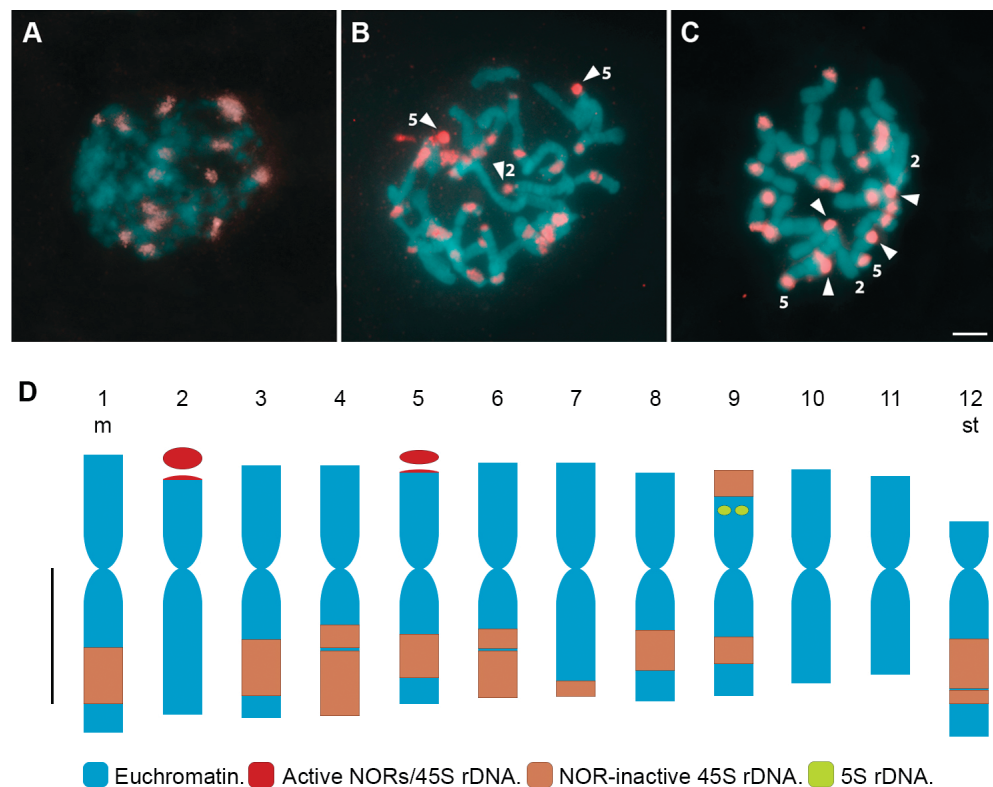


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**Fig. 4.** Cytological characterization of the wild chili pepper *Capsicum flexuosum* by means of FISH. **A**, DAPI stained interphase nucleus (blue) showing widespread 18S-25S rDNA blocks (red signals) clustered at one side. **B & C**, DAPI stained  $2n = 24$  prometaphase and metaphase chromosomes (blue), respectively, subjected to FISH with 18S-25S rDNA probe; note the widespread distribution of rDNA signals (red) mainly at the intercalary regions of chromosomes. **D**, Ideogram; note that each chromosome of the complement can be identified by morphological and/or rDNA markers; position of 5S rDNA follows Aguilera & al. (2016). — Arrowheads and numbers point out to chromosome pairs carrying NORs. Scale bars = 5  $\mu$ m.



This contribution belongs to the series “Cytogenetic characterization of the germplasm of wild chili peppers: *Capsicum flexuosum*”. This study was supported by the Agencia Nacional de Promoción Científica y Tecnológica (ANPCyT-Argentina), UNaM PICT 2014-3328, préstamo BID N° AR-L 1181.

#### SOLANACEAE

*Capsicum flexuosum* Sendtn.

$2n = 24$ , CHN. Argentina, Misiones province, Guaraní department, 20 km from the detour on national route no. 14, in direction to the Predio Guaraní, 27.0° S, 54.2° W, 15 May 2004, G.E. Barboza, F. Chiarini & E. Marini 1034 (CORD) [Fig. 4A–D].

Type, size and distribution of ribosomal loci (rDNA) in interphase nuclei added to prometaphase and metaphase chromosomes were revealed by fluorescent in situ hybridization (FISH) using a *Capsicum* derived 18S-25S (45S) rDNA probe (Grabiele, 2010) and according to the protocol of Moscone & al. (1996a). Fluorochrome staining with DAPI (4'-6-diamidino-2-phenylindole) subsequent to FISH in order to reveal nuclei and chromosome morphology was performed according to Moscone & al. (1996a). Somatic chromosome preparations and the procedure for measurements of chromosomes and their landmarks are described in Moscone & al. (1996b). Fifteen metaphase plates were analyzed and five of them were included for measurements. Asymmetry indexes:  $A_1$  and  $A_2$  (Romero Zarco, 1986);  $r > 2$  and R (Stebbins, 1971);  $i$  (centromeric mean). Abbreviations: *m*, metacentric; *st*, subtelocentric.

*Capsicum flexuosum* is a wild chili pepper native to Brazil, occurring at south (Paraná, Rio Grande do Sul, Santa Catarina) and southeast regions of this country (Minas Gerais, São Paulo), restricted to the phytogeographic domain of Mata Atlântica, but also present in Paraguay and NE Argentina at Corrientes and Misiones provinces (Zuloaga & Morrone, 1999; Stehmann & al., 2016). The species grows as a shrub (0.5–2 m), with white stellate flowers presenting greenish spots in the throat, and spherical depressed red hot fruits (Moscone & al., 2007).

