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DEVELOPMENT OF OVULE AND SEED IN STANFIELDIELLA IMPERFORATA (COMMELINACEAE)

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SUMMARY

The development of ovule and seed of *Stanfieldiella imperforata* was studied. As in all other monocotyledons studied, both integuments are dermally initiated. The seed coat of *Stanfieldiella* is formed by the outer layer of the inner, and the silicified, innermost layer of the outer integument, the other cell layers of the outer integument forming a loose cover around the seed. These testa characteristics are rather unique among the Liliatae. The commelinaceous seed is typically operculate and provided with a micropylar collar, two features also found in Zingiberales. In both taxa the ontogeny is identical, which suggests a more or less close relationship between Commelinaceae and Zingiberales.

1. INTRODUCTION

In some plant taxa germination is accomplished by the shedding of a lid-like part of the seed coat, after which the radicle protrudes through the opening.

Such a "lid" is mostly formed by a differentiation of the portions of the integuments surrounding the micropyle. It is usually referred to by the term of *operculum* (HEGELMAIER 1874, BOUHARMONT 1963); most German-speaking workers used the name *Samendeckel* (*e.g.*, HEGELMAIER 1874, NETOLITZKY 1926, BOEHM 1931). Other terms are *Propf* (TSCHIRCH 1891), *embryotège* (MIRBEL 1810), and *germinal lid* (HUMPHREY 1896). Operculate seeds are of rather common occurrence among the Monocotyledonae; NETOLITZKY (1926) reported its presence among the following taxa: Typhaceae, Pandanaceae, Sparganiaceae, Palmae, Araceae, Lemnaceae, Commelinaceae, Phylidraceae, Liliaceae, Dioscoreaceae, and Zingiberales. According to TSCHIRCH (1891) an operculum is also found in the seeds of some Potamogetonaceae, Cyperaceae, and Restionaceae. Apart from the possession of operculate seeds, the Zingiberales and Commelinaceae are characterised by an associate structure, the micropylar collar, an intrusion of the integuments into the nucellus around the embryo sac (see, *e.g.*, HUMPHREY 1896, CHIKKANAIAH 1962, 1963; ROHWEDER 1962, 1969). In a previous contribution (GROOTJEN & BOUMAN 1981) the structure and development of the operculum and micropylar collar in Zingiberaceae has been reported. The present paper is a detailed description of the ontogenesis of the two structures in Commelinaceae intended as a basis of comparison with the Zingiberales.

The ovules of the Commelinaceae are ortho- to hemianatropous, bitegmic and crassinucellate. The micropyle is sometimes formed by both integuments, but

usually the outer integument is shorter than the inner one, and often the apex of the nucellus is exposed. The epidermis of the nucellus top mostly divides by periclinal divisions to form a so-called nucellar cap 2–3 cells in thickness (DAVIS 1966). The inner integument is two-, the outer one pluri-layered (NETOLITZKY 1926). The outer integument may form a strap-shaped thickened zone running from the funicle to the micropyle; this *Intraseminalstrang* (ROHWEDER 1969) presumably functions as a conductive tissue for the growing pollen tubes.

Both integuments participate in the formation of the seed coat. The innermost layer of the outer integument ultimately has thickened radial and inner tangential walls (MAHESHWARI & BALDEV 1958), the lumina becoming filled with silica bodies (CHIKKANAIAH 1962, ROHWEDER 1969) which bodies may be facetted (GRAVIS 1898, NETOLITZKY 1926). The remainder of the outer integument consists of thin-walled cells which are squashed flat against the pericarp (MAHESHWARI & BALDEV 1958), or desiccate to form a membranous cover around the seed (CHIKKANAIAH 1962, ROHWEDER 1969). In some species the outer layers detach themselves from the rest of the seed, so that the endotesta assumes a superficial position (ROHWEDER 1969). The outer layer of the inner integument forms heavy wall thickenings; ultimately hardly any cellular structure can be discerned any longer. The innermost layer of the inner integument has moderately strong wall thickenings; the cell lumina become filled with a brownish substance (NETOLITZKY 1926, MAHESHWARI & BALDEV 1958, CHIKKANAIAH 1962).

