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Niche conservatism, divergence and polyploidy in *Senna* series *Aphyllae* (Fabaceae: Caesalpinioideae) from arid zones of South America

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This paper aims to integrate cytogenetic and ecological approaches into a phylogenetic framework to better understand the evolution and systematics of *Senna* series *Aphyllae*, an endemic group of arid and semi-arid regions of South America. We counted the chromosome numbers at the population level in three species of series *Aphyllae* using a conventional technique (GIEMSA) and *in situ* hybridization (FISH). Phylogenetic reconstructions and inference of chromosome evolution were based on four plastid DNA regions and one nuclear marker. A principal component analysis was carried out using environmental data. Finally, ecological niche models (ENM) were implemented: (1) to identify the abiotic factors that constraint the potential geographical distribution of series *Aphyllae* and (2) to understand the ecological drivers for species diversification. The basic chromosome number is $x = 12$. *Senna aphylla* showed three cytotypes with ($2n = 24$, 48 and 60) and *S. pachyrrhiza* has two cytotypes ($2n = 24$ and 48). *Senna crassiramea* appeared to be polyploid with $2n = 48$. In diploid *S. pachyrrhiza*, two 45S and three 5S rDNA sites were reported. In tetraploid *S. crassiramea*, four 45S and four 5S rDNA sites were detected. The ENM and ecological speciation tests suggest that in series *Aphyllae* speciation was sometimes associated with conservatism and sometimes with niche divergence. Our results contribute to the evolutionary knowledge of Neotropical legumes and understanding of drivers of diversification in arid regions in South America.

ADDITIONAL KEYWORDS: cytogenetics – ecological niche – phylogenetics – polyploidy.

INTRODUCTION

Speciation can occur as a result of changes associated with chromosomes, either by rearrangements of chromosomal material or changes in the number of chromosomes (Levin, 2002). Polyploidization is a mechanism that increases the number of chromosomes. It is a common and recurrent phenomenon among plants, and can promote speciation through reproductive isolation (Wood *et al.*, 2009). Moreover, polyploidization potentially offers individuals new ecological adaptations that allow them to expand the range of environmental tolerance compared with their

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diploid progenitors (Levin, 2002; Wood *et al.*, 2009; Te Beest *et al.*, 2012; Shimizu-Inatsugi *et al.*, 2017).

In Fabaceae, there are many polyploid members in different subfamilies (Stai *et al.*, 2019). Among the members of subfamily Caesalpinioideae, *Senna* Mill. includes polyploid taxa belonging to different clades, such as representatives of series *Subverrucosae* (Benth.) H.S.Irwin & Barbeny with 2*n* = 24, 56 (Randell, 1970), series *Interglandulosae* (Benth.) H.S.Irwin & Barneby [*S. surattensis* (Burm.f.) H.S.Irwin & Barneby, (2*n* = 28, 56) and *S. aversiflora* (Herb.) H.S.Irwin & Barneby (2*n* = 56); George & Bhavanandan, 1994; Matos *et al.*, 2011] and series *Bacillares* (Benth.) H.S.Irwin & Barneby [*S. rugosa* (G.Don) H.S.Irwin & Barneby (2*n* = 28–112) and *S. gardneri* (Benth.) H.S.Irwin & Barneby (2*n* = 52, 104); Biondo, Miotto & Schifino-Wittmann, 2005; Matos *et al.*, 2011; Resende *et al.*, 2014]. According to Irwin & Turner (1960) and Randell (1970), series *Subverrucosae* is the taxonomic group exhibits the greatest frequency of polyploidy in *Senna*.

Series *Aphyllae* (Benth.) H.S.Irwin & Barneby form a clade in *Senna*. The first phylogenetic studies showed that *Aphyllae* was separated into two clades (Robbiati *et al.*, 2017a). Clade A groups *Senna aphylla* (Cav.) H.S.Irwin & Barneby var. *aphylla*, *S. crassiramea* (Benth.) H.S.Irwin & Barneby, *S. rigidicaulis* (L.Bravo) H.S.Irwin & Barneby and *S. spiniflora* (Burkart) H.S.Irwin & Barneby. Clade B is formed by *S. aphylla* var. *divaricata* (Hieron.) Robbiati & Fortunato and var. *pendula* Robbiati & Fortunato, *S. acanthoclada* (Griseb.) H.S.Irwin & Barneby and *S. pachyrrhiza* (L.Bravo) H.S.Irwin & Barneby (Marazzi & Sanderson, 2010; Robbiati *et al.*, 2017a). Later, a new classification for *Senna* series *Aphyllae* based on distribution, morphology and phylogenetic evidence was proposed, recognizing five species and two varieties: *S. acanthoclada*, *S. aphylla*, *S. crassiramea*, *S. nudicaulis*, *S. pachyrrhiza* var. *pachyrrhiza*, *S. pachyrrhiza* var. *pendula* (Robbiati & Fortunato) Robbiati & Fortunato and *S. spiniflora* (Robbiati *et al.*, 2019).

Members of series *Aphyllae* are xeromorphic shrubs and subshrubs adapted to extreme climatic conditions. The leaves of adult branches are minute triangular or sublobate scales and the stem is junciform, green and photosynthetic; they inhabit rocky and sandy soils of arid and semi-arid regions of South America in the Monte and Prepuna and the Chaco subregion (Chaco biogeographic province). Several species of series *Aphyllae* show high morphological variation and the specific and varietal delimitation is sometimes difficult, mainly in geographical regions where two or more taxon are in sympatry (Robbiati, Anton & Fortunato, 2014a, Robbiati *et al.*, 2017b). Like series *Aphyllae* in South America, members of series *Subverrucosae* living in arid areas of Australia are morphologically adapted to extreme habitats and the taxonomic delimitation is difficult since they display appreciable morphological variation. Besides, polyploidization and hybridization at specific and infraspecific levels have been recognized as one cause of this morphological variability (Randell, 1970, 1989; Holman & Playford, 2000). Considering the available information on series *Subverrucosae* in Australia, we hypothesized that the high morphological variation exhibited by series *Aphyllae* in South America is a consequence of the existence of polyploid species.

Regarding speciation in series *Aphyllae*, dating and ancestral area reconstruction analyses based on molecular data suggest that the *Aphyllae* clade originated and started to diversify in the South American Transition Zone in the Late Pliocene, during the last period of Andean uplift with the aridification of South America (Robbiati *et al.*, 2017a). During this period, new ecological niches were available as a result of the Andean uplift (von Hagen & Kadereit, 2003; Bell & Donoghue, 2005; Palma, Marquet & Boric-Bargetto, 2005; Hughes & Eastwood, 2006; Pirie *et al.*, 2006; Ribas *et al.*, 2007; Antonelli *et al.*, 2009; Guerrero *et al.*, 2013). Previous investigations (Robbiati *et al.*, 2017b) revealed an association between climatic variables and part of the morphological variation in series *Aphyllae*. This might suggest that a proportion of the morphological variability in vegetative and reproductive characters represents phenotypic variation and adaptation to arid zones in mid-latitude South America. Hence, we hypothesized that the evolution of species in series *Aphyllae* was driven by ecological divergence.

The present study aimed to improve the understanding of the evolutionary mechanisms operating in series *Aphyllae* integrating cytogenetic and ecological approaches. First, we described and compared species in series *Aphyllae* based on chromosome number and size, morphology and rDNA distribution. Second, we analysed niche overlap between the species to test whether speciation was associated with or responded to evolutionary history (niche conservatism). The findings are discussed in a phylogenetic framework to help in understanding if species divergence in series *Aphyllae* was associated with polyploidization events and niche divergence, and to clarify the role of change in chromosome number in *Senna* diversification.

