Seeds and seedlings in Hanguanaceae and Flagellariaceae (Monocotyledons)

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Abstract:
TILLICH, H.-J.: Seeds and seedlings in Hanguanaceae and Flagellariaceae (Monocotyledons). – Sendtnera 3: 187–197. 1996. – ISSN 0944–0178.

The fruit in *Hanguana* is a 1-seeded berry. The seed coat is a sclerified meso-endotesta, the inner integument disappears. The endosperm is differentiated into a peripheral 1-layered aleuron region and an inner tissue of starch containing cells. The fruit in *Flagellaria* is a drupe with a massive endocarp composed of lignified stone cells. The seed coat is represented by the thick but not lignified outer periclinal cell walls of the epidermis cells of the outer integument and is firmly fixed to the fruit wall. The copious endosperm contains great amounts of compound starch grains.
The seedlings of both genera share ancestral character conditions with respect to the compact cotyledon and a leaf sequence starting with cataphylls. Using the system of DAHLGREN et al. (1985) as a base, Hanguanaceae represents a basal offshoot of Commelinales and has no relations to Asparagales. The position of Flagellariaceae as a basal group in Poales is confirmed.

Zusammenfassung:
Die Früchte von *Hanguana* sind einsamige Beeren. Die Samenschale ist als Meso-Endotesta entwickelt, das innere Integument geht zugrunde. Das Endosperm ist in eine periphere Aleuron-Schicht und ein zentrales, stärkereiches Gewebe differenziert. Die Gattung *Flagellaria* besitzt Steinfrüchte mit einem massiven, aus verholzten Steinzellen aufgebauten Endocarp. Die Samenschale besteht nur aus der dicken, aber nicht verholzten Außenwand der Epidermiszellen des äußeren Integuments und ist mit der Fruchtwand verwachsen. Das Endosperm ist reich an Stärke.
Die Keimpflanzen beider Gattungen besitzen als ursprüngliche Merkmale einen kompakten Cotyledo und eine mit Niederblättern beginnende Blattfolge. Die Hanguanaceae gehören nicht in die Asparagales sensu DAHLGREN et al. (1985), sondern passen am besten als basale Seitengruppe in die Commelinales. Die Stellung der Flagellariaceae als basale Gruppe in den Poales wird bestätigt.

Introduction

After splitting the classical family Flagellariaceae Dumortier (1829), mainly on anatomical and palynological reasons, the question arose as for the nearest relatives of

the three monogeneric families Flagellariaceae s.str., Hanguanaceae Airy Shaw (1965) and Joinvilleaceae Tomlinson & Smith (1970). While the position of Flagellariaceae s.str. and Joinvilleaceae in Poales is generally undisputed (cf. DAHLGREN et al. 1985, CHASE et al. 1995, KELLOGG & LINDER 1995, STEVENSON & LOCONTE 1995), the position of *Hanguana* was assumend in the last decade in Asparagales (DAHLGREN et al. 1985) or in the "Commelinoids" (RUDALL & CADDICK 1994, CHASE et al. 1995).

As to the structure of seeds and seedlings, the three families are known very incompletely. The only figure of an *Flagellaria* seedling is given in ARBER (1925). This very small and uncommented drawing gives no answer as to morphological details. The fruit structure of *Flagellaria* is disputed: it is reported to be a 1-seeded berry (BROWN 1902, HEPPER 1968) or a drupe (NAPPER 1971, LARSEN 1972, SMITH 1979, VILLIERS 1984, HARDEN 1993). HARRIMAN (1991) leaves the question open and describes the fruit to be "globose,..., 1–3-seeded". The *Hanguana* seedling is unknown, the seed is said to be starchless. This was an argument of DAHLGREN et al. (1985) to include *Hanguana* in the Asparagales. The fruit again is described as a drupe (LARSEN 1972) or a berry (DAHLGREN et al. 1985). HUBER et al. (1995) even propose to include again *Hanguana* into Flagellariaceae since the seed structure of *Hanguana* is maintained to indicate a relationship to *Flagellaria*.

It was demonstrated recently, that seedling structure may be an important additional source of characters for monocot systematics (TILLICH 1995). This paper aims to present data on fruit, seed and seedling structure in *Flagellaria* and *Hanguana* and to check the applicability of the results to the problems in question.

