MULTIVARIATE MORPHOMETRIC ANALYSIS OF TAXONOMIC RELATIONSHIPS IN *ELEOCHARIS TENUIS* (CYPERACEAE)

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ABSTRACT

Morphology of *Eleocharis tenuis* is traditionally recognized as being highly variable and delimited only to the level of variety. However, novel morphometric data indicate that the varieties of *E. tenuis* are unique and well delimited by four of ten characters examined in this study. Furthermore, these unique morphological entities are supported by correlating cytological data. Herein, we treat *E. tenuis* var. *verrucosa* at the rank of species, as *E. verrucosa,* to reflect the evolutionary significance of this entity. We also provide a key to distinguish *E. verrucosa* from the remaining varieties of *E. tenuis*.

RESUMEN

La morfología de *Eleocharis tenuis* se reconoce tradicionalmente como altamente variable y delimitada solo al nivel de variedad. Sin embargo, nuevos datos morfométricos indican que las variedades de *E. tenuis* son únicas y bien delimitadas por cuatro de los diez caracteres examinados en este estudio. Es más, Estas entidades morfológicas únicas están soportadas por datos citológicos correlacionados. Aquí, tratamos *E. tenuis* var. *verrucosa* en el rango de especie, como *E. verrucosa,* para reflejar el significado evolutivo de esta entidad. También aportamos una clave para distinguir *E. verrucosa* de las restantes variedades de *E. tenuis*.

INTRODUCTION

North of Mexico, North America contains a number of unresolved taxonomic relationships in the genus *Eleocharis* R. Br. (Cyperaceae), where the evolutionary convergence of morphological characters corresponds to multiple species complexes (González-Elizondo & Peterson 1997; González-Elizondo et al. 1997; Roalson & Friar 2000; Roalson et al. 2010; Smith 2001; Smith et al. 2002). Contributing to the uncertain treatment of many taxa is the limited availability of macro-level inflorescence and culm characters, where the nearly exclusive use of such characters in taxonomic analysis has led to variable degrees of success in distinguishing taxa (Catling & Hay 1993; Gregor 2003; Larson & Catling 1996; Rosen et al. 2007; Sorrie & LeBlond 2014). Such is the case with the *E. tenuis* complex (subg. *Eleocharis* ser. *Eleocharis* subser. *Truncatae* Svenson), where the use of traditional characters continues to obscure a comprehensive understanding of taxonomic relationships among its varieties (Smith 2001; Smith et al. 2002; Svenson 1929, 1932, 1947, 1953, 1957).

Svenson (1932, 1957) provided the first comprehensive description of *E. tenuis* (Willd.) Schult. and its varieties, using traditional characters of the achene and tubercle (length, width, and texture), as well as the culm (width and cross-section shape). Those initial descriptions were altered little by Smith et al. (2002) in their revisionary treatment of the species for the Flora of North America Project. Having observed subtle variations in achene and culm characteristics of each variety, Smith (2001) and Smith et al. (2002) echoed Svenson's (1932, 1957) conclusions that, although the varieties of *E. tenuis* are generally regarded as being distinct, they ultimately belong to a single, variable species of many intermediate entities.

However, novel to the treatment by Smith et al. (2002) were measurements of rhizome width for each variety: *E. tenuis* var. *tenuis* (0.4–1 mm), var. *pseudoptera* (Weath.) Svenson (1–2 mm), and var. *verrucosa* (Svenson) Svenson [(1–)1.5–2 mm]. He also noted differences in rhizome internode length: *E. tenuis* var. *tenuis* [(2–)5–10 mm], var. *pseudoptera* (5–10 mm), and var. *verrucosa* (2 mm). He successfully employed rhizome width and internodal length to distinguish between *E. tenuis* var. *tenuis* and var. *verrucosa,* and, although not expressed in his key, var. *pseudoptera* is separable from var. *tenuis* by its thicker rhizomes and from var. *verrucosa* by its longer internodes (see aforementioned values). Some authors have offered evidence suggesting that

J. Bot. Res. Inst. Texas 13(1): 71 – 81. 2019

vegetative characters of culm and rhizomes may be too variable for use in taxonomic analysis within some groups of *Eleocharis* [e.g., subg. *Limnochloa* (P. Beau. ex T. Lestib.) Torrey] (Edwards et al. 2003; Rosen et al. 2007). Nevertheless, Strandhede (1967) observed that differences in rhizome morphology of *E. mamillata* H. Lindb., *E. palustris* (L.) Roem. & Schult., and *E. uniglumis* (Link) Schult. persist when transplanted or when cultivated in common gardens. Catling (1994) successfully used rhizome characteristics to identify putative hybrids between *E. compressa* Sull. and *E. erythropoda* Steud. Regarding the *E. tenuis* complex, Smith et al. (2002) concluded that diverse rhizome morphologies existed for all member species, variation that, in some cases, exhibited extreme and distinct inter- and infraspecific differences between taxa.

Additional evidence for differentiating members of *Eleocharis* complexes has come from the combination of cytological (i.e., specimen karyotype) and morphological evidence in efforts to delimit cryptic or variable entities referable to the *E. palustris* complex (subg. *Eleocharis* ser. *Eleocharis* subser. *Eleocharis*) (Harms 1968; Smith & Gregor 2014; Strandhede 1967). With reference to the *E. tenuis* complex, cytological data has been generated for *E. compressa*, *E. elliptica* Kunth, and *E. tenuis*. However, no attempt has been made to associate these data to specimen morphology (Harms 1972; Schuyler 1977; Smith et al. 2002). Comparisons between morphological and cytological data have been hampered for varieties of *E. tenuis* due to loss of chromosome voucher specimens produced by Harms (1972) (Smith et al. 2002; pers. obs.). Fortunately, Schuyler (1977) produced complete, mature chromosome voucher specimens for the varieties of *E. tenuis* that remain available for taxonomic analysis (Figs. 1, 2). Whereas Harms (1972) and Schuyler (1977) have shown that var. *tenuis* ($2n = 24$) and var. *verrucosa* ($2n = 20$) consistently differ in chromosome number, counts for var. *pseudoptera* are variable with Harms (1972) reporting $2n = 38$ and Schuyler (1977) reporting $2n = 38$ and 39.

This study sought to identify which morphological characters best allow for distinguishing the varieties of *E. tenuis*, to determine the degree to which characters intergrade between currently recognized varieties, and to establish what correlations may exist between cytological data and morphometrically delimited taxonomic entities. Additionally, we evaluated those taxonomic ranks currently applied to the varieties of *E. tenuis*.

