ORIGINAL ARTICLE

Geographic patterns of morphological variation in *Turnera sidoides* subsp. *pinnatifida* (Turneraceae)

Viviana G. Solís Neffa

Abstract With the objective of contributing to understanding the patterns of variation within the *Turnera sidoides* complex, a detailed evaluation of morphological variation along the range of *T. sidoides* subsp. *pinnatifida* was performed. A multivariate analysis based on leaf traits and flower colour data enabled differentiation of five morphotypes. Common-garden experiments demonstrated that the morphological variants have a strong genetic basis. It was also found that the morphotypes are geographically structured along the subspecies range, display different habitat preferences, and occur in regions with different climatic regimes. Although these results are suggestive of adaptive differentiation of *T. sidoides* subsp. *pinnatifida*, comparisons between morphological and bioclimatic ordinations showed that the patterns observed cannot be fully explained by current climatic conditions. It is proposed that Miocene–Pleistocene events may explain the origin of the five morphotypes and that current climatic and ecological factors may be contributing to the maintenance of the extent and patterns of morphological differentiation in *T. sidoides* subsp. *pinnatifida*.

Keywords Morphological variation · South America · *Turnera sidoides* subsp. *pinnatifida*

Introduction

*Turnera sidoides* L. is a species suitable for addressing questions about evolution at the infraspecific level. This autopolyploid complex ($x = 7$) of perennial rhizomatous herbs has marked morphological variability and is widely distributed in southern South America. It extends over southern Bolivia and Brazil, southwestern Paraguay, Uruguay, and Argentina, reaching 39°S (Arbo 1985; Solís Neffa 2000). *T. sidoides* is an obligate outbreeder because of dystily (Arbo 1985) and genetic self-incompatibility (Solís Neffa 2000). Seeds are dispersed by gravity and ants, and tend to concentrate in localized areas. Discrete populations, ranging from less than ten to hundreds of plants, are frequently observed. Populations are usually separated from one another by a few to several kilometres (Solís Neffa 2000).

Five subspecies have been recognized on the basis of the variability of leaf shape and the indumentum (Arbo 1985). *Turnera sidoides* subsp. *pinnatifida* (Juss. ex Poir.) Arbo is clearly distinguished by its pinnatifid to pinnatisect leaves, whereas the other four subspecies have elliptic to obovate leaves and are differentiated by the type of leaf indumentum. *Turnera sidoides* subsp. *carnea* (Cambess.) Arbo has a lax indumentum; subsp. *holosericea* (Urb.) Arbo has glaucous leaves with lanate-sericeus indumentum; *T. sidoides* subsp. *integrifolia* (Griseb.) Arbo is characterized by its hirsute indumentum and subsp. *sidoides* has stellated hairs (Arbo 1985). Some subspecies also have enormous among-population variation in morphological features, which manifests, especially, in leaf size and flower colour. In addition, from observations of plants in the field and in the greenhouse it is evident that populations with similar phenotypes tend to be located in the same geographical region (pers. obs.).

The morphological variability observed in *Turnera sidoides* is suggestive of an active process of diversification in this complex. This situation offers the possibility of studying the identity and relative importance of the

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Fig. 1 Major geographical features of the *Turnera sidoides* subsp. *pinnatifida* area. The *dashed line* denotes the distribution of the *Turnera sidoides* complex and the *dotted line* the distribution of *T. sidoides* subsp. *pinnatifida*. Both distributions are based on herbarium specimens and field observations
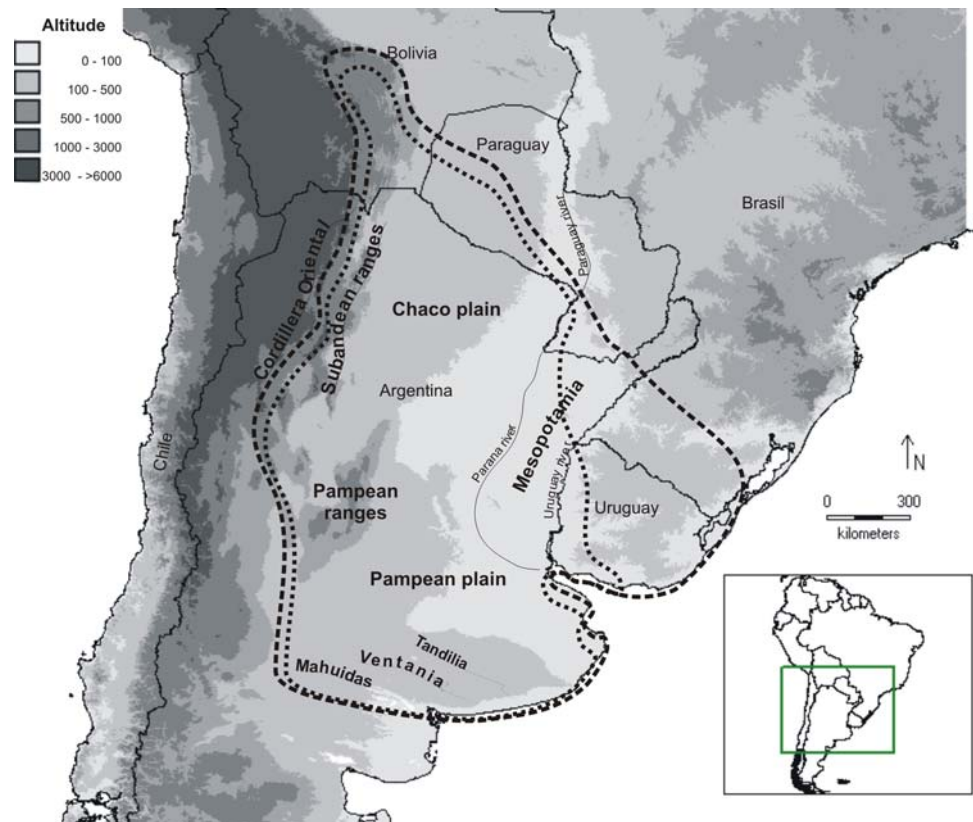
evolutionary forces that either promote or prevent differentiation between populations by using different approaches. The first step in this direction is the analysis of the extent and patterns of distribution of morphological variation, because this approach can be useful in understanding how development of the pleiotropic process, disequilibrium linkage, the environment, genetic drift, and natural selection may generate patterns of character variation within species (Armbruster 1991; Endler 1995). Despite the importance of this approach in evolutionary studies, no detailed analyses of the morphological variation of *T. sidoides* have been conducted.

In this sense, because case studies in individual subspecies can make valuable contributions to better understanding of the patterns of variation within *Turnera sidoides* complex, *T. sidoides* subsp. *pinnatifida* was selected to perform a detailed evaluation of the morphological variation along its entire distribution area. This subspecies is of particular interest, because among the subspecies of *T. sidoides* it is the most morphologically variable and has the largest geographical range.

For this purpose, in this study a multivariate analysis based on morphological data from herbarium specimens was first performed in order to determine the extent and patterns of distribution of morphological variation among 163 accessions of *T. sidoides* subsp. *pinnatifida*. In addition, because the range of this subspecies covers a wide

diversity of habitats, its morphological variation might represent phenotypic expressions affected by natural environmental conditions. Thus, to evaluate the extent to which leaf traits and flower colour variation is due to hereditary or environmental differences, transplant experiments in a common garden of individuals with distinctive morphological characteristics and collected from different geographical regions were carried out. Finally, because the climate is one of the main factors governing the distribution of plant species, a multivariate analysis based on bioclimatic variables was used to investigate the effect of climate in shaping the distribution of extant populations of *T. sidoides* subsp. *pinnatifida*.

Study area

Biogeographically, the geographic area of *Turnera sidoides* subsp. *pinnatifida* is included in the Chacoan subregion (Morrone 2000, 2006), and in the Pampeana, Espinal, Chaqueña, and Pre-Puneña provinces (Chaqueño Domain), extending into the Paranaense province (Amazónico Domain) (Cabrera 1971; Cabrera and Willink 1973). It ranges naturally in the relatively more xeric locations within the species range—southern Bolivia and Paraguay, western Uruguay, and Argentina (Solís Neffa 2000). This mostly low-lying area is surrounded by higher elevation ranges (Fig. 1). To the west, its boundary is a north-to-south

arch formed by the Cordillera Oriental, the Sierras Subandinas, and the Sierras Pampeanas. To the south, the area reaches the Mahuidas, Ventania, and Tandilia systems at approximately 39°S. This widespread latitudinal and longitudinal range comprises a great diversity of climates and ecological conditions with contrasting precipitation regimes. The rainfall decreases westwards from a maximum of around 1,200 mm along the Paraguay–Paraná rivers to a minimum of 450 mm at the western foothills. This decrease in rainfall is accompanied by the lengthening of the winter dry season from 2 to 7 months. On the eastern slopes of the first important mountain ranges, orographic rainfall which reaches a maximum at middle altitudes is produced. However, on the opposite rain-shadow slopes, precipitation drops to 100–300 mm, concentrated in 2–3 summer months and under thermal conditions, determined both by altitude and continentality. Winter frosts occur over the whole area, rather infrequently in the north-eastern part, and more frequently towards the south-west (Burgos 1970; Sarmiento 1972).

The Cordillera Oriental is a rugged terrain with steep slopes from 5,000 to 1,500 m a.s.l. limited by the Puna highlands on the western side. Mountain ranges, with a north–south direction, generate contrasting environments between eastern (wet and wooded) and western (arid) slopes. Annual precipitation varies from 350 mm on dry sites to 2,300 mm or more on wet sites. The highest rainfall levels occur between 1,000 and 1,500 m (Grau and Brown 2000). These mountain ranges are separated by low valleys (quebradas). Two types of subtropical forest cover the eastern slopes in an altitudinal gradient (Sarmiento 1972): the *Lower Montane Forest* or *Tucumano-Boliviana* rain forest (1,300–1,800 m a.s.l.), with *Blepharocalyx salicifolius* (Kunth) O. Berg., *Cinnamomum porphyrium* (Griseb.) Kosterm., *Cedrela lilloi* C. DC., *Ocotea puberula* (Rich.) Nees, and *Ficus maroma* A. Cast., as the main species; and the *Upper Temperate Montane Forest* (1,500–2,500 m a.s.l.), dominated by *Podocarpus parlatorei* Pilg., *Alnus acuminata* Kunth, and *Juglans australis* Griseb. Above the fog grasslands (2,500–3,500 m a.s.l.), there is steppe vegetation (3,500–4,700 m a.s.l. or higher). On the rain-shadow slopes, there is a semi-desert that covers the lower altitudinal belt below the high mountain steppe and the Puna cold semi-desert.

The Sierras Subandinas are placed between the Cordillera Oriental to the west and the Chaco-Pampean plain to the east, from near 10°S to 25°S. These north–south mountain systems (1,500–1,000 m a.s.l.) are transversally crossed by wide valleys that connect the ranges with the eastern plain. This area is characterized by a subtropical climate, with temperatures and precipitation that vary according to elevation and slope exposure. In most of their extent, the piedmont of the eastern slopes of the

Sierras is covered by *Subandean Piedmont Forests* (Prado 1993). There are two major types of forest within this unit, ordered in a N–S direction by a temperature gradient, and separated by a gap dividing the mountain ranges which allows the intrusion of Chaco communities into the inner dry valleys. The *"Palo Blanco"* and *"Palo Amarillo"* forest is dominated by *Calycophyllum multiflorum* Griseb., *Phyllostytlon rhamnoides* (J. Poiss.) Taub., *Anadenanthera colubrina* (Vell.) Brenan var. *cebil* (Griseb.) Altschul, *Cordia trichotoma* (Vell.) Arráb. ex Steud., *Patagonula americana* L., and *Astronium urundeuva* (Allemão) Engl. The *"Tipa"* and *"Pacará"* forest is dominated by *Tipuana tipu* (Benth.) Kuntze and *Enterolobium contortisiliquum* (Vell.) Morong. Towards the east and down the topographic gradient the forest is intermingled with Sierra or Western Chaco, with *Schinopsis marginata* Engl. forests.

The Sierras Pampeanas rise between 27°S and 33°S. The intermountain depressions are relatively flat and filled with sediments that form endorreic systems. At present, such systems are partially covered by salinas. The mountain system is within a temperate and semi-arid climate zone. The annual temperature ranges are lower than in the plain and fluctuate between 10 and 16°C. Above 2,000 m a.s.l, the lower winter temperatures cause isolated snowfalls. Precipitation increases according to elevation from 500 to 900 mm year^{-1}, with most warm rainfall concentrated in the warmer season. Two vegetational units have been described for this region: *Sierra Chaco* to the east and *Monte* to the west. In the former, main plant formations are distributed along different altitudinal belts with *Lithraea molleoides* (Vell.) Engl. and *S. marginata* forests at lower altitudes, "matorral serrano" with species of Asteraceae, for example *Heterothalamus alienus* (Spreng.) Kuntze, *Eupatorium buniifolium* Hook. ex Arn., and *Baccharis* L. spp., in the middle step, montane grasslands of *Festuca hieronymi* Hack. at higher altitudes, and palm forests of *Trithrinax campestris* (Burmeist.) Drude and Griseb. in the valleys (Luti et al. 1979). On the other hand, Monte is characterized by steppe vegetation dominated by "jarillales" (*Larrea* Cav. spp.) and "algarrobales" (*Prosopis flexuosa* DC and *P. chilensis* (Molina) Stuntz).

