Markov chain set to 100,000 trees sampled each 10th generation and with a burn-in of 1000 trees. The a priori expected number of time units between tips and root was set to 1.7, where one time unit corresponds to 50 million years, and the standard deviation
was set to 0.1. This estimate was based on the oldest unambiguously identified palm fossil (see root calibration). The a priori rate of evolution at the root node was set to 0.016 corresponding to the average of the distance among the root node and the tips divided by the number of time units (Renner 2004a, b). The a priori value of the Brownian motion parameter (nu), which determines the permitted rate change between ancestral and descendendent nodes, was set to 0.7 following the recommendations in the program manual.

The topology input to r8s was the same as in the Bayesian analysis. Branch lengths were estimated by maximum likelihood using the model of DNA substitution for the concatenated dataset found by ModelTest (GTR+gamma distribution rate). Nypa fruticans was used only to root the tree during analysis and pruned in r8s. For the PL analysis the optimal smoothing parameter (λ) was selected using a cross validation procedure (Sanderson, 2002) with eight categories among a range from 1 to 1000 for λ. The cross validation analysis indicated a smoothing factor (λ) of 32 for the M2 matrix (χ^2 = 424.1) This smoothing factor was used to calculate nodal ages in the penalized likelihood analysis. To calculate the substitution rates along the tree the Truncated-Newton algorithm (TN) was chosen. Confidence intervals were using a cut off value of four.

2.5. Fossil calibration points

As earlier stated no reliable fossils pertaining to the tribe Chamaedoreeae are known. Palm-leaves assigned to Chamaedorea (C. danai Berry), have been described from the Early Eocene in Florida (Berry, 1916), however, their identity needs to be confirmed (Uhl and Dransfield, 1996; Kaul, 1951), therefore, this fossil was not used to calibrate our dating analyses. Several related tribes in the Arrecioideas, however, have a fossil record providing potentially useful calibration points for phylogenetic dating.

2.5.1. Tribe Cocoseae

This tribe has one of the most complete fossil records within the palm family. A Cocoseae endocarp, Cocos sahnii, was reported from Kapurdi Rajasthan (Guleria et al., 1996; Kaul, 1951) in a formation dated to Lower Eocene (50–55 My; Rana et al., 2005). In addition, Cocos intertrappeanis (Patil and Uphadhye, 1984) and cf. Cocos (Sahni, 1946; Tripathi et al., 1999) have been found in Deccan Intertrappean beds. The age of these beds is not homogeneous, with deposits occurring from the Upper Cretaceous (140 My) to the Early Eocene (ca. 40 My). Dating of the fossils is therefore uncertain. The records do, however, confirm the presence of Cocoseae palms by the end of Eocene. We used this information to constrain the crown node of Cocoseae at minimum 50 My, a conservative estimate also used by Gunn (2004). The fossil record associated with subtribe Bactridinae includes two palm fruits from the Middle Oligocene in Puerto Rico, Palnocraron acrocomoides, which has been compared with Acrocomia crispa, and Bac-
RI = 0.88). The strict consensus tree recovered the tribe Chamaedoreeae (BS = 100%, DI = 20, Fig. 1) and each of the genera as monophyletic (BS = 100% in each case, DI ranged from nine steps in Gaussia to 17 steps in Hyophorbe). Two monophyletic groups were identified within Chamaedoreeae, one consisted of Hyophorbe and Wendlandiella (BS = 79%, DI = 2), and the other of Chamaedorea, Synechanthus, and Gaussia (BS = 68%, DI = 1); however, the relationship among these last three genera remains unresolved. Three lineages are recovered within Chamaedorea, the first including the clade formed by C. linearis and C. fragrans (BS = 100%, DI = 14), the second including all the species of subgenus Eleutheropetalum together with C. elegans (BS = 100%, DI = 10), and the third including all the remaining Chamaedorea species included in the analysis (BS = 53%, DI = 1).

