

# Ovules and seeds of *Barbeya* with additional arguments for an urticalean affinity of the Barbeyaceae

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## SUMMARY

*Barbeya oleoides* has a bitegmic, crassinucellate and anatropous ovule. The inner and outer integuments are about five layers thick and not vascularized. The endosperm is initially nuclear, to become cellular later. The mature seed coat is unspecialized, remains parenchymatic and is locally compressed, except for the exotesta and the tanniferous endotegmen. The exotesta is perforated by distinct crateriform holes. The embryological and anatomical seed coat characters support an urticalean affinity of the Barbeyaceae.

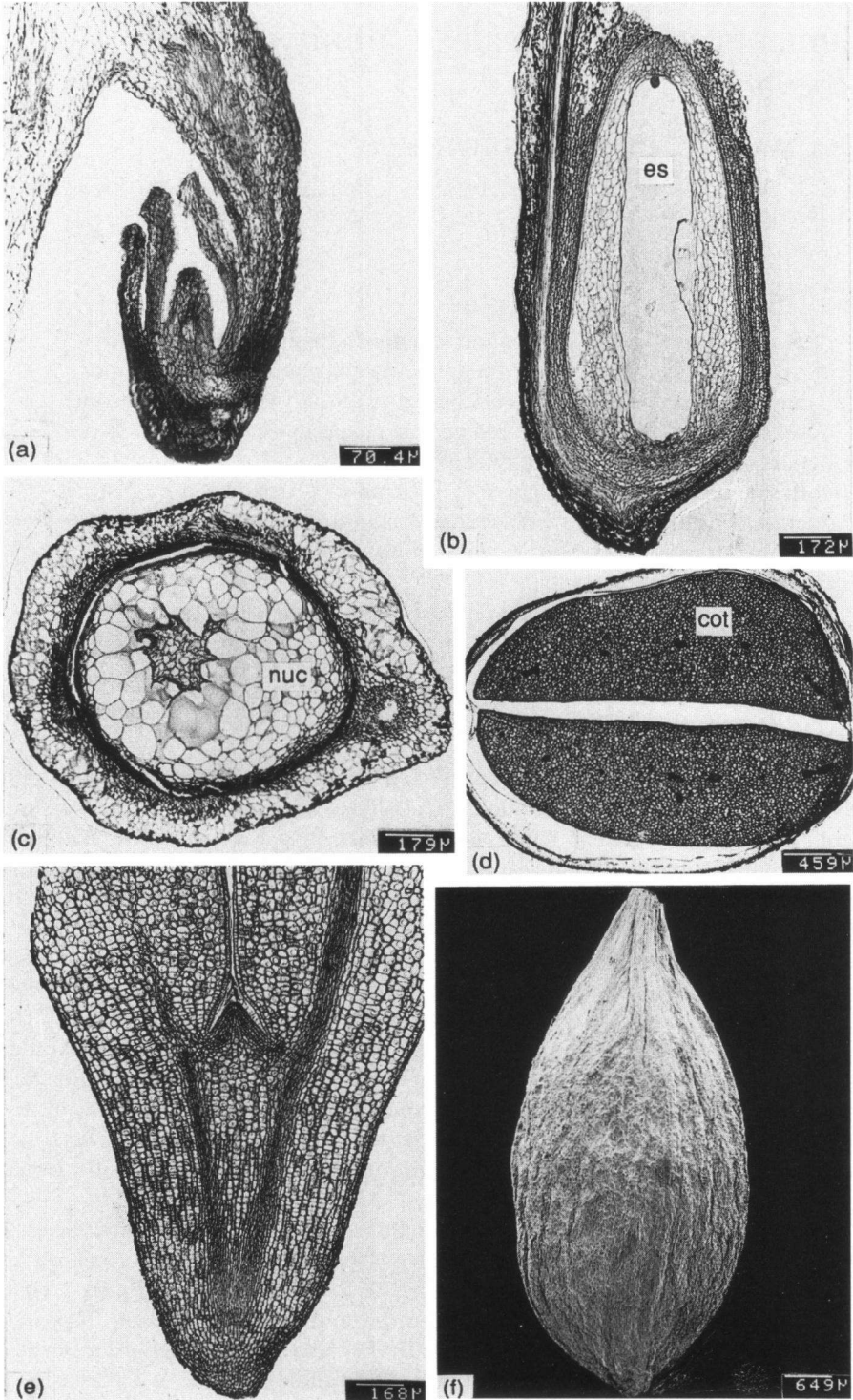
*Key-words:* Barbeyaceae, *Barbeya oleoides*, ovule, seed, taxonomic relationships, testa, Urticales.

