Bot. Bull. Acad. Sin. (1994) **35:** 105-113

Fruit structure of the genus *Pyrenacantha* Hook. (Icacinaceae) in southern Africa

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(Received August 18, 1993; Accepted March 2, 1994)

Abstract. Fruit development and structure of the three southern African species of *Pyrenacantha* were examined by scanning electron microscopy and light microscopy. Fruits of *Pyrenacantha* are laterally compressed drupes, with a pubescent outer surface. Their shape varies from more-or-less spherical in *P. kaurabassana* to ovate in *P. scandens* and *P. grandiflora*. Those of *P. grandiflora* ripen to yellow-grey, of *P. kaurabassana* to orange, and of *P. scandens* to a cream colour. The exocarp is one-cell-layered and develops solely from the outer epidermis of the ovary wall. The outer portion of the mesocarp is parenchymatous with vascular bundles and cells containing druse crystals of calcium oxalate, whereas a sclerified stone (endocarp *sensu lato*) develops from the inner portion. A one-cell-layered parenchymatous endocarp (*sensu stricto*) develops from the inner epidermis of the ovary wall. Endocarpal processes take their origin from clusters of initial cells in the mesophyll parenchyma next to the inner epidermis, and eventually project deep into the locule and seed. The presence of stomata on the surface of the processes, suggests that in addition to enlarging the surface area of the endocarp and seed, they may facilitate gas exchange between pericarp and seed. Micro-sculpturing of the surface of the endocarpal processes is of taxonomic significance at the species level. The outer surface of the mature stone is extensively pitted in a species specific pattern.

Keywords: Anatomy; Fruit; Icacinaceae; Pericarp; *Pyrenacantha*.

Introduction

The genus *Pyrenacantha* Hook., a member of the tropical/subtropical family Icacinaceae, comprises dioecious or monoecious lianas and scandent shrubs. Sleumer (1942) classifies it in the Phytocreneae, a tribe of lianas with serrate or palmately lobed leaves, fruit with the inner surface of the endocarp verrucose or spiked, and seeds with an embryo as long as the endosperm. Bailey and Howard (1941), however, consider this classification inaccurate and place *Pyrenacantha* in their group three, an informal assemblage of genera characterized by unilacunar nodes and simple vessel perforations.

Pyrenacantha consists of about 30 species, mainly African, but also extending towards Madagascar, India, and the Philippines. Tanai (1990) showed through fossil evidence that *Pyrenacantha* had also occurred in other parts of the northern hemisphere during the Paleogene. In southern Africa there are only three species, namely *P. grandiflora* Baill., *P. kaurabassana* Baill., and *P. scandens* Planch. ex Harv. These species are lianas with spicate or racemose inflorescences and inconspicuous unisexual flowers lacking a calyx. The female flowers have isomerous vestigial staminodes alternating with the petals, and the ovary is usually ellipsoid and densely pubescent, crowned by a sessile discoid or multiradiate stigma (Mendes, 1963). Species of *Pyrenacantha* play an important ethnobotanical role in Africa, with several being used for a variety of culinary, medicinal, and other traditional purposes (Watt and Breyer-Brandwijk, 1962; Akubue, Mittal, and Aguwa, 1983; Aguwa and Okunji, 1986; Mabogo, 1990). For example, during winter, leaves of *P. scandens* account for up to 85% of the edible spinaches collected for home consumption by the rural people in certain parts of KwaZulu, Natal (Cunningham, 1985).

The generic name *Pyrenacantha* was proposed by Hooker (1831), who remarked: "I have ventured to call it *Pyrenacantha* from the remarkable spinous processes which line the inside of the nut". The fruit of *Pyrenacantha* is a drupe, usually compressed and marginated, elliptic-oblong in shape, green when young, ripening to cream, orange, or red. The fruit is unusual in that it has prominent processes or prickles on the inner

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surface of the thin endocarp, which project into the interstices of the seed. Tips of processes can be sharp, round, hooked, forked, or club-shaped, and are diagnostic at the species level (Villiers, 1973).

Although the endocarpal processes have been the subject of much taxonomic interest, their development and possible function are still unknown. This paper reports on the first comparative study of the anatomical structure of the pericarp in *Pyrenacantha.* The research is aimed at elucidating the structure of the pericarp and assessing the taxonomic value of the various fruit characters.

