**Phylogenetic relationships based on morphology among the *Diospyros* (Ebenaceae) species endemic to the Mascarene Islands**

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The *Diospyros* (Ebenaceae) species which colonized the Mascarene Islands, namely Mauritius, Reunion and Rodrigues, have been decimated over the years by human settlements. Of the 14 endemic species that have been described and collected for herbaria, *Diospyros angulata* is now believed to be extinct in Mauritius. The phylogenetic relationships of the 14 *Diospyros* species were determined using maximum parsimony analysis of 35 morphological characters. This analysis separated the Mascarene *Diospyros* into two major clades, with *D. revaughanii*, *D. egrettarum* and *D. leucomelas* grouped in the same strongly supported most basal clade while the rest of the species formed the other major clade. High bootstrap values were obtained for the sister species *D. angulata* and *D. boutonania*, and the clade clustering the upland species *D. neraudii*, *D. nodosa* and *D. pterocalyx*. There was also relatively strong support for the clade comprising *D. hemiteles* and *D. melanida*, which are located in mid altitude regions. These results indicate that *Diospyros* species most probably colonized the coastal areas of Mauritius and then moved to mid altitude habitats before finally reaching the upland regions. There are also strong indications that *D. borbonica* and *D. diversifolia*, endemic to Reunion and Rodrigues, respectively, resulted from migrations from Mauritius. © 2006 The Linnean Society of London, *Botanical Journal of the Linnean Society*, 2006, 150, 307–313.

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**INTRODUCTION**

The Ebenaceae, a widespread family of woody dicot trees and shrubs, occurs mainly in tropical and subtropical regions. This family is divided into three main genera, namely *Diospyros*, *Euclea* and *Tetraclis* (Brummit, 1992). *Diospyros* species seem to be more prevalent in regions of Asia, Africa and Central to South America, while the genera *Euclea* and *Tetraclis* have been found to occur only in Madagascar, Eastern and Southern Africa (Cronquist, 1981; Ng, 1986). The genus *Diospyros*, represented by more than 350 species, is the most important both numerically and economically (Mallavadhani, Panda & Rao, 1998). Interestingly, this genus is the only representative of the Ebenaceae family in the Mascarene Islands namely, Mauritius, Reunion and Rodrigues. These islands are believed to be the results of massive submarine volcanic eruptions and are located approximately 700–900 km to the east of Madagascar in the Indian Ocean. Mauritius, the oldest of these islands, emerged some 8 million years ago (McDougall & Chamalaun, 1969), followed by Reunion which has an estimated 3 million years of existence (McDougall, 1971); the latest island to appear is the 1.5 million-year-old Rodrigues (McDougall, Upton & Wadsworth, 1965). Mauritius, situated at longitude 57°30’E and latitude 20°20’S, is some 150 km to the north-east of Reunion and 574 km to the south-west of Rodrigues. An estimated 70% of the Mascarene flora is thought to have originated from Madagascar and Africa (Cadet, 1977). While some of these species seem to closely resemble their Malagasy and African relatives, most of them have evolved to give rise to genera and species endemic to the Mascarenes (Cadet, 1984). Several vegetation surveys carried out over the years (Vaughan & Wiehe, 1937; Strahm, 1994; Page & D’Argent, 1997) have indicated that Mauritius harbours a diverse flora.
comprising at least 900 native species and ten endemic genera. The most recent survey estimates that less than 2% of the original Mauritian endemic plants survive in forest reserves and on mountain slopes (Page & D’Argent, 1997).

Prior to the arrival of Dutch settlers in 1598 Diospyros, commonly known as the ebony, were the dominant species of the native Mauritian forest (Pitot, 1905). However, during the 17th century, the Diospyros species, especially D. tessellaria, were overexploited for their excellent timber quality and black wood (Brouard, 1963). This resulted in a drastic reduction in the population sizes of these endemic plants. Over recent years, the Diospyros populations have been under severe threat due to increased demand for land space mainly for agriculture and urbanization. These species are now reduced to pockets of individuals located mostly in reserves and inaccessible areas. For the past several years, 12 endemic species have been identified in Mauritius: D. angulata Poir, D. boutoniana A.DC, D. chrysophylos Poir, D. egrettarum I.B.K. Richardson, D. hemiteles I.B.K. Richardson, D. leucomelas Poir, D. melanida Poir, D. neraudii A.DC, D. nodosa Poir, D. pterocalyx Bojer, D. revaughanii I.B.K. Richardson and D. tessellaria Poir. Unfortunately, in 2000 the last known individual of D. angulata went extinct, lowering the number of endemic Diospyros species to 11 (S. Venkatasamy, pers. observ.).

