

Embryology and systematic relationships of *Kiggelaria* (Flacourtiaceae)

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ABSTRACT

Kiggelaria L. is endemic to Africa and the only representative of tribe Pangieae (Flacourtiaceae) on the continent. Molecular genetics, phytochemistry and host-parasite relationships have indicated a relationship between this woody, pantropical tribe of Flacourtiaceae and a small, trigenic family of herbaceous, southern African endemics, the Achariaceae. In the present study, ovule and seed structure in *Kiggelaria* were investigated and compared with relevant data recently reported for Achariaceae. Support for an alliance with Achariaceae were found in the presence of anatropous, bitegmic, sessile ovules with zigzag micropyles, deep-lying embryo sacs covered by an epistase in the ovule and seed, endotestal-exotegmic seed coat layers, suspensorless embryos and sarcotestal seed with a prominent, plate-like hypostase. Unlike Achariaceae, *Kiggelaria* seeds do not have chalazal seed lids, pronounced raphal ridges, a fringe layer, or stomata in the outer epidermis of the sarcotesta. Structural dissimilarities in seeds of ornithochorous *Kiggelaria* and myrmecochorous Achariaceae were regarded as adaptations linked to different strategies for seed germination and dispersal.

INTRODUCTION

Kiggelaria L. is a widely distributed, wholly African genus of dioecious trees and shrubs. It occurs in all the provinces of the Republic of South Africa and in Lesotho, Swaziland, Namibia, south tropical Africa and tropical East Africa as far north as Mount Kilimanjaro (Killick 1976a). In these areas *Kiggelaria* represents an important floristic element of forest in the archipelago-like Afromontane Region of Endemism (White 1978). This African phytocorion is of special biogeographical and evolutionary significance because of its putative ancient status. Although the genus shows considerable variability in juvenile and mature vegetative morphology, only one polymorphic species is currently recognized, namely *Kiggelaria africana* L. (Killick 1976b).

In most modern suprageneric classification systems of Flacourtiaceae, *Kiggelaria* is placed within the tribe Pangieae. This tribe of about 10 genera has a disjunct, pantropical distribution (Wendt 1988) with most genera reported from Malaysia (Chase *et al.* 2002). *Chiangiodendron* T.Wendt occurs in southeastern Mexico (Wendt 1988), *Baileyoxylon* C.T.White is found in Australia and *Kiggelaria* is the only representative from Africa. Alternatively, *Kiggelaria* and other genera of the Pangieae have been treated as a separate family, Kiggelariaceae (Savolainen *et al.* 2000; Soltis *et al.* 2000). Within Flacourtiaceae, the Pangieae form a more or less homogeneous group that, in terms of generic content, has remained consistent in taxonomic treatments (Hutchinson 1967; Lemke 1988; Takhtajan 1997). On account of comparative macromorphology, wood anatomy, palynology and the distribution of selected classes of chemical constituents, Lemke (1988) regarded the cyanogenic, relatively unspecialized flacourtiaceous tribes Berberidopsidae, Erythrospermeae, Pangieae and Oncobeeae as

closely related. He suggested that phylogenetic relationships among them and other cyanogenic families of Violales should be examined more carefully.

A possible phylogenetic link between one such family, namely Achariaceae, a family of three monogeneric genera from southern Africa, and the tribe Pangieae, particularly *Kiggelaria*, was first suggested by the breeding behaviour of a butterfly, *Acraea horta* (Nymphalidae: Heliconiinae: Acraeini) in the botanical garden of the University of Pretoria. This was reported by Dahlgren & Van Wyk (1988; see also Steyn *et al.* 2002b). Based on evidence from molecular biology, Chase *et al.* (1996) also suggested a linkage between the herbaceous Achariaceae and the woody tribe Pangieae. More recent phylogenetic molecular data reported by e.g. Soltis *et al.* (2000) and Savolainen *et al.* (2000) have provided additional support for a close association between *Acharia* Thunb., *Guthriea* Bolus (Achariaceae) and *Kiggelaria*. The third genus, *Ceratosicyos* Nees, was not included in these investigations. Lately, Chase *et al.* (2002) proposed splitting Flacourtiaceae *sensu* Lemke (1988) and emending the circumscription of the families Achariaceae Harms and Salicaceae Mirb. Achariaceae *sensu* Chase *et al.* (2002) thus include Achariaceae Benth. & Hook.f., Pangieae (*sensu auct.*), Erythrospermeae DC. and a newly described tribe, Lindackerieae Zmarzty (in Chase *et al.* 2002: 172, 173), all cyanogenic. The non-cyanogenic tribes of Flacourtiaceae *sensu* Lemke [Flacourtieae DC., Samydeae (Vent.) Dumort., Homalieae (R.Br.) Dumort., Scolopieae Warb., Prockieae Endl., Abatieae Hook.f. and Bembicieae Warb.] have been placed with Saliceae (*Salix* L. and *Populus* L.) and Scyphostegieae (Hutch.) Zmarzty in a much enlarged Salicaceae (Chase *et al.* 2002).

