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Cytotaxonomy of *Tripogandra diuretica* and *Tripogandra glandulosa* (Commelinaceae) from NE Argentina

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Abstract

Karyotype features and microsporogenesis of *Tripogandra diuretica* (Mart.) Handlos and *Tripogandra glandulosa* (Seub.) Rohw. from 15 NE Argentina accessions are analysed via classical cytogenetics, CMA/DA/DAPI and Ag-NOR. The karyotype of *T. glandulosa* ($2n = 2x = 16$; $2m + 6sm + 8st$) is bimodal ($A_2 = 0.37$; $R = 2.65$) and asymmetrical ($A_1 = 0.63$; $i = 26.06$; $r > 2 = 0.88$) (3B-Stebbins) with a genome size of 33.28 μm; two pairs are satellited, CMA+ and NOR-actives. Another four CMA+ loci and DAPI+ universal centromeric heterochromatin occur. Microsporogenesis is normal (pollen viability short stamens = 97.8%; large = 96.7%), meiotic behaviour is regular and chromosomes pair as 8II. The karyotype of *T. diuretica* ($2n = 8x = 64$; $8m + 24sm + 32st$) is bimodal ($A_2 = 0.27$; $R = 2.54$) and asymmetrical ($A_1 = 0.60$; $i = 27.32$; $r > 2 = 0.88$) (3B-Stebbins); additionally, two pairs are satellited. Microsporogenesis is normal (short stamens = 77.8%; large = 68.5%), except for micronuclei and low pollen viability in some accessions. Meiotic behaviour is regular with 32II, acting as a cytological diploid, however the arrangement of the haploid karyotype suggests that *T. diuretica*, which also exhibits a reduction of the genome size (18.58 μm), constitutes an auto-octoploid taxa. Additionally, both species share cytological features common to karyotype orthoselection.

Keywords: *Chromosomes, cytogenetics, evolution, taxonomy,* Tripogandra

Introduction

Tripogandra Raf. is a neotropical genus with ca. 22 species (Faden 1998) characterised at historical, morphological, geographical and systematical levels by Handlos (1975). Regarding the karyology of *Tripogandra*, chromosome numbers of 14 species have been reported, with 15 different numbers ranging from $2n = 14$ (Zaman et al. 1979) to $2n = 76$ (Jones & Jopling 1972); the most common are $2n = 16$ and $2n = 32$. In addition, several basic chromosome numbers have been proposed for this genus, $x = 7$, 8, 13 and 21 (Handlos 1970a, 1975; Jones & Jopling 1972; Zaman et al. 1979) but $x = 8$ is universal (12/14 species); moreover, seven different ploidy levels, ranging from diploid to probably $9x$, have been found, with polyploidy estimated to reach ca. 79% of the chromosomally analysed taxa. The karyotypes of *Tripogandra* are known to be bimodal, asymmetrical, both with large and small metacentric and telocentric chromosomes (Jones & Jopling 1972; Handlos 1975). Meiotic behaviour analyses are few in this genus, but some contributions were made by Celarier (1955), Handlos (1970a) and Zaman et al. (1979).

In the north-eastern region of Argentina, two native, morphologically distinct species of *Tripogandra* occur: *Tripogandra diuretica* (Mart.) Handlos and *Tripogandra glandulosa* (Seub.) Rohw. They are also found in southern Brazil, Bolivia, Paraguay and Uruguay, and occur in humid soils and near streams or rivers (Bacigalupo 1964, 1967, 1996; Handlos 1975), sometimes in coexistence. *T. diuretica* as defined by Handlos (1975) is almost karyologically unknown since only two reports about its chromosome number exist (Jones & Jopling 1972; Handlos 1975). In turn, *T. glandulosa* has been chromosomally counted many times, and Zaman et al. (1979) reported the karyotype and the meiotic behaviour of a plant material described as *Tripogandra pflanzii* (G. Bruckn.) Rohw., a synonym of the former taxon.

In the present work, we report the chromosome numbers, describe the main karyotypic features and

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analyse in detail the microsporogenesis of *T. diuretica* and *T. glandulosa* from natural populations of NE Argentina, thereby delineating the cytogenetic profile for both taxa, and contributing to the knowledge of the karyological evolution of *Tripogandra*.

Materials and methods

The source of the species chromosomally studied here is presented in Table I. Voucher specimens were deposited at the herbarium of the Universidad Nacional de Misiones (MNES), and some duplicates at the herbaria of the Instituto de Botánica del Nordeste (CTES) and the Instituto de Botánica Darwinion (SI).

Protocols for mitotic pretreatment and fixation of root tip cells, fixation of floral buds, chromosome staining, karyotype description (chromosome numbers, morphology and ordering, nomenclature, satellites, asymmetry), meiotic behaviour and pollen grain fertility analysis, as well as the acquisition and management of photomicrographs, in addition to the idiogram assembly, are those described in Grabiele et al. (2005, 2009). Karyotype nomenclature follows Levan et al. (1964).

Enzyme maceration of root tips and slide preparation for differential chromosome staining procedures followed Moscone et al. (1996). In such cases, the metaphase chromosomes were observed and photographed with visible light or epifluorescence plus the appropriate filter sets under a Leica DMLS microscope coupled with the Moticam 1000 image management system.