Materials and methods

Flagellaria indica L. fruits were harvested in October 1992 from a specimen cultivated at Botanischer Garten München. It then took 18 months from sowing until germination commenced.

Hanguana fruits of an probably undescribed species were collected by J. Bogner in Sarawak (Malaysia), near Semenggoh, at March 10, 1994. They were sown at March 15, 1994, the first signs of germination were observed five weeks later. One of the seedling plants is now successfully cultivated at the Botanischer Garten München.

Alcohol material of two seedlings probably of the same species as above, received as *Hanguana malayana* (Jack.) Merr., collected by R. Brett (no. 1) at Bukit Timah Nature Reserves, Singapore, on 24.8.1993, was generously provided by P. Rudall, Royal Botanic Gardens, Kew.

Seedlings were cultivated in Petri dishes (20 × 5 cm) on moist filter paper as described earlier (TILLICH 1992, 1994). The morphological analysis was made using a Wild Stereomicroscope MZ 8 with drawing equipment. To study the anatomy of seeds handcuttings were stained with standard staining procedures. Microscopical drawings were made with a Leitz Laborlux S and drawing equipment.

Alcohol material of all investigated seeds and seedlings is stored at the seedling bank at the Institut für Systematische Botanik, München.

Results

1. *Hanguana*:

The infructescence of *Hanguana* is a spike of whitish fleshy fruits, each with a small subtending bract and the remnants of the perigon at its base (fig. 1 A). The fruit is a one-seeded berry. The seed has a basal position and archs over a globose placenta, so that the seed shape is similar to a bowl turned upside down (figs. 1 B–E). The bowl margin is modified by a small lingular extension. The hilum position is inside the "bowl" at its bottom, the micropyle position is opposite to it at the outside surface. The distance between lower and upper surface of the seed (i.e. the thickness of the bowl wall) is about 0.5 mm, the total seed diameter is 5–6 mm.

The seed coat, using CORNERS (1976) terminology, is of the meso-endotestal type. The inner integument disappears. The outer epidermis cells are filled with dark brown substances, their outer periclinal wall is mostly more or less collapsed. In the testal "mesophyll" region the tissue is totally sclerified. As can be concluded from the few visible cell lumina, the original cells might have been of fibrous shape. A strong sclerification is also found in the cell walls of the inner epidermis, but the cells seem to have been somewhat suppressed by the growing endosperm before sclerification commenced (fig. 1 G).

A remarkable character of the endosperm is its differentiation in a peripheral aleuron layer and a central starch tissue. This is in contrast to DAHLGREN et al. (1985), who maintained that the seed is free of starch. The starch grains are simple and subglobose with concentric layers.

At the beginning of the germination process the primary root pushes a small circular operculum aside and grows in length. The cotyledonary sheath, which produces only traces of chlorophyll if at all, remains extremely short and low, its position is in direct contact to the seed. The cotyledonary hyperphyll has only haustorial function. This cotyledon type was termed a compact cotyledon (TILLICH 1995) and is considered the most ancestral type in Monocotyledons. No hypocotyl, collar or collar rhizoids are discernable. The primary root is brownish in colour and covered with relatively long root hairs (fig. 2 A). Plumule development begins with some pale green cataphylls in spiral arrangement, and in the course of further shoot development the following leaves gradually change to primary foliage leaves (eophylls) with closed sheaths. The internodes of the primary axis exept the epicotyl are somewhat elongated, the degree of elongation depending on the growth conditions (cf. fig. 3).

The two first shoot-born roots (in TILLICH 1995 erroneously the term "shoot-borne" was used. The term "shoot-born" is the correct translation for the german term "sproßbürtig") have about the same diameter as the primary root and break through the bases of the first and second cataphylls in the transverse plane of the seedling, both producing a small coleorhiza (fig. 2 E). The third shoot-born root, developing some weeks later, is clearly thicker than the first roots (fig. 2 F). Primary and shoot-born roots are freely branched. A branched primary root is again a typical ancestral condition in monocots.

2. *Flagellaria*:

The *Flagellaria* fruit is a drupe with a relatively thin fleshy mesocarp and a subglobose lignified endocarp. The stone cells have only a small empty lumen, the thick lignified cell wall is passed by numerous pits (fig. 4 A). From the periphery to the center of the fruit wall the cell diameters decrease remarkably. The innermost endocarp cells are totally compressed by the growing seed, they form just a homogeneous, lignified layer of cell wall material.

