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Bolboschoenus maritimus s. l. in The Netherlands: a study of pericarp anatomy based on the work of Irene Robertus-Koster

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Electron microscope scanning results of *Bolboschoenus maritimus* (L.) Palla *sensu lato* (= *Scirpus maritimus* L.) achenes, collected from natural populations in The Netherlands and investigated earlier by Irene Robertus-Koster, are given. Speculative conclusions suggest the variability observed may be the outcome of introgression through earlier generations influenced by ecological effects not adequately understood.

Key words: achene study, *Bolboscheonus maritimus*, (Cyperaceae), The Netherlands

INTRODUCTION

Robertus-Koster (1969) reported upon the different taxonomic interpretations applied to *Scirpus maritimus* L. *s. l.*, in which taxon the inflorescence is sometimes a compact head, sometimes branched and open. These interpretations ranged from separate species to habitat forms. The problem invited further study, which this author undertook in The Netherlands, where natural populations were available for investigation. Results showed a low number of branches per inflorescence (0–2) in coastal samples where salinity was higher, compared with inland freshwater samples where inflorescences were open with clearly defined branches (4–6). The extremes were linked by transitional forms that showed gradual increase in inflorescence branch number from coastal to inland habitats. Culture experiments over two years suggested the observed differences were genotypical. External achene features of shape, size and colour were also considered. Results showed correlation in that plants from coastal, saline localities carried achenes that were predominantly light brown to brown, variable in width (but mostly broad) and in their shape in

transverse section (subtrigonous to lenticular), whereas those from inland situations were dark brown to almost black, narrower in width and triangular in transverse section.

Independently, a field study of *Bolboschoenus maritimus* (L.) Palla *s. l.* (= *Scirpus maritimus s. l.*) carried out in a southern African wetland (Verlorenvlei, Atlantic coast, W. Cape Province, S. Africa) (Browning *et al*. unpublished) produced comparable results. In this later study, achenes were subjected to electron scanning, an examination technique not used by Robertus-Koster (1969). The results revealed differences in achene surface and in pericarp anatomy in coastal and inland extremes. Robertus-Koster (1969: 196 and 197, figs. 2 and 3) had reproduced photographs of achene samples. It seemed opportune to enquire whether these samples had been preserved and if they might be made available for micro morphological investigation. Due to the kindness of Irene Robertus-Koster, eleven of her original samples of about 100 achenes were provided for a study comparable with the electron scanning carried out on achenes from Verlorenvlei.

This paper reports the findings on the achenes from The Netherlands. These support and supplement the results given by Robertus-Koster (1969). The results obtained for the southern African samples will be published in detail later as part of the complete Verlorenvlei study, in which a summary of differences observed between the achene samples from The Netherlands and South Africa will be included.

MATERIALS AND METHODS

The eleven achene samples were examined individually using a binocular microscope × 40. This revealed a degree of variability within each sample. To quantify this, 50 achenes were removed at random from each sample, except for those from Maastricht and Punt van Reide 4, where the reduced size of the sample originally received permitted removal of 20 only.

Within each sub-group of 50 or 20 achenes, those showing marked differences in outline shape and colour were separated, counted, and the percentages for each main aspect calculated: sizes were measured for each aspect.

From the eleven original samples, nine were taken as representative of coastal to inland habitats: these were used for electron microscopy. Six achenes were taken from each of the nine samples; of these six, three were mounted with abaxial surface uppermost on stubs, three were fractured according to the methods described by Browning and Gordon-Gray (1993). Whole and fractured achenes were viewed under an Hitachi S570 Scanning Electron Microscope and photographed. Ratios of exocarp to mesocarp were determined by measurement of micrographs. These were supported by binocular microscope (× 40) measurements of transverse sections (x-sections) of further achenes from each sample.

RESULTS

Results are summarised in Table 1, which is supplemented by six figures of electron micrographs (Figs. 1–6). These are representative of the nine coastal to inland habitats mentioned in Materials and Methods and named in the table. Table 1 and Figs. 1–6 should be considered in conjunction.

Two groups were found to be present, namely:

Group 1 (Table 1, except last two samples; Figs. 1–4).

Localities: Groningen West E2; Haringvliet H4; Punt van Reide R7; Biesbosch. Of these, Haringvliet and Biesbosch were tidally influenced (Robertus-Koster, 1969: 198, table 1).

Achenes: variable (see Table 1), but predominantly obovate in outline, with pericarp light brown to brown, the surface glossy and marked by regular polygonal cell outlines forming a honey-comb pattern; the polygonal cells with depressed anticlinal walls and raised outer periclinal walls. In x-section predominantly compressed subtrigonous, with exocarp cells radially elongate; ratio of exocarp depth to mesocarp depth ca. 2:1.

Group 2 (Table 1, last two samples only; Figs. 5 and 6)

Localities: Maastricht, Schoonhoven L1. Of these, Schoonhoven was tidally influenced; Maastricht, further inland and not collected by Robertus-Koster (personal communication), is not included in table 1 by Robertus-Koster (1969: 198).

Achenes: somewhat variable (see Table 1), but predominantly narrowly obovate in outline, with pericarp dark brown to black, the surface glossy (Maastricht) or dull (Schoonhoven), marked only faintly by cell outlines, the honey-comb pattern modified, sometimes imperfect; cells with anticlinal walls raised, outer periclinal walls frequently depressed. In x-section trigonous, with exocarp cells radially elongate, but less deep than in Group 1; ratio of exocarp depth:mesocarp depth ca. 1:2 (Maastricht) 1:1.4–1.7 (Schoonhoven).

