INSTITUTO POLITÉCNICO NACIONAL

CENTRO INTERDISCIPLINARIO DE INVESTIGACIÓN
PARA EL DESARROLLO INTEGRAL REGIONAL
UNIDAD DURANGO

ESTUDIO CITOGENÉTICO DE ESPECIES DE LOS GÉNEROS *Eleocharis* y *Schoenoplectus* (CYPERACEAE)

TESIS

QUE PARA OBTENER EL GRADO DE

DOCTOR EN CIENCIAS EN BIOTECNOLOGÍA

PRESENTA

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Victoria de Durango, Dgo., Julio de 2012

SIP-14-BIS

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ACTA DE REVISIÓN DE TESIS

En la Ciudad de Durango, Dgo. siendo las 14:00 horas del día 22 del mes de Junio del 2012 se reunieron los miembros de la Comisión Revisora de Tesis, designada por el Colegio de Profesores de Estudios de Posgrado e Investigación de CIIDIR-IPN DGO para examinar la tesis titulada:

Estudio citogenético de especies de los géneros *Eleocharis* y *Schoenoplectus* (*Cyperaceae*)

Presentada por el alumno:

TENA	FLORES	JORGE ALBERTO
Apellido paterno	Apellido materno	Nombre(s)

Con registro:

B	0	8	1	2	2	6

aspirante de:

DOCTORADO EN CIENCIAS EN BIOTECNOLOGÍA

Después de intercambiar opiniones los miembros de la Comisión manifestaron ***APROBAR LA TESIS***, en virtud de que satisface los requisitos señalados por las disposiciones reglamentarias vigentes.

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México, D.F. a 22 de Junio del 2012

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1.- Se designa al aspirante el tema de tesis titulado:
"Estudio citogenético de especies de los géneros de *Eleocharis* y *Schoenoplectus* (*Cyperaceae*)"

De manera general el tema abarcará los siguientes aspectos:
Citogenética
Cromosomas
Cyperaceas

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CARTA CESIÓN DE DERECHOS

En la Ciudad de **DURANGO, DGO.**, el día **22** del mes de **JUNIO** del año **2010**, la que suscribe **JORGE ALBERTO TENA FLORES** alumno del Programa de **DOCTORADO EN CIENCIAS EN BIOTECNOLOGÍA** con número de registro **B081226**, adscrito a **CIIDIR-IPN UNIDAD DURANGO**, manifiesta que es autor intelectual del presente trabajo de Tesis bajo la dirección de la **DRA. YOLANDA HERRERA ARRIETA** y del **DR. NETZAHUALCÓYOTL MAYEK PÉREZ** y cede los derechos del trabajo intitulado **ESTUDIO CITOGENÉTICO DE ESPECIES DE LOS GÉNEROS *Eleocharis* Y *Schoenoplectus (Cyperaceae)***, al Instituto Politécnico Nacional para su difusión, con fines académicos y de investigación.

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JORGE ALBERTO TENA FLORES

Nombre y firma

El presente trabajo se llevó a cabo en el Centro Interdisciplinario de Investigación para el Desarrollo Integral Regional, Unidad Durango del Instituto Politécnico Nacional, en los Laboratorios de Citogenética y de Biotecnología, bajo la dirección de la Dra. Yolanda Herrera Arrieta y del Dr. Netzahualcóyotl Mayek-Pérez

El estudio se desarrolló dentro del programa de trabajo del proyecto “Citogenética de Especies de Gramíneas y Ciperáceas con Importancia Económica en el Norte-Centro de México” (SIP 20110017)

AGRADECIMIENTOS

Fueron muchas las personas que apoyaron el desarrollo de este trabajo. Los directores, Dra. Yolanda Herrera Arrieta y Dr. Netzahuatcóyotl Mayek-Pérez, al igual que el Asesor externo, Dr. André Luis Laforga Vanzela, invirtieron incontables horas y paciencia en asesoría así como en diversas fases del establecimiento del Laboratorio de Citogenética del CIIDIR Durango, sin escatimar su tiempo y experiencia. La Dra. Norma Almaraz Abarca y la Dra. Socorro González Elizondo, miembros del Comité Tutorial, participaron también directamente en el desarrollo de la investigación, la Dra. Almaraz, como coordinadora del laboratorio de Biotecnología, aportando además espacio físico y equipo para albergar al laboratorio de Citogenética en sus primeras fases, y la Dra. González con la identificación taxonómica de las plantas así como con orientación para interpretar la información obtenida y participación en la colecta de materiales. La asesoría del Dr. Vanzela durante un mes en el CIIDIR y a través de comunicación continua posterior, así como el apoyo del Dr. Carlos Roberto Maximiano da Silva y la Dra. Vanessa Mancuso, fueron cruciales para la estandarización de las técnicas de laboratorio. La Dra. Martha González Elizondo, miembro del Comité Revisor, sugirió valiosas mejoras al manuscrito de la tesis y apoyó el desarrollo del trabajo tanto en el herbario como en campo. Muchas gracias también a los compañeros de los laboratorios de Citogenética y de Biotecnología, particularmente a Octavio Rosales Carrillo y a Claudia Silva Salas por su entusiasta ayuda tanto en el laboratorio como en la colecta de materiales, a Vanessa Arreola Heynes, y a Eli Amanda Delgado Alvarado. A los compañeros del herbario CIIDIR agradezco su ayuda, amistad y compañía: a Jorge Noriega Villa, por el trabajo de campo y de herbario y por el cuidado dedicado a la colección de las plantas en el vivero; a la M. en C. Lorena López Enriquez por el apoyo con trabajo de herbario y con la base de datos, al igual que a Daniela Corral Díaz, Lizeth Ruacho González y Flor Isela Retana Rentería; a Sergio Heynes Silerio también por colectas de material. Muy en especial quiero agradecer a la Dra. Socorro González Elizondo su paciencia, comprensión, cooperación y apoyo en todo lo relacionado con este trabajo. A los Doctores José Antonio Avila Reyes (Director) y

Marco Antonio Márquez Linares (Subdirector) del CIIDIR Dgo, por el apoyo para la realización y conclusión de mi estudio de Tesis. Gracias a la COFAA por los estímulos para investigación y a COTEPABE por la licencia de trabajo que me permitió dedicar tiempo completo al desarrollo de esta investigación.

DEDICATORIA

A la memoria de mis Padres, **María de los Ángeles** y **Benjamín**

A mis hermanos **Patricia**, **Carlos**, **Lourdes** y **Norma**

A mis dos ejemplares de *Eleocharis tenarum*, mis hijas **Georgina Angélica** y **Sandra Mayela**

A **Soco**, mi querida esposa, que a partir de ahora también es mi compañera en esta nueva aventura, mi incursión en el campo de la Citogenética

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GLOSARIO

En todos los casos, la terminología se refiere a cromosomas de organismos eucarióticos.

Agmatoploidía:	Cambio en el número de cromosomas originado por ruptura (fisión o fragmentación) de cromosomas holocinéticos, en los que cada fragmento puede llegar a ser un cromosoma independiente.
Aneuploidía:	Cambios en el número de cromosomas enteros. Es el aumento o la pérdida de uno o más pares de cromosomas, que puede dar lugar a mutaciones.
Cinetócoro:	Estructura proteica localizada en la parte externa del cromosoma, sobre el centrómero, implicada en el control del movimiento de los cromosomas durante la división celular.
Disploidia:	Condición en la cual hay aumento o disminución progresiva unitaria en el número cromosómico como resultado de rearreglos de segmentos cromosómicos y pérdidas o incorporaciones de centrómeros sin que haya un cambio en el genotipo.
Holocinético	Cromosoma con cinetócoro difuso, en el que la actividad cinética se distribuye sobre el cromosoma completo. Se ha utilizado el nombre de holocéntrico y policéntrico.
Ideograma:	Diagrama simplificado de los cromosomas metafásicos del cariotipo.
Mixoploide:	Individuo que presenta células con número diferente de cromosomas.
Mixoploidía/aneuploidía	Variación gradual en el número cromosómico en las células de un mismo individuo.
Mixoploidía/poliploidía.	Variación en el número cromosómico en las células de un mismo individuo, en el que un número proviene de la duplicación del otro.
Simploidía	Cambio en el número de cromosomas originado por fusión de cromosomas holocinéticos, en los que cada fragmento puede llegar a ser un cromosoma

	independiente.
Sinapomorfismo:	Novedad evolutiva compartida por dos o más taxones.

LISTA DE ABREVIATURAS

acc.	de acuerdo a
aff.	afín a
c., ca.	(del latin circa), alrededor o cerca de, aproximadamente
cf.	parecida a [confróntese con]
p.p.	en parte
sect.	Sección
s.l.	sensu lato = en sentido amplio
sp.	Especie
spp.	Especies
ssp.	Subespecie
subg.	Subgénero

RESUMEN

Se llevaron a cabo análisis cariomorfológicos de ocho especies de *Eleocharis* y de cuatro especies de *Schoenoplectus* (Cyperaceae) de México, basados en 49 y 11 poblaciones, respectivamente. En ambos casos se estudiaron cromosomas metafásicos de células meristemáticas de raíz con métodos de citogenética convencional. En las especies estudiadas de *Eleocharis* los números cromosómicos variaron entre 2*n* = 10 y 2*n* = 60. Se reportan por primera vez números cromosómicos para tres especies: *Eleocharis densa*, *E. reznicekii* y *E. rostellata*, y se registran nuevos números para *E. macrostachya*, *E. xyridiformis*, y para plantas del complejo de *E. montevidensis*. El mecanismo más común de variación cariotípica en las especies de *Eleocharis* estudiadas es la disploidía (ya sea por fisión o por fusión), seguida por poliploidía: la mitad de las especies son disploides (*E. densa*, *E. macrostachya*, *E. reznicekii* y *E. xyridiformis*), dos son diploides (*Eleocharis parishii* y *E. cf. montevidensis*) y tres son poliploides (*E. acicularis*, *E. montevidensis* y *E. rostellata*). No se encontró variación intraespecífica en cuanto a números cromosómicos excepto para plantas del complejo de *E. montevidensis*, pero sí se encontraron diferencias en tamaño del cariotipo entre poblaciones de ese mismo complejo y en dos formas de *E. rostellata*. La comparación del número, forma y tamaño de los cromosomas entre una especie de origen híbrido (*E. reznicekii,* 2*n* = 16) y sus parentales putativos: *E. densa* (2*n* = 16) y *E. xyridiformis* (2*n* = 28), no permitió corroborar la hipótesis de parentalidad de esas especies. En *Schoenoplectus* los números cromosómicos van de 2*n* = 38 a 2*n* = 84. Se documentan nuevos números para *Schoenoplectus acutus* (2*n* = 38 y 2*n* = 84) y para *S. americanus* (2*n* = 66). Se registra por primera vez mixoploidía para *Schoenoplectus acutus* y mixoploidía/poliploidía (variación intra-individual con poliploidía) para *Schoenoplectus*. El mecanismo más común de variación cariotípica en las especies estudiadas es la poliploidía seguida de disploidía. Tanto para *Eleocharis* como para *Schoenoplectus* se encontraron cromosomas holocinéticos, confirmándose la ausencia de constricciones primarias.

ABSTRACT

Karyomorphological analyses were carried out on eight species of *Eleocharis* and four species of *Schoenoplectus* (Cyperaceae) of Mexico, based on 49 and 11 populations, respectively. Studies in both cases were done on mitotic metaphase chromosomes from meristematic root cells using conventional cytogenetics technics. In *Eleocharis* chromosome numbers range from 2*n* = 10 to 2*n* = 60. Chromosome numbers are given for the first time for *Eleocharis densa*, *E*. *reznicekii* and *E*. *rostellata*, and new records are given for *E*. *macrostachya*, *E*. *xyridiformis*, and plants of the *E*. *montevidensis* complex. The most common mechanism of karyotype variation is disploidy (either by fission or fusion), which is present in half of the species (*E. densa*, *E. macrostachya*, *E. reznicekii*, and *E. xyridiformis*), while the two species are diploid (*Eleocharis parishii* and *E. cf. montevidensis*) and three are polyploid (*E. acicularis*, *E. montevidensis*, and *E. rostellata*). No intraspecific variation in chromosome number was found except for plants of the *E*. *montevidensis* complex; however, differences in size among chromosomes of populations of the same complex as well as between two forms of *E*. *rostellata* were found, which are related with phenotipic differences among those plants. Comparison of the karyotypes of a species of hybrid origin (*E*. *reznicekii*, 2*n* = 16) and their putative parentals: *E*. *densa* (2n = 16) and *E*. *xyridiformis* (2*n* = 28) does not resolve their relationships based only on the number, shape and size of the chromosomes. As for *Schoenoplectus*, chromosome number in the studied species range from 2*n* = 38 to 2*n* = 84. New chromosome numbers are documented for *Schoenoplectus acutus* (2*n* = 38 and 2*n* = 84) and for *S*. *americanus* (2*n* = 66). The karyotype characterization in *Schoenoplectus* allowed the discovery of mixoploidy for *Schoenoplectus acutus* and the first record of mixoploid/poliploidy (intra-individual variation including polyploidy) for the genus. The most common mechanism of karyotype variation in the studied species of *Schoenoplectus* is polyploidy followed by disploidy. For both genera, *Eleocharis* and *Schoenoplectus*, holokinetic chromosomes were found, which confirms the absence of primary constrictions.

I. INTRODUCCIÓN

La citogenética es la disciplina que estudia el comportamiento y la estructura de los cromosomas y su relación con la transmisión y recombinación de los genes. Tanto la citogenética clásica como la citogenética molecular contribuyen a los estudios taxonómicos, evolutivos y de genómica estructural y funcional (Gill y Friebe, 1998; Herrera, 2007). En investigación y en biotecnología de plantas se aplica en procesos de mejoramiento genético convencionales o biotecnológicos (Poggio *et al*., 2010). Los rasgos citológicos permiten conocer las características reproductoras y evolutivas de las especies y ayudan a clarificar el origen de híbridos y de variedades cultivadas; a revelar diferencias crípticas entre organismos estrechamente relacionados (ej. plantas cultivadas y sus parientes silvestres); y a mejorar nuestra comprensión sobre la organización y la expresión de los genes, base para el mejoramiento genético de plantas de interés económico (cultivadas, en proceso de domesticación, usadas para restauración de ecosistemas, etc.). La citogenética es también una herramienta que permite resolver interrogantes de tipo taxonómico y evolutivo, para el mejor conocimiento de la biodiversidad.

1.1. Morfología cromosómica y cariotipo

El cromosoma es el material hereditario (ADN) organizado alrededor de un esqueleto proteico, cuyas funciones son las de conservar, transmitir y expresar la información genética contenida en los genes que porta. El cariotipo es el fenotipo del complemento cromosómico, o sea, la suma y ordenamiento de todos los rasgos estructurales de los cromosomas, incluyendo número, tamaño y morfología tal como se ven en metafase mitótica. El cariotipo se visualiza generalmente después de un apropiado pre-tratamiento y tinción de las células, para hacer más visibles los cromosomas individuales. Al diagrama simplificado de los cromosomas metafásicos del cariotipo se le denomina *ideograma*, el cual se construye con el número cromosómico de la especie (Torres, sin año).

La utilidad del cariotipo – ideograma consiste en que permite la caracterización de especies, poblaciones, individuos, ecotipos, razas, etc. La cariosistemática es un área de trabajo capaz de dar respuesta a problemas inherentes a la evolución de los cromosomas, la diversificación cariotípica en el espacio y el tiempo y la consecuente especiación. Si bien las especies tienden a mantener su constitución cariotípica estable a través de las generaciones, en algunos individuos de la población es probable la ocurrencia de mutaciones espontáneas que provocan cambios de tipo estructural o numérico en el genoma (Torres, sin año).

1.2. La familia Cyperaceae

Cyperaceae es una familia de monocotiledóneas cosmopolita con cerca de 5,400 especies organizadas en 106 géneros (Govaerts *et al*., 2007) a 5,500 especies en 109 géneros (Muasya *et al*,. 2009), o hasta 122 géneros reconocidos (Bruhl, 1995); es la tercera familia más grande entre las monocotiledóneas, después de las orquídeas y de las poáceas (gramíneas). Para México se conocen 26 géneros y más de 450 especies. Las ciperáceas incluyen varios géneros cosmopolitas, como *Carex* L., con más de 2,000 especies (Reznicek, 1990) y *Eleocharis* R.Br., que comprende más de 270 especies (González-Elizondo, com. pers.).

1.2.1. Importancia económica y ambiental de Cyperaceae

Muchas especies de ciperáceas son dominantes en diversos hábitats, con frecuencia de sitios húmedos. Algunas ciperáceas se comportan como malezas o invasivas, pero en su mayor parte sirven para estabilizar y conservar el suelo y como hábitat y alimento de aves y otra fauna silvestre, como forraje para ganado y algunas, como *Eleocharis dulcis*, son usadas incluso como alimento humano. Varias especies tienen gran potencial en el manejo de malezas acuáticas y en la reducción de la contaminación (Sutton, 1984 citado por Catling y Hay, 1993). La fitorremediación es un campo emergente con gran potencial para la remoción de metales pesados de suelos y aguas contaminadas. Se basa en el uso de plantas, preferentemente

nativas, que presentan alta capacidad para acumular esos metales y removerlos de los suelos y aguas.

Entre los géneros de ciperáceas con potencial para fitorremediación destacan *Eleocharis* (Flores-Tavizón *et al*., 2003; González-Elizondo *et al*., 2005) y *Schoenoplectus* (Rchb.) Palla (Rice *et al*., 1997; Bhattacharya *et al*., 2006; Pérez-López *et al*., 2009). Ambos son géneros cosmopolitas que se encuentran bien representados en México. *Eleocharis* agrupa más de 270 especies a nivel mundial y más de 40 especies en México; es un grupo de gran complejidad taxonómica (González-Elizondo y Peterson, 1997; González-Elizondo y Tena-Flores, 2000; González-Elizondo y Reznicek, 2005; González-Elizondo *et al*., 2007, 2008, 2009; Smith *et al*., 2002; Saarela *et al*., 2010). *Schoenoplectus* agrupa alrededor de 77 especies, algunas de las cuales son el elemento dominante en vegetación acuática; varias se conocen con el nombre de "tule". Para México se conocen siete especies (González-Elizondo *et al*., 2007).

1.2.2. Citología de Cyperaceae

La familia se caracteriza por la ocurrencia de varias características citológicas cuya combinación es única a: 1) cromosomas con cinetocoro difuso, 2) meiosis pos-reduccional y 3) formación de pseudomonades, sin embargo, hay tétrada asimétrica. Final se forma un grano de polen y no cuatro como en el resto de las Angiospermas (Faulkner, 1972). El estudio de estas características citológicas atípicas revela importante información sobre la evolución de los cariotipos. Adicionalmente, en Cyperaceae se registra gran cantidad y variedad de números cromosómicos, cuya evolución cariotípica ocurre principalmente debido a agmatoploidía y simploidía (Luceño y Guerra, 1997) y a poliploidía (Håkansson, 1954; Vanzela *et al*., 2000; Da Silva *et al*., 2010).

1.2.3. Citogenética de *Eleocharis* y *Schoenoplectus*

En este trabajo se llevaron a cabo análisis citogenéticos de especies de *Eleocharis* y de *Schoenoplectus* de la región norte-centro de México (Zacatecas, Durango, Coahuila, Chihuahua). Debido a las características citológicas únicas de esta familia, su estudio citogenético arroja luz sobre aspectos taxonómicos y evolutivos y contribuye al mejor conocimiento del grupo. El trabajo es el primero en su tipo para especies mexicanas de Cyperaceae y permitió registrar los números cromosómicos y caracterización del cariotipo de ocho especies de *Eleocharis* y cuatro de *Schoenoplectus*.

Entre los resultados se presentan los manuscritos de tres artículos:

1. TENA-FLORES J.A. Cytogenetics in Cyperaceae. Artículo de revisión.

2. TENA-FLORES J.A, M.S. GONZÁLEZ-ELIZONDO, Y HERRERA-ARRIETA, N. ALMARAZ-ABARCA, N. MAYEK-PÉREZ, C.R.M. DA SILVA, AND A.L.L. VANZELA. 2012. Karyotype characterization of eight Mexican species of *Eleocharis* (Cyperaceae). Trabajo aceptado en Botanical Sciences.

