



Impact of Pleistocene geoclimatic events on the genetic structure in mid-latitude South America: insights from a phylogeographic analysis in *Turnera sidoides* complex (Passifloraceae, Turneroideae)

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6 **Impact of Pleistocene geoclimatic events on the genetic structure in mid-latitude**
7 **South American plants: insights from the phylogeography of *Turnera sidoides***
8 **complex (Passifloraceae, Turneroideae)**
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41 **Running-title:** Phylogeography of *Turnera sidoides*
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6 Aiming to shed light on the influence of Pleistocene geoclimatic events on the current
7 genetic patterns in the flora of the South American Chaqueño Phytogeographic Domain,
8 we performed phylogeographic and Bayesian ancestral area reconstruction analyses
9 based on plastid DNA sequences of *Turnera sidoides*. Lineages started to diversify 2.02
10 Mya, forming two major clades, one comprising extant populations from the western
11 boundary and the other including populations from the eastern boundary of the
12 distribution of *T. sidoides*. Major divergences within clades occurred 1.44 and 1.72
13 Mya. Ancestral area reconstruction analyses suggest a western origin of *T. sidoides* in
14 the Peripampasic Arc. Our results support the hypothesis that Pleistocene geoclimatic
15 events in the Chaqueño Domain played an important role in shaping genetic variation.
16 The establishment of the Arid Diagonal acted as a barrier to gene flow between the
17 western and eastern populations during the Pleistocene Patagonian Glaciations. After
18 this period, the orogeny of the region and Pleistocene climatic oscillations played a role
19 in the divergence of isolated ancestral populations followed by migrations to the Chaco-
20 Pampa plain.
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38 ADDITIONAL KEYWORDS: Chaqueño Phytogeographical Domain - demographic
39 history - genetic diversity - genetic patterns – phylogeography - RASP
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INTRODUCTION

Extant patterns of biodiversity are the result of complex ecological and evolutionary processes that occurred in the past (Rull, 2011). Climatic oscillations have had a great impact on the distribution of species as well and on the composition of communities and ecosystems (e.g. Schweiger *et al.*, 2008). Particularly, the Quaternary climatic fluctuations have had an influence on the majority of organisms, a hypothesis that has been extensively tested for species in the Northern Hemisphere (Hewitt, 1996). Although the Southern Hemisphere has not been exposed to extensive glaciations, considerable climate changes have affected the composition of biotic communities (Ab' Sáber, 1977; Clapperton, 1993; Iriondo, 1999).

The Chaqueño Phytogeographical Domain (Cabrera, 1971) is a mostly low-lying area that corresponds to an extremely large sedimentary basin limited by the Andes and the Brazilian cratons. It includes Uruguay, northern, central and eastern Argentina and southern areas of Brazil, Paraguay and Bolivia (Fig. 1). The process of diversification of its present flora began during the Oligocene (Spichiger *et al.*, 1995). Palaeontological, palaeoclimatic, and geological evidence indicates that a temperate climate prevailed during the Tertiary, supporting a continuous cloud forest that extended further south than it does today (Morrone, 2006; Barreda *et al.*, 2007). Cooling and aridification began during this period induced by geo-climatic events, particularly the uplift of the Andes, creating an "Arid Diagonal" which has crossed the South American continent in a SE-NW direction since the Late Miocene-Pliocene (Fig. 1). During drier periods, a pronounced aeolian activity deflated and redeposited large masses of silt and sand over the area (Iriondo, 1993). Subtropical forests became fragmented, and xeric formations expanded along the Arid Diagonal (Hinojosa & Villagrán, 1997; Villagrán & Hinojosa,

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6 1997; Morrone, 2001, 2006). Elevational shifts in vegetation occurred in the mountains;
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8 whereas in the lowlands xerophytic vegetation advanced repeatedly towards the north-
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10 east during dry, colder periods and retreated towards the south-west during humid,
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12 warmer periods (Barreda *et al.*, 2007). During the cold–dry phases, river valleys and
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14 ravines in the hilly areas and some lowland areas may have served as refugia for a flora
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16 adapted to more humid climates (Vuilleumier, 1971; Ab’Sáber, 1977). More recently, a
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18 dry period occurred concurrently with the Last Glacial Maximum (LGM); another,
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20 briefer and less severe, occurred in the Upper Holocene. After the LGM, the thawing of
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22 ice sheets on the Andes gave rise to massive fresh-water lakes in depressions of the
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24 Chaco-Pampa plain (Tapia, 1935). Such changes must have been critical in shaping the
25
26 current pattern of species distribution (Barreda *et al.*, 2007).

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28 Several studies have been conducted on the biogeography and diversification
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30 patterns of plants to analyse the consequences of past climatic changes on the South
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32 American biota (for a review see Turchetto-Zolet *et al.*, 2013). The analysis of
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34 genealogical lineages of current taxa provides a useful method to reconstruct the
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36 biogeographical history of species (Provan, Wattier & Maggs, 2005). Phylogeographic
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38 analyses allow the detection of both regional and landscape-level biodiversity patterns
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40 and enable the inference of microevolutionary processes underlying diversification and
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42 the selection of alternative evolutionary histories (Beheregaray, 2008). Nevertheless,
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44 there are few phylogeographic studies focusing on the mid-latitude South American
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46 lowlands (Fregonezi *et al.*, 2013; Anversa Segatto *et al.*, 2014; Longo *et al.*, 2014;
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48 Turchetto *et al.*, 2014; Turchetto-Zolet *et al.*, 2016). These studies did not cover the
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50 Chaqueño Domain entirely; therefore, phylogeographic studies on key species
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52 distributed over the whole area would provide the basis for more general inferences
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54 about the biogeographic implications of climate changes in this South American region.

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6 Some of the biogeographical hypotheses proposed for the Chaqueño Domain
7 (Hicken, 1918-1919; Kurtz, 1904; Frengüelli, 1950; Rambo, 1954; de la Sota, 1967)
8 have been tested using *Turnera sidoides* L. (Passifloraceae, Turneroideae), because its
9 distribution matches the Chaqueño Domain for nearly its entire extension (Fig. 1). This
10 complex of perennial rhizomatous herbs is the species of *Turnera* L. with the
11 southernmost distribution. *Turnera* comprises > 135 species in America and two in
12 Africa and appears to have started its diversification in America, *T. sidoides* being one
13 of the first lineages to diverge (Arbo, 2007; Arbo & Espert, 2009). Based on
14 biogeographic analyses, it has been suggested that the ancestor of such lineages
15 probably originated in the Chaquean subregion of the Neotropical region (Arbo &
16 Espert, 2009). Currently, *T. sidoides* is found mainly in Chaquean grasslands and open
17 forests in Bolivia, Argentina, Paraguay, Uruguay and south-eastern Brazil, growing in a
18 wide variety of habitats ranging from mountainous regions (up to 2700 m a.s.l.) to the
19 sea level and encompassing different climatic regimes (Arbo, 1985; Solís Neffa, 2000,
20 2010). A study based on the current geographical structure of plastid DNA variation in
21 restriction sites in *T. sidoides* (Speranza *et al.*, 2007) showed that plastid DNA variation
22 is highly geographically structured and reflects a pattern which is consistent with a
23 history of contraction and expansion of populations. This study constituted the first
24 attempt to interpret the influence of historical events in current distribution and
25 diversification of the mid-latitude South American lowlands flora using plastid DNA
26 data. Based on haplotype distribution three putative refugial areas associated with the
27 main orographic systems in mid-latitude South America were proposed for the complex.
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49 To shed light on the influence of past geoclimatic events on the current distribution
50 of species and genetic variability in the Chaqueño Domain, we tested the hypotheses
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6 provisionally proposed for *T. sidoides* by performing detailed phylogeographic and
7 Bayesian ancestral area reconstruction analyses based on plastid DNA sequences.
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10 11 12 13 STUDY AREA 14 15

16 From a biogeographical point of view, the geographic area of *T. sidoides* (Fig. 1) is
17 included in the Pampeana, Espinal, Chaqueña and Pre-Puneña provinces (Chaqueño
18 Domain), extending into the Paranaense province (Amazónico Domain) (Cabrera, 1971;
19 Cabrera & Willink, 1973). This widespread latitudinal and longitudinal range comprises
20 a great diversity of climates and ecological conditions with contrasting precipitation
21 regimes (Burgos, 1970; Sarmiento, 1972). To the west, its boundary is a north-to-south
22 arch (“Peripampasic arc”) formed by the Cordillera Oriental, the Sierras Subandinas,
23 and the Sierras Pampeanas. The Cordillera Oriental (5000-1500 m a.s.l.), running north-
24 south, generates contrasting environments on its eastern (wet and wooded) and western
25 (arid) slopes. The Sierras Subandinas are located between the Cordillera Oriental to the
26 west and the Chaco-Pampean plain to the east, from near 10°S to 25°S. These north-
27 south mountain systems (1500-1000 m a.s.l.) are transversally crossed by wide valleys
28 that connect the ranges with the eastern plain. This area is characterized by a subtropical
29 climate, with temperatures and precipitations that vary according to elevation and slope
30 exposure. The Sierras Pampeanas rise between 27°S and 33°S in a temperate and semi-
31 arid climate zone. Intermountain depressions form endorheic systems which are
32 relatively flat and filled with sediments. East of the Sierras Subandinas and Pampeanas,
33 the Chaco plain covers a vast area with a gentle south-east/north-west slope. The
34 Paraguay and Paraná rivers run along the eastern boundary, and four main river systems
35 (Parapetí, Pilcomayo, Bermejo and Juramento-Salado) cross the Chaco from the north-
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6 west to the south-east. The climate is warm humid in the east and semi-arid in the west
7 (Spichiger *et al.*, 1995). To the east, between the Paraná and the Uruguay rivers
8 (Mesopotamia) the area has a transitional climate towards a cold steppe to the south and
9 towards a cool tropical climate to the north (van der Sluijs, 1971; Carnevalli, 1994).
10 South of the Chaco, the Pampa plain extends from 30° to 40°S with a soft slope to the
11 east-south-east which is only interrupted by the Ventania and Tandilia hilly systems.
12 The northern part comprises a loess belt that borders a large sandy aeolian plain to the
13 south-west. Its climate is temperate-humid (Burkart, León & Movia, 1990). East of the
14 Uruguay river sediments range from Devonian to Holocenic and are deposited among
15 and over the Brazilian Shield. The resulting landscape is an undulated peneplain in
16 which the higher parts of the orographic system are called cuchillas. The most important
17 are the Cuchillas de Haedo and Grande, the main dividers of the hydrographic basins.
18 Lower forms of relief are located on the periphery of the peneplain. In southern Brazil,
19 four physiographic regions may be distinguished: Campanha, Central Depression, Serra
20 do Sudeste and Coastal Plain.
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34 35 MATERIAL AND METHODS

36 37 38 PLANT MATERIAL

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41 Leaf material from 160 individuals was sampled at 97 collecting sites (hereafter referred
42 to as populations; Fig. 1, Table S1) throughout the distribution of *T. sidoides*. The
43 geographical coordinates of each population were obtained in the field using a global
44 positioning system (GPS) unit. Five subspecies and five morphotypes were recognized
45 in *T. sidoides* on the basis of geographical distribution and variations in some
46 morphological features (Arbo, 1985; Solís Neffa, 2010); however, hybridization has
47 been documented among subspecies (Moreno *et al.*, 2015). Since difficulties in
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6 infraspecific delimitation and hybridization would influence genetic patterns and might
7 introduce biases into final phylogenetic and phylogeographic inference, infraspecific
8 taxa were not considered in this paper.
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12 Voucher specimens were deposited in the Herbarium of the Instituto de Botânica del
13 Nordeste (CTES); vouchers from Bolivian and Brazil specimens were also deposited in
14 the National Herbarium of Bolivia (LPB) and in the Herbarium of the Instituto de
15 Biociências, Universidade Federal do Rio Grande do Sul (ICN), respectively.
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21 DNA EXTRACTION AND PLASTID DNA AMPLIFICATION

