

# Reproductive morphology of the early-divergent grass *Streptochaeta* and its bearing on the homologies of the grass spikelet

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**Abstract** Reproductive morphology and development are described in the Brazilian grass *Streptochaeta spicata*, in order to assess the homologies of the characteristic grass inflorescence, termed a spikelet, and other reproductive organs. *Streptochaeta* possesses some features that are commonly found in Poaceae, including a well-differentiated embryo. It also possesses some relatively unusual, presumably derived features, such as non-plumose stigmas, which indicate that it could be insect-pollinated. It shares some features with other early-divergent grasses, such as *Pharus*, which could represent plesiomorphic conditions for grasses. The inflorescence unit in *Streptochaeta* has been interpreted as a compound branching system or pseudospikelet. The present data suggest that it is a highly modified spikelet, with a modified flower borne either on a different axis to the basal bracts (glumes) or on the same axis as the basal bracts. The three bracts below the stamens are interpreted as homologous to the lodicules. The *Streptochaeta* spikelet could be considered as morphologically intermediate between the true spikelet of grasses and reproductive units of close grass relatives.

**Keywords** Caryopsis · Grass flower · Grass spikelet · Inflorescence · Scutellum

## Introduction

The grass family Poaceae (Gramineae) is arguably the most species-rich and economically important of all plant families. The monophyly of Poaceae is clearly demonstrated by the long branch that separates it from related taxa in molecular cladistic analyses (Briggs et al. 2000; Bremer 2002; Michelangeli et al. 2003), and the family is easily recognizable by numerous highly characteristic morphological features. In particular, most grasses share a specialized bracteate partial inflorescence termed a spikelet, each spikelet containing one to many florets with a reduced or absent perianth (Clifford 1987). The family was traditionally divided into tribes and subfamilies based mainly on spikelet morphology, but more recently the Grass Phylogeny Working Group (GPWG 2001) provided a synthetic reclassification of grasses based on a combined phylogenetic analysis of both molecular and morphological data, which has facilitated re-evaluation of the homologies of the characteristic grass features. GPWG (2001) recognized 12 monophyletic subfamilies, and subsequently Sanchez-Ken et al. (2007) added a further subfamily, Micraioideae. The three earliest-divergent lineages – Anomochlooideae (*Anomochloa* and *Streptochaeta*), Pharioideae (*Pharus*) and Puelioideae (*Puelia* and *Guadella*) – are all relatively species-poor, and were previously included in the former subfamily Bambusoideae.

In all grasses except Anomochlooideae, which is sister to all other Poaceae, the typical spikelet consists of a series of distichous bracts with the two basal (proximal) set empty (the glumes) and one to many distal bracts (the

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lemmas). Each lemma subtends a contracted floral axis on which is borne a palea, two to three lodicules, the androecium and the gynoecium (Soreng and Davis 1998). The grass spikelet has been compared with a transformed stem axis with the glumes and lemmas representing modified leaf sheaths and the palea being a presumed prophyll subtending each flower (Clifford 1987). The grass flower is mostly interpreted as a monochlamydeous structure with only one perianth whorl, the lodicules (Rowlee 1898; Celakovský 1889; Arber 1929; Page 1951), though Ambrose et al. (2000) revived a dichlamydeous interpretation suggesting that the palea and possibly also the lemma have genetic features in common with the outer perianth whorl.

The reproductive structures of Anomochlooideae are highly atypical for Poaceae (Page 1951; Soderstrom 1981; Judziewicz and Soderstrom 1989). Neither *Anomochloa* nor *Streptochaeta* possesses structures that are clearly homologous with glumes, lemmas, or paleas, and thus neither can be described as possessing typical grass spikelets. Each partial inflorescence consist of solitary bisexual flowers in *Anomochloa* and bisexual flowers isolated by multiple bracts in *Streptochaeta*. Thus, in these two genera the partial inflorescence is more commonly termed as “pseudospikelet” (Page 1951; Soderstrom 1981; Soreng and Davis 1998) or a “spikelet-equivalent” (Judziewicz et al. 1999). In both the genera, the absence of an identifiable palea makes flowers appear terminal on the main sympodial axis rather than on lateral branches as in other Poaceae, in which there is a palea in the proximal, adaxial position on the branch, often interpreted as a prophyll (Clayton 1990). The pseudospikelet could be interpreted either as a synapomorphy of Anomochlooideae, or a plesiomorphy of grasses that is retained in only these two genera (Judziewicz and Soderstrom 1989).

Further questions remain about the homologies of the floral organs. The occurrence of lodicules in flowers of *Anomochloa* and *Streptochaeta* is controversial, because the homologies of the fringe of hairs below the staminal whorl in *Anomochloa* and the large, fleshy, lanceolate bracts in this position in *Streptochaeta* are not clear (Soreng and Davis 1998). However, the recent genetic study of Whipple et al. (2007) with *Streptochaeta* and two outgroups provides strong evidence that they are transformed inner tepals or lodicules. Reduction in stamen number is common in grasses and their close allies, often due to suppression of nonhomologous stamens (reviewed by Rudall and Bateman 2004; Rudall et al. 2005). However, *Streptochaeta* possesses six stamens, as in most monocots, which probably represents the plesiomorphic condition in grasses. The ovary and style are unusual in flowers of *Anomochloa* and *Streptochaeta*, and the three stigmas of *Streptochaeta* are simple, contrary to the

plumose condition present in most grasses (Soreng and Davis 1998).

In this paper, we investigate the reproductive morphology and anatomy of *Streptochaeta spicata* Schrad. ex Nees, a herbaceous grass that grows in shaded tropical forests in Brazil, and one of only three species of *Streptochaeta*. We evaluate the existing hypotheses on the homologies of the *Streptochaeta* spikelet and floral organs, and compare the development of anthers, ovary and ovules with those of other grasses, in the context of ongoing comparative studies on early-divergent grasses and their allies (Rudall et al. 2005; Sajo et al. 2007).