Ag-NOR staining to reveal the active nucleolar organiser regions (NORs) in metaphase chromosomes was carried out as described by Stack et al. (1991) with few modifications.

Fluorescent chromosome banding analysis to reveal the type and distribution of constitutive heterochromatic regions using the fluorochromes chromomycin A3, distamycin A and 4′- 6-diamidino-2-phenylindole (CMA/DA/DAPI) was done according to the procedures described in Moscone et al. (1996) and Paciolla et al. (2010).

Table I. Chromosome numbers (n, $2n$), locality and geographical coordinates, voucher specimens and herbaria of *Tripogandra* species studied.

Tripogandra diuretica (Mart.) Handlos, $n = 32$, 2, $n = 64$
Misiones Province, Capital Department, Posadas, 27°24′S, 55°53′W. MG-22. MNES, CTES, SI
Misiones Province, Capital Department, Garupá, 27°27′S, 55°49′W. MG-78. MNES
Misiones Province, San Ignacio Department, Teyú Cuaré, 27°17′S, 55°34′W. MG-38. MNES, SI
Misiones Province, San Ignacio Department, Osununú, 27°16′S, 55°34′W. MG-83. MNES
Misiones Province, San Ignacio Department, Santo Pipó, 27°06′S, 55°26′W. MG-44. MNES, CTES, SI
Misiones Province, Montecarlo Department, Montecarlo, 26°33′S, 54°46′W. MG-80. MNES
Misiones Province, L. G. San Martín Department, Puerto Rico, 26°48′S, 55°01′W MG-81. MNES
Misiones Province, Iguazú Department, Iguazú Falls, 25°41′S, 54°26′W. MG-82. MNES
Misiones Province, Apóstoles Department, San José, 27°46′S, 55°45′W. MG-77. MNES
Corrientes Province, Ituzaingó Department, Garapé, 27°36′S, 56°22′W. MG-92. MNES, SI
Tripogandra glandulosa (Seub.) Rohw., $n = 8$, $2n = 16$
Misiones Province, Capital Department, Posadas, 27°24′S, 55°53′W. MG-75. MNES
Misiones Province, Apóstoles Department, San José, 27°46′S, 55°45′W. MG-76. MNES
Misiones Province, Candelaria Department, Campo San Juan, 27°24′S, 55°36′W. MG-79. MNES
Misiones Province, Candelaria Department, Profundidad, 27°33′S, 55°42′W. MG-91, MNES
Chaco Province, San Fernando Department, near Resistencia, 27°20′S, 58°58′W. MG-29. MNES, CTES

Note: Collector: MG = Mauro Grabiele; n = gametic chromosome number; $2n$ = sporophytic chromosome number; Herbaria: MNES = Universidad Nacional de Misiones, Argentina; CTES = Instituto de Botánica del Nordeste, Argentina; SI = Instituto de Botánica Darwinion, Argentina.

Results

Fifteen natural populations from NE Argentina belonging to *T. diuretica* and *T. glandulosa* were chromosomally studied (Table I; Figure 1). The meiosis I chromosome associations and fertility analysis, the karyotype parameters, in addition to the quantitative parameters of chromosomes, are summarised in Tables II–IV, respectively. In addition, the somatic and the meiotic chromosomes subjected to different cytological approaches are shown in Figure 2. The idiogram for each taxon is shown in Figure 3.

T. diuretica is octoploid with $2n = 8x = 64$, and its karyotype is composed of 8 metacentric, 24 submetacentric and 32 subtelocentric chromosomes ($8m + 24sm + 32st$). Two chromosome pairs (5, *sm*, and 25, *st*) have a terminal macrosatellite in the short arm. The total chromosome length is 148.64 μm (18.58 μm per genome) and the mean chromosome length is 2.32 μm, ranging from 1.60 (*sm*) to 4.06 μm (*st*). The karyotype of *T. diuretica* is bimodal due to the presence of small and large chromosomes, despite a continual variation, also indicated by the interchromosomal asymmetry index (A_2) of Romero Zarco (1986) whose value is 0.27, and the longest/shortest chromosome pair ratio (R) of Stebbins (1971) whose value is 2.54. Most of the chromosomes of *T. diuretica* have a low centromeric index with a mean (i) of 27.32, which points to a tendency towards submetacentric–subtelocentric values. This, in conjunction with the intrachromosomal asymme-

