REVIEW

Systematics and evolution of the Old World Ebenaceae, a review with emphasis on the large genus *Diospyros* and its radiation in New Caledonia

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In this review article, we provide an overview of the status of research on Old World Ebenaceae with an emphasis on the large genus *Diospyros*. The well-supported phylogenetic tree obtained from nucleotide sequences of multiple regions of plastid genome gave clear insights into the subfamilial classification of Ebenaceae. It supported inclusion of previously recognized genera such as *Cargillia*, *Gunisanthus*, *Maba*, *Macreightia* and *Tetraclis* in *Diospyros*. Endemic *Diospyros* spp. of New Caledonia have multiple origins. One of these clades has *c*. 21 species that are morphologically distinct and occupy different ecological niches, but they exhibit low genetic variation, leading to a lack of phylogenetic resolution. Analyses of whole plastid genome sequences did not greatly increase resolution or support for results of our previous plastid analyses. Geographical clustering of the individuals against a background of lower sequence divergence of the whole plastid genome could be due to transfer of plastid genomes during hybridization and introgression following secondary contact. However, > 8400 filtered single nucleotide polymorphisms (SNPs) from restriction site associated DNA sequencing (RADseq) confirmed species circumscriptions for this clade and produced well-supported phylogenetic relationships, pointing to an early regional clustering among populations and species. This supported allopatric speciation with respect to macrohabitat (i.e. climatic conditions) having had a role in the initial differentiation in the group. A later, more rapid radiation involved divergence with respect to microhabitat (e.g. soil preference). Although chromosome counts indicate that *Diospyros* spp. are consistently diploids with $2n = 30$, extensive variation in genome size has been observed, which is due to an increase of repeat elements, including LTR/gypsy. In Ebenaceae, pollen is heterogeneous, and palynological synapomorphies are traced at different taxonomic levels. Several new *Diospyros* spp. have recently been identified and documented from India, Thailand, China, Africa and New Caledonia. Taxonomic revisions have been completed for the Australian species, and synonyms are reported for some New Caledonian *Diospyros* spp.

KEYWORDS: chromosome number – genome size – plastid phylogenomics – RADseq.

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INTRODUCTION

Ebenaceae *s.l.* (*sensu* APG IV, 2016; sometimes treated as Ebenaceae *s.s.* and Lissocarpaceae) are a medium-sized pantropical family with the majority of species in Asia and the Indo-Pacific region (White, 1983). The greatest morphological diversity is in Africa and Madagascar (White, 1983; Wallnöfer, 2001, 2004). The family is the source of several economically important products, the most valuable being timber (ebony) and fruit (persimmons). In addition to *Diospyros ebenum* J.Koenig ex Retz., *D. japonica* Siebold & Zucc. is also used as a timber wood; *D. oleifera* W.C.Cheng. is used as source of tannin, and *D. lotus* L., which is related to *D. kaki* L.f., is cultivated for its fruits (Fu *et al.*, 2016). Ebenaceae are also conspicuous forest component of Africa and Asia (Judd *et al.*, 2002; Christenhusz, Fay & Chase, 2017). Infrafamilial classifications of Ebenaceae *s.s.* have been proposed by de Candolle (1844), Hiern (1873), Bakhuizen (1936–1955), White (1980, 1983, 1993a) and Singh (2005).

The previous classifications based on morphological and anatomical characters have been considered to be problematic; generic and infrageneric boundaries of each system have been different and much debated. The new classification of Ebenaceae *s.l.* based on molecular phylogenetics of six regions of plastid DNA (*atpB*, *matK*, *ndhF*, *trnK* intron, *trnL* intron and *trnL-trnF* spacer; Duangjai, *et al.*, 2018) supported recognition of two subfamilies, Lissocarpoideae and Ebenoideae, and four genera, *Lissocarpa* Benth.,

Euclea L., *Royena* L. and *Diospyros* L. (Fig. 1; Berry *et al.*, 2001; Wallnöfer, 2004; APG IV, 2016).

Lissocarpa is distinguished from subfamily Ebenoideae by the absence of an indumentum, subopposite bracteoles, triporate pollen grains and an inferior ovary. Although *Lissocarpa* displays some advanced (derived) characters (inferior ovary and corona), it lacks the 12-bp deletion in *matK* that is present in all members of Ebenoideae. This molecular feature is plesiomorphic and distinguishes the rest of Ebenaceae from *Lissocarpa*. The eight *Lissocarpa* spp. have been split into two sections, *Lissocarpa* and *Enho* B.Walln. (Wallnöfer, 2004), a result compatible with the molecular results.

In earlier classifications of Ebenaceae *s.s.*, different authors recognized varying numbers of genera. de Candolle (1844) recognized eight genera in Ebenaceae *s.s.*, *Cargillia* R.Br., *Diospyros*, *Euclea*, *Gunisanthus* A.DC., *Maba* J.R.Forst. & G.Forst., *Macreightia* A.DC., *Rospidios* A.DC. and *Royena*. When compared with molecular results only two genera, *Euclea* and *Royena*, are monophyletic and independent from *Diospyros*, and the other genera of De Candolle are embedded in *Diospyros*. Hiern (1873) recognized five genera, *Diospyros*, *Euclea*, *Maba*, *Royena* and *Tetraclis* Hiern. He recognized *Tetraclis* as an independent genus because of its valvate corolla. In the plastid phylogenetic tree, it groups with the Madagascan *Diospyros* spp. and members of the *D. ferrea* (Wild.) Bakh. complex. *Maba sensu* Hiern, consisting of six sections, is a group of species with usually trimerous

Figure 1. Generic delimitation and relationships in Ebenaceae based on six plastid DNA regions with the new classification indicated (modified from Duangjai *et al.*, 2006).

flowers. However, this circumscription is polyphyletic in the plastid tree.

Bakhuizen (1936–1955) studied Ebenaceae *s.s.* extensively in Southeast Asia where only *Diospyros* occurs. He pointed out that the distinction of *Maba* from *Diospyros* is often unclear and recognized *Maba* as *Diospyros* subgenus *Maba* (J.R.Forst. & G.Forst.) Bakh. Phylogenetic results clearly show that subfamily Ebenoideae could consist of only three genera, *Euclea*, *Royena* and *Diospyros*, with the previously recognized genera (*Cargillia*, *Gunisanthus*, *Maba*, *Macreightia* and *Tetraclis*) being included in an expanded *Diospyros*. Morphological features of this subfamily are the presence of an indumentum, alternate bracteoles, tricolporate pollen grains, a superior ovary and the absence of a corona.

In Ebenoideae, pollen is generally shed as monads (permanent tetrads in two species) and is medium sized, prolate-spheroidal to subprolate and tricolporate. Palynological synapomorphies at different taxonomic levels such as subfamily, generic and subgeneric level are reported for Ebenoideae (Geeraerts *et al.*, 2009). The granular infratectum and the unique sculpturing pattern on the orbicule walls are the most discriminating pollen features for Ebenoideae (Geeraerts *et al.*, 2009).

Ebenoideae can be further subdivided into two major clades. The first consists of two genera, *Euclea* (20 species) and *Royena* (five species), which are mainly restricted to southern Africa, except for few species of the former that occur northward to the Arabian Peninsula, Socotra and the Comoro Islands. The relationship between *Euclea* and *Royena* is well supported by seed anatomy and a deletion of 69 bp in the 3′ *trnK* plastid intron. The second clade contains only *Diospyros*, which is pantropical and more diverse in its morphology and it exhibits greater DNA divergence than the other three genera in the family (including *Lissocapa*, Lissocarpoideae; Fig. 1). *Diospyros* is distinguished from the other genera in Ebenoideae by the lack of an invagination surrounding the radicle. Detailed pollen morphology has been studied in *D. kaki*, *D. virginiana* L., *D. lotus* and an interspecific hybrid between *D. virginiana* and *D. kaki*. Observations showed that pollen size, the type and the number of apertures and the microstructural characteristic of exine sculpture are important morphological traits, characteristic for each species (Grygorieva *et al.*, 2013).

