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A New Allopolyploid Species of *Saccharum* (Poaceae – Andropogoneae) from South America, with Notes on its Cytogenetics

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Abstract—Allopolyploidy is a major mode of speciation in flowering plants and particularly in the grass tribe Andropogoneae, which includes sugarcane (*Saccharum officinarum*) and relatives. A new species of *Saccharum* from South America (***S. intermedium***) is described here, supported by morphological, molecular and cytogenetic evidence. Previous molecular analyses indicated an allopolyploid origin of the new species through interspecific hybridization between *S. angustifolium* and *S. villosum*. The new taxon has intermediate morphology between the two parental species. Cytogenetic analyses of the three species were performed, including chromosome counts, meiotic regularity, and pollen viability and morphology. The new taxon is hexaploid ($2n = 60$), while the parental species are triploids ($2n = 30$), confirming the ploidy level suggested by the number of paralogues in phylogenetic trees based on low-copy nuclear genes. This represents the first chromosome count for *S. intermedium* and a new cytotype for *S. villosum.* Although both parental species are triploids, they surprisingly exhibited regular meiosis and high pollen viability, indicating they are male-fertile, as is the hexaploid new species. Data on geographic distribution and phenology is also presented, as well as a key for the South American species of *Saccharum*.

Keywords—Chromosome number, hybridization, meiotic behavior, polyploidy, *Saccharum intermedium*, sugarcane.

Hybridization, especially followed by genome duplication (i.e. allopolyploidy), is very common among flowering plants and particularly in the grass tribe Andropogoneae (Estep et al. 2014; Welker et al. 2015). Andropogoneae comprises ca. 90 genera and 1,270 species (Kellogg 2015) and includes many ecologically and economically important plants, such as sugarcane (*Saccharum officinarum* L.) and maize (*Zea mays* L.). According to Estep et al. (2014), allopolyploidy is a major mode of speciation in Andropogoneae, accounting for at least a third of all speciation events in the tribe. Hybridization and polyploidy also play a significant role in the evolutionary history of sugarcane and relatives (Hodkinson et al. 2002; Estep et al. 2014; Welker et al. 2015). Within the sugarcane clade, both *Saccharum* L. and *Miscanthus* Andersson exhibit whole genome duplications that appear to have occurred around the same time (Kim et al. 2014), although it is unclear whether there was a single shared duplication or two independent but near-simultaneous ones.

Saccharum, in the broad sense as currently accepted, comprises 35–40 species from tropics and subtropics of the world (Clayton and Renvoize 1986; Kellogg 2015). It includes caespitose perennial plants with densely pilose inflorescences formed by numerous racemes borne on a persistent central axis (Welker and Longhi-Wagner 2012; Kellogg 2015). The New World species have been placed in the distinct genus *Erianthus* Michx. by some authors (Mukherjee 1958; Molina 1981; Watson and Dallwitz 1992), which would be differentiated by the awned spikelets (versus awnless spikelets in *Saccharum* s. s.). However, phylogenetic analyses have not found strong evidence to support the segregation of *Erianthus* (Hodkinson et al. 2002; Welker et al. 2015). Members of the genus *Saccharum* are all polyploids with a huge ploidy range, but no close diploid relatives are known (D'Hont et al. 1995, 1996; Grivet et al. 1996; Hoang et al. 2015; Kellogg 2015).

The circumscription of the South American species of *Saccharum* is complex and convoluted (Welker and Longhi-Wagner 2012). The delimitation of these taxa was recently investigated by Welker et al. (2015), based on multiple low-copy nuclear genes, which confirmed the occurrence of three native species in South America: *S. angustifolium* (Nees) Trin., *S. asperum* (Nees) Steud., and *S. villosum* Steud. *Saccharum asperum* differs from the other two species by having glabrous spikelets, while *S. angustifolium* and *S. villosum* are distinct based on the shape and width of the leaf blades and the pattern of the midvein (Welker and Longhi-Wagner 2012). However, some specimens collected in southern Brazil, Argentina, and Uruguay have intermediate leaf morphology between *S. angustifolium* and *S. villosum*. These plants were called "*Saccharum* aff. *villosum*" by Welker and Longhi-Wagner (2012) and Welker et al. (2015). Based on morphology, Welker and Longhi-Wagner (2012) suggested that these specimens might be natural hybrids between *S. angustifolium* and *S. villosum*. The phylogenetic analysis based on low-copy nuclear genes presented by Welker et al. (2015) did confirm this hypothesis. All four genes investigated of "*Saccharum* aff. *villosum*" had distinct paralogous copies, some of which grouped within the *S. angustifolium* clade and others within the *S. villosum* clade. This is the expected pattern for allopolyploid species in nuclear phylogenies (Sang 2002; Estep et al. 2014; Welker et al. 2015, 2016). Therefore, molecular data confirmed the allopolyploid origin of "*Saccharum* aff. *villosum*" from the crossing of *S. angustifolium* and *S. villosum* (Welker et al. 2015). Multiple

populations of "*Saccharum* aff. *villosum*" were found in South America, many of them without any close population of *S. angustifolium* or *S. villosum* (Welker and Longhi-Wagner 2012), suggesting a longer term stability and isolation from the parental species.

Chromosome numbers and other cytogenetic data provide useful taxonomic information, especially when species are morphologically similar and their distributions overlap (Stebbins 1971; Guerra 2012; Bonasora et al. 2015). It is known that most Andropogoneae species have a base chromosome number of $x = 10$ (Gould 1956; Giussani et al. 2001; Kellogg 2015). Although $x = 10$ is also the most frequent number for *Saccharum* species, different base numbers have been reported for the genus ($x = 5, 6, 8, 9, 10$ and 12) (Sreenivasan et al. 1987; Singh et al. 1990; Hoang et al. 2015). Physical mapping of ribosomal DNA sites has enabled the determination of the base chromosome number for some species (D'Hont et al. 1998; D'Hont 2005; Hoang et al. 2015).

