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How important was polyploidy in the diversification of herbs in the Chaquean Domain? The case of the *Turnera sidoides* autopolyploid complex (Passifloraceae: Turneroideae)

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The evolutionary processes that shape the unique biodiversity of the Neotropical Chaquean Domain are still poorly understood, and choosing an appropriate model to address evolutionary questions is essential for understanding the current patterns of Chaquean diversity. We used the *Turnera sidoides* autopolyploid complex as a model to assess current and past environmental factors and the processes that shaped and maintain its intraspecific variation in the Chaquean Domain. Based on current and past niche modelling, cytogeographical and genetic divergence analyses, we provide strong evidence that the *T. sidoides* complex has been in an active process of intraspecific allopatric diversification at the diploid level since the early Pleistocene. Further diversification of the complex involved the emergence of independent polyploid series in each morphologically divergent lineage. Cytotypes in each series do not differ in diagnostic morphological traits, but are reproductively isolated and have different ecological requirements. Currently, each subspecies/morphotype and cytotype are true species according to the biological, evolutionary and ecological concepts; however, they are still considered to be the same morphological species. Our study has clarified the effect of the complex processes of diversification occurring in the Chaquean Domain on *T. sidoides* and that much diversity is overlooked when only the morphological species concept is considered when estimating the diversity of Neotropical ecoregions.

ADDITIONAL KEYWORDS: autopolyploidy – intraspecific variation – niche modelling – undiscovered diversity.

INTRODUCTION

The Neotropics, extending today from central Mexico to central Argentina, including the Caribbean (Morrone, 2013, 2014), are the most biodiverse region of the world (Antonelli & Sanmartín, 2011; Ulloa Ulloa *et al.*, 2017) and comprise extensive tropical rainforests, such as the Amazon Forest (Hoorn *et al.*, 2010; Antonelli *et al.*, 2018), but also other species-rich ecoregions and habitat types, such as seasonally dry forests, savannas and grasslands (Prado & Gibbs, 1993; Simon *et al.*, 2009; Collevatti *et al.*, 2013). There is an emerging consensus that Neotropical evolutionary history was accompanied by a succession of climatic changes and major geological events from the Neogene onwards that have modified both the continental areas and the oceans (Clapperton, 1993; Colinvaux *et al.*, 1996; Graham, 2009; Lavina & Fauth, 2016), which in turn have been critical in determining current species

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distribution patterns and creating complex species diversification scenarios (Behling, 2002; Antonelli & Sanmartin, 2011; Rull, 2011, 2020; Hughes, Pennington & Antonelli, 2013; Collevatti, Lima & Vitorino, 2020).

Aside from the rise of the Isthmus of Panama (Cody *et al.*, 2010), abiotic events of biological significance in the Neotropics include hydrological and topographic changes caused by mountain uplift (Hoorn *et al.*, 2010) and several complex global and regional climate change events that occurred in the Plio-Pleistocene (Cárdenas *et al.*, 2011; Rull, 2011, 2020; Jaramillo & Cárdenas, 2013). In some regions with high diversity, such as the Amazon Forest, speciation events have occurred since at least the early Miocene (Wing *et al.*, 2009; reviewed in Azevedo *et al.*, 2020). However, in regions of more recent origin, such as the Páramo (Madriñán, Cortés & Richardson, 2013), Cerrado (Simon *et al.*, 2009) and Chaco (Iriondo, 1993), there is a remarkable history of diversification since the late Miocene–Pliocene and mainly in the last 2–4 My (Pennington & Hughes, 2014). Since each Neotropical ecoregion possesses its own species diversity and communities (Prado & Gibbs, 1993; Pennington, Prado & Pendry, 2000, Simon *et al.*, 2009; Werneck *et al.*, 2011; Antonelli *et al.*, 2018), it is crucial to investigate the diversification history and biotic connections of each to understand the diverse processes that shaped Neotropical biodiversity (Rull, 2011, 2020; Hughes *et al.*, 2013).

The Chaquean Phytogeographical Domain (*sensu* Cabrera, 1971; Cabrera & Willink, 1973) occupies northern and central Argentina, southern Bolivia, western and central Paraguay, Uruguay, and central, south-eastern and north-eastern Brazil. Several of its main ecoregions (according to Olson *et al.*, 2001), such as the Chaco and Cerrado, and the Humid Pampa and the Uruguayan savannas, have a unique biodiversity (Miñarro & Bilenca, 2008; Simón *et al.*, 2009). However, some of these ecoregions have been overlooked in evolutionary studies, and the patterns and the processes responsible for shaping their species diversity are poorly understood (Fregonezi *et al.*, 2013; Turchetto-Zolet *et al.*, 2013, 2016; Moreno *et al.*, 2018). Due to the different ages of the ecoregions, choosing the appropriate taxonomic category is essential in diversification studies (Rull, 2020). This is true since supra-specific categories may be unreliable descriptors of biodiversity, as they may contain a wide range of internal diversity (Rull, 2012) and may underestimate recent diversification processes. Therefore, unravelling the origin and maintenance of current biodiversity in ecoregions of recent origin should preferably be addressed using species and subspecific units that represent the actual genetic variability associated with a distinct evolutionary potential (Rull, 2020).

Integrative approaches combining morphological, biogeographical, environmental and genetic data in species complexes provide information on early steps in species radiation, the colonization of new habitats and the selective pressures in different environments that shaped and maintain current diversity (Mäder *et al.*, 2013; Turchetto *et al.*, 2014; Andre *et al.*, 2015; Lagomarsino *et al.*, 2016; Xu *et al.*, 2017; Pessoa *et al.*, 2020, 2022). Such analyses improved knowledge of the evolutionary history of species and the potential processes underlying the diversification and speciation events. In the last decade, many studies have demonstrated the usefulness of species complexes for testing different evolutionary hypotheses worldwide (Schmickl *et al.*, 2010; Li *et al.*, 2014; Tomasello *et al.*, 2020). Also in South America, these complexes have recently started to be used to address species diversification in regions of recent origin (reviewed by Pinheiro, Dantas-Queiroz & Palma-Silva, 2018).

Turnera sidoides L. (Passifloraceae, Turneroideae) is a South American polyploid complex of perennial, rhizomatous and morphologically diverse herbs, originating in the early Pleistocene (Moreno *et al.*, 2018). It is naturally distributed in southern Bolivia and Brazil, south-western Paraguay, Uruguay and Argentina, reaching up to 39°S (Arbo, 1985; Solís Neffa, 2000). From a biogeographical point of view, the geographical area of the *T. sidoides* complex (Fig. 1) is mostly included in the Chaquean Domain, extending over ten of the ecoregions proposed by Olson *et al.* (2001). This makes the *T. sidoides* complex an ideal model to study the evolutionary processes operating across different ecogeographical contexts in the Chaquean Domain.

Populations of the *T. sidoides* complex show high variability in leaf shape, flower colour and indumentum, and in their current geographical distribution. They have been arranged into five subspecies (Arbo, 1985) and seven morphotypes (Solís Neffa, 2010; Moreno *et al.*, 2021). The morphotypes exhibit sufficient diagnostic traits to be treated taxonomically as subspecies, although this formal recognition has not yet been performed (Solís Neffa, 2010). An extensive hybridization programme has shown that although a low rate of F_1 hybrids can be obtained from artificial homoploid crosses among subspecies/morphotypes, and more rarely between plants of different ploidy, there are strong isolation barriers that prevent natural hybridization (Solís Neffa, 2000; Kovalsky & Solís Neffa, 2016; Roggero Luque, 2017). This was confirmed by analysing contact zones between subspecies (Moreno *et al.*, 2015) or cytotypes (Elías, 2010; Kovalsky *et al.*, 2018) and suggests that they can be considered as true biological or ecological species.

The geographical distribution of the morphological and genetic variation suggested two main centres of the species variation, one in the north-western (north-western Argentina and southern Bolivia) and the

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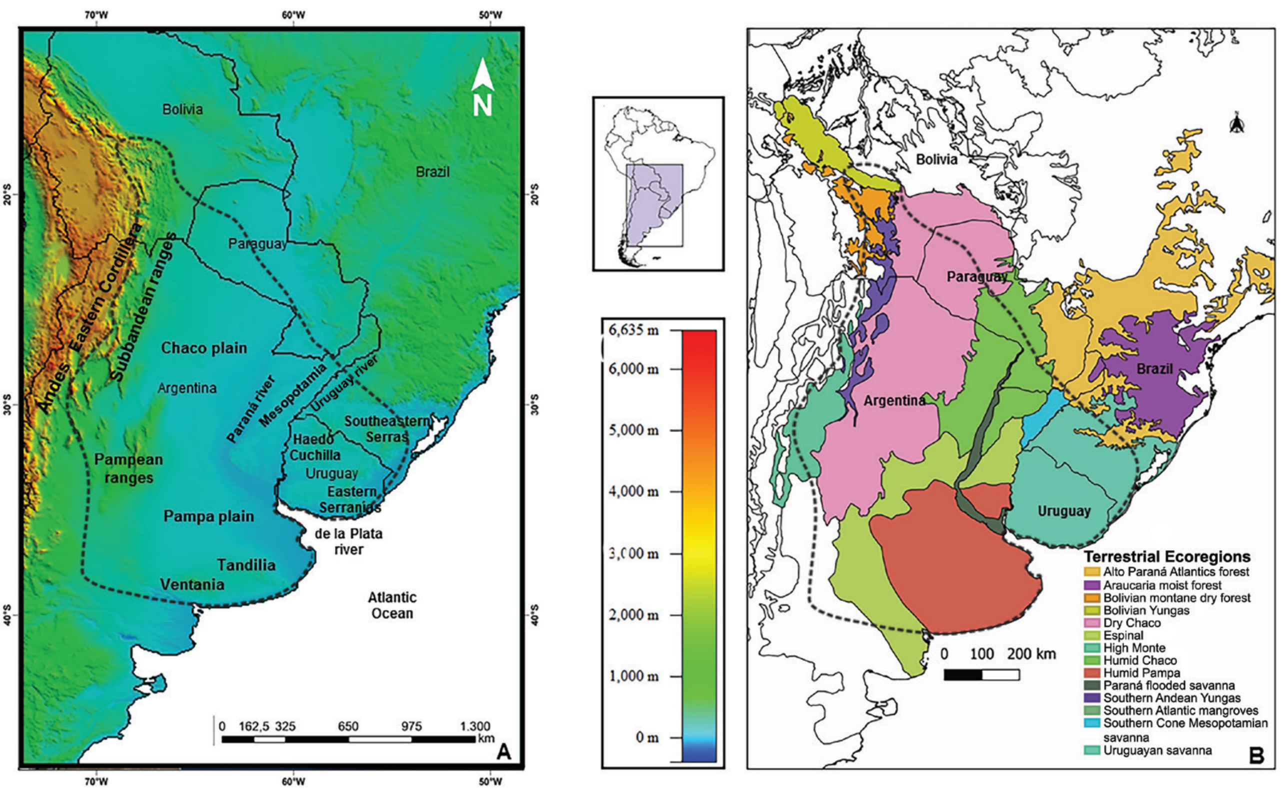

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Figure 1. Study area. A, major geographical features of the *Turnera sidoides* complex area. The dashed line denotes the distribution of the *T. sidoides* complex based on herbarium specimens and field observations. B, ecoregions included in the geographical range of *T. sidoides*.

other in the south-eastern (north-eastern Argentina, Uruguay and southern Brazil) limit of the species range (Solís Neffa *et al.*, 2004; Speranza *et al.*, 2007; Moreno *et al.*, 2018). It has been hypothesized that diversification in the *T. sidoides* complex is occurring linked to the climatic and landscape changes that have taken place in the Chaquean Domain since at least the Pleistocene (Speranza *et al.*, 2007; Moreno *et al.*, 2018). Moreover, available data suggest that current climatic and edaphic conditions are also contributing to maintain/enhance the morphological variation pattern in the *T. sidoides* complex (Solís Neffa, 2000, 2010). However, ecological niche modelling analyses performed on extensive sampling of the different lineages are still needed to support these hypotheses.

