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The pachychalazal seed of *Protorhus longifolia* (Bernh.) Engl. (Anacardiaceae) and its taxonomic significance

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Abstract. The ovule of *Protorhus longifolia* (Bernh.) Engl. is anatropous, pachychalazal, bitegmic, crassinucellar, and has a nuclear endosperm development. It is further characterized by a multilayered hypostase *s.l.* and an extension of the inner epidermis of the inner integument, (the future endotegmen), up to the periphery of the hypostase. The chalaza comprises a larger, vascularized portion towards the basis and raphal side of the ovule, associated with the hypostase, and a smaller nonvascularised portion at the antiraphal side which merges into the integuments. In the mature, exalbuminous seed the contribution of the integuments to the development of the seed-coat is negligible. The mature seed-coat is pachychalazal and consists of a large, saddle-shaped part (derived from the vascularized chalaza) which is surrounded on the sides and the antiraphe side by the smaller nonvascularized part. Walls of the hypostase cells are impregnated with lipids (probably cutin) and those of the endotegmen are lignified and abundantly pitted. The dark green cotyledons store mainly starch as nutrient reserves. Seed structure of *Protorhus longifolia* is compared with that of the pachychalazal seed of *Mangifera indica* L. (tribe Anacardieae), and the partially pachychalazal seed of four species of the tribe Spondiadeae and *Rhus lancea* L.f. (tribe Rhoeae). In contrast to *Protorhus longifolia*, the seed-coat of all these species is distinctly of dual origin, namely partly chalazal and partly integumentary. Seed morphology suggests that the Madagascan species currently treated under *Protorhus* Engl. may belong to a different genus.

Key words: Anacardiaceae; Endotegmen; Hypostase; Ovule; Pachychalaza; *Protorhus*; Rhoeae; Seed; Seed-coat; *Semecarpus*.

Introduction

The genus *Protorhus* Engl. (Anacardiaceae; tribe Rhoeae) is centred in the Malagasy Republic (henceforth referred to as Madagascar) with about 15 species (Perrier de la Bâthie, 1946), and two species in southern Africa. It is, however, doubtful whether the African members are congeneric with the Madagascan species. A detailed morphological study of the southern African tree, *Protorhus longifolia* (Bernh.) Engl., has been initiated as part of a comprehensive project on the comparative fruit and seed structure of the Anacardiaceae. Taxonomically this species is of special interest since it serves as the lectotype of the genus *Protorhus* (Farr *et al.*, 1979).

The considerable taxonomic importance of seed morphology is now well established (e.g. Corner, 1976; Netolitzky, 1926; Wunderlich, 1967). Corner (1976) convincingly showed that fruit and seed morphology is of major importance in the classification of many families of flowering plants. This also appears to be the case in the Anacardiaceae, although information on the fruit structure of this family is still scanty, very little is known about the seed-coat (Von Teichman, 1988a, b, 1990; Von Teichman and Van Wyk, 1988; Wannan and

Quinn, 1990).

In the Anacardiaceae the endocarp constitutes the main mechanical layer for protection of the embryo, and hence usually no such layers develop within the seed-coat. According to Corner (1976), the anacardiaceous seed-coat shows either little or no specialization, or only an *endotegmen* or traces of an *exotestal* state. Subsequently the seed-coat structure of a few more species was investigated. In the tribe Anacardieae, an undifferentiated seed-coat typifies the pachychalazal seed of *Mangifera indica* L. (Von Teichman *et al.*, 1988). Seed-coats of the partially pachychalazal seeds of four members of the tribe Spondiadeae are slightly more differentiated, with varying degrees of traces of an exo-, meso- and/or *endotestal* lignification (Von Teichman, 1988a, b, 1990; Von Teichman and Van Wyk, 1988). In the tribe Rhoeae, notably in *Rhus* L., *Schinus* L. and probably also *Pistacia* L., a small partial pachychalaza and a lignified *endotegmen* characterizes the seed (unpublished observations). The variation in the degree of development of the pachychalaza in all taxa hitherto studied, is of considerable taxonomic significance. In all cases the pachychalaza is associated with a remarkably similar hypostase *s.l.* (Von Teichman, 1988a), a feature considered typical of many anacardiaceous seeds (unpublished observations).

