Analysis of retrotransposon abundance, diversity and distribution in holocentric
Eleocharis (Cyperaceae) genomes

Thaíssa B. de Souza¹, Srinivasa R. Chaluvadi², Lucas Johnen¹, André Marques³, M. Socorro González-Elizondo⁴, Jeffrey L. Bennetzen² and André L. L. Vanzela¹,∗

¹Laboratory of Cytogenetics and Plant Diversity, Department of General Biology, Center for Biological Sciences, State University of Londrina, Londrina 86057–970, Paraná, Brazil, ²Department of Genetics, University of Georgia, Athens, GA 30602, USA, ³Laboratory of Genetic Resources, Campus Arapiraca, Federal University of Alagoas, Arapiraca, Brazil and ⁴CIIDIR, Instituto Politécnico Nacional, Sigma 119 Fracc. 20 de Noviembre II, 34234 Durango, Mexico

*For correspondence. E-mail andrevanzela@uel.br

Received: 9 February 2018 Returned for revision: 27 February 2018 Editorial decision: 9 April 2018 Accepted: 18 April 2018
Published electronically 4 May 2018

INTRODUCTION

The DNA C-value has been recognized as a relevant parameter for plant genome studies, especially in the context of genome evolution (Bennett and Leitch, 2011). The amount of DNA can change quickly in genomes, due to ploidy and repetitive DNA accumulation/elimination, often without any obvious taxonomic effect (Kellogg and Bennetzen, 2004; Lee and Kim, 2014). The repetitive DNA fraction can be recognized and systematically organized according to repeat structure or size, phylogenetic distribution/origin, sequence relatedness, mode of amplification and/or genomic distribution (Jurka et al., 2007; Wicker et al., 2007). Primary components of the repetitive DNA are the transposable elements (TEs), and they are particularly important because of their roles in both genome and gene functions (Lisch, 2013; Makarevitch et al., 2015). The nature and genomic representativeness of these sequences can also be used as parameters in studies of chromosome biology and karyotype organization (Marques et al., 2015; Santos et al., 2015; Ribeiro et al., 2016).

Transposable elements are the most abundant repetitive sequences in plant genomes (SanMiguel et al., 1996; Jurka et al., 2007; Heslop-Harrison and Schwarzacher, 2011). Their proportion in dicotyledonous plants has been seen to vary from >10 % of the genome in arabidopsis to approx. 62 % of retroelements in the genome of Solanum lycopersicum (Arabidopsis Genome Initiative, 2000; Paz et al., 2017). In monocotyledonous plants, such as rice, sorghum and maize, TEs routinely range from 40 to 85 % of total DNA (Meyers et al., 2001; Matsumoto et al., 2005; Schnable et al., 2009; Devos, 2010). TEs that transpose using RNA intermediates by a ‘copy-and-paste’ process (Class 1) are commonly called...
Retroelements, and those that transpose in a ‘cut-and-paste’ process (Class 2) are called DNA transposons (Jurka et al., 2007; Wicker et al., 2007). Retroelements fall into three primary categories: long terminal repeat-retrotransposons (LTR-RTs), long interspersed nuclear elements (LINEs) and short interspersed nuclear elements (SINEs). The most abundant LTR-RTs are members of either the Copia or Gypsy superfamilies (Benetzen, 2000; Heslop-Harrison and Schwarzacher, 2011; Grandbastien, 2015). LTR-RTs represent approx. 14% of the Rhynchospora pubera, Cyperaceae (Marques et al., 2015) genome, but approx. 24% and approx. 75% of the sorghum (Paterson et al., 2009) and maize (Schnable et al., 2009)
genomes, respectively. DNA transposons contribute quantitatively less to the genomes of these species, at approx. 8.8, 7.5 and 9%, respectively.

Different TE families, defined by their different vertical origins, can occur either in clusters or scattered along chromosomes (see Gaeta et al., 2010). In some species, some Gypsy and Copia families have been found to cluter in specific chromosomal regions. Centromeric retrotransposons (CRs) of the Gypsy superfamily, for example, preferentially accumulate at primary constrictions in monocentric chromosomes (Neumann et al., 2011) and near centromeric protein-binding sites along holocentric chromatids (Marques et al., 2015). Juncaceae and Cyperaceae are two families of monocotyledonous plants in which holocentric chromosomes are a synapomorphy (Greilhuber, 1995). This feature enables the parallel movement of chromatids during cell divisions in mitosis, differently from Rabl’s organization in monocentric chromosomes (Guerra et al., 2010; Marques and Pedrosa-Harand, 2016), as well as the permanence of chromosome fragments generated by chromosome fission and fusion in subsequent cell divisions (Vanzela and Colaço, 2002; Hipp et al., 2013). Information on LTR-RT distribution in plants with holocentric chromosomes is scarce. One such study shows, for example, that Copia and Gypsy retrotransposons appear as few and scattered signals on the chromosomes of Luzula elegans, a Juncaceae (Heckmann et al., 2013). An exception is the complete description of co-localization of CRRh (centromeric retrotransposon of Rhynchospora), Tyba satellite DNA (satDNA; 172 bp length) and CENH3 proteins in holocentrics of Rhynchospora Cyperaceae (Marques et al., 2015).

Holocentric chromosomes can be very tolerant of dysploidy events, and this can be observed in the wide chromosome number variation of the Cyperaceae (Da Silva et al., 2008; Roalson, 2008; Bureš and Zedek, 2014). Species of Eleocharis R. Br. (Cyperaceae) exhibit variations in holocentric chromosome numbers from 2n = 6 to 2n = 196, including dysploids and polyploids (Hoshino, 1987; Da Silva et al., 2005, 2010; Roalson, 2008). They also exhibit variation in the amount of DNA, ranging from 18 pg in E. sterneri to 0.84 pg in E. cellulosa (Zedek et al., 2010). In order to shed light on the organization of TEs in these holocentric chromosomes, we used low coverage DNA sequencing and fluorescence in situ hybridization (FISH) to investigate Eleocharis TE families for their possible distribution variance related to karyotype structure and DNA C-value. Partial assemblies of E. elegans and E. geniculata genomes were used as a database for TE discovery and FISH probe design. These experiments investigated differences in LTR-RT family accumulations and distributions, with a special focus on identifying family predominance, hotspots for co-localization and effects on karyotype diversity.

**MATERIALS AND METHODS**

**Plant materials**

A minimum of five individuals from seven Eleocharis species belonging to Eleocharis subgenus Eleocharis [E. maculosa and E. geniculata (two populations each), E. elegans, E. sellowiana, E. filiculmis, E. montana and E. niederleinii] were selected because of their known karyotype diversity (Da Silva et al., 2008, 2010), and collected in the South and South-east of Brazil (Table 1). Plants were grown in pots in the greenhouse of the Center for Biological Sciences at the State University of Londrina, and vouchers (Table 1) were deposited in the herbarium at FUEL, Brazil.

**Table 1. Information on chromosome numbers, ploidy levels and nuclear DNA content for Eleocharis species, and three species used as controls**

| Species                  | 2n     | NAN   | Mbp (2C) | Pg ± s.d. | pg (Cx) | HSD | Vouchers |
|--------------------------|--------|-------|----------|-----------|---------|-----|----------|
| E. maculosa (Vahl) Roem. & Schult. | 2n = 2x = 6     | 40 487 | 841.11   | 0.86 ± 0.04 | 0.43    | a   | 55228    |
| E. geniculata (L.) Roem. & Schult. | 2n = 2x = 10    | 56 342 | 976.42   | 1.00 ± 0.04 | 0.49    | c   | 55227    |
| E. sellowiana (Poir.) Urb. | 2n = 4x = 20       | 49 957 | 1814.12  | 1.85 ± 0.08 | 0.46    | b   | 55225    |
| E. elegans (Kunth)        | 2n = 4x = 20       | 50 048 | 1608.46  | 1.64 ± 0.07 | 0.41    | a   | 55226    |
| E. filiculmis (Kunth)     | 2n = 6x = 30       | 97 343 | 3105.15  | 3.18 ± 0.05 | 0.53    | d   | 55226    |
| E. montana (L.) Roem. & Schult. | 2n = 8x = 40    | 72 090 | 4584.27  | 4.69 ± 0.09 | 0.58    | e   | 55223    |
| E. niederleinii Boeckeler | 2n = 8x = 40       | 40 887 | 4918.74  | 5.03 ± 0.20 | 0.63    | f   | 55343    |
| R. brevisscula H. Pfeiff  | 2n = 10 >30 000   | 782.40 | 60.80    | –         | –      | –   | –        |
| R. pubera (Vahl) Boeckeler | 2n = 10 >30 000   | 3217.62 | 3.29     | –         | –      | –   | –        |
| S. lycopersicum L. ‘Stupické polní rané’ | 2n = 24 >30 000 | 1916.88 | 1.96     | –         | –      | –   | –        |

2n = diploid chromosome number; NAN, number of analysed nuclei; Mbp (2C), C-value in somatic cells in mega base pairs; pg ± s.d., average picogram value for 2C ± s.d.; pg (Cx), value in picograms for each monoploid complement; HSD (honestly significant difference), mean comparisons test, where different letters differ significantly from each other, using Tukey’s test at 5% probability.

*Seeds supplied by Jaroslav Doležel, Department of Cell Biology and Genetics, Palacky University, Czech Republic.
Genome size estimation and chromosome number counts

Measurements of DNA C-values were done with young culms using 1 mL of cold LB01 buffer plus 1 mg mL⁻¹ propidium iodide (Life Technologies), according to Doležel et al. (2007). Analyses were performed on a BD ACCURI C6 flow cytometer, in three independent estimations on different days. Solanum lycopersicum ‘Stupické polní rané’ (2C = 1.96 pg), as well as Rhynchospora pubera (2C = 3.53 pg) and R. breviiacula (2C = 0.80 pg) were used as standards (values from Doležel et al., 2007; Marques et al., 2015; Rocha et al., 2016, respectively). The 2C values were calculated as sample peak mean/standard peak means × 2C DNA amount of standard (pg). Analyses of variance (ANOVAs) were performed using the Sisvar 5.6 program, considering monoploid complement values. The GC content of the assemblies and of the TE fractions was estimated from the E. elegans and E. geniculata genomes using the Illumina R1 and R2 output files with the FastQC tool. Estimates were also done after SPAdes assembling, and after screening using the TE portions against the RepBase database. For this, investigations of GC vs. AT content were done with SED/GREP pipelines (Supplementary Data Table S3).

DNA extraction and PCR

DNA was isolated from young culms of seedlings from each species that were macerated in liquid nitrogen and treated with 2 % cetyltrimethylammonium bromide (CTAB) extraction buffer. DNA was purified with phenol:chloroform (1:1, v/v), chloroform:isoamyl alcohol (24:1, v/v) and RNase (1 mg mL⁻¹), and precipitated in 100 % absolute ethanol. Ethanol-precipitated DNAs were resuspended in 10 mm Tris–HCl pH 8. DNA concentrations were estimated using a NanoDrop 2000 Spectrophotometer (Thermo Scientific).

The LTR-RT probes were obtained by PCR using specific primers for each family with each one of the seven species as template DNA (Supplementary Data Table S2). A standard PCR [5 U µL⁻¹ Taq polymerase (0.5 µL), 10× buffer (2.5 µL), 50 mm MgCl₂ (1.5 µL), 10 mm dNTP (1 µL), 5 mm primers (2 µL each), and H₂O up to a final volume of 25 µL] was used in the following conditions: 94 °C for 2 min, 30 cycles of 94 °C for 40 s, 59 °C for 40 s and 72 °C for 1 min, and a final extension of 72 °C for 10 min. Reactions were tested using electrophoresis in an agarose gel at 3 V cm⁻¹ and stained with ethidium bromide. In the end, the reactions were found to be adequate for each primer set.

The PCR products were used in a second reaction to produce templates for Sanger sequencing, using the 3500xl Automatic Sequencer (Applied Biosystems), according to the manufacturer’s procedures. Two distinct reactions for each primer (forward and reverse) were carried out, and repeated once. The consensus sequences were obtained after alignment of these sequences, in which quality was tested with Phred/Phrap/Consed software, and these sequences were compared against GenBank (http://www.ncbi.nlm.nih.gov/blast) and against POL conserved protein cores available in the GypsyDB.

Fluorescence in situ hybridization

Fluorescence in situ hybridization was performed as described by Da Silva et al. (2008). Slides were prepared by squashing without acid hydrolysis. Probes for each LTR-RT family were obtained using a reamplification of PCR products that involved labelling with biotin-11-dUTP (Copia families) or Cy3-dUTP (Copia families). Each probe was mixed with a solution (30 µL) composed of 100 % formamide (15 µL), 50 % polyethylene glycol (6 µL), 20× SSC (3 µL), 100 ng of calf thymus DNA (1 µL), 10 % SDS (1 µL) and 100 ng of probes (4 µL). The mix was denatured at 90 °C for

In silico analyses

A small quantity of genome sequence data for E. elegans (NCBI: SRX3256858) and E. geniculata (NCBI: SRX3256859) was generated using Illumina Misex PE250. The assemblies were done with the SPAdes program considering three different K-mers, and with input files containing 5 448 113 and 1 777 302 reads, respectively (Supplementary Data Table S1). For screening the assembled sequences containing conserved stretches of TEs, sequences >150 bp in length were compared against RepBase (http://www.girinst.org/censor/) and GypsyDB (http://gydb.org/index.php/MainPage) databases. A second screening was done for conserved gag-POL regions using a local BLASTx run against a custom database containing the main families of plant TEs, grouped according to classes and families (Llorens et al., 2009): Class 1 LTR-RTs: Gypsy (Reina, Galadriel, Del, CRM, Athila and Tat) and Copia (Oryco, Retrofit, SIRE and Tork), and Class 2 transposons: CACTA, Mutator, Harbinger, hAT and Helitrons.

Because the databases do not have reference sequences from Cyperaceae, we searched for an initial cut of 60 % identity (E-value 10e-4) for conserved protein regions of gag-POL, transposase and helicase. Sequences with ≥80 % identity for integrase, RNase H and reverse transcriptase of both Copia and Gypsy members were used as templates for primer design, using the OligoPerfect™ Designer tool (http://tools.lifetechologies.com).

The similarity tree was based on reverse transcriptase fragments selected after local BLASTx, comparing E. elegans and E. geniculata sequences against GypsyDB sequences, according to E-value 10e-4, >80 % identity and >300 bp in length. Reverse transcriptase sequences were analysed with SAİE-v2.2.7 software for alignment (MAFFT and MUSCLE) and development of a maximum likelihood tree (FASTTREE) that was edited using Figtree v1.4.2.

The GC content of the assemblies and of the TE fractions was estimated from the E. elegans and E. geniculata genomes using the Illumina R1 and R2 output files with the FastQC tool. Estimates were also done after SPAdes assembling, and after screening using the TE portions against the RepBase database. For this, investigations of GC vs. AT content were done with SED/GREP pipelines (Supplementary Data Table S3).
10 min, and hybridization was performed at 37 °C during 24 h in a humid chamber. Post-hybridization washes were carried out with 70 % stringency using SSC buffer, pH 7.0. After probe detection with avidin–fluorescein isothiocyanate (FITC) conjugate, washes were performed in 4x SSC/0.2 % Tween-20, all at room temperature, and slides were mounted with 25 μL of DABCO, a solution composed of glycerol (90 %), 1,4-diaza-bicyclo (2.2.2)-octane (2.3 %), 20 mM Tris–HCl, pH 8.0 (2 %), 2.5 mM MgCl₂ (4 %) and distilled water (1.7 %), plus 1 μL of 2 μg mL⁻¹ 4,6-diamidino-2-phenylindole (DAPI).

**RESULTS**

The amount of DNA (2C) measured using at least 30 000 nuclei per species [coefficient of variation (CV) <5 %] showed a range of 0.86 ± 0.04 pg in *E. maculosa* (2n = 2x = 6) to 5.03 ± 0.20 pg in *E. niederleinii* (2n = 8x = 42). The comparative analysis of monoploid complement values (Cx) showed that typical diploids such as *E. maculosa* and *E. geniculata* (2n = 2x = 10) exhibited a Cx of approx. 0.50 pg, while larger variations were observed among polyploids. *Eleocharis sellowiana* (2n = 4x = 20) exhibited the lowest Cx value (0.41 pg), but *E. elegans* and *E. montana* presented different ploidy levels (2n = 4x = 20 and 2n = 8x = 40, respectively) with an average Cx value of 0.58 pg (Table 1; Supplementary Data Fig. S1). Other polyploid species, such as *E. geniculata* with 2n = 4x = 20 and *E. filiculmis* with 2n = 6x = 30, showed intermediate 2C values of 0.46 and 0.53 pg, respectively (Table 1). These data suggest that an increase in 1C DNA content follows an increase in ploidy levels, with a positive Pearson correlation (R² = 0.712) between ploidy and Cx values (Fig. 1A, B). Investigation with ANOVA showed that Cx values are significantly different (P <0.05). The Tukey test (HSD) allowed us to separate the species according to the highest and lowest mean values (Table 1).

To sum up, phylogenetically close species such as *E. maculosa*, *E. geniculata* and *E. sellowiana* (sect. *Eleogenus*) have a lower and similar Cx value, while *E. montana* and *E. elegans* (sect. *Eleocharis*), and *E. niederleinii* (sect. *Tenuissimae*) exhibited relatively larger amounts of DNA by chromosome complement.

In general, the mean Cx values agreed with karyotype structures. Holocentric chromosomes were observed in all the species (Supplementary Data Fig. S2), and those with similar DNA contents also have a similar chromosome number and size. We observed karyotype similarities between *E. geniculata* and *E. maculosa*, both with 2n = 10 (Supplementary Data Fig. S2B, C), and also between *E. geniculata* and *E. sellowiana*, both with 2n = 20 (Supplementary Data Fig. S2D, F). However, it is interesting to note that, except for the karyotypes of *E. maculosa* (2n = 6) and *E. niederleinii* with 2n = 42 (Supplementary Data Fig. S2A, I), the other species show similar chromosome sizes (approx. 0.1 pg per chromosome). *Eleocharis maculosa* and *E. niederleinii* showed greater karyotype asymmetry, most probably due to fission and fusion events. In the case of *E. niederleinii*, the four larger chromosomes were at least twice the size of the smaller ones, and have multivalent pairing at meiosis (data not shown). *Eleocharis montana*, with 2n = 40 and regular meiosis, also exhibited an asymmetric karyotype, but rearrangements were not noted (Supplementary Data Fig. S2H).

**Comparative genomic analysis**

A low coverage sequencing of *E. elegans* and *E. geniculata* genomes was used, which was enough for the assembly of
thousands of sequences (Supplementary Data Table S1). The search for conserved protein regions of TEs in these sequences indicated homologies on approx. 10 % of each data set. Conserved proteins of Class 1 elements were the most abundant in both genomes, accounting for 91.42 % of TE homologies in \textit{E. elegans} and 90.05 % in \textit{E. geniculata} (Fig. 2A; Supplementary Data Table S3). Of these sequences, 83.96 and 85.31 % were LTR-RTs, respectively (Fig. 2B; Supplementary Data Table S3). Class 2 elements constituted approx. 6.81 % and approx. 6.93 % of TE homologies in \textit{E. elegans} and \textit{E. geniculata}, respectively (Fig. 2B; Supplementary Data Table S3). The endogenous retrovirus (ERV) family represented from 2.40 to 3.01 % of the TE homologies in \textit{E. elegans} and \textit{E. geniculata} data sets, respectively (Fig. 2B; Supplementary Data Table S3).

