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A quantitative study of styles and achenes of terminal and basal flowers of Schoenoplectus hallii (Cyperaceae), a rare plant species of transient wetland habitats

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A QUANTITATIVE STUDY OF STYLES AND ACHENES OF TERMINAL AND BASAL FLOWERS OF *SCHOENOPLECTUS HALLII* (CYPERACEAE), A RARE PLANT SPECIES OF TRANSIENT WETLAND HABITATS

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ABSTRACT

Schoenoplectus hallii (A. Gray) S.G. Smith (Cyperaceae), a rare plant restricted to wetland habitats, is of conservation concern throughout its range. Taxonomy of the species recently has been clarified; however, quantitative descriptions of achene and flower morphology are incomplete and life history information is lacking. Because of its scarcity and the transient nature of populations, any large-scale study of the species will require the recovery of achenes from bulk soil samples and the identification and separation of the dimorphic achenes. The objectives of this study were to separate, identify and photograph the two achene types; to quantify the size and morphological differences that will be useful in separating terminal and basal achenes; and to determine the range of variability in style morphology and achene size within and among 12 populations in four states. Although each achene type varies significantly in size among populations, size differences between terminal and basal achenes are statistically significant, and the range of sizes within each achene type is larger than has been previously reported. Terminal achenes are significantly smaller in length, width, beak length, mass and surface area than basal achenes, and noticeable differences occur in surface ridging. Differences in style morphology are distinct: terminal flower styles are predominantly bifid and consistent in shape, while basal flower styles, which are six times longer than terminal styles, are trifid with a wide variety of branching patterns. Terminal and basal achenes can be separated accurately and conveniently from bulk soil samples using a series of soil sieves. The visual and quantitative descriptions provided in this study will facilitate the collection and identification of terminal and basal achenes of *S. hallii* from plants, soil and wildlife.

RESUMEN

Schoenoplectus hallii (A. Gray) S.G. Smith (Cyperaceae), una planta rara restringida a hábitats de lugares húmedos, es para ser conservada en toda su área. La taxonomía de la especie se ha clarificado recientemente; sin embargo, las descripciones cuantitativas del aquenio y la morfología floral son incompletas y falta información de su ciclo vital. Debido a su escasez y a la naturaleza transitoria de sus poblaciones, cualquier estudio a gran escala de la especie requerirá la recuperación de aquenios a partir de grandes muestras de suelo y un método adecuado de identificación y separación de los aquenios dimórficos. Por lo tanto, los objetivos de este estudio fueron separar, identificar y fotografiar los dos tipos de aquenio; cuantificar el tamaño las diferencias morfológicas que serán útiles en la separación de los aquenios terminales y basales; y determinar el rango de variabilidad en la morfología del estilo y tamaño del aquenio en y entre 12 poblaciones en cuatro estados. Aunque cada tipo de aquenio varía significativamente en tamaño entre poblaciones, las diferencias de tamaño entre aquenios terminales y basales son significativas estadísticamente, y el rango de tamaño para cada tipo de aquenio es mayor de lo que se había indicado previamente. Los aquenios terminales son significativamente más pequeños en longitud, anchura, longitud el pico, masa y área de su superficie que los aquenios basales, y hay diferencias notables en las costillas de la superficie. Las diferencias en la morfología del estilo son distintas: los estilos de las flores terminales son predominantemente bífidos y de forma constante, mientras que los estilos de las flores basales, que son seis veces más largos que los estilos de las flores terminales, son trífidos con una amplia variedad de patrones de ramificación. Los aquenios terminales y basales pueden separarse con precisión y de un modo práctico a partir de muestras de suelo voluminosas usando una serie de cribas para suelos. Las descripciones visuales y cuantitativas aportadas en este estudio facilitarán la recolección e identificación de aquenios terminales y basales de *S. hallii* a partir de plantas, suelo y de la naturaleza.

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INTRODUCTION

Schoenoplectus hallii (A. Gray) S.G. Smith is a member of the family Cyperaceae. Asa Gray (1863) described the species as *Scirpus hallii* A. Gray, based on specimens collected in Illinois. Recent work by Smith (1995) and Smith and Yatskievych (1996), however, has emphasized the breakup of the polymorphic supergenus *Scirpus* sensu lato and resulted in the acceptance of *Schoenoplectus hallii* as the appropriate binomial for the species (Smith 1995, 2002).

The known distribution of *S. hallii* prior to 1973 extended from Massachusetts to Wisconsin and Iowa on its northern boundary, westward to Kansas and Nebraska, and south to Georgia. The number of states in which populations existed declined from nine in 1973 to six in 1997 (McKenzie 1998). It has apparently been extirpated from Massachusetts and Georgia, and is now restricted to the Midwest. In 2000, *S. hallii* was reported from 14 sites in Oklahoma (Magrath 2002); however, the conservation status of the species at these sites is unclear due to the recent discovery of putative hybrids (*S. hallii* × *S. saximontanus*) at some of the sites (Smith et al. 2004). In 2002, surveys for *S. hallii* revealed new populations in several states, including Indiana (M. Homoya, pers. comm.) and Missouri (pers. obs.) and Texas (O'Kennon and McLemore 2004). In years not suitable for germination, the species may persist only in the seed bank (McKenzie 1998); therefore, it is difficult to obtain accurate estimates of the number of viable populations in any given year. Although population number and geographical distribution are not clearly defined, *S. hallii* is considered to be of conservation concern in every state in which it occurs (Beatty et al. 2004).

Schoenoplectus hallii is restricted to wetland habitats (Swink & Wilhelm 1994; McClain et al. 1997) in areas characterized by fluctuating water levels (Ostlie 1990; Ostlie & Gottlieb 1992; Robertson et al. 1994). The species is thought to have a persistent seed bank (Ostlie 1990; Ostlie & Gottlieb 1992; Robertson et al. 1994) that may contribute to its potential for population regeneration. Achenes germinate sporadically, depending on the availability of wet, exposed habitat (McKenzie 1998), resulting in wide fluctuations in population number and size from year to year (Chester 1988; Robertson et al. 1994, McClain et al. 1997). It has been suggested that because of loss of suitable wetland habitat, management of *S. hallii* sites is necessary to ensure the species' continued existence (Bowles et al. 1990; Robertson et al. 1994).

