Cytogenetic analyses as clarifying tools for taxonomy of the genus *Callisia* Loefl. (Commelinaceae)

Análisis citogenéticos como herramienta para esclarecer la taxonomía del género *Callisia* Loefl. (Commelinaceae)

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ABSTRACT

Chromosome numbers, karyotypes and meiotic behaviour in 10 populations belonging to two species of *Callisia* Loefl. from Northeast Argentina have been studied. *Callisia repens* showed a basic number of $x = 6$ and that of *C. monandra* was $x = 7$. Both species were diploids and this is the first report of the chromosome numbers for Argentinean populations: *C. repens*, $2n = 2x = 12$ and *C. monandra* $2n = 2x = 14$. The karyotype descriptions of *C. repens* (2 m + 2 sm + 6 st + 2 t) and *C. monandra* (6 m + 4 sm + 4 st) are also reported here for the first time for both species. Chromosomes behaved regularly at meiosis, pair as bivalents and produced viable pollen in the *Callisia* accessions studied. Chiasmata frequency and distribution are reported and this meiotic analysis constituted a novelty for each species. The information provided in this paper is useful for the cytogenetics diagnosis of the genus and the subtribe and contribute to understand relationships between taxa.

Keywords: Basic chromosome number, karyotype, microsporogenesis, chiasma.

RESUMEN

Se han analizado los números cromosómicos, los cariotipos y el comportamiento meiótico en 10 poblaciones pertenecientes a dos especies de *Callisia* Loefl. provenientes del Nordeste de Argentina. *Callisia repens* mostró un número básico $x = 6$ mientras que el de *C. monandra* fue $x = 7$. Ambas especies fueron diploides y éste es el primer reporte de los números cromosómicos para poblaciones Argentinas: *C. repens*, $2n = 2x = 12$ y *C. monandra* $2n = 2x = 14$. Las descripciones cariotípicas de *C. repens* (2 m + 2 sm + 6 st + 2 t) y *C. monandra* (6 m + 4 sm + 4 st) también han sido analizadas por primera vez para ambas especies. En meiosis, los cromosomas se comportaron regularmente, se aparearon como bivalentes y produjeron polen viable en ambas especies de *Callisia* estudiadas. Se analizó la frecuencia y distribución de quiasmas y este análisis meiótico también constituye una novedad para cada especie. La información que se provee en este trabajo es útil para la diagnosis citogenética del género y la subtribu así como para contribuir a la comprensión de las relaciones entre los taxa.

Palabras clave: Número cromosómico básico, cariotipo, microesporogénesis, quiasma.

INTRODUCTION

Callisia Loefl. comprises about 20 species from Southwest USA and Tropical America, of which occur in Mexico (Faden 1998, Hunt 1983). In Argentina just two are native: *Callisia repens* L. and *C. monandra* Schult. & Schult.f. This genus is included into subtribe Tradescantiinae which has undergone taxonomic reorganization because some species do not fit into the diagnostic characters of genera that compose. The most important modification in the relations between taxa of Commelinaceae was proposed by Hunt (1986), which included some of the species of Tradescantiinae into the genus *Callisia*, expanding and establishing five different sections. These sections were established according to morphological and karyological features (Hunt 1986). *Callisia repens* belongs to section *Callisia* that includes ten Neotropical species, with a basic number $x = 6$. Only four species have been karyologically analyzed: *C. fragrans* (Lindl.) Woodson ($2n = 12$), *C. gentlei* Matuda ($2n = 12$), *C. soconuscensis* Matuda ($2n = 12$), and *C. repens* $2n = 12, 24$). *Callisia repens* is distributed from Mexico and The Indies to the South of Brazil, Paraguay and North of Argentina. In Argentina, this species was collected in Tucumán, Misiones and Entre Ríos (Bacigalupo 1964). *Callisia monandra* is included in section *Leptocallisia*, whose six species are distributed from Florida, Texas, México and Tropical America, and have a basic number $x = 7$ (Tucker 1989). *Callisia monandra* is distributed in North Misiones

(Argentina), South Mexico, The Indies and Central America (Bacigalupo 1964, 1996).

