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Gourds afloat: a dated phylogeny reveals an Asian origin of the gourd family (Cucurbitaceae) and numerous oversea dispersal events

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Knowing the geographical origin of economically important plants is important for genetic improvement and conservation, but has been slowed by uneven geographical sampling where relatives occur in remote areas of difficult access. Less biased species sampling can be achieved when herbarium collections are included as DNA sources. Here, we address the history of Cucurbitaceae, one of the most economically important families of plants, using a multigene phylogeny for 114 of the 115 genera and 25 per cent of the 960 species. Worldwide sampling was achieved by using specimens from 30 herbaria. Results reveal an Asian origin of Cucurbitaceae in the Late Cretaceous, followed by the repeated spread of lineages into the African, American and Australian continents via transoceanic long-distance dispersal (LDD). North American cucurbits stem from at least seven range expansions of Central and South American lineages; Madagascar was colonized 13 times, always from Africa; Australia was reached 12 times, apparently always from Southeast Asia. Overall, Cucurbitaceae underwent at least 43 successful LDD events over the past 60 Myr, which would translate into an average of seven LDDs every 10 Myr. These and similar findings from other angiosperms stress the need for an increased tapping of museum collections to achieve extensive geographical sampling in plant phylogenetics.

Keywords: Bayesian molecular clock; biogeography; dispersal; economic plants; museomics; test of monophyly

1. INTRODUCTION

Molecular clock analyses suggest that the majority of lineages of legumes that occur on islands are younger than 30 Myr (Lavin & Beyra Matos 2008) and that plant diaspores from source areas hundreds or thousands of kilometres away regularly reach isolated Arctic islands (Alsos *et al.* 2007), island-like mountains in Eastern Africa and mountain ranges in the Northern Cape, South Africa (Galley *et al.* 2007). Striking dispersal events have also been documented for the flora of Hawaii (Wagner *et al.* 1990), the montane region of New Zealand (Winkworth *et al.* 2005) and many other island systems. Such frequent long-distance dispersal (LDD) implies that long-established views on the origin of economically important plants may need to be re-evaluated based on drastically enlarged geographical sampling. An example is the origin of *Cucumis sativus*, the cucumber. Cucumber ranks among the top 10 vegetables in world production (Chen *et al.* 2004). Until 2006, it was thought that the genus *Cucumis* had 32 species and was essentially African. Only *C. sativus* and *C. hystrix* were thought to occur naturally in India, China, Burma and Thailand (Ghebretinsae & Barber 2006). However, broader geographical species sampling revealed that *C. sativus* is closer to 13 species from Australia, India, Yunnan and Indochina than to any African species (Renner & Schaefer 2008).

Biogeographic inference for economically important plants is complicated by human transport of seeds between continents for at least 10 000 years (Smith 1997; Sanjur *et al.* 2002; Dillehay *et al.* 2007). The extent of the anthropogenic transfer, however, is difficult to work out without comprehensive phylogenetic frameworks, which can be prohibitively expensive if worldwide collecting of material is required. In the economically important plant family Cucurbitaceae, these difficulties have led to the geography of the closest relatives of watermelon (*Citrullus lanatus*), cucumber (*Cucumis sativus*), loofah (*Luffa acutangula*), bitter melon (*Momordica charantia*), chayote (*Sechium edule*), ivy gourd (*Coccinia grandis*), snake gourd (*Trichosanthes cucumerina*) and creeping cucumber (*Melothria pendula*) remaining ambiguous. Natural LDD of cucurbit diaspores may be frequent because many are adapted for transport by birds or wind, or they can withstand long periods in water (*Cayaponia*, *Fevillea*, *Hodgsonia*, *Lagenaria*, *Luffa* and *Sicana*; Ridley 1930; Whitaker & Carter 1954).

Here, we use worldwide sampling, based on museum specimens, to infer the biogeographic history of Cucurbitaceae, a family consisting of climbers or trailers of tropical and subtropical regions that are typically strongly seasonal, lacking aboveground parts during part of the year. These traits have caused cucurbits to be under-collected (Gentry 1991), resulting in dozens of species still known from only one or two collections even in the world's leading herbaria (e.g. De Wilde & Duyfjes 2007). Of the approximately 960 accepted species of Cucurbitaceae, approximately 40 per cent are endemic in the American

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continent, and the remainder occur in Africa (28%), Asia (26%), Australia (2%) and Europe (1%; Schaefer & Renner in press).

Based on chloroplast sequences from all but one of the 115 genera and 25 per cent of the 960 species, and employing specimens from 30 herbaria and up to 172 years old, we address here the following questions: did Cucurbitaceae initially diversify in Asia, in America, or in Africa and Madagascar? What are the geographical sources of the world's major Cucurbitaceae floras? Are the transoceanic geographical ranges in the genera *Cayaponia*, *Lagenaria*, *Luffa* and *Sicyos* anthropogenic or the result of natural LDD? We also use Cucurbitaceae to illustrate the still barely tapped potential of museum collections to achieve less biased geographical sampling than has traditionally been employed in tropical plant phylogenetics.