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24 Total DNA was extracted from dry leaves using the protocol of Speranza *et al.* (2007).
25 The intergenic plastid DNA regions, *trnL-trnF* and *matK5`R-matK6*, were amplified
26 using universal primers (Taberlet *et al.*, 1991; Shaw *et al.*, 2005, respectively). The
27 polymerase chain reactions (PCR) for *trnL-trnF* were carried out in a 25- μ L final
28 volume containing genomic DNA (4 ng/ μ L), primer (0.8 μ M each), dNTPs (100 μ M
29 each), MgCl₂ (1.5 mM) and Taq polymerase (1 U) in 1 \times reaction buffer. Amplification
30 conditions were: 5 min at 95 °C, one cycle of 1 min at 94 °C, 1 min at 58 °C and 2 min
31 30 s at 72 °C. The annealing temperature was decreased by 1 °C for six cycles and the
32 following 32 cycles were carried out with an annealing temperature of 52 °C, and a final
33 extension of 5 min at 72 °C. PCR amplifications for *matK5`R-matK6* were carried out in
34 a 25- μ L final volume containing genomic DNA (9 ng/ μ L), primer (0.1 μ M each),
35 dNTPs (200 μ M each), MgCl₂ (3 mM) and Taq polymerase (2 U) in 1 \times reaction buffer.
36 The amplification conditions were: 5 min at 95 °C, 31 cycles of 1 min at 95 °C, 1 min at
37 50.2 °C and 1 min at 72 °C, followed by a final extension step of 4 min at 72 °C.
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39 Amplifications were confirmed by electrophoresis in 1.4% agarose gels in 1 \times TAE
40 buffer, stained with ethidium bromide (10 mg/mL), and photographed under UV light.
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6 Forward and reverse amplification products were sequenced at Macrogen[®] (Seoul,
7 South Korea). Sequences were deposited in GenBank (accession numbers MH190460-
8 MH190783). For all subsequent analyses the two plastid DNA spacers were
9 concatenated for each individual. Chromatograms were manually edited with Chromas
10 2.33 (<http://www.techneesium.com.au>). Sequences were visually assessed and aligned
11 with MEGA 5 (Tamura *et al.*, 2011) using the Clustal W method. All the polymorphic
12 positions detected were checked in the original chromatograms for confirmation.
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14 Insertion/deletion events (indels) involving poly-T/A stretches were not considered for
15 further analyses (Kelchner, 2000). Contiguous indels were treated as single mutational
16 events (Simmons & Ochoterena, 2000). Thirty-seven representative populations were
17 randomly taken from the total, and genetic variability analyses were performed in two to
18 six individuals from each population. Little or no genetic variability was found within
19 populations for this sample. For the remaining populations only one individual per
20 population was analysed, following the recommendations of Petit and Grivet (2002).
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32 33 SEQUENCE VARIATION AND INTRASPECIFIC GENETIC VARIABILITY 34

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36 Haplotypes were determined using DnaSP 5.0 (Librado & Rozas, 2009). Standard
37 descriptive molecular diversity statistics (number of segregating sites, S , nucleotide
38 diversity, π , and haplotype diversity, h) were calculated with Arlequin 3.5.1.2 (Excoffier
39 & Lischer, 2010).
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45 GENEALOGICAL ANALYSIS 46

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48 Evolutionary relationships among haplotypes were estimated by the median-joining
49 method ($\epsilon=0$; Bandelt, Forster & Röhl, 1999) implemented in Network 4.5.0.0
50 (<http://www.fluxus-engineering.com>).
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6 Phylogenetic relationships between haplotypes were estimated using the Bayesian
7 inference method implemented in BEAST 1.8.0 (Drummond *et al.*, 2012). *Turnera*
8 *fernandezii* Arbo and *T. melochioides* Cambess. were included as outgroups. Two
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10 independent runs of 1×10^8 Markov chain Monte Carlo (MCMC) iterations were
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12 performed, sampling every 1000 generations. The initial 10% of the iterations were
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14 removed as burn-in. The HKY + I substitution model was selected using the Akaike
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16 information criterion (AIC; Kelchner & Thomas, 2007) with the software jModeltest
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18 2.1.4 (Guindon & Gascuel, 2003; Darriba *et al.*, 2012), using the Yule tree prior and a
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20 lognormal molecular clock. Tracer 1.5 (Rambaut & Drummond, 2009) was used to
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22 check for convergence of the Markov chains and adequate effective sample sizes
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24 (ESS>200). All trees generated were summarized in a maximum clade credibility tree
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26 using TreeAnnotator 1.7.1 (Drummond *et al.*, 2012) which was edited in FigTree 1.3.1
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28 (Rambaut, 2009). Statistical support was determined by assessing the Bayesian posterior
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30 probabilities (Rannala & Yang, 1996).
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34 In the absence of a fossil record for the estimation of divergence times in *T. sidoides*,
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36 we used the data obtained by Ramos-Fregonezi *et al.* (2015), which compiles rates
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38 calculated for plastid markers for shrubs with similar life traits to the *T. sidoides*
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40 complex. We used a gamma distribution prior, with a shape parameter 1.6, a scale
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42 parameter 1.6×10^{-9} as prior and, assumed an offset value of 1×10^{-9} s/s/y. Time scales
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44 were set according to Cohen & Gibbard (2016).
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46 GEOGRAPHICAL DISTRIBUTION OF GENETIC VARIABILITY

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49 To analyse the geographical structuring of the genetic variability, we generated maps
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51 with GPS Trackmaker 13.3.349 (<http://www.gpstm.com>) and QGIS (2009), combining
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53 the geographical coordinates of all populations studied here and the distribution of
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haplotypes and the identification of clades retrieved from phylogenetic and network analysis.

POPULATION GENETIC STRUCTURE

To assess patterns of genetic structure, we used GENELAND, version 4.0.3 (Guillot, Mortier & Estoup, 2005). Spatial correlation and uncorrelated allele frequency models were applied for 5 independent runs with 8,000,000 MCMC iterations and a thinning rate of 100,000 for each K value (K = 1 to 100). To determine whether genetic variation across the study area was clinal, we tested for isolation-by-distance with a Mantel test (Mantel, 1967) between genetic (Φ_{ST}) and geographical distances using Alleles in Space 1.0 (AIS; Miller, 2005). The significance of this correlation was assessed by a probability distribution with 10,000 random permutations.

DEMOGRAPHIC ANALYSES

The demographic history of *T. sidoides* was analysed using Tajima's *D* (Tajima, 1989) and Fu's *F_s* (Fu, 1997) neutrality tests with Arlequin 3.5.1.2 (Excoffier & Lischer, 2010). The Bayesian skyline plot (BSP) method (Drummond & Suchard, 2010), implemented in BEAST and Tracer 1.5, was used to estimate the dynamics of population size over time. The settings for the substitution model and substitution rate were the same as those used for the phylogenetic analysis.

ANCESTRAL AREAS RECONSTRUCTION

After areas were delimited with GENELAND, we used the Bayesian Binary Markov chain Monte Carlo (BBM), implemented in RASP 3.2 (Yu, Harris & He, 2010; Yu *et al.*, 2015) to reconstruct the possible ancestral distribution areas of *T. sidoides* on the

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6 maximum clade credibility tree previously constructed with BEAST. Two MCMC
7 chains were simultaneously performed for 50,000 cycles, and sampling frequency of
8 1000. Outgroups were removed for this analysis.
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11 12 RESULTS

13 14 SEQUENCE VARIATION AND INTRASPECIFIC GENETIC VARIABILITY

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18 The concatenated alignment resulted in a 1008-bp sequence with 40 polymorphic sites
19 (Table S2). Relatively high values of haplotype diversity ($h = 0.92$, $SD = 0.00008$) and
20 low values of nucleotide diversity ($\pi = 0.01$ $SD = 0.00019$) were observed. Haplotype
21 reconstruction for all individuals of *T. sidoides* and the outgroup resulted in 36 different
22 haplotypes. The most frequent haplotypes were H3 and H23, found in 13.54% of
23 populations; H20 found in 10.41% of the populations, was also frequent. Frequencies
24 for the remaining haplotypes ranged between 1.42 and 7.29% of the populations. From
25 the 37 populations of *T. sidoides* in which more than one individual was sampled, only
26 eight showed two haplotypes; the remaining populations showed only one haplotype.
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35 36 GENEALOGICAL ANALYSIS

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39 The haplotype network (Fig. 2) showed a clear division of *T. sidoides* in four clades.
40 The most frequent haplotypes appeared as central haplotypes, from which several less
41 frequent haplotypes derived by a single mutational step. The haplotypes corresponding
42 to the outgroup were clearly differentiated. Clade II was the only clade with a star-like
43 configuration. Clade IV was the most diverse, formed by 18 haplotypes, nine of which
44 were population-specific.
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6 The highly supported clades predicted by the Bayesian phylogenetic tree (Fig. S1)
7 recovered four main lineages that were mostly consistent with the configuration of the
8 haplotype network. Nevertheless, there were some inconsistencies between the tree and
9 the network. The most remarkable inconsistency is the position of H13, which was
10 included in clade IV in the network and in clade I in the Bayesian tree. Other haplotypes
11 showed different relations, but they were included in equivalent clades in both analyses.
12 The common ancestor of the *Turnera* spp. sampled was estimated to have lived *c.* 3.08
13 Mya during the Pliocene. The earliest divergence within *T. sidoides*, between clade IV
14 and the remaining clades, was estimated to have taken place 2.02 Mya, during the
15 Pleistocene (Fig. 3). Diversification of clade I occurred 1.72 Mya, whereas clades II and
16 III diverged *c.* 1.38 Mya. Most of the sequence divergence in the complex occurred
17 during the last 75,000 years.
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30 GEOGRAPHICAL DISTRIBUTION OF GENETIC VARIABILITY

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32 Genetic diversity and the number of exclusive haplotypes varied considerably among
33 regions (Fig. 2, Table S3). The highest haplotype diversity was found in the south-
34 eastern part of the distribution (Uruguay) and in Mesopotamia. Haplotypes belonging to
35 the four main clades were found in both regions. Sixteen haplotypes were found in
36 Uruguay, ten of which were exclusive to the area; among these, four were found only in
37 the eastern part of the south-eastern region. Twelve haplotypes were found in
38 Mesopotamia, seven of which were exclusive to this region. In the Pampean Sierras, six
39 haplotypes were found, four of which were exclusive. In the Sub-Andean Sierras six
40 haplotypes were also found, all of which were exclusive except haplotype H3. To the
41 east of Serra do Sudeste (southern Brazil), haplotype H23 was found almost exclusively.
42 This haplotype was also found eastwards on the Cuchilla Grande (eastern Uruguay) and
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6 in one population in Mesopotamia. Finally, the lowest haplotype diversity was detected
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8 in the southernmost area of the species distribution, the Ventania systems, with only two
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10 haplotypes, neither exclusive.

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12 A clear geographical structure was evident when comparing the phylogenetic
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14 arrangement of haplotypes and their geographic distribution. Clade I was found mostly
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16 in south-eastern Cuchilla Grande (Uruguay). Clade II was the only clade found in
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18 northern Serra do Sudeste (Brazil), and it was also detected in the adjacent eastern
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20 Cuchilla Grande and at both sides of Cuchilla de Haedo (Uruguay) and in Mesopotamia.
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22 Clade III was found in the eastern Cuchilla Grande and eastern Cuchilla de Haedo and
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24 in Mesopotamia. Clade IV showed the widest geographical distribution, from southern
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26 Bolivia to Argentina, reaching the southern limit of the species distribution including
27
28 south-western Uruguay. Despite the general geographical structure, some haplotypes
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30 were found in more than one area, e.g. H11 which was found in populations on the
31
32 southern limit of the species distribution and in southern Cuchilla Grande Inferior in
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34 Uruguay. Haplotype H15, was found in several populations in Mesopotamia and
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36 Pampean Sierras and in one population to the east of Cuchilla de Haedo (Uruguay).

37 38 POPULATION GENETIC STRUCTURE 39

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41 The GENELAND analysis produced four clusters (highest average posterior probability
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43 $k = 4$): (A) Cuchilla Grande Inferior (Uruguay), (B) east of Cuchilla de Haedo and east
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45 of Cuchilla Grande (Uruguay), (C) Peripampasic Arc (Sub-Andean Sierras, Pampean
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47 Sierras, Ventania and Tandilia systems, western Uruguay and the Mercedes Plateau),
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49 and (D) Eastern Serranías (Fig. S2, Table S4). No significant association between
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51 genetic and geographical distances for the complex as a whole was shown by Mantel
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53 test ($r = 0.02$; $P = 0.81$).

DEMOGRAPHIC ANALYSES

Most neutrality tests (Table 1) were not significant, suggesting that the complex and the clades follow the neutral model of evolution along time. The BSP (Fig. 4) suggested a stable effective population size for a long period followed by a gradual increase c . 75,000 years ago.

