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X-rays and virtual taphonomy resolve the first *Cissus* (Vitaceae) macrofossils from Africa as early diverging members of the genus\(^1\)

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**Running Head**

First fossil seeds of *Cissus* (Vitaceae) from Africa
Footnotes

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ABSTRACT

- **Premise of the study:** Fossilized seeds similar to *Cissus* (Vitaceae) have been recognized from the Miocene of Kenya, though some were previously assigned to the Menispermaceae. We undertake a comparative survey of extant African *Cissus* seeds to identify the fossils and consider their implications for the evolution and biogeography of *Cissus* and for African early Miocene paleoenvironments.

- **Methods:** Micro-computed tomography (µCT) and synchrotron-based X-ray tomographic microscopy (SRXTM) were used to study seed morphology and anatomy. Virtual taphonomy, using SRXTM datasets, produced digital fossils to elucidate seed taphonomy. Phylogenetic relationships within *Cissus* were reconstructed using existing and newly produced DNA sequences for African species. Paleobiology and paleoecology were inferred from African nearest living relatives.

- **Key results:** The fossils are assigned to four new *Cissus* species, related to four modern clades. The fossil plants are interpreted as climbers inhabiting a mosaic of riverine woodland and forest to more open habitats. Virtual taphonomy explains how complex mineral infill processes concealed key seed features, causing the previous taxonomic misidentification. Newly sampled African species, with seeds most similar to the fossils, belong to four clades within core *Cissus*, two of which are early diverging.

- **Conclusions:** Virtual taphonomy, combined with X-ray imaging, has enabled recognition of the first fossil *Cissus* and Vitaceae from Africa. Early divergent members of the core *Cissus* clade were present in Africa by at least the early Miocene, with an African origin suggested for the *Cissus sciaphila* clade. The
fossils provide supporting evidence for mosaic paleoenvironments inhabited by early Miocene hominoids.

**Key words**

*Cissus*; Hiwgi Formation; liana; *Menispermicarpum*; microCT; Miocene; paleoecology; seeds; SRXTM; virtual taphonomy.
The volcanic and clastic sediments of the Hiwegi Formation on Rusinga Island, Lake Victoria, Kenya have yielded a rich early Miocene flora and fauna with well over one hundred recorded species, dated to ca. 18–20 Ma (Drake et al., 1988; Andrews et al., 2009; Peppe et al., 2011). The floral assemblage contains fossilized fruits, seeds, twigs, wood, bark, and leaves (Chesters, 1957; Collinson et al., 2009; Maxbauer et al., 2013; Michel et al., 2014), and the faunal assemblage comprises numerous fossil mammals (e.g., Whitworth, 1958; Pickford, 1981; Butler, 1984; Werdelin, 2011), reptiles (e.g., Clos, 1995; Conrad et al., 2013), birds (e.g., Harrison, 1980; Rich & Walker, 1983) and invertebrates (e.g., Leakey, 1952; Verdcourt, 1963; Thackray, 1994; Pickford, 1995). The fauna also contains several genera of early hominoids (e.g., Le Gros Clark and Leakey, 1951; Andrews and Simons, 1977; Walker and Teaford, 1988; Walker et al., 1993; Harrison, 2002; McNulty et al., 2007; Harrison and Andrews, 2009; Pickford et al., 2009), including Ekembo (previously Proconsul, see McNulty et al., 2015), which mark the transition between Paleogene arboreal primates, thought to inhabit tropical forests (Andrews, 1992; Janis, 1993), and Neogene bipedal hominids, often associated with open savanna grassland (Robinson, 1963; Reed, 1997; Pickford, 2002). Study of the Hiwegi Formation flora is essential to understand the paleoenvironments in which these transitional hominoids evolved.

The fruit and seed flora was partly described by Chesters (1957) from surface-picked collections, but these lacked a sedimentological and stratigraphic context. This prompted in situ excavations at the new site of R117 (Collinson et al., 2009), where over 360 fruits and seeds were collected, including several specimens tentatively assigned to ‘cf. Cissus sp. 1 nov.’ (Vitaceae). During that study, three
other morphotypes with similarity to seeds of extant *Cissus* L. species were recognized among the collections originally studied by Chesters (1957, 1958). If these four fossil records of *Cissus* can be verified, they would constitute the first records of the Vitaceae in the flora, the earliest reported record of Vitaceae from the African continent, and could provide evidence for arid- or rainforest-adapted taxa in the African Miocene vegetation (De Santo et al., 1987; Verdcourt, 1993; Lombardi, 2000; Manchester et al., 2012a).

Recent molecular phylogenetic analyses of the genus *Cissus* (Liu et al., 2013; Rodrigues et al., 2014) showed that modern species could be assigned to several distinct clades. All African species fell within the ‘core *Cissus*’ clade, but within that were distributed in more than seven distinct clades, two of which also included Asian species (Liu et al., 2013). Many African species were missing from these phylogenies, including those with external seed morphology most similar to the putative *Cissus* fossils. If the fossils are *Cissus*, molecular study of these neglected modern species will be essential to place them in their phylogenetic context.

This paper therefore aims to: (i) confirm or refute the identification of Hiwegi Formation fossils to *Cissus* and, if confirmed, identify the clades to which the fossil species are likely related by comparing external and internal seed morphology of the fossils to extant African species; (ii) place the nearest living relatives of the fossils into the existing phylogenetic framework; and (iii) evaluate the paleoenvironmental and biogeographic significance of these fossils.
Specimens studied—The fossil fruits and seeds from the Hiwegi Formation are composed of carbonate minerals, which derive from highly alkaline ash erupted from the nephelinite-carbonatite Kisingiri volcano during the Miocene (Bestland et al., 1995; Harris and Van Couvering, 1995), and which replaced biological structures (Collinson et al., 2009). The specimens were collected from the Hiwegi Formation (for wider stratigraphic context see Drake et al., 1988; Collinson et al., 2009) by surface picking and in situ excavation and are stored in collections at the Natural History Museum, London (NHMUK, numbers prefixed V) and the National Museums of Kenya, Nairobi (KNM). Three fossil seed morphotypes in NHMUK, which Chesters (1957, 1958) had placed in the Menispermaceae (due to their bisymmetry, horseshoe-shaped curvature, and sculptured margins), were noted by Collinson to have strikingly similar exterior seed coat morphology to modern African Cissus species, as illustrated in African floras (e.g., Dewit and Willems, 1960; Descoings, 1967, 1972; Verdcourt, 1993). Collinson et al. (2009) also listed several specimens from the R117 site assigned to ‘cf. Cissus sp. 1 nov.’ (Vitaceae) based on similarity to modern Cissus seeds. All these fossils were re-examined during the current study.

Fifteen modern species of African Cissus and three species of African Cyphostemma (Planch.) Alston, which have comparable seed ornamentation to the putative Cissus fossils, were sampled from loose fruits on herbarium sheets in the Royal Botanic Gardens, Kew Herbarium (K) to study seed anatomy. The most visibly mature and undamaged specimens were selected. Additionally, fruits of Cissus dasyantha were obtained from the herbarium at the Botanic Garden Meise, Belgium (BR). Herbarium sheet information for the species sampled is provided in Appendix S1 (see Supplemental Data with the online version of this article).
Macrophotography and VP-SEM—Photographs of the specimens described by Chesters (1957, 1958) were provided by the NHMUK Photographic Unit. The smaller specimens, assigned to ‘cf. Cissus sp. 1 nov.’ by Collinson et al. (2009), were examined uncoated under a Leo 1455 vapour pressure scanning electron microscope (VPSEM) at the Imaging and Analysis Centre, NHMUK. Specimens were placed loose onto a sheet of black paper in a small tray, moved into appropriate orientation and turned over using a fine (size 00000) artist’s brush. A small amount of Blu-Tack® (Bostik, Paris, France) was used, when unavoidable, to orient specimens for apical and basal views. Images were obtained using the back scatter detector (BSD), a chamber pressure of 14–15 Pa, current of 20 kV, spot size 500 and working distance 38–39 mm. Images were adjusted uniformly for contrast and brightness using Adobe Photoshop CS2 or CS6.

Synchrotron-based X-ray tomographic microscopy (SRXTM)—The traditional method of boiling and scrubbing modern fruits to study their seeds is problematic. Depending on tissue toughness, seed features may not be revealed in a repeatable or comparable manner across different species or genera. Cutting or histological sectioning to study internal anatomy also has limitations: it is destructive, may introduce artefacts (tears, gaps), and multiple planes of section through the same specimen cannot be acquired. X-ray imaging solves these problems because multiple planes of section through a single specimen can be easily and non-destructively obtained (e.g., Smith et al., 2009). Modern Cissus and Cyphostemma fruits were scanned using SRXTM, as this technique provides the necessary quality of resolution to enable distinction of cellular details in the fruit wall and seed coat layers for systematic study and for virtual taphonomy (Smith et al., 2009; Collinson et al., 2013). The SRXTM was performed on the TOMCAT beamline at the Swiss Light
Specimens were mounted onto brass pin stubs using polyvinyl acetate glue and were scanned during one session of beamtime in July 2014. X-rays transmitted by the specimens were converted into visible light by a 300 μm-thick Ce-doped LAG scintillator screen. A microscope objective of 1.25× or 2× (depending on fruit size) magnified the projection data, which were then digitized by a high-resolution scientific CMOS camera (PCO.edge; PCO GmbH, Kelheim, Germany), giving a resultant voxel size of 3 to 5 μm. The energy was set at 17.5 keV and the exposure time per projection was 50 ms. For each scan a total of 1501 projections (2560 × 2160 pixels with PCO.edge camera) were acquired over 180°. Reconstruction algorithms were then used to combine the projections and obtain a three-dimensional volume, reconstruction was performed on a dedicated Linux PC cluster using a highly optimized routine based on the Fourier transform method and a gridding procedure (Marone et al., 2010; Marone & Stampanoni, 2012). Multiple stacked scans were used if the specimens did not completely fit within the field of view. 3D datasets were visualized, and images and videos were captured, in Avizo 8.1 (FEI Visualization Science Group, Bordeaux, France). Images were adjusted uniformly for contrast and brightness using Adobe Photoshop CS2 or CS6. Videos of digital SRXTM tomograms in transverse section (DTS) through fruits of each of these modern species are available from the Dryad Digital Repository (http://dx.doi.org/10.5061/dryad.g9r36).

Micro-computed tomography (µCT)—Externally visible ventral infolds are a characteristic feature of Cissus seeds (Chen and Manchester, 2011), but are not evident in the fossils (Fig. 1). Information on internal structure of the fossils (including holotypes) is required to test whether these characteristic ventral infolds are: (a)
genuinely absent, which would exclude affinity with Vitaceae; (b) present but externally obscured by a seed coat layer, which would indicate affinity with *Cyphostemma* (Chen and Manchester, 2011); or, (c) obscured as a consequence of taphonomic processes, such as mineral infilling during fossilization, which would support identification to *Cissus*. For holotypes and rare fossils this information must be obtained non-destructively. Therefore, three fossil specimens (V33753, V68501, V68506) from collections studied by Chesters (1957, 1958) stored in NHMUK and two specimens (R117.1981.314, R117.1981.476) from the R117 site, identified by Collinson et al. (2009) as ‘cf. *Cissus* sp. 1 nov.’, housed in KNM were scanned by μCT using a Nikon Metrology HMX ST 225 at the Imaging and Analysis Centre, NHMUK. Specimens were stabilised by inserting them into blocks of OASIS® Floral Foam (Smithers-Oasis Company, Kent, Ohio, USA) within a plastic tube. Specimens were wrapped for protection in cling film: a thin film of PVC (polyvinyl chloride) or LDPE (low density polyethylene). A voltage of 200 kV was used with a current of 180 μA, a tungsten reflection target, a 0.5 or 0.25 mm copper filter and an exposure time of 708 ms; this resulted in a voxel size of 12 μm. Four modern *Cissus* fruits scanned by SRXTM (one *C. dinklagei*, one *C. populnea*, two *C. integrifolia*) were also scanned using μCT. This duplicative scanning aimed to ensure that μCT scans of the fossils could be interpreted in the context of directly comparable scans of modern seeds (a comparison of imaging methods is provided in Appendix S2, see Supplemental Data with the online version of this article). A voltage of 125 kV was used with a current of 200 μA, a molybdenum reflection target, no filter and an exposure time of 708 ms; this resulted in a voxel size of 8 to 15 μm. μCT datasets were reconstructed using CT Pro (Nikon Metrology, Tring, UK) and were visualized in
Avizo 8.1. Images and videos were obtained as for SRXTM datasets. Videos are available from the Dryad Digital Repository (http://dx.doi.org/10.5061/dryad.g9r36).

**Virtual taphonomy**—The technique of ‘virtual taphonomy’, developed by Smith et al. (2009), solves the problem of potential variability in tissue removal with traditional boiling or scrubbing methods in seed preparation. Virtual taphonomy uses X-ray datasets to digitally remove specific tissue or cell layers from modern fruits or seeds, thereby creating digital fossils, the surfaces of which can be directly compared to real fossils to determine which layers are preserved. Digital seed infills can also be produced using this technique, mimicking the mineral infill of fruits and seeds that can occur during fossilization (Smith et al., 2009; Collinson et al., 2013). For modern *Cissus populnea* Guill. & M.Brandt, a digital infill of the space inside the inner seed coat (the endotesta) was produced (a ‘virtual fossil’), to mimic mineral infill during fossilization. The ventral infolds were then digitally infilled to mimic processes that might have led to mineral obscuring the ventral infolds during fossilization.

**Molecular phylogeny**—Phylogenetic relationships within *Cissus* and the placement of species assigned to this genus within Vitaceae were assessed using available sequence data from the plastid genome (*trnl* intron, *trnLF* spacer, *atpB-rbcL* spacer, *trnC-petN* spacer and *rps16* intron), as well as newly produced sequences of *trnLF* and *rps16* for eight African *Cissus* species with seeds most similar to the putative *Cissus* fossils, which were not represented in previous molecular phylogenetic studies (see Appendix 1). Sequence data obtained from public repositories comprise 91 *Cissus* species and 92 species from other genera of Vitaceae (see Appendix 2).

Total genomic DNA was extracted using a standard CTAB-based protocol (Doyle and Doyle, 1987) and purified using a combined caesium chloride/ethidium
bromide gradient and dialysis procedure. The trnL intron/trnL–F spacer and the rps16
intron were amplified using the primers designed by Taberlet et al. (1991) and Shaw
et al. (2005), respectively. Further details regarding the polymerase chain reactions,
amplification procedures, PCR product purifications, and cycle sequencing reactions
are provided in Appendix S3, see Supplemental Data with the online version of this
article.

Matrices (including sequences obtained from public repositories and those
produced for the present study) were aligned using MUSCLE (Edgar, 2004) in
Geneious; alignments are available from TreeBASE (https://treebase.org; study ID
18491). A phylogenetic analysis was performed on a combined matrix using the
maximum likelihood criterion as implemented in the programme RAxML v8.1.24
(Stamatakis, 2014) using the rapid bootstrap algorithm with 1000 replicates and a
search for the best-scoring tree. Divergence time estimates were obtained using the
Bayesian inference approach implemented in the package BEAST v.1.8.2
(Drummond and Rambaut, 2007). All analyses were run on the Cipres Science
Gateway portal (www.phylo.org). Further details of the phylogenetic analyses are
provided in Appendix S3, see Supplemental Data with the online version of this
article.

Calibration was performed using three fossils. The first is the oldest known
fossil securely identified to the Vitaceae family (Indovitis) from the latest
Cretaceous/earliest Paleogene Deccan traps of India (ca. 66Ma, based on
radiometric dating and biostratigraphy; Manchester et al., 2013) and was used as
calibration on the crown node of subfamily Vitoideae (calibration A), comprising all
genera of Vitaceae except the genus Leea, which is assigned to subfamily
Leeoideae. A lognormal distribution was used, which allows the age to vary (given
the uncertainty in fossil age estimation and given that a fossil’s age is considered a minimum age for a given group), with an offset value of 65 and a standard deviation of 1.0. The second calibration point comes from fossil seeds assigned to *Ampelocissus parvisemina* Chen & Manchester from the late Paleocene of North America at the Beicegel Creek locality of the Sentinel Butte Formation, Fort Union Group, North Dakota (Chen and Manchester, 2007), considered by Zetter et al. (2011) to be late Paleocene (61.7–56.8 Ma) in age based on molluscan and mammalian (Kihm and Hartman, 1991; Hartman and Kihm, 1995) biostratigraphy and pollen zonation (Nichols and Ott, 1978). It was assigned to the stem node of the clade comprising genera *Ampelocissus* Planch., *Nothocissus* (Miq.) Latiff, *Parthenocissus* Planch., *Pterisanthes* Blume, *Vitis* L., and *Yua* C.L. Li, following Nei et al. (2012) and Liu et al. (2016) (calibration B). As for the previous calibration, a lognormal distribution was used, with an offset value of 55.8 and a standard deviation of 1.0. The third calibration point is the oldest fossil unequivocally assigned to genus *Cissus*, from the Belén flora (North Coastal Peru) of the Oligocene, with a maximum age of 30–28.5 Ma based on diatom biostratigraphy (Manchester et al., 2012a). This fossil has features shared with species from Morphotype 1 and the *Cissus integrifolia* clade (see below); it was therefore assigned to the stem node of this group (calibration C) with a lognormal distribution with an offset value of 27.5 and a standard deviation of 1.0.

**Extant Cissus seed morphological information**—In addition to the sixteen SRXTM datasets of African *Cissus* seeds most similar to the Hiwegi Formation fossils, published seed illustrations and descriptions were sourced, where available, for all African *Cissus* species included in the molecular phylogeny (Table 1 and Appendix S4, see Supplemental Data with the online version of this article).
Together these data were used to determine the modern species with seeds most similar to those of the fossils and to place these nearest living relatives into the existing phylogenetic framework.

Ecological and biogeographic information—Data on the habit, habitat and biogeographic distributions of extant Cissus species were gathered for extant species in clades containing similar seed morphotypes to the fossils (Table 2). Georeferenced occurrence data from illustrated herbarium sheets were obtained from the Global Biodiversity Information Facility (GBIF, 2013) and floras, and other herbarium sheets were also used. Herbarium sheet sources are given in Appendix S5, see Supplemental Data with the online version of this article.

