Studies in the southern African species of Justicia and Siphonoglossa (Acanthaceae): seeds

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ABSTRACT

The seeds of all species of Justicia (except J. thymifolia (Nees) C.B. Cl.) and Siphonoglossa in the southern African region (as defined by the Flora of southern Africa) were examined with the Scanning Electron Microscope. A wide range of different seed surfaces were seen, many of which could be correlated with other characters to further reinforce grouping of species into sections. However, some species that on other characters, would be placed together, had widely differing seed testas. Gross surface morphology varied from smooth to colliculate, irregularly rugose, reticulate with the ridges variously arranged, wheel-shaped (radiate or ammonite-like), with a central ridge or with multicellular barbed scales. The seeds were without hairs, unlike some American species sometimes considered to belong in Justicia (Graham 1989). Micromorphology also provided useful characters, with seeds being reticulate, papillate, minutely or deeply rugose or with one to many crystals visible. Critical-point drying was sometimes helpful in viewing microstructure.

UITTREKSEL

Die sade van al die Justicia- en Siphonoglossa-spesies (behalwe J. thymifolia (Nees) C.B. Cl.) in die Suider-Afrikastreek (soos deur die Flora of southern Africa omskryf) is met die aftaselektronmikroskoop bestudeer. 'n Wye reeks verskillende saadoppervlakke is waargeneem, waarvan baie met ander kenmerke gekorreleer kon word om die groepering van spesies in seksies verder te ondersteun. Sommige spesies wat op grond van ander kenmerke saam gegroepeer sou word, het egter uiteenlopend verskillende saadtestas gehad. Makro-oppervlakmorfologie het varieer vanaf glad tot knopperig, onreëlmatig rimpelrig, netvormig met die riwwe op verskeie maniere gerangskik, wielvormig (straalvormig of ammonietvormig), met 'n sentrale rif of met meersellige stekelskubbe. Die sade was sonder hare, anders as by sommige Amerikaanse spesies wat soms as lede van Justicia beskou word (Graham 1989). Mikromorfologie het ook nuttige kenmerke opgelewer, met sade wat netvormig, gepapilleer, fyn of diep rimpelrig was of met een tot baie kristalle sigbaar. Kritieke-punt-droging was soms nuttig by waarneming van mikrostruktuur.

INTRODUCTION

Very few SEM investigations of the seed surfaces of African members of the Acanthaceae have so far been carried out. Those with relevance to the present study are briefly reported on below.

Munday (1980), in revising the southern African species of *Monechma* (Justiciae), found that, although all seeds had a smooth testa, there was variation in their size, shape and colour. A longitudinal ridge was characteristic of one species [*M. desertorum* (Engl.) C.B.Cl] and mottled colouring of another [*M. spartioides* (T. Anders.) C.B.Cl.].

In his paper on *Anisotes* (also Justiciae) Baden (1981b) found that the seeds often had a longitudinal ridge, and he described the texture as rugose, tuberculate, verrucose or smooth. In one species (*A. guineensis* Lindau) the seeds were glandular. This species was unusual in other ways also, such as having 4-porate pollen, and was placed by Baden in a separate monospecific section.

Balkwill & Getliffe Norris (1984) used the fact that the seed surface in *Hypoestes* was asperous, tuberculate or smooth in their key to species.

Balkwill, Getliffe Norris & Schoonraad (1986) used the microsculpturing of the seed surface in their investigation of *Peristrophe* and concluded that its value at present was

mainly at species level. The testa of the species of the genus was basically reticulate with minute papillae, and may or may not have tubercules bearing one, two or three layers of recoiled hooks. Details of the tubercule surface were also used.

Hedrén (1987) in his revision of *Justicia capensis* Thunb. and its tropical African allies, described the seed surface only as 'tuberculate' and did not use it to distinguish species. In his paper on the *Justicia mollugo* group in tropical Africa (1988), he described the seeds of most of the species dealt with, but did not use them in his key.

