

IAPT CHROMOSOME DATA

IAPT chromosome data 31

Karol Marhold (ed.),^{1,2}  Jaromír Kučera (ed.),¹  Camila Aguiar-Melo,³  Erton Mendonça de Almeida,⁴ 
 Lânia Isis Ferreira Alves,⁵  Tatyana V. An'kova,⁶  Fernanda Bered,^{3,7}  Kallyne Bonifácio,⁸ 
 Luana Carvalho,^{3,7}  Franco E. Chiarini,⁹  Joel M.P. Cordeiro,¹⁰  Mihai Costea,¹¹ 
 Julio Rubén Daviña,¹²  Aleksandr L. Ebel,¹³  Allan Falconi-Souto,¹⁴  Cattleya M.P. Felix,^{8,15} 
 Leonardo P. Felix,^{4,10}  Aveliano Fernández,¹⁶  Miguel Ángel García,^{17,18}  Ignacio García-Ruiz,¹⁹ 
 André dos Santos Bragança Gil,²⁰  Marcelo Guerra,²¹  Luiza Domingues Hirsch,³  Ana Isabel Honfi,¹² 
 Eliane Kaltchuk-Santos,^{3,7}  Sandra Knapp,²²  Rohit Kumar,²³  Vandna Kumari,²³  Juliana Lovo,²⁴ 
 Reinaldo F.P. Lucena,^{8,15}  Enoque Medeiros-Neto,⁴  Ana Paula Moraes,¹⁴ 
 Rodrigo Garcia Silva Nascimento,¹⁰  José Achilles Lima Neves,^{4,10}  Felipe Nollet,²⁵ 
 Regina Célia de Oliveira,²⁶  Andrés Orejuela,²⁷  Marisa Toniolo Pozzobon,²⁸ 
 Anna Verena Reutemann,¹⁶  André Rodolfo de Oliveira Ribeiro,^{26,29}  Gabriel Hugo Rua,³⁰ 
 Angeline M.S. Santos,¹⁰  Anádría Stéphanie da Silva,²⁶  Rosemere Silva,¹⁰  Ronimeire Torres da Silva,¹⁰ 
 Vijay Kumar Singhal,²³  Tatiana T. Souza-Chies,³¹  Saša Stefanović,¹⁷ 
 José Francisco Montenegro Valls,^{26,28}  Cassiano A.D. Welker³²  & Elena Yu. Zykova⁶ 

1 *Plant Science and Biodiversity Centre, Institute of Botany, Slovak Academy of Sciences, Dubravská cesta 9, 845 23 Bratislava, Slovak Republic*

2 *Department of Botany, Charles University, Benatská 2, 128 01 Praha, Czech Republic*

3 *Instituto de Biociências, Programa de Pós-Graduação em Genética e Biologia Molecular, Universidade Federal do Rio Grande do Sul, 91501-970, Porto Alegre, RS, Brazil*

4 *Department of Biological Sciences, Federal University of Paraíba, Areia, Paraíba, Brazil*

5 *Instituto Nacional do Semi Árido, INSA, Campina Grande, Paraíba, Brazil*

6 *Central Siberian Botanical Garden SB RAS, Zolotodolinskaya Str. 101, 630090 Novosibirsk, Russia*

7 *Instituto de Biociências, Departamento de Genética, Universidade Federal do Rio Grande do Sul, 91501-970, Porto Alegre, RS, Brazil*

8 *Universidade Federal da Paraíba, Centro de Ciências Exatas e da Natureza, Departamento de Sistemática e Ecologia, Laboratório de Etobiologia e Ciências Ambientais, 58.051-900, João Pessoa, Paraíba, Brazil*

9 *Instituto Multidisciplinario de Biología Vegetal (IMBIV), CONICET-UNC, Universidad Nacional de Córdoba, CC 495 Córdoba 5000, Argentina*

10 *Laboratório de Citogenética Vegetal, Departamento de Ciências Biológicas, Centro de Ciências Agrárias, Universidade Federal da Paraíba, Campus II, 58.397-000 Areia, Paraíba, Brazil*

11 *Department of Biology, University of Wilfrid Laurier, Waterloo, Ontario N2L 3C5, Canada*

12 *Programa de Estudios Florísticos y Genética Vegetal, Instituto de Biología Subtropical CONICET - Universidad Nacional de Misiones, nodo Posadas, Rivadavia 2370, 3300 Posadas, Argentina*

13 *Tomsk State University, 36 Lenin Ave., 634050, Tomsk, Russia*

14 *Center of Natural and Human Sciences, Federal University of ABC, São Bernardo do Campo, São Paulo, Brazil*

15 *Programa de Pós-Graduação em Desenvolvimento e Meio Ambiente, Centro de Ciências Exatas e da Natureza, Universidade Federal da Paraíba, Campus I, R. Tabeião Stanislau Eloy, 829, Conj. Pres. Castelo Branco III, 58033-455 João Pessoa, Paraíba, Brazil*

16 *Instituto de Botânica del Nordeste (CONICET-UNNE), C.C. 209, 3400 Corrientes, Argentina*

17 *Department of Biology, University of Toronto Mississauga, Mississauga, Ontario L5L 1C6, Canada*

18 *Royal Botanic Gardens Kew, Richmond, Surrey, TW9 3AE, U.K.*

19 *Instituto Politécnico Nacional (CIIDIR-IPN Michoacán), Justo Sierra 28, Jiquilpan, Michoacán, México, C.P. 59510*

20 *Botany Section - COBOT, Museu Paraense Emilio Goeldi - MPEG, Belém, Pará, Brazil*

21 *Laboratory of Plant Cytogenetics and Evolution - Federal University of Pernambuco, Department of Botany, Recife 50.372-970, PE, Brazil*

22 *Department of Life Sciences, Natural History Museum, Cromwell Road, London SW7 5BD, U.K.*

23 *Department of Botany, Punjabi University, Patiala-147002, Punjab, India*

24 *Biodiversity and Ecosystem Services Team, Instituto Tecnológico Vale - ITV, Belém, Pará, Brazil*

25 *Programa de Pós-Graduação em Botânica, Universidade Federal Rural de Pernambuco, Rua Dom Manoel de Medeiros, s/n, Dois Irmãos, 52.171.900, Recife, Pernambuco, Brazil*

26 *Programa de Pós-Graduação em Botânica da Universidade de Brasília, Campus Darcy Ribeiro, Brasília, DF, Brazil, CEP 70910-900*

27 *Tropical Diversity Section, Royal Botanic Garden Edinburgh, 20A Inverleith Row, Edinburgh EH3 5LR, U.K.*

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: Dorogina, O.V., Emtseva, M.V., Agafonov, A.V. & Asbaganov, S.V. 2019. IAPT chromosome data 29/1. In: Marhold, K. & al., IAPT chromosome data 30. *Taxon* 68: 880, E1–E2. | © 2020 International Association for Plant Taxonomy

- 28 *Embrapa Recursos Genéticos e Biotecnologia, Parque Estação Biológica - PqEB, Brasília, DF, Brazil, CEP 70770-917*
 29 *Universidade Federal do Ceará, Departamento de Fitotecnia, Campus do Pici, Fortaleza, CE, Brazil, CEP 60356-000*
 30 *Cátedra de Botánica Sistemática, Facultad de Agronomía, Universidad de Buenos Aires, Avenida San Martín 4453, C1417DSE Buenos Aires, Argentina*
 31 *Instituto de Biociências, Departamento de Botânica, Universidade Federal do Rio Grande do Sul, 91501-970, Porto Alegre, RS, Brazil*
 32 *Instituto de Biologia, Universidade Federal de Uberlândia, 38400-902, Uberlândia, MG, Brazil*

Author information KM, <https://orcid.org/0000-0002-7658-0844>; JK, <https://orcid.org/0000-0002-9983-7630>; CAM, <https://orcid.org/0000-0001-5984-3238>; EMdA, <https://orcid.org/0000-0001-8033-5334>; LIFA, <https://orcid.org/0000-0002-4182-911X>; TVA, <https://orcid.org/0000-0003-3661-0719>; FB, <https://orcid.org/0000-0002-5710-7170>; KB, <https://orcid.org/0000-0002-0767-9226>; LC, <https://orcid.org/0000-0002-2105-9859>; FEC, <https://orcid.org/0000-0002-6473-3129>; JMPC, <https://orcid.org/0000-0001-9833-0822>; MC, <https://orcid.org/0000-0003-3049-1763>; JRD, <https://orcid.org/0000-0002-1886-7521>; ALE, <https://orcid.org/0000-0002-7889-4580>; AFS, <https://orcid.org/0000-0002-0404-8269>; CMPF, <https://orcid.org/0000-0002-6126-3440>; LPF, <https://orcid.org/0000-0001-9202-9828>; AF, <https://orcid.org/0000-0002-1331-862X>; MAG, <https://orcid.org/0000-0002-0366-043X>; IGR, <https://orcid.org/0000-0002-2801-3802>; AdSBG, <https://orcid.org/0000-0002-0833-9856>; MG, <https://orcid.org/0000-0003-1438-9742>; LDH, <https://orcid.org/0000-0002-1868-7395>; AIH, <https://orcid.org/0000-0002-0915-2129>; EKS, <https://orcid.org/0000-0002-0002-0234>; SK, <https://orcid.org/0000-0001-7698-3945>; RK, <https://orcid.org/0000-0001-6421-6204>; VK, <https://orcid.org/0000-0002-4316-7738>; JL, <https://orcid.org/0000-0002-4506-6316>; RFPL, <https://orcid.org/0000-0002-1195-4315>; EMN, <https://orcid.org/0000-0002-8121-382X>; APM, <https://orcid.org/0000-0002-9878-3925>; RGSN, <https://orcid.org/0000-0002-7133-4994>; JALN, <https://orcid.org/0000-0003-0220-0746>; FN, <https://orcid.org/0000-0002-1362-685X>; RCdO, <https://orcid.org/0000-0001-7891-2630>; AO, <https://orcid.org/0000-0002-3511-1478>; MTP, <https://orcid.org/0000-0002-8213-9967>; AVR, <https://orcid.org/0000-0003-1043-4999>; ARdOR, <https://orcid.org/0000-0002-0085-3113>; GHR, <https://orcid.org/0000-0003-3601-786X>; AMSS, <https://orcid.org/0000-0001-8765-8291>; ASdS, <https://orcid.org/0000-0001-8023-4963>; RS, <https://orcid.org/0000-0003-1838-6362>; RTdS, <https://orcid.org/0000-0002-8362-3056>; VKS, <https://orcid.org/0000-0002-7109-7685>; TTSC, <https://orcid.org/0000-0002-1818-8748>; JFMV, <https://orcid.org/0000-0002-4586-5142>; CADW, <https://orcid.org/0000-0001-6347-341X>; EYuZ, <https://orcid.org/0000-0002-1847-5835>

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IAPT chromosome data 31/1

Lânia Isis Ferreira Alves, Allan Falconi-Souto, Enoque Medeiros-Neto, Erton Mendonça de Almeida, José Achilles Lima Neves, André dos Santos Bragança Gil, Juliana Lovo, Leonardo P. Felix & Ana Paula Moraes*

* Address for correspondence: ana.moraes@ufabc.edu.br

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All materials CHN.

IRIDACEAE Subfamily Iridoideae Tribe Trimezieae

- Neomarica candida* (Hassl.) Sprague, $2n = 18$; Brazil, São Paulo, *L.P. Felix 12802* (EAN).
Neomarica humilis (Klatt) Capell., $2n = 18$; Brazil, Pernambuco, *L.P. Felix 11001* (EAN).
Neomarica northiana (Schneev.) Sprague, $2n = 18$; Brazil, Paraíba, *L.I.F. Alves 02* (EAN).
Neomarica portosecurensis (Ravenna) Chukr, $2n = 18$; Brazil, Ceará, *L.P. Felix 13831* (EAN).
Neomarica sergipensis A.Gil & M.C.E.Amaral, $2n = 18$; Brazil, Sergipe, *L.P. Felix 12743* (EAN).
Pseudiris speciosa Chukr & A.Gil, $2n = 30$; Brazil, Bahia, *D. Cardoso & A.A. Conceição 434* (HUEFS), *L.P. Felix 14811* (EAN).
Trimezia connata Ravenna, $2n = 56$; Brazil, Pernambuco, *L.P. Felix 13151* (EAN). $2n = 82$; Brazil, Paraíba, *L.P. Felix 11966* (EAN).

Trimezia martinicensis (Jacq.) Herb., $2n = 54$; Brazil, Pernambuco, *L.P. Felix 11185* (EAN).

Trimezia sincorana Ravenna, $2n = 28$; Brazil, Bahia, *L.P. Felix 11691* (EAN). $2n = 54$; Brazil, Bahia, *J.P. Castro 119* (EAN).

IAPT chromosome data 31/2

Tatyana V. An'kova,* Elena Yu. Zykova & Aleksandr L. Ebel

* Address for correspondence: ankova_tv@mail.ru

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All materials CHN; collected in Russia; collectors: AE = A. Ebel, EZ = E. Zykova.

ASTERACEAE

- Carduus acanthoides* L., $2n = 22$; Russia, Novosibirskaya Oblast', *AE Z639* (TK); Russia, Omskaya Oblast', *AE Z640* (TK), *AE Z641* (TK).
Centaurea diffusa Lam., $2n = 18$; Russia, Altaiskii Krai, *AE Z636* (TK).
Centaurea jacea L., $2n = 44$; Russia, Novosibirskaya Oblast', *AE Z635* (TK).
Centaurea pseudomaculosa Dobrosz., $2n = 18$; Russia, Omskaya Oblast', *AE Z631*; Russia, Novosibirskaya Oblast', *AE Z634* (TK).

BRASSICACEAE

Sisymbrium officinale (L.) Scop., $2n = 14$; Russia, Altaiskii Krai, EZ Z536:3215 (NS).

CARYOPHYLLACEAE

Dianthus ×courtoisii Rchb., $2n = 30$; Russia, Tomskaya Oblast', AE Z638 (TK).

Dianthus versicolor Fisch. ex Link, $2n = 30$; Russia, Altai Republic, EZ Z331:5617 (NS).

Elisanthe noctiflora (L.) Rupr., $2n = 24$, $2n = 36$; Russia, Altaiskii Krai, EZ Z548:3215 (NS).

CHENOPODIACEAE

Chenopodium polyspermum L., $2n = 18$; Russia, Altai Republic, EZ Z392:5117 (NS), EZ Z394:6717 (NS).

FABACEAE

Caragana arborescens Lam., $2n = 16$; Russia, Novosibirskaya Oblast', EZ Z13:7617 (NS).

Vicia angustifolia L., $2n = 12$, Russia, Altai Republic, EZ Z384:5017 (NS).

OXALIDACEAE

Oxalis stricta L., $2n = 24$, Russia, Novosibirskaya Oblast', EZ Z243:0916 (NS).

PLANTAGINACEAE

Veronica arvensis L., $2n = 16$; Russia, Altai Republic, EZ Z402:2417 (NS), EZ Z401:2617 (NS).

POACEAE

Avena fatua L., $2n = 28$; Russia, Altai Republic, EZ Z440:3015 (NS). $2n = 42$; Russia, Altai Republic, EZ Z441:4615 (NS).

VITACEAE

Parthenocissus quinquefolia (L.) Planch., $2n = 40$; Russia, Altai Republic, EZ Z502:6017 (NS).

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Luana Carvalho, Cassiano A.D. Welker, Tatiana T. Souza-Chies & Eliane Kaltchuk-Santos*

* Address for correspondence: eliane.kaltchuk@ufvgs.br

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All materials CHN. Collectors: *CW* = C.A.D. Welker, *LWW* = H.M. Longhi-Wagner & C.A.D. Welker, *WKC* = C.A.D. Welker, E. Kaltchuk-Santos & L. Carvalho.

POACEAE

Eriochrysis cayennensis P.Beauv., $n = 10$; Brazil, Rio Grande do Sul, *CW* 617 (ICN), *LWW* 10863 (ICN), *WKC* 694 (HUFU).

Eriochrysis laxa Swallen, $n = 10$; Brazil, Rio Grande do Sul, *WKC* 693 (HUFU), *WKC* 695 (HUFU).

Eriochrysis villosa Swallen, $n = 10$; Brazil, Rio Grande do Sul, *CW* 541 (ICN), *WKC* 696 (HUFU).

IAPT chromosome data 31/4

Franco E. Chiarini,* Andrés Orejuela & Sandra Knapp

* Address for correspondence: franco.e.chiarini@gmail.com

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All materials CHN; collectors: *CIO* = Clara Inés Orozco, *FC* = Franco Chiarini, *GB* = Gloria Barboza, *TS* = Tiina Särkinen.

SOLANACEAE

Benthamiella graminifolia Skotts., $2n = 22$; Argentina, Neuquén, *GB* & al. 3813 (CORD).

Benthamiella patagonica Speg., $2n = 22$; Argentina, Santa Cruz, *GB* & al. 3747 (CORD).

Benthamiella pycnophylloides Speg., $2n = 22$; Argentina, Santa Cruz, *GB* & al. 3689 (CORD).

Fabiana patagonica Speg., $2n = 18$; Argentina, Jujuy, *FC* 1322 (CORD).

Fabiana punensis S.C.Arroyo, $2n = 18$; Argentina, Jujuy, *FC* 1321 (CORD).

Lycianthes heterochondra (Bitter) Bitter, $2n = 24$; Perú, Puno, *TS* & al. 4046 (BM)

Trianaea nobilis Planch. & Linden, $2n = 24$; Ecuador, Cotopaxi, *CIO* & al. 3967 (COL, QCA).

IAPT chromosome data 31/5

Julio Rubén Daviña, Aveliano Fernández & Ana Isabel Honfi*

* Author for correspondence: ahonfi@gmail.com

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All materials CHN; collector: *D* = J.R. Daviña.

AMARYLLIDACEAE

Zephyranthes candida (Lindl.) Herb., $2n = 38$; Argentina, Entre Ríos, *D* 360 (B, MNES).

Zephyranthes carinata Herb., $n = 24$ II, $2n = 48$; Argentina, Misiones, *D* 357 (B, MNES).

Zephyranthes flavissima Ravenna, $n = 7$ II, $2n = 14$; Argentina, Misiones, *D* 279 (B, CTES, G, MNES).

Zephyranthes mesochloa Herb., $2n = 12$; Argentina, Misiones, *D* 637 (MNES).

Zephyranthes minima Herb., $2n = 20$; Argentina, Corrientes, *D 348* (B, MNES).

Zephyranthes seubertii H.H.Hume, $2n = 10$; Argentina, Corrientes, *D 434* (B, CTES, MNES).

IAPT chromosome data 31/6

Cattleya M.P. Felix, Reinaldo F.P. Lucena, Leonardo P. Felix, Joel M.P. Cordeiro, Angeline M.S. Santos* & Kallyne Bonifácio

* Address for correspondence: angelinemssantos@gmail.com

Financial support from CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico) and CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior).

All materials CHN; all collections Brazil, Paraíba; vouchers in EAN (Herbarium Prof. Jayme Coelho de Moraes).

ARACEAE

Taccarum peregrinum (Schott) Engl., $2n = 34$; *J.M.P. Cordeiro 1296*.

BIGNONIACEAE

Handroanthus impetiginosus (Mart. ex DC.) Mattos, $2n = 40$; *J.M.P. Cordeiro 1343*.

CONVOLVULACEAE

Evolvulus elegans Moric., $2n = 26$; *L.P. Felix 17416*.

CYPERACEAE

Cyperus ligularis L., $2n = 50$; *L.P. Felix 17456*.

Eleocharis atropurpurea (Retz.) J.Presl & C.Presl., $2n = 20$; *L.P. Felix 16785*.

EUPHORBIACEAE

Jatropha mollissima (Pohl) Baill., $2n = 22$; *L.P. Felix 16614*.

FABACEAE

Chamaecrista hispidula (Vahl) H.S.Irwin & Barneby, $2n = 20$; *L.P. Felix 17415*.

Mimosa paraibana Barneby, $2n = 26$; *L.P. Felix 16711*.

IRIDACEAE

Alophia drummondii (Graham) R.C.Foster, $2n = 14+2Bs$; *L.P. Felix 17451*.

MALVACEAE

Waltheria indica L., $2n = 18$; *L.P. Felix 17470*.

ONAGRACEAE

Ludwigia octovalvis (Jacq.) P.H.Raven, $2n = 32$; *L.P. Felix 17440*.

ORCHIDACEAE

Prescottia plantaginea Lindl., $2n = 48$; *J.M.P. Cordeiro 1319*.

POLYGALACEAE

Polygala boliviensis A.W.Benn., $2n = 18$; *L.P. Felix 17394*.

VITACEAE

Cissus sp., $2n = 32$; *J.M.P. Cordeiro 1288*.

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Cattleya M.P. Felix, José Achilles Lima Neves, Rosemere Silva, Rodrigo Garcia Silva Nascimento, Ronimeire Torres da Silva, Felipe Nollet,* Joel M.P. Cordeiro, Reinaldo F.P. Lucena & Leonardo P. Felix

* Address for correspondence: nolletmedeiros@yahoo.com.br

Financial support from CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico), CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior), INSA (Instituto Nacional do Semiárido).

All materials CHN; vouchers in EAN (Herbário Prof. Jayme Coelho de Moraes).

ALISMATACEAE

Echinodorus grandiflorus (Cham. & Schltdl.) Micheli, $2n = 22$; Brazil, Paraíba, *L.P. Felix 10824*.

Echinodorus paniculatus Micheli, $2n = 22$; Brazil, Piauí, *L.P. Felix s.n.* (EAN 11164).

Echinodorus subalatus (Mart.) Griseb., $2n = 22$; Brazil, Piauí, *L. Feitoza 22*.

AMARANTHACEAE

Alternanthera brasiliana (L.) Kuntze, $2n = 28$; Brazil, Paraíba, *L.P. Felix 16669*.

AMARYLLIDACEAE

Griffinia angustifolia Campos-Rocha, Dutilh & Semir, $2n = 30$; Brazil, Sergipe, *L.P. Felix s.n.* (EAN 14200).

Griffinia gardneriana (Herb.) Ravenna, $2n = 20$; Brazil, Pernambuco, *L.P. Felix 12158*.

Hippeastrum stylosum Herb., $2n = 20$; Brazil, Pernambuco, *L.P. Felix 12064*.

Nothoscordum gracile (Aiton) Stearn, $2n = 19$; Brazil, São Paulo, *M. Guerra s.n.* (EAN 10154).

Nothoscordum pulchellum Kunth, $n = 5$; Brazil, Paraíba, *J.M.P. Cordeiro 1019*.

ARECACEAE

Syagrus vagans (Bondar) A.D.Hawkes, $2n = 30$; Brazil, Bahia, *V.S. Lopes 10*.

ASTERACEAE

Acmea oleracea (L.) R.K.Jansen, $2n = 78$; Brazil, Pará, *L.P. Felix 17333*.

BROMELIACEAE

Tillandsia juncea (Ruiz & Paiv.) Poir, $2n = 38$; Brazil, Paraíba, *J.M.P. Cordeiro 1266*.

CACTACEAE

Hylocereus setaceus (Salm-Dyck ex DC.) Ralf Bauer, $2n = 22$; Brazil, Paraíba, *J.M.P. Cordeiro 1265*.

FABACEAE

Chaetocalyx scandens var. *pubescens* (DC.) Rudd, $2n = 20$; Brazil, Paraíba, *L.P. Felix 16662*.

Pityrocarpa moniliformis (Benth.) Luckow & R.W.Jobson, $2n = 26$; Brazil, Bahia, *C.I. Ramalho & V.S. Lopes 135*.

HYDROCHARITACEAE

Limnobiium laevigatum (Humb. & Bonpl. ex Willd.) Heine, $2n = 28$; Brazil, Paraíba, L.P. Felix 10554.

ORCHIDACEAE

Plethrophora cultrifolia (Barb.Rodr.) Cogn., $2n = 56$; Brazil, Santa Catarina, L.P. Felix & M. Guerra 16576.

PLANTAGINACEAE

Angelonia campestris Nees & Mart., $2n = 28$; Brazil, Sergipe, J.M.P. Cordeiro, L.P. Felix, M. Almeida & J.P. Araújo 518.

POLYGONACEAE

Triplaris americana L., $2n = 22$; Brazil, Paraíba, J.M.P. Cordeiro, 1267.

SAPINDACEAE

Magonia pubescens A.St.-Hil., $2n = 30$; Brazil, Monte Alegre, J.M.P. Cordeiro 757.

VITACEAE

Cissus pulcherrima Vell., $2n = 36$; Brazil, Tocantins, J.M.P. Cordeiro 776.

IAPT chromosome data 31/8

Miguel Ángel García,* Mihai Costea, Marcelo Guerra, Ignacio García-Ruiz & Saša Stefanović

* Address for correspondence: ma.garcia@utoronto.ca

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All materials CHN.

CONVOLVULACEAE

Cuscuta africana Willd., $2n = 30$; South Africa, Western Cape, M.A. García 3920 (MA).

Cuscuta angulata Engelm., $n = 15$, $2n = 30$; South Africa, Western Cape, M.A. García 3922 (MA).

Cuscuta approximata Bab., $2n = 28$; Spain, Segovia, M.A. García 6482 (TRTE).

Cuscuta bonafortunae Costea & I.García, $2n = 30$; Mexico, Michoacán, I. García-Ruiz 8391 (CIMI, WLU).

Cuscuta brachycalyx (Yunck.) Yunck., $2n = 30$; U.S.A., California, S. Stefanović 15-22 (TRTE).

Cuscuta campestris Yunck., $2n = 60$; Serbia, Vojvodina, 03 Sep 2008, M. Šarić s.n., UTM-1569 (TRTE).

Cuscuta cephalanthi Engelm., $2n = 60$; U.S.A., Iowa, 15 Sep 2015, R. Lutz & R. Tallent s.n., UTM-1567 (TRTE).

Cuscuta chapalana Yunck., $2n = 30$; Mexico, Jalisco, I. García-Ruiz & al. 8064, UTM-1563 (CIMI, TRTE, WLU).

Cuscuta chilensis Ker Gawl., $n = 15$, $2n = 30$; Chile, 15 Jan 2011, M. Costea s.n. (WLU).

Cuscuta compacta Juss. ex Choisy, $2n = 30$; U.S.A., Texas, 03 Sep 2015, S. Stefanović s.n., UTM-1566 (TRTE).

Cuscuta corymbosa var. *grandiflora* Engelm., $2n = 30$; Mexico, Michoacán, 19 Dec 2007, M. Costea & I. García-Ruiz s.n. (CIMI, WLU).

Cuscuta costaricensis Yunck., $2n = 30$; Mexico, Jalisco, I. García-Ruiz 8052 (CIMI, WLU).

Cuscuta cotijana Costea & I.García, $2n = 30$; Mexico, Michoacán, I. García-Ruiz & al. 7560 (CIMI, NY, US, WLU).

Cuscuta desmouliniana Yunck., $2n = 30$; Mexico, Sonora, Van Devender & al. 2007-1094 (ARIZ, WLU).

Cuscuta epithymum (L.) L., $2n = 32$; Spain, Cantabria, M.A. García 6486, UTM-1573 (TRTE). $2n = 34$; Spain, Segovia, M.A. García 6483, UTM-1571 (TRTE).

Cuscuta erosa Yunck., $2n = 30$; U.S.A., Arizona, Aug 2013, J. Cowles s.n. (WLU).

Cuscuta glomerata Choisy, $n = 15$, $2n = 30$; U.S.A., Iowa, 31 Jul 2015, R. Lutz s.n., UTM-1565 (TRTE).

Cuscuta grandiflora Kunth, $2n = 30$; Peru, Cusco, 20 Aug 2007, M. Costea s.n. (WLU).

Cuscuta gronovii Willd. ex Roem. & Schult., $2n = 60$; Canada, Ontario, 26 Jul 2015, M.A. García s.n. (TRTE).

Cuscuta indecora Choisy, $2n = 30$; Mexico, Zacatecas, 14 Aug 2012, A.V. Ibarra s.n. (SAGARPA, WLU).

Cuscuta monogyna Vahl, $2n = 30$; Israel, G. Wizen s.n., UTM-1348 (TRTE).

Cuscuta obtusiflora Kunth, $2n = 30$; Mexico, Jalisco, I. García-Ruiz & al. 8256 (CIMI, WLU).

Cuscuta occidentalis Millsp., $2n = 30$; U.S.A., California, S. Stefanović 13-06 (TRTE).

Cuscuta pacifica Costea & M.A.R.Wright, $2n = 30$; U.S.A., California, S. Stefanović 15-23 (TRTE).

Cuscuta purpurata Phil., $2n = 30$; Chile, M. Muñoz 5144 (SGO, TRTE, WLU).

Cuscuta sandwichiana Choisy, $2n = 150$; U.S.A., Hawaii, Degener & Degener 36596, UTM-155 (TRTE).

Cuscuta sidarum Liebm., $2n = 30$; Mexico, Michoacán, I. García-Ruiz & al. 7584 (CIMI, WLU).

Cuscuta subinclusa Durand & Hilg., $2n = 30$; Mexico, Baja California, 01 May 2014, M. Costea s.n. (WLU).

Cuscuta tinctoria var. *floribunda* (Kunth) Costea, $n = 15$; Mexico, Temascaltepec, I. García-Ruiz & al. 8588 (CIMI, WLU).

Cuscuta tinctoria Mart. ex Engelm. var. *tinctoria*, $2n = 30$; Mexico: Michoacán, I. García-Ruiz & al. 7575 (CIMI, WLU).

Cuscuta umbrosa Beyr. ex Hook., $2n = 30$; U.S.A., Iowa, 13 Sep 2013, Watson s.n., UTM-1564 (TRTE).

Cuscuta volcanica Costea & I.García, $n = 15$; Mexico, Jalisco, I. García-Ruiz & al. 7567 (CIMI, WLU).

IAPT chromosome data 31/9

Luiza Domingues Hirsch, Fernanda Bered, Camila Aguiar-Melo, Luana Carvalho & Eliane Kaltchuk-Santos*

* Address for correspondence: eliane.kaltchuk@ufrgs.br

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All materials CHN; vouchers in ICN.

BROMELIACEAE

- Dyckia choristaminea* Mez, $2n = 50$; Brazil, Rio Grande do Sul, 26 Oct 2017, L.D. Hirsch s.n.
Dyckia hebdingii L.B.Sm., $2n = 50$; Brazil, Rio Grande do Sul, 07 Oct 2015, C.J. Breitsameter, L.D. Hirsch, F. Bered & C. Aguiar-Melo s.n.
Dyckia julianae Strehl, $2n = 50$; Brazil, Rio Grande do Sul, 20 Oct 2016, L.D. Hirsch s.n.

IAPT chromosome data 31/10

Vandna Kumari, Rohit Kumar & Vijay Kumar Singhal*

* Address for correspondence: vksinghal53@gmail.com

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All materials CHN; collectors: RK = Rohit Kumar; VK = Vandna Kumari; vouchers in PUN.

BALSAMINACEAE

- Impatiens badrinathii* Pusalkar & D.K.Singh, $n = 10$; India, Uttarakhand, RK 34392.
Impatiens devendrae Pusalkar, $n = 7$; India, Uttarakhand, RK 30727.
Impatiens leggei Pusalkar & D.K. Singh, $n = 7$; India, Uttarakhand, RK 34810.

CARYOPHYLLACEAE

- Silene gangotriana* Pusalkar, D.K.Singh & Lakshmin., $n = 36$; India, Uttarakhand, RK 34645.

FABACEAE

- Astragalus melanostachys* Benth. ex Bunge, $n = 8$; India, Uttarakhand, RK 34855.
Astragalus sanjappae L.B.Chaudhary & Z.H. Khan, $n = 8$; India, Uttarakhand, RK 34861.

POACEAE

- Agrostis griffithiana* (Hook.f.) Bor, $n = 21$; India, Himachal Pradesh, VK 34292.
Arthraxon hispidus (Thunb.) Makino, $n = 15$; India, Himachal Pradesh, VK 32475.
Brachypodium sylvaticum (Huds.) P.Beauv., $n = 18$; India, Himachal Pradesh, VK 34627.
Cymbopogon olivieri (Boiss.) Bor, $n = 20$; India, Himachal Pradesh, VK 34201.
Danthonia cachemyriana Jaub. & Spach, $n = 14$; India, Himachal Pradesh, VK 34710.