MATERIAL AND METHODS

PLANT SPECIES

Field studies were conducted between 2010 and 2015 to collect specimens and samples for chromosome and cytogenetic studies. For each taxon between one and eight populations were analysed. The species and populations are listed in Table 1. Vouchers were deposited at BAB and CORD herbaria (http://sweetgum.nybg.org/science/ih/). Despite repeated efforts to optimize germination conditions, we were unable to obtain cytological preparations for *S. acanthoclada*, *S. nudicaulis* and *S. spiniflora*.

CHROMOSOME NUMBERS, SIZE AND MORPHOLOGY

Chromosome preparations were made from root tips pre-treated with 2-mm 8-hydroxyquinoline for 4–8 h at 14 °C, fixed in ethanol–acetic acid (3:1, v/v) for 12 h, and stored at −20 °C or followed by the HCl/Giemsa staining procedure (Guerra, 1983). Meristems were isolated and squashed in a drop of 45% acetic acid. After coverslip removal for freezing, the material was stained with 2% Giemsa. To analyse chromosome morphology and total chromosome length, we selected the best mitotic metaphases that showed similar chromosome condensation (five to ten cells per individual, and two to four individuals per taxon) and measured with ImageJ software (v.1.47; NIH, USA). We used the chromosome nomenclature of Levan, Fredga & Sandberg (1964): 'm' for metacentric and 'sm' for submetacentric chromosomes.

FLUORESCENCE *IN SITU* HYBRIDIZATION

The root tips were digested with 4% cellulase plus 40% pectinase (37 °C for 2–3 h) and squashed in 45% acetic acid. Preparations were frozen in liquid nitrogen to remove the coverslip. FISH was carried out following the method of Heslop-Harrison (1991) and Cuadrado & Jouve (1994), with modifications (Vanzela *et al.*, 2002). The pTa71 probe containing the 18-5.8-26S rDNA sequence isolated from wheat (Gerlach & Bedbrook, 1979) was used to locate the 45S rDNA chromosome sites. The probe was labelled with biotin-14–dATP by nick translation (Invitrogen Life Technologies, San Diego, USA). To identify sites 5S rDNA, the DNA fragments used as probes were obtained by PCR using specific primers (Röser *et al.*, 2001), labelled with digoxigenin (DIG Nick translation mix, Roche Diagnostics, Mannheim, Germany). Preparations were incubated in 100 µg mL^{-1} RNase, post-fixed in 4% (w/v) paraformaldehyde, dehydrated in a 70–100% graded ethanol series, and air-dried. To each slide, 15 µL of hybridization mixture was added (4–6 ng/µL of the labelled probe, 50% formamide, 10% dextran sulphate, 2×SSC and 0.3% SDS), previously denatured at 75 °C for 10 min. Chromosome denaturation/hybridization was performed 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 placed in a humid chamber at 37 °C overnight. Post-hybridization washes were carried out in 2×SSC, 0.1×SSC, 2×SSC and 4×SSC/0.2% Tween 20, all at 42 °C. The 18-5.8-26S probe was detected with avidin-FITC conjugate (Sigma-Aldrich, Saint Louis, MO, USA), the 5S probe was detected with antidigoxigenin-rhodamine (Roche) and then counterstained and mounted with 25 µL of antifadeVectashield (Vector Laboratory, Peterborough, UK), containing 1.5 g/mL DAPI. Chromosome preparations were observed and photographed with a BX61 microscope (Olympus, Waltham, USA) coupled with a monochromatic camera and a digital documentation system Cytovision (Leica Biosystems, Buffalo Grove, USA).

GEOGRAPHICAL DISTRIBUTION OF CYTOTYPES

The distribution map of taxa was constructed using QGIS 'Geographic Information System' v.3.4.12 (QGIS Development Team, 2018). Selected environmental variables (see the Ecological Niche Modelling section) were used in a principal component analysis (PCA) using INFOSTAT 2.0 (Di Rienzo *et al.*, 2012) and PAST (Hammer, Harper & Ryan, 2001) to detect ecological differences for each ploidy.

Table 1. Populations sampled for *Senna* series *Aphyllae* and *Chlorocladae* detailing taxon, chromosome number (2*n*), locality and collection number and morphological features of chromosomes. All specimens were deposited at BAB and CORD herbaria

Taxa		Niche overlap		Niche equivalency (identity test)	
		D	I	D	I
Clade A					
S. aphylla	*S. crassiramea*	0.28	0.54	Different **	Different **
	S. spiniflora	0.07	0.24	Different **	Different **
S. crassiramea	*S. spiniflora*	0.10	0.30	Different **	Different **
Clade B					
S. acanthoclada	*S. pachyrrhiza*	0.68	0.89	Similar **	Similar **

Ecological Niche Modelling

Ecological niche modelling was carried out for all members of series *Aphyllae* (except for *S. nudicaulis*). This analysis was applied to determine the potential distribution and quantify niche differentiation between the species of series *Aphyllae* (Robbiati *et al.*, 2017a, 2019) using MaxEnt v.3.4.1 (Phillips, Anderson & Schapire, 2006). The georeferenced points (Supporting Information, Table S1) were compiled both during field collection and from herbarium records (BAB, CORD, CTES, LIL, LP, MCNS, SI and Z; abbreviations for herbaria follow Index Herbariorum: http://sweetgum.nybg.org/ih/). To avoid a spatial autocorrelation effect in the ENM, records that were < 1 km apart were removed using the R package 'Wallace' v.1.0.6.1 (Kass *et al.*, 2018) implemented in R v.3.6.1. After removing 382 occurrences, 346 points were retained (21 for *S. acanthoclada*, 43 for *S. aphylla*, 181 for *S. pachyrrhiza*, 51 for *S. crassiramea*, 50 for *S. spiniflora*). To characterize the environments, 19 bioclimatic variables were obtained from the WorldClim 2 database (http://www.worldclim.org) at a resolution of 2.5 arc minutes (Hijmans *et al.*, 2005) and the layers of the soil of seven edaphic variables from SoilGrids (https://www.isric.org), at the same spatial resolution. To avoid over-estimation of environmental data and consequent misleading results, we reduced some data because of multicollinearity; to this end, Pearson's correlation was performed for each species to identify pairs of bioclimatic variables with a high degree of correlation ($r > 0.6$). We selected seven climatic variables: Bio1 (annual mean temperature), Bio2 (mean diurnal range), Bio3 (isothermality), Bio7 (temperature annual range), Bio12 (annual precipitation), Bio15 (precipitation seasonality) and Bio17 (precipitation of the driest quarter) and the seven edaphic variables: Bldfie (bulk density), Clyppt (clay content), Crfvol (coarse fragments volumetric percentage), Orcdrc (soil organic carbon content), Phihox (soil pH), Sltppt (silt content) and Sndppt (sand content) that were considered biologically meaningful and directly relevant to these species. Correlation tests were performed using Infostat 2.0. Default settings were used for the MaxEnt run, including auto-features, a maximum of 500 iterations and a maximum number of background points of 10 000, with a convergence threshold of 10^{-5} and regularization multiplier 1. We used 75% random localities for model training and 25% for model testing by bootstrap with ten replicates. The ten models of each taxon were stored in ASCII raster format and imported to the QGIS to produce a strict consensus map. The area under the receiver operating characteristic curve (Peterson, Papeş & Soberón, 2008; Elith & Leathwick, 2009) values were used to evaluate the accuracy of each model prediction. Variable importance to ENMs was evaluated based on the permutation value, jackknife test and the response curves calculated by MaxEnt (Phillips *et al.*, 2017).