Balkwill & Getliffe Norris (1988) re-appraised tribal and subtribal limits within the Acanthaceae of southern Africa, and considered the lack of hairs on the seed coat to be definitive of Justiciae and some genera of Acanthoideae. However, Graham (1989) included species of *Justicia* with a variety of hair types, some even being tested by her and found to be hygroscopic (in her sect. *Anisostachya*). Graham's delimitation of *Justicia* was very wide, including a number of previously segregate genera, and she described some 20 seed types. In sect. *Sarotheca* some species had seeds with hairs having recoiled barbs similar to those seen by Balkwill *et al.* (1986) in *Peristrophe*. In sect. *Vasica* some of the Old World species had smooth testas, a character usually considered characteristic of *Monechma*, which she accepted as a distinct genus.

METHODS

Seeds of each species and subspecies of Justicia in southern Africa (except J. thymifolia), as well as Siphono-

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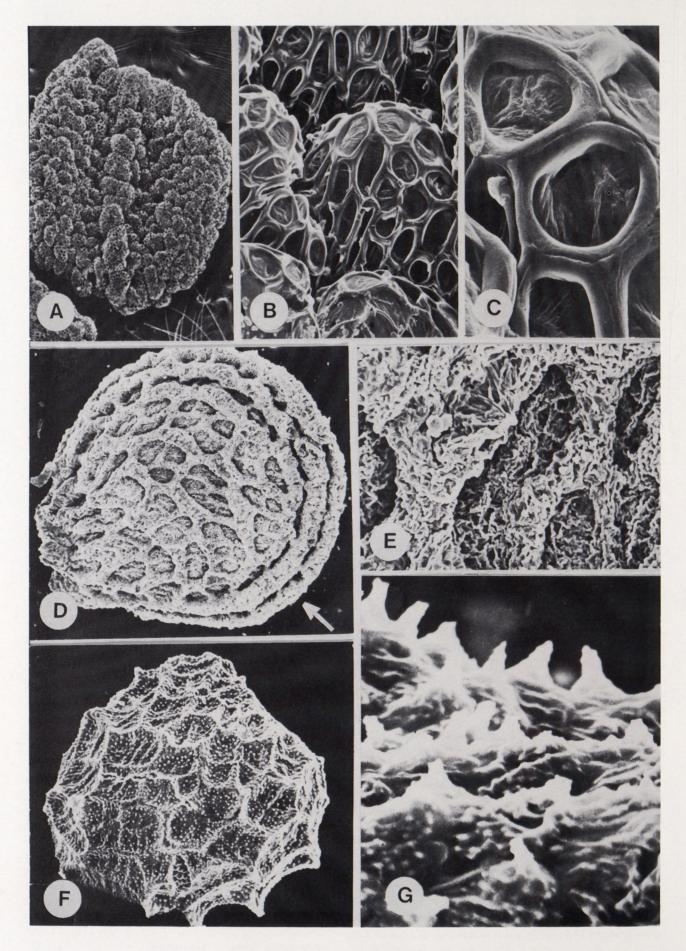


FIGURE 1.—Justicia sect. Harnieria p.p.: A-C, J. capensis: A, whole seed, × 30; B, detail of seed, × 360; C, whole seed, × 1200. Justicia sect. Rostellularia subsect. Ansellia: D-G. D, E, J. crassiradix: D, whole seed, arrow indicates semicircular marginal ridges, × 70; E, detail of seed, × 360. F, G, J. anselliana: F, whole seed (critical-point dried), × 70; G, detail of seed (critical-point dried), × 1600.

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glossa leptantha subsp. leptantha and Monechma mollissimum, were examined. Taxa covered in this study all occur in southern Africa as defined by the Flora of southern Africa, i.e. the area south of the Cunene and Limpopo Rivers. Seeds of each were removed from herbarium sheets and mounted directly onto 15 mm aluminium stubs for viewing under the SEM. Two to six seeds per taxon were viewed depending on the size of the seeds.