Homologies to conserved LTR-RT proteins of \textit{Copia} and \textit{Gypsy} superfamilies were found on 50.03 and 33.48 % of assemblies in \textit{E. elegans}, and on 49.62 and 35.11 % in \textit{E. geniculata}, and were within mapped sequences in both data sets (Fig. 2B). Of these, the Sirevirus and Athila/Tat clades were the most abundant (see Fig. 2C). BLASTx alignment using mapped TE sequences to detect conserved reverse transcriptase sequences (>80 % identity) allowed us to separate \textit{Copia} elements from \textit{Gypsy} elements (Fig. 3). However, the recognition of \textit{Copia} families was not so accurate, different from those of \textit{Gypsy} which were approx. 90 % similar (Fig. 3). One sequence of each identified TE family was chosen for primer design (Supplementary Data Table S2). When these primer pairs were used in PCR on the genomes of seven \textit{Eleocharis} species, fragments corresponding to the predicted size were observed (see Fig. S3; Supplementary Data Table S2). The alignment of the sequenced PCR products showed about 60 % identity (E-value 10e-4) with their respective members in the databases (Supplementary Data Tables S4 and S5), indicating that primer design was appropriate for each clade. The data set was also used to check the GC/AT content. The GC estimates for \textit{E. elegans} and \textit{E. geniculata} genomes showed values of 40 and 34 %, respectively. These low GC values were also observed after genome assembly, as well as in the TE fraction (Table 2).

In situ hybridization using LTR-RT probes

Fluorescence in situ hybridization assays were conducted with biotin-labelled \textit{Copia} probes that were detected with avidin–FITC (green), and \textit{Gypsy} probes labelled with Cy3-dUTP (red). \textit{Copia} and \textit{Gypsy} probes showed either scattered or clustered FISH signals, depending on the species and the LTR-RT family analysed. In some cases, probes were clustered while other species–probe combinations yielded hybridization results that were broadly scattered across the investigated genome. Figures 4 and 5, and Supplementary Data Figures S4–S9 give examples of hybridization results, with both \textit{Copia} and \textit{Gypsy} family probes. Differences within and between karyotypes were also found in the FISH profiles. \textit{Copia} probes (Fig. 4) yielded predominantly scattered signals (those dispersed along chromosomes without evidence of large clusters of the same TE family), while \textit{Gypsy} probes showed a trend toward more clustered signals (Fig. 5). Results are presented for each probe below.

Of the \textit{Copia} TEs, the Oryco probe of Sirevirus hybridized with both scattered and small clusters on chromosomes of \textit{E. maculosa} (Fig. 4A, B; Supplementary Data Fig. S4A–G) and \textit{E. geniculata} (Fig. 4C; Supplementary Data Fig. S4H–J). However, in \textit{E. maculosa}, scattered signals were seen in the sample with 2n = 6, while clustered signals appeared in the sample with 2n = 10 (Supplementary Data Fig. S4D–G). Predominantly scattered signals were observed in \textit{E. elegans} (Fig. 4D; Supplementary Data Fig. S4K–M), \textit{E. montana} (Supplementary Data Fig. S4N–P) and \textit{E. niederleinii} (Fig. 4E; Supplementary Data Fig. S4Q–S) with this probe. Remarkably, the latter three species exhibited a set of chromosomes that were only weakly labelled. The SIRE probe showed a hybridization profile scattered along chromosomes, and rare clusters (Fig. 4F–I). As seen with the Oryco probe, some chromosomes accumulated more FISH signals in relation to the others, such as in \textit{E. elegans} (Supplementary Data Fig. S5D–F), \textit{E. sellowiana} (Supplementary Data Fig. S5G–I) and \textit{E. niederleinii} (Fig. 4I; Supplementary Data Fig. S5M–O). The Tork and SIRE probes hybridized in a scattered fashion in all chromosomes, without evidence of large clusters (Fig. 4J–O). As seen before in some species, karyotypes were differentially labelled, with signals more evident in some chromosomes, such as in \textit{E. geniculata} (Supplementary Data Fig. S6G–I) and \textit{E. montana} (Supplementary Data Fig. S6M–O; see also the boxes). Interestingly, the larger chromosomes, especially those of \textit{E. niederleinii}, exhibited a higher density of hybridization signals than smaller chromosomes.

Probes for \textit{Gypsy} families, including members of the Chromovirus (Del and CRM) and Athila/Tat clades, showed more clustered signals, besides scattered signals. However, in all cases, there was great variability within and between karyotypes. The Del probe yielded a FISH profile with scattered and clustered signals, but with clear intra- and interspecific variations (Fig. 5; Supplementary Data Fig. S7). In \textit{E. maculosa} (2n = 6), signals were distributed along chromosomes, with more signals in the largest chromosome (Fig. 5A; Supplementary Data Fig. S7A–C), while in \textit{E. maculosa} (2n = 10) and \textit{E. elegans} (2n = 20) signals were homogeneously distributed (Fig. 5B, C; Supplementary Data Fig. S7D–F, J–L). \textit{Eleocharis geniculata} (Fig. 5D; Supplementary Data Fig. S7G–J), \textit{E. montana} (Supplementary Data Fig. S7M–O) and \textit{E. niederleinii} (Fig. 5E; Supplementary Data Fig. S7P–R) exhibited strong accumulations of Del homologues on almost half of the chromosomes. Similarly, the CRM probe exhibited both scattered and clustered signals, while \textit{E. maculosa} (Fig. 5F, G; Supplementary Data Fig. S8A–D), \textit{E. geniculata} (Fig. 5H; Supplementary Data Fig. S8I–K) and \textit{E. elegans} (Supplementary Data Fig. S8L–N) karyotypes yielded particularly strong CRM FISH signals on almost half of all chromosomes. In \textit{E. montana} (Fig. 5I; Supplementary Data Fig. S8O–Q) and \textit{E. niederleinii} (Fig. 5J; Supplementary Data Fig. S8R–T) strong CRM FISH signals were observed on only a few chromosomes.

Probes of the \textit{Gypsy} Athila/Tat families, in contrast to the probes of other LTR-RT families, exhibited FISH signals predominantly distributed in clusters, such as in \textit{E. maculosa} with 2n = 6 (Fig. 5K; Supplementary Data Fig. S9A–D). In \textit{E. maculosa} with 2n = 10 (Fig. 5L; Supplementary Data Fig. S9E–G), \textit{E. geniculata} (Fig. 5N; Supplementary Data Fig. S9H–J) and \textit{E. sellowiana} (Fig. 5M; Supplementary Data Fig. S9L–N), FISH signals for Athila/Tat appeared scattered along chromosomes. \textit{Eleocharis elegans}, on the other hand, showed predominantly small clustered signals on the chromosomes (Supplementary Data Fig. S9O–Q). The Athila/Tat probe exhibited a greater amount of clustered
signals in *E. montana* (Fig. 5O; Supplementary Data Fig. S9R–T), but this probe in *E. nierderleinii* showed weak and predominantly scattered signals, except for two large chromosomes that have accumulated signals along almost all of their length (Fig. 5P; Supplementary Data Fig. S9U–X). In this case, it is important to mention that this probe hybridized to only two of the four large chromosomes, while CRM and Del probes hybridized to all four.

**DISCUSSION**

*C-value and its influence on karyotype differentiation*

The amount of DNA in plants varies >2000-fold, from approx. 63 Mbp in *Genlisea aurea* to approx. 14 900 Mbp in *Paris japonica* (Bennett and Leitch, 2011). Polyploidy events, whether auto- or allopolyploidy, as well as the accumulation and elimination of repetitive DNA families and differential activity of TEs, are the main factors responsible for DNA content fluctuations (Kellogg and Bennetzen, 2004; Grover and Wendel, 2010; Bureš and Zedek, 2014). Since holocentric species seem to tolerate chromosome rearrangements, such as fission or fusion events (Luceño and Castroviejo, 1991; Da Silva et al., 2008), a combined analysis of chromosome counting and DNA content estimation is required to determine the relationship between chromosome number change and DNA content flexibility (see Bureš et al., 2013). Data obtained here for South American *Eleocharis* (subgenus *Eleocharis*) demonstrated that polyploidy is a primary evolutionary mechanism for DNA content amplification, and it is in agreement with the
strong positive correlation between the amount of DNA and polyploidy observed in Eleocharis species from Europe (Zdekov et al., 2010). However, these same authors suggested that in the subgenera Limnochloa and Zinserlingia, that exhibit karyotypes with numerous and small chromosomes, fission (agmatoploidy) may have played an additional role in DNA C-value variation.

Fluctuations in the amount of DNA are common during eukaryotic genome evolution, and they can be motivated by large- or small-scale rearrangements, such as polyploidy and dysploidy. In this sense, E. niederleinii is an interesting example because it presents 2n = 42 (Cx = 0.63 pg) and four large chromosomes derived from fissions and fusions (see Da Silva et al., 2017). Chromosomes of E. niederleinii exhibited an accumulation of AT-rich terminal heterochromatin, and the four large chromosomes showed the greatest accumulation of LTR-RT FISH signals, compared with the others. This evidence may explain the increase in Cx value in relation to the other studied species. Intraspecific variation in polyploids was detected in E. geniculata with 2n = 10 (Cx = 0.50 pg) and 2n = 20 (Cx = 0.46 pg), as well as in the dysloid E. maculosa with 2n = 6 (Cx = 0.43 pg) that exhibited a decrease in Cx value in relation to samples with 2n = 10 and Cx = 0.49 pg. As an example of interspecific variation in DNA content, we can mention the approx. 30 % difference between E. sellowiana Cx = 0.41 pg (2C = 1.64 pg) and E. elegans Cx = 0.58 pg (2C = 2.34 pg), both with 2n = 20. Although it is clear that the small-scale variations for DNA C-value in Eleocharis may involve changes in the repetitive fraction and dysploid changes, this does not seem to be a rule for sedges. In Carex, for instance, the increase in chromosome number due to rearrangements does not involve large fluctuations in the amount of DNA (Roalson et al., 2008; Chung et al., 2011, 2012). In Luzula (Juncaceae), another group with holocentric chromosomes, Bozek et al. (2012) did not find a clear correlation between chromosome number and genome sizes. The fact is that DNA C-value changes in plants may be a result of multifactorial and simultaneous events, involving numerical and structural rearrangements and activity of TEs (Grover and Wendel, 2010; Bennetzen and Wang, 2014). Good examples of the influence of LTR-RT activity on genome size were the differential amplifications of the Copia element BARE-1 in Hordeum (Vicient et al., 1999), and the Gypsy element Ogre in Vicia (Neumann et al., 2006). It is believed that the increase in plant genome sizes due to an increase in LTR-RT content could consequently lead to an increase in GC content (Šmarda et al., 2014). However, this contrasts with the low GC amounts found in the genomes of E. elegans and E. geniculata. According to Šmarda and Bureš (2012) and Šmarda et al. (2014), holocentrics can exhibit reduced recombination and repair rates, which could maintain low GC levels in comparison with plants with monocentric chromosomes. Although these data could explain the low GC amounts found in two genomes of Eleocharis, additional data from flow cytometry and molecular analysis in other species are required to confirm this trend.

Transposable elements, especially LTR-RTs, can also cause an ‘elastic effect’ in the DNA C-value, due to differential events of amplification and removal of each TE family in different chromosomes or chromosome regions (Heslop-Harrison and Schwarzacher, 2011; Tenaillon et al., 2011; Santos et al., 2015). An expectation could be created in relation to the possible tolerance of holocentric chromosomes to rearrangements (Zdekov and Bureš, 2018), especially on the role of LTR-RTs in rearranged karyotypes, such as those with even and odd numbers, irregular meiosis or with high asymmetry, which is frequently found in Cyperaceae (Da Silva et al., 2008, 2017; Roalson, 2008). It is proposed that karyotype changes could constitute a kind of genomic stress, acting in favour of differential activity of TEs (Bureš and Zdekov, 2014). Although new experiments focused on these events are necessary to elucidate this issue, this could be one explanation for the observed differences in DNA content and in the fluctuation of Copia and Gypsy families between E. elegans, E. sellowiana and E. geniculata.

Table 2. Comparison of GC content of E. elegans and E. geniculata obtained from Illumina output files, raw reads and sequences containing TE stretches against values determined by flow cytometry

| Species         | TBN   | Nucl. B | GC (r.r.) | GC (m.r.) | TBCensor | GC-TEs  | Cov  |
|-----------------|-------|---------|-----------|-----------|----------|---------|------|
| E. elegans      | 42 644 322 | 42 467 196 | 40 %     | 37.3 %    | 464 713  | 31 %    | 2.5x |
| E. geniculata   | 8 312 326  | 8 167 100  | 34 %     | 32.3 %    | 194 600  | 23.6 %  | 5.5x |
Comparative cytogenomic analysis

Recent advances in DNA sequencing technologies, bioinformatics tools and databases with more reliable annotations have provided the resources for the scientific community to broaden its understanding of the genomic composition of native species. Although it was possible for us to compare two partially sequenced genomes of *E. elegans* and *E. geniculata* to produce a TE portfolio, our effort was directed to identify and investigate mainly the sequences related to the conserved protein regions in the TEs. It is widely known that retroelements represent a large portion of monocotyledonous genomes, and LTR-RTs account for an important portion of the TE DNA in plants (Bennetzen and Wang, 2014). In maize, for instance, the percentage of LTR-RT DNA reaches approx. 75% of the total nuclear genome (Schnable et al., 2009; Devos, 2010). The annotated TEs from data sets of *E. elegans* and *E. geniculata* indicate a trend toward LTR-RTs accumulation. However, we do not consider this underprediction of their genome contribution, because the low coverage does not allow a perfect assembly of complete elements, and because the TE identification in...
native species, without good references, could lead to misidentification of many TEs.

In general terms, approx. 50% of the LTR-RTs identified in Eleocharis were Copia and approx. 35% were Gypsy. Other plant genomes have presented more pronounced differences in the superfamily abundance, such as in Musa with 25.7% Copia and 11.6% Gypsy (D’Hont et al., 2012) and Helianthus and Solanum, with a high predominance of Gypsy members (Paz et al., 2017; Qiu and ungerer, 2018). In Brachiaria and Festuca, both Poaceae, Gypsy are also most abundant (Santos et al., 2015; Křiváňková et al., 2017). In the holocentric species Luzula elegans (Juncaceae), it was found that approx. 33% of its genome is composed of Copia, with only approx. 1.1% belonging to Gypsy elements (Heckmann et al., 2013), and in Rhynchospora pubera (Cyperaceae), Copia members seem to be more abundant than Gypsy elements (Marques et al., 2015).
From the point of view of karyotypes and chromosomal biology, genomic understanding restricted to Gypsy and Copia superfamilies is somewhat superficial, because the diversity of the repetitive DNA fraction depends not only on the evaluated genomes but also on the evolutionary history, positioning and role of each repetitive sequence type [simple sequence repeats (SSRs), satDNA, TEs and others] in the genomes (Heslop-Harrison and Schwarzacher, 2011). Based on TE phylogeny (Llorens et al., 2009), the relative differences in the occurrence of Copia and Gypsy elements when comparing the genomes of E. elegans and E. geniculata indicates a predominance of sequences from the Sirevirus and Athila/Tat clades, and low occurrence of the Chromovirus clade. In addition, the variation among these families in Eleocharis suggests that, although all LTR-RTs have a similar transposition mechanism, each LTR-RT family exhibits an independent activity history and differences in the relative quantity of autonomous and non-autonomous elements, and these facts may determine their genomic distribution profile, as suggested by Bennetzen and Wang (2014).

As previously mentioned, low coverage sequencing makes it difficult to obtain large sequences, and it hinders the reconstruction of complete LTR-RTs. However, short sequence alignment (from approx. 200 to 800 bp length) against databases that contain highly conserved protein domains, especially for the POL region of the LTR-RTs, allows a secure selection of useful sequences to recognize clades and families of TEs (Langdon et al., 2000; Dixit et al., 2006), as well as for FISH probe production. Probes of highly conserved sequences from POL of Copia (Oryco, SIRE, Retrofit and Tork) and Gypsy (Del, CRM, Athila and Tat) allow detection of differences in frequency and chromosomal location of each family (see Santos et al., 2015), and this strategy was successfully applied to seven Eleocharis species. In general, chromosomes of Eleocharis showed Copia probes with a more scattered signal distribution after FISH, whereas Gypsy signals were scattered using the Del probe and more clustered with the Athila/Tat and CRM probes. This variability is especially dramatic in E. montana, where Oryco, Del and CRM probes hybridized to half of the chromosomes in this species, in contrast to what happened with the Athila/Tat probe, where clustered signals were evident on all chromosomes. The Athila/Tat probe, for example, appeared in a scattered manner in E. niederleini, especially in the two larger chromosomes that are originated by symbiodyly (for details of chromosome fusion and fission, see Da Silva et al., 2017).

The literature indicates that retroelements generally accumulate in scattered patterns across plant chromosomes, unlike satellite DNAs that form more defined clusters (Heslop-Harrison and Schmidt, 2007; Heslop-Harrison and Schwarzacher, 2011; Ribeiro et al., 2016). However, there are examples that report that Copia and Gypsy members have a heterogeneous profile, being able to be located either as scattered TEs, clustered TEs or a combination of both on some chromosomes (Belayayev et al., 2001; Gaeta et al., 2010), including holocentric chromosomes (Heckmann et al., 2013; Marques et al., 2015). FISH using Copia and Gypsy families in these Eleocharis species increases our knowledge of the location and distribution of LTR-RTs in holocentric genomes. In the single previous holocentric example, for Luzula elegans, Copia and Gypsy probes that were not specific to any LTR-RT family showed scattered FISH signals, while Gypsy also exhibited slightly clustered signals (Heckmann et al., 2013). Our data lead us to some questions. (1) Why are some LTR-RTs found more abundantly in some Eleocharis species and chromosomes than in others? (2) Why are some clustered and some not clustered? Probably, this difference is caused by such factors as the potential activity of each family in each genome, epigenetic controls in different genomes or chromosomal regions, the influence of neighbouring sequences of other natures, as well as an association with chromosomal rearrangements. The possible involvement of these factors needs to be studied further.

Our FISH results reinforce the idea that different families of LTR-RTs have independent activity on genomes and chromosomes, with different evolutionary histories and fates. As an example of this postulate, we can mention the differences in the CRM family probe distribution pattern. CRM, first named as a centromeric retrotransposon of maize, was detected in the centromere-proximal chromosome regions of several species (Neumann et al., 2011; Nunes et al., 2018), and associated with the holocentromeres of Rhynchospora (Marques et al., 2015; Rocha et al., 2016). Members of the CRM family have a terminal chromodomain in the polygenic transcript, with a zinc finger domain (HHCC) that recognizes the CENH3 protein. This chromodomain may direct its accumulation preferentially into or near centromeres, which may differentiate it from other LTR-RT families that lack this specific chromodomain (Houben et al., 2007; Neumann et al., 2011). Some chromosomes of E. maculosa, E. elegans and E. montana showed FISH signals distributed along the chromatids, agreeing with the holocentric condition. However, other chromosomes did not exhibit continuous signals using the CRM FISH probe, but rather scattered signals or as very small clusters. In addition, in species such as E. montana and E. niederleini, some chromosomes exhibited strong signals with the CRM probe, but others did not. The CRM probe used against the Eleocharis chromosomes was designed for the RNase H fraction of this family, regardless of the presence or absence of a chromodomain or CR motif, i.e. this probe was generic to the CRM clade. This could explain why the signals were also scattered and not specifically located in the holocentromeres. The holocentromeric organization observed in Rhynchospora (Marques et al., 2015) apparently does not occur in Eleocharis.

Studies on the role of LTR-RTs in the evolution of genomes and karyotype in holocentric species are still scarce when compared with organisms with monocentric chromosomes. This lack of information makes it difficult to establish evolutionary models for organisms with a large incidence of chromosomal rearrangements, such as the dysploidy commonly found in holocentrics of Cyperaceae. Nonetheless, this study presents conclusive evidence that rearranged chromosomes accumulated more LTR-RTs when compared with the others, as observed in E. niederleini. Likewise, our data showed that the polyploids with the highest Cx values have a higher density of FISH signals per chromosome than the diploids, for example in the comparison between E. montana and E. maculosa. This leads us to believe that the differential LTR-RT activity has played a secondary role in Eleocharis karyotype diversity when compared with ploidy variation.

This comparative analysis between TE activity and numerical chromosomal rearrangements is especially interesting in this case, because we compared holocentric species of
Eleocharis with different karyotype conditions: diploid, polypl oid and dysploid. Even though it is obvious that TE activity and polyploidy are the major sources of plant genomic variations (see Bennetzen, 2005; Vicent and Casacuberta, 2017), the current study showed differences in LTR-RT family fates and their contributions to DNA C-value fluctuations, associated with contrasting genomic histories. An example was the greater accumulation of FISH signals with the Athilal Tat and CRM probes of Gypsy in relation to the Copia signals, in different chromosomes and species, mainly in those chromosomes which are known to be rearranged. Hence, rapid chromosome change involving LTR-RTs seems to be an important mechanism responsible for genome differentiation in Eleocharis, subgenus Eleocharis. This contribution opens up new perspectives for studying species of other subgenera of Eleocharis, as well as species of other genera that are known to have karyotypes with numerous and small chromosomes, such as Cyperus, Carex and others, and this will help us improve our understanding of the processes involved in karyotype differentiation in Cyperaceae.

SUPPLEMENTARY DATA

Supplementary data are available online at https://academic.oup.com/aob and consist of the following. Table S1: information on genomic sequences. Table S2: information on the primers and LTR-RT families. Table S3: distribution of TEs in the genomes. Table S4: consensus sequences of the PCR products that were used as probes. Table S5: alignment of the PCR product sequences. Figure S1: data from flow cytometry. Figure S2: mitotic chromosomes Giemsa stained. Figure S3: electrophoresis gel with PCR products of the POL chain stretches. Figures S4–S9: FISH using retrotransposon probes separately for Copia and Gypsy families.

ACKNOWLEDGEMENTS

The authors thank the Brazilian agencies FINEP, Fundação Araucária, CNPq, CAPES and ProPPG-UEL for financial support, and the Laboratory of Molecular Markers and Plant Cytogenetics – UEL for their support in Sanger sequencing. The authors declare that they have no conflicts of interest in this article.

LITERATURE CITED

Arabidopsis Genome Initiative. 2000. Analysis of the genome sequence of the flowering plant Arabidopsis thaliana. Nature 408: 796–815.
Belyaev A, Raskina O, Nevo E. 2001. Chromosomal distribution of reverse transcriptase-containing retroelements in two Triteceae species. Chromosome Research 9: 129–136.
Bennett MD, Leitch IJ. 2011. Nuclear DNA amounts in angiosperms: targets, trends and tomorrow. Annals of Botany 107: 467–590.
Bennetzen JL. 2000. Transposable element contributions to plant gene and genome evolution. Plant Molecular Evolution 42: 251–269.
Bennetzen JL. 2005. Transposable elements, gene creation and genome rearrangement in flowering plants. Current Opinion in Genetics and Development 15: 1–7.
Bennetzen JL, Wang H. 2014. The contributions of transposable elements to the structure, function, and evolution of plant genomes. Annual Review of Plant Biology 65: 505–530.
Bozek M, Leitch AR, Leitch IJ, Záveská-Drábková L, Kota E. 2012. Chromosome and genome size variation in Luzula (Juncaceae), a genus with holocentric chromosomes. Botanical Journal of the Linnean Society 170: 529–541.
Bureš P, Zedek F. 2014. Holokinetid drive: centromere drive in chromosomes without centromeres. Evolution 68: 2412–2420.
Bureš P, Zedek F, Marková M. 2013. Holocentric chromosomes. In: Greilhuber J, Doležel J, Wendel J, eds. Plant genome diversity. Vienna: Springer, 181–208.
Chung KS, Weber JA, Hipp AL. 2011. Dynamics of chromosome number and genome size variation in a cytogenetically variable sedge (Carex scoparia var. scoparia, Cyperaceae). American Journal of Botany 98: 122–129.
Chung KS, Hipp AL, Roalson EH. 2012. Chromosome number evolves independently of genome size in a clade with nonlocalized centromeres (Carex: Cyperaceae). Evolution 66: 2708–2722.
Da Silva CRM, Gonzalez-Elizondo MS, Vanzela AL. 2005. Reduction of chromosome number in Eleocharis subarticulata (Cyperaceae) by multiple translocations. Botanical Journal of the Linnean Society 149: 457–464.
Da Silva CRM, Gonzalez-Elizondo MS, Vanzela ALL. 2008. Chromosome reduction in Eleocharis macalosa (Cyperaceae). Cytogenetic and Genome Research 122: 175–180.
Da Silva CRM, de Souza TB, Trevisan R, et al. 2017. Genome differentiation, natural hybridisation and taxonomic relationships among Eleocharis viridans, E. niederleinii and E. ramboana (Cyperaceae). Australian Systematic Botany 30: 183–195.
Devos KM. 2010. Grass genome organization and evolution. Current Opinion in Plant Biology 13: 139–145.
D’Hont A, Desenfeld F, Aury JM, et al. 2012. The banana (Musa acuminate) genome and the evolution of monocotyledonous plants. Nature 488: 213–217.
Dixit A, Ma KH, Ju WY, Cho EG, Park YJ. 2006. Reverse transcriptase domain sequences from Mungbean (Vigna radiata) LTR retrotransposons: sequence characterization and phylogenetic analysis. Plant Cell Reports 25: 100–111.
Doležel J, Greilhuber J, Suda J. 2007. Estimation of nuclear DNA content in plants using flow cytometry. Nature Protocols 2: 2233–2244.
Gaeta ML, Yuyama PM, Sartori D, Fungaro MHP, Vanzela ALL. 2010. Occurrence and chromosome distribution of retroelements and NUPT sequences in Coipaífera longodorsifl. Desf. (Caesalpinioideae). Chromosome Research 18: 515–524.
Grandbastien MA. 2015. LTR retrotransposons, handy hitchhikers of plant regulation and stress response. Biochimica et Biophysica Acta 1849: 403–416.
Greilhuber J. 1995. Chromosome of the monocotyledons (general aspects). In: Rudall, PJ, Cribb, PJ, Cutler, DF, Humphries, CJ, eds. Monocotyledons: systematics and evolution. Kew: Royal Botanic Gardens, 379–414.
Groover CE, Wendel JF. 2010. Recent insights into mechanisms of genome size change in plants. Journal of Botany 2010: article ID 382732. doi:10.1155/2010/382732.
Guerra M, Cabral G, Cuacos M, et al. 2010. Neocentrics and holokinetics (holocentrics): chromosomes out of the centromeric rules. Cytogenetic and Genome Research 129: 82–96.
Heckmann S, Macas J, Kumke K, et al. 2013. The holocentric species Luzula elegans shows interplay between centromere and large-scale genome organization. The Plant Journal 73: 555–565.
Heslop-Harrison JS, Schmidt T. 2007. Plant nuclear genome composition. Encyclopedia of Life Sciences doi: 10.1002/9780470015902.a0002014.
Heslop-Harrison JS, Schwarzacher T. 2011. Organisation of the plant genome in chromosomes. The Plant Journal 66: 18–33.
Hipp AL, Chung KS, Escudero M. 2013. Holocentric chromosomes. Encyclopedia of Genetics 3: 499–501.
Hoshino T. 1987. Karyomorphological studies on 6 taxa of Eleocharis in Japan. Bulletin of the Okayama University of Science 22: 305–312.
Houben A, Schroeder-Reiter E, Nagaki K, et al. 2007. CENH3 interacts with the centromeric retrotransposon Cereba and GC-rich satellites and locates to centromeric substructures in barley. Chromosoma 116: 275–283.
Jurka J, Kapitonov VV, Kohany O, Jurka MV. 2007. Repetitive sequences in complex genomes: structure and evolution. Annual Review of Genomics and Human Genetics 8: 241–259.
Kellogg EA, Bennetzen JL. 2004. The evolution of nuclear genome structure in seed plants. American Journal of Botany 91: 1709–1725.
Křivánková A, Kopeček D, Stočes Š, Doležel J, Hříbová E. 2017. Repetitive DNA: a versatile tool for karyotyping in Festuca pratensis Huds. Cyto genetic and Genome Research 151: 96–105.

