Resolving phylogenetic reconstruction in Menispermaceae (Ranunculales) using fossils and a novel statistical test

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Menispermaceae (Moonseed family) are an important family within the basal eudicots. This study is a first attempt to infer phylogenetic relationships within Menispermaceae, focusing on morphological data and using cladistic methodology. Sampling included genera from all the eight tribes usually recognized within the family, and was representative of the morphological diversity and worldwide distribution of the group. Preliminary trees were poorly resolved and were optimized using Reduced Cladistic Consensus (RCC) and NoiseSnapper. The latter program was used for the first time in phylogeny. In addition, fossils of the Eocene of the Paris Basin were included in the analysis. All three approaches were useful as they allowed for improvement of resolution. None of the tribes were found to be monophyletic except Menispermeae. We also show that fruit characters are unreliable and should not be used for classification.

KEYWORDS: cladistics, fossil, Menispermaceae, morphology, NoiseSnapper, phylogeny, Reduced Cladistic Consensus (RCC)

INTRODUCTION

The family Menispermaceae Juss. (Moonseed family) is composed of 71 genera and about 520 species (pers. estimation); it is part of the Ranunculales, in the basal eudicots (Hoot & al., 1999). According to Savolainen & al. (2000), the family is sister to Berberidaceae-Ranunculaceae, and its position is based on the following characters: dioecy, actinomorphic, trimeric, hypogyny, a heterochlamydeous, dialypetalous and oppositipetalous flower, apocarpy, and a drupaceous fruit. The geographical distribution of Menispermaceae is pantropical with a few species in temperate regions. The classification established by Diels in 1910 is still widely accepted. Fruit characters constitute the basis of Diels’ system and their utility in classification was confirmed by Barneby (1972) for Tiliacoreae and Anomospermeae. A revised system was proposed by Kessler (1993) based on a more limited study of the family.

The family is composed of 8 tribes, the valid names of which are (Forman, 1982): Menispermeae DC. (19 genera), Tiliacoreae Miers (17 genera), Anomospermeae Miers (6 genera), Coscinieae Hook. f. & Thoms. (3 genera), Tinosporeae Hook. f. & Thoms. (19 genera), Penniathae Diels (2 genera), Fibraureae Diels (4 genera), Hyperbaenae Diels (1 genus). Most authors agree on the necessity for a revision of the family. Thus, Forman (1985) wrote: “The tribes and generic delimitation of Menispermaceae need reassessment on a multidisciplinary basis, for which further data are still required.” Forman (1985) also considered merging Tinosporeae and Fibraureae, suggested earlier by Barneby (1972). According to Fairon-Demaret & Smith (2002), however, Fibraureae should be included in Coscinieae. With these concerns in mind, the present cladistic analysis is a first step toward revising these relationships and the associated nomenclature.

This study is a first attempt to infer phylogenetic relationships within Menispermaceae, focusing on morphological data and using cladistic methodology. The study is based on extant representatives from all tribes as well as Eocene fossils from the Paris Basin. Although Eocene fossils of Menispermaceae from Europe and North America are well studied (Reid & Chandler, 1933; Chandler, 1961; Collinson, 1988; Manchester, 1994; Fairon-Demaret & Smith, 2002), several newly described fossil taxa are included in the analysis (Jacques & De Franceschi, 2005). The study is also the first application of “NoiseSnapper” (Zaragüeta i Bagils & al., 2003) to improve the cladogram resolution.

MATERIALS AND METHODS

Taxon sampling. — We analysed 22 (out of 71) genera of Menispermaceae based on material present in French National Herbarium in Paris (P) (Appendix 1). We selected
two or more genera from each tribe (except the small tribe Peniantheae and the monotypic Hyperbaenaceae) to test the monophyly of the tribes, and sampled from throughout the tropics (South America, Africa, Asia, Australia). Each genus was represented by at least one species (Appendix 2) considered to be typical for that genus. As most genera have only few species, our study was reasonably representative of species diversity in the family.