East from the Sierras Subandinas and Pampeanas, the Chaco plain covers a vast area with a gentle southeast–northwest 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 northwest to southeast. The climate is warm temperate humid in the east and semiarid in the west. The highest absolute temperature of South America has been recorded in the Chaco. Mean annual temperature ranges from 18°C in the south to 26°C in the north with an absolute maximum summer temperature of 48°C and

winter frost. Annual rainfall varies from 1,300 mm in the east to less than 500 mm in the west, whereas it increases when approaching the western high mountains. Most rainfall occurs during summer and there is a long winter drought (Burgos 1970; Ramella and Spichiger 1989). Along the plain, in correlation with climatic and edaphic xericity, the vegetation changes from xeromorphic forest and thickets in the northwest (Western Chaco) to semideciduous forest in the southeast (Eastern Chaco). In the Western Chaco Forest environmental factors are more uniform and, consequently, vegetation is more homogeneous. Western Chaco is the driest, most continental sector, with the highest maxima and lowest minima in both absolute and average temperature and the longest frost periods (Prado 1993). The vegetation shows the most pronounced xeromorphy and is closely linked with the alkalinity and texture of the soils. Two different vegetation units may be distinguished in this region. In the "Quebrachal of two quebrachos" (Adamoli et al. 1972), the most widespread vegetation consists of medium-tall forest dominated by Schinopsis lorentzii (Griseb.) Engl. and Aspidosperma quebracho-blanco Schltdl. The second arboreal stratum comprises Ziziphus mistol Griseb., Geoffroea decorticans (Gillies ex Hook. & Arn.) Burkart, Prosopis alba Griseb., P. nigra (Griseb.) Hieron., P. kuntzei Harms, Cercidium praecox (Ruiz & Pav.) Burkart & Carter, Caesalpinia paraguariensis (D. Parodi) Burkart, and arboreal cacti. In the "palosantales" of Bulnesia sarmientoi Lorentz ex Griseb. the dominant species is accompanied by Tabebuia nodosa (Griseb.) Griseb., P. kuntzei, Ruprechtia triflora Griseb., Capparis L. spp, Cereus forbesii Otto ex C.F. Först., and, sometimes, S. lorentzii (Morello and Adámoli 1968, 1974; Adamoli et al. 1972).

In the transition to both Western and Eastern Chaco there is the Central Chaco Forest. Two units may be recognized in this sector: the "quebrachal" of "three quebrachos", characterized by the shared dominance of S. lorentzii, S. balansae, and A. quebracho-blanco and, the "quebrachal" of the "white quebracho", A. quebracho-blanco (Lewis and Pire 1981).

The Eastern Chaco Forest grows in the temporarily waterlogged soils of the flooded plains of the Paraguay and Paraná rivers. In this area there are two major communities (Prado 1993):

1 the Austro-Brazilian Transitional Forest, which occupies the highest topographical position in well developed and intermediate soils, and which is dominated, among others, by Tabebuia heptaphylla (Vell.) Toledo, P. americana, Gledistsia amorphoides (Griseb.) Taub., Ruprechtia laxiflora Meisn., Pisonia zapallo Griseb., A. balansae, Ceiba insignis (Kunth) P.E. Gibbs & Semir; and

2 the "Quebrachal" (quebracho woodland) of S. balansae, which is always found at a lower topographical position than the previous one, on heavy-textured soils of the saline cycle. The ground here is usually waterlogged in the rainy season and two arboreal strata can be distinguished in this forest: the higher one, which is composed of S. balansae, A. quebracho-blanco, and C. paraguariensis, and the lower by P. nigra, Acacia praecox Griseb., A. aroma Gillies ex Hook. & Arn., G. decorticans, Sideroxylon obtusifolium (Roem. & Schult.) T.D. Penn., and Z. mistol. Copernicia alba Morong ex Morong & Britton builds up the extensive palm savannas of the Eastern Chaco that are in close contact with the xeromesophilous forest of S. balansae and Astronium spp., with the xerohygrophilous forest of Prosopis spp., and with the semi-evergreen gallery forest (Spichiger et al. 1995).

The vegetation of the islands of the Paraná river, and that of the banks of the Paraguay river and their western tributaries, is usually a Gallery Forest which is an impoverished version of the Subtropical Rainforest of Misiones (Argentina), southern Brazil, and eastern Paraguay. This forest is subjected to yearly flooding by nearby rivers. The dominant species are Nectandra angustifolia (Schrad.) Nees & Mart. ex Nees, Albizia inundata (Mart.) Barneby & J.W. Grimes, Tabernaemontana catharinensis A. DC., Inga verna Willd. subsp. affinis (DC.) T.D. Penn., Geoffroea striata (Willd.) Morong, Croton urucurana Baill., Arecastrum romanzoffianum (Cham.) Becc., etc. Following the courses of the tributaries, such forests may penetrate up to 100–150 km into the Gran Chaco plains. "Selvas de ribera" (River margin forests) can be found on higher ground, which is never reached by the floods of the Parana river. This forest is dominated by Holocalyx balansae Micheli, Ficus luschnathiana (Miq.) Miq., and P. americana (Prado 1993).

South of the Chaco plain, the Pampa plain extends from 30° to 40°S. The plain has a soft slope to the east-southeast and is only interrupted by the Mahuidas, Ventania, and Tandilia systems. This uniform area consists of medium and fine sediments deposed by aeolian, fluvial, and lacustrine processes. The northern part comprises a loess belt that borders a large sandy aeolian plain to the southwest. Its climate is temperate-humid, with mean annual temperatures ranging from 13.8° in the South to 15.9° in the North. Mean annual precipitation ranges from 850 to 900 mm. The plain is covered by treeless grassland with edaphic shrub communities. Vegetation is arranged as a complex mosaic of herbaceous plant communities (Burkart et al. 1990). Species composition responds to local variations in topography and soil salinity, but has also been shaped by a century of livestock grazing which has not only modified

native species composition, but also favoured the introduction of a number of exotic species.

Eastward of the Paraguay–Paraná line, the Mesopotamia (i.e., the interfluvial area between the Paraná and Uruguay rivers) has a transitional climate in the south towards a cold steppe climate and in the north towards a cool tropical climate. Mean annual rainfall ranges from about 950 mm in the south to 1,650 mm and over in the north-east (Van der Sluijs 1971). The main vegetation types are tropical rainforest in the northeast and humid medium-tall grass savannas with remnants of depauperate subtropical forest without "quebrachos" and characterized by thorny species of *Prosopis* and *Acacia* on the better-drained lateritic soils. Humid and wet tussocky grasslands are found on poorly drained soils or soils permanently covered by a high water table, sclerophyll woodlands, and forests along the main river banks (Van der Sluijs 1971; Carnevalli 1994).

Materials and methods

Plant material was obtained during personal field collections in 163 localities from 1995–2007. Plants were randomly sampled in each locality, and some were later transported to Corrientes (Argentina). Voucher specimens were deposited in the Herbarium of the Instituto de Botánica del Nordeste (CTES), and vouchers from Bolivian specimens were also deposited in the Herbarium Nacional de Bolivia (LPB). The collections of six other herbaria (CORD, LIL, LP, SI, MVFA, MVJB) were also considered. The herbaria consulted were cited in accordance with Holmgren et al. (1990). Details of geographical position and altitude of the material studied are given in the Appendix 1.

Morphological analysis of natural populations

Samples for statistical studies were chosen to represent the range of morphological variation and geographical range of *T. sidoides* subsp. *pinnatifida*. Because flower colour and leaf traits showed high among-population variation in the herbarium material and in the field, these traits were chosen to perform the analysis. Primary (pollen size and number, number of ovules, pollen/ovule ratio, and pollen viability) and secondary (petals length and width, style length, ovary length, anthers length, number of stigma branches, and anther length/style length ratio) reproductive traits, and fruit set, were excluded from the analysis because it has previously been demonstrated that they vary among populations in relation to ploidy level and, within population, in relation to distily (Solís Neffa 2000; Panseri and Solís Neffa 2007). Besides these traits, the diameter of the corolla and the colour of the styles and stigmas were also

excluded because of their within-populations variation (Panseri et al. 2003; pers. obs.).

Four quantitative leaf traits (area, total length, average width, and maximum width) were measured on 163 herbarium specimens, which were personally collected in the field. Leaf measurements were obtained from the largest leaves of specimens using a Li-COR Li-3000 portable leaf-area measurer. For each specimen, the mean of each trait was obtained from measurements of three leaves. In addition, two multistate characters were scored and codified: the leaf shape (pinnatifid = 1, pinnately-parted = 2 and pinnatisected = 3) and the colour of the flowers (1 = yellow, 2 = salmon, 3 = light pink and 4 = orange–salmon). The colour of the flower was recorded in the field, because it was difficult to confirm flower colour from herbarium specimens. The colour of the flowers of as many individuals as possible was carefully examined at each site.

Principal coordinate analysis (PCoA) was used to assess the patterns of morphological differentiation of populations of subsp. *pinnatifida*. Each individual (specimen) measured was treated as an independent operational taxonomic unit (OTU) for the multivariate statistical test. Two quantitative (leaf length and maximum leaf width) and two qualitative variables were selected for this analysis. Leaf area and mean width were excluded from the analysis because they are correlated with leaf length and maximum leaf width, respectively. A data matrix of four variables × 163 OTUs was constructed. A discriminant analysis (DA) from a data matrix of 163 OTUs × four quantitative and qualitative variables was also performed to test the ordination obtained from PCoA by displaying the principal differences among classes in relation to a given group of variables.

The mean average, standard deviation, and range of variation of quantitative variables were calculated for each group of populations resulted from multivariate analysis (further referred to as morphotypes). To evaluate the existence of significant differences for each trait among morphotypes, one-way ANOVA at a significance level of 5% ($\alpha = 0.05$) after Bartlett's test of homogeneity was made. Also, Tukey's test (5%) was carried out to test differences between each pair of means.

Common garden experiment

Before the study began, the plants were all grown in a common garden environment at the Instituto de Botánica del Nordeste under similar soil, water and nutrient regimes for, at least, one year. The leaf traits and the colour of petals were scored for plants of selected populations representative of morphological and geographical variability. One-way ANOVA and the Tukey post hoc test (5%) were used to compare population values.
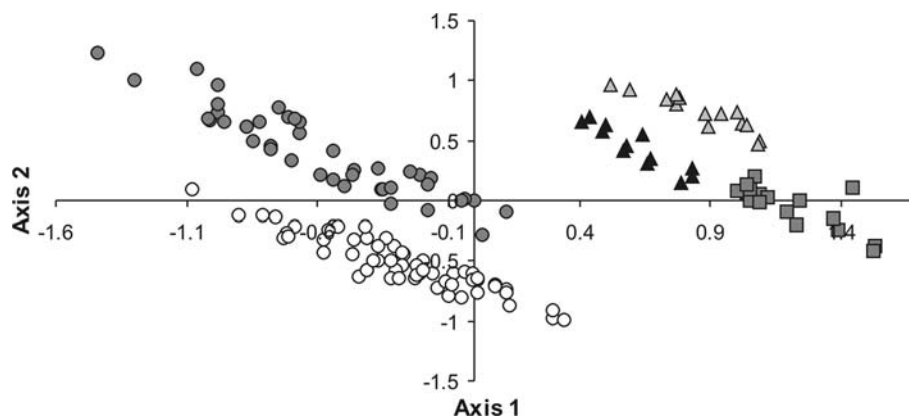
Fig. 2 PCoA ordination plot of the sample points in the plane of the first two principal axes based on morphological data. *Symbols* indicate samples with different leaf shape and flower colour: *open circles*, yellow flowers and pinnatifid leaves (*andino* morphotype); *grey circles*, salmon flowers and pinnatifid leaves (*chaqueño* morphotype);

black triangles, light-pink flowers and pinnately-parted leaves (*mesopotamico* morphotype); *light-grey triangles*, orange–salmon flowers and pinnately-parted leaves (*pampeano* morphotype); and *grey squares*, salmon flowers and pinnatisected leaves (*serrano* morphotype)

Geographical distribution

The geographical distribution of the morphotypes was analysed using a database of 326 georeferenced observations. Data were mostly obtained in the field but some were from herbarium specimens. When latitude and longitude were not available, the localities were georeferenced, whenever possible, using maps and digital gazetteers. Maps were made using Diva-Gis (Hijmans et al. 2004).

Ecological preferences of each morphotype were determined by observations in the field during collecting expeditions and by analysing the information provided on the labels of herbarium specimens.

Furthermore, in order to assess the potential effect of climate variables in shaping morphotype distributions, the following bioclimatic variables were extracted from the WorldClim data base (Hijmans et al. 2005) for each location where plants were collected: annual mean temperature, mean monthly temperature range, isothermality, temperature seasonality, maximum temperature of warmest month, minimum temperature of coldest month, temperature annual range, mean temperature of wettest quarter, mean temperature of driest quarter, mean temperature of warmest quarter, mean temperature of coldest quarter, annual precipitation, precipitation of wettest month, precipitation of driest month, precipitation seasonality, precipitation of wettest quarter, precipitation of driest quarter, precipitation of warmest quarter, and precipitation of coldest quarter.

Principal components analysis (PCA) was used to identify a smaller number of variables that account for the bulk of the variance in climates among the population locations. The calculation of the PCA was carried out on the correlation matrix estimated from the resulting standardized data set.

The mean average, standard deviation, and range of variation of bioclimatic variables were calculated for each morphotype. To evaluate the existence of significant differences for each variable among the morphotype areas, one-way ANOVA at a significance level of 5% ($\alpha = 0.05$) after Bartlett's test of homogeneity was performed. Also, Tukey's test (5%) was performed to test differences between each pair of means.

The two resulting ordinations (one based on morphological data and the other based on bioclimatic data) were compared in order to analyse the degree to which bioclimatic differences between regions explained the morphological variation among morphotypes. The comparison was performed informally by contrasting the ordination plots and by formally applying canonical correlation analysis.

All statistical analysis was performed using Infostat software, version 2009.

Results

Morphometric studies of natural populations

The PCoA results obtained suggested that there were five discrete groups (Fig. 2). The first two components of PCoA accounted for 81% (51% and 29%, respectively) of the variation. Axis 1 separated specimens with pinnatifid and larger leaves which appeared in the left quadrant from those with smaller leaves and pinnately-parted leaves which appeared in the right quadrant, and from specimens with pinnatisected leaves which had the highest scores. Axis 2 emphasized differences in the colour of the flowers thus separating specimens with yellow flowers, salmon, light pink and salmon–orange flowers. The graphic

Table 1 Contribution of the morphological variables to discriminant coordinates 1 (Axis 1) and 2 (Axis 2)

Variable	Axis 1	Axis 2
Flower colour	**0.99**	0.04
Leaf shape	0.01	**0.83**
Leaf length	−0.06	**0.37**
Maximum leaf width	0.08	0.01

Boldface indicates factor loadings of traits that most affect DA Axes 1 and 2

obtained shows the following groups: (1) specimens with pinnatifid leaves and yellow flowers (*andino* morphotype) which had negative values along both Axis 1 and 2; (2) samples with pinnatifid leaves and salmon flowers (*chaqueño* morphotype) which appeared in the upper left quadrant; sample points with pinnately-parted leaves and (3) light pink (*mesopotamico* morphotype) or (4) salmon-orange flowers (*pampeano* morphotype) which have positive values along Axis 1, and (5) samples with pinnatisected leaves and salmon flowers (*serrano* morphotype) which have the highest values along Axis 1.