Despite the vast cytogenetic knowledge of sugarcane and related species from the Old World (e.g. Panje and Babu 1960; Sreenivasan and Sreenivasan 1984; Singh et al. 1990; Iwo and Agboire 1992; Piperidis et al. 2010; Sobhakumari 2013), little information is available about the South American species of *Saccharum*. Gould (1956) reported the chromosome number $2n = 30$ for *S. angustifolium* (under the name *Erianthus angustifolius* Nees), based on material from southern Brazil. Later, Molina (1981) reported chromosome numbers of $2n =$ ca. 60 for *S. angustifolium* and *S. villosum* (under *E. angustifolius* and *E. trinii* (Hack.) Hack., respectively), based on materials from Argentina. According to Molina (1981), the high number of small chromosomes and their tendency to agglomerate on the slides hinder precise chromosome number determination of these species. Norrmann et al. (1994) confirmed the number $2n = 60$ for *S. villosum* (under *S. trinii* (Hack.) Renvoize), based on plants from Bolivia.

No cytogenetic data is currently available for the allopolyploid specimens of "*Saccharum* aff. *villosum.*" However, based on the number of paralogues in the phylogenetic nuclear trees, Welker et al. (2015) suggested that these plants should have a higher ploidy level than the two parental species. The presence in each sample of three distinct paralogues of all genes analyzed suggests that "*Saccharum* aff. *villosum*" is probably hexaploid, while *S. angustifolium* and *S. villosum* are probably triploids or tetraploids, since they had two paralogues per sample (Welker et al. 2015). Therefore, supported by morphology, molecular phylogeny, and putative ploidy level, "*Saccharum* aff. *villosum*" should be treated as a distinct taxon from *S. angustifolium* and *S. villosum*.

The aims of this paper are to (1) describe the allopolyploid specimens previously called "*Saccharum* aff. *villosum*" as a new species from South America, and (2) characterize cytogenetically (chromosome counts, meiotic behavior, and pollen viability and morphology) the new taxon and its parental species.

Materials and Methods

Specimens of the new species of *Saccharum* were collected in Argentina, Brazil, and Uruguay, from 2009 to 2013. Voucher specimens were deposited at CEN, CORD, CTES, ICN, K, and SI herbaria (acronyms according to Thiers 2016).

Cytogenetic analyses were based on individuals from six populations of "*Saccharum* aff. *villosum*", *S. angustifolium*, and *S. villosum* collected in the state of Rio Grande do Sul, southern Brazil. Voucher specimens are cited in Table 1.

For meiotic analyses, young inflorescences of each species were collected and fixed in 3:1 ethanol: acetic acid at room temperature for 12–24 h and stored at -20°C. Slides were prepared by squashing the anthers in 1% propionic carmine and were analyzed using a Zeiss Axioplan Universal photomicroscope. All chromosome counts were based on pollen mother cells (PMC) at diakinesis stage (prophase I). For meiotic behavior investigation, all available phases of meiosis I and II were analyzed.

Inflorescences at anthesis were collected for investigation of pollen viability and morphology and were fixed as described above. Pollen viability was estimated using Alexander's (1980) method in which full and well stained purple grains are considered viable. Empty or green stained pollen grains are considered non-viable. The polar axis (P) and equatorial axis (E) of mature pollen grains were measured and the P/E ratio calculated in order to determine grain shape (Erdtman 1971).

Results

A new allopolyploid species of *Saccharum* is here described, supported by morphological, molecular and cytogenetic evidence. The new taxon was previously called "*Saccharum* aff. *villosum*" and has intermediate morphology between its parental species, *S. angustifolium* and *S. villosum* (see Table 2; Fig. 1).

Chromosome number, meiotic behavior and pollen viability were investigated for "*Saccharum* aff. *villosum*" and its parents (Table 3). We found $2n = 60$ for "*Saccharum* aff. *villosum*" and $2n = 30$ for *S. angustifolium* and *S. villosum* from southern Brazil (Table 3; Fig. 2A–H). The three species exhibited meiotic stability with bivalent formation at diakinesis and metaphase I and regular disjunction and segregation in the majority of PMC analyzed. Multivalents and univalents were not found in any of the analyzed accessions. The most common abnormality was non-oriented bivalents (Fig. 2I–K).

Saccharum villosum exhibited slightly irregular meiosis with 78.9% normal PMC while *S. angustifolium* and "*Saccharum* aff. *villosum*" had 100% and 95.3% normal cells, respectively, indicating stable meiotic behavior (Table 3). Pollen stainability and morphology were used to assess pollen viability. All taxa had high pollen viability with at least 89.8% viable pollen grains (Table 3), indicating that these plants are all male-fertile. Pollen grain size was homogeneous within each species, although some grains smaller and larger than the normal ones were found in *S. villosum* (Fig. 2L).

Table 1. Voucher specimens and geographical origin of the *Saccharum* populations used for cytogenetic analyses. RS = Rio Grande do Sul state.

Species	Voucher	Locality	Latitude	Longitude
S. angustifolium	*C. A. D. Welker 666* (ICN)	Brazil, RS, Porto Alegre	30°03′28.4″S	51°07′10.6″W
	C. A. D. Welker 671 (ICN)	Brazil, RS, Porto Alegre	30°02′55.6″S	51°07′19.3″W
S. intermedium sp. nov. ("*Saccharum* aff. *villosum*")	*C. A. D. Welker 538* (ICN)	Brazil, RS, Santo Antônio das Missões	28°29′29.7″S	55°18′41.9″W
S. villosum	*C. A. D. Welker 539* (ICN)	Brazil, RS, Santo Antônio das Missões	28°31′54.7″S	55°31′55.5″W
	C. A. D. Welker 547 (ICN)	Brazil, RS, São Borja	28°38′02.5″S	55°47′19.5″W
	C. A. D. Welker 670 (ICN)	Brazil, RS, Porto Alegre	30°02′55.6″S	51°07′19.3″W

TABLE 2. Comparison of morphological and biogeographic characters of *Saccharum intermedium* sp. nov. and its parental species.