Aside from the morphological variability, polyploidy is the most outstanding feature of the *T. sidoides* complex (Fernández, 1987; Solís Neffa, 2000; Solís Neffa & Fernández, 2001; Elías, Sartor & Solís Neffa, 2011), with ploidy ranging from diploid ($2n = 2x = 14$) to octoploid ($2n = 8x = 56$), all based on $x = 7$. In particular, polyploid series from diploid to octoploid cytotypes within each subspecies/morphotype have been reported (Fernández, 1987; Solís Neffa & Fernández, 2001; Elías *et al.*, 2011; Moreno *et al.*, 2021). Since polyploidy is a major force in plant evolution that has consequences for species ecology and biogeography, plant diversification and speciation (Beaulieu *et al.*, 2008; Soltis & Soltis, 2009; Weiss-Schneeweiss *et al.*, 2013; Kellogg, 2016), the *T. sidoides* complex may allow for testing hypotheses regarding the impact of chromosome duplication on the diversification of herbaceous plants in the Chaquean Domain. Most polyploid complexes developed in the Pleistocene, when new polyploids became established under unstable environmental conditions, with obvious opportunities available during the climatic shifts (Stebbins, 1971; Soltis & Soltis, 1993; Guggisberg, 2006; Paun, Stuesey & Hörandl, 2006). Thus, a comprehensive analysis of the distribution of cytotypes and their relationship with current and past geoclimatic factors could provide information to make inferences about the evolutionary pathways and current contribution of polyploid herbaceous species to the diversity of the Chaquean Domain.

In this paper we combine biogeographical, ecological niche modelling, cytogeographical and genetic approaches to test hypotheses on the evolution of the *T. sidoides* autopolyploid complex, aiming to increase knowledge on the processes that shaped the

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current diversity in the Chaquean Phytogeographical Domain, and to test the following hypotheses: (1) current environmental factors are contributing to the maintenance of morphological differentiation of subspecies and morphotypes in the different ecoregions; (2) geological and climatic changes that occurred in the geographical range of the *T. sidoides* complex since the early Pleistocene explain the origin of subspecies/morphotypes in allopatry at the diploid level; (3) polyploid series arose independently and expanded the ranges of the already diversified subspecies and morphotypes at the diploid level; and (4) there is an enormous amount of hidden diversity associated with polyploid herbs in the Chaquean Domain.

MATERIAL AND METHODS

Study area

Turnera sidoides is primarily distributed in a low-lying area that corresponds to a huge sedimentary basin limited by the Andes and the Brazilian cratons. This widespread range comprises a great diversity of climates with contrasting precipitation regimes: xerophytic environments (200–700 mm annual precipitation) in the west and humid environments (> 1000 mm of annual precipitation) in the east. The decrease in rainfall is accompanied by a lengthening of the dry season. The area also shows a temperature decrease in a gradual north–south gradient (Iriondo & García, 1993). The 12 recognized orders of soils (Soil Survey Staff, 2014) are represented in the area of distribution of the *T. sidoides* complex.

The area is surrounded by an arc of highlands formed by the Eastern Cordillera (6400–1500 m a.s.l.) in the east and the sub-Andean (3300–1000 m a.s.l.) and the Pampean ranges (6000–500 m a.s.l.) in the west (Fig. 1A). The north–south orientation of these ranges generates contrasting environments between the east (wet and wooded) and west (arid and covered by more xeric vegetation) slopes (Ramos, Cristallini & Pérez, 2002). Between the Eastern Cordillera and the sub-Andeans ranges, a great tectonic basin forms valleys that are covered by tropical forest. East from the sub-Andean and Pampean ranges, the Chaco plain covers a vast area with a gentle north-west/south-east slope. The Paraguay and Paraná rivers run along the eastern boundary, and four main river systems (Parapetí, Pilcomayo, Bermejo and Juramento-Salado) cross the Chaco from north-west to south-east. Along the plain, and correlated with climatic and edaphic xericity, the vegetation changes from xeromorphic forest in the north-west (Dry Chaco) to semi-deciduous forest intermingled with savannas, grasslands, gallery forests and shrublands in the south-east (Humid Chaco). South of the Chaco plain, the Pampa plain is covered by treeless grassland with edaphic shrub communities. The plain is only interrupted by the Ventania and Tandilia systems (39°S, 1200–50 m a.s.l.) (Teruggi & Kilmurray, 1975; Dalla Salda, 1999).

In Mesopotamia, i.e. the interfluvial area between the Paraná and Uruguay rivers, the major vegetation types are tropical forest in the north-east, medium–tall grass savannas, tussocky grasslands, sclerophyll woodlands and forests along the main river banks (Carnevalli, 1994). The area has innumerable ponds and lakes, including the large Iberá wetland system (Popolizio, 1970, 2003). East to the Uruguay River, the landscape is an undulated peneplain in which the most important orographic systems are the Cuchillas de Haedo and Grande, Eastern Serranía in Uruguay and South-eastern Serra in southern Brazil (Fig. 1). Grasslands are the main vegetation in the entire region, although the northern area (Campos) not only differs in temperature and humidity with respect to the southern one (Pampas), but also in the flora, which has subtropical elements intermingled with temperate flora (Grela, 2004).

The lowlands and highlands were subject to different morphogenetic and climatic historical processes. A temperate climate prevailing during the Tertiary in this region supported a continuous cloud forest that extended further south than it does today (Barreda *et al.*, 2007). During the Miocene, and until the end of the Pleistocene, uplift of the mountain ranges (Mon, Monaldy & Salfity, 2005) caused the formation of a syncline in the west of the distribution area of the *T. sidoides* complex over which marine sediments and Andean deposits accumulated. Cooling and aridification began during this period, and from the Late Miocene–Pliocene the 'Arid Diagonal' crossed the South American continent in a south-east/north-west direction. During drier periods, pronounced aeolian activity deflated and redeposited large masses of silt and sand over the area (Iriondo, 1993; Iriondo & García, 1993). The arid conditions contributed to the progressive loss of the original mesic lowland elements that occupied the area (Simpson & Todzia, 1990; Hinojosa & Villagrán, 1997; Villagrán & Hinojosa, 1997). During glacial times, the significant environmental changes increased continentality (Rabassa, Coronato & Martínez, 2011).

In the Chaco plain, the current pattern of vegetation was proposed to be the result of Pleisto-Holocene alluvial dynamics of the plain and of extensive migration of the fluvial belts of the main rivers (Morello & Mateucci, 1999). During the Quaternary interglacial periods, the Chaco-Pampa plain may have been more humid than today (Barreda *et al.*, 2007). Xerophytic vegetation advanced repeatedly

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towards the north-east during the dry and colder periods and retreated towards the south-west during the humid and warmer periods. In Mesopotamia and during the Pliocene, tectonic movements and active faulting processes, which continued into the Quaternary, played an important role in shaping of the present landscape. Just after the tectonic uplift of the Mercedes plateau (140 m a.s.l.), the Uruguay and Paraná rivers took their present courses (Popolizio, 1970, 2003). In the east of the distribution area of the *T. sidoides* complex, intervals of drier climates caused a retraction and fragmentation of the Atlantic Forest and an expansion of tropical and subtropical grasslands and savannas during the Pleistocene (Rambo, 1954; Behling, 2002).

THE STUDY SYSTEM

The *T. sidoides* complex includes perennial herbs that are outbreeding due to distyly and genetic self-incompatibility (Arbo, 1985; Solís Neffa, 2000). Pollination is carried out by butterflies, small wasps and bees, and seeds are dispersed by gravity and ants. Discrete populations, ranging from fewer than ten to hundreds of plants, are separated from each other by a few to several kilometres (Solís Neffa, 2000). Five subspecies were recognized on the basis of geographical distribution and the variability of some morphological features (Arbo, 1985): *T. sidoides* subsp. *carnea* (Cambess.) Arbo, *T. sidoides* subsp. *holosericea* (Urb.) Arbo, *T. sidoides* subsp. *integrifolia* (Griseb.) Arbo, *T. sidoides* subsp. *pinnatifida* (Juss. ex Poir.) Arbo and *T. sidoides* subsp. *sidoides* (hereafter called subspecies *carnea*, *holosericea*, *integrifolia*, *pinnatifida* and *sidoides*). In addition, populations of subspecies *carnea* were grouped into two morphotypes (*grandense* and *mercedeño*) according to leaf consistency, the colour of the flowers and geographical distribution (Moreno *et al.*, 2021), and in subspecies *pinnatifida* five morphotypes (*andino*, *chaqueño*, *mesopotamico*, *pampeano* and *serrano*) have been distinguished based on the degree of incision of the leaf blade, colour of the flowers and geographical distribution (Solís Neffa, 2010).

In subspecies *carnea*, the *mercedeño* morphotype has light pink flowers and more coriaceous leaves (Fig. 2A) and mostly occurs in Mesopotamia and adjacent regions east of the Uruguay River (Fig. 3A), whereas the *grandense* morphotype (Fig. 2C) has dark pink flowers and membranaceous leaves and extends northwards into the Uruguayan savanna ecoregions and grows on stony lateritic soils (Fig. 3B). Subspecies *holosericea* has glaucous leaves with a lanate-sericeus indumentum and dark salmon flowers (Fig. 2E) and occupies a restricted area in southern Brazil and north-eastern Uruguay (Fig. 3C). Subspecies *integrifolia,* characterized by suborbicular or obovate leaves with serrate margins, a hirsute indumentum and light-pink flowers (Fig. 2G), inhabits southern Paraguay and Brazil, Argentina, and central and western Uruguay (Fig. 3D). Subspecies *sidoides* has stellate hairs and dark salmon or pink flowers (Fig. 2I) and mostly occurs on stony hills of the southern Cuchilla Grande, except for some populations found in north-eastern Cuchilla de Haedo (Uruguay) and southern Brazil (Fig. 3E). Finally, subspecies *pinnatifida* is the only one with divided leaves and covers most of the distribution area of the complex. Its *andino* morphotype, with yellow flowers and narrow pinnatifid leaves (Fig. 2K), is restricted to the Eastern Cordillera, the sub-Andean ranges, and the valleys and ravines between these ranges (Fig. 3F). The *chaqueño* morphotype characteristically has wide pinnatifid leaves and salmon flowers (Fig. 2M) and mostly grows in the Chaco forest between 20°–30°S and 63°–57°W, from nearly 500 m a.s.l in the west to 50 m a.s.l in the east. Some populations have also been detected in north-western Mesopotamia (Humid Chaco) and south-western Uruguay (Pampa grasslands) (Fig. 3G). The *mesopotamico* morphotype has pinnate leaves and light-pink flowers (Fig. 2O) and is only known from the north-eastern boundary of the range of the subspecies (Fig. 3H). The *pampeano* morphotype has pinnatifid leaves and salmon-orange flowers (Fig. 2Q) and is restricted to the Pampa plain (Fig. 3I). The *serrano* morphotype is easily recognized by its pinnatisect leaves and salmon flowers (Fig. 2S) and is common in rocky sites throughout the Pampean ranges and in the Ventania and Tandilia systems (Fig. 3J).

SAMPLING

The material analysed was obtained from field collections during 1994–2010. Overall, 403 populations were collected over almost the whole range of the *T. sidoides* complex. Plants were randomly sampled in each locality and, when possible, some of them were transferred and cultivated in a glasshouse under similar soil and culture conditions at the Instituto de Botánica del Nordeste (IBONE) in Corrientes, Argentina. Voucher specimens for each population analysed were deposited at the herbarium of IBONE (CTES), and vouchers from Bolivian and Brazilian specimens were also deposited in the National Herbarium of Bolivia (LPB) and in the Herbarium of the Instituto de Biociências, Universidade Federal do Rio Grande do Sul, Brazil (ICN), respectively. In each population leaf samples, floral buds and seeds of at least ten plants were collected. A second set of material analysed consisted of specimens from the herbaria CTES, BA, BB, BAF, CORD, JUA, LIL, SI, MVFA, MVM and MVJB (abbreviations follow Holmgren, Holmgren

