TAXONOMY, PHYLOGENY, AND INFLORESCENCE DEVELOPMENT OF THE GENUS IXOPHORUS (PANICOIDEAE: POACEAE)

Elizabeth A. Kellogg, 1 Kenneth M. Hiser, and Andrew N. Doust

Department of Biology, University of Missouri—St. Louis, One University Boulevard, St. Louis, Missouri 63121, U.S.A.

Patterns of morphological variation within the grass genus *Ixophorus* have led to uncertainty in its phylogenetic position and also to disagreement about the number of species in the genus. We use molecular phylogenetic, developmental, and morphometric approaches to address taxonomic and evolutionary problems in the genus. DNA sequence data for the gene ndhF place *Ixophorus* within the “bristle grass” clade, which includes *Setaria* and *Pennisetum*, and data for the trnL intron plus the trnL-F intergenic spacer show low levels of variation within the genus. Inflorescence development of *Ixophorus* was compared with that of several *Setaria* species to identify the stages of development that make *Ixophorus* so distinctive. *Ixophorus* is distinguished by (1) abaxially oriented tertiary axes that develop as spikelets, (2) a fixed, low number of orders of branching, (3) synchronous development of spikelets within an inflorescence, and (4) uniform elongation of primary and secondary axes late in development. However, these developmental character states are also shared with various other bristle grass clade members, making them unsuitable for circumscribing the genus. Specimens representing the geographical and morphological ranges of *Ixophorus* were used to detect groupings based on morphological variation. Principal components analyses, as well as many separate variables, weakly correlated morphological variation with latitude but supported recognition of a single species, *Ixophorus unisetus* (J. Presl) Schltdl. Common garden experiments show that much morphological variation results from plasticity. A formal taxonomic revision of the genus is presented.

*Keywords: Ixophorus, development, inflorescence, Poaceae, Setaria, morphometric, plasticity, Paniceae.*

**Introduction**

Parallelisms, reversals, continuous variation, and environmental plasticity all plague the use of morphological characters in systematics. Comparisons of adult morphologies can often be misleading because unrelated taxa may arrive at an apparently similar adult form through different developmental processes. Conversely, organisms may share similar developmental patterns and evolutionary histories but look quite different as adults because of one or a few divergences in developmental pattern.

The grass genus *Ixophorus* (J. Presl) Schltdl., a native of Mexico and Central America, presents taxonomic and phylogenetic problems arising from the interpretation of morphological characters and from variation patterns. In this study, we use a combination of molecular phylogenetics and developmental description to determine which adult characteristics are synapomorphic and which are homoplastic, thus addressing the phylogenetic problem. We use morphometrics to evaluate variation among nominal species and combine this with common garden experiments to identify the extent of environmental plasticity. This leads to an improved classification and also to a deeper understanding of the pattern of morphological diversification.

*Ixophorus* can be recognized at a distance by its open, pyramidal inflorescences with somewhat ascending branches, brown to dark reddish in color. Distinctive vegetative characters include laterally compressed, glabrous leaf sheaths, which are often purple toward the base. The spikelets are arranged in two ranks along the abaxial side of the scabrous primary inflorescence branches (fig. 1). Each spikelet shares a pedicel with a single glabrous or sometimes scabrous bristle, generally interpreted as a sterile inflorescence branch (Doust and Kellogg 2002a and references therein). The bristles vary from one to three times as long as the spikelets.

*Ixophorus* exhibits a distinctive combination of morphological characters that indicate affinities to different genera. It is a member of the speciose subfamily Panicoideae, tribe Paniceae, with which it shares bifloral, dorsiventrally compressed spikelets. Previous authors have placed species of *Ixophorus* in or near the genera *Urochloa* P. Beauv., *Panicum* L., or *Setaria* P. Beauv., depending on which morphological characters were emphasized. Presl (1830) first described *Ixophorus unisetus* as a species of *Urochloa* because of its inflorescence architecture, its mucronate upper lemma, and the long hairs at the base of each spikelet. Von Schlechtendal (1862) observed that the “hairs” are actually sterile branches, similar to those found in *Setaria*; he therefore erected the genus *Ixo- phorus*. Both Presl (1830) and von Schlechtendal (1862) observed that the lower palea of the *Ixophorus* spikelet is about equal in length to the lower lemma, although neither considered this character to be of taxonomic value. This feature is

1 Author for correspondence; e-mail tkellogg@umsl.edu.

*Manuscript received December 2003; revised manuscript received June 2004.*
found only in a few other Paniceae, including Steinchisma Raf. s.l., Otachyrium Nees, and Plagiantha Renvoize, all of which form a clade in molecular phylogenetic studies (Giussani et al. 2001; Aliscioni et al. 2003).

Recent phylogenetic studies of Panicoideae (Gómez-Martínez and Culham 2000; Duvall et al. 2001; Giussani et al. 2001; Aliscioni et al. 2003) have shown that the putative relatives of *Ixophorus* are widely separated in the phylogeny (fig. 2). Paniceae are paraphyletic and are divided into two clades characterized by different basic chromosome numbers. Both *Setaria* and *Urochloa* belong to the $x = 9$ Paniceae, while the “expanded lower palea” clade is in the $x = 10$ Paniceae. *Setaria* is in a clade with all other species having inflorescence branches modified into sterile bristles (the “bristle grass” clade); these exhibit $C_4$ photosynthesis of the NADP-ME subtype and have a single sheath surrounding each vascular bundle in the leaf (Watson and Dallwitz 1992). *Urochloa* and its relatives are members of a clade that share $C_4$ photosynthesis of the PEP carboxykinase (PCK) subtype; these have a double sheath surrounding each vascular bundle. Members of the expanded lower palea clade are all $C_3$.

