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Research Article

Integrating fossils in a molecular-based phylogeny and testing them as calibration points for divergence time estimates in Menispermaceae

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Abstract The phylogeny of extant Menispermaceae (Ranunculales) is reconstructed based on DNA sequences of two chloroplast genes (*rbc*L and *atp*B) from 94 species belonging to 56 genera. Fossilized endocarps represent 34 genera. The positions of these are inferred using 30 morphological characters and the molecular phylogeny as a backbone constraint. Nine of the thirteen nodes that are each dated by a fossil are used as calibration points for the estimates of molecular divergence times. BEAST is used to estimate stem age (121.2 Myr) and crown age (105.4 Myr) for Menispermaceae. This method does not require an input tree topology and can also account for rate heterogeneity among lineages. The sensitivity of these estimates to fossil constraints is then evaluated by a cross-validation procedure. The estimated origin for Menispermaceae is dated to the mid-Jurassic if the customary maximum age of 125 Myr for eudicots is not implemented. All constraints when used alone failed to estimate node ages in some parts of the tree. Fossils from the Palaeocene and Eocene impose strict constraints. Likewise, the use of *Prototinomiscium* as a dating constraint for Menispermaceae appears to be a conservative approach.
Key words age calibration, cross-validation, fossil, Menispermaceae, molecular scaffold, phylogeny.

The family Menispermaceae includes 72 genera (Ortiz et al., 2009, unpublished data), with approximately 520 species (Jacques et al., 2007). Large-scale phylogenetic studies of angiosperms using molecular data place Menispermaceae in the Ranunculales, sister to the Berberidaceae–Ranunculaceae clade (Soltis et al., 1997, 2000; APG, 1998; Hoot et al., 1999; Savolainen et al., 2000; APG II, 2003; Hilu et al., 2003; Kim et al., 2004; Worberg et al., 2007; Wang et al., 2009, unpublished data). Recent DNA sequence data support the monophyly of Menispermaceae (Ortiz et al., 2007; Wang et al., 2007, 2009; Hoot et al., 2009). The family is distributed throughout the tropics, with a few species occurring in temperate areas of Asia and America (e.g., Diels, 1910; Kessler, 1993). Members of the Menispermaceae are typically recognized by a frequent climbing habit, dioecious mating system, spiral phyllotaxy, unique petiole swelling, exstipulate leaves, and drupaceous fruits (Miers, 1851; Diels, 1910; Kessler, 1993). Other important features include the formation of successive cambia, a character probably linked to the climbing habit (Obaton, 1960; Mennega, 1982; Carlquist, 1988, 1996; Jacques & De Franceschi, 2007), unisexual flowers with floral parts in whorls of three and rudiments of non-functional organs of the opposite sex, especially staminodes in the female flowers (Wang et al., 2006), a condyle resulting from the development of the placental region (Miers, 1871; Diels, 1910; Dekker, 1983), a curved endocarp (Diels, 1910; Jacques et al., 2007; Ortiz et al., 2007), tricolporate pollen, and exine with a granular inner face (Thanikaimoni, 1984).

Several morphological (Jacques et al., 2007; Jacques & Bertolino, 2008) and molecular (Ortiz et al., 2007; Wang et al., 2007; Hoot et al., 2009) phylogenies have attempted to further clarify the infrafamilial relationships. The molecular phylogeny of Jacques & Bertolino (2008) is excluded from the discussion as it has been found that their results have been compromised by mislabeled samples. The molecular phylogenies (Ortiz et al., 2007; Wang et al., 2007; Hoot et al., 2009), even when there are inconsistencies among them, are congruent about the general patterns of Menispermaceae evolution. However, the latter molecular analyses and the morphological ones (Jacques et al., 2007; Jacques &

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Bertolino, 2008) exhibit incongruent evolutionary patterns.

The Menispermaceae are well represented in the fossil record (e.g., Takhtajan, 1974; Manchester et al., 2005; Jacques et al., 2007; Jacques, 2009a). The oldest known fossils of the family dates back to the Cretaceous (Takhtajan, 1974; Knobloch & Mai, 1986), namely they are several *Menispermites* leaves with often questionable identification (Jacques, 2009a) and a fossil endocarp from the Turonian of Central Europe (Knobloch & Mai, 1986). Several tribes sensu Diels (1910) are already recognized as early as the Palaeocene–Eocene boundary, with some fossils even assigned to extant genera (Reid & Chandler, 1933; Chandler, 1961; Manchester, 1994; Jacques & De Franceschi, 2005). Thirty-four Menispermaceae genera are identified in the fossil record (Jacques, 2009a) based solely on endocarp fossils. Fossil leaves are also frequent (Doria et al., 2008; Jacques, 2009a), but their identification is often problematic (Krassilov & Golovneva, 2004; Jacques, 2009a). These rich fossil records contrast with those of other families in the Ranunculales. For example, fruits and leaves of 11 genera of the Ranunculaceae are known in the fossil state (Pigg & DeVore, 2005); only a few fossil occurrences are reported for the Lardizabalaceae (Tiffney, 1993; Wilde & Frankenhäuser, 1998; Tao, 2000); the Berberidaceae are known in the fossil record through the occurrence of numerous leaves (Ramírez & Cevallos-Ferriz, 2000) and some fruits (Mai, 1987; Basilici et al., 1997); some fossil fruits of the genus *Palaeoaster* (Smith, 2001) and the fossil species *Papaveraceaepites thalmanii* Biswas 1962 in India (Kundu, 2008) are reported for the Papaveraceae; and leaves of Eupteleaceae are present in the fossil record (Tao, 2000). Other fossils related to the Ranunculales with uncertain familial affinities have also been described (Krassilov & Golovneva, 2004; von Balthazar et al., 2005). Therefore, among the early diverging eudicots, Menispermaceae are unique in having an abundant and diverse fossil record. The family is an ideal group to evaluate the impact of fossil taxa on inferred relationships (Springer, 1995; Forest et al., 2005).

Various methods are used to estimate divergence times; a complete review is provided by Rutschmann (2006). BEAST (Drummond & Rambaut, 2007) was chosen as our mode of analysis because the program does not require an input tree topology, and also accounts for rate variation. Therefore, errors associated with incorrect input topology and rate assumptions are eliminated (Wikström et al., 2001). However, it is important to point out that BEAST is not free of errors associated with tree reconstruction and errors in estimation of evolutionary model.

Obtaining calibration points from the fossil record is a crucial step in molecular dating (Wikström et al., 2001). Estimates often include multiple calibration points that are subjected to sensitivity analyses to assess the impact of each fossil on the reconstruction (Springer et al., 2003; Bremer et al., 2004; Sanderson et al., 2004). Cross-validation (Near & Sanderson, 2004; Near et al., 2005) is a technique that uses data partitioning to test the sensitivity of different calibration points. The placement of the fossil on the tree is another problem of calibration: the best way is to use synapomorphies to place fossils on the cladogram (Soltis et al., 2002). Misplaced fossils alter values of the sensitivity analysis, so this cladistic approach was applied to integrate fossils instead of relying on affinities denoted in published reports.

When analyzing fossil and extant specimens based on molecular and morphological data, a number of methods are available (Hermsen & Hendricks, 2008) including combined analysis (Kluge, 1989; Nixon & Carpenter, 1996; de Queiroz & Gatesy, 2007), the supertree approach (Schneider, 2006), and molecular scaffolding (Springer et al., 2001). The molecular scaffold approach (Manos et al., 2007) was selected to minimize the problem of missing data, which becomes severe when we incorporate a large number of Menispermaceae fossils into a cladistic analysis. Sauquet et al. (2009) successfully applied such an approach for molecular dating of Proteoideae, using palynological fossil constraints. Recently, Doyle & Endress (2010) integrated Early Cretaceous angiosperm fossils into molecular phylogenetic trees of living angiosperms.

The goals of this study are: (i) to reconstruct a phylogeny of living and fossil Menispermaceae using molecular markers (*rbc*L and *atp*B) and morphological characters; (ii) to estimate divergence times of major Menispermaceae clades; and (iii) to test the sensitivity of these estimates to different calibrations. Unlike most previous sensitivity analyses (e.g. Bremer et al., 2004; Magallón & Sanderson, 2005; Near et al., 2005), this study establishes fossil calibration points using cladistic analysis.

1 Material and methods

1.1 Taxon sampling

We sampled 78% of the genera of Menispermaceae including 56 genera and 94 species (58 and 61 sequences for the markers *rbc*L and *atp*B, respectively). Thirty-two new sequences for each gene, and data downloaded from GenBank were incorporated into this study. Our analysis includes the following genera that were not included in previous *rbc*L and/or *atp*B phylogenies: *Anisocycla*,

Anomospermum, *Antizoma*, *Aspidocarya*, *Beirnaertia*, *Caryomene*, *Disciphania*, *Elephantomene*, *Jateorhiza*, *Kolobopetalum*, *Leptoterantha*, *Rhaptonema*, *Rhigiocarya*, and *Telitoxicum*. All tribes recognized by Diels (1910) are represented. Six species were selected from Berberidaceae, Lardizabalaceae, and Ranunculaceae as outgroups based on previous studies of interfamilial relationships (Hoot et al., 1999; Kim et al., 2004; Worberg et al., 2007; Wang et al., 2009, unpublished data). A complete list of species, including voucher specimens and DNA sequence accession numbers, is available in Appendix I.

1.2 DNA extraction, amplification, and sequencing

Total genomic DNA was extracted from silica-gel dried leaves or herbarium specimens using the modified CTAB procedure of Doyle & Doyle (1987). Amplification of *rbc*L and *atp*B sequences was carried out using standard PCR protocol. The *rbc*L gene was amplified and sequenced using the 1F and 1494R primers (Chen et al., 1998) as well as the internal primers 636F (Muasya et al., 1998) and 991R (Chen et al., 1998). The *atp*B gene was amplified and sequenced using the *atp*B-S2 and *atp*B-1494R primers and the internal primers *atp*B-S611 and *atp*B-1186R (Hoot et al., 1995). The PCR products were purified using a GFX PCR DNA and Gel Band Purification Kit (Amersham Pharmacia Biotech, Piscataway, NJ, USA) then directly sequenced. Sequencing reactions were carried out using Big Dye (Perkin-Elmer, Norwalk, CT, USA) terminator cycle sequencing with an ABI 3730xl (Applied Biosystems, Foster City, CA, USA). All sequences were deposited in GenBank (see Appendix I for accession numbers).

1.3 Molecular phylogenetic analyses

Sequences were aligned manually using BioEdit (Hall, 1999), and the two datasets were combined into a common matrix.

A maximum parsimony (MP) analysis was carried out using the software TNT version 1.1 (Goloboff et al., 2003), using a traditional search with default settings except 100 replicates. Clade support was estimated using the bootstrap method (Felsenstein, 1985) with 1000 replicates and the same settings as above.

A maximum likelihood (ML) analysis was carried out using PhyML (Guindon & Gascuel, 2003), using SPR and NNI as the types of tree improvement. The GTR + I + Gamma model (general time reversible with a proportion of invariant sites and additional among-site rate variation modeled as a discrete gamma distribution, and six substitution rates; Yang, 1994) was used as the best-fit substitution model, as selected by both AIC and LRT criteria using ModelTest 3.7 (Posada & Crandall, 1998). A bootstrap analysis was carried out using 100 replicates.

Bayesian inference (BI) analysis was carried out using MrBayes 3.1.2 (Huelsenbeck & Ronquist, 2001; Ronquist & Huelsenbeck, 2003) with the GTR + I + G model. Two different analyses, each consisting of four chains, were run at the same time. Both analyses were run for 1 000 000 generations, with sampling every 200 generations. Assessment of the evolution of the log-likelihood scores against the generation time indicated that stationarity was achieved after 100 000 generations. Therefore, a burn-in of 500 trees was used. The remaining 9002 trees were loaded into PAUP* (Swofford, 1998) and a majority-rule consensus tree was constructed, with each group frequency corresponding to its posterior probability.

