

## DEVELOPMENT OF OVULE AND SEED COAT IN *CNEORUM TRICOCCON* L. (CNEORACEAE)

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### SUMMARY

The ovule primordium of *Cneorum tricoccon* is trizonate. The ovule is anatropous, bitegmic and crassinucellate with a massive parietal tissue. The inner and outer integuments are both of dermal derivation and initially 2-layered, but the inner integument later becomes about 4-layered and the outer one 4 to 5-layered. The seed coat structure is described for the first time. The fruit wall has taken over the protective function of the seed coat. The testa shows but few differentiated layers, the exotesta develops thickened outer walls, and the meso-endotestal cells are rounded and somewhat thick-walled. The other layers of the seed coat become almost completely crushed and resorbed. A distinct nucellar cuticle and nucellar remains are present at the inside of the seed coat.

The seed turns strongly campylotropous as a result of differential growth from the raphe region and by the strong enlargement of the antiraphe side. The ovule and seed characters support the Rutalean relations of the family Cneoraceae.

### 1. INTRODUCTION

The small family Cneoraceae comprises only three very similar shrubby species, placed in 2 genera. *Cneorum tricoccon* occurs in the western part of the Mediterranean region near the coast, *Cneorum trimerum* (Urban) Chodat in the mountains of eastern Cuba and *Neochamaelea pulverulenta* (Ventenat) Erdmann (= *Cneorum pulverulentum* Vent.) in the Canary Islands. The possible relationships of the family are amply discussed by STRAKA *et al.* (1976) and LOBREAU-CALLEN *et al.* (1978). The following taxa have been suggested as possible relatives: Rutales, in particular the Rutaceae and Simaroubaceae, Sapindales, Geraniales (particularly the Zygophyllaceae), Celastrales and Rhamnales. On the basis of the accumulated morphological, anatomical, embryological, karyological, palynological and phytochemical evidence the abovementioned authors have concluded that the Cneoraceae have the greatest affinity with the Rutales. Only *Cneorum tricoccon* has been investigated embryologically (DAVIS 1966), and also the knowledge of ovule and seed was scanty. The ovule was reported to be bitegmic, anatropous and crassinucellate. However, the ovule ontogeny was still unknown in detail, and the seed coat development had previously not been studied.

The present study was undertaken to ascertain whether the ovule and seed coat structure may contribute additional arguments for the taxonomic position of the Cneoraceae.

## 2. MATERIALS AND METHODS

The material of *Cneorum tricoccon* was collected in the Hortus Botanicus, University of Amsterdam and the garden of the Department of Plant Taxonomy and Geography, Agricultural University of Wageningen. The flowers and fruits of *Neochamaelea pulverulenta* (Vent.) Erdmann were received upon instigation of Professor H. Straka (Kiel) and Dr. Lobreau-Callen (Paris) from Dr. S. Laegaard (Aarhus, Denmark). Craff and Allen-Bouin mixtures were used as fixatives. Sections were cut by standard microtome techniques or by hand. Mature seeds were also embedded in epon. The following reagents were used for specific staining-tests: Phloroglucinol-HCl, Sudan IV, Ruthenium-red and IKI. The SEM-observations were made with a Cambridge Stereoscan Mark 2a. The very hard mature fruits were cracked so as to obtain the fully matured seeds.

## 3. RESULTS

### 3.1. Morphology

*Cneorum tricoccon* has a 3-loculed, syncarpous ovary with two ovules in every locule (inserted more or less above one another). The mature fruit is indehiscent, but falls easily apart into three pieces each of which mostly contains two one-seeded compartments, arisen, by the ingrowth into the locule of an oblique partition wall protruding from the dorsal side of the carpel. The orientation of the seeds is variable, but they are often situated above one another in opposite directions. The inner layer of the fruit-wall becomes multi-layered later to form the sclerified endocarp. The mesocarp, together with the endocarp, becomes lignified and the former contains crystals.

### 3.2. Ovule ontogenesis

The ovule primordia are trizonate from early stages of initiation onward (fig. 1A) and consist of a dermatogen ( $l_1$ ), a subdermatogen ( $l_2$ ) and a corpus ( $l_3$ ). The subdermatogen at first divides anticlinically only.