Table 1. Results of achene study of material from The Netherlands donated by Irene Robertus-Koster (E:M expresses ratio of exocarp depth to mesocarp depth).

Sample	Variability, outline-shape, size, colour, surface topography, x-section and E:M
Group 1	
Groningen West E2 (Fig. 1)	100% obovate; 3.0–3.4 × 2.2–2.8 mm; golden brown; smooth, glossy, cells polygonal, regular; obtusely trigonous. E:M = 2:1
Groningen West Z3	84% obovate; 3.1–3.6 × 2.2–2.7 mm; golden brown; cells polygonal; subtrigonous. 10% narrowly obovate (smaller); dark brown to black; cells faintly marked; subtrigonous. 6% elliptic; golden brown; cells polygonal; lenticular. E:M = 2:1
Haringvliet H4 (Fig. 2)	74% obovate; 3.1–3.8 × 2.4–2.8 mm; golden to ochre-brown; cells polygonal; subtrigonous. 14% elliptic; 3.5–4.1 × 2.0–2.5 mm; golden brown; cells polygonal; lenticular. 8% obovate; golden brown; cells polygonal; lenticular. 4% narrowly obovate (smaller); brown (2), black (2); cells faintly marked; subtrigonous. E:M = 2:1; but Fig. 2J = 1:1
Haringvliet D1	58% obovate; 3.4–4.3 × 2.3–2.6 mm; golden brown; cells polygonal; subtrigonous. 42% elliptic; 3.7–3.9 × 2.2–2.4 mm; golden brown; cells polygonal; lenticular. E:M = ca. 2:1
Punt van Reide R7 (Fig. 3)	70% obovate; 4.0–4.5 × 2.5–2.9 mm; ochre to kharki colour; cells polygonal, smaller on flat surfaces, larger on achene shoulders; obtusely trigonous. 30% elliptic; 3.9–4.8 × 2.1–2.7 mm; ochre to kharki colour; cells polygonal but uneven in size; subtrigonous (10), lenticular (5). E:M = ca. 1.5:1
Punt van Reide R4 (not scanned)	65% obovate; 3.1–4.3 × 2.2–2.7 mm; golden brown; cells polygonal; subtrigonous. 25% elliptic; golden brown; cells polygonal; lenticular and a few subtrigonous. 5% obovate; almost black; cells faintly polygonal; lenticular. 5% narrowly obovate; black, cells faintly marked; triangular in x-section. E:M = 2:1
Punt van Reide R6 (not scanned)	60% obovate; 3.1–3.8 × 2.1–2.5 mm; light brown to ochre; cells polygonal but variable in size; subtrigonous. 30% elliptic to obovate; 3.3–3.6 × 1.8–2.0 mm; light brown to ochre; cells polygonal but variable in size; subtrigonous. 10% elliptic; light brown to ochre; cells polygonal but variable in size; lenticular. E:M = ca. 1.5:1
Biesbosch (Fig. 4)	74% obovate; 3.0–4.0 × 2.4–2.8 mm; dark brown; cells polygonal, variable, most noticeable on abaxial ridge; subtrigonous. 20% elliptic; 3.6–4.0 × 2.0–2.5 mm; dark brown, cells polygonal, variable, most noticeable on abaxial ridge; trigonous, but not sharply angled. 6% elliptic; golden brown; cells polygonal; lenticular. E:M = 2:1
Slout Flakkee V5	50% obovate to elliptic; 4.0–4.3 × 2.0–2.4 mm; golden brown; cells polygonal appearing small; subtrigonous.

(Continues …)

Table 1. Continued.

Sample	Variability, outline-shape, size, colour, surface topography, x-section and E:M
	20% obovate; 3.1–3.9 × 2.2–2.6 mm; golden-yellow; cells polygonal; subtrigonous. 18% elliptic; golden, cells polygonal appearing small; lenticular. 10% elliptic; golden-yellow; polygonal; subtrigonous. 2% narrowly obovate; almost black; cells faintly marked; subtrigonous. E:M = 2:1
Group 2	
Maastricht (Fig. 5)	90% narrowly obovate; 3–3.5 × 1.5–1.9 mm; black; smooth, glossy, cells faintly marked; triangular in x-section. 6.6% obovate; brown-black; smooth, glossy, cells faintly marked; lenticular (1) and subtrigonous (1). 3.3% obovate; black; smooth, glossy, cells faintly marked; subtrigonous. E:M = 1:2
Schoonhoven L1 (Fig. 6)	84% narrowly obovate to obovate; 3.6–3.9 × 2.0–2.3 mm; dark brown, some pale off-white; cells mainly visible on abaxial ridge; triangular in x-section but less so than Maastricht. 8% somewhat obovate; dark brown; cells visible on abaxial ridge; triangular in x-section. 8% obovate, dark brown, cells visible on abaxial ridge; triangular in x-section. E:M = ca. 1:1.4–1.7

DISCUSSION

The most striking feature of this study of achenes from The Netherlands is their variability, which is evident in outline shape (obovate to narrowly obovate), colour (golden brown, light brown to ochre or dark brown to nearly black) and size (3–3.5 × 1.5–1.9 mm, Maastricht, to 4.0–4.5 × 2.5–2.9 mm, Punt van Reide R7). However, despite this variability, the achenes fall into two main groups, the features of which are given in Results. The most significant of the differences in the two groups are outline shape, pericarp colour and surface patterning, shape in transverse section and ratio of exocarp depth to mesocarp depth.

