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A study of ovule-to-seed development in *Ceratiosicyos* (Achariaceae) and the systematic position of the genus

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ABSTRACT

A light microscope study of developing ovules and seeds of *Ceratiosicyos laevis* (Thunb.) A.Meeuse was undertaken to augment an investigation of ovule and seed structure in Achariaceae, a tri-generic family comprising three species of herbaceous perennials endemic to southern Africa. Tests for myrmecochory suggest that seed of *Ceratiosicyos* Nees is not dispersed by ants like those of *Acharia* Thunb. and *Guthriea* Bolus. Structural differences include the absence of a raphal ridge and imbibition lid and the presence of long funicles and medium-sized embryos in *Ceratiosicyos*.

INTRODUCTION

Ceratiosicyos laevis (Thunb.) A.Meeuse is one of three species of herbaceous, dicotyledonous perennials that make up an entire family of southern African endemics, the Achariaceae. The family is regarded as highly modified and its relationships have been much debated (see e.g. Bernhard 1999; Steyn *et al.* 2001 and references therein). Traditionally, Achariaceae were placed among families belonging to Violales (Dahlgren 1980). Based on evidence from phylogenetic analyses of molecular data (Savolainen *et al.* 2000) the family is placed in Malpighiales, where it has been linked with Kiggelariaceae which consists of woody perennials from southern and East tropical Africa (*Kiggelaria* L.), Assam and Burma (*Gynocardia* R.Br.) and Sri Lanka and Malaysia (*Trichadenia* Thwaites).

In niche preferences and vegetative morphology, the three herbaceous species of Achariaceae are so diverse (Dahlgren & Van Wyk 1988) that they were placed in separate genera. Yet, in breeding habit and floral structure *Ceratiosicyos laevis* shares many characters with *Acharia tragodes* Thunb. and *Guthriea capensis* Bolus. Notable similarities include the presence of few-flowered inflorescences containing both male and female flowers, absence of rudimentary organs of the opposite sex in the unisexual flowers, sympetaly (petals loosely coherent in *Ceratiosicyos* Nees), conspicuous (yellowish), antipetalous floral glands, and anthers with broad connectives and unusual, swollen trichomes (Dahlgren & Van Wyk 1988; Bernhard 1999). In addition, recent reproductive biological studies in Achariaceae have shown that ovules and seed characters in *Acharia* Thunb. and *Guthriea* Bolus are remarkably similar (Steyn *et al.* in press). Furthermore, both genera are myrmecochorous and their seeds have the same unusual adaptations for seed germination and dispersal, namely an imbibition lid and a pronounced raphal ridge to serve as a handle for carrying the smooth seed.

For *Ceratiosicyos*, very little information is available on ovule and seed structure or seed dispersal. A brief report on ovule structure by Bernhard (1999) and a reference to seed coat structure (exotegmy, according to Dahlgren & Van Wyk 1988) suggest important embryological differences between *Ceratiosicyos* and *Acharia* or *Guthriea*. It is not known whether *Ceratiosicyos* also forms part of the herbaceous myrmecochorous flora of southern Africa—it does not bear its fruit near the ground like *Acharia* and *Guthriea* for easy collection by ants, but is a vigorously growing, nontendrilliferous twiner that reaches considerable heights along streams at the edge of Afromontane forest, particularly along the eastern escarpment of southern Africa.

For the present study we investigated embryo sac formation, mature ovule characters, ovule-to-seed development and mature seed and seed coat structure in *Ceratiosicyos*. We also tested the seed for possible dispersal by ants. Results are compared with those recently obtained on *Acharia* and *Guthriea* (Steyn *et al.* 2001; Steyn *et al.* in press) to determine the embryological characters of the family and to evaluate our findings in the light of available embryological data on *Kiggelaria* L.

MATERIAL AND METHODS

Floral buds, mature female flowers and developing fruit of *Ceratiosicyos* were collected in Eastern Cape from a population growing on the banks of the Maitland River, in the Maitland River Forest Reserve (voucher specimen: *Van Wyk 13555* PRU). Additional material that included seeds at dispersal stage was gathered at Kowyns Pass near Graskop in Mpumalanga (voucher specimen: *Steyn 24* PRE). All flowering and fruiting stages were immediately immersed and stored in a 0.1 M cacodylate-buffered solution (pH 7.4) containing 4% formaldehyde and 2.5% gluteraldehyde. Flowers and fruits were later dissected, ovules and developing seeds removed, sorted according to size and rinsed in the buffer. Dehydration and impregnation with glycol methacrylate (GMA) followed the methods of Feder &

