Taxonomic significance of pericarp and seed structure in *Heeria argentea* (Thunb.) Meisn. (Anacardiaceae), including reference to pachychalaz and recalcitrance

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*Heeria argentea* (tribe Rhoeae), a monotypic, dioecious tree, is endemic to the core area of the Cape Floristic Region. The mature exocarp consists of a uniseriate layer of palisade-like epidermal cells, interspersed with modified stomata. The mature endocarp *sensu stricto* develops solely from the inner epidermis. It is essentially two-layered and resembles the state in *Protorhus longifolius*. This endocarp is here proposed as a distinct fourth endocarpal subtype under the so-called *Anacardium*-type. The large, pachychalazal, recalcitrant seed develops from the single, anatropous, bitegmic, crassinucellate ovule. This ovule is characterized by an extensive chalaza, vascularization and Anacardiaceae-type hypostase. The pachychalazal seed coat contains abundant vascular bundles and a tanniniferous hypostase. The inner epidermis of the inner integument differentiates into an endotegmen. The contribution of the integuments towards seed coat development is negligible. Concerning characters of the disc in the female flower, the meso- and endocarp, as well as seed size, degree of pachychalaz, nutrient reserves (starch) in the chlorophyllous cotyledons and hypogeous germination, *Heeria* shows a very close phylogenetic relationship to *Protorhus longifolius*. However, fruit and seed structure clearly supports the taxonomic separation of *Heeria* from *Ozoroa*. Data also support the view that *Heeria* is a tropical relict, and the hypothesis that pachychalaz, greater seed size, as well as recalcitrant seed viability behaviour constitute ancestral seed character states. Pachychalaz is regarded as a functional adaptation for more efficient transfer of nutrients.

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ADDITIONAL KEY WORDS: — *Baronia* — drupe — flower — hypostase — ovule — *Ozoroa* — *Protorhus* — seed-coat — seedling.

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Engler (1892) included *Ozoroa* Delile in *Heeria* Meisn. and classified the combined genus in the tribe Rhoeae (= Rhoideae). Fernandes & Fernandes (1965), emphasizing flower, fruit, embryo and pollen differences, reinstated the genus *Ozoroa* for all except one species formerly placed in *Heeria*, namely *H. argentea* (Thunb.) Meisn. *Heeria* thus became monotypic. This distinction between *Ozoroa* and *Heeria* has subsequently been accepted by most workers.

*Heeria argentea* is endemic to the core area of the Cape Floristic Region. It is a small, sturdy, evergreen tree confined to the southwestern Cape, from Gordon’s Bay and Elgin in the south, northwards to Clanwilliam, Wupperthal and Worcester. Plants are locally common on mountain slopes, usually associated with rock outcrops of sandstone in fynbos vegetation. Vernacular names include Rockwood, Rock Ash, Wild Apricot, ‘Kliphout’ and ‘Klipesse’. In the past bark and leaves were used for tanning hides and skin. Gum exudates from the bark served as an ointment to draw boils and abscesses. The hard, resinous, fine-grained wood was used in general carpentry, while in earlier days it was also employed by turners and makers of musical instruments (Palmer & Pitman, 1972).

*Heeria argentea* leaves are discolorous (Fig. 1), with fine silvery white hairs below, hence the specific epithet. Principal lateral veins are conspicuously parallel and prominently raised below. This venation resembles that in *Ozoroa* as well as species of *Baronia* Baker. The dark yellow-green drupes of *H. argentea* are the size of small walnuts and differ conspicuously from the relatively small, black, raisin-like, wrinkled fruit of *Ozoroa*. Seeds are dispersed, amongst others, by rock rabbits (Marloth, 1925).

Vegetatively *Heeria* resembles not only members of *Ozoroa* but also of *Protorhus* Engl., which is phytogeographically centred in Madagascar. Previously only two species of *Protorhus* were recognized in Africa: *P. longifolia* (Bernh.) Engl., the lectotype of the genus, largely confined to the forests of the Tongaland-Pondoland Regional Mosaic (White, 1983a), and *P. namaquensis* Sprague with a remarkably disjunct distribution from *P. longifolia*, being endemic to the harsh, semi-desert lower Orange River Valley on the Namibian/South African border. Both these species have recently been studied in detail (Von Teichman, 1991a, b; 1994). The morphological differences, especially those concerning the fruit and seed, between these two species of *Protorhus*, as well as the meaningful similarities between *P. namaquensis* and species of *Ozoroa*, led to the proposal of the new combination — *Ozoroa namaquensis* (Sprague) Von Teichman & Van Wyk (Von Teichman, 1993; Von Teichman & Van Wyk, 1993, 1994b). This new classification will be followed here.

Within the Cape Floristic Region, *H. argentea* is one of a small group of apparently anomalous woody taxa of more tropical affinity than the prevailing temperate fynbos flora or associated Afromontane forest element (Van Wyk, 1990). These taxa have been interpreted as possible relict elements of an ancient tropical lowland vegetation that may have prevailed in this part of southern Africa before the onset of the present Mediterranean climate (Van Wyk, 1990). Usually monotypic or taxonomically isolated, affinities of these palaeoendemics often appear to be with taxa on Madagascar or other Gondwana fragments. With its slightly fleshy fruit and large recalcitrant seed, *H. argentea* is certainly more reminiscent of a taxon from a moist tropical forest-habitat. As suggested by Von Teichman & Van Wyk (1994a), these
seed character states, which also include pachychalazy, are often plesiomorphic in dicotyledons.