Previous karyotype analysis showed that *Callisia* species vary considerably in the number and morphology of their chromosomes and chromosome numbers of 15 species have been published, where diploid, polyploid and aneuploid cytotypes were found, with 13 different somatic chromosome numbers, going from 2n = 12 to 2n = 72 (Faden 1998). Most of the species recorded so far have at least a diploid cytotype with 2n = 12 (60%) or 2n = 14 (27%) chromosomes, 40% of the species have polyploid cytotypes and 20% have both diploid and polyploid cytotypes; some species show an aneuploid (25%) plus the euploid cytotype (Jones & Jopling 1972, Faden 1998).

Regarding the ancestral basic chromosome number of *Callisia*, Handlos (1970) and Jones & Jopling (1972) have proposed x = 6 (ca. 70% of the species recorded), although there have been found species with x = 7 and x = 8. *Callisia* species showed asymmetrical and unimodal karyotypes skewed with large chromosomes and most of the species can be recognized by its distinctive karyotype morphology (Jones & Jopling 1972). The karyotype formulas from a few of these species are known, some of which have been meiotically analyzed. However the chromosome information from populations of Argentina is not available yet and, a more detailed cytogenetic analysis is needed to the understanding of the relationships within the genus.

The aims of this work were to study the chromosome numbers, karyotypes and meiotic behaviour of *Callisia* repens and *C. monandra* in order to provide new useful tools in order to circumscribe genus *Callisia* which, despite extensive anatomical, morphological and molecular level studies, is still taxonomically confused.

MATERIAL AND METHODS

Ten populations of *Callisia repens* and *C. monandra* native from Northeast Argentina were studied (Table I). Voucher specimens were deposited at the herbarium of the Universidad Nacional de Misiones (MNES), and duplicates at Instituto de Botánica del Nordeste (CTES) and Instituto de Botánica Darwinion (SI) herbaria.

Meiotic studies were carried out in young floral buds without fixation, or fixed in absolute ethanol : glacial acetic acid (3 : 1) for at least 12 h at 4 ºC and then stored in 70% ethanol (Grabiele *et al*. 2005). Bivalent and chiasma frequencies were estimated by analysis of 30 pollen mother cells (PMCs) at diakinesis or metaphase I for each species. PMCs were stained with 2% aceto–carmine and pollen stainability was estimated by a carmine–glycerine (1 : 1) staining procedure and at least 1000 grains for each species were considered. Permanent slides were made using Venetian Turpentine solution as a mounting medium.

Mitotic studies were performed in root tips pretreated with 0.002 M solution of 8–hydroxyquinoline during 4 hat laboratory temperature, fixed in absolute ethanol: glacial acetic acid (3:1) for at least 12 h at 4 °C and stained according to the Feulgen's technique (acid hydrolysis in 1N HCl for 10 min at 60 °C followed by staining with Schiff's reagent). The meristems were macerated in a drop of 2% aceto-orcein and then squashed. Permanent slides were made using euparal as a mounting medium.

For karyotype description the chromosomes were arranged in groups according to the position of the centromere (median, m; submedian, sm; subtelocentric, st; telocentric, t) and in order of decreasing size in each class. Chromosome nomenclature followed Levan *et al*. (1964). At least ten best metaphases were selected for making idiograms.

The lengths of chromosome arms were measured on drawings made with a camera lucida (2600). Karyotype asymmetry was estimated using the Mean Centromeric Asymmetry (MCA) and Coefficien of Variation (CVCL) of Peruzzi & Eroğlu (2013), as well as the categories of Stebbins (1971).

RESULTS

All the ten populations analyzed of both *Callisia* species were diploids. However, whereas *C. repens* showed 2n = 2x = 12 chromosomes in the eight populations analyzed (Fig. 1, Table II), *Callisia monandra* showed 2n = 2x = 14 chromosomes in the two populations studied (Fig. 2, Table II). The eight populations of *C. repens* showed the karyotype formula 2 m + 2 sm + 6 st + 2 t (Fig. 5, Table II), whereas the two samples analyzed of *C. monandra* exhibited a karyotype formula which consisted in six metacentric, four submetacentric, and four telocentric chromosomes (6 m + 4 sm + 4 st) (Fig. 6, Table II). The total length of the chromosome complement for *C. repens* was 59.76 μm and that for *C. monandra* was 42.27 μm (Table II). The average chromosome length was 4.98 μm for *C. repens* and 3.02 μm for *C. monandra* (Table II). The range of variation in the average chromosome length was between 6.90 μm (metacentric, m) and 3.74 μm (telocentric, t) for *C. repens*, and 3.93 μm and 2.22 μm (both submetacentric, sm) for *C. monandra* (Table II). Considering CVCL and R indexes, *C. repens* showed a bimodal karyotype, whereas *C. monandra* showed a unimodal karyotype, since the values were CVCL: 0.25 and R: 1.84 for *C. repens* and CVCL: 0.19 and R: 1.77 for *C. monandra* (Table II). The mean centromeric index for *C. repens* (22.36) fell into the subtelocentric category (Table II), since most of their chromosomes are subtelocentric. For *C. monandra* the mean centromeric index (33.30) fit into the submetacentric group (Table II). The intrachromosomal asymmetry indexes MCA (0.68) and r>2 (0.66) indicate that *C. repens* karyotype is asymmetrical (Table II). The same indexes for *C. monandra*, MCA (0.45) and r>2 (0.57) show that this karyotype is asymmetric (Table II). Both *Callisia* species analyzed belong to the category 3A of Stebbins (1971) (Table II).

TABLE I. Chromosome numbers (2n, n), provenance and geographical coordinates of the analyzed samples of *Callisia repens* and *C. monandra*.

TABLA I. Números cromosómicos (2n, n), procedencia y coordenadas geográficas de las muestras analizadas de *Callisia repens* y *C. monandra*.

| SPECIES	2 n	n	ORIGIN AND COLLECTION NUMBER	LATITUDE	LONGITUDE
Callisia repens	12	6	ARGENTINA, Misiones, Dept. Capital, Posadas. MarG–12 (MNES, CTES)	27°24'20"S	55°53'15"O
	12	6	ARGENTINA, Misiones, Dept. Candelaria, Parque Teyú Cuaré. MarG–19 (MNES, CTES, SI)	27°16'55"S	55°35'43"O
	12	6	ARGENTINA, Misiones, Dept. Iguazú, Cataratas del Iguazú. MarG– 21 (MNES)	25°41'43"S	54°27'20"O
	12	6	ARGENTINA, Misiones, Dept. Capital, Posadas. MG–12 (MNES)	27°25'25"S	55°54'24"O
	12	6	ARGENTINA, Misiones, Dept. Capital, Posadas. MG–30 (MNES)	27°23'43"S	55°57'10"O
	12	6	ARGENTINA, Misiones, Dept. Candelaria, Parque Teyú Cuaré. MG–39 (MNES)	27°16'47"S	55°35'37"O
	12	6	ARGENTINA, Misiones, Dept. Candelaria, Osununú. MG–40 (MNES)	27°16'50"S	55°34'58"O
	12	6	ARGENTINA, Corrientes, Dept. Ituzaingó, Garapé. MG–66 (MNES)	27°35'37"S	56°18'25"O
Callisia monandra					
	14	7	ARGENTINA, Misiones, Dept. Iguazú, Cataratas del Iguazú. MarG–26 (MNES)	25°40'46"S	54°27'20"O
	14	7	ARGENTINA, Misiones, Dept. Iguazú, Cataratas del Iguazú. MarG–68 (MNES)	25°42'29"S	54°26'20"O

MarG= Marina Grabiele, MG= Mauro Grabiele.

The microsporogenesis of the two diploid *Callisia* species analyzed was normal and the meiotic behaviour was regular. In *Callisia repens* the chromosomes were associated forming almost exclusively six bivalents (97.21%) (Fig. 3, Table III), whereas in *C. monandra* the chromosomes were associated forming exclusively seven bivalents (100%) at diakinesis and metaphase I (Fig. 4, Table III), being their gametic chromosome numbers n = 6 and n = 7, respectively. In both taxa studied the meiotic statistics analysis was slightly similar. These values are presented for the first time for these two Commelinaceae species. The diakinesis and metaphase I pollen mother cells (PMCs) showed that *C. repens* and *C. monandra* had mainly ring bivalents (84.46% and 60%, respectively) and similar mean chiasmata frequencies per cell (10.92 and 12.44, respectively) (Table III). The chiasmata were mostly distal and only rarely interstitial, *C. repens*: 84.34% and *C. monandra*: 83.92% (Table III). The average of chiasmata per bivalent was high (*C. repens*: 1.85 chiasmata / bivalent and *C. monandra*: 1.78 chiasmata / bivalent). In all PMCs of *C. repens* where chromosomes associated forming six bivalents, five were always closed with two chiasmata, often distal but also interstitial sometimes, one chiasma in each chromosome arm; whereas the pair consisting of the telocentric bivalent was always open, showing a single chiasma on the long arm. The recombination index (IR) affected by the gametic number (n = 6; n = 7) and the average of chiasmata by pollen mother cell (PMC) (*C. repens*: 10.92 and *C. monandra*: 12.44) was high for the two species analyzed (*C. repens*: IR = 6 + 10.92 = 16.92; *C. monandra*: RI = 7 + 12.44 = 19.44). Both *Callisia* species have high stainability of pollen grains (*C. repens*: 92.76% and *C. monandra*: 84.66%) (Table III) and in natural conditions showed high seed production.

DISCUSSION

In this paper the chromosome numbers, karyotype structure and meiotic behaviour in 10 populations belonging to *Callisia repens* and *Callisia monandra* (Commelinaceae) from North–Eastern Argentina were studied. No inter–population variability was detected among the studied species.

Callisia repens is a weed present from Mexico and West Indies to Argentina (Hunt 1986). It is found in forest margins, moist banks, rocks, roadsides and waste grounds (Hunt 1983, 1994). The results presented in this paper showed that *C. repens* is diploid with 2n = 2x = 12 chromosomes constituting these results the first record of chromosome number for all these populations from Argentina. These results are consistent with those cited by Romeu–Pitrez *et al*. (2001) who found the same chromosome number in North–Eastern Brazilian populations. Jones & Jopling (1972) also cited *C. repens* with 2n = 12 chromosomes for populations from Mexico and Venezuela. Anderson & Sax (1936), Sax (1932), Hunt (1994), Guervin & LeCoq (1966) also cited 2n = 12 for populations of South America and others undefined localities. According to Tucker (1989) and Owens (1981) a tetraploid cytotype with 2n = 24 chromosomes for *C. repens* existed in Mexico populations. Lewis *et al*. (1967) also reported 2n =14 chromosomes for *C. repens*; however, the results presented here and most of previous papers indicate that 2n = 12 is the most common cytotype for this species.

Callisia monandra is the species with more southern extension range within the section *Leptocallisia*, and is distributed from Mexico and The Indies to South America, reaching North-Eastern Argentina. In this work, *Callisia monandra* showed to be a diploid species with 2n = 2x = 14 chromosomes. These results
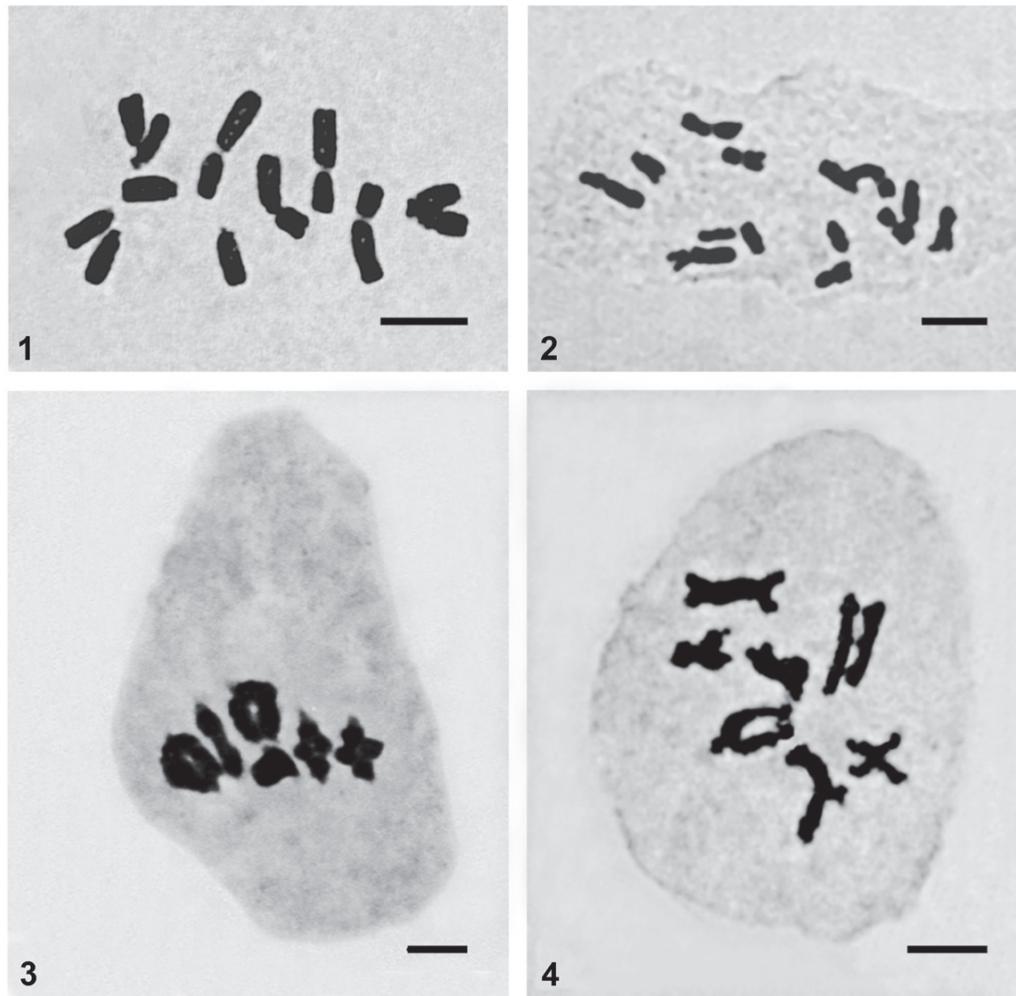
Figuras 1-4. Cromosomas somáticos (1, 2) y células madres del polen con cromosomas meióticos (3, 4). 1, *Callisia repens* (2n = 2x = 12). 2, *Callisia monandra* (2n = 2x = 14). 3, *Callisia repens*, metafase I con 6 bivalentes. 4, *Callisia monandra*, diacinesis con 7 bivalentes. Escala = 5 µm.