Our hypothesis was rooted using several outgroups. Outgroup taxa belonged to Berberidaceae (Berberis L.), the monotypic Circoeasteraceae (Circoeaster Maximowicz), Lardizabalaceae (Boquila Decne, Decaisnea Hook. f. & Thoms., Holboellia Wall., Lardizabala Ruiz & Pav., Stauntonia DC.), and Ranunculaceae (Ranunculus Hall.). To root the tree, we used two genera of Papaveraceae (Papaver L., Fumaria L.). Only tropical taxa were selected. Most families of Ranunculales were represented; thus, we tried to avoid problems caused by potential errors in Angiosperm phylogeny (Savolainen & al., 2000) that might result in incorrect familial relationships for this sister group of Menispermaceae (only represented by one sequence).

The matrix includes 32 extant taxa. Fossilized endocarps from “Le Quesnoy” outcrop in Paris Basin, dating from the Lower Eocene, were added. These fossils belong to eight genera, Anamirta Colebr., Atriecarpum Chandler, Eohypserpa Reid & Chandler, Palaeococcus Chandler, Tinomiscium Miers, Tinospora Miers and Wardenshephey Eyde, and represent the known Eocene diversity of Menispermaceae. Some fossils have been assigned to extant genera. We considered these separately in the analysis, to test the attribution of the fossils to extant genera and avoid doubtful identifications (Jacques & De Franceschi, 2005). The matrix with the fossils includes 39 taxa.

**Morphological data.** — Characters were coded (see Appendix 3) from all the parts of the plants: flowers, fruits, seeds, inflorescences, habit, leaves and pollen. Morphological information was extracted from specimen observations when possible; otherwise, data were collected in literature (for general considerations: Bentham & Hooker, 1862; Baillon, 1872; Engler & Prantl, 1894; Thanikaimoni, 1984; for American species: Krukoff & Moldenke, 1938; Krukoff & Barneby, 1970; Barneby, 1970, 1972; for Asian species: Forman, 1956, 1957, 1962, 1968, 1972, 1974, 1975, 1978, 1981, 1984, 1985; for African species: Troupin, 1962). Palynological data were found in Erdtman (1952, 1969), Kremp (1965), Thanikaimoni (1968), Kallis (1979), Nowicke & Shvarla (1981, 1982), Harlen & Ferguson (1982), Harley & Ferguson (1985). The information from the literature was standardized in order to allow character coding. Special attention was paid to fruit characters such as the shape of the endocarp, which varies considerably (Fig. 1), and the condyle. The latter is an intrusion of the endocarp into the seed cavity (Dekker, 1983) characteristic of Menispermaceae, which lacks in putatively related families. Endocarp characters of the outgroup taxa were coded as inapplicable when the fruit was not drupaceous. New observations were made on specimens from the French National Herbarium (P) for extant taxa and on specimens from palaeobotanical collections of the Muséum National d’Histoire Naturelle, Paris (Appendix 2).

The matrix (Appendix 4) is composed of 58 characters (21 binary characters, 37 unordered multi-state) and 39 taxa (32 extant, 7 fossil). All 58 characters included in the data matrix are informative. Characters 56, 57 and 58 are constant within Menispermaceae and support the monophyly of the family. Proportion of missing data in the dataset excluding fossils is 7.5%. Most herbarium specimens are male, therefore characters of fruits, seeds, and female flowers have the highest proportion of missing data, as already underlined by Krukoff & Barneby (1970). For fossils, only endocarp characters apply. Non-applicability represents 4.9% of the data. (The missing information cannot be retrieved by observation of addi-
tional specimens as species with a fleshy endocarp lack data on endocarp sclerification, etc.). In total, 12.4% of the matrix cells lack information, while half of the extant taxa feature one or more polymorphic characters due to intraspecific variability.

**Analyses.** — Tree searching was done with PAUP* 4.0b10 (Swofford, 1998), using heuristic searches with 10 random additions, TBR (Tree Bisection and Reconnection) swapping algorithm, and multrees “on”. Two different analyses were conducted: with and without fossils. Polymorphic taxa were treated as such. Bremer (1994) support was measured for each analysis.