Ecological Niche Divergence Test

To evaluate the degree of niche overlap, and to test the divergence or conservative niche hypotheses between the species of series *Aphyllae*, we applied a niche equivalence test (identity test) and niche similarity test (background test). We used ENM tools v.1.3 (Warren, Glor & Turelli, 2008, 2010) to calculate Schoener's D (Schoener, 1968) and standardized Hellinger's distance (calculated as I) to measure niche overlap between species via pairwise comparison of species. The I and D values of the niche range from 0 (two species have no overlap in the environmental space) to 1 (two species share the same environmental space). We used the identity test to assess whether the ecological niches between species are equivalent (ecological niches are interchangeable) or significantly different ($P < 0.05$). A comparison of the experimental D and calculated I for each pair of species was made with a null distribution simulation of 100 pseudo-replicates generated by random sampling from data points pooled for each pair of species. The background test assesses whether the ecological niches of any pair of species are more different than expected by chance and considers the environmental conditions available to them. Biotic regions serve as a reliable estimate of the area that is accessible to a species (Soberón & Peterson, 2005), so we chose as background the ecoregions where occurrences correspond to each species. For this purpose, the world map of terrestrial ecoregions (Olson *et al.*, 2001) was used. A background test was performed using the locations of species 1 and random points (same number of locations) from the ecoregions where the locations of species 2 occur. A null distribution was created with 100 pseudo-replicated values for each pair in both directions, and the experimental D and calculated I were compared. The overlap value between two ENMs was either above the 95% confidence interval of the null hypothesis, which supported niche conservatism or below the 95% confidence interval of the null hypothesis, supporting the niche divergence.

Phylogenetic Reconstructions and Chromosome Evolution

The phylogenetic relationships in series *Aphyllae* were inferred based on plastid loci (*rpl16*, *rps16*, *matK* and *trnL-F*) and one nuclear region (ITS) (Supporting information, Table S2). All DNA sequences analysed were retrieved from previous studies (Marazzi &

Sanderson, 2010; Robbiati *et al.*, 2017a). The species were sampled based on the availability of chromosome numbers in the Chromosome Counts Database (Rice *et al.*, 2015) and to include representatives of all clades of *Senna*. Given that *S. occidentalis* (L.) Link and *S. rugosa* have more than one chromosome number, we have used the predominant chromosome number, n = 14. Since the polyploid status is the predominant condition for *S. aphylla*, we have chosen the tetraploid number given as the most frequent chromosome number. Sequences were assembled and edited using BioEdit v.7.0.9. and were pre-aligned in Muscle (Edgar, 2004), implemented in MEGA5 (Tamura *et al.*, 2011) and manually adjusted. Each gene was analysed individually and then they were concatenated in Mesquite v.2.75 (Maddison & Maddison, 2011). For maximum likelihood (ML) analysis, the program randomized axelerated maximum likelihood (RAxML) v.8.1.11 (Stamatakis, 2014) was used, which implements a rapid hill-climbing algorithm (Stamatakis, 2006). Analyses were run searching for the best-scoring ML tree inferences under the GTR-GAMMA model. Rapid bootstrapping was performed with 1000 replications using the GTRCAT estimation to assess branch support (Stamatakis, 2006). ML analyses were run using the CIPRES Gateway Portal (http://www.phylo.org/portal2; Miller, Pfeiffer & Schwartz, 2010) using RAxML-HPC v.8 on XSEDE (8.2.4) tool. *Cassia fistula* L. was selected as the outgroup to root the tree. New chromosome counts and previously published chromosome numbers (Table 1 and Table S2 for previous counts of *Senna*) were compiled for the analysed taxa. To understand the events that led to the karyotypic diversity across the phylogenetic tree for *Senna*, we employed a statistical framework. For chromosome number (n) we applied ChromEvol implemented in RASP v.3.2. (Yu *et al.*, 2015), which was developed to model the evolution of ploidy and to test whether karyotypes evolved by polyploidy or dysploidy (Glick & Mayrose, 2014). All models were tested and compared to their likelihood values (AIC, Akaike, 1974). We set the base chromosome number as 12, the rate base number as 1, the maximal chromosome number as 120 (−10 according to RASP settings), and the minimal chromosome number as 12 (1 according to RASP settings). The base number was kept fixed and 10 000 simulations were performed.

RESULTS

Chromosome Number, Size and Morphology

Mitotic chromosome numbers for three species of series *Aphyllae* and for *Senna chloroclada* (Harms) H.S.Irwin & Barneby of series *Chlorocladae* H.S.Irwin & Barneby were obtained (Table 1; Fig. 1). For series *Aphyllae*, in all cases, the chromosome numbers were based on x = 12. First, counts were made for *S. aphylla*, which showed three cytotypes $2n$ = 24, 48 and 60. *Senna pachyrrhiza* displayed two cytotypes ($2n$ = 24 and 48), and *S. crassiramea* had only one tetraploid cytotype, $2n$ = 48. The sum of chromosome lengths in somatic nuclei ranged from 30.0 µm in diploid *S. pachyrrhiza* to 79.3 µm in polyploid *S. pachyrrhiza*, with the highest-ploidy cytotypes having the highest values. The tetraploid *S. pachyrrhiza* from Catamarca, Santa María (*Fortunato 9657*), the diploid *S. pachyrrhiza* from Córdoba, Punilla (*Fortunato 9617*) and diploid cytotypes of *S. aphylla* from the population of La Rioja, Capital (*Fortunato 9643*) displayed the shortest lengths. The analysed taxa of series *Aphyllae* showed metacentric and submetacentric chromosomes with a gradual decrease in size or similar sizes. Concerning the size of chromosomal complements in general, the polyploid cytotypes had double values in comparing diploid cytotypes. Chromosome size ranged from 0.37 to 3.20 µm and the average chromosome size was 1.31 µm. On average, the species with the largest chromosome size was the diploid *S. pachyrrhiza* (1.65 µm) and the smallest the tetraploid *S. pachyrrhiza* (0.79 µm). In diploid cytotypes, the chromosome size average was 1.46 µm and in polyploid cytotypes, was 1.22 µm. *Senna chloroclada* was reported as a diploid with $2n$ = 26 and a basic chromosome number x = 13.

Chromosomal Distribution of Ribosomal Genes (rDNA) in Diploids and Tetraploids

In diploid *S. pachyrrhiza*, two-terminal sites (one pair) in terminal regions of the short arm strongly marked with the 45S rDNA probe and three intercalary sites marked in long arms with the 5S rDNA probe were found (Fig. 2A). In parallel, in tetraploid *S. crassiramea*, four-terminal sites (two pairs) in the terminal regions of the short arm were found for the 45S rDNA and four intercalary sites in long arms for 5S rDNA (Fig. 2B).