The taxonomic history and the phylogeny thus identify several characters that might indicate phylogenetic placement of *Ixophorus*. If the inflorescence bristles of *Ixophorus* are truly sterile branches, then the genus belongs in the bristle clade, whereas if they are actually elongate hairs, then the

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**Fig. 1** *Ixophorus unisetus*. a, Portion of inflorescence branch, showing sterile bristles and cup-shaped pedicels from which spikelets have been removed. b, c, Spikelet at anthesis viewed from the abaxial (b) and adaxial (c) sides. d, Upper (pistillate) floret at anthesis. e, f, Spikelet in fruit viewed from the abaxial (e) and adaxial (f) sides. g, Upper floret in fruit showing macro and horseshoe-shaped germination flap. Reproduced from Scribner (1897).

**Fig. 2** Summary of the phylogeny of Panicoideae, based on results of Giussani et al. (2001). Possible placements of *Ixophorus* are indicated by shaded triangles.
genus may be related to Urochloa. A chromosome number based on x = 9 would argue for placement with either Setaria or Urochloa, whereas one based on x = 10 would link the genus to the expanded lower palea clade. The one reported number is 2n = 62–66 (Pohl and Davide 1971), which may indicate a base number of x = 9, although this is not certain. Leaf veins in Ixophorus are closely spaced, indicating C₄ photosynthesis, and each vein has a single bundle sheath, consistent with the NADP-ME subtype (Watson and Dallwitz 1992). In leaf anatomy and presumed photosynthetic pathway, therefore, Ixophorus is similar to Setaria and other members of the bristle clade.

In addition to the problems of generic relationships, taxonomists disagree on the number of species and varieties that should be recognized within Ixophorus. One species, Ixophorus unisetus (J. Presl) Schult., occurs throughout the range of the genus. Some authors also recognize a second species, I. pringlei (Vasey) Scribn. and a variety of it, var. minor Scribn. in west-central Mexico, at the northern limit of the genus. These northern taxa include smaller plants than I. unisetus and have been recognized as distinct by Beetle (1983) and Serna and Ferrari (2000), as well as many earlier authors (see “Taxonomic Treatment”). Differences in plant height, culm width, leaf length, leaf width, inflorescence length, number of inflorescence branches, inflorescence branch length, and bristle length were used as characters separating taxa (Vasey 1893; Scribn 1897). Other authors (Fournier 1886; Kuntze 1891; Herrmann 1911) used the number of nerves on the second (i.e., upper) glume as a species-level character within the genus. On the other hand, Hitchcock (1919), after observing a large sample of Ixophorus in the field and in the herbarium, concluded that the genus is monotypic, a conclusion followed by some recent authors (McVaugh 1983; Watson and Dallwitz 1992).

The variation observed in the field could reflect morphologically continuous variation, or conversely, covariation of multiple characters defining discrete groups. This can be tested by multivariate methods such as principal components analysis (PCA) (Naçi et al. 1998; Coronado 2003). The variation could also come from environmental plasticity, which can be tested by common garden experiments.

Here we determine the phylogenetic placement of Ixophorus within Paniceae and present a developmental description of its inflorescence. This is compared with developmental sequences of the closest relatives of Ixophorus to identify the stages common between them and those at which Ixophorus follows its own developmental trajectory. We also present results of common garden experiments and morphometric analyses describing infraspecific variation in characters of adult morphology. These data demonstrate that the genus should be considered monotypic.

**Material and Methods**

### Field Collections and Specimens

Collections were obtained from one locality in Costa Rica (July 2000), five in Nicaragua (August 2000), and seven in Mexico (October 2000). This sample represents most of the geographic range of Ixophorus. Each field collection included silica gel–dried leaf material, Caryopses collected in paper envelopes, and herbarium voucher specimens. Collections were also prepared of greenhouse-grown specimens. Vouchers were deposited in the Missouri Botanical Garden herbarium (MO), with duplicate specimens deposited at CR, MEXU, and UCAM. All specimens of Ixophorus from IBUG, MO, NY, and US were obtained and studied, a total of 411 specimens. (A list of specimens examined is available from the first author on request.)

To examine environmental plasticity, Caryopses were collected from plants in eight different localities in either Nicaragua or central Mexico. Two or three plants from each collection were grown in the University of Missouri—St. Louis greenhouse.

**Molecular Phylogenetics**

DNA was extracted from leaves of K. Hiser 20 (collected in Costa Rica), K. Hiser 32 (Nicaragua), K. Hiser 33 (Nicaragua), K. Hiser 38 (Jalisco, Mexico), and K. Hiser 38A (greenhouse grown from seeds of K. Hiser 38) using a CTAB miniprep protocol (Doyle and Doyle 1987; Giussani et al. 2001). NADH dehydrogenase (ndhF) was amplified from the specimen K. Hiser 38 using the polymerase chain reaction (PCR) and primers, as reported by Giussani et al. (2001). To evaluate possible intragenic variation, the intergenic spacer and intron region of transfer RNA Leu and tRNA Phe (trnL-F) was amplified for all five specimens with primers c and f described by Taberlet et al. (1991). The trnL-F sequence from K. Hiser 38A has been deposited in GenBank as AT571192.

PCR products were purified using either gel purification or QIAquick PCR purification columns (Qiagen, Valencia, Calif.) and quantified versus a pGEM ladder (Applied Biosystems, Foster City, Calif.). Sequencing reactions were performed using “Big-Dye” cycle sequencing, and the products were run on an ABI 377 automated sequencer (Applied Biosystems).

