OVULES AND SEEDS OF TRIGONIACEAE

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SUMMARY

Trigonia cipoensis has a trizonate ovule primordium. The mature ovule is anatropous, bitegmic and tenuinucellate. Both integuments are of dermal origin; the inner integument is strongly multiplicative, especially after fertilisation. The mature seed is densely covered by cottony testal hairs. Of the inner integument the outer layer develops into a lignified, fibrous exotegmen, whereas the inner layer is tanniniferous. Endosperm formation is initially nuclear, the tissue later becoming cellular throughout.

Ovule and seed structure indicates a relationship with the Linales and not with the Polygalales with which the family Trigoniaceae is ususally associated.

I. INTRODUCTION

The small woody family Trigoniaceae occurs in tropical lowlands and consists of 3 genera. The neotropical genus *Trigonia* is the largest with 24 species (30 according to CHANT 1978). *Trigonia* is found in more open but protected habitats such as the so-called Varzeas (periodically flooded riverine forests), gallery forests, the edges of wet forest and in disturbed areas like roadsides and edges of clearing. The palaeotropical genera *Trigoniastrum* and *Humbertiodendron* are monotypic and occur in Malaysia and Madagascar, respectively (LLERAS, 1978). Both genera are elements of the wet tropical forest. Correlated with their presence in different environments, *Trigonia* and the palaeotropical genera show a different growth habit and dispersal mechanism. *Trigonia* species are treelets, shrubs, rambling shrubs or lianas, while *Trigoniastrum* and *Humbertiodendron* are tall trees (ca. 25 m). The papilionaceous flowers of Trigoniaceae are zygomorphic with 5 or 3 unequal petals of which the dorsal one is the largest. The ovary is superior and basically trilocular, with few to numberous ovules in each locule in *Trigonia* and one or two ovules in *Trigoniastrum* and *Humbertiodendron,* respectively. The fruits of *Trigonia* are septicidal capsules and the anemo- or hydrochorous seeds are enveloped by cottony hairs and likely to be dispersed over larger distances in more open vegetation types.

Trigoniastrum and Humbertiodendron have indehiscent, trialate fruits which easily dehisce into samaras which are dispersed over a moderate distance only (LLERAS 1978). Little is known about the ovule and seed structure of *Trigonia*. The crassinucellate ovule is suspended anatropously and has a 5–6 layered inner and a 2-layered outer integument. The albuminous seeds are small and covered with long hairs (NETOLITZKY 1926, MAURITZON 1936, CORNER 1976). The anatomy of the mature seed coat has never been described before. The endosperm

formation and embryogeny were also unknown (DAVIS 1966). On morphological and anatomical grounds a relationship of the Trigoniaceae with different taxa has been suggested, mostly with families of the Polygalales: Polygalaceae, Malpighaceae, Vochysiaceae, Tremandraceae and Zygophyllaceae (HEIMSCH 1942). Alternatively, a relationship has been suggested with Dichapetalaceae and Malpighiaceae (PRANCE 1972; BRETELER 1973). LLERAS (1978) gives a complete summary of all ideas concerning the relationships of the Trigoniaceae. The chemotaxonomy of the Trigoniaceae is unknown (HEGNAUER 1966).

2. MATERIALS AND METHODS

Developing flowers and fruits of *Trigonia cipoensis* Trinta & Em. Santos were collected by M. Venturelli (University of São Paulo) at the Serra do Cipo, Minaus Gerais, Brasil in 1983 and 1984.

Herbarium specimens of developing seeds of *T. cipoensis,* collected by I. Cordeiro et al. No CFSC 7674, originated from the Botanical Department of the University of São Paulo. Dry seeds of the following species originated from the Rijksherbarium, Leiden: *Trigonia rugosa* Benth., leg. C. F. Baker, no 2554, Nicaragua; *Trigonia villosa* Aublet, leg. Leprieur no 131, Fr. Guyana; *Trigoniastrum hypoleucum* Miq., leg. Boschbouwproefstation no T. 95, Palembang, Sumatra.

