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Karyotype characterization of four Mexican species of *Schoenoplectus* (Cyperaceae) and first report of polyploid mixoploidy for the family

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Karyomorphological analysis of four species of *Schoenoplectus* (Cyperaceae) from north-central Mexico were carried out. Chromosome numbers ranged from $2n = 38$ to $2n = 84$. New records of counting are given for *Schoenoplectus acutus* var. *occidentalis* ($2n = 38$ and $2n = 84$) and *S. americanus* ($2n = 66$). Intra-individual variation in chromosome number is reported for the first time for *S. acutus*, with a rare polyploid mixoploidy with a prevalence of cells with $2n = 38$ (36 small + 2 compound, larger chromosomes) and a few cells with $2n = 84$ small, dot-shaped chromosomes, this being the first record of polyploid mixoploidy for Cyperaceae. Mean length of the diploid set ranged from 51.5 μm (*S. tabernaemontani*) to 79.5 μm (*S. acutus*). The lowest average chromosome length for the dot-shaped chromosomes was 0.69 μm (*S. acutus*) and the highest 1.62 μm (*S. tabernaemontani*); the pair of large chromosomes in *S. acutus* reached 3.17 μm. A low interchromosomal asymmetry index (A_2), 0.11 to 0.14 was found, very similar among all the species except for *S. acutus* ($A_2 = 0.30$). Absence of primary constrictions was confirmed. The most common mechanism of karyotype variation in the studied species is dysploidy, followed by polyploidy. A comparison of chromosome numbers between *Schoenoplectus* and the recently segregated *Schoenoplectiella* based on the literature reveals that *Schoenoplectus* has higher numbers ($n = 18$ to 64; $2n = 36$ to 84) than *Schoenoplectiella* ($n = 5$ to 44; $2n = 18$ to 76) as well as a higher prevalence of disploids.

Keywords: dysploidy; holocentric chromosomes; mixoploidy; polyploidy; *Schoenoplectiella*; *Scirpus*; sedges

Introduction

Schoenoplectus (Rchb.) Palla is a genus of Cyperaceae distributed almost worldwide, with about 31 species and five nothospecies, not including those that are at present considered under *Bolboschoenus* (Asch.) Palla and *Schoenoplectiella* Lye (Lye 2003). Schuyler noted since 1976 that "the genus *Scirpus* (*sensu lato*) is a diverse assemblage of unrelated species groups which eventually will be treated as different genera". The three above-mentioned genera have been segregated from *Scirpus* L. and are provisionally considered in the tribe *Fuireneae* Reichenb. ex Fenzl (Goetghebeur 1998; Muasya, Vrijdaghs, et al. 2009), although further studies are in progress to evaluate their relationships (Muasya, Vrijdaghs, et al. 2009).

Groups in *Schoenoplectus* and related genera have been distinguished based on morphology and micromorphology (Strong 1993, 1994; Bruhl 1995; Goetghebeur 1998; Pignotti and Mariotti 2004), as well as molecular data (Muasya et al. 2000; Young et al. 2002; Yano and Hoshino 2005; Jung and Choi 2010), revealing the group to be paraphyletic or polyphyletic. *Schoenoplectus* includes some difficult species complexes and is in need of a systematic revision (Smith 2002; Shiels and Monfils 2012). It is frequently found in wetlands and marshes, and some species are often the dominant element in aquatic vegetation. They have a great environmental value (for phytoremediation, as soil stabilizers, habitat and food for wild species), as well as important ethnobotanic and economic values, e.g. for *S. californicus* (C. A. Meyer) Soják, a species widely distributed in the New World (Mexican tules, Peruan and Bolivian totora). In North America their culms are used, mostly historically, for making mats, baskets, chair seats, houses, boats, and other objects. Some species are cultivated as ornamentals (Smith 2002). Five species of *Schoenoplectus* s.s. are known in Mexico, where some of them are used for craft making and phytoremediation (González et al. 2007). Except for two Japanese studies (Maeda and Uchino 2004; Yano and Hoshino 2005) very few cytogenetic studies have been performed recently on *Schoenoplectus*, and the first of them was focused on species now recognized under *Schoenoplectiella*.

Like the other members of Cyperaceae, *Schoenoplectus* has holokinetic ("holocentric") chromosomes. Although they are sometimes thought to be rare, holokinetic chromosomes are rather common among many groups of organisms, including two plant families: Cyperaceae and Juncaceae (Luceño et al. 1998). Diffuse kinetochores favor karyotype differentiation via agmatoploidy (fission), symploidy (fusion), and polyploidy (Luceño and Guerra 1996) as well as chromosome

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viability after most rearrangements (Guerra 2008). For this reason, chromosomes evolve very dynamically in Cyperaceae (Luceño and Guerra 1996; Hipp et al. 2009) and in wood-rushes (*Luzula* DC., Juncaceae) (Bozek et al. 2012), where a considerable diversity of karyotype evolution occurs.

Given that holokinetic chromosomes lack a defined centromere they have no primary constriction, so the options for analysis based on morphology of their karyotypes are greatly reduced. Karyotype parameters, such as intrachromosomal asymmetry, can only be estimated using the chromosome length; however, the high variation in chromosome number, interchromosomal asymmetry index, and presence or absence of nucleolar constrictions are useful for karyotype description.

Mexican Cyperaceae are poorly known cytologically. Here, we present karyomorphological data of four species of *Schoenoplectus* (Cyperaceae) from north-central Mexico.