Materials and Methods

Fruit structure was studied in the three southern African species of *Pyrenacantha* (Table 1). Fresh fruits in various stages of development, collected from several natural populations, were preserved in formalin-acetic acid-alcohol (FAA) (Johansen, 1940). Light microscopy (LM) and scanning electron microscopy (SEM) were used. Descriptive terminology is based on Roth (1977), except in the description of the outer epidermal wall where Radford et al. (1974) are followed. Also note that the various zones in the pericarp of the Icacinaceae are here interpreted *sensu stricto* (preferable for establishing homology). This implies that the exocarp develops from the outer, and the endocarp *s. str.* from the inner epidermal layers of the ovary wall, and/or their derivatives. The ground tissue of the ovary wall differentiates to form the mesocarp.

LM Procedures

Small pieces of preserved fruit material were dehydrated, then infiltrated and embedded in glycol methacrylate (GMA) (Feder and O'Brien, 1968). GMA sections, 3–5 μm thick, were cut on a Porter Blum MT-1 ultramicrotome, stained and, where appropriate, permanently mounted in entellan (Art. 7961, E. Merck, Darmstadt). The periodic acid-Schiff's reaction (PAS) was carried out according to Feder and O'Brien (1968),

Table 1. Species of *Pyrenacantha* studied and voucher specimens

P. grandiflora Baill.: Potgieter 4, 10, 64, 68 (PRU)[a]; Van Wyk 2042, 2043 (PRU).
P. kaurabassana Baill.: Potgieter 72; Wild 4714 (PRE)[b]; Gutheri, Mangai & Kibui 79/68 (PRE).
P. scandens Planch. ex Harv.: Potgieter 58, 59, 61 (PRU); Van Wyk 1748, 1749, 1759, 1760, 1763 (PRU).

[a]**PRU**, H. G. W. J. Schweikerdt Herbarium; [b]**PRE**, National Herbarium.

Table 2. Morphological differences in structure of mature fruit among species of *Pyrenacantha*

Character	Taxon		
	P. grandiflora	*P. scandens*	*P. kaurabassana*
Thickness of pericarp (mm)	2–2.5	3–3.5	3
Length of fruit (mm)	(21–) 23 (–25)	(14–) 17 (–18)	(18–) 20 (–23)
Shape of fruit	ovate	ovate	spherical
Sculpturing of outer stone surface	strongly verrucate with few pits	psilate with an an intermediate degree of pitting	verrucate with abundant pits
Shape of outer tangential epidermal cell walls of endocarpal processes	convex	clavate	capitate or globose
Colour of ripe fruit	yellow-grey	cream	orange
Distribution of stomata on inner surface of pericarp	whole surface	on processes and in immediate vicinity	on processes and in immediate vicinity
Degree of pubescence	least	intermediate	most
Base of pointed trichomes on exocarp	elevated	elevated	not elevated

