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ORIGINAL ARTICLE

Placement of *Kuhlmanniodendron* Fiaschi & Groppo in Lindackerieae (Achariaceae, Malpighiales) confirmed by analyses of *rbc*L sequences, with notes on pollen morphology and wood anatomy

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Abstract The phylogenetic placement of *Kuhlmanniodendron* Fiaschi & Groppo (Achariaceae) within Malpighiales was investigated with *rbc*L sequence data. This genus was recently created to accommodate *Carpotroche apterocarpa* Kuhlm., a poorly known species from the rainforests of Espírito Santo, Brazil. One *rbc*L sequence was obtained from *Kuhlmanniodendron* and analyzed with 73 additional sequences from Malpighiales, and 8 from two closer orders, Oxalidales and Celastrales, all of which were available at Genbank. Phylogenetic analyses were carried out with maximum parsimony and Bayesian inference; bootstrap analyses were used in maximum parsimony to evaluate branch support. The results confirmed the placement of *Kuhlmanniodendron* together with *Camptostylus*, *Lindackeria*, *Xylotheca*, and *Caloncoba* in a strongly supported clade (posterior probability = 0.99) that corresponds with the tribe Lindackerieae of Achariaceae (Malpighiales). *Kuhlmanniodendron* also does not appear to be closely related to *Oncoba* (Salicaceae), an African genus with similar floral and fruit morphology that has been traditionally placed among cyanogenic Flacourtiaceae (now Achariaceae). A picrosodic paper test was performed in herbarium dry leaves, and the presence of cyanogenic glycosides, a class of compounds usually found in Achariaceae, was detected. Pollen morphology and wood anatomy of *Kuhlmanniodendron* were also investigated, but both pollen (3-colporate and

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microreticulate) and wood, with solitary to multiple vessels, scalariform perforation plates and other features, do not seem to be useful to distinguish this genus from other members of the Achariaceae and are rather common among the eudicotyledons as a whole. However, perforated ray cells with scalariform plates, an uncommon wood character, present in *Kuhlmanniodendron* are similar to those found in *Kiggelaria africana* (Pangieae, Achariaceae), but the occurrence of such cells is not mapped among the angiosperms, and it is not clear how homoplastic this character could be.

Keywords Achariaceae · Cyanogenic glycosides · Lindackerieae · *Kuhlmanniodendron* · Pollen · *rbc*L · Wood anatomy

Introduction

Kuhlmanniodendron Fiaschi & Groppo is a monospecific genus recently created to accommodate *Carpotroche apterocarpa* Kuhlm., a species previously described in Flacourtiaceae by Kuhlmann (1935) on the basis of incomplete flowering and fruiting material (Fiaschi and Groppo 2008). The genus is defined by a unique set of characters, including leaves with a *Clusia*-like venation (many thin, closely spaced and parallel secondary veins), scaly trichomes, flowers with glabrous filaments, three free styles, and indehiscent fruits with a smooth surface (sometimes with vertical ribs when dried). The single species, *K. apterocarpum* (Kuhlm.) Fiaschi & Groppo, is represented by trees or tall shrubs restricted to the rainforests of Espírito Santo in eastern Brazil. This region harbors a high number of endemic species and is threatened by the rapid deforestation of the Atlantic rainforests (Morellato and Haddad 2000; Myers et al. 2000).

Kuhlmanniodendron was originally placed in the family Achariaceae, tribe Lindackerieae by Fiaschi and Groppo (2008), mainly because of some aspects of floral morphology (see below). Achariaceae was recently re-circumscribed by Chase et al. (2002) in a study of the former Flacourtiaceae, which included a large sample of the Malpighiales, based on *rbc*L sequence data. Achariaceae, traditionally constituted by a few genera of climbers, subshrubs, or acaulescent herbs restricted to southern Africa, was enlarged to accommodate genera of trees and shrubs from tropical and subtropical areas of Africa, Australia, Asia, and the Americas that were formerly placed in Flacourtiaceae, and which were found to be a polyphyletic assemblage. The polyphyly of Flacourtiaceae and its split into re-circumscribed monophyletic families has been widely accepted and confirmed by subsequent molecular studies (e.g., Davis and Chase 2004; Davis et al. 2005; Tokuoka and Tobe 2006; Wurdack and Davis 2009).

Chase et al. (2002) recognized four tribes within Achariaceae: Acharieae (3 genera, southern Africa), Pangieae (11 genera, mainly in Australia, Malesia, and Asia), Erythrospermeae (5 genera, tropical Africa, Philippines, and other Pacific and Indian archipelagos), and Lindackerieae (11 genera, tropical America and Africa). Lindackerieae included the African genera *Caloncoba* (10 species), *Camptostylus* (3 species), *Xylotheca* (3 species), and the tropical American and African *Lindackeria* (13 species), i.e., all genera that were previously synonymized under *Oncoba* by Hul and Breteler (1997) on the basis of floral and fruit similarities. Chase et al. (2002), however, chose to reinstate those genera because of the strongly supported position of *Oncoba* (now restricted to four African species) within Salicaceae, thus phylogenetically far from the Lindackeriae (Achariaceae). The position of *Oncoba* among other genera of Salicaceae was reported as an anomaly in the study of Chase et al. (2002), and the authors of that work had to check the origin of the material and re-sequence the *rbc*L gene. Placement of *Oncoba* in Salicaceae was confirmed by these authors and later by Alford (2005) with different samples, and the genus is now included in an *incertae sedis* tribal position in the family. This placement was also supported by androecium development, which is centrifugal in *Oncoba* (van Heel 1977), as in Salicaceae, but centripetal or bidirectional in Achariaceae (Bernhard and Endress 1999; for the significance of these patterns, see also Endress 2006).