The subdermal archesporium is one-celled and divides into a parietal cell and a megaspore mother-cell. The megaspore tetrad is linear and the chalazal spore becomes the functional one. Because of the rather long and twisted funicle it is very difficult to obtain longitudinal sections of ovules cut through the median plane. The ovules (and later the seeds) become oriented in an oblique direction.

The inner integument (i.i.) is initiated as a complete ring-wall before the outer integument (o.i.) which is not or hardly developed at the raphal side. Both integuments arise from 2- or 3-celled primordia and are completely of dermal derivation. Shortly after their initiation the integuments are mainly three cell layers thick but soon become 2-layered acropetally (figs. 1B, C, D). Before the ovule is mature the i.i. becomes 3-layered again by divisions in its inner layer (figs. 1E, 3A) and subsequently four-layered by divisions in the middle layer (fig. 3B). The i.i. always remains longer than the o.i., which therefore, does not contribute to the formation of the micropyle. During the first stages the ovule develops

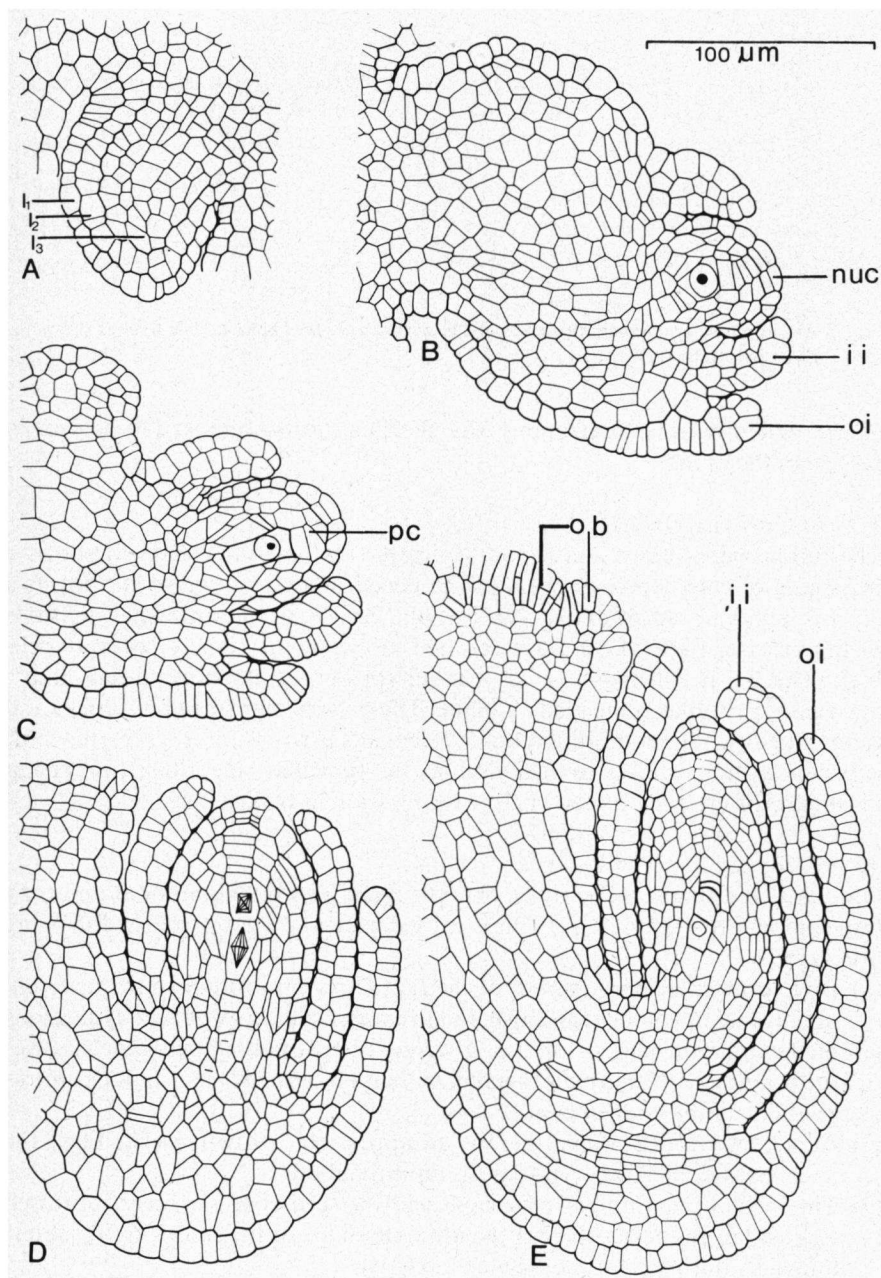


Fig. 1 A-E. Longitudinal section of developing ovules of *Cneorum tricoccon*. l<sub>1</sub>, l<sub>2</sub> and l<sub>3</sub>: dermal layer, subdermal layer and corpus, respectively.

oi = outer integument; ii = inner integument; nuc = nucellus; pc = parietal cells; ob = obturator.

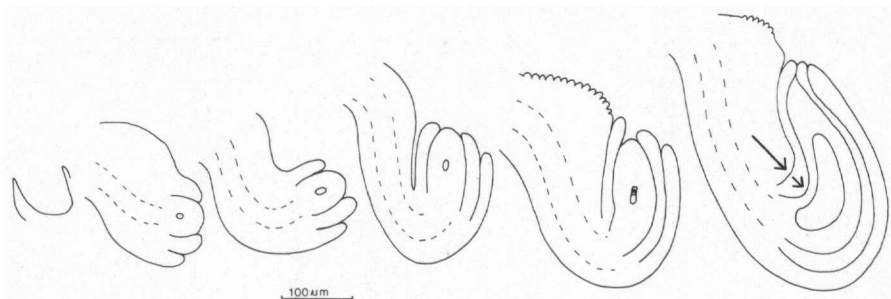


Fig. 2. Anatropous development of ovules of *Cneorum tricoccon*. The last stage figured shows the incipient campylotropy (arrows!).

