MORPHOMETRICS

Geometric morphometrics: A powerful tool for the study of shape evolution in Menispermaceae endocarps

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Abstract Within Menispermaceae, endocarp shape is highly variable and often characteristic. This study applied geometric morphometrics to the investigation of horseshoe-shaped endocarps that characterized the former Menispermeae tribe. The shape of 823 endocarp specimens, representing 66 species and 16 genera, were described on the lateral face by the means of 4 landmarks and 18 semilandmarks. The general Procrustes analysis was used to remove size and orientation of the specimens. Using thin-plate splines, we were able to visualize and describe the variation in shape for each genus and/or species. The main differences concern the symmetry/asymmetry of endocarp, the relative size of condyle, the relative length of ventral face and the concavity of ventral face. The results of a PCA reveal that for all genera except *Diploclisia*, generic variability is explained by a continuum in intra- and interspecific variability. Endocarp shape differed significantly between genera, demonstrating the potential for geometric morphometrics in fossil identification. Allometry explained only a small part of shape variation. Phylogenetic content is evaluated by comparing the results of cluster analysis with recent molecular phylogenies. Endocarp shape affinities appear to be quite different from phylogenetic relationships, demonstrating the low phylogenetic signal in endocarp shape at the family level. However, stronger variation is found in the lineages leading to modern genera. With a known phylogeny, geometric morphometrics is a good tool to understand shape evolution.

Keywords endocarp; Menispermaceae; Menispermeae; morphometrics; phylogeny; shape

■ INTRODUCTION

Menispermaceae (Ranunculales) comprise about 71 genera and 520 species (Jacques & al., 2007), most of them climbers in tropical and subtropical regions (e.g., Diels, 1910; Kessler, 1993). The species are famous for their numerous traditional uses including the preparation of curare (Krukoff & Moldenke, 1938; Phillips, 1991). Recent molecular phylogenies place Menispermaceae as sister to Berberidaceae-Ranunculaceae in the basal eudicots (APG, 1998; Soltis & al., 1997, 2000; Hoot & al., 1999; Savolainen & al., 2000; APG II, 2003; Hilu & al., 2003; Kim & al., 2004; Wang & al., 2009). The phylogenetic studies demonstrate the monophyly of Menispermaceae and aid to our understanding of the intrafamilial relationships (Jacques & al., 2007; Ortiz & al., 2007; Wang & al., 2007; Jacques & Bertolino, 2008; Hoot & al., 2009).

Horseshoe-shaped endocarps represent an important character for correct identification of Menispermaceae (Jacques & Zhou, 2008). They are known through several fossil occurrences (Chandler, 1961; Manchester, 1994; Jacques, 2009b) and have occurred on different continents through the Cenozoic (Jacques, 2009b). Some fossils have clear affinities with modern genera, such as *Diploclisia* or *Palaeosinomenium* from the Eocene of the London Clay (Chandler, 1961); others such as *Thanikaimonia* from the Eocene of the Clarno Beds have unclear affinities (Manchester, 1994).

The morphology of Menispermaceae endocarps has been studied in great detail (Forman, 1972, 1974, 1984, 1985; Lo, 1978; Thanikaimoni, 1984; Jacques, 2009a) and endocarp shape has often been used in taxonomy (Diels, 1910; Lo, 1978; Forman, 1985; Kessler, 1993); however, endocarp evolution in Menispermaceae seems more complex than primarily believed (Ortiz & al., 2007). Modern morphometric methods may enable a better understanding of the unique endocarp shape in the family.

Modern morphometrics, first developed by Bookstein & al. (1985), has the great advantage to extract size from analysis (Bookstein, 1991). Several morphometrics techniques currently exist including landmarks (Rohlf & Slice, 1990; Bookstein, 1991; Jensen, 2003), semilandmarks (Bookstein, 1997) and outline (Lohmann, 1983; McLellan & Endler, 1998; Olsson & al., 2000). In geometric morphometrics, the shape of an object is described by a set of landmarks, which should be homologous throughout the whole sample (Bookstein & al., 1985; Bookstein, 1991; Jensen, 2003; Adams & al., 2004). These landmark positions vary depending on the size, shape and orientation of each specimen (Adams & al., 2004). The analysis of landmarks should therefore begin with superimposition process in order to remove size and orientation from the analysis (Bookstein, 1986; Rohlf & Slice, 1990). The combination of landmark coordinates is then used as a descriptor of each object shape (Bookstein, 1991; Adams & al., 2004) or alternatively, the shape of an object can be described through its outline (Lohmann, 1983; McLellan & Endler, 1998; Olsson & al., 2000). Comparing outlines of different objects can be achieved via several different methods, the

most commonly used being elliptical Fourrier functions (Kuhl & Giardina, 1982; Jensen, 2003). However outline methods face two problems: firstly the lack of biological correspondence between the points from different specimen (Bookstein, 1991; Jensen, 2003), and secondly the lack of congruent results between the different methods (Rohlf, 1986; Adams & al., 2004). Semilandmarks on the other hand enable description of an object's outline using landmarks (Bookstein, 1997; Monteiro & al., 2005) and such landmarks bear only one Cartesian coordinate, namely the normal direction to the outline (Bookstein, 2002). These landmarks are able to slide along the tangent to the outline during the analysis process without any cost (Bookstein, 1997; Monteiro & al. 2005). Therefore, the small differences in the landmark positions are not taken into account, circumventing the lack of biological correspondence of those points among specimens (Monteiro & al., 2005).

The problem in botany is the common lack of homologous points, i.e., landmarks (Kores & al., 1993; Jensen, 2003). The use of semilandmarks avoids this problem as semilandmarks represent points along a contour (Bookstein, 1997; Monteiro & al., 2005). In the case of Menispermaceae endocarps, a combination of landmarks and semilandmarks can be used (Monteiro & al., 2005). Using the thin-plate splines (Bookstein, 1989, 1991) allows visualization of the differences between species and genera.

This study aims to examine the relationships between endocarp shape and evolution in Menispermaceae, despite the cladistic use of morphometric data still being controversial. The use of geometric morphometric data in cladistic is mainly based on discretisation characters from shape data (Fink & Zelditch, 1995; Zelditch & al., 2000; Swiderski & al., 2002), for example, the partial warps are explored one by one and used to sort taxa and describe the alternate states of each character (Swiderski & al., 2000). This approach is problematic in that the results of such analysis may change according the reference chosen as base for the partial warps (Adams & Rosenberg, 1998; Rohlf, 1998). Authors in favor of this method proposed that a biologically justified reference (outgroup, neonatal specimen) may limit the problem (Zelditch & al., 1998). In addition, the independent analysis of partial warps is not biologically justified, as a unique biological difference may be represented by several partial warps (Rohlf, 1998). Lastly, the univariate analysis of partial warps is unjustified, as they are statistically highly correlated (Rohlf, 1998); cladistics is based on analysis of supposedly independent characters (Farris & al., 1970). Continuous maximum likehood (Felsenstein, 1988, 2002) has been proposed as a possible way of analysing the continuous morphometric data in a phylogenetic context (Adams & al., 2004), however, the corresponding methodology has yet to be developed. Therefore, this study chose not to use morphometric data in a cladistic analysis and instead opted to compare cluster analysis with molecular phylogenies (Cardini, 2003), to enable the evolution of shape to be visualized on a molecular phylogenetic tree (Macholán, 2006).

The purpose of this study was to explore the shape differences between horseshoe-shaped endocarps in Menispermaceae using geometric morphometrics. Shape differences were analysed in a phylogenetic context to better understand the evolution of Menispermaceae endocarps and their phylogenetic relevance.

■ MATERIALS AND METHODS

Taxon sampling and specimens. — To allow for proper shape analysis, only genera sharing the same type of endocarp shape were included in the analysis. We selected 66 species in 16 genera exhibiting horseshoe-shaped endocarps (Appendix; Diels, 1910; Forman, 1968; Thanikaimoni, 1984; Jacques, 2009a). The 823 fruits measured came from specimens deposited in the herbaria BKF, KUN, MO, P and PE. Species of *Caryomene* Barneby & Krukoff, *Hyperbaena* Miers ex Benth., and *Sciadotenia* Miers were excluded from the analysis as most have been found to exhibit other types of endocarps (Jacques, 2009a). *Syrrhonema* Miers species were also excluded as the central area was found to be totally perforated unlike all other considered endocarps (Jacques, 2009a). Lack of material did not enable us to include *Echinostephia* (Diels) Domin and *Rhaptonema* Miers. *Echinostephia* was long considered as a section of *Stephania* Lour. (Diels, 1910), and shows close relationships to *Stephania*. On the other hand, *Diploclisia* Miers was included because, one of the three species, *Diploclisia affinis* (Oliv.) Diels, exhibits an endocarp shape that can be interpreted as horseshoe-shaped. The sampling is disproportionate between different species and genera as a result of more or less known species, however such disproportion did not prevent previous morphometric analyses (Cardini, 2003).

Endocarp preparation. — All endocarps were prepared according to Jacques (2009a). Fruits were first hydrated for one hour in boiling water and then overnight in cool water, before the pericarp and mesocarp of each were removed by dissection.