The micropylar collar is not always formed as a complete ring but is locally discontinuous in some species (PARKS 1935, ROHWEDER 1962, 1969). Up to now the formation of the collar has only been very summarily described.

2. MATERIALS AND METHODS

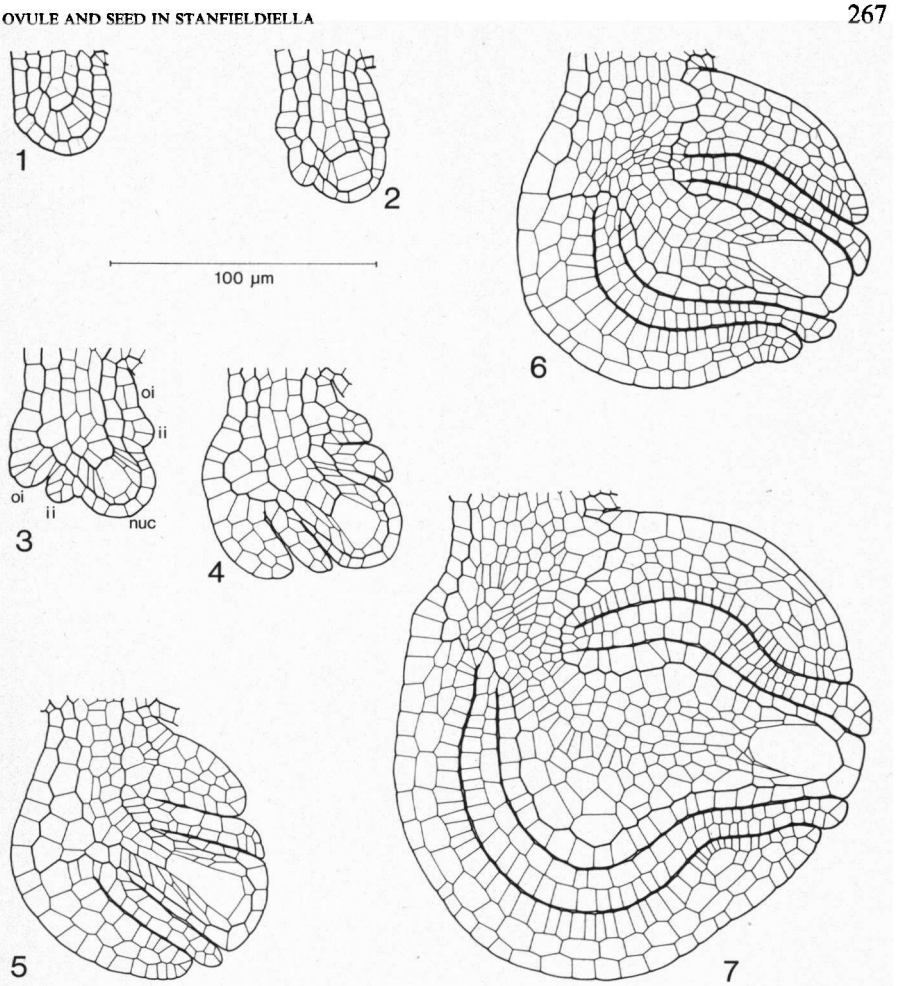
Material of *Stanfieldiella imperforata* (C.B.Cl.) Brenan (syn: *Buforrestia imperforata* C.B.Cl.) was collected in the Hortus Botanicus, Amsterdam and fixed in Allen's Modified Bouin Fluid (JOHANSEN, 1940). Young developmental stages (up to some post-fertilisation ones) were dehydrated in an ethanol/TBA series, embedded in Paraplast, 7 μm sectioned, and stained with Safranin-Astra Blue. More mature stages were dehydrated in an ethanol/NBA series, embedded in glycol-methacrylate and 3 μm sectioned by means of glass knives and PAS-stained.

For our SEM studies young, critically point-dried ovules and mature seeds were gold-palladium sputtered for 3 min and observed and photographed in a Cambridge Stereoscan Mark 2a.