3. TENA-FLORES J.A, M.S. GONZÁLEZ-ELIZONDO, Y HERRERA-ARRIETA, N. ALMARAZ-ABARCA, N. MAYEK-PÉREZ, AND A.L.L. VANZELA. 2012. Karyotype characterization of four Mexican species of *Schoenoplectus* (Cyperaceae) and first report of mixoploidy for *Schoenoplectus acutus*. Artículo de investigación (en proceso).

II. ANTECEDENTES

Las Ciperáceas son conocidas por tener una gran variación inter e intra-específica en el número de cromosomas. La mayoría de estas variaciones ha sido previamente atribuida a agmatoploidía (Da Silva *et al.,* 2008b).

Löve *et al. (*1957) propusieron *x* = 5 como el posible número básico para Cyperaceae, lo que ha sido corroborado por Vanzela *et al. (*2000) y da Silva (2005), entre otros. Los estudios citogenéticos en *Eleocharis* datan de antes de 1924, cuando Piech describió el número de cromosomas de *E. palustris* (Nijalingappa, 1973).

2.1. Los cromosomas holocinéticos

Las evidencias hasta ahora indican que los cromosomas de las especies de Cyperaceae presentan cinetócoro difuso (el centrómero no está localizado y no se evidencía en una constricción), por lo que la actividad cinética se distribuye sobre el cromosoma completo. La condición del cinetócoro difuso se considera como un sinapomorfismo del clado de las ciperáceas y las juncáceas (Greilhuber, 1995). Aunque existen reportes de cinetócoros localizados en cromosomas de algunos géneros de Cyperaceae, estos reportes han sido cuestionados y no se han confirmado. Los cromosomas con cinetócoro difuso se conocen como cromosomas holocéntricos, término no adecuado y substituido recientemente por el de cromosomas holocinéticos. Este tipo de cromosomas sin centrómero localizado fueron registrados en el género *Eleocharis* por Battaglia y Håkansson (Strandhede, 1965a). Los cromosomas con cinetócoro difuso, ya sea natural o artificialmente fragmentados, son segregados normalmente durante el ciclo celular. Esto fue demostrado por Castro *et al. (*1949) en *Luzula purpurea* (Juncaceae), por Håkansson (1954) en *E. palustris* y en *Rhynchospora pubera* por Vanzela y Colaço (2002).

2.2. Evolución del cariotipo

La evolución de los cariotipos de la familia Cyperaceae ocurre a través de agmatoploidia (fisión), simploidia (fusión) y poliploidía de los cromosomas holocinéticos. Esto fue aceptado después de numerosos trabajos realizados en el género *Carex*, el mayor en número de especies estudiadas (Davies, 1956; Luceño y Castroviejo, 1991). Las terminologías agmatoploidía, para fisión cromosómica y simploidía, para fusión cromosómica, en holocinéticos, fue revisada y propuesta por Luceño y Guerra (1997).

Vanzela *et al. (*2000) verificaron que cerca del 59% de las especies estudiadas de *Rhynchospora* son poliploides y sólo en pocas especies, por ejemplo *R. tenuis* (Vanzela *et al*., 1996) y *R. cephalotes* (Luceño *et al.*, 1998) se detectó agmatoploidía y simploidía. Esto indica que la poliploidía es un evento más importante en este grupo que en *Carex*. La evolución cariotípica para el género *Eleocharis* parece seguir un patrón mixto. Por ejemplo, da Silva (2005) menciona que la mayoría de las especies son poliploides, y Bureš (1998) describe un alto grado de poliploidía en *Eleocharis uniglumis*. Sin embargo, son comunes también las especies que presentan variaciones numéricas intra-específicas (citorazas) originadas por disploidía (agmatoploidía y simploidía). El menor número cromosómico del género (2n = 6) originado por translocaciones múltiples, ha sido registrado para *E. subarticulata* (da Silva *et al*., 2005).

A pesar de la existencia del cinetócoro difuso a lo largo de los cromosomas en mitosis, Strandhede (1965a) sugiere que en la meiosis los cromosomas holocéntricos poseen actividad cinética en regiones específicas, como fue propuesto por Bernardini (1959), lo cual no ha sido confirmado. La morfología característica de los cromosomas holocinéticos produce un comportamiento diferenciado en la meiosis. En los cromosomas holocinéticos, al contrario de los monocéntricos, la primera división puede ser ecuacional y la segunda reduccional. La orientación de las cromátidas es una restricción de la actividad cinética hasta un punto de los cromosomas donde cada cromátida hermana posee una región con esta actividad,

dependiendo de la finalización de los quiasmas (Nokkala, 1985; Camacho *et al*., 1985). De acuerdo con la posición de los quiasmas, la alineación de los bivalentes holocéntricos puede ser paralela al eje largo de la placa ecuatorial, como ocurre en algunos invertebrados (Hughes-Scharader, 1948) o bien, perpendicular al eje largo de la placa ecuatorial como normalmente ocurre en los cromosomas monocéntricos. De ese modo, en un momento de la anafase I las cromátidas hermanas presentan orientación paralela y la segregación ocurre con dos cromátidas hermanas migrando juntas para cada lado de la célula, comportamiento meiótico llamado meiosis invertida (Pazi y Plitman, 1994) o post-reduccional, a diferencia de la meiosis convencional o pre-reduccional en la que durante la anafase I la segregación se da por la separación de cromosomas homólogos (da Silva *et al*., 2005). En plantas, la meiosis invertida es muy rara, ocurriendo principalmente en Cyperaceae, en géneros como *Carex* (Wahl, 1940), *Eleocharis* (Strandhede, 1965a; Nijalingappa, 1973; Hoshino, 1987; da Silva *et al*., 2005) y *Rhynchospora* (Vanzela *et al*., 2000), así como en los géneros *Cuscuta,* de la familia Cuscutaceae (Pazi y Plitman, 1994) y *Luzula*, de la familia Juncaceae (Nordenskiöld, 1951).

Durante la alineación de los bivalentes en la meiosis, una estructura característica de los cromosomas holocéntricos puede observarse y es un arreglo cromosómico llamado “estructura en caja”, como se reporta para *R. cephalotes* (Vanzela *et al*., 2000). Otra estructura fue observada durante la meiosis invertida de *E. subarticulata*, donde durante la metafase I se observó que los cromosomas se unen por sus extremos formando un anillo, debido a translocaciones múltiples (da Silva *et al*., 2005).

Otra característica citogénetica común en Cyperaceae es la pérdida de la tétrada, donde, durante la formación de los granos de polen, tres de los cuatro núcleos provenientes de la meiosis se degeneran y solamente uno continúa el desarrollo para formar un grano de polen, a diferencia de la mayoría de las Angiospermas en donde a partir de una tétrada se originan cuatro granos de polen (Hoshino y Shimizu, 1986). En las ciperáceas el núcleo funcional se divide luego en núcleos vegetativos y

generativos (Strandhede, 1965a; Nijalingappa, 1973; Vanzela *et al*., 2000; da Silva, 2005).

La estructura de los núcleos interfásicos es otra característica citogenética que proporciona información. Los núcleos interfásicos encontrados en algunas especies de *Eleocharis* pueden presentar pequeños cuerpos fuertemente coloridos (Hoshino, 1987). Estos cuerpos pueden presentarse de manera dispersa o concentrados en las regiones de los núcleos, o ser grandes cuerpos condensados en menor cantidad. Estas señas son llamados cromocentros y se relacionan con un patrón de condensación cromosómica. En un estudio reciente (da Silva *et al*., 2010), doce especies de *Eleocharis* fueron estudiadas y de éstas solo tres especies de la sección *Limnochloa* presentaron núcleos interfásicos con cromocentros, mientras que todas las demás presentaron núcleos interfásicos difusos. Las 16 especies de *Rhynschospora* estudiadas por Luceño *et al. (*1998) apenas presentaron núcleos cromocéntricos.

2.3. Técnicas para el estudio de los cromosomas

En algunas especies los pares cromosómicos no pueden diferenciarse claramente considerando sólo sus componentes distintivos en sentido longitudinal. En estos casos se recurre a técnicas citológicas especiales para la tinción de los cromosomas que evidencian "bandas" transversales (oscuras y claras) a lo largo de los mismos y que corresponden a los distintos tipos de cromatina. En una especie dada, estas variantes de la cromatina presentan un tamaño y disposición constantes.

En la actualidad las técnicas de biología molecular más sofisticadas permiten detectar la variabilidad a nivel de ADN. Sin embargo, la caracterización cariotípica aún sigue siendo necesaria para dilucidar el rol de los cromosomas en la herencia, adaptación y evolución, aportando información importante tanto en programas de mejoramiento genético como en investigaciones taxonómicas y filogenéticas.

La citogenética molecular se remonta a los primeros experimentos de hibridación con sondas de ADN y de ARN marcadas radioactivamente (Jhon *et al*., 1969; Pardue y Gall, 1969; citados por Herrera, 2007). Sin embargo, debido a la existencia de limitaciones técnicas relacionadas con la detección de las sondas radioactivas y a la ausencia de protocolos eficientes para la clonación de secuencias de interés, los resultados de aquella época no tuvieron el auge esperado. La verdadera revolución en el campo de la citogenética se dio en los últimos 15 años. Las innovaciones técnicas en la microscopía fluorescente y el desarrollo de mejores componentes ópticos (lentes, filtros, captores) mejoraron el desempeño de los microscopios de luz transmitida (Herrera, 2007). A estos cambios se sumó la implementación progresiva de una amplia gama de métodos de hibridación con sondas fluorescentes basados en la utilización de una gran variedad de fluorocromos que abarcaban prácticamente toda la gama del espectro luminoso, desde el infrarrojo hasta el ultravioleta. El desarrollo de métodos rápidos y precisos para la marcación y detección de sondas, así como la utilización de software especializado para el tratamiento de imágenes, dieron un gran empuje a las técnicas modernas basadas en la llamada hibridación *in situ* fluorescente o FISH (por “fluorescent in situ hybridization”) utilizadas en la investigación médica como es el caso de estudiar las las características morfológicas y secuencias de ADN permite la investigación de aspectos estructurales, evolutivos y funcionales de los cromosomas, llevando descubrir las bases moleculares de las enfermedades. Por otro lado, la caracterización futura de los genomas animales y vegetales servirá sin duda para mejorar nuestra comprensión sobre la organización y la expresión de los genes, uno de los objetivos fundamentales de cualquier programa de mejoramiento genético (Herrera, 2007).

El número de cromosomas es una fuente importante de evidencias taxonómicas y, particularmente en algunos géneros, esta información ha demostrado ser valiosa en el entendimiento de la evolución de las especies (Goldblatt, 1981, citado por Espert *et al*., 2008). Sin embargo, esta debe ser aunada a otra información genética para entender la historia evolutiva de los grupos, como Hipp *et al. (*2010) han mostrado en estudios con *Carex*, donde los diferentes rearreglos cromosómicos no

necesariamente representan 'razas' monofiléticas o infraespecies, a pesar de que la evolución cariotípica juega un papel potencial en la especiación en ese género. La información citogenética proporcionada por una combinación de bandeo cromosómico y FISH, puede ser útil para comparar especies dentro de los géneros, así como de especies de diferentes géneros (Fregonezi *et al*., 2004).

El descubrimiento de las técnicas que producen patrones de bandeo en los cromosomas es uno de los acontecimientos más significativos en la citogenética. Actualmente se encuentran disponibles una serie de técnicas de bandeo de las cuales, la técnica de Bandeo C con Giemsa ha sido la más utilizada para identificar cromosomas individuales en muchas especies vegetales. Esta técnica se basa en aplicar a las preparaciones cromosómicas diferentes pretratamientos y tinción con Giemsa. Estos pretratamientos degradan el ADN cromosómico y lo extraen selectivamente de las partes eucromáticas del cromosoma, dejando que las zonas heterocromáticas se tiñan más intensamente con Giemsa. El nombre del Bandeo C se debe a que produce bandas constantes que corresponden a regiones de heterocromatina constitutiva que no se descondensan en interfase, siendo visibles tanto en mitosis como en interfase y con tamaño relativamente constante. Según los organismos pueden ser centroméricas, teloméricas o intersticiales. Este método se ha aplicado a especies de plantas con cromosomas grandes como trigo, centeno, cebada y triticale, permitiendo una identificación precisa de los cromosomas. En el caso de plantas con cromosomas pequeños como arroz, *Arabidopsis* y *Brassica*, que miden de 1 a 2 µm en metafase mitótica, se han tenido dificultades para identificar sus cromosomas por medios morfológicos. Los métodos de bandeo no han dado resultados para los cromosomas de soya y *Brassica* e incluso no han aparecido bandas en cromosomas de *Arabidopsis* y arroz (Rodríguez y Portieles, 2003).

Estudios recientes se enfocan también a relacionar el tamaño del genoma con el número cromosómico. La medición del genoma se lleva a cabo mediante diversas técnicas, la más común de las cuales es la citometría de flujo. El papel del número cromosómico y el tamaño del genoma en la diversificación del orden Cyperales y su

impacto en un contexto filogenético se discuten por Roalson *et al. (*2007), y para géneros en particular por Zedek *et al. (*2004) para *Eleocharis* y Kaur *et al. (*2012) para *Schoenus*.

2.4. Estudios citogenéticos en México

La importancia de los estudios citogenéticos para contribuir al conocimiento de las plantas Mexicanas ha sido enfatizada por Palomino (2000). Algunos ejemplos de análisis cariotípicos y citogenéticos de plantas de México son los llevados a cabo por Flores-Maya *et al. (*2010), Martínez y Palomino (1996), Mercado *et al. (*1989), Mercado-Ruaro y Delgado-Salinas (1998, 2000, 2009), Palomino and Heras (2001), Tapia-Pastrana y Gómez-Acevedo (2005), Tapia-Pastrana (2010), Tapia-Pastrana y Jiménez-Salazar (2011), y Tapia-Pastrana *et al. (*2004, 2012).

En cuanto a la citogenética de ciperáceas de México, los únicos reportes de número cromosómico previos a este trabajo son los de *Carex peucophila* Holm (Beaman *et al*., 1962) y *Fimbristylis mexicana* Palla (Kral, 1971).

III. JUSTIFICACIÓN

Con este trabajo se pretende aportar información para el mejor conocimiento de especies de ciperáceas de importancia ecológica y económica, así como generar información básica que pudiera ser aplicada en la selección y manejo de razas cromosómicas de potencial uso para fitorremediación y restauración de ecosistemas acuáticos y subacuáticos.

El mayor número de plantas que muestran potencial para usarse en proyectos de fitorremediación, principalmente de aguas contaminadas, son ciperáceas. Para el norte de México destacan las especies de los géneros *Eleocharis* y *Schoenoplectus* (tulillos y tules). Han recibido especial atención por el uso de varias especies en el manejo de malezas acuáticas y en el abatimiento de la contaminación. Sin embargo, existen aún amplios huecos en el conocimiento de estos géneros en México, cuyo estudio requiere de diversas herramientas, entre las cuales destaca la citogenética. No se han llevado a cabo estudios citogenéticos de especies de Ciperáceas mexicanas.

Se pretende también sentar las bases para el establecimiento de un laboratorio de citogenética convencional y molecular en el CIIDIR Durango, así como promover mayor interdisciplinariedad en las investigaciones que se llevan a cabo en el área de botánica del CIIDIR Durango.

IV. OBJETIVOS

Objetivo General:

Este trabajo tiene por objetivo analizar, mediante parámetros citogenéticos, especies de *Eleocharis* y *Schoenoplectus* nativas del norte-centro de México (estados de Zacatecas, Durango, Coahuila, Chihuahua) y así contribuir a su conocimiento taxonómico y evolutivo.

Objetivos Específicos:

- Determinar el número cromosómico en especies de *Eleocharis* y *Schoenoplectus* del norte-centro de México.
- Verificar el nivel de variación cariotípica y determinar los mecanismos de evolución cariotípica en dichas especies y en híbridos inter e intraespecíficos.

V. MATERIALES Y MÉTODOS

5.1 MATERIALES: Se estudiaron poblaciones de especies de *Eleocharis* y *Schoenoplectus* del norte-centro de México en los estados de Aguascalientes, Zacatecas, Durango, Coahuila y Chihuahua. Se llevaron a cabo colectas de plantas vivas para cultivar bajo condiciones de invernadero, de las cuales se obtuvieron las muestras para analizar. Los *vouchers* (un ejemplar de cada población/especie) se depositaron en el Herbario CIIDIR.

El trabajo de laboratorio se llevó a cabo en los Laboratorios de Citogenética y de Biotecnología en el CIIDIR Durango.

5.2 METODOLOGÍA: El análisis de los cariotipos se llevó a cabo mediante la técnica convencional:
Los meristemos apicales de raíz se sometieron a un pre-tratamiento con 8-hydroxiquinoleina 2mM por 24 h. Se fijaron en una mezcla de etanol: ácido acético (3 : 1, v : v) por 24 h y se almacenaron a –20 °C, o fueron usadas inmediatamente. Las muestras fueron lavadas en agua destilada, ablandadas en una solución de celulasa al 4% y pectinasa al 40% (w : v) a 37°C por 1 h, hidrolizadas en HCl 1M por 10 min a 60°C, lavadas de nuevo en agua destilada, y molidas “squashed” en una gota de ácido acético al 45%. Las placas fueron teñidas con hematoxilina al 4% y montadas con Entellan (Merck).

Los conteos de cromosomas se realizaron en al menos 20 células por muestra. La medición de los cromosomas se realizó utilizando el programa MicroMeasure 3.3, que se encuentra gratis en el sitio (http://www.colostate.edu/Depts/Biology/MicroMeasure) y los datos obtenidos se utilizaron para construir los ideogramas. Para cada muestra, se midieron de cinco a diez metafases con condensación similar. Se calculó la longitud media del cariotipo

(longitud total del diploide), así como el tamaño del cromosoma más largo y el más corto.

Las imágenes se obtuvieron con un microscopio Carl Zeiss AxioImager.Z2 equipado con una cámara Axiocam Hrc, un objetivo de inmersión en aceite Plan-Apochromat 100x/1.4 Oil, y el software AxioVs40 Rel.4.8.2.

El índice de asimetría intercromosomal fue calculado usando el índice de Romero Zarco (1986), basado en el coeficiente de dispersión de Pearson's (la relación entre la desviación estandard y el promedio de la longitud del cromosoma para cada muestra): $A_2 = s / \overline{X}$.

VI. RESULTADOS (ARTÍCULOS)

Los resultados se presentan en formato de artículo, con título y autores, como se relaciona a continuación:

1. TENA-FLORES J.A. Cytogenetics in Cyperaceae. Artículo de revisión.

2. TENA-FLORES J.A, M.S. GONZÁLEZ-ELIZONDO, Y. HERRERA-ARRIETA, N. ALMARAZ-ABARCA, N. MAYEK-PÉREZ, C.R.M. DA SILVA, AND A.L.L VANZELA. 2012. Karyotype characterization of eight Mexican species of *Eleocharis* (Cyperaceae). Trabajo aceptado en Botanical Sciences.

3. TENA-FLORES J.A, M.S. GONZÁLEZ-ELIZONDO, Y HERRERA-ARRIETA, N. ALMARAZ-ABARCA, N. MAYEK-PÉREZ, AND A.L.L. VANZELA. 2012. Karyotype characterization of four Mexican species of *Schoenoplectus* (Cyperaceae) and first report of mixoploidy for *Schoenoplectus acutus*. Artículo de investigación (en proceso).

1. Artículo de revisión:

CYTOGENETICS IN CYPERACEAE

Tena-Flores, J.A.

CIIDIR IPN, UNIDAD DURANGO

Introduction

a) Cyperaceae is a large cosmopolitan family with about 5,400 species in 106 genera (Govaerts *et al*., 2007) to 5,500 species in 109 genera (Muasya *et al*., 2009), or even 122 genera heve been recognized (Bruhl, 1995). The Cyperaceae, known as sedges, superficially resemble grasses or rushes (Fig. 1). Its representatives occur in a variety of habitats (Fig. 2), being most common in moist areas like marshes, swamps, riverbanks, ponds and sandbank environments (Goetghebeur, 1998). It includes several worldwide distributed genera, e.g., *Carex* L., one of the most species rich genera, with more than 2000 species (Reznicek 1990) or about 2100 spp. (Escudero *et al*., 2012a,b), *Eleocharis* R.Br., which comprises more than 270 species (González-Elizondo, unpubl. data) frequently found in flooded areas, and *Schoenoplectus* (Rchb.) Palla, with about 77 species (Smith, 2002).