METHODS

Approximately 950 specimens were obtained from the following herbaria for examination: ACAD, BRIT, CM, GH, ILLS, MO, NY, PH, NCU, US, VPI, and WILLI (Thiers 2016). Of these, 152 representative samples of var. *tenuis* (n = 54), var. *verrucosa* (n = 63), and var. *pseudoptera* (n = 35) were selected from across the range of *E. tenuis* sensu lato for further analysis (Fig. 3).

Ten continuous characters of the achene, spikelet, and rhizome were selected for morphometric analysis; four of these were selected for multivariate analysis (Table 1). A single measurement for each character was obtained from the middle, or near middle, of each spikelet, or from a fully developed segment of the rhizome. Measurements were made using an ocular micrometer (0.01 mm accuracy, ± 0.024 mm precision) installed on an Olympus 8–40× stereo microscope.

Morphometric analysis employed a "fixed-effects" One-Way Analysis of Variance (ANOVA) to determine significant differences between the mean values of characters. Due to the extent of variation typically exhibited by taxa with complex and variable morphology, we did not expect selected characters to exhibit normal distributions. However, we did expect characters to exhibit the mound-shaped distributions typical of natural populations. A visual assessment of each character's distribution confirmed this. Levene's Test was used to evaluate for homogeneity of variance among variables, because it is less sensitive to mound-shaped populations that do not exhibit normal distributions. Only three characters (TubL, TubW and SclW) were homogeneous in variance, but the heterogeneity of remaining characters is not surprising considering the variable nature of *E. tenuis*. All raw values were retained for analysis.

Characters exhibiting heterogeneous variance were evaluated using a Dunnett-Tukey-Kramer (DTK) test ($\alpha = 0.05$). Tukey's Honestly Significant Difference (Tukey's HSD) test ($\alpha = 0.05$) was used to detect differences between characters with homogeneous variance. Both the DTK and Tukey's HSD tests assume independent samples and mound-shaped distributions, and parametrically test for a 95% confidence that each comparison

Fig. 1. Chromosome voucher specimens for *E. tenuis* var. *tenuis* (*Schuyler 4643*, PH) on left and *E. tenuis* var. *pseudoptera* (*Schuyler et al. 4649*, PH) on right.

between variables is correct. In contrast, DTK accounts for unequal variance, while Tukey's HSD test assumes equal variance between variables.

A principal component analysis (PCA) of four statistically significant characters ($P < 0.05$) was used to identify grouping of entities and to identify characters that best distinguish the varieties (Table 1). Because characters RzmSL and RzmIL exhibited such strong correlation ($r = 0.84$), RzmSL was removed from the multivariate analysis to reduce potential bias from characters that may be genetically linked. All data were standardized (Mean = 0, Sd = 1) prior to analysis.

Strandhede (1967), and most recently Smith and Gregor (2014), showed that stoma length correlates to chromosome number for species of the *E. palustris* complex. Although stoma length may be variable relative to the maturity of a single specimen, Strandhede (1967) demonstrated that sections obtained from above-middle along a culm yield consistent measurements that are reliable for taxonomic analysis. Here, we use stoma length as a substitute measure for chromosome number to evaluate potential relationships between the cytology and morphology of *E. tenuis*. Specimens used for stomatal analysis [var. *tenuis* (n = 14), var. *verrucosa* (n = 14), and var. *pseudoptera* (n = 12)] were selected to reflect the range of morphological variation presented by each variety and were obtained from a subset of specimens used for morphometric analysis.

Sections of mature culm from each variety were boiled in distilled water for 30–60 minutes. Culm sections were halved, and a single edge-razor blade was used to remove all tissues except the epidermis. Samples of epidermis were mounted on microscope slides and viewed using a Zeiss Axioscope A.1. Images of 12 stomata per epidural sample were obtained using a Regita R6 (0.2 mm resolution) camera at 1000× magnification. An average stomatal value for each specimen was calculated from these 12 measurements and compared with

Fig. 2. Chromosome voucher specimen for *E. tenuis* var. *verrucosa* (*Schuyler et al. 4653*, PH).

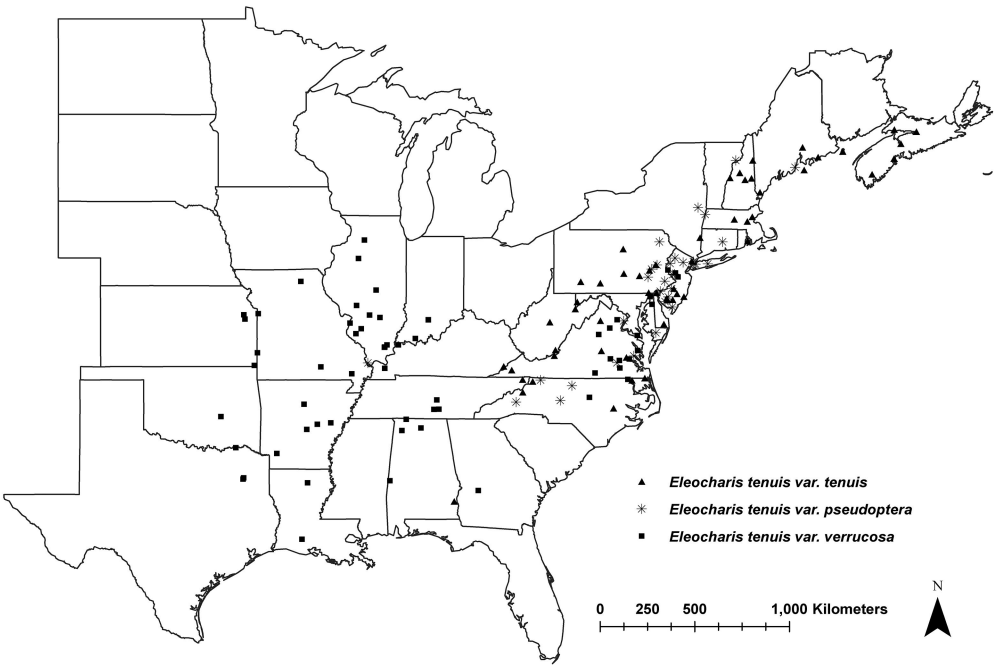

Fig. 3. Distribution of *Eleocharis tenuis* var. *tenuis*, var. *pseudoptera*, and var. *verrucosa* specimens selected for statistical analysis. Each symbol represents a single specimen.

the specimen's spatial orientation within the PCA. ImageJ (Version: 2.0.0-rc-49/1.51d) was used to measure stoma length (0.045 mm accuracy). The definition of stoma length as employed in this study, "the distance between the middle points of the stomatal ends," was obtained from Strandhede (1967).

