

## IAPT CHROMOSOME DATA

## IAPT chromosome data 40

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

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## IAPT chromosome data 40/1

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All materials CHN; all records from Brazil; all vouchers in HUCS.

#### HYMENOPHYLLACEAE

- Hymenophyllum asplenioides* (Sw.) Sw.,  $n = 36$ ; Rio Grande do Sul, F. Gonzatti & F.P. Abreu 6844.  
*Hymenophyllum fucoides* (Sw.) Sw.,  $n = 56$ ; Paraná, F. Gonzatti, F.P. Abreu & I.A. Bahima 7244.  
*Hymenophyllum magellanicum* (Desv.) Willd. ex Kunze,  $n = 27$ ; Paraná, F. Gonzatti, F.P. Abreu & I.A. Bahima 7250; Rio Grande do Sul, F. Gonzatti & F.P. Abreu 6845; Santa Catarina, F. Gonzatti, F.P. Abreu & P.L. Casa 6996.  
*Hymenophyllum megachilum* C.Presl,  $n = 13$ ; Rio Grande do Sul, F. Gonzatti & F.P. Abreu 6846; Santa Catarina, F. Gonzatti & F.P. Abreu 6992.

#### IAPT chromosome data 40/2

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All materials CHN.

#### ALSTROEMERIACEAE

- Alstroemeria piperata* A.R.Flores & J.M.Watson,  $2n = 16$ ; Chile, Valparaiso Region, G. Rojas 2590 (SGO).  
*Alstroemeria pulchra* var. *maxima* Phil.,  $2n = 16$ ; Chile, Valparaiso Region, N. Villalobos 1911 (CONC).

#### AMARYLLIDACEAE

- Miersia chilensis* Lindl.,  $2n = 22$ ; Chile, O'Higgins Region, I. Escobar 74 (CONC).  
*Phycella australis* Ravenna,  $2n = 16$ ; Chile, Biobio Region, C. Baeza & E. Thielemann 4436 (CONC).  
*Rhodolirium montanum* Phil.,  $2n = 16$ ; Argentina, Córdoba Province, 24 Feb 2023, J. Espejo s.n. (CONC).  
*Zephyranthes maculata* (L'Hér.) Nic.García,  $2n = 18$ ; Chile, Biobio Region, C. Baeza 4439 (CONC).  
*Zephyranthes monantha* (Ravenna) Nic.García,  $2n = 18$ ; Chile, Biobio Region, C. Baeza 4435 (CONC).  
*Zephyranthes* aff. *splendens* (Renjifo) Nic.García,  $2n = 18$ ; Chile, Biobio Region, C. Baeza 4447a (CONC).  
*Zephyranthes splendens* (Renjifo) Nic.García,  $2n = 18$ ; Chile, Biobio Region, C. Baeza 4435 (CONC).

#### ASTERACEAE

- Agoseris coronopifolia* (d'Urv.) K.L.Chambers,  $2n = 18$ ; Chile, Biobio Region, C. Baeza 4463 (CONC); Chile, Metropolitana Region, S. Teillier & al. 8626 (CONC).  
*Chaetanthera microphylla* (Cass.) Hook. & Arn.,  $2n = 24$ ; Chile, Biobio Region, C. Baeza 4439a (CONC).

- Chaetanthera moenchioides* Less.,  $2n = 26$ ; Chile, Biobio Region, C. Baeza 4447 (CONC).  
*Crepis capillaris* (L.) Wallr.,  $2n = 6$ ; Chile, Biobio Region, C. Baeza 4459 (CONC).  
*Crepis pulchra* L.,  $2n = 8$ ; Chile, Biobio Region, S. Teillier & al. 8642 (CONC).  
*Haplopappus bustillosianus* J.Rémy,  $2n = 10$ ; Chile, Biobio Region, S. Teillier & al. 8643 (CONC).  
*Haplopappus glutinosus* Cass.,  $2n = 10$ ; Chile, Biobio Region, S. Teillier & al. 8644 (CONC).  
*Haplopappus paucidentatus* Phil.,  $2n = 10$ ; Chile, Biobio Region, S. Teillier & al. 8645 (CONC).  
*Hieracium glaucifolium* Poepp. ex Froel.,  $2n = 18$ ; Chile, Biobio Region, C. Baeza 4455a (CONC).  
*Hypochaeris acaulis* (J.Rémy) Britton,  $2n = 8$ ;  $2n = 16$ ; Chile, Biobio Region, C. Baeza 4464a (CONC).  
*Hypochaeris apargioides* Hook. & Arn.,  $2n = 8$ ; Chile, Biobio Region, C. Baeza 4444a, 4459a (CONC).  
*Hypochaeris clarionoides* (J.Rémy) Reiche,  $2n = 8$ ; Chile, Metropolitana Region, 15 Jan 2023, J. Espejo s.n. (CONC).  
*Hypochaeris gayana* (DC.) Cabrera,  $2n = 8$ ; Chile, Biobio Region, C. Baeza 4458 (CONC).  
*Hypochaeris glabra* L.,  $2n = 10$ ; Chile, Biobio Region, C. Baeza 4444b (CONC).  
*Hypochaeris radicata* L.,  $2n = 8$ ; Chile, Biobio Region, C. Baeza & al. 4469 (CONC).  
*Hypochaeris scorzonerae* (DC.) F.Muell.,  $2n = 8$ ; Chile, Valparaiso Region, 22 Mar 2023, P. Novoa s.n. (CONC).  
*Lactuca serriola* L.,  $2n = 18$ ; Chile, Biobio Region, C. Baeza 4455 (CONC).  
*Leontodon hirtus* L.,  $2n = 8$ ; Chile, Valparaiso Region, 18 Apr 2022, P. Novoa s.n. (CONC).  
*Leontodon saxatilis* Lam.,  $2n = 8$ ; Chile, Biobio Region, 15 Jan 2021, C. Baeza s.n. (CONC).  
*Noticastrum antucense* Phil.,  $2n = 8$ ; Chile, Biobio Region, C. Baeza 4465 (CONC).  
*Notopappus prunelloides* (Poepp. ex Less.) Klingenb.,  $2n = 12$ ; Chile, Biobio Region, C. Baeza 4460 (CONC).  
*Picris echioides* L.,  $2n = 10$ ; Chile, Biobio Region, C. Baeza 4457 (CONC).

#### FABACEAE

- Lathyrus magellanicus* Lam.,  $2n = 14$ ; Chile, Biobio Region, S. Teillier & al. 8646 (CONC).

#### SALICACEAE

- Azara serrata* Ruiz & Pav. var. *serrata*,  $2n = 18$ ; Chile, Biobio Region, 20 Jan 2022, C. Baeza s.n. (CONC).

#### IAPT chromosome data 40/3

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All materials CHN.

**AMARYLLIDACEAE**

*Narcissus cuatrecasarii* Fern.Casas, M.Laínz & Ruiz Rejón,  $2n = 14$ ; Spain, *Mejías s.n.* (SEV 289807).

**ASPARGACEAE**

*Muscari armeniicum* Leichtlin ex Baker,  $2n = 18$ ; Turkey, *Mejías & Sánchez s.n.* (SEV 256836); Turkey, *Mejías & Sánchez s.n.* (SEV 256837).

**ASTERACEAE**

*Sonchus fragilis* Ball,  $2n = 18$ ; Morocco, *Ajbilou, Arroyo, Marañón & Mejías s.n.* (SEV 215728, SEV 217382).

*Sonchus masguindalii* Pau & Font Quer,  $2n = 18$ ,  $n = 9$ ; Morocco, *Arroyo & Mejías s.n.* (SEV 215733, SEV 217381).

*Sonchus nymanii* Tineo & Guss.,  $2n = 18$ ; Italy, Sicily, *Lim, Mejías & Silva IT27/11* (SEV 284705), *Lim, Mejías & Silva IT22/11* (SEV 284703).

*Sonchus pustulatus* Willk.,  $2n = 18$ ; Morocco, *Mejías s.n.* (SEV 217476, SEV 217476).

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All materials CHN; collectors: *FM* = Farhana Majid, *HK* = Hardeesh Kaur, *PR* = Poonam Rani, *SK* = Satinder Kaur.

**CACTACEAE**

*Mammillaria carnea* Zucc. ex Pfeiff.,  $n = 11$ ; India, Haryana, Panchkula, *HK s.n.* (PUN 39015).

*Mammillaria magnimamma* Haw.,  $n = 11$ ; India, Haryana, Panchkula, *HK s.n.* (PUN 39065); India, Punjab, Jalandhar, *HK s.n.* (PUN 39066).

**COMMELINACEAE**

*Murdannia nudiflora* (L.) Brenan.,  $n = 10 + 0-1B$ ; India, Himachal Pradesh, *PR s.n.* (PUN 63192, PUN 63194, PUN 63195).

*Tradescantia pallida* (Rose) D.R.Hunt.,  $n = 12 + 0-1B$ ; India, Himachal Pradesh, *PR s.n.* (PUN 63468).

**COSTACEAE**

*Costus pictus* D.Don.,  $n = 14$ ; India, Punjab, *PR s.n.* (PUN 63178).

**LAMIACEAE**

*Mentha arvensis* L.,  $n = 12$ ; India, Jammu and Kashmir, *FM s.n.* (PUN 62365).

*Scutellaria galericulata* L.,  $n = 16$ ; India, Jammu and Kashmir, *FM s.n.* (PUN 62395).

*Stachys sericea* Wall.,  $n = 16$ ; India, Jammu and Kashmir, *FM s.n.* (PUN 62405).

**MALVACEAE**

*Sida acuta* Burm.f.,  $n = 14 + 0-1B$ ; India, Himachal Pradesh, *SK s.n.* (PUN 62698, PUN 62699).

**IAPT chromosome data 40/5**

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All materials CHN.

**IRIDACEAE****Subfamily Iridoideae****Tribe Tigridieae**

*Cypella altouruguaya* Chauveau & L.Eggers,  $2n = 14$ ; Brazil, Rio Grande do Sul, *L. Eggers & O. Chauveau 716* (ICN).

*Cypella amplimaculata* Chauveau & L.Eggers,  $2n = 14$ ; Brazil, Rio Grande do Sul, *J.G. Dani & C. Guzati 280* (ICN); Brazil, Rio Grande do Sul, *J.G. Dani, C. Guzati & H.M. Buneker 375* (ICN); Brazil, Santa Catarina, *J.G. Dani, C. Guzati & H.M. Buneker 330* (ICN).

*Cypella discolor* Chauveau & L.Eggers,  $2n = 4x = 28$ ; Brazil, Rio Grande do Sul, *E.M. Stiehl-Alves, F.S. Silveira, J. Focchezatto & L.O. Tacuatiá 94* (ICN).

*Cypella fucata* Ravenna,  $2n = 14$ ; Brazil, Rio Grande do Sul, *J.G. Dani, C. Guzati, L.N. Da Silva & G. Pecoits 318* (ICN); Brazil, Rio Grande do Sul, *J.G. Dani, C. Guzati & H.M. Buneker 374* (ICN); Brazil, Rio Grande do Sul, *J.G. Dani & C. Guzati 386* (ICN).

*Cypella hauthalii* subsp. *minuticristata* Chauveau & L.Eggers,  $2n = 14$ ; Brazil, Rio Grande do Sul, *J.G. Dani, C. Guzati & H.M. Buneker 376* (ICN).

*Cypella herbertii* subsp. *herbertii* Herb.,  $2n = 14$ ; Brazil, Santa Catarina, *J.G. Dani, C. Guzati & H.M. Buneker 364* (ICN).

*Cypella luteogibbosa* Deble,  $2n = 14$ ; Brazil, Rio Grande do Sul, *E.M. Stiehl-Alves, F.S. Silveira, J. Focchezatto & L.O. Tacuatiá 96* (ICN).

*Cypella magnicristata* Deble,  $2n = 14$ ; Brazil, Rio Grande do Sul, *E.M. Stiehl-Alves, F.S. Silveira, J. Focchezatto & L.O. Tacuatiá 98* (ICN).

*Cypella pusilla* (Link & Otto) Benth. & Hook.f. ex B.D.Jacks.,  $2n = 14$ ; Brazil, Rio Grande do Sul, *T. Pastori & O. Chauveau 191* (ICN); Brazil, Rio Grande do Sul, *T. Pastori, M. Chiara-Moço, P.J.S. Silva-Filho, R. Macedo & L. Oliveira 148* (ICN); Brazil, Rio Grande do Sul, *T. Pastori, M. Longhi, C. Forgiarini & L. Nogueira 168* (ICN); Brazil, Rio Grande do Sul, Caçapava do Sul, *L. Eggers & O. Chauveau 914* (ICN).

*Gelasine* aff. *coerulea* (Vell.) Ravenna,  $2n = 14$ ; Brazil, Santa Catarina, 5 Oct 2017, *C. Valus & A. Silvério s.n.* (MBM).

*Gelasine elongata* (Graham) Ravenna,  $2n = 12$ ; Brazil, Rio Grande do Sul, L. Eggers & T.T. Souza-Chies 254 (ICN).  
*Gelasine uruguayensis* Ravenna,  $2n = 14$ ; Brazil, Rio Grande do Sul, L.P. Deble & A.S. de Oliveira-Deble 13578 (ICN).

## IAPT chromosome data 40/6

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All materials CHN; collectors: *EMA* = E.M. Almeida, *EMN* = E.M. Neto, *LPF* = L.P. Felix, *SN* = S. Nascimento; vouchers in EAN (Herbarium Prof. Jayme Coelho de Moraes).

### ARACEAE

- Anthurium andraeanum* Linden ex André,  $2n = 36$ ; Brazil, Paraíba, *LPF* 19523.  
*Anthurium bromelicola* Mayo & L.P. Felix,  $2n = 30$ ; Brazil, Paraíba, *LPF* 19524.  
*Anthurium harleyi* T.A. Pontes & Mayo,  $2n = 30 + 1B$ ; Brazil, Bahia, *LPF* 18315.  
*Anthurium ianthinopodum* (Schott ex Engl.) Nadruz & Mayo,  $2n = 30 + 2B$ ; Brazil, Espírito Santo, *EMA* 1329.  
*Anthurium minarum* Sakur. & Mayo,  $2n = 32$ ; Brazil, Minas Gerais, *LPF* 19200.  
*Anthurium pentaphyllum* (Aubl.) G. Don,  $2n = 30$ ; Brazil, São Paulo, *LPF* 16970.  
*Anthurium petrophilum* K. Krause,  $2n = 30$ ; Brazil, Bahia, *LPF* 18374.  $2n = 30 + 1B$ ; Brazil, Bahia, *E.M. Almeida* 966; Brazil, Paraíba, *SN* 150; Brazil, Pernambuco, *EMN* 49.  $2n = 30 + 2B$ ; Brazil, Bahia, *EMA* 616.  
*Anthurium raimundii* Mayo, Haigh & Nadruz,  $2n = 30 + 1B$ ; Brazil, Pernambuco, *LPF* 17818.  
*Anthurium scandens* (Aubl.) Engl.,  $2n = 48$ ; Brazil, Bahia, *LPF* 18313; Brazil, Bahia, *EMA* 1028; Brazil, Pernambuco, *LPF* 18667; Brazil, Pernambuco, *LPF* 18678.  
*Anthurium sellowianum* Kunth,  $2n = 60$ ; Brazil, São Paulo, *LPF* 16810.  
*Anthurium urvilleanum* Schott,  $2n = 30 + 8B$ ; Brazil, São Paulo, *LPF* 16998.  
*Philodendron acutatum* Schott  $2n = 32$ ; Brazil, Piauí, *EMA* 1099.  $2n = 32 + 1B$ ; Brazil, São Paulo, *LPF* 17003.  
*Syngonium podophyllum* Schott,  $2n = 24$ ; Brazil, Paraíba, *LPF* 13663.

## IAPT chromosome data 40/7

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All materials FCM; collectors: *JIM-C* = J.I. Márquez-Corro, *MS-A* = M. Sanz-Arnal, *PJ-M* = P. Jiménez-Mejías, *PM-S* = P. Muñoz-Schüler, *SM-B* = S. Martín-Bravo.

### AEXTOXICACEAE

*Aextoxicon punctatum* Ruiz & Pav.,  $2C = 2.23 \pm 0.022$  pg,  $CV = 3.60$ ; Chile, Biobío, *JIM-C* & *PM-S* 87JMC23 (UPOS).

### BLECHNACEAE

- Austroblechnum lechleri* (T. Moore) Gasper & V.A.O. Dittrich,  $2C = 23.65 \pm 0.121$  pg,  $CV = 3.22$ ; Chile, Biobío, *PM-S* & *al.* *PMS-243* (CONC, MACB).  
*Blechnum* sp.,  $2C = 17.75 \pm 0.069$  pg,  $CV = 4.03$ ; Chile, Araucanía, *SM-B* & *al.* *46bisSMB23* (CONC, MACB).  
*Parablechnum chilense* (Kaulf.) Gasper & Salino,  $2C = 27.03 \pm 0.188$  pg,  $CV = 4.48$ ; Chile, Ñuble, *SM-B* & *al.* *15SMB23* (CONC, MACB).  $2C = 26.64 \pm 0.084$  pg,  $CV = 4.03$ ; Chile, Los Lagos, *PJ-M* & *al.* *31PJM-CL23* (CONC, MACB).

### BROMELIACEAE

*Puya alpestris* (Poepp.) Gay,  $2C = 1.00 \pm 0.001$  pg,  $CV = 3.99$ ; Chile, Coquimbo, *JIM-C* & *PM-S* 82JMC23 (UPOS).

### CELASTRACEAE

*Maytenus boaria* Molina,  $2C = 2.84 \pm 0.008$  pg,  $CV = 3.22$ ; Chile, Biobío, *JIM-C* & *PM-S* 88JMC23 (UPOS).

### CUNONIACEAE

*Weinmannia trichosperma* Cav.,  $2C = 0.98 \pm 0.031$  pg,  $CV = 7.75$ ; Chile, Biobío, Concepción, *JIM-C* & *PM-S* 93JMC23 (UPOS).

### CUPRESSACEAE

*Fitzroya cupressoides* (Molina) I.M. Johnst.,  $2C = 35.28 \pm 0.141$  pg,  $CV = 4.11$ ; Chile, Los Ríos, *JIM-C* & *PM-S* 83JMC23 (CONC).

### DIOSCOREACEAE

*Dioscorea brachybotrya* Poepp.,  $2C = 1.89 \pm 0.009$  pg,  $CV = 3.77$ ; Chile, Biobío, *PM-S* & *al.* *PMS-241* (CONC, UPOS).

### EUPHORBIACEAE

*Avellanita bustillosii* Phil.,  $2C = 0.52 \pm 0.005$  pg,  $CV = 4.21$ ; Chile, Metropolitana, *MS-A* & *al.* *27MSA-CL23* (CONC, MA).

**FABACEAE**

*Astragalus cruckshanksii* (Hook. & Arn.) Griseb.,  $2C = 2.26 \pm 0.019$  pg, CV = 2.67; Chile, Coquimbo, *PM-S* & *al. PMS-197* (CONC).  
*Sophora cassioides* (Phil.) Sparre,  $2C = 1.80 \pm 0.038$  pg, CV = 3.83; Chile, Biobío, Concepción, *JIM-C* & *PM-S 86JMC23* (UPOS).

**GOMORTEGACEAE**

*Gomortega keule* (Molina) Baill.,  $2C = 2.93 \pm 0.009$  pg, CV = 3.16; Chile, Biobío, *JIM-C* & *al. 79bisJMC23* (UPOS).

**JUNCACEAE**

*Juncus microcephalus* Kunth,  $2C = 1.09 \pm 0.003$  pg, CV = 3.12; Chile, Coquimbo, *PM-S* & *al. PMS-222* (CONC, UPOS).  
*Juncus stipulatus* Nees & Meyen,  $2C = 0.93 \pm 0.015$  pg, CV = 4.22; Chile, Ñuble, *SM-B* & *al. 09SMB23* (CONC, UPOS).  
*Patosia clandestina* (Phil.) Buchenau,  $2C = 1.37 \pm 0.005$  pg, CV = 2.60; Chile, Coquimbo, *PM-S* & *al. PMS-209* (CONC, UPOS).

**LAURACEAE**

*Cryptocarya alba* (Molina) Looser,  $2C = 2.34 \pm 0.021$  pg, CV = 3.52; Chile, Biobío, *JIM-C* & *PM-S 84JMC23* (UPOS).

**MONIMIACEAE**

*Peumus boldus* Molina,  $2C = 5.18 \pm 0.079$  pg, CV = 2.87; Chile, Biobío, *JIM-C* & *PM-S 89JMC23* (UPOS).

**MYRTACEAE**

*Luma apiculata* (DC.) Burret,  $2C = 1.02 \pm 0.017$  pg, CV = 4.27; Chile, Biobío, Concepción, *JIM-C* & *PM-S 90JMC23* (UPOS).

**NOTHOFAGACEAE**

*Nothofagus obliqua* (Mirb.) Oerst.,  $2C = 1.19 \pm 0.001$  pg, CV = 3.52; Chile, Biobío, *JIM-C* & *PM-S 91JMC23* (UPOS).

**PODOCARPACEAE**

*Podocarpus salignus* D. Don,  $2C = 17.78 \pm 0.332$  pg, CV = 4.73; Chile, Biobío, Concepción, *JIM-C* & *PM-S 92JMC23* (UPOS).

**RESTIONACEAE**

*Apodasmia chilensis* (Gay) B.G. Briggs & L.A.S. Johnson,  $2C = 2.09 \pm 0.014$  pg, CV = 4.20; Chile, Los Lagos, *PJ-M* & *al. 32PJM-CL23* (CONC, UPOS).

**RUTACEAE**

*Pitavia punctata* (Ruiz & Pav.) Molina,  $2C = 1.07 \pm 0.006$  pg, CV = 3.92; Chile, Biobío, Concepción, *JIM-C* & *PM-S 85JMC23* (UPOS).

**WINTERACEAE**

*Drimys winteri* J.R. Forst. & G. Forst.,  $2C = 3.90 \pm 0.011$  pg, CV = 3.04; Chile, Biobío, *JIM-C* & *PM-S 94JMC23* (UPOS).

**IAPT chromosome data 40/8**

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

**ANACARDIACEAE**

*Anacardium occidentale* L.,  $2n = 40$ ; Brazil, Pernambuco, *L.P. Felix 17303*.

*Astronium fraxinifolium* Schott,  $2n = 30$ ; Brazil, Paraíba, *L.P. Felix 17114*.

*Mangifera indica* L.,  $2n = 40$ ; Brazil, Paraíba, *J.M.P. Cordeiro 1337*.

*Myracrodruon urundeuva* Allemão,  $2n = 30$ ; Brazil, Pernambuco, *L.P. Felix 17308*.

*Schinopsis brasiliensis* Engl.,  $2n = 28$ ; Brazil, Paraíba, *L.P. Felix 17100*.

*Schinus terebinthifolia* Raddi,  $2n = 28$ ; Brazil, Paraíba, *L.P. Felix 17496*.

**BURSERACEAE**

*Commiphora leptophloeos* (Mart.) J.B. Gillett,  $2n = 26$ ; Brazil, Paraíba, *L.P. Felix 16687*.

**CANNABACEAE**

*Celtis iguanaea* (Jacq.) Sarg.,  $2n = 20$ ; Brazil, Paraíba, *P.C. Gadelha Neto 4160*.

**LENTIBULARIACEAE**

*Utricularia quelchii* N.E. Br.,  $2n = 18$ ; Brazil, Roraima, *L.P. Felix 16222*.

**MALPIGHIACEAE**

*Amorimia septentrionalis* W.R. Anderson,  $2n = 20$ ; Brazil, Paraíba, *J.M.P. Cordeiro 1339*.

**MELIACEAE**

*Azadirachta indica* A. Juss.,  $2n = 28$ ; Brazil, Paraíba, *J.M.P. Cordeiro 1342*.

*Cedrela fissilis* Vell.,  $2n = 54$ ; Brazil, Pernambuco, *L.P. Felix 15988*.  
*Melia azedarach* L.,  $2n = 28$ ; Brazil, Paraíba, *J.M.P. Cordeiro 1368*.

**MYRTACEAE**

*Eugenia zigzag* K. Cout. & Sobral,  $2n = 22$ ; Brazil, Paraíba, *J.M.P. Cordeiro 1544*.

**ORCHIDACEAE**

*Caularthron bicornutum* Raf.,  $2n = 40$ ; Brazil, Amazonas, *L.P. Felix 16336*.

**POLYGONACEAE**

*Triplaris gardneriana* Wedd.,  $2n = 22$ ; Brazil, Paraíba, *L.P. Felix 17561*.

**RUBIACEAE**

*Randia armata* (Sw.) DC.,  $2n = 40$ ; Brazil, Rio Grande do Norte, *R. T. Queiroz 613*.

**RUTACEAE**

*Ruta chalepensis* L.,  $2n = 40$ ; Brazil, Paraíba, *J.M.P. Cordeiro 1406*.



**SAPINDACEAE**

*Filicium decipiens* (Wight & Arn.) Thwaites,  $2n = 32$ ; Brazil, Paraíba, J.M.P. Cordeiro 1407.

*Paullinia pinnata* L.,  $2n = 24$ ; Brazil, Paraíba, L.P. Felix 4777.

*Sapindus saponaria* L.,  $2n = 28$ ; Brazil, Paraíba, L.P. Felix 13946.

*Serjania salzmanniana* Schtdl.,  $2n = 24$ ; Brazil, Paraíba, J.M.P. Cordeiro 1333.

*Talisia esculenta* (A.St.-Hil.) Radlk.,  $2n = 32$ ; Brazil, Paraíba, J.M.P. Cordeiro 1338.

**SIMAROUBACEAE**

*Homalolepis bahiensis* (Moric.) Devecchi & Pirani,  $2n = 32$ ; Brazil, Paraíba, E.M. Almeida 814.

**VERBENACEAE**

*Priva bahiensis* DC. ex Schauer,  $2n = 36$ ; Brazil, Paraíba, J.M.P. Cordeiro 1525.

**IAPT chromosome data 40/9**

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All materials CHN; vouchers in UB.

**POACEAE**

*Anthropogon villosus* Nees,  $2n = 40$ ; Brazil, Distrito Federal, R.C. Oliveira & A.S. Silva 2969; Brazil, Distrito Federal, R.C. Oliveira & A.S. Silva 2971.

*Mesosetum bifarium* (Hack.) Chase,  $2n = 16$ ; Brazil, Goiás, A.R.O. Ribeiro 391.

*Mesosetum cayennense* Steud.,  $2n = 20$ ; Brazil, Goiás, A.R.O. Ribeiro & R.C. Oliveira 362; Brazil, Goiás, P.A. Reis, A.R.O. Ribeiro & J.E.Q. Faria 181.

*Mesosetum chlorostachyum* (Döll) Chase,  $2n = 8$ ; Brazil, Amazonas, R.C. Oliveira & al. 3033.

*Mesosetum elythrochaetum* (Hack.) Swallen,  $2n = 24$ ; Brazil, Goiás, A.R.O. Ribeiro 377.

*Mesosetum exaratum* (Trin.) Chase,  $2n = 26$ ; Brazil, Minas Gerais, A.R.O. Ribeiro & I.A. Conceição 351.

*Mesosetum gibbosum* Renvoize & Filg.,  $2n = 24$ ; Brazil, Bahia, A.R.O. Ribeiro & J.E.Q. Faria 408.

*Spheneria kegelii* (Müll. Hal.) Pilg.,  $2n = 20$ ; Brazil, Amazonas, R.C. Oliveira & al. 3007.

*Tatianyx arnatices* (Trin.) Zuloaga & Soderstr.,  $2n = 60$ ; Brazil, Bahia, A.R.O. Ribeiro & J.E.Q. Faria 411.

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

**EUPHORBIACEAE**

*Croton adamantinus* Müll.Arg.,  $2n = 20$ ; Brazil, Paraíba, L.P. Felix 15870.

*Croton argyrophyllus* Kunth,  $2n = 20$ ; Brazil, Bahia, L.P. Felix 15575; Brazil, Pernambuco, L.P. Felix 15636.

*Croton blanchetianus* Baill.,  $2n = 20$ ; Brazil, Paraíba, A.S. Santos 30.

*Croton campestris* A.St.-Hil.,  $2n = 20$ ; Uruguay, Maldonado, L.P. Felix 16015.

*Croton floribundus* Spreng.,  $2n = 100$ ; Brasil, Paraíba, L.P. Felix 15874; Brazil, Paraíba, L.P. Felix 15879.

*Croton glandulosus* L.,  $2n = 16$ ; Brazil, Paraíba, E.M. Almeida 1581.

*Croton grewioides* Baill.,  $2n = 20$ ; Brazil, Bahia, L.P. Felix 15559; Brazil, Pernambuco, L.P. Felix 15650.

*Croton heliotropifolius* Kunth,  $2n = 20$ ; Brazil, Paraíba, A.S. Santos 02.  $2n = 40$ ; Brazil, Ceará, L.P. Felix 14914.

*Croton hirtus* L'Hér.,  $2n = 16$ ; Brazil, Paraíba, E.M. Almeida 1582.

*Croton jacobinensis* Baill.,  $2n = 20$ ; Brazil, Paraíba, A.S. Santos 03; Brazil, Paraíba, E.M. Almeida 1579.

*Croton limae* A.P.S.Gomes, M.F.Sales & P.E.Berry,  $2n = 100$ ; Brazil, Bahia, L.P. Felix 15554.

*Croton lombardianus* Croizat.,  $2n = 20$ ; Uruguay, Maldonado, L.P. Felix 16030.

*Croton pedicellatus* Kunth,  $2n = 18$ ; Brazil, Paraíba, J.M.P. Cordeiro 1259.

*Croton pulegioides* Baill.,  $2n = 20$ ; Brazil, Bahia, L.P. Felix 15553.  $2n = 22$ ; Brazil, Paraíba, A.S. Santos 19.

*Croton rudolphianus* Müll.Arg.,  $2n = 20$ ; Brazil, Pernambuco, L.P. Felix 15662.

*Croton sellowii* Baill.,  $2n = 20$ ; Brazil, Paraíba, J.M.P. Cordeiro 600.

*Croton tricolor* Klotzsch ex Baill.,  $2n = 20$ ; Brasil, Bahia, L.P. Felix 15557.

*Croton triquetus* Lam.,  $2n = 20$ ; Brazil, Bahia, L.P. Felix 15558.  $2n = 40$ ; Brazil, Paraíba, L.P. Felix 15876.

*Croton urticifolius* Lam.,  $2n = 20$ ; Brasil, Paraíba, A.S. Santos 04.

**IAPT chromosome data 40/11**

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no. 131845/2021-7) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES; Process no. 12002011009P8/2021-03) for fellowships.

All material CHN.

#### FABACEAE

*Aldina heterophylla* Spruce ex Benth.,  $2n = 22$ ; Brazil, Amazonas, *H.C. de Lima 8201* (NYBG).

### IAPT chromosome data 40/12

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#### VOCHYSIACEAE

*Qualea grandiflora* Mart.,  $2n = 22$ ,  $n = 11$ ; *G.L.S. Silveira & al. s.n.* (UFMT 44309, UFMT 44308), *L.A.S. Nogueira & al. s.n.* (UFMT 43790, UFMT 43793, UFMT 43807, UFMT 44307, UFMT 43791).

*Qualea multiflora* Mart.,  $n = 11$ ; *L.A.S. Nogueira & al. s.n.* (UFMT 43806).

*Qualea parviflora* Mart.,  $2n = 22$ ,  $n = 11$ ; *L.A.S. Nogueira & al. s.n.* (UFMT 43792, UFMT 43789), *G.L.S. Silveira & al. s.n.* (UFMT 44042).

*Vochysia cinnamomea* Pohl,  $n = 12$ ; *L.A.S. Nogueira & al. s.n.* (UFMT 43809, UFMT 43808, UFMT 43805).

*Vochysia divergens* Pohl,  $2n = 24$ ; *G.L.S. Silveira & al. s.n.* (UFMT 44043, UFMT 44044, UFMT 44045).

### IAPT chromosome data 40/13

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All materials CHN. Collectors: *CSAM* = A.M. Cristante, E.M. Stiehl-Alves & R.M. Marchioretto; *CSVB* = A.M. Cristante, E.M. Stiehl-Alves, A. Tonetto-Vieira & R. Becker; *ECP* = L. Eggers, O. Chauveau & T. Pastori; *KSAV* = E. Kaltchuk, E.M. Stiehl-Alves & A. Vieira; *SAFS* = E.M. Stiehl-Alves, C. Forgiarini & F.S. Silveira; *SATVCH* = E.M. Stiehl-Alves, A. Tonetto-Vieira, L. Carvalho & I. Hedlund; *SATVCM* = E.M. Stiehl-Alves, A. Tonetto-Vieira, L. Carvalho & A. Mello; *TVEC* = A. Tonetto-Vieira, L. Eggers & O. Chauveau; *TVLNS* = A. Tonetto-Vieira & L. Nogueira. Voucher specimens are deposited in ICN.

#### IRIDACEAE

*Herbertia darwinii* Roitman & J.A.Castillo,  $2n = 14$ ; Brazil, Rio Grande do Sul, *TVLNS 5*.

*Herbertia* aff. *lahue* (Molina) Goldblatt,  $2n = 14$ ; Brazil, Rio Grande do Sul, *CSVB 1*, *CSVB 2*, *CSAM 12*, *KSAV 5*, *KSAV 17*, *SATVCH 137*, *SATVCH 145*, *SATVCH 162*, *SATVCH 171*.

*Herbertia lahue* (Molina) Goldblatt,  $2n = 42$ ; Brazil, Rio Grande do Sul, *KSAV 6*, *SATVCM 105*, *SATVCM 110*, *SATVCM 117*, *SATVCH 172*.  $2n = 56$ ; Brazil, Rio Grande do Sul, *KSAV 3*, *SATVCH 129*, *SATVCH 134*, *SATVCH 148*, *SATVCH 161*, *SATVCM 104*, *SATVCM 111*, *SATVCM 115*, *SATVCM 123*.

*Herbertia pulchella* Sweet,  $2n = 28$ ; Brazil, Rio Grande do Sul, *SATVCH 133*, *SATVCH 143*, *SATVCH 150*, *SATVCH 165*, *TVEC 7*.

*Herbertia* aff. *quareimana* Ravenna,  $2n = 14$ ; Brazil, Rio Grande do Sul, *SAFS 51*, *SATVCM 106*, *SATVCM 109*.

*Herbertia quareimana* Ravenna,  $2n = 28$ ; Brazil, Rio Grande do Sul, *ECP 1103*, *ECP 733A*, *SATVCM 125*, *SATVCM 126*.

## IAPT chromosome data 40 – Extended version

Karol Marhold (ed.),<sup>1,2</sup> Jaromír Kučera (ed.),<sup>1</sup> Fernanda Pessi de Abreu,<sup>3</sup> Diego Alarcón,<sup>4</sup> Erton M. Almeida,<sup>5</sup> William S. Alves,<sup>6</sup> Maria de Fátima de Araújo,<sup>7</sup> Carlos M. Baeza,<sup>8</sup> Isabela Andrade Bahima,<sup>9</sup> Ana Flávia Santos de Brito,<sup>10</sup> Érica Pereira de Campos,<sup>11</sup> Antonio Gabriel Torres Cardoso,<sup>12</sup> Daniela S. Carneiro-Torres,<sup>13</sup> Luana Crestani Carvalho,<sup>3</sup> Marcus A.N. Coelho,<sup>14</sup> Joel M.P. Cordeiro,<sup>15</sup> Pedro Costales-Maestre,<sup>16</sup> Julia Gabriele Dani,<sup>17</sup> Zoila Díaz-Lifante,<sup>16</sup> Jaime Espejo,<sup>8</sup> Leonardo P. Felix,<sup>5</sup> Joana Focchezatto,<sup>3</sup> Pablo García-Moro,<sup>18</sup> Guilherme Pecoits Goldstein,<sup>9</sup> Felipe Gonzatti,<sup>19</sup> Arneet Grewal,<sup>20</sup> Catherine Guzati,<sup>17</sup> Pedro Jiménez-Mejías,<sup>18</sup> Eliane Kaltchuk-Santos,<sup>3</sup> Hardeesh Kaur,<sup>20</sup> Satinder Kaur,<sup>20</sup> Ilia J. Leitch,<sup>21</sup> Talita K.P. Lucena,<sup>6</sup> Farhana Majid,<sup>20</sup> José Ignacio Márquez-Corro,<sup>18,21</sup> Santiago Martín-Bravo,<sup>18</sup> José A. Mejías,<sup>16</sup> Adryene Mota de Menezes,<sup>10</sup> Sahr Mian,<sup>21</sup> Paulo Muñoz-Schüler,<sup>22</sup> Rodrigo Garcia Silva Nascimento,<sup>23</sup> Sarah do Nascimento,<sup>5</sup> Felipe Nollet,<sup>23</sup> Patricio Nova,<sup>24</sup> Regina Célia de Oliveira,<sup>12</sup> Jaime Pellicer,<sup>21,25</sup> Diego N. Penneckamp,<sup>26</sup> Diego Sotero de Barros Pinangé,<sup>27</sup> Poonam Rani,<sup>20</sup> Priscila Alves dos Reis,<sup>21</sup> André Rodolfo de Oliveira Ribeiro,<sup>28</sup> Gloria Rojas,<sup>29</sup> Amanda S. Santos,<sup>5</sup> Angeline M.S. Santos,<sup>23</sup> María Sanz-Arnal,<sup>18</sup> Giulia Melilli Serbin,<sup>27</sup> Anádría Stéphanie da Silva,<sup>30</sup> Rosemere dos Santos Silva,<sup>23</sup> Andressa Dantas da Silveira,<sup>12</sup> Graciele Lurdes Silveira,<sup>31</sup> Gustavo Souza,<sup>32</sup> Tatiana T. Souza-Chies,<sup>17,33</sup> Eudes Maria Stiehl-Alves,<sup>17</sup> Luana Olinda Tacuatia,<sup>3</sup> Lu Tan,<sup>34</sup> Sebastián Teillier,<sup>35</sup> Eitel Thielemann,<sup>8</sup> Caroline Trevelin,<sup>9</sup> Ariane Tonetto Vieira<sup>3</sup> & Larissa Fonseca Andrade Vieira<sup>36</sup>

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## IAPT chromosome data 40/1

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Gustavo Pedroso de Moraes, Guilherme Pecoits Goldstein and Taciiane Schroder Jorge for the support and assistance in the analyses performed.

\* First chromosome count for the taxon.

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

### HYMENOPHYLLACEAE

#### Subfamily Hymenophylloideae

\*\**Hymenophyllum asplenioides* (Sw.) Sw.

$n = 36$ ; CHN. Brazil, Rio Grande do Sul, Cambará do Sul, 29° 10'21"S, 50°01'51"W, 17 Dec 2022, F. Gonzatti & F.P. Abreu 6844 (HUCS) [Fig. 1A,E].

\*\**Hymenophyllum fucooides* (Sw.) Sw.

$n = 56$ ; CHN. Brazil, Paraná, Campina Grande do Sul, 25°15' 16"S, 48°52'50"W, 30 Apr 2023, F. Gonzatti, F.P. Abreu & I.A. Bahima 7244 (HUCS) [Fig. 1B,F].



\**Hymenophyllum magellanicum* (Desv.) Willd. ex Kunze  
 $n = 27$ ; CHN. Brazil, Paraná, Campina Grande do Sul, 25°15' 16"S, 48°52'50"W, 30 Apr 2023, F. Gonzatti, F.P. Abreu & I.A. Bahima 7250 (HUCS); Brazil, Rio Grande do Sul, Cambará do Sul, 29°10'21"S, 50°01'51"W, 17 Dec 2022, F. Gonzatti & F.P. Abreu 6845 (HUCS); Brazil, Santa Catarina, Urupema, 27°55'19"S, 49°51'18"W, 18 Feb 2023, F. Gonzatti, F.P. Abreu & P.L. Casa 6996 (HUCS) [Fig. 1C,G].

\**Hymenophyllum megachilum* C.Presl  
 $n = 13$ ; CHN. Brazil, Rio Grande do Sul, Cambará do Sul, 29° 10'21"S, 50°01'51"W, 17 Dec 2022, F. Gonzatti & F.P. Abreu 6846 (HUCS); Brazil, Santa Catarina, Urupema, 27°55'19"S, 49°51' 18"W, 18 Feb 2023, F. Gonzatti & F.P. Abreu 6992 (HUCS) [Fig. 1D,H].

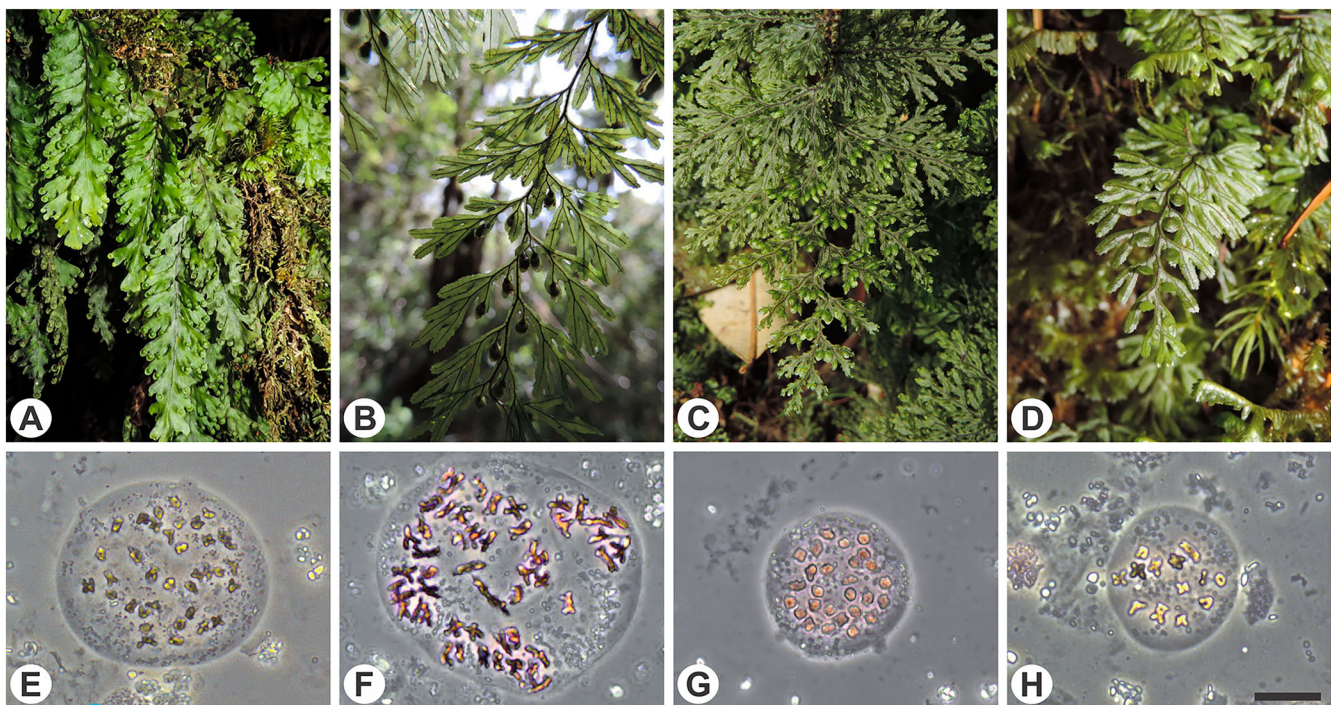
Hymenophyllaceae Mart., the only family in the order Hymenophyllales A.B.Frank, encompasses around 430 fern species distributed in two subfamilies: Trichomanoideae C.Presl, and Hymenophylloideae Burnett (PPG I, 2016). Hymenophylloideae contains the single genus *Hymenophyllum* Sm., which represents the highest species richness with approximately 250 taxa organized within 10 subgenera (Ebihara & al., 2006). Occurrences in South America have previously been registered for *H.* subg. *Globosa* (Prantl) Ebihara & K.Iwats, subg. *Hymenoglossum* (C.Presl) R.M. Tryon & A.F. Tryon, subg. *Hymenophyllum*, subg. *Mecodium* C.Presl ex Copel., subg. *Myrmecostylum* (C.Presl) Ebihara & K.Iwats., and subg. *Sphaerocionium* (C.Presl) C.Chr. (Ebihara & al., 2006). The cytogenetic data available for Hymenophyllaceae focus mainly on the taxonomy and evolution of the family (e.g., Manton, 1950; Braithwaite, 1975; Hennequin & al., 2010; Kim & Kim, 2020).

However, currently available cytogenetic data is mostly restricted to Asia and Oceania. Thus, there is a lack of surveys and specific works, especially with taxa from the New World, leaving unexplored gaps in this field of study.

For this study, chromosome number counting made use of sori fixed in ethanol : glacial acetic acid (3 : 1) for 24 h. The material was prepared for meiotic analysis following the squashing method and staining with 2% carmine-propionic acid (Manton, 1950). Counts of meiotic chromosomes were performed on diakinesis cells. For genome size estimation, the total DNA content was evaluated through flow cytometry according to the methodology described by Doležel & al. (2007). *Pisum sativum* L. 'Ctirad' (Doležel & al., 1998) and *Vicia faba* L. 'Inovec' (Doležel & al., 1992) were included as standards. The cellular lysis Ebihara buffer (Ebihara & al., 2005) was used in sample preparation. The suspension was filtered through a 35 µm nylon mesh filter and nuclei were stained with 100 µl propidium iodide. Nuclear suspensions were analyzed with a FACS Aria BD III flow cytometer, and 2C DNA estimations were calculated with the FlowPloidy R library (Smith & al., 2018).

Chromosome number and genome size data was obtained for seven populations covering four *Hymenophyllum* species (Table 1). This is the first ever chromosome number report for species occurring in South America from *H.* subg. *Myrmecostylum* and subg. *Hymenoglossum*. In terms of Brazilian populations, this study also marks the first cytogenetic data collected for *H.* subg. *Hymenophyllum* in the country. The genome sizes reported in this research represent new information, since none of the analyzed taxa had previously published data.

*Hymenophyllum asplenioides* displays a high phenotypic plasticity and its occurrence extends from Mexico to the southeast of Brazil (Stolze, 1976; Windisch, 2014; Gonzatti & Windisch, 2018).



**Fig. 1.** Species with new chromosome records and their cells in diakinesis. **A & E**, *Hymenophyllum asplenioides* ( $n = 36$ ); **B & F**, *Hymenophyllum fucoides* ( $n = 56$ ); **C & G**, *Hymenophyllum magellanicum* ( $n = 27$ ); **D & H**, *Hymenophyllum megachilum* ( $n = 13$ ). — Scale bar = 20 µm (for E–H).

The chromosome number  $n = 36$  found for *H. asplenioides* is in agreement with previous reports by Walker (1966) for specimens from Jamaica. This species belongs to *H.* subg. *Hymenoglossum*, for which the only cytogenetic data currently known is in fact from *H. asplenioides*. Moreover, this taxon showed a genome size estimate of  $2C = 38.76 \pm 0.16$  pg.

Our chromosome counts are also in agreement with those previously reported for *Hymenophyllum fucoides* for specimens from Jamaica (Walker, 1966). The haploid chromosome number for this species was  $n = 56$ , which is the highest value found for *H.* subg. *Hymenophyllum*. When it comes to genome size, *H. fucoides* had  $2C = 129.49 \pm 0.49$  pg, which is not only the largest genome size for the genus *Hymenophyllum*, but also the largest for the family Hymenophyllaceae. Before this finding, the largest genome size known was that of *Vandenboschia orientalis* (C.Chr.) Ching with  $2C = 64.23$  pg (Fujiwara & al., 2021).

*Hymenophyllum magellanicum* from *H.* subg. *Myrmecostylum* displayed a gametic chromosome number of  $n = 27$ , the first chromosome number record for this species. This is the lowest meiotic number reported for this subgenus, which previously ranged from  $n = 34$  to  $n = 72$  for *H. villosum* Colenso and *H. scabrum* A.Rich., respectively (Brownlie, 1954, 1961; Daellenbach, 1982 in Dawson, 2008). The genome size identified for *H. magellanicum* was  $2C = 36.17 \pm 0.38$  pg. Additionally, no statistically significant difference in genome size was observed between the three populations sampled for this species ( $p$ -value = 0.804).

We present here the first chromosome number counts for *Hymenophyllum megachilum*. This species is endemic to the Brazilian Atlantic Forest and belongs to *H.* subg. *Hymenophyllum*. The haploid number found is  $n = 13$  and the DNA content  $2C = 29.89 \pm 0.47$  pg. Furthermore, no statistically significant difference in genome size was found between the two sampled populations ( $p$ -value = 0.613). Three other species from the corresponding subgenus share the same chromosome number, including *H. tunbrigense* (L.) Sm. *Hymenophyllum megachilum* and *H. tunbrigense* were for a long time considered closely related taxa. However, molecular analyses have recently recognized two independent lineages (Gonzatti & al., 2023). On the other hand, the results obtained by us for *H. fucoides* ( $n = 56$ ) and *H. megachilum* ( $n = 13$ ) reflect on the vast morphological and cytogenetic diversity described for *H.* subg. *Hymenophyllum* (Ebihara & al., 2006).

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**Table 1.** Chromosome number and genome size of *Hymenophyllum* species.

Species	Subgenus	Voucher	$n$	Genome size (2C, pg)
				mean $\pm$ standard deviation
<i>H. asplenioides</i>	<i>Hymenoglossum</i>	F. Gonzatti & F.P. Abreu 6844	36	38.76 $\pm$ 0.16
<i>H. fucoides</i>	<i>Hymenophyllum</i>	F. Gonzatti, F.P. Abreu & I.A. Bahima 7244	56	129.49 $\pm$ 0.49
<i>H. magellanicum</i>	<i>Myrmecostylum</i>	F. Gonzatti & F.P. Abreu 6845	27	36.09 $\pm$ 0.13
		F. Gonzatti, F.P. Abreu & P.L. Casa 6996	27	36.30 $\pm$ 0.24
		F. Gonzatti, F.P. Abreu & I.A. Bahima 7250	27	36.07 $\pm$ 0.80
<i>H. megachilum</i>	<i>Hymenophyllum</i>	F. Gonzatti & F.P. Abreu 6846	13	30.05 $\pm$ 0.32
		F. Gonzatti, F.P. Abreu & P.L. Casa 6992	13	29.78 $\pm$ 0.59



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## IAPT chromosome data 40/2

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\* First chromosome count for the species.

### ALSTROEMERIACEAE

\**Alstroemeria piperata* A.R.Flores & J.M.Watson

$2n = 16$ , CHN. Chile, Valparaíso Region, Los Andes, Ranchillos, 1338 m, 32°55'07"S, 70°42'34"W, 09 Nov 2022, *G. Rojas 2590* (SGO) [Figs. 2A, 3A].

*Alstroemeria piperata* is endemic to Chile, occupies a very narrow range and presents serious conservation problems (Finot & al., 2018).

\**Alstroemeria pulchra* var. *maxima* Phil.

$2n = 16$ , CHN. Chile, Valparaíso Region, Petorca, alongside road to Los Molles, Route 5, at km 190, 57 m, 32°12'14.2"S, 71°30'57"W, 30 Nov 2018, *N. Villalobos 1911* (CONC) [Figs. 2B, 3B].

*Alstroemeria pulchra* constitutes a complex and includes *A. pulchra* subsp. *lavandulacea* Ehrh.Bayer and *A. pulchra* Sims subsp. *pulchra*. The latter two have  $2n = 16$  chromosomes (Baeza & al., 2018).

### AMARYLLIDACEAE

*Miersia chilensis* Lindl.

$2n = 22$ , CHN. Chile, Libertador Gral, Bernardo O'Higgins Region, Cardenal Caro, road between Paredones and San Pedro de Alcántara, 100 m, 34°41'57"S, 71°52'44"W, 03 Nov 2007, *I. Escobar 74* (CONC) [Figs. 2C, 3C].

There is a previous count by Escobar & al. (2012) for the same species, but it indicates a  $2n = 20$ , therefore, this is a new chromosomal number for this species.

*Phycella australis* Ravenna

$2n = 16$ , CHN. Chile, Biobío Region, Concepción, Hualpén, Tumbes Hills, 10 m, 36°38'S, 73°07'W, 08 Sep 2022, *C. Baeza & E. Thielemann 4436* (CONC) [Figs. 2D, 3D].

Previous counts for this species indicate the same chromosomal number (Baeza & al., 2007, 2012).

*Rhodolirium montanum* Phil.

$2n = 16$ , CHN. Argentina, Córdoba Province, Punilla, Valle Hermoso, 2714 m, 35°09'14.52"S, 70°10'36.50"W, 24 Feb 2023, *J. Espejo s.n.* (CONC) [Figs. 2E, 3E].

There is a previous count by Naranjo & Poggio (2000) for this species, also in Argentina (as *Rhodophiala rhodolirion* (Baker) Traub).

*Zephyranthes maculata* (L'Hér.) Nic.García

$2n = 18$ , CHN. Chile, Biobío Region, Yumbel, road to Yumbel railroad station, 115 m, 37°08'00"S, 72°32'00"W, 24 Sep 2022, *C. Baeza 4439* (CONC) [Figs. 2F, 3F].

*Zephyranthes monantha* (Ravenna) Nic.García

$2n = 18$ , CHN. Chile, Biobío Region, Santa Juana, km 38.6, 7 m, 37°16'35"S, 72°57'35.17"W, 12 Jun 2022, *C. Baeza 4435* (CONC) [Figs. 2G, 3G].

*Zephyranthes* aff. *splendens* (Renjifo) Nic.García

$2n = 18$ , CHN. Chile, Biobío Region, Antuco, Laguna del Laja National Park, Las Lagartijas, slopes of Antuco volcano, 2200 m, 37°24'36"S, 71°21'04"W, 07 Dec 2022, *C. Baeza 444a* (CONC) [Figs. 2H, 3H].

*Zephyranthes splendens* (Renjifo) Nic.García

$2n = 18$ , CHN. Chile, Biobío Region, Antuco, km 38, 485 m, 37°16'35"S, 72°57'35.17"W, 12 Jun 2022, *C. Baeza 4435* (CONC) [Figs. 2I, 3I].

*Zephyranthes* is a genus of Amaryllidaceae that has recently been revalidated for Chile (García & al., 2019). All species in this genus have the same chromosome number and there is enormous stability in the chromosome formula. The species indicated in this work have already been counted in other distinct populations, under the generic epithet *Rhodophiala*.

### ASTERACEAE

*Agoseris coronopifolia* (d'Urv.) K.L.Chambers

$2n = 18$ , CHN. Chile, Biobío Region, Antuco, road from Los Barros to Trapa Trapa, 1800 m, 37°31'06"S, 71°02'00"W, 2 Feb 2023, *C. Baeza 4463* (CONC); Chile, Metropolitana Region, Melipilla Province, Curacaví, Maquehua, 300 m, 33°24'S, 71°11'W, 09 Oct 2020, *S. Teillier & al. 8626* (CONC) [Figs. 2J,K, 3J].

There are previous counts of gametophytic material from Argentina of this species (Wulff, 1984; Hunziker & al., 1989). Therefore, this is the first diploid count for this species.

*Chaetanthera microphylla* (Cass.) Hook. & Arn.

$2n = 24$ , CHN. Chile, Biobío Region, Province of Biobío, Yumbel, in front of the water cup, 115 m, 37°08'00"S, 72°32'00"W, 24 Sep 2022, C. Baeza 4439a (CONC) [Figs. 2L, 3K].

*Chaetanthera moenchioides* Less.

$2n = 26$ , CHN. Chile, Biobío Region, Biobío Province, Antuco, Laguna del Laja National Park, road to Las Chilcas, 1082 m, 37°23'14.7"S, 71°24'38.3"W, 17 Dec 2022, C. Baeza 4447 (CONC) [Figs. 2M, 3L].

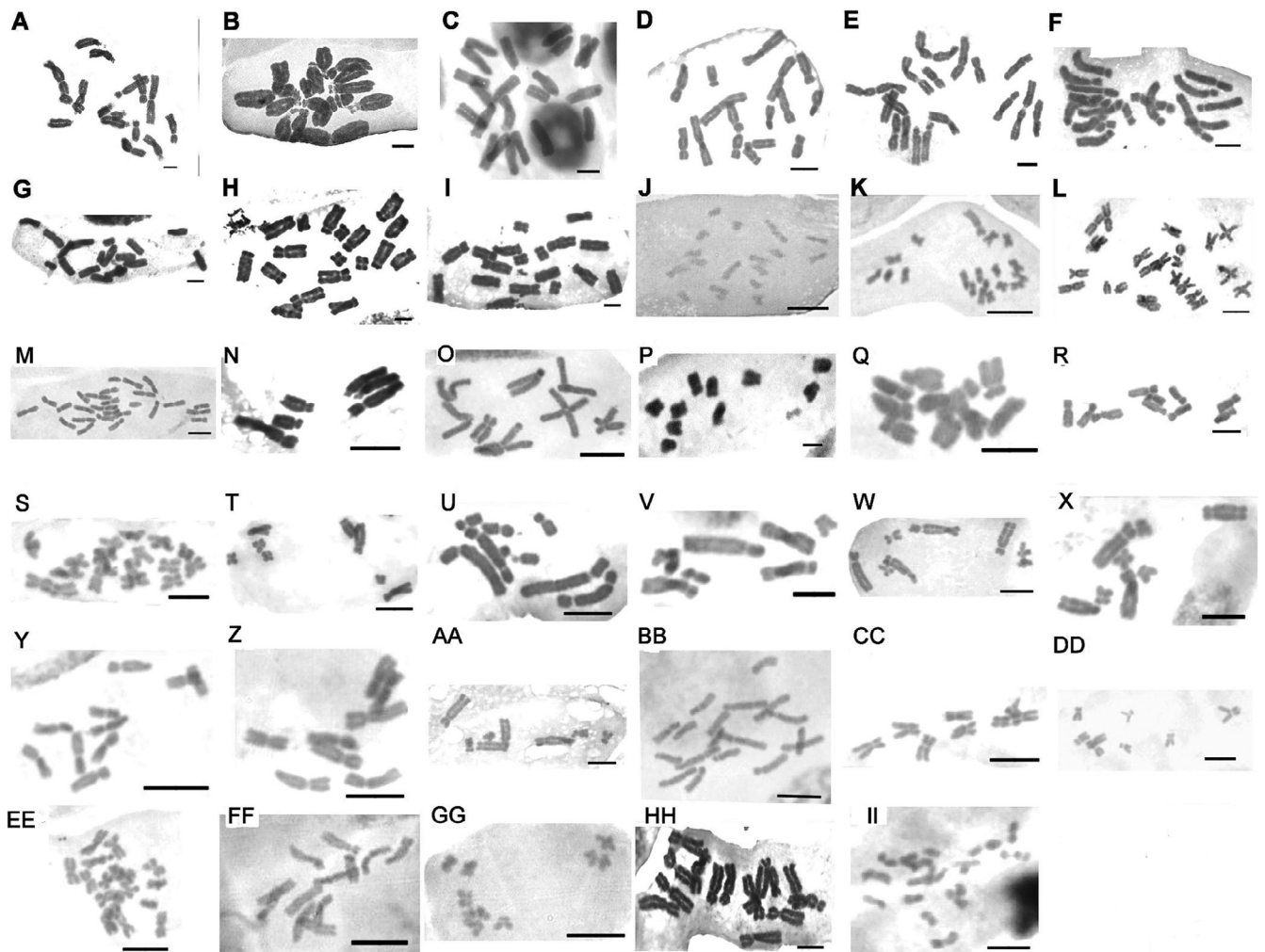
There are previous counts for both species of *Chaetanthera*, but in different populations (Baeza & Schrader, 2005; Baeza & al., 2009; Davies, 2010).

*Crepis capillaris* (L.) Wallr.

$2n = 6$ , CHN. Chile, Biobío Region, Polcura, Quelén Hill, 1260 m, 37°14'47.16"S, 71°42'12.25"W, 19 Jan 2023, C. Baeza 4459 (CONC) [Figs. 2N, 3M].

*Crepis pulchra* L.

$2n = 8$ , CHN. Chile, Biobío Region, Concepción Province, between Janequeo and San Martín Street, 18 m, 36°04'25.2"S, 73°02'04"W, 22 Nov 2022, S. Teillier s.n. 8642 (CONC) [Figs. 2O, 3N].



**Fig. 2.** A, *Alstroemeria piperata*,  $2n = 16$ ; B, *Alstroemeria pulchra* var. *maxima*,  $2n = 16$ ; C, *Miersia chilensis*,  $2n = 22$ ; D, *Phycella australis*,  $2n = 16$ ; E, *Rhodolirium montanum*,  $2n = 16$ ; F, *Zephyranthes maculata*,  $2n = 18$ ; G, *Zephyranthes monantha*,  $2n = 18$ ; H, *Zephyranthes* aff. *splendens*,  $2n = 18$ ; I, *Zephyranthes splendens*,  $2n = 18$ ; J, *Agoseris coronopifolia*,  $2n = 18$ ; K, *Agoseris coronopifolia*,  $2n = 18$ ; L, *Chaetanthera microphylla*,  $2n = 24$ ; M, *Chaetanthera moenchioides*,  $2n = 26$ ; N, *Crepis capillaris*,  $2n = 6$ ; O, *Crepis pulchra*,  $2n = 8$ ; P, *Haplopappus bustillosianus*,  $2n = 10$ ; Q, *Haplopappus glutinosus*,  $2n = 10$ ; R, *Haplopappus paucidentatus*,  $2n = 10$ ; S, *Hieracium glaucifolium*,  $2n = 18$ ; T, *Hypochaeris acaulis*,  $2n = 8$ ; U, *Hypochaeris apargioides*,  $2n = 8$ ; V, *Hypochaeris apargioides*,  $2n = 18$ ; W, *Hypochaeris clarionoides*,  $2n = 8$ ; X, *Hypochaeris gayana*,  $2n = 8$ ; Y, *Hypochaeris glabra*,  $2n = 10$ ; Z, *Hypochaeris radicata*,  $2n = 8$ ; AA, *Hypochaeris scorzonerae*,  $2n = 8$ ; BB, *Lactuca serriola*,  $2n = 18$ ; CC, *Leontodon hirtus*,  $2n = 8$ ; DD, *Leontodon saxatilis*,  $2n = 8$ ; EE, *Noticastrum antucense*,  $2n = 18$ ; FF, *Notopappus prunelloides*,  $2n = 12$ ; GG, *Picris echioides*,  $2n = 10$ ; HH, *Lathyrus magellanicus*,  $2n = 14$ ; II, *Azara serrata* var. *serrata*,  $2n = 18$ . — Scale bars = 5  $\mu$ m.



There are numerous cytological papers on both *Crepis* species, all from Europe. These are the first records of diploid chromosome number in both species in America.

*Haplopappus bustillosianus* J.Rémy

$2n = 10$ , CHN. Chile, Biobío Region, Biobío Province, Polcura, La Posta sector, 570 m, 37°17'02.74"S, 71°42'53.86"W, 19 Jan 2023, S. Teillier & al. 8643 (CONC) [Figs. 2P, 3O].

Goldblatt (1984) reports a haploid chromosome number  $n = 5$ . Therefore, this is the first diploid count for this species.

*Haplopappus glutinosus* Cass.

$2n = 10$ , CHN. Chile, Biobío Region, Biobío Province, Polcura, La Posta sector, 570 m, 37°17'02.74"S, 71°42'53.86"W, 19 Jan 2023, S. Teillier & al. 8644 (CONC) [Figs. 2Q, 3P].

There is a previous count by Baeza & Schrader (2005), of a coastal population.

*Haplopappus paucidentatus* Phil.

$2n = 10$ , CHN. Chile, Biobío Region, Biobío Province, Polcura, La Posta sector, 570 m, 37°17'02.74"S, 71°42'53.86"W, 19 Jan 2023, S. Teillier & al. 8645 (CONC) [Figs. 2R, 3Q].

Carr & al. (1999) reported the haploid number of this species. Therefore, this is the first diploid count of this species.

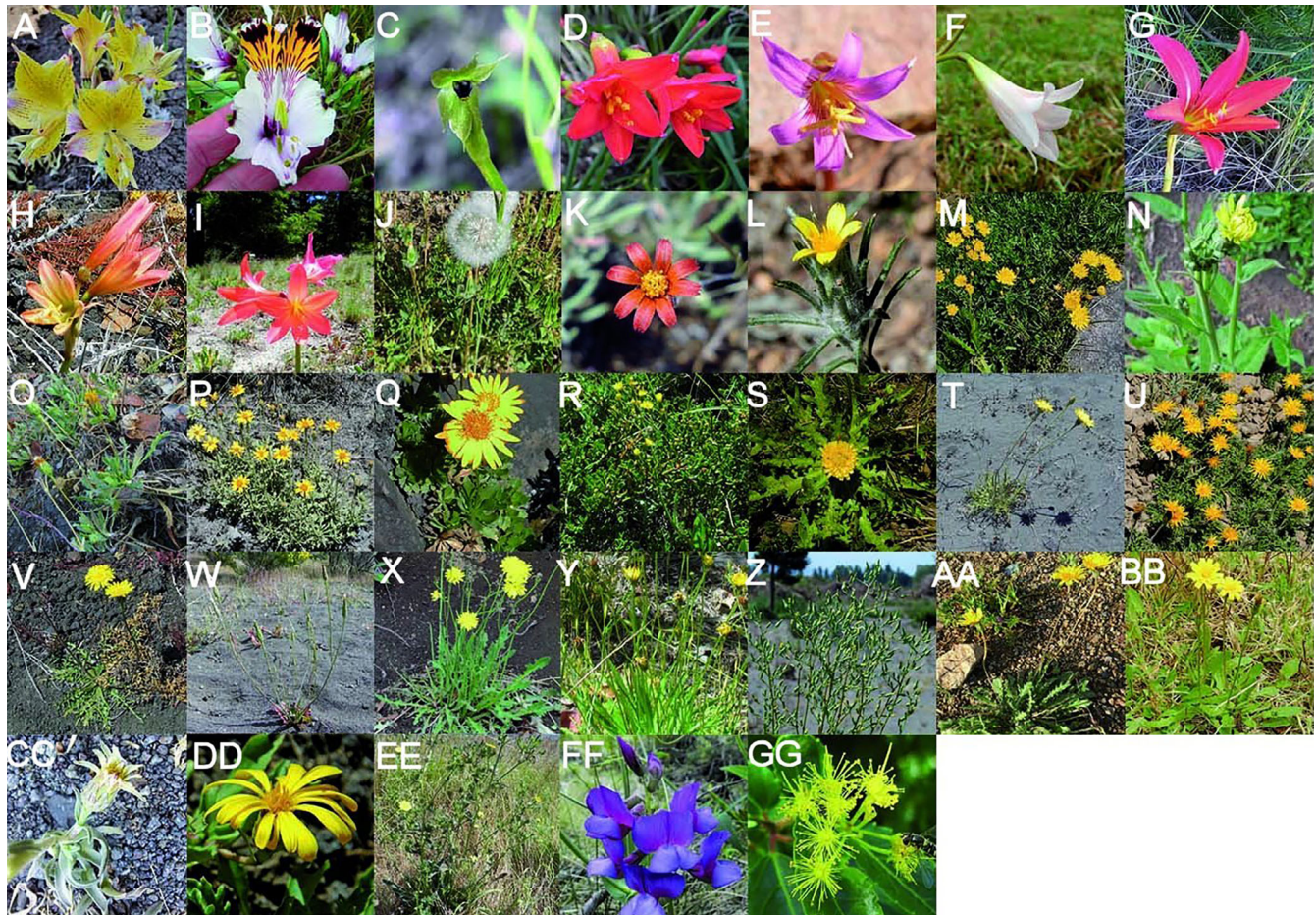
*Hieracium glaucifolium* Poepp. ex Froel.

$2n = 18$ , CHN. Chile, Biobío Region, Ralco, Pangué, Pitron, along the road, 520 m, 37°53'S, 71°31'W, 8 Jan 2023, C. Baeza 4455a (CONC) [Figs. 2S, 3R].

Wulff (1998) reported the same chromosome number for a population from Argentina.

*Hypochaeris acaulis* (J.Rémy) Britton

$2n = 8$ , CHN. Chile, Biobío Region, Antuco, route from Los Barros to Trapa Trapa, 1800 m, 37°31'06"S, 71°02'00"W, 2 Feb 2023, C. Baeza 4464a (CONC) [Figs. 2T, 3S].