## INTRODUCTION

In most current systems *Barbeya oleoides* Schweinfurt is classified in the monotypic family Barbeyaceae. *Barbeya* is a small, *Olea*-like, dioecious tree with anemophilous flowers. The dry, indehiscent fruits are surrounded by the persistent, membranous perianth segments and are wind-dispersed. *Barbeya* grows in dry forests and adjacent evergreen bushland of the slopes of the mountains facing the Red Sea and the Gulf of Aden and also occurs in similar habitats in southern Ethiopia (Friis 1993).

The status and affinities of the Barbeyaceae are still under discussion and several controversial opinions have been expressed. In his typification of 1891 Schweinfurt assigned *Barbeya* near the tribe *Ulmeae* of the Urticaceae *sensu* Bentham & Hooker. Engler (1897) raised its status and placed the genus in the subfamily Barbeyoideae of Ulmaceae. This was maintained in later versions of Engler's system (Engler & Diels 1936; Melchior 1964). The status of the taxon was raised to familial level by Rendle (1916), which opinion was adapted by most taxonomists. The affinities of *Barbeya* with the Urticales have been sustained by Hutchinson (1959), Takhtajan (1980), Cronquist (1981) and Dahlgren (1989).

Others have emphasized the differences of Barbeyaceae from the Ulmaceae, as from the order Urticales. *Barbeya* lacks stipules, cystoliths and laticiferous ducts, all characteristics generally present in the families of the Urticales, and it also deviates by having unilacunar nodes, a simple indumentum and tricolpate pollen. This induced Takhtajan (1969, 1980 and onwards) to class Barbeyaceae in a distinct order Barbeyales. On the basis of a study of trichome and pollen morphology, Tobe & Takahashi (1990) also favour the transfer of Barbeyaceae from Urticales into a separate order Barbeyales.



Thorne (1992) removed Barbeyaceae from the Urticales and placed it under 'taxa incertae sedis'. Willis (1985) suggested a distant affinity with Simmondsiaceae.

However, recent *rbcL* studies indicate Rhamnaceae (Swensen 1996), or Rhamnaceae and Dirachmaceae (Thulin *et al.* in press) as close relatives of *Barbeya*.

The structure of the ovule and seed of *Barbeya* is unknown (Davis 1966; Corner 1976), except for incomplete descriptions in Yakovlev (1981) and Takhtajan (1992). This study was undertaken to acquire lacking data and to obtain additional arguments for the assessment of the affinities of *Barbeya*.

## MATERIALS AND METHODS

The material available was poor and consisted of only a few dry developmental stages of flowers and fruits of *Barbeya oleoides*. The dry herbarium material was softened during an overnight stay in 10% ammonia. Sections were made by the standard microtome technique after embedding in glycol methacrylate. For scanning electron microscopy (SEM) untreated, dry specimens were gold- or gold/palladium sputter-coated for about 2.5 min and studied on an ISI DS 130.

Specimens examined: *Barbeya oleoides* Schweinfurth: Ethiopia, J.J.F.E. de Wilde 5038, 6413, 7199 (WAG); H.F. Mooney 9727 (WAG).

