## Ovules and seeds of Dirachma socotrana (Dirachmaceae)

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# Ovules and seeds of *Dirachma socotrana* (*Dirachmaceae*)

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**Abstract:** *Dirachma* has a bitegmic, crassinucellate and anatropous ovule with a single median provascular tegumentary bundle. The seed coat is characterized by an exotesta and an endotegmic pigment layer. Although the fruit of *Dirachma* superficially resembles that of the *Geraniaceae* s. str., the characters of ovule and seed do not support a relationship with that family. Also a relationship with *Barbeyaceae*, as suggested by recent *rbcL* studies, is not supported by seed anatomical characters. The true relationships of *Dirachma* are difficult to assess on the basis of ovule and seed characters alone. The *Rhamnaceae* may be a closer relative.

The monogeneric family *Dirachmaceae* comprises two species, *Dirachma socotrana* Schweinf. ex I. B. Balfour, endemic to the Isle of Socotra, and *D. somalensis* D. A. Link, a recently described species from central Somalia (Link 1991). Like *D. socotrana*, of which in 1967 only 30 mature trees were found with no seedlings or saplings to be seen, *D. somalensis* seems to be very rare and endangered by excessive grazing, particularly by goats (see Link 1991).

The familial status and systematic affinities of *Dirachma* are still puzzling. Several families have been mentioned as possible relatives. Balfour (1888) found *Dirachma* to resemble *Tiliaceae*, *Sterculiaceae* and the *Samydeae* group (*Flacourtiaceae*). Finally, he placed *Dirachma* in the *Geraniaceae* in the vicinity of *Vivianieae* and *Wendtieae*. This was followed by Knuth (1931). Hutchinson (1969) proposed a family status and placed *Dirachmaceae* in *Tiliales*. Willis (1985) suggested a distant connection to *Greyiaceae*. Link (1991) and Ronse Decraene & Smets (1995) pointed to floral and carpological conformities with *Malvales*, such as the presence of long, unicellular hairs on the inner surfaces of the fruit valves, as in the genera *Ceiba* and *Ochroma* (*Bombacaceae*). They concluded that the family would be best positioned at family rank in *Malvales* next to *Tiliaceae*. In most current phylogenetic systems *Dirachma* is treated as a separate family in *Geraniales* (Takhtajan 1987, Dahlgren 1989). It was treated by Thorne (1992) as a subfamily, *Dirachmoideae*, in *Geraniaceae*, and by Cronquist (1981) as a genus under *Geraniaceae*

together with *Biebersteinia, Rhynchotheca, Wendtia, Balbisia* and *Viviania*. The latter author considered it possible that some of these genera, most notably *Dirachma* and *Viviania* are not closely allied to the *Geraniaceae* because they are aberrant in one way or another. Cronquist preferred to keep them here until their relationships are more clearly established. All these suggested relationships disagree with the results of recent *rbc*L studies (B. Bremer, M. Thulin, M. Chase, unpubl. data), which indicate *Rhamnaceae, Elaeagnaceae* and *Barbeya* (*Barbeyaceae*) as close relatives to *Dirachma*, together with *Urticales*.

The structure of the reproductive organs has been described in more detail by Link (1991). The syncarpous ovary in *Dirachma socotrana* is eight-locular (in *D. somalensis* 5-locular) with the carpels connate only in the central part and each bearing one anatropous, bitegmic ovule with the micropyle in hypotropous-ventral orientation per locule. The beaked fruit is a septicidal-septifragral capsule with each locule densely packed with long unicellular trichomes originating from the inner side of the fruit valves. The hairs envelope the ellipsoid brown seed with shining testa and scant endosperm. The valves separate from base to apex, spreading umbrelliformous (Link 1991).

The structure of the ovule and seed of *Dirachma* is unknown (Davis 1966, Corner 1976), and this study was made to obtain additional arguments for the assessment of the affinities of *Dirachma*.

## Material and methods

The material available was poor and consisted of a few dry and fixed developmental stages of flowers and fruits of *Dirachma socotrana*. In addition we could use slides of flowers and fruits of *D. socotrana* made in Kew and kindly provided by Prof. P. Baas.