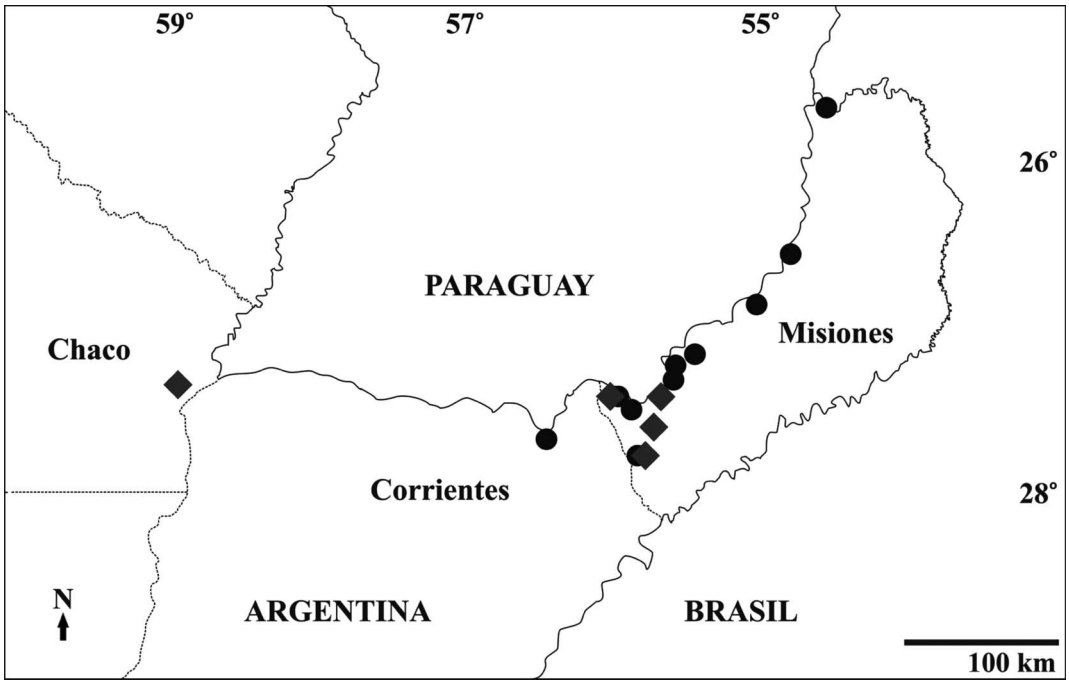

Figure 1. Map distribution of the natural populations of both species of *Tripogandra* collected in this study. ● correspond to *T. diuretica* and ■ to *T. glandulosa*.

Table II. Meiotic chromosome behaviour at diakinesis and metaphase I and pollen grains stainability of *Tripogandra* species studied.

	Tripogandra diuretica	*Tripogandra glandulosa*
Bivalents per cell ± SE	32	8
Rings	26.7 ± 0.24 (83.4%)	6.2 ± 0.21 (77.5%)
Rods	5.3 ± 0.23 (16.6%)	1.8 ± 0.21 (22.5%)
Chiasmata per cell ± SE	59.0 ± 0.20	14.3 ± 0.23
Interstitials	7.3 ± 0.25 (12.4%)	2.0 ± 0.20 (14.0%)
Distals	51.7 ± 0.24 (87.6%)	12.3 ± 0.34 (86.0%)
Chiasmata per bivalents ± SE (Q/II)	1.84 ± 0.03	1.79 ± 0.05
Recombination index (RI)	91.0	22.3
Excess chiasmata frequency (EC)	27.0	6.3
Pollen stainability in short stamens	77.8%	97.8%
Pollen stainability in large stamens	68.5%	96.7%

Note: SE = Standard error.

try index A_1 of Romero Zarco (1986) (0.60) and the proportion of chromosome pairs with arm ratio >2 of Stebbins (1971) (0.88), emphasises the asymmetry of the karyotype, which belongs to Stebbin's category 3B.

For *T. glandulosa*, $2n = 2x = 16$ and a karyotype formula of $2m + 6sm + 8st$ was found. The total chromosome length is 66.56 μm, with 33.28 μm per genome, and the mean chromosome length is 4.16 μm, ranging from 2.81 (*sm*) to 7.45 μm (*m*). The karyotype of *T. glandulosa* is bimodal ($A_2 = 0.37$; $R = 2.65$) and asymmetrical ($i = 26.06$; $A_1 = 0.63$; $r > 2 = 0.88$); it also belongs to Stebbin's category 3B.

Owing to the fact that conventional staining did not reveal the presence of satellites, fluorescent chromosome banding (CMA/DA/DAPI) and Ag-NOR staining were performed in *T. glandulosa*. Two chromosome pairs (2, *sm*, and 5, *st*) carry a terminal macrosatellite in the short arm; it is CMA-enhanced (CMA+), thus composed of CG-rich constitutive heterochromatin, and active NORs of 18S–25S rDNA. It is worth to mention that the satellite of pair No. 2 sometimes occurs as a tandemly arranged one, according to Battaglia's (1955) nomenclature. Additionally, the short arms of the chromosome pairs Nos. 4 (*sm*) and 8 (*st*) also have CMA+ bands, and the whole chromosome set displays DAPI-enhanced (DAPI+) AT-rich constitutive heterochromatic regions at the centromeres exclusively.

Since *Tripogandra* flowers have two different (short and long) sets of stamens (Owens 1981; Hunt 1983),

Table III. Karyotype parameters of *Tripogandra* species studied.