Due to the overall similarity of flowers and fruits between *Oncoba* (Salicaceae) and genera from the tribe Lindackerieae (Achariaceae), such as *Lindackeria*, *Xylotheca*, *Caloncoba*, *Camptostylus*, *Carpotroche*, and *Mayna*, we conducted here a cladistic analysis using *rbc*L sequences to verify the placement of the new genus *Kuhlmanniodendron* within the order Malpighiales, which includes Salicaceae and Achariaceae. The objective is to test whether the genus is related to the tribe Lindackerieae (Achariaceae) or *Oncoba* (Salicaceae) or yet another family in Malpighiales. We also investigate data from pollen and wood anatomy of *Kuhlmanniodendron* to better characterize this genus morphologically, and we analyze the presence of cyanogenic glycosides, a type of compound reported as rare in Salicaceae, but usually present in Achariaceae, according to Chase et al. (2002).

Materials and methods

To assess the position of *Kuhlmaniodendron* among angiosperms, the *rbc*L exon from plastid DNA was sequenced. This region has been used extensively to infer ordinal, familial, and subfamilial relationships among land plants; as a consequence, a large number of *rbc*L sequences

are available online in public databases such as GenBank (~47,000 as of December 2008), making *rbc*L probably the most appropriate sequence region for evaluating the familial position of any plant.

DNA extraction, amplification, and sequencing

Total genomic DNA was extracted from ca. 5 mg of dried leaves from a collection of *Kuhlmanniodendron apterocarpum* made in Espírito Santo, Brazil (voucher information in the "Appendix"). The Nucleon Phytopure kit (Amersham Pharmacia Biotech, Little Chalfort, Buckinghamshire, England) was used, following the manufacturer's protocol. The *rbc*L region was amplified using the primers described in Olmstead et al. (1992) and Fay et al. (1998). The PCR reaction volume (50 µL) contained 30.75 µL of water, 3 µL of pyrrolidone polyvinyl (PVP) 1%, 3 µL of 50 mM $MgCl_2$, 5 µL of *Taq* Buffer (10×), 5 µL of 10 µM dNTP, 0.25 µL of 5U/µL *Taq* polymerase, 0.25 µL of each primer, and 2 µL of DNA sample at approximately 50 ng/µL. Thermal cycling was performed in a PTC-100 Thermal Sequencer (MJ Research, Waltham, MA, USA), using initial denaturation at 95°C (2 min); followed by 33 cycles at 95°C (30 s), 57°C (1 min), and 72°C (2 min); with a final elongation at 72°C (7 min). PCR products were purified with GFX PCR columns (Amersham Biociences, Piscataway, NJ, USA), following the manufacturer's recommendations. The sequencing reaction volume was 10 µL, and it contained 3.25 µL of water, 2 µL of BigDye Terminator Ready Reaction, 0.5 µL (10 mM) of primer, and 4.25 µL of PCR product (60–150 ng of DNA). The reactions were performed in an ABI-3100 automatic sequencer (Applied Biosystems-HITACHI, Tokyo, Japan), using the same protocol of Groppo et al. (2008): 25 cycles at 96° (10 s), 50°C (15 s), and 60°C (4 min). Sequences from both strands were analyzed and the final sequence edited using the Biological Sequence Alignment Editor software (BioEdit), v.5.0.9 (copyright Tom Hall 1997–2001).

Choice of taxa

In order to identify the phylogenetic position of *Kuhlmanniodendron*, the following steps were performed for choosing the ingroup taxa. First, a MEGABLAST search with default settings was performed at GenBank (http://www.ncbi.nlm.nih.gov) using the entire *rbc*L sequence obtained. The 40 most similar sequences found were then downloaded and included in this study. Then, in order to place these sequences into a broader phylogenetic context, representatives from other plant families that have been suggested as closely related (e.g., Achariaceae and Salicaceae) were added, based on morphological and molecular affinities according to studies of Chase et al. (2002), Davis and Chase (2004), Davis et al. (2005), Tokuoka and Tobe (2006), and Wurdack and Davis (2009). A single sequence of *Brexia madagascariensis* (Celastraceae, Celastrales) was used as outgroup in all analyses, and five additional sequences from Celastrales and two from Oxalidales were also added, given their putative closer position with relation to Malpighiales (see Chase et al. 2002; Wurdack and Davis 2009; APG III 2009). Sources of all sequences (80 ingroups plus 1 outgroup) are listed in the "Appendix".

Alignment and phylogenetic analyses

Initial automated alignments of the sequences were obtained with Clustal X (Thompson et al. 1997) and checked visually. PAUP* version 4.0b10 (Swofford 2002) was used for maximum-parsimony analyses using heuristic search. All characters were unordered and equally weighted (Fitch parsimony; Fitch 1971).Searches were performed with the tree-bisection-reconnection (TBR) branch-swapping algorithm with steepest descent and multrees off options, with 1,000 random-taxon addition replicates, and with 10 trees held in each replicate. Bootstrap analyses (Felsenstein 1985) were implemented to verify support of the clades, with 10,000 pseudoreplicates (10 trees retained in each pseudoreplicate), simple addition of sequences, and subtree-pruning-regrafting (SPR) branch-swapping algorithm.

MrBayes (Huelsenbeck and Ronquist 2001) was used for the Bayesian analysis. MrModelTest 2.2 (Nylander 2004) was used to find the evolutionary model that best described the genetic region analyzed. The model chosen by the Akaike Information Criterion was then incorporated into a MrBayes block in the input file (Pol 2004; Posada and Buckley 2004). The program performed 10 simultaneous runs, each running for 5 million generations under four Metropolis-Coupled Markov Chain Monte Carlo (MCMCMC) chains, sampling every 500 generations, saving branch lengths, and using the other default settings. All runs were performed at the Computational Biology Service Unit hosted by Cornell University, USA (http://cbsuapps.tc.cornell.edu). The software Tracer (Rambaut and Drummond 2003) was used to determine when the tree sampling stabilized.