Fig. 1. Diversity of habit and inflorescences in sedges. A. *Cladium jamaicence* Crantz; B. *Cyperus seslerioides* Kunth; C. *Eleocharis cryptica* Saarela, P.M. Peterson, S. González & D.J. Rosen, the smallest sedge known to date, about 1 cm; D. *Schoenoplectus californicus* (C.A. Mey.) Soják, one of the largest sedges, reaching 4.5 m high.

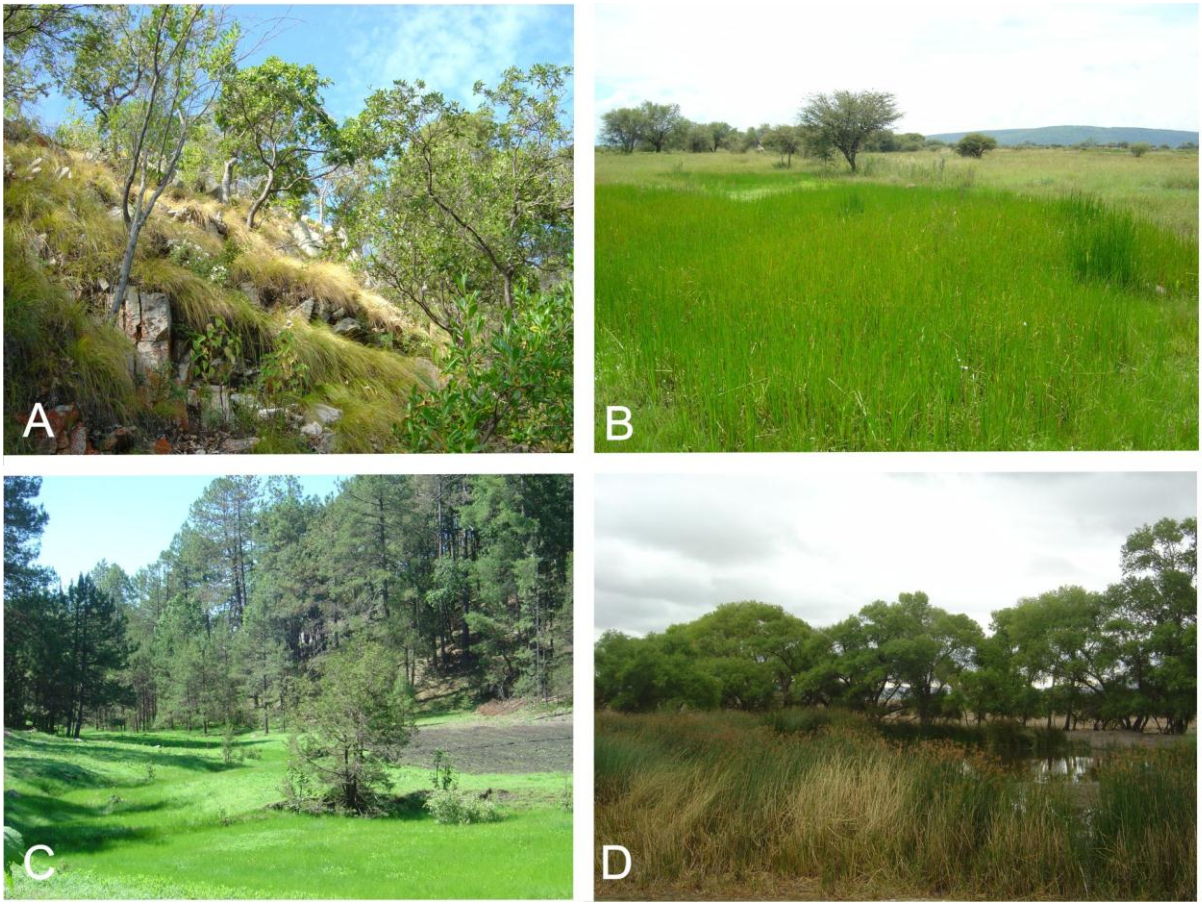

Fig. 2. Diversity of habitats of sedges. A. *Carex longissima* M.E. Jones dominant on slopes at the Sierra de La Laguna, Baja California, Mexico; B. *Eleocharis yecorensis* Roalson in temporal marshes, central Durango, Mexico; C. *Eleocharis* spp. and grasses in *Pinus* forest, Sierra Madre Occidental, Mexico; D. *Schoenoplectus californicus* with *Populus fremontii* at edges of pond, central Chihuahua, Mexico.

Eleocharis is distinguished by possessing unbranched stems, leaves reduced to basal, tubular sheaths, persistent style base, and inflorescence reduced to a simple terminal spikelet (Fig. 3) (González-Elizondo and Peterson, 1997; González-Elizondo and Tena-Flores, 2000). In spite of the easy recognition of *Eleocharis* as a genus and its prominent delimitation within Cyperaceae (Kukkonen, 1990), the species are difficult to identify and classify because of the limited number of morphological features. The supraspecific classification of *Eleocharis* has been revised and modified by Kukkonen (1990) and González-Elizondo and Peterson (1997), on the basis of the classification of Svenson (da Silva *et al*., 2008b). *Eleocharis* has been receiving attention because of the potential use of several species in aquatic weed management and in pollution abatement. Phytoremediation is an emergent field with great potential for the

removal of heavy metals from contaminated soil or water, and the use of native plants that have a high capacity to accumulate those metals and remove them from soil and water is a very convenient approach (González-Elizondo *et al*., 2005).

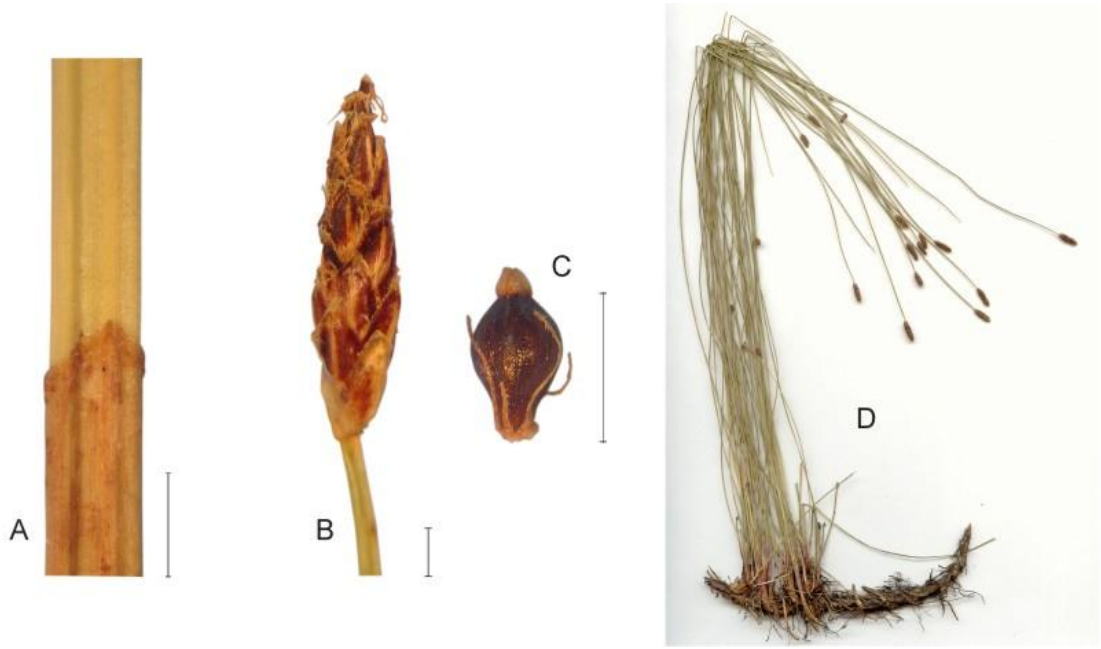

Fig. 3. Morphology of *Eleocharis*. A. Leafless sheath; B. Bractless spikelet; C. Achene, all of *Eleocharis tenarum* S. González et M. González; D. Habit of *Eleocharis ignota* S. González et Reznicek. Scale bar: A = 1 cm, B and C = 1 mm.

Schoenoplectus, a segregate of *Scirpus* s.l., includes wetland species of great environmental value (phytoremediation, soil stabilizers, habitat and food for wild species), as well as with ethnobotanic and economic value, e.g., *S. californicus*, widely distributed in the New World (Mexican tules, Peruan totora). Except for a recent Japanese study (Yano and Hoshino, 2005) very few is known on cytogenetics in *Schoenoplectus*.

b) Cytogenetics is the discipline that studies the genetic implications of the structure and behavior of chromosomes. Chromosome data relevant to plant systematics and evolution range from the number of chromosomes to details of molecular cytogenetics that are at the frontiers of current research (Stace, 2000). Over the past two decades, cytogenetic studies progressed through the information generated by classical methods, allowing establishing the first

cytogenetic models in species such as tomato, wheat and rice. At the end of the last century cytogenetic studies showed a significant improvement by implementing new techniques for the analysis of chromosomes, somatic and meiotic, including molecular cytogenetics techniques (Herrera, 2007).

c) Cytotaxonomy. The karyotype is the final result of many forces that act in the genome at structural, organizational and functional levels. The chromosome numbers, their shapes and sizes are characteristic of certain species within each genus. The use of karyological data in taxonomy, traditionally referred to as cytotaxonomy or karyosystematics, contributes to evaluate the genetic relationships among species or populations and to a better understanding of the way they diverged from each other (Guerra, 2008).

Despite being the simplest karyotype parameter, the chromosome number offers some special attractions to cytotaxonomists. It is the quickest, cheapest, and easiest way to get any substantial information about the genome of a species. The chromosome number is the best known cytotaxonomic datum for almost all families and most plant genera. Like other karyotype features it is not influenced by external conditions, developmental phases, age, etc. A lot of other karyological information can be added to this initial karyotype description, as chromosome size and morphology, karyotype symmetry, banding patterns, and chromosomal position of satellite DNAs. Chromosome counting is a relatively easy task which produces reliable and highly reproducible data, unless the chromosome number is very high (*2n* around or over one hundred). The main practical disadvantage of chromosome numbers for cytotaxonomy is the need for living material with actively growing tissues. In many taxa the chromosome number is an important landmark to distinguish monophyletic clades (Guerra, 2008).

As different examples we have in *Eleocharis*, numbers from 2*n* = 6 for *E. subarticulata* (da Silva *et al*., 2005) to 2*n* = 200 for *E. dulcis* (see Roalson, 2008) are known. Da Silva *et al*. (2008b) studied eight species of *Eleocharis*: *E. maculosa*, *E. flavescens* and *E. radicans* exhibited *2n=10*, whereas *E. geniculata* and *E. sellowiana* showed a duplicated chromosome number, with *2n=20*. An intermediate number, *2n=12*, was found in *E. minima*, where two of the chromosomes are very small. Cytotypes were found in *E. montana* with *2n=20, 40*. In this

case, the chromosomes of the cytotype with *2n=20* varied from 1.3 to 2.3 μm, and those of the cytotype with *2n=40* varied from 1.1 to 2.2 μm. Significant morphological differences were not found between the two cytotypes of *E. montana*, suggesting that the polyploidisation to *2n=40* could be a recent event, since it does not yet affect the gross morphology. The chromosome number *2n=40* was also found in *E. elegans*. Another interesting case involves *E. sellowiana* and *E. flavescens*, two species in which the morphological characters, in some instances, are not enough to distinguish them. Some differences are the transversally elliptic stems in *E. flavescens* but circular in *E. sellowiana*. Also, the upper portion of the distal sheath is not abruptly differentiated from the lower portion in *E. flavescens*, whereas in *E. sellowiana* it is abruptly differentiated (González-Elizondo *et al*., 2005). Populations of *E. sellowiana*, when slender or depauperate, may be morphologically very similar to and difficult to differentiate from *E. flavescens*. However, the karyotype of these species can be considered an additional micromorphological feature useful to taxonomy, since *E. sellowiana* possesses *2n=20* and *E. flavescens 2n=10*. The numbers recorded for the only two Mexican species studied to date are *2n=16* for *E. xyridiformis* and *2n=20* for *E. reznicekii* (Tena-Flores *et al*., unpubl. data). Vanzela *et al. (*2000), in a study about karyotype evolution and cytotaxonomy analysed 113 populations of 34 species of *Rhynchospora* (Cyperaceae). They present the chromose numbers of several species, which, as in the chromosome analysis of *Rhynchospora* through conventional staining, reveals a great variability in the size, shape and number of chromosomes. In most Cyperaceae, cytological information may be very useful in taxonomical investigations.

Despite this variability, most species have numbers multiple of 5 (da Silva *et al.*, 2008a,b). *X = 5* was proposed as the basic number for the family by Löve *et al. (*1957, cited by da Silva, 2010).

Cytogenetical distinctiveness of the Cyperaceae

Cyperaceae possess a unique combination of cytogenetical features that is highly unusual among angiosperms: (i) holocentric chromosomes, (ii) post-reductional meiosis, (iii) absence of tetrads, and (iv) karyotype evolution on the basis of agmatoploidy (fission), symploidy (fusion) and polyploidy (da Silva *et al.*, 2005, 2008b).

(i) Non-localized centromeres. Chromosomes holocentric have no primary constriction and the kinetochore is organized in a diffuse way along the chromosomes. This chromosomes were first described in insects (Schrader, 1935, cited by da Silva, 2010), and until 1954 this type of chromosome was described for sedges, in the genus *Eleocharis*, by Bataglia and Håkansson (cited by da Silva, 2010). Chromosomes holocentric have been also described in a few representatives of the families Juncaceae, Droseraceae, Cuscutaceae, Liliaceae, and Myristicaceae. Besides the plants, holocentric chromosomes are found in nematodes, protozoa, algae and several orders of insects and arachnids (Mola and Papeschi, 2006).

The diffuse kinetochore condition is a synapomorphy for the Cyperaceae. Some reports of localized kinetochores in species of *Fimbristylis*, *Eleocharis*, *Scirpus*, *Lipocarpha*, and *Cyperus* appear to be based in misinterpretation of images (Vanzela, com. pers.).

Different names are applied to this type of chromosome. Authors such as Håkansson (1958, cited by da Silva, 2010), Hoshino (1987) and Vanzela *et al. (*1996) call them holocentric chromosomes. Others (e.g., Bernardini 1959, cited by da Silva, 2010) call them polycentric. Despite the name holocentric be wrongly used, this designation is widely accepted and used.

The term holokinetic would be more correct, because the centromere is not really distributed throughout the chromosome, but the kinetic activity is. This feature leads to migration parallel of the mitotic chromosomes in anaphase, as demonstrated by Harms (1968) and Guerra *et al. (*2006). In some cases, constrictions are observed in some chromosomes. These are called nucleolar constrictions, which are related to secondary constrictions of the monocentric chromosomes (Vanzela *et al.*, 2000; da Silva *et al*., 2008a).

Chromosomes with diffuse kinetochore, when natural or artificially fragmented and / or merged, are segregated normally in cell division, both in meiosis as in mitosis. This was demonstrated in *Eleocharis palustris* (Cyperaceae) and *Rhynchospora pubera* by Vanzela and Colaço (2002). These processes of breakage and chromosomal fusion in holocentric are called agmatoploidy (breaking or fission) and simploidy (fusion), respectively (Luceño y Castroviejo, 1991; Luceño and Guerra, 1997; Luceño *et al*., 1998a). In some papers (e.g., Roalson *et al*., 2007) the term agmatoploidy is applied to both, breaking and chromosomal fusion.

Cytogenetical features, such as nucleolar constrictions, chromosome size and interphase nuclei types are useful to study many plant groups. This information is scarce in the literature on Cyperaceae because the holokinetic chromosomes, so it is not possible to compare populations and species by the use of chromosome shape (da Silva *et al*., 2008).

(ii) Post-reductional meiosis. Cyperaceae is known for the occurrence of post-reductional or reversed meiosis. The meiotic behavior was suggested the first time in *Carex* by Heilborn (1928) and Wahl (1940), cited by da Silva (2010).

This type of meiosis occurs when sister chromatids have parallel orientation in the equatorial plate at metaphase I, one for each migrating side in anaphase I. Thus, the number of chromosomes is not reduced during the first phase of meiosis, since it reduces the number of chromatids of each chromosome. In monocentric chromosomes, the sister chromatids present perpendicular orientation to the axis along the equatorial plate before anaphase I, which provides the separation of homologous chromosomes (reductional meiosis).

The alignment of bivalents in meiosis of holocentric generates a configuration known as "box structure" (Vanzela *et al*., 2000). However, the only occurrence of this structure is not evidence that meiosis is inverted. The analysis of all meiosis would be needed to confirm this behavior. Reversed meiosis was documented in *E. subarticulata*, where despite the absence of bivalents, it was clearly seen that during the first phase of meiosis, the separation of sister chromatids and thus, the number of chromosomes is not reduced during anaphase I, the reduction occurring only during the second phase of meiosis (da Silva *et al*., 2005). This meiotic behavior was first recorded for *Eleocharis* by Strandhede (1965c, 1973) and for *Rhynchospora* by Vanzela *et al*. (2000).

(iii) Absence of tetrads. Microsporogenesis in the Cyperaceae mirrors megasporogenesis in most angiosperms: three nuclei of each pollen mother cell (PMC) degenerate after failing to undergo DNA replication. With rare exceptions, each PMC consequently produces only one pollen grain in the Cyperaceae, whereas microsporogenesis in most angiosperms results in four pollen grains per PMC (Hipp *et al*., 2009).

(iv) Karyotype evolution on the basis of agmatoploidy (fission), symploidy (fusion) and polyploidy. The persistence of chromosomes originated by fusion and fission contributes to the extensive variation (inter- and intraspecific) in chromosome number in Cyperaceae. The

process of differentiation of the karyotypes and chromosomal evolution varies between groups. In *Carex*, agmatoploidy and simploidy are significantly more frequent (Roalson *et al*., 2007; Hipp *et al*., 2009), when compared with other genera such as *Rhynchospora* Vahl (Luceño *et al*., 1998a,b; Vanzela *et al*., 2000), *Eleocharis* (da Silva *et al*., 2008, 2010), and *Schoenoplectus* (Yano and Hoshino, 2005), in which polyploidy is the main cause of change in chromosome numbers.

Cytogenetics in Cyperaceae

A synopsis on the chromosome number variation on the Cyperaceae was published by Roalson (2008), where he presents 4,231 chromosome countings for the family and discuss the variation mechanisms. Here the information is updated with data that have been generated from 2002 to date, which were not included in Roalson's article with the exception of three references (Starr *et al*., 2004; Roalson *et al*., 2007; and Hipp *et al*., 2008). Although the additions to chromosome counting are relatively few, from 2002 to date the understanding on chromosome variation in sedges has advanced. Some concepts and terminology are revised here.

Cytologic studies in Cyperaceae date from before 1913, when Stout (cited by Roalson, 2008) studied the serial arrangement of the chromosomes in *Carex aquatilis*. In 1924 Heilborn studied the karyotype, species formation and phylogeny in *Carex* (Grant, 1971), and Piech described the chromosome number of *Eleocharis palustris* and *Scirpus lacustris* (da Silva, 2010). Most of the cytogenetical knowledge in Cyperaceae comes from studies in *Carex* (see Hipp *et al*., 2009; Escudero *et al*., 2012b) and *Rhynchospora* (Luceño *et al*., 1998a,b; Vanzela *et al*., 2000), as well as from studies in *Eleocharis* (Strandhede, 1965a,b, 1967; Hoshino et al., 2000; da Silva, 2005, 2010; da Silva *et al*., 2005, 2008a,b, 2010; Yano *et al*., 2004); *Schoenoplectus* (Yano and Hoshino, 2005), *Fimbristylis* (Rath and Patnaik, 1977; Yano and Hoshino, 2006), and *Scleria* (Yano and Hoshino, 2007). Chromosome variation and potential processes of chromosome evolution in Cyperaceae have been studied by da Silva (2005, 2010), da Silva *et al*. (2005, 2008a,b, 2010), Hipp *et al*. (2006, 2007), Ohkawa *et al*., 2000, Vanzela (2000), Vanzela and Guerra (2000), Vanzela and Colaco (2002), Vanzela *et al*. (1998, 2003), and reviewed by Roalson (2008), Roalson *et al*. (2007), and Hipp *et al*. (2009), the

latter concluding that chromosomes evolve more dynamically in sedges than in any other group of flowering plants.