The present study forms part of a comprehensive project on the comparative fruit and seed structure of the Anacardiaceae. We compare for the first time the pericarp and seed coat anatomy, ovule structure and germination of *Heeria argentea* with that of *Ozoroa*. We also emphasize hitherto unrecognized similarities between *H. argentea* and *P. longifolia*. Seed characteristics of *H. argentea*, i.e. the functional significance of pachychalazy and the recalcitrant seed viability behaviour, will be considered in the light of the recent hypotheses of Von Teichman & Van Wyk (1991, 1994a). The survival strategies of this tropical relict during the long dry summer, i.e. pericarp and seed coat anatomy as well as mode of germination, will also be mentioned.

**MATERIAL AND METHODS**

Floral buds, flowers and fruit of *H. argentea*, in various stages of development, were

Figures 1, 2. Fig.1. Herbarium specimen of *H. argentea* collected 5 km east of the Pakhuis Pass near Clanwilliam. Scale bar = 50 mm. Fig. 2. Fruit in various stages of development, slightly enlarged. Scale bar = 10 mm.
collected from trees in the vicinity of the Jonkershoek Forestry Station near Stellenbosch (voucher specimen: P. Vorster 2935 in PRU). Material was fixed in 2.5% glutaraldehyde or preserved in FAA. Dehydration, infiltration and embedding in glycolmethacrylate (GMA) were done according to Feder & O’Brien (1968). Composition of the monomer mixture, preparation and staining of the 3 μm-thick GMA sections, as well as the histochemical tests for fatty substances, especially cutin and lignin, were described by Von Teichman (1987). Starch grains and crystals were observed under crossed polarized filters. Staining for proteins with amido black 10B followed Bullock, Ashford & Willetts (1980).

For the SEM study of the disc in female flowers and the pericarp, material was dehydrated in ethanol, infiltrated with liquid CO₂, critical point dried, coated with gold and examined with a Jeol 840 SEM at 5kV.

RESULTS AND DISCUSSION

*Morphology of flowers and fruits*

*Heeria argentea* (henceforth referred to as *Heeria*) is dioecious. Flowers are borne in many-flowered terminal and axillary panicles. Male flowers are characterized by an articulated pedicel, three bracteoles, five free sepals and petals, five antisepalous stamens and an annular, 10-crenate, intrastaminal disc with five of the indentations more pronounced and coinciding with the position of the stamens. The gynoecium is small and vestigial.

Female flowers are cream-coloured and sweet-scented. Pedicels are articulated near the apex. Three bracteoles are present, the basal one often caducous. The calyx and corolla are five-segmented, with the petals imbricate in bud, and recurved at anthesis. Small, unicellular straight or bent trichomes, which are stiff owing to lignification, occur on the rachis, pedicel, bracteoles and abaxial surface of the calyx. Epidermal cells of the petals, especially abaxial ones, are papillate. Stamens are reduced to five free antisepalous staminodes. An orange disc, very similar to the one in male flowers, is present. The gynoecium is tricarpellate, with three styles which are free or connate only at the very base and terminated by simple, papillate stigmas. The ovary is asymmetrically subglobose, a shape also displayed by the very young fruit (Fig. 2). It is unilocular, with a single ovule attached basally and to one side. If this attachment were to be slightly higher up, it would be termed lateral. Unilocular ovaries are also present in *Protorhus longifolia*, and species of *Ozoroa* and *Baronia*. The ovary in species of *Protorhus* in Madagascar is trilocular, though functionally unilocular, as only one ovule develops into a seed (Fernandes, 1966).

In *Heeria* the disc is characterized by a well-developed, ridged cuticle (Fig. 3) and relatively thin-walled epidermal and hypodermal parenchyma cells with large nuclei and abundant cytoplasm. These characteristics are typical of such nectariferous tissue. On the contrary, in *Ozoroa paniculosa* (Sonder) R. & A. Fernandes and *O. namaquensis* the disc has a distinctly papillate surface. A pronounced similarity is noted between the discs of *Heeria* (Fig. 3) and *P. longifolia* (compare figs 15, 16 in Von Teichman & Van Wyk, 1994b), except that in *P. longifolia* stomata often occur on the level of the surrounding epidermal cells, while in *Heeria* most stomata are sunken. Cuticular ridges characterize most epidermal cells in both species (Fig. 3).

Some differences in fruit characters in *Heeria* and *Ozoroa* have been mentioned in
PERICARP AND SEED IN *HEERIA ARGENTEA*

Figure 3. Scanning electron micrograph of the disc in a female flower of *H. argentea*, showing a distinct depression on the left, a sunken stoma (white arrow) and the striate cuticle. Scale bar = 20 µm.

In size the fruit of *P. longifolia* lies between that of *Heeria* and those of most members of *Ozoroa*. Fruit shape is distinctive in all these taxa. Ripe fruit in *Heeria* are relatively large, c. 30 × 24 mm, with a slightly shrivelled, tough and leathery pericarp. They are often slightly more pronounced dorso-ventrally compressed than the largest fruit shown in Figure 2. The single, large seed has a membranous seed coat.