1.4 Fossil taxa

Menispermaceae are known in the fossil records from several organs: leaf, wood, pollen, and endocarp (Doria et al., 2008; Jacques, 2009a). Wood occurrence is very limited. The two fossil woods known from Asia (Vozenin-Serra et al., 1989; Bonde, 1997) and the one from Europe (Poole & Wilkinson, 2000) are not included in the present analysis. Menispermaceae fossil leaves are common, but lack recent taxonomic revision based on modern leaf morpho-anatomical evaluation (Jacques, 2009a). Hence, they were also omitted from this cladistic analysis. Inventories of fossil pollen of Menispermaceae are mainly restricted to Russian published reports (Doria et al., 2008; Jacques, 2009a) and as suggested by Thanikaimoni (1984), fossil pollen grains lack diagnostic characters and as a result, reliable identification is often difficult. We decided to focus on fossil endocarps as they are abundant and therefore well represented in the fossil record (Jacques, 2009a). Moreover, endocarps belonging to extant taxa have been studied extensively (Forman, 1956, 1957, 1960, 1962, 1968, 1972a, b, 1974, 1975, 1978, 1981, 1984, 1985, 1997, 2007; Thanikaimoni, 1984; Jacques, 2009b). The 34 fossils included in this study are listed in Table 1 along with stratigraphic data and absolute age of each taxon. The genera are used as operational taxonomic units for fossil taxa. Polyphyly of *Tinospora* and *Cocculus* as shown by molecular analyses (Hoot et al., 2009) can call into question the use of genera as operational taxonomic units for *Tinopsora* and *Cocculus* fossils. When compared with endocarp diversity of extant *Tinospora* (Jacques, 2009b), known fossil endocarps ascribed to *Tinospora* are of similar types and would all be coded the same way. Fossil endocarps currently ascribed to *Cocculus* were originally placed in the genus *Canticocculus* by Chandler (1961);

Table 1 Menispermaceae fossils included in the analysis

Genus	Oldest fossil record		Reference
	Age (Myr)	Locality	
Anamirta	Lutetian (43.7)	Clarno Beds, Oregon, USA	Manchester, 1994
Atriaecarpum	Lower Ypresian (55.2)	Le Quesnoy, France	Jacques & De Franceschi, 2005
Bowerbankella	Lower Lutetian (47.5)	Minster, UK	Reid & Chandler, 1933
Brueckelholzia	Serravallian (11.6)	Brückelholz, Germany	Gregor, 1977
Calycocarpum	Lutetian (43.7)	Clarno Beds, Oregon, USA	Manchester, 1994
Chandlera	Lutetian (43.7)	Clarno Beds, Oregon, USA	Manchester, 1994
Cissampelos	Burdigalian (17.7)	Rusinga, Kenya	Chesters, 1957
Cocculus	Ypresian (48.6)	Herne Bay, UK	Chandler, 1961
Curvitinospora	Lutetian (43.7)	Clarno Beds, Oregon, USA	Manchester, 1994
Cyclea	Serravallian (11.6)	Brückelholz, Germany	Gregor, 1977
Davisicarpum	Ypresian (48.6)	Sheppey, UK	Chandler, 1961
Diploclisia	Ypresian (48.6)	Bognor, UK	Chandler, 1961
Eohypserpa	Lower Ypresian (55.2)	Le Quesnoy, France	Jacques & De Franceschi, 2005
Frintonia	Ypresian (48.6)	Minster, UK	Chandler, 1961
Jateorhiza	Ypresian (48.6))	Bognor, UK	Chandler, 1964
Menispermum	Ypresian (48.6)	Bognor, UK	Chandler, 1964
Microtinomiscium	Ypresian (48.6)	Minster, UK	Reid & Chandler, 1933
Odontocaryoidea	Lutetian (43.7)	Clarno Beds, Oregon, USA	Manchester, 1994
Palaeococculus	Lower Ypresian (55.2)	Le Quesnoy, France	Jacques & De Franceschi, 2005
Palaeosinomenium	Palaeocene (55.8)	Horni Becva, Czech Republic	Knobloch, 1971
Palaeoskapha	Eocene (33.9)	Relu, China	Jacques & Guo, 2007
Parabaena	Ypresian (48.6)	Bognor, UK	Chandler, 1964
Prototinomiscium	Upper Turnonian (89.3)	Klikov-Schichtenfolge, Czech Republic	Knobloch & Mai, 1986
Rhytidocaryon	Mid-Miocene (11.6)	Orange area, Australia	Rozefelds, 1991
Sarcopetalum	Oligocene (30.0)	Glencoe, Australia	Rozefelds, 1991
Sinomenium	Oligocene (23.0)	Siberia, Russia	Takhtajan, 1974
Stephania	Burdigalian (17.7)	Rusinga, Kenya	Chesters, 1957
Syntrisepalum	Burdigalian (17.7)	Rusinga, Kenya	Chesters, 1957
Thanikaimonia	Lutetian (43.7)	Clarno Beds, Oregon, USA	Manchester, 1994
Tinomiscium	Ypresian (48.6)	Herne Bay, UK	Chandler, 1961
Tinomiscoidea	Lower Ypresian (55.2)	Le Quesnoy, France	Jacques & De Franceschi, 2005
Tinospora	Lower Ypresian (55.2)	Le Quesnoy, France	Jacques & De Franceschi, 2005
Triclisia	Burdigalian (17.7)	Rusinga, Kenya	Chesters, 1957
Wardensheppeya	Lower Ypresian (55.2)	Le Quesnoy, France	Jacques & De Franceschi, 2005

Canticocculus was then considered as an extinct section of *Cocculus* by Mai (1987). Fossil endocarps assigned to *Cocculus* are treated as a monophyletic group in this analysis.

The stratigraphy of the London Clay Formation, UK, is detailed in Collinson & Hooker (1987). One radiometric date available for the London Clay is 50.9 ± 2.9 Myr (Odin & Curry, 1985). Fossils from this formation come from many sites (Collinson & Hooker, 1987), so a single radiometric date representing all sites may not be adequate. The London Clay Formation is of Ypresian age (Collinson & Hooker, 1987), for which the younger limit is 48.6 Myr. Therefore, this is used as the minimal age for the London Clay fossils. The selected calibration age falls within the confidence interval of the radiometric age.

The age of the Clarno Nut Beds (Oregon, USA) has been estimated at 43.76 ± 0.29 Myr (Turrin in Manchester, 1994), using the Ar/Ar method. The Le Quesnoy outcrop belongs to European mammal zone MP7 (Nel et al., 1999). Rozefelds (1991) dated the Glencoe outcrop as being Oligocene (30–32 Myr). Pickford (1986) confirmed the placement of Rusinga flora in the Lower Miocene, with an estimated age of 17.9 ± 0.2 Myr.

For each genus, we retained the oldest occurrence as a constraint. For each constraint, the younger limit of its date interval was retained as the minimum age constraint.

Stratigraphic ages follow the geological timescale of Gradstein et al. (2004).

1.5 Morphological character coding

As the fossils were integrated into a molecular scaffold, only characters observable in these fossils are useful. Thus we only coded the endocarp characters. To code these characters in extant plants, personal observations, published morphological descriptions, and earlier morphology-based phylogenetic studies were used (Diels, 1910; Forman, 1956, 1957, 1960, 1962, 1968, 1972a, b, 1974, 1975, 1978, 1981, 1984, 1985, 1997, 2007; Troupin, 1962; Thanikaimoni, 1984; Jacques et al., 2007; Jacques & Bertolino, 2008; Jacques, 2009b). When no data were available for a species, the generic characters were used, leaving the

Table 2 Morphological character coding of endocarp characters in Menispermaceae

Number	Character name	Character states
1	Drupe with endocarp	0, no; 1, yes
2	Endocarp globose	0, no; 1, yes
3	Straight endocarp	0, no; 1, yes
4	Endocarp excavated lateral faces	0, no; 1, yes
5	Endocarp with large central area	0, no; 1, yes
6	Endocarp dorsi-ventrally compressed	0, no; 1, yes
7	If endocarp is not straight, its length is much bigger than width	0, no; 1, yes
8	Dorsal ridges	0, absent; 1, present
9	Number of lateral ridges on each side	0, 0; 1, 1; etc.
10	Ridges wing-shaped	0, no; 1, yes
11	Transversal ridges	0, absent; 1, present
12	Endocarp with ventral groove	0, no; 1, yes
13	Foramen	0, absent; 1, present
14	Central area partly covered by projections	0, no; 1, yes
15	If straight endocarp, keeled at apex	0, no; 1, yes
16	Type of surface	0, smooth; 1, with reticulated hollows; 2, with reticulated bumps
17	Spines on surface	0, absent; 1, present
18	Hollows on surface	0, no; 1, yes
19	The two limbs with strong dissymmetric curvature	0, no; 1, yes
20	One limb terminating outwards	0, no; 1, yes
21	If not straight endocarp, distance between the two limbs	0, small; 1, large
22	Condyle	0, absent; 1, present
23	Condyle externally conspicuous	0, no; 1, yes
24	Type of condyle	0, simple; 1, double
25	Perforated condyle	0, no; 1, yes ventrally; 2, yes on septum
26	Condyle in protruding ventral chamber	0, no; 1, yes
27	If not straight endocarp, condyle parallel to symmetry plane	0, no; 1, yes
28	If straight endocarp, condyle involving all ventral face	0, no; 1, yes
29	Seed cavity surrounding the condyle	0, no; 1, yes
30	Shape of seed cavity in transverse section	0, boat-shaped; 1, angular; 2, circular

other characters coded as unknown. For fossil endocarps, personal observations, and published descriptions were used. Thirty coded characters are presented in Table 2. Morphological coding is given in Appendix II.

1.6 Morphological phylogenetic analysis

We used the molecular scaffold approach (Springer et al., 2001; Manos et al., 2007; Hermsen & Hendricks, 2008). Results of molecular phylogenetic analyses were used to construct the scaffold. All clades found under MP, ML, and BI were included. If a clade was not recovered in all of these analyses, the relevant relationships were treated as unresolved. The morphological matrix was analyzed under MP with the software TNT version 1.1 (Goloboff et al., 2003), with tree fusing technology, and default settings, except for the 1000 replications. Fossil taxa were considered as floaters during the search. The strict consensus was constructed without collapsing branches of zero length.

1.7 Molecular dating

The calibration of the tree is part of a molecular dating analysis (Renner, 2005). First, we tried to calibrate the root of the tree. Anderson et al. (2005) proposed a stem age for Lardizabalaceae between 107 and 116 Myr old. As Lardizabalaceae were used as the rooting group, we fixed the minimal age of the root to 110 Myr, with a standard deviation of 20 Myr. The earliest reliable fossils for eudicots are tricolpate pollen grains from the Barremian (Hughes & McDougall, 1990; Doyle, 1992) and the undetected presence of such pollen grains in an earlier period is seen as unlikely (Crane et al., 1989). Therefore we used 125 Myr as the maximal age of the tree.

Other fossil-based constraints were decided according to the result of the morphological analysis. When a fossil has been assigned to a clade, the stem node or the crown node of this clade can be used as constraint. Each assumption gives a different estimate of the divergence time (Forest et al., 2005). We chose to use stem nodes as constrained points (Wikström et al., 2001; Anderson et al., 2005; Renner, 2005), as it is the most inclusive group containing all extinct and extant members of a clade (Near et al., 2005). Fossils were used as minimum age constraint.

The molecular clock was tested using PATHd8 (Britton et al., 2007). The tree obtained by ML with branch lengths was used as the input tree.

Divergence times were estimated through the Bayesian MCMC analysis implemented in BEAST version 1.4.8 (Drummond & Rambaut, 2007). This method relaxes the molecular clock through a Bayesian approach (Drummond et al., 2006) and allows for sequences showing different rates of evolution (Drummond et al., 2006; Rutschmann, 2006). This latter feature is important, and given that we used two datasets, it avoids the use of the hypothesis of a common evolution model for the two loci. The unique feature of this software is that it does not need a starting tree (Rutschmann, 2006). The tree topology and the divergence times are co-estimated together (Drummond et al., 2006). The Markov chain was run for 5 000 000 generations with sampling at every 1000 generations. The burn-in,

after evaluating for convergence, consisted of 500 samples. The analysis was carried out twice to make sure that the convergence of the Markov chain was achieved as recommended by the authors (Drummond & Rambaut, 2007). The two analyses were combined for results output. We used 10 Myr as the time unit, and the following parameters: GTR + I + Gamma model with estimated base frequencies; no fixed substitution mean rate; uncorrelated lognormal relaxed clock; Tree Prior, Speciation: Yule process; treeModel.rootHeight normal, mean = 11.0, standard deviation = 2.0, initial value = 11.0; calibration points monophyletic, uniform; unlinked parameters for the two datasets; all other settings as default.

Convergence of each chain to the target distribution was assessed using Tracer version 1.4 (Rambaut & Drummond, 2007b) and by plotting time series of the log posterior probability of sampled parameter values. The chronogram was calculated on the maximum sum of clade credibilities tree, using TreeAnnoter version 1.4.8 (Rambaut & Drummond, 2007a).

1.8 Cross-validation tests

To evaluate the influence of the different constraints on the dating, we carried out several other calculations. First, molecular dating was carried out without the maximal age of 125 Myr. We also calculated molecular dates without *Prototinomiscium*, as its assignment to Menispermaceae is sometimes regarded as tentative (Mai, 1987; Jacques, 2009a). Then we carried out two cross-validation procedures, both of them keeping the same root height prior, maximal age of 125 Myr, and *Prototinomiscium* as general constraints.

The first procedure was the fossil cross-validation developed by Near et al. (2005). The divergence estimates were calculated based on only one fossil constraint. For other nodes, the difference between estimated age and fossil age was calculated and the mean extracted. This value was calculated for each fossil in turn. This approach is called "keep-one".

The second procedure was the fossil-based model cross-validation, developed by Near & Sanderson (2004). A fossil constraint was excluded from the analysis, and the difference between its estimated age and fossil age was calculated. This value was calculated for each fossil in turn. This approach is called "leave-one".

2 Results

2.1 Molecular analyses

Table 3 summarizes principal characteristics of the sequences. The MP analysis yielded 550 equally parsimonious trees with a length of 1379 steps, consistency index of 0.590, and retention index of 0.812 including all characters (1,073, 0.473, and 0.812, respectively, when only informative characters were considered). The ML analysis yielded a single tree of $-\ln L = 12888.26966$. The 9002 trees kept after the BI were summarized in a majority rule consensus.