Boesewinkel and Bouman (1984), amongst others, stressed that a detailed knowledge of ovule morphology and the subsequent ontogeny of the seed-coat is a prerequisite for a precise interpretation of the structure of the mature seed-coat. Since published information on ovule and seed-coat structure is non-existing for *Protorhus longifolia*, it is the aim of the present paper to contribute towards our knowledge of these aspects. An attempt is made to compare aspects of the seed morphology of *Protorhus longifolia* with those of other Anacardiaceae, notably the Madagascan species of *Protorhus*.

Materials and Methods

Plant material used, details of voucher specimens and the preparation and staining of the semithin glycolmethacrylate sections correspond with those described for the pericarp of *Protorhus longifolia* (Von Teichman, 1991).

Histochemical staining for proteins was done with amido black 10B according to Bullock *et al.* (1980). In addition to the PAS (periodic acid-Schiff reaction), starch was localized with potassium iodide-iodine (Jensen, 1962) or observed with crossed polarizing filters. For the detection of callose, semithin sections were mounted in 0.1% w/v water-soluble aniline blue in 0.1 M K_3PO_4 buffer at pH 12.4, and examined with a Nikon Optiphot light microscope equipped with epifluorescence optics (450-490 nm B-2A excitation filter and 520 nm barrier filter). Fats were localized on handcut sections with sudan III and sudan IV (O'Brien and McCully, 1981).

Results

External Morphology of the Mature Seed

Removal of the relatively tough pericarp from the asymmetrically ovoid fruit reveals a single ovoid seed (Fig. 1A). A band-like funicle lies appressed to the seed-coat within a shallow groove. The pachychalazal seed is laterally slightly compressed and characterized by a striking, red-brown vascular pattern on a dark brown background in the large saddle-shaped part of the seed-coat. The latter represents the tanniferous part of the seed-coat containing the network of vascular bundles and the hypostase *s.l.* (vide Von Teichman, 1988a). Surrounding the saddle-shaped part is a narrow peripheral band of chalazal seed-coat which lacks vascular elements.

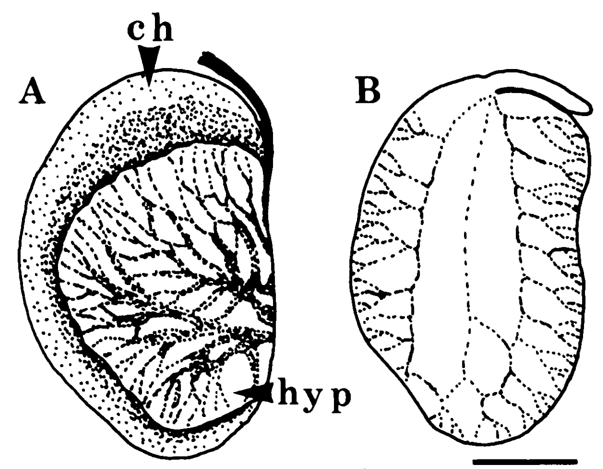

Fig. 1. Mature seed (A) and embryo (B) of *P. longifolia* in lateral view, showing their size, shape and vascularization. ch: brown, peripheral portion of the chalazal seed-coat which lacks vascular bundles and a hypostase; hyp: dark brown, saddle-shaped portion of the chalazal seed-coat with vascular tissue and hypostase. Scale bar = 3 mm.

Below the thin, membraneous seed-coat lies the embryo. It consists of a radicle, minute plumule and two thick, planoconvex cotyledons with conspicuous venation (Fig. 1B).