Female flowers in *Heeria* therefore largely resemble those in *Ozoroa* and *P. longifolia*. However, lignified trichomes and articulated pedicels seem to occur only in *Ozoroa* and *Heeria*. Concerning placentation, *Heeria* differs from both as ovule insertion is apical in *P. longifolia* and lateral in *Ozoroa*.

None of the male or female flowers we dissected had up to six perianth segments, up to ten stamens/staminodes, or styles fused basally for the greater part of their length as reported by Fernandes & Fernandes (1965). We also did not find any trilocular fruits in addition to the regular unilocular ones. A more comprehensive survey of material from the full range of the species could verify whether these features can be considered as distinctive from those in *Ozoroa* as reported by the latter authors.

**Ontogeny and anatomy of the pericarp**

At anthesis the ovary wall (Figs 4, 5) is delimited externally by the outer epidermis (future exocarp *sensu stricto*), consisting of tanniniferous epidermal cells, with a well-developed cuticle. Stomata are present and the epidermal cells show a distinct radial elongation (Fig. 4). Many anticlinal divisions take place in these epidermal cells, which are metabolically active, i.e. having large nuclei, starch and abundant cytoplasm. The ground tissue (future mesocarp) consists mainly of parenchyma, which is partly tanniniferous, especially in cells of the outer and inner layers. Many cells in the outer ground tissue contain either starch or druse crystals of calcium oxalate. In the central zone, procambial strands occur in essentially two concentric
Figures 4, 5. Transverse sections of the ovary wall of H. argentea at anthesis. Scale bar = 20 µm. Fig. 4. Outer part showing the outer epidermis, the future exocarp (oe) with stomata; as well as the adjacent parenchymatous ground tissue (future mesocarp) with secretory ducts (sd) and vascular tissue (arrows). Fig. 5. Inner part showing the inner epidermis (ie) with several cells engaged in periclinal division (future endocarp s.s.), a procambial strand (arrow) and developing secretory duct within the ground tissue (the future mesocarp). The tanniniferous outer epidermis of the abutting ovule is visible on the extreme right.

circles. Vascular tissue and associated secretory ducts develop from these procambial strands (Figs 4, 5). The inner epidermis (part of the future endocarp sensu stricto) consists of metabolically active epidermal cells, many of which show a periclinal division. This epidermis is also covered with a thin cuticle which lines the ovary locule.

In young fruit (Figs 6, 7), cells of the exocarp are still engaged in anticlinal divisions and are covered by a noticeably thicker cuticle, while cutinization of the adjacent outer tangential epidermal cell walls has also started. In this younger exocarp of Heeria a number of wide open stomata occur, each lying within a hollow which can clearly be seen in the largest fruit in Figure 2. They appear raised above the adjacent epidermis and are sometimes surrounded by a phellem-like tissue. Druse crystals are abundant in the outer mesocarp. During the Periodic Acid-Schiff Reaction (PAS)

Figures 6, 7. Transverse sections of the young pericarp of H. argentea. Scale bar = 20 µm. Fig. 6. Outer part showing the exocarp (exo) with thick cuticle. Note crystalliferous cells (asterisks) in adjacent mesocarp. Fig. 7. Inner part showing the young endocarp, comprising the inner epidermis (ie) and hypodermal layer; as well as the adjacent mesocarp with partially tanniniferous parenchyma and parts of the secretory ducts.
these crystals are dissolved, consequently only their position within the crystalliferous cells is shown in Figure 6. Numerous secretory ducts, arranged in three irregularly concentric circles, constitute the greater part of the central mesocarp. Vascular tissue is mainly closely associated with these secretory ducts. Secretions in the latter contain characteristic flat, mostly tombstone-shaped, crystal-like inclusions. However, under crossed polarized filters these inclusions do not exhibit birefringence. They stain pale purple with toluidine blue O (indicating a proteinaceous consistency), but they do not react at all during the PAS reaction. Staining for proteins with amido black 10B was also negative. Similar, probably slightly proteinaceous inclusions are present in Protorhus longifolia, but absent in Ozoroa paniculosa and O. namaquensis. Molisch (1931) associated very similar proteinaceous inclusions with the milky secretions of Mangifera indica L., Lannea grandis (Dennst.) Engl., Schinus molle L. and species of Rhus L. On injury, various tissues of P. longifolia as well as Ozoroa engleri R. Fern. & A. Fern. and O. paniculosa exude a milky latex. The innermost mesocarp consists mainly of tanniferous parenchyma (Fig. 7).

In young fruit of Heeria the endocarp (sensu stricto) comprises two cell layers, often with cells in radial rows following periclinal divisions of the initial inner epidermal cells. Already at this stage (Fig. 7) the inner epidermal cells are larger than the hypodermal layer; they are meristematic, signifying further differentiation. Small parts of the hypodermal layer have also divided periclinally, resulting locally in a three-layered endocarp.