**Fig. 3.** A, *Alstroemeria piperata*,  $2n = 16$ ; B, *Alstroemeria pulchra* var. *maxima*,  $2n = 16$ ; C, *Miersia chilensis*,  $2n = 22$ ; D, *Phycella australis*,  $2n = 16$ ; E, *Rhodolirium montanum*,  $2n = 16$ ; F, *Zephyranthes maculata*,  $2n = 18$ ; G, *Zephyranthes monantha*,  $2n = 18$ ; H, *Zephyranthes* aff. *splendens*,  $2n = 18$ ; I, *Zephyranthes splendens*,  $2n = 18$ ; J, *Agoseris coronopifolia*,  $2n = 18$ ; K, *Chaetanthera microphylla*; L, *Chaetanthera moenchioides*; M, *Crepis capillaris*; N, *Crepis pulchra*; O, *Haplopappus bustillosianus*; P, *Haplopappus glutinosus*; Q, *Haplopappus paucidentatus*; R, *Hieracium glaucifolium*; S, *Hypochaeris acaulis*; T, *Hypochaeris apargioides*; U, *Hypochaeris clarionoides*; V, *Hypochaeris gayana*; W, *Hypochaeris glabra*; X, *Hypochaeris radicata*; Y, *Hypochaeris scorzonerae*; Z, *Lactuca serriola*; AA, *Leontodon hirtus*; BB, *Leontodon saxatilis*; CC, *Noticastrum antucense*; DD, *Notopappus prunelloides*; EE, *Picris echioides*; FF, *Lathyrus magellanicus*; GG, *Azara serrata* var. *serrata*.

There are previous accounts of this species by several authors (Wulff, 1998; Weiss & al., 2003; Weiss-Schneeweis & al., 2003; Baeza, 2004).

*Hypochoeris apargioides* Hook. & Arn.

$2n = 8$ , CHN. Chile, Biobío Region, Laja, road from Route 5 to Laja, km 25, next to San Pedro Apostle Chapel Church, 50 m, 37°24' S, 72°64'W, 24 Sep 2022, *C. Baeza 4444a* (CONC); Chile, Coronel, Road to Santa Juana, km 14.7, El Llano, 41 m, 36°58'28.8"S, 73°02'16.5"W, 20 Jan 2023, *C. Baeza 4459a* (CONC) [Figs. 2U, V, 3T].

This species has been profusely analyzed cytologically because it is very abundant and widely distributed in Chile (Baeza & al., 2000, 2001, 2004, 2006; Weiss & al., 2003; Weiss-Schneeweis & al., 2003).

*Hypochoeris clarionoides* (J.Rémy) Reiche

$2n = 8$ , CHN. Chile, Valparaíso Region, Los Andes, El Juncal Park, 2331 m, 33°21'19"S, 70°19'06"W, 15 Jan 2023, *J. Espejo s.n.* (CONC) [Figs. 2W, 3U].

There is a previous count carried out by Weiss & al. (2003) for a population of the Santiago metropolitan region.

\**Hypochoeris gayana* (DC.) Cabrera

$2n = 8$ , CHN. Chile, Biobío Region, Polcura, Quelén Hill, 1260 m, 37°14'47.16"S, 71°42'12.25"W, 19 Jan 2023, *C. Baeza 4458* (CONC) [Figs. 2X, 3V].

This species is very polymorphic and has an Andean distribution, both in Chile and Argentina (Urtubey & al., 2019).

*Hypochoeris glabra* L.

$2n = 10$ , CHN. Chile, Biobío Region, Laja, road from Route 5 to Laja, km 25, next to San Pedro Apostle Chapel Church, 50 m, 37°24' S, 72°46'W, 24 Sep 2022, *C. Baeza 4444b* (CONC) [Figs. 2Y, 3W].

There is a previous count for Chile by Baeza & al. (2004).

*Hypochoeris radicata* L.

$2n = 8$ , CHN. Chile, Biobío Region, Antuco, Laguna del Laja National Park, Los Barros, Andean Plain, 2200 m, 37°24'36"S, 71°21'04"W, 3 Feb 2023, *C. Baeza & al. 4469* (CONC) [Figs. 2Z, 3X].

There are previous counts by Baeza & al. (2000, 2001, 2004).

*Hypochoeris scorzonerae* (DC.) F.Muell.

$2n = 8$ , CHN. Chile, Valparaíso, Balmaceda upload, road to La Pólvara, 40 m, 33°06'36.1"S, 71°37'42.3"W, 22 Mar 2023, *P. Novoa s.n.* (CONC) [Figs. 2AA, 3Y].

There is a previous count by Weiss & al. (2003).

*Lactuca serriola* L.

$2n = 18$ , CHN. Chile, Biobío Region, Ralco, Pangué, Pitron, 520 m, 37°53'S, 71°31'W, 08 Jan 2023, *C. Baeza 4455* (CONC) [Figs. 2BB, 3Z].

There are numerous studies and counts of this species in Europe. This count is the first for a population in America.

*Leontodon hirtus* L.

$2n = 8$ , CHN. Chile, Valparaíso Region, Valparaíso, Plazoleta Unimarc Villa Fundadores, 20 m, 33°07'S, 71°34'W, 18 Apr 2022, *P. Novoa s.n.* (CONC) [Figs. 2CC, 3AA].

There are counts in Europe for this species, but not from America. Miceli & Garbari (1976) and Garbari (1979) reported  $2n = 22$

chromosomes, while Natarajan (1977, 1978) reported  $2n = 24$ . This new  $2n = 8$  count constitutes a ploidy level never reported for this species.

*Leontodon saxatilis* L.

$2n = 8$ , CHN. Chile, Biobío Region, Coronel, road to Santa Juana, km 14.7, El Llano, 41 m, 36°58'28.8"S, 73°02'16.5"W, 15 Jan 2021, *C. Baeza s.n.* (CONC) [Figs. 2DD, 3BB].

For Europe there are counts by Izuzquiza & Feliner (1991) and Vogt & Oberprieler (1993). This is the first count for America.

\**Noticastrum antucense* Phil.

$2n = 18$ , CHN. Chile, Biobío Region, Antuco, Laguna del Laja National Park, Lagunillas, 1000 m, 37°23'42.87"S, 71°25'34.88"W, 2 Feb 2023, *C. Baeza 4465* (CONC) [Figs. 2EE, 3CC].

*Notopappus prunelloides* (Poepp. ex Less.) Klingenb.

$2n = 12$ , CHN. Chile, Biobío Region, Antuco, Los Barros, 1479 m, 37°31'39.2"S, 71°12'38.5"W, 2 Feb 2023, *C. Baeza 4460* (CONC) [Figs. 2FF, 3DD].

There is a previous account by Baeza & Schrader (2005) as *Grindelia prunelloides* (Poepp. ex Less.) A.Dr. Bartoli & Tortosa.

*Picris echioides* L.

$2n = 10$ , CHN. Chile, Biobío Region, Hualpén, Rocuant Island, 2 m, 36°43'38.3"S, 73°04'58"W, 12 Jan 2023, *C. Baeza 4457* (CONC) [Figs. 2GG, 3EE].

There are numerous counts for European and U.S.A. populations. This is the first count for South America.

**FABACEAE**

*Lathyrus magellanicus* Lam.

$2n = 14$ , CHN. Chile, Biobío Region, Polcura, Mañihuales, 1040 m, 37°15'18.07"S, 71°43'36.24"W, 19 Jan 2023, *S. Teillier & al. 8646* (CONC) [Figs. 2HH, 3FF].

There are previous counts by Moore (1981) and Seijo & Fernández (2003).

**SALICACEAE**

\**Azara serrata* Ruiz & Pav. var. *serrata*

$2n = 18$ , CHN. Chile, Biobío Region, Coronel, road to Santa Juana, km 14.7, El Llano, 20 m, 36°58'S, 73°02'W, 20 Jan 2022, *C. Baeza s.n.* (CONC) [Figs. 2II, 3GG].

There are only gametophytic counts ( $n = 9$ ) for *Azara serrata* var. *fernandeziana* (Gay) Reiche (Sanders & al., 1983; Spooner & al., 1987), a taxon endemic to the Juan Fernandez archipelago.

**METHODS**

Rhizome roots or germinated seeds (1–2 cm length) obtained from individuals in each population and held in a greenhouse, were cut and pretreated with a solution of 8-hydroxyquinoline (2 mM) for 24 h at 4°C or in a 1% colchicine solution for 2 h at 18°C in the dark and then 2 h at 4°C. These samples were subsequently fixed with a fresh solution of ethanol/acetic acid (3 : 1) for 24 h. Squash preparations from root tips were made using an acid hydrolysis pretreatment with HCL 0.5 N during 20 min at 42°C. After washing in distilled water, the material was stained with 1% orcein solution. Metaphase chromosome plates were photographed using a Zeiss Axioskop microscope, with an incorporated video camera (Baeza & al., 2018).



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## IAPT chromosome data 40/3

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\* First chromosome count for the species.

\*\* First gametophytic chromosome count for the species.

**AMARYLLIDACEAE**

*Narcissus cuatrecasatii* Fern.Casas, M.Lainz & Ruiz Rejón

$2n = 14$ , CHN. Spain, Mancha Real, Sierra Mágina, climb to Peña del Águila from Mancha Real, 1200 m, 13 Apr 2000, *Mejías s.n.* (SEV 289807) [Fig. 4A].

Like various published chromosome counts (Fernández-Casas & al., 1973; Löve & Kjellqvist, 1973; Ruiz Rejón & Sañudo, 1976), we have also found the somatic number  $2n = 14$  (Fig. 4A). In some of these reports, morphological characteristics of the chromosomes of the karyotype are also provided; however, to the best of our knowledge, a full description of the karyotype including idiogram formula and asymmetry indexes, as we provide here, has not been published before. The apparent chromosome size ranges from 3.51 to 11.05  $\mu\text{m}$ ; therefore, most of the chromosomes have been found to be medium large, but one large pair and one medium-small pair have also been detected. The total length of the haploid chromosome complement is 46.25  $\mu\text{m}$ . According to their morphology, chromosomes can be grouped as follows: 2 metacentric (M; pair 1), 2 metacentric (m; pair 5), 8 submetacentric (sm; pairs 2, 3, 6, 7), two of them possess satellites (pair 7), and 2 submetacentric-subtelocentric (sm-st; pair 4) (Fig. 5A). Thus, the idiogram formula is  $2M + 2m + 6sm + 2sm^{\text{sat}} + 2sm\text{-st}$ . The asymmetry is of type 2B, and asymmetry coefficients are  $A_1 = 0.45$  and  $A_2 = 0.31$ .

**ASPARAGACEAE**

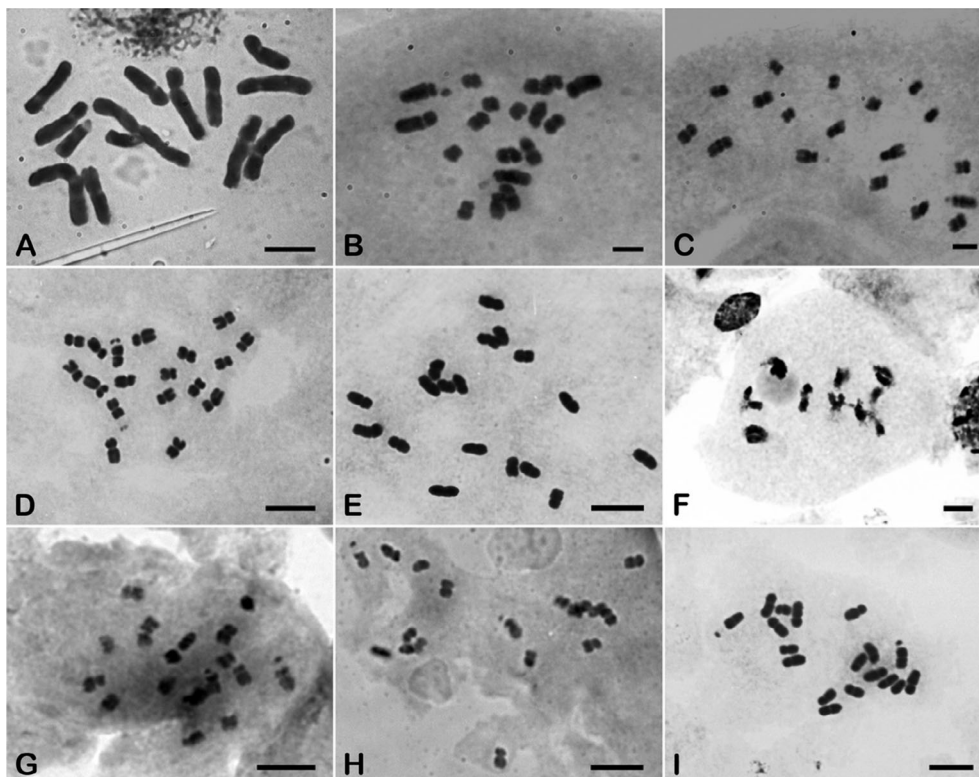
*Muscari armeniacum* Leichtlin ex Baker

$2n = 18$ , CHN. Turkey, Giresum Province, Asarcik Köyü, outskirts of the village, 1700 m,  $40^{\circ}24'39.1''\text{N}$ ,  $38^{\circ}23'45.5''\text{E}$ , 13 May 2010, *Mejías & Sánchez s.n.* (SEV 256836) [Fig. 4B]; Turkey, Bolu Province, Ulaşlar Köyü, pine forest by the road D-100, 1500 m,  $40^{\circ}49'38.4''\text{N}$ ,  $32^{\circ}21'20.7''\text{E}$ , 15 May 2010, *Mejías & Sánchez s.n.* (SEV 256837) [Fig. 4C].

In the two populations studied, the somatic chromosome number was  $2n = 18$  (Fig. 4B,C); therefore, they should be considered

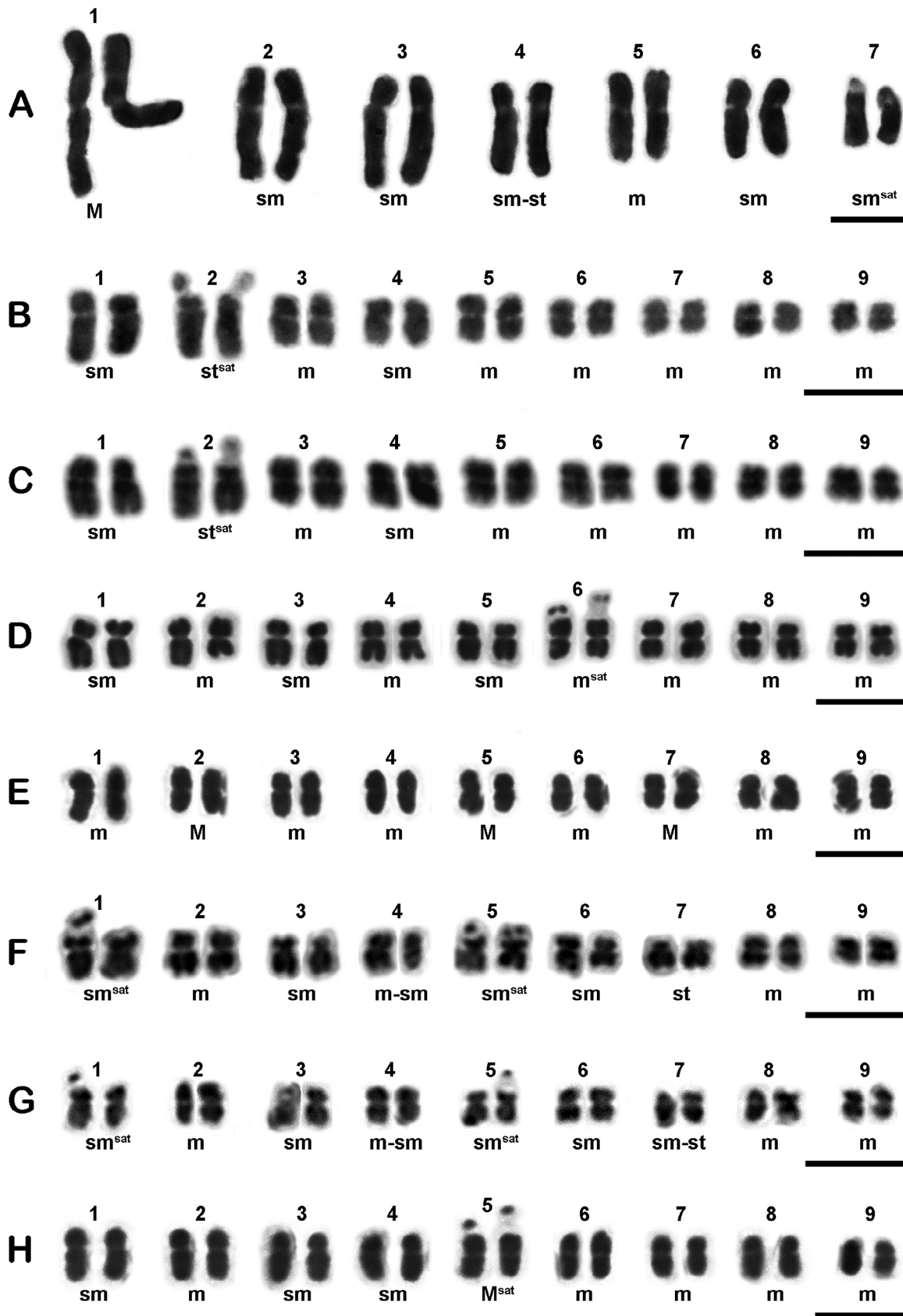
as diploid (Demirci Kayıran & Özhatay, 2017; Kiran & al., 2020). Most chromosome countings have reported the same number for this eastern Mediterranean species (e.g., Karlén, 1984; Özhatay & Johnson, 1996; Demirci Kayıran & Özhatay, 2017; Kiran & al., 2020; Uysal & al., 2022), but some others have indicated the tetraploid number  $2n = 4x = 36$  (e.g., Stuart, 1970; Özhatay & Johnson, 1996), or even the presence of B chromosomes (Özhatay & Johnson, 1996). Other chromosome numbers have been reported for cultivated material (Saito & Matsuzawa, 1969; Rice & al. 2015).

In our observations, the apparent chromosome length of *Muscari armeniacum* ranges from 2.22 to 6.36  $\mu\text{m}$  (total length of haploid chromosome complement: 34.43  $\mu\text{m}$ ) in the population from Asarcik and from 2.21 to 5.32  $\mu\text{m}$  (total length of haploid chromosome complement: 32.08  $\mu\text{m}$ ) in the one from Ulaşlar; thus, they can be classified as medium-short (pairs 3 to 9) and medium-large (pairs 1 and 2). Chromosomes of both populations are grouped as follows: 12 metacentric (m; pairs 3, 5, 6, 7, 8, 9), 4 submetacentric (sm; pairs 1, 4) and 2 subtelocentric (st; pair 2), which are satellite (Fig. 5B,C). The resulting idiogram formula is  $12m + 4sm + 2st^{\text{sat}}$ . The asymmetry found is of type 2B, and asymmetry coefficients are  $A_1 = 0.33$  and  $A_2 = 0.37$  in the first population and  $A_1 = 0.30$  and  $A_2 = 0.27$  in the second one. The present idiogram formula clearly agrees with those reported by Dermicki Karyan & Özhatay (2017) in five diploid populations from Turkey, although they did not detect satellite chromosomes in three of the populations. Kiran & al. (2020) and Uysal & al. (2022) reported the formulas  $2M + 14m + 2sm$  and  $14m + 4sm + 2st^{\text{sat}}$  respectively, which, in general, show karyotypes of somewhat more symmetrical chromosomes. Karlén (1984) also reported a haploid idiogram for diploid *M. armeniacum* showing quite similar chromosome morphology, without subtelocentric chromosomes. As far as we know, no karyomorphological analyses in polyploid material of this species have been published.



**Fig. 4.** Mitotic metaphases and meiotic diakinesis in *Narcissus*, *Muscari* and *Sonchus* species. **A**, *N. cuatrecasatii* ( $2n = 14$ ); **B & C**, *M. armeniacum* ( $2n = 18$ ); **D**, *Sonchus fragilis* ( $2n = 18$ ); **E**, *S. masquindalii* ( $2n = 18$ ); **F**, *S. masquindalii* ( $n = 9$ ); **G & H**, *S. nymanii* ( $2n = 18$ ); **I**, *S. pustulatus* ( $2n = 18$ ). — Scale bars = 5  $\mu\text{m}$ .





**Fig. 5.** Karyograms for *Narcissus*, *Muscari* and *Sonchus* species. **A**, *N. cuatrecasarii* ( $2n = 14$ ); **B & C**, *M. armeniacum* ( $2n = 18$ ); **D**, *Sonchus fragilis* ( $2n = 18$ ); **E**, *S. masguindalii* ( $2n = 18$ ); **F & G** *S. nymanii* ( $2n = 18$ ); **H**, *S. pustulatus* ( $2n = 18$ ). — Scale bars = 5  $\mu\text{m}$ .

Differences in chromosome morphology suggest possible karyotype morphological and genetic shifts due to evolutionary processes in changing habitats (Uysal & al., 2022), but they can also reflect misidentifications of plant material, since *Muscari* subg. *Botryanthus* is a taxonomically complex group, where imprecise species delimitation could lead to incorrect chromosome reports (Karlén, 1984; Kiran & al., 2020; Uysal & al., 2022). According to taxonomic indications of Pinar & al. (2018) and Uysal & al. (2022), the material here studied should be identified as *M. armeniacum*. Leaf features and raceme density of our material clearly correspond to *M. armeniacum*, although bulb scales are not clearly dark brown. Karyotype equivalences with others members from the same group (mostly with *M. aucheri*, *M. botryoides* and *M. vanensis*) have been found while doing this research (Karlén, 1984; Demirci Kayıran & Özhatay, 2017; Kiran & al. 2020; Uysal & al., 2022); in fact, karyological data are sometimes confusing, which reinforces the need of a sound phylogenetic and taxonomic revision of the *Muscari* subg. *Botryanthus* (Dizkirici & al., 2019).

## ASTERACEAE

### *Sonchus fragilis* Ball

$2n = 18$ , CHN. Morocco, Mts. Gorgues (Beni-Hosmar), S Tetouan, limestone cliffs, 475 m, 17 Apr 1999; *Ajbilou, Arroyo, Marañón & Mejías s.n.* (SEV 215728, SEV 217382) [Fig. 4D].

The somatic chromosome number found,  $2n = 18$  (Fig. 4D), agrees with a previous report for plants from another location in the Beni Hosmar range (Vogt & Oberprieler, 2008) of this narrow endemic, which is restricted to the mountainous environments of the city of Tetouan (Morocco). The apparent size of the chromosomes in the karyotype ranged from 1.56 to 2.70  $\mu\text{m}$ ; therefore, they can be classified as small and medium-small ones. The total length of the haploid chromosome complement is 18.82  $\mu\text{m}$ . According to their morphology, chromosomes can be grouped as follows: 12 metacentric (m; pairs 2, 4, 6, 7, 8, 9), two of them being satellite (pair 6), and 6 submetacentric (sm; pairs 1, 3, 5) (Fig. 5D). The idiogram formula is  $10m + 2m^{\text{sat}} + 6sm$ . The asymmetry is of type 2A, and asymmetry coefficients are  $A1 = 0.30$  and  $A2 = 0.15$ . *Sonchus fragilis* chromosome morphology shows great similarity to that of *S. pustulatus* Willk. (as can be checked below), another species of the same section (*S. sect. Pustulati* Boulos), which is consistent with the close relationship between the two taxa (Silva & al., 2015). It is remarkable that *S. fragilis* shows only a single metacentric satellite pair, as does Moroccan *S. pustulatus* material.

### \*\**Sonchus masquindalii* Pau & Font Quer

$2n = 18$ ,  $n = 9$ , CHN. Morocco, Al-Hoceïma, Asfisha beach, limestone fissures by the sea,  $35^{\circ}12'35''\text{N}$ ,  $03^{\circ}54'05''\text{E}$ , 24 Apr 1999, *Arroyo & Mejías s.n.* (SEV 215733, SEV 217381) [Fig. 4E,F].

The somatic chromosome number found,  $2n = 18$  (Fig. 4E), agrees with a previous count in material of this endemic species to the Moroccan Bakkoya Region. As expected, chromosome number of plants from the westernmost part of the distribution area (here) agrees with that from the easternmost part (Vogt & Oberprieler, 2008). We also observed that the chromosomes regularly form 9 bivalents in meiosis ( $n = 9$ ) (Fig. 4F). In somatic metaphase, the apparent size of the chromosomes varies from 1.81 to 3.01  $\mu\text{m}$ ; therefore, they are small and medium-small. The total length of the haploid chromosome complement is 19.81  $\mu\text{m}$ . The chromosomes can be grouped as follows: 6 metacentric (M; pairs 2, 5, 7); and 12 metacentric (m; pairs 1, 3, 4, 6, 8) (Fig. 5E). Therefore, the idiogram formula is  $6M + 12m$ , and no satellite chromosome has been found. The

Alas asymmetry type of the karyotype is 1A, and asymmetry coefficients are  $A1 = 0.20$  and  $A2 = 0.16$ , which denotes a remarkable karyotype symmetry among the species of the genus *Sonchus* L. (Mejías & Andrés, 2004), including those from the same group (*S. sect. Pustulati* Boulos).

### \**Sonchus nymanii* Tineo & Guss.

$2n = 18$ , CHN. Italy, Sicily, road from Alia to Roccapalumba, farmed fields, 520 m,  $37^{\circ}46'31.6''\text{N}$ ,  $13^{\circ}40'12.6''\text{E}$ , 3 May 2011, *Lim, Mejías & Silva IT27/11* (SEV 284705) [Fig. 4G]; Italy, Sicily, Scillato-Caltavuturo, grassland in damp soils next to cultivated fields, 400 m,  $37^{\circ}50'34.4''\text{N}$ ,  $13^{\circ}54'14.3''\text{E}$ , 3 May 2011, *Lim, Mejías & Silva IT22/11* (SEV 284703) [Fig. 4H].

As far as we know, these are the first chromosome counts for *Sonchus nymanii*, a taxon that was described to designate as a separate species an assemblage of rhizomatous perennial plants growing in clay soils of crop areas in central Sicily, with great morphological similarity to the annual/biennial species *S. asper* (L.) Hill (Tineo & Gussone; Gussone, 1844: 860). The two populations studied showed the somatic number  $2n = 18$  (Fig. 4G,H), which agrees with several previous studies in *S. asper* (Mejías & Andrés, 2004; Mejías & al., 2012). The number also reveals the diploid level of the plants, as well as *S. asper*, since  $x = 9$  has repeatedly been proposed as the basic number of genus *Sonchus* (Boulos, 1972; Mejías & Andrés, 2004; Mejías & al., 2018).

The apparent size of the chromosomes in the karyotypes varies between 0.74 and 1.62  $\mu\text{m}$ , with a total length of the haploid chromosome complement of 10.78  $\mu\text{m}$ , in the plants from Alia and between 0.79 and 1.70  $\mu\text{m}$ , with a total length of the haploid chromosome complement of 11.56  $\mu\text{m}$ , in those from Scillato-Caltavuturo, all of them being small. The chromosomes can be grouped as follows: 4 metacentric (m; pairs 2, 9); 4 metacentric-submetacentric (m-sm; pairs 4, 8); 8 submetacentric (sm; pairs 1, 3, 5, 6), 4 of which are satellite (pairs 1, 5); and 2 subtelo-centric (st; pair 7) in the first population (Fig. 5F) or 2 submetacentric-subtelo-centric (sm-st; pair 7) in the second population (Fig. 5G). Therefore, the idiogram formula is  $4m + 4m\text{-sm} + 4sm + 4sm^{\text{sat}} + 2st$  in the population from Alia and  $4m + 4m\text{-sm} + 4sm + 4sm^{\text{sat}} + 2sm\text{-st}$  in the plants from Scillato-Caltavuturo. The asymmetry is of type 2B in both cases, and asymmetry coefficients are  $A1 = 0.44$  and  $A2 = 0.24$  in the first population and  $A1 = 0.41$  and  $A2 = 0.21$  in the second one. All these traits show a clear similarity of the karyotypes studied with those found in common plants of *Sonchus asper* (Mejías & Andrés, 2004).

In both *Sonchus asper* and *S. nymanii*, the achenes are highly compressed, show marginal wings and bear few ribs (commonly three) on each fruit side, a set of features that is currently considered characteristic and privative of *S. asper* s.l., or even of *S. sect. Asperi* Boulos within *Sonchus*. In addition to the life form, the plants studied here differ from typical *S. asper* in the presence of quite showy flower heads, a trait also highlighted in the protologue of *S. nymanii* (“Differt a *S. aspero*; floribus duplo majoribus”; Gussone, 1844: 860) that should make easier the recognition of this species. The diploid condition and the achene morphology allow to reject the possibility that the plants studied represent a race of *S. arvensis* L., a rhizomatous polyploid taxon ( $2n = 36$ ,  $54 [4x, 6x]$ ) that also colonizes moist soils, preferably clay and loam soils, in higher latitudes of the Northern Hemisphere.

Despite all these arguments, the taxonomic identity of *Sonchus nymanii* is not currently recognized and the name is considered a synonym of *S. asper* subsp. *glaucescens* (Jord.) Ball, a putative biennial form in *S. asper* (Boulos, 1973: 165–166) hardly distinguishable from typical annual plants. The material studied here came from

two nearby localities from central Sicily (Italy), one of which (Alia) is cited in the protologue of the name (Gussone, 1844: 860) and, therefore, is a type locality. This fact reinforces the certainty that we are dealing with the plants designated as *S. nymanii* by V. Tineo and G. Gussone.

*Sonchus pustulatus* Willk.

$2n = 18$ , CHN. Morocco, Talambote electrical power station, limestone cliffs and rocks, 150 m, 13 Aug 2003, *Mejías s.n.* (SEV 217476, SEV 217476) [Fig. 4I].

We found the somatic number  $2n = 18$  (Fig. 4I), as reported in several previous publications for both Moroccan and Spanish plants (Stebbins & al., 1953; Talavera & al., 1984; Mejías, 1988; Vogt & Oberprieler, 2008) of this Baetic-Rifean endemic (Silva & al., 2015). However, it is the first time that chromosome morphology has been analyzed in Moroccan material. In our observations, the apparent chromosome size ranges from 1.66 to 2.90  $\mu\text{m}$ ; thus, they can be classified as small and medium-small. The total length of the haploid chromosome complement is 20.95  $\mu\text{m}$ . These chromosomes can be grouped as follows: 2 metacentric (M; pair 5), which are satellite, 10 metacentric (m; pairs 2, 6, 7, 8, 9) and 6 submetacentric (sm; pairs 1, 3, 4) (Fig. 5H). The resulting idiogram formula is:  $2M^{\text{sat}} + 10m + 6\text{sm}$ , the asymmetry is of type 1A, and the karyotype asymmetry coefficients are  $A1 = 0.28$  and  $A2 = 0.15$ . The comparison between our results and those provided by Mejías (1988) clearly shows the high similarities between the karyotypes of plants from both sides of the Mediterranean Sea; the most notable difference between them lies in the number of satellite chromosomes: two pairs in the Iberian plants (one strongly metacentric plus one submetacentric pair), and one metacentric pair in the material from northern Morocco.

## METHODS

Mitotic chromosome numbers were examined in meristematic cells obtained from germinating seeds and grown adult plant root tips. The samples were pretreated with 2 mM 8-hydroxyquinoline for 3.5 h at room temperature, fixed in Farmer's solution, absolute ethanol : glacial acetic acid (3 : 1, v/v), and stored at  $-20^\circ\text{C}$  until use. Pretreated material was stained in alcoholic hydrochloric acid-carminum for 48–72 h under the heat provided by an incandescent lamp. Slide preparations were performed by squashing in a drop of 45% acetic acid. For meiotic chromosomes, flower heads buds of four plants were fixed in Farmer's solution in the wild. The procedure for staining and squashing the material was the same as in the previous case. The best slides were photographed on a ZEISS AXIOPHOT photomicroscope with a ZEISS AXIOCAM 305 camera. Adobe Photoshop CS6 v.13 and ImageJ v.1.53k (Schneider & al., 2012) were used for the elaboration and measure of the karyograms.

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## IAPT chromosome data 40/4

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- \* First report of a new cytotype for the species.
- \*\* First report of B chromosomes for the species.
- # First chromosome count for the species from India.

Cytological investigations were carried out from immature unopened flower buds fixed in Carnoy's fixative (ethylalcohol : chloroform : glacial acetic acid, 6 : 3 : 1, v/v/v) for 24 h at room temperature and then transferred to 70% alcohol and squashed in 1% acetocarmine.

### CACTACEAE

# *Mammillaria carnea* Zucc. ex Pfeiff.

$n = 11$ , CHN. India, Haryana, Panchkula, 30°41'18.78"N, 76°59'39.822"E, 496 m, 30 Mar 2021, *H. Kaur s.n.* (PUN 39015) [Fig. 6A].

An accession collected from Panchkula (Haryana) showed the presence of 11 bivalents at metaphase I [Fig. 6A]. Previously, Remski (1954) recorded the chromosome number of  $n = 11$  from the U.S.A. The chromosome number of  $n = 11$  is published here as the first meiotic report for the species from India.

# *Mammillaria magnimamma* Haw.

$n = 11$ , CHN. India, Haryana, Panchkula, 30°41'18.78"N, 76°59'39.822"E, 496 m, 27 Mar 2021, *H. Kaur s.n.* (PUN 39065); India, Jalandhar, Punjab, 31°17'31.23"N, 75°34'05.008"E, 233 m, 20 Apr 2022, *H. Kaur s.n.* (PUN 39066) [Fig. 6B,C].

Two accessions collected from Panchkula (Haryana) and Jalandhar (Punjab) showed the presence of 11 bivalents at diakinesis and metaphase I [Fig. 6B,C]. The present chromosome count is in agreement with the previous reports of Katagiri (1953) and Remski (1954) from Japan and the U.S.A., respectively.

### COMMELINACEAE

\*\**Murdannia nudiflora* (L.) Brenan

$n = 10 + 0-1B$ , CHN. India, Himachal Pradesh, Sirmaur, 31°49'02.136"N, 77°18'09.936"E, 1552 m, 8 Jul 2018, *P. Rani s.n.* (PUN 63192); India, Himachal Pradesh, Palampur, 32°06'34.214"N, 76°32'08.880"E, 1472 m, 11 Sep 2018, *P. Rani s.n.* (PUN 63194); India, Himachal Pradesh, Chamunda, 31°49'02.136"N, 77°18'09.936"E, 1662 m, 12 Sep, 2018, *P. Rani s.n.* (PUN 63195) [Fig. 6D,E].

Three accessions collected from different localities (Sirmaur, Palampur, Chamunda) of Himachal Pradesh showed the presence of B-chromosome along with 10 bivalents at diakinesis ( $n = 10 + 1B$ ) [Fig. 6D,E]. Previously, there were no reports of B chromosome for this species.

\*\**Tradescantia pallida* (Rose) D.R.Hunt.

$n = 12 + 0-1B$ , CHN. India, Himachal Pradesh, Nadaun, 31°41'11.0364"N, 76°31'17.04"E, 555 m, 20 Sep 2019, *P. Rani s.n.* (PUN 63468) [Fig. 6F].

An accession collected from Nadaun (Himachal Pradesh) revealed the presence of a B chromosome along with 12 bivalents at anaphase I [Fig. 6F]. This is the first record of a B chromosome for this species.

### COSTACEAE

\**Costus pictus* D.Don

$n = 14$ , CHN. India, Punjab, Patiala, 30°21'36.072"N, 76°27'06.3612"E, 256 m, 4 Apr 2019, *P. Rani s.n.* (PUN 63178) [Fig. 6G,H].

The presence of small-sized 14 bivalents in PMCs was observed in metaphase I and anaphase I, illustrating that the plant bears a gametic chromosome number of  $n = 14$  [Fig. 6G,H]. Earlier, Venkatasubban (1946) reported  $2n = 36$  as the chromosome number based on specimens from Sri Lanka. Later, Vovides & Lascurain (1995) reported the chromosome number of  $2n = 18$  from specimens from southern Veracruz, Mexico. The present report presents a newly detected dysploid cytotype for *Costus pictus* with a chromosome number of  $2n = 4x = 28$ . The present finding is the first report of a meiotic chromosome count for the species.

### LAMIACEAE

# *Mentha arvensis* L.

$n = 12$ , CHN. India, Jammu and Kashmir, Ganderbal, 34°17'40.7184"N, 75°13'00.8112"E, 2039 m, 22 Jul 2017, *F. Majid s.n.* (PUN 62365) [Fig. 6I,J].

An accession collected from Ganderbal (Jammu and Kashmir) showed 12 bivalents at diakinesis and metaphase I, illustrating that the plant has a gametic chromosome number of  $n = 12$  [Fig. 6I,J]. Previously, the chromosome number of  $n = 12$  was recorded by Ouweneel (1968) from the Netherlands. The present finding is the first report of chromosome count from India for this species.

# *Scutellaria galericulata* L.

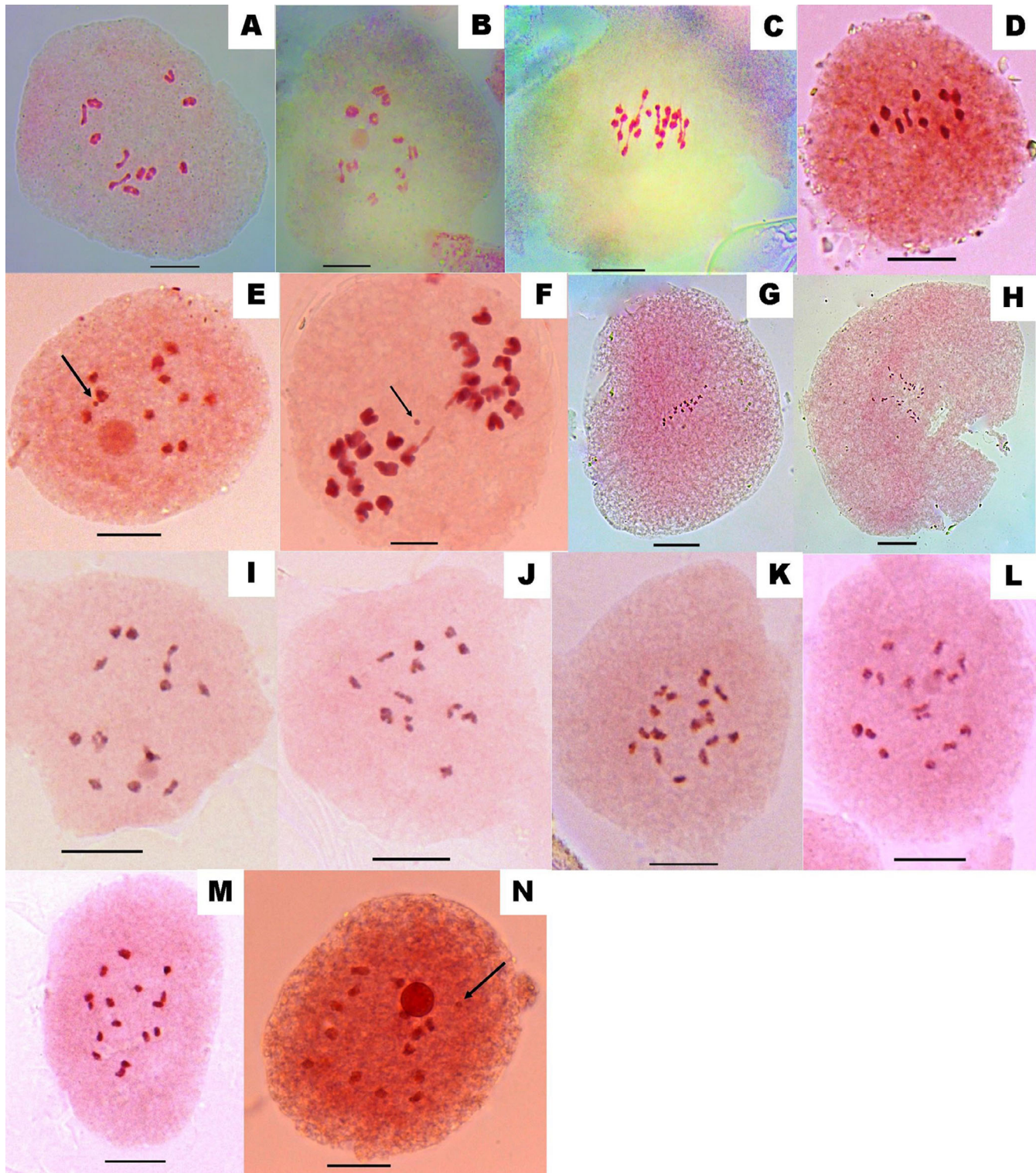
$n = 16$ , CHN. India, Jammu and Kashmir, Baramulla, 34°18'28.5228"N, 74°14'21.0048"E, 1859 m, 18 Aug 2017, *F. Majid s.n.* (PUN 62395) [Fig. 6K].

An accession collected from Baramulla (Jammu and Kashmir) revealed the presence 16 bivalents at metaphase I [Fig. 6K]. This is the first report of chromosome number for this species from India. The chromosome number of  $n = 16$  was been previously reported by Gadella & Kliphuis (1967; Netherlands), Morton (1973; Great Britain), Pogan & al. (1980; Poland), Váchová & Feráková (1980), Gill (1981; Canada) and Dmitrieva (2000; Belarus).

\**Stachys sericea* Wall.

$n = 16$ , CHN. India, Jammu and Kashmir, Ganderbal, 34°18'02.7468"N, 74°14'21.0048"E, 2610 m, 22 Jul 2017, *F. Majid s.n.* (PUN 62405) [Fig. 6L,M].





**Fig. 6.** A, *Mammillaria carnea*: PMC showing 11 bivalents at metaphase-I; B & C, *Mammillaria magnimamma*: B, PMC showing 11 bivalents at diakinesis; C, PMC showing 11 bivalents at metaphase-I; D & E, *Murdannia nudiflora*, male meiotic course: D, PMC showing 10 bivalents at diakinesis; E, PMC showing small-sized B chromosome at diakinesis (arrow); F, *Tradescantia pallida*, PMC showing B chromosome at anaphase-I (arrow); G & H, *Costus pictus*: G, PMC showing 14 bivalents at metaphase-I; H, PMC showing 14 bivalents at anaphase-I; I & J, *Mentha arvensis*: I, Meicyte showing 12 bivalents at diakinesis; J, PMC showing 12 bivalents at metaphase-I; K, *Scutellaria galericulata*, PMC showing 16 bivalents at metaphase-I; L & M, *Stachys sericea*: L, PMC showing 16 bivalents at diakinesis; M, PMC showing 16 bivalents at metaphase-I; N, *Sida acuta*, PMC showing small-sized B chromosome at diakinesis (arrow). — Scale bars = 10  $\mu$ m.

An accession collected from Baramulla (Jammu and Kashmir) revealed the presence of 16 bivalents at diakinesis and metaphase I [Fig. 6L,M]. The chromosome number of  $n = 16$  is reported here as a new cytotype for the species. However, the chromosome number of  $n = 15$  has been reported by Gill (1970, 1984), Saggoo (1983), Saggoo & Bir (1983) and Malik & al. (2017) (Western Himalayas).

## MALVACEAE

\*\**Sida acuta* Burm.f.

$n = 14 + 0-1B$ ; CHN. India, Himachal Pradesh, Una, 31°28' 43.68"N, 76°16'30.0072"E, 369 m, 17 Oct 2017, *S. Kaur s.n.* (PUN 62698); India, Himachal Pradesh, Kangra, 32°05'59.2944"N, 76°16'8.7744"E, 733 m, 18 Oct 2017, *S. Kaur s.n.* (PUN 62699) [Fig. 6N].

Two accessions, collected from Una and Kangra, District of Himachal Pradesh, revealed the presence of one B-chromosome along with 14 bivalents ( $n = 14 + 0-1B$ ) at diakinesis [Fig. 6N]. This is the first-ever report of B-chromosome in *Sida acuta*.

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## IAPT chromosome data 40/5

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\* First chromosome count for the taxon.

\*\* First chromosome count from an unexplored region, providing new data for the taxon.

# First record of polyploidy within the genus.

## IRIDACEAE

### Subfamily Iridoideae

#### Tribe Tigridieae (Clade A)

\**Cypella altouruguayana* Chauveau & L.Eggers

$2n = 14$ ; CHN. Brazil, Rio Grande do Sul, Trindade do Sul, 27° 31'01.0"S, 52°58'53.0"W, 18 Aug 2012, *L. Eggers & O. Chauveau* 716 (ICN) [Fig. 7A,E].

\**Cypella amplimaculata* Chauveau & L.Eggers

$2n = 14$ ; CHN. Brazil, Rio Grande do Sul, Porto Alegre, Morro Santana, 30°03'09.0"S, 51°07'26.0"W, 20 Oct 2022, *J.G. Dani & C. Guzati* 280 (ICN); Brazil, Rio Grande do Sul, Passo Fundo, 28°14'12.0"S, 52°19'46.0"W, 6 Nov 2022, *J.G. Dani, C. Guzati & H.M. Bunecker* 375 (ICN); Brazil, Santa Catarina, Lages, 27°49' 16.0"S, 50°16'54.0"W, 1 Nov 2022, *J.G. Dani, C. Guzati & H.M. Bunecker* 330 (ICN) [Fig. 7B,F].

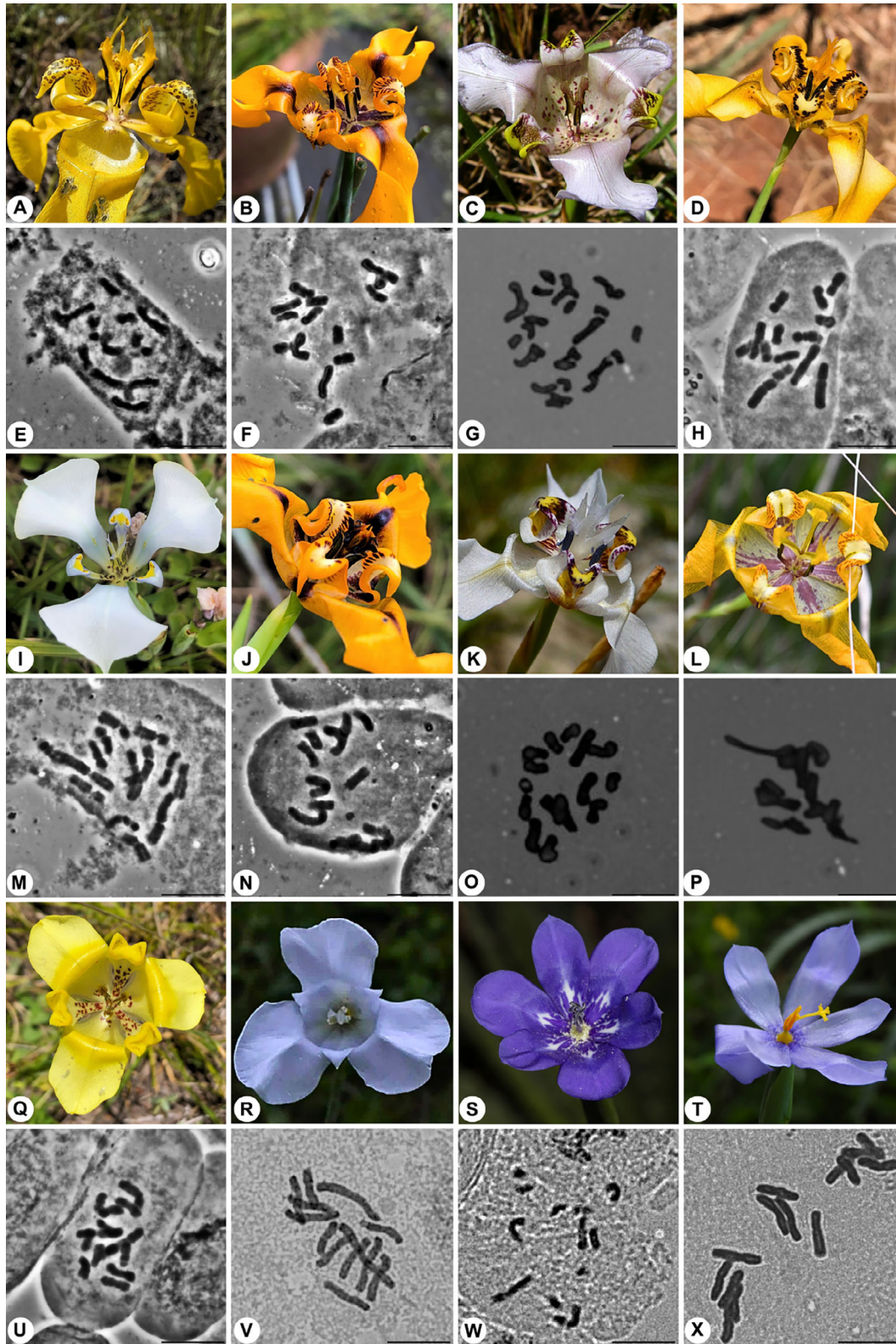
\*# *Cypella discolor* Ravenna

$2n = 4x = 28$ ; CHN. Brazil, Rio Grande do Sul, Quaraí, 30°11' 43.8"S, 56°29'19.6"W, 14 Nov 2013, *E.M. Stiehl-Alves, F.S. Silveira, J. Focchezatto & L.O. Tacuatiá* 94 (ICN) [Fig. 7C,G].

\*\**Cypella fucata* Ravenna

$2n = 14$ ; CHN. Brazil, Rio Grande do Sul, Caçapava do Sul, 30° 33'18.0"S, 53°12'44.0"W, 26 Oct 2022, *J.G. Dani, C. Guzati, L.N. Da Silva, G. Pecoits* 318 (ICN); Brazil, Rio Grande do Sul, Passo Fundo, 28°14'12.0"S, 52°19'46.0"W, 6 Nov 2022, *J.G. Dani, C. Guzati & H.M. Bunecker* 374 (ICN); Brazil, Rio Grande do Sul, Porto Alegre, Morro São Pedro, 11 Nov 2022, *J.G. Dani & C. Guzati* 386 (ICN) [Fig. 7D,H].





**Fig. 7.** Species with new chromosome records and their mitotic cells in metaphase. **A & E**, *Cypella altouruguayana* ( $2n = 14$ ); **B & F**, *Cypella ampli-maculata* ( $2n = 14$ ); **C & G**, *Cypella discolor* ( $2n = 4x = 28$ ); **D & H**, *Cypella fucata* ( $2n = 14$ ); **I & M**, *Cypella hauthalii* subsp. *minuticristata* ( $2n = 14$ ); **J & N**, *Cypella herbertii* subsp. *herbertii* ( $2n = 14$ ); **K & O**, *Cypella luteogibbosa* ( $2n = 14$ ); **L & P**, *Cypella magnicristata* ( $2n = 14$ ); **Q & U**, *Cypella pusilla* ( $2n = 14$ ); **R & V**, *Gelasine coerulea* ( $2n = 14$ ); **S & W**, *Gelasine elongata* ( $2n = 12$ ); **T & X**, *Gelasine uruguayensis* ( $2n = 14$ ). — Scale bars: E–H, M–P & U–X, 10  $\mu\text{m}$ . Photos: H.M. Buneker (L); J.G. Dani (A, B, I, Q); M. Grings (C, K, S); C. Guzati (D, J); A. Silvério (R); A. González (T).

The results of this research confirm the chromosome number previously documented for Piratini (Brazil, Rio Grande do Sul), Pinheiro Machado (Brazil, Rio Grande do Sul) and Punta del Este (Uruguay, Maldonado) samples, as reported by Goldblatt & Takei (1997) and Moraes & al. (2015).

\**Cypella hauthalii* subsp. *minuticristata* Chauveau & L.Eggers  
2n = 14; CHN. Brazil, Rio Grande do Sul, Passo Fundo, 08°14' 12.0"S, 52°19'46.0"W, 6 Nov 2022, J.G. Dani, C. Guzati & H.M. Buneker 376 (ICN) [Fig. 7L,M].

The chromosome number here reported is the same as one previously recorded for *Cypella hauthalii* subsp. *opalina* Ravenna from Garruchos (Argentina, Misiones) and Santo Antônio das Missões (Brazil, Rio Grande do Sul) as reported by Goldblatt & Takei (1997) and Moraes & al. (2015), respectively.

\*\**Cypella herbertii* (Herb.) Herb. subsp. *herbertii*  
2n = 14; CHN. Brazil, Santa Catarina, Urubici, 28°08'24.0"S, 49°38'36.0"W, 4 Nov 2022, J.G. Dani, C. Guzati & H.M. Buneker 364 (ICN) [Fig. 7J,N].

The observed chromosome number corroborates the data reported by Moares & al. (2015) for plants from Rio Grande do Sul.

\**Cypella luteogibbosa* Deble  
2n = 14; CHN. Brazil, Rio Grande do Sul, Quaraí, 30°11'43.8"S, 56°29'19.6"W, 14 Nov 2013, E.M. Stiehl-Alves, F.S. Silveira, J. Focchezatto & L.O. Tacuatiá 96 (ICN) [Fig. 7K,O].

\**Cypella magnicristata* Deble  
2n = 14; CHN. Brazil, Rio Grande do Sul, Quaraí, 30°11'43.8"S, 56°29'19.6"W, 14 Nov 2013, E.M. Stiehl-Alves, F.S. Silveira, J. Focchezatto & L.O. Tacuatiá 98 (ICN) [Fig. 7L,P].

\**Cypella pusilla* (Link & Otto) Benth. & Hook.f. ex B.D.Jacks.  
2n = 14; CHN. Brazil, Rio Grande do Sul, São Gabriel, 28°08' 24.0"S, 49°38'36.0"W, 19 Mar 2015, T. Pastori & O. Chauveau O 191 (ICN); Brazil, Rio Grande do Sul, São Gabriel, 30°34'38.0"S, 54°01'28.0"W, 27 Oct 2014, T. Pastori, M. Chiara-Moço, P.J.S. Silva-Filho, R. Macedo & L. Oliveira 148 (ICN); Brazil, Rio Grande do Sul, Caçapava do Sul, 30°32'46.0"S 53°29'50.0"W, 12 Nov 2014, T. Pastori, M. Longhi, C. Forgiarini & L. Nogueira 168 (ICN); Brazil, Rio Grande do Sul, Caçapava do Sul, 30°50' 26.0"S, 53°30'00.0"W, 19 Apr 2014, L. Eggers & O. Chauveau 914 (ICN) [Fig. 7Q,U].

## IRIDACEAE

### Subfamily Iridoideae

#### Tribe Tigridieae (Clade B)

\**Gelasine* aff. *coerulea* (Vell.) Ravenna  
2n = 14; CHN. Brazil, Santa Catarina, Água Doce, 26°42'38.0" S, 51°36'27.0"W, 5 Oct 2017, C. Valus & A. Silvério s.n. (MBM) [Fig. 7R,V].

\*\**Gelasine elongata* (Graham) Ravenna  
2n = 12; CHN. Brazil, Rio Grande do Sul, Porto Alegre, 30°03' 13.0"S, 51°07'03.0"W, 9 Oct 2007, L. Eggers & T.T. Souza-Chies 254 (ICN) [Fig. 7S,W].

The results of this study confirm the chromosome number previously documented by Goldblatt (1982), Kenton & Rudall (1987) and Bennett & Leitch (2012).

\*\**Gelasine uruguaiensis* Ravenna

2n = 14; CHN. Brazil, Rio Grande do Sul, Aceguá, 7 Nov 2011, L.P. Deble & A.S. de Oliveira-Deble 13578 (ICN) [Fig. 7T,X].

The findings of this work corroborate the chromosome count previously reported by Ravenna (1984).

Iridaceae Juss., a monocot family, is taxonomically classified into seven subfamilies (Goldblatt & al., 2008). Among them, Iridoideae, the subject of the present work, stands out as the exclusive subfamily encompassing species within the Neotropical region, which is the second major center of biodiversity (Goldblatt & al., 2008; Goldblatt & Manning, 2008). The morphology and size of chromosomes demonstrate notable diversity among this family, with frequent observations of bimodal karyotypes and asymmetry, especially in Iridoideae (Goldblatt & Takei, 1997; Souza-Chies & al., 2012). Polyploidy and dysploidy are also important for the evolution of the family, playing a crucial role in the diversity of karyotypes (Goldblatt & Takei, 1997).

There is a scarcity of cytogenetic data for the South American genera, particularly for *Cypella* Herb. and *Gelasine* Herb. Within *Cypella*, the chromosome number and genome size have been elucidated for only 5 out of approximately 30 species (Goldblatt & Takei, 1997; Ravenna, 1981; Goldblatt, 1982; Kenton & Heywood, 1984; Kenton, 1990; Moraes & al., 2015). Likewise, this information is known for merely three out of seven species of *Gelasine* (Goldblatt, 1982; Ravenna, 1984; Kenton & Rudall, 1987; Bennett & Leitch, 2012; Moraes & al., 2015).

Mitotic analyses were performed with at least five plant bulbs from each population. Roots tips were pretreated with a solution of 8-hydroxyquinoline for 4 h at 15°C and subsequently fixed in fresh ethanol : glacial acetic acid solution (3 : 1). Slides were prepared using a modified protocol derived from the conventional squash technique (Schwarzacher & Leitch, 1994). Root tips were submitted to an enzymatic digestion (macerozyme 1%, cellulase 2% and pectinase 20%) for 9 min and squashed with a drop of 45% acetic acid. Slides were stained with Giemsa. Metaphases with the best chromosome viewing were selected for counting. All analyses were performed using a Zeiss Axioplan microscope equipped with a digital image capture system (AxioVision Zeiss software).

Fresh leaves from five specimens per accession were used for genome size estimation following the methodology proposed by Doležel & al. (2007). *Solanum lycopersicum* L. 'Stupické polní rané' and *Vicia faba* L. 'Inovec' (Doležel & al., 1992) were selected for internal standards (2C = 1.96 pg and 26.09 pg, respectively). Standard and sample plants were chopped together in 1 ml of cold nuclear-isolation Ebihara buffer (Ebihara & al., 2005). The suspension was filtered through a 30 µm mesh nylon filter, and nuclei were stained with 50 µl propidium iodide. Nuclear suspensions of 5000 nuclei of each sample were analyzed in a FACS Aria BD III flow cytometer.

Cytological data were obtained from 13 populations of eight species of Tigridieae. Results are presented in Table 2. This is the first chromosomal number record for seven species of *Cypella*: *C. altouruguaya*, *C. amplimaculata*, *C. discolor*, *C. hauthalii* subsp. *minuticristata*, *C. luteogibbosa*, *C. magnicristata*, *C. pusilla*, and *Gelasine* aff. *coerulea*. Chromosome counts and genome sizes from an unexplored part of the distribution area of the Tigridieae were also obtained for four species: *Cypella fucata*, *C. herbertii* subsp. *herbertii*, *Gelasine elongata* and *G. uruguaiensis*. Moreover, this is the first report of genome size estimates for *Cypella pusilla*, *Gelasine* aff. *coerulea* and *G. uruguaiensis*.



All species and accessions of *Cypella* studied presented the same base chromosome number  $x = 7$  in agreement with all remaining taxa previously studied. Since now, all *Cypella* species investigated were diploid with  $2n = 14$ . Our analysis unveiled a polyploid species: *C. discolor*, a tetraploid with  $2n = 4x = 28$ . This discovery is especially intriguing as it suggests the only occurrence of polyploidy in *Cypella*.

Each examined species of *Gelasine* studied presented only diploid individuals, and no other cytotypes were observed. Previous studies on *Gelasine* established that both *G. coerulea* and *G. uruguaiensis* presented the same basic chromosome number  $x = 7$ , consistent with our data. Interestingly, *G. aff. coerulea* is a diploid with  $2n = 14$ , but has a higher DNA content than previously reported by Moraes & al. (2015) for *G. coerulea* ( $2C = 11.32$  pg and  $2C = 5.05$  pg, respectively). A more detailed karyotype analysis and new collections are necessary for a better understanding of this difference. Among the species within the genus, *G. elongata* stands out by presenting a reduced chromosome count of  $2n = 12$ , in accordance with earlier records.

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**Table 2.** Chromosome numbers and genome sizes in *Cypella* and *Gelasine* species.

Taxa	$2n$	Voucher	Genome size (2C, pg) mean $\pm$ standard deviation
<b>Tigridieae (Clade A): <i>Cypella</i> Herb.</b>			
<i>C. altouruguaya</i>	14	<i>L. Eggers &amp; O. Chauveau 716</i>	–
<i>C. amplimaculata</i>	14	<i>J.G. Dani, C. Guzati &amp; H.M. Buneker 330</i> <i>J.G. Dani, C. Guzati &amp; H.M. Buneker 375</i> <i>J.G. Dani &amp; C. Guzati 280</i>	–
<i>C. discolor</i>	$4x = 28$	<i>E.M. Stiehl-Alves, F.S. Silveira, J. Focchezatto &amp; L.O. Tacuatiá 94</i>	–
<i>C. fucata</i>	14	<i>J.G. Dani, C. Guzati &amp; H.M. Buneker 374</i> <i>J.G. Dani, C. Guzati, L.N. Da Silva &amp; G. Pecoits 318</i> <i>J.G. Dani &amp; C. Guzati 386</i>	3.89 $\pm$ 0.08 3.76 $\pm$ 0.03 3.78 $\pm$ 0.09
<i>C. hauthalii</i> subsp. <i>minuticristata</i>	14	<i>J.G. Dani, C. Guzati &amp; H.M. Buneker 376</i>	–
<i>C. herbertii</i> subsp. <i>herbertii</i>	14	<i>J.G. Dani, C. Guzati &amp; H.M. Buneker 364</i>	–
<i>C. luteogibbosa</i>	14	<i>E.M. Stiehl-Alves, F.S. Silveira, J. Focchezatto &amp; L.O. Tacuatiá 96</i>	–
<i>C. magnicristata</i>	14	<i>E.M. Stiehl-Alves, F.S. Silveira, J. Focchezatto &amp; L.O. Tacuatiá 98</i>	–
<i>C. pusilla</i>	14	<i>T. Pastori, M. Longhi, C. Forgiarini &amp; L. Nogueira 168</i> <i>L. Eggers &amp; O. Chauveau 914</i> <i>T. Pastori &amp; O. Chauveau 191</i> <i>T. Pastori, M. Chiara-Moço, P.J.S. Silva-Filho, R. Macedo &amp; L. Oliveira 148</i>	3.61 $\pm$ 0.07 3.67 $\pm$ 0.02 3.59 $\pm$ 0.02 3.43 $\pm$ 0.03
<b>Tigridieae (Clade B): <i>Gelasine</i> Herb.</b>			
<i>G. aff. coerulea</i>	14	<i>C. Valus &amp; A. Silvério s.n.</i>	11.32 $\pm$ 0.01
<i>G. elongata</i>	12	<i>L. Eggers &amp; T.T. Souza-Chies 254</i>	3.46 $\pm$ 0.01
<i>G. uruguaiensis</i>	14	<i>L.P. Deble &amp; A.S. de Oliveira-Deble 13578</i>	16.90

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## IAPT chromosome data 40/6

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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

*Anthurium andraeanum* Linden ex André

\*\* $2n = 36$ , CHN. Brazil, Paraíba, Areia, 06°58'19"S, 35°42'58"W, 526 m, 7 Sep 2022, *L.P. Felix 19523* (EAN) [Fig. 8A].

*Anthurium andraeanum* is the ornamental species of *Anthurium* most widely cultivated, either as an indoor plant or for the production of inflorescences for cutting. The count of  $2n = 30$  predominates in the vast majority of previous numerical records for this species (Sheffer & Kamemoto, 1976; Sheffer & Croat, 1983;

Petersen, 1989; Marutani & al., 1993). Our count for a single individual from the state of Paraíba diverged from all previous counts reported for *A. andraeanum*.

*Anthurium bromelicola* Mayo & L.P. Felix subsp. *bromelicola*  
 $2n = 30$ , CHN. Brazil, Paraíba, Areia, 06°58'19"S, 35°42'58"W, 526 m, 7 Sep 2022, *L.P. Felix 19524* (EAN) [Fig. 8B].

*Anthurium bromelicola*, a rare epiphytic species exclusive to bromeliads, endemic to the Northeast Region of Brazil, reported in the states of Pernambuco, Alagoas and Bahia. It has two recognized subspecies: *A. bromelicola* subsp. *bromelicola* from the states of Pernambuco and Alagoas, and *A. bromelicola* subsp. *bahiense* Mayo & J.G. Jardim, with exclusive occurrence in the Atlantic Forest of the southern region of the state of Bahia. The only previous chromosomal record for *A. bromelicola* subsp. *bromelicola* with  $2n = 30$  (Vilar & al., 2017) was confirmed in this study, and there are no chromosomal records known for *A. bromelicola* subsp. *bahiense*.

*Anthurium harleyi* T.A. Pontes & Mayo  
\* $2n = 30 + 1B$ , CHN. Brazil, Bahia, Morro do Chapéu, 11°35'29"S, 41°12'28"W, 14 Dec 2019, *L.P. Felix 18315* (EAN) [Fig. 8C].

*Anthurium harleyi* is a rupicolous plant found in the sandstone outcrops of the Chapada Diamantina in the state of Bahia, a region exceptionally rich in endemism. The species was recently described based on materials collected in the municipality of Morro do Chapéu, the same location where the material analyzed here was collected. It is closely related to *A. erskinei* Mayo, from which it differs in having longer inflorescences, shorter and wider leaves, a shorter spathe, and larger flowers (Pontes & al., 2019). Like other *Anthurium* species, the karyotype consists of submetacentric and metacentric chromosomes, with the presence of a smaller B chromosome.

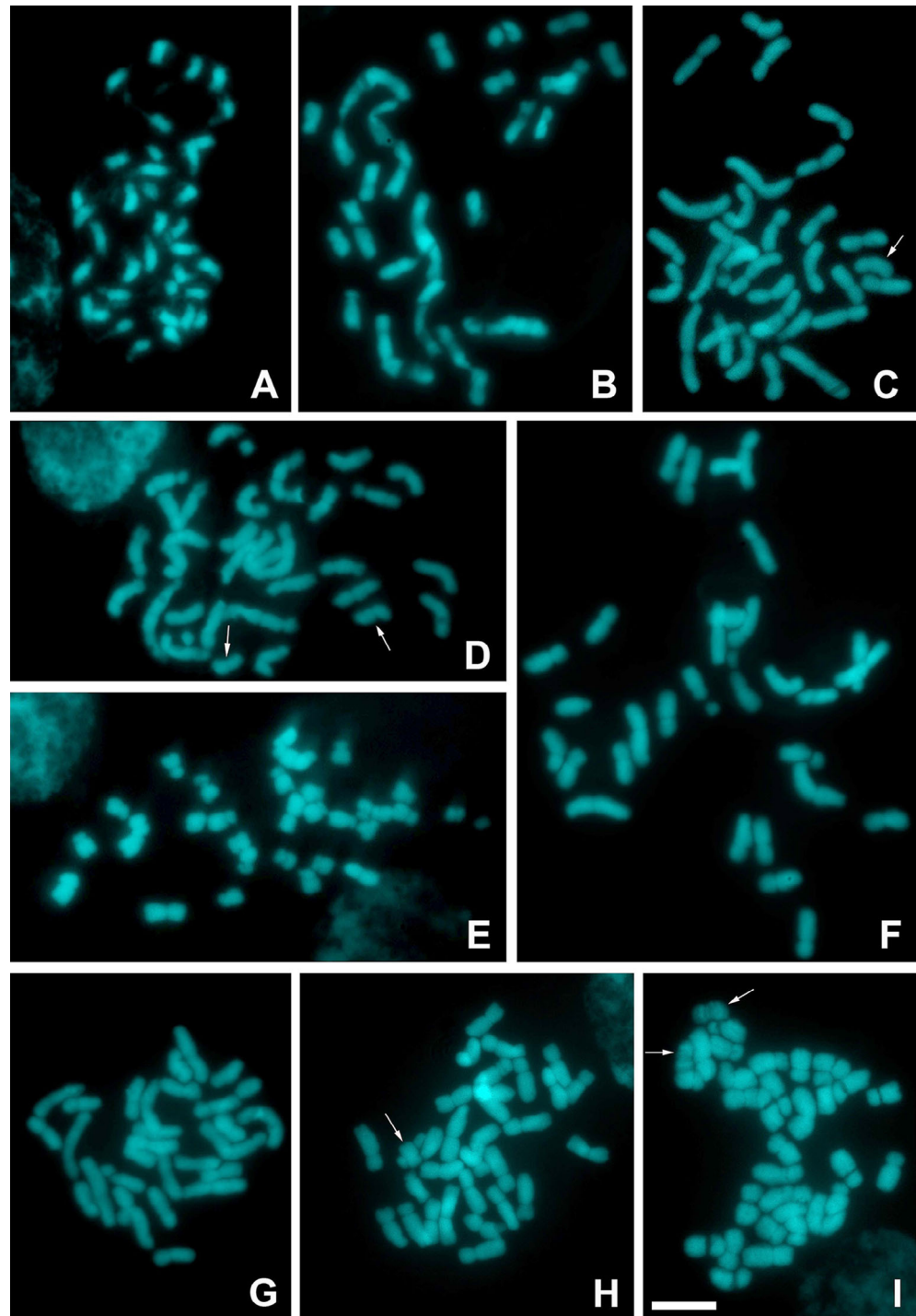
*Anthurium ianthinopodum* (Schott ex Engl.) Nadruz & Mayo  
\* $2n = 30 + 2B$ , CHN. Brazil, Espírito Santo, Itaguaçu, 19°04'32"S, 40°50'34"W, 150 m, 7 Feb 2015, *E.M. Almeida 1329* (EAN) [Fig. 8D].

*Anthurium ianthinopodum* is an epiphytic species endemic to the Atlantic Forest of the states of Bahia and Espírito Santo. It is morphologically related to *A. longifolium* G. Don, from which it differs by having upright leaves with a leaf blade of acute base and whitish fruits vinaceous at the apex and pink to reddish at the base, while *A. ianthinopodum* has pendant leaves with a leaf blade of obtuse base and completely red berries. It has a chromosome number of  $2n = 30 + 2B$  and a symmetrical karyotype primarily composed of metacentric and submetacentric chromosomes.

*Anthurium minarum* Sakur. & Mayo  
\* $2n = 32$ , CHN. Brazil, Minas Gerais, Diamantina, 18°02'07.4"S, 43°47'20.4"W, 1245 m, 30 Sep 2021, *L.P. Felix 19200* (EAN) [Fig. 8E].

*Anthurium minarum* has a restricted distribution in the Southeast Region of Brazil, occurring in the states of Minas Gerais, Rio de Janeiro, and São Paulo, particularly in rocky outcrops in the state of Minas Gerais (Sakuragui & Mayo, 1999). Cytogenetically, the species is characterized by having  $2n = 32$ , a karyotype mainly composed of submetacentric chromosomes and the presence of rare acrocentric chromosomes, including the NOR (Nucleolus Organizer Region) chromosome pair. There are no previous chromosome counts known for this species.

*Anthurium pentaphyllum* (Aubl.) G. Don  
 $2n = 30$ , CHN. Brazil, São Paulo, Ubatuba, 23°26'13"S, 45°04'08"W, 15 Jan 2017, *L.P. Felix 16970* (EAN) [Fig. 8F].



**Fig. 8.** Chromosome complements belonging to the species: **A**, *Anthurium andraeanum*,  $2n = 36$ ; **B**, *Anthurium bromelicola*,  $2n = 30$ ; **C**, *Anthurium harleyi*,  $2n = 30 + 1B$ ; **D**, *Anthurium ianthinopodum*,  $2n = 30 + 2B$ ; **E**, *Anthurium minarum*,  $2n = 32$ ; **F**, *Anthurium pentaphyllum*,  $2n = 30$ ; **G**, *Anthurium petrophilum*,  $2n = 30$ ; **H**, *Anthurium petrophilum*,  $2n = 30 + 1B$ ; **I**, *Anthurium petrophilum*,  $2n = 30 + 2B$ . — Arrows indicate B chromosomes. Scale bar in I = 10  $\mu\text{m}$ .

*Anthurium pentaphyllum* is a species with a wide distribution in Brazilian territory and other countries in Central and South America (from Costa Rica to the Guianas and Peru). It belongs to *A.* sect. *Dactylophyllum* (Schott) Engl., mainly characterized by deeply lobed, palmatisect, or completely digitate leaves, free to the base (Croat & Carlsen, 2013). The chromosome count of  $2n = 60$  prevails in most of its numerical records (Sheffer & Kamemoto, 1976; Sheffer & Croat, 1983; Vilar & al., 2017), which differs from our count. However, in Vilar & al. (2017), in addition to the count of  $2n = 60$ ,

a number of  $2n = 30$  was also observed in material collected in the state of Paraíba in the Northeast Region of Brazil. Our record for a population in the Southeast Region of Brazil indicates that the diploid cytotype, as well as the tetraploid cytotype, also has a wide geographic distribution. *Anthurium pentaphyllum* and *A. sinuatum* Benth. ex Schott are morphologically and genetically similar (Andrade & al., 2009), and these numerical differences may be related to difficulties in taxonomic delimitation between these two species or to events of auto- or allopolyploidy.



*Anthurium petrophilum* K.Krause

$2n = 30$ , CHN. Brazil, Bahia, Morro do Chapéu, 11°33'09"S, 41°09'27"W, 1017 m, Dec 2019, *L.P. Felix 18374* (EAN) [Fig. 8G].

\*\* $2n = 30 + 1B$ , CHN. Brazil, Bahia, Santa Terezinha, 12°51'04"S, 39°28'51"W, 669 m, 19 Jan 2014, *E.M. Almeida 966* (EAN); Brazil, Paraíba, São Joao do Tigre, 08°06'07"S; 36°37'52"W, 4 Aug 2011, *S. Nascimento 150* (EAN); Brazil, Pernambuco, Pesqueira, 08°21'28"S, 36°41'47"W, 654 m, 14 Nov 2011, *E.M. Neto 49* (EAN) [Fig. 8H].

\*\* $2n = 30 + 2B$ , CHN. Brazil, Bahia, Palmeiras, 12°31'44"S, 41°33'32"W, 697 m, 10 Aug 2012, *E.M. Almeida 616* (EAN) [Fig. 8I].

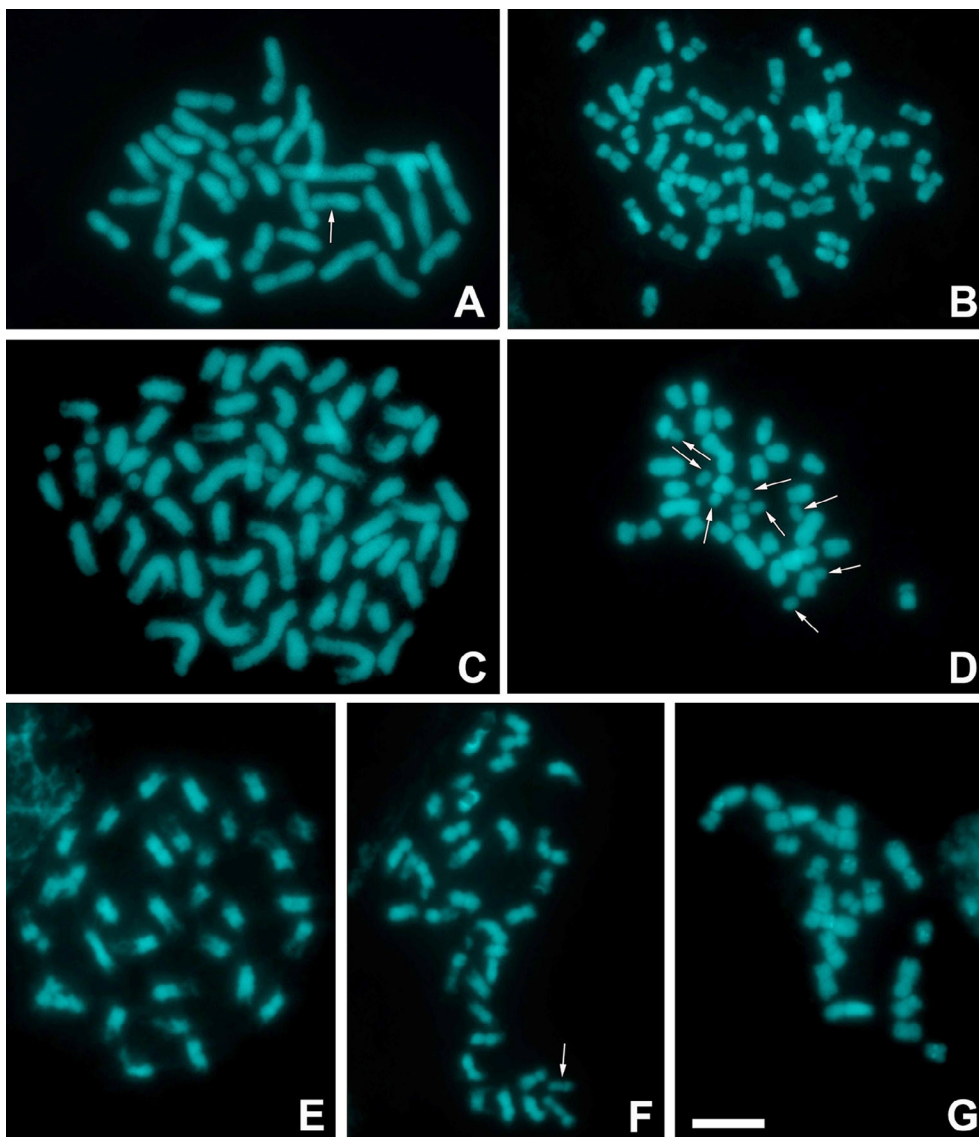
An endemic species of Brazil with recorded occurrences in the Northeast and Southeast regions, in the states of Pernambuco, Bahia, Minas Gerais (Coelho & al., 2023), and Paraíba. These are primarily rupicolous plants that typically grow on rocky outcrops in the crevices of rocks. The previous count of  $2n = 30$  conducted by Vilar & al. (2017) for two populations in Paraíba and Pernambuco was confirmed here only for the population in the state of Bahia. Our counts

of  $2n = 30 + 2B$  for an individual from Palmeiras, Bahia, and  $2n = 30 + 1B$  for several individuals from two populations in Paraíba and one population in Pernambuco suggest that this is the most common cytotype for the species.

*Anthurium raimundii* Mayo, Haigh & Nadruz

\* $2n = 30 + 1B$ , CHN. Brazil, Pernambuco, Brejo da Madre de Deus, 08°08'45"S, 36°22'16"W, 636 m, 20 Oct 2018, *L.P. Felix 17818* (EAN) [Fig. 9A].

*Anthurium raimundii* is reported in the states of Espírito Santo, Bahia, and Sergipe (Coelho & al., 2023). Its occurrence in Pernambuco expands its distribution area to the mountain forests in the northern part of the state of Alagoas. It has a chromosomal number of  $2n = 30 + 1B$ , with a symmetrical karyotype composed of metacentric and submetacentric chromosomes, in addition to an euchromatic B chromosome that is not very differentiated from the other chromosomes in the complement. The species was described just over 12 years ago based on materials collected in the southern part of the state of Bahia (Haigh & al., 2011).



**Fig. 9.** Chromosome complements belonging to the species: **A**, *Anthurium raimundii*,  $2n = 30 + 1B$ ; **B**, *Anthurium scandens*,  $2n = 48$ ; **C**, *Anthurium sellowianum*,  $2n = 60$ ; **D**, *Anthurium urvilleanum*,  $2n = 30 + 8B$ ; **E**, *Philodendron acutatum*,  $2n = 32$ ; **F**, *Philodendron acutatum*,  $2n = 32 + 1B$ ; **G**, *Syngonium podophyllum*,  $2n = 26$ . — Arrows indicate B chromosomes. Scale bar in G = 10  $\mu$ m.

*Anthurium scandens* (Aubl.) Engl.

$2n = 48$ , CHN. Brazil, Bahia, Morro do Chapeu, 11°35'29"S, 41°12'28"W, 14 Dec 2019, *L.P. Felix 18313* (EAN); Brazil, Bahia, Santa Terezinha, 12°50'51"S, 39°28'50"W, 719 m, 19 Jan 2014, *E.M. Almeida 1028* (EAN); Brazil, Pernambuco, Bonito, 08°30'23"S, 35°43'30"W, 782 m, 11 Jul 2021, *L.P. Felix 18667* (EAN); Brazil, Pernambuco, Bonito, 08°30'23"S, 35°43'30"W, 782 m, 11 Jul 2021, *L.P. Felix 18678* (EAN) [Fig. 9B].

*Anthurium scandens* is an epiphytic species that occurs in the Antilles and the American continent, from southern Mexico to Brazil (Sheffer & al., 1980), where it is reported in the Northern, Northeastern, Southeastern, and Southern regions (Coelho & al., 2023). Our count of  $2n = 48$  confirms all previous counts for the species (Sheffer & Kamemoto, 1976; Sheffer & Croat, 1983; Vilar & al., 2017). *Anthurium scandens* has a remarkably symmetrical karyotype with a predominance of submetacentric chromosomes, as well as some metacentric chromosome pairs.

*Anthurium sellowianum* Kunth

\*\* $2n = 60$ , CHN. Brazil, São Paulo, Cubatão, 23°53'24"S, 46°28'58"W, 12 Nov 2017, *L.P. Felix 16810* (EAN) [Fig. 9C].

*Anthurium sellowianum* is a terrestrial habitat plant endemic to the Atlantic Forest in the states of Minas Gerais, Rio de Janeiro, São Paulo, Paraná and Santa Catarina (Coelho & al., 2023). Most of the chromosome numbers reported for this species by Petersen (1989) are  $2n = 48$ , which differs from the number observed in the present study. However, despite the divergent counts, the presence of two chromosome pairs with a Nucleolus Organizer Region (NOR) and the formation of quadruplets with four chromosomes of similar size and morphology are consistent with a recent tetraploid origin.

*Anthurium urvilleanum* Schott

\* $2n = 30 + 8B$ , CHN. Brazil, São Paulo, Caraguatatuba, 23°34'56"S, 45°27'34"W, 736 m, 11 Nov 2017, *L.P. Felix 16998* (EAN) [Fig. 9D].

*Anthurium urvilleanum* is an epiphytic plant endemic to the Atlantic Forest with recorded occurrences in the southeastern and southern regions of Brazil, in the states of Rio de Janeiro, São Paulo, Paraná and Santa Catarina (Coelho & al., 2023). There are no previous chromosomal records known for this species, which presented  $2n = 38$  for the only individual analyzed. However, the presence of eight small chromosomes and similar size indicates that these are B chromosomes. This is, so far, the highest number of B chromosomes recorded for the genus *Anthurium*. The highest number of B chromosomes in *Anthurium* to date was reported by Cotias-de-Oliveira & al. (1999) for several individuals from the same population of *A. affine* Schott with  $2n = 30 + 1-4B$ .

*Philodendron acutatum* Schott

$2n = 32$ , CHN. Brazil, Piauí, Pedro II, 04°19'57"S, 41°26'48"W, 19 Mar 2014, *E.M. Almeida 1099* (EAN) [Fig. 9E].

\*\* $2n = 32 + 1B$ , CHN. Brazil, São Paulo, Caraguatatuba, 23°34'56"S, 45°27'34"W, 736 m, 16 Nov 2017, *L.P. Felix 17003* (EAN) [Fig. 9F].

*Philodendron acutatum* has a wide distribution from northern South America to southeastern Brazil. It is a hemiepiphytic species, morphologically characterized by having leaves with a sagittate or cordate base and an acuminate or acute apex of variable size (Sakuragui & al., 2005). Material of this species was long identified as *P. imbe* Schott ex Kunth, a morphologically distinct taxon, most

likely extinct in the wild (Mayo & Sakuragui, 2011). Our chromosome count of  $2n = 32$  differs from older counts for the species with  $2n = 34$  (Petersen, 1989), but it confirms the more recent count by Vilar & al. (2017) for a population in the state of Pernambuco in the Northeast Region of Brazil. Our count of  $2n = 32 + 1B$  for a specimen from the state of São Paulo is unprecedented for the species.

*Syngonium podophyllum* Schott

$2n = 26$ , CHN. Brazil, Paraíba, Areia, 06°58'19"S, 35°42'78"W, 10 Mai 2023, *L.P. Felix 13663* (EAN) [Fig. 9G].

*Syngonium podophyllum* is reported in Mexico, Central America, and the northern part of South America. It is a species that is difficult to delimit due to its considerable morphological variability (Croat, 1981). It is widely cultivated as an ornamental plant and, in some cases, considered an invasive species (Brunel, 2009). Two different chromosome numbers have been reported for this species:  $2n = 24$  (Ghosh & al., 2001) and  $2n = 26$  (Vilar & al., 2017), with only the latter confirmed in our study. Morphologically variable and numerically unstable species can exhibit variations in ploidy levels, as seen in *Lachenalia* Jacq. (Kleynhans & Spies, 1999), or numerical variations due to dysploidy, as in *Lychnophora* Mart. (Mansanares & al., 2002). Populations of the same species with different chromosome numbers are reproductively isolated and constitute distinct biological species that can eventually differentiate morphologically (Rieseberg & Willis, 2007).

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## IAPT chromosome data 40/7

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Here we report genome size estimations from several endemic or subendemic vascular plant species of Chile that were collected during January–February of 2023, with vouchers stored at CONC, UPOS, MA and MACB herbaria. These reports constitute a significant contribution to the scarce knowledge of genome sizes for the South American flora, which is still poorly known even for endangered and/or restricted endemic species, and evolutionarily isolated lineages. We provide the first genome size estimation for 22 species, 14 genera and 4 families, which are endemic to the “Chilean Winter Rainfall-Valdivian Forests” biodiversity hotspot (Arroyo & al., 2004). This information is indicated in the species name as follows:

\* First estimation for the species.

\*\* First estimation for the genus.

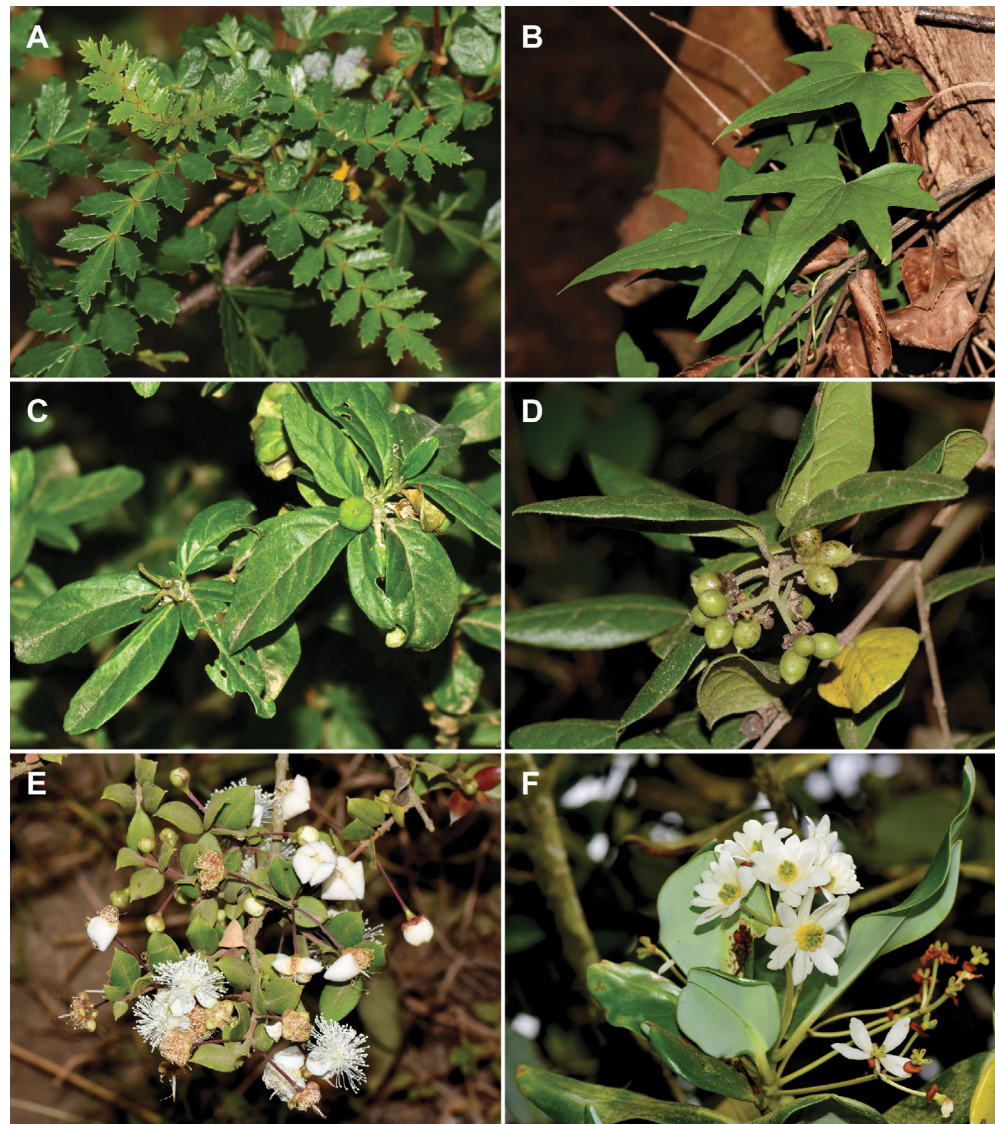
\*\*\* First estimation for the family.

## FLOW CYTOMETRY

Fresh leaves were analyzed together with different internal standard plants: *Oryza sativa* ‘IR 36’ (2C = 1.00 pg; Bennett & Smith, 1991), *Solanum lycopersicum* ‘Stupické’ (2C = 1.98 pg; Doležel & al., 1992), *Petroselinum crispum* ‘Champion Moss Curled’ (2C = 4.50 pg; Obermayer & al., 2002), *Pisum sativum* ‘Ctirad’ (2C = 9.09 pg; Doležel & al., 1998). We used different isolation buffers for processing the samples: General Purpose Buffer (GPB, Loureiro & al., 2007) supplemented with 3% PVP-40 (Pellicer & al., 2021), Lysis buffer LB01 (Doležel & al., 1989), Galbraith’s buffer (Galbraith & al., 1983), Ebihara’s buffer (Ebihara & al., 2005), and CyStain PI OxProtect buffer (Sysmex). These have been indicated for each specimen. For most of the buffers, we added 1 ml of buffer to the target sample and the internal standard, then chopped together the leaves with a razor blade, added another 1 ml of the buffer, and filtered it through a 30 µm pore size CellTrics filter (Sysmex). Finally, we added 100 µl of propidium iodide (PI, 1 mg/ml; Sigma) and incubated for 10 to 30 min. For the CyStain PI OxProtect (Sysmex) buffer, we followed the same protocol, but with 500 µl of buffer before chopping, then 1 ml after chopping, filtering, and finally adding 500 µl into the filter. After this, the samples were analyzed using a CyFlow Space cytometer (Sysmex) fitted with a Cobolt Samba laser (532 nm). The flow histograms were analyzed using FloMax v.2.9 software (Sysmex). We ran the samples through the cytometer at least three times and stopped after the target sample and the standard had reached at least 800 nuclei per fluorescence peak.

All the information will be uploaded to the Plant DNA C-values Database (Pellicer & Leitch, 2019), and the summary table can be found on GitHub. Pictures of some of the species have been included in Fig. 10.





**Fig. 10.** Representative photos of some of the studied species. **A**, *Weinmannia trichosperma*, Chile, Biobío; **B**, *Dioscorea brachybotrya*, Chile, Biobío; **C**, *Avellanita bustillosii*, Chile, Los Ríos; **D**, *Peumus boldus*, Chile, Biobío; **E**, *Luma apiculata*, Chile, Biobío; **F**, *Drimys winteri*, Chile, Biobío. — Photos by J.I. Márquez-Corro.

#### AEXTOXICACEAE

\*\*\**Aextoxicon punctatum* Ruiz & Pav.

2C =  $2.23 \pm 0.022$  pg, CV = 3.60 (GPB). [Cultivated] Chile, Biobío, Concepción, University of Concepción, 25 m, 36°49'41.0" S, 73°02'12.2"W, 5 Feb 2023, *Márquez-Corro & Muñoz-Schüler 87JMC23* (UPOS). Propagules originally provided by the University of Concepción nursery, collected from Chile, Biobío, Concepción, 36°50'24"S, 73°01'29"W.

This is the first estimation for the monotypic family Aextoxicaceae. Its chromosome number was reported to be  $2n = 32$  (Goldblatt, 1976). The only other family in the order Berberidopsidales, the also species-poor Berberidopsidaceae (2 genera, 3 species) has been reported to have  $2n = 42$  chromosomes and  $2C = 0.52$  pg for *Berberidopsis corallina* Hook.f. (Hanson & al., 2001), estimating a 6x ploidy. Comparing our results, it seems that *Aextoxicon* Ruiz & Pav. could have undergone several anagenetic polyploidization events with later diploidization, maybe combined with a strong accumulation of repetitive elements. Nevertheless, more karyological studies are required in these families to properly assess ploidy levels.

#### BLECHNACEAE

\*\**Austroblechnum lechleri* (T.Moore) Gasper & V.A.O.

Dittrich

2C =  $23.65 \pm 0.121$  pg, CV = 3.22 (Ebihara). Chile, Biobío, Polcura, Polcura mountain range, path to Frutillar, 943 m, 37°15' 53.1"S 71°44'03.9"W, 20 Jan 2023, *Muñoz-Schüler & al. 56.PMS.ENE* (CONC, MACB).

First genome size estimation for this Chilean subendemic genus. The 2C values obtained here are similar to the average of the family reported so far (Pellicer & Leitch, 2020).

\**Blechnum* sp.

2C =  $17.75 \pm 0.069$  pg, CV = 4.03 (Ebihara). Chile, Araucanía, Malleco, Nahuelbuta National Park, "Estero de los Gringos" trail, 1258 m, 37°48'45.2"S, 73°00'45.8"W, 22 Jan 2023, *Martín-Bravo & al. 46bisSMB23* (CONC, MACB).

This is the first genome size reported for a South American *Blechnum* L., and also similar to the average estimation of the genus (Pellicer & Leitch, 2020).

\*\**Parablechnum chilense* (Kaulf.) Gasper & Salino

2C = 27.03 ± 0.188 pg, CV = 4.48 (Ebihara). Chile, Ñuble, Chillán, Termas de Chillán, Aguas Calientes valley, 2081 m, 36°54'23.4"S, 71°22'27.9"W, 18 Jan 2023, *Martín-Bravo & al. 15SMB23* (CONC, MACB).

2C = 26.64 ± 0.084 pg, CV = 4.03 (Ebihara). Chile, Los Lagos, Llanquihue, camino a la laguna Sofia, 85 m, 41°35'34.3"S, 72°41'18.3"W, 27 Jan 2023, *Jiménez-Mejías & al. 31PJM-CL23* (CONC, MACB).

These two estimations are the first for *Parablechnum* C.Presl. Although the genus has been reported to have a labile karyotype (Gasper & al., 2016), this species has been reported to have 2n = 66 chromosomes (Jara-Seguel & al., 2006).

#### BROMELIACEAE

\**Puya alpestris* (Poepp.) Gay

2C = 1.00 ± 0.001 pg, CV = 3.99 (GPB). [Cultivated] Chile, Biobío, Concepción, University of Concepción, 18 m, 36°49'39.8" S, 73°02'15.8"W, 5 Feb 2023, *Márquez-Corro & Muñoz-Schüler 82JMC23* (UPOS). Specimen originally transplanted from the wild in the 1940s, collected from Chile, Coquimbo, Limari, 30°40'15"S, 71°38'34"W.

This is the first genome size estimation for the species. It displays similar values as the four previous reports for the genus (Pellicer & Leitch, 2020).

#### CELASTRACEAE

\*\**Maytenus boaria* Molina

2C = 2.84 ± 0.008 pg, CV = 3.22 (GPB). Chile, Biobío, Concepción, University of Concepción, 44 m, 36°49'44.4"S, 73°02'07.6"W, 05 Feb 2023, *Márquez-Corro & Muñoz-Schüler 88JMC23* (UPOS).

This is the first genome size estimation for the genus, and the first to a South American Celastraceae lineage. Although the value is somewhat similar to the family average, this family has been reported (Pellicer & Leitch, 2020) to vary from 2C = 0.37 to 8.80 pg, showing remarkable changes along the lineages that may be indicative of high ploidy level variation.

#### CUNONIACEAE

\*\*\**Weinmannia trichosperma* Cav.

2C = 0.98 ± 0.031 pg, CV = 7.75 (Galbraith). [Cultivated] Chile, Biobío, Concepción, University of Concepción, 26 m, 36°49'41.0"S, 73°02'13.3"W, 5 Feb 2023, *Márquez-Corro & Muñoz-Schüler 93JMC23* (UPOS). Propagules originally provided by the University of Concepción nursery, collected from Chile, Biobío, Concepción, 36°50'24"S, 73°01'29"W. [Fig. 10A]

This is the first genome size estimation for the family Cunoniaceae. Although the coefficient of variation (CV) is quite high, similar values have been obtained in an unpublished work (J. Pellicer, personal observation). This value is close to the single known report for the close family Elaeocarpaceae, in which *Crinodendron patagua* Molina was reported to have 2n = 16 and 2C = 0.60 pg (Hanson & al., 2005).

#### CUPRESSACEAE

*Fitzroya cupressoides* (Molina) I.M.Johnst.

2C = 35.28 ± 0.141 pg, CV = 4.11 (GPB). [Cultivated] Chile, Biobío, Concepción, University of Concepción, 18 m, 36°49'40.5" S, 73°02'15.5"W, 5 Feb 2023, *Márquez-Corro & Muñoz-Schüler*

*83JMC23* (UPOS). Specimen from vegetative cutting originally provided by the Austral University of Chile nursery, collected from Chile, Los Ríos, Ranco, 40°11'41"S, 73°26'07"W.

Second genome size estimation for the Patagonian cypress, the largest tree in South America. It is an endangered (EN; Hechenleitner & al., 2005), tetraploid (2n = 44; Zonneveld, 2012) species with a highly restricted distribution in Chile and Argentina. The first estimation was reported by Zonneveld (2012), indicating a similar average value of 2C = 35 pg.

#### DIOSCOREACEAE

\**Dioscorea brachybotrya* Poepp.

2C = 1.89 ± 0.009 pg, CV = 3.77 (LB01). Chile, Biobío, Polcura, Polcura mountain range, path to Frutillar, 943 m, 37°15'53.1" S, 71°44'03.9"W, 20 Jan 2023, *Muñoz-Schüler & al. 54.PMS.ENE* (CONC, UPOS). [Fig. 10B]

This is the first *Dioscorea* L. from South America ever estimated. Our report indicates *D. brachybotrya* as a polyploid species based on similar estimates in a recent study of the genus (Viruel & al., 2019). However, more karyological studies should be carried out to elucidate the complex ploidy system in this lineage of yams.

#### EUPHORBIACEAE

\*\**Avellanita bustillosii* Phil.

2C = 0.52 ± 0.005 pg, CV = 4.21 (GPB). [Cultivated] Chile, Los Ríos, Lago Ranco, Pitruico, Diego N. Penneckamp's private botanical garden, 192 m, 25 Jan 2023, *Sanz-Arnal & al. 27MSA-CL23* (CONC, MA). Seeds originally provided by the Jardín Botánico Nacional (JBN), collected from Chile, Metropolitana, Paine, proximities of Laguna de Aculeo, 33°50'S, 70°56'W. [Fig. 10C]

This estimation is the first for this monotypic genus endemic to Chile. This critically endangered species (Hechenleitner & al., 2005) also has the lowest genome size registered for the family so far (Pellicer & Leitch, 2020). This sample was analyzed using tomato and rice as standards. Here, only the one using rice is indicated since it displayed better results, but the results using tomato were similar: 2C = 0.52 ± 0.005 pg, CV = 4.31.

#### FABACEAE

*Astragalus cruckshanksii* (Hook. & Arn.) Griseb.

2C = 2.26 ± 0.019 pg, CV = 2.67 (GPB). Chile, Coquimbo, Cordillera Doña Ana, Minera El Indio, 3350 m, 29°48'54.8"S, 70°01'19.6"W, 13 Jan 2023, *Muñoz-Schüler & al. 10.PMS.ENE* (CONC).

There is a previous report on the genome size of *Astragalus cruckshanksii* from Neuquén (Argentina) by Dopchiz & al. (1995). In their study, they found 2n = 28 and 2C = 2.88 pg, although they measured significantly fewer nuclei (3 replicates of 60 nuclei). These results may indicate a latitudinal difference in the species that needs further investigation.

*Sophora cassioides* (Phil.) Sparre

2C = 1.80 ± 0.038 pg, CV = 3.83 (GPB). [Cultivated] Chile, Biobío, Concepción, University of Concepción, 18 m, 36°49'39.2" S, 73°02'14.2"W, 5 Feb 2023, *Márquez-Corro & Muñoz-Schüler 86JMC23* (UPOS). Propagules originally collected from Chile, Biobío, Concepción, 36°49'40"S, 73°02'09"W.

The genome size of this diploid species has been previously estimated to be 2C = 1.61 pg (Espejo & al., 2016). Our results are similar to those reported.



**GOMORTEGACEAE**

\*\*\**Gomortega keule* (Molina) Baill.

2C = 2.93 ± 0.009 pg, CV = 3.16 (GPB). Chile, Biobío, Concepción, patches of native forest close to the petrol station on the Itata highway, before Juan Chico, 323 m, 36°44'51.0"S, 72°54'40.1"W, 3 Feb 2023, *Márquez-Corro & al. 79bisJMC23* (UPOS).

This species has only been studied karyologically ( $2n = 42$ ; Baeza & al., 2001; Oginuma & Tobe, 2006), and this is the first genome size estimation for the species, which is the only representative of the family. The queule is a tree species endemic to a highly restricted area in the coastal range around Concepción in regions Maule, Ñuble and Biobío in central southern Chile. It is considered a living fossil with great evolutionary distinctiveness, as the lineage is included within the early divergent Laurales and has been dated to the Cretaceous (Renner, 2005). However, this palaeoendemic is endangered (EN; Echeverría & Campos, 2019) and threatened by habitat destruction due to extensive reforestation with exotic tree species. Recent massive wildfires that affected Central Chile during the summer of 2023 destroyed several wild queule individuals, so its conservation status is likely to have significantly deteriorated (only around 1000 mature individuals were known; Echeverría & Campos, 2019).

**JUNCACEAE**

\**Juncus microcephalus* Kunth

2C = 1.09 ± 0.003 pg, CV = 3.12 (LB01). Chile, Coquimbo, Tulahuén, Río Grande valley, beyond Cuesta del Toro, 1623 m, 30°57'37.6"S, 70°31'20.7"W, 15 Jan 2023, *Muñoz-Schüler & al. 35.PMS.ENE* (CONC, UPOS).

This report falls within the average value of the genus. It is the first genome size estimation for the species and, together with the estimation of *J. stipulatus* below, it constitutes the first for *Juncus* L. in South America.

\**Juncus stipulatus* Nees & Meyen

2C = 0.93 ± 0.015 pg, CV = 4.22 (GPB). Chile, Ñuble, Chillán, Termas de Chillán, Aguas Calientes valley, 2151 m, 36°54'13.2"S, 71°22'36.6"W, 18 Jan 2023, *Martín-Bravo & al. 09SMB23* (CONC, UPOS).

This estimation is the first for the species, displaying values similar to the genus average.

\*\**Patosia clandestina* (Phil.) Buchenau

2C = 1.37 ± 0.005 pg, CV = 2.60 (LB01). Chile, Coquimbo, Doña Ana mountain range, Mine "El Indio", 3350 m, 29°48'54.8"S, 70°01'19.6"W, 13 Jan 2023, *Muñoz-Schüler & al. 22.PMS.ENE* (CONC, UPOS).

First record for this Andean endemic, monotypic genus. The estimation is slightly lower than the family average (Pellicer & Leitch, 2020). The relationships within the family have not yet been clarified (Drábková & Čestmír, 2007), and further molecular phylogenetic and genomic studies are needed to understand the evolution of this genus, which is a key component of high Andean wetland vegetation, in which it forms very distinctive cushions.

**LAURACEAE**

\**Cryptocarya alba* (Molina) Looser

2C = 2.34 ± 0.021 pg, CV = 3.52 (GPB). Chile, Biobío, Concepción, University of Concepción, 18 m, 36°49'39.3"S, 73°02'13.8"W, 5 Feb 2023, *Márquez-Corro & Muñoz-Schüler 84JMC23* (UPOS). Specimen of natural origin that already existed when the Ottmar Wilhelm Building was built in the 1930s.

This is the first genome size estimation for this genus, and for all the early diverging lineages of Lauraceae (Cryptocaryae). This family has been largely studied karyologically but there is a lack of information on the Cryptocaryae, where different ploidy levels were reported (e.g., Oginuma & Tobe, 2006).

**MONIMIACEAE**

\*\**Peumus boldus* Molina

2C = 5.18 ± 0.079 pg, CV = 2.87 (GPB). Chile, Biobío, Concepción, University of Concepción, 42 m, 36°49'46.3"S, 73°02'04.7"W, 5 Feb 2023, *Márquez-Corro & Muñoz-Schüler 89JMC23* (UPOS). [Fig. 10D]

First estimation for the species and Chilean subendemic genus. Within Monimiaceae, it is more than twice the size of the genus *Mollinedia* Ruiz & Pav. (Pellicer & Leitch, 2020). This result points to polyploidization in *Peumus* Molina, as its known chromosome number is  $2n = 78$ , whereas *Mollinedia* has been reported to have different ploidy levels  $2n = 36, 38, 180$  (Oginuma & Tobe, 2006). Therefore, one could assume that the already estimated genome sizes in *Mollinedia* belong to the lowest registered chromosome numbers, around  $2n = 38$ , which must be confirmed.

**MYRTACEAE**

\*\**Luma apiculata* (DC.) Burret

2C = 1.02 ± 0.017 pg, CV = 4.27 (GPB). Chile, Biobío, Concepción, University of Concepción, 45 m, 36°49'46.2"S, 73°02'04.1"W, 5 Feb 2023, *Márquez-Corro & Muñoz-Schüler 90JMC23* (UPOS). [Fig. 10E]

First estimation for this species and Chilean subendemic genus. The 2C values agree with the mean reported for the family (Pellicer & Leitch, 2020). The values reported in the family vary in what looks like different ploidy levels, even within the genus (e.g., *Psidium* L.). This lineage requires further phylogenetic and genomic studies, as the family still needs nomenclatural rearrangements (Vasconcelos & al., 2017).

**NOTHOFAGACEAE**

\*\*\**Nothofagus obliqua* (Mirb.) Oerst.

2C = 1.19 ± 0.001 pg, CV = 3.52 (OXPRO). Chile, Biobío, Concepción, University of Concepción, 45 m, 36°49'46.2"S, 73°02'04.1"W, 5 Feb 2023, *Márquez-Corro & Muñoz-Schüler 91JMC23* (UPOS).

First estimation for this family. *Nothofagus* Blume chromosome number has been reported to be very stable, with this species showing the genus' most frequent number of  $2n = 26$  (Jara-Seguel & al., 2014). Values of 2C including or close to the one reported here have been testified for the close families of Fagaceae, Juglandaceae or Betulaceae (Pellicer & Leitch, 2020).

**PODOCARPACEAE**

\**Podocarpus salignus* D. Don

2C = 17.78 ± 0.332 pg, CV = 4.73 (OXPRO). Chile, Biobío, Concepción, University of Concepción, 56 m, 36°49'46.7"S, 73°02'03.8"W, 5 Feb 2023, *Márquez-Corro & Muñoz-Schüler 92JMC23* (UPOS).

Similar 2C values have been reported for the genus, although they tend to be slightly higher (Pellicer & Leitch, 2020). This is the first estimation of this vulnerable species (Hechenleitner & al., 2005) endemic to Chile, and for South American *Podocarpus* L'Hér. ex Pers.



**RESTIONACEAE**

\*\**Apodasmia chilensis* (Gay) B.G.Briggs & L.A.S.Johnson

2C = 2.09 ± 0.014 pg, CV = 4.20 (LB01). Chile, Los Lagos, Llanquihue, cove north of Punta Metri, 7 m, 41°35'19.6"S, 72°42'05.0"W, 27 Jan 2023, Jiménez-Mejías & al. 32PJM-CL23 (CONC, UPOS).

First record for this Chilean endemic species, and for the South American representatives of the family (Pellicer & Leitch, 2020). *Gaimardia australis* Gaudich. and *Apodasmia chilensis* are the only two South American native and endemic restiads (POWO, 2023). Therefore, this data is important to understand in future studies whether different ploidy levels may be associated with an extension of the genus' natural range.

**RUTACEAE**

\*\**Pitavia punctata* (Ruiz & Pav.) Molina

2C = 1.07 ± 0.006 pg, CV = 3.92 (GPB). [Cultivated] Chile, Biobío, Concepción, University of Concepción, 18 m, 36°49'40.5" S, 73°02'12.8"W, 5 Feb 2023, Márquez-Corro & Muñoz-Schüler 85JMC23 (UPOS). Seeds originally provided by Arauco S.A. Forestry Company nursery, collected from Chile, Biobío, Concepción, 37°06'48"S, 73°09'01"W.

First estimation for this Critically Endangered (Rivera Caniulao, 2021), Chilean endemic, monotypic genus. This species has been reported to have 2n = 36 chromosomes (Stace & al., 1993).

**WINTERACEAE**

\**Drimys winteri* J.R.Forst. & G.Forst.

2C = 3.90 ± 0.011 pg, CV = 3.04 (GPB). [Cultivated] Chile, Biobío, Concepción, University of Concepción, 18 m, 36°49'38.1" S, 73°02'13.1"W, 5 Feb 2023, Márquez-Corro & Muñoz-Schüler 94JMC23 (UPOS). Propagules originally provided by the University of Concepción nursery, collected from Chile, Biobío, Concepción, 36°50'24"S, 73°01'29"W. [Fig. 10F]

This is the first estimation for the species, and the second for the genus. *Drimys winteri* presents an almost twice as large genome as *D. vickeriana* A.C.Sm. (Pellicer & Leitch, 2020). This is probably due to the different ploidy levels present in the genus, as *D. winteri* has been reported to have 2n = 86, but also 2n = 26 chromosomes have been counted in *Drimys* J.R.Forst. & G.Forst. (Ehrendorfer & al., 1968).

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- IAPT chromosome data 40/8**
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- Methods for chromosome analysis according to Cordeiro & al. (2020).
- \* First chromosome count for the species.  
\*\* First chromosome count for the genus.
- ANACARDIACEAE**  
*Anacardium occidentale* L.  
2n = 40, CHN. Brazil, Pernambuco, Taquaritinga do Norte, 07°54'11"S, 36°02'39"W, 28 Jan 2018, *L.P. Felix 17303* (EAN) [Fig. 11A].  
*Astronium fraxinifolium* Schott  
2n = 30, CHN. Brazil, Paraíba, Maturéia, 07°15'54"S, 37°22'33"W, 2 Dec 2017, *L.P. Felix 17114* (EAN) [Figs. 11B, 13A].  
*Mangifera indica* L.  
2n = 40, CHN. Brazil, Paraíba, Sertãozinho, 06°44'06"S, 35°27'30"W, 3 Jun 2018, *J.M.P. Cordeiro 1337* (EAN) [Fig. 11C].  
*Myracrodruon urundeuva* Allemão  
2n = 30, CHN. Brazil, Pernambuco, Taquaritinga do Norte, 07°54'11"S, 36°02'39"W, 28 Jan 2018, *L.P. Felix 17308* (EAN) [Fig. 11D].  
*Schinopsis brasiliensis* Engl.  
2n = 28, CHN. Brazil, Paraíba, São José dos Cordeiros, 07°28'08"S, 36°53'47"W, 30 Nov 2017, *L.P. Felix 17100* (EAN) [Figs. 11E, 13B].  
*Schinus terebinthifolia* Raddi  
2n = 28, CHN. Brazil, Paraíba, João Pessoa, 07°09'13"S, 34°52'58"W, 28 Jun 2018, *L.P. Felix 17496* (EAN) [Fig. 11F].
- BURSERACEAE**  
*Commiphora leptophloeos* (Mart.) J.B. Gillett  
2n = 26, CHN. Brazil, Paraíba, Areia, 06°58'03"S, 35°42'58"W, 18 Jul 2017, *L.P. Felix 16687* (EAN) [Figs. 11G, 13C].
- CANNABACEAE**  
*Celtis iguanaea* (Jacq.) Sarg.  
2n = 20, CHN. Brazil, Paraíba, Areia, 06°57'49"S, 35°44'34"W, 9 Jun 2017, *P.C. Gadelha Neto 4160* (EAN) [Fig. 11H].
- LENTIBULARIACEAE**  
*Utricularia quelchii* N.E.Br.  
2n = 18, CHN. Brazil, Roraima, Monte Roraima, 05°13'51"N, 60°43'46"W, 21 Feb 2017, *L.P. Felix 16222* (EAN) [Figs. 11I, 13D].
- MALPIGHIACEAE**  
*Amorimia septentrionalis* W.R. Anderson,  
2n = 20, CHN. Brazil, Paraíba, Sertãozinho, 06°44'06"S, 35°27'30"W, 16 Jun 2018, *J.M.P. Cordeiro 1339* (EAN) [Figs. 11J, 13E].
- MELIACEAE**  
*Azadirachta indica* A.Juss.  
2n = 28, CHN. Brazil, Paraíba, Serra Branca, 07°29'00"S, 36°29'54"W, 30 Jun 2018, *J.M.P. Cordeiro 1342* (EAN) [Fig. 11K].

\**Cedrela fissilis* Vell.

$2n = 54$ , CHN. Brazil, Pernambuco, Taquaritinga do Norte, 07°54'18"S, 36°01'47"W, 13 Oct 2016, *L.P. Felix 15988* (EAN) [Figs. 11L, 13F].

*Melia azedarach* L.

$2n = 28$ , CHN. Brazil, Paraíba, Areia, 06°58'03"S, 35°42'58"W, 23 Jul 2018, *J.M.P. Cordeiro 1368* (EAN) [Fig. 12A].

## MYRTACEAE

\**Eugenia zigzag* K.Cout. & Sobral

$2n = 22$ , CHN. Brazil, Paraíba, Sertãozinho, 06°44'06"S, 35°27'30"W, 18 Dec 2021, *J.M.P. Cordeiro 1544* (EAN) [Figs. 12B, 13G].

## ORCHIDACEAE

*Caularthron bicornutum* Raf.

$2n = 40$ , CHN. Brazil, Amazonas, Novo Airão, 03°03'11"S, 60°46'07"W, 26 Feb 2017, *L.P. Felix 16336* (EAN) [Fig. 12C].

## POLYGONACEAE

\**Triplaris gardneriana* Wedd.

$2n = 22$ , CHN. Brazil, Paraíba, Serra Branca, 07°29'46"S, 36°43'52"W, 30 Jun 2018, *L.P. Felix 17561* (EAN) [Figs. 12D, 13H].

## RUBIACEAE

\**Randia armata* (Sw.) DC.

$2n = 40$ , CHN. Brazil, Rio Grande do Norte, Martins, 06°04'25"S, 37°55'17"W, 2 Feb 2006, *R.T. Queiroz 613* (EAN) [Figs. 12E, 13I].

## RUTACEAE

*Ruta chalepensis* L.

$2n = 40$ , CHN. Brazil, Paraíba, Sertãozinho, 06°44'06"S, 35°27'30"W, 15 Dec 2018, *J.M.P. Cordeiro 1406* (EAN) [Fig. 12F].

## SAPINDACEAE

*Filicium decipiens* (Wight & Arn.) Thwaites

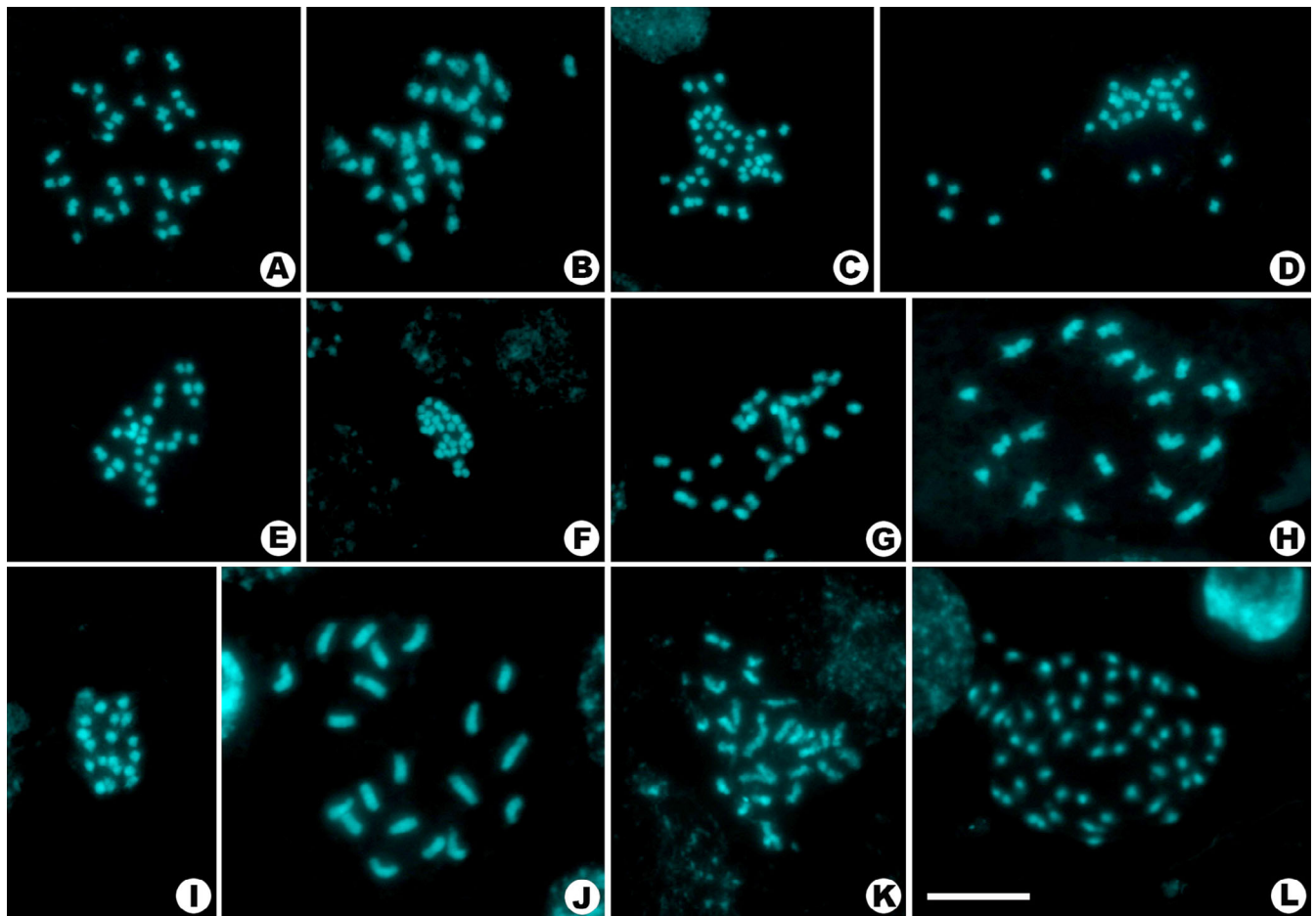
$2n = 32$ , CHN. Brazil, Paraíba, Areia, 06°58'03"S, 35°42'58"W, 15 Dec 2018, *J.M.P. Cordeiro 1407* (EAN) [Fig. 12G].

*Paullinia pinnata* L.

$2n = 24$ , CHN. Brazil, Paraíba, Areia, 06°58'03"S, 35°42'58"W, 17 Feb 1992, *L.P. Felix 4777* (EAN) [Fig. 12H].

*Sapindus saponaria* L.

$2n = 28$ , CHN. Brazil, Paraíba, Areia, 06°58'03"S, 35°42'58"W, 2 Oct 2012, *L.P. Felix 13946* (EAN) [Fig. 12I].



**Fig. 11.** Chromosome number of tropical plants: **A**, *Anacardium occidentale*,  $2n = 40$ ; **B**, *Astronium fraxinifolium*,  $2n = 30$ ; **C**, *Mangifera indica*,  $2n = 40$ ; **D**, *Myracrodruon urundeuva*,  $2n = 30$ ; **E**, *Schinopsis brasiliensis*,  $2n = 28$ ; **F**, *Schinus terebinthifolia*,  $2n = 28$ ; **G**, *Commiphora leptophloeos*,  $2n = 26$ ; **H**, *Celtis iguanaea*,  $2n = 20$ ; **I**, *Utricularia quelchii*,  $2n = 18$ ; **J**, *Amorimia septentrionalis*,  $2n = 20$ ; **K**, *Azadirachta indica*,  $2n = 28$ ; **L**, *Cedrela fissilis*,  $2n = 54$ . — Scale bar = 10  $\mu$ m.



\**Serjania salzmanniana* Schldtl.

$2n = 24$ , CHN. Brazil, Paraíba, Mamanguape, 06°44'32"S, 35°08'31"W, 1 Jun 2018, *J.M.P. Cordeiro 1333* (EAN) [Figs. 12J, 13J].

*Talisia esculenta* (A.St.-Hil.) Radlk.

$2n = 32$ , CHN. Brazil, Paraíba, Sertãozinho, 06°44'06"S, 35°27'30"W, 3 Jun 2018, *J.M.P. Cordeiro 1338* (EAN) [Fig. 12K].

#### SIMAROUBACEAE

*Homalolepis bahiensis* (Moric.) Devecchi & Pirani

$2n = 32$ , CHN. Brazil, Paraíba, Barra de Santana, 07°29'17"S, 36°03'04"W, 22 Dec 2013, *E.M. Almeida 814* (EAN) [Fig. 12L].

#### VERBENACEAE

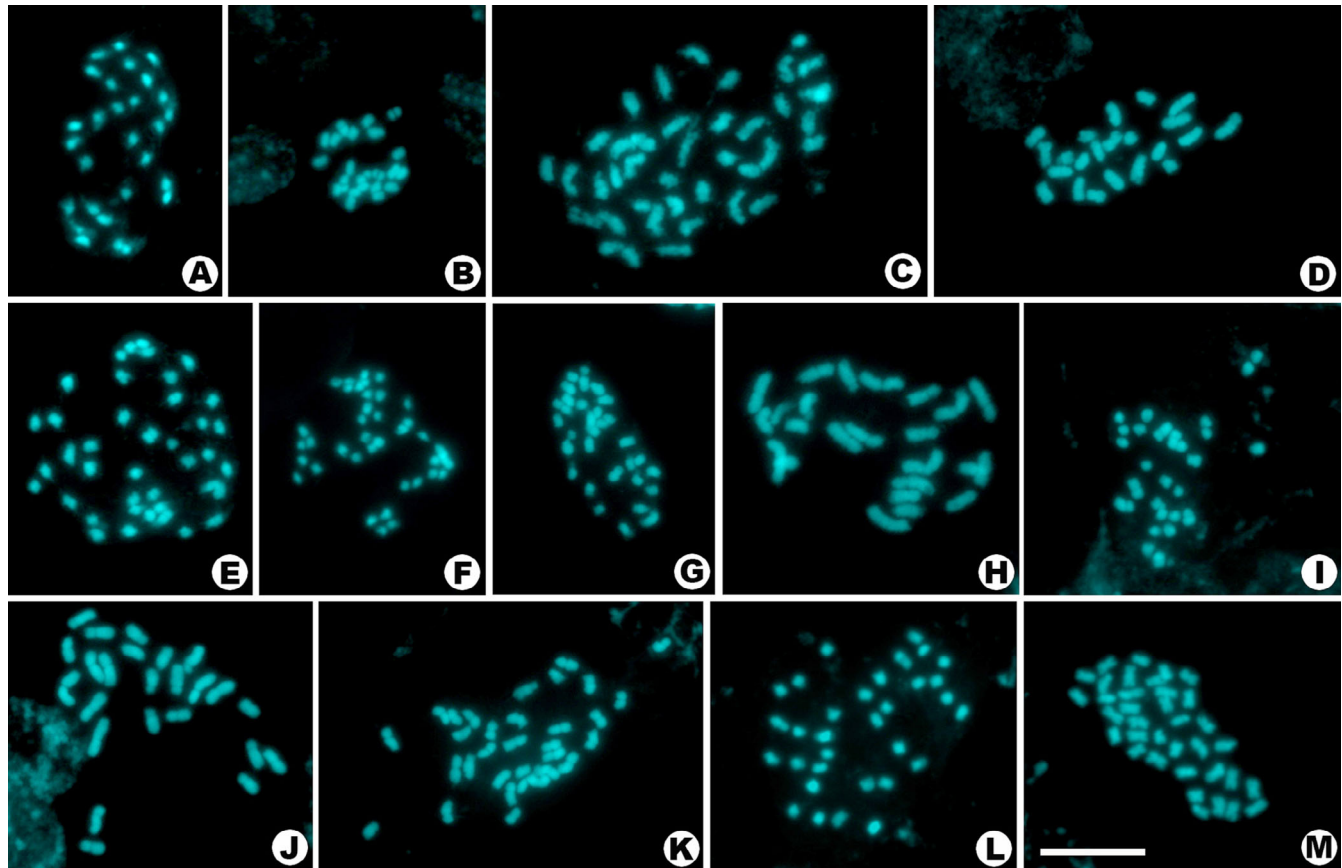
\**Priva bahiensis* DC. ex Schauer

$2n = 36$ , CHN. Brazil, Paraíba, Sertãozinho, 06°44'06"S, 35°27'30"W, 16 Jul 2021, *J.M.P. Cordeiro 1525* (EAN) [Figs. 12M, 13K].

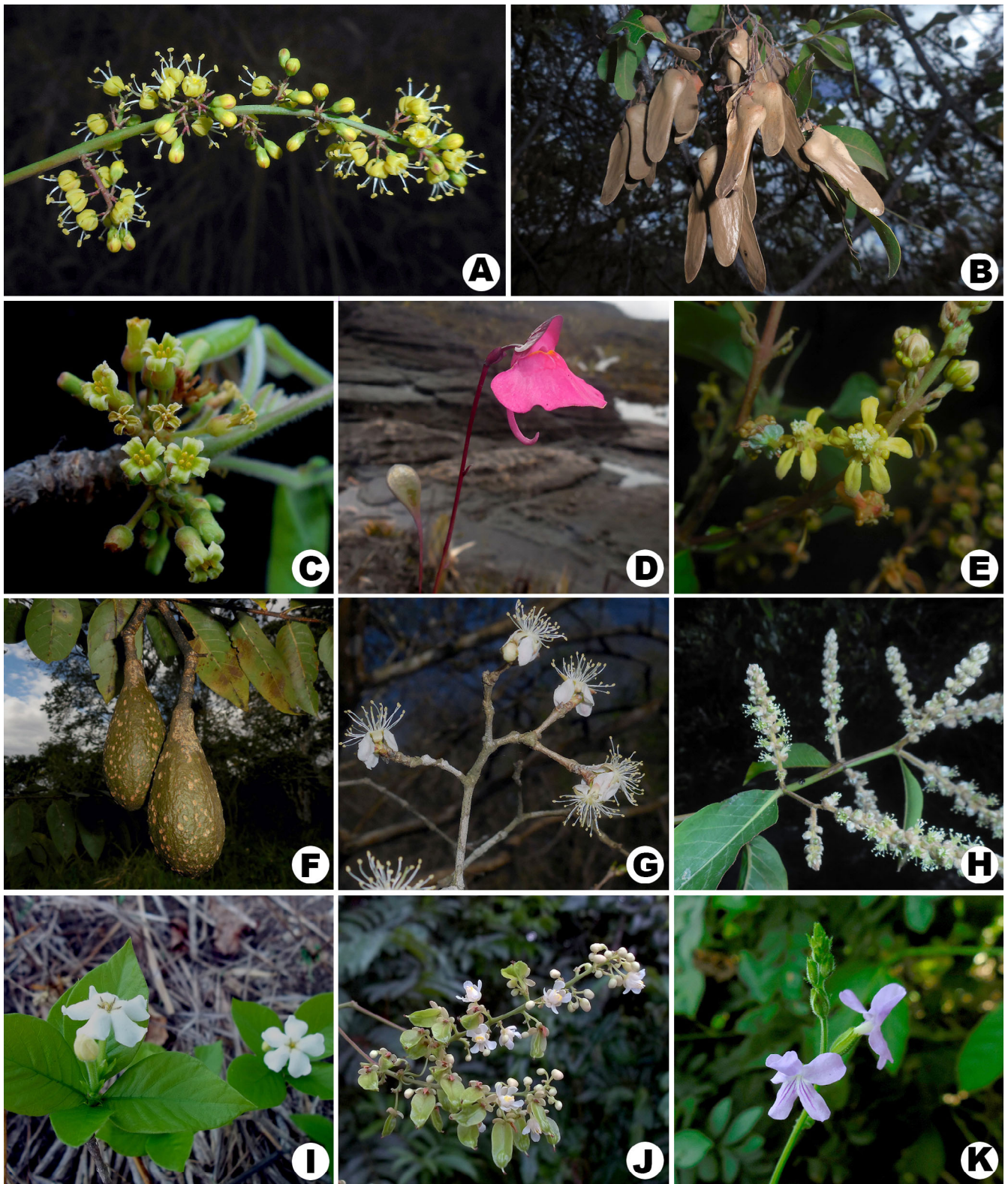
In the present work we analyzed the chromosome number of 25 tropical plant species from Brazil (Figs. 11, 12), of which 11 are new (Fig. 13), including those for the genus *Amorimia* W.R. Anderson ( $2n = 20$ ). Tropical regions are home to the greatest diversity of plants, especially due to climatic conditions that combine high temperatures and abundant rainfall (Wiens & Donoghue, 2004).

However, more incident UV-B radiation and soils generally poor in nutrients may cause deleterious DNA-damage and cell size reduction, respectively. In this sense, tropical regions exhibit environmental pressures that select species with lower DNA content when compared to temperate regions (Ohri, 2008). Genome size generally follows a proportional variation with polyploidy or chromosome size in a plant group (Soltis & al., 2003; Souza & al., 2019). Thus, it is expected that tropical plants have, in general, smaller chromosomes compared to plants from regions with lower-temperature climates. In fact, among 25 tropical species analyzed here, most of them have relatively small chromosomes (<2  $\mu\text{m}$ ) and only one species had a chromosome number greater than  $2n = 40$  (*Cedrela fissilis* Vell.,  $2n = 54$ ).

In our analyses, the largest samples correspond to the families Anacardiaceae and Sapindaceae, which are included, together with Burseraceae, Meliaceae, Rutaceae and Simaroubaceae, in the order Sapindales. In total, 17 species of that order had chromosome records presented here, with new records for *Astronium fraxinifolium* ( $2n = 30$ ), *Schinopsis brasiliensis* ( $2n = 28$ ), *Commiphora leptophloeos* ( $2n = 26$ ), *Cedrela fissilis* ( $2n = 54$ ) and *Serjania salzmanniana* ( $2n = 24$ ). In Sapindales, dysploidy appears to be the most important karyotypic change at family level diversification, while polyploidy stands out especially in Meliaceae and Rutaceae (Guimarães & Forni-Martins, 2022). However, the wide variation in chromosome number between genera within families of Sapindales



**Fig. 12.** Chromosome number of tropical plants: **A**, *Melia azedarach*,  $2n = 28$ ; **B**, *Eugenia zigzag*,  $2n = 22$ ; **C**, *Caularthron bicornutum*,  $2n = 40$ ; **D**, *Triplaris gardneriana*,  $2n = 22$ ; **E**, *Randia armata*,  $2n = 40$ ; **F**, *Ruta chalepensis*,  $2n = 40$ ; **G**, *Filicium decipiens*,  $2n = 32$ ; **H**, *Paullinia pinnata*,  $2n = 24$ ; **I**, *Sapindus saponaria*,  $2n = 28$ ; **J**, *Serjania salzmanniana*,  $2n = 24$ ; **K**, *Talisia esculenta*,  $2n = 32$ ; **L**, *Homalolepis bahiensis*,  $2n = 32$ ; **M**, *Priva bahiensis*,  $2n = 36$ . — Scale bar = 10  $\mu\text{m}$ .



**Fig. 13.** Species with new chromosome records: **A**, *Astronium fraxinifolium*; **B**, *Schinopsis brasiliensis*; **C**, *Commiphora leptophloeos*; **D**, *Utricularia quelchii*; **E**, *Amorimia septentrionalis*; **F**, *Cedrela fissilis*; **G**, *Eugenia zigzag*; **H**, *Triplaris gardneriana*; **I**, *Randia armata*; **J**, *Serjania salzmanniana*; **K**, *Priva bahiensis*. — Photos: Joel M.P. Cordeiro.



makes it difficult to establish the basic ancestral chromosome number for that order. Raven (1975) proposed  $x = 7$ , with rapid evolution to  $x = 14$  and later  $x = 13$ , justified by the frequent occurrence of these numbers in Anacardiaceae, Burseraceae, Meliaceae, Simaroubaceae and Sapindaceae. More recently, Guimarães & Forni-Martins (2022) suggested  $x = 6$ , with derivations for  $x = 5$  and  $x = 7$  in the basal clades, while the families with the highest number of species would be derived from a common polyploid ancestor with  $x = 14$ .

Among the new chromosome records for the other families, stand out *Amorimia septentrionalis*, *Eugenia zigzag* and *Utricularia quelchii*. *Amorimia* was the only Neotropical genus of the Malpighioid clade (Malpighiaceae) without chromosome records (Almeida, 2018). The number  $2n = 20$  for *A. septentrionalis* presented here is similar to that found in closely related genera, especially *Malpighia* L. and *Mascagnia* (DC.) Bertero (Rice & al., 2015). *Eugenia zigzag* (Myrtaceae) is a recently described species (Sobral & al., 2018), known so far only by the type material from a dry forest (Caatinga) in a single municipality in Bahia State. The record of  $2n = 22$  confirms the predominance of this chromosome number for most representatives of *Eugenia* L. (Costa & Forni-Martins, 2006; Rice & al., 2015). *Utricularia quelchii*, in turn, stands out for being endemic to the Pantepuys in South America, a region with few plants analyzed in terms of chromosome number due to difficult access and limitations in their cultivation. *Utricularia quelchii* exhibited  $2n = 18$  unlike most species of *Utricularia* L., which commonly have higher chromosome numbers (Rice & al., 2015).

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#### IAPT chromosome data 40/9

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\* First chromosome count for the species.

\*\* First chromosome count for the genus.

#### POACEAE

\*\**Arthropogon villosus* Nees

$2n = 40$ , CHN. Brazil, Distrito Federal, Brasília, Fercal, 15.5075°S, 47.9491666°W, 834 m, 27 Nov 2014, R.C. Oliveira & A.S. Silva 2969 (UB) [Fig. 14A]; Brazil, Distrito Federal, Brasília, Fercal, 15.5475°S, 47.9538889°W, 923 m, 27 Nov 2014, R.C. Oliveira & A.S. Silva 2971 (UB).

*Mesosetum bifarium* (Hack.) Chase

$2n = 16$ , CHN. Brazil, Goiás, Alto Paraíso de Goiás, Buddhist center of Chapada dos Veadeiros, close to Serra da Cobra, 14.1502778°S, 47.5891666°W, 1171 m, 6 Jun 2014, A.R.O. Ribeiro 391 (UB) [Fig. 14B].

*Mesosetum cayennense* Steud.

$2n = 20$ , CHN. Brazil, Goiás, Pirenópolis, Serra dos Pirineus State Park, 15.7975°S, 48.8122222°W, 1196 m, 19 May 2014, A.R. O. Ribeiro & R.C. Oliveira 362 (UB) [Fig. 15A]; Brazil, Goiás, Flores de Goiás, 12 km from the roundabout of flowers, right side of BR, 14.8972222°S, 46.9511111°W, 21 Mar 2013, P.A. Reis, A.R.O. Ribeiro & J.E.Q. Faria 181 (UB).

\**Mesosetum chlorostachyum* (Döll) Chase

$2n = 8$ , CHN. Brazil, Amazonas, Manaus, Cabeceira do São José, a tributary of the Rio Negro, Chácara Nossa Senhora de Fátima, Careiro da Várzea, 03.0208334°S, 60.1597222°W, 27 m, 28 Apr 2015, R.C. Oliveira & al. 3033 (UB) [Fig. 14C,D].

*Mesosetum elythrochaetum* (Hack.) Swallen

$2n = 24$ , CHN. Brazil, Goiás, Alto Paraíso de Goiás, National Park Chapada dos Veadeiros, 14.1708334°S, 47.8275°W, 1006 m, 3 Jun 2014, A.R.O. Ribeiro 377 (UB) [Fig. 15B].



\**Mesosetum exaratum* (Trin.) Chase

$2n = 26$ , CHN. Brazil, Minas Gerais, Serra do Cipó, MG 010, towards Alto Palácio, 19.2888889°S, 43.5922222°W, 23 Nov 2013, A.R.O. Ribeiro & I.A. Conceição 351 (UB) [Fig. 16A,B].

\**Mesosetum gibbosum* Renvoize & Filg.

$2n = 24$ , CHN. Brazil, Bahia, Rio de Contas, road to Jussiape, ca. 1 km of Rio de Contas, 13.5925°S, 41.7986111°W, 1100 m, 19 Apr 2015, A.R.O. Ribeiro & J.E.Q. Faria 408 (UB) [Fig. 14E].