as if it would become anatropous, the nucellus turning almost 180 degrees in respect of the raphe.

### 3.3. The mature ovule

The full-grown ovule is anatropous, bitegmic and crassinucellate. It shows a beginning of campylotropy (fig. 2). The nucellus is very massive and forms a distinct nucellar cap of about 4 cells high. Periclinal and anticlinal divisions of the primary parietal cell have resulted in a parietal tissue of over 10 cells high. The o.i. is still two-layered. The micropyle is formed by the i.i. and is orbicular to slit-like in the median plane. The provascular strand of funicle and raphe already shows some differentiated elements. An obturator is present which is hardly distinguishable from the o.i. at the funicular side. Starch is present in all parts of the ovule already before fertilisation has taken place.

### 3.4. Seed and seed coat development

Gradually the shape of the ovule changes from a seemingly normal anatropy into campylotropy (figs. 2, 4). This is caused by a combination of the following processes:

- (1) By a local intercalary growth of the raphal region just above the insertion of the inner integument and also, but to a lesser extent, by periclinal divisions of the adjacent part of the i.i., a wedge of tissue is formed which causes the bending of the nucellus together with its embryo sac (e.s.) and the antiraphal side of the integuments.
- (2) As a consequence of (1), there is an appreciable growth in length of the nucellus and the integuments at the antiraphal side.
- (3) The locules remain relatively small and the seed coat is closely adpressed against the endocarp, so that the ultimate shape of the seed is partly determined by the inner form of the locule cavity.