## RESULTS

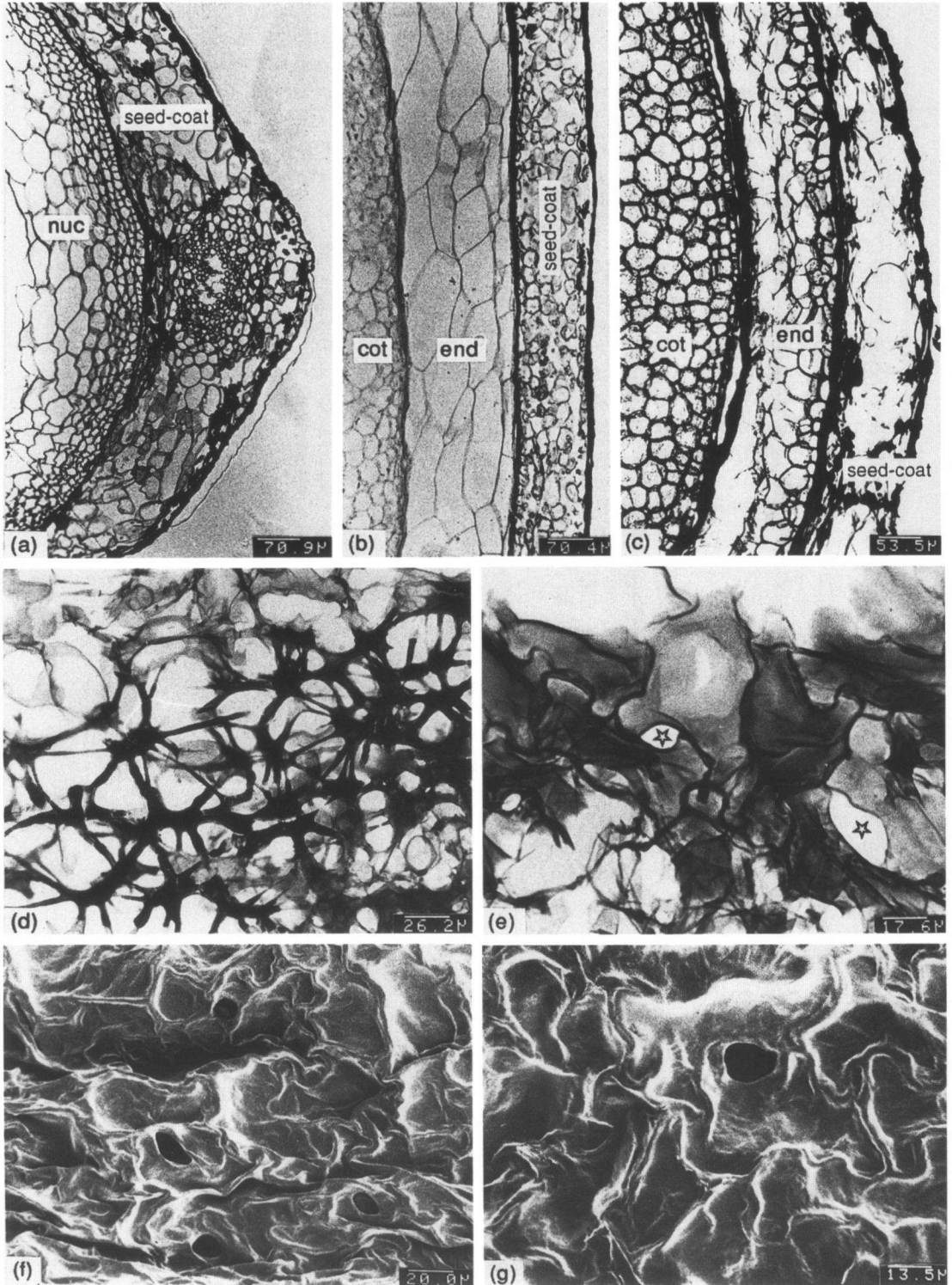
### *Ovule development*

The gynoecium of *Barbeya oleoides* consists mostly of only one carpel, with a single, epitropous ovule. The ovule is bitegmic, anatropous, crassinucellate and circular in cross section (Fig. 1a-c). The nucellus is large, with about five parietal layers and a nucellar cap of up to five cells thick. The embryo sac is initially deeply situated. The chalaza is relatively large and measures about one-third to half of the length of the young ovule. The inner integument is five- to six-layered, the outer integument has four to five layers. The micropyle is formed by the apex of the inner integument and has a long endostome. The raphe bundle is amphicribal, is surrounded by an interrupted sheath of tanniferous cells, and already has differentiated spiral xylem elements. The bundle fans out in the chalaza. There is no tegumentary bundle.

### *Seed development and mature seed*

The fruit is dry, indehiscent and has a thin pericarp. The mature seed is ellipsoid in shape, tapers towards the hilar-micropylar end, and measures  $7 \times 3 \times 3$  mm (Fig. 1f). The embryo is straight with two plano-convex cotyledons and a short radicle and hypocotyl (Fig. 1d,e). The orientation of the cotyledons is irregular and does not conform to the medium plane of the seed. The embryo fills most of the seed. The endosperm is initially nuclear (Fig. 1b), to become cellular later. Its cells are large and thin-walled. In the mature seed about two to four cell layers remain. The walls of the

**Fig. 1.** *Barbeya oleoides*, light microscopical photographs of sections of ovule, developing and mature seeds (a-e). (a): Longitudinal section of a young ovule. (b): Longitudinal section of a developing seed with globular embryo and endosperm in the nuclear stage. (c and d): Cross-section of a developing and mature seed with thick, plano-convex cotyledons. (e): Length section of a mature embryo. (f): SEM photograph of a seed. Abbreviations: cot=cotyledon; es=embryo sac; nuc=nucellus.



endosperm cells locally show slight reticulate thickenings. Embryo and endosperm contain aleuron grains. In the mature seed, the nucellus has become partly resorbed, and is present as a thin layer of compressed cell walls with a distinct cuticle.