Schoenoplectus hallii exhibits amphicarpy (Bruhl, 1994), as do other members of the section *Supini* (i.e., *S. erectus* and *S. saximontanus* in North America), and achenes produced in multi-flowered spikelets near the tip of stems (hereafter designated as "terminal" achenes or flowers) have been illustrated and/or described by various authors (Gleason & Cronquist 1963; Mohlenbrock 1963; Steyermark 1963; Radford et al. 1964; Mohlenbrock 1976; Kolstad 1986; Yatskievych 1999; Smith 2002). Although achene size is estimated in the taxonomic literature, and varies among authors, a quantitative measure of variation in achene size from a representative sample of populations across the range of the species is lacking. Basal achenes, which are produced by solitary pistillate flowers enclosed within the leaf sheaths at culm bases, are less adequately illustrated and described than terminal achenes. With the exception of a photograph of one basal achene (Schuyler 1969) no photographs or illustrations have been published. In addition, no quantitative description of variation in basal achene size within and among populations exists. Quantitative data establishing the size and morphology of the basal achenes are important for the correct separation and identification of seed bank components and are essential for the development of life history studies that estimate the relative reproductive contribution of basal achenes to population size.

In early summer before achenes have been produced, style lobe number is the morphological character most useful in separating *S. hallii* from *S. saximontanus*, because of their similar vegetative appearance. As far as we are aware, there has been no large-scale study of style size and morphology in *S. hallii*. Recently, *S. hallii* and *S. saximontanus* were found growing in mixed populations in Oklahoma (Magrath 2002); therefore, it would seem prudent to determine if style lobe number is constant throughout a number of widely distributed populations of *S. hallii* in areas where *S. saximontanus* is not sympatric to confound the issue.

The objectives of this study were **1)** to develop a simple procedure for separating, collecting and identifying achenes from bulk soil samples; **2)** to photograph the two achene types; **3)** to quantify the size and morphological differences that will be useful in separating terminal and basal achenes; and **4)** to determine the range of variability in style morphology and achene size within and among 12 populations in four states (IL, IN, KY, and MO).

MATERIALS AND METHODS

When possible, living plants were collected *in situ* and transferred to the greenhouse at Southern Illinois University, Edwardsville, IL. Plants were potted in a 75%-sand:25%-potting soil mixture in 10 × 10 × 11 cm pots, covered with a plastic bag to decrease transpirational water loss, and placed in flats in 5 cm of standing water. The organic content of the mixture (%OM = 2.5%) was approximately that of soil at the study sites (mean %OM = 2.6±0.5%, analyses conducted by Alvey Labs, Belleville, IL). Plants were given identifying numbers and mature terminal achenes (and basal achenes, when present) were collected and saved for measurement. Otherwise, basal achenes were collected as they matured.

Site names, acronyms, approximate locations (exact locations are not listed to protect population sites) and dates of collection are listed in Table 1. At three sites that were without plants (SP, FO and KY), but were known to have had populations of *S. hallii* within the past 5 years, soil cores were collected to provide a seed source. In all cases, plants or cores were collected at regular intervals along transects from across the known extent of the population. To ensure accurate identification of achene types, initial collections were of terminal and basal achenes that were attached to the parent plant. After achene identification was established, others were collected from soil samples. Whenever possible, pairs of terminal and basal achenes were selected, using a random number table, from 15 plants and photographed, measured and weighed in the laboratory. For sites without plants, soil sieves (U.S. Standard by Fisher Scientific Co) #16 (1.18 mm mesh), #18 (1.00 mm) and #20 (0.841 mm) were used to separate basal and terminal achenes. Identification was verified using a dissecting microscope. As some achenes that appeared to be healthy and mature fragmented when pressed between the thumb and forefinger, all achenes were given this preliminary test before being measured (Baskin et al. 2003).

Achene photographs were made using a Nikon CoolPix 995 digital camera mounted on a Nikon dissecting microscope (Model SMZ800) fitted with a fiber optic ring-light. Length, width, and beak measurements were taken at a magnification of 40X using a Leitz compound microscope (Model Laborlux S) and a Bausch and Lomb ocular micrometer that was calibrated with a Leitz stage micrometer. Achene beak length was measured from the point where tangential lines drawn at the top of the achene and alongside the

TABLE 1. Names, acronyms and county and state locations of 12 *Schoenoplectus hallii* sites and types of samples collected for this study.

Site	State	County	Date and Sample Collected
Ebken (EB)	IL	Mason	2000 Plants and soil
Fornoff (FO)	IL	Mason	2000 Soil only
Sand Pond (SP)	IL	Mason	2000 Soil only
Wemker (WK)	IL	Mason	2000 Plants and soil
Indiana (IN)	IN	Daviess	2002 Plants and soil
Kentucky (KN)	KN	Christian	2000 Soil only
Baptist Camp (BC)	MO	Scott	2000 Plants and soil
Howell County (HC)	MO	Howell	2002 Plants and soil
Petite Isle (PI)	MO	Scott	2002 Plants and soil
Sherer (SH)	MO	Scott	1999–2001 Plants and soil
Waterman (WM)	MO	Scott	2002 Plants and soil
West Vaco (WV)	MO	Scott	2002 Plants and soil

beak intersected (Fig. 1). As beak length varied considerably, but contributed little to achene area, achene surface area was calculated as width × (achene length–beak length). Mass was determined using a Mettler analytical balance (Model AT 261 Delta Range). To illustrate the contrast in size, one pair of terminal and basal achenes were photographed with attached styles (Fig. 2A). To illustrate variability in basal and terminal achene morphology, three of each achene type were photographed in the same field-of-view (Fig. 2B). Also, three achenes of each type were photographed in cross-section (Fig. 2C) and one pair of achenes was photographed showing the achenes in frontal (Fig. 2D) and profile (Fig. 2E) views.