Where fresh fruiting material was available from the nursery (National Botanical Institute, Pretoria), seeds were also dehydrated, fixed and critical-point dried by the following method:

- specimens were dehydrated either in series of ascending concentrations of alcohol or, more usually, in two changes of 2,2-Dimethoxypropane (DMP) for up to two hours;
- 2, they were then transferred to 100% acetone for five minutes;
- 3, critical point drying was done with liquid CO_2 evaporated off at 40°C and 80–90 atmospheres (critical point for CO_2 is at 31,3°C and 72,8 atmos.);
- 4, the dried specimens were stored in a desiccator with silica gel, or immediately mounted on aluminium stubs for coating.

All seeds were glow-discharge coated with metallic gold in an Eiko sputter coater. They were then viewed with an MSM 4 Hitachi-Akashi (desk top model) SEM at kv 15 and photographed.

Delimitation and names of sections are according to Graham (1989).

RESULTS AND DISCUSSION

Results of critical-point drying: it was noted in many instances that material that had not been critical-point dried showed structures of the testa such as radial cell walls, ornamentation of these walls, and crystals in the testa, better than fresh, critical-point dried material. This was due to the collapse of the outermost cell walls, which made it easier to see underlying structures. On the other hand, structures such as fine papillae were sometimes only visible when the material had been pre-treated before viewing. This was the case with *J. anselliana* (Nees) T. Anders. (Figure IG), and it was felt that critical-point drying would be a valuable procedure for those species which, like *J. anselliana*, had 'amorphous' testas, as found in sects. *Ansellia* and *Justicia* p.p.

A wide variety of seed surface types was seen, sometimes peculiar to the species, but in at least some cases correlating with characters used to demarcate sections.

Sect. Rostellularia subsect. Ansellia p.p. (J. crassiradix Burkill & Clarke, J. anselliana (Nees) T. Anders., J. anagalloides (Nees) T. Anders.): in J. crassiradix the seed has a gross pattern of reticulate ridges and depressions on the sides, whereas around the margin of the seeds the ridges form a roughly semicircular pattern (Figure 1D, arrowed). The testa of two other southern African species, J. anselliana (Figure 1F) and J. anagalloides, which on the basis of other characters should probably be placed with J. crassiradix in subsect. Ansellia, do not show this semicircular pattern. Instead, the ridges are randomly placed on the seed surface. At higher magnifications, when they were viewed without having been critical-point dried, the seeds of all three species had an irregularly rugose surface (Figure 1E). When seed of J. anselliana was critical-point dried, the cells in the depressions between the ridges were found to each have a centrally situated papilla (Figure 1G), while the cells on the ridge were without papillae, having instead a minutely verrucose surface. The papillae were longer than wide, with an acute or truncate apex. It was not possible to establish whether these papillae were present in the other two species, as fresh material was not available.

Sect. Justicia p.p. (J. orchioides L.f., J. cuneata Thunb., J. guerkeana Schinz, J. platysepala (S. Moore) P.G. Mey., J. thymifolia (Nees) C.B. Cl.): rugose testas are also present in all examined species of Sect. Justicia except J. bolusii C.B. Cl. (Figure 2A, B). Only the first four species could be examined, as seeds of J. thymifolia were not available. This irregularly rugose pattern is apparent at both low and high magnifications. Seeds of J. guerkeana were also critical-point dried for viewing. In this case, unlike J. anselliana, the irregularly rugose pattern remained, and no papillae were seen.

Sect. Justicia p.p. (J. bolusii C.B. Cl.): this species (Figure 2C, D), which on other characters might be considered to occupy an isolated position among the southern African species of the genus, also has very atypical seeds. These are almost smooth, being only very slightly rugose even at high magnifications (Figure 2D) and compare well with *Monechma mollissimum* (Nees) P.G. Mey., which was also viewed for purposes of comparison (Figure 2E, F). However, it was decided that, despite similarities in the seeds and capsules, J. bolusii could not be transferred to *Monechma*. It differs from *Monechma* in its 'spicate' inflorescence and its tricolporate pollen with entire margocolpi (Immelman 1989).