	Tripogandra diuretica	*Tripogandra glandulosa*
x	8	8
$2n$	64	16
Ploidy level	$8x$	$2x$
Karyotype formula	$8m + 24sm + 32$st	$2m + 6sm + 8st$
Basic karyotype formula	$1m + 3sm + 4st$	$1m + 3sm + 4st$
Total chromosome length	148.64 μm	66.56 μm
Haploid chromosome length	74.31 μm	33.28 μm
Genome size (μm)	18.58 μm	33.28 μm
Mean chromosome length	2.32 μm	4.16 μm
Maximum chromosome length	4.06 μm (*st*)	7.45 μm (m)
Minimum chromosome length	1.60 μm (*sm*)	2.81 μm (*sm*)
Mean centromeric index	27.32	26.06
Intrachromosomal asymmetry index A_1	0.60	0.63
Interchromosomal asymmetry index A_2	0.27	0.37
Ratio of longest/shortest chromosome pair	2.54	2.65
Proportion of chromosome pairs with arm ratio >2	0.88	0.88
Stebbins' category	3B	3B

Note: x = basic chromosome number; $2n$ = sporophytic chromosome number.

meiotic studies were carried out for both. Results pointed out that, within the same floral bud, the short stamens always exhibited the most advanced meiotic stage, both in *T. diuretica* and *T. glandulosa*.

In the case of the diploid *T. glandulosa*, whose haploid number is $n = 8$, the pollen mother cells (PMCs) at diakinesis and metaphase I presented chromosomes associated as eight bivalents exclusively. The mean number of chiasmata per PMC (Q/PMC) was 14.3, and most of the bivalents formed rings (77.5%; rods, 22.5%) with distal chiasmata (86.0%; interstitials, 14.0%) showing a mean of 1.79 chiasmata per bivalent, even though the *st* chromosomes presented a mean of 1.63, with the highest rod bivalents per cell (1.5/1.8). The value of the recombination index (RI) of Darlington (1939), affected by n and by Q/PMC, is low (RI = 8 + 14.3 = 22.3), unlike the excess chiasmata (EC) frequency of Burt and Bell (1987): EC = Q/PMC – n = 6.3.

The meiotic behaviour of *T. glandulosa* was regular, and microsporogenesis was normal, producing viable pollen grains in both set of stamens (>96%). Additionally, all the accessions of *T. glandulosa* presented a high seed production under natural conditions.

In the octoploid *T. diuretica*, the haploid number is $n = 32$, and in the PMC at diakinesis and metaphase I the chromosomes associated as 32 bivalents exclusively. The mean of Q/PMC was 59.0, and most of the bivalents formed rings (83.4%; rods, 16.6%) with distal chiasmata (87.6%; interstitials, 12.4%) showing a mean of 1.84 chiasmata per bivalent. The RI value is high (91.0) and so is the EC frequency (27.0).

Furthermore, in the PMC at anaphase I of this species, differential separation of bivalents was observed, those of the small chromosomes separating first and moving to the poles, while large bivalents were still aligned on the metaphase plate; meiosis I, however, is regular, resulting in balanced dyads. Some accessions of *T. diuretica* showed few PMCs at telophase II, tetrads and microspores with 1 > 2 > 3 chromosomes not integrated in the nucleus, which probably caused a lower pollen grain stainability, but a regular meiosis II in most of the accessions studied. *T. diuretica* has dimorphic pollen, with the smallest grains in the short stamens. The pollen grains in the long stamens were never released, and differential stainability among accessions was found when opening the anthers manually, ranging from 30.0 to 90.0%, averaging 68.5%. The pollen grains in the short stamens also showed a differential stainability among accessions, ranging from 40.0 to 90.0%, averaging 77.8%. In both types of stamens, microsporogenesis was normal in most of the accessions studied. Moreover, all the accessions of *T. diuretica* presented a high seed production under natural conditions.

Discussion

For the first time, natural populations from NE Argentina of *T. diuretica* ($2n = 64$) and *T. glandulosa* ($2n = 16$) were chromosomally studied.

Since Handlos (1970b, 1975) separated the herbarium specimens of *Tripogandra elongata* (G. Mey.) Woodson (synonyms included) in *Tripogandra serrulata* (Vahl) Handlos and *T. diuretica* (Mart.) Handlos, only two chromosome count reports refer to the latter taxon. Jones and Jopling (1972) found $2n = 62 + 1$B in accessions of *T. diuretica* from Buenos Aires, Argentina, and $2n = 64$ from those of Rio de Janeiro, Brazil. Also, Handlos (1975)

Table IV. Quantitative parameters of chromosomes of *Tripogandra* species studied.