SYSTEMATICS

Definitions of lateral, ventral, dorsal, apical and basal views and seed height, width and dorsiventral dimension are given in Appendix S6 (see Supplemental Data with the online version of this article).

Family—Vitaceae Juss. 1789.

Genus—Cissus L. 1753.

Species—Cissus crenulata (Chesters) Adams, Collinson, S.Y. Smith & Bamford comb. nov.

Basionym—Menispermicarpum crenulatum Chesters 1957 (pl. 19, figs. 19, 20).

Emended diagnosis—Seed bilaterally symmetrical, 19 mm in dorsiventral dimension, laterally flattened, sub-oval to D-shaped in lateral view, narrowly elliptical and 7 mm wide in apical and basal views, and elliptical in ventral view. Center of
lateral face crossed by four pronounced, long ridges radiating from adjacent to the ventral infolds; longest ridge almost as long as seed; curved ridge, 2–3 mm from dorsal and basal margins of lateral faces, delineates faceted marginal area; prominent median ridge (rib perichalaza) extends from beneath ventral infolds, around base, over dorsal margin, and almost full length of apical margin as far as ventral infolds. Upper portion of ventral surface concave, forming acute angle with long axis of seed; pair of very deep, narrow ventral infolds present.

**Holotype**—Seed—V33753 (Fig. 1A–D; µCT DTS video available from the Dryad Digital Repository, http://dx.doi.org/10.5061/dryad.g9r36).

**Excluded specimens**—KNMP-RU7787 (8 specimens with field number 60*52, formerly P. B. 8, designated as paratypes of *Menispermicarpum crenulatum* by Chesters, 1957).

**Type locality**—Rusinga Island, Lake Victoria, Kenya.

**Geological horizon and age**—Hiwegi Formation, early Miocene.

**Repository**—Natural History Museum, London, U.K.

**Description**—The seed is bilaterally symmetrical around a prominent median perichalazal rib (Fig. 1B, C), laterally flattened with a height/width ratio of 1.7 (Fig. 1A, B), sub-oval to D-shaped in lateral view (Fig. 1A), 19 mm in the dorsiventral dimension and 11 mm in height, narrowly elliptical and 7 mm wide in apical (Fig. 1B) and basal (Fig. 1C) views, and elliptical in ventral view (Fig. 1D). The seed apex is rounded, with no indication of an apical notch or chalazal grooves (Fig. 1A-C), and the seed narrows to the ventral margin (Fig. 1A-C). The seed ornamentation is most clearly visible on one lateral face (Fig. 1A). A curved ridge, 2–3 mm from the basal and dorsal margins of the lateral faces, delineates a faceted outer margin with facets spaced at 2.5 to 4.5 mm (Fig. 1A). There are short ridges (ca. 2 mm long), roughly
perpendicular to the long curved ridge, within the outer margin, which define the facets (Fig. 1A). There are at least four ridges crossing the center of the lateral face, with one long (10 mm) ridge perpendicular to the ventral surface, and three curved ridges, which radiate away from the ventral surface towards the basal margin, abutting the curved ridge (Fig. 1A). The reverse lateral face is partly obscured by mineral encrustation in the holotype (the only specimen), making the ornamentation less clear. Nevertheless, there are clearly at least three ridges crossing the center of the face radiating from the ventral surface, with one longer and more pronounced than the others. In lateral view, the upper portion of the ventral surface is nearly straight for three-quarters of its length but the basal-most part is indented, forming an angle of ca. 65° with the long axis of the seed (Fig. 1A). The seed narrows into a rounded point on the ventral surface, possibly equivalent to the beak in typical Vitaceae (see fig. 1 in Chen and Manchester, 2011). The lateral flattening, near-straight ventral surface and sub-oval, or near elliptical, outline give the seed a very different shape from typical Vitaceae genera (e.g. Chen and Manchester, 2011).

Externally the ventral infolds are only tentatively identifiable from a pink mineral infill from the apical and ventral views (Fig. 1B, D). However, in μCT digital transverse section (DTS), a pair of very deep (4.5 mm) and narrow (0.5 mm) ventral infolds are clearly delineated by a very thin gap (black in Fig. 2C) between the inferred outer surface of the endotesta and the mineral infill of the infolds (outlined in solid yellow in Fig. 2D).

Comments—Seeds of modern Cissus integrifolia (Baker) Planch. are very similar to the holotype of C. crenulata, being narrow, laterally flattened and sub-oval in lateral view with a line of bisymmetry passing through a median longitudinal rib perichalaza and having a similar seed coat ornamentation. However, the greater
number of ridges across the lateral faces and different orientation of the ventral surface relative to the long axis of the seed in *Cissus integrifolia* support the recognition of a separate species. Chesters (1957) listed P. B. 8 as a paratype of *Menispermicarpum crenulatum*. Currently the number KNMP-RU7787 (P. B. 8) includes eight specimens, which have here been transferred to *Cissus andrewsii* sp. nov. (see below). Therefore, *Cissus crenulata* is represented only by a single specimen.

**Species**—*Cissus andrewsii* Adams, Collinson, S.Y. Smith, & Bamford sp. nov.

**Etymology**—The species epithet “*andrewsii*” is named in honor of Dr. Peter Andrews, in recognition of his extensive work on the Kenyan Miocene and the invaluable support he provided, which enabled one of us (Collinson) to undertake fieldwork on Rusinga and Mfangano Islands in 1980-1981.

**Diagnosis**—Seeds bilaterally symmetrical, 16–19 mm in dorsiventral dimension, laterally flattened, sub-oval in lateral view, narrowly elliptical and 5–8 mm wide in apical and basal views, elliptical in ventral view. Centers of lateral faces ornamented by 4–5 short ridges in radial pattern; curved ridge 1–2.5 mm from margins of lateral faces extends around most of seed and delineates faceted to reticulate marginal area; prominent median ridge (rib perichalaza) extends from base of ventral surface, around base, over dorsal surface, and almost full length of apical surface. Upper portion of ventral surface forms acute angle with long axis of the seed. Pair of deep, wide ventral infolds present.

**Holotype hic designatus**—Seed—V68501 (Fig. 1E–H; µCT DTS video available from the Dryad Digital Repository, http://dx.doi.org/10.5061/dryad.g9r36).

**Type locality**—Rusinga Island, Lake Victoria, Kenya.
**Paratypes**—V68500 (Fig. 1I–J); V68502 (Fig. 1K–L); KNMP-RU7787 (8 specimens with field number 60*52, formerly P. B. 8, previously listed as paratypes of *Menispermicarpum crenulatum* by Chesters, 1957; re-examined for this study by Bamford).

**Geological horizon and age**—Hiwegi Formation, early Miocene.


**Description**—Seeds are bisymmetrical around a prominent median perichalazal rib (Fig. 1H, J) and vary from 16 to 19 mm in dorsiventral dimension, 11 to 15 mm in height and 5 to 8 mm in width (Fig. 1E–L). Seeds are laterally flattened with height/width ratios of 1.9-2.4 and are narrow in apical and basal views (Fig. 1G, H, J, L). The seed apex is rounded, with no indication of an apical notch or chalazal grooves (Fig. 1E-G), and narrows to the ventral margin (Fig. 1F, G). The ventral surface is slightly rounded, not straight (Fig. 1F, K). The lateral surfaces have a curved ridge, delimiting a sculptured outer margin, which extends from approximately half way along the apical margin, around the dorsal margin, and fully along the basal margin (Fig. 1F). The sculptured marginal band is facetted, facets spaced at 2 to 3.5 mm, and ridged with ridges sometimes fusing to form a reticulum (Fig. 1F, I, K). The central areas of the lateral faces are ornamented by four to five short (2–3 mm) ridges in a radial arrangement (Fig. 1F). Where undamaged, the upper portion of the ventral surface forms an angle of 52 to 55 degrees with the long axis of the seed (Fig. 1F, K). The ventral infolds are not visible on the outside of the seed (Fig. 1E, G, L) but by using µCT, deep (2.5 mm), broad (0.6 mm) ventral infolds can be identified in V68501 (Fig. 2F) by differences in X-ray attenuation (grey level) due to variation in
mineral density and mineral texture in the infold infills. There is a very clear gap (black in Fig. 2E) demarcating the inferred original position of the endotesta outer surface in the areas away from the infolds, where endotesta would have been originally thicker based on observations in modern seeds. Some additional outer mineral (possibly representing exotesta or fruit wall remnants) is also present in the holotype (Fig. 2E).

**Comments**—In her unpublished thesis, Chesters (1958) assigned the specimen shown in Fig. 1E-H (V68501) to *Menispermicarpum crenulatum*, here revised to *Cissus crenulata*, but there are clear differences between *C. crenulata* and V68501, supporting assignment of this specimen, and other similar specimens, to a new species. Although there is now only a single specimen of *Cissus crenulata* (making it impossible to assess intraspecific variation), there are eleven specimens of *C. andrewsii*, all with consistent morphology distinct from that of the single specimen of *C. crenulata*. In *Cissus andrewsii* the central portion of the lateral faces is crossed by short ridges in a radial pattern, unlike *C. crenulata*, and the lateral faces have a greater number of short transverse ridges in the outer margins, some of which fuse to form a reticulum. In addition, the curved ridge, delineating the sculptured margin from the central flat area, extends further down the ventral surface and the ventral margin is curved not straight. *Cissus andrewsii* is therefore more similar to seeds of modern *Cissus dasyantha* Gilg & M.Brandt than *C. integrifolia*, but is sufficiently different from these extant species to warrant assignment to a new species, and differs in several ways from the fossil *C. crenulata*.

**Species**—*Cissus rusingensis* Adams, Collinson, S.Y. Smith, & Bamford sp. nov.
Synonymy—‘cf. *Cissus* sp. 1 nov.’ in Collinson et al. (2009).

Etymology—The epithet “rusingensis” refers to the type locality on Rusinga Island from which the specimens were collected during in situ excavations.

Diagnosis—Seeds bilaterally symmetrical, 7–8 mm in dorsiventral dimension, slightly laterally flattened, sub-rounded to pyriform in lateral view, broadly elliptical in apical, basal and ventral views. Lateral faces ornamented by ridges and reticula with 7–11 wide muri radiating to margins and enclosing lumina in central area; prominent median ridge (rib perichalaza) extends from ventral margin, around base, over dorsal surface, and almost full length of the apex. Upper portion of ventral surface forms acute angle with long axis of the seed. Pair of deep, very wide ventral infolds present.

Holotype hic designatus—Seed—R117.1981.314 (Fig. 1M–O; µCT DTS video available from the Dryad Digital Repository, http://dx.doi.org/10.5061/dryad.g9r36).

Type locality—R117 site (see Collinson et al., 2009), Rusinga Island, Lake Victoria, Kenya.

Paratypes—Seeds—KNMP-RU9647 (field number R117.1981.422); R117.1981.476 (Fig. 1P–R; µCT DTS video available from the Dryad Digital Repository, http://dx.doi.org/10.5061/dryad.g9r36); R117.1981.604 (Fig. 1S–U); R117.1981.605 (Fig. 1V–X).

Geological horizon and age—Grit Member, Hiwegi Formation, early Miocene.


Description—Seeds are bisymmetrical around a prominent median perichalazal rib (Fig. 1N–O, Q–R, T–U, W–X) and they vary from 7 to 8 mm in dorsiventral dimension, 5 to 6 mm in height, and 3 to 4 mm in width (Fig. 1M–X). The
seeds are laterally flattened with a height/width ratio varying between 1.5 and 1.8 and are broadly elliptical in apical and basal views (Fig. 1N, O, Q, R, T, U, W, X). The seeds have a rounded apex (Fig. 1M, P, S, V) but are variable in their lateral shape (Fig. 1M, P). The perichalazal rib extends around less of the specimen in R117.1981.476 (Fig. 1P) and R117.1981.604 (Fig. 1S), resulting in a reduced lateral width higher up the seed, a longer ventral projection and a more pyriform shape. By contrast, the holotype (Fig. 1M) and R117.1981.605 (Fig. 1V) are both sub-rounded with only small ventral projections. The lateral surfaces have a reticulate ornamentation with one or two centrally positioned lumina (Fig. 1M, P, S, V) and 7 to 11 muri or ridges ranging in width from 0.2 to 0.6 mm (e.g., Fig. 1M). Some ridges radiate to the edges of the specimen and join up with the strong perichalazal rib producing marginal lumina (Fig. 1O, X). Others terminate before reaching the margin without forming a reticulum (bottom right in Fig. 1S; top left in Fig. 1V), resulting in a radiating pattern of marginal ridges and unenclosed marginal depressions. The upper portion of the ventral surface forms an angle of 35 to 55 degrees with the long axis of the seed (Fig. 1M, P, S, V). The ventral infolds, although not externally visible (Fig. 1N, Q, T, W), are readily identifiable in the holotype by differences in contrast and mineral density in µCT scans (Fig. 2G). A distinct pale area (highlighted yellow in Fig. 2H) marks the position of the embryo cavity, while the deep (1.5 mm) and very broad (0.75 mm) ventral infolds are infilled with mineral with lower X-ray attenuation and hence darker grey colour (Fig. 2H).

Comments—*Cissus rusingensis* is distinctive in lacking a distinct curved ridge delineating a marginal region and in having muri in the central area of the lateral faces forming a reticulum. This species is very similar to seeds of modern *Cissus lebrunii* Dewit, but *C. rusingensis* has fewer muri and at most two (rather than three)
Species—Cissus psilata Adams, Collinson, S.Y. Smith, & Bamford sp. nov.

Etymology—The epithet “psilata” derives from the ancient Greek adjective “psilós”, meaning “bare” or “smooth”, and refers to the lack of seed coat ornamentation on the smooth surface of the lateral faces.

Diagnosis—Seed bilaterally symmetrical, 7.5 mm in dorsiventral dimension, sub-rounded in lateral view, broadly elliptical in apical, basal, and ventral views. Seed smooth, lacks external ornamentation (psilate), except for prominent median ridge (rib perichalaza) that extends all around dorsal surface, over apex, and almost full length of ventral surface. Base of ventral surface concave, forming acute angle with long axis of seed. Pair of very short, narrow ventral infolds present.

Holotype hic designatus—Seed—V68506 (Fig. 1Y-BB; µCT DTS video available from the Dryad Digital Repository, http://dx.doi.org/10.5061/dryad.g9r36).

Type locality—Rusinga Island, Lake Victoria, Kenya.

Geological horizon and age—Hiwegi Formation, early Miocene.

Repository—Natural History Museum, London, UK.

Description—Seed inferred to be originally bilaterally symmetrical around a prominent median perichalazal rib, although the single specimen is slightly deformed (Fig. 1Z, AA), probably due to abnormal development in life or distortion during fossilization. The seed is sub-rounded in lateral view (Fig. 1Y), 7.5 mm in dorsiventral dimension and 6 mm in height, and broadly elliptical in apical and basal (Fig. 1Z, AA) views, 4.5 mm in width. The seed is not laterally flattened and has an inflated morphology with a height/width ratio of 1.3. The perichalazal rib is ca. 0.3–0.45 mm thick around the dorsal and basal margins (Fig. 1Y, Z) but thicker at the seed base.
(ca. 0.8 mm), forming a strong point (Fig. 1Y, AA), equivalent to the beak in typical Vitaceae (see fig. 1 in Chen and Manchester, 2011). The lateral surfaces are smooth (Fig. 1Y). The upper portion of the ventral surface forms an angle of 45 degrees with the long axis of the seed (Fig. 1Y). The ventral infolds are not visible on the outside of the seed (Fig. 1Z, BB). µCT scans of the holotype (Fig. 2I) show very little internal information compared to the other fossils, except for two very short (less than 0.5 mm) grooves at the base of the ventral surface (Fig. 2J), which correspond to the position of the ventral infolds characteristic of Cissus.

Comments—In her unpublished thesis, Chesters (1958) suggested that V68506 could be assigned to the genus Menispermicarpum as a new, but never published, species. However, the fossil morphology differs from endocarps of Menispermaceae. It is almost identical to seeds of modern Cissus petiolata Hook.f., differing in the greater extent of the rib perichalaza on the dorsal surface. In modern Cissus petiolata seeds, the ventral infolds are very shallow and short which, in combination with mineralization effects, can explain the very limited evidence for this diagnostic feature in V68506 (Fig. 2J).

RESULTS

Identification of fossils to genus Cissus—The lack of cellular and tissue detail in the fossils (Fig. 2C-J), and the complexities of mineralization during fossilization, make it difficult to judge if the external morphology of a fossil represents the external morphology of a living equivalent. The SRXTM videos of modern Cissus seeds (available from the Dryad Digital Repository, http://dx.doi.org/10.5061/dryad.g9r36) show that the inner and outer surfaces of the endotesta are parallel to one another and hence a mineral infill of the endotesta will
have a very similar external morphology to a mineral replacement of the endotesta itself. Therefore, it is justifiable to compare the external surface of the Hiwgei Formation fossils with that of modern seeds for purposes of identification. These comparisons show that several modern African *Cissus* species have seeds with almost identical shape and ornamentation to the Hiwgei Formation fossil seeds (see *Phylogenetic context of seed morphotypes*). However, the ventral infolds that characterize modern *Cissus* seeds are not visible on the fossils. The taphonomy of the fossils may explain the absence of these key features.

A novel approach in virtual taphonomy was used to produce a digital infill of the endotesta of a modern *Cissus* seed (Fig. 3A). This showed all the characters typical of *Cissus* seeds (i.e., a long linear chalaza, ‘perichalaza’; a thickened ridge of seed coat along the perichalaza ‘perichalazal rib’; deep, narrow, linear ventral infolds). Digital infilling of the ventral infolds produced a digital fossil comparable to the real Hiwgei Formation fossils (Fig. 3B). This virtual taphonomy suggests a two-stage fossilization process with an initial infilling of the endotesta (and perhaps some mineral replacement of organic tissues) followed by a later stage of infilling of the ventral infolds. µCT imaging supports this hypothesis as mineral-infilled ventral infolds can be recognized (Fig. 2C-J) in digital sections through the fossils (albeit more clearly in some specimens than others).