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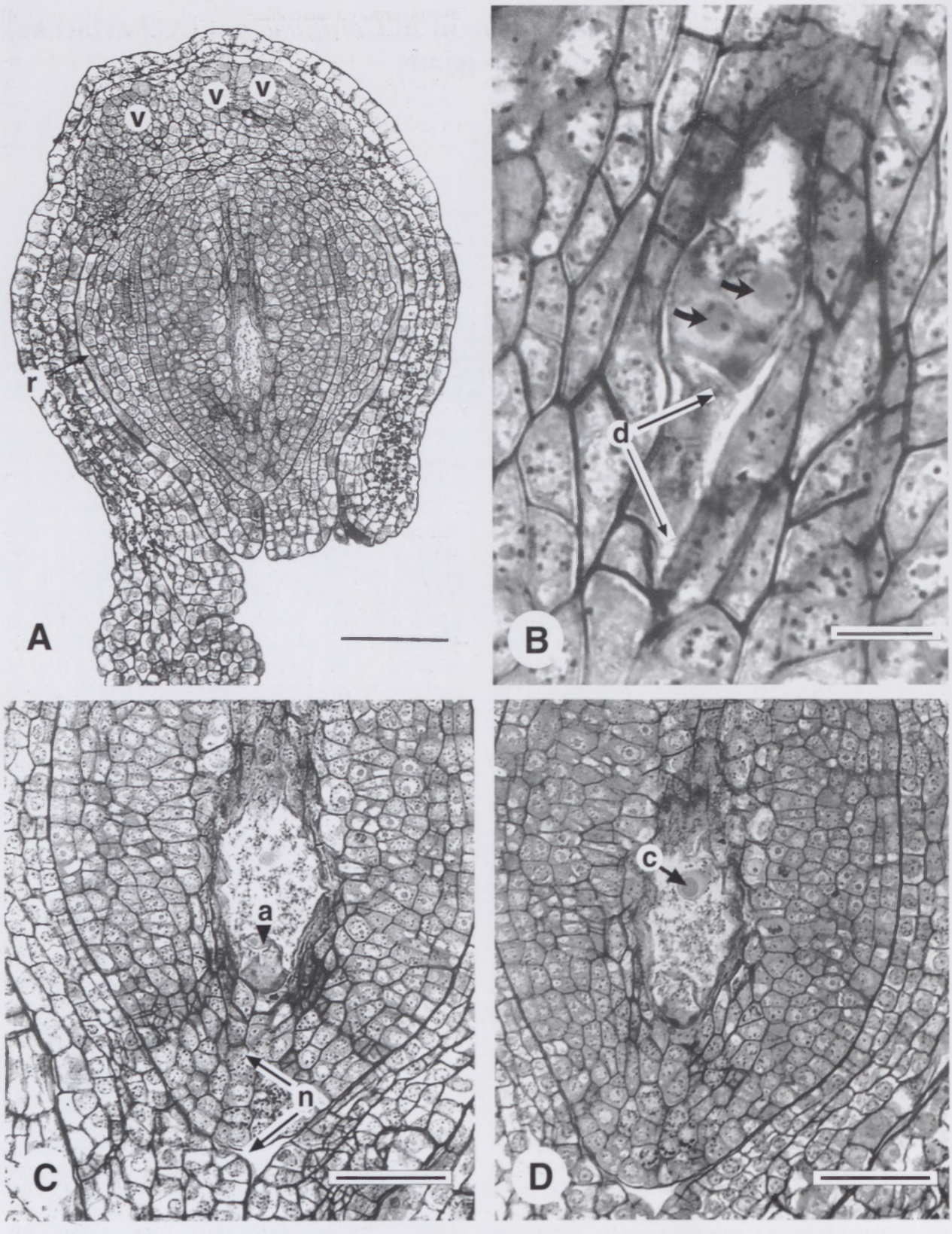

FIGURE 1.—Ovule and embryo sac of *Ceratiosicyos* as seen in sagittal section. A, mature ovule; B, early stage in development of bisporic embryo sac; C, D, consecutive sections of same ovule as in A to show details of mature embryo sac. a, egg cell; c, central cell nucleus; curved arrows indicate megaspore nuclei in chalazal dyad cell; d, disintegrating micropylar dyad cell; n, derivatives of nucellus epidermis; r, outer epidermis of inner integument; v, vascular bundles in chalaza. Scale bars: A, 100 µm; B, 10 µm; C, D, 50 µm.

O'Brien (1968). A selection of impregnated structures was individually imbedded in GMA, hardened in the oven at about 58°C and sectioned transversely and sagittally. Selected sections were stained with the periodic acid/Schiff reaction and counterstained with toluidine blue O by using the protocols of O'Brien & McCully (1981).

Tests for myrmecochory: at the collection site on the Kowyns Pass, dehiscing capsules were carefully removed

from the plants, the seeds collected and immediately strewn onto the trails or near the nests of ants of varying sizes found at the collection site. Mature capsules were stored in air-tight bags overnight and taken to Pretoria where seeds, removed from capsules that had in the meantime split open, were again offered to ants. Dried-out seeds with tuberculate surfaces were also offered to ants.

RESULTS

Placentation and orientation of ovules

The hypogynous female flowers of *Ceratiosicyos* contain elongated, pentagonal ovaries borne on gynophores. The ovaries are unilocular and usually contain 7–15 anatropous ovules that are borne singly on five parietal placentae. The latter regions are not ridged and run longitudinally along the inner surface of the ovary wall, opposite the five median carpel traces. Ovules from five placentae are so arranged that they form a single row in the narrow locule and this alignment is maintained throughout ovule-to-seed development. The alignment is achieved by the ability to vary the orientation of the ovules and the lengths of the funicles—some ovules point upwards, others downwards and the funicles may be short or more than twice the length of the ovular body to place the ovules neatly in a single row.

Structure of mature ovule

Mature ovules are anatropous, bitegmic, crassinucellate structures (Figure 1A) with an ovoid shape and ± 520 µm long (funicle excluded). The integuments are multilayered, the outer consists of four to five layers in its central part, while the inner is up to seven layers thick in this region. The outer epidermis of the inner integument is very conspicuous—the cells are four to six times longer than any other cell in the ovule with the exception of the embryo sac. On the antiraphal side of the ovule, the tip of the outer integument increases in thickness by tangential divisions in its inner epidermal layer. In pre-fertilization stages of ovules, the outer integument is as long as the inner integument so that the micropyle canal is formed by the inner integument only. The raphe is not ridged. The vascular bundle of the raphe branches as soon as it enters the chalaza, but the integuments are not vascularized (Figure 1A).

The mature embryo sac is about one-third the length of the nucellar cylinder and lies in the centre of massive nucellar tissue. About six layers of nucellus cells cover the embryo sac on all sides. Below the micropyle, at least three of these layers result from periclinal divisions of the nucellus epidermis (Figure 1C, D). The nucellus apex is slightly attenuate, but does not protrude into the micropyle.

The embryo sac develops from the chalazal dyad cell while the micropylar dyad cell degenerates (Figure 1B). During the second meiotic division the chalazal dyad is not partitioned by a transverse cell wall so that both megaspore nuclei are included in the same cell (Figure 1B). After two mitotic divisions an eight-nucleate, bisporic embryo sac of the *Allium* Type is formed. The mature embryo sac contains many starch grains. A small egg apparatus occurs below the parietal nucellar tissue (Figure 1C). The short neck regions of the synergids contain a filiform apparatus. A large central cell nucleus lies in about the central part of the embryo sac, while three ephemeral antipodal cells (not shown) develop in the elongated and narrow chalazal base (Figure 1A) of the embryo sac.