The mature exocarp consists of a single layer of extensively radially elongated epidermal cells (Fig. 8) containing starch grains. This palisade-like epidermis is covered by a thick (about 12 μm) cuticle, while the outer tangential and outer parts of the radial cell walls are strongly cutinized (Fig. 8). Presence of starch in the exocarp is strikingly similar to that in Ozoroa namaquensis. Protorhus longifolia has a very similar palisade-like exocarp, but without starch. The older exocarp contains irregularly scattered depressions, each with a central pore derived from a modified stoma. Some of these pores are covered by a secretion of unknown composition, whereas others eventually develop a prominent lenticel-like plug. It is suspected that these modified stomata may function as extrafloral nectaries, at least during earlier stages of fruit development. Modified stomata functioning as extrafloral nectaries, or with at least

![Figures 8, 9. Transverse sections of the mature pericarp. Fig. 8. Sudan black B-stained section showing the massive cutinization of the outer parts of the cell wall of the palisade-like exocarp (exo) and a thick cuticle; outer layers of the mesocarp visible below the exocarp. Folds in the thin GMA-sections are artifacts. Scale bar = 100 μm. Fig. 9. The inner part of the pericarp, showing the two-layered endocarp (on the right) and adjacent secondarily thickened cells of the mesocarp. Scale bar = 10 μm.](https://academic.oup.com/botlinnean/article-abstract/122/4/335/2607947)
a secretory function, have previously been reported in the pericarp of members of *Ozoroa* (Von Teichman & Van Wyk, 1993, 1994b) and also occur in *Protorhus longifolia*. Lenticels developing in the place of stomata are known in the exocarp of *Sclerocarya birrea* (A. Rich.) Hochst. subsp. *caffra* (Sond.) Kokwaro (Von Teichman & Robbertse, 1986a). Modified stomata which, amongst others, characterize the mature exocarp in *Heeria*, also occur in *Ozoroa paniculosa* and *O. namaquensis*, while in the tropical taxon *Protorhus longifolia*, they are relatively scarce.

In the mature mesocarp the walls of the parenchyma cells show a distinct secondary thickening and lignification to varying degrees. It is particularly pronounced in the layers adjacent to the endocarp. These brachysclereids are responsible for the tough leathery texture of the mature pericarp. Presence of crystalliferous cells in the mature mesocarp differs only with regard to the outer layers. In *P. longifolia* and especially in *Heeria*, crystals of calcium oxalate occur abundantly in the outer mesocarp, while in *Ozoroa* crystals are practically absent in that zone.

The mature endocarp comprises sclereids and is essentially two-layered (Figs 9, 10). A cuticle covers the outer tangential cell walls of the innermost layer of sclereids, thus lining the fruit locule. Tanniniferous substances occur predominantly in these innermost sclereids. Lignification occurs in the hypodermal sclereids of the endocarp, but is negligible in the innermost ones.

In *Protorhus longifolia* the endocarp is also two-layered. However, in contrast to *Heeria* the sclereids of the innermost macrosclereid layer are very well developed compared to the adjacent (hypodermal) small brachysclereids. In *Heeria* the sclereids in the two layers differ only slightly in size (Figs 9, 10). Unlike in *Ozoroa*, the endocarp in *Heeria* and *P. longifolia* does not have a so-called crystalliferous layer. In *Heeria* no crystals were observed in the endocarp, whereas in *P. longifolia* a few scattered ones are present in the hypodermal layer of small brachysclereids.

In *O. paniculosa* and *O. namaquensis* derivatives of the inner epidermis of the ovary wall differentiate into a very characteristic, stratified, four-layered endocarp *sensu stricto*. At maturity this comprises consecutive layers of outermost (i.e. lining the fruit locule) macrosclereids, which exhibit a pronounced radial elongation, osteosclereids (typified by a capitate part and cell wall flutes), brachysclereids, and a layer of crystalliferous sclereids.

Considering the pericarp of the taxa under discussion, the overall picture is similar to that exhibited by the flower. A uniseriate exocarp *s.s.* of radially elongated palisade-like parenchyma with thick cuticle and outer cutinized parts, as well as

![Figure 10. Transverse section showing part of the entire mature pericarp. Note palisade-like exocarp on the left (the cuticle and cutinized parts unstained), the mesocarp with numerous large secretory ducts and the thin, 2-layered endocarp, on the extreme right. Scale bar = 200 µm.](https://academic.oup.com/botlinnean/article-abstract/122/4/335/2607947)
modified stomata is characteristic of all these taxa. The presence of starch in the exocarp is shared by *Heeria* and *Ozoroa*. On the other hand, presence of tombstone-like inclusions in the secretions, and a two-layered endocarp are characters shared only by *Heeria* and *P. longifolia*. Mesocarp and exocarp features are also very similar in *Heeria* and *P. longifolia*. In both these two taxa the relative contribution of the exocarp is considerably greater than in *Ozoroa*.

A distinctly layered (stratified) endocarp *s.s.*, as in *Ozoroa*, represents the *Anacardium*-type endocarp *sensu* Wannan & Quinn (1990). Under the *Anacardium*-type these authors recognize three endocarpal sub-types (or taxon groups) in the tribe *Rhoeae*. An endocarp of four discrete layers (i.e. three palisade-like sclereid layers and a fourth crystalliferous layer), as in *Ozoroa, Rhus, Schinus* L. and seven other genera, forms Group A. Group B taxa have an endocarp of two or three distinct layers of thin-walled parenchyma. Group C taxa have an endocarp comprising irregularly orientated sclereids, i.e. an unstratified endocarp. However, endocarp structure both in *Heeria* and *P. longifolia* does not neatly fit any of these sub-types or Groups, because although distinctly stratified, only two layers of sclereids occur in the mature endocarp. Therefore, it is here proposed as a fourth *Rhoeae* endocarpal sub-type (Group D), i.e. also derived from the Group A-type. An ontogenetic study of the pericarp in *Rhus lancea* L. f. (Von Teichman & Robbertse, 1986b) showed that the mature, four-layered endocarp is also derived (by periclinal divisions) from the inner epidermis of the ovary wall. This endocarp therefore conforms to the definition of an endocarp in the strict sense.