The seed coat is relatively unspecialized and not multiplicative. Shortly after fertilization, the borderline between testa and tegmen fades and both integuments constitute a 10–12-layered seed coat (Fig. 2a–c). The cells of the outer layer of the testa and of the inner layer of the tegmen are both puzzle-piece-shaped. In surface view the exotesta is perforated and shows distinct crateriform holes between the anticlinal walls (Fig. 2e–g). The developing seed coat is tanniferous. The tannin is especially present in the outer and adjacent testal layer and in the endotegmen. Except for the endotegmen, of which the inner periclinal and radial walls become slightly thickened, all layers remain thin-walled and parenchymatous with intercellulars. The hypodermal layer of the testa resembles an actinenchyma (Fig. 2d). Locally, the parenchyma of the seed coat becomes compressed and partly resorbed during the maturation of the seed.

The chalaza stays behind during development and occupies about one sixth of the length of the seed. The outer layers of the chalaza are aerenchymatous. The chalazal plexus is separated from the embryo by a tanniferous hypostase, which is continuous with the tanniferous endotegmen. The raphal bundle is large, amphicribal, has developed a distinct rhexigenous cavity and differentiated ring and spiral elements (Figs 1c and 2a).

#### *The fruit wall*

The pericarp of *Barbeya* consists of a one-layered outer and inner epidermis and about 10 middle layers. The middle layers are parenchymatous with intercellulars, and become compressed in the later phases of seed maturation. The hypodermal layer facing the endocarp differs from the other parenchymatic layers by having smaller and more square and more tanniferous cells. The inner hypodermal cells do not contain crystals as in some other taxa of the Urticales. The cells of the epicarp remain small and have thickened outer periclinal walls with a distinct cuticle. The endocarp forms the main mechanical layer. The cells enlarge, elongate radially and develop strongly thickened, layered and pitted walls. The cells of the mature endocarp are palisade-like on length-section and cross-section of the fruit, but appear stellate at tangential sections. Their anticlinal walls are stronger undulated at both ends of the palisade and more straight at the middle. All layers of the fruit wall may contain tannins. Longitudinal sclerenchyma fibres are present near smaller and around larger vascular bundles. The dorsal bundle is amphicribal.