	S. angustifolium	*S. intermedium* sp. nov. ("*Saccharum* aff. *villosum*")	*S. villosum*
Leaf blades: shape, indument, width	Linear, glabrous, 2–6 mm wide	Lanceolate, glabrous or pilose, 3–6 mm wide	Lanceolate, generally pilose, 7–20 mm wide
Midvein of the blades	Width of the midvein > or = distance from midvein to leaf margin, midvein conspicuous up to the apex of the blade	Width of the midvein < distance from midvein to leaf margin, midvein inconspicuous in the upper portion of the blade	Width of the midvein < distance from midvein to leaf margin, midvein inconspicuous in the upper portion of the blade
Margins of the blades	Strongly scabrous	Scabrous	Scabrous
Habitat	Dry grasslands	Marshlands and wet grasslands	Marshlands and wet grasslands
Geographical distribution	Colombia and Venezuela to Argentina, Brazil, and Uruguay	Argentina, Brazil, and Uruguay	Mexico to Argentina, Brazil, and Uruguay

Regarding pollen morphology, all taxa were classified as oblate spheroidal (P/E = 0.87–1.0). The polar axis (P) for all species varied from 44.32 to 45.97 μm and the equatorial axis (E) ranged between 45.09 and 46.37 μm. We found no direct relationship between ploidy level and pollen size. The smallest values of pollen measurements were found in *Saccharum villosum* (Table 3).

DISCUSSION

Molecular analyses of the new species here described, based on five regions of four low-copy nuclear genes (*apo*1, *d*8, *ep*2-*ex*7, *ep*2-*ex*8, and *rep*1), were recently published by our research group (Welker et al. 2015). Multiple accessions of "*Saccharum* aff. *villosum*," *S. angustifolium*, and *S. villosum* were included in that phylogeny, as well as many samples of other species of *Saccharum* and other genera of Andropogoneae. Phylogenetic trees inferred from nuclear genes are useful to understand the origin of allopolyploid taxa, because they produce characteristic double-labeled tree topologies in which the allopolyploid species appear twice. In such trees, some paralogous copies of the hybrid taxon group with each parental species (Sang 2002; Estep et al. 2014; Welker et al. 2016). The phylogenetic analyses of Welker et al. (2015) confirmed the hybrid origin of "*Saccharum* aff. *villosum*" between *S. angustifolium* and *S. villosum*, since some of its paralogues grouped within the *S. angustifolium* clade and others within the *S. villosum* clade (Welker et al. 2015). The lower bootstrap support for most nodes of the trees when "*Saccharum* aff. *villosum*" specimens were included, compared to the trees without them, corroborates the hybrid origin of these plants (Funk 1985; McDade 1992; Welker et al. 2015).

The morphology of "*Saccharum* aff. *villosum*" is also consistent with the molecular evidence of its hybrid origin, as previously suggested by Welker and Longhi-Wagner (2012). The leaf blades of "*Saccharum* aff. *villosum*" are similar to those of *S. villosum* (lanceolate blades with narrow midvein, which is inconspicuous in the upper portion of the blade), but the blades are narrower, with width similar to those of *S. angustifolium* (Table 2; Fig. 1). Killeen (1990) reported that, when burned, populations of *S. villosum* flower en masse and produce exserted inflorescences within a few weeks, while unburned populations bloom irregularly during several months of the year and produce inflorescences that are included or only slightly exserted from the spathe. The same characteristic is observed in "*Saccharum* aff. *villosum*" populations.

The cytogenetic analyses performed in this paper agree with the hypothesis suggested by nuclear DNA sequences about the polyploid status of "*Saccharum* aff. *villosum*" and provided some clues about the evolutionary history of these plants and their parents. As mentioned earlier, two different chromosome numbers were described for *Saccharum angustifolium*, $2n = 30$ and $2n = 60$ (Gould 1956; Molina 1981). In our study, all plants analyzed of both populations of this species have $2n = 30$, corroborating Gould's (1956) results for Brazilian specimens. Molina (1981) and Norrmann et al. (1994) reported $2n = 60$ for *S. villosum* from Argentina and Bolivia, respectively. Our analysis of individuals from three populations showed that Brazilian specimens of *S. villosum* have $2n = 30$, which is a new cytotype for this species. We also provide the first chromosome count for "*Saccharum* aff. *villosum*" and, different from its parental species from Brazil, it has $2n = 60$. As the most common base chromosome number for the tribe Andropogoneae and for *Saccharum* is $x = 10$ (Gould 1956; Giussani et al. 2001; Kellogg 2015), we can deduce that Brazilian populations of *S. angustifolium* and *S. villosum* are triploids ($2n = 3x = 30$) and "*Saccharum* aff. *villosum*" is hexaploid ($2n = 6x = 60$). The cytogenetic analyses thus confirmed the suggestion of Welker et al. (2015) about the ploidy level of these species, based on the number of paralogues in the phylogenetic nuclear trees.

Polyploidy is common in Andropogoneae and the ploidy level can range from triploid to 18-ploid (Gould 1956). Two or more chromosome numbers as well as polyploid series have been reported for species of *Saccharum* and related genera (Gould 1956; Bir and Sahni 1985; Singh et al. 1990; Peichoto et al. 2011; Rounsaville et al. 2011). More than 20 cytotypes of *Saccharum spontaneum* L. are reported from India, with chromosome numbers ranging from $2n = 40$ to $2n = 128$ (Panje and Babu 1960; Sobhakumari 2013). Taking into account the present study and information from the literature (Gould 1956; Molina 1981; Norrmann et al. 1994), two cytotypes are found in *Saccharum angustifolium* and *S. villosum* ($2n = 30$ and $2n = 60$), although each population of these species is apparently composed of individuals of only one cytotype.