The dry material was softened overnight in 10% ammonia and sections were made by the standard microtome technique after embedding in either paraffin wax or in glycol methacrylate. For scanning electron microscopy (SEM) untreated or fixed specimens were gold- or gold/palladium sputter-coated for about 2.5 min and studied on an ISI DS 130.

Specimens examined: *Dirachma socotrana* Schweinfurth ex I. B. Balfour: (1) Socotra, Jebel Rughid, Smith & Lavranos s.n. (L), (2) Socotra, Jebel Rughid, Smith & Lavranos 210 (WAG).

## Results

**Ovule development.** Shortly after the beginning of anatropous curvature, the integuments initiate and the subdermal archespore differentiates and cuts off parietal cells (Fig. 1A). The inner integument (i.i.) is initiated as a dermal, circular rim of two to three cells thick. The outer integument (o.i.) is initiated in the median plane by periclinal divisions of both the dermal and subdermal layer (Fig. 1A). The o.i. is poorly developed at the raphal side (Fig. 1A, B).

The mature ovule is anatropous, bitegmic and crassinucellate. In the almost mature ovule the curvature has become nearly 180° (Fig. 1B). The ovule is laterally flattened and in median longitudinal section almost circular. Compare the transmedian section (Fig. 1C), in which the ovule is more slender and has a thinner o.i. The nucellus is relatively large. The parietal tissue is about six-layered, the nucellar cap
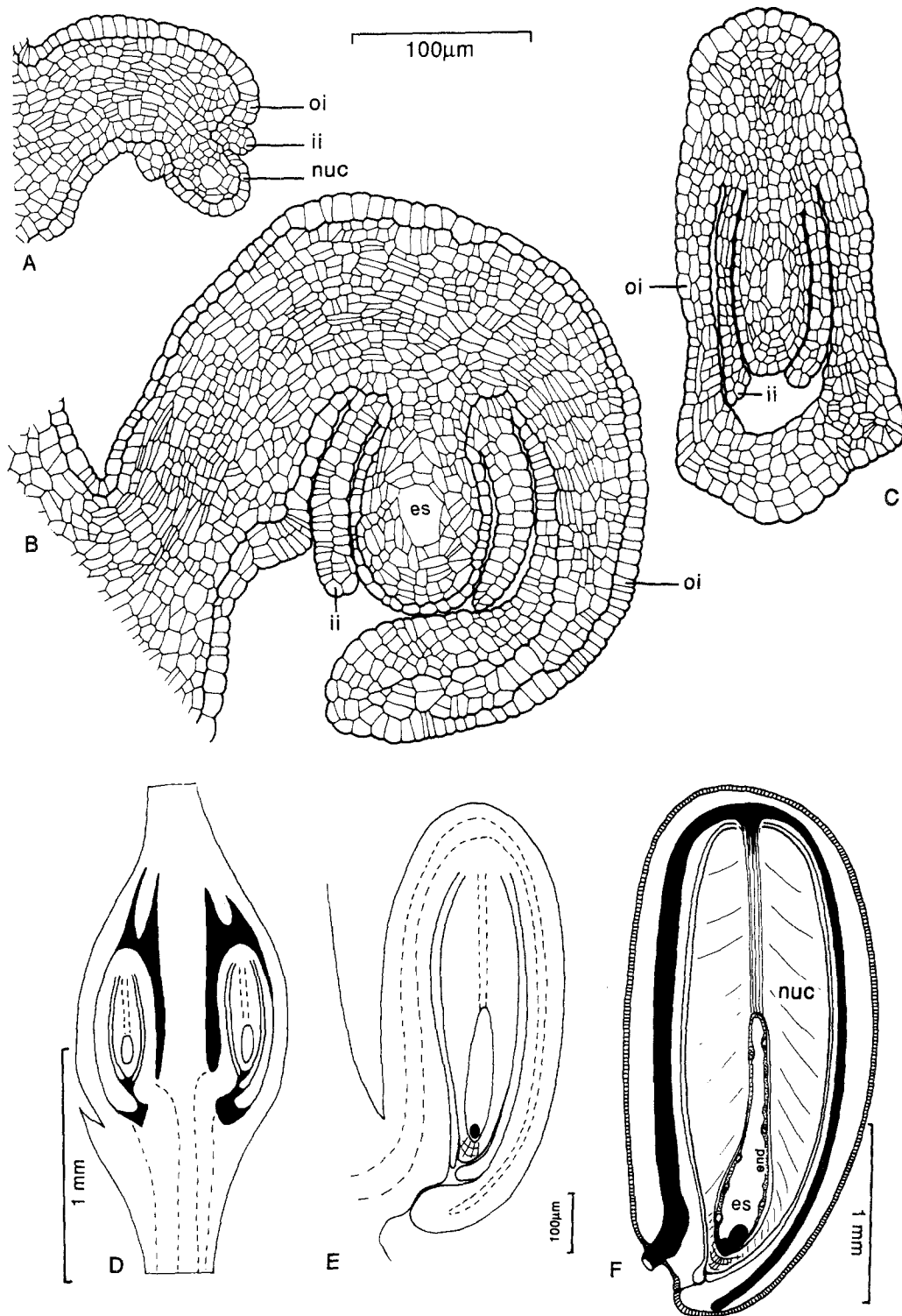
Fig. 1. *Dirachma socotrana*. Line drawings of ovules (*A–C*), developing ovary (*D*) and seeds (*E–F*). *A* Ovule shortly after initiation of integuments; *B*, *C* almost mature ovule, median section, with starting development of median vascular bundle and transmedian section with thin o.i.; *D*, *E* early developing ovary and its enlarged developing seed; *F* developing seed (median section) with early stages of globular embryo and endosperm, very large nucellus and well-developed vascular bundle (black). – *es* Embryo sac, *end* endosperm, *nuc* nucellus, *ii* inner integument, *oi* outer integument