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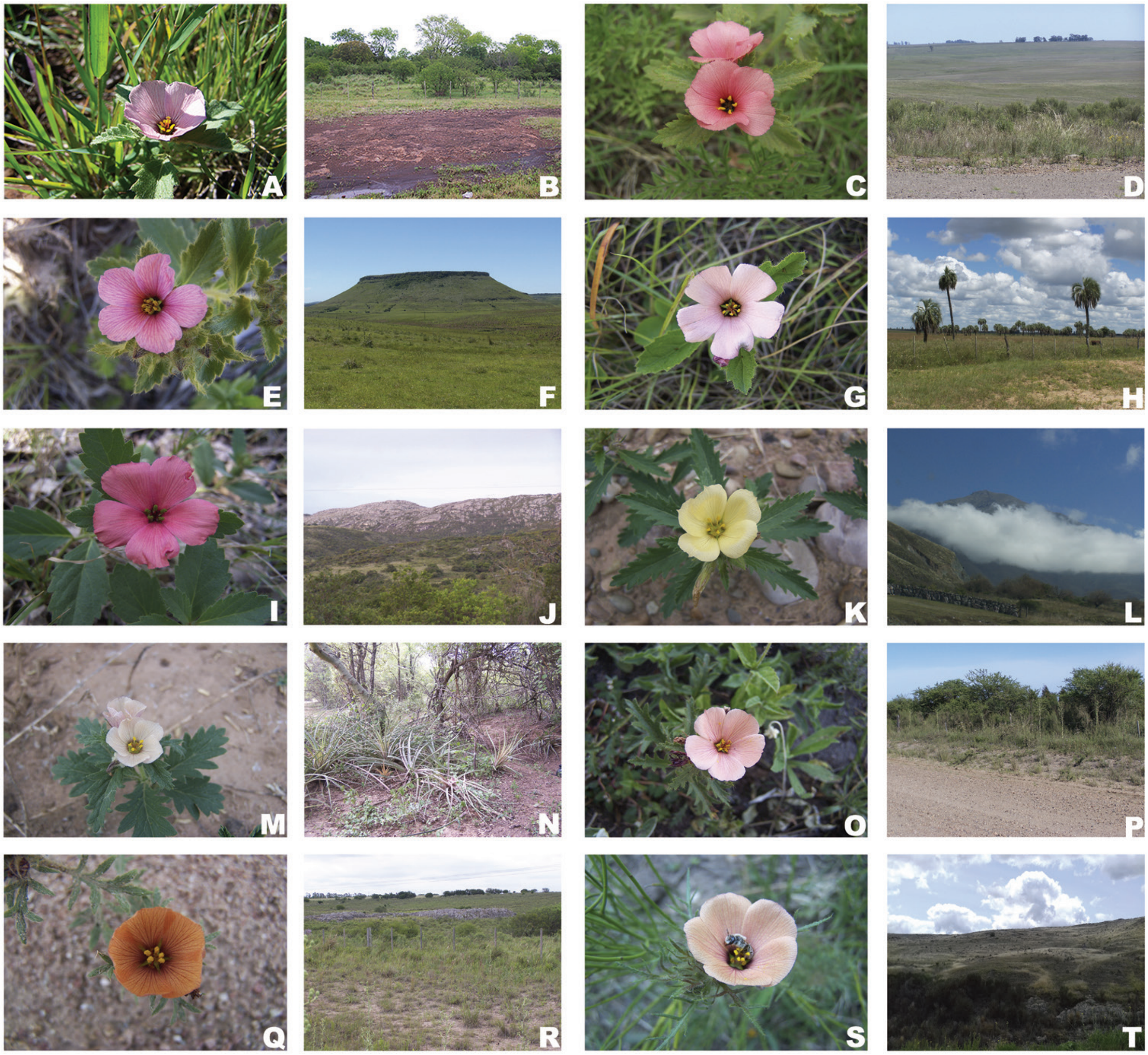

Figure 2. *Turnera sidoides* complex and the diversity of habitats that inhabit. A–D, *T. sidoides* subsp. *carnea*. A–B, *mercedeño* morphotype. C–D, *grandense* morphotype. E–F, *T. sidoides* subsp. *holosericea*. G–H, *T. sidoides* subsp. *integrifolia*. I–R, *T. sidoides* subsp. *pinnatifida*. I–J, *T. sidoides* subsp. *sidoides.* K–L, *andino* morphotype. M–N, *chaqueño* morphotype. O–P, *mesopotamico* morphotype. Q–R, *pampeano* morphotype. S–T, *serrano* morphotype. A, C, E, G, I, K, M, O, Q, S, blooming individuals of each subspecies/morphotype. B, outcrops of basaltic rocks in the Mercedes platau. D, pampa grasslands of southern Brazil. F, flat hills of northern Uruguay. H, palm groves of *Butia yatay* in the sandy hills of north-eastern Argentina. J, Eastern Serranía (Uruguay). L, mountain grassland in the sub-Andean ranges (north-western Argentina). N, Dry Chaco forest in southern Bolivia. P, thorny vegetation in the Mesopotamia plain. R, Pampa grasslands. T, Ventania System.

& Barnett, 1990). Details of the studied material are given in the Supporting Information (Appendix S1). Due to the distinctive, genetically determined traits of each morphotype and the segregation of their geographical distributions, subspecies and morphotypes will be treated for all subsequent analyses as if they all belonged to the same taxonomic category.

ECOLOGICAL DIFFERENTIATION OF SUBSPECIES AND MORPHOTYPES

To analyse whether current climate and ecological factors are contributing to the distribution patterns of the subspecies/morphotypes of the *T. sidoides* complex, a principal components analysis (PCA) was performed on 638 occurrence data obtained from georeferenced field

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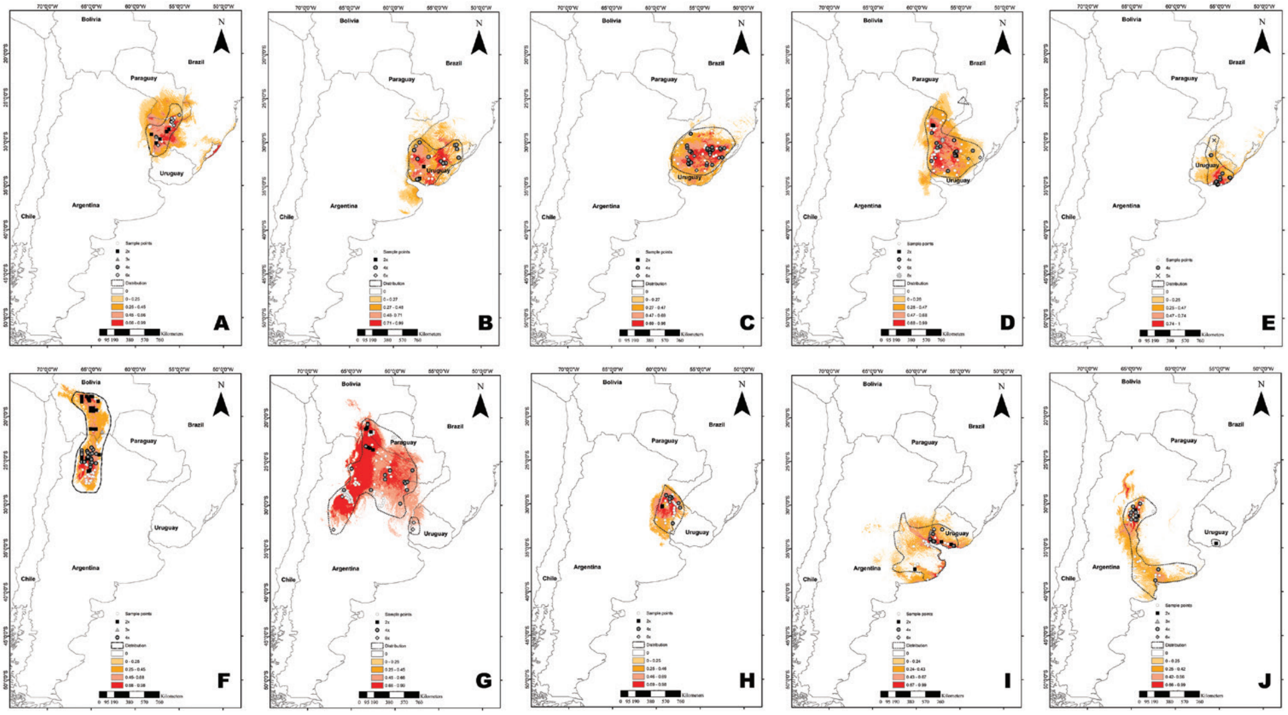

Figure 3. Spatial projections of subspecies, morphotypes and cytotypes of the *Turnera sidoides* complex under current environmental niches. A–B, *T. sidoides* subsp. *carnea*. A, *mercedeño* morphotype. B, *grandense* morphotype. C, *T. sidoides* subsp. *holosericea*. D, *T. sidoides* subsp. *integrifolia*. E, *T. sidoides* subsp. *sidoides*. F–J, *T. sidoides* subsp. *pinnatifida*. F, *andino* morphotype. G, *chaqueño* morphotype. H, *mesopotamico* morphotype. I, *pampeano* morphotype. J, *serrano* morphotype. The dashed lines denotes the distribution of the subspecies and morphotypes.

collections and from herbaria specimens (Supporting Information, S1). The occurrence data were previously subjected to rigorous checking for duplicates and inconsistencies related to geographical distribution (e.g. coordinate typing errors) as proposed by Hijmans *et al.* (2017).

For the PCA, 19 bioclimatic variables under present climatic conditions were obtained from the CHELSA (Karger *et al*., 2016, 2017), which has high resolution and adds the effect of elevation. Also, four biophysical variables (pH and clay, sand and silt content) were obtained from the ISRIC—World Soil Information database (http://soilgrids1km.isric.org) at 30 arcsec resolution (*c*. 1 km) (Supporting Information, Table S2). For edaphic data we calculated a weighted mean from five depths (0–5, 5–15, 15–30, 30– 60, 60–100 cm) to generate a single value for the first 1 m of soil for each layer, and then resampled the data to 30 arc sec resolution to match the CHELSA dataset, using QGis 3.4.2-Madeira (QGIS Development Team, 2018).

The mean average, standard deviation and range of variation of the bioclimatic and soil variables were calculated for each subspecies/morphotype. Since variables can be highly correlated (Graham, 2003), Pearson's correlation coefficients between environmental variables were estimated. Thus, the following five correlated bioclimatic variables (with $\rho > 0.70$) were removed from the PCA: maximum temperature of warmest month (BIO 5), minimum temperature of coldest month (BIO 6), mean temperature of driest quarter (BIO 9), mean temperature of warmest quarter (BIO 10) and mean temperature of coldest quarter (BIO 11). Calculation of the PCA was done on the correlation matrix estimated from the resulting standardized data set. Moreover, to evaluate the existence of significant differences among subspecies/morphotypes, a one-way multivariate analysis of variance (MANOVA) with *post-hoc* Hotelling analysis to identify homogeneous subsets was performed. A series of separate ANOVAs after Bartlett's test of homogeneity were used to investigate the nature of among-subspecies/morphotype variation for each of the environmental variables alone. Tukey's test was used to test differences between each pair of means. The significance level for all tests was $\alpha = 0.05$. These statistical analyses were performed using Infostat v.2016 (Di Rienzo *et al.*, 2016).

Ecological niche modelling (ENM) under current climatic and soil conditions was performed to analyse the potential distribution for the complex and each subspecies/morphotype. For this analysis, the

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occurrence points used for final models were separated by 5 km to reduce the effect of spatial autocorrelation due to geographical clustering. After that, 594 points of occurrence were selected. To determine the area 'M' of mobility of each taxon (area to be modelled) according to the BAM diagram (Soberón & Peterson, 2005, Barve *et al.*, 2011), polygons were clipped to a buffer zone of 200 km around the distribution points recorded for both the whole *T. sidoides* complex and for each morphotype. The 19 bioclimatic and the four soil variables described in the PCA were included in the analysis. ENMs were performed using MaxEnt (Phillips, Anderson & Schapire, 2006; Phillips *et al.*, 2017) in the R software package 'DISMO' (Hijmans *et al.*, 2017). MaxEnt was run using default settings: random test percentage = 30, convergence threshold = 0.00001, maximum iterations = 1000, averaged across ten runs and the autofeatures option. We utilized the lowest value of probability of occurrence, obtained among the trustable points of presence used in the model for each subspecies/morphotype, as the threshold value for each prediction. Finally, to assess the predictive power of ENMs, we used the area under the receiver operator curve (AUC) and Kohen's kappa statistics (KAPPA), with k-fold partitioning of occurrence data. AUC and KAPPA > 0.7 indicate that models could predict occurrences with high accuracy. The niche overlap statistical framework was employed to compare niches among subspecies/morphotypes using the metric D of proportional similarity of the distribution (Schoener, 1970). This metric ranges from 0, representing 'no overlap', to 1, representing 'complete overlap'. In addition, niche equivalence and similarity tests (Warren, Glor & Turelli, 2008; Broennimann *et al.*, 2012) were used to assess the niche divergence or conservatism among subspecies/morphotypes, and among cytotypes of the complex. A niche equivalence test was applied to estimate whether the ecological niches of the subspecies/morphotypes and cyotypes of the *T. sidoides* complex are significantly different and whether the niche spaces are interchangeable. The niche equivalence was determined by comparing the niche overlap values (D) of the subspecies/morphotypes and cytotypes under study to a null distribution of 1000 overlap values. If the niche overlap value fell outside the 95% of the null hypotheses, the equivalency of the two niches could be rejected. A niche similarity test compares the niche overlap of one range randomly distributed over its background, while keeping the other unchanged (1→2), and then carries out the reciprocal comparison (1←2). For the similarity test, $P > 0.05$ was considered to indicate that niches were no more similar than expected by chance. These analyses were performed in R software v.3.6.2 (R Development Core Team, 2019) and niche equivalency and similarity tests were run using the 'Ecospat' package (Di Cola *et al.*, 2017).