The application of DA to the same data set subjected to PCoA revealed that the first two DA axes accounted for 99.91% of the total among-units variation. Most of the data variability was explained by the first DA axis (99.03% of the variance), which weighted flower colour, while the characters that best discriminated along the second axis (0.88% of the variation) were leaf maximum width and length. The absolute values of the coefficients of the standardized discriminant functions are shown in Table 1. The plot of the first two discriminant coordinates (Fig. 3) show that the first axis separated quite well samples of the *andino* morphotype in its negative half, followed by those of the *chaqueño* and *serrano* morphotypes scattered in the middle part of the plot; whereas samples of the *mesopotamico* and *pampeano* morphotypes had the highest scores. The classification function of discriminant analysis

indicates that the probability of the samples being correctly assigned to each group was 98%.

Figures 4 and 5 show the range of flower colour and leaf shape among the groups of natural populations resulting from multivariate analysis. The average values and standard deviation of the quantitative traits analysed are summarized in Table 2. One-way ANOVA showed that the means of all the variables differed significantly among morphotypes (Table 2). The boxplots of quantitative leaf traits illustrate the broad variation in most morphotypes (Fig. 6).

Common garden experiments

The differences in leaf shape and size, and the colour of the flowers that were evident among natural populations, persisted under greenhouse conditions (Fig. 7), although cultivated plants showed more vigorous growth. Flowers are ephemeral and thus exhibited more intense colours when they opened early in the morning and became discoloured early in the afternoon before they closed. A temporal change in flower colour intensities was observed in both field and cultivated plants. The means of all the variables differed significantly among morphotypes (Table 3).

Geographical distribution and ecological preferences

The spatial distribution of morphotypes is shown in Fig. 8. In this figure it may be appreciated that the morphotypes occur in geographically separated areas. Although the distribution areas of some morphotypes partially overlapped geographically, in the contact zones each form was restricted to its particular ecological niche. Individuals of different morphotypes were never found in simpatry. The morphotypes also display different habitat preferences and were collected from a wide range of vegetation types (Fig. 4). The *andino* morphotype occurs in southern Bolivia and northwestern Argentina, between 17°–27°S and

Fig. 3 Ordination plot of the sample points in the plane of the first two discriminant coordinates based on morphological data. *Symbols* indicate different morphotypes: *open circles*, *andino* morphotype; *grey circles*, *chaqueño* morphotype; *black triangles*, *mesopotamico* morphotype; *light grey triangles*, *pampeano* morphotype; and *grey squares*, *serrano* morphotype
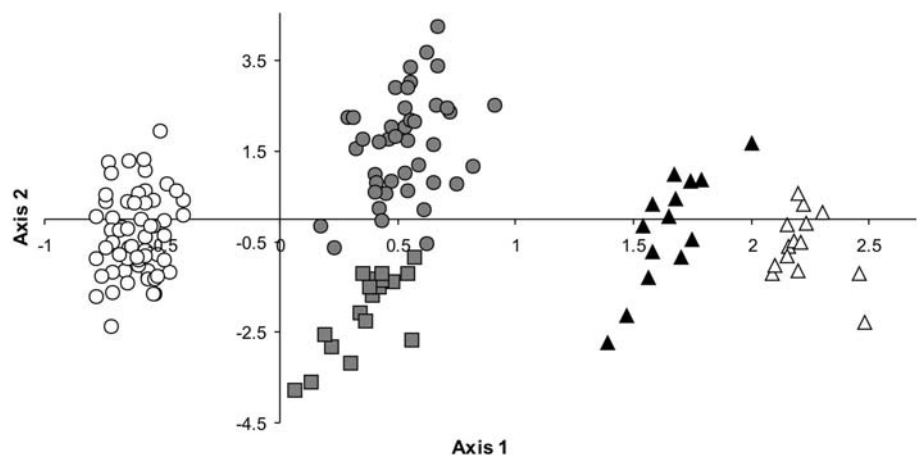
Fig. 4 Morphological and
environmental variation among
morphotypes of *Turnera
sidoides* subsp. *pinnatifida*.
a, b, *andino* morphotype.
a Individual with yellow
flowers. **b**, Mountain grassland
in the Sierras Subandinas.
c, d *chaqueño* morphotype.
c Individual with salmon flower.
d Chaco forest.
e, f *mesopotamico* morphotype.
e light-pink flower. **f** Thorny
vegetation in the Mesopotamia
plain. **g, h** *pampeano*
morphotype. **g** Orange–salmon
flower. **h** Pampean plain.
i, j *serrano* morphotype.
i Salmon flower. **j** Sierras
Pampeanas

Fig. 5 Leaf shape variation
among morphotypes of *Turnera
sidoides* subsp. *pinnatifida*
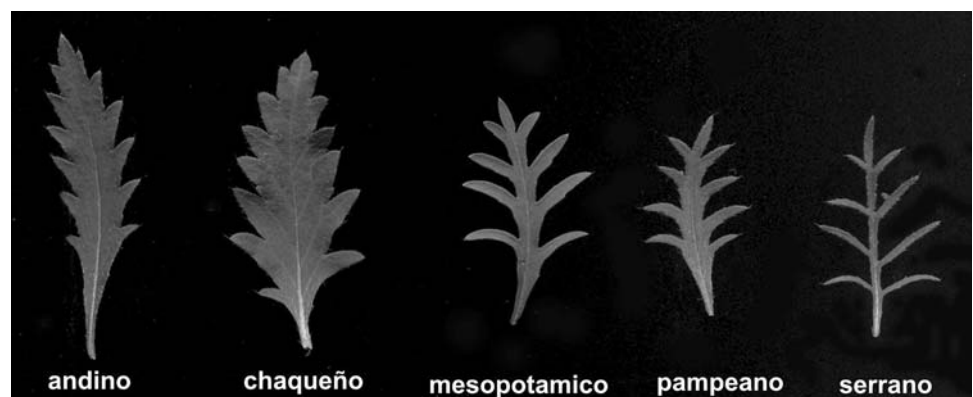
Table 2 Leaf and floral traits analysed in natural populations of each morphotype of *Turnera sidoides* subsp. *pinnatifida*

Morphotype	Flower colour	Leaf				
		Shape	Area (mm^2)	Length (mm)	Average width (mm)	Maximum width (mm)
Andino	Yellow	Pinnatifid	2.00^b ± 0.63	5.49^b ± 1.66	0.37^b ± 0.08	1.00^{b,c} ± 0.21
			0.75–3.34	2.17–8.53	0.20–0.60	0.73–1.37
Chaqueño	Salmon	Pinnatifid	2.59^a ± 1.03	6.00^a ± 2.00	0.47^a ± 0.39	1.34^a ± 0.34
			1.18–5.28	2.87–8.90	0.30–1.03	0.85–2.23
Mesopotamico	Light pink	Pinnately parted	1.56^c ± 0.60	4.09^c ± 1.11	0.37^{a,b} ± 0.11	1.06^b ± 0.22
			0.83–3.89	2.80–5.53	0.27–0.77	0.80–1.83
Pampeano	Orange–salmon	Pinnately parted	1.50^c ± 0.49	4.88^{b,c} ± 1.28	0.34^b ± 0.29	1.01^{b,c} ± 0.20
			0.93–2.66	3.63–6.47	0.20–2.03	0.73–1.60
Serrano	Salmon	Pinnatisected	1.05^d ± 0.43	4.01^c ± 1.24	0.26^b ± 0.06	0.88^c ± 0.24
			0.59–2.20	2.70–6.10	0.20–0.37	0.60–1.63
F (ANOVA)			48.79**	19.16**	7.00**	47.72**

Basic statistic: average, standard deviation, and range of variation of each quantitative leaf trait

Results of ANOVA for each quantitative leaf variable are shown in the last row (**$P < 0.05$). Within a column, different letters indicate significant differences (Tukey post-hoc test for multiple comparisons of means, $P < 0.05$)


Fig. 6 Boxplots of leaf area, length, mean width, and maximum width for morphotypes of *Turnera sidoides* subsp. *pinnatifida*. The boxplots portray the mean, median, interquartile range, adjacent values (*lines*), and outliers (*dots*) of the quantitative leaf traits analysed. *and*, *andino* morphotype; *cha*, *chaqueño* morphotype; *mes*, *mesopotamico* morphotype; *pam*, *pampeano* morphotype; and *ser*, *serrano* morphotype
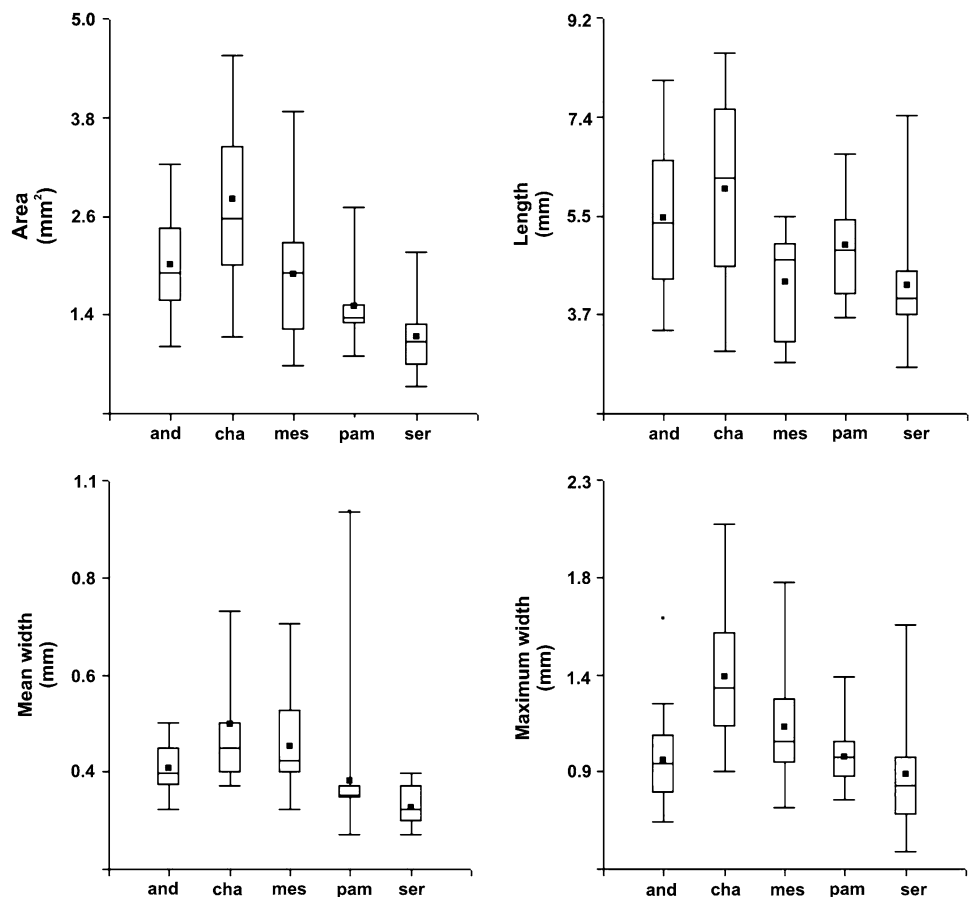
63°–66°W, and from 591 to 2,638 m a.s.l. (Fig. 8). It is widespread in the Subandean Piedmont Forest of the temperate and lower subtropical valleys between the Cordillera Oriental and the Sierras Subandinas, and in mountain grasslands at higher altitudes (Fig. 4a), but does not extend into the Tucumano-Boliviana rainforest that separates the ranges of the *andino* and *chaqueño* morphotypes. The *chaqueño* morphotype grows in clayed soils of

Fig. 7 Plants of the different morphotypes of *Turnera sidoides* subsp. *pinnatifida* cultivated under similar greenhouse conditions: **a**, *andino* morphotype; **b**, *chaqueño* morphotype; **c**, *mesopotamico* morphotype; **d**, *pampeano* morphotype; **e**, *serrano* morphotype
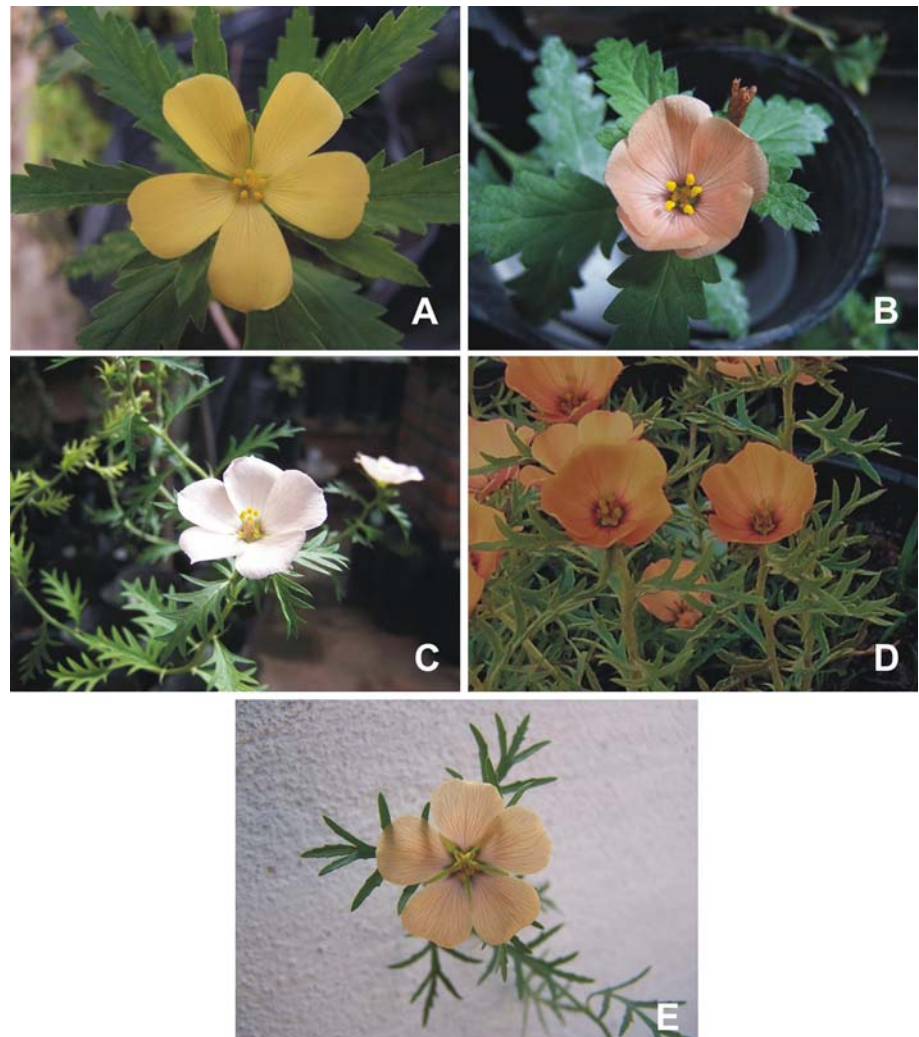