The sequence of ndhF for Ixophorus unisetus has been deposited in GenBank as AT623749. Sequences were assembled into contigs and edited using Sequencher, version 3.1, (Gene Codes Corporation, Ann Arbor, Mich.). These were then aligned to the data set of Giussani et al. (2001) using Clustal W (Thompson et al. 1994) and further aligned manually with Se-Al, version 1 (Rambaut 1996). The ndhF data were analyzed with PAUP, version 4.0b10 (Swoford 2001). Initial parsimony searches used 1000 random addition sequences with TBR branch swapping, but with the MulTrees option turned off, and identified a set of trees of length 1453 steps. These were then used as starting trees in a maximum parsimony heuristic search with MulTrees turned on. Character states were treated as unordered, and gaps were treated as missing data. Full heuristic bootstrap analyses using 500 replicates were performed using the same parameters as the initial analysis (Felsenstein 1985), but with MaxTrees set to 500. The ndhF analysis placed Ixophorus in the bristle grass clade (see “Results”), so subsequent analyses focused solely on this group.

For more rigorous analysis of relationships within the bristle clade, the Ixophorus sequence for ndhF was aligned to sequences for 25 members of the bristle grass clade, obtained from Giussani et al. (2001) and from Doust and Kellogg...
In addition, we obtained new sequences for *Cenchrus agrimonioides* Trin. (AY623745; voucher Morden 1534, University of Hawaii), *C. pilosus* Kunth (AY623746; voucher K. Hiser 57, MO), *Setaria italica* (L.) P. Beauv. (AF499140; voucher PI 315090, Doust 1361, MO), and *Setaria grisebachii* E. Fourn. (AF499141; voucher PI 229164, Doust 1363, MO). Urochloa acuminata (Renvoize) Morrone & Zuloaga, *Panicum repens* L., and *Panicum virgatum* L. were included as outgroup taxa, following Giussani et al. (2001). Data were submitted to MrModeltest (Nylander 2002) to determine the best-fit model of evolution for maximum likelihood analyses. This found that the best-fit model assumed bases at equal frequency, two substitution types, a TR ratio of 1.8696, and no among-site rate variation. Using these settings, the data were analyzed by maximum likelihood with 10 random addition sequences. Data were also analyzed using maximum parsimony with heuristic searches, uninformative characters excluded, gaps treated as missing data, characters equally weighted, 1000 random addition sequences, and MaxTrees set to increase automatically as necessary. Support was assessed by nonparametric bootstrap analysis with 500 replicates and the same parameters.

**Inflorescence Development**

Vegetative meristems and developing inflorescences were dissected from freshly harvested greenhouse-grown plants and fixed in either FAA (formalin-acetic acid–70% ethanol, 10 : 5 : 85 v/v/v) or PFA/glutaraldehyde (phosphate-buffered 4% paraformaldehyde followed by phosphate-buffered 4% glutaraldehyde). FAA-fixed specimens were hydrated in an ethanol series (70%, 50%, 30%, 15%, H2O). FAA and PFA/glutaraldehyde specimens were then infused with osmium tetroxide (OsO4) following the OTOTO (or OTO) method of Murphy (1978). This treatment consisted of overnight fixation in 1% OsO4, six washes with deionized (DI) water, 30 min in fresh 1% thiocarbohydrazide (TCH); six DI water washes, 1 h in 1% OsO4, six DI water washes, 30 min in 1% TCH; six DI water washes, 1 h in 1% OsO4, and a final six DI water washes. For some specimens, the final TCH and OsO4 steps were omitted. Specimens were then dehydrated using an ethanol series (15%, 30%, 50%, 70%, 80%, 90%, 95%, 100%, 100%) and were critical-point dried in an SPI Jumbo critical-point drier. Some specimens required sputter-coating with gold using a Polaron E5000 sputter-coater (Quorum Technologies, Hailsham, U.K.) to reduce charging. Specimens were photographed using a Hitachi S450 or an Amray AMR1000 scanning electron microscope at 20 kV.

**Morphological Variation**

Measurements for morphometric study were taken from 95 plants, including 50 herbarium specimens, 25 field collections made for this study, and 20 greenhouse-grown plants (collection information listed by Hiser [2002]). We chose specimens from throughout the geographical and morphological ranges of *Ixophorus*, on which all or most of the characters could be measured. Of the 75 field-collected specimens, 46 were from Central America and southern Mexico and 29 were from central Mexico, in the general region in which *Ixophorus pringlei* is postulated to be endemic. Five of the greenhouse plants were grown from Central American caryopses and 15 from Mexican caryopses.

Twenty-four characters, including six vegetative, eight inflorescence, and 10 spikelet characters were measured (21 continuous) or counted (three meristic) (table 1). Eighteen characters were recorded as length measurements for one sample per specimen, two were mean values of multiple measurements, three were meristic, and one was a ratio. Length measurements of structures greater than 1 cm long were made with a standard metric ruler. Characters less than 1 cm long were measured using an ocular micrometer on a dissecting microscope at 20× magnification. For the three meristic characters (number of inflorescence branches, number of bristles per branch, and number of nodes along the mature culm), one of each structure was counted per specimen. Bract : caryopsis is a ratio of the lengths of these two structures. Anther length represents the mean of six anthers measured from two spikelets per plant. Bristle length is the mean length of ten bristles from different parts of a single inflorescence. For all spikelet characters, two spikelets were dissected and measured per plant, and the recorded values are the means of these.