Styles were excised from flowers of each type, floated on water and measured. For statistical analysis, measurements were made on terminal styles from 15 plants from eight populations, but for basal styles, which are scarce and difficult to collect intact, measurements were limited to 15 plants from one population. Others from eight additional populations were observed for style morphology, but not measured. To illustrate some of the variation in style morphology observed in basal flowers, drawings were made to scale (Fig. 3B–D).

When data sets for terminal and basal achenes were combined for analysis, variances were significantly different ($P<0.001$) between achene types for all dimensions; therefore, analysis by two-way ANOVA was rejected and data for each achene type were analyzed separately by one-way ANOVA. Equal variance was verified using Levene's Test and data were log transformed, when necessary, to ensure normal distribution. Comparisons of mean values for achene characteristics among sites were determined by Bonferroni's t-test. Terminal style measurements, for which data could not be successfully transformed, were analyzed by Kruskal-Wallace one-way ANOVA on ranks, followed by Tukey's t-test for comparison of means. Pooled differences in dimensions between achene types were determined by t-test, assuming unequal variances. When matched pairs of terminal and basal achenes were available within a population, statistical comparisons were made using a paired-samples t-test. All statistical procedures were calculated using SPSS 11.4 (SPSS, Inc. 2002) and are in accordance with Sokal and Rohlf (1981). Principle ANOVA statistics, means, and P-values) were reported when differences were statistically significant.

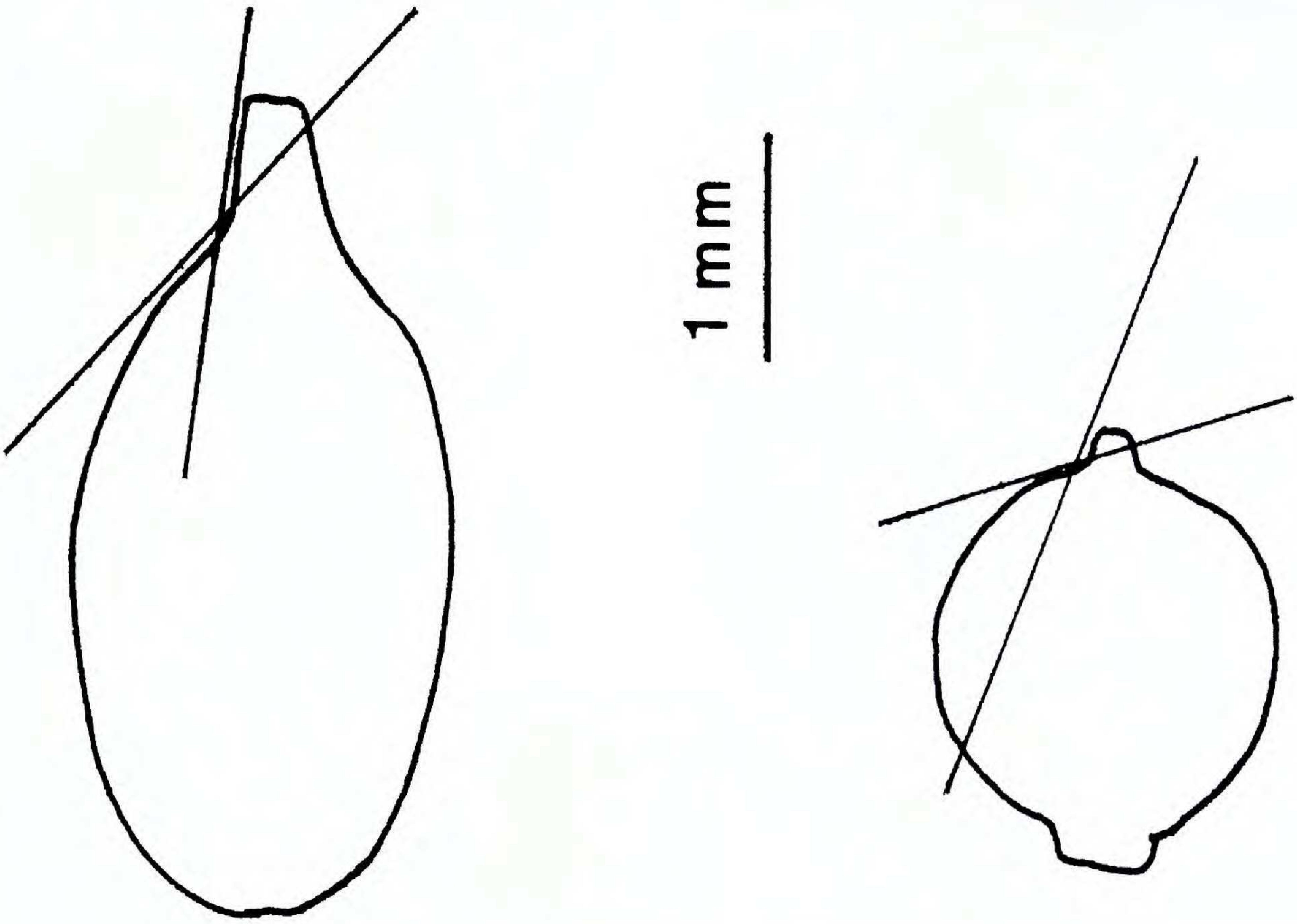

Fig. 1. Method for determining beak length in basal (left) and terminal (right) achenes of *Schoenoplectus hallii*. Drawn to scale as indicated in the figure.

RESULTS

Comparisons among populations

Terminal achenes.—Terminal achenes varied significantly in every dimension (length, width, beak length, mass and surface area) among populations (Table 2, Fig. 4A–E). Achenes from HC were significantly larger than those from all other populations, with the exception of beak length (Table 3). There was a wide range in terminal achene size and mass (Table 4), even with the removal of the extremely large mean values for HC achenes, which, when included, raised the upper limit for every character.

Terminal styles.—All terminal styles examined were bifid, with the exception of the one 4-parted style from HC. There was a much wider range in style length compared to the range for any achene character (Table 4). There were significant differences in style length among populations (Table 2): the styles of the WK population were longer than those from any other site and the styles from the SH site significantly longer than all except those from WK ($P<0.05$). There were no significant differences in style lengths among the other six sites.

