

## IAPT chromosome data 36

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**DOI** <https://doi.org/10.1002/tax.12809>

First published as part of this issue. See online for details.

All materials for the chromosome column should be submitted electronically to: Karol Marhold, karol.marhold@savba.sk. The full version of this contribution is available in the online edition of TAXON appended to this article. The following citation format is recommended: Korobkov, A.A., Kotseruba, V.V. & Krivenko, D.A. 2019. IAPT chromosome data 30/4. In: Marhold, K. & Kučera, J. (eds.) & al., IAPT chromosome data 30. *Taxon* 68: 882, E1–E2.

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## IAPT chromosome data 36/1

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This study was supported by Agencia Nacional de Promoción Científica y Técnica (ANPCyT) grant nos. PICT-2016-1637 and -2017-4203, by Universidad Nacional de Misiones (UNaM) 16Q1240-PI MX1205-Programa de Cooperación Científico-Tecnológica between Ministerio de Ciencia, Tecnología e Innovación Productiva de la República Argentina (MINCYT), Consejo Nacional de Ciencia y Tecnología de México (CONACyT, Project 191711) and Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), and with a doctoral fellowship from CONICET to ACGA and OARM.

All materials CHN.

## AMARYLLIDACEAE

*Habranthus cardenasianus* Traub & I.S.Nelson,  $2n = 24$ ; Argentina, Salta, 10 Oct 2013, J.R. Daviña, A.I. Honfi & E.J. Martínez 666 (MNES).

## IAPT chromosome data 36/2

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This research was carried out within the framework of the “Grasses and grass-endophyte interactions: genomics and ecological adaptation” project funded by the Spanish Aragon Government (grant LMP82\_21) and the Spanish Aragon Government and European Social Fund Bioflora research group grant A01-20R. The field expedition to the Ecuadorian paramos was supported by a European and Spanish Government (SEPIE) Erasmus+KA107 mobility grant (2019-1-ES01-KA107-062605) between the Universidad de Zaragoza (Spain) and the Universidad Técnica Particular de Loja (Ecuador) and by a University of Zaragoza-Santander Ph.D. fellowship to MFMA. Permission to collect *Festuca* samples in the Ecuadorian paramos was given by the Ministry of Environment, Water and Ecological Transition of Ecuador (MAE-DNB-CM-2015-0016).

All materials CHN; collected in Ecuador.

## POACEAE

*Festuca andicola* Kunth,  $2n = 42$ ; M.F. Moreno & al. F90\_i (HUTPL 14037).

*Festuca caldasii* (Kunth) Kunth,  $2n = 28$ ; M.F. Moreno & al. F98\_j (HUTPL 14054).

*Festuca chimborazensis* subsp. *micacochensis* Stančič,  $2n = 42$ ; M.F. Moreno & al. F58\_j (HUTPL 14065).

*Festuca subulifolia* Benth.,  $2n = 42$ ; M.F. Moreno & al. F60\_i (HUTPL 14099).

## IAPT chromosome data 36/3

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## FABACEAE

## Tribe Dipterygeae

*Dipteryx lacunifera* Ducke,  $2n = 16$ , CHN. Brazil, Piauí, S.C. Silva & M. Lenara (TEPB 205).

## IAPT chromosome data 36/4

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All materials CHN.

## POACEAE

*Paspalum carinatum* Humb. & Bonpl. ex Flüggé,  $2n = 40$ ; Brazil, Goiás, J.F.M. Valls, M.W.S. Sousa & C.A.D. Welker 16606 (CEN).

*Paspalum compressifolium* Swallen,  $2n = 40$ ; Brazil, Paraná, J.F.M. Valls, M.W.S. Sousa, C.A.D. Welker & A.P. Fávoro 16474 (CEN).

*Paspalum divergens* Döll,  $2n = 60$ ; Brazil, Rio de Janeiro, M.P. Araújo, A.C. Petry & J.F.M. Valls 331 (CEN).

*Paspalum fimbriatum* Kunth,  $2n = 20$ ; Brazil, Piauí, M.W.S. Sousa 135 (UB).

*Paspalum foveolatum* Steud.,  $2n = 40$ ; Brazil, Goiás, J.F.M. Valls, R.C. Oliveira, C. Silva, C.O. Moura & A.C.V. Berto 16189 (CEN); Brazil, Goiás, J.F.M. Valls & M.L. Paulo 17026 (CEN).

*Paspalum giuliettiae* Pimenta, G.H.Rua & R.P.Oliveira,  $2n = 40$ ; Brazil, Bahia, J.F.M. Valls 17012 (CEN).

*Paspalum juergensii* Hack.,  $2n = 20$ ; Brazil, Rio Grande do Sul, J.F.M. Valls, L.N. da Silva & E. Valduga 16981 (CEN); Brazil, Santa Catarina, J.F.M. Valls, M.W.S. Sousa, C.A.D. Welker & A.P. Fávoro 16460 (CEN).

*Paspalum lenticulare* Kunth,  $2n = 20$ ; Brazil, Tocantins, J.F.M. Valls, M.W.S. Sousa & C.A.D. Welker 16548 (CEN);  $2n = 40$ ; Brazil, Goiás, J.F.M. Valls, M.W.S. Sousa & C.A.D. Welker 16497 (CEN); Brazil, Tocantins, J.F.M. Valls, M.W.S. Sousa & C.A.D. Welker 16553 (CEN).

*Paspalum maculosum* Trin.,  $2n = 40$ ; Brazil, Bahia, J.F.M. Valls, M.W.S. Sousa & C.A.D. Welker 16586 (CEN).

*Paspalum mandiocanum* Trin.,  $2n = 60$ ; Brazil, Paraná, J.F.M. Valls, M.W.S. Sousa, C.A.D. Welker & A.P. Fávoro 16348, 16468 (CEN).

*Paspalum multicaule* Poir.,  $2n = 20$ ; Brazil, Goiás, J.F.M. Valls, H.M. Longhi-Wagner, R.C. Oliveira, C.A.D. Welker & D.M. Ramos 16623 (CEN).

*Paspalum notatum* Flüggé,  $2n = 40$ ; Brazil, Distrito Federal, J.F.M. Valls & M.W.S. Sousa 16774 (CEN).

*Paspalum pauciciliatum* (Parodi) Herter,  $2n = 40$ ; Brazil, São Paulo, J.F.M. Valls, M.W.S. Sousa, C.A.D. Welker & A.P. Fávoro 16311 (CEN).

*Paspalum plicatulum* Michx.,  $2n = 40$ ; Brazil, Paraná, J.F.M. Valls, M.W.S. Sousa, C.A.D. Welker & A.P. Fávoro 16355 (CEN).

*Paspalum redondense* Swallen,  $2n = 20$ ; Brazil, Paraná, J.F.M. Valls, M.W.S. Sousa, C.A.D. Welker & A.P. Fávero 16417 (CEN).  
*Paspalum regnellii* Mez,  $2n = 40$ ; Brazil, Paraná, J.F.M. Valls, M.W.S. Sousa, C.A.D. Welker & A.P. Fávero 16395 (CEN).  
*Paspalum scalare* Trin.,  $2n = 20$ ; Brazil, Goiás, J.F.M. Valls, H.M. Longhi-Wagner, R.C. Oliveira, C.A.D. Welker & D.M. Ramos 16652 (CEN).  
*Paspalum thrasyoides* (Trin.) S.Denham,  $2n = 40$ ; Brazil, Goiás, J.F.M. Valls, M.W.S. Sousa & C.A.D. Welker 16509 (CEN).

## IAPT chromosome data 36/5

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All materials CHN; collectors: *D* = J.R. Daviña, *H* = A.I. Honfi, *Hojs* = D.H. Hojsgaard, *S* = M.A. Sader, *SN* = V. Solis Neffa, *W* = M. Worthington.

### POACEAE

#### Subfamily Panicoideae, Tribe Paspaleae

*Paspalum arundinellum* Mez,  $2n = 50$ ; Argentina, Misiones Province, 22 Jul 1993, *H* 297 (MNES); Argentina, Formosa Province, Formosa, 2 Jun 2000, *H* & *D* 1107 (CTES, MNES); *H* & *D* 1108 (CTES, MNES, SI). Paraguay, Cordillera Department, 1 Dec 2000, *D* & *H* 495 (CTES, MNES, SI).  
*Paspalum buckleyanum* Vasey,  $2n = 40$ ; Argentina, Salta Province, 31 Mar 2012, *H* 1586 (MNES); Argentina, Formosa Province, 2 Apr 2012, *H* 1600 #1 (MNES).  $2n = 50$ ; Argentina, Formosa Province, 2 Apr 2012, *H* 1600 #1, 2, 3, 3' (MNES); Argentina, Corrientes Province, 20 Feb 2015, *H* 1736 (MNES); 7 Dec 2015, *H* 2127 (MNES).  
*Paspalum commune* Lillo,  $2n = 40$ ;  $n = 20$ ; Argentina, Jujuy Province, 20 Jan 2002, *SN* 681 (BAA, CTES, MNES, SI, US); Argentina, Tucumán Province, 1 Apr 2012, *H* 1585 (MNES).  
*Paspalum compressifolium* Swallen,  $2n = 40$ ; Argentina, Misiones Province, 15 Feb 2011, *H* 1535 #1 (MNES); 10 Oct 2017, *H* & *D* 2276 (MNES).  
*Paspalum conjugatum* P.J.Bergius,  $2n = 40$ ; Argentina, Misiones Province, 9 Apr 2006, *H* 1299 (MNES). Puerto Rico, Aibonito, 4 Aug 2009, *W* 35705 (CTES, UTEP); 16 Jul 2014, *W* 37480 (CTES, UTEP).  
*Paspalum conspersum* Schrad.,  $n = 30$ ; Argentina, Misiones Province, 21 Apr 2001, *Hojs* 175 (CTES, MNES); 10 Mar 2001, *H* 1143 (MNES).  
*Paspalum dedecae* Quarín,  $2n = 40$ ; Argentina, Misiones Province, 21 Sep 1995, *H* 649 (CTES, MNES, SI).  
*Paspalum denticulatum* Trin.,  $n = 20$ ; Argentina, Misiones Province, 31 Dec 2002, *Hojs* 256 (CTES, MNES, SI).  
*Paspalum ellipticum* Döll,  $2n = \text{ca. } 60$ ; Argentina, Misiones Province, 21 Jan 1995, *H* 648 (CTES, CORD, MNES).

*Paspalum glaucescens* Hack.,  $2n = 40$ ; Argentina, Misiones Province, 17 Dec 2005, *S* 89 (MNES).  
*Paspalum guenoarum* Arechav.,  $2n = 40$ ; Argentina, Misiones Province 3 Feb 1993, *H* 223 (CTES, CORD, MNES); 4 Mar 1993, *D* 161 (MNES).  
*Paspalum inaequivalve* Raddi,  $n = 30$ ; Argentina, Misiones Province, 8 Nov 2001, *Hojs* 221 (MNES).  
*Paspalum intermedium* Munro ex Morong & Britton,  $2n = 40$ ; Argentina, Misiones Province, 23 Oct 1993, *H* 387 (MNES).  
*Paspalum ionanthum* Chase,  $2n = 40$ ;  $n = 20$ ; Argentina, Corrientes Province, 5 Jan 2006, *S* 85 (MNES).  
*Paspalum malacophyllum* Trin.,  $2n = 40$ ; Argentina, Córdoba Province, 22 Sep 2003, *Hojs* 325 (MNES).  
*Paspalum pauciciliatum* (Parodi) Herter,  $2n = 40$ ; Argentina, Misiones Province, 29 Jan 1992, *H* 167 (MNES).  
*Paspalum plicatum* Michx.,  $2n = 40$ ; Argentina, Misiones Province, 15 Feb 2011, *H* & *D* 1533 (MNES). Paraguay, Central Department, 8 Mar 2004, *H* & *D* 1238 (MNES).  $n = 20$ ; Paraguay, Central Department, 26 Apr 2001, *H* & *D* 1121 (MNES); Paraguay, Presidente Hayes Department, 5 Mar 2006, *H* & *D* 1278 (MNES).  
*Paspalum regnellii* Mez,  $2n = 40$ ; Argentina, Misiones Province, 19 Mar 1991, *H* 120 (MNES); 30 Mar 1991, *H* 131B (MNES).  
*Paspalum rufum* Nees ex Steud.,  $2n = 40$ ; Argentina, Corrientes Province, 30 Apr 2010, *H* 1462 (MNES).  
*Paspalum simplex* Morong ex Britton,  $2n = 40$ ; Argentina, Misiones Province, 12 Feb 2012, *H* 2560 (MNES).  
*Paspalum unispicatum* (Scribn. & Merr.) Nash,  $2n = 40$ ; Argentina, Córdoba Province, 3 Jan 2006, *S* 88 (MNES); Argentina, Salta Province, 1 Apr 2012, *H* 1590 (MNES).