Table 3 Leaf and floral traits analysed in plants of each morphotype of *Turnera sidoides* subsp. *pinnatifida* growing under greenhouse conditions

Morphotype	N	Flower colour	Leaf				
			Shape	Area (mm^2)	Length (mm)	Average width (mm)	Maximum width (mm)
Andino	62	Yellow	Pinnatifid	3.55^b ± 0.49	5.87^{a,b} ± 1.30	0.63^b ± 0.07	1.39^b ± 0.14
				2.88–4.48	4.40–9.60	0.50–0.70	1.20–1.60
Chaqueño	52	Salmon dark pink	Pinnatifid	4.73^a ± 1.30	6.13^a ± 0.75	0.77^a ± 0.15	1.93^a ± 0.30
				2.96–8.26	4.80–7.50	0.60–1.10	1.30–2.60
Mesopotamico	15	Light pink	Pinnately parted	2.95^{b,c} ± 0.80	4.95^c ± 0.71	0.59^b ± 0.15	1.47^b ± 0.29
				1.96–4.45	3.75–6.10	0.40–0.80	1.00–2.00
Pampeano	18	Orange–salmon	Pinnately parted	2.33^{c,d} ± 0.53	5.58^{b,c} ± 0.93	0.35^c ± 0.08	1.28^b ± 0.23
				1.77–3.19	5.30–7.90	0.30–0.50	0.90–1.50
Serrano	16	Salmon	Pinnatisected	1.68^d ± 0.51	4.99^c ± 1.16	0.33^c ± 0.08	1.25^b ± 0.34
				0.80–2.82	3.40–7.00	0.20–0.50	0.80–2.00
F (ANOVA)				29.95**	5.30**	37.28**	18.57**

Basic statistic: average, standard deviation, and range of variation of each quantitative leaf trait

Results of ANOVA for each quantitative leaf variable are shown in the last row (**$P < 0.05$). Within a column, different letters indicate significant differences (Tukey post-hoc test for multiple comparisons of means, $P < 0.05$)

Fig. 8 Distribution of morphotypes across the *Turnera sidoides* subsp. *pinnatifida* range, based on altitudinal data. *Shading* represents altitudinal gradient (m a.s.l.)
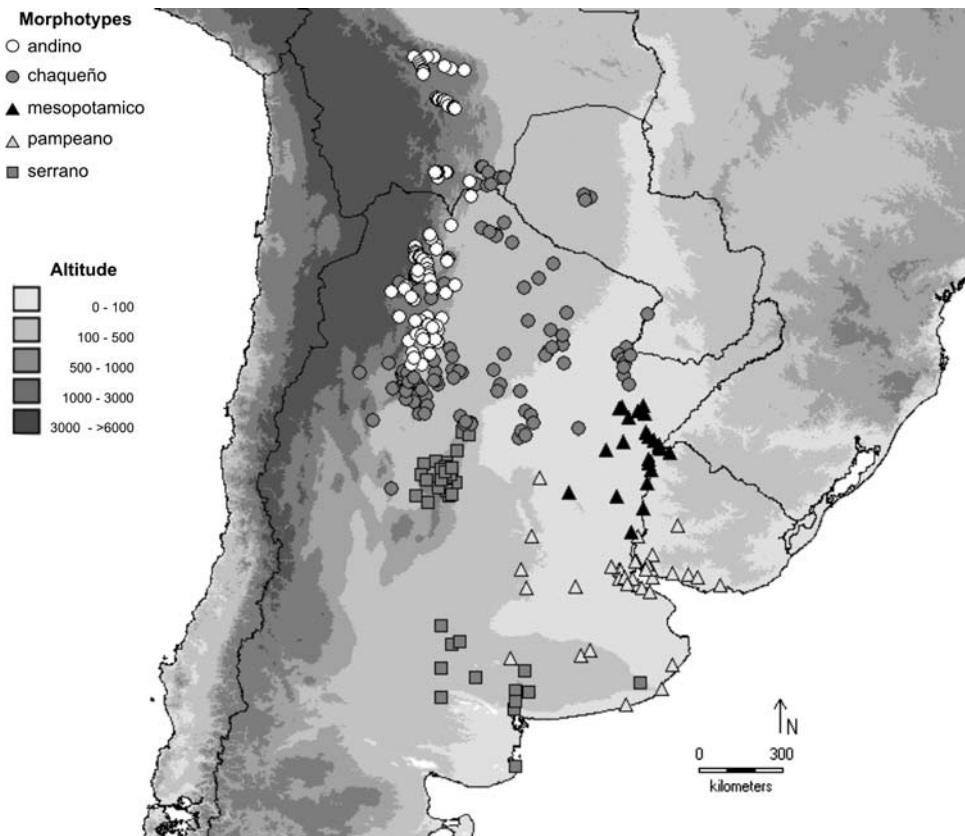
the Chaco plains of Bolivia, Paraguay, and Argentina (Fig. 4b), between 20°–31°S and 58°–66°W, in altitudes that vary from 20 m a.s.l. at the eastern limit to 850 m a.s.l. at the western boundary (Fig. 8). In Argentina, this morphotype grows mostly in the "quebrachales" of Western and Eastern Chaco forests, whereas in Bolivia it occurs in the Pilcomayo District (Chaco Boreal Occidental Sector, according to Navarro and Maldonado 2002). Some populations were also detected in clayed soils of northwestern Mesopotamia and southwestern Uruguay. The *mesopotamico* morphotype is only known from the northeastern subspecies boundary, from 28° to 33°S and from 57° to 60°W and at altitudes up to 100 m a.s.l. (Fig. 8) and was mostly detected on outcrops of sandstones frequently associated with woodland vegetation dominated by *Propopis* (Fig. 4c) in central Mesopotamia and northwestern Uruguay. The *pampeano* morphotype was restricted to grasslands in the deep humic soils of the Pampean aeolian plain of Argentina and Uruguay (Fig. 4d), between 33° and 39°S and 54° and 62°W, at altitudes that vary from 2 to 200 m (Fig. 8). With a slightly disjunct distribution, the *serrano* morphotype was found in rocky sites throughout the Sierras Pampeanas (Fig. 4e), with Sierra Chaco vegetation, between 29°and 31°S and 58°34′and 66°19′W, at altitudes from 350 to 1,400 m a.s.l., and in the Tandilia,

Ventania, and Mahuidas systems from 36° to 40°S 60° to 64°W, and from 100 to 300 m a.s.l. (Fig. 8).

The morphotypes also inhabit regions with different climatic regimes. The bioclimatic traits that characterize the geographical area of each morphotype are summarized in Appendix 2. Multivariate analysis (PCA) based on bioclimatic data showed that the first two components accounted for 79% of the total variance. The loadings of each variable to components 1 and 2 are shown in Table 4. Most of the bioclimatic data variability (43%) was explained by the first ordination axis (Fig. 9). This axis represented a major gradient in annual mean temperature, maximum temperature of the warmest month, minimum temperature of coldest month, mean temperature of the wettest quarter, mean temperature of the warmest quarter, mean temperature of the coldest quarter, annual precipitation, and precipitation seasonality. The lowest values on the first axis correspond to the *andino* and *serrano* morphotypes sample points whereas the highest ones correspond to the *chaqueño* and *pampeano* morphotypes, followed by the sample points of the *mesopotamico* morphotype, which are scattered over the right part of the ordination axis. The second component, accounting for the remaining 36% of the variance was closely related to the mean temperature of the driest quarter, mean

Table 4 Contribution of the bioclimatic variables to components 1 (Axis 1) and 2 (Axis 2)

Bioclimatic variable	Axis 1	Axis 2
Annual mean temperature	**0.28**	**0.26**
Mean monthly temperature range	−0.20	−0.04
Isothermality	−0.23	−0.18
Temperature seasonality	0.16	−0.21
Maximum temperature of warmest month	**0.28**	−0.09
Minimum temperature of coldest month	**0.27**	−0.19
Temperature annual range	−0.01	0.12
Mean temperature of wettest quarter	**0.29**	−0.17
Mean temperature of driest quarter	0.24	**−0.34**
Mean temperature of warmest quarter	**0.30**	−0.12
Mean temperature of coldest quarter	0.24	**−0.33**
Annual precipitation	**0.28**	0.19
Precipitation of wettest month	0.14	−0.11
Precipitation of driest month	0.21	**0.37**
Precipitation seasonality	**−0.24**	**−0.30**
Precipitation of wettest quarter	0.16	−0.12
Precipitation of driest quarter	0.21	**0.37**
Precipitation of warmest quarter	0.19	−0.07
Precipitation of coldest quarter	**0.24**	**0.32**

Boldface indicates factor loadings of traits that most influence PC axes 1 and 2

temperature of the coldest quarter, precipitation of the driest month, precipitation seasonality, precipitation of the driest quarter, and, precipitation of the coldest quarter. This axis separated the *andino* and *chaqueño* morphotypes from the *serrano, pampeano*, and *mesopotamico* ones. One-way ANOVA showed that, except for the annual temperature range, the precipitation of the wettest quarter, and the precipitation of the warmest quarter, the means of all the

bioclimatic variables differed significantly among geographical morphotype ranges (Appendix 2). The bioclimatic traits that best characterized the geographical area of the morphotypes are represented in Figs. 10 and 11.

By comparing ordinations based on morphological and bioclimatic data, it was evident that morphotypes with pinnatifid leaves (*andino* and *chaqueño*) grow in areas with higher precipitation seasonality and lower precipitation in the driest period, in contrast with those with pinnatisected (*serrano*) or pinnately-parted (*mesopotamico* and *pampeano*) leaves. In addition, morphotypes with narrower leaves (*andino* and *serrano*) grow in the coldest and driest areas, in contrast with morphotypes with wider leaves (*chaqueño, mesopotamico,* and *pampeano*). The *chaqueño* morphotype exhibited a wider range of variation of quantitative leaf traits than the other morphotypes, in accordance with its wider climatic range. Plants of this morphotype appeared to be more vigorous at the more humid sites of the east (Eastern Chaco) than in drier sites of the west (Western Chaco). No relationship was found by comparing the patterns of flower colour variation and the bioclimatic data. Canonical correlation analysis showed an intermediate degree of coincidence between ordinations, resulting from averaging medium correlation in the direction of the first canonical variates ($r^2 = 0.48$) with law correlation in the direction of the second one ($r^2 = 0.34$).

Discussion

The multivariate analysis based on morphological data was helpful in revealing the existence of five morphotypes in *Turnera sidoides* subsp. *pinnatifida*. Although the ranges of variation of the quantitative leaf traits overlap partially, by combining these traits with leaf shape and the colour of the

Fig. 9 Ordination plot of the sample points in the plane of the first two principal components (in PCA) based on bioclimatic data. *Symbols* indicate different morphotypes: *open circles, andino* morphotype; *grey circles, chaqueño* morphotype; *black triangles, mesopotamico* morphotype; *light grey triangles, pampeano* morphotype; and *grey squares, serrano* morphotype
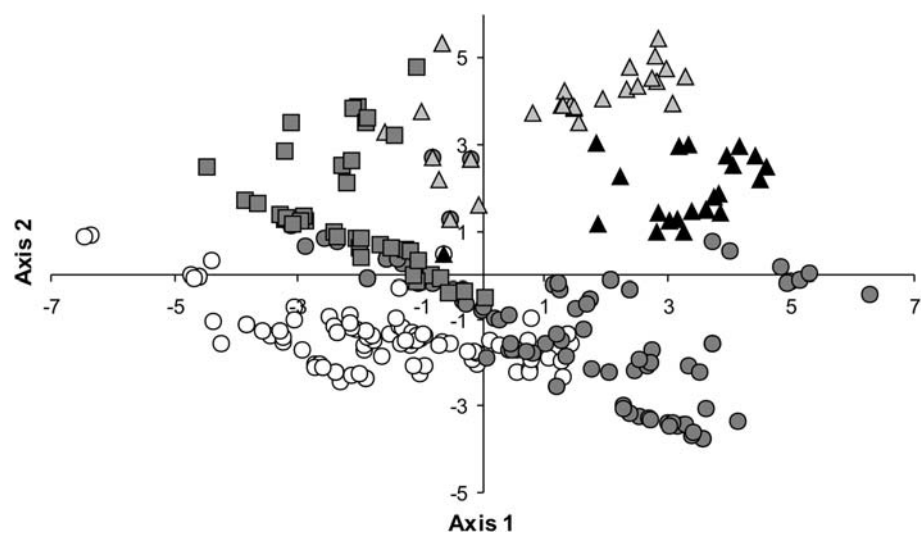
Fig. 10 Distribution of morphotypes across *Turnera sidoides* subsp. *pinnatifida* range based on bioclimatic analysis. **a** Annual mean temperature (°C). **b** Mean temperature of wettest quarter (°C). **c** Mean temperature of driest quarter (°C). **d** Mean temperature of warmest quarter (°C). *Symbols* indicate different morphotypes: *open circles*, *andino* morphotype; *grey circles*, *chaqueño* morphotype; *black triangles*, *mesopotamico* morphotype; *light grey triangles*, *pampeano* morphotype; and *grey squares*, *serrano* morphotype. *Shading* represents temperature gradient
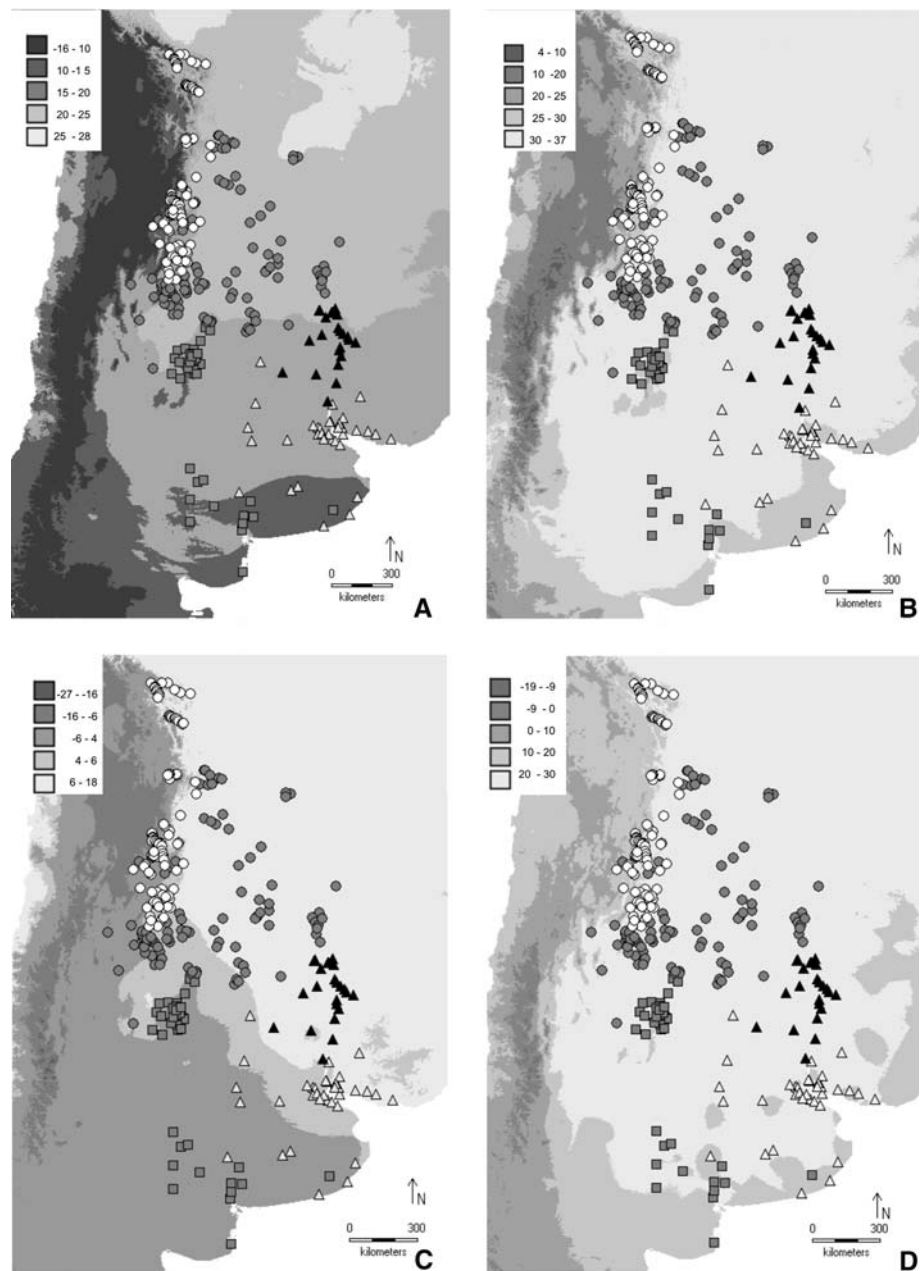

