

Comparative structure of ovules and seeds in *Rafflesiaceae*

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Abstract: The genera of the *Rafflesiaceae* show a marked diversity in the structure of their ovules and seeds. Evolutionary trends are recognizable in ovule orientation and number of integuments. A change from anatropous ovules in *Apodantheae* and *Mitragastemoideae* towards incomplete anatropy in *Rafflesieae* and orthotropy in *Cytineae* occurs, next to a change from bitegmic ovules in *Apodantheae* towards unitegmy with rudimentary outer integuments in *Rafflesieae* and *Cytineae* and full unitegmy in *Mitragastemoideae*. — The differences in ovule structure are clearly reflected in the seeds. The seeds are essentially exotegmic, have very small embryos and an oily endosperm. — Seed structure strongly confirms the existing subfamilial classification and supports additional arguments for the generic status of *Apodanthes*. It does not support a separate status of the genus *Berlinianche*. In *Rafflesiaceae*, seed micromorphology is only of limited use at the species level. As far as known seed dispersal is endo- or exozoochorous in all genera.

The family *Rafflesiaceae*, and especially the type genus *Rafflesia*, has received much attention owing to its endangered occurrence, parasitic way of life, the fascinating and, in some species, huge flowers, and its intriguing flower and fruit biology.

Depending on the taxonomic point of view, the *Rafflesiaceae* comprises five to ten genera and about 40 to 50 species. The traditional familial classification by HARMS (1935) was emended by MEIJER (1993) as follows:

Subfam. *Mitragastemoideae* is monogeneric and parasitizes mostly *Fagaceae* (MEIJER & VELDKAMP 1993). *Mitragastema* has one polytypic Asian species recorded from India to S China, Japan, Taiwan, and Malesia and one American species occurring from Mexico to Guatemala and NW Colombia, only discovered in 1934.

Subfam. *Rafflesioideae* has three tribes.

Tribe *Rafflesieae* with *Rafflesia*, having about 14 species in Malesia, *Sapria* with one to three species in SE Asia, and *Rhizanthes* with two species in W Malesia. All members have *Tetrastigma* (*Vitaceae*) as host plants.

Tribe *Apodantheae* with *Apodanthes*, having one or several species in tropical America, mainly parasitizing *Flacourtiaceae*, *Pilostyles* with about 18 species in tropical America, one species in Persia and one to three in Western Australia, and *Berlinianche* with two species in East Africa. *Pilostyles* and *Berlinianche* species are often very specific parasites on *Mimosaceae*, *Fabaceae*, or *Caesalpiniaceae*.

Tribe Cytineae with two genera, *Cytinus* and *Bdallophyton*. *Cytinus* has two species in the Mediterranean parasitizing *Cistus* and six species in S Africa and Madagascar on various woody host plants, including *Asteraceae* and *Rutaceae*. One of the Madagascan species was considered as a separate genus, *Botryocytinus*, by WATANABE (1936). *Bdallophyton* has two species in Central America, parasitizing on *Burseraceae* and other hosts.

Differences in opinion exist on the taxonomic level of the suprageneric taxa, not on their mutual delimitation. By some taxonomists the *Mitrastemoideae*, *Apodantheae*, and *Cytineae* have been or are still considered as separate families: *Mitrastemonaceae* MAKINO 1911 (see CRONQUIST 1981), *Apodanthaceae* VAN TIEGHEM 1898 (see suggestion by TAKTHAJAN 1980) and *Cytinaceae* BRONGNIART 1824 (see FARR & al. 1979). There is also discord about the justification of the genera *Berlinianche*, which is closely related to *Pilostyles*, and *Botryocytinus*, which may be merged with *Cytinus*.

The embryological and seed anatomical knowledge of the *Rafflesiaceae* is scattered. Most papers deal with one species only. Classical studies are those of SOLMS-LAUBACH (1874, 1898) and ERNST & SCHMID (1913) on *Rafflesia*, and of ENDRISS (1902) on *Pilostyles*. Short reviews on the embryology have been given by DAVIS (1966) and YAKOVLEV (1981), on seed anatomy by CORNER (1976) and TAKTHAJAN (1988). Embryologically the family is of special interest for the occurrence of bitegmic and unitegmic ovules, and of anatropous and orthotropous ovules. The ovule is tenuinucellate in all taxa studied. The archesporial cell functions directly as megasporangium mother cell, and no parietal cells are formed. The tetrads are linear or T-shaped and the chalazal megasporangium develops into a *Polygonum* type of embryo sac. The mature embryo sac is 7-nucleate by fusion of the polar nuclei before fertilization. Fertilization is porogamous. Endosperm formation is initially nuclear in *Rafflesia*, *Pilostyles*, and *Cytinus*. Cell wall formation starts after eight to sixteen free nuclei are formed. However, in *Mitrastema* the endosperm is ab initio cellular. The first division of the zygote is transverse and embryogeny probably conforms to the Solanad type.

The seeds of all species are relatively small with one mechanical layer in the seed coat, one, sometimes locally two, layers of endosperm and a small, undifferentiated embryo, in some cases even without a recognizable protoderm. The small dimensions of the embryos repeatedly have led to misinterpretations by description of the endosperm body as embryo. In the older literature this was the case in *Cytinus* by BRONGNIART (1824) and BROWN (1834), in *Pilostyles* by KARSTEN (1858) and even more recently by KUMMEROW (1962).

The fruits of all the genera may be described as berries. Data from literature on seed dispersal are poor and often speculative because of lack of field observations (KUIJT 1969).

In this paper, ovules and seeds of representatives of the genera of *Rafflesiaceae* are described as far as material was available. The data are supplemented and compared with the existing literature.

Material and methods

Material was sampled from spirit collections or herbarium sheets of the herbaria of AMD, BKF, BO, C, CA, CAY, CR, F, GH, HBG, K, L, MO, P, PRE, S, SPF, TAN, U, and USA, or collected in the field.

For standard light microscopy, material was dehydrated by means of a normal butyl-alcohol series, embedded in glycolmethacrylate, sectioned at 5 µm with glass knives and stained with periodic acid-Schiff's reagent and toluidine blue (O'BRIEN & McCULLY 1981). Material obtained from herbarium specimens was pretreated by soaking for several hours in 10% ammonia. Whole mounts were prepared by bleaching and embedding of seeds in Faure (100 ml distilled water, 60 g arabic gum, 40 g glycerol, and 100 g chloral hydrate). Hand cut sections were specifically stained with aniline sulphate, Sudan IV, iodine in potassium iodide and nigrosine dye.

For scanning electron microscopic studies, seeds were sputter-coated with gold-palladium for three minutes and observed by use of a Cambridge Stereoscan Mark 2A at 10 KV or an ISI DS 130 at 9 KV. Ovules were dehydrated in an ethyl alcohol series and critical point dried with liquid CO₂. Seeds covered with dust or fungal hyphae were soaked in a 10% ammonium solution and cleaned by ultrasonic treatment.

Results

Rafflesia R. BROWN (Rafflesieae). Ovules (Fig. 1 a, b). The mature ovules of *Rafflesia micropylora* are about 700 µm long, with a micropylar and raphal part about 280 and 160 µm in width, respectively, and a funicle about 90 µm in width. Ovules are basically anatropous, however, often irregularly or incompletely curved due to the reduction of the outer integument, as a result of which the apical part of the ovule is not bound to the raphe. The nucellus is tenuinucellate without parietal cells. The embryo sac is directly covered by a more dense-staining nucellar epidermis. The cells of the nucellar epidermis at the flanks of the embryo sac are one-layered. However, at the apex they are radially stretched and often periclinally divided forming a nucellar cap up to three cells high. The embryo sac conforms to the *Polygonum* type.

The inner integument is initiated by a ring of dermal cells and becomes two cells thick only, except for its apex, where the micropylar canal is closed by pericinal divisions of the inner layer. Cells of the inner and outer layer already clearly differ in the mature ovule. The inner layer is about 15 cells in circumference, the cells are small, cubic in cross section about 15 µm deep, and somewhat elongated in length. Inner cell wall and adjoining nucellar walls both have a thin cuticle. The outer layer of the inner integument is about ten cells in circumference, the cells are distinctly enlarged, more or less cubic at longitudinal and cross sections, with bulging outer pericinal walls. Anticinal walls are about 60 µm high.

The outer integument is reduced, visible as a broad, insignificant rim proximal to the basis of the inner integument, and appears in sections as a zone, three to four cells thick, of periclinally divided, small, protoplasm-dense dermal cells.

Seeds (Figs. 1 c-f, 2 a-f). The seeds of *Rafflesia rochussenii* are 735–900 µm in length, 340–400 µm in width (mean 825 × 370 µm). Seeds are more or less J- or irregularly-shaped, depending on the extent of the anatropous curvature of their ovules.

Seeds are composed of two unequal parts: The distal part contains the embryo and endosperm and is covered by the derivatives of the inner integument (tegmen). The exotegmen forms the protective layer. Exotegmic cells are more or less arranged in longitudinal rows, polygonal in surface view, with a diameter up to 120 µm and about 90 µm deep. Tegmic cells become smaller towards the micropylar and chalazal ends of the seed. Exotegmic cells are U-shaped in section with strongly thickened