Materials and methods

Mitotic metaphase chromosomes were studied from root meristematic cells. Eleven populations representing four species of *Schoenoplectus* were collected in seven localities of north-central Mexico, in the state of Durango. Voucher specimens were deposited in herbarium CIIDIR at the Centro Interdisciplinario de Investigación para el Desarrollo Integral Regional, Instituto Politécnico Nacional, in Durango, Mexico. Samples were cultivated in the greenhouse of CIIDIR. Data of the studied taxa, localities of collection and voucher specimens are presented in Table 1.

The karyotypical analyses were performed with root tips pre-treated in 2 mM 8-hydroxyquinoline for 24 h and fixed in absolute ethanol:acetic acid (3:1, v:v) for 24 h, and stored at –20°C until use. Root tips were washed in distilled water, digested for 1 h in a mixture of 4% (w:v) cellulase and 40% (w:v) pectinase at 37°C, further hydrolyzed in 1 M HCl for 10 min at 60°C, washed again in distilled water, and squashed in a drop of 45% acetic acid. The cover slips were removed after freezing in liquid nitrogen. Slides were stained in 4% hematoxylin and permanent slides mounted in Entellan (Merck KGaA, Darmstadt, Germany). Chromosome counts were made in at least 20 cells. Mean lengths of the karyotype (the total diploid length) and of the shortest and longest chromosome of the complement were calculated. Chromosome measurements were carried out using the freeware computer application MicroMeasure software version 3.3 for Windows (Colorado State University, Dept of Biology, USA)(http://www.colostate.edu/Depts/Biology/MicroMeasure) and the data were used to assemble ideograms. For each sample, from 5 to 10 metaphase spreads with similar condensation were measured.

All images were acquired with a Carl Zeiss AxioImager.Z2 microscope (ZEISS. Oberkochen, Germany) equipped with an Axiocam Hrc camera and AxioVs40 Rel.4.8.2 software (ZEISS. Oberkochen, Germany). Interchromosomal asymmetry was calculated using the Romero-Zarco (1986) index based on Pearson's dispersion coefficient (the ratio between the standard deviation (s) and the mean of chromosome length ($\bar{X}$) for each sample):

$$A_2 = \frac{s}{\bar{X}} \tag{1}$$

Results

The karyotypes of 11 populations corresponding to four species of *Schoenoplectus* section *Schoenoplectus* from

Table 1. Studied taxa, localities of collection, voucher specimens and diploid number.

Species	Geographical coordinates of localities and voucher number	2*n*
S. acutus	24°12′01″ N, −104°29′07″ W (O. Rosales 4024, 4025)	38
S. acutus	24°12′01″ N, −104°29′07″ W (O. Rosales 4024, 4025)	84
S. americanus	24°08′41″ N, −104°27′13″ W (S. Gonzalez 7816	66
S. californicus	24°28′25″ N, −104°43′03″ W (O. Rosales 4057, 4059)	68
	24°26′13″ N, −104°41′52″ W (O. Rosales 4062)	
	24°08′41″ N, −104°27′13″ W (S. Gonzalez 7817)	
S. tabernaemontani	23°55′28″ N, −104°32′43″ W (J. Tena S-2)	42
	23°55′53″ N, −104°33′17″ W (J. Tena S-4)	
	23°54′58″ N, −104°32′08″ W (O. Rosales 4072)	
	23°55′28″ N, −104°32′43″ W (O. Rosales 4075)	

Table 2. Chromosome count and size, and interchromosomal asymmetry index in Mexican species of *Schoenoplectus*.

Species of *Schoenoplectus*	2*n*	Total length (μm)	Long[a] Short[a] (μm)		A_2	Figures
S. tabernaemontani	42	51.49	1.62	0.87	0.13	1A
S. americanus	66	70.10	1.44	0.76	0.14	1B
S. californicus	68	67.86	1.35	0.70	0.14	1C
S. acutus	84	79.46	1.18	0.69	0.11	1D
S. acutus	38	54.12	3.17	1.0	0.30	1E

[a]Average of the length of the longest and shortest chromosomes.

Table 3.   Chromosome numbers for species and hybrids of *Schoenoplectus* s.s. as compared with previous reports. Disploid mixoploidy is indicated by *; polyploid mixoploidy is indicated by **. Numbers separated by a dash represent an intraspecific series recorded in a reference; by a comma represent variation given in a reference (including this work); and by a semicolon represent intraspecific variation given in different references.