Apart from data provided by molecular phylogenetics, phytochemistry and host-parasite relationships, support for an alliance between Pangieae *sensu auct.* and Achariaceae Harms has rarely been offered. Steyn *et al.* (2002a, b) pointed out that the development and structure of mechanical seed coat layers in Achariaceae closely match those of *Kiggelaria* and *Hydnocarpus* Gaertn.

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(Pangieae) as described by Van Heel (1979). This resemblance suggests that a detailed comparison of ovule and seed characters might reveal additional similarities between *Kiggelaria* and Achariaceae. However, embryological data on *Kiggelaria* obtainable from literature were scant and ambiguous; we therefore re-investigated ovule and seed structure in *Kiggelaria africana*. The results of our study are given in the present report.

MATERIAL AND METHODS

Open flowers and developing fruit were collected from female trees in a dioecious, natural population of *Kiggelaria africana* growing in the National Botanical Garden, Pretoria, South Africa. To facilitate penetration of chemicals into ovules and seeds, the thick ovary wall was partly removed and developing seeds were removed from the locule. All material was fixed and stored in a 0.1 M cacodylate buffered solution containing 4% formaldehyde and 2.5% glutaraldehyde. Following the methods of Feder & O'Brien (1968), material was dehydrated in an alcohol series and impregnated with glycol methacrylate (GMA). All material was imbedded in GMA, sectioned transversely or longitudinally at 2–3 μm and subsequently stained with the periodic acid/Schiff reaction and toluidine blue O (pH 4.4) by using the protocols of O'Brien & McCully (1981).

RESULTS

Placentation and orientation of ovules

The unilocular, thick-walled and densely pubescent ovaries in female flowers (Figure 1) contain many ovules borne singly or in pairs on four or five parietal placentas. The sessile ovules are supplied by vascular strands connected to the large vascular bundles lying opposite the petals with their conspicuous, adaxial nectary glands

(adnate adaxial basal scales, according to Chase *et al.* 2002). The position of the placental bundles in relation to the petals suggests that they represent the marginal bundles of the congenitally fused carpels. During the maturation of ovules, the space in the locule becomes restricted. The ovules are pressed against each other and the ovary wall, and pushed out of alignment with the micropyles pointing in all possible directions.

Structure of mature ovule

Ovules are anatropous, bitegmic, crassinucellate and ovoid in shape ($\pm 530 \times 400 \mu\text{m}$) when the flowers open. Sagittal sections show that there is no funicle and the raphe is pronounced (Figure 2A). The vascular bundle of the raphe branches as it enters the overgrown chalaza, but the ramifications do not enter the integuments. Both integuments are multi-layered. The outer is thicker than the inner, especially at its distal rim where it overtops the inner integument to take part in the formation of the slightly zigzag micropyle canal (Figure 2A, B). At anthesis, the outer and inner epidermal layers of the outer integument are separated by about four layers of thin-walled, isodiametric mesophyll cells containing many, small starch grains and occasionally showing periclinal as well as anticlinal divisions. Periclinal divisions also occur in the inner epidermal cells of this integument, whereas the cells of the outer epidermis and the epidermis of the chalaza mostly divide anticlinally to form a layer of radially flattened cells (Figure 2A, B). The inner integument is about five layers thick and its cells are smaller than those of the outer integument, except for the outer epidermal cells of the inner integument which are elongate in the direction of the longitudinal axis of the ovule. A distinct cuticle layer separates the integuments from each other.