ANCESTRAL AREA RECONSTRUCTION

Ancestral area reconstructions (Figs 3, S3) showed the Peripampasic Arc (C) as the area with the most diversification events. This area also showed the highest probability to be the ancestral area (C: 82.99%) for *T. sidoides*. The analysis suggested several range expansions and vicariance events as central in shaping the current distribution pattern found in *T. sidoides*. Taking C as the ancestral area, the earliest events in the diversification of the species were dispersals from this area to the Cuchilla Grande Inferior (A), followed by vicariance between the two regions (node 67) (60.78% probability). These events resulted in the divergence of two major phylogroups: one, grouping clades I, II and III (node 50), with an ancestral area in Cuchilla Grande Inferior (A: 73.45%); and another including only clade IV (node 66), with an ancestral area in Peripampasic Arc (C: 99.71%). After the initial divergence, the history of this species has been characterized mainly by processes of diversification within the wide range of the C area, and by range expansion events from the Cuchilla Grande Inferior to its surrounding areas. Early diversification in the east included dispersal events from the Cuchilla Grande Inferior (A; node 50) towards the Eastern Serranías (D) and a subsequent subdivision of both areas (1.72 Mya). This expansion resulted in the differentiation of clade I in A and the ancestor of clades II and III which later dispersed from D to B with subsequent vicariance (node 49; D: 46.90%, 1.38 Mya).

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6 Diversification along the Peripampasic Arc (C) (node 66), involved sympatric
7 diversification processes in clade IV. The two earliest groups diverged 1.44 Mya, one of
8 them (node 51) included two of the most ancestral haplotypes of clade IV (H6 and
9 H11). These two haplotypes, which diverged from each other 470,000 years ago are
10 located at the base of clade IV when data were analysed as a network. The other group
11 (node 65) is more diverse and includes 15 haplotypes which started diversifying *c.* 1.09
12 Mya (Fig. 3).
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19 20 DISCUSSION 21

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23 The genetic variation found in *T. sidoides* provides useful genealogical information and
24 its distribution offers strong evidence that the Pleistocene geomorphological and
25 climatic events occurred in the Chaqueño Phytogeographical Domain have had have
26 significant implications on the patterns of genetic variation in the *T. sidoides* complex.
27 Besides the three refugial centres hypothesized by Speranza *et al.* (2007) associated
28 with the main orographic systems in mid-latitude South America (the elevations of the
29 western side of the area and the Cuchilla de Haedo and the Eastern Serranías systems on
30 the eastern side), our results suggest that other putative refugia for *T. sidoides* would
31 also have existed. The finding of genetically differentiated groups descending from
32 different ancestral populations, the occurrence of areas with high genetic diversity, the
33 presence of several exclusive haplotypes in a single region and the high levels of
34 haplotype and nucleotide diversity are considered strong evidence of the existence of
35 refugial areas (Avice, 2000; Provan, Wattier & Maggs, 2005; Eidesen *et al.*, 2007;
36 Ehrich, Alsos & Brochmann, 2008; Bennet & Provan, 2008). Considering that, the other
37 refugia for *T. sidoides* would be located at the Chaco region, the southern Cuchilla
38 Grande Inferior and in the Mesopotamia, consistent with earlier interpretations that the
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6 ravines in the hilly areas of Argentina and Uruguay and some lowland areas may have
7 served as refugia for a flora adapted to more humid climates during the cold-dry phases
8 (Ab'Sáber, 1977; Haffer, 1982). Moreover, contact zones between clades would be
9 located north-west and south-east of the Iberá system, respectively.
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14 The lineages found today in *T. sidoides* started to split *c.* 2.02 Mya, with the
15 divergence of the major eastern and western clades. Further major divergences within
16 these clades occurred at 1.44 and 1.72 Mya. This time frame coincides with the
17 Pleistocene (2.59 M to 15,000 years ago), suggesting that the evolutionary history of *T.*
18 *sidoides* is closely tied to remarkable climatic and landscape changes that took place in
19 the Chaqueño Phytogeographical Domain during this period (Iriondo, 1999; Barreda *et*
20 *al.*, 2007), as also suggested for other South American plant species (Mäder *et al.*, 2013;
21 Longo *et al.*, 2014; Reck-Kortmann *et al.*, 2014; Turchetto *et al.*, 2014; Acosta *et al.*,
22 2017; Camps *et al.*, 2017). Based on biogeographic analyses it has been hypothesized
23 that *Turnera* originated in the Chaquean region, with subsequent vicariance and
24 dispersal events to other Neotropical regions (Arbo & Espert, 2009). The ancestral area
25 reconstruction based on BBM performed in *T. sidoides* supports such a hypothesis. The
26 presence of ancestral haplotypes (H3, H6, H11 and H15) in the highlands of the western
27 and south-eastern limits of the area of *T. sidoides* suggests an ancestral continuous
28 distribution of populations along the ranges of the Peripampasic arc. Such a connection
29 has also been proposed to explain the presence of species of Andean origin in the
30 Uruguayan and Argentinean Pampas and southern Brazil (Kurtz, 1904; Hicken, 1918-
31 1919; Frengüelli, 1950; Morello, 1958; de la Sota, 1967; Crisci *et al.*, 2001; Morrone,
32 2001; Ferretti, González & Pérez-Miles, 2012). The final Andes uplift produced a
33 continuous arid vegetation belt, called the Arid Diagonal, which crosses the continent in
34 a south-west/north-east direction. The centre of the Arid Diagonal occupied a nearly
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6 constant position during the glacial periods of the Quaternary (Villagrán & Hinojosa,
7 1997) and, it would have acted as an important barrier to gene flow between eastern and
8 western populations of *T. sidoides* leading to the diversification of the two first main
9 clades identified in the phylogenetic analyses by the time of the Pleistocene Patagonian
10 Glaciations. Subsequent lineage diversification in both clades coincides with cold marine
11 isotope stages (Cohen and Gibbard, 2016), suggesting that cooling and drying during
12 glaciation periods in the Pleistocene may have caused the isolation and divergence of
13 ancestral populations of *T. sidoides* due to the retreat and range shift of the ancient
14 widely distributed subtropical flora of southern South America.
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24 In the eastern part of the distribution of *T. sidoides*, the metamorphic formations
25 were temporarily isolated during the Pleistocene and Holocene by marine incursions
26 (Rambo, 1954), affecting population dynamics and leading to the diversification of
27 plant species distributed near the coastal plain of Uruguay and Brazil (Mäder *et al.*,
28 2013; Longo *et al.*, 2014). These events could also have promoted the isolation of
29 populations of *T. sidoides* and the allopatric differentiation for clade I. Moreover, the
30 relatively low haplotype variability detected in southern Brazil is consistent with a
31 sudden expansion event (Schaal *et al.*, 1998). In this region, intervals of drier climates
32 during the Pleistocene caused a retraction and fragmentation of the Atlantic Forest
33 (Rambo, 1954) and the expansion of tropical and subtropical grasslands and savannas
34 (Behling & Lichte, 1997). Such vegetation changes would have favoured the
35 diversification of clade II and the expansion of clade III. In addition, our results of BBM
36 analysis also support the proposal of Speranza *et al.* (2007) that the ravines from the
37 Cuchilla de Haedo system may have acted as a suitable dispersion route for this species.
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6 To the west, isolation of *T. sidoides* populations in the Sub-Andean and Pampean
7 Sierras and the Ventania system and their dispersion to the surrounding Chaco-Pampa
8 plain could have promoted the divergence of clade IV. These isolation and colonization
9 events would have been associated with the orogeny of the region and the Pleistocene
10 climatic oscillations. Most of the uplifting of the Andean and pre-Andean mountain
11 chains that surround the isolated valleys has occurred since the Miocene and even as
12 recently as the Pleistocene (Simpson, 1975). Elevational vegetation shifts may most
13 likely have caused population isolation and the differentiation of groups of populations
14 occurring at the temperate valleys. In the Pampean Sierras, palaeontological and
15 geological evidence supports the presence of tropical or subtropical vegetation in the
16 Miocene. Nevertheless, the general aridification caused a gradual evolution to more
17 xerophytic vegetation and to an adaptation of biotopes to higher elevations (Luti *et al.*,
18 1979). These changes may have been sufficient to act as significant barriers,
19 determining the fixation of the exclusive haplotypes of *T. sidoides* from the Pampean
20 Sierras. The lowlands have also been subjected to significant changes in climate
21 (Iriondo, 1999) and vegetation (Spichiger *et al.*, 1995; Prieto, 1996) that may have
22 caused repeated vicariance events leading to the differentiation of haplotypes found in
23 the eastern Chaco (H4, H5, H14 and H30) and the Pampa (H19 and H18).
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41 In Mesopotamia, where contact zones among extant haplotypes occur, marine
42 incursions covered much of the area during the Pliocene. Also, owing to the rise of the
43 Andes, the Brazilian shield was fragmented along large faults causing the elevation and
44 depression of different areas. Subsequent to the rising of the Mercedes plateau, the
45 Uruguay and Paraná rivers changed their direction towards their present location and the
46 Iberá wetland developed (Popolizio, 1970). Sediments filled and levelled this landscape
47 during the Quaternary. Based on palaeontological samples, Barreda *et al.* (2007)
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6 describe climatic conditions in the early Pleistocene in this area as more arid than the
7 Miocene optimum with the presence herbaceous communities by the mid Pleistocene.
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9 Later, more humid warm-temperate palm and mimosoid forests would have developed
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11 in the area during the last interglacial. In this scenario, groups of populations of *T.*
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13 *sidoides* could have been confined to different environments, where exclusive
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15 haplotypes of different linages may have been fixed. The sharing of some haplotypes
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17 among the Mercedes Plateau, Brazil (H23) and Uruguay (H20, H27), supports the
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19 hypothesis of an ancestral connection between the biota of these regions (de la Sota,
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21 1967; Crisci *et al.*, 2001). The Paraná and Uruguay rivers may have acted as important
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23 biogeographical corridors for *T. sidoides* populations, as also suggested for a number of
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25 plant and animal groups (Bonetto, 1961; Bonetto & Drago, 1968; Popolizio, 1970;
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27 Rabinovich & Rapoport, 1975; Ringuet, 1975; Menalled & Adámoli, 1995; Spichiger
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29 *et al.*, 1995; Nores, Cerana & Serra, 2005, Arzamendia & Giraud, 2009). Moreover,
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31 the establishment of the current course of the Paraná river and the development of the
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33 Iberá wetlands would have restricted some populations to sandy soils of the old Paraná
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35 delta, leading to differentiation and fixation of H28 and H29 to the north of the Iberá
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37 system.

38 39 CONCLUSIONS

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42 The distribution of genetic variation found in *Turnera sidoides* provides useful
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44 genealogical information and offers strong evidence that the Pleistocene
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46 geomorphological and climatic events occurred in the Chaqueño Phytogeographical
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48 Domain could have had significant implications on the patterns of genetic variation in
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50 the *T. sidoides* complex. Analyses of plastid DNA sequences showed four well-defined
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52 clades within *T. sidoides*. The non-random association of haplotypes in space detected
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6 in this species may be derived from vicariance as a result of extended periods of
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8 isolation among groups of populations followed by migrations to surrounding areas.
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10 Our current understanding of the climate and vegetation history of the area is consistent
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12 with the present pattern of distribution of the haplotypes.
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14 Populations of *T. sidoides* started to split by the time of Pleistocene Patagonian
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16 Glaciations (c. 2.02 Myr), with the divergence of two major clades, one (clade IV)
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18 comprising populations from the western boundaries of *T. sidoides*, and the other one
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20 including populations of the east of the species range. Major divergences within these
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22 clades occurred at 1.44 and 1.72 Mya, probably associated to cool and dry periods of
23
24 Pleistocene.
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11 SUPPORTING INFORMATION

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14 Additional Supporting Information may be found in the online version of this article:
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17 **Figure S1.** Bayesian inference tree of *Turnera sidoides* based on two plastid DNA
18 intergenic sequences. Nodes with posterior probabilities greater or equal to 0.7 are
19 represented with thicker lines. Numbers above branches indicate node numbers;
20 numbers below branches show estimates for the ages (in millions of years). The vertical
21 bars to the right of the tree indicate the principal clades identified in the analysis.
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27 **Figure S2.** Results from GENELAND analysis based on plastid DNA data for four
28 genetic clusters. The number of each population is indicated in the map, as well as the
29 cluster for which the posterior probability was high.
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34 **Figure S3.** Possible migration routes between and within geographical areas, according
35 to BBM results. Areas: (A) Cuchilla Grande Inferior (Uruguay); (B) east of Cuchilla de
36 Haedo and east of Cuchilla Grande (both Uruguay); (C) Peripampasic Arc; and (D)
37 Eastern Serranías. The times that speciation within an area has occurred is represented
38 by 4×, 5× and 16×, so 4× = four times, 5× = five times, 16× = 16 times.
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47 **Table S1.** Details for the 97 populations of *Turnera sidoides* analyzed, including
48 collector, number of collection, population code, sampling place, geographical
49 coordinates, elevation and sample size. Plastid DNA haplotypes found at each
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6 population and number of clade at which each haplotype belongs. Collectors: E, Elías;
7 H, Hosjsgaard; M, Moreno; P, Panseri; S, Seijo; SN, Solís Neffa.
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10 **Table S2.** Characteristics and summary statistics calculated for each plastid DNA
11 region studied in *Turnera sidoides*. Ts = transitions; Tv = transversions; S =
12 polymorphic sites; π = nucleotide diversity; h = haplotype diversity; SD = standard
13 deviation.
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19 **Table S3.** Haplotype constitution of clades and geographic location of each haplotype.
20 Clade (percentage of populations that are included in each haplogroup); H = haplotype
21 (percentage of populations in which the haplotype was observed); N° = number of
22 population; N_p = total of populations; N_i = total of individuals.
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28 **Table S4.** Results of individual-based Bayesian clustering analysis for GENELAND.
29 Each individual is coloured according to the cluster from which presents the highest the
30 posterior probability.
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FIGURE LEGENDS

Figure 1. Major geographical features of the distribution area of *Turnera sidoides*. AD= Arid Diagonal; IS= Iberá System; MP= Mercedes Plateau; CH= Cuchilla de Haedo; CG= Cuchilla Grande; CGI= Cuchilla Grande Inferior; SS= Serra do Sudeste. The red dashed line denotes the distribution of the *T. sidoides* complex. Each sample location is represented by a circle. The green solid line represents the limits of the Phytogeographical Chaqueño Domain.

