

Infrageneric Classification of *Eleocharis* (Cyperaceae) Revisited: Evidence from the Internal Transcribed Spacer (ITS) Region of Nuclear Ribosomal DNA

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ABSTRACT. The goals of this study were to elucidate phylogenetic relationships in *Eleocharis* (Cyperaceae) using the nuclear ribosomal internal transcribed spacer (ITS) region, and compare this phylogeny with three infrageneric classifications. Thirty *Eleocharis* species and eight outgroups were analyzed using maximum parsimony and maximum likelihood analyses. These analyses place *Websteria* within the *Eleocharis* clade. The sister group of *Eleocharis* has traditionally been inferred to be either members of the Abildgaardieae or Scirpeae. These analyses do not show strong support for *Bulbostylis* (Abildgaardieae) or *Schoenoplectus* (Scirpeae) as the sister lineage to *Eleocharis*. The ITS results are not entirely congruent with any of the classifications. There is support for a monophyletic *Eleocharis* subgenus *Limnochloa* and subgenus *Zinserlingia* sensu González-Elizondo and Peterson, but many of the subdivisions of *Eleocharis* subgenus *Eleocharis* are para- or polyphyletic. Morphological characters inferred to have multiple origins by the ITS data, and qualitative traits such as texture, shape and color of nuts and scales, and shape and texture of sheath apices have been used to define classification units in *Eleocharis* and this appears to have played a major role in the confusion surrounding relationships in the genus.

The purpose of this study was to estimate phylogenetic relationships in the genus *Eleocharis* R. Br. (Cyperaceae) using the nuclear ribosomal (nr) DNA internal transcribed spacer (ITS) region and compare this hypothesis of relationships to previous infrageneric classifications in the genus.

Eleocharis includes about 200 species and are circumboreal in distribution (González-Elizondo and Peterson 1997). Originally included in the genus *Scirpus* L., *Eleocharis* is well demarcated (Kukkonen 1990), and circumscribed by the following characters: inflorescence of a single terminal spikelet, leaves basal and reduced to tubular sheaths, and base of style persistent as a tubercle (González-Elizondo and Peterson 1997). Some authors recognize one or more of three segregate genera, *Websteria* S. H. Wright, *Chillania* Roiv., and *Egleria* L. T. Eiten (Eiten 1964; Bruhl 1995; González-Elizondo and Peterson 1997). Seberg (1985) presented rationale for including *Chillania* within *Eleocharis*. Tucker (1987) questioned the recognition of *Websteria*, and included it as a section of *Eleocharis*. *Egleria* has been consistently considered to be a distinct genus, but is as similar to *Eleocharis* as *Chillania* and *Websteria*.

Eleocharis has been traditionally placed in tribe Scirpeae Kunth ex Dumort., but the relationships among the various portions of *Scirpus* s.l., including *Bolboschoenus* (Ascherson) Palla, *Isolepis* R. Br., *Schoenoplectus* (Reichb.) Palla, *Scirpus* s.s., and *Triphorum* Pers., and related genera and tribes are

unknown (Bruhl 1995). Bruhl (1995) included 27 genera in tribe Scirpeae, but *Bulbostylis* Kunth and *Fimbristylis* Vahl, often considered part of Scirpeae and close relatives of *Eleocharis* (Goetghebeur 1985; Kukkonen 1990), are placed in the Abildgaardieae Lye along with *Abildgaardia* Vahl, *Crosslandia* V. W. Fitzgerald, *Nelmesia* Veken, *Nemum* Desv., and *Tylocarya* Nelmes. In 30 morphology-based analyses (24 cladistic; 6 phenetic) in which different combinations of morphological data sets are included, Bruhl's (1995) Scirpeae are polyphyletic in 23 of them, paraphyletic in six, and monophyletic in only one analysis. Bruhl notes that the Scirpeae, as he defines it, is the most poorly supported of the groups recognized in his treatment, and uses it primarily as a "conservative and convenient means of dealing with these genera" (Bruhl 1995).

In the first published molecular phylogenetic study in the Cyperaceae (Plunkett et al. 1995) based on the chloroplast gene *rbcl*, *Eleocharis* (*E. pauciflora* (Lightf.) Link) was placed sister to a clade consisting of *Scirpus* (actually *Schoenoplectus*) and *Cyperus* L., with this clade of three taxa forming a polytomy with *Oxychloë* Philippi, *Eriophorum* L., and a *Carex* L./*Kobresia* Willd. clade. A much-expanded analyses of relationships in the Cyperales based on *rbcl* (Muasya et al. 1998), infers a portion of *Schoenoplectus* to be the sister group of *Eleocharis*. Unfortunately, most branches in this analysis have low boot-

strap support, including the branch placing *Schoenoplectus* and *Eleocharis* together.

A plethora of infrageneric classifications within *Eleocharis* have been suggested over the last two centuries (Torrey 1836; Kunth 1837; Bentham and Hooker 1883; Clarke 1900, 1902, 1908; Beauverd 1921; Svenson 1929, 1934, 1937, 1939; Zinserling 1935; Blake 1939; Koyama 1961; Egorova 1976, 1980, 1981; Egorova and Khoi 1980; Kukkonen 1990; González-Elizondo and Peterson 1997). The rationale for most of these reclassifications is not stated. The most recent of these (González-Elizondo and Peterson 1997) follows a phylogenetic and phenetic study of relationships using morphological characters (González-Elizondo et al. 1997). The González-Elizondo and Peterson classification (1997), the classification presented in the only world-wide monograph by Svenson (1939), and Kukkonen's (1990) classification are contrasted in Fig. 1.

MATERIALS AND METHODS

Plant Samples. Samples of taxa were selected from live material at Rancho Santa Ana Botanic Garden (RSA), and herbarium material at RSA, TAES, and UC. Voucher information and GenBank accession numbers are listed in Table 1. Outgroup choice was based on groups hypothesized to be related to *Eleocharis* (Goetghebeur 1985; Bruhl 1995; Kukkonen 1990; Muasya et al. 1998) and other genera of the Cyperaceae as available, including *Amphiscirpus*, *Bulbostylis*, *Fuirena*, *Schoenoplectus*, and *Websteria*. All infrageneric groups defined by González-Elizondo and Peterson (1997) were sought for these analyses, but section *Disciformes* and series *Multicaules* were unavailable.

DNA Sequencing. DNA was isolated using a modified 2X CTAB buffer method (Doyle and Doyle 1987; Porter 1997). Templates of the nrITS region were prepared using a 1:1 ratio of primers "ITS5i" (5'-AGG TGA CCT GCG GAA GGA TCA TT-3') and "ITS4i" (5'-GGT AGT CCC GCC TGA CCT GG-3'). Polymerase chain-reaction (PCR) amplifications follow the procedures described by Baldwin (1992) and Baldwin et al. (1995).