3. RESULTS

3.1. Development of ovule up to the fertilisation stage

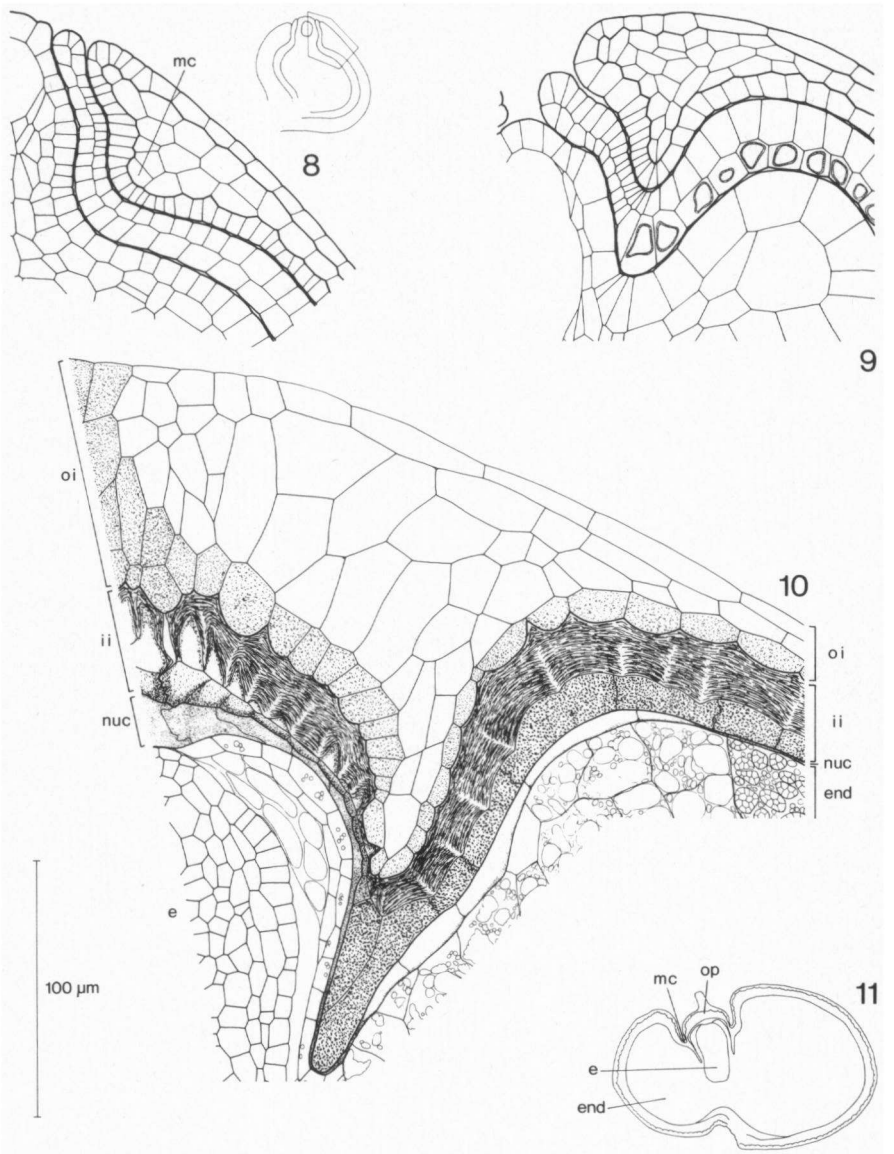
The ovules of *S. imperforata* are initiated on two axial placentae in each of the three locules, but the ovules of the two rows push in between one another to form a single row of alternatingly oriented ovules. The ovule primordium is trizonate



Stanfieldiella imperforata. Figs. 1–6: Longitudinal sections of developing ovules, showing integument initiation and hemi-anatropous curvature. Both integuments are fully of dermal origin (Fig. 3). Fig. 7: L.s. of mature ovule. Note the exposed apex of the nucellus, and the periclinal divisions in the middle layers of the outer integument (oi).
ii = inner integument, nuc = nucellus.