flowers, the morphotypes can be unequivocally distinguished. The *andino* morphotype has yellow flowers and narrow pinnatifid leaves, the *chaqueño* morphotype characteristically has wide pinnatifid leaves and salmon flowers, the *mesopotamico* morphotype has pinnately parted leaves and light-pink flowers, the *pampeano* morphotype has pinnately parted leaves and salmon-orange flowers, and the *serrano* morphotype is easily recognized by pinnatisect leaves and salmon flowers.

Common garden experiments have been widely used to test hypotheses on the relative influence of the environment and genetic factors on the morphology of plants that inhabit different habitats (Schaal et al. 1991; Oliva et al. 1993;

Quiroga et al. 2002). In *Turnera sidoides* subsp. *pinnatifida*, although shifts in quantitative leaf traits were observed among field-collected plants and those cultivated under the same greenhouse conditions, the relative differences among morphotypes of characters expressing leaf size were mostly retained. Moreover, populations of the five morphotypes also maintained the leaf shape and the colour of the flowers observed in the field, thus indicating that the morphological differences among morphotypes have a strong genetic basis.

When analysed on a large geographical scale, patterns of variation became much more obvious, the morphological variants being geographically structured along the range of

Fig. 11 Distribution of morphotypes across *Turnera sidoides* subsp. *pinnatifida* range based on bioclimatic analysis. **a** Annual precipitation (mm). **b** Precipitation of driest month (mm). **c** Precipitation seasonality (mm). **d** Precipitation of driest quarter (mm). *Symbols* indicate different morphotypes: *open circles*, *andino* morphotype; *grey circles*, *chaqueño* morphotype; *black triangles*, *mesopotamico* morphotype; *light grey triangles*, *pampeano* morphotype; and *grey squares*, *serrano* morphotype. *Shading* represents precipitation gradient
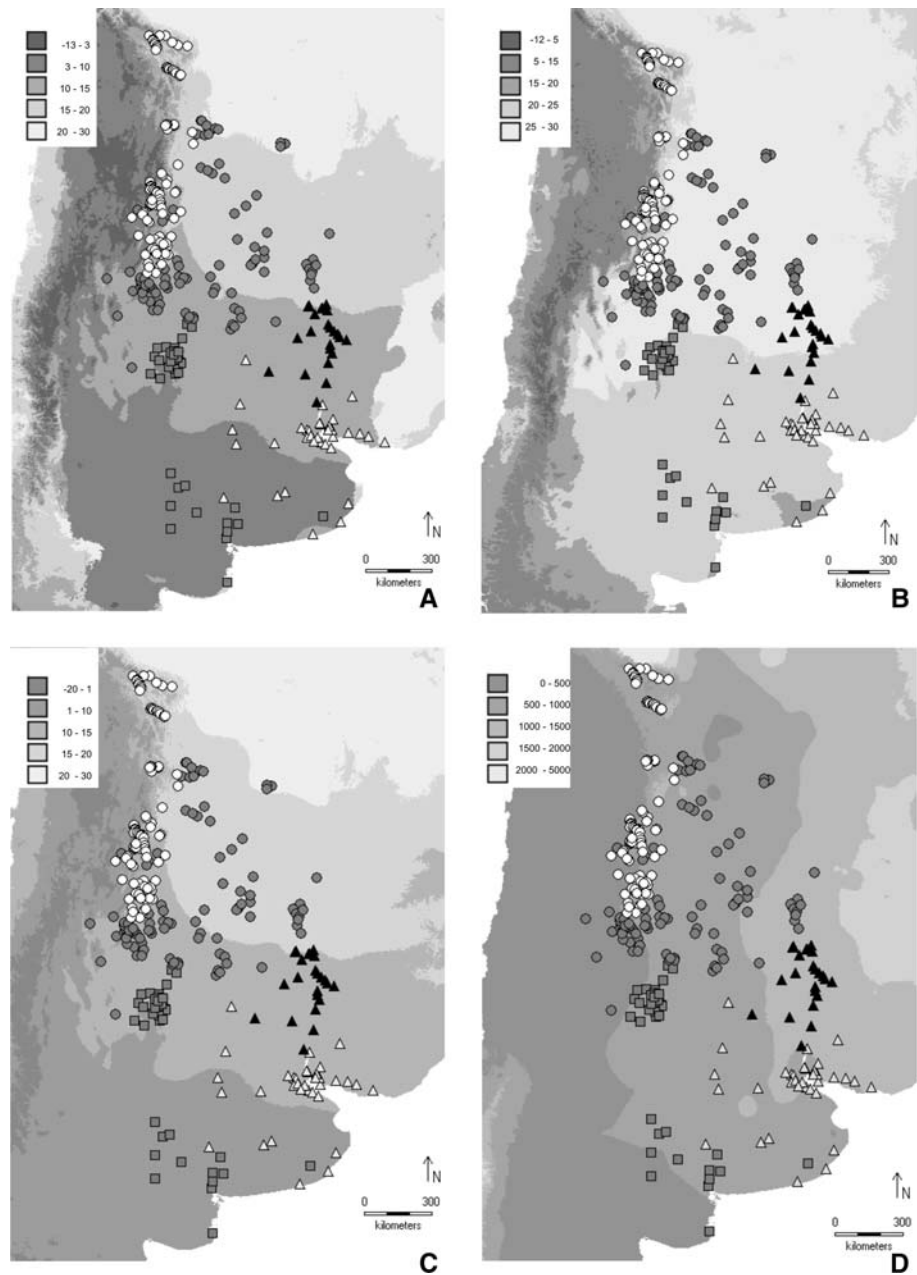
T. sidoides subsp. *pinnatifida*. Currently, the habitats of the *andino* and *serrano* morphotypes are the mountain regions, although the former reaches higher altitudes. In contrast, the other three morphotypes occur in the lowlands, in different soil conditions. Such environmental differences among morphotypes seem to be critical factors determining morphotype distribution on a local scale. The five morphotypes also occur in habitats with different vegetation cover. Except for the *pampeano* morphotype, which grows mostly in grasslands, and the *andino* morphotype, which occurs in the Subandean Piedmont Forest and in mountain grasslands, the *chaqueño, mesopotamico*, and *serrano*

morphotypes grow in woodlands dominated by different arboreal species.

Factors that affect the distribution and abundance of populations on a large geographical scale include how organisms relate to their environment (MacArthur 1984). Historical factors, for example geographical barriers and/or lack of sufficient dispersal opportunities, also affect species distributions. Current climate is considered one of the main factors explaining the distribution pattern of many plant species, because it acts either directly through physiological constraint on growth and reproduction or indirectly through ecological factors such as competition for

resources (Shao and Halpin 1995). The area of *T. sidoides* subsp. *pinnatifida* is characterized by a subtropical to temperate climate, which varies from humid in the east (annual precipitation >1,600 mm) to semi-arid and arid (<400 mm) in the west and south-west. Rainfall increases again (>2,300 mm) on the eastern slopes of the Subandean ranges. The region also shows a temperature decrease with a gradual north–south gradient (Cabrera and Willink 1973; Iriondo and García 1993). Within this wide range of climatic conditions, the PCA based on bioclimatic data showed that the morphotypes of *T. sidoides* subsp. *pinnatifida* occur in different climates. The *andino* and *serrano* morphotypes grow in colder and drier regions and with higher precipitation seasonality than the *pampeano* and *mesopotamico* ones. The climate that characterizes the area of the *chaqueño* morphotype varies from humid in the east, for example the *pampeano* and *mesopotamico* morphotypes, to semi-arid and arid in the west, for example the *serrano* morphotype, although with higher precipitation seasonality than these three morphotypes.

Comparison of the leaf traits and bioclimatic data revealed an interesting tendencies toward larger leaf area in regions with higher precipitation seasonality and toward wider leaves in the warmest and wettest areas of *T. sidoides* subsp. *pinnatifida*. The variation patterns of leaf traits along the area of *T. sidoides* subsp. *pinnatifida* are in agreement with the trend toward smaller leaf size in subtropical South American woody vegetation types (Sarmiento 1972). The differences observed between populations living in different habitats could be the consequence of either a relatively plastic genome interacting with different environments or an adaptation to such different environments. The facts that the morphological differences among morphotypes of *T. sidoides* subsp. *pinnatifida* have a genetic basis and that morphotypes occur in different environments suggest adaptive differentiation of leaf traits in *T. sidoides* subsp. *pinnatifida*.

Flower colour was geographically structured in *Turnera sidoides* subsp. *pinnatifida*, varying with a gradual west–east gradient from yellow to light pink and orange. However, colour variation in relation to climate is unclear and the pattern of variation could not be explained by any of the variables analysed. Thus, this floral trait may be associated with other environmental factors such as soil types or UV radiation. Alternatively, it has been proposed that variation in flower colour may be the result of the adaptive response to divergent selective pressures generated by pollinators (Meléndez-Ackerman et al. 1997; Niove Jones and Reithel 2001). In this sense, it has been demonstrated that yellow and salmon flowers of *T. sidoides* subsp. *pinnatifida* are different in colour to bees and that flowers differing in colour are visited by different pollinators (Benítez-Vieyra et al. 2007). Thus, divergent selective pressures generated by pollinators could contribute to the differentiation of the flower colour of the morphotypes. This mechanism may be especially important in the maintenance of morphotype distribution in the contact zones.

The intermediate degree of coincidence among morphological and bioclimatic ordinations suggests that the patterns of morphological variation along the subspecies range cannot be fully explained by the current climatic conditions. In this sense, because the areas in which the morphotypes currently occur were subjected to different morphogenetic and climatic historical processes from the Miocene onwards, current biogeographical patterns of morphotypes may be explained in terms of Miocene–Pleistocene events.

The differentiation of vegetation that would later give rise to the present flora of the *Turnera sidoides* subsp. *pinnatifida* geographical range began during the Oligocene (Spichiger et al. 1995). Paleontological, paleoclimatological, 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. Cooling and aridification began in the Oligocene, and, simultaneously with the climatic changes induced by the uplift of the Andes during the Miocene, the forest became fragmented (Morrone 2001, 2006). At the time of the Quaternary, climatic oscillations caused advances and retreats of glaciers at higher latitudes, altitudinal shifts and cyclical coalescence and separation of vegetational zones in the mountains, and a series of dry and humid periods in the lowlands (Iriondo and García 1993). A dry period occurred at the end of the Pleistocene, together with the large glacial maximum (LGM), and a briefer and less severe one in the Upper Holocene (Iriondo and García 1993). During the drier periods, a pronounced aeolian activity deflated and redeposited large masses of silt and fine sand over most of the area (Popolizio 1982, 2003; Iriondo 1992; Iriondo and García 1993). The vegetation cover of the region has not been stable since the LGM owing to oscillations in the balance between xerophytic and tropical and subtropical types of vegetation. The former has advanced repeatedly towards the NE during the dry and colder periods and retreated towards the SW during the humid and warmer climates (Popolizio 1970, 2003; Iriondo 1992). During the cold–dry phases, the river valleys and ravines in the hilly areas of Argentina, Uruguay, and Rio Grande do Sul, and some lowland areas, may have served as refugia for a flora adapted to more humid climates (Vuilleumier 1971; Ab'Sáber 1977). Such geomorphological and climatic events could have had significant effects on the distribution and abundance of populations of *T. sidoides* subsp. *pinnatifida*, disrupting the subspecies range and confining some populations to a number of isolated regions with particular selective pressures. This

isolation hypothesis is supported by the geographic structure of cpDNA haplotypes of *T. sidoides*, which, it has been proposed, are fixed in three putative refugial areas associated with the main orographical systems in the species area (Speranza et al. 2007). In this scenario, the morphotypes of *T. sidoides* subsp. *pinnatifida* would have originated by divergent evolution from an ancestral form in those refugia and, after the amelioration of the climate, the differentiated populations may have recolonized the area from such refugia up to their current limits.