is initially one-layered. The nucellar apex is rich in starch grains. The i.i. is mainly two-layered and still has not grown over the nucellar apex (Fig. 1B). In the median plane the o.i. is about ten-layered, but in the transmedian plane it is only three- to five-layered. The thickness of the o.i. in the median plane is connected with the developing, medially-situated vascular bundle which consists of the raphal bundle and its extension into the o.i. (Fig. 1B). The part of the o.i. where the provascular bundle is present may be of subdermal origin. The o.i. is weakly developed at the raphal side. The upper part of the o.i. derives its thickness from strong periclinal divisions which form cell rows in its inner layer. The o.i. grows over the nucellar top, so that a zig-zag micropyle is formed (Fig. 1B). The embryo sac is probably of the Polygonum type, the three antipodal cells degenerate soon after fertilization.

**Seed development and mature seed.** Shortly after fertilization the developing seed becomes longitudinally elongated. It remains flat in median plane into maturity (Figs. 1D-F; 3F, G; 4D).

The raphal bundle is collateral and has an outer phloem arch with the vessels in groups; the xylem has differentiated into spiral elements. This vascular bundle runs below the margins of the seed and ends in the top of the o.i. (Figs. 1F, 3F, 4D). In the chalaza the bundle undulates. At maturity, a constriction is present in the short and thick funicle, suggesting an abscission layer facilitating rupture.

In Fig. 1F the globular stage of the embryo is shown together with a small amount of nuclear endosperm against the wall of the embryo sac, which has enlarged at the expense of the nucellus. The mature embryo is straight with the flat cotyledons in the median plane of the seed. The endosperm is never abundant, becomes cellular later and forms a layer of about six to eight cells at maturity (Fig. 2F). The nucellus has become very massive and is provided with a cuticle, which is more distinct at the chalazal part (Fig. 1E, F). The parietal tissue becomes up to ten-layered and is initially rich in starch grains. An indistinct small nucellar cap is formed, which seemingly never becomes more than three-layered. The embryo sac is connected with the vascular tissue in the chalaza by means of a strand of longitudinally elongated nucellar cells. This strand apparently functions in the transport of nutrients. The nucellus is almost completely crushed at maturity (Fig. 2F).

The mainly two- to three-layered i.i. overgrows the nucellar apex, (Fig. 1E) its inner layer becomes tanniniferous, and later its cells become somewhat flattened and thick-walled. Especially in the inner layer of the i.i. unidentified bodies are present situated against the cell walls. The endotegmen is the only layer of the i.i. which contributes to the formation of the mature seed coat: the other cells enlarge and become crushed (Fig. 2A–F).