Robertus-Koster (1969) likewise distinguished two groups from her study of more comprehensive samples. She recognised 'fruits … small, broad, brown, semi-spherical to slightly triangular [in outline], with great variability within and between the samples', (compare our Results, Group 1) and 'fruits; narrow [in outline], dark brown to almost black, in cross-section sharply triangular. The variability of the fruits within a sample and between samples of different places is small.' (Robertus-Koster 1969: 200, English summary; compare our Results, Group 2).

Robertus-Koster related her group with small, brown, semi-spherical achenes to plants that carried compact inflorescences with a small number of inflorescence branches (0–2) which grew in coastal or tidal (usually more saline) habitats. Her group with dark, almost black, narrowly-triangular achenes was representative of plants with open branched (4–6) inflorescences from inland (usually less saline to fresh water) habitats. She was careful to stress that the groups, representing extremes, were linked by transitional forms so that, at least in morphological characteristics, there was intergradation between what she termed paramorphs *compactus* (Group 1) and *maritimus* (Group 2).

From our study of the samples, we are able to add for each of these extremes, further details of achene structure revealed by electron scanning, namely achene surface features and ratio of exocarp to mesocarp depths (see Results, Table 1 and Figs. 1–6). We are also able to support the major distinctions between Groups 1 and 2 from the results of the independent study carried out at

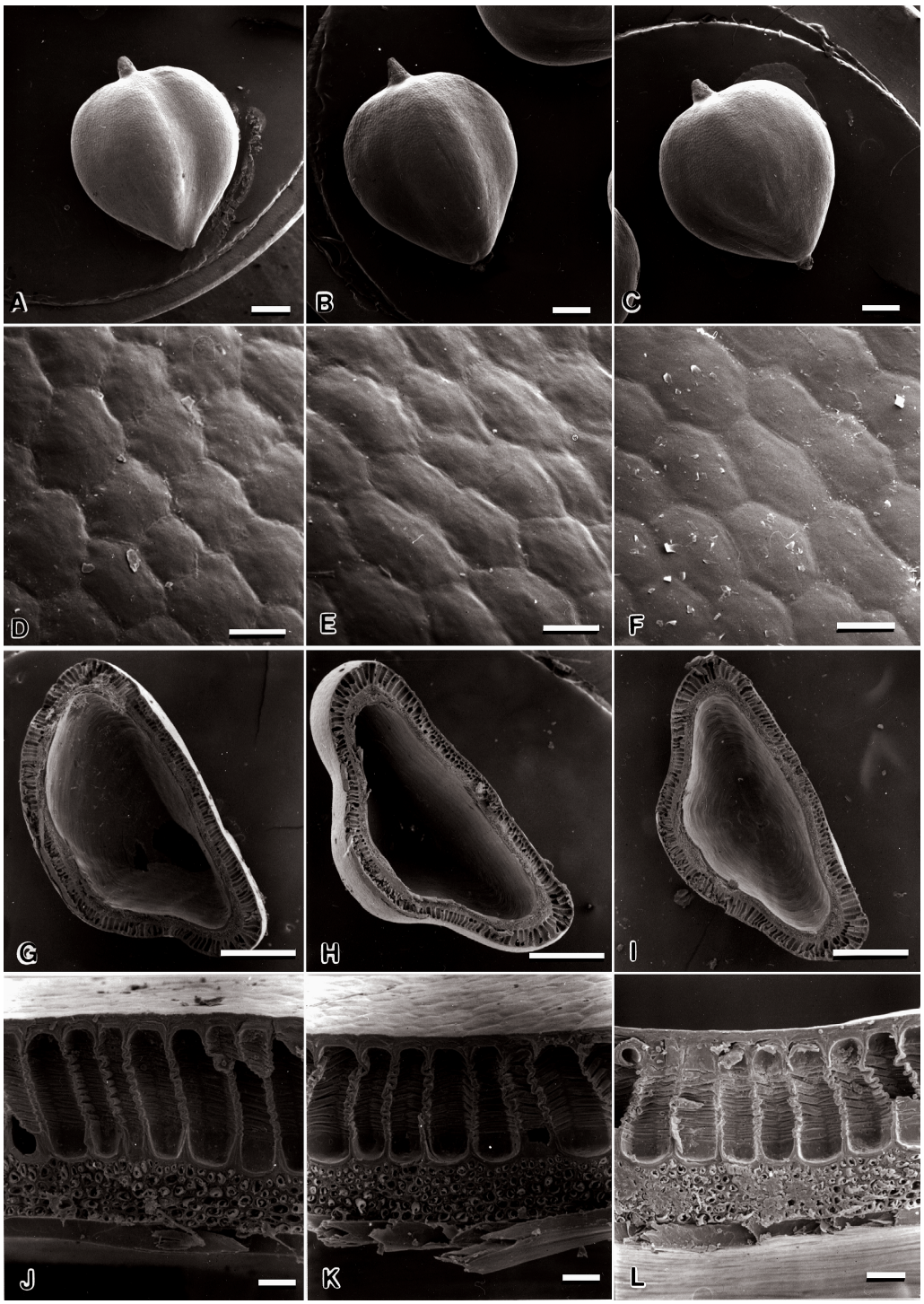

Fig. 1. *Bolboschoenus maritimus* (L.) Palla, Groningen West E2. — A–C: Achene abaxial view (× 20, scale bar = 500 μm). — D–F: Exocarp surface (× 600, scale bar = 25 μm). — G–I: Achenes in transverse section (× 40, scale bar = 500 μm). — J–L: Detail of pericarp structure in transverse section (× 400, scale bar = 25 μm).