3.2. Analysis of PRK

PRK sequences ranged from 531 base pairs in Gaussia maja to 638 base pairs in Manicaria saccifera. The aligned matrix consisted of 706 characters of which 47 characters
were excluded from analysis due to difficulties in assessing homology in alignment. Of the remaining 659 characters, 140 (21.2%) were potentially parsimony informative. The MP analysis of PRK sequences resulted in 30 MP trees (length = 245 steps, CI = 0.75, RI = 0.92). The strict consensus tree recovered Chamaedoreeae, *Hyophorbe*, *Gaussia*, and *Chamaedorea* as monophyletic (Fig. 2A). The relationships among genera were resolved with *Hyophorbe* as sister to the other four Chamaedoreeae genera (BS = 100%, DI = 6). *Wendlandiella* was placed as sister to the remaining three genera from the Americas (BS = 83) which were resolved with *Synechanthus* as sister to *Gaussia* and *Chamae-

![Fig. 2. (A) Strict consensus tree of the 30 most parsimonious trees resulting from an analysis of the nuclear region PRK (659 bases, 140 potentially informative characters, length = 245 steps, CI = 0.75, RI = 0.92). (B) Strict consensus tree of the eight most parsimonious trees resulting from an analysis of the nuclear region RPB2 (927 bases, 141 potentially informative characters, length = 268 steps, CI = 0.69, RI = 0.83). BS values are shown above the branches and DI values different than one below the branches.](http://example.com/fig2.png)
edorea with BS = 67%. Within the genus Chamaedorea, subgenus Eleutheropetalum was recovered as monophyletic (BS = 85%, DI = 2). Subgenus Stephanostachys was paraphyletic with C. pedunculata and C. pinnatifrons while subgenera Chamaedorea and Chamaedoropsis were both polyphyletic.

3.3. Analysis of RPB2

The RPB2 sequences ranged from 666 base pairs in Roystonea oleracea to 823 base pairs in Gaussia princeps. The aligned matrix consisted of 992 characters of which 65 were excluded from analysis due to difficulties in assessing homology. Of the remaining 927 characters, 141 (15.2%) were potentially parsimony informative. Analysis of RPB2 sequences resulted in eight MP trees (length = 268 steps, CI = 0.69, RI = 0.83). The tribe Chamaedoreeae and all its genera were resolved as monophyletic with a similar topology as in the PRK analysis but with slightly higher BS support (Fig. 2B). Chamaedorea subgenus Eleutheropetalum and Stephanostachys were both recovered as monophyletic with BS = 81% and 95%, respectively.

3.4. Combined analysis of plastid and nuclear sequences

The combined matrix of plastid and nuclear DNA sequences included 7395 characters, of which 239 were excluded a priori. Of the remaining 756 characters, 482 were parsimony informative. The MP analysis (Fig. 3) resulted in 85 trees (length = 906 steps, CI = 0.69, RI = 0.86). The topology of the Bayesian 50% majority rule consensus tree was identical to that of the MP analysis, except for the node (C. seifrizii, C. brachypoda), (C. linearis, C. fragrans), which was collapsed in the Bayesian tree. In both analyses all genera were recovered as monophyletic with high support (BS = 100%, PP = 1.0). Hyophorbe was placed as sister to the remaining members of the tribe (BS = 98%, DI = 9, PP = 1). The relationships within Hyophorbe were fully resolved with BS support of 80–94% at internal nodes. Wendlandiella was recovered as sister to the other three genera from the Americas (BS = 99%, DI = 9, PP = 1.0). Synechanthus was resolved as sister to a (Gaussia, Chamaedorea) clade with moderate support (BS = 72%, DI = 2, PP = 0.57).

Within Chamaedorea a limited number of species groups were recovered with high BS support, but the relationships among these groups were mostly unresolved. Chamaedorea microspadix was placed as sister to the remaining Chamaedorea species (BS = 65%, DI = 1, PP = 0.71). Subgenus Stephanostachys was recovered as paraphyletic. Chamaedorea elegans was firmly placed as sister to subgenus Eleutheropetalum (BS = 98%, DI = 10, PP = 1). Otherwise clades did not correspond to the subgenera previously defined in the genus.

Although the PHT showed incongruence among all partitions, the Partition Bremer support indicated only few nodes with a high level of incongruence. At the generic level, the placement of Gaussia as sister to Chamaedorea had low support by nuclear regions (PBS = 1 + 1) and it is slightly rejected by plastid data (PBS = 1). The position of Wendlandiella is strongly supported by nuclear data (PBS = 6 + 5) but slightly rejected by plastid data (PBS = 2). Otherwise incongruence related mostly to internal nodes in the genus Chamaedorea, most notably the clade formed by subgenus Stephanostachys together with C. pinnatifrons, C. costaricana, and C. pedunculata, where nuclear data support the clade with a PBS of 3.5 + 2.8 while plastid data reject it with a PBS of −5.3.