In the ripe fruit the seed coat is crushed and firmly fused with the fruit wall. The only discernable structure of the testa are the thick, colourless outer cell walls of the outer epidermis of the outer integument (fig. 4 A). In the uniform endosperm cells starch is stored in great amount. The starch grains are relatively small but of different size, their outline being angular rather than rounded (fig. 4 B). These grains are members of compound super-grains which desintegrate in microscopical cuttings. This is in contrast to HAMANN (1961), who has mentioned for *Flagellaria* simple starch grains.

The hard, lignified endocarp is a serious barrier for the embryo. Only one of the two stone kernels harvested in the greenhouse germinated after one year and a half. As to the seedling structure only early developmental stages of this one seedling could be observed, since the plant was soon infected by fungi. The root pole breaks through the top of endocarp opposite the basal remnants of the flower axis. The seedling possesses a compact, pale cotyledon very similar to *Hanguana*. Differing from that genus the collar is well developed and consists of large cells which produce but a few collar rhizoids. The first plumular leaf is a folded, green cataphyll (fig. 4 C).

Discussion

Fruits, seeds and seedlings of *Hanguana* and *Flagellaria* are distinguished by a number of characters. This is in agreement with anatomical and palynological evidence and supports the separation of *Hanguana* from Flagellariaceae and even from Poales. All available evidence about *Hanguana* points out, that the nearest living relatives of *Hanguana* are probably the Commelinaceae. The Botanischer Garten München, by the courtesy of P. Rudall recently received two young plants of *Hanguana* from Singapore, collected by R. Brett. These plants are not to distinguish from the seedling plant grown in our laboratory from seeds collected by J. Bogner in Sarawak. These three plants are very dissimilar to *H. malayanum* (Jack.) Merr. and certainly represent an undescribed species. They amazingly resemble in habit certain Commelinaceae with broad and petioled leaves, as, for instance, *Palisota*.

Berries are missing in Poales (the unusual fruit of the bambusoid genus *Melocanna* needs morphological reconsideration), but are met with in some Commelinaceae, e.g. *Palisota*. Furthermore, a sclerified testal seed coat is missing in Poales. In Restionaceae and Centrolepidaceae the seed coat is tegmic and formed by the inner layer of the inner integument (KIRCHER 1986). The seeds of Flagellaria (and probably Joinvillea) and Poaceae, firmly enclosed in drupes or caryopses, possess a strongly reduced seed coat. In Commelinales, on the other hand, manifold forms of seed coat occur (cf. GIULIETTI et al. 1987, DANILOVA et al. 1995). In the few Commelinaceae members thoroughly investigated, both integuments contribute to the seed coat, but the principal protecting layer is formed by the inner layers of the outer integument (MAHESHWARI & BALDEV 1958, GROOTJEN 1983, GROOTJEN & BOUMAN 1981). The testal seed coat is found again in *Hanguana* (see fig. 1 G).

The *Hanguana* seedling represents a very ancient type. This is obvious by the combination of a compact, achlorophyllous cotyledon, a robust, freely branching primary root, an indiscernible root collar and a foliation sequence starting with a number of cataphylls. Primitive character states of seedling organs are also scattered in Commelinales, though combined with more derived organ structures. Some Commelinaceae have a compact cotyledon, but the collar is of special construction, and the leaf sequence starts with eophylls (*Cyanotis*, *Weldenia*). In *Cartonema* the collar is inconspicuous and the primary root is well developed, but the cotyledon is of the far derived *Bromus* type and the seedling develops a short mesocotyl. *Hanguana* in this context is best placed as a basal offshoot of the Commelinales.

STEVENSON & LOCONTE found *Hanguana* to be a sister group of Zingiberales, mainly due to some shared apomorphies, as for instance petioled leaves, silica bodies or inaperturate pollen grains. It is true that these characters are predominant in Zingiberales, but they are by no means absent from Commelinales. Petioled leaves and silica bodies are met with in some Commelinaceae, inaperturate pollen is reported in *Abolboda* (ERDTMAN 1971). Additionally, the missing perisperm, the differentiation of the endosperm in aleuron and starch containing tissues, and also the hypogynous flower of *Hanguana* strongly argue against a Zingiberalean relationship. The affiliation of *Hanguana* to Zingiberales as resulting from a cladistic analysis seems to be due to a simplification of the character matrix in order to make it readable for the computer.