The consensus trees were obtained with RadCon (Thorley & Page, 2000), which not only provides the strict component consensus tree (Wilkinson, 1994) but also the Reduced Cladistic Consensus (RCC). The RCC method recursively prunes taxa: first, one by one; then, two by two, and so on. It computes the reduced strict consensus at each stage, the RCC being retained when resolution is improved. Conditions for computation of RCC trees are unambiguity, nonredundancy and informativeness (Wilkinson, 1994). To avoid loss of information due to pruned taxa, all RCC are presented in a profile including the n-taxon statements common to every fundamental trees. RadCon subsequently orders the RCC trees according to the number of taxa and information content. In this paper, RCC trees are numbered according to RadCon ordering. The RCC must be interpreted carefully, however, since monophyly cannot be deduced from a RCC because of the unknown position of the pruned taxa (Fig. 2). We can only conclude that the taxa within a group, for a given RCC, are closer to each other than they are to taxa outside the group.

The fit of each character was evaluated a posteriori from all most parsimonious trees using the beta version of NoiseSnapper (Zaragüeta i Bagils & al., 2003). This study is the first application of the NoiseSnapper method in empirical phylogenetic investigation. NoiseSnapper is a statistical test; it measures the fit of a character to a given tree using the retention index (RI) and can be downloaded at http://lis.snv.jussieu.fr/apps/ noisesnapper. The null hypothesis is that each character fits to a tree as if it was random character. If the null hypothesis is rejected, the fit of the character is significantly better than the fit of a random character. This method determines a retention index threshold, below which each character behaves as if it was randomly coded. If the search yields several most parsimonious trees, NoiseSnapper may help to select the tree(s) having the best overall character fit. The parsimony criterion is equivalent to the maximization of synapomorphies, or as a corollary, the minimization of homoplasies. The tree(s) having the highest proportion of primary homology statements retained as secondary homology provide reference for this term, as detected by NoiseSnapper, may be preferred. Here, we used NoiseSnapper to reduce the number of most parsimonious trees. For comparison, we also used successive weighting as implemented in PAUP (reweight according to RI, using the maximum value if more than one tree).

The RCC and NoiseSnapper methods address two different aspects of the analysis (Fig. 3), RCC focusing on taxa whereas NoiseSnapper applies to characters. In order to determine whether similar trees exist among the most parsimonious trees, we calculated the pairwise distance between the trees, using “symmetric difference” (REF) as implemented in PAUP (Swofford, 1998).

![Fig. 2. Monophyly cannot be deduced from RCC. Tree a is the ternary RCC of the three fundamental trees presented in b, c, and d. Group B + C seems to be monophyletic in the RCC, yet may be paraphyletic (b), monophyletic (c) or polyphyletic (d).](image-url)

![Fig. 3. Comparison of RadCon and NoiseSnapper analysis. Software is in ellipses, data are in boxes. RadCon reorganizes fundamental trees by selecting taxa. NoiseSnapper selects fundamental trees using RI of each character.](image-url)
**RESULTS**

**Analysis without fossils.** — The analysis yielded 17 most parsimonious trees (365 steps, consistency index CI = 0.389, retention index RI = 0.487). The strict consensus (Fig. 4) of these trees was poorly resolved. Menispermaceae appear to be monophyletic but the relationships within the family and with other families are not clear. In addition, branch support for external nodes to the Menispermaceae was low (Bremer index = 1). Just a few groups appeared: (Arcangelisia, Coscinium), (Menispermum, Cocculus), (Abuta, Chondrodendron), (Disciphania, Tiliacora, Triclisia) and Cissampelideae sensu Bentham & Hooker 1862 (Sarcopetalum, Stephania, Cissampelos).