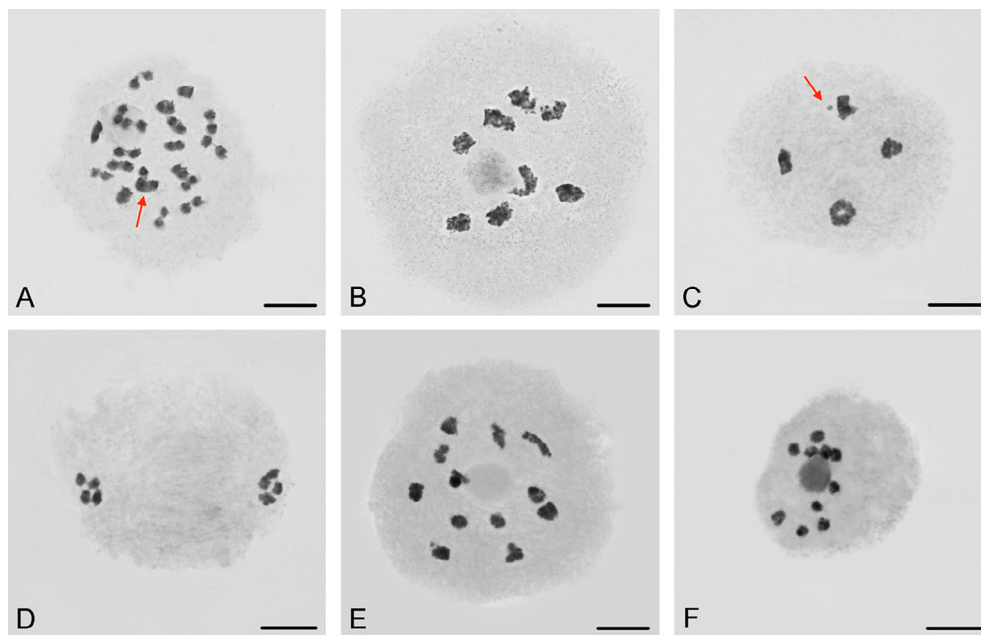
\*\**Spheneria kegelii* (Müll.Hal.) Pilg.

$2n = 20$ , CHN. Brazil, Amazonas, Manaus, Campina do INPA, 15.9786111°S, 47.9552778°W, 25 Apr 2015, R.C. Oliveira & al. 3007 (UB) [Fig. 14F].

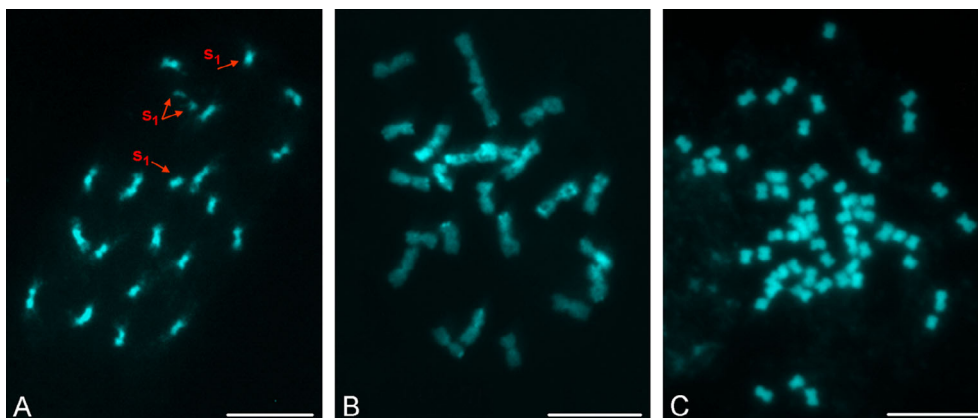
*Tatianyx arnacites* (Trin.) Zuloaga & Soderstr.

$2n = 60$ , CHN. Brazil, Bahia, Rio de Contas, road to Jussiape, ca. 1 km of Rio de Contas, 13.5925°S, 41.7986111°W, 1100 m, 19 Apr 2015, A.R.O. Ribeiro & J.E.Q. Faria 411 (UB) [Fig. 15C].

In Poaceae, Panicoideae is the most diverse subfamily in the tropical region, comprising 3325 species (GPWG, 2001; Soreng & al., 2015, 2017, 2022; Burke & al., 2016; Huang & al., 2022). In Panicoideae, the basic chromosome numbers of  $x = 9$  and  $x = 10$  are the most frequent, but the basic chromosome numbers of  $x = 3$  to 8,  $x = 11$ ,  $x = 12$  and  $x = 14$  have also been reported (Celarier & Paliwal, 1957; Rao, 1975; De Wet, 1987; GPWG, 2001; Hilu, 2004; Morrone & al., 2006; Sede & al., 2010; Goldblatt & Johnson, 2023). The chromosome numbers of  $2n = 6$  and  $2n = 8$  ( $x = 3$ ,

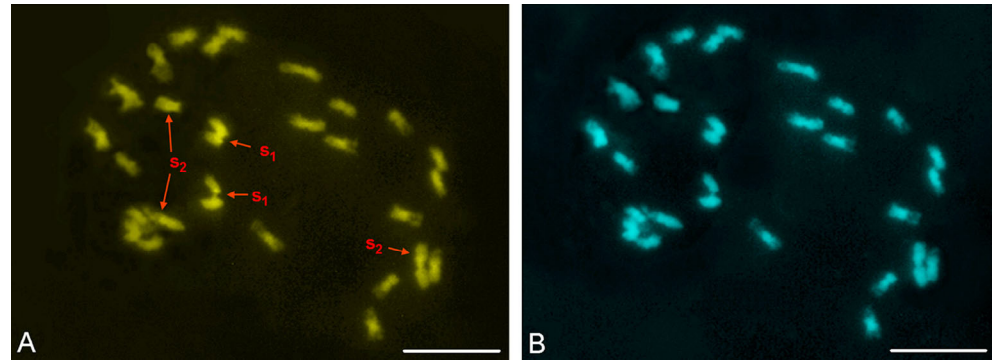


**Fig. 14.** Meiotic cells of species of the subtribe Arthropogoninae (*Arthropogon*, *Mesosetum*) and Paspalinae (*Spheneria*). **A**, *A. villosus*, R.C. Oliveira & A.S. Silva 2969, diakinesis with 20 bivalents ( $2n = 40$ ), arrow indicates overlapping of two bivalents; **B**, *M. bifarium*, A.R.O. Ribeiro 391, diakinesis with 8 bivalents ( $2n = 16$ ); **C & D**, *M. chlorostachyum*, R.C. Oliveira & al. 3033: **C**, diakinesis with 4 bivalents ( $2n = 8$ ), arrow indicates satellite associated with a chromosome pair (bivalent); **D**, Anaphase I showing segregation of 4 chromosomes to each pole ( $2n = 8$ ); **E**, *M. gibbosum*, A.R.O. Ribeiro & J.E.Q. Faria 408, diakinesis with 12 bivalents ( $2n = 24$ ); **F**, *S. kegelii*, R.C. Oliveira & al. 3007, diakinesis with 10 bivalents ( $2n = 20$ ). — Scale bars = 10  $\mu$ m.



**Fig. 15.** Mitotic cells of species of the subtribe Arthropogoninae (*Mesosetum*, *Tatianyx*) showing chromosomes stained with DAPI. **A**, *M. cayennense*, A.R.O. Ribeiro & R.C. Oliveira 362, metaphase with  $2n = 20$  chromosomes, arrows indicate a satellited chromosome pair (S1); **B**, *M. elytrochaetum*, A.R.O. Ribeiro 377, metaphase with  $2n = 24$  chromosomes; **C**, *T. arnacites*, A.R.O. Ribeiro & J.E.Q. Faria 411, metaphase with  $2n = 60$  chromosomes. — Scale bars = 10  $\mu$ m.

**Fig. 16.** Mitotic cell of *Mesosetum exaratum* (subtribe Arthropogoninae), A.R.O. Ribeiro & I.A. Conceição 351, showing metaphase with  $2n = 26$  chromosomes. **A**, Chromosomes stained with CMA (yellow), arrows indicate the satellited chromosome pairs (S1, S2); **B**, Chromosomes stained with DAPI (blue). — Scale bars = 10  $\mu$ m.



$x = 4$ ) are rare and the lowest recorded among the tropical grasses, being exclusively reported in *Iseilema* Andersson (tribe Andropogoneae) and *Mesosetum* Steud. (tribe Paspaleae, subtribe Arthropogoninae) (Celarier & Paliwal, 1957; Rao, 1975; De Wet, 1987; Morrone & al., 2012; Ribeiro & al., 2015, 2019; Sousa & al., 2017).

The majority of the genera of the subtribe Arthropogoninae have records of chromosome numbers that are multiples of  $x = 10$ , which is also the basic chromosome number of the tribe Paspaleae (Morrone & al., 2012). Many species of Arthropogoninae and Paspaleae have a restricted geographical distribution or are still little known, regarding their morphology, cytology, molecular genetics and reproduction, notably in *Arthropogon* Nees, *Mesosetum*, *Spheneria* Kuhlm., and *Tatianyx* Zuloaga & Soderstr. (Filgueiras, 1986, 1989; Morrone & al., 2012; Filgueiras & al., 2015; Silva & al., 2016; Oliveira & al., 2019a,b; Ribeiro & al., 2023a,b; Silva & al., 2023a,b). In *Mesosetum*, there are two basic chromosome numbers recorded,  $x = 4$  ( $2n = 8, 16, 24$  and  $32$ ), as well as  $x = 10$  ( $2n = 20$  and  $60$ ), highlighting that  $x = 4$  is not found in other genera of the subtribe (Ribeiro & al., 2015; Sousa & al., 2017; Ribeiro & al., 2019). The chromosome numbers in other genera of Arthropogoninae are known only for *Homolepis aturensis* (Kunth) Chase ( $2n = 20$ ), *H. isocalyctia* (G.Mey.) Chase ( $2n = 40$ ), *Phanopyrum gymnocarpon* (Elliott) Nash ( $2n = 40$ ), and *T. arnatices* (Trin.) Zuloaga & Soderstr. ( $2n = 60$ ) (Gould & Soderstrom, 1967; Pohl & Davidse, 1971; Kessler & Hatch, 1984; Morrone & al., 1995).

This study was performed in 11 accessions belonging to nine species of the subtribes Arthropogoninae and Paspalinae. The voucher specimens were deposited in the herbarium UB. The chromosome numbers were determined by the analysis of at least 10 cells with a good chromosome spreading, according to methods described by Ribeiro & al. (2015).

For the meiotic analysis, the samples were taken from young inflorescences and immersed in the fixative solution 3 : 1 (ethanol : acetic acid, v/v) for 24 h at room temperature and stored in 70% ethanol solution at 4°C, according to Ribeiro & al. (2015).

For the mitotic analysis, the root tips were pretreated with 2 mM 8-hydroxyquinoline for 10 h at 10°C, fixed in Carnoy solution (ethanol/acetic acid, 3 : 1, v/v) for 2–24 h at room temperature and then stored at –20°C. The samples were digested with enzymatic solution containing 2% cellulase (Onozuka-SERVA Electrophoresis, Heidelberg, Germany) and 20% pectinase (Sigma-Aldrich, Darmstadt, Germany) for 90 min at 37°C. The slides and the chromosomes were prepared with acetic acid 60% (aqueous solution, v/v), using the spreading technique based on Ruban & al. (2014). The CMA/DAPI staining was performed in the best slides, according to the method described by Cabral & al. (2014). The slides were aged for 3 days,

stained with CMA (chromomycin A3) 0.5 mg/ml for 1 h and restained with DAPI (4',6-diamidino-2-phenylindole) 2 mg/ml for 30 min.

The images of meiotic chromosomes were acquired and analyzed by the Leica DM 750 microscope and software Leica Application Suite (v4.5). The images of mitotic chromosomes were captured using the Leica DMRB fluorescent microscope equipped with the CoHu digital camera and the Leica Q-FISH software.

The chromosome numbers were determined in 11 new accessions belonging to nine species of tribe Paspaleae, eight of which belong to the subtribe Arthropogoninae and one of which belongs to the subtribe Paspalinae. First chromosome counts are reported here for five species: *Arthropogon villosus* ( $2n = 40$ ), *Mesosetum chlorostachyum* ( $2n = 8$ ), *M. exaratum* ( $2n = 26$ ), *M. gibbosum* ( $2n = 24$ ), and *Spheneria kegelii* ( $2n = 20$ ). These results are the first records of chromosome numbers in the genera *Arthropogon* (subtribe Arthropogoninae) and *Spheneria* (subtribe Paspalinae).

The chromosome number of  $2n = 26$  ( $x = 13$ ) found in *Mesosetum exaratum* is here registered for the first time in the subfamily Panicoideae. *Mesosetum exaratum* is endemic to Brazil and this basic chromosome number of  $x = 13$  is rare and cited only for eight genera of Poaceae, of which six belong to the subfamily Pooideae, one to Aristidoideae, and one to Chloridoideae. In *M. exaratum* (accession A.R.O. Ribeiro & I.A. Conceição 351) two pairs of the proximal Nucleolus Organizer Region (NOR) were identified, visible as distended regions (Fig. 16A,B). This centromeric NORs were positively stained with the fluorochrome CMA (CMA+) and negatively stained with the fluorochrome DAPI (DAPI–), a common pattern in plants (Roa & Guerra, 2012). Proximal NORs may be related to centric fission/fusion processes (Souza & al., 2012) resulting in the origin of  $x = 13$ . In *Deschampsia* P.Beauv. (subfamily Pooideae), for example,  $x = 13$  arose by a reduction from the secondary basic number of  $x = 14$ , which in turn originated by polyploidy from  $x = 7$  (Cardone & al., 2009; Amosova & al., 2015).

In Arthropogoninae, our chromosome counts of  $2n = 40$  in *Arthropogon villosus* and  $2n = 60$  in *Tatianyx arnatices* and the previous records of  $2n = 20$  in *Homolepis aturensis*,  $2n = 40$  in *H. isocalyctia* and  $2n = 40$  in *Phanopyrum gymnocarpon* (Gould & Soderstrom, 1967; Pohl & Davidse, 1971; Kessler & Hatch, 1984; Morrone & al., 1995) support  $x = 10$  as the basic chromosome number of the subtribe Arthropogoninae and tribe Paspaleae, corroborating the molecular phylogeny of Morrone & al. (2012).

*Arthropogon* comprises five species recognized by the paniculiform inflorescence and laterally compressed spikelets (Filgueiras, 1982; Filgueiras & al., 2001, 2015; POWO, 2023; Ribeiro & al., 2023a). *Arthropogon villosus* is a perennial species that has a geographical distribution restricted to Bolivia and Brazil, which is

distinguished by the convolute leaf sheaths, leaf blades 2.6–10.0 mm wide, and usually the fire-induced flowering (Filgueiras, 1982; Filgueiras & al., 2001, 2015; Ribeiro & al., 2023a), as observed in the accession *R.C. Oliveira & A.S. Silva 2971*. Exceptionally, there are no burnt leaves in *R.C. Oliveira & A.S. Silva 2969*, which does not appear to have the fire-induced flowering. In both accessions the same chromosome number ( $2n = 40$ ) and regular pairing in 20 bivalents (Fig. 14A) was registered, suggesting tetraploidy with apparent process of cytological diploidization, as described by Li & al. (2021).

*Spheneria* is a monospecific genus represented by *S. kegelii*, which is restricted to Brazil, Guyana, and Suriname (POWO, 2023; Ribeiro & al., 2023b). *Spheneria kegelii* has an inflorescence composed of 2–3 racemiform branches, which are alternate on the inflorescence axis. The accession analyzed (*R.C. Oliveira & al. 3007*) is typically diploid with pairing in 10 bivalents ( $2n = 20$ ) (Fig. 14F).

The chromosomes of *Tatianyx arnaces* ( $2n = 60$ ) were for the first time observed in mitosis in the present study (Fig. 15C), confirming the previous chromosome counting of another accession made at meiosis by Morrone & al. (1995).

*Mesosetum chlorostachyum* and *M. gibbosum* are endemic to South America (Filgueiras, 1986, 1989; Filgueiras & al., 2015; Silva & al., 2023a) and their chromosome numbers were recorded for the first time in this study. *Mesosetum chlorostachyum* is an annual species restricted to the Amazon forest in Brazil and Venezuela, while *M. gibbosum* is a perennial species occurring only in the Cerrado of the states of Bahia and Tocantins (Filgueiras, 1986, 1989; Filgueiras & al., 2015; Silva & al., 2023a). In *M. chlorostachyum*, the pairing in four bivalents ( $2n = 8$ ) (Fig. 14C) is compatible with diploidy. In *M. gibbosum*, the pairing in 12 bivalents ( $2n = 24$ ) (Fig. 14E) suggests hexaploidy with evident cytological diploidization (Li & al., 2021).

In *Mesosetum*, the chromosome numbers determined by the present study corroborate the cytological data obtained in previous works (Gould, 1966; Gould & Soderstrom, 1967; Pohl & Davidse, 1971; Davidse & Pohl, 1972; Kessler & Hatch, 1984; Morrone & al., 2006; Sede & al., 2010; Silva & al., 2012; Ribeiro & al., 2015; Sousa & al., 2017; Ribeiro & al., 2019), supporting at least three chromosomal lineages with distinct basic chromosome numbers in the genus: the first with  $x = 10$ , including three species (*M. cayennense*, *M. ferrugineum*, *M. rottboellioides* (Kunth) Hitchc.), the second with  $x = 4$ , comprising 14 species (*M. alatum* Filg., *M. anuum* Swallen, *M. ansatum* (Trin.) Kuhl., *M. bifarium*, *M. chaseae* Lucas, *M. chlorostachyum*, *M. compressum* Swallen, *M. elytrachaeatum*, *M. gibbosum*, *M. loliiforme* (Hochst. ex Steud.) Chase, *M. longiaristatum* Filg., *M. pappophorum* (Nees) Kuhl., *M. pittieri* Hitchc., *M. sclerochloa* (Trin.) Hitchc.), and the third monospecific with  $x = 13$  represented by *M. exaratum*.

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## IAPT chromosome data 40/10

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\* First chromosome count for the species

\*\* New cytotype for the species

## EUPHORBIACEAE

*Croton adamantinus* Müll.Arg.

\* $2n = 20$ , CHN. Brazil, Paraíba, Areia, Pau-ferro Forest, edge of the lane past the chapel, 06°58'27"S, 35°44'17"W, 645 m, 1 Mar 2016, *L.P. Felix 15870* (EAN) [Figs. 17A, 19A].

*Croton adamantinus* is a shrubby species, endemic to Brazil, with occurrence restricted to the Northeast (except Maranhão and Alagoas) and Southeast (Minas Gerais) regions, associated with the Caatinga domain, in semi-arid vegetation (Caruzo & al., 2020). The present record of the occurrence of this species in Paraíba suggests that *C. adamantinus* also adapts to environments in the humid forest domain of the Atlantic Forest. The count of  $2n = 20$  coincides with most chromosome records for the genus.

*Croton argyrophyllus* Kunth

$2n = 20$ , CHN. Brazil, Bahia, Itaberaba, BR 424, 12°31'15"S, 40°07'04"W, 231 m, 3 May 2015, *L.P. Felix 15575* (EAN)

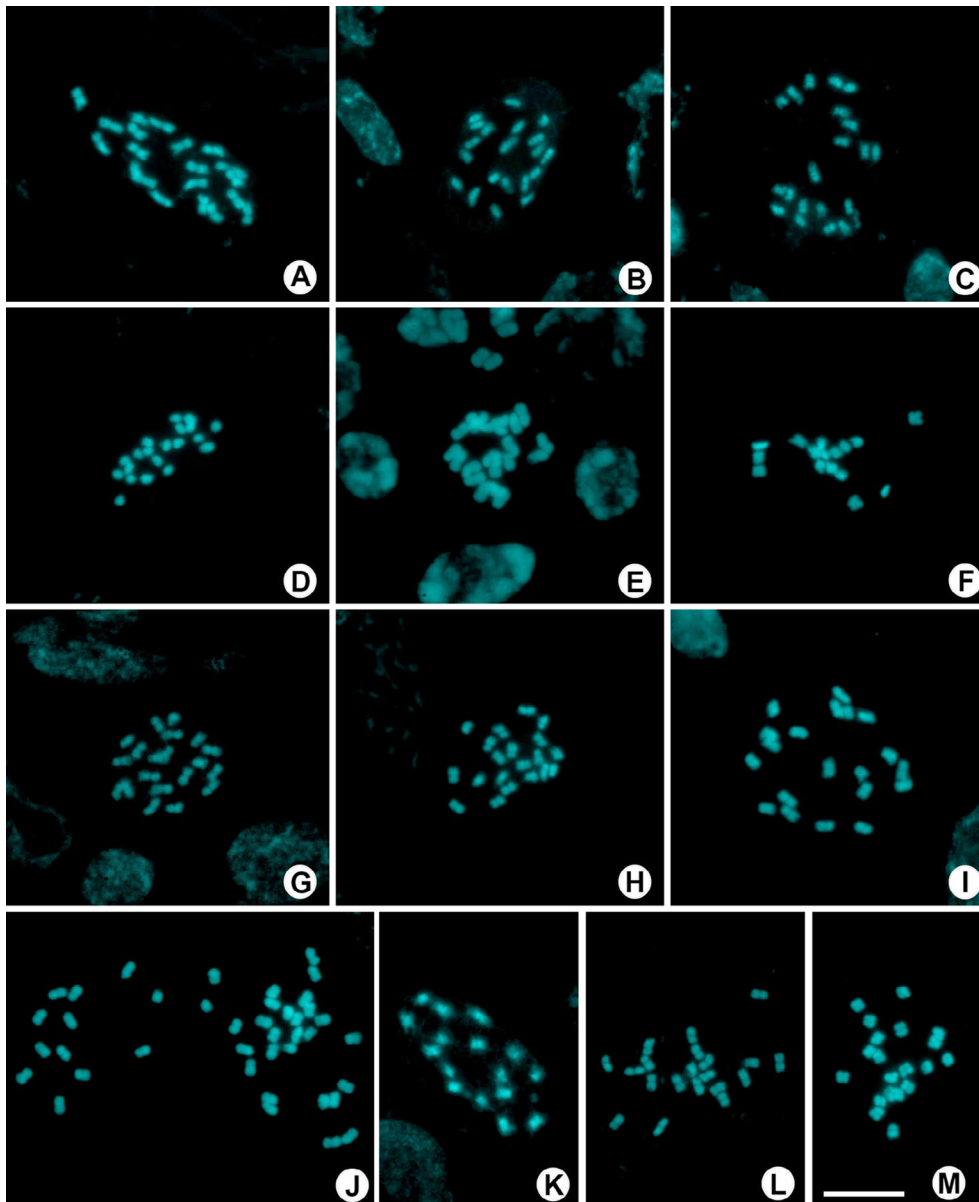
[Figs. 17B, 19B]; Brazil, Pernambuco, Brejo da Madre de Deus, Tabocas Small Farm, 08°09'07"S, 36°19'38"W, 502 m, 29 Jun 2015, *L.P. Felix 15636* (EAN) [Fig. 17C].

*Croton argyrophyllus* occurs in South America in Colombia, Venezuela, Peru, Bolivia and Paraguay (Caruzo & al., 2020). In Brazil there are records for the Northeast Region (except Rio Grande do Norte) and in the North Region (Roraima, Rondônia) in the Caatinga and Amazon domains. The present record of  $2n = 20$  confirms the two counts previously reported for the species (Pôrto & al., 2014).

*Croton blanchetianus* Baill.

$2n = 20$ , CHN. Brazil, Paraíba, Esperança, Timbaúba Farm, over rocky outcrop, 07°01'01"S, 35°52'49"W, 640 m, 16 Jun 2015, *A.S. Santos 30* (EAN) [Figs. 17D, 19C].

*Croton blanchetianus* is an endemic species of Brazil with distribution restricted to the Northeast (except Maranhão) and Southeast (Minas Gerais) regions, occurring exclusively in the Caatinga



**Fig. 17.** Mitotic metaphase.

A, *Croton adamantinus*,  $2n = 20$ ; B & C, *Croton argyrophyllus*,  $2n = 20$ ; D, *Croton blanchetianus*,  $2n = 20$ ; E, *Croton campestris*,  $2n = 20$ ; F, *Croton glandulosus*,  $2n = 16$ ; G & H, *Croton grewiooides*,  $2n = 20$ ; I, *Croton heliotropiifolius*,  $2n = 20$ ; J, *Croton heliotropiifolius*,  $2n = 40$ ; K, *Croton hirtus*,  $2n = 16$ ; L & M, *Croton jacobinensis*,  $2n = 20$ .

domain (Caruzo & al., 2020). The present record of  $2n = 20$  confirms previous counts for the species (Pôrto & al., 2014; Rice & al., 2015).

*Croton campestris* A.St.-Hil.

\* $2n = 20$ , CHN. Uruguay, Maldonado, Punta de Punta Ballena, 34°53'46"S, 55°02'16"W, 61 m, 14 Oct 2016, *L.P. Felix 16015* (EAN) [Fig. 17E].

*Croton campestris* is a species distributed only in South America, with records for Brazil, Bolivia and Paraguay (Jørgensen & al., 2014). For Brazil, its occurrence has been confirmed for the North (Tocantins), Northeast (except Maranhão, Alagoas and Sergipe), Midwest (Goiás, Mato Grosso do Sul) and Southeast (except São Paulo) regions. The present occurrence record for Uruguay suggests a wider distribution of the species in South America. Our count of  $2n = 20$  for this species coincides with most chromosome records of the genus.

*Croton floribundus* Spreng.

\*\* $2n = 100$ , CHN. Brazil, Paraíba, Areia, Pau-ferro Forest, 06°58'27"S 35°44'17"W, 646 m, 1 Mar 2016, *L.P. Felix 15874* (EAN) [Figs. 18H, 19J]; Brazil, Pernambuco, Taquaritinga do Norte, Hill

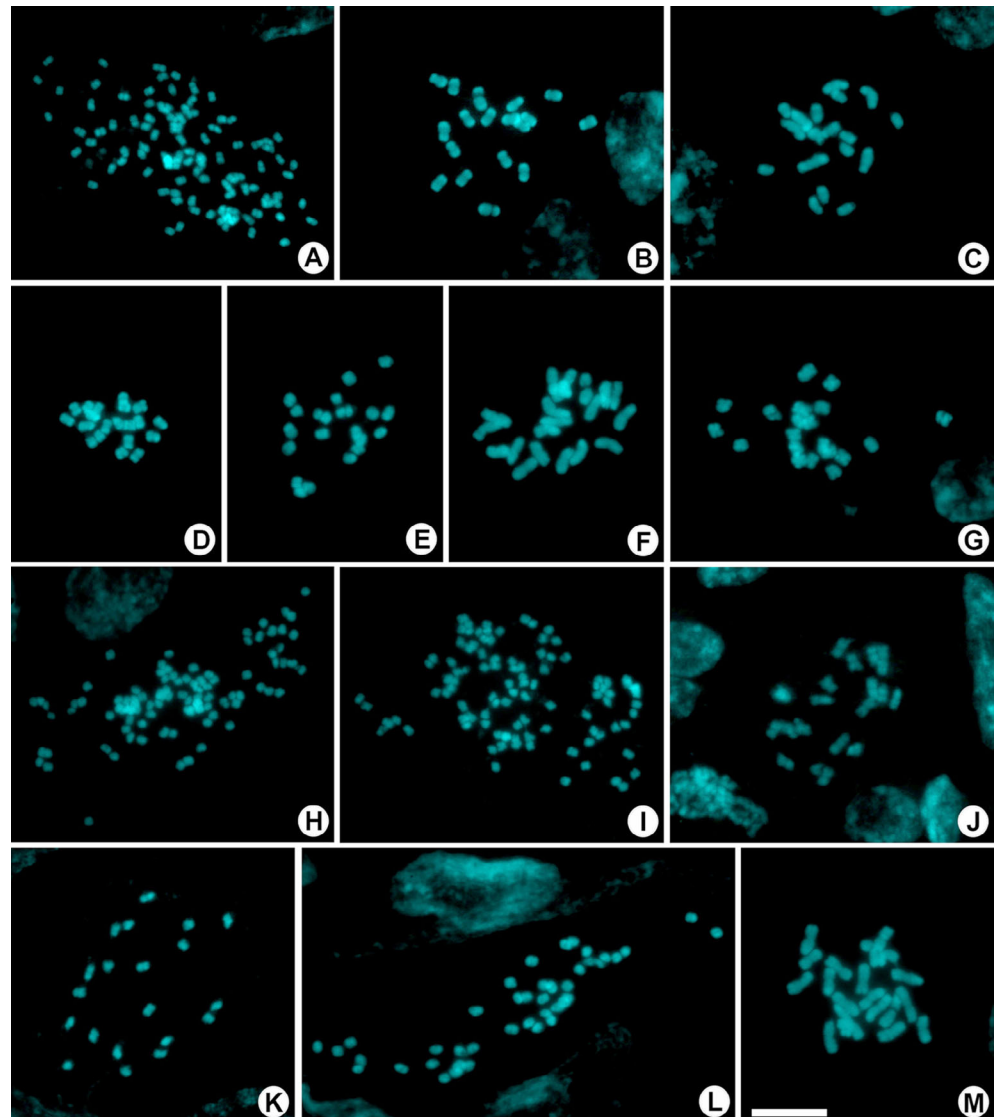
of antennas, 07°54'28"S 36°01'39"W, 1100 m, 13 May 2016, *L.P. Felix 15879* (EAN) [Fig. 18I].

*Croton floribundus* is a tree species, found in environments of the Atlantic Forest, distributed only in South America, with records for Brazil and Paraguay (Lima & Pirani, 2003; Paña-Chocarro & al., 2010). In Brazil, it was reported for the Northeast (except Maranhão, Piauí, Rio Grande do Norte and Sergipe), Central-West (except Goiás), Southeast and South (Paraná) regions. Our count of  $2n = 100$  is unprecedented and differs from the previous record of  $2n = 112$  for this species (Silvestrini & al., 2013).

*Croton glandulosus* L.

$2n = 16$ , CHN. Brazil, Paraíba, Jacaraú, Timbó, Riacho do meio, 06°36'53"S, 35°17'28"W, 192 m, 5 Mar 2016, *E.M. Almeida & G.F. Pessoa 1581* (EAN) [Fig. 17F].

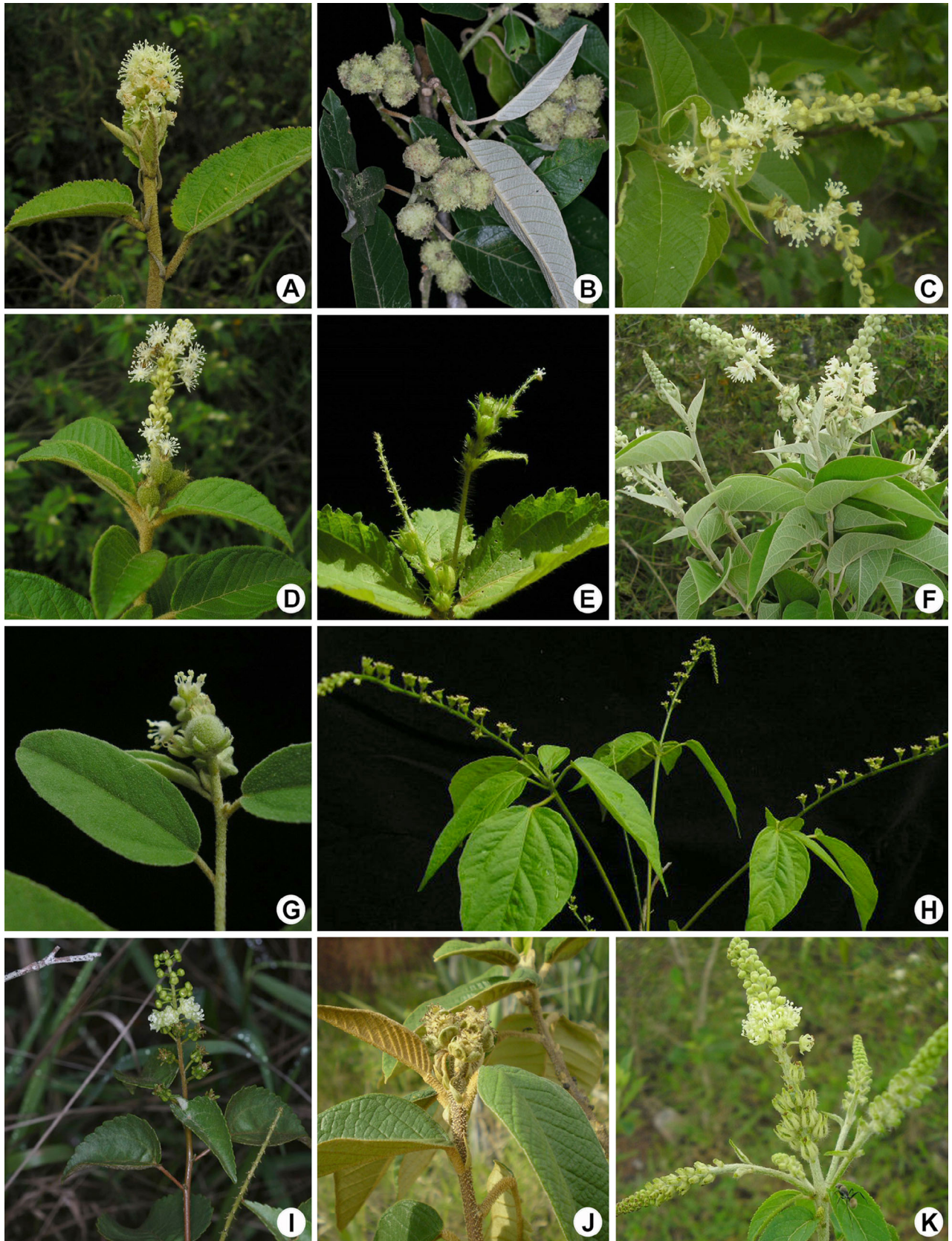
*Croton glandulosus* is a species widely distributed throughout the Neotropics, from the southern United States and Mexico to Central America, the Antilles and tropical South America (Standley & Steyermark, 1949). They are subshrubby ruderal plants occurring in different environments, such as Cerrado, coastal vegetation, dry and humid forests (Silva & al., 2010) in virtually all types of



**Fig. 18.** Mitotic metaphase.

A, *Croton limae*,  $2n = 100$ ;  
 B, *Croton lombardianus*,  $2n = 20$ ;  
 C, *Croton pedicellatus*,  $2n = 18$ ;  
 D, *Croton pulegioidorus*,  $2n = 20$ ;  
 E, *Croton pulegioidorus*,  $2n = 22$ ;  
 F, *Croton rudolphianus*,  $2n = 20$ ;  
 G, *Croton sellowii*,  $2n = 20$ ;  
 H & I, *Croton floribundus*,  
 $2n = 100$ ; J, *Croton tricolor*,  
 $2n = 20$ ; K, *Croton triqueter*,  
 $2n = 20$ ; L, *Croton triqueter*,  
 $2n = 40$ ; M, *Croton urticifolius*,  
 $2n = 20$ .





**Fig. 19.** Species with chromosome count. **A**, *Croton adamantinus*; **B**, *Croton argyrophyllus*; **C**, *Croton blanchetianus*; **D**, *Croton heliotropiifolius*; **E**, *Croton hirtus*; **F**, *Croton jacobinensis*; **G**, *Croton pedicelatus*; **H**, *Croton pulegioidorus*; **I**, *Croton rudolphianus*; **J**, *Croton floribundus*; **K**, *Croton urticifolius*. — Photos: A, C, D, F & K: Amanda S. Santos; B, E & G–J: Leonardo P. Felix.

vegetation in the country from the Amazon to the Pampas Gaúchos in Rio Grande do Sul. Our count of  $2n = 16$  confirms previously reported counts for this species (Pôrto & al., 2014; Rice & al., 2015).

*Croton grewoides* Baill.

\* $2n = 20$ , CHN. Brazil, Bahia, Barra da Estiva, Capão da volta, road to Ibicoara, 13°26'49"S, 31°29'20"W, 1 May 2015, *L.P. Felix 15559* (EAN) [Fig. 17G]; Brazil, Pernambuco, Pesqueira, Chucuru Reserve, 08°19'15"S, 36°43'12"W, 1122 m, 30 Jun 2015, *L.P. Felix 15650* (EAN) [Fig. 17H].

*Croton grewoides* is a species that has an occurrence record only for Bolivia and Brazil, in Brazil it has a restricted distribution in the Northeast (except Maranhão) and Southeast (Minas Gerais) regions, in Caatinga environments (Jørgensen & al., 2014). The  $2n = 20$  record for the species coincides with most chromosomal records for *Croton*.

*Croton heliotropiifolius* Kunth

\*\* $2n = 20$ , CHN. Brazil, Paraíba, Areia, Chã de Jardim, 06°57'50"S, 35°44'46"W, 22 Oct 2014, *A.S. Santos 02* (EAN) [Figs. 17I, 19D].

$2n = 40$ , CHN. Brazil, Ceará, Iguatu, 06°22'55"S, 39°07'04"W, 435 m, 16 Apr 2014, *L.P. Felix & E.M. Almeida 14914* (EAN) [Fig. 17J].

*Croton heliotropiifolius* is a species distributed throughout the Northeast Region of Brazil, in addition to the Southeast (Minas Gerais) and Midwest (Goiás) regions, occurring in the Caatinga, Atlantic Forest, Cerrado and Amazon domains, also with records of occurrence in Peru (Macbride, 1951). Of the two populations analyzed, one was diploid with  $2n = 20$  and the other tetraploid with  $2n = 40$ . Of these counts, only the last one coincided with the previous chromosomal record for the species (Pôrto & al., 2014).

*Croton hirtus* L'Hér.

$2n = 16$ , CHN. Brazil, Paraíba, Jacaraú, Timbó, Riacho do meio, 06°36'53"S, 35°17'28"W, 192 m, 5 Mar 2016, *E.M. Almeida & G.F. Pessoa 1582* (EAN) [Figs. 17K, 19E].

*Croton hirtus* is widely distributed throughout the tropical regions of the world, occurring in Africa, Oceania, Central America and South America (Jørgensen & al., 2014). In Brazil, the species occurs in the North (Pará, Tocantins), Northeast (all states), Midwest (Goiás), Southeast (Minas Gerais, São Paulo) and South (Paraná, Santa Catarina) regions. The present count of  $2n = 16$  confirms previous counts for the species (Soontornchainaksaeng & al., 2003).

*Croton jacobinensis* Baill.

$2n = 20$ , CHN. Brazil, Paraíba, Areia, Chã de Jardim, 06°57'50"S, 35°44'46"W, 641 m, 22 Oct 2014, *A.S. Santos 03* (EAN) [Figs. 17L, 19F]; Brazil, Paraíba, Jacaraú, Timbó, Riacho do meio, 06°36'53"S, 35°17'28"W, 192 m, 5 Mar 2016, *E.M. Almeida & G.F. Pessoa 1579* (EAN) [Fig. 17M].

*Croton jacobinensis* is an endemic species of Brazil with exclusive distribution in the Northeast (except Alagoas and Sergipe) and Southeast (Minas Gerais) regions with exclusive occurrence in the Caatinga domain. Our count of  $2n = 20$  for two populations confirms the previous chromosomal record for the species (Pôrto & al., 2014).

*Croton limae* A.P.S.Gomes, M.F.Sales & P.E.Berry

\* $2n = 100$ , CHN. Brazil, Bahia, Jussiape, road to Ibicoara, 13°27'10"S, 41°32'20"W, 1062 m, 1 May 2015, *L.P. Felix 15554* (EAN) [Fig. 18A].

*Croton limae* is a tree or shrub species endemic to Brazil, with occurrence records exclusive to the Northeast Region (except Maranhão, Rio Grande do Norte and Sergipe) in the Caatinga phytogeographic domain (Caruzo & al., 2020). The present record of  $2n = 100$  is unprecedented and, together with the count for *C. floribundus*, constitutes one of the highest chromosome numbers reported for the genus.

*Croton lombardianus* Croizat

\* $2n = 20$ , CHN. Uruguay, Maldonado, Punta de Punta Ballena, 34°22'50"S 54°40'36"W, 61 m, 15 out 2016, *L.P. Felix 16030* (EAN) [Fig. 18B].

*Croton lombardianus* is an endemic species of Uruguay, which occurs in dry and desert environments (Govaerts & al., 2000). The record of  $2n = 20$  constitutes one of the most common chromosome numbers reported for the genus.

*Croton pedicellatus* Kunth

$2n = 18$ , CHN. Brazil, Paraíba, Sertãozinho, Canafistula Small Farm, 06°44'06"S, 35°27'30"W, 130 m, 25 Jan 2018, *J.M.P. Cordeiro 1259* (EAN) [Figs. 18C, 19G].

*Croton pedicellatus* is a subshrub species with occurrence records for Mexico and South America (Bolivia, Brazil, Colombia, Peru). In Brazil, it is distributed across the five regions, in Caatinga, Atlantic Forest and Cerrado environments (Macbride, 1951; Forzza, 2010; Villaseñor Ríos, 2016). The record of  $2n = 18$  coincides with the previous chromosome record for this species (Pôrto & al., 2014).

*Croton pulegioides* Baill.

$2n = 20$ , CHN. Brazil, Bahia, Jussiape, road to Ibicoara, 13°22'47"S, 41°33'23"W, 816 m, 1 May 2015, *L.P. Felix 15553* (EAN) [Fig. 18D].

\*\* $2n = 22$ , CHN. Brazil, Paraíba, Campina Grande, São José da Mata Forest, 07°11'02"S, 35°59'01"W, 667 m, 16 Jun 2015, *A.S. Santos 19* (EAN) [Figs. 18E, 19H].

*Croton pulegioides* is endemic to Brazil, restricted to the Northeast (except Rio Grande do Norte, Maranhão, Sergipe and Alagoas), Southeast (Minas Gerais) and Midwest (Goiás) regions in the Caatinga and Cerrado domains (Forzza, 2010). The record of  $2n = 20$  coincides with the previous count reported by Porto & al. (2014) for the state of Paraíba. However, our count of  $2n = 22$ , also for Paraíba, is new for the species and corresponds to ascending dysploidy possibly mediated by centric fission.

*Croton rudolphianus* Müll.Arg.

\* $2n = 20$ , CHN. Brazil, Pernambuco, Buíque, Parma Catimbal, 08°35'37"S, 37°12'18"W, 811 m, 1 Jul 2015, *L.P. Felix 15662* (EAN) [Figs. 18F, 19I].

*Croton rudolphianus* is an endemic species of Brazil, restricted to the Northeast (except Rio Grande do Norte and Maranhão) and Southeast (Minas Gerais) regions, exclusively in vegetation of rocky outcrops of the Caatinga. The record of  $2n = 20$  for the species coincides with most chromosomal records of the genus.

*Croton sellowii* Baill.

\* $2n = 20$ , CHN. Brazil, Paraíba, Mamanguape, Rebio Guaribas, 06°43'06"S, 35°12'44"W, 211 m, 27 Nov 2014, *J.M.P. Cordeiro 600* (EAN) [Fig. 18G].

*Croton sellowii*, a terrestrial plant of shrubby habit, is an endemic species of Brazil, with occurrence restricted to the Northeast Region (Paraíba, Pernambuco, Sergipe, Alagoas, Bahia), in vegetation of rocky outcrops in the Caatinga and Atlantic Forest domains.



*Croton tricolor* Klotzsch ex Baill.

\* $2n = 20$ , CHN. Brasil, Bahia, Barra da Estiva, Capão da volta, road to Ibicoara, 13°26'49"S, 31°29'20"W, 1154 m, 1 May 2015, L.P. *Felix 15557* (EAN) [Fig. 18J].

*Croton tricolor* is distributed throughout South America (Bolivia, Brazil). In Brazil, it occurs in the Northeast (except Maranhão), Southeast (Minas Gerais, São Paulo) and South (Paraná) regions, in the Caatinga, Cerrado and Atlantic Forest domains. The record of  $2n = 20$  for the species coincides with most chromosomal records of the genus.

*Croton triqueter* Lam.

\* $2n = 20$ , CHN. Brazil, Bahia, Barra da Estiva, Capão da volta, road to Ibicoara, 13°26'49"S, 31°29'20"W, 1154 m, 1 May 2015, L.P. *Felix 15558* (EAN) [Fig. 18K].

\*\* $2n = 40$ , CHN. Brazil, Paraíba, Areia, Center of Agrarian Sciences, Federal University of Paraíba, 06°58'12"S, 35°42'47"W, 579 m, 2 Mar 2016, L.P. *Felix 15876* (EAN) [Fig. 18L].

*Croton triqueter* is widely distributed throughout South America, with records for Brazil, Bolivia, Peru and Venezuela (Macbride, 1951; Jørgensen & al., 2014). In Brazil, it occurs in the Caatinga, Atlantic Forest, Cerrado and Pantanal domains. The two chromosome numbers reported here,  $2n = 20$  and 40, are unprecedented for the species and compatible with chromosomal evolution by polyploidy for the genus *Croton*.

*Croton urticifolius* Lam.

$2n = 20$ , CHN. Brasil, Paraíba, Areia, Chã de Jardim, 06°57'50"S, 35°44'46"W, 641 m, 19 Nov 2014, A.S. Santos 04 (EAN) [Figs. 18M, 19K].

*Croton urticifolius* can be found in Bolivia and Brazil. In Brazil, it occurs in the Northeast (except Maranhão) and Southeast (except São Paulo) regions, in the Caatinga, Cerrado and Atlantic Forest domains. The record of  $2n = 20$  for the species coincides with most chromosomal records of the genus and with the previous record for this species (Pôrto & al., 2014).

*Croton* is the second-largest genus of the Euphorbiaceae family and includes about 1100 species distributed throughout the tropical and subtropical regions of the American continent, Africa and Madagascar (Stevens, 2001; Judd & al., 2009). In Brazil, 300 species of the genus are found and 230 of them (84%) are endemic (Caruzo & al., 2020). They are mainly shrubs and subshrubs, or even trees and herbaceous plants, characterized by lactescent wood, glands at the leaf base, narrow inflorescence and unisexual flowers (Webster, 1993; Barry & al., 2005; Carneiro-Torres & al., 2011). The genus is poorly known karyologically, with chromosome numbers known for only 47 species (4.2% of the genus), with records of  $2n = 16$ , 18, 20, 28, 30, 38, 40, 64 and 120 (Pôrto & al., 2014; Rice & al., 2015). Most of these species (65%) have  $2n = 20$ , suggesting  $x = 10$  as the basic haploid number of the genus (Miller & Webster, 1966).

The cytogenetic analysis followed the protocol by Guerra & Souza (2002). Root tips were pretreated in 8-hydroxyquinoline for 24 h at 10°C, fixed in ethanol-acetic acid (3 : 1; v/v) for 2 h at room temperature and stored in a freezer at -20°C. To prepare the slides, the material was digested with an enzymatic solution containing 2% cellulase (Onozuka) and 20% pectinase (Sigma) (w/v) for 1 h at 37°C. Then, the slides were prepared by crushing method, in a drop of 45% acetic acid, and the coverslips were removed in liquid nitrogen. The slides were stained with 10 µl of DAPI (4',6-diamidino-

2-phenylindole – 2 µg/ml) for 30 min, and mounted in McIlvaine/glycerol buffer medium and subsequently analyzed and photographed in a Zeiss epifluorescence photomicroscope, with camera Axio Cam MRC5 video camera using Axiovision v.4.8 software (Carl Zeiss Microscopy, Jena, Germany).

Among the 19 *Croton* species analyzed here, 14 had  $2n = 20$ , while 5 had other chromosome numbers, with  $2n = 16$  for *C. glandulosus* and *C. hirtus*,  $2n = 18$  for *C. pedicellatus* and  $2n = 100$  for *C. floribundus* and *C. limae*. Unpublished chromosome counts are being registered for 10 species: *C. adamantinus*, *C. campestris*, *C. grewoides*, *C. lombardianus*, *C. rudolphianus*, *C. sellowii* and *C. tricolor*, all with  $2n = 20$ , in addition to *C. triqueter* ( $2n = 20$  and 40), *C. limae* and *C. floribundus* ( $2n = 100$ ). Intraspecific numerical variations were observed in *C. heliotropifolius* ( $2n = 20$  and 40), *C. pulegioidorus* ( $2n = 20$  and 22) and *C. triqueter* ( $2n = 20$  and 40). The predominance of records of  $2n = 20$  described in the present work corroborates the hypothesis of  $x = 10$  as the basic number of the genus and the predominance of the diploid level in most species of *Croton* (Miller & Webster, 1966). However, counts of  $2n = 20$  and 40 and  $2n = 100$  reveal the occurrence of intraspecific and interspecific polyploidy in the genus. On the other hand, the records of  $2n = 16$ , 18 and 22 reveal the occurrence of interspecific dysploidy, while  $2n = 20$ , 22 in *C. pulegioidorus* is an intraspecific dysploid variant.

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## IAPT chromosome data 40/11

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Pessoal de Nível Superior (CAPES; Process no. 12002011009P8/2021-03) for fellowships.

Methods for chromosome counts follow Guerra & Souza (2002).

\* First chromosome count for the species.

## FABACEAE

\**Aldina heterophylla* Spruce ex Benth.

$2n = 22$ , CHN. Brazil, Amazonas, Presidente Figueiredo, 02°00' 58"S, 59°54'26"W, 18 Feb 2016, *Haroldo C. de Lima 8201* (NYBG) [Fig. 20].

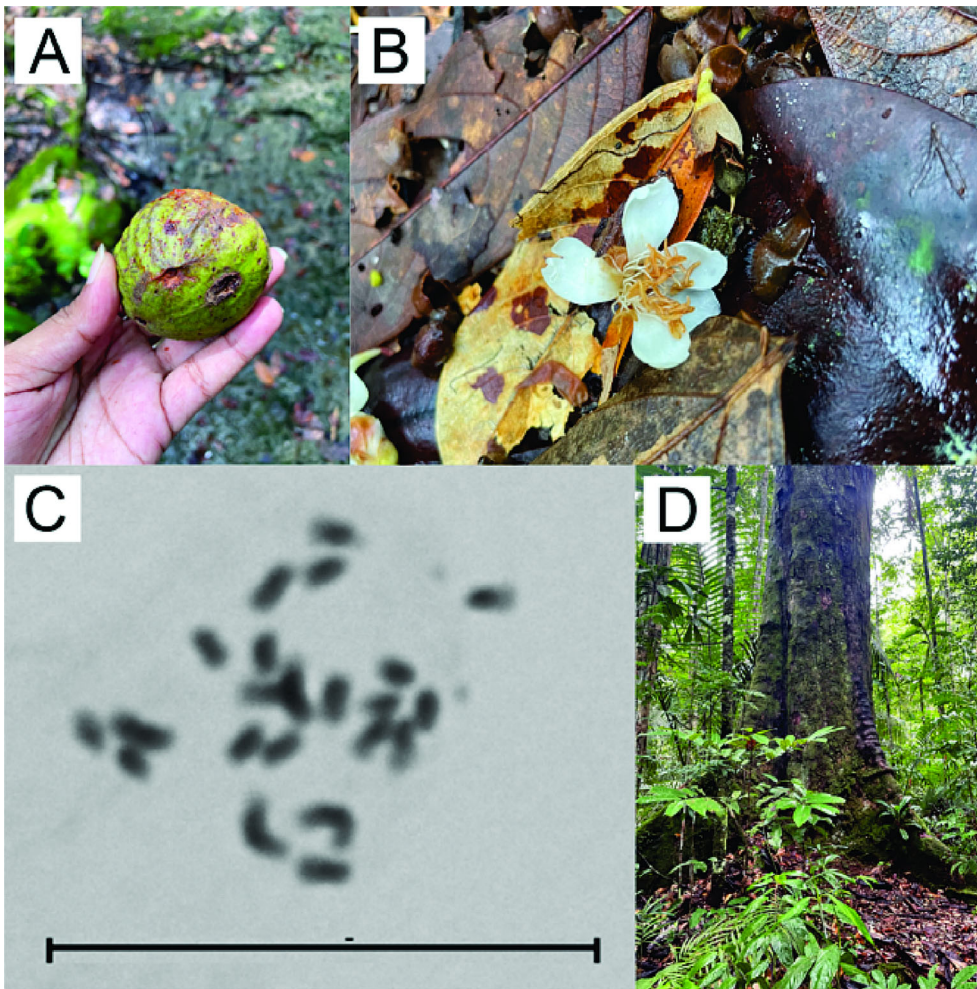
Fabaceae is the third-largest family within angiosperms, encompassing approximately 765 genera and 19,500 species (LPWG, 2017). Legume species hold significant economic and ecological importance (Bruneau, 2013; Zhao, 2021). Within the Amazonian context, this family assumes a crucial role in the nutrient-poor white-sand soils, renowned for their arid climatic conditions and diminutive forests (Mayle & Power, 2008; Flores & Holmgren, 2021).

Among the most prevalent evolutionary lineages in these ecosystems is the genus *Aldina* Endl., comprising 20 species (Choi & al., 2022). In addition to their presence in the white-sand forests in western Amazon, *Aldina* species can also be found in other Amazonian vegetation formations, such as in both flooded and non-flooded ecosystems (Junk & al., 2011). Traditionally, *Aldina* has been categorized within the Swartzieae tribe, owing to its radial flowers that showcase an intact calyx and numerous stamens (Ramos & al., 2016). Nevertheless, recent phylogenetic analyses employing chloroplast markers have recently repositioned *Aldina* within the Andira clade, situated in the Dalbergieae tribe (Cardoso & al., 2015; Ramos & al., 2016; Choi & al., 2022).

Taxonomic, morphological, and ecological studies concerning the genus have been expanding (Ramos & al., 2016). However, the chromosome numbers in this genus have not been investigated until now. Thus, our count for an Amazonian species of *Aldina*, *A. heterophylla*, which revealed the number of  $2n = 22$  also marks the first chromosomal record for the genus. Intriguingly, this count aligns with that of the sister group, the genus *Andira* Lam. Notably, two species within *Andira*, *A. humilis* Mart. ex Benth. ( $2n = 22$ ) (Forni-Martins & al., 1995), and *A. inermis* (W.Wright) Kunth ex DC. ( $2n = 22$ ) (Bawa, 1973), present the same chromosomal number.

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**Fig. 20.** *Aldina heterophylla*: A, Habitat; B, Flower; C, Fruit; D, Metaphase,  $2n = 22$ . — Scale bar = 20  $\mu\text{m}$ . Photos: Adryene Mota de Menezes.

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#### IAPT chromosome data 40/12

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\* First chromosome count for the species.



**VOCHYSIACEAE***Qualea grandiflora* Mart.

$2n = 22$ ,  $n = 11$ , CHN. Brazil, Mato Grosso State, Federal University of Rondonópolis, 16°27'46.56"S, 54°34'49.40"W, 19 Jan 2019, G.L.S. Silveira & al. s.n. (UFMT 44309, UFMT 44308); 5 Feb 2018, L.A.S. Nogueira & al. s.n. (UFMT 43790, UFMT 43793, UFMT 44307, UFMT 43791), 30 Apr 2018, L.A.S. Nogueira & al. s.n. (UFMT 43807).

*Qualea multiflora* Mart.

$n = 11$ , CHN. Brazil, Mato Grosso State, Federal University of Rondonópolis, 16°27'46.56"S, 54°34'49.40"W, 30 Apr 2018, L.A.S. Nogueira & al. s.n. (UFMT 43806).

*Qualea parviflora* Mart.

$2n = 22$ ,  $n = 11$ , CHN. Brazil, Mato Grosso State, Federal University of Rondonópolis, 16°27'46.56"S, 54°34'49.40"W, 4 Oct 2016, L.A.S. Nogueira & al. s.n. (UFMT 43792); 20 Jul 2018, G.L.S. Silveira & al. s.n. (UFMT 44042); 5 Feb 2018, L.A.S. Nogueira & al. s.n. (UFMT 43789).

*Vochysia cinnamomea* Pohl

$n = 12$ , CHN. Brazil, Mato Grosso State, Federal University of Rondonópolis, 16°27'46.56"S, 54°34'49.40"W, 25 Apr 2018, L.A.S. Nogueira & al. s.n. (UFMT 43809), 30 Apr 2018, L.A.S. Nogueira & al. s.n. (UFMT 43808, UFMT 43805).

\**Vochysia divergens* Pohl

$2n = 24$ , CHN. Brazil, Mato Grosso State, Federal University of Rondonópolis, 16°27'46.56"S, 54°34'49.40"W, 18 Jul 2018, G.L.S. Silveira & al. s.n. (UFMT 44043), 23 Jul 2018, G.L.S. Silveira & al. s.n. (UFMT 44044, UFMT 44045).

Vochysiaceae A.St.-Hil. is a family composed of trees and shrubs, included in the order Myrtales as a sister group of Myrtaceae

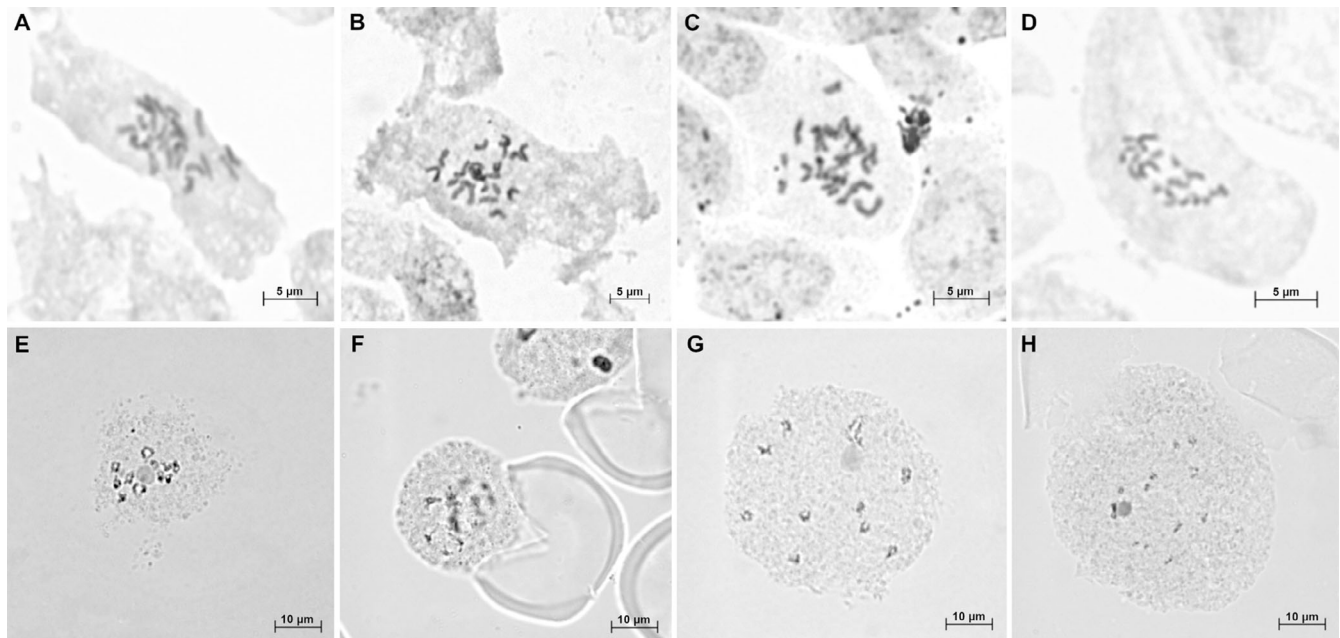
Juss. (Berger & al., 2016; APG IV, 2016), a clade with strong Gondwanan representation for most of its families. Although previous phylogenetic studies greatly improved our understanding of intergeneric and interspecific relationships within the order, our understanding of interfamilial relationships still remains unresolved; hence, we also lack a robust time-calibrated chronogram to address hypotheses (e.g., biogeography and diversification rates). In Brazil, Vochysiaceae comprises nearly 160 species, distributed in six genera (*Callisthene* Mart., *Erisma* Rudge, *Qualea* Aubl., *Ruizterania* (Stafleu) Marc.-Berti, *Salvertia* A.St.-Hil., *Vochysia* Aubl.) (Carmo-Oliveira & Morretes, 2009; Azevedo & al., 2015; Shimizu & Gonçalves, 2017; Shimizu & al., 2019).

Pioneering works on chromosome numbers in Vochysiaceae include Goldblatt (1979, *Vochysia lomatoxylla* Standl.,  $2n = 22$ ), Berry (1987, *Vochysia venezuelana* Stafleu,  $2n = 24$ ), and Barbosa (1999, *Vochysia cinnamomea* and *Vochysia tucanorum* Mart.,  $2n = 24$ ). While the most recent study was conducted by Yamagishi-Costa & al. (2018), including samples of the genera *Callisthene*, *Qualea*, *Salvertia* and *Vochysia*, from Goiás, Mato Grosso, Minas Gerais and São Paulo states, resulting in  $2n = 22$  and  $n = 11$  for *Callisthene* and *Qualea*, and  $2n = 24$  and  $n = 12$  for *Salvertia* and *Vochysia*.

Although Vochysiaceae is well represented in Brazil, being easily found in floristic inventories, there are few reports on chromosome numbers for species occurring in the Cerrado domain. Therefore, this study was carried out to fill this gap and contribute to the knowledge of native Cerrado species.

To our knowledge this is the first report on the chromosome number of *Vochysia divergens*, which is a relevant information for taxonomic and evolutionary studies.

In the present research *Qualea grandiflora* (Fig. 21A,B) and *Q. parviflora* (Fig. 21C) presented 22 chromosomes, and *Vochysia divergens* presented 24 chromosomes (Fig. 21D) (Table 3). The meiotic analysis revealed 11 bivalents in *Qualea grandiflora* (Fig. 21E), *Q. multiflora* (Fig. 21F) and *Q. parviflora* (Fig. 21G) and 12 bivalents



**Fig. 21.** Mitotic and meiotic cells of Vochysiaceae species. A–D, C-metaphases: A & B, *Qualea grandiflora*; C, *Qualea parviflora*; D, *Vochysia divergens*. E–H, Meiotic cells: E, *Qualea grandiflora* (diakinesis); F, *Qualea multiflora* (diplotene); G, *Qualea parviflora* (diakinesis); H, *Vochysia cinnamomea* (diakinesis). — Image capturing performed in light microscope, with 100× magnification.



in *Vochysia cinnamomea* (Fig. 21H) (Table 3). For *Qualea multiflora* and *Vochysia cinnamomea*, the mitotic analysis was not performed due to unavailable fruits and seeds or due to lack of germination under the experimental conditions, and in *V. divergens*, the meiotic cells did not allow the counting of bivalents.

Thus, for the *Qualea* species, the chromosome number is  $2n = 22$ , with  $n = 11$ , and for the *Vochysia* species, the chromosome number is  $2n = 24$  and  $n = 12$ .

The present study confirms the results found by previous authors, indicating that the chromosome numbers are stable in *Qualea* and *Vochysia* species, and suggests that the chromosome evolution in terms of numeric variation is conserved in these genera, as previously stated by Yamagishi-Costa & al. (2018). Furthermore, to our knowledge this study was the first survey of Vochysiaceae in the region, contributes to the genetic knowledge on species native to Brazil and provides additional information on species occurring in the Cerrado domain, which demonstrates the importance of these analyses for taxonomic and evolutionary studies.

### MATERIALS AND METHODS

Plant samples of Vochysiaceae species were collected between the years of 2017 and 2019 in a Cerrado sensu stricto fragment, located in the Universidade Federal de Rondonópolis, municipality of Rondonópolis, Mato Grosso State, Brazil. The voucher specimens were registered and incorporated into the collection of the Universidade Federal de Mato Grosso Herbarium (UFMT Herbarium, Process 02000.002662/2003-09, MMA-SEPRO) (Table 3).

The fruits and seeds sampled were stored in paper bags at  $-10^{\circ}\text{C}$ , while the inflorescences were fixed in Falcon with methanol and propionic acid solution (3 : 1) and stored at  $-4^{\circ}\text{C}$ .

The seed germination took place in a BOD incubator under a temperature of  $27^{\circ}\text{C}$  and photoperiod of 12 h, until the emergence of roots (15–45 days). The root tips were collected and pretreated with 2 mM 8-hydroxyquinoline for 4 h, washed in distilled water, fixed in ethanol and acetic acid solution (3 : 1) and stored at  $-4^{\circ}\text{C}$ . After a minimum of 24 h, the fixative solution was replaced by 70% ethanol.

Slides for the mitotic analysis were prepared according to the squash technique (Belling, 1921), using Schiff's reagent for 1 h and 30 min. The slides were evaluated in a light microscope (Carl Zeiss, Axio Lab A1) coupled with a microcamera (AxioCam ERc 5s). Images of C-metaphases were captured using the AxioVision software.

For the meiotic analysis, slides were prepared with anthers from fixed young inflorescences according to Fukui & Nakayama (1996), in 60% acetic acid, 2% Triton X-100 and 1% carmine-propionic acid staining. The slides were analyzed in light microscope (Carl Zeiss,

Axio Lab A1 or Nikon Eclipse E200) coupled with a microcamera (AxioCam ERc 5s). Representative images were captured using the AxioVision software. Some images were captured using a cellphone camera (Samsung Galaxy J7 Prime), an adaptor (Pereira, 2019) and a micrometric slide for reference.

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**Table 3.** Registration numbers and chromosome numbers ( $n$ ,  $2n$ ) of the sampled Vochysiaceae species.

Species	Registration numbers	$n$	$2n$
<i>Qualea grandiflora</i> Mart.	UFMT 44309; UFMT 44308; UFMT 43790; UFMT 43793; UFMT 43807; UFMT 44307; UFMT 43791	11	22
<i>Qualea multiflora</i> Mart.	UFMT 43806	11	–
<i>Qualea parviflora</i> Mart.	UFMT 43792; UFMT 44042; UFMT 43789	11	22
<i>Vochysia cinnamomea</i> Pohl	UFMT 43809; UFMT 43808; UFMT 43805	12	–
<i>Vochysia divergens</i> Pohl	UFMT 44043; UFMT 44044; UFMT 44045	–	24

**Yamagishi-Costa, J., Barbosa, A.R., Shimizu, G.H., Yamamoto, K. & Forni-Martins, E.R.** 2018. Chromosome numbers and the systematics of tribe Vochysieae (Vochysiaceae). *Acta Bot. Bras.* 32: 314–320. <https://doi.org/10.1590/0102-33062017abb0354>

## IAPT chromosome data 40/13

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- \* New chromosome number (cytotype) for the species.
- \*\* First chromosome count from an unexplored part of the distribution area of the taxon.

### IRIDACEAE

#### Subfamily: Iridoideae

#### Tribe: Tigridieae

\*\**Herbertia darwinii* Roitman & J.A.Castillo

$2n = 14$ , CHN. Brazil, Rio Grande do Sul, Alegrete, 29°08'51.0" S, 56°01'49.7"W, 131 m, 2 Nov 2021, *A. Tonetto-Vieira & L. Noqueira* 5 (ICN).

\*\**Herbertia* aff. *lahue* (Molina) Goldblatt

$2n = 14$ ; Brazil, Rio Grande do Sul, São Jerônimo do Sul, 30°07'43.03"S, 51°51'20.38"W, 38 m, 8 Nov 2018, *E. Kaltchuk, E.M. Stiehl-Alves & A. Vieira* 5 (ICN); Brazil, Rio Grande do Sul, São Jerônimo do Sul, 30°10'48.40"S, 51°23'33.29"W, 38 m, 16 Nov 2018, *E. Kaltchuk, E.M. Stiehl-Alves & A. Vieira* 17 (ICN); Brazil, Rio Grande do Sul, Canguçu, 31°17'00.6"S, 53°02'60.0"W, 376 m, 8 Nov 2019, *E.M. Stiehl-Alves, A. Tonetto-Vieira, L. Carvalho & I. Hedlund* 137 (ICN); Brazil, Rio Grande do Sul, Canguçu, 30°41'37.0"S, 53°23'35.0"W, 376 m, 9 Nov 2019, *E.M. Stiehl-Alves, A. Tonetto-Vieira, L. Carvalho & I. Hedlund* 145 (ICN); Brazil, Rio Grande do Sul, Santana da Boa Vista, 30°45'20.3"S, 53°09'15.2"W, 190 m, 10 Nov 2019, *E.M. Stiehl-Alves, A. Tonetto-Vieira, L. Carvalho & I. Hedlund* 162 (ICN); Brazil, Rio Grande do Sul, Lavras do Sul, 30°46'06.1"S, 53°55'26.8"W, 418 m, 11 Nov 2019, *E.M. Stiehl-Alves, A. Tonetto-Vieira, L. Carvalho & I. Hedlund* 171 (ICN); Brazil, Rio Grande do Sul, Caçapava do Sul, 30°41'34"S, 53°23'32"W, 26 Oct 2018, *A.M. Cristante, E.M. Stiehl-Alves, A. Tonetto-Vieira & R. Becker* 1 (ICN); Brazil, Rio Grande do Sul, Caçapava do Sul, 30°53'42"S, 53°25'48"W, 27 Oct 2018, *A.M. Cristante, E.M. Stiehl-Alves,*

*A. Tonetto-Vieira & R. Becker* 2 (ICN); Brazil, Rio Grande do Sul, Caçapava do Sul, 30°47'58"S, 53°11'23"W, 3 Dec 2017, *A.M. Cristante, E.M. Stiehl-Alves & R.M. Marchioretto* 12 (ICN) [Fig. 22A].