Table 3 Principal characteristics of *rbc*L and *atp*B sequences in extant Menispermaceae

	*rbc*L	*atp*B	All
Length (bp)	1392	1407	2799
Number of Menispermaceae sequences	94	88	94
Proportion of A	27.2	29.0	28.1
Proportion of C	19.6	19.8	19.7
Proportion of G	24.9	23.3	24.1
Proportion of T	28.3	27.9	28.1
Number of variable sites	334	340	674
Percent of variable sites	24.0	24.2	24.08
Number of informative sites	211	181	392
Percent of informative sites	15.2	12.9	14.0

Results from these different analyses are generally congruent (Fig. 1). Some nodes are not resolved under MP. Relationships are slightly different in the Tiliacoreae under ML.

2.2 Phylogenetic relationships

The monophyly of Menispermaceae is strongly supported (100/100/100). We found three major clades in Menispermaceae. Clade 1 includes Coscinieae (sensu Diels, 1910) and Expanded Tinosporeae sensu Ortiz et al. (2007), and confirms the inclusion of *Tinomiscium* in Expanded Tinosporeae (Wang et al., 2009, unpublished data; Hoot et al., 2009). Clade 2 includes Expanded Tiliacoreae and Expanded Anomospermeae sensu Hoot et al. (2009), and *Diploclisia* and *Limacia*. Clade 3 includes *Menispermum* and *Sinomenium* and is sister to all other Menispermaceae. The position of Clade 3 is similar to what Hoot et al. (2009) found with *rbc*L and *atp*B, but differs from results using other cpDNA markers, where Clade 3 is sister to Clade 2 (Ortiz et al., 2007; Wang et al., 2007).

In Expanded Tinosporeae we found a strongly supported (98/100/100) *Aspidocarya–Disciphania–Parabaena* clade. Contrary to Hoot et al. (2009) *Penianthus* is not monophyletic, but our sequence for *Penianthus longifolius* is different from the sequence used by Hoot et al. (2009). The alternative placement of *Penianthus patulinervis* in our study is weak. *Tinospora* is confirmed as polyphyletic (Hoot et al., 2009; Ortiz et al., 2009, unpublished data).

Tiliacoreae are only moderately supported (<50/72/98), and infratribal relationships are poorly resolved (Fig. 1). Relationships within Clade C differ from previous studies (Ortiz et al., 2007; Hoot et al.,

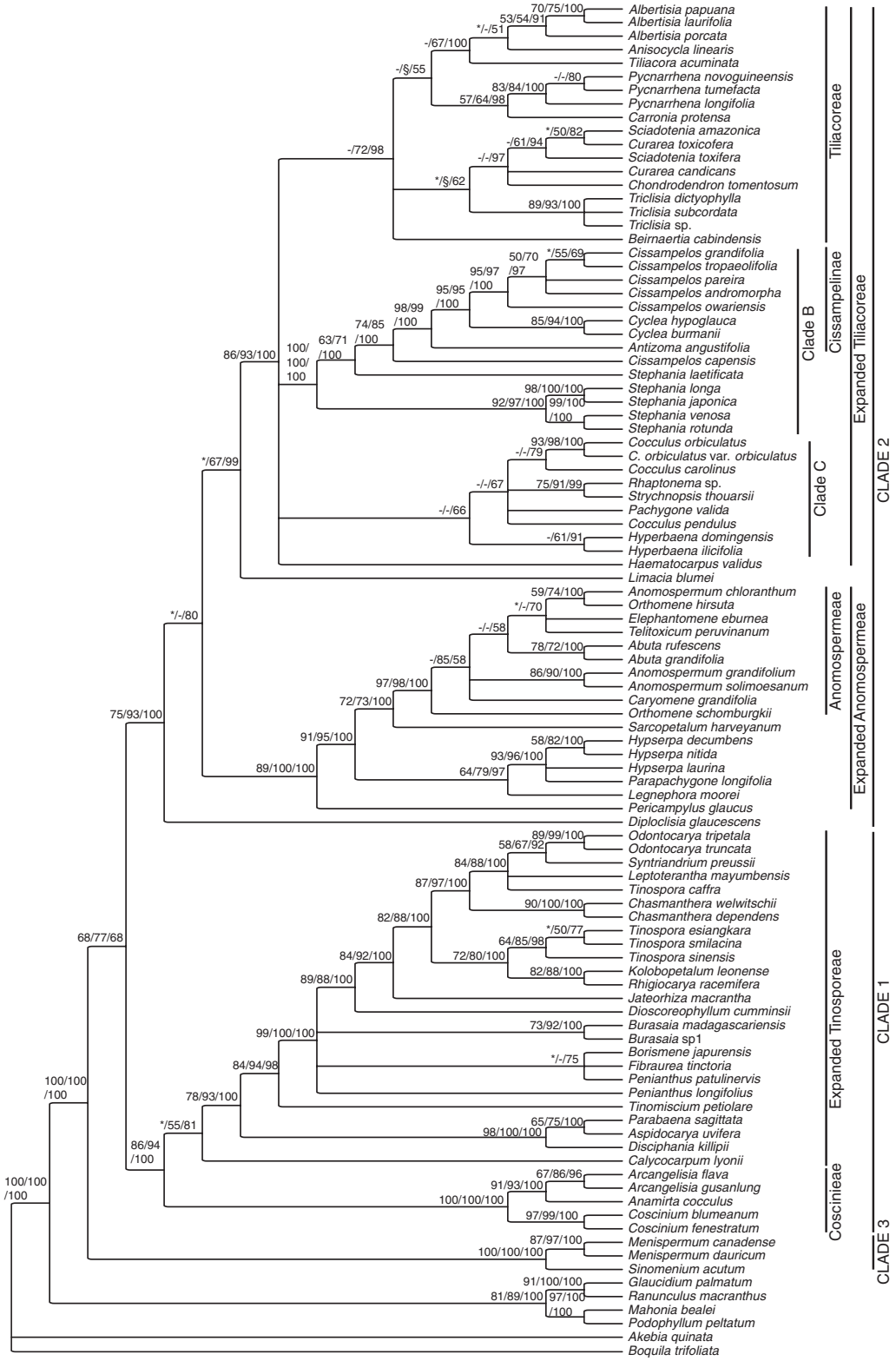

Fig. 1. Majority rule consensus of 9002 Bayesian inference trees. Branch support is indicated over the branch in the following order: maximum parsimony bootstrap value/maximum likelihood bootstrap value/Bayesian posterior probability (×100). Tribes and subtribes are according to Diels (1910), except Expanded Tinosporeae (Ortiz et al., 2007), Expanded Anomospermeae, and Expanded Tiliacoreae (Hoot et al., 2009). –, Value below 50; *Nodes are not retrieved in maximum parsimony analysis; §Two nodes are resolved differently under maximum likelihood.

2009), but are poorly resolved and weakly supported (Fig. 1).

2.3 Position of fossil taxa

Positions of four taxa (*Davisicarpum*, *Jateorhiza*, *Thanikaimonia*, and *Tinomiscoidea*) are not fully resolved; they are placed equally parsimoniously at several positions. Therefore those taxa were excluded from further analysis. Twenty-six taxa were included in the subsequent analysis; 56 equally parsimonious trees (187 steps) were retrieved and a strict consensus tree was constructed (Fig. 2). Fossil taxa are generally found near their proposed affinities. Important exceptions are the *Cocculus* fossil that is found in Clade 3 (and not near the modern *Cocculus* in Clade C), and the *Parabaena* fossil that is found sister to *Calycocarpum lyonii*.

Here, we detail all the possible placements of taxa that are ambiguously placed in our analysis. *Davisicarpum* is found as sister to Expanded Tiliacoreae, sister to all Menispermaceae, sister to Clade 1, sister to Clades 1 and 2, or sister to Anomospermeae. *Jateorhiza* and *Tinomiscoidea* are found in different positions in Expanded Tinosporeae. *Thanikaimonia* is found in various positions in Clade B, as sister to Anomospermeae, or near *Diploclisia glaucescens*.

We used Mesquite version 1.6 (Maddison & Maddison, 2006) to reconstruct character evolution (Fig. 3). The straight endocarp is a synapomorphy of Expanded Tinosporeae, but has independently evolved in other clades. The dorsal ridges have been lost several times independently. The transversal ridges evolved several times independently. Their function remains unknown, although Jacques & Bertolino (2008) suggested a potential role in strengthening the endocarp structure. The ventral perforation of the condyle is a synapomorphy of Clade 1. The only exception is *Chandlera*, which has a condyle without perforation. In Clade 2, the orientation of the condyle changed several times towards a condyle not parallel to the symmetry plane.

2.4 Dating constraints

Fossils can potentially be used to constrain the ages of 13 nodes in molecular dating (Fig. 2; Table 4). Because of the lack of resolution within Tiliacoreae (Fig. 1), the constraint point M in the Tiliacoreae was set at the base of this clade. Similarly, because of the lack of resolution within Clade C (Fig. 1), *Palaeococculus* was used to constrain point L and not one of the included group, that is, daughter node. Nodes were selected based on the criterion that minimal age assigned to one node should be strictly older than any minimal age assigned to its descendant nodes (Near & Sanderson, 2004). Only nine nodes were selected (Table 4). As *Prototinomiscium* is the oldest fossil occurrence of the family, we decided to use it as the minimal age of the whole family (Fig. 2, point A), even if it could have been used to constrain the younger node C.

2.5 Divergence time estimates

Using PATHd8, the molecular clock was accepted on 55 nodes and rejected on 43 nodes. Therefore the molecular clock for the entire dataset is rejected. A chronogram of Menispermaceae using all 10 constraints and reconstructed with BEAST is shown in Fig. 4. Main divergence estimates are presented in Table 5. The stem of the Menispermaceae is estimated as Aptian, and the crown group began to diversify in the Albian.

2.6 Cross-validation tests

When no maximal age is considered, the Menispermaceae stem group goes back as far as Bajocian (mid-Jurassic) and the crown group is estimated as Valanginian (Table 5). The deep nodes are generally given older estimates when all constraints are included than when only some of them are included, but derived nodes show no strong differences. When *Prototinomiscium* is excluded from the analysis, estimates are similar to those obtained when all constraints are included (Table 5). The results of cross-validation tests are presented in Table 6. The "leave-one" procedure shows that the model tends to underestimate the age of two points (D, *Tinospora*; L, *Palaeococculus*), and to "overestimate" the age of other points. The "keep-one" procedure shows similar mean absolute error (from 14 to 19 Myr), but differences in mean error, with three calibrations overestimating other points (D, *Tinospora*; E, *Palaeoskapha*; and L, *Palaeococculus*). The differences between mean absolute error and mean error for all calibration points (Table 6) show that all points "overestimate" some node ages and underestimate others.

3 Discussion

3.1 Phylogenetic relationships

The phylogeny presented here (Fig. 1) is highly congruent with earlier molecular phylogenies using *ndh*F (Ortiz et al., 2007), *mat*K, and *trn*L-F (Wang et al., 2007), and *atp*B and *rbc*L (Hoot et al., 2009).

The main difference between our results and those generated previously (Ortiz et al., 2007; Wang et al., 2007) lies in the placement of Clade 3 (*Menispermum* and *Sinomenium*). In our study, this clade is sister to all other Menispermaceae, although with low support (Fig. 1), whereas it is sister to Clade 2 in the analysis of Wang et al. (2007) and in the analysis of Ortiz

