

**EMBRYO AND CARYOPSIS MORPHOLOGY OF DANTHONIID  
GRASSES (ARUNDINOIDEAE: POACEAE):  
IMPORTANT CHARACTERS FOR THEIR SYSTEMATICS?**

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**ABSTRACT**

Embryo and/or caryopsis morphology in 27 species in 22 genera of danthonioid grasses is reinvestigated for use in a phylogenetic study. Embryo characters are too conservative to reveal phylogenetic relationships among the tribes of the Arundinoideae. However, data presented here and in the literature are used to show that embryo data are useful at subfamily and higher level and shown to be largely consistent with phylogenetic hypotheses generated with molecular data.

Caryopsis morphology is shown to be far less conservative but may nevertheless be useful in conjunction with other characters. *Anisopogon avenacea*, at present included in the Arundinoideae by Clayton and Renvoize (1986), is shown to have embryo characters resembling those of the Pooideae and this was corroborated by its caryopsis morphology.

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## INTRODUCTION

Within the Poaceae, five subfamilies have been recognized in recent classifications (Campbell 1985, Dahlgren, Clifford and Yeo 1985, Watson, Clifford and Dallwitz 1985, Clayton and Renvoize 1986): The Bambusoideae, Pooideae, Panicoideae, Chloridoideae and Arundinoideae. Recent phylogenetic studies which have focused on determining the basal subfamily to the grasses, have given much attention to the subfamilies Pooideae and Bambusoideae (e.g. Davis and Soreng 1993, Cummings et al. 1994). This emphasis may have contributed to the neglect of the other subfamilies, in particular the Arundinoideae, which is known to be a taxonomically difficult group (Stebbins & Crampton 1961, Renvoize 1986, Conert 1987). Clayton and Renvoize (1986) characterized the Arundinoideae as generally having a fairly simple spikelet structure, slender microhairs, cuneate lodicules, arundinoid embryo (as defined by Reeder 1957), and typically non-kranz anatomy. However, the lack of a synapomorphy for the subfamily, or even for a large subset of it, has led authors like Kellogg and Campbell to consider the Arundinoideae to be polyphyletic. The subfamily was first recognized by Tateoka (1957), who included 17 tribes in it. Renvoize's (1981) treatment of the Arundinoideae includes 72 genera distributed among eight tribes. Later (Renvoize 1986, Clayton and Renvoize 1986), several of these were transferred to other subfamilies, leaving in the Arundinoideae 4 tribes - Arundineae (including members of the Danthonieae and Cortaderieae), Thysanolaeneae, Micraireae and Aristideae (Table 1). These tribes had been recognized on the

basis of a phenetic study of anatomical and morphological characters. Conert (1987), defined the subfamily in a narrower sense, based on reproductive biology. He included the tribes Arundineae (including *Thysanolaena*), Cortaderieae and Danthonieae. The difficulty in establishing the taxonomic boundaries of the subfamily Arundinoideae has been asserted by Clayton (1981) to lie in the fact that the subfamily has been reduced to dismembered fragments around an extinct core. Conert (1987), on the other hand, indicated that the classification of the Arundinoideae is still based on spikelet morphology where much convergence has occurred. Therefore, it is necessary to use other characters and approaches to understand the relationships better.

Embryological characters have proven to be of great importance in grass systematics, particularly at tribal and subfamilial level (Reeder 1957). Both Bruns (1892) and van Tieghem (1897) investigated the taxonomic significance of the grass embryo. Van Tieghem was the first to discover that grass embryos could be classified as either panicoid (P) or festucoid (F). However, their work was overlooked until referred to by Yakolev (1950) and Reeder (1957). Reeder (1957) found four characters most valuable for a systematic study: elongation of the vascular system, presence or absence of the epiblast, presence or absence of a notch between scutellum and coleorhiza, and position of the margins of the embryonic leaves. These four sets of characters enabled Reeder to separate six groups: festucoid, panicoid, chloridoid-eragrostoid, bambusoid, oryzoid, and arundinoid-danthonioid.

Numerous attempts have been made to establish precise

homologies of these embryonic structures with foliage leaves (Brown 1960, Guignard and Mestre 1970). However, Clayton and Renvoize (1986) argued that it may be just as likely that these are novel organs evolved to meet the special needs of germination.

Reeder (1957) argued that the embryo assist in suggesting relationships of anomalous genera, especially when employed in connection with other histological and cytological information. However, in contrast to the subfamilies Pooideae, Panicoideae and Chloridoideae, which are commonly recognized as monophyletic groups, the Arundinoideae appear to be polyphyletic and thus may not be uniform with respect to embryological characters. Until now only a few species of the tribe Danthonieae (sensu Conert 1971), which is a large group of genera with an austral center of distribution, have been investigated (Kinges 1961, Decker 1964). Reeder (1957) investigated the following genera (number of species examined in brackets): *Cortaderia* (1), *Danthonia* (6), *Phragmites* (1), *Sieglingia* (1), *Lamprothyrsus* (1). However, he does not say which species were examined. As *Danthonia* s.l., as used by Reeder, appears to be polyphyletic (e.g. Kellogg & Campbell 1987), Reeder's data may not be of any value. Thus the Arundinoideae need to be reinvestigated, in order to test whether embryogeny may be used in a phylogenetic study.

**Table 1.** The genera of the Arundinoideae (Poaceae) according to Clayton and Renvoize (1986). Supraspecific classification follows Clayton and Renvoize (1986) except that *Karroochloa* and *Merxmüllera* are separated from *Rytidosperma*. Genera investigated in this study are marked with an asterix. Genera marked with a plus (+) have only been investigated for caryopsis characters.

<i>Tribolium*</i>	<i>Pentaschistis*</i>	<i>Amphipogon</i>
<i>Urochlaena*</i>	<i>Pentameris*</i>	<i>Crinipes*</i>
<i>Elytrophorus</i>	<i>Pseudopentameris*</i>	<i>Nematopoa</i>
<i>Prionanthium</i>	<i>Rytidosperma*</i>	<i>Leptagrostis</i>
<i>Spartochloa+</i>	<i>Phaenanthoecium*</i>	<i>Dichaetaria</i>
<i>Notochloe</i>	<i>Alloeochoete*</i>	<i>Cortaderia</i>
<i>Zenkeria+</i>	<i>Monachather*</i>	<i>Lamprothyrsus</i>
<i>Piptophyllum</i>	<i>Pyrrhanthera</i>	<i>Arundo</i>
<i>Styppeiochloa</i>	<i>Dregeochloa*</i>	<i>Hakonechloa</i>
<i>Chionochloa*</i>	<i>Centropodia</i>	<i>Molinia</i>
<i>Danthonia*</i>	<i>Danthonidium+</i>	<i>Phragmites</i>
<i>Chaetobromus*</i>	<i>Anisopogon*</i>	<i>Gynerium</i>
<i>Plinthanthesis*</i>	<i>Diplopogon</i>	
THYSANOLAENEAE		
<i>Thysanolaeneae</i>		
MICRAIREAE		
<i>Micraira</i>		
ARISTIDEAE		
<i>Sartidia</i>	<i>Aristida</i>	<i>Stipagrostis</i>

In respect of the non-embryo characters of the caryopsis, Clifford and Watson (1977:31) observed that "mature grass grains exhibit a wide diversity of shapes and sizes, which could be extensively used for identifying them".

Some early observations were made by Reeder (1957), who proposed that the relative size of the embryo in relation to the seed as a whole has some systematic value. Furthermore, in an extensive study of the Gramineae, Kinges (1961) also found the shape of the hilum to be a useful taxonomic character. He noted that the hilum was small and roundish in the panicoid species and longish in the festucoid species. At a generic level the shape

of the hilum has also been used by Zotov (1963) to separate *Rytidosperma* from *Danthonia*. However, this treatment was criticized by Jacobs (1982), who showed that caryopsis characters did not support such a division.

In recent years there has been more reference to caryopsis characters, especially in the definition of higher taxa (Campbell 1985). At a generic level, shape and surface sculpturing of the caryopsis were also found to be useful by Barker (1986) to separate *Pseudopentameris* and *Pentameris*. In addition, caryopsis morphology was used in the classification of the Triticeae (Terrell and Peterson 1993).

In this project some of the caryopsis characters previously found to be useful for classification have been re-investigated across a broader spectrum of genera in the Arundineae and the phylogenetic implications of variation in the embryo proper (i.e. embryogeny) and caryopsis morphology are evaluated.

## **MATERIALS AND METHODS.**

### **1. Embryo characters.**

Embryo characters of 25 species in 19 genera of the subfamily Arundinoideae were examined (Table 1). In addition, the embryo characters of three other subfamilies - Panicoideae, Eragrostoidae and Pooideae - were investigated for comparative purposes.

In this study mature grass caryopses were either freshly collected in the field or extracted from herbarium material. The preparation of the embryos for microscopic study largely followed Reeder's (1957) method. However, the details of the method were not mentioned by Reeder (1957) or Decker (1964) and thus had to be found experimentally. These details were found to be important to optimize the quality of the microscopic preparations, to enable an unambiguous interpretation of the characters.