Basal achenes.—Basal achenes were more varied in shape than terminal achenes (Fig. 2B); however, there were significant differences among populations for every basal achene character measured (Table 5). Unlike the case with terminal achenes, in which those from HC were significantly larger than those from all other sites, no single population had consistently larger basal achenes (Fig. 5A–E, Table 6).

Comparisons between terminal and basal achenes and styles.—Pooled means for every

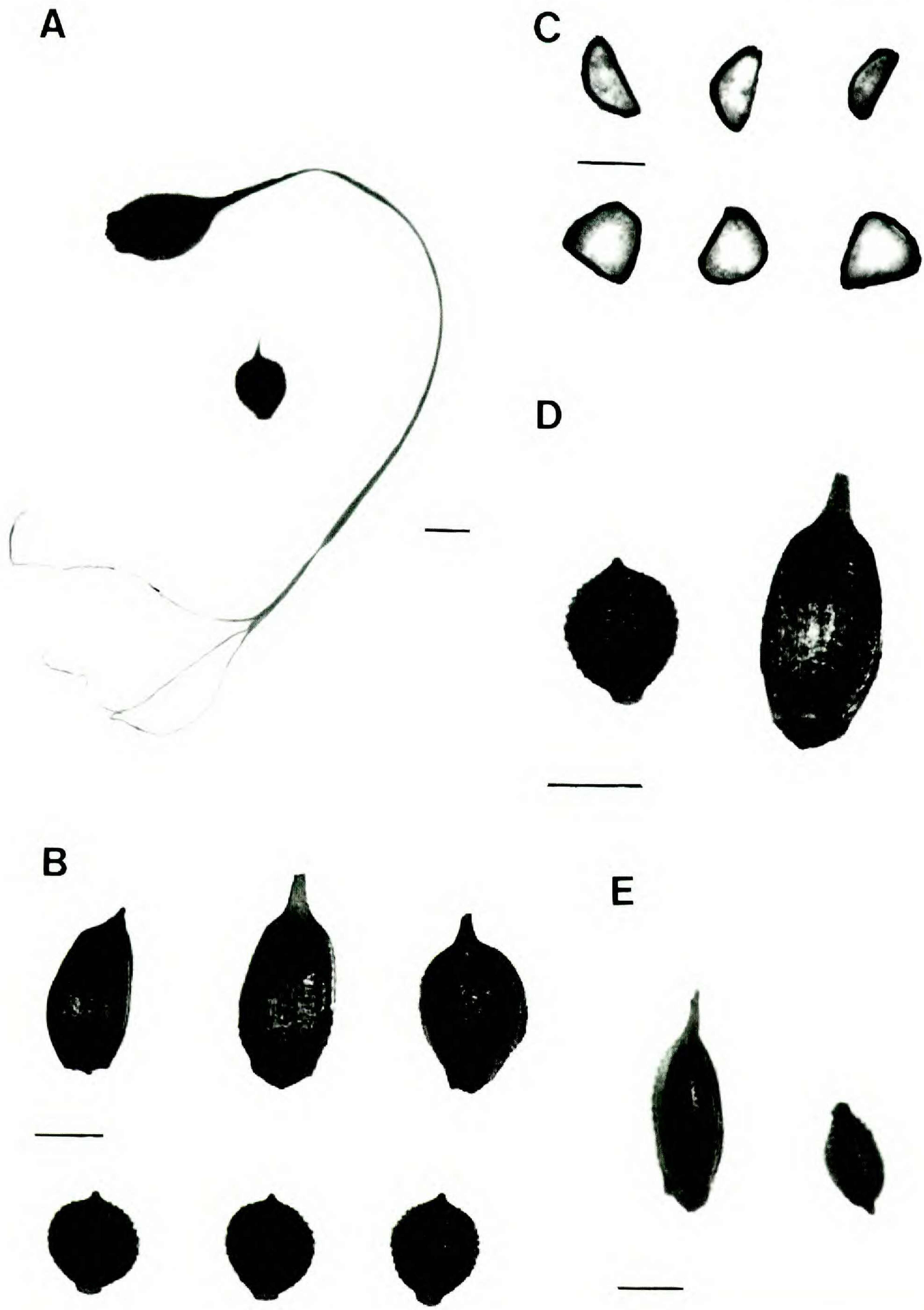

Fig. 2 A–E. A. Basal (top) and terminal achenes of *Schoenoplectus hallii* with attached styles; B. adaxial view of basal achenes (top) and terminal achenes of *S. hallii*; C. cross sectional view of terminal (top) and basal achenes of *S. hallii*; D. close-up of terminal (left) and basal achenes of *S. hallii*; and E. profile view of basal (left) and terminal achenes of *S. hallii*. Bars on all photographs are 1 mm in length.