Figure 2. Left, geographical ranges and collecting places of *Turnera sidoides*. Haplotypes found at each collecting site are represented with different numbers and coloured according to the clade to which they belong. Right, evolutionary relationships among haplotypes of *T. sidoides* plastid DNA using a median-joining network approach. Each circle represents a haplotype. The circle sizes are proportional to haplotype frequency. The lines linking the haplotypes represent relationships between haplotypes and crossed lines are substitutions inferred in the branches. mv = median vectors. The clades identified are represented in the same colors as in the map.

Figure 3. Combined results from the phylogenetic and biogeographical analysis. (A), Areas: (A) Cuchilla Grande Inferior (Uruguay); (B) East of Cuchilla de Haedo, East of Cuchilla Grande (Uruguay) and part of Iberá system; (C) Peripampasic Arc (Sub-Andean Sierras + Pampean Sierras + Ventania and Tandilia systems + western of Uruguay and south of Corrientes province); and (D) Eastern Serranías. * indicates areas with a probability <5 %. (B), Bayesian inference tree of *T. sidoides* based on two plastid

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6 DNA intergenic sequences. Posterior probabilities of all nodes are indicated between
7 parentheses. Numbers above branches indicate node numbers; numbers to the right of
8 the node show estimates for the ages (in millions of years). The pie charts on the nodes
9 show the most likely ancestral areas as reconstructed by Bayesian Binary Markov chain
10 Monte Carlo (BBM). The processes suggested by the BBM analysis for each node are
11 show by a complete circle (dispersion) and a dashed circle (vicariance). Marine isotopic
12 stages (MIS) according to Cohen & Gibbard (2016) are indicated in red. Current
13 distributions are indicated in parenthesis before the haplotype names.

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22 **Figure 4.** Bayesian skyline plot for *Turnera sidoides* plastid DNA showing the effective
23 fluctuation in population size over time, the thick solid line representing the mean
24 estimates and the shaded area the 95% confidence interval.
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Table 1. Neutrality statistics calculated for *Turnera sidoides* and for each of the clades founded in the network of haplotypes. **D** = Tajima's D; **F_s** = Fu's F_s.

	<i>T. sidoides</i>	Clade I	Clade II	Clade III	Clade IV
D	0.20	0.78	0,11	-1,54*	-0,88
	$P = 0,648$	$P = 0.78$	$P = 0.58$	$P = 0.02$	$P = 0.22$
F_s	-3.80	0.05	-0.37	-1.44	-4.51
	$P = 0.21$	$P = 0.52$	$P = 0.42$	$P = 0.12$	$P = 0.07$

*significant $P < 0.05$

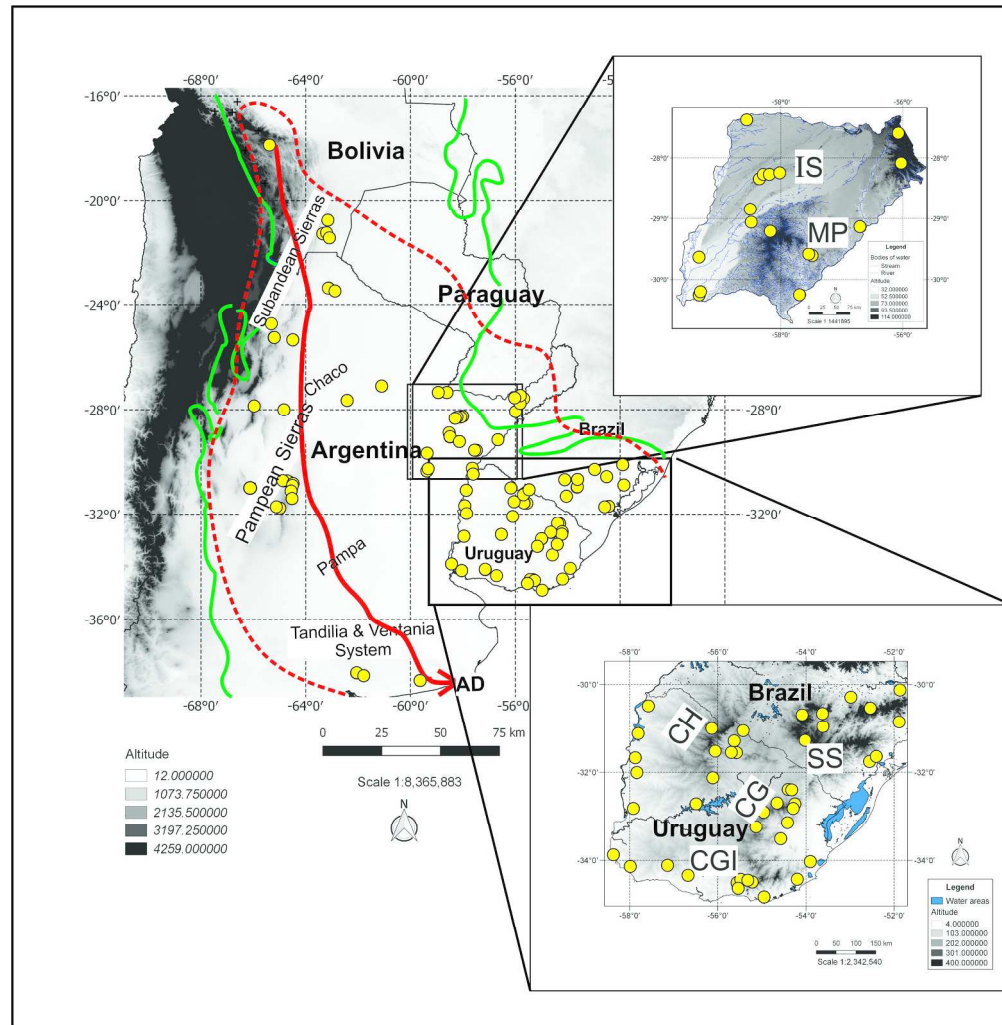


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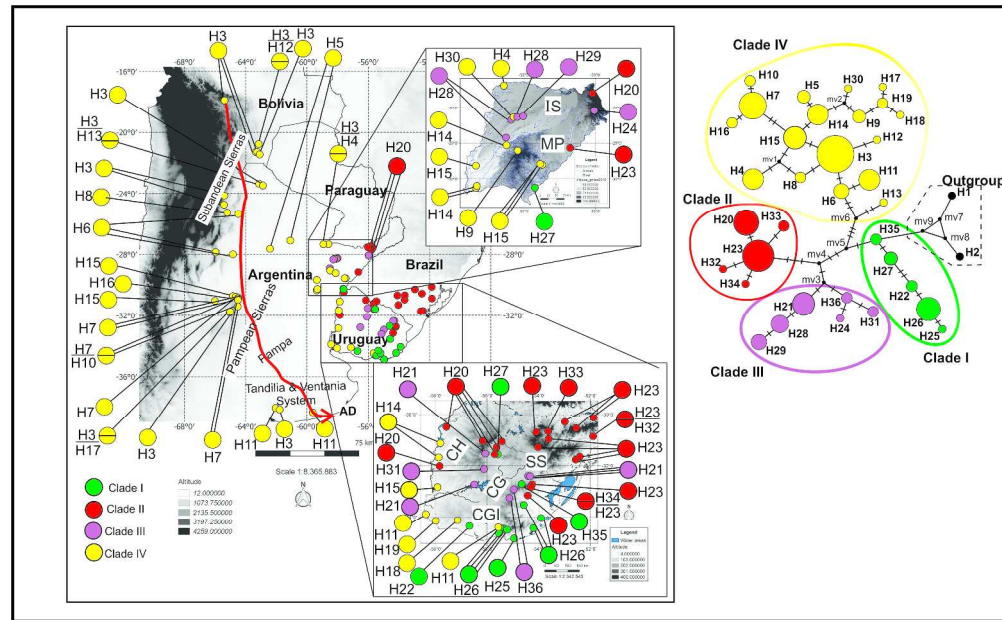


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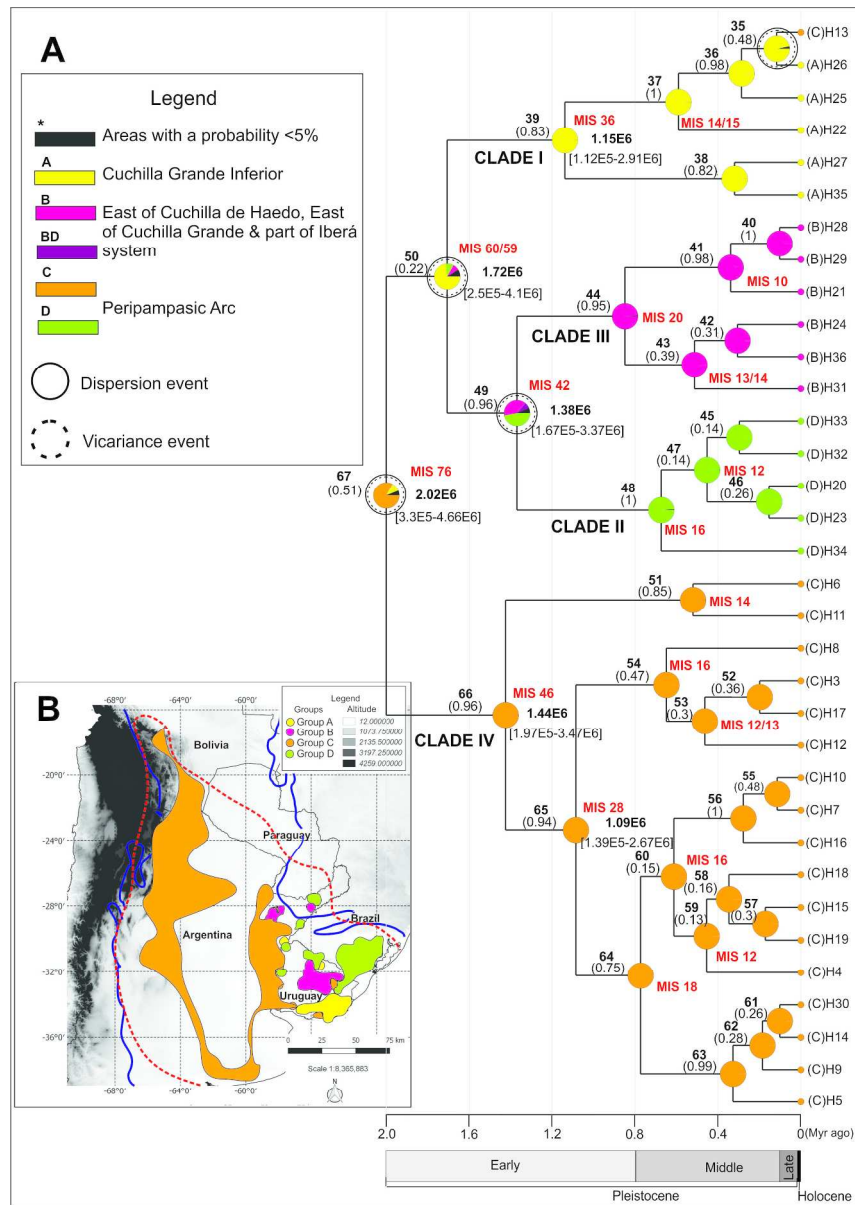