Fresh seeds were briefly treated in boiling water in order to kill the embryos and prevent subsequent growth. Herbarium material was first soaked overnight in water in order that the embryos could expand fully. The embryos were dissected out and passed manually through a dehydration series consisting of: 2 baths 70%, 80%, 95%, 100% ethanol, 2 baths N-propanol and 2 baths N-butanol. The embryos were left in each bath for 10 minutes. Thereafter they were placed in a wax bath and left for 12 hours to allow the wax to penetrate the tissue adequately. While the wax was still fluid the embryos had to be positioned in such a way that some embryos could later be sectioned transversely and



others longitudinally in a sagittal plane. The wax blocks were cooled, trimmed and then serially sectioned on a Leitz Wetzlar rotary microtome to a thickness between 6 - 10 microns. Sections were placed on microscope slides which had been treated with Haupt's adhesive. The best results were obtained when the sections were first placed on a water bath (30-35° C) so that the material stretched sufficiently to remove any "crinkles" and then placed onto the microscope slide.

The sections were passed through a staining sequence (Johansen 1940): xylene (2 baths, 5 minutes each), 2-methoxy-ethanol (1-2 minutes), 96% ethanol (2 baths, 2 minutes each), safranin (30 minutes), distilled water (brief rinse), 2-methoxy-ethanol (1-2 minutes), fast green (brief rinse), clove oil rinse [clove oil: 100% ethanol: xylene, 2:1:1 by volume] (2-3 minutes), 96% ethanol (2 baths, 2-5 minutes each), N-butanol (2 baths, 1-2 minutes each), and xylene (2 baths, 5 minutes each). The slides were mounted with DPX and covered with a cover slip. Slides were viewed using light microscopy on a Zeiss standard 25 microscope and drawn using a *camera lucida*.

Photographs were taken on a Zeiss Axioskop microscope, using PAN F 50 film.

## **2. Caryopsis morphology.**

Five caryopses of each species (where less material was available the number of specimens investigated is in brackets) were investigated under a Zeiss Stemi SV 6 dissection microscope.

Photographs were taken on a Zeiss microscope, using PAN F film.

**Table 2.** A list of taxa examined for embryo and/or caryopsis characters, with vouchers. Vouchers are deposited at the Australian National Herbarium (CANB); the Bolus Herbarium (BOL), University of Cape Town, South Africa; Royal Botanic Gardens, Kew (K).

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#### ARUNDINOIDEAE

##### Arundineae

- Alloeochaete geniculata* C.E. Hubbard, Brummitt 9767, (K)  
*A. gracillima* C.E. Hubbard, Phillips 56, (K)  
*Anisopogon avenacea* R.Br., Linder 5674, (BOL)  
*Chaetobromus dregeanus* Nees, Verboom 73, (BOL)  
*C. involucratus* (Schr.) Nees, Verboom 65, (BOL)  
*Chionochloa cheesemannii* (Hack.) Zotov, Druce 210204, (CANB)  
*Crinipes abyssinicus* (A. Rich.) Hoechst., De Wilde & Gilbert 206, (K)  
*Danthonia carphoides* DC., no voucher  
*D. pallida* DC., no voucher  
*Danthonidium gammiei* (Bhide) C.E. Hubbard, Ambo 6887, (K)  
*Dregeochloa pumila* (Nees) Conert, Williamson 2609, (BOL)  
*Karoochloa* sp., no voucher  
*Merxmuellera dura* (Stapf) Conert, (BOL)  
*Merxmuellera rufa* (Nees) Conert, no voucher  
*Monachather paradoxus* Steud., Burbidge 1170, (CANB)  
*Pentameris macrocalycina* (Steud.) Schweick., no voucher  
*Pentaschistis aristoides* (Thunb.) Stapf, no voucher  
*P. minor* (Ball and Hubb.) Ball. & Hubb., Phillips 79, (BOL)  
*P. papillosa* (Steud.) Linder, no voucher  
*Phaenanthoecium kostlinii* C.E. Hubbard, Friis et al. 2189, (K)  
*Plinthanthesis rodwayi* Steud., Linder 5704, (BOL)  
*Pseudopentameris macrantha* (Schr.) Conert, no voucher  
*Rytidosperma corinum* Steud., Druce 402035, (CANB)  
*Schismus inermis* (Stapf) C.E. Hubbard, Muir 3509, (BOL)  
*Spartochloa scirpoidea* (Steud.) C.E. Hubbard, Knight 350, (CANB)  
*Tribolium uniolae* (Spreng.) Nees, no voucher  
*Urochlaena pusilla* Nees, no voucher  
*Zenkeria elegans*, Trin., Sauliere 838, (K)  
*Zenkeria* sp., Rice 60, (K)

#### CHLORIDOIDEAE

##### Eleusininae

- Eleusine coracana* (L.) Gaertn., no voucher

#### POOIDEAE

##### Poeae

- Briza maxima* L., no voucher

#### PANICOIDEAE

##### Digitariinae

- Pennisetum setaceum* Rich., no voucher
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## RESULTS

**EMBRYO CHARACTERS.**— The median saggittal sections revealed three embryo characters (Fig. 1 a, b). Cross-sections of the embryo through the region of the coleoptile revealed a fourth character (Fig. 1 c, d).

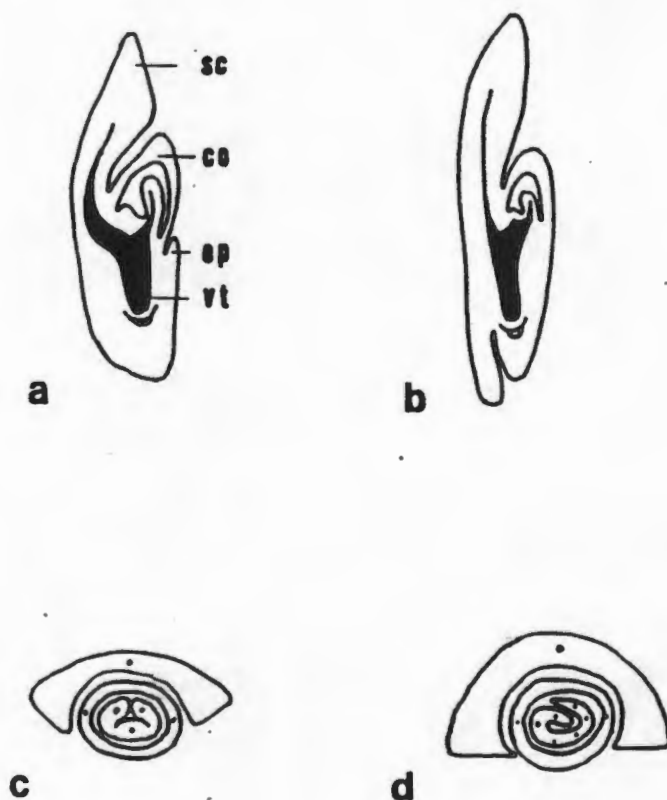


Fig. 1. Median sagittal sections of grass embryos showing the different states: a: presence of epiblast, short embryonic mesocotyl internode, lack of a scutellum cleft. b: lack of epiblast, long embryonic mesocotyl internode, presence of scutellum cleft.

Cross-sections of the embryo through the region of the coleoptile. c: embryonic leaf margins overlap. d: embryonic leaf margins do not overlap. co, coleoptile; ep, epiblast; sc, scutellum; vt, vascular trace. Fig. 1 after Decker 1964.

All but one species investigated in this study and belonging to the tribe Arundineae sensu Clayton and Renvoize (1986), are

characterized by the presence of a long embryonic mesocotyl internode, the lack of an epiblast, the presence of a scutellum cleft and non-overlapping leaf margins (Table 3, 4; Fig. 2A, B). *Anisopogon avenacea* is the only arundinoid species that is characterized by a short embryonic mesocotyl internode, the presence of an epiblast, the lack of a scutellum cleft and non-overlapping leaf margins (Table 4, Fig. 2C). These character states resemble those of *Briza maxima* (Poeae, Pooideae) (Table 3, 4; Fig. 2D).

*Eleusine coracana* (Eleusininae, Chloridoideae) differs from the arundinoid species investigated only by the presence of an epiblast, whereas *Pennisetum setaceum* (Digitariinae, Panicoideae) differs from the arundinoids by the presence of overlapping leaf margins (Table 3, 4).

For comparative purposes, data for the other subfamilies, obtained from various published studies, are included in Table 4.