Despite the polyploid condition of the three species of *Saccharum* analyzed here, all of them exhibited regular meiotic behavior producing a highly fertile pollen population. As expected, *S. villosum* had the lowest pollen viability as a reflection of its slight meiotic instability (Table 3). Several studies of meiotic behavior for sugarcane and relatives are available, but analysis of meiosis in wild species of *Saccharum* is limited (Burner 1991). However, data reported in the literature have shown normal meiosis with prevalence of bivalent association

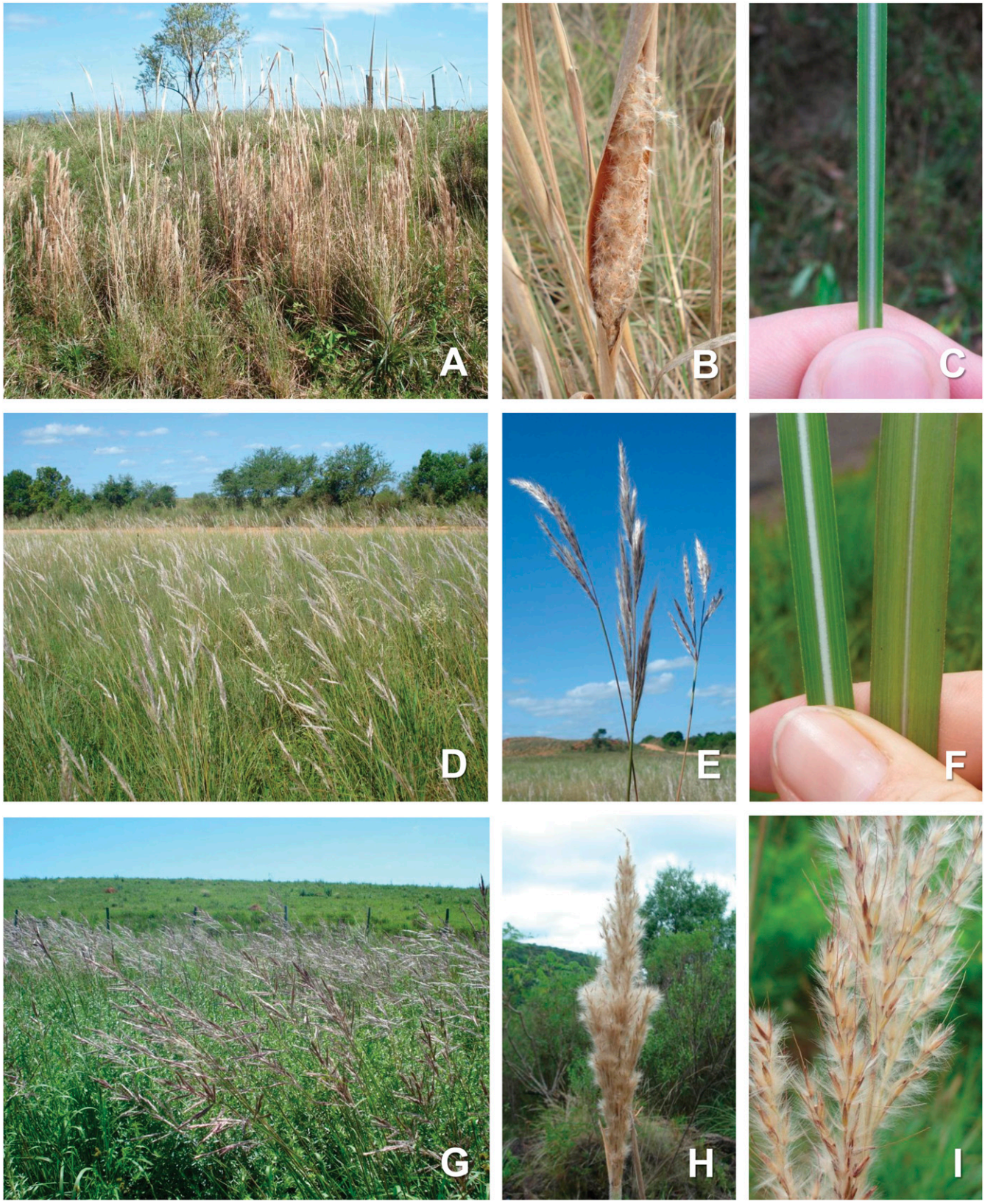

FIG. 1. Photographs of *Saccharum* species in their habitat. *S. angustifolium*. A. Habit. B. Inflorescence. C. Portion of the leaf blade. *S. intermedium* sp. nov. ("*Saccharum* aff. *villosum*"). D. Habit. E. Inflorescences. F. Portion of the leaf blade (left), together with portion of the leaf blade of *S. villosum* (right). *S. villosum*. G. Habit. H. Inflorescence. I. Detail of the inflorescence. Photos: C. A. D. Welker and H. M. Longhi-Wagner.

at diakinesis and metaphase I for most species of *Saccharum*. Occasional univalents or multivalent formation has been described as well as laggards and micronuclei in tetrads which probably contribute to the formation of aneuploid gametes in some species (Bremer 1961; Sreenivasan and Jagathesan 1975; Sreenivasan and Sreenivasan 1984; Piperidis et al. 2010). Even

TABLE 3. Chromosome numbers and cytogenetic analyses of meiotic stability in pollen mother cells and pollen viability in *Saccharum* species. Measurements of pollen grain axes and morphological classification according to Erdtman (1971). [a]Number of cells analyzed (number of individuals). [b]Percentage of normal cells. [c]Percentage of viable pollen grains (stained pollen grains). [d]Average measurement of 20 mature pollen grains per individual.