The only other modern genus including seeds similar to *Cissus* is *Cyphostemma*, some seeds of which may be laterally flattened and have somewhat similar ornamentation. Unlike *Cissus* (Fig. 4A) the ventral infolds on extant *Cyphostemma* seeds are externally concealed by extra layers of endotestal sclereids (Chen and Manchester, 2011; Fig. 4B). If the fossils were originally *Cyphostemma* seeds with the endotesta infilled by mineral during initial stages of fossilization, then
the spaces enclosed by the endotesta over the ventral infolds would have been infilled at the same time as the main area within the endotesta. However, µCT images of the fossils show mineral infills of the ventral infolds that are distinct from other mineral infill within the endotesta (Fig. 2C-H) indicating infill during a later stage of fossilization.

The combined data show that four African fossil seed morphotypes conform to the genus *Cissus* in all characters that can be determined from the fossils. The complex taphonomy and concealment of key features diagnostic of the genus explain the original taxonomic misidentification to the Menispermaceae.

*Phylogenetic relationships within Cissus*—In our maximum likelihood analysis, species of *Cissus* are found in three distinct groups (Fig. 5; Appendix S7, see Supplemental Data with the online version of this article): the *Cissus striata* clade (81%/0.94 [hereafter, bootstrap support/Bayesian posterior probabilities]), the *Cissus trianae* clade (<50%/0.25), and the core *Cissus* clade (60%/1.0), as previously identified by Rodrigues et al. (2014). Within the core *Cissus* clade in our analysis, the early-diverging lineages comprise only African species (Fig. 5). The African species *Cissus barbeyana*, *C. sagittifera* and *C. floribunda* form a clade (the *Cissus barbeyana* clade; 100%/1.0) sister to the remainder of the core *Cissus* clade, in which the *Cissus integrifolia* clade (*C. integrifolia* + *C. populnea*; 100%/1.0) is sister to two main clades that comprise the rest of the species in genus *Cissus*. In the first of these two main clades (100%/1.0) only eight of 35 species in our analysis are not African; whilst only seven of the 49 species in the second clade are African (Fig. 5). The topologies and support values obtained with the maximum likelihood (Fig. 5) and Bayesian analyses (Appendix S8, S9, and S10, see Supplemental Data with the online version of this article) do not present well-supported topological discrepancies.
The divergence time analyses estimated that the core *Cissus* clade diverged 57.9 Ma (highest posterior density (HPD) 55.9–64.0 Ma; 95% HPD intervals of age estimates are provided in Appendix S11, see Supplemental Data with the online version of this article), in the late Paleocene (Appendix S8, see Supplemental Data with the online version of this article), and started to diversify 41.5 Ma (HPD 31.6–51.0 Ma), in the late middle Eocene (Appendix S9, see Supplemental Data with the online version of this article).

**Phylogenetic context of seed morphotypes**—Seeds of four distinct *Cissus* species are now recognized from the early Miocene Hiwegi Formation. Given that claims of Vitaceae pollen from the Oligocene of Cameroon remain unconfirmed (Salard-Cheboldaeff, 1978, 1981; Muller, 1981), these seeds represent the first confirmed fossil record of Vitaceae and of the genus *Cissus* in Africa. The four fossil species represent distinct morphotypes, which are also found in seeds of extant species (Table 1), as revealed by SRXTM imaging of modern fruits and by a literature survey of published seed descriptions. The phylogenetic position of these extant species provides a phylogenetic context for the early Miocene fossils.

**Morphotype 1**—*Cissus crenulata* (Fig. 1A-E) has laterally flattened, sub-oval seeds with a faceted marginal area, several long radiating lateral ridges and two marginal ridges. The extant species *Cissus integrifolia* (Fig. 6D, 7A) and *C. populnea* (Fig. 2A, 6E) share this distinctive morphotype (Table 1). These two species are recovered in our phylogenetic analyses with strong support (100% BS) as a distinct clade, the *Cissus integrifolia* clade, which is one of the two earliest-diverging clades in core *Cissus* (Fig. 5). It is, therefore, likely that *Cissus crenulata* was a member of the early diverging *Cissus integrifolia* clade.

**Morphotype 2**—*Cissus andrewsii* has seeds that are laterally flattened and
have a ridged and faceted marginal area where ridges sometimes fuse to form a reticulum, two prominent marginal ridges, and short radiating lateral ridges. The extant species *Cissus barbeyana* (Fig. 6B) and *C. dasyantha* (Fig. 7B and fig. 13J in Dewit and Willems, 1960) are most similar to *C. andrewsii*, in that they are slightly laterally flattened (H:W ratio of 1.3-1.5) with a similarly ridged and faceted marginal reticulum, two marginal ridges and short radiating lateral ridges (Table 1). Although the sampled specimen of *Cissus dasyantha* failed to amplify material for phylogenetic analysis, *C. barbeyana* was found in the basal clade of core *Cissus*, the *Cissus barbeyana* clade, with *C. floribunda* and *C. sagittifera* (Fig. 5). Seeds of *Cissus floribunda* (Fig. 6A) and *C. sagittifera* (fig. 14 in Descoings, 1967) also possess the Morphotype 2 characters, (except that *C. sagittifera* lacks the ridged and faceted marginal area). This suggests that Morphotype 2 is characteristic of the basal *Cissus barbeyana* clade and that *C. andrewsii* can be placed in this clade. Future work determining if *Cissus dasyantha* is in the *C. barbeyana* clade would test this hypothesis.

**Morphotype 3—Cissus rusingensis** has slightly laterally flattened seeds and ridges forming a complete to incomplete reticulum across the lateral faces. Extant species sharing these features (Table 1) include: *Cissus lebrunii* (fig. 13K in Dewit and Willems, 1960), *C. oreophila* (pl. 39, fig. 11 in Descoings, 1972), *C. sciaphila* (Fig. 6H), *C. smithiana* (Fig. 6G) and *C. tiliifolia* (Fig. 7C and Verdcourt, 1993). *Cissus sciaphila* and *C. tiliifolia* are found together in the *C. sciaphila* clade (*C. lebrunii* did not amplify), with five other African species (*C. aphyllantha*, *C. polyantha*, *C. quarrei*, *C. rhodotricha*, *C. smithiana*) and the Asian/Australasian species, *C. adnata* and *C. rostrata* (Fig. 5). Seed descriptions suggest these species share most, if not all, of the external morphological features of Morphotype 3 (Appendix S4, see...
Cissus polyantha seeds are very variable in surface ornamentation from smooth to extensively ridged (pl. 36, figs. 11-12 in Descoings, 1972; fig. 13H in Dewit and Willems, 1960), also seen in our sampling of two specimens (Appendix S1, see Supplemental Data with the online version of this article). However, one illustration of a ridged specimen (fig. 13H in Dewit and Willems, 1960) falls into Morphotype 3. Morphotype 3 is characteristic of almost all of the species belonging to the Cissus sciaphila clade (and occurs in at least one collection of C. polyantha). This suggests that Cissus rusingensis was an early Miocene member of this clade. Cissus auricoma has seeds of Morphotype 3 but is a member of the C. microdonta-C. auricoma clade (Fig. 5). This being the only species in that clade with Morphotype 3 seeds suggests parallel evolution of seed morphology in this case, although further sampling of both DNA and seed morphology would be needed to confirm this.

Morphotype 4—Seeds of Cissus psilata are sub-rounded with smooth lateral faces almost identical to seeds of extant C. petiolata (Table 1; Fig. 6M, 7D). Cissus petiolata is found in a clade with seven solely African species (Fig. 5): C. aralioides, C. oliveri, C. polita, C. rondoensis, C. rotundifolia, C. sylvicola, C. welwitschii. Of these, Cissus aralioides (Fig. 6L), C. rotundifolia, C. sylvicola and C. welwitschii have Morphotype 4 seeds (Appendix S4, see Supplemental Data with the online version of this article). Cissus oliveri, with coarsely pitted seeds and very strong radial and lateral ribs (Verdcourt, 1993), and C. rondoensis, with seeds bearing two to three, faint transverse ridges (Verdcourt, 1993), lack the smooth seed coat typical of Morphotype 4. These two species occupy contrasting habitats to the rest of the clade (Table 2), so contrasting seed morphology may reflect different environmental pressures.
Extant species in the *Cissus pseudoguerkeana*-C. *albiporcata* clade, for which seed descriptions were available, also share seed Morphotype 4: *C. faucicola* and *C. quadrangularis* have smooth seeds (Appendix S4, see Supplemental Data with the online version of this article), and *C. cactiformis* also has the smooth seed coat (Verdcourt, 1993). Furthermore, the majority of species in the *Cissus elongata*-C. *subtetragona* clade (Fig. 5) share Morphotype 4 characters (Appendix S4, see Supplemental Data with the online version of this article). This evidence suggests that Morphotype 4 occurs throughout the broader *Cissus petiolata*-C. *albiporcata* clade (Fig. 5), and suggests that *C. psilata* belongs in this clade.

Further morphological and molecular analyses, with expanded geographic and taxonomic sampling (including those species that failed to amplify for this study), are needed to fully evaluate the systematic significance of seed morphotypes. This study focused on modern African species to place the African Miocene fossils in context. Based on extant species with morphologically comparable seeds, and their phylogenetic position, species from four clades of *Cissus* (or from the ends of their stem lineages) were present during the early Miocene in East Africa. These species were related to the two early-diverging clades of core *Cissus* (the *Cissus integrifolia* and *C. barbeyana* clades), and two later-diverging clades with mainly African species today, the *C. sciaphila* clade and the *C. petiolata*-C. *albiporcata* clade (Fig. 6).

**Fossil plant biology and ecology**—Habit and habitat data (Table 2) for nearest living relatives of the fossil *Cissus* (Fig. 6) can be used to consider the likely paleobiology and paleoecology of the fossil plants. In the *Cissus integrifolia* clade, the plants are lianas or herbaceous climbers in wide-ranging habitats, from evergreen forest and woodland to bushland and savanna grassland. Members of the *Cissus barbeyana* clade are herbaceous to woody lianas and predominantly occupy
rainforest, gallery and riverine forest fringes. Species in the *Cissus sciaphila* clade are also herbaceous to woody climbers in rainforests or are scrambling shrubs or woody climbers in drier deciduous forest and woodland. The extant species of the *Cissus aralioides-C. albiporcata* clade are herbaceous to woody lianas or climbing shrubs, a number of which are succulent (*C. aralioides, C. cactiformis, C. petiolata, C. quadrangularis, C. rotundifolia, C. sylvicola*). They occur in riverine forest and wooded savanna to rocky outcrops, xerophilic thickets in *Acacia* mixed bushland and grassland (Table 2). The two members of the *Cissus petiolata* sub-clade with different seeds (see previous section) also differ in their habitat preferences, being most often found in moist rainforests.

These modern ecologies suggest that a diversity of climbers (either herbaceous or woody or both) were present in the early Miocene on Rusinga Island. These climbers may have occupied gallery or riverine forest (*Cissus andrewsii* related to the *C. barbeyana* clade) and evergreen forest, through woodland to savanna (*C. rusingensis* related to the *C. sciaphila* clade and *C. crenulata* related to the *C. integrifolia* clade). The plant producing the *Cissus psilata* seeds may have been somewhat succulent and hence able to occupy arid habitats as well as riverine forest or savanna, based on the habitats of a number of related extant species in the *C. aralioides-C. albiporcata* clade.

**DISCUSSION**

*Homology of the ventral surface in Vitaceae seeds*—The current convention for descriptive terminology of Vitaceae seeds (Chen and Manchster 2011) and fossil *Cissus* seeds (Manchester et al., 2012b) has been followed here (Appendix S6, see Supplemental Data with the online version of this article) to allow
for ease of comparison with their work. However, an alternative interpretation is possible, particularly for the strongly flattened seeds (e.g. Fig. 1A-L; Fig. 2A-D; Fig. 3; Fig. 6A-F), whereby the ventral surface incorporates both ventral and apical sensu Appendix S6 herein (see Supplemental Data with the online version of this article) and the ventral grooves are short occupying less than half of the dimension of that surface. This alternative seed orientation is followed in all the floras to which we refer in this paper (i.e. the modern seed illustrations in Fig. 6 are all rotated 90 degrees clockwise). These two alternative homologies would best be investigated by a developmental study.

**Cissus origins, phylogeny and Miocene diversity in Africa**—Relationships among genera in the Vitaceae are mostly comparable between our study and the most recent previous studies of *Cissus* (Liu et al., 2013, 2016; Rodrigues et al., 2014), with limited support for the backbone of the trees (but see Wen et al., 2013 and Zhang et al., 2015). *Cissus* species are found in three distinct clades in all analyses, identified by Rodrigues et al. (2014) as the *Cissus striata* clade (Clade III of Liu et al., 2013), the *C. trianae* clade (Clade V of Liu et al., 2013) and the core *Cissus* clade, which contains most of the species. As in Liu et al. (2013), the earliest-diverging clades comprise mainly African species, although this is more evident in our study in which the first branches are exclusively African species. This topology provides support for an African origin for the genus, as Liu et al. (2013) also concluded.

The new African fossils are entirely consistent with an African origin for *Cissus*. The notable similarities in seed morphology between extant *Cissus* species and Hiwegi Formation fossils across multiple seed morphotypes suggest the presence of four clades or members of their stem lineages, indicating diversity of
Cissus, by the early Miocene in Africa. Based on the divergence times (Appendix S9, see Supplemental Data with the online version of this article), it appears that all four clades originated much earlier than the ca. 18 Ma age of the Miocene fossils: 41.5 Ma (HPD 31.5-51.0 Ma) for the split of the Cissus barbeyana clade from the rest of core Cissus; 36.0 Ma (HPD 27.6-44.7 Ma) for the divergence of the C. integrifolia clade; 24.6 Ma (HPD 18.1-32.1 Ma) for the divergence of the C. sciaphila clade; and 22.8 Ma (HPD ca. 16.6-30.0 Ma) for the divergence of the C. aralioides-C. albipor cata clade. The time-calibrated phylogeny (Appendix S9, see Supplemental Data with the online version of this article) therefore suggests that several ghost lineages of Cissus await discovery in the fossil record. The new African Miocene seeds, and those from the Oligocene of Peru (see Comparison with other fossil Cissus below), provide fossil evidence consistent with the suggested phylogenetic history of Cissus.

**Comparison with other fossil Cissus**—The fossil record of the grape family (Vitaceae) extends back to the latest Cretaceous or earliest Paleogene in central India (Manchester et al., 2013) and to the Paleogene in North America and Europe (e.g., Manchester, 1994; Fairon-Demaret and Smith, 2002; Chen and Manchester, 2007; Collinson et al., 2012). However, for Cissus, the oldest, and only other, currently recognised examples are from the late early Oligocene Belén flora of northern Peru (Manchester et al., 2012b). This flora contains two Cissus species: *Cissus willardii* Berry and *Cissus lombardii* Manchester, Chen, & Lott. *Cissus willardii* is small and globose with smooth lateral faces and large ventral infolds (fig. 2A-Q in Manchester et al., 2012b), unlike any of the fossils described above from Rusinga Island.
Conversely, *Cissus lombardii* shares some characteristics with African Miocene *C. crenulata* and modern *C. integrifolia* (seed Morphotype 1), being bilaterally symmetrical, laterally flattened, elliptical in lateral view with a pronounced median perichalazal rib and a faintly faceted marginal area defined by a marginal ridge on each lateral face (fig. 3A-N in Manchester et al., 2012b). This suggests that *Cissus lombardii* might be related to the *C. integrifolia* clade, extending its fossil record to the early Oligocene (30-28.5 Ma). However, *Cissus lombardii* is smaller in all dimensions than *C. crenulata* and *C. integrifolia*, and lacks the distinct ornamenting ridges that cross the lateral faces in seeds of these species. The specimens of *Cissus lombardii* from the Belén flora are internal casts, which could result in a more subdued surface ornamentation than if the fossils were seeds themselves. However, as has been shown in extant *Cissus* seeds in this study using SRXTM (SRXTM videos of modern *Cissus* seeds (available from the Dryad Digital Repository, http://dx.doi.org/10.5061/dryad.g9r36), the inner surface of the endotesta closely parallels the outer surface, which would result in a similar pattern of ornamentation whether a fossil is an internal cast or a replacement of the endotesta itself. There is no indication of even faint ridges across the lateral faces in *Cissus lombardii* (fig. 3A-B in Manchester et al., 2012b), suggesting that the ornamentation of the original seed was significantly different from Morphotype 1. The portion of the ventral surface containing the ventral infolds is “more or less planar (not markedly concave)” in *Cissus lombardii* (Manchester et al., 2012b, pg. 936), rather than weakly to strongly concave as in *C. integrifolia* and *C. crenulata*. Therefore, *Cissus lombardii*, is clearly distinct from the African Miocene species.

**Biogeographic implications**—The position of previously unsampled modern African species near the base of the phylogeny (Fig. 5) reinforces the African origin
for the core *Cissus* clade, suggested by Liu et al. (2013). The new African Miocene fossil *Cissus crenulata* has seed Morphotype 1 as do both extant species of the *C. integrifolia* clade, confirming the presence of early-divergent members of the core *Cissus* clade in Africa by at least the Miocene.

The two modern species outside Africa in the *Cissus sciaphila* clade (*C. rostrata* and *C. adnata*) are advanced within this clade. Six of the seven modern African species in this clade are characterized by seeds of Morphotype 3, and the seventh also includes specimens with this seed morphotype. The new African Miocene fossil *Cissus rusingensis* also has seeds of Morphotype 3. These data suggest that this clade originated in Africa. Dispersal during or after the Neogene to Australasia resulted in the modern pantropical intercontinental disjunct distribution.

The calibrated phylogeny presented here (Appendix S9, see Supplemental Data with the online version of this article) suggests that the divergence of Australasian species occurred near the end of the Pliocene at 2.7 Ma (HPD 0.8–5.5 Ma) differing from, although within the error of, the late Miocene estimate of 7.8 Ma (HPD 3.0–15.1 Ma) made by Liu et al. (2013).