Plant material was fixed in formalin, acetic acid and alcolhol (F.A.A.) and dehydrated in an ethanol/n-butyl alcohol series, embedded in glycerol metacrylate, sectioned at 5–10 μm with glass knives, stained with the PAS reaction and counterstained with aqueous methylene blue. Dry fruits and seeds were softened during an overnight stay in 10% ammonia solution and subsequently dehydrated and infiltrated with methacrylate. Phloroglucinol-HCl, Sudan IV, ruthenium red and Iodine in potassium iodide solutions were used for identification of lignins, fats, pectins and starch. The occurrence of tannins in prepared slides is commonly indicated by the presence of dark red or reddish-brown precipitate (JOHANSEN 1940). For scanning electron microscopy (SEM) specimens were gold/palladium sputter-coated for about $2\frac{1}{2}$ minute and studied on an ISI DS 130.

3. RESULTS

Trigonia cipoensis has a three locular ovary with more than 10 epitropous ovules in each locule. The dehiscent fruits are septicidal and contain several seeds in each locule.

3.1. Ovule development
The ovule primordium is trizonate *(fig. 1A, B)*. Shortly after the initation of the integuments about two cells of the second layer of the nucellar apex differentiate into archespores *(fig. 1C–G)*. The archespores serve directly as megaspore
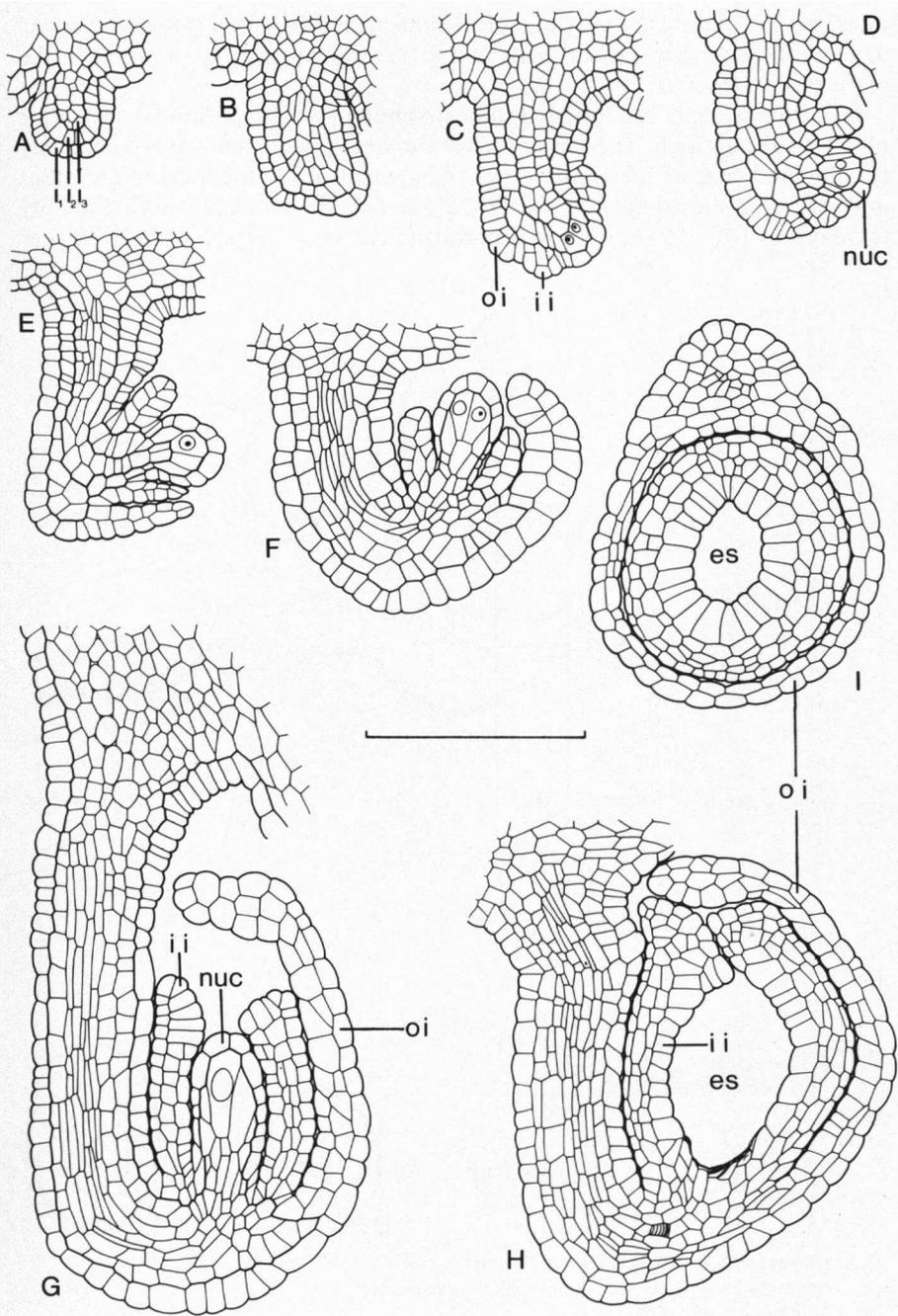
Fig. 1. *Trigonia cipoensis.*
A–G: Longitudinal sections of developing ovules;
H,I: Longitudinal section and cross section of mature ovule.
l_1, l_2 and l_3: dermal layer, subdermal layer and corpus, respectively; es = embryo sac; nuc = nucellus; ii = inner integument; oi = outer integument. The bar indicates 100 μm.