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PDF Proof

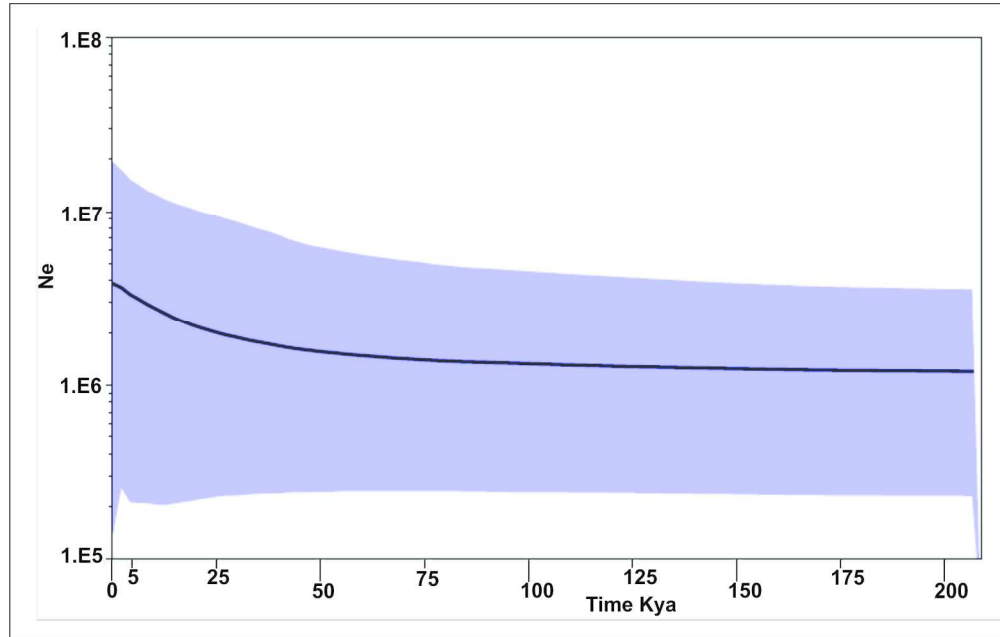


Figure 4. Bayesian Skyline Plot for *Turnera sidoides* cpDNA showing the effective fluctuation in population size over time, the thick solid line representing the mean estimates and the shaded area the 95% confidence interval.

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F_s	-3.80 <i>P</i> = 0.21	0.05 <i>P</i> = 0.52	-0.37 <i>P</i> = 0.42	-1.44 <i>P</i> = 0.12	-4.51 <i>P</i> = 0.07

*significant *P* < 0.05

PDF Proof

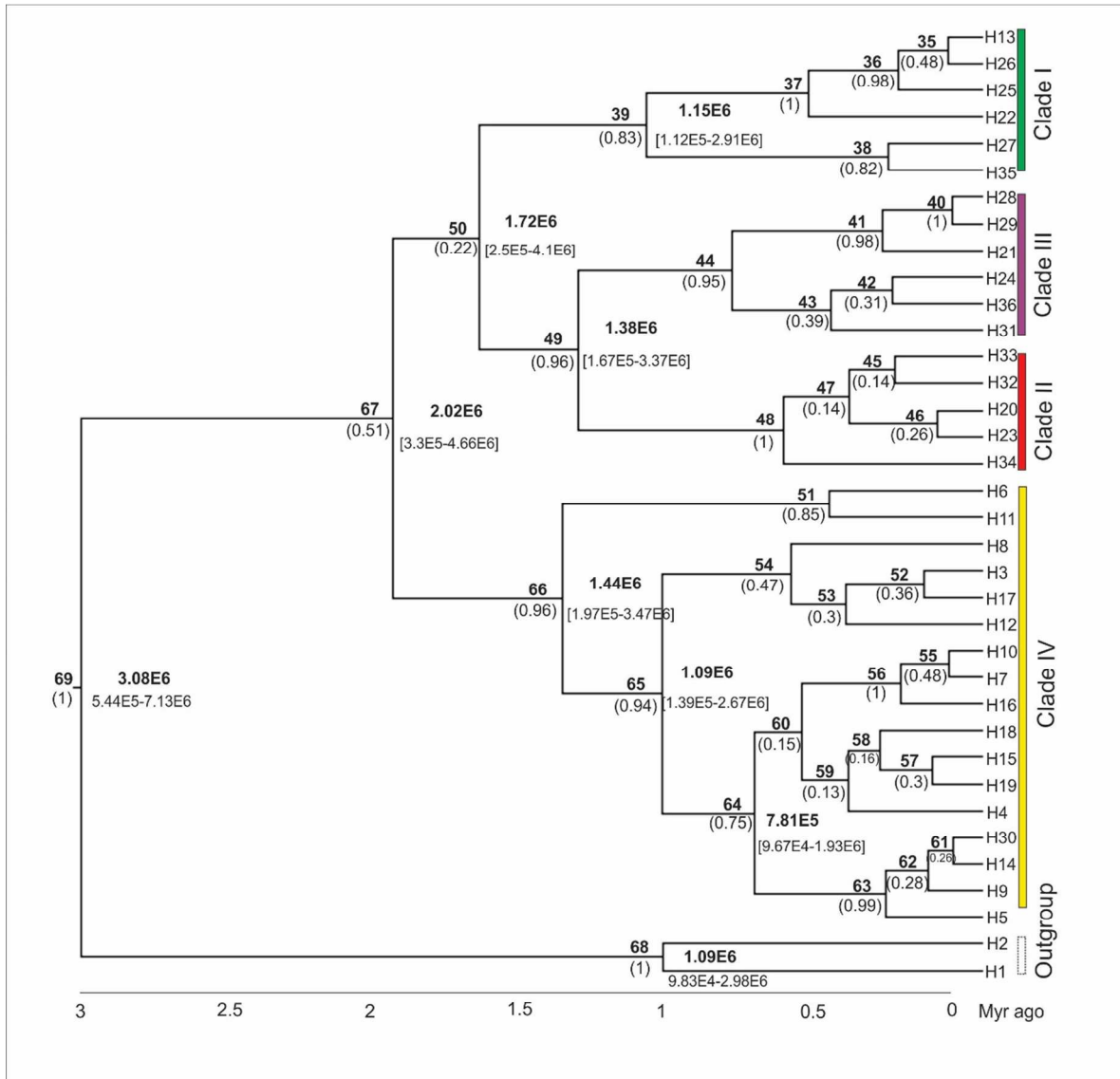


Figure S1. Bayesian inference tree of *Turnera sidoides* based on two cpDNA intergenic sequences. Posterior probabilities of nodes are represented below branches inside parentheses. Numbers above branches indicate node numbers; numbers to the right of principal nodes show estimates for the ages in millions of years, and inside brackets shows the highest posterior density interval. The vertical bars to the right of the tree indicate the principal clades identified in the analysis.

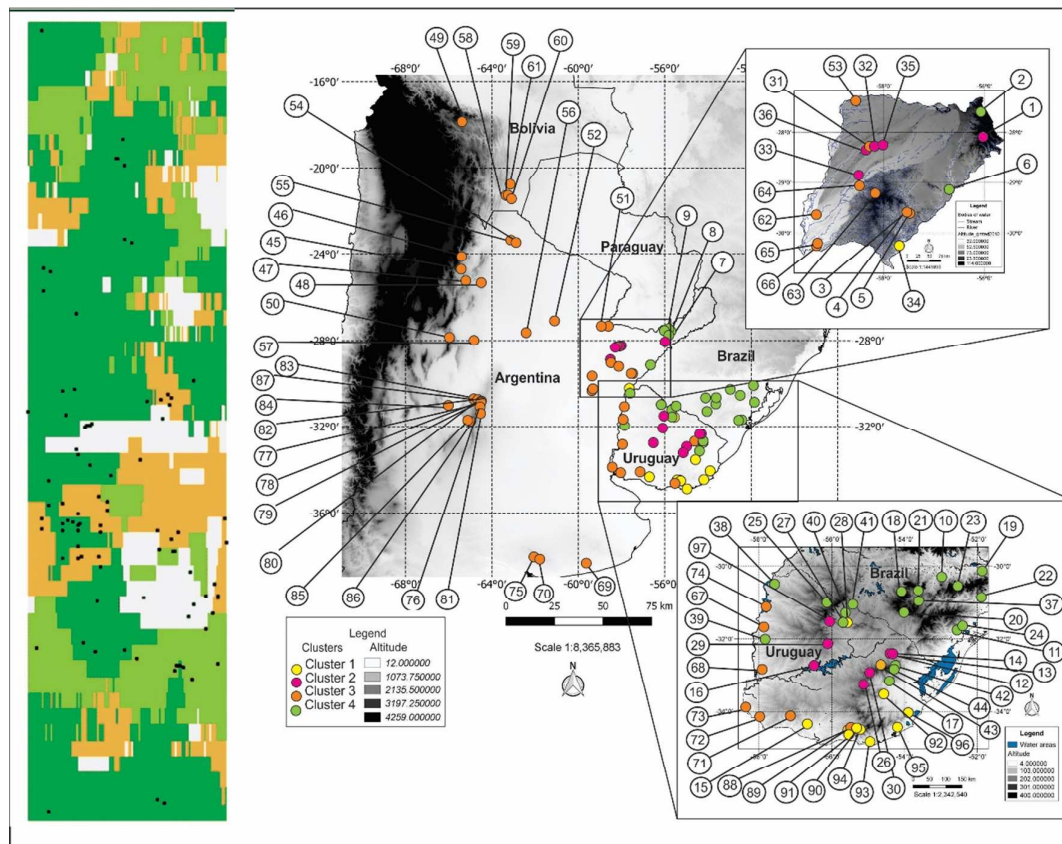


Figure S2. Results from GENELAND analysis based on cpDNA data for four genetic clusters. Each number's population is indicated in the map, as well as the cluster for which the posterior probability was high.

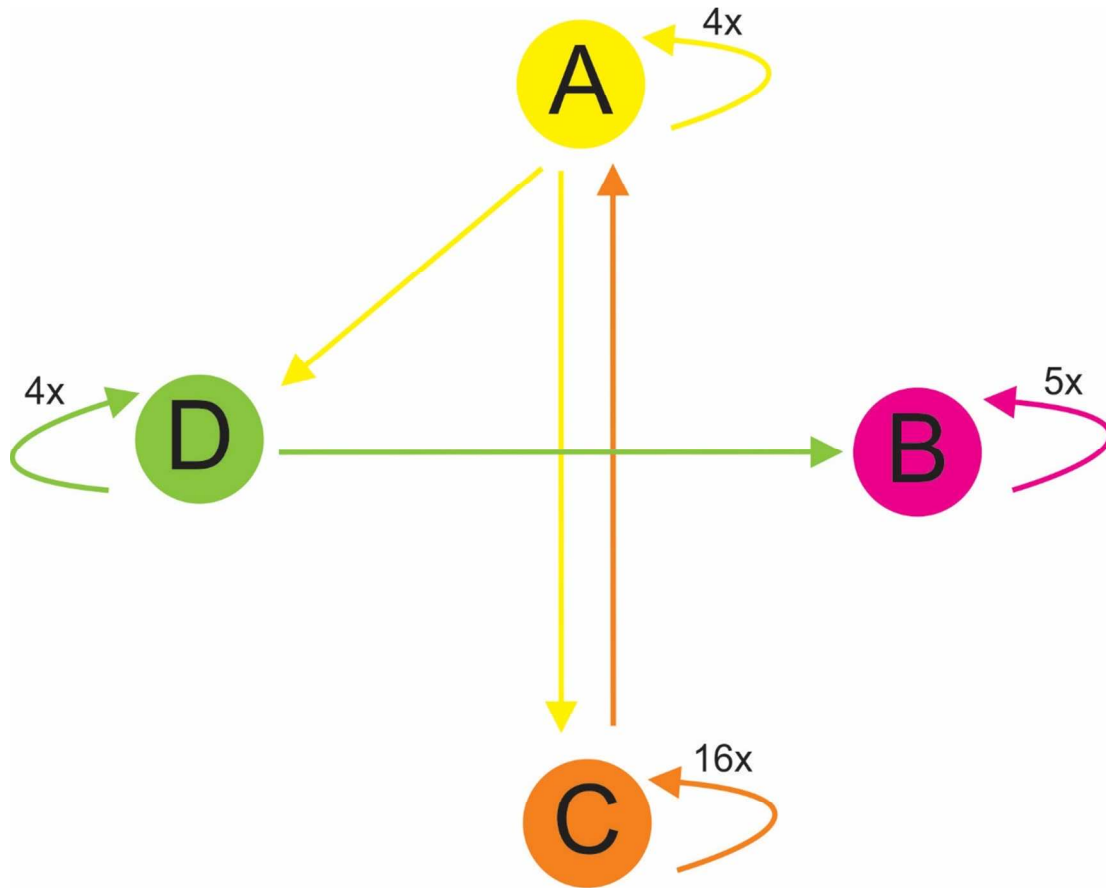


Figure S3. Possible migration routes between and within geographical areas, according to BBM results. Areas: (A) Cuchilla Grande Inferior (Uruguay), (B) east of Cuchilla de Haedo and east of Cuchilla Grande (both Uruguay), (C) Peripampasic Arc, and (D) Eastern Serranias. The times that speciation within an area has occurred is represented by 4 \times , 5 \times and 16 \times , so 4 \times = four times, 5 \times = five times, 16 \times = sixteen times.