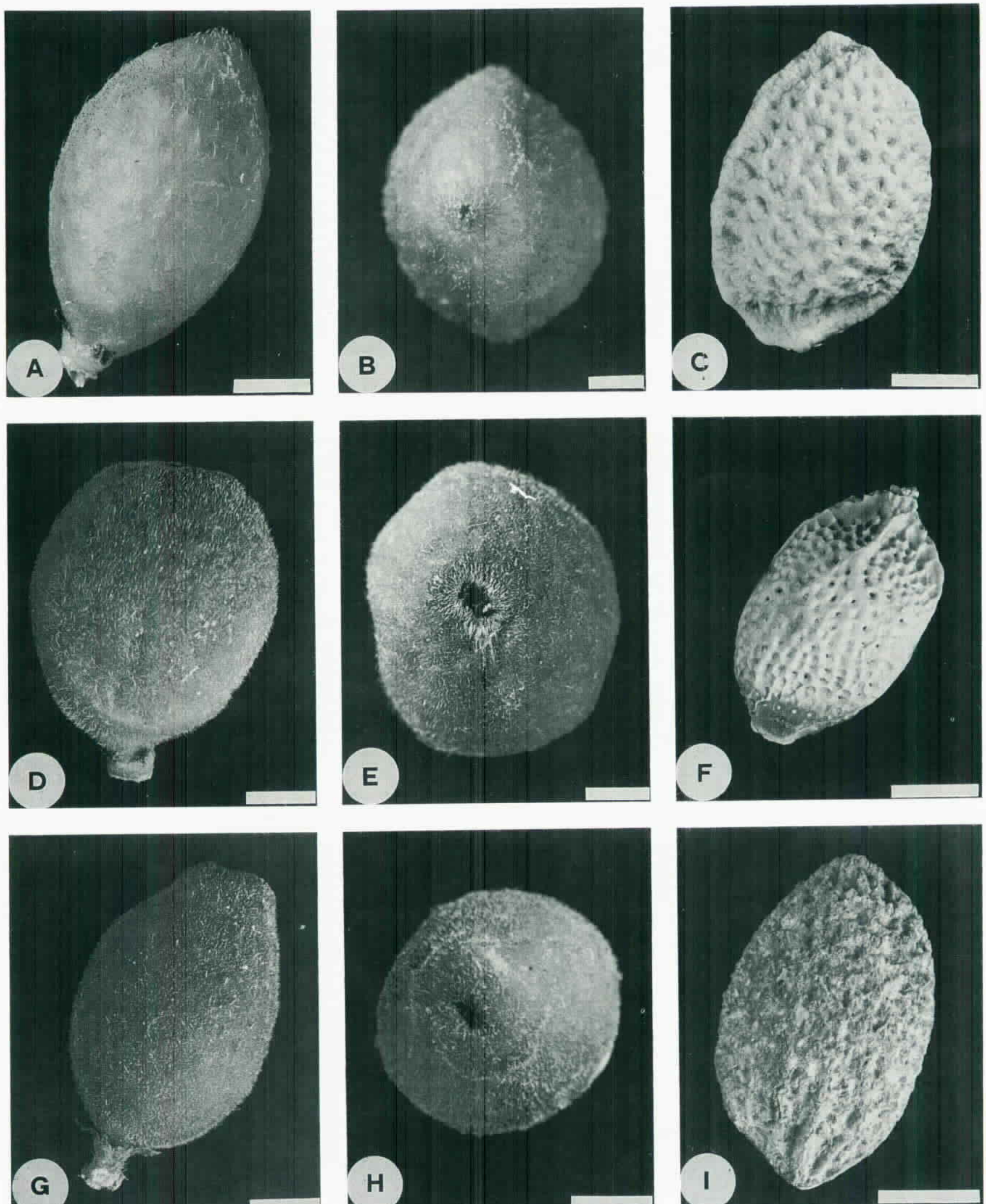

Fig. 1. Mature fruit of three species of *Pyrenacantha*. **A–C**) photograph of *P. grandiflora* showing **A**) lateral view; **B**) apical view; **C**) pitted stone. **D–F**) photograph of *P. kaurabassana* showing **D**) lateral view; **E**) apical view; **F**) pitted stone. **G–I**) photograph of *P. scandens* showing; **G**) lateral view; **H**) apical view; **I**) pitted stone. Scale bar = 4 mm.

using 0.5% dinitrophenyl hydrazine in 15% acetic acid (for 30 min) as blocking agent. Following the PAS reaction, some sections were counterstained for two minutes in 0.05% toluidine blue O (Feder and O'Brien, 1968) in a benzoate buffer at pH 4.4 (Sidman, Mottla and Feder, 1961). For further histochemical interpretation, GMA sections were treated with Sudan black B (O'Brien and McCully, 1981) and phloroglucinol (Johansen, 1940).

SEM Procedures

Fruit fragments were dehydrated, infiltrated with liquid carbon dioxide, subjected to critical-point drying, sputter-coated with gold, and examined with a Jeol JSM 840 SEM at 8 kV.

Results

Macromorphology of the Fruit

Fruits of *Pyrenacantha* are laterally compressed drupes, with a pubescent outer surface. The degree of pubescence varies in the southern African members, with *P. grandiflora* the least (Fig. 1, A & B) and *P. kaurabassana* the most pubescent (Fig. 1, D & E). Fruit shape tends to vary from more-or-less spherical as in *P. kaurabassana* (Fig. 1, D) to ovate as in *P. grandiflora* (Fig. 1A), and *P. scandens* (Fig. 1G). Mature fruits of *P. grandiflora* ripen to yellow-grey, those of *P. kaurabassana* to orange, and of *P. scandens* to a cream colour (Table 2).