Geographical Distribution of Cytotypes

The diploid area overlaps with the tetraploid and pentaploid areas. The geographical distribution of each taxon and ploidy is presented in Figure 3. Although a distribution pattern cannot be detected, polyploid cytotypes had a more northern and western distribution. In the PCA, the first three axes explained 87.3% of the total variance and were projected on a two-dimensional plane to observe the relationships among samples. The three first axes were retained. For PC1, which explains 43.4% of the variability, the most important variable was Bio3 (Eigenvector = 0.34),

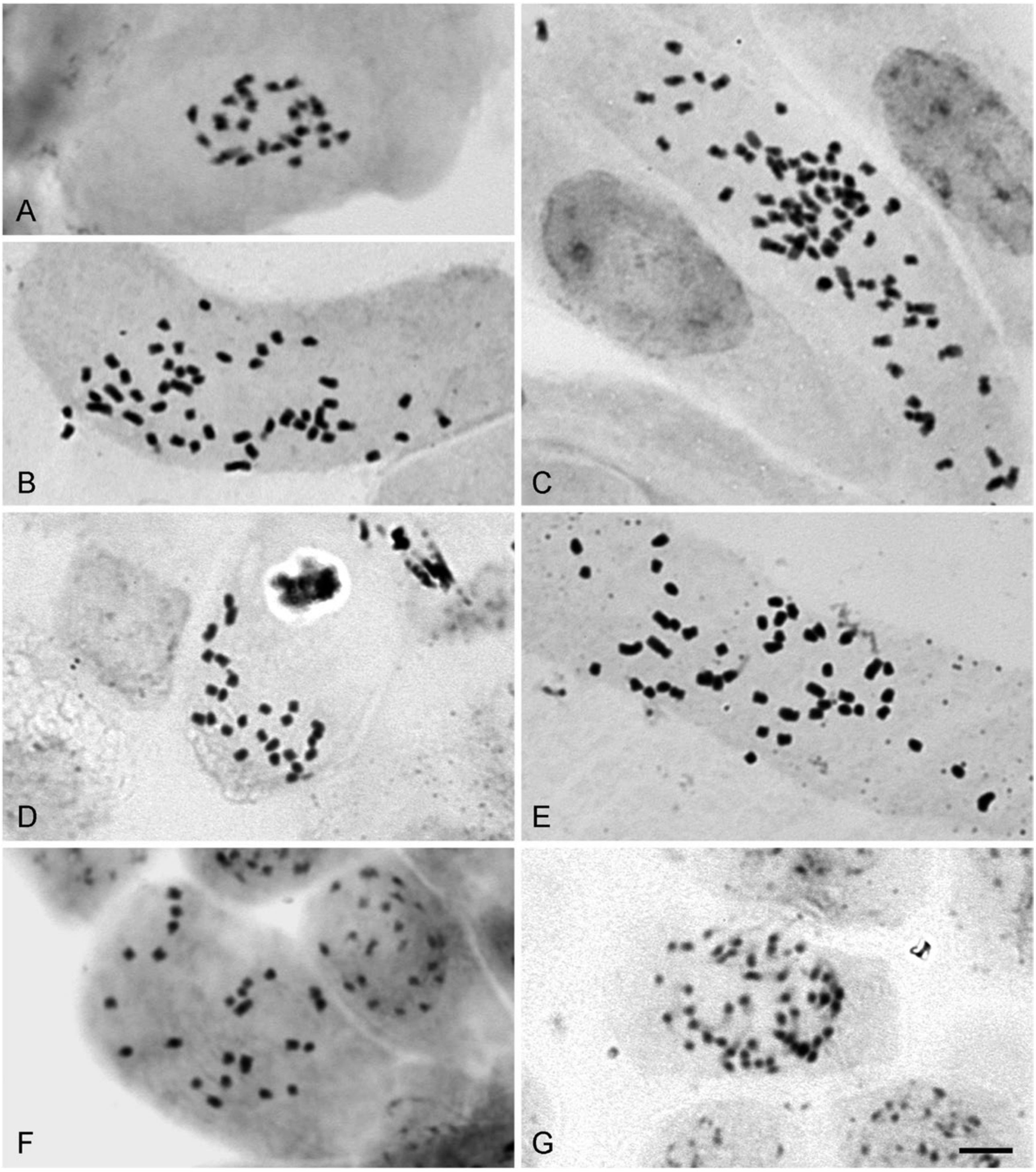

Figure 1. Mitotic metaphase chromosomes coloured by the conventional technique (Giemsa): A, *Senna aphylla* diploid (*Fortunato 9913*). B, *S. aphylla* tetraploid (*Fortunato 9935*). C, *S. aphylla* pentaploid (*Fortunato 9651*). D, *S. chloroclada* (*Fortunato 10169*). E, *S. crassiramea* (*Fortunato 9654*). F, *S. pachyrrhiza* diploid (*Fortunato 9617*). G, *S. pachyrrhiza* tetraploid (*Fortunato 9657*). All the same scale: 5 µm.

followed by Bio7 (Eigenvector = 0.33). For PC2, which explains 33.9% of the variability, the most important variables were Bio12 (Eigenvector = 0.40) and Bldfie (Eigenvector = 0.40), and for PC3, which explains 10% of the variability, the most important variables were Bio2 (Eigenvector = 0.73) and Bio7 (Eigenvector = 0.35).

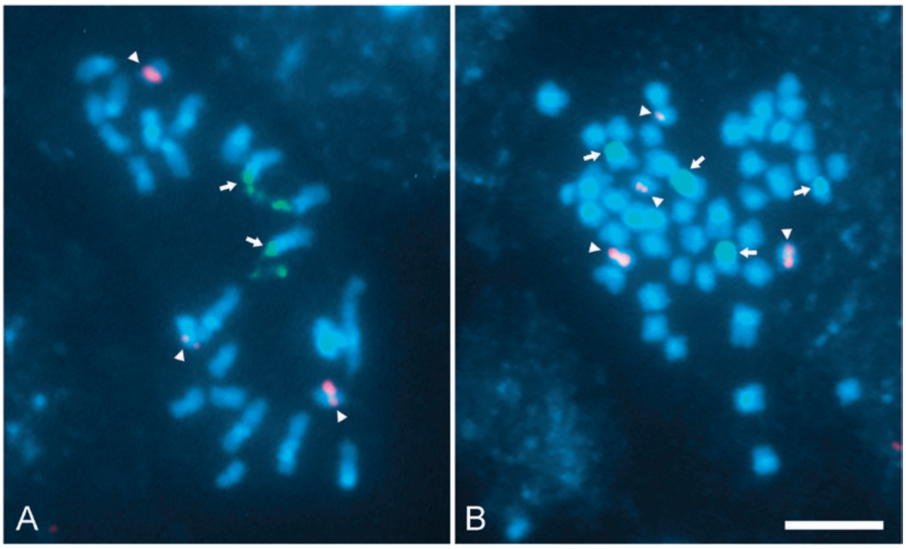

Figure 2. FISH technique. Arrows indicate 18-5.8-26S rDNA signals (green) and triangles indicate 5S rDNA signals (red) in two species of *Senna* series *Aphyllae*. A, *S. pachyrrhiza* (*Fortunato 9646*); B, *S. crassiramea* (*Fortunato 9654*). Scale 5 µm.

According to the two first axis (Fig. 4) for *S. aphylla*, the diploid cytotypes appear grouped in the left upper quadrant in the scatterplot and are associated with Sndppt. Two tetraploid samples of *S. aphylla* are located in the left lower quadrant and explained by Bio17 and Bio12, and the other tetraploid sample of *S. aphylla* is grouped with tetraploids of *S. pachyrrhiza* and one pentaploid of *S. aphylla*, in the right upper quadrant. This group is explained by Bio15 and Crfvol. The other pentaploid sample of *S. aphylla* is located in the left upper quadrant distanced of diploid cytotypes of *S. aphylla* and explained by Bio7. The two samples of diploid cytotypes of *S. pachyrrhiza* appeared gathered in the right lower quadrant and are explained by Clyppt and Sltppt. Finally, the tetraploid cytotypes of *S. crassiramea* are grouped in the right upper quadrant and explained by Bio3 and Orcdrc. The plot of PC1 vs. PC3 (Fig. 4) displayed a similar clustering pattern between the samples to that of PC1 vs. PC2, the main difference being that the two diploid samples of *S. pachyrrhiza* appeared separated, and one of them (Catamarca, Tinogasta, *Fortunato 9646*) was located near one pentaploid sample of *S. aphylla* (La Rioja, Capital, *Fortunato 9642*). The plot of PC2 vs. PC3 did not provide a clustering pattern (figure not shown).