- Festuca valesiaca* Schleich. ex Gaudin, $n = 28$; India, Himachal Pradesh, VK 34671.
Lolium rigidum Gaudin, $n = 7$; India, Himachal Pradesh, VK 34681.
Microstegium ciliatum (Trin.) A.Camus, $n = 14$; India, Himachal Pradesh, VK 34210.
Microstegium nudum (Trin.) A.Camus, $n = 14$; India, Himachal Pradesh, VK 34211.
Muhlenbergia himalayensis Hack. ex Hook.f., $n = 10$; India, Himachal Pradesh, VK 34738.
Pennisetum lanatum Klotzsch, $n = 14$; India, Himachal Pradesh, VK 34268.
Stipa brandisii Mez, $n = 12$; India, Himachal Pradesh, VK 34693.
Trisetum micans (Hook.f.) Bor, $n = 14$; India, Himachal Pradesh, VK 34728.

IAPT chromosome data 31/11

Anna Verena Reutemann, Julio Rubén Daviña, Gabriel Hugo Rua & Ana Isabel Honfi*

* Address for correspondence: ahonfi@gmail.com

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All materials CHN; collectors: D = J.R. Daviña, H = A.I. Honfi, R = G.H. Rua.

POACEAE

- Paspalum alnum* Chase, $2n = 12$; Argentina, Corrientes, H & D 1703 (MNES), H & D 1704 (MNES).
Paspalum conspersum Schrad., $2n = 60$; Argentina, Misiones, H & D 1119 (MNES), H & D 1143 (MNES).
Paspalum equitans Mez, $2n = 20$; Argentina, Misiones, H & D 1447 (MNES).
Paspalum fasciculatum Wild. ex Flügge, $2n = 20$; Argentina, Formosa, R 307 (BAA).
Paspalum glaucescens Hack., $2n = 40$; Argentina, Misiones, H & D 1109 (MNES).
Paspalum ionanthum Chase, $n = 20$; Paraguay, Cordillera, H & D 1177 (MNES).
Paspalum maculosum Trin., $2n = 20, 40$; Argentina, Misiones, H & D 1445 (MNES).
Paspalum malacophyllum Trin., $2n = 40$; Argentina, Salta, H & D 1448 (MNES).
Paspalum notatum var. *saurae* Parodi, $2n = 20$; Argentina, Santa Fe, H & D 1453 (MNES).
Paspalum notatum Flügge var. *notatum*, $2n = 40$; Argentina, Misiones, H 220 (CTES, MNES); Argentina, Santa Fe, H & D 1304 (MNES); Argentina, Misiones, H & D 1603 (MNES).
Paspalum pauciciliatum (Parodi) Herter, $2n = 40$; Argentina, Misiones, H & D 465 (CTES, MNES).
Paspalum paucifolium Swallen, $2n = 40$; Paraguay, Paraguari, H & D 1294 (MNES).

Paspalum quarinii Morrone & Zuloaga, $2n = 20$; Argentina, Misiones, *H & D 1190* (CTES, MNES, SI).

Paspalum regnellii Mez, $2n = 40$; Argentina, Misiones, *H & D 1118* (MNES).

IAPT chromosome data 31/12

André Rodolfo de Oliveira Ribeiro,* Anádría Stéphanie da Silva, Marisa Toniolo Pozzobon, José Francisco Montenegro Valls & Regina Célia de Oliveira

* Address for correspondence: *andre_rodolf@hotmail.com*

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All materials CHN.

POACEAE

Mesosetum bifarium (Hack.) Chase, $2n = 8$; Brazil, Goiás, *J.F.M. Valls & al. 15927, J.F.M. Valls & al. 15933* (CEN). $2n = 16$, Brazil, Goiás, *A.S. Silva & al. 235* (UB).

Mesosetum cayennense Steud., $2n = 20$; Brazil, Tocantins, *R.C. Oliveira & al. 2848* (UB).

Mesosetum chaseae Lucas, $2n = 24$; Brazil, Mato Grosso, *R.C. Oliveira & al. 2772* (UB).

Mesosetum compressum Swallen, $2n = 16$; Brazil, Pernambuco, *A.S. Silva & D.B. Crisci 274* (UB).

Mesosetum elytrochaetum (Hack.) Swallen, $2n = 24$; Brazil, Goiás, *R.C. Oliveira & al. 2722* (UB).

Mesosetum filgueirasii R.C.Oliveira & Valls, $2n = 16$; Brazil, Tocantins, *J.F.M. Valls & al. 16273* (paratype: CEN, UB).

Mesosetum longiaristatum Filg., $2n = 24$; Brazil, Goiás, *J.F.M. Valls & al. 15812, J.F.M. Valls & al. 15841* (CEN).

Mesosetum pappophorum (Nees) Kuhl., $2n = 8$; Brazil, Goiás, *J.F.M. Valls & al. 15949* (CEN).

Mesosetum sclerochloa (Trin.) Hitchc., $2n = 16$; Brazil, Goiás, *J.F.M. Valls & al. 15937* (CEN).

IAPT CHROMOSOME DATA

IAPT chromosome data 31 – Extended version

Karol Marhold (ed.),^{1,2}  Jaromír Kučera (ed.),¹  Camila Aguiar-Melo,³  Erton Mendonça de Almeida,⁴ 
 Lânia Isis Ferreira Alves,⁵  Tatyana V. An'kova,⁶  Fernanda Bered,^{3,7}  Kallyne Bonifácio,⁸ 
 Luana Carvalho,^{3,7}  Franco E. Chiarini,⁹  Joel M.P. Cordeiro,¹⁰  Mihai Costea,¹¹ 
 Julio Rubén Daviña,¹²  Aleksandr L. Ebel,¹³  Allan Falconi-Souto,¹⁴  Cattleya M.P. Felix,^{8,15} 
 Leonardo P. Felix,^{4,10}  Aveliano Fernández,¹⁶  Miguel Ángel García,^{17,18}  Ignacio García-Ruiz,¹⁹ 
 André dos Santos Bragança Gil,²⁰  Marcelo Guerra,²¹  Luiza Domingues Hirsch,³  Ana Isabel Honfi,¹² 
 Eliane Kaltchuk-Santos,^{3,7}  Sandra Knapp,²²  Rohit Kumar,²³  Vandna Kumari,²³  Juliana Lovo,²⁴ 
 Reinaldo F.P. Lucena,^{8,15}  Enoque Medeiros-Neto,⁴  Ana Paula Moraes,¹⁴ 
 Rodrigo Garcia Silva Nascimento,¹⁰  José Achilles Lima Neves,^{4,10}  Felipe Nollet,²⁵ 
 Regina Célia de Oliveira,²⁶  Andrés Orejuela,²⁷  Marisa Toniolo Pozzobon,²⁸ 
 Anna Verena Reutemann,¹⁶  André Rodolfo de Oliveira Ribeiro,^{26,29}  Gabriel Hugo Rua,³⁰ 
 Angeline M.S. Santos,¹⁰  Anádría Stéphanie da Silva,²⁶  Rosemere Silva,¹⁰  Ronimeire Torres da Silva,¹⁰ 
 Vijay Kumar Singhal,²³  Tatiana T. Souza-Chies,³¹  Saša Stefanović,¹⁷
 José Francisco Montenegro Valls,^{26,28}  Cassiano A.D. Welker³²  & Elena Yu. Zykova⁶ 

- 1 *Plant Science and Biodiversity Centre, Institute of Botany, Slovak Academy of Sciences, Dubravská cesta 9, 845 23 Bratislava, Slovak Republic*
- 2 *Department of Botany, Charles University, Benatska 2, 128 01 Praha, Czech Republic*
- 3 *Instituto de Biociências, Programa de Pós-Graduação em Genética e Biologia Molecular, Universidade Federal do Rio Grande do Sul, 91501-970, Porto Alegre, RS, Brazil*
- 4 *Department of Biological Sciences, Federal University of Paraíba, Areia, Paraíba, Brazil*
- 5 *Instituto Nacional do Semi Árido, INSA, Campina Grande, Paraíba, Brazil*
- 6 *Central Siberian Botanical Garden SB RAS, Zolotodolinskaya Str. 101, 630090 Novosibirsk, Russia*
- 7 *Instituto de Biociências, Departamento de Genética, Universidade Federal do Rio Grande do Sul, 91501-970, Porto Alegre, RS, Brazil*
- 8 *Universidade Federal da Paraíba, Centro de Ciências Exatas e da Natureza, Departamento de Sistemática e Ecologia, Laboratório de Etobiologia e Ciências Ambientais, 58.051-900, João Pessoa, Paraíba, Brazil*
- 9 *Instituto Multidisciplinario de Biología Vegetal (IMBIV), CONICET-UNC, Universidad Nacional de Córdoba, CC 495 Córdoba 5000, Argentina*
- 10 *Laboratório de Citogenética Vegetal, Departamento de Ciências Biológicas, Centro de Ciências Agrárias, Universidade Federal da Paraíba, Campus II, 58.397-000 Areia, Paraíba, Brazil*
- 11 *Department of Biology, University of Wilfrid Laurier, Waterloo, Ontario N2L 3C5, Canada*
- 12 *Programa de Estudios Florísticos y Genética Vegetal, Instituto de Biología Subtropical CONICET - Universidad Nacional de Misiones, nodo Posadas, Rivadavia 2370, 3300 Posadas, Argentina*
- 13 *Tomsk State University, 36 Lenin Ave., 634050, Tomsk, Russia*
- 14 *Center of Natural and Human Sciences, Federal University of ABC, São Bernardo do Campo, São Paulo, Brazil*
- 15 *Programa de Pós-Graduação em Desenvolvimento e Meio Ambiente, Centro de Ciências Exatas e da Natureza, Universidade Federal da Paraíba, Campus I, R. Tabeião Stanislau Eloy, 829, Conj. Pres. Castelo Branco III, 58033-455 João Pessoa, Paraíba, Brazil*
- 16 *Instituto de Botánica del Nordeste (CONICET-UNNE), C.C. 209, 3400 Corrientes, Argentina*
- 17 *Department of Biology, University of Toronto Mississauga, Mississauga, Ontario L5L 1C6, Canada*
- 18 *Royal Botanic Gardens Kew, Richmond, Surrey, TW9 3AE, U.K.*
- 19 *Instituto Politécnico Nacional (CIIDIR-IPN Michoacán), Justo Sierra 28, Jiquilpan, Michoacán, México, C.P. 59510*
- 20 *Botany Section - COBOT, Museu Paraense Emilio Goeldi - MPEG, Belém, Pará, Brazil*
- 21 *Laboratory of Plant Cytogenetics and Evolution - Federal University of Pernambuco, Department of Botany, Recife 50.372-970, PE, Brazil*
- 22 *Department of Life Sciences, Natural History Museum, Cromwell Road, London SW7 5BD, U.K.*
- 23 *Department of Botany, Punjabi University, Patiala-147002, Punjab, India*
- 24 *Biodiversity and Ecosystem Services Team, Instituto Tecnológico Vale - ITV, Belém, Pará, Brazil*
- 25 *Programa de Pós-Graduação em Botânica, Universidade Federal Rural de Pernambuco, Rua Dom Manoel de Medeiros, s/n, Dois Irmãos, 52.171.900, Recife, Pernambuco, Brazil*
- 26 *Programa de Pós-Graduação em Botânica da Universidade de Brasília, Campus Darcy Ribeiro, Brasília, DF, Brazil, CEP 70910-900*
- 27 *Tropical Diversity Section, Royal Botanic Garden Edinburgh, 20A Inverleith Row, Edinburgh EH3 5LR, U.K.*
- 28 *Embrapa Recursos Genéticos e Biotecnologia, Parque Estação Biológica - PqEB, Brasília, DF, Brazil, CEP 70770-917*
- 29 *Universidade Federal do Ceará, Departamento de Fitotecnia, Campus do Pici, Fortaleza, CE, Brazil, CEP 60356-000*
- 30 *Cátedra de Botánica Sistemática, Facultad de Agronomía, Universidad de Buenos Aires, Avenida San Martín 4453, C1417DSE Buenos Aires, Argentina*
- 31 *Instituto de Biociências, Departamento de Botânica, Universidade Federal do Rio Grande do Sul, 91501-970, Porto Alegre, RS, Brazil*
- 32 *Instituto de Biologia, Universidade Federal de Uberlândia, 38400-902, Uberlândia, MG, Brazil*

Author information KM, <https://orcid.org/0000-0002-7658-0844>; JK, <https://orcid.org/0000-0002-9983-7630>; CAM, <https://orcid.org/0000-0001-5984-3238>; EMdA, <https://orcid.org/0000-0001-8033-5334>; LIFA, <https://orcid.org/0000-0002-4182-911X>; TVA, <https://orcid.org/0000-0003-3661-0719>; FB, <https://orcid.org/0000-0002-5710-7170>; KB, <https://orcid.org/0000-0002-0767-9226>; LC, <https://orcid.org/0000-0002-2105-9859>; FEC, <https://orcid.org/0000-0002-6473-3129>; JMPC, <https://orcid.org/0000-0001-9833-0822>; MC, <https://orcid.org/0000-0003-3049-1763>; JRD, <https://orcid.org/0000-0002-1886-7521>; ALE, <https://orcid.org/0000-0002-7889-4580>; AFS, <https://orcid.org/0000-0002-0404-8269>; CMPF, <https://orcid.org/0000-0002-6126-3440>; LPF, <https://orcid.org/0000-0001-9202-9828>; AF, <https://orcid.org/0000-0002-1331-862X>; MAG, <https://orcid.org/0000-0002-0366-043X>; IGR, <https://orcid.org/0000-0002-2801-3802>; AdSBG, <https://orcid.org/0000-0002-0833-9856>; MG, <https://orcid.org/0000-0003-1438-9742>; LDH, <https://orcid.org/0000-0002-1868-7395>; AIH, <https://orcid.org/0000-0002-0915-2129>; EKS, <https://orcid.org/0000-0002-0002-0234>; SK, <https://orcid.org/0000-0001-7698-3945>; RK, <https://orcid.org/0000-0001-6421-6204>; VK, <https://orcid.org/0000-0002-4316-7738>; JL, <https://orcid.org/0000-0002-4506-6316>; RFPL, <https://orcid.org/0000-0002-1195-4315>; EMN, <https://orcid.org/0000-0002-8121-382X>; APM, <https://orcid.org/0000-0002-9878-3925>; RGSN, <https://orcid.org/0000-0002-7133-4994>; JALN, <https://orcid.org/0000-0003-0220-0746>; FN, <https://orcid.org/0000-0002-1362-685X>; RCdO, <https://orcid.org/0000-0001-7891-2630>; AO, <https://orcid.org/0000-0002-3511-1478>; MTP, <https://orcid.org/0000-0002-8213-9967>; AVR, <https://orcid.org/0000-0003-1043-4999>; ARdOR, <https://orcid.org/0000-0002-0085-3113>; GHR, <https://orcid.org/0000-0003-3601-786X>; AMSS, <https://orcid.org/0000-0001-8765-8291>; ASdS, <https://orcid.org/0000-0001-8023-4963>; RS, <https://orcid.org/0000-0003-1838-6362>; RTdS, <https://orcid.org/0000-0002-8362-3056>; VKS, <https://orcid.org/0000-0002-7109-7685>; TTSC, <https://orcid.org/0000-0002-1818-8748>; JFMV, <https://orcid.org/0000-0002-4586-5142>; CADW, <https://orcid.org/0000-0001-6347-341X>; EYuZ, <https://orcid.org/0000-0002-1847-5835>

IAPT chromosome data 31/1

Lânia Isis Ferreira Alves, Allan Falconi-Souto, Enoque Medeiros-Neto, Erton Mendonça de Almeida, José Achilles Lima Neves, André dos Santos Bragança Gil, Juliana Lovo, Leonardo P. Felix & Ana Paula Moraes*

* Address for correspondence: ana.moraes@ufabc.edu.br

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* The chromosome numbers ascertained here are different from those reported by other authors (Goldblatt & Takei, 1997; Chukr & Giulietti, 2008).

** Species with intra-specific cytotype variation.

IRIDACEAE

Subfamily Iridoideae

Tribe Trimezieae

Neomarica candida (Hassl.) Sprague

$2n = 18$, CHN. Brazil, São Paulo, Ilha Comprida, 24°43'55.92" S, 47°32'28.39"W, 6 m, 13 Jul 2010, *L.P. Felix 12802* (EAN) [Fig. 1A].

The species is a terricolous herb, with underground stem rhizome-like, leaves multiple, dorsiventrally flattened, linear, distichously arranged, dark green, flowering stem winged, leafy-like and longer than leaves, rhipidia with short peduncle, flowers white with brown to purple stripes.

Neomarica humilis (Klatt) Capell.

$2n = 18$, CHN. Brazil, Pernambuco, Bonito, 08°28'25.74"S, 35°43'42.21"W, 433 m, 28 Jul 2005, *L.P. Felix 11001* (EAN) [Fig. 1B].

The species is a terricolous herb, with underground stem rhizome-like, vertical, leaves multiple, dorsiventrally flattened, linear, distichously arranged, light green, flowering stem winged, leafy-like, longer and narrower than leaves, rhipidia with

conspicuous peduncles, flowers small, predominantly whitish to cream with yellowish proximal region, distal purple stripes.

Neomarica northiana (Schneev.) Sprague

$2n = 18$, CHN. Brazil, Paraíba, Campina Grande, 07°13'44.67"S, 35°52'51.00"W, 516 m, 16 Feb 2010, *L.I.F. Alves 02* (EAN) [Fig. 1C].

The species is a robust terricolous herb, with underground stem rhizome-like, vertical, with multiple leaves, dorsiventrally flattened, linear, distichously arranged, dark green, glossy, flowering stem winged, leafy-like, shorter than leaves, rhipidia with short and long peduncles, flowers predominantly white, with yellowish proximal region, with blue to violet stripes.

Neomarica portosecurensis (Ravenna) Chukr

$2n = 18$, CHN. Brazil, Ceará, Maranguape, 03°53'29.63"S, 38°41'50.60"W, 180 m, 28 Oct 2005, *L.P. Felix 13831* (EAN) [Fig. 1D].

The species is a terricolous herb, with underground stem rhizome-like, vertical, leaves multiple, dorsiventrally flattened, linear, distichously arranged, light green, rhipidia with short peduncle, large yellow flowers.

Neomarica sergipensis A.Gil & M.C.E.Amaral

$2n = 18$, CHN. Brazil, Sergipe, Areia Branca, Serra de Itabaina, 10°44'17.02"S, 37°20'07.94"W, 203 m, 23 Oct 2005, *L.P. Felix 12743* (EAN) [Fig. 1E].

The species is a terricolous herb, with underground stem rhizome-like, vertical, delicate, leaves multiple, dorsiventrally flattened, linear, distichously arranged, opaque green, flowering stem winged, leafy-like, narrower than leaves, rhipidia with conspicuous peduncles, flowers predominantly white with yellow proximal region and brown and purple stripes.

Pseudiris speciosa Chukr & A.Gil

$2n = 30$, CHN. Brazil, Bahia, Mucugê, PARNA da Chapada Diamantina, Serra do Esbarrancado, 12°43'51.0"S, 41°30'33.0"W, 1500 m, 16 Apr 2005, rupestrian field, *D. Cardoso & A.A. Conceição*

434 (HUEFS) [Fig. 1F]; Brazil, Bahia, Mucugê, 13°00'31.65"S, 41°22'01.39"W, 982 m, 12 Oct 2012, *L.P. Felix 14811* (EAN) [Fig. 1G].

The species is a terricolous herb, with underground stem, vertical, corm-like, wrapped in a fibrous tunic, leaves multiple, dorsiventrally flattened, linear, flowering stem wingless, longer than leaves, delicate purple flowers.

*****Trimezia connata* Ravenna**

* $2n = 56$, CHN. Brazil, Pernambuco, Brejo da Madre de Deus, Bitury Forest, 08°11'42.00"S, 36°23'35.15"W, 982 m, 09 Oct 2010, *L.P. Felix 13151* (EAN) [Fig. 1H].

$2n = 82$, CHN. Brazil, Paraíba, Areia, 06°58'18.46"S, 35°42'24.39"W, 982 m, 09 Oct 2010, *L.P. Felix 11966* (EAN) [Fig. 1I].

The species is a terricolous herb, with underground stem corm-like, wrapped in a fibrous tunic, two leaves, dorsiventrally flattened, linear, flowering stem wingless, yellow flowers.

***Trimezia martinicensis* (Jacq.) Herb.**

$2n = 54$, CHN. Brazil, Pernambuco, Brejo da Madre de Deus, 08°08'30.72"S, 36°21'22.53"W, 692 m, 09 Oct 2010, *L.P. Felix 11185* (EAN) [Fig. 1J].

The species is a terricolous herb, with underground stem corm-like, wrapped in a fibrous tunic, leaves multiple, dorsiventrally flattened, linear leaves, similar in length to flowering stems, wingless flowering stem, yellow small flowers.

*****Trimezia sincorana* Ravenna**

$2n = 28$, CHN. Brazil, Bahia, Morro do Chapéu, 11°31'57.72"S, 41°09'07.51"W, 1066 m, 27 Mar 2007, *L.P. Felix 11691* (EAN) [Fig. 1K].

* $2n = 54$, CHN. Brazil, Bahia, Morro do Chapéu, 11°33'33.72"S, 41°10'11.35"W, 1051 m, 14 Oct 2012, *J.P. Castro 119* (EAN) [Fig. 1L].

The species is a terricolous herb, with underground stem corm-like, wrapped in a fibrous tunic, long linear leaves, wingless flowering stem, shorter than leaves, predominantly yellow flowers, light brown spotted inflorescence with 1–3 rhipidia.

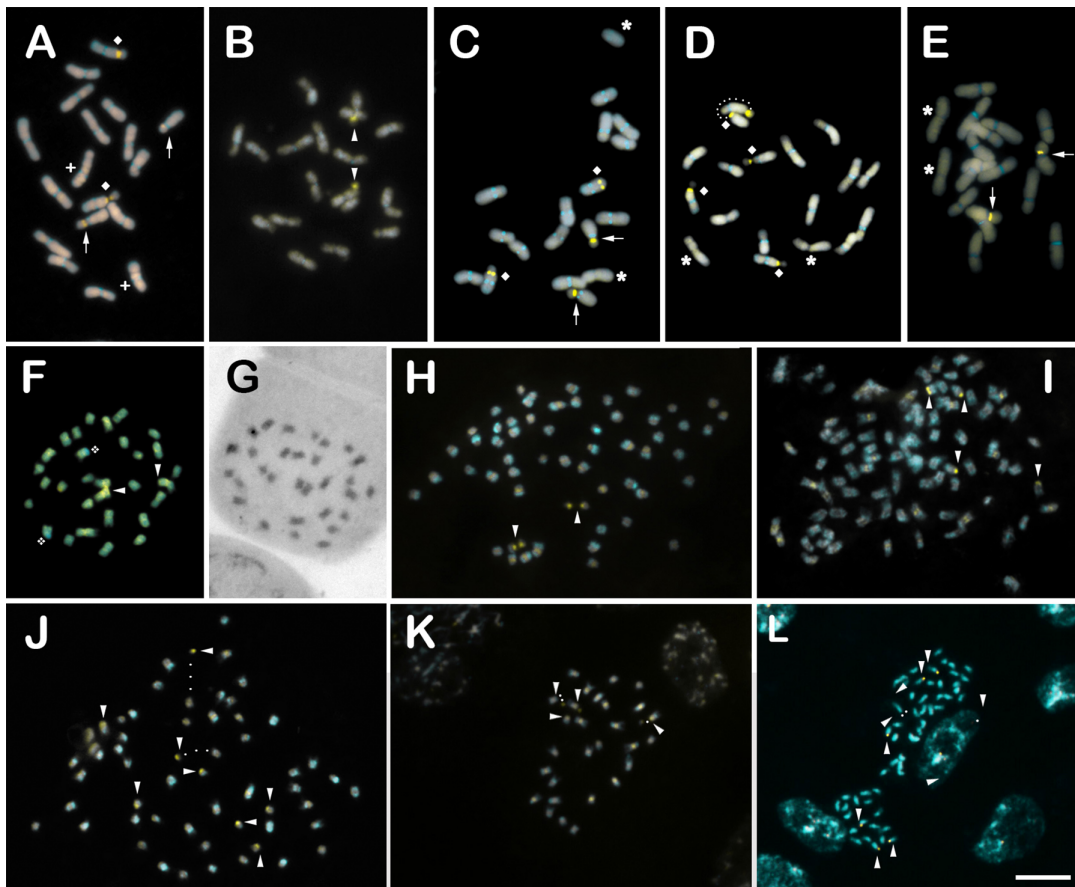


Fig. 1. Karyotypes in Trimezieae species from *Neomarica*, *Pseudiris*, and *Trimezia*. **A**, *Neomarica candida*, $2n = 18$; **B**, *N. humilis*, $2n = 18$; **C**, *N. northiana*, $2n = 18$; **D**, *N. portosecurensis*, $2n = 18$; **E**, *N. sergipensis*, $2n = 18$; **F & G**, *Pseudiris speciosa*, $2n = 30$; **H**, *Trimezia connata*, $2n = 56$; **I**, *T. connata*, $2n = 82$; **J**, *T. martinicensis*, $2n = 54$; **K**, *T. sincorana*, $2n = 28$; **L**, *T. sincorana*, $2n = 54$. — The metaphases presented after the CMA/DAPI banding with CMA⁺ bands (CG-rich blocks) presented in yellow and DAPI⁺ bands (AT-rich blocks) in blue. The metaphase stained with Giemsa (**G**) is presented in black and white. The + symbols in **A** indicate chromosomes with an interstitial DAPI⁺ band, the asterisks in **C**, **D**, and **E** indicate chromosomes without any heterochromatic band, the ∙ symbols in **F** indicate chromosomes with terminal DAPI⁺ band, and the ♦ symbols in **A**, **C**, and **D** indicate chromosomes with interstitial CMA⁺ bands. The arrows in **A**, **C**, and **E** indicate chromosomes with proximal CMA⁺ bands on the short arm and proximal DAPI⁺ bands on the long arm, and the arrowheads in **B**, **F** and **H–L** indicate terminal CMA⁺ bands. The dots in **D**, **J**, **K** and **L** represent distended regions of the chromosomes, linking the main part of the chromosome with the satellite. Bar in **L** indicates 10 μm.

The taxonomic entities often differ in terms of chromosome number and morphology; in the same way they could differ in overall body morphology, anatomy, and physiology (Guerra, 2008, 2012). Differences in karyotype characters are caused by different chromosome numbers and structural changes (Stebbins, 1971; Schubert & Lysak, 2011), and these modifications could be detected by a variety of cytogenetic techniques such as chromosome counting and chromosome banding, by detecting the distribution and classification (AT- or CG-rich) of heterochromatin blocks (Schweizer, 1976). The data obtained by these techniques usually provides an useful element for taxonomic delimitations.

Iridaceae Juss. is remarkable by the high diversity of karyotypes, in terms of both chromosome number and morphology, often presenting asymmetric karyotypes, a character commonly considered to be apomorphic (Stebbins, 1971). Asymmetric karyotypes result from recurrent chromosome changes such as fission/fusion, which also causes dysploidy, a chromosome number variation also common in Iridaceae (Goldblatt & Takei, 1997; Moraes & al.,

2015). Regarding chromosome numbers, Iridaceae is a relatively well-studied plant family, with data available for more than 65% of its c. 2030 species. The two most frequent chromosome numbers in Iridaceae are $2n = 14$ and 28 (base chromosome number $x = 7$), but high ploidy levels such as $2n = 14x = 96$ may also be found (see Goldblatt, 1982; Goldblatt & Henrich, 1987; Moraes & al., 2015). However, from the Neotropical region, the second-largest center of Iridaceae diversity, the chromosome data is poorly known, with available counts for less than 10% of Neotropical species (140 out the approx. 1944 species).

Among the seven Iridaceae subfamilies, only Iridoideae occurs in the Neotropics. Within the Iridoideae's five lineages, only the tribes Tigridae and Trimezieae are endemic to this important biodiversity region (Goldblatt & al., 2008). Trimezieae was recently the subject of a broad taxonomic treatment that resulted in the formal recognition of five generic-level monophyletic entities: *Pseudotrimezia* R.C.Foster (including some species traditionally treated under *Trimezia* Salisb. ex Herb.), *Neomarica* Sprague,

Table 1. Chromosome number and CMA/DAPI banding pattern for Trimezieae species.

Taxa	Voucher	Origin [City, State]	$2n$ [Fig.]	Chromosome banding*		Reference(s) [#]
				CMA ⁺ DAPI ⁻	CMA ⁻ DAPI ⁺	
<i>Neomarica</i> Sprague						
<i>N. candida</i> (Hassl.) Sprague	<i>L.P. Felix 12802</i>	Ilha Comprida, SP	18 [1A]	2p + 2i	18p + 2i	18 (A07; A11; M15; PW)
<i>N. humilis</i> (Klatt) Capell.	<i>L.P. Felix 11001</i>	Bonito, PE	18 [1B]	2p	16p	18 (A11; G12; PW)
<i>N. northiana</i> (Schneev.) Sprague	<i>L.I.F. Alves 02</i>	Campina Grande, PB	18 [1C]	2p + 2i	16p	18 (GT82; GT97; AF07; TRB08; G12; PW) 22 (K02)
<i>N. portosecurensis</i> (Ravenna) Chukr	<i>L.P. Felix 13831</i>	Maranguape, CE	18 [1D]	4i	16p	18 (G12; PW)
<i>N. sergipensis</i> A.Gil & M.C.E.Amaral	<i>L.P. Felix 12743</i>	Areia Branca, SE	18 [1E]	2t	18p	18 (G12; PW)
<i>Pseudiris</i> Chukr & A.Gil						
<i>P. speciosa</i> Chukr & A.Gil	<i>L.P. Felix 14811</i>	Mucugê, Chapada Diamantina, BA	30 [1F]	20p + 2t	2t	30 (G08, PW)
	<i>D. Cardoso & A.A. Conceição, 434</i>	PARNA da Chapada Diamantina, Serra do Esbarrancado	30 [1G]			
<i>Trimezia</i> Salisb. ex. Herbert						
<i>T. connata</i> Ravenna	<i>L.P. Felix 13151</i>	Brejo da Madre de Deus, PE	56 [1H]	56p + 2t (st)		56 (PW)
	<i>L.P. Felix 11966</i>	Areia, PB	82 [1I]	82p + 4t	28t	82 (A11; PW)
<i>T. martinicensis</i> (Jacq.) Herb.	<i>L.P. Felix 11185</i>	Brejo da Madre de Deus, PE	54 [1J]	8t (2st)		54 (KH84; AF07; A11; PW)
<i>T. sincorana</i> Ravenna	<i>L.P. Felix 11691</i>	Morro do Chapéu, BA	28 [1K]	4t (3st) + 28p		28 (G12; PW)
	<i>L.P. Felix 119</i>	Morro do Chapéu, BA	54 [1L]	6t		54 (PW)

* Band position: i, interstitial; p, proximal; t, terminal; (st), satellite (the number of satellited chromosomes is indicated in parentheses)

[#] References: GT82, Goldblatt (1982); KH84, Kenton & Heywood (1984); GT97, Goldblatt & Takei (1997); K02, Kalaiselvi (2002); A07, Alves & Felix (2007); G08, Gil & al. (2008); TRB08, De Tullio & al. (2008); A11, Alves & al. (2011); G12, Gil (2012); M15, Moraes & al. (2015); PW, Present work.

Deluciris A.Gil & Lovo (a recently described genus), *Pseudiris* Chukr & A.Gil and *Trimezia* (Lovo & al., 2018). Within Iridoideae, tribe Trimezieae is the tribe with the least-studied karyotypes. The present study aims to minimize this gap by presenting a karyotype analysis based on chromosome counting and banding, for selected species from the three genera: *Neomarica* (five species), *Pseudiris* (one species) and *Trimezia* (three species). Methods for chromosome preparation and Giemsa staining (only for *P. speciosa* collected by D. Cardoso & A.A. Conceição 434) followed Guerra & Souza (2002), while CMA/DAPI banding (for the remaining vouchers) followed Schweizer (1976). The best metaphases were photographed under a Zeiss Axio Microscope coupled with an Axio Cam MRC5 camera and software Axiovision v.4.8. All metaphases were homogeneously edited for brightness and contrast using Adobe Photoshop CS3 v.10.