Early development of endosperm and embryo

Fertilization is porogamous in *Ceratiosicyos* and endosperm formation is nuclear. After entering the micropyle, the tip of the pollen tube swells and stains darkly with PAS and toluidine blue (Figure 2A). During the initial stages of embryo sac enlargement (Figure 2A), free endosperm nuclei become arranged in a single layer alongside the embryo sac wall. When the growing seed has reached a size of ± 5 × 2.5 mm, the first cell walls are laid down between adjacent endosperm nuclei, and the embryo sac then gradually becomes filled, layer upon layer, with thin-walled endosperm cells.

The zygote remains inactive during the nuclear stage of endosperm formation. The first division of the zygote was not seen, but pro-embryos in the tetrad stage of development (Figure 2B) were found before the endosperm started to become cellular. These four-celled pro-embryos are T-shaped which shows that the apical cell (ca) has divided in a vertical plane and the basal cell (cb) transversely (Figure 2B). The two daughter cells of ca then both divide obliquely (Figure 2C) so that a bicellular, wedge-shaped epiphysis (e) is formed in the apical tier during the quadrant stage of the pro-embryo (i.e. when the derivatives of ca comprise four cells). The epiphysis later forms the shoot apex, whereas the remaining cells of the quadrant form the cotyledons (Natesh & Rau 1984: 390). The pro-embryo is globular in shape and has no suspensor. Below the cells of the quadrant, the uppermost derivative of cb is a discoidal cell (h), designated 'hypophysis' by Hanstein (1870). The hypophysis later forms the initials of the root cortex and the root cap (Crété 1963).

In Johansen's (1950) classification of embryogenic types, T-shaped pro-embryos are characteristic of both the Onagrad Type and Asterad Type, but it is only in the latter type that the basal cell (cb) contributes significantly to the formation of the embryo proper. *Ceratiosicyos* embryos have no suspensors, all derivatives of the basal cell are incorporated into the embryo proper which indicates an Asterad Type embryo. The presence of the epiphysis also points towards the Asterad Type—it is in taxa conforming to this type that an epiphysis is formed during the quadrant stage (Natesh & Rau 1984: 386, fig. 8.6). The lack of a suspensor places the embryo of *Ceratiosicyos* in the *Penaea* variation of the Asterad Type (Natesh & Rau 1984: 390, 414).

Development and structure of the seed coat

The seed coat of *Ceratiosicyos* is mainly derived from the outer integument. This integument forms the sarcotestal layers and the outer, wavy layer of sclereids that protrude peak-like into the sarcotesta (Figures 3D; 5D).