Species	N	References	2n	References
S. acutus var. *acutus*	19	Schuyler (1976)	36; 38	Harriman (1981); Smith (2002)
	20	Hicks (1929)	42	Löve and Löve (1981)
S. acutus var. *occidentalis*			38, 84**	This work
S. americanus			66	This work
	39	Hicks (1929); Schuyler (1976)	78	Cited by Smith (2002) and Yano and Hoshino (2005)
S. americanus x *S. pungens*	43–47	Schuyler (1976)		
	50–64	Hicks (1929)		
S. californicus ssp. *californicus*	34	Schuyler (1976)	68	Cited by Smith (2002)
	32, 34	Heiser (1979)	64, 68	Cited by Yano and Hoshino (2005)
			68	this work
S. californicus ssp. *tatora* (Kunth) T. Koyama	35	Heiser (1979)		
S. deltarum (Schuyler) Soják	39	Schuyler (1976)		
S. heterochaetus (Chase) Soják	18(19)	Hicks (1929)		
	19	Schuyler (1976)		
S. lacustris (L.) Palla	(20)21(22)*	Otzen (1962)	38	Tanaka (1940)
	21	Mehra and Sachdeva 1975, cited in Roalson (2008)	42	Löve and Kjellqvist (1973)
	38, 40, 42	Tanaka (1938, 1939)	76, 77	Tarnavschi 1948, cited in Roalson (2008)
			40, 42	Kamari et al. (2000)
			38, 42, 80	Dai et al. (2010)
			42	Reese 1957; Sorsa 1963; Skalinska and Pogan; Löve and Kjellqvist 1973; Hindakova 1976, all cited in Roalson (2008).
			80	Sharma and Sarkar 1967–1968; Sharma 1970; Sanyal and Sharma 1972, all cited in Roalson (2008)
S. lacustris x *S. tabernaemontani*	(18–20)21 (22)*	Otzen (1962)		
S. lacustris x *S. triqueter*	(18–20)21 (22)*	Otzen (1962)		
S. litoralis (Schrad.) Palla	40	Nijalingappa et al. 1978 in Roalson (2008)	36	Baquar (1969)
	39, 42	Bir et al. 1990 1991 in Roalson (2008)		
	42, 78, 80, 84	Dai et al. (2010)		
S. nipponicus (Makino) Soják			74	Yano and Hoshino (2005)
			76	Tanaka (1948, cited in Yano and Hoshino 2005)
S. pungens (Vahl) Palla	38	Hicks (1929)	74	Harriman 1981
	39	Schuyler (1976)		
	(37–)39*	Otzen (1962)		
S. pungens var. *longispicatus* (Britton) S.G. Sm.			78	Löve and Löve (1981)
S. subterminalis (Torr.) Soják	37	Schuyler (1976)	72	in Smith (2002)
S. tabernaemontani	20(21)22*	Otzen (1962)	42	Håkansson (1928); Wulff (1938); Hindakova (1976); Arohonka (1982); Kozhevnikov et al. (1986); Stoeva (1987); Javurkova-Jarolimova (1992); Hoshino et al. (1993); Montgomery et al. (1997); all cited in Roalson (2008)
	21	Hicks (1929)	42	Harriman (1981)
	21	Schuyler (1976)	42	Löve and Löve (1981)

(*Continued*)

Table 3.   (*Continued*).

Species	N	References	2n	References
			42	de Lange et al. (2004)
			42	Yano and Hoshino (2005)
			42	this work
			38	Pólya (1949) in Moore (1982)
			44	Wulff (1938) in Moore (1982)
S. tabernaemontani x triqueter	(19,20)21 (22,23)*	Otzen (1962)		
S. torreyi (Olney) Palla	35	Schuyler (1976)	42	Löve and Löve (1981)
S. triqueter (L.) Palla	(20)21(22)*	Otzen (1962)	38	Clapham et al. (1987)
	21	Bir et al. 1993 in Rich and Fitzgerald (2002)	40	Tanaka 1942, 1948; Fang 1992, all cited in Roalson (2008)
			40, 42	Dai et al. (2010)
			41, 42	Hoshino et al. (1993)

north-central Mexico were analyzed. The diploid numbers, the total length expressed in μm, the average of the length of the longest and shortest chromosomes, and the rate of asymmetry are given in Table 2. Chromosome numbers in the plants studied here range from $2n = 38$ to $2n = 84$. The numbers $2n = 42$ for *S. tabernaemontani* (C. C. Gmelin) Palla and $2n = 68$ for *S. californicus* confirm previous reports. Numbers documented for the first time are $2n = 66$ for *S. americanus* (Pers.) Volkart ex Schinz & R. Keller, and $2n = 38$ and $2n = 84$ for *Schoenoplectus acutus* (Muhl. ex Bigelow) Á. Löve & D. Löve var. *occidentalis* (S. Watson) S. G. Smith (Table 3).

As shown in Figure 1, mitotic metaphase chromosomes are dot-like, characteristic of *Schoenoplectus sensu lato*, with the exception of a pair of larger chromosomes found in the $2n = 38$ cells of *S. acutus*.

Mean length of the diploid set ranged from 51.5 μm (*S. tabernaemontani*) to 79.5 μm (*S. acutus*). The lowest average chromosome length for the dot-shaped chromosomes was 0.69 μm (*S. acutus*) and the highest 1.62 μm (*S. tabernaemontani*); the pair of large chromosomes in *S. acutus* reaches 3.17 μm.