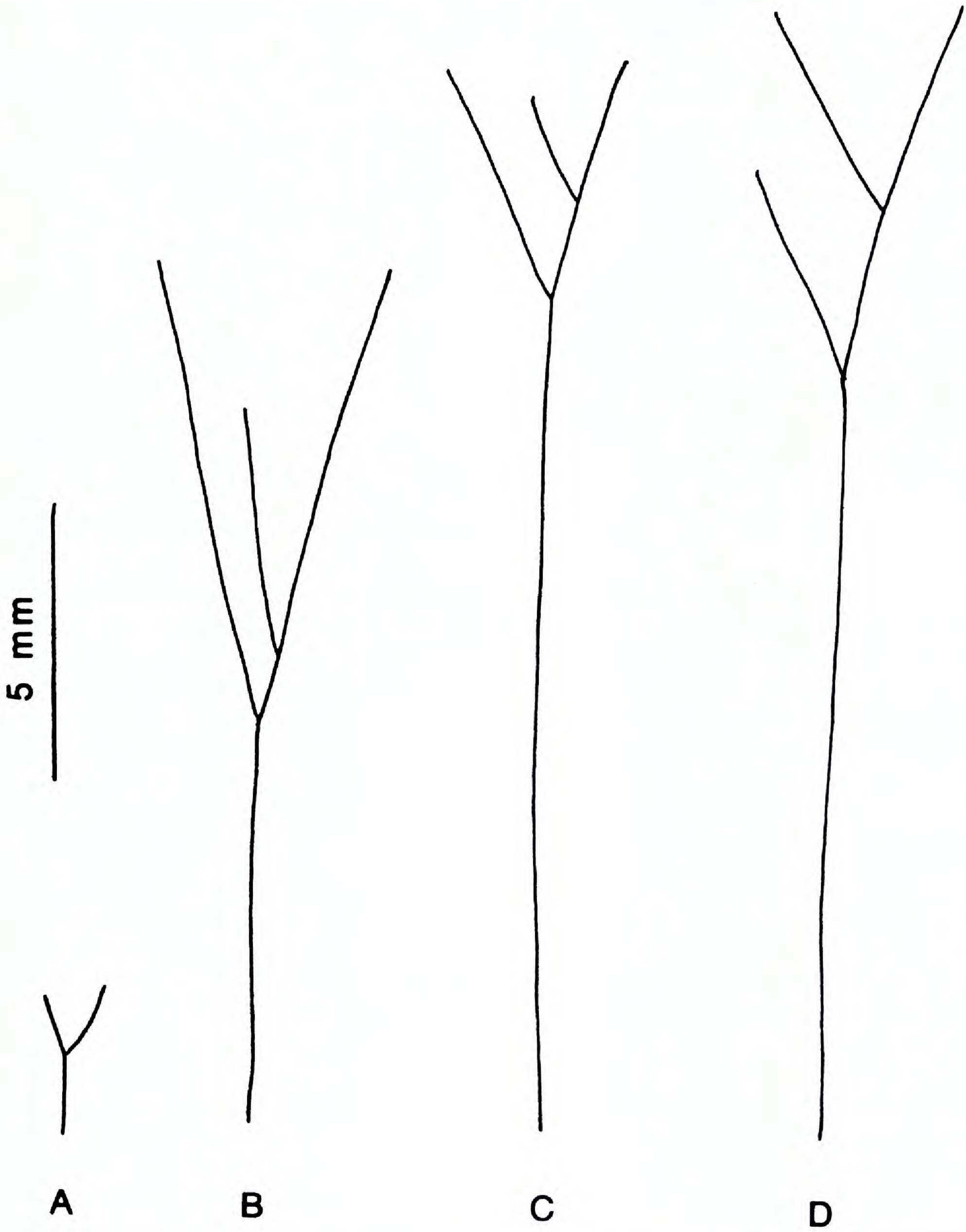

Fig. 3 A–D. Representative styles of A. terminal and B–D. basal flowers of *Schoenoplectus hallii*.

dimension measured were significantly larger for basal compared to terminal achenes (Table 4, $P<0.001$, df = 289 for each comparison). Within-population comparisons for the nine sites for which achene pairs were available indicated that in seven populations (EB, WK, IN, BC, SH, WM, WV), basal achenes were significantly larger for all dimensions. At HC, area was not significantly different between terminal and basal achenes ($P\leq0.536$, df

TABLE 2. Summary ANOVA statistics for terminal achenes and styles of *Schoenoplectus hallii* from 12 population sites.

Character	Source of Variation	SS	df	MS	F	*P*
Length	Between pops	2.457	11	0.223	28.656	<0.001
	Within pops	1.310	168	0.008		
Width	Between pops	3.241	11	0.295	45.384	<0.001
	Within pops	1.091	168	0.006		
Beak length	Between pops	0.056	11	0.005	10.136	<0.001
	Within pops	0.085	168	0.001		
Area	Between pops	23.54	11	2.132	61.447	<0.001
	Within pops	5.829	168	0.035		
Mass	Between pops	4.092	11	0.372	26.263	<0.001
	Within pops	2.380	168	0.014		
Style	Between pops	24.163	11	3.452	15.980	<0.001
	Within pops	27.193	168	0.216		

= 11), and at PI, width for terminal and basal achenes did not differ significantly ($P \leq 0.348$, df = 14).

All terminal achenes examined had prominent transverse ridging extending the width of the achene surface as recently reported for *S. hallii* achenes from Oklahoma populations (Magrath 2002). In contrast, transverse ridging was less conspicuous and did not extend the entire width of basal achenes, which had obvious vertical ridges. The cross-sectional shape of achenes was generally as described by Yatskievych (1999) ("unequally biconvex in cross-section, sometimes slightly concave on 1 side" and "unequally 3-angled," for terminal and basal achenes, respectively); however, both achene types occasionally varied from these descriptions. Some terminal achenes were plano-convex as illustrated in Figure 2 C.

Styles of basal flowers were approximately 6 times longer than those of terminal flowers (Fig. 2A, Table 4), and exhibited a wide variety of structural morphologies. Styles of all terminal flowers examined were bifid (Fig. 3), with the exception of one style from HC that had four style lobes (not shown). All styles from basal flowers were trifid with a variety of branching patterns, some of which are illustrated in Fig. 3B–D.