**Reduced Cladistic Consensus.** — After calculating the strict RCC profile (including seven trees), the third reduced consensus (Fig. 5A) shows a more resolved structure. The only pruned taxon is Anamirta. Fibraurea and Burasaia occupy a basal position. An internal group (number 1) with Pycnarrhena, Limacia, Tinospora, Arcangelisia, Coscinium, Menispermum, Cocculus, Sarcopetalum, Stephania and Cissampelos appeared. The sixth consensus (Fig. 5B) shows another internal group (number 2) with Abuta, Chondrodendron, Hyperbaena, Disciphania, Tiliacora, Triclisia. Other consensuses of the profile are not discussed here, because they only show slight differences with the first (the strict consensus), the third and the sixth reduced consensus.

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**Fig. 4.** Strict consensus of the analysis without fossils.
Trees analysis. — The calculation of tree-to-tree distances shows three groups of trees (Fig. 6): one of 10 trees, a second of 6 and only one with an intermediate structure. The strict consensus of each tree group is well-resolved (Fig. 7), differing mostly by the position of Anamirta and the paraphyly or monophyly of the internal group 2. At least one of these positions is artifactual.

Character analysis. — The analysis of random characters using NoiseSnapper shows that all trees but one have 20 “random characters.” One tree, named here “least random tree”, has only 18 “random characters” (Fig. 8). All characters that are considered random are presented in Table 1. The “least random tree”, according to NoiseSnapper analysis shows the monophyly the
Fig. 7. A, consensus of the first set of fundamental trees; B, consensus of the second set of fundamental trees.

Table 1. Characters considered random under NoiseSnapper analysis.

Characters considered random on all trees
2 Form of sepal extremity
3 Form of the sepals
4 Type of sepals
6 Internal sepals petaloid
26 Size of stigma
31 Type of endocarp sclerification
35 Form of condyle
37 Perforation of condyle
39 Endosperm
43 Divarication of cotyledons
44 Bracts
45 Degree of composition of inflorescence
46 Type of inflorescence
49 Form of the leaf
52 Position of apertures
58 Join of carpel

Characters considered random on the least random tree but not on all trees
12 Join of stamens
14 Number of locules per anther

Characters considered random
7 Number of petals in male flower
10 Nectaries
12 Join of stamens
14 Number of locules per anther
27 Form of the fruit
34 Endocarp ornamentation
41 Form of the embryo
48 Pubescence (at inflorescence)

*A character is considered as random when its RI is not significantly higher than the RI of a random character (Zaragüeta i Bagils & al., 2003).
internal groups 1 and 2; the basal position of *Fibraurea*
and *Burasaisia*; *Anamirta* has the most basal position in
the family.

The analysis after successive weighting by PAUP
resulted in three most parsimonious trees and a strict
consensus tree (Fig. 9). It differs from the “least random
tree” by the position of *Anamirta* and the non-monophyly
of group 2.

**Analysis with fossils.** — When fossils were included
in the analysis, 297 most parsimonious trees were ob-
tained (366 steps, CI = 0.388, RI = 0.508). The consensus
tree (Fig. 10) is well resolved and its structure is the same
as the one of the “least random tree”. However, Menisper-
meae are not resolved in this analysis.

**RCC: a more sensitive consensus method.** —
The RCC method allows for more thorough investigation
of phylogenetic trees, but interpretation of RCCs should
be cautious because they contain less information than
the original trees (see Fig. 2). The advantage of the RCC,
however, is that it is always unambiguous, which does not
hold for Adams’ consensus (Wilkinson, 1994).

In the third consensus (Fig. 5A) of the RCC profile
*Anamirta* is the only genus to be pruned. Therefore, the
interpretation is obvious: although the position of *Ana-
mirta* is ambiguous, the relationships between the other
taxa are clear. Group 1 appears to be monophyletic but
*Anamirta* may or may not be part of it.