The affiliation of *Hanguana* to Asparagales, as proposed by DAHLGREN et al. (1985) is now refuted, since it was shown that the cell walls contain phenolic compounds (HARRIS & HARTLEY 1980, RUDALL & CADDICK 1994) and the endosperm contains starch.

The seedling of *Flagellaria* is in part of ancient structure with respect to the compact, achlorophyllous cotyledon and the leaf sequence beginning with cataphylls. But, in contrast to *Hanguana*, the root collar is conspicuously developed and produces some collar rhizoides, and the primary root has an only trifling growth. A seedling with such a character combination could serve well as a basic model for the derivation of the Poacean seedling. This would include the following processes:

1. Development of a coleoptile
2. Total reduction of the primary root
3. Transformation of the collar to a coleorhiza of the first shoot-born root

It is noteworthy in this connection, that regarding to SACK (1994) the stomata complex of *Flagellaria* also represents a preliminary stage to those of Poaceae in that the guard cells are much less specialized than in Poaceae.

The seedlings of Centrolepidaceae and Restionaceae investigated so far are similar to each other but have a very different construction type compared to *Flagellaria* or Poaceae (KIRCHER 1986, TILLICH 1995). They resemble Juncaceae or Typhaceae seedlings rather than Poaceae.

Acknowledgements

I am indebted to Dr. Paula Rudall, Royal Botanic Gardens, Kew, and Joseph Bogner, Botanischer Garten München. They provided generously fruits, seeds, seedlings or young plants of *Hanguana* and *Flagellaria*, respectively. Without their unselfish help and permanent interest this work would have been impossible.

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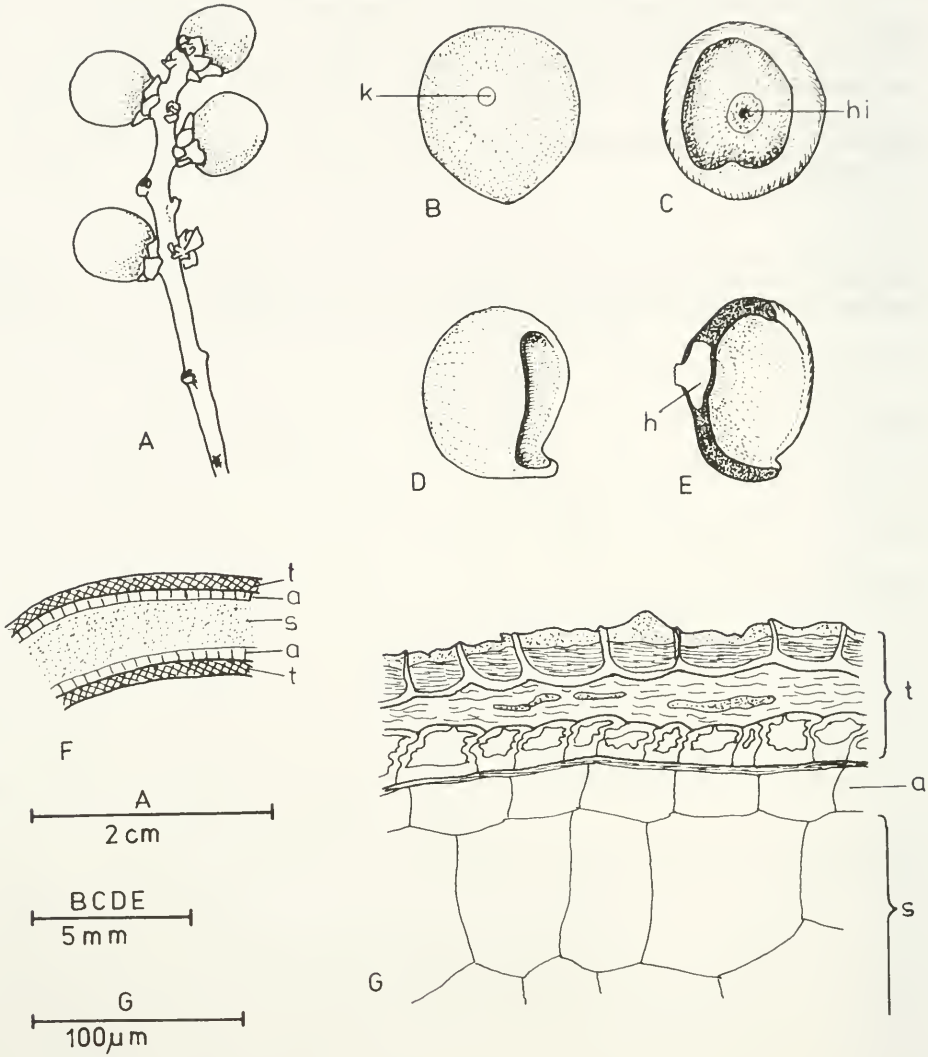