THE GENUS *DIOSPYROS*

Diospyros comprises > 500 species distributed in the tropics and subtropics worldwide. Phylogenetic results support two clades successively sister to a large internally unresolved clade in this genus (Fig. 2). Although relationships within the last clade remain unclear, nine well-supported clades (I–Q) were observed in the plastid phylogenetic tree. As mentioned earlier, infrageneric classifications of *Diospyros* (de Candolle, 1844; Hiern, 1873; Bakhuizen, 1936–1955; White, 1980, 1993a; Singh, 2005) are not compatible with the results based on molecular data. Clade F corresponds to subgenus *Hierniodendron* Bakh. and is sister to the rest of *Diospyros*. Clade G contains *Diospyros* subgenus *Cargillia* (R.Br.) Bakh. [*D. australis* (R.Br.) Hiern and *D. pentamera* (Woolls & F.Muell.) ex F.Muell.] from Australia and five New Caledonian species (*D. balansae* Guillaumin, *D. brassica* F.White, *D. macrocarpa* Hiern, *D. margaretae* F.White and *D. oubatchensis* Kosterm.). Their close relationship is supported by a combination of biogeographic and morphological evidence. *Diospyros borneensis* Hiern (clade I) is sister to clade J and K. Clade J includes two species, *D. fulvopilosa* H.R.Fletcher and *D. mollis* Griff., both restricted to South-east Asia. Clade K comprises nine Asian species (Duangjai *et al.*, 2006) and a widespread African species, *D. mespiliformis* Hochst. ex A.DC. (White, 1988). Long-distance dispersal played a role for these African-Asian connections. Clade L contains the *D. ferrea* complex (including Madagascan *Maba*) and all Madagascan *Diospyros* spp. (including *Tetraclis*). The *D. ferrea* complex has been referred to as the genus *Maba sensu* de Candolle or *Diospyros* sections *Ferreola* Hiern and *Cupulifera* Bakh. Most have small fruits, which may be relatively easily dispersed across water barriers by migratory birds (Pannell & White, 1988; White, 1993a). Clade M consists of three subclades (Duangjai *et al.*, 2006). The first two subclades comprise only African species and are distributed in western Africa, mainly in the Guineo-Congolian region (White, 1978). The third subclade contains the South American species. Clade N contains five temperate or subtemperate species, for which phylogenetic relationships are supported by morphological characters (Morton *et al.*, 1996). Fruits in this group are edible and three species have been brought into cultivation: persimmon (*D. kaki*), date plum (*D. lotus*) and common persimmon (*D. virginiana*).

Polyploidy is reported in *D. kaki* (2*n* = 60, 90 and 135) and *D. virginiana* (2*n* = 60 and 90; Tamura *et al.*, 1998, and references therein). The other three species *D. glandulosa* Lace, *D. glaucifolia* Metc. and *D. lotus* (Tamura *et al.*, 1998, and references therein) are diploid, 2*n* = 30. Clade O contains three African species [*D. abyssinica* (Hiern) F.White, *D. pseudomespilus* Mildbr. and an unidentified species] and, although this clade is well supported by molecular data, shared morphological characters are still unclear. Species of clade P are characterized by a combination of the reddish inner bark and ruminate endosperm. Clade
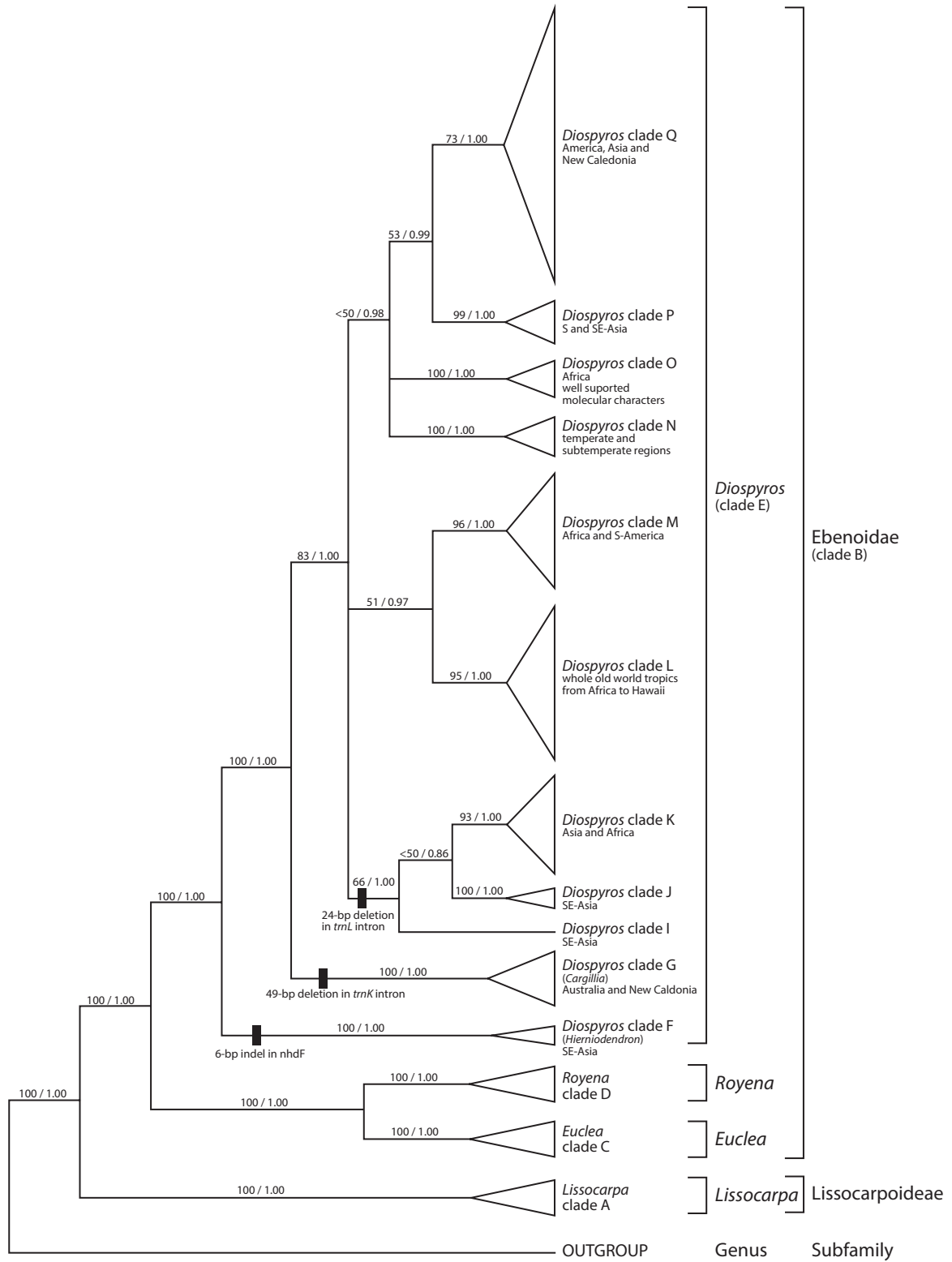
Figure 2. Schematic diagram summarizing relationships among well-supported clades in Ebenaceae (modified from Duangjai *et al.*, 2006).

Q does not show any obvious unifying morphological features.