Digital imaging. — All endocarps were viewed laterally and only one lateral face was used, i.e., when the dorsal face was upwards and the stylar end of endocarp was orientated left (Fig. 1). The majority of endocarps were pictured under

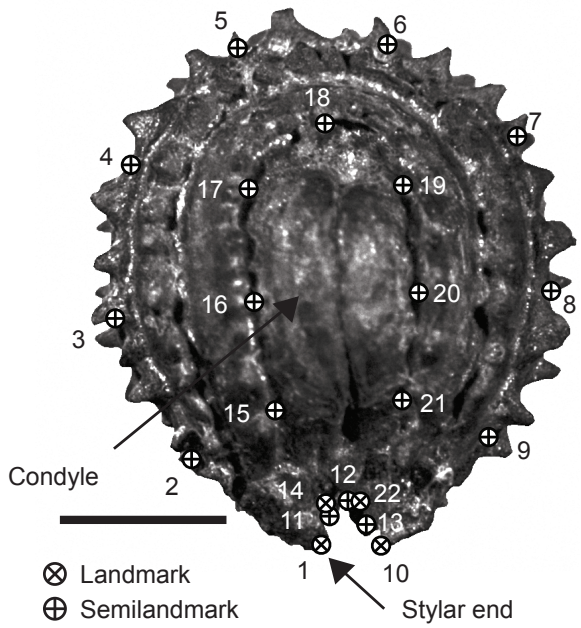

Fig. 1. Positions of landmarks and semilandmarks plotted on *Cyclea hypoglauca* (PE0103673). Scale bar: 1 mm.

stereoscopic microscope, coupled with a digital camera and digitizing software (Act-2U). Very large endocarps (*Diploclisia glaucescens* (Bl.) Diels, *Pachygone valida* Diels) were digitized using the close-up option of a Nikon Coolpix 4300.

Landmarks. — Endocarp shape was digitized using tpsDig (Rohlf, 2006a). Four landmarks were plotted on each specimen using the landmark tool. The first landmark was at the stylar end of limb, the second was at the opposite end of limb, the third was at the stylar-side intersection of condyle dorsal border with ventral margin and the fourth was at the other-side intersection of the same lines. All landmarks were of type I (Bookstein, 1991). Dorsal face, ventral face and dorsal border of condyle were drawn by hand using the 'curve drawing tool'. Dorsal face was described with 10 points, ventral face with 5 points and dorsal condyle border with 9 points, and the dorsal and ventral face shared the same end points (landmarks 1 and 2, Fig. 1). All points, except end points, were analysed as semilandmarks, and there was a total of 4 landmarks and 18 semilandmarks.

Landmarks should fulfil the requirements of being homologous points (Bookstein & al., 1985; Bookstein, 1991; Jensen, 2003), and lines should also be homologous. We think that, for dorsal face and ventral face, the homology hypothesis is highly reliable. Points and lines involving the condyle raise the question of the condyle homology in all those species. The condyle is a special structure in Menispermaceae, even if lacking in some genera (Miers, 1871; Diels, 1910; Troupin, 1962; Forman, 1974, 1975, 1978, 1981; Dekker, 1983; Thanikaimoni, 1984; Jacques, 2009a). The condyle may derive from the placenta (Miers, 1871; Dekker, 1983) and often represents a depression or invagination in the endocarp (Dekker, 1983; Jacques, 2009a). As no clear evidence challenges the homology of condyle in Menispermaceae, we considered this structure as homologous in the whole family. Lateral ridges could not be used: they vary from one to two in those genera (one in most genera, two in *Cissampelos* and *Cyclea*), and we were not able to find the homologies: is the single one homologous to the inner one or the outer one, is it the same for all genera? As the general shape of those ridges is constrained by dorsal face and condyle, the induced lack of information should not be so important.

Another problem is to deal with landmarks derived from a curve. Indeed, there is a lack of correspondence between the points on the curve (Monteiro & al., 2005). As explained in the introduction, the use of semilandmarks limits this problem: those points are allowed to slide along a vector (Bookstein, 1997; Monteiro & al., 2004). Following Monteiro & al. (2005), the slider vectors were defined as the chord between previous and next points on the curve using tpsUtil (Rohlf, 2006b).

Geometric morphometrics. — All specimens were aligned through general Procrustes analysis with semilandmarks being allowed to slide. Consensus shapes were combined and partial warps and partial warp scores were calculated using tpsRelw (Rohlf, 2007a). The consensus shape of each genus was then calculated and visualized on the form of a thin-plate spline using both thin-plate spline (Rohlf, 2007a) and overall consensus as references. The thin-plate spline is considered a powerful tool to map the deformation in shape from one object to another (Bookstein, 1991; Cardini, 2003; Adams & al., 2004).

Statistical analyses. — A principal component analysis (PCA) of partial warp scores was carried out to explore the distribution of species in the shape space. This method was chosen because some of the test species were represented by only one specimen. Canonical variance analysis (CVA) and multivariate analysis of variance (MANOVA) were used to test the shape differences between genera with more than one specimen, using all partial warp scores as variables. For the MANOVA, a nested model was analysed, with the species included in the genera. The CVA plot allowed the position of the group centroids along the canonical variate axes to be viewed, together with the corresponding 95% confidence ellipses (Gittins, 1985). PCA and CVA were carried out using the software PAST (Hammer & al., 2001), and the MANOVA was conducted using SPSS v.13.0 (SPSS Science). For genera with a large number of samples (i.e., *Cocculus*, *Cyclea*, *Menispermum*, *Pericampylus*, *Sinomenium*, *Stephania*), a regression multivariate test of shape (as described by the partial warp scores), onto centroid size, was undergone using tpsRegr (Rohlf, 2007b) in order to test allometry. A similar test was carried out on log-transformed centroid size, as this value has been found in some cases to better describe the allometric relationship (Cardini, 2003).

Phylogenetic analyses. — Two cluster analyses were carried out on the partial warp scores using PAST (Hammer & al., 2001). In the first analysis, the genera were used as OTUs (operational taxonomic units) and in the second analysis the species were used as OTUs. UPGMA algorithm and Euclidean distances were used to calculate all partial warp scores and the resulting tree for generic OTUs was compared with an average phylogenetic configuration derived from the most recent molecular analyses (Ortiz & al., 2007; Wang & al., 2007; Hoot & al., 2009; Jacques & al., under work). These analyses have slightly different samplings but are congruent for species and/or genera included in the present study. *Limaciopsis*, *Spirospermum* and *Strychnopsis* were not included in this configuration as data on the placements of the first two in molecular phylogenies was lacking, and the placement of the later is poorly resolved (Hoot & al., 2009). This average configuration (assuming all genera were monophyletic) was then used to explore the shape evolution of the horseshoe-shaped endocarps using tpsTree (Rohlf, 2007c), without any branch length. The shape of the nodes was estimated using the squared change parsimony procedure (Maddison, 1991; McArdle & Rodrigo, 1994). The tpsTree software (Rohlf, 2007c) only slightly differs from the algorithm of McArdle & Rodrigo (1994) which proposed an analytical solution to the problem of calculating the ancestral state of a continuous character, estimating the state of each internal node as the average of the values of the three nodes around it.

■ RESULTS

Shape differences. — All specimens were aligned, and their consensus is presented in Fig. 2A. Consensus for all genera using thin-plate splines is shown in Figs. 2–3. Principal characteristics of each genus are summarized in Table S1 in

the Electronic Supplement to the online version of this article. *Cissampelos* exhibited a relatively more elongated shape with narrower width, and the ventral face was relatively less symmetric than the consensus (Fig. 2B). In *Cocculus* the condyle was relatively more contracted than in the consensus (Fig. 2C). *Cyclea* exhibited a relatively narrower and more concave ventral face than in the consensus (Fig. 2D). In *Diploclisia affinis*, the condyle was larger and the general shape was more round (Fig. 2E). *Diploclisia glaucescens* showed a more elongated shape and a narrower condyle than the consensus (Fig. 2F). *Hypserpa* exhibited a smaller asymmetric condyle than the consensus (Fig. 2G). *Legnephora* had a condyle with ventral ends closer than the consensus and an asymmetric ventral face (Fig. 2H). In *Limacia*, the condyle was larger, and the ventral face largely convex (Fig. 3A). *Limaciopsis* had a strongly asymmetric ventral face and this asymmetry was also observed on the condyle (Fig. 3B). *Menispermum* had a larger condyle than the consensus and a concave ventral face (Fig. 3C). *Pachygone* showed a more constricted condyle and a concave ventral face compared to the consensus (Fig. 3D). *Pericampylus* was found