Despite the apparent normality and heterogeneity of data, as demonstrated through visual inspections of histograms, the Shapiro-Wilk test and Levene's test, nonparametric methods of statistical analysis were chosen to analyze stomatal data due to the small number of samples collected. Specifically, the Kruskal-Wallis rank sum test ($\alpha = 0.05$) was used to test for similarity between groups and the Dunn test ($\alpha = 0.05$) was employed to identify significant differences between groups.

RESULTS

Morphometric analysis of ten continuous characters yielded notable differences in specimen morphology (Table 2). All characters but one, SclW, were significantly different ($P < 0.05$) between at least one pair of varietal entities. Two characters of the achene, AcnL and TubW, as well as all rhizome characters, RzmW, RzmSL, and RzmIL, were distinct among all varieties of *E. tenuis*.

PCA of four continuous characters explained 80.07% of the variation observed for 152 representative specimens of E. *tenuis* sensu lato and indicated a clear separation of two distinct groups (Fig. 4). Loading values and a vector map indicate that AcnL and RzmIL provided the strongest separation of taxonomic groups along dimension 1 of the PCA, while TubW provided the strongest separation of groups along dimension 2 (Table 3). Character RzmW accounted for the separation of groups nearly equally along both dimensions of the PCA (Table 3).

Of Schuyler's (1977) chromosome vouchers, var. *verrucosa* (*Schuyler et al. 4653,* PH) appeared centrally within its group as recognized by the PCA (Fig. 4). In contrast, his chromosome vouchers for var. *tenuis* (*Schuyler 4643,* PH) and var. *pseudoptera* (*Schuyler et al. 4649,* PH) fell closer to the margins of their respective entities, with that of var. *tenuis* falling where specimens of var. *tenuis* intergrade with those of var. *pseudoptera*

Table 1. Ten characters selected for statistical analysis. Character symbols used for analysis are enclosed in parenthesis. Characters used for multivariate analysis are denoted by an asterisk.

Achene length from base to tubercle (AcnL)*
Achene width at its widest point (AcnW)
Achene length from base to its widest point (AcnLM)
Tubercle length from base to apex (TubL)
Tubercle width at its widest point (TubW)*
Scale length from base to apex (SclL)
Scale width at its widest point (SclW)
Rhizome width at its widest point (RzmW)*
Rhizome internode length from node to node (RzmIL)*
Rhizome scale length from base to apex (RzmSL)

Table 2. Means ± 1 standard deviation, and ranges (mm) for statistically evaluated characters. Character symbols are those provided in Table 1. ETT = *Eleocharis tenuis* var. *tenuis,* ETP = var. *pseudoptera,* ETV = var. *verrucosa;* n = 152. Different uppercase letters differ significantly (DTK: $P < 0.05$) and different lowercase letters differ significantly (Tukey HSD: $P < 0.05$).

Character	ETT	ETP	ETV
AcnL	0.76 ±0.078 ^{A}	0.88 ±0.091 ^{B}	0.71 ±0.058 ^{C}
	(0.60–0.91)	(0.72–1.10)	(0.58–0.91)
AcnW	0.60 ±0.072 ^{A}	0.71 ±0.070 ^{A}	0.50 ±0.041 ^{B}
	(0.43–0.76)	(0.56–0.84)	(0.50–0.72)
AcnLM	0.47 ±0.066 ^{A}	0.54 ±0.063 ^{A}	0.44 ±0.039 ^{B}
	(0.36–0.70)	(0.38–0.67)	(0.34–0.58)
TubL	0.21 ±0.054 ^{a}	0.18 ±0.067 ^{b}	0.16 ±0.051 ^{b}
	(0.12–0.36)	(0.05–0.37)	(0.05–0.29)
TubW	0.32 ±0.047 ^{a}	0.42 ±0.057 ^{b}	0.37 ±0.042 ^{c}
	(0.22–0.43)	(0.26–0.58)	(0.26–0.50)
SclL	2.01 ±0.29 ^{A}	2.39 ±0.29 ^{A}	1.97 ±0.22 ^{B}
	(1.39–2.69)	(1.82–3.25)	(0.91–1.63)
SclW	1.27 ±0.18 ^{a}	1.29 ±0.27 ^{a}	1.21 ±0.18 ^{a}
	(0.96–1.78)	(0.62–1.92)	(0.91–1.63)
RzmW	0.83 ±0.16 ^{A}	1.14 ±0.24 ^{B}	1.64 ±0.26 ^{C}
	(0.58–1.22)	(0.82–1.90)	(1.11–2.33)
RzmIL	4.67 ±1.19 ^{A}	6.19 ±1.54 ^{B}	1.95 ±0.64 ^{C}
	(2.63–8.00)	(3.25–10.0)	(0.88–4.38)
RzmSL	5.56 ±1.13 ^{A}	6.83 ±1.70 ^{B}	2.78 ±0.49 ^{C}
	(3.75–7.88)	(3.75–10.2)	(1.38–4.63)

(Fig. 4). Overall, specimens selected for stomatal analysis comprehensively reflected the range of morphological variation presented by each respective entity.

Analysis indicates that stoma length is statistically significant ($P < 0.05$) between all varietal entities (Table 4). *Eleocharis tenuis* var. *verrucosa* was least variable in stoma length with a standard deviation of ±4.66 mm. Stomatal lengths for both var. *tenuis* and var. *pseudoptera* exhibited standard deviations of ±5.86 mm and ±5.33 mm, respectively. Typical values of stoma length for varieties *tenuis, pseudoptera,* and *verrucosa* were 32.33 mm, 47.97 mm, and 39.98 mm, respectively.