Pair	$s(\mu m) \pm SE$	$l(\mu m) \pm SE$	$c(\mu m) \pm SE$	i	LR%	Type
Tripogandra glandulosa						
1	3.14 ± 0.06	4.31 ± 0.06	7.45 ± 0.06	42.15	22.39	m
2	0.87 ± 0.01	2.28 ± 0.06	3.15 ± 0.06	27.62	9.47	sm-sat
3	0.86 ± 0.02	2.09 ± 0.06	2.95 ± 0.06	29.15	8.86	*sm*
4	0.85 ± 0.02	1.96 ± 0.03	2.81 ± 0.04	30.25	8.44	*sm*
5	0.83 ± 0.02	4.3 ± 0.06	5.13 ± 0.06	16.18	15.41	st-sat
6	0.82 ± 0.05	3.72 ± 0.06	4.54 ± 0.06	18.06	13.64	*st*
7	0.83 ± 0.02	3.01 ± 0.06	3.84 ± 0.06	21.61	11.54	*st*
8	0.80 ± 0.03	2.61 ± 0.06	3.41 ± 0.06	23.46	10.25	*st*
Tripogandra diuretica						
1	0.91 ± 0.02	1.17 ± 0.06	2.08 ± 0.06	43.75	2.80	*m*
2	0.85 ± 0.05	1.00 ± 0.02	1.85 ± 0.04	45.95	2.49	*m*
3	0.81 ± 0.05	0.99 ± 0.06	1.80 ± 0.05	45.00	2.42	*m*
4	0.78 ± 0.03	0.97 ± 0.06	1.75 ± 0.06	44.57	2.35	*m*
5	0.85 ± 0.04	1.71 ± 0.06	2.56 ± 0.06	33.20	3.46	sm-sat
6	0.71 ± 0.04	1.67 ± 0.06	2.38 ± 0.06	29.83	3.20	*sm*
7	0.69 ± 0.05	1.53 ± 0.06	2.22 ± 0.06	31.08	3.00	*sm*
8	0.62 ± 0.04	1.53 ± 0.06	2.15 ± 0.06	28.84	2.89	*sm*
9	0.61 ± 0.02	1.44 ± 0.04	2.05 ± 0.03	29.76	2.76	*sm*
10	0.61 ± 0.03	1.38 ± 0.03	1.99 ± 0.01	30.65	2.68	*sm*
11	0.61 ± 0.05	1.29 ± 0.04	1.90 ± 0.03	32.11	2.56	*sm*
12	0.59 ± 0.05	1.28 ± 0.06	1.87 ± 0.05	31.55	2.51	*sm*
13	0.54 ± 0.04	1.26 ± 0.05	1.80 ± 0.01	30.00	2.42	*sm*
14	0.52 ± 0.02	1.26 ± 0.04	1.78 ± 0.01	29.21	2.39	*sm*
15	0.54 ± 0.05	1.12 ± 0.04	1.66 ± 0.03	32.53	2.23	*sm*
16	0.49 ± 0.01	1.11 ± 0.03	1.60 ± 0.02	30.63	2.15	*sm*
17	0.63 ± 0.03	3.43 ± 0.06	4.06 ± 0.06	15.52	5.47	*st*
18	0.64 ± 0.04	3.06 ± 0.06	3.70 ± 0.06	17.30	5.00	*st*
19	0.58 ± 0.01	2.91 ± 0.03	3.49 ± 0.06	16.62	4.70	*st*
20	0.56 ± 0.03	2.90 ± 0.06	3.46 ± 0.06	16.18	4.66	*st*
21	0.59 ± 0.04	2.36 ± 0.06	2.95 ± 0.06	20.00	3.97	*st*
22	0.59 ± 0.05	2.26 ± 0.06	2.85 ± 0.01	20.70	3.84	*st*
23	0.58 ± 0.01	2.16 ± 0.01	2.74 ± 0.01	21.17	3.69	*st*
24	0.58 ± 0.02	2.12 ± 0.02	2.70 ± 0.02	21.48	3.63	*st*
25	0.53 ± 0.04	2.00 ± 0.05	2.53 ± 0.03	20.95	3.40	st-sat
26	0.54 ± 0.02	1.88 ± 0.05	2.42 ± 0.03	22.31	3.26	*st*
27	0.47 ± 0.03	1.80 ± 0.06	2.27 ± 0.06	20.70	3.05	*st*
28	0.47 ± 0.03	1.68 ± 0.03	2.15 ± 0.02	21.86	2.89	*st*
29	0.48 ± 0.01	1.55 ± 0.03	2.03 ± 0.03	23.65	2.73	*st*
30	0.45 ± 0.02	1.52 ± 0.02	1.97 ± 0.01	22.84	2.65	*st*
31	0.40 ± 0.01	1.41 ± 0.05	1.81 ± 0.06	22.10	2.43	*st*
32	0.38 ± 0.03	1.35 ± 0.01	1.73 ± 0.04	21.97	2.32	*st*

Note: s = mean short arm length; l = mean long arm length; c = mean chromosome length; i = mean centromeric index; LR% = relative chromosomes length; SE = standard error.

reported $2n = 64$ for this taxon. Moreover, Saura (1948) found $n = 30$ and $2n = 60$ in a plant material collected near Buenos Aires, described in detail as *Tradescantia elongata* Meyer (synonym of *T. diuretica*) by Ing. Agr. Lorenzo R. Parodi. According to Handlos' (1970a) opinion, this count may be referred to *Tradescantia fluminensis* Vell., a common species in that area and a usual cytotype for this taxon. However, we analysed in detail the original article of Saura (1948) and concluded that the somatic and meiotic chromosome counts refer to *T. diuretica*, although inaccurate because of technique and ploidy level limitations. On the other hand, the chromosome counts of *Tradescantia elongata* G. Mey., $2n = $ c.50 (Simmonds 1954) and *T. elongata* (Meyer) Woodson, $n = 24$ and $2n = 48$ (Lewis et al. 1967) refer to *T. serrulata*, according to Handlos (1970a). Besides, Hunt (1994) reported an octoploid cytotype $2n = 64$ for *T. serrulata*, which, added to that of *T. diuretica*, are unique in *Tripogandra*.