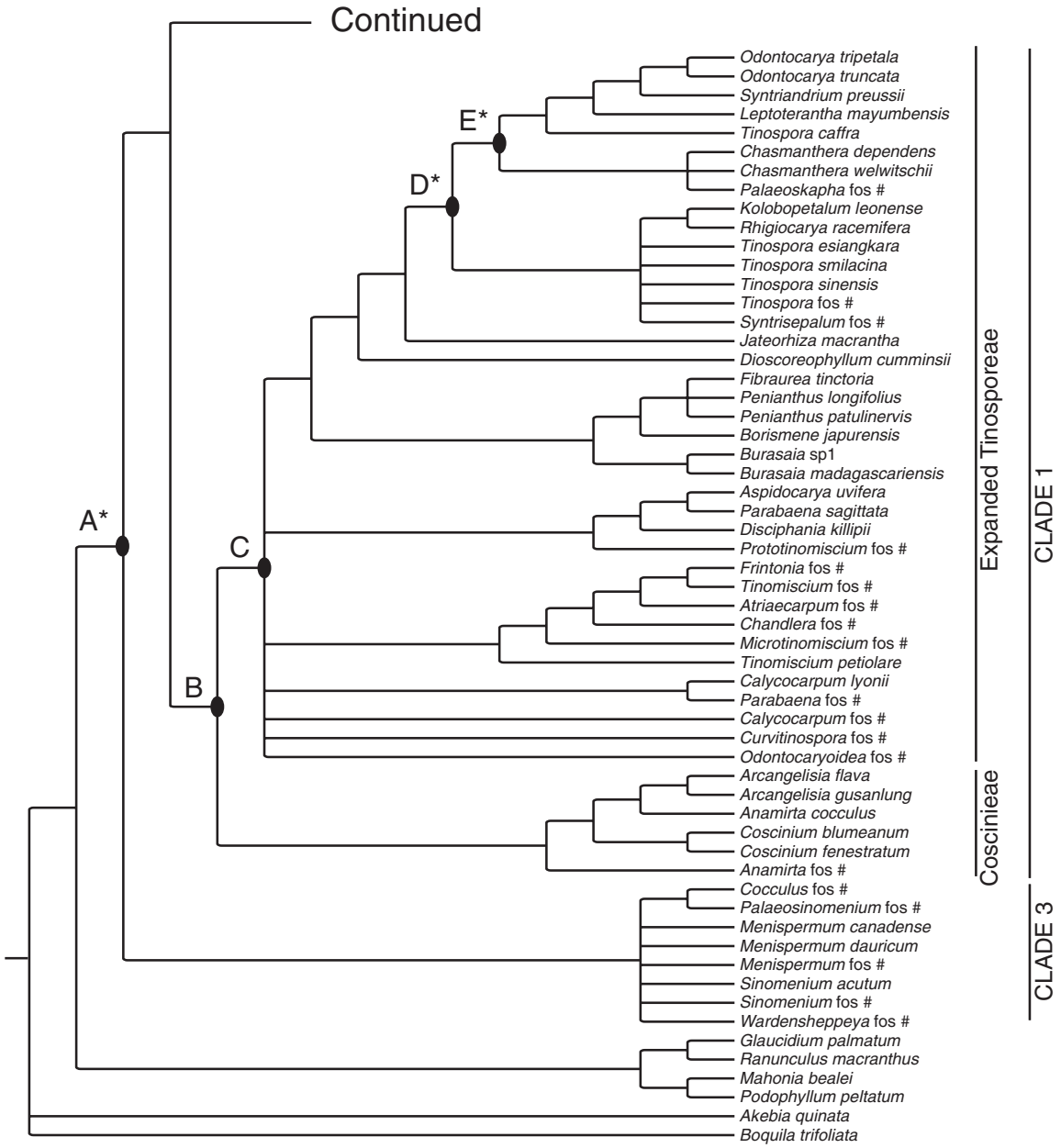

Fig. 2. Consensus tree of morphological analysis of extant and extinct Menispermaceae, using a molecular scaffold. Points on the nodes refer to possible constraint nodes (see Table 4). *Node was selected as constraint for divergence time estimation; fos #, A fossil taxon.

et al. (2007). The latter study included only species of *Menispermum*. In this study, the placement of Clade 3 is congruent with that of Hoot et al. (2009), which was based on the same markers used in this study. In some chronograms reconstructed during the cross-validation, Clade 3 is found as sister to Clade 2, and in one case to Clade 1. This incongruence may be a result of the poor diversity of Clade 3 (consisting of only three species), which can lead to a sampling effect (Satta et al., 2000; Kopp & True, 2002; Rokas et al., 2003; Rokas & Carroll, 2005). The sequences used here may also not be variable enough to resolve relationships at that level. Based on a geometric morphometrics analysis, Jacques & Zhou (2010) show that Clade 3 has horseshoe-shaped endocarps that clearly differ in shape from other horseshoe-shaped endocarps occurring in this family.

The placement of *Calycocarpum* differs from Hoot et al. (2009). Using *atp*B and *rbc*L, they found *Calycocarpum* to be sister to Coscinieae, whereas we found it sister to the remaining Expanded Tinosporeae. Both

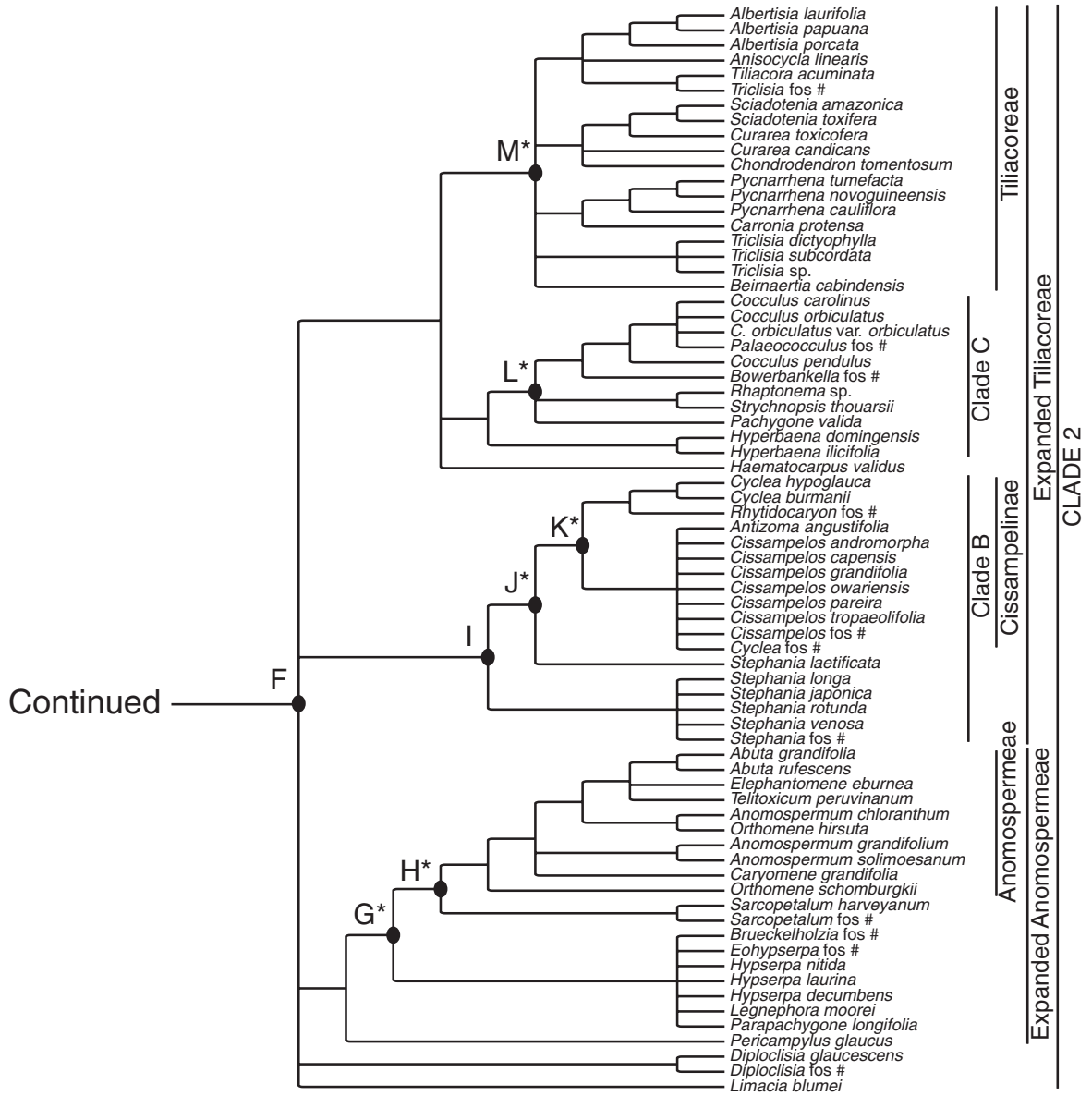

Fig. 2. Continued.

of the placements lack strong support. Our placement agrees with that found by Ortiz et al. (2007) using *ndh*F.

Expanded Tinosporeae (Fig. 1) were first proposed by Ortiz et al. (2007). The genus *Tinomiscium* was found to be outside of Expanded Tinosporeae by Ortiz et al. (2007). However, the sequence used in their analysis was from herbarium material and resulted in ambiguous readings (Ortiz, personal observation, 2007). *Tinomiscium* is now placed within Expanded Tinosporeae due to the inclusion of a new specimen (Ortiz et al., 2009, unpublished data). Therefore, of the hypotheses suggested by Hoot et al. (2009), we can exclude the one with considerable molecular variation in the genus *Tinomiscium*. Wang et al. (2007) and Hoot et al. (2009) reported a similar position for *Tinomiscium*. In addition to the genera included by Hoot et al. (2009) in Expanded Tinosporeae, we add the genus *Aspidocarya* (Fig. 1). The genus *Tinospora* is not monophyletic in our analyses (Fig. 1) thus confirming the results from other studies (Hoot et al., 2009; Ortiz et al., 2009, unpublished data; Wang et al., 2009, unpublished data). A novel relationship found in our study is the clade formed by *Aspidocarya*, *Disciphania,* and *Parabaena*, with strong support in MP, ML, and BI analyses (Fig. 1).

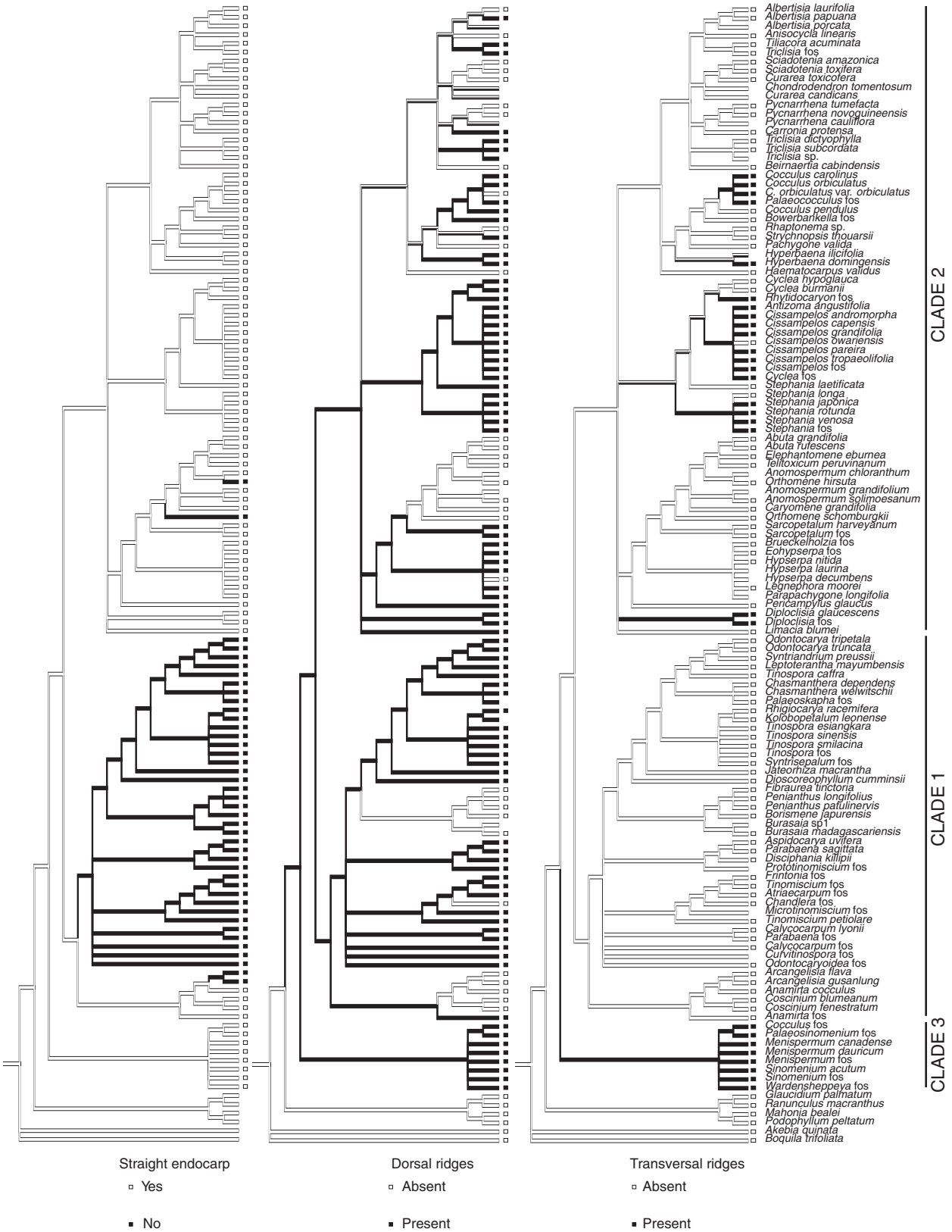

Fig. 3. Reconstruction of character evolution using Mesquite (Maddison & Maddison, 2006). fos, A fossil taxon.