The PCR products were electrophoresed in an 0.8% agarose gel in a 0.5× TBE (pH 8.3) buffer, and subsequently stained with ethidium bromide to confirm a single product and purified using the PEG precipitation procedure (Johnson and Soltis 1995).

Sequencing was performed using an Applied Biosystems Model 373A Automated DNA Sequenc-

ing System and run on 6% polyacrylamide gels (Sequagel-6, National Diagnostics). Direct cycle-sequencing of purified template DNAs followed manufacturers specifications, using the PRISM[™] DyeDeoxy[™] Terminator Kit (Perkin Elmer). Sequencing of the ITS region made use of primers ITS5i, ITS4i, "ITS2" (5'-GCT GCG TTC TTC ATC GAT GC-3'), and "ITS3" (5'-GCA TCG ATG AAG AAC GCA GC-3'). The four sequencing primers provide sequences for overlapping fragments that collectively cover the entire spacer and 5.8S rDNA regions along both strands.

Automated DNA sequencing chromatograms were proofed, edited, and contigs were assembled using Sequencher 3.0 (Gene Codes Corporation, Inc.). The sequences were truncated to include only ITS1, 5.8S, and ITS2. Identification of the terminal ends of ITS1 and ITS2 were based on comparisons with other species of Cyperaceae (Roalson 1997). The sequences were initially aligned using Clustal W 1.4 (Thompson et al. 1994) using a gap cost:gap extension cost ratio of 10:5, followed by manual editing of the alignment (Baum et al. 1994).

Phylogenetic Analyses. Both maximum parsimony (MP) and maximum likelihood (ML) analyses were performed using PAUP* 4.0d64 (Swofford pers. comm.). Both analyses were performed using heuristic searches (acctrans; 100 random addition cycles; TBR branch swapping; steepest descent) due to the size of the data set under study. The Hasegawa-Kishino-Yano (1985) model of evolution with rate heterogeneity was used in the ML analysis and was optimized to the data set (transition/transversion ratio estimated; base frequencies estimated; proportion of invariant sites estimated; variable sites set as discrete approximation to the gamma distribution with the shape parameter estimated). Internal branch support for the parsimony analyses was estimated using 10,000 "fast heuristic" bootstrap replicates (Felstenstein 1985; Hillis and Bull 1993; Swofford PAUP* 4.0), and decay index (Bremer 1988; Donoghue et al. 1992).

RESULTS

The aligned ITS data set was 653 bp long with 373 variable sites, of which 279 were potentially parsimony-informative. The length of the unaligned ITS-5.8s region varied from 533 to 619 bp. Alignment resulted in 75 gaps, ranging in size from 1 to 25 bp. Seventeen were potentially phylogenetically informative. Alignment became difficult with some

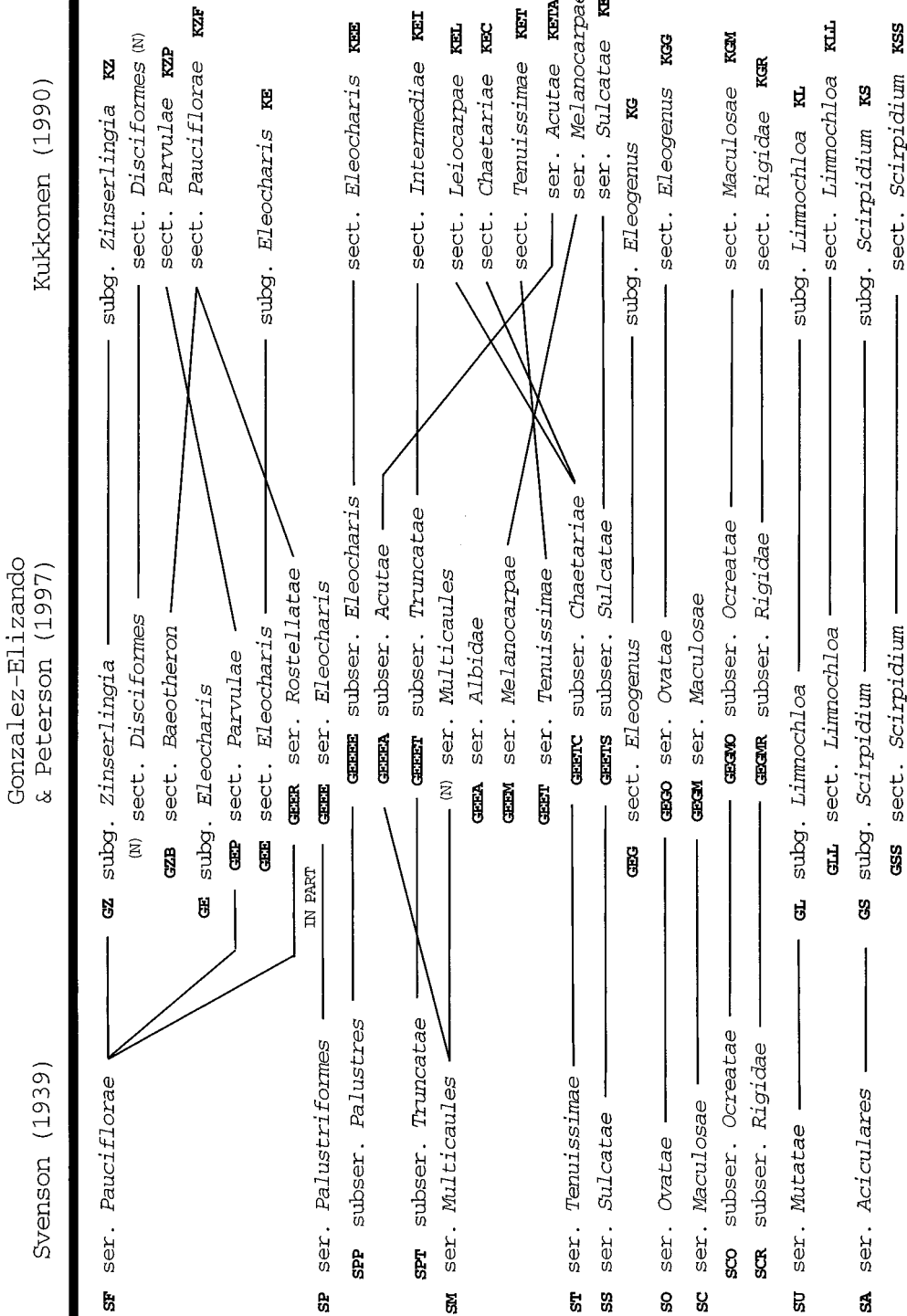


FIG. 1. Comparison of three infrageneric classifications of *Eleocharis*. Letter codes are associated with each taxon name and the codes are used in Figs. 4-6 to look at congruence of these classifications with the ITS phylogeny. Letter codes are independent for each classification. The symbol "(N)" refers to those groups not sampled in these analyses. Lines connect groups under different classifications which cover the same species.