\*\**Herbertia lahue* (Molina) Goldblatt

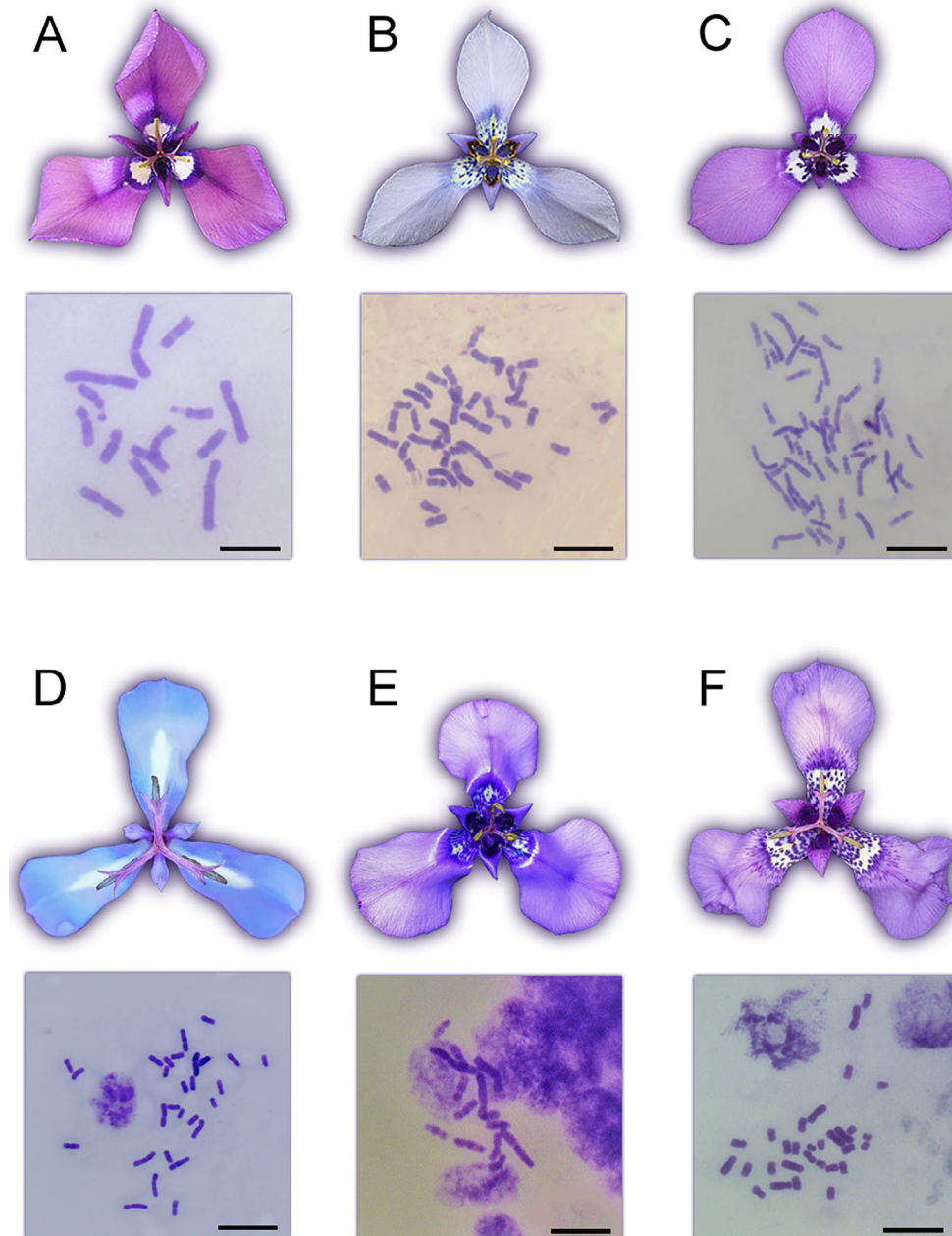
$2n = 42$ , CHN. Brazil, Rio Grande do Sul, São Jerônimo do Sul, 30°07'43.03"S, 51°51'20.38"W, 38 m, 8 Nov 2018, *E. Kaltchuk, E.M. Stiehl-Alves & A. Vieira* 6 (ICN); Brazil, Rio Grande do Sul, Santo Antonio das Missões, 28°32'42.17"S, 54°33'19.39"W, 284 m, 23 Oct 2019, *E.M. Stiehl-Alves, A. Tonetto-Vieira, L. Carvalho & A. Mello* 105 (ICN); Brazil, Rio Grande do Sul, Santo Antonio das Missões, 28°33'44.4"S, 55°09'37.3"W, 284 m, 24 Oct 2019, *E.M. Stiehl-Alves, A. Tonetto-Vieira, L. Carvalho & A. Mello* 110 (ICN); Brazil, Rio Grande do Sul, Itaqui, 29°00'22.7"S, 56°03'38.7"W, 105 m, 25 Oct 2019, *E.M. Stiehl-Alves, A. Tonetto-Vieira, L. Carvalho & A. Mello* 117 (ICN); Brazil, Rio Grande do Sul, Lavras do Sul, 30°46'06.1"S, 53°55'26.8"W, 418 m, 11 Nov 2019, *E.M. Stiehl-Alves, A. Tonetto-Vieira, L. Carvalho & I. Hedlund* 172 (ICN) [Fig. 22B].

\*\**Herbertia lahue* (Molina) Goldblatt

$2n = 56$ , CHN. Brazil, Rio Grande do Sul, São Miguel das Missões, 28°32'42.24"S, 54°33'18.93"W, 291 m, 23 Oct 2019, *E.M. Stiehl-Alves, A. Tonetto-Vieira, L. Carvalho & A. Mello* 104 (ICN); Brazil, Rio Grande do Sul, São Jerônimo do Sul, 30°08'55.32"S, 51°54'19.94"W, 38 m, 8 Nov 2018, *E. Kaltchuk, E.M. Stiehl-Alves & A. Vieira* 3 (ICN); Brazil, Rio Grande do Sul, Santo Antonio das Missões, 28°33'44.4"S, 55°09'37.3"W, 284 m, 24 Oct 2019, *E.M. Stiehl-Alves, A. Tonetto-Vieira, L. Carvalho & A. Mello* 111 (ICN); Brazil, Rio Grande do Sul, Itaqui, 28°59'50.6"S, 56°05'50.00"W, 105 m, 25 Oct 2019, *E.M. Stiehl-Alves, A. Tonetto-Vieira, L. Carvalho & A. Mello* 115 (ICN); Brazil, Rio Grande do Sul, Alegrete, 29°50'50.7"S, 55°52'12.2"W, 195 m, 26 Oct 2019, *E.M. Stiehl-Alves, A. Tonetto-Vieira, L. Carvalho & A. Mello* 123 (ICN); Brazil, Rio Grande do Sul, Cristal, 30°54'35.4"S, 52°04'22.4"W, 93 m, 7 Nov 2019, *E.M. Stiehl-Alves, A. Tonetto-Vieira, L. Carvalho & I. Hedlund* 129 (ICN); Brazil, Rio Grande do Sul, Canguçu, 31°21'44.1"S, 52°48'25.8"W, 8 Nov 2019, 423 m, *E.M. Stiehl-Alves, A. Tonetto-Vieira, L. Carvalho & I. Hedlund* 134 (ICN); Brazil, Rio Grande do Sul, Caçapava do Sul, 30°49'58.80" S, 53°30'14.40"W, 255 m, 9 Nov 2019, *E.M. Stiehl-Alves, A. Tonetto-Vieira, L. Carvalho & I. Hedlund* 148 (ICN); Brazil, Rio Grande do Sul, Santana da Boa Vista, 30°45'20.3"S, 53°09'15.2" W, 190 m, 10 Nov 2019, *E.M. Stiehl-Alves, A. Tonetto-Vieira, L. Carvalho & I. Hedlund* 161 (ICN) [Fig. 22C].

\*\**Herbertia pulchella* Sweet

$2n = 28$ ; CHN. Brazil, Rio Grande do Sul, Canguçu, 31°21'48.0"S, 52°44'48.0"W, 376 m, 8 Nov 2019, *E.M. Stiehl-Alves, A. Tonetto-Vieira, L. Carvalho & I. Hedlund* 133 (ICN); Brazil, Rio Grande do Sul, Canguçu, 31°16'49.0"S, 52°42'04.0"W, 376 m, 8 Nov 2019, *E.M. Stiehl-Alves, A. Tonetto-Vieira, L. Carvalho & I. Hedlund* 143 (ICN); Brazil, Rio Grande do Sul, Caçapava do Sul, 30°49'58.80"S, 53°30'14.40"W, 255 m, 9 Nov 2019, *E.M. Stiehl-Alves, A. Tonetto-Vieira, L. Carvalho & I. Hedlund* 150 (ICN); Brazil, Rio Grande do Sul, Santana da Boa Vista, 30°47'58.02"S, 53°11'23.96"W, 190 m, 10 Nov 2019, *E.M. Stiehl-Alves, A. Tonetto-Vieira, L. Carvalho & I. Hedlund* 165 (ICN); Brazil, Rio Grande do Sul, Santana do Livramento, 30°46'40.70"S, 53°08'27.7"W, 26 Nov 2019, *A. Tonetto-Vieira, L. Eggers & O. Chauveau* 7 (ICN) [Fig. 22D].



**Fig. 22.** Photomicrographs of mitotic metaphases with their respective representative flowers of *Herbertia* species, stained with Giemsa, all at the same scale. **A**, *H. aff. lahue* ( $2n = 14$ ); **B**, *H. lahue* ( $2n = 42$ ); **C**, *H. lahue* ( $2n = 56$ ); **D**, *H. pulchella* ( $2n = 28$ ); **E**, *H. aff. quareimana* ( $2n = 14$ ); **F**, *H. quareimana* ( $2n = 28$ ). — Scale bars = 5  $\mu\text{m}$ .

*\*Herbertia aff. quareimana* Ravenna

$2n = 14$ , CHN. Brazil, Rio Grande do Sul, São Nicolau, 28°12' 29.52"S, 55°19'13.80"W, 95 m, 23 Oct 2019, *E.M. Stiehl-Alves, C. Forgiarini & F.S. Silveira 51* (ICN); Brazil, Rio Grande do Sul, São Miguel das Missões, 28°32'44.04"S, 54°33'14.01"W, 284 m, 23 Oct 2019, *E.M. Stiehl-Alves, A. Tonetto-Vieira, L. Carvalho & A. Mello 106* (ICN); Brazil, Rio Grande do Sul, Santo Antonio das Missões, 28°31'24.01"S, 55°14'2.11"W, 24 Oct 2019, *E.M. Stiehl-Alves, A. Tonetto-Vieira, L. Carvalho & A. Mello 109* (ICN) [Fig. 22E].

*\*\*Herbertia quareimana* Ravenna

$2n = 28$ , CHN. Brazil, Rio Grande do Sul, Santana do Livramento, 30°47'16.1"S, 55°38'02.8"W, 6 Nov 2012, *L. Eggers, O. Chauveau & T. Pastori 1103* (ICN); Brazil, Rio Grande do Sul,

Uruguaiana, 29°53'22.2"S, 57°07'16.5"W, 5 Nov 2012, *L. Eggers, O. Chauveau & T. Pastori 733A* (ICN); Brazil, Rio Grande do Sul, Alegrete, 29°51'27.6"S, 55°53'06.6"W, 140 m, 26 Oct 2019, *E.M. Stiehl-Alves, A. Tonetto-Vieira, L. Carvalho & A. Mello 125* (ICN); Brazil, Rio Grande do Sul, Alegrete, 29°59'02.79"S, 55°58'31.6"W, 195 m, 26 Oct 2019, *E.M. Stiehl-Alves, A. Tonetto-Vieira, L. Carvalho & A. Mello 126* (ICN) [Fig. 22F].

*Herbertia* Sweet is a small genus (eight species) belonging to the Clade A of Tigridaeae (Chauveau & al., 2012), that comprises perennial, herbaceous and bulbous geophytic plants, generally with violet flowers (Goldblatt & Manning, 2008). *Herbertia* species show great vegetative similarity, but some have high floral polymorphism. Although floral characters have been used as diagnostics within the genus, tenuous limits of interspecific differentiation and a large



intraspecific polymorphism result in taxonomic uncertainties (Chauveau & al., 2012; Stiehl-Alves & al., 2016, 2017). In this sense, karyotypic characters of *Herbertia* need to be investigated, since they have also been useful for elucidating the relationships of various taxa being potentially informative in Iridaceae (Goldblatt & Takei, 1997).

According to literature, Clade A has the base chromosome number  $x = 7$  and ploidy series in several species (Moraes & al., 2015). Six of the eight recognized *Herbertia* species have chromosome counts with four ploidy levels reported:  $2x$ ,  $4x$ ,  $6x$  and  $8x$ . Intraspecific polyploid series have been found for *H. lahue* and *H. pulchella* (Winge, 1959; Kenton & Heywood, 1984; Goldblatt & Takei, 1997; Roitman & Castillo, 2004; Moreno & al., 2009; Moraes & al., 2015).

*Herbertia* occurs naturally in grasslands in the south of the Neotropical region (Goldblatt & Manning, 2008), with seven species endemic to the northeast of the Pampean province. The Pampean province (hereafter Pampas) is part of the Chacoan biogeographical domain (Morrone, 2017) and is a species-rich ecosystem characterized by the predominance of grassland vegetation, with Poaceae, Asteraceae, Fabaceae, and Cyperaceae as the most dominant families (Andrade & al., 2019).

Efforts have been made by our team to delineate a clearer picture of the patterns of geographic distribution and evolutionary relationships of *Herbertia* species and thus understand the impact of polyploidy on the genus' evolution. Therefore, cytogenetic data for *Herbertia* have been obtained for several populations throughout the distribution area. For chromosome counting, root tips were pretreated with 8-hydroxyquinoline for 24 h at 8°C and fixed with 3 : 1 ethanol : glacial acetic acid. Slides were prepared according to Moraes & al. (2015) and Alencar & al. (2018). Chromosomes obtained for the *Herbertia* populations in the present study are represented by the image of the flower of each *Herbertia* species (Fig. 22A–F).

In the present study, chromosome numbers are presented for 38 populations of four *Herbertia* species: *H. darwinii*, *H. lahue*, *H. pulchella* and *H. quareimana*. Chromosome numbers have been described in the literature for the four species investigated; however, such data are relatively old and, moreover, taxonomic changes occurred in the genus. Furthermore, the chromosome counts are restricted to a few points of occurrence, most of them outside Brazil. In view of the existence of intraspecific cytotypes in the genus, evaluation of a greater number of populations is necessary.

*Herbertia darwinii* is an endemic species of the Pampas distributed along the triple border of Brazil, Argentina, and Uruguay, in an area of less than 40,000 km<sup>2</sup>. The chromosome number  $2n = 14$  found here for one population of *H. darwinii* is the same as reported previously in samples from another four populations of this species (Roitman & Castillo, 2004; Moreno & al., 2009; Moraes & al., 2015). So far, only diploid plants have been found for this species, even in populations located outermost of its geographic distribution.

*Herbertia lahue* is the only species with a wide distribution, occurring throughout the Pampas and on the borders with Chaco province (Chacoan dominion), Araucaria Forest province (Paraná dominion), and Atacama province (part of South American transition zone). There are also records of populations of *H. lahue* in the southern U.S.A., Australia, and Japan, but these seem to represent recent introductions (Goldblatt & Manning, 2008). Twenty-three populations of *H. lahue* sampled in southern Brazil were investigated. The cytotype ( $2n = 2x = 14$ ) was found in samples from nine populations named *H. aff. lahue*. Other cytotypes previously reported for *H. lahue* were also observed in the present study, and hexaploids ( $2n = 6x = 42$ ) and octoploids ( $2n = 8x = 56$ ) were recorded

respectively in five and nine populations. The chromosome number  $2n = 42$  was previously reported in samples from Argentina (Moreno & al., 2009), Brazil (Moraes & al., 2015), Chile (Kenton & Heywood, 1984; Goldblatt & Takei, 1997), and Uruguay (Moreno & al., 2009), whereas the chromosome number  $2n = 56$  was previously reported in populations from Argentina (Moreno & al., 2009) and Brazil (Moraes & al., 2015). The spatial distribution of *H. lahue* populations in the Pampas is complex, ranging from mixed populations, where the three ploidy levels are found in sympatry, as well as parapatric and allopatric distributed populations (Stiehl-Alves & al., 2016; Martins & al., 2020).

*Herbertia pulchella* is a species that is distributed in an area of about 100,000 km<sup>2</sup> located in southern Brazil and Uruguay, in the Pampas. In this study, seven populations of *H. pulchella* were studied and a chromosome number  $2n = 28$  was recorded for all samples. Chromosome number  $2n = 28$  was also described by Moraes & al. (2015) in samples from four populations from Rio Grande do Sul (Brazil). Older studies (Winge, 1956; Kenton & Heywood, 1984; Goldblatt & Takei, 1997) reported other chromosome numbers for this species ( $2n = 14$ ,  $2n = 42$ ), which has motivated us to expand the sampling of this species to search for populations unequivocally identified as *H. pulchella*. Despite the effort, only tetraploid plants have been registered.

*Herbertia quareimana* is a species endemic to the Pampas, near the border of Brazil with Argentina and Uruguay, covering an area of about 48,000 km<sup>2</sup>. Seven populations of *H. quareimana* sampled in Rio Grande do Sul (Brazil) were investigated here, and three of them presented the chromosome number  $2n = 2x = 14$ . This is the first report of a diploid cytotype for *H. quareimana*. The remaining four populations had  $2n = 4x = 28$ . Tetraploid *H. quareimana* was also reported for other Brazilian populations by Ravenna (1989) and Moraes & al. (2015), as well as for populations located in Argentina and Uruguay (Moreno & al., 2009). Geographic data obtained through collection expeditions carried out by our time since 2006 indicate that diploid and tetraploid populations are geographically isolated from each other.

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## IAPT chromosome data 40 – Extended version

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## IAPT chromosome data 40/1

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Gustavo Pedroso de Moraes, Guilherme Pecoits Goldstein and Taciane Schroder Jorge for the support and assistance in the analyses performed.

\* First chromosome count for the taxon.

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

### HYMENOPHYLLACEAE

#### Subfamily Hymenophylloideae

\*\**Hymenophyllum asplenoides* (Sw.) Sw.

*n* = 36; CHN. Brazil, Rio Grande do Sul, Cambará do Sul, 29° 10'21"S, 50°01'51"W, 17 Dec 2022, F. Gonzatti & F.P. Abreu 6844 (HUCS) [Fig. 1A,E].

\*\**Hymenophyllum fucooides* (Sw.) Sw.

*n* = 56; CHN. Brazil, Paraná, Campina Grande do Sul, 25°15' 16"S, 48°52'50"W, 30 Apr 2023, F. Gonzatti, F.P. Abreu & I.A. Bahima 7244 (HUCS) [Fig. 1B,F].

\**Hymenophyllum magellanicum* (Desv.) Willd. ex Kunze  
 $n = 27$ ; CHN. Brazil, Paraná, Campina Grande do Sul, 25°15' 16"S, 48°52'50"W, 30 Apr 2023, F. Gonzatti, F.P. Abreu & I.A. Bahima 7250 (HUCS); Brazil, Rio Grande do Sul, Cambará do Sul, 29°10'21"S, 50°01'51"W, 17 Dec 2022, F. Gonzatti & F.P. Abreu 6845 (HUCS); Brazil, Santa Catarina, Urupema, 27°55'19"S, 49°51'18"W, 18 Feb 2023, F. Gonzatti, F.P. Abreu & P.L. Casa 6996 (HUCS) [Fig. 1C,G].

\**Hymenophyllum megachilum* C.Presl  
 $n = 13$ ; CHN. Brazil, Rio Grande do Sul, Cambará do Sul, 29° 10'21"S, 50°01'51"W, 17 Dec 2022, F. Gonzatti & F.P. Abreu 6846 (HUCS); Brazil, Santa Catarina, Urupema, 27°55'19"S, 49°51' 18"W, 18 Feb 2023, F. Gonzatti & F.P. Abreu 6992 (HUCS) [Fig. 1D,H].

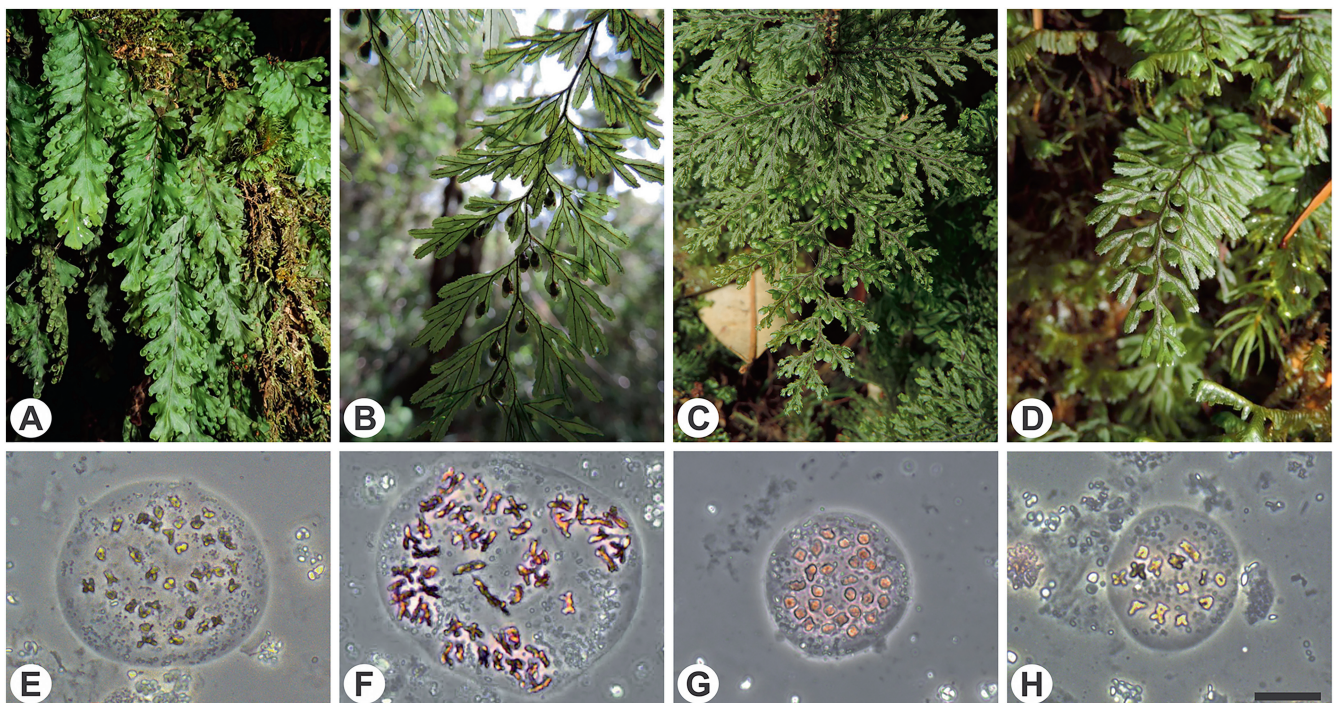
Hymenophyllaceae Mart., the only family in the order Hymenophyllales A.B.Frank, encompasses around 430 fern species distributed in two subfamilies: Trichomanoideae C.Presl, and Hymenophylloideae Burnett (PPG I, 2016). Hymenophylloideae contains the single genus *Hymenophyllum* Sm., which represents the highest species richness with approximately 250 taxa organized within 10 subgenera (Ebihara & al., 2006). Occurrences in South America have previously been registered for *H.* subg. *Globosa* (Prantl) Ebihara & K.Iwats, subg. *Hymenoglossum* (C.Presl) R.M. Tryon & A.F. Tryon, subg. *Hymenophyllum*, subg. *Mecodium* C.Presl ex Copel., subg. *Myrmecostylum* (C.Presl) Ebihara & K.Iwats., and subg. *Sphaerocionium* (C.Presl) C.Chr. (Ebihara & al., 2006). The cytogenetic data available for Hymenophyllaceae focus mainly on the taxonomy and evolution of the family (e.g., Manton, 1950; Braithwaite, 1975; Hennequin & al., 2010; Kim & Kim, 2020).

However, currently available cytogenetic data is mostly restricted to Asia and Oceania. Thus, there is a lack of surveys and specific works, especially with taxa from the New World, leaving unexplored gaps in this field of study.

For this study, chromosome number counting made use of sori fixed in ethanol : glacial acetic acid (3 : 1) for 24 h. The material was prepared for meiotic analysis following the squashing method and staining with 2% carmine-propionic acid (Manton, 1950). Counts of meiotic chromosomes were performed on diakinesis cells. For genome size estimation, the total DNA content was evaluated through flow cytometry according to the methodology described by Doležel & al. (2007). *Pisum sativum* L. 'Ctirad' (Doležel & al., 1998) and *Vicia faba* L. 'Inovec' (Doležel & al., 1992) were included as standards. The cellular lysis Ebihara buffer (Ebihara & al., 2005) was used in sample preparation. The suspension was filtered through a 35 µm nylon mesh filter and nuclei were stained with 100 µl propidium iodide. Nuclear suspensions were analyzed with a FACS Aria BD III flow cytometer, and 2C DNA estimations were calculated with the FlowPloidy R library (Smith & al., 2018).

Chromosome number and genome size data was obtained for seven populations covering four *Hymenophyllum* species (Table 1). This is the first ever chromosome number report for species occurring in South America from *H.* subg. *Myrmecostylum* and subg. *Hymenoglossum*. In terms of Brazilian populations, this study also marks the first cytogenetic data collected for *H.* subg. *Hymenophyllum* in the country. The genome sizes reported in this research represent new information, since none of the analyzed taxa had previously published data.

*Hymenophyllum asplenioides* displays a high phenotypic plasticity and its occurrence extends from Mexico to the southeast of Brazil (Stolze, 1976; Windisch, 2014; Gonzatti & Windisch, 2018).



**Fig. 1.** Species with new chromosome records and their cells in diakinesis. **A & E**, *Hymenophyllum asplenioides* ( $n = 36$ ); **B & F**, *Hymenophyllum fucoides* ( $n = 56$ ); **C & G**, *Hymenophyllum magellanicum* ( $n = 27$ ); **D & H**, *Hymenophyllum megachilum* ( $n = 13$ ). — Scale bar = 20 µm (for E–H).



The chromosome number  $n = 36$  found for *H. asplenoides* is in agreement with previous reports by Walker (1966) for specimens from Jamaica. This species belongs to *H.* subg. *Hymenoglossum*, for which the only cytogenetic data currently known is in fact from *H. asplenoides*. Moreover, this taxon showed a genome size estimate of  $2C = 38.76 \pm 0.16$  pg.

Our chromosome counts are also in agreement with those previously reported for *Hymenophyllum fucoides* for specimens from Jamaica (Walker, 1966). The haploid chromosome number for this species was  $n = 56$ , which is the highest value found for *H.* subg. *Hymenophyllum*. When it comes to genome size, *H. fucoides* had  $2C = 129.49 \pm 0.49$  pg, which is not only the largest genome size for the genus *Hymenophyllum*, but also the largest for the family Hymenophyllaceae. Before this finding, the largest genome size known was that of *Vandenboschia orientalis* (C.Chr.) Ching with  $2C = 64.23$  pg (Fujiwara & al., 2021).

*Hymenophyllum magellanicum* from *H.* subg. *Myrmecostylum* displayed a gametic chromosome number of  $n = 27$ , the first chromosome number record for this species. This is the lowest meiotic number reported for this subgenus, which previously ranged from  $n = 34$  to  $n = 72$  for *H. villosum* Colenso and *H. scabrum* A.Rich., respectively (Brownlie, 1954, 1961; Daellenbach, 1982 in Dawson, 2008). The genome size identified for *H. magellanicum* was  $2C = 36.17 \pm 0.38$  pg. Additionally, no statistically significant difference in genome size was observed between the three populations sampled for this species ( $p$ -value = 0.804).

We present here the first chromosome number counts for *Hymenophyllum megachilum*. This species is endemic to the Brazilian Atlantic Forest and belongs to *H.* subg. *Hymenophyllum*. The haploid number found is  $n = 13$  and the DNA content  $2C = 29.89 \pm 0.47$  pg. Furthermore, no statistically significant difference in genome size was found between the two sampled populations ( $p$ -value = 0.613). Three other species from the corresponding subgenus share the same chromosome number, including *H. tunbrigense* (L.) Sm. *Hymenophyllum megachilum* and *H. tunbrigense* were for a long time considered closely related taxa. However, molecular analyses have recently recognized two independent lineages (Gonzatti & al., 2023). On the other hand, the results obtained by us for *H. fucoides* ( $n = 56$ ) and *H. megachilum* ( $n = 13$ ) reflect on the vast morphological and cytogenetic diversity described for *H.* subg. *Hymenophyllum* (Ebihara & al., 2006).

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**Table 1.** Chromosome number and genome size of *Hymenophyllum* species.

Species	Subgenus	Voucher	$n$	Genome size (2C, pg)
				mean $\pm$ standard deviation
<i>H. asplenoides</i>	<i>Hymenoglossum</i>	<i>F. Gonzatti &amp; F.P. Abreu 6844</i>	36	38.76 $\pm$ 0.16
<i>H. fucoides</i>	<i>Hymenophyllum</i>	<i>F. Gonzatti, F.P. Abreu &amp; I.A. Bahima 7244</i>	56	129.49 $\pm$ 0.49
<i>H. magellanicum</i>	<i>Myrmecostylum</i>	<i>F. Gonzatti &amp; F.P. Abreu 6845</i>	27	36.09 $\pm$ 0.13
		<i>F. Gonzatti, F.P. Abreu &amp; P.L. Casa 6996</i>	27	36.30 $\pm$ 0.24
		<i>F. Gonzatti, F.P. Abreu &amp; I.A. Bahima 7250</i>	27	36.07 $\pm$ 0.80
<i>H. megachilum</i>	<i>Hymenophyllum</i>	<i>F. Gonzatti &amp; F.P. Abreu 6846</i>	13	30.05 $\pm$ 0.32
		<i>F. Gonzatti, F.P. Abreu &amp; P.L. Casa 6992</i>	13	29.78 $\pm$ 0.59



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## IAPT chromosome data 40/2

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\* First chromosome count for the species.

### ALSTROEMERIACEAE

\**Alstroemeria piperata* A.R.Flores & J.M.Watson

$2n = 16$ , CHN. Chile, Valparaíso Region, Los Andes, Ranchillos, 1338 m, 32°55'07"S, 70°42'34"W, 09 Nov 2022, *G. Rojas 2590* (SGO) [Figs. 2A, 3A].

*Alstroemeria piperata* is endemic to Chile, occupies a very narrow range and presents serious conservation problems (Finot & al., 2018).

\**Alstroemeria pulchra* var. *maxima* Phil.

$2n = 16$ , CHN. Chile, Valparaíso Region, Petorca, alongside road to Los Molles, Route 5, at km 190, 57 m, 32°12'14.2"S, 71°30'57"W, 30 Nov 2018, *N. Villalobos 1911* (CONC) [Figs. 2B, 3B].

*Alstroemeria pulchra* constitutes a complex and includes *A. pulchra* subsp. *lavandulacea* Ehr.Bayer and *A. pulchra* Sims subsp. *pulchra*. The latter two have  $2n = 16$  chromosomes (Baeza & al., 2018).

### AMARYLLIDACEAE

*Miersia chilensis* Lindl.

$2n = 22$ , CHN. Chile, Libertador Gral, Bernardo O'Higgins Region, Cardenal Caro, road between Paredones and San Pedro de Alcántara, 100 m, 34°41'57"S, 71°52'44"W, 03 Nov 2007, *I. Escobar 74* (CONC) [Figs. 2C, 3C].

There is a previous count by Escobar & al. (2012) for the same species, but it indicates a  $2n = 20$ , therefore, this is a new chromosomal number for this species.

*Phycella australis* Ravenna

$2n = 16$ , CHN. Chile, Biobío Region, Concepción, Hualpén, Tumbes Hills, 10 m, 36°38'S, 73°07'W, 08 Sep 2022, *C. Baeza & E. Thielemann 4436* (CONC) [Figs. 2D, 3D].

Previous counts for this species indicate the same chromosomal number (Baeza & al., 2007, 2012).

*Rhodolirium montanum* Phil.

$2n = 16$ , CHN. Argentina, Córdoba Province, Punilla, Valle Hermoso, 2714 m, 35°09'14.52"S, 70°10'36.50"W, 24 Feb 2023, *J. Espejo s.n.* (CONC) [Figs. 2E, 3E].

There is a previous count by Naranjo & Poggio (2000) for this species, also in Argentina (as *Rhodophiala rhodolirion* (Baker) Traub).

*Zephyranthes maculata* (L'Hér.) Nic.García

$2n = 18$ , CHN. Chile, Biobío Region, Yumbel, road to Yumbel railroad station, 115 m, 37°08'00"S, 72°32'00"W, 24 Sep 2022, *C. Baeza 4439* (CONC) [Figs. 2F, 3F].

*Zephyranthes monantha* (Ravenna) Nic.García

$2n = 18$ , CHN. Chile, Biobío Region, Santa Juana, km 38.6, 7 m, 37°16'35"S, 72°57'35.17"W, 12 Jun 2022, *C. Baeza 4435* (CONC) [Figs. 2G, 3G].

*Zephyranthes* aff. *splendens* (Renjifo) Nic.García

$2n = 18$ , CHN. Chile, Biobío Region, Antuco, Laguna del Laja National Park, Las Lagartijas, slopes of Antuco volcano, 2200 m, 37°24'36"S, 71°21'04"W, 07 Dec 2022, *C. Baeza 444a* (CONC) [Figs. 2H, 3H].

*Zephyranthes splendens* (Renjifo) Nic.García

$2n = 18$ , CHN. Chile, Biobío Region, Antuco, km 38, 485 m, 37°16'35"S, 72°57'35.17"W, 12 Jun 2022, *C. Baeza 4435* (CONC) [Figs. 2I, 3I].

*Zephyranthes* is a genus of Amaryllidaceae that has recently been revalidated for Chile (García & al., 2019). All species in this genus have the same chromosome number and there is enormous stability in the chromosome formula. The species indicated in this work have already been counted in other distinct populations, under the generic epithet *Rhodophiala*.

### ASTERACEAE

*Agoseris coronopifolia* (d'Urv.) K.L.Chambers

$2n = 18$ , CHN. Chile, Biobío Region, Antuco, road from Los Barros to Trapa Trapa, 1800 m, 37°31'06"S, 71°02'00"W, 2 Feb 2023, *C. Baeza 4463* (CONC); Chile, Metropolitana Region, Melipilla Province, Curacaví, Maquehua, 300 m, 33°24'S, 71°11'W, 09 Oct 2020, *S. Teillier & al. 8626* (CONC) [Figs. 2J,K, 3J].

There are previous counts of gametophytic material from Argentina of this species (Wulff, 1984; Hunziker & al., 1989). Therefore, this is the first diploid count for this species.

*Chaetanthera microphylla* (Cass.) Hook. & Arn.

$2n = 24$ , CHN. Chile, Biobío Region, Province of Biobío, Yumbel, in front of the water cup, 115 m, 37°08'00"S, 72°32'00"W, 24 Sep 2022, C. Baeza 4439a (CONC) [Figs. 2L, 3K].

*Chaetanthera moenchioides* Less.

$2n = 26$ , CHN. Chile, Biobío Region, Biobío Province, Antuco, Laguna del Laja National Park, road to Las Chilcas, 1082 m, 37°23'14.7"S, 71°24'38.3"W, 17 Dec 2022, C. Baeza 4447 (CONC) [Figs. 2M, 3L].

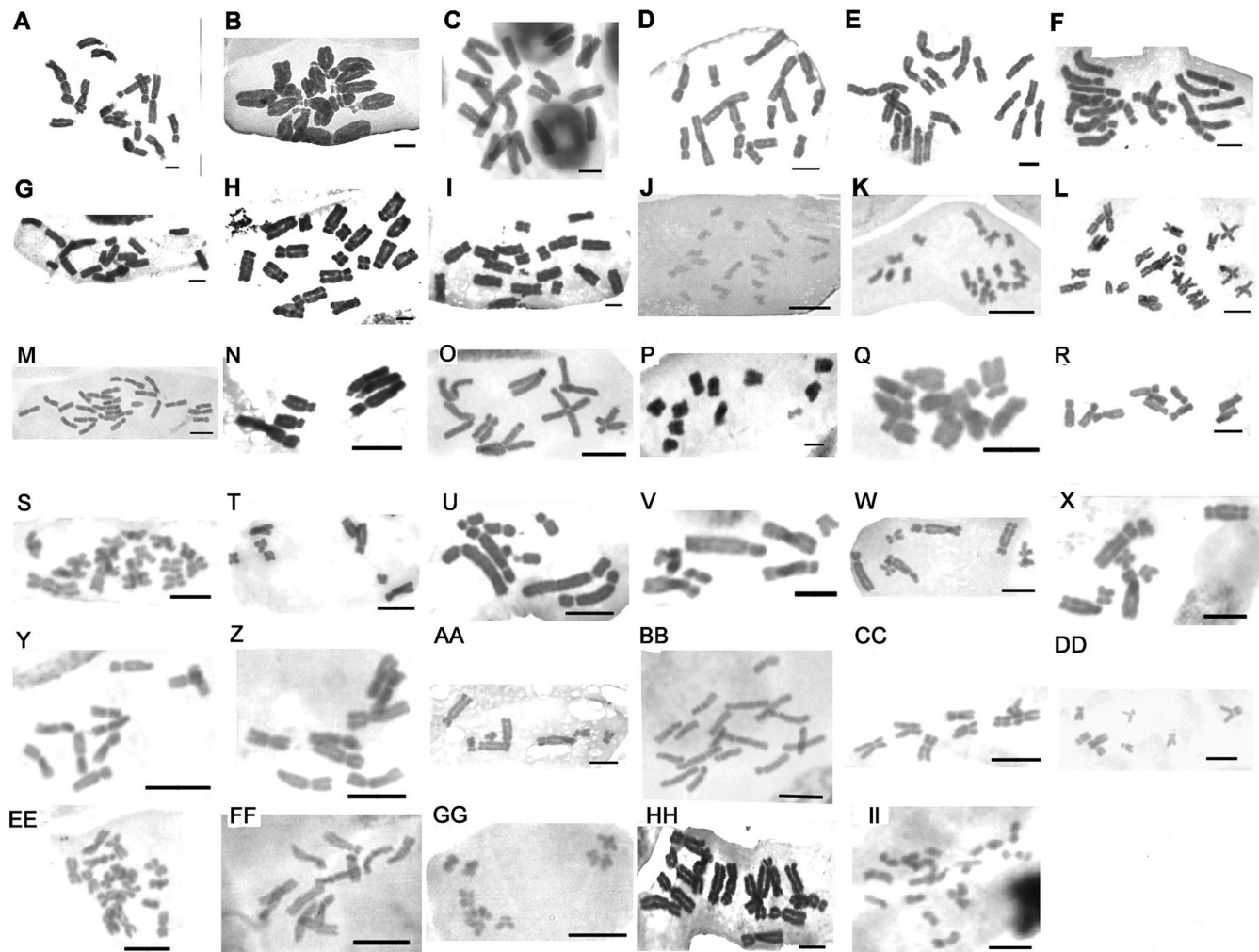
There are previous counts for both species of *Chaetanthera*, but in different populations (Baeza & Schrader, 2005; Baeza & al., 2009; Davies, 2010).

*Crepis capillaris* (L.) Wallr.

$2n = 6$ , CHN. Chile, Biobío Region, Polcura, Quelén Hill, 1260 m, 37°14'47.16"S, 71°42'12.25"W, 19 Jan 2023, C. Baeza 4459 (CONC) [Figs. 2N, 3M].

*Crepis pulchra* L.

$2n = 8$ , CHN. Chile, Biobío Region, Concepción Province, between Janequeo and San Martín Street, 18 m, 36°04'25.2"S, 73°02'04"W, 22 Nov 2022, S. Teillier s.n. 8642 (CONC) [Figs. 2O, 3N].



**Fig. 2.** A, *Alstroemeria piperata*,  $2n = 16$ ; B, *Alstroemeria pulchra* var. *maxima*,  $2n = 16$ ; C, *Miersia chilensis*,  $2n = 22$ ; D, *Phycella australis*,  $2n = 16$ ; E, *Rhodolirium montanum*,  $2n = 16$ ; F, *Zephyranthes maculata*,  $2n = 18$ ; G, *Zephyranthes monantha*,  $2n = 18$ ; H, *Zephyranthes* aff. *splendens*,  $2n = 18$ ; I, *Zephyranthes splendens*,  $2n = 18$ ; J, *Agoseris coronopifolia*,  $2n = 18$ ; K, *Agoseris coronopifolia*,  $2n = 18$ ; L, *Chaetanthera microphylla*,  $2n = 24$ ; M, *Chaetanthera moenchioides*,  $2n = 26$ ; N, *Crepis capillaris*,  $2n = 6$ ; O, *Crepis pulchra*,  $2n = 8$ ; P, *Haplopappus bustillosianus*,  $2n = 10$ ; Q, *Haplopappus glutinosus*,  $2n = 10$ ; R, *Haplopappus paucidentatus*,  $2n = 10$ ; S, *Hieracium glaucifolium*,  $2n = 18$ ; T, *Hypochaeris acaulis*,  $2n = 8$ ; U, *Hypochaeris apargioides*,  $2n = 8$ ; V, *Hypochaeris apargioides*,  $2n = 18$ ; W, *Hypochaeris clarionoides*,  $2n = 8$ ; X, *Hypochaeris gayana*,  $2n = 8$ ; Y, *Hypochaeris glabra*,  $2n = 10$ ; Z, *Hypochaeris radicata*,  $2n = 8$ ; AA, *Hypochaeris scorzonerae*,  $2n = 8$ ; BB, *Lactuca serriola*,  $2n = 18$ ; CC, *Leontodon hirtus*,  $2n = 8$ ; DD, *Leontodon saxatilis*,  $2n = 8$ ; EE, *Noticastrum antucense*,  $2n = 18$ ; FF, *Notopappus prunelloides*,  $2n = 12$ ; GG, *Picris echioides*,  $2n = 10$ ; HH, *Lathyrus magellanicus*,  $2n = 14$ ; II, *Azara serrata* var. *serrata*,  $2n = 18$ . — Scale bars = 5  $\mu$ m.



There are numerous cytological papers on both *Crepis* species, all from Europe. These are the first records of diploid chromosome number in both species in America.

*Haplopappus bustillosianus* J.Rémy

$2n = 10$ , CHN. Chile, Biobío Region, Biobío Province, Polcura, La Posta sector, 570 m,  $37^{\circ}17'02.74''S$ ,  $71^{\circ}42'53.86''W$ , 19 Jan 2023, S. Teillier & al. 8643 (CONC) [Figs. 2P, 3O].

Goldblatt (1984) reports a haploid chromosome number  $n = 5$ . Therefore, this is the first diploid count for this species.

*Haplopappus glutinosus* Cass.

$2n = 10$ , CHN. Chile, Biobío Region, Biobío Province, Polcura, La Posta sector, 570 m,  $37^{\circ}17'02.74''S$ ,  $71^{\circ}42'53.86''W$ , 19 Jan 2023, S. Teillier & al. 8644 (CONC) [Figs. 2Q, 3P].

There is a previous count by Baeza & Schrader (2005), of a coastal population.

*Haplopappus paucidentatus* Phil.

$2n = 10$ , CHN. Chile, Biobío Region, Biobío Province, Polcura, La Posta sector, 570 m,  $37^{\circ}17'02.74''S$ ,  $71^{\circ}42'53.86''W$ , 19 Jan 2023, S. Teillier & al. 8645 (CONC) [Figs. 2R, 3Q].

Carr & al. (1999) reported the haploid number of this species. Therefore, this is the first diploid count of this species.

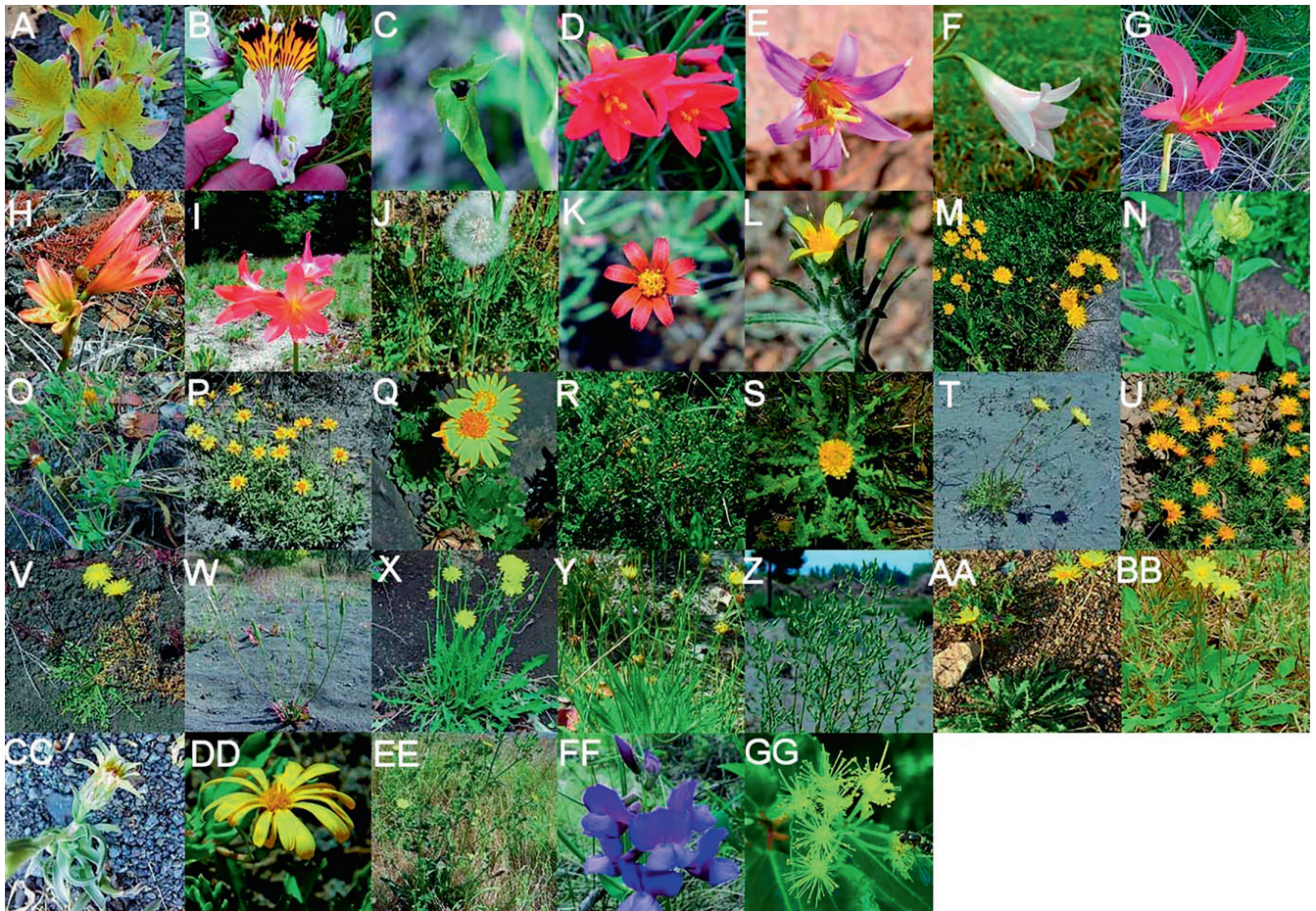
*Hieracium glaucifolium* Poepp. ex Froel.

$2n = 18$ , CHN. Chile, Biobío Region, Ralco, Pangué, Pitrilon, along the road, 520 m,  $37^{\circ}53'S$ ,  $71^{\circ}31'W$ , 8 Jan 2023, C. Baeza 4455a (CONC) [Figs. 2S, 3R].

Wulff (1998) reported the same chromosome number for a population from Argentina.

*Hypochaeris acaulis* (J.Rémy) Britton

$2n = 8$ , CHN. Chile, Biobío Region, Antuco, route from Los Barros to Trapa Trapa, 1800 m,  $37^{\circ}31'06''S$ ,  $71^{\circ}02'00''W$ , 2 Feb 2023, C. Baeza 4464a (CONC) [Figs. 2T, 3S].



**Fig. 3.** A, *Alstroemeria piperata*,  $2n = 16$ ; B, *Alstroemeria pulchra* var. *maxima*,  $2n = 16$ ; C, *Miersia chilensis*,  $2n = 22$ ; D, *Phycella australis*,  $2n = 16$ ; E, *Rhodolirium montanum*,  $2n = 16$ ; F, *Zephyranthes maculata*,  $2n = 18$ ; G, *Zephyranthes monantha*,  $2n = 18$ ; H, *Zephyranthes* aff. *splendens*,  $2n = 18$ ; I, *Zephyranthes splendens*,  $2n = 18$ ; J, *Agoseris coronopifolia*,  $2n = 18$ ; K, *Chaetanthera microphylla*; L, *Chaetanthera moenchioides*; M, *Crepis capillaris*; N, *Crepis pulchra*; O, *Haplopappus bustillosianus*; P, *Haplopappus glutinosus*; Q, *Haplopappus paucidentatus*; R, *Hieracium glaucifolium*; S, *Hypochaeris acaulis*; T, *Hypochaeris apargioides*; U, *Hypochaeris clarionoides*; V, *Hypochaeris gayana*; W, *Hypochaeris glabra*; X, *Hypochaeris radicata*; Y, *Hypochaeris scorzonerae*; Z, *Lactuca serriola*; AA, *Leontodon hirtus*; BB, *Leontodon saxatilis*; CC, *Noticstrum antucense*; DD, *Notopappus prunelloides*; EE, *Picris echioides*; FF, *Lathyrus magellanicus*; GG, *Azara serrata* var. *serrata*.



There are previous accounts of this species by several authors (Wulff, 1998; Weiss & al., 2003; Weiss-Schneeweiss & al., 2003; Baeza, 2004).

*Hypochaeris apargioides* Hook. & Arn.

$2n = 8$ , CHN. Chile, Biobío Region, Laja, road from Route 5 to Laja, km 25, next to San Pedro Apostle Chapel Church, 50 m, 37°24' S, 72°64'W, 24 Sep 2022, *C. Baeza 4444a* (CONC); Chile, Coronel, Road to Santa Juana, km 14.7, El Llano, 41 m, 36°58'28.8"S, 73°02' 16.5"W, 20 Jan 2023, *C. Baeza 4459a* (CONC) [Figs. 2U, V, 3T].

This species has been profusely analyzed cytologically because it is very abundant and widely distributed in Chile (Baeza & al., 2000, 2001, 2004, 2006; Weiss & al., 2003; Weiss-Schneeweiss & al., 2003).

*Hypochaeris clarionoides* (J.Rémy) Reiche

$2n = 8$ , CHN. Chile, Valparaíso Region, Los Andes, El Juncal Park, 2331 m, 33°21'19"S, 70°19'06"W, 15 Jan 2023, *J. Espejo s.n.* (CONC) [Figs. 2W, 3U].

There is a previous count carried out by Weiss & al. (2003) for a population of the Santiago metropolitan region.

\**Hypochaeris gayana* (DC.) Cabrera

$2n = 8$ , CHN. Chile, Biobío Region, Polcura, Quelén Hill, 1260 m, 37°14'47.16"S, 71°42'12.25"W, 19 Jan 2023, *C. Baeza 4458* (CONC) [Figs. 2X, 3V].

This species is very polymorphic and has an Andean distribution, both in Chile and Argentina (Urtubey & al., 2019).

*Hypochaeris glabra* L.

$2n = 10$ , CHN. Chile, Biobío Region, Laja, road from Route 5 to Laja, km 25, next to San Pedro Apostle Chapel Church, 50 m, 37°24' S, 72°46'W, 24 Sep 2022, *C. Baeza 4444b* (CONC) [Figs. 2Y, 3W].

There is a previous count for Chile by Baeza & al. (2004).

*Hypochaeris radicata* L.

$2n = 8$ , CHN. Chile, Biobío Region, Antuco, Laguna del Laja National Park, Los Barros, Andean Plain, 2200 m, 37°24'36"S, 71° 21'04"W, 3 Feb 2023, *C. Baeza & al. 4469* (CONC) [Figs. 2Z, 3X].

There are previous counts by Baeza & al. (2000, 2001, 2004).

*Hypochaeris scorzonerae* (DC.) F.Muell.

$2n = 8$ , CHN. Chile, Valparaíso, Balmaceda upload, road to La Pólvora, 40 m, 33°06'36.1"S, 71°37'42.3"W, 22 Mar 2023, *P. Novoa s.n.* (CONC) [Figs. 2AA, 3Y].

There is a previous count by Weiss & al. (2003).

*Lactuca serriola* L.

$2n = 18$ , CHN. Chile, Biobío Region, Ralco, Pangué, Pitron, 520 m, 37°53'S, 71°31'W, 08 Jan 2023, *C. Baeza 4455* (CONC) [Figs. 2BB, 3Z].

There are numerous studies and counts of this species in Europe. This count is the first for a population in America.

*Leontodon hirtus* L.

$2n = 8$ , CHN. Chile, Valparaíso Region, Valparaíso, Plazoleta Unimarc Villa Fundadores, 20 m, 33°07'S, 71°34'W, 18 Apr 2022, *P. Novoa s.n.* (CONC) [Figs. 2CC, 3AA].

There are counts in Europe for this species, but not from America. Miceli & Garbari (1976) and Garbari (1979) reported  $2n = 22$

chromosomes, while Natarajan (1977, 1978) reported  $2n = 24$ . This new  $2n = 8$  count constitutes a ploidy level never reported for this species.

*Leontodon saxatilis* L.

$2n = 8$ , CHN. Chile, Biobío Region, Coronel, road to Santa Juana, km 14.7, El Llano, 41 m, 36°58'28.8"S, 73°02'16.5"W, 15 Jan 2021, *C. Baeza s.n.* (CONC) [Figs. 2DD, 3BB].

For Europe there are counts by Izuzquiza & Feliner (1991) and Vogt & Oberprieler (1993). This is the first count for America.

\**Noticastrum antucense* Phil.

$2n = 18$ , CHN. Chile, Biobío Region, Antuco, Laguna del Laja National Park, Lagunillas, 1000 m, 37°23'42.87"S, 71°25'34.88"W, 2 Feb 2023, *C. Baeza 4465* (CONC) [Figs. 2EE, 3CC].

*Notopappus prunelloides* (Poepp. ex Less.) Klingenb.

$2n = 12$ , CHN. Chile, Biobío Region, Antuco, Los Barros, 1479 m, 37°31'39.2"S, 71°12'38.5"W, 2 Feb 2023, *C. Baeza 4460* (CONC) [Figs. 2FF, 3DD].

There is a previous account by Baeza & Schrader (2005) as *Grindelia prunelloides* (Poepp. ex Less.) Adr. Bartoli & Tortosa.

*Picris echioides* L.

$2n = 10$ , CHN. Chile, Biobío Region, Hualpén, Rocuant Island, 2 m, 36°43'38.3"S, 73°04'58"W, 12 Jan 2023, *C. Baeza 4457* (CONC) [Figs. 2GG, 3EE].

There are numerous counts for European and U.S.A. populations. This is the first count for South America.

**FABACEAE**

*Lathyrus magellanicus* Lam.

$2n = 14$ , CHN. Chile, Biobío Region, Polcura, Mañihuales, 1040 m, 37°15'18.07"S, 71°43'36.24"W, 19 Jan 2023, *S. Teillier & al. 8646* (CONC) [Figs. 2HH, 3FF].

There are previous counts by Moore (1981) and Seijo & Fernández (2003).

**SALICACEAE**

\**Azara serrata* Ruiz & Pav. var. *serrata*

$2n = 18$ , CHN. Chile, Biobío Region, Coronel, road to Santa Juana, km 14.7, El Llano, 20 m, 36°58'S, 73°02'W, 20 Jan 2022, *C. Baeza s.n.* (CONC) [Figs. 2II, 3GG].

There are only gametophytic counts ( $n = 9$ ) for *Azara serrata* var. *fernandeziana* (Gay) Reiche (Sanders & al., 1983; Spooner & al., 1987), a taxon endemic to the Juan Fernandez archipelago.

**METHODS**

Rhizome roots or germinated seeds (1–2 cm length) obtained from individuals in each population and held in a greenhouse, were cut and pretreated with a solution of 8-hydroxyquinoline (2 mM) for 24 h at 4°C or in a 1% colchicine solution for 2 h at 18°C in the dark and then 2 h at 4°C. These samples were subsequently fixed with a fresh solution of ethanol/acetic acid (3 : 1) for 24 h. Squash preparations from root tips were made using an acid hydrolysis pretreatment with HCL 0.5 N during 20 min at 42°C. After washing in distilled water, the material was stained with 1% orcein solution. Metaphase chromosome plates were photographed using a Zeiss Axioskop microscope, with an incorporated video camera (Baeza & al., 2018).

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## IAPT chromosome data 40/3

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\* First chromosome count for the species.

\*\* First gametophytic chromosome count for the species.

**AMARYLLIDACEAE**

*Narcissus cuatrecasatii* Fern.Casas, M.Lainz & Ruiz Rejón

$2n = 14$ , CHN. Spain, Mancha Real, Sierra Mágina, climb to Peña del Águila from Mancha Real, 1200 m, 13 Apr 2000, *Mejías s.n.* (SEV 289807) [Fig. 4A].

Like various published chromosome counts (Fernández-Casas & al., 1973; Löve & Kjellqvist, 1973; Ruiz Rejón & Sañudo, 1976), we have also found the somatic chromosome number  $2n = 14$  (Fig. 4A). In some of these reports, morphological characteristics of the chromosomes of the karyotype are also provided; however, to the best of our knowledge, a full description of the karyotype including idiogram formula and asymmetry indexes, as we provide here, has not been published before. The apparent chromosome size ranges from 3.51 to 11.05  $\mu\text{m}$ ; therefore, most of the chromosomes have been found to be medium large, but one large pair and one medium-small pair have also been detected. The total length of the haploid chromosome complement is 46.25  $\mu\text{m}$ . According to their morphology, chromosomes can be grouped as follows: 2 metacentric (M; pair 1), 2 metacentric (m; pair 5), 8 submetacentric (sm; pairs 2, 3, 6, 7), two of them possess satellites (pair 7), and 2 submetacentric-subtelocentric (sm-st; pair 4) (Fig. 5A). Thus, the idiogram formula is  $2M + 2m + 6sm + 2sm^{\text{sat}} + 2sm\text{-st}$ . The asymmetry is of type 2B, and asymmetry coefficients are  $A_1 = 0.45$  and  $A_2 = 0.31$ .

**ASPARAGACEAE**

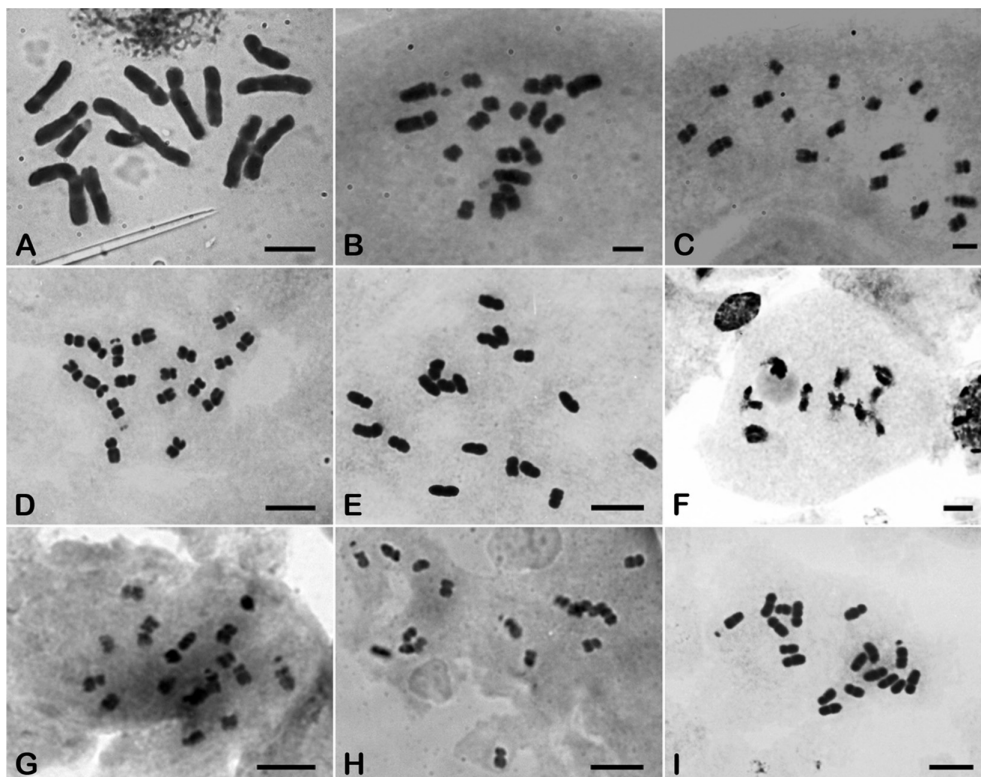
*Muscari armeniacum* Leichtlin ex Baker

$2n = 18$ , CHN. Turkey, Giresun Province, Asarcik Köyü, outskirts of the village, 1700 m, 40°24'39.1"N, 38°23'45.5"E, 13 May 2010, *Mejías & Sánchez s.n.* (SEV 256836) [Fig. 4B]; Turkey, Bolu Province, Ulaşlar Köyü, pine forest by the road D-100, 1500 m, 40°49'38.4"N, 32°21'20.7"E, 15 May 2010, *Mejías & Sánchez s.n.* (SEV 256837) [Fig. 4C].

In the two populations studied, the somatic chromosome number was  $2n = 18$  (Fig. 4B,C); therefore, they should be considered

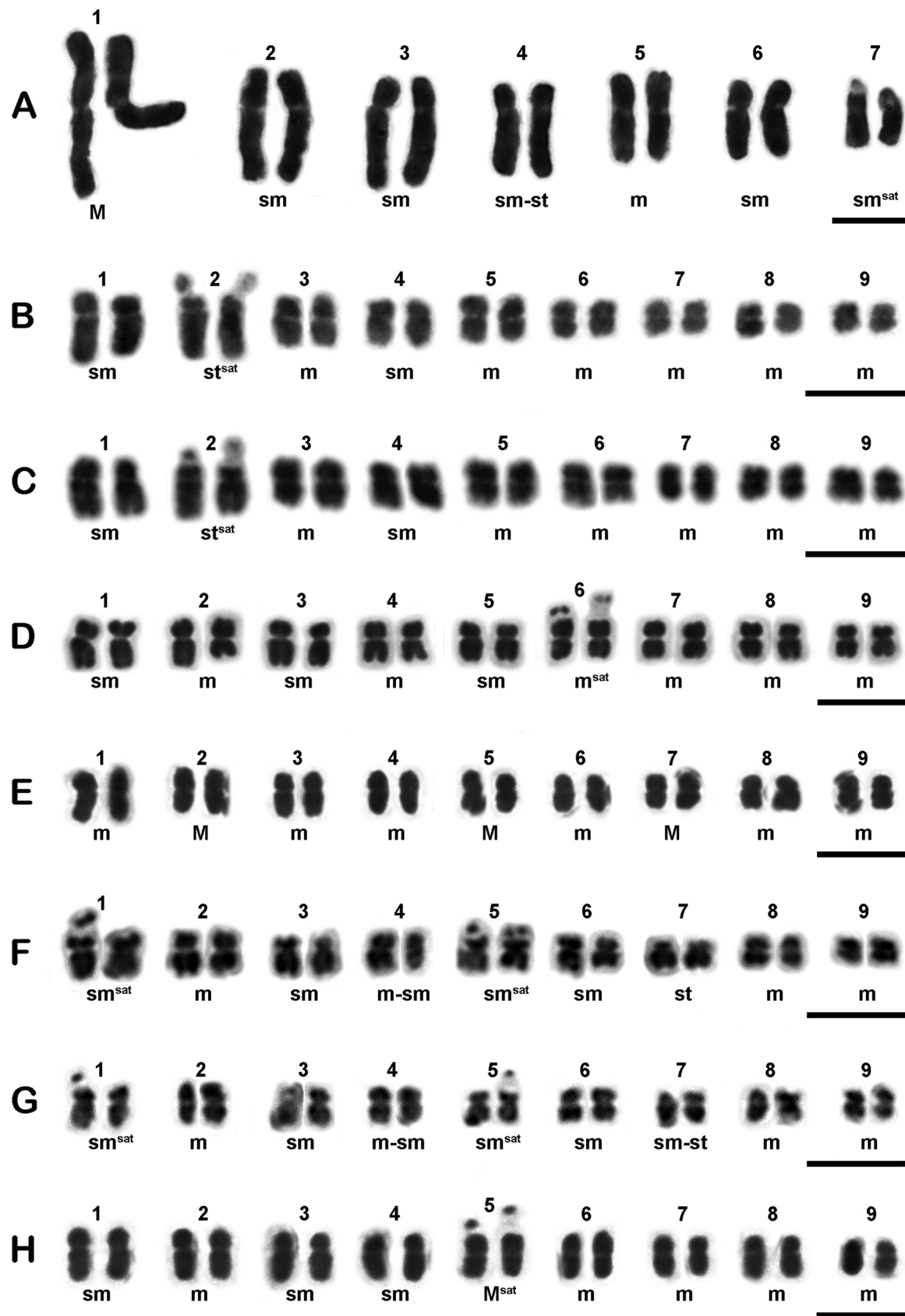
as diploid (Demirci Kayıran & Özhatay, 2017; Kiran & al., 2020). Most chromosome countings have reported the same number for this eastern Mediterranean species (e.g., Karlén, 1984; Özhatay & Johnson, 1996; Demirci Kayıran & Özhatay, 2017; Kiran & al., 2020; Uysal & al., 2022), but some others have indicated the tetraploid number  $2n = 4x = 36$  (e.g., Stuart, 1970; Özhatay & Johnson, 1996), or even the presence of B chromosomes (Özhatay & Johnson, 1996). Other chromosome numbers have been reported for cultivated material (Saito & Matsuzawa, 1969; Rice & al. 2015).

In our observations, the apparent chromosome length of *Muscari armeniacum* ranges from 2.22 to 6.36  $\mu\text{m}$  (total length of haploid chromosome complement: 34.43  $\mu\text{m}$ ) in the population from Asarcik and from 2.21 to 5.32  $\mu\text{m}$  (total length of haploid chromosome complement: 32.08  $\mu\text{m}$ ) in the one from Ulaşlar; thus, they can be classified as medium-short (pairs 3 to 9) and medium-large (pairs 1 and 2). Chromosomes of both populations are grouped as follows: 12 metacentric (m; pairs 3, 5, 6, 7, 8, 9), 4 submetacentric (sm; pairs 1, 4) and 2 subtelocentric (st; pair 2), which are satellite (Fig. 5B,C). The resulting idiogram formula is  $12m + 4sm + 2st^{\text{sat}}$ . The asymmetry found is of type 2B, and asymmetry coefficients are  $A_1 = 0.33$  and  $A_2 = 0.37$  in the first population and  $A_1 = 0.30$  and  $A_2 = 0.27$  in the second one. The present idiogram formula clearly agrees with those reported by Dermicki Karyan & Özhatay (2017) in five diploid populations from Turkey, although they did not detect satellite chromosomes in three of the populations. Kiran & al. (2020) and Uysal & al. (2022) reported the formulas  $2M + 14m + 2sm$  and  $14m + 4sm + 2st^{\text{sat}}$  respectively, which, in general, show karyotypes of somewhat more symmetrical chromosomes. Karlén (1984) also reported a haploid idiogram for diploid *M. armeniacum* showing quite similar chromosome morphology, without subtelocentric chromosomes. As far as we know, no karyomorphological analyses in polyploid material of this species have been published.