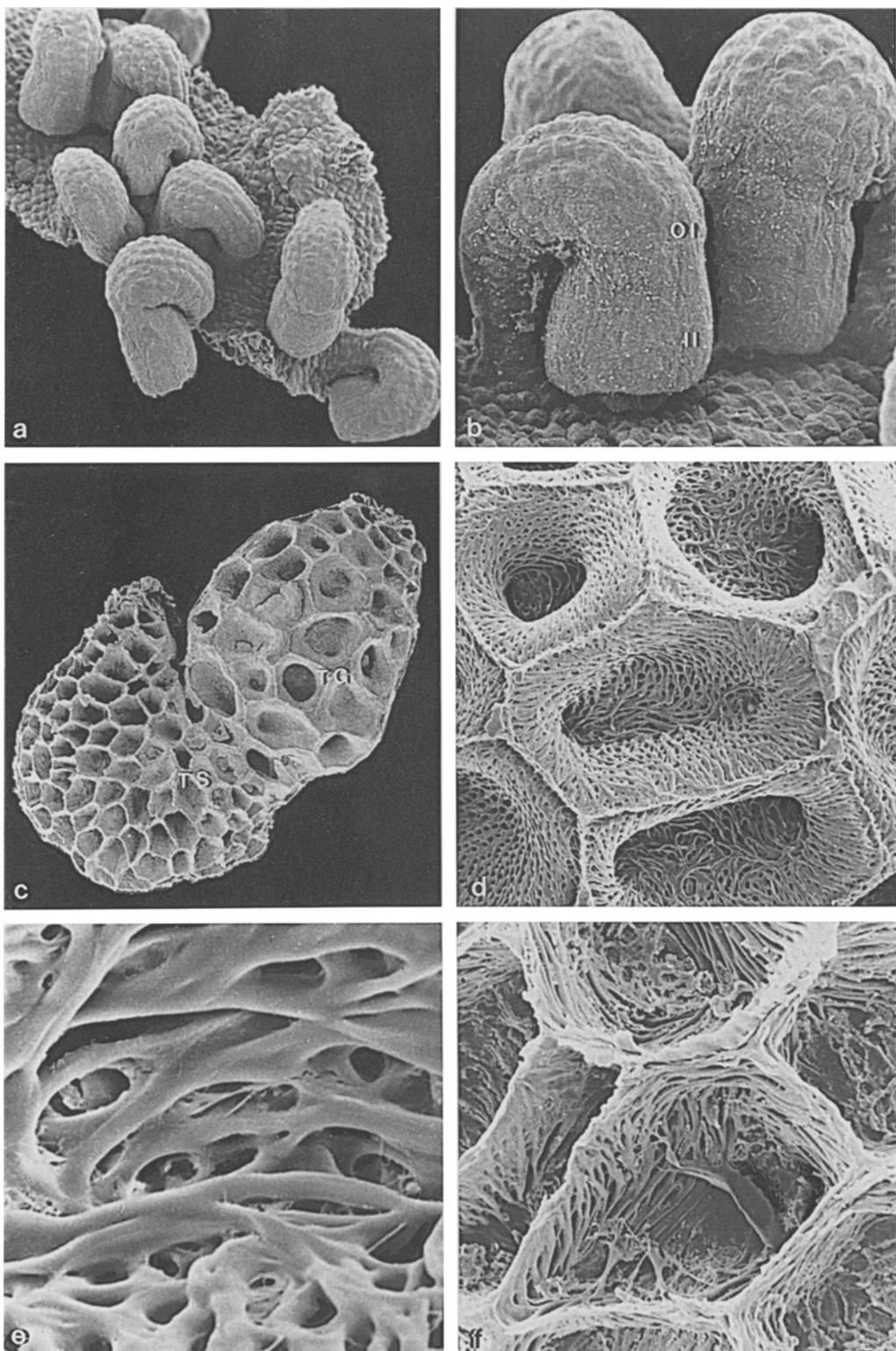


Fig. 1. Ovules and seeds of *Rafflesia*. *a, b* *Rafflesia micropylora*, developing ovules showing anatropy and reduced outer integument, $\times 80$ and $\times 190$. *c-f* *Rafflesia gadutensis*, *c* mature seed, outer pericinal walls removed, $\times 70$; *d* and *e* detail of the tegmic part, $\times 285$ and $\times 2200$; *f* detail of testal part of the seed coat, $\times 470$. — *II* Inner integument, *OI* outer integument, *TG* tegmic part, *TS* testal part of the seed

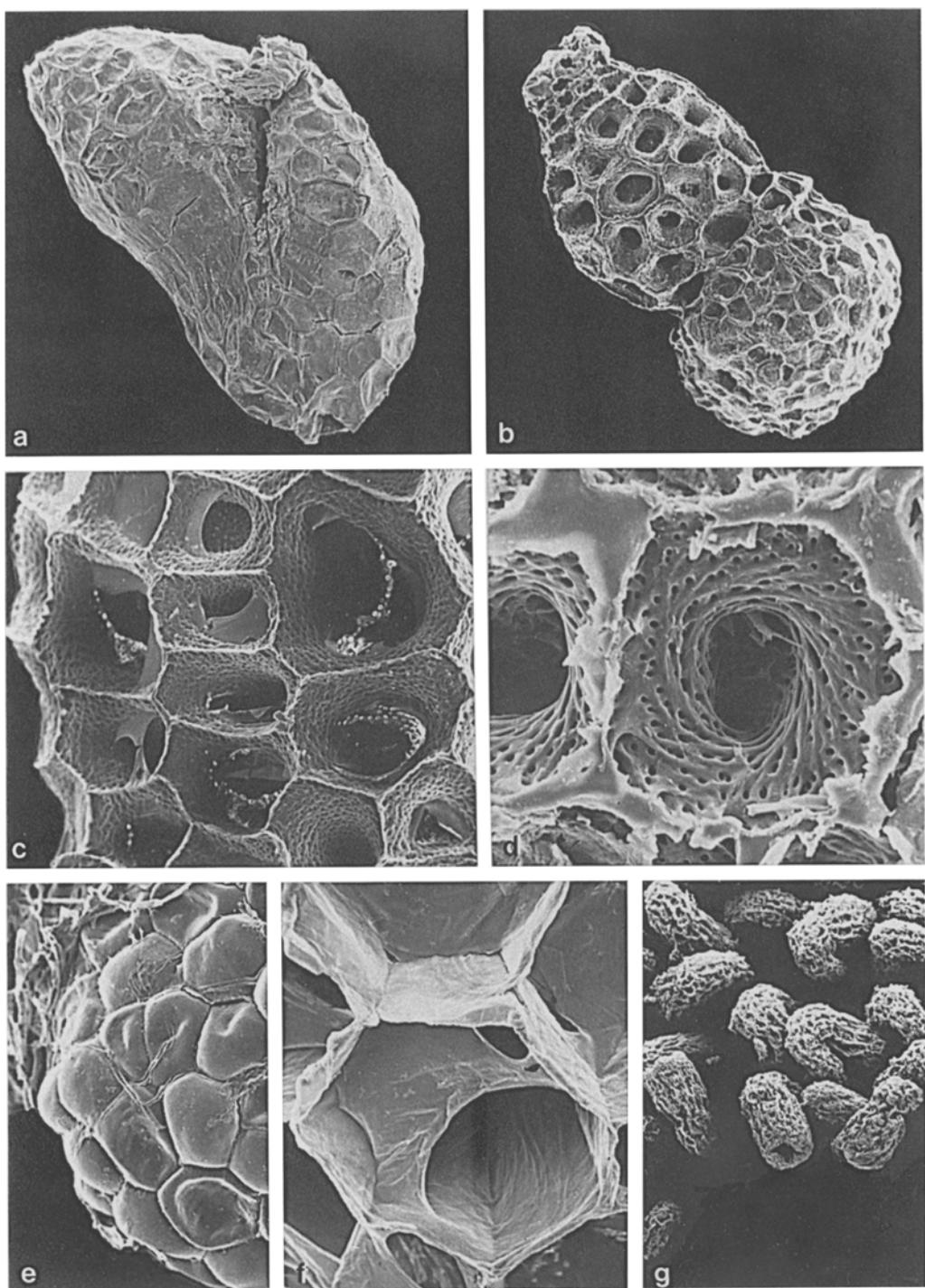


Fig. 2. Seeds of *Rafflesia rochusenii*. a, b mature seeds before and after removal of the outer walls, $\times 95$ and $\times 105$; c, d details of tegmic part, $\times 265$ and $\times 850$; e, f details of testal part, $\times 315$ and $\times 635$. g *Sapria himalayensis*, ovules, $\times 95$

and pitted anticlinal and inner periclinal walls. Pits are mostly simple, sometimes branched, with the inner side of these walls often with a spiral structure. Outer periclinal walls of the exotegmen are thin and slightly sunken in, or sometimes cracked or collapsed in the dry seed, with raised anticlinal boundaries. The cell lumina contain tanniniferous substances. In the fully mature seed, a yellowish, compact and cutinized pellicle about 20 µm thick and without a distinct cell pattern lies under the exotegmen. From developmental stages, this pellicle appears to be developed from the thickened outer, and especially inner periclinal walls of the endotegmen. The pellicle has a simple perforation at its chalazal side and is not continuous in the micropyle. The micropyle is lined with parenchyma cells of the inner layer of the inner integument.

The proximal part of the seed is mostly somewhat smaller and consists of the tissue of the rudimentary outer integument (testa), the chalaza, the raphe and a part of the funicle. Outer testal cells are smaller, up to 80 µm in diameter, and have only slightly thickened anticlinal and inner periclinal walls. The outer periclinal walls are thin, mostly bulging outwards in fixed seeds and collapsed in dried seeds.

Endosperm is one-layered in the mature seed and envelopes the embryo. Endosperm cells are about 30 to 40 cells in number, oily, thin-walled, also the outer walls, and of about the same size and appearance as the cells of the embryo.

The embryo is small, without differentiation of cotyledons and apical meristems and still without dermatogen initials. The embryo contains two juxtaposed basal cells and an apical part of three to five tiers of four cells each. No distinct suspensor is present. The arrangement of the embryonal cells is often more irregular.

The ovules and seeds of other *Rafflesia* species observed have essentially the same structure. No important differences could be observed, although small variation exists in seed size and in the structure of the inner surface of the exotegmic cell walls. This is in accordance with the data from literature (ERNST & SCHMID 1913 on *R. patma* and OLAH 1960 on *R. arnoldii* and *R. gadutensis*).

Specimens observed. *Rafflesia micropylora* MEIJER: Sumatra, W. DE WILDE 13954 (L); W. DE WILDE & B. DE WILDE-DUIJFJES 16566 (L); W. DE WILDE & B. DE WILDE-DUIJFJES 19272B (L); Borneo, HALLIER s.n. (BO); RIJKSEN s.n. (L); *R. gadutensis* MEIJER: Sumatra, J. MACDOUGAL 4681 (MO); MEIJER s.n. (AMD); *R. keithii* MEIJER: Sabah, MEIJER 1992 s.n.; *R. rochussenii* TEIJSM. & BINNEND.: Java, ADER s.n. (BO).

Rhizanthes DUMORT. (*Rafflesieae*). Seeds (Fig. 3 a-f). The mature seeds of *Rhizanthes* and according to the data of SOLMS-LAUBACH (1874, 1898) also the ovules, fully agree in structure with those of *Rafflesia*, no important differences could be discerned. Seeds of *Rhizanthes zippelii* are 630–800 µm in length and 280–350 µm in width (mean 750 × 320 µm).

Specimens observed. *Rhizanthes zippelii* (BLUME) SPACH.: Sumatra, MEIJER 17005 (BO); *R. lowii* (BECC.) HARMS: Sumatra, W. DE WILDE & B. DE WILDE-DUIJFJES 19272 A (L).

Sapria GRIFFITH (*Rafflesieae*). Ovules (Fig. 2 g). The ovules of *Sapria himalayana* closely resemble those of *Rafflesia* and *Rhizanthes*. The ovules are tenuinucellate, irregularly or incompletely anatropous with a reduced outer integument and a two-layered inner integument. Immature ovules at the stage of megasporangium mother cell are about 210 × 105 µm in size, the funicle is about 55 µm in diameter.

Mature fruits and seeds were not available for study.