Liu et al. (2013) argued that transoceanic long distance dispersal, rather than terrestrial mammalian dispersal, was the most likely explanation for pantropical intercontinental disjunctions in *Cissus*, because *Cissus* fruits are fleshy and, although in some instances dispersed by mammals, are predominantly bird-dispersed, enabling long-distance transport by bird migration. Multiple large islands across the Indian Ocean may have facilitated an out-of-Africa migration by acting as migratory "stepping stones", as invoked for dispersal of other vitaceous genera (e.g., *Cayratia*; Lu et al., 2013).
The distributions of modern species in all four clades containing nearest living relatives to the fossils extend across Africa from East to West (Table 2). The fossil seeds suggest that the clades containing these living relatives may have had their origins in East Africa with subsequent spread to the rest of the continent. However, additional African fossil records of *Cissus* are needed to document dispersal patterns.

**Paleoenvironmental implications**—Previous paleoenvironmental reconstructions from the Hiwegi Formation have inferred a habitat mosaic inhabited by early hominoids, such as *Ekembo* (see McNulty et al., 2015). Evidence for mosaic habitats comes from gastropod (Verdcourt, 1963; Pickford, 1995) and mammal faunas (e.g., Andrews and Van Couvering, 1975), paleosols (Retallack et al., 1995) and paleobotany (Collinson et al., 2009; Maxbauer et al., 2013; Michel et al., 2014).

Collinson et al. (2009) concluded that the overall paleoenvironmental signal, considering the evidence from plants, mammals, gastropods and paleosols, was one of “mixed habitats dominated by woodlands, with waterside environments and small patches of forest big enough to support forest faunas” (p. 161); this is very similar to the possible fossil *Cissus* paleoecologies based on nearest living relatives (see *Fossil plant paleobiology and paleoecology*).

The vegetation in which *Cissus andrewsii*, *C. crenulata* and *C. psilata* lived cannot be inferred from associated fossils, as the specimens were surface-picked. However, the context of the *Cissus rusingensis* fossils is well understood as they derive from in situ excavations of plant litter assemblages at the R117 site (Collinson et al., 2009). These litter assemblages were interpreted to have accumulated under a continuous canopy in deciduous, broad-leaved woodland bordering a river, based on the fossil fruits and seeds and their taphonomy (Collinson et al., 2009). *Cissus*
rusingensis is therefore known to have inhabited a closed riverine woodland, consistent with interpretations made for this species from inferred near living relatives. Recent studies have revealed temporal paleoenvironmental changes through the Hiwegi Formation (Michel et al., 2013, 2014; Garrett et al., 2015) and have suggested that interpretations of mosaic paleoenvironments may be based on time-averaged faunal and floral assemblages that conflate separate, more homogeneous habitats. These studies suggest that more open, drier woodland habitats low in the Hiwegi Formation (e.g., Grit Member) gave way to dense, closed canopy forest further up (e.g., Fossil Bed and Kibanga Members), with early hominoid fossils recovered from both paleoenvironments (Garrett et al., 2015). Since the stratigraphic context of Cissus andrewsii, C. crenulata and C. psilata are unknown and they are not associated with C. rusingensis, the four new species of Cissus described may or may not have existed contemporaneously. Despite this uncertainty, it is known from in situ excavations in the Fruit and Nut Bed (Collinson et al., 2009) and stratigraphically associated leaf assemblages (Maxbauer et al., 2013) that a riverine mosaic habitat of woodland and forest existed during the deposition of the Grit Member of the lower Hiwegi Formation. The paleoecology of the new fossil species of Cissus, inferred from living relatives and supported (for Cissus rusingensis) by associated fossils, provides new evidence for mosaic landscapes on Rusinga Island during the early Miocene, ranging from gallery or riverine forest to woodland, bushland and savanna.


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Verdcourt, B. 1963. The Miocene non-marine mollusca of Rusinga Island, Lake 

195, 1-149. A.A. Balkema, Rotterdam, Netherlands.


Table 1. Seed morphotypes of selected single-seeded, modern African *Cissus* species based on internal and external morphology obtained from SRXTM datasets and descriptions and illustrations in floras (Dewit and Willems, 1960; Descoings, 1972; Verdcourt, 1993). H = height, W = width.

<table>
<thead>
<tr>
<th>Seed morphotype</th>
<th>Species</th>
<th>Description</th>
</tr>
</thead>
</table>
| 1 (Figs. 6D, 6E, 7A) | *C. integrifolia*; *C. populnea* | - Two-layered, thick fruit wall, with a denser outer layer and more porous inner layer  
- Seeds laterally flattened (H:W ratio of 1.5–1.8 in median DTS), elongate in the dorsiventral dimension  
- Obvious break in seed coat near the chalaza  
- Outer endotesta consists of thin layer with different X-ray attenuation (possibly high mineral content)  
- Dense and thin seed coat  
- Seed surface with two marginal ridges, one on each lateral face, a ridged and faceted marginal area, long ridges radiating from ventral margin across lateral faces |
| 2 (Figs. 6B, 7B) | *C. barbeyana*; *C. dasyantha* | - Thin to moderately thick fruit wall  
- Seeds slightly laterally flattened (H:W ratio of 1.3–1.5 in median DTS), elongate in the dorsiventral dimension  
- Indistinct break in seed coat near the chalaza  
- Endotesta has uniform X-ray attenuation  
- Dense and moderately thick seed coat  
- Seed surface with two marginal ridges; a ridged and faceted marginal area, where ridges sometimes fuse to form a reticulum; short ridges radiating across part of the lateral faces |
| 3 (Figs. 6H, 7C) | *C. lebrunii*; *C. sciaphila*; *C. tiliifolia* | - Thin fruit wall  
- Seeds not, or slightly, laterally flattened (H:W ratio of 1.0–1.6 in median DTS), short in the dorsiventral dimension  
- Indistinct break in seed coat near the chalaza  
- Endotesta has uniform X-ray attenuation  
- Thin seed coat of variable texture  
- Seed surface covered in ridges, forming a complete to incomplete reticulum across the lateral faces |
| 4 (Figs. 6M, 7D) | *C. petiolata* | - Thick fruit wall  
- Seeds laterally flattened (H:W ratio of 1.5-1.8 in median DTS), short in the dorsiventral dimension  
- Thickened chalaza with no break in seed coat  
- Endotesta has uniform X-ray attenuation  
- Dense and thick seed coat  
- Seed surface smooth. |
<table>
<thead>
<tr>
<th>Species</th>
<th>Distribution</th>
<th>Habit</th>
<th>Habitat</th>
<th>Sources</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. adnata</em> Roxb.</td>
<td>Australasia; E, SE, and S Asia</td>
<td>Scrambling or climbing shrub, or woody liana, up to 10 metres</td>
<td>Primary lowland monsoon forests, riparian forest, disturbed and semi-open scrub and deciduous forest, shrubland and thickets</td>
<td>1, 2, 3, 4</td>
</tr>
<tr>
<td><em>C. albiporcarta</em> Masinde &amp; L.E.Newton</td>
<td>E Africa</td>
<td>Climber</td>
<td>Bushland in rocky areas</td>
<td>3, 5</td>
</tr>
<tr>
<td><em>C. aphyllantha</em> Gilg</td>
<td>E Africa</td>
<td>Shrub, scrambler or woody climber, 1 to 4 meters tall</td>
<td><em>Acacia</em> scrub or desert thornbush, scrubby woodland, rocky outcrops</td>
<td>3, 5</td>
</tr>
<tr>
<td><em>C. aralioides</em> (Welw. ex Baker) Planch.</td>
<td>C, E and W Africa</td>
<td>Vigorous, succulent liana or herbaceous climber, a strong, lofty climber, up to 25 meters</td>
<td>Coastal and riverine evergreen forest, rainforest, coastal bushland, <em>Acacia</em> bushland, grassland, thickets</td>
<td>3, 5, 6, 7, 8, 9</td>
</tr>
<tr>
<td><em>C. barbeyana</em> De Wild. &amp; T.Durand</td>
<td>C and W Africa</td>
<td>Herbaceous to woody, low, small liana</td>
<td>Rainforest, forest clearings, forest fragments and clusters</td>
<td>3, 6, 7, 9</td>
</tr>
<tr>
<td><em>C. cactiformis</em> Gilg</td>
<td>E and S Africa</td>
<td>Succulent climber or scrambler, 1.2 to 4.5 meters long</td>
<td>Woodland, mixed bushland, usually in stony places, rock domes</td>
<td>3, 5</td>
</tr>
<tr>
<td><em>C. dasyantha</em> Gilg &amp; M.Brandt</td>
<td>W Africa</td>
<td>Liana, up to 6 meters</td>
<td>Occasionally flooded forest, gallery forests</td>
<td>3, 7</td>
</tr>
<tr>
<td><em>C. faucicola</em> Wild &amp; R.B.Drumm.</td>
<td>E Africa</td>
<td>Herbaceous climber, several meters long</td>
<td>Evergreen rainforest, especially edges by waterfalls and in grassy clearings</td>
<td>5, 8</td>
</tr>
<tr>
<td><em>C. floribunda</em> (Baker) Planch.</td>
<td>E Africa</td>
<td>Thin woody liana or climber, several meters long</td>
<td>Rainforest, dense moist tropical forest, disturbed forest or forest edge</td>
<td>4, 10</td>
</tr>
<tr>
<td><em>C. integrifolia</em> (Baker) Planch.</td>
<td>C and E Africa</td>
<td>Herbaceous vigorous climber to 5 meters, reaching tops of trees or thicket-forming</td>
<td>Evergreen riverine forest, woodland, bushland and savanna grassland, often on rocky scarps</td>
<td>3, 5, 7, 8, 11</td>
</tr>
<tr>
<td><em>C. lebrunii</em> Dewit</td>
<td>C Africa</td>
<td>Herbaceous climber</td>
<td>Rainforest</td>
<td>3, 7</td>
</tr>
<tr>
<td><em>C. oliveri</em> (Engl.) Gilg ex Engl.</td>
<td>C and E Africa</td>
<td>Herbaceous to woody climbing shrub, up to 6 meters long</td>
<td>Riverine and gallery forest, marshy areas, papyrus swamps, wet grassland with scattered trees</td>
<td>3, 5, 7</td>
</tr>
<tr>
<td><em>C. oreophila</em> Gilg &amp; M.Brandt</td>
<td>C and W Africa</td>
<td>Large herbaceous liana</td>
<td>Gallery and riverine forest, forest edges, swamp areas</td>
<td>3, 4, 6, 9</td>
</tr>
<tr>
<td><em>C. petiolata</em> Hook.f.</td>
<td>C, E and W Africa</td>
<td>Large, somewhat succulent liana,</td>
<td>Riverine forest, occasionally flooded forest edges,</td>
<td>3, 5, 6, 7, 7</td>
</tr>
<tr>
<td>Species</td>
<td>Distribution</td>
<td>Life Form</td>
<td>Habitat</td>
<td>References</td>
</tr>
<tr>
<td>----------------------------------------------</td>
<td>---------------------------------------</td>
<td>----------------------------------------</td>
<td>---------------------------------------------------------------------------------------------------</td>
<td>------------</td>
</tr>
<tr>
<td>C. phymatocarpa Masinde &amp; L.E.Newton</td>
<td>E Africa</td>
<td>Vigorous climber or scrambler, to 10 meters</td>
<td>Thickets, Acacia mixed bushland, rocky ground with scattered trees and shrubs, woody/shrub savanna</td>
<td>8, 9, 11</td>
</tr>
<tr>
<td>C. polita Desc.</td>
<td>E Africa</td>
<td>Herbaceous liana, 1.5 to 3 meters</td>
<td>Calcareous hills and plateaux; dry, deciduous seasonal forest; savanna grassland with dry forest</td>
<td>4, 10</td>
</tr>
<tr>
<td>C. polyantha Gilg &amp; M.Brandt</td>
<td>C, E and W Africa</td>
<td>Herbaceous to woody climber/ liana, up to 15 meters</td>
<td>Riverine and gallery rainforest, rocky hollows in grassland and thickets</td>
<td>3, 5, 6, 7, 9</td>
</tr>
<tr>
<td>C. populnea Guill. &amp; Perr.</td>
<td>C, E and W Africa</td>
<td>Bushy liana, to 4.5 meters</td>
<td>Wooded savanna, rocky outcrops and scree, bushland, lowland forest edges</td>
<td>3, 5, 6, 7, 9</td>
</tr>
<tr>
<td>C. pseudoguerkeana Verdc.</td>
<td>E Africa</td>
<td>Spreading herb, at least 60cm long</td>
<td>Woodland, low shrubs on sand, swampy places</td>
<td>3, 5</td>
</tr>
<tr>
<td>C. quadrangularis L.</td>
<td>Arabia; C, E, N, S, and W Africa; SE and S Asia</td>
<td>Succulent bushy liana 1 to 15 meters long, or succulent climbing shrub</td>
<td>Xerophilic thickets, thorny savanna, Acacia woodland, grassland, riverine thicket, coastal forest edges</td>
<td>3, 5, 8, 9, 10</td>
</tr>
<tr>
<td>C. quarrei Dewit</td>
<td>C and E Africa</td>
<td>Erect herb or herbaceous climber, 0.6 to 1 meter tall</td>
<td>Riverine vegetation, Brachystegia woodland</td>
<td>3, 5, 7, 8</td>
</tr>
<tr>
<td>C. rhodotricha (Baker) Desc.</td>
<td>E Africa</td>
<td>Scrambling and climbing strong liana or erect shrub</td>
<td>Rocky outcrops, deciduous seasonal forest, wooded savanna</td>
<td>10</td>
</tr>
<tr>
<td>C. rondoensis Verdc.</td>
<td>E Africa</td>
<td>Herbaceous to semi-woody climber to around 4 meters</td>
<td>Moist, (semi-) evergreen forest, dense forest thicket</td>
<td>3, 4, 5</td>
</tr>
<tr>
<td>C. rostrata (Miq.) Korth. ex Planch.</td>
<td>SE Asia</td>
<td>Climber/ liana, up to 10 to 15 meters</td>
<td>Fringe and understorey forest; forest river banks, peat swamp/ marshy forest</td>
<td>3, 4, 12</td>
</tr>
<tr>
<td>C. rotundifolia Vahl</td>
<td>Arabia; C, E and S Africa</td>
<td>Succulent, herbaceous to woody, vigorous climber/ liana, up to 5 meters</td>
<td>Dry woodland and bush, thorny savanna, Acacia scrub, bushland, thickets, dry forest and forest edges particularly on rocky outcrops</td>
<td>3, 5, 7, 8</td>
</tr>
<tr>
<td>C. sagittifera Desc.</td>
<td>E Africa</td>
<td>Creeping or climbing, thin liana</td>
<td>Woodland edges, limestone cliffs, quartzite outcrops</td>
<td>3, 4, 10</td>
</tr>
<tr>
<td>C. sciaphila Gilg</td>
<td>E Africa</td>
<td>Woody climber/ liana, 3 to 12 meters long or shrubby</td>
<td>Lowland riverine forest fringes, woodland slopes above river valleys</td>
<td>3, 5, 8</td>
</tr>
<tr>
<td>C. smithiana (Baker) Planch.</td>
<td>C and W Africa</td>
<td>Large liana/ climber</td>
<td>Rainforest, forest galleries and edges</td>
<td>3, 7, 9</td>
</tr>
<tr>
<td>C. sylvicola Masinde &amp; L.E.Newton</td>
<td>E Africa</td>
<td>Herbaceous, somewhat succulent and fleshy liana, up to 12 meters</td>
<td>Evergreen forest, also forest on rocky hills, coralline limestone and thicket</td>
<td>3, 5</td>
</tr>
<tr>
<td>C. tiliifolia Planch.</td>
<td>C and E Africa</td>
<td>Herbaceous to woody climber, up to 10 meters</td>
<td>Forest and thickets in swampy areas, particularly near lake shores, swampy grassland</td>
<td>3, 5</td>
</tr>
<tr>
<td>C. welwitschii (Baker) Planch.</td>
<td>C and E Africa</td>
<td>Vigorous, woody climber, 2 to 9 meters long, or shrubby</td>
<td>Semi-evergreen bushland, riverine fringes, thickets and termite mounds in Brachystegia woodland, rocky outcrops</td>
<td>3, 5, 8</td>
</tr>
</tbody>
</table>
Appendix 1. Voucher information and GenBank accession numbers of *Cissus* species, for which new sequences were produced for the present phylogenetic analysis. Species; *trnLF*, *rps16*, *trnC-petN*, *atpB-rbcL*; voucher information; Kew DNA Bank accession number.

- **Cissus barbeyana** De Wild. & T.Durand; KX131178; -; -; -; Lisowski, S. 16406 (K); 31957.
- **Cissus bosseri** Desc.; KX131174; -; -; KX131172; Phillipson, P.B. & Rabesihanaka, S. 3140 (K); 31965.
- **Cissus petiolata** Hook.f.; KX131175; -; -; -; Luke, P.A. & WRQ 9365 (K); 31952.
- **Cissus polyantha** Gilg & M.Brandt; KX131176; -; -; -; Deighton, F.C. 5208 (K); 31955.
- **Cissus populnea** Guill. & Perr.; KX131179; -; -; -; Daramola, B.O. 221 (K); 31968.
- **Cissus rondoensis** Verdc.; -; -; -; Bidgood, S., Abdallah, R. & Vollesen, K. 1553 (K); 31961.
- **Cissus smithiana** (Baker) Planch.; JX476858; JX476547; JX476673; JX476429.
- **Cissus albiporcata** Masinde & L.E. Newton; JF437304; JX476548; JF437201; JX476430.
- **Cissus amazonica** Lindel.; JX476859; JX476549; JX476674; JX476431.
- **Cissus anisophylla** Lombardi; AB235010; JX476550; JX476675; JX476432.
- **Cissus annamica** Gagnep.; -; -; JX476676; -.