Forty specimens were measured for all 24 characters. Occasional missing values were replaced with the mean for the measurements of those that were measured. Mean bristle length and the lengths of the first two inflorescence internodes varied as much within single specimens as among all specimens, so these characters were excluded, as was bract : caryopsis ratio, which could only be measured on a few specimens (see “Results”). Preliminary analyses indicated that spikelet characters varied little, so they were excluded, and an additional 55 specimens were measured for the remaining 11, mostly vegetative, characters.

PCAs used SPSS, version 10.1 (SPSS 2001). Each analysis was performed with and without Varimax rotation. Without rotation, the program defines the first component as that explaining the highest percentage of variation and the second component as orthogonal to the first and explaining the highest percentage of the remaining variation. The Varimax option optimizes the percentage variation explained between the first two components, while maintaining the same total percentage explained by the two as the unrotated solution. In many analyses, rotation improves interpretability of the results (Quinn and Keogh 2002). However, we observed little difference between rotated and unrotated solutions, so only unrotated solutions are shown here.

Two data sets were analyzed. The first used the 40 specimens for all 20 included characters. The second used 95 specimens for the 11 nonspikelet characters. We also conducted an analysis that included only the characters by which Scribner (1897) differentiated *I. pringlei* from *I. munisetus* (plant height, leaf length, leaf width, inflorescence length, inflorescence branch length, and number of inflorescence branches).

**Results**

**Phylogenetic Placement**

*Ixophorus* was placed unambiguously as a member of the bristle clade in the x = 9 Paniceae, with a bootstrap value of
100% (fig. 3). TrnL-F sequences from five plants of Ixophorus, collected in Nicaragua, Costa Rica, and Mexico, were all identical, indicating that there is little variation among members of I. unisetus s.l. in terms of the chloroplast.

The maximum likelihood tree for the bristle clade alone was topologically virtually identical to one of the maximum parsimony trees (fig. 4). Parsimony analyses found 12 trees on two islands, with 162 steps, consistency index (CI) = 0.67, and retention index (RI) = 0.83. Pennisetum + Cenchrus form a well-supported clade (parsimony bootstrap = 86%). Setaria falls into two clades, one with a bootstrap of 100% and the other, which includes Ixophorus, with low support. Within the latter Setaria clade, I. unisetus and Setaria grisebachii are sister to each other, although with low support (54%), and together they are sister to Setaria lachnea and a strongly supported clade of Setaria italica, Setaria verticillata, and Setaria viridis. Paspalidium and Stenotaphrum are consistently placed together, but their position is not stable within the clade.

Leaf Anatomy and Chromosome Number

Freehand sections of leaves of Ixophorus showed that the veins were close together and had only a single bundle sheath. We were unable to obtain precise chromosome counts.

### Inflorescence Development and Adult Morphology

The vegetative meristem produces distichous leaf primordia (fig. 5a). After the transition to flowering, the primary axis of the inflorescence elongates, and secondary axis primordia appear spirally along it (fig. 5b). Once all secondary axis primordia have been initiated, these elongate (fig. 5c) and initiate tertiary axis primordia in two abaxial rows (fig. 5d). During this phase, the secondary axis becomes flattened and widened dorsiventrally (figs. 5e, 6a).

The tertiary axis primordia divide in a plane parallel to the secondary axis, yielding two distinct primordia (fig. 6b, 6c). This division proceeds acropetally along the secondary axis, and the inner primordium (closer to the midline of the secondary axis) that results is slightly wider and less elongate than the outer (lateral) primordium (fig. 6d, 6e). The inner primordium becomes a spikelet, while the outer primordium becomes the bristle lateral to it (fig. 6f).

Differentiation of spikelets is more or less synchronous throughout the inflorescence (fig. 7a). First, the spikelet meristem extends and glume primordia appear laterally (fig. 7b). The meristem then produces two floret meristems, each producing a lemma primordium (fig. 7c). The lower floret produces a single gyroecial primordium surrounded by three stamen primordia (fig. 7d). The stamens eventually overcome...
the gynoecium (fig. 7e), which never develops. In the upper
floret, the same four organ primordia appear (fig. 7e), but in
this case the gynoecial primordium survives while the three
stamens do not develop. During organ differentiation, all
glomer, lemmas, and paleas of the spikelet form but remain
relatively diminutive (fig. 7b–7e). As the florets continue
to develop, the lemmas, paleas, and glumes expand to enclose
the floral organs, and the adjacent bristles elongate (fig. 8a).
Bristles are described as glabrous by most authors, but all in
this study were actually minutely scabrous.

Late in development, the primary and secondary axes elon-
gate to produce the adult inflorescence (fig. 8b). All spikelets
develop completely, except for a few at the bases of the low-
est secondary axes; individual bristles develop in this posi-
tion, but without their associated spikelets (fig. 8c). Each
secondary axis is terminated by a bristle that is slightly long-
er and thicker than those associated with spikelets. Mature
spikelets consist of two florets, the lower of which is stami-
nate and the upper pistillate.

The putative bristle described in Urhochoa is indeed a mac-
rohair (fig. 9) and is thus quite distinct from the bristles in Ix-
ophor. A bristle early in development is shown in figures 7b
and 9. Comparison of the two figures shows that the bristle is
appreciably more than 100 μm thick even at this stage of de-
velopment and is clearly made up of many cells. The macro-
hair, in contrast, is much less than 100 μm in thickness and is
comprised of no more than two or three cells. Because the
bristle is a sterile branch, it is homologous to the entire axis
in figure 9. The upper lemma of Ixophorus does have a short
mucro and is ornamented, similar to the palea (fig. 10a, 10b).