Pollen morphology

Pollen samples from the same specimen used to sequence DNA (Fiaschi et al. 2000, CEPEC) were acetolyzed according to Erdtman (1960). For light microscopy (LM), pollen grains were mounted in glycerin jelly and sealed with paraffin. For scanning electron microscopy (SEM), acetolyzed pollen grains were rinsed in an ethanol series up to 100%, pipetted onto specimen stubs, air dried, coated with a heavy metal (gold), and examined and photographed

with a LEO 1430 VP scanning electron microscope. The measurements given in the descriptions represent the average of the dimensions of 25 pollen grains of the specimen examined. The palynological terminology follows that of Punt et al. (2007).

Wood anatomy

The wood samples were prepared following traditional methods in wood anatomy (Johansen 1940). Wood blocks were softened in boiling water with glycerol (20%) for 2 h, and the sections were obtained with a sliding microtome using C-type knives. The cuts were bleached with warm sodium hypoclorite, stained with aqueous safranin (1%), and mounted in synthetic resin (Enthelan) on permanent slides. Macerations were prepared with a simplified Franklin's procedure (Franklin 1945), stained with safranin and observed on temporary slides. The wood anatomy terminology follows the suggestions of the IAWA Committee (1989).

Cyanogenic glycosides

The presence of cyanogenic glycosides in *Kuhlmanniodendron apterocarpum* was verified using a picrosodic paper test according to the methodology described in Costa (1961). Approximately 3 g of dry leaves from each of two herbarium sheets (D.A. Folli 3725 and 3729) were used. Both vouchers are deposited at Herbário do Departamento de Botânica, Instituto de Biociências, Universidade de São Paulo (Herbarium SPF).

Results

Phylogenetic analyses

The aligned matrix comprised a total of 1,405 characters: 825 invariable, 244 variable but parsimony-uninformative, and 336 parsimony-informative. The Akaike Information Criterion implemented in MrModelTest chose the GTR+I+G evolutionary model as the best fit for the *rbc*L gene. The burn-in value was set to 4,000 tree samplings, reflecting 2 million generations, i.e., long after the analysis was considered to have stabilized (by inspection of effective sample sizes and standard deviation of split frequencies). Parsimony analysis resulted in 348 most parsimonious trees with 1,773 steps, consistency index (CI) = 0.43 (0.39 excluding uninformative characters), and retention index (RI) = 0.59.

Figure 1 shows the majority-rule consensus tree with posterior probabilities (PP) estimated using Bayesian inference. Bootstrap percentages (BP) are also shown for clades recovered in the majority-rule consensus tree of the bootstrap analysis. Bayesian analysis showed a better resolution (more resolved clades) when compared with the majority-rule bootstrap consensus trees. This can be noted in Fig. 1, where many clades obtained using Bayesian methodology do not exhibit the 50% bootstrap minimum percentage, even in clades with PP as high as 0.99. Given its better resolution, we choose to discuss our results on the basis of the Bayesian tree, but we are aware that sometimes Bayesian methods can produce inflated support values and consequently spurious clades (see Suzuki et al. 2002; Simmons et al. 2004). Apart from these differences in the resolution of the trees obtained in each methodology, incongruences (conflicting clades) were not noted in the resulting trees when both analyses were compared.

In the following sections, support will be referred to as strong for a posterior probability (PP) $\geq$0.91 or a bootstrap percentage (BP) $\geq$88%. These values have been shown to represent minimum values required for a 95% confidence interval of a node under certain circumstances (Zander 2004). Other arbitrarily defined intervals of support values will be referred to as moderate (76–87% for BP and 0.85–0.90 for PP), weak (63–75% for BP and 0.75–0.84 for PP), and ambiguous (<63% for BP and <0.75 for PP).

Malpighiales appeared as a strongly (PP = 1 and BP = 99%) supported monophyletic group. The bulk of Malpighiales was grouped in an ambiguous clade (PP = 0.56), sister to groups such as Phyllanthaceae and Clusiaceae. This ambiguous clade is characterized by a large basal polytomy that includes a strongly supported clade in the Bayesian analysis (PP = 0.96) but not present in the boostrap tree, and comprises representatives of Achariaceae, Lacistemataceae, Salicaceae, Goupiaceae, Violaceae, and Passifloraceae. Achariaceae emerged as monophyletic (PP = 0.94), as did Lindackerieae within Achariaceae (PP = 0.99), forming a strong clade (PP = 1, BP = 86%) together with Erythrospermeae. *Kuhlmanniodendron* appeared as a member of Lindackerieae in a strongly supported clade (PP = 0.99) that also included *Camptostylus*, *Xylotheca*, and *Caloncoba*. *Oncoba* appeared as sister to *Dovyalis* (PP = 0.59), together with other salicaceous genera, and far from the Lindackerieae.

