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ORIGINAL ARTICLE

# Late Cretaceous–Early Eocene origin of yams (*Dioscorea*, Dioscoreaceae) in the Laurasian Palaearctic and their subsequent Oligocene–Miocene diversification

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## ABSTRACT

**Aim** *Dioscorea* (Dioscoreaceae) is a predominantly pantropical genus (< 600 species) that includes the third most important tropical tuber crop and species of pharmacological value. Fossil records from both the Northern and Southern Hemispheres were used to test hypotheses about the origin of the genus *Dioscorea*, and to examine potential macroevolutionary processes that led to its current distribution.

**Location** Pantropical distribution.

**Methods** Divergence times were estimated using the most comprehensive phylogeny of the group published to date based on plastid sequences and fossil calibrations, applying a relaxed-clock model approach. Ancestral areas and range shifts were reconstructed using time-stratified likelihood-based models, reflecting past continental connectivity and biogeographical models incorporating the spatial range of fossils.

**Results** Fossil-informed biogeographical analysis supported colonization of the Nearctic by ancient yam lineages from the western Palaearctic and subsequent migration to the South. Most of the pantropical South American, African and Southeast Asian lineages experienced a relatively recent diversification in the Oligocene–Miocene. Long-distance dispersals were inferred for the colonizations of the New World, Africa and Madagascar.

**Main conclusions** *Dioscorea* likely originated between the Late Cretaceous and the Early Eocene in the Laurasian Palaearctic, followed by possible dispersal to South America via the Eocene North Atlantic Land Bridge.

**Keywords**

**biogeography, dispersal-extinction-cladogenesis model, fossil constrains, Laurasian origin, N-S American Long-Distance Dispersal, Palaearctic – Nearctic colonization, pantropical distribution, phylogenetic dating, Thulean – Beringian land bridges, yams**

## INTRODUCTION

Plate tectonics and climatic oscillations have shaped the vicariance, extinction and long-distance dispersal events that have resulted in the contemporary distributions of pantropical angiosperm lineages since their diversification in the Early Cretaceous (Magallón & Sanderson, 2001). Climatic conditions have varied dramatically from warm environments in the Late Mesozoic and Early Cenozoic (Palaeocene–Eocene) to a cooler climate in the Oligocene–Miocene, followed by the Quaternary glaciations (Zachos *et al.*, 2001; Morley, 2003), with dramatic influence on the distribution of tropical taxa (Antonelli *et al.*, 2009; Bartish *et al.*, 2011).

Biogeographical origins of pantropical groups that diversified after the split of Pangea (*c.* 180 Ma) have been inferrred to be either Laurasia (e.g. Davis *et al.*, 2002; Antonelli *et al.*,

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2009; Baker & Couvreur, 2013) or Gondwana (e.g. Barker *et al.*, 2007; Renner *et al.*, 2010; Bartish *et al.*, 2011). These studies estimated the beginning of their respective divergences to the Mid-Late Cretaceous or Early Palaeocene, before the Laurasian break up (55 Ma), or concomitant with some of the successive Gondwanan (180–30 Ma) splits. It has also been proposed that most of the Laurasian groups migrated from Eurasia to North America (or *vice versa*) via some of the North Atlantic Land Bridges (NALBs; Tiffney, 1985) or the early Beringian Land Bridge (BLB; Tiffney, 1985; Brikiatis, 2014), which lasted from the Late Cretaceous to the Mid Palaeocene (Brikiatis, 2014). Specifically, the North Atlantic De Geer route (71–63 Ma) was coincident with the hyperthermal Mid Maastrichtian Event (69 Ma) and the Earliest Danian Warming (EDW, 65.5 Ma) (Brikiatis, 2014), whereas the North Atlantic Thulean route (57 Ma, 56 Ma) was formed during a period of global warming in the Late Palaeocene Thermal Maximum (LPTM, 56 Ma) (Tiffney, 1985; Sanmartín *et al.*, 2001; Zachos *et al.*, 2001; Brikiatis, 2014), and the BLBs (65 Ma, 58 Ma) were contemporary, respectively, to the EDW and LPTM (Brikiatis, 2014). These land bridges allowed the exchange of boreotropical floras at high latitudes in the Northern Hemisphere, as indicated by a wealth of fossil deposits of shared plants and animals in Eurasia and N America (Tiffney, 1985; Morley, 2003). An additional dispersal hypothesis to the NALBs or BLBs migrations is the early long-distance colonization of South America from Laurasian North America in the Late Cretaceous, as shown for several stem lineages of palms (Baker & Couvreur, 2013).

The disappearance of the NALBs and BLBs (56–50 Ma) (Tiffney, 1985) together with a decrease in temperatures by Mid Eocene (45 Ma), after the Early Eocene Climatic Optimum (EECO, 52 Ma; Zachos *et al.*, 2001), forced the boreotropical species to move southwards to the tropical belt. These migrations occurred in parallel with the main Eurasian – N American vicariance events in temperate organisms of Laurasian origin (Tiffney, 1985; Sanmartín *et al.*, 2001; Brikiatis, 2014). By contrast, in the Southern Hemisphere the divergence times and colonization routes for tropical Gondwanan groups are not always consistent with the plate tectonic sequence and patterns of climatic change. A lack of vicariance events concomitant with the post-LPTM/EECO boundary, and the unsuitably low temperatures for many tropical plants in the Australia-Antarctica-South America corridor in the Mid Eocene (52–45 Ma) decreases the probability of the tropical colonizations (Bartish *et al.*, 2011). Nevertheless, the discovery of Late Cretaceous or Early Cenozoic fossil records of Myrtaceae in Antarctica (Poole *et al.*, 2003) and of Mid-Late Eocene palm fossils in Patagonia (Zucol *et al.*, 2010), together with other biogeographical evidence, suggests a Gondwanan origin of some tropical plant groups, either in East Gondwana (e.g. Monimiaceae, Renner *et al.*, 2010) or in West Gondwana (e.g. Sapotaceae, Bartish *et al.*, 2011 in Africa; Malpighiaceae, Davis *et al.*, 2002 in South America).

Alternatively, long-distance colonization of the New World from Africa has been proposed through past land connections across a series of islands (96–76 Ma; Morley, 2003), or else by earlier short-distance dispersals shortly after the West Gondwanan break up (105–80 Ma; Raven & Axelrod, 1974; McKenna, 1981). More recently, some palaeopantropical groups in the Holarctic region could have migrated via the second Late Tertiary BLB connecting Asia and North America in the Late Miocene and Early Pliocene (Sanmartín *et al.*, 2001; Donogue & Smith, 2004). Additionally, long-distance dispersals have been advocated to explain the relatively recent colonization of long isolated areas (Duangjai *et al.*, 2009), like transatlantic dispersals between South America and Africa (Dick *et al.*, 2003; Renner, 2004).