The five studied *Neomarica* species have $2n = 18$ (Table 1; Fig. 1A–E), which is in agreement with previous studies (Goldblatt & Takei, 1997; Alves & Felix, 2007; De Tullio & al., 2008; Alves & al., 2011; Gil, 2012; Moraes & al., 2015). The stable chromosome number in *Neomarica* reflects the karyotype stability described for this genus in the literature (Goldblatt, 1982; Kenton & Heywood, 1984). Interestingly, this is the only lineage recovered as monophyletic in molecular phylogeny, presenting also a very cohesive morphology (corresponding to the Fluminensis clade by Lovo & al., 2018). For the monotypic *P. speciosa* Chukr & A.Gil, the chromosome number also agrees with data previously published by Gil & al. (2008), $2n = 30$ (Table 1; Fig. 1F–G). Contrasting with *Neomarica*, in *Trimezia* the chromosome numbers vary considerably among species and also among populations, as observed in *T. connata* and *T. sincorana* (Table 1). *Trimezia connata* presents two cytotypes: a putative hexaploid with $2n = 82$ (Fig. 1I), as previously observed by Alves & al. (2011) (Table 1), and a putative tetraploid cytotype with $2n = 56$ (Fig. 1H), a new count for this species. Also for *T. sincorana*, two ploidy levels were found: the diploid cytotype $2n = 28$ (Fig. 1K), which agrees with a previous one found by Gil (2012), and the polyploid cytotype $2n = 54$ (Fig. 1L), which represents a new chromosome number for the species. We hypothesize that the cytotypes $2n = 82$ of *T. connata* and $2n = 54$ of *T. sincorana* could represent polyploid cytotypes with subsequent descending dysploidy (from 84 to 82 in the former and from 56 to 54 in the latter) (Table 1). Finally, our results for *T. martinicensis*, $2n = 54$ (Fig. 1J), agree with those published by Alves & Felix (2007), Chukr & Giulietti (2008), Moreno & al. (2009) and Alves & al. (2011) (Table 1), and also could represent a polyploid cytotype with descending dysploidy. Our results, despite representing a small fraction of *Trimezia*, reflect the huge diversity of chromosome numbers found in this genus, with several polyploid and dysploid series, as reported in the literature: $2n = 26, 28, 40, 54, 60, 76, 80$ and 82 (Goldblatt, 1982; Kenton & Heywood, 1984; Goldblatt & Takei, 1997; Alves & al., 2011; Moraes & al., 2015). These results reinforce the important role of dysploidy in the evolutionary history of Iridaceae, as already suggested by Moraes & al. (2015) after analysing the karyotype evolution within the exclusive Neotropical lineages Tigridaeae-Trimezieae.

The CMA/DAPI (chromomycin A3/4',6-diamidino-2-phenylindole) chromosome banding revealed two types of heterochromatic blocks: CG (guanine-cytosine)-rich, identified as bright CMA⁺ bands (yellow blocks), and AT (adenine-thymine)-rich, when bright with DAPI (DAPI⁺ bands, blue blocks). The pattern of heterochromatic CMA⁺ and DAPI⁺ distribution reveals an important difference among genera (Table 1): while *Neomarica* presented more frequently pericentromeric DAPI⁺ bands (Fig. 1A–E),

Pseudiris and *Trimezia* (both belonging to the Martinicensis clade, Lovo & al., 2018) presented more frequently proximal CMA⁺ bands (Fig. 1F–L), revealing that different repetitive DNA sequences colonized the pericentromeric and terminal regions among genera in accordance with the lineages recovered by the phylogeny and recognized as genus in the new classification for the tribe (Lovo & al., 2018). Three *Neomarica* species, *N. northiana*, *N. portosecurensis*, and *N. sergipensis*, possess one chromosome pair without any heterochromatic band (see asterisks in Fig. 1C–E), while the remaining eight chromosome pairs possess different combinations of CMA⁺ and/or DAPI⁺ bands. The proximal DAPI⁺ bands were found in the five analyzed *Neomarica* species, but *N. candida* showed also one chromosome pair with an interstitial DAPI⁺ band (see + in Fig. 1A). In *Neomarica*, the CMA⁺ heterochromatic blocks varied between two and four: (1) two terminal bands on the long arm in *N. humilis* (see arrowheads in Fig. 1B); (2) two proximal bands in the short arm in *N. sergipensis* (see arrows in Fig. 1E), and (3) four bands in *N. candida* (Fig. 1A), *N. northiana* (Fig. 1C) and *N. portosecurensis* (Fig. 1D). In these three last species, the four CMA⁺ bands were placed, two at the proximal region on the short arm and two at interstitial position on the long arm (see arrows in Fig. 1A and C), but in *N. portosecurensis* (Fig. 1D), the four bands were placed in the interstitial region on the long arm and are frequently distended, possibly associated with the nuclear organizing region (NOR) (see ♦ in Fig. 1A, C and D). *Pseudiris speciosa* ($2n = 30$) has proximal tiny CMA⁺ heterochromatic sites in approx. 20 chromosomes (Fig. 1F), plus two terminal CMA⁺ bands (see arrowheads in Fig. 1F) and also, two terminal DAPI⁺ bands (see ∴ in Fig. 1F). In *Trimezia*, the banding pattern was diverse and informative (Table 1). *Trimezia connata* possess two terminal and distended CMA⁺ bands in the diploid cytotype (see arrowheads in Fig. 1H) and four terminal CMA⁺ bands in the polyploid cytotype (see arrowheads in Fig. 1J). On both cytotypes, proximal CMA⁺ bands could be detected in almost all chromosomes. The species *T. martinicensis* presented eight terminal CMA⁺ bands, with two bands frequently distended constituting the satellite (see dots in Fig. 1J). In *T. sincorana*, the two cytotypes also differ in the number of terminal CMA⁺ bands: while $2n = 28$ possess four small terminal CMA⁺ bands, usually distended (see dots in Fig. 1K), the polyploid cytotype with $2n = 54$ possess six terminal CMA⁺ bands (see dots in Fig. 1L). The variation in the number of heterochromatic bands indicated that in *T. sincorana* there is no direct relationship between cytotype ploidy level and number of heterochromatic blocks, reflecting the process of diploidization and reorganization of the genome after polyploid and dysploid events. However, in *T. connata* such relationship is conserved. It is important to note that despite the detected karyotype diversity in *Trimezia* species, it is not associated with morphological discontinuities, and the occurrence of cryptic species could not be discarded. The results of chromosome banding contrast with the traditionally assumed karyotypic constancy and reveal, even for *Neomarica*, very diverse and informative karyotypes. Such characters should be explored in future taxonomic studies, allowing to differ between cryptic species and recent diverging populations.

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IAPT chromosome data 31/2

Tatyana V. An'kova,* Elena Yu. Zykova & Aleksandr L. Ebel

* Address for correspondence: ankova_tv@mail.ru

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- * First chromosome count from a Russian accession.
- First chromosome count from a Siberian accession.

ASTERACEAE

Carduus acanthoides L.

* $2n = 2x = 22$, CHN. Russia, Novosibirskaya Oblast', Katkovskii village, Kudryashovskii bor, pine forest, 55°10'30"N, 82°43'54"E, 13 Aug 2018, *A.L. Ebel Z639* (TK); Russia, Omskaya Oblast', Pyatiletka village, roadside, 54°55'41"N, 72°56'12"E, 16 Aug 2018, *A.L. Ebel Z640* (TK); Russia, Omskaya Oblast', Odesskii Raion, Generalovka village, roadside, 54°16'06"N, 73°09'39"E, 18 Aug 2018, *A.L. Ebel Z641* (TK).

Carduus acanthoides is of European origin. As an alien plant, it is distributed in North America and in several Asian countries. By now, the species is widespread in the southern regions of western Siberia from the Kurgan region in the west to the Kemerovo region in the east. Within Siberia, this species is most widespread in the Altai Territory. Being a plant not grazed by cattle, *C. acanthoides* is a typical pasture weed. In the foothills of the North and West Altai (Altai Territory) and in the Kuznetsk Basin (Kemerovo Region), *C. acanthoides* sometimes forms continuous thickets on intensively exploited pastures (Ebel & al., 2017).

Centaurea diffusa Lam.

• $2n = 2x = 18$; CHN. Russia, Altaiskii Krai, Kulundinskii Raion, 12 km north-west of Kulunda village, field edge, 52°40'03"N, 78°50'37"E, 22 Aug 2018, *A.L. Ebel Z636* (TK).

The natural range of this species is located in Europe and Asia Minor. It is native to southeastern Europe and occasionally adventive in central and western Europe. In Siberia, the species is found as invasive plant in the plains steppe regions of the Altai Territory, less often in the Kemerovo Region. *Centaurea diffusa* is characterized by a significant polymorphism that determines its resistance to drought and other factors. It is a pioneer species in degraded lands or broken pastures (Vinogradova & Kupriyanov, 2016).

Centaurea jacea L.

$2n = 4x = 44$; CHN. Russia, Novosibirskaya Oblast', Moshkovskii Raion, vicinity of Koshevo village, roadside, 55°15'47"N, 83°30'49"E, 26 Aug 2018, *A.L. Ebel Z635* (TK).

This species of European-Mediterranean origin is actively spreading in the southern regions of Siberia. In Siberia, this species was sown as honey or ornamental plant; later, it penetrated the fields as a weed together with the seeds of crops. Currently, the species is distributed along transport routes. It grows in meadows, among shrubs, as ruderal in urban areas, and along roads. In some areas, this species has successfully settled in river valleys (Vinogradova & Kupriyanov, 2016).

Centaurea pseudomaculosa Dobrosz.

• $2n = 2x = 18$; CHN. Russia, Omskaya Oblast', Omsk city, wasteland, 54°52'54"N, 73°22'30"E, 16 Aug 2018, *A.L. Ebel Z631* (TK); Russia, Novosibirskaya Oblast', Karasukskii Raion, Michailovka village, steppe mowing, 53°39'05"N, 78°03'46"E, *A.L. Ebel Z634* (TK).

This is a species of European origin; by now, it is widespread in the southern regions of Siberia. The spread of *C. pseudomaculosa* to Siberia began in the second half of the 20th century. In recent decades, the species has a tendency to expand its range in Siberia. *Centaurea pseudomaculosa* is able to invade natural communities, which is observed in some steppe and forest-steppe regions in southern Siberia (Vinogradova & Kupriyanov, 2016).

BRASSICACEAE

Sisymbrium officinale (L.) Scop.

$2n = 2x = 14$; CHN. Russia, Altaiskii Krai, Sovetskii Raion, Shulgin Log village, roadside, 52°11'N, 85°50'E, 01 Aug 2015, *E. Zykova Z536:3215* (NS).

CARYOPHYLLACEAE

Dianthus ×courtoisii Rchb.

* $2n = 2x = 30$; CHN. Russia, Tomskaya Oblast', Tomskii Raion, Voronino village, cemetery, 56°33'38"N, 85°13'07"E, 01 Sep 2018, *A.L. Ebel Z638* (TK).

Spontaneous hybrid between native *Dianthus superbus* L. and alien *D. barbatus* L.

Dianthus versicolor Link

$2n = 2x = 30$; CHN. Russia, Altai Republic, Turochakskii Raion, about 7 km from Artybash village, bank of the Biya River, roadside, 51°46'N, 87°15'E, 15 Aug 2017, *E. Zykova Z331:5617* (NS).

Elisanthe noctiflora (L.) Rupr.

$2n = 3x = 24$, $2n = 4x = 36$; CHN. Russia, Altaiskii Krai, Sovetskii Raion, Shulgin Log village, roadside, 52°11'N, 85°50'E, 01 Aug 2015, *E. Zykova Z548:3215* (NS).

CHENOPODIACEAE

Chenopodium polyspermum L.

$2n = 2x = 18$; CHN. Russia, Altai Republic, Turochakskii Raion, Altai state reserve, Kartash cordon, homestead, 51°46'N, 87°23'E, 14 Aug 2017, *E. Zykova Z392:5117* (NS); Russia, Altai Republic, Gorno-Altai city, Shosseina street, wasteland, 51°58'N, 85°55'E, 22 Aug 2017, *E. Zykova Z394:6717* (NS).

FABACEAE

Caragana arborescens Lam.

$2n = 2x = 16$; CHN. Russia, Novosibirskaya Oblast', Novosibirsk, Akademgorodok, Central Siberian Botanical Garden,

abandoned area, 54°59'N, 83°00'E, 15 Oct 2017, *E. Zykova Z313:7617* (NS).

Vicia angustifolia L.

$2n = 2x = 12$, Russia, Altai Republic, Maiminskii Raion, Maima village, roadside, 52°02'N, 85°54'E, 12 Aug 2017, *E. Zykova Z384:5017* (NS).

OXALIDACEAE

Oxalis stricta L.

* $2n = 4x = 24$, CHN. Russia, Novosibirskaya Oblast', Novosibirsk, Akademgorodok, Novosibirsk State University campus, along the path, 54°59'N, 83°00'E, 18 Sep 2016, *E. Zykova Z243:0916* (NS).

PLANTAGINACEAE

Veronica arvensis L.

* $2n = 2x = 16$, CHN. Russia, Altai Republic, Choiskii Raion, Sovetskoe village, gravel blade, 52°02'N, 86°36'E, 29 Jun 2017, *E. Zykova Z402:2417* (NS); Russia, Altai Republic, Turochakskii Raion, Iogach village, stadium, 51°46'N, 87°15'E, 29 Jun 2017, *E. Zykova Z401:2617* (NS).

POACEAE

Avena fatua L.

• $2n = 4x = 28$, CHN. Russia, Altai Republic, Maiminskii raion, Maima village vicinities, arable land near the custom post, 52°02'N, 85°54'E, 01 Aug 2015, *E. Zykova Z440:3015* (NS).

• $2n = 6x = 42$, CHN. Russia, Altai Republic, Chemalskii Raion, Anos village, wasteland, 51°29'N, 85°56'E, 16 Aug 2015, *E. Zykova Z441:4615* (NS).

VITACEAE

Parthenocissus quinquefolia (L.) Planch.

• $2n = 4x = 40$, CHN. Russia, Altai Republic, Gorno-Altai city, Protochnaya street, wasteland, 51°58'N, 85°55'E, 18 Aug 2017, *E. Zykova Z502:6017* (NS).

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IAPT chromosome data 31/3

Luana Carvalho, Cassiano A.D. Welker, Tatiana T. Souza-Chies & Eliane Kaltchuk-Santos*

* Address for correspondence: eliane.kaltchuk@ufrgs.br

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Pessoal de Nível Superior, Brazil – finance code 001). We acknowledge the laboratory technicians Letícia Gal and Pedro Erê Disconzi Brum for their help in sample preparation and handling of the cytometer to obtain genome size data of this work.

* First chromosome count for the taxon.

** First chromosome count from an unexplored part of the distribution area of the taxon.

POACEAE

Subfamily Panicoideae

Tribe Andropogoneae

***Eriochrysis cayennensis* P.Beauv.

$n = 10$, CHN. Brazil, Rio Grande do Sul, Cidreira, 30°08'07.5"S, 50°11'19.7"W, 28 Feb 2013, C.A.D. Welker 617 (ICN); Brazil, Rio Grande do Sul, Osório, 29°52'28.2"S, 50°05'39.2"W, 12 Dec 2011, H.M. Longhi-Wagner & C.A.D. Welker 10863 (ICN); Brazil, Rio Grande do Sul, São Borja, 28°38'02.5"S, 55°47'19.5"W, 23 Jan 2018, C.A.D. Welker, E. Kaltchuk-Santos & L. Carvalho 694 (HUFU) [Fig. 2A].

The chromosome number found is in agreement with the previous reports by Pohl & Davidse (1971) and Killeen (1990) for specimens from Costa Rica and Bolivia, respectively.

***Eriochrysis laxa* Swallen

$n = 10$, CHN. Brazil, Rio Grande do Sul, São Borja, 28°38'02.5"S, 55°47'19.5"W, 23 Jan 2018, C.A.D. Welker, E. Kaltchuk-Santos & L. Carvalho 693 (HUFU), C.A.D. Welker, E. Kaltchuk-Santos & L. Carvalho 695 (HUFU) [Fig. 2B].

The same chromosome number was reported by Killeen (1990) for plants from Bolivia.

**Eriochrysis villosa* Swallen

$n = 10$, CHN. Brazil, Rio Grande do Sul, São Borja, 28°38'02.5"S, 55°47'19.5"W, 22 Jan 2013, C.A.D. Welker 541 (ICN), 23 Jan 2018, C.A.D. Welker, E. Kaltchuk-Santos & L. Carvalho 696 (HUFU) [Fig. 2C].

Poaceae is one of the largest families of flowering plants, comprising about 768 genera and 11,500 species in 12 subfamilies (Soreng & al., 2017). The grass genus *Eriochrysis* P.Beauv. belongs to the subfamily Panicoideae, tribe Andropogoneae, and includes ca. 7–12 species mainly from the New World, with a few species in Africa and India (Clayton & Renvoize, 1986; Watson & Dallwitz, 1992; Kellogg, 2015; Soreng & al., 2017). The number of accepted species in the genus is controversial and highlights the complex circumscription of these taxa (Welker & al., 2016). Six species are referred to from Brazil: *Eriochrysis cayennensis*, *E. filiformis* (Hack.) Filg., *E. holcooides* (Nees) Kuhl., *E. laxa*, *E. villosa*, and *E. warmingiana* (Hack.) Kuhl. (Welker & al., 2012; Flora do Brasil, 2019). Polyploidy and hybridization have been reported in grass evolution as important events for speciation. At least one-third of the Andropogoneae species resulted from allopolyploidy, with a remarkably high number of independent allopolyploidization events (Estep & al., 2014). Cytogenetic data for *Eriochrysis* taxa are scanty and restricted to chromosome number determination for three species. Considering the three species investigated in the present study, chromosome number is available for *E. cayennensis* (based on specimens from Costa Rica and Bolivia) and *E. laxa* (from Bolivia) (Pohl & Davidse, 1971; Killeen, 1990). Until now, there are no chromosome counts for Brazilian specimens of *Eriochrysis*. Data regarding genome size and meiotic stability are very important to understand

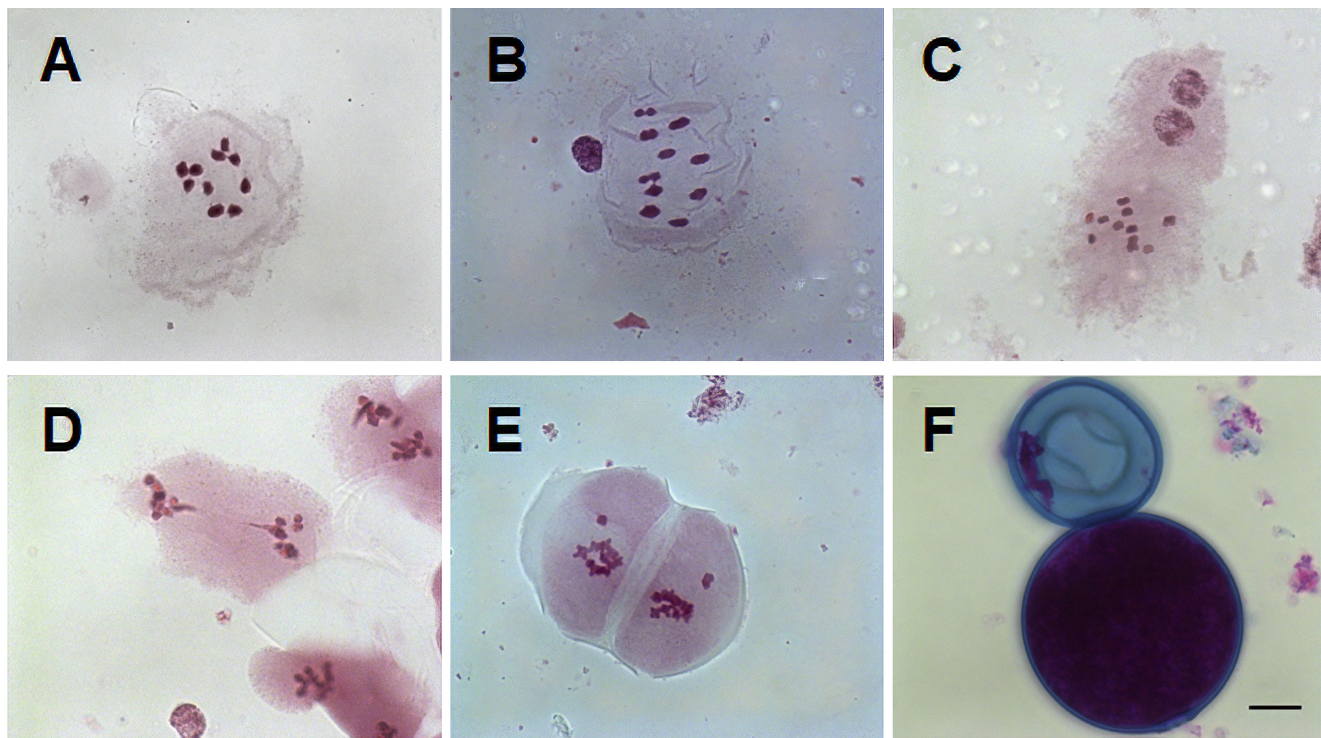


Fig. 2. Meiotic cells of *Eriochrysis* species. **A**, *E. cayennensis*, diakinesis with 10 bivalents; **B**, *E. laxa*, metaphase I with 10 bivalents; **C**, *E. villosa*, diakinesis with 10 bivalents; **D**, Chromosome bridge in anaphase I of *E. cayennensis*; **E**, Non-oriented chromosomes in metaphase II of *E. laxa*; **F**, Viable and unviable pollen grains of *E. villosa*. — Scale bar = 10 μm .

Table 2. Chromosome numbers, analyses of meiotic behavior and pollen viability, and genome size in *Eriochrysis* species.

Species	<i>n</i>	Meiosis I and II		Meiotic index (MI)%	Pollen viability			P (μm) ^d	E (μm) ^d	RatioP/E	Pollen morphology	Genome size (pg)
		N ^a	% ^b		N ^a	% ^c	2C					
<i>E. cayennensis</i>	10	1195 (5)	99.58	100	1526 (3)	99.74	41.53	40.93	1.01	Prolate spheroidal	3.33	
<i>E. laxa</i>	10	591 (2)	99.83	100	3772 (8)	97.67	40.70	40.01	1.02	Prolate spheroidal	3.42	
<i>E. villosa</i>	10	1659 (4)	100	100	1072 (2)	99.81	42.44	41.78	1.02	Prolate spheroidal	3.46	

Measurements of pollen grain axes and morphological classification according to Erdtman (1971).

a – Number of cells analyzed (number of individuals); b – Percentage of normal cells; c – Percentage of viable pollen grains (stained pollen grains); d – P = pollen polar axis and E = pollen equatorial axis, average measurement of 20 mature pollen grains per individual

the evolution of the genus within the tribe Andropogoneae. However, such data are not available in the literature.

Aiming to contribute with new cytotaxonomic information for species of the genus *Eriochrysis*, this study presents the chromosome numbers, meiotic behavior, pollen viability and morphology, as well as DNA content (2C) for three species from Brazil.

For meiotic analyses, young inflorescences of each species were collected and fixed in 3 : 1 ethanol : acetic acid at room temperature for 12–24 h and stored at –20°C. Slides were prepared by squashing the anthers in 2% propionic carmine. Chromosome counts were performed on pollen mother cells at diakinesis or metaphase I. For meiotic behavior investigation, all available phases of meiosis I and II were analyzed. Meiotic indexes (MI) were calculated using the formula: MI = (number of normal tetrads / total of tetrads) × 100. Inflorescences at anthesis were collected for investigation of pollen viability and morphology and were fixed as described above. Pollen viability was estimated using Alexander's (1980) method. The polar axis (P) and equatorial axis (E) of mature pollen grains were measured and the P/E ratio calculated in order to determine grain shape (Erdtman, 1971). For genome size analysis, approximately 25 mg of leaf tissue from standard (*Pisum sativum* L. 'Ctirad' 2C = 9.09 pg; Doležel & al., 2007) and from each species were chopped in 1 ml of cold nuclear-isolation buffer LB01. The suspension was filtered through a 40 μm mesh nylon filter, and nuclei were stained with 50 μl propidium iodide. The DNA content of 10,000 stained nuclei for each sample was estimated using FACSARIA II (Becton Dickinson, Franklin Lakes, New Jersey, U.S.A.) flow cytometer.

In this work, cytological data were obtained from five populations of three *Eriochrysis* species. Cytogenetic results are presented in Table 2. This is the first report of chromosome counts for *Eriochrysis* specimens from Brazil. *Eriochrysis cayennensis* and *E. laxa* presented the same chromosome number ($n = x = 10$, Fig. 2A and B respectively) previously reported for plants from Costa Rica and Bolivia (Pohl & Davidse, 1971; Killeen, 1990), indicating that the Brazilian specimens analyzed are also diploid. *Eriochrysis villosa* had its chromosome number determined for the first time, being diploid ($n = x = 10$) as well (Fig. 2C).

The three species exhibited highly stable meiotic behavior (Table 2) with bivalent pairing and regular segregation. The most frequent abnormalities were chromosome bridges (anaphase I/II) (Fig. 2D) and non-oriented chromosomes (metaphase I/II) (Fig. 2E). Pollen stainability and morphology were used to assess pollen viability. All taxa presented high meiotic indexes (MI) and pollen viability with at least 97.67% viable pollen grains (Table 2), indicating that these plants are all male-fertile. The pollen morphology was the same for all species, prolate spheroidal (Fig. 2F).

Genome size was very similar in all species (Table 2), ranging from 3.33 pg (*E. cayennensis*) to 3.46 pg (*E. villosa*). This is the first report of genome size estimates for the genus *Eriochrysis*.

In a recent phylogenetic study based on low-copy nuclear genes, Welker & al. (2016) suggested that all New World species of *Eriochrysis* are diploid, since these taxa had only a single sequence per locus for all nuclear genes analyzed. Thus, the chromosome numbers presented here confirm the ploidy level suggested by Welker & al. (2016). Among the *Eriochrysis* samples analyzed by Welker & al. (2016), the only exception was the specimens identified as *E. aff. laxa*, which had two distinct paralogues in the nuclear phylogenetic trees. These plants have intermediate morphology between *E. laxa* and *E. villosa* (Welker & al., 2012) and were confirmed as natural hybrids between the two species (Welker & al., 2016). Based on the number of paralogues in the nuclear gene trees, the *E. aff. laxa* specimens are probably polyploid (Welker & al., 2016). Taking into account the data of the parental species presented here, the collection and analysis of *E. aff. laxa* specimens through cytogenetic tools may bring more information to clarify their allopolyploid origin.

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IAPT chromosome data 31/4

Franco E. Chiarini,* Andrés Orejuela & Sandra Knapp

* Address for correspondence: franco.e.chiarini@gmail.com

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* First chromosome count for the species.

SOLANACEAE

**Benthamiella graminifolia* Skottsbo.

$2n = 22$, CHN. Argentina, Neuquén, Pehuenches, Parque Provincial Tromen, 31 Jan 2013, G. Barboza, F. Chiarini & S. Knapp 3813 (CORD).

**Benthamiella patagonica* Speg.

$2n = 22$, CHN. Argentina, Santa Cruz, Río Chico, Laguna El Salital, 25 Jan 2013, G. Barboza, F. Chiarini & S. Knapp 3747 (CORD).

Benthamiella pycnophylloides Speg.

$2n = 22$, CHN. Argentina, Santa Cruz, Deseado, 3.7 km al N de Fitz Roy, 21 Jan 2013, G. Barboza, F. Chiarini & S. Knapp 3689 (CORD).

**Fabiana patagonica* Speg.

$2n = 18$, CHN. Argentina, Jujuy, Tumbaya, Salinas Grandes, 23°38'29.3"S, 65°50'24"W, 25 Jan 2017, F. Chiarini 1322 (CORD).

**Fabiana punensis* S.C.Arroyo

$2n = 18$, CHN. Argentina, Jujuy, Tumbaya, Salinas Grandes, 23°38'29.3"S, 65°50'24"W, 25 Jan 2017, F. Chiarini 1321 (CORD).

**Lycianthes heterochondra* (Bitter) Bitter

$2n = 24$, CHN. Perú, Puno, Sandia, 1.5–2 km from Sandia on road to Cuyocuyo, 14°23'19"S, 69°28'13"W, T. Särkinen, A. Matthews & P. González 4046 (BM).

**Trianaea nobilis* Planch. & Linden

$2n = 24$, CHN. Ecuador, Cotopaxi, San Francisco de la Pampa, desde Naranjito rumbo a la cabaña de la Reserva Otonga, C.I. Orozco 3967 (COL, QCA).

We present here chromosome numbers and karyotype features of seven species from four genera of Solanaceae. Mitotic chromosomes were examined in root tips obtained from germinating seeds. Roots were pretreated in saturated p-dichlorobenzene in water for 2 h at room temperature, fixed in 3 : 1 ethanol : acetic acid, washed in distilled water, digested 45 min at 37°C with Pectinex SP ULTRA (Novozymes, Bagsværd, Denmark), and squashed in a drop of 45% acetic acid. Slides were stained with Giemsa (Guerra, 1983). At least ten metaphases of each species were photographed with phase contrast in a Zeiss Axiophot microscope. Photographs were used to take the following measurements for each chromosome pair: s (short arm), l (long arm), and c (average total chromosome length). The arm ratio ($r = l / s$) was then calculated and used to classify the chromosomes as recognized by Levan & al. (1964). In addition, total haploid chromosome length of the karyotype (TL) based on the mean chromosome lengths was calculated. Karyotype asymmetry was estimated using Romero Zarco's (1986) indices (A_1 = intrachromosomal asymmetry index, and A_2 = interchromosomal asymmetry index).

The location and number of rDNA sites were determined by fluorescent *in situ* hybridization (FISH) using two probes: pTa71 containing the 18S-5.8S-26S gene of wheat (Gerlach & Bedbrook, 1979) labelled with biotin-14-dATP (BioNick, Invitrogen, Carlsbad, California, U.S.A.) and a 5S rDNA fragment obtained by PCR from *Solanum stuckertii* Bitter using the primers 5S rDNA-3 (5'-GTG CTT GGG CGA GAG TAG TA-3') and 5SrDNA-4 (5'-GGT GCG TTA GTG CTG GTATG-3'; Fulneček & al., 1998), and then labelled with digoxigenin-11-dUTP (DigNick, Roche, Penzberg, Germany). FISH protocol was according to Schwarzach & Heslop-Harrison (2000), with minor modifications as documented in Deanna & al. (2018).

Figures 3–5 show the range of chromosomes morphologies found. The location and number of rDNA sites are shown in Fig. 4. Idiograms representing the haploid complement were based on the mean values for each species (Fig. 5).

Six of these chromosome counts are first reports for those taxa, while the remaining count confirms previously published data. Chromosome features are summarized in Table 3. All species were presumed diploid, and they have chromosomes of medium size ($c =$ from 2.34 to 5.45 μm), falling within the range expected for the family (Badr & al., 1997).

Benthamiella Speg. is a genus with 12 species centred in Patagonia, and together with *Combera* and *Pantacantha* forms the tribe Benthamielleae (Hunziker, 2001). Counts for *Benthamiella* suggest that $x = 11$ is the basic number for this genus. This number is shared with the other two genera of Benthamielleae, and chromosome morphology of *Combera* Sandwith and *Pantacantha* Speg. (Moscone, 1989) is similar to that of the three *Benthamiella* species reported here, which supports the affinities found in molecular phylogenies (Olmstead & al., 2008).