The ovoid, relatively small nucellus with enclosed embryo sac, lies in the centre of the ovule, occupying

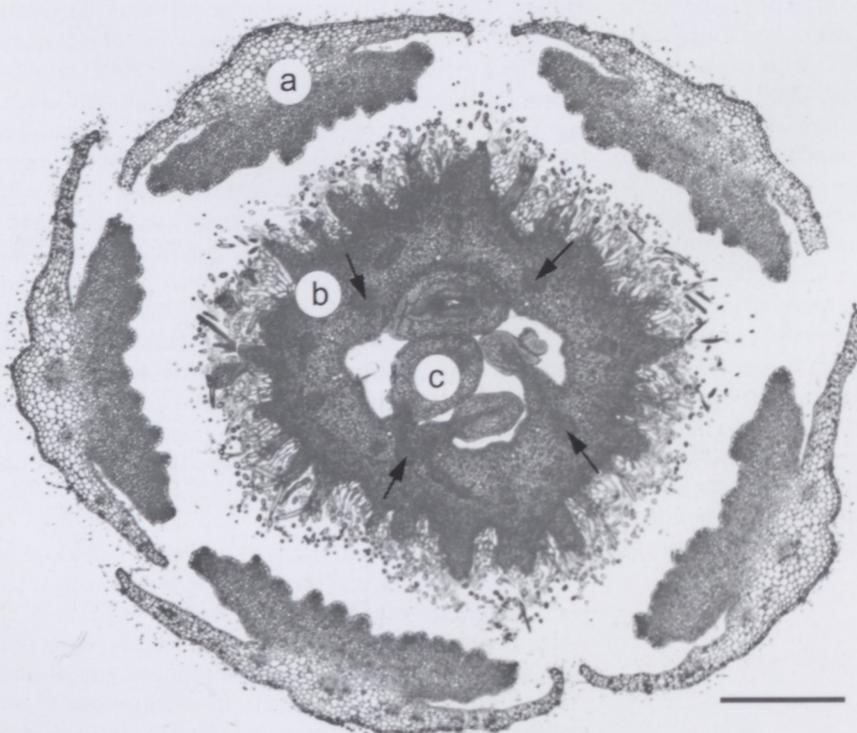


FIGURE 1.—Female flower of *Kiggelaria* in *t/s* (sepals removed). a, petal with adnate adaxial nectary gland; b, ovary with parietal placentae in antepetalous position; c, ovule. Note arrows indicating large vascular bundles that supply ovules. Scale bar: 500 μm .

about one-third of its length (Figure 2A, B). The micropylar nucellus consists of about eight cell layers and the nucellus epidermis contributes to the formation of this

tissue by dividing once, periclinally. Below the nucellus epidermis, the parietal nucellus stains darkly on account of the thicker walls and large numbers of starch grains in