Dioscoreaceae have a characteristic pantropical distribution with most taxa concentrated within intertropical latitudes (Knuth, 1924; Burkill, 1960; Dahlgren *et al.*, 1985; Huber, 1998; Fig. 1). Dioscoreaceae belong to the order Dioscoreales together with Burmanniaceae and Nartheciaceae based on combined analyses of morphological and molecular data (Caddick *et al.*, 2002a,b). The latter classification subsumed the former Taccaceae, Stenomeridaceae and Trichopodaceae as early-diverging lineages of the enlarged Dioscoreaceae. More recent papers (Merckx *et al.*, 2006, 2010) suggested that the achlorophyllous Thismiaceae and *Afrothismia*, together with Taccaceae and Trichopodaceae, form a clade sister to Dioscoreaceae s. s., whereas *Stenomeris* might be nested within it. These authors reconstructed a nuclear phylogeny of Dioscoreales that showed the early split of Narthecieae and the sister relationship of Burmanniaceae and Dioscoreaceae plus allies [(Dioscoreaceae, (*Afrothismia*, (Trichopodae, (Taccaceae, Thismiaceae))))]. By contrast, Taccaceae were reconstructed as sister to Burmanniaceae/Dioscoreaceae in the plastid angiosperm tree of Magallón *et al.* (2015). Caddick *et al.* (2002a) recognized four genera within Dioscoreaceae, the hermaphrodite yam allies *Stenomeris*, *Tacca* and *Trichopus*, and the large dioecious yam genus *Dioscorea* (Huber, 1998; Wilkin *et al.*, 2005). However, the phylogenetic positions of mycoheterothropic *Thismia* and *Afrothismia* based on nuclear and mitochondrial sequences disagree with previous classifications (Merckx *et al.*, 2010), suggesting that Dioscoreales sensu Caddick *et al.* (2002a) is not monophyletic. Generally, species of the Dioscoreaceae s. l. group (Dioscoreaceae, Taccaceae, Thismiaceae, Stenomeridaceae, Trichopodaceae, *Afrothismia*) are geophytes possessing underground rhizomes and/or tubers with aerial stems which usually have a climbing habit (*Dioscorea, Trichopus, Stenomeris*), or more rarely have a stemless basal rosette (*Tacca*) (Wilkin, 2001). *Dioscorea* species are generally characterized by their winged seeds and capsular, six-seeded fruits; however, the current monophyletic circumscription of *Dioscorea s.l.* includes small genera initially segregated from it based on their wingless seeds (*Borderea*, *Epipetrum*, *Nanarepenta*, *Tamus*), samaroid (*Rajania*) or berry (*Tamus*) fruits or tuber morphology (*Testudinaria*) (Caddick *et al.*, 2002a; Wilkin *et al.*, 2005). A preliminary plastid phylogeny of *Dioscorea* identified seven to eight main lineages (Wilkin *et al.*, 2005), greatly simplifying the earlier

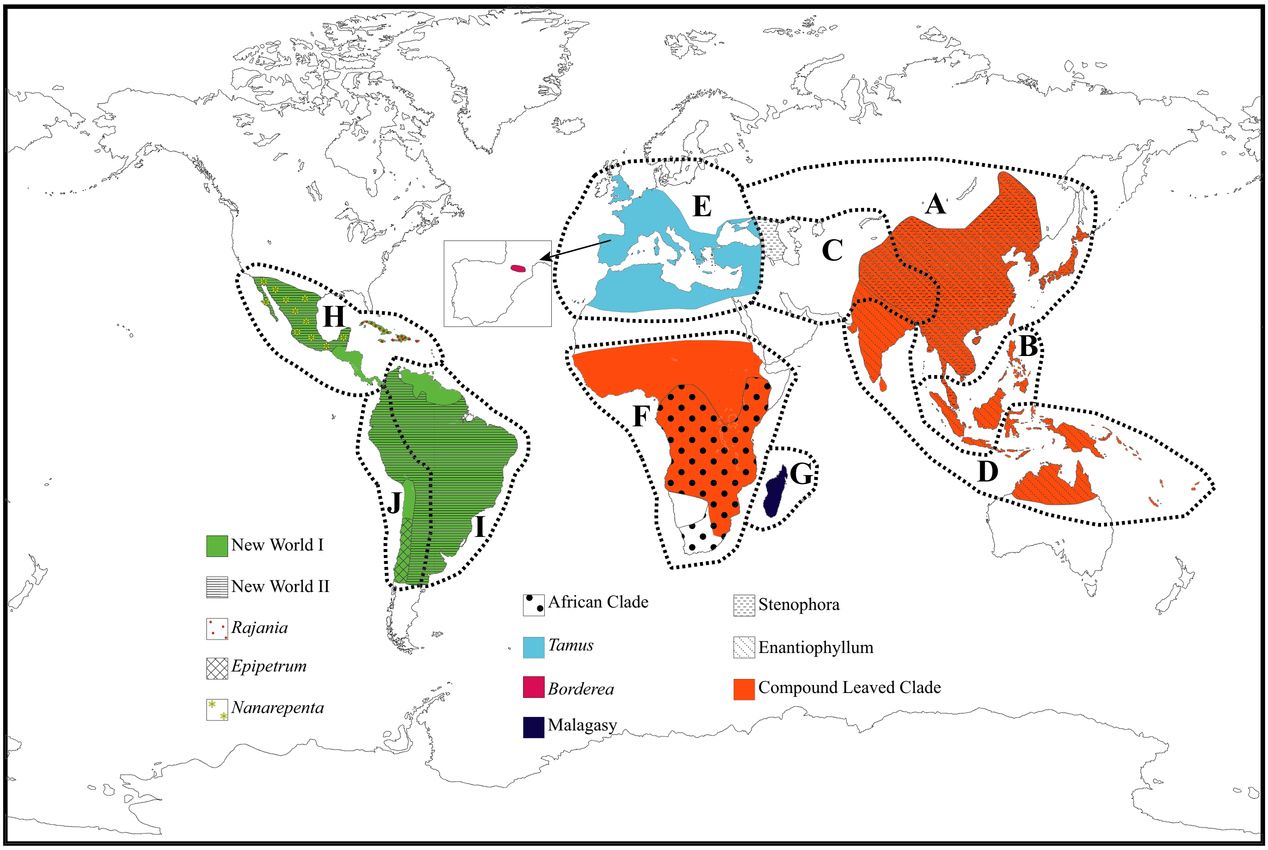


**Figure 1** Geographical distribution of *Dioscorea* lineages (see Table S1 for more detailed information on species' distributions). Dashed lines indicate the boundaries of the operational areas used in the biogeographical analysis: A, East Palaearctic [continental Asia including Temperate China-Japan, Tropical Asia (border isthmus of Kra), northern India (Burma), south China, Indochina, north-west Thailand]; B, Sundaland [Malay Peninsula (border Isthmus of Kra) and Borneo, Java, Sumatra (north-west of Wallace line)]; C, Irano-Turanian-Himalayan [from the Zagros Mountains and the Caucasus in the west through the Iranian plateau, the Hindu Kush, Tien Shan and Kunlun Shan Mountains to the Altay Mountains in the northeast, and the Tibetan Plateau and the Himalayan Mountain range in the southeast]; D, Indoaustralia [Subhimalayan India (including Sri Lanka and Bangladesh), Australasia (south-east of Wallace's line)]; E, West Palaearctic [Circum-Mediterranean (including Macaronesia) extended to Europe up to western Urals]; F, sub-Saharan African [tropical Africa (including sub-Saharan tropical Africa, Socotra, Zimbabwe, Angola, Mozambique) and South Africa]; G, Madagascar; H, Nearctic-Mesoamerica [Mexico, Central America, West Indies]; I, Neotropics [N South America (including Amazonia and the Guiana Shield) and south-east South America (including south-east Brasil, Argentina, Paraguay, south-east Bolivia)]; J, Andes [including northern, central and southern Andes and Mediterranean Chile]. The Indoaustralia area (D) includes the former east Gondwanan territories where the ancestors of the early-diverging hermaphrodite clade presumably split.

classifications of this megadiverse genus (Knuth, 1924; Burkill, 1960).