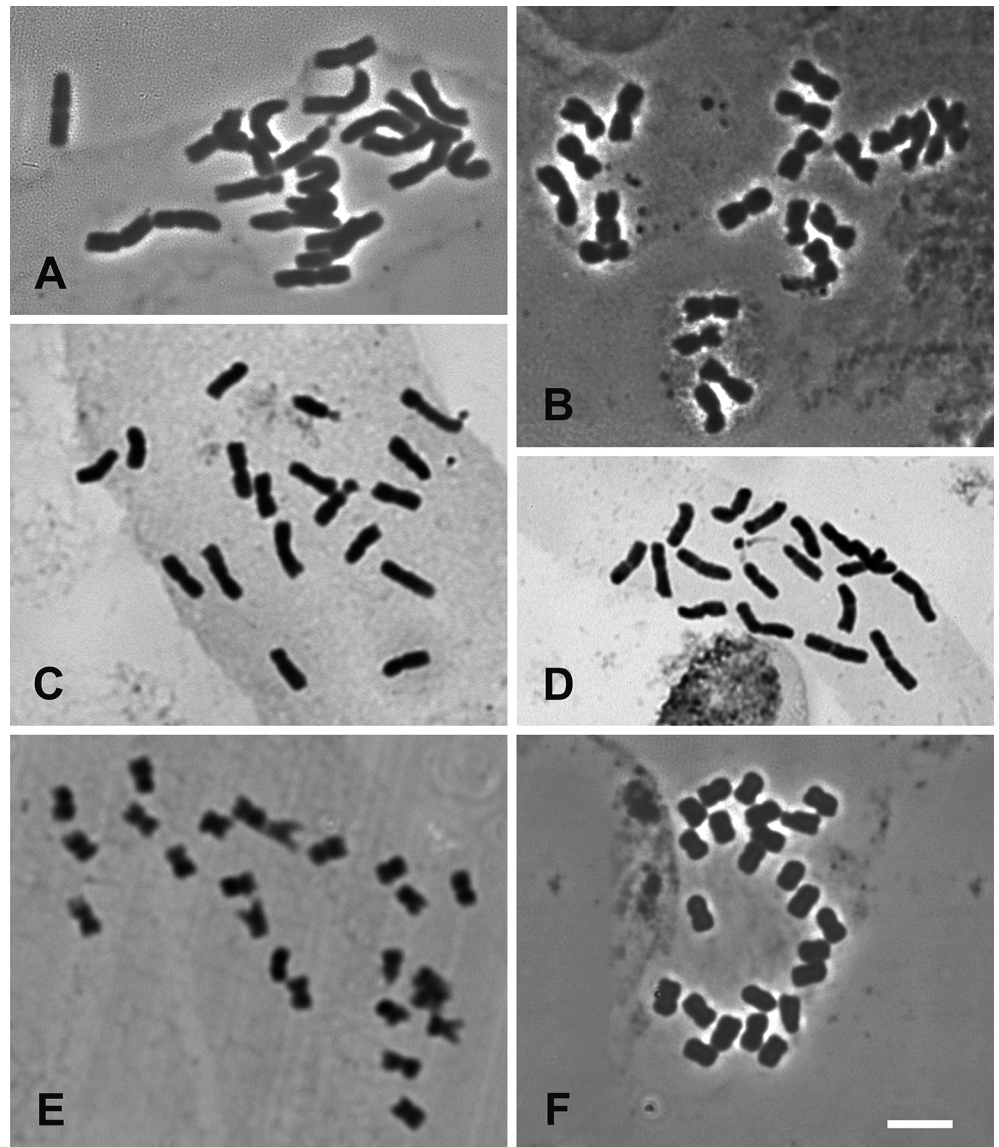


Fig. 3. Photomicrographs of mitotic metaphases of Solanaceae species, stained with Giemsa, all at the same scale. **A**, *Benthamiella graminifolia*; **B**, *B. patagonica*; **C**, *Fabiana punensis*; **D**, *F. patagonica*; **E**, *Lycianthes heterochondra*; **F**, *Trianaea nobilis*. — Scale bar 5 μ m.

Fabiana Ruiz & Pav. comprises 15 species exclusive to the Andes of South America (Hunziker, 2001). Karyotypes here are the first documented for the genus. Our counts, together with previous reports from other three species (Moscone, 1992; Acosta & al., 2006) suggest that $x = 9$ is the basic number for the genus, similar to numbers found in its close relative, *Calibrachoa* Cerv., also with $x = 9$ (Acosta & al., 2006). This suggests that $x = 9$ is the base number for the *Petunia* clade (consisting of *Fabiana*, *Calibrachoa* and *Petunia* Juss. [with $x = 7$]).

The count of $2n = 24$ and chromosome morphology of *Trianaea nobilis*, of the Solandreae (Orejuela & al., 2017), are congruent with the data previously found in two other members of the tribe (Piovano, 1989; Acosta & Moscone, 2000) and fit with the general pattern of the “ $x = 12$ clade” where Solandreae are placed (see Särkinen & al., 2013). The tribe includes ca. 60 Neotropical species, mostly lianas, scandent shrubs, or epiphytes, but few chromosome data exist because of the difficulties of collecting these plants flowering in the forest canopy.

In general, the karyotypes presented here are typically symmetrical (Fig. 3), with r values varying from 1.58 in *F. punensis* to 1.25 in *T. nobilis*. All species have a majority of m (metacentric) chromosomes and a few sm (submetacentric) chromosomes (Table 3, Fig. 3). Subtelocentric and telocentric chromosomes were not detected. Values of A_1 are relatively low, showing there are no big differences between chromosome arms in each complement. Low values of A_2 (0.096–0.148) indicate that none of the species has notable size differences among chromosomes within a single complement. One chromosome pair with secondary constrictions (i.e., satellites) is present in all species.

A survey of 18S-5.8S-26S rDNA loci number for 749 angiosperm species (Roa & Guerra, 2012) indicated that the most frequent numbers of sites per diploid karyotype were two and four, and that they most often occur at terminal positions (45%), usually within the short arms, and frequently on telocentric chromosomes where they usually occupy the whole arm. Most Solanaceae follow this general trend (Acosta & al., 2016; Chiarini & al., 2016, 2018), so

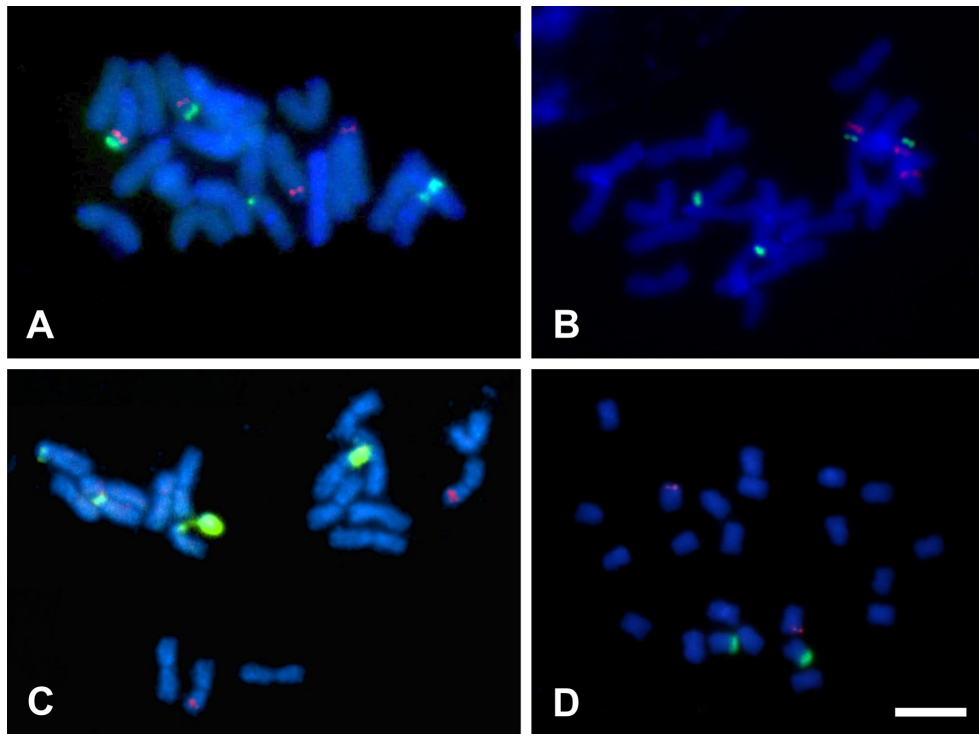


Fig. 4. Fluorescence *in situ* hybridization with 5S (red signals) and 18S-5.8S-26S rDNA (green signals) probes in Solanaceae species. **A**, *Benthamiella graminifolia*; **B**, *B. pycnophylloides*; **C**, *Fabiana patagonica*; **D**, *Trianaea nobilis*. — All pictures at the same scale. Scale bar 5 μ m.

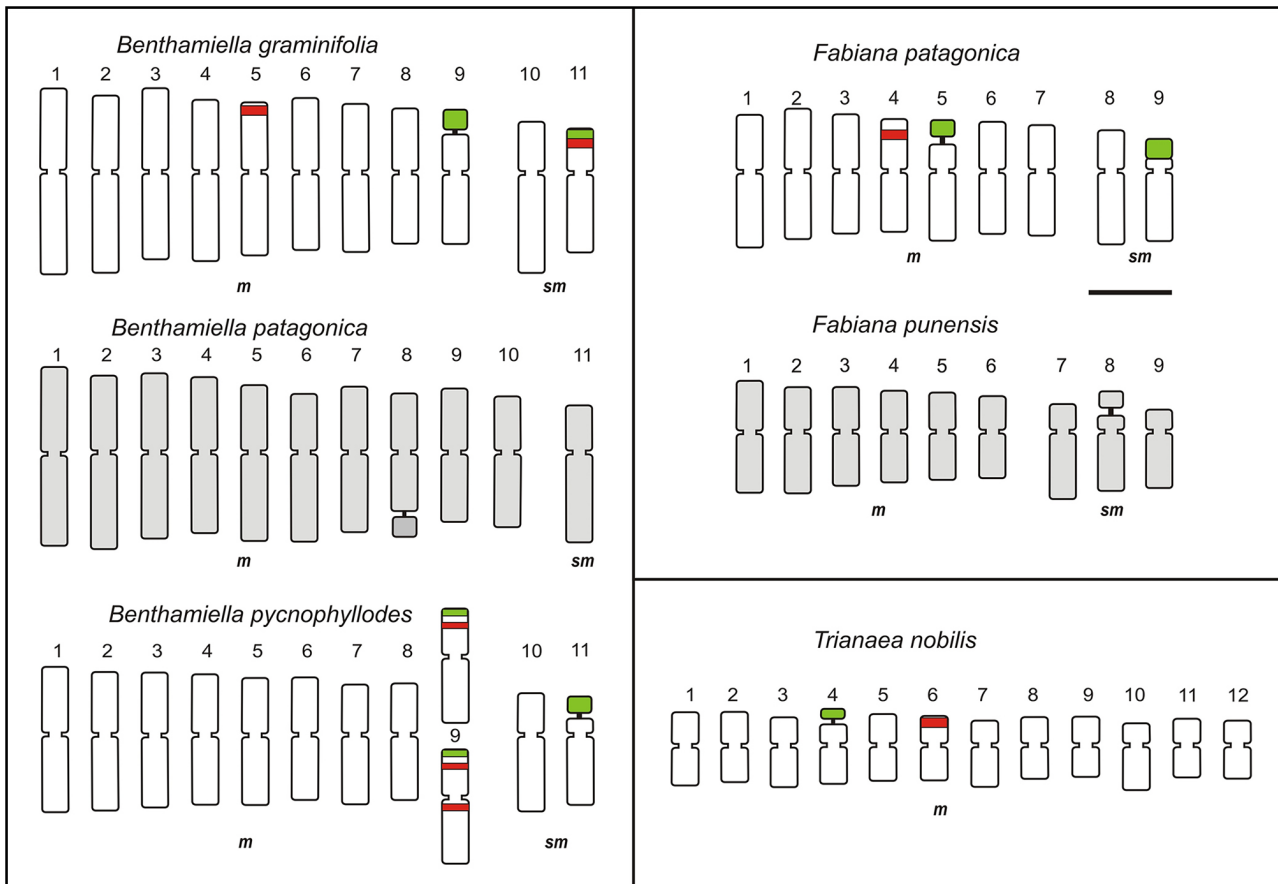


Fig. 5. Idiograms for Solanaceae accessions, based on mean values. All chromosomes at the same scale, ordered by categories, from metacentric (*m*) to submetacentric (*sm*). Red blocks: 5S rDNA loci; green blocks: 18S-5.8S-26S loci. In *B. pycnophylloides*, both homologues are represented to show the heterophism. Idiograms in gray represent species studied only with classical technique. — Scale bar 3 μ m.

Table 3. Chromosome data of the Solanaceae species studied.

Species	2n	Formula	TL	c	r	A ₁	A ₂
<i>Benthamiella graminifolia</i>	22	9m + 2sm	59.03 ± 6.80	5.37 ± 0.62	1.36 ± 0.05	0.291	0.148
<i>Benthamiella patagonica</i>	22	10m + 1sm	59.92 ± 4.86	5.45 ± 0.44	1.30 ± 0.06	0.253	0.126
<i>Benthamiella pycnophylloides</i>	22	9m + 2sm	54.62 ± 3.95	4.96 ± 0.17	1.47 ± 0.18	0.259	0.113
<i>Fabiana patagonica</i>	18	7m + 2sm	38.25 ± 2.20	4.25 ± 0.24	1.50 ± 0.10	0.276	0.124
<i>Fabiana punensis</i>	18	6m + 3sm	30.10 ± 3.38	3.34 ± 0.38	1.58 ± 0.12	0.295	0.139
<i>Trianaea nobilis</i>	24	12m	28.13 ± 2.12	2.34 ± 0.18	1.25 ± 0.04	0.173	0.096

TL = total haploid chromosome length of the karyotype in μm ± standard deviation; c = average total chromosome length in μm ± standard deviation; r = average arm ratio ± standard deviation; A₁ = intrachromosomal asymmetry index; A₂ = interchromosomal asymmetry index.

Benthamiella, *Fabiana* and *Trianaea* Planch. & Linden are not exceptional in the family. Another general trend in angiosperms here confirmed is that both the number and localization of 18S-5.8S-26S and 5S rDNA loci are homoplastic and independent from one another (see Chiarini & al. 2018 for references).

Benthamiella pycnophylloides is remarkable for the heteromorphism observed in the 5S rDNA loci. Similar polymorphisms have been reported in other plant families and also in *Solanum* (Chiarini, 2014). Heterozygous karyotypes with odd number of rDNA arrays may be attributed to reproductive self-incompatibility (Vaio & al., 2005), but no reproductive studies are available for *Benthamiella* to date. Further research is needed to understand the causes underpinning these observations.

Our data are useful for distinguishing species and to characterize some clades within a large family such as the Solanaceae, where chromosome morphology has already proven to be a useful approach (Acosta & al., 2006, 2016; Olmstead & al., 2008; Chiarini & al., 2018). More counts and karyotypes, especially in early-branching Solanaceae clades, are needed in order to establish firm hypotheses of chromosome evolution across this economically important family.

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IAPT chromosome data 31/5

Julio Rubén Daviña, Aveliano Fernández & Ana Isabel Honfi*

* Author for correspondence: ahonfi@gmail.com

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Methods are described in Daviña & Fernández (1989) and Daviña (2001).

- * First chromosome count for the species.
- First gametic chromosome count for the species.
- # New cytotype for the species.
- First meiotic behaviour study for the species.

AMARYLLIDACEAE

The Amaryllidaceae family comprises 860 species in 59 genera with mainly tropical distribution (Meerow & Snijman, 1998). The Hippeastreae Herb. ex Sweet tribe has a major centre of diversification in central Chile and western Andean Argentina and a second centre in eastern Brazil and northeastern Argentina (Meerow & Snijman, 1998; Arroyo-Leuenberger & Dutilh, 2008).

The genus *Zephyranthes* Herb. comprises approximately 50 native species, mainly from tropical and subtropical America, that occur from the SE of the United States of America and the Caribbean to Patagonia (Meerow & Snijman, 1998). There are 11 species in Argentina (Arroyo-Leuenberger, 1996). In this study, *Zephyranthes* species originating from Argentina were cytogenetically investigated.

Zephyranthes candida (Lindl.) Herb.

$2n = 38$, CHN. Argentina, Entre Ríos Province, Concepción del Uruguay Department, Camping Banco Pelay, 32°27'S, 58°12'W, 13 Mar 1997, Daviña 360 (B, MNES) [Figs. 6F, 7B].

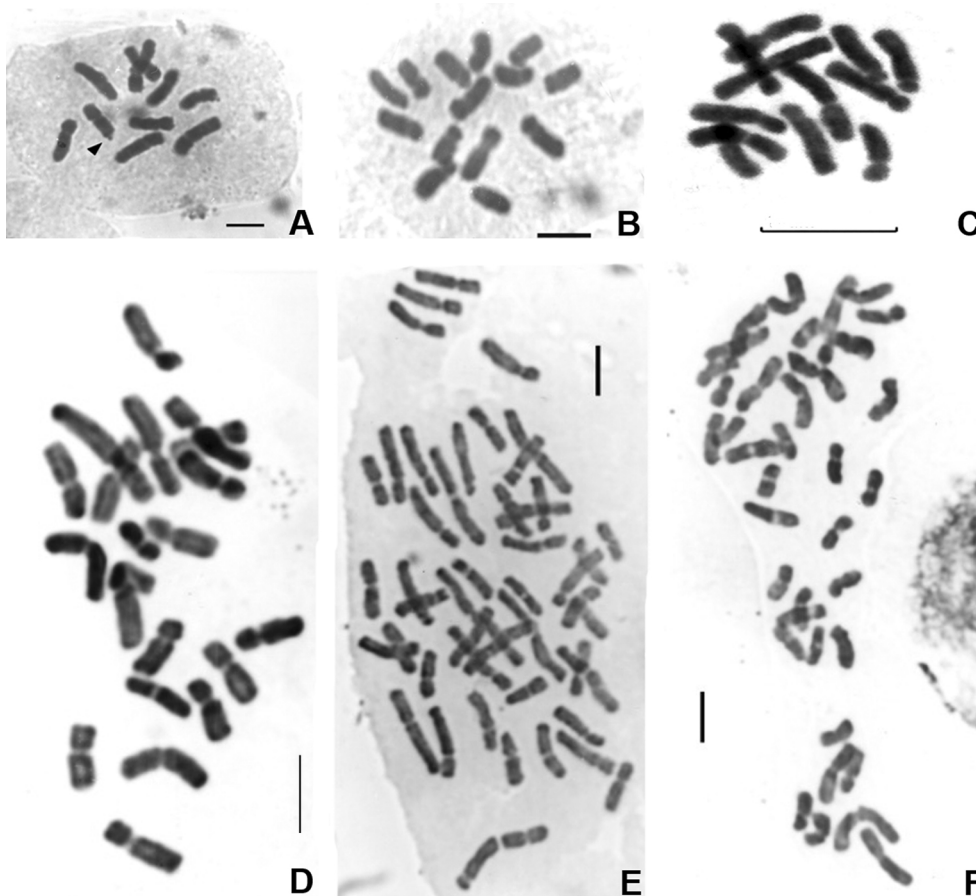


Fig. 6. Mitotic metaphase. **A**, *Zephyranthes seubertii*, $2n = 10$; **B**, *Z. flavissima*, $2n = 14$; **C**, *Z. mesochloa*, $2n = 12$; **D**, *Z. minima*, $2n = 20$; **E**, *Z. carinata*, $2n = 48$; **F**, *Z. candida*, $2n = 38$. — Scale bars = 5 μm .

The somatic chromosome number of this accession agrees and confirms the findings made by Naranjo (1969) of $2n = 38$. The diversity of karyotypes found in this taxon is interesting since the plants with $2n = 38$ can be constituted by $32m + 6sm$ (Yokouchi, 1963; Naranjo, 1969), $36sm + 2st$ (Raina & Koshoo, 1971), $6m + 26sm + 6st$ (Vij & al., 1982) or $24m + 12sm + 2st$ as described here. The $2n = 38$ of *Z. candida* originated either as an amphiploid formed by the combination of $x = 6$ and $x = 7$ (Naranjo, 1969) or as a hexaploid cytotype based on $x = 6$ plus two additional chromosomes (Sato, 1938; Yokouchi, 1963, 1965).

Zephyranthes carinata Herb.

• $2n = 8x = 48$, CHN. Argentina, Misiones Province, Oberá Department, Panambi, 27°43'S, 54°54'W, 12 Nov 1996, *Daviña* 357 (B, MNES) [Figs. 6E, 7C, 8B].

Zephyranthes carinata is a polyploid species with different chromosome numbers that vary from $2n = 4x = 24$ to $2n = 67 + 1B$. The number most frequently found is $2n = 48$ and is considered to be an octoploid based on $x = 6$. The karyotype of $20m + 24sm + 4st$ found in our Argentinian accession clearly differs from the previously reported karyotype of $28sm + 20st$ by Yokouchi (1964). Meiotic behaviour in the material studied here was regular, and pollen mother cells (PMCs) at metaphase I have 24 bivalents and pollen viability of

88.55%. Our results confirm the allopolyploidy suggested by Yokouchi (1964) for this octoploid cytotype.

Zephyranthes flavissima Ravenna

* $n = 7II$, $2n = 2x = 14$, CHN. Argentina, Misiones Province, San Pedro Department, Moconá Province Park, 27°09'S, 53°54'W, 18 Dec 1992, *Daviña, Honfi & Seijo* 279 (B, CTES, G, MNES) [Figs. 6B, 8A, 9C].

Zephyranthes flavissima is one of the diploid species of the genus, with $2n = 2x = 14$, with the basic number $x = 7$. The karyotype is formed by $2m + 10sm + 2st$ chromosomes. This karyotype formula agrees with Naranjo's (1969) report. For the first time, the meiotic behaviour was analyzed here, and in all the PMCs at metaphase I, chromosome pairing was regular with formation of 7 bivalents (II). The frequency of ring bivalents per cell was 7. The average frequency of chiasmata per PMC was 14, as a result of the presence of one chiasma in each chromosome arm. The pollen viability was estimated at 99.6%.

Zephyranthes mesochloa Herb.

$2n = 2x = 12$, CHN. Argentina, Misiones Province, Capital Department, Garupá, 27°27'S, 55°51'W, 26 Sep 2010, *Daviña & Honfi* 367 (MNES) [Fig. 6C, 9B].

In the diploid population of *Z. mesochloa* ($2n = 2x = 12$), the karyotype has a formula of $4m + 4sm + 4st$. We confirm the chromosome number, ploidy level and karyotype that was reported as for *Z. aff. mesochloa* by Daviña & Fernández (1989). The

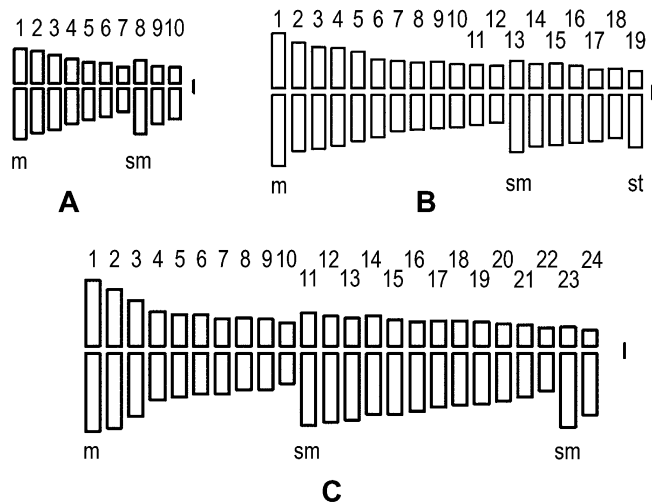


Fig. 7. Idiograms. **A**, *Zephyranthes minima*, $2n = 4x = 20$, $7m + 3sm$; **B**, *Z. candida*, $2n = 2x = 38$, $12m + 6sm + 1st$; **C**, *Z. carinata*, $2n = 8x = 48$, $10m + 12sm + 2st$. — Scale bars = 1 μ m.

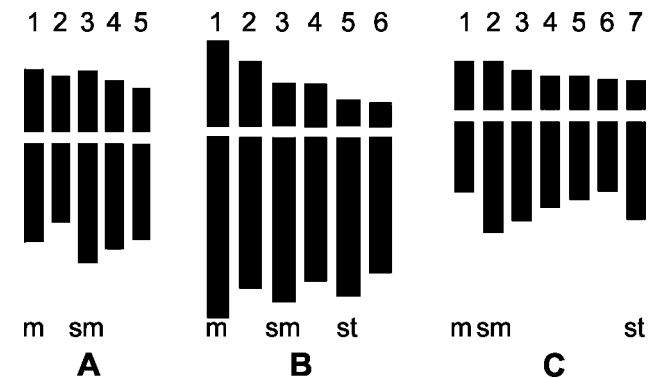


Fig. 9. Idiograms. **A**, *Zephyranthes seubertii*, $2n = 10$, $2m + 3sm$; **B**, *Z. mesochloa*, $2n = 12$, $2m + 2sm + 2st$; **C**, *Z. flavissima*, $2n = 14$, $1m + 5sm + 1st$. — Scale bars = 1 μ m.

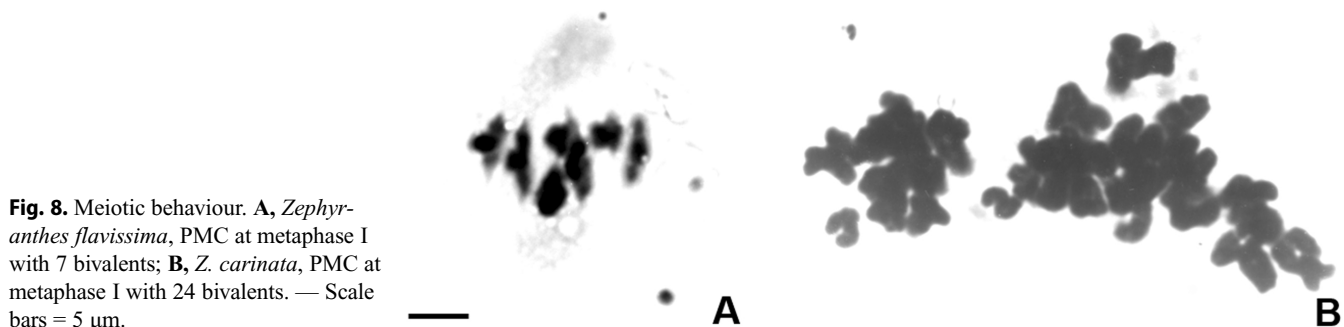


Fig. 8. Meiotic behaviour. **A**, *Zephyranthes flavissima*, PMC at metaphase I with 7 bivalents; **B**, *Z. carinata*, PMC at metaphase I with 24 bivalents. — Scale bars = 5 μ m.

karyotype formula found here differs from that reported by Greizerstein & Naranjo (1987) ($2m + 10sm$).

Zephyranthes minima Herb.

$2n = 4x = 20$, CHN. Argentina, Corrientes Province, Capital Department, Corrientes, 27°28'S, 58°49'W, 15 Mar 1996, *Daviña* 348 (B, MNES) [Fig. 6D, 7A].

Zephyranthes minima is reported here for the first time for the Corrientes Province; its geographical distribution was considered as confined only to the Buenos Aires, Córdoba and Entre Ríos Provinces (Arroyo-Leuenberger, 1997). The chromosome number observed in all individuals was $2n = 4x = 20$, being tetraploid with the basic number $x = 5$. The karyotype is formed by $14m + 6sm$ chromosomes and in agreement with Naranjo (1974).

Zephyranthes seubertii H.H.Hume

*●#■ $2n = 2x = 10$, CHN. Argentina, Corrientes Province, Paso de los Libres Department, Ruta N 123, Río Miriñay, 29°37'S, 57°15'W, 24 Mar 2000, *Daviña* & *Seijo* 434 (B, CTES, MNES) [Fig. 6A, 9A].

For the first time, we report here the diploid cytotype for *Z. seubertii*, collected in northeastern Argentina, with $2n = 2x = 10$ chromosomes, with the basic number $x = 5$. This new basic chromosome number corresponds to the lowest chromosome number published so far for the genus and for the Amaryllidaceae family. The karyotype formula is constituted by 2 metacentric pairs and 3 submetacentric pairs. This finding proved the existence of $x = 5$ for the genus *Zephyranthes*, earlier proposed by Daviña & Fernández (1989), based on meiotic behaviour analysis of several *Zephyranthes* polyploids.

From the review of the chromosomal numbers reported for the species of *Zephyranthes*, it appears that all of them integrate to a dysploid series, constituted by three basic chromosome numbers, $x = 5$ (Daviña & Fernández, 1989; Daviña, 2001), $x = 6$ and $x = 7$ (Darlington & Wylie, 1955). The only species with $x = 5$ are *Z. minima* and *Z. seubertii*, here reported. The basic number $x = 7$ is found only in *Z. flavissima*, in its diploid and without other conspecific cytotypes. On the other hand, $x = 6$ occurs in diploid species such as *Z. mesochloa* and polyploid species such as *Z. candida* and *Z. carinata*.

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IAPT chromosome data 31/6

Cattleya M.P. Felix, Reinaldo F.P. Lucena, Leonardo P. Felix, Joel M.P. Cordeiro, Angeline M.S. Santos* & Kallyne Bonifácio
* Address for correspondence: angelinemssantos@gmail.com

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Methods for chromosome analysis are according to Guerra & Souza (2002).

- * First chromosome count for the species.
** New cytotype for the species.

ARACEAE

**Taccarum peregrinum* (Schott) Engl.

$2n = 34$, CHN. Brazil, Paraíba, Serra Banca, Serra do Jatobá, 07°21'48"S, 36°44'34"W, 20 Apr 2018, *J.M.P. Cordeiro* 1296 (EAN) [Figs. 10A, 12A].

Our count of $2n = 34$ is in agreement with the only previously reported count for this genus (Rice & al., 2014). The species is distinct by having the largest chromosomes and karyotype formed by a majority of acrocentric chromosomes and a minority of metacentric and submetacentric ones. Heterochromatic regions were restricted to the terminals of two pairs of acrocentric chromosomes.

BIGNONIACEAE

Handroanthus impetiginosus (Mart. ex DC.) Mattos

$2n = 40$, CHN. Brazil, Paraíba, Serra Banca, Serra do Jatobá, 07°29'50"S, 36°40'47"W, 01 Jul 2018, *J.M.P. Cordeiro 1343* (EAN) [Figs. 11A, 12D].

This species occurs in Mexico, Bolivia and Brazil (Villaseñor Ríos, 2016). It is a tree species typical of semi-humid environments, widely used as an ornamental plant. It has a symmetrical karyotype with predominance of metacentric and submetacentric chromosomes and heterochromatin rich in GC (guanine-cytosine) distributed in the terminals of one chromosomal pair and in the proximal region of another pair. Our count of $2n = 40$ is in agreement with all previous records for the species (Piazano, 1998).

CONVOLVULACEAE

**Evolvulus elegans* Moric.

$2n = 26$, CHN. Brazil, Paraíba, Serra Banca, Serra do Jatobá, 07°29'09"S, 36°40'47.3"W, 19 Apr 2018, *L.P. Felix 17416* (EAN) [Figs. 11B, 12G].

This species is distributed exclusively in South America, occurring mainly in rupicolous environments (Ooststroom, 1934). Its $2n = 26$ karyotype is composed of metacentric and submetacentric chromosomes and heterochromatin rich in GC in proximal regions, restricted to one chromosomal pair. A new count is presented here, it is in agreement with most counts previously reported for the genus (Pitrez & al., 2008).

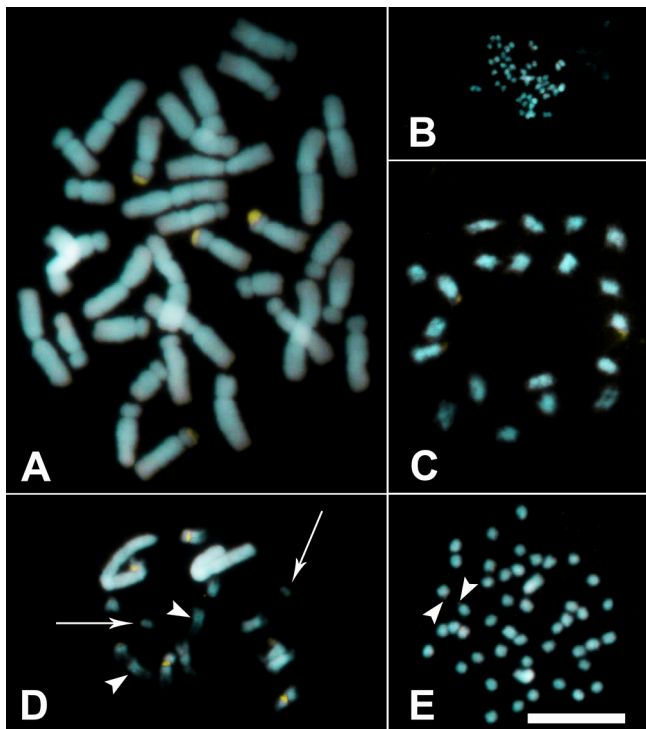


Fig. 10. Metaphases of species collected in Serra do Jatoba. **A**, *Taccarum peregrinum* ($2n = 34$); **B**, *Cyperus ligularis* L. ($2n = 50$); **C**, *Eleocharis atropurpurea* ($2n = 20$); **D**, *Alophia drummondii* ($2n = 14+2Bs$), arrows: B chromosomes, arrow-heads: DAPI punctiform bands; **E**, *Prescottia plantaginea* ($2n = 48$), arrow-heads: inconsistent CMA bands. — Scale bar in E = 10 μm .

CYPERACEAE

**Cyperus ligularis* L.

$2n = 50$, CHN. Brazil, Paraíba, Serra Banca, Serra do Jatobá, 07°29'09"S, 36°40'47.3"W, 19 Apr 2018, *L.P. Felix 17456* (EAN) [Figs. 10B, 12B].