ECOLOGICAL NICHE MODELLING

We obtained predictions of potential distribution for species of series *Aphyllae* based on climatic and soil variables that set the limits of the ecological niche (Fig. 5). The area under the receiver operating characteristic curve values indicated that all models had a high predictive ability, as it was > 0.9. The points of occurrence of *S. aphylla* and *S. crassiramea* showed that they are partially sympatric, both being allopatric with respect to *S. spiniflora*. *Senna acanthoclada* and *S. pachyrrhiza* are sympatric. For *S. acanthoclada* the variables that most contributed to the ENM model (Supporting Information, Fig. S1) were Bio7 (temperature annual range), Bio15 (precipitation seasonality), Orcdrc (soil organic carbon content) and Bio3 (isothermality). The potential distribution area comprises *c.* 580 357 km^2. The potential distribution extends to areas where the temperature annual range (Bio7) is *c.* 25–40 °C. Suitability was high in areas where precipitation seasonality (Bio15) is *c.* 60%, and in soils with low values of organic carbon content and Crfvol (coarse fragments volumetric percentage). For *S. aphylla* the variables Orcdrc, Bio7 and Bio17 (precipitation of driest quarter) contributed mainly to the ENM model (Supporting Information, Fig. S2). The area comprising the potential distribution for this species is *c.* 186 846 km^2. The potential distribution extends to areas where the temperature annual range (Bio7) is *c.* 31–40 °C. Suitability was high in areas where annual precipitation (Bio12) is *c.* 200 mm and precipitation of driest quarter (Bio17) is low. This species inhabits soils with low organic carbon content. The potential distribution extends mainly to the central area of Argentina. For *S. crassiramea* the variables Bio17, Bio3, Bio15, Bio1 (annual mean temperature) and Bio12 contributed mainly to the ENM model (Supporting Information, Fig. S3). The potential distribution area covers almost 155 536 AQ8 km^2. The potential distribution extends to areas where the variables Bio1, Bio3 and Bio15 are in the range of 5.5–19 °C, 50–65 and 90–155%, respectively.

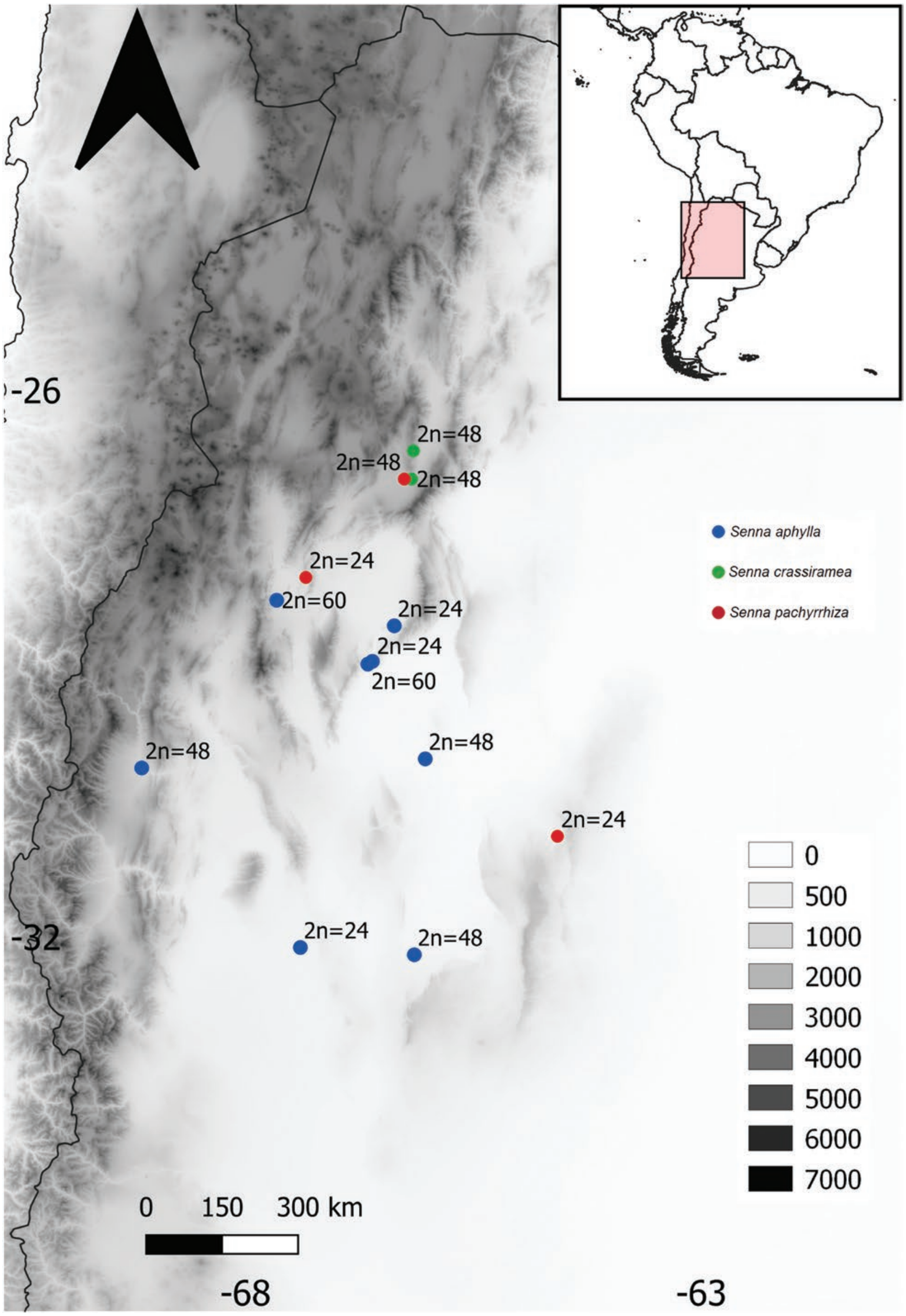

Figure 3. Localities map of studied populations of *Senna aphylla*, *S. crassiramea* and *S. pachyrrhiza*. For each population, the chromosome number is indicated.

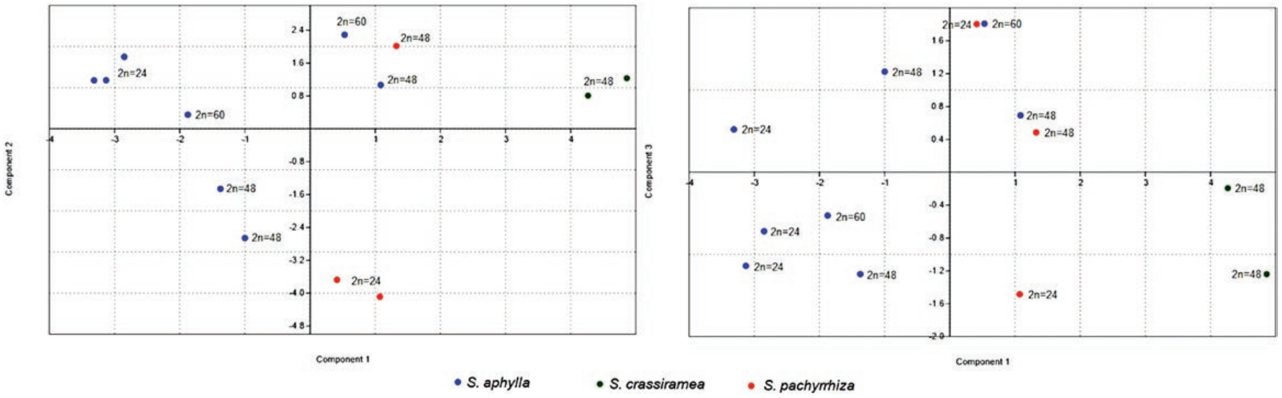

Figure 4. A Scatter plot of the first three coordinates from principal components analysis (PCA) based on environmental variables of the populations of the samples of *Senna* series *Aphyllae* in this study.

