Numerical and karyomorphometric variation in *Eichhornia* Kunth (Pontederiaceae) species

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Abstract

The genus *Eichhornia* Kunth (Pontederiaceae) popularly known in Brazil as aguapé or Jacinto d'água in Portugal and Angola (water hyacinth) is represented by six species of aquatic, rhizomatous and floating plants, which occur preferably in slow stream rivers or freshwater ponds. Its species have great ecological importance and may serve as food for fish and aquatic herbivorous animals, besides having great importance in the disposal of industrial waste, being used as rivers and lakes anti-pollutant, removing pollutants such as heavy metals. This study aimed to characterize the species *Eichhornia crassipes*, *E. diversifolia*, *E. heterosperma* and *E. paniculata* through cytogenetic techniques to identify karyotype polymorphisms that contribute as cytological marks and to understand the genus evolution. karyotype details were described in this study, including the type of interphase nucleus, morphology and pattern of prophase condensation, chromosomes size, heterochromatin disposition and 45S and 5S rDNA sites. All investigated species showed symmetrical karyotypes with small metacentric chromosomes and interphase nuclei of areticulate and semi-reticulate type. Chromosome numbers range between $2n = 16$ to $2n = 32$ in *E. paniculata* and *E. crassipes*, respectively, $2n = 30$ in *E. heterosperma* and $2n = 28$ in *E. diversifolia*. Heterochromatin blocks with strong marking for chromomycyn (CMA^+) occurred in co-located terminal positions with 45S rDNA sites, with two signs in *E. diversifolia* and *E. heterosperma*, four in *E. paniculata* and six in *E. crassipes*. Two 5S rDNA sites were located in a chromosome pair in *E. paniculata* terminal region, and for the other species the location of the two sites was in the pericentromeric region. In the *E. heterosperma* species, the 5S rDNA was located in the largest chromosome pair. Chromosome evolution inferences for *Eichhornia* genus were discussed in this study, with emphasis on the events of inversion and disploidy, to explain the variation in ribosomal DNA sites and the non-collinearity of 5S rDNA sites.

Keywords: Chromosome banding. Disploidy. Inversion. FISH. CMA/DAPI. Water hyacinth.

Resumo

O gênero *Eichhornia* Kunth (Pontederiaceae), popularmente conhecido no Brasil como aguapé e como jacinto-d'água em Portugal e Angola (*water hyacinth*), é composto por seis espécies de plantas aquáticas, rizomatosas e flutuantes, que ocorrem preferencialmente em rios de correnteza lenta ou lagoas de água doce. Suas espécies possuem grande importância ecológica, podendo servir como alimento para peixes e animais herbívoros aquáticos, além de desempenharem um papel relevante na remoção de resíduos industriais, sendo utilizadas como agentes antipoluentes em rios e lagos, removendo poluentes como metais pesados. Este estudo teve como objetivo caracterizar as espécies *Eichhornia crassipes*, *E. diversifolia*, *E. heterosperma* e *E. paniculata* por meio de técnicas citogenéticas para identificar polimorfismos cariotípicos que possam servir como marcadores citológicos e contribuir para a compreensão da evolução do gênero. Foram descritos detalhes do cariótipo, incluindo o tipo de núcleo interfásico, morfologia e padrão de condensação na prófase, tamanho dos cromossomos, disposição da heterocromatina e sítios de DNAr 45S e 5S. Todas as espécies investigadas apresentaram cariótipos simétricos com cromossomos pequenos e metacêntricos, além de núcleos interfásicos do tipo reticulado e semi-reticulado. O número cromossômico variou de 2n = 16 em *E. paniculata* a 2n = 32 em *E. crassipes*, sendo 2n = 30 em *E. heterosperma* e 2n = 28 em *E. diversifolia*. Blocos de heterocromatina fortemente marcados pela cromomicina (CMA+) ocorreram em posições terminais co-localizadas com os sítios de DNAr 45S, com dois sinais em *E. diversifolia* e *E. heterosperma*, quatro em *E. paniculata* e seis em *E. crassipes*. Dois sítios de DNAr 5S foram localizados em um par cromossômico na região terminal de *E. paniculata*, enquanto para as demais espécies os dois sítios estavam na região pericentromérica. Em *E. heterosperma*, o DNAr 5S estava localizado no maior par cromossômico. Inferências sobre a evolução cromossômica no gênero *Eichhornia* foram discutidas neste estudo, com ênfase nos eventos de inversão e disploidia para explicar a variação nos sítios de DNA ribossomal e a não colinearidade dos sítios de DNAr 5S.

Palavras-chave: Bandeamento cromossômico. Disploidia. Inversão. FISH. CMA/DAPI. Aguapé.

Variación numérica y cariomorfométrica en especies de *Eichhornia* Kunth (Pontederiaceae)

Resumen

El género *Eichhornia* Kunth (Pontederiaceae), conocido popularmente en Brasil como aguapé y como jacinto de agua en Portugal y Angola (*water hyacinth*), está representado por seis especies de plantas acuáticas, rizomatosas y flotantes, que se encuentran preferentemente en ríos de corriente lenta o lagunas de agua dulce. Sus especies tienen una gran importancia ecológica, ya que pueden servir como alimento para peces y animales herbívoros acuáticos, además de desempeñar un papel importante en la eliminación de desechos industriales, siendo utilizadas como agentes antipolución en ríos y lagos para eliminar contaminantes como los metales pesados. Este estudio tuvo como objetivo caracterizar las especies *Eichhornia crassipes*, *E. diversifolia*, *E. heterosperma* y *E. paniculata* mediante técnicas citogenéticas para identificar polimorfismos cariotípicos que puedan servir como marcadores citológicos y contribuir a la comprensión de la evolución del género. Se describieron

detalles del cariotipo, incluyendo el tipo de núcleo en interfase, morfología y patrón de condensación en profase, tamaño de los cromosomas, disposición de la heterocromatina y sitios de ADN ribosomal 45S y 5S. Todas las especies investigadas presentaron cariotipos simétricos con cromosomas pequeños y metacéntricos, además de núcleos interfásicos de tipo reticulado y semi-reticulado. El número cromosómico varió de 2n = 16 en *E. paniculata* a 2n = 32 en *E. crassipes*, siendo 2n = 30 en *E. heterosperma* y 2n = 28 en *E. diversifolia*. Bloques de heterocromatina fuertemente marcados por cromomicina (CMA+) se encontraron en posiciones terminales colocalizadas con los sitios de ADN ribosomal 45S, con dos señales en *E. diversifolia* y *E. heterosperma*, cuatro en *E. paniculata* y seis en *E. crassipes*. Se localizaron dos sitios de ADN ribosomal 5S en un par cromosómico en la región terminal de *E. paniculata*, mientras que en las demás especies los dos sitios estaban en la región pericentromérica. En *E. heterosperma*, el ADN ribosomal 5S estaba ubicado en el par cromosómico más grande. En este estudio se discutieron inferencias sobre la evolución cromosómica en el género *Eichhornia*, con énfasis en los eventos de inversión y disploidía para explicar la variación en los sitios de ADN ribosomal y la no colinearidad de los sitios de ADN ribosomal 5S.

Palabras clave: Bandeo cromosómico. Disploidía. Inversión. FISH. CMA/DAPI. Jacinto de agua.

Introduction

The Pontederiaceae family is a small family of monocot plants, consisting of aquatic species with a wide geographic distribution. In 2018, the family was reclassified, indicating that *Eichhornia*, *Monochoria*, and *Pontederia* form a monophyletic group, leading to the inclusion of *Eichhornia* species within the *Pontederia* genus (Pellegrini et al. 2018). The *Eichhornia* Kunth (Pontederiaceae Kunth) genus comprises six species distributed predominantly in the Neotropics (Eckenwalder and Barrett 1986, Barrett and Graham 1997, Pellegrini et al. 2018). In Brazil, there is the presence of six species, of which four (*E. crassipes* (Mart.) Solms, *E. diversifolia* (Vahl) Urb., *E. heterosperma* Alexander and *E. paniculata* (Spreng.) Solms) are found in the Northeastern region (Amaral 2014).

Some species, such as *E. crassipes*, *E. azurea*, *E. paniculata* and *E. heteropserma* may multiply in an exaggerated way, covering most of the surface of rivers and freshwater ponds, causing impacts on aquatic ecosystems (Villabona-González et al. 2011; Ndimele et al. 2011). However, they may be useful for the ecological dynamics of other organisms, serving as a food source for fish and aquatic animals, in addition to absorbing heavy metals, cleaning up springs (Pott & Pott 2002; Joly 2002; Stanley et al. 2007).

Eichhornia has been widely investigated concerning its reproductive system by systematists through phylogeny, botany and molecular markers. Some representatives have heterostyly and size differences in flowered parts, in which the stamen and stigma have different lengths (Barrett 1988; Graham et al. 1998; Ness et al. 2011, Pellegrini et al. 2018). However, it has been little explored cytogenetically, as karyotypic data are scarce and refer only to chromosome number determination (Fedorov 1969; Goldblatt 1981, 1985; Eckenwalder & Barrett 1986; Goldblatt & Johnson 1990, 1991; Pedrosa et al. 1999).

In Pontederiaceae there is great variation among diploid chromosome numbers of $2n = 14$ to $2n = 80$ and basic chromosome numbers $x = 7$, 8 and 15, with numerical chromosomal variations, such as aneuploidy, polyploidy and disploidy being mentioned in the literature (Eckenwalder &

Barrett 1986; Leitch et al. 2010). For many taxonomists, chromosome count is the only used parameter due to being easy to observe and obtain, and thus it has been important in the study of intra- and interspecific relations and of botanical identification (Candan 2013; Weiss-Schneeweiss & Schneeweiss 2013).

Except for *E. crassipes* and *E. paniculata*, which had their interphase nuclei and chromosome morphology described by Pedrosa et al. (1999), no other mitotic cytogenetic parameter besides the counting of the number of chromosomes was conducted in the *Eichhornia* genus. The reason for the lack of data for this genus and for Pontederiaceae family as a whole may be linked to the small size of the chromosomes reported by Leitch et al. (2010), but may also be related to the lack of work with botanical description for the genus, preventing species identification and their geographic distribution in the continents, although the genus had a higher occurrence in the American continent (Eckenwalder & Barrett 1986, Pellegrini et al. 2018).

Haploid chromosome numbers described to the genus are $n = 8, 15, 16$, where $x = 8$ is considered the original family basic number (Barrett 1978). This author considered polyploid the species with haploid number higher than $n = 8$, but it is not known for sure how extensive variations in the genus chromosome numbers occurred. Therefore, in this study four species of *Eichhornia* were comparatively characterized by number, size, mitotic chromosomes morphology, heterochromatin distribution with CMA and DAPI fluorochromes and location of 5S and 45S rDNA regions. The main object was to detect numerical and structural variations and better understand the evolutionary patterns of karyotypes and associated cytogenetic events.

Material and Methods

Plant material

Eichhornia crassipes, *E. diversifolia*, *E. heterosperma* and *E. paniculata* species were kept in the experimental garden of the Laboratory of Plant Cytogenetics of Federal Rural University of Pernambuco (UFRPE), Pernambuco, Brazil, to collect root tips and observation flowers. Exsicates were deposited in the Professor Vasconcelos Sobrinho Herbarium, PEUFR of UFRPE.

Conventional staining

For mitotic analysis, root tips were collected and pre-treated with 8-hydroxyquinoline 2 mM for 24 h at 10 °C. The roots were fixed in Carnoy solution 3:1 (v/v) for 24 h at room temperature and stored in a freezer at - 20 °C. The conventional staining followed the protocol of Guerra and Souza (2002). Root tips were hydrolyzed in HCl 5N for 20 min, smashed in 45% acetic acid and then frozen in liquid nitrogen for the removal of glass slides. The slides were stained with 2% Giemsa for 10 min and mounted with Entellan (Merck).

CMA and DAPI staining

Preparations were made according to Schweizer and Ambros (1994), with the modifications of Guerra and Souza (2002). Root tips were washed twice in distilled water and digested in enzyme solution at 37°C, which contained 2% cellulase and 20 % pectinase, until the roots were soft. Subsequently, the roots were washed in distilled water and slides were prepared as described above for conventional staining. The best slides were kept for at least three days prior to staining.

Afterwards, they were stained with 0.5 mg/mL CMA_3 for 1 h and with 2µg/mL DAPI for 30 min. After that, they were mounted with glycerol/McIlvaine.

Fluorescent in situ hybridization - FISH

For slides preparation, the procedure was used as described for staining with CMA/DAPI. The D2 clone of *Lotus japonicus* with 500-bp (Pedrosa et al. 2002) and the R2 clone of *Arabidopsis thaliana* with 6.5-kb (Wanzenböck et al. 1997) were used as probes to locate 5S and 45S rDNA sites, respectively. For probes marking, DNA was isolated by mini-prep technique, using the Invisorb Kit and following manufacturer's recommendations. Probes were marked with Cy3-dUTP (Amersham) through Nick Translation with a Nick Translation Mix kit (Roche). FISH followed the protocol of Pedrosa et al. (2002) with modifications. The hybridization mixture consisted of: 50% formamide, 10% dextran sulfate, 2 x SSC and 2-5 ng/µl of probe. The denaturing process previously occurred with the probe for 5 min at 75°C and with the slides for 10 min at 75°C. After addition of the probe solution on the slides, this set was denatured again in the same conditions of time and temperature and *overnight* hybridized at 37°C. The slides were counterstained with DAPI (2 µg/mL) in Vectashield medium (Vector).

Image analysis and chromosome measurements

The interphase and metaphase complete nuclei obtained with conventional staining were captured in a Leica DM 2500 light microscope with Leica DFC 345FX camera via Leica CW4000 software. The classification of the interphase nucleus was made according to Guerra (1985b). Chromosomes measurement was based on 10 metaphases using the Micromeasure 3.3 version program. Idiograms were assembled and edited with the help of Microsoft Office PowerPoint program and the adopted nomenclature for chromosome morphology was the one suggested by Guerra (1988a). The images from CMA/DAPI banding and FISH were adjusted in brightness and contrast with the help of Paint Shop Pro 5 and Adobe Photoshop CS3 programs.

Results

The chromosome number varied between $2n = 16$ for *E. paniculata*, $2n = 28$ for *E. diversifolia*, $2n = 30$ for *E. heterosperma* and $2n = 32$ for *E. crassipes* (Figure 2). One to two pairs of satellites chromosomes were observed, which were more easily seen when the NOR were distended, as observed in *E. diversifolia* (Figures 2c, 3d and 3f) and *E. heterosperma* (Figure 3h).

The analysis of the decondensed chromatin structure allowed recognizing semi-reticulated type nuclei for *E. diversifolia* (Figure 2f) and *E. heterosperma* species, whose chromocenters were small and irregular, with light staining of the diffuse chromatin. In *E. crassipes* and *E. paniculata* (Figure 2e), areticulate type nuclei with slightly less stained diffuse chromatin and irregular and more stained chromocenters were observed in the different analyzed cells. The prophase condensation pattern was of proximal type for the four species, with a more intense staining being observed in the early condensed pericentromeric region, as shown in *E. paniculata* (Figure 2g). Chromosomes morphology was predominantly of metacentric type (Fig. 4 and Table 1). However, there were submetacentric chromosomes, such as in *E. crassipes*, which were not included by the Micromeasure software because it worked with means, and due to the high level of condensation of chromosomes, clear delineation of the centromere was impossible. Chromosome measurements have indicated

chromosomal gradual differences in size between the largest and the smallest chromosome in the species (Table 1), in addition to the clearly visible presence of very small chromosomes the metaphase (Figure 2). Although *E. heterosperma* had the largest chromosome complement, the chromosomes were not in the maximum state of condensation as in other species (Table 2). The average length of the chromosome sets of these species are presented in Table 2.

The technique that used the base-specific fluorochromes chromomycin and DAPI revealed for all species $CMA^+/DAPI^-$ bands or regions located in the terminal region of chromosomes (Figure 3). *E. paniculata* species showed four CMA^+ bands (Figure 3a); *E. diversifolia* showed two CMA^+ bands corresponding to the satellites (Figure 3d) and *E. heterosperma* showed two CMA positive distal bands CMA (Figure 3g). *E. crassipes* species showed a larger quantity of CMA bands, with a total of six terminals bands (Figure 3i).

The number and size of the observed bands in metaphase chromosomes were confirmed on interphase nuclei, which maintained a part of the condensed CMA heterochromatin. One exception, however, was *E. crassipes*, whose bands were observed more diffusely in interphase nuclei (Figure 3i).

In all species, 45S rDNA sites were located at the end of chromosomes, corresponding to the secondary constriction and CMA^+ bands. In *E. diversifolia* (Figure 3f) and *E. heterosperma* (Figure 3h), two hybridization signals were observed. In *E. paniculata* (Figure 3c) and *E. crassipes* (Figure 3l) were observed four and six co-located markings between CMA^+ blocks and 45S rDNA, respectively. The 5S rDNA sites were located in only one chromosome pair in all investigated species, but on different chromosomes than those with the 45S ribosomal DNA. However, there were differences in the location of 5S rDNA signals in homologous pairs. In *E. paniculata* (Figure 3b), the signal was terminal, with the two signals being of the same size. For the other species, the 5S rDNA signals were of pericentromeric position, having polymorphisms in relation to the probe signal size. The comparative ideogram of Figure 4 suggests possible fission-fusion events that may have contributed to karyotype differentiation along the speciation process.

Discussion

The structure of interphase nuclei in *Eichhornia* genus was of semi-reticulate and areticulate type. According to Guerra (2000), these nuclear types are usually found in karyotypes with small chromosomes that have early condensed heterochromatin or euchromatin in the proximal or subterminal region of chromosomes. Areticulate nuclei are also commonly observed in various genera, including *Costus* (Guerra 1988b), *Koelreuteria* (Urdampilleta et al. 2005), *Cuscuta* (García & Martín 2007) and *Pennisetum* (Fajardo et al. 2010), with chromocenters corresponding, in general, in number and size to CMA heterochromatin blocks (Guerra 1985a; Grif 2000). Although the nuclear structure in the interspecific level is generally constant (Guerra 1985b), there can be variations in tissues of the same species or between nuclei of different species of the same genus (Guerra 1987), as seen in *Passiflora* (Guerra 1985b) and *Cardiospermun* (Urdampilleta et al. 2012) and in *Tripogandra* species (Marques et al. 2010). Perhaps this may be a possible explanation for *E. paniculata* and *E. crassipes* having a half-reticulate nucleus, as previously shown by Pedrosa et al. (1999), and *E. diversifolia* and *E. heterosperma* having areticulate nucleus, as shown in this study.

Chromosome numbers in the species were relatively low, ranging from $2n = 16$ in *E. paniculata* to the double, $2n = 32$ in *E. crassipes*, with confirmed results from the observation of several complete

metaphase with well-spread chromosomes. This finding differs considerably from previous counts reported by Cook (1998), who observed $2n = 30$ and $2n = 58$. Perhaps this disagreement is related to counting or species identification errors. In the present study, only *E. heterosperma* had a compatible chromosome number with the basic number $n = 15$ found by Eckenwalder & Barrett (1986) and Barrett (1988), showing $2n = 30$ with the presence of two satellite chromosomes. In this present study, it was difficult to distinguish small chromosomes satellites with the only use of conventional staining, mainly because the secondary constriction was higher stretched. That information was only possible with the use of chromomycyn and FISH. Maybe that's the reason for the disagreement with the number found in the literature. Difficulty in chromosome count was also reported for the *Vellozia bahiana* and *V. glabra.* species. In the first case, there was disagreement with the chromosome number found and literature data; and for *V. glabra* the chromosome count was variable ($2n$ = 14-18), probably due to the presence of large satellites of similar size to the chromosome arms (Melo et al. 1997).

This study confirms the report of Leitch et al. (2010) of small to very small chromosomes for a family, since according to Guerra (2000), small chromosomes are smaller than 3µm, and the present study revealed a variation of 0.73 µm in *E. crassipes* to 2.94 µm for *E. heterosperma*. The chromosomes showed similar size for all studied subjects, not exceeding 2 µm of difference between the largest and the smallest chromosome of each species. Furthermore, centromeric indexes revealed metacentric chromosomes for all species. Even with the presence of submetacentric chromosomes (as seen in some metaphases of *E. crassipes*), these are reduced in number, not compromising the karyotype symmetry of *Eichhornia* genus.

The bands revealed by CMA/DAPI double staining were similar in all species, since they are present in the terminal regions with little variation for band size. Although equilocal bands are common in the proximal region of some species with small chromosomes, such as the Fabaceae family, for example (Guerra 2000), the results of this study also suggest the conservation of terminal bands in *Eichhornia*, since these blocks show the structural and functional similarity of these regions between studied species. The terminal position of heterochromatin is frequent in some plant groups with small chromosomes, such as *Manihot* (Carvalho & Guerra 2002), *Citrus* (Rutaceae) (Carvalho et al. 2005; Moraes et al.2007), Asteraceae (Torrell et al., 2003; Fregonezi et al. 2004; Moraes & Guerra 2010), among others.

The low heterochromatin amount suggests a plesiomorphic condition for the genus. According to Guerra et al. (2000), there is an apparent correlation between heterochromatin richness and apomorphic characters. Although *Eichhornia* and other Pontederiaceae family representatives have derived external characteristics, such as the presence of inflorescence, evolution does not involve all organs at the same time and many changes in the genome may not reflect in the morphological characters, or may simply be a response to biotic and abiotic factors in the environment. Furthermore, heterochromatin is mainly composed of satellite DNA (Kubis et al. 1998; Guerra 2004) with non-coding sequences (Hemleben et al. 2007), as well as transposable elements (Bennetzen 2000; Redi et al. 2001; Lippman et al. 2004; Slotkin & Martienssen 2007; Kejnovsky et al. 2012). These variable elements have a preference for specific locations of the chromosome where they have less deleterious effect (Kejnovsky et al. 2012), such as in heterochromatin regions, due to the fact that this genomic region have a high level of condensation.

The observations of this study showed low loci interspecies variation of 45S rDNA, which only varied from two to four sites, while the 5S rDNA remained constant in the number of two sites. A

small amount of the rDNA in the species may be related to the highly repetitive nature and the absence of allelic homologous recombination (Roa & Guerra 2012) and non-allelic (Pedrosa et al. 2006), with the possibility of errors during the replication of unequal translocations, what makes it vulnerable to the loss or gain of copies (Kobayashi et al. 2011).

Detection of 45S rDNA sites in the same position of CMA^+ heterochromatin confirms that only a fraction of rDNA copies are transcribed and that heterochromatin stabilizes repetitive sequences, because a high copy number in the genome would lead to instability (Kobayashi 2011). Furthermore, the position of 45S rDNA sites in the terminal region may be associated with the protection of telomeres and the occurrence of structural rearrangements without deleterious effects (Roa & Guerra 2012).

The chromosomal region encoding the 5S rRNA is variable between angiosperms (Wiche et al. 2011), and in this study the 5S rDNA sites were apparently preserved, because for each species only two signals on different chromosomes than those who had the 45S rDNA were observed. Usually, 5S and 45S rDNA vary among them in location and number. In some species, the change of its location suggests that structural rearrangements have occurred (Guerra 1988; Hamon et al. 2009), which may also be related to speciation (Heslop-Harrison 2000). In *Eichhornia*, loci position difference between diploid species and possible tetraploids may suggest the occurrence of rearrangements such as fusion and inversion. Moreover, the occurrence of a large chromosome pair in *E. heterosperma* may have arisen from the fusion of two chromosomes. On the other hand, the presence of 5S rDNA sites in the pericentromeric regions may be due to paracentric inversion of the terminal region to near the centromere.

Referring to the basic number of Pontederiaceae family (x = 8), Barrett (1988) considers tetraploid all *Eichhornia* species with more than $2n$ = 16. In the case of *E. diversifolia* and *E. heterosperma*, both with two 45S rDNA sites co-located with CMA^+, it seems to be, in fact, tetraploids that suffer descendent disploidy over time in their karyotypes. *E. crassipes* was considered an autopolyploid due to meiotic irregularities and frequency in the formation of multivalent (Isa et al. 2013). *E. crassipes* seems as an older polyploid derived from a representative diploid (*E. paniculata* or *E. paradoxa*). In general, a newer polyploid must have the double of bands present in the ancestor diploid (Roa & Guerra, 2012), but the reduction of these sequences, according to Weiss and Maluszynska (2000) may be characteristic of an old polyploid, that is, this species is probably diploiding (Leitch & Bennett 1997). The absence of duplicated 5S rDNA sites in *E. crassipes* is also suggestive of the formation of a paleopolyploid (Melo & Guerra 2003).

The combination of cytogenetic techniques was significant in characterizing the studied species. Karyotypic data revealed in this study are useful for understanding the differentiation and consequent interspecific evolution within *Eichhornia*. Species representative idiogram suggests the occurrence of chromosomal inversion events with terminal 5S rDNA site transfer, as noted in *E. paniculata* to the pericentromeric region that occurred in the other polyploidy species, which was followed by descending disploidy events. On the other hand, the occurrence of species with numbers that do not represent multiples of $x = 8$ can be explained by independent inversion and fusion events on different chromosomes, resulting in descending disploidy or fusion involving chromosomes that carry 5S rDNA sites, which are possibly numbered four in the species that were considered polyploid.

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Figure 1. Illustrative photographs of the species of the genus *Eichhornia*: *E. crassipes* (a, f), *E. paniculata* (b, c), E. *diversifolia* (d, e) e *E. heterosperma* (g, h).

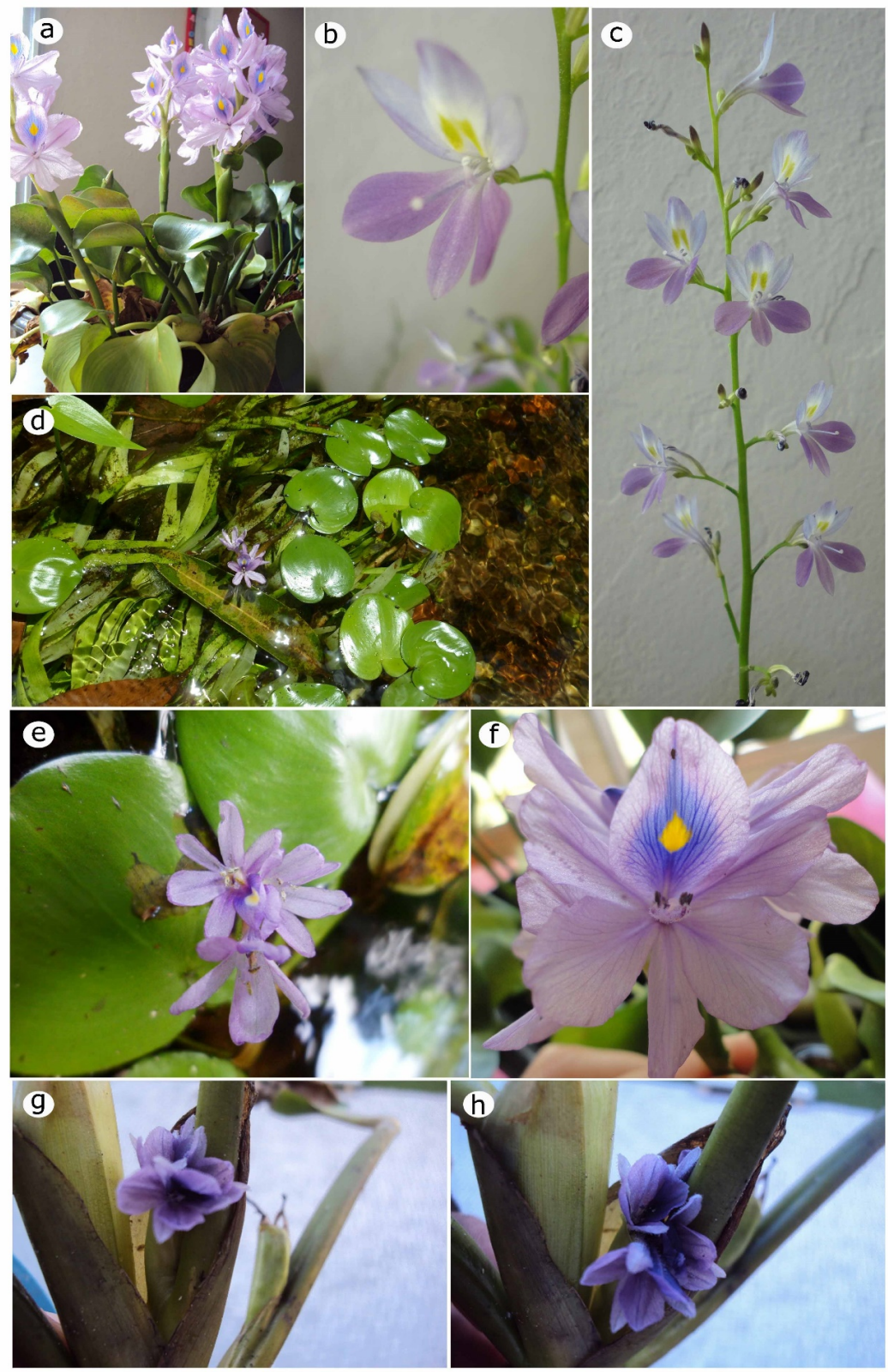


Figure 2. Conventional staining of chromosomes of the *Eichhornia* species. a) *E. crassipes* (2*n* = 32), b) *E. paniculata* (2*n* = 16), c) *E. diversifolia* (2*n* = 28), d) *E. heterosperma* (2*n* = 30), e and f) semi reticulated and reticulated nucleus of *E. paniculata* and *E. diversifolia* respectively and prophase chromosomes of the *E. paniculata.* The inserts show morphological aspects of the enlarged chromosomes. In (c) the insert shows a chromosome satellited with distended NOR and arrow indicates the satellited homologous chromosome. Bar represents 5μm.

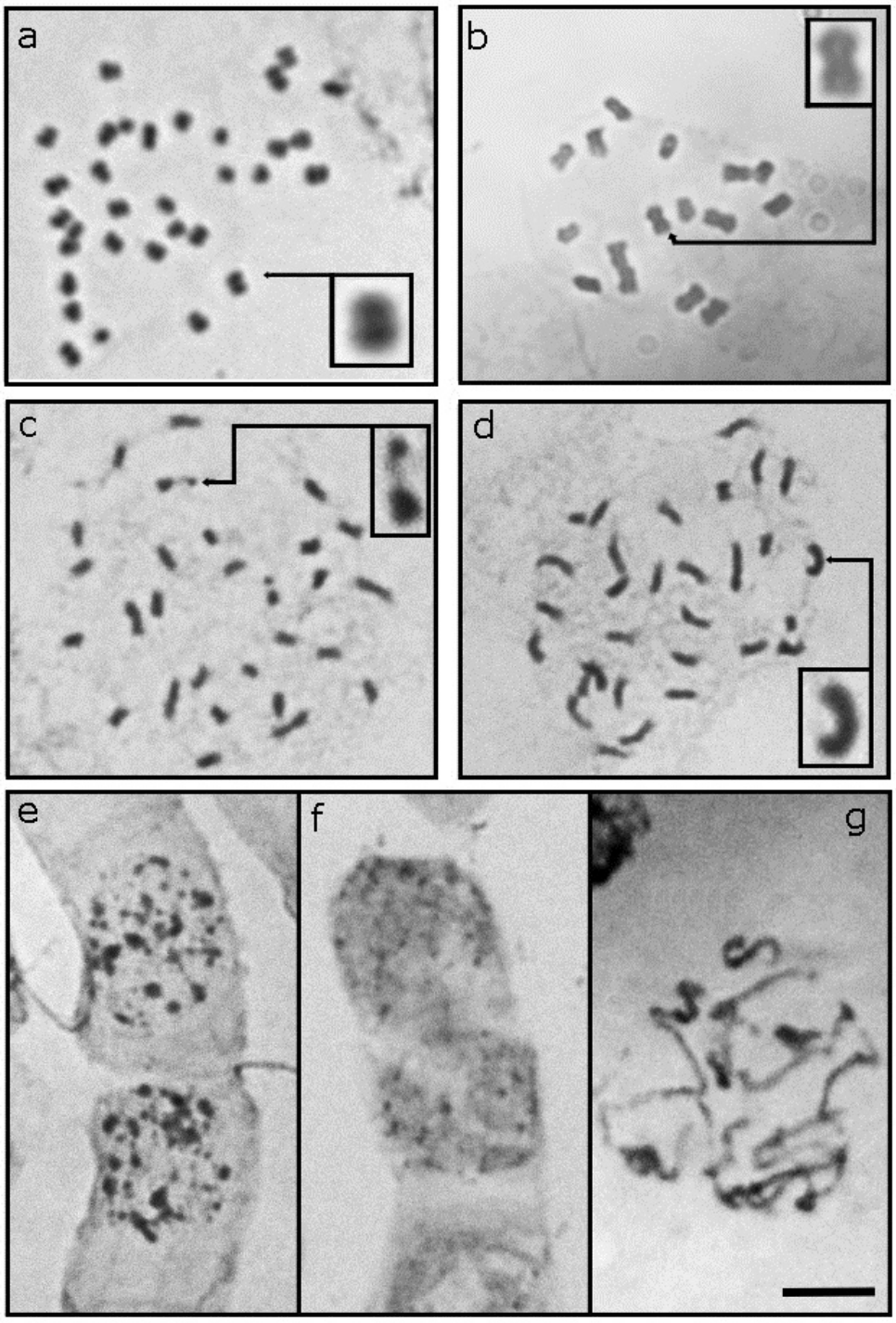


Figure 3. Heterochromatic bands CMA$^+$ and rDNA sites in chromosomes of *Eichornia*. a,b,c) *E. paniculata* $2n = 16$) four CMA$^+$ bands two major and two minor (a), two terminals sites of 5S rDNA (b), four sites of 45S rDNA terminals (c). d,e,f) *E. diversifolia* $2n = 28$, two CMA$^+$ bands (d), two 5S rDNA pericentromeric sites (e), two terminals sites of 45S rDNA (f). g,h,k) *E. heterosperma* $2n = 30$, two bands of CMA$^+$ (g), two terminal 45S rDNA sites (h), two sites of 5S rDNA in pericentromeric position (k) and i,j,l) *E. crassipes* $2n = 32$, six terminal bands CMA$^+$(i), two pericentromeric 5S rDNA sites (j), l) four terminals 45S rDNA. Arrows indicate CMA$^+$ bands. Arrow heads indicate 5S rDNA sites. Bars represent 5µm.

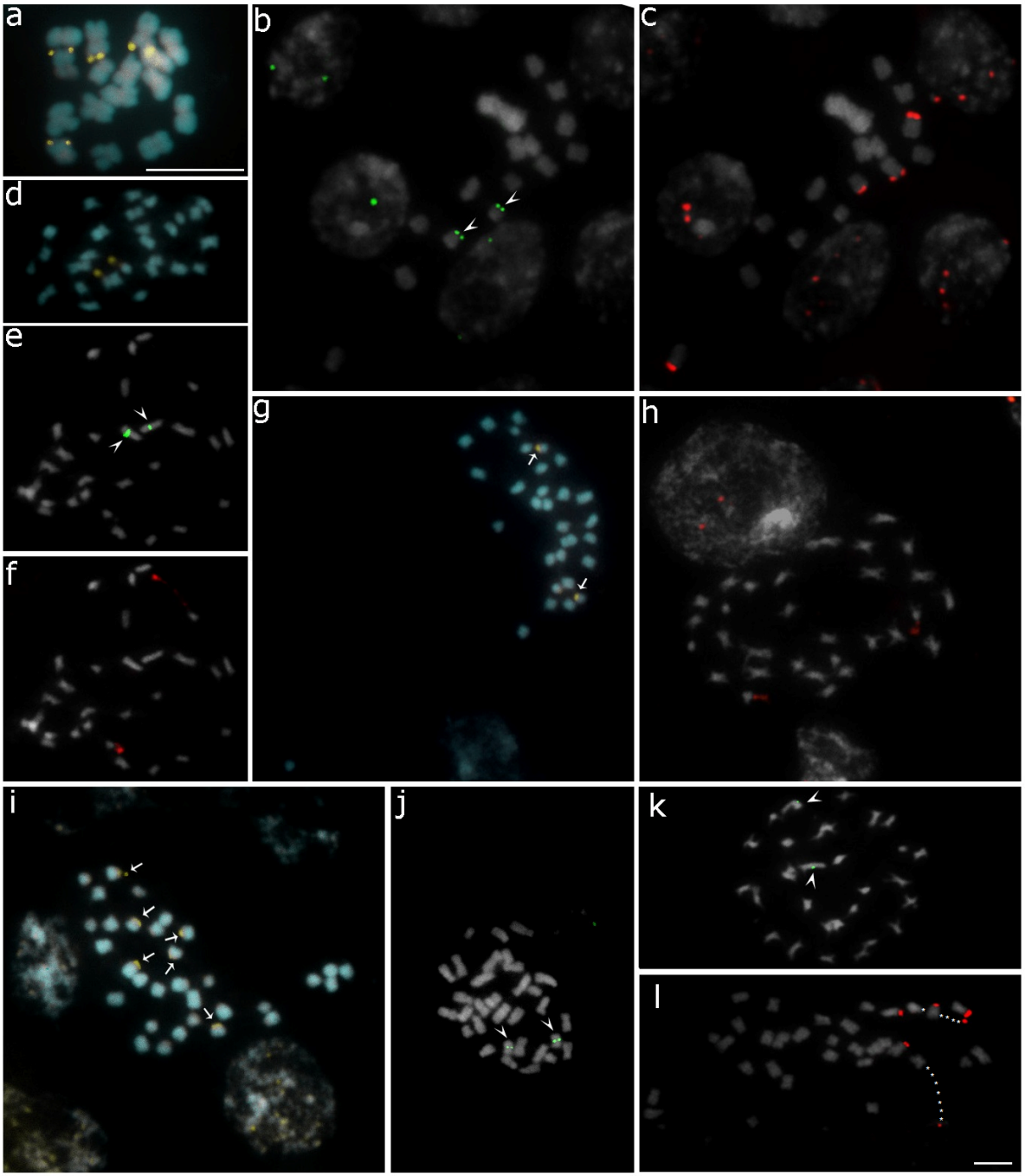
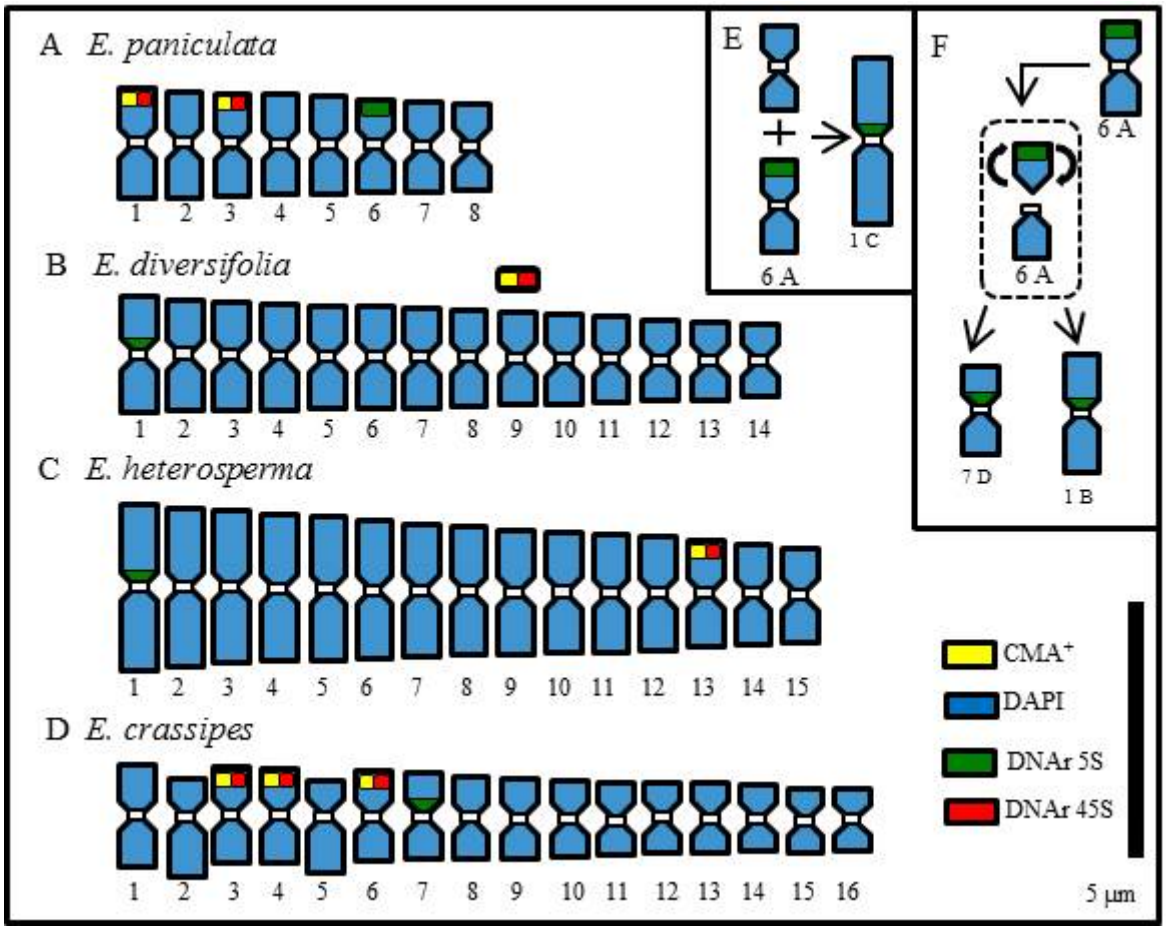


Figure 4. Schematic representation of the chromosomes of the *Eichhornia* species. *E. paniculata* (A), *E. diversifolia* (B), *E. heterosperma* (C) e *E. crassipes* (D). Highlighted the representation of possible chromosome fusion (E) in *E. heterosperma* and fission and then inversion in *E. diversifolia* (1B) and *E. crassipes (*7D).



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Table 1. Morphometric characterization of the species *Eichhornia*. PC (chromosome pair), CTC (total length of each chromosome), BL (long arm), BC (short arm), R (ratio), IC (centromeric index), TC (type of chromosome), M (metacentric). Amounts in µm

PC	*E. crassipes*					*E. heterosperma*					*E. diversifolia*					*E. paniculata*					TP
	CTC	BL	BC	R	IC	CTC	BL	BC	R	IC	CTC	BL	BC	R	IC	CTC	BL	BC	R	IC	
1	1,61±0,23	0,88±0,18	0,72±0,12	1,25	0,45	2,94±0,44	1,57±0,22	1,37±0,24	1,15	0,47	2,18±0,29	1,16±0,17	1,01±0,14	1,15	0,47	1,78±0,19	0,94±0,10	0,84±0,11	1,13	0,47	M
2	1,46±0,20	0,83±0,16	0,63±0,08	1,32	0,44	2,57±0,34	1,39±0,21	1,18±0,15	1,18	0,46	1,90±0,20	1,03±0,14	0,87±0,09	1,19	0,46	1,66±0,17	0,88±0,09	0,78±0,10	1,13	0,47	M
3	1,40±0,18	0,81±0,12	0,59±0,08	1,40	0,42	2,33±0,28	1,21±0,15	1,12±0,14	1,08	0,48	1,77±0,17	0,95±0,11	0,82±0,08	1,16	0,46	1,59±0,17	0,84±0,11	0,75±0,08	1,12	0,47	M
4	1,34±0,15	0,71±0,09	0,64±0,08	1,12	0,47	2,21±0,29	1,20±0,18	1,01±0,15	1,19	0,46	1,67±0,19	0,88±0,09	0,79±0,12	1,13	0,47	1,52±0,16	0,81±0,09	0,71±0,08	1,14	0,47	M
5	1,29±0,13	0,70±0,08	0,58±0,07	1,22	0,45	2,15±0,28	1,15±0,16	1,00±0,14	1,16	0,46	1,58±0,17	0,84±0,11	0,74±0,07	1,13	0,47	1,45±0,15	0,76±0,08	0,68±0,08	1,12	0,47	M
6	1,25±0,13	0,68±0,09	0,58±0,08	1,18	0,46	2,08±0,24	1,09±0,12	0,99±0,13	1,10	0,48	1,49±0,12	0,82±0,09	0,67±0,06	1,23	0,45	1,38±0,14	0,74±0,07	0,64±0,09	1,18	0,46	M
7	1,22±0,13	0,69±0,10	0,52±0,06	1,33	0,43	2,00±0,22	1,08±0,13	0,92±0,12	1,18	0,46	1,43±0,12	0,76±0,07	0,67±0,07	1,15	0,47	1,29±0,10	0,68±0,06	0,60±0,06	1,14	0,47	M
8	1,18±0,12	0,65±0,08	0,53±0,07	1,23	0,45	1,94±0,20	1,01±0,11	0,93±0,09	1,10	0,48	1,37±0,12	0,74±0,07	0,63±0,06	1,17	0,46	1,18±0,10	0,62±0,06	0,55±0,05	1,13	0,47	M
9	1,14±0,12	0,62±0,08	0,52±0,05	1,20	0,46	1,90±0,18	1,00±0,11	0,90±0,09	1,12	0,47	1,31±0,12	0,70±0,07	0,61±0,05	1,15	0,47						M
10	1,10±0,12	0,60±0,08	0,51±0,06	1,18	0,46	1,81±0,14	0,97±0,09	0,84±0,08	1,16	0,46	1,23±0,11	0,66±0,07	0,58±0,05	1,14	0,47						M

Table 1. Morphometric characterization of the species *Eichhornia*. PC (chromosome pair), CTC (total length of each chromosome), BL (long arm), BC (short arm), R (ratio), IC (centromeric index), TC (type of chromosome), M (metacentric). Amounts in µm

PC	*E. crassipes*					*E. heterosperma*					*E. diversifolia*					*E. paniculata*					TP
	CTC	BL	BC	R	IC	CTC	BL	BC	R	IC	CTC	BL	BC	R	IC	CTC	BL	BC	R	IC	
11	1,07±0,12	0,59±0,07	0,49±0,07	1,23	0,45	1,75±0,15	0,92±0,08	0,82±0,08	1,12	0,47	1,17±0,10	0,63±0,06	0,54±0,06	1,18	0,46						M
12	1,03±0,11	0,57±0,08	0,46±0,06	1,25	0,45	1,67±0,15	0,88±0,08	0,79±0,08	1,11	0,47	1,12±0,09	0,58±0,05	0,54±0,05	1,09	0,48						M
13	0,97±0,09	0,52±0,05	0,45±0,05	1,16	0,46	1,58±0,13	0,83±0,07	0,75±0,07	1,12	0,47	1,05±0,09	0,56±0,05	0,50±0,05	1,13	0,47						M
14	0,90±0,07	0,47±0,04	0,43±0,05	1,11	0,48	1,49±0,11	0,79±0,08	0,70±0,05	1,12	0,47	0,94±0,09	0,51±0,06	0,43±0,05	1,18	0,46						M
15	0,83±0,06	0,44±0,04	0,39±0,04	1,14	0,47	1,30±0,11	0,69±0,08	0,61±0,06	1,15	0,47											M
16	0,73±0,10	0,39±0,05	0,34±0,05	1,16	0,46																M

± Equivalent to the standard deviation

Table 2. List of species of the genus *Eichhornia* with synonyms, total chromatin length, chromosome numbers (of this study and previous counts) and references.

		Present study		Previous counts		
Especies	Synonyms [1]	2*n*	Total chromatin length in µm	*n*	2*n*	References
E. crassipes (Mart.) Solms	*Pontederia crassipes* Mart., *Pontederia crassipes* Roem. & Schult., *Pontederia elongata* Balf., *Eichhornia cordifolia* Gand., *Eichhornia crassicaulis* Schltr., *Eichhornia speciosa* Kunth, *Heteranthera formosa* Miq., *Piaropus crassipes* (Mart.) Raf., *Piaropus mesomelas* Raf., *Piaropus tricolor* Raf., *Pontederia azurea* Sw., *Pontederia crassicaulis* Schltr.,	32	154.84	9	32	Fedorov, 1969; Goldblatt, 1981; 1985; Goldblatt &Johnson, 1990; 1991; Pedrosa et al. 1999.
E. diversifolia (Vahl) Urb.	*Eichhornia natans* var. *pauciflora* (Seub.) Solms, *Eichhornia pauciflora* Seub., *Heteranthera cordata* Vahl, *Heteranthera diversifolia* Vahl, *Heteranthera grandiflora* Klotzsch, *Piaropus diversifolius* (Vahl) P. Wilson	28	168.99	15		Eckenwalder & Barrett, 1986.
E. heterosperma Alexander	*Eichhornia venezuelensis* Velásquez	30	248.44	15		Eckenwalder & Barrett, 1986.
E. paniculata (Spreng.) Solms	*Cabanisia martiana* (Seub.) Schltdl., *Pontederia paniculata* Spreng., *Eichhornia cordifolia* A. Rich., *Eichhornia martiana* Seub., *Eichhornia meyeri* A. G. Schulz, *Eichhornia tricolor* Seub., *Eichhornia martiusiana* (Roem. & Schult.) Walp., *Piaropus paniculatus* (Spreng.) Small, *Pontederia tricolor* C. Mart. ex Seub., *Pontederia martiana* Seub., *Pontederia martiusiana* Roem. & Schult..	16	98.94	-	16	Fedorov, 1969; Pedrosa et al. 1999.

[1] Synonyms according to the Missouri Botanical Garden