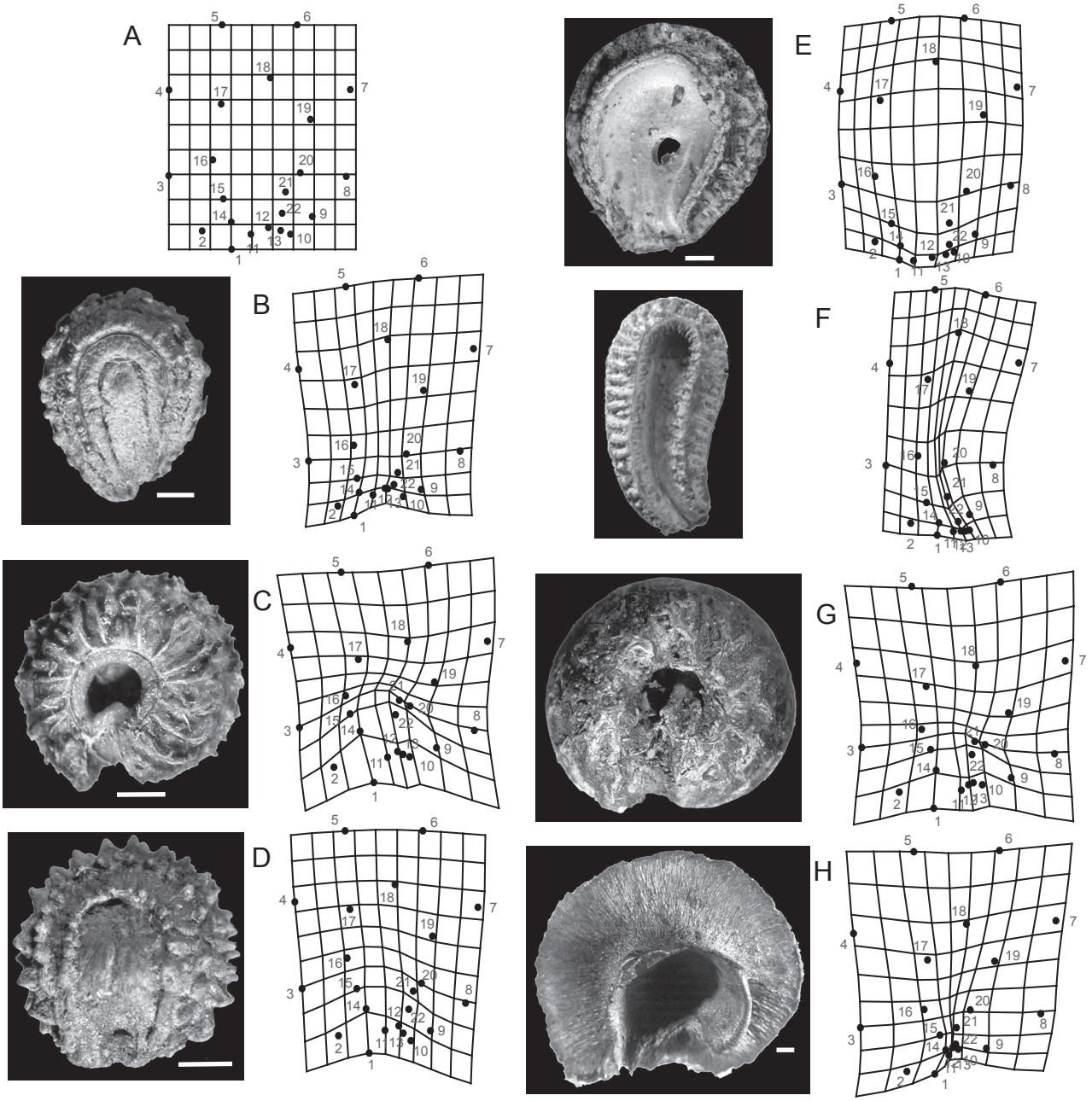

Fig. 2. Endocarps and thin-plate splines of the genus consensus, based on comparison with the overall consensus. **A,** whole consensus; **B,** *Cissampelos pareira* (PE 01070033); **C,** *Cocculus orbiculatus* (KUN 0164684); **D,** *Cyclea racemosa* (KUN 0164909); **E,** *Diploclisia affinis* (KUN 0165404); **F,** *D. glaucescens* (KUN 0165387); **G,** *Hypserpa nitida* (PE 01070940); **H,** *Legnephora minutiflora* (*M.S. Clemens 8682*, P). Scale bars: 1 mm.

to exhibit a larger ventral face compared to dorsal face (Fig. 3E). In *Sarcopetalum*, the endocarp was more dorso-ventrally compressed with narrower width compared to the consensus (Fig. 3F). *Sinomenium* had a slightly asymmetric endocarp with a ventral face relatively larger (Fig. 3G). *Spirospermum* exhibited a strongly asymmetric endocarp with a narrow dorsal rib compared to the consensus (Fig. 3H). In *Stephania*, the condyle was larger than in the consensus, and the endocarp showed a lateral constriction slightly upper ventral face (Fig. 3I). *Strychnopsis* had a strongly asymmetric endocarp (Fig. 3J).

The first two PCA axes (Fig. 4) represented the highest variability of the sampling. Axis 1 represented 42.11% of variability

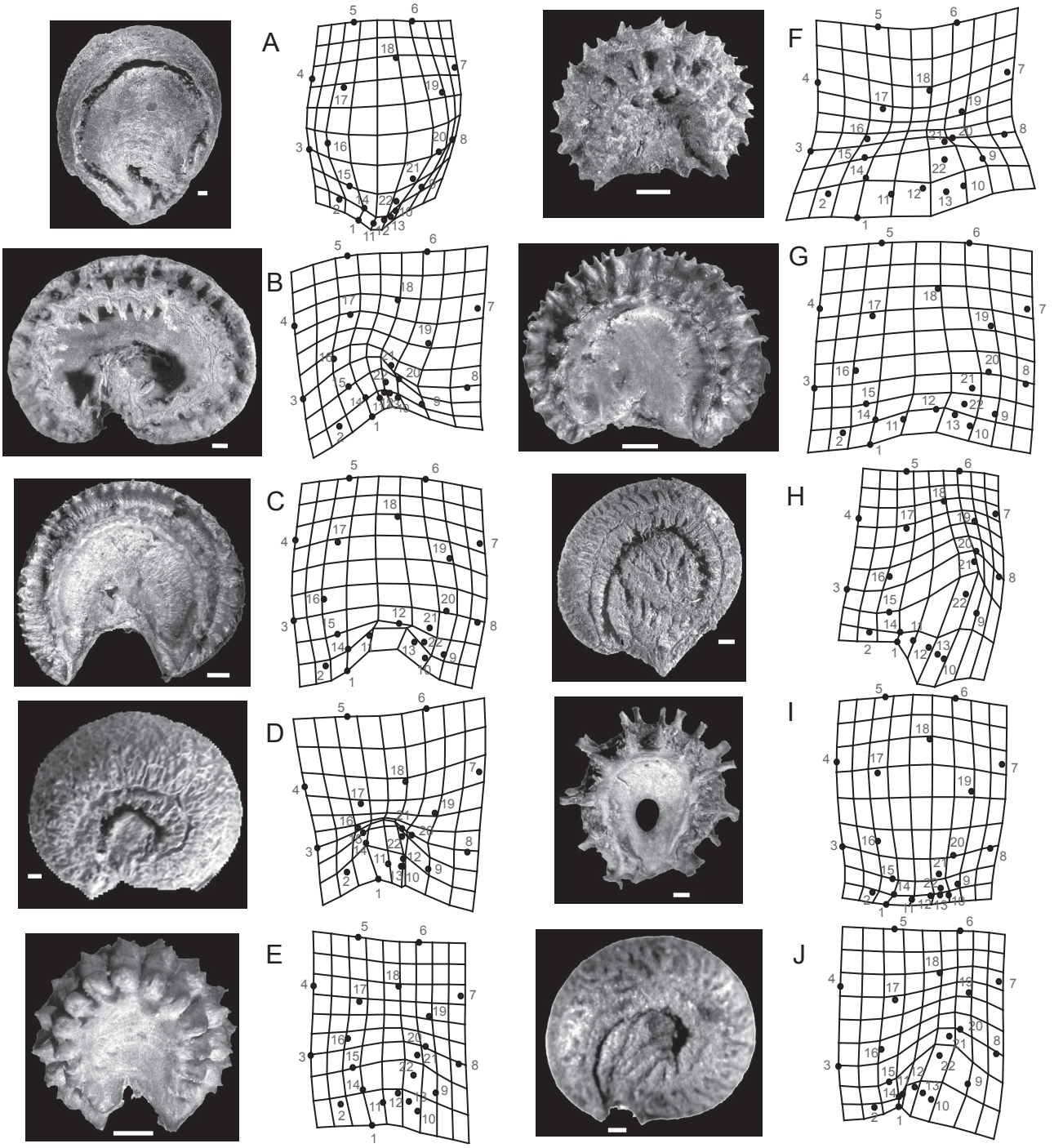

Fig. 3. Endocarps and thin-plate splines of the genus consensus, based on comparison with the overall consensus. **A,** *Limacia scandens* (*L. Pierre 1643*, P); **B,** *Limaciopsis loagensis* (*Tisserant 1426*, P); **C,** *Menispermum dauricum* (PE 01071135); **D,** *Pachygone valida* (PE 01071394); **E,** *Pericampylus glaucus* (KUN 0165157); **F,** *Sarcopetalum harveyanum* (*M.J. v. Balgooy 1561*, P); **G,** *Sinomenium acutum* (KUN 0165594); **H,** *Spirospermum penduliflorum* (*Boivin 1834*, P); **I,** *Stephania hernandifolia* (KUN 0166116); **J,** *Strychnopsis thouarsii* (*C. Birkinskaw 575*, P). Scale bars: 1 mm.