Variation in chromosome number and mechanisms of evolution of the karyotype

A considerable variation in the chromosome number in Cyperaceae has been recorded. A total of 4,231 counts in 31 genera and 851 species of Cyperaceae were recorded by Roalson (2008), most of them (approximately 2,447 or 58%) made in *Carex*. Even so, only approximately 16% of the recognized species in the family have been counted, which still limits our ability to make broad-scale assessments of chromosome evolution in the family (Roalson, 2008). *Carex* has the largest nonpolyploid chromosome radiation (2*n* = 12 - 124) (Escudero *et al.*, 2012b).

For *Schoenoplectus*, chromosome number ranges from 2*n* = 10 (Schuyler 1969) to 2*n* = 128 (Hicks 1928, cited by Yano & Hoshino, 2005), with chromosome number peaks at 21 (in seven species), 19 and 39 (five species each), and 37 (four species) (Roalson, 2008). For four Mexican species studied by Tena-Flores *et al. (*2012b), polyploidy followed by dysploidy is the most common mechanism of karyotype variation; …

The importance of the karyotypic information to understand the evolution and phylogeny of plants and as an aid in the taxonomy has been widely exemplified by Grant (1971). The new techniques available in the last two decads have allowed the development of the cytogenetics and molecular cytogenetics, which are producing a wealth of new data of enormous taxonomic and evolutionary importance; for these to reach their full impact it is essential that they are fully integrated with traditional cytological data (Stace, 2000).

Intraspecific variations have been identified in some populations of *E. geniculata*, with *2n = 10, 20* (Sanyal and Sharma, 1972), *E. uniglumis* with *2n = 46, 78-82* and *E. palustris 2n = 14-17, 38, 39* (Strandhede, 1965a,b, 1967; Bures, 1998; Bures *et al*., 2004), *E. acicularis* f. *longiseta* with *2n = 20, 21* (Yano *et al*., 2004), and *E. maculosa* with *2n = 10, 8, 7, 6* (da Silva *et al*., 2008a). In the last case, da Silva *et al. (*2008a) showed which cromosomes fused from

the 2*n* = 10 citorace to form the remaining reduced numbers, and discuss symploidy as an important mechanism in karyotype differentiation in *Eleocharis*.

Although intra-specific variation in chromosome numbers in sedges is common (e.g., Bures *et al*., 2004; da Silva *et al*., 2008 a,b, among others), intra-individual variation is extremely rare, and it usually implicates aneuploidy. Chromosome number variation, both inter-specific and intra-specific, is very high in *Carex*, and even a high intraindividual variation has been found for *Carex disticha* (Luceño, 1992).

In *Schoenus* from New Zealand chromosome numbers range from 2*n* = 8 to c. 2n = 90, and the variation in the chromosome number may be caused by both agmatoploidy/symploidy and polyploidy (Kaur *et al*., 2012). The first report of this fenomenon for *Schoenoplectus* was given by Maeda and Uchino (2004), who found inter- and intra-individual variations in chromosome number, a mixoploidy/aneuploidy in the root-tip cells of three species: chromosome number varied from 2n = 68 to 74 in *Schoenoplectus gemmifer*, 2n = 32 to 39 in *S. mucronatus* and 2n = 37 to 44 in *S. triangulatus*, respectively. This kind of mixoploidy/polyploidy is reported also by Tena-Flores *et al. (*2012b) for *Schoenoplectus acutus* var. *occidentalis*, with 2*n* = 38 and 2*n* = 84 in the same individual, which represent two cell lines, with 2*n* = 84 derived of 2*n* = 38 with a karyotype size equivalent to one of 42 small chromosomes.

Carex exhibit remarkable agmatoploid chromosome series between and within species. This chromosomal diversity is due in large part to the structure of the holocentric chromosomes: fragments that would not be heritable in organisms with monocentric chromosomes have the potential to produce viable gametes in organisms with holocentric chromosomes. The rapid rate of chromosome evolution in the genus and high species diversification rate in Cyperales together suggest that chromosome evolution may play an important role in the evolution of species diversity in *Carex*. Yet the other genera of the Cyperaceae and their sister group, the Juncaceae, do not show the degree of chromosomal variation found in Carex, despite the fact that diffuse centromeres are a synapomorphy for the group. Moreover, fission and fusion apparently account for the majority of chromosome number changes in *Carex*, with relatively little duplication of whole chromosomes, whereas polyploidy is relatively important in the other sedge genera (Hipp *et al.*, 2009).

Schoenoplectus has chromosome number peaks at *19, 21, 39* (Roalson 2008). Putative natural hybrids in *Schoenoplectus* were found to have intermediate chromosome numbers of the putative parents (Yano and Hoshino, 2005; Yano *et al*., 2010).

Molecular cytogenetics

This synthetic new approach derives of the combination of cytogenetical and molecular information. The main techniques used are fluorescence in-situ hybridization (FISH), GISH, and C-banding (Drets, 2002). The technique of FISH has allowed the identification of a large number of different landmarks for physical chromosome mapping and fine karyotype comparison among related species, cultivars or populations.

Da Silva *et al. (*2008) studied repetitive DNA and chromosome organization in *Eleocharis.* The C-CMA_3/DAPI banding showed that the studied species have noDAPI bands, suggesting that AT-rich sequences seem not to be important in the karyotype organization. However, most of the species showed fine terminal C-CMA_3 bands (GC-rich blocks), with low fluorescence intensity, but varying in the number and size of the bands. *E. flavescens* and *E. maculosa*, both with 2*n*=10, exhibited four C-CMA_3 signals, two signals being slightly larger and two smaller. *E. sellowiana* with 2*n*=20 exhibited ~10 C-CMA_3 blocks, eight being more conspicuous and two fine. *E. geniculata* with 2*n*=20 showed four C-CMA_3 blocks, two being larger and terminal and two smaller and interstitial. Interestingly, these interstitial blocks were found in the largest pair. *E. elegans* and *E. montana*, both with 2*n*=40, showed tenuous C-CMA_3^+ bands always in one chromosome pair. *Eleocharis acutangula*, the species with the smallest chromosomes, presented the largest number of C-CMA_3^+ bands, with ~20 interstitial and terminal bands. In spite of the very small number of treated species for the chromosome banding, these data indicate that heterochromatin rich in GC is very important in chromosome organization in *Eleocharis*, whereas AT-rich segments represent a repetitive DNA family that perhaps appears sporadically in a few species, with a punctual importance. They conclude that these results show that despite of the reduction in the number of morphological and cytogenetical features (considering the absence of primary constrictions), the karyotypes of *Eleocharis* accumulate differences in the size and number of chromosomes between species and populations as well as in the size and number of GC-rich bands and 45S rDNA sites.

Hence, cytogenetics can be considered a good tool for taxonomic and evolutionary studies in Cyperaceae.

Genome size and karyotype

The role of chromosome number and genome size in the diversification of Cyperales and their impact in a phylogenetic context is discussed by Roalson *et al. (*2007) and Chung *et al. (*2012).

Measurement of genome size is a relatively new technique used to estimate the overall size of a genome by means of a variety of techniques (Roalson *et al*., 2007), from which the most used is flow cytometry. The DNA amount in the unreplicated gametic nucleus of an organism is referred to as its C-value, irrespective of the ploidy level of the taxon (Bennett and Leitch, 2010).

A comparative analysis between genome sizes and chromosome numbers in the Cyperales is presented by Roalson *et al. (*2007), revealing that genome sizes in the order range between 108 and approximately 5415 megabasepairs (Mbp), with many species having very small genomes, a few with genomes considerably larger, and an average of 498 Mbp. The authors contrast two alternative hypothesis to correlate the genome size and chromosome number with the mechanism of genome repatterning: with agmatoploidy as the primary mechanism, chromosome number changes without concurrent change in genome size, but if polyploidy or some type of quantitative aneuploidy is occurring, we would expect a positively correlated increase in genome size. They found instead that as chromosome numbers increase, genome sizes get smaller, which is also a result of agmatoploidy. As preliminary hypothesis, they propose that as fission events occur, there is some loss of breakpoint end DNA before new telomeres cap off the new chromosome ends; so, as genome becomes more fragmented (continually increasing the number of chromosomes), genome sizes might continually decrease as more breakpoint locations occur, each potentially losing some DNA.

For ten species of *Schoenus* from New Zealand, Kaur *et al. (*2012) found chromosome numbers ranged from $2n = 8$ to c. $2n = 90$. The same authors note that with the exception of the new values obtained here for *Schoenus* and one previously published value for *Eleocharis*

(Bennett & Leitch, 2010), the species in the seven genera in which measurements have been made typically have low C values (< 3.0 pg/2C nucleus) with a narrow range of variation in each genus.

Chung *et al. (*2012) found that in *Carex*, chromosome number evolves independently of genome size, and that this influence the high chromosome number variation in the genus and its tremendous diversity.

As for *Eleocharis*, a positive correlation between genome size and chromosome number was found by Zedek *et al. (*2010), which confirms the occurrence of polyploidy in the genus; however, these authors also report small genome sizes correlated with high chromosome counts, which reveals that agmatoploidy is the most important process determining chromosome number in the subgenera *Limnochloa* and *Zinserlingia*.

Conclusions

The chromosome number changes in Cyperaceae occur mainly for agmatoploidy, symploidy, and translocations, followed by polyploidy or usually by agmatoploidy/poliploidy within many genera. Duplications of the chromosome number that are not associated to an equivalent increase in the length of the karyotype do not correspond to a real polyploidy but to a special kind of agmatoploidy (Grant, 1981), a mechanism very common in sedges. Fission, fusion and translocations account for the majority of chromosome number changes in *Carex*, the largest genus in the family, with relatively little duplication of whole chromosomes. Polyploidy, on the other hand, is relatively important in the other genera of sedges.

Fragmentation increases the chromosome number, thus providing greater variability and better adaptation capability (several authors, cited by Mola and Papeschi, 2006). Hence, the high morphologic variation found in many species of Cyperaceae may in part derive of the chromosome number changes by agmatoploidy and polyploidy.

The role that chromosomal changes play in evolutionary diversification—ranging from race formation through speciation—remains debated: the classic view holding that chromosome structural changes are directly responsible for reproductive isolation and thus the primary agent for speciation, and a more recent view that posits chromosome structural changes are not

directly responsible for reproductive isolation but instead reduce gene flow to allow adaptive differences or genic incompatibilities to become fixed within populations or among neighboring populations (several authors, cited by Roalson *et al.*, 2007). Recent studies on *Carex* (Escudero *et al.*, 2012b) reveal that the effects of environment on chromosome number reflect selection for recombination rates, hence chromosome evolution may explain a component of sedge diversity not just through its effect on reproductive isolation, but also by promoting ecological diversification (Hipp, 2012).

Less than 20% of the species of Cyperaceae have been citologically studied, most of them in North America -not including Mexico-, Europe, Japan, Nepal(Hoshino) and Brazil. Cytology, cytogenetics and molecular cytogenetics produce data of enormous taxonomic and evolutionary importance. More information is needed to better understand the natural world.

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Table 1. Molecular cytogenetics in Cyperaceae

A summary

Author, year	Theme	Taxonomical group	Geographical region	Still to do
Bureš, 1998	Polyploidy	*Eleocharis uniglumis*	C, SE Europe	All other genera
Bureš *et al*., 2004	Cytogeography	*Eleocharis palustris*	Europe	All other genera
Da Silva, 2005; 2010	cytogenetics, evolution	*Eleocharis*	Brazil	Most other genera
Da Silva *et al*., 2005; 2008a; 2010	cytogenetics, cytotaxonomy	*Eleocharis*	Brazil	Most other genera
Da Silva *et al*., 2008b; 2010	cytogenetics, cytotaxonomy	*Eleocharis*	Brazil	Most other genera
Escudero *et al*., 2006	Cytogenetics	*Carex sect. Spirostachyae*	Eurasia	All other genera
Guerra *et al*., 2006	Cytogenetics	*Rhynchospora*	Brazil	Most other genera
Hipp *et al*., 2007	cytogenetics, evolution	*Carex*	North America	All other genera
Hipp *et al*., 2009	Karyotype evolution	*Carex*	North America	All other genera
Hoshino, 1987	Karyomorphology	Eleocharis	Japan	Few genera
Hoshino *et al*., 2000	Cytology	Several	Nepal	Evolution/taxonomy
Luceño y Castroviejo, 1991	Karyotype evolution	*Carex*	Spain	All other genera
Luceño *et al*., 1998a, b	Cytotaxonomy	*Rhynchospora*	Brazil	Most other genera
Ohkawa *et al*., 2000	cytogenetics, cytotaxonomy	*Eleocharis*	Japan	Few genera
Roalson *et al*. 2007	Karyotype evolution (a review)	Cyperales	worldwide	
Roalson 2008 (it does not include 2002 to present nor molecular cytogenetics)	Chromosome number variation (a review)	Cyperaceae	worldwide	2002 to present + molecular cytogenetics
Vanzela, 2000; Vanzela and Guerra 2000; Vanzela and Colaço. 2002	Karyotype evolution	*Rhynchospora*	Brazil	Most other genera
Vanzela *et al*., 1996	Karyomorphology	*Rhynchospora*	Brazil	Most other genera
Vanzela *et al*., 1998; 2000	cytogenetics, cytotaxonomy	*Rhynchospora*	Brazil	Most other genera
Yano and Hoshino 2006 a	karyotype evolution	*Fimbristylis*	Japan	Few genera
Yano and Hoshino 2006 b	Cytogenetics	*Eleocharis kamtschatica*	Japan	Few genera
Yano and Hoshino 2006 c	Karyomorphology	*Schoenus*	Japan	Few genera
Yano and Hoshino 2007	Karyomorphology	*Scleria*	Japan	Few genera
Yano *et al*., 2004	molecular cytogenetics	*Eleocharis*	Japan	Few genera
Yano *et al*., 2007	Cytogenetics	*Carex*	Japan	Few genera
Yano *et al*., 2010	cytology of hybrid	*Schoenoplectus*	Japan	Few genera

Table 2. Examples of variation in chromosome number in genera of Cyperaceae

	N	2*n*	Main mechanism of karyotypic evolution
Carex 1	40, 41	78, 80, 81, 82, 83	**aneuploidy**
Carex 2	31; 40	62; 80	polyploidy
Eleocharis 1	8 19, 20 23	16 38, 39, 40, 41, 42 46, 47, 48, 49, 50, 51, 54	**aneuploidy**
Eleocharis 2	5, 10	10, 20, 40	Polyploidy
Fimbristylis	**5**, 10, 20	**10**, 20, 40, 60	Polyploidy
Lipocarpha	13, **20**	26, **40**	Polyploidy
Pycreus	21, 25, 40	42, 50, 80	Polyploidy
Rhynchospora	5, 10, 13	10, 20, 26	Polyploidy
Schoenoplectus1 *Schoenoplectus 2* *mixoploidía*	15, **21, 22**	32, 38, 40, **42, 44**	polyploidy + aneuploidy

Table 3. Chromossome number in Cyperaceae recorded from 2002 to date.

Species	n	2*n*	Fuente
Carex			
C. capillacea Boott		c.60	De Lange et al., 2004
C. carsei Petrie		60	De Lange et al., 2004
C. colensoi Boott		c. 60-64	De Lange et al., 2004
C. comans Bergg.		40	De Lange et al., 2004
C. diandra Schrank		c.60	De Lange et al., 2004
C. dipsacea Bergg.		c. 74	De Lange et al., 2004
C. echinata Murr.		c.58	De Lange et al., 2004
C. flaviformis Nelmes		c.64	De Lange et al., 2004
C. forsteri Wahl.		60	De Lange et al., 2004
C. fretalis Hamlin		c. 60-64	De Lange et al., 2004
C. hectori Petrie		64-68	De Lange et al., 2004
C. kaloides Petrie		c. 78-84	De Lange et al., 2004
C. kermadecensis Petrie		c.60	De Lange et al., 2004
C. litorosa Bailey		48	De Lange et al., 2004
C. muelleri Petrie		c. 70	De Lange et al., 2004
C. petriei Cheeseman		c. 60-62	De Lange et al., 2004
C. resectans Cheeseman		58-60	De Lange et al., 2004
C. sectoides (Kuk.) Edgar		64-68	De Lange et al., 2004
Desmoschoenus			
D. spiralis (A.Rich.) Hook.f.		30	De Lange et al., 2004
Eleocharis			
E. acuta R.Br.		20	De Lange et al., 2004
E. acutangula (Roxb.) Schult.		54	Da Silva et al., 2008b, 2010
E. aff. *rostellata* Torr.		60	Tena et al., 2012unpubl data
E. bonariensis Ness		20	Da Silva et al., 2010
E. brasiliensis Boeck		~54	Da Silva et al., 2008b
E. capillaceae Kunth		10	Da Silva et al., 2010
E. cf. *montevidensis* Kunth 1		20	Tena et al., 2012unpubl data
E. cf. *montevidensis* Kunth 2		20	Tena et al., 2012unpubl data
E. cf. *montevidensis* Kunth 3		10	Tena et al., 2012unpubl data
E. contracta Maury		20	Da Silva et al., 2010
E. debilis Kunth		30	Da Silva et al., 2010
E. densa Benth.		16	Tena et al., 2012unpubl data
E. dulci (Burm. f.) Trin. ex Henschel.		ca. 196	Yano et al., 2004
E. filiculmis Kunth.		30	Da Silva et al., 2010
E. flavescens (Poir.) Urb.		10	Da Silva et al., 2010
E. geniculata (L.) Roem & Schult.		20	Da Silva et al., 2008b
E. geniculata (L.) Roem. & Schult.		10,20	Da Silva et al., 2010

Species	n	2*n*	Fuente
E. gracilis R.Br.		20	De Lange et al., 2004
E. interstincta (Vahl) Roem and Schult.		40,52	Da Silva et al., 2010
E. laeviglumis R. Trevis. and Boldrini		60	Da Silva et al., 2010
E. liesneri S. González and Reznicek		50	Da Silva et al., 2010
E. loefgreniana Boeck.		20	Da Silva et al., 2010
E. macrostachya Britton		28	Tena et al., 2012unpubl data
E. maculosa (Vahl) Roem. & Schult.		6, 7, 8, 10	Da Silva et al., 2008, 2010
E. minima Kunth		12	Da Silva et al., 2008b
E. minima Kunth		20, 30, 34	Da Silva et al., 2010
E. montana (Kunth) Roem & Schult.		10	Da Silva et al., 2008b
E. montana (Kunth) Roem. and Schult.		40	Da Silva et al., 2010
E. nana Kunth		20	Da Silva et al., 2010
E. neozelandica Kirk		30	De Lange et al., 2004
E. niederleinii Boeck.		20	Da Silva et al., 2010
E. obtusertigona (Lindl. and Ness) Steud.		52	Da Silva et al., 2010
E. ochrostachys (Steud.)		74	Yano et al., 2004
E. parbula (Roem. & Schult.) Link ex Bluff.		10	Yano et al., 2004
E. plicarhachis (Griseb.) Svenson		54	Da Silva et al., 2010
E. pusilla R.Br.		30	De Lange et al., 2004
E. radicans (Poir.) Kunth. Enum.		10	Da Silva et al., 2008b
E. reznicekii S. González, D.J. Rosen, R. Carter and P.M. Peterson		16	Tena et al., 2012unpubl data
E. rostellata Torr.		60	Tena et al., 2012unpubl data
E. sellowiana Kunth		10,20	Da Silva et al., 2008b, 2010
E. sphacelata R.Br.		100	De Lange et al., 2004
E. subarticulata (Ness) Boeck.		6	Da Silva et al., 2005, 2008b, 2010
E. subarticulata (Ness) Boeck.	3		Da Silva et al. 2008
E. viridans Kúk. ex Osten		20,40	Da Silva et al., 2010
E. xiridiformis Fernald & Brack.		28	Tena et al., 2012unpubl data
Eleocharis sp. 1		40	Da Silva et al., 2010
Eleocharis sp. 2		10	Da Silva et al., 2010
Eleocharis sp. 3		42	Da Silva et al., 2010
Fimbristylis			
F. autumnalis (L.) Roem. et Schult.		10	Yano & Hoshino, 2006
F. complanata (Retz.) Link		16	Yano & Hoshino, 2006
F. cymosa R.Br.		52	Yano & Hoshino, 2006
F. dichotoma (L.) Vahl f. *annua* (All.) Ohwi		20	Yano & Hoshino, 2006
F. dichotoma (L.) Vahl f. floribunda (Miq.) Ohwi		30, 15II	Yano & Hoshino, 2006
F. diphylloides Makino		10	Yano & Hoshino, 2006
F. ferruginea (L.) Vahl var. *anpinensis* (Hayata) H.Y.Liu		10	Yano & Hoshino, 2006