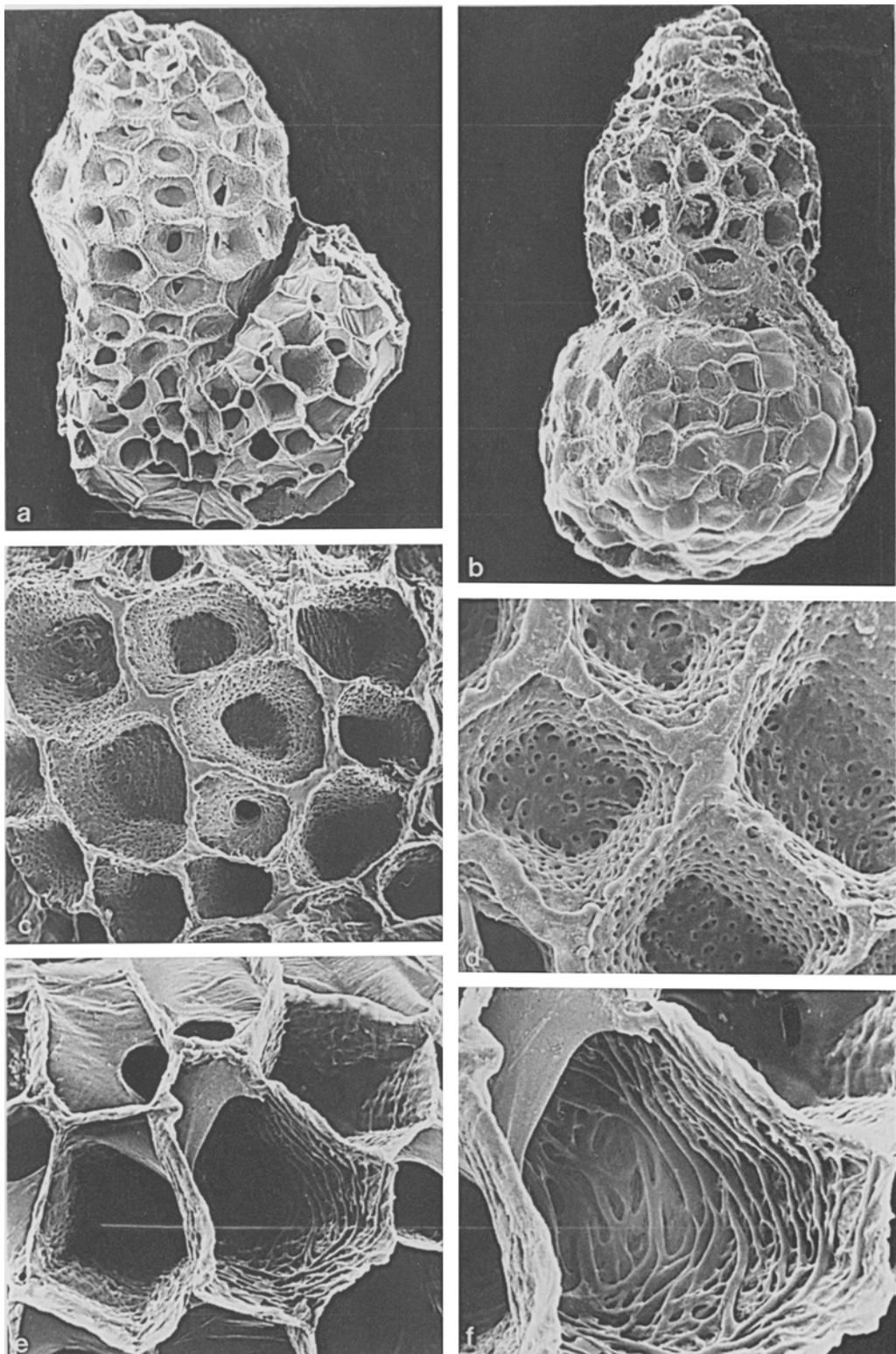


Fig. 3. Seeds of *Rhizanthes zippelii*. *a, b* mature seeds, $\times 115$ and $\times 135$; *c, d* detail of tegmic part, $\times 240$ and $\times 600$; *e, f* detail of testal part, some cells with fragments of the outer periclinal walls, $\times 470$ and $\times 890$

Specimens observed. *Sapria himalayana* GRIFFITH: Thailand, HENNIPMAN 3007 (BKF); BEUSEKOM 18-1219 (L); BEUSEKOM & GEESINK 3715 (L).

***Apodanthes* Poit. (*Apodantheae*).** Ovules. The mature ovules of *Apodanthes caseariae* are anatropous, bitegmic, tenuinucellate, and about $290 \times 160 \times 135 \mu\text{m}$ in size. Megasporogenesis results in a linear triad, the chalazal megaspore developing into the embryo sac. Nucelli mostly contain three rows of subdermal cells at their base. The nucellar epidermis is one-layered and about ten cells in circumference. Apical cells are slightly stretched radially, but do not divide periclinally.

The inner integument is two-layered along its whole length. Both layers count about ten cells in circumference. The inner layer consists of small, densely protoplasmic cells, about $10 \mu\text{m}$ in width and elongated in the length direction of the ovule. The cells bordering the endostome are large and papillate. The outer layer consists of more or less cubic cells, about $20 \mu\text{m}$ in width, with large vacuoles. Cells above the level of the nucellar apex are radially stretched and help to close the micropyle. A distinct cuticular layer occurs between the nucellus and inner integument.

The outer integument is one-layered, sometimes locally two-layered at the flanks, about 18 cells in circumference. The funicle and adjacent raphe are of a very unusual structure. Funicular and raphal tissues are composed of very few cells showing intercellulars and are not bound by a continuous epidermis at the inner side.

Seeds (Fig. 4 a–c). The seeds of *Apodanthes caseariae* are $425–560 \mu\text{m}$ in length, $320–380 \mu\text{m}$ in width (mean $510 \times 350 \mu\text{m}$), with a length : width ratio of 1.5. Seeds are mostly more or less oval-shaped with the endostome hidden in the funicular groove. Testa cells are more isodiametric, papillose near the micropyle and chalaza and collapse around the middle of the mature seed and locally reflect the pattern of the exotegmen. Anticlinal boundaries are locally sunken. The cuticle sometimes has short local striae.

The testa is composed of one layer of thin-walled, tanniniferous cells. The outer layer of the inner integument is differentiated into a one-layered exotegmen. The exotegmic cells are more or less cubic in shape, about $50 \mu\text{m}$ in width, with tanniniferous plasm and degenerated nuclei. The cells are U-shaped owing to strongly thickened antecinal and inner pericinal walls with many, long and narrow pits. The outer wall facing the testa is thin. The inner layer of the tegmen is fully compressed, with a thin cuticular layer between tegmen and endosperm.

The embryo is small, without dermal initials, and surrounded by one layer of endosperm, containing abundant oil and protein bodies.

Specimens observed. *Apodanthes caseariae* Poit.: Costa Rica, LUIS GÓMEZ 19447 (MO); Panama, NEE 6776 (MO); Colombia, DE BRUIJN 1093 (U); French Guyana, DE GRANVILLE 1700 (CAY); Brasil, Y. MEXIA 4590 (GH).

***Pilostyles* GUILL. (*Apodantheae*).** Ovules (Fig. 5 a, b). The ovules of *Pilostyles* resemble those of *Apodanthes* in general characters. The mature ovules of *Pilostyles ingae* are anatropous, bitegmic, and tenuinucellate. They vary in length from $230–270 \mu\text{m}$, in width from $110–140 \mu\text{m}$ (mean $245 \times 120 \mu\text{m}$), with a length : width ratio of 2.0. Ovules are initiated by pericinal divisions of the subdermal, placental layer. The ovule primordium is initially formed by a single row of subdermal cells, covered by a one-layered epidermis, the subdermal part becoming more-rowed by divisions parallel to the longitudinal axis at the chalazal part, however not so in the basal

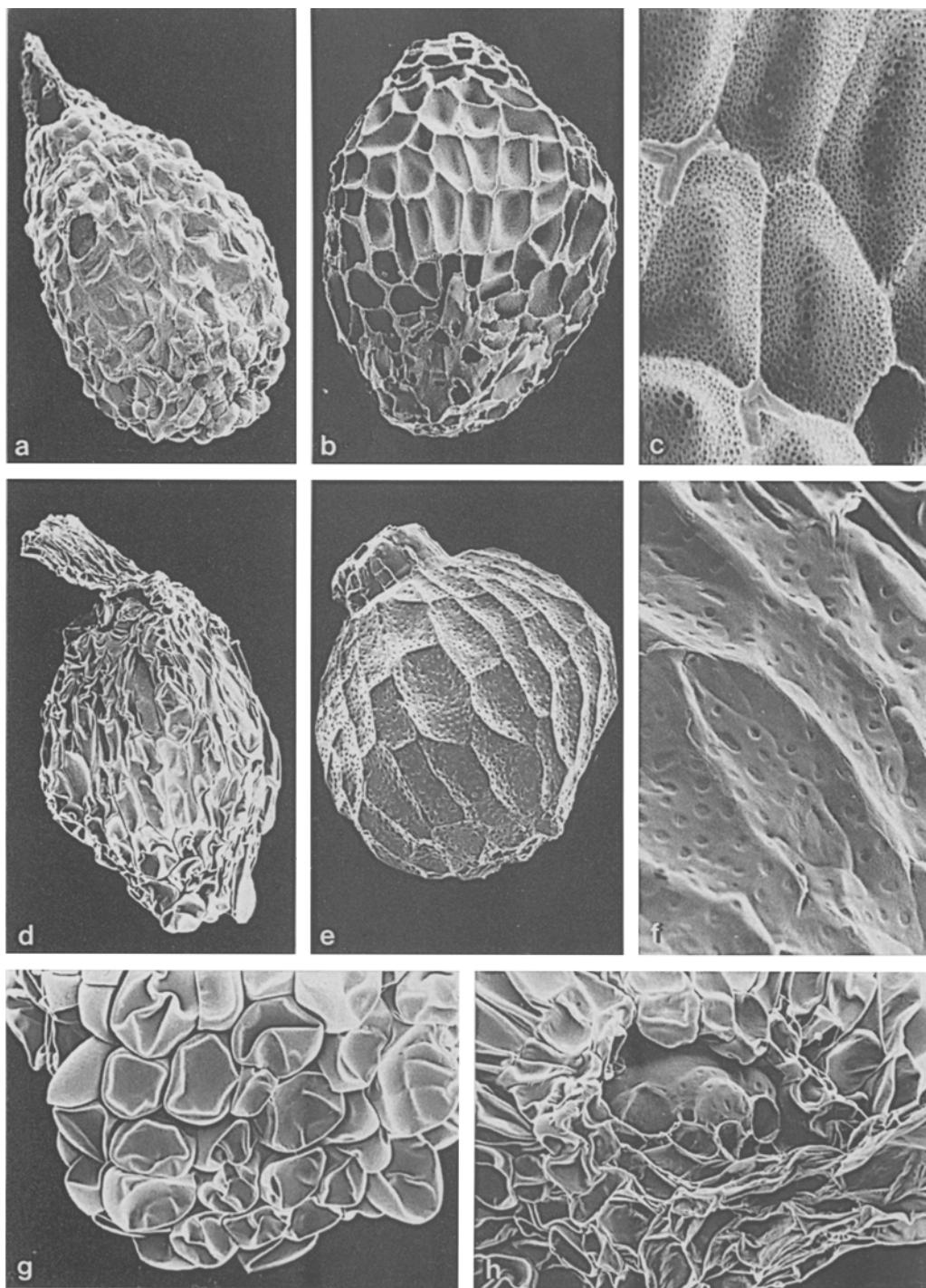


Fig. 4. Ovules and seeds of *Apodantheae*. a-c *Apodanthes caseariae*; a seed with testa, $\times 105$; b seed after removal of the testa, $\times 135$; c detail of tegmic cells, $\times 580$. d-h *Berlinianche aethiopica*; d, e seed with and without testa, showing surface of testa and tegmen, respectively, $\times 100$ and $\times 130$; f detail tegmen, $\times 630$; g, h details of chalaza and micropyle, $\times 350$ and $\times 270$