The interpretation of the sixth consensus of the RCC
profile is more complicated (Fig. 5B) because 10 taxa
are pruned. As a consequence it is difficult to conclude
that the observed groups are monophyletic. The internal
group 2 appears to be monophyletic, but could also be
paraphyletic or even polyphyletic depending on its true
relationship to the pruned taxa. In this case, we may
conclude that *Abuta* is closer to *Triclisia* than *Anomo-
spermum*, which was not shown in the strict consensus.
Moreover, it appears that *Anamirta* is probably not part
of the group 1.

The RCC analysis clarified relationships within Menis-
permaceae and revealed the presence of two groups that
were not resolved in the strict consensus. The third and
sixth consensus structure suggests that the position of
*Anamirta* is ambiguous and is probably not part of groups
1 and 2 but occupies an external position.

**Tree pairwise analysis.** — The tree-to-tree com-
parsions rendered two sets of trees and an intermediate
one. These two sets differ most sharply on the position of
*Anamirta*, which may have either an external or internal
position as previously detected by the RCC approach. In
the first set consensus (Fig. 7A) *Anamirta* is in external
position and group 2 is paraphyletic, which is compatible
with the sixth RCC. The second set consensus (Fig. 7B) is
compatible with the third RCC, which includes *Anamirta*
in group 1.

**NoiseSnapper: selecting a tree among most
dparsimonious trees.** — NoiseSnapper allows char-
acters with good fit to be distinguished from those that
fit the tree as random characters (Zaragüeta i Bagils &
al., 2003). Its purpose is to estimate the fit of individual
characters; it allows for comparing a set of most parsi-
onious trees. We propose that the trees which mini-
mize the number of “random characters” be retained.
The more characters that fit the tree, the more accurate
the tree.

A single tree, among the seventeen most parsimoni-
os ones, contained the most characters having good fit
(Fig. 8) was completely resolved. Groups 1 and 2 are mon-
ophyletic and *Anamirta* has an external position, which

**DISCUSSION**

**Methodological aspects.** — The first analysis
yielded a poorly resolved consensus tree, which was not
informative for relationships within Menispermaceae.
Strict component consensus is a generalization of funda-
mental trees; it encompasses more relationships than
the ones that actually exist in the fundamental trees. To
explore these trees in greater detail, we carried out three
additional independent analyses using the RCC method,
tree-to-tree distance, and NoiseSnapper.
is consistent with the results of the 6th RCC analysis. Furthermore, this tree takes an intermediate position as determined by the tree-to-tree comparisons (Fig. 8).

The analysis with reweighted characters (Fig. 9) indicates that other relationships among Menispermaceae are incongruent with the “least random tree” and the sixth RCC tree. However, bias may have been introduced reweighting of characters. In contrast, the tree selected by NoiseSnapper is based on the original, unmodified assumptions (unreweighted matrix). As NoiseSnapper

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**Fig. 10.** Strict consensus tree of analysis integrating the fossils. Fossil genera are in bold. A, Anomospermeae; C, Cosciniae; F, Fibraureae; H, Hyperbaenae; L, Tiliacoreae; M, Menispermeae; P, Peniantheae; T, Tinospermeae.
does not perturb data, we favor the use of NoiseSnapper rather than reweighting methods.

**Fossils: new information.** — Since fossils have new and original combinations of characters they may modify the inferred phylogeny. The strict consensus of the trees found in the analysis with fossils (Fig. 10) is better resolved than the one obtained in the analysis without fossils, even if there are many more most-parsimonious trees (297; compare 17 without fossils). This high number of trees is due to the high proportion of unknown characters among the fossil taxa. *Palaeococcus* and *Wardensheppeya* can be placed equally with *Cissampelos* and *Cocculus*, as all their observable informative characters are shared with *Cissampelos* and *Cocculus*. The introduction of new taxa, even when only partially known, improves the resolution. This result is completely compatible with previous results obtained by RCC, tree-to-tree distance and NoiseSnapper.

**Improving the resolution.** — We investigated the problem of poor tree resolution in two different ways by exploring the equi-parsimonious trees and by adding fossil data. The first approach consisted of exploring trees or characters in greater detail and demonstrated the usefulness of the RCC and NoiseSnapper methods in increasing the accuracy of the most parsimonious trees. The second approach is widely used in phylogenetic analyses and consists of adding new taxa. Both approaches helped to better infer the phylogeny of Menispermaceae; congruence of the results is an indication of their relevance.