*Dioscorea* includes numerous economically important species. Some have been used by the pharmaceutical industry as sources of diosgenin, a precursor for the synthesis of steroid drugs (Liu *et al.*, 2008). Edible yam species, characterized by their starch-rich annual tubers, collectively constitute the third most important tropical tuberous crop globally (Asiedu & Sartie, 2010). Despite the evolutionary and economic importance of yams, no divergence time or biogeographical studies have been conducted. The standing biogeographical hypothesis is that of Burkill (1960), who proposed an 'Eastern' origin for Dioscoreaceae based on the distribution of the putatively ancestral hermaphrodite genera in the 'Nepenthes Arc' of tropical Asia (Madagascar, Seychelles, Sri Lanka, India, south China, Malesia, north-east Australia and New Caledonia). Burkill's hypothesis was also supported by the East Asian distribution of *Dioscorea* sect. *Stenophora*, with one known fossil in Europe. *Dioscorea* sect. *Stenophora* species have rhizomes instead of tubers, interpreted by Burkill to be the ancestral condition in the genus. Previous phylogenetic studies by Wilkin *et al.* (2005) confirmed that *Dioscorea* sect. *Stenophora* is an early branching lineage in *Dioscorea*. A Laurasian origin would fit with the current distribution of the early-diverging hermaphrodite *Stenomeris* (Malaysia, North Borneo, Philippines (cf. Huber, 1998); see Appendix S1 in Supporting Information, Fig. 1) and *Tacca* species (SE Asia; see Appendix S1), west of Wallace's Line. However, the following features all favour a potential origin of yams in Gondwana: 1) the inferred Gondwanan origin of the sister to Dioscoreaceae (Burmanniaceae) (Merckx *et al.*, 2008, 2010); 2) the presence of 'ancestral' $x = 9/10$ chromosome base numbers in the African and American species (Martín & Ortiz, 1963); and 3) the East Gondwanan-type distribution of *Trichopus* (Nepenthes Arc-type: Madagascar, India) and *Tacca pro parte* species (India, Australasia; see Appendix S1, Fig. 1). Burkill (1960) also invoked the rifting of the Atlantic in the diversification of *Dioscorea*, considering that the yam genus was already established in Gondwana by the end of the Cretaceous.

The aim of this study is to reconstruct a comprehensive phylogeny of Dioscoreaceae sensu stricto (e.g. *Dioscorea*) and close allies (*Stenomeris*, *Trichophus*, *Tacca*) including representatives from all the main lineages. We also reconstruct the spatio-temporal evolution of this narrowly circumscribed family using newly studied and reliable Dioscoreaceae and Taccaceae fossils to estimate divergence times, as well as a likelihood-based biogeographical analysis of *Dioscorea* that incorporates both palaeogeographical information and the spatial range of ancestral lineages using fossil-constrained models. This allows us to test the two competing hypotheses about the biogeographical origin of yams (Laurasia versus Gondwana) and the potential migration routes used to achieve their current pantropical distribution. Our study provides insights into the biogeographical patterns of one of the most ancient and economically important pantropical plant groups, based on reliable fossils dated to the Eocene (Potonié, 1921; Gregor, 1983).

## MATERIALS AND METHODS

### Taxon sampling and DNA sequencing

Plant samples used in this study are detailed in Appendix S1. Taxon sampling was enlarged to include groups that were underrepresented in previous phylogenetic studies (Caddick *et al.*, 2002a,b; Wilkin *et al.*, 2005). One hundred and thirty five taxa of *Dioscorea s.l.* (100% of its nine lineages; *c.* 25% of the total), six species of *Tacca* (40%), two of *Trichophus* (100%), and one of *Stenomeris* (50%) were included (see Appendix S1; Fig. 1). To this, we added two representatives of Burmanniaceae (*Burmannia* spp.), sister to Dioscoreaceae, and one representative of the Nartheciaceae, sister to Burmanniaceae and Dioscoreaceae (*Metanarthecium luteoviride*; see Appendix S1).

Four plastid DNA markers, including three coding genes (*atp*B, *mat*K, *rbc*L) and the *trn*LF (*trn*L intron–*trn*L exon–*trn*L/*trn*F spacer) region were used for this family-wide analysis. These markers were selected to provide phylogenetic resolution at different levels, from basal family level (*rbc*L) to shallow species level (*trn*LF) relationships. Although we used only genes from the plastid genome, an unpublished phylogeny based on the the low-copy nuclear coding gene *Xdh* provides support for the phylogeny presented here with plastid markers; however, lack of sampling for some *Dioscorea* lineages prevented us for using this *Xdh* data set. Moreover, given the old age estimates for most *Dioscorea* lineages inferred here, it is unlikely that they are affected by coalescence and maternal inheritance biases. Procedures for DNA amplification, sequencing and alignment are described in Appendix S2. The concatenated data set consisted of 4474 aligned positions of which 2955 corresponded to coding regions; 817 were potentially-parsimony informative (18.3%). In all, the data set included 147 samples: 96 were fully sequenced for the four markers and the remaining 51 were sequenced for three markers, with incomplete sequences coded as missing data (see Appendix S1).

### Phylogenetic and dating analyses, fossil calibrations

Bayesian and maximum parsimony searches were performed for the combined data set using, respectively, MrBayes 3.1.2 and Paup 4.0 beta10 (see Appendix S2). Absolute divergence times within Dioscoreaceae were estimated using a Bayesian relaxed-clock approach implemented in Beast 1.5.2 (Drummond & Rambaut, 2007). The concatenated data set was run under the selected GTR+I+G substitution model, a Yule tree prior, and an uncorrelated lognormal molecular clock (see Appendix S2).