Appendix 2. Species included in the phylogenetic analysis of family Vitaceae for which sequences were obtained from GenBank, with a particular focus on genus *Cissus*. Species; *trnLF*, *rps16*, *trnC-petN*, *atpB-rbcL*.
1254 JX476551; JX476677; JX476433. **Cissus apendiculata** Lombardi; JX313413; -; -; -.
1255 **Cissus aphyllantha** Gilg; JX476862; JX476553; JX476679; JX476435. **Cissus**
1256 **araguainensis** Lombardi; JX313415; -; -; -; **Cissus aralioides** (Welw. ex Baker)
1257 Planch.; JF437305; -; JF437202; -. **Cissus assamica** (M.A. Lawson) Craib;
1258 JF437307; JX476559; JF437204; JX476441. **Cissus auricoma** Desc.; JX476866;
1259 JX476560; JX476682; JX476442. **Cissus bahiensis** Lombardi; JX313416; -; -; -.
1260 **Cissus biformifolia** Standl.; -; JX476562; JX476684; JX476444. **Cissus**
1261 **blanchetiana** Planch.; JX313417; -; -; -. **Cissus cactiformis** Gilg.; JX476868;
1262 JX476563; JX476685; JX476445. **Cissus campestris** (Baker) Planch.; JX313418; -;
1263 -; -; **Cissus cardiophylla** Standley; EF179080; -; -; -. **Cissus cornifolia** (Baker)
1264 Planch.; JF437308; JX476567; JF437205; JX476449. **Cissus decidua** Lombardi;
1265 JX313419; -; -; -. **Cissus descoingsii** Lombardi; JX313420; -; -; -. **Cissus diffusa**
1266 (Miq.) Amshoff; JX476871; JX476569; JX476689; JX476451. **Cissus diffusiflora**
1267 (Baker) Planch.; JX476872; JX476570; -; JX476452. **Cissus discolor** Blume;
1268 JF437309; -; JF437206; -. **Cissus duartea** Planch.; JX313421; -; -; -. **Cissus**
1269 ** elongata** Roxb.; -; JX476573; JX476691; JX476455. **Cissus erosa** Rich.;
1270 HM585942; HM585802; JX476693; HM585526. **Cissus faucicola** Wild &
1271 R.B.Drumm.; JX476874; JX476576; JX476694; JX476458. **Cissus floribunda**
1272 (Baker) Planch.; JX476875; JX476577; JX476695; JX476459. **Cissus gongyloides**
1273 (Burch. ex Baker) Planch.; JX476877; JX476579; JX476697; JX476461. **Cissus**
1274 **granulosa** Ruiz & Pav.; JX476880; JX476582; JX476700; JX476464. **Cissus**
1275 ** hastata** Miq.; AB235012; -; JX476701; JX476465. **Cissus hypoglaucua** Durras;
1276 JX476881; JX476583; JX476702; JX476466. **Cissus incisa** (Nutt.) Des Moul. Ex
1277 S.Watson; HM585944; HM585804; -; HM585528. **Cissus integrifolia** (Baker)
1278 Planch.; JX476882; JX476584; JX476703; JX476467. **Cissus javana** DC. ;
1279 JX476883; JX476585; JX476704; JX476468. **Cissus lanea** Desc.; JX476884;
1280 JX476586; JX476705; JX476469. **Cissus leucophleus** (Scott-Elliot) Suess.;
1281 JX476885; JX476587; JX476706; JX476470. **Cissus madecassa** Desc.; JX476886;
1282 JX476588; JX476707; JX476471. **Cissus microcarpa** Vahl.; JX476888; JX476590;
1283 JX476709; JX476472. **Cissus microdonta** Vahl.;
1284 JX476474. **Cissus neei** Croat; JX313424; -; -; -; **Cissus nodosa** Blume;
1285 HM585945; JX476592; JX476711; JX476475. **Cissus obliqua** Ruiz & Pav.;
1286 JX476890; JX476593; JX476712; JX476476. **Cissus oblonga** (Benth.) Planch.;
1287 EF179083; -; -; -; **Cissus oliveri** Gilg. ex Engl.; JX476892; JX476595; JX476714;
1288 JX476477. **Cissus paraensis** Lombardi; JX313427; -; -; -; **Cissus paulinifolia**
1289 Vell.; JX313426; -; -; -; **Cissus penninervis** (F.Muell.) Planch.; AF300300; -; -;
1290 **Cissus pentaclada** Jackes; EF179084; -; -; -; **Cissus phymatocarpa** Masinde &
1291 L.E. Newton; JF437311; JX476596; JF437209; JX476479. **Cissus pileata** Desc.;
1292 JX476893; JX476597; JX476715; JX476480. **Cissus polita** Desc.; JX476894;
1293 JX476598; JX476716; JX476481. **Cissus producta** Afzel.; JF437312; JX476600;
1294 JX476718; JX476483. **Cissus pseudoguerkeana** Verdc.; JX476896; JX476601;
1295 JX476719; JX476484. **Cissus pseudoverticillata** Verdc.; JX476897; JX476602;
1296 JX476720; JX476485. **Cissus pulcherrima** Vell.; JX313429; -; -; -; **Cissus**
1297 quadrangularis L.; JF437313; JX476603; JF437211; JX476486. **Cissus quarrei**
1298 Dewit; JX476899; JX476605; JX476722; JX476488. **Cissus reniformis** Domin.;
1299 EF179086; -; -; -; **Cissus repanda** Vahl; JX476900; JX476607; JX476724;
1300 JX476490. **Cissus repens** Lam.; HM585946; -; -; HM585530. **Cissus rhodotricha**
1301 (Baker) Desc.; JX476902; JX476609; JX476727; JX476492. **Cissus rhombifolia**
1302 Vahl; JX476905; JX476612; JX476729; JX476495. **Cissus rostrata** Korth.ex
1303 Planch.; AB235016; -; JX476731; JX476497. **Cissus rotundifolia** (Forssk.) Vahl;
Cissus rubiginosa Welw. ex Bak. 1304
Cissus sagittifera Desc.;
Cissus sciaphila Gilg; JF437316;
Cissus serroniana (Glaz.) Lombardi; JX313430;
Cissus simsiana Roem. & Schult.; JX476910; JX476620; JX476734;
Cissus spinosa Cambess.; JX313435;
Cissus stipulata Vell.; JX313436;
Cissus striata Ruiz & Pav.; AB235017;
Cissus sulcicaulis (Baker) Planch.;
Cissus surinamensis Desc.; JX313439;
Cissus sylvicola Masinde & L.E.Newton;
Cissus tiliacea Kunth;
Cissus trianae Planch.; JX313441;
Cissus trifoliata (L.) L.; JX476926;
Cissus trothae Gilg & M. Brandt; JF437318;
Cissus tuberosa Moc. & Sesse ex DC.;
Cissus tweediana (Baker) Planch.;
Cissus ulmifolia (Baker) Planch.; JX476928; JX476642;
Cissus verticillata (L.) Nicolson & C.E. Jarvis;
Cissus vinosa Jackes; EF179090;
Cissus welwitschii (Baker) Planch.; JX476934; JX476651; JX476537.
Cissus wenshanensis C.L. Li; HM585949; HM585533. Ampelocissus acapulcensis (Kunth) Planch.; JF437281; JX476543; JF437172.
Ampelocissus africana (Lour.) Merr. ; JQ182553; JQ182603; JQ182448. Ampelocissus ascendiflora Latiff ;
Ampelocissus costaricensis Lundell; AB234911.
Ampelocissus elephantina Planch.; HM585932; HM585792; HM585516.
Ampelocissus erdwendbergiana Planch.; JF437282; JX476544; JF437173; -.

Ampelocissus filipes Planch.; AB234982; -; -; - Ampelocissus gracilis Planch.;

AB234983; -; -; - Ampelocissus javalensis (Seem.) W.D. Stevens & A. Pool;

AB234984; -; -; - Ampelocissus obtusata (Welw. ex Baker) Planch.; JQ182556;

JQ182612; -; JQ182457. Ampelocissus thyrsiflora (Blume) Planch.; JQ182546;

JQ182593; -; JQ182438. Ampelopsis bodinieri (H. Lév. & Vaniot) Rehder;

JF437284; JX476545; JF437175; JX476427. Ampelopsis cantoniensis Planch.

HM585933; HM585793; JX476667; HM585517. Ampelopsis chaffanjonii (H. Lev.) Rehder;

JF437286; -; -; -; - Ampelopsis delavayana Planch.; HM223253; -; -; - Ampelopsis rubifolia (Wall.) Planch.;

JF437293; JX476546; JF437186; JX476428. Cayratia acris F. Muell.; EF179070; -; -; - Cayratia clematidea (F. Muell.) Domin.;

EF179072; -; -; - Cayratia cordifolia C.Y. Wu ex C.L. Li; HM585934; HM585794;

JX476668; HM585518. Cayratia debilis (Baker) Suess.; JF437296; -; -; - Cayratia eurynema B.L. Burtt; EF179073; -; -; - Cayratia gracilis (Guill. & Perr.) Suess.;

JF437297; -; -; - Cayratia imerinensis (Baker) Desc.; HM585936; HM585796;

JX476669; HM585520. Cayratia japonica (Thunb.) Gagnep.; HM585937; -; -; -

HM585521. Cayratia maritima Jackes; EF179074; -; -; - Cayratia mollissima Gagnep.; HM585938; HM585798; JX476671; HM585522. Cayratia pedata Gagnep.; AB235005; -; -; - Cayratia saponaria (Seem. Ex Benth.) Domin.;

EF179075; -; -; - Cayratia trifolia (L.) Domin; HM585940; -; JX476672;

HM585524. Cayratia triternata (Baker) Desc.; HM585941; -; -; - Clematicissus angustissima (F. Muell.) Planch.; EF179091; -; -; - Clematicissus opaca (F. Muell) Jackes & Rossetto; JX476935; JX476652; JX476767; JX476538. Cyphostemma adenocaule (A. Rich.) Wild & R.B. Drumm.; JX476936; JX476653; JX476768;
JX476539. *Cyphostemma bainesii* (Hook.f.) Desc.; AB235025; -; -; -.

1355 *Cyphostemma duparquetii* (Planch.) Desc.; JF437324; -; JF437222; -.

1356 *Cyphostemma horombense* Desc.; HM585950; -; -; - *Cyphostemma jiguu*

1357 Verdc.; JX476937; JX476655; JX476769; JX476540. *Cyphostemma*

1358 *kilimandscharicum* (Gilg) Wild & R.B.Drumm.; JF437327; -; -; - *Cyphostemma*

1359 *mappia* (Lam.) Galet; AB235026; -; -; - *Cyphostemma maranguense* (Gilg) Desc.;

1360 JF437329; -; JF437227; -. *Cyphostemma montagnacii* Desc.; AB235027; -

1361 JF437228; -. *Cyphostemma simulans* (C.A. Sm.) Wild & R.B. Drumm; HM585952;

1362 -; -; HM585536. *Leea aculeata* Blume; AB235087; -; -; - *Leea guineensis* G. Don.;

1363 -; JX476657; JF437235; JX476541. *Leea indica* (Burm.f.) Merr.; HM585953; -

1364 JX476771; HM585537. *Leea macrophylla* Roxb. ex Hornem. & Roxb.; JF437335;

1365 JX476659; JF437237; -. *Leea spinea* Desc.; HM585955; -; -; - *Nothocissus*

1366 *spicifera* (Griff.) Latiff; JF437336; JX476660; JF437239; -. *Parthenocissus*

1367 *chinensis* C.L. Li; HM223263; HM223320; JF437240; HM223373. *Parthenocissus*

1368 *henryana* (Hemsl.) Graebn. ex Diels & Gilg; HM223272; HM223329; JF437244;

1369 HM223383. *Parthenocissus heptaphylla* (Buckl.) Britton ex Small; HM223256; -; -

1370 -. *Parthenocissus himalayana* Planch.; AB235034; -; -; - *Parthenocissus*

1371 *laetevirens* Rehder; HM223267; -; -; - *Parthenocissus quinquefolia* (L.) Planch.;

1372 HM223275; HM223332; JF437246; HM223386. *Parthenocissus suberosa* Hand.-

1373 Mazz.; HM223273; HM223330; JF437247; HM223384. *Parthenocissus*

1374 *tricuspidata* (Sieb. & Zucc.) Planch.; HM223274; HM223331; JF437248;

1375 HM223385. *Parthenocissus vitacea* (Knerr.) Hitchc.; HM223295; -; -; -

1376 *Pterisanthes eriopoda* Planch.; -; JX476661; -; - *Pterisanthes heterantha* M.

1377 Laws; AB235045; AB234965; -; AB234930. *Pterisanthes stonei* Latiff; AB235046;

1378 JX476662; -; -. *Rhoicissus digitata* Gilg & Brandt.; AB235047; -; -; - *Rhoicissus*
rhomboidea Planch.; AB235049; -; -; -. Rhoicissus tomentosa (Lam.) Wild & R.B.

Drumm; JF437342; JX476663; JF437251; -. Rhoicissus tridentata (L.f.) Wild &

R.B. Drumm.; JF437341; JX476664; JF437250; -. Tetrastigma glabratum Planch.;

HM585995; -; -; -. Tetrastigma hemsleyanum Diels & Gilg; HM586000; HM585860;

-; HM585584. Tetrastigma lanyuense C.E. Chang; HM586009; HM585869;

JF437257; HM585593. Tetrastigma laxum Merr.; HM586017; -; -; -. Tetrastigma

lenticellatum Planch.; HM586019; -; -; -. Tetrastigma loheri Gagnep.; HM586021;

-; -; -. Tetrastigma obtectum (Wall.) Planch; HM586029; HM585888; -; HM585614.

Tetrastigma pachyphyllum (Hemsl.) Chun; HM586032; HM585891; JF437259;

HM585616. Tetrastigma petraeum Jackes; EF179094; -; -; -. Tetrastigma

pyriforme Gagnep.; HM586039; -; -; -. Tetrastigma sichouense C.L.Li;

HM586047; -; -; -. Tetrastigma triphyllum (Gagnep.) W.T. Wang; HM586061;

HM585919; -; HM585646. Tetrastigma voinerianum Pierre ex Gagnep.;

HM586067; -; -; -. Vitis aestivalis Michx. ; HM586070; HM585928; -; HM585655.

Vitis betulifolia Diels & Gilg; JF437352; JX476665; JF437269; -. Vitis flexuosa

Thunb.; HM586071; HM585929; -; HM585656. Vitis heyneana Roem. & Schult;

JF437354; JX476666; JF437273; -. Vitis labrusca L.; JX507364; JX507361;

JX507362; JX507360. Vitis mengziensis C.L. Li; HM223276; HM223333;

JF437270; HM223387. Vitis popenoei J.L. Fennell; HM586072; HM585930;

JF437276; HM585657. Vitis riparia Michx; JF437357; -; JF437277; -. Vitis

rotundifolia Michx.; HM586073; HM585931; -; HM585658. Vitis thunbergii Siebold & Zucc.; AB235082; -; -; -. Vitis vinfera L.; -; -; -; -. Vitis vulpina L.; JQ182566;

JQ182622; -; JQ182467. Yua austro-orientalis (F.P. Metcalf) C.L. Li; AB235085; -;

-; -. Yua thomsoni (M.A. Lawson) C.L. Li; HM223277; HM223335; -; HM223389.
Fig. 1. External morphology of fossilized *Cissus* seeds from the Hiwegi Formation, Rusinga Island, Kenya. (A) Lateral, (B) apical, (C) basal, (D) ventral views of the *Cissus crenulata* (Chesters) comb. nov. holotype (V33753). (E) Ventral, (F) lateral, (G) apical, (H) basal views of the *Cissus andrewsii* sp. nov. holotype. (I) Lateral and (J) basal views of a paratype of *C. andrewsii* (V68500), and (K) lateral and (L) apical views of another paratype (V68502), demonstrating intraspecific variation in seed size, shape, ornamentation and basal/apical width. (M) Lateral, (N) basal and (O) apical views of the *Cissus rusingensis* sp. nov. holotype (R117.1981.314). (P, S, V) Lateral, (Q, T, W) basal and (R, U, X) apical views of paratypes of *C. rusingensis* (R117.1981.476, R117.1981.604, R117.1981.605 respectively), illustrating intraspecific variation in seed shape (sub-rounded to pyriform), the number of muri and enclosed lumina, the extent of the perichalazal rib and the length of the basal projection. (Y) Lateral, (Z) apical, (AA) basal, (BB) ventral views of the *Cissus psilata* sp. nov. holotype (V68506). Scale bar is 10 mm in 1 mm increments.

Fig. 2. Ventral infolds revealed in a seed of modern African *Cissus populnea* Guill. & Perr. by SRXTM (A–B) and in fossil seeds from the Hiwegi Formation, Rusinga Island, Kenya (C–J), assigned to *Cissus* herein, by μCT. (A, B) SRXTM digital transverse section (DTS) through modern *C. populnea*, infill of the endotesta in yellow in (B) highlighting the position of the ventral infolds. (C, D) μCT DTS through the holotype (V33753) of *Cissus crenulata* comb. nov., with (D) showing inferred position of the ventral infolds (solid yellow lines) and the margins of the endotesta (dotted yellow lines). (E, F) μCT DTS through the holotype (V68501) of *Cissus andrewsii* sp. nov., with (F) showing infill of the inferred endotesta in yellow,
highlighting two parallel, broad ventral infolds. (G, H) μCT DTS through the holotype (R117.1981.314) of *Cissus rusingensis* sp. nov., with (H) showing infill of inferred embryo cavity within endotesta in yellow, highlighting two parallel, very broad ventral infolds. (I, J) μCT DTS through holotype (V68506) of *Cissus psilata* sp. nov., with (J) showing inferred position of pair of very short, narrow parallel ventral infolds (solid yellow lines). All μCT sections obtained from near ventral part of seeds, where ventral infolds were most likely to be evident, if concealed externally, based on their position in modern *Cissus* seeds. All scale bars are 1 mm.

Fig. 3. Virtual taphonomy using digital fossils produced from an SRXTM dataset of a modern fruit of *Cissus populnea* Guill. & Perr. Specimen oriented in oblique ventral/apical view to best display ventral infolds. (A) Produced by digitally infilling space inside endotesta of seed, showing externally conspicuous pair of ventral infolds, thereby mimicking mineral seed infill processes during fossilization. (B) Produced by digitally concealing ventral infolds in (A) thereby mimicking taphonomic processes that could conceal ventral infolds by mineral infill. Scale bar is 1 mm.