Sects. Raphidospora p.p. (J. campylostemon (Nees T. Anders.), Tyloglossa p.p. (J. flava (Vahl) Vahl, J. kirkiana T. Anders.), Rostellularia subsect. Ansellia p.p. (J. minima A. Meeuse) and Harnieria p.p. (J. capensis Thunb.): seeds of a number of species of Justicia from various sections of the genus (listed above) are basically colliculate or rugose with slightly protruding radial cell walls forming a reticulate surface pattern. However, if other characters of the species are taken into consideration, they are not necessarily related, and there are also many differences in detail in both the macro- and micro-patterns. J. capensis (Figure 1A–C) and J. minima (Figure 3A–C) have a randomly colliculate macro-pattern and, at high magnifications, show a simple reticulate micro-pattern. J. campylostemon (Figure 4A-C) has an irregularly vermiculate macro-pattern, and the radial cell walls are ornamented with a double row of minute projections, which are more clearly visible without critical-point drying (Figure 4C). J. flava, J. kirkiana, and J. petiolaris have characteristic macro-patterns (see below) and crystals visible in the testa.

Sect. Tyloglossa (J. flava (Vahl) Vahl, J. kirkiana T. Anders. and J. petiolaris (Nees) T. Anders.): these three

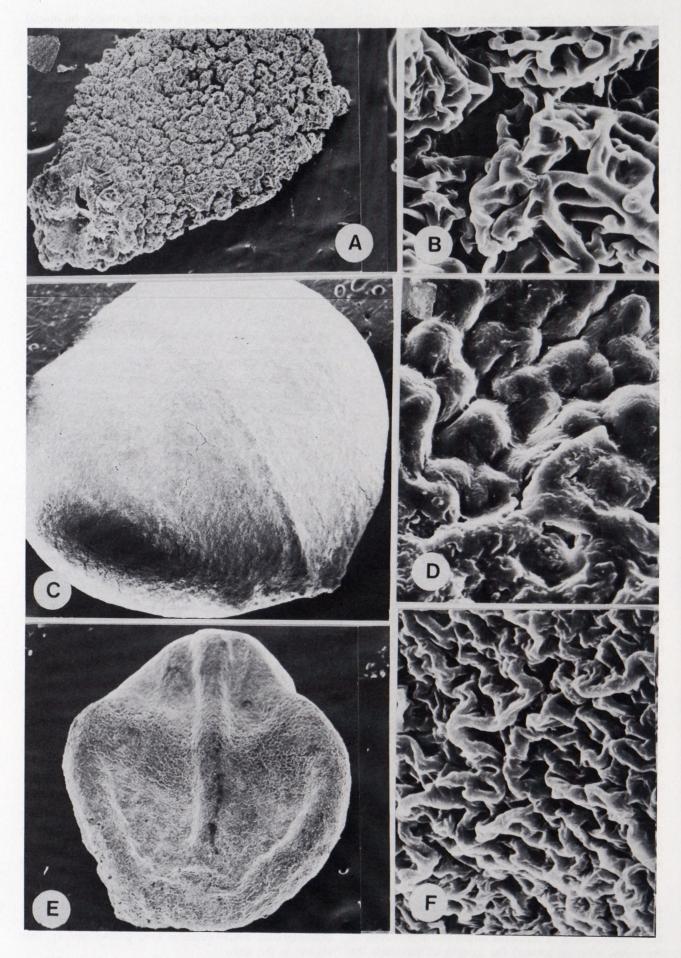


FIGURE 2.—Justicia sect. Justicia: A-D. A, B, J. orchioides: A, whole seed, × 55; B, detail of seed, × 1900. C, D, J. bolusii: C, whole seed (composite figure), × 25; D, detail of seed, × 1800. E, F, Monechma mollissimum: E, whole seed, × 60; F, detail of seed, × 2300.