## IAPT chromosome data 36/6

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All materials CHN; collectors: *D* = J.R. Daviña, *H* = A.I. Honfi, *OR* = O.A. Rodríguez Mata; vouchers at MNES.

### AMARYLLIDACEAE

*Habranthus brachyandrus* (Baker) Sealy,  $2n = 24$ ; Argentina, Misiones Province, 27 Mar 2017, *OR* 2; 8 Jan 2017, *OR* 3; 8 Jan 2017, *OR* 4; 19 Apr 2017, *OR* 5; 10 Jan 2017, *OR* 7.  
*Hippeastrum striatum* (Lam.) H.E.Moore,  $2n = 22$ ; Argentina, Misiones Province, 21 Apr, 2017, *OR* 13; 7 Apr 2017, *OR* 14.  $2n = 55$ ; Argentina, Buenos Aires Province, 2 Feb 2017, *OR* 8. Argentina, Misiones Province, 21 Nov 2006, *D* 609; 30 Apr 2015, *H* 2046; 8 Nov 2015, *H* 2104A; 14 Apr 2007, *H* 1311; 3 May 2014, *H* 1712; 10 Jan 2017, *OR* 6; 2 Feb 2017, *OR* 11; 7 Apr 2017, *OR* 12; 29 Sep 2018, *OR* 15; 29 Sep 2018, *OR* 16; 29 Sep 2018, *OR* 17; 29 Sep 2018, *OR* 18; 7 Oct 2018, *OR* 19.

## IAPT chromosome data 36 – Extended version

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## IAPT chromosome data 36/1

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This study was supported by Agencia Nacional de Promoción Científica y Técnica (ANPCyT) grant nos. PICT-2016-1637 and -2017-4203, by Universidad Nacional de Misiones (UNaM) 16Q1240-PI MX1205-Programa de Cooperación Científico-Tecnológica between

Ministerio de Ciencia, Tecnología e Innovación Productiva de la República Argentina (MINCYT), Consejo Nacional de Ciencia y Tecnología de México (CONACYT, Project 191711) and Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), and with a doctoral fellowship from CONICET to ACGA and OARM.

Methods are described in Daviña (2001) and Barba-González & al. (2010).

\* First chromosome count for the species.

## AMARYLLIDACEAE

Hippeastreae (Amaryllidaceae) is a controversial clade (tribe) with mainly tropical distribution (Meerow, 2010; Garcia & al., 2019). The tribe includes the genus *Habranthus* Herb., commonly named “rain lilies”, whose species are principally distributed in South America (Arroyo, 1990; Meerow & Snijman, 1998; Howard, 2001; Amaral, 2011; Garcia & al., 2019). Species of this genus are prized for their ornamental and phytochemical potential. *Habranthus cardenasianus* Traub & I.S.Nelson, is a species distributed in Bolivia and northwest Argentina and grows in sandy soils in deciduous forest (Roitman & al., 2006). In this work, the chromosome characterization of *H. cardenasianus* originating from Argentina was investigated.

*Habranthus cardenasianus* Traub & I.S.Nelson

\* $2n = 24$ , CHN. Argentina, Province of Salta, Campo Quijano Department, Rosario de Lerma, 24°54'S 65°38'W, 10 Oct 2013, Daviña, Honfi & Martínez 666 (MNES). [Fig. 1A]

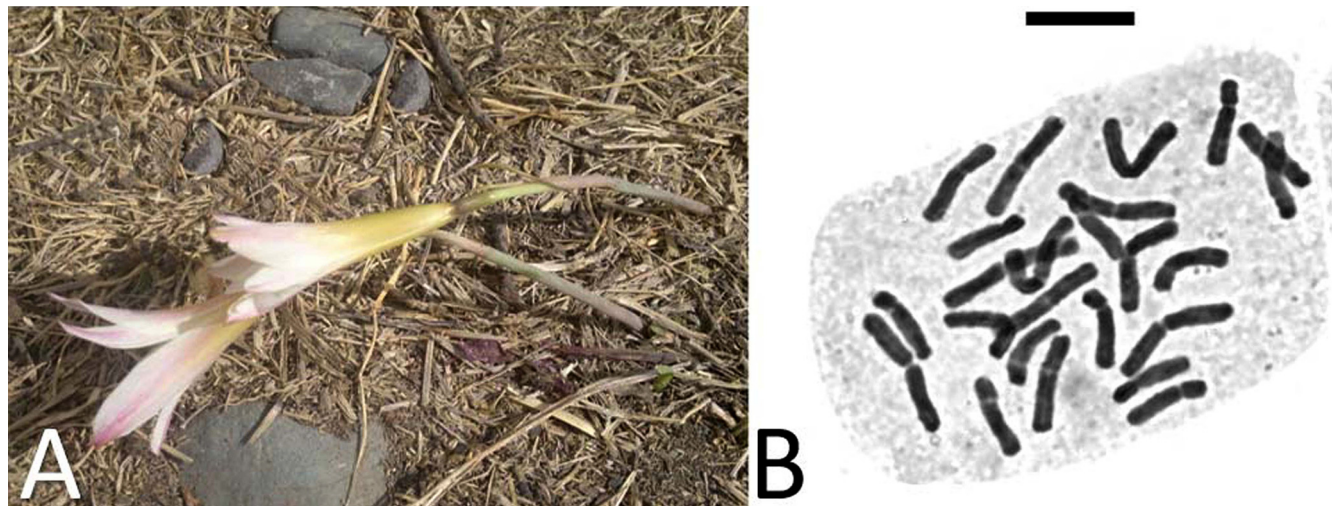
*Habranthus cardenasianus* has  $2n = 4x = 24$  chromosomes (Fig. 1B), and a basic chromosome number of  $x = 6$ , which agrees with Flory & Flagg (1959). The karyotypic formula consists in 12 metacentric (m) and 12 submetacentric (sm) chromosomes (Fig. 2). The genomic size is 213.73  $\mu\text{m}$ . The mean centromeric index (i) is 36.77 and the mean chromosomal length is 8.90  $\mu\text{m}$ , which indicate that it is a slightly asymmetric karyotype because it belongs to Stebbins's (1971) category 2A and because they have intrachromosomal ( $A_1$ ) and interchromosomal asymmetry ( $A_2$ ) values of 0.386 and 0.203, respectively, according to Romero Zarco (1986). For the first time, molecular techniques were applied to this species. The heterochromatic region observed is  $\text{CMA}^+ \text{DAPI}^-$  and is located on the short arms of metacentric chromosomes 9 and 10, the long arm of metacentric chromosome 1 and the short arms of submetacentric chromosomes 23 and 24. The amount of constitutive heterochromatin rich in GC is 1.68% of the genome (Figs. 2, 3A,B).

Fluorescent in situ hybridization showed the hybridization sites of the ribosomal DNA probes. The presence of active ribosomal genes (rDNA 18S/26S) was located on the short arms of chromosomes 9 and 10 (m), on the long arm of chromosome 1 (m) and on the short arms of submetacentric chromosomes 23 and 24. All signals were located in the terminal position. The 5S rDNA probe hybridized at six locations. Signals were observed in the terminal position of the long arms of the submetacentric chromosomes 13, 14, 19 and 20, and in the intercalary position of chromosomes 23 and 24 (Figs. 2, 3C).

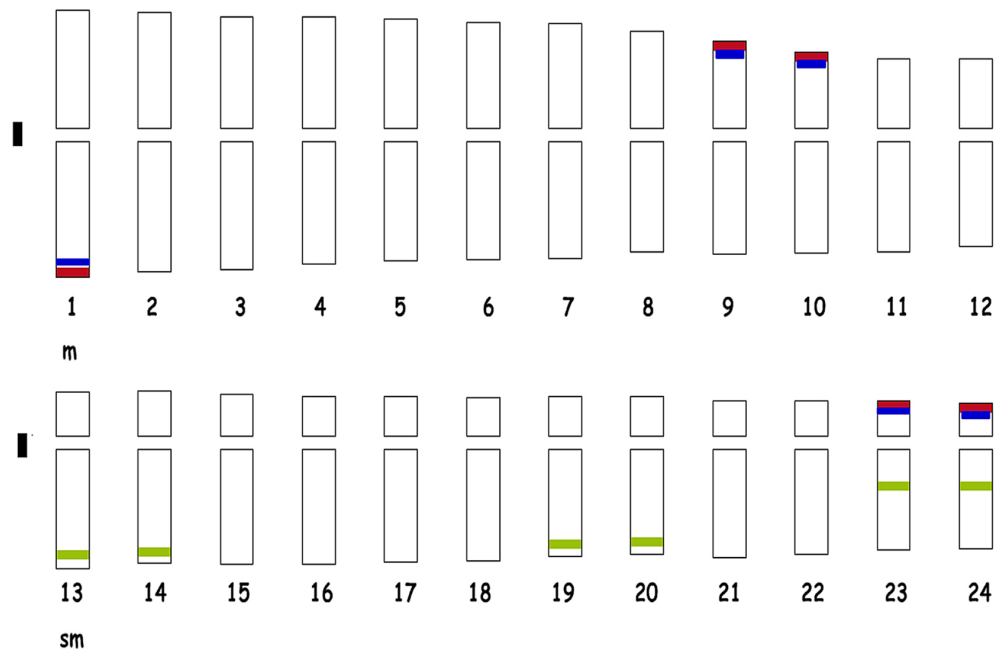
There are few studies of chromosomal banding patterns such as fluorescent in situ hybridization (Barros e Silva & Guerra, 2010; Felix & al., 2011). The hybridization sites of ribosomal DNA banding and patterns of constitutive heterochromatin could be used to characterize species or cytotypes. These contributions to the cytogenetics of *H. cardenasianus* add to the few molecular cytogenetics studies in the Hippeastreae clade. Furthermore, karyotype and constitutive heterochromatin characterization contribute to the taxonomical identity of this controversial species. Some works suggest that *H. cardenasianus* could be considered a synonym of *H. robustus* Herb. (Arroyo-Leuenberger, 2009), a species with two recorded chromosome numbers ( $2n = 12, 48$ ) (Gianini Aquino & al., 2020). However, in all the studied accessions of *H. robustus*, only a diploid cytotype was unfailingly found (Gianini Aquino & al., 2020). Ongoing studies about cyto-embryology, genetic systems and morphology of *Habranthus* species will shed light on the taxonomical status of both species.

## METHODS

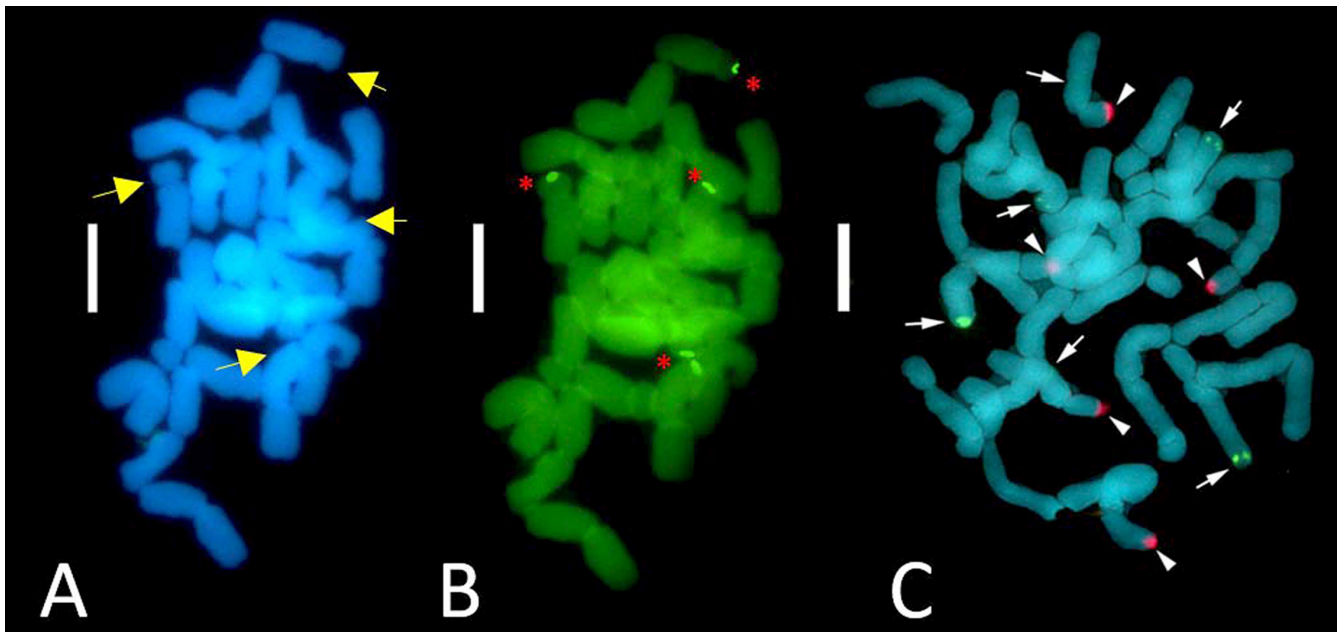
The conventional cytogenetic and triple sequential CMA/DA/DAPI staining techniques were applied according to Daviña (2001). Fluorescent in situ hybridization was performed using two different probes, (i) clone pTa71, which contains the 45S fragment of wheat ribosomal DNA (Gerlach & Bedbrook, 1979), and (ii) clone pTa794, which contains the wheat ribosomal DNA 5S fragment (Gerlach & Dyer, 1980), according to Barba-Gonzalez & al. (2010).