DISCUSSION

Rhizome characters exhibited the most perceptible differences between *E. tenuis* var. *verrucosa*, and the other varieties *tenuis* and *pseudoptera.* The typically thick rhizomes presented by var. *verrucosa*, 1.64 (±0.26) mm allow for clear and consistent separation from the typically thin rhizomes of var. *tenuis,* 0.83 (±0.16) mm and narrower rhizomes of var. *pseudoptera,* 1.14 (±0.24) mm. Uniformity in rhizome scale length and internode length for var. *verrucosa*, 2.78 (±0.49) mm and 1.95 (±0.64) mm, respectively, delimit it from the more variable, and typically greater values of both var. *tenuis* and var. *pseudoptera*, exhibiting rhizome scale lengths of 5.56

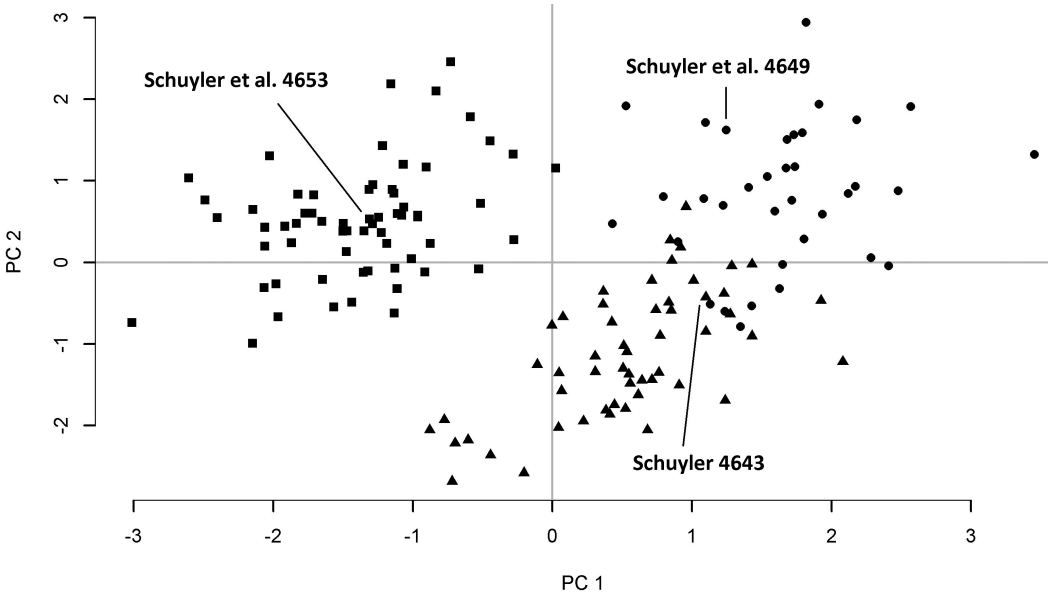

Fig. 4. Scatterplot of the first two principal components (Dim. 1 and Dim. 2) of a principal components analysis, using four morphological characters (AcnL, TubW, RzmW, and RzmIL) measured from 152 specimens of *Eleocharis tenuis* var. *tenuis* (triangles), var. *pseudoptera* (circles), and var. *verrucosa* (squares). Chromosome vouchers for var. *tenuis* (*Schuyler 4643,* PH), var. *pseudoptera* (*Schuyler et al. 4649,* PH) and var. *verrucosa* (*Schuyler et al. 4653,* PH), are identified.

Table 3. Eigenvalues, percent variance, and loading values of five characters along the first two principal components (dimensions) of a PCA performed on a cumulative 152 specimens of *Eleocharis tenuis* var. *tenuis,* var. *pseudoptera,* and var. *verrucosa*. Character symbols are those provided in Table 1.

	Dimension 1	Dimension 2
Eigenvalues	1.900	1.305
Percent variance	47.456	32.616
AcnL	0.604	0.118
TubW	0.082	0.774
RzmW	-0.450	0.401
RzmIL	0.763	-0.012

Table 4. Means ± 1 standard deviation and ranges (mm) for stomatal length, as well as chromosome counts reported by Schuyler (1977) for *Eleocharis tenuis* var. *tenuis* (ETT), var. *pseudoptera* (ETP), and var. *verrucosa* (ETV). Different uppercase letters differ significantly (Dunn: $P < 0.05$).

ETT	ETP	ETV
32.33 ±5.86 [A]	47.97 ±5.33 [B]	39.98 ±4.66 [C]
(19.05–39.71)	(42.16–59.34)	(34.71–51.49)
$2n = 24$	$2n = 38$ & 39	$2n = 20$

(±1.13) mm and 6.83 (±1.70) mm, respectively, and internode lengths of 4.67 (±1.19) mm and 6.24 (±1.75) mm, respectively. Although achene length and tubercle width of var. *verrucosa* are morphometrically distinct from those characters in var. *tenuis* and var. *pseudoptera*, the small differences of 1–2 mm limit their practical use in identification.

Achene length and tubercle width are responsible for much of the separation apparent in the PCA

between varieties *tenuis* and *pseudoptera*, i.e., achene lengths of 0.76 (±0.08) mm and 0.88 (±0.09), respectively, and tubercle widths of 0.32 (±0.05) mm and 0.42 (±0.06) mm, respectively. Yet, these characters do not clearly delimit var. *tenuis* from var. *pseudoptera* and the PCA recognizes the two varieties to be widely variable groups where specimens intergrade along the upper and lower limits of their morphological variation. Although morphometrically distinct, rhizome width, scale length and internode length for varieties *tenuis* and *pseudoptera* do not totally delimit the two groups from one another. In contrast, the PCA indicates that no specimens of var. *verrucosa* appear to exhibit characters intermediate with var. *tenuis* or var. *pseudoptera*.

Interestingly, increasing numbers of chromosomes correlate with increasing stomatal lengths for varieties *tenuis* and *pseudoptera*, i.e., $2n = 24$ and $2n = 38$, 39, respectively, and 32.33 mm to 47.97 mm, respectively. This pattern is consistent with observations that var. *tenuis* and var. *pseudoptera* originate from the same ancestral diploid ($2n = 12$) (Harms 1972). Here, we note that Schuyler's (1977) report of $2n = 39$ for var. *pseudoptera* is obviously not an exact multiple of this ancestral diploid. After close examination of Schuyler's photographed chromosome squash, archived with the voucher specimen used in this study (*Schuyler et al. 4649,* PH), we concluded that it clearly contains a haploid count of 19 chromosomes ($2n = 38$). Schuyler (1977) likewise writes of observing many specimens with a haploid count of 19 chromosomes which is consistent with Harms' (1972) observations for the variety. Yet we cannot render his report of $2n = 39$ as erroneous since Schuyler included photographs of two chromosome squashes with a haploid count of 20 chromosomes in his 1977 publication. Both authors suspected hybridization as a possible source for this discrepancy, with Schuyler (1977) identifying hybrids between var. *pseudoptera* and var. *tenuis,* and Harms (1972) suspecting hybrids between var. *pseudoptera* and *E. elliptica*. We have not discussed hybrids here, partly due to the absence of an adequate number of voucher specimens, and until further cytological data is obtained, this point may not be clarified. Still, cytological and morphological evidence from Harms (1972), Schuyler (1977), and the present study all suggest that varieties *tenuis* and *pseudoptera*, and perhaps *E. elliptica*, share complex, possibly close taxonomic relationships.