Five nodes in the tree of Dioscoreaceae plus the sister Burmanniaceae (Fig. 2, see Fig. S1 in Appendix S3) were calibrated using Dioscoreaceae fossils and secondary age estimates from a monocot family-wide analysis (see below). All fossils selected here met the necessary criteria for calibrating phylogenies: reliable stratigraphy and characters that permit an unambiguous taxonomic assignment. For all fossil calibrations, we selected the upper (younger) bound of the geological period in which the fossil was found, and used it as a minimum age constraint to calibrate the stem node of the clade to which the fossil is assigned (cf. Magallón & Sanderson, 2001), using a lognormal distribution prior. The only exception was the *Tacca* fossil, which was assigned to the crown node of this lineage based on the existence of shared synapomorphies between the fossilized seed and the seeds of the extant members of this clade (cf. Magallón & Sanderson, 2001), that is, reniform and striate seeds. *Dioscorea* sect. *Stenophora* is represented in the fossil record by a fossilized leaf of *Dioscoroides lyelli* (Wat.) Fritel, from the Early Eocene. This material dates to the Cuisian stage of the Ypresian age (52–48.2 Ma) at the Paris basin (Potonié, 1921). It shows affinities to extant Stenophora taxa such as a sinuate leaf margin, membranaceous texture, and degree of curvature of the primary veins (Burkill, 1960) and thus, provides a minimum constraint of $48.2 \pm 1.0$ Ma (LogNormal Prior mean = 48.2, SD = 0.008) for the stem node of Stenophora (Node C, Fig. 3; see Fig. S2 in Appendix S3). A seed fossil from the Upper Eocene (33.9–37.2 Ma) attributed to *Tacca* found in Putschirn (Czech Republic; Gregor, 1983) was assigned to the crown node of *Tacca* (LogNormal Prior mean = 35.85, SD = 0.028) (Node D, Fig. 3). *Dioscorea* sect. *Lasiophyton* (Fig. 2) is known from a fossilized leaflet from the Mid Oligocene (*c.* 27.2 Ma) attributed to *D. wilkinii* Pan, which was found in the high plateaus of north-western Ethiopia (Jacobs *et al.*, 2005; Pan *et al.*, 2014). This fossil

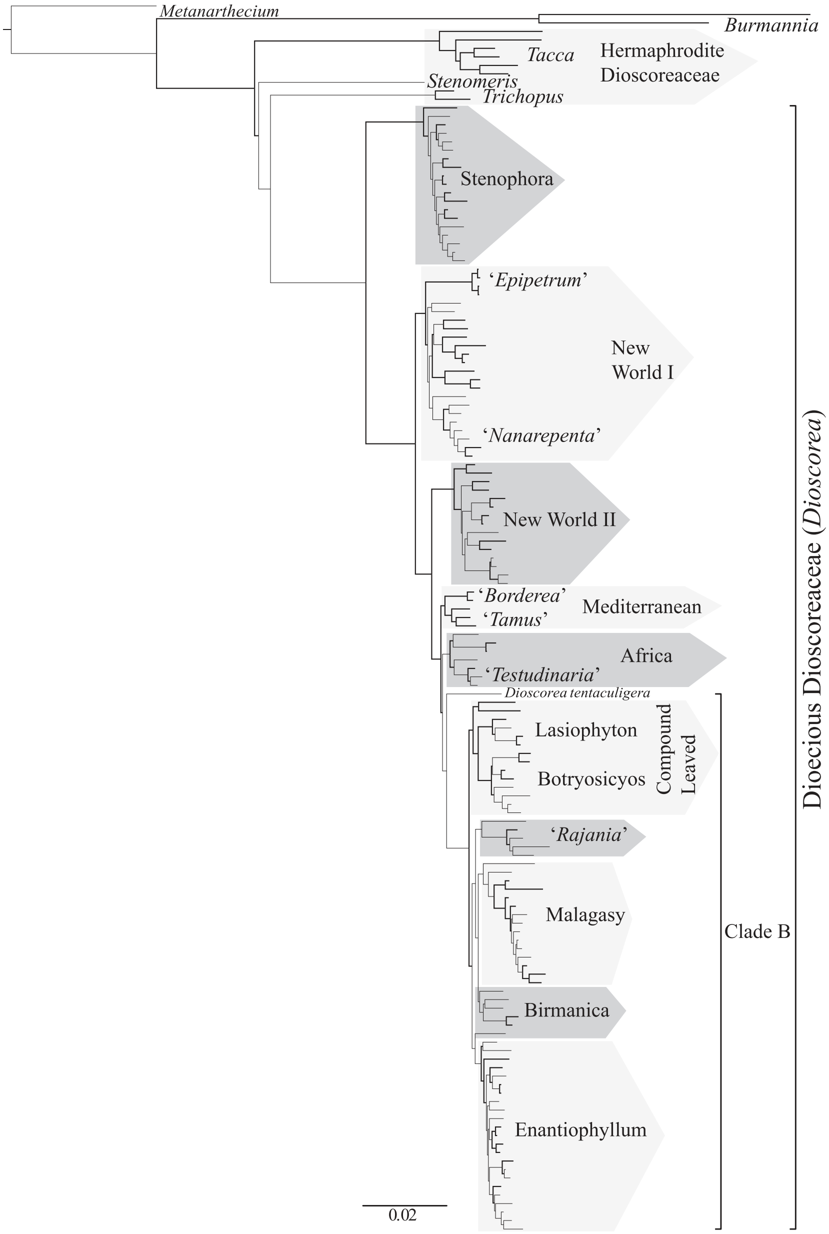
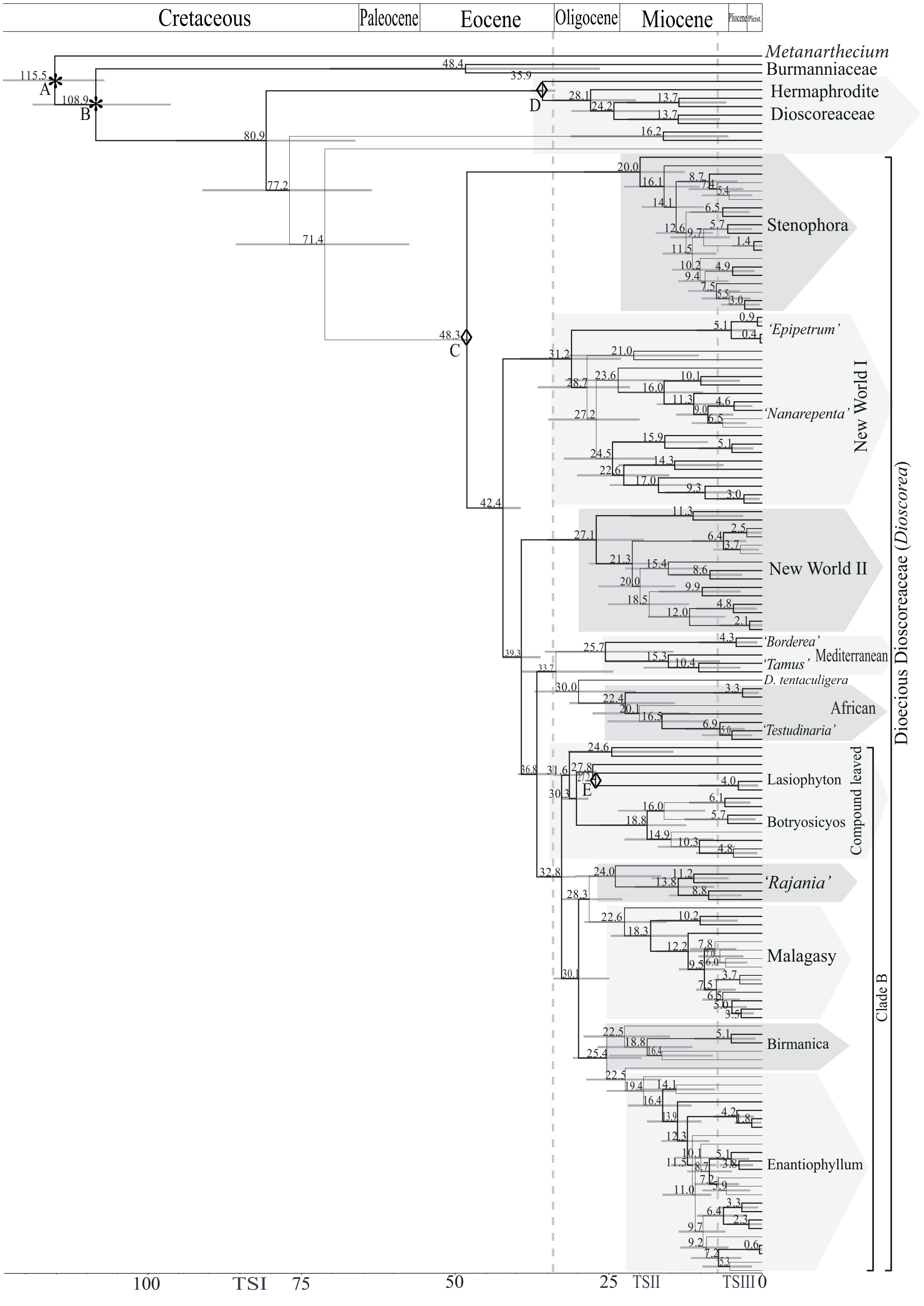