A species with a wide distribution in the Neotropics, without previous chromosome count. The present record of $2n = 50$ is in agreement with some previous counts for the genus *Cyperus* (Roalson, 2008). The species has small chromosomes, some with the length less than 1 μm , without definite morphology, probably due to the occurrence of holocentric chromosomes (with diffuse centromere) characteristic for the family Cyperaceae (Guerra, 2016).

Eleocharis atropurpurea (Retz.) J.Presl & C.Presl.

$2n = 20$, CHN. Brazil, Paraíba, Serra Banca, Serra do Jatobá, 07°29'09"S, 36°40'47.3"W, 05 Jul 2017, *L.P. Felix 16785* (EAN) [Fig. 10C].

This species has a cosmopolitan distribution, being generally associated with humid environments. Its occurrence in the outcrop is related to the tanks or cauldrons that accumulate water during the rainy season. Our count of $2n = 20$ coincides with all counts previously reported for this species.

EUPHORBIACEAE

**Jatropha mollissima* (Pohl) Baill.

$2n = 22$, CHN. Brazil, Paraíba, Serra Banca, Serra do Jatobá, 07°29'46"S, 36°44'05"W, 05 Jul 2017, *L.P. Felix 16614* (EAN) [Figs. 11C, 12H].

This species has a restricted distribution to South America, occurring in the phytogeographic domains of the Cerrado, Caatinga and Amazon. Our report of $2n = 22$ is a new count, but it coincides with most chromosome numbers previously reported for this genus (Dehgan & Webster, 1979). Its karyotype was characterized by mainly metacentric and submetacentric chromosomes, with 1.5 to 3.0 μm in length and CMA (fluorochrome chromomycin A3) bands located at the terminals of five chromosomes, one of which is heteromorphic.

FABACEAE

***Chamaecrista hispidula* (Vahl) H.S.Irwin & Barneby

$2n = 20$, CHN. Brazil, Paraíba, Serra Banca, Serra do Jatobá, 07°29'09"S, 36°40'47.3"W, 19 Apr 2018, *L.P. Felix 17415* (EAN) [Figs. 11D, 12I].

This species is widely distributed in the Neotropics, occurring in anthropic areas, Caatinga, Rock Field, Cerrado, Restinga and Amazon Savannah. Our count of $2n = 20$ diverges from the only previous count for this species of $n = 7$ (Coleman & DeMenezes, 1980) referred to as *Cassia tetraphylla* Martyn, a supposed nomenclatural synonym of *C. hispidula* (Rice & al., 2014). *Chamaecrista* (L.) Moench is a variable genus, and the occurrence of intraspecific dysploidy is related to the formation of cryptic species difficult to be delimited morphologically (Souza & Benko-Iseppon, 2004). It has a karyotype formed by chromosomes with small pericentromeric CMA bands except for a small chromosomal pair with CMA⁺/DAPI⁻ terminal bands of more intense brightness.

Mimosa paraibana Barneby

$2n = 26$, CHN. Brazil, Paraíba, Serra Banca, Serra do Jatobá, 07°29'46"S, 36°44'05"W, 05 Jul 2017, *L.P. Felix 16711* (EAN) [Figs. 11E, 12J].

Endemic in the northeast region of Brazil, occurs in the phyto-geographical domains of the Caatinga and Atlantic Forest. Our count

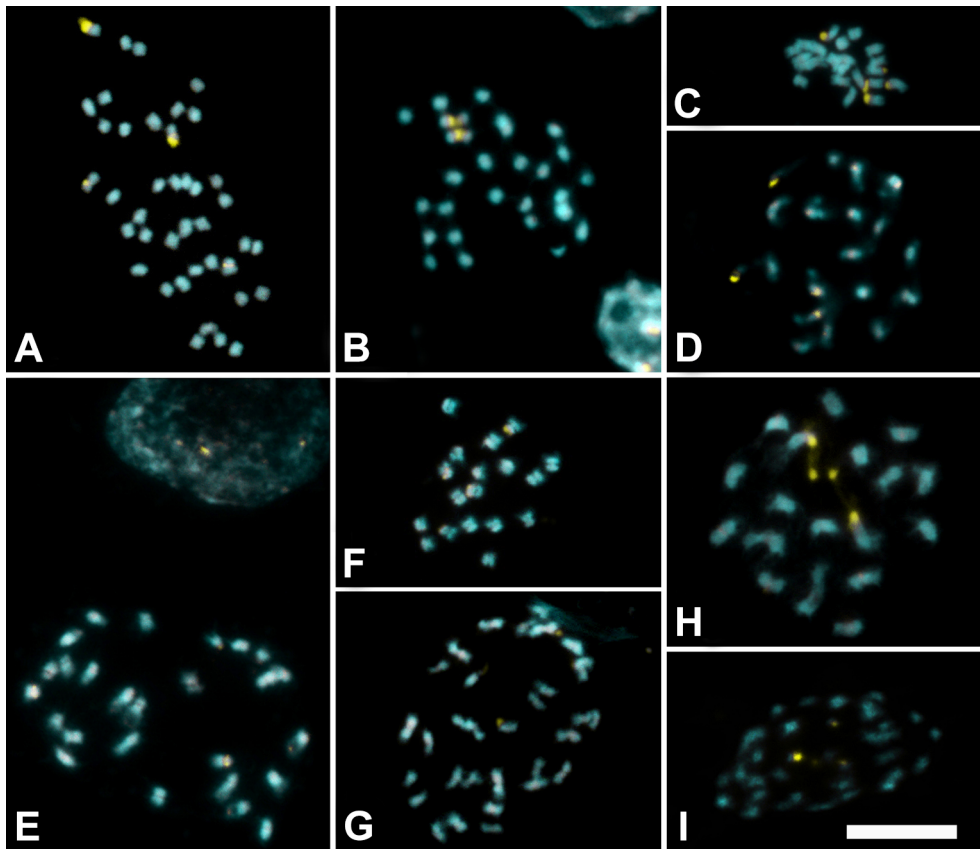


Fig. 11. Mitotic metaphases of species collected in Serra do Jatoba. **A**, *Handroanthus impetiginosus* ($2n = 40$); **B**, *Evolvulus elegans* ($2n = 26$); **C**, *Jatropha mollissima* ($2n = 22$); **D**, *Chamaecrista hispidula* ($2n = 20$); **E**, *Mimosa paraibana* ($2n = 26$); **F**, *Waltheria indica* ($2n = 18$); **G**, *Ludwigia octovalvis* ($2n = 32$); **H**, *Polygala boliviensis* ($2n = 18$); **I**, *Cissus* sp. ($2n = 32$). — Scale bar in I = 10 μm .

of $2n = 26$ is in agreement with the single previous count for the species (Santos & al., 2012). It has a karyotype formed by metacentric, submetacentric and acrocentric chromosomes, with a pair of larger terminal CMA bands and several small bands distributed in the terminals of the short and long arms of several chromosomes.

IRIDACEAE

***Alophia drummondii* (Graham) R.C.Foster

$2n = 14+2Bs$, CHN. Brazil, Paraíba, Serra Banca, Serra do Jatobá, $07^{\circ}29'09''S$, $36^{\circ}40'47.3''W$, 19 Apr 2018, *L.P. Felix 17451* (EAN) [Figs. 10D, 12C].

It is a species with a large distribution in the Neotropics, characterized by a large numerical chromosome variation, with records ranging from $2n = 14$ to $2n = 56$, including a count of $2n = 14+1B$ (Alves & al., 2011). The only individual analyzed here presented a karyotype formed by a pair of large acrocentric chromosomes and the others with less than half the size of the larger pair. It contains GC-rich heterochromatin located in the proximal portion of two minor chromosomal pairs and TA (adenine-thymine)-rich punctiform bands in the pericentromeric region of a small chromosomal pair.

MALVACEAE

***Waltheria indica* L.

$2n = 18$, CHN. Brazil, Paraíba, Serra Banca, Serra do Jatobá, $07^{\circ}29'09''S$, $36^{\circ}40'47.3''W$, 19 Apr 2018, *L.P. Felix 17470* (EAN) [Figs. 11F, 12K].

Ruderal and cosmopolitan plants with a wide distribution in the Neotropics. This species occurs typically in anthropic areas, Caatinga, Rock Field, Cerrado, Ciliary Forest or Gallery, Ombrophilous Forest and Restinga. Its karyotype consists mainly of metacentric

chromosomes with one pair of larger CMA terminal bands and one smaller proximal pair. Our count of $2n = 18$ diverges from all previous counts for the species, from materials obtained from other continents (Wilkins & Chappill, 2002; Rice & al., 2014).

ONAGRACEAE

Ludwigia octovalvis (Jacq.) P.H.Raven

$2n = 32$, CHN. Brazil, Paraíba, Serra Banca, Serra do Jatobá, $07^{\circ}29'09''S$, $36^{\circ}40'47.3''W$, 19 Apr 2018, *L.P. Felix 17440* (EAN) [Fig. 11G].

These are herbaceous to shrub plants, with a wide distribution in the Neotropics where it occurs in anthropic areas, in the Caatinga, Amazonian Campinarana, Varzea Field, Cerrado, Ciliary Forest, Terra Firme Forest, Semideciduous Seasonal Forest, Ombrophilous Forest, Mangrove and Restinga. Its karyotype consists of metacentric and submetacentric chromosomes measuring between 1.5 and 3.0 μm with two pairs of terminal CMA bands.

ORCHIDACEAE

**Prescottia plantaginea* Lindl.

$2n = 48$, CHN. Brazil, Paraíba, Serra Banca, Serra do Jatobá, $07^{\circ}21'48''S$, $36^{\circ}44'34''W$, 20 Apr 2018, *J.M.P. Cordeiro 1319* (EAN) [Figs. 10E, 12E,F].

Endemic to Brazil, it is a typical species of rocky outcrops. Its karyotype with $2n = 48$ is in agreement with most counts previously reported for the genus (Felix & Guerra, 2005). Its karyotype has a chromosome pair larger than the other chromosomes. In addition, it has a small amount of constitutive heterochromatin restricted to two small CMA bands located in the terminal region of a chromosomal pair, probably corresponding to NORs (nucleolus organizing regions).

POLYGALACEAE

**Polygala boliviensis* A.W.Benn.

$2n = 18$, CHN. Brazil, Paraíba, Serra Banca, Serra do Jatobá, 07° 29' 09"S, 36° 40' 47.3"W, 19 Apr 2018, L.P. Felix 17394 (EAN) [Fig. 11H].

An annual herbaceous plant, endemic in northeast Brazil, occurs in the Caatinga and Restinga vegetation. The genus is highly variable in terms of chromosome numbers with records from $2n = 16$ to $2n = 76$ (Rice & al., 2014). Our count of $2n = 18$ is in agreement with

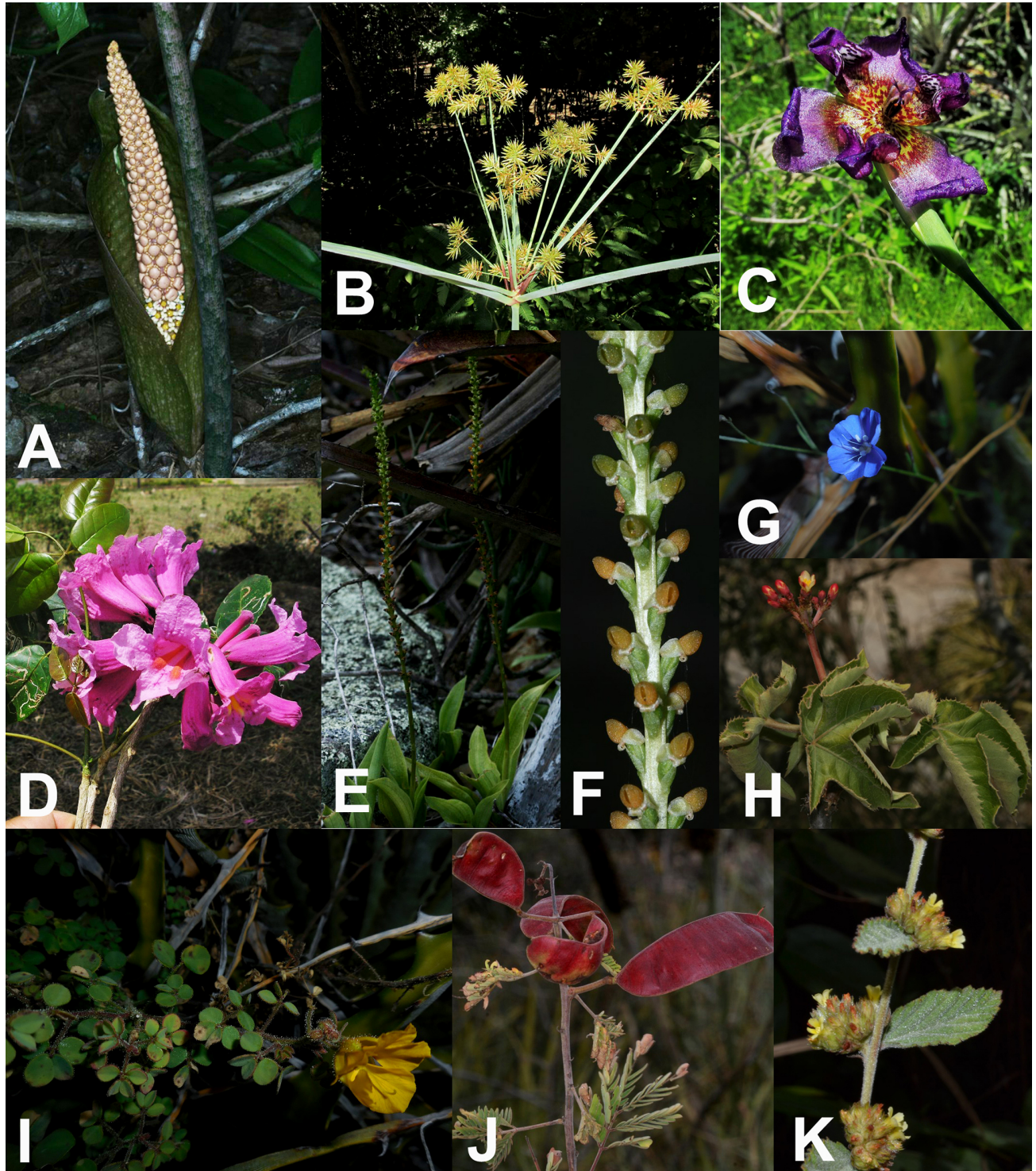


Fig. 12. Partial sampling of species analyzed. **A**, *Taccarum peregrinum*; **B**, *Cyperus ligularis*; **C**, *Alophia drummondii*; **D**, *Handroanthus impetiginosus*; **E & F**, *Prescottia plantaginea*; **G**, *Evolvulus elegans*; **H**, *Jatropha mollissima*; **I**, *Chamaecrista hispida*; **J**, *Mimosa paraibana*; **K**, *Waltheria indica*. — Photos: Leonardo P. Felix and Joel M.P. Cordeiro.

a single count for the genus, published by Raven & al. (1965), for *P. fishiae* Parry. It has a karyotype exhibiting metacentric and sub-metacentric chromosomes, ranging in size from 2.5 to 5.0 μm , with a single pair of long distended CMA bands on the prometaphasic chromosomes.

VITACEAE

Cissus sp.

$2n = 32$, CHN. Brazil, Paraíba, Serra Banca, Serra do Jatobá, 07°21'48"S, 36°44'34"W, 20 Apr 2018, *J.M.P. Cordeiro 12288* (EAN) [Fig. 111].

The genus *Cissus* comprises climbing plants with tendrils, with chromosome numbers varying from $2n = 20$ to $2n = 80$ (Rice & al., 2014). Our count for *Cissus* sp. diverges from all previous counts for the genus. The presence of a pair of distended CMA terminal bands is compatible with the presence of heterochromatic NORs.

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IAPT chromosome data 31/7

Cattleya M.P. Felix, José Achilles Lima Neves, Rosemere Silva, Rodrigo Garcia Silva Nascimento, Ronimeire Torres da Silva, Felipe Nollet,* Joel M.P. Cordeiro, Reinaldo F.P. Lucena & Leonardo P. Felix

* Address for correspondence: nolletmedeiros@yahoo.com.br

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* First chromosome count(s) for the genus.

** First chromosome count for the species.

- New cytotype for the species.

ALISMATACEAE

Echinodorus grandiflorus (Cham. & Schltdl.) Micheli

$2n = 22$, CHN. Brazil, Paraíba, Ipororoca, Fazenda Macacos, 06°49'47"S, 35°14'50"W, 04 Oct 1997, *L.P. Felix 10824* (EAN) [Fig. 13B].

Echinodorus paniculatus Micheli

$2n = 22$, CHN. Brazil, Piauí, Campo Maior, 04°49'40"S, 42°10'07"W, 10 Aug 2004, *L.P. Felix s.n.* (EAN 11164) [Fig. 13C].

Echinodorus subalatus (Mart.) Griseb.

$2n = 22$, CHN. Brazil, Piauí, Campo Maior, 04°49'40"S, 42°10'07"W, 25 Mar 2004, *L. Feitoza 22* (EAN) [Fig. 13A].

AMARANTHACEAE

***Alternanthera brasiliana* (L.) Kuntze

$2n = 28$, CHN. Brazil, Paraíba, Areia, Junco, 06°52'46"S, 35°47'31"W, 27 Jul 2017, *L.P. Felix 16669* (EAN) [Fig. 14A].

AMARYLLIDACEAE

**Griffinia angustifolia* Campos-Rocha, Dutilh & Semir

$2n = 30$, CHN. Brasil, Sergipe, Carmópolis, 10°38'53"S, 36°59'20"W, 04 Jul 1998, *L.P. Felix s.n.* (EAN 14200) [Fig. 13D].

**Griffinia gardneriana* (Herb.) Ravenna

$2n = 20$, CHN. Brazil, Pernambuco, Águas Belas, 09°06'41"S, 37°07'23"W, 24 Mar 2008, *L.P. Felix 12158* (EAN) [Fig. 13E].

**Hippeastrum stylosum* Herb.

$2n = 22$, CHN. Brazil, Pernambuco, Taquaritinga do Norte, subida para a Torre, 07°54'11"S, 36°02'39"W, *L.P. Felix 12064* (EAN) [Fig. 13F].

Nothoscordum gracile (Aiton) Stearn

$2n = 19$, CHN. Brazil, São Paulo, São Paulo, Campus da Universidade de São Paulo, 21°10'03"S, 47°51'06"W, 22 Jan 1998, M. Guerra s.n. (EAN 10154) [Fig. 13G].

Nothoscordum pulchellum Kunth

$n = 5$, CHN. Brazil, Paraíba, Areia, Centro de Ciências Agrárias, 06°58'13"S, 33°42'47"W, J.M.P. Cordeiro 1019 (EAN) [Fig. 13H].

ARECACEAE

Syagrus vagans (Bondar) A.D.Hawkes

$2n = 30$, CHN. Brazil, Bahia, Senhor do Bonfim, 10°27'41"S, 40°11'22"W, 12 Jan 2006, V.S. Lopes 10 (EAN) [Fig. 13J].

ASTERACEAE

Acmella oleracea (L.) R.K.Jansen

$2n = 78$, CHN. Brazil, Pará, Mercado Ver o Peso, 01°27'18"S, 48°30'09"W, 26 Mar 2018, L.P. Felix 17333 (EAN) [Fig. 14D].

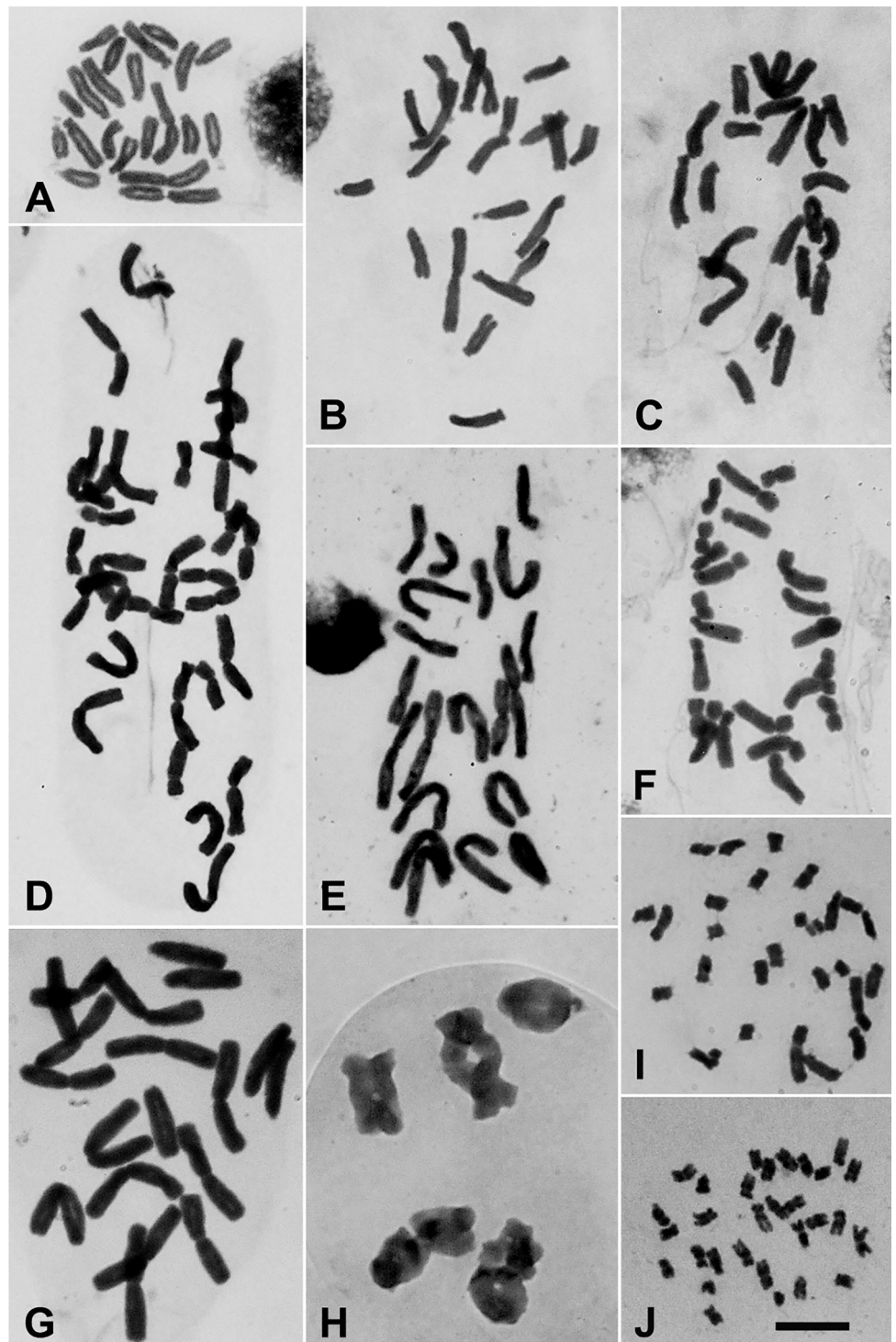


Fig. 13. A, *Echinodorus subulatus*, $2n = 22$; B, *E. grandiflorus*, $2n = 22$; C, *E. paniculatus*, $2n = 22$; D, *Griffinia angustifolia*, $2n = 30$; E, *G. gardneriana*, $2n = 20$; F, *Hippeastrum stylosum*, $2n = 20$; G, *Nothoscordum gracile*, $2n = 19$; H, *N. pulchellum*, $n = 5$; I, *Limnobium laevigatum*, $2n = 28$; J, *Syagrus vagans*, $2n = 30$. — Scale bar = 10 μm .

BROMELIACEAE

• *Tillandsia juncea* (Ruiz & Paiv.) Poir

$2n = 38$, CHN. Brazil, Paraíba, Sertãozinho, Sítio Canafistuala, 06°45'05"S, 35°26'32"W, 19 Mar 2018, *J.M.P. Cordeiro 1266* (EAN) [Fig. 14B].

CACTACEAE

• *Hylocereus setaceus* (Salm-Dyck ex DC.) Ralf Bauer

$2n = 22$, CHN. Brazil, Paraíba, Sertãozinho, Sítio Canafistuala, 06°45'05"S, 35°26'32"W, 19 Mar 2018, *J.M.P. Cordeiro 1265* (EAN) [Fig. 14E].

FABACEAE

* *Chaetocalyx scandens* var. *pubescens* (DC.) Rudd

$2n = 20$, CHN. Brazil, Paraíba, Areia, Junco, 06°52'46"S, 35°47'31"W, 27 Jul 2017, *L.P. Felix 16662* (EAN) [Fig. 14F].

* *Pityrocarpa moniliformis* (Benth.) Luskow & R.W. Jobson

$2n = 26$, CHN. Brazil, Bahia, Jacobina, 11°10'50"S, 40°31'06"W, 11 Aug 2006, *C.I. Ramalho & V.S. Lopes 135* (EAN) [Fig. 14C].

HYDROCHARITACEAE

Limnobium laevigatum (Humb. & Bonpl. ex Willd.) Heine

$2n = 28$, CHN. Brazil, Paraíba, Guarabira, Piripiri, 06°51'17"S, 35°29'24"W, 14 Jan 2004, *L.P. Felix 10554* (EAN) [Fig. 13I].

ORCHIDACEAE

* *Plectrophora cultrifolia* (Barb.Rodr.) Cogn.

$2n = 56$, CHN. Brazil, Santa Catarina, Barra Velha, 25°21'10"S, 48°53'13"W, 05 Jun 2017, *L.P. Felix & M. Guerra 16576* (EAN) [Fig. 14J].

PLANTAGINACEAE

** *Angelonia campestris* Nees & Mart.

$2n = 28$, CHN. Brazil, Sergipe, Serra da Guia, Poço Redondo, 09°47'54"S, 37°41'25"W, 31 Oct 2014, *J.M.P. Cordeiro, L.P. Felix, E.M. Almeida & J.P. Araújo 518* (EAN) [Fig. 14G].

POLYGONACEAE

Triplaris americana L.

$2n = 22$, CHN. Brazil, Paraíba, Areia, 06°52'46"S, 35°47'31"W, 02/04/2018, *J.M.P. Cordeiro 1267* (EAN) [Fig. 14H].

SAPINDACEAE

Magonia pubescens A.St.-Hil.

$2n = 30$, CHN. Brazil, Monte Alegre, Goiás, 13°27'01"S, 47°04'58"W, 19 Jan 2015, *J.M.P. Cordeiro 757* (EAN) [Fig. 14K].

VITACEAE

** *Cissus pulcherrima* Vell.

$2n = 36$, CHN. Brazil, Aurora do Tocantins, Tocantins, 12°42'47"S, 46°24'28"W, 19 Jan 2015, *J.M.P. Cordeiro 776* (EAN) [Fig. 14I].

Chromosome features, such as number, morphology, centromere position and secondary constrictions, can be used as taxonomic criteria and/or phylogenetic signs in groups of plants and animals (Kamel & al., 2014; Vershina & Lukhtanov, 2017). The use of conventional staining techniques from classical cytogenetics allows analyzing these features, especially chromosome numbers, the most widely used cytotoxic parameter (Guerra, 2008). Hematoxylin

has been widely used because it strongly stains the chromosomes due to its affinity with DNA (Guerra, 1999). The use of this technique made possible to observe variations in chromosome morphology between different genotypes of *Cicer arietinum* (Kordi & al., 2006), the occurrence of heterozygosity in *Citrus clementina* (Germana & al., 2013) and the chromosome number variation of *Panax ginseng* (Khrolenko & al., 2012).

We followed Guerra & Souza (2002) for chromosome analysis. Root tips were pretreated with 8-hydroxyquinoline (0.002 M) for 20 h at 10°C, fixed in absolute ethanol : glacial acetic acid (3 : 1 v/v) for 2 to 24 h at room temperature and stored at -20°C. To prepare the slides, the roots were washed twice in distilled water and hydrolyzed in HCl (5 N) for 20 minutes. They were then squashed in 60% acetic acid and frozen in liquid nitrogen for remove the coverslip, and subsequently stained with 1% hematoxylin, air dried and mounted with Entellan. The best metaphases were captured with an Axiocam MRC 5 video camera using Axiovision v.4.8 software. The images have been edited for brightness and contrast with Adobe Photoshop CS3 version 10.0.

Of the 21 species analyzed from 14 families, new chromosome numbers are recorded here for the genera *Griffinia* Ker Gawl. (for *G. angustifolia* and *G. gardneriana*; Fig. 13D,E), *Hippeastrum* Herb (for *H. stylosum*; Fig. 13F), *Pityrocarpa* (Benth.) Britton & Rose (for *P. moniliformis*; Fig. 14C) and *Plectrophora* H. Focke (for *P. cultrifolia*; Fig. 14J). We present new chromosome counts for 10 species, including: *Syagrus vagans* (Fig. 13J), *Alternanthera brasiliensis* (Fig. 14A), *Chaetocalyx scandens* var. *pubescens* (Fig. 14F), *Angelonia campestris* (Fig. 14G), and *Cissus pulcherrima* (Fig. 14I). New cytotypes are recorded here for *Tillandsia juncea*, with $2n = 38$ (Fig. 14B) and *Hylocereus setaceus*, with $2n = 22$ (Fig. 14E) that diverge from all previous counts.

Echinodorus subalatus, *E. grandiflorus* and *E. paniculatus* are aquatic or underwater species widely distributed in the Neotropics, from Mexico to the southern and southeastern Regions of Brazil (Haynes & Holm-Nielsen, 1994). The genus is characterized by strong chromosome number stability, with a record of a single triploid cytotype for two populations of *E. tenellus* (Mart. ex Schult. & Schult.f.) Buchenau in the state of São Paulo, Brazil (Costa & Forni-Martins, 2004). Our counts of $2n = 22$ (Fig. 14A,B,C, respectively) for the species analyzed here are in agreement with all previous counts for these species (Costa & Forni-Martins, 2003; Costa & al., 2006; Feitoza & al., 2009, 2010).

There is no chromosome record for the genus *Griffinia*. The present counts for *G. angustifolia* with $2n = 30$ (Fig. 13D) and for *G. gardneriana* with $2n = 20$ (Fig. 13E) are the first karyological data for the genus *Griffinia*. The genus is considered endemic for Brazil, referred to the northern, northeastern, midwest and southeastern regions (Dutilh & Oliveira, 2015). *Griffinia angustifolia* has recently been described for the Caatinga biome (a semi-arid vegetation of the northeastern region of Brazil), with restricted distribution to the states of Bahia, Sergipe and Alagoas (Rocha & al., 2017). This species is regularly flowering throughout the year. On the other hand, *G. gardneriana* has a broader distribution, sometimes forming large populations, presenting larger and showier flowers, flowering only after the first rains (Alves-Araújo & al., 2009).

Hippeastrum stylosum is an endemic species from the northeastern region of Brazil, being recorded in all the states of that region, occurring in several environments, from rocky coastal cliffs, Atlantic Forest (rainforest) to rocky outcrops in semi-arid vegetation (Alves-Araújo & al., 2009). Our count of $2n = 22$ (Fig. 13F) is in agreement

with all previous counts for the species (Rice & al., 2014), as well as for several other species of the genus (Poggio & al., 2007; Pitrez & al., 2014).

The genus *Nothoscordum* Kunth has disjunct distribution from North to South America (Meerow, 2012). It is characterized by large chromosomes, with numbers varying from $2n = 8$ to $2n = 20$ and karyotypes formed by mainly metacentric and acrocentric chromosomes (Souza & al., 2012). Our count of $2n = 19$ for *N. gracile* (Fig. 13G) is in agreement with most previous counts for the species (Sato, 1980). This species is a hybrid with apomictic reproduction, resulting from recurrent and independent crossings between *N. nudicaule* (Lehm.) Guagl. and *N. macrostemon* Kunth (Souza & al., 2012). *Nothoscordum pulchellum* with $n = 5$ (Fig. 13H) is a diploid species with only one previous count of $2n = 10$ (Guerra & Felix, 2000). This species is endemic from Brazil with occurrence registered for the northeastern, central-western and southeastern regions.

Limnobiium Schimp. is a Neotropical genus with two species (Bove, 2015). *Limnobiium laevigatum* is a species with a broad distribution in the Neotropics, from Mexico to Uruguay and Argentina (Díaz-Miranda & al., 1981). It is floating or fixed immerse, stoloniferous, and with unisexual flowers (Bove, 2015). Our count of $2n = 28$ (Fig. 13I) is in agreement with all previous counts for the species, including Feitoza & al.'s (2009) for another population in the state of Paraíba.