TABLE 1. Voucher information for taxa sampled. GenBank accession numbers follow voucher information.

Species	Voucher
<i>Bulbostylis funkii</i> (Steud.) Clarke	E. H. Roalson 1384 (RSA); AF190616
<i>B. juncoides</i> (Vahl) Kükenth.	E. H. Roalson 1350 (RSA); AF190615
<i>Eleocharis acuta</i> R. Br.	A. E. Orchard 5316 (RSA); AF190582
<i>E. acutisquamata</i> Buckl.	T. H. Perry 1003-1A (TAES); AF190591
<i>E. albida</i> Torr.	L. G. Polasek 11 (TAES); AF190592
<i>E. atropurpurea</i> (Retz.) J. & K. Presl	T. R. Van Devender 93-1305 (RSA); AF190605
<i>E. baldwinii</i> (Torr.) Chapman	E. S. Nixon & S. D. & G. Jones 15776 (TAES); AF190599
<i>E. cellulosa</i> Torr.	F. R. Walker 2833 (TAES); AF190603
<i>E. dulcis</i> (Burm. f.) Trin.	S. R. Hill 17451 (TAES); AF190604
<i>E. engelmannii</i> Steud.	T. Starbuck 2913 (TAES); AF190593
<i>E. equisetoides</i> (Ell.) Torr.	L. C. Anderson 9505 (RSA); AF190584
<i>E. flavescens</i> (Poir.) Urban	J. Whipple 2768 (RSA); AF190586
<i>E. geniculata</i> (L.) Roem. & Schult.	E. H. Roalson 1371 (RSA); AF190589
	E. H. Roalson 1368 (RSA); AF190590
<i>E. interstincta</i> (Vahl) Roem. & Schult.	G. Crow 7493 (RSA); AF190611
<i>E. melanocarpa</i> Torr.	S. D. & G. Jones 3139 (TAES); AF190594
<i>E. microcarpa</i> Torr.	S. L. Orzell & E. L. Bridges 8369 (TAES); AF190600
<i>E. montevidensis</i> Kunth	V. W. Steinmann 873 (RSA); AF190608
<i>E. mutata</i> (L.) Roem. & Schult.	E. J. Lott 4116 (RSA); AF190587
<i>E. obtusa</i> (Willd.) Schult.	S. D. Jones 2133 (TAES); AF190595
<i>E. pachycarpa</i> Desv.	L. Ahart 5161 (TAES); AF190596
<i>E. pallens</i> S. T. Blake	D. J. McGillivray 2782 (RSA); AF190610
<i>E. palustris</i> (L.) Roem. & Schult.	S. D. & G. Jones 1409 (TAES); AF190602
<i>E. parishii</i> Britton	S. L. Hatch 6004 (TAES); AF190612
<i>E. parvula</i> (Roem. & Schult.) Link ex Bluff var. <i>anachaeta</i> (Torr.) Svenson	S. L. Orzell & E. L. Bridges 11323 (TAES); AF190597
	R. F. Thorne 60716 (RSA); AF190583
<i>E. pauciflora</i> (Lightf.) Link	G. K. Helmkamp s.n. (RSA); AF190606
<i>E. quinqueflora</i> (Hartmann) O. Schwarz	M.-A. Thiebaud s.n. (RSA); AF190607
<i>E. radicans</i> (A. Dietr.) Kunth	S. Boyd 8354 (RSA); AF190609
<i>E. robbinsii</i> Oakes	L. C. Anderson 9670 (RSA); AF190588
<i>E. rostellata</i> (Torr.) Torr.	W. R. Carr 10743 (TAES); AF190598
<i>E. tortilis</i> (Link) Schult.	L. E. Brown & W. Fleming 12186 (TAES); AF190601
<i>E. vivipara</i> Link	J. B. Nelson 732 (RSA); AF190614
<i>E. yecorensis</i> E. H. Roalson	E. H. Roalson 1385 (RSA); AF190585
<i>Fuirena simplex</i> Vahl	T. R. Van Devender s.n. (RSA); AF190617
<i>Scirpus acutus</i> Bigelow var. <i>occidentalis</i> (S. Wats.) Beetle "Schoenoplectus"	R. F. Thorne 54937 (RSA); AF190619
<i>S. americanus</i> Pers. "Schoenoplectus"	C. R. Annable 289 (RSA); AF190621
<i>S. californicus</i> (C. Mey.) Steud. "Schoenoplectus"	S. D. White 4733 (RSA); AF190620
<i>S. nevadensis</i> S. Wats. "Amphiscirpus"	G. Helmkamp s.n. (RSA); AF190618
<i>Websteria confervoides</i> (Poir.) S. Hooper	B. Bosserman s.n. (UC); AF190613

outgroup taxa (particularly *Amphiscirpus*, *Fuirena*, and *Schoenoplectus*).

Forty most-parsimonious trees (length = 1143 steps, CI = 0.53, RI = 0.67, RC = 0.36) were found from parsimony analyses of the ITS-5.8s *Eleocharis* data set. The strict consensus of these trees is presented in Fig. 2.

The ML analysis, while not run to completion, was run for more than 144 hours, completing

325,906 rearrangements. Three trees ($-ln = 6046.43276$) were found (Fig. 3). These trees are congruent with the parsimony strict consensus except for one branch (Figs. 2, 3). Parsimony places *E. baldwinii* and *E. microcarpa* (*Chaetariae*) as a grade leading to the clade containing *E. atropurpurea*, *E. flavescens*, and *E. geniculata*, whereas ML places *E. baldwinii* and *E. microcarpa* as a clade sister to the *E. atropurpurea*, *E. flavescens*, and *E. geniculata* clade.