PLOIDY ESTIMATION AND CYTOGEOGRAPHY

The ploidy of 108 populations (Supporting Information, Table S1) was estimated by meiotic chromosome counting and flow cytometry according to the methods described in Kovalsky & Solís Neffa (2012) and others were summarized from previous contributions (Table S1, Appendix S1) (Fernández, 1987; Solís Neffa & Fernández, 2001; Solís Neffa *et al.*, 2004; Speranza *et al.*, 2007; Elías *et al.*, 2011; Kovalsky & Solís Neffa, 2012; Kovalsky *et al.*, 2018; Moreno *et al.*, 2021).

The geographical distribution of ploidy was analysed based on geo-referenced points obtained during the field sampling. Maps were made using the software QGis 3.4.2-Madeira (QGIS Development Team, 2018). The ecological preferences of each cytotype were determined from observations in the field during collection trips and by analysing the distribution of ploidy in the area of occurrence of each subspecies/morphotype derived from ENM under the current environment.

GENETIC DIVERGENCE AND TIME DIVERGENCE ESTIMATES

To evaluate the genetic divergence and estimate the divergence times among the subspecies/morphotypes of the *T. sidoides* complex and to test the origins of multiple independent polyploid, a subset of 77 sampled diploid and polyploid individuals, representative of all subspecies/morphotypes, was genetically analysed using sequences of the plastid DNA regions *trnL–trnF* and *matK–trnK* previously obtained (Moreno *et al.*, 2018) and deposited in the GenBank (accession numbers in Supporting Information, Table S1). Sequences were concatenated for all the analyses, manually edited with Chromas 2.33 (http://www.technelysium.com.au) and aligned with the Clustal W method implemented in MEGA 5 (Tamura *et al.*, 2011). The polymorphic positions detected in the alignment were corroborated in the original chromatograms. All poly-T/A stretches were eliminated from the analyses since the mutational dynamics that rules the insertion/deletion events (indels) involved in poly-T/A stretches cannot be assessed (Aldrich *et al.*, 1988; Kelchner, 2000). Also, all contiguous indels were treated as one mutational event (Simmons & Ochoterena, 2000).

Haplotypes were determined using DnaSP 5.0 (Librado & Rozas, 2009). Standard descriptive molecular diversity statistics (number of segregating sites, S; nucleotide diversity, π; and haplotype diversity, h) were calculated with Arlequin 3.5.1.2 (Excoffier & Lischer, 2010). Population genetic structure was assessed by analysis of molecular variance (AMOVA, Excoffier, Smouse & Quattro, 1992) using the same software. To elucidate the levels of population structuring, AMOVA

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was based on groups of populations according to the subspecies/morphotypes. Evolutionary relationships among haplotypes were estimated by the median-joining method (ε = 0; Bandelt, Forster & Röhl, 1999) implemented in Network 4.5.0.0 (http://www.fluxus-engineering.com) for the complex as a whole. Absolute divergence times between samples were estimated using the Bayesian inference method implemented in BEAST 1.8.0 (Drummond *et al.*, 2012). *Turnera fernandezii* Arbo and *T. melochioides* Cambess. were included as outgroups based on Arbo, Gonzalez & Sede (2015), Moreno *et al.* (2018) and Rocha *et al.* (2019). Two independent runs of 1×10^8 Markov chain Monte Carlo (MCMC) iterations were performed, sampling every 1000 generations. The initial 10% of the iterations were removed as burn-in. To define the molecular clock to be used, we calculated the marginal likelihood of the data under an uncorrelated relaxed log normal clock (URLC) and strict clock (SC) using the stepping stone sampling (Xie *et al.*, 2011) and path sampling methods (Lartillot & Philippe, 2006) in the software BEAST 1.8.0 (Drummond *et al.*, 2012). We then used these marginal likelihoods to calculate a Bayes factor (BF) (Jeffreys, 1935) for each comparison and interpreted the results according to the standard reported by Kass & Raftery (1995). The GTR+G substitution model was selected using the corrected Akaike information criterion (AICc; Kelchner & Thomas, 2007) with the software jModeltest 2.1.4 (Guindon & Gascuel, 2003; Darriba *et al.*, 2012), using the Yule tree prior and a log-normal molecular clock. Tracer 1.5 (Rambaut & Drummond, 2009) was used to check for convergence of the Markov chains and adequate effective sample sizes (ESS > 200). All trees generated were summarized in a maximum clade credibility tree using TreeAnnotator 1.7.1 (Drummond *et al.*, 2012), which was edited in FigTree 1.3.1 (Rambaut, 2009). Statistical support was determined by assessing the Bayesian posterior probabilities (Rannala & Yang, 1996). In the absence of fossil records, we used the mean nucleotide substitution rates for plastid markers calculated for shrubs and herbaceous plants with a generation time of up to 3 years (Ramos-Fregonezi *et al.*, 2015) as a model for estimating the divergence times among samples of the *T. sidoides* complex. We used a mean substitution rate of 1.60×10^{-9} per site per year, with a gamma distribution prior, with a shape parameter of 1.60 and a scale parameter of 1.60×10^{-9} as prior, and we assumed an offset value of 1×10^{-9} substitutions per site per year. Time scales were set according to Cohen *et al.* (2013).

To test the multiple independent origin of polyploid series in different lineages of the complex, each cluster or subcluster corresponding to the different subspecies/morphotypes of the *T. sidoides* complex was inspected to detect diploids and polyploids with the same (or closely derived) haplotype, assuming that tetraploids arose from diploids having the same plastid DNA haplotype (Segraves *et al.*, 1999). The minimum of independent origins was inferred by counting the number of branches with different haplotypes that had diploids and tetraploids in co-occurrence, and the maximum number of origins was determined by the number of different plastid DNA haplotypes harboured by polyploids. Clusters composed of only polyploids and having a particular haplotype (without diploids with related haplotypes) were considered to have arisen from an independent origin from an extinct or an as yet uncollected diploid.

POLYPLOIDY AND RANGE EXPANSION

To make inferences about the possible distributions of cytotypes in the past, a further ENM analysis under different past climatic scenarios was done for the whole *T. sidoides* complex, and separately for diploid, tetraploid and hexaploid cytotypes. For modelling, the 19 bioclimatic variables for current climate obtained from CHELSA (Karger *et al.*, 2017), and the database for historical weather obtained from PaleoClim (Otto-Bliesner *et al.*, 2006; Hill, 2015; Fordham *et al.*, 2017; Karger *et al.*, 2017; Brown *et al.*, 2018) were used, both with a 2.5 arc-min resolution. This was possible since the two bases are equivalent, having the same temperature and precipitation data, from which annual, quarterly, monthly and range averages are summarized in 19 bioclimatic variables. The models were built on the current climatic conditions and then projected onto the mid-Pliocene warm period (3.2 Mya), Marine Isotope Stage (MIS) 19 (*c.* 787 kya), Last Interglacial (*c.* 130 kya), Last Glacial Maximum (LGM) (*c.* 21 kya) and mid-Holocene (8.3–4.2 kya).

RESULTS

ECOLOGICAL DIFFERENTIATION OF SUBSPECIES AND MORPHOTYPES

Multivariate analysis (PCA) based on bioclimatic data showed that subspecies and morphotypes of the *T. sidoides* complex inhabit regions with different environments and that the first two components accounted for 71.8% of the total variance. The loadings of each variable to components 1 and 2 are shown in Supporting Information Table S2. Most of the environmental data variability (43.7%) was explained by the first ordination axis (Fig. S1). This axis represented a major gradient in annual precipitation, precipitation of the driest month, precipitation seasonality, precipitation of the driest quarter, precipitation of the coldest quarter, soil pH and clay/sand content. Three groups were recognized. The

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first, composed of the *andino*, *chaqueño* and *serrano* morphotypes, was in the left extreme of the first axis with the highest values of soil pH, content and precipitation seasonality. The second, composed of the *grandense* and *mercedeño* morphotypes and subspp. *holosericea* and *sidoides*, was on the other extreme with the highest values of clay content, annual precipitation, precipitation of the driest month and precipitation of the coldest quarter. The *pampeano* and *mesopotamico* morphotypes, and subspecies *integrifolia*, composed the third group and lay between the two formerly described groups. The second component (28.1%) was mostly explained by isothermality, precipitation of the wettest month, precipitation of the wettest quarter and precipitation of the warmest quarter. In this axis, all the subspecies/morphotypes analysed were further spatially separated into each group. MANOVA and ANOVA showed that all the bioclimatic and edaphic variables differ significantly among subspecies/morphotypes (Table S3).

ENMs under current climatic and edaphic conditions predicted different suitable areas for each subspecies/morphotype of the *T. sidoides* complex (Fig. 3). The AUC (0.80 in subsp. *holosericea* and 0.90 in the remaining subspecies/morphotypes) and KAPPA statistics (0.60: *andino* and *serrano* morphotypes, 0.70: subspp. *holosericea* and *integrifolia* and *mesopotamico* and *pampeano* morphotypes, 0.80: *chaqueño* morphotype, and 0.90: subsp. *sidoides* and *grandense* and *mercedeño* morphotypes) indicated that most models performed well at predicting current subspecies/morphotype distributions. The predicted areas, although congruent with the respective points of occurrence of the subspecies/morphotype, extended slightly beyond the current ranges of each one (Fig. 3). The percentages of contribution of each bioclimatic and edaphic variable to the ENM are shown in Supporting Information Table S4. The projections onto current climatic conditions showed that, although the areas with high probability of occurrence for the *mercedeño* and *grandense* morphotypes and subspp. *holosericea*, *integrifolia* and *sidoides* partially overlap in the Uruguayan savanna ecoregions, most of these subspecies/morphotypes are segregated within this ecoregion extending towards the neighbouring ones. The areas with higher probability of occurrence for the other subspecies/morphotypes of the complex were associated with different ecoregions. For the *mercedeño* morphotype (Fig. 3A), these areas are along both banks of the Uruguay River (north-east of Espinal and north-west of the Uruguayan savanna ecoregions), extending north-eastwards into the Mesopotamian savannas and Atlantic Forest ecoregions. The most suitable areas for the *grandense* morphotype (Fig. 3B) and subsp. *holosericea* (Fig. 3C) are in the Uruguayan savanna ecoregion associated with elevated areas of southern Brazil (Serra do Sudeste) and Uruguay (Cuchilla de Haedo and Eastern Serranías), although the area of the *grandense* morphotype also extends further south towards the Cuchilla Grande. Highest suitabilities for subsp. *integrifolia* (Fig. 3D) are along both banks of the Uruguay River, mostly in the north-west of the Uruguayan savanna ecoregion, but also west of the Iberá wetland system (Humid Chaco); and for subsp. *sidoides* (Fig. 3E) they are restricted to the Eastern Serrania (south-east of the Uruguayan savanna ecoregion). The most suitable area for subspecies *pinnatifida* was independently modelled for each morphotype. The best predicted areas for each one were: the southern Andean Yungas for the *andino* morphotype (Fig. 3F); the Dry Chaco (north-western and central Argentina, and southern Bolivia) and Humid Chaco (north-eastern Argentina) for the *chaqueño* morphotype (Fig. 3G); the Humid Chaco (central Mesopotamia, south-west of the Iberá system) for the *mesopotamico* morphotype (Fig. 3H); the south-eastern grasslands of the Humid Pampa ecoregion associated with the de la Plata River (in Argentina and Uruguay) for the *pampeano* morphotype (Fig. 3I); and the Pampean ranges (south-west of Dry Chaco) for the *serrano* morphotype (Fig. 3J). All the most suitable areas modelled were consistent with the present geographical distribution of the subspecies/morphotypes.

Despite the overlapping ranges at a geographical scale, the subspecies/morphotypes of the *T. sidoides* complex differed in their ecological preferences and did not overlap at a local scale due to the heterogeneity and patchy arrangements of habitats. In the north-western area of the complex, *andino*, *chaqueño* and *serrano* morphotypes co-occur. However, each occupies segregated geographical and ecological areas. In central Mesopotamia, some contact/overlapping zones were detected associated with the ecotone among Humid Chaco, Espinal and Mesopotamian savanna ecoregions (Supporting Information, Fig. S2). In such zones, *chaqueño* and *mesopotamico* morphotypes occur in alkaline soils, the *mercedeño* morphotype grows on red lateritic clays and on outcrops of silicified red sandstone and basalt, and subsp. *integrifolia* grows on sandy soils. Niche overlap of the subspecies/morphotypes of the *T. sidoides* complex in environmental space (Table S5) was significantly lower than the null hypothesis of niche equivalency ($P < 0.05$) for most subspecies/morphotypes analysed. These results indicate that the bioclimatic niches of each subspecies/morphotype differ more among each other than expected by chance. The highest D values ($D = 0.50$–0.60) were detected among the *grandense* morphotype, subspp. *holosericea* and *integrifolia*, and the *pampeano* morphotype.