**Morphometrics**

Three morphological characters—first internode length,
second inflorescence internode length, and mean bristle

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**Fig. 3** The ndhF phylogeny of Panicoideae showing strong support for Ixophorus as a member of the bristle clade. One of 18,000 equally parsimonious trees, length = 1453 steps, consistency index = 0.436, retention index = 0.778. Branches that collapse in the strict consensus are indicated by an asterisk. Bootstrap support values (500 replicates) are indicated above the branches. Some strongly supported major clades (e.g., Andropogoneae, Digitaria, Panicum s.s.) have been collapsed into a single terminal taxon for clarity, but their bootstrap values are shown.
length—varied as much within individuals as among all specimens and so were discarded. The embryo : caryopsis ratio was also omitted because not enough specimens had mature fruits for measurement.

PCA using 40 specimens and the 20 included characters produced five components with eigenvalues greater than 1, which together explained 80% of the total variance in the matrix. Characters related to overall plant size, including culm width, leaf length, number of inflorescence branches, inflorescence length, and inflorescence branch length, all had high coefficients on the first component, which explained 40% of the variance. The second component reflected mostly variation in spikelet characters, notably length of the second glume and second lemma, and explained 21% of the variance. Plant height, number of nodes, and anther length loaded heavily on the third component.

Scatter plots of PC1 versus PC2 and PC1 versus PC3 do not show any clear groups of specimens (fig. 11). We labeled the specimens according to geographic origin, with plants from Central America plus Chiapas in one group (open squares) and plants from central Mexico in another (filled triangles). Although the very smallest plants (left side of scatter plots) are from Mexico and the largest (right side of plots) are from Central America, the two geographic groups overlap extensively.

PCA using all specimens and 11 vegetative and inflorescence characters found two components with eigenvalues greater than 1.0. The first component explains 65% of the variance. Plant height, number of nodes, and anther length loaded heavily on the third component.

Superimposed on the topology are branch lengths from one of the 12 equally parsimonious trees (length = 162 steps, consistency index = 0.67, retention index = 0.83). Numbers below the branches are parsimony bootstrap values based on 500 replicates.

Fig. 4  Phylogeny of the bristle clade based on ndhF data. The topology shown is the maximum likelihood topology; ln likelihood=5025.5734. Superimposed on the topology are branch lengths from one of the 12 equally parsimonious trees (length = 162 steps, consistency index = 0.67, retention index = 0.83). Numbers below the branches are parsimony bootstrap values based on 500 replicates.
variance, and the second component explains a further 9%. Remaining components had eigenvalues less than 1; each explained less than 6% of the variation, and none separated groups of specimens. As in the previous analysis, the first component appears to be a general size factor affecting the overall robustness of the plant, and the second largely reflects plant height and the number of stem nodes produced, similar to the third component of the analysis with all characters. Similar to the previous analysis, plots of specimens on the first two component axes did not show any discrete groups, although there was a general geographic trend of increasing size from north to south (low values to high values on the first component) (fig. 12).

Included in the PCAs are a number of field-collected plants plus their greenhouse-grown offspring. Plants grown from Central American seed (open squares) were large, similar to their parents (filled squares), and fall near each other in multivariate space. Plants grown from seed from Jalisco (open triangles), however, were notably larger than their parents (filled triangles) and fell near the Central American plants in the PCAs.

The characters used by Scribner to distinguish species in *Ixophorus* all covaried, and all gave high loadings along the first principal component, with coefficients of 0.609–0.877 (40 specimens, all characters) and 0.708–0.941 (95 specimens, nonspikelet characters). Analysis of those characters

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**Fig. 5** *Ixophorus unisetus* early inflorescence development. *a*, Vegetative meristem with distichous leaf primordia. *b*, Elongated primary inflorescence axis soon after transition to reproductive phase, with spirally arranged secondary axis primordia initiated. *c*, Elongated secondary axes with tertiary axis primordia beginning to initiate. *d, e*, Initiation of tertiary axis primordia abaxially along secondary axes. *lf* = leaf; *vm* = vegetative meristem; 1 = primary axis; 2 = secondary axis; 3 = tertiary axis; scale bar = 100 μm.
alone produced results similar to those of the previous PCAs (not shown).

**Discussion**

**Implications of Phylogenetic Placement**

The *ndhF* phylogeny of the Panicoideae was similar to that reported by Giussani et al. (2001), with comparable bootstrap values and branch lengths. Some authors have doubted the monophyly of bristle-bearing grasses (Clayton and Renvoize 1986), but morphological (Zuloaga et al. 2000) and molecular phylogenetic studies have confirmed that they do indeed form a clade (Gómez-Martínez and Culham 2000; Giussani et al. 2001; Doust and Kellogg 2002a, 2002b) and thus that the bristle is synapomorphic. Inclusion of *Ixophorus* within the bristle clade reinforces this interpretation, even though its exact position in the clade is not resolved by our data.

Freehand sections of leaves of *Ixophorus* showed that the veins were close together and had only a single bundle sheath; both characters are diagnostic for C₄ NADP-ME photosynthesis (Hattersley and Watson 1976). Our

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**Fig. 6** *Ixophorus unisetus* inflorescence development. a, Dorsiventrally compressed secondary axis with tertiary axes initiated. b, Entire inflorescence with tertiary axes fully differentiated. c, Close-up of branch showing tertiary axis fission beginning with basal primordia. d, Close-up of branch showing early spikelet-bristle differentiation. e, Close-up of branch showing bristle primordium beginning to elongate. f, Inflorescence branch with spikelets and bristles differentiated and spikelet structures beginning to develop. 2 = secondary axis; 3 = tertiary axis primordium; b = bristle primordium; s = spikelet primordium; scale bar = 100 µm.
observations of leaf anatomy also confirmed that *Ixophorus* shares C4 NADP-ME anatomy with all other members of the clade. As in previous studies (e.g., Doust and Kellogg 2002a), relationships of the various subclades within the bristle clade are unresolved by our data.