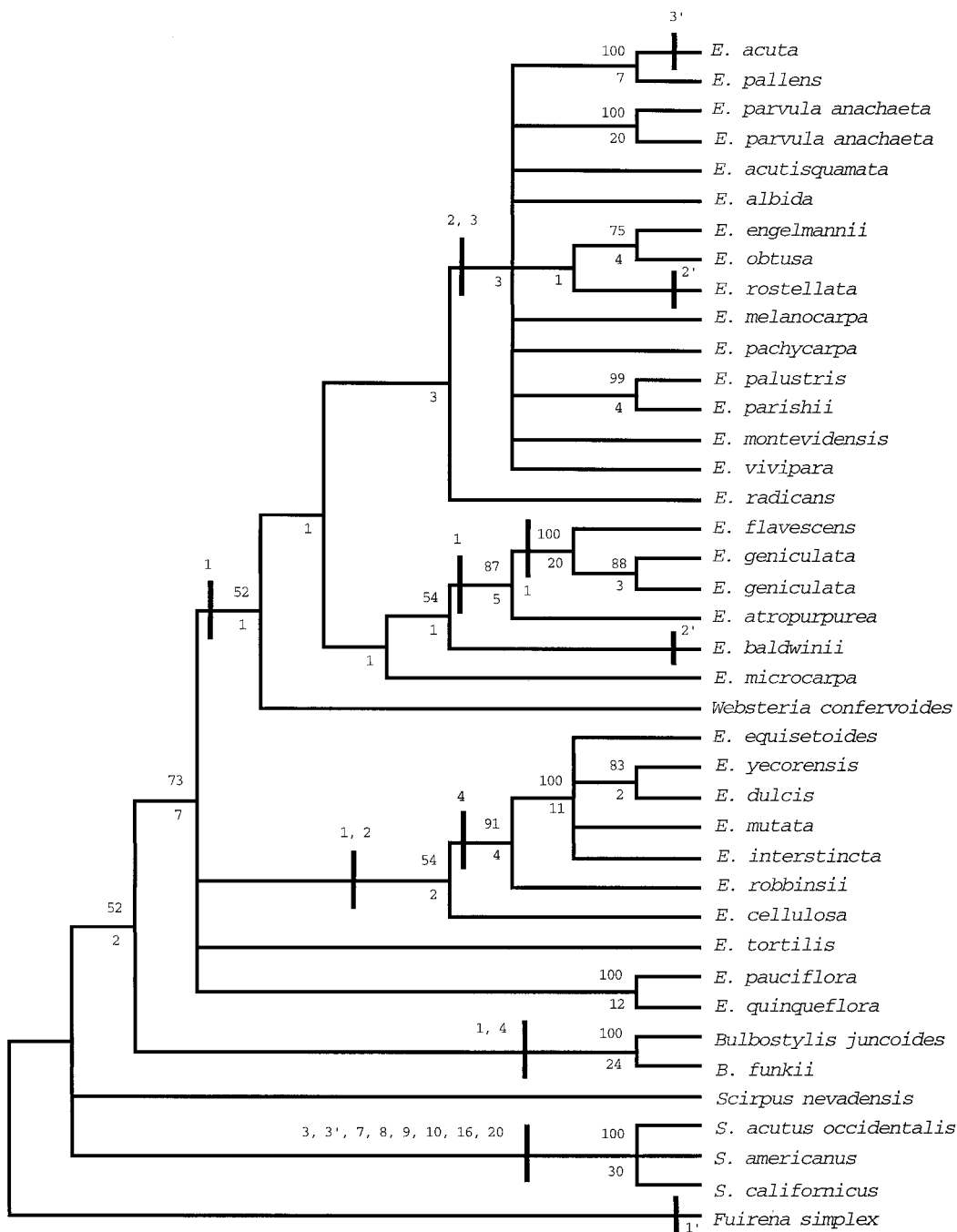


FIG. 2. Analysis of relationships within *Eleocharis*. Strict (maximum parsimony) consensus tree of 40 most parsimonious trees of 1143 steps (CI = 0.53; RI = 0.67; RC = 0.36). Numbers above branches refer to bootstrap percentages. Branches without bootstrap percentages noted had values <50%. Numbers below the branches refer to decay values. Bars across branches refer to gap locations. Numbers associated with the bars refer to gap size in bp. Those numbers with a "' " refer to those gaps superimposed onto a second location.

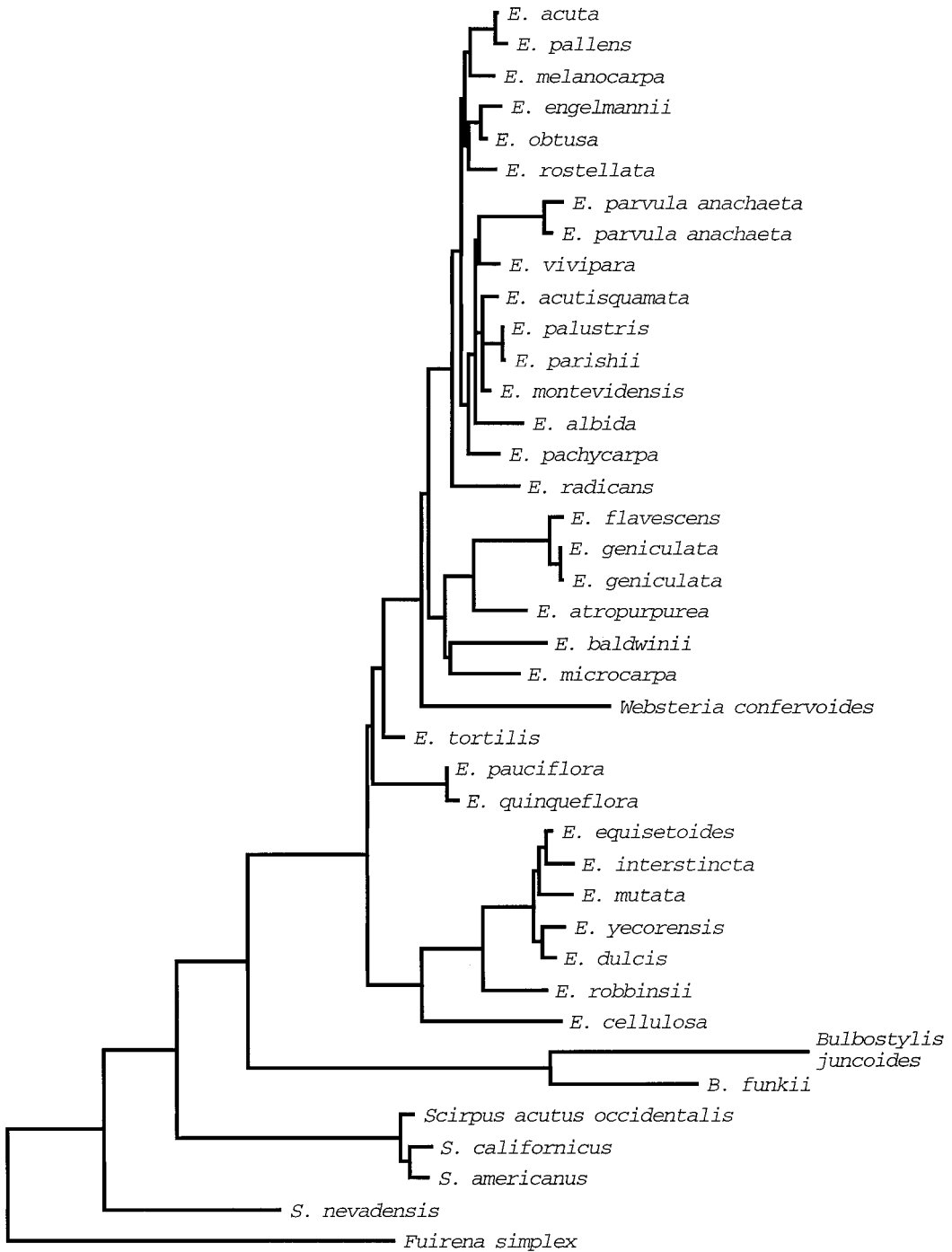


FIG. 3. Analysis of relationships within *Eleocharis*. Maximum likelihood tree ($-\ln = 6046.43276$).