Morphological studies of the Mascarene Diospyros species were carried out as early as 1804 (Richardson, 1880), and these were identified and classified into 14 distinct species, 12 of which were found to be endemic to Mauritius, one to Reunion and another to Rodrigues. Although the Mascarene Diospyros species have been collected for herbaria, no information is available on their colonization patterns or adaptive radiation. As these species are endangered, there is an urgency to understand the biology of the remaining Diospyros species. Findings are likely to be invaluable for conservation and reforestation. Furthermore, the proven good quality of the timber of the Diospyros species, together with potential medicinal properties (Mallavadhani et al., 1998), strengthens the need to study these species at different levels.

The purpose of this study was to analyse selected morphological characters with a view to establishing the phylogenetic relationships within the Mascarene Diospyros species.

**MATERIAL AND METHODS**

For each species, 35 stable morphological characters were selected based on their presence in all the species and their potential for phylogenetic informativeness (Table 1). In the case of D. angulata measurements were taken from plant materials we collected in 2000, while other characters were obtained from herbarium (Mauritius Sugar Industry Research Institute: MSIRI) specimens as this species is now believed to be extinct in the wild. Data, which could not be obtained from live specimens of D. borbonica (Reunion), were complemented with measurements from herbarium (MSIRI) specimens. Measurements for D. kaki, which is a native of Asia, have been used as outgroup, as adult plants of this species are found in Mauritius with flowers and fruits that were readily available at the time of study.

The morphological characters (12 vegetative and 23 reproductive) were scored and a data matrix was constructed for each of the 14 species (Appendix). This morphological data matrix contained two taxa that were polymorphic for one character and 3% of missing data.

All characters were treated as independent, unordered and of equal weight. The phylogenetic analysis was performed with PAUP 4.0b. The most parsimonious trees were found using a heuristic search with 100 random stepwise additions and MULTREES option. Branch lengths for the trees were calculated using the ACCCTRAN (accelerated transformation optimization) option in PAUP. A strict consensus tree was then constructed from the most parsimonious trees obtained. Bootstrap analyses (100) using simple stepwise additions were conducted to examine the relative level of support for individual clades of the cladograms (Felsenstein, 1985).

**RESULTS**

Cladastic analysis of the morphological data generated five most parsimonious trees with a length of 125, a consistency index (CI) of 0.57, a homoplasy index (HI) of 0.43, a retention index (RI) of 0.63 and a rescaled consistency index (RC) of 0.36. One of the five trees was arbitrarily selected and shown with the synapomorphies along the branches in Figure 1.

The 14 Diospyros species are divided into two major clades. Clade 1 consists of D. chrysophylos, D. tessellaria, D. angulata, D. boutoniana, D. borbonica, D. diversifolia, D. neraudii, D. nodosa, D. pterocalyx, D. hemiteles and D. melanida. Clade 2 is made up of D. revaughanii, D. egrettarum and D. leucomelas. The relative positions of the species in clade 1 vary slightly among the five trees, while the order of clade 2 species is identical for all the cladograms. In three of the cladograms, D. chrysophylos and D. tessellaria are grouped as sister species in a clade which collapsed in the other two trees. Diospyros angulata and D. boutoniana are represented as sister species in all five trees and their relative positions only differ in one cladogram. The order of the minor clade consisting of D. neraudii, D. nodosa and D. pterocalyx is similar in
all the cladograms and these species are shown to be among the last to have evolved. *Diospyros hemitites* and *D. melanida* are also indicated as recent species except for one cladogram, where both sister species are shown to have appeared just before *D. chrysophyllos* and *D. tessellaria*. *Diospyros borbonica* in Reunion Island and *D. diversifolia* in Rodrigues Island seem to have emerged after the lineage consisting of *D. angulata* and *D. boutoniana* in all five trees. Figure 1 also provides some information on the accumulated morphological changes for each of the 14 Mascarene *Diospyros* species.

Clade 2 is supported by a cordate leaf base, leathery leaf texture, intermediate petiole thickness, shortest petiole length, cupuliform-cylindrical male flower calyx shape, aggregate female flower cluster, 7–8
stigmas, 20–24 stamnodes and 6–7 fruit calyx lobes (characters 8, 10, 11, 12, 15, 21, 25, 26 and 30, respectively). Within clade 2, the species *D. egrettarum* and *D. leucomelas* can be grouped together by the shortest fruit length and a calyx height of 10–15 mm (characters 29 and 31, respectively).