Presl placed *Ixophorus* in *Urochloa* based on perceived similarities to *U. panicoides*, the only other *Urochloa* species in his treatment. He inferred that both taxa had bristles in the inflorescence. We show that this is an erroneous assessment of homology, confirming the observation of von Schlechtendal (1862). Presl also noted the similarity in inflorescence architecture between *Ixophorus* and *Urochloa*, but the inflorescence type appears in other members of the bristle clade and thus is not diagnostic for *Urochloa*.

The molecular phylogeny shows that the mucronate upper lemma is homoplasious and does not unite *Ixophorus* with *Urochloa*. The expanded lower palea is likewise homoplasious between *Ixophorus* and the expanded lower palea clade of Paniceae (Giussani et al. 2001). The upper lemma of *Ixophorus*, however, does have a short mucro (fig. 10a, 10b), similar to that found in some species of *Urochloa* (fig. 10c–10e) (Watson and Dallwitz 1992).

**Fig. 7** *Ixophorus unisetus* spikelet development. *a*, Early developing spikelets and bristles. *b*, Spikelet-bristle pairing, showing the 1 : 1 pairing on a slightly elongated pedicel and differentiated glumes. *c*, Early spikelet development. *d*, Lower floret development. Three androecial primordia surround the single gynoecial primordium, while organs of upper floret are just beginning to differentiate. *e*, Upper floret development. Androecial primordia overtake the gynoecial primordium of the lower floret, while the upper floret gynoecium is beginning to overtake its three androecial primordia. *spi* = spikelet; *bri* = bristle; *g1* = lower glume; *g2* = upper glume; *l1* = lower lemma; *l2* = upper lemma; *f1* = lower floret; *f2* = upper floret; *p1* = lower palea; *p2* = upper palea; *a* = anther; *g* = gynoecium. Scale bars: in *a*, 500 μm; in *b*, 100 μm; in *c*–*e*, 50 μm.
Many taxa in the bristle clade have five or more orders of branching (not shown; Doust and Kellogg 2002a, 2002b). However, members of *Setaria* subg. *Ptychophyllum* (e.g., *Setaria palmifolia*, *S. barbata*) have relatively few orders of branching, similar to *Ixophorus*, which consistently exhibits only four orders of branching.

In many species of *Setaria*, spikelets can be found simultaneously in all stages of development (fig. 13e), and asynchronous development often correlates with high orders of branching. As in *Ixophorus*, however, spikelet and bristle development are largely synchronous throughout the inflorescence in subg. *Ptychophyllum* (fig. 13f).

Late in development, the primary and secondary axes of *Ixophorus* elongate to their adult proportions, yielding the characteristic open inflorescence with elongated branches (fig. 8b). *Setaria* species exhibit a broad range between contracted primary and secondary axes (fig. 13c) and open inflorescences, the latter being like that of *Ixophorus* (fig. 13d). However, none have quite such uniform elongation as *Ixophorus*, and therefore *Setaria* species never exhibit uniformly placed spikelets and bristles like those along *Ixophorus* inflorescence branches. Bristles of *Ixophorus* are described as glabrous by most authors, but all in this study were actually minutely scabrous, as also noted by Sohns (1953). Sohns (1953) reported stamens with small, empty anthers occasionally occurring in the upper floret, but we have not seen these.

*Ixophorus* also differs from all other species of Panicoideae in that the lower floret always develops before the upper one in the spikelet. In every other species of Panicoideae that has been examined developmentally, the upper floret develops well before the lower (LeRoux and Kellogg 1999; Doust and Kellogg 2002a).

![Fig. 8 Ixophorus unisetsus late inflorescence development.](a) Inflorescence showing glumes expanded to enclose the more developed florets, with secondary axes still contracted and appressed to the primary axis. (b) Mature inflorescence with elongated primary and secondary axes. (c) Mature secondary axis with stigmas emerged. \(lg = \) lower glume; \(ug = \) upper glume. Scale bars: in \(a\), 500 \(\mu\)m; in \(b\), 2 cm; in \(c\), 4 mm.

**Comparative Inflorescence Development**

We compared development of *Ixophorus* with the detailed developmental descriptions of *Setaria*, *Pennisetum*, and *Cenchrus* published by Doust and Kellogg (2002a). Early inflorescence development of *Ixophorus* is similar to that of other members of the bristle clade in that the primary inflorescence axis produces secondary meristems in a spiral phylloxy. At the stage of tertiary meristem formation, *Ixophorus* looks similar to all known species of *Setaria* but quite different from members of the *Pennisetum/Cenchrus* clade. This is consistent with the phylogenetic placement of *Ixophorus* outside the strongly supported *Pennisetum/Cenchrus* group.

Most species of *Setaria* develop tertiary axis primordia laterally with respect to the secondary axis (fig. 13a), but the basal inflorescence branches of *Setaria barbata* (Lam.) Kunth (fig. 13b) are similar to those of *Ixophorus*, in which tertiary axis primordia form along the abaxial sides of secondary axes (cf. fig. 6a). This character state is not obvious in adult *S. barbata* inflorescences (fig. 13d), perhaps because the secondary axes do not broaden during spikelet and bristle development, as they do in *Ixophorus*. Species of *Paspalidium* Stapf also have abaxial spikelets at maturity, but we do not have developmental data on any of them.