In the early stages of seed maturation the tops of the o.i. and i.i. contain star-shaped crystals, which are also present in other floral parts. The o.i. is initially six- to ten-layered and becomes very thick later, especially at the seed-margins where the vascular bundle runs. At first, this thickening is mainly the result of cell division (Fig. 2A-C), but later of a combination of cell enlargement and the formation of many large intercellular spaces, so that the o.i. becomes strongly aerenchymatous (Figs. 2E, 3F). Gradually, also numerous starch grains develop in the o.i. (Fig. 2E), which disappear later and are probably used up during the formation of the exotesta in the final stages of seed maturation. Finally, the aerenchyma is crushed, so that the contribution of the o.i. to the seed coat consists of the exotesta and a few, thin-walled
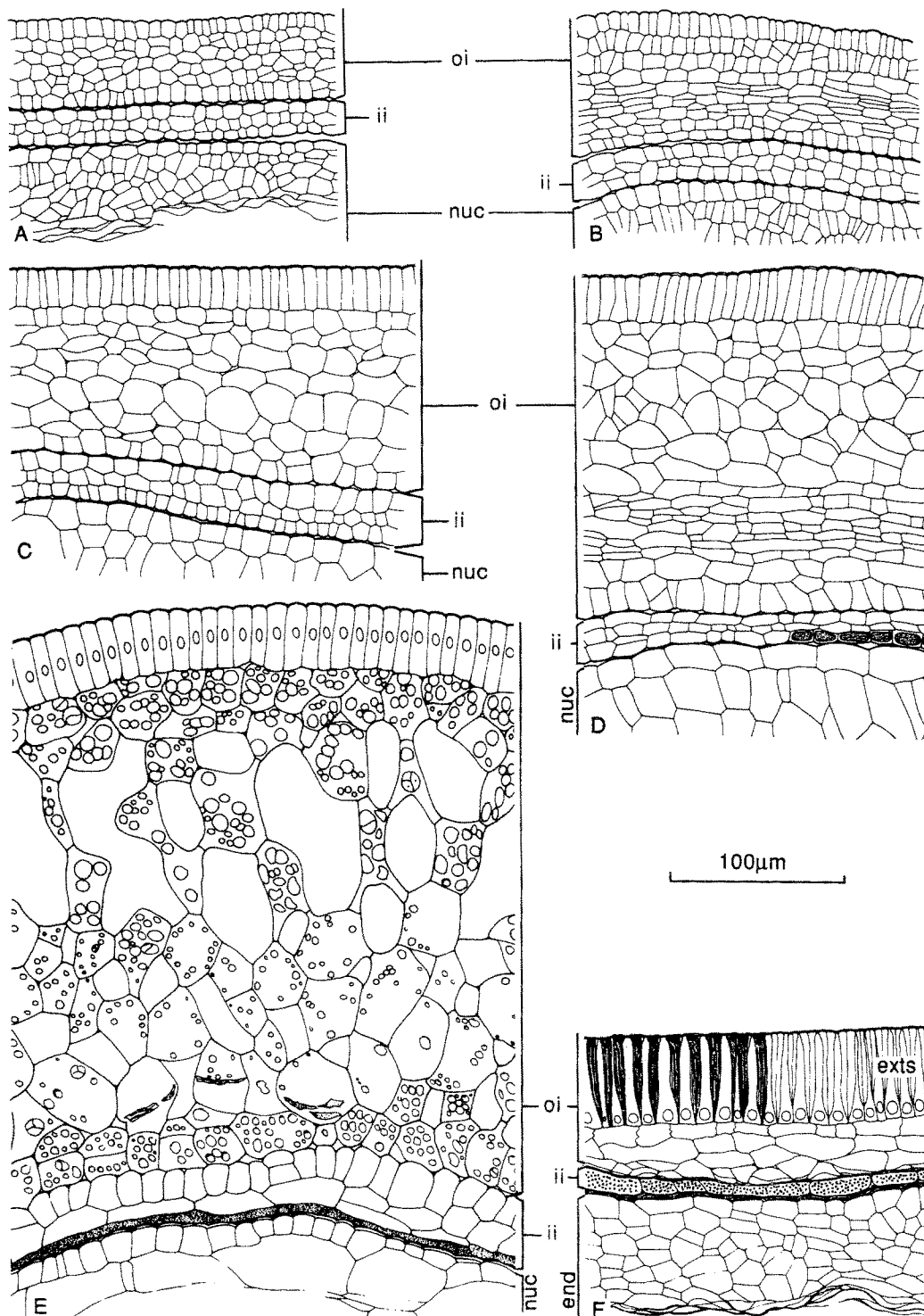
Fig. 2. *Dirachma socotrana.