Pollen morphology

Pollen of *Kuhlmanniodendron apterocarpum* is medium sized (P 28.0 ± 1.58 µm × E 29.7 ± 1.58 µm), oblate spheroidal (P/E = 0.94), and 3-colporate (Fig. 2a–c). The amb is subtriangular to subcircular (angulaperturate) with a smooth membrane in the ectoaperture and a lalongate endoaperture. There is a thickening in nexine under the lalongate endoapertures (costa). The exine is ca. 2.0 µm thick, simplicolumellate, and its analysis under LM

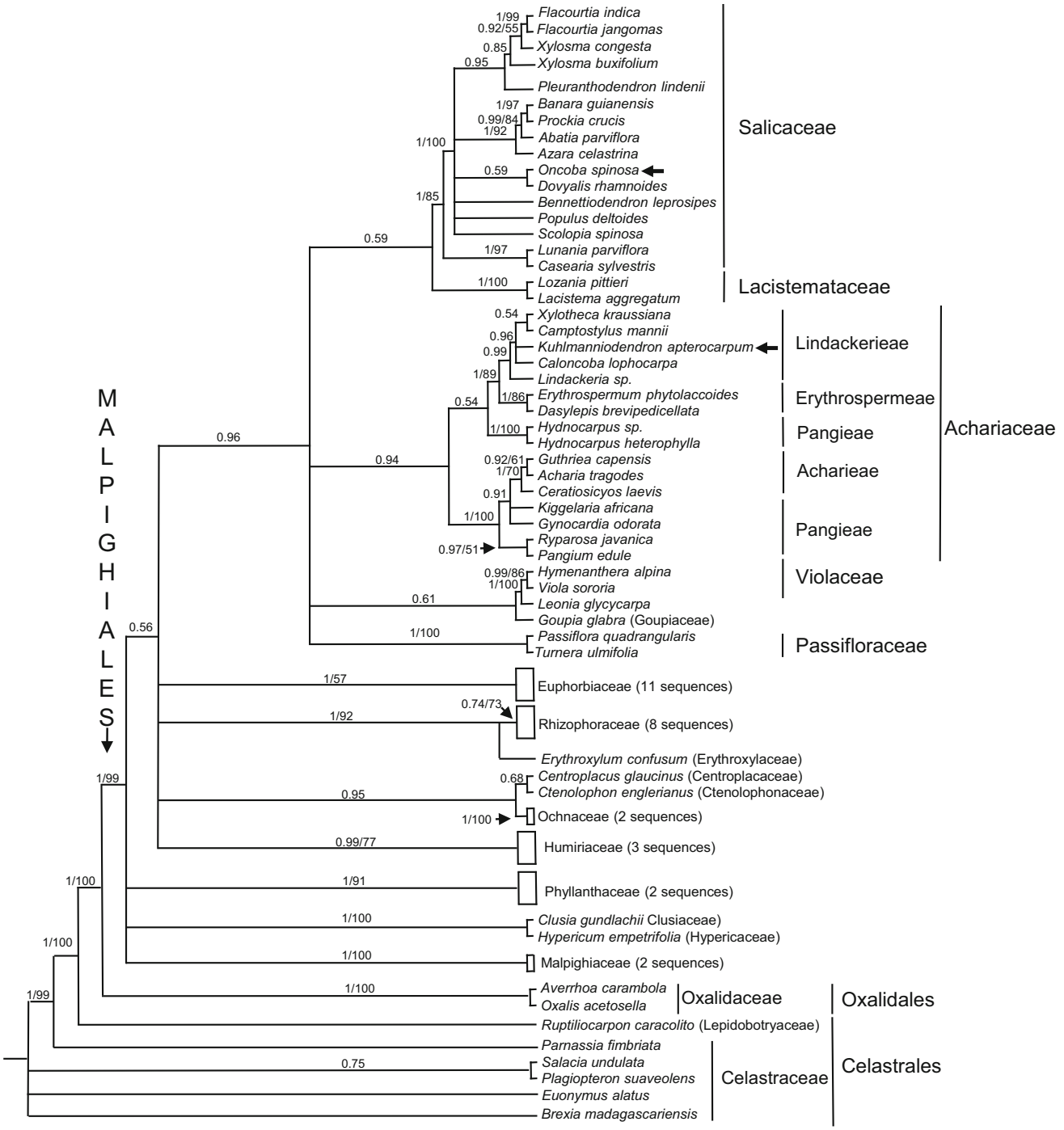

Fig. 1 Majority-rule consensus tree estimated using Bayesian inference resulting from *rbc*L analysis of Malpighiales. Posterior probabilities (≥50%) are shown *above the branches*. Bootstrap percentages (only for branches in agreement with those obtained in the Bayesian analysis) are given after posterior probabilities; when only a number appears supporting a clade it refers to Bayesian posterior probabilities. Families and tribes (only for those of Achariaceae) are given. The positions of *Kuhlmanniodendron* and *Oncoba* are indicated by *arrows* after their names

revealed a microreticulate (homobrochate) surface, with circular lumina decreasing in size towards the apertures. The same exine surface under SEM shows perforations around 0.5 μm but differing in size and shape; tectum is smooth on the apocolpium, and wavy to rugulate on the mesocolpus. The sexine is thicker than the nexine.