Species	Chromosome number ($2n$)	Meiosis I and II		Pollen viability		Pollen polar axis (P) (μm)[d]	Pollen equatorial axis (E) (μm)[d]	P/E ratio	Pollen morphology
		N[a]	%[b]	N[a]	%[c]				
S. angustifolium	30	149 (5)	100	2,060 (6)	98.1	45.85	46.37	0.99	Oblate spheroidal
S. intermedium sp. nov. ("*Saccharum* aff. *villosum*")	60	159 (3)	95.3	1,997 (4)	98.7	45.97	45.75	1.00	Oblate spheroidal
S. villosum	30	55 (2)	78.9	1,782 (4)	89.8	44.32	45.09	0.98	Oblate spheroidal

interspecific *Saccharum* hybrids have meiotic behavior similar to their parents exhibiting mostly bivalents and very few univalents at metaphase I (Nair 1975; Sreenivasan et al. 1987). According to Burner (1991), other genera considered closely related to *Saccharum,* such as *Erianthus*, *Miscanthus*, and *Narenga* Bor, show regular meiosis with fewer meiotic irregularities than sugarcane clones.

Although both *S. angustifolium* and *S. villosum* populations from Brazil are triploids, they surprisingly showed regular meiosis and high pollen viability (Table 3). Meiotic behavior in triploids, in most cases, is widely variable because of the inability of the three chromosome sets to pair and divide adequately during meiosis (Ramsey and Schemske 1998; Rounsaville et al. 2011). It is well known that meiosis in triploids is characterized by irregular pairing with presence of univalents, bivalents and trivalents at diakinesis I; unequal segregation and laggards are found in anaphases I and II resulting in unbalanced gametes and plants with reduced fertility (Caponio et al. 2012; Farco and Dematteis 2014). In *Stenodrepanum bergii* Harms (Fabaceae), different triploid plants showed various degrees of pollen fertility, which may be attributed to particular genotypes (Caponio et al. 2012). Triploids in spinach (*Spinacia oleracea* L., Amaranthaceae), *Arabidopsis thaliana* (L.) Heynh. (Brassicaceae), and *Chamerion angustifolium* (L.) Holub (Onagraceae) can be relatively fertile although less than diploids (Comai 2005). *Miscanthus sinensis* Andersson, a taxon closely related to *Saccharum*, has diploid ($2n = 38$) and triploid cytotypes ($2n = 57$). The triploid plants have reduced fertility with low pollen viability, seed set and seed germination. The limited progeny are predominantly aneuploid with low fecundity (Rounsaville et al. 2011).

Triploids have been described in *Saccharum*, but there are few data about their meiotic stability and pollen viability (Gould 1956). Norrmann and Quarín (1987) reported the maintenance of permanent odd polyploidy in a triploid species of Andropogoneae [*Andropogon ternatus* (Spreng.) Nees] by simultaneous formation of gametes with $n = 10$ and $n = 20$. In this species, one-half of the pollen grains carry only one 10-chromosome genome and the other half carry two genomes, but only grains with $n = 20$ are functional in the fertilization process. The embryo sac always develops from a megaspore with 10 chromosomes in this species, thus producing an egg cell with $n = 10$. Therefore, *A. ternatus* is a sexual triploid that accomplishes the stability of its odd polyploid level by transmitting one genome through the egg cell and two genomes through the sperm nucleus (Norrmann and Quarín 1987). Although the present study indicated regular meiosis and high pollen viability in *Saccharum angustifolium* and *S. villosum*, the occurrence of a similar process to that of *Andropogon ternatus* in triploid species of *Saccharum* cannot be completely discarded. Piperidis et al. (2010) confirmed by genomic in situ hybridization (GISH) previous results (Bremer 1961) that showed the occurrence of $2n + n$ transmission in backcrossed progeny between *S. officinarum* and *S. spontaneum*. Several hypotheses have been proposed to explain the formation of $2n$ gametes, such as formation of unreduced egg cells and chromosome doubling through endoduplication either at the dyad or tetrad stage (Sreenivasan et al. 1987). A detailed investigation using different methodological strategies is needed to elucidate the origin and maintenance of triploidy in *Saccharum angustifolium* and *S. villosum*.

The evolutionary history of "*Saccharum* aff. *villosum*" and its parental species is complex. Welker et al. (2015) found that some specimens of "*Saccharum* aff. *villosum*" are genetically more similar to *S. angustifolium*, and others to *S. villosum*. Hybridization therefore has probably occurred more than once in the formation of the hybrids, with different contributions from both *S. angustifolium* and *S. villosum*. The higher ploidy level (hexaploid) of "*Saccharum* aff. *villosum*" compared to both parental species (triploids in populations from Brazil) suggests an allopolyploid origin through interspecific hybridization between *S. angustifolium* and *S. villosum* followed by genome duplication, and/or by hybridization involving unreduced gametes (Welker et al. 2015). Considering that Brazilian populations of both *S. angustifolium* and *S. villosum* are triploids, it is reasonable to expect the production of unreduced gametes. However, taking into account the meiotic stability of these species and the apparent homogeneity in the size of pollen grains, unreduced gametes seem to be rare. Since hexaploid populations of *S. angustifolium* and *S. villosum* occur in Argentina (Molina 1981), it is possible that Argentinian specimens of "*Saccharum* aff. *villosum*" have originated from hybridization events not involving genome duplication or unreduced gametes. Additional cytogenetic analysis, including GISH and studies on megasporogenesis and megagametogenesis, may bring new insights into the evolutionary history of "*Saccharum* aff. *villosum*" and the genome contribution of each parental species in the formation of this allopolyploid taxon.