Chromosome number and stomatal lengths for var. *verrucosa* do not correspond to the patterns of positive correlation between stomatal lengths and chromosome number observed for varieties *tenuis* and *pseudoptera* or for the *E. palustris* complex as identified by Strandhede (1967) and Smith and Gregor (2014). With var. *verrucosa* exhibiting fewer chromosomes ($2n = 20$) than var. *tenuis* ($2n = 24$), one would expect the stomatal lengths of var. *verrucosa* (39.98 mm) to be less than that of var. *tenuis* (32.33 mm). Harms (1972) proposed a unique origin for var. *verrucosa*, a $2n = 10$ ancestral diploid, to explain cytological differences between var. *verrucosa* and the remaining varieties of *E. tenuis*. The absence of a correlation between stoma length and chromosome number among all varieties of *E. tenuis* offer support for his hypothesis.

CONCLUSION

In 1972, Harms proposed *Eleocharis verrucosa* as a distinct species on the basis of cytology. Although his work has been corroborated by Schuyler (1977), neither author provided supporting morphological evidence and instead relied on Svenson's (1932, 1957) diagnoses to support elevating the entity to that of species. Our study has drawn upon the critical contributions of Svenson (1929, 1932, 1947, 1953, 1957), Harms (1968, 1972), Schuyler (1977), Smith (2001), Smith et al. (2002), and Smith & Gregor (2014), and has used specimen cytology to support the morphological recognition of *E. verrucosa* as a unique entity. Specifically, morphological and cytological evidence presented here indicate that *E. tenuis* var. *verrucosa* is distinct from varieties *tenuis* and *pseudoptera* based on criteria set forth by the phenetic species concept, the biological species concept, and the evolutionary species concept. Consequently, we suggest that the current taxonomic rank applied to *E. tenuis* var. *verrucosa* does not appropriately reflect the evolutionary significance of this entity. We follow Harms (1972) in treating it at the rank of species, as *E. verrucosa*. Additionally, we present a key to distinguish *E. verrucosa* from the remaining varieties of *E. tenuis*.

Eleocharis verrucosa (Svenson) L.J. Harms, Amer. J. Bot. 59(5):483. 1972. TYPE: U.S.A. Missouri: Cedar Gap, 22 May–3 Jun 1911, *O.E. Lansing, Jr. 3040* (HOLOTYPE: GH!).

KEY TO *ELEOCHARIS VERRUCOSA* AND THE VARIETIES OF *E. TENUIS* (SUBG. *ELEOCHARIS* SER. *ELEOCHARIS* SUBSER. *TRUNCATAE* SVENSON)

1. Rhizomes (1.1–)1.4–1.9(–2.3) mm wide, appearing thick relative to short internode lengths of (0.9–)1.3–2.6(–4.4) mm, scales of rhizome (1.4–)2.3–3.3(–4.6) mm long; tubercles typically ¾ the width of achene, greatly depressed, rarely pyramidal; achenes 0.6–0.7(–0.9) mm in length to base of tubercle by 0.5(–0.7) mm wide, coarsely (to finely) rugose to cancellate at 10× [of e. PA and NJ south to GA, west to e. NE, OK, and TX] _____ **E. verrucosa**
1. Rhizomes 0.6–1.4(–1.9) mm wide, appearing delicate or slender relative to long internode lengths of (2.6–)3.5–7.7(–10.0) mm, scales of rhizome (3.7–)4.4–8.5(–10.2) long; tubercles typically less than ¾ the width of achene, pyramidal to depressed; achenes (0.6–)0.7–1.1 mm in length to base of tubercle by (0.4–)0.5–0.8 mm wide, finely rugulose to finely cancellate at 10×.
   2. Culms bluntly angled to smooth, seldom deeply sulcate, to 0.5 mm wide; rhizomes delicate to slender, 0.6–1.0(–1.2) mm wide, with longer internodes (2.6–)3.5–5.9(–8.0), scales of rhizome (3.7–)4.4–6.7(–7.9) mm in length; tubercles pyramidal, rarely depressed (0.2–)0.3–0.4 mm wide; achene obovate occasionally nearing an orbicular shape (0.6–)0.7–0.8(–0.9) mm long by (0.4–)0.5–0.7 mm wide, rugulose to finely cancellate at 10× [of e. KY and w. NC northeast to N.S.]_____ **E. tenuis** var. **tenuis**
   2. Culms sharply angled, usually deeply sulcate, to 0.8 mm wide; rhizomes slender, (0.8–)0.9–1.4(–1.9) mm wide, with longer internodes (3.2–)4.6–7.7(–10) mm, scales of rhizome (3.7–)5.1–8.5(–10.2) mm in length; tubercles depressed, rarely pyramidal (0.3–)0.4–0.6 mm wide; achene elongate obovate to faintly spatulate, (0.7–)0.9–1.1 mm long by 0.6–0.8 mm wide, obscurely to finely rugulose at 10× [of w. NC northeast to se. ME, disjunct to s. IL) _____ **E. tenuis** var. **pseudoptera**

SPECIMENS OF *ELEOCHARIS TENUIS* VAR. *TENUIS* EXAMINED
*Denotes stomata length voucher specimen
†Denotes A.E. Schuyler chromosome voucher specimen