**Figure 2** Bayesian consensus tree of Dioscoreaceae showing all compatible partitions, based on the combined plastid DNA sequence data. Clades with bootstrap support values of 75–100% or posterior probability support values of 0.95–1.0 are marked with black branches.

provides a minimum constraint of 27.2 ± 0.1 Ma (LogNormal Prior mean = 27.23, SD = 0.002) for the *D. dregeana*/*D. dumetorum* stem node (Node E, Fig. 3; Iles *et al.*, 2015). Additional fossilized records of potential yams (e.g. leaves of *Dioscoreites giganteus* Andr. and a capsule of *Dioscoreae-carpum marginatum* Andr.) from the Early Oligocene (*c.* 33 Ma) were found in Hungary (Andreánszky, 1959); however, uncertainties in their attributions to extant Dioscoreaceae lineages precluded their use for nodal calibration. Putative *Dioscorea*-like fin-winged fruits from the Late Eocene–Early Oligocene (*c.* 34 Ma) found in the Florissant Formation in Colorado (Manchester, 2001) could not be confidently assigned to Dioscoreaceae.

To estimate the age of origin for the most recent common ancestor of yams, we used a secondary calibration approach in which we dated representatives of all main Dioscoreaceae

**Figure 3** BEAST maximum clade credibility tree of Dioscoreaceae based on the plastid data set, showing node mean ages and 95% highest posterior density (HPD) intervals for nodes with > 50% posterior support (grey bars). Diamonds represent fossil calibrations and asterisks secondary calibrations: Nodes A (crown node of Dioscoreales) and B (crown node of Discoreaceae + Burmanniaceae) were calibrated using Magallón *et al.* (2015) secondary age estimates; Nodes C (stem node of *D.* sect. *Stenophora*), D (crown node of *Tacca*) and E (stem node of *D.* sect. *Lasiophyton*) were calibrated using the fossil record (see text). Vertical discontinuous grey lines separate the three time slices (TSI-TSIII) used in the biogeographical stratified (M1) DEC analysis. Clades with bootstrap support values of 75–100% or Posterior Probability support values of 0.95–1.0 are marked with wider branches.


Cretaceous
Paleocene
Eocene
Oligocene
Miocene
Pliocene
Pleist.
*Metanarthecium*
Burmanniaceae
Hermaphrodite Dioscoreaceae
Stenophora
*'Epipetrum'*
*'Nanarepenta'*
New World I
New World II
*'Borderea'*
*'Tamus'*
Mediterranean
*D. tentaculigera*
African
*'Testudinaria'*
Lasiophyton
Botryosicyos
Compound leaved
*'Rajania'*
Malagasy
Birmanica
Enantiophyllum
Clade B
Dioecious Dioscoreaceae (*Dioscorea*)
A
B
C
D
E
115.5
108.9
80.9
77.2
71.4
48.4
35.9
48.3
42.4
39.3
36.8
33.7
32.8
31.2
28.1
24.2
20.0
100
TSI
75
50
25
TSII
TSIII
0

lineages and of sister family Burmanniaceae (*Burmannia biflora*, *B. longiflora*) based on Magallón *et al.* (2015). The stem node of Dioscoreaceae s. s. (Node B, Fig. 3) was calibrated using the estimated divergence of Dioscoreaceae at 108 ± 10 Ma, whereas the stem node of Dioscoreaceae + Burmanniaceae (Node A, Fig. 3) was calibrated using the estimate of 115 ± 4.5 Ma, assigned as normal prior distributions for the respective nodes.

### Ancestral range reconstruction analysis

Ten operational areas were defined based on the present distribution ranges of extant yam lineages (Fig. 1; see Appendix S1) and palaeogeography/plate tectonic history (cf. Bartish *et al.*, 2011; Buerki *et al.*, 2011). The likelihood-based dispersal-extinction-cladogenesis (DEC) model (Ree *et al.*, 2005) implemented in Lagrange 20130526 (Ree & Smith, 2008) was used to infer global extinction and dispersal rates and ancestral range inheritance scenarios for each node in the maximum clade credibility tree obtained from Beast. Widespread ranges were constrained to combinations of two ancestral areas (AAs), assuming that ancestors were not more widespread than their extant descendants (Sanmartín, 2003). Analyses were run imposing the fossils' AAs to the corresponding nodes following Meseguer *et al.* (2015). We tested two alternative dispersal models: an unconstrained model (M0) in which dispersal rates were assumed equal among areas, and a stratified model (M1) with three time slices reflecting changes in continental connectivity relevant in plant biogeography (see Appendix S1). TSI (Early Eocene–Early Oligocene, 55–34 Ma) captures the connectivity through the NALB and the climatic LPTM/EECO boundary; TSII (Early Oligocene–Late Miocene, Tortonian–Messinian, 34–7 Ma) reflects the shift to dispersal through the BLB and the opening of the Red Sea strait; TSIII (Late Miocene to present, 7–0 Ma) captures the Central American connection and the accretion of SE Asia, and ends with the current continental configuration.