The endosperm is initially nuclear, the subsequent wall formation taking place centripetally (fig. 3E). The nucellus is gradually crushed by the e.s. but in its outer part the cells enlarge and some divisions still take place. Ultimately the remaining outer layers are crushed (fig. 3D, E). The nucellar remnants are cover-

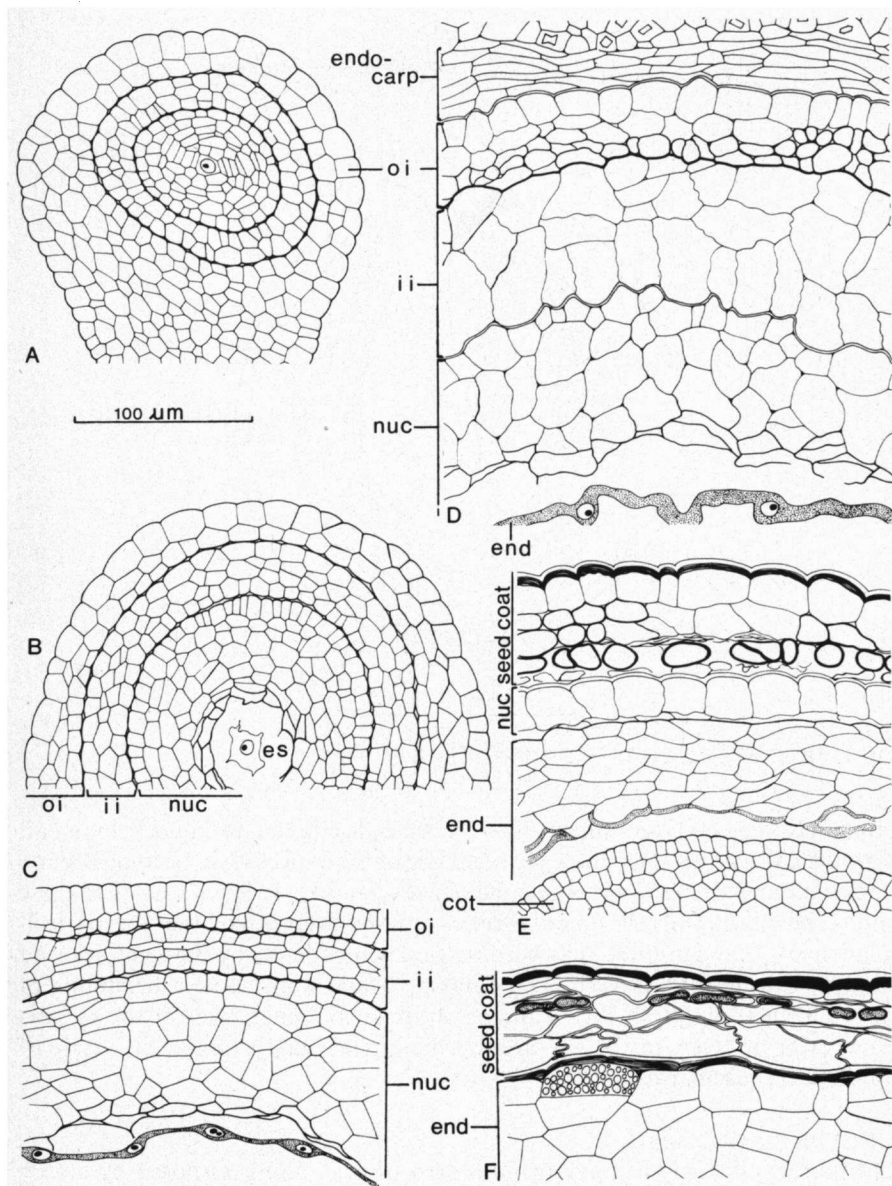


Fig. 3. *Cneorum tricoccon*. Ovules (A,B) in cross section and developing seed coat in cross section (C-F).

es = embryo sac; end = endosperm; cot = cotyledon.

ed by a conspicuous cuticle. The i.i. remains about 4-layered. Its cells enlarge substantially and are ultimately crushed and mainly resorbed (fig. 3D, E). The o.i. also becomes multilayered by divisions of its inner layer (fig. 3C). It becomes