DISCUSSION

A.C. Martin, a U.S. Fish and Wildlife Service biologist stated that when identifying achenes encountered in the stomachs of wildlife, his staff found the description of *Scirpus* achenes in the taxonomic literature was unhelpful in separating species (Martin 1943). Although he stated that achenes of some species are distinctive enough to present no problem in identification, "the difficulty and danger involved in attempting to distinguish some of the other species important to wildlife frequently necessitated noncommittal conservatism." These troublesome species included what is now known as *Schoenoplectus hallii*, and, although descriptions of its achenes are far more comprehensive now than in 1943, no taxonomic reference adequately describes the variation of achene size and shape existing within the species. Our data clarify and illustrate the distinctions between achene types and expand the range of achene dimensions cited in the current published literature. While several publications (Beetle 1942; Magrath 2002; Schuyler 1969; Smith & Yatskievych 1996; Smith 2002) stated clearly that *S. hallii* exhibits amphicarpy, many

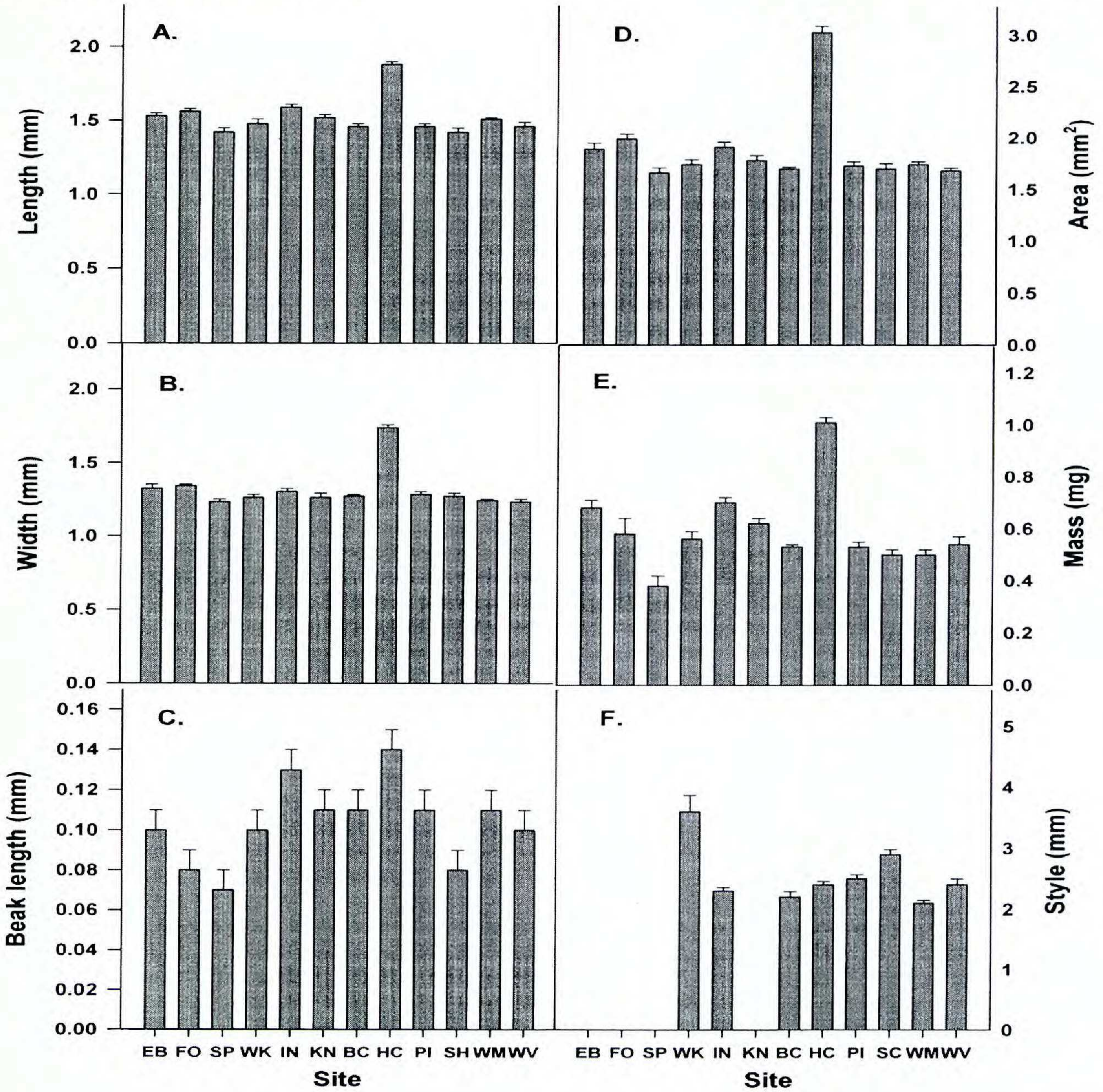

FIG. 4 A–F. A. Length, B. width, C. beak length, D. area, and E. mass of terminal achenes of *Schoenoplectus hallii* from 12 populations, and F. style length of terminal flowers of *S. hallii* from 8 populations. Each bar represents the mean ± SE of 15 measurements.

did not (Mohlenbrock 1963; Radford et al. 1964; Steyermark 1963; Kolstad 1986; Rolfsmeier 1995). Of those that recognize variation in floral types, only three provided estimates of basal achene size (Schuyler 1969; Yatskievych 1999; Smith 2002), and none presented data to quantify differences between terminal and basal achenes, nor any measure of the variance in size within achene type across a range of populations.

Although there is general agreement between our achene-size data and published reports, there are notable differences. With the exception of the three cases where size of basal achenes was specifically addressed (i.e., Schuyler 1969; Yatskievych 1999; Smith 2002), we assumed that all published estimates referred to terminal achenes. Six authors listed the following estimates of length for terminal achenes: 1.3–1.7 mm (Yatskievych 1999); 1.5–2.0 mm (Mohlenbrock 1976); "up to" 1.5 mm (Kolstad 1986); 1.3–1.5 mm (Gleason & Cronquist 1963); "less than" 0.4 mm (Robertson et al. 1994); and 1.3–1.7 mm (Smith 2002). There are three estimates of achene width in the literature: 1 mm (Beetle 1942),

TABLE 3. Significant differences in mean length, width, beak length, beak length and mass of terminal achenes among 12 populations of *Schoenoplectus hallii*. All *P* values calculated by Bonferroni's t-test.