## RESULTS

### Phylogenetic relationships

Figures 2 (see Fig. S1 in Appendix S3) and 3 (see Fig. S2 in Appendix S3) show the results of MrBayes and Beast analyses respectively. Maximum parsimony analysis gave a similar topology and only bootstrap support values are indicated in Fig. 2 (see Fig. S1 in Appendix S3). Nine major clades, consistent with previous studies but with better resolution and clade support, were recovered within a monophyletic *Dioscorea*. MrBayes and beast topologies were highly congruent, except for the resolution of the following splits, the branching order of the weakly supported early-diverging lineages [(*Tacca*(*Stenomeris*(*Trichopus*,rest))), Fig. 2 vs. (*Tacca* (*Trichopus*(*Stenomeris*,rest))), Fig. 3], and of the strongly supported African and Mediterranean clades [(Mediterranean (African(*D.tentaculigera*,rest))), Fig. 2 vs. (Mediterranean/African+*D. tentaculigera*), Fig. 3]. The earliest diverging *Dioscorea* clades (i.e. Stenophora, New World (NW) I and II, and to a lesser extent Mediterranean+Africa), also showed strong to moderate support. Branching order of clades within the more recently evolved Clade B showed successively the compound leaved (CL), *Rajania* – Malagasy and Enantiophyllum+Birmanica clades (Fig. 3); these clades had weak support but included strongly supported subclades (Figs 2 & 3). The Birmanica group was resolved as paraphyletic, with *D. birmanica* sister to the Enantiophyllum clade (Fig. 3). All the former *Dioscorea*-type segregate genera (*Borderea*, *Epipetrum*, *Nanarepenta*, *Rajania, Tamus*, *Testudinaria*) were resolved as monophyletic with high support.

### Divergence time and biogeographical analyses

According to the Beast analysis (Fig. 3, see Fig. S2 in Appendix S3), the divergence of the hermaphrodite Dioscoreaceae lineages, *Tacca, Trichopus* and *Stenomeris* in the Late Cretaceous (Campanian, 80.9 (95% HPD: 66.5–95.8), 77.2 (63.7–91.4) and 71.4 (57.7–85.9) Ma respectively), considerably pre-dates the split of the dioecious *Dioscorea* lineage, which is estimated as Mid Eocene (Lutetian, 48.3 (47.6–49.1) Ma). Divergence times for most *Dioscorea* lineages range between the Late Oligocene–Early Miocene and the Pliocene, but some recently evolved species originated in the Pleistocene (Fig. 3). The earliest diverging lineages in *Dioscorea* (Mediterranean+Africa, 33.7 (24.3–39.2); CL, 31.6 (33.8–39.9); NW I, 31.2(23.1–39.5); NW II, 27.1 (19.5–35.1); *Rajania* + *D. trifida*, 24.0 (16.6–30.6)) diverged in the Oligocene. The remaining Clade B lineages and Stenophora (Malagasy, 22.6 (15.6–29.1); Birmanica p.p., 22.5 (15.1–29.2); Stenophora, 20.0 (12.8–29.1); Enantiophyllum, 19.4 (14.1–26.4)) diverged in the Miocene. The formerly segregate genera (now *Dioscorea*-type) subclades diverged in the Miocene (e.g. *Tamus*, 15.3 (6.9–25.7); *Rajania*, 13.8 (7.9–20.8); *Nanarepenta*, 11.3 (6.4–17.2); *Testudinaria*, 6.9 (3.0–12.7)) or in the Pliocene (*Epipetrum*, 5.1 (1.4–10.8); *Borderea*, 4.3 (0.8–10.4).

The two biogeographical DEC models with fossil AA constraints (M0 and M1) gave similar results (Fig. 4, Appendices S1, S3 Fig. S3), but reconstructed distinct biogeographical scenarios for the most recent common ancestor (MRCA) of *Dioscorea* (Node 132). Because the stratified M1 model showed a better fit to the data than the unconstrained M0 model (−ln likelihood 281.4 versus 316.9, respectively; likelihood ratio test, $P = 0.001$), we will refer to the results from this model hereafter (but see comments below and in the Discussion). The global estimated dispersal rate for the M1 model (*dis*: 0.0136947) was three times higher than the estimated extinction rate (*ext*: 0.00465982).

An Eocene Palaearctic 'AE' and 'CE' distribution was inferred for the MRCA of *Dioscorea* in the M1 and M0 models, respectively (Node 132, Fig. 4, see Fig. S3 in Appendix S3). A dispersal event from Asia to South America during the Palaeocene–Eocene transition is invoked to