**Morphology of the ovule**

*Heeria argentea* is characterized by a single bitegmic, anatropous and crassinucellar ovule (Fig. 11). Noteworthy is the extended chalaza, which is characterized by an extensive vascularization and a hypostase *s.l.* The distinction between a hypostase *s.s.* and hypostase *s.l.* in the ovules of the Anacardiaceae is suggested by Von Teichman (1988a). This hypostase comprises, as in previously studied species of the Anacardiaceae, contiguous, thin-walled parenchyma cells, the vacuoles filled with tanniferous substances. In the three or four cell layers of the hypostase bordering onto the embryo sac, deposits of tanniferous substances are much less dense. This area probably facilitates the symplastic transport of nutrients into the embryo sac from the adjacent vascular elements and parenchyma cells. Starch grains are present in the latter.

A well-developed raphe (Fig. 11) with an amphicribral vascular bundle, and a tanniferous outer epidermis covered by a thin cuticle also characterize this ovule. Cells of the inner epidermis of the inner integument (*i.e.* *ii* in Fig. 11), especially at the antiraphal side, are metabolically active, *i.e.* they have large nuclei and dense cytoplasm. This indicates the future differentiation and meristematic activity in this part of the ovule.

A comparison of the ovule of *Heeria* with those of *Protorhus longifolia, Ozoroa paniculosa* and *O. namaquensis* (Von Teichman 1991b, 1993 & 1994), is quite informative. These ovules represent a structural continuum regarding the degree of development of the chalaza. The latter is characterized by the hypostase *s.l.* (henceforth referred to as hypostase), which represents the ‘Anacardiaceae-type’ of hypostase which has previously also been found in the tribes Anacardieae,
Spondiadeae and other members of Rhoeae. Most previous studies on the seed of the Anacardiaceae are summarized by Von Teichman (1991c). In *P. longifolia* the chalaza is so extensive that the ovule is described as pachychalazal (compare fig. 2 in Von Teichman, 1991b). Similarities between this ovule and the very young seed of *Heeria* (Fig. 12) are so striking that the close phylogenetic alliance of these two taxa cannot be overlooked. A 'bent' embryo sac has also, until now, been found only in *Heeria* and *P. longifolia*. The hypostase in *Heeria* is, however, much bigger than that in the ovules of the two species of *Ozoroa*, where it merely comprises a plate-like group of cells.

The latter type of hypostase also occurs in the ovules of *Rhus lancea* (Rhoeae), species of *Lannea* A. Rich., *Sclerocarya* Hochst. and *Harpephyllum* Bernh. ex Krauss.

Figures 11, 12. Scale bar = 400\,\mu m. The ovule and young seed of *Heeria argentea* reconstructed from several series of longitudinal sections. Fig. 11. The ovule at anthesis. Fig. 12. The very young seed, collected two weeks after anthesis. The chalaza (ch), embryo sac (es), funicle (fun), hypostase (hyp), inner epidermis of the inner integument (ie ii), inner integument (ii), nucellus (nuc), nucellar epidermis (ne), outer epidermis (oe), outer integument (oi), raphe (ra), secretory duct (sd), and vascular tissue (vt) are illustrated.
(Von Teichman, 1991c), as well as Operculicarya decaryi H. Perrier of the Spondiadeae (Von Teichman, 1992). Species of Spondias L. and Tapirira Aubl., also of the Spondiadeae, could probably be added as they also have partially pachychalazal seeds. However, their ovules still need to be studied (Von Teichman, 1990, 1991c). Concerning placentation, Heeria differs from both taxa, as ovule insertion is apical in P. longifolia and lateral in the species of Ozoroa.

**Ontogeny and structure of the seed, with notes on germination**

Longitudinal sections of the very young seed (Fig. 12) clearly show the bent embryo sac, the secondary growth of the chalaza with hypostase, as well as the chalazal tissues bordering onto the ie ii (the inner epidermis of the inner integument) at the antiraphal side. In P. longifolia a meristematic layer adjacent to the actively dividing ie ii was described. The similarities between the very young seeds of Heeria and P. longifolia (compare Fig. 3 in Von Teichman, 1991b) are very striking. Corner (1976) described the secondary growth in the chalaza during the development of pachychalazal seeds. Therefore, it is suggested that in both taxa this type of basipetal intercalary meristem is responsible for the secondary growth in the chalazal region adjacent to the integuments. Heeria is an example of this growth taking place mainly after fertilization, while in Mangifera indica (Von Teichman, Robbertse & Schoonraad, 1988) and in P. longifolia the ovule is already pachychalazal at anthesis.