**Table 3.** List of the characters and their states.

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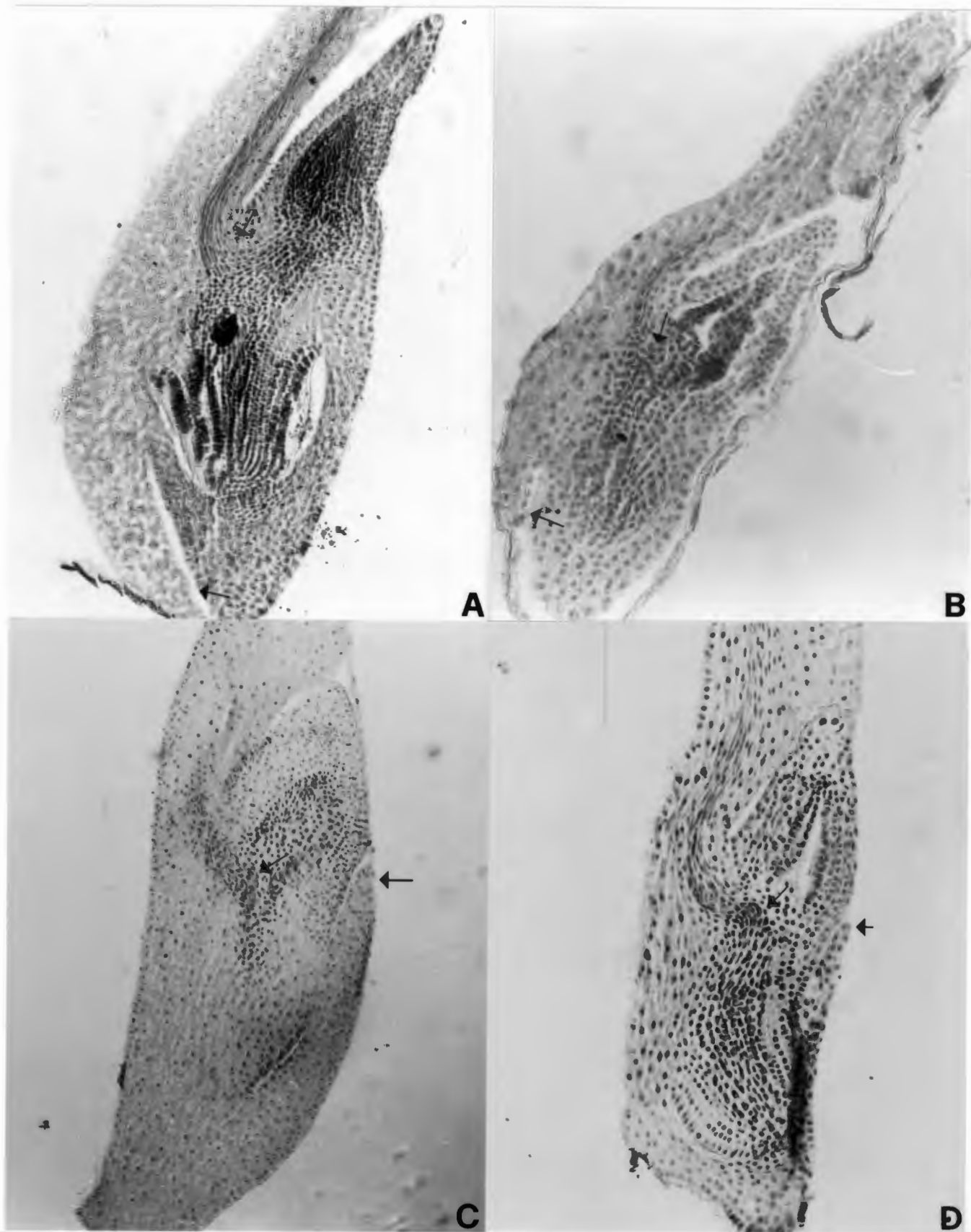
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1.	Embryonic mesocotyl internode: 0 = long, short = 1
2.	Epiblast: 0 = present, 1 = absent
3.	Scutellar tail: 0 = present, 1 = absent
4.	Embryonic leaf margins: 0 = meeting, 1 = overlapping

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**Table 4.** States of the embryo characters for grasses investigated.  
[a = Kellogg and Campbell (1987); 9 = variable within a taxon.]

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SUBFAMILY				
Species	1	2	3	4
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ARUNDINOIDEAE				
<i>Crinipes abyssinicus</i>	0	1	0	0
<i>Alloeochoete geniculata</i>	0	1	0	0
<i>Alloeochoete gracillima</i>	0	1	0	?
<i>Phaenanthoecium kostlinii</i>	0	1	0	?
<i>Schismus inermis</i>	0	1	0	0
<i>Plinthanthesis rodwayi</i>	0	1	0	0
<i>Karroochloa</i> sp.	0	1	0	0
<i>Chaetobromus dregeanus</i>	0	1	0	0
<i>Chaetobromus involucratus</i>	?	?	?	0
<i>Dregeochloa pumila</i>	0	1	0	?
<i>Rytidosperma corinum</i>	0	1	0	0
<i>Pentaschistis minor</i>	0	1	0	0
<i>Pentaschistis aristoides</i>	0	1	0	0
<i>Pentaschistis papillosa</i>	0	1	0	0
<i>Tribolium uniola</i>	?	1	0	0
<i>Merxmuellera rufa</i>	0	1	0	0
<i>Merxmuellera dura</i>	0	1	0	0
<i>Pseudopentameris macrantha</i>	0	1	0	0
<i>Urochlaena pusilla</i>	0	1	0	0
<i>Pentameris macrocalycina</i>	0	1	0	0
<i>Danthonia carphoides</i>	0	1	0	0
<i>Danthonia pallida</i>	0	1	0	0
<i>Chionochloa cheesemannii</i>	0	1	0	0
<i>Monachather paradoxa</i>	0	1	0	?
<i>Anisopogon avenacea</i>	1	0	1	0
BAMBUSOIDEAE <sup>a</sup>	1	9	9	1
POOIDEAE <sup>a</sup>	1	9	1	0
<i>Briza maxima</i>	1	0	1	0
CHLORIDOIDEAE <sup>a</sup>	0	9	0	9
<i>Eleusine coracana</i>	0	0	0	0
PANICOIDEAE <sup>a</sup>	9	1	0	9
<i>Pennisetum setaceum</i>	0	1	0	1
CENTOTHECOIDEAE <sup>a</sup>	0	1	0	9
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**Fig. 2. Median sagittal sections of grass embryos. A, *Monachather paradoxus* (Arundinoideae) (100X), arrows indicate long vascular internode and scutellum cleft. B, *Crinipes abyssinicus* (Arundinoideae) (200X), arrows indicate long vascular internode and scutellum cleft. C, *Anisopogon avenacea* (Arundinoideae) (100X), arrows indicate short vascular internode and epiblast. D, *Briza maxima* (Pooideae) (100X) arrows indicate short vascular internode and epiblast.**



CARYOPSIS CHARACTERS.- Variation in the shape of the caryopsis is shown in Figure 3 and 4. Elliptic shapes were the most common among the arundinoid species investigated, followed by obovate and intermediate shapes (Table 5). *Anisopogon avenacea* was the only species with a remarkable lorate shape (Fig. 3A, 4B), whereas *Monachather paradoxus* differed from the others by its turbinate shape (Fig. 3F). In the majority of species the length of the caryopsis lay between 0.5-4.0 mm (Table 5). *Anisopogon avenacea* was exceptionally long at 6.1-8.2 mm. *Pseudopentameris macrantha*, at 5.1-6.1 mm, just met the lower end of the range of *A. avenacea*, but was larger than the majority of species investigated.

Widths were between 0.25 and 1.25 mm and this was only exceeded in *Monachather paradoxus*, which ranged between 1.2-1.6 mm (Table 5).

Under low magnification only three conspicuous differences in the surface sculpturing could be observed: Rugulose (Fig. 3A, B, C, E), dull and smooth (Fig. 3F), shiny and smooth. The first two were the most common. Only *Karoochloa* sp. and *Schismus inermis* had shiny caryopses (Table 5).

*Anisopogon avenacea* and *Pentameris macrocalycina* were the only two species with a villose apex (Fig. 4 A, B). All others were glabrous. In addition, both species were the only ones observed with a flattened apex. In all species the apex was developed into an appendage whose shape and size varied between the species and the genera. In many cases it was a thin membranous structure as in *Alloeochaete gracillima* (Fig. 4 F). In others such as *Anisopogon avenacea* and *Danthonia pallida* it was somewhat thicker and hardened (Fig. 4 B, E).