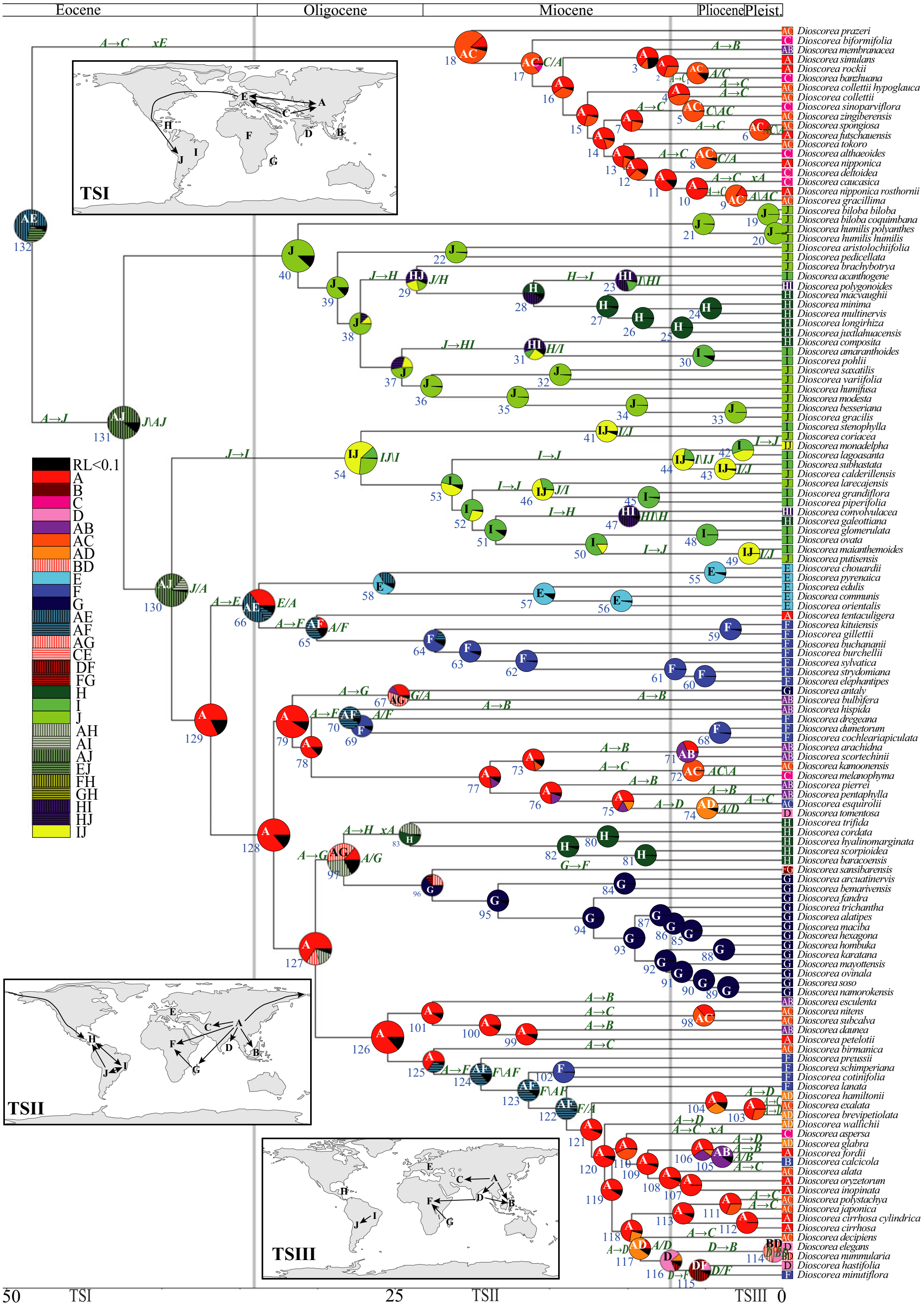


**Figure 4** Spatio-temporal evolution of Dioscoreaceae as inferred by Lagrange using the DEC stratified (M1) model. Pie charts at nodes indicate the relative probabilities for alternative ancestral ranges. Node numbers are indicated below branches. Vertical grey lines separate the three time slices (TSI-TSIII) used in the Lagrange analysis. Inset maps represent the palaeogeographical configuration of continents in each time slice, with main inferred dispersal events represented by arrows. Inferred dispersal (x→y), vicariance (x/x) and peripheral isolate speciation (x\xy) events are represented also in the tree.

explain the widespread transcontinental distribution (AJ) of the ancestor of the non-Stenophora *Dioscorea* clade (Node 131; Fig. 4). According to models M1 and M0, the colonization of the Neotropics by *Dioscorea* ancestors likely occurred through the Western Palaearctic and the Nearctic (AE or CE, AJ; nodes 132, 131, 130; Fig. 4, see Fig. S3 in Appendix S3). A continental range expansion from Eastern Palaearctic to the Irano-Turanian-Himalayan area was inferred to explain the widespread Asian (AC) distribution of the ancestor of Stenophora in the Early Miocene (Node 18; Fig. 4). An extinction in area E, Europe, was also reconstructed in this period.

Dispersal from Eastern to Western Palaearctic is inferred to be responsible for the widespread AE distribution of the ancestor of the African+Mediterranean clade in the mid Eocene (Node 66; Fig. 4). This was followed by a vicariance event that gave rise to the divergence of the Western Palaearctic (Mediterranean) clade during the Eocene/Oligocene transition (E, Node 58; Fig. 4). The ancestors of its sister clade in Asia dispersed to Africa in the Early Oligocene (AF, Node 65; Fig. 4), followed by a split between the Eastern Palaearctic *D. tentaculigera* and an African lineage (F, Node 64, Fig. 4), which diversified during the Mid Oligocene. Independent dispersal events from Asia to Madagascar and Africa in the Oligocene would explain the widespread distributions of the ancestors of some CL lineages (AG, AF, Nodes 67, 70; Fig. 4). Similarly, the present distribution of *Rajania* and the Malagasy lineages within Clade B are explained by separate dispersal events to the Caribbean region and Madagascar during the Oligocene (AG, H, G; Nodes 97, 83, 96; Fig. 4), whereas a Miocene migration from Asia to Africa might account for the widespread distribution of the ancestors of Enantiophyllum clade (AF; Nodes 124–122; Fig. 4). Scenarios of recolonization of Africa (F) and neocolonization of Sundaland and Indoaustralia (BD) were inferred to have occurred as recently as the Pliocene (Nodes 114, 115; Fig. 4).

## DISCUSSION

### A new phylogeny of yams supports a Laurasian origin and the role of the North Atlantic Land Bridge in the Early Tertiary migration of angiosperms

The extensively sampled phylogeny presented here provides a solid framework for evolutionary studies of the dioecious *Dioscorea* lineages. However, uncertainty still persists regarding the divergence order of the hermaphrodite lineages, which are otherwise embedded within the strongly supported Dioscoreaceae clade (Figs. 2 & 3). Beast results support the divergence of the sister *Dioscorea* and *Stenomeris*, consistent with previous studies (Caddick *et al.*, 2002a,b; Wilkin *et al.*, 2005). Further evidence from Merckx *et al.* (2006, 2010) suggests a close relationship of these hermaphrodite lineages to the pantropical mycotrophic Thismiaceae.

Relationships among major clades of *Dioscorea* are mostly in agreement with previous studies (Caddick *et al.*, 2002a; Wilkin *et al.*, 2005). New findings include the nested position of the central Chilean endemic *Epipetrum* within the NW I clade, the early divergence of the 'compound-leaved' lineage within Clade B, and the resolution of the paraphyletic Birmanica group as the closest relatives of the most recently evolved members of the Enantiophyllum clade (Figs 2 & 3).

Our biogeographical study lends support to an ancestral Laurasian distribution for yams. The inferred Palaeartic origin of the group in the M1 (AE, Fig. 4) and M0 (CE, see Fig. S3 in Appendix S3) models agrees well with those inferred for other pantropical angiosperm groups (e.g. Baker & Couvreur, 2013; Buerki *et al.*, 2013, 2014) in the northern mid-latitude megathermal belt (Morley, 2003). However, our biogeographical scenario also suggests a widespread distribution of ancestral yams in eastern Laurasia-Gondwana (the '*Nepenthes* arc' of Clarke, 1997). This scenario is compatible with the 'interplate dispersal path' hypothesis suggested by Morley (2003) (e.g. dispersal from Asia towards the south (tropical belt) as the climate became cooler in the Eocene–Oligocene transition) for megathermal plant groups that radiated in the Mid-to-Late Cretaceous in this area.

The transcontinental distribution exhibited by the ancestors of the non-Stenophora *Dioscorea* clade (AJ, Node 131, Fig. 4) might be explained by either a long-distance dispersal event or by migration across one of the land bridges that were present during the Early Tertiary, the NALB and the BLB (Tiffney, 1985; Brikiatis, 2014). During the Palaeocene–Eocene thermal maximum (PETM, Zachos *et al.* 2008), a boreotropical vegetation belt, composed by evergreen broad-leaved and deciduous taxa, extended across the Holarctic (Tiffney, 1985; Morley, 2003). The dramatic cooling event that followed the Eocene–Oligocene transition (Zachos *et al.* 2008) led to the extinction of many of these megathermal plants in Europe and North America, some of which migrated to tropical regions in the south (Mai, 1995; Kubitzki & Krutzsch, 1996; Morley, 2003). The discovery of an Early Eocene (Upper Ypresian, 52–48.2 Ma) *Dioscorea lyelli* seed fossil from Central France (Potonié, 1921) supports the presence of yams in Europe during the PETM. Range expansion from Asia to South America could therefore be explained by land dispersal across the NALB in the Eocene (55–50 Ma, Tiffney, 1985), followed by dispersal from North America to South America, presumably facilitated by occasional island chains (e.g. proto-Greater Antilles, cf. Antonelli *et al.*, 2009). The latter is further supported by two samaroid-fruited Mexican species resolved as sister to Stenophora in our *rbc*L and *atp*B data sets (results not shown because the subtree does not fit the 3-genes threshold criterion). A similar hypothesis has been proposed for other pantropical plant groups (e.g. Davis *et al.*, 2002; Antonelli *et al.*, 2009; Baker & Couvreur, 2013). An alternative route to North America is through Africa, which had at that time collided with Europe but had not yet undergone the climatic aridification that started in the Neogene.