## Materials and methods

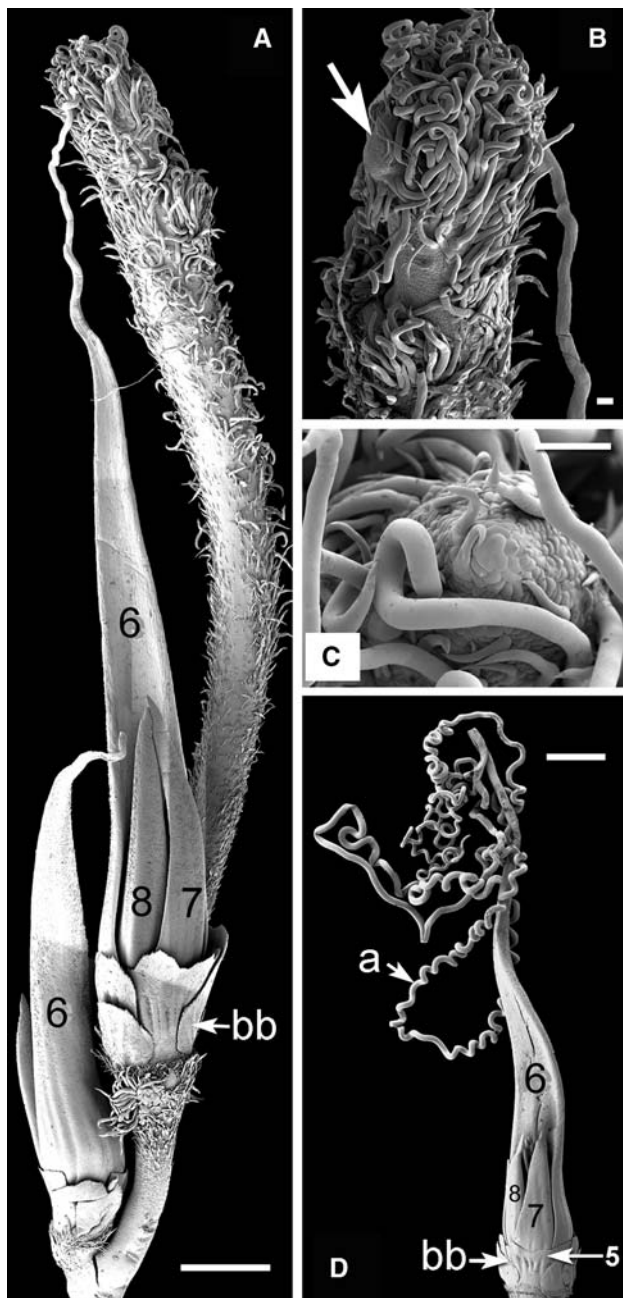
Material was collected from plants in their natural habitat in Rio Grande do Sul, Brazil, by Hilda Longhi-Wagner. Vouchers were deposited at the Herbário do Departamento de Botânica da Universidade Federal do Rio Grande do Sul (*Streptochaeta spicata* Schrad.: HLW 9371, 9372, 10087, 10097, 10234, ICN).

Flowers were fixed in formalin-acetic alcohol (FAA) and stored in 70% ethanol. For light microscopy, flowers were embedded in paraplast using standard methods (Johansen 1940) and serially sectioned at ca 13 µm thickness using a rotary microtome. For light microscopy (LM), sections were mounted onto microscope slides, stained in safranin and Alcian blue, dehydrated through an ethanol series to 100% ethanol, transferred to HistoClear, and mounted in DPX mounting medium (distrene, with dibutyl phthalate and xylene). Slides were examined using a Leica DMLB photomicroscope fitted with a Zeiss AxioCam digital camera. For scanning electron microscope (SEM) examination, fixed spikelets and florets were carefully dissected in 70% ethanol and then dehydrated in an ethanol series to 100% ethanol. Then they were dried at critical point using a Bal-Tec 030 critical point dryer, mounted onto pin stubs, coated with platinum using an Emitech K550 sputter coater, and examined using a Hitachi cold field emission SEM S-4700 at 2 kV.

## Observations

### Morphology of inflorescence and pseudospikelet (or spikelet-equivalent)

In *Streptochaeta spicata*, the inflorescence axis bears several short-pedicellate pseudospikelets arranged more or less spirally in a 2/5 phyllotaxy. The axis apex is covered by a tuft of hairs and has an aborted bud in a subapical position (Fig. 1a–c). Although some authors report the



**Fig. 1** **a** SEM entire rachis with two young pseudospikelets and sterile apical region. **b** SEM rachis apex with aborted bud in a subapical position indicated by *white arrow*. **c** SEM subapical aborted bud. **d** SEM young spikelet. Bracts 6–8 and basal bract 5 numbered (see Fig. 2b, d). *a* awn, *bb* basal bracts. *Scale a, d* 1 mm; *b, c* 100  $\mu$ m

presence of 12 bracts, all pseudospikelets examined here consisted of 11 bracts that are similar in texture but differ in size and shape. The pseudospikelet axis (below the basal node) contains two vascular bundles (Fig. 2c). Bracts 1–5 (the basal bracts, sometimes interpreted as glumes) are attached to a basal node (Figs. 1d, 2b, d). Bracts 1–2 are smaller, scale-like and positioned on the adaxial side of the

pseudospikelet, with bract 1 on the left and bract 2 on the right (Fig. 2d). Bract 3 lies laterally on the left, bract 4 is adaxially placed, and bract 5 is more or less opposite to bract 4 and overlapped by it on the right (Fig. 2d). None of the five basal bracts have axillary buds in pseudospikelets examined here.

Above the basal node there is a bractless internode (Fig. 2f) containing four vascular bundles (Fig. 2d) supplying bracts 6–11, which are attached at the pseudospikelet level that is here termed as the “flower node” (Fig. 2f, k). At the flower node, bract 6 is on the adaxial side of the spikelet-equivalent (in the opposite sector to bract 5) with its back facing the inflorescence axis (Fig. 2k, m); it bears an extensive awn that becomes entangled in the hairs of the rachis apex (Fig. 1a, d). At anthesis, pseudospikelets become entangled by their awns to the rachis apex and are dispersed together. Bracts 7 and 8 form a whorl, with bract 7 overlapping bract 8 in the opposite direction to bracts 4 and 5 (compare Fig. 2d and k). These bracts (7–8) lie on the abaxial side of the pseudospikelet opposite bract 6, to which they eventually become basally fused during late development (Fig. 2m). Above bracts 7–8, three bracts (9–11) form another whorl and overlap each other to the left in a direction opposite to bracts 7–8 (compare Fig. 2m and k). The last two whorls (7–8 and 9–11) are spirally arranged, though the outer one (7–8) presumably lacks a member.