In addition to these, new species have been identified and documented. *Diospyros albiflora* Alston has been recently recorded from Bangladesh (Sultana *et al.*, 2010). *Diospyros udaiyanii* P.S.Udayan, a new species from the Western Ghats, India, was reported by Udayan *et al.* (2015). *Diospyros cleistantha* O.Lachenaud & G.E.Schatz occurs in the Atlantic regions of Cameroon, and *D. subargentea* O.Lachenaud, Dauby & G.E.Schatz is endemic to west-central Gabon (Lachenaud *et al.*, 2017). *Diospyros leei* Y.Liu, S.Shi & Y.S.Huang (Huang *et al.*, 2015) and *D. microcalyx* D.X.Nong, Y.D.Peng & L.Y.Yu (Nong *et al.*, 2017) are new species reported from limestone areas in Guangxi, China. *Diospyros phengklaii* Duangjai, Sinbumroong & Suddee is a new species from south-western Thailand (Duangjai, Sinbumroong & Suddee, 2018). Two new species, *D. hequetiae* G.E.Schatz, Lowry & Fleurot and *D. rufotomentosa* G.E.Schatz & Lowry, have been reported from New Caledonia (Schatz & Lowry, 2018). Taxonomic revisions have been made for Australian *Diospyros* and 22 species (21 native and one naturalized) are recognized. Six species have been described as new: *D. granitica* Jessup, *D. peninsularis* Jessup, *D. pluviatilis* Jessup, *D. rheophila* Jessup, *D. uvida* Jessup and *D. yandina* Jessup, and new combinations have been made for *D. hemicycloides* (F.Muell. ex Benth.) Jessup (based on *Maba hemicycloides* F.Muell. ex Benth.) and *D. laurina* (R.Br.) Jessup (based on *Maba laurina* R.Br.) by Jessup (2014). Nomenclatural notes have been published on Malagasy *Diospyros* (Schatz & Lowry, 2011a). A new name, *D. boiviniana* (Baill.) G.E.Schatz & Lowry (previously *Olax boiviniana* Baill.), was proposed by Schatz & Lowry (2011b) for the endemic species in the Seychelles. Synonymies were reported by Schatz & Lowry (2018) for the following New Caledonian species, *D. calciphila* F.White (including *D. inexplorata* F.White), *D. glans* F.White (including *D. neglecta* F.White), *D. revolutissima* F.White (including *D. erudita* F.White), *D. samoensis* A.Gray (including *D. olen* Hiern) and *D. vieillardii* (Hiern) Kosterm. (including *D. fastidiosa* F.White *and D. nebulosa* F.White).

NEW CALEDONIAN *DIOSPYROS*

New Caledonia is an island group located in the south-western Pacific *c.* 1300 km east of Australia, consisting of a main island, Grande-Terre (*c*. 16 000 km²), Iles Belep (in the north), Iles des Pins (in the south), Loyalty Islands (in the east) and several other smaller islands. It is one of the 34 biodiversity hotspots (Lowry *et al.*, 2004) and nearly 75% of the native flora is endemic (Morat *et al.*, 2012), the fourth highest percentage found on islands (Lowry, 1998). Among these endemic

taxa, 62–98 genera and three families, Amborellaceae, Oncothecaceae and Phellinaceae, are endemic (Morat *et al.*, 2012; Pillon, Barrabé & Buerki, 2017).

Diospyros colonized New Caledonia via long-distance dispersal at least four times during the last 25 Myr (Duangjai *et al.*, 2009). Three of these colonization events gave rise to only a small set of species (one to five species each; Turner *et al.*, 2013a). However, another event gave rise to a clade of > 20 closely related species that took advantage of all vegetation types in the archipelago, except mangroves (Turner *et al.*, 2013b; Fig. 3). Most of these closely related species are morphologically and ecologically clearly differentiated, and only a few of them occur in local sympatry. Several of the species are point endemics (Fig. 4).

The first group of New Caledonian *Diospyros* spp., *D. balansae*, *D. brassica*, *D. macrocarpa*, *D. margaretae* and *D. oubatchensis*, forms a clade with Australian species (clade II, Fig. 3). The other two widespread New Caledonian species, *D. samoensis* (including *D. olen*; Schatz & Lowry, 2018) and *D. fasciculosa* (F.Muell.) F.Muell. are found throughout the southern Pacific and are not sister species; they represent two colonization events (clade XI, Fig. 3). The third group is the largest clade, with > 20 closely related species. One of the closest relatives of this latter group is *D. ferrea*, a widespread species found in Africa and the whole Indian Ocean and western South Pacific region. These *c.* 21 species belong to *Diospyros* section *Maba* (J.R.Forst. & G.Forst.) V.Singh & V.S.Kumar, the *D. ferrea* complex (Duangjai *et al.*, 2006), and are relatively uniform in their morphology, but exceedingly variable in New Caledonia, resulting in several discrete species having been recognized. White (1993b) hypothesized that allopatric speciation may have played an important role, promoted by the great diversity of steep physical gradients in New Caledonia.