Fig. 4. Comparative tissue organisation in *Cissus* (A) and *Cyphostemma* (B) showing how the endotesta obscures ventral infolds in *Cyphostemma*. (A-B) SRXTM digital transverse sections through modern fruits. (A) *Cissus populnea* Guill. & Perr. (B) *Cyphostemma maranguense* (Gilg) Desc. Scale bars in (A) and (B) are 1 mm.

Fig. 5. Phylogenetic tree of core *Cissus* clade obtained using maximum likelihood criteria, implemented in RAxML (Stamatakis, 2014), based on plastid DNA sequence data of Vitaceae, with particular focus on genus *Cissus*. Bootstrap values and
posterior probabilities are indicated at nodes. Clades and seed morphotypes referred to in text are indicated; species placed in seed morphotypes based on internal and external morphology are shown with solid colour highlight, and those tentatively placed in seed morphotypes based on external illustrations and descriptions are distinguished by coloured outline; distribution ranges indicated after species name (Af, Africa; As, Asia; Au, Australia; N, Neotropics); species for which new DNA sequences were produced are highlighted with an asterisk; species for which no data on seed morphology could be found are denoted by a question mark. Scale bar shows degree of genetic change (nucleotide substitutions per site) as distance on the phylogram.

Fig. 6. Major clades recognised in Fig. 5 with representative modern (A-B, D-E, G-H, J-M, O-W) and fossil (C, F, I, N) seed morphologies. Modern images all rotated 90 degrees clockwise from the original source. Seed Morphotype 1 (A-C) characterises the *Cissus barbeyana* clade; Morphotype 2 (D-F) the *C. integrifolia* clade; Morphotype 3 (G-I) the *C. sciaphila* clade and Morphotype 4 (L-N) the *C. petiolata* clade. Smooth seeds, somewhat similar to Morphotype 4, also characterise the wider clade (in which the *C. petiolata* clade is basal) indicated by the dotted grey line.

Sources of modern images are as follows: (A) *C. floribunda* (fig. 15.13 in Descoings, 1967); (B) *C. barbeyana* (fig. 13E in Dewit and Willems, 1960); (D) *C. integrifolia* (fig. 13F in Dewit and Willems, 1960); (E) *C. populnea* (pl. 34, fig. 11 in Descoings, 1972); (G) *C. smithiana* (pl. 52, fig. H in Dewit and Willems, 1960); (H) *C. sciaphila* (fig. 6.1c in Verdcourt, 1993); (J) *C. bosseri* (fig. 10.8 in Descoings, 1967); (K) *C. leucophlea* (fig. 14.13 in Descoings, 1967); (L) *C. aralioides* (fig. 13C in Dewit and Willems, 1960); (M) *C. petiolata* (fig. 13A in Dewit and Willems, 1960); (O) *C. elongata* (fig.
1478 171.10 in Chen et al., 2007); (P) *C. subtetragona* (fig. 171.2 in Chen et al., 2007); (Q) *C. cactiformis* (fig. 9.8 in Verdcourt, 1993); (R) *C. quadrangularis* (pl. 29, fig. 10 in Descoings, 1972); (S) *C. cornifolia* (pl. 47, fig. 12 in Descoings, 1972); (T) *C. pileata* (fig. 13.9 in Descoings, 1967); (U) *C. repens* (fig. 2B in Jackes, 1988); (V) *C. hastata* (fig. 5C in Jackes, 1988); (W) *C. diffusiflora* (pl. 44, fig. 11 in Descoings, 1972).

1483 Seeds are not to scale and are all shown in lateral view, except (O) and (P), which are shown in apical view.

1486 Fig. 7. SRXTM digital transverse sections through fruits of modern African *Cissus* using representative specimens to illustrate typical features of each seed morphotype listed in Table 1. (A) Morphotype 1, *Cissus integrifolia* Guill. & Perr. (B) Morphotype 2, *Cissus dasyantha* Gilg & M.Brandt (C) Morphotype 3, *Cissus tiliifolia* Planch. (D) Morphotype 4, *Cissus petiolata* Hook.f.. Transverse sections were obtained from near ventral part of fruits to best show features of ventral infolds and characteristics of seed coat layers. All scale bars are 1 mm.
Figure 3

A

short, deep, narrow ventral infolds

B

infolds digitally concealed
**Figure 6**

- **Cissus barbayana clade**
  Central, East & West Africa

- **Cissus integrifolia clade**
  Central, East & West Africa

- **Cissus sciaphila clade**
  Central, East & West Africa; Southeast Asia; Australia

- **Other African Cissus**

- **Cissus petiolata clade**
  Central, East, South & West Africa; Arabia

  - **C. elongata - C. subtetragona clade**
    Australasia

  - **C. pseudoguerkeana - C. albiporcata clade**
    C, E, N, S and W Africa; Arabia; SE and S Asia

- **Other African Cissus**
  (+1 Australian species)

- **Australasian Cissus**

- **Cissus diffusiflora**
  Central, East and West Africa

- **Neotropical Cissus**
  (+1 Asian species)
Appendix S1. Herbarium sheet for seed morphotypes of sixteen modern species of African *Cissus* and three species of *Cyphostemma* studied. All specimens sampled from the Royal Botanic Gardens, Kew Herbarium (K), except *C. dasyantha* (from the Botanic Gardens Meise Herbarium (BR), Belgium). References to literature illustrating seeds of these species are also provided.

<table>
<thead>
<tr>
<th>Species</th>
<th>Collector (collector number)</th>
<th>Country/ region of collection</th>
<th>Seed morphotype (see text and Table 1 for explanation of morphotypes)</th>
<th>Literature with seed Illustrations of this species</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Cissus barbeyana</em> De Wild. &amp; T.Durand</td>
<td>S. Lisowski (16406)</td>
<td>Zaire (Congo)</td>
<td>2</td>
<td>fig. 13e in Dewit and Willems (1960); pl. 42, fig. 10 in Descoings (1972)</td>
</tr>
<tr>
<td><em>Cissus bosseri</em> Desc.</td>
<td>P.B. Phillipson &amp; S. Rabesihanaka (3140)</td>
<td>Mascarene Islands</td>
<td>Unlike fossils, shares some features with Morphotype 1 but lacks ornament on lateral faces</td>
<td>fig. 10 in Descoings (1967)</td>
</tr>
<tr>
<td><em>Cissus dasyantha</em> Gilg &amp; M.Brandt</td>
<td>J. Louis (13410)</td>
<td>Congo</td>
<td>2</td>
<td>fig. 13j in Dewit and Willems (1960)</td>
</tr>
<tr>
<td><em>Cissus dinklagei</em> Gilg &amp; M.Brandt</td>
<td>A.J.M. Leeuwenberg (5207)</td>
<td>Masok (Tanzania)</td>
<td>Unlike fossils, has very variable seed morphology in floral illustrations, shares some features with Morphotype 1 but differs in that the most prominent ridge is central not marginal</td>
<td>fig. 13g in Dewit and Willems (1960); pl. 40, fig. 10 in Descoings (1972)</td>
</tr>
<tr>
<td><em>Cissus integrifolia</em> (Baker) Planch.</td>
<td>C.M. Taylor, R.E. Gereau &amp; J. Lovett (8517a)</td>
<td>Iringa (Tanzania)</td>
<td>1</td>
<td>fig. 13f in Dewit and Willems (1960)</td>
</tr>
<tr>
<td><em>Cissus integrifolia</em> (Baker) Planch.</td>
<td>C.M. Taylor, R.E. Gereau &amp; J. Lovett (8517b)</td>
<td>Iringa (Tanzania)</td>
<td>1</td>
<td>fig. 13f in Dewit and Willems (1960)</td>
</tr>
<tr>
<td><em>Cissus lebrunii</em> Dewit</td>
<td>B.A. Nkongmenec (1592)</td>
<td>Cameroon</td>
<td>3</td>
<td>fig. 13k in Dewit and Willems (1960)</td>
</tr>
<tr>
<td><em>Cissus oreophila</em> Gilg &amp; M.Brandt</td>
<td>W.J. Harley (693A)</td>
<td>Liberia</td>
<td>Unlike fossils, as this is a two-seeded specimen</td>
<td>pl. 39, fig. 11 in Descoings (1972)</td>
</tr>
<tr>
<td><em>Cissus petiolata</em> Hook.f.</td>
<td>L.P.A. &amp; W.R.Q. (9365)</td>
<td>Tanzania</td>
<td>4</td>
<td>fig. 13a in Dewit and Willems (1960); pl. 33, fig. 11 in Descoings (1972); fig. 3 in Verdcourt (1993)</td>
</tr>
<tr>
<td><em>Cissus pileata</em> Desc.</td>
<td>H. Humbert (18981)</td>
<td>Madagascar</td>
<td>Unlike fossils, shares some features with Morphotype 3, but has only very irregular ridges on the lateral faces that do not form a reticulum</td>
<td>fig. 13 in Descoings (1967)</td>
</tr>
<tr>
<td><em>Cissus planchoniana</em> Gilg</td>
<td>W. Robyns (606)</td>
<td>Congo</td>
<td>Unlike fossils, shares some features with Morphotype 2 but has long pronounced ridges radiating right across the lateral faces</td>
<td>pl. 43, fig. 11 in Descoings (1972)</td>
</tr>
<tr>
<td><em>Cissus polyantha</em> Gilg &amp; M.Brandt</td>
<td>F.C. Deighton (5208)</td>
<td>Kokoru, Gaura (Sierra Leone)</td>
<td>Unlike fossils, seeds very variable in surface ornament in floral illustrations and SRXTM videos, from smooth to extensively ridged, including specimens that fall into Morphotype 3</td>
<td>fig. 13h in Dewit and Willems (1960); pl. 36, fig. 11 in Descoings (1972)</td>
</tr>
<tr>
<td><em>Cissus polyantha</em></td>
<td>M. Sacande, L. Sanou, Burkina Faso</td>
<td></td>
<td>Unlike fossils, seeds very variable in surface</td>
<td>fig. 13h in Dewit and Willems (1960); pl. 36, fig. 11 in Descoings (1972)</td>
</tr>
<tr>
<td>Specimen</td>
<td>Study</td>
<td>Location</td>
<td>Seed Count</td>
<td>Reference</td>
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<td>----------</td>
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<tr>
<td><em>Cissus populnea</em> Guill. &amp; Perr.</td>
<td>R.A.A. Oldeman (350)</td>
<td>Tehini</td>
<td>1</td>
<td>pl. 34, fig. 10 in Descoings (1972)</td>
</tr>
<tr>
<td><em>Cissus rondoensis</em> Verdc.</td>
<td>S. Bidgood, R. Abdallah &amp; K. Vollesen (1553)</td>
<td>Tanzania</td>
<td></td>
<td>Unlike fossils, descriptions in Verdcourt (1993) state that seeds are essentially smooth but have two to three transverse ridges.</td>
</tr>
<tr>
<td><em>Cissus sciaphila</em> Gilg</td>
<td>B. Mhoro (UMBC287)</td>
<td>Tanzania</td>
<td>3</td>
<td>fig. 6 in Verdcourt (1993)</td>
</tr>
<tr>
<td><em>Cissus smithiana</em> (Baker) Planch.</td>
<td>R.P. Hulstaert (416)</td>
<td>Congo</td>
<td></td>
<td>Unlike fossils, as this is a two-seeded specimen</td>
</tr>
<tr>
<td><em>Cissus tiliifolia</em> Planch.</td>
<td>G. Eilu (240)</td>
<td>Uganda</td>
<td>3</td>
<td>n/a</td>
</tr>
<tr>
<td><em>Cyphostemma adenocaule</em> (Steud. ex A.Rich.) Desc. ex Wild &amp; R.B.Drumm.</td>
<td>H. Ern, B. Leuenberger, H. Scholz, U. Scholz &amp; W. Schwarz (1446)</td>
<td>Togo</td>
<td></td>
<td>pg. 533, fig. h in Dewit and Willems (1960); pl. 32, fig. 11 in Descoings (1972)</td>
</tr>
<tr>
<td><em>Cyphostemma maranguense</em> (Gilg) Desc.</td>
<td>C.G. Rogers (2)</td>
<td>Kenya</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Appendix S2. Comparison of tissue and cellular detail revealed by SRXTM and routine μCT in a modern fruit of Cissus populnea Guill. & Perr., where the digital transverse section (DTS) is through the part of the seed distal to the external ventral infolds. (A) SRXTM and (B) μCT DTS with labelled identifiable seed tissues (storage and chalazal tissues, testa, fruit wall), the distinction between endotesta and exotesta can be made using the SRXTM DTS but this distinction is not as clear using routine μCT. (C) SRXTM and (D) μCT DTS at higher magnification with labelled tissue layers as for (A) and (B), showing the greater cellular detail, ability to distinguish layers in the seed coat and the distribution of phases of different X-ray attenuation in the fruit in SRXTM. White boxes in (A) and (B) outline the areas shown at higher magnification in (C) and (D). Scale bars in (A) and (B) are 1 mm and in (C) and (D) are 0.5 mm.
Appendix S3. Further details of the phylogenetic analyses.

Total genomic DNA was extracted from 0.2 g of herbarium specimen material using a modified version of the 2× cetyltrimethyl ammonium bromide (CTAB) method (Doyle and Doyle, 1987). The total DNA was purified using a caesium chloride/ethidium bromide gradient (1·55 g/mL) followed by a dialysis procedure for long-term storage in the DNA & Tissue Collections at RBG Kew (http://apps.kew.org/dnabank/homepage.html). The trnL intron and trnL–F spacer were amplified in two reactions using primers c/d and e/f, respectively (Taberlet et al., 1991). The rps16 intron was amplified using the primers rps16F and rps16R (Shaw et al., 2005). The polymerase chain reactions (PCRs) for the above markers were performed in 25 μL volumes, containing 12.5 μL of DreamTaq PCR Master Mix (2x) (4 mM MgCl₂; Thermo Fisher Scientific, Waltham, Massachusetts, USA), 4 μL TBT-PAR (trehalose, bovine serum albumin (BSA), and polysorbate-20 (Tween-20); Samarakoon et al., 2013), 0.5 μL of each primer (100 ng/μL) and 1 μL of DNA template. A 'long and cold' programme was used with an initial denaturation at 80°C for 5 mins, followed by 35 cycles of 95 °C for 1 min, 50°C (trnL-F) or 48 °C (rps16) for 1 min and 65 °C for 5 mins, completed with a final extension of 65°C for 4 mins. All amplifications were performed on a 9700 GeneAmp thermocycler (ABI, Warrington, UK). All PCR products were purified with either the QIAquick PCR kit (Qiagen, Hilden, Germany) or the Nucleospin Extract II kit (Machery-Nagel, Düren, Germany), following the manufacturer protocols. Cycle sequencing reactions were performed in 5 μL reactions using 0.5 μL of BigDye® Terminator cycle sequencing chemistry (v3.1; ABI, Warrington, UK) and the same primers as for PCR. Complementary strands were sequenced on an ABI3730 automated sequencer and then assembled and aligned in Geneious (Biomatters Limited, Auckland, New Zealand). The newly produced DNA sequences are available from GenBank (see GenBank accession numbers in Appendix 1 of main paper).
Publically available sequences (Appendix 2) were downloaded using Geneious 7.0.2 (Biomatters Limited, Auckland, New Zealand) and matrices (including sequences produced for the present study) were aligned using MUSCLE (Edgar, 2004) in Geneious. Alignments used to produce the phylogeny are available at from TreeBASE (https://treebase.org; study 18491). A phylogenetic analysis was performed using the maximum likelihood criterion as implemented in the programme RAxML v8.1.24 (Stamatakis, 2014) using the rapid bootstrap algorithm with 1000 replicates and a search for the best-scoring tree. The analysis was run on the Cipres Science Gateway portal (www.phylo.org). Divergence time estimates were obtained using the Bayesian inference approach implemented in the package BEAST v.1.8.2 (Drummond and Rambaut, 2007). The plastid regions were combined and the GTR+I+G evolutionary model was applied to the resulting matrix. An uncorrelated relaxed molecular clock with a lognormal distribution of rates and a birth-death speciation model were used. Two analyses were run on the Cipres Science Gateway portal (www.phylo.org) for 50 million generations each, sampling one tree every 1000th generation. Parameter convergence was confirmed using the software Tracer v1.6 (Rambaut et al., 2014). Following the combination of these two runs using LogCombiner v1.8.2 (Drummond and Rambaut, 2007), a maximum clade credibility tree with median branch lengths and 95% highest posterior density (HPD) interval on nodes was reconstructed using TreeAnnotator 1.8.2 (Drummond and Rambaut, 2007), after an initial burn-in period of five million generations had been removed (10%).
Appendix S4. Seed morphotypes for all seeds from clades within core *Cissus* (Fig. 5) containing African species (fifty-two species in total); those highlighted in grey were studied by SRXTM.