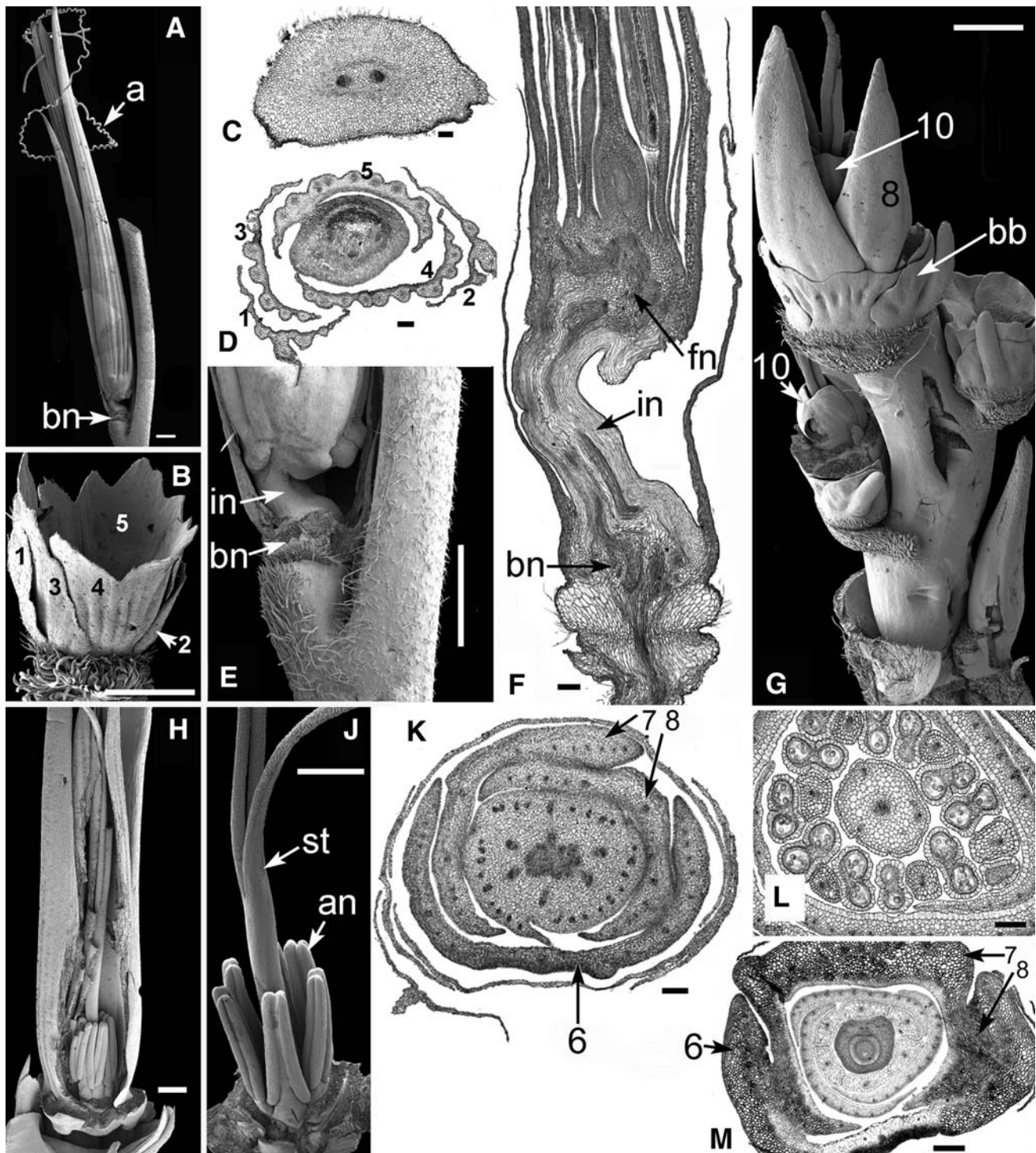
All bracts at the flower node (6–11) are long (ca 1–2 cm) and coriaceous, and supplied by many vascular bundles; they form a hard, more or less tubular structure that surrounds the fertile organs. Fertile organs consist of six stamens and a single ovary that bears a single solid style and three non-plumose stigmas (Fig. 2j). Each stamen is supplied by a single vascular bundle; the ovary bears three vascular bundles that each pass into one of the stigmas (Fig. 2l).

Contrary to some previous authors (see “Discussion”) we never found an “awned palea” (bracts 7–8), nor a “rudimentary palea bract” opposite bracts 7–8, nor an “extra bract” above and behind bracts 7–8.

### Pseudospikelet ontogeny (Fig. 3)

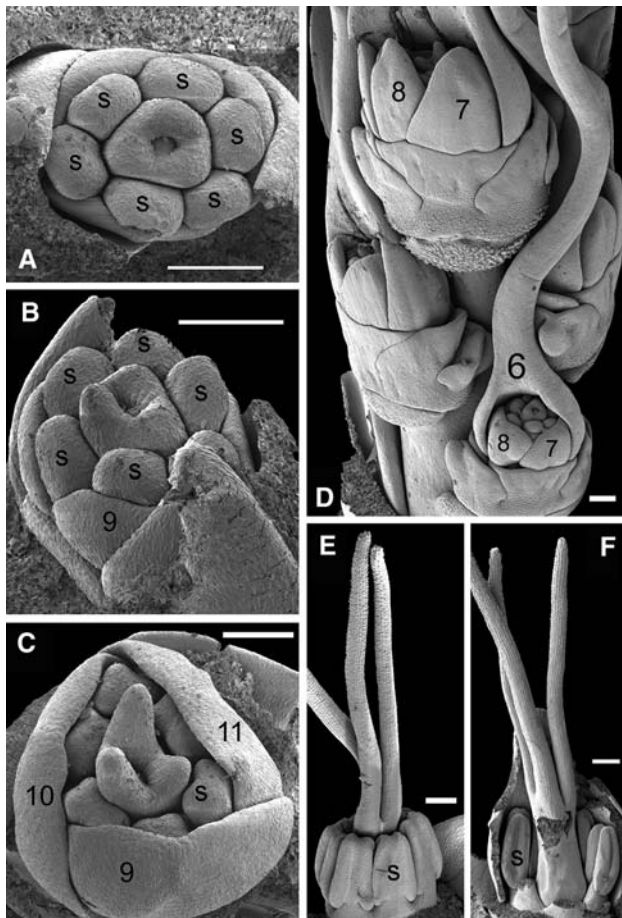
The bracts of the basal node are relatively well-developed at an early stage, while primordia are still initiating at the flower node (Fig. 3d). At the flower node, bract 6 has a long tip that will develop into the awn (Fig. 3d). Bracts 9–11 develop after bracts 7–8, and are followed by the six stamen primordia, which initiate simultaneously (Fig. 3a–c). A single whorl of three carpel primordia is also visible at this time. Bracts 6–11 rapidly enlarge and completely enclose the reproductive structures (Fig. 3d). The bracts of