2. MATERIAL AND METHODS

(a) *Taxon sampling*

We generated 126 sequences, representing 32 species from seven genera not sampled in previous studies (*Anangia*, *Cucumeropsis*, *Gomphogyne*, *Hodgsonia*, *Papuasicyos*, *Pseudosicydium* and *Zanonia*). GenBank accession numbers (EU436320–EU436422) and vouchers for newly sequenced taxa are listed in table 1 in the electronic supplementary material. Accession numbers and voucher information for additional Cucurbitaceae sequences from our earlier studies are given in Zhang *et al.* (2006), Kocyan *et al.* (2007), Schaefer *et al.* (2008a) and Nee *et al.* (submitted). Fourteen sequences from Genbank were included to represent *Indomelothria* (EF065456), *Neochmanandra* (EF065484–86), *Urceodiscus* (EF065464) and *Zehneria* (EF065485, EF065489, EF065491–493, EF065497, EF065499–500 and EF065502). This resulted in a sampling of 114 of the 115 genera currently recognized in Cucurbitaceae (Schaefer & Renner in press). The only genus of Cucurbitaceae not yet sequenced is *Khmeriosicyos* W. J. de Wilde & B. Duyfjes, which is only known from the Cambodian type collection. Judging from morphology, it is expected to group with other Asian Benincaseae. As outgroups, we used 15 species of the Cucurbitales families Anisophyllaceae, Begoniaceae, Coriariaceae, Corynocarpaceae, Datisceae and Tetramelaceae, based on Zhang *et al.* (2006). Of these, the Begoniaceae, Datisceae and Tetramelaceae, with the Cucurbitaceae, constitute a morphologically and molecularly well-defined clade, traditionally called the core Cucurbitales (Zhang *et al.* 2006).

(b) *DNA extraction, amplification, sequencing and alignments*

Total genomic DNA was isolated from herbarium specimens or, more rarely, silica-dried leaves with a commercial plant DNA extraction kit (NucleoSpin, MACHEREY-NAGEL, Düren, Germany), following the manufacturer's manual. We amplified the *rbcl* and *matK* genes, the *trnL* intron and the *trnL-F* and *rpl20-rps12* intergenic spacers. Polymerase chain reactions (PCRs) were performed with the standard protocol and primers described in Kocyan *et al.* (2007), and products were purified with the Wizard SV PCR clean-up kit (Promega GmbH, Mannheim, Germany). Cycle sequencing was performed with BigDye Terminator cycle sequencing kits on an ABI Prism 3100 Avant automated sequencer (Applied Biosystems, Foster City, California, USA). Sequences were edited with SEQUENCHER v. 4.6 (Gene Codes, Ann Arbor,

Michigan, USA) and aligned by eye, using MACCLADE v. 4.06 (Maddison & Maddison 2003).

The data matrices comprised 245 ingroup species plus 15 outgroup species. The lengths of the individual loci were 1356 aligned nucleotides for the *rbcl* gene, 1195 for the *matK* gene, 667 for the tRNA-Leu (*trnL*) intron (after exclusion of a poly A run and a highly variable microsatellite region), 803 for the tRNA-Leu–tRNA-Phe (*trnL-F*) intergenic spacer and 1010 for the *rpl20-rps12* intergenic spacer. The combined dataset comprised 5031 aligned nucleotides.

(c) *Phylogenetic analysis*

Maximum-likelihood (ML) tree searches and ML bootstrap searches were performed using RAXML v. 7.0.3 (Stamatakis *et al.* 2008; available at <http://phylobench.vital-it.ch/raxml-bb/>) and GARLI v. 0.951 (Zwickl 2006; available at www.bio.utexas.edu/faculty/antisense/garli/Garli.html). RAXML and GARLI searches relied on the GTR+ Γ +I model (six general time-reversible substitution rates, assuming gamma rate heterogeneity and a proportion of invariable sites), with model parameters estimated over the duration of specified runs. Analyses in RAXML were run both with the combined unpartitioned data and with a model that partitioned the *rbcl* gene from the remaining non-coding regions. GARLI does not allow data partitioning. The data matrix and trees have been deposited in TREEBASE (www.treebase.org; study number S2210).

(d) *Molecular clock analysis*

Estimation of divergence times relied either on a strict clock or on a Bayesian relaxed clock with autocorrelated rates (Thorne *et al.* 1998). Very short ('zero-length') branches are known to cause problems for time estimation algorithms, and we therefore reduced their number by using the best-scoring ML tree for 147 taxa instead of the full 260-taxon tree. The clock tree was rooted on Coriariaceae and Corynocarpaceae, instead of Anisophyllaceae, because the latter are extremely rich in autapomorphies, contributing towards rate heterogeneity near the base.

For the Bayesian approach, we used BASEML from the PAML package (Yang 2007) and MULTIDIVTIME (Thorne *et al.* 1998; Thorne & Kishino 2002) in LAGOPUS, an R package written by Heibl & Cusimano (2008). LAGOPUS checks the input data for consistency, automates the assignment of constraints to nodes and connects the executables of the mentioned software packages in a pipeline. Model parameters for the 147-taxon matrix were estimated in BASEML, and branch lengths and their variance then calculated in ESTBRANCHES, all under the F84+G model (the only model implemented in MULTIDIVTIME). Priors for MULTIDIVTIME were as follows: based on outgroup fossils (below), the prior on the mean age of the root node was set to 84 Myr, with an equally large standard deviation. The prior on the substitution rate at the root was set to the value obtained by dividing the median distance between the root and the tips in the ESTBRANCHES phylogram by 84 Myr. This yielded a rate of 0.0009 substitutions per site and million years [S/(S \times Myr)]. The prior for the Brownian motion parameter, which controls the magnitude of autocorrelation along the descending branches of the tree, was set to 1.11, with a standard deviation of the same size. Markov chain Monte Carlo (MCMC) samples were drawn for every 100th generation up to one million generations, with a burn-in of 100 000 cycles. Confidence in node ages was assessed using the 95 per cent credibility intervals calculated by MULTIDIVTIME.