Ontogeny and Anatomy of the Seed

The unilocular ovary contains a single anatropous, crassinucellar, bitegmic and pachychalazal ovule. The ovule is suspended apically from the ovary wall on the funicle. Dissections and examination of serial sections of the ovule at anthesis (Fig. 2) reveal the following salient features:

(i) well-developed raphe with an amphicribral vascular bundle and secretory ducts adjoining the peripheral phloem;
(ii) tanniferous outer epidermis covered by a thin cuticle;
(iii) well-developed outer and inner integuments of which only the basal portion surrounds the nucellus;
(iv) the chalaza is unusual in that it is considerably extended towards the micropyle, thereby replacing the integuments as the principal container for the now slightly curved nucellus. The nucellar epidermis which is distinguished from the rest of the nucellus by the embryonic appearance of its cells, is covered by a cuticle;
(v) the embryo sac seems laterally displaced by a conspicuous, multilayered hypostase *s.l.* which forms a cup-like cell plate. Embryo sacs of flowers from the Pretoria locality showed a pronounced measure of degeneration, i.e. only four out of ten were normal. The reason for this probably is that Pretoria lies outside the natural geographical range of *P. longifolia*; and
(vi) an unusual feature is the extension of the inner epidermis of the inner integument (the future endotegmen) right up to the periphery of the hypostase towards the base of the ovule. The cells of this epidermis are meristematically active and engaged in anticlinal cell divisions. It is covered by a cuticle which abuts on the cuticle of the nucellar epidermis.

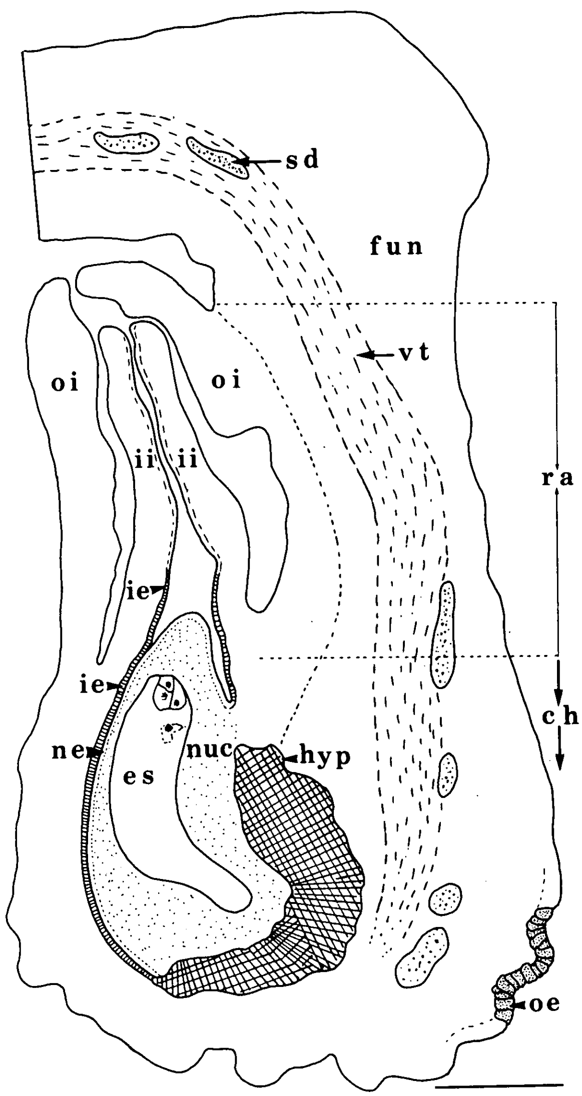

Fig. 2. Longitudinal section of the ovule at anthesis, illustrating its structure. ch: chalaza; es: embrio sac; fun: funicle; hyp: hypostase *s.l.*; ie: inner epidermis of inner integument and chalaza = future endotegmen; ii: inner integument; ne: nucellar epidermis; nuc: nucellus; oe: outer epidermis of the ovule; oi: outer integument; ra: raphe; sd: secretory ducts; vt: vascular tissue. Scale bar = 0.25 mm.