Table S1. Details for the 97 populations of *Turnera sidoides* analyzed, including voucher specimen, population code, sampling place, geographical coordinates, altitude, and sample size. cpDNA haplotypes found at each population and number of clade at which each haplotype belongs. Collectors: E, Elías; H, Hosjsgaard; M, Moreno; P, Panseri; S, Seijo; SN, Solís Neffa

Population	Herbarium, voucher specimen (population code)	Sampling place	Geographical coordinates	Altitude (m.a.s.l.)	Sample size	Clade	Haplotype
1	CTES, S s/n (P14)	Argentina, Corrientes, route 147	28°04'34.20"S 55°59'1.80"W		1	I	H24
2	CTES, SN <i>et al.</i> s/n (S ₂₀₃)	Argentina, Corrientes, Ituzaingó	27°33'44"S 56°01'40.68"W	-	1	II	H20
3	CTES, SN & S 960 (S ₂₁₅)	Argentina, Corrientes, Mercedes	29°33'44"S 57°30'40"W	66	3	IV	H15
4	CTES, SN <i>et al.</i> 2222 (S ₄₃₀)	Argentina, Corrientes, Mercedes	29°33'0.00"S 57°32'15.50"W	69	1	IV	H15
5	CTES, SN <i>et al.</i> 2223 (S ₄₃₁)	Argentina, Corrientes, Mercedes	29°33'45.70"S 57°31'7.10"W	57	1	IV	H15
6	CTES, P <i>et al.</i> 17 (Tur 1)	Argentina, Corrientes, San Martín	29°10'34"S 56°38'34"W	-	1	II	H23
7	CTES, S s/n (P11)	Argentina, Misiones	27°41'56.01"S 55°48'0.80"W	-	1	II	H20
8	CTES, SN <i>et al.</i> 986 (S ₂₀₈)	Argentina, Misiones, Profundidad	27°33'00"S 55°43'00"W	-	2	II	H20
9	CTES, S s/n (3.5)	Argentina, Misiones, Profundidad	27°27'33.80"S 55°49'18.10"W	-	1	II	H20
10	ICN 158608 (6_10 P1)	Brasil, RS, Cachoeira do Sul	30°17'46.90"S 52°57'56.70"W	106	2	II	H23
11	ICN 158569 (3_10 P10)	Brasil, RS, Encruzilhada do Sul	31°44'7.50"S 52°32'44.3"W	58	2	II	H23
12	CTES, SN <i>et al.</i> 271 (S ₄₈)	Uruguay, Cerro Largo, Bañado de Medina	32°23'00"S 54°21'00"W	-	1	III	H21

Table S1. *Continued*

Population	Herbarium, voucher specimen (population code)	Sampling place	Geographical coordinates	Altitude (m.a.s.l.)	Sample size	Clade	Haplotype
13	CTES, SN <i>et al.</i> 278 (S49)	Uruguay, Cerro Largo	32°23'08"S 54°22'08"W	-	1	III	H21
14	CTES, SN <i>et al.</i> 395 (S ₉₆)	Uruguay, Cerro Largo, Bañado de Medina	32°22'56"S 54°20'22"W	-	1	III	H21
15	CTES, SN & S 2109 (S ₃₉₀)	Uruguay, San José, route 23 km 129	34°20'00"S 56°42'00"W	179	2	I	H22
16	CTES, SN 551 (S ₁₅₆)	Uruguay, Tacuarembó, route 5 km 257	32°45'9"S 56°30'57"W	101	2	III	H21
17	CTES, M <i>et al.</i> s/n (P4uru)	Uruguay, Treinta y Tres	33°9'39.20"S 54°23'6.80"W	-	1	II	H23
18	ICN 158603 (5_10 P11)	Brasil, RS, RS 473, from Lavras do Sul to Sao Gabriel	30°39'40.50"S 54°05'44.20"W	315	1	II	H23
19	SN s/n (6_10 P4)	Brasil, RS, BR 290	30°07'17"S 51°51'34"W	-	1	II	H23
20	ICN 158579 (4_10 P5)	Brasil, RS, Bagé	31°18'24"S 54°01'52.60"W	254	1	II	H23
21	ICN 158594 (5_10 P6)	Brasil, RS, Caçapava do Sul	30°40'30.40"S 53°37'07"W	339	2	II	H33
22	ICN 158555 (2_10 P1)	Brasil, RS, Camaquá	30°51'36.70"S 51°51'01.10"W	51	2	II	H23 & H32
23	ICN 158556 (3_10 P1)	Brasil, RS, Encruzilhada do Sul	30°32'22.30"S 52°30'56.90"W	390	2	II	H23
24	ICN 158568 (3_10 P9)	Brasil, RS, Pelotas	31°41'39.90"S 52°25'30.80"W	35	1	II	H23
25	CTES, SN & S 2025 (S ₃₆₆)	Uruguay, Artigas, route 30 km 207,5	30°59'56.70"S 56°07'0.52"W	316	1	II	H20
26	CTES, M <i>et al.</i> s/n (P10uru)	Uruguay, Florida	33°13'50.70"S 55°8'25.10"W	-	1	III	H36
27	CTES, SN & S 2051 (S ₃₇₂)	Uruguay, Rivera, Cuñá Pirú	31°31'45"S 55°35'08"W	1321	1	II	H20

Table S1. *Continued*

Population	Herbarium, voucher specimen (population code)	Sampling place	Geographical coordinates	Altitude (m.a.s.l.)	Sample size	Clade	Haplotype
28	CTES, SN & S 2052 (S ₃₇₃)	Uruguay, Rivera, Cerro Miriñaque	31°32'15.50"S 55°38'17"W	180	2	I	H27
29	CTES, SN & S 2073 (S ₃₇₉)	Uruguay, Tacuarembó, stream Quebrada Grande	32°05'34"S 56°06'13"W	145	2	III	H31
30	CTES, <i>M et al.</i> s/n (P9uru)	Uruguay, Treinta y Tres	32°56'8.40"S 54°58'1.10"W	-	1	III	H36
31	CTES, SN s/n (P5)	Argentina, Corrientes, Santa Rosa, route 118 km 54	28°16'47.40"S 58°12'52.40"W	69	1	IV	H30
32	CTES, SN s/n (P2)	Argentina, Corrientes, Santa Rosa, route 118 km 72	28°14'8.40"S 58°2'49.40"W	72	3	III	H28
33	CTES, SN s/n (P8)	Argentina, Corrientes,	28°54'26.60"S 58°31'05"W	65	1	III	H28
34	CTES, SN & S 961 (S ₂₂₀)	Argentina, Corrientes, Monte Caseros	30°14'57.20"S 57°37'25"W	-	1	I	H27
35	CTES, SN s/n (P4)	Argentina, Corrientes, Santa Rosa, route 118 km 61	28°16'39.1"S 58°8'21.1"W	69	4	III	H29
36	CTES, SN s/n (Tabay)	Argentina, Corrientes, Tabay	28°19'4.20"S 58°16'59.50"W	75	1	III	H28
37	ICN 158583 (4_10 P9)	Brasil, RS, Rochas de Bagé	30°56'56.50"S 53°38'9.40"W	197	1	II	H23
38	CTES, SN & S 2016 (S ₃₆₁)	Uruguay, Artigas, route 30 km 6	30°28'24"S 57°35'49"W	90	1	II	H20
39	CTES, SN & S 2141 (S ₄₀₃)	Uruguay, Paysandú, route 3 km 449	31°41'35.40"S 57°53'23.40"W	50	1	II	H20
40	CTES, SN & S 2040 (S ₃₆₈)	Uruguay, Rivera, route 30 and route 5	31°14'48"S 55°39'45"W	-	1	II	H20

Table S1. *Continued*

Population	Herbarium, voucher specimen (population code)	Sampling place	Geographical coordinates	Altitude (m.a.s.l.)	Sample size	Clade	Haplotype
41	CTES, SN & S 2043 (S ₃₇₀)	Uruguay, Rivera, route 27 km 14	31°02'44"S 55°28'50"W	-	1	II	H20
42	CTES, M <i>et al.</i> s/n (P6uru)	Uruguay, Cerro Largo	32°41'3.50"S 54°12'35.10"W	-	1	II	H23
43	CTES, M <i>et al.</i> s/n (P8uru)	Uruguay, Cerro Largo, stream Sarandí del Quebracho	32°42'51.10"S 54°37'19.30"W	-	2	I	H35
44	CTES, M <i>et al.</i> s/n (P5uru)	Uruguay, Treinta y Tres	32°45'22.30"S 54°13'37.30"W	-	2	II	H23 & H34
45	CTES, SN <i>et al.</i> 959 (S ₂₁₂)	Argentina, Jujuy, Capital, Palpalá	24°15'18"S 65°12'28"W	1104	1	IV	H3
46	CTES, SN <i>et al.</i> 827 (S ₁₈₂)	Argentina, Jujuy, Yala	24°07'31"S 65°23'50"W	1467	1	IV	H3
47	CTES, SN <i>et al.</i> 839 (S ₁₈₇)	Argentina, Salta, Capital, El Milagro	24°43'12"S 65°24'33"W	1246	2	IV	H8
48	CTES, SN <i>et al.</i> 924 (S ₂₀₀)	Argentina, Salta, Metán	25°20'29.5"S 64°28'28"W	523	2	IV	H6
49	CTES, LPB, SN <i>et al.</i> 1378 (S ₂₆₁)	Bolivia, Cochabamba	17°54'01"S 65°20'58"W	2124	1	IV	H3
50	CTES, SN s/n (S ₇₁)	Argentina, Catamarca, Ambato	27°51'40.09"S 65°56'44.84"W	-	1	IV	H6
51	CTES, SN 306 (S ₃₂)	Argentina, Chaco, 1° de Mayo, Colonia Benítez	27°20'00"S 58°56'00"W	52	6	IV	H3 & H4
52	CTES, SN 1994 (S ₃₁₅)	Argentina, Chaco, Las Breñas	27°05'11"S 61°05'09"W	103	2	IV	H5
53	CTES, SN 496 (S ₈₆)	Argentina, Corrientes, San Cosme	27°19'00"S 58°35'00"W	50	4	IV	H4