RADSEQUENCING FOR RADIATING *DIOSPYROS* SPECIES IN NEW CALEDONIA

Explosive radiation featuring rapid opportunistic morphological and ecological diversification is reported for some islands (Glor, 2010). AFLP results did not show any significant grouping according to ecological (edaphic, climatic, elevational), geographical or morphological factors (Turner *et al.*, 2013b). Therefore, restriction site associated DNA sequencing (RADseq) was attempted to resolve the phylogenetic relationships of 18 species (out of *c*. 21) in this rapidly radiating *Diospyros* group (clade III, Fig. 3; Paun *et al.*, 2016). Thousands of SNPs derived from RAD loci assembled *de novo* from Illumina reads gave a completely resolved tree, which was not the case in previous analyses using multiple DNA loci (Duangjai
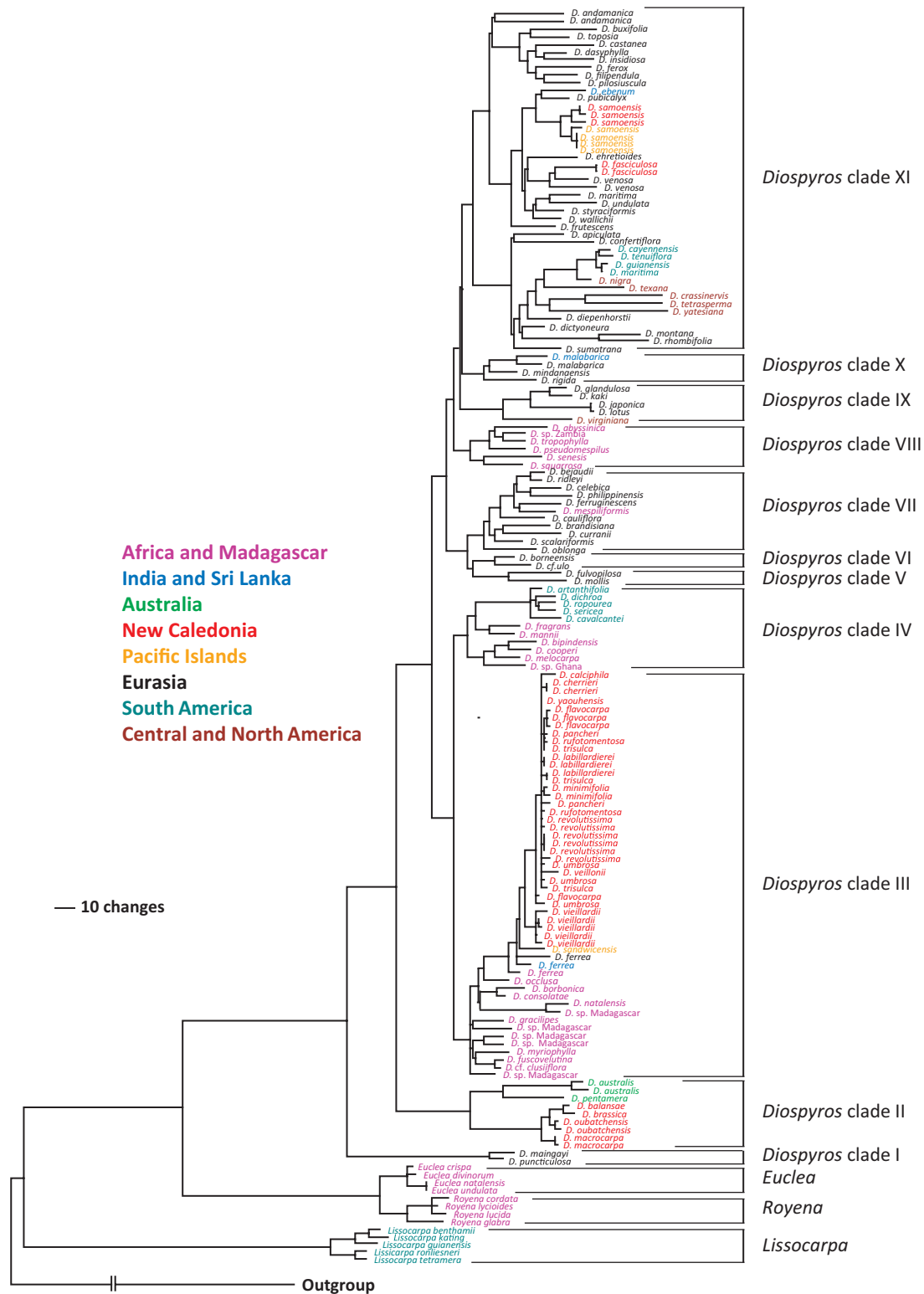
Figure 3. Plastid tree for the pantropical genus *Diospyros*, with an emphasis on the radiation and biogeographic origins of the New Caledonian endemic species (modified from Duangjai *et al.*, 2009).
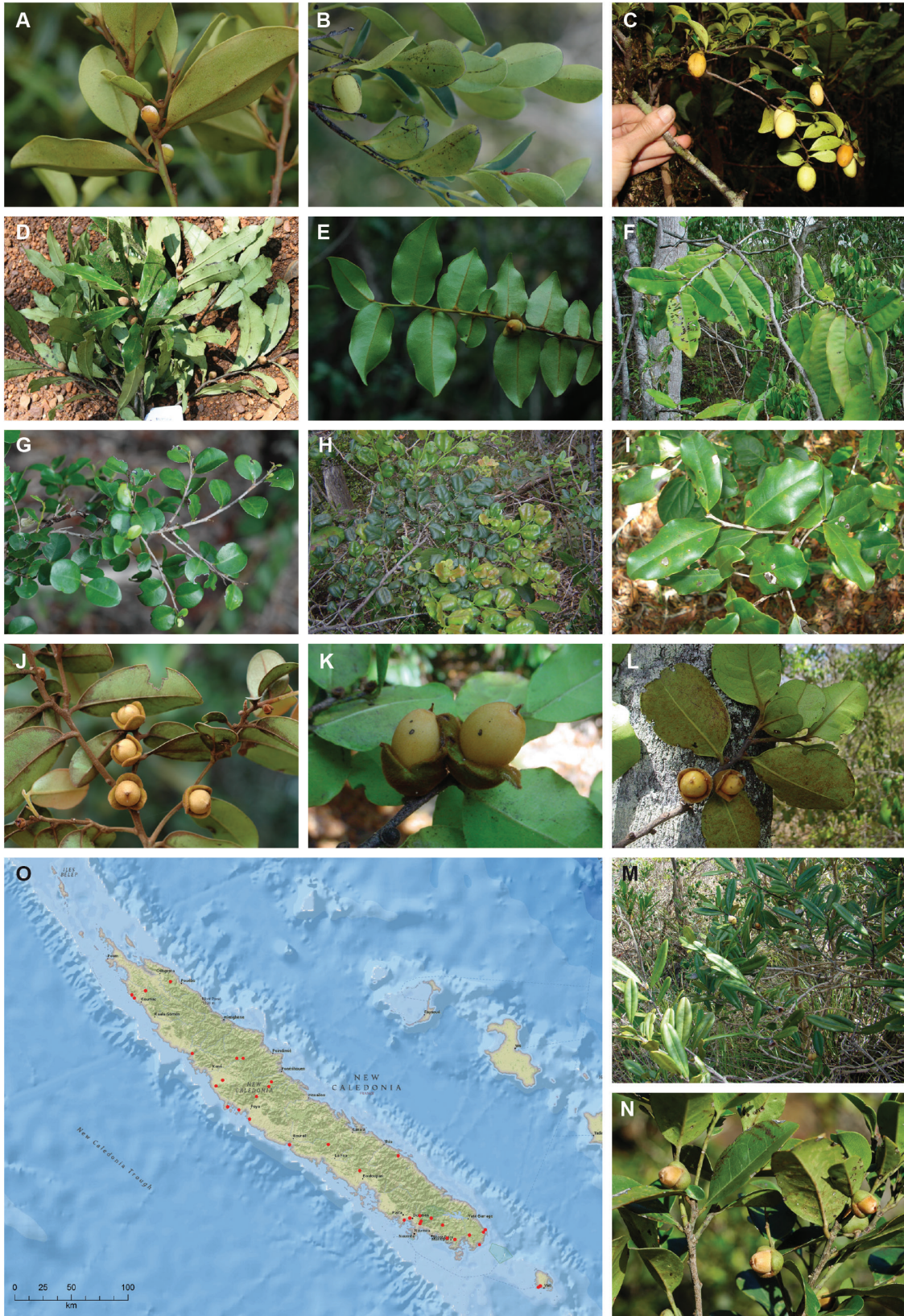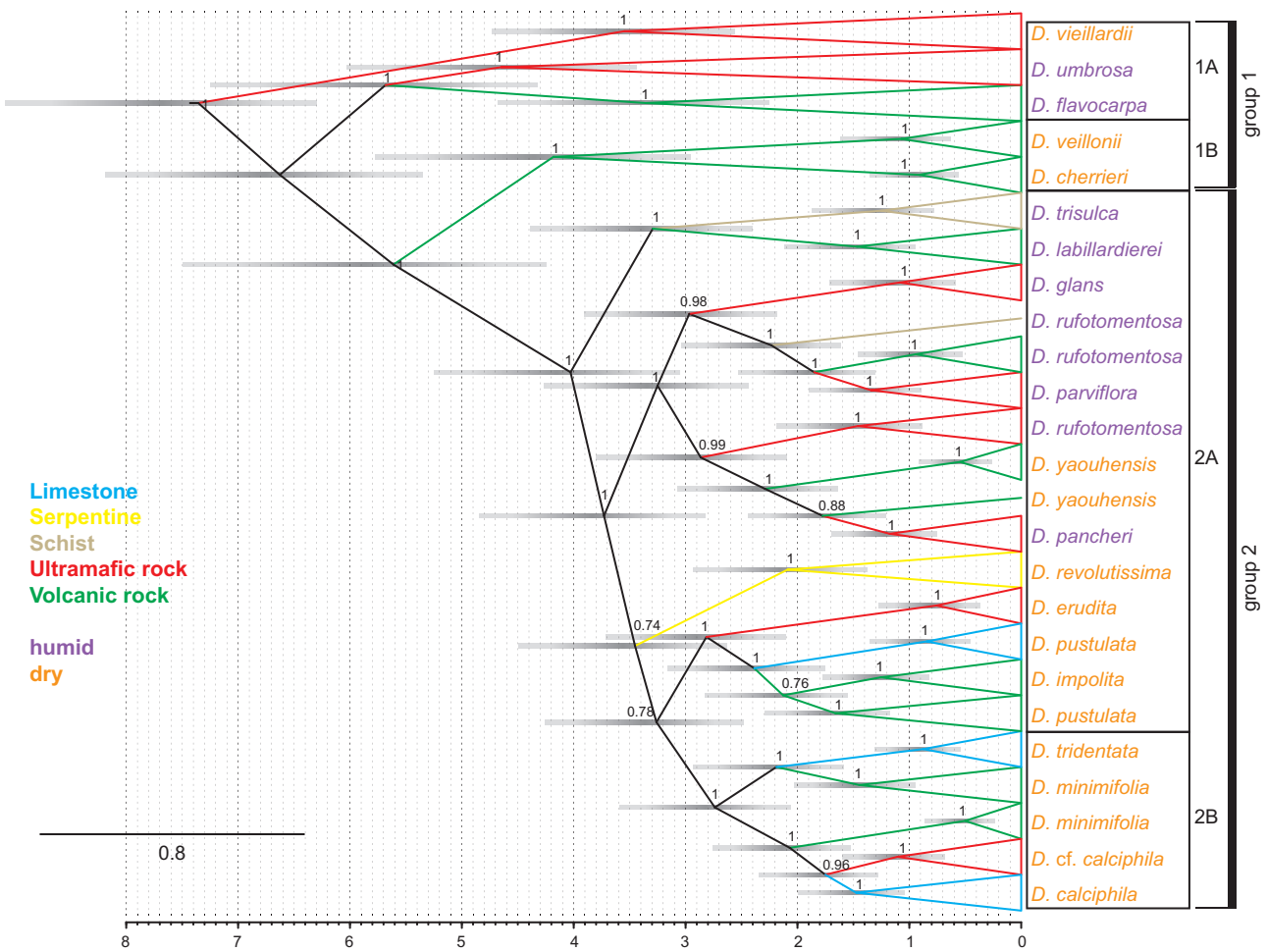
Figure 5. Phylogenetic tree based on RADseq. Clades are colour-coded according to the soil types; moisture level is represented at the tip of the clades, purple-humid, orange-dry (modified from Turner, 2014).