The main karyotype features of *T. diuretica* are described here for the first time. In a plant material from Brazil, Roa Ovalle (2007) found a comparable karyotype formula as that reported here for *T. diuretica* ($8m + 24sm + 32st$), with $6m + 20sm + 38a$ (a = acrocentric = st), four chromosome pairs (*sm* or *a*) carrying terminal CMA+ bands in their short arms and DAPI+ bands in the centromeres of the whole set.

As regards *T. glandulosa*, the same cytotype reported here ($2n = 16$) was previously reported for Argentinian accessions from Buenos Aires [as *T. pflanzii* (Bruckn.) Rohw.; Celarier 1955], Córdoba (as *Tradescantia radiata* Clarke; $n = 8$; Cocucci 1956) and Entre Rios and Catamarca (Jones & Jopling 1972). *T. glandulosa* has been chromosomally counted many times, but the cytotype $2n = 16$ is exclusive for this species [Holzer 1952 as *Descantaria pflanzii* G. Bruckn.; Lewis et al. 1967; Le Coq & Guervin 1968; Zaman et al. 1979 as *T. pflanzii* (Bruckn.) Rohw.; Handlos 1975; Romeu Pitrez et al. 2001].

The main karyotypic features of *T. glandulosa* are likewise described here in detail for the first time. Celarier (1955) and Lewis et al. (1967) presented a vague illustration of the karyotype of *T. pflanzii*, which coincides with our description for *T. glandulosa*. On the other hand, Zaman et al. (1979) gave a more detailed description of the karyotype of *T. pflanzii*, which resulted to be bimodal and asymmetrical; despite using a different classification, if the mean values for each chromosome are arranged according to the nomenclature employed here, then a karyotype formula of $2m + 6sm + 8st$, and a mean chromosome length of 71.98 μm are obtained. Thus, the main karyotype features and formula as well as the morphometric parameters of the chromosomes of *T. pflanzii* described by Zaman et al. (1979) are identical to those depicted here for *T. glandulosa*. Additionally, *T. glandulosa* exhibited two pairs of satellited chromosomes, one (No. 2, *sm*) shorter than the other (No. 5, *st*), the same as in the Bolivian accession of *T. pflanzii* (Lewis et al. 1967). Moreover, in our plant material MG-75 belonging to *T. glandulosa* and in agreement with our results, Roa Ovalle (2007) found a karyotype formula of $2m + 6sm + 8a$, in addition to four chromosome pairs (*sm* or *a*) carrying terminal CMA+ bands in their short arms, and C bands in the centromeres of the whole

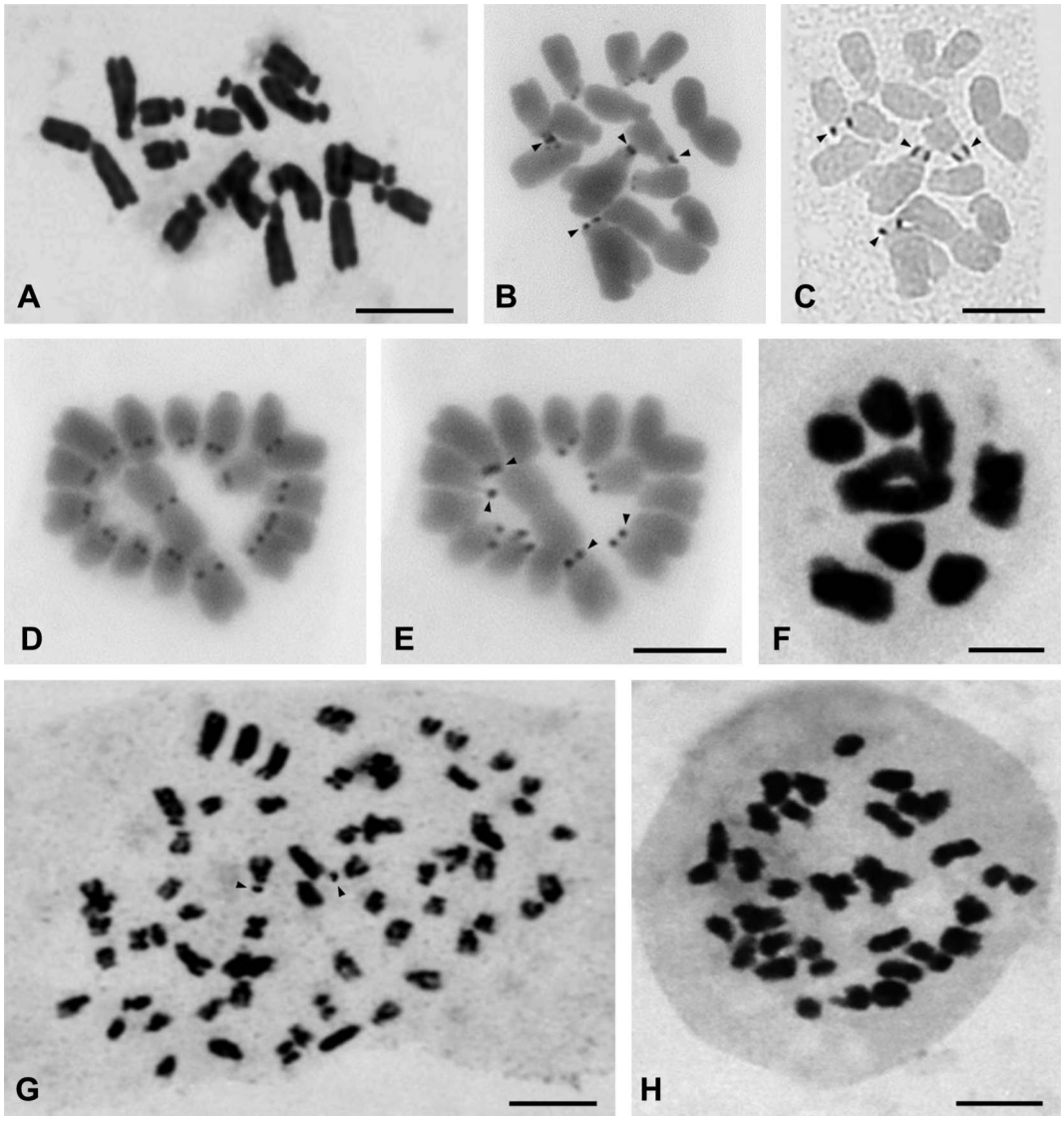

Figure 2. Photomicrographs of somatic and meiotic chromosomes of *Tripogandra*. (A) Mitotic metaphase of *T. glandulosa* showing $2n = 2x = 16$ chromosomes. (B–C) Somatic metaphase of *T. glandulosa* submitted to CMA/DA fluorescent banding and subsequently to Ag-NOR staining respectively; four pairs of chromosomes with terminal CMA+ CG-rich constitutive heterochromatin in their short arms were revealed and arrowheads point out those two pairs with CMA+ bands that were also NOR-actives. (D–E) Somatic metaphase plate of *T. glandulosa* submitted to CMA/DA/DAPI fluorescent banding showing an exclusively centromeric DAPI+ AT-rich constitutive heterochromatin genomic pattern and terminal CMA+ bands respectively; arrowheads point out macrosatellites. (F) PMC at diakinesis of *T. glandulosa* with 8 II. (G) Somatic metaphase of *T. diuretica* showing $2n = 8x = 64$ chromosomes; arrowheads point out macrosatellites. (H) PMC at diakinesis of *T. diuretica* with 32 II. B, D and E are inverted in the colour that they appear in the microscope. Scale bars = 5 μm.

set, which actually correspond to the DAPI+ bands described here. He also found a correspondence between CMA+ bands and 18S–25S rDNA after fluorescent *in situ* hybridisation (FISH; Roa Ovalle 2007), although just two loci per genome behaved as active NORs in our study.

As expected, the genome size and the mean chromosome length of the octoploid *T. diuretica* (18.58 μm, and 2.32 μm, respectively) are smaller (approximately half) than those of the diploid *T. glandulosa* (33.28 μm and 4.16 μm, respectively), probably caused by transposable elements variation associated with polyploidisation, rather than evident heterochromatin changes, in the evolutionary history of the two taxa.

T. diuretica and *T. glandulosa* share the basic chromosome number ($x = 8$), and unexpectedly possess related karyotype parameters associated to the bimodality and the asymmetry of a same basic karyotype formula ($1m + 3sm + 4st$) as well as DAPI+ bands located exclusively at the centromeres of the whole set of chromosomes, suggesting that karyotype orthoselection processes may be involved in delineating the karyological evolution of both taxa.

Regarding the karyotype descriptions performed in *Tripogandra* (Celarier 1955; Handlos 1970a and