**Fig. 1.** A, *Habranthus cardenasianus*, plant collected from the study area (photo: A.I. Honfi); B, Mitotic metaphase with conventional staining,  $2n = 24$ . — Scale bar = 10  $\mu\text{m}$ .



**Fig. 2.** Idiogram of the complete set of *Habranthus cardenasianus* chromosomes,  $2n = 24$  (12m + 12sm). CMA<sup>+</sup>/DAPI<sup>-</sup> heterochromatic bands (in blue) on chromosomes 9 and 10 (m), and 23 and 24 (sm) on the short arm and on chromosome 1 (m) on the long arm. The rDNA ribosomal genes (18S/26S; in red) colocalize with the constitutive heterochromatin bands on the same chromosomes. The 5S rDNA genes (in green) are located on chromosomes 13, 14, 19, 20, 23 and 24 (sm) on the long arm. Scale bars = 1 μm.



**Fig. 3.** *Habranthus cardenasianus* mitotic metaphase,  $2n = 24$ , CMA/DA/DAPI sequential banding. **A**, Arrows indicate the DAPI<sup>-</sup> sites on chromosomes (sm and m); **B**, CMA<sup>+</sup>/DAPI<sup>-</sup> bands are observed on five chromosomes: three metacentric (2 located on the short arm, 1 on the long arm) and two submetacentric (on the short arm, asterisks); **C**, Fluorescent in situ hybridization (FISH) where the chromosomes carrying 5S ribosomal genes (green, indicated with arrows), located in the short arms of submetacentric chromosomes, are observed. Arrowheads indicate 45S ribosomal gene signals (red), located on the short arms of two metacentric chromosomes, two submetacentric chromosomes and on the long arm of a metacentric chromosome. — Scale bars = 10 μm.

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## IAPT chromosome data 36/2

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This research was carried out within the framework of the “Grasses and grass-endophyte interactions: genomics and ecological adaptation” project funded by the Spanish Aragon Government (grant LMP82\_21) and the Spanish Aragon Government and European Social Fund Bioflora research group grant A01-20R. The field expedition to the Ecuadorian paramos was supported by a European and Spanish Government (SEPIE) Erasmus+KA107 mobility grant (2019-1-ES01-KA107-062605) between the Universidad de Zaragoza (Spain) and the Universidad Técnica Particular de Loja (Ecuador) and by a University of Zaragoza-Santander Ph.D. fellowship to MFMA. Permission to collect *Festuca* samples in the Ecuadorian paramos was given by the Ministry of Environment, Water and Ecological Transition of Ecuador (MAE-DNB-CM-2015-0016).

## POACEAE

*Festuca andicola* Kunth

$2n = 42, x = 6$ , CHN. Ecuador: Loja, Saraguro, Cerro de Arcos, 3.563037°S, 79.463008°W, 3650 m; 21 May 2018, *M.F. Moreno & al.* *F90\_i* (HUTPL 14037).

*Festuca caldasii* (Kunth) Kunth

$2n = 28, x = 4$ , CHN. Ecuador: Loja, Catamayo, route Las Chinchas–Tambara, 3.967127°S, 79.515866°W, 2050 m; 26 May 2018, *M.F. Moreno & al.* *F98\_i* (HUTPL 14054).

*Festuca chimborazensis* subsp. *micacochensis* Stančík

$2n = 42, x = 6$ , CHN. Ecuador: Chimborazo, Riobamba, route Chimborazo–Guaranda, 1.44243°S, 78.93002°W, 4138 m; 30 Sep 2017, *M.F. Moreno & al.* *F58\_i* (HUTPL 14065).

*Festuca subulifolia* Benth.

$2n = 42, x = 6$ , CHN. Ecuador: Chimborazo, Riobamba, route Chimborazo–Guaranda, 2.17711°S, 78.51006°W, 3472 m, 1 Oct 2017, *M.F. Moreno & al.* *F60\_i* (HUTPL 14099).

The Ecuadorian Andean paramos are considered hotspots of global biodiversity, hosting about 6.7% of the world’s endemic plants (Myers & al., 2000). The most abundant plant communities of these paramunean ecosystems are the pajonales, extensive grazed communities dominated by cold seasonal grasses adapted to the high Andean climate (Sklenář & Ramsay, 2001). Among the most frequent pajonal grasses are species of the genus *Festuca*. This genus is the largest of the pooid subtribe Loliinae Dumort. and consists of more than 600 species distributed in temperate and mountainous regions of both hemispheres (Catalán, 2006). Approximately a quarter of the South American *Festuca* species are endemic to the North Andean region. Stančík (2004) recognized 23 species of *Festuca* in Ecuador and Stančík & Peterson (2007)

53 in the paramos of the northern Andes. *Festuca* species show a uniform chromosome base number of  $x = 7$ , and ploidy levels range from diploids to dodecaploids (Catalán, 2006). Phylogenetic studies have detected the existence of two main clades within *Festuca* and the Loliinae, the Broad-leaved (BL) and the Fine-leaved (FL) lineages, each containing different South American sublineages (Catalán & al., 2004, 2007; Catalán, 2006; Inda & al., 2008; Minaya & al., 2017; Moreno-Aguilar & al., 2020). Cytogenetic works have shown that all Southern Hemisphere *Festuca* species analyzed so far are polyploids (Dubcovsky & Martínez, 1992; Catalán, 2006; Šmarda & Stančík, 2006); however, a large number of *Festuca* species from South America have not been studied chromosomally yet.

Here we report new chromosome counts for four *Festuca* species from the Ecuadorian paramos: *Festuca caldasii* (Kunth) Kunth is a tetraploid with  $2n = 28$  chromosomes, whereas *F. andicola* Kunth, *F. chimborazensis* subsp. *micacochensis* Stančík and *F. subulifolia* Benth. are hexaploids with  $2n = 42$  chromosomes. The chromosomal study was performed following the protocol of Jenkins & Hasterok (2007). Chromosome counting was performed in two to five metaphasic cells per individual using DAPI-stained meristematic root cells; the staining was performed with the DAPI fluorescent marker (4', 6-diamino-2-phenylindole), and counting was done using a Motic BA410 fluorescence microscope. Chromosome numbers of *F. caldasii* and *F. chimborazensis* subsp. *micacochensis* are given here for the first time. The chromosome-based hexaploid level detected in *F. chimborazensis* subsp. *micacochensis* agrees with its inferred ploidy from genetic analysis of nuclear genes (Díaz-Pérez & al., 2014). Šmarda & Stančík (2006) reported different chromosome numbers and ploidy levels for *F. andicola* and *F. subulifolia* (tetraploids with  $2n = 28$ ); thus, our chromosome data provide new ploidy levels for these species.

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#### IAPT chromosome data 36/3

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\* First chromosome count for the species.

#### FABACEAE

##### Tribe Dipterygeae

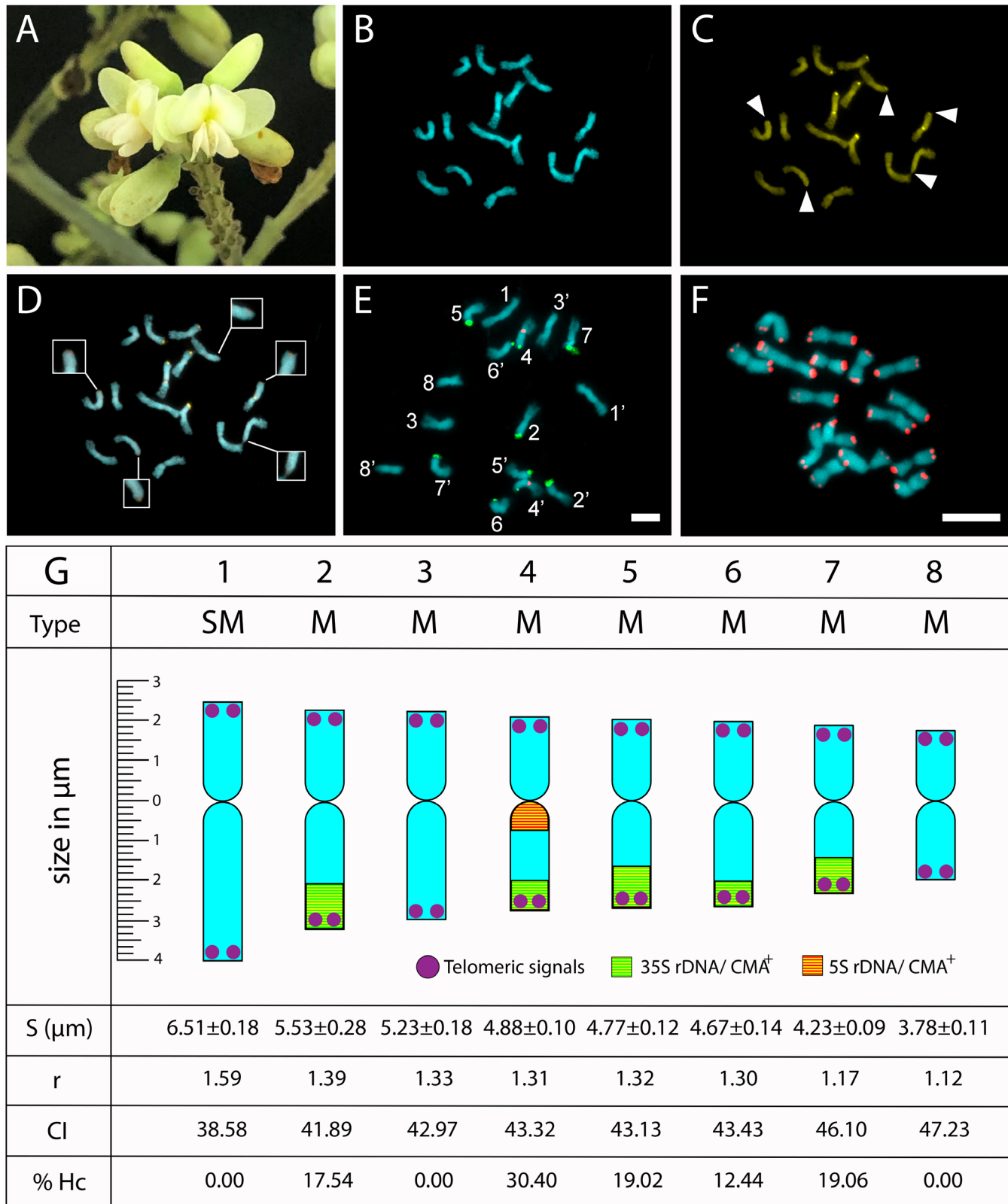
\**Dipteryx lacunifera* Ducke

$2n = 16$ , CHN. Brazil, Piauí, Bom Jesus, 09°04'57.2"S, 44°19'41.8"W, 298 m, 7 Dec 2021, S.C. Silva & M. Lenara (TEPB 205). [Fig. 4]

The genus *Dipteryx* Schreb. belongs to the tropical tribe Dipterygeae Polhill, family Leguminosae-Papilionoideae, and it comprises ~12 species dispersed through South and Central America (Ducke, 1948). The species commonly contains chemical compounds as coumarins, isoflavones, triterpenoids, fatty acids and furanocoumarin diterpenoids (Nakano & Suárez, 1970; Nakano & al., 1979; Godoy & al., 1989; Vieira Júnior & al., 2007), whose properties have aroused interest from food and cosmetic industry (Mendes & Silveira, 1994; Jang & al., 2003).