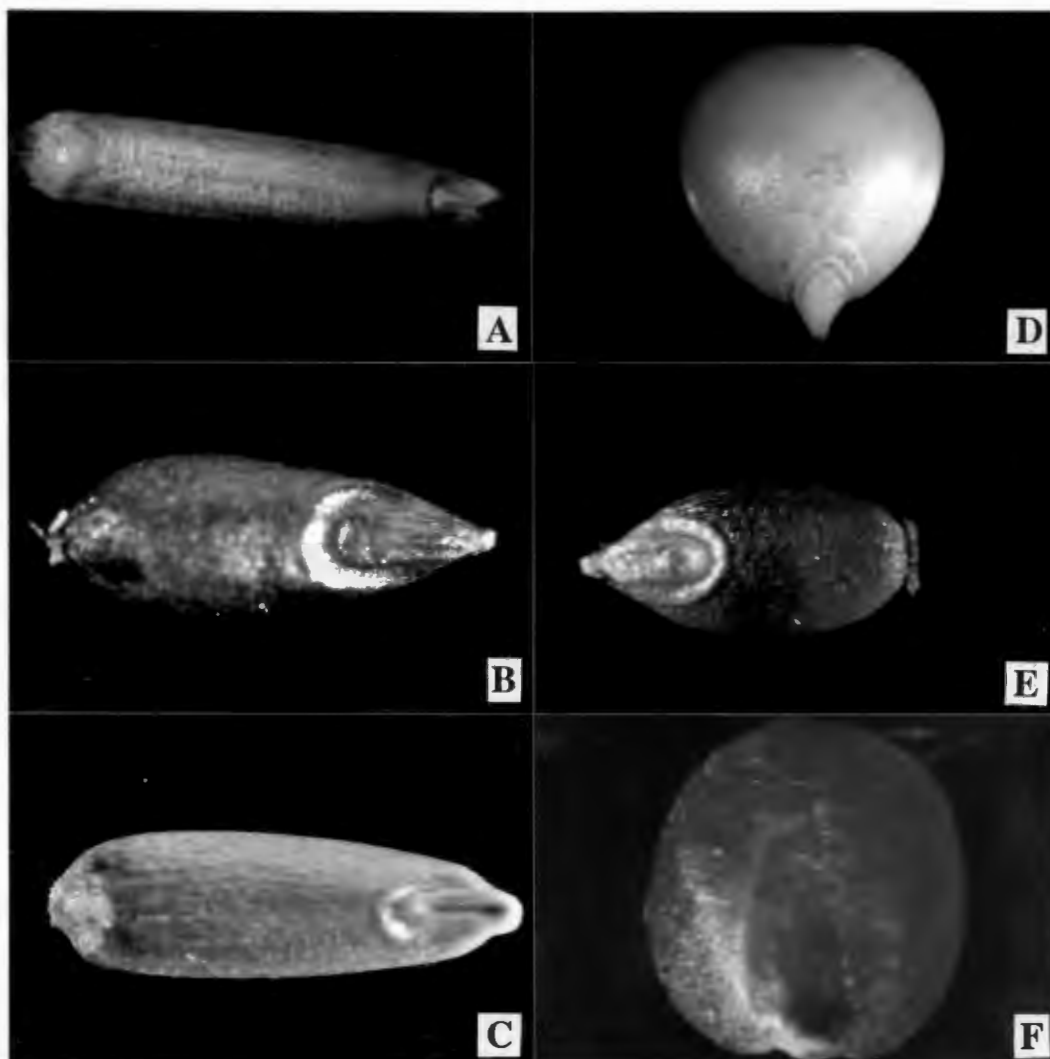


Fig. 3. Dorsal views of caryopses. A, *Anisopogon avenacea* (A) (36X). B, *Merxmuellera rufa* (A) (100X). C, *Pseudopentameris macrantha* (A) (60X). D, *Briza maxima* (P) (60X). E, *Plinthanthesis rodwayi* (A) (100X). F, *Monachather paradoxus* (A) (150X). (A)=Arundinoideae; (P)=Pooideae.



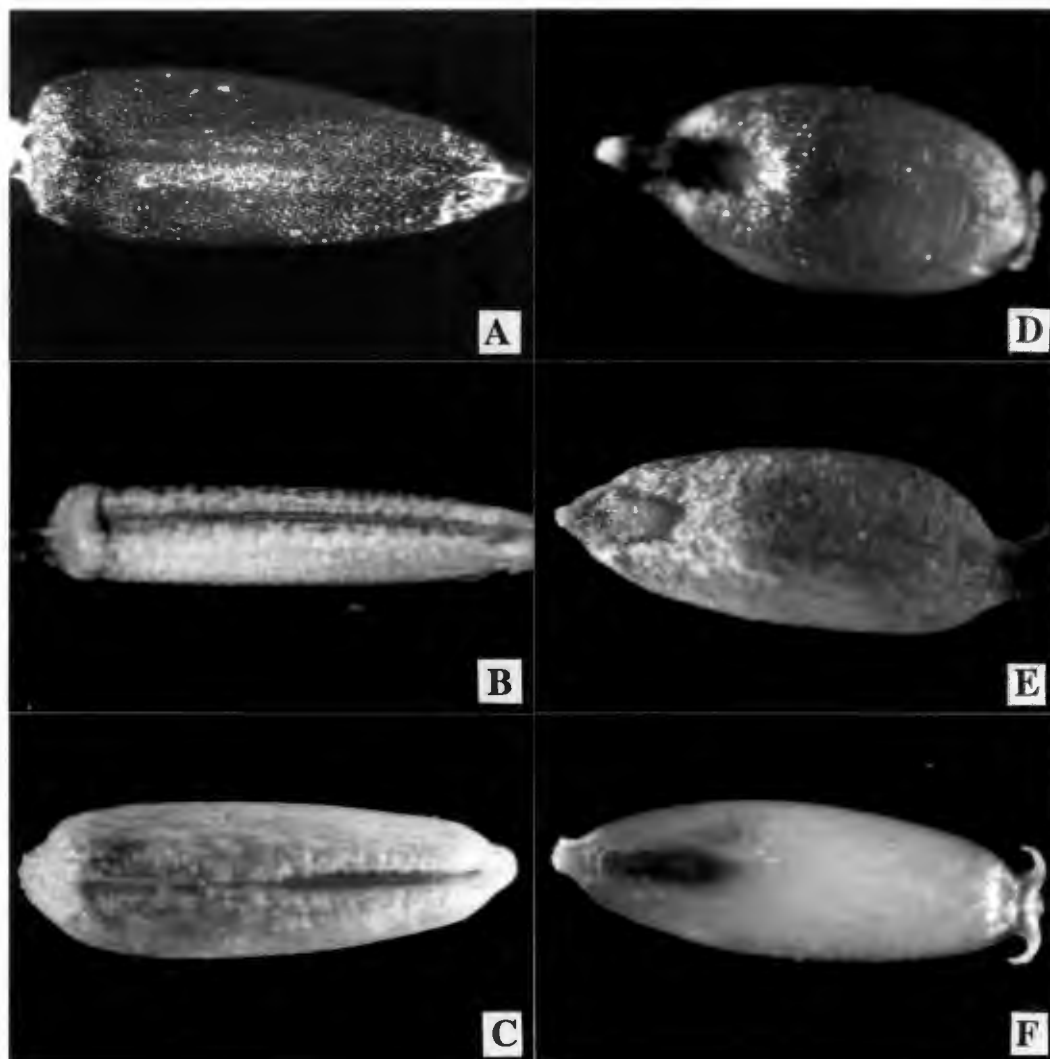


Fig. 4. Ventral views of caryopses, Arundinoideae. A, *Pentameris macrocalycina* (100X). B, *Anisopogon avenacea* (36X). C, *Pseudopentameris macrantha* (60X). D, *Plinthanthesis rodwayi* (100X). E, *Danthonia carphoides* (150X). F, *Alloochaete geniculata* (100X).

Table 5. Morphological variation in selected characters. (Abbreviations as follows: lor = lorate; el = ellipsoid; rug = rugulose; d/s = dull and smooth; shin = shiny; lan = lanceolate; obov = obovate; cu = cuneiform; ov = ovate; obl = oblong; p = punctiform; turb = turbinate.

Species	Shape	Length (mm)	Width (mm)	Surf
<i>Anisopogon avenacea</i>	lor	6.1-8.2	1.5-2.0	rug
<i>Danthonidium gammiei</i>	el	2.4-2.9	0.5-0.7	rug
<i>Phaenanthoecium kostlinii</i>	el/lan	1.1-1.9	0.4-0.5	d/s
<i>Pseudopentameris macrantha</i>	obov	5.1-6.1	1.3-1.6	d/s
<i>Chaetobromus involucatus</i>	el	2.7-3.1	0.8-0.9	d/s
<i>Merxmuellera rufa</i>	obov	2.2-2.7	0.8-1.1	rug
<i>M. dura</i>	obov	3.2-3.6	0.8-1.1	d/s
<i>Schismus inermis</i>	obov/el	0.9	0.5	shin
<i>Tribolium uniolae</i>	el	1.5-1.7	0.7-0.8	rug
<i>Urochlaena pusilla</i>	el	1.1-1.6	0.5-0.7	d/s
<i>Karoochloa sp.</i>	el	1.1-1.7	0.5-0.8	shin
<i>Danthonia pallida</i>	obov	2.1-2.6	0.8-1.1	rug
<i>D. carphoides</i>	obov	1.5-1.9	0.7-1.0	d/s
<i>Pentameris macrocalycina</i>	obov/cu	3.4	1.2	rug
<i>Pentaschistis minor</i>	el	1.2-1.4	0.4	rug
<i>P. aristoides</i>	el/obov	2.7-3.3	0.8-0.9	d/s
<i>Zenkeria sp.</i>	el	2.1-2.4	0.6-0.7	rug
<i>Z. elegans</i>	el	1.8-1.9	0.4-0.5	rug
<i>Alloeochaete gracillima</i>	lor/el	1.7-2.4	0.4-0.5	d/s
<i>A. geniculata</i>	el	2.4-2.8	0.7-0.9	d/s
<i>Monachather paradoxus</i>	turb	1.3-1.8	1.8	d/s
<i>Spartochloa scirpoidea</i>	lor	1.6	1.0	rug
<i>Rytidosperma corinum</i>	obov	1.4-1.7	0.7-0.8	d/s
<i>Chionochloa cheesemannii</i>	el/obov	2.4-2.8	0.7-0.8	d/s
<i>Crinipes abyssinicus</i>	el	2.1-2.3	0.6	rug
<i>Plinthanthesis rodwayi</i>	obov	1.2-1.3	0.8	rug
<i>Dregeochloa pumila</i>	obov	1.9-2.0	1.0-1.1	?

Table 5 continued.