3.5. Divergence time analysis

The combined M2 matrix of seven DNA regions (matK, ndhF, trnD-trnT, rps16 intron, trnL-trnF, PRK, and RPB2) included 8480 characters, of which 477 were excluded a priori. Of the remaining 8003 characters, 887 (11.1%) were potentially parsimony informative. The MP analysis recovered 192 most parsimonious trees (length = 2400 steps, CI = 0.523, RI = 0.790). The relationships among members of Chamaedorea were the same in the analysis of the M1 and M2 matrices.

Penalized likelihood analysis of the M2 matrix estimated the stem of Chamaedorea to 71.9 My, whereas the crown node of tribe Chamaedorea was estimated to 53.2 My. The crown age for each genus was estimated to 20.1 My for Chamaedorea, 19.3 My for Synechanthus, 17.9 My for Gaussia, and 11.7 My for Hyophorbe (Table 1, Figs. 4 and 5). Analyses performed on the complete data matrix (reversing the exclusion of 12 Chamaedorea species to improve the balance of the taxon sample; results not shown) gave an identical value of the smoothing factor (λ = 32) and divergence time estimates for members of Chamaedorea varied between 0.5 and 1.7 My from the results obtained using the M2 matrix.

The Bayesian dating analysis, where the age of the root was freely estimated rather than fixed, in general resulted in older divergence time estimates than PL for all nodes. The age of the crown of subfamily Arecoideae was estimated to be 81.4 ± 4.8 My. The stem node of Chamaedorea was estimated to be 79.5 My old and the crown node 50 My. The crown nodes of the Chamaedorea genera were estimated to 21.2 My for Chamaedorea, 19.5 My for Synechanthus, 20.8 My for Gaussia, and 14.0 My for Hyophorbe (Table 1, Fig. 5). The age estimates for the deeper nodes in the phylogeny were slightly younger when all taxa were included, while those of the more recent nodes in general were slightly older (around 1 My older).

4. Discussion

4.1. Phylogenetic relationships of the tribe Chamaedorea

The present study is the first to provide a full resolution of the generic relationships among members of Chamaedorea based on molecular data. Analyses based in plas-
tid DNA sequences (Cuenca and Asmussen-Lange, 2007; the present paper) recovered *Hyophorbe* and *Wendlandiella* as sister genera, with moderate support (Fig. 1). Analyses based on nuclear DNA sequences placed *Hyophorbe* as sister of the remaining chamaedoroid genera, and *Wendlandiella* as sister to the other American genera within the tribe (Fig. 2; Thomas et al., 2006). This same topology is obtained when plastid and nuclear DNA data are combined (Fig. 3). On the combined analysis both, *Hyophorbe* as sister to the remaining genera of Chamaedoreae (BS = 98%) and *Wendlandiella* as sister to the remaining American genera, are recovered with high bootstrap support (98% and 99%, respectively, Fig. 3). We therefore consider the position of these genera fully resolved. The relationships among *Gaussia*, *Synechanthus*, and *Chamaedorea* are more problematic. The two studies cited above recovered either a polytomy among the three genera or placed *Gaussia* as sister to *Synechanthus* with low support. Our findings indicate that *Gaussia* and *Chamaedorea* are sister genera (Fig. 3) with moderate bootstrap support in the combined analysis of all data sets (BS = 72%). The association between *Chamaedorea* and *Gaussia* is striking,
since the two genera seem very different in their habitat, size, leaf, and root morphology, and reproductive system.

A similar result, however, is obtained when plastid and morphological data are combined (Argelia Cuenca, unpublished data).

This study also provides full resolution at species level in Hyophorbe and Gausia. We recovered Hyophorbe lagenicaulis, endemic to Mauritius, as sister to all other Hyophorbe species, followed by H. verschaffeltii from Rodrigues Island sister to the remaining three species. This result disagrees slightly with the monographic work of Hyophorbe by Moore (1978) who considered H. verschaffeltii to be the species with most ancestral character states. In our phylogeny Hyophorbe indica, an endemic to the youngest of the Mascarene Islands, Reunion, is embedded in a clade formed by two species occurring on Mauritius (H. amaricaulis and H. vaughanii), perhaps reflecting a recent migration from that island. For Gausia the findings presented here correspond to those of previous studies (Cuenca and Asmussen-Lange, 2007). The genus consists of two highly supported clades, corresponding to the two-continental species G. maya and G. gomez-pompea, once placed in the genus Opsiantra, and the three Caribbean species, respectively. Opsiantra and Gausia were united by Quero and Read (1986) as they found fruit size and shape to be the single consistent morphological difference between the two species groups. Although our results suggest that the continental and Caribbean species form two independent lineages, the weak morphological differentiation does not justify their separation into distinct genera.