Length	P	Beak	P	Area	P	Width	P	Mass	P
HC vs All	<0.001	IN vs FO	<0.001	HC vs All	<0.001	HC vs All	<0.001	HC vs All	<0.001
FO vs SP	<0.001	vs SP	<0.001	FO vs SP	<0.001	SH vs SP	≤0.014	SP vs All	≤0.040
vs SH	≤0.002	vs SH	<0.001	vs WK	≤0.019	SH vs WV	≤0.018	IN vs BC	≤0.007
IN vs SP	<0.001	HC vs EB	≤0.002	vs BC	≤0.002	–	–	vs PI	≤0.009
vs BC	≤0.006	vs FO	<0.001	vs PI	≤0.016	–	–	vs SH	<0.001
vs SH	<0.001	vs SP	<0.001	vs SH	≤0.002	–	–	vs WM	<0.001
vs PI	≤0.018	vs WK	≤0.003	vs WM	≤0.002	–	–	–	–
vs WV	<0.011	vs SH	,0.001	vs WV	,0.001	–	–	–	–

TABLE 4. Pooled means ± SE and range of values for length, width, beak length, area and mass for terminal (T) and basal (B) achenes, and styles of terminal achenes of *Schoenoplectus hallii* from 12 population sites. *Omitting extreme values from Howell County **Mean values of basal styles from SH only.

	T Mean	T Range	T Range*	B Mean	B range
Length	1.52±0.01	1.14–2.7	1.14–1.76	2.30±0.03	1.20–3.11
Width	1.32±0.02	0.88–1.88	0.88–1.50	1.43±0.01	1305–1.93
Beak length	0.11±0.00	0.04–0.22	0.04–0.18	0.34±0.03	0.11–0.90
Area	1.88±0.03	1.12–3.32	1.12–2.42	2.81±0.04	1.64–4.32
Mass	0.60±0.01	0.23–1.13	0.23–0.95	1.68±0.04	0.85–2.83
Style	3.25+0.06	1.30–6.00	1.30–6.00	17.07±0.89**	12.00–22.00**

1.2–1.5 mm (Radford et al. 1964) and 1.2–1.3mm (Smith 2002). With the exception of the measurement reported by Robertson et al. (1994), which undoubtedly refers to beak length, as previously stated in Schuyler (1969), rather than achene length, all measures of terminal achenes are within a reasonable range; however, the incomplete dimensions given in any single source create uncertainty in identification or confirmation of achene identity. As we have demonstrated, size can vary significantly between sites, and in exceptional cases (e.g., HC) the variation is so extreme that the achenes of *S. hallii* could be misidentified if size, as described in the existing taxonomic literature, was included in the criteria for identification. As it is not customary for authors of taxonomic literature to indicate the sample sizes upon which their estimates are made, it is possible that some are based on a single achene, although most cite a range in size, which implies more than one measurement. In any case, it is time that a clearer delineation of the range of dimensions in achenes becomes available in the published literature.

Relying solely on information in the taxonomic literature, it is possible that someone examining the seed bank of *S. hallii* could fail to recognize basal achenes as belonging to the species, and thus underestimate the potential population size or the contribution of basal achenes to it. Only three authors described basal achene size: Yatskievych (1999) states that they are "slightly larger" than terminal achenes, Smith (2002) stated a range from 1.7–2.5 mm long and 1.0–1.3 mm wide, and Schuyler (1969) published a photograph of one terminal and basal achene pair. While the photograph is the most useful representation of the appearance of basal achenes currently available in the published literature, neither the verbal nor the visual description is adequate to understand the

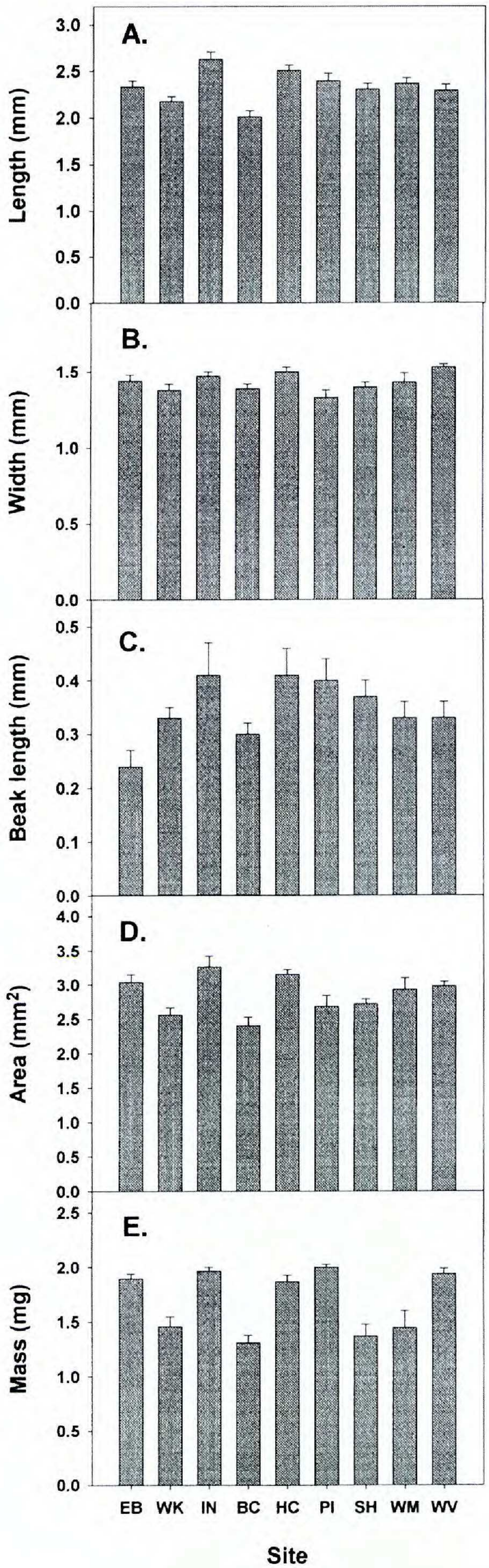

Fig. 5 A–E. A. Length, B. width, C. beak length, D. area, and E. mass of basal achenes of *Schoenoplectus hallii* from 9 populations. Each bar represents the mean ± SE of 15 measurements.

Table 5. Summary ANOVA statistics for basal achenes of *Schoenoplectus hallii* from nine population sites.