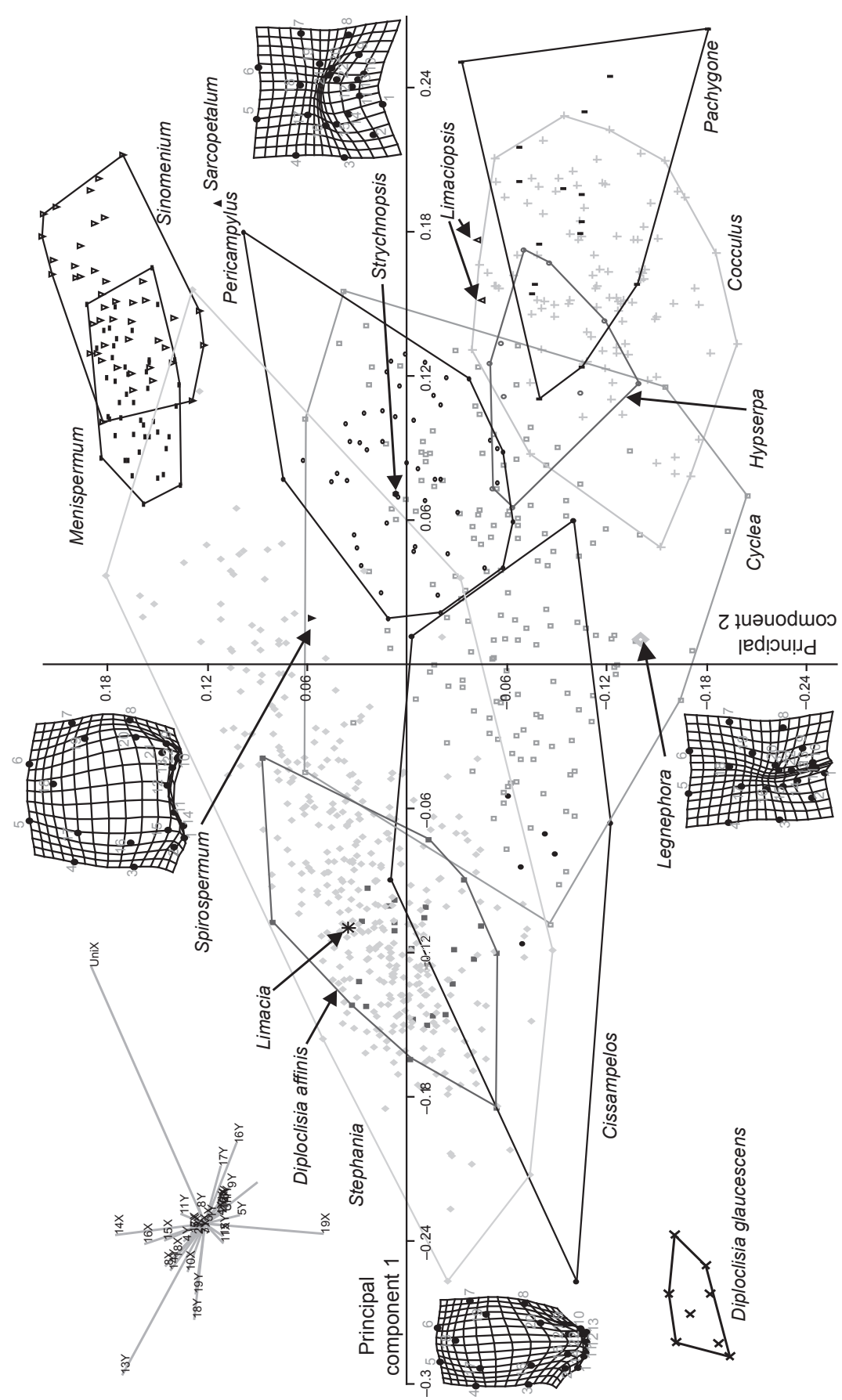

Fig. 4. Principal component analysis (PCA) showing the distribution of specimens according to first (42.11 % of variability) and second (24.07 % of variability) principal components. Hulls only represent the genus limits without any statistical meaning. Graphs along axes are thin-plate splines of deformation observed along these axes. The biplot shows the contributions of the different descriptors.

and axis 2 represented 24.07% of variability. Both axes can be described as a constriction/dilatation of the condyle and, to a lesser extent, the whole endocarp. Axis 1 corresponds to a dorsi-ventral constriction/dilatation while axis 2 corresponds to a stylar-chalazian constriction/dilatation. *Diploclisia* was clearly separated in two clusters, one for *D. affinis* and the other for *D. glaucescens*. Some genera, like *Menispermum* or *Sinomenium*, including genera containing a large number of specimens, showed a low variability. Other genera, such as *Stephania*, *Cissampelos* or *Cyclea* had a higher variability. The details of PCAs at the species level are presented for *Stephania* and *Cyclea*, which included several species (Fig. 5). Most of *Stephania* species group together (Fig. 5A). *Stephania laetificata* was separated from the rest, but with only one specimen; *S. tetrandra* and *S. delavayi* were the most different species in this genus. In *Cyclea* (Fig. 5B), *C. varians* was the most distinct species,

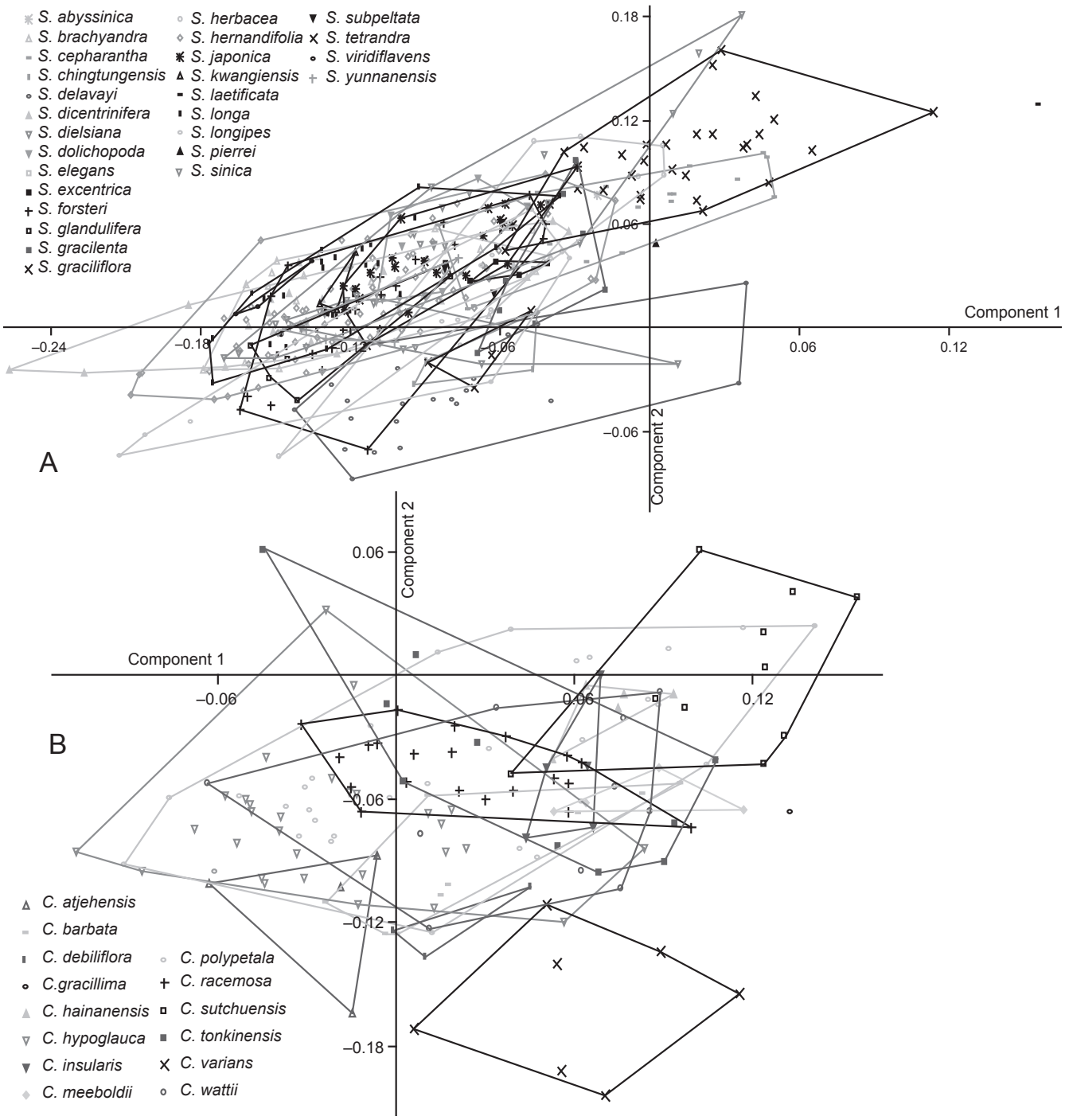

Fig. 5. Details of species distributions in the principal component analysis. **A,** *Stephania*; **B,** *Cyclea*.

but a continuum of variation between the species existed. As demonstrated in *Cyclea* and *Stephania*, the infrageneric variability can be described as a continuum. This is maybe less true for *Cissampelos*, but low number of specimens limits the analysis (Appendix). The results of the MANOVA upon a nested model revealed that the hypothesis of similarity between genera was rejected, as was the similarity-between-species hypothesis (Table 1). The CVA (Fig. 6) shows the dispersion of the groups. *Cissampelos* was found to occupy a wide space and shows high shape variability. Several groups can be distinguished: *Menispermum* and *Sinomenium*; *Diploclisia glaucescens*, *Stephania* and *D. affinis*; *Cyclea* and *Pericampylus*; *Cocculus* and *Pachygone*; *Hypserpa* links *Cocculus* and *Cyclea*. On CVAs at the species level for *Stephania* and *Cyclea* (data not shown), only *S. tetrandra* clearly differed from other *Stephania*, and no *Cyclea* species clearly differed from the others.

Allometry. — Results of multivariate regression tests are presented in Table 2. In each genus about 90% of the shape differences cannot be explained by size, therefore the hypothesis of allometry was rejected.

Shape evolution. — Cluster analysis (Fig. 7) revealed several groupings: *Menispermum*, *Sinomenium* and *Sarcopetalum*; *Spirospermum* and *Strychnopsis*; *Pachygone*, *Hypserpa*, *Cocculus* and *Limaciopsis*; *Legnephora* and *Cissampelos*; *Limacia*, *Diploclisia affinis* and *Stephania*; *Pericampylus* and *Cyclea*. Comparison with molecular average configuration (Fig. 7), showed many differences. The molecular clade comprising *Pericampylus*, *Sarcopetalum*, *Hypserpa* and *Legnephora* was not found, with these genera occurring in different groups in the cluster analysis. Moreover the close relationships between *Cissampelos*, *Cyclea* and *Stephania* were not so clear in the cluster analysis. However, some closely related genera exhibited relatively similar endocarp shapes, for example *Sinomenium* and *Menispermum*; *Cocculus* and *Pachygone*.

Cluster analysis at the species level (Fig. S1 in the Electronic Supplement) revealed that most of the *Stephania* species

Table 1. Results of MANOVA using species nested in genera.