Suitability was high in areas where the variable Bio12 is *c.* 200 mm and Bio17 is low. For *S. pachyrrhiza* the major contributions to the ENM model were made by Bio7, Crfvol, Bio1 and Bio3 (Supporting Information, Fig. S4). The potential distribution area comprises *c.* 892 840 km^2. The potential distribution extends to areas where the variables Bio1, Bio3 and Bio7 are in the range of 10–21 °C, 40–60% and 24–30 °C, respectively. Suitability was high in areas where the variable values Bio17 and coarse fragments volumetric percentage are low. Finally, for *S. spiniflora* the variables that most contribute to the ENM model were Bio7, Bio15, Sltppt (silt content), Bio3 and Bio1 (Supporting information, Fig. S5). The potential distribution area covers almost 392 186 km^2. The potential distribution extends to areas where the variables Bio1, Bio7 and Bio3 are in the range of 17–23 °C and 26–27 °C and 40–60%, respectively. Suitability was high in areas where the values of variable Bio15 are low and values of silt content are medium.

NICHE CHARACTERISTICS

The results of the niche identity test showed that the null hypothesis was not rejected for *S. acanthoclada* and *S. pachyrrhiza* indicating that ENMs between these taxa were interchangeable (Table 2). For *S. aphylla*, *S. crassiramea* and *S. spiniflora* the null hypothesis was rejected for each pair. In the background test (Table 3), the null hypothesis of the similarity test could not be rejected between *S. acanthoclada* and *S. pachyrrhiza*, niche similarities of which were greater than expected by chance. *Senna aphylla* and *S. crassiramea* also showed similar niche spaces that were more similar than expected by chance, but only in one direction. For *S. aphylla* and *S. spiniflora*, the background test was significant, showing lower *D* and *I* values than expected by the null model in both directions. Furthermore, for the *S. crassiramea* and *S. spiniflora*, the background test was also significant, showing a lower *D* value than expected by the null model in both directions and a lower *I* value than expected by the null model only in one direction.

PHYLOGENETIC RECONSTRUCTIONS AND CHROMOSOME EVOLUTION

The reconstructions of ChromEvol are shown in Supplementary Information, Figure S6. This analysis indicated $n = 14$ ($P = 1$) as the most likely ancestral haploid number of *Senna*. For the ancestor of *S. kurtzii* (Harms) H.S.Irwin & Barneby and the series *Aphyllae* clade, the most likely ancestral karyotype was inferred as $n = 12$ but with low probability ($P = 0.40$). For series *Aphyllae* the chromosome number $n = 24$ was inferred as the most likely, again insignificantly ($P = 0.51$). The model favoured by ChromEvol was CONST_RATE_DEMI model (log-likelihood = –45.98 and AIC = 97.97). Variations in chromosome number are assumed to be linked to descending (frequency = 10.2) and ascending (frequency = 3.8) dysploidy and to a lower frequency of duplications (frequency = 1.8). These events were inferred with an expectation > 0.5.

DISCUSSION

CHROMOSOME NUMBER VARIATION IN *SENNA* AND ITS EVOLUTIONARY IMPLICATIONS IN SERIES *APHYLLAE*

Our results provide further evidence that the basic chromosome number for *Senna* is $x = 14$ and that for series *Aphyllae* plus the related *S. kurtzii* is $x = 12$; dysploidy and polyploidy event have had important implications in the evolution of this group. The chromosome events of dysploidy occurred independently in different clades of *Senna* such as $x = 12$ in *S. atomaria* (L.) H.S.Irwin & Barneby and $x = 13$ in *S. spectabilis* (DC.) H.S.Irwin & Barneby

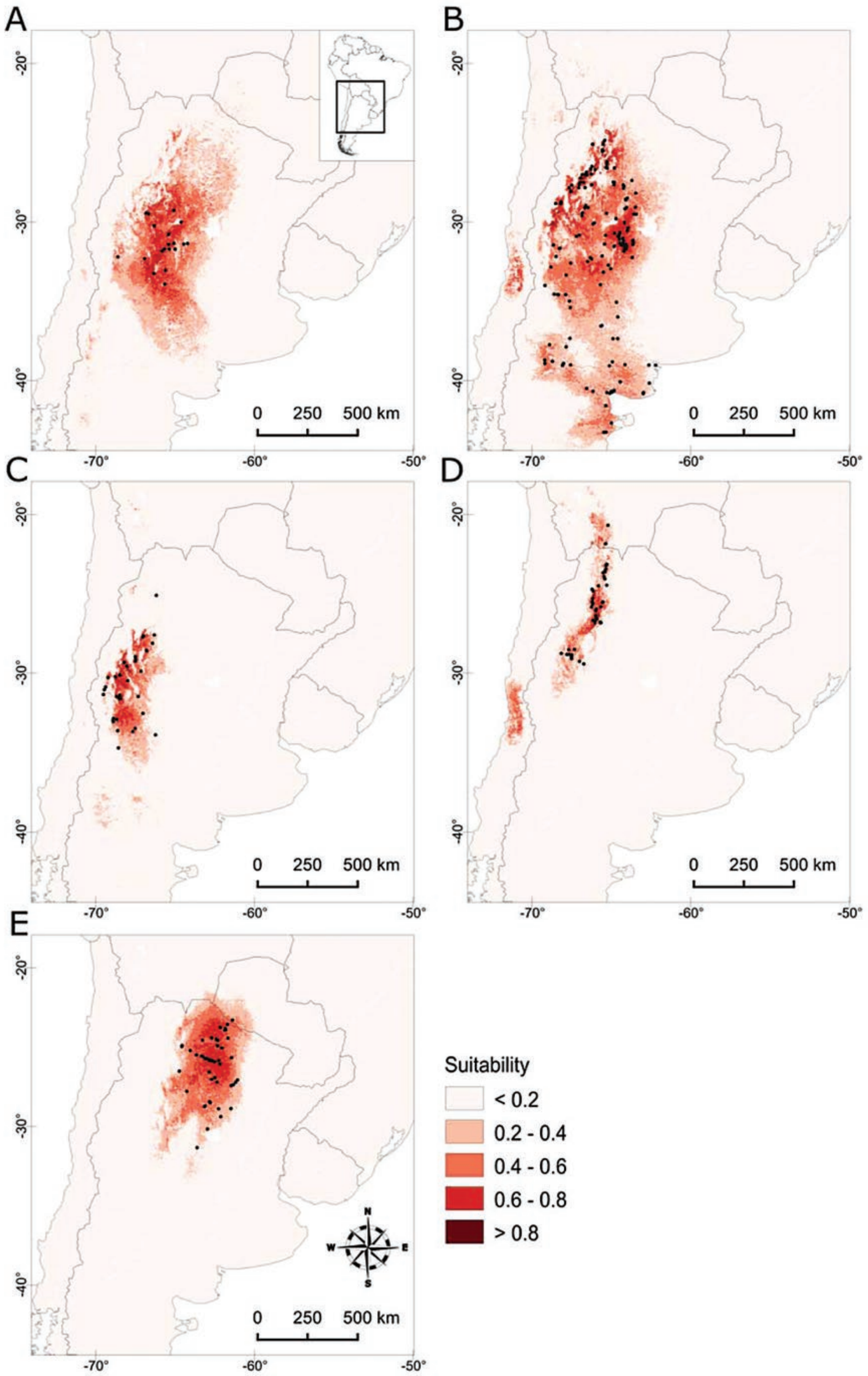

Figure 5. Distribution of suitable niches of the species of *Senna* series *Aphyllae*. A, *S. acanthoclada*; B, *S. pachyrrhiza*; C, *S. aphylla*; D, *S. crassiramea* and E, *S. spiniflora*. The suitability value represents the predicted distribution probability (in logistic value) for current climatic conditions.