**Fig. 2** **a.** SEM mature spikelet. **b.** SEM five basal bracts attached to axis, numbered from inside outwards, as in **d**; remainder of pseudospikelet removed. **c.** Light micrograph (LM) of transverse section of axis with two vascular bundles. **d.** LM transverse section of five basal bracts surrounding the remainder of pseudospikelet. **e.** SEM basal node. **f.** LM of longitudinal section of pseudospikelet. **g.** SEM partially dissected developing inflorescence with bracts 7–8 just starting to envelope reproductive parts. **h.** SEM partially dissected pseudospikelet showing stamen with short filaments surrounding

style/stigmas. **j.** SEM pseudospikelet with sterile bracts removed to show six stamens surrounding style. **k.** LM transverse section of flower node. **l.** LM transverse section of flower showing six anthers surrounding solid style. **m.** LM transverse section near the base of anthetic flower showing bracts 6, 7 and 8 basally fused; bracts 9–11 surrounding ovary with a single ovule. **a.** awn, **an** anther, **bb** basal bracts, **bn** basal node, **fn** flower node, **in** internode, **st** style/stylodia (all SEM, except LM in **c**, **d**, **f**, **k–m**). Scale **a**, **b**, **e** 1 mm, **c–f**, **k–m** 100  $\mu$ m, **g**, **h**, **j** 500  $\mu$ m

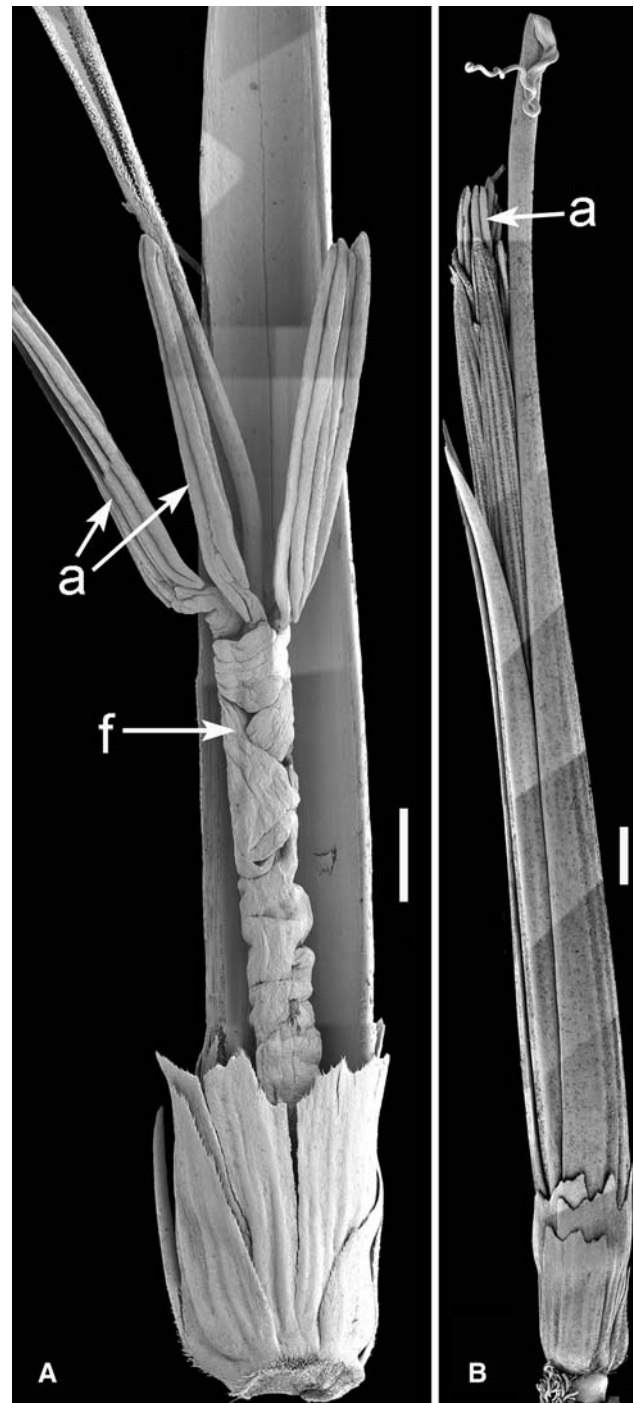


**Fig. 3** Pseudospikelet ontogeny (SEM). **a–c** Successive developmental stages of flower ontogeny, showing six developing stamens surrounding ovary with three stylodia. Central basal ovule just visible in **a**. **d** Developing inflorescence. **e, f** Older stages of floral ontogeny than in **a–c**. Bracts numbered as in text. *s* stamen. Scale 100  $\mu$ m

the basal node grow minimally, whereas the bracts of the flower node (6–11) grow extensively (Fig. 4b).

**Stamen development (Fig. 4)**

At early stages male and female parts develop synchronously. When microsporocytes are present inside the anthers, an ovule primordium is initiating within the ovary (Fig. 5d). However, the gynoecium enlarges before the anthers, pushing the three stigmas outside the spikelet when the anthers are still enclosed at the base (Figs. 2h, 3e–f). The anthers never hang free, as in most grasses. When the anthers reach the spikelet top, an early fruit is already developing. Initially, the stamen filaments are short and free, keeping the anthers inside the spikelet even when the pollen grains are mature inside the anthers; at this stage, the three stigmas are outside the spikelet. The filaments eventually twist together into a tube that elongates and pushes the anthers to the top of the spikelet (Fig. 4a).