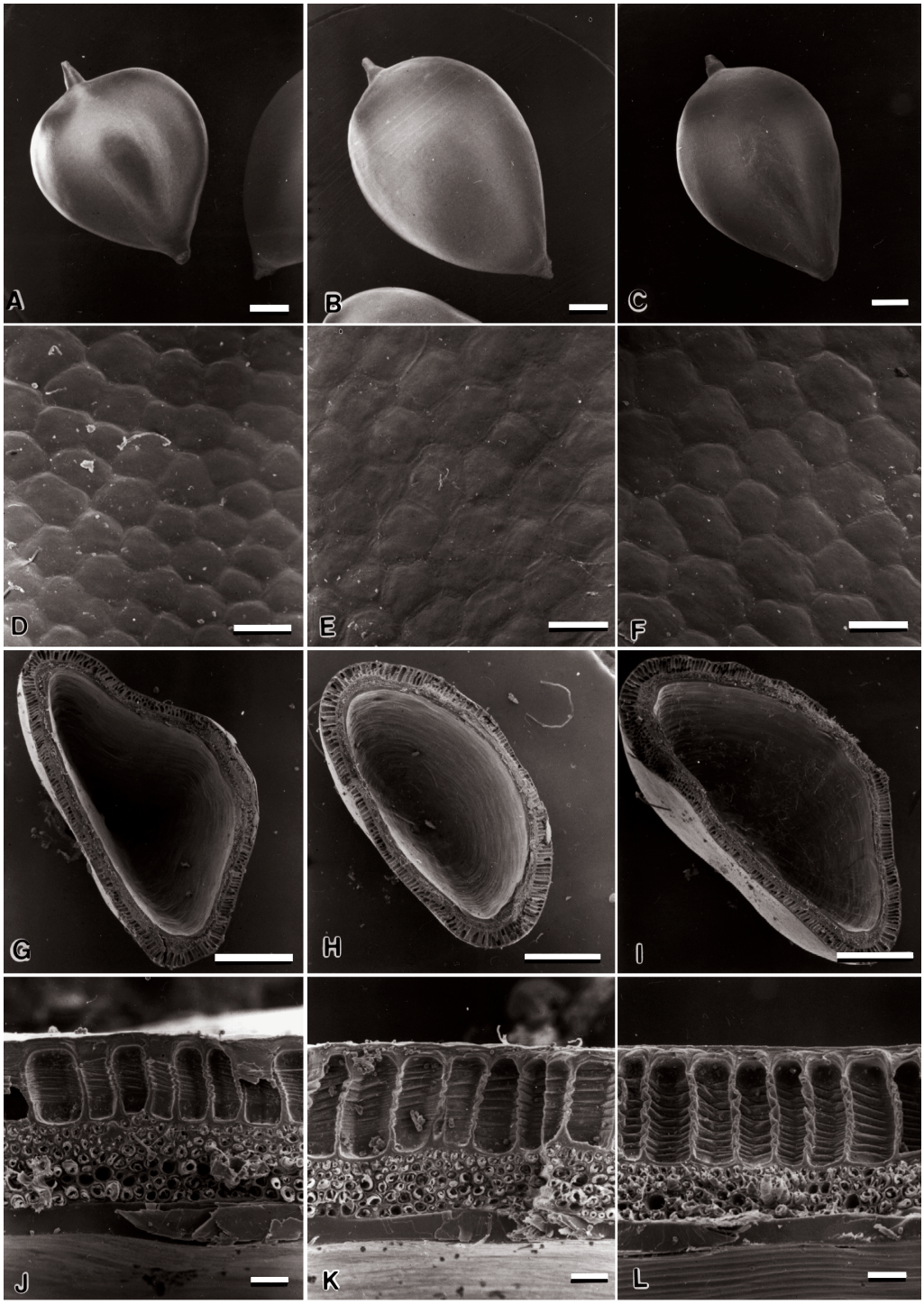

Fig. 2. *Bolboschoenus maritimus* (L.) Palla, Haringvliet H4. — A–C: Achene abaxial view (× 20, scale bar = 500 μm). — D–F: Exocarp surface (× 600, scale bar = 25 μm). — G–I: Achenes in transverse section (× 40, scale bar = 500 μm). — J–L: Detail of pericarp structure in transverse section (× 400, scale bar = 25 μm).