Concerning the *andino* morphotype, the altitudinal shift of the vegetation zones as a consequence of the Andes uplift (Simpson 1975) may have restricted populations to the temperate valleys limited by the Cordillera Oriental and Sierras Subandinas. This morphotype would have expanded its range from these valleys toward the subtropical valleys and the mountain grasslands. Two physical barriers would have limited the eastward expansion of this morphotype into the Chaco plains: the establishment of more xeric conditions and the settlements of clayed soil. In Sierras Pampeanas, the climatic changes induced by the Andes uplift gradually yielded arid environments and more xeric vegetation (Luti et al. 1979). These changes might be enough to act as significant barriers, triggering local adaptation and subsequent differentiation of the *serrano* morphotype. This morphotype would have evolved in the central part of the Sierras Pampeanas, followed by dispersal throughout these sierras and toward the Ventania and Tandilia systems through the Mahuidas corridor. Because the distribution of the *chaqueño* morphotype is associated with the quebracho forest in the Chaco plain, its evolutionary history may be directly related to the dynamics of this type of vegetation. The patterns of vegetation of the Chaco have been proposed to be the result of the Pleisto–Holocenic alluvional dynamics of the plains and of the intensive migration of the fluvial belts of the main rivers (Iriondo 1995; Morello and Mateucci 1999). The Chaco plain is a great sedimentary basin limited by the Andes and the Brazilian craton. The Andean uplift led to the formation of a syncline, which was first occupied by two Miocene marine transgressions that, after their withdrawal, left marine sediments over which Andean deposits were accumulated (Tapia 1935). The dry climate during the Lower Holocene caused the mobilization and deposition of sand and thick loess transported by northerly winds. The Middle Holocene was characterized by humid, subtropical, and tropical climates and by the development of fluvial belts and widespread pedogenesis. During the Upper Holocene, warm and humid conditions were replaced again by a semiarid climate. Winds generated by an anticyclone deflated the Holocene soils, depositing a thin silt and sand layer that currently covers the entire region (Popolizio 1982, 2003; Iriondo 1992; Iriondo and García 1993).

Before the settlement of the present loamy salty and alkaline substratum colonized by xeromorphic flora, the vegetation of the Chaco plain should have been cerrado-like or a mesophilous forest. The saline soils and a highly seasonal climate would effectively limit the westward expansion of the Paranaense forest, although the immigration of non-Chacoan species is made possible by a net of gallery forests (Spichiger et al. 2004). The initial differentiation of the *chaqueño* morphotype probably took place in the northwestern boundary of the Chaco plain. From this northwestern boundary, the range expansion eastward and southward of the Chaco plain might have occurred concomitantly with the migration of the river channels and the subsequent colonization of the paleo-channels by the quebracho forest. To the south, the Pampa plain consists of medium and fine sediments deposited by aeolian, fluvial, and lacustrine processes. The northern part comprises a loess belt that borders a large sandy aeolian plain to the southwest. The typical loess of the region was deposited during the dry, cold periods of the Quaternary. At that time, the vegetation oscillated from psammophytic, gramineous, or shrubby steppes during the cold, dry periods to humid grasslands and pond environments during more humid conditions (Prieto 1996). Under these conditions, populations of the *pampeano* morphotype may have evolved and diversified. Finally, tectonic movements and active faulting processes, which continued into the Quaternary, played an important role in the shaping of the present landscape in the Mesopotamia. During the Pliocene, marine ingressions covered much of the southern Mesopotamia and, 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). The *mesopotamico* morphotype probably originated around this area.

Consequently, historical processes are likely to be important in explaining the origin of the five morphotypes, while the current climatic and ecological factors may be contributing to the maintenance of the morphological differentiation and the pattern of distribution of the morphotypes of *Turnera sidoides* subsp. *pinnatifida*.

Conclusions and prospects

The multivariate analysis based on leaf traits and flower colour data performed in this study enabled differentiation of five morphotypes in *Turnera sidoides* subsp. *pinnatifida* Common-garden experiments also demonstrated that the morphological differences among morphotypes have a strong genetic basis. The morphological variants are geographically structured along the subspecies range, occurring in habitats with different climatic regimes, vegetation

cover, and soil types. Although morphological characters and habitat preference are suggestive of adaptive differentiation of *T. sidoides* subsp. *pinnatifida*, the intermediate degree of coincidence between morphological and bioclimatic ordinations suggests that the present-day biogeographical patterns of the morphotypes are only partially affected by the current climatic conditions. The Miocene–Pleistocene events which occurred in the subspecies area may explain the origin of the five morphotypes, and the current climatic and ecological factors may be contributing to maintenance of the morphological differentiation and the patterns of distribution of the morphotypes of *T. sidoides* subsp. *pinnatifida*. We are currently analysing the regional variation in the *T. sidoides* complex by using ploidy levels distribution and a phylogeographic approach in order to gain further insights into the evolution of *T. sidoides* complex.

Appendix 1

Detailed information about the material of *Turnera sidoides* subsp. *pinnatifida* examined in this study.

ARGENTINA. Buenos Aires: Las Palmas, −34.0833 −59.1667, 25 asl, *Boelke 4970* (SI). Ituzaingo, −34.6667 −58.6667, 28 asl, *Holmberg 116* (SI). Guaminí, −37.0333 −62.4167, 95 asl, Nicora 4191 (SI). Anchorena, −34.4833 −58.4833, 0 asl, *Refniez 182* (BAF). Azul, −36.7833 −59.8500, 136 asl, *Puyssegur s/n* (CORD). Bahía Blanca, −38.7167 −62.2833, 38 asl, S_{33}, *Solís Neffa 22* (CTES). Napostá, −38.4333 −62.2500, 181 asl, *Verettoni and Lamberto 3162* (BB). Campana, Campana, −34.1769 −58.9208, 2 asl, *Hunziker 1041* (CORD). Coronel Suárez, El Pantanoso, −37.4666 −61.9166, 234 asl, S_{238}, *Seijo 2915* (CTES). Villa Arcadia, −37.4666 61.9166, 234 asl, S_{249}, *Seijo s/n*. Escobar, Garín, −34.4233 −58.7619, 13 asl, *Lanfrandi 443* (SI). General Pueyrredón, Mar del Plata, −38.000, −57.5500, 38 asl, *Pelosi 24* (SI). Pilar, −34.4836 −58.9319, 18 asl, *Del Viso 667* (SI). Tornquist, Sierra de la Ventana, −38.1500 −61.800, 250 asl, *Hunziker 612* (CORD). **Catamarca**: Quebrada de Cébila, −32.7833 −67.0333, 112 asl, S_{240}, *Seijo and Solís Neffa 3017* (CTES). Ambato, −28.2167 −65.8667, 726 asl, S_{71}, *Solís Neffa et al. 344* (CTES). Sierra de Ambato, El Rodeo, −28.2167 −65.8667, 1,300 asl, *Hunziker 20784* (CORD).

La Rinconada, Sierra de Graciana, −27.9500 −65.8667, 1,144 asl, *Saravia Toledo et al. 12856* (CTES). 1 km W from Los Varela, −27.9500 −65.8667, 1,144 asl, *Saravia Toledo et al. 12942* (CTES). La Puerta, −28.1667 −65.8000, 873 asl, *Saravia Toledo et al. 13047* (CTES). 2 km S from El Bolson, −27.9167 −65.8833, 1,229 asl, *Saravia Toledo et al. 13090* (CTES). 0.5 km N from Los Varela, −27.9500 −65.8667, 1,144 asl, *Saravia Toledo et al. 13144* (CTES). Divisoria de Cuenca (Los Puestos y Singuil), N from El Bolson, −27.8167 −65.8667, 1,170 asl, *Saravia Toledo et al. 13258* (CTES). Sierra de Graciana, −27.8833 −65.7167, 1,281 asl, *Saravia Toledo et al. 13282* (CTES). La Paz, La Brea, −29.2000 −65.1667, 218 asl, *Brizuela 462* (CTES). Between San Antonio de la Paz and Icaño, −28.9333 −65.1000, 257 asl, *Hunziker 17162* (CORD). Ancasti, San Antonio, −27.8667, −67.2667, 2,035 asl, *Tatter 4306* (CORD). Between Maná and Las Caleras, towards San Francisco, −28.4500 −65.2500, 566 asl, *Hunziker 15748* (CORD). Andalgalá, El Suncho, −29.200 −65.3667, 305 asl, *Jörgensen 1615* (SI/BAF/BA). Ea. Las Rosas, −27.6000 −66.3167, 1,700 asl, *Filipovich 54* (CORD). El Alto, Sierra Ancasti, 9 km S from Dique de Coyogasta, −28.8167 −65.500, 870 asl, *Hunziker 17264* (CORD). Capital, Choya, −28.4333 −65.7833, 541 asl, *Guaglianone and Ulibarri 843* (CORD). **Córdoba**: Falda de San Esteban, Sierra Chica, −30.9167 −64.5500, 1,007 asl, *Kurtz 2817* (CORD). General Paz, −31.1333 −64.1500, 513 asl, *Stuckert 385* (CORD). General Roca, between Burmeister and Onagoity, −34.7500 −63.6, 128 asl, *Di Fulvio 21380* (CORD). Río Seco, −29.900 −63.7167, 315 asl, *Stuckert 2368* (CORD). Achala, Punta Sierra, −30.8000 −65.1667, 413 asl, *Stuckert 10716* (CORD). Río I, Ea. San Teodoro, −31.3333 −63.6167, 242 asl, *Stuckert 11892* (CORD). Pan de Azúcar, Sierra Chica, −31.2500 −64.4333, 1,760 asl, *Kurtz 4444* (CORD). Colón, Sierra Chica (E slope), −31.1667 −64.3333, 729 asl, *Subils 651* (CORD). Calamuchita, between Santa Rosa and Yacanto, −32.0500 −65.0500, 745 asl, *Gutiérrez 39* (CORD). Capital, Ciudad Universitaria, −31.1667 −64.1833, 383 asl, S_{87}, *Solís Neffa and Seijo 985* (CTES). Los Altos, −31.1667 −64.3000, 701 asl, *Kurtz 584* (CORD). Altos Sud, ca. Córdoba, −31.400 −64.1833, 383 asl, *Stuckert 5025* (CORD). San Vicente, −31.8500 −65.4333, 392 asl, *Kurtz 583* (CORD). Colón, Casa Bamba, −31.3500 −64.4000, 555 asl, *Stuckert 23894* (CORD). Ischilín, Ongamira, −30.7667 −64.4333, 1,334 asl, *Stuckert 16557* (CORD). Marcos Juárez, Marcos Juárez, −32.700 −62.1000, 108 asl, *Stuckert 17856* (CORD). Punilla, Capilla del Monte, −30.8500 −64.5166, 979 asl, S_{234}, *Solís Neffa and Seijo 966* (CTES). −30.8500 −64.5166, 979 asl, S_{235}, *Solís Neffa and Seijo 967* (CTES). −30.8500 −64.5166, 979 asl, S_{236}, *Solís Neffa and Seijo*

968 (CTES). −30.8500 −64.5166, 79 asl, S$_{237}$, *Solís Neffa and Seijo 969* (CTES). Carlos Paz, −31.3833 −64.5167, 661 asl, S$_{112}$, *Solís Neffa 486* (CTES). Los Cocos, −30.9167 −64.5000, 1,221 asl. *Pedersen 9935* (CTES). Copina, −31.5667 −64.7000, 1,494 asl, *Hosseus 522* (CORD). Cuchi Corral, −30.9833 −64.6000, 934 asl, *von Rentzell 18832* (SI). Tanti Viejo, −31.3333 −64.6000, 996 asl, *Novara 128* (CORD). La Falda, −31.0833 −64.5000, 952 asl, *Hunziker 8449* (CORD). Santa María, Dique Los Molinos, −31.8500 −64.3833, 460 asl, S$_{115}$, *Solís Neffa s/n.* Near Toledo, −31.5667 −64.0167, 367 asl, *Hunziker 16571* (CORD). Alta Gracia, −31.6667 −64.4333, 515 asl, *Hunziker 1271* (CORD). Despeñaderos, −31.8167 −64.3000, 445 asl, *Krapovickas 6521* (SI/CORD). Sierras Grandes, between Salsacate and Los Gigantes, −31.3167 −65.0833, 910 asl, *Luti 1255* (CORD). Río Cuarto, Moldes, −33.6333 −64.6000, 341 asl, *Stuckert 21402* (CORD). Río Cuarto, −33.1333 −64.3500, 425 asl, *de Loos 3749* (CORD). San Alberto, Despeñaderos, Olmedo, Mina Clavero, −31.7167 −65.000, 915 asl, *Stuckert 10574* (CORD). Totoral, Totoral, −30.8167 −63.7167, 233 asl, *Stuckert 8541* (CORD). Unión, Idiazábal, −32.8000 −63.0333, 143 asl, *Subils 2489* (CORD). Canales, −33.5500 −62.8833, 109 asl, *Roth 21885* (CORD). Tulumba, Cerro Colorado, −30.4000 −64.1167, 668 asl, *Hunziker 14496* (CORD). Río Seco, Tulumba, −29.9000 −63.7167, 315 asl, *Hunziker 7789* (CORD). **Corrientes:** Capital, Molina Punta, −27.4667 −58.9000, 58 asl, S$_9$, *Schinini 29142* (CTES). Curuzú Cuatiá, route to Parada Acuña, −29. 9167 −57.9667, 57 asl, S$_{84}$, *Solís Neffa 387* (CTES). Avalos stream, 31 km S from Perugorria, −29.3333 −58.6167, 49 asl, *Schinini 21648* (CTES). Empedrado, Estancia La Yela, −27.9500 −58.8000, 56 asl, *Pedersen 6409* (CTES). Sombrerito stream and route 12, −27.7000 −58.7667, 61 asl, *Schinini 13857* (CTES). Esquina, route 126, towards Sauce, −30.2294 −58.8333, 51 asl, S$_{418}$, *Solís Neffa et al. 2201* (CTES). 26 Km SE from Libertador, −30.3333 −59.3333, 32 asl, *Krapovickas et al. 27425* (CTES). Lavalle, Yataytí, −29.0166 −58.8833, 58 asl, S$_{36}$, *Schinini et al. 29142* (CTES). Mercedes, route 123 km 89, −29.0100 −58.3333, 75 asl, S$_{31}$, *Solís Neffa et al. 307* (CTES). Route 123 km 65, −29.1000 −58.3300, 98 asl, S$_{30}$, *Solís Neffa et al. 44* (CTES). Route 119 km 89, −31.8333 −62.0500, 98 asl, S$_{85}$, *Solís Neffa 388* (CTES). Route 119 km 65, −29.1000 −58.3300, 98 asl, S$_{213}$, *Solís Neffa and Seijo 977* (CTES). Route 119 km 97, −31.8333 −62. 5000, 98 asl, S$_{223}$, *Solís Neffa and Seijo 978* (CTES). Route 123, −29.1000 −58.3300, 98 asl, S$_{314}$, *Solís Neffa et al. 1990* (CTES). Route 119, 7 km S from Mercedes, −29.1000 −58.3333, 75 asl, *Tressens et al. 2252* (CTES). Pay-Ubre Grande stream and road to Ita Cora, −28.9333 −58.1500, 66 asl, *Tressens et al. 2274* (CTES). 6 km S from F. Yofre, −29.1000, −58.3333, 74 asl, *Tressens et al. 2531* (CTES).