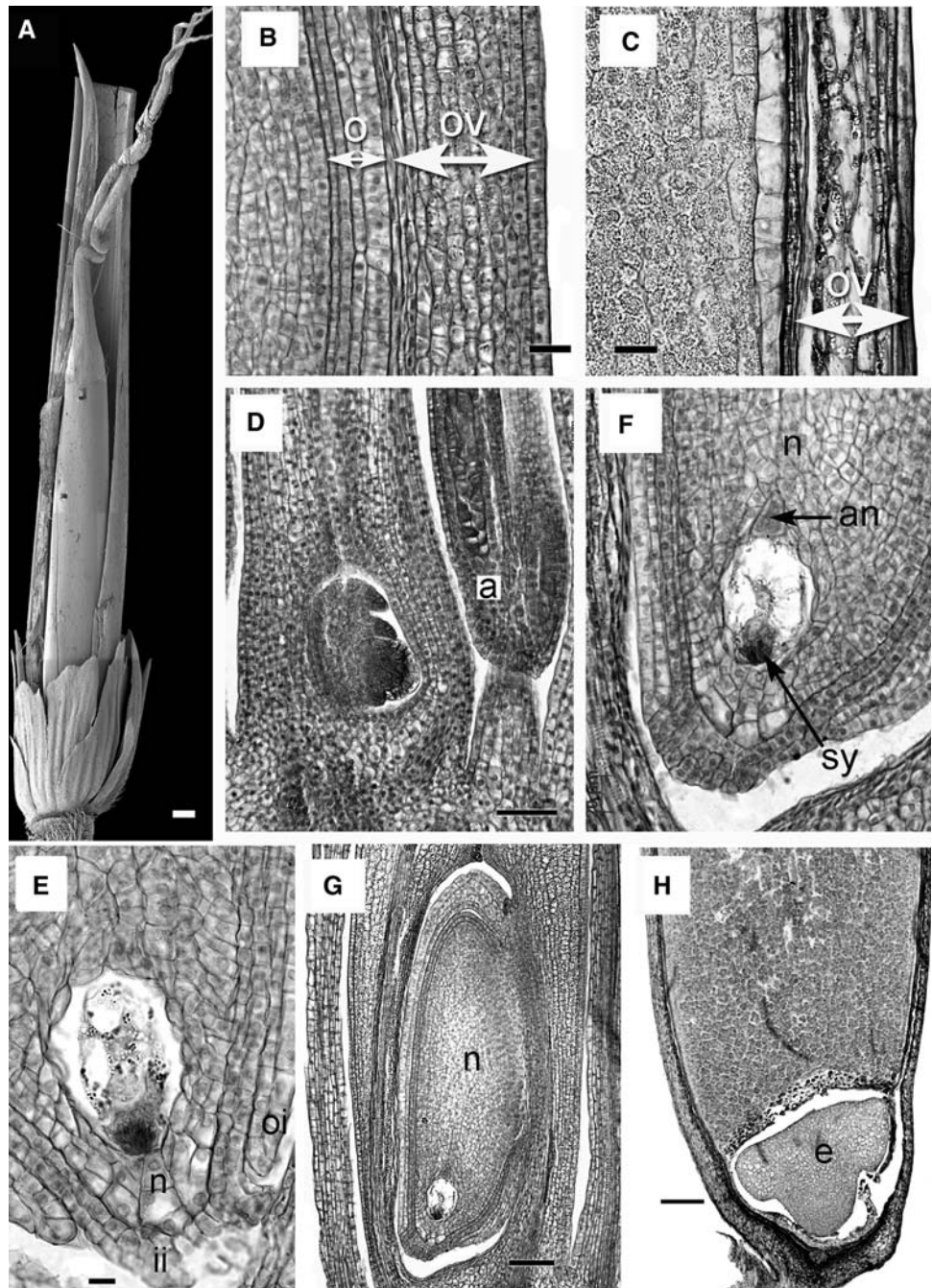
**Fig. 4** **a** Dissected pseudospikelet with anthers just protruding. **b** Mature spikelet. *a* anther, *f* fused filaments. Scale 1 mm

**Ovary and ovule development (Figs. 5, 6)**

The ovary is sessile and fusiform. The ovary wall is of approximately similar thickness throughout, though at the point of attachment to the pedicel the ventral side is thicker than the dorsal (Fig. 5g). The ovary wall consists of 7–8 cell layers including the outer and inner epidermis (Fig. 5b).



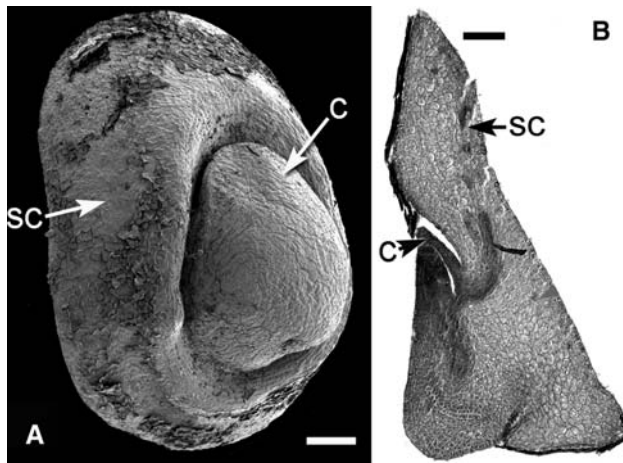
**Fig. 5** Caryopsis development. **a** SEM mature caryopsis in dissected pseudospikelet. **b** LM of longitudinal section of ovary and ovule wall. **c** LM of longitudinal section of caryopsis wall. **d** LM of longitudinal section of flower with young ovule and stamen. **e, f** LM of longitudinal section of embryo sac and micropyle. **g** LM of longitudinal section of ovule. **h** LM of longitudinal section of fruit with embryo. *a* anther, *an* antipodals, *e* embryo, *ii* inner integument, *n* nucellus, *o* ovule wall, *oi* outer integument, *ov* ovary wall, *sy* synergids. Scale 50  $\mu\text{m}$  in **a, d, e, g, h**; 20  $\mu\text{m}$  in **b, c**; 100  $\mu\text{m}$  in **f**



The single ovule arises in the basal position of the locule (Fig. 3a) but further growth leads to its curvature, becoming hemianatropous with its funiculus fused to the dorsal carpel wall (Fig. 5d). Subsequently, the ovule undergoes gradual curvature towards the base of the ovary, becoming anatropous at the megagametophyte stage (Fig. 5g). Following megagametophyte formation, the nucellus enlarges, especially at the chalazal region, where it is massive (Fig. 5g). At this stage, the region of ovular attachment to the placenta is more than three-quarters the length of the ovule, and there is no well-defined funiculus. Both integuments are initiated

simultaneously (Fig. 5d) and extend beyond the nucellus, though only the inner integument forms the micropyle (Fig. 5e). Both integuments are mostly two-layered, though the outer one becomes two to four-layered on the ventral side and five-layered at the chalazal end (Fig. 5g).

Inside the ovule primordium, a hypodermal archesporial cell gives rise directly to the megasporocyte without cutting off parietal cell (the tenuinucellate condition). The megasporocyte undergoes meiosis to form a tetrad of megaspores. The functional chalazal megaspore develops into a seven-celled, eight-nucleate megagametophyte with a



**Fig. 6** **a** SEM mature embryo. **b** LS mature embryo (LM). **c** coleoptile, **sc** scutellum. Scale 100  $\mu$ m

globular egg cell, two pear-shaped synergid cells with filiform apparatus (Fig. 5e, f), three antipodal cells (Fig. 5f), and a central cell with two polar nuclei.

Following fertilization, the primary endosperm nucleus divides earlier than the zygote (Fig. 5f), and endosperm development conforms to the “Nuclear type”. In the mature caryopsis the endosperm is large and the embryo is small, with an extensive free scutellum (Fig. 5h). Both cell layers of the outer integument and the outer cell layer of the inner integument become disorganized as the caryopsis enlarges. The cells of the inner layer of the inner integument enlarge anticlinally and show deposition of a densely staining material (Fig. 5c). As the caryopsis enlarges, these cells become sclerified first at the micropylar region and subsequently all round the seed. Both the outer and the inner pericarp epidermis remain intact and the cells of its median layers enlarge periclinally, resulting in a multi-layered tissue that together with the epidermal cells covers the seed coat, which is formed only by the inner layer of the inner integument (Fig. 5b–c). The mature caryopsis is enveloped by the coriaceous lemma, paleas and lodicules (Fig. 5a).