## DISCUSSION

Recent *rbcL* studies (Thulin *et al.* in press) have questioned the close relationship between *Barbeya oleoides* (Barbeyaceae) and Urticales and indicate Rhamnaceae and Dirachmaceae as the closest relatives of Barbeyaceae. In a study on ovule and seed structure, Boesewinkel & Bouman (1997) provided additional arguments which sustain

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**Fig. 2.** *Barbeya oleoides*, light microscopical photographs of sections of seeds (a–e). (a): Cross-section of a seed shortly after fertilization, showing nucellar tissue and amphicribal raphe bundle. (b and c): Longitudinal section of a developing seed and cross-section of a mature seed, respectively. (d and e): Sagittal sections of the subdermal and epidermal layer of the seed coat showing perforations. SEM photographs of the seed surface (f and g). Abbreviations: cot=cotyledon; end=endosperm; nuc=nucellus; \*=perforation.

the relation between Dirachmaceae and Rhamnaceae. Dirachmaceae and Rhamnaceae are both characterized by laterally flattened seeds, with one median tegumentary bundle and an exotestal palisade. However, as shown in this paper, the Barbeyaceae clearly deviate by having, for example, indehiscent fruits, an unspecialized seed coat, a non-differentiated exotesta and the lack of an antiraphal bundle.

Ovule and seed characters of *Barbeya oleoides* match those of Urticales and support the classical opinion that the family Barbeyaceae belongs to, or at least is closely related to, the Urticales. The ovule of *Barbeya* is bitegmic, and not unitegmic (Willis 1985; Mabberley 1987; Takhtajan 1992), nor 'apparently unitegmic' as stated by Cronquist (1981) and Watson & Dallwitz (1996). As is the case in the Barbeyaceae, all families of the Urticales have unspecialized seed coats of which either testa or tegmen, or both, may become crushed during development (Chernik 1982). In particular, the presence of perforations in the exotesta is very typical for taxa of this order and occurs in genera of Celtidaceae, Ulmaceae, Cannabaceae, Moraceae and Urticaceae (Takaso & Tobe 1990). The origin of the perforations in *Barbeya* agrees with that described for taxa of undisputed urticalean affinity.

Perforated exotestas are uncommon among the flowering plants. Single perforations may resemble stomata. Stomata are known on the seed coats of a number of families (Boesewinkel & Bouman 1984), but always show the normal differentiation with guard cells. Perforations of 'normal' testal cells are less known. Intercellular-like splitting at the corners of anticlinal walls are known in Taccaceae (Bouman 1995). Exotestal perforations by splitting along the anticlinal walls seems to be of restricted occurrence in angiosperms and have only been recorded from Urticales until now. Comparable splitting of the endotestal layer has been described in *Carpolobia* (Polygalaceae) (Verkerke 1985).

The fruit wall anatomy of *Barbeya* conforms to the type generally described in Urticales. As far as known (Prakash, Bohm & Maze 1979; Takhtajan 1992), the fruit wall of Cannabaceae, Moraceae, Urticaceae and Ulmaceae all have a one-layered endocarp as main mechanical layer. Fossil fruits of urticalean taxa are mainly known by their endocarp remains (Collinson 1989).

Also, anatomical, palynological (Dickison & Sweitzer 1970) and phytochemical characters (Friis 1993) indicate an urticalean affinity of the Barbeyaceae.

A relation between Barbeyaceae and Simmondsiaceae as suggested by Willis (1985) seems highly unlikely and is not supported by ovule and seed characters.

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