Effect	λ_{Wilks}	*F*	df	P
Genus	0.000	15.418	11,849.4	0[a]
Species (genus)	0.000	4.086	23,440.0	0[a]

[a] Smaller that the smallest value displayed by the software.

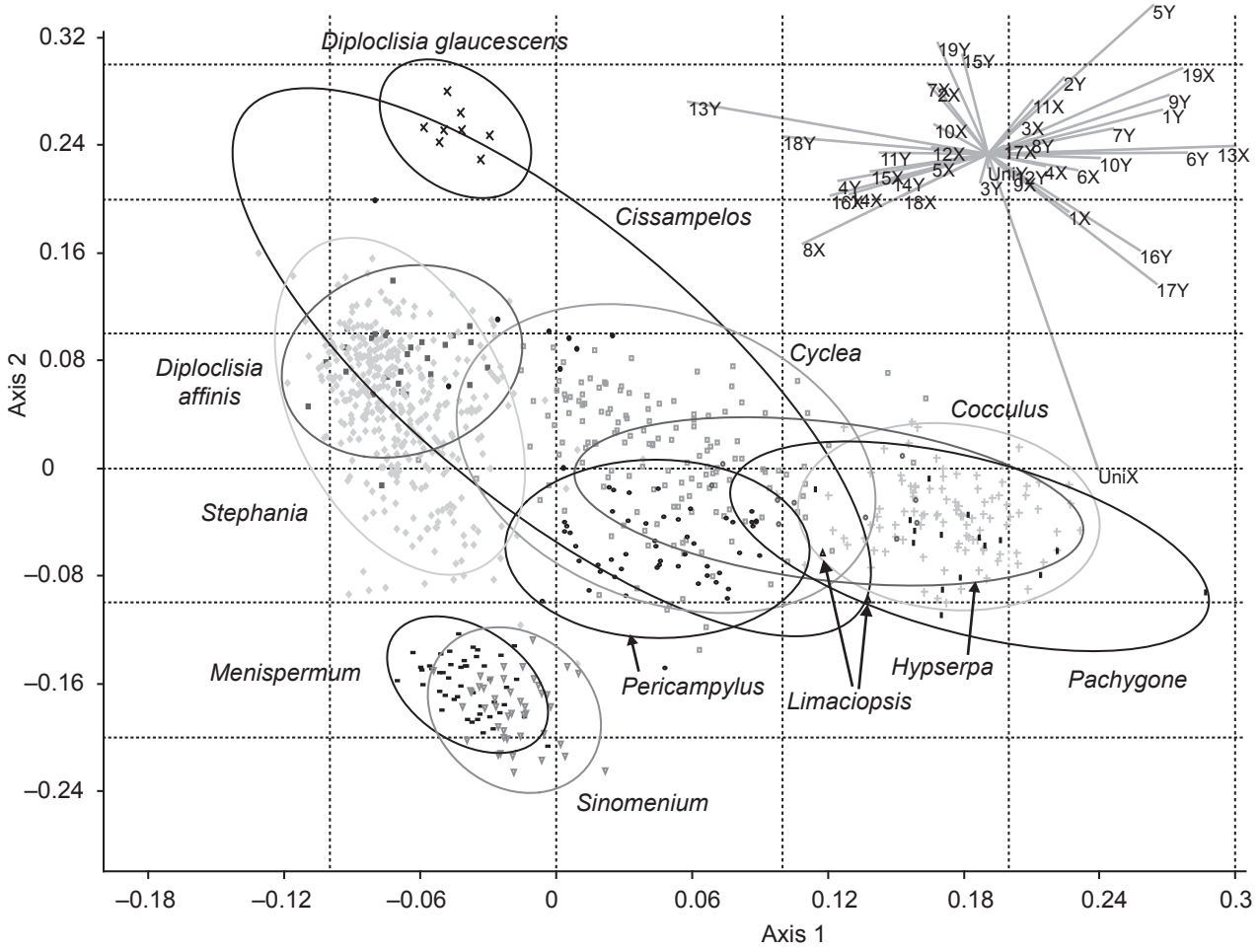

Fig. 6. Canonical variation analysis (CVA) showing distribution of genera with more than one specimen. Ellipses represent 95% confidence area. The biplot shows the contributions of the different descriptors.

Table 2. Results of multivariate regression tests of shape onto centroid size and log-transformed centroid size.

Genus	Number of specimens	Centroid size		Log-transformed centroid size	
		Percentage unexplained	*P* value	Percentage unexplained	*P* value
Cocculus	99	94.62	0	94.38	0
Cyclea	149	97.12	0	97.23	0
Menispermum	46	89.86	0	89.65	0
Pericampylus	48	96.63	0.0080	96.76	0.0145
Sinomenium	44	96.86	0.0654	96.78	0.0505
Stephania	381	97.70	0	97.76	0

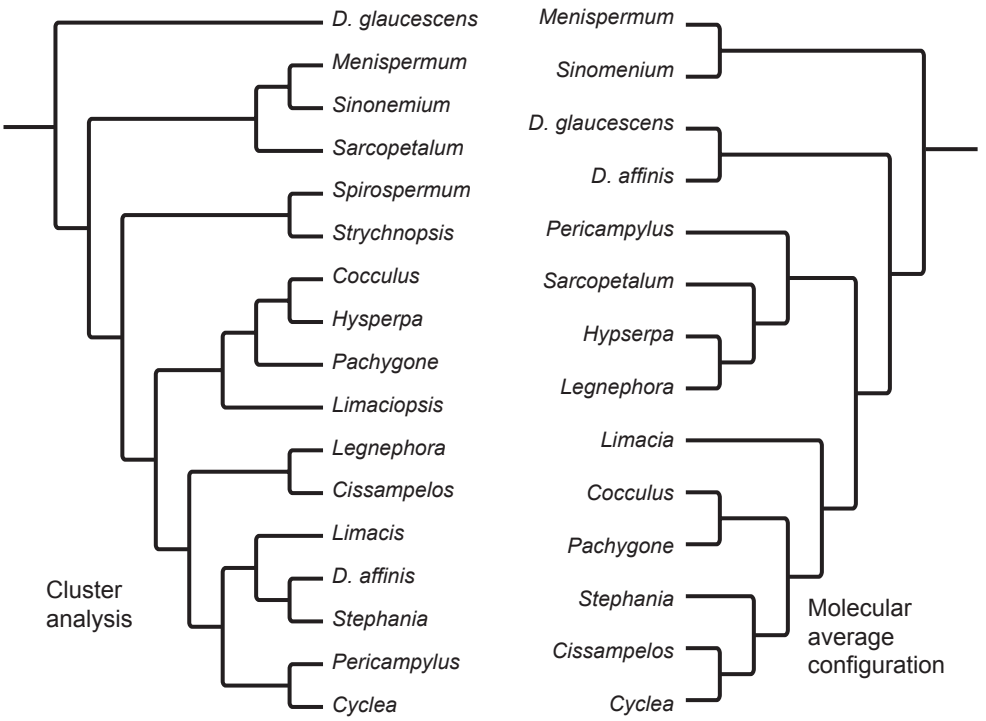

Fig. 7. Comparison of cluster analysis dendrogram (left) and molecular average configuration (right). Note that positions of *Limaciopsis* and *Spirospermum* are not known in molecular phylogenies; that of *Strychnopsis* is poorly resolved.

could be grouped together, as could the *Cyclea* species. *Sinomenium* and *Menispermum* group together but *Cissampelos* is dispersed in the dendogram. *Diploclisia* is nested in *Stephania*, while *Pericampylus* is nested in *Cyclea*.

When shape evolution was reconstructed in a molecular framework (Fig. 8), we were able to investigate the endocarp shape of ancestors. The common ancestor of all horseshoe-shaped endocarps showed a larger condyle compared to the consensus shape. The evolution along the deep nodes showed only slight differences; the main differences were seen in generic lineages and in some lineages grouping two genera. The common ancestor of *Menispermum* and *Sinomenium* appeared more oblique than the consensus, with a more concave ventral face. The common ancestor of *Hypserpa* and *Legnephora* had a smaller condyle and a shorter ventral face than the consensus. The common ancestor of *Pachygone* and *Cocculus*, finally, had a more constricted condyle than the consensus.

■ DISCUSSION

Taxonomy and shape. — Thin-plate splines (Figs. 2–3) of generic consensus, based on comparison to the overall consensus, allowed us to characterize the main differences amongst genera in terms of symmetry/asymmetry of endocarp, relative size of condyle, relative length of ventral face and concavity of ventral face. The CVA (Fig. 6) and MANOVA (Table 1) demonstrated that the differences between genera were statistically significant. On CVAs at species level for *Stephania* and *Cyclea*, only one species could be differentiated, justifying the comparison of consensus shapes between genera. According to PCA (Fig. 4), the most important variations to discriminate between specimens concerned the condyle which can be more or less constricted in dorsi-ventral axis and/or in stylar-chalazian axis.