## Continental range expansions in early *Dioscorea* lineages

Burkill (1960) hypothesized that Stenophora originated north of the Tethys, and dispersed and radiated through Europe and Asia during the Eocene and Oligocene, with a secondary colonization of the Himalayas. Our results confirm an early colonization of the Stenophora lineage from Asia towards the newly emerged Himalayan mountains in the Miocene (AC), followed by colonization of South East Asia (AB) in the Pliocene (Figs. 3 & 4). A similar scenario of range expansion followed by diversification is inferred for the ancestors of the NW I and II lineages, which migrated to the north (Caribbean) and east (SE South America), during the Middle Eocene–Oligocene (Figs. 3 & 4), probably facilitated by the absence of most of the Andean range (Taylor, 1991).

A westward dispersal event in the Late Eocene is inferred to explain the widespread Asian-European distribution of the ancestor of the African-Mediterranean clade (Node 66; Figs. 3 & 4); this could have been favoured by the closing of the Turgai Strait between these two continents *c.* 30 Ma ago (Sanmartín *et al.*, 2001). A later dispersal to sub-Saharan Africa (Node 65, Figs. 3 & 4) probably occurred through the Indian accreted subcontinent and the islands of the eastern Tethys (TSII, Fig. 4). This was concomitant with the global climate cooling that inhibited most interplate dispersals of megathermal plants and likely caused the extinction of the boreotropical flora in the former Laurasian northern latitudes (Morley, 2003). However, Dioscoreaceae fossils provide evidence that tropical yams likely persisted in central Europe until the Early Oligocene (33 Ma; Andreánszky, 1959).

An Early Oligocene widespread Asian distribution is reconstructed for the ancestor of Clade B (Figs. 3 & 4). Later colonization of Africa was inferred for the ancestor of the Lasiophyton clade, corroborated by the finding of the *D. wilkinii* macrofossil in the Mid Oligocene in Ethiopia (Jacobs *et al.*, 2005; Pan *et al.*, 2014). The current distribution of *Rajania* in the Caribbean region could be explained by a dispersal event through Africa (e.g. Dick *et al.*, 2003; Renner, 2004; see Fig. S3 in Appendix S3) or via the Early Tertiary Bering Land Bridge I (BLB-I; Sanmartín *et al.*, 2001; Fig. 4). The latter route, supported by the M1 model (Fig. 4), is more likely: warmer climatic conditions permitted trans-Beringian exchange for warm temperate plants until the end of the Eocene, when global cold and dry conditions locked this route (Tiffney, 1985; Sanmartín *et al.*, 2001).

## Recent diversification of extant *Dioscorea* lineages

Most divergence events within the main *Dioscorea* lineages occurred in the Oligocene and Miocene on a world-wide scale (TSII-III; Fig. 4). The pan-Himalayan Stenophora clade radiated in this area, and the most recent lineages only dispersed to the west (Caucasus) and east (temperate SE China and Japan) in Miocene–Pliocene times (Figs. 3 & 4). Similar pre-Messinian migrations have been proposed for other temperate and continental plants in central and southern Asia (e.g. Von Hagen & Kadereit, 2001; Buerki *et al.*, 2011).

Several Oligocene and Miocene radiations within the NW I clade resulted in the earliest diverging Chilean (*Epipetrum*) lineages (Fig. 4). By contrast, the more recently evolved ones likely dispersed to northern South America and to Central America and Mexico in the Oligocene, leading later to the evolution of members of the *Nanarepenta* clade (Fig. 4). The inferred colonizations from Chile and the Andes to north-west South America and Central America in the Oligocene and Early Miocene are compatible with the palaeogeographical configuration of South America and the existence of land corridors before the main uplift of the Andean Cordillera (Taylor, 1991; Antonelli *et al.*, 2009). The Mid-to-Late Miocene colonization and radiation of the *Nanarepenta* clade in Mexico (11.3–4.6 Ma) was concomitant with the spread of some South American biotas to Central and North America (Head *et al.*, 2012) and viceversa (Bacon *et al.*, 2013; Elmer *et al.*, 2013), all predating the definitive closure of the Isthmus of Panama (3.5 Ma; Briggs, 1994). Most of the Oligocene–Miocene dispersals inferred within the NW II clade were confined to South America and those colonizations likely followed the same routes as in the NW I clade (Fig. 4).

Our results support the hypothesis that Madagascar was colonized once in Mid Oligocene times (Chattian, 28.3 Ma) from Asia (Fig. 4b; Warren *et al.*, 2010), contrary to the African origin documented for many other plants (e.g. Weeks & Simpson, 2007). The ancestor of the Malagasy clade arose in the Late Oligocene and the lineage rapidly diverged, probably favoured by the abundance and diversity of suitable ecological niches in the Miocene and Pliocene. The ancestor of *Tamus* colonized the Canary Islands probably by a long-distance dispersal from the coast of north-west Africa (Sanmartín *et al.*, 2008). The Mid-to-Late Miocene divergence of *D. orientalis* (J. Thièbaut) Caddick & Wilkin in the eastern Mediterranean region supports its expansion before the establishment of the Mediterranean climate conditions in the Pliocene (Suc, 1984). Finally, the origin of the Birmanica+Enantiophyllum clade was placed in E Asia in the Late Oligocene and most of its descendant lineages were inferred to have spread in this region through the Miocene (Fig. 4). However, a series of dispersals to other surrounding areas occurred within the clade, mostly to sub-Saharan Africa and to the Irano-Turanian-Himalayan region in the Miocene and Pliocene (Figs. 3 & 4).

The number of biogeographical speciation events in yams decreased during the Quaternary (TSIII, Fig. 4). The low number of species of extra-tropical yams (e.g. *Epipetrum*, *Borderea*) is probably associated with their adaptation to arid and alpine glacial conditions and potential extinction of some non-tolerant congeners (Segarra-Moragues & Catalán, 2002; Viruel *et al.*, 2012, 2014). By contrast, most of the remaining tropical lineages experienced greater diversification and dispersal (Fig. 4).

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## SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

**Appendix S1** Supplementary Tables. List of taxa and dispersal rate matrices.
**Appendix S2** Expanded Materials and Methods.
**Appendix S3** Supplementary Figures. MrBayes and Beast trees, Lagrange M0 model.

## BIOSKETCH

The research team is formed by experts on systematics and evolution of Dioscoreaceae and monocots, on the development of new analytical methods in historical biogeography, and on the spatio-temporal evolution of various plant families.

Author contributions: P.W., P.C., L.R. conceived and designed the experiment. J.V., F.F., L.R. performed the experiments. J.V., P.C., I.S. analysed the data. P.C., J.V., P.W., I.S., L.R., F.F., J.G.S.M. wrote the paper. The authors declare no conflict of interest.

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