Species	Apex	Embr/ Caryps (%)	Hilum Shape	Hilum/ Caryps (%)
<i>Anisopogon avanacea</i>	vil	13-15	lin	79-95
<i>Danthonidium gammiei</i>	gla	34-40	lin	67-77
<i>Phaenanthoecium kostlinii</i>	gla	16-30	lin	52-72
<i>Pseudopentameris macrantha</i>	gla	25-31	lin	78-90
<i>Chaetobromus involucratus</i>	gla	42-47	lin	74-87
<i>Merxmuellera rufa</i>	gla	36-42	el	14-22
<i>M. dura</i>	gla	24-33	el	49-53
<i>Schismus inermis</i>	gla	33-43	el	13-17
<i>Tribolium uniolae</i>	gla	36-43	el	19-20
<i>Urochlaena pusilla</i>	gla	38-57	el	19-20
<i>Karoochloa</i> sp.	gla	45-67	el	19-24
<i>Danthonia pallida</i>	gla	42-49	ov	19-24
<i>D. carphoides</i>	gla	41-56	el	20-26
<i>Pentameris macrocalycina</i>	vil	30	el	40
<i>Pentaschistis minor</i>	gla	32-43	el	29-43
<i>P. aristoides</i>	gla	27-33	obl	43-67
<i>Zenkeria</i> sp.	gla	23-24	obl/el	52-74
<i>Zenkeria elegans</i>	gla	21-26	obl	68-81
<i>Alloeochaete gracillima</i>	gla	24-26	el	20-26
<i>A. geniculata</i>	gla	40-44	obl	27-29
<i>Monachather paradoxus</i>	gla	50-80	el	25
<i>Spartochloa scirpoidea</i>	gla	27	p	0.9
<i>Rytidosperma corinum</i>	gla	48-58	el/obl	21-24
<i>Chionochloa cheesemannii</i>	gla	45-55	el	34-39
<i>Crinipes abyssinicus</i>	gla	26-31	el/lan	28-36
<i>Plinthanthesis rodwayi</i>	gla	28-41	obl	30-44
<i>Dregeochloa pumila</i>	gla	61-79	el	21

The size of the embryo as a percentage of the caryopsis length was mostly greater than 20% (except in *Phaenanthoecium kostlinii*, where it varied from 16-30%), but there was often rather large variation within species. In *Anisopogon avenacea*, on the other hand, variation was limited and here the size of the embryo did not exceed 15% of the length of the caryopsis (Fig. 3 A, Table 5). The small embryo of *Briza maxima* (Pooideae) is shown for comparative purposes (Fig. 3 D).

The hilum shape was mostly elliptic and only in five of the species investigated was it linear (Fig. 4). *Spartochloa* was the only species with a punctiform hilum (Table 5). In species with linear hilum its length ranged from 52-95 % of the caryopsis. In those with oblong to elliptic hilum it ranged from 13-81 % (Table 5). Variability in the compression of the hilum was also noted and examples are shown in Figure 4.

#### **Descriptions of caryopses.**

*Zenkeria elegans* Trin.- 1.8-1.9 X 0.4-0.5 mm; elliptic; reddish brown; rugulose; dorsal surface rounded; ventral surface flat; apex glabrous, 2-way ridge; embryo 21-26% of caryopsis length; hilum oblong; hilum 66-81% of caryopsis length.

*Zenkeria sp..* (2) - 2.1-2.4 X 0.6-0.7 mm; elliptic; eddish brown; rugulose; dorsal surface rounded; ventral surface flat; apex glabrous, 2-way ridge; embryo 23-24% of caryopsis length, hilum oblong to elliptical; hilum 52-74% of caryopsis length.

*Danthonidium gammiei* (Bhide) C.E. Hubbard.- 2.4-2.9 X 1.5-

2.0 mm; elliptic; pale brown; rugulose; dorsal surface rounded, ventral surface flat; apex glabrous, developed; embryo 34-40% of caryopsis length; hilum linear, grooved; hilum 67-77% of caryopsis length.

*Crinipes abyssinicus* (A.Rich.) Hoechst.- 2.1-2.3 X 0.6 mm; elliptical to obovate; reddish brown; rugulose; no compression (round in transverse section); apex glabrous, 2-way ridge; embryo 26-31% of caryopsis length; hilum elliptical to lanceolate; hilum 28-36% of caryopsis length.

*Alloeochaete geniculata* C.E. Hubbard.- 2.4-2.8 X 0.7-0.9 mm; elliptic; pale brown to yellow; dull and smooth; dorsal surface rounded, ventral surface flat; apex glabrous, 2-way ridge; embryo 40-44% of caryopsis length; hilum oblong to elliptical; hilum 27-29% of caryopsis length.

*Alloeochaete gracillima* C.E. Hubbard.- 1.7-2.4 X 0.4-0.5 mm; elliptic to lorate; pale brown to yellow; dull and smooth; dorsal surface rounded, ventral surface flat; apex glabrous, 2-way ridge; embryo 24-26% of caryopsis length; hilum elliptical; hilum 20-26% of caryopsis length.

*Phaenanthoecium kostlinii* (A. Rich.) C.E. Hubbard.- 1.1-1.9 X 0.5-0.7 mm; elliptical to lanceolate; yellow; dull and smooth; dorsal surface rounded, ventral surface flat; apex glabrous, developed; embryo 16-30% of caryopsis length; hilum linear; hilum 52-72% of caryopsis length.

*Plinthanthesis rodwayi* Steud.- 1.2-1.3 X 0.8 mm; obovate; dark brown; rugulose; no compression (round in transverse section); apex glabrous, 2-way ridge; embryo 28-41% of caryopsis length; hilum elliptical, grooved; hilum 30-44% of caryopsis length.

*Spartochloa scirpoideae* (Steud.) C.E. Hubbard. (1) - 1.6 X 1.0 mm; lorate; dark brown; rugulose; three-lobed in transverse section; apex glabrous; embryo 27% of caryopsis length; hilum punctiform; hilum 0.9% of caryopsis length.

*Monachather paradoxus* Steud. (4) - 1.3-1.8 X 1.2-1.6 mm; turbinate; yellow; dull and smooth; no compression; apex glabrous, 2-way ridge; embryo 50-80% of caryopsis length; hilum elliptical, grooved; hilum 25% of caryopsis length.

*Rytidosperma corinum* Steud.- 1.4-1.7 X 0.7-0.8 mm; obovate; yellow; smooth and dull; dorsal surface rounded, ventral surface flat; apex glabrous, 2-way ridge; embryo 48-58% of caryopsis length; hilum elliptical to ovate; hilum 21-24% of caryopsis length.

*Chionochloa cheesemannii* Zotov.- 2.4-2.8 X 0.7-0.8 mm; elliptic to obovate; yellow; dull and smooth; no compression (elliptic in transverse section); apex glabrous, 2-way ridge; embryo 34-39% of caryopsis length; hilum elliptical; hilum 34-39% of caryopsis length.

*Anisopogon avenacea* R.Br.- 6.1-8.2 X 1.5-2.0 mm;

lorate; light brown; rugulose; dorsal surface rounded, ventral surface flat; apex villose, 2-way ridge; embryo 13-15% of caryopsis length; hilum linear, grooved; hilum 79-95% of caryopsis length.

*Pseudopentameris macrantha* (Schrud.) Conert. (4) - 5.1-5.6 X 1.3-1.6 mm; obovate to elliptical; light brown; dull and smooth; apex glabrous, undeveloped; embryo 25-31% of caryopsis length; hilum linear, deeply grooved; hilum 78-90% of caryopsis length.

*Pentameris macrocalycina* (Steud.) Schweick. (1) - 3.4 X 1.2 mm; fruit an achene; obovate to cuneate; dark brown; rugulose; no compression (round in transverse section); apex flattened, villose, 2-way ridge; embryo 30% of caryopsis length; hilum elliptical; hilum 40% of caryopsis length.

*Merxmuellera dura* (Stapf) Conert.- 3.2-3.6 X 0.8-1.1 mm; obovate; yellow to light brown; rugulose; dorsal surface rounded, ventral surface compressed; apex glabrous, 2-way ridge; embryo 24-33% of caryopsis length; hilum elliptic; hilum 49-53% of caryopsis length.

*Merxmuellera rufa* (Nees) Conert.- 2.2-2.7 X 0.8-1.1 mm; obovate; dark brown; rugulose; dorsal surface rounded, ventral surface compressed; apex glabrous, 2-way ridge; embryo 36-42% of caryopsis length; hilum elliptic, compressed; hilum 14-22% of caryopsis length.



*Tribolium uniolae* (Spreng.) Nees.- 1.5-1.7 X 0.7-0.8 mm; elliptic; yellow to light brown; rugulose; dorsal surface rounded, ventral surface flat; apex glabrous, 2-way ridge; embryo 36-43% of caryopsis length; hilum elliptical; hilum 19-20% of total caryopsis length.

*Urochlaena pusilla* Nees.- 1.1-1.6 X 0.5-0.7 mm; elliptic; yellow to light brown; rugulose; dorsal surface rounded, ventral surface compressed; apex glabrous, 2-way ridge; embryo 38-57% of caryopsis length; hilum elliptic; hilum 19-20% of caryopsis length.

*Karroochloa* sp.- 1.1-1.7 X 0.5-0.8 mm; elliptic; yellow to light brown; shiny and smooth, dorsal surface rounded, ventral surface flat; apex glabrous, 2-way ridge; embryo 45-67% of caryopsis length; hilum elliptic; hilum 19-24% of caryopsis length.

*Danthonia pallida* DC.- 2.1-2.6 X 0.8-1.1 mm; obovate; light brown; rugulose; dorsal surface rounded, ventral surface flat; apex glabrous, 2-way ridge; embryo 42-49% of caryopsis length; hilum ovate, grooved; hilum 19-24% of caryopsis length.

*Danthonia carphoides* DC.- 1.5-1.9 X 0.7-1.0 mm; obovate; yellow; dull and smooth; dorsal surface rounded, lower third of ventral face flat, upper third of ventral surface round; apex glabrous, 2-way ridge; embryo 41-56% of caryopsis length; hilum elliptic; hilum 20-26% of caryopsis length.



*Chaetobromus involucratus* (Ball and Hubb.) Ball and Hubb.- 2.7-3.1 X 0.8-0.9 mm; elliptic; yellow; dull and smooth; dorsal surface rounded, ventral surface compressed along hilar region; apex glabrous, 2-way ridge; embryo 42-47% of caryopsis length; hilum linear, grooved; hilum 74-87% of caryopsis length.