The mature embryo is about one-seventh the length of the caryopsis and has a large scutellum (Fig. 6). The vascular supply to the scutellum and embryonic leaves diverges from the common point and there is no epiblast. The coleoptile is a conical structure and poses a small opening on the side away from the scutellum and a small slit on opposite side.

## Discussion

Inflorescence and pseudospikelet morphology – a revised interpretation

Morphologists such as Celakovský (1889), Arber (1929), Page (1951) and Soderstrom (1981) have postulated

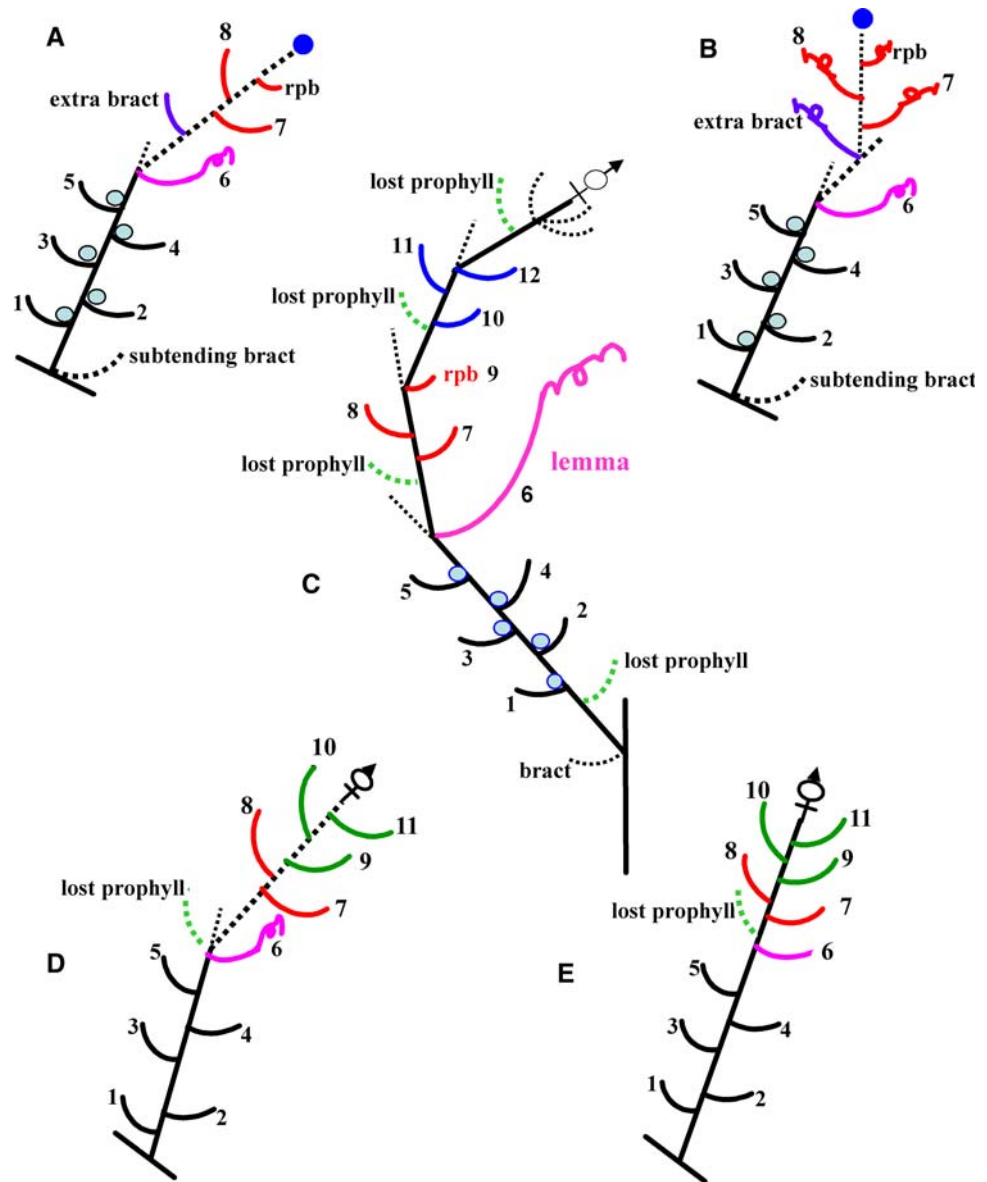
different hypotheses to interpret the *Streptochoaeta* spikelet (Fig. 7). Both Arber (1929) and Page (1951) reported axillary buds on bracts 1–5 (those attached to the basal node), and concluded that these bracts are fertile and homologous with the awned bract 6 (the lemma). Page (1951) also reported a “rudimentary palea bract” opposite the paleas (bracts 7–8). In two spikelets she found paleas with a short curled awn, and interpreted these structures as modified lemmas. She also reported an “extra bract” above and behind the paleas (bracts 7–8), and regarded the lodicules as homologous to the inner perianth of the flower, a view later supported by Whipple et al. (2007). Thus, Page (1951) interpreted the *Streptochoaeta* spikelet as possessing a basal branch with six potentially fertile bracts. Of these, bract 6 (the awned bract) subtends a branch that could be either (1) a complete flower without the anterior member of the outer perianth (represented by the “rudimentary palea bract”) with the “extra bract” representing either a lemma or a prophyll (Fig. 7a), or (2) a branch with two sterile bracts (the paleas, which she reinterpreted as lemmas, including the “rudimentary palea bract”) and a flower lacking the outer perianth, lemma and prophyll. In this case, the “extra bract” would be homologous to the lemma and the spikelet would represent a reduced compound branching system (Fig. 7b).

Based primarily on Page’s (1951) observations, Soderstrom (1981) agreed that the *Streptochoaeta* spikelet possesses five fertile bracts that are homologous to each other and to bract 6 (the lemma), and that bracts 7–8 (the paleas) and “the rudimentary palea bract” (bract 9) are on a different axis from bracts 1–6 and bracts 10–12. Assuming that the branching pattern repeats itself, he suggested that a flower lacking a perianth is on a different axis subtended by bract 12; the floral axis would be protected by bracts 10–12, and the prophyll (palea) and lodicules have been lost during evolution. Thus, Soderstrom (1981, page 41) interpreted the *Streptochoaeta* spikelet as a “highly modified branching system made up of three orders of pseudospikelet” (Fig. 7c).