While the three ML trees are not represented in the 40 most parsimonious, the differences in trees are minimal and do not involve well-supported branches.

Well supported nodes are found to support many relationships (Fig. 2), though support for branching structure is poor for "deep" branches. Gaps were not included in the phylogenetic analyses, but were superimposed on the ML tree as a measure of additional clade support. Seventeen of the 75 gaps lend additional support to branching structure of the ML tree (Fig. 3). Nine of these gaps were associated with branches leading to outgroup taxa. Three clades with strong bootstrap/decay support were also supported by gaps as well as three other clades (Fig. 2).

DISCUSSION

The results of this study allow us to address the following three aspects of *Eleocharis* evolution. First, *Eleocharis* displays morphological specialization and reduction, which has led to controversy surrounding sister relationships of the genus. This controversy will continue to be the case, even in light of molecular evidence. ITS data support some aspects of infrageneric classification, while they fail to support others. Morphological characters used to support previous classifications follow a similar pattern, with some characters being relatively homoplasy-free and are synapomorphies for clades, and others are homoplastic on the ITS phylogeny. It should be noted that support of relationships within *Eleocharis* is dependant on sampling. Because of the size of the genus, sampling is a necessity, and further sampling may alter weakly supported branches. Well supported branches (bootstrap $\geq 70\%$; clades defined by indels) are not expected to change with a sampling increase. Taxonomic groups described in the discussion as para- or polyphyletic are in reference to the current phylogenetic hypothesis presented in Fig. 2. It should be noted that many clades in this analysis do not have strong statistical support, and therefore are only a preliminary hypothesis of relationships.

The ongoing discussion of accepting paraphyletic taxa above the species level has not come to a conclusion (Cronquist 1987; Donoghue and Cantino 1988; Rieseberg and Brouillet 1994; Brummitt 1996, and others), but paraphyletic, and especially polyphyletic, superspecific taxa are generally not accepted (Donoghue and Cantino 1988). It is our con-

vention that infrageneric taxa should be monophyletic, and thereby reflect evolutionary relationships.

Sister Relationships. Several conclusions can be drawn from these analyses regarding sister relationships to *Eleocharis*. First, while the bootstrap support for *Websteria* being nested within *Eleocharis* is weak (52%), this is bolstered by a synapomorphic gap placing *Websteria* within *Eleocharis* (Fig. 2). The other two genera (both monotypic) that share a strong morphological resemblance to *Eleocharis*, *Chillania* (*C. pusilla* Roiv.) and *Egleria* (*E. fluctuans* L. T. Eiten), were not available for this study. *Chillania* is known only from the type collection in 1929, and the most recent material available of *Egleria* for this study was a paratype from 1912 (RSA). Their status, therefore, cannot be resolved at this time. It should be noted, however, that the morphological conditions found (branching culms; spikelets with a single flower) in these three genera are also found in *Eleocharis*. If anything, these genera only form the extreme end of a morphocline. Some suggest that the dimorphous branching culms of *Websteria* and *Egleria* are qualitatively different from the culms found in *Eleocharis* species that branch when growing in submerged conditions (González-Elizondo and Peterson 1997). We consider the specialized branching structure of these two segregate genera to be a stabilized extreme of this habitat-induced pattern. Extreme forms of culm branching can be found in the aquatic species of *Eleocharis*, *E. caillei* Hutchinson ex Hutchinson & Dalziel, *E. naumanniana* Boeckeler, and *E. subtilissima* Nelmes from Africa, and *E. egleroides* S. González & Reznicek from Venezuela (Nelmes 1952; González-Elizondo and Reznicek 1996). *Eleocharis caillei* and *E. naumanniana*, along with *E. capillacea* Kunth from Brazil, have spikes bearing a single flower, as is the condition for *Websteria* and *Chillania*. It should be noted that when Eiten (1964) described *Egleria*, she suggested that both *Egleria* and *Websteria* evolved from within *Eleocharis*. This is supported by the analyses presented here, at least for *Websteria*. The only difference becomes one of generic circumscription philosophy, not evolutionary relationships.

These analyses suggest *Bulbostylis* to be the most closely related lineage to *Eleocharis*, of the lineages included in this analysis, although this is weakly supported (bootstrap of 52%). Suggestions that *Schoenoplectus* (Bruhl 1995; Muasya et al. 1998) is the sister to *Eleocharis* are not supported. In addition to branch placement, ITS structure suggests *Schoenoplectus* (at least the representatives sampled) to be more distantly related to *Eleocharis*. Align-