Structure of the ovary Wall

The one-cell-layered outer epidermis of the ovary wall contains stomata, as well as normal (long, straight, pointed) and globular trichomes. Trichome bases are implanted between ordinary epidermal cells. The future mesocarpal zone (ground tissue) comprises two distinct zones, namely an inner and an outer zone. The outer zone contains vascular bundles and actively dividing parenchyma cells. The inner zone comprises five to six cell layers that lack intercellular spaces. The inner epidermis of the ovary wall is one-cell-layered, with cells containing large nuclei.

Ontogeny and Structure of the Pericarp

The exocarp of *Pyrenacantha* is one-cell-layered and develops solely from the outer epidermis. Globular trichomes and short hook-like trichomes as well as long, thick-walled pointed trichomes (normal hairs), all unicellular, are present on the outer epidermis. In *P. scandens* and *P. grandiflora* the long pointed trichomes have an elevated base; this is not the case in *P. kaurabassana*. Stomata are found on the outer pericarp surface of all the investigated species (Fig. 3F).

The mesocarp is parenchymatous and develops from the ground tissue of the ovary wall. It consists of an outer and an inner zone. The outer zone contains large vascular bundles as well as cells with druse crystals of calcium oxalate, and the inner initial cells from which the processes arise. Later in development, this zone lignifies to become the hard stone of the fruit. The endocarp (*s. str.*) originates from the inner epidermis of the ovary wall and remains one-cell-layered, comprising thin-walled and parenchymatous cells.

Endocarpal processes (stone and endocarp *s. str.*) develop towards the fruit locule from clusters of initial (meristematic) cells located in the mesophyll parenchyma next to the inner epidermis (Fig. 4A). These initial cells enlarge radially towards the inner epidermis. As a consequence, the inner epidermis is pushed into the fruit locule towards the developing seed (Fig. 4B). As cell division and radial elongation proceed, the bulges elongate into processes (Fig. 2 A & 4C). The processes have distinct inner and outer tissue zones. The outer zone, consisting of five to six layers of sub-epidermal cells, eventually acquires thick, lignified walls, and is continuous with the lignified mesocarpal zone (the stone). Staining with Sudan black B showed that a thin cuticle covers the inner epidermis. The inner zone consists of mostly thin-walled, non-lignified, radially elongated cells. Cells in this section tend to be loosely arranged, with relatively large intercellular spaces, and are continuous with the parenchymatous mesocarp (Fig. 2B).

Processes are already evident in the very young ovary, even before anthesis. They usually develop faster than the seed and eventually grow almost to the centre of the locule (Fig. 3A). The processes do not actually penetrate the seed-coat, but merely distort the shape of the seed. Hence, when the pericarp is removed from the mature fruit, the seed has cavities corresponding to the processes of the endocarp (Fig. 3C). The general shape of the processes is cylindrical with a rounded apex, and they are macroscopically very similar in the three species investigated.

The microscopic surface sculpture of the processes, however, differs substantially among the species. Processes in *P. kaurabassana* have epidermal cells in which the outer periclinal portion tends to be capitate or globular (Fig. 2, D & G). Outer epidermal cell walls are slightly convex in *P. grandiflora* (Fig. 2, C & F), whereas those of *P. scandens* are more or less clavate (Fig. 2, E & H).

Stomata are present on the processes and the inner surface of the pericarp (Fig. 2C & 3E). In *P. grandiflora*, stomata occur both on processes and on the whole inner surface of the pericarp. This is in contrast to *P. scandens* and *P. kaurabassana*, where stomata are located only on the processes and their immediate vicinity. At anthesis no stomata are present on locule walls; they clearly develop at a later stage. Perforations which may be