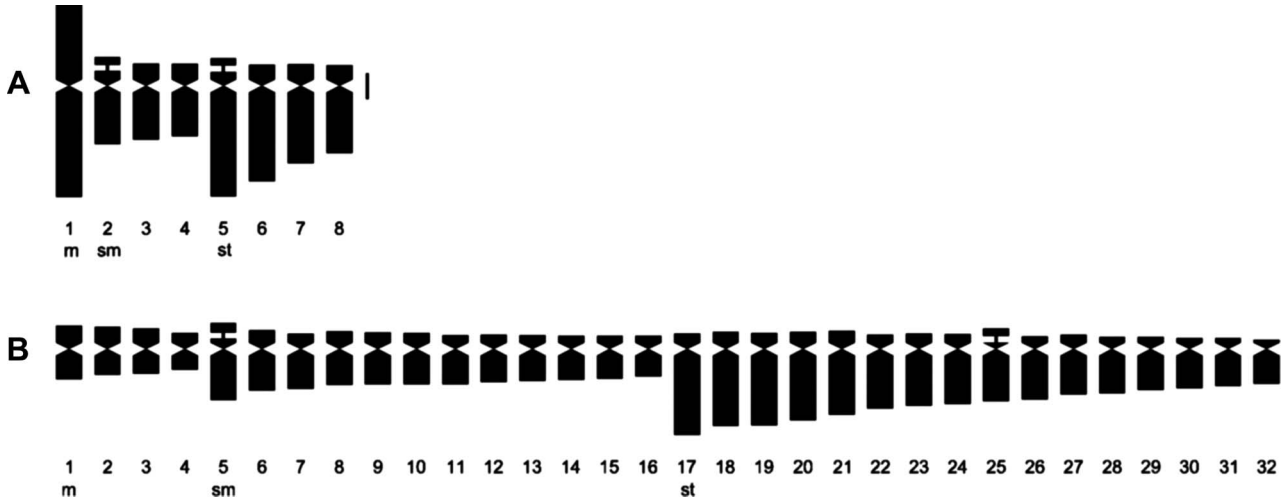

Figure 3. Idiograms of both species of *Tripogandra* studied. (A) Karyotype of *T. glandulosa* ($2m + 6sm + 8st$). (B) Karyotype of *T. diuretica* ($8m + 24sm + 32st$). Scale bar = 1 μm.

references therein; Jones & Jopling 1972; Zaman et al. 1979), most are superfluous, do not follow the criteria of Levan et al. (1964) or are of doubtful affiliation to a precise taxonomical entity. For these and other reasons, the karyological evolution of the genus remains unsolved.

Considering the meiotic chromosome pairing at diakinesis and metaphase I of *T. glandulosa* and *T. diuretica*, similarities were found between both diploid and octoploid species. Chromosomes associated exclusively as bivalents, with a high mean value of chiasmata per bivalent and of PMCs, in addition to the prevalence of ring bivalents and distal chiasmata per PMC in both taxa. Furthermore, the amount of total meiotic intra- and inter-chromosomal recombination, estimated via the RI and the EC, both being measures of the amount of genetic recombination of any taxon, pointed out that the meiotic system of the diploid *T. glandulosa* promotes less total recombination than that of the octoploid *T. diuretica* in agreement with previous analyses in other Commelinaceae (*Commelina*; Grabiele et al. 2009).

Moreover, the chromosome association at meiosis I and the meiotic behaviour of *T. glandulosa* are in accordance with those described for *T. pflanzii* by Zaman et al. (1979) and disagree with those of Celarier (1955), who additionally to eight bivalents found some non-homologous associations, laggard chromosomes at anaphase I and telophase I, and micronuclei in dyads in the latter taxon. The meiotic behaviour of *T. glandulosa* was regular and its microsporogenesis was normal, producing viable, monomorphic pollen grains, as previously reported by Poole and Hunt (1980) for this species.

The differential separation of bivalents at anaphase I observed in *T. diuretica* was previously reported for several *Tripogandra* species by Handlos (1970a). What is more, *T. diuretica* presented dimorphic pollen grains in agreement with earlier observations of Bacigalupo (1967). According to Handlos (1975), the large stamens of *T. diuretica* act as staminodes and its pollen grains are sterile (Bacigalupo 1967; Handlos 1975). However, in our study, pollen grain viability between 30.0 and 90.0% was found in the staminodes of *T. diuretica* when opening its anthers manually. The octoploid *T. diuretica* behaved as a cytological diploid in agreement with previous observations in polyploid taxa of *Commelina* (Grabiele et al. 2005). A diploidised meiotic behaviour in polyploids is almost exclusive to allopolyploids; however, it can also take place in autopolyploids through genetic control of the chromosomal pairing which enhances bivalent formation (Watanabe 1983 and references therein). So, considering the diploidised meiotic behaviour, and the number and distribution of CMA+ loci, it is impossible to elucidate the probable origin of the polyploidy in *T. diuretica*. However, the arrangement of the haploid karyotype into eight groups of four chromosomes each suggests an auto-octoploid condition for this taxon.

T. diuretica and *T. glandulosa* share a similar geographical distribution and habitat, but differ widely in morphological features, making it easy to distinguish them. In addition, the cytogenetic approaches, applied by us and others, show that both taxa also have in common major karyotype features, probably associated to orthoselection processes occurring in *Tripogandra*, as well as a regular and diploidised meiotic behaviour with a normal microsporogenesis and seed production. Thus, at the cytological level, *T. diuretica* and *T. glandulosa* mainly differ at their ploidy levels and in chromosomal features related to polyploidisation (i.e. mean chromosome length and genome size).

Although polyploidy is widespread in the Commelinaceae family (Grabiele et al. 2005), octoploidy

is not a common condition, even within *Tripogandra*. From an evolutionary point of view, octoploidy became a stable condition in *T. diuretica*, associated to a diploidised meiotic behaviour, which facilitated its present distribution.

The detailed cytological analysis of *T. diuretica* and *T. glandulosa* performed here, summed to the high incidence of polyploidy in the genus, suggest that the latter has played an important evolutionary role in the speciation of *Tripogandra*.

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