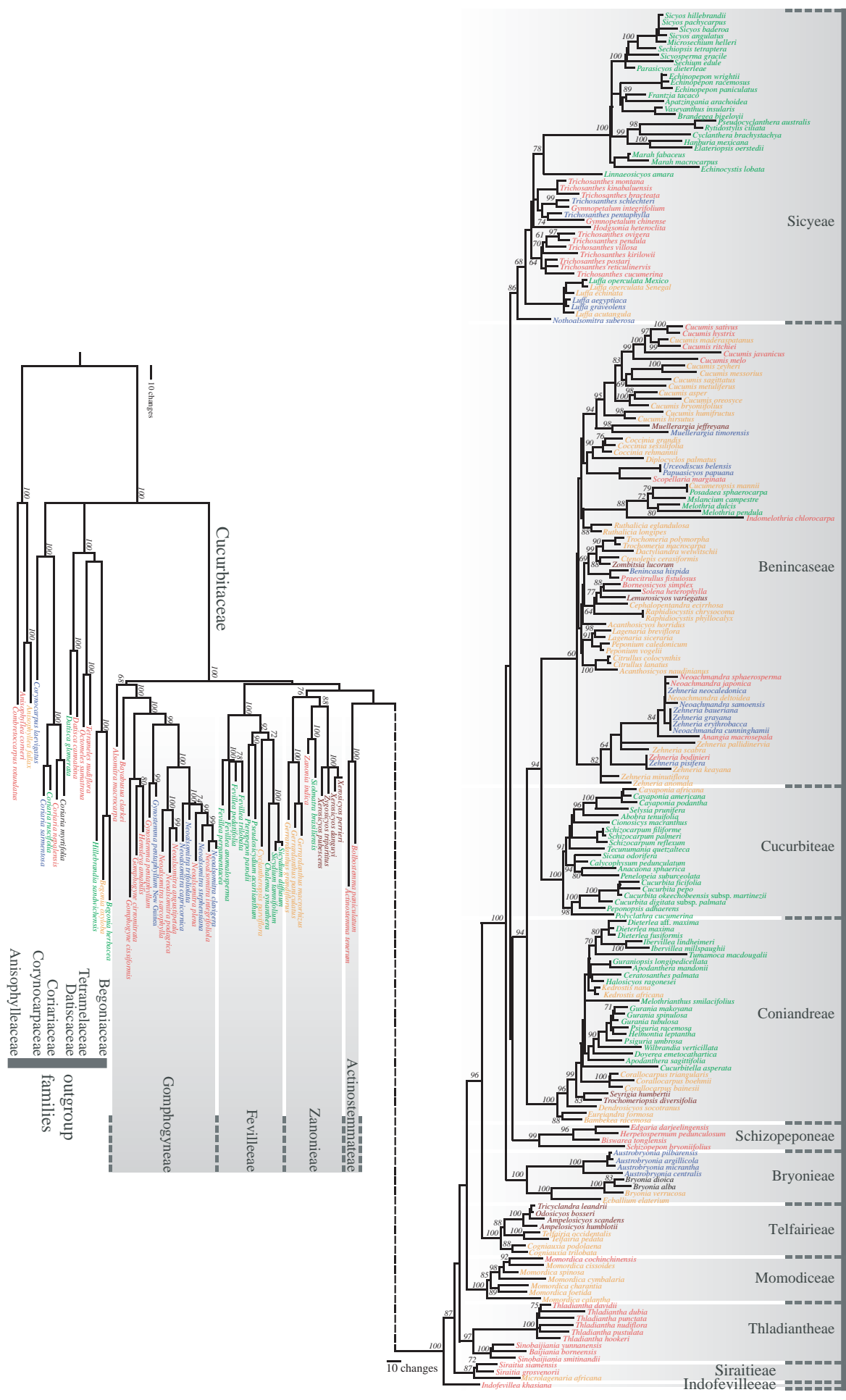


Figure 1. (Caption overleaf.)

Figure 1. (*Overleaf*). Best ML tree for Cucurbitaceae and relatives found with combined chloroplast gene, spacer and intron sequences (5031 nucleotides) analysed under a GTR+ Γ +I model with unlinked partitions for coding and non-coding regions. Likelihood bootstrap values greater than 60% are given at the nodes. Rooting follows Zhang *et al.* (2006). The geographical occurrence of genera is colour coded as follows: green, America (including Galapagos, Hawaii and the Caribbean); yellow, mainland African; brown, Madagascar; red, Asia; blue, Australia/New Guinea/Polynesia; black, Europe.