About three weeks after anthesis the very young seed displays a pronounced lateral intercalary meristem at the antiraphal side of the chalaza (Figs. 3 and 4). This meristem comprises the extension of the inner epidermis of the inner integument, and the adjacent chalazal cell layer. Through this meristematic activity the chalaza gradually expands peripherally and eventually virtually surrounds the large, saddle-like part of the chalaza which contains the hypostase and vascular network (Fig. 1A). Figure 5 is a transverse section of that part of the young pachychalazal seed-coat which contains the tanniferous hypostase. To the outside of the hypostase lies the chalazal parenchyma with vascu-

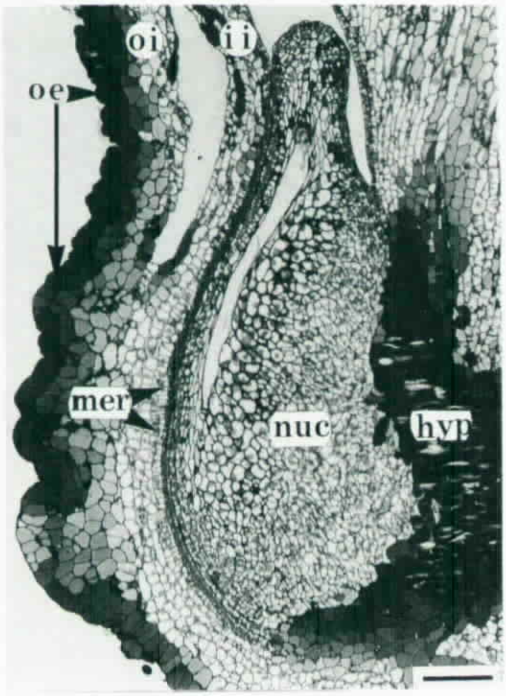

Fig. 3. Longitudinal section of a part of the very young seed showing a degenerated embryo sac within the nucellus. nuc: nucellus; hyp: hypostase; ii: inner integument; mer: the meristematic layer in the chalaza; oe: outer epidermis; oi: the outer integument. Scale bar = 100 μm.

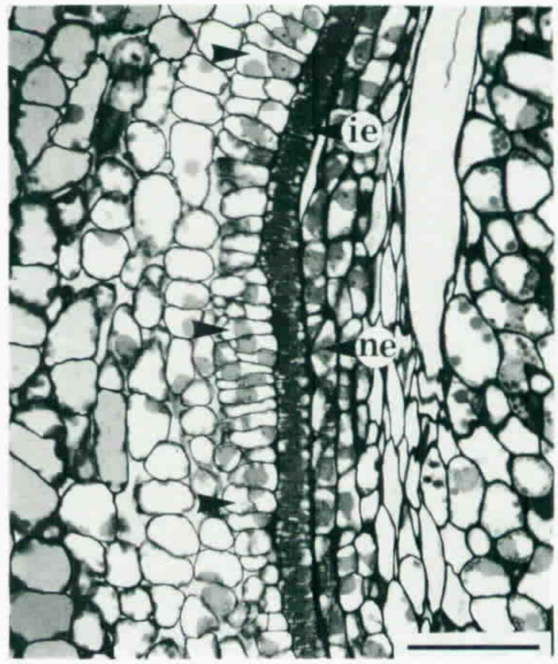

Fig. 4. Higher magnification of a small part of Figure 3 showing the meristematic layer in the chalaza (arrow heads). ie: adjacent meristematic inner epidermis (= future endotegmen); ne: embryonic nucellar epidermis. Note the thin, unstained cuticular layer between the cells of these two epidermal layers. Scale bar = 50 μm.

lar bundles and secretory ducts, a zone of hypodermal parenchyma and outer epidermis. The nucellar and endosperm tissues lie to the inside of the hypostase.