Within clade 1, the node leading to *D. angulata* and *D. boutoniana* is supported by the broadest leaf, thickest petiole, and longest leaf, petiole, flower calyx, fruit and fruit calyx (characters 6, 11, 5, 12, 14, 29 and 31). The clade consisting of *D. neraudii*, *D. nodosa* and *D. pterocalyx* is typified by nearly black bark and 16–26 stamens (characters 3 and 18), while the fruit calyx of *D. pterocalyx* is characterized by very pronounced wings (character 35). The species *D. chrysophyllos* and *D. tessellaria* share a number of similarities, namely, 9–15 stamens, the smallest corolla diameter in both male and female flowers, 2–10 stamnodes in the female flowers and the presence of dense hairs on the surface of the flower calyx (characters 18, 16, 24, 26 and 27). *Diospyros tessellaria* is, however, quite distinct and can be separated from the other species by having the smallest number of corolla lobes and fruit calyx lobes (characters 17 and 30), very fragrant flowers, fleshy fruits and no wings on the fruit calyx (characters 28, 34 and 35). *Diospyros chrysophyllos*, *D. tessellaria* and *D. boutoniana* have an ovoid male calyx (character 15), while *D. tessellaria*, *D. boutoniana* and *D. angulata* have characteristic black barks (character 1). *Diospyros diversifolia*, *D. hemiteles*, *D. melanida*, *D. neraudii*, *D. nodosa* and *D. pterocalyx* all have a solitary male flower cluster (character 13), while only the species *D. neraudii*, *D. nodosa* and *D. pterocalyx* are characterized by the absence of hairs on the surface of the flower calyces (character 27). *Diospyros borbonica*, together with *D. diversifolia*, *D. hemiteles*, *D. melanida*, *D. neraudii*, *D. nodosa* and *D. pterocalyx*, have cupuliform male and female flower calices in common (characters 15 and 22). *Diospyros hemiteles* and *D. melanida* typically have the largest male and female flower corolla (characters 16 and 24) and 20–24 stamnodes in the female flowers (character 26). *Diospyros melanida* can be distinguished by its greyish green leaves and largest female flower calyx diameter (characters 4 and 23). *Diospyros hemiteles*, on the other hand, exhibits the highest number of corolla lobes (character 17).

Figure 2 represents a strict consensus tree with bootstrap values of more than 50%. Bootstrap estimates were relatively good for some basal nodes, but low bootstrap values for the other nodes and the ambiguity in the exact order of some species resulted in the polytomies observed in the consensus tree.

According to Figure 2, there is strong support that the species *D. revaughanii*, *D. egrettarum* and *D. leucomelas* are among the most ancient and are morphologically different from the rest of the Mascarene species. Moreover, the upland species
D. neraudii, D. nodosa and D. pterocalyx are shown to be closely related. The consensus tree also suggests that D. hemiteles and D. melanida, which grow in the same habitat, can be considered as sister species while D. angulata and D. boutoniana seem to share many similarities. However, D. chrysophylllos, D. tessellaria, D. borbonica and D. diversifolia could not be placed in any exact order.

**DISCUSSION**

The Mascarene Diospyros species are all dioecious and can easily be differentiated from each other by their leaves and tree stature. The leaf colour and structures of the Diospyros species in Mauritius often vary regardless of the types of habitat. However, D. diversifolia has the smallest and narrowest leaves, which could be an adaptation to arid areas. Indeed, D. chrysophylllos, D. tessellaria, D. borbonica and D. diversifolia could have been the first pioneering species of Mauritius. Diospyros revaughanii has colonized areas ranging from low altitude habitats to marshy lands on the central plateau. This species has also shown a certain phenotypic plasticity in that it occurs either as a tree or as a shrub in the upland marshy areas. As its fruits are sweet scented and sticky, they may have been picked by or stuck to birds, thus dispersing the seeds over broader distances. Diospyros egrettarum is the only true coastal species, with fragmented populations occurring only on the eastern lowland regions and on a 25 ha islet (Ile aux Aigrettes) off the south-east coast of Mauritius. It is noteworthy that we have recorded the occurrence of leaky dioecy in individuals from two populations of the multistemmed D. egrettarum. Indeed, the same plant was observed to produce distinct male and female flowers on separate stems. Furthermore, these leaky
dioecious plants generated seeds that had a germination rate of approximately 30% (unpublished data). Therefore, the mechanism of leaky dioecy could have been a strategy to ensure that a single pioneer Diospyros plant had the ability to generate fertile seeds and establish a reproducibly viable population.