U.S.A. ALABAMA: Henry Co.: 2 May 1972, *Kral 46203* (MO). **DELAWARE.** Millsboro, 8 Jul 1884, *Commons s.n.* (PH). Greenbank, 20 Jun 1884, *Commons s.n.* (PH). **MASSACHUSETTS.** Stoneham, Spot Pond, 9 Jul 1912, *Crane 988* (NY)*; Natick, 5 Jul 1908, *Heatley & Wiegand s.n.* (NY). **Worchester Co.:** 27 Jul 1947, *Gates s.n.* (NCU). **MARYLAND. Garret Co.:** 26 Jun 1947, *Allard 12352* (US)*. **MAINE. Penobscot Co.:** 4 Aug 1916, *Fernald & Long 12809* (PH)*. **Washington Co.:** 15 Jul 2001, *Hill 34026* (ILLS)*. **York Co.:** 20 Jul 1928, *True & Fogg, Jr. 34* (PH). **Knox Co.:** 28 Jun 1958, *Rossbach 4432* (NCU). **NORTH CAROLINA. Gates Co.:** 26 Jun 1958, *Ahles & Duke 44745* (NCU)*. **Ashe Co.:** 7 Jul 1966, *Radford 44910* (BRIT). **Avery Co.:** 24 Jul 1958, *Ahles & Duke 47417* (NCU). **Greene Co.:** 11 Jul 1958, *Radford 36651* (NCU). **Ashe Co.:** 7 Jul 1966, *Radford 44910* (US). **NEW HAMPSHIRE. Carroll Co.:** 26 Jul 1932, *True & Fogg, Jr. 12* (PH)*; 2 Sep 1936, *Weatherby 6867* (US). **Coos Co.:** 23 Aug 1926, *True & Fogg, Jr. 11* (PH). **Grafton Co.:** 3 Aug 1917, *Fernald 15508* (CM); 27 Jul 1932, *True & Fogg, Jr. 37a* (PH). **NEW JERSEY.** Bergen **Co.:** 20 Jun 1935, *Svenson 7885* (PH). **Burlington Co.:** 26 May 1976, *Schuyler 4643* (PH)*†; 1 Jul 1937, *True & Fogg, Jr. 4169* (PH). **Cumberland Co.:** 10 Jun 1934, *Long 43535* (PH); 12 Jul 1935, *Long 46633* (PH). **Ocean Co.:** 13 Jul 1914, *Long 10294* (PH). **Salem Co.:** 2 Jul 1935, *Long 46387* (PH). **NEW YORK. Dutchess Co.:** 12 Jun 2014, *Naczi & Dorey 15394* (NY)*. **KENTUCKY. Letcher Co.:** 1 Jul 1935, *Braun 1087* (US)*. **PENNSYLVANIA. Bedford Co.:** 1 Jul 1945, *Berkheimer 6195* (CM)*. **Berks Co.:** 18 Jun 1962, *Schaeffer, Jr. 65879* (PH). **Centre Co.:** 25 Jun 1958, *Henry s.n.* (CM). **Chester Co.:** 22 Jun 1929, *Svenson 3453* (US). **Lebanon Co.:** 27 Jun 1954, *Berkheimer 16291* (PH). Lehigh **Co.:** 4 Jul 1918, *Pretz 9438* (PH). **Lycoming Co.:** 30 Jun 1959, *Wahl 19149* (PH). **Perry Co.:** 7 Jul 1935, *Adams & Adams 2112* (US). **Westmoreland Co.:** 4 Jul 1959, *Henry s.n.* (CM). **RHODE ISLAND.** Rhode Island, 1800s, *Olney 309* (US). **TENNESSEE. Sullivan Co.:** 17 Jun 1934, *Underwood 989* (US)*. **VIRGINIA. Charles City Co.:** 3 Jul 1966, *Svenson 432* (VPI)*; Beahm's Gap and the headwaters of the N. Fork of Thornton River, 10 Jun 1936, *Camp 1441* (NY); between northwest Norfolk Co. and Mayock N.C., 25 May 1983, *Britton & Small s.n.* (NY). **Giles Co.:** 28 Jul 1937, *Fogg, Jr. 12736* (PH). **Powhatan Co.:** 4 Jun 1976, *Corcoran & Diggs, Jr. 632* (WILLI). **Scott Co.:** 19 Aug 1979, *Peake 671* (WILLI).**WEST VIRGINIA. Monroe Co.:** 14 Jul 2003, *Wieboldt & Wieboldt 11265* (VPI)*; Cranberry Summit, 15 Jul 1877, *Guttenberg 3094* (CM). **Tuker Co.:** 16 Jul 1951, *Allard 19957* (US). **CANADA: NOVA SCOTIA. Colchester Co.:** 18 Jul 1920, *Bean & White 20159* (PH). **Cumberland Co.:** 1 Aug 1953, *Schofield 3550* (ACAD). **Hants Co.:** 18 Aug 1954, *Smith et al. 12508* (ACAD). **Lunenburg Co.:** 22 Aug 1954, *Smith et al. 12772* (ACAD). **Shelburne Co.:** 13 Aug 1954, *Smith et al. 12149* (ACAD)*†. **NEW BRUNSWICK. Charlotte Co.:** 27 Jul 1927, *Weatherby & Weatherby 5693* (US).

SPECIMENS OF *ELEOCHARIS TENUIS* VAR. *PSEUDOPTERA* EXAMINED
*Denotes stomata length voucher specimen
†Denotes A.E. Schuyler chromosome voucher specimen

U.S.A. CONNECTICUT. Middlesex Co.: 5 Jul 1916, *Chambelain s.n.* (NY)*; Crystal Lake, 28 Jun 1924, *Bennett s.n.* (NY). **DELAWARE. Newcastle Co.:** 30 Jun 1929, *Svenson 3457* (PH)*. **ILLINOIS. Alexander Co.:** 31 May 1993, *Basinger & Ketzner 5283* (ILLS)*. **MAINE. Knox Co.:** 27 Jun 1962, *Rossbach 5817* (NY)*. **MARYLAND**. **Somerset Co.:** 24 Jun 2014, *Naczi et al. 15523* (NY)*. **MASSACHUSETTS. Berkshire Co.:** 15 Jul 1917, *Churchill 184* (MO)*. **NEW JERSEY. Hunterdon Co.:** 14 Jun 1976, *Schuyler et al. 4649* (PH)*†. **Cumberland Co.:** 21 Jun 1926, *Bright 13283* (CM). **Morris Co.:** 30 Jun 1957, *Hoiberg 635* (NCU). **Cape May Co.:** 21 Jun 1919, *Long 21582* (PH). **Warren Co.:** 17 Jun 1952, *Schaeffer, Jr. 38683* (PH). **Hunterdon Co.:** 30 Jun 1937, *Long 50323* (PH). **Sussex Co.:** 13 Jul 1976, *Schuyler et al. 4702* (PH). **Burlington Co.:** 2 Jul 1937, *Fogg, Jr. 12237* (PH). **Salem Co.:** 7 Jul 1934, *Fogg, Jr. 7054* (PH). **NEW YORK. Bronx Co.:** 12 Jul 1901, *Burnham 117* (NY); Yonkers, Jul 1888, *Howe s.n.* (NY). **Queens Co.:** 16 Jul 1916, *Pennell 2552* (PH); Staten Island, 20 Jun 1930, *Svenson 3496* (PH). Long Island, 18 Jul 1924, *Ferguson 3052* (NY)*. **NORTH CAROLINA. Alleghany Co.:** 23 Jun 2009, *Poindexter 09-696* (NCU)*. **Davidson Co.:** 7 Jun 1975, *Wickland 313* (NCU). **Buncombe Co.:** 11 Jun 1977, *Rothrock 1194* (NCU). **Rockingham Co.:** 8 Jun 1961, *Radford 43834* (NCU). **PENNSYLVANIA. Berks Co.:** 24 Jun 1943, *Berkheimer 3800* (CM)*. **Lehigh Co.:** 23 Jun 1918, *Pretz 9408* (BRIT). **Berks Co.:** 24 Jun 1943, *Berkheimer 3788* (CM). **Montgomery Co.:** 18 Jun 1965, *Wherry s.n.* (PH). **Berks Co.:** 17 Jun 1953, *Schaeffer, Jr. 43160* (PH). **Lackawanna Co.:** 27 Jun 1946, *Glowenke 6811* (PH).

