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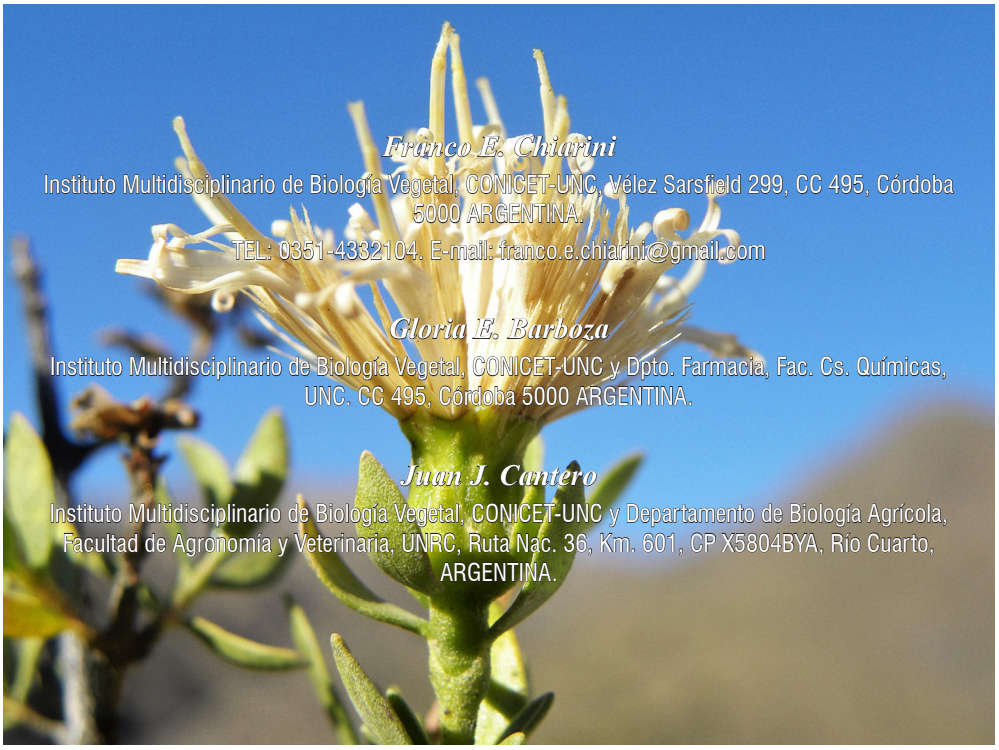
The chromosomes of the rare and endemic genus *Famatinanthus* (Famatinanthoideae, Asteraceae)

Los cromosomas del raro y endémico género *Famatinanthus* (Famatinanthoideae, Asteraceae)



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Abstract

Classical staining and *in situ* fluorescent hybridization with probes for the 18–5.8–26S and 5S genes were performed in *Famatinathus decussatus* (endemic from Argentina), in order to know its main chromosomal characteristics and to compare them to related genera. The species is a paleopolyploid with 2n = 54, likely originated from x=9, and its karyotype features are conservative: one pair of 5S signals and two of 18–5.8–26S per complement were found. All the data are congruent with the basal position of this species in the Asteraceae phylogenies.

Keywords: Chromosomes, endemism, *Famatinanthus decussatus*, polyploidy, r*DNA*.

Resumen

Los cromosomas del raro y endémico género *Famatinanthus*. Se aplicaron tinción convencional e hibridación *in situ* fluorescente con sondas para los genes de 18–5.8–26S y 5S en *Famatinathus decussatus* (endémica de Argentina), con el fin de conocer su características cromosómicas principales y compararlas con géneros relacionados. La especie es un paleopoliploide con 2n = 54, probablemente originado a partir de x=9; sus caracteres cariotípicos son conservados: se encontraron un par de señales de 5S por complemento y dos de 18–5.8–26S. Los datos son congruentes con la posición basal que esta especie ocupa en las filogenias de Asteraceae.

Palabras clave: Cromosomas, endemismo, *Famatinanthus decussatus*, poliploidía, r*DNA*.