Longitudinal and transverse sections (henceforth referred to as ls and ts respectively) of small seeds of Heeria (Figs 13, 14) clearly demonstrate the origin of the mature seed coat (Fig. 15). In Figure 13 the vertical line indicates the plane of the transverse section shown in Figure 14. Between the nucellar epidermis (covered with

![Figure 13. A slightly older seed of Heeria argentea than the one shown in Figure 12. The chalaza (ch), embryo (e), endosperm (endos), funicle (fun), hypostase (hyp), inner epidermis of the inner integument (ie ii), inner integument (ii), nucellus (nuc), nucellar epidermis (ne), outer integument (oi), raphe (ra), secretory duct (sd), slit (sl) and vascular tissue (vt) are illustrated. The vertical line indicates the plane of the transverse section shown in Figure 14. Scale bar = 1 mm.](https://academic.oup.com/botlinnean/article-abstract/122/4/335/2607947)
a cuticle) and the ie ii (covered with its own cuticle) a slit is present (sl in Figs 13 & 14); the ie ii is metabolically active. This is logical as in larger seeds an endotegmen differentiates at the antiraphal side from this ie ii. Adjacent to the ie ii lies the unvascularized chalazal tissue.

Figure 15A shows a fruit of Heeria during seed germination, a seed with protruding radicle is shown in Figure 15B, while C, D and E show similar mature seeds. An insignificantly small part of the seed coat (near point of radicle protrusion/funicle attachment) develops from the integuments. This is the integumentary seed coat. On the lower side, i.e. the antiraphal side of the seed, the chalazal seed coat is light brown, owing to tanniniferous substances in the outer epidermis, as well as the absence of the hypostase. By far the largest part of the chalazal seed coat develops from the chalazal tissues including the hypostase at the raphal side of the ovule/very young seed. This part is darker owing to the presence of the hypostase. The vascularization in this part of the seed coat is easily seen externally (Fig. 15C–E). This is also the case in P. longifolia (compare Fig. 1A in Von Teichman, 1991b).

Anatomically the ripe chalazal seed coat at the antiraphal side is c. 80 μm to 100 μm thick, comprising (from the outside inwards): remnants of the outer epidermis and parenchyma cells; the endotegmen; a cuticular layer which consists of the two adjacent cuticles of the ie ii and nucellar epidermis, and lastly the totally

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Figures 14, 15. Transverse section (TS) of young seed and the morphology of mature fruit and seed of Heeria argentea. Fig. 14. TS of seed similar to the one shown in Fig. 13. It illustrates the endosperm (endos), hypostase (hyp), inner epidermis of inner integument (ie ii), nucellus (nuc), nucellar epidermis (ne), outer epidermis (oe), secretory duct (sd), slit (sl) and vascular tissue (vt). Scale bar = 1 mm. Fig. 15. Fruit during seed germination (A), showing the radicle and fruit stalk remnants. A germinating seed with radicle and seed coat which has burst open (B). The seed with funicle in (C) is shown in lateral view; note the difference in colour in the seed coat, i.e. the upper portion is darker with whitish stripes, denoting the vascular bundles within the chalazal seed coat with associated hypostase. Seeds (D & E) illustrate the chalazal seed coat with hypostase and extensive vascularization. In seed (F) the seed coat has been removed to show the two large cotyledons. Scale bar = 20 mm.
squashed remnants of the nucellus and endosperm. The relatively weakly developed endotegmen, which differentiated from the endosperm, has pitted, lignified secondary radial and tangential cell walls.

The vascularized chalazal seed coat (Fig. 16) is thicker, i.e. c 180 μm, and consists of the following: the slightly squashed outer epidermis; mostly tanniniferous parenchyma; the xylem of the vascular tissue consisting mainly of tracheoids (Sensu Schmid, 1986) with helical secondary thickenings. Then follows the hypostase consisting of thin-walled, contiguous, tanniniferous parenchyma cells. As shown in Figure 16, in some of these cells the tannin-like substances leached out; however, here the thickness and staining of the cell walls is shown more clearly. Adjacent and to the inside of the hypostase tissue, are the remnants of nucellus and endosperm.

As shown in Figures 13 and 14, the thin peripheral edge of the hypostase overlaps the endotegmen with its cuticular layer. This was also seen in P. longifolia, the seed coat of which is also characterized by an endotegmen in the non-vascularized part of the chalazal seed coat. In the two species of Ozoroa, as well as in Rhus lancea, the endotegmen characterizes the integumentary seed coat, which in these partially pachychalazal seeds constitutes the larger part of the seed coat. In the relatively small seeds of these taxa the pachychalazal seed coat, with tanniniferous hypostase, manifests externally as a relatively small, dark brown, saddle-like patch. In these taxa, the transitional region, i.e. between chalazal and integumentary parts of the seed coat, the edge of the hypostase also overlies the endotegmen and cuticular layer.

The similarities in seed coat structure in Heeria and P. longifolia are therefore very significant. In the physiologically ripe seeds of P. longifolia, the cell walls of the hypostase cells are, however, impregnated with lipids, probably cutin. In the two species of Ozoroa these cell walls reacted positively not only for lipids (cutin), but also for callose.