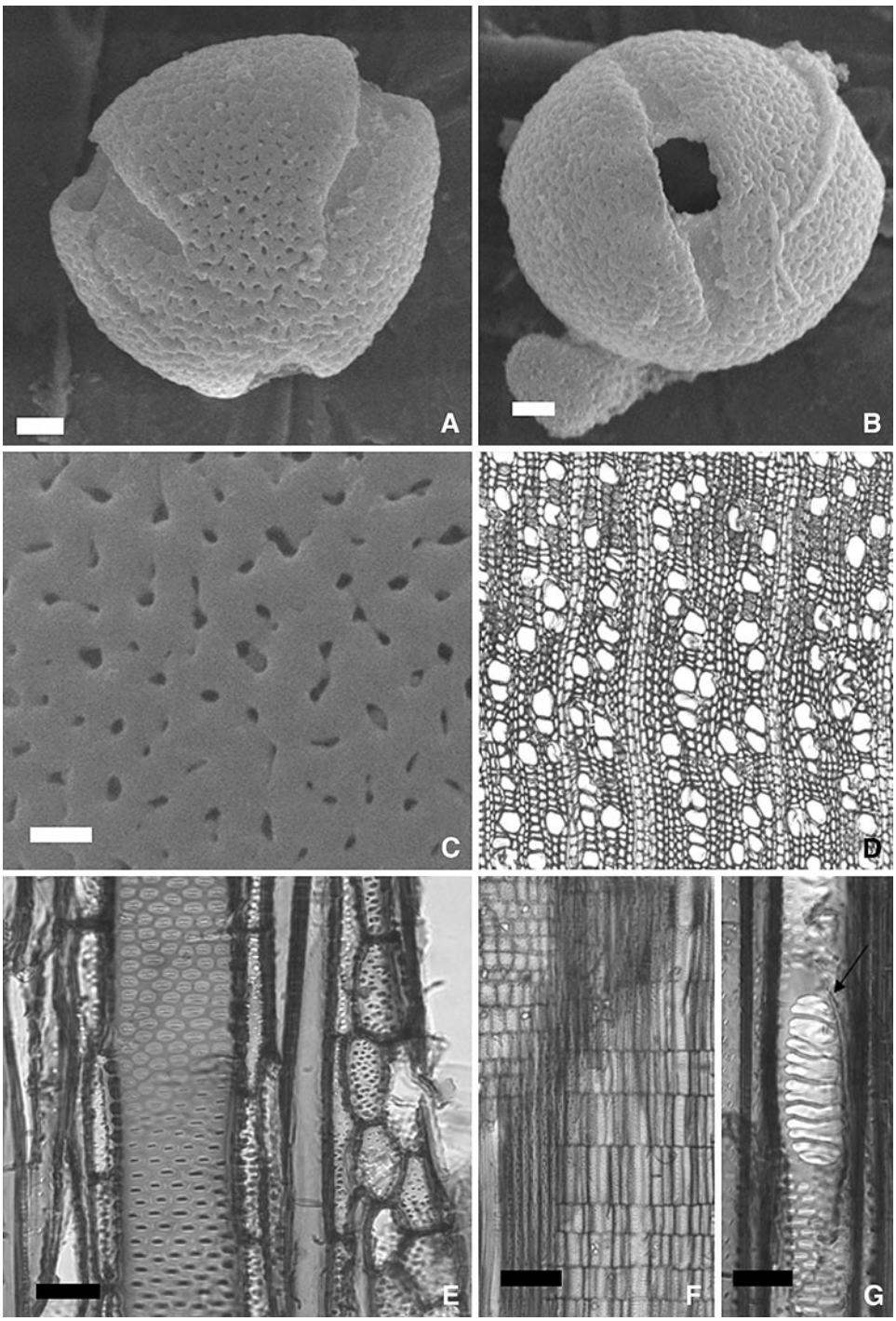

Fig. 2 Pollen (**a**–**c**) and wood anatomy (**d**–**h**) in *Kuhlmanniodendron apterocarpum*. **a** Pollen, full view. **b** Aperture. **c** Detail of the sculpture (microreticulate). **d** Wood, transverse view. **e** Longitudinal tangential view, intervessel pitting alternate. **f** Wood, radial view, rays composed of upright/square cells. **g** Radial, perforated ray cell with scalariform perforation plate (*arrow*). *Scale bars* **a**, **b** = 2 μm; **c** = 1 μm; **d**, **e**, **f** = 100 μm; **g** = 30 μm

Wood anatomy

The wood (Fig. 2d–g) of *Kuhlmanniodendron apterocarpum* is whitish diffuse-porous and has weakly distinct growth ring boundaries marked by flattened fibers. Vessels are distributed at a frequency of 20–40 per mm^2 (mean 35 ± 8 vessels/mm^2) and are mainly solitary, but groups of two to four can occur. Solitary vessel outline is angular and the tangential diameter is 50–100 μm (mean 75 ± 12 μm). Vessel elements are long (mean 1,550 ± 26 μm). The majority of perforation plates are scalariform with 20–40 bars, some have 10–20 bars, and rarely some are reticulate. Intervessel pits are alternate and large, measuring more than 10 μm (8–16 μm), while ray-vessel pits have much reduced borders and pits are round to angular or horizontal. Axial parenchyma is diffuse and rare, with five to eight cells per strand. Fibers are thick walled, very long (mean 3,595 ± 287 μm), the majority septate, with minutely

bordered pits. Rays have two distinct sizes and occur in a frequency higher than 12 rays/mm. They are often more than 1 mm and 20–100 cells in height. Uniseriate rays are composed of square and upright cells while multiseriate rays have a few strands of procumbent cells mixed in with the predominantly upright/square cells. Perforated ray cells are present and present scalariform to reticulate perforation plates. Prismatic calcium oxalate crystals occur singly or grouped in ray cells.

Cyanogenic glycosides

Both samples presented a positive result when tested for the presence of cyanogenic glycosides in the leaves, with picrosodic papers turning from yellow into light red.

Discussion

Malpighiales has been one of the most difficult groups to recognize morphologically, with unclear nonmolecular synapomorphies (Wurdack and Davis 2009). Despite recent progress on the understanding of the circumscription of the order (Chase et al. 2002; Tokuoka and Tobe 2006; Wurdack and Davis 2009) and on its position in the phylogeny of the angiosperms (Chase et al. 1993; Savolainen et al. 2000a, b, Soltis et al. 2000; Wurdack and Davis 2009 and references therein), internal groups, especially the most inclusive ones, remain unresolved. However, some larger internal groups, such as the clade constituted by Salicaceae, Lacistemataceae, Achariaceae, Violaceae, and Passifloraceae (and Goupiaceae when sampled) have arisen in different studies (Davis et al. 2005; Tokuoka and Tobe 2006; Wurdack and Davis 2009), but were not present in the study of Chase et al. (2002), which, in addition to using only *rbc*L sequences as here, did not implement Bayesian methodology. Wurdack and Davis (2009) named this group the "parietal clade," as this type of placentation in the ovary is very common among its members, as in *Kuhlmanniodendron* (Fiaschi and Groppo 2008). On the other hand, clades such as Centroplaceaeceae plus Ctenolophonaceae (ambiguous here) as sister to Ochnaceae (PP = 0.95, but not present in the bootstrap majority-rule consensus tree), and the large ambiguous (PP = 0.56) group represented by the "Salicaceae until Humiriaceae" (as drawn in Fig. 1) are not corroborated by the studies of Tokuoka and Tobe (2006) and Wurdack and Davis (2009), which used more terminals and sequenced regions.