To translate relative times into absolute times, the Bayesian clock relied on the following simultaneous constraints. (i) The age of core Cucurbitales (the root node) was constrained to maximally 84 Myr, based on the earliest fossils of the sister group of Cucurbitales, the Fagales (Herendeen *et al.* 1995). However, we also performed two runs in which the root node was unconstrained or constrained to minimally 84 Myr. This yielded age estimates for the Cucurbitaceae crown group that were older than the oldest angiosperm fossils (132 Myr). In general, relaxed molecular clocks will yield reliable ages only with at least one minimal and one maximal constraint, the latter preferentially at or near the root (Thorne *et al.* 1998). (ii) The age of the split between *Datisca* and *Octomeles/Tetrameles* was set to minimally 68 Myr old or, in an alternative run, to minimally 65.5 Myr old, based on the fossil wood of *Tetrameleoxylon prenudiflora* from the Deccan intertrappean beds at Mohgaonkalan in India (Lakhanpal & Verma 1965; Lakhanpal 1970). These beds have been dated to the Maastrichtian or Late Maastrichtian (Khajuria *et al.* 1994; Kar *et al.* 2003), and we therefore used either the midpoint of the Maastrichtian (68 Myr) or the Maastrichtian/Palaeocene border (65.5 Myr). (iii) The crown group of Cucurbitaceae was set to minimally 65 Myr or, alternatively, 55.8 Myr, based on the seeds from the Palaeocene Felpham flora (Collinson 1986; Collinson *et al.* 1993). These dates span the upper and lower boundary of the Palaeocene. (iv) The split between *Limnaeosicyos*, with tetracolpate-reticulate pollen, and the remaining New World Sicyeae (Schaefer *et al.* 2008a), which usually have polycolpate pollen, was set to minimally 33.9 Myr or, alternatively, 23 Myr, based on *Hexacolpites echinatus* pollen from the Oligocene of Cameroon, which is the oldest hexacolpate Sicyeae-type pollen (Salard-Cheboldaef 1978; Muller 1985). Polycolpate pollen is not found in other Cucurbitaceae except the African *Neoachmandra peneyana* (Van der Ham & Pruessapan 2006). The Oligocene epoch ranges from 33.9 to 23 Myr, and the stratum containing *Hexacolpites* has not been precisely dated; therefore, in alternative analyses, we used the upper or lower boundary as minimal constraints. (v) The split between the Hispaniola endemics *Anacaona* and *Penelopeia* was set to maximally 20 or 15 Myr, based on the age of Dominican amber, which was produced by tropical trees and provides a proxy for the presence of tropical forest on that island; the amber age is estimated as 15–20 Myr (Iturralde-Vinent & MacPhee 1996).

To explore the sensitivity of the Bayesian clock to the various priors, we performed alternative MCMC runs in which we tested (a) the effect of a more clock-like Brownian motion parameter of 0.4 instead of 1.11, (b) the effect of using up to four data partitions, thereby allowing the genes and spacer regions to have different rates, and (c) the effects of varying the age constraints. For the latter exploration, we ran an analysis in which all constraints were set to the lowest age boundaries, another in which all constraints were set to the highest age boundaries, and four analyses that used the minimum age for one of the constraints and the maximum age for the remaining constraints. All other parameters, such

as root rate and MCMC chain length, were constant between these six runs. Finally, we performed a run (d) with mean ages for four constraints, namely 66.8 Myr (constraint ii), 60.4 Myr (iii), 28.5 Myr (iv) and 17.5 Myr (v).

For the strict clock approach, *rbcL* branch lengths were calculated under a GTR+ Γ +I+ clock model on the preferred ML topology. The tree was imported into PAUP, rooted on *Coriaria* (Zhang *et al.* 2006), and branch lengths were then calculated under the 'enforce clock' option. The distance between a calibration node and the present was divided by the age of the calibration node to obtain a substitution rate, and this rate was then used to calculate the age of divergence events of interest. As calibration nodes, we used either the age of the earliest Cucurbitaceae seeds (constraint iii) or the oldest Sicyeae-type pollen (constraint iv).

(e) *Biogeographic analysis*

For a dispersal-variance analysis, we used the 147-taxon dataset also used for the clock runs and coded the distribution ranges of all species in a binary matrix in MACCLADE. The species were recorded as present in one of five regions: Asia; Europe; Africa (including Madagascar); America (including Caribbean, Galapagos, and Hawaii); and Australia (including New Guinea and Polynesia). We then used the parsimony-based approach implemented in DIVA v. 1.1 (Ronquist 1996, 1997) to infer vicariance and dispersal events. The maximum number of areas simultaneously occupied by hypothetical ancestral lineages was experimentally constrained to 4, 3 or 2 because it is unlikely that an ancestral species would have ranged over several continents.

3. RESULTS

Herbarium material yielded suitable DNA in more than 95 per cent of the cases, even for 50–100-year-old collections.

The highest-scoring ML tree obtained for the 260-taxon dataset (figure 1) shows Cucurbitaceae highly supported as monophyletic and family relationships similar to those found by Zhang *et al.* (2006) with a much larger amount of sequence data. Within Cucurbitaceae, there are five main clades (figure 1), namely: (i) a group of approximately 100 genera traditionally treated as subfamily Cucurbitoidae (Kosteletzky 1833) and usually subdivided into several tribes (below); (ii) a clade of Asian genera, including *Alsomitra*, *Bayabusua* and *Nealsomitra* that corresponds to the tribe Gomphogyneae of Bentham & Hooker (1867); (iii) a clade of one African and five Neotropical genera, including *Fevillea* and *Sicydium*, that corresponds to the tribe Fevilleae of Bentham & Hooker (1867); (iv) a clade of a few genera from Madagascar, continental Africa, Asia and South America corresponding to the tribe Zanonieae of Blume (1826); and (v) a clade consisting of the two Asian genera *Actinostemma* and *Bolbostemma*.