TAXONOMIC TREATMENT

Saccharum intermedium Welker & Peichoto, sp. nov.—TYPE: BRAZIL. Rio Grande do Sul: Santo Antônio das Missões, BR-285 to São Borja, near km 608, 28°29′29.7″S, 55°18′41.9″W, 22 Jan 2013, *C. A. D. Welker 538* (holotype: ICN!; isotype: CTES!).

Saccharum intermedium differs from *S. angustifolium* by its lanceolate leaf blades with narrow midvein, which is inconspicuous in the upper portion of the blade (in *S. angustifolium*, the blades are linear, the midvein is broad compared to blade width and is conspicuous up to the apex of the blade).

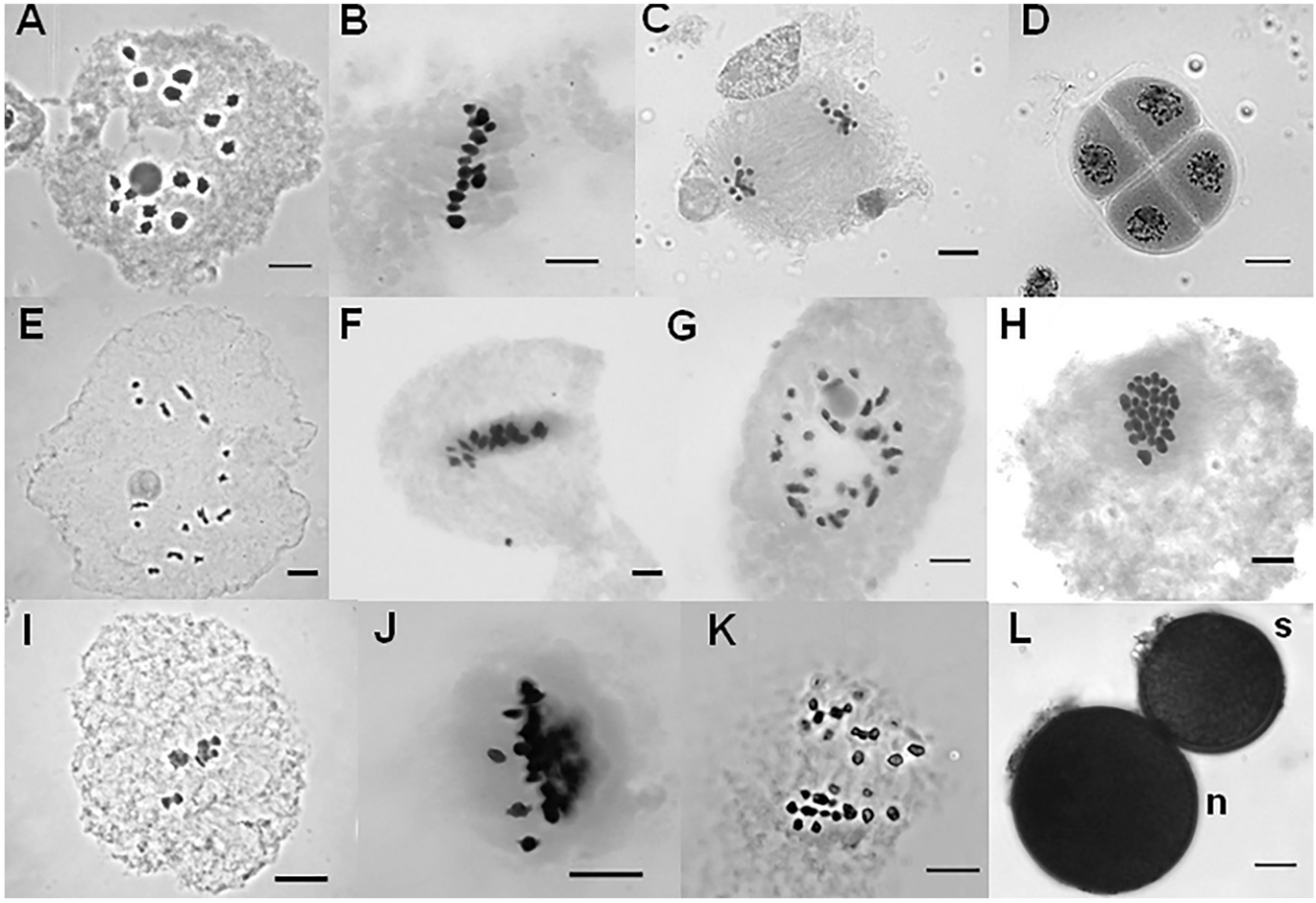

FIG. 2. Meiotic analysis in *Saccharum* species. A–D. *S. angustifolium*. A. Diakinesis with 15 bivalents. B. Metaphase I. C. Anaphase I. D. Tetrad. E–F. *S. villosum*. E. Diakinesis with 15 bivalents. F. Metaphase I. G–H. *S. intermedium* sp. nov. ("*Saccharum* aff. *villosum*"). G. Diakinesis with 30 bivalents. H. Metaphase I in polar view. I–L. Abnormalities in meiosis. I. Non-oriented bivalents in *S. villosum*. J. Metaphase with bivalents outside the plate in *S. angustifolium*. K. Anaphase I with chromosomes lagging in *S. angustifolium*. L. Two stained pollen grains of *S. villosum*, (n) normal size, (s) small pollen grain. Scale bars = 10 μm.

Saccharum intermedium is also similar to *S. villosum*, but differs by its narrower leaf blades (3–6 vs. 7–20 mm wide).