* Developing and mature seed coats. *A–D* Longitudinal section of developing seed coats, with antiraphal vascular bundle (*B* and *D*) and start of stretching of exotestal cells (*C* and *D*), (*A* and *B* enlarged from 1 *E*, and *D* enlarged from 1 *F*); *E* cross section of seed coat shortly before maturity with large intercellulars and numerous starch grains in o.i. (same stage as 3 *F*); *F* mature seed coat with well-developed exotesta and tanniniferous endotegmen. – *end* Endosperm, *nuc* nucellus, *ii* inner integument, *oi* outer integument, *exts* exotesta, *ls* longitudinal section.
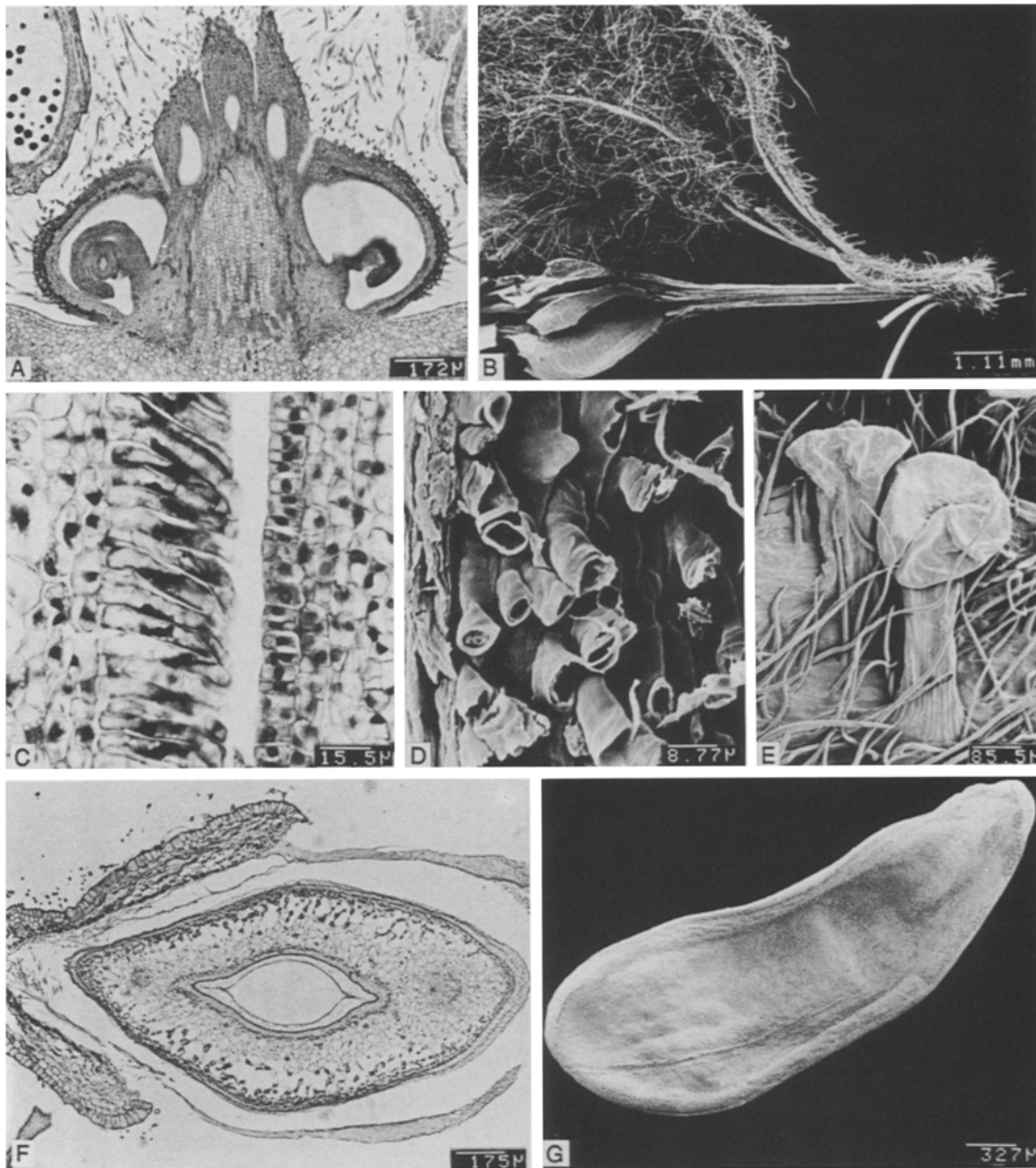
Fig. 3. *Dirachma socotrana*. Light microscopical (*A, C* and *F*) and SEM (*B, D, E* and *G*) photographs. *A* L.s. of ovary with two locules, and one down-pointing ovule (same as 1 *B*); *B* dehisced fruit with awns connected to top of central column, broken fibre bundles visible below top; *C* starting development of intralocular hairs; *D* bases of broken mature, thick-walled intralocular hairs; *E* glands on developing fruit wall; *F* cross section of immature developing seed, surrounded by fruit valve and still thin-walled intralocular hairs; *G* immature seed, air-dried (same stage as *F*)