Species	n	2*n*	Fuente
F. ferruginea (L.) Vahl var. *sieboldii* (Miq. ex Franch. et Sav.) Ohwi		10	Yano & Hoshino, 2006
F. longispica Steud. var. *boninensis* Ohwi		30	Yano & Hoshino, 2006
F. longispica Steud. var. *hahajimensis* (Tuyama) Ohwi		30	Yano & Hoshino, 2006
F. miliacea (L.) Vahl		10	Yano & Hoshino, 2006
F. ovata (Burm. fil.) Kern		20	Yano & Hoshino, 2006
F. pierotii Miq.		42	Yano & Hoshino, 2006
F. sericea (Poir.) R.Br.		44	Yano & Hoshino, 2006
F. subbispicata Ness et Meyen		10	Yano & Hoshino, 2006
F. velata R.Br.		24	Yano & Hoshino, 2006
F. verrucifera (Maxim.) Makino		10	Yano & Hoshino, 2006
Machaerina			
M. articulata (R.Br.) T.Koyama [*Baumea articulata* (R.Br.) Blake]		24	De Lange et al., 2004
M. complanata (Berggr.) T.Koyama [*Baumea complanata* (Bergg.) Blake]		c.50	De Lange et al., 2004
M. sinclairii (Hook.f.) Koyama		c.30	De Lange et al., 2004
Morelotia			
M. affinis (Brong.) Blake		46	De Lange et al., 2004
Rhyncospora			
R. asperula		18	Arguelho et al., 2012 Unpubl. data
R. austro-brasilensis		18	Arguelho et al., 2012 Unpubl. data
R. breviscula		10	Arguelho et al., 2012 Unpubl. data
R. ciliata		10	Arguelho et al., 2012 Unpubl. data
R. corymbosa		18	Arguelho et al., 2012 Unpubl. data
R. gigantea		18	Arguelho et al., 2012 Unpubl. data
R. globosa		36, 45, 58	Arguelho et al., 2012 Unpubl. data
R. junciformis		18	Arguelho et al., 2012 Unpubl. data
R. marisculus		36	Arguelho et al., 2012 Unpubl. data
R. nervosa		10, 20, 30	Arguelho et al., 2012 Unpubl. data
R. pubera		10, 12	Arguelho et al., 2012 Unpubl. data
R. tenerrima		20	Arguelho et al., 2012 Unpubl. data
R. tenuis		4, 5	Arguelho et al., 2012 Unpubl. data
R. tenuis Link		4, 8	Vanzela et al., 2003
R. tenuis Link	3		Da Silva et al., 2008
R. velutina		10	Arguelho et al., 2012 Unpubl. data

Species	n	2*n*	Fuente
Schoenoplectus			
S. acutus		38,84	Tena et al., unpubl data
S. americanus		66	Tena et al., unpubl data
S. gemmifer C. Sato, T.Maeda & Uchino		76	Yano & Hoshino, 2005
S. hondoensis (Ohwi) Soják		38	Yano & Hoshino, 2005
S. juncoides (Roxb.) Palla		74	Yano & Hoshino, 2005
S. komarovii (Roshev.) Soják	19	38	Yano & Hoshino, 2005
S. lineolatus (Franch. & Sav.) T. Koyama	37	74	Yano & Hoshino, 2005
S. mucronathus (L.) Palla		38	Yano & Hoshino, 2005
S. multisetus Hayasaka & C. Sato		70	Yano & Hoshino, 2005
S. nipponicus (Makino) Soják		74	Yano & Hoshino, 2005
S. otarui (Ohwi) Holub	21	42, 44	Yano & Hoshino, 2005
S. tabernaemontani (Gmel.) Palla		42	De Lange et al., 2004
S. triangulatus (Roxb.) Soják		42	Yano & Hoshino, 2005
S. triqueter (L.) Palla		42	Yano & Hoshino, 2005
S. X *trapezoideus* (Koids.) Hayasaka & H. Oshashi		43	Yano & Hoshino, 2005
S. X *uzenensis* (T. Koyama) Hayasaka & H. Oshashi		58	Yano & Hoshino, 2005
Schoenus			
S. apogon Roem. & Schult.		8, 4II	N. Kaur et al., 2012
S. apogon Roem. et Schult. var. apogon		8	De Lange et al., 2004
S. apogon var. caespitans (Petrie) Edgar		8	De Lange et al., 2004
S. brevifolius R.Br.		90	N. Kaur et al., 2012
S. caespitans (Petrie) Edgar		8	N. Kaur et al., 2012
S. carsei Cheesem.		60	N. Kaur et al., 2012
S. carsei Cheeseman		c.60	De Lange et al., 2004
S. concinnus (Hook.f.) Hook.f.		c.68	De Lange et al., 2004
S. concinnus Hook.f.		c.68	N. Kaur et al., 2012
S. fluitans Hook.f.		10, 5II	N. Kaur et al., 2012
S. maschalinus Roem. & Schult.		10	N. Kaur et al., 2012
S. maschalinus Roem. et Schult.		10	De Lange et al., 2004
S. nitens (R.Br.) Hook.f.		74	De Lange et al., 2004
S. nitens (R.Br.) Roem. & Schult.		74	N. Kaur et al., 2012
S. pauciflorus (Hook.f.) Hook.f.		28, 56	De Lange et al., 2004
S. pauciflorus (Hook.f.) Hook.f.		28, 14II, 56	N. Kaur et al., 2012
S. tendo (Hook.f) Hook.f.		70	N. Kaur et al., 2012
Tetraria			
T. capillaris (F.Muell.) J.M.Black		20	De Lange et al., 2004

2. Artículo de investigación: Karyotype characterization of eight Mexican species of *Eleocharis* (Cyperaceae).

Trabajo aceptado en Botanical Sciences.

KARYOTYPE CHARACTERIZATION OF EIGHT MEXICAN SPECIES OF *ELEOCHARIS* (CYPERACEAE)

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Abstract: Karyotypes of 49 populations belonging to eight species of Mexican *Eleocharis* (Cyperaceae) are described. Chromosome numbers for *Eleocharis densa*, *E. reznicekii*, and *E. rostellata* are reported for the first time and new numbers are reported for *E. macrostachya*, *E. xyridiformis*, and the *E. montevidensis* complex. Numbers range from $2n = 10$ to $2n = 60$. Dysploidy is the most common mechanism of karyotype variation, which has been detected in four species (*E. densa*, *E. macrostachya*, *E. reznicekii*, and *E. xyridiformis*). Two species are diploid (*Eleocharis parishii* and *E. cf. montevidensis*) and three are polyploid (*E. acicularis*, *E. montevidensis*, and *E. rostellata*). Except for specimens of *E. montevidensis* complex, no intraspecific variation in chromosome number was found. However, differences in the chromosome sizes were found among populations of that complex and in *E. rostellata*. Mean lengths of diploid set ranged from 12.96 in *E. montevidensis* to 178.25 μm in *E. rostellata* and the average of chromosomes sizes varied from 0.97 in *E. montevidensis* to 6.01 μm in *E. xyridiformis*. These two taxa presented an extreme interchromosomal asymmetry A_2: 0.12 and 0.43. Absence of primary constrictions was confirmed. Taxonomical implications of the karyological data are discussed.

Key words: chromosome, cytotaxonomy, dysploidy, holocentric, sedges.

Resumen: Se describen los cariotipos de 49 poblaciones de ocho especies de *Eleocharis* (Cyperaceae) de México. Se reportan por primera vez números cromosómicos para *Eleocharis densa*, *E. reznicekii* y *E. rostellata*, así como nuevos números para *E. macrostachya*, *E. xyridiformis* y plantas del complejo de *E. montevidensis*. Los números cromosómicos van de $2n = 10$ a $2n = 60$. El mecanismo más común de variación cariotípica es la disploidía, presente en la mitad de las especies (*E. densa*, *E. macrostachya*, *E. reznicekii* y *E. xyridiformis*). Dos especies son diploides (*Eleocharis parishii* y *E. cf. montevidensis*) y tres son poliploides (*E. acicularis*, *E. montevidensis* y *E. rostellata*). No se encontró variación intraespecífica en cuanto a números cromosómicos excepto para plantas del complejo de *E. montevidensis*, pero se encontraron diferencias en tamaño entre poblaciones de ese mismo complejo y en *E. rostellata*. Las longitudes medias del cariotipo van de 12.96 a 178.25 μm (en una variante de *E. montevidensis* y en *E. rostellata*, respectivamente); los promedios de longitud de los cromosomas van de 0.97 μm en *E. montevidensis s.l.* a 6.01 μm en *E. xyridiformis*, especies que también presentan los extremos de asimetría intercromosomal A_2: 0.12 y 0.43, respectivamente. Se confirma la ausencia de constricciones primarias. Se discuten las implicaciones taxonómicas de los datos cariológicos.

Palabras clave: ciperáceas, citotaxonomía, cromosoma, disploidía, holocéntrico.

Cyperaceae is the third largest family of monocots, with about 5,400 species in 106 genera (Govaerts *et al*., 2007) to 5,500 species in 109 genera (Muasya *et al*., 2009). Known as sedges, its representatives occur in a variety of habitats, being most common in moist areas. It includes several worldwide distributed genera, e.g., *Carex* L., with more than 2,000 species

(Reznicek, 1990) and *Eleocharis* R.Br., which comprises more than 270 species (González-Elizondo, unpubl. data). Many species of *Eleocharis* are important forage for livestock, a few are used as human food, and several have potential use in aquatic weed management and in pollution abatement (Sutton, 1984 cited by Catling & Hay, 1993). The use of native plants that have a high capacity to accumulate metals and remove them from soil and water (phytoremediation) is a very convenient approach (González-Elizondo *et al*., 2005).

Eleocharis is distinguished by having unbranched stems, leaves reduced to basal, tubular sheaths, inflorescence reduced to a simple terminal spikelet, floral traits very reduced in number and size, and achene with a persistent stylobase (González-Elizondo and Peterson, 1997; González-Elizondo and Tena-Flores, 2000). In spite of the easy recognition of *Eleocharis* as a genus and its prominent delimitation within Cyperaceae (Kukkonen, 1990), the species are difficult to identify and classify because of the limited number of morphological features (Smith *et al.*, 2002). This limitation is increased because several traits are strongly variable among related species and, in some cases, the convergence is common and many morphological features are not phylogenetically informative (González-Elizondo and Tena-Flores, 2000). The supraspecific classification of *Eleocharis* has been revised and modified by Kukkonen (1990) and González-Elizondo and Peterson (1997) on the basis of the classification of Svenson (1929). Four subgenera are recognized but recent phylogenies (Roalson and Friar, 2000; Roalson *et al*., 2010) reveal that subgenus *Eleocharis* is paraphyletic.

Cyperaceae possess a unique combination of cytogenetical features: holokinetic ("holocentric") chromosomes, possibility of inverted meiosis, and pseudomonad development (asymmetric tetrads). These features favor karyotype differentiation for agmatoploidy

(fission), symploidy (fusion), and polyploidy (Luceño and Guerra, 1996; Da Silva *et al*., 2005, 2008b).

Since holokinetic chromosomes have no primary constriction, the main feature useful to identify the chromosome morphology, the options for analysis based on morphology of the karyotype are greatly reduced in sedges. Because the lack of centromere, 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 have been useful for cytotaxonomical diagnosis (e.g., Da Silva *et al.*, 2008b, 2010).

The ability of holokinetic chromosomes to migrate parallel in the cell divisions is due to kinetic activity distributed throughout the chromosome, which favors the maintenance of chromosome rearrangements, such as fissions and fusions, and chromosomes viability after most rearrangements. For this reason, chromosomes evolve more dynamically in sedges than in any other group of flowering plants (Hipp *et al*., 2009).

The use of karyological data in taxonomy, traditionally referred to as cytotaxonomy or karyosystematics, contributes to evaluate the genetic relationships among species or populations and to a better understanding of the way they diverged from each other (Guerra, 2008). The importance of cytogenetical studies to contribute to the knowledge of Mexican plants has been addressed by Palomino (2000). Some examples of karyotypical and cytogenetical analyses of Mexican plants are those of Flores-Maya *et a*l. (2010), Martínez and Palomino (1996), Mercado *et al. (*1989), Mercado-Ruaro y Delgado-Salinas (1998, 2000, 2009), Palomino and Heras (2001), Tapia-Pastrana and Gómez-Acevedo (2005), Tapia-Pastrana (2010), Tapia-Pastrana and Jiménez-Salazar (2011), and Tapia-Pastrana *et al. (*2004,

2012). Chromosomes of Mexican sedges are almost entirely unknown. Chromosome numbers have been reported for *Carex peucophila* Holm (Beaman *et al*., 1962) and *Fimbristylis mexicana* Palla (Kral, 1971).

A considerable variation in the chromosome number in Cyperaceae has been recorded, from $2n = 4$ for *Rhynchospora tenuis* (Vanzela *et al*., 1996) to $2n = 216$ for *Eleocharis dulcis* (Patnaik and Guru, 1968, cited by Roalson, 2008). Chromosome counting in *Eleocharis palustris* dates from 1924, by Piech (Da Silva, 2010) and cytological studies in the genus have been made by Strandhede (1965a,b, 1967), Hoshino *et al*. (2000), Bureš *et al*. (2004), Yano *et al*. (2004), and da Silva *et al*. (2005, 2008a,b, 2010), among others. Yet, most members of the family (about 84%) remain cytologically unexplored (Roalson, 2008). The aim of this study was to contribute to the knowledge of Mexican *Eleocharis*. The karyotypes of 49 populations of eight species of *Eleocharis* from north-central Mexico were analyzed and the taxonomical implications of the karyological data are discussed.

Materials and methods

Mitotic metaphase chromosomes were studied from root meristematic cells. Forty-nine samples representing eight species of *Eleocharis* were collected in 35 different localities of north-central Mexico, most of them in the state of Durango. Voucher specimens were deposited in herbarium CIIDIR. Data of the studied taxa, localities of collection and voucher specimens are given in Table 1. Plants were raised in the nursery of CIIDIR (Centro Interdisciplinario de Investigación para el Desarrollo Integral Regional, Instituto Politécnico Nacional) in Durango, Mexico, from materials transplanted from the wild. Root-tips were

taken from actively growing plants. The root tips were pre-treated in 2mM 8-hydroxyquinoline for 24 h, fixed in ethanol: acetic acid (3:1, v:v) for 24 h, and stored at –20 °C or immediately used. For the conventional staining, the root tips were softened in 4% cellulase plus 40% pectinase at 37 °C for 1 h, hydrolyzed in 1M HCl for 10 min at 60 °C, and squashed in a drop of 45% acetic acid. Slides were stained in 4% hematoxylin and mounted with Entellan (Merck). Chromosome counts were made in at least 20 cells per sample. Chromosome measurements were made using the freeware computer application MicroMeasure version 3.3, available on the Internet at http://www.colostate.edu/Depts/Biology/MicroMeasure. For each sample, from 5 to 10 metaphase spreads with similar condensation were measured. Mean lengths of the karyotype (the total diploid length) and of the shortest and longest chromosome of the complement were calculated. Slides were acquired with a Carl Zeiss AxioImager.Z2 microscope equipped with an Axiocam Hrc camera, objective Plan-Apochromat 100x/1.4 Oil, and AxioVs40 Rel.4.8.2 software. Ideograms were draw from the chromosome measurements. Interchromosomal asymmetry was calculated using the Romero Zarco (1986) index based on Pearson's dispersion coefficient (the ratio between the standard deviation and the mean of chromosome length for each sample): $A_2 = s / \overline{X}$.

Table 1. Studied taxa, localities of collection, voucher specimens, chromosome number (2*n*), and Figures. Initials for collectors are: C.S. = Claudia Silva, J.T. = Jorge Tena, O.R. = Octavio Rosales, S.G. = Socorro González, S.H. = Sergio Heynes.

Species	**Localities, geographic coordinates and voucher number**	**2*n***	**Figure**
E. acicularis (L.) Roem. & Schult.	Buenos Aires, DGO; 23°42'29"N 105°43'26"W (*J.T. 003*) Puentecillas, DGO; 23°40'21"N 105°27'23"W (*S.G. 7823*) Sn Jose de Gracia, DGO; 24°28'25"N 104°43'03"W (*O.R. 4060*) El Carmen, DGO; 24°16'46"N 104°4'07"W (*O.R. 4067*)	20	1 D
E. densa Benth.	Cd. Durango al NE, DGO 24°11'46"N 104°29'18"W (*J.T. 007 a*; *J.T. 007 b*) Cd. Durango al NE, DGO 24°11'N 104°29'W (*S.H. 00a*)	16	1 B
E. macrostachya Britton	Carr Mezquital, DGO; 23°53'06"N 104°29'59"W (*J.T. 007c*) Ej. Abraham González, DGO; 24°13'23"N 104°30'36"W (*S.G.7782 a*; *S.G.7782 b*) Refugio Salcido, DGO; 23°54'43"N 104°31'52"W (*S.G.7832*)	28	1 H
E. cf. *montevidensis* Kunth 1	Jiménez del Teul, ZAC; 23°22'42"N 103°53'52"W (*O.R. 4031a*) Cd. Durango al NE, DGO; 24°12'01"N 104°29'07"W (*O.R. 4023*) Villanueva, ZAC 22°24'12"N 92°29'08"W (*O.R. 4013*) Canatlán, DGO; 24°31'58"N 104°48'19"W (*O.R. 4051*) Sn Jose de Gracia, DGO; 24°28'25"N 104°43'03"W (*O.R. 4041*) Plan de Ayala, DGO; 23°54'58"N 104°30'01"W (*O.R. 4071*)	20	1 G
E. cf. *montevidensis* Kunth 2	Jiménez del Teul, ZAC; 23°22'42"N 103°53'52"W (*O.R. 4031b*) Canatlán, DGO; 24°31'58"N 104°48'19"W (*O.R. 4050*) Málaga, DGO; 24°13'55"N 104°29'42"W (*O.R. 4019*) Plan de Ayala, DGO; 23°54'58"N 104°32'08"W (*O.R. 4070*)	20	1 F
E. cf. *montevidensis* Kunth 3	Canatlán, DGO; 24°31'58"N 104°48'19"W (*O.R. 4049*) Canatlán, DGO; 24°31'58"N 104°48'19"W (*O.R. 4054a*)	10	1 E
E. parishii Britton	Sn Jose de Gracia, DGO; 24°28'25"N 104°43'03"W (*O.R. 4058*) Ej. Abraham González, DGO; 24°13'22"N 104°30'37"W (*S.G.7783*; *S.G.7784*) Refugio Salcido, DGO; 23°55'28"N 104°32'43"W (*O.R.4078*) Cd. Durango al NE, DGO; 24°11'45"N 104°29'18"W (*J.T. 006*) Rancho El Coro, DGO; (23°53'08"N 104°3001"W (*J.T. 001*) Refugio Salcido, DGO; 23°54'42"N 104°31'48"W (*J.T. 002*) Ej 27 Nov, DGO; 24°12'38"N 104°29'59"W (*S.G. 7794*) Ej 27 Nov, DGO; 24°12'42"N 104°30'08"W (*S.G. 7807*) Málaga, DGO; 24°08'37"N 104°26'42"W (*S.G.7811*)	10	1 A
E. reznicekii S. González, D.J. Rosen, R. Carter and P.M. Peterson	Mezquital, DGO; 23°53'08"N 104°30'01"W (*J.T. 005*) Mezquital, DGO; 23°53'06"N 104°29'59"W (*J.T. 006*) Felipe Angeles, DGO; 23°55'54"N 104°33'44"W (*O.R. 4026*) Refugio Salcido, DGO; 23°55'28"N 104°32'43"W (*O.R.4076*; *O.R.4077*) Refugio Salcido, DGO; 23°55'40"N 104°32'56"W (*J.T. 1ra*)	16	1 C
E. rostellata Torr.	Málaga, DGO; 24°13'55"N 104°29'42"W (*O.R.4017*) Ej 27 Nov, DGO; 24°12'42"N 104°30'08"W (*S.G. 7806*) Rincón de Ramos, AGS; 22°11'22"N 102°17'44 W (*C.S. 28*)	60	1 J
E. aff. *rostellata* Torr.	Málaga, DGO; 24°08'40"N 104°26'35"W (*S.G. 7810*)	60	1 K
E. xyridiformis Fernald & Brack.	Mezquital, DGO; 23°53'08"N 104°30'01"W (*J.T. 005a*) Rancho El Coro, DGO; (23°53'08"N 104°3001"W (*J.T. 005s1*) Cd. Durango al NE, Dgo-Torr, DGO 24°11'46"N 104°29'18"W (*J.T. 006s6*) Cd. Durango al NE, DGO; 24°12'01"N 104°29'07"W (*O.R. 4021*; *O.R. 4022*) Mezquital, DGO; 23°29'15"N 104°23'06"W (*O.R. 4068*) Málaga, DGO; 24°13'55"N 104°29'42"W (*O.R. 4018*)	28	1 I

Results

Karyotypes of eight species of *Eleocharis* from Mexico present holokinetic chromosomes, without primary constrictions (Figure 1), and only nucleolar constrictions were observed, as in *E. densa* (Figure 1B). Chromosome numbers, mean of diploid set length, highest and lowest average chromosome length, and interchromosomal asymmetry index are presented in Table 2. The chromosome number is registered here for the first time for *E. densa* ($2n = 16$), *E. reznicekii* ($2n = 16$), *E. rostellata* ($2n = 60$), and a variant of *E. montevidensis* (*E.* cf. *montevidensis* 3) with $2n = 10$. New numbers are reported for *E. macrostachya* ($2n = 28$) and *E. xyridiformis* ($2n = 28$). Numbers range from $2n = 10$ to $2n = 60$ (Figure 1). Mean length of the karyotype (diploid set) ranges from 12.96 μm (*E.* cf. *montevidensis* 3) to 178.24 μm (*E. rostellata*). The lowest average chromosome length was 1.03 μm (*E.* cf. *montevidensis* 3) and the highest 6.01 μm for *E. xyridiformis* (Table 2).