In PCA (Fig. 4), *Diploclisia glaucescens* occupied a position clearly separated from other groups, including *D. affinis*

from the same genus. This result matched previous work revealing that *D. glaucescens* is characterized by a hairpin-shaped endocarp and not a horseshoe-shaped endocarp (Jacques, 2009a). A difference in endocarp type was clearly identified by the geometric morphometric method used in the present study. For other genera examined, specimens were more or less on all the generic space and no clear separation could be seen between infrageneric groups (Fig. 5). The generic variability therefore corresponded to a continuum in intra- and interspecific variability. The variability of *Menispermum* and *Sinomenium* was smaller than that found in genera such as *Cissampelos*, *Cocculus*, *Cyclea* and *Stephania*. *Menispermum* was represented by two species and *Sinomenium* by only one species whereas other genera were represented respectively by 4, 3, 14 and 27 species. The generic variability may be explained in part by the species diversity, i.e., different species correspond to variations of a same theme. However, *Pericampylus*, also represented by only one species, shows a high variability, therefore generic variability could not be reduced to species richness. Endocarp shape, although appropriate for discriminating between most genera (Figs. 4 and 6), was not reliably sufficient in discriminating between species. Other

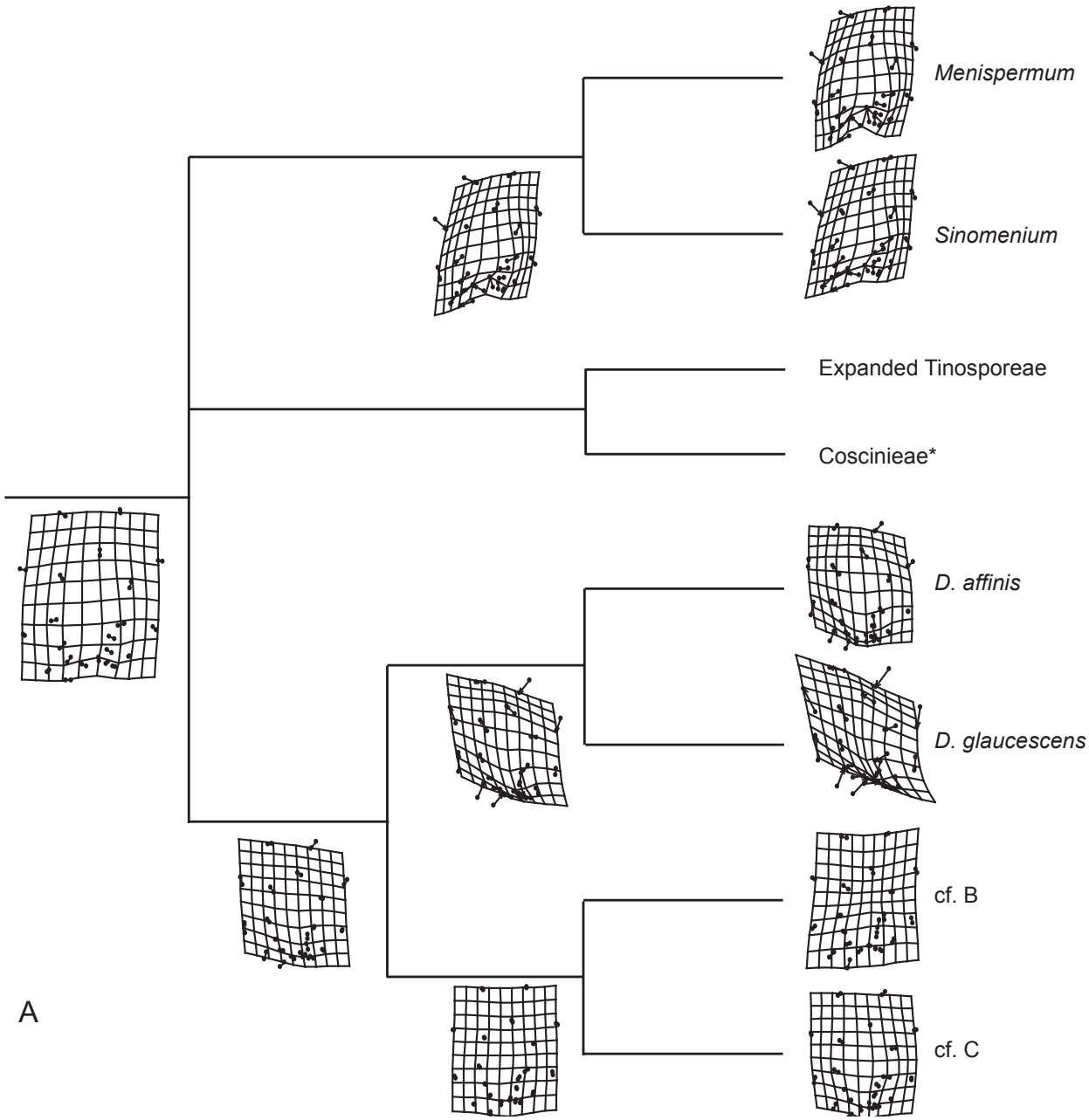

Fig. 8A. Shape evolution of horseshoe-shaped endocarps in Menispermaceae, reconstructed on the molecular average configuration. Groups without horseshoe-shaped endocarp were added after the analysis. * indicates groups with curved endocarps. The tree has been cut in several parts for practical purposes only.

endocarp characters, such as ornamentation, have been found to be more useful for this purpose (Forman, 1974; Lo, 1982; Jacques, 2009a).

As sampling was not the same for all species (Appendix), intraspecific variability could not easily be compared. However, for species with a similar number of specimens, such as *D. affinis* (28 specimens), *Pericampylus* (49 specimens) and *Sinomenium* (43 specimens), the variability was quite similar (Fig. 4).

Allometry. — As genera correspond to quite homogenous groups (as shown by specific PCA and CVA for *Stephania* and *Cyclea*), the test for allometry at genus level was justified. This test was performed on six genera and for all of them no more than 5% to 11% of shape differences could be explained by

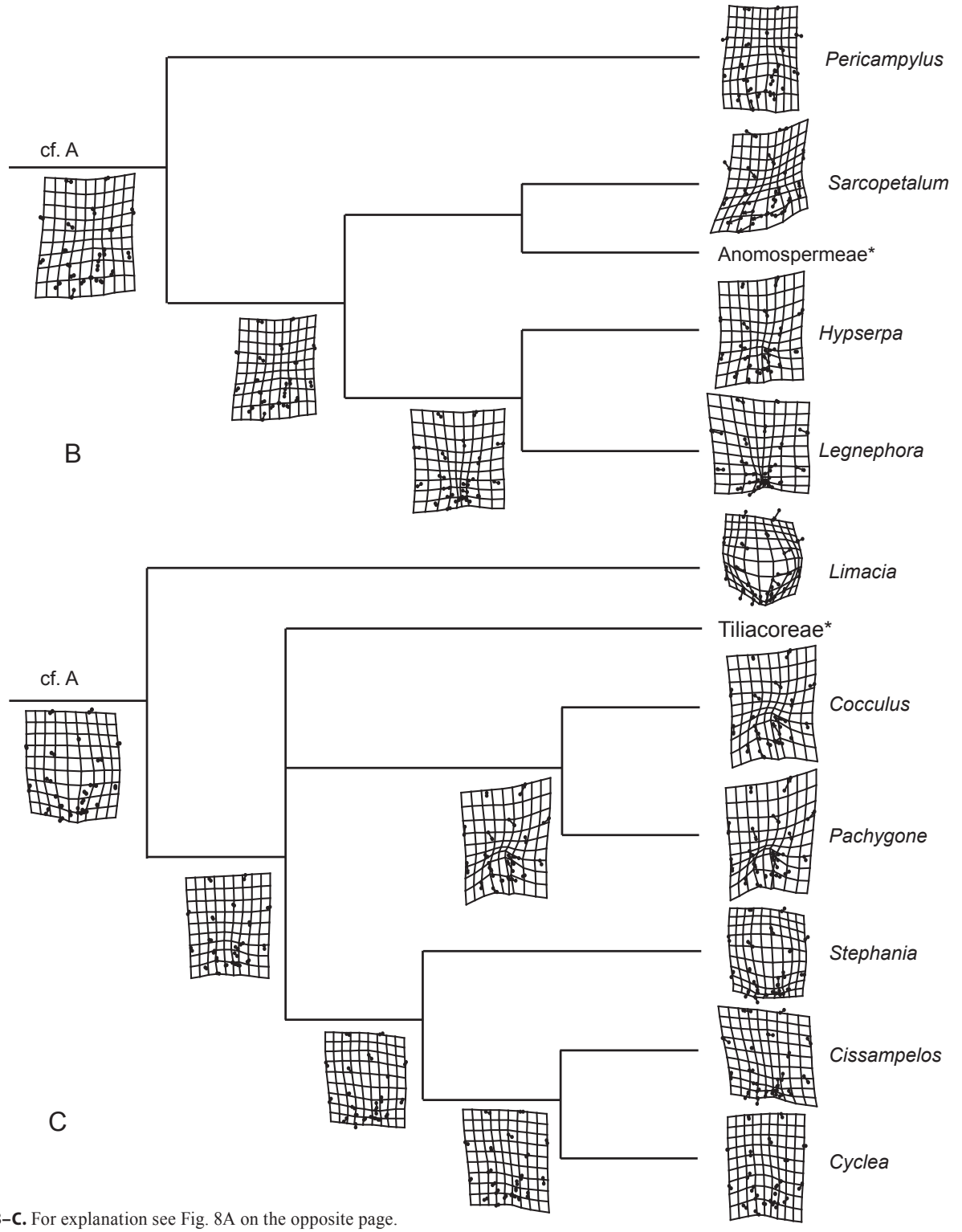

Fig. 8B–C. For explanation see Fig. 8A on the opposite page.

size variation using a linear model or a log-transformed model (Table 2). The effect of size on shape variation was therefore negligible. Allometry may have only a marginal role in endocarp shape evolution.