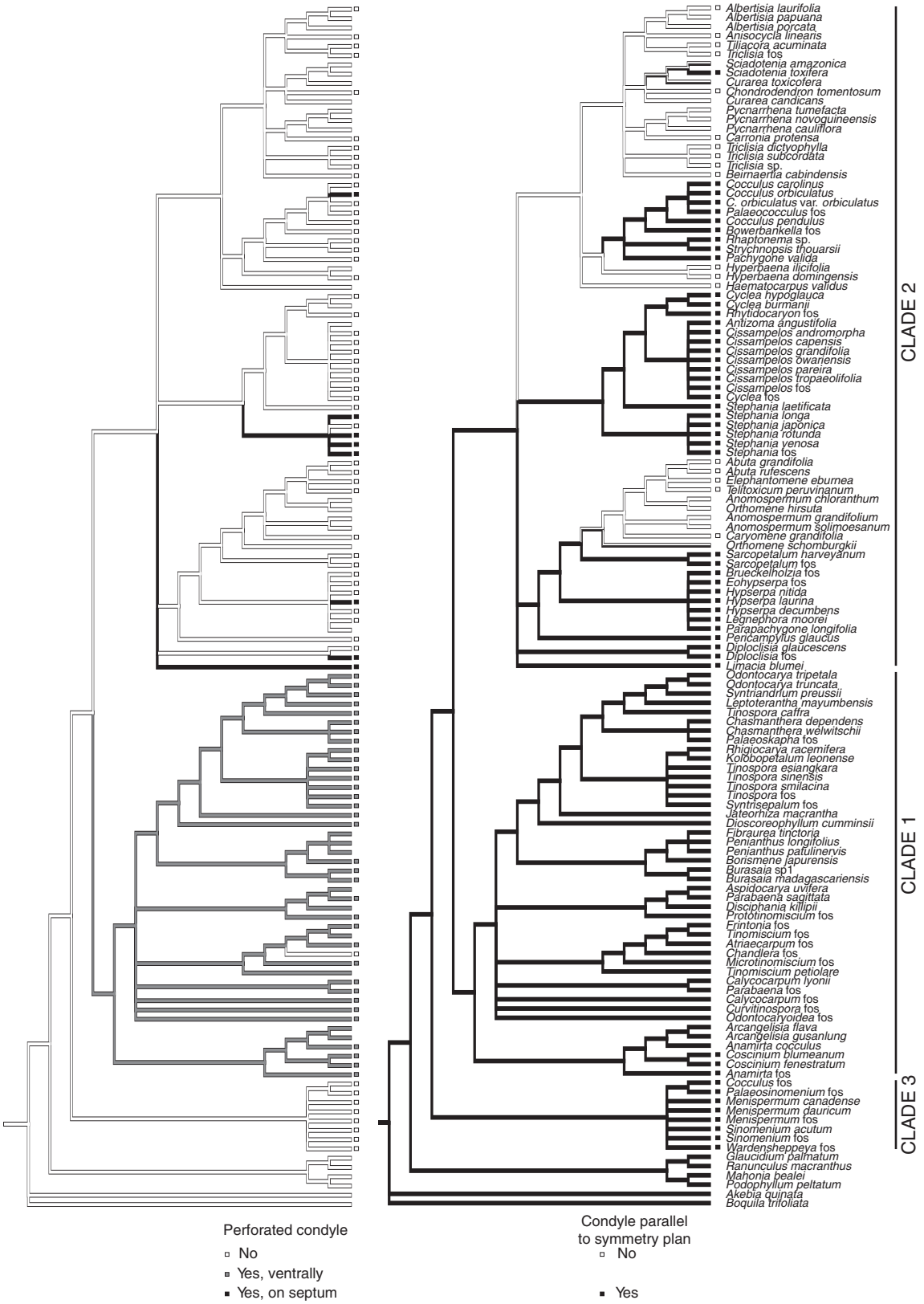

Fig. 3. Continued.

Table 4 Nodes potentially useful in molecular dating of Menispermaceae fossils and nodes actually selected

Node	Fossils potentially useful (Myr)	Oldest fossil	Selected constraint (Myr)
A	*Cocculus* (48.6), *Palaeosinomenium* (55.8), *Menispermum* (23.0), *Sinomenium* (23.0), *Wardensheppeya* (55.2)	*Palaeosinomenium* (55.8), [*Prototinomiscium* (89.3)]†	89.3†
B	*Anamirta* (43.7), *Curvitinospora* (43.7)	*Anamirta* (43.7), *Curvitinospora* (43.7)	—
C	*Atriaecarpum* (55.2), *Calycocarpum* (43.7), *Chandlera* (43.7), *Frintonia* (48.6), *Microtinomiscium* (48.6), *Odontocaryoidea* (43.7), *Parabaena* (48.6), *Prototinomiscium* (89.3), *Tinomiscium* (48.6)	*Atriaecarpum* (55.2), [*Prototinomiscium* (89.3)]†	—
D	*Syntrisepalum* (17.7), *Tinospora* (55.2)	*Tinospora* (55.2)	55.2
E	*Palaeoskapha* (33.9)	*Palaeoskapha* (33.9)	33.9
F	*Diploclisia* (48.6)	*Diploclisia* (48.6)	—
G	*Brueckelholzia* (11.6), *Eohypserpa* (55.2)	*Eohypserpa* (55.2)	55.2
H	*Sarcopetalum* (30.0)	*Sarcopetalum* (30.0)	30.0
I	*Stephania* (17.7)	*Stephania* (17.7)	—
J	*Cyclea* (11.6), *Cissampelos* (17.7)	*Cissampelos* (17.7)	17.7
K	*Rhytidocaryon* (11.6)	*Rhytidocaryon* (11.6)	11.6
L	*Bowerbankella* (48.6), *Palaeococculus* (55.2)	*Palaeococculus* (55.2)	55.2
M	*Triclisia* (17.7)	*Triclisia* (17.7)	17.7

†As *Prototinomiscium* is the oldest known fossil, we decided to use it as a constraint for the whole family. —, node not selected as constraint.

However, those genera have long branches, and their inferred affinities could be an artifact of long branch attraction.

The Anomospermeae formed a strongly supported monophyletic clade (Fig. 1), confirming the results of Ortiz et al. (2007) and Hoot et al. (2009). The polyphyletic character of the genus *Anomospermum* (Ortiz et al., 2007) is confirmed. *Anomospermum chloranthum* is a member of section *Anomospermum*, whereas *A. grandifolium* and *A. solimoesanum*, sister groups in this study, are members of section *Elissarhena*. The reduction of *Elissarhena* to a section of *Anomospermum* by Barneby & Krukoff (1971) should be reconsidered. The genus *Orthomene* is found to be polyphyletic (Fig. 1), confirming earlier results by Ortiz et al. (2007).

The Expanded Tiliacoreae of Hoot et al. (2009) are monophyletic. Our analysis resulted in the inclusion of two other genera in this clade (*Anisocycla* and *Antizoma*; Fig. 1). The placement of *Triclisia* as sister to the American Tiliacoreae (*Chondrodendron*, *Curarea*, and *Sciadotenia*) differs from that of Ortiz et al. (2007) and from that of Hoot et al. (2009). However, the position of *Triclisia* in the present study is weakly supported in MP and BI, and different in ML. *Tiliacora* is sister to *Albertisia* (Fig. 1), whereas Ortiz et al. (2007) found *Tiliacora* as sister to *Albertisia* and *Anisocycla*; Hoot et al. (2009) found *Tiliacora* sister to *Albertisia* and *Triclisia*, but did not include *Anisocycla* in their analysis. Interestingly, an Asian species was sampled in the present study, whereas Ortiz et al. (2007) sampled only African species.

Only four South American genera belong to the tribe Tiliacoreae (*Chondrodendron*, *Curarea*, *Sciadotenia*, and *Ungulipetalum*). *Ungulipetalum* is poorly understood and could not be included in this analysis. The remaining three genera form a monophyletic group (Fig. 1) as in the study by Ortiz et al. (2007). Our results favor a South American Tiliacoreae clade. This clade was found by Hoot et al. (2009) only in the combined analysis of *atp*B, *rbc*L, and *ndh*F, but not when the latter dataset was not included.

Cissampelos is polyphyletic, with the species *C. capensis* being separated from the others. This species is placed near *Antizoma angustifolia*, and has been considered to belong to *Antizoma* by some authors (Diels, 1910). Its inclusion in *Antizoma* would make this genus paraphyletic. The genus *Stephania* is monophyletic with 56% bootstrap support based on internal transcribed spacer sequences (Hong et al., 2001). In the present study it is paraphyletic, as in Hoot et al. (2009), with the African species *S. laetificata* and the sampled Asian species being placed in different clades, but with low support (Fig. 1). From a strict nomenclatural point of view, *S. laetificata* is included in another genus *Perichasma* Miers (Kundu & Guha, 1977). The sequences of *S. laetificata* have big gaps that could have induced some artifact in the reconstruction.

3.2 Placement of the fossils

Positions of several fossil taxa (Fig. 2) correspond to the affinities that have previously been suggested, namely: *Anamirta*, *Atriaecarpum*, *Cissampelos*, *Eohypserpa*, *Frintonia*, *Menispermum*, *Microtinomiscium*, *Palaeococculus*, *Palaeosinomenium*, *Palaeoskapha*, *Sarcopetalum*, *Sinomenium*, *Stephania*, *Syntrisepalum*, *Tinospora*, *Tinomiscium*, and *Wardensheppeya*. The positions of some other fossil taxa differ from their suggested affinities. Those taxa are worthy of a more developed discussion.

Scott (1956), followed by Manchester (1994), suggested *Parabaena* as a possible living relative of *Chandlera*. Our analysis indicates that *Chandlera* may be related to *Tinomiscium*, and indeed, both taxa share

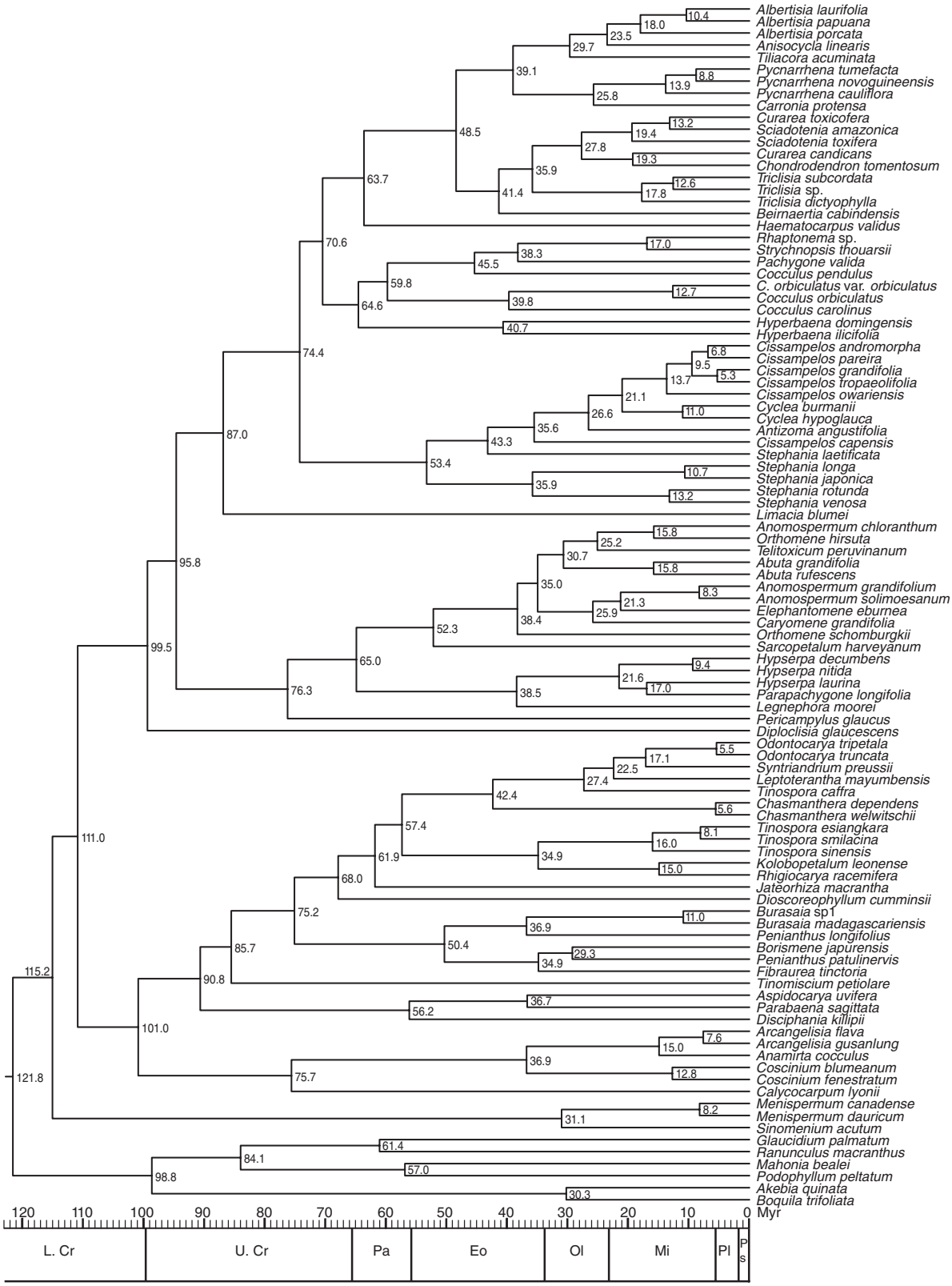

Fig. 4. Chronogram of Menispermaceae calculated using all non-redundant constraint nodes. Numbers near the nodes refer to mean age of the nodes (Myr). Eo, Eocene; L. Cr, Lower Cretaceous; Mi, Miocene; Ol, Oligocene; Pa, Palaeocene; Pl, Pliocene; Ps, Pleistocene; U. Cr, Upper Cretaceous.