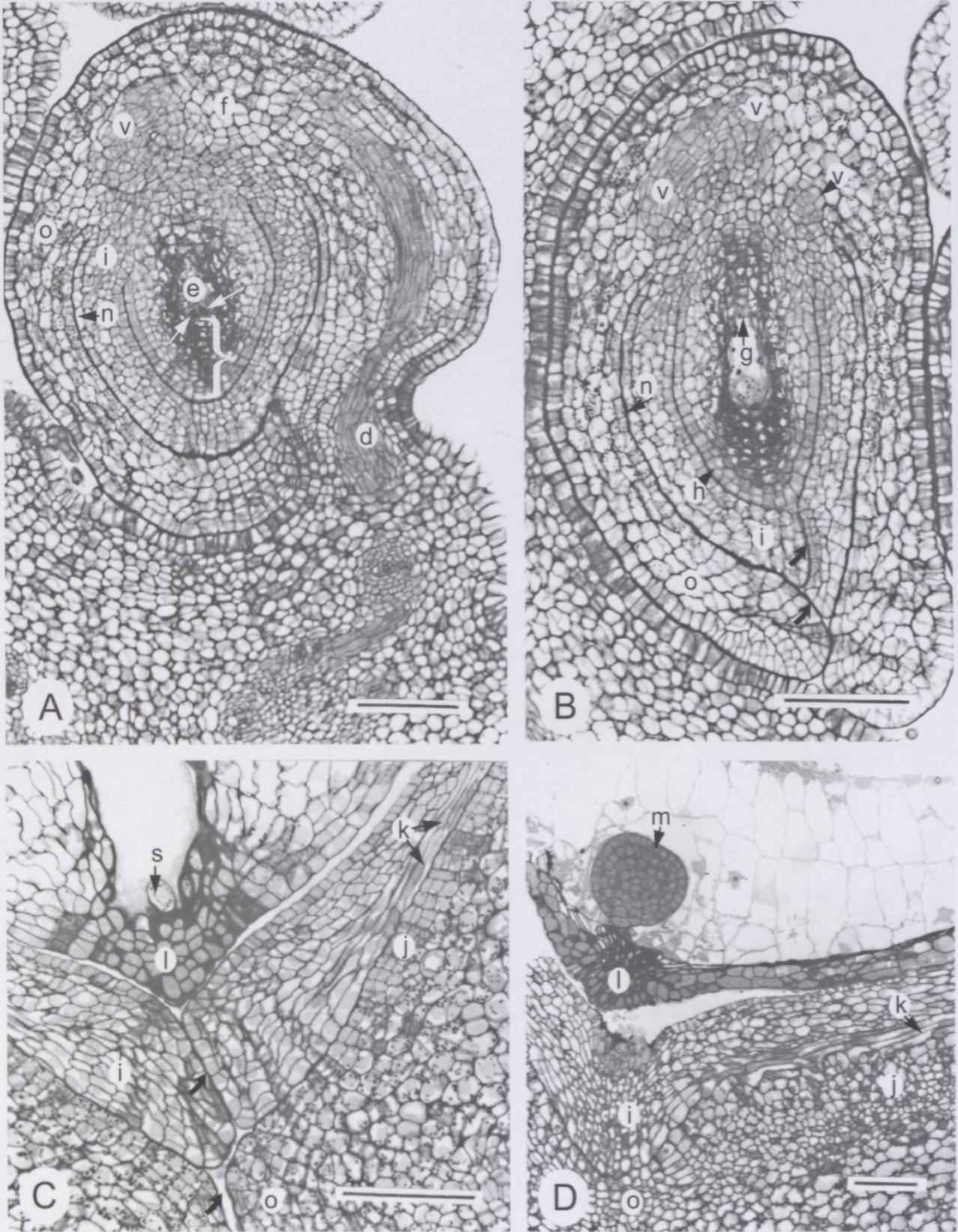


FIGURE 2.—Ovule and developing seed of *Kiggelaria*. A, structure of mature ovule in sagittal section. Note thick nucellus cap (white bracket) and filiform apparatus of synergids (white arrows); B, mature ovule in median l/s showing zigzag micropyle; C, micropylar part of young seed during resting stage of zygote; D, suspensorless embryo in micropylar part of developing seed. Curved arrows in B & C indicate zigzag micropyle; d, raphe; e, embryo sac; f, chalaza; g, antipodal cells; h, nucellus epidermis; i, inner integument; j, inner epidermis of testa; k, outer epidermis of tegmen; l, epistase; m, young embryo; n, outer epidermis of inner integument; o, outer integument; s, zygote; v, vascular bundle. Scale bars: 100 μ m.