*Schismus inermis* P. Beauv. (3) - 0.9 X 0.5 mm; obovate; yellow; shiny and smooth; dorsal surface rounded, ventral surface flat; apex glabrous, 2-way ridge; embryo 33-43% of caryopsis length; hilum elliptic; hilum 13-17% of caryopsis length.

*Pentaschistis aristoides* (Thunb.) Stapf.- 2.7-3.3 X 0.8-0.9 mm; elliptic to obovate; yellow to light brown; smooth and dull; no compression (round in transverse section); apex glabrous, 2-way ridge; embryo 27-33% of caryopsis length; hilum oblong; hilum 43-67% of caryopsis length.

*Pentaschistis minor*.- 1.2-1.4 X 0.4 mm; elliptic; yellow; rugulose; dorsal surface rounded, ventral surface concave; apex glabrous, 2-way ridge; embryo 32-43% of caryopsis length; hilum elliptic to lanceolate; hilum 29-43% of caryopsis length.

*Dregeochloa pumila* (Nees) Conert. (2) - 1.9-2.0 X 1.0-1.1 mm; obovate; fruit an achene (separable pericarp); yellow to light brown; rugulose; dorsal surface rounded, ventral surface concave; apex glabrous, 2-way ridge; embryo 61-79% of caryopsis length; hilum elliptic, grooved?; hilum 21% of caryopsis length.

## DISCUSSION

### 1. EMBRYO MORPHOLOGY.

All arundinoid species investigated (except *Anisopogon avenacea*, which is discussed separately), exhibited no variability in their embryo characters (Table 4). These were characterized by a long embryonic mesocotyl internode, the lack of an epiblast, the presence of a scutellum cleft and non-overlapping leaf margins (Table 4, Fig. 2 A, B).

The lack of variation observed in the embryo characters implies that these characters will not assist in distinguishing the tribes, despite the likelihood that they are polyphyletic (Davis and Soreng 1993, Barker et al. in press, Kellogg and Linder, in press).

The data presented here and in the literature, however, can be used to show that embryo characters are phylogenetically informative at a subfamilial and higher taxonomic level.

In contrast to earlier approaches such as Reeder (1957) who treated the entire embryo as a single character, individual characters of the embryo are treated here separately (Kellogg and Campbell 1987).

**SCUTELLUM CLEFT.** - All arundinoid species investigated in this study possess a scutellum cleft (Table 4). Exceptions have been recorded by Clayton and Renvoize 1986, Kellogg and Campbell 1987 for the genera *Sartidia*, *Chionochoa* and *Merxmuellera*. In the case of the latter two genera this has not been confirmed in the present study (Table 4). There are two possibilities which may account for this: Either the embryo structures have been

misinterpreted, or the scutellum cleft is variable within these genera. Unfortunately, the information for *Chionochloa* and *Merxmuellera* in Kellogg and Campbell (1987) is from the database of Watson et al. (1985) and thus of unknown origin so that it is unverifiable.

*Merxmuellera* may be paraphyletic or even polyphyletic (Verboom et al. in press). This was a consequence of the lack of a synapomorphy for the genus and the observation that some species such as *M. rufa* and *M. arundinacea* have retained more plesiomorphic integument and ovule features than some of the others such as *M. dura*. In the present study, however, *M. dura* and *M. rufa* do not show any differences in their embryo features (Table 4). These appear therefore to be uninformative at a species level. However, only a wider sampling of species within the genus will substantiate or refute the proposition that this character is variable.

In the small genus *Sartidia*, the lack of a scutellum cleft has been used (in addition to some differences in the spikelet structure) to distinguish it from the other two genera at present included in the Aristideae. Here it appears that the embryo structure may be informative at generic level.

The scutellum cleft appears to be present in most species of the PACC (Panicoideae, Arundinoideae, Chloridoideae, Centothecoideae) clade as well as in some genera placed by Clayton and Renvoize (1986) in the Bambusoideae.

The phylogenetic implications of this character distribution are uncertain, since the Bambusoideae is still unresolved and there is only weak evidence for rooting the Poaceae among a paraphyletic bambusoid group (Kellogg and Linder, in press). In

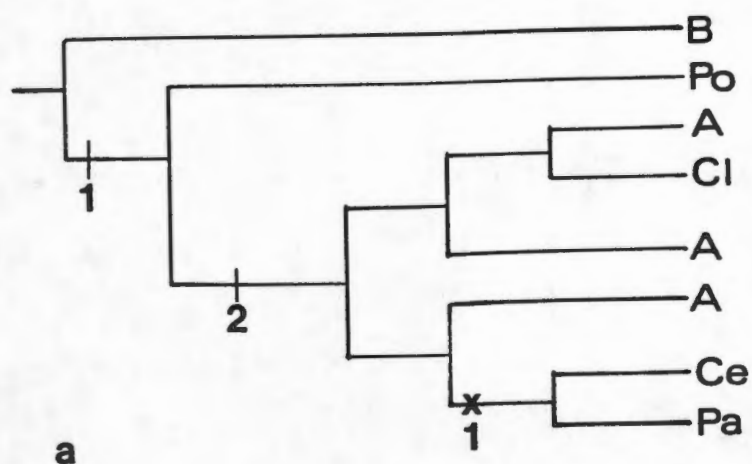
addition variability of this character within the highly heterogeneous Bambusoideae is unknown. Also, in the putative sister group of the Poaceae, the Joinvilleaceae (Doyle et al. 1992, Kellogg and Linder, in press) the embryo is undifferentiated.

EPIBLAST. - The Chloridoideae differs from Danthonieae, Arundineae and Aristideae only by the presence of an epiblast (Table 4, Reeder 1957, Kinges 1961, Decker 1964). However, this subfamily was shown to be variable in this character (Reeder 1957, Decker 1964). For example, some members of the Eragrostideae (Chloridoideae), such as *Myriostachia* and *Blepharidachne* lack the epiblast (Reeder 1957, Decker 1964). Reeder (1957) records that even within the genus *Spartina* (Cynodonteae, Chloridoideae) the presence of the epiblast is variable. He indicated that early workers such as Bruns (1892) had attached undue taxonomic importance to the epiblast. Nevertheless, Reeder himself attributes some taxonomic significance to it. However, it appears to be a rather homoplastic character showing variation also within the subfamily Bambusoideae and Pooideae (Decker 1964, Clayton and Renvoize 1986). *Arctophila* (Poeae, Pooideae) and *Dupontia* (Poeae, Pooideae), two monotypic genera found in the Arctic lack the epiblast (Decker 1964). Examples from the tribe Triticeae (Pooideae) include the genera *Elymus*, *Hystrix* and *Secale* (Decker 1964). However, it remains unknown how variable the epiblast is within the genera. Although it may not be consistent within the subfamilies and therefore could be termed "erratic" (Clayton and Renvoize 1986), it may provide information at a lower taxonomic

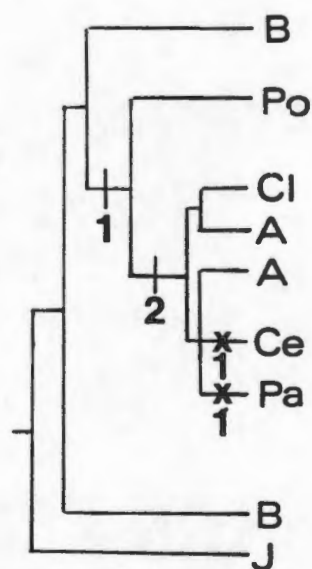
level. Overall, the lack of an epiblast appears to be relatively rare in the Pooideae (Reeder 1957, Kinges 1961, Decker 1964).

However, the epiblast appears not to be variable in all subfamilies (Table 4, Reeder 1957, Kinges 1961, Decker 1964). Reeder (1957) found that in the Panicoideae the epiblast is always absent and this also appears to be so in all the arundinoid species investigated, except *A. avenacea* (Table 4). In contrast in the Centothecoideae all genera so far studied possess an epiblast (Reeder 1962, Kellogg and Campbell 1987). Therefore the epiblast appears to be a reliable character for distinguishing the subfamilies Centothecoideae and Panicoideae, which otherwise share the same embryo states (Table 3, 4).

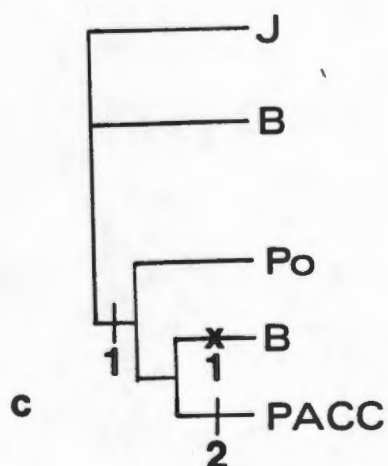
Arguments in respect of the uncertainty of phylogenetic implications of the character distribution of the epiblast are similar to those for the scutellum cleft. In addition, the epiblast occurs twice in the PACC clade. However, the two subfamilies in which it occurs are apparently not as closely related to one another as they are to others of the PACC clade (Fig. 5 a, b). Barker et al. (in press) therefore suggest two independent reversals of this character, assuming that the presence of the epiblast is the plesiomorphic state. For the reasons discussed above such an assumption appears unfounded. So far it can only be stated that the epiblast does not reveal relationships among the subfamilies in the PACC clade. Also at tribal level it appears not to be useful in the polyphyletic Arundinoideae, since it does not distinguish the tribes (Table 4). Evidence so far suggests the epiblast to be of limited use, though it may have value if considered in conjunction with other characters.



a



b



c

Fig. 5. Embryo characters mapped onto cladograms generated with molecular data set (a, c), and combined (morphology and molecular) data set (b).- Cladograms extracted and simplified from: a. Barker et al. (in press). - b. Kellogg and Linder (in press). - c. Davies and Soreng (1993). A, Arundinoideae; B, Bambusoideae; Ce, Centothecoideae; Cl, Chloridoideae; J, Joinvilleaceae; Pa, Panicoideae; Po, Pooideae. 1, presence of non-overlapping embryonic leaf margins; 2, presence of long embryonic mesocotyl internode; X, reversal.