The interchromosomal asymmetry index (A_2) was very low (0.11 to 0.14) except for the $2n = 38$ cells of *S. acutus*.
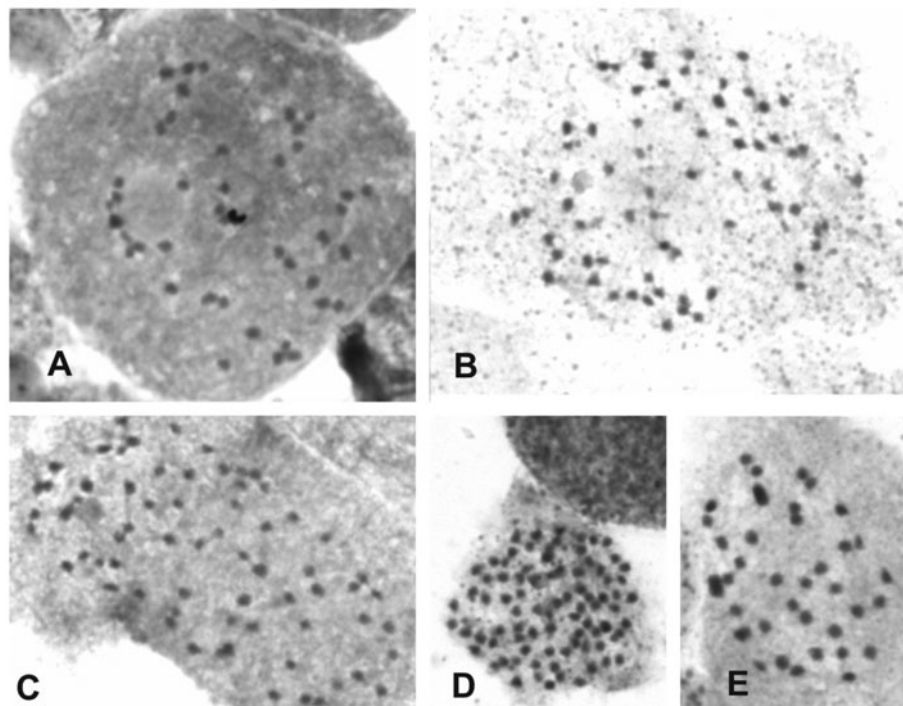

Figure 1.   Mitotic metaphases of four species of *Schoenoplectus*. (A) *S. tabernaemontani* $2n = 42$; (B) *S. americanus* $2n = 66$; (C) *S. californicus* $2n = 68$; (D) *S. acutus* $2n = 84$; *S. acutus* $2n = 38$. Scale bar = 10 μm.
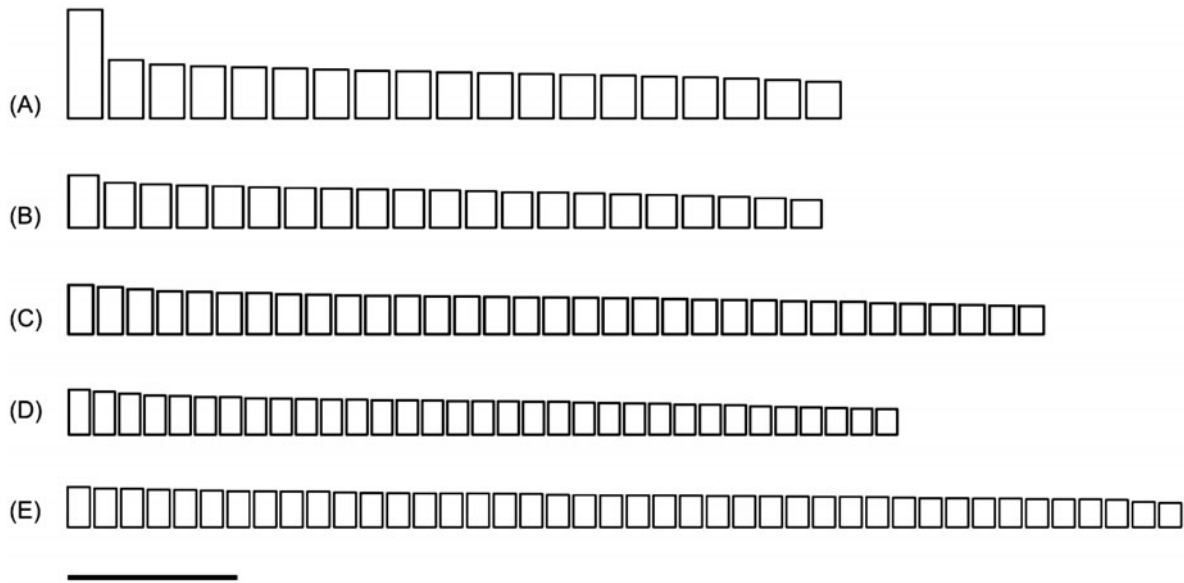
Figure 2.   Haploid ideograms for four species of *Schoenoplectus*. All the ideograms represent the haploid set. Note that all species exhibit chromosomes decreasing gradually in size except for the longest pair found in *S. acutus*: (A) *S. acutus* 2*n* = 38; (B) *S. tabernaemontani* 2*n* = 42; (C) *S. americanus* 2*n* = 66; (D) *S. californicus* 2*n* = 68; (E) *S. acutus* 2*n* = 84. Scale bar = 5 μm.

Figure 2 shows the ideograms of the haploid complement for every karyotype. Most of them, regardless of the number of chromosomes, show a size that decreases gradually. The four species show a low rate of interchromosomal asymmetry $A_2 < 0.14$ with the exception of the 2*n* = 38 cells of *S. acutus*.

Discussion

Among the species of *Schoenoplectus*, karyotypes differ mainly in chromosome number, which range from 2*n* = 36 to 84. Our results for the studied species (2*n* = 38 to 84) cover almost the whole range for the genus, with both extremes found in a single individual of *S. acutus* var. *occidentalis*.

Most of the chromosomes are dot-shaped and very homogeneous in size. They are small according to the categories of Stebbins (1938), with the exception of the long pair in *S. acutus*. The total length of the diploid set is in a relatively short range, from 51.5 to 79.5 μm. The interchromosomal asymmetry index (A_2) is also very low (0.11–0.14) and very similar among all the species, except for the dimorphic karyotypes of the mixoploid *S. acutus* ($A_2 = 0.30$).