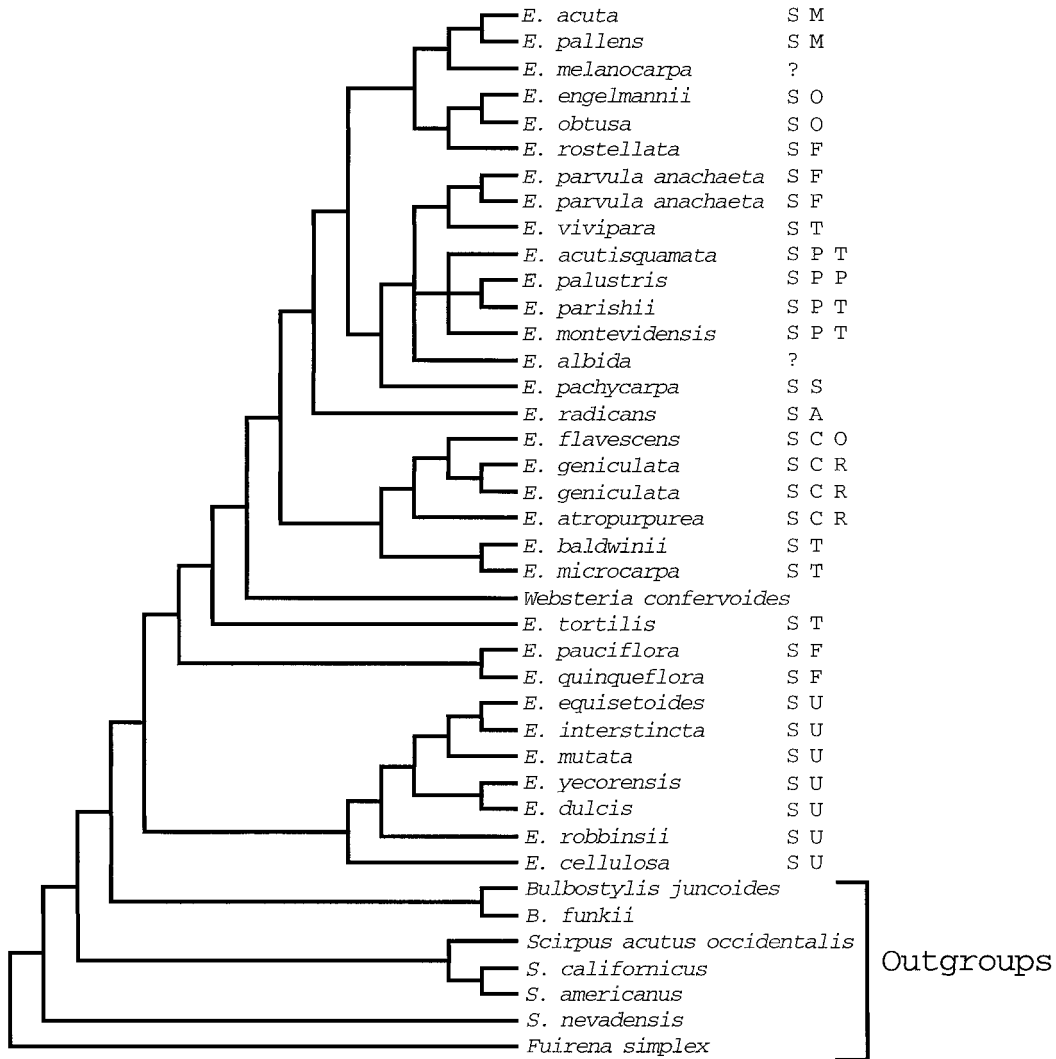


FIG. 4. Svenson (1939) classification groups superimposed onto the ML tree. Question marks refer to those taxa referred to as having uncertain placement in the classification. Letter codes refer to classification groups illustrated in Fig. 1.

ment of *Schoenoplectus* with *Eleocharis* was difficult and required the imposition of 23 or 24 gaps (in total 113/114/121 bp) in the *Schoenoplectus* sequences in order to align it with the *Eleocharis* sequences, as opposed to the imposition of only 16 or 17 gaps (in total 32/35 bp) in the *Bulbostylis* sequences. This does not preclude the possibility that some unsampled *Schoenoplectus* species may share more recent common ancestry with *Eleocharis* than *Bulbostylis*, especially in view of diverse opinions about the limits of *Schoenoplectus* and putatively related genera.

Infrageneric Classifications. The only worldwide revision of *Eleocharis* was completed by Svenson (1929, 1932, 1934, 1937, 1939). In this classification, he erected nine series with two series each having two subspecies (Fig. 1). When contrasted with the ITS ML tree (Fig. 4), the series *Pauciflorae* and *Tenuissimae* are polyphyletic and all other series are monophyletic, given the current sampling. Two of the four subspecies (*Rigidae* and *Truncatae*) are paraphyletic with respect to their associated subspecies in the series (*Ocreatae* and *Palustres*).

In the classification presented by Kukkonen

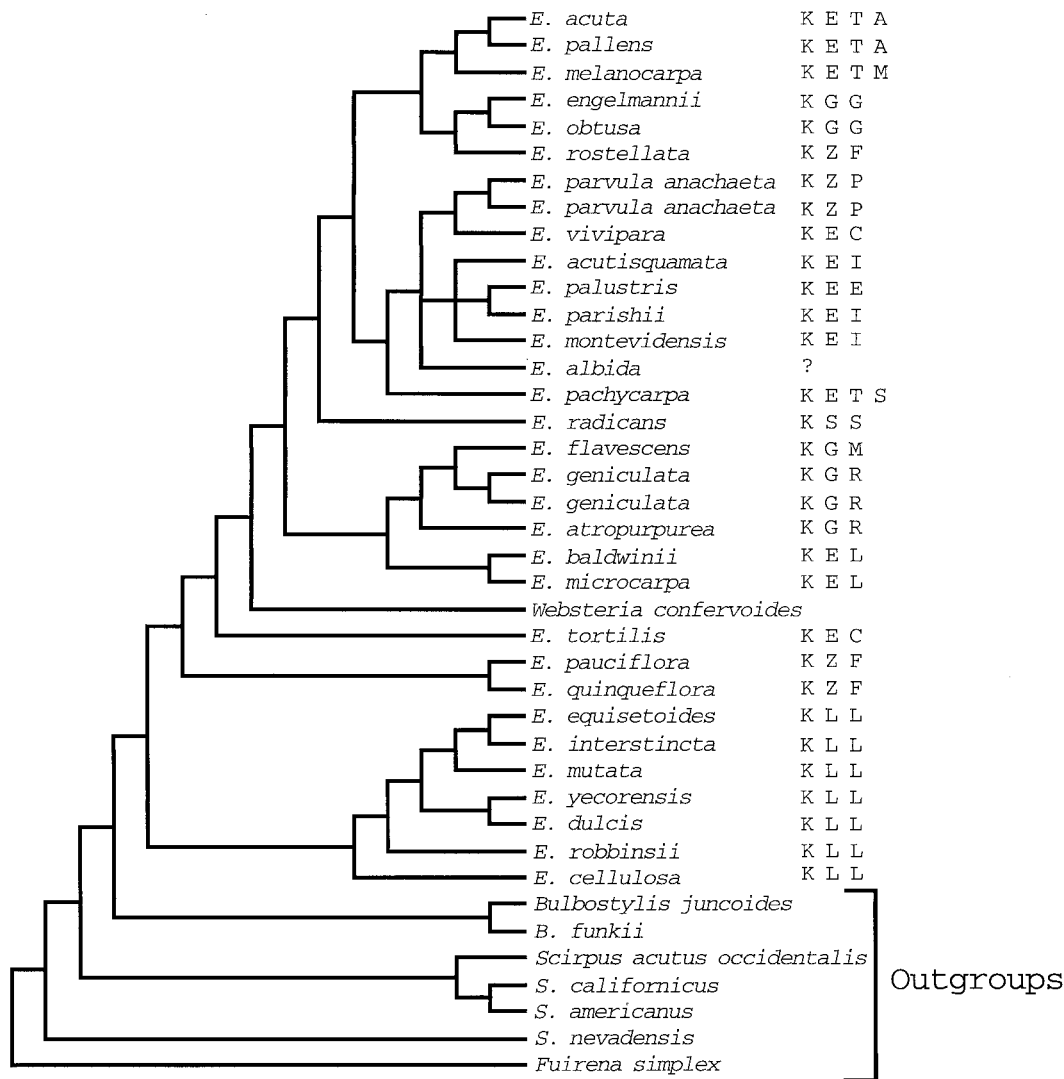


FIG. 5. Kukkonen (1990) classification groups superimposed onto the ML tree. Question marks refer to those taxa referred to as having uncertain placement in the classification. Letter codes refer to classification groups illustrated in Fig. 1.