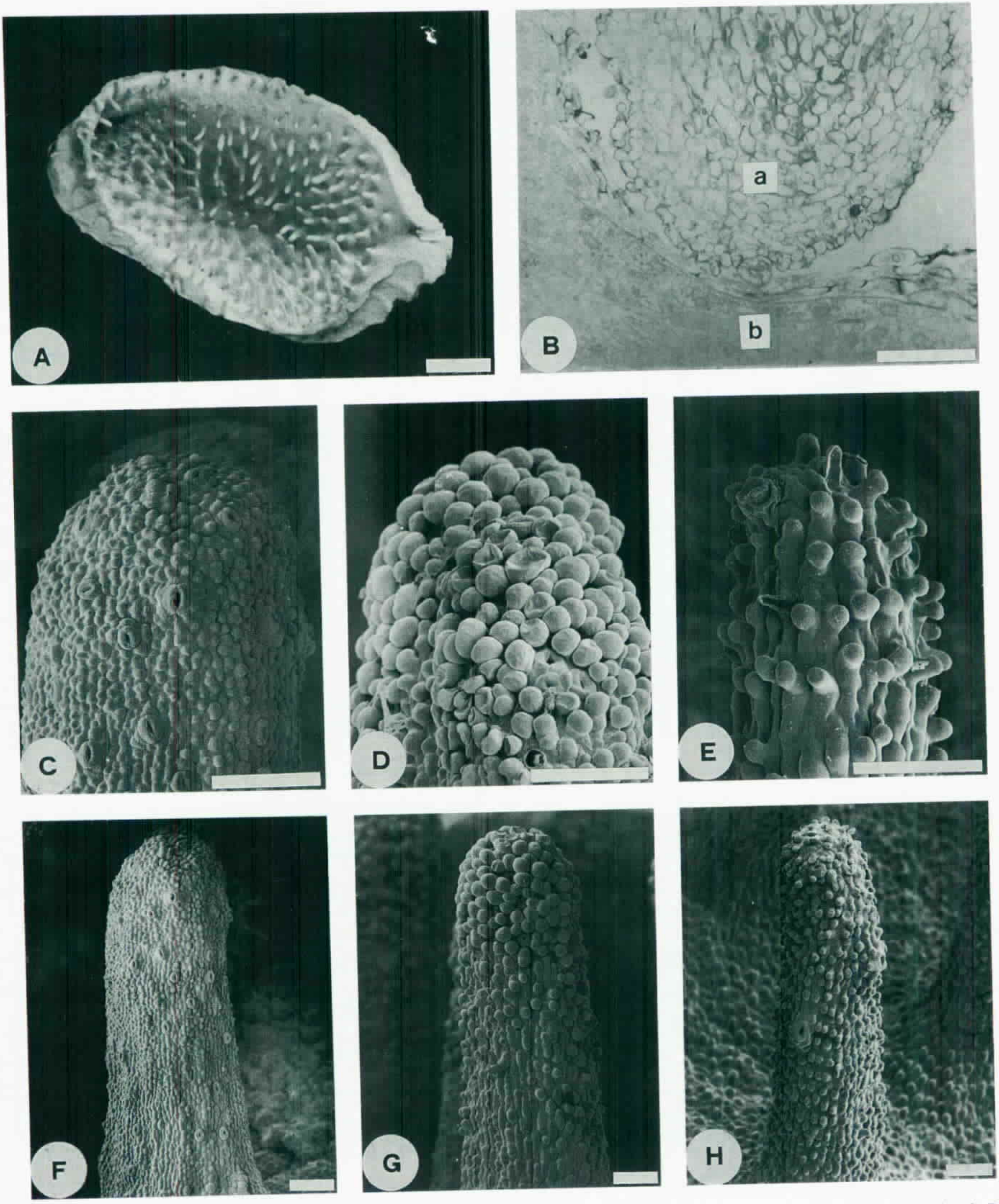

Fig. 2. A) Photograph of *Pyrenacantha kaurabassana* showing the inner surface of the stone; **B**) light micrograph of *P. kaurabassana*, showing an endocarpal process (**a**) in close contact with the seed (**b**); **C** & **F**) scanning electron micrographs of endocarpal processes of *P. grandiflora*; **D** & **G**) endocarpal processes of *P. kaurabassana*; **E** & **H**) endocarpal processes of *P. scandens*. Scale bars of A = 2 mm, B = 10 μm, C–H = 100 μm.