Introducción

Famatinanthus Ariza & S. E. Freire is a recently described genus of Asteraceae endemic to a restricted area in the province of La Rioja, Argentina (Freire *et al*., 2014). Its single species, *F. decussatus* (Hieron.) Ariza & S. E. Freire, was formerly placed within *Aphyllocladus* Wedd. However, analyses of floral characters (i.e. corollas, anthers, style, achenes), stem anatomy, trichomes and pollen (Freire *et al*., 2014), indicate that the species has a combination of features that do not correspond with the circumscription of *Aphyllocladus* or any other genus within the Mutisioideae tribe. Consequently, a new genus was proposed to accommodate it. The singularities of *F. decussatus* not only involve morpho-anatomical features; in fact, in a molecular phylogenetic study using 14 chloroplast DNA loci, Panero *et al*. (2014) found that the recently named genus has the two chloroplast inversions present in all Asteraceae except the nine genera of the subfamily Barnadesioideae and concluded that it is sister to the Mutisioideae–Asteroideae clade that represents more than 99% of Asteraceae. These characteristics made *Famatinanthus* to diserve a subfamily only for itself. Considering this background it is important to know the chromosomal characteristics of the species.

From the cytological point of view, chromosome counts provide a key tool in studies of systematics, phylogeny and evolution, and they are especially useful for understanding speciation and hybridization (Stebbins, 1971). Structural and quantitative characteristics of karyotypes have been significant in evolutionary and taxonomic studies in many angiosperm groups (Stebbins, 1971), being decisive in establishing linkage groups and natural classifications. (e.g. Weiss-Schneeweiss *et al*., 2003; Pellicer *et al*., 2010). The FISH procedure allows homologous chromosomes in a complement to be differentiated and permits the comparison among related species (Heslop-Harrison, 2000; Garcia *et al*., 2007; Leitch *et al*., 2008). The procedure also provides information

on genome organization and allows chromosomal evolutionary questions to be addressed (Chacón *et al*., 2012; Pellicer *et al*., 2010; Chiarini *et al*., 2014). The most common molecular-cytogenetic markers are ribosomal genes (5S and 18–5.8–26S rDNA), which are abundant and highly conserved in all higher plant species (Schmidt & Heslop-Harrison, 1998). Variations in the number, signal intensity and position of rDNA loci seem to be common in several plant groups (e.g. Datson & Murray, 2006; Urdampilleta *et al*., 2013), suggesting their mobility. Among related species, the number and location of rDNA loci may be conserved or vary considerably among populations with different ploidy levels (e.g. Adachi *et al*., 1997; Lan *et al*., 2011). Considering this background, the aim of our work was to describe the basic cytogenetic features of *Famatinathus*, together with the patterns of 18–5.8–26S and 5S genes, in order to compare them to related genera.

Materials and Methods

Seeds were bulked from plants in several natural populations in **La Rioja province**, Famatina Dpt. (S28º52'00.9'' W67º41'28.1'', *G. Barboza et al. 4268*; S28º50'28'' W67º40'56.3'', *G. Barboza et al. 4270*; S28º39'58.2'' W67º42'05.5'', *G. Barboza et al. 4271*). Voucher specimens are deposited at the Herbarium of the National University of Córdoba (CORD).