However, contrary to the observations of Arber (1929) and Page (1951) on the same species – *Streptochoaeta spicata*, we found no axillary buds in the five basal bracts in any pseudospikelets. Thus, we found no evidence to support the basal node as an inflorescence branch. Also contrary to Page’s (1951) description, we did not find a “rudimentary palea bract”, nor an “extra bract”, nor an awned palea (bracts 7–8) in any spikelets. It is difficult to explain this significant discrepancy. Page (1951) observed material from 12 greenhouse-grown plants, of which six originated from a single collection and the other six from seeds produced by the first group; thus, the different structures that she observed could have resulted from an inherited teratology, possibly representing natural



**Fig. 7** Different interpretations of *Streptochaeta* pseudospikelet (see text). **a, b** Page (1951) described several features that were not observed here, including axillary buds on bracts 1–5, a “rudimentary palea bract” (rpb) opposite bracts 7–8, an “extra bract” above bracts 7–8 and awned bracts 7–8. She interpreted bract 6 as subtending either **a** a complete flower without the anterior member of the outer perianth (represented by the rpb), or **b** a branch with two sterile bracts (including the rpb) and a flower. **c** Soderstrom (1981) proposed that a flower lacking a perianth is in a different axis subtended by bract 12. **d, e**. Our observations suggest two further interpretations, in both of which bracts 1–5 of the basal node represent glumes. **d** A modified flower is borne on a different axis to the basal bracts, in the axil of bract 6 (the awned one). Bracts 7–8 represent the outer perianth with a missing inner member, and bracts 9–11 represent the inner perianth. This agrees with Celakovsky’s (1889) interpretation. **e** As in **d**, except the flower is borne on the same axis as the basal bracts, but on a different node. *rpb* rudimentary palea bract



variation. Our wild-collected material from several distantly located plants lacked such “abnormal” structures. Furthermore, we found no pseudospikelets with more than 11 bracts, despite reports of 12 bracts by some authors (Judziewicz et al. 1999; Whipple and Schmidt 2006; Whipple et al. 2007).

Our observations show that bract 6 (the awned bract) belongs, together with bracts 7–11, to a different node from the basal bracts (1–5), and is irrigated by the same vascular complex as the reproductive parts. Regarding ovule attachment, bract 6 always lies opposite the free side of the ovule, like the lemma in other grasses, though bract 6 is adaxial, contrary to the abaxial lemma of other grasses. In contrast to a “true” grass spikelet, with a single palea on the same side of the ovule attachment, the *Streptochaeta* pseudospikelet has two bracts in this position (bracts 7–8).

Although with a missing adaxial member, bracts 7–8 overlap each other in a clockwise spiral that lies in the opposite direction to the basal node bracts 4–5. Above bracts 7–8, bracts 9–11 also form a whorl overlapping each other in a counter-clockwise direction. The same arrangement (spirally overlapping whorls) of bracts 7–8 and 9–11 (in the same spiral) suggests that these two alternating cycles are borne on a different branch to the basal bracts (1–5), and lie in the axil of bract 6, though earlier ontogenetic stages are necessary to confirm this.

Our results suggest two further interpretations for the *Streptochaeta* pseudospikelet. In both the cases, bracts 1–5 of the basal node represent glumes; they are empty as are the glumes of grasses. (1) The *Streptochaeta* pseudospikelet contains a modified flower that is borne on a different axis to the basal bracts (1–5), in the axil of a lemma (the



awned bract 6). Bracts 7–8 represent the outer perianth with a missing member, and bracts 9–11 represent the inner perianth (Fig. 7d). (2) Alternatively, the *Streptochaeta* pseudospikelet contains a modified flower that is borne on the same axis as the basal bracts (1–5), but on a different node. Bracts 6–8 representing the outer perianth members, and bracts 9–11 the inner perianth members (Fig. 7e). In both the cases the abaxial prophyll is missing.

Our first (preferred) model (Fig. 7d) agrees with Celakovský's (1889) interpretation of the *Streptochaeta* spikelet. The second (Fig. 7e) agrees with Ambrose et al.'s (2000) interpretation of the grass lodicules as modified petals, and paleas and lemma as modified sepals. In either case, the *Streptochaeta* pseudospikelet is not a compound structure and cannot be termed a pseudospikelet as defined by Soderstrom (1981). Our interpretations of the *Streptochaeta* pseudospikelet are consistent with derivation of the grass flower from an ancestral monocot flower with a perianth containing two whorls of tepals, perhaps similar to the perianths of *Joinvillea* or *Ecdeiocolea* (Rudall et al. 2005), though this became highly modified with the origin of grasses (Zanis 2007). The highly condensed grass spikelet compares with the condensed pseudanthial inflorescences of other anemophilous Poales (Linder and Rudall 2005).

Bracts 7–8 were interpreted as halves of a single structure (the paleas) by Arber (1929). However, they originate from different primordia and are supplied by separate vasculature, though they eventually fuse to each other and to the lemma at later developmental stages. Paleas and lemmas are novel structures found only in grasses and have been interpreted as sepals or prophylls (Clifford 1987), though their homology to leaves, bracts, or perianth organs in other monocots remains uncertain (Arber 1929; Cocucci and Anton 1988; Rudall et al. 2005; Zanis 2007).

Contrary to most grasses, in which the lodicules are scalelike and function in opening up the floret (Clayton and Renvoize 1986), in *Streptochaeta* there are three coriaceous structures in the position of the lodicules. We interpret these as homologous to the inner tepals, in agreement with the Whipple et al. (2007)'s data, and with the interpretation postulated for different grasses (Celakovský 1889; Rowlee 1898; Arber 1929; Page 1951; Judziewicz and Soderstrom 1989; Ambrose et al. 2000; Rudall et al. 2005). We interpret the basal bracts of the *Streptochaeta* spikelet as glumes. The term "glume" is defined as a sterile bract at the base of the spikelet (GPWG 2001), and interpreted as a modified leaf sheath. Most grasses bear only two glumes, being abaxial, adaxial or lateral according to the grass group. In *Streptochaeta* there are five glumes spirally arranged on the basal node of the spikelet, the first two being attached adaxially.