*Dipteryx lacunifera* Ducke (= *Coumarouna lacunifera* Ducke) is popularly known as Gurgueia's nut, donkey's nut or garampara (Fig. 4A). In Brazil, it can be found in the Amazon forest, Northeast and Central-North regions, mainly in Maranhão and Piauí states.





**Fig. 4.** Flower morphology and chromosome characterization of *Dipteryx lacunifera*. **A**, Flowers; **B**, Chromosome complement ( $2n = 16$ ) stained with DAPI (in blue); **C**, Chromosomes stained with CMA (in yellow), the arrowheads point to weak CMA<sup>+</sup> terminal bands; **D**, DAPI and CMA overlay, inserts evidence weak CMA<sup>+</sup> bands; **E**, 5S and 35S ribosomal DNA localization by FISH (red and green signals, respectively); **F**, Identification of telomeres (in red); **G**, Idiogram presenting chromosome sizes in  $\mu\text{m}$  (S), arm ratios (r), centromere indexes (CI), percentage of heterochromatin per chromosome (% Hc) and chromosome morphologies (SM, submetacentric; M, metacentric). — Scale bars in F and G: 5  $\mu\text{m}$ .

Previous studies indicate the use of its nuts as a supplement in human nutrition due to their quantity of fatty acids and proteins (Carvalho & al., 2008; Cavalcante & al., 2015). Some studies have also reported the potential of this plant in biodiesel production, wood industry and as a natural antibiotic (Ayres & al., 2008; Araújo & al., 2010).

For this analysis, Gurgueia's nut seeds were collected in Bom Jesus (Piauí/Brazil) and after mechanic scarification germinated in greenhouse. The root tips were pretreated with 2 mM 8-hydroxyquinoline (8HQ) for 24 h at 10°C, fixed in Carnoy solution (ethanol/acetic acid, 3 : 1, v/v) for 2–24 h at room temperature and then stored at –20°C. The samples were digested with enzymatic solution containing 2% cellulase (Onozuka-SERVA Electrophoresis, Heidelberg, Germany) and 20% pectinase (Sigma-Aldrich, Darmstadt, Germany) for 2 h at 37°C, and the chromosomes were prepared using the air-drying technique (De Carvalho & Saraiva, 1993). The best slides were selected using a solution of 2 µg/ml DAPI in 50% glycerol and then destained in Carnoy solution and 96% ethanol, for 30 min and overnight, respectively. The CMA/DAPI staining was performed according to the method described by Vaio & al. (2018). The slides were aged for 3 days, stained with CMA 0.1 mg/ml for 30 min and mounted in glycerol/McIlvaine buffer pH 7.0 (1 : 1; v/v) containing 2.5 mM MgCl<sub>2</sub> and 1 µg/ml DAPI.

For fluorescent in situ hybridization (FISH), the pre-treatment of the slides, hybridization mix and probe denaturation were performed according to the protocol described by Fonsêca & al. (2010). Probes for 5S ribosomal DNA (from the clone D2 of *Lotus japonicus*; Pedrosa & al., 2002) and 25–28S, 5.8S and 18S ribosomal DNA (from plasmid pTa71; Gerlach & Bedbrook, 1979) cited here as 35S rDNA, were both obtained by miniprep with the Qiagen Mini-Prep Kit and labelled by nick translation (Thermo Scientific or Invitrogen, Waltham, Massachusetts, U.S.A.) with Cy3-dUTP (Amersham, GE Healthcare, Illinois, U.S.A.) and Alexa-dUTP (Thermo Fisher, Waltham, Massachusetts, U.S.A.), respectively. For the detection of telomeres, an *Arabidopsis*-type telomeric oligo probe labelled with Cy3 (Macrogen, Seoul, South Korea) was used following the methodology of ND-FISH (nondenaturing FISH) described by Cuadrado & al. (2009). The images were captured using a Leica DM5500B microscope coupled to a Leica DFC345 FX camera equipped with LAS AF software v.2.5. The chromosomes from the five best complete metaphases were analysed and measured, and the chromosomal arm ratio (r) and the centromeric index (CI) were used in the classification of each chromosome as metacentric (M), submetacentric (SM) or acrocentric (A), following the nomenclature proposed by Guerra (1986). We also estimated the proportion of heterochromatin per chromosome based on the sum of CMA<sup>+</sup>/DAPI<sup>–</sup> bands measured using Adobe Photoshop.

All the analysed samples presented the same chromosome number,  $2n = 16$  (Fig. 4B–F), varying in chromosome size, from 3.78 to 6.51 µm, and the total haploid chromosome length was 39.6 µm. The identification of the submetacentric morphology in pair 1 and the metacentric in the others, allowed the establishment of the karyological formula  $7M + 1SM$ . The chromosome pairs were ordered from 1 to 8 and organized according to the decreasing length of the short arm (Fig. 4G). Most CMA<sup>+</sup> bands were located in terminal regions of chromosome pairs 2–7 (Fig. 4G). In pair 4, a CMA<sup>+</sup> band was also observed in the proximal region of the long arm. Regarding the ribosomal DNA sites, *Dipteryx lacunifera* presented one 5S ribosomal DNA site in the proximal region of chromosome pair 4, which presented an average size of 0.82 µm (Fig. 4E,G). Additionally, five 35S ribosomal DNA sites were located in the terminal regions of the long arms of the chromosome pairs 2–7, ranging in size from 0.58

(in pair 6) to 0.96 µm (in pair 2) (Fig. 4E,G). All the ribosomal DNA sites were colocalized with CMA<sup>+</sup> bands (Fig. 4G). The telomeric signals were detected in all chromosomes, although with different intensities among chromosomes (Fig. 4F). The proportion of heterochromatin per chromosome pair varied from 12.44% in pair 6 to 30.4% in pair 4, while chromosome pairs 1, 3 and 8 presented no CMA<sup>+</sup> bands nor 5S or 35S ribosomal DNA sites (Fig. 4E,G).

Our results demonstrate the chromosome number of *Dipteryx lacunifera* as  $2n = 16$  and brings a complete cytogenetical characterization that can be applied to other species of the genus in future studies, helping to shed light on the chromosome organization and evolution of the group.

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## IAPT chromosome data 36/4

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Financial support from Conselho Nacional de Desenvolvimento Científico e Tecnológico/CNPq (Research fellowship 310026/2018-0) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior/ CAPES.

\* First chromosome count for the species.

### POACEAE

*Paspalum carinatum* Humb. & Bonpl. ex Flügge  
 $2n = 40$ , CHN. Brazil, Goiás, São Domingos, 13°16'52.80"S, 46°03'19.60"W, 849 m, 13 Dec 2016, *J.F.M. Valls, M.W.S. Sousa & C.A.D. Welker 16606* (CEN).

*Paspalum compressifolium* Swallen  
 $2n = 40$ , CHN. Brazil, Paraná, Castro, 24°46'51.03"S, 50°00'56.23"W, 981 m, 23 Nov 2016, *J.F.M. Valls, M.W.S. Sousa, C.A.D. Welker & A.P. Fávero 16474* (CEN).

\**Paspalum divergens* Döll  
 $2n = 60$ , CHN. Brazil, Rio de Janeiro, Quissamã, Parque Nacional da Restinga de Jurubatiba, 22°10'34.70"S, 41°24'57.40"W, 8 m, 28 Jun 2018, *M.P. Araújo, A.C. Petry & J.F.M. Valls 331* (CEN).

*Paspalum fimbriatum* Kunth  
 $2n = 20$ , CHN. Brazil, Piauí, Monte Alegre do Piauí, 09°45'26.65"S, 45°18'22.04"W, 456 m, 18 May 2019, *M.W.S. Sousa 135* (UB).

*Paspalum foveolatum* Steud.  
 $2n = 40$ , CHN. Brazil, Goiás, Alexânia, Condomínio Porto do Sol, 16°11'33.20"S, 48°29'49.20"W, 882 m, 24 Apr 2019, *J.F.M. Valls & M.L. Paulo 17026* (CEN). Brazil, Goiás, Flores de Goiás, 14°54'37.85"S, 46°57'19.55"W, 530 m, 16 Mar 2016, *J.F.M. Valls, R.C. Oliveira, C. Silva, C.O. Moura & A.C.V. Berto 16189* (CEN).

\**Paspalum giuliettiae* Pimenta, G.H.Rua & R.P.Oliveira  
 $2n = 40$ , CHN. Brazil, Bahia, Lençóis, Poço do Diabo, 12°27'42.30"S, 41°24'52.02"W, 672 m, 22 Dec 2018, *J.F.M. Valls 17012* (CEN).

*Paspalum juergensii* Hack.  
 $2n = 20$ , CHN. Brazil, Rio Grande do Sul, Lagoa Vermelha, 28°21'37.90"S, 51°27'51.00"W, 795 m, 22 Nov 2018, *J.F.M. Valls, L.N. da Silva & E. Valduga 16981* (CEN). Brazil, Santa Catarina, Ponte Alta, 27°31'48.30"S, 50°22'11.70"W, 851 m, 22 Nov 2016, *J.F.M. Valls, M.W.S. Sousa, C.A.D. Welker & A.P. Fávero 16460* (CEN).

*Paspalum lenticulare* Kunth  
 $2n = 20$ , CHN. Brazil, Tocantins, Natividade, 11°42'23.20"S, 47°36'22.70"W, 352 m, 10 Dec 2016, *J.F.M. Valls, M.W.S. Sousa & C.A.D. Welker 16548* (CEN).  
 $2n = 40$ , CHN. Brazil, Goiás, Alto Paraíso de Goiás, 14°25'37.80"S, 47°30'30.60"W, 955 m, 8 Dec 2016, *J.F.M. Valls, M.W.S. Sousa & C.A.D. Welker 16497* (CEN); Brazil, Tocantins, Natividade, 11°39'58.40"S, 47°26'41.60"W, 322 m, 10 Dec 2016, *J.F.M. Valls, M.W.S. Sousa & C.A.D. Welker 16553* (CEN).

*Paspalum maculosum* Trin.  
 $2n = 40$ , CHN. Brazil, Bahia, Barreiras, 11°59'30.81"S, 45°35'10.89"W, 730 m, 12 Dec 2016, *J.F.M. Valls, M.W.S. Sousa & C.A.D. Welker 16586* (CEN).

*Paspalum mandiocanum* Trin.  
 $2n = 60$ , CHN. Brazil, Paraná, Tunas do Paraná, 24°05'04.90"S, 49°06'00.20"W, 923 m, 17 Nov 2016, *J.F.M. Valls, M.W.S. Sousa, C.A.D. Welker & A.P. Fávero 16348* (CEN); Brazil, Paraná, Carambeí, 24°52'17.70"S, 50°02'23.60"W, 1074 m, 23 Nov 2016, *J.F.M. Valls, M.W.S. Sousa, C.A.D. Welker & A.P. Fávero 16468* (CEN).

*Paspalum multicaule* Poir  
 $2n = 20$ , CHN. Brazil, Goiás, Alto Paraíso de Goiás, 14°10'41.90"S, 47°30'40.50"W, 1286 m, 27 Mar 2017, *J.F.M. Valls, H.M. Longhi-Wagner, R.C. Oliveira, C.A.D. Welker & D.M. Ramos 16623* (CEN).

*Paspalum notatum* Flügge  
 $2n = 40$ , CHN. Brazil, Distrito Federal, Brasília, Parque Ecológico Bernardo Sayão, 15°50'25.10"S, 47°48'54.50"W, 1087 m, 17 Jan 2018, *J.F.M. Valls & M.W.S. Sousa 16774* (CEN).

*Paspalum pauciciliatum* (Parodi) Herter  
 $2n = 40$ , CHN. Brazil, São Paulo, Itapetinga, 23°27'54.20"S, 47°57'50.80"W, 616 m, 16 Nov 2016, *J.F.M. Valls, M.W.S. Sousa, C.A.D. Welker & A.P. Fávero 16311* (CEN).

*Paspalum plicatum* Michx.  
 $2n = 40$ , CHN. Brazil, Paraná, Curitiba, 25°24'18.60"S, 49°21'49.30"W, 981 m, 18 Nov 2016, *J.F.M. Valls, M.W.S. Sousa, C.A.D. Welker & A.P. Fávero 16355* (CEN).

*Paspalum redondense* Swallen

$2n = 20$ , CHN. Brazil, Paraná, Cândói, 25°26'02.75"S, 51°48'30.80"W, 960 m, 20 Nov 2016, J.F.M. Valls, M.W.S. Sousa, C.A.D. Welker & A.P. Fávero 16417 (CEN).