Clades (ii–v) have been treated as subfamily Nhandir-oboideae (an illegitimate name) or Zanonioideae

(a taxonomic synonym of Fevilleoideae), but this subfamily is not supported as monophyletic by our data. Clade (i), Cucurbitoideae, can be divided into geographically or morphologically more homogeneous groups that correspond to the traditional tribes *Herpetospermeae*, *Bryoniae*, *Sicyeae*, *Coniandreae*, *Benincaseae* and *Cucurbitaeae*, plus a few clades of similar phylogenetic depth that have not traditionally been ranked as tribes, such as the Asian *Thladiantha* and *Baijiania*, the Asian/African *Siraïtia* and *Microlagenaria*, the African/Asian *Momordica*, the African *Telfairia*, *Cogniauxia*, a group of Madagascan genera and the Himalayan *Indofevillea*, which is sister to all remaining Cucurbitoideae (figure 1). Well-known genera found to be poly- or paraphyletic include *Citrullus* (must include *Acanthosicyos*), *Ampelosicyos* (must include *Tricyclandra* and *Odosicyos*), *Gomphogyne* (must include *Hemsleya*), *Xerosicyos* (must include *Zygosityos*), *Apodanthera*, *Psiguria* and *Trichosanthes*.

MULTIDIVTIME dating runs with a Brownian motion parameter of 0.4 instead of 1.11, yielding barely different estimates for the ingroup nodes of interest. Runs that allowed uncoupled rates for the two genes and the spacers also yielded essentially identical estimates, and final runs therefore modelled the data under a single model. The estimates from the run in which all constraints were set to their lowest boundaries differed significantly from those obtained when all constraints were set to their highest boundaries (Wilcoxon signed-rank test, $p=0.0085$; see fig. 1c,d in the electronic supplementary material). Among the test runs in which one constraint was set to the minimum age and the others to the maximum age, only two yielded significantly different results: the *Tetrameleoxylon* fossil set to the minimum age and the Felpham flora seed set to the minimum age (see fig. 1e in the electronic supplementary material). However, all results were within the 95 per cent confidence intervals of the estimates obtained when the constraints were set to mean ages (see fig. 1a,b in the electronic supplementary material).

The substitution rates obtained under a strict clock model calibrated with either the seed or the pollen fossil (§2) were 0.00018 S/(S×Myr) (oldest Sycieae-type pollen, constraint (iv)) or 0.00030 S/(S×Myr) (earliest Cucurbitaceae seeds, constraint (iii)). An average rate of 0.00024 S/(S×Myr) yielded absolute times that for the most part were older than those obtained with the relaxed clock model (see table 2 in the electronic supplementary material that lists the ages obtained with the relaxed clock and with the strict clock model). The following discussion focuses on the relaxed clock estimates because they provide 95 per cent confidence intervals as a measure of uncertainty.

The split between the two genera of Begoniaceae, *Begonia* and *Hillebrandia*, is estimated as 29 (41–18) Myr old, roughly the age of the Hawaiian archipelago (ca 30 Myr), where *Hillebrandia* is endemic (Clement et al. 2004). The deepest split in the Cucurbitaceae is ca 63 (69–61) Myr old, while crown group Cucurbitoideae are 53 (60–48) Myr old, Gomphogyneae 56 (63–51) Myr, Fevilleeae 46 (55–37) Myr and the *Actinostemma/Bolbostemma* clade 52 (59–44) Myr. The Madagascan *Xerosicyos* clade (split *Zanonia*–*Xerosicyos*) appears to be 49 (57–40) Myr old; the likewise Madagascan *Ampelosicyos* clade is 29 (39–19) Myr old. The Madagascar/

Southeast Asia disjunction between the two species of *Muellerargia* is only ca 12 (18–7) Myr old. The South America/Asia disjunction in the *Zanonia* clade (*Siolmatra*–*Zanonia* split), finally, is 24 (38–11) Myr old, and the South America/Africa disjunction in the *Fevillea* clade (*Chalema*–*Cyclantheropsis* split) 41 (51–31) Myr. Other estimates of specific interest (*Introduction*; see table 2 in the electronic supplementary material) concern *Cayaponia* (stem age 10 (17–5) Myr), *Luffa* (stem age 35 (41–31) Myr), *Sicyos* (stem age 8 (13–4) Myr) and the *Lagenaria* crown group (8 (14–3) Myr; figure 2).

The DIVA analysis yielded Asia as the most likely region of origin of the Cucurbitaceae (figure 3a). From there, at least five lineages reached Africa, and 12 lineages reached Australia. No fewer than seven lineages independently reached the American continent, and no fewer than 13 lineages dispersed from Africa to Madagascar. Some disjunctions are best explained as secondary dispersals from Africa back to Asia (*Coccinia* and *Momordica*). A few lineages reached Africa via LDD from America (*Cayaponia africana*, *Cucumeropsis*, perhaps *Cyclantheropsis* and *Kedrostis*).