Monte Caseros, Mocoretá, Curuzú stream, −30.0500 −57.8167, 53 asl, S$_{219}$. Mocoretá, towards Juan Pujol, −30.4024 −57.0161, 74 asl, S$_{424}$, *Solís Neffa et al. s/n.* Mocoreta river, −30.6333 −57.9667, 25 asl, *Ahumada et al. 3911* (CTES). Dpt. San Cosme, −27.3167 −58.5833, 50 asl, S$_{86}$, *Solís Neffa 496* (CTES). Sauce, Chacra El Timbó, 3 Km N from Sauce, −30.0833 −58.7667, 36 asl, *Ahumada* et al. *1340* (CTES). **Chaco**: Alte. Brown, Pampa del Infierno, −26.5358 −61.1405, 126 asl, S$_{201}$, *Solís Neffa et al. 935* (CTES). Bermejo, Las Palmas, −27.0667 −58.7000, 64 asl, *Jörgensen 2551* (SI). Chacabuco, Charata, −27.2167 −61.2000, 85 asl, *Martinez Crovetto 10432* (CTES). General Güemes, Castelli, −26.9499 −60.6166, 103 asl, S$_{239}$, *Schinini and Urbani 35770* (CTES). Independencia, 4 km SE from Avia Terai, −26.7000 −60.7333, 98 asl, *Schinini 19475* (CTES). 1° de Mayo, Colonia Benítez, −27.3333 −58.9333, 52 asl, S$_{32}$, *Solís Neffa 306* (CTES). 9 de Julio, Las Breñas, −27.0833 −61.0833, 103 asl, S$_{315}$, *Solís Neffa 1994* (CTES). 12 de Octubre, General Pinedo, −27.3167 −61.2833, 93 asl, *Schulz 8910* (CTES). 25 de Mayo, Tres Palmas. *Caplan 27* (CTES). General Pinedo, −27.3167 −61.2833, 78 asl, Schultz 8910 (CTES). **Entre Ríos**: Paraná, −31.7333 −60.5333, 76 asl, *Jozani 148* (SI). Route 1, between Los Conquistadores and Chajarí, −30.7008 −58.1906, 76 asl, S$_{423}$, *Solís Neffa et al. 2217* (CTES). Colón, port, −32.2167 −58.1333, 2 asl, S$_{34}$, *Solís Neffa et al. 309.* Federacion, Chajari, −30.7667 −57.9833, 34 asl, *Cabrera 12366* (LP). **Formosa**: Laishi, route 11, 18 km NE from Bermejo river, −26.0000 −58.0000, 66 asl, S$_{24}$, *Arbo 2697* (CTES). Bermejo, Laguna Yema, −24.2500 −61.2500, 153 asl, *Schinini and Pire 24251* (CTES). Matacos, route 81 km 1591, −24.3972 −61.0500, 171 asl, S$_{168}$, *Solís Neffa et al. 583* (CTES). **Jujuy**: Capital, Yala, graveyard, −24.1252 −65.3988, 1,467 asl, S$_{182}$, *Solís Neffa et al. 827* (CTES). Lozano, −24.1333 −65.3833, 1,414 asl, S$_{184}$, *Solís Neffa et al. 829* (CTES). Palpalá, −24.2783 −65.1983, 1,113 asl, S$_{181}$, *Solís Neffa and Seijo 959* (CTES). −24.2500 −65.2000, 1,104 asl, S$_{212}$, *Solís Neffa and Seijo 959* (CTES). Termas de Reyes, −24.1667 −65.4833, 1,754 asl, Arbo 695 (CTES). Reyes −24.1667 −65.3667, 1,364 asl, *Schreiter 266* (BA). El Carmen, "El Cadillal" airport, −24.3730 −65.0930, 918 asl, S$_{180}$, *Solís Neffa and Seijo s/n.* Pampa Blanca, −24.55 −65.0833, 698 asl, *Schinini et al. 19532* (CTES). Tumbaya, Volcán, −23.9269 −65.4633, 2,095 asl, S$_{211}$, *Solís Neffa et al. 1469* (CTES). 2 km N from Volcán, −23.8988 −65.4622, 2,098 asl, S$_{290}$, *Solís Neffa et al. 1467* (CTES). 13 km S from Volcán, −23.9863 −65.4536, 1,615 asl, S$_{291}$, *Solís Neffa et al. 1472* (CTES). Chilcayre, −23.85 −65.4667, 2,250 asl, *Kiesling 871* (LP) Dr. M. Belgrano, towards Tiraxi, −24.0491 −65.4222, 2,094 asl, S$_{292}$, *Solís Neffa et al. 1472* (CTES). Ledesma, Calilegua, −23.7727 −64.7758, 491 asl, S$_{171}$, *Solís Neffa et al. 717* (CTES). Route 34, 4 km S

from Fraile Pintado, −23.9333 −64.7833, 414 asl, Schinini 19536 (CTES). Valle Grande, Huacanqui, −23.5500 −64.9666, 1,379 asl, S$_{172}$, *Solís Neffa* et al. *722* (CTES). 17 km from Valle Grande, −23.5605 −64.9666, 1,379 asl, S$_{173}$, *Solís Neffa* et al. Pueblo, −23.4667 −64.9833, 2,313 asl, *Villa* et al. *848* (LIL). Santa Bárbara, 6 km NW from El Fuerte, −24.2269 −64.3944, 1,140 asl, S$_{174}$, *Solís Neffa* et al. *767* (CTES). 7 km NW from El Fuerte, −24.2889 −64.4850, 1,313 asl, S$_{177}$, *Solís Neffa* et al. *790* (CTES). Route 23 towards Santa Clara, −24.2672 −64.4333, 1,391 asl, S$_{175}$, *Solís Neffa* et al. *779* (CTES). −24.2888 −64.4580, 1,313 asl, S$_{176}$. *Solís Neffa* et al. *779* (CTES). −24.2855 −64.4580, 1,313 asl, S$_{178}$, *Solís Neffa* et al. *788* (CTES). 12 km NW from El Fuerte, 24.2980 −64.4847, 1,338 asl, S$_{179}$, *Solís Neffa* et al. *820* (CTES). **La Pampa:** Parque Luro, between Santa Rosa and General Acha, −36.6167 −64.283, 175 asl, *Burkart 20474* (SI). General Acha, −37.3833 −64.6000, 252 asl, *Monticelli 46* (SI). Cuchillo-Co, −38.3333 −64.6167, 241 asl, *Solbrig 171* (SI). **La Rioja**: San Martín, −31.5833 −66.2500, 450 asl, *Stuckert 17038* (CORD). −29.4333 −66.85, 497 asl, S$_{25}$, *Aguirre 2697* (CTES). General Belgrano, between Chamical and Chañarazo, −30.3035 −66.3167, 452 asl, *Biurrum 2926* (CTES). Independencia, road 79 km 219, −30.5354 −66.2802, 512 asl, *Pensiero* et al. *7423* (CTES). **Rio Negro**: Pichi Mahuida, Juan de Garay, −38.8667 −64.5667, 103 asl, M. *Crovetto and Piccini 7033* (CTES). Route 22 km 890, −39.0167 −64.0833, 59 asl, *Burkart 15852* (SI). Juan de Garay, −38.8667 −64.5667, 103 asl, *Martinez Crovetto* et al. *7033* (CTES). **Salta:** Anta, 20 km W from Joaquin V.Gonzalez, −25.0833 −64.1833, 378 asl, *Schinini 19519* (CTES). Candelaria, Candelaria river, −26.1000 −65.1000, 979 asl, *Cuezzo* et al. *3462* (LIL). Capital, airport, −24.8388 −65.4777, 1,247 asl, S$_{185}$, *Solís Neffa* et al. *837* (CTES). Arenales river, −24.8105 −65.4222, 1,195, asl S$_{186}$, *Solís Neffa* et al. *836* (CTES). B° El Milagro, −24.7200 −65.4091, 1,246 asl, S$_{187}$, *Solís Neffa* et al. *839* (CTES). Towards San Lorenzo, El Tipal, −24.7525 −65.4705, 1,353 asl, S$_{188}$, *Solís Neffa* et al. *845* (CTES). Road to Salta km 1578, −24.0800 −65.2166, 1140, S$_{193}$, *Solís Neffa* et al. *903* (CTES). Road to Salta km 1554, −24.7450 −65.0361, 880 asl, S$_{194}$, *Solís Neffa* et al. *904* (CTES). Coronel Moldes, La Hoyada, −25.8500 −65.1833, 1,115 asl, *Burkart 13155* (SI). Chicoana, La Zanja, −25.1500 −65.7500, 2,332 asl, S$_{189}$, *Solís Neffa* et al. *852* (CTES). 10 km N from Quebrada de Escoipe, −25.1000 −65.7667, 2,700 asl, *Krapovickas* et al. *36081* (CTES). Cachi, Cuesta del Obispo, route 33 km 46, −25.1833 −65.0800, 2,566 asl, S$_{190}$, *Solís Neffa* et al. *892* (CTES). Route 33 km 42, −25.1833 −65.7833, 2,256 asl, S$_{191}$, *Solís Neffa* et al. *895* (CTES). M.M. de Güemes, Cabeza de Buey, −24.7736 −65.0347, 757 asl, S$_{195}$, *Solís Neffa* et al. *907* (CTES). Route 9 km 1551, −24.9091

−65.9841, 757 asl, S$_{196}$, *Solís Neffa* et al. *907* (CTES). Metán, route 9 km 1490, −25.2463 −64.9196, 737 asl, S$_{198}$, *Solís Neffa* et al. *911* (CTES). Route 5, 6 km W from Castellanos river, −25.0008 −64.5218, 644 asl, S$_{199}$, *Solís Neffa* et al. *918* (CTES). Route 16 km 467, −25.3413 −64.4743, 523 asl, S$_{200}$, *Solís Neffa* et al. *924* (CTES). 1 km E from de las Piedras river, −25.3000 −64.9000, 685 asl, *Krapovickas and Cristobal 46*613 (CTES). 20 km S from Metán, −25.4833 −64.9500, 802 asl, *Krapovickas* et al. *17407* (CTES). Rosario de la Frontera, −25.0000 −64.0000, 791 asl, S$_{74}$, *Solís Neffa* et al. *356* (CTES). Horcones, −25.7167 −64.9000, 702 asl, *Cabrera* et al. *15943* (LP). Oran, Orán, −23.1333 −64.3333, 336 asl, *Spegazzini s.n.* (BAB, CTES). Rivadavia, route 81, 19 km E from Sola, −23.4786 −62.8794, 194 asl, S$_{118}$, *Solís Neffa 505* (CTES). 1 km SE from Sola, −23.4786 −62.8794, 242 asl, S$_{169}$, *Solís Neffa* et al. *983* (CTES). Pluma de Pato, −23.3725 −63.0980, 237 asl, S$_{311}$, *Solís Neffa* et al. *1986* (CTES). Estación Murillo, −23.4730 −63.8913, 222 asl, S$_{312}$, *Solís Neffa* et al. *1988* (CTES). Los Blancos, −23.0833 −63.5980, 216 asl, S$_{313}$, *Solís Neffa* et al. *1989* (CTES). Route 81, 21 km SE from Coronel Juan Sola (Murillo), −23.4667 −62.8833, 220 asl, *Schinini 19573* (CTES). Gral. J. de San Martín, Dragones, −23.2494 −63.3463, 259 asl, S$_{310}$, *Solís Neffa* et al. *1979* (CTES). Juramento, −25.1500 −64.9500, 811 asl, *Krapovickas* et al. *30444* (CTES). 4 km W from Antill, −26.1167 −64.600, 498, *Krapovickas* et al. *30479* (CTES). **San Luis**: Pedernera, 6 km S from Las Isletas, −33.7833 −65.3000, 444 asl, *Anderson 1369* (CORD). Divisadero, −33.1667 −66.1500, 1,157 asl, *Galander 3686* (CORD). Coronel Pringles, ca. Fraga, −33.5000 −65.8000, 665 asl, *Hunziker 13137* (CORD). Villa Mercedes, −33.8500 −65.2667, 424 asl, *Burkart 10917* (SI). **Santa Fe**: Between Hersilia and E. Rams, −30.0000 −61.8500, 89 asl, *Krapovickas 759* (CTES). Logroño, −29.5000 −61.7000, 62 asl, *Krapovickas 801* (CTES). Rafaela, −31.2667 −61.4833, 90 asl, *Leon 973* (SI). Castellanos, Sunchales, −30.9333 −61.5667, 86 asl, *Cristobal and Krapovickas 2491* (CTES). 9 de Julio, Tostado, Rio Salado, −29.2333 −61.7667, 64 asl, *Job 1075* (LP). San Cristobal, Ceres, −29.8833 −61.9500, 84 asl, *Kuntze s.n.* (CORD). Dpt. Vera, Margarita, −29.6666 −60.2500, 73 asl, *Krapovickas 38*176 (CTES). **Santiago del Estero:** Capital, near Santiago del Estero, −27.7833 −64.2667, 181 asl, *Argañaraz 3* (LP). Moreno, −28.0250 −62.0750, 149 asl, S$_{69}$, *Solís Neffa* et al. *316* (CTES). Between Yuchan and Quimili, −27.8000 −62.9667, 158 asl, *Pedersen 11858* (CTES). Guasayán, road 64, 12 km from Santa Catalina, −28.1333 −64.7863, 424 asl, S$_{70}$, *Solís Neffa* et al. *332* (CTES). Jiménez, road 34 4 km N from Las Cejas. *Krapovickas and Cristobal 46606* (CTES). Between Fortín Inca and Bandera, −29.1167 −61.9500, 67 asl, *Alonso* et al. *576* (LP/SI). Rivadavia, 10 km W from La