Table 5 Divergence time estimates for major clades of Menispermaceae

Node	All constraints	No maximal age	No *Prototinomiscium*	C as *Prototinomiscium*
Menispermaceae stem	121.8 (115.6–125.0)	170.7 (141.4–198.7)	121.5 (114.9–125.0)	118.7 (108.0–125.0)
Menispermaceae crown	115.2 (103.3–124.4)	139.0 (111.0–171.2)	117.0 (104.6–125.0)	112.2 (99.1–124.9)
Clade 3 crown	31.1 (7.8–61.8)	36.6 (9.0–73.1)	32.5 (7.9–64.3)	25.0 (6.7–49.2)
Clades 1 and 2 stem	111.0 (101.3–124.2)	135.2 (107.8–166.0)	115.1 (102.5–125.0)	‡
Clade 1 crown	101.0 (87.0–115.8)	117.4 (92.4–114.6)	103.0 (86.9–121.5)	99.5 (90.4–111.6)
Coscinieae crown	36.9 (14.1–64.4)	43.6 (16.9–75.4)	36.4 (14.4–62.0)	31.9 (10.6–57.7)
Exp. Tinosporeae crown†	90.8 (77.8–105.1)	102.8 (81.1–124.9)	92.2 (76.9–107.1)	75.0 (89.3–101.9)
Clade 2 crown	99.5 (84.8–114.3)	116.5 (88.8–143.9)	100.4 (85.1–117.0)	86.1 (61.2–108.8)
Exp. Anomospermeae stem	95.8 (80.5–109.0)	111.7 (87.3–140.7)	96.8 (81.6–113.3)	81.4 (54.6–92.7)
Exp. Anomospermeae crown	76.3 (60.7–93.0)	85.3 (61.7–109.6)	76.9 (60.5–94.3)	58.8 (39.1–83.4)
Anomospermeae stem	52.3 (36.3–68.3)	58.4 (40.4–79.1)	53.5 (38.6–69.3)	39.1 (24.3–55.4)
Anomospermeae crown	38.4 (25.1–51.7)	43.4 (28.4–61.5)	39.9 (25.3–53.8)	28.6 (17.2–40.4)
Exp. Tiliacoreae stem	87.0 (71.4–101.7)	100.3 (76.5–126.4)	88.1 (72.6–103.5)	71.7 (50.7–95.9)
Exp. Tiliacoreae crown	74.4 (62.1–89.1)	83.4 (64.0–104.9)	75.1 (61.8–88.7)	57.7 (38.5–77.2)
Clade B crown	53.4 (37.9–68.8)	62.4 (43.3–80.1)	53.8 (39.7–69.4)	41.5 (28.5–58.4)
Tiliacoreae crown	48.5 (32.2–65.1)	55.1 (35.6–76.2)	49.6 (32.0–68.4)	36.2 (23.4–51.1)
Clade C crown	64.6 (56.4–75.3)	69.1 (56.3–83.8)	66.2 (56.4–77.9)	39.6 (21.5–58.3)

†As *Calycocarpum lyonii* is sometimes reconstructed as sister to Coscinieae, it is not included in Expanded (Exp.) Tinosporeae in this table. ‡Clade 3 is sister to Clade 2 in this analysis, therefore Clades 1 and 2 do not share the same stem node. Ages are in Myr; 95% confidence intervals are given in brackets.

a similar general endocarp shape (Fig. 2). However, *Chandlera* is unique in having the endocarp with a lacunae system and thus differs from all extant Menispermaceae (Scott, 1956; Manchester, 1994).

The *Parabaena* fossils are sister to *Calycocarpum lyonii* (Fig. 2). Only one extant *Parabaena* species is included in this analysis. Thus, the diversity of this genus (Jacques, 2009b) is not well represented. Inclusion of more extant *Parabaena* species may change the results.

Chandler (1961) described the genus *Canticocculus*, which Mai (1987) considered as a section of *Cocculus*. *Canticocculus* differs from *Cocculus* by its subparallel limbs (Chandler, 1961; Mai, 1987). Our results suggest that it may be related to *Sinomenium* and *Menispermum* (Fig. 2), thus in disagreement with Mai's view. Mai (1987) described the fossils that he assigned to *Cocculus* as having a foramen, a character present in *Sinomenium* and *Menispermum* but absent in living *Cocculus*. This character could not be verified on the British specimens examined by one of us (FJ) due to the incompleteness of the material.

Table 6 Results of cross-validation tests for estimated origin for Menispermaceae

	Keep-one		Leave-one
	Mean error	Mean absolute error	
D, *Tinospora*	7.7	13.8	−14.3
E, *Palaeoskapha*	3.4	14.8	3.2
G, *Eohypserpa*	−1.7	19.1	3.0
H, *Sarcopetalum*	−7.0	18.3	22.8
J, *Cissampelos*	−10.6	17.6	25.3
K, *Rhytidocaryon*	−10.1	17.3	24.3
L, *Palaeococculus*	2.1	17.6	−11.5
M, *Triclisia*	−10.4	17.5	31.5

All values are in Myr.

The *Triclisia* fossils from Rusinga described by Chesters (1957) seem to be closer to *Tiliacora*, even though the two genera have the same general morphology of endocarp and show only small differences (Jacques, 2009b).

Brueckelholzia was described by Gregor (1977) as having potential affinities with Tiliacoreae or possibly Menispermeae. In our analysis, *Brueckelholzia* groups with *Hypserpa*, *Legnephora*, and *Parapachygone* (Fig. 2). The latter three genera were included in the former Menispermeae. The hypothesis of Tiliacoreae affinities is therefore rejected.

One fossil species of *Cyclea* was described by Gregor (1977). Our results show that it is more closely related to *Cissampelos* than to *Cyclea* (Fig. 2).

The Australian fossil *Rhytidocaryon* shows affinities with *Cyclea* (Fig. 2). Mueller (1876) proposed some affinities with *Hypserpa*, *Limacia*, *Cocculus*, or *Sarcopetalum*. Rozefelds (1991) concluded that it was not closely related to any of the Australian genera. *Cyclea* is not distributed in Australia (Forman, 2007). Moreover, *Cyclea* endocarps are generally small (Jacques, 2009b), whereas *Rhytidocaryon* endocarps are large and of similar size to *Haematocarpus* (Mueller, 1876).

Eight fossil taxa were not placed on the molecular scaffold. For some of them, like *Thanikaimonia* and *Tinomiscoidea*, only locule casts are available, and therefore many characters are lacking. The observable characters are not sufficient to resolve their placement.

With few exceptions, it is difficult to find strict morphological synapomorphies for clades in Menispermaceae. A straight endocarp with ventral condyle perforation belongs to Expanded Tinosporeae. However,

for most clades, it is mostly a combination of characters that allows group recognition.

3.3 Divergence time estimates

The stem age of Menispermaceae is estimated between 115.6 and 125.0 Myr (Table 5), and the crown age between 103.3 and 124.4 Myr (Table 5), whereas Anderson et al. (2005), studying the basal eudicots, estimated these ages between 105 and 116 Myr, and between 70 and 80 Myr, respectively. Wikström et al. (2001) gave an estimated stem age of Menispermaceae of 103 to 113 Myr. Our results indicate much older ages, placing the crown origin of Menispermaceae before the Early–Late Cretaceous border. The order Ranunculales, to which Menispermaceae belong, is the first diverging lineage from the eudicots (APG, 2003). If we accept the origin of eudicots from the Barremian, based on tricolpate pollen fossil (Hughes & McDougall, 1990; Doyle, 1992), early divergence times of Ranunculales families are possible. This is consistent with the idea based on fossil evidence that major angiosperm lineages diverged in a short time interval (Hickey & Doyle, 1977; Lidgard & Crane, 1988; Crane & Lidgard, 1989; Crane et al., 1995; Wikström et al., 2001). Menispermaceae are older than many eudicot families, such as Euphorbiaceae whose origin was estimated to be between 69 and 71 Myr; Rubiaceae, 61–64 Myr; and Rosaceae, 76 Myr (Wikström et al., 2001). The diversification of Ranunculales at the family level is therefore older than many other clades in eudicots.

The major clades of Menispermaceae emerged during the Late Cretaceous (Fig. 4). This is congruent with the palaeobotanical evidence, which shows that, during this epoch, numerous angiosperm fossils have characteristics of extant families (Stewart, 1983; Crepet et al., 2004; Friis et al., 2006).

The diversification of Expanded Tinosporeae is estimated to have occurred during the Late Cretaceous (Fig. 4), which would explain their relative abundance during the Palaeocene and Eocene (Reid & Chandler, 1933; Chandler, 1961; Manchester, 1994; Jacques & De Franceschi, 2005).

Similar to Tinosporeae, representatives of the former tribe Menispermeae are also often present in the Palaeocene and Eocene (Reid & Chandler, 1933; Chandler, 1961; Manchester, 1994; Jacques & De Franceschi, 2005). The present study, as well as previous molecular analyses (Ortiz et al., 2007; Wang et al., 2007), reconstructs this tribe as polyphyletic. However, differently from the above cited studies, in this study some taxa are recovered in a basal or almost basal position (Figs. 1, 4). However, some tribes are uncommon in the fossil record, such as Tiliacoreae and Anomospermeae. The divergence time of tribe Anomospermeae is estimated at approximately 52.3 Myr, with a crown age of 38.4 Myr (Table 5). This is suggestive of an Eocene diversification of this tribe. Newly described fossil leaves, found in the Palaeocene of Colombia, and included in the genus *Menispermites*, show some similarities with some extant genera of the Anomospermeae and Tiliacoreae (Doria et al., 2008). However, the authors do not use the term "affinities". The confidence interval for divergence of the stem lineage begins in the Late Cretaceous. The Colombian fossils, if confirmed as Anomospermeae, could represent early stages in the evolution of Anomospermeae. The Tiliacoreae started to diversify during the Eocene, 48.5 Myr (32.2–65.1 Myr; Table 5).

The divergence between the two *Menispermum* species, *M. canadense* and *M. dauricum*, which exhibit an Eastern Asian–Eastern North American disjunction, is estimated at 8.2 Myr (Fig. 4). This date is older than the one estimated with internal transcribed spacer sequences (2.35 Myr; Lee et al., 1996), and than the date reported by Xiang et al. (2000) at less than 0.28 Myr. The latter authors only used *rbc*L sequences and did not find any substitution between either species of *Menispermum*. We also used *atp*B sequences, which show four nucleotide substitutions between these two species. The age estimated in the present study corresponds to the usual Late Miocene–Pliocene age found for the divergence time of species showing an Eastern Asian–Eastern North American disjunction (Xiang et al., 2000; Donoghue et al., 2001). This divergence time is congruent with a Beringian pathway hypothesis (Donoghue et al., 2001). Our result stresses the importance of using larger amounts of data in molecular dating (Sanderson, 2003; Renner, 2005).

Wikström et al. (2001) listed four potential origins of error in age estimates: calibration points; "noise"; rate variations that invalidate the model of evolution; and tree topology. The use of the software BEAST, which does not need an input tree (Drummond et al., 2006; Drummond & Rambaut, 2007), minimizes the error due to the input of a wrong topology. However, potential errors might be introduced due to fossil (mis)identifications as well as selection of calibration points. It is widely known that a fossil only provides a minimal age (Doyle & Donoghue, 1993; Wikström et al., 2001; Renner, 2005), and its placement on the stem or crown nodes of a clade modifies age estimates (Forest et al., 2005). An advantage of the use of Bayesian approaches to estimate divergence time is that it gives confidence intervals for the ages that account for the errors in the estimation of branch length (Renner, 2005). Similarly, the BEAST approach handles datasets with different models of evolution (Drummond et al., 2006;

Rutschmann, 2006). This latter aspect of the BEAST approach becomes relevant in our study for it improves the fit of the model to our dataset. However, it is important to keep in mind that our results strongly rely on the parameters and models used. Using a cladistic analysis to place the fossils, that is, calibration points, as in the present study (Fig. 2), limits the problems of fossil identification. It also limits the problem of deciding whether to use a fossil to constrain a stem age or a crown age. The cross-validation process is a way to analyze how each calibration point influences the results of divergence time estimation.

3.4 Sensitivity analysis

Analyzing the data without any maximal age clearly increases the age estimates of deep nodes (stem node back to mid-Jurassic), but is less influential on derived nodes (Table 5). Thus, when no maximal is included, the age of Menispermaceae, stem or crown, is estimated as being older than the 125 Myr age of the first fossil record of eudicots (Hughes & McDougall, 1990; Doyle, 1992). Many divergence time estimates reconstructed on all angiosperms also give inconsistencies between molecular estimates and fossil data, with molecular estimates being far older (Soltis et al., 2002). One possible explanation is the existence of gaps in the fossil record (Soltis et al., 2002), but in the case of eudicots, those gaps seem unlikely (Crane et al., 1989). The incongruencies may also be due to the strong constraint of calibration points or to differences in evolution rates between deep branches and derived groups (Wikström et al., 2001). Inconsistencies about the age of the eudicots are discussed in greater detail by Anderson et al. (2005). In some of our analyses, the results tend to underestimate ages for nodes towards the terminals. In this case, an explanation might be the sparse taxonomic sampling (Wikström et al., 2003). Including more sequences representing infrageneric diversity might increase branch length near terminals.

If the *Prototinomiscium* constraint for the whole family is excluded from the analysis, some divergence estimates are slightly older than when it is included (Table 5). However, overall estimates with and without *Prototinomiscium* are very similar. This indicates that *Prototinomiscium* is not the strongest constraint in the analysis. Slightly older ages can be explained by the Bayesian nature of the analysis. Because values result from a sampling procedure of a stochastic process, small differences are expected between replicates of the same file.