mother cells, but mostly only one of them develops into a mature embryo sac. The nucellus remains rather small and is already completely resorbed in the mature ovule (*fig. 1H, I*).

Both integuments are initiated almost simultaneously as circular rims of 2 to 3 dermal cells high. The rim of the future inner integument (ii) is completely circular, but that of the future outer integument (oi) is incomplete and does not develop at the raphal side (*fig. 1G, H*). At first both integuments are mainly two-layered (*fig. 1D–F*). The inner integument becomes secondarily thicker
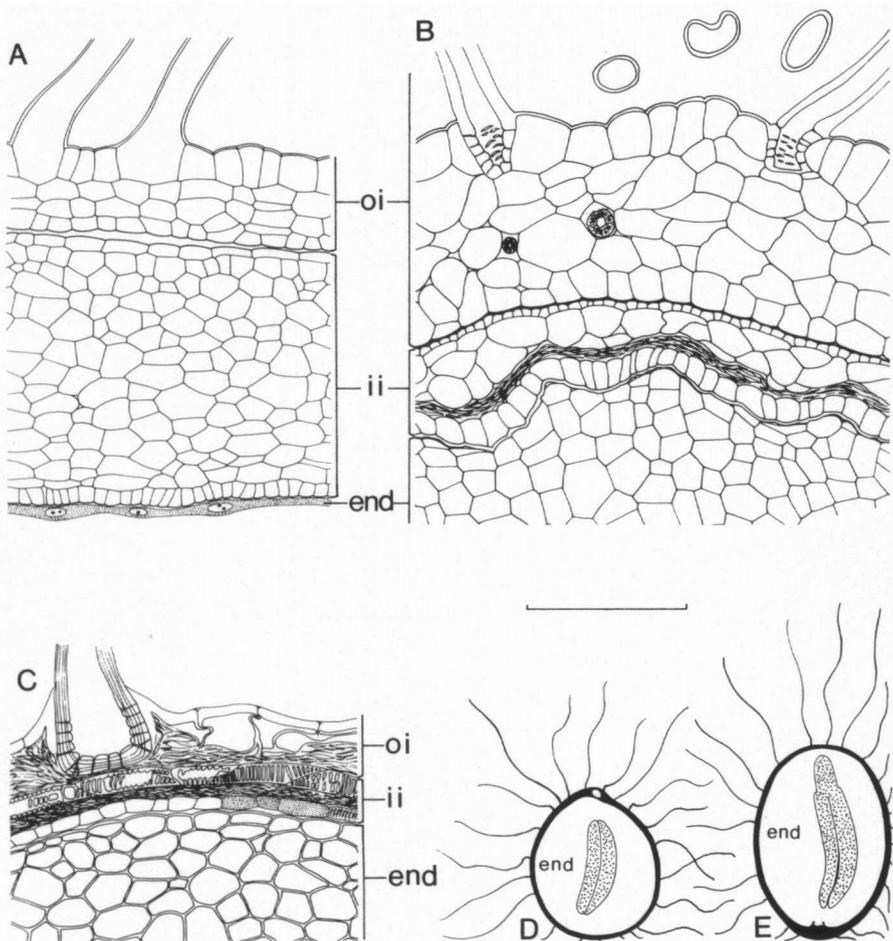