VERMONT. ESSEX **Co.:** 4 Jul 1963, *Seymour 21203* (BRIT)*. **VIRGINIA. Dinwiddie Co.:** 13 Jun 1940, *Fernald & Long 11978* (NY)*. **Fairfax Co.:** 31 May 1930, *Hasselbring s.n.* (NCU). **James City Co.:** 12 Jul 2001, *Townsend 2594* (VPI).

SPECIMENS OF *ELEOCHARIS TENUIS* VAR. *VERRUCOSA* EXAMINED
*Denotes stomata length voucher specimen
†Denotes A.E. Schuyler chromosome voucher specimen
‡Denotes type specimen

U.S.A. ALABAMA. Lauderdale Co.: 4 Jun 1968, *Kral 31044* (BRIT)*; Joe Wheeler Wildlife Refuge, Sec 25 T.4.S. R.3.W., 20 May 1980, *Meigs 550* (BRIT). **Sumter Co.:** 28 Apr 1968, *McDaniel 10534* (BRIT). Franklin **Co.:** 17 May 1968, *Kral 30606* (BRIT). **ARKANSAS. Lee Co.:** 7 Jun 1968, *McDaniel 10669* (BRIT)*. **Lonoke Co.:** 25 Apr 1977, *Kral 59746* (BRIT). **Hempstead Co.:** 5 May 1998, *Kral 87584* (BRIT). **Saline Co.:** 4 May 2004, *Witsell 04-250* (NY). **Van Buren Co.:** 12 Jun 2005, *Witsell 05-647* (NY). **GEORGIA. Sumter Co.:** 22 Mar 1997, *Morris 6795* (BRIT)*. **ILLINOIS. Bond Co.:** 13 Jun 1950, *Evers 23884* (ILLS). **Fayette Co.:** 22 May 1951, *Evers 28923* (ILLS). **Lee Co.:** 13 Jun 2000, *Phillippe 31673* (ILLS). **Macon Co.:** 22 Jun 1915, *Clokey 2373* (NY). **Macoupin Co.:** 28 May 1884, *Robertson 9865* (ILLS). **Monroe Co.:** 10 Jun 1992, *Phillippe & Gehlhausen 20182* (ILLS)*. **Pope Co.:** 21 May 1991, *Jones 6889* (US). **Saline Co.:** 27 May 1992, *Phillippe et al. 20072* (ILLS). **Stark Co.:** 28 Jun 1900, *Chase 643* (BRIT); 5 Jun 1956, *Buser 6722* (ILLS). **Washington Co.:** 11 Jun 1992, *Phillippe & Gehlhausen 20203* (ILLS). **INDIANA. Posey Co.:** 30 Jun 1939, *Kriebel 8185* (NY)*. **Lawrence Co.:** 30 May 1937, *Kriebel 5210* (NY); 26 Jun 1935, *Kriebel 3681* (NY). **Spencer Co.:** 10 Jun 1929, *Deam 46803* (PH). **KANSAS. Cherokee Co.:** 2 Jun 1964, *Harms & Kolstad 1277* (NY). **Douglas Co.:** 17 May 2001, *Morse 5783* (BRIT)*; 7 Jun 2000, *Freeman 14773* (BRIT). **KENTUCKY. Graves Co.:** 18 May 1990, *McKinney & Hamilton 4146* (BRIT)*. **LOUISIANA. Ouachita Parish:** 15 May 1959, *Kral 8904* (BRIT)*. **Acadia Parish:** 7 Apr 1936, *Harper 3469* (NY). **MARYLAND. St. Mary's Co.:** 31 May 1959, *Benedict, Jr. 6295* (VPI)*. **Cecil Co.:** 6 Jun 1934, *Herlinhy s.n.* (US). **Kent Co.:** 18 Jun 1959, *Benedict, Jr. 6302* (VPI). **MISSOURI. Adair Co.:** 26 May 1970, *Conrad 5612* (CM). **Barton Co.:** 5 Jun 1996, *Timme 12879b* (MO). **Stoddard Co.:** 31 May 2000, *Brant et al. 4385* (MO)*. County Unknown: Montier, 15 May 1894, *Bush 589* (NY); St. Louis, 31 May 1878, *Eggert s.n.* (NY); Dodson, 11 Jun 1904, *Bush 2014a* (US); Wright, Jun 1911, *Lansing, Jr. 3040* (GH)‡. **NEW JERSEY. Somerset Co.:** 14 Jun 1976, *Schuyler et al. 4653* (PH)*†. **Mercer Co.:** Jul 1911, *Mackenzie 4910* (NY). **NORTH CAROLINA. Hertford Co.:** 30 May 1958, *Ahles & Duke 41647* (NCU)*. **Wake Co.:** 17 May 1959, *Radford 42703* (NCU). **OKLAHOMA. Hughes Co.:** 3 May 1946, *Hammon s.n.* (BRIT)*. **PENNSYLVANIA. Bucks Co.:** 17 Jul 1925, *True 79* (PH). **TENNESSEE. Cannon Co.:** 20 May 1974, *Kral 52802* (BRIT). **Coffee Co.:** 28 May 1942, *Kriebel 9801* (BRIT); 2 Jun 1938, *Svenson 8715* (US). **Franklin Co.:** 31 May 1962, *DeSelm & Shanks 30648* (BRIT). **TEXAS. Wood Co.:** 7 Jun 1969, *Correll 37423* (NY)*. **Lamar Co.:** 19 May 1963, *Correll & Correll 27476* (BRIT). **Wood Co.:** 24 Apr 1942, *Lundell & Lundell 11338* (BRIT). **VIRGINIA. Amelia Co.:** 9 Jun 1993, *Wieboldt & Stevens 8593* (VPI). **Charles City Co.:** 5 Jun 1949, *Mikula 694* (WILLI). **Culpeper Co.:** 5 Jun 1996, *Stevens 25288* (VPI). **Dinwiddie Co.:** 8 Jun 1938, *Fernald & Long 8103* (US). **Greene Co.:** 10 Jun 1972, *Wieboldt 943* (WILLI). **Mecklenburg Co.:** 26 Jun 1990, *Wieboldt 7251* (VPI). **Middlesex Co.:** 24 Jun 1981, *North 329* (WILLI). **Prince William Co.:** 22 Jun 2005, *Townsend 3437* (VPI)*. **Sussex Co.:** 13 May 1960, *Kral 10213* (BRIT).