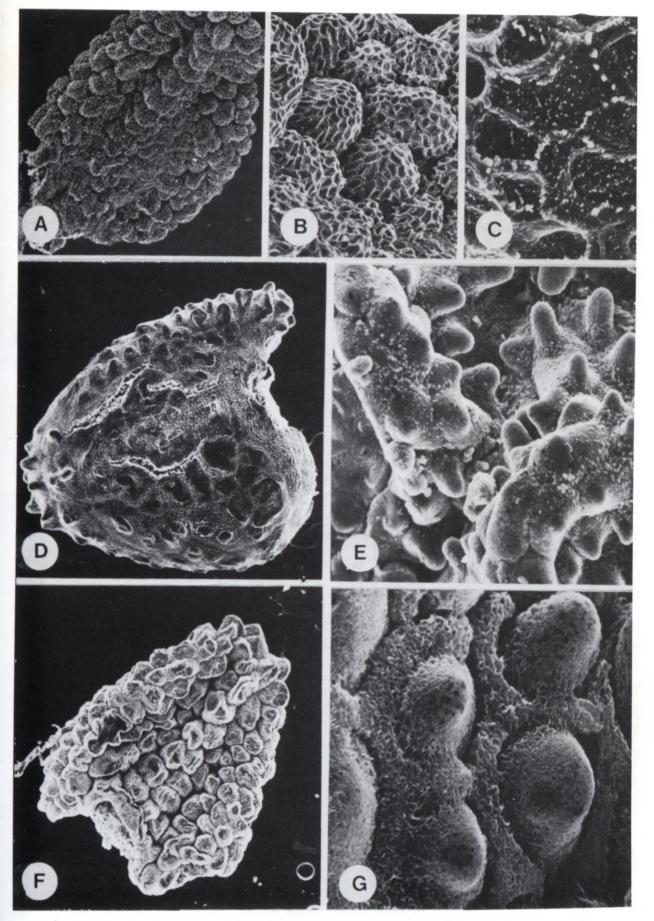


FIGURE 3.—A-C, Justicia minima: A, whole seed, × 50; B, detail of seed, × 200; C, detail of seed, × 1200. Justicia sect. Harnieria p.p.: D-G. D, E, J. protracta subsp. protracta: D, whole seed, × 50; E, detail of seed, × 1400. F, G, J. parvibracteata: F, whole seed, × 70; G, detail of seed, × 3500.

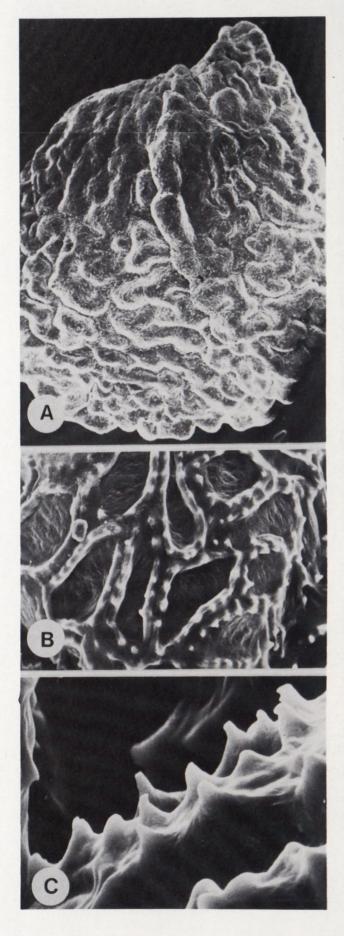


FIGURE 4.—Justicia sect. Raphidospora: A–C, J. campylostemon: A, whole seed (composite picture), \times 32; B, detail of seed, \times 725; C, detail of seed, \times 3000.

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species (Figure 5) form a well-defined section. They all have a characteristic reticulate micro-pattern on the seed surface and one large or many small crystals visible as cubic or rectangular projections below the surface of the outer wall (Figure 5B, D, F). Such crystals were not seen in any other Justicia species examined. These crystals are scarcely visible in critical-point dried material, but are clearly seen when the material is viewed without pretreatment. The two yellow-flowered species (J. flava and J. kirkiana) have a highly characteristic macro-pattern of raised segments or bosses (colliculae) (Figure 5C, E), termed 'ammonite-like' by Graham (1989), but which could also be interpreted as three segmented rows or as segments radiating from a central 'hub' like spokes of a wheel. This pattern is not present in any of the subspecies of the blueflowered J. petiolaris, where the gross pattern of the testa is sometimes colliculate, and sometimes rather irregularly rugose, often having a central longitudinal ridge.