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PLOIDY ESTIMATION AND CYTOGEOGRAPHY

In each subspecies/morphotype, different cytotypes were recorded. Except for the *mercedeño* morphotype, in which diploids, triploids and tetraploids occur on sandstone outcrops with woodland vegetation on the Mercedes plateau or in subtropical grasslands south and east of the Iberá wetland system (including hexaploids) (Fig. 3A), the cytotypes of each series are usually geographically segregated and occur in different environments. The *grandense* morphotype (Fig. 3B) has two diploid populations in the plains adjacent to the south-eastern slope of the Cuchilla de Haedo, whereas tetraploids grow in stony soils of the ravines and in prairies of the Cuchilla Grande del Este (Uruguay), and Serra de Sudeste (southern Brazil). In subsp. *holosericea* (Fig. 3C), two diploid populations occur on the eastern slopes of the Cuchilla de Haedo. Tetraploids and hexaploids are more frequent and inhabit the stony ravines of the Cuchilla de Haedo and Cuchilla Grande and prairies with flat hills in Uruguay and hilly systems in southern Brazil. In subsp. *integrifolia* (Fig. 3D) there is a polyploid series from diploid to octoploid. Diploids occur on the sandy ridges of Mesopotamia frequently associated with open grasslands and palm groves of *Butia yatay* (Mart.) Becc. and in the sandstones of the crystalline basements in northern Uruguay. The most common habitat of tetraploids includes the valley of the Uruguay River, although they may also grow in the stony ravines of the basaltic slopes of the Cuchilla de Haedo. Hexaploids and the only known octoploid were found in the north-eastern part of the range of the subspecies. Subspecies *sidoides* is the only subspecies composed only of tetraploids; these occur in central Uruguay and in the stony hills of Eastern Serranías. The only exception is a pentaploid found in grasslands of the hilly systems of southern Brazil (Fig. 3E).

In subsp. *pinnatifida*, diploids of the *andino* morphotype (Fig. 3F) are mostly distributed in temperate Andean valleys from 23°S to 29°S (1467–782 m a.s.l.), whereas tetraploid populations are found in lower subtropical valleys or in mountain grasslands (up to 2566 m a.s.l.). Diploid–tetraploid contact zones were detected in two large inter-Andean valleys, the Quebrada de Humahuaca and the Lerma valley. In the *chaqueño* morphotype (Fig. 3G), diploids are restricted to the north-western boundary of the Dry Chaco, close to diploids of the *andino* morphotype. Tetraploids extend throughout the Dry and Humid Chaco plains, whereas hexaploids occur in the Dry Chaco and in the south-eastern area of the morphotype. In the *mesopotamico* morphotype (Fig. 3H), diploids (the first report here), tetraploids and hexaploids are distributed on stony soils of the Mercedes plateau and in north-western Uruguay. In the *pampeano* morphotype (Fig. 3I), diploids (the first report here), tetraploids and hexaploids occur on deep humic soils of the grasslands at the south-eastern extreme of the Humid Pampa ecoregion. Diploids and hexaploids of the *serrano* morphotype (Fig. 3J) were found in only two localities each, the former at higher elevations (up to 1600 m a.s.l.) than the latter. Tetraploids extend along deep valleys over the whole area.

Analysis of the cytotypes in the complex as a whole (Fig. 4D) shows that the tetraploid is the most widely distributed cytotype, covering the whole distribution area of the species, except in the north-western extreme. Diploids are distributed in both the north-western and south-eastern centres of variation of the *T. sidoides* complex. In the north-west, diploid populations are the only ones found in the inter-Andean valleys (2580–782- m a.s.l.) and western lowlands of the Dry Chaco (850–194 m a.s.l). However, in the south-east, diploids are rare among tetraploid populations at a geographical scale. Hexaploids, in general, showed a peripheral distribution and are mainly concentrated in the south-eastern centre. Connecting these two centres of variation, along the ranges that bordered the south-western Chaco-Pampean lowlands, are diploids and tetraploids of the *serrano* and *pampeano* morphotypes in the ranges (Pampean, Ventania and Tandilia) and the surrounding lowlands, respectively.

Only in a few localities, contact zones between tetraploid populations of two different subspecies were registered. All the contact zones between different subspecies so far identified corresponded to tetraploid populations or populations with higher ploidy levels (V. G. Solís Neffa, pers. obs.), except one diploid–tetraploid contact zone in Uruguay (Moreno *et al.*, 2015).

GENETIC DIVERGENCE AND TIME DIVERGENCE ESTIMATES

The descriptive molecular diversity statistics estimated for *T. sidoides* complex are summarized in Supporting Information Table S6. The concatenated alignment resulted in a 1012-bp sequence with 28 polymorphic sites. Haplotype reconstruction for all individuals of the *T. sidoides* complex and the outgroup resulted in 31 haplotypes, two of them (H1 and H2) only present in the outgroup (Tables S1 and S6; Fig. 5). Relatively high values of haplotype diversity (h = 0.71–1.0) were detected in most subspecies/morphotypes, except the *andino* and *mesopotamico* morphotypes (h = 0.50). Also, low values of nucleotide diversity (π = 0.01–0.25) were observed in all subspecies/morphotypes. The most frequent haplotypes were H29 and H3, found in 15.19% and 12.66% of the samples, respectively. Twenty out of the 29 haplotypes found in *T. sidoides* complex were specific to subspecies/morphotypes (Tables S1 and S6).

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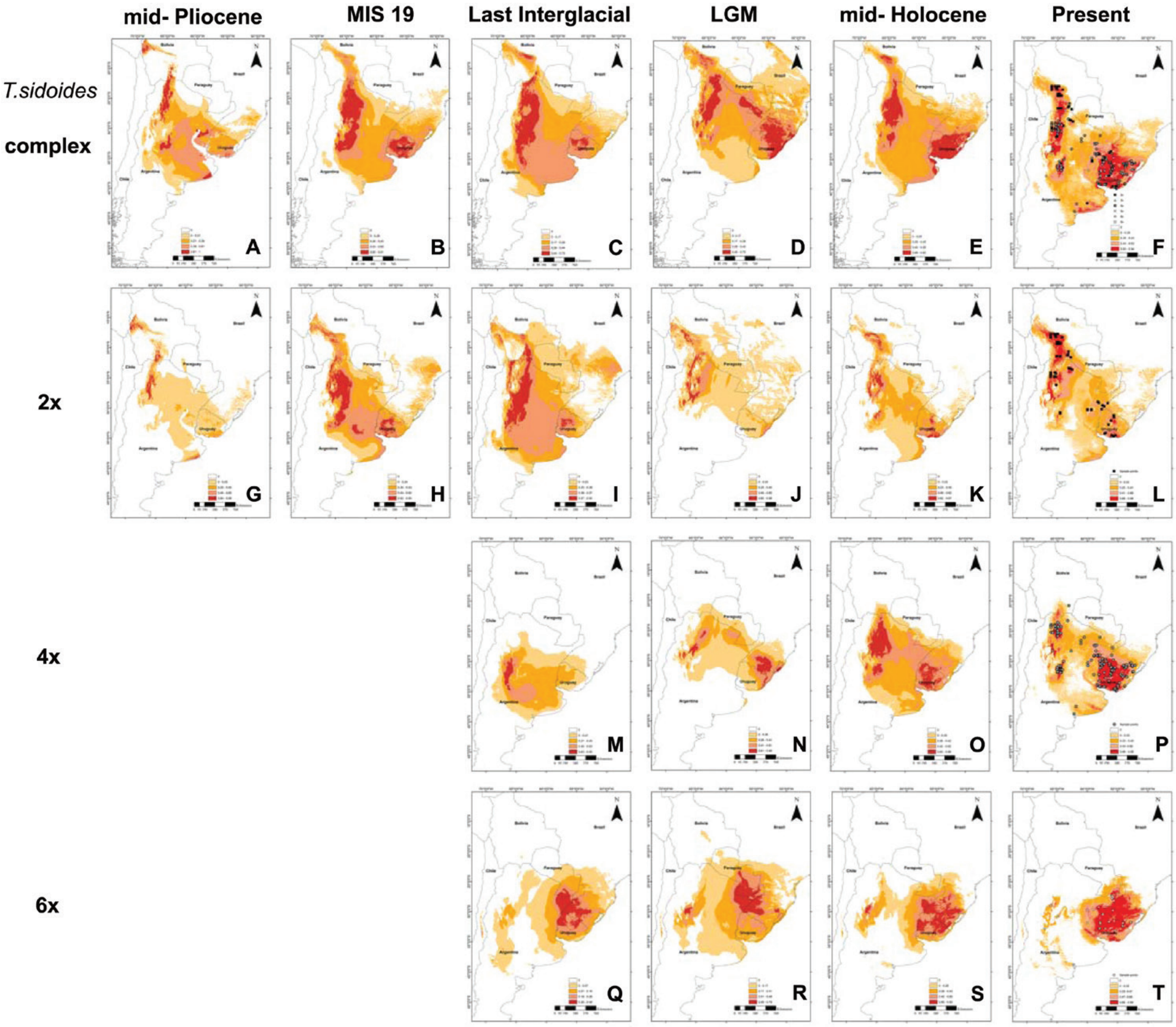

Figure 4. Spatial projections of climatic niches of the *Turnera sidoides* complex from the mid-Pliocene to current scenarios. A–F, *T. sidoides* complex. G–L, diploid populations. M–P, tetraploid populations. Q–T, hexaploid populations. A, G, mid-Pliocene warm period (3.2 Mya). B, H, MIS 19 (*c*. 787 kya). C, I, M, Q, Last Interglacial (*c*. 130 kya). D, J, N, R, Last Glacial Maximum (*c*. 21 kya). E, K, O, S, mid-Holocene (8.3–4.2 kya). F, L, P, T, current conditions.

At the infraspecific level, the *serrano* and *chaqueño* morphotypes had the highest number of exclusive haplotypes (four haplotypes each; in contrast, the *mercedeño* morphotype was the only one without any specific haplotypes. At the cytotype level, exclusive haplotypes were found in diploids of subspp. *holosericea* and *integrifolia* and in the *andino*, *mesopotamico* and *serrano* morphotypes. Also, they were found in tetraploids and/or hexaploids of the other subspecies/morphotypes (Supporting Information, Table S1). Usually, the same, or directly derived haplotypes, were found in diploids and polyploids within each subspecies/morphotype. The AMOVAs were statistically significant and revealed that most of the variation was explained by differences within subspecies/morphotypes (Table S7).

Genealogical relationships of plastid DNA haplotypes are shown in Figure 5. The haplotype network revealed that the outgroup haplotypes were clearly differentiated from the ingroup, and that the latter split into two large haplogroups. The most frequent haplotypes (H3 and H29) appeared as central haplotypes in the network, from which several less frequent haplotypes derived. Nine haplotypes formed clade I, and included samples of all the subspecies, except those of subsp. *pinnatifida*. In this haplogroup, haplotype H14 (found in tetraploids of subspp.