Mitotic chromosomes were examined in root tips obtained from seeds germinated in Petri dishes. Root tips were pre-treated in saturated p-dichlorobenzene in water for 2 h at room temperature, fixed in 3:1 ethanol/ acetic acid, washed in distilled water, digested with PECTINEX ® (45 min at 37 °C), and squashed in a drop of 45% acetic acid. After coverslip removal in liquid nitrogen, the slides were air dried and stored at –20 °C. Some of these slides were used for classical staining with Giemsa. The reamining stored slides were used for determining the location and number of rDNA sites by FISH. One of the probes was the pTa71 containing the 18-5.8-26S rDNA (Gerlach & Bedbrook, 1979) labeled with biotin-14-dATP (BioNick; Invitrogen, Carlsbad, USA). For the 5S rDNA, a probe was obtained from the genome of *Prionopsis ciliata* by PCR (Moreno *et al*., 2012) labeled with digoxigenin-11-dUTP (Roche Diagnostics). The FISH procedure was in accordance with Schwarzacher & Heslop-Harrison (2000), with minor modifications. The preparations were incubated in 100 lg/ml RNase, post-fixed in 4 % (w/v) paraformaldehyde, dehydrated in a 70–100% graded ethanol series, and air-dried. On each slide 15 µ1 of hybridization mixture was added (3 ng/µl of probe, 100% formamide, 50% dextran sulfate, 20 x SSC and 10% SDS), previously denatured at 70°C for 10 min. Chromosome denaturation/ hybridization was done at 90°C for 10 min, 48°C for 10 min, and 38°C for 5 min using a thermal cycler (Mastercycler, Eppendorf, Hamburg, Germany), and slides were placed in a humid chamber at 37°C overnight. The probes were detected with avidin-FITC conjugate and antidigoxigenin-rodamine conjugate and counterstained and mounted with 25 µl antifade (Vectashield Vector Lab., Burlingame, USA), containing 2ng/µl of DAPI.

Results

Seeds resulted inviable in most collected capitula. Only accession G.B. 4271 showed a good germination rate, thus the analyzed cells were all from this sample and they presented 2n = 54 (Fig. 1). Chromosomes are small compared to related genera of Asteraceae with 2n = 54, being the average length c = 1.75 ± 0.21 µm and the total

haploid lenght of LT = 94.05 ± 2.95 µm (Table 1). The chromosomes are homogeneous in size, with a ratio between the largest and the shortest of the complement ca. 1.87. Notable secondary constrictions were detected in two chromosome pairs in most of the metaphases (Fig. 1A). Centromeres were difficult to visualize, and the high number of chromosomes hindered identifying the homologue pairs.

FISH technique with 18-5.8-26S probe evidenced four loci in terminal position, coinciding with the secondary constrictions visualized with the conventional staining, while the 5S probe showed two terminal signals per cell (Fig. 1B), located in asynteny respect to the chromosomes bearing 18-5.8-26S.

Discussion

Chromosome number. As well as most basal Asteraceae, *Famatinanthus* resulted to be a high polyploid with a hypothetical basic number x = 9. Polyploids with chromosome numbers derived from x = 9 have been reported for Barnadesioideae (Wulff, 1990), Mutisioideae (Ward, 1983; Waisman *et al*., 1984; Grau, 1987) and Stifftioideae (Gibbs & Ingram, 1982) (Table 1). In fact, this number has been pointed out as basic for the entire family, which is congruent with the basal position of *Famatinanthus* within Asteraceae (Panero *et al*., 2014). Semple & Watanabe (2009) hypothesized that multiple downward dysploid events from polyploids based on x = 9 account for nearly all the base numbers reported for Mutisieae, the numbers x = 8 to 32 would be the result of a long dysploid series from x2 = 27. In this sense, *Famatinanthus* is remarkable by conserving the paleopolyploid number.

Recurrent polyploidy. Polyploidy is important for many aspects described in several review articles (e.g. Soltis *et al*., 2003; Hegarty & Hiscock, 2008; Leitch & Leitch, 2008; Van de Peer *et al*., 2009). Autopolyploidy is a common phenomenon and it is regarded as frequent in angiosperms (Ramsey & Schemske, 1998). Within Asteraceae, Barker *et al*. (2008) revealed at least three ancient whole-genome duplications: a first one (shared by Mutisioideae, Carduoideae, Cichorioideae and Helianthoideae) placed near the origin of the family just prior to the rapid radiation of its tribes, and two independent genome duplications near the base of the tribes Mutisieae and Heliantheae. We can speculate that the genome duplication near the base of Mutisieae also affected *Famatinanthus* since they are sister clades. Thus, *Famatinanthus* would be an ancient polyploid.