If *Prototinomiscium* is included as calibration point C, ages of Clade 1 are generally older, and ages of Clades 2 and 3 are younger than in the first analysis (Table 5). All the other nodes are then "overestimated" (mean error and mean absolute error, 16.6 Myr). In this case, *Prototinomiscium* only constrained Clade 1. Constraining the whole family with *Prototinomiscium* is therefore not a strong constraint at the family level.

If we consider other calibration points, all tend to "overestimate" some points and underestimate others (Table 6). This suggests that all points tend to constrain older ages in some parts of the tree, but fail in giving estimates old enough in all parts of the tree. Our results confirm previous analyses showing that a single calibration is often problematic in estimating divergence times (Kress et al., 2001; Renner & Meyer, 2001; Soltis et al., 2002). For Menispermaceae, as for angiosperms and all seed plants, estimated ages are older when constraints are applied than when they are not (Magallón & Sanderson, 2005). The incompleteness of the fossil record (Soltis et al., 2002) may explain this. A fossil only provides a minimal age (Doyle & Donoghue, 1993; Wikström et al., 2001; Renner, 2005) but the actual node age is always older than the age of the fossil calibrating it. Therefore the fossil underestimates the age of its "own" node and may also underestimate other node ages. Some fossils were rejected as they give low estimates (Table 4). We measured the mean difference between fossil age and divergence time estimates for the rejected calibration points and found 42.5 Myr when all constraints were included and 14.3 Myr when only J (*Cissampelos*) was kept (as it generally gave the lowest estimates). Furthermore, if some fossil leaves described from the Palaeocene by Doria et al. (2008) were confidently assigned to Anomospermeae, then the age of this group would be clearly older than what we estimated, and *Sarcopetalum* would be shown to underestimate the age of node H.

The maximal age constraint for the whole family and the minimal age fossil constraints represent strong constraints. A possible explanation is that the rate of evolution is higher in basal branches than it is in derived branches. Several causes can account for differences in substitution rates (Bromham, 2009). Among them are the generation time and habit (Smith & Donoghue, 2008), high or low level energy environments (Davies et al., 2004), the number of DNA replication events per generation (Bartosch-Harild et al., 2003), and population size (Lynch, 2007).

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Appendix I

List of specimens, accession numbers and morphological coding. After each species sampled, the voucher specimen is indicated followed by the accession numbers of *rbc*L, then *atp*B. When the voucher is different for the two sequences, a new voucher is indicated before the second accession number. The vouchers only correspond to molecular sequences, not to morphological data.

Menispermaceae: ***Abuta grandifolia*** **(Mart.) Sandwith**, Balslev 60630, DQ099443, Ecuador, C Ott 63 (MJG), FJ026398; ***Abuta rufescens*** **Aubl.**, Peru, Ortiz & al. 226 (MO), HQ260756, HQ260812; ***Albertisia laurifolia*** **Yamamoto**, China, Hong YP 99371 (PE), HQ260757, HQ260813; ***Albertisia papuana*** **Becc.**, cult. Bogor, M Chase 1315 (K), FJ026399 cult. Bogor, F Jacques 10 (P), EU526982; ***Albertisia porcata*** **Breteler**, Gabon, McPherson 16678 (MO), HQ260758, HQ260814; ***Anamirta cocculus*** **(L.) Wight & Arn.**, cult. Meise, D Aplin S4042 (BR), EU526983; Thailand, Wang H-C 1003 (PE), HQ260815; ***Anisocycla linearis*** **Pierre ex Diels**, Madagascar, Hong-Wa & al. 466 (MO), HQ260759, HQ260816; ***Anomospermum chloranthum*** **Diels**, Costa Rica., Ortiz & Aguilar 324 (MO), HQ260760, HQ260817; ***Anomospermum grandifolium*** **Eichl.**, Peru, Ortiz & al. 243 (MO), HQ260761, HQ260818; ***Anomospermum solimoesanum*** **(Moldenke) Krukoff & Barneby**, Ecuador, Ortiz & Vargas 198 (MO), HQ260762, HQ260819; ***Antizoma angustifolia*** **(Burch.) Mies ex Harv. & Sond.**, Blomberg 583, DQ099437, no *atp*B available; ***Arcangelisia flava*** **(L.) Merr.**, cult. Kepong, F Jacques 26 (P), HQ260763, EU526980; ***Arcangelisia gusanlung*** **H.S. Lo**, China, Hong YP 99406 (PE), HQ260764, HQ260820; ***Aspidocarya uvifera*** **Hook. f. & Thoms.**, China, Hong YP 99190 (PE), HQ260765, HQ260821; ***Beirnaertia cabindensis*** **(Exell & Mendonca) Troupin**, Gabon, Walters & Niangadouma 1267 (MO), HQ260766, HQ260822; ***Borismene japurensis*** **(Mart.) Barneby**, cult. Meise, D Aplin S4033 (BR), EU526984, EU526979; ***Burasaia apetala*** **Capuron ex Westerhaus**, Madagascar, A Westerhaus 241 (UWM), FJ026464, FJ026404; ***Burasaia madagascariensis*** **Thou.**, Madagascar, Rabenantoandro & al. 1262 (MO), HQ260767, HQ260823; ***Calycocarpum lyonii*** **Nutt. ex A. Gray**, USA, Ortiz & al. 335 (MO), HQ260768, HQ260824; ***Carronia protensa*** **(F. Muell.) Diels**, Australia, van der Werff & Gray 17049 (MO), HQ260769, HQ260825; ***Caryomene grandifolia*** **Barneby & B.A. Krukoff**, Peru, Zárate 2136 (MO), HQ260770, HQ260826; ***Chasmanthera dependens*** **Hochst.**, Thulin 6769, DQ099445, cult. in California State University, Chico, S Hoot 08–1 (UWM), FJ026407; ***Chasmanthera welwitschii*** **Troupin**, cult. Meise, D Aplin S4040 (BR), EU526985, HQ260827; ***Chondrodendron tomentosum*** **Ruiz & Pav.**, Peru, Ortiz & Vásquez 217 (MO), HQ260771, HQ260828; ***Cissampelos andromorpha*** **DC.**, Peru, Ortiz & al. 302 (MO), HQ260772, HQ260829; ***Cissampelos capensis*** **Thunb.**, South Africa, E van Jaarsveld 13831 (NBG), FJ026471, FJ026411; ***Cissampelos grandifolia*** **Triana & Planch.**, Ecuador, C Ott 53 (MJG), FJ02642, FJ026412; ***Cissampelos owariensis*** **Beauv. ex DC.**, cult. Meise, D Aplin S4039 (BR), EU526986; EU526978; ***Cissampelos pareira*** **L.**, AF197590, AF197613; ***Cissampelos tropaeolifolia*** **DC.**, Ecuador, C Ott 5 (MJG), FJ026475, FJ026415; ***Cocculus carolinus*** **(L.) DC.,** USA, Ortiz & Pruski 349 (MO), HQ260773, HQ260830; ***Cocculus orbiculatus*** **(L.) DC**, China, Hong YP H419 (PE), HQ260774, HQ260831; ***Cocculus orbiculatus*** **var.** ***orbiculatus***, L12642, no *atp*B available; ***Cocculus pendulus*** **(J.R. & G. Forst.) Diels**, Pakistan, D De Franceschi s.n. (P), EU526987, EU526975; ***Coscinium blumeanum*** **Miers ex Hook. f & Thoms.**, cult. Kepong, F Jacques 27 (P), HQ260775, EU526974; ***Coscinium fenestratum*** **Colebr.**, Sri Lanka, M Chase 17404 (K), FJ026479, FJ026419; ***Curarea candicans*** **(L.C. Richard ex DC.) Barneby & Krukoff**, Guyana, Torke 310 (MO), HQ260776, HQ260832; ***Curarea toxicofera*** **(Wedd.) Barneby & Krukoff**, Ecuador, C Ott 61 (MJG), FJ026480, FJ026420; ***Cyclea burmannii*** **Miers**, Sri Lanka, M Chase 17394 (K), FJ026481, FJ026481; ***Cyclea hypoglauca*** **(Schauer) Diels**, China, Chen ZD & al. 9812108 (PE), HQ260777, HQ260833; ***Dioscoreophyllum cumminsii*** **(Stapf) Diels**, cult. Meise, D Aplin S4049 (BR), EU526988, EU526972;

***Diploclisia glaucescens* (Bl.) Diels**, cult. South China Bot Gard, Hong YP 99403 (PE), HQ260778, HQ260834; ***Disciphania killipii* Diels**, Peru, Ortiz & Zárate 310 (MO), HQ260779, HQ260835; ***Elephantomene eburnea* Barneby & Krukoff**, Peru, Ortiz & al. 237 (MO), HQ260780, HQ260836; ***Fibraurea tinctoria* Lour.**, cult. Bogor, F Jacques 04 (P), HQ260781, EU526970; ***Haematocarpus validus* Bakh. f. ex Forman**, Himalayas, M Chase 1321 (K), FJ026486, FJ026426; ***Hyperbaena domingensis* (DC.) Benth.**, Ecuador, van der Werff & al. 19586 (MO), HQ260782, HQ260837; ***Hyperbaena illicifolia* Standl.**, Mexico, E Lott (NY), FJ026487, FJ026427; ***Hypserpa decumbens* (Benth.) Diels**, Australia, van der Werff 17057 (MO), HQ260783, HQ260838; ***Hypserpa laurina* (F. Muell.) Diels**, Australia, S Gleed 2 (Johnstone Regional Herbarium), FJ026489, FJ026429; ***Hypserpa nitida* Miers ex Benth.**, China, Hong YP 99378 (PE), HQ260784, HQ260839; ***Jateorhiza macrantha* (Hook. f.) Exell & Mendonca**, Cameroon, Kenfack & Zapfack 2039 (MO), HQ260785, HQ260840; ***Kolobopetalum leonense* Hutchinson & Dalziel**, Ghana, Schmidt & al. 3435 (MO), HQ260786, HQ260841; ***Legnephora moorei* (F. Muell.) Miers**, Australia, van der Werff & Gray 17053 (MO), HQ260787, HQ260842; ***Leptoterantha mayumbensis* (Exell) Troupin**, Democratic Republic of Congo, Ewango 3005 (MO), HQ260788, HQ260843; ***Limacia blumei* (Boerl.) Diels**, cult. Bogor, F Jacques 07 (P), EU526989, EU526968; ***Menispermum canadense* L.**, AF190437, AF093384; ***Menispermum dauricum* DC.**, AF190436; cult. Beijing, Hong YP 99095 (PE), HQ260844; ***Odontocarya tripetala* Diels**, Peru, Ruiz 5601 (MO), HQ260789, HQ260845; ***Odontocarya truncata* Standl.**, Costa Rica, Hammel & Perez 22567 (MO), HQ260790, HQ260846; ***Orthomene hirsuta* (Krukoff & Moldenke) Barneby & Krukoff**, Peru, Ortiz & al. 308 (MO), HQ260791, HQ260847; ***Orthomene schomburgkii* (Miers) Barneby & Krukoff**, Brazil, W Thomas & al. 12197 (MO), FJ026495, FJ026435; ***Pachygone valida* Diels**, China, Hong YP 99247 (PE), HQ260792, HQ260848; ***Parabaena sagittata* Miers ex Hook. f. & Thoms.**, China, Hong YP H346 (PE), HQ260793, HQ260849; ***Parapachygone longifolia* (E.M. Bailey) Forman**, Australia, S Gleed 4 (Johnstone Regional Herbarium), FJ026498, FJ026438; ***Penianthus longifolius* Miers**, Cameroon, Sweeney & al. 1436 (MO), HQ260794, HQ260850; ***Penianthus patulinervis* Hutch. & Dalziel**, Ghana, M Merello & al. 1415 (MO), FJ026500, FJ026440; ***Pericampylus glaucus* (Lam.) Merr.**, Ryding 671, DQ099442, FJ026441; ***Pycnarrhena longifolia* (Decne. ex Miq.) Becc.**, cult. Bogor, F. Jacques 15 (P), EU526993, EU526965; ***Pycnarrhena tumefacta* Miers**, cult. Bogor., M Chase 1323 (K), FJ026502, FJ026442; ***Pycnarrhena novoguineensis* Miq.**, Australia, Gray 8794 (MO), HQ260795, HQ260851; ***Rhaptonema* sp.**, Madagascar, McPherson 18854 (MO), HQ260796, HQ260852; ***Rhigiocarya racemifera* Miers**, Cameroon, Kenfack 1655 (MO), HQ260797, HQ260853; ***Sarcopetalum harveyanum* F. Muell.**, Australia, van der Werff 17058 (MO), HQ260798, HQ260854; ***Sciadotenia amazonica* Eichl.**, Peru, Ortiz & Zárate 264 (MO), HQ260799, HQ260855; ***Sciadotenia toxifera* Krukoff & A.C. Sm.**, Peru, Ortiz & al. 231 (MO), HQ260800, HQ260856; ***Sinomenium acutum* (Thunb.) Rehder & E.H. Wilson**, China, Hong YP H006 (PE), HQ260801, HQ260857; ***Stephania japonica* (Thunb.) Miers**, Australia, I Solomon 681 (PERTH), FJ026507, FJ026447; ***Stephania laetificata* (Miers) Benth.**, Central African Republic, D Harris 4964 (E), FJ026508, FJ026448; ***Stephania longa* Lour.**, China, Hong YO H101 (PE), HQ260802, HQ260858; ***Stephania rotunda* Lour.**, cult. Meise, FJ026509, FJ026449; ***Stephania venosa* (Bl.) Spreng.**, cult. Bogor, F Jacques 01 (P), EU526996; EU526963; ***Strychnopsis thouarsii* Baill.**, Madagascar, Schatz & al. 3728 (MO), HQ260803, HQ260859; ***Syntriandrium preussii* Engl.**, MK 8407 (PE), HQ260804, HQ260860; ***Telitoxicum peruvianum* Moldenke**, Peru, Ortiz & al. 218 (MO), HQ260805, HQ2608061; ***Tiliacora acuminata* (Lam.) Hook. f. & Thoms.**, cult. Bogor, F Jacques 11 (P), EU526997, HQ260862; ***Tinomiscium petiolare* Hook. f. & Thoms.**, China, Hong YP H142 (PE), HQ260806, HQ260863; ***Tinospora caffra* (Miers) Troupin**, L37923, L37933; ***Tinospora esiangkara* (F.M. Bailey) Forman**, Australia, Gray 8927 (MO), HQ260807, HQ260864; ***Tinospora sinensis* (Lour.) Merr.**, Thailand, Wang HC 109 (PE), HQ260808, no *atp*B available; ***Tinospora smilacina* Benth.**, Australia, Gray 8798 (MO), HQ260809, HQ260865; ***Triclisia dictyophylla* Diels**, Cameroon, Kenfack & Zapfack 2038 (MO), HQ260810, HQ260866; ***Triclisia* sp.**, Madagascar, A Westerhaus 254 (UWM), FJ026517, FJ026457; ***Triclisia subcordata* Oliv.**, Ghana, Kenfack 2101 (MO), HQ260811, HQ260867.