About eight weeks after anthesis (Fig. 6) differentiation of the endotegmen at the antiraphal side commences. Abundantly pitted lignified secondary cell walls now characterize parts of this cell layer. Towards the periphery of the hypostase, where the endotegmen differentiates a little later, the nucellar epidermis remains distinct for somewhat longer (Fig. 6).

In the mature seed-coat, about three months after anthesis, the entire endotegmen is fully differentiated, whereas the adjacent nucellar epidermis is more or less squashed, as are the remnants of the inner nucellus and endosperm. In the mature seed, furthermore, the chalazal seed-coat at the antiraphal side displays a very distinct cuticular layer (cuticles of the nucellar epidermis and of the endotegmen) as well as cutinization of the outer anticlinal cell walls of the now squashed nucellar epidermis cells. While this cuticular layer lies on the inside of the endotegmen, one notices on the outside only a few layers of more or less squashed, partly tanniferous hypodermal parenchyma cells and an outer, tanniferous, epidermis with a thin cuticle.

At maturity, the larger part of the chalazal seed-coat, i.e. that which contains the hypostase (Fig. 1A), consists of the following tissues:

(i) outer epidermis (as described above);
(ii) hypodermal, partly tanniferous parenchyma cells containing an extensive network of vascular bundles and more or less squashed secretory ducts. The xylem consists mostly of tracheoids (*sensu* Schmid, 1986) with helical secondary cell wall thickenings.
(iii) hypostase comprising thin-walled, tanniferous parenchyma cells which are contiguous and fit into each other like pieces of a jigsaw-puzzle, although their walls now exhibit a distinct impregnation with lipids; and
(iv) squashed remnants of the nucellus and endosperm.

The cuticles of the nucellar epidermis and endotegmen merge into the periphery of the hypostase. It is

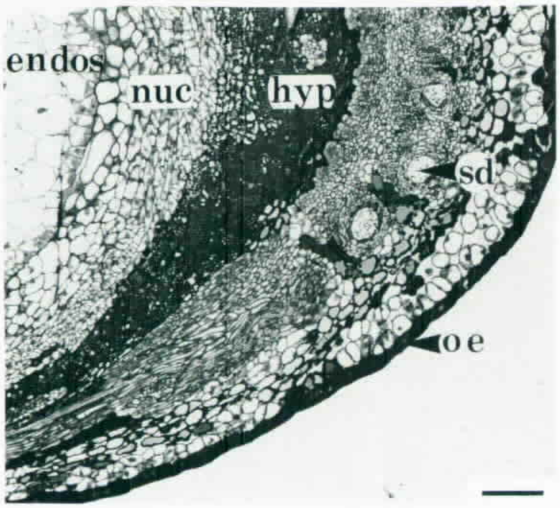

Fig. 5. Transverse section of the chalazal seed-coat of a young seed. hyp: hypostase; nuc: nucellus; endos: cellular endosperm; sd: partially tanniferous parenchyma with vascular bundles and secretory ducts; oe: hypodermal parenchyma and tanniferous outer epidermis. Scale bar = 100 μm.

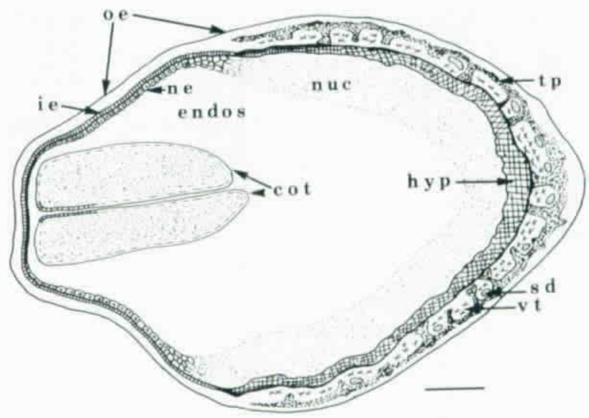

Fig. 6. Line drawing of a transverse section of the seed-coat eight weeks after anthesis. endos: endosperm; cot: two cotyledons; hyp: hypostase; ie: inner epidermis (endotegmen); ne: nucellar epidermis; nuc: nucellus; oe: outer epidermis; sd: secretory ducts; vt: vascular tissue. The antiraphal side is the part nearest to the young cotyledons. Scale bar = 0.5 mm.

therefore suspected that the lipids in the hypostase cell walls are probably also cutin. The entire seed-coat therefore has a lipidic (cutinic) layer serving as effective protection against dehydration in the mature, physiologically ripe seed. Crystals of calcium oxalate which occur abundantly in the pericarp, were not observed in the seed-coat.