It is interesting to note that D. leucomelas and D. egrettarum, which exhibit a high degree of morphological similarity, are both located in close proximity in the remnant forests on the east coast of Mauritius. However, most of the populations of D. leucomelas are found in mid altitude areas. On the other hand, D. chrysophylllos, which exhibits the closest morphological resemblance to D. tessellaria, has been encountered in a few low to high altitude regions as isolated individuals. Diospyros tessellaria is the most widely distributed species, indicating adaptation to most of the ecological conditions of Mauritius in that it has been able to establish viable populations in regions of low, mid and high altitude. This broad distribution and significant population size is certainly linked to the fact that the fruits of D. tessellaria are fragrant and fleshy enough to be eaten and dispersed by the endemic bat, Pteropus niger. It should be noted that D. tessellaria is the only Diospyros species in the Mascarenes that bears fleshy and fragrant mature fruits, while the rest of the Mascarene species produce fruits that remain hard even when they are mature.

Diospyros boutoniana and D. angulata are the two species that have the broadest leaves in this genus. Although D. angulata and D. boutoniana have been found to be morphologically quite close, they do not share the same ecological habitats. Diospyros boutoniana occurs mostly in upland forests with only a few individuals inhabiting low and mid altitude areas, while the only plant representing D. angulata was located in a mid altitude region. However, as D. angulata was down to one single female individual, it is difficult to ascertain its real geographical distribution. Diospyros borbonica, which is endemic to Reunion Island, has been reported to occur only on the south-east coast of this island (Bosser et al., 1981), while D. diversifolia is endemic to Rodrigues Island and can be found as very fragmented populations in several locations. Given that Mauritius is the oldest Mascarene island (8 million years), and based on the phylogenetic analysis, it is tempting to speculate that the Diospyros group evolved and speciated in Mauritius until at some point in time, one species moved to the coastal region of Reunion Island (3 million years old) to give rise to D. borbonica. This event may have been followed by another migration to produce D. diversifolia in Rodrigues Island (1.5 million years old). The species D. hemiteles and D. melanida, which appear to be closely related (Figs 1, 2), are known to occur only in mid altitude regions. Despite the fact that the group D. leucomelas, D. tessellaria, D. hemiteles and D. melanida all occur in very close proximity in mid altitude habitats, they have a staggered flowering period so that no two species flower at the same time. It is therefore not surprising that hybrids have never been observed among these species, which are sometimes only a few metres apart.

The high altitude species most likely emerged to colonize the humid habitats on the central plateau of Mauritius. Diospyros pterocalyx, D. neraudii and D. nodosa all occupy the same niche in the upland forests of Mauritius. Like the above mid altitude species, they have developed reproductive barriers so as to remain as distinct species.

In essence, the phylogenetic trees obtained from morphological data support the notion that colonization of the Diospyros group most probably started in the coastal areas. Then, with speciation and adaptive radiation, the species moved to the mid altitude regions and finally, the upland species arose to colonize the humid habitats. The seeds of D. tessellaria, on the other hand, were most probably dispersed by the Pteropus niger (and other endemic bats, now extinct) some distance away from the mother plant, explaining the wide distribution of D. tessellaria over the whole island. Unfortunately, little is known of the eating habits of the now extinct herbivore species such as the flightless dodo (Raphus cucullatus), the red rail (Aphanapteryx bonasia) and giant land tortoises (Cylindraspis inepta, Cylindraspis triserrata), which could have contributed to the dispersal of Diospyros seeds to different niches.

Homoplasmy in some character traits from both vegetative and reproductive parts of Diospyros species has created ambiguities in the relative positions of a few species in the phylogenetic tree. It is hoped that future molecular analyses will clarify the effects of parallel or convergent evolution, and provide evidence on whether or not the shared derived characters arose independently in the different Diospyros species as adaptations to a common environment.

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APPENDIX

The morphological characters of the 14 Diospyros species listed were scored as described in the text and arranged in a data matrix for phylogenetic reconstruction:

| Species       | Characters | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 |
|---------------|------------|---|---|---|---|---|---|---|---|---|----|----|----|----|----|----|----|----|----|----|----|----|
| D. kaki       | 11112211001102000??01’012112110202 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| D. angulata   | 2000340100220012121011011212132011 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| D. borbonica  | 102023020001012111010121222211 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| D. chrysophylllos | 00212222000102101001100001122211 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| D. diversifolia | 1020000110021300100201221122211 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| D. egrettarum | 1130230011001212121211121110112211 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| D. hemiteles  | 102111221001100323021013031111012211 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| D. leucomelas | 113023001100121212121012120112211 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| D. melanida   | 105212121001100321201203311122211 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| D. neraudii   | 0010110200011022111110012212112111 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| D. nodosa     | 0,1111111200011211111101011212121011 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| D. pterocalyx | 0111001201011001111100101211110110 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| D. revaughanii| 0,1113022201110021211011112121211011 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| D. tessellaria| 20012200001001010011110000001010202 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |