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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 ranged from $2n = 10$ to $2n = 60$. Dysploidy was 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 μm in *E. montevidensis* to 178.25 μm in *E. rostellata* and the average of chromosomes sizes varied from 0.97 μm 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 (*E. 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* 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 (Catling and 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). Holokinetic condition favors karyotype differentiation for agmatoploidy (fission), symploidy (fusion), and polyploidy (Luceño and Guerra, 1996; Da Silva *et al*., 2005, 2008b).

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).

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, the interchromosomal asymmetry index, and the presence or absence of nucleolar constrictions had been useful for cytotaxonomical diagnosis (e.g., Da Silva *et al*., 2008b, 2010).

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 al*. (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* (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. However, 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 (Centro Interdisciplinario de Investigación para el Desarrollo Integral Regional, Instituto Politécnico Nacional). Data of the studied taxa, localities of collection and voucher specimens are given in Table 1. Plants were raised in the nursery of CIIDIR 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 ºC or immediately used. For the conventional staining, the root tips were softened in 4% cellulase plus 40% pectinase at 37 ºC for 1 h, hydrolyzed in 1M HCl for 10 min at 60 ºC, 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. Chromo-

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	*2n*	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 José de Gracia, Dgo., 24° 28′2 5″ 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 José de Gracia, Dgo., 24° 28′ 25″ N, 104° 43′ 03″ W (O.R. 4058) Ejido 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° 30′ 01″ 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 Romos, 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° 30′ 01″ W (J.T. 005s1) Cd. Durango al NE, 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

some measurements were made using the freeware computer application MicroMeasure version 3.3. 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 microscope (Carl Zeiss AxioImager.Z2) 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 (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

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 number, mean of diploid set length, the highest and the 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) ($2n = 10$). 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 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.

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 $2n = 6$ for *E. subarticulata* (Da Silva *et al*., 2005) to $2n = 216$ for *E. dulcis* (Roalson, 2008). In this study, chromosome numbers ranged from $2n = 10$ to $2n = 60$, 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 that 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 *Eleocharis 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 $2n = 20$ (Yano *et al*., 2004; Roalson, 2008), as well as $2n = 20$ for *E. montevidensis* (Roalson, 2008), and $2n = 10$ for *E. parishii* (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 *Eleocharis xyridiformis* and one medium-large pair in *E. macrostachya*. In these two hexaploids with $2n = 28$ the medium-large chromosomes could have been originated by fusion of four chromosomes (two-by-two) from the original $2n = 30$. The smallest chromosomes were found in the diploid *E.* cf. *montevidensis* 3, with $2n = 10$. 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* according 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 *E. montevidensis* s.l. and in *E.* aff. *rostellata*, species that belong to the subgenus *Eleocharis*.

Interchromosomal asymmetry A_2 (Romero, 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 $2n = 16$ 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 $2n = 20$ (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