(*fig. 1*) and is initiated by periclinal divisions in the third cell layer of the placenta. The subdermal archespore cell divides into a primary parietal cell and a megaspore mother cell at an early stage of development (*fig. 1*); the primary parietal cell divides once again anticlinally (*fig. 3*) and the parietal tissue becomes squashed flat already in the pre-fertilisation phase of ovular development (*fig. 6*). After the meiotic division of the megaspore mother cell (*fig. 5*) the chalazal derivative cell develops into an embryo sac of the *Polygonum* type.

The inner integument (ii) is initiated in the dermal layer of the ovule primor-



Stanfieldiella imperforata. Formation of operculum and micropylar collar. Figs. 8–10: Longisections of developing seeds. The micropylar collar (mc) is initiated in the middle layers of the apex of the outer integument (oi). In Fig. 10, note the thin zone in the inner integument (ii). The parts of the integuments distally of it form, together with the epistase, the operculum (op). Fig. 11: L.s. of ripe seed. The outer integument, except its silicified inner layer, is detached, leaving the micropylar collar discernable as a circular groove around the operculum.
e = embryo; end = endosperm; nuc = nucellus.

dium, starting from the future longest flank (*figs. 2, 3*). The ii is two cell layers thick and consists of cells rich in cytoplasm which remain of a smaller size at the zone where later the micropylar collar is initiated (*fig. 7*).

The outer integument (oi) originates almost simultaneously with the ii and is also of dermal origin and first initiated at the longer flank of the ovule (*fig. 3*). At this side the oi is three cell layers thick but becomes 5–6 layered at the other, short side (*figs. 5–7*). By the time of fertilisation the apical portions of the oi undergo periclinal divisions (*fig. 7*). The oi remains shorter than the ii (*figs. 7, 13e*). The cells of the innermost layer of the oi remain smaller and are richer in cytoplasm than the cells of the other layers. At the shorter flank of the ovule the dermal cells of the oi are more or less papilliferous (*fig. 12a*).

The full-grown ovule is hemie-anatropous (*fig. 7*). The nucellus is asymmetrical by differential growth of especially the subdermal layer (*figs. 6, 7*). The core of the nucellus between chalaza and embryo sac consists of snall cells rich in cytoplasm. The cells of the nucellar apex are somewhat enlarged an papilliferous, and remain exposed (*figs. 7, 13e*).

3.2. Post-fertilisation development

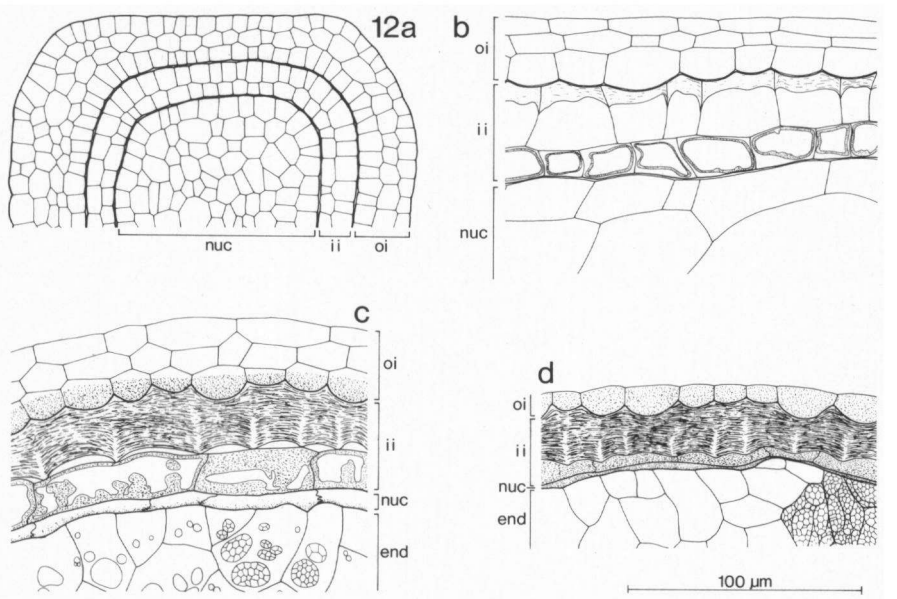
After fertilisation the nucellus top is gradually overgrown by the integuments and the micropyle becomes closed (*figs. 8–10*). Since the forming micropylar collar inhibits an outgrowth of its apical part, the developing seed increases in girth chiefly in its basal part (*fig. 11*). The nucellus is almost completely resorbed. Apart from a structure-less layer underneath the seed-skin (*fig. 12d*) vestiges of the nucellus persist near the micropyle and the chalaza (*figs. 10, 11*), where they form the epi- and hypostase, respectively. The initially nuclear endosperm turns cellular. The majority of the endosperm cells contain simple and composite starch grains (*figs. 10, 12*); below the seed coat lie scattered peripheral endosperm cells which do not contain starch grains but a fatty substance (*fig. 10*). The full-grown embryo is relatively small and cylindric in shape.