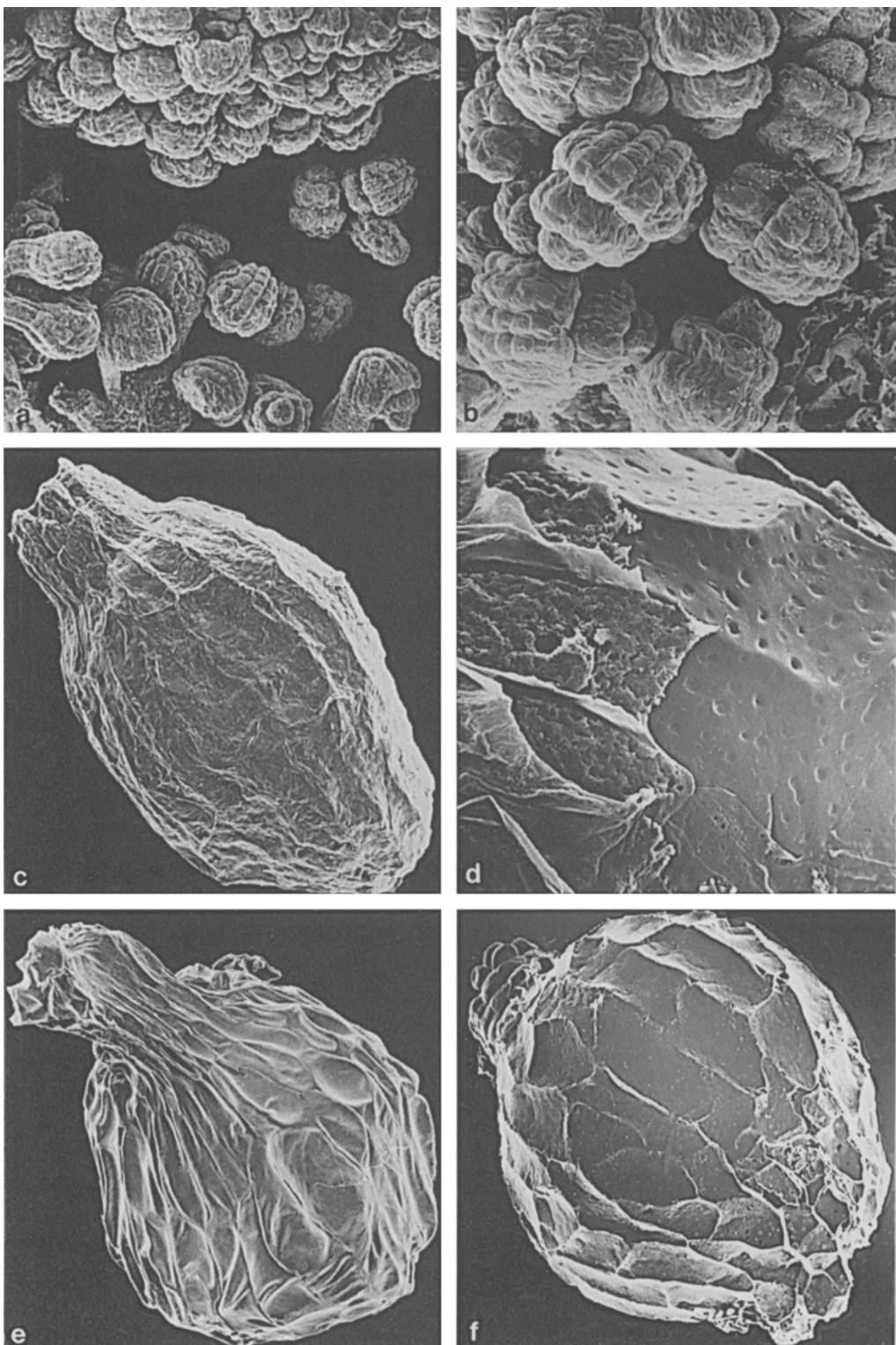


Fig. 5. Ovules and seeds of *Pilostyles*. *a, b* *Pilostyles ingae*, ovarian placenta with developing ovules showing initiation of inner and outer integuments, $\times 100$ and $\times 220$. *c, d* *Pilostyles ulei*, mature seed and detail of seed coat with testa removed to show pitted tegmen, $\times 250$ and $\times 1050$. *e* *Pilostyles thurberi*, $\times 160$. *f* *Pilostyles hamiltonii*, $\times 145$

and apical parts. The single archesporial cell directly functions as megasporangium, forming a linear or T-shaped tetrad, with the chalazal megasporangium functioning to form a *Polygonum* type embryo sac. The embryo sac is covered by a one-layered nucellar epidermis of about six to eight cells in circumference. The apical cells of the nucellus are slightly enlarging, but not dividing.

The inner integument is initiated shortly before or almost simultaneously with the outer one by cross or oblique divisions in a ring of one or two underlying, dermal cells. The inner integument is two-layered, with cells of the outer layer enlarging, more or less cubic in cross section and about 10 µm in width, and cells of the inner layer smaller and more elongated. The funicle contains large intercellular spaces.

The outer integument is about 16 cells in circumference, and only one, or locally two layers thick. The cells are elongated in the longitudinal direction of the ovule, about 20 µm in width and distinctly larger than the cells of the outer ovular tissues. Outer periclinal walls are convex. The micropyle of the mature ovule is formed by both the inner and outer integument.

Seeds (Figs. 5 c–f, 6 a–f). The seeds of *Pilostyles ulei* are 290–370 µm in length, 190–250 µm in width (mean 330 × 215 µm) with a length : width ratio of 1.5. Seeds are almost circular in shape with a somewhat nozzle-shaped micropylar and a small chalazal extension. During fruit development, the seeds become closely surrounded by the expanding placental tissue.

Cells of the one-layered testa are relatively large, slightly tanniniferous, thin-walled, with their outer tangential wall bulging in fresh seeds, however, collapsing in dry seeds. Testa cells at the chalaza are tangentially stretched up to about 70 µm, with outer cell wall smooth, without cuticular ornamentation. The testa cells press into the tegmen, forming the facets on the outer surface of the tegmen. Both the outer and inner layer of the tegmen are thickened and form the mechanical layer of the seed. Cell walls are strongly thickened all around, with broad pits. Exotegmic cells are more or less cubic in cross section, about 30–40 µm in thickness, smaller towards the micropyle and chalaza. Cells of the endotegmen are much smaller, about 15 µm in thickness, and elongated in the longitudinal direction of the seed, with a thin cuticular layer between tegmen and endosperm.

The embryo is surrounded by a one- or two-layered endosperm. The outermost cells of the endosperm are larger. The cells are thin-walled and provided with oil and protein bodies.

The embryo is small, without differentiation of cotyledons, without dermatogen initials and mostly about five cells high. The apical cells may be divided longitudinally.

The seeds of the other *Pilostyles* species studied have essentially the same structure, but may differ slightly in shape, size, and funicular appendage. Mature seeds vary in shape from almost globular to elliptic with a length : width ratio of 1.0 in *P. goyazensis* to 1.5 in *P. blanchetii* and *P. caulotreti*. Mean seed size varies from about 325 × 230 µm in *P. ingae*, 325 × 280 µm in *P. mexicana*, 345 × 250 µm in *P. pringlei*, 350 × 335 µm in *P. goyazensis*, 390 × 265 µm in *P. blanchetii*, 400 × 270 µm in *P. caulotreti*, 450 × 325 µm in *P. thurberi* to 525 × 375 µm in *P. hamiltonii*.

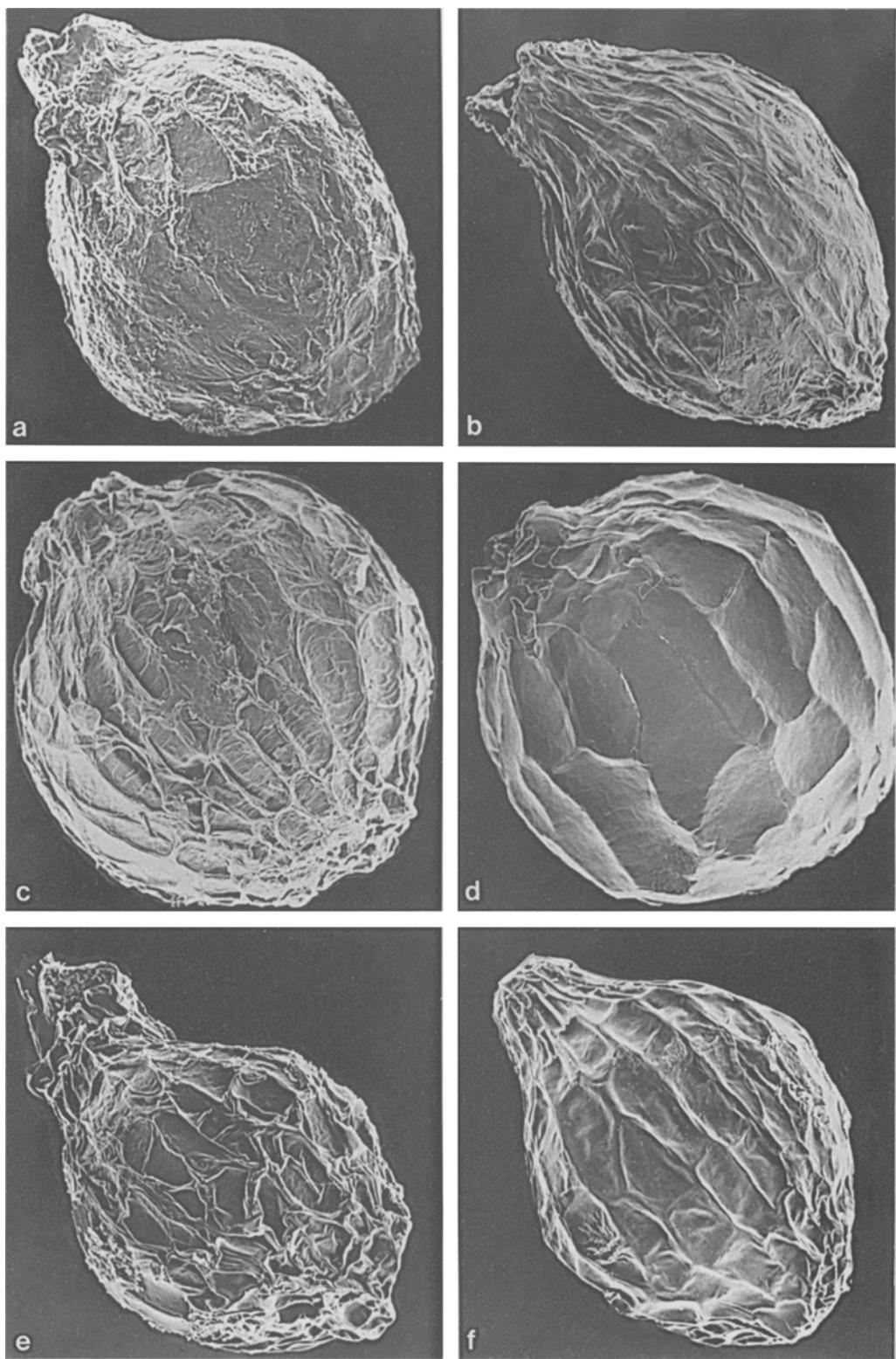


Fig. 6. Mature seeds of *Pilostyles* species. *a* *Pilostyles blanchetti*, $\times 200$. *b* *Pilostyles cau-*
lotreti, $\times 185$. *c, d* *Pilostyles goyazensis*, seeds showing testa and exotegmen, $\times 180$ and
 $\times 205$. *e* *Pilostyles mexicana*, $\times 190$. *f* *Pilostyles pringlei*, $\times 195$