The presence of *Oncoba* in Salicaceae (and far from achariaceous genera, see further discussion below) in the present study is not surprising, given the same sequence was used in the study of Chase et al. (2002). However, while in this latter study *Oncoba* appears as sister to *Flacourtia*, in the present study *Oncoba* is closer to *Dovyalis*, while *Flacourtia* is closer to *Xylosma* here and in the studies of Tokuoka and Tobe (2006). These differences can be explained by both different sampling and methodology, since Chase et al. (2002) included more genera of Salicaceae than here and used only maximum parsimony analysis, while Tokuoka and Tobe (2006) used more regions (three plastidial and one nuclear) and parsimony as well. Still, the grouping of *Flacourtia*, *Dovyalis*, and *Xylosma* (plus *Oncoba*, *Trimeria,* and *Pleuranthodendron* in the study of Chase et al. 2002) appears as constant in the different studies of Chase et al. (2002), Tokuoka and Tobe (2006), and Wurdack and Davis (2009).

Although not the focus of the present work, the number of taxa of Achariaceae sampled here made possible the verification of the circumscription of its tribes as stated by Chase et al. (2002). Lindackerieae, Erythrospermeae, and Achatieae appear to be monophyletic, but the position of species of *Hydnocarpus* (Pangieae) closer to the clade of Lindackerieae and Erythrospermeae than other members of Pangieae requires further investigation. Additionally, the remaining Pangieae members appear in a paraphyletic group close to Achatieae (see Fig. 1). The grouping of *Hydnocarpus* plus Lindackerieae and Erythrospermeae obtained here is ambiguous (PP = 0.56) but is also present in the consensus tree of Chase et al. (2002). On the other hand, the remaining Pangieae members (including *Pangium*, the type genus) appear here in a strongly supported clade (PP = 1, BP = 100%), as they do in Chase et al. (2002) in the consensus tree and with a BP = 99%. The grouping of *Hydnocarpus* (Pangieae) with *Carpotroche* (Lindackerieae) plus *Erythrospermum* (Erythrospermeae), and *Kiggelaria* plus *Pangium* (both Pangieae) with *Acharia* (Achatieae) in the study of Wurdack and Davis (2009), all of them with strong support, corroborates the nonmonophyletism of Pangieae, despite the fact that only these six genera of Achariaceae have been sampled. Studies with more terminals of Achariaceae are needed to clarify the circumscription of tribes Achatieae and Pangieae within Achariaceae.

Kuhlmanniodendron was tentatively placed in the tribe Lindackerieae of Achariaceae by Fiaschi and Groppo (2008), mainly because of floral features, such as the possession of sepals and petals asymmetrically arranged and distinct from each other, three imbricate sepals, petals more numerous and larger than sepals, without an adaxial scale (encountered in tribes Achatieae, Erythrospermeae, and Pangieae), many stamens with anthers more or less linear, disk glands absent, and the unilocular ovary with parietal placentation. Although the fruits of *Kuhlmanniodendron* do not bear the wings, bristles, or spines that are typical of many Lindackerieae, some longitudinal ribs can be seen in dried material (see Fiaschi and Groppo 2008).

Kuhlmanniodendron apterocarpum would have been assigned as an *Oncoba* species according to the treatment of Hul and Breteler (1997). These authors merged *Caloncoba*, *Lindackeria*, *Xylotheca*, *Camptostylus*, *Paraphyadanthe*, and *Mayna* under *Oncoba*, arguing that the generic division proposed by Gilg (1925) based on fruit surface, typology and size of the inflorescences, and shape of the placenta was unsatisfactory. Before the publication of this treatment, however, van Heel (1977) had already pointed out several differences in the development of flowers and fruits between *Oncoba* and genera such as *Caloncoba*, *Lindackeria*, *Dasylepis*, *Camptostylus*, among others. These differences included the presence of atropous ovules in *Oncoba* (vs. anatropous in all other genera); the embryo sac forming extensions upwards into the nucellar cap (*Oncoba*) versus downwards into the chalaza accompanied by a ring or cup of tracheids (*Caloncoba*, *Camptostylus*, and *Lindackeria*); and the sclereid layer comprising one (*Oncoba*) versus two or more cell layers in all other genera analyzed (*Caloncoba*, *Camptostylus*, *Dasylepis*, *Scottellia*, *Berberidopsis*, *Lindackeria*, and *Peterodendron*).