4. DISCUSSION

(a) *The geographical origins of the world's regional Cucurbitaceae floras*

Our results suggest that Cucurbitaceae initially diversified in Asia (specifically, the region north of the Tethys) sometime in the Late Cretaceous. This fits with the observation that India contains more deeply divergent lineages of Cucurbitaceae than any other similar-sized geographical area (Chakravarty 1946, 1959; this study). Of the family's Late Cretaceous radiations, two clades (the Gomphogyneae and the Actinostemmataeae) are now almost restricted to subtropical Asia (figure 1). A third clade, Fevilleeae, is mainly Neotropical except for a small African 'extension', *Cyclantheropsis*. The ancestors of Fevilleeae were probably more widely distributed in the Laurasian tropics and reached the American continent by dispersing across a still-narrow Atlantic (figure 3b; seeds of Fevilleeae are wind- and water-dispersed). *Cyclantheropsis* must result from a back dispersal from South America to Africa in the Middle Eocene. The ancestors of the fourth ancient clade, Zanonieae, apparently reached the African continent early and from there dispersed to Madagascar (the Early Eocene *Xerosicyos* lineage; figure 3b). Later, in the Oligocene, at least two LDD events brought the *Siolmatra* lineage to America and the *Zanonia* lineage back to tropical Asia (figure 3c). The fifth and last ancient clade, the Cucurbitoideae, diversified partly in Asia (e.g. *Thladiantha*, *Siraïtia*, *Trichosanthes*), and partly in Africa (e.g. *Momordica*, *Cucumis*, *Coccinia*, *Kedrostis*). The cucumber (*Cucumis sativus*) and its closest relatives (not all included in the present study) evolved from a common ancestor ca 3 (6–1) Myr ago. The wax gourd *Benincasa* and its sister group *Praecitrullus*, an important vegetable in parts of India, apparently split only 5 (10–1) Myr ago. Further dispersals from Africa back to Asia are present within *Momordica*, *Coccinia*, *Kedrostis* and *Corallocarpus* (figure 3c).

The native European cucurbit flora consists only of *Bryonia*, with 10 species (Volz & Renner 2008), and its monotypic sister *Ecballium*, which probably represent a lineage that spread along the Tethys border from Asia to