*Paspalum regnellii* Mez

$2n = 40$ , CHN. Brazil, Paraná, Guarapuava, 25°21'40.80"S, 51°21'06.60"W, 1081 m, 20 Nov 2016, J.F.M. Valls, M.W.S. Sousa, C.A.D. Welker & A.P. Fávero 16395 (CEN).

*Paspalum scalare* Trin.

$2n = 20$ , CHN. Brazil, Goiás, Alto Paraíso de Goiás, 14°03'05.60"S, 47°30'44.20"W, 1453 m, 27 Mar 2017, J.F.M. Valls, H.M. Longhi-Wagner, R.C. Oliveira, C.A.D. Welker & D.M. Ramos 16652 (CEN).

\**Paspalum thrasyoides* (Trin.) S.Denham

$2n = 40$ , CHN. Brazil, Goiás, Alto Paraíso de Goiás, 14°10'47.20"S, 47°30'42.60"W, 1268 m, 8 Dec 2016, J.F.M. Valls, M.W.S. Sousa & C.A.D. Welker 16509 (CEN).

Polyploidy plays an important role in grass evolution and is well documented among *Paspalum* species, where it is predominant (Ortiz & al., 2013) and appears mainly at the tetraploid level, generally linked to apomixis (Bonasora & al., 2018), and sometimes showing a geographic concentration of plants of a certain ploidy level in distinct areas or ecosystems (Pozzobon & Valls, 2000). Chromosome counting, in this case, is essential to certify the ploidy level, contributing to the knowledge of the mode of reproduction of different species. The identification of sexuality allows us to weave strategies for the conservation of wild populations and for the incorporation of elite accessions to genetic improvement programs.

Meiotic chromosome numbers were determined from pollen mother cells, following Sousa & al. (2017). Young inflorescences were collected in the field or under cultivation in a greenhouse, and fixed in ethanol: glacial acetic acid (3 : 1) for 24 h at room temperature and stored at 4°C in 70% ethanol until analysis. The anthers were macerated and stained with 2% acetic carmine. Cells in diakinesis or metaphase I were used for chromosome counting.

Our results from Brazilian accessions confirm previous counts of  $2n = 20$  for *Paspalum fimbriatum* from the United States (Florida), Dominican Republic, Trinidad and Venezuela (Banks, 1964; Gould & Soderstrom, 1967; Davidse & Pohl, 1972, 1974), *P. juergensii* from Argentina, Bolivia, Brazil, Paraguay (Burson & Bennett, 1971b; Pozzobon & al., 2008; Hojsgaard & al., 2009; Sede & al., 2010; Reutemann & al., 2020) and Uruguay (Vaio & al., 2007 – flow cytometry estimations), *P. multicaule* from Costa Rica, Venezuela and several Brazilian states (Pohl & Davidse, 1971; Davidse & Pohl, 1974; Berto & al., 2020), as well as for the Brazilian endemic species *P. redondense* (Pozzobon & al., 2013; Galdeano & al., 2016) and *P. scalare* (Pozzobon & al., 2008; Berto & al., 2020).

For other species, the number  $2n = 40$  is in agreement with previous reports, as for the annual species of the informal group Plicatula, *Paspalum foveolatum* (Sousa & al., 2020). The documented chromosome number of  $2n = 40$  for *P. carinatum* from Central Brazil is in agreement with the oldest record, from Nicaragua (Davidse & Pohl, 1972), while the species also showed  $2n = 20$  in samples from Venezuela (Davidse & Pohl, 1978), and  $2n = 30$  from the state of Bahia, Brazil (Peñaloza & al., 2008).

As documented here from the northernmost population analyzed so far, the tetraploid level ( $2n = 40$ ), generally associated with reports

of irregular meiotic behavior of Argentine, Brazilian, and Uruguayan populations, clearly predominates in the apomictic *Paspalum pauciciliatum* (Nuñez, 1952; Bashaw & Forbes, 1958; Fernandes & al., 1968, 1974; Bashaw & al., 1970; Pozzobon & al., 2000, 2008; Speranza, 2009), with additional references to a count of  $2n = ca. 38$  and to the report attributed to Fernandes & al. (1974) of “a diploid cytotype with  $2n = 20$ ”, actually resulting from misinformation (Morrone & al., 2006).

*Paspalum maculosum* shows an interesting case of sympatry of diploid and tetraploid individuals, as reported, for example, from Argentina, Corrientes, 17 km south of Santo Tomé (Norrman & al., 1989,  $2n = 20, 40$ ), Bolivia, Santa Cruz, Ñuflo de Chávez, respectively at 10 and 9 km south of Concepción to Lomerio (Norrman & al., 1994,  $2n = 40$ ; Sede & al., 2010, 10 II), as well as reports of  $2n = 20$  or 40 from different counties of Rio Grande do Sul State in Brazil (Rodrigues & al., 2001; Pozzobon & al., 2008), and reference to a mixed population of Misiones, Argentina (Reutemann & al., 2019). Unlike previous records geographically linked to the Amazon or La Plata river basins, the count of  $2n = 40$  reported here is from a population in the state of Bahia, in the São Francisco River basin.

*Paspalum notatum*, documented with  $2n = 40$  from a Nature Conservation Unit in the Brazilian Federal District, has been consistently characterized as an apomictic tetraploid species, including a low number of counts of  $2n = 20$ . Such diploids, for some time recognized as *P. sauræ* (Parodi) Parodi, are eventually found in sympatric populations with the widespread tetraploids (Daurelio & al., 2004). Diploid counts from natural populations are typically concentrated in Argentina (Quarín, 1977), but have also been reported in Rio Grande do Sul State, in Brazil, where, however, the diploid “Pensacola” Bahia grass is often cultivated in pastures, from where it can escape to natural grassland areas (Pozzobon & Valls, 1997), a subject that deserves further investigation (Dahmer & al., 2008; Fachinetto & al., 2018).

Although less intensively studied than *Paspalum notatum* and subject to varying interpretations of its morphological circumscription, which may cast doubt on associated records of chromosome counts, *P. plicatulum* shows a similar situation, with full predominance of tetraploids, such as the one documented here from Paraná State in Brazil, and a few diploid counts reported from Argentina, Brazil and Mexico (Honfi & al., 1990; Pozzobon & al., 2000; Morrone & al., 2006). A single colchicine-induced tetraploidization event of the diploid Argentine accession originally reported as *Honfi 14* (Honfi & al., 1990; Sartor & al., 2009; Weihmüller & al., 2014) triggered a promising forage breeding program involving the induced tetraploid as a female parent in crosses with several apomictic tetraploid species of the informal group Plicatula (Aguilera & al., 2015; Novo & al., 2016, 2019; Saraiva & al., 2021), emphasizing the good prospects for the continuous search for new sexual diploids.

Also a member of the informal group Plicatula, *Paspalum compressifolium* typically shows large populations of morphologically homogeneous tetraploid individuals, as the one documented here, with  $2n = 40$ , in addition to two rare reports of diploid counts from the Brazilian state of Rio Grande do Sul and the adjacent province of Misiones in Argentina, and a single hexaploid count from the Brazilian state of Paraná (Quarín & al., 1996; Hojsgaard & al., 2009).

In previous reports, some conspecific apomictic tetraploids and their occasional sexual diploid counterparts, eventually sympatric, were segregated at the species level, such as *Paspalum notatum*/*P. sauræ* or *P. alnum*/*P. hexastachyum*, a trend now reversed as the mechanisms that regulate the evolution of polyploidy and the

expression of apomixis are better understood (Quarín, 1974; Quarín & Hanna, 1980; Quarín & al., 2001; Daurelio & al., 2004; Ortiz & al., 2013). A similar situation is apparent in the *Plicatula* group, with respect to *P. lenticulare*, recorded as predominantly tetraploid in Bolivia, Paraguay and several Brazilian states (Norrman & al., 1994; Espinoza & al., 2001; Hojsgaard & al., 2009; Marcón & al., 2018; Gonçalves & al., 2021). Diploid cytotypes of this species, usually showing reduced sizes of plants, leaves and spikelets, were identified as *P. limbatum* Henrard, with  $2n = 20$  reported from Argentina, Bolivia and Paraguay (Norrman & al., 1994; Espinoza & al., 2001; Pozzobon & al., 2008; Hojsgaard & al., 2009; Reutemann & al., 2020), but there are also reports of  $2n = 40$ , from Brazil and Paraguay (Adamowski & al., 2005; Pozzobon & al., 2008). Synonymization of *P. limbatum* under *P. lenticulare* has been proposed (Oliveira & Valls, 2008) and is being followed in recent cytogenetic documents (Ortiz & al., 2013; Galdeano & al., 2016; Novo & al., 2019). Therefore, previous counts of  $n = 10$  or  $2n = 20$  are now recorded for *P. lenticulare*, also including a recent one from a very narrow-leaved form from the state of Mato Grosso do Sul, Brazil (Gonçalves & al., 2021). Here we add three more counts for this variable species, including another diploid from the state of Tocantins.

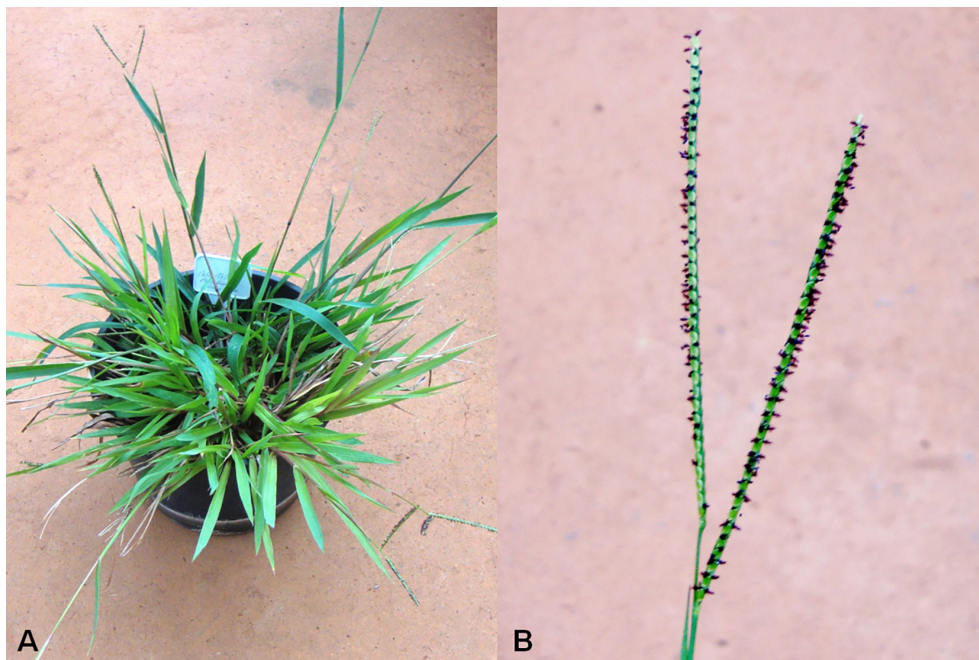
Both counts for *Paspalum mandiocanum* yielded  $2n = 60$ . So far, counts at the pentaploid and hexaploid levels correspond mainly to *P. mandiocanum* var. *mandiocanum* ( $2n = 50$ ) and *P. mandiocanum* var. *subaequiglume* Barreto ( $2n = 60$ ). Four counts from Misiones Province, Argentina show  $2n = 60$ , and are attributed to *P. mandiocanum* var. *subaequiglume* (Honfi & al., 1990; Hojsgaard & al., 2009). The same level of ploidy is demonstrated by seven accessions from the adjacent state of Rio Grande do Sul, Brazil (Burson & Bennett, 1971a; Fernandes & al., 1974; Pozzobon & al., 2008), from where

the variety was described and where it is quite frequent (Barreto, 1965), as well as by two accessions from the states of Santa Catarina and Paraná, in the southern region of Brazil. Pentaploid counts are mainly from more tropical environments, such as the department of Amambay in Paraguay, and a nearby location in Mato Grosso do Sul State, Brazil, or from coastal locations in Santa Catarina and Rio Grande do Sul states (Honfi & al., 1990; Pozzobon & al., 2000, 2008). However, for some specimens, the variety identification is not provided or the count does not match the identified variety. *Paspalum mandiocanum* certainly deserves further sampling and investigation, including the potential segregation of the two cytotypes as distinct species.

This work presents chromosome counts obtained, for the first time, for *Paspalum giuliettiae* and *P. thrasyooides*, with  $2n = 40$ , and for *P. divergens*, with  $2n = 60$  chromosomes.