**Fig. 4.** Mitotic metaphases and meiotic diakinesis in *Narcissus*, *Muscari* and *Sonchus* species. **A**, *N. cuatrecasatii* ( $2n = 14$ ); **B & C**, *M. armeniacum* ( $2n = 18$ ); **D**, *Sonchus fragilis* ( $2n = 18$ ); **E**, *S. masguindalii* ( $2n = 18$ ); **F**, *S. masguindalii* ( $n = 9$ ); **G & H**, *S. nymanii* ( $2n = 18$ ); **I**, *S. pustulatus* ( $2n = 18$ ). — Scale bars = 5  $\mu\text{m}$ .





**Fig. 5.** Karyograms for *Narcissus*, *Muscari* and *Sonchus* species. **A**, *N. cuatrecasasii* ( $2n = 14$ ); **B & C**, *M. armeniacum* ( $2n = 18$ ); **D**, *Sonchus fragilis* ( $2n = 18$ ); **E**, *S. masquindalii* ( $2n = 18$ ); **F & G** *S. nymanii* ( $2n = 18$ ); **H**, *S. pustulatus* ( $2n = 18$ ). — Scale bars = 5  $\mu$ m.

Differences in chromosome morphology suggest possible karyotype morphological and genetic shifts due to evolutionary processes in changing habitats (Uysal & al., 2022), but they can also reflect misidentifications of plant material, since *Muscari* subg. *Botryanthus* is a taxonomically complex group, where imprecise species delimitation could lead to incorrect chromosome reports (Karlén, 1984; Kiran & al., 2020; Uysal & al., 2022). According to taxonomic indications of Pinar & al. (2018) and Uysal & al. (2022), the material here studied should be identified as *M. armeniacum*. Leaf features and raceme density of our material clearly correspond to *M. armeniacum*, although bulb scales are not clearly dark brown. Karyotype equivalences with others members from the same group (mostly with *M. aucheri*, *M. botryoides* and *M. vanensis*) have been found while doing this research (Karlén, 1984; Demirci Kayıran & Özhatay, 2017; Kiran & al. 2020; Uysal & al., 2022); in fact, karyological data are sometimes confusing, which reinforces the need of a sound phylogenetic and taxonomic revision of the *Muscari* subg. *Botryanthus* (Dizkirici & al., 2019).

## ASTERACEAE

### *Sonchus fragilis* Ball

$2n = 18$ , CHN. Morocco, Mts. Gorgues (Beni-Hosmar), S Tetouan, limestone cliffs, 475 m, 17 Apr 1999; *Ajbilou, Arroyo, Marañón & Mejías s.n.* (SEV 215728, SEV 217382) [Fig. 4D].

The somatic chromosome number found,  $2n = 18$  (Fig. 4D), agrees with a previous report for plants from another location in the Beni Hosmar range (Vogt & Oberprieler, 2008) of this narrow endemic, which is restricted to the mountainous environments of the city of Tetouan (Morocco). The apparent size of the chromosomes in the karyotype ranged from 1.56 to 2.70  $\mu\text{m}$ ; therefore, they can be classified as small and medium-small ones. The total length of the haploid chromosome complement is 18.82  $\mu\text{m}$ . According to their morphology, chromosomes can be grouped as follows: 12 metacentric (m; pairs 2, 4, 6, 7, 8, 9), two of them being satellite (pair 6), and 6 submetacentric (sm; pairs 1, 3, 5) (Fig. 5D). The idiogram formula is  $10m + 2m^{\text{sat}} + 6sm$ . The asymmetry is of type 2A, and asymmetry coefficients are  $A1 = 0.30$  and  $A2 = 0.15$ . *Sonchus fragilis* chromosome morphology shows great similarity to that of *S. pustulatus* Willk. (as can be checked below), another species of the same section (*S. sect. Pustulati* Boulos), which is consistent with the close relationship between the two taxa (Silva & al., 2015). It is remarkable that *S. fragilis* shows only a single metacentric satellite pair, as does Moroccan *S. pustulatus* material.

### \*\**Sonchus masquindalii* Pau & Font Quer

$2n = 18$ ,  $n = 9$ , CHN. Morocco, Al-Hoceïma, Asfisha beach, limestone fissures by the sea,  $35^{\circ}12'35''\text{N}$ ,  $03^{\circ}54'05''\text{E}$ , 24 Apr 1999, *Arroyo & Mejías s.n.* (SEV 215733, SEV 217381) [Fig. 4E,F].

The somatic chromosome number found,  $2n = 18$  (Fig. 4E), agrees with a previous count in material of this endemic species to the Moroccan Bokkoya Region. As expected, chromosome number of plants from the westernmost part of the distribution area (here) agrees with that from the easternmost part (Vogt & Oberprieler, 2008). We also observed that the chromosomes regularly form 9 bivalents in meiosis ( $n = 9$ ) (Fig. 4F). In somatic metaphase, the apparent size of the chromosomes varies from 1.81 to 3.01  $\mu\text{m}$ ; therefore, they are small and medium-small. The total length of the haploid chromosome complement is 19.81  $\mu\text{m}$ . The chromosomes can be grouped as follows: 6 metacentric (M; pairs 2, 5, 7); and 12 metacentric (m; pairs 1, 3, 4, 6, 8) (Fig. 5E). Therefore, the idiogram formula is  $6M + 12m$ , and no satellite chromosome has been found. The

Alas asymmetry type of the karyotype is 1A, and asymmetry coefficients are  $A1 = 0.20$  and  $A2 = 0.16$ , which denotes a remarkable karyotype symmetry among the species of the genus *Sonchus* L. (Mejías & Andrés, 2004), including those from the same group (*S. sect. Pustulati* Boulos).

### \**Sonchus nymanii* Tineo & Guss.

$2n = 18$ , CHN. Italy, Sicily, road from Alia to Roccapalumba, farmed fields, 520 m,  $37^{\circ}46'31.6''\text{N}$ ,  $13^{\circ}40'12.6''\text{E}$ , 3 May 2011, *Lim, Mejías & Silva IT27/11* (SEV 284705) [Fig. 4G]; Italy, Sicily, Scillato-Caltavuturo, grassland in damp soils next to cultivated fields, 400 m,  $37^{\circ}50'34.4''\text{N}$ ,  $13^{\circ}54'14.3''\text{E}$ , 3 May 2011, *Lim, Mejías & Silva IT22/11* (SEV 284703) [Fig. 4H].

As far as we know, these are the first chromosome counts for *Sonchus nymanii*, a taxon that was described to designate as a separate species an assemblage of rhizomatous perennial plants growing in clay soils of crop areas in central Sicily, with great morphological similarity to the annual/biennial species *S. asper* (L.) Hill (Tineo & Gussone; Gussone, 1844: 860). The two populations studied showed the somatic number  $2n = 18$  (Fig. 4G,H), which agrees with several previous studies in *S. asper* (Mejías & Andrés, 2004; Mejías & al., 2012). The number also reveals the diploid level of the plants, as well as *S. asper*, since  $x = 9$  has repeatedly been proposed as the basic number of genus *Sonchus* (Boulos, 1972; Mejías & Andrés, 2004; Mejías & al., 2018).

The apparent size of the chromosomes in the karyotypes varies between 0.74 and 1.62  $\mu\text{m}$ , with a total length of the haploid chromosome complement of 10.78  $\mu\text{m}$ , in the plants from Alia and between 0.79 and 1.70  $\mu\text{m}$ , with a total length of the haploid chromosome complement of 11.56  $\mu\text{m}$ , in those from Scillato-Caltavuturo, all of them being small. The chromosomes can be grouped as follows: 4 metacentric (m; pairs 2, 9); 4 metacentric-submetacentric (m-sm; pairs 4, 8); 8 submetacentric (sm; pairs 1, 3, 5, 6), 4 of which are satellite (pairs 1, 5); and 2 subtelocentric (st; pair 7) in the first population (Fig. 5F) or 2 submetacentric-subtelocentric (sm-st; pair 7) in the second population (Fig. 5G). Therefore, the idiogram formula is  $4m + 4m\text{-sm} + 4sm + 4sm^{\text{sat}} + 2st$  in the population from Alia and  $4m + 4m\text{-sm} + 4sm + 4sm^{\text{sat}} + 2sm\text{-st}$  in the plants from Scillato-Caltavuturo. The asymmetry is of type 2B in both cases, and asymmetry coefficients are  $A1 = 0.44$  and  $A2 = 0.24$  in the first population and  $A1 = 0.41$  and  $A2 = 0.21$  in the second one. All these traits show a clear similarity of the karyotypes studied with those found in common plants of *Sonchus asper* (Mejías & Andrés, 2004).

In both *Sonchus asper* and *S. nymanii*, the achenes are highly compressed, show marginal wings and bear few ribs (commonly three) on each fruit side, a set of features that is currently considered characteristic and privative of *S. asper* s.l., or even of *S. sect. Asperi* Boulos within *Sonchus*. In addition to the life form, the plants studied here differ from typical *S. asper* in the presence of quite showy flower heads, a trait also highlighted in the protologue of *S. nymanii* (“Differt a *S. aspero*; floribus duplo majoribus”; Gussone, 1844: 860) that should make easier the recognition of this species. The diploid condition and the achene morphology allow to reject the possibility that the plants studied represent a race of *S. arvensis* L., a rhizomatous polyploid taxon ( $2n = 36, 54 [4x, 6x]$ ) that also colonizes moist soils, preferably clay and loam soils, in higher latitudes of the Northern Hemisphere.

Despite all these arguments, the taxonomic identity of *Sonchus nymanii* is not currently recognized and the name is considered a synonym of *S. asper* subsp. *glaucescens* (Jord.) Ball, a putative biennial form in *S. asper* (Boulos, 1973: 165–166) hardly distinguishable from typical annual plants. The material studied here came from

two nearby localities from central Sicily (Italy), one of which (Alia) is cited in the protologue of the name (Gussone, 1844: 860) and, therefore, is a type locality. This fact reinforces the certainty that we are dealing with the plants designated as *S. nymanii* by V. Tineo and G. Gussone.

*Sonchus pustulatus* Willk.

$2n = 18$ , CHN. Morocco, Talambote electrical power station, limestone cliffs and rocks, 150 m, 13 Aug 2003, *Mejías s.n.* (SEV 217476, SEV 217476) [Fig. 4I].

We found the somatic number  $2n = 18$  (Fig. 4I), as reported in several previous publications for both Moroccan and Spanish plants (Stebbins & al., 1953; Talavera & al., 1984; Mejías, 1988; Vogt & Oberprieler, 2008) of this Baetic-Rifean endemic (Silva & al., 2015). However, it is the first time that chromosome morphology has been analyzed in Moroccan material. In our observations, the apparent chromosome size ranges from 1.66 to 2.90  $\mu\text{m}$ ; thus, they can be classified as small and medium-small. The total length of the haploid chromosome complement is 20.95  $\mu\text{m}$ . These chromosomes can be grouped as follows: 2 metacentric (M; pair 5), which are satellite, 10 metacentric (m; pairs 2, 6, 7, 8, 9) and 6 submetacentric (sm; pairs 1, 3, 4) (Fig. 5H). The resulting idiogram formula is:  $2M^{\text{sat}} + 10m + 6sm$ , the asymmetry is of type 1A, and the karyotype asymmetry coefficients are  $A1 = 0.28$  and  $A2 = 0.15$ . The comparison between our results and those provided by Mejías (1988) clearly shows the high similarities between the karyotypes of plants from both sides of the Mediterranean Sea; the most notable difference between them lies in the number of satellite chromosomes: two pairs in the Iberian plants (one strongly metacentric plus one submetacentric pair), and one metacentric pair in the material from northern Morocco.

## METHODS

Mitotic chromosome numbers were examined in meristematic cells obtained from germinating seeds and grown adult plant root tips. The samples were pretreated with 2 mM 8-hydroxyquinoline for 3.5 h at room temperature, fixed in Farmer's solution, absolute ethanol : glacial acetic acid (3 : 1, v/v), and stored at  $-20^\circ\text{C}$  until use. Pretreated material was stained in alcoholic hydrochloric acid-carmin for 48–72 h under the heat provided by an incandescent lamp. Slide preparations were performed by squashing in a drop of 45% acetic acid. For meiotic chromosomes, flower heads buds of four plants were fixed in Farmer's solution in the wild. The procedure for staining and squashing the material was the same as in the previous case. The best slides were photographed on a ZEISS AXIOPHOT photomicroscope with a ZEISS AXIOCAM 305 camera. Adobe Photoshop CS6 v.13 and ImageJ v.1.53k (Schneider & al., 2012) were used for the elaboration and measure of the karyograms.

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## IAPT chromosome data 40/4

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- \* First report of a new cytotype for the species.
- \*\* First report of B chromosomes for the species.
- # First chromosome count for the species from India.

Cytological investigations were carried out from immature unopened flower buds fixed in Carnoy's fixative (ethylalcohol : chloroform : glacial acetic acid, 6 : 3 : 1, v/v/v) for 24 h at room temperature and then transferred to 70% alcohol and squashed in 1% acetocarmine.

### CACTACEAE

# *Mammillaria carnea* Zucc. ex Pfeiff.

$n = 11$ , CHN. India, Haryana, Panchkula, 30°41'18.78"N, 76°59'39.822"E, 496 m, 30 Mar 2021, *H. Kaur s.n.* (PUN 39015) [Fig. 6A].

An accession collected from Panchkula (Haryana) showed the presence of 11 bivalents at metaphase I [Fig. 6A]. Previously, Remski (1954) recorded the chromosome number of  $n = 11$  from the U.S.A. The chromosome number of  $n = 11$  is published here as the first meiotic report for the species from India.

# *Mammillaria magnimamma* Haw.

$n = 11$ , CHN. India, Haryana, Panchkula, 30°41'18.78"N, 76°59'39.822"E, 496 m, 27 Mar 2021, *H. Kaur s.n.* (PUN 39065); India, Jalandhar, Punjab, 31°17'31.23"N, 75°34'05.008"E, 233 m, 20 Apr 2022, *H. Kaur s.n.* (PUN 39066) [Fig. 6B,C].

Two accessions collected from Panchkula (Haryana) and Jalandhar (Punjab) showed the presence of 11 bivalents at diakinesis and metaphase I [Fig. 6B,C]. The present chromosome count is in agreement with the previous reports of Katagiri (1953) and Remski (1954) from Japan and the U.S.A., respectively.

### COMMELINACEAE

\*\**Murdannia nudiflora* (L.) Brenan

$n = 10 + 0-1B$ , CHN. India, Himachal Pradesh, Sirmaur, 31°49'02.136"N, 77°18'09.936"E, 1552 m, 8 Jul 2018, *P. Rani s.n.* (PUN 63192); India, Himachal Pradesh, Palampur, 32°06'34.214"N, 76°32'08.880"E, 1472 m, 11 Sep 2018, *P. Rani s.n.* (PUN 63194); India, Himachal Pradesh, Chamunda, 31°49'02.136"N, 77°18'09.936"E, 1662 m, 12 Sep, 2018, *P. Rani s.n.* (PUN 63195) [Fig. 6D,E].

Three accessions collected from different localities (Sirmaur, Palampur, Chamunda) of Himachal Pradesh showed the presence of B-chromosome along with 10 bivalents at diakinesis ( $n = 10 + 1B$ ) [Fig. 6D,E]. Previously, there were no reports of B chromosome for this species.

\*\**Tradescantia pallida* (Rose) D.R.Hunt.

$n = 12 + 0-1B$ , CHN. India, Himachal Pradesh, Nadaun, 31°41'11.0364"N, 76°31'17.04"E, 555 m, 20 Sep 2019, *P. Rani s.n.* (PUN 63468) [Fig. 6F].

An accession collected from Nadaun (Himachal Pradesh) revealed the presence of a B chromosome along with 12 bivalents at anaphase I [Fig. 6F]. This is the first record of a B chromosome for this species.

### COSTACEAE

\**Costus pictus* D. Don

$n = 14$ , CHN. India, Punjab, Patiala, 30°21'36.072"N, 76°27'06.3612"E, 256 m, 4 Apr 2019, *P. Rani s.n.* (PUN 63178) [Fig. 6G,H].

The presence of small-sized 14 bivalents in PMCs was observed in metaphase I and anaphase I, illustrating that the plant bears a gametic chromosome number of  $n = 14$  [Fig. 6G,H]. Earlier, Venkatasubban (1946) reported  $2n = 36$  as the chromosome number based on specimens from Sri Lanka. Later, Vovides & Lascurain (1995) reported the chromosome number of  $2n = 18$  from specimens from southern Veracruz, Mexico. The present report presents a newly detected dysploid cytotype for *Costus pictus* with a chromosome number of  $2n = 4x = 28$ . The present finding is the first report of a meiotic chromosome count for the species.

### LAMIACEAE

# *Mentha arvensis* L.

$n = 12$ , CHN. India, Jammu and Kashmir, Ganderbal, 34°17'40.7184"N, 75°13'00.8112"E, 2039 m, 22 Jul 2017, *F. Majid s.n.* (PUN 62365) [Fig. 6I,J].

An accession collected from Ganderbal (Jammu and Kashmir) showed 12 bivalents at diakinesis and metaphase I, illustrating that the plant has a gametic chromosome number of  $n = 12$  [Fig. 6I,J]. Previously, the chromosome number of  $n = 12$  was recorded by Ouweneel (1968) from the Netherlands. The present finding is the first report of chromosome count from India for this species.

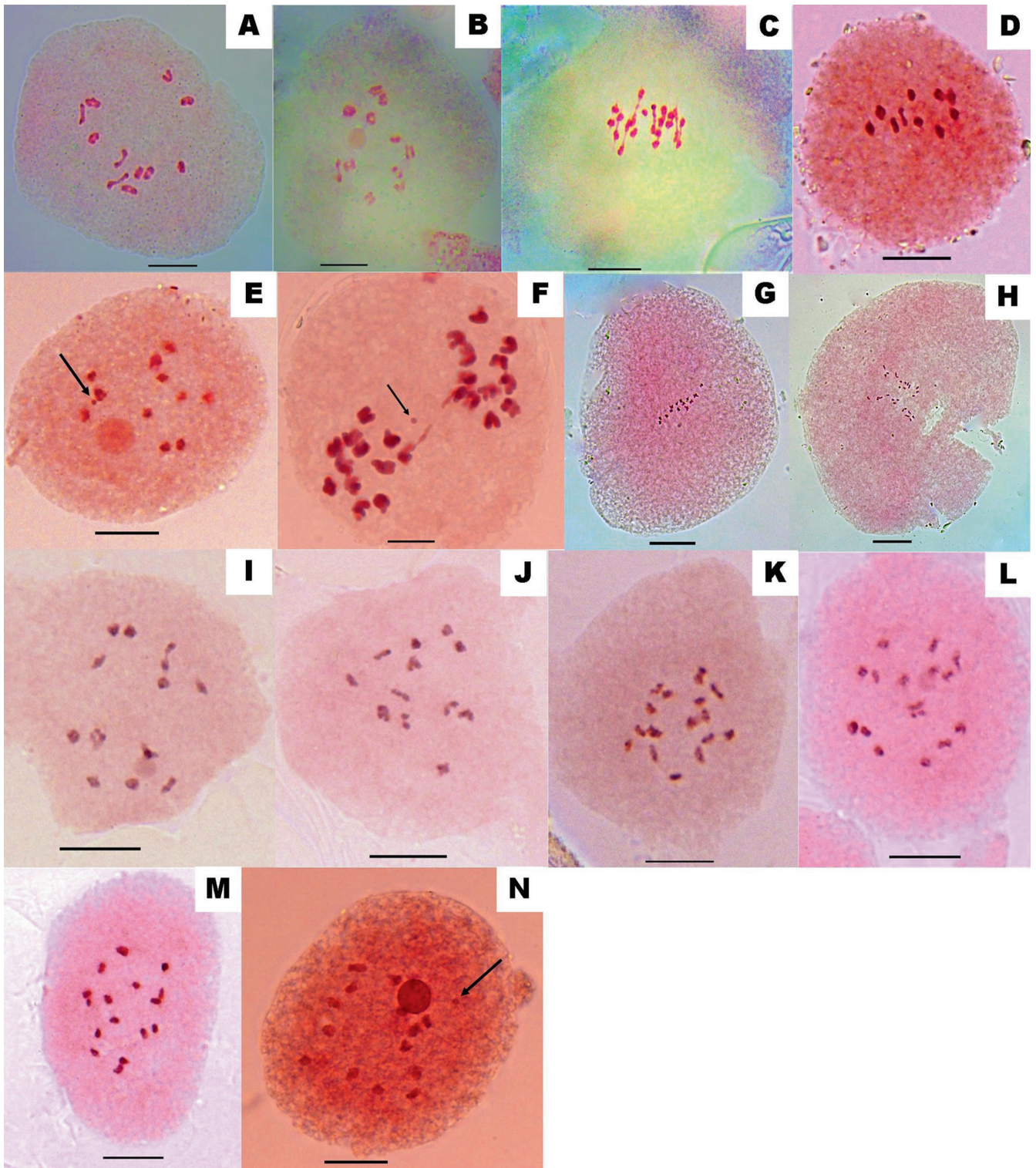
# *Scutellaria galericulata* L.

$n = 16$ , CHN. India, Jammu and Kashmir, Baramulla, 34°18'28.5228"N, 74°14'21.0048"E, 1859 m, 18 Aug 2017, *F. Majid s.n.* (PUN 62395) [Fig. 6K].

An accession collected from Baramulla (Jammu and Kashmir) revealed the presence 16 bivalents at metaphase I [Fig. 6K]. This is the first report of chromosome number for this species from India. The chromosome number of  $n = 16$  was been previously reported by Gadella & Kliphuis (1967; Netherlands), Morton (1973; Great Britain), Pogan & al. (1980; Poland), Váchová & Feráková (1980), Gill (1981; Canada) and Dmitrieva (2000; Belarus).

\**Stachys sericea* Wall.

$n = 16$ , CHN. India, Jammu and Kashmir, Ganderbal, 34°18'02.7468"N, 74°14'21.0048"E, 2610 m, 22 Jul 2017, *F. Majid s.n.* (PUN 62405) [Fig. 6L,M].



**Fig. 6.** A, *Mammillaria carnea*: PMC showing 11 bivalents at metaphase-I; B & C, *Mammillaria magnimamma*: B, PMC showing 11 bivalents at diakinesis; C, PMC showing 11 bivalents at metaphase-I; D & E, *Murdannia nudiflora*, male meiotic course: D, PMC showing 10 bivalents at diakinesis; E, PMC showing small-sized B chromosome at diakinesis (arrow); F, *Tradescantia pallida*, PMC showing B chromosome at anaphase-I (arrow); G & H, *Costus pictus*: G, PMC showing 14 bivalents at metaphase-I; H, PMC showing 14 bivalents at anaphase-I; I & J, *Mentha arvensis*: I, Meiocyte showing 12 bivalents at diakinesis; J, PMC showing 12 bivalents at metaphase-I; K, *Scutellaria galericulata*, PMC showing 16 bivalents at metaphase-I; L & M, *Stachys sericea*: L, PMC showing 16 bivalents at diakinesis; M, PMC showing 16 bivalents at metaphase-I; N, *Sida acuta*, PMC showing small-sized B chromosome at diakinesis (arrow). — Scale bars = 10  $\mu$ m.



An accession collected from Baramulla (Jammu and Kashmir) revealed the presence of 16 bivalents at diakinesis and metaphase I [Fig. 6L,M]. The chromosome number of  $n = 16$  is reported here as a new cytotype for the species. However, the chromosome number of  $n = 15$  has been reported by Gill (1970, 1984), Saggoo (1983), Saggoo & Bir (1983) and Malik & al. (2017) (Western Himalayas).

#### MALVACEAE

\*\**Sida acuta* Burm.f.

$n = 14 + 0-1B$ ; CHN. India, Himachal Pradesh, Una, 31°28'43.68"N, 76°16'30.0072"E, 369 m, 17 Oct 2017, *S. Kaur s.n.* (PUN 62698); India, Himachal Pradesh, Kangra, 32°05'59.2944"N, 76°16'8.7744"E, 733 m, 18 Oct 2017, *S. Kaur s.n.* (PUN 62699) [Fig. 6N].

Two accessions, collected from Una and Kangra, District of Himachal Pradesh, revealed the presence of one B-chromosome along with 14 bivalents ( $n = 14 + 0-1B$ ) at diakinesis [Fig. 6N]. This is the first-ever report of B-chromosome in *Sida acuta*.

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#### IAPT chromosome data 40/5

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\* First chromosome count for the taxon.

\*\* First chromosome count from an unexplored region, providing new data for the taxon.

# First record of polyploidy within the genus.

#### IRIDACEAE

##### Subfamily Iridoideae

##### Tribe Tigridieae (Clade A)

\**Cypella altouruguaya* Chauveau & L.Eggers

$2n = 14$ ; CHN. Brazil, Rio Grande do Sul, Trindade do Sul, 27°31'01.0"S, 52°58'53.0"W, 18 Aug 2012, *L. Eggers & O. Chauveau* 716 (ICN) [Fig. 7A,E].

\**Cypella amplimaculata* Chauveau & L.Eggers

$2n = 14$ ; CHN. Brazil, Rio Grande do Sul, Porto Alegre, Morro Santana, 30°03'09.0"S, 51°07'26.0"W, 20 Oct 2022, *J.G. Dani & C. Guzati* 280 (ICN); Brazil, Rio Grande do Sul, Passo Fundo, 28°14'12.0"S, 52°19'46.0"W, 6 Nov 2022, *J.G. Dani, C. Guzati & H.M. Buneker* 375 (ICN); Brazil, Santa Catarina, Lages, 27°49'16.0"S, 50°16'54.0"W, 1 Nov 2022, *J.G. Dani, C. Guzati & H.M. Buneker* 330 (ICN) [Fig. 7B,F].

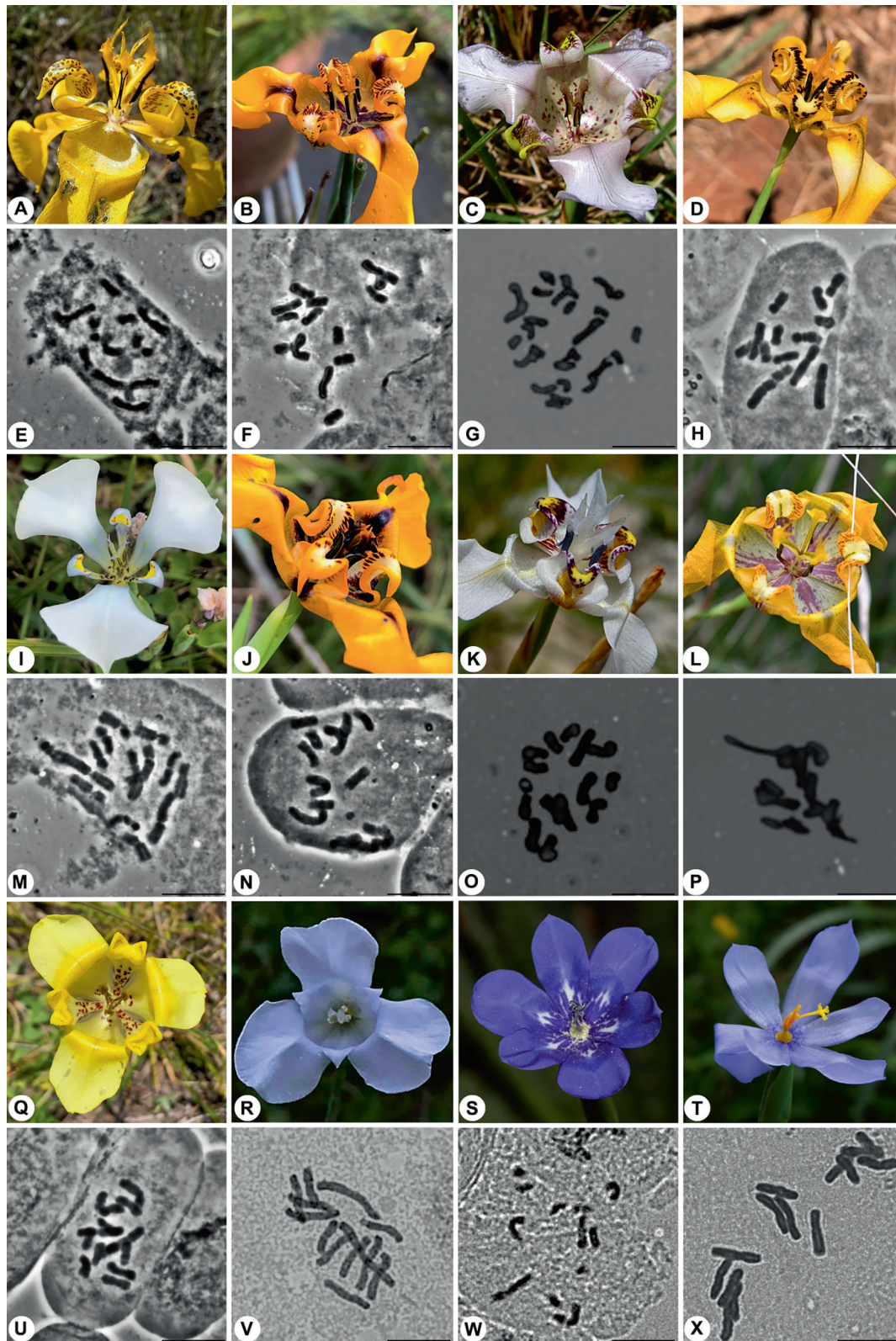
\*# *Cypella discolor* Ravenna

$2n = 4x = 28$ ; CHN. Brazil, Rio Grande do Sul, Quaraí, 30°11'43.8"S, 56°29'19.6"W, 14 Nov 2013, *E.M. Stiehl-Alves, F.S. Silveira, J. Focchezatto & L.O. Tacuatia* 94 (ICN) [Fig. 7C,G].

\*\**Cypella fucata* Ravenna

$2n = 14$ ; CHN. Brazil, Rio Grande do Sul, Caçapava do Sul, 30°33'18.0"S, 53°12'44.0"W, 26 Oct 2022, *J.G. Dani, C. Guzati, L.N. Da Silva, G. Pecoits* 318 (ICN); Brazil, Rio Grande do Sul, Passo Fundo, 28°14'12.0"S, 52°19'46.0"W, 6 Nov 2022, *J.G. Dani, C. Guzati & H.M. Buneker* 374 (ICN); Brazil, Rio Grande do Sul, Porto Alegre, Morro São Pedro, 11 Nov 2022, *J.G. Dani & C. Guzati* 386 (ICN) [Fig. 7D,H].





**Fig. 7.** Species with new chromosome records and their mitotic cells in metaphase. **A & E**, *Cypella altouruguaya* ( $2n = 14$ ); **B & F**, *Cypella ampli-maculata* ( $2n = 14$ ); **C & G**, *Cypella discolor* ( $2n = 4x = 28$ ); **D & H**, *Cypella fucata* ( $2n = 14$ ); **I & M**, *Cypella hauthalii* subsp. *minuticristata* ( $2n = 14$ ); **J & N**, *Cypella herbertii* subsp. *herbertii* ( $2n = 14$ ); **K & O**, *Cypella luteogibbosa* ( $2n = 14$ ); **L & P**, *Cypella magnicristata* ( $2n = 14$ ); **Q & U**, *Cypella pusilla* ( $2n = 14$ ); **R & V**, *Gelasine coerulea* ( $2n = 14$ ); **S & W**, *Gelasine elongata* ( $2n = 12$ ); **T & X**, *Gelasine uruguayensis* ( $2n = 14$ ). — Scale bars: E–H, M–P & U–X, 10  $\mu$ m. Photos: H.M. Buneker (L); J.G. Dani (A, B, I, Q); M. Grings (C, K, S); C. Guzati (D, J); A. Silvério (R); A. González (T).

The results of this research confirm the chromosome number previously documented for Piratini (Brazil, Rio Grande do Sul), Pinheiro Machado (Brazil, Rio Grande do Sul) and Punta del Este (Uruguay, Maldonado) samples, as reported by Goldblatt & Takei (1997) and Moraes & al. (2015).

\**Cypella hauthalii* subsp. *minuticristata* Chauveau & L.Eggers  
2n = 14; CHN. Brazil, Rio Grande do Sul, Passo Fundo, 08°14' 12.0"S, 52°19'46.0"W, 6 Nov 2022, J.G. Dani, C. Guzati & H.M. Buneker 376 (ICN) [Fig. 7L,M].

The chromosome number here reported is the same as one previously recorded for *Cypella hauthalii* subsp. *opalina* Ravenna from Garruchos (Argentina, Misiones) and Santo Antônio das Missões (Brazil, Rio Grande do Sul) as reported by Goldblatt & Takei (1997) and Moraes & al. (2015), respectively.

\*\**Cypella herbertii* (Herb.) Herb. subsp. *herbertii*  
2n = 14; CHN. Brazil, Santa Catarina, Urubici, 28°08'24.0"S, 49°38'36.0"W, 4 Nov 2022, J.G. Dani, C. Guzati & H.M. Buneker 364 (ICN) [Fig. 7J,N].

The observed chromosome number corroborates the data reported by Moraes & al. (2015) for plants from Rio Grande do Sul.

\**Cypella luteogibbosa* Deble  
2n = 14; CHN. Brazil, Rio Grande do Sul, Quaraí, 30°11'43.8"S, 56°29'19.6"W, 14 Nov 2013, E.M. Stiehl-Alves, F.S. Silveira, J. Focchezatto & L.O. Tacuatiá 96 (ICN) [Fig. 7K,O].

\**Cypella magnicristata* Deble  
2n = 14; CHN. Brazil, Rio Grande do Sul, Quaraí, 30°11'43.8"S, 56°29'19.6"W, 14 Nov 2013, E.M. Stiehl-Alves, F.S. Silveira, J. Focchezatto & L.O. Tacuatiá 98 (ICN) [Fig. 7L,P].

\**Cypella pusilla* (Link & Otto) Benth. & Hook.f. ex B.D.Jacks.  
2n = 14; CHN. Brazil, Rio Grande do Sul, São Gabriel, 28°08' 24.0"S, 49°38'36.0"W, 19 Mar 2015, T. Pastori & O. Chauveau O 191 (ICN); Brazil, Rio Grande do Sul, São Gabriel, 30°34'38.0"S, 54°01'28.0"W, 27 Oct 2014, T. Pastori, M. Chiara-Moço, P.J.S. Silva-Filho, R. Macedo & L. Oliveira 148 (ICN); Brazil, Rio Grande do Sul, Caçapava do Sul, 30°32'46.0"S 53°29'50.0"W, 12 Nov 2014, T. Pastori, M. Longhi, C. Forgiarini & L. Nogueira 168 (ICN); Brazil, Rio Grande do Sul, Caçapava do Sul, 30°50' 26.0"S, 53°30'00.0"W, 19 Apr 2014, L. Eggers & O. Chauveau 914 (ICN) [Fig. 7Q,U].

## IRIDACEAE

### Subfamily Iridoideae

#### Tribe Tigridieae (Clade B)

\**Gelasine* aff. *coerulea* (Vell.) Ravenna  
2n = 14; CHN. Brazil, Santa Catarina, Água Doce, 26°42'38.0"S, 51°36'27.0"W, 5 Oct 2017, C. Valus & A. Silvério s.n. (MBM) [Fig. 7R,V].

\*\**Gelasine elongata* (Graham) Ravenna  
2n = 12; CHN. Brazil, Rio Grande do Sul, Porto Alegre, 30°03' 13.0"S, 51°07'03.0"W, 9 Oct 2007, L. Eggers & T.T. Souza-Chies 254 (ICN) [Fig. 7S,W].

The results of this study confirm the chromosome number previously documented by Goldblatt (1982), Kenton & Rudall (1987) and Bennett & Leitch (2012).

\*\**Gelasine uruguaiensis* Ravenna  
2n = 14; CHN. Brazil, Rio Grande do Sul, Aceguá, 7 Nov 2011, L.P. Deble & A.S. de Oliveira-Deble 13578 (ICN) [Fig. 7T,X].

The findings of this work corroborate the chromosome count previously reported by Ravenna (1984).

Iridaceae Juss., a monocot family, is taxonomically classified into seven subfamilies (Goldblatt & al., 2008). Among them, Iridoideae, the subject of the present work, stands out as the exclusive subfamily encompassing species within the Neotropical region, which is the second major center of biodiversity (Goldblatt & al., 2008; Goldblatt & Manning, 2008). The morphology and size of chromosomes demonstrate notable diversity among this family, with frequent observations of bimodal karyotypes and asymmetry, especially in Iridoideae (Goldblatt & Takei, 1997; Souza-Chies & al., 2012). Polyploidy and dysploidy are also important for the evolution of the family, playing a crucial role in the diversity of karyotypes (Goldblatt & Takei, 1997).

There is a scarcity of cytogenetic data for the South American genera, particularly for *Cypella* Herb. and *Gelasine* Herb. Within *Cypella*, the chromosome number and genome size have been elucidated for only 5 out of approximately 30 species (Goldblatt & Takei, 1997; Ravenna, 1981; Goldblatt, 1982; Kenton & Heywood, 1984; Kenton, 1990; Moraes & al., 2015). Likewise, this information is known for merely three out of seven species of *Gelasine* (Goldblatt, 1982; Ravenna, 1984; Kenton & Rudall, 1987; Bennett & Leitch, 2012; Moraes & al., 2015).

Mitotic analyses were performed with at least five plant bulbs from each population. Roots tips were pretreated with a solution of 8-hydroxyquinoline for 4 h at 15°C and subsequently fixed in fresh ethanol : glacial acetic acid solution (3 : 1). Slides were prepared using a modified protocol derived from the conventional squash technique (Schwarzacher & Leitch, 1994). Root tips were submitted to an enzymatic digestion (macerozyme 1%, cellulase 2% and pectinase 20%) for 9 min and squashed with a drop of 45% acetic acid. Slides were stained with Giemsa. Metaphases with the best chromosome viewing were selected for counting. All analyses were performed using a Zeiss Axioplan microscope equipped with a digital image capture system (AxioVision Zeiss software).

Fresh leaves from five specimens per accession were used for genome size estimation following the methodology proposed by Doležel & al. (2007). *Solanum lycopersicum* L. 'Stupické polní rané' and *Vicia faba* L. 'Inovec' (Doležel & al., 1992) were selected for internal standards (2C = 1.96 pg and 26.09 pg, respectively). Standard and sample plants were chopped together in 1 ml of cold nuclear-isolation Ebihara buffer (Ebihara & al., 2005). The suspension was filtered through a 30 µm mesh nylon filter, and nuclei were stained with 50 µl propidium iodide. Nuclear suspensions of 5000 nuclei of each sample were analyzed in a FACS Aria BD III flow cytometer.

Cytological data were obtained from 13 populations of eight species of Tigridieae. Results are presented in Table 2. This is the first chromosomal number record for seven species of *Cypella*: *C. altouruguaya*, *C. amplimaculata*, *C. discolor*, *C. hauthalii* subsp. *minuticristata*, *C. luteogibbosa*, *C. magnicristata*, *C. pusilla*, and *Gelasine* aff. *coerulea*. Chromosome counts and genome sizes from an unexplored part of the distribution area of the Tigridieae were also obtained for four species: *Cypella fucata*, *C. herbertii* subsp. *herbertii*, *Gelasine elongata* and *G. uruguaiensis*. Moreover, this is the first report of genome size estimates for *Cypella pusilla*, *Gelasine* aff. *coerulea* and *G. uruguaiensis*.



All species and accessions of *Cypella* studied presented the same base chromosome number  $x = 7$  in agreement with all remaining taxa previously studied. Since now, all *Cypella* species investigated were diploid with  $2n = 14$ . Our analysis unveiled a polyploid species: *C. discolor*, a tetraploid with  $2n = 4x = 28$ . This discovery is especially intriguing as it suggests the only occurrence of polyploidy in *Cypella*.

Each examined species of *Gelasine* studied presented only diploid individuals, and no other cytotypes were observed. Previous studies on *Gelasine* established that both *G. coerulea* and *G. uruguaiensis* presented the same basic chromosome number  $x = 7$ , consistent with our data. Interestingly, *G. aff. coerulea* is a diploid with  $2n = 14$ , but has a higher DNA content than previously reported by Moraes & al. (2015) for *G. coerulea* ( $2C = 11.32$  pg and  $2C = 5.05$  pg, respectively). A more detailed karyotype analysis and new collections are necessary for a better understanding of this difference. Among the species within the genus, *G. elongata* stands out by presenting a reduced chromosome count of  $2n = 12$ , in accordance with earlier records.

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**Table 2.** Chromosome numbers and genome sizes in *Cypella* and *Gelasine* species.

Taxa	$2n$	Voucher	Genome size (2C, pg) mean ± standard deviation
<b>Tigridieae (Clade A): <i>Cypella</i> Herb.</b>			
<i>C. altouruguayana</i>	14	<i>L. Eggers &amp; O. Chauveau 716</i>	–
<i>C. amplimaculata</i>	14	<i>J.G. Dani, C. Guzati &amp; H.M. Buneker 330</i> <i>J.G. Dani, C. Guzati &amp; H.M. Buneker 375</i> <i>J.G. Dani &amp; C. Guzati 280</i>	–
<i>C. discolor</i>	$4x = 28$	<i>E.M. Stiehl-Alves, F.S. Silveira, J. Focchezatto &amp; L.O. Tacuatiá 94</i>	–
<i>C. fucata</i>	14	<i>J.G. Dani, C. Guzati &amp; H.M. Buneker 374</i> <i>J.G. Dani, C. Guzati, L.N. Da Silva &amp; G. Pecoits 318</i> <i>J.G. Dani &amp; C. Guzati 386</i>	3.89 ± 0.08 3.76 ± 0.03 3.78 ± 0.09
<i>C. hauthalii</i> subsp. <i>minuticristata</i>	14	<i>J.G. Dani, C. Guzati &amp; H.M. Buneker 376</i>	–
<i>C. herbertii</i> subsp. <i>herbertii</i>	14	<i>J.G. Dani, C. Guzati &amp; H.M. Buneker 364</i>	–
<i>C. luteogibbosa</i>	14	<i>E.M. Stiehl-Alves, F.S. Silveira, J. Focchezatto &amp; L.O. Tacuatiá 96</i>	–
<i>C. magnicristata</i>	14	<i>E.M. Stiehl-Alves, F.S. Silveira, J. Focchezatto &amp; L.O. Tacuatiá 98</i>	–
<i>C. pusilla</i>	14	<i>T. Pastori, M. Longhi, C. Forgiarini &amp; L. Nogueira 168</i> <i>L. Eggers &amp; O. Chauveau 914</i> <i>T. Pastori &amp; O. Chauveau 191</i> <i>T. Pastori, M. Chiara-Moço, P.J.S. Silva-Filho, R. Macedo &amp; L. Oliveira 148</i>	3.61 ± 0.07 3.67 ± 0.02 3.59 ± 0.02 3.43 ± 0.03
<b>Tigridieae (Clade B): <i>Gelasine</i> Herb.</b>			
<i>G. aff. coerulea</i>	14	<i>C. Valus &amp; A. Silvério s.n.</i>	11.32 ± 0.01
<i>G. elongata</i>	12	<i>L. Eggers &amp; T.T. Souza-Chies 254</i>	3.46 ± 0.01
<i>G. uruguaiensis</i>	14	<i>L.P. Deble &amp; A.S. de Oliveira-Deble 13578</i>	16.90



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## IAPT chromosome data 40/6

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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

*Anthurium andraeanum* Linden ex André

\*\* $2n = 36$ , CHN. Brazil, Paraíba, Areia, 06°58'19"S, 35°42'58"W, 526 m, 7 Sep 2022, *L.P. Felix 19523* (EAN) [Fig. 8A].

*Anthurium andraeanum* is the ornamental species of *Anthurium* most widely cultivated, either as an indoor plant or for the production of inflorescences for cutting. The count of  $2n = 30$  predominates in the vast majority of previous numerical records for this species (Sheffer & Kamemoto, 1976; Sheffer & Croat, 1983;

Petersen, 1989; Marutani & al., 1993). Our count for a single individual from the state of Paraíba diverged from all previous counts reported for *A. andraeanum*.

*Anthurium bromelicola* Mayo & L.P. Felix subsp. *bromelicola*  
 $2n = 30$ , CHN. Brazil, Paraíba, Areia, 06°58'19"S, 35°42'58"W, 526 m, 7 Sep 2022, *L.P. Felix 19524* (EAN) [Fig. 8B].

*Anthurium bromelicola*, a rare epiphytic species exclusive to bromeliads, endemic to the Northeast Region of Brazil, reported in the states of Pernambuco, Alagoas and Bahia. It has two recognized subspecies: *A. bromelicola* subsp. *bromelicola* from the states of Pernambuco and Alagoas, and *A. bromelicola* subsp. *bahiense* Mayo & J.G. Jardim, with exclusive occurrence in the Atlantic Forest of the southern region of the state of Bahia. The only previous chromosomal record for *A. bromelicola* subsp. *bromelicola* with  $2n = 30$  (Vilar & al., 2017) was confirmed in this study, and there are no chromosomal records known for *A. bromelicola* subsp. *bahiense*.

*Anthurium harleyi* T.A. Pontes & Mayo

\* $2n = 30 + 1B$ , CHN. Brazil, Bahia, Morro do Chapéu, 11°35'29"S, 41°12'28"W, 14 Dec 2019, *L.P. Felix 18315* (EAN) [Fig. 8C].

*Anthurium harleyi* is a rupicolous plant found in the sandstone outcrops of the Chapada Diamantina in the state of Bahia, a region exceptionally rich in endemism. The species was recently described based on materials collected in the municipality of Morro do Chapéu, the same location where the material analyzed here was collected. It is closely related to *A. erskinei* Mayo, from which it differs in having longer inflorescences, shorter and wider leaves, a shorter spathe, and larger flowers (Pontes & al., 2019). Like other *Anthurium* species, the karyotype consists of submetacentric and metacentric chromosomes, with the presence of a smaller B chromosome.

*Anthurium ianthinopodum* (Schott ex Engl.) Nadruz & Mayo

\* $2n = 30 + 2B$ , CHN. Brazil, Espírito Santo, Itaguaçu, 19°04'32"S, 40°50'34"W, 150 m, 7 Feb 2015, *E.M. Almeida 1329* (EAN) [Fig. 8D].

*Anthurium ianthinopodum* is an epiphytic species endemic to the Atlantic Forest of the states of Bahia and Espírito Santo. It is morphologically related to *A. longifolium* G. Don, from which it differs by having upright leaves with a leaf blade of acute base and whitish fruits vinaceous at the apex and pink to reddish at the base, while *A. ianthinopodum* has pendant leaves with a leaf blade of obtuse base and completely red berries. It has a chromosome number of  $2n = 30 + 2B$  and a symmetrical karyotype primarily composed of metacentric and submetacentric chromosomes.

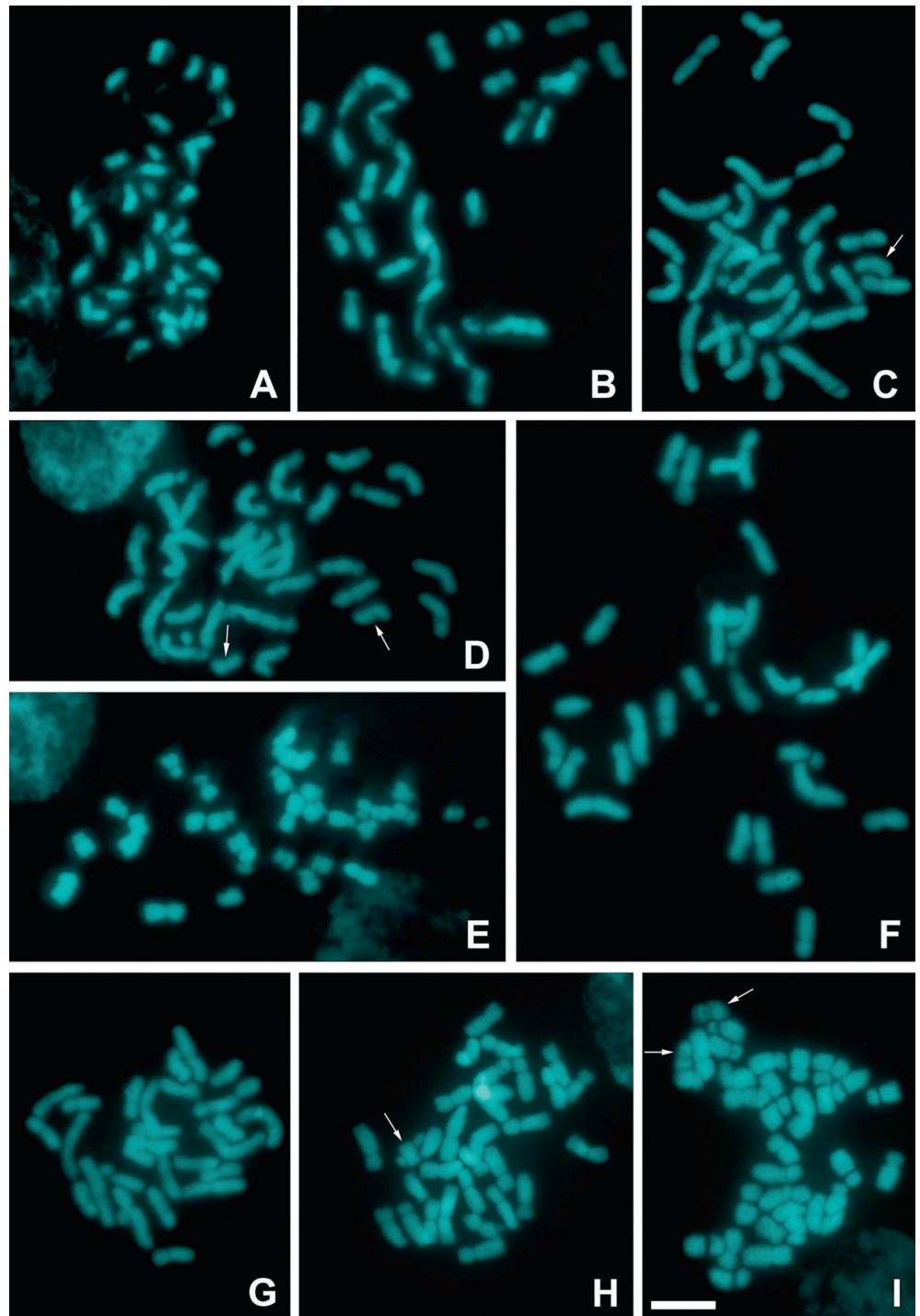
*Anthurium minarum* Sakur. & Mayo

\* $2n = 32$ , CHN. Brazil, Minas Gerais, Diamantina, 18°02'07.4"S, 43°47'20.4"W, 1245 m, 30 Sep 2021, *L.P. Felix 19200* (EAN) [Fig. 8E].

*Anthurium minarum* has a restricted distribution in the Southeast Region of Brazil, occurring in the states of Minas Gerais, Rio de Janeiro, and São Paulo, particularly in rocky outcrops in the state of Minas Gerais (Sakuragui & Mayo, 1999). Cytogenetically, the species is characterized by having  $2n = 32$ , a karyotype mainly composed of submetacentric chromosomes and the presence of rare acrocentric chromosomes, including the NOR (Nucleolus Organizer Region) chromosome pair. There are no previous chromosome counts known for this species.

*Anthurium pentaphyllum* (Aubl.) G. Don

$2n = 30$ , CHN. Brazil, São Paulo, Ubatuba, 23°26'13"S, 45°04'08"W, 15 Jan 2017, *L.P. Felix 16970* (EAN) [Fig. 8F].



**Fig. 8.** Chromosome complements belonging to the species: **A**, *Anthurium andraeanum*,  $2n = 36$ ; **B**, *Anthurium bromelicola*,  $2n = 30$ ; **C**, *Anthurium harleyi*,  $2n = 30 + 1B$ ; **D**, *Anthurium ianthinopodum*,  $2n = 30 + 2B$ ; **E**, *Anthurium minarum*,  $2n = 32$ ; **F**, *Anthurium pentaphyllum*,  $2n = 30$ ; **G**, *Anthurium petrophilum*,  $2n = 30$ ; **H**, *Anthurium petrophilum*,  $2n = 30 + 1B$ ; **I**, *Anthurium petrophilum*,  $2n = 30 + 2B$ . — Arrows indicate B chromosomes. Scale bar in I = 10  $\mu$ m.

*Anthurium pentaphyllum* is a species with a wide distribution in Brazilian territory and other countries in Central and South America (from Costa Rica to the Guianas and Peru). It belongs to *A.* sect. *Dactylophyllum* (Schott) Engl., mainly characterized by deeply lobed, palmatisect, or completely digitate leaves, free to the base (Croat & Carlsen, 2013). The chromosome count of  $2n = 60$  prevails in most of its numerical records (Sheffer & Kamemoto, 1976; Sheffer & Croat, 1983; Vilar & al., 2017), which differs from our count. However, in Vilar & al. (2017), in addition to the count of  $2n = 60$ ,

a number of  $2n = 30$  was also observed in material collected in the state of Paraíba in the Northeast Region of Brazil. Our record for a population in the Southeast Region of Brazil indicates that the diploid cytotype, as well as the tetraploid cytotype, also has a wide geographic distribution. *Anthurium pentaphyllum* and *A. sinuatum* Benth. ex Schott are morphologically and genetically similar (Andrade & al., 2009), and these numerical differences may be related to difficulties in taxonomic delimitation between these two species or to events of auto- or allopolyploidy.

*Anthurium petrophilum* K.Krause

$2n = 30$ , CHN. Brazil, Bahia, Morro do Chapeu, 11°33'09"S, 41°09'27"W, 1017 m, Dec 2019, *L.P. Felix 18374* (EAN) [Fig. 8G].

\*\* $2n = 30 + 1B$ , CHN. Brazil, Bahia, Santa Terezinha, 12°51'04"S, 39°28'51"W, 669 m, 19 Jan 2014, *E.M. Almeida 966* (EAN); Brazil, Paraíba, São Joao do Tigre, 08°06'07"S; 36°37'52"W, 4 Aug 2011, *S. Nascimento 150* (EAN); Brazil, Pernambuco, Pesqueira, 08°21'28"S, 36°41'47"W, 654 m, 14 Nov 2011, *E.M. Neto 49* (EAN) [Fig. 8H].

\*\* $2n = 30 + 2B$ , CHN. Brazil, Bahia, Palmeiras, 12°31'44"S, 41°33'32"W, 697 m, 10 Aug 2012, *E.M. Almeida 616* (EAN) [Fig. 8I].

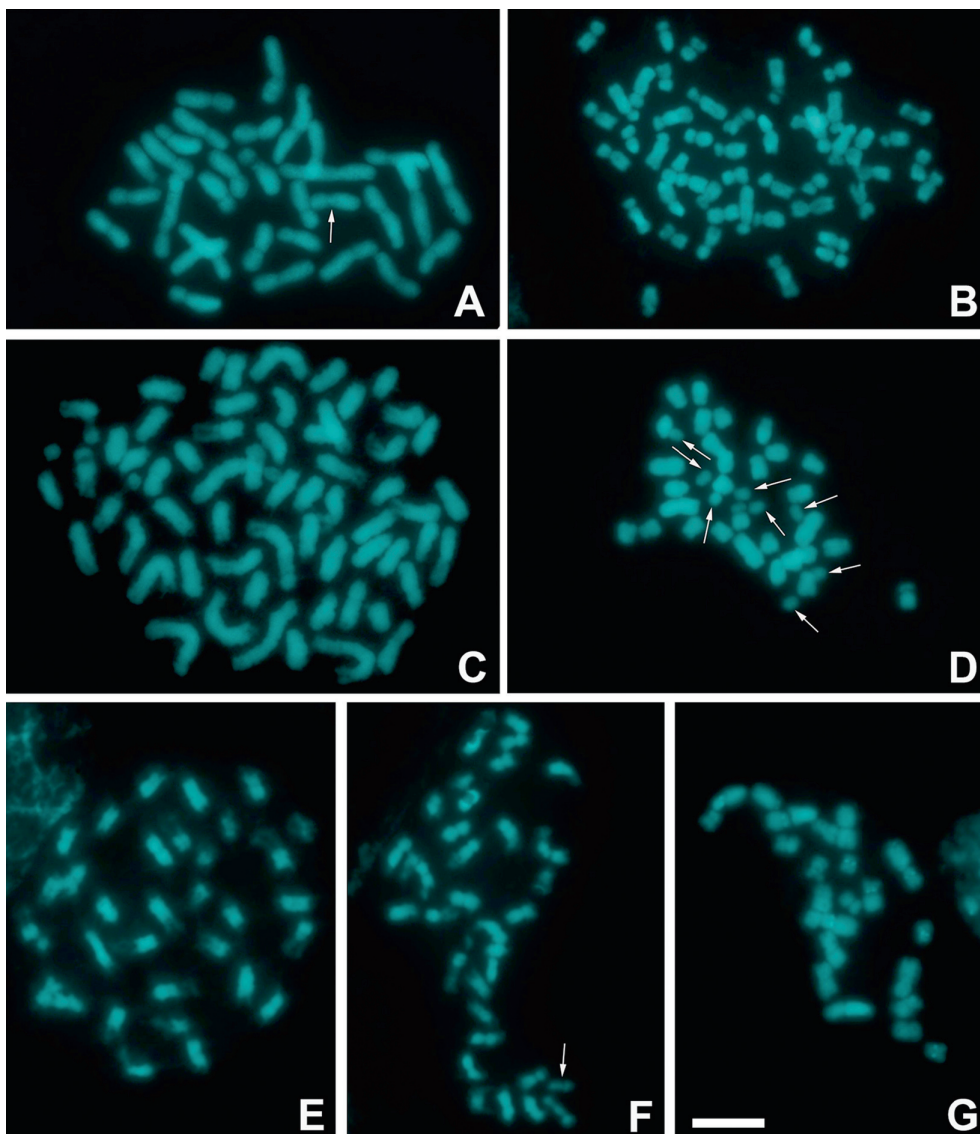
An endemic species of Brazil with recorded occurrences in the Northeast and Southeast regions, in the states of Pernambuco, Bahia, Minas Gerais (Coelho & al., 2023), and Paraíba. These are primarily rupicolous plants that typically grow on rocky outcrops in the crevices of rocks. The previous count of  $2n = 30$  conducted by Vilar & al. (2017) for two populations in Paraíba and Pernambuco was confirmed here only for the population in the state of Bahia. Our counts

of  $2n = 30 + 2B$  for an individual from Palmeiras, Bahia, and  $2n = 30 + 1B$  for several individuals from two populations in Paraíba and one population in Pernambuco suggest that this is the most common cytotype for the species.

*Anthurium raimundii* Mayo, Haigh & Nadruz

\* $2n = 30 + 1B$ , CHN. Brazil, Pernambuco, Brejo da Madre de Deus, 08°08'45"S, 36°22'16"W, 636 m, 20 Oct 2018, *L.P. Felix 17818* (EAN) [Fig. 9A].

*Anthurium raimundii* is reported in the states of Espírito Santo, Bahia, and Sergipe (Coelho & al., 2023). Its occurrence in Pernambuco expands its distribution area to the mountain forests in the northern part of the state of Alagoas. It has a chromosomal number of  $2n = 30 + 1B$ , with a symmetrical karyotype composed of metacentric and submetacentric chromosomes, in addition to an euchromatic B chromosome that is not very differentiated from the other chromosomes in the complement. The species was described just over 12 years ago based on materials collected in the southern part of the state of Bahia (Haigh & al., 2011).



**Fig. 9.** Chromosome complements belonging to the species: **A**, *Anthurium raimundii*,  $2n = 30 + 1B$ ; **B**, *Anthurium scandens*,  $2n = 48$ ; **C**, *Anthurium sellowianum*,  $2n = 60$ ; **D**, *Anthurium urvilleanum*,  $2n = 30 + 8B$ ; **E**, *Philodendron acutatum*,  $2n = 32$ ; **F**, *Philodendron acutatum*,  $2n = 32 + 1B$ ; **G**, *Syngonium podophyllum*,  $2n = 26$ . — Arrows indicate B chromosomes. Scale bar in G = 10  $\mu$ m.



*Anthurium scandens* (Aubl.) Engl.

$2n = 48$ , CHN. Brazil, Bahia, Morro do Chapéu, 11°35'29"S, 41°12'28"W, 14 Dec 2019, *L.P. Felix 18313* (EAN); Brazil, Bahia, Santa Terezinha, 12°50'51"S, 39°28'50"W, 719 m, 19 Jan 2014, *E.M. Almeida 1028* (EAN); Brazil, Pernambuco, Bonito, 08°30'23"S, 35°43'30"W, 782 m, 11 Jul 2021, *L.P. Felix 18667* (EAN); Brazil, Pernambuco, Bonito, 08°30'23"S, 35°43'30"W, 782 m, 11 Jul 2021, *L.P. Felix 18678* (EAN) [Fig. 9B].

*Anthurium scandens* is an epiphytic species that occurs in the Antilles and the American continent, from southern Mexico to Brazil (Sheffer & al., 1980), where it is reported in the Northern, Northeastern, Southeastern, and Southern regions (Coelho & al., 2023). Our count of  $2n = 48$  confirms all previous counts for the species (Sheffer & Kamemoto, 1976; Sheffer & Croat, 1983; Vilar & al., 2017). *Anthurium scandens* has a remarkably symmetrical karyotype with a predominance of submetacentric chromosomes, as well as some metacentric chromosome pairs.

*Anthurium sellowianum* Kunth

$**2n = 60$ , CHN. Brazil, São Paulo, Cubatão, 23°53'24"S, 46°28'58"W, 12 Nov 2017, *L.P. Felix 16810* (EAN) [Fig. 9C].

*Anthurium sellowianum* is a terrestrial habitat plant endemic to the Atlantic Forest in the states of Minas Gerais, Rio de Janeiro, São Paulo, Paraná and Santa Catarina (Coelho & al., 2023). Most of the chromosome numbers reported for this species by Petersen (1989) are  $2n = 48$ , which differs from the number observed in the present study. However, despite the divergent counts, the presence of two chromosome pairs with a Nucleolus Organizer Region (NOR) and the formation of quadruplets with four chromosomes of similar size and morphology are consistent with a recent tetraploid origin.

*Anthurium urvilleanum* Schott

$*2n = 30 + 8B$ , CHN. Brazil, São Paulo, Caraguatatuba, 23°34'56"S, 45°27'34"W, 736 m, 11 Nov 2017, *L.P. Felix 16998* (EAN) [Fig. 9D].

*Anthurium urvilleanum* is an epiphytic plant endemic to the Atlantic Forest with recorded occurrences in the southeastern and southern regions of Brazil, in the states of Rio de Janeiro, São Paulo, Paraná and Santa Catarina (Coelho & al., 2023). There are no previous chromosomal records known for this species, which presented  $2n = 38$  for the only individual analyzed. However, the presence of eight small chromosomes and similar size indicates that these are B chromosomes. This is, so far, the highest number of B chromosomes recorded for the genus *Anthurium*. The highest number of B chromosomes in *Anthurium* to date was reported by Cotias-de-Oliveira & al. (1999) for several individuals from the same population of *A. affine* Schott with  $2n = 30 + 1-4B$ .

*Philodendron acutatum* Schott

$2n = 32$ , CHN. Brazil, Piauí, Pedro II, 04°19'57"S, 41°26'48"W, 19 Mar 2014, *E.M. Almeida 1099* (EAN) [Fig. 9E].

$**2n = 32 + 1B$ , CHN. Brazil, São Paulo, Caraguatatuba, 23°34'56"S, 45°27'34"W, 736 m, 16 Nov 2017, *L.P. Felix 17003* (EAN) [Fig. 9F].

*Philodendron acutatum* has a wide distribution from northern South America to southeastern Brazil. It is a hemiepiphytic species, morphologically characterized by having leaves with a sagittate or cordate base and an acuminate or acute apex of variable size (Sakuragui & al., 2005). Material of this species was long identified as *P. imbe* Schott ex Kunth, a morphologically distinct taxon, most

likely extinct in the wild (Mayo & Sakuragui, 2011). Our chromosome count of  $2n = 32$  differs from older counts for the species with  $2n = 34$  (Petersen, 1989), but it confirms the more recent count by Vilar & al. (2017) for a population in the state of Pernambuco in the Northeast Region of Brazil. Our count of  $2n = 32 + 1B$  for a specimen from the state of São Paulo is unprecedented for the species.

*Syngonium podophyllum* Schott

$2n = 26$ , CHN. Brazil, Paraíba, Areia, 06°58'19"S, 35°42'78"W, 10 Mai 2023, *L.P. Felix 13663* (EAN) [Fig. 9G].

*Syngonium podophyllum* is reported in Mexico, Central America, and the northern part of South America. It is a species that is difficult to delimit due to its considerable morphological variability (Croat, 1981). It is widely cultivated as an ornamental plant and, in some cases, considered an invasive species (Brunel, 2009). Two different chromosome numbers have been reported for this species:  $2n = 24$  (Ghosh & al., 2001) and  $2n = 26$  (Vilar & al., 2017), with only the latter confirmed in our study. Morphologically variable and numerically unstable species can exhibit variations in ploidy levels, as seen in *Lachenalia* Jacq. (Kleynhans & Spies, 1999), or numerical variations due to dysploidy, as in *Lychnophora* Mart. (Mansanares & al., 2002). Populations of the same species with different chromosome numbers are reproductively isolated and constitute distinct biological species that can eventually differentiate morphologically (Rieseberg & Willis, 2007).

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## IAPT chromosome data 40/7

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Here we report genome size estimations from several endemic or subendemic vascular plant species of Chile that were collected during January–February of 2023, with vouchers stored at CONC, UPOS, MA and MACB herbaria. These reports constitute a significant contribution to the scarce knowledge of genome sizes for the South American flora, which is still poorly known even for endangered and/or restricted endemic species, and evolutionarily isolated lineages. We provide the first genome size estimation for 22 species, 14 genera and 4 families, which are endemic to the “Chilean Winter Rainfall-Valdivian Forests” biodiversity hotspot (Arroyo & al., 2004). This information is indicated in the species name as follows:

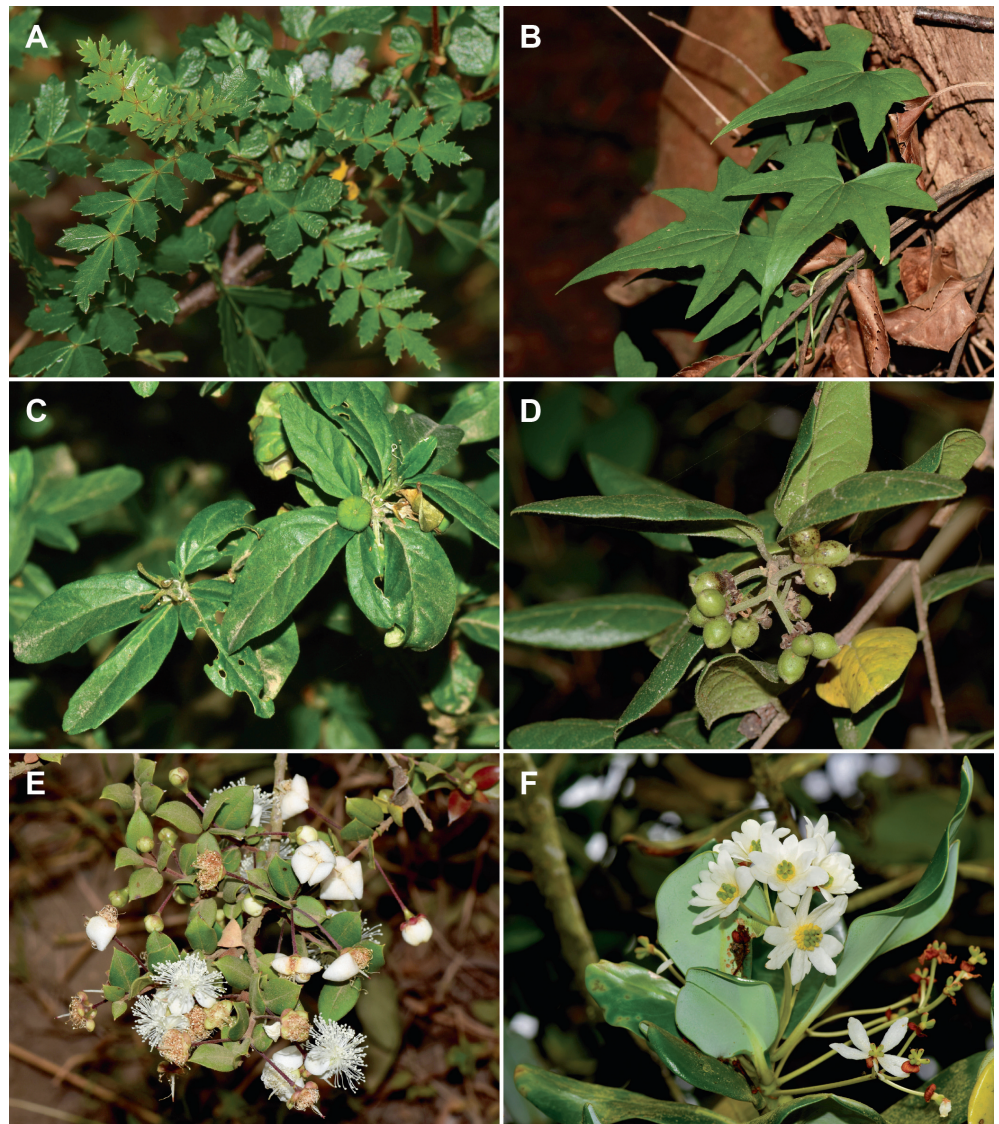
- \* First estimation for the species.
- \*\* First estimation for the genus.
- \*\*\* First estimation for the family.