Our study does not support the subgeneric division of Chamaedorea proposed by Hodel (1992) based on flower and inflorescence morphology. A few of Hodel’s subgenera, however, appear to constitute natural entities. Subgenus Eleutheropetalum is supported as monophyletic with 98% BS support. Subgenus Stephanostachys was recovered as paraphyletic with C. pinnatifrons, C. pedunculata, and C. costaricana, which is consistent with plastid DNA sequences, but not with nuclear data, which recovered Stephanostachys as monophyletic (see Thomas et al., 2006). Chamaedorea nicrospadix from subgenus Moreniella is placed as sister to the remaining Chamaedorea species with low support (BS = 65%, PP = 0.71), a result also obtained by Thomas et al. (2006). Otherwise resolution within Chamaedorea was low. A polytomy of five clades was recovered (Fig. 3), two of which were well supported. The two South American species C. linearis and C. fragrans were recovered as sisters, a result which is consistent with previous phylogenetic analyses (Cuenca and Asmussen-Lange, 2007; Thomas et al., 2006).

4.2. Biogeography of Chamaedorea

The results of our analyses placed the stem of the Chamaedorea at the end of the Cretaceous, from the Campanian to the Maastrichtian (70–85 My old; Table 1, Figs. 4 and 5), whereas its crown group was placed in the Lower Eocene (50 My, Table 1, Figs. 4 and 5). To explain the current distribution of the Chamaedorea, a West Gondwanan origin for the tribe has been suggested, with subsequent diversification following the Gondwana break-up and extinction of species in the bridging places such as Africa (Moore, 1973a,b, 1978; Uhl and Dransfield, 1987). The separation of the African and South American plates occurred by the end of the Albion (96 My), but it is known that plant dispersals were frequent across the newly formed Atlantic until the early Tertiary, possibly through a series of islands that remained emergent throughout this period (Morley, 2000). As expected, the basal diversification of the tribe (node A, Fig. 5) is clearly too young (40–60 My, Table 1) to be caused by the Gondwana break-up. A number of interplate dispersal routes for megathermal angiosperms, however, have been proposed to exist during the early terrestrial (Palaeocene–Eocene), facilitated by the combination of warm climates and relative proximity of the major continents (Morley, 2003; Muellner et al., 2006; Pennington and Dick, 2004). These include (1) a trans-oceanic dispersal route either along the mid-oceanic Rio Grande ridge, which could have been above sea level until the Oligocene (Morley, 2000), or along the more southern Walvis ridge and (2) a Boreotropical dispersal route across a North Atlantic land bridge connecting Europe and North America, as suggested for, e.g., Malphigiaeae (Davis et al., 2002). Both of these passageways could in principle have facilitated exchange of ancestral Chamaedoreaceae between the Old and the New World within the time-frame indicated by our dating estimates for the basal split in the tribe. These findings do not contradict the hypothesis stated by Moore (1978) that Hyophorbe is a lineage with a long evolutionary history whose ancestors possibly migrated to the Mascarene Islands from Africa or Madagascar, where they are now extinct. There is, however, no evidence for the presence of Hyophorbe or any other member of the Chamaedorea in Africa.

Trans-Atlantic dispersal between Africa and South America is well in line with the Amazon distribution of Wendlandiella. Under such a scenario there would have

Table 1

Ages in million years estimated for members of Chamaedoreeae based on a combined analysis of two nuclear DNA regions (PRK and RPB2) and five plastid DNA regions (maK, ndhF, rps16 intron, trnL-trnF, and trnD-trnT) and two dating methods implemented