Chromosome size. There is a phenomenon called genome downsizing whereby polyploids do not have a genome size exactly equal to the multiple of their diploid progenitors but which is somewhat smaller, as a result of a removal of redundant DNA (Leitch & Bennett, 2004). *Famatinanthus* has small chromosomes compared to hexaploid members of Barnadesiodeae and Mutisioideae (Table 1). Only species of Doniophyton have c and LT values minor than *F. decussatus*. Genome size is related to the size of single chromosomes (e.g. Garnatje *et al*., 2004), and therefore the relatively small size of the chromosomes of *F. decussatus* would be due to the longer time that the species have had from its polyploidization to rearrange its genome and eliminate redundant elements.

rDNA sites. Regarding Asteraceae species related to *Famatinanthus*, FISH experiments with 18-5.8-26S probe were conducted only in the Mutisioideae species

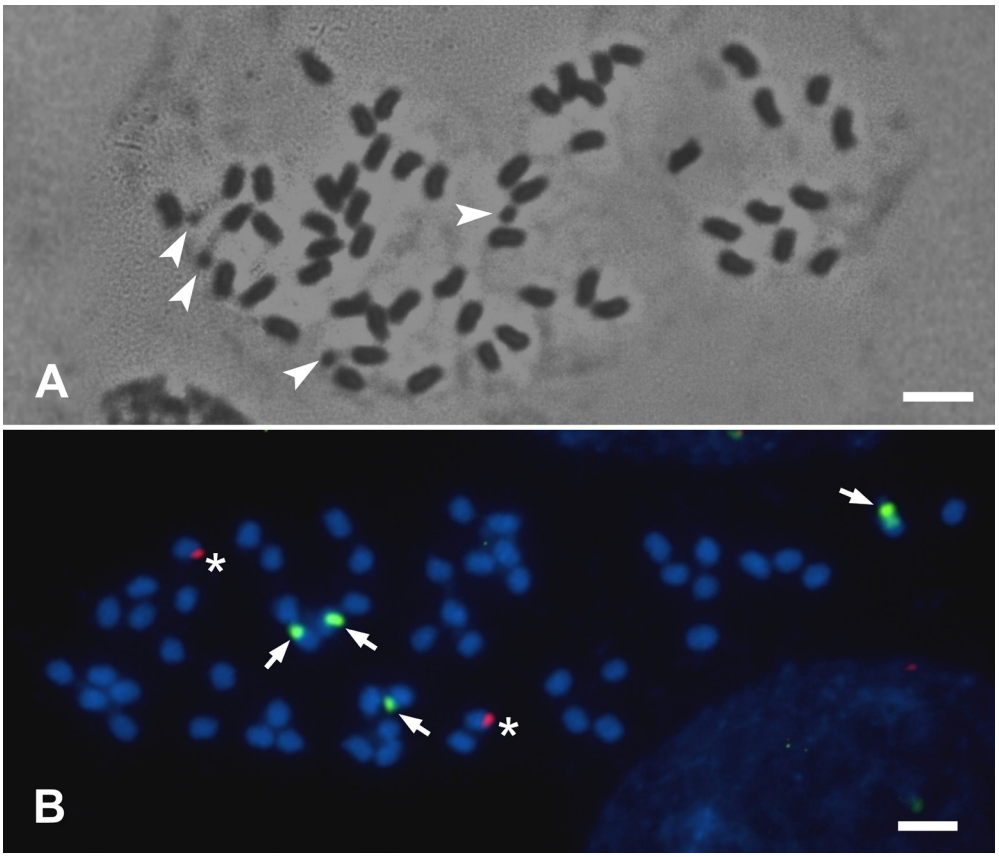

Fig. 1. Metaphasic chromosomes of *Famatinanthus decussatus* with (A) Giemsa staining and (B) FISH technique. Arrowheads point to NORs, arrows point to 18-5.8-26S loci (green signals) and asterisks indicate 5S loci (red signals). Bars represent 3 μm.