ACKNOWLEDGMENTS

We would like to thank the herbaria cited for loan specimens and all associated staff for their patience, assistance in answering questions, and for supplying quality material for study. We are especially grateful to GH for loaning us a type specimen for direct analysis. James Madison University graciously provided financial assistance to offset the cost of publication. Richard Carter and Arthur Haines provided helpful review of the final manuscript, David McLeod and Mike Renfro provided support as the MS thesis committee for the first author, Kristopher Kubow provided essential guidance with microscopy, S. Galen Smith and Alfred E. Schuyler provided helpful correspondence, and Fred Huber provided access and support for field observations on federal lands in Virginia. Finally, special thanks are extended to Thomas F. Wieboldt for helpful discussions and valued insight.

REFERENCES

Catling, P.M. 1994 *Eleocharis compressa* × *Eleocharis erythropoda,* a new natural hybrid spike rush from Ontario. Canad. J. Bot. 72:837–842.

Catling, P.M. & S.G. Hay. 1993. The hybrid origin of *Eleocharis macounii.* Rhodora 95:85–96.

Edwards, A.L., D.W. Lee, & J.H. Richards. 2003. Responses to a fluctuating environment: Effects of water depth on growth and biomass allocation in *Eleocharis cellulosa* Torr. (Cyperaceae). Canad. J. Bot. 81:964–975.

González-Elizondo, M. & P.M. Peterson. 1997. A classification of and key to the supraspecific taxa in *Eleocharis* (Cyperaceae). Taxon 46:433–449.

González-Elizondo, M., P.M. Peterson, & I. Granzow-de La Cerda. 1997. A cladistic and phenetic analysis of the Pauciflorae group of *Eleocharis* (Cyperaceae). BioLlania Edición Esp. No. 6:341–368.

Gregor, T. 2003. *Eleocharis mamillata*—distribution and infraspecific differentiation. Folia Geobot. 38:49–64.

Harms, L.J. 1968. Cytotaxonomic studies in *Eleocharis* subser. *Palustres*: Central United States taxa. Amer. J. Bot. 55:966–974.

Harms, L.J. 1972. Cytotaxonomy of the *Eleocharis tenuis* complex. Amer. J. Bot.59:483–487.

Larson, B.M.H. & P.M. Catling. 1996. The separation of *Eleocharis obtusa* and *Eleocharis ovata* (Cyperaceae) in eastern Canada. Canad. J. Bot. 74:238–242.

Roalson, E.H. & E.A. Friar. 2000. Infrageneric classification of *Eleocharis* (Cyperaceae) revisited: Evidence from the internal transcribed spacer (ITS) region of nuclear ribosomal DNA. Syst. Bot. 25:323–336.

Roalson, E.H., C.E. Hinchliff, R. Trevisan, & C.R.M. da Silva. 2010. Phylogenetic relationships in *Eleocharis* (Cyperaceae): C4 photosynthetic origins and patterns of diversification in the spikerushes. Syst. Bot. 35:257–271.

Rosen, D.J., S.L. Hatch, & R. Carter. 2007. Infraspecific taxonomy and nomenclature of *Eleocharis acutangula* (Cyperaceae). J. Bot. Res. Inst. Texas 1:875–888.

Schuyler, A.E. 1977. Chromosome observations on some eastern North American *Eleocharis* (Cyperaceae). Brittonia 29:129–133.

Smith, S.G. 2001. Taxonomic innovation in North American *Eleocharis* (Cyperaceae). Novon 11:241–257.

Smith, S.G., J.J. Bruhl, M.S. Gonzalez-Elizondo, & F.J. Menapace. 2002. *Eleocharis*. In: Flora of North America Editorial Committee, eds. 1993+. Flora of North America north of Mexico, Vol. 23, Magnoliophyta: Commelinidae (in part): Cyperaceae. Oxford University Press, New York and Oxford, U.SA. and UK. Pp. 60–120.

Smith, S.G. & T. Gregor. 2014. North American distribution of *Eleocharis mamillata* (Cyperaceae) and confusion with *E. macrostachya* and *E. palustris*. Rhodora 116:163–186.

Sorrie, B.A. & R.J. LeBlond. 2014. *Eleocharis fallax* (Cyperaceae): Recent collections and morphological comparison with *E. ambigens* and *E. monteviensis*. Phytoneuron 2014-7:1–7.

Strandhede, S.O. 1967. *Eleocharis* subser. *Eleocharis* in North America. Taxonomical comments and chromosome numbers. Bot. Not. 120:355–368.

Svenson, H.K. 1929. Monographic studies in the genus *Eleocharis*. Rhodora 31:121–135; 167–191.

Svenson, H.K. 1932. Monographic studies in the genus *Eleocharis*–II. Rhodora 34:193–203; 215–227.

Svenson, H.K. 1947. The group of *Eleocharis palustris* in North America. Rhodora 49:61–67.

Svenson, H.K. 1953. The *Eleocharis obtusa-ovata* complex. Rhodora 55:1–6.

Svenson, H.K. 1957. Scirpeae (continuatio). North Amer. Fl. 18:505–556.

Thiers, B. 2016. Index herbariorum: A global directory of public herbaria and associated staff. New York Botanical Garden's Virtual Herbarium. http://sweetgum.nybg.org/science/ih/.