Sect. Betonica (J. betonica L. and J. montissalinarum A. Meeuse): both these species have a macropattern of short sinuous ruminate ridges (Figure 6). The ridges are often drawn out into dentate structures which either have an areolate micro-pattern on their surface, as in J. betonica (Figure 6B) or they are irregularly striate as in J. montis-salinarum (Figure 6D). When broken, these teeth can be seen to be hollow.

Sect. Raphidospora p.p. (J. glabra Koenig ex Roxb.): the most complex pattern observed was that of J. glabra, which has seeds quite unlike those of any other Justicia species (Figure 7A-E). It is probable, on the basis of inflorescence and pollen characters, that it should be grouped with J. campylostemon, in sect. Raphidospora, but the seed testas of the two species are quite different. The testa of J. glabra is drawn out into numerous long flat multicellular scales (Figure 7B) with each cell of the scale producing a sharp retrorse barb (Figure 7C-E). The scales are probably a means of seed dispersal, analogous to the barbed scales on the fruit of Bidens pilosa (Asteraceae). J. campylostemon, on the other hand, has the seeds covered with irregular vermiculate ridges, each of which has a reticulate micro-pattern on the surface. Along each margin of the raised ridges of the reticulations there is a row of small, blunt, tooth-like projections (Figure 4B, C).

Sect. Harnieria p.p. (J. protracta (Nees) T. Anders., J. odora (Forssk.) Vahl, J. parvibracteata Immelman, J. dinteri S. Moore) as well as Siphonoglossa leptantha (Nees) Immelman subsp. leptantha: these species have a colliculate macro-pattern. They also share a micro-pattern where each cell is defined with a single large rounded papilla in the centre of each cell (Figures 3D-G; 8). These papillae are visible in material that was not pre-treated before mounting, as well as in critical-point dried material of S. leptantha subsp. leptantha (Figure 8A). Material of Siphonoglossa viewed without pre-treatment had an irregularly rugose testa.

In *J. dinteri* two kinds of capsule are regularly produced, a four-seeded normal capsule and a one-seeded smaller capsule with four irregularly toothed wings. The seeds from each type of capsule were compared, and the testa found to be similar, though the seeds in the one-seeded capsules were larger (Figure 8B, D, F, cf. Figure 8C, E).