Caespitose perennials, with short, thick rhizomes. Culms 95–170 cm tall, 0.3–1 cm diam, erect, the pith not sappy or sweet; nodes antrorsely bearded or glabrous. Leaf sheaths pruinose, sparsely to densely pilose, especially near the ligule, less commonly glabrous. Ligules 3–6 mm long, membranous-ciliate. Blades (22–)28–70 cm long, 3–6 mm wide at middle, rigid, flat, lanceolate, widest at middle, narrowed to the base forming a long pseudopetiole, glabrous or sparsely pilose on both faces, or densely pilose abaxially and glabrous adaxially, the margins scabrous; midvein 0.7–1 mm wide, whitish, prominent, narrow compared to blade width, inconspicuous in the upper portion of the blade. Peduncle glabrous to densely pilose below the inflorescence. Inflorescence (12–)20–35 cm long, densely pilose, whitish to purplish or pinkish, contracted to slightly open, partially or totally included in the spathe or exserted, bearing numerous racemes on a persistent central axis. Racemes differentiated into nodes and internodes, disarticulating at the nodes. Spikelets paired at each node of the rachis, one sessile and one pedicelled, homogamous, the pedicelled spikelet falling off first at maturity, the sessile spikelet falling off together with a rachis internode and the pedicel. Rachis internodes 3.5–5(–7) mm long, pedicels of the pedicelled spikelets 3–4 mm long, both with trichomes 3–7 mm long on the margins. Spikelets 6–7 mm long (excluding awns), the callus densely bearded with trichomes 6–10 mm long. Lower glume 5.5–6.5 mm long, chartaceous, two-keeled, flat to weakly convex on back, sparsely to densely pilose on back and on the margins, especially on the upper portion, trichomes 3–6 mm long. Upper glume 5–6 mm long, chartaceous, one-keeled, sparsely to densely pilose on back and on the margins, especially on the upper portion, trichomes 2–4 mm long, rarely glabrous. Lower floret sterile, hyaline; lemma 4.5–5.5 mm long, ciliate on the margins, awnless; palea lacking. Upper floret bisexual, hyaline; lemma 3.5–4.5 mm long (excluding the awn), ciliate on the margins and the apex, the apex generally bidentate with awn 8–15 mm long; palea 2.2–3 mm long, ciliate on the margins and the apex. Lodicules 2, 0.7–1 mm long, glabrous or ciliate on the apex. Stamens 2, anthers 1–1.5 mm long. Caryopsis 2.5–3 mm long. Figures 1D–F, 3, and 4.

Etymology—The specific epithet refers to the intermediate leaf morphology of the new taxon between its parental species, *Saccharum angustifolium* and *S. villosum*.

Chromosome Number—$2n = 6x = 60$ (Fig. 2G–H).

Distribution and Habitat—Argentina, Brazil, and Uruguay (Fig. 4), in marshlands and wet grasslands.

Phenology—Collected with flowers and fruits from December to March.

FIG. 3. *Saccharum intermedium* sp. nov. A. Basal portion of the plant. B. Inflorescence. C. Pair of spikelets. D. Lower glume. E. Upper glume. F. Lower lemma. G. Upper lemma (awn partially removed). H. Apex of upper lemma (awn partially removed). I. Upper palea. J. Lodicule. K. Ovary and stamens. L. Developing caryopsis. Drawn from the type specimen (*C. A. D. Welker 538*) by Liliana Gómez and Anelise Scherer.