The growth in girth of the seed is somewhat retarded at the *Intraseminalstrang*, so that a shallow constriction is formed (*fig. 13a, b, d*). The two flat faces of the seed (*fig. 13a*) are the result of the mutual compression of the seeds; the terminal seeds, accordingly, have only one flat side (*fig. 13b*).

3.3 Formation of the seed-coat

Some time after the fertilisation has taken place a tannin-like substance is deposited along the walls of the cell lumina of the innermost layer of the ii (*figs. 9, 12b*) ultimately to fill an appreciable portion of the lumen (*fig. 12c*) before the cell layer is compressed (*figs. 10, 12d*).

Owing to the pressure exerted by the cells of the oi, the outer layer of the ii acquires a typical, scalloped appearance in cross section (*fig. 12b*). The nuclei of these cells migrate to and come to lie against the outer wall where a pectinose layer free of lignin is deposited (*fig. 12b*) which substance gradually fills almost the whole lumen (*fig. 12c*). By the slight thickening of the radial walls also the inner surface gets a scalloped appearance in section; here, however, it is mainly



Stanfieldiella imperforata. Formation of the seed coat. Cross-sections of mature ovule (fig. 12a), developing seeds (12b, c), and ripe seed (12d). The principal layers of the testa are formed out of the outer layer of the inner integument (ii) and the inner layer of the outer integument (oi). The other layers of the oi become detached (fig. 12d).

determined by the cell structure of the concerning layer. In the mature (*fig. 12d*) seed the cell lumina are squashed flat. The radial cell walls cannot easily be discerned, so that it is difficult to recognise the cellular build-up in this seemingly acellular and homogeneous layer.

The innermost layer of the oi does not or hardly form thickened cell walls. The cell lumina gradually become filled with a granular, solid and colourless substance rich in silica (*figs. 12c, d*). The remaining layers of the oi, consisting of large and very thin-walled cells, are compressed as the seed matures, and what is left of these cells, together with the outer tangential walls of the siliciferous cells, become detached as a loose membranous cover (*fig. 12d*), so that the silica layer is directly exposed (*figs. 13f, 14a, b*). For this reason the seeds assume a greyish colour at least when dry.

The principal mechanical layers of the testa are, accordingly, the outer layer of the inner integument (forming the exotegmen) and the inner layer of the outer integument (constituting the endotesta).

The silica bodies show a great diversity in structure (*figs. 14e, f*) and may be of taxonomic importance.

3.4. Formation of micropylar collar and operculum

The micropylar collar (mc) is initiated at about the time of fertilisation by periclinal divisions in the middle layers of the apical rim of the oi (*fig. 7*).

By a repeated mitotic activity within these layers followed by cell stretching in the inner layer of the ii the mc penetrates more deeply (*figs. 8–10*). In *Stanfieldiella* it is initiated as a complete ring surrounding the top of the embryo sac. At the inside of the mc (facing the embryo sac) the ii differentiates in a fashion somewhat different from that in the other parts of the developing seed. The innermost cell layer of the ii also becomes partially compressed and tanniferous but the deposited substance differs from that formed in the other parts of the seed coat. In the outer cell layer of the ii rather solid cell wall thickenings are formed but they do not ultimately fill up the lumina almost completely as happens elsewhere. About half-way up the inner face of the mc the ii remains very thin. The oi develops exactly as in other parts of the seed coat, *i.e.*, the inner cell layer silicifies also around the micropyle (*fig. 10*), the remaining cell layers becoming detached. The mc, therefore, is discernible on the outside as a groove in the seed coat around the operculum (*figs. 13a, c*). This groove can be rather deep in other species (*figs. 14c, d*).