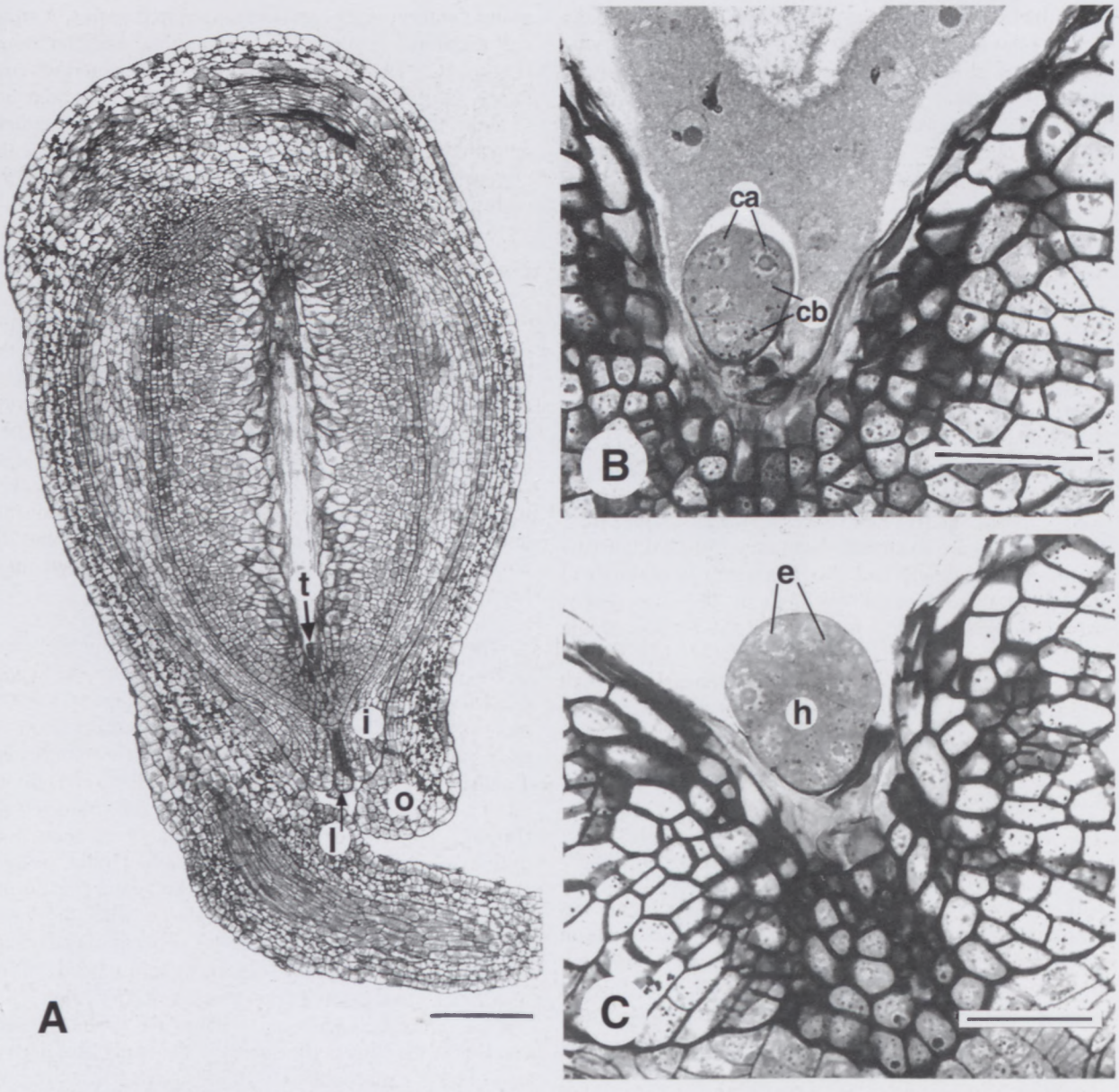

FIGURE 2.—Early development of endosperm and embryo in *Ceratiosicyos*. A, developing seed during resting stage of zygote; B, T-shaped pro-embryo; C, suspensorless pro-embryo during quadrant stage. ca, apical cell after vertical division; cb, basal cell after transverse division; e, epiphysis; h, hypophysis; i, inner integument; l, bulge of inner integument; o, outer integument participating in formation of micropyle canal; t, pollen tube in elongating embryo sac. Scale bars: A, 200 µm; B–C, 50 µm.

The inner integument contributes a few layers of periclinally elongated fibres to the seed coat. During seed coat development the cuticle between the outer and inner integument gradually disappears so that a study of the mature seed coat alone does not show which part each integument plays in the formation of the seed coat.

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

In pre-fertilization stages, the outer integument consists of about five cell layers (Figure 1A), except at its rim where the number of layers increases through tangential divisions of the inner epidermis. The cells in the rim remain meristematic and in early post-fertilization stages the distal part of the outer integument grows beyond the inner integument to take part in the formation of the micropyle (Figure 2A). In developing seeds a layer of actively dividing endotestal cells (s) can be seen inside the developing sarcotesta (Figure 3A, B, D). These cells are the derivatives of the inner epidermis of the outer integument. At first, the derivatives lie in radial rows and the layer is of even thickness (Figure 3A, B). When the seed has reached its final length of ± 6 mm, the endotestal layer becomes wavy and starts forming projections into the sarcotesta (Figure 3D). The endotestal cells later develop into closely packed, thick-walled sclereids with starch grains and single, large crystals of calcium oxalate in some of the cells, but the crystal-containing cells do not form a continuous layer. At seed dispersal stage, the contents of the endotestal sclereids stain intensely with toluidine blue, indicating the presence of phenolic substances (Figures 3C; 4A, B).

The epidermis and mesophyll of the outer integument form a sarcotesta that envelops the whole seed, including the chalaza and raphe. The raphal region is not pronounced, i.e. a raphal ridge is not formed (Figures 4A; 5D). The raphal bundle lies imbedded in thin-walled,