Specimens observed. *Pilostyles berterii* GUILL.: Bolivia, J. N. ROSE 18849 (GH); *P. blanchetii* (GARDN.) R. BR.: Brasil, IRWIN & al. 21129 (MO); ULE 2699 (HBG); *P. caulotreti* HOOK. f.: Venezuela, LIESNER & GONZALEZ 10950 (MO); *P. goyazensis* ULE: Brasil, IRWIN & al. 11544 (MO); IRWIN & al. 14480 (MO); *P. hamiltonii* C. A. GARDNER: Australia, GRIMWADE ex. (L); L. DEBUHT 4183; *P. haussknechtii* BOISS.: Persia, J. BORNMÜLLER s.n. (L); *P. ingae* (KARSTEN) HOOK. f.: Brazil, VENTURELLI & al. s.n. (SPF); *P. mexicana* (BRAND.) ROSE: Mexico, BREEDLOVE 27233 (MO, CA); Honduras, WILLIAMS & MOLINA 11955 (MO); *P. pringlei* (S. WATS.) ROSE: Mexico, PRINGLE 1949 (S, GH); *P. thurberi* A. GRAY: USA, R. J. BARR 273 (MO); W. MEIJER s.n. (MO); *P. ulei* S.-LAUB.: Brasil, IRWIN & al. 14322 (MO); ULE 3096, 3097 (HBG).

Berlinianche (HARMS) VATTIMO (Apodantheae). Seeds (Fig. 4 d-h). The seeds of *Berlinianche aethiopica* are 460–560 µm in length, 300–420 µm in width (mean 510 × 385 µm), with a length : width ratio of 1.3. Seeds are somewhat irregular in shape, with a persistent funicle and a chalazal swelling composed of papillate testa cells.

The testa is tanniniferous, thin-walled and one-layered only. Testa cells are elongated, up to four times their width at the middle of the seed. Anticinal boundaries are sunken and the cuticle is smooth. The tegmen is 40 to 65 µm in thickness and has two mechanical layers; an outer layer of large, more or less isodiametric cells, 25 to 45 µm thick, and an inner layer of smaller, elongated cells about 15 to 20 µm thick. Cells of the tegmen are thickened all around, with broad, sometimes branched pits. The surface of the tegmen is distinctly impressed by the testa cells, and shows pits.

The embryo and endosperm are as in *Pilostyles*.

The seeds agree in general structure with those of *Pilostyles* species.

Specimens observed. *Berlinianche aethiopica* (WELW.) DE VATTIMO: Zambia, RICHARDS s.n. (K); Zimbabwe, MONRO 457 (K); WILD 3842 (MO, K).

Cytinus L. (Cytineae). Ovules (Fig. 7 a). The ovules are embedded in a colourless, mucilaginous substance, produced by the epidermal layer of the placentae and funicles. The mature ovules of *Cytinus rubra* are small, vary in length from 180 to 220 µm, in width from 60 to 85 µm (mean 200 × 70 µm), with a length : width ratio of 2.9. Ovules are fully orthotropic and have a tenuinucellate nucellus without parietal cells. The nucellar epidermis is one-layered, about seven cells in circumference, and does not form a nucellar cap. Embryo sacs are of the *Polygonum* type.

The inner integument is dermal in origin and initially two-layered, overgrowing the nucellus and forming a distinct micropylar canal. The inner integument later becomes three-layered by periclinal divisions of its inner layer. Cells of the outer layer enlarge and count about 11 cells in circumference.

The outer integument is arrested in its development, dermal in initiation, two-layered, and forms an irregular rim at the ovular base. The outer integument contributes to the formation of the mucilage.

Seeds (Figs. 7 b-f, 8 a, b). During fruit development, the seeds become embedded in the tissues of the placentae and funicles. Seeds in the fruit are about halfway enveloped by the irregularly lobed, parenchymatous outer integument. However, they become detached from it during seed release.

The seeds of *Cytinus hypocistis* are 180–220 µm in length, 130–150 µm in width (mean 190 × 140 µm), with a length : width ratio of 1.4. Seeds are urceolate with micropyle and hilar scar at opposite sites.

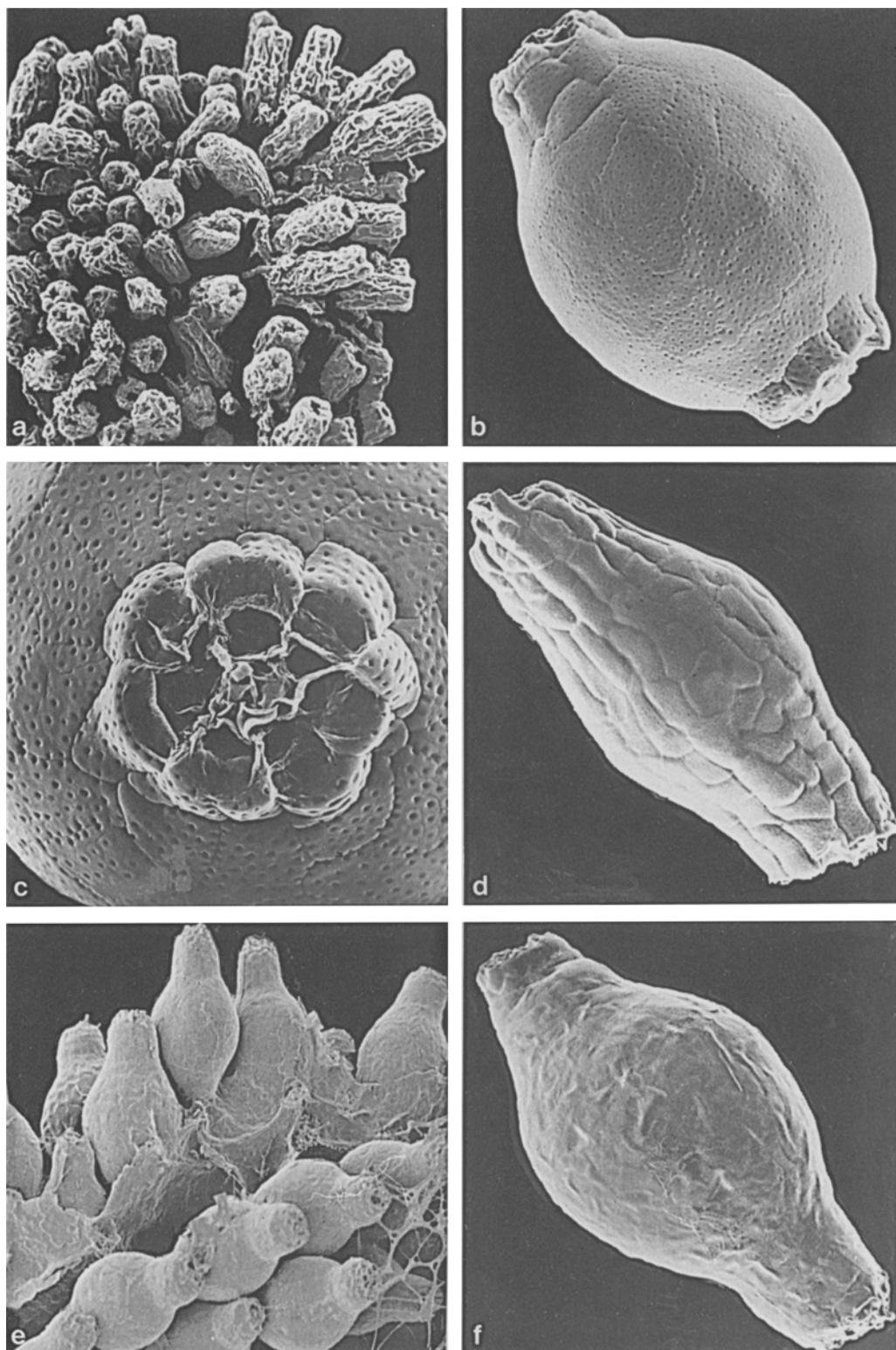


Fig. 7. Ovule and seeds of *Cytinus*. *a–c* *Cytinus hypocistis*; *a* ovules, $\times 140$; *b* seed, $\times 440$; *c* detail of the micropyle, $\times 880$. *d* *Cytinus* spec., $\times 205$. *e, f* *Cytinus glandulosus* seeds on placenta and seed in detail, $\times 50$ and $\times 150$