Upon germination, due to the internal pressure exerted by the embryo, the seed coat ruptures along the weakest zone, *i.e.*, where the ii is thinnest, half-way the side of the mc facing the embryo (*fig. 10*), the part situated distally of the zone of rupture thus forming the operculum (*fig. 13d*), which accordingly consists of the epistase and the micropylar portions of the integuments.

4. DISCUSSION

Both integuments of *Stanfieldiella* are dermally initiated. As pointed out previously (GROOTJEN & BOUMAN 1981) presumably all monocotyledons have integuments of dermal derivation, which may be taken as an argument pleading in favour of the derived status of this taxon.

The nucellar apex of *Stanfieldiella* and of other Commelinaceae (DAVIS 1966) is exposed at the time of fertilisation. This is a rare condition and has been recorded in *Sterculiaceae*, (see VENKATA RAO 1953) and *Euphorbiaceae* (SINGH & PAL 1968).

Both integuments participate in the formation of the testa, the mechanical layers being formed by the outer layer of the inner integument (exotegmen sensu CORNER 1976) and the inner layer of the outer integument (endotesta). The importance of the first-mentioned layer was underestimated by ROHWEDER (1969). The lumina of the cells of the innermost oi layer are siliciferous, and GRAVIS (1898) mentions also silicified cell walls. The remaining oi layers become compressed and become detached from the seed. These testa characteristics are almost unique among the Liliatae. According to NETOLITZKY (1926) in this assembly an endotesta occurs in Typhaceae, some Araceae, Pontederiaceae, the Zingiberales, and, perhaps, the Eriocaulaceae. In conjunction with an exotegmen an endotesta has been recorded for Xyridaceae, Taccaceae and Dioscoreaceae. In the latter taxon the innermost outer integument layer also forms a crystal layer. A seed coat whose outer layers become detached from the remainder of the seed has been reported to occur in two other families of the

Farinosae, *viz.*, the Xyridaceae and Eriocaulaceae (NETOLITZKY 1926). Since hardly any details are known of the ontogeny of the seed coat in these taxa and in other families of the Farinosae, additional studies are certainly required.

The micropylar collar – a characteristic feature of the commelinaceous seed – is initiated in the middle layers of the apical part of the outer integument. During the later intrusion also cell stretching plays a role. Both the interpretation given by CHIKKANAIAH (1962), who says that the micropylar collar is a derivative of the nucellus, the integuments only becoming passively involved, and that of ROHWEDER (1969), who maintains that the micropylar collar is formed by a marginal meristem at the inside of the outer integument, are erroneous.

An operculum and associated micropylar collar have, apart from their occurrence in Commelinaceae, been recorded in the Zingiberales. The two closely connected structures are of a more complex origin and structure in the latter assembly: in the Zingiberales also the hilar portion of the raphe is incorporated in the operculum. The ovular anatropy of the Zingiberales results in a partly dermal and partly subdermal origin of the operculum and micropylar collar (GROOTJEN & BOUMAN 1981), and the operculum is in this group much more strongly developed and separated from the rest of the seed by a preformed rupturing layer of thin-walled cells. The initiation of the micropylar collar proceeds in an identical fashion in both groups, however. Another corresponding character is the presence of a nucellar cap (DAVIS 1966). In *Stanfieldiella* this is less strongly developed than in other Commelinaceae, however (compare MCCOOLUM 1939, MAHESHWARI & BALDEV 1958, CHIKKANAIAH 1962, RAO 1968). The presence of an aril, characteristic of the Zingiberales, has been reported for the commelinaceous *Dichorisandra*. HAMANN (1962) has already pointed out the resemblances between the Commelinaceae and the Zingiberales, *viz.*, the reduction of the androecium, the partly similar morphology of the perianth, the presence of an operculum, the structure of the stomatal apparatus, and, finally, a rather similar mode of seed germination. Owing to their more highly specialised conducting tissues the Commelinaceae cannot be directly progenitorial in respect of the Zingiberales, but the similar mode of development of the mc in both taxa provides an additional indication of their taxonomic affinity.