et al., 2009; Turner *et al.*, 2013a) and genome-wide fingerprinting analyses (AFLP; Turner *et al.*, 2013b). Despite the relatively large number of SNPs obtained by Paun *et al.* (2016), relationships inferred for *Diospyros* were, however, not always well-supported. The reason for this may lie in the limited number of generations since the extreme bottleneck associated with the initial long-distance dispersal event to New Caledonia, followed by an explosive radiation within a short time interval. Additional processes may have blurred the phylogenetic signal in this rapidly radiating group, in particular introgression, which could have been common during some episodes of speciation in this group (Paun *et al.*, 2016).

Grande-Terre, the main island of New Caledonia, is split by a mountain range into humid, south-eastern and dry, north-western parts (Maitrepierre, 2012). The inferred phylogenetic relationships and analyses of niche evolution point to an initial, but fairly slow, divergence (group 1, Fig. 5) with respect to these climatic conditions along the major clade (deep split on the backbone of the phylogenetic tree). A latter more rapid phase of the radiation was found to be driven by microhabitat, in particular soil type. Modelling studies have suggested that the divergence with respect to macrohabitats is indeed the first expected stage for rapid radiation (Gavrilets & Losos, 2009; Glor, 2010). In *Diospyros*, this pattern could also be merely the

Figure 4. A–N, Examples of *Diospyros* species from New Caledonia and O, a map of New Caledonia with collection points. A, *D. vieillardii*; B, *D. umbrosa*; C, *D. flavocarpa*, D, *D. labillardierei*; E, *D. pancheri*, F, *D. veillonii*; G, *D. minimifolia*; H, *D. pustulata*; I, *D. cherrieri*; J, *D. rufotomentosa*; K, *D.perplexa*; L, *D. yaouhensis*; M, *D. revolutissima*; N, *D. glans*; O, Map of New Caledonia with sampling localities (from Turner *et al.*, 2013a).
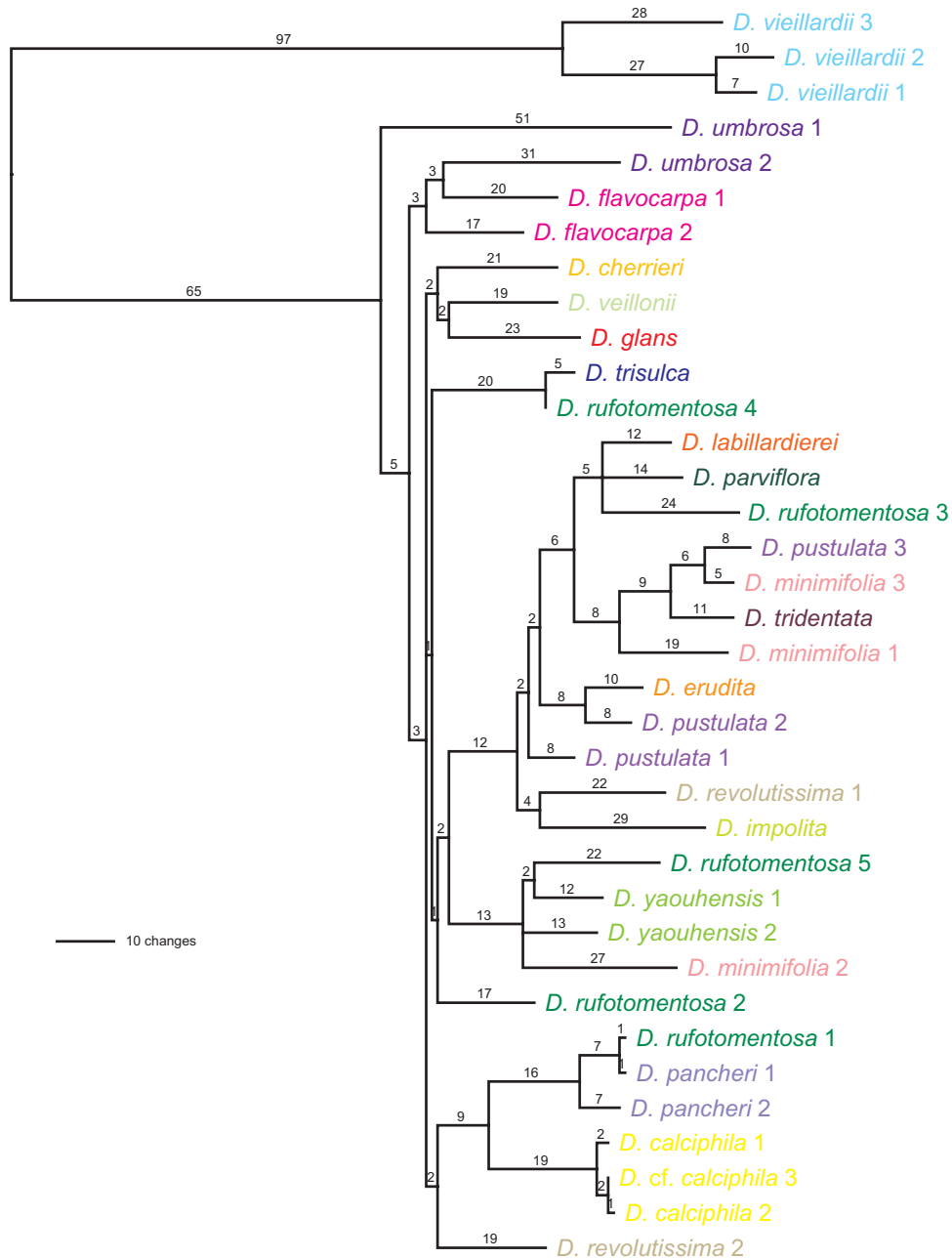
Figure 6. Tree based on whole plastid genomes in which species failed to form unique groups (modified from Turner *et al.*, 2016).

result of allopatric differentiation, with isolation by distance promoted by a geographical barrier between the dry and wet areas that is difficult to cross.