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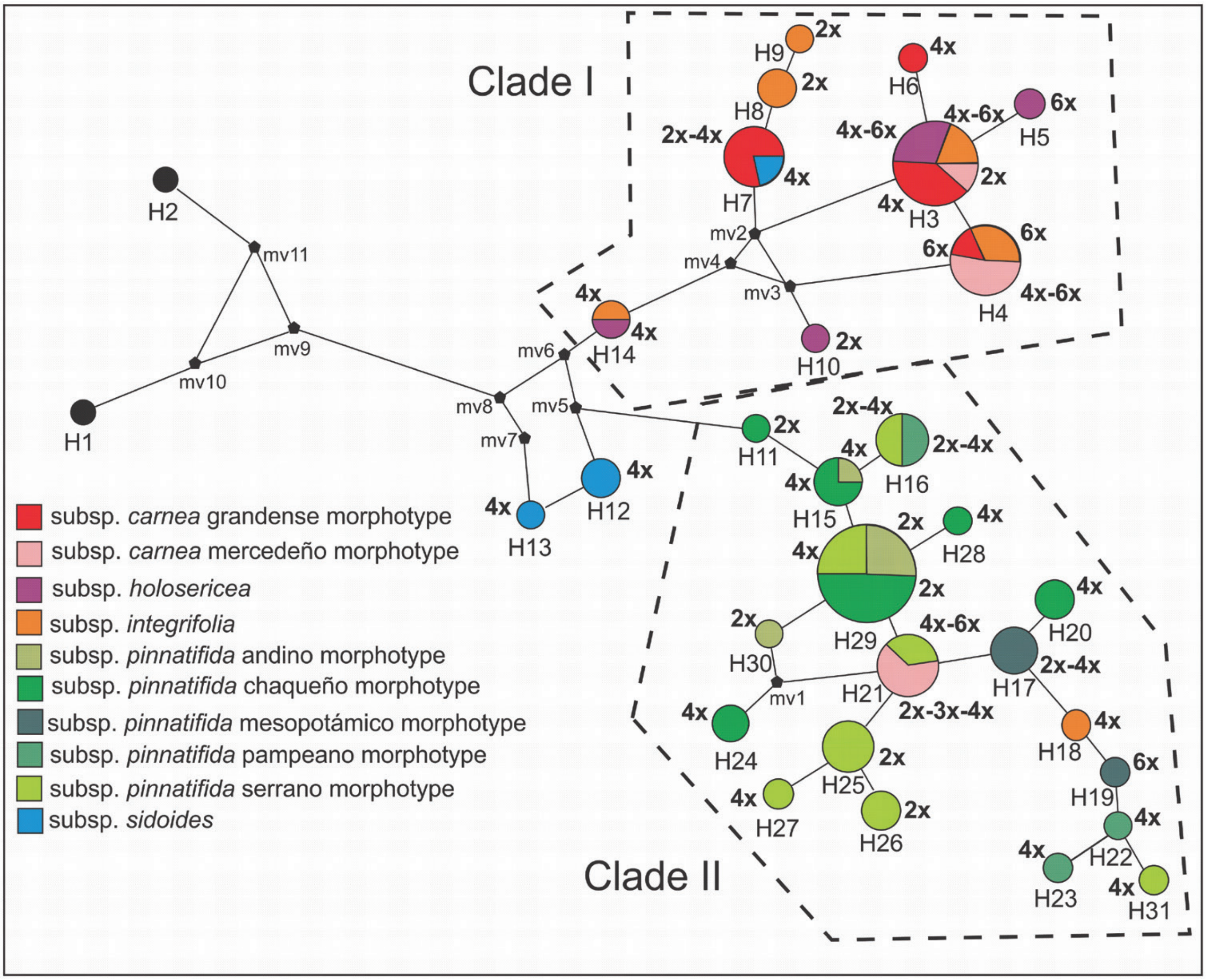

Figure 5. Evolutionary relationships among haplotypes of the *Turnera sidoides* complex plastid DNA using a median-joining network approach. Each circle represents a haplotype. Circle sizes are proportional to haplotype frequency. The lines linking haplotypes represent relationships between them. The clades identified are represented in different colours. The outgroup is represented in black. Mv: median vectors.

holosericea and *integrifolia*) was basal and less frequent in relation to haplotype H3, which was detected in diploids and tetraploids of the *mercedeño* morphotype, in tetraploids of the *grandense* morphotype, and in tetraploids and hexaploids of subspp. *holosericea* and *integrifolia*. Clade II was the most diverse, despite being mostly composed of subsp. *pinnatifida*. The only exceptions were one population of the *mercedeño* morphotype and one of subsp. *integrifolia*. Sixteen of the 18 haplotypes detected in this clade were exclusive of the morphotypes of subsp. *pinnatifida*. One central haplotype (H29) was shared by diploids of the *andino* and *chaqueño* morphotypes and by tetraploids of the *serrano* one. All the other haplotypes derived, directly or indirectly, from this central haplotype. Exceptions were the basal H11 found in a diploid population of the *chaqueño* morphotype and H15 shared by tetraploids of the *andino* and *chaqueño* morphotypes. All subspecies and morphotypes presented haplotypes belonging to only one clade; with the exception of two populations, one of the *mercedeño* morphotype and the other of subsp. *integrifolia*, which showed haplotypes belonging mainly to clade I, but also H21 (*mercedeño* morphotype) and H18 (subsp. *integrifolia*) of clade II.

The clades predicted by the Bayesian phylogenetic tree (Supporting Information, Fig. S3) were consistent with the configuration of the haplotype network. The origin of the *T. sidoides* complex lineage was estimated at the early Pleistocene (1.54 Mya), and the earliest divergence within the species, between clades I and II, was estimated as early as 1.17 Mya. Diversification since 0.89 Mya in clade I corresponded to subspecies/

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morphotypes restricted to the south-eastern centre of variation. In clade II, an early split (1.11 Mya) of the lineage that corresponds to subsp. *sidoides* (of the south-eastern centre) was observed. Further splits, since 0.96 Mya, recovered in clade II, corresponded to the morphotypes of subsp. *pinnatifida*. In each main lineage, many cladogenetic events occurred in the last 0.5 My. However, it is from the last 0.25 My that the greatest diversification occurred, with the emergence of the most extant polyploid populations.

The haplotype network (Fig. 5) and the Bayesian tree (Supporting Information, Fig. S3) showed that polyploid series appeared independently several times in different subspecies/morphotypes. Clade I has independent lineages with diploids, tetraploids and hexaploids in subspp. *carnea*, *integrifolia* and *holosericea*. Clade II has diploids and polyploids (tetraploids and hexaploids) in each independent lineage of all the morphotypes of subsp. *pinnatifida*.

POLYPOIDY AND RANGE EXPANSION

The results of ENM analysis under different past and current climatic scenarios are presented first considering all populations of the *T. sidoides* complex and then for diploid, tetraploid and hexaploid cytotypes independently (Fig. 4). Climatic layers for ENM under past climatic conditions are available for the Pliocene (*c*. 3.3 Mya), the mid-Pliocene warm period (3.2 Mya) and from MIS 19 (*c*. 787 kyr) to the late Holocene (4.2–0.3 kya). Since the lineage split of the *T. sidoides* complex was estimated at 1.5 Mya, none of the projected models can provide appropriate information to clearly understand the early steps of diversification of the complex. However, with this in mind, we assayed the ENM from the mid-Pliocene warm period (3.2 Mya) as the best available approach to infer the most suitable areas for the ancestral lineage that gave rise to the *T. sidoides* complex.

Under this scenario, four most suitable areas for the ancestral lineage (Fig. 4A) were detected during the mid-Pliocene. The most extensive was located in the north-western centre of variation of the current species range (north-western Argentina and south-western Bolivia) along the Eastern Cordillera and sub-Andean ranges, extending somewhat further to the north-west than the current boundary. The other, less extensive, areas were predicted in the Pampean ranges, in the Mesopotamia and in the south-eastern extreme of the current distribution of the complex.

The distribution models for MIS 19 (Fig. 4B) showed two large most suitable areas, one of them in the north-west (extending along the Subandean and Pampean ranges) and the other in the south-east (Uruguayan savanna) that are coincident with the two current centres of morphological and genetic diversity of the species.

These two areas remained almost stable during the Interglacial period, although with a slight expansion of the north-western area and a retraction of the south-eastern one (Fig. 4C). For the LGM (Fig. 4D), projections identified a retraction of the western suitable area of the complex and a range expansion of the eastern one towards north-eastern Uruguay and southern Brazil. Also, the model identified a further suitable area in the Chaco lowlands, connecting both centres of variation of the complex, along the present day west–east channels of the Pilcomayo and Bermejo Rivers. During the mid-Holocene (Fig. 4E), projections predicted a retraction of the eastern suitable area for the species and the disappearance of the suitable area in the Chaco. The climatically favourable area in the north-west is now fragmented (Fig. 4F), whereas in the east, the suitable area extended throughout Uruguay and southern Brazil (Uruguayan savanna ecoregion).

Considering each cytotype of the complex individually, the ENM including exclusively all diploid populations showed that the most suitable climatic area during the Pliocene was restricted to a narrow area at the north-western boundary of the current species distribution (Fig. 4G). Analysis suggested an expansion of this area southwards during MIS 19, and identified two other smaller suitable areas in the Pampean ranges and at the south-eastern limit of the current species distribution (Fig. 4H). In the Interglacial period (Fig. 4I), the north-western area expanded northward, and it retreated in the south-east. Modelling for the LGM (Fig. 4J) predicted a strong retraction for the diploids over the whole area, only a narrow suitable area remaining along the Sierras Subandinas. The models suggested a progressive re-expansion of climatically favourable areas for diploids from the mid-Holocene (Fig. 4K) to the present (Fig. 4L), at both the north-western and the south-eastern boundaries for the species. Currently, the area of major suitability of diploid occurrence, although fragmented, is similar to that predicted for the complex as a whole in the north-western centre of variation. However, at the south-eastern boundary the predicted area for diploids is much smaller than that predicted for the complex.

Models for tetraploids and hexaploids were made from the Last Interglacial to the present to infer the impact of the last climatic shifts on the polyploid series. During the Last Interglacial period, models for tetraploids (Fig. 4M) predicted a narrow area of climatic suitability in the Pampean ranges. For the LGM (Fig. 4N), the model showed that the suitable area in the north-west became fragmented but identified a new large area of high suitability at the south-eastern extreme of the complex range

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(in southern Brazil). In the mid-Holocene (Fig. 4O), models predicted two large areas of climatic suitability for tetraploids, one in the north-western centre somewhat shifted towards the Chaco lowland compared to diploids, and the other, at the south-eastern boundary, which has remained almost stable since the LGM. The latter area continued to expand up to the present day and is currently the largest and most suitable area for tetraploids (Fig. 4P). However, the model revealed a reduction and fragmentation of the suitable area in the north-west.

Finally, the distribution models for hexaploids identified a favourable climatic area in Mesopotamia (Argentina), south-eastern Brazil and north-western Uruguay in the Last Interglacial period (Fig. 4Q). During the LGM, models predicted a northward shift of this area (Fig. 4R) and identified a second, smaller suitable area in the Pampean ranges. These areas were maintained in the mid-Holocene, although in the south-east this shifted southwards again toward south-eastern Brazil and Uruguay (Fig. 4S). At present, the model predicts a single large and continuous area in the south-east, covering almost all of Uruguay, southern Brazil and Mesopotamia (Fig. 4T).

DISCUSSION

The evolutionary processes that shaped the unique biodiversity of the southern Neotropical ecoregions (Miñarro & Bilenca, 2008) are still largely unknown (Rull, 2020). Here, we provide insights into the patterns and the processes associated with the origin and maintenance of the great intraspecific variation in the *T. sidoides* complex. In particular, this model was analysed to assess the role that autopolyploidy may have played in the diversification and range expansion of herbs in the Chaquean Domain.

The results provided strong evidence for the existence of many lineages evolving in parallel polyploid series that may fit the requirements of true species according to the biological, evolutionary or ecological species concepts (Solís Neffa, 2000; Elías, 2010; Roggero Luque, 2017). In this way, we provide information on the broader issue of how environmental changes and climatic oscillations during the Quaternary (Iriondo & García, 1993; Villagrán & Hinojosa, 1997; Gregory-Wodzicki, 2000) affected the evolutionary history of autopolyploid herbaceous plants adapted to the different ecoregions in the mid-latitude Neotropics. Moreover, the *T. sidoides* complex has highlighted the huge diversity existing in this type of autopolyploid complex, which is overlooked when estimates are made base on only the morphological species concept.

CURRENT DISTRIBUTION AND ECOLOGICAL NICHE PREFERENCES

Environmental heterogeneity has been proposed to be an important diversity-generating factor in some Neotropical species complexes (Svenning, 2001; Perret *et al.*, 2007; Turchetto *et al.*, 2014; Aguilar *et al.*, 2020). The *T. sidoides* complex shows a marked morphological variation along its wide latitudinal and longitudinal range (Arbo, 1985; Solís Neffa, 2000, 2010; Moreno *et al.*, 2021) in the environmentally heterogeneous Chaquean Domain (Cabrera & Willink, 1973; Iriondo & García, 1993). In this Domain, variation in geological substrate, climate and soil conditions contribute to the diversity of vegetation types (Carnevalli, 1994; Prado & Gibbs, 1993; Morello & Mateucci, 1999; Waechter, 2002). Here, the geographical segregation observed for most subspecies/morphotypes of the *T. sidoides* complex could be related to significant differential ecological preferencies among the subspecies/morphotypes, mainly in precipitation regime and soil conditions (pH and clay/sand content). Despite the ecogeographical overlap detected here and in previous studies (Solís Neffa, 2000; Moreno *et al.*, 2015) for some subspecies in the south-eastern centre of variation of the *T. sidoides* complex, ENM analysis revealed that the ecological niche differs significantly among the areas of the subspecies/morphotypes. These results showed that the abiotic environmental factors underlying each particular habitat in the southern ecoregions of the Chacoan Domain (Prado & Gibbs, 1993; Carnevalli, 1994; Morello & Mateucci, 1999; Waechter, 2002) are shaping, at least in part, the geographical distribution of the infraspecific diversity in the *T. sidoides* complex.