Syagrus Mart. presents the main center of its diversity in Brazil, which has 57 of the 65 species from the genus. *Syagrus vagans* is an endemic species of Brazil, registered for Bahia (northeastern) and Minas Gerais (southeastern) States. It is included in the group of plants with solitary stipe, underground, characterized by petioles with serrated margin (Noblick, 2017). The chromosome number $2n = 32$ has been previously recorded for all analyzed species of the genus (Röser, 1994), all diverging from our count of $2n = 30$

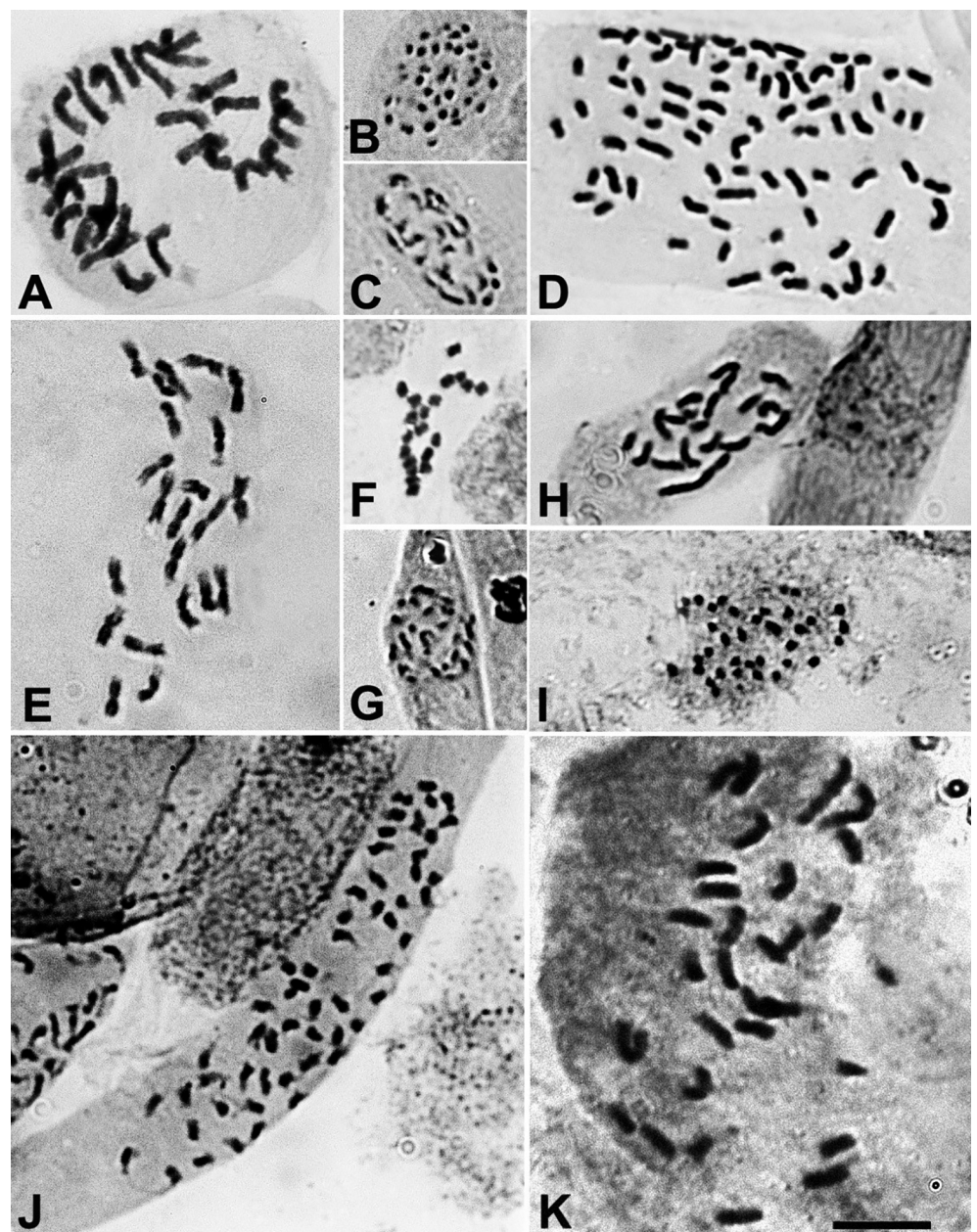


Fig. 14. A, *Alternanthera brasiliana*, $2n = 28$; B, *Tillandsia juncea*, $2n = 38$; C, *Pityrocarpa moniliformis*, $2n = 26$; D, *Acmella oleracea*, $2n = 26$; E, *Hylocereus setaceus*, $2n = 78$; F, *Chaetocalyx scandens* var. *pubescens*, $2n = 20$; G, *Angelonia campestris*, $2n = 28$; H, *Triplaris americana*, $2n = 22$; I, *Cissus pulcherrima*, $2n = 36$; J, *Plectrophora cultrifolia*, $2n = 56$; K, *Magonia pubescens*, $2n = 30$. — Scale bar = 10 μ m.

(Fig. 13J), the only species of *Syagrus* with underground stipe analyzed.

For *Alternanthera brasiliensis*, $2n = 28$ was counted (Fig. 14A). It is a ruderal species with a wide distribution in the Neotropics, occurring in all regions of Brazil (Senna, 2015). This is the first chromosome number record for the species and is in agreement with the previous record of *A. tenella* Colla (Pitrez & al., 2014), a related species, also ruderal and widely distributed in the Neotropics (Burger, 1983).

Tillandsia juncea has a disjunct distribution, with populations in the Andes, Central America, the Caribbean and eastern Brazil (González & al., 2013; Forzza & al., 2015). The present count with $2n = 38$ (Fig. 14B) was confirmed in at least three cells from a single individual, and diverged from all previous counts for the species: $2n = 96$ (Rice & al., 2014) and $2n = 50$ (Brown & Gilmartin, 1989). Maybe the discrepancy is associated with taxonomic misidentification or strong evolutionary divergence of the present sample in relation to the plants from Central America and Mexico.

Pityrocarpa was segregated from *Piptadenia* Benth. by presenting unarmed branches or branches with slightly stipular spines (Jobson & Luckow, 2007). The genus does not have a previous chromosome number record. However, our count of $2n = 26$ for *P. moniliformis* (Fig. 14C) in a population from Bahia, Brazil, is in agreement with the only three records for the related genus *Piptadenia* (Alves & Custodio, 1989; Santos & al., 2012). *Pityrocarpa moniliformis* is an endemic species from Brazil, occurring only in the northeastern and southeastern regions (Morim, 2015).

Acmella oleracea has a restricted distribution in Brazil, occurring in other countries only as cultivated plant (Jansen, 1985; Mondin & al., 2015). The present count of $2n = 78$ (Fig. 14D) confirms at least one previous count for the species (Mathew & Mathew, 1988) and for *A. alba* (L'Hér.) R.K.Jansen (Jansen & Stuessy, 1980), which is considered a derivative form.

Hylocereus setaceus has a restricted distribution to South America, occurring in Brazil (North, Northeastern, Midwest and Southeastern), Paraguay, southwestern Bolivia and northern Argentina (Bauer, 2003; Zappi & al., 2017). The present count of $2n = 22$ (Fig. 14E) diverges from two previous records for the species, including $2n = 44$ reported by Castro & al. (2013) from another population in the state of Paraíba. With the exception of the subfamily Pereskioideae, high incidence of intraspecific polyploidy is characteristic for the family Cactaceae (Castro & al., 2016).

Chaetocalyx scandens var. *pubescens* also has no previous chromosome record. Our count of $2n = 20$ (Fig. 14F) is in agreement with those for two other species of *Chaetocalyx* DC. collected in Argentina and three other species of the genus *Aeschynomene* L. (Vanni, 1983). The subspecies has a Neotropical distribution, occurring in Mexico, the Antilles, Venezuela, Colombia and Brazil (Rudd, 1958), from the latter is referred to the northern, northeastern and southern regions (Lima, 2015).

Angelonia campestris is an herbaceous species endemic to the Caatinga and Cerrado vegetation of the northern region of Brazil (Souza & Hassemer, 2015). Previous chromosome records are known for only three other species of *Angelonia* Bonpl., all with $2n = 20$ (Subramanian & Pondmud, 1987). Our count of $2n = 28$ (Fig. 14G) for a wild population in northeastern Brazil differs from all previous counts for the genus.

Triplaris americana is a widely distributed species in South America and Panama (Brandbyge, 1986), being referred to Brazil for the midwest, southeastern and southern regions (Melo, 2015). The present record of $2n = 22$ (Fig. 14H) confirms two previous

counts for the genus: *T. americana* (Bawa, 1973) and *T. weigeltiana* (Rchb.) Kuntze (Rice & al., 2014).

Cissus pulcherrima is an endemic species from Brazil, previously referred only for the northeastern and southeastern regions (Lombardi, 2000). The count of $2n = 36$ (Fig. 14I) is the first one for a population in the state of Tocantins of the northern region, as well as the record of its occurrence in Tocantins. However, its occurrence in limestone outcrops that border between Tocantins and Bahia indicates that the species may have a wider distribution related to calcareous environments. Our count diverges from all previous counts for the genus, including the only record for a Brazilian species, *C. decidua* Lombardi, with $2n = 34$ (Pitrez & al., 2014; Cordeiro & al., 2017).

The genus *Plectrophora* is a small genus from the subtribe Oncidiinae with approximately 10 Neotropical species (Chase & al., 2015), of which five occur in Brazil, while *P. cultrifolia* occurs in several countries of South America including Brazil (Barros & al., 2015). Our count of $2n = 56$ (Fig. 14J) for this species constitutes the first chromosome record for the genus and is in agreement with most counts for other genera from Oncidiinae (Felix & Guerra, 2000).

Magonia pubescens is the only known species for the genus *Magonia* A.St.-Hil. These are arboreal plants, occurring in Bolivia and Brazil, especially in Cerrado vegetation and Semideciduous Seasonal Forest (Somner & al., 2015). Our count of $2n = 30$ (Fig. 14K) is in agreement with all previous counts for the species (Forni-Martins & al., 1995).

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IAPT chromosome data 31/8

Miguel Ángel García,* Mihai Costea, Marcelo Guerra, Ignacio García-Ruiz & Saša Stefanović

* Address for correspondence: ma.garcia@utoronto.ca

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* First chromosome count for the species.

** New cytotype for the species.

CONVOLVULACEAE

Cuscuta africana Willd.

* $2n = 30$, CHN. South Africa, Western Cape, Outeniqua Pass, near small resting area on road N9-12 towards George, 33°53'26.88"S, 22°24'11.52"E, 28 Nov 2007, *M.A. García 3920* (MA-877241) [Figs. 15A, 18A].

Cuscuta angulata Engelm.

* $n = 15$, $2n = 30$, CHN. South Africa, Western Cape, De Hoop Nature Reserve, sand road towards Potberg Nature Reserve, 34°25'50.52"S, 20°42'27.72"E, 04 Dec 2007, *M.A. García 3922* (MA-877242) [Figs. 15B,C, 18B].

Cuscuta approximata Bab.

$2n = 28$, CHN. Spain, Segovia Province, Maderuelo, Hoces del Riaza Natural Park, near Linares water reservoir, 41°32'18.47"N, 03°33'27.73"W, 01 Jul 2015, *M.A. García 6482* (TRTE) [Fig. 15D].

Cuscuta bonafortunae Costea & I.García

* $2n = 30$, CHN. Mexico, Michoacán, Municipio Zamora: Libramiento Sur-Sureste de Zamora, 19°56'59"N, 102°15'07"W, 1740 m, *I. García-Ruiz 8391* (CIMI, WLU) [Figs. 15E, 18C].

Cuscuta brachycalyx (Yunck.) Yunck.

* $2n = 30$, CHN. U.S.A., California, Mariposa Co., Yosemite NP, Wawona, near hotel, 07 Jun 2015, *S. Stefanović 15-22* (TRTE) [Fig. 15F].

Cuscuta campestris Yunck.

** $2n = 60$, CHN. Serbia, Vojvodina Province, Lukićevo, 45°20'40"N, 20°30'30"E, 03 Sep 2008, *M. Šarić s.n.*, UTM-1569 (TRTE) [Figs. 15G, 18D]. Voucher grown from seeds in UTM greenhouse.

Cuscuta cephalanthi Engelm.

2n = 60, CHN. U.S.A., Iowa, Williams Prairies Preserve, 15 Sep 2015, R. Lutz & R. Tallent s.n., UTM-1567 (TRTE) [Fig. 15H]. Voucher grown from seeds at UTM greenhouse.

Cuscuta chapalana Yunck.

*2n = 30, CHN. Mexico, Jalisco, Tlajomulco, between Poterillos and Trojes, 19 Dec 2007, I. García-Ruiz & al. 8064, UTM-1563 (CIMI, TRTE, WLU) [Figs. 15I, 18E]. Voucher grown from seeds at UTM greenhouse.

Cuscuta chilensis Ker Gawl.

*n = 15, 2n = 30, CHN. Chile, La Campana National Park, 32°55'55.8"S, 71°04'52.4"W, 15 Jan 2011, M. Costea s.n. (WLU) [Figs. 15J, 18F].

Cuscuta compacta Juss. ex Choisy

2n = 30, CHN. U.S.A., Texas, Taylor Co., 03 Sep 2015, S. Stefanović s.n., UTM-1566 (TRTE) [Fig. 15K]. Voucher grown from seeds at UTM greenhouse.

Cuscuta corymbosa var. *grandiflora* Engelm.

*2n = 30, CHN. Mexico, Michoacán, San Pedro Tesistan, 20°13'23"N, 103°24'35.4"W, 19 Dec 2007, M. Costea & I. García-Ruiz s.n. (CIMI, WLU). [Figs. 15L, 18G].

Cuscuta costaricensis Yunck.

*2n = 30, CHN. Mexico, Jalisco, La Manzanilla, La Rosa Amarilla, 18 Dec 2007, I. García-Ruiz 8052 (CIMI, WLU) [Figs. 16A, 18H].

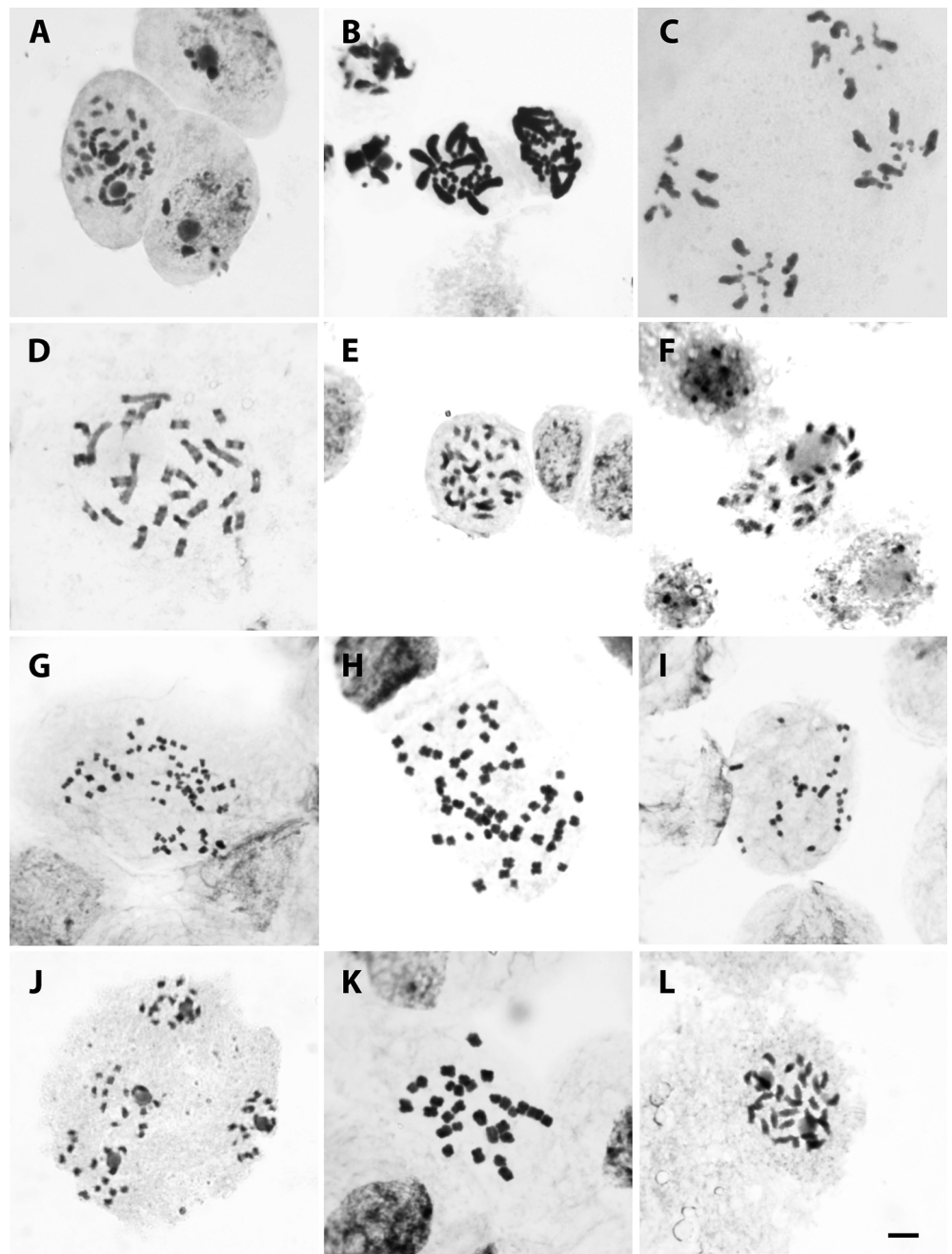


Fig. 15. Mitotic or meiotic chromosomes of *Cuscuta* species.

A, *C. africana*; B, *C. angulata*; C, Telophase II of *C. angulata*; D, *C. approximata*; E, *C. bona-fortunae*; F, *C. brachycalyx*; G, *C. campestris*; H, *C. cephalanthi*; I, *C. chapalana*; J, Telophase II of *C. chilensis*; K, *C. compacta*; L, *C. corymbosa* var. *grandiflora*. — Scale bar = 5 μ m.

Cuscuta cotijana Costea & I.García

* $2n = 30$, CHN. Mexico, Michoacán, Cotija, Los Gallineros, 16 Feb 2007, *I. García-Ruiz & al.* 7560 (CIMI, NY, US, WLU) [Fig. 16B]. Chromosomes counted from seedlings.

Cuscuta desmouliniana Yunck.

* $2n = 30$, CHN. Mexico, Sonora, Cañon Los Anegados, 28°02'09"N, 111°08'05"W, 18 Nov 2007, *Van Devender & al.* 2007-1094 (ARIZ, WLU) [Fig. 16C]. Chromosomes counted from seedlings.

Cuscuta epithymum (L.) L.

$2n = 32$, CHN. Spain, Cantabria Province, Puerto Palomera descending to Saja, 43°05'20.70"N, 04°14'49.45"W, 11 Jul 2015, *M.A. García 6486*, UTM-1573 (TRTE) [Fig. 16D].

* $2n = 34$, CHN. Spain, Segovia Province, Maderuelo, Hoces del Riaza Natural Park, near Linares water reservoir, 41°32'18.47"N, 03°33'27.73"W, 01 Jul 2015, *M.A. García 6483*, UTM-1571 (TRTE) [Fig. 16E].

Cuscuta erosa Yunck.

* $2n = 30$, CHN. U.S.A., Arizona, Santa Rita Experimental Range, foothills of the Santa Rita, Aug 2013, *J. Cowles s.n.* (WLU) [Figs. 16F, 18I]. Chromosomes counted from seedlings.

Cuscuta glomerata Choisy

$n = 15$, $2n = 30$, CHN. U.S.A., Iowa, Williams Prairie, 31 Jul 2015, *R. Lutz s.n.*, UTM-1565 (TRTE) [Fig. 16G]. Voucher grown from seeds at UTM greenhouse.

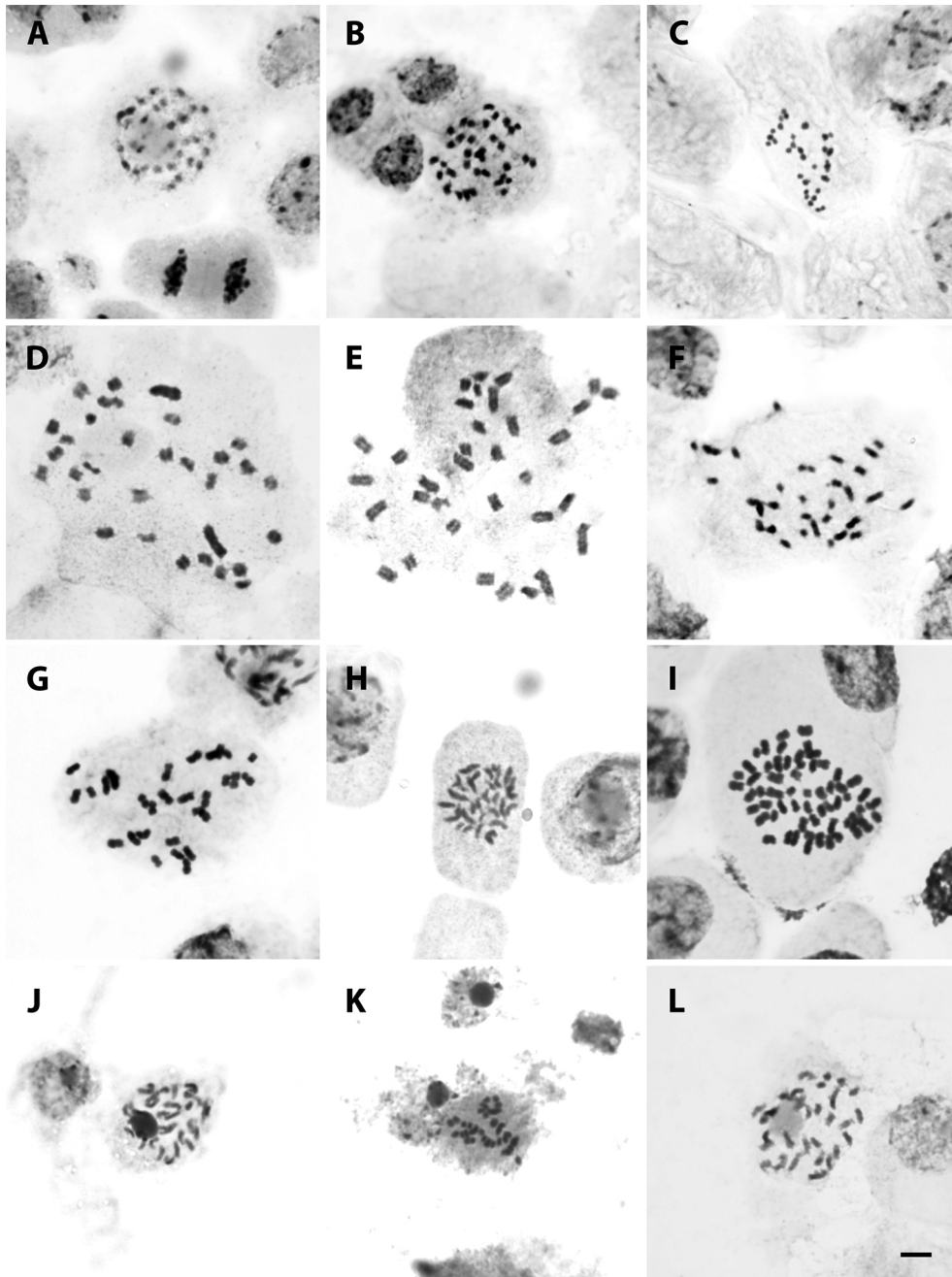


Fig. 16. Mitotic chromosomes of *Cuscuta* species. **A**, *C. costaricensis*; **B**, *C. cotijana*; **C**, *C. desmouliniana*; **D & E**, *C. epithymum*; **F**, *C. erosa*; **G**, *C. glomerata*; **H**, *C. grandiflora*; **I**, *C. gronovii*; **J**, *C. obtusiflora*; **K**, *C. occidentalis*; **L**, *C. pacifica* var. *pacifica*. — Scale bar = 5 μ m.

Cuscuta grandiflora Kunth

* $2n = 30$, CHN. Peru, Cusco Region, entrance to archaeological site Tipón, 13°33'45.54"S, 71°47'57.62"W, 20 Aug 2007, *M. Costea s.n.* (WLU) [Figs. 16H, 18J].

Cuscuta gronovii Willd.

$2n = 60$, CHN. Canada, Ontario, Caledon, Terra Cotta, Credit River, 26 Jul 2015, *M.A. García s.n.* (TRTE) [Figs. 16I, 18K].

Cuscuta indecora Choisy

$2n = 30$, CHN. Mexico, Zacatecas, Municipio Luis Moya, 22°26'35.70"N, 102°13'41.68"W, 14 Aug 2012, *A.V. Ibarra s.n.* (WLU) [Fig. 17A]. Voucher grown from seeds at UTM greenhouse.

Cuscuta monogyna Vahl

$2n = 30$, CHN. Israel, Kursi, 32°49'34.9"N, 35°38'59.6"E, 2012, *G. Wizen s.n.*, UTM-1348 (TRTE) [Fig. 17B]. Voucher grown at UTM greenhouse from seeds.

Cuscuta obtusiflora Kunth

* $2n = 30$, CHN. Mexico, Jalisco, 2–3 km N of San Diego, *I. García-Ruiz & al. 8256* (CIMI, WLU) [Figs. 16J, 18L].

Cuscuta occidentalis Millsp.

* $2n = 30$, CHN. U.S.A., California, San Diego Co., Anza-Borrego Desert State Park, Hwy S2, mile 11.5 W side, 21 Apr 2013, *S. Stefanović 13-06* (TRTE) [Fig. 16K].

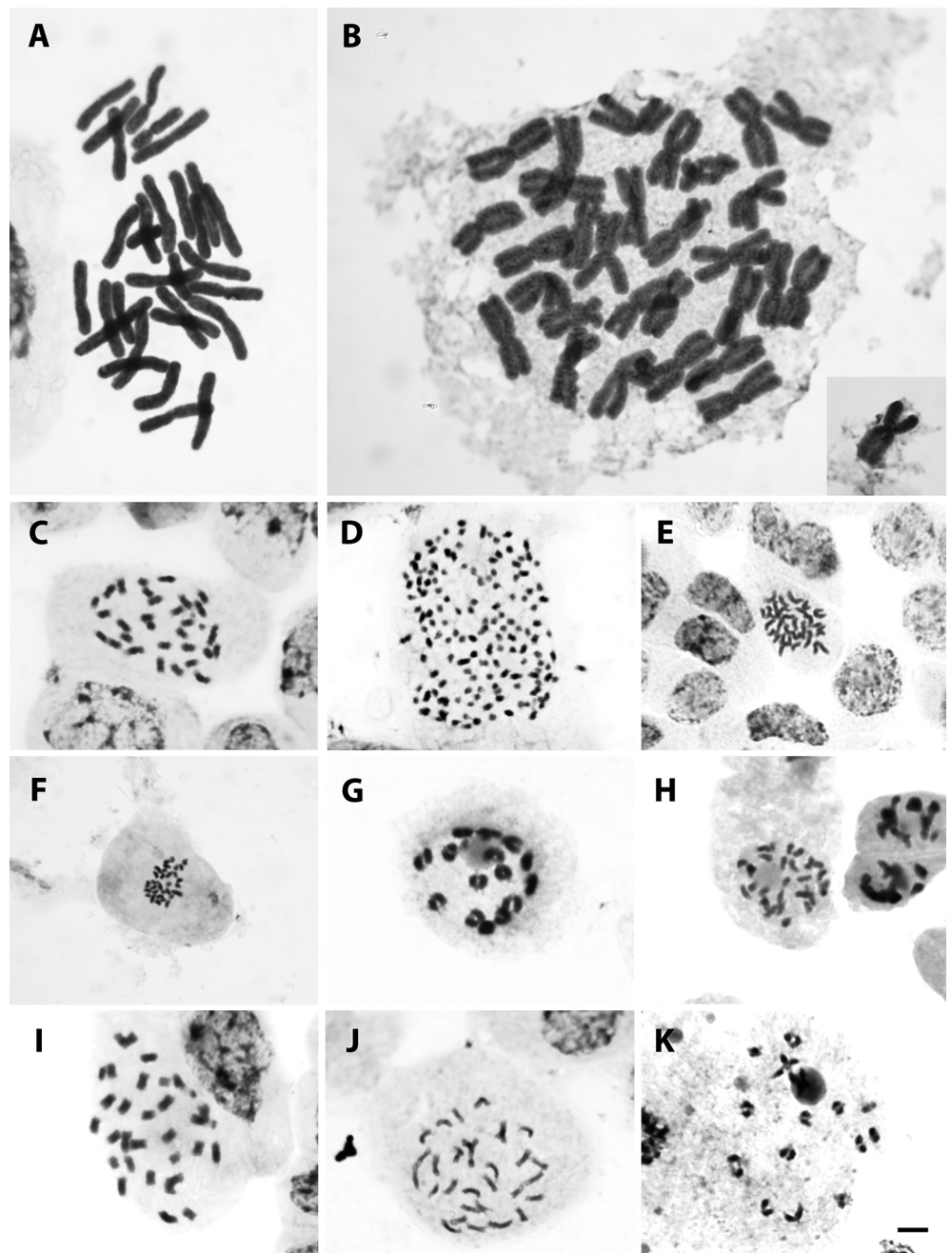


Fig. 17. Mitotic or meiotic chromosomes of *Cuscuta* species.

A, *C. indecora*; **B**, *C. monogyna* (inset: one chromosome lying at some distance from the main group); **C**, *C. purpurata*; **D**, *C. sandwichiana*; **E**, *C. sidarum*; **F**, *C. subinclusa*; **G**, Diakinesis of *C. tinctoria* var. *floribunda*; **H**, *C. tinctoria* var. *tinctoria*; **I**, *C. umbrosa*; **J**, *C. volcanica*; **K**, Diakinesis of *C. volcanica*. — Scale bar = 5 μ m.



Fig. 18. Flowers of *Cuscuta* species. **A**, *C. africana*; **B**, *C. angulata*; **C**, *C. bonafortunae*; **D**, *C. campestris*; **E**, *C. chapalana*; **F**, *C. chilensis*; **G**, *C. corymbosa* var. *grandiflora*; **H**, *C. costaricensis*; **I**, *C. erosa* (photo: Jillian Cowles); **J**, *C. grandiflora*; **K**, *C. gronovii*; **L**, *C. obtusiflora*; **M**, *C. pacifica* var. *pacifica*; **N**, *C. purpurata* (photo: Sergio Moreira & Melica Muñoz Schick); **O**, *C. sidarum*; **P**, *C. subinclusa*; **R**, *C. tinctoria* var. *tinctoria*; **S**, *C. tinctoria* var. *floribunda*; **T**, *C. volcanica*. — All photographs by the authors except for those indicated otherwise.

Cuscuta pacifica Costea & M.A.R. Wright var. *pacifica*

* $2n = 30$, CHN. U.S.A., California, Solano Co., San Pablo Bay NWR, along Hwy 37, 09 Jun 2015, *S. Stefanović 15-23* (TRTE) [Figs. 16L, 18M].

Cuscuta purpurata Phil.

* $2n = 30$, CHN. Chile, Region III, Quebrada al S de Bahía Salado, 13 Oct 2010, *M. Muñoz 5144*, UTM-1433 (SGO, TRTE, WLU) [Figs. 17C, 18N]. Voucher grown from seeds at UTM greenhouse.

Cuscuta sandwichiana Choisy

* $2n = 150$, CHN. U.S.A., Hawaii, Hawaii Island (Big Island), Punaluu beach, 20 Dec 1984, *Degener & Degener 36596*, UTM-155 (TRTE) [Fig. 17D]. Voucher grown from seeds at UTM greenhouse.

Cuscuta sidarum Liebm.

* $2n = 30$, CHN. Mexico, Michoacán, Aquila, 18°35'52.43"N, 103°30'5.17"W, 22 Feb 2007, *I. García-Ruiz & al. 7584* (CIMI, WLU) [Figs. 17E, 18O].

Cuscuta subinclusa Durand & Hilg.

* $2n = 30$, CHN. Mexico, Baja California Norte, Hills on the W side of Road 1, N of San Vicente, 31°25'41.16"N, 116°19'22.96"W, 589 m, 01 May 2014, *M. Costea s.n.* (WLU) [Figs. 17F, 18P].

Cuscuta tinctoria var. *floribunda* (Kunth) Costea

* $n = 15$, CHN. Mexico, Temascaltepec, E de La Peña, on road to Temascaltepec, 27 Dec 2012, *I. García-Ruiz & al. 8588* (CIMI, WLU) [Fig. 17G, 18S].