Ideograms of the haploid complement showed variable karyotypes in which most species exhibit chromosomes decreasing gradually in size, independently of the chromosome numbers (Figure 2). Karyotypes of four species have a low A_2 interchromosomal asymmetry index (< 0.17), with chromosomes of about the same size, decreasing very gradually. *Eleocharis densa* and *E. reznicekii* showed $A_2 = 0.23$-0.25, with two medium and six gradually decreasing small pairs of chromosomes, whereas *E. macrostachya* and *E. xyridiformis* present a higher A_2 (0.34-0.43), with two large and twelve pairs of medium to small chromosomes gradually decreasing.

Table 2. Chromosome count and size in Mexican species of *Eleocharis*. Total length is calculated on the diploid set. [1] larger chromosome/smaller chromosome. [2] chromosome numbers recorded for the first time. [3] new numbers reported for the first time. A_2 interchromosomal asymmetry index.

Species of *Eleocharis*	2*n*	Total length (µm)	L/S [1] (µm)	A_2
E. parishii	10	26.99	3.34 - 2.15	0.131
E. densa [2]	16	40.25	3.92 - 1.93	0.245
E. reznicekii [2]	16	38.71	3.58 - 1.74	0.226
E. acicularis	20	40.63	2.72 - 1.46	0.169
E. cf. ***montevidensis*** 1 [3]	20	54.67	3.56 - 2.02	0.144
E. cf. ***montevidensis*** 2 [3]	20	31.59	1.99 - 1.25	0.123
E. cf. ***montevidensis*** 3 [3]	10	12.96	1.60 - 1.03	0.133
E. macrostachya [3]	28	67.01	5.08 - 1.55	0.337
E. xyridiformis [3]	28	80.67	6.01 - 1.61	0.429
E. rostellata [2]	60	159.28	3.70 - 1.70	0.165
E. aff. ***rostellata*** [2]	60	116.03	2.60 - 1.30	0.151

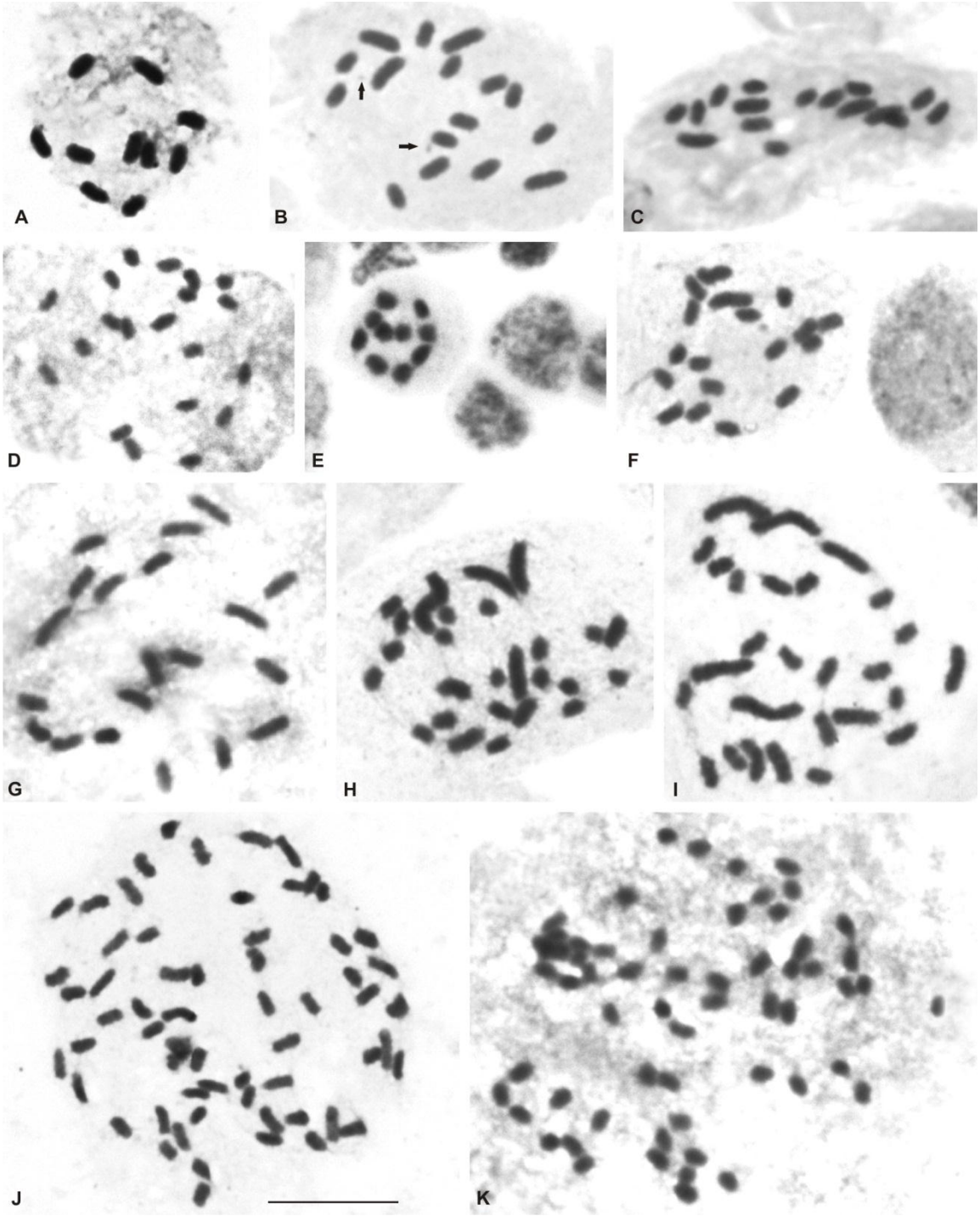

Figure 1. Mitotic metaphase in species of *Eleocharis.* (A) *E. parishii* 2*n* = 10; (B) *E. densa* 2*n* = 16, arrow point out nucleolar constriction; (C) *E. reznicekii* 2*n* = 16; (D) *E. acicularis* 2*n* = 20; (E) *E*. cf. *montevidensis*3 2*n* = 10; (F) *E*. cf *montevidensis*2 2*n* = 20; (G) *E.* cf. *montevidensis*1 2*n* = 20; (H) *E. macrostachya* 2*n* = 28; (I) *E. xyridiformis* 2*n* = 28; (J) *E. rostellata* 2*n* = 60; (K) *E*. aff. *rostellata* 2*n* = 60. Scale bar = 10 µm.

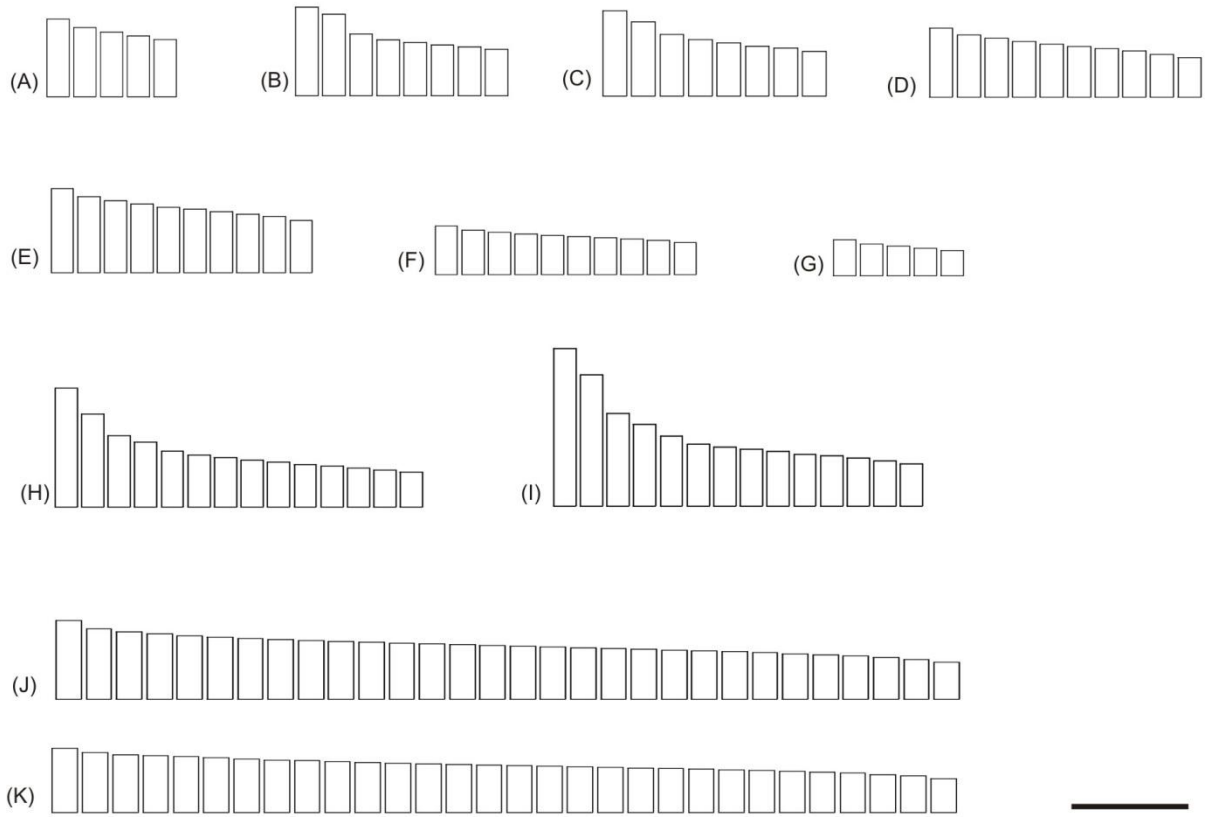

Figure 2. Ideograms of the studied species. All the ideograms represent the haploid set. Note that all species exhibit chromosomes decreasing gradually in size. (A) *E. parishii* 2*n* = 10; (B) *E. densa* 2*n* = 16; (C) *E. reznicekii* 2*n* = 16; (D) *E. acicularis* 2*n* = 20; (E) *E.* cf. *montevidensis*1 2*n* = 20; (F) *E.* cf. *montevidensis*2 2*n* = 20; (G) *E.* cf. *montevidensis*3 2*n* = 10; (H) *E. macrostachya* 2*n* = 28; (I) *E. xyridiformis* 2*n* = 28; (J) *E. rostellata* 2*n* = 60; (K) *E.* aff. *rostellata* 2*n*= 60. Scale Bar = 5µm.

Discussion

Karyotypes differ among the studied species in a combination of traits including chromosome number, total length of the diploid set, chromosomes length, asymmetry indices, and mechanisms of variation.

Chromosome numbers. A wide range of chromosome numbers have been registered for *Eleocharis*, from 2*n* = 6 for *E. subarticulata* (Da Silva *et al*., 2005) to 2*n* = 216 for *E. dulcis* (Patnaik and Guru, 1968, acc. Roalson, 2008). In this study, chromosome numbers ranged from 2*n* = 10 to 2*n* = 60, a broad spectrum considering the low number of taxa analyzed (Table 2). Results in this work, with chromosome numbers that are multiples of 5 or suggesting dysploidy from multiples of that number, are consistent with x = 5 as the basic number for *Eleocharis*.

For the first time, numbers are given for *E. densa*, *E. reznicekii*, *E. rostellata*, and a variant of *E. montevidensis* (*E.* cf. *montevidensis* 3). New numbers are reported for *E. macrostachya* and *E. xyridiformis*, as well as for plants of the *E. montevidensis* complex. Chromosomal counts confirm previous reports for *E. acicularis* with 2*n* = 20 (Tanaka, 1948 cited by Yano *et al*., 2004; Yano *et al.*, 2004; several authors cited by Roalson, 2008), as well as 2*n* = 20 for *E. montevidensis* (Kessler, 1986 cited by Roalson, 2008), and 2*n* = 10 for *E. parishii* (Raven 1965 cited by Roalson, 2008).

Karyotype length. Chromosomes exhibited from small to medium-small sizes, considering the Stebbins (1938) standards, ranging from 1.03 to 6.01 μm, with only two medium-large pairs (>

5 μm) in *E. xyridiformis* and one medium-large pair in *E. macrostachya*. In these two hexaploids with 2*n* = 28 the medium-large chromosomes could have been originated by fusion of four chromosomes (two-by-two) from the original 2*n* = 30. The smallest chromosomes were found in the diploid *E.* cf. *montevidensis* 3, with 2*n* = 10. A more fine division in the small chromosomes category for *Eleocharis* was proposed by Yano *et al*. (2004), who recognized very small (< 1.1 μm) and larger (1.4-4.3 μm) chromosomes. The first case applies to species of the section *Limnochloa* and the second one to species of sections *Pauciflorae* and *Eleocharis* [subgenera *Zinserlingia* p.p. and *Eleocharis* acc. González-Elizondo and Peterson, 1997]. Very small and numerous chromosomes distinguishing *Limnochloa* have been confirmed by da Silva *et al*. (2008b, 2010). In the present study no species of *Limnochloa* were included, but some chromosomes lower than 1.4 μm are present in *Eleocharis montevidensis* s.l. and in *E. aff. rostellata*, species that belong to the subgenus *Eleocharis*.

Interchromosomal asymmetry A_2 (Romero Zarco, 1986) describes the variation in chromosome length in a complement. In general terms, the karyotypes found in this study have a low interchromosomal A_2 index, with chromosomes decreasing gradually in size (Figure 2, Table 2). *Eleocharis densa* and *E. reznicekii* with 2*n* = 16 could be considered dysploids in relation to basic number *x* = 5 (see Da Silva *et al*., 2008b, 2010) with six small and two small-medium pairs of chromosomes (A_2 = 0.23-0.25), a karyotype that seems derived by chromosome fusions from a 2*n* = 20 (Figure 2B, C). Comparatively, the most asymmetric karyotypes, with the highest difference between the longest and the shortest chromosomes were found in *E. xyridiformis* (6.01/1.61) and *E. macrostachya* (5.08/1.55), with A_2 indices of 0.43 and 0.34, respectively (Table 2). No association between total karyotype length and asymmetry was found.

Intraspecific variation. Variation in chromosome number among populations of the same taxon was found only in *Eleocharis montevidensis s.l.* in which most of the studied plants are $2n = 20$ and only one is $2n = 10$. A similar example of polyploidy has been reported for *E. geniculata* $2n = 10, 20$ (Sanyal and Sharma, 1972 cited by Roalson, 2008). Intraspecific variations in chromosome number involving few chromosomes have been recorded for species of *Eleocharis* elsewere, e.g., for *E. acicularis* $2n = 36\text{-}38, 50\text{-}58$ (Hicks, 1929, cited by Roalson, 2008), *E. acicularis* f. *longiseta* $2n = 20, 21$ (Yano *et al*., 2004), *E. maculosa* $2n = 10, 8, 7, 6$ (Da Silva *et al*., 2008a), *E. palustris* $2n = 14\text{-}17, 38, 39$, and *E. uniglumis* $2n = 46, 78\text{-}82$ (Strandhede, 1965a,b, 1967), *E. palustris* $2n = 15\text{-}42$ (Bureš *et al*., 2004), and *E. xyridiformis* $2n = 18\text{-}20$ (Harms, 1968). All these examples reinforce that mechanisms of karyotype differentiation in the genus which include dysploidy (fission and/or fusion) and polyploidy.

Given the morphological diversification in the plants identified as *Eleocharis montevidensis* and several differences with the "typical" *E. montevidensis*, the plants studied here are considered as part of a complex. Three cytotypes are recognized: *E. cf. montevidensis* 1 (Figures 1G, 2E), with $2n = 20$ and large karyotype (54.67 μm), *E. cf. montevidensis* 2 (Figures 1F, 2F), with $2n = 20$ and medium size karyotype (31.59 μm), and *E. cf. montevidensis* 3 (Figures 1F, 2G), with $2n = 10$ and small karyotype (12.96 μm). Although the karyotype of *E. cf. montevidensis* 3 has the same diploid number as *E. parishii*, a closely related species, it differs in having chromosomes half sized in relation of those of *E. parishii*: longer chromosome 1.60 vs 3.34 μm, shorter 1.03 vs 2.15 μm, and total length of the complement 12.96 vs 26.99 μm (Table 2). Morphological traits of the plants allow to

separating to *E. cf. montevidensis* 3 from *E. parishii*: cuspid at apex of upper sheath short and thick (vs long and thin); spikelets ovate (vs ovate-lanceolate to lanceolate); glumes ovate, almost black, broadly hyalino marginated (vs lanceolate, paler); and a thicker, obovate achene with short pyramidal stylobase (vs narrowly pyramidal to lanceolate stylobase). As currently circumscribed, *E. montevidensis* includes at least three elements that need further study and could represent undescribed taxa or taxa that have been included as synonyms of *E. montevidensis*. The complex, distributed from the United States to southern South America, needs taxonomic revision.

Intraspecific differences in size were also found in *Eleocharis rostellata*. All the studied samples are $2n = 60$, but among those that can be confidently identified as *E. rostellata* two groups of chromosomes were found: small-medium, 1.90-4.30 μm with a total length of the complement of 178 μm (Figures 1J, 2J) and small to small-medium (1.50-3.10 μm); besides, a smaller variant with chromosomes 1.30-2.60 μm and a total length of μm 116 μm (Figures 1K, 2K) differs in some morphological external features, such as filiform culms and thinner and darker glumes, which possibly representing an incipient species which is called here *Eleocharis aff. rostellata*.

Karyotypic variation. The most common mechanism of karyotype variation in the species studied here was dysploidy, occurring in four of the eight species (*Eleocharis densa*, *E. macrostachya*, *E. reznicekii*, and *E. xyridiformis*). Two other taxa are diploid (*E. parishii* and *E. cf. montevidensis* 3), and three polyploid: *E. acicularis* and *E. cf. montevidensis*, which are tetraploids, and *E. rostellata*, a dodecaploid.

Eleocharis displays a large variation in karyotype and genome sizes (Zedek *et al*., 2010) and the occurrence of polyploidy and agmatoploidy/symploidy have been well documented (Da Silva *et al*., 2008a, b). Despite its variability, most species of *Eleocharis* have numbers multiple of 5 (Da Silva *et al*., 2008a, b), the number proposed as the basic number for the family by Löve *et al*. (1957, cited by Da Silva, 2010). Polyploidy has been found as an important mechanism of evolution in this genus (Hoshino, 1987; Yano *et al*., 2004; Da Silva *et al*. 2008b) as in eudicotiledons in general (Stebbins, 1971; Soltis and Soltis, 1999). In some groups, chromosome evolution has proceeded from higher to lower numbers, as found by Hipp *et al*. (2007) for *Carex* sect. *Ovales* and confirmed by Mayrose *et al*. (2010) using probabilistic models. However, for *Eleocharis* dysploidy has been as important as polyploidy.