Shape affinities and phylogenetic relationships. — Comparison of the cluster analysis with molecular phylogeny revealed important differences (Fig. 7). The clade of *Pericampylus*, *Sarcopetalum*, *Hyperspa* and *Legnephora* was not found in the shape analysis but these four genera were found in various clusters. The morphological affinities between *Legnephora* and *Pericampylus*, underlined by Diels (1910) and Forman (1982), were not retrieved in the present analysis of endocarp shape. The position of *Strychnopsis* and *Spirospermum* could not be compared to molecular phylogenies as they have not been included in such analyses yet. On the shape basis, the two genera endemic to Madagascar were grouped in one cluster (Fig. 7). The clade comprising *Cissampelos*, *Cyclea* and *Stephania*, even though well defined on molecular and morphological basis (Ortiz & al., 2007; Wang & al., 2007; Jacques & Bertolino, 2008; Hoot & al., 2009), was not found in the cluster analysis (Fig. 7). This group was already recognized by Baillon (1872) and Bentham & Hooker (1862) added *Sarcopetalum* which was corroborated by Jacques & Bertolino (2008) using morphological cladistics. However, *Sarcopetalum* was treated separately by Diels (1910) and Baillon (1872) underlined its affinities with *Menispermum* or *Sinomenium*. Molecular analyses (Ortiz & al., 2007; Hoot & al., 2009) excluded *Sarcopetalum* from the Cissampelideae.

On the other hand, some clusters correspond to the molecular clade: the clade of *Menispermum* and *Sinomenium* has been found in molecular analysis (Wang & al., 2007; Hoot & al., 2009) and cluster analysis (Fig. 7). *Sinomenium* was separated from *Menispermum* by Diels (1910), and the monophyly of this group was underlined by Thanikaimoni (1984). *Hypserpa* and *Limacia* were grouped by Miers (1871) in the Hypserpeae, which were found monophyletic by morphological cladistic analysis (Jacques & Bertolino, 2008) but show no close affinity in the current morphometric analysis (Fig. 7) or in molecular analysis (Ortiz & al., 2007).

At the species level (Fig. S1 in the Electronic Supplement), our results suggest that the closest shape affinities are not always among cogeneric species. For example, *Stephania abyssinica*, *S. laetificata* and *S. tetrandra* did not group with other *Stephania* species. The taxonomic status of *S. laetificata*, also found quite different in PCA (Fig. 5), is under discussion: the most recent molecular phylogeny (Hoot & al., 2009) resolved it in a paraphyletic relationship with other *Stephania* species and some authors considered it as belonging to *Perichasma* (Kundu & Guha, 1977).

The phylogenetic signal in endocarp shape appears to be very low. This could be explained by (1) erroneous molecular phylogenies, (2) retention of plesiomorphic states in endocarp shape, or (3) convergent evolution. The molecular phylogenetic data used in this paper are based on several plastid markers; including *ndhF* (Ortiz & al., 2007), *matK* and *trnL-F* (Wang & al., 2007), *rbcL* and *atpB* (Hoot & al., 2009), and the use of several markers may reduce errors in phylogenetic reconstruction (Cummings & al., 1995; Rosenberg & Kumar, 2001; Rokas & Carroll, 2005). However, it should be considered that all available sequences are from chloroplastic genome. Analysis of convergent evolution is complex due to the fact that nothing is known about the biological role of the curved endocarp in Menispermaceae. Very little is known about Menispermaceae fruit dispersal, which may occur endozoochorically via spider monkeys (Link & di Fiore, 2006), gorillas (Watts, 1984), lemurs (Sussman, 1977), galagos (Molez-Verrière, 2000) and tapirs (Henry & al., 2000). Furthermore, nothing is known about a possible evolutionary link between endozoochory and endocarp morphology. We cannot, therefore, reject the idea of a possible convergent evolution of endocarp shape in response to dispersal selective pressure. The fossil record of Menispermaceae horseshoe-shaped endocarp is rich and the diversification of shape seems to be old (Jacques, 2009b), therefore fossil records gives no clear indication of the ancestral endocarp morphology.

Taxonomy versus phylogeny. — Endocarp shape in Menispermaceae is useful in identification of specimens and for determining affinities of fossils (Jacques & Zhou, 2008). At the generic level, endocarp shape seems to be well defined (Fig. 4); for higher taxonomic units, however, endocarp shape is less characteristic (Fig. 8). Our study shows that endocarp shape in Menispermaceae is of low value to reconstruct phylogenetic relationships at family level.

Limit of the method. — The non-grouping of *Cissampelos* and *Cyclea* in the cluster analysis was striking since these two genera, have been found to exclusively share two lateral ridges on each side (Jacques, 2009a). In our large-scale morphometric analysis, it was difficult to account for this character, however, since the points were expected to be homologous (Bookstein & al., 1985; Bookstein, 1991; Jensen, 2003), and the geometric morphometric method cannot deal with missing data (Adams & al., 2004).

This study used a different number of specimens for each genus. This was partly due to the fact that some genera include many species (*Cyclea*, *Stephania*), while others are monospecific, e.g., *Sinomenium* and *Spirospermum* (Appendix). Although this may have affected the results of statistical analyses the use of a nested model for MANOVA limited this problem.

Shape evolution according to phylogeny. — Much information can be derived from the shape evolution reconstruction on the molecular-based phylogeny (Fig. 8). The shape of the common ancestor appeared very similar to the consensus shape, and only slight evolution occurred along the backbone. On the other hand, in generic lineages and some lineages leading to two genera, evolution was more obvious. This may explain the low phylogenetic signal present in endocarp shape. We have also to keep in mind that several clades contain other endocarp shapes as well (Fig. 8), for example straight endocarps in Expanded Tinosporeae and hairpin-shaped endocarp in Anomospermeae and Tiliacoreae. The current analysis does not provide a comprehensive picture of endocarp evolution in Menispermaceae. According to Hoot & al.'s (2009) phylogeny, the curved endocarps may represent a plesiomorphic state in Menispermaceae, therefore some plesiomorphic characteristics may explain certain groupings in the cluster analysis.

Furthermore, hairpin-shaped endocarps, with more elongated and reduced condyle (Jacques, 2009a), seems to have evolved several times from curved endocarps, in *Diploclisia glaucescens*, Anomospermeae and Tiliacoreae (Fig. 8).

Thanikaimoni (1984) suggested an evolution of horseshoe-shaped endocarps towards a reduction of the internal cavity. The current analysis (Fig. 8) shows this trend for *Menispermum*, *Sinomenium*, *Stephania*, and, to a lesser extent, *Pericampylus*. However, our analysis reveals the opposite trend (towards an increase of the internal cavity) in other lineages such as *Cocculus* and *Pachygone*.

Convergent evolution also occurred for other characters. Evolution towards concave ventral face occurred in several lineages (e.g., *Legnephora*, *Menispermum*, *Legnephora*; Fig. 8), as did evolution towards convex ventral face (e.g., *Diploclisia affinis*, *Limacia*, *Stephania*; Fig. 8). Asymmetrical endocarps appeared several times, e.g., in the *Pachygone*+*Cocculus* clade, in *Sinomenium* and in *Legnephora* (Fig. 8).

In conclusion, the endocarp shape evolution in Menispermaceae shows some trends but cluster analysis did not match the phylogenetic tree. It should be noted that the cluster analysis was based on an overall comparison of endocarps. Separating endocarp shape into different characters may lead to better results. Attempts have been made to include shape in cladistic analysis uses partial warps as characters (Fink & Zelditch, 1995; Zelditch & al., 2000; Swiderski & al., 2002). However, this has been questioned by several workers (Adams & Rosenberg, 1998; Rohlf, 1998). In this study, we demonstrate that despite difficulties reconstructing a phylogeny using shape, geometric morphometrics can help us to understand the shape evolution based on existing phylogenies.

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Appendix. List of studied specimens. The numbers in brackets after a genus name refer to the number of recognized species in the genus. The numbers in brackets after a specimen refer to the number of studied endocarps from the specimen.

