The seed structure and taxonomic relationships of *Hypseocharis* Remy

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SUMMARY

Hypseocharis has a bitegmic, crassinucellate and anatropous ovule. Shortly after fertilization the ovule becomes campylotropous by a bulge formed by the inner integument at the raphal side. The seed coat is characterized by a crystalliferous endotesta and an exotegmen consisting of stellately lobed cells. The seed structure corresponds to that of the Geraniaceae s.s. The loculicidal capsule of *Hypseocharis* does not show the specialized method of dispersal of the beaked fruits of Geraniaceae s.s. The andine genus *Hypseocharis* may be a relic of the ancestors of modern Geraniaceae that lived in the Gondwana continent before the separation of South America and Africa. This study indicates that *Hypseocharis* has to be placed in Geraniaceae, not in Oxalidaceae.

Key-words: campylotropy, exotegmen, Geraniaceae, Oxalidaceae, seed anatomy.

INTRODUCTION

The genus *Hypseocharis* Remy consists of eight species all of which occur in the sub-alpine zone of the Andes from Peru through Bolivia into North Argentina at altitudes of about 2000–4000 m (Knuth 1930). According to Gutte (1986) the average temperature of the subalpine zone in Peru is ±8–10°C and frost is possible from 3000 m upwards.

Macbride (1949) has suggested that *Hypseocharis pimpinellifolia,* the type species, is polymorphic and that the other described plants are variants. According to Willis (1985) the genus *Hypseocharis* consists of acaulescent perennial herbs with a thick tap root. The gynoecium has a simple, filiform style with capitate stigma and many axile, biseriate and anatropous ovules. The fruit is a tardily and irregularly loculicidal capsule. The embryo is cochlear and the endosperm scanty. *Hypseocharis* differs from Geraniaceae in the lack of stipules, the numerous ovules and seeds, and the loculicidal capsule, and from Oxalidaceae in the completely connate styles with capitate stigma (Willis 1985). Its ovules and seeds are not or poorly known.

Hypseocharis was first described as a new genus by Remy in 1847 and placed in the Geraniaceae. Later the opinions concerning the relationships of *Hypseocharis* changed and a placement under Oxalidaceae or in a separate family, Hypseocharitaceae, was favoured, see Knuth (1908, 1931), and the discussion of his opponent Hallier (1923). However, the position of *Hypseocharis* still remained doubtful and the genus hovered

between Geraniacae and Oxalidaceae. According to Cronquist (1981), the genus *Hypseocharis* connects the Geraniaceae and Oxalidaceae. Oltmann (1971) has remarked that there is still uncertainty concerning the relationships of *Hypseocharis*, but on palynological grounds he considered the genus related to Oxalidaceae. Macbride (1949) stated that the genus *Hypseocharis* is not clearly a member of the Oxalidaceae and that its position is uncertain.

This study describes the ovule and seed structure of *Hypseocharis* to clarify its relationships.

MATERIALS AND METHODS

Flowers and developing fruits of *Hypseocharis pimpinellifolia* Remy were collected by O. Buchtien in Bolivia (La Paz) in 1909 (Herbarium AMD). A mature fruit with seeds of *H. tridentata* (Griseb.) was collected by H. Ellenberger in Peru (Cusco) in 1957 (Herbarium U).

For light microscopy this dry material was softened during an overnight stay in 10% ammonia solution and subsequently dehydrated in an ethanol/*n*. butyl alcohol series, embedded in glycol methacrylate, sectioned at 5–10 μm with glass knives, stained with the PAS reaction and counterstained with aqueous methylene blue.

Phloroglucin-HCl, Sudan IV, ruthenium red, iodine in potassium iodide and nigrosine solutions were used for the light microscopical identification of respectively lignins, fats, pectins, starch and storage proteins. For scanning electron microscopy dry specimens were gold/palladium sputter-coated for about 2·5 min and studied on an ISI DS 130.

RESULTS

Hypseocharis pimpinellifolia has a five-locular ovary with about 12 epitropous ovules per locule. The loculicidal capsule of *H. tridentata* contains many seeds. Only the more lower ovules become fully mature, while the remains of the upper ones persist in the mature fruit. The material did not allow for a detailed study of ovule development, because of the poor quality of the dry material. Nevertheless, it could be established that both integuments are initially two-layered, and that they become three-layered by periclinal divisions of the inner layer. The ovule of *Hypseocharis pimpinellifolia* is anatropous, bitegmic and crassinucellate (Fig. 1a). The large nucellus is provided with a small dermal cap. A cuticle is already present between nucellus and inner integument (ii). The outer layer of the outer integument (oi) starts to store tannins. The zig-zag micropyle is formed by both integuments. The amphicribral funicular bundle is distinct and starts to differentiate.