Sections of the funicular/raphal part of immature seeds show that the two integuments form only a very small part of the seed-coat. In the mature seed, the contribution of the integuments to the development of the seed-coat is negligible.

In the mature seed the cotyledons are dark green and consist mostly of storage parenchyma packed with starch grains. Negligible amounts of lipids and proteins are also present. The vascular bundles, which are clearly visible externally (Fig. 1B), are associated with secretory ducts.

Discussion and Conclusions

A comparison of the pachychalazal ovule of *Protorhus longifolia* with the basal pachychalazal ovule of *Mangifera indica* (Von Teichman *et al*., 1988) reveals that the latter is significantly different by being unitegmic, with a rudimentary integument. However, in *Mangifera indica* the integument forms the peripheral, integumentary part of the pachychalazal seed-coat, which is undifferentiated. The similarities between the seed-coat of *Mangifera indica* and *Anacardium occidentale* L. (both tribe Anacardieae) as well as the presence of an extensive chalaza in other members of this tribe, have already been pointed out (Von Teichman *et al*., 1988).

All the hitherto investigated species of the tribe Spondiadeae are characterized by a pendulous, bitegmic ovule. These ovules differ from that of *Protorhus longifolia* in having:

a much shorter inner integument;

a knee-like funicular protuberance;

a hypostase *sensu stricto* (Von Teichman, 1988a).

In the Spondiadeae, the two integuments develop into the more pronounced *integumentary* part of the partially pachychalazal seed-coat. However, this integumentary seed-coat lacks a mechanical layer (Von Teichman, 1988a, b, 1990; Von Teichman and Van Wyk, 1988).

The basal, bitegmic ovule of *Rhus lancea* L.f. (tribe Rhoeae) shows the same differences from the ovule of *Protorhus longifolia* as those mentioned above. However, in the partially pachychalazal seed of *Rhus lancea*, as well as other closely related members of the Rhoeae, the inner integument develops into an *endotegmen* (unpublished observations), rather similar to that found in *Protorhus longifolia*, assigned to the same tribe.

In all species of the Anacardiaceae investigated in detail, the ovules and seeds show the following similarities:

(i) ovules are anatropous, crassinucellate, with nuclear endosperm development;
(ii) seeds being exalbuminous;
(iii) raphe bundle amphicribral, the xylem consisting mostly of tracheoids with helical secondary wall thickenings;
(vi) hypostase, as described for *Protorhus longifolia*, always associated with an extensive chalaza in all its gradations.

Typical for many anacardiaceous seed-coats, except *Mangifera indica*, is the distinct cuticular layer on the inside of the inner epidermis of the inner integument, or its continuation as in *Protorhus longifolia*. This cuticular layer always merges into the periphery of the hypostase.

Among the hitherto investigated Anacardiaceae, *Protorhus longifolia* is unique in that practically the entire seed-coat is of chalazal origin. In *Mangifera indica* and in members of the Spondiadeae and Rhoeae, the seed-coat is distinctly of dual origin, namely partly chalazal and partly integumentary. The combination of a complete pachychalaza and an endotegmen, as in *Protorhus longifolia*, seems unusual. Although detailed information on the seeds of only a very small fraction of the about 60-80 genera of the Anacardiaceae is currently available, the diversity and potential taxonomic value of its seed-coat structure is obvious.