![Fig. 9 Macrohair from Urochloa mutica. Scale bar = 100 \(\mu\)m](c)
Ixophorus is also unusual in being monoecious, with the upper floret in the spikelet forming only a gynoecium and the lower floret only stamens. We do not know of other panicoïds with this particular pattern of sex expression. While the lower floret is either staminate or sterile in most panicoïds, the upper one is generally bisexual. Other monoeccious species bear staminate and pistillate flowers in separate inflorescences (e.g., Zea), or in separate parts of the same inflorescence (e.g., Heteropogon contortus, Coix).

High loadings of the taxonomically important characters along PC1 indicate that these characters are largely correlated. This indicates that the characters that Scribner used to differentiate taxa within Ixophorus may really be expressions of a single underlying genetic character affecting general plant size.

Taxonomic Treatment

History

In 1830, C. B. Presl published a treatment of the grasses by J. S. Presl, who described Urochloa uniseta from a specimen prepared by Thaddeus Haenke in Mexico, likely collected during an excursion inland from Acapulco. Trinianus (1834), who had probably not seen Haenke's specimen, referred it to Panicum section Setaria, naming it Panicum unisetum (Presl) Trin. in a footnote. Steudel (1855) later provided a description for Trinianus's P. unisetum.

Von Schlechtendal (1862) established the genus Ixophorus, based on its winged lower palea and its viscid and smooth bristles. (Sohns [1953] noted occasional antrorse hairs at the bases of the bristles and stated that the bristles are heavily cutinized, not viscid.) Von Schlechtendal recognized Ixophurus unisetus (Presl) Schltdl., based on Presl's description, and
Ixophorus schiedeanum Schltdl., based on a specimen collected in 1834 by Schiede near Atlacomulco, Estado de Mexico, Mexico. He noted that the latter shares all characteristics of *I. unisetus* but is more delicate, that the bristles are thinner and longer, and that the relative glume sizes differ slightly.

Fournier (1886) separated *Setaria* from *Panicum* and included von Schlechtendal’s *Ixophorus* species as *Setaria unisetata* (Presl) Fourn. and *Setaria schiedeana* (Schltdl.) Fourn. Fournier also named *Setaria cirrosa*, which he separated on the basis of larger size, and *Setaria effusa*, which supposedly differed in its nine-veined upper glume. Fournier also described *S. uniseta* var. “vaginis pubescentibus,” but no authors subsequent to Fournier have taken up the name, and we have seen no specimens with pubescent sheaths.

Kuntze (1891) thought that the name *Setaria* was illegitimate, based on an earlier lichen genus of the same name, so he transferred Fournier’s four species of *Setaria* to the new genus *Chamaeraphis*. The grass genus *Setaria* was later formally conserved, rendering the name *Chamaeraphis* superfluous. For the same reason, Nash (1895) transferred four species of *Setaria* s.s. (*Setaria glauca*, *Setaria verticillata*, *Setaria italica*, and *Setaria viridis*; see “Excluded Names”) to *Ixophorus*, which was the earliest legitimate name he could find to replace *Setaria*.

Vasey (1893), apparently unaware of earlier epithets, described *Panicum palmeri* Vasey and *Panicum pringlei* Vasey, based on collections in Mexico by Palmer and Pringle in the late 1880s. *P. palmeri* is clearly a specimen of *I. unisetus*, while *P. pringlei* is a much shorter plant with shorter, narrower leaves, more vegetative branching near the base, and a shorter inflorescence with fewer branches, each with fewer

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**Fig. 11** Scatter plots of principal components analysis. These show no evidence for clear groups of specimens.

**Fig. 12** Scatter plot of principal components analysis of field- and greenhouse-grown specimens, against a backdrop of all field collections of *Ixophorus*. Differences between the Mexican and Central American specimens that were evident in the field were lost when accessions were grown in a common greenhouse environment.
spikelets along it, and bearing longer bristles. Beal (1896) referred *P. pringlei* to *Panicum schiedeana* Steud. ex Beal and retained *P. palmeri* Vasey for the larger species.

Scribnier (1897) was the only nineteenth-century author to follow von Schlectendal’s recognition of *Ixophorus*. He revised the genus and synonymized *I. schiedeana* Schltdl. with *I. unisetus*. Like Vasey and Beal, he also recognized a smaller species, naming it *I. pringlei* (Vasey) Scrib., and added a variety of it, *I. pringlei* var. *minor* Scrib., based on a specimen (Palmer 1256) even smaller than *I. pringlei*. Scribnier also published plates of *I. unisetus* (fig. 1) and *I. pringlei*, which are the first published illustrations of *Ixophorus*.

Herrmann (1911) placed four species of *Setaria* in a new section, *Setaria* sect. *Ixophorus*. Two of his species, *S. schiedeana* Fourn. and *S. cirrosa* Fourn., had an 11-veined second glume but varied in size. He distinguished *S. effusa* Fourn. and *Setaria polynesia* R.A.W. Herrm. as having second glumes with nine and 15 veins, respectively, which are characteristics not consistent with our observations. Vasey also states that *Panicum (=Ixophorus) pringlei* has a nine-veined upper glume. All specimens seen for the current study have 11 veins, including each of Vasey’s syntypes for *P. pringlei*, *Pringle 2423*, *Pringle 2047*, and *Palmer 1256*.