A short time after fertilization the ii becomes thickened by very local periclinal divisions of the middle layers at the raphal side (Fig. 1b). By repeated divisions a narrow, radially stretched bulge is formed, which extends into the embryo sac (es) perpendicular to its longitudinal axis (Figs 1c and 3a). This narrow bulge stays in contact with the seed coat at the lateral sides of the seed and assumes the aspect of a septum when its cells are crushed in later stages.

Simultaneously with this process of indenting the es, the antiraphal side is stretched whereas the raphal side remains relatively short. As a result of these developments the es
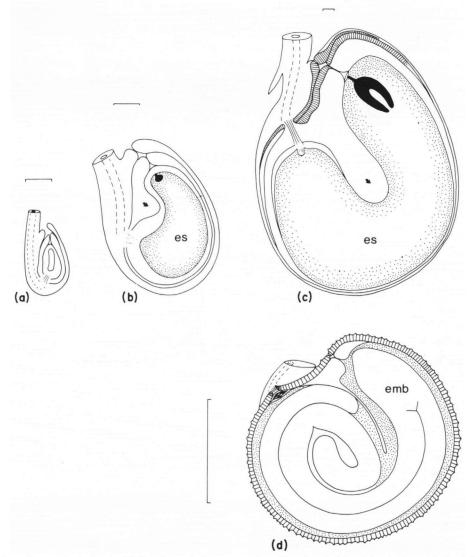
Fig. 1. *Hypseocharis pimpinellifolia,* schematical representation of l.s. of ovule (a) and developing seeds (b–d); the arrow points at bulge of inner integument; measure is 100 μm; (d) *H. tridentata,* l.s. of mature seed with cochlear embryo; measure 1 mm. es = embryo sac; emb = embryo.

curves and the seed ultimately becomes strongly campylotropous. In the mature seed the bulge is crushed into a small series of cell remains (Fig. 1d).

The embryo is still relatively small when the developing seed has become nearly campylotropous (Fig. 1c). At this stage the seed coat starts to differentiate in the micropylar region. The developing embryo grows and adapts the bent shape of the es. When the embryo is about half the length of the es, the seed coat is almost completely differentiated. The cotyledons grow out in such a way that they become spiral, seen in longitudinal section, and that the embryo becomes cochlear (Fig. 1d). In cross-section the cotyledons are plano-convex (Fig. 3c) and are not always evenly situated within the seed (Fig. 4c).
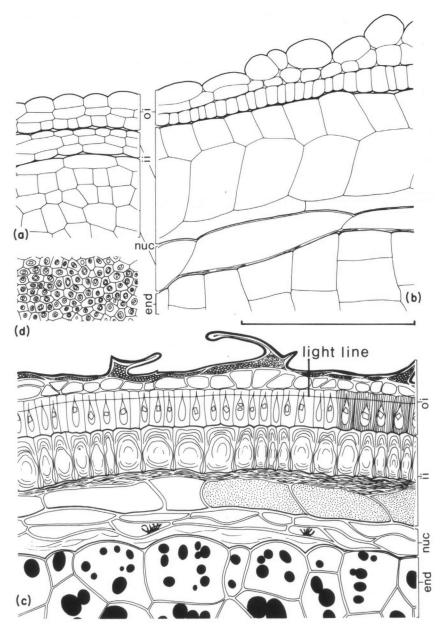
Fig. 2. (a–b) *H. pimpinellifolia*, l.s. of developing seed coats; (c) *H. tridentata*, l.s. of mature seed coat showing endotestal crystal cells with light line; (d) *H. tridentata*, tangential section in the plane of the endotesta. Measure is 100 μm. ii = inner integument; oi = outer integument; nuc = nucellus; end = endosperm.