<table>
<thead>
<tr>
<th></th>
<th>PL analysis</th>
<th>Bayesian dating</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chamaedorea (stem)</td>
<td>71.9 (64.6–74.4)</td>
<td>79.5 (70.4–89.2)</td>
</tr>
<tr>
<td>Chamaedorea</td>
<td>53.2 (38.7–54.3)</td>
<td>50.0 (39.9–60.0)</td>
</tr>
<tr>
<td>Hyophorbe</td>
<td>11.7 (5.6–15.4)</td>
<td>14.0 (7.5–23.5)</td>
</tr>
<tr>
<td>Wendlandiella (stem)</td>
<td>45.7 (32.5–49.6)</td>
<td>44.0 (34.1–54.5)</td>
</tr>
<tr>
<td>Synechanthus</td>
<td>19.3 (11.7–23.7)</td>
<td>19.5 (12.2–28.8)</td>
</tr>
<tr>
<td>Gausia</td>
<td>17.9 (10.2–22.6)</td>
<td>20.8 (12.7–30.4)</td>
</tr>
<tr>
<td>Chamaedorea</td>
<td>20.1 (12.7–24.9)</td>
<td>21.2 (13.9–30.1)</td>
</tr>
</tbody>
</table>

The ages correspond to the crown group of each clade, except where it is indicated otherwise. Confidence intervals obtained from the Penalized Likelihood analysis, as well 95% probability intervals from the Bayesian analysis are indicated for each node.
been an ancestral distribution of the American Chamaedoreeae in South America, followed by an early dispersal to North America of the ancestor of the *Synechanthus–Gaussia–Chamaedorea* clade. Independent redispersals from North to South would then have occurred in *Synechanthus warscewiczianus* and at least two subclades of *Chamaedorea* (*C. linearis–C. fragrans* clade; *C. pinnatifrons*; the latter not included in our taxon sample but shown by Thomas et al. (2006) and Cuenca and Asmus-Lange (2007) to belong to a clade different from the first two species). Alternatively, a Boreotropical dispersal route would assume a north hemispherical ancestral distribution of the American Chamaedoreeae with an early migration of the ancestor of *Wendlandiella* into South America. Again independent redispersals of *Synechanthus* and *Chamaedorea* species to South America would be
required. The two solutions are equally parsimonious when Chamaedoreeae is regarded in isolation, but assuming a Gondwana origin of the entire Arecoideae subfamily, as suggested by Moore (1973b), and a sub basal position of Chamaedoreeae within this subfamily, the former solution may seem more likely. An alternative hypothesis includes dispersal through Antarctica into southern Africa; however, this seems improbable in view of the ancient split between Africa and Australasia (Davis et al., 2002; Morley, 2000). No matter which scenario is favoured an early arrival and radiation of Chamaedoreeae in North America is strongly indicated by our results.

4.3. Hyophorbe

The Mascarene Islands are a group of volcanic islands of recent geological formation located 700–1400 km east from Madagascar. The age of Mauritius, the oldest of the Mascarene Islands, has been estimated to ca. 7.5 million years (McDougall and Chamalaun, 1969), however, it has been suggested that Rodrigues Island could be at least as old as Mauritius, with an age between 8 and 10 million years (Austin et al., 2004). Reunion, the third of the Mascarene Islands, is younger, formed around 3 My ago. If *Hyophorbe* arose in the Mascarenes, then the crown group of this genus should be younger than the archipelago. Bayesian analysis indicate an age of 14 My (95% = 7.5–23.5 My) for the crown group of *Hyophorbe* while PL suggest an age of 11.7 My (5.6–15.4 My; Table 1). Both estimates are older than the Mascarenes although the lower 95% confidence interval boundary just falls within the assumed age of the islands. If *Hyophorbe* is older than the Mascarenes then it could be explained by at least two non-exclusive scenarios. The first one involves independent migration of the ancestor of *H. lagenicaulis* to Mauritius followed by dispersal of the ancestor of the

Fig. 5. Cronogram for members of the tribe Chamaedoreeae. Relevant nodes with their 95% probability interval obtained in multidivtime in grey. The full circles indicate the age estimated using PL for each node. The small arrows indicate nodes collapsed in the consensus tree from the parsimony analysis. The ruler at the top of the figure indicate ages in My and the geological timescale. Node A indicates the separation of *Hyophorbe* from the American genera. Node B indicates the separation of the South American species of *Chamaedorea* from their Central American relatives.
remaining *Hyophorbe* species to Rodrigues. The second assumes the occurrence of the ancestral population of *Hyophorbe* on islands that are currently submerged. The Reunión hotspot, which created the Mascarene Islands 8 My ago, started its activity around 65 My and there is some evidence that the Mascarene plateau once formed an archipelago much bigger than the Mascarenes today. Most of these islands are now placed 10–40 m below sea level, and only small islets remain emerged (Austin et al., 2004; Turner and Klaus, 2005). It has been proposed that these now submerged islands have played an important role in the colonization of the Mascarenes, constituting places where old groups that are now endemics to these islands could have occurred (see for example the case of the *Phelsuga* gekos, Austin et al., 2004; and the dodo, Shapiro et al., 2002). These observations stress the potential risk of using the age of volcanic islands for phylogenetic dating.