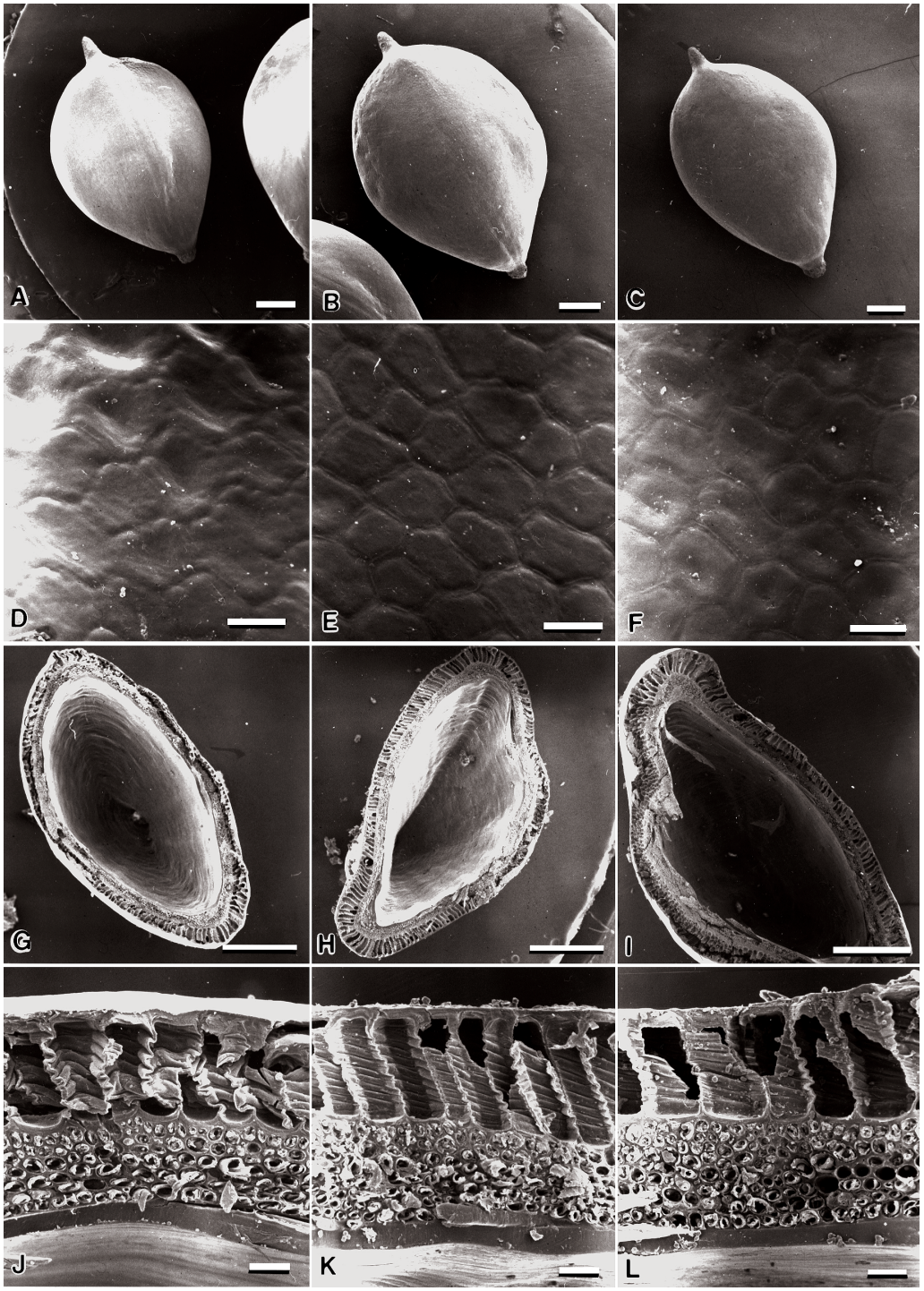

Fig. 3. *Bolboschoenus maritimus* (L.) Palla, Punt van Reide R7. — A–C: Achene abaxial view (× 20, scale bar = 500 μm). — D–F: Exocarp surface (× 600, scale bar = 25 μm). — G–I: Achenes in transverse section (× 40, scale bar = 500 μm). — J–L: Detail of pericarp structure in transverse section (× 400, scale bar = 25 μm).