<table>
<thead>
<tr>
<th>Species</th>
<th>Clade in phylogeny (Figs. 5 &amp; 6)</th>
<th>Seed morphology</th>
<th>Seed illustration and/or description in literature</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Cissus adnata</em> Roxb.</td>
<td><em>C. sciaphila</em> clade</td>
<td>3</td>
<td>fig. 3B and 3C and p. 489 in Jackes (1988) p. 701 in Lu (1993); fig. 172.7 and 172.8 and p. 185 in Chen et al. (2007)</td>
</tr>
<tr>
<td><em>Cissus albioporcarata</em> Masinde &amp; L.E.Newton</td>
<td><em>C. pseudoguerkeana-</em> <em>C. albioporcarata</em> clade</td>
<td>no published illustration or description found</td>
<td></td>
</tr>
<tr>
<td><em>Cissus annamica</em> Gagnep.</td>
<td><em>C. elongata-</em> <em>C. subtetragona</em> clade</td>
<td>no published illustration or description found</td>
<td></td>
</tr>
<tr>
<td><em>Cissus aphyllantha</em> Gilg</td>
<td><em>C. sciaphila</em> clade</td>
<td>3</td>
<td>p. 38 in Verdcourt (1993)</td>
</tr>
<tr>
<td><em>Cissus aralioides</em> (Welw. ex Baker) Planch.</td>
<td><em>C. petiolata</em> clade</td>
<td>4</td>
<td>fig. 13C and p. 548 in Dewit and Willems (1960); pl. 30, figs. 10-12 and p. 88 in Descoings (1972); fig. 11.10 and p. 47 in Verdcourt (1993)</td>
</tr>
<tr>
<td><em>Cissus auricoma</em> Desc.</td>
<td><em>C. microdonta-</em> <em>C. auricoma</em> clade</td>
<td>3</td>
<td>figs. 12.6 and 12.7 and p. 89 in Descoings (1967)</td>
</tr>
<tr>
<td><em>Cissus barbeyana</em> De Wild. &amp; T.Durand</td>
<td><em>C. barbeyana</em> clade</td>
<td>2</td>
<td>fig. 13E and p. 525 in Dewit and Willems (1960); pl. 42, fig. 10 and p. 118 in Descoings (1972)</td>
</tr>
<tr>
<td><em>Cissus bosseri</em> Desc.</td>
<td><em>C. microdonta-</em> <em>C. auricoma</em> clade</td>
<td>unlike fossils</td>
<td>fig. 10.8 and p. 83-84 in Descoings (1967)</td>
</tr>
<tr>
<td><em>Cissus cactiformis</em> Gilg</td>
<td><em>C. pseudoguerkeana-</em> <em>C. albioporcarata</em> clade</td>
<td>4</td>
<td>fig. 9.8 and p. 40 in Verdcourt (1993)</td>
</tr>
<tr>
<td><em>Cissus cornifolia</em> (Baker) Planch.</td>
<td><em>C. cornifolia-</em> <em>C. trothae</em> clade</td>
<td>unlike fossils</td>
<td>p. 519 in Dewit and Willems (1960); pl. 47, figs. 11-12 and p. 132 in Descoings (1972); fig. 8.9 and p. 36 in Verdcourt (1993)</td>
</tr>
<tr>
<td><em>Cissus dasyantha</em> Gilg &amp; M.Brandt</td>
<td>unknown</td>
<td>2</td>
<td>fig. 13J and p. 543 in Dewit and Willems (1960)</td>
</tr>
<tr>
<td><em>Cissus diffusa</em> (Miq.) Amshoff</td>
<td><em>C. elongata-</em> <em>C. subtetragona</em> clade</td>
<td>no published illustration or description found</td>
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</tr>
<tr>
<td><em>Cissus diffusiflora</em> (Baker) Planch.</td>
<td><em>C. diffusiflora</em> clade</td>
<td>unlike fossils</td>
<td>p. 528-529 in Dewit and Willems (1960); pl. 44, figs. 11-12 and p. 124 in Descoings (1972); p. 24 in Verdcourt (1993)</td>
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<tr>
<td><em>Cissus dinklagei</em> Gilg &amp; M.Brandt</td>
<td>unknown</td>
<td>variable seed morphology</td>
<td>fig. 13G and p. 515 in Dewit and Willems (1960); pl. 40, fig. 10 and p. 114 in Descoings (1972)</td>
</tr>
<tr>
<td><em>Cissus elongata</em> Roxb.</td>
<td><em>C. elongata-</em> <em>C. subtetragona</em> clade</td>
<td>4</td>
<td>fig. 171.10 and p. 186 in Chen et al. (2007)</td>
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<td>Species</td>
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</tr>
<tr>
<td>---------------------------------</td>
<td>------------------------------------------</td>
<td>-------------------------------</td>
<td></td>
</tr>
<tr>
<td>Cissus floribunda (Baker) Planch.</td>
<td>C. barbeyana clade</td>
<td>figs. 15.12 and 15.13 and p. 136 in Descoings (1967)</td>
<td></td>
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<tr>
<td>Cissus integrifolia (Baker) Planch.</td>
<td>C. integrifolia clade</td>
<td>fig. 13F and p. 514 in Dewit and Willems (1960); p. 16 in Verdcourt (1993)</td>
<td></td>
</tr>
<tr>
<td>Cissus javana DC.</td>
<td>C. elongata-C. subtetragona clade</td>
<td>unlike fossils</td>
<td></td>
</tr>
<tr>
<td>Cissus lanea Desc.</td>
<td>C. microdonta-C. auricoma clade</td>
<td>no published illustration or description found</td>
<td></td>
</tr>
<tr>
<td>Cissus lebrunii Dewit</td>
<td>unknown</td>
<td>fig. 13K and p. 543 in Dewit and Willems (1960)</td>
<td></td>
</tr>
<tr>
<td>Cissus madecassa Desc.</td>
<td>C. microdonta-C. auricoma clade</td>
<td>figs. 13.4 and 13.5 and p. 112 in Descoings (1967)</td>
<td></td>
</tr>
<tr>
<td>Cissus microdonta (Baker) Planch.</td>
<td>C. microdonta-C. auricoma clade</td>
<td>figs. 15.4 and 15.5 and p. 100 in Descoings (1967)</td>
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<tr>
<td>Cissus oreophila Gilg &amp; M.Brandt</td>
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<td>pl. 39, fig. 11 and p. 112 in Descoings (1972)</td>
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<tr>
<td>Cissus pentaclada Jackes</td>
<td>C. cornifolia-C. trothae clade</td>
<td>fig. 3D and p. 491 in Jackes (1988)</td>
<td></td>
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<tr>
<td>Cissus petiolata Hook.f.</td>
<td>C. petiolata clade</td>
<td>fig. 13A and p. 515 in Dewit and Willems (1960); pl. 33, fig. 11 and p. 97 in Descoings (1972); fig. 3.9 and p. 16 in Verdcourt (1993)</td>
<td></td>
</tr>
<tr>
<td>Cissus phymatocarpa Masinde &amp; L.E.Newton</td>
<td>C. pseudoguerkeana-C. albiporcata clade</td>
<td>no published illustration or description found</td>
<td></td>
</tr>
<tr>
<td>Cissus pileata Desc.</td>
<td>C. cornifolia-C. trothae clade</td>
<td>figs. 13.9 and 13.10 and p. 119 in Descoings (1967)</td>
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<tr>
<td>Cissus planchoniana Gilg</td>
<td>unknown</td>
<td>p. 520 in Dewit and Willems (1960); pl. 43, figs. 11-12 and p. 120 in Descoings (1972); p. 23-24 in Verdcourt (1993)</td>
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<tr>
<td>Cissus polita Desc.</td>
<td>C. petiolata clade</td>
<td>no published illustration or description found</td>
<td></td>
</tr>
<tr>
<td>Cissus polyantha Gilg &amp; M.Brandt</td>
<td>C. sciaphila clade</td>
<td>variable seed morphology, including specimen like Morphotype 3</td>
<td></td>
</tr>
<tr>
<td>Cissus populnea Guill. &amp; Perr.</td>
<td>C. integrifolia clade</td>
<td>p. 531 in Dewit and Willems (1960); pl. 34, figs. 10 and 11 and p. 100 in Descoings (1972); p. 19 in Verdcourt (1993)</td>
<td></td>
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<tr>
<td>Species</td>
<td>Clade</td>
<td>Status</td>
<td>References</td>
</tr>
<tr>
<td>---------------------------------------------</td>
<td>--------------------------------------</td>
<td>------------------------------------------------------------------------</td>
<td>---------------------------------------------------------------------------</td>
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<tr>
<td><em>Cissus producta</em> Afzel.</td>
<td><em>C. cornifolia-C. trothae</em> clade</td>
<td>unlike fossils</td>
<td>fig. 13B and p. 524 in Dewit and Willems (1960); pl. 46, fig. 10 and p. 130 in Descoings (1972); p. 22 in Verdcourt (1993)</td>
</tr>
<tr>
<td><em>Cissus pseudoguerkeana</em> Verdc.</td>
<td><em>C. pseudoguerkeana-C. albiporcarta</em> clade</td>
<td>no published illustration or description found</td>
<td></td>
</tr>
<tr>
<td><em>Cissus quadrangularis</em> L.</td>
<td><em>C. pseudoguerkeana-C. albiporcarta</em> clade</td>
<td>4</td>
<td>p. 513 in Dewit and Willems (1960); p. 91 in Descoings (1967); pl. 29, figs. 10 and 11 and p. 87 in Descoings (1972); p. 41 in Verdcourt (1993)</td>
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<tr>
<td><em>Cissus quarrei</em> Dewit</td>
<td><em>C. sciaphila</em> clade</td>
<td>3</td>
<td>p. 537 in Dewit and Willems (1960); p. 32 in Verdcourt (1993)</td>
</tr>
<tr>
<td><em>Cissus reniformis Domin</em></td>
<td><em>C. elongata-C. subtetragona</em> clade</td>
<td>4</td>
<td>figs. 2F-G in Jackes (1988)</td>
</tr>
<tr>
<td><em>Cissus rhodotricha</em> (Baker) Desc.</td>
<td><em>C. sciaphila</em> clade</td>
<td>3</td>
<td>figs. 12.15 and 12.16 and p. 116 in Descoings (1967)</td>
</tr>
<tr>
<td><em>Cissus rostrata</em> (Miq.) Korth. ex Planch.</td>
<td><em>C. sciaphila</em> clade</td>
<td>3</td>
<td>p. 326 in Yeo et al. (2012)</td>
</tr>
<tr>
<td><em>Cissus rotundifolia</em> Vahl</td>
<td><em>C. petiolata</em> clade</td>
<td>4</td>
<td>p. 519 in Dewit and Willems (1960); p. 21 in Verdcourt (1993)</td>
</tr>
<tr>
<td><em>Cissus rubiginosa</em> (Welw. ex Baker) Planch.</td>
<td><em>C. cornifolia-C. trothae</em> clade</td>
<td>unlike fossils</td>
<td>pl. 53, fig. E and p. 540 in Dewit and Willems (1960); pl. 31, figs. 12 and 13 and p. 92 in Descoings (1972); fig. 4E and p. 26 in Verdcourt (1993)</td>
</tr>
<tr>
<td><em>Cissus sagittifera</em> Desc.</td>
<td><em>C. barbeyana</em> clade</td>
<td>2</td>
<td>figs. 14.8 and 14.9 and p. 130 in Descoings (1967)</td>
</tr>
<tr>
<td><em>Cissus sciaphila</em> Gilg</td>
<td><em>C. sciaphila</em> clade</td>
<td>3</td>
<td>figs. 5.10 and 6.1 and p. 30 in Verdcourt (1993)</td>
</tr>
<tr>
<td><em>Cissus smithiana</em> (Baker) Planch.</td>
<td><em>C. sciaphila</em> clade</td>
<td>3</td>
<td>pl. 52, fig. H and p. 534 in Dewit and Willems (1960); pl. 32, fig. 11 and p. 94 in Descoings (1972)</td>
</tr>
<tr>
<td><em>Cissus subtetragona</em> Planch.</td>
<td><em>C. elongata-C. subtetragona</em> clade</td>
<td>4</td>
<td>fig. 171.2 and p. 185 in Chen et al. (2007)</td>
</tr>
<tr>
<td><em>Cissus tiliifolia</em> Planch.</td>
<td><em>C. sciaphila</em> clade</td>
<td>3</td>
<td>p. 35 in Verdcourt (1993)</td>
</tr>
<tr>
<td><em>Cissus trothae</em> Gilg &amp; M.Brandt</td>
<td><em>C. cornifolia-C. trothae</em> clade</td>
<td>no published illustration or description found</td>
<td></td>
</tr>
</tbody>
</table>
Appendix S5. List of herbarium sheets used to provide habit and habitat data for modern *Cissus* species (source 4 in Table 2 of main paper). Herbarium abbreviations: DSM (University of Dar es Salam Herbarium, Tanzania); G (Geneva General Herbarium); IFAN (Institut Fondamental d’Afrique Noire Herbarium); K (Royal Botanic Gardens, Kew Herbarium); L (National Herbarium of the Netherlands at Leiden University); MO (Missouri Botanical Garden Herbarium); P (Muséum National d’Histoire Naturelle Herbarium, Paris); TAN (Tsimbazaza Herbarium, Madagascar).