### 4.4. Connections between North and South America

As earlier stated, our analyses indicate that the first diversification of the Neotropical Chamaedoreae members occurred with the separation of the lineage leading to *Wendlandiella* from that leading to *Synechanthus*, *Gaussia*, and *Chamaedorea* (Fig. 5). *Wendlandiella* is a monotypic genus distributed in the western Amazon region, whereas *Chamaedorea* and *Synechanthus* occur in both North America and western South America. *Gaussia* is exclusively North American. The basal diversification of the Neotropical genera corresponds in time to the Eocene, around 45 My ago (Figs. 4 and 5). This is consistent with the hypothesis of a pathway connecting South and North America when the proto Antilles collided with the Bahamas plate, and with the principal emergence of the islands in the Middle Eocene. This hypothesis suggested that the recently emerged Antilles could form a fragmented migratory pathway between both land masses around 50 My ago (Graham, 2003; Morley, 2003; Pennington and Dick, 2004). Otherwise the observed pattern of cladogenesis showed at least two subsequent interplate migrations. These include (1) a dispersal of *Synechanthus warscewiczi-anus* to the Choco region and western Ecuador and (2) migration of the (*Chamaedorea linearis, C. fragrans*) clade to western South America. With exception of *C. pauciflora*, also part of the (*C. linearis, C. fragrans*) clade (Thomas et al., 2006), the phylogenetic position of the other *Chamaedorea* species endemics to South American is not known at present. While interplate dispersal of *Synechanthus* could have been a recent event, the *Chamaedorea* species endemic to South America form a clade that is clearly too young (18.3 My, 95% interval = 12–26 My) to be explained by a migration through the Middle Eocene bridge (ca. 50 My ago) or by the Aves ridge bridge (ca. 35–33 My ago). *Chamaedorea* is too old to have used the Panamanian isthmus (ca. 3 My). This result mirrors those of several other studies indicating that a significant dispersal took place between Mesoamerica and South America prior to the closure of the Isthmus of Panama. Examples are Neotropical trees (Cavers et al., 2003), Valerianaceae plants (Bell and Donoghue, 2005), hummingbirds (García-Moreno et al., 2006), frogs (Crawford, 2003), reptiles (Savage, 1982), artropods (Zeh et al., 2003), and primary (Bermingham and Martin, 1998) and secondary (Concheiro-Pérez et al., 2007) fresh water fishes. Since these migrations in many cases involve groups that are not able to tolerate salt water, a dry-land connection between the continents during the Miocene has been inferred (Bermingham and Martin, 1998; Zeh et al., 2003; Concheiro-Pérez et al., 2007). Our results point in the same direction, even though no geological evidence for a connection has been produced so far. In addition, recent independent dispersals must have occurred in species such as *C. pinnatifrons*, the distribution of which includes both sides of the Panama isthmus (Fig. 3).

### 4.5. Diversification of *Gaussia* in the Caribbean

The colonization of the Greater Antilles from the central America by *Gaussia* is marked by the divergence of the clade formed by *G. princeps, G. attenuata*, and *G. spirituana* from the continental species ca. 20 My ago (Fig. 5). A physical connection between Cuba and the Yucatan Peninsula during the Early Eocene has been proposed (Graham, 2003), however, this event is clearly too old to explain the presence of *Gaussia* in the Greater Antilles. In the same way, the separations of Cuba from Hispaniola and of Hispaniola and Puerto Rico in the Early to Middle Miocene are also too old to explain the cladogenesis events among *Gaussia* species. Instead, the phylogenetic relationships among the three members of *Gaussia* occurring in the Greater Antilles could be explained by a migration via stepping stone, from the Eastern part of Cuba (Pinar del Rio, *G. princeps*) to the central part of the island (*G. spirituana, Sierra de Jatibonico*) and from there to Puerto Rico and possibly Hispaniola (*G. attenuata*).