The endosperm is *ab initio* nuclear and later becomes centripetally cellular, at first around the embryo. It is not completely resorbed and at least one layer is left around the embryo in the mature seed. Locally it remains multilayered, for example at the flanks of the seed (Fig. 3c) and around the root of the embryo. The walls of the endosperm are thin.
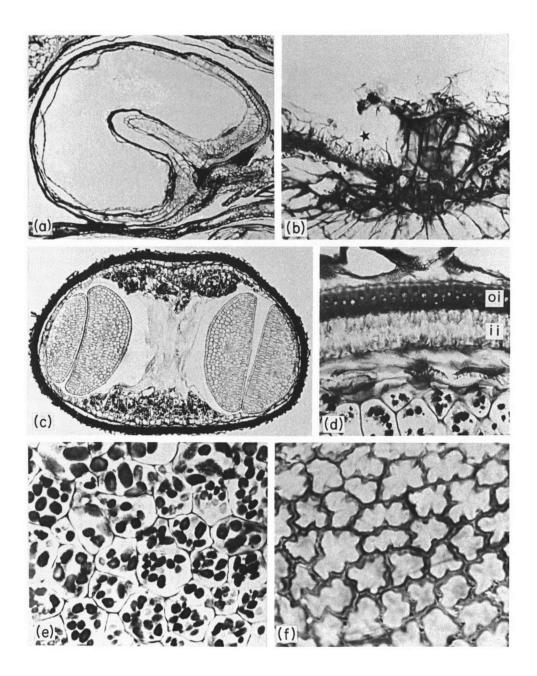
Fig. 3. Light microscopical photographs. (a) *H. pimpinellifolia*, l.s. of developing seed (compare Fig. 1c); (b) Detail of Fig. 3a: chalazal part of nucellus showing wall ingrowths; (c) *H. tridentata*, cross-section of mature seed with planoconvex cotyledons; (d) *H. tridentata*, l.s. of mature seed coat; (e) *H. tridentata*, endosperm with starch grains; (f) *H. tridentata*, tangential section in the plane of the exotegmen showing stellately lobed exotegmic cells. ii = inner integument; oi = outer integument.
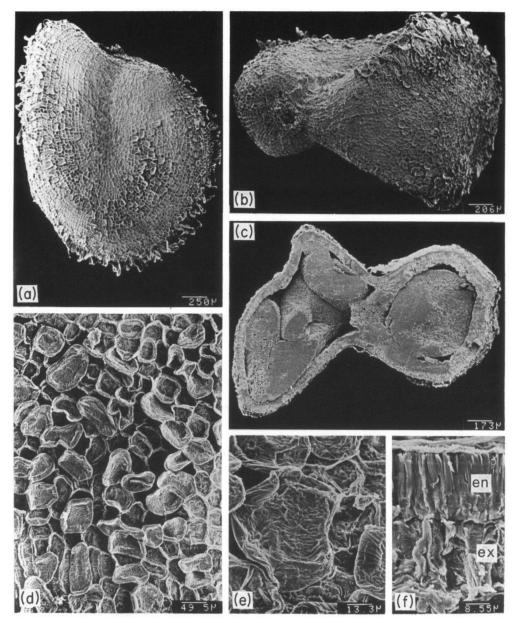
Fig. 4. *H. tridentata*, SEM photomicrographs. (a,b) Mature seeds with papillae in side and hilar view; (c) cross-section of seed; (d,e) surface view of seed coat and detail; (f) l.s. of exotegmen (ex) and endotesta (en).

The nucellar tissue is gradually resorbed at the inner side, but the nucellar cap remains visible for a long time. Even in the mature seed the nucellar remains form a crushed layer bordered by a pronounced cuticle (Fig. 2a–c). At the stage indicated by Figs 1c and 3a the cells of the inner boundary of the remaining nucellar tissue show, mainly at the chalazal side, marked ingrowths of cell wall material (Fig. 3b). In the mature seed the crushed cells of the nucellar base form a small brown tissue that closes the chalazal opening as a

Table 1. Comparison of ovule and seed characters of *Hypseocharis* with those of Geraniaceae, Oxalidaceae and Linaceae

	Hypseocharis	Geraniaceae	Oxalidaceae	Linaceae
Inner integument	three-layered	three-layered	three- or multilayered	strongly multiplicative
Outer integument	three-layered	three-layered	three- to multilayered	two–three layered
Exotegmen	stellate, unlignified palisade-cells	stellate, unlignified palisade-cells	fibrous, lignified cells	fibrous, lignified cells
Endotesta	crystal cells	crystal cells	crystal cells	absent
Embryo	cochlear	curved	straight	straight
Endosperm	scanty	absent	copious	copious
Seed	campylotropous	campylotropous	anatropous	anatropous
Fruit type	loculicidal capsule	beaked schizocarp	loculicidal capsule or berry	septicidal capsule or drupe

hypostase (Fig. 1d). With the exception of the micropylar tips, which become multilayered by periclinal divisions of their inner layers (Figs 1b, c and 2a, b), the ii and oi remain three-layered during further development.