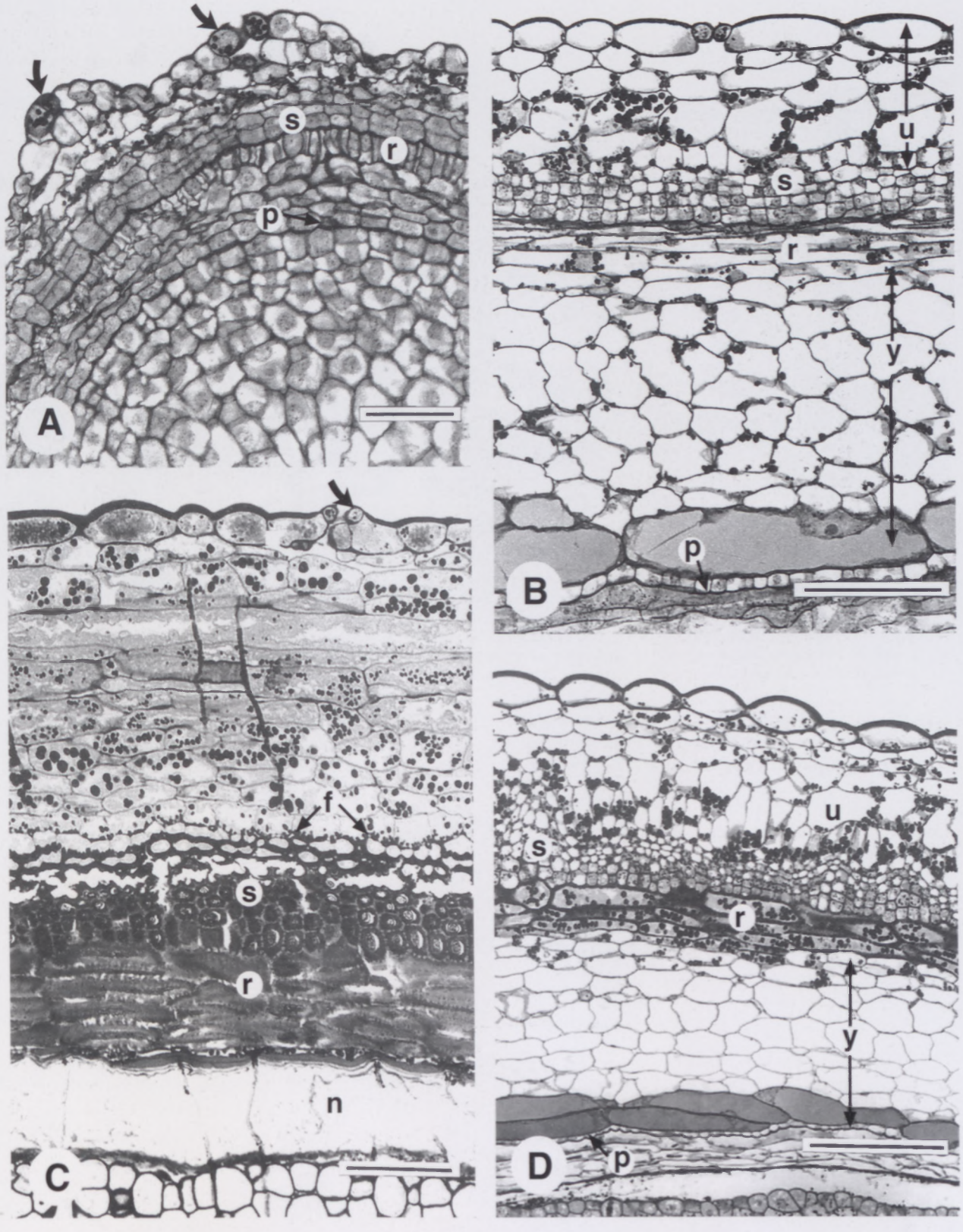

FIGURE 3.—Development and structure of seed coat in *Ceratiosicyos*. A, t/s young seed coat just after fertilization; B, l/s seed coat during first stages of pro-embryo formation; C, l/s mature seed coat of dispersed seed; D, l/s seed coat during maturation of fibrous exotegmen. Curved arrows indicate position of stomata; n, nucellus cell remains; p, inner epidermis of tegmen; r, outer epidermis of tegmen with anticlinal divisions; s, derivatives of inner epidermis of testa; u, sarcotesta; y, mesophyll of tegmen. Scale bars: A, 50 µm; B, C, 100 µm; D, 200 µm.

parenchymatous, sarcotestal tissue that, especially in this area, contains large numbers of starch grains (Figure 4A). Stomata, not seen in the outer epidermis of the ovule, were found at regular intervals in the developing (Figure 3A, B) and mature (Figure 3C) epidermis of the sarcotesta.

When the seeds are dispersed, the cells of the innermost layer of the sarcotesta have developed small, fibrillar protuberances on their inner tangential walls (Figures 3C; 4A, B). The fringe-like wall ingrowths are strongly PAS-positive and also stain dark blue with toluidine blue. The fringe layer possibly represents a layer of transfer

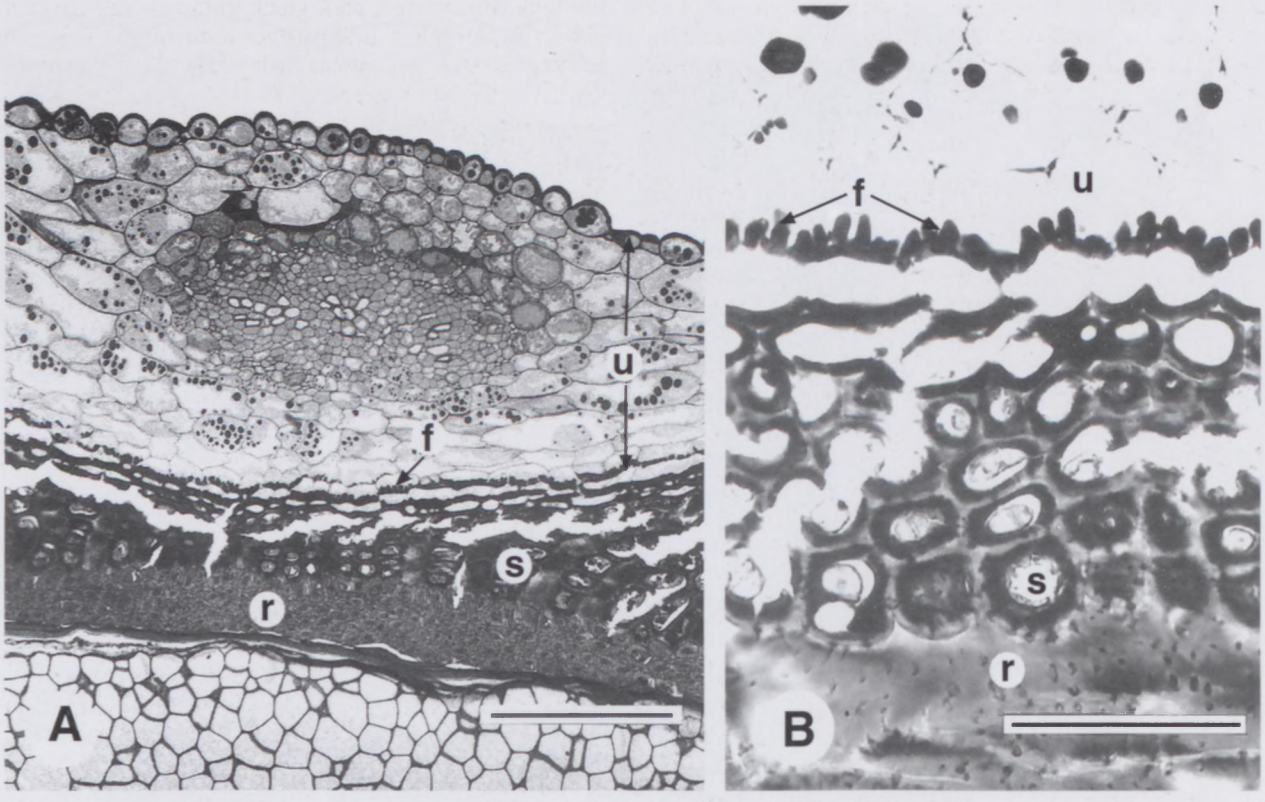

FIGURE 4.—Development and structure of seed coat of *Ceratiosicyos* (continued): A, raphe and underlying seed coat layers in a median t/s of seed; B, t/s fringe layer and adjacent cell layers seen at higher magnification than in Figure 3C. f, fringe layer; r, fibres of exotegmen; s, derivatives of inner epidermis of testa; u, sarcotesta. Scale bars: A, 200 µm; B, 50 µm.

cells (Gunning & Pate 1969), often found in reproductive structures for the short-distance transport of solutes (Johri & Ambegaokar 1984: 29, fig. 1.13A–F).