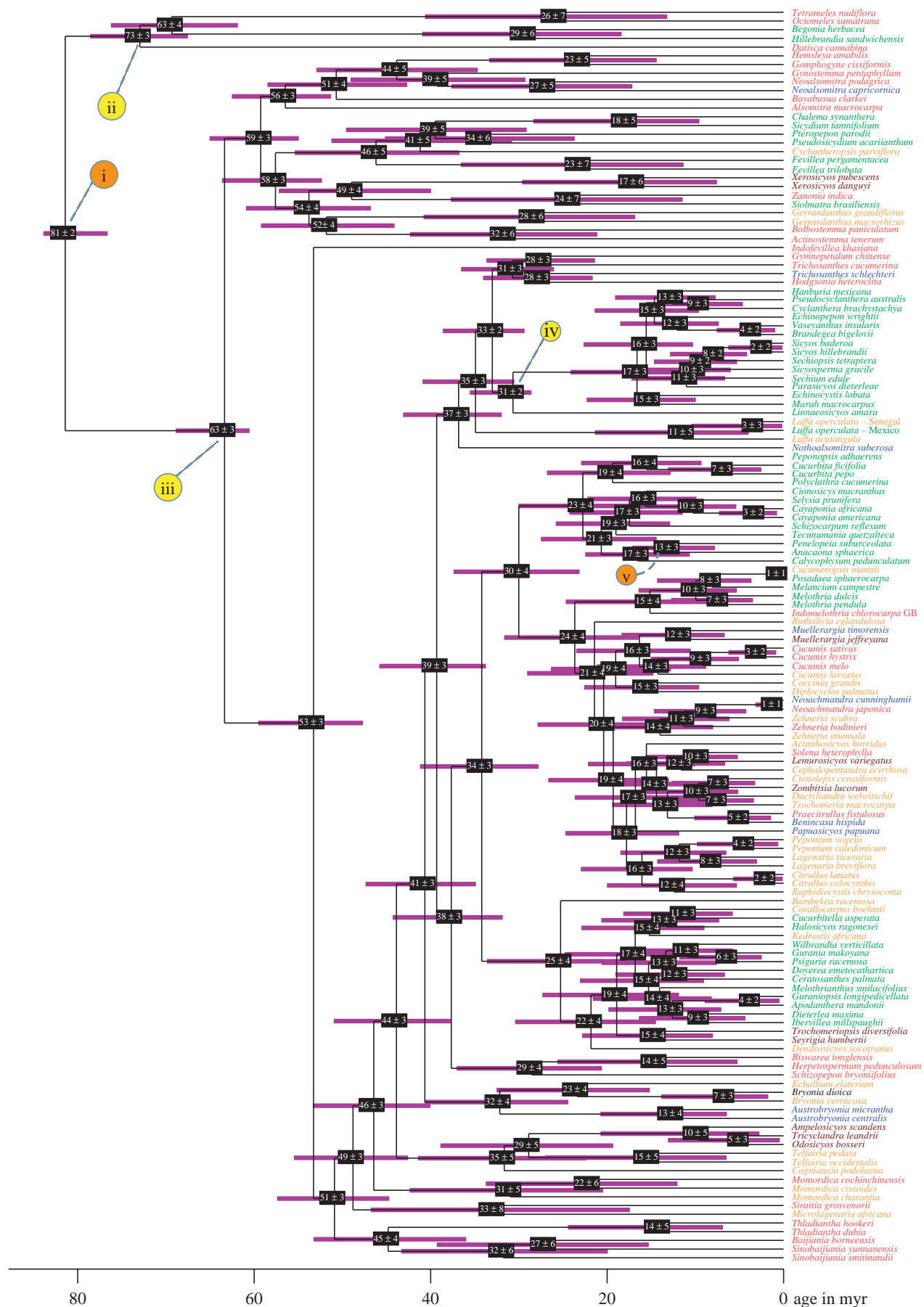


Figure 2. Chronogram obtained for Cucurbitaceae under a Bayesian autocorrelated rates relaxed clock model applied to the combined data (5031 nucleotides) and calibrated with three minimal (yellow) and two maximal (orange) constraints as in run (d) of §2(d). Age estimates with their 95% confidence ranges shown in purple. Rooting follows Zhang *et al.* (2006). Green, America (including Galapagos, Hawaii and the Caribbean); yellow, mainland Africa; brown, Madagascar; red, Asia; blue, Australia/New Guinea/Polynesia; black, Europe.

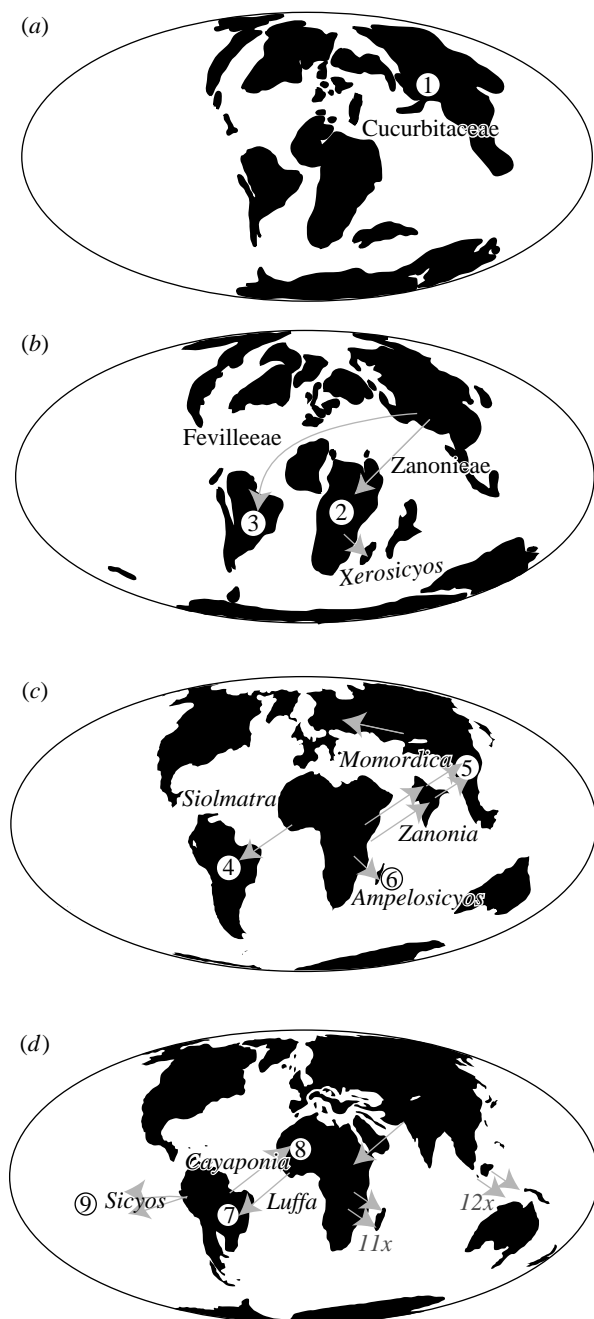


Figure 3. The biogeographic history of Cucurbitaceae as inferred from the statistical approach described in the text (coastlines drawn after [Smith *et al.* 1994](#)). (a) Late Cretaceous, *ca* 70 Myr ago; (1) origin of the Cucurbitaceae in Asia. (b) Palaeocene/Eocene, 60–40 Myr ago; (2) the ancestor of the Zanonieae reaches Africa, the ancestor of the *Xerosicyos* lineage reaches Madagascar, and (3) the ancestor of Fevilleae reaches South America. (c) Oligocene, *ca* 30 Myr ago; (4) the ancestor of the *Siolmatra* lineage disperses over the Atlantic into South America, (5) the ancestors of *Momordica cochinchinensis* and *Zanonieae indica* independently disperse from Africa to Southeast Asia, and (6) the ancestor of the *Ampelosicyos* lineage reaches Madagascar. (d) Middle Miocene, *ca* 10 Myr ago; (7) the ancestors of Neotropical *Luffa* disperse from Africa to the Americas, (8) the ancestor of *Cayaponia africana* disperses from South America to West Africa, and (9) ancestors of several *Sicyos* species groups spread from South America to Hawaii, Galapagos, New Zealand and Australia.

the Mediterranean 32 (41–24) Myr ago. The remaining cucurbit species that occur in Europe are the result of recent introductions (*Echinocystis lobata*, *Sicyos angulatus*

and *Thladiantha dubia*) or casual escapes from cultivation (*Citrullus lanatus*, *Cucumis melo*, *C. sativus* and *Cucurbita pepo*). The closest extant relative of *Bryonia* and *Ecballium* is the Australian genus *Austrobryonia* (four species), which may have reached Australia from Asia *ca* 13 (21–6) Myr ago ([Schaefer *et al.* 2008b](#)).