Little is known about pollinators and fruit dispersal in these species. Most species are dioecious and have fleshy fruits. The fruits of other *Diospyros* spp. present on the island are eaten by birds; fruits of *D. fasciculosa* are, for example, dispersed by the red-bellied fruit-dove, *Ptilinopus greyii* (Tassin, Boissenin & Barré,

2010). Since there are no fossils available pertaining to this New Caledonian group of *Diospyros*, dating estimates were obtained by secondary calibration, taking into account the age of the split between *D. vieillardii* and the rest of the group (7.2 Mya) so that it conforms to a previous date obtained for this split (Turner *et al.*, 2013a, Fig. 5). The molecular clock analysis resulted in a slightly older age for the split of *D. vieillardii* from the rest of the group, estimated
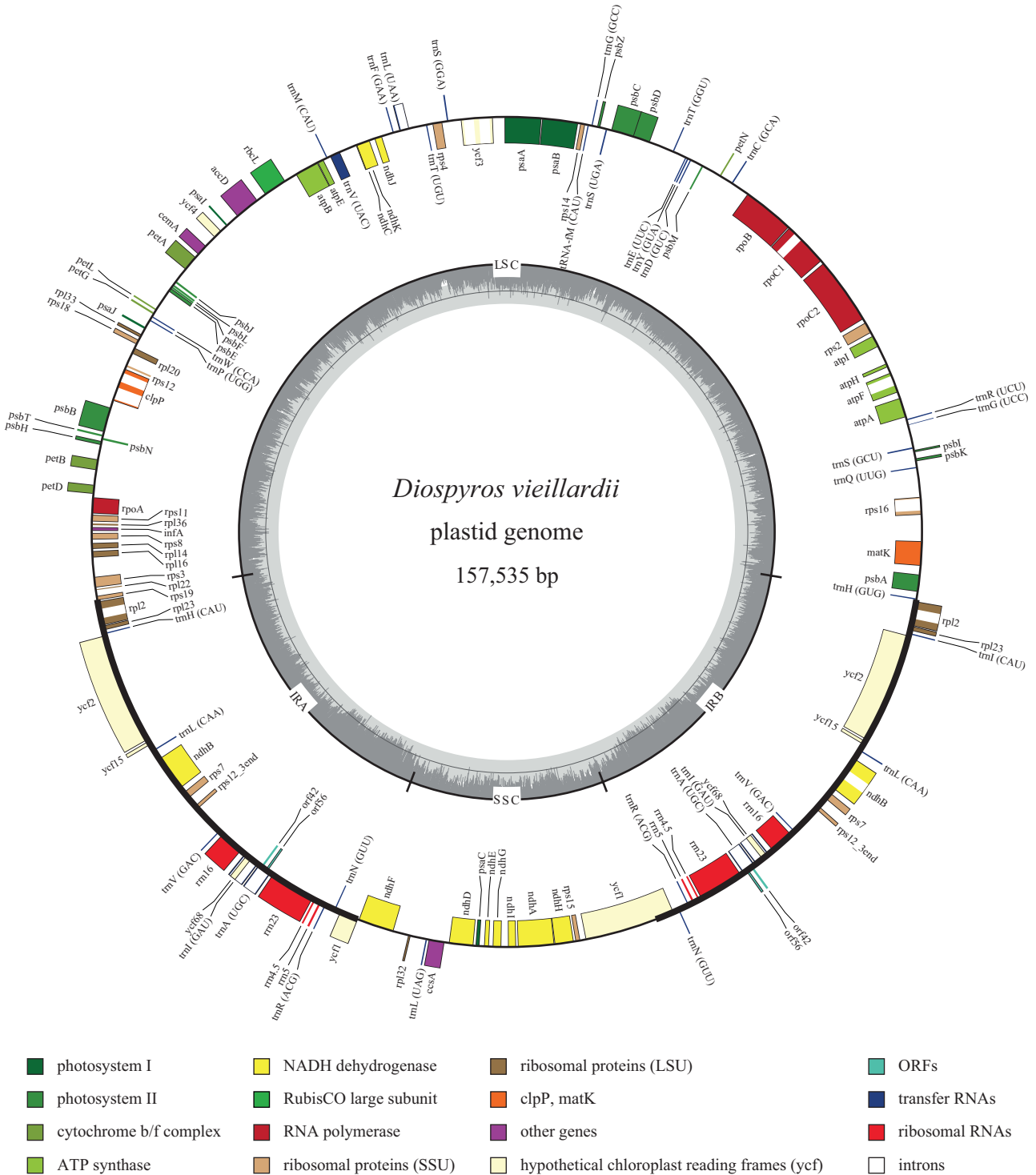
Figure 7. Plastid genome of *Diospyros vieillardii* (Turner *et al.*, 2016).

at 7.4 Mya, with a wide 95% confidence interval of 2.7 Ma. The next divergence [i.e. *D. flavocarpa* (Vieill. ex P.Parm.) F.White/*D. umbrosa* F.White from the rest of the species] took place *c*. 6.6 Mya. The clade with

D. cherrieri F.White and *D. veillonii* F.White separated from the rest *c*. 5.6 Mya. The rapidly radiating group (group 2, Fig. 5) started to diversify *c*. 4 Mya. Clade 2B is a young group, *c*. 2.7 Myr old. Most speciation
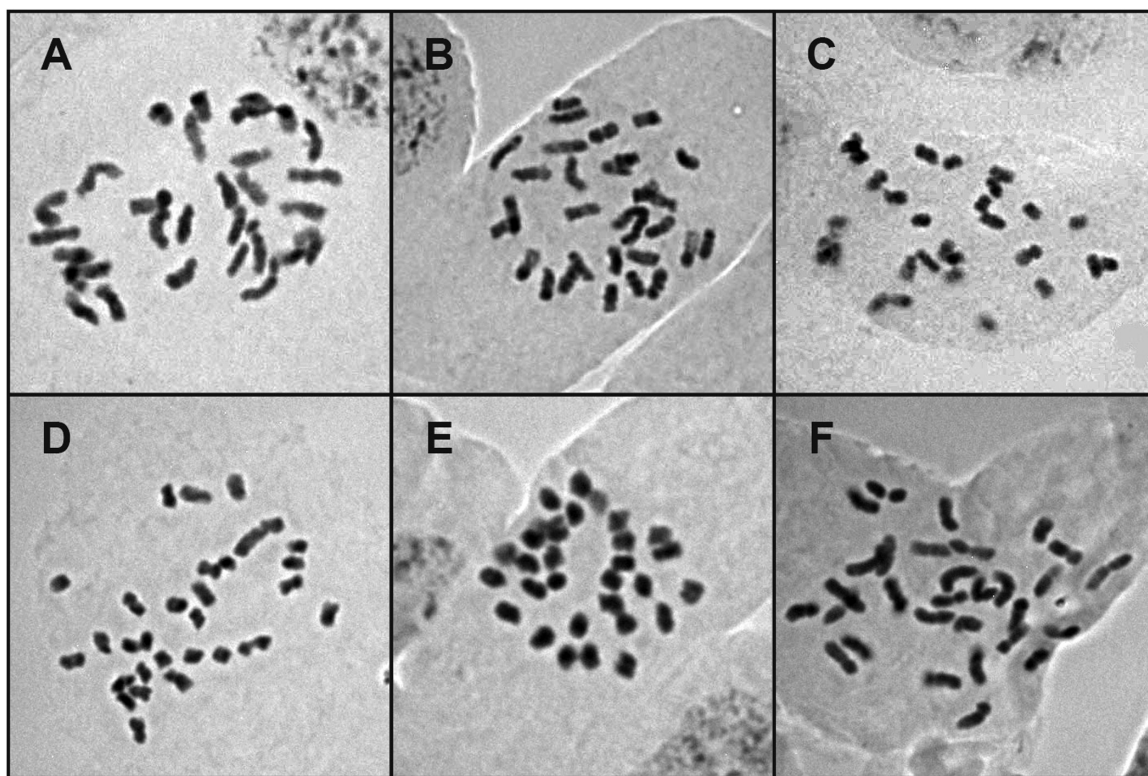
Figure 8. Chromosome counts for *Diospyros* species, 2*n* = 30. A, *D. pustulata*; B, *D. veillonii*; C, *D. macrocarpa*; D, *D. inconstans*; E, *D. texana* and F, *D. pentamera*.

events in this New Caledonian group seem to have taken place between 3.5 and 1.5 Mya.