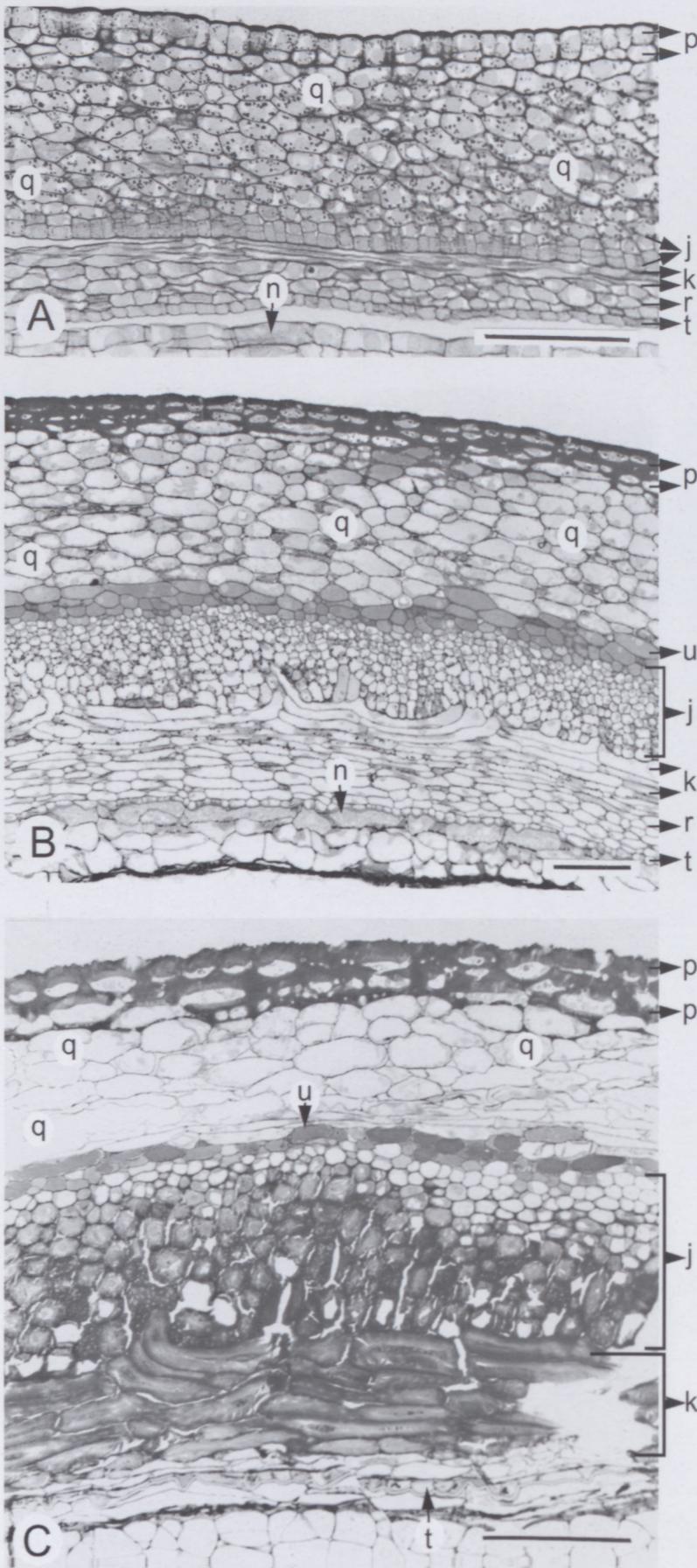


FIGURE 3.—Seed coat development in *Kiggelaria*. A, zygote stage of embryo; B, when endosperm becomes cellular; C, dispersed seed. j, inner epidermis of testa; k, outer epidermis of tegmen; n, nucellus epidermis; p, outer epidermis of testa; q, succulent mesophyll of sarcotesta; r, mesophyll of tegmen; t, inner epidermis of tegmen; u, darkly stained layer of sarcotesta. Scale bars: 100 μ m.

the cells. The inner layers of the lateral nucellus and the column-like nucellus below the embryo sac (postament, according to Shamrov 1998) show a similar staining reaction.

The pear-shaped embryo sac of the mature ovule thus lies deeply imbedded in nucellar tissue. The narrow chalazal end contains three small antipodal cells (Figure 2B). The nucleus of the central cell lies in the centre,

adjacent to the wall, whereas the egg apparatus occupies the wider, micropylar region. The position of the two synergids with darkly stained, well-formed filiform apparatus (Figure 2A), clearly indicates the distal limits of the embryo sac. Reports that the tip of the embryo sac of *Kiggelaria* breaks through the nucellus tissue and enters the micropyle (Johri *et al.* 1992) are not substantiated by the present study.

Seed and seed coat development

Fertilized ovules enlarge rapidly and endosperm formation precedes embryo development. During the free-nuclear stage of the endosperm, the zygote remains in a resting phase directly below the micropyle (Figure 2C) and is separated from the endostome by the thick-walled remnants of the nucellus cap that form an epistase. This tissue plugs the micropyle (Bouman 1984) and it persists in later stages of embryo development (Figure 2D).

The embryogeny of *Kiggelaria* was not studied in detail; pro-embryos were not found in our material and the embryo could not be typified. When the seed reaches its final size of about 6 mm, the free-nuclear endosperm becomes cellular. The embryo then is in the early heart-shaped stage and has no suspensor (Figure 2D). By the time the capsule splits open to release the ripe, bright orange-red seeds, the embryo lies in the centre of the endosperm, has thin, spatulate cotyledons and is of medium size, i.e. it extends about halfway up into the endosperm.