The chromosome numbers for *Schoenoplectus* s.s., complemented by previous reports, are presented in Table 3. A wide variation exists in the haploid chromosome number in *Schoenoplectus* worldwide, which ranges from *n* = 19 to 42(-64), with chromosome number peaks at 39 (in four species) and 21 (three species) (Figure 3A). Diploid numbers range from 2*n* = 36 to 2*n* = 84, with peaks at 40 and 42 (Figure 3B). The highest haploid chromosome numbers so far reported for the genus correspond to putative hybrids between *S. americanus* and *S. pungens*: *n* = 50–64 is based on a sole record given for a robust form of *S. americanus* (Hicks 1929) which is at present considered as a hybrid with *S. pungens*: *S.* × *contortus* (Eames) S. G. Smith. A record of *n* = *c*.43–47 was also given for the hybrid *S. americanus* × *S. pungens* (Schuyler 1976). Except for the extrapolations of *n* = 64 (e.g. the reference to 2*n* = 38 to 2*n* = 84(-128) for *Schoenoplectus* in Roalson 2008), no reports have been found of 2*n* > 84.

A record of 2*n* = 20 for *Schoenoplectus litoralis* (Schrader) Palla (Sarkar et al. 1976) may come from a misidentification, given that: (a) *S. litoralis* belongs to *Schoenoplectus* s.s., a group where the known numbers range otherwise between 2*n* = 36 and at least 2*n* = 84; (b) the other numbers recorded for *S. litoralis* are 2*n* = 42, 78, 80, 84 (Dai et al. 2010). Numbers recorded for *Schoenoplectus* ranging from 2*n* = 10 to 2*n* = 76 belong to species currently recognized under *Schoenoplectiella*, a recently segregated genus (Lye 2003) that includes several annual amphicarpous plants. A comparison of chromosome numbers between *Schoenoplectus* and *Schoenoplectiella* based on literature records (Figure 3A, B) reveals that *Schoenoplectus* has higher numbers (*n* = 18 to 64; 2*n* = 36 to 84) as well as a higher prevalence of disploids (see Table 3) than *Schoenoplectiella* (*n* = 5 to 44; 2*n* = 18 to 76) (Table 4).

a)   Schoenoplectus acutus

Schoenoplectus acutus includes two varieties: *S. acutus* var. *acutus*, from Canada, USA, and probably Eurasia (Smith 2002), and *S. acutus* var. *occidentalis*, known from Canada and western USA to central Mexico
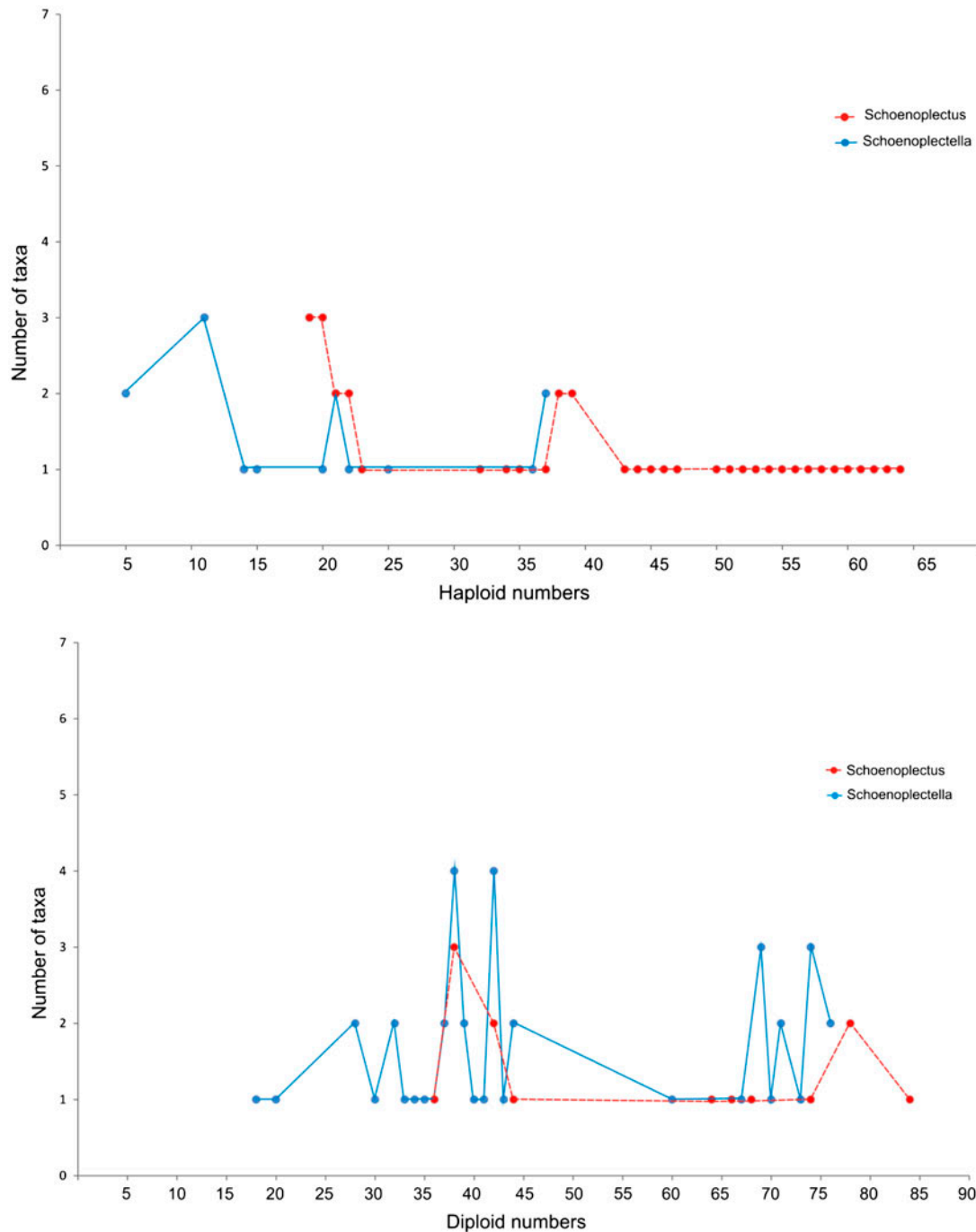
Figure 3.   (Color online) Chromosome number variations for *Schoenoplectus* s.s. and *Schoenoplectiella*. Data on *Schoenoplectus* are based on Table 2; for *Schoenoplectiella* data are based on the literature, mainly Hicks (1929), Yano and Hoshino (2005), and Roalson (2008).