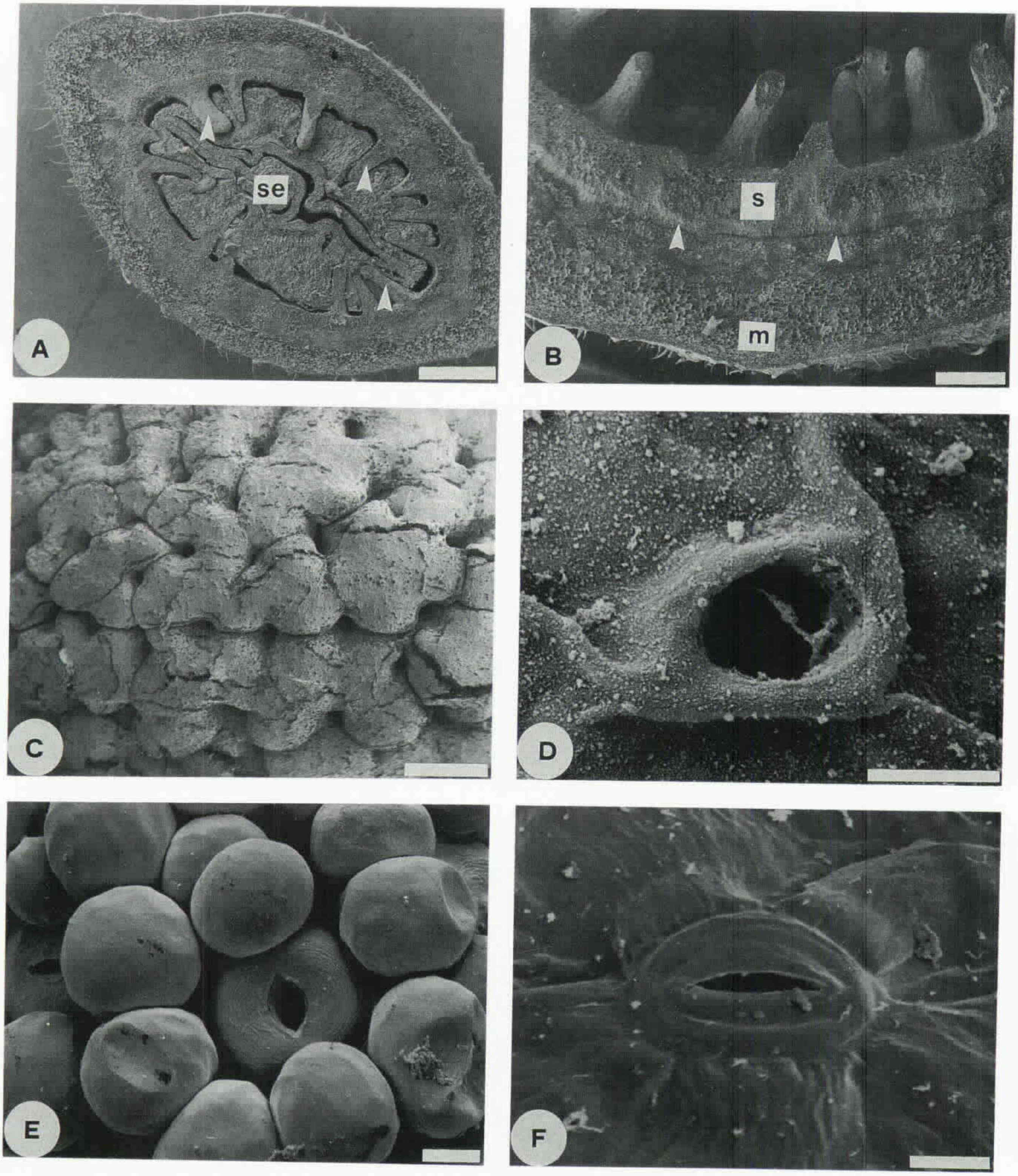

Fig. 3. A) Transverse section of fruit structure of *Pyrenacantha grandiflora* showing seed (**se**) penetrated by endocarpal processes (some arrowed); **B**) enlarged portion of the pericarp showing line of division (arrowed) between inner sclerified stone (**s**) and outer parenchymatous mesocarp (**m**); **C**) seed cavities left by fruit processes; **D**) perforation on the seed surface. Stomata of *P. kaurabassana*. **E**) stoma on process; **F**) stoma on fruit surface. Scale bars of A–C = 1 mm, D–F = 10 μm.