Fig. 1. *Hanguana* spec. A: infructescence (collected by J. Bogner in Sarawak). B–G: seed (collected by R. Brett in Bukit Timah Nature Reserves, Singapore). B–E: seed shape, the seed is shown after detachment of the seedling plant. B: seed from above, showing the germination pore. C: seed from below, showing the hilum position. D: seed in lateral view, note the small lingular extension of the seed margin at bottom right. E: seed in median section, note the low thickness of the seed and the position of the haustorium. F: schematical cross section of the seed. G: cross section of the seed coat and adjacent endosperm. a: aleuron layer. h: haustorium. hi: hilum. k: germination pore. s: starch containing endosperm. t: testa.

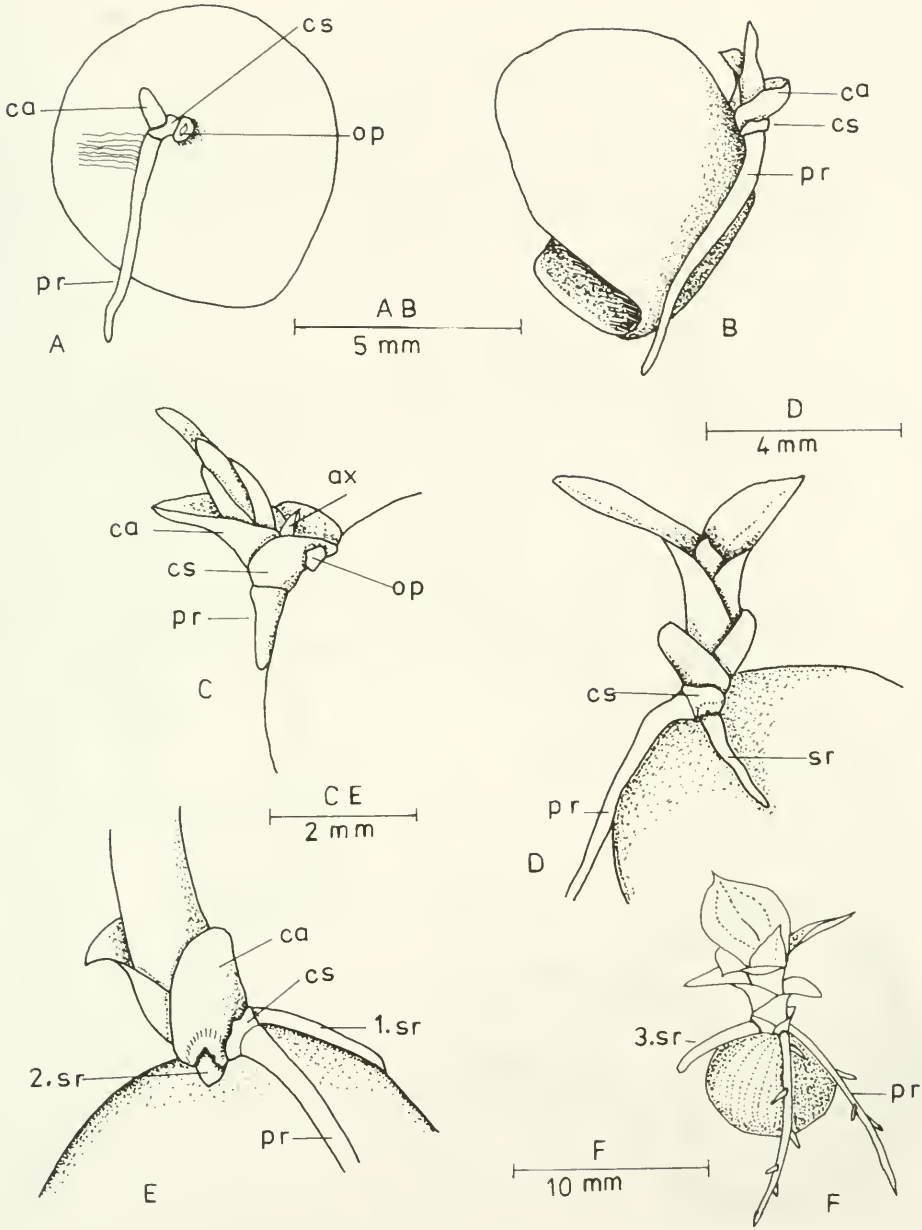

Fig. 2. *Hanguana* spec. Seedlings grown from seeds collected by J. Bogner in Sarawak. A, B, D–F: the same plant in successive stages of development. A, B: early stages of development, note