Fig. 2. *Trigonia cipoensis* (A,B,D,E) and *Trigonia rugosa* (C).
A,B: Longitudinal and cross section of developing seed coat;
C   : L.s. of mature seed coat;
D,E: Outline of seed in cross and longitudinal sections.
end = endosperm; ii = inner integument; oi = outer integument; bar indicates 100 μm

when it has overgrown the nucellus (*fig. 1G*). At first the inner cell layer divides periclinally and these divisions are followed by periclinal divisions in the thus formed middle layer, so that the ii becomes 4-layered. The outer integument overgrows the ii and becomes locally 3-layered by periclinal divisons within the inner layer.

During ovule development tannins are formed in the outer layer of the oi and the raphe. The curvature of the ovule is initially caused by mitotic divisions at the convex side of the ovule (*fig. 1C, D*).

3.2. The fully developed ovule
The ovule is anatropous, bitegmic and tenuinucellate. The nucellus has dissappeared completely, so that the embryo sac is in direct contact with and surrounded by the ii (*fig. 1H, L*). The embryo sac contains starch, is 8-nucleate and probably of the *Polygonum* type.

The ii has become 4–5 layered by periclinal divisions within the middle layer. The cells of the inner layer are enlarged and have assumed a somewhat endothelial character. The oi has remained 2–3 layered and its outer layer is rich in tannins.

The zig-zag micropyle is formed by both integuments. The endostome is more or less orbicular to slit-like in cross section and the exostome is slit-like in the median plane. The funicular bundle is distinct and starts to develop differentiated elements. The dermal cells of the funicle facing the micropyle are somewhat papilliferous and may function as an obturator.

3.3. Seed development and mature seed
After fertilisation the ovule increases in size, but the overall shape of the ovule does not change much. The endosperm is nuclear and becomes cellular at a later stage (*fig. 2A, B*). Before the seed is mature, orbicular unidentified crystallike bodies arise at the inside of the endosperm (*fig. 4C*). In the mature seed the walls of the endosperm are slightly thickened and pectinaceous. The endosperm cells contain one large or several small, unidentified globules, strongly reminiscent of those found in the endosperm of Vochysiaceae. The endosperm is rich in fats and poor in starch.

Of the early embryogeny only a stage of a filamentous proembryo of 7 cells high could be observed (*fig. 4D*).