*Paspalum giuliettiae* (Fig. 5) is endemic to the Chapada Diamantina plateau, in the state of Bahia, and has been documented in sites between  $11^{\circ}30'25''S$  and  $13^{\circ}13'44''S$ , in the municipalities of Morro do Chapéu, Mucujê and Ibicoara. It closely morphologically resembles *P. pumilum* Nees (Pimenta & al., 2013), a widely dispersed species, described from coastal Bahia, so far always reported at the diploid level, with  $2n = 20$ , from Costa Rica and several Brazilian states (Burson & Bennett, 1971a; Pohl & Davidse, 1971; Fernandes & al., 1974; Morrone & al., 1995; Pozzobon & al., 2000, 2008; Peña-loza & al., 2008). Our collection site ( $12^{\circ}27'42''S$ , in the municipality of Lençóis) is geographically intermediate, and yielded  $2n = 40$ , establishing yet another diagnostic difference for *P. pumilum*.

*Paspalum thrasyooides* is a member of the *P.* subg. *Harpostachys* (Trin.) S. Denham, of which nine other species also showed the predominant tetraploid level, with  $2n = 40$ , besides rare diploid and a few other counts (Denham, 2005). This first cytogenetic information



**Fig. 5.** *Paspalum giuliettiae*. **A**, Plant transplanted directly from the natural collection site in the municipality of Lençóis, state of Bahia (J.F.M. Valls 17012), to a screen house in Brasília, Distrito Federal, Brazil, from where samples were taken for chromosome counting; **B**, Inflorescence at the end of anthesis, showing the long insertion interval of the inflorescence branches, a characteristic that distinguishes the tetraploid *P. giuliettiae* from the morphologically close diploid *P. pumilum*. — Photos: José F.M. Valls.

about *P. thrasyooides* is associated with irregular meiotic behavior, which may indicate that the common conspecificity of sexual diploids and apomictic tetraploids in *Paspalum* extends to this subgenus, as already confirmed for *P. foliiforme* S.Denham, also a member of *Harpostachys*, under the synonym *Thrasya petrosa* (Trin.) Chase (Acuña & al., 2005).

*Paspalum divergens* is a Brazilian endemic, as well as *P. giuliettiae* described from the state of Bahia, but showing a clear preference for more coastal and maritime environments. Although collected in Olinda, state of Pernambuco, as early as 1935 (Pickel 3773), knowledge about its distribution was only expanded as of 2010, with an increase in collections in the northeastern states of Sergipe, Pernambuco and Rio Grande do North (Maciel & al., 2009). However, the specimen counted here for the first time, with  $2n = 60$  chromosomes, also documents a southern extension, towards Quissamã, in the state of Rio de Janeiro. The species has been assigned to the informal group Caespitosa (Maciel & al., 2009; Delfini & al., 2019), where the compiled chromosome counts correspond to diploid, triploid and tetraploid levels (Pozzobon & al., 2013; Delfini & al., 2019), so the hexaploid level shown by *P. divergens* is very peculiar.

Although the germplasm studied here is predominantly tetraploid and possibly with apomictic reproduction, its evaluation can help in the selection of promising materials for use in breeding and, consequently, in obtaining new forage cultivars.

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## IAPT chromosome data 36/5

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This study was supported by Agencia Nacional de Promoción Científica y Técnica (ANPCyT) grant no. PICT-2017-4203 and Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), and a postdoctoral fellowship from CONICET to AVR and doctoral fellowships to MCP and FE.

- \* First gametic chromosome count for the cytotype.
- \*\* First somatic chromosome count for a specimen from Argentina.
- ▼ New cytotype for the species.
- First somatic chromosome count for a specimen from Paraguay.

## POACEAE Subfamily Panicoideae Tribe Paspaleae

### • *Paspalum arundinellum* Mez

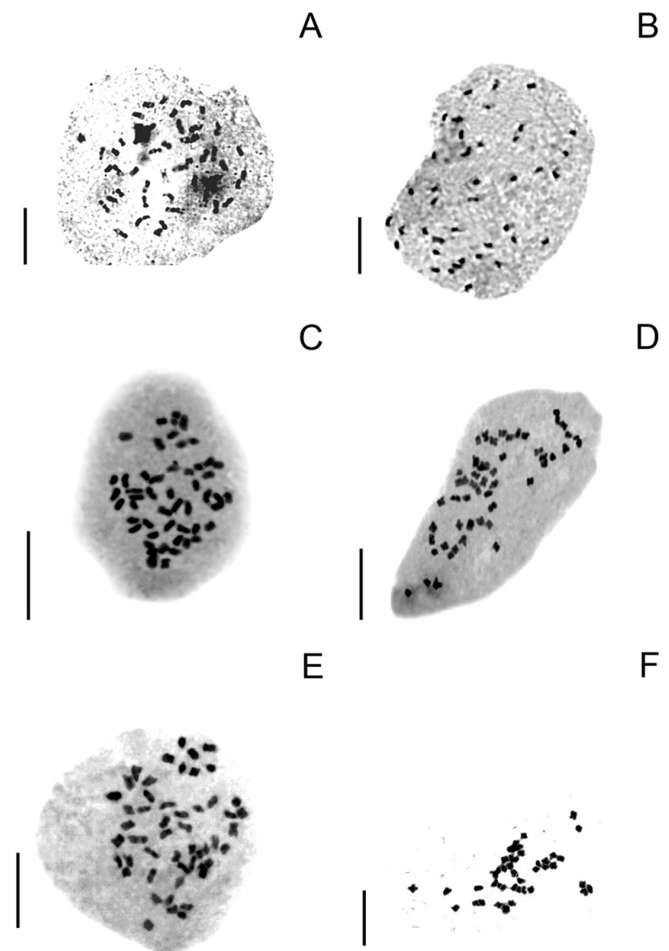
$2n = 50$ , CHN. Argentina, Formosa Province, National Route 11, 5 km S of Clorinda, 2 Jun 2000, *A.I. Honfi* & *J.R. Daviña* 1107 (CTES, MNES); *A.I. Honfi* & *J.R. Daviña* 1108 (CTES, MNES, SI); Argentina, Misiones Province, Capital Department, Nemesio Parma, 22 Jul 1993, *A.I. Honfi* 297 (MNES) [Fig. 6A]. Paraguay, Cordillera Department, between Tobatí and Caacupé, 1 Dec 2000, *J.R. Daviña* & *A.I. Honfi* 495 (CTES, MNES, SI).

This pentaploid cytotype ( $2n = 5x = 50$ ) has already been reported in other Argentinian locations (Honfi & al., 1990; Hojsgaard & al., 2009; Reutemann & al., 2020) but not for Paraguay.

### *Paspalum buckleyanum* Vasey

$2n = 40$ , CHN. Argentina, Salta Province, National Route 9, 31 Mar 2012, *A.I. Honfi* 1586 (MNES); Argentina, Formosa Province, Las Lomitas, 2 Apr 2012, *A.I. Honfi* 1600 #4 (MNES) [Fig. 6B].

$2n = 50$ , CHN. Argentina, Formosa Province, Las Lomitas, 2 Apr 2012, *A.I. Honfi* 1600 #1, 2, 3, 3' (MNES); Argentina,



**Fig. 6.** Mitotic chromosomes. **A**, *Paspalum arundinellum*,  $2n = 50$  (*A.I. Honfi* & *J.R. Daviña* 1107); **B**, *P. buckleyanum*,  $2n = 40$  (*A.I. Honfi* 1600 #4); **C–E**, *P. buckleyanum*,  $2n = 50$ : **C**, *A.I. Honfi* 1600 #2; **D**, *A.I. Honfi* 1600 #3; **E**, *A.I. Honfi* 1736; **F**, *P. compressifolium*,  $2n = 40$  (*A.I. Honfi* & *J.R. Daviña* 2276). — Scale bars = 10  $\mu\text{m}$ .



Corrientes Province, Route 24, 20 Feb 2015, *A.I. Honfi 1736* (MNES); Argentina, Corrientes Province, Route 29, 7 Dec 2015, *A.I. Honfi 2127* (MNES). [Fig. 6C,D,E]

The studied individuals of *Paspalum buckleyanum* from the *A.I. Honfi 1600* population confirm the occurrence of different ploidy levels within a species. Several previous works report that the species is a polyploid agamic complex, with sexual diploids in Paraguay, and a series of polyploids comprising tetraploids, pentaploids, hexaploids and heptaploids, which occur in Paraguay and to the north from Argentina (Burson 1997; Hojsgaard & al., 2009; Rua & al., 2010; Sartor & al., 2011). Interestingly, despite its odd condition, pentaploids are commonly found in natural populations, probably because they are facultative apomicts (Burson, 1997).

*\*Paspalum commune* Lillo

$n = 20$ , CHN. Argentina, Jujuy Province, P.N. Calilegua, Ruta Prov. 83, El Mirador, 20 Jan 2002, *V. Solis Neffa 681* (BAA, CTES, MNES, SI, US). [Fig. 7A,B]

$2n = 40$ , CHN. Argentina Tucumán Province, Ruta Nacional 9, between Tucumán and Salta, 1 Apr 2012, *A.I. Honfi 1585* (MNES).

This species belongs to the informal group Virgata of *Paspalum* (Chase, 1929; Zuloaga & Morrone, 2005). Our counts of  $2n = 40$  agree with Hunziker & al. (1998), Morrone & al. (2006) and Hojsgaard & al. (2009). Meiotic behavior is frequently regular in the studied accessions. The most frequent chromosomal associations at diakinesis and metaphase I were the bivalents, whereby 20 bivalents (20 II) that segregated equally to the poles were often observed

(81.81%). However, other chromosome associations were also found in low frequencies, i.e., 18 II + 1 IV (5.45%); 15 II + 1 IV + 1 VI, 17 II + 1 VI (3.63% each); or 13 II + 2 IV + 1 VI, 1 I + 12 II + 1 III + 3 IV, 16 II + 2 IV (1.81% each) [Fig. 7A,B, Table 1]. In these last cases, the chromosomes migrated to the poles as laggards and sometimes a bridge was also observed. Meiotic behavior indicates that this species is an allotetraploid, with heterozygous translocations involving at least three chromosome pairs.

*\*\*Paspalum compressifolium* Swallen

$2n = 40$ , CHN. Argentina, Misiones Province, San Ignacio Department, Teyú Cuaré, 15 Feb 2011, *A.I. Honfi 1535#1* (MNES); Argentina, Misiones Province, Capital Department, Posadas, margins of Mártires Stream, 10 Oct 2017, *A.I. Honfi & J.R. Daviña 2276* (MNES). [Fig. 6F]

This is the first report of tetraploids in a material of *Paspalum compressifolium* from Argentina (Fig. 6F). Quarin & al. (1996) studied the genetic systems of diploids, tetraploids and hexaploids ( $2n = 20, 40, 60$ ) from Brazil. In Argentina, diploid cytotypes from Posadas and Teyú Cuaré, Misiones, were also reported (Hunziker & al., 1998; Hojsgaard & al., 2009). Our new count of  $2n = 40$  in individuals from Posadas and Teyú Cuaré indicates that the species might have a multiploid ( $2x$  and  $4x$ ) contact zone at these localities 100 km distant from each other.

*Paspalum conjugatum* P.J.Bergius

$2n = 40$ , CHN. Argentina, Misiones Province, Montecarlo Department, Colonia Guatambú, 9 Apr 2006, *A.I. Honfi 1299* (MNES). Puerto Rico, Aibonito, Cordillera Central, 4 Aug 2009, *M. Worthington 35705* (CTES, UTEP); Puerto Rico, Cordillera Central, 16 Jul 2014, *M. Worthington 37480* (CTES, UTEP).

Our counts confirm the somatic chromosome number  $2n = 40$  for the studied accessions as it was reported for other materials by several authors (Hojsgaard & al., 2009, and references therein). This is a cosmopolitan species with several ploidy levels, and the tetraploid cytotype is the most frequent ploidy level found in natural populations.

*Paspalum conspersum* Schrad.