Outgroups: Berberidaceae: *Mahonia bealei* (Fortune) Carrière, L12657.2; AF197611.1. *Podophyllum peltatum* L., AF1975591.1; AF197612.1. **Lardizabalaceae:** *Akebia quinata* Decne, L12627; AF209523.1. *Boquila trifoliata* Decne, L37915.1; L37925.1. **Ranunculaceae:** *Glaucidium palmatum* Siebold & Zucc., AF093723.1; AF093375.1. *Ranunculus macranthus* Scheele, DQ069502.1; DQ069346.1.

Appendix II Morphological coding of extant and fossil Menispermaceae. Character codings are written in order from character 1 to character 30.

Operational taxonomic unit	Type	Coding
Abuta grandifolia	Extant	100000100?000??10000010000?02
Abuta rufescens	Extant	1000?0100?000??10000010000?02
Albertisia laurifolia	Extant	1000?0100?000??20000010000?02
Albertisia papuana	Extant	1000?0110?000??0000000???????2
Albertisia porcata	Extant	100??0????????????00?0???????2
Anamirta cocculus	Extant	1100?0000?000??200???11110??12
Anisocycla linearis	Extant	1000?0100?000??000000110000?02
Anomospermum chloranthum	Extant	1000?01?????0?????00?????????2
Anomospermum grandifolium	Extant	1000?01???????????00?????????2
Anomospermum solimoesanum	Extant	1000?0100?000??00000?????????2
Antizoma angustifolia	Extant	1001?0?1??1000????00?111?01??1
Arcangelisia flava	Extant	1110?0?00?000?0000???0???????2
Arcangelisia gusanlung	Extant	1110?0?00?000?0000???0???????2
Aspidocarya uvifera	Extant	1010?1?121000?1?10???0???????0
Beirnaertia cabindensis	Extant	1000?0100?000??100000110000?02
Borismene japurensis	Extant	1010?0?00?000?0000???11010?012
Burasaia apetala	Extant	1010?0??????0?0??????1??1????0
Burasaia madagascariensis	Extant	1010?0?00?000?0?10???11010?010
Calycocarpum lyonii	Extant	1010?0?10?000?1010???11110?110
Carronia protensa	Extant	1000?01110000??000010110000?02
Caryomene grandifolia	Extant	1000?0100?000??001000110000?02
Chasmanthera dependens	Extant	1010?0?111000?1000???11011?110
Chasmanthera welwitschii	Extant	1010?0?111000?1000???11011?100
Chondrodendron tomentosum	Extant	1000?01????0??????00?110000??2
Cissampelos andromorpha	Extant	10000001201000?000000111001?01
Cissampelos capensis	Extant	10010011201000?000000111001?01
Cissampelos grandifolia	Extant	100?00?1201000?000000111001?01
Cissampelos owariensis	Extant	10010011200000?000000111001?01
Cissampelos pareira	Extant	10010001201000??00000111001?01
Cissampelos tropaeolifolia	Extant	100?00?1201000?000000111001?01
Cocculus carolinus	Extant	10010001101001?000100111001?01
Cocculus orbiculatus	Extant	10010001101001?000100111201?01
Cocculus orbiculatus var. *orbiculatus*	Extant	10010000101001?000100111001?01
Cocculus pendulus	Extant	10010001000001??00100111001?01
Coscinium blumeanum	Extant	1100?0000?000??000??0101101?12
Coscinium fenestratum	Extant	1100?0000?000??000???101101?12
Curarea candicans	Extant	1000?0?????????0??00?1???????2
Curarea toxicofera	Extant	100??0100?00???00?00?1???????2
Cyclea burmannii	Extant	100?00012000?1?00000111?01?01
Cyclea hypoglauca	Extant	10000001200001?01000111001?01
Dioscoreophyllum cumminsii	Extant	1010?0?100000?1?10???11010?010
Diploclisia glaucescens	Extant	10010011101000?000110111001?01
Disciphania killipii	Extant	1010?1?131000?0000???0???????0
Elephantomene eburnea	Extant	1000?0100?000??10000010000?02
Fibraurea tinctoria	Extant	1010?0?00?010?0000???0???????2
Haematocarpus validus	Extant	100000100?000??000???1?0?00?02
Hyperbaena domingensis	Extant	1000?0110?100??200000110000?02
Hyperbaena illicifolia	Extant	100000?1???00????0?00111?00?02
Hypserpa decumbens	Extant	100?00000??001?000000111?0?1?02
Hypserpa laurina	Extant	10010001100?001??00000111201?02
Hypserpa nitida	Extant	100100010000?1?20000111001?02
Jateorhiza macrantha	Extant	1010?0?10?000?1000???11010?010
Kolobopetalum leonense	Extant	1010?0????000?1?10???11011?110
Legnephora moorei	Extant	10011001110000?010101111001?01
Leptoterantha mayumbensis	Extant	1010?0?121000?1000???11011?100
Limacia blumei	Extant	10001001000001?000000111201?01
Menispermum canadense	Extant	10011001101010?000001111001?01
Menispermum dauricum	Extant	10011001101010?000001111001?01
Odontocarya tripetala	Extant	1010?0?121000?10?0???11011?010
Odontocarya truncata	Extant	1010?0?121000?10?0???11011?010
Orthomene hirsuta	Extant	1010?0?00?000?0000???00??????2
Orthomene schomburgkii	Extant	1010?0?00?000?0100???0???????2
Pachygone valida	Extant	100000000?0001?100100111001?02
Parabaena sagittata	Extant	1010?0?130000?1?10???11010?110
Parapachygone longifolia	Extant	1?0??0?1?????????????1?1?01???
Penianthus longifolius	Extant	1010?0?00?010?0000???0???????2
Penianthus patulinervis	Extant	1010?0?00?010?0000???0???????2

Continued.

Appendix II Continued.

Operational taxonomic unit	Type	Coding
Pericampylus glaucus	Extant	10011001200000?01000111001?01
Pycnarrhena longifolia	Extant	1100000????000???000?0???????2
Pycnarrhena tumefacta	Extant	1100?0?00?000?????00?10??????2
Pycnarrhena novoguineensis	Extant	110000000?0000?0000000???????2
Rhaptonema sp.	Extant	100000000?000????0?0?111001?02
Rhigiocarya racemifera	Extant	1010?0?1A0000?1?10???11011?110
Sarcopetalum harveyanum	Extant	10011001100010?01000111001?01
Sciadotenia amazonica	Extant	100??0000?00??????0001??????02
Sciadotenia toxifera	Extant	1001?0000?000?????000111?01?02
Sinomenium acutum	Extant	10011001101010?000001111001?01
Stephania japonica	Extant	10011001101000?000001111001?01
Stephania laetificata	Extant	10011001100000?001001111001?01
Stephania longa	Extant	10011001100000?01000111201?01
Stephania rotunda	Extant	10011001101000?000001111201?01
Stephania venosa	Extant	10011001101000??0000?111201?01
Strychnopsis thouarsii	Extant	10001001100001?200101111001?02
Syntriandrium preussii	Extant	1010?0?130000?1000???11011?010
Telitoxicum peruvianum	Extant	1000?0100?000??100000100000?02
Tiliacora acuminata	Extant	1000?01110000??200010111000?02
Tinomiscium petiolare	Extant	1010?1?100000?1001???0???????0
Tinospora caffra	Extant	1010?0?110000?1000???11010?010
Tinospora esiangkara	Extant	1010?0?100000?1?00???11010?010
Tinospora sinensis	Extant	1010?0?100000?1?00???11010?010
Tinospora smilacina	Extant	1010?0?100000?1?00???11?10?010
Triclisia dictyophylla	Extant	1000?01100000??000010111000?02
Triclisia sp.	Extant	1000001????00???00010111000?02
Triclisia subcordata	Extant	1000?011?0000??000010111000?02
Mahonia bealei	Outgroup	0??????00?00???0?????0????????
Podophyllum peltatum	Outgroup	0??????00?00???0?????0????????
Akebia quinata	Outgroup	0??????00?00???0?????0????????
Boquila trifoliata	Outgroup	0??????00?00???0?????0????????
Glaucidium palmatum	Outgroup	0??????00?00???0?????0????????
Ranunculus macranthus	Outgroup	0??????00?00???0?????0????????
Anamirta Colebr. 1822	Fossil	1100?0010?000??000??0111101?12
Atriaecarpum Chandler 1978	Fossil	1010?1?10?000?0201???11110?000
Bowerbankella Reid & Chandler 1933	Fossil	1001?0010?000??2001??111001?02
Brueckelholzia Gregor 1977	Fossil	10011001100001?000101111001?01
Calycocarpum Nutt. ex Torr. & Gray 1838	Fossil	1010?0?10?000?1000???11110??10
Chandlera Scott 1954	Fossil	1010?1?00?000?0001???11100?000
Cissampelos L. 1753	Fossil	10010011201000?000000111001?01
Cocculus DC. 1817	Fossil	10011001101012?000101111001?0?
Curvitinospora Manchester 1994	Fossil	1010?0?10??00?1?????1111??010
Cyclea Arn. ex Wight 1840	Fossil	10000001201000?000000111001?01
Davisicarpum Chandler 1961	Fossil	10001001000002?000000111001?01
Diploclisia Miers 1851	Fossil	10011011101000?000010111201?01
Eohypserpa Reid & Chandler 1933	Fossil	10010001000001?000000111001?02
Frintonia Chandler 1961	Fossil	1010?1?10?000?0?10???11110?000
Jateorhiza Miers 1849	Fossil	1010?0?10?000?1000???11010?010
Menispermum L. 1735	Fossil	10011001101010?000001111001?01
Microtinomiscium Reid & Chandler 1933	Fossil	1010?1?1???00?0??1???11110?010
Odontocaryoidea Scott 1954	Fossil	1010?0?10?000?1001???11110?110
Palaeococculus Chandler 1961	Fossil	10010001101002?000100111001?01
Palaeosinomenium Chandler 1961	Fossil	10011001101010?000101111001?01
Palaeoskapha Jacques & Guo 2007	Fossil	1010?0??11000?1000???1111??110
Parabaena Miers 1851	Fossil	1010?0?10?000?1?10???11110?110
Prototinomiscium Knobloch & Mai 1984	Fossil	1010?0?100?00?1200???01110?110
Rhytidocaryon Mueller 1876	Fossil	10000001201001??00000111001?0?
Sarcopetalum F. Muell. 1860	Fossil	10011001100010?01000111001?01
Sinomenium Diels 1910	Fossil	10011001101010?000001111001?01
Stephania Lour. 1790	Fossil	10011001101000?000?00111201?01
Syntrisepalum Chesters 1957	Fossil	1010?0?10?000?1200???11011??10
Thanikaimonia Manchester 1994	Fossil	10010001???000????00?111001?01
Tinomiscium Miers ex Hook. f. & T. Thomson 1855	Fossil	1010?1?100000?0200???0???????0
Tinomiscoidea Reid & Chandler 1933	Fossil	1010???????00?1??????11?1???10
Tinospora Miers 1851	Fossil	101010?10?000?120????11010?010
Triclisia Benth. 1862	Fossil	1000?01110000??200000111000?02
Wardensheppeya Eyde 1970	Fossil	10011001101010?000001111001?01

?, unknown or not applicable; A, {0/1}. See Table 2 for definitions of characters and character states.