Contribution of the outer integument (testa) to the seed coat

The outer integument is strongly multiplicative and all cell layers contribute to the formation of the mature seed coat. The outer epidermis divides periclinally once or twice (Figure 3A) and the resulting epidermal tissue eventually acquires unevenly thickened cell walls to form a collenchymatous pellicle (Figure 3B, C). This protective layer also covers the chalaza and raphal region of the seed and contains no stomata. The cells of the mesophyll below the epidermis of the integument, raphe and chalaza divide in various planes to develop into the multi-layered, thin-walled, succulent tissue of the sarcotesta (Figure 3A–C). In the ripe seed, the contents of the innermost cells of the sarcotesta reacts strongly with PAS and toluidine blue. These dark-staining cells form a layer that separates the sarcotesta from the mechanical tissue (Figure 3B, C). It is in this position that a fringe layer occurs in the three genera of the Achariaceae (Steyn *et al.* 2002a, b), but such a layer is absent in *Kiggelaria*.

The outer integument contributes to the protective, mechanical tissue of the seed coat. This tissue has a dual derivation and consists of two sub-layers. The outer layer originates from the inner epidermis of the outer integument; the inner layer from the outer epidermis of the inner integument (see further on). The cells of the inner epidermis start dividing periclinally just after fertilization to form radial rows of cells (Figures 2C; 3A). The rows later become disrupted, because the outer cells undergo further divisions in various planes, the inner cells increase in size and the developing exotegmic fibres

start intruding into the endotestal layers (Figures 2D; 3B, C). At seed dispersal stage, the large cells of the endotesta have developed into thick-walled, isodiametric sclereids (Figure 3C). The outer, smaller cells of the endotesta remain relatively thin-walled and separate the sclereids from the layer of dark-staining sarcotestal cells (Figure 3C). At the chalazal end of the seed, the layers of relatively thin-walled, endotestal cells continue into the chalaza, surround the vascular tissue and separate the latter from a thin, plate-like layer of lignified cells that represents a hypostase (not illustrated).

Contribution of the inner integument (tegmen) to the seed coat

The inner sub-layer of the mechanical tissue is exotegmic and originates from the outer epidermis of the inner integument. The cells divide periclinally and cells are formed centripetally (Figures 2C; 3A). The derivatives rapidly stretch in a direction parallel to the longitudinal axis of the seed (Figures 2C, D; 3B, C). At this stage the cuticle between the two developing sub-layers is still distinct. When the endosperm becomes cellular, the tips of the elongated exotegmic cells start intruding into the adjacent sub-layer of endotestal tissue (Figures 2D; 3B) and the cuticle becomes disrupted. At seed dispersal stage the endotegmic sub-layer has matured into thick-walled, longitudinally orientated fibres (Figure 3C). This sub-layer does not continue into the chalaza.

The mesophyll and inner epidermis of the inner integument do not play a significant role in the structure of the mature seed coat; the layers disintegrate when the endosperm tissue matures (Figure 3C). The cuticle between the nucellus and inner epidermis of the inner integument, so prominent in seeds of *Guthriea* (Steyn *et al.* 2001), is thin. A pigment layer that, according to Van Heel (1979), differentiates on the inside of the sclereid layer in *Kiggelaria* (also *Hydnocarpus*) and persists when the mesophyll disintegrates later on, was not seen during this investigation.

DISCUSSION

For most of the 80–95 genera traditionally placed in Flacourtiaceae (e.g. Hutchinson 1967; Lemke 1988) and recently split in two groups to expand the families Achariaceae and Salicaceae (Chase *et al.* 2002), embryological characters are completely unknown. The meagre, scattered data used to compile accounts of Flacourtiaceae in compendia dealing with comparative embryology (e.g. Davis 1966; Johri *et al.* 1992) provide no support for the 'sweeping taxonomic changes' proposed by Chase *et al.* (2002). However, two embryological studies do suggest that such changes might have merit. Firstly, a recent study on members of Flacourtiaceae *sensu lato* with a multi-whorled androecium (Bernhard & Endress 1999), showed that the stamens are initiated centrifugally in Flacourtiaceae and Sclopiaceae (non-cyanogenic tribes) as reported for *Populus* in Salicaceae Mirb. (Kaul 1995). In Erythrospermeae, Oncobae (except *Oncoba*) and Pangieae (cyanogenic tribes) initiation is centripetal or simultaneous. Achariaceae, however, are characterized by a single whorl of 3–5 stamens (Dahlgren & Van Wyk 1988; Bernhard

TABLE 1.—Comparison of selected ovule and seed characters in *Kiggelaria* and Achariaceae Harms