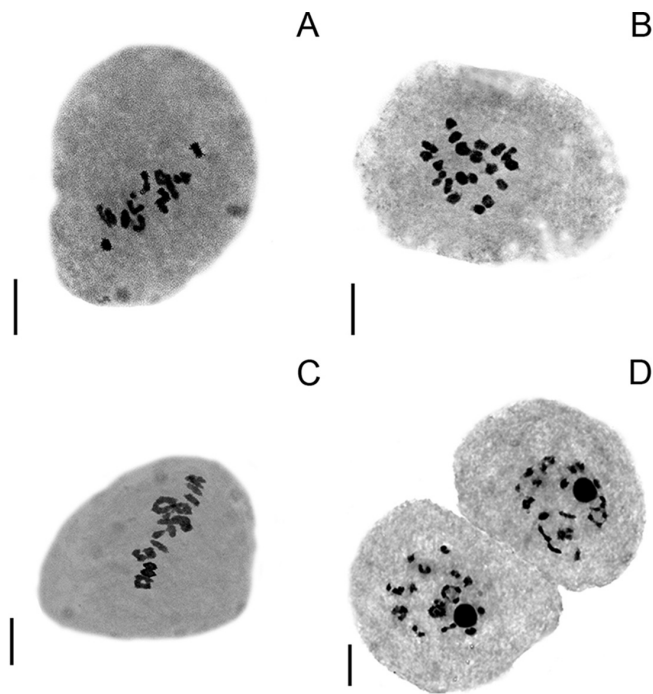
$n = 30$ , CHN. Argentina, Misiones Province, General San Martín Department, 3 de Mayo Stream and Paraná River, 21 Apr 2001, *D.H. Hojsgaard 175* (CTES, MNES); Argentina, Misiones Province, Port of Puerto Rico, on Paraná River, 10 Mar 2001, *A.I. Honfi 1143* (MNES).

The somatic chromosome count confirms the previous ploidy reports for this self-fertile species (Quarin & Hanna, 1980; Hojsgaard & al., 2009; Reutemann & al., 2019). In all pollen mother cells, the chromosomes behave as 30 bivalents at diakinesis and metaphase I (Table 1). Quarin & Hanna (1980) observed the same meiotic behavior in a single plant.

*Paspalum dedecae* Quarin

$2n = 40$ , CHN. Argentina, Misiones Province, Capital Department, Zaimán Stream swamps, 21 Sep 1995, *A.I. Honfi 649* (CTES, MNES, SI).

This is a multiploid South American species, with diploid and tetraploid cytotypes. Diploids are found in Brazil (Honfi & al., 1990; Quarin & Burson, 1991; Pozzobon & al., 2000), whilst the tetraploids occur in south Brazil and northeastern Argentina (Hojsgaard & al., 2009). In nature, this is a rare species, specialized to grow in swamps and marshes.



**Fig. 7.** Meiotic chromosomes. **A & B**, *Paspalum commune*,  $n = 20$  (*V. Solis Neffa 681*): **A**, Pollen mother cell (PMC) at diakinesis with 13 II + 2 IV + 1 VI; **B**, *P. commune*,  $n = 20$  (*V. Solis Neffa 681*), PMC at metaphase I with 20 II; **C**, *P. denticulatum*,  $n = 20$  (*D.H. Hojsgaard 256*), PMC at metaphase I with 10 II + 5 IV; **D**, *P. plicatulum*,  $n = 20$  (*A.I. Honfi & J.R. Daviña 1278*), PMC at metaphase I with 16 II + 2 IV. — Scale bars = 10  $\mu$ m.

*Paspalum denticulatum* Trin.

$n = 20$ , CHN. Argentina, Misiones Province, Capital Department, Posadas, 31 Dec 2002, *D.H. Hojsgaard 256* (CTES, MNES, SI). [Fig. 7C]

Our gametic chromosome count for *Paspalum denticulatum* agrees with a previous report made by Quarin & Burson (1991). The meiotic chromosome pairing suggests that the species is a segmental allotetraploid (Table 1).

▼ *Paspalum ellipticum* Döll

$2n = 60$ , Argentina, Misiones Province, Capital Department, Zaimán Stream swamps, 21 Jan 1995, *A.I. Honfi 648* (CORD, CTES, MNES).

Our mitotic chromosome count for *Paspalum ellipticum* is a new cytotype for the species. Previous reports comprised three cytotypes for the species, rare diploids and tetraploids ( $n = 20$  II) from Brazil and frequent octoploids from Brazil and northern Argentina (Fernandes & al., 1974; Morrone & al., 2006; Souza Chies & al., 2006; Hojsgaard & al., 2009). Furthermore, the hexaploid individual studied here was sampled at the same collection locality as the octoploid individual reported by Hojsgaard & al. (2009). Thus, this species represents a complex with  $2x$ ,  $4x$ ,  $6x$  and  $8x$  ploidy levels.

*Paspalum glaucescens* Hack.

$2n = 40$ , CHN. Argentina, Misiones Province, Candelaria Department, Bonpland, 17 Dec 2005, *M.A. Sader 89* (MNES). [Fig. 8A]

This species belongs to the Plicatula group of *Paspalum*, which comprises several important forage species that are native in South America. In this group, tetraploid is the most frequent chromosome number reported for nearly all the species. However, in *P. glaucescens*, diploids are found inhabiting northern Argentina and southern Brazil, and tetraploids and hexaploids occur in central and southern Brazil. Our tetraploid count for the species agrees with previous reports made by Pozzobon & al. (2000) on Brazilian materials, by Hojsgaard & al. (2009) on Paraguayan materials, and by Morrone & al. (2006) and Reutemann & al. (2019, 2020) on Argentinian materials.

*Paspalum guenoarum* Arechav.

$2n = 40$ , CHN. Argentina, Misiones Province, Capital Department, Santa Inés, 3 Feb 1993, *A.I. Honfi 223* (CORD, CTES, MNES); Argentina, Misiones Province, Candelaria Department, Bonpland, 4 Mar 1993, *J.R. Daviña 161* (MNES). [Fig. 8B]

Our count for this taxon is in concordance with all previous reports for this species (i.e., Pozzobon & al., 2000; Hojsgaard & al., 2009 and references therein). Like *Paspalum glaucescens*, *P. guenoarum* belongs to the Plicatula group, where tetraploidy is associated to apomixis as a reproduction mode, and few tetraploids were found to provide a source of genetic variability for forage improvement in this species (Aguilera & al., 2011; Novo & al., 2017).

*Paspalum inaequivalve* Raddi

$n = 30$ , CHN. Argentina, Misiones Province, Candelaria Department, Santa Ana, Yabebiry Stream, 8 Nov 2001, *D.H. Hojsgaard 221* (MNES).

The meiotic behavior of *Paspalum inaequivalve* was regular in all studied pollen mother cells. Chromosomes paired forming 30 bivalents (30 II) at diakinesis and metaphase I, suggesting the cytotype is an allohexaploid (Table 1). This species belongs to the Corcovadensia group of *Paspalum* (Pozzobon & al., 2000) or to the Inaequivalvia group (Zuloaga & Morrone, 2005). In addition, *P. inaequivalve* reproduces sexually, and the ploidy report in this work agrees with previous data (Honfi & al., 1990; Quarin & Burson, 1991; Hunziker & al., 1998; Hojsgaard & al., 2009).

*Paspalum intermedium* Munro ex Morong & Britton

$2n = 40$ , CHN. Argentina, Misiones Province, Capital Department, Villa Lanús 23 Oct 1993, *A.I. Honfi 387* (MNES). [Fig. 8C]

*Paspalum intermedium* has diploid and tetraploid populations across its geographical range, each ploidy showing a segregated latitudinal distribution (Karunaratne & al., 2018). Our count of  $2n = 4x = 40$  chromosomes agrees with the cytogeographical distribution of tetraploids in South America, particularly in the north of Argentina.

*Paspalum ionanthum* Chase

$2n = 40$ ,  $n = 20$ , CHN. Argentina, Corrientes Province, San Cosme Department, San Cosme, 5 Jan 2006, *M.A. Sader 85* (MNES).

In *Paspalum ionanthum*, the meiotic behavior was regular in all the studied pollen mother cells (Table 1). Chromosomes associated as 20 II at diakinesis and metaphase I, and segregated normally to the poles. The chromosome pairing behavior indicates an allotetraploid origin for this species. Burson & Bennett (1970) and Quarin & Hanna (1980) reported similar meiotic analyses. This species belongs to the Notata group of *Paspalum*, whose species have a valuable use as a forage in South America.

**Table 1.** Meiotic chromosome pairing in seven *Paspalum* species.

Species and vouchers	Ploidy level	No. of PMCs	Average chromosomal associations (and range) per PMCs at diakinesis/metaphase I				
			I	II	III	IV	VI
<i>P. commune</i> (Solis Neffa 681)	4x	55	0.018 (0–1)	19.254 (12–20)	0.018 (0–1)	0.218 (0–3)	0.090 (0–1)
<i>P. conspersum</i> (Hojsgaard 175)	6x	76		30			
<i>P. denticulatum</i> (Hojsgaard 256)	4x	12		10		5	
<i>P. inaequivalve</i> (Hojsgaard 221)	6x	23		30			
<i>P. ionanthum</i> (Sader 85)	4x	22		20			
<i>P. plicatum</i> (Honfi & Daviña 1278)	4x	14	0.071 (0–1)	14.78 (10–20)		2.57 (0–5)	
<i>P. regnellii</i> (Hojsgaard 171)	4x	29		20			

PMC, pollen mother cell; I, univalents; II, bivalents; III, trivalents, IV, tetravalents, VI, hexavalents.

*Paspalum malacophyllum* Trin.

$2n = 40$ , CHN. Argentina, Córdoba Province, Punilla Department, Huerta Grande, 22 Sep 2003, D.H. Hojsgaard 325 (MNES).

Our counts of  $2n = 40$  agree with previous reports on this native American species. Germplasm accessions from northern Argentina, Brazil, Bolivia and Paraguay are exclusively tetraploids (Hojsgaard & al., 2008, 2009; Zilli & al., 2014 and references therein).

*Paspalum pauciciliatum* (Parodi) Herter

$2n = 40$ , CHN. Argentina, Misiones Province, Capital Department, Posadas, 29 Jan 1992, A.I. Honfi 167 (MNES).

This species is phylogenetically related to *Paspalum dilatatum* within the Dilatata group, and our chromosome count agrees with all previous reports (Honfi & al., 1990; Morrone & al., 2006; Hojsgaard & al., 2009).

*Paspalum plicatum* Michx.

$2n = 40$ , CHN. Argentina, Misiones Province, San Ignacio Department, Teyú Cuaré, 15 Feb 2011, A.I. Honfi & J.R. Daviña 1533 (MNES). Paraguay, Central Department, Limpio, margins of

Paraguay River, 8 Mar 2004, A.I. Honfi & J.R. Daviña 1238 (MNES).

$n = 20$ , CHN. Paraguay, Central Department, San Lorenzo 26 Apr 2001, A.I. Honfi & J.R. Daviña 1121 (MNES); Paraguay, Presidente Hayes Department, on National Rout 9, near Villa Hayes, 5 Mar 2006, A.I. Honfi & J.R. Daviña 1278 (MNES). [Fig. 7D]

The informal group Plicatula within the genus *Paspalum* comprises several species allied to *P. plicatum* (Brownseed *Paspalum*). In this group, the most frequent cytotype detected among its species is the tetraploid one, and diploids are rarely found. Our count of  $2n = 40$  for *P. plicatum* agrees with this situation, and corroborates previous works (e.g., Honfi & al., 1990; Hojsgaard & al., 2009; Novo & al., 2019). Chromosomal behavior during meiosis was complex, with varying frequencies of bivalents and quadrivalents. For instance, in the Honfi 1121 accession we observed 20 II or 12 II + 4 IV at diakinesis or metaphase I in pollen mother cells, while in Honfi 1278, the most frequently observed chromosomal configurations were 14 II + 3 IV and 16 II + 2 IV (Fig. 7D, Table 1).

*Paspalum regnellii* Mez

$2n = 40$ ,  $n = 20$ , CHN. Argentina, Misiones Province, Candelaria Department, Bonpland, 19 Mar 1991, A.I. Honfi 120 (MNES); Argentina, Misiones Province, Eldorado Department, Salto Küppers, 30 Mar 1991, A.I. Honfi 131B (MNES). [Fig. 8D]

Our count agrees with the chromosome number and meiotic behavior previously reported for this taxon, a sexual self-fertile tetraploid species (Norrman, 1981; Honfi & al., 1990; Pagliarini & al., 1998; Hojsgaard & al., 2009).

*Paspalum rufum* Nees ex Steud.

$2n = 40$ , CHN. Argentina, Corrientes Province, Route 123 and Miriñay River, 30 Apr 2010, A.I. Honfi 1462 (MNES).

There are diploid, triploid and tetraploid cytotypes in *Paspalum rufum* (Quarin & al., 1998; Sartor & al., 2011). Our tetraploid report for this species agrees with previous works; however, our count is the first report of tetraploids from Corrientes (Quarin & al., 1998; Siena & al., 2008; Sartor & al., 2011).