An undifferentiated seed-coat, the presence of starch in the nutrient reserves, and relatively large (overgrown) mature seed (e.g. *Mangifera indica, Anacardium occidentale, Tapirira* Aubl. and *Protorhus longifolia*), seem to be associated with plants from a tropical habitat. On the other hand, an endotegmen, presence of mainly lipids and proteins as nutrient reserves, and relatively smaller seeds (e.g. members of Spondiadeae and *Rhus* spp.), occur more often in species favouring an extra-tropical habitat. This suggests that *Protorhus longifolia* may represent a palaeoendemic and tropical relict. White (1983) lists *Protorhus longifolia* as one of the endemic species of the Tongaland-Pondoland Regional Mosaic, although its range also extends into the afromontane forests of Swaziland and the eastern and northeastern Transvaal.

From the scanty literature it is clear that the fruit shape of *Protorhus longifolia*, as described previously (Von Teichman, 1991), as well as its seed and embryo structure, is markedly different from that of the Madagascan species of *Protorhus*. Seeds of the latter members have been described as ovate or ovate-lanceolate, with the cotyledons auriculate at the base, more or less resinous and moulded into a single mass ruminated by numerous large inclusions of gum-like resin (Perrier de la Bâthie, 1946). Strongly ruminate and resinous cotyledons were also found in a species of *Semecarpus* L.f. of the tribe Semecarpeae (unpublished observations). Although the endocarp structure of *Protorhus longifolia* (Von Teichman, 1991) complies to the so-called *Anacardieae*-type described by Wannan and Quinn (1990), the pericarp as such suggests an affinity of *Protorhus* with the genus *Semecarpus*.

The present study therefore not only contributes towards the knowledge of *Protorhus longifolia*, but it may also be useful to assess the circumscription, taxonomic position as well as phylogenetic affinities of the genus *Protorhus*. At this stage it may be speculated that *Protorhus longifolia* is not only a relictual member of the Rhoeae in Africa, (considering the endotegmen), but also a taxon showing affinities with members of the tribes Anacardieae (pachychalazal seed) as well as Semecarpeae (pericarp structure). There are also indications that the Madagascan species currently treated under *Protorhus* may belong to a different genus. More detailed information on the seed structure of the latter taxa is desirable before any final conclusions can be made.

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漆樹科 *Protorhus longifolia* (Bernh.) Engl. 的厚合點型種子及其分類上的意義

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Protorhus longifolia (Bernh.) Engl. 的胚珠是倒生的，具厚合點的 (pachychalazal)，具雙珠被的，具厚珠心的 (crassinucellate)，而胚乳的發育是無壁式的。除這些基本性狀外，它還具有一若干層細胞厚的承珠盤 (hypostase) 和一其內表皮延伸至承珠盤邊緣的內珠被。胚珠的合點 (chalaza) 包括兩部份，較大的部份具維管束，包含胚珠的基部及種脊這一側，與承珠盤相接；較小的部份不具維管束，位於種脊的對側。成熟種子中不含胚乳。種皮主要是由合點衍生而來，由珠被發育而來的部份極少。成熟的種皮兩側被一大而且呈鞍狀的構造所包覆，此構造係由合點中具維管束的部位發育出；另外種脊的對側較小且無維管束組織。承珠盤的細胞胞壁充滿了脂肪（或爲角皮質 (cutin)）。內種皮的細胞胞壁則木質化且具多數的穿孔。子葉暗綠色，以澱粉爲主要貯藏物。文中亦將 *Protorhus longifolia* 的種子與 *Mangifera indica* L. (屬 Anacardieae 族)的厚合點型種子，與四個 Spondiadeae 族的種及 *Rhus lancea* L.f. (屬 Rhoeae 族) 的部份厚合點型種子進行比較，顯示這些種均與 *Protorhus longifolia* 不同，它們的種皮係部份由合點，部份由珠被發育而成。此外，種子形態的資料顯示馬達加斯加島上的 *Protorhus* 種可能應置於另一個屬中。