Figures 1-4. Somatic chromosomes (1, 2) and pollen mother cells with meiotic chromosomes (3, 4). 1, *Callisia repens* (2n = 2x = 12). 2, *Callisia monandra* (2n = 2x = 14). 3, *Callisia repens*, metaphase I with 6 bivalents. 4, *Callisia monandra*, diakinesis with 7 bivalents. Scale bars = 5 µm.

constitute the first record of chromosome number for these southernmost populations from Argentina. The chromosome number 2n = 14 exhibited here is consistent with those cited by Jones & Jopling (1972, as *Aploleia monandra* (Sw.) H.E.Moore), who counted 2n = 14 chromosomes in populations from Mexico, while Romeu-Pitrez *et al*. (2001) obtained the same chromosome number for one Brazilian population.

In *Callisia* the diploid species are predominant, considering that it was recorded 2n = 12, 14 and 16 chromosomes in *C. fragrans*, *C. macdougallii* Miranda, *C. elegans* Alexander ex H.E.Moore, *C. soconuscensis* Matuda, *C. multiflora* (M.Martens & Galeotti) Standl., *C. laui* (D.R.Hunt) D.R.Hunt, *C. gentlei* Matuda, *C. warszewicziana* (Kunth & Bouché) D.R.Hunt, *C. tehuantepecana* Matuda,

C. filiformis M.Martens & Galeotti) D.R.Hunt, *C. graminea* (Small) G.C.Tucker, *C. rosea* (Vent.) D.R.Hunt, *C. cordifolia* (Sw.) E.S.Anderson & Woodson, *C. monandra* and *C. repens*, and only a few are known to be tetraploid and hexaploid species (Darlington 1929, Anderson & Sax 1936, Sax 1932, Giles 1942, Guervin & Lecoq 1965, 1966, Heitz 1968, 1969, Handlos 1970, Jones & Jopling 1972, Begum & Zaman 1980, Alam & Sharma 1984, Weryszko-Chmielewska 1988, Beltrao & Guerra 1990, Hunt 1994, Singh 1995). In the present analysis the 10 studied populations of *Callisia repens* (2n = 12) and *C. monandra* (2n = 14) were diploid.

The karyotype formula of *Callisia repens* consisted in 2 metacentric, 2 submetacentric, 6 subtelocentric and 2 telocentric chromosomes, constituting these results the first

karyotype description for the species, where morphometric parameters of the karyotype were performed. Anderson & Sax (1936) mentioned that *Callisia repens* has six pairs of medium sized chromosomes, of which two of them exhibited submedian constrictions whereas the other four have terminal or subterminal constrictions, however, these authors did not carry out any statistical measurements to arrive at such conclusions. Both samples of *Callisia monandra* analyzed exhibited a karyotype formula which consisted in six metacentric, four submetacentric, and four telocentric chromosomes. In previous studies in *C. monandra*, no descriptions of the karyotype were carried out considering the analysis of morphometric parameters. Therefore, the karyotype formula from both *Callisia* species presented here, are not possible to being compared with other sources. In this sense, all the karyotype features presented in this paper for *Callisia repens* and *C.*

monandra constituted a novelty for the species.

Although the two *Callisia* species analyzed in the present work are slightly morphologically similar, mainly when young specimens are being compared (Faden 1998), cytologically, *Callisia repens* and *C. monandra*, can be distinguished by chromosome numbers and their karyotype formulae.

Centromere positions as well as comparative chromosome size were used as criteria of karyotype symmetry in some plant species, especially in Commelinaceae; and bimodality, a clear-cut separation into larger and smaller chromosomes within a complement (Jones & Jopling 1972), can be seen in only a few genera of this family (Jones & Jopling 1972, Grabiele *et al*. 2012). Symmetrical complements are rare in the family and most of the genera have karyotypes which are decidedly asymmetrical, though to varying degrees (Jones & Jopling

Table II. Karyotype parameters in *Callisia* species. Total chromosome length (TCL); mean chromosome length (c); maximum and minimum chromosome length (c max, c min); mean centromeric index (i); proportion of chromosome pairs with arm ratio > 2 (r > 2); largest/smallest chromosome ratio (R); ratio of shortest/longest pair (S/L); Mean Centromeric Asymmetry (MCA); Coefficient of Variation (CVCL). Chromosome abbreviations: m, metacentric; sm, submetacentric; st, subtelocentric; t, telocentric.

Tabla II. Parámetros cariotípicos en las especies de *Callisia*. Longitud total del complemento (LTC); longitud cromosómica media (c); longitud cromosómica máxima y mínima (c max, c min); índice centromérico medio (i); proporción de pares cromosómicos con relación entre brazos > 2 (r > 2); relación entre la longitud del par mayor y menor del complemento (R); entre la longitud del par más corto y más largo (S/L); e índice de asimetría centromérica (MCA) y coeficiente de variación (CVCL). Abreviatura de los cromosomas: m, metacéntrico; sm, submetacéntrico; st, subtelocéntrico; t, telocéntrico.

	C. repens	*C. monandra*
2n	12	14
x	6	7
Karyotype formula	2 m + 2 sm + 6 st + 2 t	6 m + 4 sm + 4 st
TCL	59.76 μm	42.27 μm
c	4.98 μm	3.02 μm
c max	6.90 μm	3.93 μm
c min	3.74 μm	2.22 μm
i	22.36	33.30
MCA	0.68	0.45
CVCL	0.25	0.19
R	1.84	1.77
r>2	0.66	0.57
S/L	0.54	0.61
Stebbin's category	3A	3A

Table III. Meiotic chromosome association at diakinesis and metaphase I of *Callisia repens* and *C. monandra*.

Tabla III. Asociación cromosómica meiótica en diacinesis y metafase I de *Callisia repens* y *C. monandra*.

	C. repens	*C. monandra*
Bivalents per cell ± SE		
Rings	0.92 ± 0.06	2.78 ± 0.14
Rods	5.00 ± 0.00	4.22 ± 0.14
Total	5.92	7
Chiasmata per cell ± SE		
Interstitials	1.71 ± 0.26	2 ± 0.23
Distals	9.21 ± 0.28	10.44 ± 0.26
Total	10.92 ± 0.06	12.44 ± 0.29
Chiasmata per bivalent ± SE	1.85 ± 0.01	1.78 ± 0.04
Pollen stainability (%)	92.76	84.66
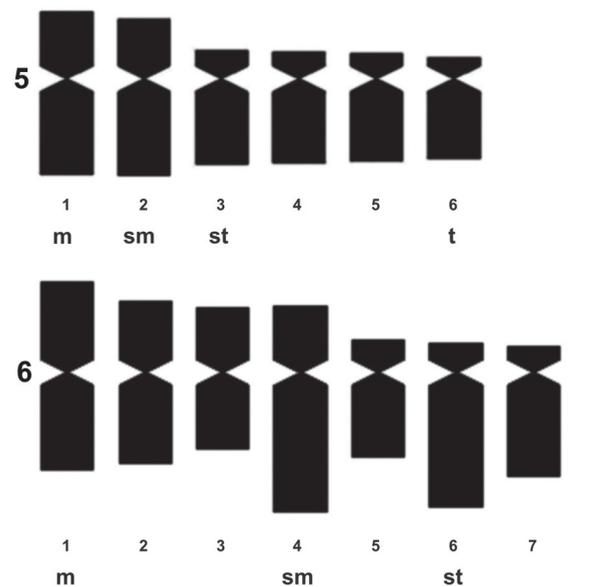
FIGURES 5-6. Idiograms. 5, *Callisia repens* (2n = 2 m + 2 sm + 6 st + 2 t). 6, *Callisia monandra* (2n = 6 m + 4 sm + 4 st). Scale bars = 1 μm.