(1990), five subgenera, 13 sections, and three series are defined (Fig. 1). When these taxa are examined in light of the ITS phylogenetic hypothesis (Fig. 5), subgenera *Eleocharis*, *Zinserlingia*, and *Eleogenus* are polyphyletic, sections *Chaetariae*, *Pauciflorae*, and *Tenuissimae* are polyphyletic, and sections *Intermediae* and *Rigidae* are paraphyletic. This classification supports a monophyletic subgenus *Limnochloa*, series *Acutae*, and sections *Eleogenus* and *Leiocarpae*. Subgenus *Scirpidium*, sections *Eleocharis*, *Maculosae*, and *Parvulae*, and series *Sulcatae* cannot be assessed

for monophyly given the present sampling, and series *Melanocarpae* was not sampled.

The most recent infrageneric classification proposed in *Eleocharis* is that by González-Elizondo and Peterson (1997). Circumscribed are 4 subgenera, 7 sections, 8 series, and 7 subseries (Fig. 1). This classification, as with the others, is congruent with the ITS phylogeny at certain levels (Fig. 6): subgenera *Zinserlingia* and *Limnochloa*, series *Maculosae* and *Ovatae*, and subseries *Acutae* are monophyletic in the ITS analysis. Subgenus *Eleocharis*, sections *Eleocharis*

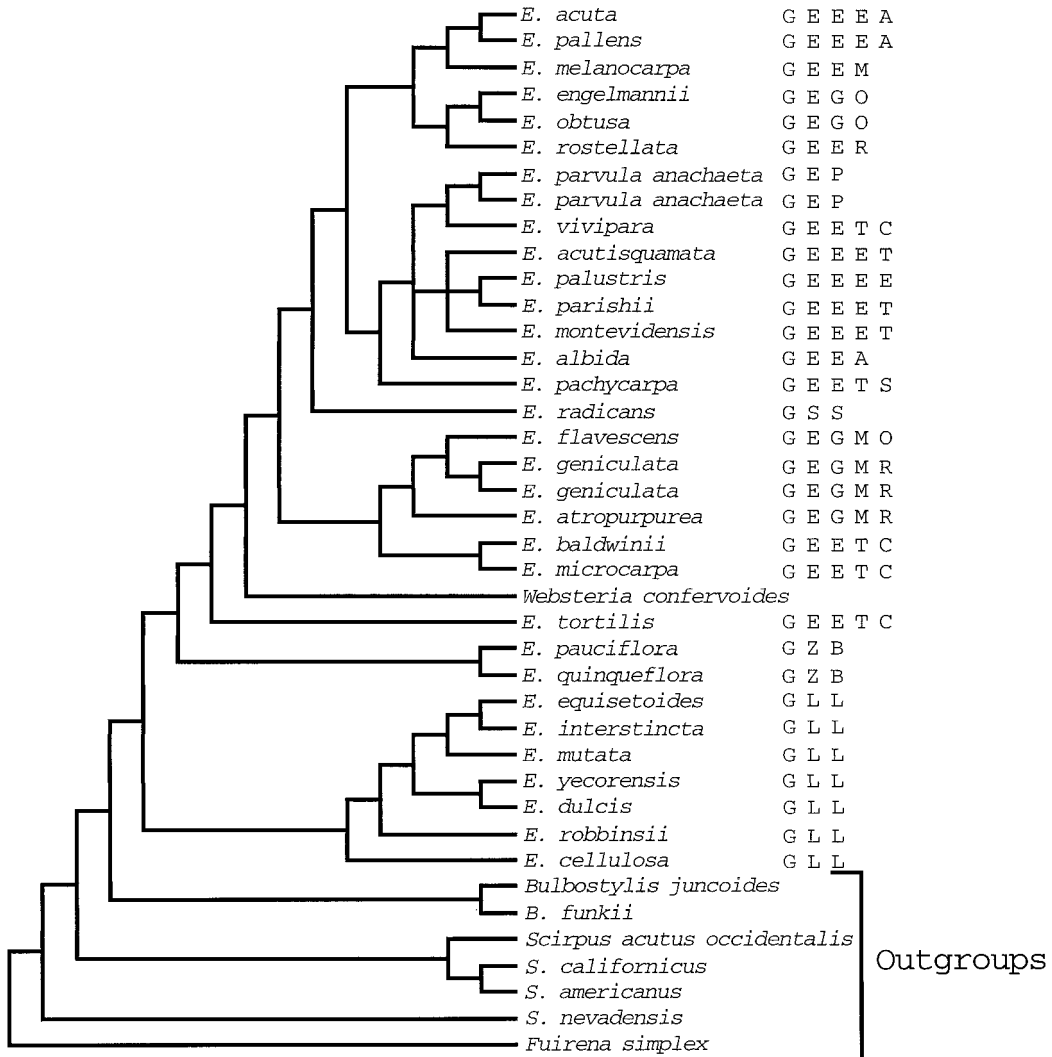


FIG. 6. González-Elizondo and Peterson (1997) classification groups superimposed onto the ML tree. Letter codes refer to classification groups illustrated in Fig. 1.

and *Eleogenus*, series *Eleocharis*, *Tenuissimae*, *Ovatae* and *Maculosae*, and subseries *Truncatae*, *Chaetariae*, and *Rigidae* are para-/polyphyletic in the ITS analysis.

Each of these classifications has a certain level of agreement with the ITS phylogeny. One grouping which is congruent among all of the classifications and the ITS data is the *Limnochloa/Mutatae* clade. Svenson (1939) presented the least complex of the three classifications discussed here. In his monograph, he does not state presumed relationships among series. This, in addition to the relative simplicity of Svenson's classification, effectively mini-

mized non-monophyletic groups in comparison to Kukkonen's (1990) and González-Elizondo and Peterson's (1997). The series *Pauciflorae* and *Tenuissimae* in Svenson's 1939 classification are the most discordant part of his classification in relation to the ITS analysis. Kukkonen (1990) reorganized Svenson's (1939) *Pauciflorae* and moved the portion he placed in section *Parvulae* to subgenus *Eleocharis*. González-Elizondo and Peterson (1997), based on morphological studies of Svenson's *Pauciflorae*, further excluded several species and placed them in series *Rostellatae* of subgenus *Eleocharis*. These two reorganizations of classification units agree with the ITS