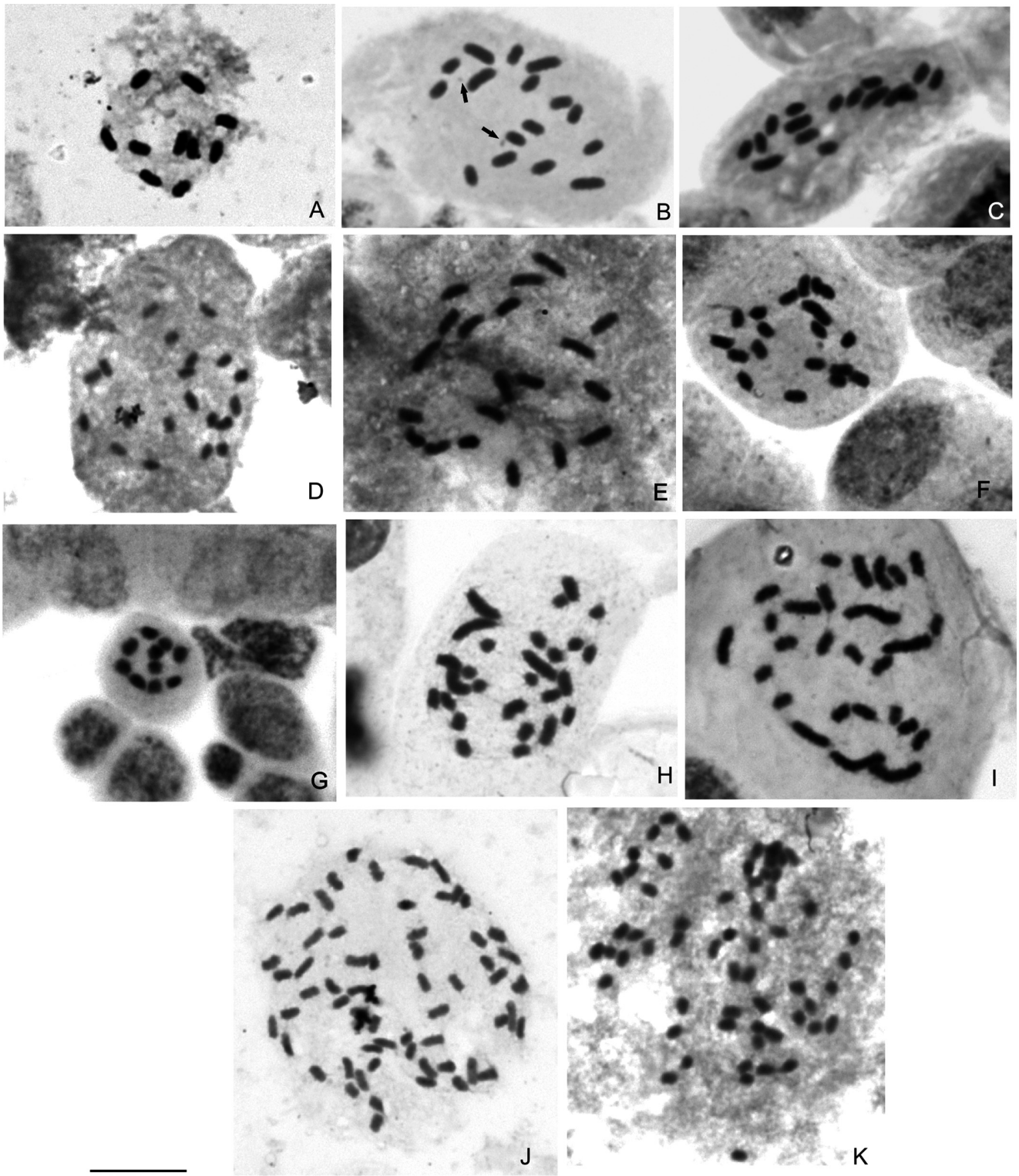

Figure 1. Mitotic metaphase in species of *Eleocharis*. (A) *Eleocharis parishii* 2*n* = 10; (B) *E. densa* 2*n* = 16, arrow point out nucleolar constriction; (C) *E. reznicekii* 2*n* = 16; (D) *E. acicularis* 2*n* = 20; (E) *E.* cf. *montevidensis*3 2*n* = 10; (F) *E.* cf *montevidensis*2 2*n* = 20; (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.