FIGURAS 5-6. Idiogramas. 5, *Callisia repens* (2n = 2 m + 2 sm + 6 st + 2 t). 6, *Callisia monandra* (2n = 6 m + 4 sm + 4 st). Escala = 1 μm.

1972). In this sense, both taxa of the same genus analyzed here showed some differences regarding to this. While *C. repens* showed a bimodal asymmetrical karyotype, the karyotype of *C. monandra* was shown to be unimodal and asymmetrical. Bimodal karyotypes were demonstrated for *Tripogandra* species, other member of Commelinaceae familiy (Grabiele *et al*. 2012). This congruence is not a surprise, because *Callisia* is included into subtribe *Tradescantiinae* Rohweder together with *Tripogandra* and *Tradescantia* (Rohweder 1956). Mean values obtained for the shortest/longest pair ratio (S/L) and the Coefficient of Variation (CVCL) indicate that there is little variation in the sizes of the chromosomes in *C. monandra*, so the greatest variation is that for *C. repens* karyotype.

According to the Mean Centromeric Asymmetry (MCA), *Callisia monandra* indicated trend of metacentric chromosomes (as shown by the results of karyotype studied), whereas in *C. repens*, the MCA value was higher than in *C. monandra*, which is according to the highest number of subtelocentric and telocentric chromosomes than to the number of metacentric and submetacentric chromosomes. The karyotype of *C. repens* was the most asymmetrical of the two species analyzed, and exhibited the greatest variation in length among the chromosomes. The differences between the chromosome arms are moderate in *C. monandra* while in *C. repens* tends to be from moderate to high. Some moderate degrees of karyotype asymmetry and gradual differences in chromosome sizes were also shown in *Commelina* species, another member of the Commelinaceae family (Grabiele *et al*. 2005).

The results obtained from the analysis of the meiotic behaviour are given for the first time for *Callisia monandra*, whereas the chiasmata frequency and distribution constitute a novelty for *C. repens* and *C. monandra*. The regular behaviour in the PMCs of *C. repens* confirmed that the basic number of the species is x = 6 and these data are consistent with those of Jones & Jopling (1972), who also proposed x = 6 as the ancestral basic chromosome number for the entire genus *Callisia*. Handlos (1970) and Anderson & Sax (1936) also proposed the ancestral basic chromosome number x = 6 for the genus. However, in *Callisia monandra*, both the exclusive association of seven bivalents in the PMCs and the regular behaviour, together with previous information (Jones & Jopling 1972, which mentioned this species under the name *Aploleia monandra*), confirmed that the basic number of this species is x = 7.

Chromosomal characteristics such as size and volume chromosomal and quantity of DNA, allow inferring about the karyotype evolution of *Callisia*. Guervin *et al*. (1975) observed a polyploid series with 2n = 12, 24 and 48 for *C. repens*, *C. multiflora* and *C. insignis*, respectively. The increase in chromosome number corresponded to a reduction in the chromosome size, which was related to a trade–off associated with polyploidy. Bergamo (2003), analyzing *Callisia* and *Tripogandra* species suggested an ancestral basic number x = 8, currently conserved in *C. warszewicziana* and *Tripogandra*.

The clades of section *Callisia* (x = 6) and one other that group most species of section *Leptocallisia* (x = 7) have undergone independent dysploid reductions (Bergamo 2003). Another dysploid reduction would have occurred in *C. gracilis* (x = 7) of section *Leptocallisia*, which appears in the clade of *Tripogandra* demonstrating that the genus is polyphyletic. Thereby, according to Bergamo (2003) in the genus, the chromosomal loss would be more common than chromosomal gain.