Cuscuta tinctoria Mart. ex Engelm. var. *tinctoria*

** $2n = 30$, CHN. Mexico: Michoacán, Venustiano Carranza, San Pedro, 20 Feb 2007, *I. García-Ruiz & al. 7575* (CIMI, WLU) [Fig. 17H, 18R].

Cuscuta umbrosa Beyr. ex Hook.

* $2n = 30$, CHN. U.S.A., Iowa, Cerro Gordo Co., 13 Sep 2013, *Watson s.n.*, UTM-1564 (TRTE) [Fig. 17I]. Voucher grown from seeds at UTM greenhouse.

Cuscuta volcanica Costea & I.García

* $2n = 30$, $n = 15$, CHN. Mexico, Jalisco, Mazamitla, road to Santa Maria del Oro, 17 Feb 2007, *I. García-Ruiz & al. 7567* (CIMI, WLU) [Figs. 17J,K, 18T].

Flower buds were collected and fixed in the field, stained in darkness with 4% Wittman's hematoxylin for at least 24 hours and mounted and squashed in 45% acetic acid. Alternatively, stems, seedlings, or flower meristems were prefixed in 8-hydroxyquinoline 0.002 M for 24 hours at 10°C, fixed in Carnoy's solution for 24 h at room temperature, hydrolysed in HCl 5N for 20 minutes, washed in distilled water, and squashed and mounted in 45% acetic acid. The slides were frozen in liquid nitrogen to remove the coverslip, air-dried and chromosomes stained with 1% hematoxylin for a few seconds, washed with distilled water, air-dried and permanent mounts prepared in Canada balsam. All materials, CHN. Those specimens grown from seeds and used for DNA extraction and further

studies are indicated with UTM (University of Toronto Mississauga) followed by collection number.

Cuscuta (dodders) is one of the most diverse groups of obligate parasitic plants, comprising about 200 species (Costea & al., 2015), many of which have a substantial economic and ecological importance (Costea & Tardif, 2006; Costea & Stefanović, 2009). Systematics of the genus has been challenging in the past because of rampant hybridization and the lack of morphological characters, but several recent molecular studies have provided an explicit and robust phylogenetic framework for this group (García & al., 2014). Albeit limited, the currently available evidence indicates that *Cuscuta* may represent the genus with the broadest karyotypic and genome size diversity among all angiosperms. First, it includes species not only with regular monocentric but also with holocentric chromosomes (Pazy & Plitmann, 1994; García, 2001), a feature found in a very few phylogenetically scattered groups of plants. Also, dodders exhibit significant karyotype variation and diversity in chromosome size, with reported chromosome numbers varying from $2n = 8$ to $2n = 60$ (Pazy & Plitmann, 1995; García & Castroviejo, 2003). In addition, the genus has a high variation in nuclear DNA content (McNeal & al., 2007).

For our study, counts were obtained from 32 taxa (31 species plus one variety) belonging to the four subgenera of *Cuscuta*. New counts are provided for 22 species and new cytotypes for 3 species. All the images are shown at the same magnification to visualize the diversity in chromosome size in the genus. Holocentric chromosomes are present in species of *Cuscuta* subg. *Cuscuta*, in this study represented by *C. approximata* and *C. epithymum*. The cytotype of *C. epithymum* with $2n = 32$ chromosomes was reported by García & Castroviejo (2003) and is here illustrated for the first time. The karyotype is bimodal, with one pair of chromosomes clearly longer than the rest (Fig. 16D). We report a new cytotype for this species with $2n = 34$ chromosomes, having chromosomes of similar size (Fig. 16E).

Two species of the South African *Cuscuta* subg. *Pachystigma* (Engelm.) Baker & C.H. Wright have been studied: *C. africana* and *C. angulata*. The subgenus was previously cytogenetically unknown and is analysed here for the first time. These two species have $2n = 30$ monocentric chromosomes and strongly asymmetrical karyotypes, with two pairs of longer chromosomes in *C. africana* and 5 pairs in *C. angulata* (Fig. 15A–C).

Previous studies on species of *Cuscuta* subg. *Monogynella* (Des Moul.) Peter, Engl. & Prantl showed that the group is cytogenetically characterised by large monocentric chromosomes (Pazy & Plitmann, 1995; García & Castroviejo, 2003). We report $2n = 30$ chromosomes for *C. monogyna* (Fig. 17B) in accordance with previous counts on plants from Israel (Pazy & Plitmann, 1995) and Spain (García & Castroviejo, 2003). Additionally, $2n = 28$ chromosomes were reported for Iranian specimens (Aryavand, 1987).

Most of the species sampled for this study belong to *Cuscuta* subg. *Grammica* (Lour.) Peter, Engl. & Prantl, a group that represents about 3/4 of the species diversity of the genus. Two species in the subgenus, *C. indecora* and *C. coryli* Engelm., both included in *Cuscuta* sect. *Indecorae* (Costea & al., 2015) were previously reported as having $2n = 30$ large chromosomes (Fogelberg, 1938). We here confirm the chromosome count for *C. indecora* and show the photograph of a mitotic metaphase (Fig. 17A). The chromosome size in this species is remarkably larger compared to any other species of *Cuscuta* subg. *Grammica* analysed in this study. We have sampled representatives of 10 out of the 15 sections recognised for the subgenus, and only

Cuscuta sect. *Indecorae* has such large chromosomes. Most species in *Cuscuta* subg. *Grammica* have either $2n = 30$ or $2n = 60$ chromosomes, but we report for the first time $2n = 150$ (decaploid) chromosome number for the Hawaiian endemic *C. sandwichiana* (Fig. 17D), a species of probable allopolyploid origin (Stefanović & Costea, 2008; García & al., 2014). This finding is remarkable because the highest known chromosome number for the genus was $2n = 60$.

Hybridization and polyploidy have likely played a significant role in *Cuscuta* species diversification (Stefanović & Costea, 2008; Costea & Stefanović, 2010; García & al., 2014, 2018). The enormous variation in chromosome type, number, size, and DNA amounts, along with a well-resolved phylogeny at multiple levels, makes *Cuscuta* a great model to study genome and chromosome evolution in plants generally, and the transition from monocentric to holocentric chromosomes in particular.

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IAPT chromosome data 31/9

Luiza Domingues Hirsch, Fernanda Bered, Camila Aguiar-Melo, Luana Carvalho & Eliane Kaltchuk-Santos*

* Address for correspondence: eliane.kaltchuk@ufrgs.br

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* First chromosome count for the taxon.

BROMELIACEAE

Subfamily Pitcairnioideae

**Dyckia choristaminea* Mez

$2n = 50$, CHN. Brazil, Rio Grande do Sul, Barra do Ribeiro, 30°18'52"S, 51°30'14"W, 228 m, 26 Oct 2017, L.D. Hirsch s.n. (ICN) [Fig. 19].

**Dyckia hebdingii* L.B.Sm.

$2n = 50$, CHN. Brazil, Rio Grande do Sul, Barra do Ribeiro, 30°18'52"S, 51°30'21"W, 239 m, 07 Oct 2015, C.J. Breitsameter, L.D. Hirsch, F. Bered & C. Aguiar-Melo s.n. (ICN) [Fig. 20].

**Dyckia julianae* Strehl

$2n = 50$, CHN. Brazil, Rio Grande do Sul, Barra do Ribeiro, 30°18'52"S, 51°30'14"W, 228 m, 20 Oct 2016, L.D. Hirsch s.n. (ICN) [Fig. 21].

Bromeliaceae is one of the most diverse families of the Neotropics (Benzing, 2000; Martinelli & al., 2008; Givnish & al., 2011), comprising approximately 75 genera and 3552 species (Gouda & al., 2018). *Dyckia* Schult. & Schult.f. is a large genus of the Pitcairnioideae subfamily (Givnish & al., 2007; Krapp & al., 2014), with 171 species (Gouda & al., 2018). Twenty-eight species of *Dyckia* have been described for the southernmost portion of Brazil, of which several are endemics (Strehl, 2004). *Dyckia choristaminea*, *D. hebdingii* and *D. julianae* are endemic to southern Brazil with restricted distribution, often occurring in sympatry (Smith & Downs, 1974; Strehl, 2004). Hirsch & al. (2019), analyzing sympatric populations of these three species through SSR markers, found an intermediate molecular profile for *D. julianae* when compared to the other two species. Such data strongly suggests a hybrid origin for *D. julianae* resulting from an ancient crossing between *D. hebdingii* and *D. choristaminea*. To better understand this issue, other approaches have to

be used, including cytogenetic analysis. In Bromeliaceae, cytogenetic studies have so far been carried out for approximately 10% of the species. The basic chromosome number of this family is $x = 25$ (Marchant, 1967), with prevalence of diploid species. Chromosome counts for *Dyckia* are reported for only 14 species (Rice & al., 2015), most of them presenting $2n = 50$. Considering the three species investigated in the present study, no previous data have been found. Determination of chromosome number/ploidy level and evaluation of meiotic stability are especially important when hybrid status is investigated, since homoploid or allopolyploid pathways give rise to individuals with different ploidy levels and irregular meiosis is expected.

Aiming to shed some light on the occurrence of hybridization events among these three *Dyckia* species, a cytogenetic characterization was carried out. Therefore, the goals of the present study were: (1) to determine chromosome numbers; (2) to analyze the meiotic behavior and pollen viability, and (3) to estimate the genome size of the three studied species.

The chromosome number and meiotic behavior were analyzed by young inflorescences previously fixed (3 : 1 ethanol : acetic acid). Anthers were squashed in 1% propionic carmine for slides preparation. Chromosome counts were performed in diakinesis. All available phases of meiosis I and II were analyzed for meiotic stability. Meiotic indexes were calculated from 200 tetrads per plant using the formula: $MI = (\text{number of normal tetrads} / \text{total of tetrads}) \times 100$. Pollen viability estimation and pollen morphology evaluation were carried

out by Alexander method (Alexander, 1980). Categorization of pollen morphology followed the classification of Erdtman (1971). For genome size analysis, approximately 25 mg of leaf tissue from standard (*Zea mays* L. 'CE-777' $2C = 5.43$ pg; Doležel & al., 2007) and from each species was chopped in 1 ml of cold nuclear-isolation buffer LB01. The suspension was filtered through a 40 μm mesh nylon filter, and nuclei were stained with 50 μl propidium iodide. The DNA content of 10,000 stained nuclei for each sample was estimated using a FACSAria II (Becton Dickinson, Franklin Lakes, New Jersey, U.S.A.) flow cytometer.

Cytogenetic data obtained for the three sympatric species of *Dyckia* are presented in Table 4 and discussed taking into account the recent study of our team (Hirsch & al., 2019) involving molecular markers and hybridization of these species.

All species, *Dyckia choristaminea*, *D. hebdingii* and *D. julianae* have their chromosome number determined for the first time, all being diploids with $2n = 2x = 50$ (Figs. 19A, 20A, 21A). Only three tetraploid species (*D. argentea*, *D. lorentziana*, *D. remotiflora*) have been reported for this genus (Sharma & Ghosh, 1971; Baracho & Guerra, 2000; Cotias-de-Oliveira & al., 2004). The chromosomes are small, as generally found in bromeliads, and similar in size among the three species.

The genome sizes for the three species were very similar, around 1.7 pg (Table 4), corroborating the same ploidy level of them. DNA content is available only for *D. choristaminea*; Moura & al. (2018) reported $2C = 1.78$ pg for this species, therefore an equivalent result. Bromeliaceae species present a narrow range for DNA content, with

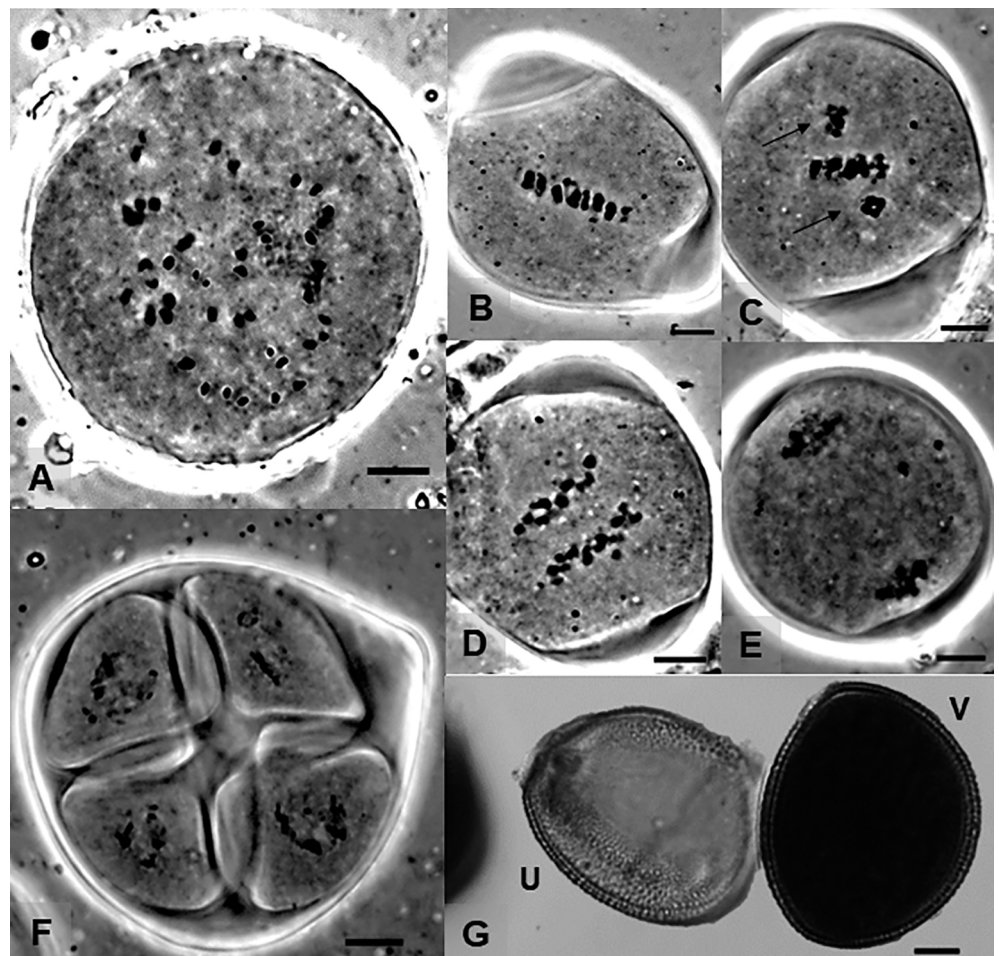


Fig. 19. Cytogenetic analysis of *D. choristaminea*. **A**, Diakinesis with 25 bivalents; **B**, Metaphase I; **C**, Metaphase I with non-oriented chromosomes (arrows); **D**, Anaphase I; **E**, Telophase I; **F**, Tetrade; **G**, Viable pollen (V) and unviable (U) pollen grains. — Scale bar = 5 μm .

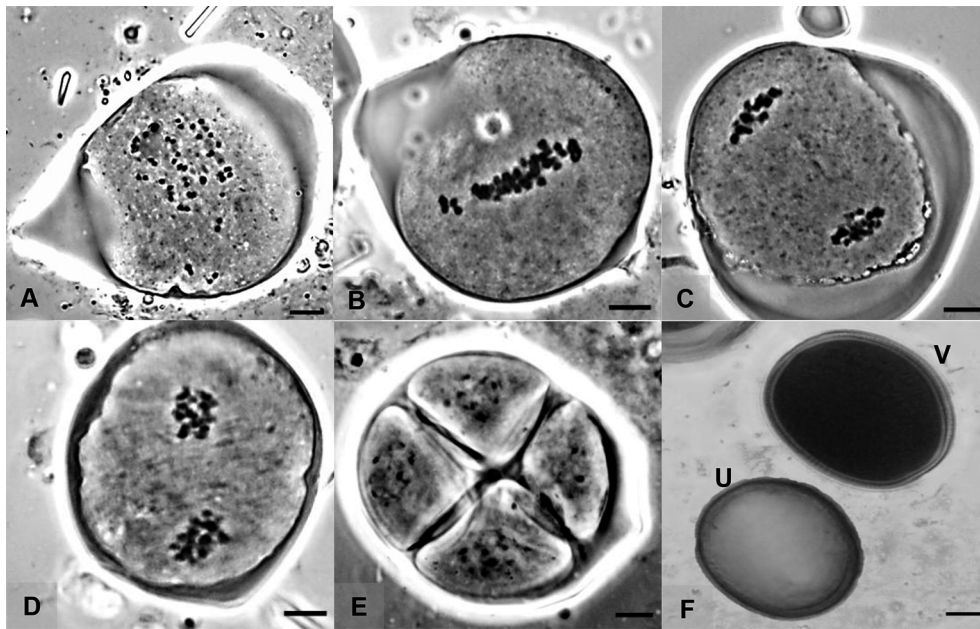


Fig. 20. Cytogenetic analysis of *D. hebdingii*. **A**, Diakinesis with 25 bivalents; **B**, Metaphase I; **C**, Anaphase I; **D**, Telophase I; **E**, Tetrads; **F**, Viable pollen (V) and unviable (U) pollen grains. — Scale bar = 5 μ m.

2C values between 0.6 and 2.52 pg (Angiosperm DNA C-values Database; Bennett & Leitch, 2012).

Concerning meiotic behavior, the three species showed high stability with bivalents pairing and regular segregation at anaphase I and II. Elevated meiotic indexes (MI) and pollen viability for all species reinforce the regular meiotic behavior. The lowest viability (92.64%) was observed in *D. hebdingii*. The pollen morphology was subprolate for all species. Of the species studied, *D. choristaminea* showed the largest pollen, whereas *D. hebdingii* had the smallest ones (Table 4).

As mentioned above, Hirsch & al. (2019) showed that *D. juliana* has an intermediate molecular profile when compared to *D. hebdingii* and *D. choristaminea* (both with pure molecular profiles), raising questions about the origin of this pattern. One of the possibilities involves hybridization. *Dyckia juliana* could be a contemporary hybrid between the other two species or a newly discovered established taxon originated by hybrid speciation. Interspecific crosses can produce two kinds of hybrids: homoploids and allopolyploids. Depending on the hybrid status of *D. juliana*, cytogenetics results can result in interesting hints to clarify these evolutionary pathways.

In this study, we observed that *D. juliana* has the same chromosome number as *D. hebdingii* and *D. choristaminea* ($2n = 50$). The

same chromosome number by itself does neither confirm nor discard the possibility of hybridization. Nevertheless, in this case, allopolyploidy is refuted. Possibly, we are facing homoploid hybridization, a situation in which parental species and hybrid have the same chromosome number. Despite a homoploid hybridization scenario, surprisingly, *D. juliana* has a regular meiosis and produces highly fertile pollen grains. Although the three species have the same chromosomal number, depending on the similarity of the parental species, an instable meiosis is expected, considering the inability of the chromosome sets to pair and segregate adequately (Ramsey & Schemske, 1998; Rounsaville & al., 2011). Although the present study clearly shows that *D. juliana* is not an allopolyploid, more than one hypothesis can be raised to explain its origin: (1) *Dyckia juliana* may have had a hybrid origin in sufficient time to overcome compatibility problems and present disomic behavior; (2) *Dyckia hebdingii* and *D. choristaminea* could have very similar and compatible chromosomes, reducing errors at meiosis, once they are closely related (Krapp & al., 2014) species and occur in sympatry (Strehl, 2004); (3) The intermediate molecular profile obtained for *D. juliana* may be due to a reason other than hybridization, such as retention of ancestral polymorphism. To elucidate these questions and better understand the genome contribution of each parental species in the formation of this taxon, other analyzes are necessary, such as *in situ* hybridization (FISH and GISH).

Table 4. Cytogenetic analysis.

Species	Chromosome number <i>n</i>	Meiosis I and II		Meiotic index %	Pollen viability		Pollen grains N	P (μ m) Mean	E (μ m) Mean	P/E Mean	Pollen morphology	Genome size 2C (pg)
		N	%		N	%						
DC	25	11 (2053)	99.90	100	10 (5078)	98.88	10 (200)	30.43	24.04	1.27	Subprolate	1.67
DH	25	23 (1780)	99.89	100	10 (5109)	92.64	10 (200)	26.70	21.75	1.23	Subprolate	1.72
DJ	25	19 (2105)	99.95	100	10 (5542)	93.28	10 (200)	28.93	23.68	1.22	Subprolate	1.75

DC = *D. choristaminea*; DH = *D. hebdingii*; DJ = *D. juliana*

N, Number of individuals (number of analyzed cells); P, Polar diameter; E, Equatorial diameter

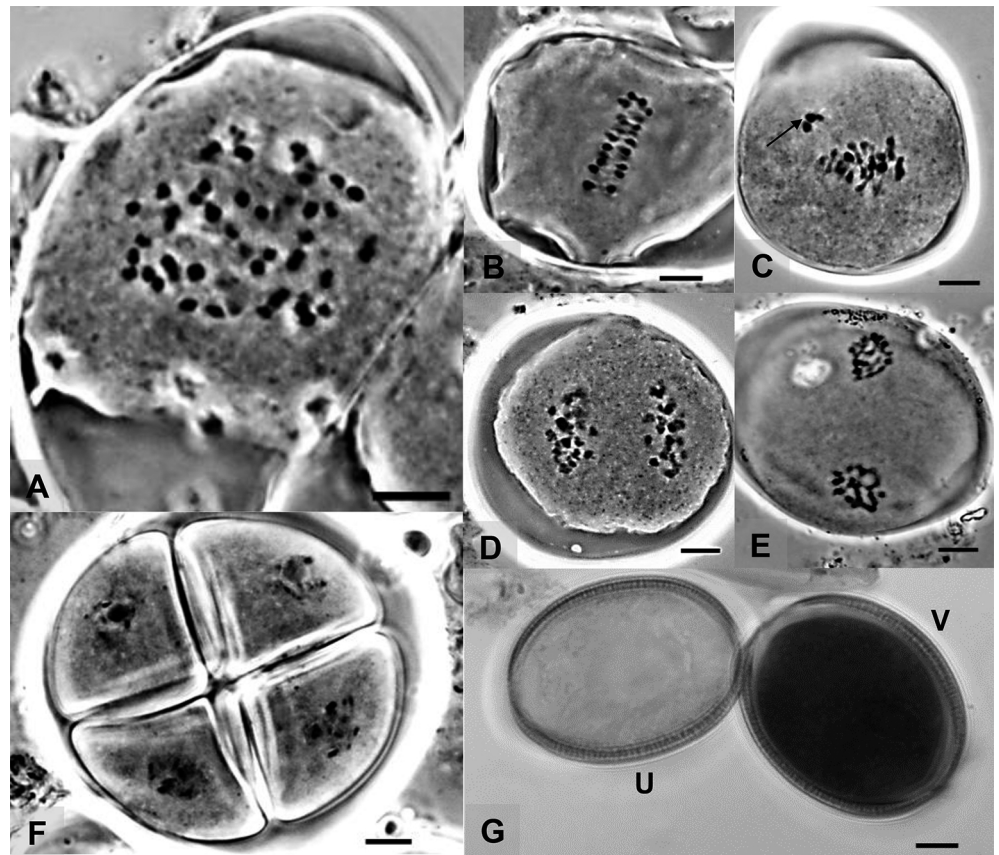


Fig. 21. Cytogenetic analysis of *D. juliana*. **A**, Diakinesis with 25 bivalents; **B**, Metaphase I; **C**, Metaphase I with non-oriented bivalents (arrow); **D**, Anaphase I; **E**, Telophase I; **F**, Tetrad; **G**, Viable pollen (V) and unviable (U) pollen grains. — Scale bar = 5 μ m.

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Vandna Kumari, Rohit Kumar & Vijay Kumar Singhal*

* Address for correspondence: vkshinghal53@gmail.com

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- * First chromosome count for the species.
- ** New chromosome report (cytotype) for the species.
- First chromosome count for an Indian accession.

BALSAMINACEAE

**Impatiens badrinathii* Pusalkar & D.K.Singh

$n = 10$, CHN. India, Uttarakhand, Uttarkashi, Har-ki-Dun Trek, 31°07'57.35"N, 78°23'32.12"E, 3300 m, isolated individuals in moist places along streams and grassy slopes, *Rohit Kumar 34392* (PUN 61649) [Fig. 22A].

**Impatiens devendrae* Pusalkar

$n = 7$, CHN. India, Uttarakhand, Uttarkashi, Rarhi, 30°46'15.67"N, 78°15'17.19"E, 2230 m, shaded or partly shaded moist places in forests and along forest edges, *Rohit Kumar 30727* (PUN 60222) [Fig. 22B].

**Impatiens leggei* Pusalkar & D.K.Singh

$n = 7$, CHN. India, Uttarakhand, Uttarkashi, Har-ki-Dun, 31°08'51.20"N, 78°25'25.85"E, 3600 m, moist, open or partly shaded places along streams in meadows, *Rohit Kumar 34810* (PUN 61666) [Fig. 22C].

CARYOPHYLLACEAE

**Silene gangotriana* Pusalkar, D.K.Singh & Lakshmin.

$n = 36$, CHN. India, Uttarakhand, Uttarkashi, Near Gomukh glacier, 30°55'58.85"N, 79°04'22.59"E, 3900 m, sandy places along

river banks, on rock walls and open dry slopes, *Rohit Kumar 34645* (PUN 61603) [Fig. 22D].

FABACEAE

***Astragalus melanostachys* Benth. ex Bunge

$n = 8$, CHN. India, Uttarakhand, Uttarkashi, Near Gomukh glacier, 30°55'44.56"N, 79°04'38.65"E, 3900 m, open dry, sandy or gravely slopes, *Rohit Kumar 34855* (PUN 61713) [Fig. 22E].

Previously, $2n = 12$ was reported for this species (Ashraf & Gohil, 1986, 1988; Kumar & Singhal, 2011).

**Astragalus sanjappae* L.B.Chaudhary & Z.H.Khan

$n = 8$, CHN. India, Uttarakhand, Uttarkashi, Near Gomukh glacier, 30°55'44.56"N, 79°04'38.65"E, 3900 m, open dry, sandy or gravely slopes, *Rohit Kumar 34861* (PUN 61719) [Fig. 22F].

POACEAE

***Agrostis griffithiana* (Hook.f.) Bor

$n = 21$, CHN, India, Himachal Pradesh, Kullu, Kothi, 32°18'52.77"N, 77°11'24.76"E, 2500 m, mountain slopes, *Vandna Kumari 34292* (PUN 61496) [Fig. 22G].

Previously, $2n = 28$ was reported for this species (Mehra & Sood, 1974; Parkash, 1979; Mehra, 1982).

***Arthraxon hispidus* (Thunb.) Makino

$n = 15$, CHN. India, Himachal Pradesh, Kullu, Malana Village, 32°03'45"N, 77°15'38"E, 2652 m, along road sides, among crops in fields, *Vandna Kumari 32475* (PUN 61379) [Fig. 22H].

The species is having several base numbers ($x = 5, 8, 9, 13, 19$). Previous reports for the species are $2n = 10$ (Sindhe & al., 1975), $2n = 18$ (Gosavi & Yadav, 2011), $2n = 36$ (Gould & Soderström, 1970; Pohl & Davidse, 1971; Mehra & Sharma, 1975; Dujardin, 1979), $2n = 38$ (Gill & al., 1980) and $2n = 40$ (Avdulov, 1931; Sindhe, 1967). So, based on $x = 5$, the present gametic count is the first report of hexaploid cytotype for the species.

***Brachypodium sylvaticum* (Huds.) P.Beauv.

$n = 18$, CHN. India, Himachal Pradesh, Kullu, Kothi, 32°18'52.77"N 77°11'24.76"E, 2400 m, along slopes and along roadsides, *Vandna Kumari 34627* (PUN 61358) [Fig. 22I].

Previous reports for the species were $2n = 14, 18, 42$ (Mehra & Sunder, 1969), $2n = 18$ (Tischler, 1934; Larsen, 1960; Mehra & al., 1968; Saxena & Gupta, 1970; Mehra & Remanandan, 1973; Mehra & Sharma, 1975, 1977; Strid & Franzén, 1981; Baltisberger & Leuchtmann, 1991; Dobeš & al., 1997; Lövkvist & Hultgård, 1999; Gupta & al., 2014), $2n = 18, 28, 44, 56$ (Kozuharov & Petrova, 1973) and $2n = 28$ (Mehra & Sood, 1974). The present meiotic count of $2n = 36$ is the first report of $4x$ cytotype for the species.

***Cymbopogon olivieri* (Boiss.) Bor

$n = 20$, CHN. India, Himachal Pradesh, Kullu, Palchan, 32°18'35"N, 77°10'31"E, 2400 m, moist places and along roads, *Vandna Kumari 34201* (PUN 61405) [Fig. 22J].

Previous reports for the species were $2n = 20$ (Gupta, 1969, 1970; Quraish & Faruqi, 1979). The present meiotic count of the species is the first report of $4x$ cytotype for the species.

**Danthonia cachemyriana* Jaub. & Spach

$n = 14$, CHN. India, Himachal Pradesh, Kullu, Jalori Pass, 31°32'14"N, 77°22'26"E, 3223 m, dry rocky slopes, rock crevices, *Vandna Kumari 34710* (PUN 61259) [Fig. 22K].

***Festuca valesiaca* Schleich. ex Gaudin

$n = 28$, CHN. India, Himachal Pradesh, Kullu, Bijali Mahadev, 31°55'30"N, 77°09'01"E, grassy mountain slopes, subalpine meadows, grasslands, roadsides, *Vandna Kumari 34671* (PUN 61320) [Fig. 22L].

Previous reports for the species were $2n = 14$ (Baksay, 1958; Nazarova & Goukasian, 1995; Schreiner, 2000; Probatova & al., 2008), $2n = 28$ (Felföldy, 1947; Mehra & Remanandan, 1973; Mehra

& Sharma, 1977), $2n = 42$ (Gagnieu & Braun, 1959; Chopanov & Yurtsev, 1973; Mizianty & Pawlus, 1982; Koul & Gohil, 1991; Probatova & al., 1991). This is the first report of $8x$ cytotype for the species.

●*Lolium rigidum* Gaudin

$n = 7$, CHN, India, Himachal Pradesh, Kullu, Jari, 31°59'44"N, 77°14'04"E, 1600 m, fields, *Vandna Kumari 34681* (PUN 61330) [Fig. 22M].