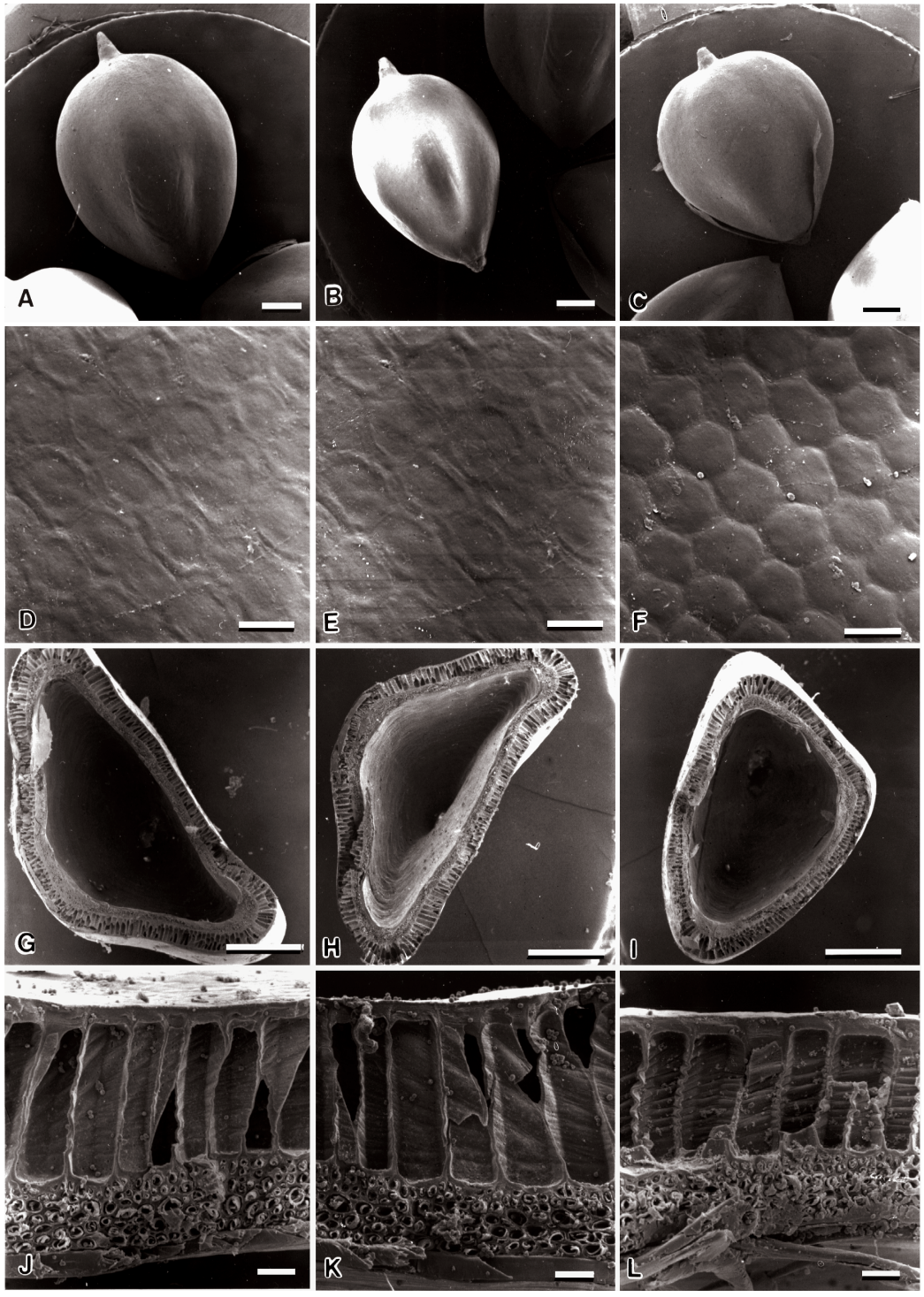

Fig. 4. *Bolboschoenus maritimus* (L.) Palla, Biesbosch. — A–C: Achene abaxial view (× 20, scale bar = 500 μm). — D–F: Exocarp surface (× 600, scale bar = 25 μm). — G–I: Achenes in transverse section (× 40, scale bar = 500 μm). — J–L: Detail of pericarp structure in transverse section (× 400, scale bar = 25 μm).