hypodermal layers (Fig. 2F). The exotestal cells gradually stretch radially into palisade cells. These cells become tanniniferoùs in early stage, have a centrally situated nucleus, and their walls become secondarily thickened, except for the inner periclinal one and a part of the outer wall. At the cell base a probably tanniniferous globule, staining strongly with Safranine, is present (Fig. 2F).

By the crushing of the nucellus, i.i. and o.i. more space becomes available for the mature embryo and endosperm. The immature seed measures $6 \times 3 \times 0.8$ mm, which probably approximate the dimensions of the fully mature seed.

**Other embryological characters.** The surface of the anther is characterized by linear cuticular striae. The outer epidermis is relatively large-celled, somewhat
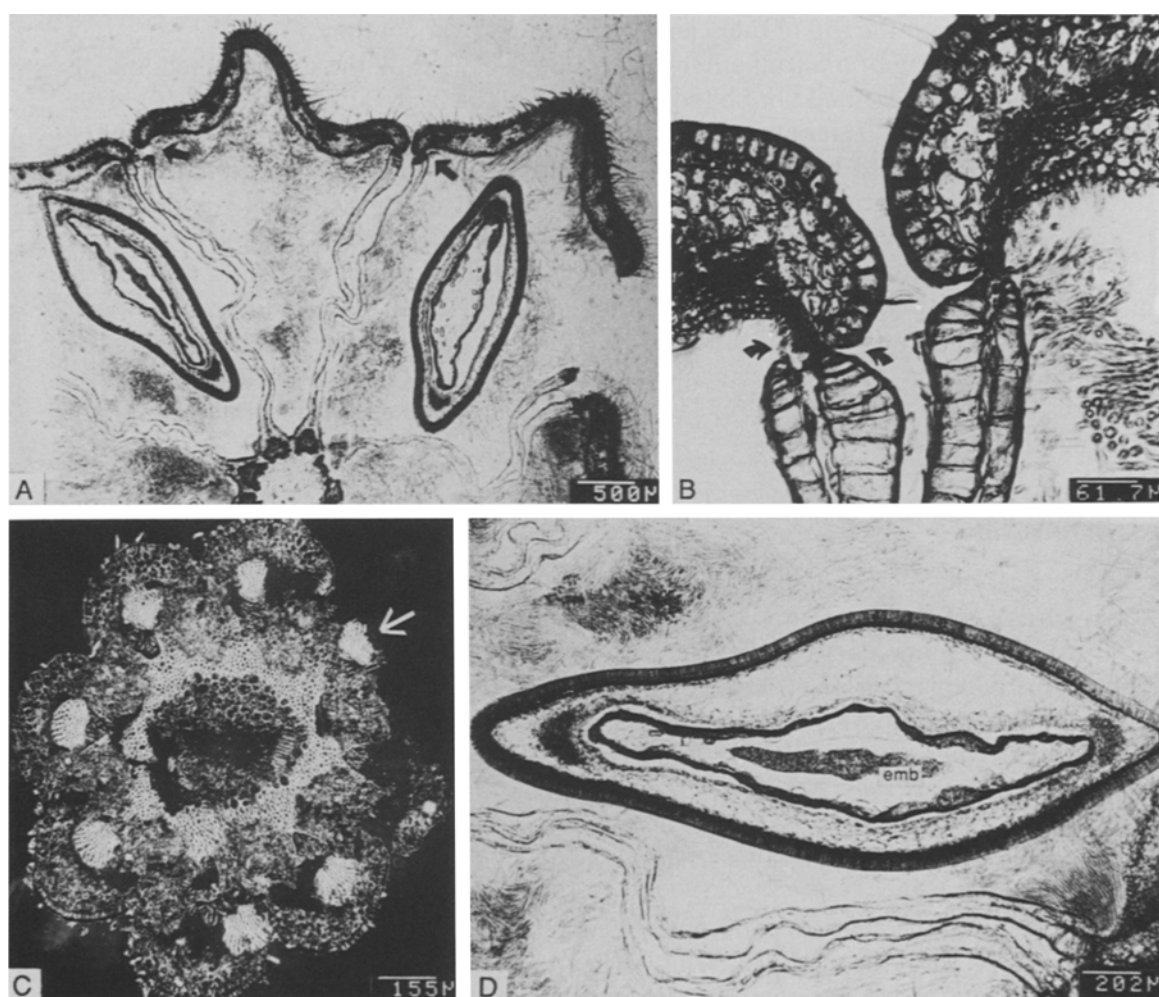