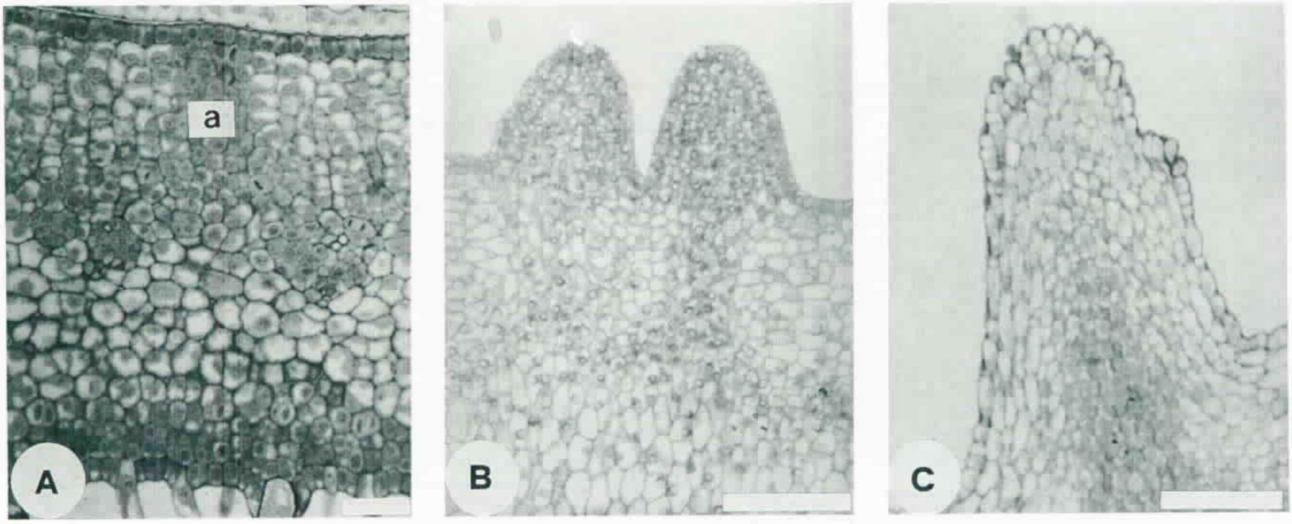

Fig. 4. Light micrographs of stages in the initial development of the endocarpal processes in *Pyrenacantha kaurabassana*. **a**) cluster of initial cells. Scale bar = 10 μm.

analogous to stomata were noted on the seed-coat, usually next to the intruding processes (Fig. 3D).

The outer surface of the mature stone (after removal of the fleshy exo- and mesocarp) is extensively pitted and shows a distinctive pattern. Specific differences are found both in the surface sculpturing and in the density of the pits. The smallest number of pits was observed in *P. grandiflora*, where the surface sculpture is strongly verrucate (Fig. 1C). In *P. kaurabassana* pits are more numerous than in the other two species and the surface is verrucate (Fig. 1F). In *P. scandens*, the surface sculpture tends to be rather smooth, with an intermediate number of pits (Fig. 1I). The pits always correspond to the number of processes that line the inside of the stone. The spongy mesocarp tissue inside the processes extends through the pits, and covers the outside of the stone (Fig. 3B).

Discussion

In *Pyrenacantha*, the sclerified part of the mesocarp together with the parenchymatous endocarp *s. str.* (inner epidermis) forms the stone (so-called endocarp *sensu lato* of some authors). In terms of a narrow-sense interpretation (*see* Materials and Methods) the fleshy part of the drupe comprises the exo- and outer mesocarp.

The presence of endocarp (*s. l.*) intrusions is not exclusive to either *Pyrenacantha* or the Icacinaceae. The endocarp of *Antidesma* L. (Euphorbiaceae), which is characteristically foveolate-reticulate inside, shows many similarities to that of several genera of the Icacinaceae, e.g. *Rhyticaryum* Beccari, *Natsiatum* Buch.-Ham. and *Iodes* Blume (Radcliffe-Smith, 1987; Carter and Smith, 1988). Reid and Chandler (1933) have found distinctive patterns with respect to the arrangement of the external pits and to the shape and size of the internal processes in members of the Icacinaceae. In the thick-walled endocarp of *Phytocrene* Wall. the processes consist only of low moulds, or may not be developed at all, as in some species. The thin-walled endocarp of *Chlamydocarya* Baill. has prominent vertical-sided processes which are longitudinally elongated and arranged in irregular longitudinal rows. The processes of *Miquelia* Meissn. are conical, and in *Polycephalium* Engl. irregular rows of circular processes occur in low mounds (Scott and Barghoorn, 1957).