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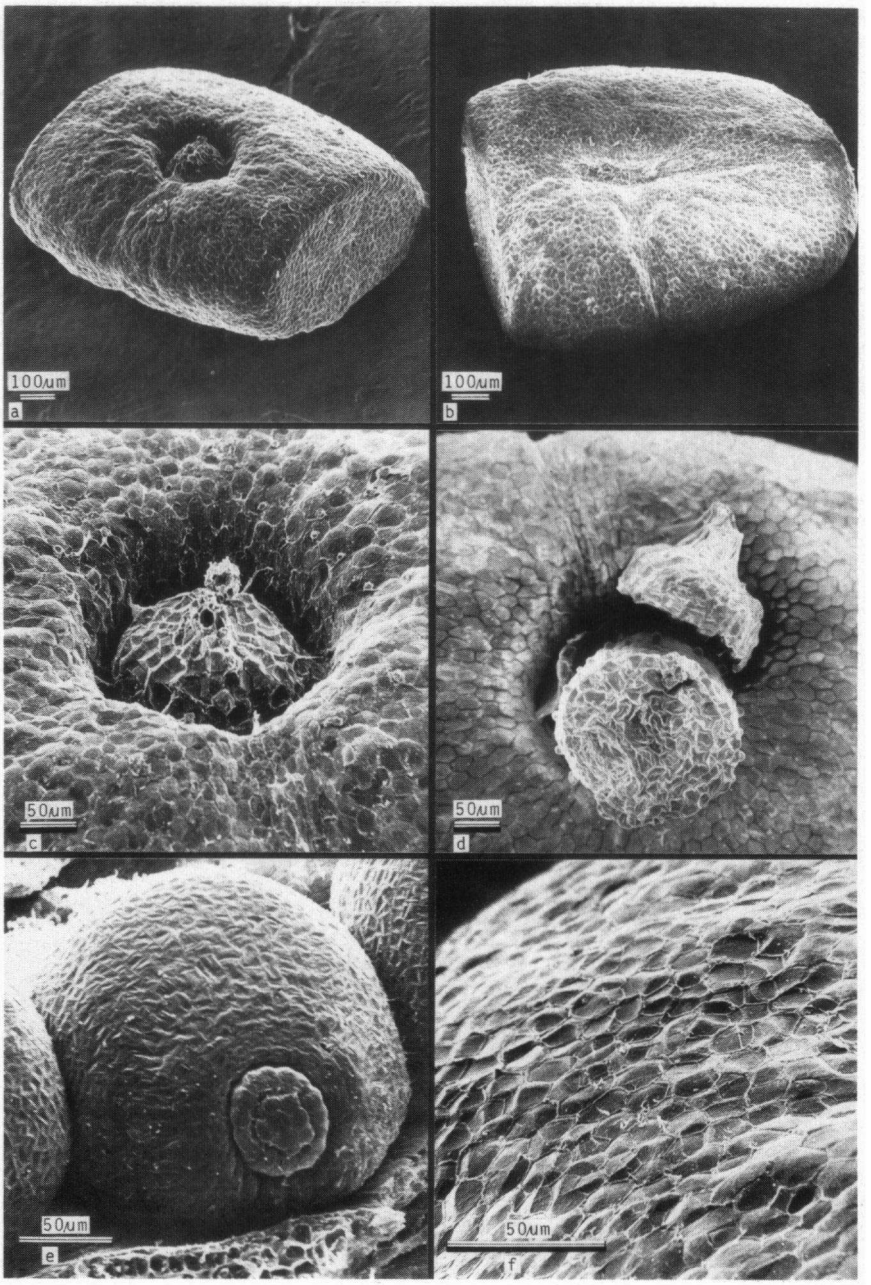

Fig. 13. SEM-photomicrographs of *Stanfieldiella imperforata*.
a, b seeds as seen from the micropylar and hilar side, respectively.
c detail of operculum.
d germinating seed: the operculum is pushed away by the embryo.
e critically-point-dried ovule. Note the exposed nucellus-apex.
f detail of seed coat.