Langdon T, Seago C, Mende M, et al. 2000. Retrotransposon evolution in diverse plant genomes. Genetics 156: 313–325.

Lee SI, Kim NS. 2014. Transposable elements and genome size variations in plants. Genomics and Informatics 12: 87–97.

Lisch D. 2013. How important are transposons for plant evolution? Nature Reviews Genetics 14: 49–61.

Llorens C, Muñoz-Pomer A, Bernad L, Botella H, Moya A. 2009. Network dynamics of eukaryotic LTR retroelements beyond phylogenetic trees. Biology Direct 4: 41. doi: 10.1186/1745-6150-4-41.

Lucero M, Castroviejo S. 1991. Agmatoploidy in Carex laevigata (Cyperaceae). Fusion and fission of chromosomes as the mechanism of cytot genetic evolution in Iberian populations. Plant Systematics and Evolution 177: 149–159.

Makarevitch I, Waters AJ, West PT, et al. 2015. Transposable elements contribute to activation of maize genes in response to abiotic stress. PLoS Genetics 11:e1004915.

Marques A, Pedrosa-Harand A. 2016. Holocentromere identity: from the typical mitotic linear structure to the great plasticity of meiotic holocentromeres. Chromosoma 125: 669–681.

Marques A, Ribeiro T, Neumann P, et al. 2015. Holocentromeres in Rhynchospora are associated with genome-wide centromere-specific repeat arrays interspersed among euchromatin. Proceedings of the National Academy of Sciences, USA 112: 13633–13638.

Matsumoto T, Wu J, Kanamori H, Jin Y. 2006. The map-based sequence of the rice genome. Nature 436: 793–800.

Meyers BC, Tingey SV, Morgante M. 2001. Abundance, distribution, and transcriptional activity of repetitive elements in the maize genome. Genome Research 11: 1660–1676.

Neumann P, Kobližková A, Navrátilová A, Macas J. 2006. Significant expansion of Vicia pannonica genome size mediated by amplification of a single type of giant retroelement. Genetics 173: 1047–1056.

Neumann P, Navrátilová A, Kobližková A, et al. 2011. Plant centromeric retrotransposons: a structural and cytogenetic perspective. Mobile DNA 2 4. doi: 10.1186/1759-8753-2-4.

Nunes RC, Orozo-Arias S, Crouzillat D, et al. 2018. Structure and distribution of centromeric retrotransposons at diploid and allotetraploid Coffea centromeric and pericentromeric regions. Frontiers in Plant Science 9: 175. doi: 10.3389/fpls.2018.00175.

Paterson AH, Bowers JE, Bruggmann R, et al. 2009. The Sorghum bicolor genome and the diversification of grasses. Nature 457: 551–556.

Paz RC, Kozaczek ME, Rosl HG, Andino NP, Sanchez-Puerta MV. 2017. Diversity, distribution and dynamics of full-length Copia and Gypsy LTR retroelements in Solanum lycopersicum. Genetica 145: 417–430.

Qiu F, Ungerer MC. 2018. Genomic abundance and transcriptional activity of diverse gypsy and copia long terminal repeat retrotransposons in three wild sunflower species. BMC Plant Biology 18: 6. doi: 10.1186/s12870-017-1223-z.

Ribeiro T, Marques A, Novák P, et al. 2016. Centromeric and non-centromeric satellite DNA organisation differs in holocentric Rhynchospora species. Chromosoma 126: 325–335.

Roalson EH. 2008. A synopsis of chromosome number variation in the Cyperaceae. Botanical Review 74: 209–393.

Rocha DM, Marques A, Andrade CG, et al. 2016. Developmental programmed cell death during asymmetric microsporogenesis in holocentric species of Rhynchospora (Cyperaceae). Journal of Experimental Botany 67: 5391–5401.

Sanmiguel P, Tikhonov A, Jin Y, et al. 1996. Nested retrotransposons in the intergenic regions of the maize genome. Science 274: 765–768.

Santos FC, Guyot R, Do Valle CB, et al. 2015. Chromosomal distribution and evolution of abundant retrotransposons in plants: gypsy elements in diploid and polyploid Bracharia forage grasses. Chromosome Research 23: 571–582.

Schnable PS, Ware D, Fulton RS, et al. 2009. The B73 maize genome: complexity, diversity, and dynamics. Science 326: 1112–1115.

Šmarda P, Bureš P. 2012. The variation of base composition in plant genomes. In: Wendel F, Greilhuber J, Doležel J, Leitch IJ, eds. Plant genome diversity. Vienna: Springer, 209–235.

Šmarda P, Bureš P, Horová I, et al. 2014. Ecological and evolutionary significance of genomic GC content diversity in monocots. Proceedings of the National Academy of Sciences, USA 15: E4096–E4102.

Tenaillon ML, Hufford MB, Gaut BS, Ross-Ibarra J. 2011. Genome size and transposable element content as determined by high-throughput sequencing in maize and Zea luxurians. Genome Biology and Evolution 3: 219–229.

Vanezha A.L, Colaco W. 2002. Mitotic and meiotic behavior of γ irradiated holocentric chromosomes of Rhynchospora pubera (Cyperaceae). Acta Scientiarum 24: 611–614.

Viscint CM, Casacuberta JM. 2017. Impact of transposable elements on polyploid plant genomes. Annals of Botany 2: 195–207.

Viscint CM, Suoniemi A, Ananthawat-Jónsson K, et al. 1999. Retrotransposon BARE-1 and its role in genome evolution in the genus Hordeum. The Plant Cell 11: 1769–1784.

Wicker T, Sabot F, Hua-Van A, et al. 2007. A unified classification system for eukaryotic transposable elements. Nature Reviews Genetics 8: 973–982.

Zedek F, Bureš P. 2018. Holocentric chromosomes: from tolerance to fragmentation to colonization of the land. Annals of Botany 121: 9–16.

Zedek F, Smerda J, Šmarda P, Bureš P. 2010. Correlated evolution of LTR retrotransposons and genome size in the genus Eleocharis. BMC Plant Biology 10: 265.