All the seeds of the Anacardiaceae are exalbuminous. In Heeria, the large cotyledons (Fig. 15F) are chlorophyllous and store mainly starch. Unlike in Sclerocarya birrea subsp. caffra, where germination has been studied in detail (Von Teichman, Small & Robbertse, 1986), germination data on Heeria, P. longifolia and Ozoroa paniculosa (Von Teichman, 1988b), are not based on extensive experiments.
However, they are significant enough to be mentioned here. *Protorhus longifolia* and *Heeria* exhibit a hypogeal germination, while in *O. paniculosa* it is epigeal. Concerning the relative evolutionary status of the modes of germination, two authors (Burtt, 1991; Clifford, 1991) came to contrasting conclusions. However, their ideas will not be evaluated/discussed in this paper. According to Fahn & Cutler (1992:38) hypogeal, i.e. 'cryptogeal', "germination provides an efficient mechanism for protection of the developing shoot. The selective pressures which have led to cryptogeal germination can be attributed to fire and/or to seasonally arid climates as for instance the Mediterranean type of climate of California". This surely applies to *Heeria* as well. The findings of Rizzini (1965), who examined seedling development of cerrado woody plants, i.e. in the Brazilian wooded savanna, are discussed by Von Teichman (1988b). Here it suffices to note that the association of larger, heavier, animal-distributed seeds with hypogeal germination, as noted by Rizzini, holds true for *P. longifolia* and *Heeria*. Fruit of *Heeria* ripen at the end of April and can germinate with the first rains in late autumn. Notwithstanding their tough, leathery, very resinous and therefore odorous pericarp, fruit are eaten by rats and rock rabbits, who act as dispersal agents.

**CONCLUSIONS**

Following the transfer of *Protorhus namaquensis* to the genus *Ozoroa* (Von Teichman & Van Wyk, 1994b), *P. longifolia* remains the only member of *Protorhus* in Africa. Phytogeographically, *Protorhus* is centred in Madagascar, where it comprises about 20 species (Mabberley, 1987). Fruit shape of *P. longifolia* differs markedly from that of the Madagascan species (Von Teichman, 1991a). A detailed morphological study of the fruit of these Madagascan taxa is essential and is likely to provide useful, if not decisive evidence towards a more refined generic demarcation. Such a study should include the genus *Baronia*. Engler (1892) recognized one species, *B. tarantana* Baker, from the forests of Madagascar and remarked that phylogenetically it is allied to *Protorhus*. Kokwaro & Gillett (1980:745), in agreement with Fernandes (1966), observed that *Baronia*, although included in *Rhus* by Perrier de la Bâthie (1946), "... seems to be closer to *Ozoroa* than *Heeria* is". Perrier de la Bâthie (1946) placed three species of *Baronia* together with *R. natalensis* Bernh. into the genus *Rhus*. Data on fruit structure in *P. longifolia*, *Heeria* and *Ozoroa*, suggest that the gross fruit morphology of *Baronia* (cf. figs 7 and 8 in Perrier de la Bâthie, 1946) is different from all these taxa, and that neither fruit nor leaf morphology shows any similarities between these taxa and those in *Rhus*.

A preliminary study of pollen by means of light and scanning electron microscopy (J.J.M. van der Merwe, pers. comm.) revealed a striated exine in *H. argentea*, *P. longifolia* and some of those species of *Ozoroa* confined to the arid, semi-desert areas of the northwestern Cape and Namibia, namely *O. concolor* (C. Presl ex Sond.) De Winter and *O. dispar* (C. Presl) R. Fern. & A. Fern. On the other hand, members of *Ozoroa* from the savanna regions of southern Africa, amongst others *O. paniculosa*, all have a punctate exine sculpture. It should therefore be borne in mind that *Ozoroa*, as currently defined, is a heterogeneous taxon consisting of two palynologically distinct sub-groups.

Ovule and seed characteristics shared by *Heeria*, *Protorhus longifolia* as well as the two species of *Ozoroa* are the following: anatropous, bitegmic, crassinucellar ovule with a
nuclear endosperm development; amphicribal vascular bundle in the raphe; tracheoids with spiral thickening in the vascularized pachychalazal seed coat; Anacardiaceae-type hypostase in the pachychalaza; presence of an endotegmen and associated cuticular layer (as discussed); and the exalbuminous nature of the mature seed. Table 1 summarizes the differences between these taxa regarding some female flower and pericarp characteristics, as well as features of the ovule, the seed, and mode of germination. According to Yakovlev & Zhukova (1980) the occurrence of chlorophyll in the embryo is of taxonomic significance and species within a genus do not differ regarding this character state. Therefore all species of Ozoroa will be characterized by a non-chlorophyllous embryo (see also Table 1).

These data do not only strongly support the taxonomic separation of Heeria from all the species of Ozoroa advocated by Fernandes & Fernandes (1965), but also emphasize the close phylogenetic relationship between Heeria and P. longifolia.