Character	Source of Variation	SS	df	MS	F	P
Length	Between groups	2.5755.929	8	0.322	5.537	<0.001
	Within populations		102	0.058		
Width	Between groups	0.4141.969	8	0.052	2.680	0.010
	Within populations		102	0.019		
Area	Between groups	0.3411.401	8	0.043	3.106	0.004
	Within populations		102	0.014		
Area	Between groups	7.246	8	0.906	5.416	<0.001
	Within populations	17.066	102	0.167		
Mass	Between groups	8.584	8	1.068	11.204	<0.001
	Within populations	9.727	102	0.096		

Table 6. Significant differences in mean length, width, mass and beak length of basal achenes among nine populations of *Schoenoplectus hallii*. All *P* values calculated by Bonferonni's t-test.

Length	P	Mass	P
BC vs IN	≤0.003	BC vs EB	<0.001
Vs HC	<0.001	vs IN	≤0.014
vs PI	<0.001	vs HC	<0.001
Width	*P*	vs PI	<0.001
PI vs WV	≤0.009	vs WV	<0.001
Beak	*P*	PI vs SH	<0.001
EB vs HC	≤0.006	vs WV	<0.001

magnitude and constancy of the differences between achene types, nor the potential of using size to separate achenes. Mean size and mass data, including values for the unusually wide terminal achenes at the HC population, indicate that basal achenes average 1.5× longer, 1.1× wider, 1.5× greater in area and 2.8× heavier than terminal achenes (Table 4).

Although data in the present study indicated that there was significant variation in morphology among basal achenes, they easily can be distinguished from terminal achenes, and at any site lacking *S. saximontanus* or *S. erectus* they can be identified easily to species. Except for the mixed populations in OK and KS, there are no known sites where *S. hallii* co-occurs with the species with which its basal achenes might easily be confused. Although we processed from 20–100 soil cores from each of the 12 sites to determine the potential seed bank for *S. hallii*, we did not find any mature achenes of any other species that resembled *S. hallii* basal achenes.

Various authors have noted that transverse or horizontal ridges are present on the surface of achenes of *S. hallii* (Beetle 1942; Gleason & Cronquist 1963; Magrath 2002; Radford et al. 1964; Schuyler 1969; Mohlenbrock 1976; Kolstad 1986; Robertson et al. 1994; Rolfsmeier 1995; Yatskievych 1999). Our observations confirm the presence of prominent horizontal ridges on all terminal achenes examined; however, they are absent or incomplete on basal achenes, which have conspicuous vertical ridges. Although prominent horizontal ridges are limited to terminal achenes, this character is universally attributed to achenes in *S. hallii*.

Cross-sectional shape is often used to separate terminal achenes in *S. hallii*, described as "unequally biconvex in cross section" by Yatskievych (1999), from terminal achenes in

S. saximontanus, which have a distinctly 3-angled shape (Yatskievych 1999). Our study corroborates the general regularity of this feature in *S. hallii* throughout the 12 populations studied; however, as is the case with basal achenes, there are exceptions (Fig. 2C).

Because all published studies of *S. hallii* are confined to the species' taxonomy or occurrence (distribution and rarity or abundance in various regions), which required no large-scale seed collection, no authors have suggested any methods for separating achenes from bulk soil samples. The results of our study indicate that it is possible to separate terminal and basal achenes from soil samples using a series of soil sieves. Surface litter and a large soil fraction can be separated from achenes by using sieves of various sizes, depending upon the type of soil and the nature of the litter. For example, if a sample contains considerable extraneous organic matter, one can use a #5 sieve to remove litter, a #14 sieve to remove coarse particles if the soil is sandy, and then sift the soil through sieves #16, #18 and #20 to separate achenes by type. The majority of the basal achenes will not pass through the #16 sieve, and the few that do are always retained by the #18 sieve. A small proportion of the terminal achenes remain in the #18 sieve (as was the case with many of HC achenes) with the majority of achenes passing through into the #20 sieve. In our study, none of the achenes passed through the #20 sieve into the next sieve. If the soil is predominantly silt or fine loam, all soil passes through the #20, which facilitates the final collection and counting of achenes. If soil is composed of coarse sand, larger grains can be filtered out of the sample using the #14 sieve and finer particles of sand will pass through the #18 sieve; however, it is impossible to remove all coarse sand from achenes using sieves. Final separation of achenes from sand must be done manually. Although this final process requires some manual separation of achenes and soil using a dissecting microscope, the processing of bulk samples of soil for seeds is greatly facilitated using the graduated sieve method described. For future seed bank and germination studies requiring large numbers of achenes, this method will be useful. Based on assumptions of a long-lived seed bank, populations of *S. hallii* are often listed as "extant" if plants existed at the site during the previous 25 years (McKenzie 1998); therefore, seed bank studies may be essential to the correct assessment of the species' status.

Unlike terminal flowers, which have uniformly bifid styles, with the exception of the single 4-parted style from HC, basal flowers have trifid styles as shown in Fig. 2A and 3B–D. Most references to the number of divisions present in *S. hallii* styles indicate the presence of bifid styles (Steyermark 1963; Kolstad 1986; Rolfsmeier 1995; Smith 2002), with only Yatskievych (1999) noting "stigmas 2 (rarely 3 in basal spikelets)." Although all basal styles had trifid branching, this only became apparent in some cases when the styles were floated on a film of water, which allowed shorter style lobes to separate from the main branch of the style (Fig. 3–D). Styles of basal flowers, as previously indicated by Yatskievych (1999), are longer than those of terminal flowers (Table 4, Figs. 2A and 3A–D). Our data indicate that this difference is significant (statistics not shown, see Table 4 for mean values) with no overlap in size between the two style types.

In summary, significant variation in achene size and shape occurs within and among populations; identification and separation of terminal and basal achenes can be accomplished rapidly using a series of sieves; transverse ridging is not a regular or prominent feature of basal achenes; and in the vast majority of cases, a bifid style is a consistent character in the terminal flowers (we noted one exception). All basal styles examined were trifid. Data from this study provide visual and quantitative information that will facilitate the collection and identification of terminal and basal achenes of *S. hallii* from plants, soil and wildlife and contribute to an accurate assessment of the species' conservation status.

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