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

After fertilization the conspicuously elongated outer epidermal cells of the inner integument (Figure 1A) initially divide anticlinally to form a single layer of densely packed, radially elongated meristematic cells (Figure 3A). While the first divisions of the pro-embryo are taking place, these meristematic cells of the tegmen divide periclinally once or twice to form three to four layers of cells that are stretched in a direction parallel to the longitudinal axis of the seed (Figure 3B). At this stage the cuticle between the developing endotesta and exotegmen starts to disappear, but in longitudinal sections of immature seeds the boundary between the two layers is clear because of the difference in the orientation of the cells (Figure 3B, D). When the seed reaches its final size, the

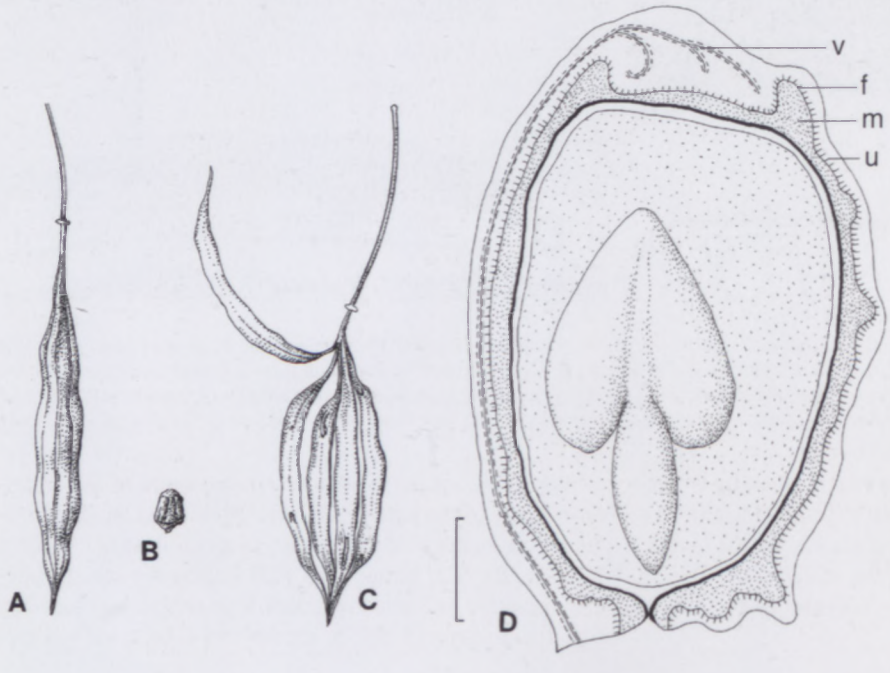

FIGURE 5.—Fruit and seed of *Ceratiosicyos*. A, mature, 5-valved capsule, × 0.8; B, dispersed seed, × 0.8; C, dehisced fruit, showing funicles of varying lengths left on the placentae, × 0.8; D, l/s seed. f, fringe layer; m, mechanical layers; u, sarcotesta; v, vascular bundle branching in chalaza. Scale bar: 1 mm. Artist: G. Condy.

exotegmic cells contain many starch grains and the outer elements have started maturing into fibres (Figure 3D). At seed dispersal stage the fibres have thick, lignified walls with simple pits and contain no starch (Figures 3D; 4A, B).

The mesophyll and inner epidermis of the tegmen do not play a significant role in the structure of the mature seed coat. The thin-walled mesophyll tissue initially shows divisions in various planes so that the layers increase in number (Figure 3B). The innermost layer of mesophyll cells becomes conspicuous by their large size and darkly staining properties (Figure 3B, D). At first, the inner epidermis of the tegmen keeps pace with the growth of the seed by dividing anticlinally so that a layer of small, densely packed cells is formed (Figure 3B). Eventually, when the seeds are dispersed, all layers inside the exotegmic fibres are obliterated and a structureless pellicle remains between the fibres and the flattened cells of the nucellar tissue (Figures 3C; 4A).

Macromorphology of the fruit and seed and tests for myrmecochory

Mature fruit consists of 50–90 mm long, 5-valved capsules (Figure 5A, C) that are thin-walled and light green at seed dispersal stage. Five to twelve stalked seeds are arranged in a single row, sometimes packed close to one another in the locule. When the valves split open (not very forcefully), the seeds break off, leaving their funicles of various lengths attached to the centre of the valves (Figure 5C). Seeds are ovoid to short-cylindrical, 6.0–6.5 mm long (Figure 5B), dark green to brown and covered with a thin, succulent and translucent sarcotesta. The seed surface becomes tuberculate when the sarcotesta dries out, reflecting the projections formed by the underlying mechanical layers (Figure 5D). At seed dispersal, the axile embryo (sensu Martin 1946: 520) is of medium size (i.e. it occupies about three-quarters of the length of the endosperm). It lies straight in the seed, has thin, spatulate cotyledons and a well-formed radicle (Figure 5D).

Tests for myrmecochory were negative. If ant–seed interactions are not species specific as claimed by Slingsby & Bond (1981) and found by Steyn *et al.* (in press), our results suggest that *Ceratiosicyos* seeds are not dispersed by ants.

DISCUSSION

Differences between Ceratiosicyos *and* Acharia *and* Guthriea

A comparison of ovule and seed characters in *Ceratiosicyos* with those of *Guthriea* and *Acharia* (Table 1) shows that the three genera are fundamentally very similar in characters usually regarded as of taxonomic importance (see No. 1, 2, 8–12, 14, 16 & 17). The structure of the integuments is also comparable to a large degree, although *Ceratiosicyos* lacks the peculiar zigzag micropyle (see No. 5) that characterizes ovules and seeds of *Guthriea* and *Acharia*. The short outer integument in the *Ceratiosicyos* ovule possibly does not denote an important structural difference with the other two genera, because this integument overtops the inner after fertiliza-