Table S1. *Continued*

Population	Herbarium, voucher specimen (population code)	Sampling place	Geographical coordinates	Altitude (m.a.s.l.)	Sample size	Clade	Haplotype
54	CTES, SN <i>et al.</i> 1986 (S ₃₁₁)	Argentina, Salta, Pluma de Pato	23°22'21"S 63°05'53"W	237	1	IV	H3
55	CTES, SN <i>et al.</i> 1988 (S ₃₁₂)	Argentina, Salta, Estación Morillo (Solá)	23°28'23"S 62°53'29"W	222	4	IV	H3 & H13
56	CTES, SN <i>et al.</i> 316 (S ₆₉)	Argentina, Santiago del Estero, Moreno	27°38'00"S 62°25'00"W	-	1	IV	H4
57	CTES, SN <i>et al.</i> 332 (S ₇₀)	Argentina, Santiago del Estero, Guasayan	27°59'53.13"S 64°48'39.90"W	-	1	IV	H4
58	CTES, LPB, SN <i>et al.</i> 1025 (S ₂₅₀)	Bolivia, Tarija, Gran Chaco	21°17'32"S 63°20'55"W	374	4	IV	H3
59	CTES, LPB, SN <i>et al.</i> 1505 (S ₃₀₁)	Bolivia, Tarija, Gran Chaco	21°17'35"S 63°18'37"W	385	4	IV	H3
60	CTES, LPB, SN <i>et al.</i> 1508 (S ₃₀₂)	Bolivia, Tarija, Gran Chaco	21°27'33"S 63°06'44"W	337	1	IV	H3
61	CTES, LPB, SN <i>et al.</i> 1971 (S ₃₀₉)	Bolivia, Tarija, Gran Chaco	20°45'46"S 63°07'55"W	675	2	IV	H3 & H12
62	CTES, SN <i>et al.</i> 2140 (S ₄₁₅)	Argentina, Corrientes	29°39'53.40"S 59°21'40.40"W	44	1	IV	H15
63	CTES, SN <i>et al.</i> 2141 (S ₂₂₃)	Argentina, Corrientes, Mercedes	29°14'7.4"S 58°07'28.6"W	80	3	IV	H9
64	CTES, SN <i>et al.</i> 2138 (S ₃₁₄)	Argentina, Corrientes, Mercedes	29°01'37"S 58°29'15"W	-	2	IV	H14
65	CTES, SN <i>et al.</i> s/n (S ₄₁₈)	Argentina, Corrientes, Sauce	30°13'45.9"S 59°19'20.7"W	38	2	IV	H14

Table S1. *Continued*

Population	Herbarium, voucher specimen (population code)	Sampling place	Geographical coordinates	Altitude (m.a.s.l.)	Sample size	Clade	Haplotype
66	CTES, SN <i>et al.</i> s/n (S ₄₁₉)	Argentina, Corrientes	30°19'0.05"S 59°21'48.50"W	32	1	IV	H14
67	CTES, SN & S 2140 (S ₄₀₂)	Uruguay, Paysandú, stream Quebrada Grande	31°58'15.80"S 57°51'3.40"W	48	1	IV	H14
68	CTES, SN & S 2138 (S ₄₀₀)	Uruguay, Río Negro, route 24 km 44,5	32°50'38.20"S 57°57'19.20"W	43	1	IV	H15
69	CTES, H 378 (S ₃₃₅)	Argentina, Buenos Aires, San Cayetano	38°20'00"S 59°37'00"W	98	2	IV	H11
70	CTES, S s/n (S ₃₄₂)	Argentina, Buenos Aires, Tornquist, El Pantanoso	38°03'00"S 62°02'00"W	-	1	IV	H3
71	CTES, SN & S 2097 (S ₃₈₇)	Uruguay, Colonia	34°06'14.70"S 57°06'31.50"W	-	1	IV	H18
72	CTES, SN & S 2117 (S ₃₉₃)	Uruguay, Colonia, Conchillas	34°10'03.70"S 58°02'33.20"W	27	2	IV	H19
73	CTES, SN & S 2133 (S ₃₉₆)	Uruguay, Punta Gorda	33°54'42"S 58°25'04"W	2	1	IV	H11
74	CTES, SN & S 2009 (S ₃₅₅)	Uruguay, Salto	31°04'07"S 57°50'19"W	34	1	IV	H14
75	CTES, S 2915 (S ₂₄₈)	Argentina, Buenos Aires, Tornquist, Villa Arcadia	38°08'21.89"S 61°47'38.61"W	234	1	IV	H11
76	CTES, E 11 (S ₃₂₈)	Argentina, Córdoba, La Cumbre	30°59'10.60"S 64°30'22"W	1133	1	IV	H7
77	CTES, SN & S 966 (S ₂₃₄)	Argentina, Córdoba, Punilla, Capilla del Monte	30°51'45"S 64°30'40.7"W	1007	4	IV	H7

Table S1. *Continued*

Population	Herbarium, voucher specimen (population code)	Sampling place	Geographical coordinates	Altitude (m.a.s.l.)	Sample size	Clade	Haplotype
78	CTES, SN & S 967 (S ₂₃₅)	Argentina, Córdoba, Punilla, Capilla del Monte, hill Uritorco	30°51'57.50"S 64°29'30"W	1615	4	IV	H7 & H10
79	CTES, E s/n (S ₃₁₉)	Argentina, Córdoba, Punilla, Capilla del Monte, hill Uritorco	30°51'45.10"S 64°29'42.71"W	1168	3	IV	H7 & H10
80	CTES, E 6 (S ₃₂₆)	Argentina, Córdoba, Punilla, Capilla del Monte, hill Las Gemelas	30°53'12.30"S 64°29'52.10"W	1249	1	IV	H7
81	CTES, SN 486 (S ₁₁₂)	Argentina, Córdoba, Carlos Paz	31°24'01.93"S 64°31'01.14"W	661	1	IV	H7
82	CTES, SN & S 969 (S ₂₃₇)	Argentina, Córdoba, Capilla del Monte	30°50'39"S 64°28'34"W	979	1	IV	H7
83	CTES, E s/n (S ₃₄₅)	Argentina, Córdoba, Cruz del Eje	30°44'00"S 64°48'00"W	449	1	IV	H15
84	CTES, E 2 (S ₃₄₇)	Argentina, Córdoba, La Higuera	31°0'80"S 65°0.60'20.50"W	612	1	IV	H15
85	CTES, E 4 (S ₃₄₉)	Argentina, Córdoba, Mina Clavero	31°44'0.40"S 65°00'26.10"W	890	2	IV	H3 & H17
86	CTES, E 5 (S ₃₄₈)	Argentina, Córdoba, Nono	31°47'49"S 65°00'23"W	861	1	IV	H3
87	CTES, E 1 (S ₃₄₆)	Argentina, Córdoba, San Marcos Sierra	30°47'0.35"S 64°38'34.20"W	635	2	IV	H16
88	CTES, SN 2000 (S ₃₃₈)	Uruguay, Lavalleja, Aguas Blancas	34°30'46.70"S 55°20'53"W	-	3	IV	H11
89	CTES, SN 2147 (S ₄₀₆)	Uruguay, Canelones	34°38'21"S 55°32'10"W	57	1	I	H26
90	CTES, SN 1999 (S ₃₃₇)	Uruguay, Lavalleja, Aguas Blancas	34°30'46"S 55°21'15"W	-	3	I	H26
91	CTES, SN 2148 (S ₄₀₇)	Uruguay, Lavalleja	34°30'7"S 55°26'58"W	76	1	I	H26

Table S1. *Continued*

Population	Herbarium, voucher specimen (population code)	Sampling place	Geographical coordinates	Altitude (m.a.s.l.)	Sample size	Clade	Haplotype
92	CTES, M <i>et al.</i> s/n (P3uru)	Uruguay, Lavalleja	33°32'55.20"S 54°34'58.60"W	-	1	I	H26
93	CTES, SN <i>et al.</i> 51 (S ₁₀₁)	Uruguay, Maldonado	34°52'52.53"S 54°58'25.89"W	-	1	I	H25
94	CTES, M <i>et al.</i> s/n (P2uru)	Uruguay, Maldonado	34°32'44.20"S 55°15'17.70"W	-	1	I	H26
95	CTES, SN 2173 (S ₄₁₁)	Uruguay, Rocha	34°27'19.40"S 54°10'58.60"W	59	1	I	H26
96	CTES, SN 2175 (S ₄₁₂)	Uruguay, Rocha	34°03'17.70"S 53°53'26.70"W	28	2	I	H26
97	CTES, SN 2063 (S ₃₇₈)	Uruguay, Tacuarembó	31°31'08"S 56°2'13"W	206	3	III	H21

Table S2. Characteristics and summary statistics calculated for each cpDNA region studied in *Turnera sidoides*. Ts = transitions; Tv = transversions; *S* = polymorphic sites; π = nucleotide diversity; *h* = haplotype diversity; SD = standard deviation.

cpDNA Region	bp	Ts	Tv	Indels	<i>S</i>	π (DE)	<i>h</i> (SD)	Number of haplotypes
<i>trnL-trnF</i>	259	0	1	8	9	0.00076 (0.00015)	0.191 (0.038)	9
<i>matK5'-matK6</i>	749	20	9	2	31	0.00738 (0.00027)	0.906 (0.010)	25
Concatenated sequenced	1008	20	10	10	40	0.00569 (0.00019)	0.920 (0.00008)	34

Appendix S3. Haplotype constitution of clades and geographic location of each haplotype. Clade; H = haplotype (percentage of populations in which the haplotype was observed); N° = number of population; N_p = total of populations; N_i = total of individuals.

Clade (%)	H (%)	N° (N _p / N _i)	Geographic location
I	H22 (1.42)	15 (1 / 2)	Uruguay (San José)
	H25 (1.42)	93 (1 / 1)	Uruguay (Maldonado)
	H26 (7.29)	89, 90, 91, 92, 94, 95, 96 (7 / 10)	Uruguay (Canelones, Lavalleja, Maldonado and Rocha)
	H27 (2.08)	28, 34 (2 / 3)	Uruguay (Rivera) and Argentina (Corrientes)
	H35 (1.42)	43 (1 / 2)	Uruguay (Cerro Largo)
II	H20 (10.42)	2, 7, 8, 9, 25, 27, 38, 39, 40, 41 (10 / 11)	Argentina (Corrientes and Misiones) and Uruguay (Artigas, Rivera and Paisandú)
	H23 (13.54)	6, 10, 11, 17, 18, 19, 20, 22, 23, 24, 37, 42, 44 (13 / 16)	Argentina (Corrientes), Brasil (RS, Cachoeira do Sul, Encruzilhada do Sul, Bagé, Camaquá, Pelotas and Rochas de Bagé) and Uruguay (Cerro Largo and Treinta y Tres)
	H32 (1.42)	22 (1 / 1)	Brasil (RS, Camaquá)
	H33 (1.42)	21 (1 / 2)	Brasil (RS, Caçapava do Sul)
	H34 (1.42)	44 (1 / 1)	Uruguay (Treinta y Tres)
	H21 (5.21)	12, 13, 14, 16, 97 (5 / 8)	Uruguay (Cerro Largo and Tacuarembó)
III	H24 (1.42)	1 (1 / 1)	Argentina (Corrientes)
	H28 (3.13)	32, 33, 36 (3 / 5)	Argentina (Corrientes)
	H29 (1.42)	35 (1 / 4)	Argentina (Corrientes)
	H31 (1.42)	29 (1 / 2)	Uruguay (Tacuarembó)
	H36 (2.08)	26, 30 (2 / 2)	Uruguay (Florida)
	H3 (13.54)	45, 46, 49, 51, 54, 55, 58, 59, 60, 61, 70, 81, 82 (13 / 23)	Argentina (Buenos Aires, Chaco, Córdoba, Jujuy y Salta) and Bolívia (Cochabamba and Tarija)
H4 (2.08)	51, 53 (2 / 7)	Argentina (Chaco and Corrientes)	
H5 (2.08)	52, 56 (2 / 3)	Argentina (Chaco and Santiago del Estero)	
H6 (3.13)	48, 50, 57 (3 / 4)	Argentina (Catamarca, Salta, and Santiago del Estero)	
H7 (7.29)	72, 73, 74, 75, 76, 77, 78 (7 / 13)	Argentina (Córdoba)	
H8 (1.42)	47 (1 / 2)	Argentina (Salta)	
H9 (1.42)	63 (1 / 3)	Argentina (Corrientes)	
H10 (2.08)	74, 75 , (2 / 2)	Argentina (Córdoba)	

	H11 (4.17)	69, 71, 86, 87 (4 / 7)	Argentina (Buenos Aires) and Uruguay (Lavalleja and Punta Gorda)
	H12 (1.42)	61 (1 / 1)	Bolivia (Tarija)
	H13 (1.42)	55 (1 / 1)	Argentina (Salta)
	H14 (5.21)	64, 65, 66, 67, 88 (5 / 7)	Argentina (Corrientes) and Uruguay (Paisandú and Salto)
	H15 (7.29)	3, 4, 5, 62, 68, 79, 80 (7 / 9)	Argentina (Córdoba and Corrientes) and Uruguay (Río Negro)
	H16 (1.42)	83 (1 / 2)	Argentina (Córdoba)
	H17 (1.42)	81 (1 / 1)	Argentina (Córdoba)
	H18 (1.42)	84 (1 / 1)	Uruguay (Colonia)
	H19 (1.42)	85 (1 / 2)	Uruguay (Colonia)
	H30 (1.42)	31 (1 / 1)	Argentina (Corrientes)

Table S4. Results of individual-based Bayesian clustering analysis for GENELAND. Each individual is coloured according to the cluster from which presents the highest the posterior probability.