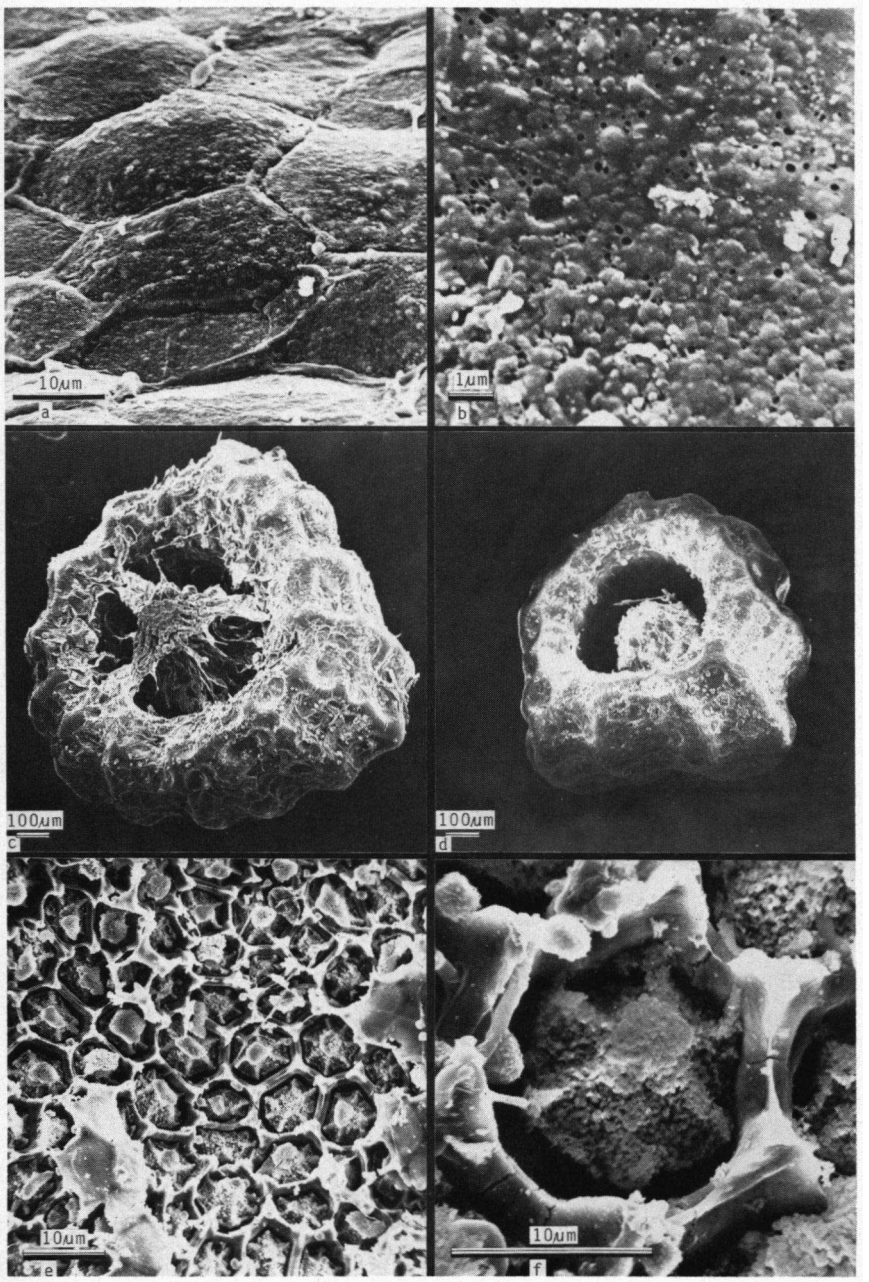

Fig. 14a, b. SEM-photomicrographs of *Stanfieldiella imperforata*. Details of the testa. The silicified innermost layer of the outer integument is directly exposed.
Fig. 14c, d. Seeds of *Tripogandra amplexicaule* and *T. pflanzii*, respectively. Note the deep micropylar collar around the operculum.
Fig. 14e, f. Details of seed coat of *T. pflanzii*. The silica bodies in the inner layer of the oi are faceted; the radial cell walls of this layer are thickened.