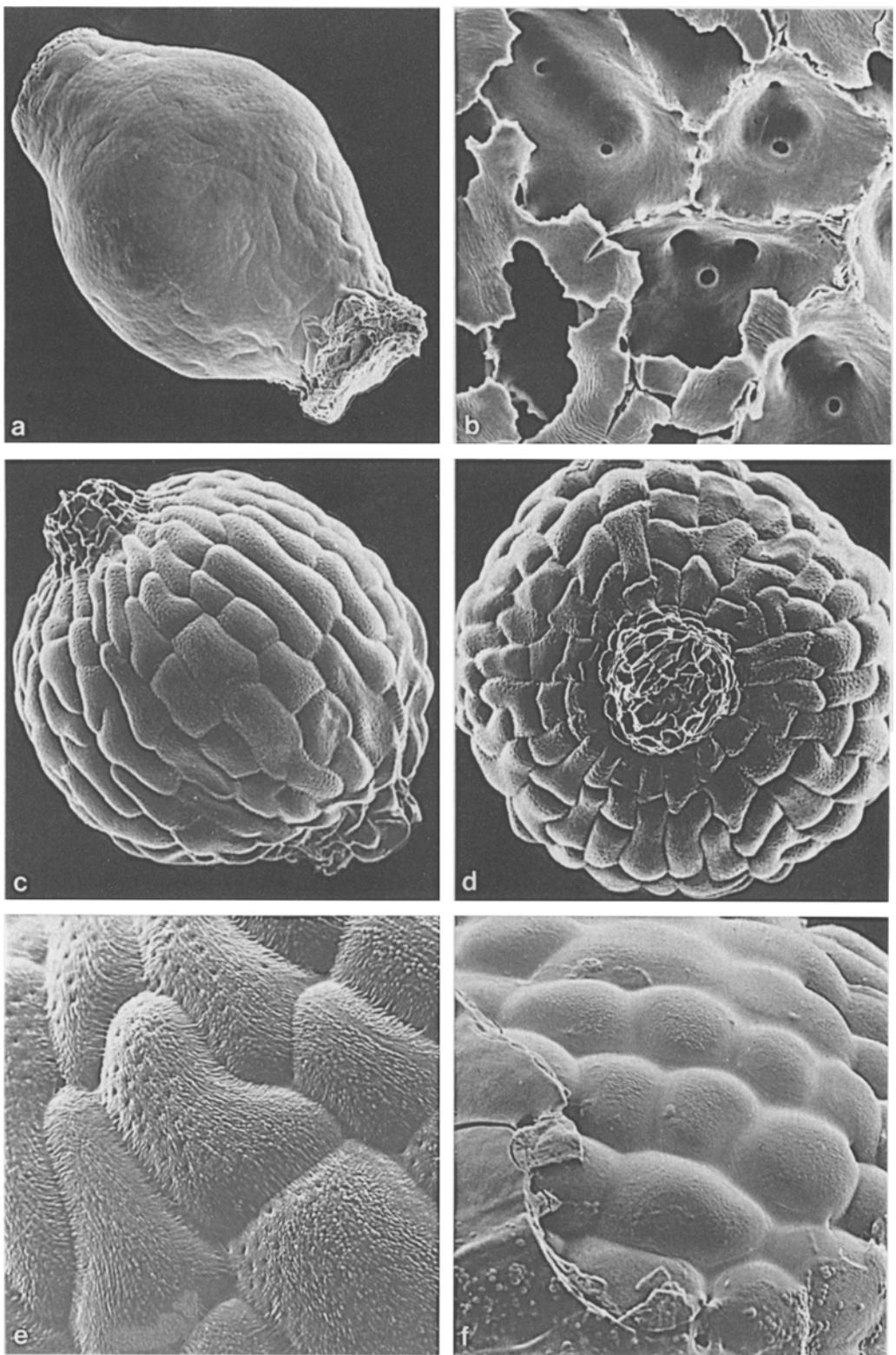


Fig. 8. Seeds of Cytineae. *a, b* *Cytinus capensis*, seed and detail of tegmen after partial removal of the outer walls, $\times 180$ and $\times 890$. *c–e* *Bdallophyton oxylepis*, seed, micropyle, and detail of seed coat, respectively, $\times 225$, $\times 230$, and $\times 1200$. *f* *Bdallophyton americanum*, detail of seed coat, $\times 400$

The outer layer of the mature seed is sclerotic, composed of one layer of cells only, about 15 cells in circumference, and represents the exotegmen. The exotegmic cells are thickened all around and have cell walls with simple pits. Tegmic cells are irregular to polygonal in surface view, measuring about $25 \times 30 \mu\text{m}$. Outer periclinal walls have about 50 pits. Anticlinal walls are straight or slightly curved with sunken anticlinal boundaries. Cells become smaller towards the micropyle and hilum. The exotegmic layer is about $20 \mu\text{m}$ thick. The inner tegmic layer(s) and nucellus are fully compressed in the mature seed. During seed maturation the intraovarial tissues become mucilaginous and solidify into a brown more compact mass.

The endosperm is thin-walled, contains oil and protein bodies and closely adheres to the embryo.

The mature embryo is small, about six layers long and, by longitudinal divisions of the basal cells, composed of about ten or more cells in length. The embryo has an irregular cellular arrangement and sometimes shows dermal initials by periclinal division of the cells (see figs. in GUZOWSKA 1964 and BERNARD 1903).

The seeds of the other *Cytinus* species differ from each other in shape, size, cell pattern, and micromorphology. Seeds of southern African species differ from those of the Mediterranean ones by a more irregular pattern of the testa cells, by more curved anticlinal walls and a distinctly larger size. Seeds of all species have a more rounded central part. However, the micropylar and hilar extensions are more pronounced in the African species. The seeds of the two Mediterranean species closely resemble each other and differ mainly in size.

Mean seed size in *C. ruber* is $210 \times 115 \mu\text{m}$, in *C. capensis* $385 \times 245 \mu\text{m}$, in *C. glandulosus* $565 \times 265 \mu\text{m}$, and in *C. baroni* $420 \times 195 \mu\text{m}$. The length : width ratios are 1.8, 1.6, 2.1, and 2.2, respectively. The seed surface of *C. glandulosus* is locally marked with a faint pattern of cuticular striae, that of *C. spec.* with a distinct striated sculpture.

No seeds were available of the Madagascan taxon (*Botryo*)*cytinus baroni*.

Specimens observed. *Cytinus capensis* MARLOTH: South Africa, MARLOTH 10065 (PRE); *C. glandulosus* JUMELLE: Madagascar, H. PERRIER DE LA BATHIE 15260 (P, type); D'ALLEIZETTE (L); *C. hypocistis* LINN.: Portugal, DE NAUTET s.n. (K); *C. ruber* (LINN.) FRITSCH: Mallorca, VERKERKE s.n. (AMD); *C. spec.*: Madagascar, P. LOWRY 4063 (TAN).

***Bdalophyton* EICHLER (*Cytineae*).** Ovules. Ovules were not seen. Sections of young developing seeds indicate orthotropous and unitegmic organization. The integument is two-layered, around the micropyle more-layered. In developing seeds no indication for the presence of a rudimentary outer integument as seen in *Cytinus* was found. Epidermal cells of the placentae and inner walls of the fruit are slimy as in *Cytinus*.

Seeds (Fig. 8 c–e). The seeds of *Bdalophyton oxylepis* are $300\text{--}400 \mu\text{m}$ in length, $275\text{--}330 \mu\text{m}$ in width (mean $365 \times 300 \mu\text{m}$), with a length : width ratio of 1.2. Seeds are globular in shape, or slightly broadly elliptical. Seeds lack a raphe; the hilum and micropyle are at the opposite sides. The micropyle is a more-layered, funnel-like protuberance with a distinct micropylar canal. The hilar side of the seed is more irregular, somewhat flattened, in other cases with a basal extension, with a small hilar scar.

The outer cell layer of the seed coat is about $40 \mu\text{m}$ in thickness and functions as the main mechanical layer of the seed. The cell walls are strongly thickened all

around and regularly pitted. Pit canals in the outer walls are narrow and often branched. Pits in the outer periclinal wall are more numerous, about twice as many as in the inner periclinal one. The inner layer with flat cells is mostly compressed in the mature seed coat, present locally as a thin layer with slightly thickened walls. The seed coat is delimited against the endosperm by a distinct cuticular layer.

The outer cell layer is composed of about 26 cells in circumference and about 20 cells at the level of the micropyle. Testa cells are arranged in more or less distinct longitudinal rows. The cells in the middle region of the seed are often elongated, at most three times their width. Outer cell walls are convex, have a distinct cuticular sculpture, and do not collapse at maturity. Anticlinal walls are straight. The central field has granular or short linear foldings, however, in more elongated cells with more linear foldings oriented in the length direction of the cells. Foldings at the periphery are short, linear, and perpendicular to the anticlinal walls. The cuticle is relatively thin and reflects the underlying pits. Anticlinal boundaries are sunken. Seeds are embedded in a mucous substance. In dry fruits the mucilage is present as an extra, thin, dried-up layer on the seed surface.

The endosperm is thin-walled, with oil and protein bodies.

Seeds of *Bdallophyton americanum* closely resemble those of *B. oxylepis* in general morphology. Mean seed size is about $435 \times 340 \mu\text{m}$, with a length : width ratio of 1.3. Anticlinal cell walls of the exotegmen are more straight and the cuticular sculpture is less distinct.

Specimens observed. *Bdallophyton americanum* (R. Br.) HARMS: Mexico, HITCHCOCK & STANFORD 7317 (GH); Nicaragua, W. D. STEVENS 21780 (MO); Costa Rica, S. SALAS s.n. (CR); *B. oxylepis* (B. L. ROBINSON) HARMS: Mexico, PRINGLE 4373 (K, GH).

Mitragastema MAKINO (Mitragastoideae). Ovules. The mature ovules of *Mitragastema yamamotoi* are anatropous and unitegmic, small about $190 \times 120 \mu\text{m}$ in size, with a length : width ratio of 1.6.

The single integument is two-layered. The cells of the outer layer are relatively large, more or less isodiametric and have polyphenolic compounds at an early stage. The outer layer is about ten cells in circumference and seven to nine cells from micropyle to chalaza. The cells of the inner layer are smaller and often more elongated. Cells of the nucellar epidermis disintegrate during megasporogenesis and have fully or almost fully disappeared at the mature embryo sac stage.

The mature ovules of *Mitragastema matudae* are $220-270 \mu\text{m}$ in length and $120-165 \mu\text{m}$ in width (mean $235 \times 140 \mu\text{m}$), with a length : width ratio of 1.7.

Seeds (Fig. 9 a-e). Probably by lack of sufficient pollination, seed set is sometimes very low in *Mitragastema* (WATANABE 1936). In the material from southern Japan, the seeds collected from fruits were all sterile and without embryos. The testa cells do not increase in number during seed development, and the ovule only slightly increases in size. Mature seeds of *Mitragastema yamamotoi* are $265-315 \mu\text{m}$ in length and $195-230 \mu\text{m}$ in width (mean $305 \times 215 \mu\text{m}$), with a length : width ratio of 1.4. Almost mature seeds of *Mitragastema matudae* have a mean size of $280 \times 190 \mu\text{m}$.

The seed coat is mainly composed of the outer testal layer; the inner layer is only represented by remnants pressed against the exotesta and along the inside of the micropylar canal. The inner walls of the exotesta cells become strongly thickened and the outer walls remain thin. The cells are strongly tanniniferous.