This diploid taxon based on  $x = 12$  displays medium-sized to large chromosomes, with lengths ranging from 7.10 ( $m$ ) to 10.26  $\mu\text{m}$  ( $m$ ), a mean of 8.58  $\mu\text{m}$  and 103.00  $\mu\text{m}$  per haploid genome. The karyotype, 11  $m + 1 st$ , is unimodal ( $A_2 = 0.10$ ;  $R = 1.45$ ) and symmetrical ( $A_1 = 0.25$ ;  $r > 2 = 0.08$ ;  $i = 42.14$ ) and belongs to the category 2A of Stebbins. Pairs number 2 ( $m$ ) and 5 ( $m$ ), which carry the active nucleolar organizer regions (NORs), display a terminal macrosatellite in their short arms (Fig. 4B–D).

FISH of 18S-25S rDNA to interphase nuclei of *C. flexuosum* revealed an extensive number of ribosomal blocks (12–15) of different size and clustered at one side of the nucleus, as usual in *Capsicum* (Fig. 4A). In addition, the prometaphase and metaphase 18S-25S rDNA FISH pattern of this taxon is consistent with ten chromosome pairs that embrace thirty ribosomal signals of different size. Twelve signals were observed at terminal regions (p2, 5, 9; q4, 6, 7) and eighteen at intercalary positions of large arms (q1, 3, 4, 5, 6, 8, 9, 12) (Fig. 4B, C). The smallest chromosome pairs, nos. 10 ( $m$ ) and 11 ( $m$ ), are deprived of rDNA loci (Fig. 4B–D). Furthermore, four of those 18S-25S rDNA FISH signals occurred at the expected active NOR chromosome pairs nos. 2 and 5, respectively (Fig. 4B, C). Supplementary 18S-25S rDNA loci are NOR-inactive and actually correspond to the typical CMA enhanced (CMA+) highly GC-rich constitutive heterochromatin found in *C. flexuosum* and throughout *Capsicum* (Grabiele, 2010; Scaldaferrò & al., 2013; Grabiele & al., unpub.). The 18S-25S ribosomal fraction comprises 19.18  $\mu\text{m}$  (18.62%) of the haploid genome of *C. flexuosum* and the ratio of euchromatin to rDNA in this taxon is 4.37:1.

The cytological characterization of the wild hot chili pepper *C. flexuosum* performed here by means of a *Capsicum*-derived 18S-25S rDNA FISH probe resulted in a highly detailed chromosomal map for this taxon. For the first time each *C. flexuosum* chromosome can be further recognized via different markers, either morphological and/or related to rDNA (Fig. 4D).

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Mitotic chromosomes were examined in root tips of seedlings. Method is described in Smirnov (1968). Chromosome numbers in literature were checked using IPCN (Goldblatt & Johnson, 1979+).

The study was financially supported by the Russian Foundation for Basic Research (grants 14-04-01415-a and 15-34-20513 mol\_a\_ved), D.I. Mendeleev Scientific Fund Program of Tomsk State University, National Natural Science Foundation of China (grant 31270269), and the Youth Innovation Promotion Association Foundation of CAS.

\* First chromosome count for species

#### APIACEAE

*Eryngium campestre* L.

$2n = 14$ , CHN. France, Region Midi-Pyrénées, Department Tarn, 1 km S of Escoussens, dry meadow, 43°29'N, 02°12'E, 26 Sep 2015, A. Erst & I. Kuzmin AEK 15 (NS) [Fig. 5C].

#### ASTERACEAE

*Dittrichia graveolens* (L.) Greuter

$2n = 18$ , CHN. France, Region Midi-Pyrénées, Department Haute-Garonne, Toulouse, near Cité de l'espace, waste places, 43°35'N, 01°29'E, 23 Oct 2015, A. Erst & I. Kuzmin AEK 255 (NS).

*Erigeron canadensis* L.

$2n = 18$ , CHN. France, Region Midi-Pyrénées, Department Haute-Garonne, Toulouse, Balma, small landfill site, 43°35'N, 01°29'E, 16 Oct 2015, A. Erst & I. Kuzmin AEK 191 (NS).

*Inula conyzae* (Griess.) Meikle

$2n = 32$ , CHN. France, Region Midi-Pyrénées, Department Tarn, 2 km W of Labruguière, 43°32'N, 02°13'E, 4 Oct 2015, A. Erst & I. Kuzmin AEK 098 (NS) [Fig. 5D].

*Senecio inaequidens* DC.

$2n = 40$ , CHN. France, Region Midi-Pyrénées, Department Haute-Garonne, Toulouse, Balma, small landfill site, 43°35'N, 01°29'E, 16 Oct 2015, A. Erst & I. Kuzmin AEK 187 (NS).

#### CARYOPHYLLACEAE

*Silene vulgaris* (Moench) Garcke

$2n = 24$ , CHN. France, Region Midi-Pyrénées, Department Tarn, 2 km W of Labruguière, roadside thickets of grass, 43°32'N, 02°13'E, 18 Oct 2015, A. Erst & I. Kuzmin AEK 226 (NS).

*Spergula rupicola* (Lebel) G.López

$2n = 36$ , CHN. France, Region Bretagne, Department Ille-et-Vilaine, Saint-Malo, ruderal plants community on the coast of the English Channel, 48°39'N, 02°01'E, 25 Oct 2015, A. Erst & I. Kuzmin AEK 268 (NS).