## FLOW CYTOMETRY

Fresh leaves were analyzed together with different internal standard plants: *Oryza sativa* ‘IR 36’ (2C = 1.00 pg; Bennett & Smith, 1991), *Solanum lycopersicum* ‘Stupické’ (2C = 1.98 pg; Doležel & al., 1992), *Petroselinum crispum* ‘Champion Moss Curled’ (2C = 4.50 pg; Obermayer & al., 2002), *Pisum sativum* ‘Ctirad’ (2C = 9.09 pg; Doležel & al., 1998). We used different isolation buffers for processing the samples: General Purpose Buffer (GPB, Loureiro & al., 2007) supplemented with 3% PVP-40 (Pellicer & al., 2021), Lysis buffer LB01 (Doležel & al., 1989), Galbraith’s buffer (Galbraith & al., 1983), Ebihara’s buffer (Ebihara & al., 2005), and CyStain PI OxProtect buffer (Sysmex). These have been indicated for each specimen. For most of the buffers, we added 1 ml of buffer to the target sample and the internal standard, then chopped together the leaves with a razor blade, added another 1 ml of the buffer, and filtered it through a 30 µm pore size CellTrics filter (Sysmex). Finally, we added 100 µl of propidium iodide (PI, 1 mg/ml; Sigma) and incubated for 10 to 30 min. For the CyStain PI OxProtect (Sysmex) buffer, we followed the same protocol, but with 500 µl of buffer before chopping, then 1 ml after chopping, filtering, and finally adding 500 µl into the filter. After this, the samples were analyzed using a CyFlow Space cytometer (Sysmex) fitted with a Cobolt Samba laser (532 nm). The flow histograms were analyzed using FloMax v.2.9 software (Sysmex). We ran the samples through the cytometer at least three times and stopped after the target sample and the standard had reached at least 800 nuclei per fluorescence peak.

All the information will be uploaded to the Plant DNA C-values Database (Pellicer & Leitch, 2019), and the summary table can be found on GitHub. Pictures of some of the species have been included in Fig. 10.





**Fig. 10.** Representative photos of some of the studied species. **A**, *Weinmannia trichosperma*, Chile, Biobío; **B**, *Dioscorea brachybotrya*, Chile, Biobío; **C**, *Avellanita bustillosii*, Chile, Los Ríos; **D**, *Peumus boldus*, Chile, Biobío; **E**, *Luma apiculata*, Chile, Biobío; **F**, *Drimys winteri*, Chile, Biobío. — Photos by J.I. Márquez-Corro.

#### AEXTOXICACEAE

\*\*\**Aextoxicon punctatum* Ruiz & Pav.

2C =  $2.23 \pm 0.022$  pg, CV = 3.60 (GPB). [Cultivated] Chile, Biobío, Concepción, University of Concepción, 25 m,  $36^{\circ}49'41.0''$  S,  $73^{\circ}02'12.2''$  W, 5 Feb 2023, *Márquez-Corro & Muñoz-Schüler 87JMC23* (UPOS). Propagules originally provided by the University of Concepción nursery, collected from Chile, Biobío, Concepción,  $36^{\circ}50'24''$  S,  $73^{\circ}01'29''$  W.

This is the first estimation for the monotypic family Aextoxiceae. Its chromosome number was reported to be  $2n = 32$  (Goldblatt, 1976). The only other family in the order Berberidopsidales, the also species-poor Berberidopsidaceae (2 genera, 3 species) has been reported to have  $2n = 42$  chromosomes and  $2C = 0.52$  pg for *Berberidopsis corallina* Hook.f. (Hanson & al., 2001), estimating a 6x ploidy. Comparing our results, it seems that *Aextoxicon* Ruiz & Pav. could have undergone several anagenetic polyploidization events with later diploidization, maybe combined with a strong accumulation of repetitive elements. Nevertheless, more karyological studies are required in these families to properly assess ploidy levels.

#### BLECHNACEAE

\*\**Austroblechnum lechleri* (T.Moore) Gasper & V.A.O.

Dittrich

2C =  $23.65 \pm 0.121$  pg, CV = 3.22 (Ebihara). Chile, Biobío, Polcura, Polcura mountain range, path to Frutillar, 943 m,  $37^{\circ}15'53.1''$  S  $71^{\circ}44'03.9''$  W, 20 Jan 2023, *Muñoz-Schüler & al. 56.PMS.ENE* (CONC, MACB).

First genome size estimation for this Chilean subendemic genus. The 2C values obtained here are similar to the average of the family reported so far (Pellicer & Leitch, 2020).

\**Blechnum* sp.

2C =  $17.75 \pm 0.069$  pg, CV = 4.03 (Ebihara). Chile, Araucanía, Malleco, Nahuelbuta National Park, “Estero de los Gringos” trail, 1258 m,  $37^{\circ}48'45.2''$  S,  $73^{\circ}00'45.8''$  W, 22 Jan 2023, *Martín-Bravo & al. 46bisSMB23* (CONC, MACB).

This is the first genome size reported for a South American *Blechnum* L., and also similar to the average estimation of the genus (Pellicer & Leitch, 2020).



\*\**Parablechnum chilense* (Kaulf.) Gasper & Salino

2C = 27.03 ± 0.188 pg, CV = 4.48 (Ebihara). Chile, Ñuble, Chillán, Termas de Chillán, Aguas Calientes valley, 2081 m, 36°54'23.4"S, 71°22'27.9"W, 18 Jan 2023, *Martín-Bravo & al. 15SMB23* (CONC, MACB).

2C = 26.64 ± 0.084 pg, CV = 4.03 (Ebihara). Chile, Los Lagos, Llanquihue, camino a la laguna Sofia, 85 m, 41°35'34.3"S, 72°41'18.3"W, 27 Jan 2023, *Jiménez-Mejías & al. 31PJM-CL23* (CONC, MACB).

These two estimations are the first for *Parablechnum* C.Presl. Although the genus has been reported to have a labile karyotype (Gasper & al., 2016), this species has been reported to have 2n = 66 chromosomes (Jara-Seguel & al., 2006).

#### BROMELIACEAE

\**Puya alpestris* (Poepp.) Gay

2C = 1.00 ± 0.001 pg, CV = 3.99 (GPB). [Cultivated] Chile, Biobío, Concepción, University of Concepción, 18 m, 36°49'39.8"S, 73°02'15.8"W, 5 Feb 2023, *Márquez-Corro & Muñoz-Schüler 82JMC23* (UPOS). Specimen originally transplanted from the wild in the 1940s, collected from Chile, Coquimbo, Limari, 30°40'15"S, 71°38'34"W.

This is the first genome size estimation for the species. It displays similar values as the four previous reports for the genus (Pellicer & Leitch, 2020).

#### CELASTRACEAE

\*\**Maytenus boaria* Molina

2C = 2.84 ± 0.008 pg, CV = 3.22 (GPB). Chile, Biobío, Concepción, University of Concepción, 44 m, 36°49'44.4"S, 73°02'07.6"W, 05 Feb 2023, *Márquez-Corro & Muñoz-Schüler 88JMC23* (UPOS).

This is the first genome size estimation for the genus, and the first to a South American Celastraceae lineage. Although the value is somewhat similar to the family average, this family has been reported (Pellicer & Leitch, 2020) to vary from 2C = 0.37 to 8.80 pg, showing remarkable changes along the lineages that may be indicative of high ploidy level variation.

#### CUNONIACEAE

\*\*\**Weinmannia trichosperma* Cav.

2C = 0.98 ± 0.031 pg, CV = 7.75 (Galbraith). [Cultivated] Chile, Biobío, Concepción, University of Concepción, 26 m, 36°49'41.0"S, 73°02'13.3"W, 5 Feb 2023, *Márquez-Corro & Muñoz-Schüler 93JMC23* (UPOS). Propagules originally provided by the University of Concepción nursery, collected from Chile, Biobío, Concepción, 36°50'24"S, 73°01'29"W. [Fig. 10A]

This is the first genome size estimation for the family Cunoniaceae. Although the coefficient of variation (CV) is quite high, similar values have been obtained in an unpublished work (J. Pellicer, personal observation). This value is close to the single known report for the close family Elaeocarpaceae, in which *Crinodendron pataguna* Molina was reported to have 2n = 16 and 2C = 0.60 pg (Hanson & al., 2005).

#### CUPRESSACEAE

*Fitzroya cupressoides* (Molina) I.M.Johnst.

2C = 35.28 ± 0.141 pg, CV = 4.11 (GPB). [Cultivated] Chile, Biobío, Concepción, University of Concepción, 18 m, 36°49'40.5"S, 73°02'15.5"W, 5 Feb 2023, *Márquez-Corro & Muñoz-Schüler*

*83JMC23* (UPOS). Specimen from vegetative cutting originally provided by the Austral University of Chile nursery, collected from Chile, Los Ríos, Ranco, 40°11'41"S, 73°26'07"W.

Second genome size estimation for the Patagonian cypress, the largest tree in South America. It is an endangered (EN; Hechenleitner & al., 2005), tetraploid (2n = 44; Zonneveld, 2012) species with a highly restricted distribution in Chile and Argentina. The first estimation was reported by Zonneveld (2012), indicating a similar average value of 2C = 35 pg.

#### DIOSCOREACEAE

\**Dioscorea brachybotrya* Poepp.

2C = 1.89 ± 0.009 pg, CV = 3.77 (LB01). Chile, Biobío, Polcura, Polcura mountain range, path to Frutillar, 943 m, 37°15'53.1"S, 71°44'03.9"W, 20 Jan 2023, *Muñoz-Schüler & al. 54.PMS.ENE* (CONC, UPOS). [Fig. 10B]

This is the first *Dioscorea* L. from South America ever estimated. Our report indicates *D. brachybotrya* as a polyploid species based on similar estimates in a recent study of the genus (Viruel & al., 2019). However, more karyological studies should be carried out to elucidate the complex ploidy system in this lineage of yams.

#### EUPHORBIACEAE

\*\**Avellanita bustillosii* Phil.

2C = 0.52 ± 0.005 pg, CV = 4.21 (GPB). [Cultivated] Chile, Los Ríos, Lago Ranco, Pitruico, Diego N. Penneckamp's private botanical garden, 192 m, 25 Jan 2023, *Sanz-Arnal & al. 27MSA-CL23* (CONC, MA). Seeds originally provided by the Jardín Botánico Nacional (JBN), collected from Chile, Metropolitana, Paine, proximities of Laguna de Aculeo, 33°50'S, 70°56'W. [Fig. 10C]

This estimation is the first for this monotypic genus endemic to Chile. This critically endangered species (Hechenleitner & al., 2005) also has the lowest genome size registered for the family so far (Pellicer & Leitch, 2020). This sample was analyzed using tomato and rice as standards. Here, only the one using rice is indicated since it displayed better results, but the results using tomato were similar: 2C = 0.52 ± 0.005 pg, CV = 4.31.

#### FABACEAE

*Astragalus cruckshanksii* (Hook. & Arn.) Griseb.

2C = 2.26 ± 0.019 pg, CV = 2.67 (GPB). Chile, Coquimbo, Cordillera Doña Ana, Minera El Indio, 3350 m, 29°48'54.8"S, 70°01'19.6"W, 13 Jan 2023, *Muñoz-Schüler & al. 10.PMS.ENE* (CONC).

There is a previous report on the genome size of *Astragalus cruckshanksii* from Neuquén (Argentina) by Dopchiz & al. (1995). In their study, they found 2n = 28 and 2C = 2.88 pg, although they measured significantly fewer nuclei (3 replicates of 60 nuclei). These results may indicate a latitudinal difference in the species that needs further investigation.

*Sophora cassioides* (Phil.) Sparre

2C = 1.80 ± 0.038 pg, CV = 3.83 (GPB). [Cultivated] Chile, Biobío, Concepción, University of Concepción, 18 m, 36°49'39.2"S, 73°02'14.2"W, 5 Feb 2023, *Márquez-Corro & Muñoz-Schüler 86JMC23* (UPOS). Propagules originally collected from Chile, Biobío, Concepción, 36°49'40"S, 73°02'09"W.

The genome size of this diploid species has been previously estimated to be 2C = 1.61 pg (Espejo & al., 2016). Our results are similar to those reported.

**GOMORTEGACEAE**

\*\*\**Gomortega keule* (Molina) Baill.

2C =  $2.93 \pm 0.009$  pg, CV = 3.16 (GPB). Chile, Biobío, Concepción, patches of native forest close to the petrol station on the Itata highway, before Juan Chico, 323 m,  $36^{\circ}44'51.0''\text{S}$ ,  $72^{\circ}54'40.1''\text{W}$ , 3 Feb 2023, *Márquez-Corro & al. 79bisJMC23* (UPOS).

This species has only been studied karyologically ( $2n = 42$ ; Baeza & al., 2001; Oginuma & Tobe, 2006), and this is the first genome size estimation for the species, which is the only representative of the family. The queule is a tree species endemic to a highly restricted area in the coastal range around Concepción in regions Maule, Ñuble and Biobío in central southern Chile. It is considered a living fossil with great evolutionary distinctiveness, as the lineage is included within the early divergent Laurales and has been dated to the Cretaceous (Renner, 2005). However, this palaeoendemic is endangered (EN; Echeverría & Campos, 2019) and threatened by habitat destruction due to extensive reforestation with exotic tree species. Recent massive wildfires that affected Central Chile during the summer of 2023 destroyed several wild queule individuals, so its conservation status is likely to have significantly deteriorated (only around 1000 mature individuals were known; Echeverría & Campos, 2019).

**JUNCACEAE**

\**Juncus microcephalus* Kunth

2C =  $1.09 \pm 0.003$  pg, CV = 3.12 (LB01). Chile, Coquimbo, Tulahuén, Río Grande valley, beyond Cuesta del Toro, 1623 m,  $30^{\circ}57'37.6''\text{S}$ ,  $70^{\circ}31'20.7''\text{W}$ , 15 Jan 2023, *Muñoz-Schüler & al. 35.PMS.ENE* (CONC, UPOS).

This report falls within the average value of the genus. It is the first genome size estimation for the species and, together with the estimation of *J. stipulatus* below, it constitutes the first for *Juncus* L. in South America.

\**Juncus stipulatus* Nees & Meyen

2C =  $0.93 \pm 0.015$  pg, CV = 4.22 (GPB). Chile, Ñuble, Chillán, Termas de Chillán, Aguas Calientes valley, 2151 m,  $36^{\circ}54'13.2''\text{S}$ ,  $71^{\circ}22'36.6''\text{W}$ , 18 Jan 2023, *Martín-Bravo & al. 09SMB23* (CONC, UPOS).

This estimation is the first for the species, displaying values similar to the genus average.

\*\**Patosia clandestina* (Phil.) Buchenau

2C =  $1.37 \pm 0.005$  pg, CV = 2.60 (LB01). Chile, Coquimbo, Doña Ana mountain range, Mine “El Indio”, 3350 m,  $29^{\circ}48'54.8''\text{S}$ ,  $70^{\circ}01'19.6''\text{W}$ , 13 Jan 2023, *Muñoz-Schüler & al. 22.PMS.ENE* (CONC, UPOS).

First record for this Andean endemic, monotypic genus. The estimation is slightly lower than the family average (Pellicer & Leitch, 2020). The relationships within the family have not yet been clarified (Drábková & Čestmír, 2007), and further molecular phylogenetic and genomic studies are needed to understand the evolution of this genus, which is a key component of high Andean wetland vegetation, in which it forms very distinctive cushions.

**LAURACEAE**

\*\**Cryptocarya alba* (Molina) Looser

2C =  $2.34 \pm 0.021$  pg, CV = 3.52 (GPB). Chile, Biobío, Concepción, University of Concepción, 18 m,  $36^{\circ}49'39.3''\text{S}$ ,  $73^{\circ}02'13.8''\text{W}$ , 5 Feb 2023, *Márquez-Corro & Muñoz-Schüler 84JMC23* (UPOS). Specimen of natural origin that already existed when the Ottmar Wilhelm Building was built in the 1930s.

This is the first genome size estimation for this genus, and for all the early diverging lineages of Lauraceae (Cryptocaryae). This family has been largely studied karyologically but there is a lack of information on the Cryptocaryae, where different ploidies were reported (e.g., Oginuma & Tobe, 2006).

**MONIMIACEAE**

\*\**Peumus boldus* Molina

2C =  $5.18 \pm 0.079$  pg, CV = 2.87 (GPB). Chile, Biobío, Concepción, University of Concepción, 42 m,  $36^{\circ}49'46.3''\text{S}$ ,  $73^{\circ}02'04.7''\text{W}$ , 5 Feb 2023, *Márquez-Corro & Muñoz-Schüler 89JMC23* (UPOS). [Fig. 10D]

First estimation for the species and Chilean subendemic genus. Within Monimiaceae, it is more than twice the size of the genus *Mollinedia* Ruiz & Pav. (Pellicer & Leitch, 2020). This result points to polyploidization in *Peumus* Molina, as its known chromosome number is  $2n = 78$ , whereas *Mollinedia* has been reported to have different ploidies  $2n = 36, 38, 180$  (Oginuma & Tobe, 2006). Therefore, one could assume that the already estimated genome sizes in *Mollinedia* belong to the lowest registered chromosome numbers, around  $2n = 38$ , which must be confirmed.

**MYRTACEAE**

\*\**Luma apiculata* (DC.) Burret

2C =  $1.02 \pm 0.017$  pg, CV = 4.27 (GPB). Chile, Biobío, Concepción, University of Concepción, 45 m,  $36^{\circ}49'46.2''\text{S}$ ,  $73^{\circ}02'04.1''\text{W}$ , 5 Feb 2023, *Márquez-Corro & Muñoz-Schüler 90JMC23* (UPOS). [Fig. 10E]

First estimation for this species and Chilean subendemic genus. The 2C values agree with the mean reported for the family (Pellicer & Leitch, 2020). The values reported in the family vary in what looks like different ploidies, even within the genus (e.g., *Psidium* L.). This lineage requires further phylogenetic and genomic studies, as the family still needs nomenclatural rearrangements (Vasconcelos & al., 2017).

**NOTHOFAGACEAE**

\*\*\**Nothofagus obliqua* (Mirb.) Oerst.

2C =  $1.19 \pm 0.001$  pg, CV = 3.52 (OXPRO). Chile, Biobío, Concepción, University of Concepción, 45 m,  $36^{\circ}49'46.2''\text{S}$ ,  $73^{\circ}02'04.1''\text{W}$ , 5 Feb 2023, *Márquez-Corro & Muñoz-Schüler 91JMC23* (UPOS).

First estimation for this family. *Nothofagus* Blume chromosome number has been reported to be very stable, with this species showing the genus' most frequent number of  $2n = 26$  (Jara-Seguel & al., 2014). Values of 2C including or close to the one reported here have been testified for the close families of Fagaceae, Juglandaceae or Betulaceae (Pellicer & Leitch, 2020).

**PODOCARPACEAE**

\**Podocarpus salignus* D. Don

2C =  $17.78 \pm 0.332$  pg, CV = 4.73 (OXPRO). Chile, Biobío, Concepción, University of Concepción, 56 m,  $36^{\circ}49'46.7''\text{S}$ ,  $73^{\circ}02'03.8''\text{W}$ , 5 Feb 2023, *Márquez-Corro & Muñoz-Schüler 92JMC23* (UPOS).

Similar 2C values have been reported for the genus, although they tend to be slightly higher (Pellicer & Leitch, 2020). This is the first estimation of this vulnerable species (Hechenleitner & al., 2005) endemic to Chile, and for South American *Podocarpus* L'Hér. ex Pers.

**RESTIONACEAE**

\*\**Apodasmia chilensis* (Gay) B.G.Briggs & L.A.S.Johnson

2C = 2.09 ± 0.014 pg, CV = 4.20 (LB01). Chile, Los Lagos, Llanquihue, cove north of Punta Metri, 7 m, 41°35'19.6"S, 72°42'05.0"W, 27 Jan 2023, Jiménez-Mejías & al. 32PJM-CL23 (CONC, UPOS).

First record for this Chilean endemic species, and for the South American representatives of the family (Pellicer & Leitch, 2020). *Gaimardia australis* Gaudich. and *Apodasmia chilensis* are the only two South American native and endemic restiads (POWO, 2023). Therefore, this data is important to understand in future studies whether different ploidy levels may be associated with an extension of the genus' natural range.

**RUTACEAE**

\*\**Pitavia punctata* (Ruiz & Pav.) Molina

2C = 1.07 ± 0.006 pg, CV = 3.92 (GPB). [Cultivated] Chile, Biobío, Concepción, University of Concepción, 18 m, 36°49'40.5" S, 73°02'12.8"W, 5 Feb 2023, Márquez-Corro & Muñoz-Schüler 85JMC23 (UPOS). Seeds originally provided by Arauco S.A. Forestry Company nursery, collected from Chile, Biobío, Concepción, 37°06'48"S, 73°09'01"W.

First estimation for this Critically Endangered (Rivera Caniulao, 2021), Chilean endemic, monotypic genus. This species has been reported to have 2n = 36 chromosomes (Stace & al., 1993).

**WINTERACEAE**

\**Drimys winteri* J.R.Forst. & G.Forst.

2C = 3.90 ± 0.011 pg, CV = 3.04 (GPB). [Cultivated] Chile, Biobío, Concepción, University of Concepción, 18 m, 36°49'38.1" S, 73°02'13.1"W, 5 Feb 2023, Márquez-Corro & Muñoz-Schüler 94JMC23 (UPOS). Propagules originally provided by the University of Concepción nursery, collected from Chile, Biobío, Concepción, 36°50'24"S, 73°01'29"W. [Fig. 10F]

This is the first estimation for the species, and the second for the genus. *Drimys winteri* presents an almost twice as large genome as *D. vickeriana* A.C.Sm. (Pellicer & Leitch, 2020). This is probably due to the different ploidy levels present in the genus, as *D. winteri* has been reported to have 2n = 86, but also 2n = 26 chromosomes have been counted in *Drimys* J.R.Forst. & G.Forst. (Ehrendorfer & al., 1968).

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- Methods for chromosome analysis according to Cordeiro & al. (2020).
- \* First chromosome count for the species.  
\*\* First chromosome count for the genus.
- ANACARDIACEAE**  
*Anacardium occidentale* L.  
 $2n = 40$ , CHN. Brazil, Pernambuco, Taquaritinga do Norte, 07° 54'11"S, 36°02'39"W, 28 Jan 2018, *L.P. Felix 17303* (EAN) [Fig. 11A].
- \**Astronium fraxinifolium* Schott  
 $2n = 30$ , CHN. Brazil, Paraíba, Maturéia, 07°15'54"S, 37°22'33"W, 2 Dec 2017, *L.P. Felix 17114* (EAN) [Figs. 11B, 13A].
- Mangifera indica* L.  
 $2n = 40$ , CHN. Brazil, Paraíba, Sertãozinho, 06°44'06"S, 35°27'30"W, 3 Jun 2018, *J.M.P. Cordeiro 1337* (EAN) [Fig. 11C].
- Myracrodruon urundeuva* Allemão  
 $2n = 30$ , CHN. Brazil, Pernambuco, Taquaritinga do Norte, 07° 54'11"S, 36°02'39"W, 28 Jan 2018, *L.P. Felix 17308* (EAN) [Fig. 11D].
- \**Schinopsis brasiliensis* Engl.  
 $2n = 28$ , CHN. Brazil, Paraíba, São José dos Cordeiros, 07°28'08"S, 36°53'47"W, 30 Nov 2017, *L.P. Felix 17100* (EAN) [Figs. 11E, 13B].
- Schinus terebinthifolia* Raddi  
 $2n = 28$ , CHN. Brazil, Paraíba, João Pessoa, 07°09'13"S, 34°52'58"W, 28 Jun 2018, *L.P. Felix 17496* (EAN) [Fig. 11F].
- BURSERACEAE**  
\**Commiphora leptophloeos* (Mart.) J.B.Gillett  
 $2n = 26$ , CHN. Brazil, Paraíba, Areia, 06°58'03"S, 35°42'58"W, 18 Jul 2017, *L.P. Felix 16687* (EAN) [Figs. 11G, 13C].
- CANNABACEAE**  
*Celtis iguanaea* (Jacq.) Sarg.  
 $2n = 20$ , CHN. Brazil, Paraíba, Areia, 06°57'49"S, 35°44'34"W, 9 Jun 2017, *P.C. Gadelha Neto 4160* (EAN) [Fig. 11H].
- LENTIBULARIACEAE**  
\**Utricularia quelchii* N.E.Br.  
 $2n = 18$ , CHN. Brazil, Roraima, Monte Roraima, 05°13'51"N, 60°43'46"W, 21 Feb 2017, *L.P. Felix 16222* (EAN) [Figs. 11I, 13D].
- MALPIGIACEAE**  
\*\**Amorimia septentrionalis* W.R.Anderson,  
 $2n = 20$ , CHN. Brazil, Paraíba, Sertãozinho, 06°44'06"S, 35°27'30"W, 16 Jun 2018, *J.M.P. Cordeiro 1339* (EAN) [Figs. 11J, 13E].
- MELIACEAE**  
*Azadirachta indica* A.Juss.  
 $2n = 28$ , CHN. Brazil, Paraíba, Serra Branca, 07°29'00"S, 36° 29'54"W, 30 Jun 2018, *J.M.P. Cordeiro 1342* (EAN) [Fig. 11K].

\**Cedrela fissilis* Vell.

$2n = 54$ , CHN. Brazil, Pernambuco, Taquaritinga do Norte, 07°54'18"S, 36°01'47"W, 13 Oct 2016, *L.P. Felix 15988* (EAN) [Figs. 11L, 13F].

*Melia azedarach* L.

$2n = 28$ , CHN. Brazil, Paraíba, Areia, 06°58'03"S, 35°42'58"W, 23 Jul 2018, *J.M.P. Cordeiro 1368* (EAN) [Fig. 12A].

**MYRTACEAE**\**Eugenia zigzag* K.Cout. & Sobral

$2n = 22$ , CHN. Brazil, Paraíba, Sertãozinho, 06°44'06"S, 35°27'30"W, 18 Dec 2021, *J.M.P. Cordeiro 1544* (EAN) [Figs. 12B, 13G].

**ORCHIDACEAE***Caularthron bicornutum* Raf.

$2n = 40$ , CHN. Brazil, Amazonas, Novo Airão, 03°03'11"S, 60°46'07"W, 26 Feb 2017, *L.P. Felix 16336* (EAN) [Fig. 12C].

**POLYGONACEAE**\**Triplaris gardneriana* Wedd.

$2n = 22$ , CHN. Brazil, Paraíba, Serra Branca, 07°29'46"S, 36°43'52"W, 30 Jun 2018, *L.P. Felix 17561* (EAN) [Figs. 12D, 13H].

**RUBIACEAE**\**Randia armata* (Sw.) DC.

$2n = 40$ , CHN. Brazil, Rio Grande do Norte, Martins, 06°04'25"S, 37°55'17"W, 2 Feb 2006, *R.T. Queiroz 613* (EAN) [Figs. 12E, 13I].

**RUTACEAE***Ruta chalepensis* L.

$2n = 40$ , CHN. Brazil, Paraíba, Sertãozinho, 06°44'06"S, 35°27'30"W, 15 Dec 2018, *J.M.P. Cordeiro 1406* (EAN) [Fig. 12F].

**SAPINDACEAE***Filicium decipiens* (Wight & Arn.) Thwaites

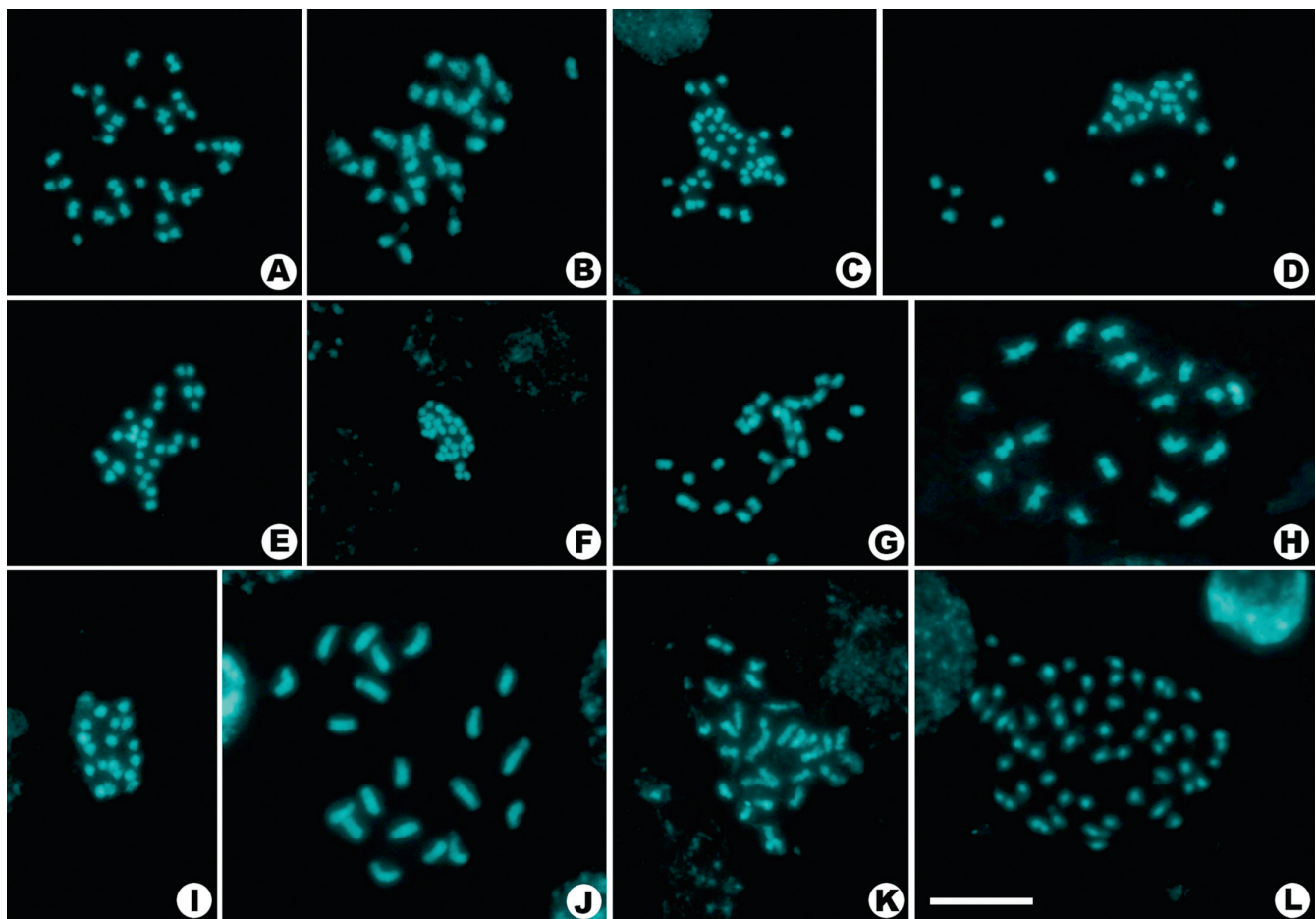
$2n = 32$ , CHN. Brazil, Paraíba, Areia, 06°58'03"S, 35°42'58"W, 15 Dec 2018, *J.M.P. Cordeiro 1407* (EAN) [Fig. 12G].

*Paullinia pinnata* L.

$2n = 24$ , CHN. Brazil, Paraíba, Areia, 06°58'03"S, 35°42'58"W, 17 Feb 1992, *L.P. Felix 4777* (EAN) [Fig. 12H].

*Sapindus saponaria* L.

$2n = 28$ , CHN. Brazil, Paraíba, Areia, 06°58'03"S, 35°42'58"W, 2 Oct 2012, *L.P. Felix 13946* (EAN) [Fig. 12I].



**Fig. 11.** Chromosome number of tropical plants: **A**, *Anacardium occidentale*,  $2n = 40$ ; **B**, *Astronium fraxinifolium*,  $2n = 30$ ; **C**, *Mangifera indica*,  $2n = 40$ ; **D**, *Myracrodruon urundeuva*,  $2n = 30$ ; **E**, *Schinopsis brasiliensis*,  $2n = 28$ ; **F**, *Schinus terebinthifolia*,  $2n = 28$ ; **G**, *Commiphora leptophloeos*,  $2n = 26$ ; **H**, *Celtis iguanaea*,  $2n = 20$ ; **I**, *Utricularia quelchii*,  $2n = 18$ ; **J**, *Amorimia septentrionalis*,  $2n = 20$ ; **K**, *Azadirachta indica*,  $2n = 28$ ; **L**, *Cedrela fissilis*,  $2n = 54$ . — Scale bar = 10  $\mu\text{m}$ .

\**Serjania salzmanniana* Schldtl.

$2n = 24$ , CHN. Brazil, Paraíba, Mamanguape, 06°44'32"S, 35°08'31"W, 1 Jun 2018, *J.M.P. Cordeiro 1333* (EAN) [Figs. 12J, 13J].

*Talisia esculenta* (A.St.-Hil.) Radlk.

$2n = 32$ , CHN. Brazil, Paraíba, Sertãozinho, 06°44'06"S, 35°27'30"W, 3 Jun 2018, *J.M.P. Cordeiro 1338* (EAN) [Fig. 12K].

#### SIMAROUBACEAE

*Homalolepis bahiensis* (Morici.) Devecchi & Pirani

$2n = 32$ , CHN. Brazil, Paraíba, Barra de Santana, 07°29'17"S, 36°03'04"W, 22 Dec 2013, *E.M. Almeida 814* (EAN) [Fig. 12L].

#### VERBENACEAE

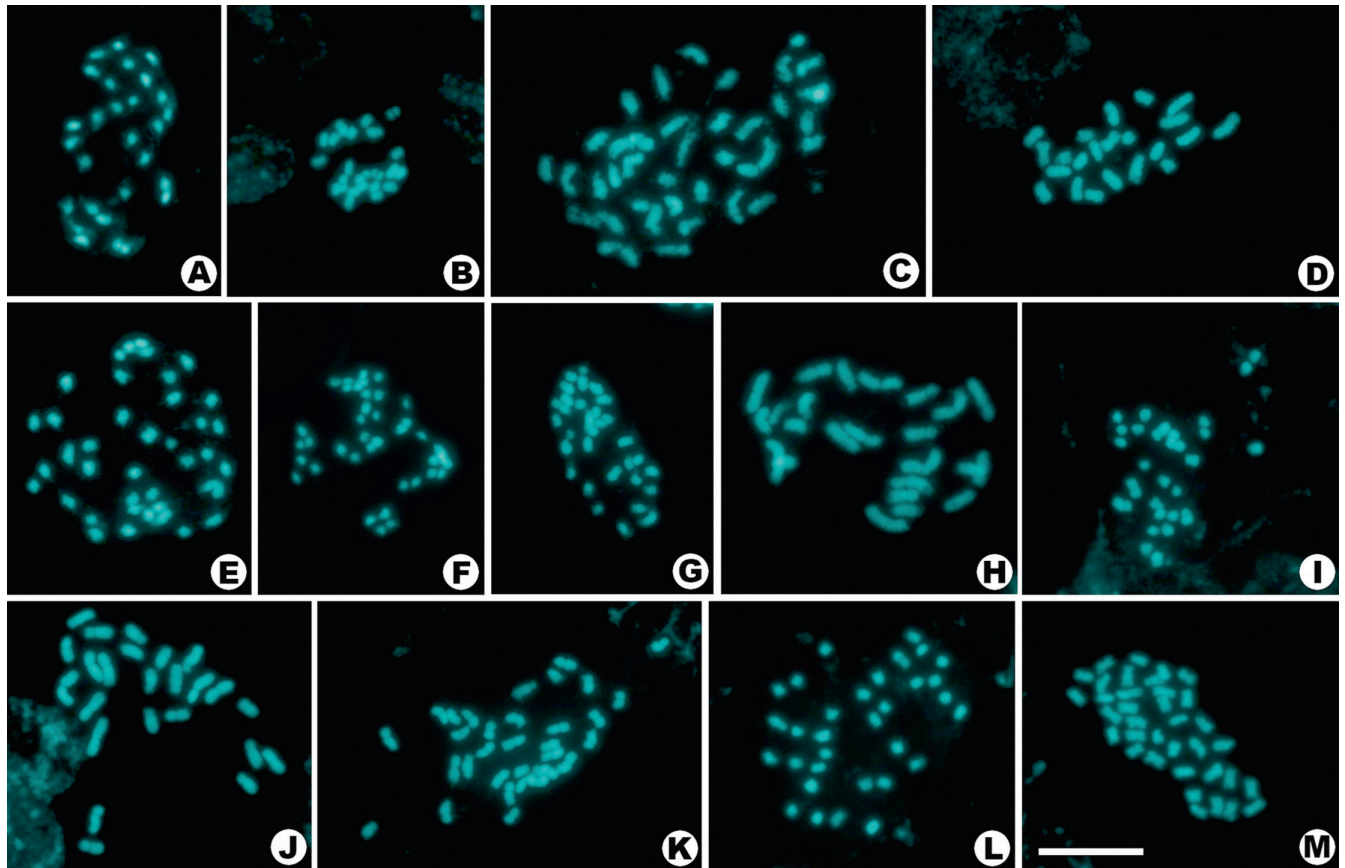
\**Priva bahiensis* DC. ex Schauer

$2n = 36$ , CHN. Brazil, Paraíba, Sertãozinho, 06°44'06"S, 35°27'30"W, 16 Jul 2021, *J.M.P. Cordeiro 1525* (EAN) [Figs. 12M, 13K].

In the present work we analyzed the chromosome number of 25 tropical plant species from Brazil (Figs. 11, 12), of which 11 are new (Fig. 13), including those for the genus *Amorimia* W.R. Anderson ( $2n = 20$ ). Tropical regions are home to the greatest diversity of plants, especially due to climatic conditions that combine high temperatures and abundant rainfall (Wiens & Donoghue, 2004).

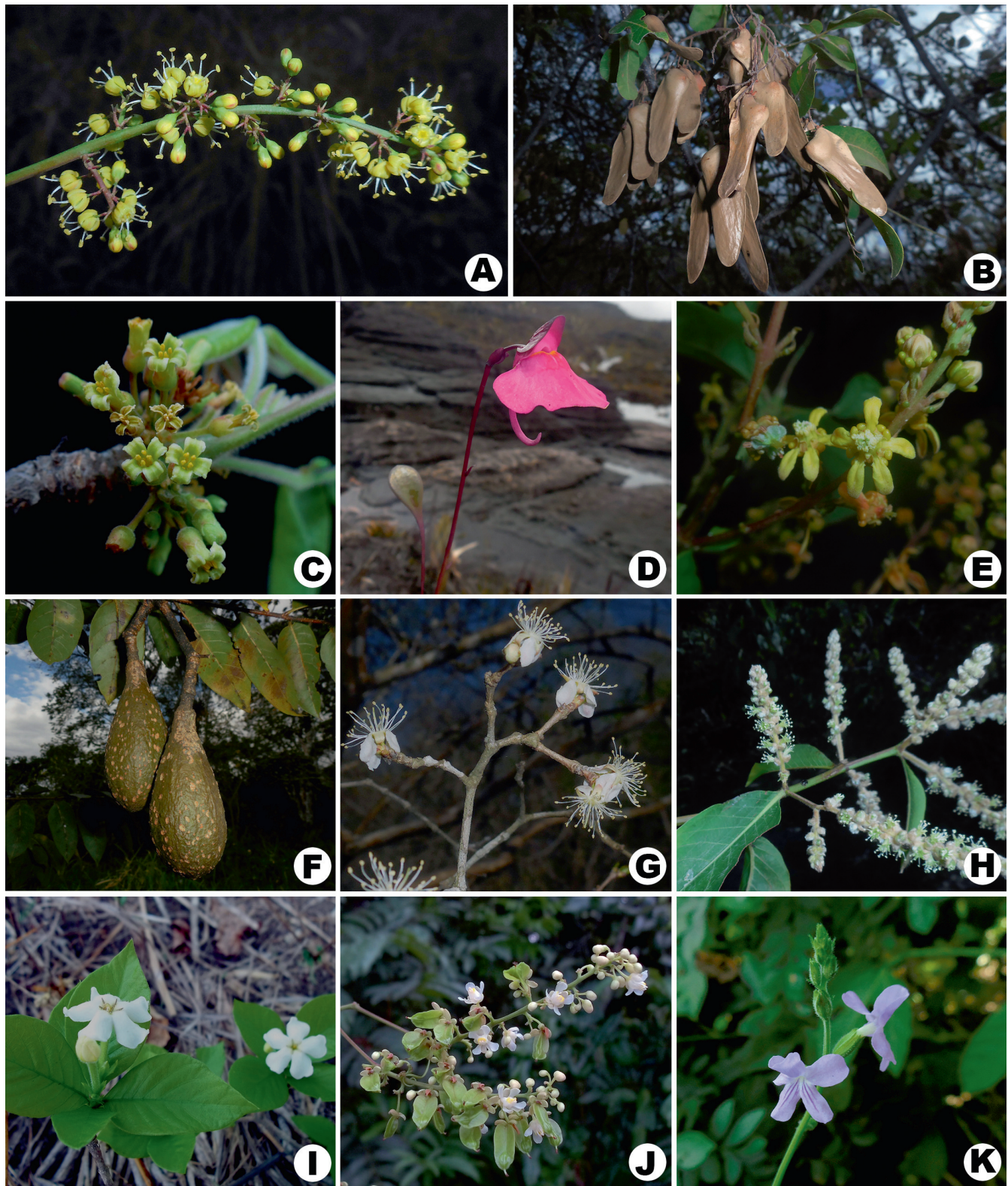
However, more incident UV-B radiation and soils generally poor in nutrients may cause deleterious DNA-damage and cell size reduction, respectively. In this sense, tropical regions exhibit environmental pressures that select species with lower DNA content when compared to temperate regions (Ohri, 2008). Genome size generally follows a proportional variation with polyploidy or chromosome size in a plant group (Soltis & al., 2003; Souza & al., 2019). Thus, it is expected that tropical plants have, in general, smaller chromosomes compared to plants from regions with lower-temperature climates. In fact, among 25 tropical species analyzed here, most of them have relatively small chromosomes (<2  $\mu\text{m}$ ) and only one species had a chromosome number greater than  $2n = 40$  (*Cedrela fissilis* Vell.,  $2n = 54$ ).

In our analyses, the largest samples correspond to the families Anacardiaceae and Sapindaceae, which are included, together with Burseraceae, Meliaceae, Rutaceae and Simaroubaceae, in the order Sapindales. In total, 17 species of that order had chromosome records presented here, with new records for *Astronium fraxinifolium* ( $2n = 30$ ), *Schinopsis brasiliensis* ( $2n = 28$ ), *Commiphora leptophloeos* ( $2n = 26$ ), *Cedrela fissilis* ( $2n = 54$ ) and *Serjania salzmanniana* ( $2n = 24$ ). In Sapindales, dysploidy appears to be the most important karyotypic change at family level diversification, while polyploidy stands out especially in Meliaceae and Rutaceae (Guimarães & Forni-Martins, 2022). However, the wide variation in chromosome number between genera within families of Sapindales



**Fig. 12.** Chromosome number of tropical plants: **A**, *Melia azedarach*,  $2n = 28$ ; **B**, *Eugenia zigzag*,  $2n = 22$ ; **C**, *Caularthron bicornutum*,  $2n = 40$ ; **D**, *Triplaris gardneriana*,  $2n = 22$ ; **E**, *Randia armata*,  $2n = 40$ ; **F**, *Ruta chalepensis*,  $2n = 40$ ; **G**, *Filicium decipiens*,  $2n = 32$ ; **H**, *Paullinia pinnata*,  $2n = 24$ ; **I**, *Sapindus saponaria*,  $2n = 28$ ; **J**, *Serjania salzmanniana*,  $2n = 24$ ; **K**, *Talisia esculenta*,  $2n = 32$ ; **L**, *Homalolepis bahiensis*,  $2n = 32$ ; **M**, *Priva bahiensis*,  $2n = 36$ . — Scale bar = 10  $\mu\text{m}$ .





**Fig. 13.** Species with new chromosome records: **A**, *Astronium fraxinifolium*; **B**, *Schinopsis brasiliensis*; **C**, *Commiphora leptophloeos*; **D**, *Utricularia quelchii*; **E**, *Amorimia septentrionalis*; **F**, *Cedrela fissilis*; **G**, *Eugenia zigzag*; **H**, *Triplaris gardneriana*; **I**, *Randia armata*; **J**, *Serjania salzmannaiana*; **K**, *Priva bahiensis*. — Photos: Joel M.P. Cordeiro.



makes it difficult to establish the basic ancestral chromosome number for that order. Raven (1975) proposed  $x = 7$ , with rapid evolution to  $x = 14$  and later  $x = 13$ , justified by the frequent occurrence of these numbers in Anacardiaceae, Burseraceae, Meliaceae, Simaroubaceae and Sapindaceae. More recently, Guimarães & Forni-Martins (2022) suggested  $x = 6$ , with derivations for  $x = 5$  and  $x = 7$  in the basal clades, while the families with the highest number of species would be derived from a common polyploid ancestor with  $x = 14$ .

Among the new chromosome records for the other families, stand out *Amorimia septentrionalis*, *Eugenia zigzag* and *Utricularia quelchii*. *Amorimia* was the only Neotropical genus of the Malpighioid clade (Malpighiaceae) without chromosome records (Almeida, 2018). The number  $2n = 20$  for *A. septentrionalis* presented here is similar to that found in closely related genera, especially *Malpighia* L. and *Mascagnia* (DC.) Bertero (Rice & al., 2015). *Eugenia zigzag* (Myrtaceae) is a recently described species (Sobral & al., 2018), known so far only by the type material from a dry forest (Caatinga) in a single municipality in Bahia State. The record of  $2n = 22$  confirms the predominance of this chromosome number for most representatives of *Eugenia* L. (Costa & Forni-Martins, 2006; Rice & al., 2015). *Utricularia quelchii*, in turn, stands out for being endemic to the Pantepuys in South America, a region with few plants analyzed in terms of chromosome number due to difficult access and limitations in their cultivation. *Utricularia quelchii* exhibited  $2n = 18$  unlike most species of *Utricularia* L., which commonly have higher chromosome numbers (Rice & al., 2015).

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#### IAPT chromosome data 40/9

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\* First chromosome count for the species.

\*\* First chromosome count for the genus.

#### POACEAE

\*\**Arthropogon villosus* Nees

$2n = 40$ , CHN. Brazil, Distrito Federal, Brasília, Fercal, 15.5075°S, 47.9491666°W, 834 m, 27 Nov 2014, R.C. Oliveira & A.S. Silva 2969 (UB) [Fig. 14A]; Brazil, Distrito Federal, Brasília, Fercal, 15.5475°S, 47.9538889°W, 923 m, 27 Nov 2014, R.C. Oliveira & A.S. Silva 2971 (UB).

*Mesosetum bifarium* (Hack.) Chase

$2n = 16$ , CHN. Brazil, Goiás, Alto Paraíso de Goiás, Buddhist center of Chapada dos Veadeiros, close to Serra da Cobra, 14.1502778°S, 47.5891666°W, 1171 m, 6 Jun 2014, A.R.O. Ribeiro 391 (UB) [Fig. 14B].

*Mesosetum cayennense* Steud.

$2n = 20$ , CHN. Brazil, Goiás, Pirenópolis, Serra dos Pirineus State Park, 15.7975°S, 48.8122222°W, 1196 m, 19 May 2014, A.R.O. Ribeiro & R.C. Oliveira 362 (UB) [Fig. 15A]; Brazil, Goiás, Flores de Goiás, 12 km from the roundabout of flowers, right side of BR, 14.8972222°S, 46.9511111°W, 21 Mar 2013, P.A. Reis, A.R.O. Ribeiro & J.E.Q. Faria 181 (UB).

\**Mesosetum chlorostachyum* (Döll) Chase

$2n = 8$ , CHN. Brazil, Amazonas, Manaus, Cabeceira do São José, a tributary of the Rio Negro, Chácara Nossa Senhora de Fátima, Careiro da Várzea, 03.0208334°S, 60.1597222°W, 27 m, 28 Apr 2015, R.C. Oliveira & al. 3033 (UB) [Fig. 14C,D].

*Mesosetum elythrochaetum* (Hack.) Swallen

$2n = 24$ , CHN. Brazil, Goiás, Alto Paraíso de Goiás, National Park Chapada dos Veadeiros, 14.1708334°S, 47.8275°W, 1006 m, 3 Jun 2014, A.R.O. Ribeiro 377 (UB) [Fig. 15B].

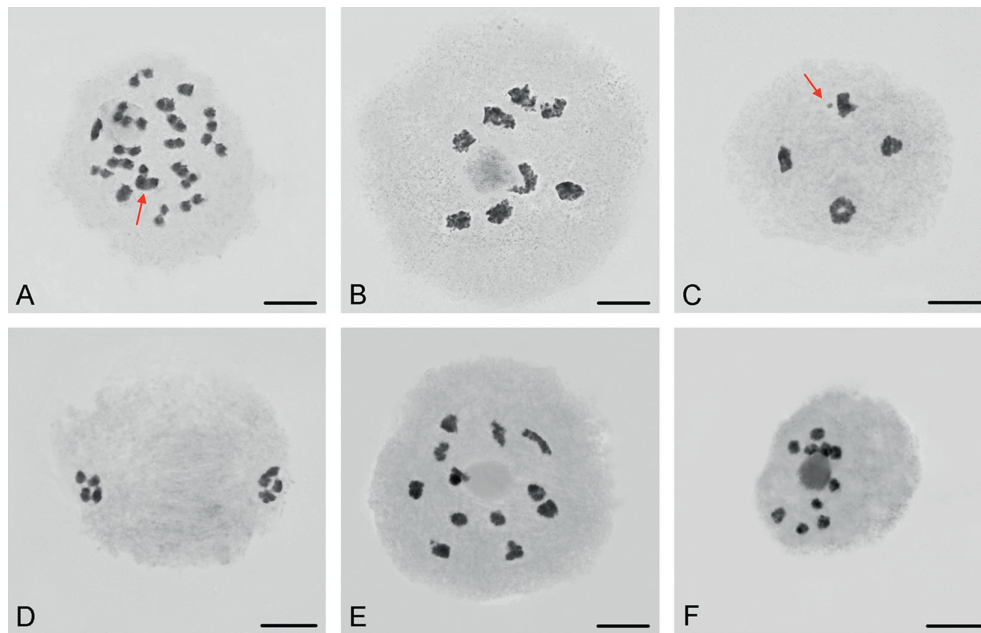
\**Mesosetum exaratum* (Trin.) Chase  
 $2n = 26$ , CHN. Brazil, Minas Gerais, Serra do Cipó, MG 010, towards Alto Palácio, 19.2888889°S, 43.5922222°W, 23 Nov 2013, A.R.O. Ribeiro & I.A. Conceição 351 (UB) [Fig. 16A,B].

\**Mesosetum gibbosum* Renvoize & Filg.  
 $2n = 24$ , CHN. Brazil, Bahia, Rio de Contas, road to Jussiape, ca. 1 km of Rio de Contas, 13.5925°S, 41.7986111°W, 1100 m, 19 Apr 2015, A.R.O. Ribeiro & J.E.Q. Faria 408 (UB) [Fig. 14E].

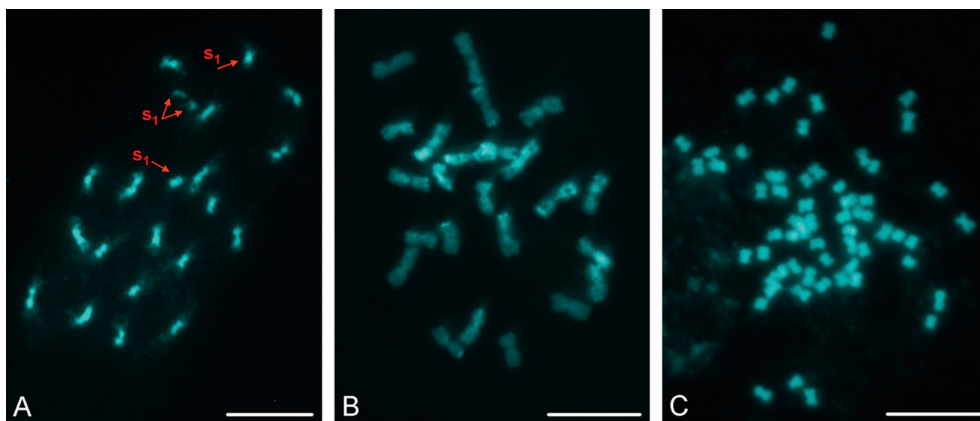
\*\**Spheneria kegelii* (Müll.Hal.) Pilg.  
 $2n = 20$ , CHN. Brazil, Amazonas, Manaus, Campina do INPA, 15.9786111°S, 47.9552778°W, 25 Apr 2015, R.C. Oliveira & al. 3007 (UB) [Fig. 14F].

*Tatianyx arnacites* (Trin.) Zuloaga & Soderstr.  
 $2n = 60$ , CHN. Brazil, Bahia, Rio de Contas, road to Jussiape, ca. 1 km of Rio de Contas, 13.5925°S, 41.7986111°W, 1100 m, 19 Apr 2015, A.R.O. Ribeiro & J.E.Q. Faria 411 (UB) [Fig. 15C].

In Poaceae, Panicoideae is the most diverse subfamily in the tropical region, comprising 3325 species (GPWG, 2001; Soreng & al., 2015, 2017, 2022; Burke & al., 2016; Huang & al., 2022). In Panicoideae, the basic chromosome numbers of  $x = 9$  and  $x = 10$  are the most frequent, but the basic chromosome numbers of  $x = 3$  to 8,  $x = 11$ ,  $x = 12$  and  $x = 14$  have also been reported (Celarier & Paliwal, 1957; Rao, 1975; De Wet, 1987; GPWG, 2001; Hilu, 2004; Morrone & al., 2006; Sede & al., 2010; Goldblatt & Johnson, 2023). The chromosome numbers of  $2n = 6$  and  $2n = 8$  ( $x = 3$ ,



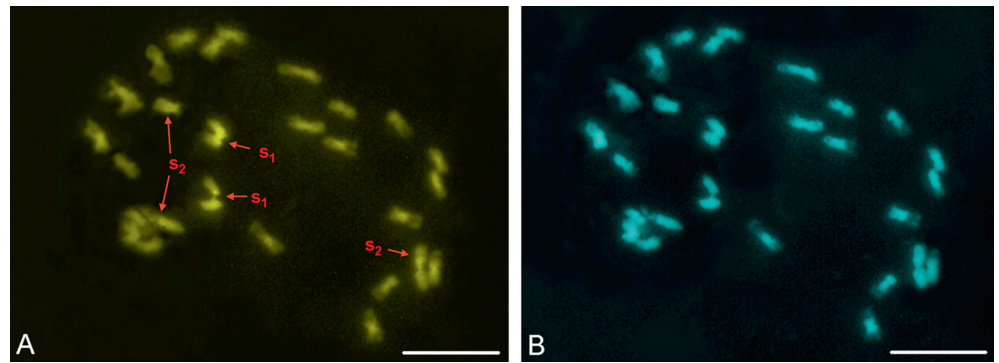
**Fig. 14.** Meiotic cells of species of the subtribes Arthropogoninae (*Arthropogon*, *Mesosetum*) and Paspalinae (*Spheneria*). **A**, *A. villosus*, R.C. Oliveira & A.S. Silva 2969, diakinesis with 20 bivalents ( $2n = 40$ ), arrow indicates overlapping of two bivalents; **B**, *M. bifarium*, A.R.O. Ribeiro 391, diakinesis with 8 bivalents ( $2n = 16$ ); **C & D**, *M. chlorostachyum*, R.C. Oliveira & al. 3033: **C**, diakinesis with 4 bivalents ( $2n = 8$ ), arrow indicates satellite associated with a chromosome pair (bivalent); **D**, Anaphase I showing segregation of 4 chromosomes to each pole ( $2n = 8$ ); **E**, *M. gibbosum*, A.R.O. Ribeiro & J.E.Q. Faria 408, diakinesis with 12 bivalents ( $2n = 24$ ); **F**, *S. kegelii*, R.C. Oliveira & al. 3007, diakinesis with 10 bivalents ( $2n = 20$ ). — Scale bars = 10  $\mu$ m.



**Fig. 15.** Mitotic cells of species of the subtribe Arthropogoninae (*Mesosetum*, *Tatianyx*) showing chromosomes stained with DAPI. **A**, *M. cayennense*, A.R.O. Ribeiro & R.C. Oliveira 362, metaphase with  $2n = 20$  chromosomes, arrows indicate a satellited chromosome pair (S1); **B**, *M. elytrochaetum*, A.R.O. Ribeiro 377, metaphase with  $2n = 24$  chromosomes; **C**, *T. arnacites*, A.R.O. Ribeiro & J.E.Q. Faria 411, metaphase with  $2n = 60$  chromosomes. — Scale bars = 10  $\mu$ m.



**Fig. 16.** Mitotic cell of *Mesosetum exaratum* (subtribe Arthropogoninae), A.R.O. Ribeiro & I.A. Conceição 351, showing metaphase with  $2n = 26$  chromosomes. **A**, Chromosomes stained with CMA (yellow), arrows indicate the satellited chromosome pairs (S1, S2); **B**, Chromosomes stained with DAPI (blue). — Scale bars = 10  $\mu\text{m}$ .



$x = 4$ ) are rare and the lowest recorded among the tropical grasses, being exclusively reported in *Iseilema* Andersson (tribe Andropogoneae) and *Mesosetum* Steud. (tribe Paspaleae, subtribe Arthropogoninae) (Celarier & Paliwal, 1957; Rao, 1975; De Wet, 1987; Morrone & al., 2012; Ribeiro & al., 2015, 2019; Sousa & al., 2017).

The majority of the genera of the subtribe Arthropogoninae have records of chromosome numbers that are multiples of  $x = 10$ , which is also the basic chromosome number of the tribe Paspaleae (Morrone & al., 2012). Many species of Arthropogoninae and Paspaleae have a restricted geographical distribution or are still little known, regarding their morphology, cytology, molecular genetics and reproduction, notably in *Arthropogon* Nees, *Mesosetum*, *Spheneria* Kuhl., and *Tatianyx* Zuloaga & Soderstr. (Filgueiras, 1986, 1989; Morrone & al., 2012; Filgueiras & al., 2015; Silva & al., 2016; Oliveira & al., 2019a,b; Ribeiro & al., 2023a,b; Silva & al., 2023a,b). In *Mesosetum*, there are two basic chromosome numbers recorded,  $x = 4$  ( $2n = 8, 16, 24$  and  $32$ ), as well as  $x = 10$  ( $2n = 20$  and  $60$ ), highlighting that  $x = 4$  is not found in other genera of the subtribe (Ribeiro & al., 2015; Sousa & al., 2017; Ribeiro & al., 2019). The chromosome numbers in other genera of Arthropogoninae are known only for *Homolepis aturensis* (Kunth) Chase ( $2n = 20$ ), *H. isocalycia* (G.Mey.) Chase ( $2n = 40$ ), *Phanopyrum gymnocarpon* (Elliott) Nash ( $2n = 40$ ), and *T. arnaces* (Trin.) Zuloaga & Soderstr. ( $2n = 60$ ) (Gould & Soderstrom, 1967; Pohl & Davidse, 1971; Kessler & Hatch, 1984; Morrone & al., 1995).

This study was performed in 11 accessions belonging to nine species of the subtribes Arthropogoninae and Paspalinae. The voucher specimens were deposited in the herbarium UB. The chromosome numbers were determined by the analysis of at least 10 cells with a good chromosome spreading, according to methods described by Ribeiro & al. (2015).

For the meiotic analysis, the samples were taken from young inflorescences and immersed in the fixative solution 3 : 1 (ethanol : acetic acid, v/v) for 24 h at room temperature and stored in 70% ethanol solution at 4°C, according to Ribeiro & al. (2015).

For the mitotic analysis, the root tips were pretreated with 2 mM 8-hydroxyquinoline for 10 h at 10°C, fixed in Carnoy solution (ethanol/acetic acid, 3 : 1, v/v) for 2–24 h at room temperature and then stored at –20°C. The samples were digested with enzymatic solution containing 2% cellulase (Onozuka-SERVA Electrophoresis, Heidelberg, Germany) and 20% pectinase (Sigma-Aldrich, Darmstadt, Germany) for 90 min at 37°C. The slides and the chromosomes were prepared with acetic acid 60% (aqueous solution, v/v), using the spreading technique based on Ruban & al. (2014). The CMA/DAPI staining was performed in the best slides, according to the method described by Cabral & al. (2014). The slides were aged for 3 days,

stained with CMA (chromomycin A3) 0.5 mg/ml for 1 h and restained with DAPI (4',6-diamidino-2-phenylindole) 2 mg/ml for 30 min.

The images of meiotic chromosomes were acquired and analyzed by the Leica DM 750 microscope and software Leica Application Suite (v.4.5). The images of mitotic chromosomes were captured using the Leica DMRB fluorescent microscope equipped with the Cohu digital camera and the Leica Q-FISH software.

The chromosome numbers were determined in 11 new accessions belonging to nine species of tribe Paspaleae, eight of which belong to the subtribe Arthropogoninae and one of which belongs to the subtribe Paspalinae. First chromosome counts are reported here for five species: *Arthropogon villosus* ( $2n = 40$ ), *Mesosetum chlorostachyum* ( $2n = 8$ ), *M. exaratum* ( $2n = 26$ ), *M. gibbosum* ( $2n = 24$ ), and *Spheneria kegelii* ( $2n = 20$ ). These results are the first records of chromosome numbers in the genera *Arthropogon* (subtribe Arthropogoninae) and *Spheneria* (subtribe Paspalinae).

The chromosome number of  $2n = 26$  ( $x = 13$ ) found in *Mesosetum exaratum* is here registered for the first time in the subfamily Panicoideae. *Mesosetum exaratum* is endemic to Brazil and this basic chromosome number of  $x = 13$  is rare and cited only for eight genera of Poaceae, of which six belong to the subfamily Pooideae, one to Aristidoideae, and one to Chloridoideae. In *M. exaratum* (accession A.R.O. Ribeiro & I.A. Conceição 351) two pairs of the proximal Nucleolus Organizer Region (NOR) were identified, visible as distended regions (Fig. 16A,B). This centromeric NORs were positively stained with the fluorochrome CMA (CMA+) and negatively stained with the fluorochrome DAPI (DAPI–), a common pattern in plants (Roa & Guerra, 2012). Proximal NORs may be related to centric fission/fusion processes (Souza & al., 2012) resulting in the origin of  $x = 13$ . In *Deschampsia* P.Beauv. (subfamily Pooideae), for example,  $x = 13$  arose by a reduction from the secondary basic number of  $x = 14$ , which in turn originated by polyploidy from  $x = 7$  (Cardone & al., 2009; Amosova & al., 2015).

In Arthropogoninae, our chromosome counts of  $2n = 40$  in *Arthropogon villosus* and  $2n = 60$  in *Tatianyx arnaces* and the previous records of  $2n = 20$  in *Homolepis aturensis*,  $2n = 40$  in *H. isocalycia* and  $2n = 40$  in *Phanopyrum gymnocarpon* (Gould & Soderstrom, 1967; Pohl & Davidse, 1971; Kessler & Hatch, 1984; Morrone & al., 1995) support  $x = 10$  as the basic chromosome number of the subtribe Arthropogoninae and tribe Paspaleae, corroborating the molecular phylogeny of Morrone & al. (2012).

*Arthropogon* comprises five species recognized by the paniculiform inflorescence and laterally compressed spikelets (Filgueiras, 1982; Filgueiras & al., 2001, 2015; POWO, 2023; Ribeiro & al., 2023a). *Arthropogon villosus* is a perennial species that has a geographical distribution restricted to Bolivia and Brazil, which is

distinguished by the convolute leaf sheaths, leaf blades 2.6–10.0 mm wide, and usually the fire-induced flowering (Filgueiras, 1982; Filgueiras & al., 2001, 2015; Ribeiro & al., 2023a), as observed in the accession *R.C. Oliveira & A.S. Silva 2971*. Exceptionally, there are no burnt leaves in *R.C. Oliveira & A.S. Silva 2969*, which does not appear to have the fire-induced flowering. In both accessions the same chromosome number ( $2n = 40$ ) and regular pairing in 20 bivalents (Fig. 14A) was registered, suggesting tetraploidy with apparent process of cytological diploidization, as described by Li & al. (2021).

*Spheneria* is a monospecific genus represented by *S. kegelii*, which is restricted to Brazil, Guyana, and Suriname (POWO, 2023; Ribeiro & al., 2023b). *Spheneria kegelii* has an inflorescence composed of 2–3 racemiform branches, which are alternate on the inflorescence axis. The accession analyzed (*R.C. Oliveira & al. 3007*) is typically diploid with pairing in 10 bivalents ( $2n = 20$ ) (Fig. 14F).

The chromosomes of *Tatianyx arnatices* ( $2n = 60$ ) were for the first time observed in mitosis in the present study (Fig. 15C), confirming the previous chromosome counting of another accession made at meiosis by Morrone & al. (1995).

*Mesosetum chlorostachyum* and *M. gibbosum* are endemic to South America (Filgueiras, 1986, 1989; Filgueiras & al., 2015; Silva & al., 2023a) and their chromosome numbers were recorded for the first time in this study. *Mesosetum chlorostachyum* is an annual species restricted to the Amazon forest in Brazil and Venezuela, while *M. gibbosum* is a perennial species occurring only in the Cerrado of the states of Bahia and Tocantins (Filgueiras, 1986, 1989; Filgueiras & al., 2015; Silva & al., 2023a). In *M. chlorostachyum*, the pairing in four bivalents ( $2n = 8$ ) (Fig. 14C) is compatible with diploidy. In *M. gibbosum*, the pairing in 12 bivalents ( $2n = 24$ ) (Fig. 14E) suggests hexaploidy with evident cytological diploidization (Li & al., 2021).

In *Mesosetum*, the chromosome numbers determined by the present study corroborate the cytological data obtained in previous works (Gould, 1966; Gould & Soderstrom, 1967; Pohl & Davidse, 1971; Davidse & Pohl, 1972; Kessler & Hatch, 1984; Morrone & al., 2006; Sede & al., 2010; Silva & al., 2012; Ribeiro & al., 2015; Sousa & al., 2017; Ribeiro & al., 2019), supporting at least three chromosomal lineages with distinct basic chromosome numbers in the genus: the first with  $x = 10$ , including three species (*M. cayennense*, *M. ferrugineum*, *M. rottboellioides* (Kunth) Hitchc.), the second with  $x = 4$ , comprising 14 species (*M. alatum* Filg., *M. annum* Swallen, *M. ansatum* (Trin.) Kuhl., *M. bifarium*, *M. chaseae* Luces, *M. chlorostachyum*, *M. compressum* Swallen, *M. elytrochaetum*, *M. gibbosum*, *M. loliiforme* (Hochst. ex Steud.) Chase, *M. longiaristatum* Filg., *M. pappophorum* (Nees) Kuhl., *M. pittieri* Hitchc., *M. sclerochloa* (Trin.) Hitchc.), and the third monospecific with  $x = 13$  represented by *M. exaratum*.

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## IAPT chromosome data 40/10

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\* First chromosome count for the species

\*\* New cytotype for the species



## EUPHORBIACEAE

*Croton adamantinus* Müll.Arg.

\* $2n = 20$ , CHN. Brazil, Paraíba, Areia, Pau-ferro Forest, edge of the lane past the chapel, 06°58'27"S, 35°44'17"W, 645 m, 1 Mar 2016, *L.P. Felix 15870* (EAN) [Figs. 17A, 19A].