The cells of the developing ii enlarge. The cells of its inner layer become flattened and tanniniferous, those of the middle layer cubic to isodiametric and those of the outer layer, palisade-like by anticlinal divisions (Fig. 2b). The latter contain a more densely stained cytoplasm. Around the micropyle the cells of the outer layer show some periclinal divisions, so that it becomes slightly multilayered. In the mature seed the cells of the inner layer of the ii may or may not be crushed and are sometimes tanniniferous (Fig. 2c). The cells of the middle layer are crushed and their remains are visible between the inner and outer layers of the ii. The cells of the outer layer of the ii become sclerotic (Figs 2c, 3d, 3f and 4f). The primary walls (Fig. 3f) react PAS positive, whereas most of the secondary wall thickenings seem to be of a pectic nature. The unevenly dried pectic substances are probably the cause of the tangled appearance of these cells (without clear wall thickenings) especially in the SEM (Fig. 4f), but also in the light microscope (Fig. 3d). In the middle of the radial walls of the exotegmic cells the pectic wall thickenings are probably less well developed (Fig. 2c). In cross-section the exotegmic cells have a distinct stellate appearance strongly reminiscent of that of the Geraniaceae (Fig. 3d).

Both integuments are in close contact and are not easily separable. The cells of the testa also enlarge during development (Figs 1a, b). The cells of the inner layer become palisade-like by anticlinal divisions and their plasma stains darker. The cells of the two outer layers become more or less rounded and form small intercellulars. The first signs of wall thickening in the inner layer are visible around the micropyle as thickened inner tangential and basal radial walls. Here also the endotesta becomes multi-layered. Ultimately, the endotestal cells become strongly sclerotized crystal-cells with layered wall thickenings and a "light line" (Figs 2c, 3d and 4f). The lumen of the cells is drop-shaped, contains a small crystal and ends as a narrow channel in the upper part of the cell. In cross-section these cells are polygonal (Fig. 2d). They do not react with any specific colour test, but a reaction is difficult to see because of the dark appearance of the cells, probably caused by the presence of tannins. Above the lightline, which is also visible in the SEM, the wall thickening contain less or no tannins.

   The cells of the middle layer are not or scarcely thickened and partly crushed. The outer cells of the testa enlarge considerably and part of these cells, mainly in the median plane, grows out into papillose extrusions (Fig. 4a and b). These cells contain small starch grains, while the outer wall is pressed against the inner one (Figs 2c and 4d, e). When the seeds are placed in water the extrusions do not exhibit any special behaviour. No stomata were found in the seed coat.

   The broadened tips of the ii are not crushed in the mature seed. Their cells are slightly thick-walled and tanniniferous.

   The seed of *H. tridentata* measures about 2 mm in the median plane. The cochlear embryo is rich in fats and proteins. The endosperm contains numerous, large starch grains and is poor in fats and proteins. No part of the seed coat is lignified.

DISCUSSION

The developing seed of *Hypseocharis* becomes increasingly campylotropous by the strong ingrowth of a bulge, which originates on the raphal side of the ii through periclinal divisions of the middle layers. Most ovule and seed characters of *Hypseocharis*, including its type of campylotropy, correspond closely with those of the Geraniaceae s.s. (namely *Geranium, Erodium, Pelargonium, Monsonia* and *Sarcocaulon*) but not with those of the Oxalidaceae (Boesewinkel 1979, 1985, Bouman 1974, Corner 1976). See also Table 1.

   In *Hypseocharis* the embryo is cochlear with spirally folded planoconvex cotyledons in the longitudinal orientation. Geraniaceae s.s. differ in the curvature of the embryo because the cotyledons are straight in longitudinal section but strongly folded in cross-section. These foldings of the cotyledons have the advantage of a large cotyledonary surface after germination. In *Hypseocharis* the bulge runs perpendicular to the es but in *Geranium* it becomes oblique, running ultimately more or less in the longitudinal direction of the seed. Another difference between *Hypseocharis* and *Geranium* is the wall ingrowths in the inner boundary of the nucellar base of the developing seed of *Hypseocharis*. Probably these ingrowths facilitate the transport of nutrients to the embryo from the narrow chalazal opening, from the nucellar tissue or from the integuments.

   The aforementioned striking correspondences between ovule and seed structure of *Hypseocharis* and of Geraniaceae render a close relationship between these taxa very likely.

   Hypseocharis is not closely related to the Oxalidaceae, nor does it connect Geraniaceae with Oxalidaceae. In the case of *Hypseocharis*, the ovule and seed structure appear to be better indicators of a relationship than the character sets hitherto used (Knuth 1908, 1931; Willis 1985). This is because ovules and seeds show relatively little variability and many families have a distinctive ovule and seed structure.

   The small seeds of *Hypseocharis* are probably dispersed by the wind. The papillose outgrowths of the seed coat enlarge the surface and may represent a weakly developed (or rudimentary) wing. These extrusions apparently do not function in relation to water because they do not show a special behaviour when placed in water.