Since the variation in vegetative morphological traits in the *T. sidoides* complex has a genetic basis and is spatially structured, it was suggested that the diagnostic traits of the subspecies/morphoypes (leaf indumentum and shape and the degree of incision of the leaf blade) would constitute adaptive traits (Solís Neffa, 2010; Moreno *et al.*, 2021). In addition, wide variation in stomata size and density was detected in the complex (Solís Neffa, 2000), a trait suggested to be associated with different water regimes and conditions of evapotranspiration and solar radiation (Bertolino, Caine & Gray, 2019). Our results for the *T. sidoides* complex suggest that the complex pattern of abiotic factors, at both regional and local scales, would favour the maintenance of various combinations of adaptive traits in the different subspecies/morphotypes and probably promote intraspecific diversification. However, further analyses are needed to understand the contribution of biotic and abiotic factors to the distribution patterns of the subspecies/morphotypes of the *T. sidoides* complex at a local scale.

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Regarding variation in the reproductive traits of the *T. sidoides* complex, variation in flower colour is the most outstanding (Arbo, 1985) and geographically structured, with a gradual north-west to south-east variation from yellow to orange and light pink to dark pink (Solís Neffa, 2000, 2010; Moreno *et al.*, 2021). Variation in this floral trait was suggested to be associated with environmental factors such as soil types or UV radiation or, alternatively, to be the result of the adaptive response to divergent selective pressures generated by pollinators (Solís Neffa, 2010). In this sense, yellow and salmon flowers of subsp. *pinnatifida* are perceived differently by bees, and different pollinators visit flowers varying in colour (Benítez-Vieyra *et al.*, 2007). This suggests that biotic factors may also be contributing to the maintenance of the subspecies and morphotype differentiation. Furthermore, the subspecies/morphoypes vary in the levels of cyanide released, which was proposed would provide different tolerances to herbivory (Solís Neffa, Faloci & Seijo, 2003). These few examples serve as a working hypothesis to analyse the effects of biotic factors that may be contributing to the maintenance of the morphological differentiation of the *T. sidoides* complex.

INITIAL DIVERGENCE OF THE COMPLEX AT THE DIPLOID LEVEL AND RANGE EXPANSION OF POLYPLOIDS

We integrated cytogeographical and genetic divergence data with past niche modelling analysis to provide insights into the geographical context of the processes driving divergence and range expansion in the *T. sidoides* complex. Our survey provides new informative data to interpret current patterns of distribution and diversification of the *T. sidoides* polyploid complex.

According to the projected model for the mid-Pliocene (*c.* 3.3–3.0 Mya), four areas were zones of highest climatic suitability for a possible ancestor of the *T. sidoides* complex. The main one, in the north-west of the current range of the species, follows the direction of the Eastern Cordillera and the sub-Andean ranges, and the three smaller ones in the Pampean ranges, in Mesopotamia and, in the south-east of the current range, along the Serranías del Este and the nearby lowlands. These regions were coincident with the proposed refugial centres for the *T. sidoides* complex based on plastid genome diversity (Speranza *et al.*, 2007, Moreno *et al.*, 2018). Among them, the main orographic systems in the north-west of the current area have been proposed as the ancestral geographical area for the *T. sidoides* complex (Moreno *et al.*, 2018). Geomorphological events and aridity (Villagrán & Hinojosa, 1997; Rabassa *et al.*, 2011) that took place between the suitable areas in the north-west and south-east of the ancestral area of the *T. sidoides* complex were suggested to become important barriers to plant dispersal (Simpson, 1975; Collevatti *et al.*, 2020). The spread of large areas of semi-arid conditions in the Chaquean Domain concomitant with the final uplift of the Andes (Gregory-Wodzicki, 2000) would also have acted as a barrier to gene flow among populations of the *T. sidoides* complex, promoting the divergence between both main lineages at *c.* 1.17 Mya by the time of the Great Patagonian Glaciations. The results of genetic divergence analyses showed that further diversification of lineages in the two main clades of the *T. sidoides* complex mostly coincides with colder periods, supporting the notion that cooling and drying during Pleistocene glaciations may have caused the isolation and divergence of ancestral *T. sidoides* complex populations (Moreno *et al.*, 2018). Although these climatic events were also suggested to have contributed to the evolution and diversification of other Chaquean plant species (Catalano *et al.*, 2008; Chiarini *et al.*, 2018; Turchetto *et al.*, 2014; Scaldaferro, Barboza & Acosta, 2018) our analysis is the first to demonstrate their influence on infraspecific diversification of an autopolyploid complex in the southern ecoregions of the Chaquean Domain.

Nevertheless, the predicted distribution of diploids during interglacial periods (MIS 19 and Last Interglacial) suggests a more continuous area of diploids than at present, probably along the most elevated terrains as proposed by Moreno *et al.* (2018), and the pattern of diploid distribution described here for each subspecies/morphotype analysed. The subsequent evolutionary history of the complex (from the LGM to the present day) mostly involved range expansion of the allopatrically differentiated populations to their current limits. During the mid-Holocene, the warmer and wetter climate would have promoted the westward reduction of the area of diploids and the range expansion of tetraploids. Semi-arid conditions during the Late Holocene (Iriondo & García, 1993) may have determined the progressive retraction of the suitable area for diploids, whereas the subhumid climate period would have favoured the persistence of diploids in the inter-Andean valleys.

In the north-western centre of variation of the *T. sidoides* complex, the valleys and ravines along the hilly areas (where most extant diploids occur) would have had more stable environments, serving as refuges for populations during the cold–dry phases, whereas during warmer periods they persisted in the highlands (Vuilleumier, 1971; Ab'Sáber, 1977; Werneck, 2011). The mountain systems may have also provided suitable ecological conditions for the survival of diploids since the origin of the complex. The mountain barriers and elevational shift of the vegetation zones during glacial times (Simpson, 1975; Collevatti *et al.*, 2020) may have

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restricted diploids to the temperate valleys limited by the Eastern Cordillera and sub-Andean ranges, leading to differentiation of the *andino* morphotype. In contrast, tetraploids of this morphotype may have been able to colonize the subtropical valleys and the mountain grasslands. The establishment of more xeric conditions and the settlement of clay soils (Iriondo, 1993; Iriondo & García, 1993) would have limited the eastward expansion of this morphotype into the Chaco plains (Solís Neffa, 2010). However, the finding of some diploid populations of the *chaqueño* morphotype at the western limit of this region supports the hypothesis that some lowland areas may have also served as centres of diploid diversification as previously proposed for the *T. sidoides* complex and other species (Speranza *et al.*, 2007; Moreno *et al.*, 2018). The warmer conditions in the lowlands during the mid-Holocene (Werneck, 2011; Contreras & Zucol, 2019; Ramos *et al.*, 2020) would have favoured the origin and the range expansion of tetraploids from the north-western boundary, towards the east and south of the Chaco plain, concomitant with the migration of the channels of the Pilcomayo, Bermejo, Juramento-Salado and subsidiary rivers and the subsequent colonization of the palaeochannels by the quebracho woodland (Iriondo, 1993; Morello & Mateucci, 1999). The spatial discontinuities and the progressive changes in the Pampean ranges associated with the uplift of the Andes (Luti *et al.*, 1979; Gregory-Wodzicki, 2000) might be enough to act as significant barriers, triggering local adaptation and subsequent differentiation of the *serrano* morphotype (Solís Neffa, 2010). The diploid palaeodistribution model suggests that this morphotype may have evolved in the central part of the Pampean ranges since MIS 19, followed by tetraploid dispersal throughout these ranges and toward the Ventania and Tandilia systems since the Last Interglacial.

The south-eastern centre of variation of the *T. sidoides* complex was a highly suitable area for diploids during the interglacial periods, concomitant with the earliest diversification of the complex. After that period the favourable area for diploids declined, but suitability for polyloids was predicted to increase after the Last Interglacial in central Mesopotamia, northern and eastern Uruguay and southern Brazil, currently the most suitable area for polyploids of the *T. sidoides* complex. The current distribution of subspecies/morphotypes and cytotypes suggests that the subspecies/morphotypes of the *T. sidoides* complex would have differentiated in previously identified refugia in the south-eastern centre (Speranza *et al.*, 2007; Moreno *et al.*, 2018), i.e. subsp. *holosericea* and the *grandense* morphotypes in Cuchilla de Haedo system, subsp. *sidoides* in the Serrania del Este, and subspp. *integrifolia* and *carnea* and the *mesopotamico* morphotype of subsp. *pinnatifida* associated with the dynamics of the Paraná and Uruguay Rivers and to the tectonic uplift of the Mercedes Plateau (Popolizio, 1970). Finally, in the sedimentary Pampean plains, the *pampeano* morphotype may have evolved and diversified. Our results also suggest that polyploids from different genetic origins were able to colonize the newer areas that became available in this centre after the amelioration of the climate.

MULTIPLE ORIGINS OF POLYPLOID SERIES

Turnera sidoides is one of the few well-studied autopolyploid complexes in South America (Solís Neffa, 2000; Solís Neffa & Fernández, 2001, 2002; Elias *et al.*, 2011; Kovalsky & Solís Neffa, 2012, 2016; Roggero Luque *et al.*, 2015; Kovalsky *et al.*, 2018). In this work, ploidy determinations were doubled and the area of sampling was significantly enlarged compared to previous studies (Solís Neffa & Fernández, 2001; Solís Neffa *et al.*, 2004; Elias *et al.*, 2011). The analysis of ploidy considering the whole complex revealed a higher frequency and distribution of tetraploids, the cytotype that gives continuity to the complex throughout its distribution area. This is true for the extant populations and may have been the pattern since the mid-Holocene as inferred from the niche modelling analysis. In contrast, although diploids were found in almost all environments typical of each subspecies/morphotype, their distribution ranges are currently restricted (except for the *andino* morphotype). Likewise, polyploids with higher levels were found in disjunct, usually marginal, areas of the complex. These patterns of cytotype frequencies and distribution support the classification of *T. sidoides* as a mature polyploid complex as initially proposed (Solis Neffa, 2000), following the criteria of Stebbins (1971).

The idea that autopolyploid formation is a rare event was implicit in much of the literature prior to the last decade of the 20th century, but it is now recognized that multiple origins typify polyploid plant species (Soltis & Soltis, 1993, 1999; Segraves *et al.*, 1999). However, recurrent autopolyploidy has been well documented in a few examples, and most cases corresponds to plants from temperate regions of the Northern Hemisphere (Soltis & Soltis, 1993; Segraves *et al.*, 1999; Melichárková *et al.*, 2020; Šingliarová, Zozomová-Lihová & Mráz, 2019). In the *T. sidoides* complex, the lineage arrangement observed in the Bayesian tree showed that polyploid series (diploids to tetraploids or hexaploids) appeared independently several times in all the subspecies/morphotypes. Lineage-exclusive haplotypes found in diploid and polyploid populations were strong evidence of direct diploid–polyploid ancestry and were interpreted as independent polyploidization events. Polyploids with unique haplotypes, not directly related to any of the

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haplotypes detected in the diploids analysed, suggest an additional independent origins of polyploids. These results clearly demonstrated that recurrent autopolyploidy in the *T. sidoides* complex is remarkable and is one of the highest reported for plants (Servick *et al.*, 2015).

The recurrent and polytopic autopolyploidy has been demonstrated here to be important in structuring the geographical distribution of the *T. sidoides* complex, as it was previously reported in a few other temperate autopolyploid complexes, e.g. *Heuchera grossulariifolia* Rydb. (Segraves *et al.*, 1999), *Biscutella laevigata* L. (Parisod & Besnard, 2007), *Solidago altissima* L. (Halverson *et al.*, 2008), *Galax urceolata* (Poir.) Brummitt (Servick *et al.*, 2015), *Ranunculus kuepferi* Greuter & Burdet (Cosendai *et al.*, 2011) and *Pilosella rhodopea* (Griseb.) Szeląg (Šingliarová *et al.*, 2019). Based on these results we can speculate that this phenomenon could be occurring in other herbaceous species of the Chaquean Domain with autopolyloid series, such as those in *Mimosa* L. (Seijo, 1999; Seijo & Fernández, 2001), *Crotalaria* L. (Almada, Daviña & Seijo, 2006), *Paspalum* L. (Urbani, Quarin & Espinoza, 2002), *Solanum* L. (Scaldaferro *et al.*, 2012; Chiarini *et al.*, 2018), *Turnera* L. (Fernández, 1987), *Zephyranthes* Herb. (Greinzerstein & Naranjo, 1987; Daviña & Fernández, 1989) and *Stemodia* L. (Sosa, Seijo & Fernández, 2009), in which additional studies are necessary to understand more deeply the infraspecific diversity associated with polyploidy.