Table 2. Identity test of niche equivalency. A statistically significant value denotes a pair of species that are ecologically distinct (*$P \leq 0.05$, **$P \leq 0.01$, NS $P > 0.05$). The niche overlap column shows the results of the metrics *D* and *I* obtained for each pair of taxa

Taxa		Niche overlap		Niche similarity (background test)			
		D	I	D		I	
a	b			a → b	b → a	a → b	b → a
Clade A							
S. aphylla	*S. crassiramea*	0.28	0.54	NS	Similar **	NS	Similar *
	S. spiniflora	0.07	0.24	Different **	Different **	Different **	Different **
S. crassiramea	*S. spiniflora*	0.10	0.30	Different **	Different **	Different **	NS
Clade B							
S. acanthoclada	*S. pachyrrhiza*	0.68	0.89	Similar **	Similar **	Similar **	Similar **

(clade III), $x = 12$ in *S. multijuga* (Rich.) H.S.Irwin & Barneby (clade VI), $x = 12$ in *S. hirsuta* (L.) H.S.Irwin & Barneby (clade VIIb) (Cordeiro & Felix, 2018) and for *S. chloroclada* $2n = 26$ (Fig. 1D; Table S2). The last species belongs to series *Chloracladae* and inhabits the Chaco biogeographic province (Irwin & Barneby, 1982). The ancestral chromosome number analysis with ChromEvol indicates that, despite the lack of data, there was more than one independent dysploidy event in *Senna*. However, this is a provisional conclusion because of incomplete chromosome numbers available to date (*c.* 20%).

Polyploidy and Different Cytotypes in *Senna* Series *Aphyllae*

According to the studied species in series *Aphyllae*, the ploidy state of a species could be a phenomenon associated with its evolutionary history. *Senna aphylla* shows variation in ploidy at the intraspecific level ($2n = 24$, 48 and 60). It inhabits sandy and rocky soils in the northern areas of the Monte (north of 35° S), which are characterized by mountain ranges and foothills connected by often isolated valleys or bolsons (Robbiati *et al.*, 2017a, 2019). The plants exhibit considerable morphological variation, which led to different interpretations of the specific boundaries (Bravo, 1978; Robbiati *et al.*, 2014b; Robbiati *et al.*, 2019). Based on the populations assessed, *S. aphylla* is composed of diploid and polyploid plants. Moreover, our findings showed that populations with pentaploid plants are rare, and probably originated from hybrid derivation, from diploid/hexaploid crosses, although hexaploid populations were not found. Furthermore, according to the distribution of *S. aphylla*, we cannot rule out the occurrence of several independent polyploidy and hybridization events between cytotypes, especially in the region where the pentaploids are found. *Senna pachyrrhiza* included diploid and tetraploid plants. This widely distributed species inhabits the Monte extension, south-western Dry Chaco, and its ecotone with the Espinal, growing in sandy and clay soils (Robbiati *et al.*, 2017a). It also shows a high morphological variation (Robbiati *et al.*, 2017a). On the other hand, morphological studies revealed intermediate individuals between *S. aphylla* and *S. pachyrrhiza* (Robbiati *et al.*, 2017a). Thus, we cannot discard interspecific hybridization processes between them in regions where they co-occurred. Finally, chromosome counts revealed that *S. crassiramea* is a tetraploid species, which exhibits great morphological variation in its small distribution area. It inhabits southern Bolivia and north-western Argentina, growing in mountainous slopes on rocky soils in northern Monte and Prepuna biogeographic provinces (Robbiati *et al.*, 2017a). Among members of series *Aphyllae*, this entity is the only one that grows on arid slopes of the Sierras Pampeanas and the Precordillera (Robbiati *et al.*, 2019).

The duplication of 45S rDNA could suggest that tetraploid plants may have an autopolyploid origin and the localization of 5S and 45S rDNA concur with other studies of *Senna* spp. (Pellerin, Waminal & Kim, 2019). The variation of rDNA 5S loci number is not proportional between diploid and tetraploid cytotypes, and this could be a consequence of a diploidization process in autopolyploids. There is evidence that the development of a diploidization process (in autopolyploids) reduces the origin of unviable gametes since it removes interspersed repetitive DNA material. These processes include progressive loss of rDNA sites after the duplication. This loss could be related to the age of the polyploids (Wendel, 2000; Winterfeld & Röser, 2007; Roa & Guerra, 2012). In consequence, these conclusions may indicate that in *S. crassiramea* the chromosome duplication could be relatively old. However, in *S. aphylla* and *S. pachyrrhiza* polyploidy remains uncertain, and it is not clear if they were

Table 3. Background test of niche similarity. A statistically significant value denotes a pair of species that are ecologically distinct or similar (${}^{*}P \leq 0.05$, ${}^{**}P \leq 0.01$, NS $P > 0.05$). The niche overlap column shows the results of the metrics D and I obtained by comparing respective ENMS for each pair of taxa. Background tests were performed between taxa in both directions, represented as a → b and b → a

Taxon	Chromosome number (2*n*)	Locality, voucher number	Morphological features of chromosomes				
			Mean chromosome length and range (μm)	Prevalent morphology	45S rDNA	5S rDNA	Total complement length (μm)
S. aphylla	48	La Rioja, Chamical, *Fortunato 9635* San Juan, Iglesia, *Fortunato 9939* San Luis, Ayacucho, *Fortunato 9902*	1.31 (0.37–2.57)	m, sm			67.75
	60	La Rioja, Capital, *Fortunato 9642* Catamarca, Tinogasta, *Fortunato 9651*	1.08 (0.66–2.10)	m, sm			72.03
	24	La Rioja, Capital, *Fortunato 9643* La Rioja, Capital, *Fortunato 9645* San Juan, 25 de Mayo, *Fortunato 9913*	1.28 (0.54–3.20)	m, sm			34.75
S. crassiramea	48	Catamarca, Belén, *Fortunato 9654* Catamarca, Santa María, *Fortunato 9666*	1.27 (0.75–2.47)	m, sm	4	4	58.71
S. pachyrrhiza	48	Catamarca, Santa María, *Fortunato 9657*	0.71 (0.49–2.71)* * possible measurement data	m, sm			79.3
	24	Catamarca, Tinogasta, *Fortunato 9646*	1.65 (0.74–2.62)	m, sm	2	3	30.01
		Córdoba, Punilla, *Fortunato 9617*		m, sm			
S. chloroclada		Formosa, Bermejo, *Fortunato 10169*	1.21 (0.69–2.57)	m, sm			30.47

produced by a loss of rDNA sites during polyploidization processes or if they are auto- or allopolyploid.