TABLE 1.—A comparison of ovule and seed characters in Achariaceae

No.	Character	*Ceratiosicyos*	*Guthriea* and *Acharia*
1.	Ovule position and number	Parietal, 7–15 ovules on 5 placentas.	Parietal, 15–35 ovules on five placentas (*Guthriea*); 4 ovules on 4 placentas (*Acharia*).
2.	Ovule type	Anatropous, bitegmic, crassinucellate.	Anatropous, bitegmic, crassinucellate.
3.	Outer integument	Multi-layered, as long as inner in ovule, overtops inner after fertilization to form exostome, not lobed distally.	Multi-layered, longer than inner in ovule and seed to form exostome, lobed at distal rim.
4.	Inner integument	Multi-layered, outer epidermal cells conspicuously long.	Multi-layered, outer epidermal cells conspicuously long.
5.	Micropyle canal	Straight, formed by inner integument in ovule, both integuments in seed.	Zigzag, formed by both integuments in ovule and seed.
6.	Raphe	Not pronounced.	Pronounced to form a ridge.
7.	Funicle	Variable in length, often long in ovule and seed.	Practically absent, ovules and seed sessile.
8.	Nucellus cap formation	Epidermis divides periclinally to add to parietal nucellus.	Epidermis divides periclinally to add to parietal nucellus.
9.	Embryo sac	Bisporic, 8-nucleate, *Allium* Type.	Bisporic, 8-nucleate, *Allium* Type.
10.	Seed type	Anatropous, sarcotestal, endospermous.	Anatropous, sarcotestal, endospermous.
11.	Seed size	Medium, 6.0–6.7 mm long.	Medium, 5–6 mm long.
12.	Embryo type	*Penaea* variation of Asterad Type, epiphysis present in quadrant stage.	*Penaea* variation of Asterad Type, epiphysis present in quadrant stage.
13.	Embryo size	Medium, ± 4 mm.	Small, < 2 mm.
14.	Presence of stomata	In outer epidermis of sarcotesta.	In outer epidermis of sarcotesta.
15.	Presence of trichomes	Absent.	Present as unicellular hairs on seed surface.
16.	Sarcotestal structure	Comprises uni-layered outer epidermis and chlorenchymatous mesophyll of outer integument, raphe and chalaza; innermost cells form fringe layer.	Comprises bi-layered outer epidermis with hairs, hypodermis and chlorenchymatous mesophyll of outer integument, raphe and chalaza; innermost cells form fringe layer.
17.	Mechanical layers in seed	Endotestal sclereids + longitudinal exotegmic fibres.	Endotestal sclereids + longitudinal exotegmic fibres.
18.	Chalazal seed lid	Absent.	Present.
19.	Dispersal mechanism	Autochory, elaiosome absent.	Autochory + myrmecochory, elaiosome present.

tion. The inner integument of *Ceratiosicyos* bulges into the micropyle canal (Figure 2A). The bulging cells might have been mistaken for a nucellar beak by Dahlgren & Van Wyk (1988). The present investigation also showed that the mechanical seed coat layer in *Ceratiosicyos* is, like those of the other two genera, of dual origin (endotestal-exotegmic) and not purely exotegmic as previously reported (Dahlgren & Van Wyk 1988).

Many of the differences between *Ceratiosicyos* and the other two genera can possibly be attributed to specific adaptations for seed dispersal and germination (see No. 6, 7, 13, 15, 18 & 19). *Ceratiosicyos* is not myrmechorous, the ovule therefore lacks the pronounced raphe that, in *Acharia* and *Guthriea*, eventually forms a ridge-like part of the sarcotestal elaiosome (see No. 6 & 19). The presence of unicellular hairs on the seed surface of *Guthriea* and *Acharia* (see No.15) possibly also relates to myrmechory, since openings left by broken-off trichome bases would allow ant-attracting substances to rapidly reach the seed surface (Steyn *et al.* in press).

Instead of a pronounced raphe, ovules and seeds of *Ceratiosicyos* might have developed long funicles as an adaptation to seed dispersal (see No. 7). By varying the lengths of the funicles, the seeds can be manipulated into a single, vertical row in the narrow, elongated locule. This arrangement may be necessary for rapid splitting of the fruit by distributing pressure on the valves evenly along the length of the locule. In the two diplochorous (autochorous + myrmecochorous) genera (see No. 19) the seeds are sessile in the short-cylindrical capsules and pressure on the valves is applied by the swollen elaiosomes. Also, the capsules remain within the covering of persistent corolla tubes to protect developing seeds with their soft elaiosomes (Steyn *et al.* in press). *Ceratiosicyos* is autochorous; its seeds need less protection and developing capsules rapidly outgrow the protective covering of the corolla tubes.

Seed size in genera of the Achariaceae does not differ significantly, but *Ceratiosicyos* has a much larger embryo than the other two genera (see No. 11 & 13). We propose that the small embryo has been the causal factor for the formation of the unique seed lid in the other two genera. This device would allow water and air to enter the seed through the unsclerified cells in the rim of the lid during the slow maturation (12 weeks) of the embryo in the hydrated seed (Steyn *et al.* in press). The much larger embryo of *Ceratiosicyos* possibly did not require such an adaptation.

Martin (1946) reasoned that smallness in embryos, as compared to size of the endosperm, is representative of a primitive state in seeds of angiosperms, and, conversely, that embryos which become well developed before dormancy, reflect a higher evolutionary rank. For dicotyledons, Corner (1976: 48) also regarded small embryos as primitive and considered simplification through loss of structures or cell layers as an indication of an advanced state in seeds. However, considered on its own, it is usually difficult, if not impossible, to tell which of the two embryo states—small or large—is the more primitive for a particular taxon. One reason for this difficulty is that embryo (and seed) size, like so many other characters, are subject to homoplasy (parallelism, convergence and character state reversals). Unfortunately homoplasy is rampant among seed plants, thus considerably limiting the reliability of outgroup comparisons to establish polarity (Cronquist 1988). Compared to the other members of the family, *Ceratiosicyos* shows a notable trend towards simplification of the seed through the loss of several cell layers and structures, e.g. trichomes, a bi-layered testa epidermis, a hypodermis, a crystal-containing layer inside the fringe layer, perisperm and the reduction of the chalazal region with seed lid. These reductions, together with the larger embryo, may indicate that *Ceratiosicyos* is more advanced than *Guthriea* and *Acharia*. On the other hand, indications are that Kiggelariaceae may well be the sister group of Achariaceae (see further on). Both *Kiggelaria africana* and *Ceratiosicyos laevis* share a medium-sized embryo and rather unspecialized seed. coat, states that may be the plesiomorphic ones in Achariaceae. Relatively small embryos and a rather elaborate seed coat occur in *Acharia tragodes* and *Guthria capensis*, both having specialized myrmecochorous seeds. Therefore *Ceratiosicyos laevis*, with its lianeous habit and mesic forest habitat, could just as well be the more primitive member of the family. *Acharia tragodes* (semi-woody shrublet) is confined to the xerophytic thicket vegetation of the Eastern Cape, with *Guthria capensis* (rosulate herb) confined to temperate grassland and karroid vegetation at high altitude.