*Croton adamantinus* is a shrubby species, endemic to Brazil, with occurrence restricted to the Northeast (except Maranhão and Alagoas) and Southeast (Minas Gerais) regions, associated with the Caatinga domain, in semi-arid vegetation (Caruzo & al., 2020). The present record of the occurrence of this species in Paraíba suggests that *C. adamantinus* also adapts to environments in the humid forest domain of the Atlantic Forest. The count of  $2n = 20$  coincides with most chromosome records for the genus.

*Croton argyrophyllus* Kunth

$2n = 20$ , CHN. Brazil, Bahia, Itaberaba, BR 424, 12°31'15"S, 40°07'04"W, 231 m, 3 May 2015, *L.P. Felix 15575* (EAN)

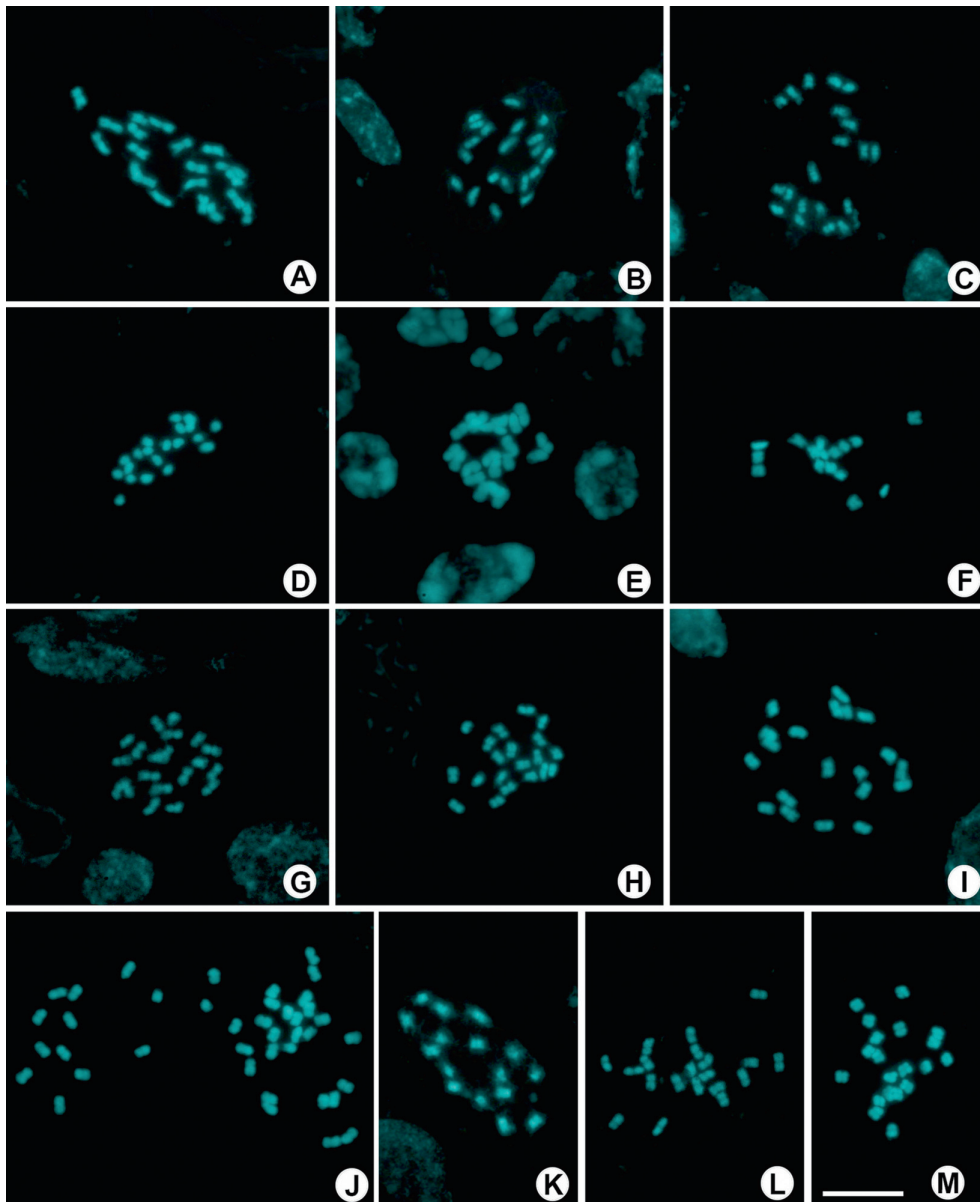
[Figs. 17B, 19B]; Brazil, Pernambuco, Brejo da Madre de Deus, Tabocas Small Farm, 08°09'07"S, 36°19'38"W, 502 m, 29 Jun 2015, *L.P. Felix 15636* (EAN) [Fig. 17C].

*Croton argyrophyllus* occurs in South America in Colombia, Venezuela, Peru, Bolivia and Paraguay (Caruzo & al., 2020). In Brazil there are records for the Northeast Region (except Rio Grande do Norte) and in the North Region (Roraima, Rondônia) in the Caatinga and Amazon domains. The present record of  $2n = 20$  confirms the two counts previously reported for the species (Pôrto & al., 2014).

*Croton blanchetianus* Baill.

$2n = 20$ , CHN. Brazil, Paraíba, Esperança, Timbaúba Farm, over rocky outcrop, 07°01'01"S, 35°52'49"W, 640 m, 16 Jun 2015, *A.S. Santos 30* (EAN) [Figs. 17D, 19C].

*Croton blanchetianus* is an endemic species of Brazil with distribution restricted to the Northeast (except Maranhão) and Southeast (Minas Gerais) regions, occurring exclusively in the Caatinga



**Fig. 17.** Mitotic metaphase.

A, *Croton adamantinus*,  $2n = 20$ ; B & C, *Croton argyrophyllus*,  $2n = 20$ ; D, *Croton blanchetianus*,  $2n = 20$ ; E, *Croton campestris*,  $2n = 20$ ; F, *Croton glandulosus*,  $2n = 16$ ; G & H, *Croton grewiooides*,  $2n = 20$ ; I, *Croton heliotropiifolius*,  $2n = 20$ ; J, *Croton heliotropiifolius*,  $2n = 40$ ; K, *Croton hirtus*,  $2n = 16$ ; L & M, *Croton jacobinensis*,  $2n = 20$ .

domain (Caruzo & al., 2020). The present record of  $2n = 20$  confirms previous counts for the species (Pôrto & al., 2014; Rice & al., 2015).

*Croton campestris* A.St.-Hil.

\* $2n = 20$ , CHN. Uruguay, Maldonado, Punta de Punta Ballena, 34°53'46"S, 55°02'16"W, 61 m, 14 Oct 2016, *L.P. Felix 16015* (EAN) [Fig. 17E].

*Croton campestris* is a species distributed only in South America, with records for Brazil, Bolivia and Paraguay (Jørgensen & al., 2014). For Brazil, its occurrence has been confirmed for the North (Tocantins), Northeast (except Maranhão, Alagoas and Sergipe), Midwest (Goiás, Mato Grosso do Sul) and Southeast (except São Paulo) regions. The present occurrence record for Uruguay suggests a wider distribution of the species in South America. Our count of  $2n = 20$  for this species coincides with most chromosome records of the genus.

*Croton floribundus* Spreng.

\*\* $2n = 100$ , CHN. Brazil, Paraíba, Areia, Pau-ferro Forest, 06°58'27"S 35°44'17"W, 646 m, 1 Mar 2016, *L.P. Felix 15874* (EAN) [Figs. 18H, 19J]; Brazil, Pernambuco, Taquaritinga do Norte, Hill

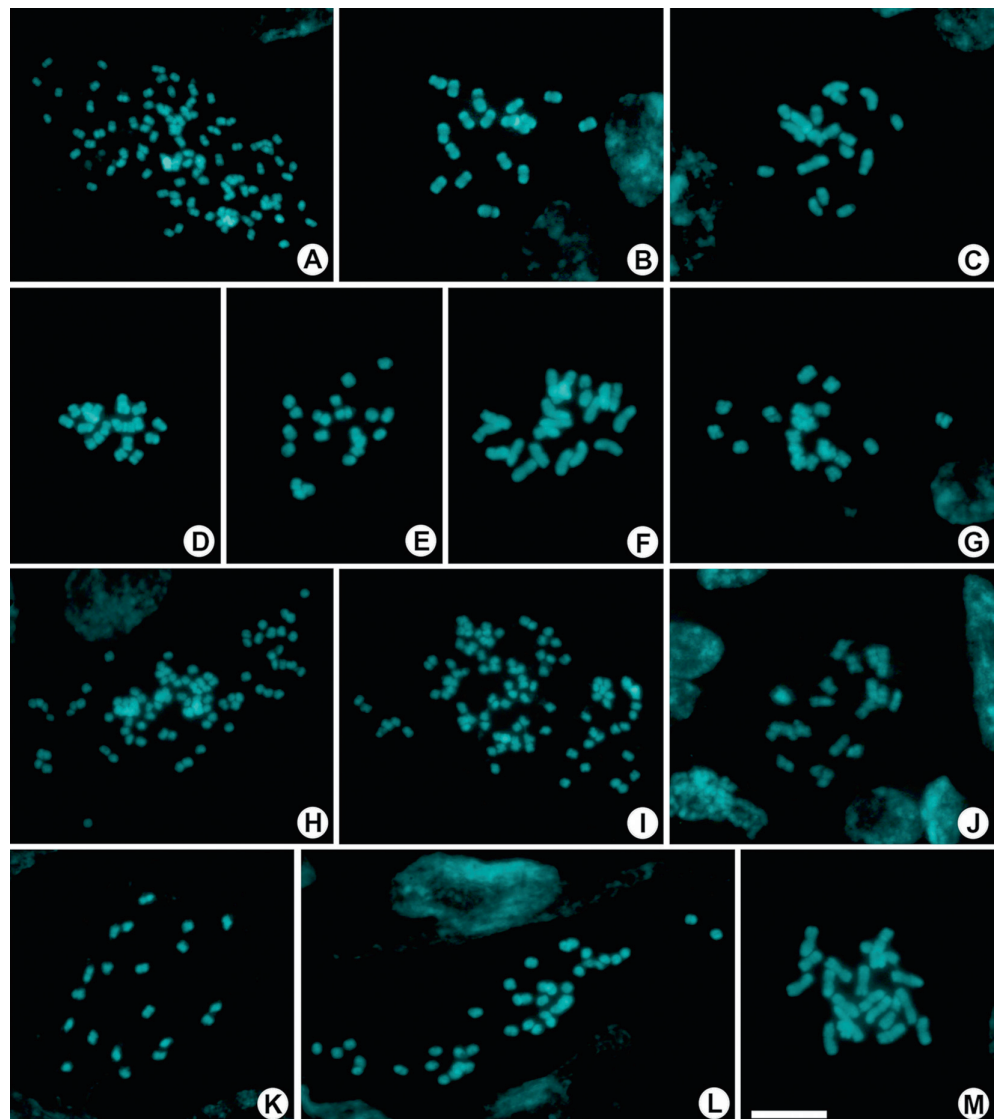
of antennas, 07°54'28"S 36°01'39"W, 1100 m, 13 May 2016, *L.P. Felix 15879* (EAN) [Fig. 18I].

*Croton floribundus* is a tree species, found in environments of the Atlantic Forest, distributed only in South America, with records for Brazil and Paraguay (Lima & Pirani, 2003; Paña-Chocarro & al., 2010). In Brazil, it was reported for the Northeast (except Maranhão, Piauí, Rio Grande do Norte and Sergipe), Central-West (except Goiás), Southeast and South (Paraná) regions. Our count of  $2n = 100$  is unprecedented and differs from the previous record of  $2n = 112$  for this species (Silvestrini & al., 2013).

*Croton glandulosus* L.

$2n = 16$ , CHN. Brazil, Paraíba, Jacaraú, Timbó, Riacho do meio, 06°36'53"S, 35°17'28"W, 192 m, 5 Mar 2016, *E.M. Almeida & G.F. Pessoa 1581* (EAN) [Fig. 17F].

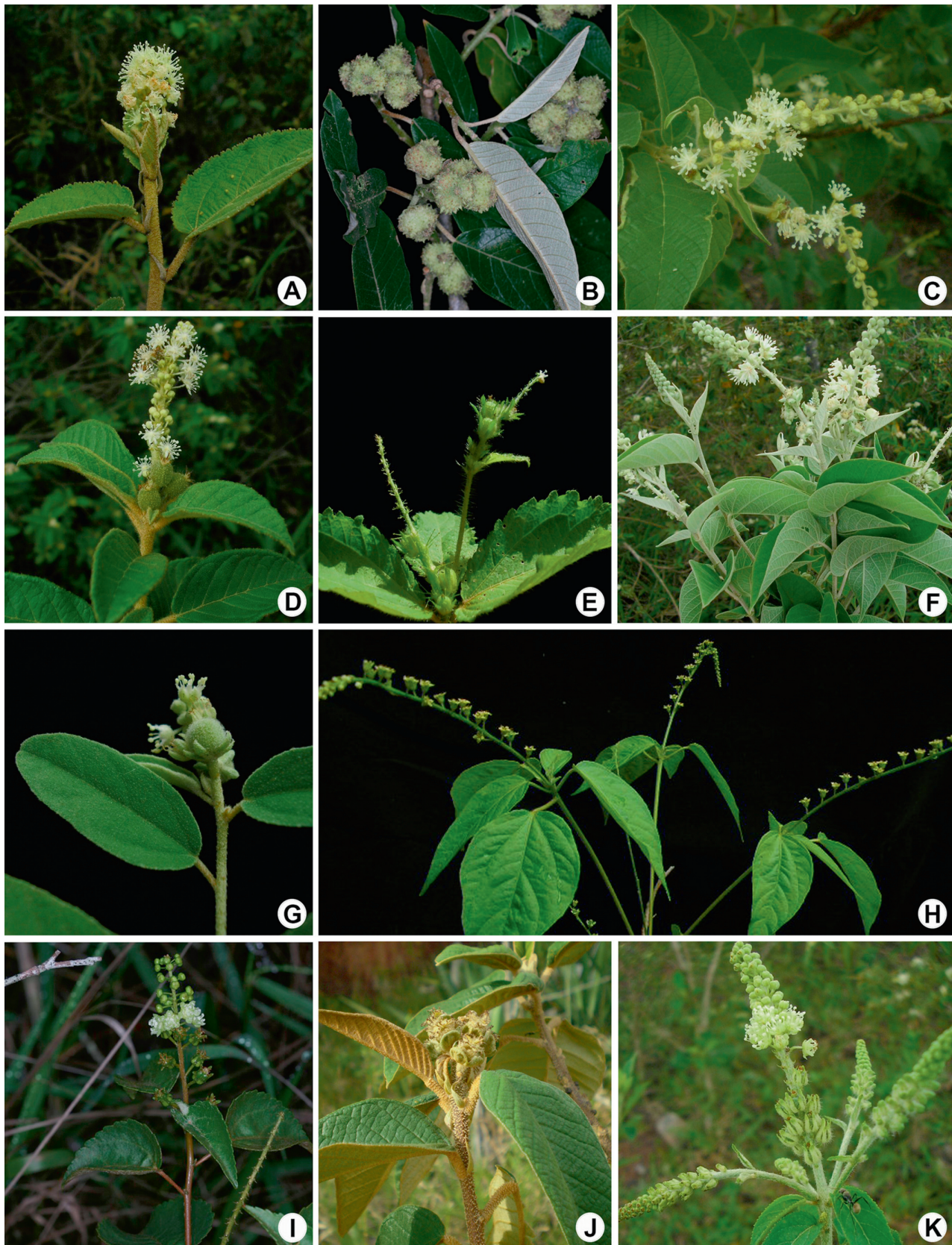
*Croton glandulosus* is a species widely distributed throughout the Neotropics, from the southern United States and Mexico to Central America, the Antilles and tropical South America (Standley & Steyermark, 1949). They are subshrubby ruderal plants occurring in different environments, such as Cerrado, coastal vegetation, dry and humid forests (Silva & al., 2010) in virtually all types of



**Fig. 18.** Mitotic metaphase.

A, *Croton limae*,  $2n = 100$ ;  
 B, *Croton lombardianus*,  $2n = 20$ ;  
 C, *Croton pedicellatus*,  $2n = 18$ ;  
 D, *Croton pulegioidorus*,  $2n = 20$ ;  
 E, *Croton pulegioidorus*,  $2n = 22$ ;  
 F, *Croton rudolphianus*,  $2n = 20$ ;  
 G, *Croton sellowii*,  $2n = 20$ ;  
 H & I, *Croton floribundus*,  
 $2n = 100$ ; J, *Croton tricolor*,  
 $2n = 20$ ; K, *Croton triqueter*,  
 $2n = 20$ ; L, *Croton triqueter*,  
 $2n = 40$ ; M, *Croton urticifolius*,  
 $2n = 20$ .





**Fig. 19.** Species with chromosome count. **A**, *Croton adamantinus*; **B**, *Croton argyrophyllus*; **C**, *Croton blanchetianus*; **D**, *Croton heliotropiifolius*; **E**, *Croton hirtus*; **F**, *Croton jacobinensis*; **G**, *Croton pedicelatus*; **H**, *Croton pulegioidorus*; **I**, *Croton rudolphianus*; **J**, *Croton floribundus*; **K**, *Croton urticifolius*. — Photos: A, C, D, F & K: Amanda S. Santos; B, E & G–J: Leonardo P. Felix.



vegetation in the country from the Amazon to the Pampas Gaúchos in Rio Grande do Sul. Our count of  $2n = 16$  confirms previously reported counts for this species (Pôrto & al., 2014; Rice & al., 2015).

*Croton grewiooides* Baill.

\* $2n = 20$ , CHN. Brazil, Bahia, Barra da Estiva, Capão da volta, road to Ibicoara, 13°26'49"S, 31°29'20"W, 1 May 2015, *L.P. Felix 15559* (EAN) [Fig. 17G]; Brazil, Pernambuco, Pesqueira, Chucuru Reserve, 08°19'15"S, 36°43'12"W, 1122 m, 30 Jun 2015, *L.P. Felix 15650* (EAN) [Fig. 17H].

*Croton grewiooides* is a species that has an occurrence record only for Bolivia and Brazil, in Brazil it has a restricted distribution in the Northeast (except Maranhão) and Southeast (Minas Gerais) regions, in Caatinga environments (Jørgensen & al., 2014). The  $2n = 20$  record for the species coincides with most chromosomal records for *Croton*.

*Croton heliotropiifolius* Kunth

\*\* $2n = 20$ , CHN. Brazil, Paraíba, Areia, Chã de Jardim, 06°57'50"S, 35°44'46"W, 22 Oct 2014, *A.S. Santos 02* (EAN) [Figs. 17I, 19D].

$2n = 40$ , CHN. Brazil, Ceará, Iguatu, 06°22'55"S, 39°07'04"W, 435 m, 16 Apr 2014, *L.P. Felix & E.M. Almeida 14914* (EAN) [Fig. 17J].

*Croton heliotropiifolius* is a species distributed throughout the Northeast Region of Brazil, in addition to the Southeast (Minas Gerais) and Midwest (Goiás) regions, occurring in the Caatinga, Atlantic Forest, Cerrado and Amazon domains, also with records of occurrence in Peru (Macbride, 1951). Of the two populations analyzed, one was diploid with  $2n = 20$  and the other tetraploid with  $2n = 40$ . Of these counts, only the last one coincided with the previous chromosomal record for the species (Pôrto & al., 2014).

*Croton hirtus* L'Hér.

$2n = 16$ , CHN. Brazil, Paraíba, Jacaraú, Timbó, Riacho do meio, 06°36'53"S, 35°17'28"W, 192 m, 5 Mar 2016, *E.M. Almeida & G.F. Pessoa 1582* (EAN) [Figs. 17K, 19E].

*Croton hirtus* is widely distributed throughout the tropical regions of the world, occurring in Africa, Oceania, Central America and South America (Jørgensen & al., 2014). In Brazil, the species occurs in the North (Pará, Tocantins), Northeast (all states), Midwest (Goiás), Southeast (Minas Gerais, São Paulo) and South (Paraná, Santa Catarina) regions. The present count of  $2n = 16$  confirms previous counts for the species (Soontornchainaksaeng & al., 2003).

*Croton jacobinensis* Baill.

$2n = 20$ , CHN. Brazil, Paraíba, Areia, Chã de Jardim, 06°57'50"S, 35°44'46"W, 641 m, 22 Oct 2014, *A.S. Santos 03* (EAN) [Figs. 17L, 19F]; Brazil, Paraíba, Jacaraú, Timbó, Riacho do meio, 06°36'53"S, 35°17'28"W, 192 m, 5 Mar 2016, *E.M. Almeida & G.F. Pessoa 1579* (EAN) [Fig. 17M].

*Croton jacobinensis* is an endemic species of Brazil with exclusive distribution in the Northeast (except Alagoas and Sergipe) and Southeast (Minas Gerais) regions with exclusive occurrence in the Caatinga domain. Our count of  $2n = 20$  for two populations confirms the previous chromosomal record for the species (Pôrto & al., 2014).

*Croton limae* A.P.S.Gomes, M.F.Sales & P.E.Berry

\* $2n = 100$ , CHN. Brazil, Bahia, Jussiapé, road to Ibicoara, 13°27'10"S, 41°32'20"W, 1062 m, 1 May 2015, *L.P. Felix 15554* (EAN) [Fig. 18A].

*Croton limae* is a tree or shrub species endemic to Brazil, with occurrence records exclusive to the Northeast Region (except Maranhão, Rio Grande do Norte and Sergipe) in the Caatinga phytogeographic domain (Caruzo & al., 2020). The present record of  $2n = 100$  is unprecedented and, together with the count for *C. floribundus*, constitutes one of the highest chromosome numbers reported for the genus.

*Croton lombardianus* Croizat

\* $2n = 20$ , CHN. Uruguay, Maldonado, Punta de Punta Ballena, 34°22'50"S 54°40'36"W, 61 m, 15 out 2016, *L.P. Felix 16030* (EAN) [Fig. 18B].

*Croton lombardianus* is an endemic species of Uruguay, which occurs in dry and desert environments (Govaerts & al., 2000). The record of  $2n = 20$  constitutes one of the most common chromosome numbers reported for the genus.

*Croton pedicellatus* Kunth

$2n = 18$ , CHN. Brazil, Paraíba, Sertãozinho, Canafistula Small Farm, 06°44'06"S, 35°27'30"W, 130 m, 25 Jan 2018, *J.M.P. Cordeiro 1259* (EAN) [Figs. 18C, 19G].

*Croton pedicellatus* is a subshrub species with occurrence records for Mexico and South America (Bolivia, Brazil, Colombia, Peru). In Brazil, it is distributed across the five regions, in Caatinga, Atlantic Forest and Cerrado environments (Macbride, 1951; Forzza, 2010; Villaseñor Ríos, 2016). The record of  $2n = 18$  coincides with the previous chromosome record for this species (Pôrto & al., 2014).

*Croton pulegiodorus* Baill.

$2n = 20$ , CHN. Brazil, Bahia, Jussiapé, road to Ibicoara, 13°22'47"S, 41°33'23"W, 816 m, 1 May 2015, *L.P. Felix 15553* (EAN) [Fig. 18D].

\*\* $2n = 22$ , CHN. Brazil, Paraíba, Campina Grande, São José da Mata Forest, 07°11'02"S, 35°59'01"W, 667 m, 16 Jun 2015, *A.S. Santos 19* (EAN) [Figs. 18E, 19H].

*Croton pulegiodorus* is endemic to Brazil, restricted to the Northeast (except Rio Grande do Norte, Maranhão, Sergipe and Alagoas), Southeast (Minas Gerais) and Midwest (Goiás) regions in the Caatinga and Cerrado domains (Forzza, 2010). The record of  $2n = 20$  coincides with the previous count reported by Porto & al. (2014) for the state of Paraíba. However, our count of  $2n = 22$ , also for Paraíba, is new for the species and corresponds to ascending dysploidy possibly mediated by centric fission.

*Croton rudolphianus* Müll.Arg.

\* $2n = 20$ , CHN. Brazil, Pernambuco, Buíque, Parma Catimbal, 08°35'37"S, 37°12'18"W, 811 m, 1 Jul 2015, *L.P. Felix 15662* (EAN) [Figs. 18F, 19I].

*Croton rudolphianus* is an endemic species of Brazil, restricted to the Northeast (except Rio Grande do Norte and Maranhão) and Southeast (Minas Gerais) regions, exclusively in vegetation of rocky outcrops of the Caatinga. The record of  $2n = 20$  for the species coincides with most chromosomal records of the genus.

*Croton sellowii* Baill.

\* $2n = 20$ , CHN. Brazil, Paraíba, Mamanguape, Rebio Guaribas, 06°43'06"S, 35°12'44"W, 211 m, 27 Nov 2014, *J.M.P. Cordeiro 600* (EAN) [Fig. 18G].

*Croton sellowii*, a terrestrial plant of shrubby habit, is an endemic species of Brazil, with occurrence restricted to the Northeast Region (Paraíba, Pernambuco, Sergipe, Alagoas, Bahia), in vegetation of rocky outcrops in the Caatinga and Atlantic Forest domains.

*Croton tricolor* Klotzsch ex Baill.

\* $2n = 20$ , CHN. Brasil, Bahia, Barra da Estiva, Capão da volta, road to Ibicoara, 13°26'49"S, 31°29'20"W, 1154 m, 1 May 2015, *L.P. Felix 15557* (EAN) [Fig. 18J].

*Croton tricolor* is distributed throughout South America (Bolivia, Brazil). In Brazil, it occurs in the Northeast (except Maranhão), Southeast (Minas Gerais, São Paulo) and South (Paraná) regions, in the Caatinga, Cerrado and Atlantic Forest domains. The record of  $2n = 20$  for the species coincides with most chromosomal records of the genus.

*Croton triqueter* Lam.

\* $2n = 20$ , CHN. Brazil, Bahia, Barra da Estiva, Capão da volta, road to Ibicoara, 13°26'49"S, 31°29'20"W, 1154 m, 1 May 2015, *L.P. Felix 15558* (EAN) [Fig. 18K].

\*\* $2n = 40$ , CHN. Brazil, Paraíba, Areia, Center of Agrarian Sciences, Federal University of Paraíba, 06°58'12"S, 35°42'47"W, 579 m, 2 Mar 2016, *L.P. Felix 15876* (EAN) [Fig. 18L].

*Croton triqueter* is widely distributed throughout South America, with records for Brazil, Bolivia, Peru and Venezuela (Macbride, 1951; Jørgensen & al., 2014). In Brazil, it occurs in the Caatinga, Atlantic Forest, Cerrado and Pantanal domains. The two chromosome numbers reported here,  $2n = 20$  and 40, are unprecedented for the species and compatible with chromosomal evolution by polyploidy for the genus *Croton*.

*Croton urticifolius* Lam.

$2n = 20$ , CHN. Brasil, Paraíba, Areia, Chã de Jardim, 06°57'50"S, 35°44'46"W, 641 m, 19 Nov 2014, *A.S. Santos 04* (EAN) [Figs. 18M, 19K].

*Croton urticifolius* can be found in Bolivia and Brazil. In Brazil, it occurs in the Northeast (except Maranhão) and Southeast (except São Paulo) regions, in the Caatinga, Cerrado and Atlantic Forest domains. The record of  $2n = 20$  for the species coincides with most chromosomal records of the genus and with the previous record for this species (Pôrto & al., 2014).

*Croton* is the second-largest genus of the Euphorbiaceae family and includes about 1100 species distributed throughout the tropical and subtropical regions of the American continent, Africa and Madagascar (Stevens, 2001; Judd & al., 2009). In Brazil, 300 species of the genus are found and 230 of them (84%) are endemic (Caruzo & al., 2020). They are mainly shrubs and subshrubs, or even trees and herbaceous plants, characterized by lactescent wood, glands at the leaf base, narrow inflorescence and unisexual flowers (Webster, 1993; Barry & al., 2005; Carneiro-Torres & al., 2011). The genus is poorly known karyologically, with chromosome numbers known for only 47 species (4.2% of the genus), with records of  $2n = 16$ , 18, 20, 28, 30, 38, 40, 64 and 120 (Pôrto & al., 2014; Rice & al., 2015). Most of these species (65%) have  $2n = 20$ , suggesting  $x = 10$  as the basic haploid number of the genus (Miller & Webster, 1966).

The cytogenetic analysis followed the protocol by Guerra & Souza (2002). Root tips were pretreated in 8-hydroxyquinoline for 24 h at 10°C, fixed in ethanol-acetic acid (3 : 1; v/v) for 2 h at room temperature and stored in a freezer at -20°C. To prepare the slides, the material was digested with an enzymatic solution containing 2% cellulase (Onozuka) and 20% pectinase (Sigma) (w/v) for 1 h at 37°C. Then, the slides were prepared by crushing method, in a drop of 45% acetic acid, and the coverslips were removed in liquid nitrogen. The slides were stained with 10 µl of DAPI (4',6-diamidino-

2-phenylindole – 2 µg/ml) for 30 min, and mounted in McIlvaine/glycerol buffer medium and subsequently analyzed and photographed in a Zeiss epifluorescence photomicroscope, with camera Axio Cam MRC5 video camera using Axiovision v.4.8 software (Carl Zeiss Microscopy, Jena, Germany).

Among the 19 *Croton* species analyzed here, 14 had  $2n = 20$ , while 5 had other chromosome numbers, with  $2n = 16$  for *C. glandulosus* and *C. hirtus*,  $2n = 18$  for *C. pedicellatus* and  $2n = 100$  for *C. floribundus* and *C. limae*. Unpublished chromosome counts are being registered for 10 species: *C. adamantinus*, *C. campestris*, *C. grewoides*, *C. lombardianus*, *C. rudolphianus*, *C. sellowii* and *C. tricolor*, all with  $2n = 20$ , in addition to *C. triqueter* ( $2n = 20$  and 40), *C. limae* and *C. floribundus* ( $2n = 100$ ). Intraspecific numerical variations were observed in *C. heliotropifolius* ( $2n = 20$  and 40), *C. pulegioides* ( $2n = 20$  and 22) and *C. triqueter* ( $2n = 20$  and 40). The predominance of records of  $2n = 20$  described in the present work corroborates the hypothesis of  $x = 10$  as the basic number of the genus and the predominance of the diploid level in most species of *Croton* (Miller & Webster, 1966). However, counts of  $2n = 20$  and 40 and  $2n = 100$  reveal the occurrence of intraspecific and interspecific polyploidy in the genus. On the other hand, the records of  $2n = 16$ , 18 and 22 reveal the occurrence of interspecific dysploidy, while  $2n = 20$ , 22 in *C. pulegioides* is an intraspecific dysploid variant.

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## IAPT chromosome data 40/11

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Pessoal de Nível Superior (CAPES; Process no. 12002011009P8/2021-03) for fellowships.

Methods for chromosome counts follow Guerra & Souza (2002).

\* First chromosome count for the species.

## FABACEAE

\**Aldina heterophylla* Spruce ex Benth.

$2n = 22$ , CHN. Brazil, Amazonas, Presidente Figueiredo, 02°00' 58"S, 59°54'26"W, 18 Feb 2016, *Haroldo C. de Lima 8201* (NYBG) [Fig. 20].

Fabaceae is the third-largest family within angiosperms, encompassing approximately 765 genera and 19,500 species (LPWG, 2017). Legume species hold significant economic and ecological importance (Bruneau, 2013; Zhao, 2021). Within the Amazonian context, this family assumes a crucial role in the nutrient-poor white-sand soils, renowned for their arid climatic conditions and diminutive forests (Mayle & Power, 2008; Flores & Holmgren, 2021).

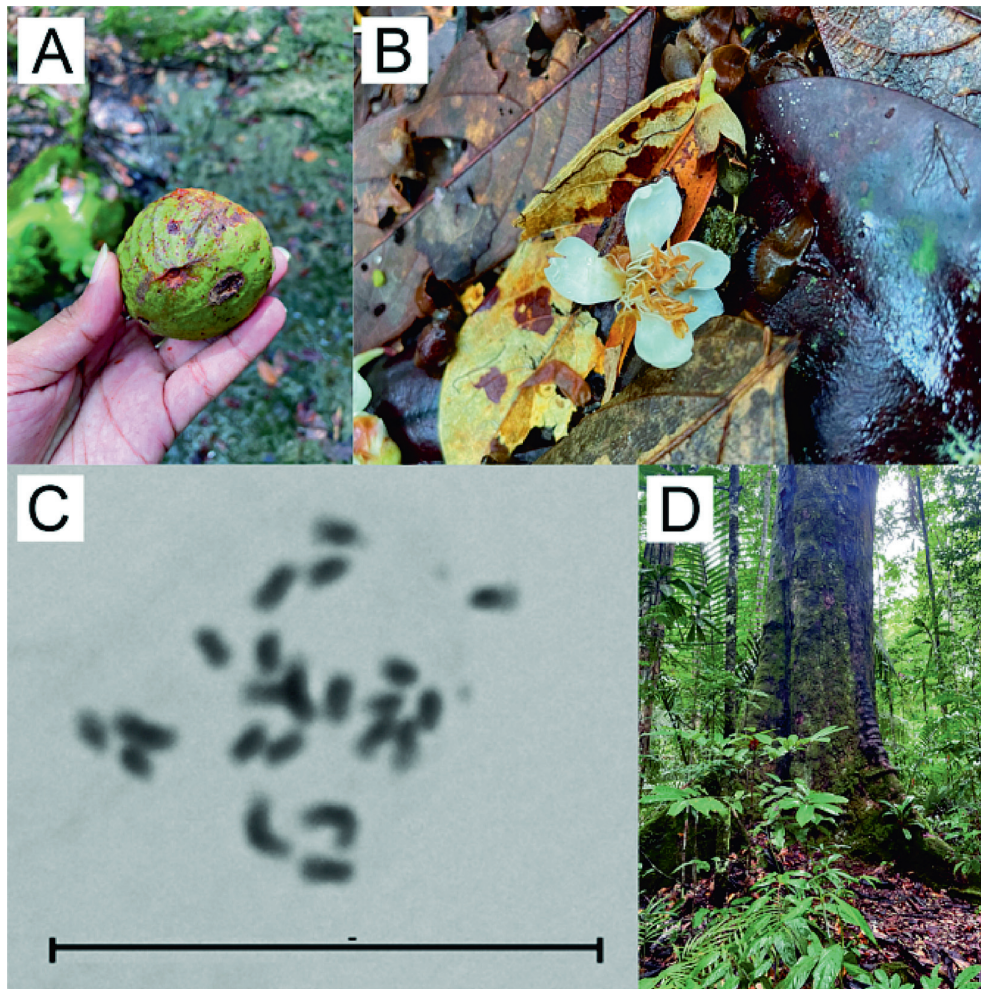
Among the most prevalent evolutionary lineages in these ecosystems is the genus *Aldina* Endl., comprising 20 species (Choi & al., 2022). In addition to their presence in the white-sand forests in western Amazon, *Aldina* species can also be found in other Amazonian vegetation formations, such as in both flooded and non-flooded ecosystems (Junk & al., 2011). Traditionally, *Aldina* has been categorized within the Swartzieae tribe, owing to its radial flowers that showcase an intact calyx and numerous stamens (Ramos & al., 2016). Nevertheless, recent phylogenetic analyses employing chloroplast markers have recently repositioned *Aldina* within the Andira clade, situated in the Dalbergieae tribe (Cardoso & al., 2015; Ramos & al., 2016; Choi & al., 2022).

Taxonomic, morphological, and ecological studies concerning the genus have been expanding (Ramos & al., 2016). However, the chromosome numbers in this genus have not been investigated until now. Thus, our count for an Amazonian species of *Aldina*, *A. heterophylla*, which revealed the number of  $2n = 22$  also marks the first chromosomal record for the genus. Intriguingly, this count aligns with that of the sister group, the genus *Andira* Lam. Notably, two species within *Andira*, *A. humilis* Mart. ex Benth. ( $2n = 22$ ) (Forni-Martins & al., 1995), and *A. inermis* (W.Wright) Kunth ex DC. ( $2n = 22$ ) (Bawa, 1973), present the same chromosomal number.

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**Fig. 20.** *Aldina heterophylla*: A, Habitat; B, Flower; C, Fruit; D, Metaphase,  $2n = 22$ . — Scale bar = 20  $\mu\text{m}$ . Photos: Adryene Mota de Menezes.

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#### IAPT chromosome data 40/12

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\* First chromosome count for the species.

## VOCHYSIACEAE

*Qualea grandiflora* Mart.

$2n = 22$ ,  $n = 11$ , CHN. Brazil, Mato Grosso State, Federal University of Rondonópolis, 16°27'46.56"S, 54°34'49.40"W, 19 Jan 2019, G.L.S. Silveira & al. s.n. (UFMT 44309, UFMT 44308); 5 Feb 2018, L.A.S. Nogueira & al. s.n. (UFMT 43790, UFMT 43793, UFMT 44307, UFMT 43791), 30 Apr 2018, L.A.S. Nogueira & al. s.n. (UFMT 43807).

*Qualea multiflora* Mart.

$n = 11$ , CHN. Brazil, Mato Grosso State, Federal University of Rondonópolis, 16°27'46.56"S, 54°34'49.40"W, 30 Apr 2018, L.A.S. Nogueira & al. s.n. (UFMT 43806).

*Qualea parviflora* Mart.

$2n = 22$ ,  $n = 11$ , CHN. Brazil, Mato Grosso State, Federal University of Rondonópolis, 16°27'46.56"S, 54°34'49.40"W, 4 Oct 2016, L.A.S. Nogueira & al. s.n. (UFMT 43792); 20 Jul 2018, G.L.S. Silveira & al. s.n. (UFMT 44042); 5 Feb 2018, L.A.S. Nogueira & al. s.n. (UFMT 43789).

*Vochysia cinnamomea* Pohl

$n = 12$ , CHN. Brazil, Mato Grosso State, Federal University of Rondonópolis, 16°27'46.56"S, 54°34'49.40"W, 25 Apr 2018, L.A.S. Nogueira & al. s.n. (UFMT 43809), 30 Apr 2018, L.A.S. Nogueira & al. s.n. (UFMT 43808, UFMT 43805).

\**Vochysia divergens* Pohl

$2n = 24$ , CHN. Brazil, Mato Grosso State, Federal University of Rondonópolis, 16°27'46.56"S, 54°34'49.40"W, 18 Jul 2018, G.L.S. Silveira & al. s.n. (UFMT 44043), 23 Jul 2018, G.L.S. Silveira & al. s.n. (UFMT 44044, UFMT 44045).

Vochysiaceae A.St.-Hil. is a family composed of trees and shrubs, included in the order Myrtales as a sister group of Myrtaceae

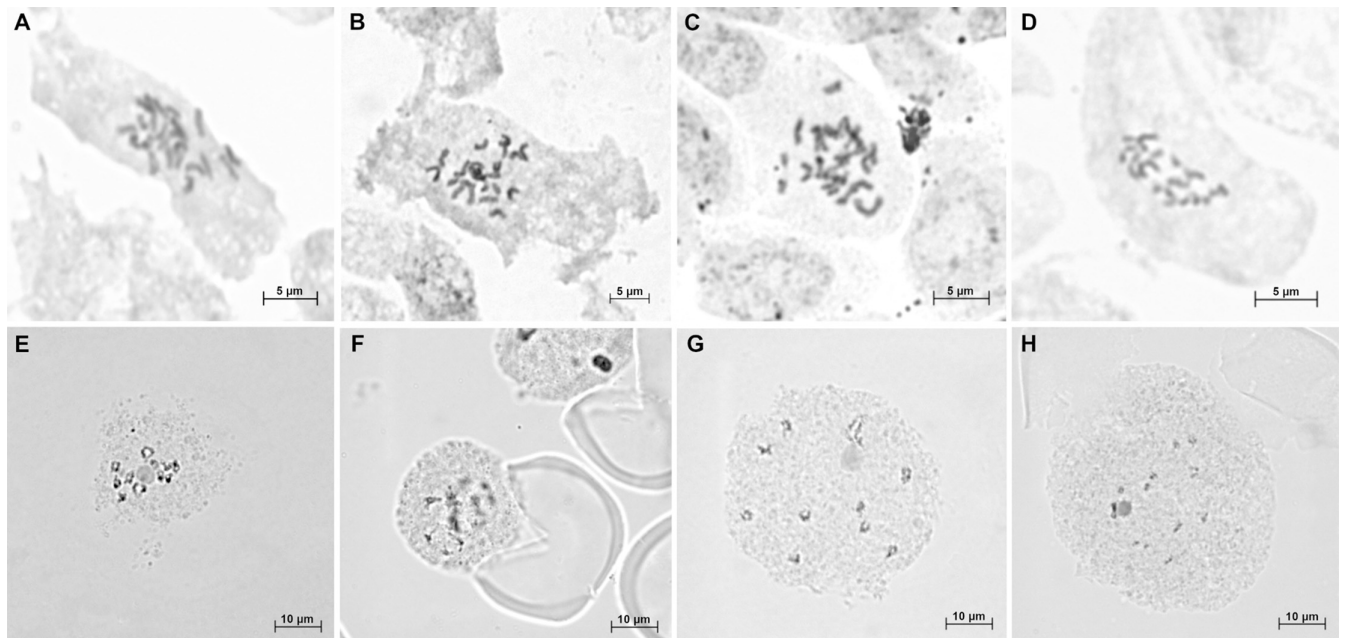
Juss. (Berger & al., 2016; APG IV, 2016), a clade with strong Gondwanan representation for most of its families. Although previous phylogenetic studies greatly improved our understanding of intergeneric and interspecific relationships within the order, our understanding of interfamilial relationships still remains unresolved; hence, we also lack a robust time-calibrated chronogram to address hypotheses (e.g., biogeography and diversification rates). In Brazil, Vochysiaceae comprises nearly 160 species, distributed in six genera (*Callisthene* Mart., *Erismia* Rudge, *Qualea* Aubl., *Ruizterania* (Stafleu) Marc.-Berti, *Salvertia* A.St.-Hil., *Vochysia* Aubl.) (Carmo-Oliveira & Morretes, 2009; Azevedo & al., 2015; Shimizu & Gonçalves, 2017; Shimizu & al., 2019).

Pioneering works on chromosome numbers in Vochysiaceae include Goldblatt (1979, *Vochysia lomatophylla* Standl.,  $2n = 22$ ), Berry (1987, *Vochysia venezuelana* Stafleu,  $2n = 24$ ), and Barbosa (1999, *Vochysia cinnamomea* and *Vochysia tucanorum* Mart.,  $2n = 24$ ). While the most recent study was conducted by Yamagishi-Costa & al. (2018), including samples of the genera *Callisthene*, *Qualea*, *Salvertia* and *Vochysia*, from Goiás, Mato Grosso, Minas Gerais and São Paulo states, resulting in  $2n = 22$  and  $n = 11$  for *Callisthene* and *Qualea*, and  $2n = 24$  and  $n = 12$  for *Salvertia* and *Vochysia*.

Although Vochysiaceae is well represented in Brazil, being easily found in floristic inventories, there are few reports on chromosome numbers for species occurring in the Cerrado domain. Therefore, this study was carried out to fill this gap and contribute to the knowledge of native Cerrado species.

To our knowledge this is the first report on the chromosome number of *Vochysia divergens*, which is a relevant information for taxonomic and evolutionary studies.

In the present research *Qualea grandiflora* (Fig. 21A,B) and *Q. parviflora* (Fig. 21C) presented 22 chromosomes, and *Vochysia divergens* presented 24 chromosomes (Fig. 21D) (Table 3). The meiotic analysis revealed 11 bivalents in *Qualea grandiflora* (Fig. 21E), *Q. multiflora* (Fig. 21F) and *Q. parviflora* (Fig. 21G) and 12 bivalents



**Fig. 21.** Mitotic and meiotic cells of Vochysiaceae species. A–D, C-metaphases: A & B, *Qualea grandiflora*; C, *Qualea parviflora*; D, *Vochysia divergens*. E–H, Meiotic cells: E, *Qualea grandiflora* (diakinesis); F, *Qualea multiflora* (diplotene); G, *Qualea parviflora* (diakinesis); H, *Vochysia cinnamomea* (diakinesis). — Image capturing performed in light microscope, with 100× magnification.



in *Vochysia cinnamomea* (Fig. 21H) (Table 3). For *Qualea multiflora* and *Vochysia cinnamomea*, the mitotic analysis was not performed due to unavailable fruits and seeds or due to lack of germination under the experimental conditions, and in *V. divergens*, the meiotic cells did not allow the counting of bivalents.

Thus, for the *Qualea* species, the chromosome number is  $2n = 22$ , with  $n = 11$ , and for the *Vochysia* species, the chromosome number is  $2n = 24$  and  $n = 12$ .

The present study confirms the results found by previous authors, indicating that the chromosome numbers are stable in *Qualea* and *Vochysia* species, and suggests that the chromosome evolution in terms of numeric variation is conserved in these genera, as previously stated by Yamagishi-Costa & al. (2018). Furthermore, to our knowledge this study was the first survey of Vochysiaceae in the region, contributes to the genetic knowledge on species native to Brazil and provides additional information on species occurring in the Cerrado domain, which demonstrates the importance of these analyses for taxonomic and evolutionary studies.

### MATERIALS AND METHODS

Plant samples of Vochysiaceae species were collected between the years of 2017 and 2019 in a Cerrado sensu stricto fragment, located in the Universidade Federal de Rondonópolis, municipality of Rondonópolis, Mato Grosso State, Brazil. The voucher specimens were registered and incorporated into the collection of the Universidade Federal de Mato Grosso Herbarium (UFMT Herbarium, Process 02000.002662/2003-09, MMA-SEPRO) (Table 3).

The fruits and seeds sampled were stored in paper bags at  $-10^{\circ}\text{C}$ , while the inflorescences were fixed in Falcon with methanol and propionic acid solution (3 : 1) and stored at  $-4^{\circ}\text{C}$ .

The seed germination took place in a BOD incubator under a temperature of  $27^{\circ}\text{C}$  and photoperiod of 12 h, until the emergence of roots (15–45 days). The root tips were collected and pretreated with 2 mM 8-hydroxyquinoline for 4 h, washed in distilled water, fixed in ethanol and acetic acid solution (3 : 1) and stored at  $-4^{\circ}\text{C}$ . After a minimum of 24 h, the fixative solution was replaced by 70% ethanol.

Slides for the mitotic analysis were prepared according to the squash technique (Belling, 1921), using Schiff's reagent for 1 h and 30 min. The slides were evaluated in a light microscope (Carl Zeiss, Axio Lab A1) coupled with a microcamera (AxioCam ERc 5s). Images of C-metaphases were captured using the AxioVision software.

For the meiotic analysis, slides were prepared with anthers from fixed young inflorescences according to Fukui & Nakayama (1996), in 60% acetic acid, 2% Triton X-100 and 1% carmine-propionic acid staining. The slides were analyzed in light microscope (Carl Zeiss,

Axio Lab A1 or Nikon Eclipse E200) coupled with a microcamera (AxioCam ERc 5s). Representative images were captured using the AxioVision software. Some images were captured using a cellphone camera (Samsung Galaxy J7 Prime), an adaptor (Pereira, 2019) and a micrometric slide for reference.

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**Table 3.** Registration numbers and chromosome numbers ( $n$ ,  $2n$ ) of the sampled Vochysiaceae species.

Species	Registration numbers	$n$	$2n$
<i>Qualea grandiflora</i> Mart.	UFMT 44309; UFMT 44308; UFMT 43790; UFMT 43793; UFMT 43807; UFMT 44307; UFMT 43791	11	22
<i>Qualea multiflora</i> Mart.	UFMT 43806	11	–
<i>Qualea parviflora</i> Mart.	UFMT 43792; UFMT 44042; UFMT 43789	11	22
<i>Vochysia cinnamomea</i> Pohl	UFMT 43809; UFMT 43808; UFMT 43805	12	–
<i>Vochysia divergens</i> Pohl	UFMT 44043; UFMT 44044; UFMT 44045	–	24



Yamagishi-Costa, J., Barbosa, A.R., Shimizu, G.H., Yamamoto, K. & Forni-Martins, E.R. 2018. Chromosome numbers and the systematics of tribe Vochysieae (Vochysiaceae). *Acta Bot. Bras.* 32: 314–320. <https://doi.org/10.1590/0102-33062017abb0354>

## IAPT chromosome data 40/13

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\* New chromosome number (cytotype) for the species.

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

### IRIDACEAE

#### Subfamily: Iridoideae

#### Tribe: Tigridiaceae

\*\**Herbertia darwinii* Roitman & J.A.Castillo

$2n = 14$ , CHN. Brazil, Rio Grande do Sul, Alegrete, 29°08'51.0" S, 56°01'49.7"W, 131 m, 2 Nov 2021, *A. Tonetto-Vieira & L. Noogueira* 5 (ICN).

\*\**Herbertia* aff. *lahue* (Molina) Goldblatt

$2n = 14$ ; Brazil, Rio Grande do Sul, São Jerônimo do Sul, 30°07'43.03"S, 51°51'20.38"W, 38 m, 8 Nov 2018, *E. Kaltchuk, E.M. Stiehl-Alves & A. Vieira* 5 (ICN); Brazil, Rio Grande do Sul, São Jerônimo do Sul, 30°10'48.40"S, 51°23'33.29"W, 38 m, 16 Nov 2018, *E. Kaltchuk, E.M. Stiehl-Alves & A. Vieira* 17 (ICN); Brazil, Rio Grande do Sul, Canguçu, 31°17'00.6"S, 53°02'60.0"W, 376 m, 8 Nov 2019, *E.M. Stiehl-Alves, A. Tonetto-Vieira, L. Carvalho & I. Hedlund* 137 (ICN); Brazil, Rio Grande do Sul, Canguçu, 30°41'37.0"S, 53°23'35.0"W, 376 m, 9 Nov 2019, *E.M. Stiehl-Alves, A. Tonetto-Vieira, L. Carvalho & I. Hedlund* 145 (ICN); Brazil, Rio Grande do Sul, Santana da Boa Vista, 30°45'20.3"S, 53°09'15.2"W, 190 m, 10 Nov 2019, *E.M. Stiehl-Alves, A. Tonetto-Vieira, L. Carvalho & I. Hedlund* 162 (ICN); Brazil, Rio Grande do Sul, Lavras do Sul, 30°46'06.1"S, 53°55'26.8"W, 418 m, 11 Nov 2019, *E.M. Stiehl-Alves, A. Tonetto-Vieira, L. Carvalho & I. Hedlund* 171 (ICN); Brazil, Rio Grande do Sul, Caçapava do Sul, 30°41'34"S, 53°23'32"W, 26 Oct 2018, *A.M. Cristante, E.M. Stiehl-Alves, A. Tonetto-Vieira & R. Becker* 1 (ICN); Brazil, Rio Grande do Sul, Caçapava do Sul, 30°53'42"S, 53°25'48"W, 27 Oct 2018, *A.M. Cristante, E.M. Stiehl-Alves,*

*A. Tonetto-Vieira & R. Becker* 2 (ICN); Brazil, Rio Grande do Sul, Caçapava do Sul, 30°47'58"S, 53°11'23"W, 3 Dec 2017, *A.M. Cristante, E.M. Stiehl-Alves & R.M. Marchioreto* 12 (ICN) [Fig. 22A].

\*\**Herbertia lahue* (Molina) Goldblatt

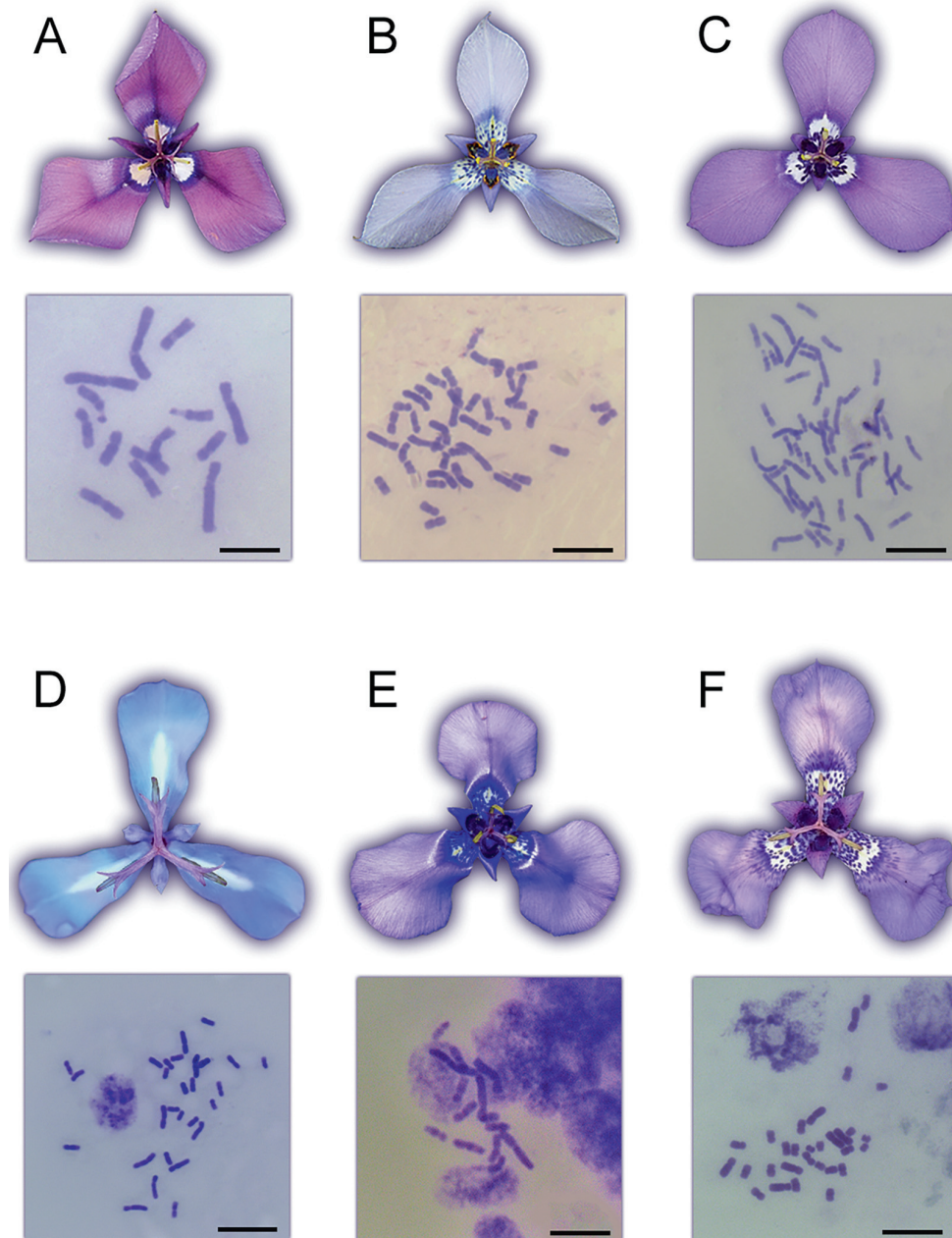
$2n = 42$ , CHN. Brazil, Rio Grande do Sul, São Jerônimo do Sul, 30°07'43.03"S, 51°51'20.38"W, 38 m, 8 Nov 2018, *E. Kaltchuk, E.M. Stiehl-Alves & A. Vieira* 6 (ICN); Brazil, Rio Grande do Sul, Santo Antonio das Missões, 28°32'42.17"S, 54°33'19.39"W, 284 m, 23 Oct 2019, *E.M. Stiehl-Alves, A. Tonetto-Vieira, L. Carvalho & A. Mello* 105 (ICN); Brazil, Rio Grande do Sul, Santo Antonio das Missões, 28°33'44.4"S, 55°09'37.3"W, 284 m, 24 Oct 2019, *E.M. Stiehl-Alves, A. Tonetto-Vieira, L. Carvalho & A. Mello* 110 (ICN); Brazil, Rio Grande do Sul, Itaqui, 29°00'22.7"S, 56°03'38.7"W, 105 m, 25 Oct 2019, *E.M. Stiehl-Alves, A. Tonetto-Vieira, L. Carvalho & A. Mello* 117 (ICN); Brazil, Rio Grande do Sul, Lavras do Sul, 30°46'06.1"S, 53°55'26.8"W, 418 m, 11 Nov 2019, *E.M. Stiehl-Alves, A. Tonetto-Vieira, L. Carvalho & I. Hedlund* 172 (ICN) [Fig. 22B].

\*\**Herbertia lahue* (Molina) Goldblatt

$2n = 56$ , CHN. Brazil, Rio Grande do Sul, São Miguel das Missões, 28°32'42.24"S, 54°33'18.93"W, 291 m, 23 Oct 2019, *E.M. Stiehl-Alves, A. Tonetto-Vieira, L. Carvalho & A. Mello* 104 (ICN); Brazil, Rio Grande do Sul, São Jerônimo do Sul, 30°08'55.32"S, 51°54'19.94"W, 38 m, 8 Nov 2018, *E. Kaltchuk, E.M. Stiehl-Alves & A. Vieira* 3 (ICN); Brazil, Rio Grande do Sul, Santo Antonio das Missões, 28°33'44.4"S, 55°09'37.3"W, 284 m, 24 Oct 2019, *E.M. Stiehl-Alves, A. Tonetto-Vieira, L. Carvalho & A. Mello* 111 (ICN); Brazil, Rio Grande do Sul, Itaqui, 28°59'50.6"S, 56°05'50.00"W, 105 m, 25 Oct 2019, *E.M. Stiehl-Alves, A. Tonetto-Vieira, L. Carvalho & A. Mello* 115 (ICN); Brazil, Rio Grande do Sul, Alegrete, 29°50'50.7"S, 55°52'12.2"W, 195 m, 26 Oct 2019, *E.M. Stiehl-Alves, A. Tonetto-Vieira, L. Carvalho & A. Mello* 123 (ICN); Brazil, Rio Grande do Sul, Cristal, 30°54'35.4"S, 52°04'22.4"W, 93 m, 7 Nov 2019, *E.M. Stiehl-Alves, A. Tonetto-Vieira, L. Carvalho & I. Hedlund* 129 (ICN); Brazil, Rio Grande do Sul, Canguçu, 31°21'44.1"S, 52°48'25.8"W, 8 Nov 2019, 423 m, *E.M. Stiehl-Alves, A. Tonetto-Vieira, L. Carvalho & I. Hedlund* 134 (ICN); Brazil, Rio Grande do Sul, Caçapava do Sul, 30°49'58.80" S, 53°30'14.40"W, 255 m, 9 Nov 2019, *E.M. Stiehl-Alves, A. Tonetto-Vieira, L. Carvalho & I. Hedlund* 148 (ICN); Brazil, Rio Grande do Sul, Santana da Boa Vista, 30°45'20.3"S, 53°09'15.2" W, 190 m, 10 Nov 2019, *E.M. Stiehl-Alves, A. Tonetto-Vieira, L. Carvalho & I. Hedlund* 161 (ICN) [Fig. 22C].

\*\**Herbertia pulchella* Sweet

$2n = 28$ ; CHN. Brazil, Rio Grande do Sul, Canguçu, 31°21'48.0"S, 52°44'48.0"W, 376 m, 8 Nov 2019, *E.M. Stiehl-Alves, A. Tonetto-Vieira, L. Carvalho & I. Hedlund* 133 (ICN); Brazil, Rio Grande do Sul, Canguçu, 31°16'49.0"S, 52°42'04.0"W, 376 m, 8 Nov 2019, *E.M. Stiehl-Alves, A. Tonetto-Vieira, L. Carvalho & I. Hedlund* 143 (ICN); Brazil, Rio Grande do Sul, Caçapava do Sul, 30°49'58.80"S, 53°30'14.40"W, 255 m, 9 Nov 2019, *E.M. Stiehl-Alves, A. Tonetto-Vieira, L. Carvalho & I. Hedlund* 150 (ICN); Brazil, Rio Grande do Sul, Santana da Boa Vista, 30°47'58.02"S, 53°11'23.96"W, 190 m, 10 Nov 2019, *E.M. Stiehl-Alves, A. Tonetto-Vieira, L. Carvalho & I. Hedlund* 165 (ICN); Brazil, Rio Grande do Sul, Santana do Livramento, 30°46'40.70"S, 53°08'27.7"W, 26 Nov 2019, *A. Tonetto-Vieira, L. Eggers & O. Chauveau* 7 (ICN) [Fig. 22D].



**Fig. 22.** Photomicrographs of mitotic metaphases with their respective representative flowers of *Herbertia* species, stained with Giemsa, all at the same scale. **A**, *H. aff. lahue* ( $2n = 14$ ); **B**, *H. lahue* ( $2n = 42$ ); **C**, *H. lahue* ( $2n = 56$ ); **D**, *H. pulchella* ( $2n = 28$ ); **E**, *H. aff. quareimana* ( $2n = 14$ ); **F**, *H. quareimana* ( $2n = 28$ ). — Scale bars = 5  $\mu\text{m}$ .

*\*Herbertia aff. quareimana* Ravenna

$2n = 14$ , CHN. Brazil, Rio Grande do Sul, São Nicolau, 28°12' 29.52"S, 55°19'13.80"W, 95 m, 23 Oct 2019, *E.M. Stiehl-Alves, C. Forgiarini & F.S. Silveira 51* (ICN); Brazil, Rio Grande do Sul, São Miguel das Missões, 28°32'44.04"S, 54°33'14.01"W, 284 m, 23 Oct 2019, *E.M. Stiehl-Alves, A. Tonetto-Vieira, L. Carvalho & A. Mello 106* (ICN); Brazil, Rio Grande do Sul, Santo Antonio das Missões, 28°31'24.01"S, 55°14'2.11"W, 24 Oct 2019, *E.M. Stiehl-Alves, A. Tonetto-Vieira, L. Carvalho & A. Mello 109* (ICN) [Fig. 22E].

*\*\*Herbertia quareimana* Ravenna

$2n = 28$ , CHN. Brazil, Rio Grande do Sul, Santana do Livramento, 30°47'16.1"S, 55°38'02.8"W, 6 Nov 2012, *L. Eggers, O. Chauveau & T. Pastori 1103* (ICN); Brazil, Rio Grande do Sul,

Uruguaiana, 29°53'22.2"S, 57°07'16.5"W, 5 Nov 2012, *L. Eggers, O. Chauveau & T. Pastori 733A* (ICN); Brazil, Rio Grande do Sul, Alegrete, 29°51'27.6"S, 55°53'06.6"W, 140 m, 26 Oct 2019, *E.M. Stiehl-Alves, A. Tonetto-Vieira, L. Carvalho & A. Mello 125* (ICN); Brazil, Rio Grande do Sul, Alegrete, 29°59'02.79"S, 55°58'31.6"W, 195 m, 26 Oct 2019, *E.M. Stiehl-Alves, A. Tonetto-Vieira, L. Carvalho & A. Mello 126* (ICN) [Fig. 22F].

*Herbertia* Sweet is a small genus (eight species) belonging to the Clade A of Tigridaeae (Chauveau & al., 2012), that comprises perennial, herbaceous and bulbous geophytic plants, generally with violet flowers (Goldblatt & Manning, 2008). *Herbertia* species show great vegetative similarity, but some have high floral polymorphism. Although floral characters have been used as diagnostics within the genus, tenuous limits of interspecific differentiation and a large

intraspecific polymorphism result in taxonomic uncertainties (Chauveau & al., 2012; Stiehl-Alves & al., 2016, 2017). In this sense, karyotypic characters of *Herbertia* need to be investigated, since they have also been useful for elucidating the relationships of various taxa being potentially informative in Iridaceae (Goldblatt & Takei, 1997).

According to literature, Clade A has the base chromosome number  $x = 7$  and ploidy series in several species (Moraes & al., 2015). Six of the eight recognized *Herbertia* species have chromosome counts with four ploidy levels reported:  $2x$ ,  $4x$ ,  $6x$  and  $8x$ . Intraspecific polyploid series have been found for *H. lahue* and *H. pulchella* (Winge, 1959; Kenton & Heywood, 1984; Goldblatt & Takei, 1997; Roitman & Castillo, 2004; Moreno & al., 2009; Moraes & al., 2015).

*Herbertia* occurs naturally in grasslands in the south of the Neotropical region (Goldblatt & Manning, 2008), with seven species endemic to the northeast of the Pampean province. The Pampean province (hereafter Pampas) is part of the Chacoan biogeographical domain (Morrone, 2017) and is a species-rich ecosystem characterized by the predominance of grassland vegetation, with Poaceae, Asteraceae, Fabaceae, and Cyperaceae as the most dominant families (Andrade & al., 2019).

Efforts have been made by our team to delineate a clearer picture of the patterns of geographic distribution and evolutionary relationships of *Herbertia* species and thus understand the impact of polyploidy on the genus' evolution. Therefore, cytogenetic data for *Herbertia* have been obtained for several populations throughout the distribution area. For chromosome counting, root tips were pre-treated with 8-hydroxyquinoline for 24 h at  $8^{\circ}\text{C}$  and fixed with 3 : 1 ethanol : glacial acetic acid. Slides were prepared according to Moraes & al. (2015) and Alencar & al. (2018). Chromosomes obtained for the *Herbertia* populations in the present study are represented by the image of the flower of each *Herbertia* species (Fig. 22A–F).

In the present study, chromosome numbers are presented for 38 populations of four *Herbertia* species: *H. darwinii*, *H. lahue*, *H. pulchella* and *H. quareimana*. Chromosome numbers have been described in the literature for the four species investigated; however, such data are relatively old and, moreover, taxonomic changes occurred in the genus. Furthermore, the chromosome counts are restricted to a few points of occurrence, most of them outside Brazil. In view of the existence of intraspecific cytotypes in the genus, evaluation of a greater number of populations is necessary.

*Herbertia darwinii* is an endemic species of the Pampas distributed along the triple border of Brazil, Argentina, and Uruguay, in an area of less than 40,000 km<sup>2</sup>. The chromosome number  $2n = 14$  found here for one population of *H. darwinii* is the same as reported previously in samples from another four populations of this species (Roitman & Castillo, 2004; Moreno & al., 2009; Moraes & al., 2015). So far, only diploid plants have been found for this species, even in populations located outermost of its geographic distribution.

*Herbertia lahue* is the only species with a wide distribution, occurring throughout the Pampas and on the borders with Chaco province (Chacoan dominion), Araucaria Forest province (Paraná dominion), and Atacama province (part of South American transition zone). There are also records of populations of *H. lahue* in the southern U.S.A., Australia, and Japan, but these seem to represent recent introductions (Goldblatt & Manning, 2008). Twenty-three populations of *H. lahue* sampled in southern Brazil were investigated. The cytotype ( $2n = 2x = 14$ ) was found in samples from nine populations named *H. aff. lahue*. Other cytotypes previously reported for *H. lahue* were also observed in the present study, and hexaploids ( $2n = 6x = 42$ ) and octoploids ( $2n = 8x = 56$ ) were recorded

respectively in five and nine populations. The chromosome number  $2n = 42$  was previously reported in samples from Argentina (Moreno & al., 2009), Brazil (Moraes & al., 2015), Chile (Kenton & Heywood, 1984; Goldblatt & Takei, 1997), and Uruguay (Moreno & al., 2009), whereas the chromosome number  $2n = 56$  was previously reported in populations from Argentina (Moreno & al., 2009) and Brazil (Moraes & al., 2015). The spatial distribution of *H. lahue* populations in the Pampas is complex, ranging from mixed populations, where the three ploidy levels are found in sympatry, as well as parapatric and allopatric distributed populations (Stiehl-Alves & al., 2016; Martins & al., 2020).

*Herbertia pulchella* is a species that is distributed in an area of about 100,000 km<sup>2</sup> located in southern Brazil and Uruguay, in the Pampas. In this study, seven populations of *H. pulchella* were studied and a chromosome number  $2n = 28$  was recorded for all samples. Chromosome number  $2n = 28$  was also described by Moraes & al. (2015) in samples from four populations from Rio Grande do Sul (Brazil). Older studies (Winge, 1956; Kenton & Heywood, 1984; Goldblatt & Takei, 1997) reported other chromosome numbers for this species ( $2n = 14$ ,  $2n = 42$ ), which has motivated us to expand the sampling of this species to search for populations unequivocally identified as *H. pulchella*. Despite the effort, only tetraploid plants have been registered.

*Herbertia quareimana* is a species endemic to the Pampas, near the border of Brazil with Argentina and Uruguay, covering an area of about 48,000 km<sup>2</sup>. Seven populations of *H. quareimana* sampled in Rio Grande do Sul (Brazil) were investigated here, and three of them presented the chromosome number  $2n = 2x = 14$ . This is the first report of a diploid cytotype for *H. quareimana*. The remaining four populations had  $2n = 4x = 28$ . Tetraploid *H. quareimana* was also reported for other Brazilian populations by Ravenna (1989) and Moraes & al. (2015), as well as for populations located in Argentina and Uruguay (Moreno & al., 2009). Geographic data obtained through collection expeditions carried out by our time since 2006 indicate that diploid and tetraploid populations are geographically isolated from each other.

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