SEQUENCING WHOLE PLASTID GENOMES OF *DIOSPYROS* SPECIES ENDEMIC TO NEW CALEDONIA

Whole plastid genome (plastome) sequencing has become affordable and practical, and this has been employed to generate more highly resolved phylogenetic trees in some groups (Yang *et al.*, 2013; Barrett *et al.*, 2014). The phylogenetic tree obtained from whole plastid genomes (Fig. 6; Turner *et al.*, 2016) is similar both in resolution and structure to the phylogenetic tree based on that of combined plastid and low-copy nuclear markers (Turner *et al.*, 2013a). From this tree, we can see that the species fail to form clades, which could suggest plastid capture or ancestral polymorphisms, the latter unlikely given the genetic bottleneck expected with a long-distance dispersal event. In cases of recently radiating species groups, in particular following an extreme bottleneck associated with the arrival of *Diospyros* in New Caledonia, the plastid genome appears to be insufficient for inference of phylogenetic relationships.

PLASTID GENOMES IN *DIOSPYROS*

The plastid genomes of the New Caledonian *Diospyros* spp. (Turner *et al.*, 2016) were the first fully sequenced plastid genomes of Ebenaceae reported in literature. Shortly after the New Caledonian species, the plastome sequence of *D. blancoi* A.DC. was published by Jo *et al.* (2016), and Fu *et al.* (2016) published plastome sequences of *D. kaki*, *D. lotus*, *D. oleifera*, *D. glaucifolia* and *D. 'Jinzaoshi'* ined. The plastome sequences of two more *Diospyros* species, *D. dumentorum* W.W.Sm. and *D. strigosa* Hemsl., were published by Yu *et al.* (2017).

The size (*c.* 157 kb) and gene order of the plastid genome of *D. vieillardii* was found to be similar to that of *Camellia sinensis* (L.) Kuntze, which is also a member of order Ericales (Turner *et al.*, 2016; GenBank KC143082.1; Fig. 7). The overall AT content of the plastid genomes of *Diospyros* is ~63%, leading to a GC content of ~ 37%, which is similar to those of other angiosperms, including *Camellia* (Yang *et al.*, 2013).

The complete plastome size in *Diospyros* ranges from 157 322 (*D. 'Jinzaoshi'* ined.) to 157 845 bp (*D. dumetorum*) in length and consists of a large single-copy region of 86 817 (*D. rufotomentosa*) to 87 192 bp (*D. blancoi*) and a small single-copy region of 18 167 (*D. 'Jinzaoshi'* ined.) to 18 536 bp (*D. kaki*), which are
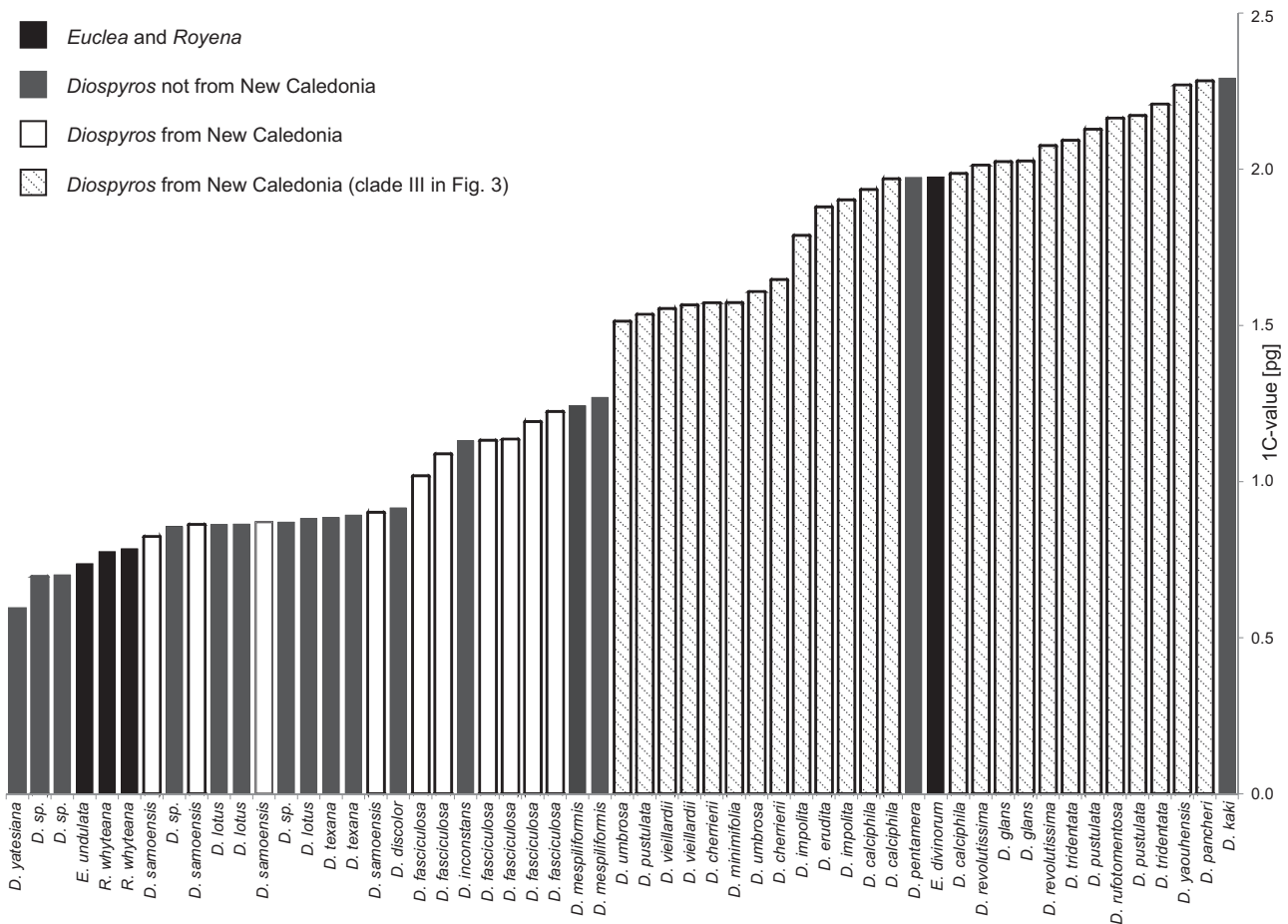
Figure 9. Genome size (1C values in pg) of *Diospyros* (modified from Turner, 2014) Phylogenetic tree based on plastid genome together with genomic proportions of repeated elements for few species of *Diospyros.*

separated by a pair of 26 003 (*D.* '*Jinzaoshi'* ined.) to 26 206 bp (*D. dumetorum*) long, inverted repeat regions. The plastome contains 113 genes, of which 79 are protein-coding genes, 30 are tRNA genes and four are rRNA genes. Sixteen genes contain one intron and two genes have two introns. Forty-five simple sequence loci were identified from the genome.