**Character analysis.** — The characters considered as random pertain to different parts of the plant (Table 1). The randomness of those used to describe flowers clearly corroborates Barneby’s assumption (1970): “It appears that the menispermaceous flower [...] is unsuited by its very plasticity to form the basis of a generic classification.” He also discussed the androecium: “It is likely that the relatively rapid and repetitive modification of the androecium is connected with the selective pressure exerted by pollinators” (Barneby 1972, under the hypothesis of entomogamy). As a confirmation, the characters of the androecium behave as “random” (under NoiseSnapper analysis) on most of the trees, and are highly homoplasic. In our phylogeny, the fusion of stamens (character 12) appears independently three times inside the Menispermaceae.

The characters of inflorescence are also homoplasic. For example, inflorescence is known to be variable in *Odontocarya* Miers (Barneby, 1970), some species having simple racemes whereas in others they have become composed at least three different times. Leaf form is also very homoplasic, as expected for a character strongly dependent on ecological conditions (Givnish & Vermeij, 1976; Goodwillie & al. 2004).

More surprisingly, a few characters of the fruit are homoplasic and fit the tree as bad as random characters on the NoiseSnapper analysis. Type of fruit sclerification is currently being studied and seems to be more complex than first believed (Fig. 1). The form of the condyle appears homoplasic, having a retention index as low as a random one. This is consistent with Dekker’s (1983) interpretation of this character: “There exists some confusion about defining the condyle, mainly by lack of a good description of this structure.” The presence of endosperm and the divarication of cotyledons are also homoplasic, in spite of constituting the basis of the classification of the Menispermaceae of Diels (1910).

**Phylogenetic information.** — Clade support can only be assessed in this analysis without the fossils. Since the fossils have too many unknown characters, measurements of character support is not possible (the maximum number of trees kept in memory is reached). The Bremer support index is very low in the analysis without fossils (maximum value found is 3).

The monophyly of the family is confirmed in the strict consensus, being supported by characters 22 (anthers on staminodes), 29 (type of endocarp), 53 (reticulation of pollen) and 56 (number of ovules per carpel). The sclerified endocarp and the single ovule per carpel are often cited in the diagnoses of the family. As the minimal age of Menispermeae dates back to the Cretaceous (Knobloch & Mai, 1986; Magallón & al., 1999), some character states might have been lost or evolved in some taxa included in the family.

Except for Menispermeae and the monotypic Hyperbaenaceae, no tribe described by Diels (1910) or Kessler (1993) is monophyletic. The hippocrepiform endocarp is a synapomorphy of Menispermaceae. Considering that the characters used by Diels (1910) are highly homoplasic as revealed by NoiseSnapper, the non-congruence of his classification and our phylogeny is not surprising.

The tribe Fibraeaceae is paraphyletic in the basal part of the Menispermaceae clade. The straight endocarp present in all Fibraeaceae could be a plesiomorph character-state in the family. Furthermore, *Tinominiscium* and *Atriaecarpum* might possibly be united.

In internal group 2 tribal status of the genus *Hyperbaena* is questionable. Since it is resolved between *Chondrodendron* and *Triclisia*, both members of Tiliacoreae, there is no reason to maintain this tribe.

The relationships of *Anamirta* in the most external subclade of the Menispermaceae is also questionable; this seems to be a highly derived taxon. Its position is sustained by characters 15 (dehiscence, extrorse), 26 (size of stigma, thick), and 38 (curve-shaped seed) under DELTRAN or ACCTRAN optimization. Character 26, however, is random under NoiseSnapper. Our analysis suggests that the position of *Anamirta* is largely uncertain.