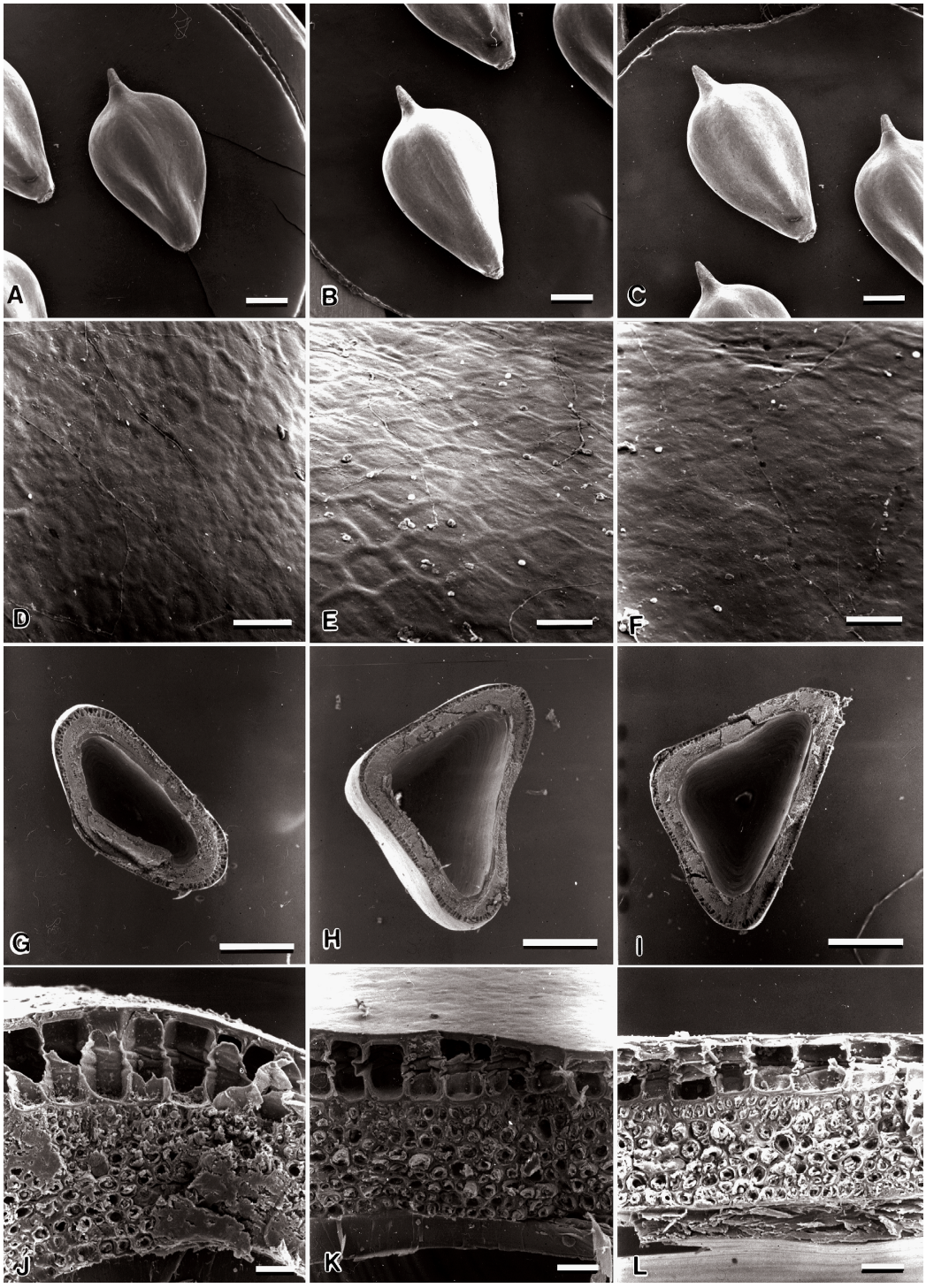

Fig. 5. *Bolboschoenus maritimus* (L.) Palla, Maastricht. — A–C: Achene abaxial view (× 20, scale bar = 500 μm). — D–F: Exocarp surface (× 600, scale bar = 25 μm). — G–I: Achenes in transverse section (× 40, scale bar = 500 μm). — J–L: Detail of pericarp structure in transverse section (× 400, scale bar = 25 μm).

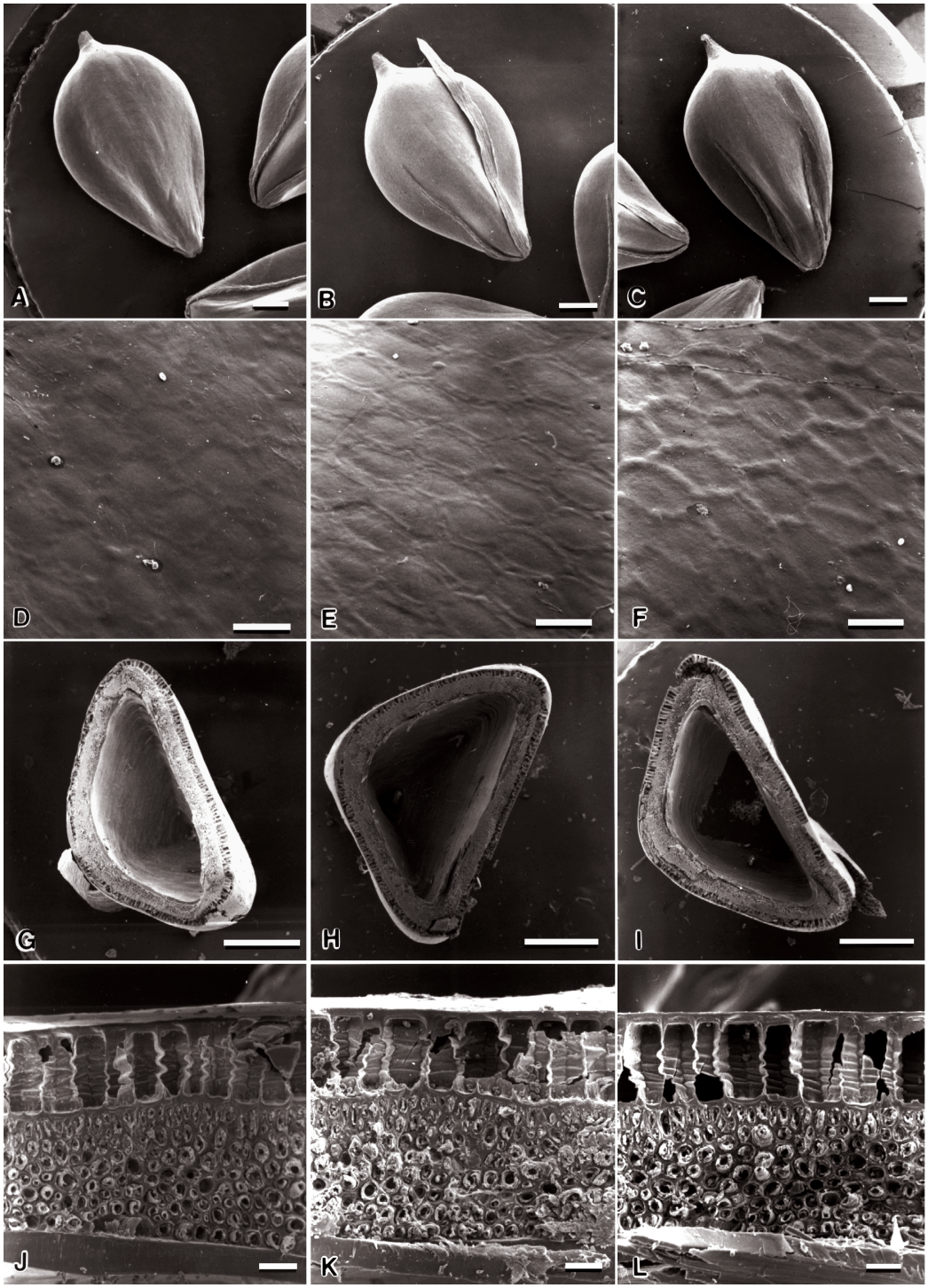

Fig. 6. *Bolboschoenus maritimus* (L.) Palla, Schoonhoven L1. — A–C: Achene abaxial view (× 20, scale bar = 500 μm). — D–F: Exocarp surface (× 600, scale bar = 25 μm). — G–I: Achenes in transverse section (× 40, scale bar = 500 μm). — J–L: Detail of pericarp structure in transverse section (× 400, scale bar = 25 μm).