(Michoacan), where it grows in marshes and edges of lakes and streams, sometimes in alkaline habitats. *Schoenoplectus acutus* is part of a taxonomically difficult group that includes *S. lacustris*, *S. tabernaemontani*, and *S. heterochaetus* (Smith 1995), and has been considered as part of *S. lacustris*, a highly polymorphic and widely distributed species distributed in Europe, the Mediterranean region and Africa. This taxonomically difficult group is in need of a taxonomic revision worldwide (González-Elizondo et al. 2007).

Previously known chromosome numbers for *S. acutus* are $n = 19$, 20 and $2n = 36$, 38, and 42, all of them for *S. acutus* var. *acutus* (see Table 3). Here, we report an extremely rare polyploid mixoploidy for *S. acutus* var. *occidentalis*, with $2n = 38$ and $2n = 84$ in the same individual. These numbers represent two cell lines in which $2n = 84$ may be derived from a $2n = 38$. The last line presents a highly asymmetric karyotype with 36 small, dot-shaped $+ 2$ larger chromosomes. The largest pair represent compound chromosomes, each corresponding in

Table 4.   Chromosome numbers recorded for *Schoenoplectiella*. Disploid mixoploidy is indicated by *.

Species	N	References	2n	References
S. articulata (L.) Lye	15	Rath and Patnaik 1972, 1974, cited in Roalson (2008)	20	Subramanian 1988, cited in Roalson (2008)
			32	Sharma and Bal 1956; Sharma and Sarkar 1967–1968; Sharma 1970; Sanyal and Sharma 1972, all cited in Roalson (2008)
S. erecta (Poir.) Lye? (misidentification?)	37	Mehra and Sachdevain 1971, 1975; Rath and Patnaik 1975, all cited in Roalson (2008)		
S. erecta subsp. *raynalii* (Schuyler) Beentje	5	Schuyler (1969)		
S. gemmifera (C. Sato, T. Maeda & Uchino) Hayas.			68–74*	Maeda and Uchino (2004)
			76	Yano and Hoshino (2005)
S. hallii (A. Gray) Lye	11	Schuyler (1969)		
S. hondoensis (Ohwi) Soják			38	Yano and Hoshino (2005)
S. hotarui (Ohwi) Holub	21	Yano and Hoshino (2005)	42, 44	Yano and Hoshino (2005)
	22	Belaeva and Siplivinsky in Roalson (2008)		
S. juncoides (Roxb.) Lye	37	Belaeva and Siplivinsky in Roalson (2008)	ca 70	Skottsberg 1955 in Roalson (2008)
			76	Nijalingappa 1972 in Roalson (2008)
			74	Yano and Hoshino (2005)
S. komarovii (Roshev.) Soják	19	Yano and Hoshino (2005)	38	Yano and Hoshino (2005)
S. lateriflora (J. F. Gmel.) Lye	11	in Roalson (2008)	30	In Roalson (2008)
S. lineolata (Franch. & Sav.) J. Jung & H. K. Choi	37	Yano and Hoshino (2005)	42	Tanaka (1940)
			ca 60	Kozhevnikov et al. (1986), in Yano and Hoshino (2005) and in Roalson (2008)
			74	Yano and Hoshino (2005)
S. mucronata (L.) J. Jung & H. K. Choi	21	Mehra and Sachdeva 1975 in Roalson (2008)	32–39*	Maeda and Uchino (2004)
	22	Seven references in Roalson (2008)	38	Yano and Hoshino (2005)
			42	Four references in Roalson (2008)
			44	Two references in Roalson (2008)
S. multiseta (Hayas. & C. Sato) Hayas.			70	Yano and Hoshino (2005)
S. purshiana (Fernald) Lye var. *purshiana*	19	Schuyler (1972)		
S. purshiana var. *williamsii* (Fernald) Hayas.	19	Schuyler (1972)		
S. roylei (Nees) Lye	11	Bir et al. 1981, 1986, 1988 in Roalson (2008)		
	44	Bir et al. 1985 in Roalson (2008)		
	11, 44	Bir et al. 1990, 1991 in Roalson (2008); Bir et al. 1992		
S. saximontana (Fernald) Lye	25	Schuyler (1969)		
S. senegalensis (Steud.) Lye			28	In Roalson (2008)
S. smithii (A. Gray) Hayasaka [with several infraspecific taxa]	20	In Roalson (2008)	18	In Roalson (2008)
S. supina (L.) Lye	5	Silvestre 1980 in Roalson (2008)	28	Sharma and Bal 1956; Nijalingappa 1972; Sanyal and Sharma 1972 in Roalson (2008)
	14	Various in Roalson (2008)		
S. triangulata (Roxb.) J. Jung & H. K. Choi			37–44*	Maeda and Uchino (2004)
			42	Yano and Hoshino (2005)
			44	Dai et al. (2010) [cited as *S. mucronatus* var. *robustus*]
S. wallichii (Nees) Lye	36	Belaeva and Siplivinsky 1975 in Roalson (2008)		