Results of the phylogenetic analyses of *rbc*L sequences performed by Chase et al. (2002) corroborated the structural data of van Heel (1977) and Bernhard and Endress (1999). Molecular data have not only shown that *Oncoba* is not closely related to other genera of Lindackerieae, but that it should instead be placed in another family, namely Salicaceae. In fact, there are no traces of cyanogenic glycosides in *Oncoba*—these compounds are usually present in Achariaceae, where their presence has been confirmed for many genera of the tribes Pangieae and Erythrospermeae (see Chase et al. 2002) and now in *Kuhlmanniodendron* (Lindackerieae), which tested positive for these kind of compounds according to our results. Cyanogenic glycosydes are rare in Salicaceae, occurring, for example, in *Banara* (Spencer and Seigler 1985; Webber and Miller 2008). Furthermore, genera from Salicaceae bear leaves with salicoid teeth, which are absent in Achariaceae (van Heel 1977). Thus, macromorphological similarities that linked *Oncoba* to the Lindackerieae are better interpreted as the result of convergence. The close relationship between *Caloncoba* and *Camptostylus* advocated by van Heel (1977) based on floral anatomical features was confirmed by molecular data from Chase et al. (2002). In this phylogenetic study the two genera were placed in a well-supported clade together with *Xylotheca*, which had not been included in van Heel's (1977) study. Other characteristics that link *Caloncoba* and *Camptostylus*, as well as the closely related genus *Lindackeria*, are the presence of an absorbing cavity in the chalaza of the seeds, which is surrounded by a whorl of chalazal vascular bundles, and the absence of endosperm (van Heel 1977, p. 363). Since *Kuhlmanniodendron* appears to be closely related to *Caloncoba*, *Camptotylus*, *Lindackeria*, and *Xylotheca* (present study), it is probable that the floral developmental and anatomical features shared by these genera are also found in this new genus. The same features of floral development may occur in *Carpotroche* and *Grandidiera*, two groups that appeared close to *Lindackeria* in the study of Alford (2005). Studies on the floral development and anatomy of *Kuhlmanniodendron*, as well as on the remaining exclusively Neotropical genera of Lindackerieae (*Carpotroche* and *Mayna*), are desirable.

According to Miller (1975), the genera of Oncobeae (sensu Gilg 1925) are scarcely distinguishable in terms of wood anatomy. This circumscription of Oncobeae includes 18 American and African genera plus *Oncoba*, but today the genera of this tribe are not only divided in tribes Lindackerieae and Erythrospermeae of Achariaceae, but also, as we have seen for *Oncoba*, positioned in another family of Malpighiales (Salicaceae), and even in an uncertain position in eudicots such as *Berberidopsis* (Berberidopsidaceae, see Soltis et al. 2000). Thus, the affirmation of Miller (1975) regarding the gross wood anatomy of the *Oncobeae sensu* Gilg (1925) is probably a reflection of a relative lack of specialized structures in the wood of these plants, as observed in our data for *Kuhlmanniodendron*. Wood with solitary to multiple vessels, scalariform perforation plates, alternate intervessel pitting, and rays of two sizes composed of square and/or upright cells is common in many eudicots. Otherwise, the presence of perforated ray cells with scalariform plates in *Kuhlmanniodendron*, similar to those found in *Kiggelaria africana* (Chalk and Chattaway 1933), is not a common character. The occurrence of perforated ray cells has not been mapped among the angiosperms, and it is not clear how homoplastic this character is. So it is desirable that this cryptic character be observed in Malpighiales.

The same pattern occurs with pollen morphology, where 3-colporate, reticulate pollen is widely distributed among the eudicots (see Doyle 2005). According to genera sampled by Erdtman (1952), which included representatives of *Acharia*, *Caloncoba*, *Ceratosicyos*, *Erythrospermum*, *Guthriea*, *Hydnocarpus*, *Kiggelaria*, *Lindackeria*, and *Xylotheca*, achariaceous pollen has shared morphological features; however, the group needs more extensive study to cover the possible variations.

It is expected that more accurate phylogenies of the members of Achariaceae could clarify not only the position of *Kuhlmanniodendron*, but also of the remaining genera of the family, guiding studies of vegetative and reproductive morphology and development.

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Appendix

Vouchers and literature citations or database accession numbers for taxa included in this study. Family (in bold), Genbank accession number, reference or source.