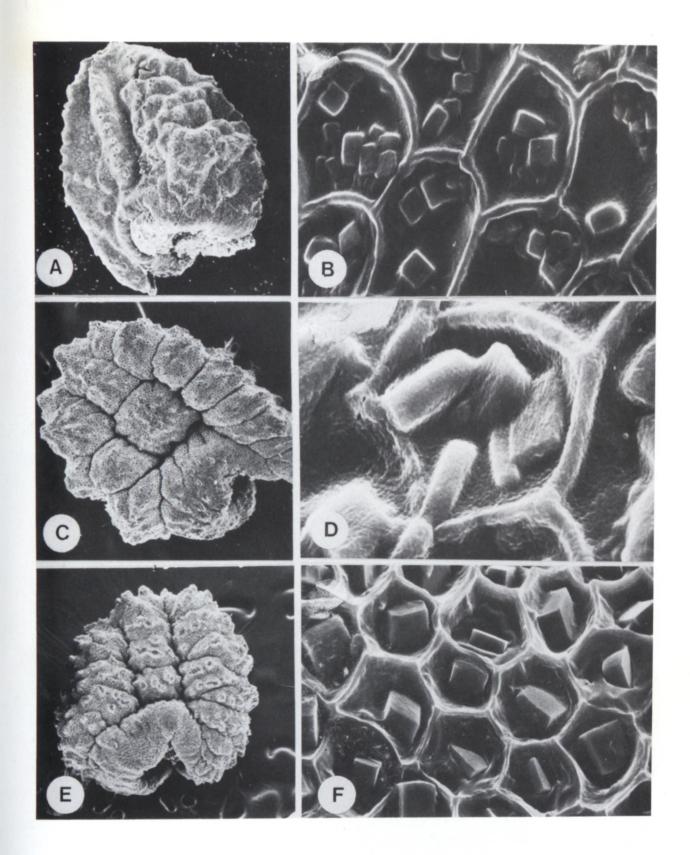


FIGURE 5.—Justicia sect. Tyloglossa: A-F. A,B,D, J. petiolaris: A, subsp. petiolaris, whole seed, × 30; B, subsp. incerta, detail of seed, × 2700; D, subsp. petiolaris, detail of seed, × 50. C, F, J. kirkiana: C, whole seed, × 50; F, detail of seed, × 2000. E, J. flava, whole seed, × 50.

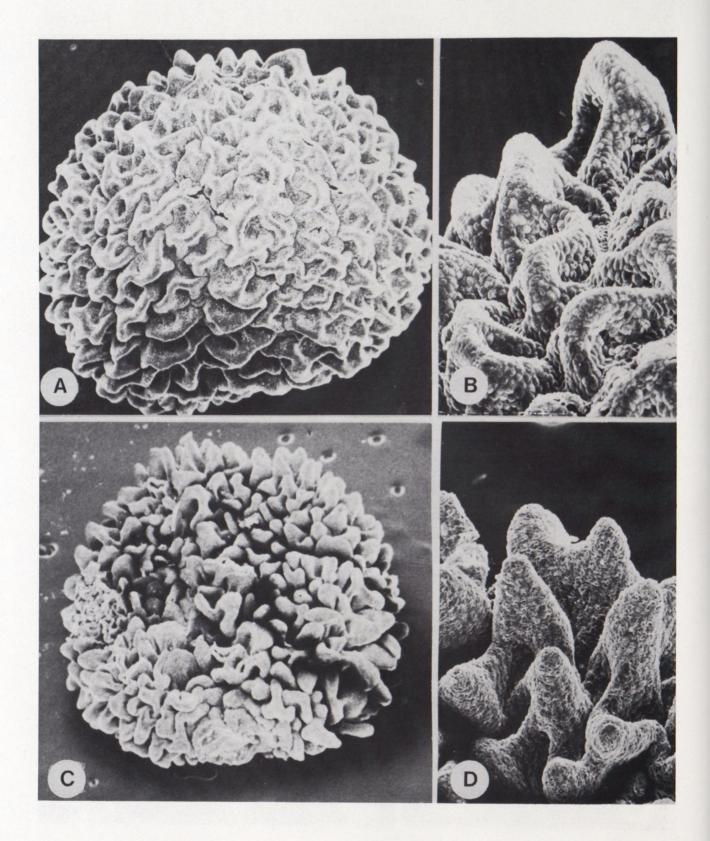


FIGURE 6.—Justicia sect. Betonica: A-D. A, B, J. betonica: A, whole seed, × 32; B, detail of seed, × 70. C, D, J. montis-salinarum: C, whole seed, × 50; D, detail of seed, × 220.