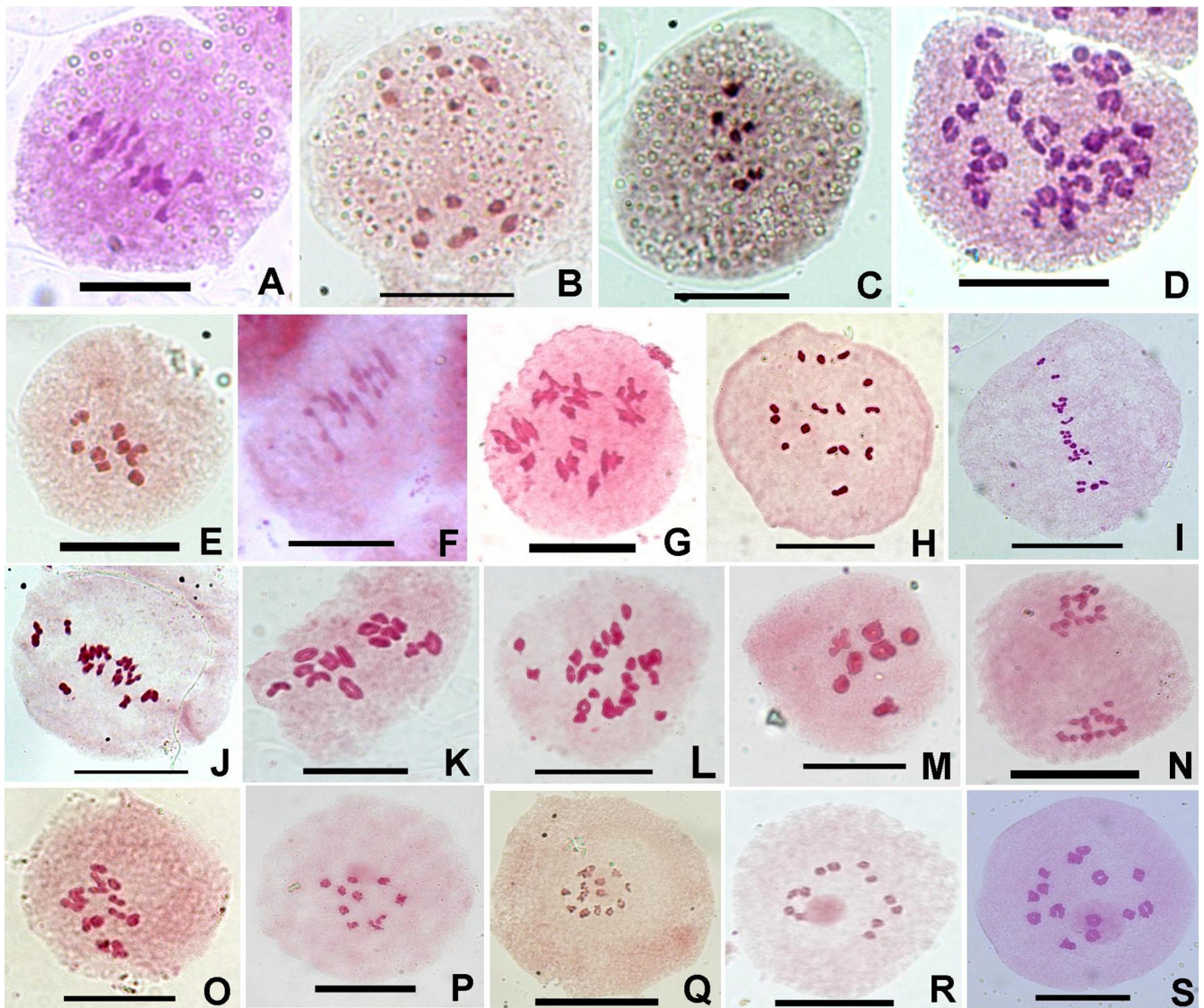


Fig. 22. Meiosis: **A**, *Impatiens badrinathii*, PMC showing 10 bivalents at metaphase I, $n = 10$ (PUN 61649); **B**, *I. devendrae*, PMC showing equal distribution of 7 : 7 chromosomes at anaphase I, $n = 7$ (PUN 60222); **C**, *I. leggei*, PMC showing 7 bivalents at metaphase I, $n = 7$ (PUN 61666); **D**, *Silene gangotriana*, PMC showing 36 bivalents at metaphase I, $n = 36$ (PUN 61603); **E**, *Astragalus melanostachys*, PMC showing 8 bivalents at metaphase I, $n = 8$ (PUN 61713); **F**, *A. sanjappae*, PMC showing 8 bivalents at metaphase I, $n = 8$ (PUN 61719); **G**, *Agrostis griffithiana*, PMC showing 21 bivalents at metaphase I, $n = 21$ (PUN 61496); **H**, *Arthraxon hispidus*, PMC showing 15 bivalents at metaphase I, $n = 15$ (PUN 61379); **I**, *Brachypodium sylvaticum*, PMC showing 18 bivalents at metaphase I, $n = 18$ (PUN 61358); **J**, *Cymbopogon olivieri*, PMC showing 20 bivalents at metaphase I, $n = 20$ (PUN 61405); **K**, *Danthonia cachemyriana*, PMC showing 14 bivalents at metaphase I, $n = 14$ (PUN 61259); **L**, *Festuca valesiaca*, PMC showing 28 bivalents at metaphase I, $n = 28$ (PUN 61320); **M**, *Lolium rigidum*, PMC showing 7 bivalents at metaphase I, $n = 7$ (PUN 61330); **N**, *Microstegium ciliatum*, PMC showing equal distribution of 14 : 14 chromosomes at anaphase I, $n = 14$ (PUN 61414); **O**, *M. nudum*, PMC showing 14 bivalents at metaphase I, $n = 14$ (PUN 61415); **P**, *Muhlenbergia himalayensis*, PMC showing 10 bivalents at diakinesis, $n = 10$ (PUN 61186); **Q**, *Pennisetum lanatum*, PMC showing 14 bivalents at metaphase I, $n = 14$ (PUN 61472); **R**, *Stipa brandisii*, PMC showing 12 bivalents at diakinesis, $n = 12$ (PUN 61242); **S**, *Trisetum micans*, PMC showing 14 bivalents at diakinesis, $n = 14$ (PUN 61277). — Scale bars = 10 μ m.

The diploid chromosome count of $2n = 14$ was reported from other parts of the world (Fernandes & Queirós, 1969; Queirós, 1973; Chopanov & Yurtsev, 1976; Luque & al., 1984; Kozuharov & Petrova, 1991).

*****Microstegium ciliatum* (Trin.) A. Camus**

$n = 14$, CHN, India, Himachal Pradesh, Kullu, Bijali Mahadev, $31^{\circ}55'30''\text{N}$, $77^{\circ}09'01''\text{E}$, open lands and along shady banks, roadsides, *Vandna Kumari 34210* (PUN 61414) [Fig. 22N].

Previous reports for the species were $2n = 20, 60, 70, 80$ (Mehra, 1982), $2n = 40$ (Celarier, 1956; Chen & Hsu, 1962; Khosla & Mehra, 1973; Mehra & Sharma, 1975; Mehra & Kalia, 1976), $2n = 60$ (Reeder & Soderström, 1968; Mehra & Kalia, 1976), $2n = 72$ (Mehra & Sood, 1974). The present meiotic count is the first report of $4x$ cytotype for the species.

*****Microstegium nudum* (Trin.) A. Camus**

$n = 14$, CHN, India, Himachal Pradesh, Kullu, Parvati Valley, Sheela Village, $32^{\circ}00'23''\text{N}$, $77^{\circ}22'44''\text{E}$, 2650 m, moist mountainsides and shady places, *Vandna Kumari 34211* (PUN 61415) [Fig. 22O].

*****Muhlenbergia himalayensis* Hack. ex Hook. f.**

$n = 10$, CHN, India, Himachal Pradesh, Kullu, Parvati Valley, Palchan, $32^{\circ}18'35''\text{N}$, $77^{\circ}10'31''\text{E}$, 2400 m, moist mountainsides and shady places, *Vandna Kumari 34738* (PUN 61186) [Fig. 22P].

The tetraploid chromosome count of $2n = 40$ was reported by several authors for this species (Mehra & Sharma, 1972; Kaur & al., 2011b; Gupta & al., 2014).

*****Pennisetum lanatum* Klotzsch**

$n = 14$, CHN, India, Himachal Pradesh, Kullu, Parvati Valley, Tosh, $32^{\circ}03'08''\text{N}$, $77^{\circ}26'58''\text{E}$, 2400 m, sandy waste places and dry mountain slopes, *Vandna Kumari 34268* (PUN 61472) [Fig. 22Q].

Several chromosome numbers were reported for this species, namely $2n = 18$ (Mehra & Remanandan, 1973), $2n = 36$ (Ahsan & al., 1994), $2n = 36, 42$ (Kaur & al., 2017) and $2n = 42$ (Kaur & al., 2011a). The present meiotic count is the first report of $4x$ cytotype for the species.

****Stipa brandisii* Mez**

$n = 12$, CHN, India, Himachal Pradesh, Kullu, Parvati Valley, Malana Village, $32^{\circ}03'45''\text{N}$, $77^{\circ}15'38''\text{E}$, 2652 m, rocky slopes, open places *Vandna Kumari 34693* (PUN 61242) [Fig. 22R].

****Trisetum micans* (Hook. f.) Bor**

$n = 14$, CHN, India, Himachal Pradesh, Kullu, Jalori Pass, $31^{\circ}32'14''\text{N}$, $77^{\circ}22'26''\text{E}$, 3223 m, along roadsides, grassy slopes *Vandna Kumari 34728* (PUN 61277) [Fig. 22S].

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IAPT chromosome data 31/11

Anna Verena Reutemann, Julio Rubén Daviña, Gabriel Hugo Rua & Ana Isabel Honfi*

* Address for correspondence: ahonfi@gmail.com

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Methods are described in Hojsgaard & al. (2009).

- * First chromosome count for an Argentinian accession of the species.
- First chromosome count for a Paraguayan accession of the species.

POACEAE

The genus *Paspalum* comprises approximately 300 mostly American species (Morrone & al., 2012). Polyploidy is very frequent (80%) in *Paspalum* species, and tetraploidy is the most common ploidy level. In this study, chromosome numbers of *Paspalum* species from Argentina and Paraguay were studied.

Paspalum almum Chase

$2n = 12$, CHN. Argentina, Corrientes, Capital, Santa Ana de los Guácaras, 11 Dec 2013, *Honfi & Daviña 1703* (MNES); Argentina, Corrientes, Ruta Nac. 12 and Arroyo Santa María, 11 Dec 2013, *Honfi & Daviña 1704* (MNES) [Fig. 23A].

The somatic chromosome number of these accessions agrees and confirms the findings by Quarin (1974), Honfi & al. (1990) and later reports. This taxon has an infrequent basic chromosome number in *Paspalum* ($x = 6$), and the diploid cytotype was once considered as a different species.

Paspalum conspersum Schrad.

$2n = 60$, CHN. Argentina, Misiones, General San Martín, Puerto Rico, Paraná River coast, 30 Oct 2000, *Honfi & Daviña 1119* (MNES) [Fig. 23B]; Argentina, Misiones, General San Martín, Puerto Rico Port, 10 Mar 2001, *Honfi & Daviña 1143* (MNES).

Paspalum conspersum is a hexaploid species with $2n = 60$ chromosomes, based on $x = 10$, the most frequent basic chromosome number of the genus. Two cytotypes have been reported for the species, however, $6x$ is the only cytotype found in northern Argentina. Our counts agree with previous reports made by Honfi & al. (1990), Pagliarini & al. (1999), Pozzobon & al. (2007), and references therein.

Paspalum equitans Mez

$2n = 20$, CHN. Argentina, Misiones, Capital, Bañado Zaimán, 23 Oct 2010, *Honfi & Daviña 1447* (MNES).

Here we confirm the chromosome number and ploidy level of *P. equitans*, a very rare species that inhabits swampy areas. Our count confirms previous chromosome reports (Quarin & Norrmann, 1987; Pozzobon & al., 2008) and flow cytometry analyses made by Galdeano & al. (2016).

Paspalum fasciculatum Willd. ex Flügge

$2n = 20$, CHN. Argentina, Formosa, Pilcomayo, road to Puerto Pilcomayo, *Rua 307* (BAA) [Fig. 23C].

Paspalum glaucescens Hack.

$2n = 40$, CHN. Argentina, Misiones, Candelaria, Bonpland, Balneario road, 19 Mar 1991, *Honfi 109* (MNES) [Fig. 23D].

Paspalum ionanthum Chase

• $n = 20$, CHN, Paraguay, Cordillera, Tobatí, 23 Mar 2003 *Honfi & Daviña 1177* (MNES) [Fig. 23E].

This is the first report of the chromosome number of *Paspalum ionanthum* from Paraguay. All individuals studied represent

tetraploids with the basic chromosome number $x = 10$. Meiotic behavior was mainly regular with 20 bivalents in pollen mother cells at diakinesis and metaphase I.

Paspalum maculosum Trin.

$2n = 20, 40$, CHN. Argentina, Misiones, Capital, Villa Lanús, Bañado Zaimán, 17 Nov 2017, *Honfi & Daviña 1445* (MNES) [Fig. 23F].

The studied natural population of *P. maculosum* is formed by coexisting diploid and tetraploid individuals. The same combination of ploidy levels within populations was also found in other *Paspalum* species, for example in *P. buckleyanum* Vasey (sub *P. alcalinum* Mez), *P. denticulatum* Trin. and *P. rufum* Nees ex Steud. (Sartor & al., 2011). This is the first report of a $2x/4x$ mixed population of this taxon.

Paspalum malacophyllum Trin.

$2n = 40$, CHN. Argentina, Salta, Route 9, between Salta and Metán, $24^{\circ}55'57''S$, $64^{\circ}59'11''W$, 27 Sep 2009, *Honfi & Daviña 1448* (MNES) [Fig. 23G].

The studied accession belongs to the tetraploid cytotype, which is the most frequent ploidy level in *P. malacophyllum*. Our report agrees with previous ones (Hojsgaard & al., 2008 and references therein)

Paspalum notatum var. *saurae* Parodi

$2n = 20$, CHN. Argentina, Santa Fe, Ruta 19, Colastiné, $31^{\circ}39'56.3''S$, $60^{\circ}37'09''W$, 28 Mar 2010, *Honfi & Daviña 1453* (MNES).

We report a new locality for the diploid cytotype of this taxon, which has a considerable importance for forage breeding purposes. In general, diploidy is not common in natural populations of this species.

Paspalum notatum Flügge var. *notatum*

$2n = 40$, CHN. Argentina, Misiones, Capital, Santa Inés, 03 Feb 1993, *Honfi 220* (CTES, MNES); Argentina, Santa Fe, Santa Fe, 30 Sep 2006, *Honfi & Daviña 1304* (MNES) [Fig. 23H]; Argentina, Misiones, Capital, Posadas, 03 Apr 2012, *Honfi & Daviña 1603* (MNES).

Tetraploidy is the common ploidy level of this polymorphic species. Our counts agree with several previous reports (Pozzobon & Valls, 1997; D'auelio & al., 2004; Gates & al., 2004 and references therein).

Paspalum pauciciliatum (Parodi) Herter

$2n = 40$, CHN. Argentina, Misiones, Guaraní, El Soberbio, 24 Nov 1993, *Honfi & Daviña 465* (CTES, MNES).

This species is very frequent in the province of Misiones, Argentina, and the chromosome number found here is the only known for the species.

Paspalum paucifolium Swallen

$2n = 40$, CHN. Paraguay, Paraguari, Ruta 1, between Carapeguá and Villa Florida, 24 km before Caapucú, 06 Mar 2006, *Honfi & Daviña 1294* (MNES) [Fig. 23I].

This count confirms a previous report by Burson (1997) on a Paraguayan accession of this species, however it disagrees with Hojsgaard & al. (2009), who report a diploid cytotype from Paraguay. The presence of two or more ploidy levels within a

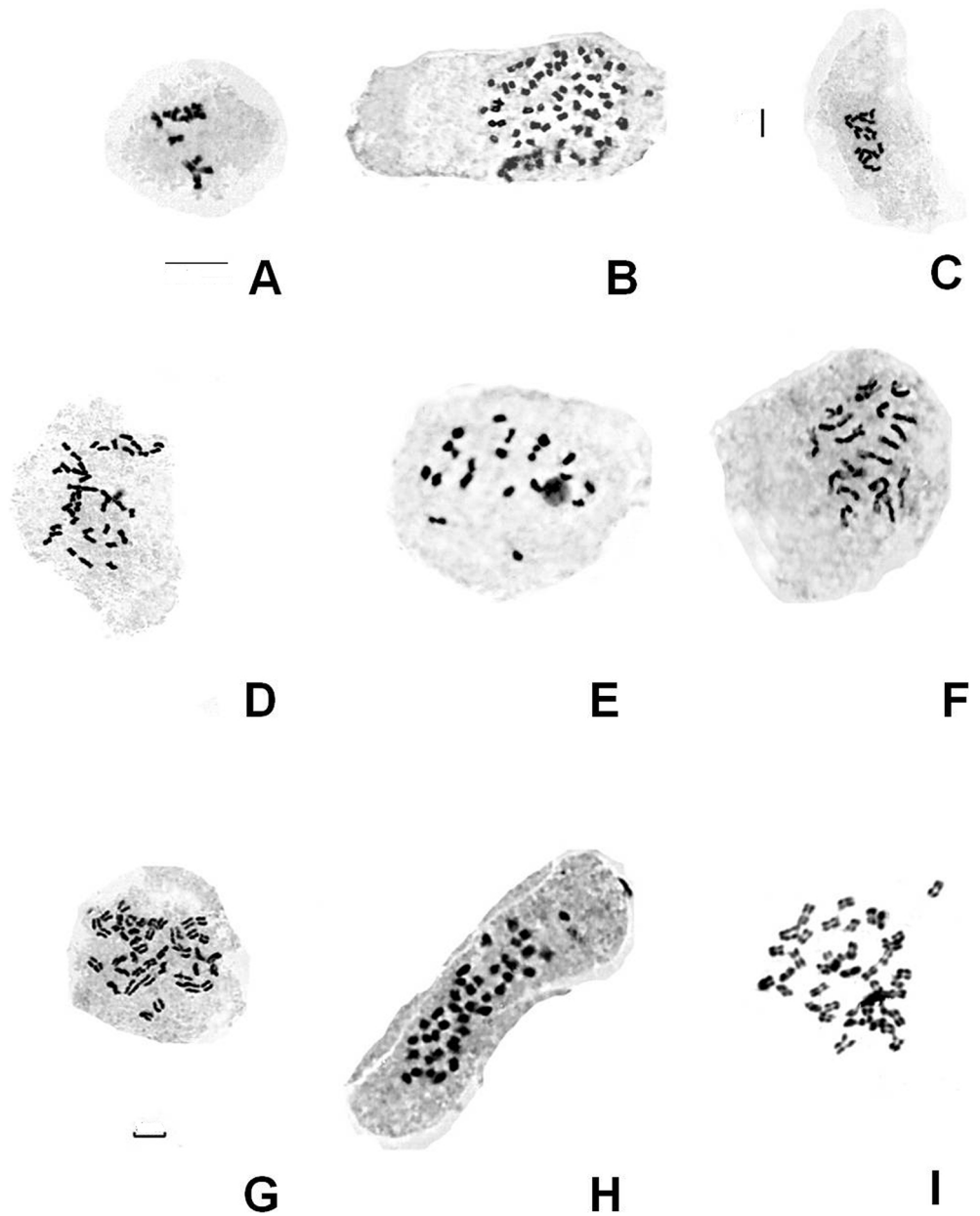


Fig. 23. Mitotic (A–D, F–H) and meiotic chromosomes (E). **A**, *Paspalum alnum*, $2n = 12$ (Honfi & Daviña 1704); **B**, *P. conspersum*, $2n = 60$ (Honfi & Daviña 1119); **C**, *P. fasciculatum*, $2n = 20$ (Rua 307); **D**, *P. glaucescens*, $2n = 40$ (Honfi 109); **E**, *P. ionanthum*, $n = 20$ II (Honfi & Daviña 1177); **F**, *P. maculosum*, $2n = 20$ (Honfi & Daviña 1445); **G**, *P. malacophyllum*, $2n = 40$ (Honfi & Daviña 1448); **H**, *P. notatum* var. *notatum*, $2n = 40$ (Honfi & Daviña 1304); **I**, *P. paucifolium*, $2n = 40$ (Honfi & Daviña 1294). — Scale bars = 5 μm (C & G), 10 μm (A, B, D–F, H, I).

species and also a polyploid series is common in *Paspalum* species.

Paspalum quarinii Morrone & Zuloaga

$2n = 20$, CHN. Argentina, Misiones, Candelaria, Santa Ana, 01 Apr 2003, Honfi & Daviña 1190 (CTES, MNES, SI).

Diploids, triploids and tetraploids were reported for this taxon (Quarin & Norrmann, 1987; Honfi & al., 1990; Pozzobon & al., 2008; Hojsgaard & al., 2009).

Paspalum regnellii Mez

$2n = 40$, CHN. Argentina, Misiones, General San Martín, Puerto Rico Port, 30 Oct 2000, Honfi & Daviña 1118 (MNES).

This species has a narrow geographical distribution, which comprises central and southern Brazil, northeastern Argentina and eastern Paraguay. This species is associated to red soils and disturbed

areas. All Brazilian and Argentinian accessions studied to date are tetraploids based on $x = 10$. Our count agrees with previous chromosome reports (Norrmann, 1981; Honfi & al., 1990; Pagliarini & al., 1998; Hojsgaard & al., 2009).

Almost 50% of *Paspalum* species have a known chromosome number; however, new counts are important for finding diploid materials. Diploid species of *Paspalum* reproduce by sexual means, while polyploids are mainly apomictic. For this reason, findings of new diploid genotypes contribute as a source of variability for forage breeding purposes and *ex situ* germplasm conservation.

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IAPT chromosome data 31/12

André Rodolfo de Oliveira Ribeiro,* Anádría Stéphanie da Silva, Marisa Toniolo Pozzobon, José Francisco Montenegro Valls & Regina Célia de Oliveira

* Address for correspondence: andre_rodolfo@hotmail.com

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* First chromosome count for the species.

** New cytotype for the species.

POACEAE

Mesosetum bifarium (Hack.) Chase

* $2n = 8$, CHN. Brazil, Goiás, Flores de Goiás, low area east of GO-114 hwy., ca. 25 km from BR-020, 14°42'44.8"S, 46°59'55.3"W, 23 Jan 2014, *J.F.M. Valls & al.* 15927 (CEN); Brazil, Goiás, Flores de Goiás, ca. 22 km south of the city's main square, east of GO-114 hwy., 14°38'34.9"S, 46°59'27.9"W, 23 Jan 2014, *J.F.M. Valls & al.* 15933 (CEN) [Fig. 24A].

* $2n = 16$, CHN. Brazil, Goiás, Teresina de Goiás, Poço Encantado, secondary track, bordering the river, 13°52'32"S, 47°15'40"W, 23 May 2011, *A.S. Silva & al.* 235 (UB) [Fig. 24B].

Mesosetum cayennense Steud.

$2n = 20$, CHN. Brazil, Tocantins, Natividade, 4 km of Natividade, on the way to Paraíso waterfall, 11°39'21"S, 47°41'53"W, 25 Mar 2013, *R.C. Oliveira & al.* 2848 (UB) [Fig. 24C].

Mesosetum chaseae Luces

* $2n = 24$, CHN. Brazil, Mato Grosso, Poconé, 16°20'10"S, 56°18'06"W, 25 May 2012, *R.C. Oliveira & al.* 2772 (UB) [Fig. 24D].

Mesosetum compressum Swallen

* $2n = 16$, CHN. Brazil, Pernambuco, Pesqueira, Ororubá mountain range, 08°21'28"S, 36°41'47"W, 09 Aug 2014, *A.S. Silva & D.B. Crisci* 274 (UB) [Fig. 24E].

Mesosetum elythrochaetum (Hack.) Swallen

* $2n = 24$, CHN. Brazil, Goiás, Alto Paraíso de Goiás, São Jorge village, 14°11'05"S, 47°50'53"W, 29 Jan 2012, *R.C. Oliveira & al.* 2722 (UB) [Fig. 24F].

Mesosetum filgueirasii R.C.Oliveira & Valls

* $2n = 16$, CHN. Brazil, Tocantins, Rio da Conceição, Garganta, 11°22'21.99"S, 46°34'01.52"W, 09 May 2016, *J.F.M. Valls & al.* 16273 (paratype: CEN, UB) [Fig. 25C].

Mesosetum longiaristatum Filg.

* $2n = 24$, CHN. Brazil, Goiás, São Domingos, Terra Ronca State Park, 13°39'15"S, 46°19'00"W, 16 May 2013, *J.F.M. Valls & al. 15812* (CEN) [Fig. 24G]; Brazil, Goiás, São Domingos, Terra Ronca State Park, from GO-108 hwy., on the way to Angélica cave, 13°30'52"S, 46°23'19"W, 16 May 2013, *J.F.M. Valls & al. 15841* (CEN).

Mesosetum pappophorum (Nees) Kuhl.

* $2n = 8$, CHN. Brazil, Goiás, Flores de Goiás, 9.15 km west and north of the bridge over the Paranã river, along the dirt road parallel to the river, 14°23'49.9"S, 47°01'43.0"W, 23 Jan 2014, *J.F.M. Valls & al. 15949* (CEN) [Fig. 24H].

Mesosetum sclerochloa (Trin.) Hitchc.

$2n = 16$, CHN. Brazil, Goiás, Flores de Goiás, outskirts of the city, on the dirt road to Alvorada, 14°27'13.8"S, 47°01'43.0"W, 23 Jan 2014, *J.F.M. Valls & al. 15937* (CEN) [Fig. 24I].

METHODS

This study was performed in 12 accessions belonging to nine species of the genus *Mesosetum* Steud.

The chromosome numbers were determined by the analysis of at least 10 meicytes with good chromosome spreading, according to methods described by Ribeiro & al. (2015). Samples were taken from young inflorescences and immersed in fixative solution

(ethanol : acetic acid 3 : 1; v/v) for 24 h at room temperature and stored in 70% ethanol solution at 4°C.

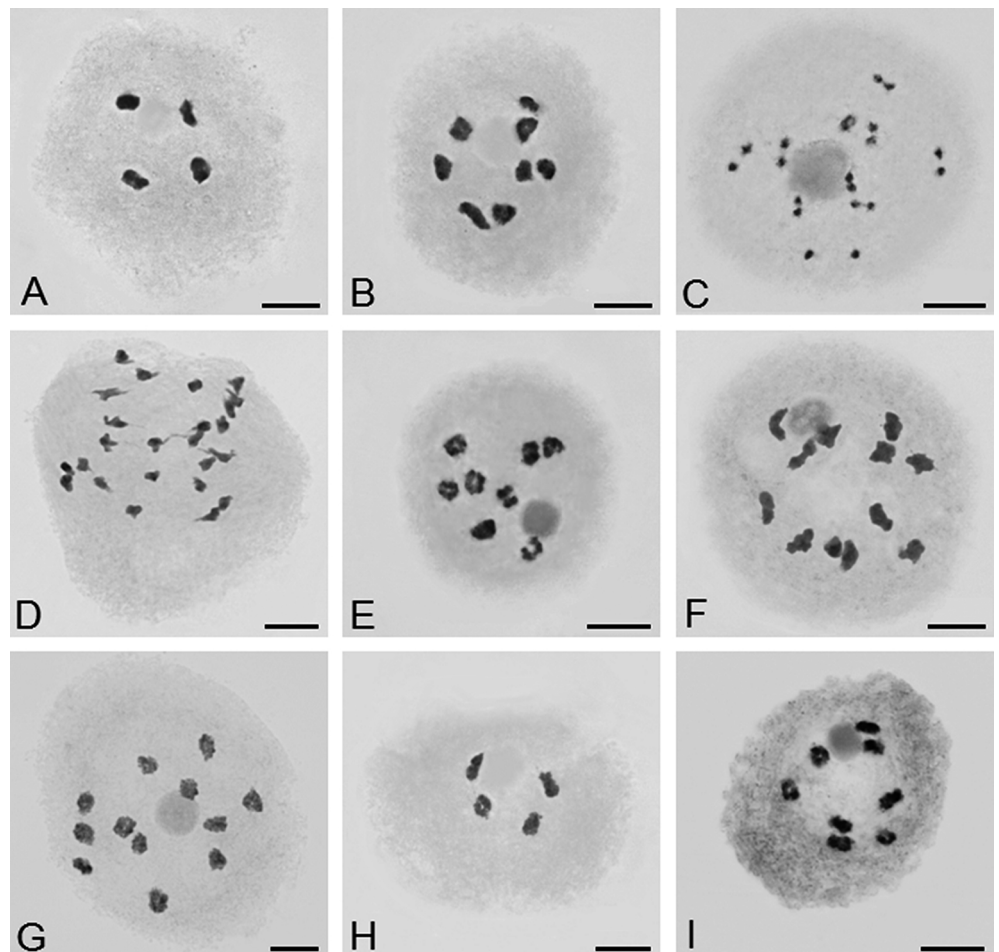
Image capture and chromosome analysis were performed using a Zeiss Axiophot microscope (Zeiss, Germany) and Axiovision Software (v.4.8.2), as well as a Leica DM 750 microscope and software Leica Application Suite (v.4.5).

Mesosetum is a Neotropical genus and contains 27 species distributed from southern Mexico to northeast Argentina, mainly in open habitats (Filgueiras, 1989; Filgueiras & al., 2015; Silva & al., 2016; Oliveira & al., 2019a,b). The centre of diversity of the genus is located in Brazil, where 24 species occur in natural ecosystems (Filgueiras, 1989; Filgueiras & al., 2015; Silva & al., 2016; Oliveira & al., 2019a,b).

According to the phylogenetic tree proposed by Morrone & al. (2012), *Mesosetum* is a member of the tribe Paspaleae ($x = 10$), subtribe Arthropogoninae. Although it belongs to a clade with $x = 10$, and the type of the genus name, *M. cayennense*, shows $2n = 20$ (Sousa & al., 2017), the basic chromosome number $x = 4$ (initially interpreted as $x = 8$) occurs in most of its species (Gould, 1966; Gould & Soderstrom, 1967; Pohl & Davidse, 1971; Davidse & Pohl, 1972; Sede & al., 2010; Silva & al., 2012; Ribeiro & al., 2015; Sousa & al., 2017).

First chromosome counts are reported here for five species: *Mesosetum bifarium* ($2n = 8$, $2n = 16$, Fig. 24A,B), *M. compressum* ($2n = 16$, Fig. 24E), *M. elytrochaetum* ($2n = 24$, Fig. 24F), *M. filgueirasii* ($2n = 16$, Fig. 25C), and *M. pappophorum* ($2n = 8$,

Fig. 24. Meiotic cells of *Mesosetum* species. **A**, *Mesosetum bifarium*, diakinesis with 4 bivalents ($2n = 8$), *J.F.M. Valls & al. 15933*; **B**, *Mesosetum bifarium*, diakinesis with 8 bivalents ($2n = 16$), *A.S. Silva & al. 235*; **C**, *M. cayennense*, diakinesis with 9 bivalents and 2 univalents ($2n = 20$), *R.C. Oliveira & al. 2848*; **D**, *M. chaseae*, anaphase I showing segregation of 12 chromosomes to each pole ($2n = 24$), *R.C. Oliveira & al. 2772*; **E**, *M. compressum*, diakinesis with 8 bivalents ($2n = 16$), *A.S. Silva & D.B. Crisci 274*; **F**, *M. elytrochaetum*, diakinesis with 12 bivalents ($2n = 24$), *R.C. Oliveira & al. 2722*; **G**, *M. longiaristatum*, diakinesis with 12 bivalents ($2n = 24$), *J.F.M. Valls & al. 15812*; **H**, *M. pappophorum*, diakinesis with 4 bivalents ($2n = 8$), *J.F.M. Valls & al. 15949*; **I**, *M. sclerochloa*, diakinesis with 8 bivalents ($2n = 16$), *J.F.M. Valls & al. 15937*. — Scale bars: 10 μ m.



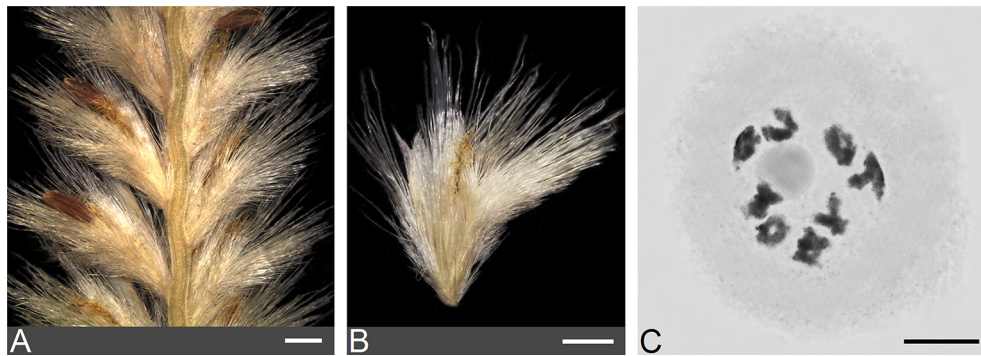


Fig. 25. Inflorescence and meiotic cell of *Mesosetum filgueirasii*. **A**, Inflorescence segment showing the rachis, *J.F.M. Valls & al. 16338* (holotype); **B**, Spikelet, *J.F.M. Valls & al. 16338* (holotype); **C**, Diakinesis with 8 bivalents ($2n = 16$), *J.F.M. Valls & al. 16273* (paratype). — Scale bars: A & B, 1 mm; C, 10 μ m.

Fig. 24H). Two cytotypes were observed in *M. bifarium*, one diploid (Fig. 24A), with $2n = 8$ chromosomes, the other tetraploid (Fig. 24B), with $2n = 16$, documenting once again the occurrence of polyploidy in the genus.

New cytotypes were detected for *M. chaseae* ($2n = 24$) and *M. longiaristatum* ($2n = 24$). In *M. chaseae*, the chromosome number $2n = 16$ was previously reported by Silva & al. (2012) for ten accessions with predominant normal pairing in eight bivalents, while our accession *R.C. Oliveira & al. 2772* ($2n = 24$) showed high frequency of multiple associations at diakinesis, which allowed the exact determination of the chromosome number only at anaphase I (Fig. 24D). In *M. longiaristatum*, the previous results obtained by Ribeiro & al. (2015) showed diploids ($2n = 8$) with normal pairing in four bivalents, while the present results show hexaploids ($2n = 24$) with normal pairing in 12 bivalents (Fig. 24G) for two new accessions of this species.

Our results for two new accessions from the Brazilian states of Tocantins and Goiás confirmed the chromosome numbers of *M. cayennense* ($2n = 20$, Fig. 24C) and *M. sclerochloa* ($2n = 16$, Fig. 24I), previously determined by Sousa & al. (2017).

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