   In contrast to *Hypseocharis*, modern Geraniaceae have strongly beaked fruits with an advanced and complicated type of fruit discharge (Yeo 1984). According to Yeo three main types of seed discharge occur in Geraniaceae, namely (1) seed ejection, with ballistic expulsion of the seed from the mericarp, (2) carpel projection, with forcible discharge of the mericarp containing the seed, whilst the mericarp becomes separated from the awn,

and (3) the *Erodium*-type, in which the seed containing mericarp is thrown off together with the attached awn, the latter becoming helically coiled. These principal seed-dispersal mechanisms are all found in *Geranium*, but they are not matched in the other genera of the family, which have what is structurally an *Erodium*-type of discharge. On the grounds of its general prevalence and its function, the *Erodium*-type might be held to be primitive (Yeo 1984).

With regard to its fruit and seed structure, *Hypseocharis* shows primitive character-states of the ancestors of the Geraniaceae s.s. The number of ovules and seeds is less reduced and the fruit structure is more original. Within each fruit locule only the lower ovules develop into fully mature seeds. This is a trend to the situation in Geraniaceae with only a single basal seed remaining in each fruit locule. Also the planoconvex cotyledons and the vestiges of the endosperm might be original.

According to Yeo (1984) the family Geraniaceae s.s. is divisible into two groups on the combined basis of distribution and taxonomy. One part is characteristic of the Afro-Arabian land mass, which formed a continent through much of the Tertiary era. *Pelargonium, Monsonia, Sarcocaulon* and *Erodium* subgenus *Erodium* are mainly or exclusively African. The other part of the family is characteristic of the North-Temperate continents, but has various extensions and outliers. The North-Temperate taxa are *Geranium*, the diversity of which reaches a peak towards the end of the Mediterranean basin, and *Erodium* subgenus *Barbata*, that is mainly found in the Mediterranean Basin.

Yeo (1984) proposes that the splitting up of the genera, combined with the diversification in fruit types, occurred early in the history of the family. This provides the time required for the more extensive groups to spread over the continents and speciate to the extent that they have. The abundance of Geraniaceae in the Old World, and especially in Africa, leads to the conclusion that modern Geraniaceae s.s. arose mainly after the separation of South America from Africa. By this method of reasoning *Hypseocharis* must be a descendent of the ancestral Geraniaceae that occurred in Gondwana before the separation or at least before the time that distance made exchange impossible. *Hypseocharis*, then, represents a relic of a somewhat extreme, subalpine environment. In Africa the ancestors of Geraniaceae have become extinct.

The question remains whether *Biebersteinia, Wendtia, Viviania* and *Dirachma* are geraniaceous. They do not have the characteristic beaked fruits of Geraniaceae. Their seed structure undoubtedly will assist in answering these questions.

Biebersteinia may be geraniaceous because there is an exotegmen with stellate-like cells, which have a complicated morphology. No endotesta exists and the seed is not or hardly campylotropous (Corner 1976). Corner (1976) concludes that this woody taxon represents a more original condition compared with Geraniaceae s.s. Grisebach (1877) considers *Hypseocharis* related to *Biebersteinia* on account of its fruit structure.

Viviania has a curved embryo (Knuth 1931) but the seed coat is exotestal and differs strongly from that of Geraniaceae (Boesewinkel, unpublished). *Dirachma* is certainly not related to Geraniaceae, because the seed is exotestal and not campylotropous. There is one integumentary vascular bundle at the antiraphal side (Boesewinkel, unpublished).

Geraniaceae s.s. with their very efficient seed or fruit dispersal are clearly the most successful representatives of modern Geraniaceae.

Behnke (1982) has detected morphological similarities between the plastids of *Connarus, Averrhoa* and *Oxalis* (the latter two Oxalidaceae), which have P-type sieve element plastids. However, *Biophytum* (also Oxalidaceae) and *Hypseocharis* contain S-type plastids. These results are in contradiction to those from the study of Oxalidaceous seeds

(Boesewinkel 1985). A separation of *Averrhoa* and *Oxalis* from *Biophytum* is difficult to understand because they all clearly belong to Oxalidaceae. The last two genera show a unique type of autochorous seed dispersal, in which the ejecting mechanism is situated in the seed coat (Boesewinkel & Bouman 1984). A relationship between *Biophytum* and *Hypseocharis* is likewise in contradiction to the results of the present study.

In conclusion, the data presented in this paper strongly favour a placement of *Hypseocharis* in the Geraniaceae.

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