The ii becomes about 15 cell layers thick by further divisions of the middle layers and is mainly responsible for the enlargement of the developing seed (*fig. 2A, 4E*). The inner layer of the ii becomes tanniniferous and shows many anticlinal divisions. The cells of its outer layer become stretched in the longitudinal direction of the seed (*fig. 2A, 4F*). At a later stage, when the endosperm has already become cellular, the enlarged cells of the middle layers are almost completely crushed (*fig. 2B*). Between ii and endosperm a conspicuous cuticle has developed which reacts strongly with Sudan IV (*fig. 2B*). Mature seeds of *T. cipoensis, T. rugosa* and *T. villosa* could be observed. The seed coat anatomy of these species is very similar. In the seed the middle layers of the ii are complete-
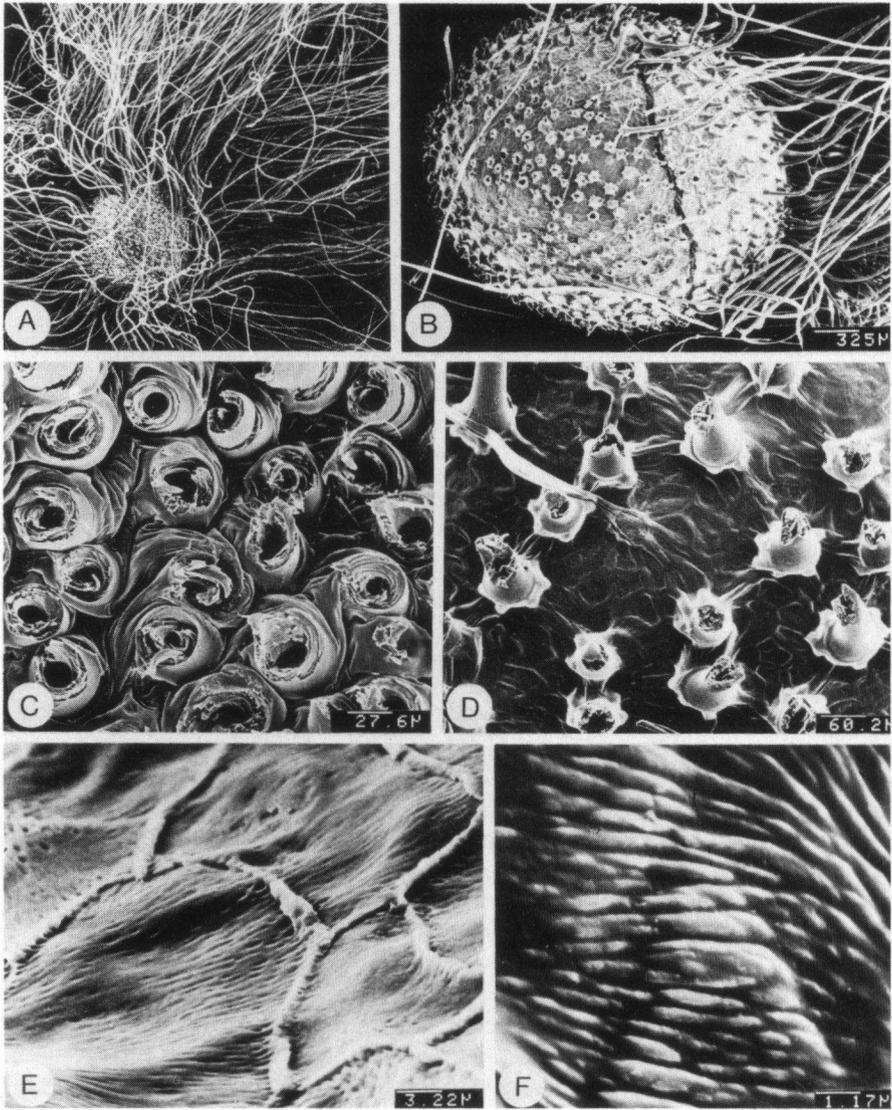
Fig. 3. SEM photographs of mature seeds.
A,B: Seeds of *Trigonia cipoensis* and *T. rugosa,* hairs partly removed;
C,D: *T. cipoensis* and *T. rugosa:* details of seed coat;
E,F: *T. rugosa:* inside and detail of outside of pigment layer of inner integument, showing periclinal cells walls with parallel ridges.
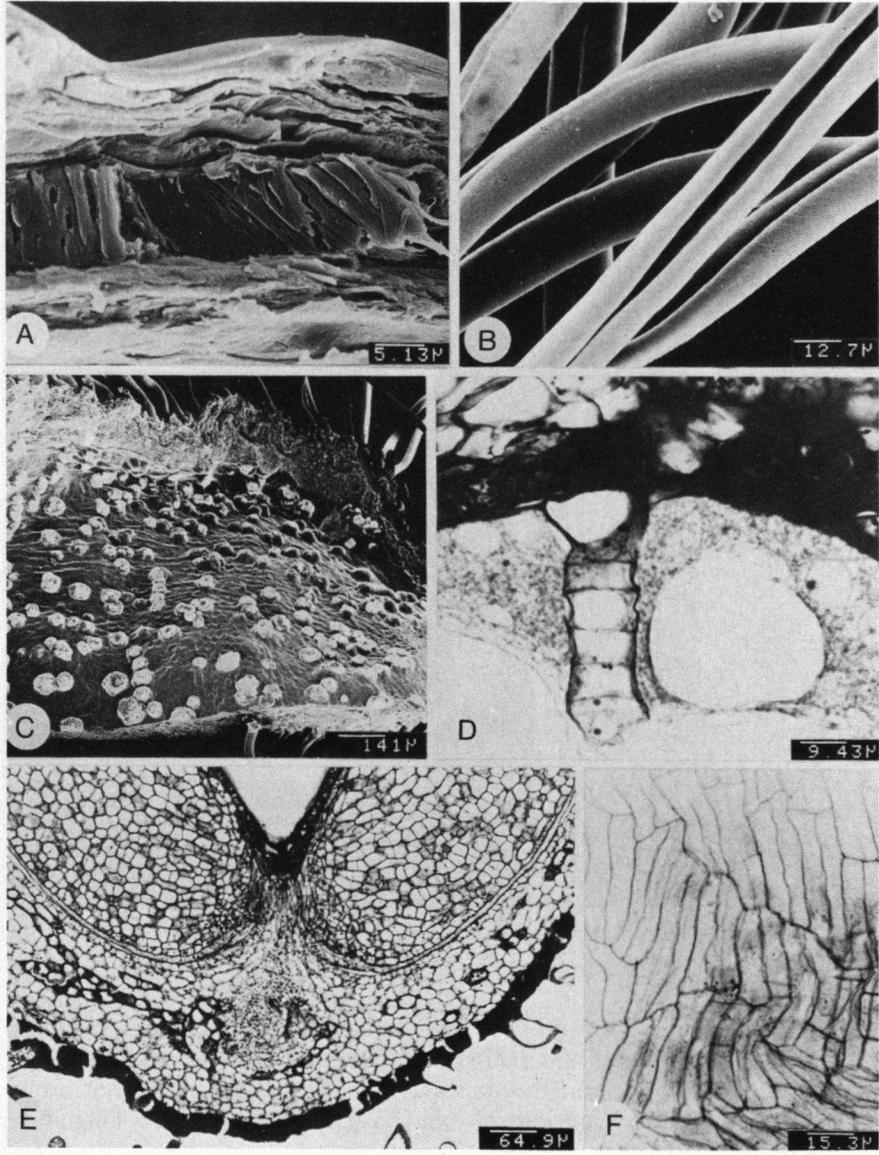
Fig. 4. *Trigonia cipoensis* SEM (4A–C) and LM (4D–F) photographs
A: Cross section of the mature seed coat with exotegmic layer;
B: Seed coat hairs showing furrows;
C,D: Developing seed. C: Inside of endosperm with crystal-like bodies;
D: Filamentous proembryo;
E: Chalazal part of developing seed with thick ii;
F: Surface view of early exotegmen showing the formation of longitudinally stretched cells