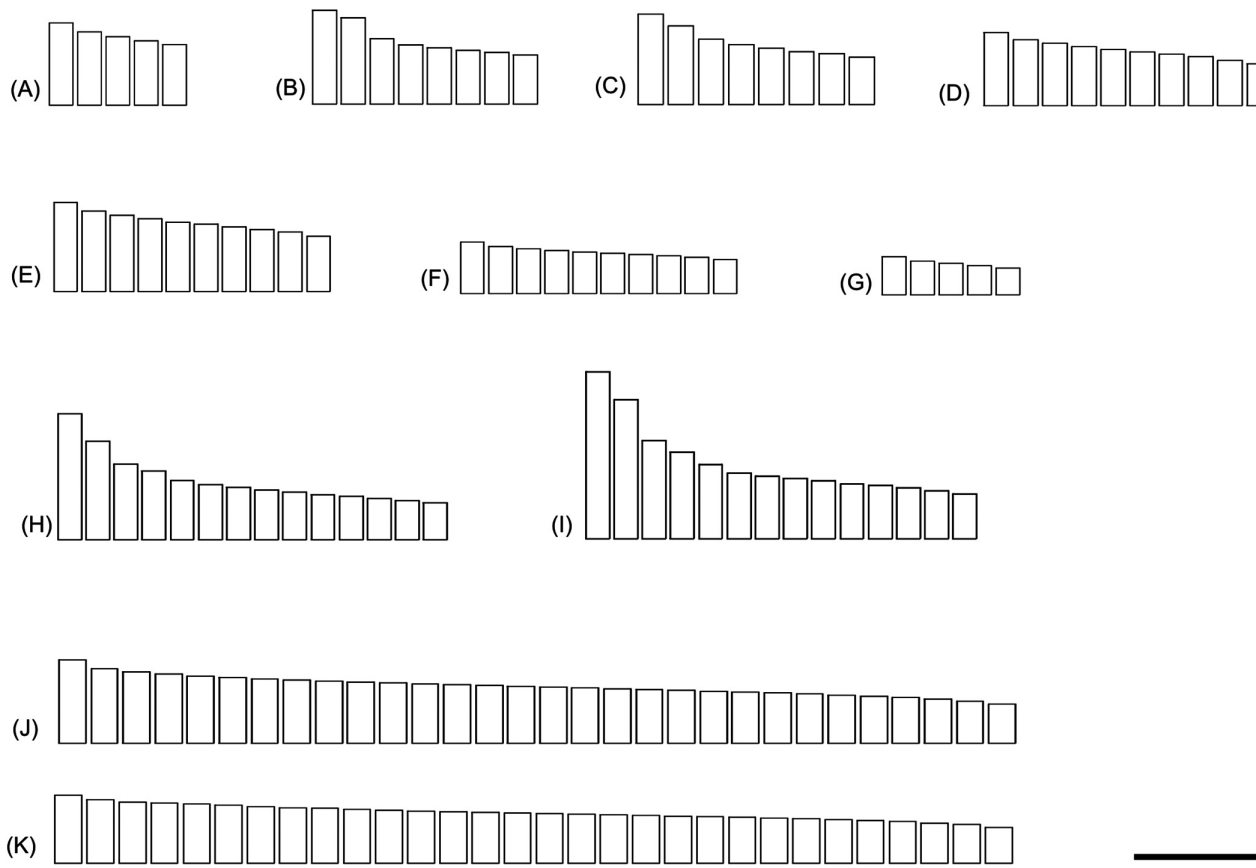

Figure 2. Ideograms of the studied species. All the idiograms represent the haploid set. Note that all species exhibit chromosomes decreasing gradually in size. (A) *Eleocharis parishii* 2*n* = 10; (B) *E. densa* 2*n* = 16; (C) *E. reznicekii* 2*n* = 16; (D) *E. acicularis* 2*n* = 20; (E) *E.* cf. *montevidensis*1 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 = 5 *µ*m.

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 2*n* = 20 and only one is 2*n* = 10. A similar example of polyploidy has been reported for *E. geniculata* 2*n* = 10, 20 (Sanyal and Sharma, 1972). Intraspecific variations in chromosome number involving few chromosomes have been recorded for species of *Eleocharis* elsewere, e.g., for *E. acicularis* 2*n* = 36-38, 50-58 (Hicks, 1929), *E. acicularis* f. *longiseta* 2*n* = 20, 21 (Yano *et al*., 2004), *E. maculosa* 2*n* = 10, 8, 7, 6 (Da Silva *et al*., 2008a), *E. palustris* 2*n* = 14-17, 38, 39, and *E. uniglumis* 2*n* = 46, 78-82 (Strandhede, 1965a,b, 1967), *E. palustris* 2*n* = 15-42 (Bureš *et al*., 2004), and *E. xyridiformis* 2*n* = 18-20 (Harms, 1968). All these examples reinforce that mechanisms of karyotype di-

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

fferentiation 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 μm *vs* 3.34 μm, shorter 1.03 μm *vs* 2.15 μm, and total length of the complement 12.96 μm *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 *E.* 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*). Another two 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). 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 angiosperms 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 *E. 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 may have 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* reflects 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 $2n = 28$. Different numbers have been registered for both species: *E. macrostachya* with unstable polyploid numbers ranging around $2n = 38$ and *E. xyridiformis* with $2n = 18$-20, the cytotype 19-chromosome is 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 $2n =$ 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: $2n = 18$ for variant a and $2n = 38$ 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*, 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 ($2n = 20$) 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. Reports of *E. acicularis* $2n = 56$ (36-38, 50-58; Hicks, 1929) suggest 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 have 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 $2n = 16$, whereas *E. macrostachya* and *E. xyridiformis* (that also were suggested as putative parents) are both $2n = 28$. 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 "*E. palustris* complex". *Eleocharis palustris* has been recorded with $2n = 16$ (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.

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; however, 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 and field studies to better understand the taxonomy and the evolutionary relationships of this complex genus.

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