Except for *Tinospora*, all fossils were placed near their hypothetical affinities. Thus, the taxonomic interpretations of the fossils are supported by our results.
CONCLUSION

Our cladistic analysis confirms the need for a reassessment of the Menispermaceae. The naturalness of one tribe only, Menispermeae, characterized by its horseshoe-shaped endocarp, could be shown. Due to the weak support of the groups found in this study, no new taxonomy is proposed. More detailed morphological studies (of wood anatomy, for example) and molecular analysis might lead to an improved classification of Menispermaceae.

In this study, fossils have shown highly useful to clarify the phylogeny of Menispermeae, allowing an improvement of the resolution of the consensus. We have also shown how RCC and NoiseSnapper can be used to more accurately analyze a set of trees that, at first, yield a poorly resolved strict consensus.

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LITERATURE CITED


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<td>Elephantomene Barneye &amp; Krukoff</td>
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Appendix 2. List of specimens examined. All specimens kept in P.

Species: origin; voucher.


Appendix 3. Characters used in the morphological data matrix.

Flower

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<thead>
<tr>
<th>Character</th>
<th>Description</th>
<th>Values</th>
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<td>01</td>
<td>Number of sepals in the male flower</td>
<td>0 = 0; 1 = 4; 2 = 6; 3 = 9; 4 = n (more than 9); 5 = 8; 6 = 5; 7 = 2.</td>
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<tr>
<td>02</td>
<td>Form of the sepal extremity</td>
<td>0 = acute; 1 = obtuse.</td>
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<tr>
<td>03</td>
<td>Form of the sepals</td>
<td>0 = length/width ≥ 1; 1 = length/width &lt; 1.</td>
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<td>04</td>
<td>Type of sepals</td>
<td>0 = foliaceous; 1 = fleshy.</td>
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<td>05</td>
<td>Relative size of internal sepals to other sepals</td>
<td>0 = longer; 1 = equal; 2 = smaller.</td>
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<tr>
<td>06</td>
<td>Internal sepals petaloid</td>
<td>0 = yes; 1 = no.</td>
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<tr>
<td>07</td>
<td>Number of petals in male flower</td>
<td>0 = 0; 1 = 3; 2 = 6; 3 = 1; 4 = 5; 5 = 4.</td>
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<tr>
<td>08</td>
<td>Form of petals</td>
<td>0 = length/width ≥ 1; 1 = length/width &lt; 1; 2 = cuneate (with apical lobe curved outwards and lateral lobes curved inwards).</td>
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<td>09</td>
<td>Relative size of the petals to the sepals</td>
<td>0 = smaller; 1 = equal; 2 = longer; 3 = rudimentary.</td>
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<tr>
<td>10</td>
<td>Nectaries</td>
<td>0 = absent; 1 = present.</td>
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<tr>
<td>11</td>
<td>Number of stamens relative to merosity</td>
<td>0 = n; 1 = 1; 2 = 2; 3 = 0.5; 4 = 1.5.</td>
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</table>
Appendix 3. Continued.