<table>
<thead>
<tr>
<th>Species</th>
<th>Habit</th>
<th>Habitat</th>
<th>Region/ Country of Collection</th>
<th>Collector (Collection Number)</th>
<th>Herbarium (Herbarium Barcode)</th>
<th>Accession Number</th>
<th>Web Link</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Cissus adnata</em> Roxb.</td>
<td>Scrambler</td>
<td>Remnant primary forest on south facing slope</td>
<td>Yunnan, China</td>
<td>Li Heng (9095)</td>
<td>(Unknown)</td>
<td>(Unknown)</td>
<td><a href="http://www.tropicos.org/Specimen/1402887">http://www.tropicos.org/Specimen/1402887</a></td>
</tr>
<tr>
<td>(Unknown)</td>
<td>Forest margin</td>
<td>Yunnan, China</td>
<td>Zhan Huo Tsi (91-109)</td>
<td>MO (MO-162633)</td>
<td>4250721</td>
<td><a href="http://www.tropicos.org/Specimen/1344197">http://www.tropicos.org/Specimen/1344197</a></td>
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</tr>
<tr>
<td>Climber</td>
<td>Riparian forest</td>
<td>Sarawak, Malaysia</td>
<td>Abang Mohtar et al. (S49581)</td>
<td>MO (MO-162639)</td>
<td>3844467</td>
<td><a href="http://www.tropicos.org/Specimen/1344238">http://www.tropicos.org/Specimen/1344238</a></td>
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</tr>
<tr>
<td>Climbing woody vine (stem very corky)</td>
<td>Semi-open scrub forest</td>
<td>Sri Lanka</td>
<td>F.R. Fosberg et al. (50818)</td>
<td>MO (MO-162634)</td>
<td>2213855</td>
<td><a href="http://www.tropicos.org/Specimen/1344199">http://www.tropicos.org/Specimen/1344199</a></td>
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<tr>
<td>(Unknown)</td>
<td>Deciduous forest</td>
<td>Northern Thailand</td>
<td>T. Shimizu et al. (T10433)</td>
<td>MO (MO-162637)</td>
<td>2580082</td>
<td><a href="http://www.tropicos.org/Specimen/1344231">http://www.tropicos.org/Specimen/1344231</a></td>
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</tr>
<tr>
<td>Woody vine (10-15m long)</td>
<td>Primary evergreen seasonal broad-leaved closed lowland forest on very steep slopes</td>
<td>Thanh Hoa, Vietnam</td>
<td>L.V. Averyanov, P.K. Lôc, D.T. Doan &amp; N.T. Vinh (3880)</td>
<td>MO (Unknown)</td>
<td>(Unknown)</td>
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</tr>
<tr>
<td>Vine (common)</td>
<td>Primary evergreen seasonal broad-leaved closed lowland forest on very steep slopes</td>
<td>Thanh Hoa, Vietnam</td>
<td>L.V. Averyanov, P.K. Lôc, D.T. Doan &amp; N.T. Vinh (3939)</td>
<td>MO (Unknown)</td>
<td>(Unknown)</td>
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<tr>
<td>(Unknown)</td>
<td>Monsoon forest</td>
<td>Sumbawa, Indonesia</td>
<td>J. Elbert (3667)</td>
<td>L (L 0746186)</td>
<td>L.2334734</td>
<td><a href="http://medialib.naturalis.nl/file/id/L.2334734/format/large">http://medialib.naturalis.nl/file/id/L.2334734/format/large</a></td>
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</tr>
<tr>
<td>Evergreen woody climber</td>
<td>Open, disturbed thicket along a seasonal stream with secondary growth in a destroyed primary, evergreen, seasonal, hardwood forest</td>
<td>Chiang Mai, Thailand</td>
<td>J.F. Maxwell (01-295)</td>
<td>L (L 0761745)</td>
<td>L.3928867</td>
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<td></td>
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<tr>
<td>Woody climber</td>
<td>Open, degraded, disturbed, fire-prone</td>
<td>Champasak, Laos</td>
<td>J.F. Maxwell (98-1041)</td>
<td>L (L 0762737)</td>
<td>L.4254525</td>
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<tr>
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<td>L No.</td>
<td>Reference</td>
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<td>---------------------------------------------------------------------------</td>
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<tr>
<td>Climber Riparian forest</td>
<td>thicket bordering deciduous secondary growth forest</td>
<td>Borneo, Indonesia</td>
<td>A. Kostermans</td>
<td>L (L 0672603)</td>
<td>L.2334780 <a href="http://medialib.naturalis.nl/file/id/L.2334780/format/large">http://medialib.naturalis.nl/file/id/L.2334780/format/large</a></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Climbing-scrambling vine</td>
<td>Open areas in evergreen forest and open rocky thickets</td>
<td>Saraburi, Thailand</td>
<td>J.F. Maxwell</td>
<td>L (L 0194871)</td>
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<td>Deciduous woody climber</td>
<td>Open degraded mixed deciduous secondary growth, margins of evergreen and deciduous seasonal forest, canopy to 25m</td>
<td>Ubon Ratchathani, Thailand</td>
<td>Martin Greijmans</td>
<td>L (L 0761507)</td>
<td>L.3928674 <a href="http://medialib.naturalis.nl/file/id/L.3928674/format/large">http://medialib.naturalis.nl/file/id/L.3928674/format/large</a></td>
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<td>Vine Thicket</td>
<td>deciduous secondary growth, margins of evergreen and deciduous seasonal forest, canopy to 25m</td>
<td>Saraburi, Thailand</td>
<td>J.F. Maxwell</td>
<td>L (L 0761883)</td>
<td>L.4254130 <a href="http://medialib.naturalis.nl/file/id/L.4254130/format/large">http://medialib.naturalis.nl/file/id/L.4254130/format/large</a></td>
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<td>Creeper Dipterocarp forest</td>
<td>mixed lowland forest</td>
<td>Borneo, Indonesia</td>
<td>M.M.J. van Balgooy</td>
<td>L (L 0672608)</td>
<td>L.2334775 <a href="http://medialib.naturalis.nl/file/id/L.2334775/format/large">http://medialib.naturalis.nl/file/id/L.2334775/format/large</a></td>
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<td>Slender climber Mixed lowland forest</td>
<td>Much disturbed primary forest ca. 40m high, on alluvial soil near a river. Terrain flat to gently sloping.</td>
<td>Sulawesi, Indonesia</td>
<td>E.F. de Vogel</td>
<td>L (L 0483122)</td>
<td>L.2334730 <a href="http://medialib.naturalis.nl/file/id/L.2334730/format/large">http://medialib.naturalis.nl/file/id/L.2334730/format/large</a></td>
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<td>Solitary climber, 1m high</td>
<td>Woody climber Partly open, alluvial area along a small stream in degraded mixed evergreen and deciduous hardwood forest with much bamboo, in overall deciduous dipterocarp-oak and pine, seasonal forest</td>
<td>Chiang Mai, Thailand</td>
<td>J.F. Maxwell</td>
<td>L (L 0762949)</td>
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<td>Shrubby creeper Sandstone outcrop in broadleaf scrub</td>
<td>much disturbed primary forest ca. 40m high, on alluvial soil near a river. Terrain flat to gently sloping.</td>
<td>Northern Territory, Australia</td>
<td>Martensz &amp; Schodde</td>
<td>L (L 0746142)</td>
<td>L.2334721 <a href="http://medialib.naturalis.nl/file/id/L.2334721/format/large">http://medialib.naturalis.nl/file/id/L.2334721/format/large</a></td>
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<td>Climber (5 m)</td>
<td>Forest edge with clearing in remnant primary forest</td>
<td>Sumatra, Indonesia</td>
<td>W.J.J.O. de Wilde and B.E.E. de Wilde-Duyfjes</td>
<td>L (L 0746262)</td>
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<td>Climber (ca. 8m)</td>
<td>Forest edge in primary</td>
<td>Sumatra, Indonesia</td>
<td>W.J.J.O. de Wilde</td>
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<td>Phitsanulok, Thailand</td>
<td>Mixed deciduous forest</td>
<td>Kai Larsen, T. Smitinand &amp; E. Warncke (877)</td>
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<td>Sumbawa, Indonesia</td>
<td>Monsoon forest, busy and light</td>
<td>J. Elbert (4204)</td>
<td>L0746182</td>
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<td>Ranong, Thailand</td>
<td>Bushes by forest stream</td>
<td>A.F.G. Kerr (16877)</td>
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<td>Sulawesi, Indonesia</td>
<td>Solitary climber, 8m high</td>
<td>E.F. de Vogel (5592)</td>
<td>L0483124</td>
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<td>Kanchanaburi, Thailand</td>
<td>Partly shaded areas in the mixed evergreen &amp; deciduous forest, base of limestone cliffs, rugged limestone terrain</td>
<td>J.F. Maxwell (93-923)</td>
<td>L0762963</td>
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<td>Luzon, Philippines</td>
<td>Vine</td>
<td>H.G. Gutierrez (61-75)</td>
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<td>Borneo, Malaysia</td>
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<td>Abang Mohtar et al. (S.49581)</td>
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<td>Antananarivo, Rainforest</td>
<td>Cissus Vine</td>
<td>L.C. Barnett</td>
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| floribunda  
(Baker) Planch. | Forest | Madagascar | Laurence J. Dorr (187) | (MO-163568) | /177642 |
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<td>Tropical moist forest</td>
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<td>George E. Schatz (2405)</td>
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<td>Liana</td>
<td>Rainforest towards a small lake</td>
<td>Antsiranana, Madagascar</td>
<td>Armand Rakotozafy &amp; Jeannine I. Raharilala (2295)</td>
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<td>Liana (4m in height)</td>
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<td>C. Rakotovao &amp; et al. (3148)</td>
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<td>Liana</td>
<td>Dense rainforest</td>
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<td>N.M. Andrianjafy &amp; et al. (670)</td>
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<td>Slender liana (to ca. 10m long)</td>
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<td>S.T. Malcomber (1288)</td>
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<td>Cissus oreophila Gilg &amp; M.Brandt</td>
<td>Climber</td>
<td>Riverine forest border</td>
<td>Gabon</td>
<td>Adriaan M. Louis (1019)</td>
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<td>(Unknown)</td>
<td>Forest regrowth remains on the edge of a Sphagnum pond</td>
<td>Brazzaville, Congo</td>
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<td>Liana</td>
<td>Swamp region</td>
<td>Brazzaville, Congo</td>
<td>Bernard M. Descoings (6988)</td>
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<td>Herbaceous vine (20-25m)</td>
<td>Dense primary forest with large trees and vines</td>
<td>Equatorial Guinea</td>
<td>Manuel Fidalgo de Carvalho (3596)</td>
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<td>Climber</td>
<td>Forest edge</td>
<td>Ivory Coast</td>
<td>J. Bokdam (2785)</td>
<td>MO (MO-163217)</td>
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<td>Creeping herb</td>
<td>Secondary forest</td>
<td>Ivory Coast</td>
<td>C. Geerling &amp; J. Bokdam (2471)</td>
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<td>(Unknown)</td>
<td>Savanna edge and hill forest regrowth</td>
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<td><strong>Cissus polita</strong></td>
<td><strong>Desc.</strong></td>
<td>(Unknown) Hills and limestone plateau, seasonal deciduous forest</td>
<td>Antsiranana, Madagascar</td>
<td>H. Humbert (19215)</td>
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<td>Liana (3m)</td>
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<td>F. Ratoivoson (1234)</td>
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<td>Liana (3m height)</td>
<td>Dry forest on sandy soil</td>
<td>Antsiranana, Madagascar</td>
<td>Andriamalala Rakotondrafara (400)</td>
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<td>Liana</td>
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<td>Stéphan Rakotonandrasana (938)</td>
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<td>Climbing shrub</td>
<td>Rocky sandstone woodland</td>
<td>Antsiranana, Madagascar</td>
<td>H. Perrier de la Bâthie (6185)</td>
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<td>Liana (1.5m)</td>
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<td>L. Nusbaumer &amp; P. Ranirison (1128)</td>
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<td>Liana</td>
<td>Savanna grassland with residual groves of dry deciduous forest</td>
<td>Antsiranana, Madagascar</td>
<td>M. Bardot-Vaucoulon &amp; G. Véné (1607)</td>
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<td><strong>Cissus rondoensis</strong></td>
<td>Herbaceous climber to ca. 4m</td>
<td>Moist evergreen forest</td>
<td>Lindi, Tanzania</td>
<td>G. Sally Bidgood, R.D. Abdallah &amp; K.B. Vollesen (1553)</td>
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<td>Semi-woody climber to 3m tall</td>
<td>Dense forest thicket</td>
<td>Lindi, Tanzania</td>
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<td>Liana</td>
<td>Secondary forest</td>
<td>North Maluku, Indonesia</td>
<td>Tjut Jul Fatisa Bangun, Mary Merello, Iska Gushilman, Idris Haris &amp; Roji Mahroji (11)</td>
<td>MO (MO-2702487)</td>
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<td>Liana (at 1m high)</td>
<td>Disturbed forest</td>
<td>North Maluku, Indonesia</td>
<td>Lalao Andriamahefarivo, Deby Aritiani, Richard Razakamalala &amp; Bahar Fabanyo (137)</td>
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<td>Climber</td>
<td>Foothill primary rain forest</td>
<td>Negeri Sembilan, Malaysia</td>
<td>Eric Gardette (E.G 2112)</td>
<td>L (L 0761437)</td>
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<th>Climber</th>
<th>Secondary forest, flatland</th>
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<td>Aban Gibot (94480)</td>
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<td>Scrambling shrub</td>
<td>River banks in rainforest seral growths</td>
<td>New Guinea, Indonesia</td>
<td>L.J. Brass (13064)</td>
<td>L (L 0745287)</td>
<td>L.2333803</td>
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<td>Vine</td>
<td>Secondary growth in area intermediate between heath and dipterocarpaceous forests</td>
<td>Borneo, Malaysia</td>
<td>Ding Hou (391)</td>
<td>L (L 0672375)</td>
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<td>Tall woody climber (30 feet)</td>
<td>River bank in disturbed primary forest</td>
<td>Borneo, Malaysia</td>
<td>P.S. Ashton (S.18398)</td>
<td>L (L 0672365)</td>
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<td>Climber (6m tall)</td>
<td>Forest edge in primary peat swamp and marshy forest on flat land</td>
<td>Sumatra, Indonesia</td>
<td>W.J.J.O. de Wilde and B.E.E. de Wilde-Duyfjes (20538)</td>
<td>L (L 0745311)</td>
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<td>Climber</td>
<td>Rainforest (Eucalyptus deglupta dominant)</td>
<td>Sulawesi, Indonesia</td>
<td>M.M.J. van Balgooy (3421)</td>
<td>L (L 0745292)</td>
<td>L.2333797</td>
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<td>F.H. Endert (3387)</td>
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<td>Soft climber</td>
<td>Riverside vegetation in mixed lowland forest</td>
<td>Borneo, Brunei</td>
<td>M.J.E. Coode (6443)</td>
<td>L (L 0672388)</td>
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<tr>
<td>Twining climber</td>
<td>Lowland dipterocarp primary rain forest</td>
<td>Negeri Sembilan, Malaysia</td>
<td>Eric Gardette (E.G 1643)</td>
<td>L (L 0762757)</td>
<td>L.4254436</td>
<td><a href="http://medialib.naturalis.nl/file/id/L.4254436/format/large">http://medialib.naturalis.nl/file/id/L.4254436/format/large</a></td>
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<tr>
<td>Climber</td>
<td>Secondary regrowth forest in disturbed lowland rainforest</td>
<td>Borneo, Malaysia</td>
<td>C.E. Ridsdale (2130)</td>
<td>L (L 0672384)</td>
<td>L.2333826</td>
<td><a href="http://medialib.naturalis.nl/file/id/L.2333826/format/large">http://medialib.naturalis.nl/file/id/L.2333826/format/large</a></td>
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<tr>
<td>Climber (4m long)</td>
<td>Riverside in forest</td>
<td>Borneo, Malaysia</td>
<td>Asik Mantor (118747)</td>
<td>L (L 0672382)</td>
<td>L.2333828</td>
<td><a href="http://medialib.naturalis.nl/file/id/L.2333828/format/large">http://medialib.naturalis.nl/file/id/L.2333828/format/large</a></td>
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<tr>
<td>Climber (20m above ground)</td>
<td>Lowland mixed forest</td>
<td>Palawan, Philippines</td>
<td>D.D. Soejarto, D.A. Madulid, F. Gaerian, E. Sagcal, O. Fernando (8861)</td>
<td>L (L 0761399)</td>
<td>L.3298568</td>
<td><a href="http://medialib.naturalis.nl/file/id/L.3298568/format/large">http://medialib.naturalis.nl/file/id/L.3298568/format/large</a></td>
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<tr>
<td>Climber</td>
<td>Disturbed open area on evergreen forest margins</td>
<td>Singapore</td>
<td>J.F. Maxwell (83-9)</td>
<td>L (L 0745318)</td>
<td>L.2333866</td>
<td><a href="http://medialib.naturalis.nl/file/id/L.2333866/format/large">http://medialib.naturalis.nl/file/id/L.2333866/format/large</a></td>
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<tr>
<td>Climber</td>
<td>River side in secondary forest</td>
<td>Borneo, Malaysia</td>
<td>A.A. Nordin (86041)</td>
<td>L (L 0672367)</td>
<td>L.2333843</td>
<td><a href="http://medialib.naturalis.nl/file/id/L.2333843/format/large">http://medialib.naturalis.nl/file/id/L.2333843/format/large</a></td>
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<tr>
<td>Climber</td>
<td>Undergrowth on open bank of a stream</td>
<td>New Guinea, Indonesia</td>
<td>L.J. Brass (12397)</td>
<td>L (L 0745286)</td>
<td>L.2333804</td>
<td><a href="http://medialib.naturalis.nl/file/id/L.2333804/format/large">http://medialib.naturalis.nl/file/id/L.2333804/format/large</a></td>
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<tr>
<td>Climber (to 60 feet)</td>
<td>Near a stream in primary forest</td>
<td>Borneo, Malaysia</td>
<td>J.A.R. Anderson and Ilias bin Paie (S.28316)</td>
<td>L (L 0672373)</td>
<td>L.2333836</td>
<td><a href="http://media.lib.naturalis.nl/file/id/L.2333836/format/large">http://media.lib.naturalis.nl/file/id/L.2333836/format/large</a></td>
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<tr>
<td>Climber (6 m)</td>
<td>Primary riverine forest</td>
<td>Borneo, Indonesia</td>
<td>J.P. Moga and W.J.J.O. de Wilde (3766)</td>
<td>L (L 0672370)</td>
<td>L.2333840</td>
<td><a href="http://media.lib.naturalis.nl/file/id/L.2333840/format/large">http://media.lib.naturalis.nl/file/id/L.2333840/format/large</a></td>
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<tr>
<td>Climber</td>
<td>Undulating disturbed forest</td>
<td>Borneo, Malaysia</td>
<td>Maidil Amin &amp; Matin Amin (69988)</td>
<td>L (L 0672386)</td>
<td>L.2333824</td>
<td><a href="http://media.lib.naturalis.nl/file/id/L.2333824/format/large">http://media.lib.naturalis.nl/file/id/L.2333824/format/large</a></td>
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<tr>
<td>Climber</td>
<td>Riverine forest</td>
<td>Moluccas, Indonesia</td>
<td>M.M.J. van Balgooy (4676)</td>
<td>L (L 0745289)</td>
<td>L.2333800</td>
<td><a href="http://media.lib.naturalis.nl/file/id/L.2333800/format/large">http://media.lib.naturalis.nl/file/id/L.2333800/format/large</a></td>
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<tr>
<td>Climber (2m high)</td>
<td>Mixed dipterocarp forest</td>
<td>Borneo, Malaysia</td>
<td>H. Othman, Rantai &amp; Jugal (56497)</td>
<td>L (L 0761832)</td>
<td>L.4254181</td>
<td><a href="http://media.lib.naturalis.nl/file/id/L.4254181/format/large">http://media.lib.naturalis.nl/file/id/L.4254181/format/large</a></td>
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<tr>
<td>Climber</td>
<td>Riverside of fast flowing river with steep banks, almost precipitous in places, in primary forest</td>
<td>Borneo, Malaysia</td>
<td>J.A.R. Anderson &amp; Ilias bin Paie (S.28876)</td>
<td>L (L 0672374)</td>
<td>L.2333835</td>
<td><a href="http://media.lib.naturalis.nl/file/id/L.2333835/format/large">http://media.lib.naturalis.nl/file/id/L.2333835/format/large</a></td>
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<tr>
<td>Climber (ca. 5m long)</td>
<td>Forest hillsides</td>
<td>Borneo, Malaysia</td>
<td>T. Sawan (125281)</td>
<td>L (L 0762871)</td>
<td>L.4254355</td>
<td><a href="http://media.lib.naturalis.nl/file/id/L.4254355/format/large">http://media.lib.naturalis.nl/file/id/L.4254355/format/large</a></td>
</tr>
<tr>
<td>Climber</td>
<td>Lowland dipterocarp primary rain forest</td>
<td>Negeri Sembilan, Malaysia</td>
<td>Eric Gardette (E.2255)</td>
<td>L (L 0762759)</td>
<td>L.4254438</td>
<td><a href="http://media.lib.naturalis.nl/file/id/L.4254438/format/large">http://media.lib.naturalis.nl/file/id/L.4254438/format/large</a></td>
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<td>Cissus sagittifera</td>
<td>(Unknown)</td>
<td>Cracks and fissures in quartzite</td>
<td>Fianarantsoa, Madagascar</td>
<td>P (Unknown)</td>
<td>(Unknown)</td>
<td><a href="http://www.tropicos.org/Specimen/177505">http://www.tropicos.org/Specimen/177505</a></td>
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<tr>
<td>Climbing liana</td>
<td>Woodland edge</td>
<td>Mahajanga, Madagascar</td>
<td>H. Perrier de la Bâthie (12540)</td>
<td>P (Unknown)</td>
<td>(Unknown)</td>
<td><a href="http://www.tropicos.org/Specimen/177497">http://www.tropicos.org/Specimen/177497</a></td>
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<tr>
<td>(Unknown)</td>
<td>Base of a limestone cliff</td>
<td>Toliara, Madagascar</td>
<td>Bernard M. Descoings (2443)</td>
<td>MO (MO-163554)</td>
<td>2211717</td>
<td><a href="http://www.tropicos.org/Specimen/1354431">http://www.tropicos.org/Specimen/1354431</a></td>
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Appendix S6. Definitions of lateral, ventral, dorsal, basal, apical views and the dorsiventral dimension, height and width of the seeds used in the systematic descriptions of the fossils.
Appendix S7. Phylogenetic tree of the Vitaceae family obtained using the maximum likelihood criteria, as implemented in RAxML (Stamatakis, 2014), based on plastic DNA sequence data of Vitaceae, with a particular focus on genus Cissus. Bootstrap values and posterior probability values are indicated on the nodes. Positions of the core Cissus (see Fig. 5 in the main text) and Cissus striata clades are indicated, and the Cissus trianae clade of Rodrigues et al. (2014) is only weakly supported in this tree. Scale bar shows degree of genetic change (nucleotide substitutions per site) as distance on the phylogram.
Appendix S8. Chronogram of the Vitaceae family obtained using the Bayesian approach implemented in the software BEAST with two Vitaceae fossils as calibration points (indicated by asterisks; see text for details), with a particular focus on genus Cissus. Ages in millions of years (Ma) are indicated on the nodes. Scale is in millions of years.
Appendix S9. Chronogram of the core *Cissus* clade obtained using the Bayesian approach implemented in the software BEAST with the fossil *Cissus* from the Oligocene Belen flora used as a calibration point (indicated by asterisk; see text for details). Ages in millions of years (Ma) are indicated on the nodes. Scale is in millions of years.
Appendix S10. Maximum credibility tree obtained for the Vitaceae family from the Bayesian analysis as implemented in the software BEAST. Posterior probability values are indicated at the nodes. Scale is in millions of years.
Appendix S11. Chronogram obtained using the Bayesian approach implemented in the software BEAST and three Vitaceae fossils as calibration points. Bars on nodes are the 95% highest posterior probability intervals on the age estimates. Scale is in millions of years.