The *Callisia* species studied here are clearly based on x = 6 (*C. repens*) and x = 7 (*C. monandra*), the two most common basic chromosome numbers in the genus. Hunt (1986) considering the morphological features and the chromosome numbers divided the species of *Callisia* into five sections; *C. monandra* along with 5 other species belongs to section *Leptocallisia*, which species are characterized by having a basic chromosome number x = 7. The remaining sections have a basic number x = 6, as section *Callisia*, which includes *Callisia repens*; with the exception of sections *Brachyphylla* (x = 6, 8) and *Hadrodemas* (x = 8). Thereby, the gametic number n = 7 confirms the basic number x = 7 proposed for *C. monandra* (Jones & Jopling 1972, as *Aploleia monandra*), which is probably derived from x = 6, since x = 6 is the most represented basic chromosome number of the genus (Anderson & Sax 1936, Handlos 1970, Jones & Jopling 1972). Furthermore, the observation of a regular meiotic chromosomal behaviour and the exclusive association of 7 bivalents in all the PMCs analyzed do not provide conclusive data on the origin of this basic number. Therefore, probably the karyotype of *C. monandra* is derivative with respect to the ancestral karyotype considered for the genus, and the higher occurrence of metacentric chromosomes could be explained by chromosomal fission phenomena followed by translocations, something that was previously proposed for the genus *Gibasis*, also belonging to Commelinaceae (Kenton 1981). Also, the direction of the dysploidy, can only be explained when these numbers are plotted against a robust phylogenetic tree. However, more detailed analysis of the karyotype through classical and molecular cytogenetics are necessary in *C. monandra* and the closest species with x = 6, 8 to resolve the origin of x = 7.

Callisia repens and *Callisia monandra* exhibit sympatric distribution and reach the southernmost area of the geographical range of the whole genus. They have been also morphologically confused (Faden 1998). Therefore, the detailed cytogenetic analysis presented here is of great importance for solving taxonomic problems between these two species; and further, in future studies it would be interesting to include karyotype analysis of all species of the genus *Callisia*. In this sense, the knowledge of apparently simple cytogenetic characteristics of a species, such as chromosome number, basic number, meiotic behaviour of chromosomes, the gametic number and fertility of individuals, can contribute to better understand the complex patterns of morphological variation and to help define taxonomic boundaries between species.

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APPENDIX: Quantitative parameters of chromosomes. s: mean short arm length, l: mean long arm length, c: mean chromosome length, i: mean centromeric index, SE: standard error.

APÉNDICE: Parámetros cuantitativos de los cromosomas: s: longitud promedio del brazo corto, l: longitud promedio del brazo largo, c: longitud promedio cromosómica, i: índice centromérico, SE: error estándar.

PAIR	s (µm) ± SE	l (µm) ± SE	c (µm) ± SE	i	TYPE
Callisia repens					
1	2.75 ± 0.03	4.15 ± 0.07	6.90 ± 0.08	39.85	m
2	2.33 ± 0.02	4.21 ± 0.08	6.54 ± 0.09	35.62	sm
3	0.86 ± 0.05	3.48 ± 0.03	4.34 ± 0.07	19.81	st
4	0.58 ± 0.00	3.61 ± 0.05	4.19 ± 0.05	13.84	st
5	0.62 ± 0.02	3.54 ± 0.07	4.16 ± 0.09	14.90	st
6	0.38 ± 0.00	3.36 ± 0.05	3.74 ± 0.06	10.16	t
Callisia monandra					
1	1.73 ± 0.06	1.86 ± 0.10	3.59 ± 0.15	48.26	m
2	1.36 ± 0.10	1.76 ± 0.03	3.12 ± 0.10	43.52	m
3	1.21 ± 0.07	1.46 ± 0.10	2.67 ± 0.16	45.32	m
4	1.25 ± 0.07	2.68 ± 0.15	3.93 ± 0.16	31.78	sm
5	0.61 ± 0.04	1.62 ± 0.08	2.22 ± 0.12	27.27	sm
6	0.54 ± 0.04	2.58 ± 0.15	3.12 ± 0.14	17.28	st
7	0.49 ± 0.01	2.00 ± 0.13	2.49 ± 0.12	19.69	st