12 Stamens connate. 0 = no; 1 = yes; 2 = in central column.
13 Stamens and petals adnate. 0 = no; 1 = yes.
14 Number of locules per anther. 0 = 1; 2 = 2; 4 = 3; but apparently 4.
15 Anther dehiscence. 0 = introrse; 1 = extrorse; 2 = lateral.
16 Orientation of the anther. 0 = vertical; 1 = oblique; 2 = horizontal.
17 Pistiloides in the male flower. 0 = no; 1 = yes; 2 = fertile carpels.
18 Number of sepals in the female flower. 0 = 0; 1 = 1; 2 = 6; 3 = 9; 4 = n; 5 = 4; 6 = 5; 7 = 2.
19 Number of petals in the female flower. 0 = 0; 1 = 1; 2 = 6; 3 = 9; 4 = n; 5 = 5; 6 = 4.
20 Symmetry of the female flower. 0 = actinomorph; 1 = zygomorph.
21 Staminodes in the female flower. 0 = no; 1 = yes; 2 = fertile stamens (hermaphrodite flower).
22 Anthers on the staminodes. 0 = no; 1 = yes.
23 Number of carpels. 0 = 1; 1 = always 3; 2 = 3 or 6; 3 = 4 or 5; 4 = n; 5 = 2.
24 Length of style. 0 = no style; 1 = short; 2 = long (of same size as ovary or longer).
25 Form of stigma. 0 = laciniate; 1 = entire; 2 = lobed.
26 Fruit shape. 0 = spherical; 1 = oblong; 2 = reniform.
27 Position of style scar. 0 = at base; 1 = not far from base; 2 = lateral.
28 Type of endocarp. 0 = fleshy endocarp; 1 = sclerified endocarp.
29 Form of endocarp. 0 = horseshoe-shaped; 1 = bent; 2 = invaginated; 3 = straight.
30 Type of endocarp sclerification. 0 = crustaceous; 1 = woody.
31 Number of dorsal crests of endocarp. 0 = 0; 1 = 1; 2 = 2.
32 Number of lateral crests at each side. 0 = 0; 1 = 1; 2 = 2; 3 = 3.
33 Endocarp ornamentation. 0 = smooth; 1 = transverse ridges; 2 = non-structured ornamentation.
34 Form of condyle. 0 = absent; 1 = flat; 2 = spherical; 3 = boat-shaped.
35 Type of condyle. 0 = simple; 1 = double.
36 Condyle perforate. 0 = no; 1 = yes.

Seed

38 Shape of the seed. 0 = straight; 1 = horseshoe-shaped; 2 = bent; 3 = reniform.
39 Endosperm (albumen). 0 = absent; 1 = present.
40 Endosperm ruminate. 0 = no; 1 = yes.
41 Shape of the embryo. 0 = horseshoe-shaped; 1 = curved; 2 = straight. May be different of seed shape when albumen is abundant.
42 Type of cotyledons. 0 = foliaceous; 1 = fleshy.
43 Cotyledons divaricate. 0 = no; 1 = yes.

Inflorescence

44 Bracts. 0 = absent; 1 = present.
45 Degree of composition of the inflorescence. 0 = isolated flower; 1 = simple; 2 = compound.
46 Type of inflorescence. 0 = raceme; 1 = spike; 2 = cyme; 3 = glomerule.

Others characters

47 Habit 0 = bush; 1 = tree; 2 = climber; 3 = herbaceous.
48 Pubescence (on inflorescence). 0 = glabrous; 1 = pubescent. All trichomes were assumed as homologous, appearing similar under binocular.

Leaf

49 Form of the leaf. 0 = entire, not lobed; 1 = entire, lobed; 2 = compound.
50 Nervation of leaves. 0 = pinnate; 1 = palmatifoliate; 2 = palmate.

Pollen

51 Type of pollen. 0 = inaperturate; 1 = colpate; 2 = colporate; 3 = porate.
52 Position of aperture. 0 = fossaperturate; 1 = angulaperturate; 2 = spiraperturate.
53 Reticulation of pollen. 0 = fine; 1 = intermediate; 2 = coarse.
54 Link of reticulation. 0 = irregular; 1 = pentagonal; 2 = hexagonal.
55 Margin of colpus. 0 = absent; 1 = diffuse; 2 = narrow.

Additional characters (not informative inside Menispermaceae)

56 Number of ovules per carpel. 0 = 1; 1 = n.
57 Type of mesocarp. 0 = sclerified; 1 = fleshy.
58 Carpel fused. 0 = free; 1 = joined.
## Appendix 4. Character matrix.

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<td>Tinospora baikei Hook &amp; Thom.</td>
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</table>

### Character matrix applicable to the fossils

<table>
<thead>
<tr>
<th>Characters (matrix of characters applicable to the fossils)</th>
<th>23</th>
<th>3333</th>
<th>33</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>90</td>
<td>2345</td>
<td>67</td>
</tr>
</tbody>
</table>

Note: A = 0/1; B = 0/2; C = 1/2; D = 1/3; E = 2/3; F = 0/1/5; N = non applicable; ? = indeterminate.