that the cotyledonary sheath is very small in relation to seed size. C: young seedling showing a bud in the cotyledon axil. D: the first shoot-born root has emerged. E: the second shoot born-root emerges breaking through a small coleorhiza. F: branching of older roots, including the primary root begins. ax: prophyll of the bud in the cotyledon axil. ca: first cataphyll. cs: cotyledonary sheath. op: operculum. pr: primary root. sr: shoot-born root.

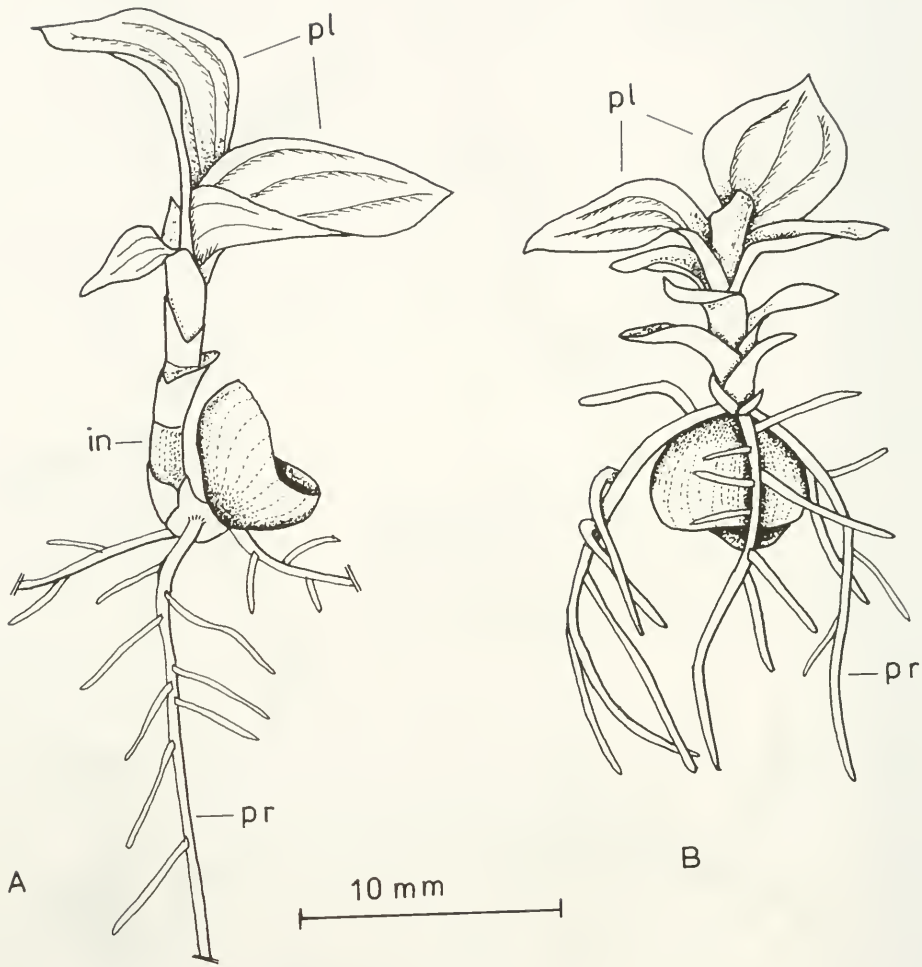

Fig. 3: *Hanguana* spec. A: seedling grown in soil under natural conditions, collected by R. Brett in Bukit Timah Nature Reserves, Singapore. Details of cotyledon and shoot base structure are not recognizable in this late stage of development. B: seedling grown in permanent light condition and from storage material of the seed only, seed collected by J. Bogner in Sarawak. in: elongated internode. pl: primary foliage leaves (eophylls). pr: primary root.