According to Levin (1975), niche differentiation between polyploid and diploid progenitors is required for a long-term coexistence and the establishment, success and persistence of polyploids. In general, polyploids display changes in their morphology and physiology causing niche differentiation between plant populations that show variation in ploidy (Ramsey & Schemske, 1998, 2002). Also, some evidence suggests that polyploid species, given their broader environmental tolerance, differs in the geographical distributions compared to diploids (Stebbins, 1971; Brochmann *et al.*, 2004; Buggs & Pannell, 2007; Meimberg *et al.*, 2009). Our results display a differentiation in niche requirements between diploids and polyploids. For *S. aphylla* the few diploid samples shared a similar ecological niche among them and this was different from that of the polyploids which present a wide range of niche requirements. For *S. pachyrrhiza* the diploid and tetraploid cytotypes also displayed different ecological niches. Moreover, comparing the diploid cytotypes of *S. aphylla* and *S. pachyrrhiza,* it is clear that they also showed different niche requirements. In parallel, the polyploids in *S. crassiramea* displayed different niche requirements than those in *S. aphylla* and *S. pachyrrhiza*. However, to confirm this trend it is necessary to carry out a more exhaustive sampling in series *Aphyllae* and to apply modern technology, e.g. flow cytometry, to discover cytogeographic patterns.

ECOLOGICAL NICHE MODELS FOR SERIES *APHYLLAE*

The incorporation of soil variables to perform niche modelling in a new taxonomic classification of series *Aphyllae* based on phylogenetic and morphological evidence (Robbiati *et al.*, 2019) complements a previous ecological study (Robbiati *et al.*, 2017b). The results of ENM showed that the importance of climatic variables was similar to those found previously by Robbiati *et al.* (2017a), even though there were differences in soil variables. According to Warren *et al.* (2008), rejecting the niche equivalency null hypothesis means that environmental niche differentiation has occurred in association with speciation events. Moreover, they suggest that failure to reject the null hypothesis in the background test indicates that the data are such that there is insufficient power to make inferences regarding niche evolution. Our results of the identity and similarity test showed that the niches between *S. acanthoclada* and *S. pachyrrhiza* responded to clear phylogenetic niche conservatism, whereas *S. aphylla*, *S. crassiramea*, and *S. spiniflora* are divergent. The phylogenetic niche conservatism is the tendency of closely related lineages to retain ancestral ecological characteristics over time. This phenomenon suggests that the taxa that have speciated through niche conservatism experience population fragmentation and allopatry, rather than ecological differences between populations (Wiens 2004; Kozak & Wiens, 2006). Although *S. acanthoclada* and *S. pachyrrhiza* show a current sympatric distribution, the ancestral populations could have experienced past fragmentation and allopatry caused by the Quaternary climatic change, and a recent demographic expansion. Both taxa originated in the South American Transition Zone during the mid-Pleistocene (1.26 Mya) (Robbiati *et al.*, 2017a). For *S. pachyrrhiza,* Robbiati *et al.* (2021) revealed a recent demographic expansion in the Monte desert. Considering that ecological niche divergence between related species can be a sign of ecological specialization (Schluter, 2001), the niche divergence showed by *S. aphylla, S. crassiramea* and *S. spiniflora* might have been the result of ecological speciation caused by divergent selection and adaptation. Species with overlapping geographical distribution may have experienced parapatric speciation or may have become parapatric as a consequence of secondary geographic contact and niche partitioning between them after allopatric speciation occurred (Diamond, 1977; Lynch, 1989). *Senna aphylla* and *S. crassiramea* are partially sympatric in the Monte biogeographic province. Its desert has a heterogeneous topography with several isolated valleys and bolsons, and there are differences in seasonal precipitation and drought (Abraham *et al.*, 2009; Labraga & Villalba, 2009). This landscape heterogeneity could promote parapatric speciation via local adaptation and niche divergence. Both species are morphologically different, especially in branch thickening. Robbiati *et al.* (2017a) suggested that the acquisition of thickened branches in *S. crassiramea* may have favoured its establishment in drier habitats. Also, it is well-documented that polyploidy provides a clear mechanism for sympatric speciation (Schluter, 2001). Since *S. aphylla* and *S. crassiramea* showed polyploidy, another possible explanation is that the polyploidy could have promoted sympatric speciation as a result of immediate post-zygotic reproductive isolation of the neopolyploid population from that of the progenitor(s). On the other hand, *S. spiniflora* is distributed exclusively in the xerophilous forest of the southern Chaco biogeographic province in an adjacent area of the Monte desert (Robbiati *et al.*, 2017a). Considering its geographical distribution, *S. spiniflora* could have experienced parapatric speciation adapting to grow in saline soils given that the Chaco flora is considered a Pleistocene relict established in salty soils (Iriondo, 1993).

CONCLUSIONS

Senna series *Aphyllae* originated when the Andean peaks were already formed and diverged mainly during the Quaternary climatic change. Under this scenario, and taking into account the results of ENMs based on the niche overlap performed here, we concluded that the evolutionary history of series *Aphyllae* could have been driven by both allopatric speciation promoted by niche conservatism, and/or ecological speciation, reflected in niche divergence. In parallel, polyploidy could have influenced the speciation mechanism of series *Aphyllae*, adapting the species to the extreme environmental condition of the arid and semi-arid zones, since polyploids are considered to be well adapted to aridity (Winterfeld, Schneider & Röser, 2009). Furthermore, the fact that polyploidy occurred during periods of climate change (Alix *et al.*, 2017) could also represent a survival strategy (Cai *et al.*, 2019; Levin, 2019). However, it remains unclear whether the presence and frequency of the hybridization processes at inter- and intraspecific levels have played a role in the evolutionary history of series *Aphyllae*. To answer these questions, molecular studies are required such as next-generation sequencing to get a better insight into the formation of the polyploids.

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DATA AVAILABILITY

AQ9 The data underlying this article are available in [repository name, e.g. the GenBank Nucleotide Database] at [URL], and can be accessed with [unique identifier, e.g. accession number, deposition number].

AQ10

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Table S1. Localities, vouchers and coordinates used in the ecological niche modelling
Table S2. Chromosome records of species of *Senna* used to trace ancestral reconstruction character analysis, their respective references and voucher information, and GenBank accessions (*rpl16*, *rps16*, *matK*, *trnL-F*, ITS)
Figure S1. The percentage contribution and permutation importance of environmental variables, and jackknife test of contribution of variables, in modelling habitat distribution of *Senna acanthoclada*. Response curves of the most important environmental variables in habitat distribution of the *S. acanthoclada* model.
Figure S2. The percentage contribution and permutation importance of environmental variables, and jackknife test of contribution of variables, in modelling habitat distribution of *Senna aphylla*. Response curves of the most important environmental variables in habitat distribution of the *S. aphylla* model.
Figure S3. The percentage contribution and permutation importance of environmental variables, and jackknife test of contribution of variables, in modelling habitat distribution of *Senna crassiramea*. Response curves of the most important environmental variables in habitat distribution of the *S. crassiramea* model.
Figure S4. The percentage contribution and permutation importance of environmental variables, and jackknife test of contribution of variables, in modelling habitat distribution of *Senna pachyrrhiza*. Response curves of the most important environmental variables in habitat distribution of the *S. pachyrrhiza* model.
Figure S5. The percentage contribution and permutation importance of environmental variables, and jackknife test of the contribution of variables, in modelling habitat distribution of *Senna spiniflora*. Response curves of the most important environmental variables in habitat distribution of the *S. spiniflora* model.
Figure S6. Chromosome number evolution in *Senna* inferred from ChromEvol indicated by the pies with different colours and proportions. Basic chromosome numbers are indicated before the species names.