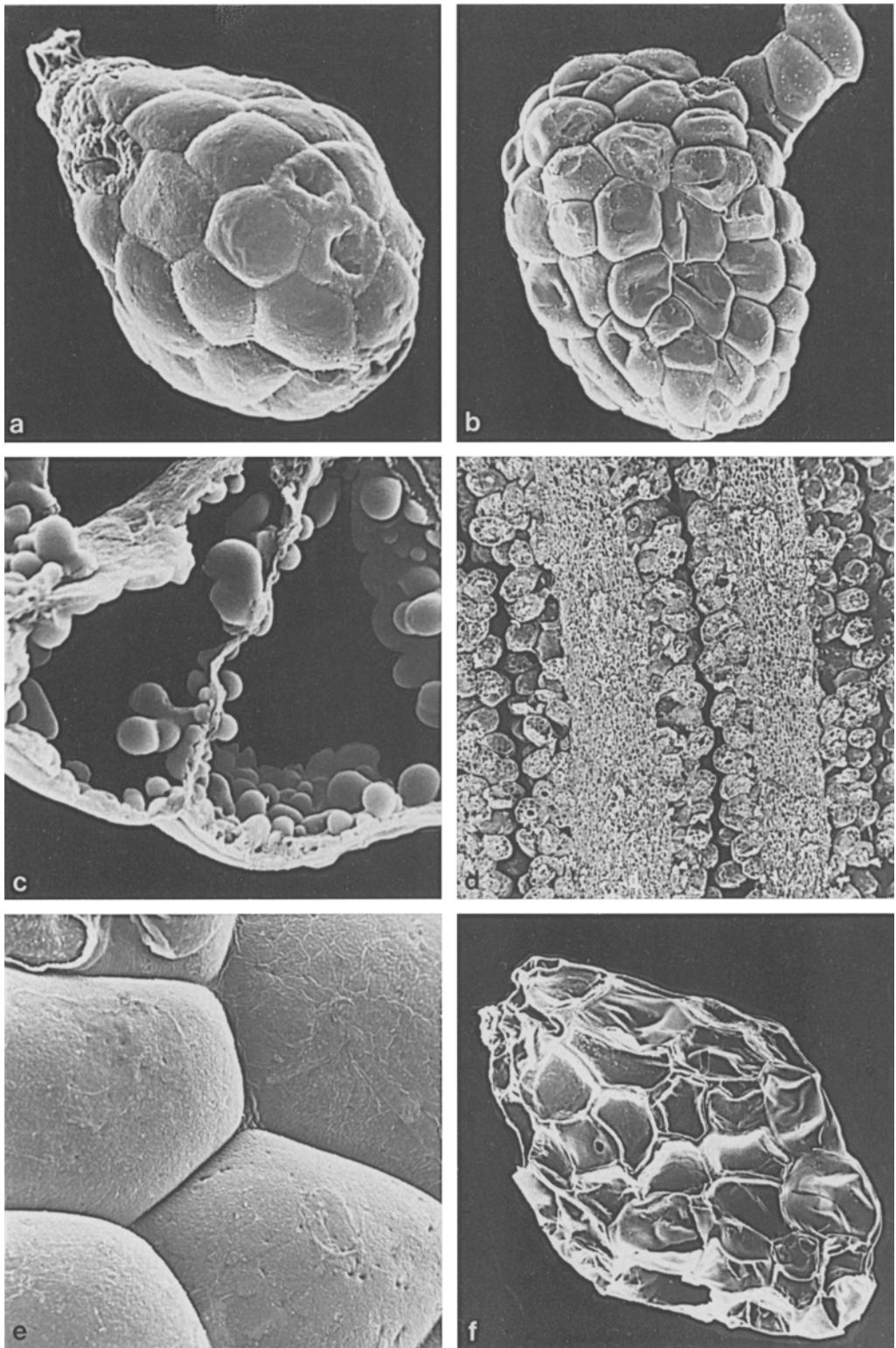


Fig. 9. Ovules and seeds of *Mitrastema*. *a–e* *Mitrastema yamamotoi*; *a, b* seeds, probably sterile, $\times 470$ and $\times 270$; *c* cross section of the outer layer of a developing seed showing tannin bodies, $\times 2950$; *d* placentae with numerous developing seeds, $\times 35$; *e* detail of seed coat, $\times 1600$. *f* seed of *Mitrastema matudae*, $\times 260$

The record of WATANABE (1936) on ab initio cellular endosperm is confirmed. The embryo does not exceed the four-celled stage and is surrounded by about 10 to 15 endosperm cells containing oil.

Specimens observed. *Mitrasistema yamamotoi* MAKINO: New Guinea, VINK 16402 (L); VAN ROYEN 11039 (L); VAN ROYEN 15092 (L); Sabah, CHEW & CORNER RSNB 5746 (L); Japan, T. TAKASO s.n. (T, AMD); Cambodia, forest officer s.n. (L); Sumatra, STOMPS s.n. (AMD); *M. matudae* (YAMAMOTO) MEIJER: Mexico, BREEDLOVE 34366 (CA); Guatemala, P. C. STANDLEY 91455 (F).

Notes on dispersal. Information about seed dispersal in the *Rafflesiaceae* is scarce and scattered in the literature. Very few data are based on field observations and several opinions on the type or the agent of dispersal involved are only suggestions or even mere speculations.

Generally, seed and fruit structure of angiosperms are well adapted to their dispersal agents. The morphological characters of the fruits of all the genera of the *Rafflesiaceae* clearly indicate a zoolochorous dispersal. The fruits are always many-seeded berries and sometimes brightly colored. The seeds are embedded in a fleshy, slimy or even sticky pulp. In *Apodantheae* the outer seed coat layer contributes to this parenchymatous mass also. In all the genera the seed is protected by a mechanical layer of thick-walled cells, often in combination with a tanniniferous content.

Our present knowledge and understanding of germination and the host-parasite relationships of the *Rafflesiaceae* has not improved much since the experiments by HEINRICHER (1928) in *Cytinus*. As obligate parasites, *Rafflesiaceae* depend on the limited number of their specific hosts, so effective dispersers must meet the demand of a more directed transport. For successful "seedling" establishment it is required that the seeds be brought into the very close vicinity of the tissues of the host. The seeds of all *Rafflesiaceae* are relatively small with undifferentiated embryos and these seeds do not seem to have enough reserves to grow through thicker barks. Probably, in some cases the disperser has to make a wound in the root (or stem) to achieve host inoculation.

In *Rafflesia arnoldii*, a flower bud needs about nine to sixteen months before flowering, and the development of the pollinated ovary into a mature fruit takes half a year at least (MEIJER 1958). Flowers of *Rafflesia* and *Rhizanthes* can develop on very small roots of the host plants, possibly close to the point of original infection (W. MEIJER, unpubl. obs.). The fruit of *Rafflesia* is a giant berry with a dull, blackish-brown and bark-like outer surface, which makes the fruit extremely cryptic against the leaf litter of the ground (EMMONS & al. 1991). In *Rafflesia* and *Rhizanthes*, the seeds are embedded in a whitish, sticky and edible pulp. The fruit of *Sapria* has not been described until now.

Epizoochorous dispersal by the feet of mammals passing along the regular game trails and crushing the fruits has been suggested in the older scientific publications (ERNST & SCHMID 1913, JUSTESSEN 1922, WINKLER 1927). In the more popular literature impressive animals like elephants, tapirs and wild pigs have been nominated as dispersers. According to MEIJER (1958, 1983), wild pigs and smaller mammals like ground squirrels, other rodents, scaly anteaters and pangolins are plausible dispersers. Old fruits and unfertilized older ovaries of *Rafflesia* are often connected with termite nests. He reported that in the Bukittinggi reserve and the

Mount Sago area in West Sumatra, wild pigs and ground squirrels in search of worms, termites and ant nests greatly disturb the topsoil and may thus uncover the roots of *Tetrastigma*. Ground squirrels eat fruits of *Rafflesia* and may also dig into the soil and injure roots with their sharp claws, or stems when climbing about the lianas. The relationship between *Rafflesia* and squirrels was substantiated by later observations in 1983 by MEIJER, LAUMONIER, and ROSS (MEIJER, pers. comm.) on ground squirrels opening flower buds and eating away anthers. By field observations on *Rafflesia keithii*, fruits in a disturbed, secondary forest in Sabah, Malaysia, EMMONS & al. (1991) recently verified that squirrels (*Callosciurus*) and treeshrews (*Tupaia*) potentially serve as dispersers.

JUSTESEN (1922) reported that fruits are often opened at their base by termites and ants and assumed that they may transport the seeds into the earth. However, termites are purely dystrophic (VAN DER PIJL 1969), and *Rafflesia* seeds do not have edible parts reminiscent of elaiosomes. Termites and ants may play a role by attracting animals which get the sticky seeds around their mouth during ant-eating. MEIJER (unpubl.) observed that the contents of young fruits of *Rhizanthes* disappeared a few days after he made incisions in them to check for their ripeness, always from the lower side of the developing fruit.

Summarising, we have to conclude that seed dispersal in *Rafflesia* is still mysterious, and in *Rhizanthes* and *Sapria* is fully unknown. Animals eating the ripe fruits of *Rafflesia* and also digging into the soil in search of edible roots, subterranean fruits or termites and ant nests have to be considered as the most probable dispersers of *Rafflesia*.

The fruits of the *Apodantheae* are relatively small and generally do not become more than about twice the size of their ovaries. The fruits are rather inconspicuous, become yellow, orange or purple at maturity and remain enveloped at their base by the brownish bracts and petals. Depending on the host species, the mean number of ovules per ovary varies from 70 to 120 in *Pilostyles hamiltonii* (DELL & BURBIDGE 1981). An infected *Adesmia* shrub may bear about 2000 to 10 000 fruits of *Pilostyles berterii*, each containing about 360 seeds (KUMMEROW 1962). Unfortunately, most of the statements on dispersal for *Pilostyles* are also based on assumptions, not on field observations. Animals mentioned as possible dispersers are birds, mammals, ants, and termites.

The genera of the *Apodantheae* clearly differ from those of the other *Rafflesiaceae* in their exposition of flowers and fruits. In the *Rafflesieae*, *Cytineae*, and *Mitramitoideae*, flowers mostly sprout from the root stock of the host, so fruits are easily accessible for terrestrial animals. In *Apodanthes*, *Pilostyles*, and *Berlinianche*, the flowers appear on the twigs and stems of the hosts. It is questionable whether this difference in the exposition of fruits implies any differences in the kind of dispersers and in the location of host infection. Without doubt birds play a role in dispersal (ULE 1915). Birds seem the most obvious dispersers capable of carrying the small sticky seeds endo- or exochorously and achieving stem infections. The fruit characters of most *Apodantheae* clearly agree with the ornithochorous syndrome. GÓMEZ (1983) reports that the yellow or orange fruits of *Apodanthes caseariae* are eaten by Troupis birds (*Thraupis*). Observations on host infection in *Apodantheae* are not available, and the data on the distribution of the endoparasite within its hosts are contradictory. Anatomical sections showed that parasitic tissue is entirely absent

in the roots of *Dalea emoryi*, a host of *Pilostyles thurberi*. According to KUIJT (1969), the distribution of *Pilostyles* flowers on the host indicates a court of infection on the branches, not on the roots. The growth of the endophytic system of *Pilostyles* keeps pace with that of the apices of the host, since flower formation takes place at a rather uniform distance below the shoot apices. In *Pilostyles hamiltonii*, the most recent flowers are borne on woody stems usually one growth season old, but occasionally older (DELL & BURBIDGE 1981).