The highest ploidy level (12-ploid) and the longest length in this study were found in *Eleocharis rostellata*. No previous data had been published on the chromosome number nor karyotype structure for this highly variable and widely distributed species known from North America and South America. The series *Rostellatae* accommodates species characterized by firm and shiny sheaths, culms 10-220 cm long, flattened, wiry, sometimes arching to decumbent; spikelets ovoid-lanceolate to spindle-shaped, acute, often proliferous, and achene obtusely trigonous to plano-convex, prolongated at the apex and continuous with the conic to lanceolate stylobase (González-Elizondo and Peterson, 1997). The chromosome number and karyotype length confirm the distinctiveness of this species or species complex. The important role that karyotypes play in the acclimation of plants has been pointed out by Mayrose *et al*. (2010) and Wang *et al*. (2011), who indicate 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. The dodecaploid karyotype of *E. rostellata* mirrors the wide adaptability of this

species both to acid and to strongly alkaline habitats, being the last the most common habitat for the populations of *E. rostellata* studied here.

Eleocharis macrostachya and *E. xyridiformis* were both found to be 2*n* = 28. Different numbers have been registered for both species: *E. macrostachya* with unstable polyploid numbers ranging around 2*n* = 38 and *E. xyridiformis* with 2*n* = 18-20, the 19-chromosome cytotype trisomic for one of the long chromosomes (Harms, 1968). Cytotaxonomical and morphological studies suggested that *E. macrostachya* may be a diploid-polyploid complex, at least partly of hybrid origin (Smith *et al*., 2002) with 2*n* = 10, 16, 18, 19, and 38.

Eleocharis macrostachya is an extremely variable taxon and *E. xyridiformis* has been synonymized under it or has been recognized as a species with karyotypical and morphological differences (Harms, 1968). Smith *et al*. (2002) noted that *E. xyridiformis* (treated by them as "variant a" of *E. macrostachya*) "almost certainly deserves taxonomic recognition, perhaps as a species". They differ in several morphological features and, for the plants revised by them, in chromosome numbers: 2*n* = 18 for "variant a" and 2*n* = 38 for "variant b" or "typical" *E. macrostachya*. Although under both criteria species boundaries are diffuse among the plants treated in this study as *E. macrostachya* and *E. xyridiformis*, we consider that more information, including a broader sampling and using different taxonomic approaches is needed to better understand the limits of *E. macrostachya*.

Eleocharis acicularis, which belongs to subgenus *Scirpidium* (González-Elizondo and Peterson, 1997), has a karyotype very similar to some plants of the *E. montevidensis* complex with the same diploid number (2*n* = 20) and similar general aspect. No differences were found between subgenera in this study. Our data are in accordance to phylogenetic analyses (Roalson and Friar, 2000; Yano *et al*., 2004) that indicate that *Scirpidium* is a monophyletic group

nested into a paraphyletic subgen. *Eleocharis* and that it could be considered a sister group to the rest of this clade (Roalson and Hinchcliff, 2007; Roalson *et al*., 2010). These similarities could suggest a karyotype conservation for this species. According to Guerra (2008), the chromosome number can be a plesiomorphic characteristic of a large clade or a recurrent trait which arose independently in two or more clades. On the other hand, reports of *E. acicularis* 2*n* = 56 (36-38, 50-58) (Hicks, 1929 cited by Roalson, 2008) mirror the highly polymorphic nature of the complex identified worldwide as *E. acicularis*. The plants analyzed during this study have relatively coarse rhizomes and culms (0.3-0.4 mm wide) and spikelets more than 12-flowered but other plants that also key to *E. acicularis* (which died in the nursery and were not analyzed during this study) have slender rhizomes, capillary culms and few-flowered spikelets. Recognition of varieties in *E. acicularis* is premature pending a worldwide taxonomic revision of subg. *Scirpidium* (Smith *et al.*, 2002), and the plants studied in this work are considered as part of that complex.

Interspecific hybridization may be a widely overlooked evolutionary phenomenon in *Eleocharis* and may play a significant role in its diversification (Košnar *et al*., 2010). Karyological data in the present study do not support the hypothesis of the hybrid origin of *E. reznicekii* suggested by González-Elizondo *et al.* (2007). At least, no intermediate chromosome numbers were found between the putative parents, as in Bureš (1998) studies. In this study, *E. reznicekii* and one of its putative parents (*E. densa*) are 2*n* = 16, whereas *E. macrostachya* and *E. xyridiformis* (that also were suggested as putative parents) are both 2*n* = 28. To test the hybrid origin hypothesis, molecular and/or cytogenetical analyses are required (González-Elizondo *et al*., 2007). Because of the perennial mat-forming habit, long, horizontal rhizomes; mostly bifid styles; and biconvex, blunt angled, yellow to brown achenes almost

smooth at 30x, *E. reznicekii* is classified into *Eleocharis* subg. *Eleocharis*, sect. *Eleocharis* (González-Elizondo and Peterson, 1997), which also includes the "*Eleocharis palustris* complex". *Eleocharis palustris* has been recorded with *2n* = 16 (several authors cited in Roalson, 2008), as in *E. densa* and *E. reznicekii*.

The karyotype features examined are useful to distinguish among the taxa studied. Chromosome structure and ploidy status as well as the interspecific variation of karyotypes (cytotypes) provide indicators of the genetic similarity between populations or species (Palomino, 2000), but caution should be applied in the interpretation of the results. As Hipp *et al*. (2010) have noticed for *Carex*, different chromosome rearrangements not necessarily represent monophyletic 'races' or infraspecies, in spite that karyotype evolution is a potential player in the speciation in that genus.

Therefore, although karyotype features can be considered a good tool to distinguish species in sedges and karyotypical differences have an excellent potential to be used in evolutionary studies, they shall be interpreted in combination with other taxonomic characters. Additional karyotype studies as well as cytogenetical analyses are needed for *Eleocharis*, along with morphological studies and field work to better understand the taxonomy and the evolutionary relationships of this complex genus.

Acknowledgments

We are indebted to Octavio Rosales Carrillo and Claudia Silva Salas for their help in the field and the laboratory, to Martha González Elizondo, Lorena López Enriquez, Jorge Noriega Villa and Vanessa Mancuso for assistance, and to COFAA and EDI for stimuli for research. A

licence from COTEPABE is gratefully acknowledged by the first author. Two anonymous reviewers provided helpful suggestions to improve the manuscript.

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Copia de aceptación del MS1213; Botanical Sciences

3. Artículo de investigación (en proceso):

Karyotype characterization of four Mexican species of *Schoenoplectus* (Cyperaceae) and first report of mixoploidy for *Schoenoplectus acutus.*
Este artículo sera enviado para revision para su publicación a Botanical Sciences.

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

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Karyomorphological analysis of metaphase chromosomes from meristematic root cells were performed for four species of *Schoenoplectus* (Cyperaceae) from 11 populations in north-central Mexico. Chromosome numbers range from 2*n* = 38 to 2*n* = 84. New records of counting are given for *Schoenoplectus acutus* (2*n* = 38 and 2*n* = 84) and for *S. americanus* (2*n* = 66). Intra-individual variation in chromosome number was found in *S. acutus*, which a rare mixoploidy with prevalence of cells with 2*n* = 38 (36 small + 2 compound, larger chromosomes) and a few cells with 2*n* = 84 small, dot-shaped chromosomes, being this the first record of mixoploid/poliploidy for that species. Polyploidy followed by dysploidy is the most common mechanism of karyotype variation in the studied species. Absence of primary constrictions was confirmed. Mean length of the diploid set ranges 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. A low interchromosomal asymmetry index (A_2), 0.11 to 0.14 was found, very similar among all the species except for the dimorphic karyotypes of *S. acutus* (A_2 = 0.30).

Key words: chromosome, dysploidy, holocentric, mixoploidy, polyploidy, sedges.

Resumen

Caracterización cariotípica de cuatro especies Mexicanas de *Schoenoplectus* (Cyperaceae) y primer registro de mixoploidía para *Schoenoplectus acutus*. Se llevaron a cabo análisis cariomorfológicos de cromosomas metafásicos de células meristemáticas de raíz de cuatro especies de *Schoenoplectus* (Cyperaceae) provenientes de 11 poblaciones del norte-centro de México. Los números cromosómicos van de 2*n* = 38 a 2*n* = 84. Se documentan nuevos números cromosómicos para *Schoenoplectus acutus* (2*n* = 38 y 2*n* = 84) y para *S. americanus* (2*n* = 66). Se registra por primera vez variación intra-individual en el número cromosómico de *S. acutus*, una rara mixoploidía/poliploidía en la que pocas células presentan 2*n* = 84 con cromosomas pequeños en forma de punto, mientras que las células prevalentes presentan 2*n* = 38 (36 cromosomas pequeños + 2 grandes). El mecanismo más común de variación cariotípica en las especies estudiadas es la poliploidía seguida de disploidía. Se confirma la ausencia de constricciones primarias. La longitud media del complemento diploide va de 51.5 µm (*S. tabernaemontani*) a 79.5 µm (*S. acutus*). La menor longitud promedio de los cromosomas es 0.69 µm (*S. acutus*) y la mayor 1.62 µm (*S. tabernaemontani*), a excepción del par de cromosomas largos de *S. acutus* que alcanzan 3.17 µm. El índice de asimmetría intercromosomal (A_2) es muy bajo (0.11- 0.14) y muy similar entre todas las especies a excepción del cariotipo dimórfico de *S. acutus* (A_2 = 0.30).

Palabras clave: ciperáceas, cromosoma, disploidía, holocéntrico, mixoploidía, poliploidía.

Schoenoplectus (Rchb.) Palla (Cyperaceae) is a morphologically diverse, almost worlwide distributed genus of about 39 (Govaerts *et al.*, 2007) to 77 species (Smith, 2002), segregate from the polyphyletic *Scirpus* s.l. (Strong, 1993, 1994). *Schoenoplectus* includes some difficult species complexes (Smith, 2002) and four sections are recognized for the genus: *Actaeogeton*, *Malacogeton*, *Schoenoplectus* and *Supini* (Smith and Hayasaka, 2001 cited by Yano and Hoshino, 2005).

The species of *Schoenoplectus* are frequently found in wetlands and marshes, and some 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., *S. californicus*, 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). Seven species are known for 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 the genus *Schoenoplectus*.

Materials and methods

Mitotic metaphase chromosomes were studied from root meristematic cells. Eleven populations representing four species of *Schoenoplectus* were collected in seven different 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 2mM 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 used. Root tips were washed in distilled water, digested for 1 h in a mixture 4% (w:v) cellulase and 40% (w:v) pectinase at 37°C, further hydrolyzed in 1N 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 freexing in liquid nitrogen. Slides were stained in 4% hematoxylin and permanent slides mounted in Entellan (Merck). Chromosome counts were made in at least 20 cells for sample. Chromosome measurements were made using the freeware computer application

MicroMeasure 3.3 software, (http://www.colostate.edu/Depts/Biology/MicroMeasure) and the data used to assemble ideograms. For each sample, from 5 to 10 metaphase spreads with similar condensation were measured. Mean lengths of the karyotype (the total diploid length) and of the shortest and longest chromosome of the complement were calculated. All images were acquired with a Carl Zeiss AxioImager.Z2 microscope equipped with an Axiocam Hrc camera, objective Plan-Apochromat 100x/1.4 Oil, and AxioVs40 Rel.4.8.2 software. Interchromosomal asymmetry was calculated using the Romero Zarco (1986) index based on Pearson's dispersion coefficient (the ratio between the standard deviation and the mean of chromosome length for each sample): $A_2 = s / \overline{X}$.

Results

The karyotype of 11 populations corresponding to four species of *Schoenoplectus* section *Schoenoplectus* from north-central Mexico was analyzed. The data of the taxa, localities of the studied species and the vouchers are given in Table 1.

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

Species	Localities, geographical coordinates and voucher number	2*n*	Figure
S. acutus	24°12'01"N 104°29'07"W (O. Rosales 4024, 4025)	38	1 E
S. acutus	24°12'01"N 104°29'07"W (O. Rosales 4024, 4025)	84	1 D
S. americanus	24°08'41"N 104°27'13"W (S. Gonzalez 7816	66	1 B
S. californicus	24°28'25"N 104°43'03"W (O. Rosales 4057, 4059) 24°26'13"N 104°41'52"W (O. Rosales 4062) 24°08'41"N 104°27'13"W (S. González 7817)	68	1 C
S. tabernaemontani	23°55'28"N 104°32'43"W (J. Tena Sitio 2) 23°55'53"N 104°33'17"W (J. Tena Sitio 4) 23°54'58"N 104°32'08"W (O. Rosales 4072) 23°55'28"N 104°32'43"W (O. Rosales 4075)	42	1 A

Chromosome numbers range from 2*n* = 38 to 2*n* = 84. Numbers documented for the first time in this study are 2*n* = 66 for *S. americanus* (Pers.) Volkart ex Schinz & R. Keller, and 2*n* = 38 and 2*n* = 84 for *Schoenoplectus acutus* (Muhl. ex Bigelow) Á.Löve

& D.Löve var. *occidentalis* (S. Watson) S.G. Smith. The numbers 2*n* = 42 for *S. tabernaemontani* (C. C. Gmelin) Palla and 2*n* = 68 for *S. californicus* (C. A. Meyer) Soják confirm previous reports (see Table 2).

Table 2. Chromosome numbers for the studied species, including previous reports. New records given here are indicated by *****.

Species	*N*	Reference	2*n*	Reference
S. acutus	ca. 19	(Schuyler, 1976)	36	(Harriman, 1981)
			42	(Löve & Löve, 1981)
			38*	(this work)
			84*	(this work)
S. americanus	39	(Otzen, 1962)	78	(cited by Smith, 2002 and by Yano & Hoshino, 2005)
	ca 19	(Schuyler, 1976)	66*	(this work)
S. americanus x *S. pungens*	ca 43-47	(Schuyler, 1976)		
S. californicus ssp. *californicus*	ca. 34	(Schuyler, 1976)	68	(cited by Smith, 2002)
	32, 34	(Heiser, 1979)	64, 68	(cited by Yano & Hoshino, 2005)
			68	(this work)
S. californicus ssp. *tatora* (Kunth) T.Koyama	35	(Heiser, 1979)		
S. tabernaemontani	21	(Otzen, 1962)	42	(Håkansson, 1928; Wulff, 1938; Hindakova, 1976; Arohonka,1982; Kozhevnikov *et al.*, 1986; Stoeva, M. P., 1987; Javurkova-Jarolimova, 1992; Hoshino *et al*., 1993; Montgomery *et al*., 1997; all in Roalson, 2008)
	ca 21	[S. validus] (Schuyler, 1976)	42	[S.validus] (Harriman, 1981)
			42	(de Lange *et al*., 2004)
			42	(Yano and Hoshino, 2005)
			42	(this work)

Intra-individual variation in chromosome number was found in *S. acutus*, a rare mixoploidy with prevalence of cells with 2*n* = 38 (36 small + 2 compound, larger chromosomes) and a few cells with 2*n* = 84 small, dot-shaped chromosomes, being this the first record of mixoploidy for that species and of mixoploid/poliploidy for the genus.

As shown in Figure 1, mitotic metaphase chromosomes are small and dot-like, as reported by Maeda and Uchino (2004) for Japanese *Schoenoplectus*, with the exception of the pair of larger chromosomes found in the 2n = 38 cells of *S. acutus*.

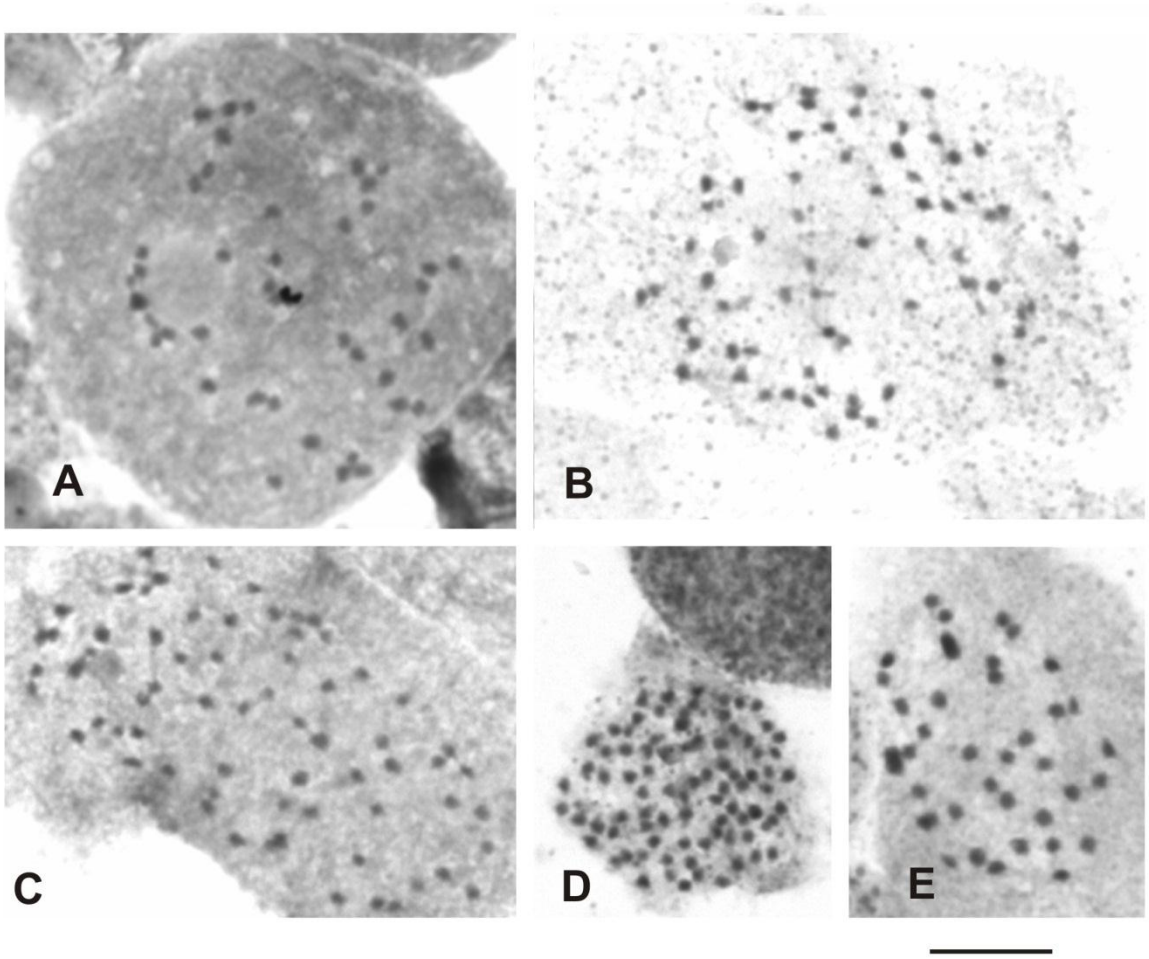

Figure 1. Mitotic metaphases of four species of *Schoenoplectus*. (A) *S. tabernaemontani* 2*n* = 42; (B) *S. americanus* 2*n* = 66; (C) *S. californicus* 2*n* = 68; (D) *S. acutus* 2*n* = 84; *S. acutus* 2*n* = 38. Scale bar = 10µm.

Mean length of the diploid set ranges 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) is very low (0.11 to 0.14) except for the 2*n* = 38 cells of *S. acutus*. Table 3 shows the number of chromosomes of the diploid set for the studied species, the total length expressed in µm, the average of the length of the longest and shortest chromosomes, as well as the rate of asymmetry.

Figure 2 shows the ideograms of the haploid complement for every karyotype. All 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*.