EMBRYONIC LEAF MARGINS. - The Centothecoideae and Panicoideae differ from the tribes in the Arundinoideae and Chloridoideae by the presence of overlapping leaf margins. Kellogg and Campbell (1987) found this character to be variable within both subfamilies. Reeder (1957) and Decker (1964), on the other hand, found no variation. Here, it has to be pointed out again that none of the authors recorded the species that they investigated. In addition, Kellogg and Campbell (1987) noted neither the genera investigated nor the frequency of variation of this character in the Centothecoideae. Due to the lack of such valuable data Kellogg and Campbell's study appears worthless. Although some variability in this character cannot be excluded, it is most likely to be rare (Reeder 1957, Decker 1964).

Overlapping leaf margins are otherwise only present in the Bambusoideae (sensu Clayton and Renvoize 1986) and are apparently not variable (Reeder 1962), whereas non-overlapping leaf margins are found in the Pooideae, Chloridoideae and Arundinoideae (sensu Clayton and Renvoize 1986). If this character is derived it may lend support to hypotheses generated with morphological and molecular data which suggest that the Pooideae are closer to the PACC clade than the Bambusoideae are to this clade (Barker et al. in press, Kellogg and Linder in press, Fig. 5 a, b).

In the PACC clade overlapping leaf margins are present in the Centothecoideae and Panicoideae. Davies and Soreng (1993) found the Centothecoideae to be more closely related to the Arundinoideae and Chloridoideae than to the Panicoideae. However, the presence of overlapping leaf margins in these two subfamilies may rather give support to the recent hypothesis of

Barker et al. (in press, based on *rbcL* sequence data) which suggests that these two subfamilies are sister taxa (Fig. 5 a).

**EMBRYONIC MESOCOTYL INTERNODE.**- A long embryonic mesocotyl internode is found in all members of the PACC clade, whereas it is short in the Bambusoideae and Pooideae (Table 4, Fig. 2 D). Except for the Panicoideae (Kellogg and Campbell 1987) this character appears to be present in all the taxa of the PACC clade so far investigated. Although Kellogg and Campbell (1987) do not mention which taxa show variation or its frequency, variability appears to be rare, since a large number of genera investigated by Reeder (1957) and Decker (1964) showed none. The vascular internode is the most difficult character to observe on a microscopic preparation and this may account for some of the anomalies (Reeder 1962, Clayton and Renvoize 1986).

In contrast, the presence of a short embryonic mesocotyl internode is found in the Bambusoideae and Pooideae and is apparently not variable (Reeder 1957, Decker 1964, Kellogg and Campbell 1987). In the Bambusoideae, Reeder (1957) emphasizes that all taxa (although variable in the presence of the epiblast and the state of the scutellum) possess a short embryonic mesocotyl internode and overlapping leaf margins. Thus the presence of a long mesocotyl internode is unique to the PACC clade.

This character separates the Pooideae and Bambusoideae from the PACC clade. If this character is derived it is congruent with the clear division of the PACC clade suggested by Davis and Soreng (1993) (Fig. 5 a-c).



*Anisopogon avenacea* which is currently included in the Danthonieae by Watson and Clifford (1976) and Renvoize (1981), has typical non-arundinoid/danthonioid characters. A short embryonic mesocotyl internode, the presence of an epiblast, the lack of a scutellum cleft and non-overlapping leaf margins clearly bring it in close association with the Pooideae (Fig. 2 C, D). Previously Watson and Dallwitz (1988) included *Anisopogon avenacea* in the [Stipeae, Arundinoideae]. However, some evidence from *rbcL* sequence data exists for a [Stipeae, Pooideae] grouping (Barker et al., in press). Such a clade has also been retrieved by restriction site data (Davies and Soreng 1993), and morphological data (Kellogg and Watson 1993). Although embryo data are mostly uninformative at a tribal or lower level, they clearly place *Anisopogon avenacea* among the Pooideae and therefore corroborate the above findings. Based on ovular features it has also been suggested by Verboom et al. (in press) that *Anisopogon avenacea* has most likely been misplaced.

## 2. CARYOPSIS MORPHOLOGY: HOW INFORMATIVE IS IT?

The characters have been tabulated for comparison (Table 5). With one exception, the observations made were insufficient to recognize groups within the arundinoids. Some suggestions, however, have been made as to which characters may be 'typical' or 'atypical' for the species investigated. This has been done by comparison to the caryopses of the Triticeae (Pooideae) for which in a recent study numerous data were generated. In previous studies very little attention was given to variation of characters, particularly the quantitative ones, within species which may have led to erroneous conclusions (e.g. Zotov 1963). It appears therefore appropriate to discuss the characters separately to indicate their usefulness in a phylogenetic study of the Arundinoideae.

Shape.- An elliptic or obovate shape was most common among the arundinoid species investigated (Table 5, Fig. 3, 4). The shape appeared to be variable in *Pentameris macrocalycina*. Unfortunately in this study only one seed was available, which had an obovate to cuneate shape (Fig. 4 A). Barker (1986), however, described the seed as ellipsoid. Although interpretation of shapes is somewhat difficult due to 'intermediate' forms, the photograph in Barker (1986) suggests that the shape is variable within this species. This may be of particular importance since Barker (1986) places emphasis on the shape of the caryopsis as an useful character in the classification of *Pentameris* and *Pseudopentameris*. Barker does not say how many caryopses of each species he investigated. Although seed material is often difficult to obtain, it appears

neccessary to investigate at least 5 to 10 seeds of each species to detect possible intraspecific variation.

In contrary, it was noted that unlike all other specimens investigated, *Pentameris macrocalycina* has a flat apex (Fig. 4 A). This is a character which is at least also present in *P. obtusifolia*, *P. dregeana*, *P. thuarii* var. *thuarii* (Barker 1986). The shape of the apex as a useful taxonomic character has previously been pointed out by others such as Sendulsky et al. (1987). However, the investigation of other species in *Pentameris* is needed to support the present evidence that this character may be useful in the classification of *Pentameris*.

Size.- The majority of species appears to be characterized by a fairly small size and small variation range (Table 5). In contrast *Anisopogon avenacea* and *Pseudopentameris macrantha* differ from the other species both by their particularly large size and a large range of variation (Table 5). It was noted that caryopses in the Triticeae (Pooideae) are also relatively large (ranging from 2.5-11 mm) and vary considerably in size (Terrell and Peterson 1993).

*Monachather paradoxus* sets itself apart from the others less by its length than by its large width. The unusual size and shape of *Monachather paradoxus* and *Anisopogon avenacea* may weakly support their placement outside the arundinoids as proposed by several authors such as Barker et al. (in press) and Verboom et al. (in press). However, *Pseudopentameris macrantha* also has a fairly large seed, with an upper range in size coinciding with the lower end of *A. avenacea* (Table 5). Clayton and Renvoize noted that *A. avenacea* closely resembles and may even have

affinities to *P. macrantha*. However, size may here only superficially suggest a relationship, since the embryo characters in section 1 do not support any affinity. In addition, the ranges shown for the Triticeae and Arundineae overlap. Therefore seed size by itself appears unreliable and may have use only if employed in conjunction with other characters.

In contrast, seed shape and size was found to be useful to distinguish two main groups within the Triticeae (Terrell and Peterson 1993).

At a genus level Zotov (1963) used mean caryopsis length as one of the key characters to distinguish *Danthonia* from *Notodanthonia*. It has already been shown by Jacobs (1982) that variability between the species did not support such a separation (see also Table 5). The data presented here show in addition that variation also exists within species (Table 5). Among the genera there is a lack of discontinuity in both lengths and widths (e.g. *Tribolium*, *Karroochloa* and *Urochlaena*). The range seen is often less than that found within a genus (e.g. *Alloeochate*, *Merxmuellera*, *Pentaschistis*). Both of these facts indicate the limited use of this data for a phylogenetic study at generic level.

Appendages of the apex.- *Pentameris macrocalycina* and *Anisopogon avenacea* were the only two species with a villose apex (Fig. 4 A, B). Conert (1971) claimed that *Pentameris* could easily be separated from *Pseudopentameris* both by the leaf anatomy and caryopsis structure. The rarity of apical hairs among arundinoids has proven to be a useful character to delimit *Pentameris* and this has been confirmed by Barker (1986).

However, apical hairs are also present in *A. avenacea*. This character is otherwise characteristically found in the Pooids (Tsvelev 1976, Terrell and Peterson 1993 (data for Triticeae)). It may therefore be argued that the present evidence from the embryo characters suggesting placement of *Anisopogon avenacea* among the Pooids may additionally be corroborated by the presence of apical hairs.

Apart from *Pseudopentameris macrantha* all species here investigated showed the presence of an ovary appendage in which differences in shape and size were seen (photos). However, too little material was investigated to establish its importance for classification.