Cissampelos L. [22], ***C. fasiculata*** Benth.: *Oldeman 545* (1; P); ***C. mucronata*** A. Rich.: *J.C. Bille 3059* (1; P); ***C. owariensis*** DC.: *W. Robyns 1153* (1; P); ***C. pareira*** L.: PE 01070033 (5), *Bourgeau 2444* (2; P). ***Cocculus*** DC. [8], ***C. carolinus*** DC.: *E.J. Palmer 22109* (1; P); ***C. laurifolius*** DC.: KUN 0457033 (1), KUN 0164613 (3), KUN 0164615 (1), KUN 0164642 (5), KUN 0164655 (5), PE 01070063 (4), PE 01070091 (5), PE 01070092 (5), PE 01070061 (3); ***C. orbiculatus*** (L.) DC.: KUN 0797160 (2), KUN 0164916 (3), KUN 0164796 (3), KUN 0164872 (3), KUN 0164791 (2), KUN 0164739 (3), KUN 0164899 (3), KUN 0164895 (3), KUN 0797359 (2), KUN 0166980 (4), KUN 0164684 (3), KUN 0164835 (3), KUN 0165293 (1), KUN 0164731 (2), KUN 0164842 (3), KUN 0164728 (2), KUN 0164901 (1), KUN 0166291 (1), KUN 0165296 (3), KUN 0164715 (3), KUN 0164716 (3), KUN 0164683 (3), KUN 0164768 (3), KUN 0753117 (4), KUN 0164770 (2), KUN 0166298 (2), *J. Cavalerie 984* (1; P); ***C. pendulus*** (J.R. & G. Frost) Diels: *D. De Franceschi s.n.* (1; P). ***Cyclea*** Arn. ex Wight. [29], ***C. atjehensis*** Forman: BKF 115319 (4); ***C. barbata*** Miers: BKF 123850 (4), PE 01036724 (4), *L. Pierre 1261* (1; P); ***C. debiliflora*** Miers: KUN 0165042 (3); ***C. gracillima*** Diels: KUN 0575895 (1); ***C. hainanensis*** Merr.: PE 01036770 (5); ***C. hypoglauca*** (Schauer) Diels: KUN 0164957 (3), KUN 0164968 (3), KUN 0164962 (1), KUN 0164959 (3), KUN 0164983 (1), KUN 0164988 (3), KUN 01648989 (5), KUN 0165016 (3), PE 01036737 (3), PE 01036743 (5); ***C. insularis*** (Makino) Hatusima: PE 01041229 (5); ***C. meeboldii*** Diels: KUN 0166862 (1), KUN 0164980 (3); ***C. polypetala*** Dunn: KUN 0164936 (5), KUN 0164946 (1), KUN 0164935 (4), KUN 0164948 (5), KUN 0164949 (4), PE 01036765 (6), PE 01036768 (6); ***C. racemosa*** Oliv.: KUN 0164909 (3), KUN 0165005 (4), KUN 0164942 (5), KUN 0164910 (5), PE 01036789 (4); ***C. sutchuensis*** Gagnep.: KUN 0165064 (1), KUN 0165125 (2), KUN 0165111 (2), PE 01036832 (2), PE 1036815 (3); ***C. tonkinensis*** Gagnep.: KUN 0165096 (3), KUN 0165088 (2), KUN 0165060 (2), KUN 0165095 (3); ***C. varians*** **Craib**: BKF 42928 (3), BKF 81693 (4); ***C. wattii*** Diels: KUN 0165031 (1), KUN 0166889 (2), KUN 0165055 (2), KUN 0165058 (5). ***Diploclisia*** Miers [3], ***D. affinis*** (Oliv.) Diels: KUN 0165404 (5), KUN 0165406 (1), KUN 0165402 (2), KUN 0165394 (5), KUN 0165392 (1), PE 01070858 (5), PE 01070885 (5), PE 01070879 (4); ***D. glaucescens*** (Bl.) Diels: KUN 0166313 (2), KUN 0165387 (2), PE 0107913 (3), *E. Poilane 7640* (1; P). ***Hypserpa*** Miers [8], ***H. nitida*** Miers ex Benth.: KUN 0165338 (2), KUN 0166319 (2), PE 01070940 (3), PE 01071203 (3), PE 01071184 (1), *H. Bon 55665* (1; P). ***Legnephora*** Miers [5], ***L. minutiflora*** (K. Schum.) Diels: *M.S. Clemens 8682* (1; P). ***Limacia*** Lour. [3], ***L. scandens*** Lour.: *L. Pierre 1643* (1; P). ***Limaciopsis*** Engl. [1], ***L. loagensis*** Engl.: *R. Letouzey 12245* (1; P), Tisserant 1426 (1; P). ***Menispermum*** L. [2], ***M. canadense*** L.: KUN 0165280 (2), *J. Miller & al. 5500* (8; MO), S.D. Swanson 2328 (11; MO), *Michaux s.n.* (1; P); ***M. dauricum*** DC.: KUN 0165236 (2), KUN 0165256 (1), PE 01071135 (5), PE 01071343 (5), PE 01604656 (2), PE 01071064 (4). ***Pachygone*** Hook. f. & T. Thomson [9], ***P. nitida*** **Pierre** ex Gagnep.: *Thorel 2104* (1; P); ***P. odorifera*** Miers: *Poilane 4666* (1; P); ***Pachygone ovata*** (Poir.) Hook. f. & T. Thomson: *W. Arnott s.n.* (1; P); ***P. sinica*** Diels: KUN 0165235 (2); ***P. valida*** Diels: KUN 0165277 (1), KUN 0165261 (2), KUN 0165263 (1), KUN 0165259 (1), PE 01071394 (3), PE 01071393 (3). ***Pericampylus*** Miers [2], ***P. glaucus*** (Lam.) Merr.: KUN 0165157 (3), KUN 0166970 (3), KUN 0165795 (2), KUN 0757369 (2), KUN 0165713 (2), KUN 0165784 (5), KUN 0166895 (1), KUN 0165136 (1), KUN 0165833 (2), KUN 0166969 (4), KUN 0165750 (2), KUN 0165767 (3), KUN 0166335 (3), KUN 0165780 (4), KUN 0164816 (5), KUN 0165166 (2), KUN 0165823 (4), *F. Jacques 67* (1; P). ***Sarcopetalum*** F. Muell. [1], ***S. harveyanum*** F. Muell.: *M.J. v. Balgooy 1561* (P). ***Sinomenium*** Diels [1], ***S. acutum*** (Thunb.) Rhed. & Wils.: KUN 0165594 (3), KUN 0165600 (4), KUN 0165624 (2), KUN 0165650 (2), KUN 0165642 (3), KUN 0165631 (3), KUN 0166848 (2), KUN 0575891 (3), KUN 0166336 (5), KUN 0165610 (2), KUN 0165565 (2), KUN 0165604 (3), KUN 0166855 (2), KUN 01655585 (2), KUN 0165572 (2), KUN 0165595 (4). ***Spirospermum*** Thou. [1], ***S. penduliflorum*** Thou.: *Boivin 1834* (1; P). ***Stephania*** Lour. [66], ***S. abyssinica*** (Dill. & Rich.) Walp.: *Quartis-Dillon & Petit s.n.* (1; P); ***S. brachyandra*** Diels: KUN 0165978 (3), KUN 0165589 (1), KUN 0165581 (3), KUN 0165977 (2), PE 01036094 (5), PE 01036026 (4), PE 01036225 (5); ***S. cepharantha*** Hayata: KUN 0165544 (3), KUN 0165552 (3), KUN 0165555 (5), PE 01036056 (5), PE 01071999 (5); ***S. chingtungensis*** H.S. Lo: KUN 0165550 (4); ***S. delavayi*** Diels: KUN 0165305 (2), KUN 0165524 (2), KUN 0165518 (3), KUN 0165486 (1), KUN 0165511 (4), KUN 0165478 (2), PE 01036206 (3), PE 01036215 (5); ***S. dicentrinifera*** H.S. Lo: KUN 0165444 (1), KUN 0165454 (1), KUN 0165442 (1), KUN 0165437 (1), KUN 0165453 (5), KUN 0165503 (5), KUN 0165449 (6), KUN 0165452 (4); ***S. dielsiana*** Wu: KUN 0165464 (4), KUN 0165465 (4), PE 01036167 (3); ***S. dolichopoda*** Diels: KUN 0165408 (4), KUN 0812011 (2); ***S. elegans*** Hook. f. & T. Thomson: KUN 0166258 (1); ***S. excentrica*** H.S. Lo: PE 01036133 (4); ***S. forsteri*** (DC.) A. Gray: KUN 0166402 (5), KUN 0166263 (5), KUN 0166401 (5), KUN 0166249 (2), KUN 0166250 (4); ***S. glandulifera*** Miers: PE 01036136 (6); ***S. gracilenta*** Miers: KUN 0166404 (3), PE 01036140 (5); ***S. graciliflora*** **Yamamoto**: KUN 0166217 (1), KUN 0166252 (1), KUN 0166237 (1), KUN 0166254 (1); ***S. herbacea*** Gagnep.: KUN 0166191 (2), KUN 0166208 (1), KUN 0166849 (4); ***S. hernandifolia*** (Willd.) Walp.: KUN 0166029 (4), KUN 0166157 (1), KUN 0167000 (2), KUN 0166153 (1), KUN 0166173 (2), KUN 0166143 (3), KUN 0166004 (3), KUN 0771830 (4), KUN 0166155 (5), KUN 0166016 (4), KUN 0166179 (2), KUN 0166195 (2), KUN 0166030 (4), KUN 0166005 (7), KUN 0166036 (6), KUN 0166142 (3), KUN 0166126 (2), KUN 0166116 (3), KUN 0166140 (4), KUN 0166109 (4), KUN 0166133 (4), KUN 0166007 (3), KUN 0166099 (3), KUN 0166093 (3), KUN 0166112 (4); ***S. japonica*** (Thunb.) Miers: KUN 0166068 (3), KUN 0166075 (1), KUN 0166070 (3), KUN 0166074 (2), PE 01036240 (5), PE 01036232 (3), PE 01036259 (6); ***S. kwangiensis*** H.S. Lo: KUN 0166090 (3); ***S. laetificata*** (Miers) Benth.: *R. Letouzey 2989* (1; P). ***S. longa*** Lour.: KUN 0166050 (1), KUN 0166054 (1), KUN 0166059 (5), KUN 0165913 (2), KUN 0166047 (3), KUN 0166063 (5), PE 01036475 (6), PE 01036466 (5), PE 01036457 (4); ***S. longipes*** H.S. Lo: KUN 0165935 (3), KUN 0165964 (3), KUN 0165958 (2), KUN 0165954 (2), KUN 0165957 (2), KUN 0165947 (1), KUN 0165942 (5); ***S. pierrei*** Diels: Poilane 17870 (1; P); ***S. sinica*** Diels: KUN 0165911 (2), KUN 0165908 (5), KUN 0575870 (3), KUN 0165910 (5), KUN 0575875 (4), PE 01036399 (3), PE 01036418 (1), *Farges 41* (1; P); ***S. subpeltata*** H.S. Lo: KUN 0165926 (2); ***S. tetrandra*** S. Moore: KUN 0165916 (2), KUN 0165904 (3), KUN 0165896 (3), KUN 0165902 (4), KUN 0165870 (3), KUN 0165895 (2), KUN 0165899 (3), PE 01036579 (4), PE 01036561 (4); ***S. viridflavens*** H.S. Lo & M. Yang: KUN 0165891 (3); ***S. yunnanensis*** H.S. Lo: KUN 0165840 (1), KUN 0165858 (2), KUN 0165843 (3), KUN 0165857 (1). ***Strychnopsis*** Baill. [1], ***S. thouarsii*** Baill.: *C. Birkinskaw 575* (1; P).