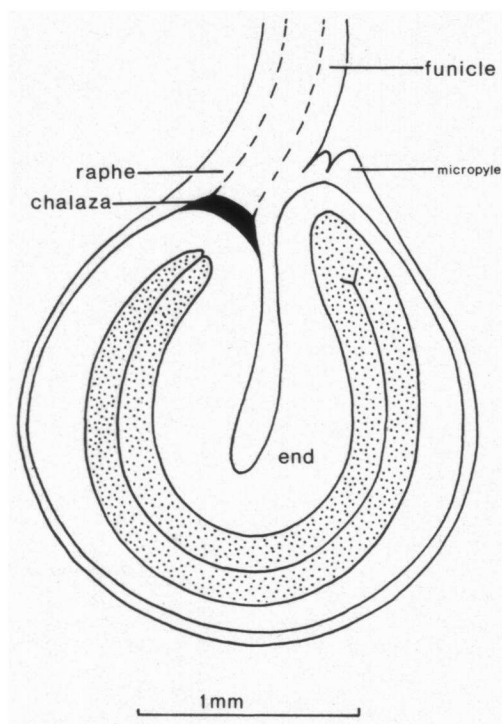


Fig. 4. Median section of campylotropous seed of *Cheorom tricocon*.

ultimately 4–5 cell layers thick (fig. 3D). All cells enlarge during development. The cell walls of the inner and a part of those of the middle layer become slightly thickened and the cells become subglobose (fig. 3E). It is striking that these endo- and mesotestal cells do not form a continuous layer, and that the intercellular spaces between these cells become so much widened by the separation of the cells during their growth. The outer periclinal walls show a beginning of cell wall-thickening (fig. 3E). Tannins occur in the inner layer of the i.i., the outer layer or layers of the o.i., around the raphe bundle and at the inside of the chalazal vascular tissue.

### 3.5. The mature seed

The mature, strongly campylotropous seed (figs. 4, 5A) is enclosed by a very thick and lignified fruit-wall. The seed coat is but little differentiated. At the outside the seed coat is covered by a thick cuticle, strongly reacting with Sudan IV (figs. 3F, 5B, C). The outer periclinal walls of the seed coat show secondary wall thickenings probably of a pectinous nature. The outer cells of the seed coat contain tannins. With the exception of the outer cells and the slightly thick-walled and subglobose meso- and endotestal cells with much enlarged intercellular spaces, the cell layers of the seed coat are completely crushed or resorbed

(fig. 3F). The endo- and mesotestal cells react positively with Ruthenium red. The anticlinal cell walls of the inner layer of the i.i. show very faint strap-shaped or reticulate wall-thickenings (fig. 3F). The greater part of the seed coat becomes strongly pressed against the endocarp and when the seed is taken out of the locule, parts of the seed coat often tear off and rest against the endocarp. The funicular and raphal bundles and the surrounding tissues break away from the seed (fig. 5A). The splitting may take place between the o.i. and i.i. In this case the o.i. remains attached, and the subglobose cells become visible at the inside of the endocarp (fig. 5D). The remaining inner part of the seed coat, with remnants of the i.i., remains around endosperm and embryo (fig. 5E). When the splitting takes place in the crushed nucellar cell layer at the inside of the seed-coat, the endosperm is covered by the nucellar cell walls (fig. 5F). In this case the whole seed coat is left behind in the locule.

The nucellar cuticle reacts strongly with Sudan IV. The single raphal bundle is complex, mainly amphicribal and divides fanwise at the chalaza. The integuments are not vascularized. Around the raphal bundle there is a layer of tanniferous cells. At the inside of the chalazal vascular complex a hypostase is present consisting of somewhat thick-walled and tannin-containing cells weakly reacting with Sudan IV. This tissue closes the chalazal opening.