However, observations of MEIJER (unpubl.) on *Pilostyles thurberi* in the Sonoran Desert indicate that the flowers of this parasite progressively appear further from the roots during the life cycle of the host. Other authors also contradict the opinion that parasitism occurs through stem infection. The more recent observations of DELL & BURBIDGE (1981) on *Pilostyles hamiltonii* suggest that root stocks may be infected. A number of the 15 papilionoid host species of this Australian representative rely on regeneration from extensive root stocks. The presence of *Pilostyles* on one and a half year-old shoots suggests that *Pilostyles* survives fire and disturbance within the root stock and may grow up within the generating *Daviesia* stems. ULE (1915) believes that ground-living fowl or small mammals are the dispersers and that seeds voided by these animals will germinate on the soil to form a mycelial mat which, upon reaching a suitable host, may cause infection. KUMMEROW (1962) states that each year numerous seeds will fall on the ground and suggests that a burrowing rat, *Octodon degu*, a strong inhibitor of the habitat of *Pilostyles berterii*, carries the seeds underground, where they will germinate, stimulated by root exudations of the host. RUTHERFORD (1966) described in his Ph.D. thesis that the fruits of *Pilostyles thurberi* can drop below the host plant and that fruits are eaten by Jack rabbits. Seeds were found in their droppings. According to DELL & BURBIDGE (1981) ants are likely dispersal agents of the seeds of the Australian *Pilostyles* species. MEIJER (unpubl.) observed that fruits of *Pilostyles thurberi* might open on the plant during the summer. Harvester ants collect and bring to their nests all kind of fruits, seeds and droppings from animals. This way they may bring seeds of the host plant as well as the parasite together inside cool, moist, subterranean sites. This could explain why flower buds of *Pilostyles thurberi* occur close to the soil level in young plants and only higher up in older ones and that they are in most cases all of one sex.

Considering the widespread distribution of the *Apodantheae* and the diverse habitats, it may be expected that the taxa will show a range of fruit consumers and potential dispersers. In fact SUZUKI (1975) reported chimpanzees eating *Pilostyles* spec. in open woodlands of western Tanzania during the dry season.

Even in *Cytineae*, the data on dispersal and host infection are fragmentary and inadequate. The fruit of *Cytinus* is a berry with numerous seeds embedded in a sweet, mucilaginous and viscid pulp. According to the oldest record of ASCHERSON (1864) seed dispersal is achieved by birds. KRUPKO (in GUZOWSKA 1966) suggests the participation of snails in the dispersal of seeds of *Cytinus hypocistis* on Cyprus. BARGAGLI (in BARONI 1900) records ants invading fruits of *Cytinus hypocistis* and suggests ants as possible dispersers. The fleshy rudiments of the outer integument may serve as an elaiosome (KUIJT 1969). In future studies, ants certainly deserve special attention. Ants are important in the habitats of *Cytinus*, the maquis and garrigue vegetations of the Mediterranean and fynbos of southern Africa (BOND &

SLINGSBY 1983). Ants are among others involved in the dispersal of the seeds of *Cistus*, the host of *Cytinus*. *Cistus* seeds may be transported by *Messor* ants over five to twenty meters (MÜLLER 1933). Ants are also very active on the flowers of *Cistus monspeliensis*. They remove parts of the corollas, which can be found in large number on the ants garbage places (G. OOSTERMEIJER, pers. comm.). By burying seeds of *Cytinus hypocistis* near the roots of *Cistus*, HEINRICHER (1917, 1928) succeeded for the first time to achieve experimental infection in *Rafflesiaceae*. Because, with some rare exceptions, *Cytinus* invades mainly the roots of its host (FORSTMEIER & al. 1983), root infection is most likely in nature.

The data on seed dispersal in *Bdalophyton* are scarce. The fruits are mostly concrecent at their base. According to GÓMEZ (1983), ripe fruits of *Bdalophyton americanum* often show the signs of sharp incisors, and the seeds are carried around by rodents.

For the seed dispersal of *Mitrastema* we have to rely on the findings of WATANABE (1936–1937). The fruits of *Mitrastema* develop in three months after flowering. A large percentage of the ovaries develop into sterile fruits with embryoless seeds. The mature fruit of *Mitrastema yamamotoi* is a sweet berry, about 2 cm long and orange-pink in colour. The upper part of the fruit, composed of style and stigma, is lifted by its swelling contents. Especially on rainy days, the placentas with numerous, small, yellow seeds appear. The fruit lid may fall apart during heavy rains and seeds may become rinsed out and dispersed to some extent. WATANABE never observed animals consuming the fruits, but described fruits damaged by gluttony, showed bill imprints in the fruit remains, and supposed zoothorous dispersal, most likely by birds. In feeding experiments with *Zosterops japonicus*, the pollinator of *Mitrastema*, and with canary he demonstrated that *Mitrastema* seeds pass through the intestinal duct unharmed. Without dispersal of seeds by birds, it would be difficult to explain the dispersal of *Mitrastema* to many small islands around the southern islands of Japan and around the Malay archipelago.

Summarizing, we can state that all taxa seem to have primarily a zoothorous type of dispersal; exochorously on the feet or bills of birds or on the snouts of mammals, endozoochorously by passing the digestive tract of animals, or myrmechorously by ants. The variety in seed dispersers for *Rafflesiaceae* recorded in the literature reflects the wide geographic distribution of the family and the diversity of the different habitats of the members of the *Rafflesiaceae*. Considering the small size of the seeds, secondary dispersal of seeds by rain wash seems possible, but not very effective.

Discussion

The ovules of the members of the *Rafflesiaceae* show two interesting evolutionary trends resulting into a further reduction of the seed structure, viz. a change from anatropy towards orthotropy and a change from bitegmy to unitegmy. The most primitive type, the anatropous and bitegmic ovule, is found in the tribe *Apodantheae*; however, the outer integument in all three genera is one cell thick only (RUTHERFORD 1970), a character state seldomly found in seed plants. The most derived type, the orthotropous and unitegmic ovule is known from *Cytineae*. Rudiments of the outer integument are known from the ovules of the genus *Cytinus* (PONZI & PIZZOLONGO 1982), but are absent in *Bdalophyton*. In the tribe *Rafflesieae*, both trends are seen.

The outer integument is still initiated, but does not envelop the inner one and remains as a rudiment on the ovule and seed. As a consequence of this, the shape of the ovules is irregular, more or less intermediate between the anatropous and orthotropous condition. The ovules in *Mitrastemoideae* are unitegmic and anatropous.

The ovules of all members of the *Rafflesiaceae* are dizonate, i.e., initiated in the subdermal layer of the placenta. The subdermal tissue is mostly restricted to one single axial row of cells. The ovules never show any sign of vascular or pro-vascular tissue. The diversity in the structure of the ovules clearly supports the existing generic grouping at the tribal level.

The differences in ovule structure are reflected in the morphology of the seeds. The ovules of *Cytinus*, *Mitrastema*, and *Pilostyles* are among the smallest known. In all species studied, the ovules almost do not, or only slightly, increase in size during seed development. The space for the reduced embryo and the amount of endosperm is created mainly by the resorption of the nucellar and inner tegumentary layer. Assuming that the unitegmic condition in *Mitrastema* is the result of the reduction of an outer integument also, the seeds of all *Rafflesiaceae* may be interpreted as basically exotegmic. In *Apodantheae* the exotegmen is covered by an undifferentiated one-layered testa, in the three other tribes the exotegmen represents the outermost layer of the seed. The structure of the exotegmic cells differs between the tribes.

At the generic level the seed structure is of limited use. Morphologically dissimilar genera like *Rafflesia* and *Rhizanthes* (MEIJER & VELDKAMP 1988) have practically identical seeds. The seeds of these genera show only small variations in size and in the structure of the exotegmic cells. Since *Sapria* is morphologically more similar to *Rafflesia* than *Rhizanthes*, we expect that the seeds of this genus will be very similar to those of *Rafflesia*. Within the *Apodantheae*, the exotegmen provides an additional argument for the generic status of *Apodanthes*. In *Apodanthes*, the cell wall thickenings are U-shaped, reminding of those in *Rafflesieae*, whereas in *Pilostyles* and *Berlinianche* the cells are thickened all around. Seed structure essentially does not differ between *Berlinianche* and *Pilostyles* species, including its Australian and Mideastern representatives. Therefore, seed morphology does not provide additional indications for the generic status of *Berlinianche*. Although the seeds of *Bdallophyton* and *Cytinus* have the same basic, orthotropous structure, they show distinct differences in testal pattern, in the absence or presence of the rudimentary outer integument, and in structural details.

In *Rafflesiaceae* seed micromorphology is of restricted use as a taxonomic tool at the species level. Only species of *Cytinus* can be identified on basis of seed characters. Future research is required to prove whether the differences between the seeds of *Pilostyles* species described in this paper may be of help at the level of sections or species.

The systematic position of the *Rafflesiaceae* among angiosperms is still under discussion. In all current systems the *Rafflesiaceae* s.l. are grouped with the *Hydnoraceae* in a separate order *Rafflesiales*. At the supraordinal level, *Rafflesiales* are grouped in the *Magnolianae* near the *Aristolochiaceae* in the systems of DAHLGREN (1991), in the *Rafflesianae* near the *Aristolochiales* of the *Magnolianae* in TAKTHAJAN (1980), in the *Rafflesianae* near the *Piperinae* of the *Magnolianae* in THORNE (1992)

or, alternatively, in the *Rosidae* near the *Santalales*, *Proteales*, and *Celastrales* in CRONQUIST (1981). The position of *Rafflesiaceae* in the *Rosidae* is mainly based on supposed relationships with the parasitic families of the *Santalales*.

The extreme reductions of the vegetative characters of these plants and the often specialized adaptations of the reproductive organs greatly hamper discussions on the phylogenetic relationships of parasitic plant families. Considering the above described trends in the evolution of the ovule and seed, the ancestors of the *Rafflesiaceae* must have had bitegmic, anatropous ovules and endospermous seeds with minute embryos and exotegmic seed coats. *Aristolochiaceae* share these characters and have exotegmic fibres. CORNER (1976) makes, in spite of some transitions, a sharp distinction between seeds with an exotegmic palisade and those with exotegmic fibres. For this reason he excludes the affinity with *Aristolochiaceae* and relates *Rafflesiaceae* with *Piperaceae*, *Saururaceae*, and even *Podostemaceae*. However, seeds as small as in *Rafflesiaceae* never can accommodate fibres by lack of length. Shortening of exotegmic fibres into cuboid or palisade sclerotic cells can occur at the chalaza and at the endostome within the same seed, and the difference in shape of tegmic cells also exists between a number of closely related families (CORNER 1976, DAHLGREN 1991). The extant taxa of the *Santalales* show a series of extreme ovule reductions (BOUMAN 1984) and a set of embryological characters which are very different from those of the *Rafflesiaceae* (COCUCCI 1983). Although still speculative, the relation of *Rafflesiaceae* with *Aristolochiaceae*, as already suggested by such early 19th century botanists as BRONGNIART (1824) and BROWN (1834), seems the best defensible one.

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