Chaptalia nutans, 2n = 50, 100 (Fregonezi *et al*., 2004) with two and four pairs of signals per complement, respectively, and also in two species of *Chaetanthera* (Baeza *et al*., 2005a), where 1 and 3 pairs of signals in diploids with 2n = 22 were found. Regarding the 5S, data in species more or less related to *Famatinanthus* are known only for *Chaetanthera* (Baeza *et al*., 2005a) in which 3 and 4 pairs of 5S signal in diploid species with 2n = 22 have been found. This situation evidenced the different pathways that the rDNA loci took since the two lineages split from each other. The great variability in amount and position of rDNA loci in related species is a fact already known (Hasterok *et al*., 2006; Heslop-Harrison & Schwarzacher, 2011; Chacón *et al*., 2012; Morales *et al*., 2012). For these rapid changes of copy number and chromosomal location of rDNA, different types of transposable elements have been postulated as responsible (Raskina *et al*., 2004a, b; Belyayev *et al*., 2005; Altinkut *et al*., 2006; Datson & Murray, 2006).

In higher eukaryotes, the 18-5.8-26S rDNA and 5S rDNA loci are transcribed by different RNA polymerases and

usually located in different positions of chromosomes (Srivastava & Schlessinger, 1991). Colocalization of 18-5.8-26S and 5S rDNA loci has commonly been reported in animals (Dobigny *et al*., 2003) but it is less frequent in plants (e.g. Garcia *et al*., 2007, 2009b; Abd El-Twab & Kondo, 2006; Chang *et al*., 2009). A dominant linked rDNA genotype was found within three large groups of Asteraceae (García *et al*., 2010): Anthemideae, Gnaphalieae and in the "Heliantheae alliance" (Asteroideae). The remaining five tribes of the Asteroideae displayed canonical non linked arrangement of rDNA, and also the remaining 12 subfamilies, with separate organisation. *Famatinanthus* fits perfectly into this scheme, with the 5S and 18-5.8-26S loci in separate chromosomes. The results of García *et al*. (2010) indicate that nearly 25% of Asteraceae species may have developed unusual linked arrangement of rRNA genes, and the 5S gene integration within the 35S unit might have repeatedly occurred during plant evolution, and probably once in Asteraceae.

Genome downsizing, a phenomenon referred before, also affects rDNA genes. During the polyploidization process, gene reordering and gene silencing may occur (Stebbins, 1985; Soltis *et al*., 2003; Leitch & Bennett, 2004; Pires *et al*., 2004). In Solanaceae, for example, an analysis of the 18-5.8-26S rDNA of Nicotiana indicated that parental loci were initially maintained in newly formed polyploids, although the sequences within a locus might be subject to concerted evolution, and over periods greater than 1 myr, individual loci would disappear (Kovařík *et al*., 2008). In Asteraceae, a similar situation was found in Tragopogon (Buggs *et al*., 2009; Chester *et al*., 2012).

Given that Famatinanthus is a hexaploid, more than a pair of 5S signals per complement could be expected. Instead, only a pair was found, which would be an indication of an ancient polyploidy. It has been found that the number of loci of 5S is subject to dynamic, rapid, changes (cfr. sup.). Loss of gene copies might be taking place at these loci, as demonstrated in members of other several plant families (i.e. Kotseruba et al. 2003; Renny-Byfield *et al*., 2012). A similar situation was observed in polyploid species in other Asteraceae genera such as Brachyscome (Asteroideae, Adachi *et al*., 1997), Artemisia (Asteroideae, Garcia *et al*., 2009a; Pellicer *et al*., 2013), Xeranthemum (Carduoideae, Garnatje *et al*., 2004), Tragopogon (Cichorioideae, Buggs *et al*., 2009; Chester *et al*., 2012), in which the number of 18–5.8–26S sites seemed to evolve faster than the 5S sites, as they do not increase with the successive genome additions. By constrast, genome sizes of some polyploid taxa present additivity, although data pointing to genome upsizing in older polyploids are limited (Garcia *et al*., 2009; Leitch *et al*., 2008).

FISH studies with rDNA probes would be desirable in genera more closely related to Famatinanthus (e.g. Barnadesia, Duseniella, Dasyphyllum) in order to achieve a more complete picture of chromosomal evolution in basal Asteraceae.