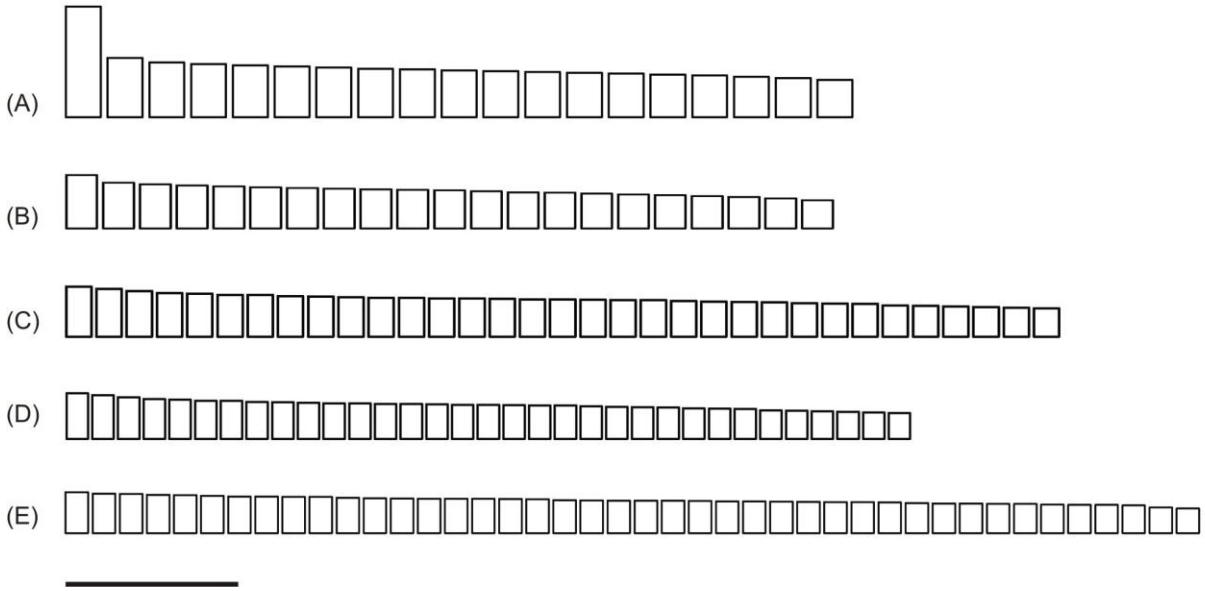

Figure 2. Haploid Ideograms for four species of *Schoenoplectus*. All the ideograms represent the haploid set. Note that all species exhibit chromosomes decreasing gradually in size except for the longest pair found in *S. acutus*: (A) *S. acutus* 2*n* = 38; (B) *S. tabernaemontani* 2*n* = 42; (C) *S.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

Table 3. Chromosome count and size, and interchromosomal asymmetry index in Mexican species of *Schoenoplectus.*

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

Discussion

a) *Schoenoplectus acutus* var. *occidentalis*

Schoenoplectus acutus is distributed from Canada to Mexico and probably Eurasia, since some specimens from Eurasia identified as *S. lacustris* (L.) Palla are probably *S. acutus* (Smith, 2002). It includes two varieties: *S. acutus* var. *acutus*, from Canada, the United States, and probably Eurasia, and *S. acutus* var. *occidentalis*, known from Canada and western United States to central Mexico (Michoacán), where it grows in marshes and edges of lakes and streams, sometimes in saline habitats (González-Elizondo *et al*., 2007).

Schoenoplectus acutus and *S. tabernaemontani* are part of a species complex and have been considered as part of a highly polymorphic and widely distributed species: *Schoenoplectus lacustris* (L.) Palla, which is 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).

An extremely rare mixoploidy/polyploidy was found for *S. acutus* var. *occidentalis*, with 2*n* = 38 and 2*n* = 84 in the same individuals. These numbers represent two cell lines, in which 2*n* = 84 is derived of a 2*n* = 38 with a karyotype size equivalent to one of 42 small chromosomes. The 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 real polyploidy but to a special kind of agmatoploidy (Grant, 1981).

Although intra-specific variation in chromosome numbers in sedges is common (e.g., Bures *et al*., 2004; da Silva *et al*., 2008 a, b, among others), intra-individual variation is extremely rare, and it usually implicates aneuploidy. The first report of this fenomenon for *Schoenoplectus* was given by Maeda and Uchino (2004), who found inter- and intra-individual variations in chromosome number, a mixoploidy/aneuploidy in the root-tip cells of three species: chromosome number varied from 2n = 68 to 74 in *Schoenoplectus gemmifer*, 2n = 32 to 39 in *S. mucronatus* and 2n = 37 to 44 in *S. triangulatus*, respectively.

The mixoploidy/polyploidy found here, with prevalence in the same individual of 2*n* = 38 and some cells with 2*n* = 84, 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 during more than a half of the year. Several authors (cited by Mola and Papeschi, 2006) report that the increasing of chromosome number by fragmentation provides a greater variability and better adaptation capability. Similar conclusions have been found 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. Since genomic attributes are strongly linked to the adaptation capability of the plants, the chromosome condition found here may be part of the search for an adaptative pathway to the severe environmental conditions where these plants grow. The fact that they were collected on 2010, a particularly dry year, could also influence the chromosomal changes going on. This kind of findings have an important role in the search and selection of strains with adaptative capabilities to rough environments, particularly on places where climate change tends to accentuate frequency and length of drough. This appears to be the first report of intra-individual variation in chromosome number in *Schoenoplectus*.

As for the 2*n* = 38 line, it presents a highly asymmetric karyotype with 36 small, dot-shaped + 2 larger chromosomes. The larger pair represent compound chromosomes, each with a size equivalent to three of the smaller ones (3.17 vs. 1.0 µm, respectively). Dimorphic chromosomes have been recorded for the closely related *S. lacustris* by Tanaka since 1940, who also found two compound chromosomes thought to be the equivalent of three small chromosomes, as well as by Kamari *et al*. (2000) for a cytotype of *S. lacustris* with 2*n* = 40 from the Danube plain, in which two chromosomes are much longer than the others. In *S. acutus* var. *occidentalis* the cells with 2*n* = 38 have a karyotype length equivalent to 2*n* = 42 small chromosomes, which directly doubles to 84 in the new ploidy level.

Previously known chromosome numbers for *S. acutus* are *n* = 19 and 2*n* = 36, 42 (see Table 2). Among those numbers, *n* = ca. 19 (Schuyler, 1976) and 2*n* = 42 (Löve and Löve, 1981) are the most related to the ones reported in this work. *Schoenoplectus acutus* shows the lowest average chromosome length (0.69 µm) among the species studied here.

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 spings in central Durango, Mexico.

The record here of 2*n* = 66 is the first for *S. americanus*. Countings of *n* = ca 19, 39, and 2*n* = 78 had been reported for the species, as well as *n* = ca 43-47 for *S. americanus* x *S. pungens* (see Table 2 for sources).

Schoenoplectus californicus

This species from warm to temperate to cold climates is distributed from the SW of the United States to Argentina and Chile, as well as some Pacific islands (Hawaii and others); introduced to New Zeland. It is one of the dominant elements of the aquatic vegetation in Mexico, forming communities locally called “tulares” (González-Elizondo *et al.*, 2007).

The counting reported here of 2*n* = 68 for *S. californicus* confirms the reports cited by Smith (2002) and Yano & Hoshino (2005). Other numbers previously given for the species are *n* = 32, 34 (Heiser, 1979) and 2*n* = 64 (cited by Yano & Hoshino, 2005). For *S. californicus* ssp. *tatora* (Kunth) T.Koyama, the Bolivian and Peruan totora, Heiser (1979) reported *n* = 35.

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.

The number 2*n* = 42 for *S. tabernaemontani* confirms previous reports for the species (Table 2). Among the studied species, this had the lowest chromosome number and the highest average chromosome length (1.62 µm).

Schoenoplectus tabernaemontanii is the correct name for many plants that were previously known as *Scirpus validus* Vahl or *Schoenoplectus validus* (Vahl) A. Löve et D. Löve, as well as for Mexican plants mistakely identified as *S. lacustris*, species with which is closely related (González-Elizondo *et al*., 2007). The remarkable stability in the chromosome numbers reported for *S. tabernaemontani* worlwide (*n* = 21, 2*n* = 42, see Table 2) supports its recognition to the specific level, particularly considering that it belongs to a family in which chromosomic unstability is almost the rule.

The number of chromosomes has been reported for 27 species of *Schoenoplectus* (various authors, cited by Yano and Hoshino, 2005, and Roalson, 2008). A wide variation exists in the diploid numbers in the genus, which range from 2*n* = 10 (Schuyler 1969) to 2*n* = 128 (Hicks 1928, cited by Yano & Hoshino (2005). Haploid chromosome number in *Schoenoplectus* worlwide ranges from *n* = 5 to 44, with chromosome number peaks at 21 (in seven species), 19 and 39 (five species each), and 37 (four species) (Roalson, 2008).

The numbers found for the species studied here range from 2*n* = 42 (*S. tabernaemontani*) to 2*n* = 84 (*S. acutus var. occidentalis*), being in general terms in the group of high numbers. The chromosome numbers for the studied species, including previous reports, are given in Table 2.

Among the studied species, karyotypes differ mainly in number. Most of them are small, dot-shaped, and very homogeneus in size, with the exception of the longest pair in *S. acutus*. The interchromosomal asymmetry index (A_2) is very low (0.11- 0.14) and very similar among all the species except for the dimorphic karyotypes of *S. acutus* (A_2 = 0.30). The total length of the diploid set is also in a relatively short range, from 51.5 to 79.5 µm.

The basic chromosome number *x* = 5 traditionally accepted for sedges, is difficult to estimate based in haploid numbers ranging from *n* = 19 to *n* = 47, and in diploids 2*n* = 36 to 84, unless complex mechanisms of agmatoploidy and symploidy occur previous and following poliploidization events.

For *Schoenoplectus*, Yano and Hoshino (2005) studied the chromosomal evolution and the molecular phylogeny of 14 species of Japan, finding that the chromosomal evolution in the studied species has been caused more by polyploidy than for aneuploidy. They concluded that based in the fact that the species they studied have high chromosome numbers with almost equal chromosome sizes, which is the same situation found in our work. The variation largely distributed around 20 and 40 reported by Roalson (2008) is interpreted by him as a possible indication of polyploidy with subsequent aneuploidy. In the species studied here, polyploidy followed by dysploidy is the most common mechanism of karyotype variation.

None of the species studied, as expected for Cyperaceae, presented primary constrictions in the chromosomes.

Acknowledgments

Octavio Rosales Carrillo provided valuable help with the collection of samples as well as with lab work, along with Claudia Silva Salas and Vanessa Arreola Heynes. Financial support for the project came from SIP-IPN and stimuli for research from COFAA and EDI. A licence from COTEPABE is gratefully acknowledged by the first

author.

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VII. DISCUSIÓN GENERAL

Se describen los cariotipos de ocho especies de *Eleocharis* y de cuatro especies de *Schoenoplectus* (Cyperaceae) de México, en el primer caso basado en 49 poblaciones y en el segundo en 11. Los análisis cariomorfológicos en ambos casos se basan en el estudio de cromosomas metafásicos de células meristemáticas de raíz.

Se reportan por primera vez números cromosómicos para tres especies (*Eleocharis densa*, *E. reznicekii* y *E. rostellata*), así como el registro de nuevos números para *E. macrostachya*, *E. xyridiformis*, y plantas del complejo de *E. montevidensis*, así como para *Schoenoplectus acutus* y *S. americanus*.

En las especies estudiadas de *Eleocharis* los números cromosómicos varían entre 2*n* = 10 y 2*n* = 60. A nivel mundial, se registra una considerable variación en el número y tamaño cromosómico de especies de este género, siendo sus extremos 2n = 6 (da Silva *et al*., 2005) y ca. 196 (Hoshino, 1987). El mecanismo más común de variación cariotípica encontrado aquí es la disploidía, presente en la mitad de las especies, mientras que dos especies son diploides y tres son poliploides. No se encontró variación intraespecífica en cuanto a números cromosómicos excepto para plantas del complejo de *E. montevidensis*, pero sí se encontraron diferencias en tamaño entre poblaciones de ese mismo complejo y en *E. rostellata*. Las variaciones intraespecíficas en número de cromosomas en *Eleocharis* han sido previamente reportadas para poblaciones de *E. geniculata*, con 2*n* = 10 y 20 (Sanyal y Sharma, 1972; Nijalingappa, 1973), *E. palustris* con 2*n* = 15 y 16 (Strandhede, 1965a) y *E. acicularis* f. *longiseta*, 2*n* = 20 y 21, siendo esta última considerada por Yano *et al*. (2004) como un mixoploide prevalentemente originado por fragmentación de un cromosoma de cariotipo conteniendo 2n = 20. Este tipo de fragmentación también puede ser el origen de un mixoploide en *E. atropurpurea* con 2n = 20 y 21 (Nijalingappa, 1973). Para *Eleocharis maculosa* s.l. se han registrado 2n = 8, 7 y 6, siendo los tres últimos originados por simploidia a partir de la raza cromosómica con 2n = 10 (da Silva *et al.*, 2008a).

Otros ejemplos de variación intraespecífica en *Eleocharis* han sido reportados para *E. atropurpurea* 2n = 20 y 21 y *E. kamtschatica* 2n = 44 y 46 (Yano *et al*., 2004); *E. kamtschatica* con siete aneuploides continuos desde 41 a 47 (Yano y Hoshino, 2006). Los mecanismos de cambio son principalmente la fusión y fragmentación cromosómica (como en *E. atropurpurea* y *E. kamtschatica,* respectivamente), o fusión y translocación (por ej. en *E. maculosa*). Adicionalmente, se han registrado para *Eleocharis acicularis* 2n = 36-38, 50-58 (Hicks, 1929, citado por Roalson, 2008); *E. acicularis* f. *longiseta* 2n = 20, 21 (Yano *et al.*, 2004); *E. palustris* 2n = 14-17, 38, 39, y *E. uniglumis* 2n = 46, 78-82 (Strandhede, 1965a,b, 1967), *E. palustris* 2n = 15-42 (Bureš *et al.*, 2004), y *E. xyridiformis* 2n = 18-20 (Harms, 1968). Todos esos ejemplos refuerzan que los mecanismos de diferenciación cariotípica en *Eleocharis* incluyen disploidía (fisión y/o fusión) y poliploidía.

La hibridación interespecífica puede jugar un papel significativo en la diversificación de *Eleocharis* (Smith *et al.*, 2002; Košnar *et al*., 2010). La comparación de los cariotipos de una especie de origen híbrido (*E. reznicekii,* 2*n* = 16) y sus parentales putativos: *E. densa* (2*n* = 16) y *E. xyridiformis* (2*n* = 28) durante este trabajo no permitió resolver sus relaciones con base solamente en el número, forma y tamaño de los cromosomas. Diversos estudios (p. ej. Yano y Hoshino, 2005, con *Schoenoplectus* de Japón) han revelado que los híbridos naturales presentan un número cromosómico intermedio al de los parentales putativos. Esto no resulta así en el caso de *E. reznicekii*, de manera que se requiere un estudio de cromosomas meióticos y apoyo con bandeos o FISH para resolver las probables relaciones de parentesco entre los taxa involucrados.

La caracterización cariotípica en *Schoenoplectus* permitió el descubrimiento de mixoploidía para *Schoenoplectus acutus* y el primer registro de mixoploidía/poliploidía (variación intra-individual incluyendo poliploidía) para el género. Los números cromosómicos en las especies estudiadas van de 2*n* = 38 a 2*n*

= 84. Se documentan nuevos números cromosómicos para *Schoenoplectus acutus* (2*n* = 38 y 2*n* = 84) y para *S. americanus* (2*n* = 66).

El mecanismo más común de variación cariotípica en las especies estudiadas de *Eleocharis* es disploidía (ya sea fisión o fusión), seguida por poliploidía, mientras que para *Schoenoplectus* es la poliploidía seguida de disploidía. Esto último coincide con los resultados de Hoshino (2005), quienes estudiaron la evolución cromosómica y la filogenia molecular de 14 especies de *Schoenoplectus* de Japón, encontrando que la evolución cromosómica ha sido causada más por poliploidía que por aneuploidía.

En términos generales, los aumentos en número cromosómico ocurren ya sea en series graduales (aneuploidía) o por duplicación (poliploidía). Cuando los aumentos se derivan de fisión (ruptura) de cromosomas holocinéticos, el proceso se denomina agmatoploidía (Luceño y Castroviejo, 1991; Luceño and Guerra, 1997; Luceño *et al.*, 1998a), que es un tipo de anueuploidía en la que cada fragmento del cromosoma tiene la posibilidad de actuar como un cromosoma independiente, a diferencia de la aneuploidía de cromosomas con centrómero definido en la que usualmente los fragmentos son inviables.

En cuanto a la poliploidía, Grant (1981) ha hecho notar que la aparente poliploidía en la que el juego total de cromosomas holocinéticos se duplica mediante fisión sin que ocurra una duplicación real del material genético, puede considerarse más bien como un tipo especial de agmatoploidía. Este es el tipo de poliploidía que se presenta en las especies estudiadas aquí, ya que aunque se llega a observar un aumento en la longitud del cariotipo, este aumento no corresponde al equivalente de una duplicación. Este fenómeno es tratado en el presente trabajo (al igual que en el resto de la literatura referente a citogenética de Cyperaceae) como poliploidía, aunque tal vez debería aplicarse el término “pseudopoliploidía”.

Tanto para *Eleocharis* como para *Schoenoplectus* se confirma la ausencia de constricciones primarias, encontrándose cromosomas holocinéticos.

VIII. CONCLUSIONES

Debido a las características citológicas únicas de las ciperáceas, el estudio citogenético de sus elementos arroja luz sobre aspectos taxonómicos y evolutivos y contribuye al mejor conocimiento del grupo. El presente trabajo es el primero en su tipo para especies mexicanas de Cyperaceae y permitió registrar los números cromosómicos y caracterización del cariotipo de ocho especies de *Eleocharis* y cuatro de *Schoenoplectus*.

Los caracteres del cariotipo pueden ser una buena herramienta para distinguir especies en ciperáceas y tienen muy buen potencial para usarse en estudios evolutivos, pero dada la variación intra-específica que se presenta en los grupos estudiados, es conveniente que la información citogenética sea interpretada en combinación con otros caracteres taxónomicos. Se requiere también llevar a cabo investigaciones más amplias en la citogenética de ciperáceas, junto con estudios morfológicos y estudio de las poblaciones en campo para comprender mejor la taxonomía y las relaciones evolutivas entre los grupos. Lo anterior contribuirá al conocimiento de los componentes del entorno natural y proveerá mejores herramientas para su manejo y conservación.

Los descubrimientos de anomalías cromosómicas ligadas a condiciones ambientales extremas, como los encontrados para una forma de *Eleocharis rostellata* en ambientes acuáticos ácidos, y la mixoploidía-poliploidía encontrada para *Schoenoplectus acutus* var. *occidentalis* de ambientes acuáticos en sitios alcalinos con época seca larga, presentan un valioso potencial de aplicación en la selección de razas o líneas genéticas con capacidades adaptativas a ambientes extremos. Esto es particularmente útil en la búsqueda de soluciones para cubrir suelos en ecosistemas de climas secos y semisecos a la luz de los impactos del cambio climático en situaciones, como ocurre para el norte de México, en que éste tiende a acentuar la frecuencia y la extensión de los períodos de sequía.

IX. RECOMENDACIONES Y SUGERENCIAS

Continuar con esta línea de investigación, ya que uno de los objetivos planteados fue el establecimiento del Laboratorio de Citogenética en CIIDIR Dgo., el cual se inició durante este trabajo y se encuentra en proceso.

Ampliar los estudios citogenéticos mediante la aplicación de diferentes técnicas: además de la citogenética convencional, aplicar técnicas de bandeo y de citogenética molecular, particularmente la de FISH, con el fin de complementar los resultados aquí presentados. Debido a impedimentos para conseguir los reactivos, estas técnicas se encuentran en proceso de ser montadas.

Muchas especies están pendientes de ser colectadas, entre ellas *Eleocharis arsenifera*, *E. cryptica*, y *E. tenarum*, entre otras, por las cuales el autor siente especial interés. Su estudio permitirá completar aún más la información referente a los números y los mecanismos de evolución cromosómica de las especies de *Eleocharis* mexicanos.

Extender la investigación a otros géneros de Ciperáceas de México. Dados los particulares mecanismos de evolución cromosómica en esta familia, su estudio es una importante fuente de información para la comprensión de la taxonomía, la biogeografía y la evolución de este grupo de plantas.

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