Fig. 4. *Dirachma socotrana*. Light microscopical photographs of the fruits. *A*, *B* Cross section with one empty and two seed containing locules, arrows point to rupture zones (enlarged in *B*) facilitating bursting of fruit. *C* Dark field, cross section above the level of the locules, arrow points to one of the carpellar, dorsal fibre bundles. In the middle central column more or less circular and also consisting of thick-walled cells. *D* Cross section of immature seed with flat embryo

tanniniferous and has clearly thickened outer periclinal walls with a cuticle. Between the epidermal cells thick-walled hairs are present, which are of the same type as those on the other floral parts. The endothecium has branched wall thickenings, mainly oriented periclinally. There are two to three middle layers, compressed in the mature anther. Locally, there are star-shaped crystals in the outer wall, of the same type as in other flower parts. The tapetum is binuclear and of the secretory type. The arrangement of the microspores is tetrahedral. The mature tricolporate pollen grains contain numerous starch grains, so that the number of nuclei is difficult to assess.

**Fruit**. The beaked, schizocarpic fruit superficially resembles that of *Geraniaceae*. However, it is different because after dehiscence the mericarps do not completely envelop the seed, which is embedded in unicellular hairs growing out from the inner sides of the valves (Figs. 3F, 4A). The fruit valves separate acropetally, but remain connected with the top of the sclerenchymatous stylar column (Fig. 4C).

The opening of the fruit, after drying out, is caused by the outward bending of longitudinally oriented fibre bundles situated in the fruit valves (Figs. 3B, 4C). The lifting of the firm fruit valves from the more fragile, non-fused and original double septae is facilitated by the presence of rupture zones. These longitudinal rupture zones at the junction of valves and septae consist of constrictions composed of smaller, tanniniferous and brittle cells (Fig. 4A, B).

The extension of the intralocular hairs into long unicellular trichomes starts after fertilization (Fig. 3C, D). Shortly before maturity these hairs adhere and form a membranaceous layer around the seed (Fig. 3F). When mature they are thick-walled, become separated and constitute a woolly mass. The whole fruit is densely covered with thick-walled, unicellular hairs interspersed with capitate, dermal glands (Fig. 3E).

## Discussion

The well-differentiated and flattened ovule of *Dirachma* has some special characters such as a very large nucellus, especially in somewhat later stages of development, and a median tegumentary bundle. The o.i. is probably partly of subdermal derivation in the median plane. In the transmedian plane its derivation is almost certainly dermal.

Fruit morphology and supposed diaspore release superficially resemble those of the *Geraniaceae*. Apparently, this has been the main reason for relating *Dirachma* to the *Geraniaceae*. Actual seed dispersal has never been observed, but LINK (1991) speculated that the valves may be dispersed as anemochorous, one-seeded diaspores. It is also possible that the small, flat seeds are separately dispersed anemochorously.