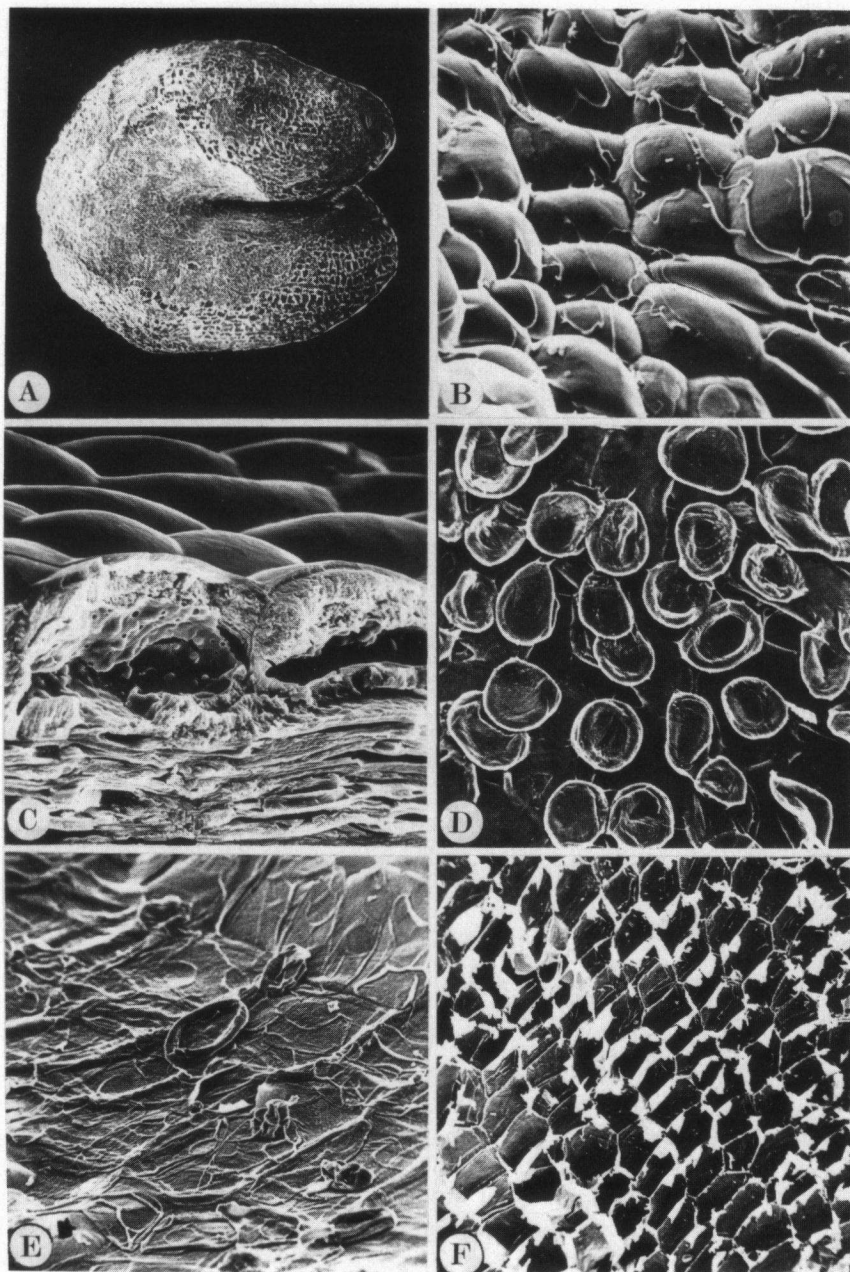
The cellular endosperm and embryo are rich in lipids and poor in starch. The cotyledons are situated in the transmedian plane. Mature seeds partly or wholly without seed coat have the same light colour as the endosperm in places where the seed coat was torn off.

The curved seed is about 2 mm long and 1 mm broad. The seed of *Neochamaelea pulverulenta* shows much resemblance with that of *Cneorum tricoccon*. The mature seed is also campylotropous. The seed coat has a slightly thickened exotesta, subglobose meso- and endotestal cells are also present, and at the inside of the crushed seed coat a distinct nucellar cuticle is present.

#### 4. CONCLUSIONS

The taxa which previously were suggested as relatives of the Cneoraceae belong to the Rutales, Sapindales, Geraniales, Celastrales or Rhamnales. It is remarkable that in recent systems of classification these orders are usually considered as more or less related. Both HEGNAUER and MEEUSE (both in P. G. WATERMAN 1983) derive the Rutales directly from a magnolioid ancestral stock, especially the chemical pointers being very cogent, but several other data appear to be relevant (see MEEUSE 1983). The relatively primitive ovular characteristics of the Rutales do not contradict, but rather support this suggested relationship. This disagreement with CRONQUIST's (1981) view (viz. a rosalean origin of the Rutales and some other most probably or possibly related orders) does not effect the taxonomic position of the Cneoraceae as we shall see (closest to Rutaceae within Rutales). Despite some controversies there is a consensus of opinion concerning the relationships of the Cneoraceae.