The inflorescence of *Streptochaeta* is determinate as in other grasses (Kellogg 2001). Its axis bears an aborted bud

that is probably subapical, though we cannot rule out the possibility that it is a displaced apical bud. The *Streptochaeta* inflorescence probably originates in the same way as the inflorescence of Andropogoneae – *Coelorachis aurita* and *Heteropogon contortus* (Le Roux and Kellogg 1999), in which the inflorescence meristem gives rise to a single axis. However, spikelet development in *Streptochaeta* does not entirely conform to the patterns in Andropogoneae (Le Roux and Kellogg 1999) or *Pharus* (Sajo et al. 2007), in which the glumes are very young when the reproductive parts are initiated. In *Streptochaeta* the glumes (bracts 1–5) are completely formed at this stage.

#### Gynoecium morphology

The ovary of *Streptochaeta* closely resembles that of other grasses, including the early-divergent grass *Pharus*, in possessing a unilocular ovary supplied by three vascular bundles (Sajo et al. 2007). In both *Streptochaeta* and *Pharus* the ovary is initiated as three separate primordia, thus supporting Philipson's (1985) conclusion that the grass gynoecium is pseudomonocarpellary. However, *Streptochaeta* differs from *Pharus* in possessing a "solid" style with a stylar transmitting tissue, as in many other grasses (e.g. Arber 1934; Li and You 1991), rather than a "hollow" style as in *Pharus* and many other Poales, including *Ecdeiocolea* (Rudall et al. 2005). Since solid styles are relatively uncommon in Poales (though present in *Flagellaria*; Rudall, personal observation), the presence of a solid style could be a significant synapomorphy for grasses, though optimization could be ambiguous at the basal node.

#### Ovule and embryo sac

The length of ovule integuments varies between different species of Poaceae. In *Streptochaeta* both the integuments grow beyond the nucellus but only the inner forms the micropyle, whereas in some Bambusoideae and Ehrhartoideae the outer integument encloses at most two-thirds of the ovule (Bhanwra 1988; Bhanwra et al. 2001). Unitegmic and ategmic ovules occur in some Bambusoideae (Hari Gopal and Mohan Ram 1987). In *Streptochaeta*, the outer integument degenerates after fertilization, and the inner layer of the inner integument shows deposition of dark-staining material, as in *Pharus* (Sajo et al. 2007) and some Arundinoideae, Pooideae, and Chloridoideae (Bhanwra 1988). This layer produces a mechanical coat as the caryopsis develops.

As in the early-divergent grass *Pharus* (Sajo et al. 2007), the ovule is basal at early stages, and becomes anatropous at the megagametophyte stage. It is tenuinucellate, as in many other Poaceae and Poales (Aulbach-Smith and Herr

1984; Rudall 1997; Sajo et al. 2004) and the nucellus epidermis on the micropylar region undergoes periclinal divisions, as described for some grasses (Bhanwra et al. 1991, 2001). *Streptochaeta* also possess the plesiomorphic condition of three antipodals, in contrast to many other grasses, in which antipodals are proliferated.

#### Embryo and endosperm

The highly differentiated grass embryo possesses a prominent outgrowth termed a scutellum, which is normally interpreted as a modified cotyledon (Fig. 6). The scutellum is apparently unique to grasses, and is absent from related taxa such as *Ecdiocola* (Rudall et al. 2005). All grasses possess a scutellum, including other early-divergent taxa such as *Anomochloa* (Judziewicz and Soderstrom 1989) and *Pharus* (Sajo et al. 2007). In *Streptochaeta* the embryo is highly differentiated, and possesses a small cleft between the scutellum and the coleorhiza but lacks a distinct epiblast, as in the panicoid embryo type in Reeder's (1957) classification. However, there is no distinct elongation between the point of divergence of the scutellum bundle and the coleoptile, so in this respect it resembles Reeder's (1957) festucoid embryo type. The coleoptile possesses a small opening on the side away from the scutellum and a small slit on the opposite side (Fig. 6). The first opening is probably the point through which the first leaf of the plumule protrudes at germination. The second opening could represent the marginal attachment of the coleoptile, though Reeder (1953) suggested that the coleoptile is not closed, and homologised it to a leaf.

#### Conclusions

*Streptochaeta* possesses some features that are commonly found in Poaceae, such as a female gametophyte of the *Polygonum* type, nuclear endosperm and a well-differentiated embryo, and also some unusual (presumably derived) features. For example, in contrast to most wind-pollinated grasses, which have a reduced perianth and plumose stigmas, *Streptochaeta* possesses non-plumose stigmas, suggesting that it could be insect-pollinated, as suggested by Soderstrom and Calderón (1971) for some other tropical herbaceous grasses. The stamens mature before the gynoecium in most Poales, contrasting with the protogynous condition in *Streptochaeta*.

*Streptochaeta* also shares some features with *Pharus* (Sajo et al. 2007), perhaps representing the plesiomorphic conditions in these early-divergent grasses. For example, the gynoecium in *Streptochaeta* initiates as three primordial carpels, and is therefore clearly a pseudomonocarpellary structure, as in *Pharus*, supporting the hypothesis that even

when monoculular at maturity, the gynoecium of some grasses retains features of a tricarpellary ancestry (Philipson 1985; Rudall et al. 2005), though this feature is difficult to distinguish in some relatively derived grasses (Maze et al. 1971, Le Roux and Kellogg 1999). The basal position of the ovule early in ontogeny in *Streptochaeta* is unusual within Poaceae, but resembles the condition in *Pharus*.

Finally, *Streptochaeta* differs from other grasses in that its inflorescence unit lacks structures clearly homologous to glumes, lemma and palea, and is commonly interpreted as a compound branching system or pseudospikelet (Page 1951; Soderstrom 1981). We found no evidence supporting Page and Soderstrom's interpretation, and regard the *Streptochaeta* unit as a spikelet, as in other grasses, although it is highly modified. It could represent either a modified flower on the same axis as the basal bracts (glumes), or a modified flower on a different axis to the glumes. The spikelet in *Streptochaeta* and *Anomochloa* has been described as lacking lodicules and petals (Soderstrom 1981; Soreng and Davis 1998; GPWG 2001; Kellogg 2001; Zanis 2007), but we agree with the interpretation of Whipple and Schmidt (2006) and Whipple et al. (2007) that the three bracts below the stamens are homologous to the lodicules and correspond to the inner tepal whorl of outgroups. In *Anomochloa*, a ring of hairs in the position of the lodicules could represent modified lodicules (Arber 1929), though more detailed study of its spikelet is needed to clarify this controversial interpretation. The *Streptochaeta* spikelet possesses two bracts below the lodicules (or inner tepals), and in the same arrangement as them. These two bracts (7–8) together with the awned bract 6 could represent an outer tepal whorl, in which case fusion of bracts 7–8 would produce the single palea typical of a true grass spikelet. In this respect the *Streptochaeta* spikelet could be considered as morphologically intermediate between the true spikelets of grasses and reproductive units of close grass relatives.

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