No.	Character	<i>Kiggelaria</i>	<i>Acharia</i> , <i>Guthriea</i> and <i>Ceratiosicyos</i> (Steyn <i>et al.</i> 2002a, b)
1.	Ovule position and number	Parietal, multi-ovular.	Parietal, multiovular in <i>Guthriea</i> and <i>Ceratiosicyos</i> , tetra-ovular in <i>Acharia</i> .
2.	Ovule type	Anatropous, bitegmic, crassinucellate.	Anatropous, bitegmic, crassinucellate.
3.	Outer integument	Multilayered, overtops inner in ovule and seed.	Multilayered, shorter than inner in ovule (<i>Ceratiosicyos</i>), overtops inner in ovule and seed (<i>Acharia</i> , <i>Guthriea</i>).
4.	Inner integument	Multilayered, outer epidermal cells not conspicuously elongate in 1/s of ovule, first divisions periclinal.	Multilayered, outer epidermal cells conspicuously elongate in 1/s of ovule, first divisions anticlinal, then periclinal.
5.	Micropyle canal	Zigzag, formed by both integuments in ovule and seed.	Zigzag, formed by both integuments in ovule and seed (<i>Acharia</i> , <i>Guthriea</i>); straight, formed by inner integument in ovule, both integuments in seed (<i>Ceratiosicyos</i>).
6.	Raphe	Pronounced in ovule, not forming a ridge in seed.	Pronounced to form a ridge in ovule and seed (<i>Acharia</i> , <i>Guthriea</i>); not pronounced in <i>Ceratiosicyos</i> .
7.	Funicle	Absent, ovule and seed sessile.	Variable in length (<i>Ceratiosicyos</i>) or absent (<i>Acharia</i> , <i>Guthriea</i>).
8.	Nucellus cap	Comprises derivatives of nucellus epidermis and parietal cell, separates embryo sac from endostome, eventually forms epistase.	Comprises derivatives of nucellus epidermis and parietal cell, separates embryo sac from endostome, eventually forms epistase.
9.	Embryo sac	Monosporic, eight-nucleate, <i>Polygonum</i> Type (Johri <i>et al.</i> 1992, requires confirmation).	Bisporic, eight-nucleate, <i>Allium</i> Type.
10.	Seed type	Anatropous, sarcotestal, endospermous.	Anatropous, sarcotestal, endospermous.
11.	Hypostase	Present as plate-like layer of lignified cells in mature seed.	Present as plate-like layer of thin-walled cells with phenolic substances (<i>Ceratiosicyos</i>) or crystals (<i>Acharia</i> , <i>Guthriea</i>) in mature seed.
12.	Perisperm	Absent.	Present in <i>Acharia</i> and <i>Guthriea</i> , absent in <i>Ceratiosicyos</i> .
13.	Seed size	Medium, \pm 6 mm long.	Medium, \pm 6 mm long.
14.	Embryo type	Unknown, suspensorless.	<i>Penaea</i> variation of Asterad Type, suspensorless.
15.	Embryo size	Medium, \pm 4 mm.	Medium, \pm 4 mm (<i>Ceratiosicyos</i>), small, $<$ 2 mm (<i>Acharia</i> , <i>Guthriea</i>).
16.	Epidermis of sarcotesta	Multilayered collenchymatous pellicle, hairs and stomata absent.	Unilayered, glabrous with stomata (<i>Ceratiosicyos</i>), multilayered, collenchymatous pellicle, hairs and stomata present (<i>Acharia</i> , <i>Guthriea</i>).
17.	Mesophyll of sarcotesta	Succulent outer layers, fringe layer absent in inner layer.	Succulent outer layers, inner layer forms fringe layer.
18.	Mechanical layers in seed	Endotestal sclereids + exotegmic longitudinal fibres.	Endotestal sclereids + exotegmic longitudinal fibres.
19.	Chalazal seed lid	Absent.	Absent in <i>Ceratiosicyos</i> , present in <i>Acharia</i> , <i>Guthriea</i> .
20.	Dispersal mechanism	Autochory + ornithochory.	Autochory (<i>Ceratiosicyos</i>), autochory + myrmecochory (<i>Acharia</i> , <i>Guthriea</i>).

1999). Secondly, an earlier work on seed coat structure by Corner (1976) indicated that the family should be divided in two groups, namely a *Flacourtia* group (*Casearia* Jacq., *Flacourtia* L'Hér., *Idesia* Maxim., *Oncoba* Forssk.) and a *Hydnocarpus* group (*Hydnocarpus*, *Kiggelaria*, *Pangium* Reinw., *Scaphocalyx* Ridl.).