Surface structure.- Due to the low magnification, surface topology provided only limited information. The rare occurrence of shiny caryopses in *Karroochloa* sp. and *Schismus inermis* is, however, noteworthy (Table 5). Close relationships between the genera *Karroochloa*, *Schismus* and *Tribolium* have been suggested by several authors such as Conert and Tuerpe (1974). The presence of the shiny caryopsis has long been thought to place *Karroochloa* closer to *Schismus* than to *Tribolium*. However, a revision of *Schismus* and *Karroochloa* that is at present in preparation has indicated that *Schismus* may be more closely related to *Tribolium* (Scott, pers. comm). If this view finds general acceptance, the shiny caryopsis would not anymore be indicative of close relationship. However, further investigation of the seed surface of *Tribolium* may provide more insight as to how phylogenetically informative this character is.

Barker (1986) found that Scanning Electron Microscope (SEM)



views of the sculpturing of the coat did reveal important characters at a generic level, which he used in the classification of *Pentameris* and *Pseudopentameris*. It is therefore possible that SEM may also be useful to reveal relationships among the other genera.

Embryo.- The ranges of embryo size within the same species, were found to be fairly large (Table 5). For example, the embryo of *Plinthanthesis rodwayi* ranges in size from 28 to 41 % of the whole caryopsis. The embryo of *Chionochoa cheesemannii* ranges from 45 to 55 % of the whole caryopsis. Early studies such as Reeder's (1957) and Kinges' (1961) do not account for such variation. Reeder (1957) noted that the embryo is "relatively" small in typical festucoid species, whereas it is "relatively" large in the true panicoids (Reeder 1957: 758). Reeder (1957) only provided sketches of the proportions of the embryo to the whole caryopsis to explain the terms "relatively" large or small. Kinges (1961) expanded on this terminology and classified the embryo as small (size of embryo  $< 1/3$  of caryopsis), medium (size of embryo  $> 1/3$  and  $< 1/2$  of caryopsis) or large (size of embryo  $> 1/2$  of caryopsis). It appears that the authors were not aware of the extent of variation, possibly due to the low number of seeds investigated. [There is no statement of how many seeds were investigated].

The lack of documentation of such variation within species (and most likely also between species) led Kellogg and Campbell (1987), who based their study on secondary data (i.e. Watson data base), to recognize two discrete states, i.e. large and small. They assigned "small" to the genera *Phaenanthoecium* (16-30 %) and

*Pseudopentameris* (25-31 %), "large" to *Plinthanthesis* (28-41 %). The numbers in brackets indicate data found in this study (Table 5), from which it can be seen these states overlap. Therefore, the assignment of discrete states here is meaningless (see also Stevens 1991).

Although this study too was based on only five or fewer specimens per species, *A. avenacea* was found to have an unusually small embryo (only 13-15 % of the caryopsis length), more typical of the pooids (Kinges 1961, Fig. 3 A, D). All but *Danthonidium gammiei* had a lower range of 20% or greater (Table 5, Fig. 3 B, C, E, F). This may suggest the existence of a discontinuity between *A. avenacea* and the other species. The minor gap of 1 % between the upper range of *A. avenacea* and the lower range of *Danthonidium gammiei* is insufficient to suggest two discrete states. Nevertheless, it is possible that the lower range may be due to an exceptional case (immature embryo). The paucity of material made it impossible to re-investigate this point.

Despite the small gap between *A. avenacea* and the other species, the small size and range of the embryo is very unusual within this group and may provide additional support for placing *A. avenacea* outside the Arundineae, most likely among the Pooids.

*Monachather paradoxus* (50-80 %) and *Dregeochloa pumila* (61-79 %) had unusually large embryos. Both species are thought to belong outside the Danthonieae sensu Barker et al. (Barker et al. in press; Verboom et al. in press). Conert (1971:303) states that characters of the mature caryopsis, such as the embryo, of *Dregeochloa* (originally *Danthonia*) *pumila*, would provide the most important means of distinguishing it from other species of *Danthonia*. In the two species observed here, the upper ranges

lie below those of *Dregeochloa pumila* while only a 5 % difference lies between *D. pumila* and *D. carpha*. However, as very few species of *Danthonia* and few specimens of *D. pumila* were investigated, it appears premature to reach any conclusion on the validity of Conert's observation.

Large embryos are also present in *Karroochloa* sp. and *Urochlaena pusilla* and they overlap with the ranges of *D. pumila*, possibly suggesting a relationship. Yet, there is recent evidence from embryological (Verboom et al. in press), anatomical and gross morphological data (De Wet 1954, 1956, 1960) that *D. pumila* should be placed outside the "core" danthonioids (as defined by Verboom et al. and including *Karroochloa* and *Urochlaena*).

It is apparent that, because of its variability within species (possibly due to environmental influences), embryo size is of limited value in resolving relationships within the arundinoids, i.e. at generic level.

An exception appears to be *A. avenacea*, which exhibits a 'typical' pooid embryo. This suggests that embryo size may have some value at subfamilial level.

Hilum.- The majority of seeds had an elliptic hilum, whereas a linear or punctiform hilum was rare (Table 5, Fig. 4). Kinges (1961) found the hilum to be small and roundish in the panicoid and longish in the festucoid species. Sendulsky et al. (1987) mentioned the predominant presence of the linear hilum in the Bambusoideae and Pooideae, which they interpreted as a primitive feature. However, they pointed out that the linear hilum was also found in advanced genera such as *Mesosetum*



(Panicoideae), suggesting the possibility of evolutionary reversal.

In *A. avenacea* the linear hilum may lend further support to its placement among the Pooideae, because of all the other caryopsis characters that it shares with the pooids.

*Danthonidium* and *Phaenanthoecium* are two little known, monotypic genera. *P. kostlinii* is thought to be a segregate from *Rytidosperma*, from which it differs by its short glumes and the long hilum (Clayton and Renvoize 1986). Similarly *D. gammiei* appears to share characters with *Rytidosperma*, but also with *A. avenacea* (Clayton and Renvoize 1986). The relationships to *Rytidosperma* suggested by the linear hilum need to be corroborated by other characters.

At a quantitative level, the length of the hilum has been used by Zotov (1963) to separate *Rytidosperma* from *Danthonia*. He claimed that the mean hilum length of *Danthonia* exceeded 50 % of the length of the caryopsis, whereas *Rytidosperma* has a mean value smaller than 33 %. The ranges of hilum length (Table 5), however, show that such separation is not justified. This has also been demonstrated by Jacobs (1982). In addition, the ranges found in this study demonstrate that variation within species may be rather large. Therefore, taking means of quantitative data may obscure overlapping states and lead to erroneous interpretations.

## CONCLUSION

Earlier treatments, such as Reeder (1957) and Clayton and Renvoize (1986), considered the embryo as a single character. Here the embryo characters are treated as separate evolutionary entities. The embryo characters were shown to differ in their degree of conservation and are therefore useful in inferring phylogenetic relationships at different taxonomic levels.

The embryonic mesocotyl internode appears to be the most conservative embryo character within the subfamilies. The short embryonic mesocotyl internode is present in the Pooideae and Bambusoideae. In contrast, a long embryonic mesocotyl internode is exclusively found in the PACC clade. If this character is derived it corroborates this clade which was generated by molecular data (Davies and Soreng 1993).

Overlapping leaf margins are conservative in the Bambusoideae. In the Pooideae, Chloridoideae and the arundinoid tribes the embryonic leaf margins do not overlap. This shared character supports the hypothesis based on molecular data (Barker et al., in press; Kellogg and Linder, in press) that the Pooideae are the sister group to the PACC clade. In the Centothecoideae and Panicoideae, this character state is likely to be a reversal and at the same time lends support to the hypothesis that these two subfamilies are sister taxa (Barker et al., in press).

The epiblast appears to be most variable in the Bambusoideae. Its level of phylogenetic informativeness is still uncertain. This is due to its variability in the Bambusoideae and its reappearance in the PACC clade, i.e. in two subfamilies that are apparently not related. So far it appears to be of

little use at subfamily and tribal level.

The scutellum cleft is absent in the Pooideae and mostly present in the PACC clade. As for the epiblast, variability in the Bambusoideae and the lack of embryo characters in the putative outgroup to the Poaceae, the Joinvilleaceae, make it difficult to assess the importance of this character in a phylogenetic study.

The data presented here for the Arundinoideae indicate that *Anisopogon avenacea* has embryo characters which brings it in close association with the Pooideae. Evidence for the misplacement of *Anisopogon avenacea* has also been produced by others (Verboom et al in press).

Quantitative caryopsis characters such as length of the embryo, the seed and the hilum, which were found to be taxonomically discriminating in earlier studies (Reeder 1957, Kings 1961, Zotov 1963) were found to exhibit great variability within species. This led to the characters being uninformative at generic level. However, at subfamily or higher level seed size and the length of the embryo appear to have some taxonomic value.

Qualitative data such as presence of apical hairs, shape of hilum and surface sculpturing appeared less variable within species and appear useful at generic level. In some cases it was shown that the presence of a shared character did not appear to reflect phylogenetic relationships. It therefore appears that the characters investigated here are not very conservative. Nevertheless they appear informative if used in conjunction with other characters.

This was shown for *Anisopogon avenacea* which differed in almost all its caryopsis characters from the majority of species investigated. The characteristics of the caryopsis of *A. avenacea* are 'typical' of the Pooideae. Seed morphology therefore corroborates the suggested phylogenetic relationships based on embryo characters.

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