According to the most recent studies of STRAKA *et al.* (1976) and LOBREAU





CALLEN *et al.* (1978) the Cneoraceae must be included in the order Rutales and placed in the neighbourhood of the families Rutaceae, Simaroubaceae and Meliaceae (see also GERSHENZON & MABRY 1983). In the classifications of THORNE (1983) and DAHLGREN (1983) the Cneoraceae are placed between Rutaceae and Simaroubaceae, and between Pteroxylaceae and Simaroubaceae respectively.

Burseraceae, Meliaceae and Simaroubaceae have a rather different seed structure. The Burseraceae are characterized by a somewhat tracheidal endotesta, and the Meliaceae exhibit several ovular and seed specialisations such as pachychalazy and arils and the seed coat is exotegmic. Because of these characteristics these families do not seem to be so closely related with Rutaceae and Cneoraceae. The ovule and seed characters of Simaroubaceae are rather diverse in structure but they are not strongly reminiscent of those of Cneoraceae.

The present study reveals that the ovular and seed characters of the Cneoraceae link up fairly well with those of the Rutaceae. The crassinucellate bitegmic ovule of *Cneorum tricoccon* is rather large and retains many of the characteristics of the more primitive angiospermous ovules. It resembles the rutalean ovule in its massive parietal tissue, the nucellar cap and the deep-seated embryo sac. The dermal initiation and initially two-layered integuments point to a more derived condition of the family. Although a subdermally initiated outer integument occurs in Rutaceae, the Rutoideae have a dermal outer integument. The slightly multiplicative nature of the i.i. and the enlargement of the its cells during the early stages of seed development are in agreement with a Rutalean affinity of the Cneoraceae. The crushed seed coat and the strong campylotropy of the seed (called amphitropy by Straka) also constitute derived features (apomorphies). The reduced seed coat of Cneoraceae is most probably connected with the taking over of the protective function by the strongly developed endo- and mesocarp of the indehiscent fruit. Accordingly, it is likely that the seed coat anatomy of the Cneoraceae is derived from an originally more complicated structure. The slightly thick-walled meso- and endotestal cells may represent a reduced sclerotic meso- and endotesta. A similar trend in endotestal reduction showing a loss of wall-thickenings, the formation of intercellulars and the subsequent separation of individual cells is found in polygalaceous taxa with indehiscent fruits (VERKERKE, in the press). Also the faint tracheidal wall-thickenings in the inner layer of the tegmen of the mature seed may be a relic of an originally tracheidal tegmen.

Campylotropy of seeds is rather rare, but in Rutaceae it occurs in some genera, e.g. in *Ruta* and *Thamnosma*. The subfamily Rutoideae, especially the tribe Ru-

Fig. 5. SEM photomicrographs of *Cneorum tricoccon*.

- A: Mature seed with incomplete seed coat and without funicle and raphe. ( $\times 17$ );
- B: Seed coat in surface view showing zones of contact with endocarp ( $\times 500$ );
- C: Transverse section of crushed seed coat with thickened outer periclinal walls ( $\times 1000$ );
- D: Subglobose cells as seen against the inner side of the locule after rupturing of the seed coat during the removal of the seed ( $\times 500$ );
- E: Inner part of the seed coat, left behind around the seed, showing remnants of the ii and the outlines of nucellar cells ( $\times 500$ );
- F: Nucellar cell walls on seed after removal of the seed from the fruit locule cavity ( $\times 100$ ).

teae, shows the greatest resemblance with the Cneoraceae in ovule and seed characters. Especially the ovule and seed of *Ruta* resemble those of *Cneorum* very closely. The ovule of *Ruta graveolens* is slightly campylotropous (its partly crushed seed-coat is exotestal), has somewhat thick-walled endo- and mesotestal cells and exhibits a tracheidal nature of the inner layer of the inner integument (BOESEWINKEL 1977: figs. 1 and 2b, d).

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