size to about three of the smaller, dot-shaped ones (3.17 versus 1.0 μm, respectively). Dimorphic chromosomes had been also recorded for this species by Hicks (1929), who mentioned one larger chromosome among the $n = 20$, and for Schuyler (1976), who reported for *S. acutus* and for the related *S. heterochaetus* "one meiotic unit which is about three times larger" than the others. For *S. lacustris*, another closely related species, Tanaka (1940) found two compound chromosomes, each the equivalent of three small chromosomes, and Kamari et al. (2000) found a cytotype of *S. lacustris* with $2n = 40$ in which two chromosomes are much longer than the others. In *S. acutus* var. *occidentalis* the cells with $2n = 38$ have a karyotype length of $2n = c.42$ small chromosomes, which double to 84 in the new ploidy level.

Schoenoplectus acutus shows the lowest average chromosome length (0.69 μm) among the species studied here. The length of the $2n = 38$ set is 54 μm, whereas that for the $2n = 84$ set is 79.5 μm. This kind of duplication of the number of chromosomes that is not associated to an equivalent increase in the length of the karyotype does not correspond to a true polyploidy but to a special kind of agmatoploidy (Heilborn 1924; Grant 1981). The dysploidy and the non-multiploid (disploid) polyploidy in *Scirpus* s.l. was interpreted by Hicks (1929) as derived from hybridization, which is common in the genus.

b) *Schoenoplectus americanus*

This widely distributed species, known from temperate regions from southern Canada to Chile, is a common element in aquatic vegetation in different ecosystems in Mexico (González-Elizondo et al. 2007). It has good tolerance to contamination and grows efficiently in artificial wetlands under greenhouse conditions (Pérez-López et al. 2009). The plants studied here grow in marshy areas at the edges of springs in central Durango, Mexico.

The record here of $2n = 66$ is the first for *S. americanus*. Counts of $n = 39$ and $2n = 78$ had been reported for the species, as well as $n = 43–47$ and $n = 50–64$ for *S. americanus* × *S. pungens* (see Table 3 for sources). *Schoenoplectus americanus*, *S. deltarum* and *S. pungens*, all with $n = 39$, are closely related and cytologically cohesive species (Schuyler 1976).

c) *Schoenoplectus californicus*

This species from warm to temperate to cold climates is distributed from the SW of the USA to Argentina and Chile, as well as some Pacific islands, and introduced to New Zealand. It is one of the dominant elements of the aquatic vegetation in Mexico, forming communities locally called "tulares" (González-Elizondo et al. 2007).

The count reported here of $2n = 68$ for *S. californicus* confirms the reports cited by Smith (2002) and Yano and Hoshino (2005). Other numbers previously given for the species are $n = 32, 34$ (Heiser 1979) and $2n = 64$ (cited by Yano and Hoshino 2005). For *S. californicus*

ssp. *tatora* (Kunth) T. Koyama, the Bolivian and Peruan totora, Heiser (1979) reported $n = 35$.

d) *Schoenoplectus tabernaemontani*

This semicosmopolitan species from warm–temperate regions is widely distributed in Mexico, forming "tulares", as *S. californicus* does. It is dominant in different ecosystems, including halophylous vegetation. *Schoenoplectus tabernaemontanii* is the correct name for many plants previously known as *Scirpus validus* Vahl and *Schoenoplectus validus* (Vahl) A. Löve et D. Löve, as well as for Mexican plants mistakenly identified as *S. lacustris*, a species with which is closely related (González-Elizondo et al. 2007).

The number $2n = 42$ for *S. tabernaemontani* confirms previous reports. The remarkable stability in the chromosome numbers reported for *S. tabernaemontani* worldwide in more than a dozen works ($n = 21, 2n = 42$) (see Table 3), supports its recognition at the specific level, particularly considering that it belongs to a family in which chromosome instability is almost the rule. A few variations are those reported by Hicks (1929), $n = (20)21(22)$. In a study of North American *Scirpus*, Hicks (1929) notes this stability ("metaphase plates in all cases show 21 chromosomes"), as compared with the other studied species. Among the species studied in this work, *S. tabernaemontani* had the lowest chromosome number and the highest average chromosome length (1.62 μm).