### 4.6. Conclusion

The present study provides resolution of the phylogenetic relationships among the genera of tribe Chamaedoreae, albeit with low support for the sister relationship between *Gaussia* and *Chamaedorea*. The disjunction between the ancestor of *Hyophorbe* and the ancestor of the Neotropical genera of the tribe is found to be too young to be caused by a Gondwanan vicariance followed by displacement of *Hyophorbe* to the Mascarenes. Inter plate dispersal during the Late Eocene is needed to explain the presence of *Hyophorbe* in the Mascarene Islands. Evidence for Eocene inter-continental dispersal in Chamaedoreae match the findings in other recent studies of the palm family, e.g., within the Cocoseae (Gunn, 2004) and the Ceroxylaceae (Trénel et al., 2007). The estimated age for the crown node of *Hyophorbe* in combination with data on the geology of the Mascarene Islands suggest that the radiation of *Hyophorbe* may have taken place on
islands in the Indic Ocean now submerged predating the creation of the present day Mascarene archipelago.

Latitudinal migration has played an important role shaping the diversity and biogeography of the American Chamaedoreeae, as for several other plant and animal groups. At least two independent dispersals between North and South America predated the closure of the Isthmus of Panama are needed to explain the current distribution of the tribe: one during the Middle Eocene and the other in the Miocene. Species such as Syncnethanthes warscewiczianus and Chamaedorea pinnatifrons could have reached South America after the isthmus was formed. To explain the presence of Gaussia in the Caribbean Islands, we suggest a west–east stepping stone/sweepstake dispersal process starting in the Central America and ending in Puerto Rico. While the traditional interpretation of the Chamaedoreeae assumes a west Gondwana origin with subsequent dispersal to North America, our results make it equally possible to interpret the group as a primarily boreotropical element. Under this scenario, the present day distribution of Chamaedoreeae may reflect a retraction following the demise of the boreotropical palaeobiome resulting from Mid-Tertiary global cooling. A strong northern bias in the biogeography of the Syncnethanthes–Gaussia–Chamaedorea assemblage, together with a possible leaf macrofossil (C. danai) indicating an even more northerly distribution of chamaedoroid palms in the Eocene, is compatible with such interpretation.

Acknowledgments

We thank the Fairchild Tropical Botanic Garden, Biological Station Los Tuxtlas, Mexico, and Royal Botanical Gardens, Kew, where most of the material used in this study was obtained. Thanks to Carl Lewis for providing DNA samples and unpublished sequences of Hyophorbe. We thank Julissa Roncal, Ole K. Hansen, and Vinnie Deichmann for assistance in the laboratory. Thanks to Philipp Trenel for valuable discussions during the development of this project and to Scott Zona and William Baker for their comments to this manuscript. We specially thank Madeleine M. Harley for sharing with us unpublished information about the fossil record in Arecoideae, together with an extensive reference list on this topic. This project was funded by CONACyT Mexico and a Ph.D. scholarship from the Faculty of Life Sciences, University of Copenhagen, to Argelia Cuenca; and a grant from the Danish Natural Science Research Council to Conny Asmussen-Lange. The participation of F. Borchsenius was funded by the Danish Natural Science Research Coun-

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ympev.2007.10.010.

References


Francisco-Ortega, J., Jansen, R.K., Santos-Guerra, A., 1996. Chloroplast DNA evidence of colonization, adaptive radiation, and hybridization of the tribe: one during the Middle Eocene and the other in the Miocene. Species such as Syncnethanthes warscewiczianus and Chamaedorea pinnatifrons could have reached South America after the isthmus was formed. To explain the presence of Gaussia in the Caribbean Islands, we suggest a west–east stepping stone/sweepstake dispersal process starting in the Central America and ending in Puerto Rico. While the traditional interpretation of the Chamaedoreeae assumes a west Gondwana origin with subsequent dispersal to North America, our results make it equally possible to interpret the group as a primarily boreotropical element. Under this scenario, the present day distribution of Chamaedoreeae may reflect a retraction following the demise of the boreotropical palaeobiome resulting from Mid-Tertiary global cooling. A strong northern bias in the biogeography of the Syncnethanthes–Gaussia–Chamaedorea assemblage, together with a possible leaf macrofossil (C. danai) indicating an even more northerly distribution of chamaedoroid palms in the Eocene, is compatible with such interpretation.

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