Achariaceae versus families in Malpighiales

A detailed comparison of ovule and seed characters in Achariaceae and the 36 families placed in Malpighiales (including many families traditionally placed in Violales) by Savolainen *et al.* (2000), is hampered by a lack of comparable data for many of the families (Davis 1966; Johri *et al.* 1992; Nandi *et al.* 1998). Achariaceae seem generally well placed in Malpighiales and fit in comfortably among those families previously regarded as part of Violales *sensu* Dahlgren (1980). Similarities include bitegmic, anatropous, crassinucellate ovules, parietal nucellus partly formed by nucellus epidermis derivatives, both integuments participating in formation of micropyle canal, nuclear endosperm becoming copious in the seed and a medium-sized to large embryo lying straight in the seed.

Some of the characters observed in Achariaceae are rare for Malpighiales, namely a bisporic *Allium* Type embryo sac, suspensorless Asterad Type embryos, protective seed layers containing endotestal sclereids and exotegmic fibres, and a sarcotesta. A number of characters shared by *Guthriea* and *Acharia*, such as the zigzag micropyle, distally lobed outer integument, perisperm and small embryo is also uncommon for the order. A zigzag micropyle and Asterad embryos conforming to the *Penaea* variation only occur in Violaceae (Davis 1966), while lobed integuments and perisperm are found in Scyphostegiaceae (Van Heel 1976; Johri *et al.* 1992) and sarcotestal seeds in Passiflorales (Nandi *et al.* 1998).

A possible phylogenetic link between Achariaceae and Flacourtiaceae (tribe Pangieae, particularly *Kiggelaria africana*) was first suggested by the breeding behaviour of a butterfly. Several butterflies in the sub-

tribe Acraeina (subfamily Acraeinae, tribe Acraeini), including the common garden acraea (*Acraea horta*), utilize as larval food plants, members of a closely knit group of plant families traditionally classified in the order Violales (notably Achariaceae, Flacourtiaceae, Passifloraceae and Turneraceae), all containing a unique group of toxic compounds known as cyclopentenoid cyanogenic glucosides (Seigler 1975; Dahlgren 1980; Cronquist 1981; Takhtajan 1997; Kroon 1999). In their natural habitat larvae of *Acraea horta* feed mainly on *Kiggelaria africana*, a species containing gynocardin as major cyanogenic glucoside (Jaroszewski & Olafsdottir 1987; Raubenheimer & Elsworth 1988). The larvae selectively sequester and store some of the gynocardin, which are passed on to all other stages in the life cycle, supplemented by apparent self-synthesis (Raubenheimer 1987, 1989). Accumulation of this toxin is believed to render the insects unpalatable to predators. Previously gynocardin has been isolated from the seed of *Gynocardia odorata* R.Br., another member of the tribe Pangieae (Coburn & Long 1966). When live plants of *Ceratiosicyos laevis* and *Guthriea capensis* were introduced into the botanical garden at the University of Pretoria in the mid-1980s, both were immediately selected by *Acraea horta* for egg deposition; larvae emerged and butterflies were raised (Dahlgren & Van Wyk 1988). This observation led to a chemical study of *Ceratiosicyos laevis*, the first of its kind on a member of the Achariaceae, resulting in the identification of gynocardin as one of the principal cyanogenic glucosides in this species (Jensen & Nielsen 1986); its presence in *Guthriea capensis* is suspected.

Based on evidence from molecular biology, Chase *et al.* (1996) also suggested a linkage between the herbaceous Achariaceae and the woody tribe Pangieae. This tribe included amongst others, *Gynocardia* R.Br., *Hydnocarpus* Gaertn., *Kiggelaria* L., *Pangium* Reinw. and *Trichadenia* Thwaites (Lemke 1988). In the circumscription of Soltis *et al.* (2000), Kiggelariaceae include *Pangium*, *Hydnocarpus*, and *Kiggelaria*. Our results show that Achariaceae agree closely with the latter two genera as far as seed development and seed coat structure are concerned. Van Heel (1979) found the seeds of *Hydnocarpus* and *Kiggelaria* sarcotestal with undulating endotestal-exotegmic mechanical layers, a dominant endotestal layer of sclereids, an outer integument that is initially short, but overtops the inner during seed formation and a cuticle that disappears early so that the dual nature of the protective layer is masked and the erroneous impression given that the seeds are pachychalazal. These characters are so similar to the characters we found in Achariaceae that they can be regarded as strong support for a linkage between Achariaceae and Kiggelariaceae.

In *Kiggelaria* the embryo is of medium size, as found in *Ceratiosicyos*. It is perhaps noteworthy that seeds with a small embryo, a fleshy raphe and a conspicuous notched cuticle between the tegmen and nucellus as reported for *Guthriea* (Steyn *et al.* 2001) also occur in *Berberidopsis* Hook.f. (Van Heel 1979). This taxon, previously also included in the Flacourtiaceae (Lemke 1988), is currently regarded as a relict with an unclear taxonomic position (Savolainen *et al.* 2000).

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