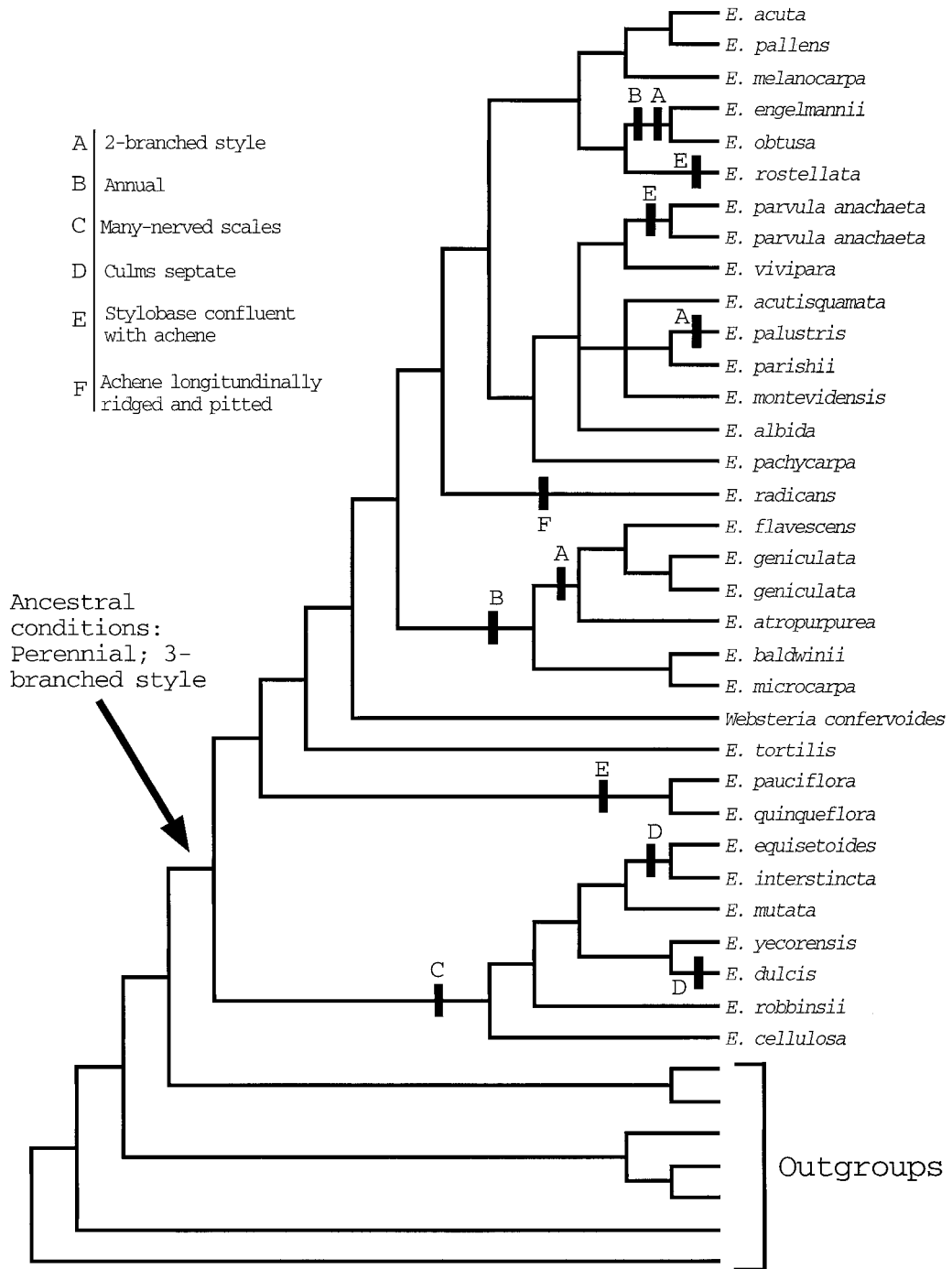


FIG. 7. Morphological characters superimposed onto the ML tree.

analysis and create a monophyletic subgenus *Zinserlingia*. The ITS data provide evidence that the *Tenuissimae*/*Chaetariae* are polyphyletic. This is one of the largest groups in the genus (41 species; González-Elizondo and Peterson 1997), and needs to be reevaluated in light of additional sampling. When defined by González-Elizondo and Peterson (1997), they do note this collection of species to be a "diverse group".

Morphological Evolution. Given the incongruence between the current classification and the ITS phylogeny, a question can be raised as to what relationships suggested by the ITS data are supported by morphological variation. Associated with the publication of the González-Elizondo and Peterson (1997) classification were phenetic and phylogenetic analyses of morphological variation. In the morphology-based phylogenetic study, representatives of all infrageneric taxa were included except one series (*Ovatae*) and two subseries (*Acutae* and *Ocreatae*). While the results of their cladistic analysis are not congruent with the results herein, neither are they congruent with their classification. In fact, it is unclear how the phylogenetic and phenetic studies impacted the classification. If the infrageneric taxa of González-Elizondo and Peterson (1997) are superimposed onto their phylogenetic tree, one subgenus is paraphyletic (*Eleocharis*), one section is paraphyletic (*Eleocharis*), and two series are polyphyletic (*Eleocharis* and *Tenuissimae*). Section *Eleogenus* could not be properly tested for monophyly on this tree since only one subseries of one of the series was included (series *Ovatae* and subseries *Ocreatae* are missing).

One of the major areas of incongruence between the ITS phylogeny and the classifications of Kukkonen and González-Elizondo and Peterson is the entangling of subgenus/section *Eleogenus* and subgenus *Eleocharis*. The primary characters separating *Eleogenus* from *Eleocharis* are annual duration in combination with 2-branched stigmas vs. annual or perennial duration with 3-branched stigmas. It is perhaps not surprising that these characters show homoplasy, given the variation in number of stigma branches within species (e.g., *E. quadrangulata* with two or three-branched stigmas), and the presence of several groups of perennials with two-branched stigmas (i.e. subseries *Eleocharis*). There is also some question as to the accuracy in assessment of duration (annual vs. perennial), as noted by González-Elizondo and Peterson (1997).

Several other morphological characters are superimposed on the ML tree (Fig. 7) including the

presence of septate culms, nuts with a confluent stylobase, many-nerved scales, and nuts with longitudinal ridges and pits. Given the distribution of character states in the phylogeny, the inferred ancestral condition is that of a perennial with 3-branched styles. Most of the characters show multiple changes in state, inferring homoplasy in these characters (given the ITS phylogeny). For instance, 2-branched styles show three independent origins, annual duration in two, and stylobase confluent with the nut in three origins. Characters inferred to have multiple origins by the ITS data, and qualitative traits such as texture, shape and color of nuts and scales, and shape and texture of sheath apices have been commonly used to define classification units in *Eleocharis*.

Several of the infrageneric taxa (regardless of rank) appear to be monophyletic, as assessed by the ITS phylogeny. These include the subgenera *Zinserlingia* and *Limnochloa* as currently defined (González-Elizondo and Peterson 1997), a clade containing portions of subseries *Chaetariae* and section *Eleogenus* excluding series *Ovatae*, and a clade containing the rest of subgenera *Eleocharis* and *Scirpidium* (Fig. 7). Large groups such as subseries *Chaetariae* (41 species), *Truncatae* (19 species), and *Eleocharis* (20 species) need much broader sampling given their combined coverage of nearly half of the species in the genus and their potential for polyphyly (i.e., *Chaetariae*). In an effort to achieve a classification which reflects evolutionary relationships, assessment of other genic regions and broader sampling of large infrageneric taxa is required to corroborate the ITS results. In addition, morphological variation needs to be reassessed in light of the implications of relationship made by the molecular phylogenetic data.

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