Achariaceae. *Acharia tragodes* Thunb. AJ418795, Chase et al. (2002); *Caloncoba lophocarpa* Gilg AJ418796, Chase et al. (2002); *Camptostylus mannii* Gilg AJ418797, Chase et al. (2002); *Ceratosicyos laevis* A.Meeuse AB233841, Tokuoka and Tobe (2006); *Dasylepis brevipedicellata* Chipp AB233842, Tokuoka and Tobe (2006); *Erythrospermum phytolaccoides* Gardner AJ418798, Chase et al. (2002); *Guthriea capensis* Bolus AJ402965, Savolainen et al. (2000b); *Gynocardia odorata* R.Br. AJ402957, Savolainen et al. (2000b); *Hydnocarpus heterophylla* Blume AJ418799, Chase et al. (2002); *Hydnocarpus* sp. AF206783, Soltis et al. (2000); *Kiggelaria africana* L. AF206786, Soltis et al. (2000); *Kuhlmanniodendron apterocarpum* (Kulhm.) Fiaschi and Groppo GU929701, this paper, Fiaschi et al. 2000 (Herbarium CEPEC), *Lindackeria* sp. AJ418800, Chase et al. (2002); *Pangium edule* Reinw. AJ418801, Chase et al. (2002); *Ryparosa javanica* (Blume) Kurz ex Koord. & Valeton AJ418802, Chase et al. (2002); *Xylotheca kraussiana* Hochst. AJ418803, Chase et al. (2002). **Celastraceae**. *Brexia madagascariensis* Thouars ex Ker.-Gawl. L11176, Morgan and Soltis (1993); *Euonymus alatus* (Thunb.) Siebold L13184, Chase et al. (1993); *Parnassia fimbriata* K.D.Koenig L01939, Soltis et al. (1990); *Plagiopteron suaveolens* Griff. AJ235787, Savolainen et al. (2000a); *Salacia undulata* Cambess. AJ402998, Savolainen et al. (2000b). **Centroplacaceae**. *Centroplacus glaucinus* Pierre AY663646, Wurdack et al. (2005). **Clusiaceae**. *Clusia gundlachi* Stahl Z75673, Fay et al. (1997); **Ctenolophonaceae**. *Ctenolophon englerianus* Mildbr. AJ402940, Savolainen et al. (2000b). **Euphorbiaceae**. *Blumeodendron tokbrai* Kurz ex J.J.Sm. AJ418805, Chase et al. (2002); *Elateriospermum tapos* Blume AY794873, Wurdack et al. (2005); *Klaineanthus gaboniae* Pierre ex Prain AY794869, Wurdack et al. (2005); *Manihot esculenta* Crantz AB233880, Tokuoka and Tobe (2006); *Manihot grahamii* Hook. AY794875, Wurdack et al. (2005); *Maprounea guianensis* Aubl. AJ418810, Chase et al. (2002); *Moultonianthus leembruggianus* (Boerl. & Kourd.) Steenis AY794982, Wurdack et al. (2005); *Pogonophora schomburgkiana* Miers ex Benth. AY88185, Davis et al. (2005); *Suregada boiviniana* Baill. AY88189, Davis et al. (2005); *Syndyophyllum occidentale* (Airy Shaw) Welzen AY794967, Wurdack et al. (2005); *Thyrsanthera suborbicularis* Pierre ex Gagnep. AY794984, Wurdack et al. (2005). **Goupiaceae**. *Goupia glabra* Aubl. AJ235780 Savolainen et al. (2000a). **Humiriaceae**. *Humiria balsamifera* Aubl. AB233889, Tokuoka and Tobe (2006); *Saccoglottis* sp. AB233890 Tokuoka and Tobe (2006); *Vantanea guianensis* Aubl. Z75679, Fay et al. (1997). **Hypericaceae**. *Hypericum empetrifolium* Willd. AF206779, Soltis et al. (2000). **Lacistemataceae**. *Lacistema aggregatum* Rusby AF206787, Soltis et al. (2000); *Lozania pittieri* L.B.Sm., AJ418804, Chase et al. (2002). Lepidobotryaceae. *Ruptiliocarpon caracolito* Hammel & N.Zamora AJ402997, Savolainen et al. (2000b). **Malpighiaceae**. *Byrsonima crassifolia* (L.) Kunth L01892, Albert et al. (1992); *Malpighia glabra* L. AB233900, Tokuoka and Tobe (2006). **Ochnaceae**. *Ochna serrulata* Walp. Z75273, Chase et al. (1993); *Ouratea duparquetiana* Baill. Z75684, Fay et al. (1997). **Oxalidaceae**. *Averrhoa carambola* L. L14692, Price and Palmer (1993); *Oxalis acetosella* L. FJ670181, Wurdack and Davis (2009). **Passifloraceae**. *Passiflora quadrangularis* (Baill.) H.Perrier AF206802, Soltis et al. (2000); *Turnera ulmifolia* L. Z75691, Fay et al. (1997). **Phyllanthaceae**. *Margaritaria tetracocca* (Baill.) G.L.Webster Z75675, Fay et al. (1997); *Phyllanthus liebmannianus* Müll.Arg. Z75676, Fay et al. (1997). **Rhizophoraceae**. *Blepharistemma membranifolium* (Miq.) Ding Hou AF006761, Setoguchi et al. (1999); *Bruguiera gymnorhiza* (L.) Savigny AB233927, Tokuoka and Tobe (2006); *Cassipourea ceylanica* Alston AF127674, Schwarzbach and Ricklefs (2000); *Cassipourea elliptica* (Sw.) Poir. AF127672, Schwarzbach and Ricklefs (2000); *Cassipourea guianensis* Aubl. AF127673, Schwarzbach and Ricklefs (2000); *Dactylopetalum ellipticifolium* Arénes AF129129, Setoguchi et al. (1999); *Erythroxylum confusum* Britton L13183, Chase et al. (1993); *Macarisia emarginata* Scott-Elliot AF129130, Setoguchi et al. 1999; *Sterigmapetalum guianense* Steyerm. AF127671, Schwarzbach and Ricklefs (2000). **Salicaceae**. *Abatia parviflora* Ruiz & Pav. AF206726, Soltis et al. (2000); *Azara celastrina* D.Don AJ418820, Chase et al. (2002); *Banara guianensis* Aubl. AJ402923, Savolainen et al. (2000b); *Bennettiodendron leprosipes* (Clos) Merr. AJ418821, Chase et al. (2002); *Casearia sylvestris* Sw. AF206746, Soltis et al. (2000); *Dovyalis rhamnoides* (Burch. Ex DC.) Burch. Ex Harv. & Sond. Z75677, Fay et al. (1997); *Flacourtia indica* (Burm.f.) Merr. AB233933, Tokuoka and Tobe (2006); *Flacourtia jangomas* Ateud. AF206768, Soltis et al. (2000); *Lunania parviflora* Spruce

ex Benth. AB233936, Tokuoka and Tobe (2006); *Oncoba spinosa* Forssk. AJ418823, Chase et al. (2002); *Pleuranthodendron lindenii* (Turcz.) Sleumer AJ418832, Chase et al. (2002); *Populus deltoides* Marshall AJ418829, Chase et al. (2002); *Prockia crucis* L. AJ418831, Chase et al. (2002); *Scolopia spinosa* Warb. AJ418833, Chase et al. (2002); *Xylosma buxifolium* A.Gray AJ418834, Chase et al. (2002), *Xylosma congesta* (Lour.) Merr. AB233938, Tokuoka and Tobe (2006). **Violaceae**. *Hymenanthera alpina* Oliv. Z75692, Fay et al. (1997); *Leonia glycycarpa* Z75693, Fay et al. (1997); *Viola sororia* Willd. L11674; Olmstead et al. (1992).

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