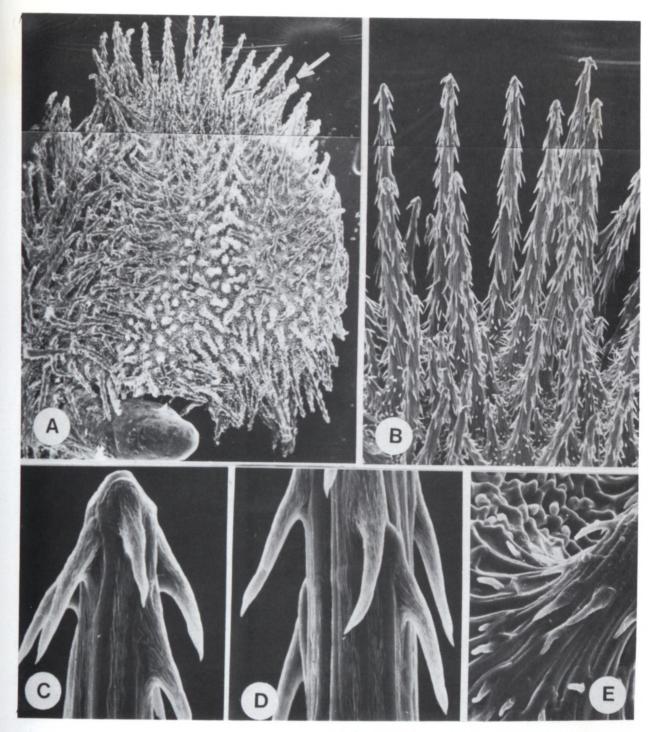


FIGURE 7.—*Justicia* sect. *Raphidospora*: A–E, *J. glabra*: A, whole seed (composite picture), × 55; B, scales on seed (composite picture), × 190; C, apex of scale, × 1800; D, shaft of scale, × 1800; E, base of scale, × 725.

One other species was examined, which on flower and pollen characters possibly belongs in sect. *Harnieria*. This was *J. capensis* Thunb. (Figure 1A–C), which was placed in this section by Graham (1989). The seeds, however, are different from the papillate seeds of the other species in the section, being colliculate with a strongly reticulate micro-pattern.

CONCLUSION

Both macro- and micro-pattern of the seed testa may be considered of major importance in placing many species in their sections, and delimiting some sections in *Justicia*, but need to be used with caution as they do not always correlate with other features. They are also suggestive of a relationship between some species of *Justicia* sect. *Harnieria* and *Siphonoglossa*.

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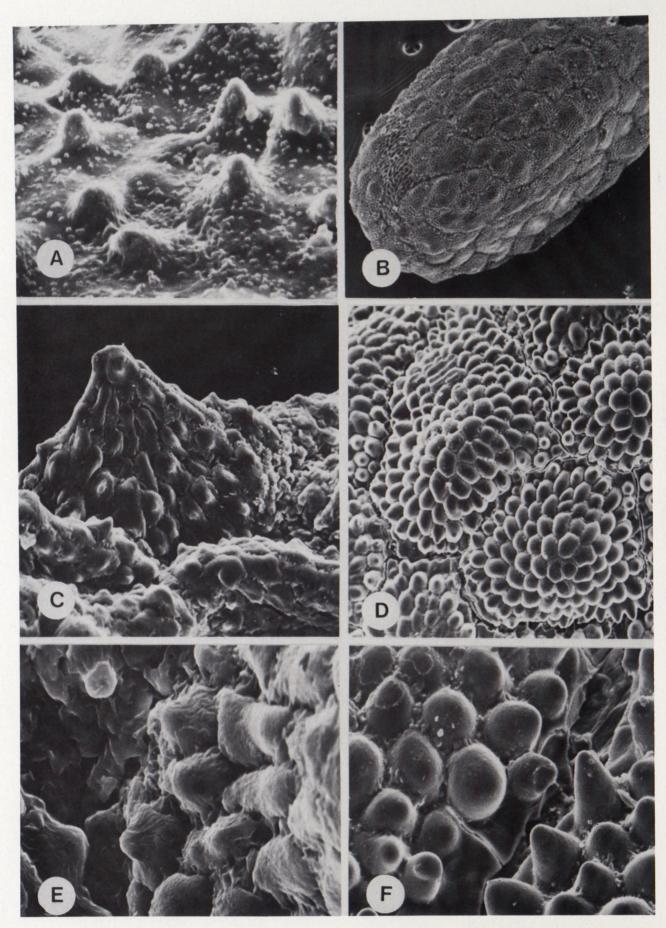


FIGURE 8.—Siphonoglossa leptantha subsp. leptantha: A, detail of seed (critical-point dried), × 2000. Justicia sect. Harnieria p.p.: B-F, J. dinteri: B, whole seed from one-seeded capsule, × 60; C-F, detail of seed, C, from four-seeded capsule, × 900; D, from one-seeded capsule, × 370; E, from four-seeded capsule, × 3000; F, from one-seeded capsule, × 1000.

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