Fig. 5. *T. cipoensis*. Dehisced fruit with some seeds. Length of the fruit ± 3,5 cm.

ly crushed (*fig. 2C, 4A*). The cells of the inner tannin-containing layer have become tangentially flattened and their slightly thickened cell walls show a barred structure at both the inner and the outer side (*fig. 3E, F*). The cells of the fibrous exotegmen have enlarged considerably, and have developed tracheoid, lignified wall thickenings. In surface view the exotegmic layer shows imprints of the endotestal cells. The individual exotegmic cells cannot be clearly discerned, but some pits are visible.

The oi becomes thicker by divisions in the inner and middle layers (*fig. 2A*). Clusters of cells in the oi, especially the cells of the outer epidermal layer, contain tannins (*fig. 4E*). All or some of the epidermal cells grow out into unicellular hairs (*fig. 2A*) which may obtain a length of upto 5 mm. When the endosperm has become completely cellular the oi is 6–7 layered, and has reached about

its maximum thickness (*fig. 2B*). The cells of the middle layers are enlarged, parenchymatic and show some intercellulars. Subglobose, echinate bodies have developed in some of its cells. These bodies consist of more or less radially oriented, crystal-like elements around a more open, orbicular centre. The hairs have become thick-walled over their entire length. Their basal parts show layered and strongly pitted walls (*fig. 2B, C*). In the mature seed of *T. rugosa* and *T. cipoensis* the oi is nearly completely crushed (*fig. 2C, 4A*). As a result of this crushing the bases of the hairs come to ly against the exotegmen. The outer walls of the testa cells have become strongly thickened and contain much pectic substances. The walls of the hairs are strongly lignified. Both testa cells and hairs are tanniniferous.

The mature seeds of *T. cipoensis*, *T. rugosa* and *T. villosa* consist of a seed body with dimensions of about 2 mm, surrounded by a voluminous cover of white, cottony hairs (*fig. 3A, B*). The hair cover is denser on the seed of *T. cipoensis* (*fig. 3C*) than on that of *T. rugosa* (*fig. 3D*). In *T. cipoensis* and *T. rugosa* a large part of this indumentum is sometimes longitudinally and unilaterally furrowed (*fig. 4B*). In cross section the hairs appear indented (*fig. 2B*).

The raphal bundle is amphicribral and complex. The embryo is linear and has flat cotyledons with a differentiating palisade layer at their adaxial sides (*fig. 2D, E*). The embryo and endosperm of *T. rugosa* are rich in fats but poor in starch.

The mature fruits of *Trigonia* dehisce between the carpel margins into 3 valves. The axile placenta of the fruits splits into 6 separate strings which remain connected pair-wise with the apex of the fruit valves (*fig. 5*).

Of *Trigoniastrum hypoleucum* only developing seeds were available. Each of the three locules is one-seeded. The seeds are much larger than they are in *Trigonia* and laterally flattened, their shape corresponding with that of the locule. The anatomy of the seed coat resembles that of *Trigonia* very much and only deviates in details. The oi is about 10 cells thick. The outer layer is provided with thick-walled, pitted hairs as in *Trigonia,* but lack a furrow. The cells of the middle layer are tanniniferous; they remain small and have a more irregular shape. The ii is multiplicative, with an inner pigment layer. The middle layer of strongly enlarged isodiametric cells is in a state of compression. The exotegmen cells are longitudinally stretched but not thickened at this stage.

According to NG (1972) the velvety seed of *Trigoniastrum*, measuring 17 × 5 × 1,2 mm is exalbuminous, obovate and flat. The cotyledons are flat. The shape of the seed agrees with that of the carpel cavity. The germination is epigaeic.