Hitchcock (1919) had seen many more specimens than any previous author when he revised the genus. He concluded, “Only recently has there been sufficient material to confirm the judgment that all the forms belong to one species” (p. 549). Except for floristic works, he was the only author after Trinius to recognize but a single species within *Ixophorus*.

**Taxonomy**


Fig. 13 Development of *Setaria* species. a, *Setaria grisebachii*, tertiary axes are initiated dichotomously by the secondary axis meristem. b, *Setaria barbata*, tertiary axes are initiated along the abaxial sides of the secondary axes. c, *Setaria verticillata*, contracted primary and secondary inflorescence axes. d, *Setaria barbata*, open inflorescence. e, *Setaria grisebachii* showing spikelets at all stages of development. f, *Setaria palmifolia* showing synchronous spikelet development. Scale bars: in a, 50 µm; in b, 100 µm; in c–d, 2 cm; in e, 100 µm; in f, 500 µm.


Plants monoecious annuals or short-lived perennials, caespitose, erect to spreading. Culms 15–150 cm tall, 1–10 cm thick, dry to somewhat succulent, longitudinally grooved, glabrous. Leaves linear; vernation conduplicate; sheaths open, glabrous, compressed laterally, often purple streaked basally; ligules membranous, fringed, 1.0–1.5 mm long; blades flat, 5–60 cm long, 0.4–4 cm wide; midvein often white; minute hairs immediately above ligule; margins strigillose. Inflorescences open, pyramidal panicles consisting of primary axis bearing 4–40 spirally arranged branches; primary axes and branches minutely scabrous. Branches ascending to divergent, spikelike, to 7 cm long, two-ranked spikelets borne abaxially; pedicels less than 1 mm long, cuplike, bearing a single bristle; bristles 4–12 mm long, scabralose, pale brown to black. Spikelets dorsiventrally compressed, 3–4 mm long, disarticulating below the glumes. Lower glumes orbicular to triangular, 3-nerved, one-fourth to one-third as long as upper glumes; upper glumes acute, 11-nerved, slightly shorter than lower lemma, often purple or green. Florets two, lower floret staminate, upper floret pistillate; lower lemma acute, 5-nerved, as long as spikelet; lower paleas hyaline, about as long as upper glume, accrescent, forming a winged structure clasping the upper floret at maturity; upper lemma flattened, rugose, indurate, papillate, apex mucronate, horseshoe-shaped scar (“germination flap”) prominent, margin wrapping around upper palea margin; upper paleas flattened, indurate, papillate; stamens three, anthers ca. 2 mm long, orange; stigmas plumose, bright red. Caryopsis oblong, obtuse, dorsiventrally compressed; embryo about one-third as long as caryopsis.

Fig. 14 Map showing approximate distribution of Ixophorus unisetus in Mexico and Central America. Collections from localities in South America, the Caribbean, and elsewhere in the world are presumed to represent cultivated plants. Stars = collecting sites for this study; squares = locations of type specimens; circles = locations of other herbarium specimens. 1 = type location, I. unisetus; 2 = Ixophorus pringlei var. minor; 3 = type location, Ixophorus palmeri; 4 = type location, I. pringlei.
**Distribution and Phenology**

*Ixophorus* occurs from central Mexico through Central America (fig. 14) at altitudes from sea level to ca. 1800 m, primarily on disturbed soils in open areas, under dry to somewhat moist conditions. It is also widely cultivated and escaped in areas as disparate as Costa Rica, Colombia, Venezuela, Bolivia, Cuba, Hawaii, and even Fiji. Cultivated specimens were collected in the United States in the late nineteenth century and in parts of Central America in the early years of the twentieth century. Because of its widespread use for forage, its original native range is not certain.

*Ixophorus* is a preferred forage grass for many livestock. On collecting trips for this study, it was never found more than 10 m from a road, despite surveys of many open grassy areas away from roads. This might reflect grazing pressure, with *Ixophorus* relegated to vacant lots, roadsides, enclosures, and other areas inaccessible to grazing animals. It could also reflect a preference for disturbed habitats and germination in open soil.

*Ixophorus* produces both vegetative and reproductive growth from about June to December. In the greenhouse, it was found to be a short-day (long-night) plant, requiring at least 12 h of darkness to initiate flowering. Under proper light conditions, the primary inflorescence axis emerges 4–6 wk after vegetative growth commences. Tillers are added throughout the rest of the season, so that plants with inflorescences in various stages of flowering and fruiting are found from late July to December. Anthesis begins with stigma presentation, followed by anther presentation, with some overlap between the two stages on a single inflorescence.

**Excluded Names**


**Acknowledgments**

We thank Peter Stevens and Gerrit Davidse for help with taxonomy and nomenclature. They also provided helpful comments on the manuscript, as did Mary Barkworth, Janet Barber, Mark Beilstein, J. Hugo Cota Sanchez, Liliana Giussani, Bee Gunn, Simon Malcomber, Patrick Sweeney, Alberto Vicentini, and two anonymous reviewers. Allison Miller, Ricardo Rueda, J. Gabriel Sanchez-Ken, Marcela Diaz, and Ana-Carolina Gómez helped with fieldwork, and Kathy Upson provided greenhouse support. Emilie Bass and Michael Gadberry produced the DNA sequences, and Trisha Consiglio helped with the map. Funding was provided by National Science Foundation grant DEB 98302511 to E. A. Kellogg and by the International Center for Tropical Ecology, the Missouri Botanical Garden, the Organization for Tropical Studies, and the E. Desmond Lee and Family Endowment.

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