Pop number	Pop code	Cluster 1	Cluster 2	Cluster 3	Cluster 4
1	P14	0.14084507	0.521126761	0.154929577	0.183098592
2	s203_1	0.225352113	0.098591549	0.126760563	0.549295775
3	s215_38	0.070422535	0.281690141	0.563380282	0.084507042
	s215_61	0.070422535	0.281690141	0.563380282	0.084507042
	s215_104	0.070422535	0.281690141	0.563380282	0.084507042
4	s430_226	0.070422535	0.281690141	0.563380282	0.084507042
5	s431_235	0.070422535	0.281690141	0.563380282	0.084507042
6	Tur	0.225352113	0.098591549	0.126760563	0.549295775
7	P11	0.225352113	0.098591549	0.126760563	0.549295775
8	s208_1	0.225352113	0.098591549	0.126760563	0.549295775
	s208_3	0.225352113	0.098591549	0.126760563	0.549295775
9	s3_5_4	0.225352113	0.098591549	0.126760563	0.549295775
10	6_10_P1_1	0.225352113	0.098591549	0.126760563	0.549295775
	6_10_P1_4	0.225352113	0.098591549	0.126760563	0.549295775
11	3_10_P10_1	0.225352113	0.098591549	0.126760563	0.549295775
	3_10_P10_3	0.225352113	0.098591549	0.126760563	0.549295775
12	s48	0.14084507	0.521126761	0.154929577	0.183098592
13	s49	0.14084507	0.521126761	0.154929577	0.183098592
14	s96	0.14084507	0.521126761	0.154929577	0.183098592
15	s390_2	0.563380282	0.098591549	0.154929577	0.183098592
	s390_11	0.563380282	0.098591549	0.154929577	0.183098592
16	s156_1	0.14084507	0.521126761	0.154929577	0.183098592
	s156_3	0.14084507	0.521126761	0.154929577	0.183098592
17	P4uru	0.225352113	0.098591549	0.126760563	0.549295775
18	5_10_P11_4	0.225352113	0.098591549	0.126760563	0.549295775
19	6_10_P4_2	0.225352113	0.098591549	0.126760563	0.549295775
20	4_10_P5_4	0.225352113	0.098591549	0.126760563	0.549295775
21	5_10_P6_1	0.225352113	0.098591549	0.126760563	0.549295775
	5_10_P6_3	0.225352113	0.098591549	0.126760563	0.549295775
22	2_10_P1_1	0.225352113	0.098591549	0.126760563	0.549295775
	2_10_P1_3	0.225352113	0.098591549	0.126760563	0.549295775
23	3_10_P1_1	0.225352113	0.098591549	0.126760563	0.549295775
	3_10_P1_5	0.225352113	0.098591549	0.126760563	0.549295775
24	3_10_P9_2	0.225352113	0.098591549	0.126760563	0.549295775
25	s366_4	0.225352113	0.098591549	0.126760563	0.549295775
26	P10_6uru	0.14084507	0.521126761	0.154929577	0.183098592
27	s372_1	0.225352113	0.098591549	0.126760563	0.549295775
28	s373_3	0.563380282	0.098591549	0.154929577	0.183098592
	s373_6	0.563380282	0.098591549	0.154929577	0.183098592
29	s379_1	0.14084507	0.521126761	0.154929577	0.183098592

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	s379_8	0.14084507	0.521126761	0.154929577	0.183098592
30	P9_16uru	0.14084507	0.521126761	0.154929577	0.183098592
31	P5_2	0.070422535	0.281690141	0.563380282	0.084507042
32	P2_5	0.14084507	0.521126761	0.154929577	0.183098592
	P2_8	0.14084507	0.521126761	0.154929577	0.183098592
	P2_11	0.14084507	0.521126761	0.154929577	0.183098592
33	P8	0.14084507	0.521126761	0.154929577	0.183098592
34	s220_1	0.563380282	0.098591549	0.154929577	0.183098592
35	P4_1	0.14084507	0.521126761	0.154929577	0.183098592
	P4_2	0.14084507	0.521126761	0.154929577	0.183098592
	P4_3	0.14084507	0.521126761	0.154929577	0.183098592
	P4_4	0.14084507	0.521126761	0.154929577	0.183098592
36	Tabay	0.14084507	0.521126761	0.154929577	0.183098592
37	4_10_P9_2	0.225352113	0.098591549	0.126760563	0.549295775
38	s361_15	0.225352113	0.098591549	0.126760563	0.549295775
39	s403_3	0.225352113	0.098591549	0.126760563	0.549295775
40	s368_2	0.225352113	0.098591549	0.126760563	0.549295775
41	s370_1	0.225352113	0.098591549	0.126760563	0.549295775
42	P6_3uru	0.225352113	0.098591549	0.126760563	0.549295775
43	P8_2uru	0.563380282	0.098591549	0.154929577	0.183098592
	P8_3uru	0.563380282	0.098591549	0.154929577	0.183098592
44	P5_1uru	0.225352113	0.098591549	0.126760563	0.549295775
	P5_3uru	0.225352113	0.098591549	0.126760563	0.549295775
45	s212_7	0.070422535	0.281690141	0.563380282	0.084507042
46	s182_30	0.070422535	0.281690141	0.563380282	0.084507042
47	s187_8	0.070422535	0.281690141	0.563380282	0.084507042
	s187_28	0.070422535	0.281690141	0.563380282	0.084507042
48	s200_7	0.070422535	0.281690141	0.563380282	0.084507042
	s200_17	0.070422535	0.281690141	0.563380282	0.084507042
49	s261_1	0.070422535	0.281690141	0.563380282	0.084507042
50	s71	0.070422535	0.281690141	0.563380282	0.084507042
51	s32_1	0.070422535	0.281690141	0.563380282	0.084507042
	s32_3	0.070422535	0.281690141	0.563380282	0.084507042
	s32_4	0.070422535	0.281690141	0.563380282	0.084507042
	s32_5	0.070422535	0.281690141	0.563380282	0.084507042
	s32_6	0.070422535	0.281690141	0.563380282	0.084507042
	s32_7	0.070422535	0.281690141	0.563380282	0.084507042
52	s315_3	0.070422535	0.281690141	0.563380282	0.084507042
	s315_7	0.070422535	0.281690141	0.563380282	0.084507042
53	s86_7	0.070422535	0.281690141	0.563380282	0.084507042
	s86_23	0.070422535	0.281690141	0.563380282	0.084507042
	s86_28	0.070422535	0.281690141	0.563380282	0.084507042
	s86_30	0.070422535	0.281690141	0.563380282	0.084507042
54	s311_4	0.070422535	0.281690141	0.563380282	0.084507042
55	s312_1	0.070422535	0.281690141	0.563380282	0.084507042
	s312_4	0.070422535	0.281690141	0.563380282	0.084507042

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	s312_6	0.070422535	0.281690141	0.563380282	0.084507042
	s312_8	0.070422535	0.281690141	0.563380282	0.084507042
56	s69	0.070422535	0.281690141	0.563380282	0.084507042
57	s70	0.070422535	0.281690141	0.563380282	0.084507042
58	s250_11	0.070422535	0.281690141	0.563380282	0.084507042
	s250_14	0.070422535	0.281690141	0.563380282	0.084507042
	s250_22	0.070422535	0.281690141	0.563380282	0.084507042
	s250_27	0.070422535	0.281690141	0.563380282	0.084507042
59	s301_2	0.070422535	0.281690141	0.563380282	0.084507042
	s301_4	0.070422535	0.281690141	0.563380282	0.084507042
	s301_7	0.070422535	0.281690141	0.563380282	0.084507042
	s301_8	0.070422535	0.281690141	0.563380282	0.084507042
60	s302_3	0.070422535	0.281690141	0.563380282	0.084507042
61	s309_3	0.070422535	0.281690141	0.563380282	0.084507042
	s309_6	0.070422535	0.281690141	0.563380282	0.084507042
62	s415_5	0.070422535	0.281690141	0.563380282	0.084507042
63	s223_1	0.070422535	0.281690141	0.563380282	0.084507042
	s223_7	0.070422535	0.281690141	0.563380282	0.084507042
	s223_21	0.070422535	0.281690141	0.563380282	0.084507042
64	s314_5	0.070422535	0.281690141	0.563380282	0.084507042
	s314_13	0.070422535	0.281690141	0.563380282	0.084507042
65	s418_1	0.070422535	0.281690141	0.563380282	0.084507042
	s418_4	0.070422535	0.281690141	0.563380282	0.084507042
66	s419_1	0.070422535	0.281690141	0.563380282	0.084507042
67	s402_1	0.070422535	0.281690141	0.563380282	0.084507042
68	s400_1	0.070422535	0.281690141	0.563380282	0.084507042
69	s335_31	0.070422535	0.281690141	0.563380282	0.084507042
	s335_37	0.070422535	0.281690141	0.563380282	0.084507042
70	s342_1	0.070422535	0.281690141	0.563380282	0.084507042
71	s387_2	0.070422535	0.281690141	0.563380282	0.084507042
72	s393_1	0.070422535	0.281690141	0.563380282	0.084507042
	s393_7	0.070422535	0.281690141	0.563380282	0.084507042
73	s396_2	0.070422535	0.281690141	0.563380282	0.084507042
74	s355_4	0.070422535	0.281690141	0.563380282	0.084507042
75	s248_1	0.070422535	0.281690141	0.563380282	0.084507042
76	s328_3	0.070422535	0.281690141	0.563380282	0.084507042
77	s234_118	0.070422535	0.281690141	0.563380282	0.084507042
	s234_119	0.070422535	0.281690141	0.563380282	0.084507042
	s234_120	0.070422535	0.281690141	0.563380282	0.084507042
	s234_123	0.070422535	0.281690141	0.563380282	0.084507042
78	s235_1	0.070422535	0.281690141	0.563380282	0.084507042
	s235_179	0.070422535	0.281690141	0.563380282	0.084507042
	s235_181	0.070422535	0.281690141	0.563380282	0.084507042
	s235_184	0.070422535	0.281690141	0.563380282	0.084507042
79	s319_155	0.070422535	0.281690141	0.563380282	0.084507042
	s319_169	0.070422535	0.281690141	0.563380282	0.084507042

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3		s319_172	0.070422535	0.281690141	0.563380282	0.084507042
4	80	s326_14	0.070422535	0.281690141	0.563380282	0.084507042
5	81	s112	0.070422535	0.281690141	0.563380282	0.084507042
6	82	s237_1	0.070422535	0.281690141	0.563380282	0.084507042
7	83	s345_15	0.070422535	0.281690141	0.563380282	0.084507042
8	84	s347_1	0.070422535	0.281690141	0.563380282	0.084507042
9	86	s348_3	0.070422535	0.281690141	0.563380282	0.084507042
10	85	s349_G2_3	0.070422535	0.281690141	0.563380282	0.084507042
11		s349_G3_1	0.070422535	0.281690141	0.563380282	0.084507042
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13	87	s346_1	0.070422535	0.281690141	0.563380282	0.084507042
14		s346_2	0.070422535	0.281690141	0.563380282	0.084507042
15	88	s338_18	0.098591549	0.295774648	0.535211268	0.070422535
16		s338_27	0.098591549	0.295774648	0.535211268	0.070422535
17		s338_39	0.098591549	0.295774648	0.535211268	0.070422535
18	89	s406_1	0.563380282	0.098591549	0.154929577	0.183098592
19	90	s337_14	0.563380282	0.098591549	0.154929577	0.183098592
20		s337_54	0.563380282	0.098591549	0.154929577	0.183098592
21		s337P1_17	0.563380282	0.098591549	0.154929577	0.183098592
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23	91	s407_1	0.563380282	0.098591549	0.154929577	0.183098592
24	92	P3uru	0.563380282	0.098591549	0.154929577	0.183098592
25	93	s101_3	0.563380282	0.098591549	0.154929577	0.183098592
26	94	P2_7uru	0.563380282	0.098591549	0.154929577	0.183098592
27	95	s411_12	0.563380282	0.098591549	0.154929577	0.183098592
28	96	s412_1	0.563380282	0.098591549	0.154929577	0.183098592
29		s412_13	0.563380282	0.098591549	0.154929577	0.183098592
30						
31	97	s378_1	0.14084507	0.521126761	0.154929577	0.183098592
32		s378_4	0.14084507	0.521126761	0.154929577	0.183098592
33		s378_12	0.14084507	0.521126761	0.154929577	0.183098592
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