Verlorenvlei in southern Africa, namely, differences in achene outline shape, surface texture and markings and exocarp/mesocarp depth ratios. There was also a difference in achene colour, the achenes from inland freshwater habitats being noticeably darker than those from the coast, but no southern African examples were nearly black, so that the contrast between coastal and inland extremes was not as well marked as in the two groups from The Netherlands. Some differences between the set of samples from The Netherlands and the set from southern Africa warrant more detailed discussion, namely:

1. The variability within the The Netherlands samples.
2. The longer lengths of the The Netherlands achenes. The ranges recorded as length (including projecting apex) × width, in mm, were:
   The Netherlands 3.0–4.8 × 1.8–2.9
   Verlorenvlei 2.6–3.5 × 1.7–2.8
   The lengths of achenes from Punt van Reide R7 (4.0–4.5) were not matched at Verlorenvlei where maximum recorded lengths were 3.1–3.5 for one coastal sample (no. 814).

In connection with these differences, we believe species sympatry for the two areas must be taken into account. Widely separated geographically and with different geological, climatic and biotic histories, we consider it unlikely that the western Cape Province has undergone the extent of habitat disturbance experienced in the area of collection in The Netherlands. We speculate that through past generations, introgression may have been more active in The Netherlands than in the Cape Province, resulting in increased variability within the taxon known as *Bolboschoenus maritimus s. l.* in the former country, as compared with the same taxon in the latter. Our deductions have been based on the information that follows.

For the Cape Province *Bolboschoenus maritimus s. l.* is known and *B. glaucus* (Lam.) S. G. Smith (Smith 1995: 101) only tentatively. *Bolboschoenus glaucus* is a species of eastern (and western?) tropical Africa, Madagascar, India and southwest Asia (not authoritatively known for Europe, except perhaps the eastern Mediterranean zone?). Achenes of African plants of *B. glaucus* (studied as *B. maritimus*, Entity 2, see Browning & Gordon-Gray 1993: 314, fig. 2A, B and D and also in subsequent unpublished work) were found to have a very narrow exocarp, the cells not radially elongated, and the exocarp:mesocarp depth ratio about 1:4 to 1:6. The pericarp surface showed only faint traces of cell outlines, the cells not arranged in a defined 'honey-comb' pattern. Achenes were also of small size (2.4–3.3 × 1.8–2.2 mm) (Browning & Gordon-Gray 1993: 316, table 1). These features are perhaps reflected in the achenes from Verlorenvlei as compared with the achenes from The Netherlands which are larger (see also Browning *et al*. 1995: 442, fig. 4, where a putative *B. maritimus* × *glaucus* achene is illustrated).

In Europe, in addition to the variable *Bolboschoenus maritimus*, there has now been reported *B. yagara* (Ohwi) A. E. Kozhevnikov and putative hybrids between these species (Browning *et al*. 1996: 129). In eastern Asia (Eurasia?) there is *B. affinis* (Roth) Drobov, which Norlindh (1972) regarded as differing from *B. maritimus* at subspecific level only. Achenes from Maastricht (Fig. 5) are narrowly obovate in outline and triangular in transverse section; the exocarp in surface view shows raised anticlinal walls. These are characters of *B. yagara* and this relationship is borne out also in the exocarp:mesocarp depth ratio of the Maastricht achenes (compare Browning *et al*. 1996 fig. 1I–L with Fig. 5 of the present paper). The Schoonhoven achenes (Figs. 6) are wider than those from Maastricht and have a pericarp anatomy resembling that of putative *B. maritimus* × *yagara* hybrids (Browning *et al*. 1996, fig. 2A–L). The very large achenes from Punt van Reide R7 (The Netherlands) resemble *B. affinis* in their shape in transverse section (majority not markedly nor strongly trigonous, some lenticular) and in their colour (ochre to khaki, not dark brown).

CONCLUSIONS

Introgression through past generations may have contributed to the extensive variability in achene morphology in *Bolboschoenus maritimus s. l.* observed in The Netherlands, as compared with

the relative uniformity observed for achenes of the same taxon in a southern African wetland. In this speculated genetic interchange, species sympatry must have been influential, as also extent of habitat disturbance, perhaps as major factors. However, this is by no means an adequate explanation and does not effectively account for the differences observed in pericarp anatomy for coastal and inland achenes in both The Netherlands and southern Africa. That these structural differences in achenes are correlated with differences in inflorescence form, which in turn are related to salinity levels in the microhabitat, suggests there are in operation ecological effects, as yet it would seem, poorly understood, that act upon, either directly, or more covertly by gradual selection, changes brought about by generic recombination.

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