Many of the characters of ovule, seed and fruit are specialized, e.g., the simple seed coat and the complex fruit structure. The exotestal seed coat and the anatropous seed of *Dirachma* are very much different from those of the *Geraniaceae* s. str. (*Geranium, Erodium, Pelargonium, Monsonia, Sarcocaulon* and *Hypseocharis*) which are always characterized by a crystal-containing endotesta, stellate exotegmic cells and a campylotropous seed with a curved embryo (BOESEWINKEL 1979, 1988). A close relationship between *Geraniaceae* s. str. and *Dirachma* is therefore unlikely. The fruit shape of *Dirachma* must have arisen independently of that of the *Geraniaceae*.

Also, there is no support for a relationship of *Dirachma* with one of the other genera doubtfully placed in the *Geraniales* s. l., such as *Balbisia, Wendtia, Viviania, Rhynchotheca* and *Biebersteinia*. The flat ovule of *Dirachma* with its median vascular bundle differs from the ovules of these genera. Furthermore, *Balbisia, Wendtia* and *Viviania* have a more or less campylotropous ovule and seed and a curved embryo. In general, these genera have tannin-rich seed coats with little differentiation and lack a palisade exotesta (BOESEWINKEL 1997). Because the ovules and seeds of these genera are also different from those of the *Geraniaceae* s. str., the conclusion is supported that the family *Geraniaceae* is restricted to the genera *Geranium, Erodium, Pelargonium, Monsonia, Sarcocaulon*, and *Hypseocharis*, which are characterized by the same diagnostic seed characters. This conclusion is in agreement with the results of *rbcL* analysis in *Geraniales* (PRICE & PALMER 1993).

Ovule and seed characters are not decisive in indicating the true relationships of *Dirachmaceae*. An exotesta is of rather common occurrence in angiosperm seeds, and therefore not very useful in the assessment of relationships. Often suggested relationships of *Dirachma* are *Tiliales* (or *Malvales*), especially *Bombacaceae*, and *Greyiaceae* (see LINK 1991). These relationships are not supported by the seed anatomy. *Tiliales* (*Malvales*) and *Bombacaceae* are generally characterized by an exotegmic palisade (CORNER 1976). In *Greyia sutherlandii* HOOK. & HARV. the seed coat develops from both integuments and the outer epidermal layers of the integuments persist in the form of two fibre layers (DAHLGREN & WIJK 1988).

We could find only one other family, like *Dirachma*, characterized by laterally flattened seeds, with one median integumentary bundle and an exotestal palisade, viz. the *Rhamnaceae* (CORNER 1976). In cross section the developing seed of *Zizyphus mauritiana* LAMK. corresponds much with that of *Dirachma*. According to MEDAN (1985) a dry, dehiscing fruit is basic in *Rhamnaceae*, from which both drupes and more specialized explosive capsules have evolved. The similarities between seeds and fruits of *Dirachmaceae* and *Rhamnaceae* may be an indication of a relationship between these families.

Recent *rbcL* studies (B. BREMER, M. THULIN & M. CHASE, unpublished data) indicate *Rhamnaceae, Barbeyaceae, Elaeagnaceae* and *Urticales* as closer relatives of *Dirachma*. However, the ovule and seed characters confirm the classical opinion that *Barbeya* belongs in the *Urticales*. In particular the perforated and aerenchymatous testa without mechanical layers is very typical for the taxa of this order (TAKASO & TOBE 1990). The ovule of *Barbeya* is bitegmic, and not 'apparently unitegmic', as stated by CRONQUIST (1981). Also anatomical, morphological and palynological characters indicate an urticalean affinity of the *Barbeyaceae* (DICKISON & SWEITZER 1970).

*Elaeagnaceae* resemble *Dirachma* and *Rhamnaceae* in a thick-walled, sometimes palisade-like exotesta, and an aerenchymatous mesotesta. However, the seeds lack a tegumentary bundle.

In summary, the family *Dirachmaceae* is difficult to relate to another taxon on the basis of its ovule and seed structure, and it has a more or less isolated position. The *Rhamnaceae* may be one of its closer relatives.

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