Villiers's (1973) study in Cameroon showed that the processes of ten *Pyrenacantha* species differ substantially in macromorphological appearance, and he subsequently used these differences to distinguish between some of the species. We have shown that the micromorphology of the processes may provide further characters that could be used for diagnostic purposes. It is possible that by using only macromorphological characters, previous authors could have lumped different species together incorrectly. In this regard micromorphological characters such as the surface sculpture of the processes could be significant in the taxonomy of the group.

The function of the endocarpal processes remains unclear, with no suggestions in the literature. We suspect that the processes may serve to enlarge the internal surface of the endocarp and external surface of the seed. Minute perforations in the seed surface (perhaps analogous to stomata) usually occur in close proximity to the processes. The loose outer parenchymatous mesocarp tissue extends from within the processes to the outer surface of the stone, forming a zone which covers the stone. These features, together with the presence of stomata on the outside of the processes, suggest that these structures facilitate gas exchange between the pericarp and the seed.

Internal stomata (lining the fruit locule) are not unusual, and their occurrence has been reported in such diverse families as Ericaceae, Resedaceae and Liliaceae (Roth, 1977). Roth (1977) noted that stomata develop either in the outer epidermis only or on both surfaces of the pericarp. According to Fischer (1929a, b), stomata in the outer epidermis may function normally, whereas inside the fruit they could either be transformed into hydathodes or play a role in aeration. These comments further strengthen our case for a possible gas exchange role for the processes.

Trichomes are often of considerable taxonomic significance (Metcalfe and Chalk, 1979; Roth, 1977). This is also the case in the southern African members of *Pyrenacantha.* The presence of an elevated trichome base provides a good taxonomic character for separating *P. kaurabassana* from *P. scandens* and *P. grandiflora.* It seems that the short hook-like trichomes may have a dual function — facilitating fruit dispersal and reducing transpiration in the young fruit. The function of the large, pointed trichomes is unclear.

The macromorphology of the endocarpal processes in *Pyrenacantha* provides a good basis for taxonomic distinction, and has been successfully used for this purpose. The present study has shown that, despite a rather similar macromorphology, the micromorphological structure of the processes provides additional diagnostic characters. These findings offer considerable scope for detailed anatomical studies of the fruit structure of other members of the genus *Pyrenacantha.*

Acknowledgements. We thank Mss. E. du Plessis and M. Mössmer for critically reading and improving the manuscript, Ms. A. Veltman for technical assistance, and Dr. I. von Teichman for useful discussions on the various interpretations of pericarp structure. This research was supported by grants from the Foundation for Research Development and from the University of Pretoria.

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南非產 *Pyrenacantha* Hook.（茶茱萸科）的果實構造

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本研究以掃描電子顯微鏡及光學顯微鏡探討南非產 *Pyrenacantha* 屬三種植物的果實發育及構造。本屬果實爲二側扁平的核果，外表面有毛。果實的外形在 *P. kaurabassana* 爲圓形，在 *P. scandens* 及 *P. grandiflora* 爲卵圓形。果熟時 *P. grandiflora* 的果呈黃灰色， *P. kaurabassana* 的果橙色， *P. scandens* 的則爲米色。外果皮僅一層細胞厚，係由子房的外表皮發育而來。中果皮的外區爲含維管束的薄壁組織，亦含草酸鈣簇狀結晶；內區則爲厚壁化的硬核。狹義的內果皮爲一層薄壁細胞，係由子房壁內表皮發育而來。內果皮上的突起是由鄰近內表皮的一團薄壁始源細胞而來，最終會深入腔室及種子。這些突起的表面有氣孔的分布，顯示突起除了增加內果皮及種子的表面積外，也有助於果皮及種子的氣體交換。這些內果皮突起上的微細雕紋在種間有分類上的價值。而各種植物成熟的核的外表面上滿佈的孔洞皆呈固定之類型。

關鍵詞：解剖學；果實；茶茱萸科；果皮； *Pyrenacantha* 。