African Cucurbitaceae (25 genera) are the result of five dispersals from Asia to Africa and two from America to Africa (in the genera *Cucumeropsis* and *Cayaponia*). The watermelon (*Citrullus lanatus*) and its sister species (*C. colocynthis*) apparently evolved from a common ancestor as recently as 2 (6–0.1) Myr ago. The lineage leading to the cucumber tree, *Dendrosicyos socotranus*, endemic on Socotra, some 350 km off the Arabian peninsula, is estimated as 22 (30–14) Myr old, while the Socotra archipelago is only some 10 Myr old ([Ghebreab 1998](#)). *Dendrosicyos* thus seems to be an island relict of a progenitor lineage that went extinct on the mainland. This example of a species that is twice as old as the island on which it occurs cautions against using geological calibrations in molecular clock dating. Another supposed example of a species being older than the island on which it lives, that of the Hawaiian *Hillebrandia sandwicensis* ([Clement *et al.* 2004](#)), is not supported by our data (see table 2 in the electronic supplementary material).

Madagascar has 16 native Cucurbitaceae genera with 50 species in total. From our data it appears that Cucurbitaceae reached Madagascar at least 13 times, apparently always from the African mainland, and that these 13 ancestors then underwent local radiations, giving rise to today's 50 species. Using Madagascar as a stepping stone, one of these clades, *Peponium*, later reached the Seychelles (the endemic species there has not yet been sequenced).

South America has approximately 350 species of Cucurbitaceae in 47 genera that all descend from five LDD events, mostly from Africa to South America. These involved the ancestors of Cucurbitaceae, Sicyinae, a clade of Coniandreae, the *Melothria* clade and a subclade of *Luffa*. Based on the tree topology ([figure 1](#)), *Luffa* originated in the Old World or Australia, and one species then reached the New World by LDD from Africa across the Atlantic as suggested by [Heiser & Schilling \(1988\)](#). The fruit is dry with fibrous tissue and probably well adapted to floating ([Ridley 1930](#)). The Neotropical *Melothria* clade ([figure 1](#)) appears to have crossed the Pacific because the sister group of *Melothria*, *Indomelothria*, is endemic in Southeast Asia. Today's pumpkin and squash species (*Cucurbita* spp. in the Cucurbitaceae) apparently originated in Central or South America, and the genus *Cucurbita* split from its sister clade, *Peponopsis*, only some 16 (23–9) Myr ago. North American Cucurbitaceae, finally, all descend from seven expansions of Central and South American lineages that occurred at widely different times ([figure 2](#); see table 2 in the electronic supplementary material).

The indigenous Australian Cucurbitaceae flora consists of 30 species in 12 genera of which two are endemic: *Nothoalsomitra*, a liana species of Queensland's rainforests; and *Austrobryonia*, four species of trailers or creepers in the dry regions of (mostly) Central Australia. This low Australian species diversity is in marked contrast with the minimally 12 independent dispersal events into Australia ([figures 1 and 3d](#)). The largest Australian 'radiation' comprises only four species ([Schaefer *et al.* 2008b](#)), even

though the ecological conditions in Australian rainforests and bushland are similar to those in Southeast Asian forests and African bushland, where cucurbit diversity is much higher.

Overall, Cucurbitaceae underwent at least 43 successful LDD events over the past 60 Myr, which would translate into an average of seven LDDs every 10 Myr. These events seem to have occurred throughout the evolutionary past of the family, rather than being clustered at a particular geological time (see table 2 in the electronic supplementary material). The most striking case of a rapid radiation following LDD is *Sicyos*, which reached Hawaii only *ca* 2 (6–0.1) Myr ago (figure 3*d*) and now has 15 Hawaiian species. *Sicyos* species often grow in seabird colonies (www.botany.hawaii.edu/gradstud/eijzenga/OIRC/lehua.htm#Vegetation), and the hooked barbs on the fruits may help external dispersal on bird feathers. Another extreme case is *Muellerargia*, the closest relative of *Cucumis* (Renner & Schaefer 2008), which comprises just two species, one endemic in Madagascar, and the other in northeast Australia and Timor.

(b) Using museomics to achieve less biased taxon sampling

Results of this study may have implications for the conservation of wild Cucurbitaceae gene pools for future crop improvement; this is almost certainly true for *Luffa* and *Cucumis*. They also show that the gourd family, which appears to be of Asian origin, has undergone numerous natural LDD events, prior to anthropogenic LDD, and that these events have played a large role in the build-up of local cucurbit floras. More generally, this study illustrates the great potential of herbarium material for generating broad phylogenetic frameworks at relatively low costs and in a time-efficient manner (no lengthy permit procedures). With rapidly improving molecular techniques for DNA isolation from tiny fragments of ancient collections, it is now possible to study the phylogeny of worldwide lineages without time-consuming and difficult field trips. And broad geographical sampling in turn is the precondition for assessing the full extent of LDD in the angiosperms at different geological times and across different ocean basins. Given that habitat destruction in the centres of cucurbit diversity (Madagascar, Southeast Asia, West Africa and Central America) is extremely high, the use of herbarium material also may soon be the only option for future research on the phylogenetics of this plant group.

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