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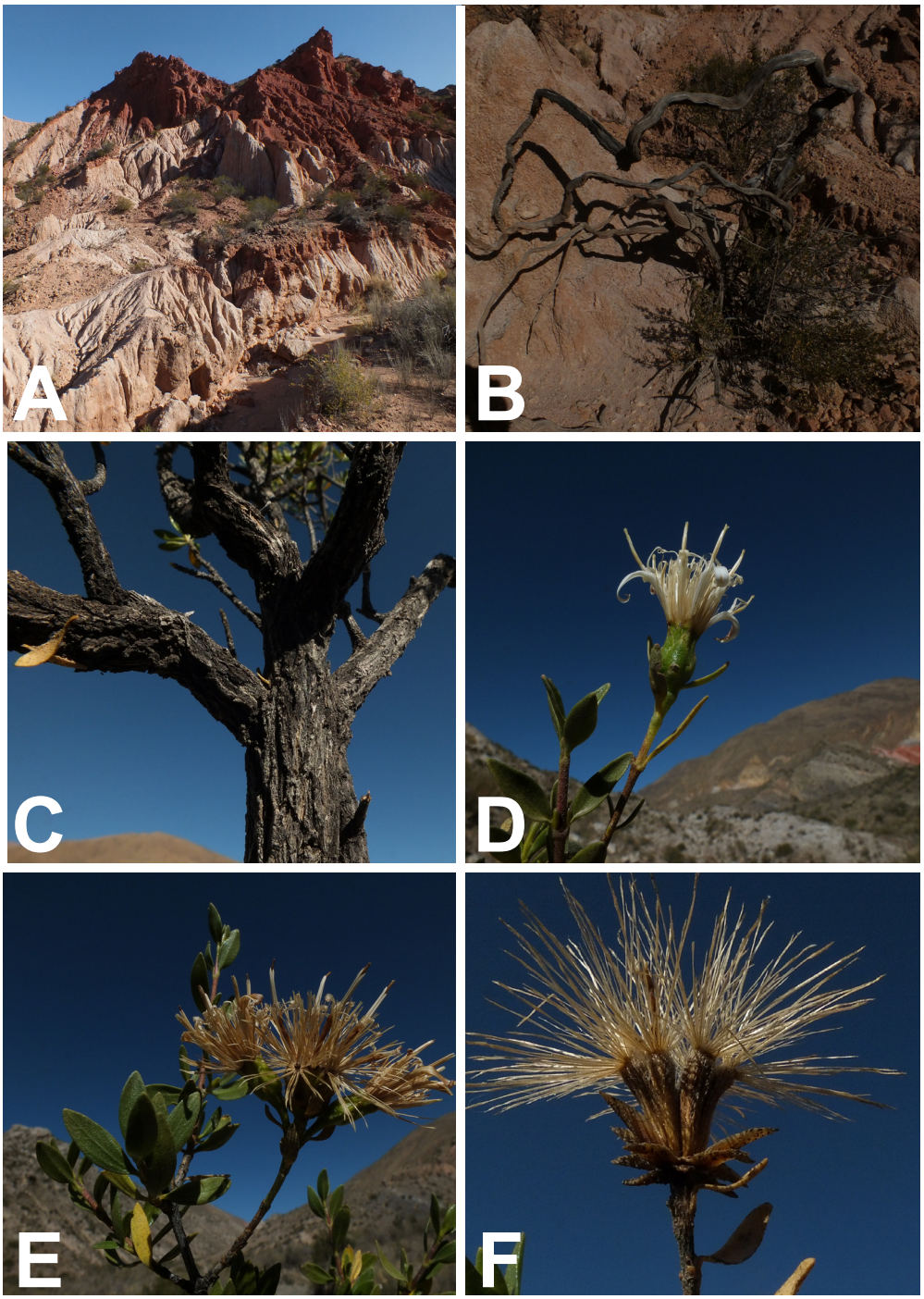

Fig. 2. *Famatinanthus decussatus*. A. Hábitat; B. Planta mostrando las raíces; C. Tallo; D. Capítulo; E. Capitulecencia; F. Cipselas. (Fotografías *G. Barboza, J. Cantero; R. Deanna & S. Leiva 4270*, CORD, HAO).