The present study supports the conclusions of Fernandes (1966) who argues that, based on the number of locules in the ovary, Protorhus (possibly three-locular in Madagascar only) can be considered to be primitive and that Ozoroa, Heeria and

<table>
<thead>
<tr>
<th>Character</th>
<th>P. longifolia</th>
<th>Heeria</th>
<th>Ozoroa</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Female flower:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Disc</td>
<td>smooth with cuticular ridges</td>
<td>smooth with cuticular ridges</td>
<td>distinctly papillate</td>
</tr>
<tr>
<td>B. Pericarp:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exocarp</td>
<td>starch absent</td>
<td>starch present</td>
<td>starch present</td>
</tr>
<tr>
<td>Mesocarp with tombstone-shaped crystal-like inclusions</td>
<td>present</td>
<td>present</td>
<td>absent</td>
</tr>
<tr>
<td>Endocarp</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(a) number of layers</td>
<td>two</td>
<td>two</td>
<td>four</td>
</tr>
<tr>
<td>(b) crystalliferous layer</td>
<td>absent</td>
<td>absent</td>
<td>present</td>
</tr>
<tr>
<td>(c) relative thickness</td>
<td>thin</td>
<td>thin</td>
<td>thick</td>
</tr>
<tr>
<td>C. Ovule:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insertion</td>
<td>apical</td>
<td>unilateral-basal</td>
<td>lateral</td>
</tr>
<tr>
<td>Pachychalazal</td>
<td>present</td>
<td>absent</td>
<td>absent</td>
</tr>
<tr>
<td>Hypostase: size</td>
<td>very extensive</td>
<td>extensive</td>
<td>small</td>
</tr>
<tr>
<td>D. Seed:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Relative size</td>
<td>large</td>
<td>large</td>
<td>small</td>
</tr>
<tr>
<td>Seed coat (in ripe seed)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(a) degree of pachychalazal</td>
<td>pachychalazal</td>
<td>pachychalazal</td>
<td>pachychalazal</td>
</tr>
<tr>
<td>(b) contribution of integuments in seed coat development</td>
<td>insignificant</td>
<td>insignificant</td>
<td>very important</td>
</tr>
<tr>
<td>(c) impregnation of cell walls of hypostase</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(i) callose</td>
<td>absent</td>
<td>absent</td>
<td>present</td>
</tr>
<tr>
<td>(ii) cutin</td>
<td>present</td>
<td>absent</td>
<td>present</td>
</tr>
<tr>
<td>Embryo colour</td>
<td>chlorophyllous</td>
<td>chlorophyllous</td>
<td>white</td>
</tr>
<tr>
<td>Principal nutrient reserves</td>
<td>starch</td>
<td>starch</td>
<td>lipid and protein</td>
</tr>
<tr>
<td>E. Mode of germination</td>
<td>hypogeal</td>
<td>hypogeal</td>
<td>(epigeal O. paniculosa)</td>
</tr>
</tbody>
</table>
Baronia (unilocular) may have been derived from a three-locular, Protorhus-like ancestor. Baronia remained in Madagascar, while Protorhus reached Africa, where it became unilocular and also gave rise to Heeria and Ozoroa, possibly through intermediates which are now extinct. Evidence from fruit and seed structure therefore supports the proposed relictual status of Heeria as a possible link between members of the Anacardiaceae in Africa and Madagascar.

Heeria complies with the definition of a relict as circumscribed by amongst others White (1983b), namely a taxon that is taxonomically isolated, ecologically specialized and restricted in its distribution. This supports the view of Van Wyk (1990) that Heeria might be a tropical relict of a generally extinct, moist, subtropical rain forests which existed in southern Africa and have been partially replaced by the temperature fynbos flora in the Cape Floristic Region. It is suggested that, during past climatic changes, Heeria adapted significantly; and that the large, chlorophyllous and recalcitrant embryo which germinates hypogoeally represents the crucial survival adaptation.

This study of the fruit and seed of Heeria argentea also revealed micromorphological survival adaptations which ensure the protection of the embryo, i.e. the strong cutinization in the exocarp, the number of plugged modified stomata, abundance of tanniniferous substances in the pericarp and seed coat, and endotegmen with associated cuticular layer. The habitat, e.g. rock crevices on mountain slopes and passes, deep valleys and riversides sets the present ‘scene’ for the survival of Heeria.

The hypotheses of Von Teichman & Van Wyk (1991, 1994a) suggest that pachychalazy with associated greater seed size and the recalcitrant seed viability behaviour may be regarded as ancestral character states in dicotyledons. Data presented here strongly support these hypotheses. Protorhus longifolia with pachychalazy already present in the ovule might be considered more ancestral than Heeria. Heeria with its large recalcitrant seed certainly is ancestral, while Ozoroa with so much smaller, partially pachychalazal seeds (with an orthodox seed viability) is considered derived. Considering nutrient reserves in the exalbuninuous seeds, the presence of starch as main component, which is associated with a predominantly tropical habitat, a woody habit and greater seed size, is regarded as more ancestral. Starch occurs in seeds of Mangifera indica, P. longifolia and Heeria. Tapirira guianensis Aubl. occurring in tropical America (Von Teichman, 1990), with relatively large partially pachychalazal seeds and larger, saddle-shaped pachychalaza in the mature seed coat, seems to be intermediate, as starch and fats are both very important. Species of Ozoroa, Rhus and Operculicarya with much smaller seeds, store mainly lipids (comprising a higher energy reserve) and are considered more derived.

As suggested by Von Teichman & Van Wyk (1994a), pachychalazy represents a functional adaptation for speedy transfer of nutrients, respiratory gases and hormones from the parent plant into the developing seed. This transfer, via the extensive vascular network in the pachychalaza, is more direct than during the development of integumentary seeds, where cuticular layers constitute a barrier to the direct symplastic translocation of nutrients etc. (compare fig. 1 in Von Teichman & Van Wyk, 1994a).
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