The significance of the chromosome number in Schoenoplectus

Intraspecific differences in chromosome number may or may not be correlated with phenotypic variations (Bir et al. 1992). For a disploid *S. lacustris* with $2n = 38, 40, 42$, Tanaka (1938) found that $2n = 38$ is associated with a normal form of the species; 40 with a variegated form *pictus*; and 42 with a variegated form *zebrinus*. Schuyler (1967), on the other hand, mentions that the gradual increases in chromosome number, a common situation in the Cyperaceae, would not, hypothetically, cause morphological divergence and discontinuity; he found chromosome numbers consistent for several species of *Scirpus* and considered them as a taxonomic characteristic useful to delineate species and to indicate relationships among them. As for intraspecific variation in chromosome number, Schuyler (1976) notes that further study on morphological and ecological differentiation of cytotypes may have bearing on our understanding of speciation.

Among the genera segregated from *Schoenoplectus*, a tendency to smaller, more numerous chromosomes is seen in *Bolboschoenus*, e.g. $2n = 104, 110$ (Tanaka 1937 in Roalson 2008; Löve and Löve 1981), whereas *Schoenoplectiella* has lower numbers, with *Schoenoplectus* s.s. having, in general terms, intermediate sizes and a higher number of chromosomes (Figure 3A, B). Already

in 1929 Hicks noted that the "*Scirpus lacustris* group" [*Schoenoplectus*] had larger chromosomes than the "*Scirpus maritimus* group" [*Bolboschoenus*]. Since low chromosome numbers represent a primitive condition whereas high numbers and small chromosomes represent derived conditions (Stebbins 1950; Sharma 1964; Schuyler 1967), it could be suggested that the species of *Schoenoplectiella* are the most primitive in the group, which is reinforced by the fact that that genus includes the annual species, a feature associated by Stebbins (1938) with primitiveness. However, in recent molecular studies (Muasya, Simpson, et al. 2009) *Schoenoplectiella* is resolved into a strongly supported clade that includes perennial tropical *Schoenoplectus* sharing a lateral spikelet morphology.

As for *Scirpus*, Schuyler (1967) found a lack of hybridization among species with low chromosome numbers and a progressive increase in the amount and complexity of hybridization among species with higher numbers, which was interpreted as due to those plants representing a series of hybrid complexes.

The basic chromosome number $x = 5$ traditionally accepted for Cyperaceae is difficult to corroborate for *Schoenoplectus* in which haploid numbers range from $n = 19$ to $n = 47$, and diploids $2n = 36$ to 84[-128], unless complex mechanisms of agmatoploidy and symploidy occur before and after polyploidization events.

Mixoploidy

Intra-individual variation in chromosome number (mixoploidy) has been recorded for many organisms. Polyploid mixoploidy appears to be the most common mixoploidy for Angiosperms (e.g. Ranjbar et al. 2011). However, all previous reports for sedges correspond to disploid mixoploidies. The first report of this phenomenon for *Schoenoplectus* was by Otzen (1962), who detected intraclonal chromosomal variability in four species and three hybrids of *Schoenoplectus* (see Table 3) and one of *Schoenoplectiella*. Maeda and Uchino (2004) also found disploid mixoploidy in the root-tip cells of three species of *Schoenoplectus* s.l., which has recently been transferred to *Schoenoplectiella*. In those, the chromosome number varied as follows: $2n = 68$ to 74 in *Schoenoplectus gemmifer* [*Schoenoplectiella gemmifera*], $2n = 32$ to 39 in *S. mucronatus* [*Schoenoplectiella mucronata*], and $2n = 37$ to 44 in *S. triangulatus* [*Schoenoplectiella triangulata*]. In this work we report the first polyploid mixoploidy for Cyperaceae: a prevalence of cells with $2n = 38$ (36 small + 2 compound, larger chromosomes) and a few cells with $2n = 84$ small, dot-shaped chromosomes, found in *Schoenoplectus acutus* var. *occidentalis*.

It has been proposed that the increasing chromosome number by fragmentation provides a greater variability and better adaptation capability (Mola and Papeschi 2006). Similar conclusions have been drawn by Mayrose et al. (2010) and Wang et al. (2011), who note that when plants are exposed to a large variety of abiotic stresses, their karyotypes or genomes tend to evolve to polyploidy suitable for adverse environments. Mixoploidy in *S. acutus* var. *occidentalis* may be a response to the harsh environment where these plants grow: temporarily flooded wetlands in alkali flats in central Durango, on the Mexican highlands, which are dry for more than half the year.

The chromosomal evolution and the molecular phylogeny of 14 species of *Schoenoplectus* s.l. of Japan were studied by Yano and Hoshino (2005). Based on the fact that the groups of species they studied have high and low chromosome numbers with almost equal chromosome sizes, they concluded that the chromosomal evolution in those species has been caused more by polyploidy than by dysploidy (considered by them as aneuploidy). The variation in chromosome number largely distributed around 20 and 40 reported by Roalson (2008) was interpreted by him as a possible indication of polyploidy with subsequent dysploidy (Roalson referred to dysploidy as aneuploidy, which does not occur in groups with holokynetic chromosomes).

Conclusion

For the species studied here, karyotype variation occurs mainly through dysploidy (numeric variation due to rearrangements), with polyploidy appearing as a secondary pathway. As expected for Cyperaceae and for any group with holokinetic chromosomes, none of the species studied presented primary constrictions in the chromosomes. All the studied species exhibit dot-shaped chromosomes decreasing gradually in size, a feature shared by the species of *Schoenoplectus*, *Schoenoplectiella* and *Bolboschoenus*.

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