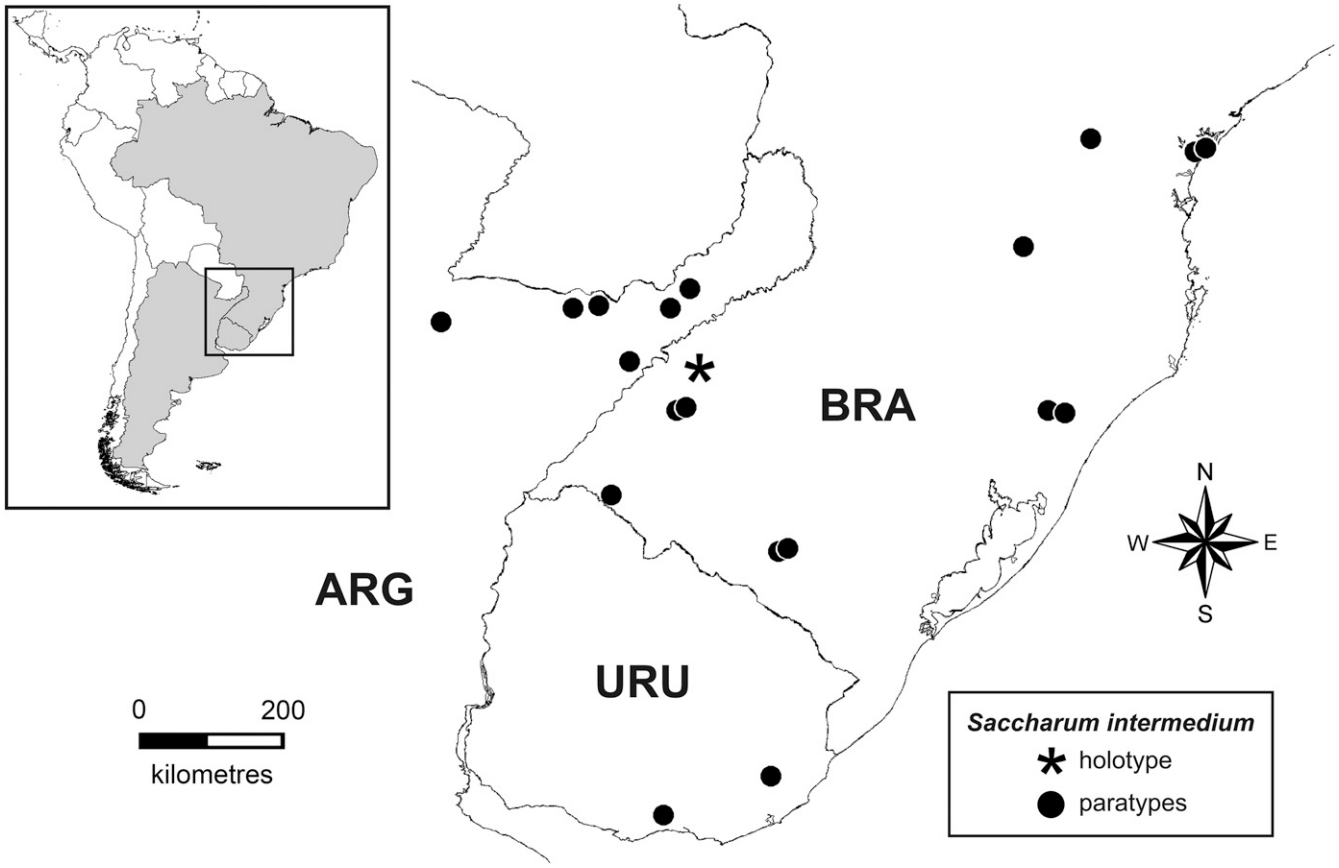

Fig. 4. Geographical distribution of *Saccharum intermedium* sp. nov. ARG = Argentina, BRA = Brazil, URU = Uruguay.

***Additional Specimens Examined*—**ARGENTINA. Corrientes: Empedrado, ruta nacional 12, near km 986, 27°52′05.7″S, 58°45′44.6″W, 58 m, 26 Jan 2013, *C. A. D. Welker* & *M. C. Peichoto 556* (CTES, ICN); Ituzaingó, near represa Yaciretá, 27°33′12.5″S, 56°39′46.9″W, 86 m, 28 Jan 2013, *C. A. D. Welker* & *M. C. Peichoto 605* (CEN, CTES, ICN); ruta nacional 12, near km 1227, 27°37′14.1″S, 56°57′26.9″W, 78 m, 01 Mar 2013, *M. C. Peichoto 285* (CTES); Santo Tomé, ruta nacional 14, 28°27′31.3″S, 56°06′32.3″W, 84 m, 27 Jan 2013, *C. A. D. Welker* & *M. C. Peichoto 573* (CEN, CTES, ICN, K, SI). Misiones: Candelaria, ruta provincial 103, 8 km W from Mártires, 27°25′18.2″S, 55°25′55.9″W, 110 m, 11 Feb 2013, *M. C. Peichoto 262* (CTES); Leandro N. Alem, ruta nacional 14, 27°41′00.8″S, 55°39′11.6″W, 127 m, 27 Jan 2013, *C. A. D. Welker* & *M. C. Peichoto 584* (CORD, CTES, ICN).

BRAZIL. Paraná: Palmeira, near rio Salto, 25°21′12.5″S, 49°55′15.9″W, 824 m, 18 Dec 2012, *C. A. D. Welker 520* (ICN); Pontal do Sul, PR-412 to Pontal do Paraná, 25°34′22.8″S, 48°21′19.9″W, 19 Dec 2011, *C. A. D. Welker 377* (CTES, ICN), *378* (CTES, ICN). Rio Grande do Sul: Jaquirana, RS-010, 29°06′09.1″S, 50°26′18.4″W, 13 Jan. 2009, *H. M. Longhi-Wagner & C. A. D. Welker 10650* (ICN); Jaquirana to Passo do S, 29°05′42.2″S, 50°24′09.3″W, 13 Jan. 2009, *H. M. Longhi-Wagner & C. A. D. Welker 10659* (ICN); Lavras do Sul to Bagé, RS-473, 30°59′12.0″S, 54°07′55.0″W, 13 Jan. 2010, *C. A. D. Welker 276* (ICN), *277* (ICN); Quaraí, BR-293, near km 439, 30°24′03.0″S, 56°25′36.2″W, 17 Dec 2009, *C. A. D. Welker 238* (ICN); São Borja to Santiago, BR-287, 28°58′37.3″S, 55°31′10.0″W, 21 Dec 2010, *H. M. Longhi-Wagner & C. A. D. Welker 10839* (ICN), *10840* (ICN). Santa Catarina: Caçador, SC-302, near km 102, Fazenda Amoreira, 26°50′50.2″S, 50°51′13.6″W, 1100 m, 17 Dec 2012, *C. A. D. Welker 502* (CTES, ICN).

URUGUAY. Canelones: near Soca, 34°40′45.0″S, 55°44′52.0″W, 07 Mar 2013, *C. A. D. Welker 641* (ICN). Rocha: Velázquez, ruta 13 to Castillos, 34°03′27.0″S, 54°13′42.6″W, 95 m, 05 Mar 2013, *C. A. D. Welker 630* (CTES, ICN).

***Note*—**The specimens belonging to this new species were called "*Saccharum* aff. *villosum*" by Welker and Longhi-Wagner (2012) and Welker et al. (2015).

Key to *Saccharum* Species from South America

1. Culms 2–5 cm in diameter, the pith sappy and sweet; spikelets awnless; introduced and cultivated species (sugarcane) ..... *S. officinarum*
1. Culms 0.3–1 cm in diameter, the pith not sappy or sweet; spikelets awned, awn 5–15 mm long; native species 2
   2. Glumes of spikelets glabrous, only scabrous toward the apex ..... *S. asperum*
   2. Glumes of spikelets sparsely to densely pilose, at least near the margins 3
      3. Leaf blades 7–20 mm wide ..... *S. villosum*
      3. Leaf blades 2–6 mm wide 4
         4. Leaf blades linear; width of the midvein > or = distance from midvein to leaf margin, midvein conspicuous up to the apex of the blade; occurs in dry grasslands ..... *S. angustifolium*
         4. Leaf blades lanceolate; width of the midvein < distance from midvein to leaf margin, midvein inconspicuous in the upper portion of the blade; occurs in marshlands and wet grasslands ..... *S. intermedium*

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