*Paspalum simplex* Morong ex Britton

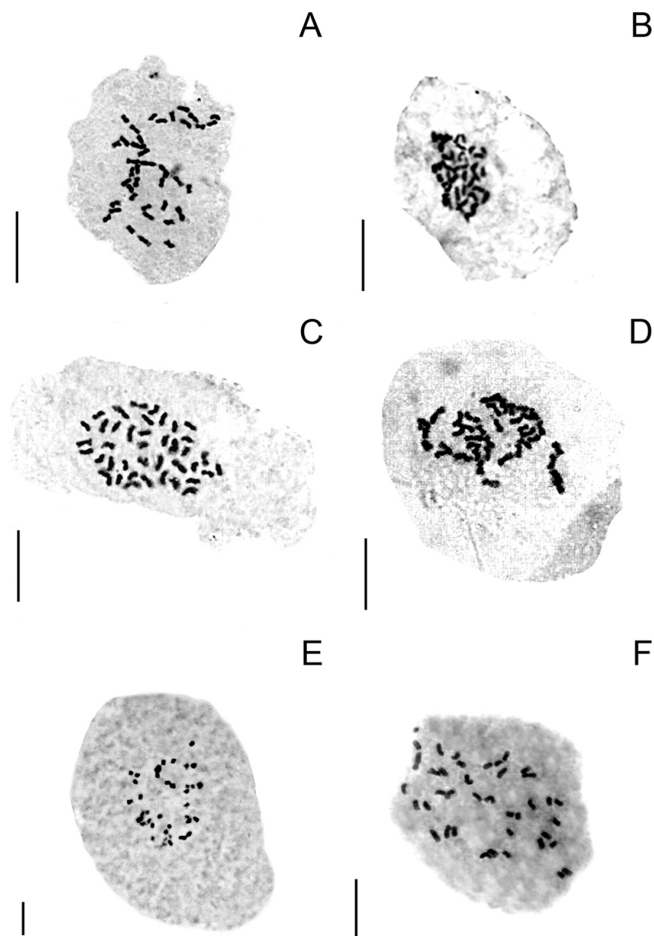
$2n = 40$ , CHN. Argentina, Misiones Province, Capital Department, Parada Leis, 12 Feb 2012, A.I. Honfi 2560 (MNES).

*Paspalum simplex* is considered a high-quality forage grass distributed throughout the phytogeographic Chaco region in South America (Urbani & al., 2002). Diploid, triploid, tetraploid and hexaploid cytotypes were found in Argentina, while tetraploids and hexaploids were found in Paraguay (Hojsgaard & al., 2009; Rivarola & al., 2016), and in Brazil and Bolivia only tetraploids occur (Pozzobon & al., 2000; Urbani & al., 2002). Unexpectedly, the *P. simplex* plant reported here was collected in Misiones Province, and is tetraploid. Our count agrees with previous studies and is the first report for this species from Misiones, Argentina.

*Paspalum unispicatum* (Scribn. & Merr.) Nash

$2n = 40$ , CHN. Argentina, Córdoba Province, Punilla Department, Uritorco, 3 Jan 2006, M.A. Sader 88 (MNES); Argentina, Salta Province, Campo Quijano, 1 Apr 2012, A.I. Honfi 1590 (MNES). [Fig. 8E,F]

This is a species with perennial, rhizomatous plants belonging to the informal group Decumbentes. It grows in fields, along roadsides and river ravines and on modified soils (Zuloaga & Morrone, 2005; Denham & Zuloaga, 2007). The accessions Sader 88 and Honfi



**Fig. 8.** Mitotic chromosomes. **A**, *Paspalum glaucescens*,  $2n = 40$  (M.A. Sader 89); **B**, *P. guenoarum*,  $2n = 40$  (J.R. Daviña 161); **C**, *P. intermedium*,  $2n = 40$  (A.I. Honfi 387); **D**, *P. regnellii*,  $2n = 40$  (A.I. Honfi 131B); **E & F**, *P. unispicatum*,  $2n = 40$ : **E**, M.A. Sader 88; **F**, A.I. Honfi 1590. — Scale bars = 10  $\mu\text{m}$ .

1590 were tetraploids ( $2n = 4x = 40$ ), agreeing with previous reports for the species (Burson, 1997; Hojsgaard & al., 2009; Galdeano & al., 2016). Burson (1997) reported a tetraploid accession from grasslands of Uruguay, Hojsgaard & al. (2009) reported tetraploid plants from Salta and Jujuy in Argentina, and Galdeano & al. (2016) reported diploids, triploids and tetraploids from north Argentina, particularly from Chaco and Corrientes. The tetraploid plants studied here came from central and northwest Argentina, pointing to a probable widespread distribution of the tetraploid cytotype in southern South America.

All the chromosome numbers reported here for *Paspalum* species have a base chromosome number  $x = 10$ . Natural populations can be monoploid if only a single cytotype is found, or multiploid when two or more distinct ploidy levels occur in the population. We found a multiploid population in *P. buckleyanum*, and a possible occurrence of multiple cytotypes in the same locality in *P. compressifolium*. Both species are fodder grasses in natural grasslands, and a valuable genetic resource for domestication. Since diploids of *Paspalum* reproduce sexually while polyploids reproduce either by sexual means or by apomixis (Ortiz & al., 2013), the polyploid plants reported here open up possibilities for reproductive and agronomic studies. In this work, we broaden the cytological knowledge in species of *Paspalum* from Argentina and Paraguay as a contribution to ongoing phylogenetic, reproductive and biogeographical studies. The chromosome numbers of the studied accessions will be incorporated to the database of ex situ germplasm collection.

#### METHODS

Chromosome numbers were analyzed following the methodology described in Hojsgaard & al. (2009) and Honfi & al. (2021). Young inflorescences for meiotic studies were fixed in ethanol : glacial acetic acid (3:1) for 24 h at room temperature and then stored at 4°C in 70% ethanol until analysis. Anthers were macerated in a glass slide, and stained with 2% acetocarmine. Permanent slides were made using Venetian Turpentine Solution. In each case, selected microsporocyte cells at specific stages of meiosis were analyzed. Chromosome associations were assessed in at least 10 cells at diakinesis or metaphase I per accession.

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### IAPT chromosome data 36/6

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This study was supported by Agencia Nacional de Promoción Científica y Técnica (ANPCyT) grant nos. PICT-2016-1637, 2017-4203, by Universidad Nacional de Misiones (UNaM) 16Q1240-PI and Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) and a doctoral fellowship from CONICET to OARM and ACGA.

Chromosomes were counted according to the method of Daviña & Fernández (1989) and Daviña (2001).

▼ New cytotype for the species.

### AMARYLLIDACEAE

Amaryllidaceae family comprise 860 species and 59 genera with mainly tropical distribution (Meerow & Snijman, 1998) with the characteristics of ornamental plants. The Hippeastreae tribe has a major center of diversification in central Chile and western Andean Argentina and a second center in eastern Brazil and northeastern Argentina (Meerow & Snijman, 1998; Arroyo-Leuenberger & Dutilh, 2008). In this work, populations of *Habranthus* and *Hippeastrum* species from Argentina were cytogenetically studied.

*Habranthus brachyandrus* (Baker) Sealy

$2n = 4x = 24$ , CHN. Argentina, Misiones Province, Capital Department, Posadas, 27°21'S, 55°53'W, 27 Mar 2017, *O.A. Rodríguez Mata 2* (MNES); Argentina, Misiones Province, Capital Department, Posadas, 25°22'S, 55°53'W, 8 Jan 2017, *O.A. Rodríguez Mata 3* (MNES); Argentina, Misiones Province, Capital Department, Posadas, 25°22'S, 55°53'W, 8 Jan 2017, *O.A. Rodríguez Mata 4* (MNES); Argentina, Misiones Province, Capital Department, Posadas, 27°37'S, 55°53'W, 10 Jan 2017, *O.A. Rodríguez Mata 7* (MNES); Argentina, Misiones Province, Iguazú Department, 25°36'S, 54°34'W, 19 Apr 2017, *O.A. Rodríguez Mata 5* (MNES). [Fig. 9A]



**Fig. 9.** Mitotic chromosomes with conventional staining; mitotic metaphase. **A**, *Habranthus brachyandrus*,  $2n = 24$  (*Rodríguez Mata 2*); **B**, *Hippeastrum striatum*,  $2n = 22$  (*Rodríguez Mata 14*); **C**, *H. striatum*,  $2n = 55$  (*Rodríguez Mata 11*). — Scale bars = 10  $\mu$ m.

This genus has several basic numbers:  $x = 6, 7, 9, 11, 13$  and  $15$  (Schnack & Covas, 1947; Coe, 1954; Darlington & Wylie, 1955; Naranjo, 1969, 1974). The basic chromosome number  $x = 6$  is the most frequent one and has the greatest amount of polyploids and aneuploids. In the different provenances of *Habranthus brachyandrus* analyzed, our count is in agreement with previous reports (Flory & Flagg, 1958; Daviña & Honfi, 2018).

*Hippeastrum striatum* (Lam.) H.E.Moore

$2n = 2x = 22$ , CHN. Argentina, Misiones Province, Capital Department, Posadas, 27°37'S, 55°53'W, 21 Apr 2017, *O.A. Rodríguez Mata 1*, *O.A. Rodríguez Mata 3* (MNES); Argentina, Misiones Province, Oberá Department, 27°29'S, 54°54'W, 7 Apr 2017, *O.A. Rodríguez Mata 14* (MNES). [Fig. 9B]

▼  $2n = 5x = 55$ , CHN. Argentina, Buenos Aires Province, Capital Department, 34°59'S, 58°39'W, 2 Feb 2017, *O.A. Rodríguez Mata 8* (MNES); Argentina, Misiones Province, Candelaria Department, 27°21'S, 55°36'W, 14 Apr 2007, *A.I. Honfi 1311* (MNES); Argentina, Misiones Province, Candelaria Department, 27°22'S, 55°39'W, 3 May 2014, *A.I. Honfi 1712* (MNES); Argentina, Misiones Province, Candelaria Department, 27°21'S, 55°36'W, 8 Nov 2015, *A.I. Honfi 2104A* (MNES); Argentina, Misiones Province, Candelaria Department, 27°22'S, 55°39'W, 30 Apr 2015, *A.I. Honfi 2046* (MNES); Argentina, Misiones Province, Capital Department, Posadas, 27°37'S, 55°53'W, 10 Jan 2017, *O.A. Rodríguez Mata 6* (MNES); Argentina, Misiones Province, Capital Department, Posadas, 27°22'S, 55°53'W, 2 Feb 2017, *O.A. Rodríguez Mata 11* (MNES); Argentina, Misiones Province, Capital Department, Posadas, 27°22'S, 55°53'W, 7 Apr 2017, *O.A. Rodríguez Mata 12* (MNES); Argentina, Misiones Province, Capital Department, Posadas, 27°37'S, 55°09'W, 29 Sep 2018, *O.A. Rodríguez Mata 15* (MNES); Argentina, Misiones Province, Capital Department, Posadas, 27°37'S, 55°53'W, 29 Sep 2018, *O.A. Rodríguez Mata 16* (MNES); Argentina, Misiones Province, Capital Department, Posadas, 27°22'S, 55°53'W, 29 Sep 2018, *O.A. Rodríguez Mata 17* (MNES); Argentina, Misiones Province, Capital Department, Posadas, 27°36'S, 55°53'W, 29 Sep 2018, *O.A. Rodríguez Mata 18* (MNES); Argentina, Misiones Province, Capital Department, Posadas, 27°39'S, 55°53'W, 7 Oct 2018, *O.A. Rodríguez Mata 19* (MNES); Argentina, Misiones Province, Oberá Department, 27°29'S, 55°08'W, 21 Nov 2006, *J.R. Daviña 609* (MNES). [Fig. 9C]

In mitosis,  $2n = 2x = 22$  chromosomes were observed in two accessions, while in the other accessions, the study of mitotic chromosomes indicated that they belong to a new pentaploid cytotype for the species, with  $2n = 5x = 55$  chromosomes. This cytotype of *Hippeastrum striatum* is a new polyploid for the genus.

The species of the genus *Hippeastrum* present a great karyotypic stability and a low occurrence of polyploidy (Naranjo & Andrada, 1975; Meerow, 1984). The number of chromosomes of the new pentaploid cytotype agrees with the basic chromosome number  $x = 11$  for the genus (Inariyama, 1937; Sato, 1938; Goldblatt, 1976).

The chromosome counts made in these species from different accessions contribute to the cytogeographic location of the populations, as

well as, to the incorporation of new genotypes to the ex situ germplasm bank for future uses in genetic improvement of ornamental bulb plants.

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