The multiple polyploidization events detected in the *T. sidoides* complex are probably related to the innate characteristic of many diploid populations to produce unreduced gametes (Panseri, Seijo & Solís Neffa, 2008; Kovalsky & Solís Neffa, 2012, 2016), but also to the ability of polyploids to survive and to establish in nature. Unilateral and bilateral sexual polyploidization are processes that continue to produce autopolyploids frequently in the natural populations of the complex (Kovalsky *et al.*, 2018). These results confirmed the prediction of Ramsey & Schemske (1998). These authors, working on experimental plants, predicted that species that were high-rate non-reduced gamete producers would be likely to produce autopolyploids frequently in nature. However, the results presented here disagree with a second prediction of the authors who considered that most autopolyploids may not persist in the populations for long. The high frequency of autopolyploids found in the *T. sidoides* complex is probably related to their ability to multiply asexually by rhizomes and the perennial life cycle (Solís Neffa, 2000). These characters would avoid the need for sexual reproduction to ensure the establishment of nascent autopolyploids (Kovalsky *et al.*, 2018). Moreover, the similar ecological preferences of diploids and polyploids (in their origin) may also promote the survival and establishment of autopolyploids at higher frequencies than many other non-clonal polyploids, and maintain (or increase) the genetic variation of extant and extinct diploid populations. Thus, the *T. sidoides* complex is an example of how recurrent polyploid formation may have been an important force in the evolutionary history of polyploid herbaceous plants in the marginal ecoregions of the western and south-eastern Neotropics.

EVOLUTIONARY SIGNIFICANCE OF POLYPLOID SERIES

When morphological species are studied cytologically in detail across their geographical ranges, as we did with the *T. sidoides* complex, polyploid populations are often detected in them (see Soltis *et al.*, 2010). Moreover, some cases of polyploidization in a species may involve multiple origins of polyploidy, as revealed in our genetic divergence analysis of the *T. sidoides* complex. Multiple independent origins of polyploids allow the genetic diversity of diploid parents to be transferred to the autopolyploid gene pool, increasing its genetic diversity. This has been proposed to promote the long-term survival and adaptation of polyploid lineages (Soltis & Soltis, 1993, 1999; Husband, 2004; Parisod, Holderegger & Brochmann, 2010). However, the consequences that multiple independent autopolyploid origins have on the successful establishment and persistence of autopolyploid lineages are still under debate.

Previous analyses on different subspecies/morphotypes of the *T. sidoides* complex showed that diploids and polyploids cannot be distinguished from each other using taxonomically diagnostic traits (Solís Neffa, 2000, 2010), but that they exhibit significant variation in reproductive and vegetative microcharacters and quantitative traits (Elías, 2010; Panseri, 2012). Changes associated with genome size variation and the gigas effect induced by chromosome doubling have also been recorded in the polyploid series of the complex (Solís Neffa, 2000). All these chromosome doubling-induced changes may have had an adaptive significance that promoted the expansion of the geographical range that diploids of each distinct lineage of the *T. sidoides* complex had, as has been proposed for other polyploid complexes (Soltis *et al.*, 2007).

A direct relationship between any character and increased fitness of individuals is not easy to demonstrate and is not the focus of this work. However, cytogeographical analysis of the *T. sidoides* complex (this study; Solís Neffa & Fernández, 2001; Solís Neffa *et al.*, 2004; Elías *et al.*, 2011) showed that, despite the fact that polyploid series of each subspecies/morphotype inhabit the same ecoregion, the cytotypes tend to be spatially segregated at a more local scale.

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A clear example was initially demonstrated in the *serrano* morphotype of subsp. *pinnatifida* (Elías *et al.*, 2011), in which diploids and polyploids inhabit the same ecorregion, but are segregated along elevational and bioclimatic gradients at a regional/local scale. The geographical segregation of diploids from their polyploid derivatives as observed here in the polyploid series of the *T. sidoides* complex appears to be the rule for autopolyploid species (Soltis *et al.*, 2007; Karunarathne *et al.*, 2018). The spatial segregation between diploids and tetraploids is often accompanied by different niche preferences that indirectly support the existence of differences in the adaptive traits to abiotic factors between cytotypes. In this sense, the wider distribution of tetraploids compared to their related diploids in each polyploid series of the *T. sidoides* complex supports the hypothesis that autopolyploids may have been more effective colonizers of new ecological niches.

Despite biotic niche divergence in autopolyploids being poorly studied, examples include the evolution of floral traits that promote assortative mating within cytotypes of *Chamerion angustifolium* L. (Holub.) (Kennedy *et al.*, 2006), differential herbivory among cytotypes in *Heuchera grossulariifolia* Rydb. (Thompson, Nuismer & Merg, 2004) and the association of specific mycorrhizal symbionts with different cytotypes of *Gymnadenia conopsea* (L.) R.Br. (Těšitelová *et al.*, 2013). In the *T. sidoides* complex, differences in floral microcharacters (Elías, 2010; Panseri, 2012), in cyanide content (Solís Neffa *et al.*, 2003) and in leaf microcharacters (Solís Neffa, 2000) between cytotypes of each polyploid series were proposed to be promoting or maintaining niche divergence within polyploid series. However, further studies are needed to understand the relationship between variation in any of these traits with specific biotic factors in each particular niche. Ongoing studies on the evolutionary dynamics of diploid–tetraploid contact zones in the *T. sidoides* complex will improve knowledge on the biotic and abiotic factors associated with the spatial segregation of cytotypes and polyploid range expansion.

AUTOPOLYPLOIDY AND SPECIES DIVERSITY ESTIMATION IN THE NEOTROPICS

Many angiosperm taxa, such as the *T. sidoides* complex, comprise multiple autopolyploids or presumed autopolyploid cytotypes. However, an autopolyploid has rarely been formally named and has been considered to represent a distinct species from its diploid parents. Soltis *et al.* (2007) proposed that many ‘unnamed’ autopolyploids meet the requirements of multiple species concepts, including the biological, morphological and evolutionary species concepts. Compared to the diploid parent, many autopolyploids, such as the complex analysed here, have distinct geographical ranges, can be distinguished by their macro- or micro-characters, and show pre- and post-zygotic reproductive isolation from their diploid progenitors and/or each other. Consequently, plant diversity based on taxonomic names underestimates the number of biological entities in nature.

In our species model, ten lineages (subspecies and morphotypes) clearly distinguishable on the basis of diagnostic morphological traits (Arbo, 1985; Solís Neffa, 2010; Moreno *et al.*, 2021) and reproductively isolated at the diploid level (Solís Neffa, 2000; Roggero Luque, 2017) are taxonomically recognized under a single specific name, the *T. sidoides* complex. In addition, in each of these lineages, there are polyploid series with multiple origins and with marked reproductive isolation and ecological niche segregation, behaving as good biological/ecological/evolutionary species despite the fact that low gene flow between cytotypes has been inferred from analysis of molecular markers (Moreno *et al.*, 2015) and the presence of triploids in diploid–tetraploid contact zones (Elías *et al.*, 2011; Kovalsky *et al.*, 2018). Thus, the *T. sidoides* complex demonstrates that biodiversity estimated from taxonomic diversity is clearly underestimated for plants. This is especially relevant for herbaceous plants inhabiting ecoregions with the highest frequency of species composed of autopolyploid series. In particular, several studies in herbaceous members of Asteraceae, Fabaceae, Plantaginaceae, Poaceae and Solanaceae (Urbani *et al.*, 2002; Almada *et al.*, 2006; Sosa *et al.*, 2009; Chiarini *et al.*, 2018; Via do Pico *et al.*, 2019) have revealed that in temperate regions with savannas and grasslands, such as in the Chaco, Humid Pampa and Uruguayan savanna ecoregions, the frequency of polyploidy, in many cases combined with a diversity of reproductive systems (Karunarathne *et al.*, 2018), is much higher than in warmer regions (such as in the Atlantic Forest and Amazonia).

Although it may seem operationally convenient to include morphologically similar cytotypes under a single species name, this practice obscures understanding of the evolutionary proceses and hampers conservation. Not considering multiple autopolyploid lineages as independent evolutionary units would greatly underestimate the diversity of the ecoregions of the southern Neotropics, with important consequences for their conservation and restoration.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's website.

Figure S1. PCA ordination plot of the subspecies and morphotypes in the plane of the first two principal component axes based on the selected bioclimatic and soil data. BIO 1: annual mean temperature, BIO 2: mean diurnal range, BIO 3: isothermality, BIO 4: temperature seasonality, BIO 7: temperature annual range, BIO 8: mean temperature of wettest quarter, BIO 12: annual precipitation, BIO 13: precipitation of wettest month, BIO 14: precipitation of driest month, BIO 15: precipitation seasonality, BIO 16: precipitation of wettest quarter, BIO 17: precipitation of driest quarter, BIO 18: precipitation of warmest quarter and BIO 19: precipitation of coldest quarter.

Figure S2. Distribution of subspecies/morphotypes and cytotypes of the *Turnera sidoides* complex in central Mesopotamia. The subspecies/morphotype are represented with different colours: pink = *mercedeño* morphotype, orange = subspecies *integrifolia*, green = *chaqueño* morphotype and blue-green = *mesopotamico* morphotype. Ploidy is indicated with different symbols: square = diploid, triangle = triploid, circle = tetraploid and diamond = hexaploid.

Figure S3. Bayesian inference tree of the *Turnera sidoides* complex based on two plastid DNA regions. Nodes with posterior probabilities ≥0.5 are represented with thicker lines. Posterior probabilities are shown above branches. Numbers above branches indicate estimates for the ages (in Mya). Next to each terminal tip there are indicated the subspecies/morphotype of the sample with different colours. Ploidy is indicated with different symbols: square = diploid, triangle = triploid, circle = tetraploid, and diamond = hexaploid. Numbers at the end of each terminal tip are sample population IDs (see Supporting Information, Table S1).

Appendix S1. Detailed information about the material of the *Turnera sidoides* complex used in ecological niche modelling analyses. *New ploidy estimates.

Table S1. Detailed information about the populations of the *Turnera sidoides* complex analysed in this study. Collector abbreviations: A, Arbo MM; C, Cristóbal C; De, Dematteis M; E, Elías; G, Gonzáles A; H, Hojsgaard D; K, Krapovickas A; M, Moreno EMS; P, Panseri A; Sch, Schinini A; Se, Seijo G; SN, Solís Neffa VG; U, Urbani M.

Table S2. Bioclimatic and edaphic variables analysed in the geographical area of each subspecies and morphotypes of the *Turnera sidoides* complex. BIO 1: annual mean temperature; BIO 2: mean diurnal range; BIO 3: isothermality; BIO 4: temperature seasonality; BIO 5: maximum temperature of warmest month; BIO 6: minimum temperature of coldest month; BIO 7: temperature annual range; BIO 8: mean temperature of wettest quarter; BIO 9: mean temperature of driest quarter; BIO 10: mean temperature of warmest quarter; BIO 11: mean temperature of coldest quarter; BIO 12: annual precipitation; BIO 13: precipitation of wettest month; BIO 14: precipitation of driest month; BIO 15: precipitation seasonality; BIO 16: precipitation of wettest quarter; BIO 17: precipitation of driest quarter; BIO 18: precipitation of warmest quarter; BIO 19: precipitation of coldest quarter.

Table S3. Contribution of the bioclimatic and edaphic variables to components 1 (PC 1) and 2 (PC 2).

Table S4. Percentage of contribution of the bioclimatical and edaphic variables analysed to the ENM of each subspecies/morphotypes and ploidy of the *Turnera sidoides* complex. Bold type depicts variables that most influence the models.

Table S5. Results of niche similarity tests for the subspecies and morphotypes of the *T. sidoides* complex.

Table S6. Characteristics and summary statistics estimated for the plastid DNA regions studied in the *Turnera sidoides* complex. Ts: transitions; Tv: transversions; Subs: substitutions; Ind: indels; *S*: polymorphic sites; π: nucleotide diversity; *h*: haplotype diversity; SD: standard deviation.

Table S7. Hierarchical distribution analysis of genetic variation estimated by AMOVA between and within subspecies and morphotypes of the *Turnera sidoides* complex with plastid DNA sequences. D.f. = degrees of freedom.

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