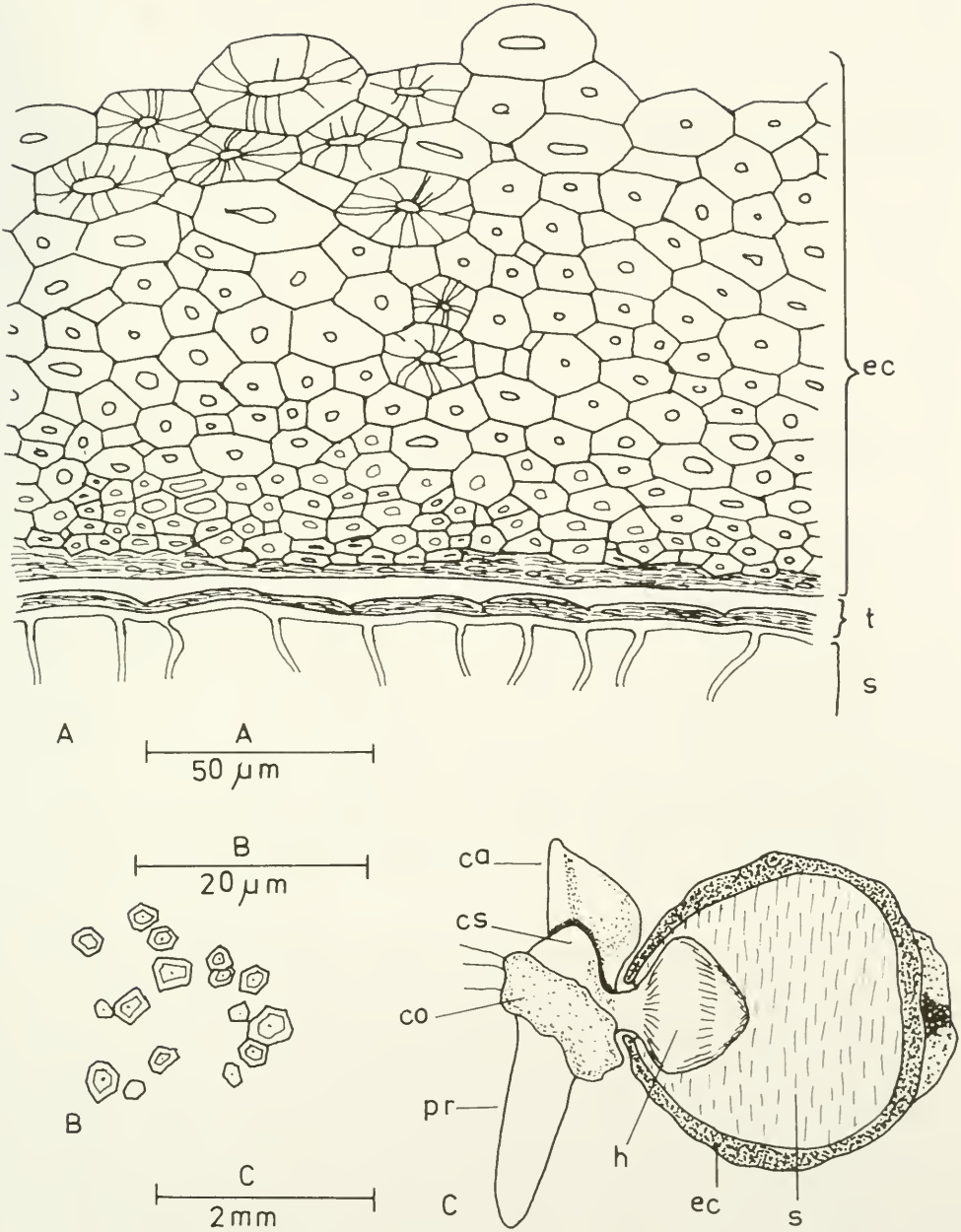

Fig. 4. *Flagellaria indica*. A: cross section of the endocarp and adjacent seed coat and endosperm. The pit canals are indicated only in a few stone cells. B: starch grains from endosperm. C: young seedling, the endocarp and endosperm shown in median longisection.