4. DISCUSSION

In *Trigonia* the fruits are dehiscent and the seeds are adapted to aerial or hydrochorous dispersal (LLERAS 1978). In *T. spruceana* the capsule dehisces at the top and at least a moderate wind is required to carry the seed away. Of the

24 species of *Trigonia* only two have echinate trichomes, so that hydrochory is probably only found in these species. *Trigonia* is the largest and according to its fruit and seed structure, also the most primitive genus of the Trigoniaceae. According tot LLERAS (1978), *Humbertiodendron* and *Trigoniastrum* are anatomically close to one another. In *Trigoniastrum* and *Humbertiodendron* (PERRIER & LEANDRI 1955) the somewhat reduced seeds remain enclosed in the winged fruit, which may fall apart into 3 samaras, which act as the dispersal units. The seeds of *Trigoniastrum* have reticulately villous hairs; those of *Humbertiodendron* are glabrous.

The take-over of the protective function of the seed coat by the fruit wall in non-dehiscent fruits is a common trend among angiosperms and indicates a more derived condition of *Trigoniastrum* and *Humbertiodendron* in respect of *Trigonia*.

The ovule and seed anatomy of *Trigonia cipoensis* is very similar to that of *Linum* (BOESEWINKEL 1980a) and related taxa. They share several characters: the ovule is trizonate, the integuments are dermal and initially two- or three-layered only, and the ii becomes thicker by repeated divisions of the middle layers, these divisons often rendering the ii very thick, especially during the early stages of seed development. In contrast to MAURITZON's (1936) observations on *Trigonia nivea* and *T. parviflora*, the ovules of *T. cipoensis* are tenuinucellate. This character needs confirmation in other species. MAURITZON (1936) studied dry herbarium material and his illustration was made from a not precisely median section. On the other hand, also within the genus *Linum* both character states can be found. The seed coat structure of *Trigonia* also corresponds with that of *Linum, Erythroxylum* and *Humiria* (BOESEWINKEL 1980a, b, 1985).

Especially the characters of the inner integument are strikingly similar. The middle layers become crushed and the inner layer develops into a tanniniferous, pigmented layer, with slightly thickened cell walls. The fibrous exotegmen is strongly lignified and forms the principal mechanical layer of the seed coat. The outer layer of the outer integument differentiates in various ways in the Trigoniaceae and the families of the Linales. These differences are clearly attributable to differences in seed dispersal, however. The above-mentioned correspondences in ovule and seed structure are an indication of a relationship between Trigoniaceae and the Linales. This is apparently also the case with most other families of the Polygalales sensu CRONQUIST (1981), such as the Vochysiaceae, Malpighiaceae and Tremandraceae, but not with the Polygalaceae. The family Polygalaceae (incl. of Xanthophyllaceae) differs from the majority of the 'Polygalales' in its ovule and seed structure (VERKERKE 1985). Also according to CORNER (1976), the family Trigoniaceae cannot be classified near the Polygalaceae if the Trigoniaceae have exotegmic fibres.

The more primitive representative of the Polygalaceae are characterised by crassinucellate ovules, with a subdermal, vascularized outer integument and a 2-layered inner integument. The endotesta is the main protective layer of the seed coat.

With the exception of the family Polygalaceae I agree with HEIMSCH (1942),

who is of the opinion that the Polygalaceae, Trigoniaceae, Tremandraceae, Malpighiaceae, Vochysiaceae and also Zygophyllaceae are interrelated and, although more specialized in general habit, floral structure and wood anatomy, these families are supposed to be related to the Linaceae, Erythroxylaceae and Humiriaceae. The often suggested relationship of the Trigoniaceae with the Dichapetalaceae (BRETELER 1973) is not supported by the ovule and seed structure (BOESEWINKEL 1980c). Because of its derived seed structure the relationships of the Dichapetalaceae are difficult to assess from the seed morphology and anatomy alone. Also CRONQUIST's (1981) ultimate derivation of the Linales and Polygalales from ancestral Rosales is not supported by the seed architecture. The families of the Rosales are mainly mesotestal and not exotegmic. A derivation of Linales and Polygalales through Sapindales or Rutales from a magnolialean-ranunculalean stock seems to be more probable (WATERMAN & GRUNDON 1984).

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