CHROMOSOME NUMBERS AND GENOME SIZE IN *DIOSPYROS*

CHROMOSOME NUMBERS

The basic chromosome number in *Diospyros* is $2n = 2x = 30$, and most of the species appear to be diploid (White, 1993b; Tamura *et al.*, 1998; Yang *et al.*, 1999). There are some reports of polyploids, mostly from cultivated species (*D. rhombifolia* Hemsl. 4*x*, *D. ebenum* 6*x*, *D. kaki* 6*x* and 9*x*, *D. virginiana* 6*x* and 9*x*; Tamura *et al.*, 1998; Yang *et al.*, 1999). New Caledonian *Diospyros* spp. (*D. calciphila* F.White,

D. fasciculosa, *D. flavocarpa*, *D. minimifolia* F.White*, D. parviflora* (Schltr.) Bakh.; White had a broad view of *D. parviflora*, and therefore we cannot say if it is real *D. parviflora s.s.* or *D. rufotomentosa* or even another species]*, D. samoensis* [*D. olen*]*, D. umbrosa, D. vieillardii* and *D. yaouhensis* (Schltr.) Kosterm.) were reported to be diploids (White, 1993b). Further counts for the New Caledonian species *D. macrocarpa*, *D. pustulata* F.White *and D. veillonii* and for the non-New-Caledonian species *D. inconstans* Jacq., *D. pentamera*, *D. texana* Scheele and *D. yatesiana* Standl. also showed them to be diploid $2n = 30$ (Fig. 8; Turner *et al.*, 2013a).

GENOME SIZE

Measurements of genome size showed differences among the New Caledonian *Diospyros* spp. *Diospyros samoensis* (*D. olen*) has 1C = 0.86pg, the smallest genome of the New Caledonian *Diospyros* spp. examined, followed by *D. fasciculosa* with 1C = 1.13pg.
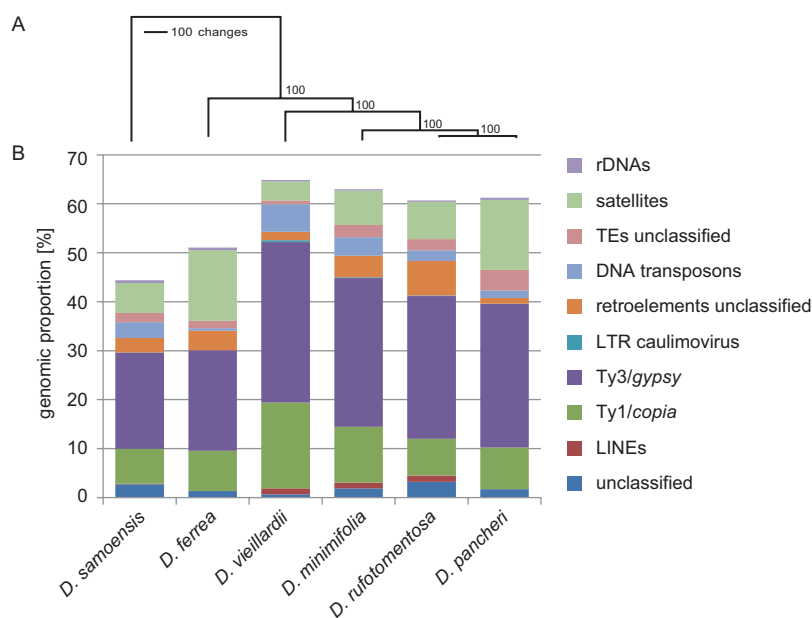
Figure 10. Phylogenetic tree based on plastid genomes with genomic proportions of repeated elements for six *Diospyros* species. Genome size (1C values in pg) of *Diospyros* (modified from Turner, 2014).

Species from the group III (Fig. 9) revealed up to nearly three-fold larger genome sizes compared to *Diospyros* spp. from other groups. Chromosome counts showed no indication of polyploidy in this group. The increase in genome size in these species led us to investigate the repeat elements of these genomes (Turner *et al.*, unpubl. data). Whole genome sequencing using next-generation sequencing techniques showed that the larger genomes generally contain more copies of the Ty3/gypsy repeat elements, as observed in other plant groups (tobacco, Renny-Byfield *et al.*, 2013; sunflower, Natali *et al.*, 2013). The number of TEs is higher in the genomes of the endemic New Caledonian species (mean of 52.3%) compared to *D. samoensis* (*D. olen*; 34.4%). Besides alterations in transposable element content, differences in the number of reads annotated as tandem repeats (satDNA) were also observed (Fig. 10). *Diospyros rufotomentosa* and *D. pancheri* Kosterm. (1C values = 2.2–2.3 pg) differ slightly in the number of repetitive elements, with the satellite-elements contributing to most of the variation between these two species (Turner *et al.*, unpubl. data).

RECENT NOVELTIES AND SYNONYMIES IN NEW CALEDONIAN *DIOSPYROS*

Schatz & Lowry (2018) described two new species, *D. hequetiae*, thus far not sequenced in any phylogenetic analysis, and *D. rufotomentosa*, previously included in the widespread *D. parviflora*.

The AFLP results (Turner *et al.*, 2013b) indeed showed two distinct groups in the *D. parviflora* complex, one corresponding to all populations sampled of the newly described species (*D. rufotomentosa*), thus confirming its segregation and new status. From our molecular results, the proposed synonymy of *D. calciphila* and *D. inexplorata* as proposed by Schatz & Lowry (2018) seems to be supported (Paun *et al.*, 2016). However, the molecular results available so far (Duangjai *et al.*, 2009; Turner *et al.*, 2013a,b; Paun *et al.*, 2016; Turner *et al.*, 2016) do not support inclusion of *D. erudita* in *D. revolutissima* as proposed by Schatz & Lowry (2018). Inclusion of *D. olen* into *D. samoensis* as proposed by Schatz & Lowry (2018) also has support from molecular results (Fig. 3; Dunangjai *et al.*, 2009; Turner *et al.*, 2013a). At this point, it is important to mention that *D. samoensis/olen* is part of a series of closely allied species extending from southern India and Sri Lanka to Australia, New Caledonia, Fiji, Samoa, Tonga and Vanuatu, also including *D. ebenum*, *D. hebecarpa* A.Cunn. ex Benth., *D. insularis* Bakh., *D. novoguineensis* Bakh. and *D. pubicalyx* Bakh. (*D. montana* Roxb.). To confirm this, a detailed study with material of the mentioned species representing their whole distribution would be needed. In the same paper, Schatz & Lowry (2018) put *D. fastidiosa* (only known from the type collection from Aoupinié) and *D. nebulosa* (only known from the type collection from Panié) in synonymy with *D. vieillardii*. *Diospyros vieillardii* is a distinct species in all molecular analyses (Duangjai *et al.*, 2009; Turner *et al.*, 2013a; Paun *et al.*,

2016). The specimen used by Schatz & Lowry (2018) to determine *D. fastidiosa* (=*D. vieillardii*) falls within *D. flavocarpa* in all molecular analyses (Turner *et al.*, 2013a,b; Paun *et al.*, 2016; Turner *et al.*, 2016, Fig. 6). Therefore, we reject the synonymy of *D. fastidiosa* and *D. nebulosa.* Further investigations are needed to clarify this. Schatz & Lowry (2018) hypothesized interspecific hybrids both in the field and herbarium. In all cases, the two presumed parental species of the putative hybrids are known to occur in close proximity. From our molecular results we can neither confirm nor reject their hypotheses. Controlled garden experiments are needed to confirm this.

CONCLUSIONS

Sanger DNA sequencing, next generation RADseq and analysis of plastid genomes can help improve the morphology-based taxonomy at subfamily and generic level, as illustrated here for the large pantropical genus *Diospyros*. There is now a much better understanding of the evolutionary dynamics in the family and of the biogeography of *Diospyros*. The origin and ecological adaptation of New Caledonian *Diospyros* spp. is now better understood based on analyses of SNPs from the RADseq data. Polyploidy has been shown not to play a major role in the diversification of this genus, but genome size change has been substantial, due to the activity of transposable elements. How genome size change is related to the ecology of these species remains poorly understood and should be investigated in greater detail. Reproductive biology of these species is also poorly studied, due to the fact that they are dioecious, long-lived trees often bearing their flowers at great height. Since most of the next generation work has been done only on the New Caledonian *Diospyros* spp., further molecular work on the South and Southeast Asian species will be needed to give a deeper understanding of evolution in pantropical *Diospyros*. More molecular investigation is also needed to confirm the recently published synonymy of New Caledonian *Diospyros* spp., especially for *D. vieillardii*.

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