Corner (1976) mistakenly interpreted the seeds of all investigated members of his *Hydnocarpus* group as pachychalazal with no contribution of the integuments towards the formation of mechanical seed coat layers. Van Heel's (1974, 1979) studies showed that the seed coat of only *Pangium* is pachychalazal; *Erythrospermum* Lam., *Hydnocarpus* and *Kiggelaria* have seed coats with endotestal-exotegmic mechanical layers, but the integumental derivation and dual origin of the mechanical layers are masked by the early disappearance of the cuticle boundary. In *Caloncoba* Gilg and *Camptostylus* Gilg (Lindackerieae) the cuticle separating the inner and outer integument also vanishes entirely during seed formation (Van Heel 1977). Results obtained during the present study confirm Van Heel's (1979) observations on *Kiggelaria*. Seed coat structure in Pangieae, Erythrospermeae and Lindackerieae therefore corresponds to Achariaceae Harms as described by Steyn *et al.* (2002a, b), providing embryological support for emending the circumscription of Achariaceae as proposed by Chase *et al.* (2002).

A second embryological character of *Kiggelaria* that needed clarification is the shape of the embryo sac. Davis (1966) reported that the embryo sac of *Kiggelaria* does not become much elongated, while Johri *et al.* (1992) found the embryo sac in *Kiggelaria* similar to that of Azara Ruiz & Pav. (= *Arechavaletaia* Speg.) with an embryo sac breaking through the nucellus and reaching the exostome of the micropyle. An 'extra-nucellar embryo sac' (Johri *et al.* 1992: 549) also occurs in *Flacourtia* (Johri *et al.* 1992) and approaches the state described for Salicaceae Mirb. where the tip of the embryo sac comes into contact with the integument (Chamberlain 1897; Maheshwari & Roy 1951; Johri *et al.* 1992). This type of elongated embryo sac may well be characteristic for Salicaceae *sensu* Chase *et al.* (2002). Our results on *Kiggelaria* show that the tip of the embryo sac, clearly indicated by the filiform apparatus of the synergids, remains covered by the nucellus cap (Figure 2C, D) as reported for Achariaceae (Steyn *et al.* 2001, 2002a, b). The nucellus cap later forms an epistase, separating the zygote and embryo from the micropyle. The presence of an epistase is another clear indication that the tip of the embryo sac stays inside the nucellus. An epistase is only known in a few angiosperm families (Bouman 1984) and was recently reported for Achariaceae (Steyn *et al.* 2001, 2002a).

A detailed comparison of ovule and seed structure in *Kiggelaria* and Achariaceae (Table 1) shows additional noteworthy similarities, such as the contribution of the nucellus epidermis to nucellus cap formation, sessile ovules with zigzag micropyles (not *Ceratosicyos*), suspensorless embryos and sarcotestal seeds. The latter two characters may be important markers for Achariaceae *sensu* Chase *et al.* (2002); the embryo of at least *Idesia* (Flacourtiaceae) has a long suspensor (Johri *et al.* 1992),

while sarcotestal seeds were also reported for *Caloncoba* Gilg and *Camptostylus* Gilg (Lindackerieae) by Van Heel (1977). In the recently circumscribed Salicaceae the seeds are not sarcotestal, but are often arillate (Chase *et al.* 2002).

With regard to ovule characters, *Kiggelaria* seems closer to *Acharia* and *Guthriea* than to *Ceratosicyos* (Table 1: Nos 3, 5, 6 & 7). Some structural dissimilarities between *Kiggelaria* and Achariaceae Harms (see Nos 6, 16 & 17) can possibly be linked to specific adaptations for seed dispersal (see Steyn *et al.* 2002a for details); *Kiggelaria* seeds are bird-dispersed (Palmer & Pitman 1972) whereas *Acharia* and *Guthriea*, but not *Ceratosicyos*, are adapted to dispersal by ants. It is noteworthy that seeds of *Kiggelaria* and *Ceratosicyos*, both having relatively large embryos, lack the seed lid found in seeds of *Acharia* and *Guthriea*. The absence of such a lid in *Kiggelaria* supports our hypothesis (Steyn *et al.* 2002a, b) that this peculiar device is a specific adaptation to seed germination for the small, slow-maturing embryos in seeds of *Acharia* and *Guthriea*.

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