Isleta, −29.9667 −62.1333, 79 asl, *Hunziker 10453* (CORD). Choya, route 157 between Frías and Tapso, −28.6500 −65.1500, 315 asl, *Hunziker 25638* (CORD). Between Forres and Robles, −27.8833 −63.9667, 153, *Bartlett 19730* (SI). Brig. Juan Felipe Ibarra, 51 km NE from Añatuya, towards Quimili, −28.4667 −62.8333, 107 asl, *Krapovickas and Cristóbal 46183* (CTES). Rio Hondo, Termas de Rio Hondo, airport, −27.4833 −64.8667, 248 asl, *Krapovickas et al. 17378* (CTES). Robles, Beltran, −27.8333 −64.0667, 157 asl, Maldonado 515 (LP). Silipica, Arraga, −28.0667 −64.2333, 160 asl, *Renolfi 182* (CTES). Copo, Pampa de los Guanacos, −26.2333 −61.8500, 149 asl, *Spegazzini 10565* (BAF). **Tucumán**: Burruyacú, −26.500, −64.7500, 497 asl, *Stuckert 12581* (CORD). El Puestito, −26.400 −64.7833, 743 asl, *Venturi 7501* (SI). Alto de Medina, −26.3667 −65.0500, 1,657 asl, *Venturi 2688* (SI). Capital, El Cadillal, Parque de las Americas, −26.6833 −65.2667, 685 asl, *Krapovickas et al. 27136* (CTES). Los Puestos, −27.2833 −65.000, 300 asl, *Krapovickas et al. 27146* (CTES). Leales, La Florida, route 9, −27.2333 −65.5667, 359 asl, *Krapovickas et al. 17361* (CTES). Trancas, route 9, 7 km S from Trancas, −26.2167 −65.2833, 782 asl, S_{28}, *Krapovickas and Cristóbal 46241* (CTES). Tapia, −26.6000 −65.3000, 711 asl, S_{72}, *Solís Neffa et al. 350* (CTES).Towards San Pedro de Colalao, −26.2333 −65.4833, 1,087 asl, S_{73}, *Solís Neffa et al. 352* (CTES).

BOLIVIA. Cochabamba: Carrasco, towards Cochabamba, −17.7422 −64.8614, 2,295 asl, S_{258}, *Solís Neffa et al. 1363* (CTES, LPB). Copachuncho, −17.7255 −65.0847, 2,580 asl, S_{259}, *Solís Neffa et al. 1366* (CTES, LPB). 4 km NW from Mizque, −17.7310 −65.5224, 2,153 asl. S_{260}, *Solís Neffa et al. 1377* (CTES, LPB). 5 km NW from Mizque, −17.1003 −65.3494, 2,124 asl, S_{261}, *Solís Neffa et al. 1378* (CTES, LPB). 4 km SW from Mizque, −17.9168 −65. 3386, 2,128 asl, S_{262}, *Solís Neffa et al. 1379* (CTES, LPB). 3 km SW from Mizque, −17.9255 −65.3427, 2,045 asl, S_{263}, *Solís Neffa et al. 1380* (CTES, LPB). 10 km SE from Mizque. −17.9986 −65.2908, 2,199 asl, S_{265}, *Solís Neffa et al. 1385* (CTES, LPB). 24 km SE from Mizque, −18.0741 −65.2688, 2232, S_{266}, *Solís Neffa et al. 1390* (CTES, LPB). 38 km S from Mizque, −18.1703 −65.2111, 2,364 asl, S_{267}, *Solís Neffa et al. 1396* (CTES, LPB). 48 km S from Mizque, −18.2236 −65.2019, 2,331 asl, S_{268}, *Solís Neffa et al. 1398* (CTES, LPB). 131 km S from Mizque, −18.3025 −65.2219, 2,147 asl, S_{269}, *Solís Neffa et al. 1403* (CTES, LPB). **Chuquisaca**: 85 km SE from Sucre, −19.0664 −64.8025, 2,540 asl, S_{270}, *Solís Neffa et al. 1405* (CTES, LPB). 87 km SE from Sucre. −19.0766 −64.8063, 2,638 asl, S_{271}, *Solís Neffa et al. 1406* (CTES, LPB). 95 km SE from Sucre. −19.0600 −64.7717, 2,391 asl, S_{272}, *Solís Neffa et al. 1407* (CTES, LPB). 101 km SE from Sucre. −19.0822 −64.7316, 2,399, S_{273}, *Solís Neffa et al. 1409* (CTES, LPB). 109 km SE from Sucre. −19.1191 −64.6866, 2,504 asl, S_{274}, *Solís Neffa et al. 1412* (CTES, LPB). 128 km SE from Sucre. −19.1644 −64.5650, 2,277 asl, S_{275}, *Solís Neffa et al. 1413* (CTES, LPB). 137 km SE from Sucre. −19.1486 −64.5158, 2,046 asl, S_{276}, *Solís Neffa et al. 1415* (CTES, LPB). 148 km SE from Sucre. −19.1958 −64.4605, 2,063 asl, S_{277}, *Solís Neffa et al. 1416* (CTES, LPB). 1 km NW from Alcala. −19.2958 −64.4058, 2,298 asl, S_{278}, *Solís Neffa et al. 1417* (CTES, LPB). 11 km SE from Padilla. −19.3630 −64.2508, 2,059 asl, S_{279}, *Solís Neffa et al. 1419* (CTES, LPB). 20 km SE from Padilla. −19.3916 −64.2350, 2,258 asl, S_{280}, *Solís Neffa et al. 1423* (CTES, LPB). 24 km SE from Padilla. −19.3797 −64.2150, 2,375 asl, S_{281}, *Solís Neffa et al. 1427* (CTES, LPB). Azero, 10 km SE from EE "El Salvador", −20.6514 −63.1319, 704 asl, S_{294}, *Solís Neffa and Seijo 1484* (CTES, LPB). 11.5 km SE from EE "El Salvador", −20.6819 −63.1105, 704 asl, S_{295}, *Solís Neffa and Seijo 1485* (CTES, LPB). 5.3 km SE from EE "El Salvador". −20.6819 −63.1105, 704 asl, S_{296}, *Solís Neffa and Seijo 1486* (CTES, LPB). 64.6 km E from Boyuibe, −20.4447 −62.7433, 520 asl, S_{306}, *Solís Neffa et al. 1962.* towards Carandaity, −21.5758 −63.2763, 850 asl, S_{251}, *Solís Neffa et al. 1033* (CTES, LPB). −20.6588 −63.1261, 705 asl, S_{253}, *Solís Neffa et al. 1042.* −21.3958 −63.2136, 797 asl, S_{254}, *Solís Neffa et al. 1048* (CTES, LPB). 800 m from EE El Salvador, −21.7500 −63.216, 500 asl, S_{252}, *Solís Neffa et al. 1035* (CTES, LPB). **Santa Cruz:** Florida, 5 km W from Samaipata. −18.1619 −63.9144, 1,359 asl, S_{255}, *Solís Neffa et al. 1327* (CTES, LPB). Caballero, 2 km W from Comarapa, −18.0175 −64.338, 1,884 asl, S_{256}, *Solís Neffa et al. 1346* (CTES, LPB). 2 km W from Comarapa. **Tarija**: 50 km E from Tarija, −21.4381 −64.4719, 2,400 asl, S_{282}, *Solís Neffa et al. 1436* (CTES, LPB). O′Connor, between Tarija and Entre Ríos, −21.4633 −64.5013, 2,251 asl, S_{283}, *Solís Neffa et al. 1438* (CTES, LPB). −21.4472 −64.4838, 2,403 asl, S_{284}, *Solís Neffa et al. 1441* (CTES, LPB). −21.4497 −64.7544, 1,992 asl, S_{285}, *Solís Neffa et al. 1449* (CTES, LPB). 22 km SW from Tarija, −21.4641 −64.8244, 1,992 asl, S_{286}, *Solís Neffa et al. 1450* (CTES, LPB). 27 km SW from Tarija, −21.4497 −64.7544, 2,522 asl, S_{287}, *Solís Neffa et al. 1463* (CTES, LPB). Cercado, 7 km from San Jacinto lake, −21.6433 −64.7669, 1,920 asl, S_{289}, *Solís Neffa et al. 1466* (CTES, LPB). Gran Chaco, 9 km N from Caraparí, −21.7519 −63.7286, 591 asl, S_{293}, *Solís Neffa and Seijo 1481* (CTES, LPB). 6.3 km E from Caiza, −21.8188 −63.5330, 559 asl, S_{297}, *Solís Neffa et al. 1498* (CTES, LPB). 45.8 km E from Caiza, −21.8608 −63.1872, 561 asl, S_{298}, *Solís Neffa et al. 501* (CTES, LPB). 14.5 km E from Villa Montes, −21.2930 −63.3475, 364 asl, S_{300}, *Solís Neffa et al. 1504* (CTES, LPB). 18.4 km E from Villa Montes, −21.2930 −63.3102, 385 asl, S_{301},

Solís Neffa et al. *1505* (CTES, LPB). 27 km E from Villa Montes, −21.2922 −63.3486, 374 asl, S_{250}, *Solís Neffa* et al. *1025*. 52.2 km E from Villa Montes, −21.4591 −63.1122, 337 asl, S_{302}, *Solís Neffa* et al. *1508* (CTES, LPB). 95.9 km E from Villa Montes, −21.6036 −62.7508, 317 asl, S_{303}. *Solís Neffa* et al. *1510* (CTES, LPB). 38 km E from Ibibobo, −21.6308 −62.6361, 303 asl, S_{304}, *Solís Neffa* et al. *1511*. Cordillera, 6,8 km W from Carandaity, −20.7628 −63.1319, 675 asl, S_{307}, *Solís Neffa* et al. *1968*. −20.7500 −63.0667, 616 asl, S_{309}, *Solís Neffa* et al. *1968*.

PARAGUAY. Boquerón: Filadelfia, −23.9667 −57.2833, 63 asl, *Krapovickas and Cristóbal 44236* (CTES). *Schinini* et al. *21232* (CTES). Colonia Fernheim, −22.2500 −59.8333, 111 asl, *August 117* (CTES). Campo Aroma. −22.1800 −60.0600, 100 asl, *Mereles and Degen 4833* (CTES).

URUGUAY. **Artigas**: route 30 and Cuaró stream, −30.4369 −57.2947, 61 asl, S_{125}, *Solís Neffa* et al. *980* (CTES). **Canelones:** route 8 km 56, −34.6703 −55.7169, 40 asl, S_{404}, *Solís Neffa* and Seijo *2142* (CTES). Cerro de los Mosquitos, −34.6833 −55.6833, 21 asl, *Montoro 1003* (MVFA). Santa Lucía, −34.4533 −56.3906, 34 asl, *Marchesi 1290* (MVFA). **Colonia**: Balneario Fomento, −34.4355 −57.2533, 12 asl, S_{382}, *Solís Neffa and Seijo 2077* (CTES). Riachuelo, −34.4466 −57.7155, 4 asl, S_{384}, *Solís Neffa and Seijo 2085* (CTES). −34.1880 −57.8789, 30 asl, S_{414}, *Solís Neffa and Seijo 2113* (CTES). Road 21 and Miguelete stream, −34.1835 −57.9105, 50 asl, S_{391}, *Solís Neffa and Seijo 2115* (CTES). Road to Conchillas, −34.1625 −58.0213, 51 asl, S_{392}, *Solís Neffa and Seijo 2116* (CTES). Conchillas, −34.1677 −58.0425, 27 asl, S_{393}, *Solís Neffa and Seijo 2117* (CTES).

Nueva Palmira, −33.9380 −58.2205, 13 asl, S_{394}, *Solís Neffa and Seijo 2118* (CTES). Punta Gorda, E from Rincón de Darwin, −33.9159 −58.4136, 35 asl, S_{395}, *Solís Neffa and Seijo 2122* (CTES). Colonia Suiza, −34.3167 −57.2167, 25 asl, *Berro 8425* (MVFA). **Florida**: route 7 km 114, Mansavillagra stream, −33.7308 −55.4533, 173, S_{148}, *Solís Neffa* et al. *542* (CTES). **Paysandú**: route 3 and Quebrada Grande stream, −31.9710 −57.8509, 48 asl, S_{402}, *Solís Neffa and Seijo 2140* (CTES). Est. Experimental Fac. Agronomía, −32.3333 −58.0333, 61 asl, *Marchesi* 14208 (MVFA). **Río Negro**: route 24 km 44.5, −32.3132 −57.9553, 43 asl, S_{400}, *Solís Neffa and Seijo 2138* (CTES). Route 25 towards Young, −32.7608 −57.9553, 43 asl, S_{401}, *Solís Neffa and Seijo 2425*, −33.7333 −57.8333, 76 asl, *Marchesi 20654* (MVFA). Playa Ubisi, N from Fray Bentos, −33.1325 −58.2956, 15 asl, *Del Puerto* et al. *5678* (MVFA). **Salto**: Road to Constitución, −31.0686 −57.8386, 34 asl, S_{355}, *Solís Neffa and Seijo 2009* (CTES). Espinillar, −31.0741 −57.7577, 61 asl, *del Puerto* et al. *2155* (MVFA). **San José:** Rincón del Pino, −34.4833 −56.85, 31 asl, *Izaguirre* et al. *9572* (MVFA). San José, −34.3375 −56.7136, 45 asl, *Lombardo 9430* (MVJB). **Soriano**: towards Villa Soriano, −33.5132 −58.1942, 14 asl, S_{397}, *Solís Neffa and Seijo 2134* (CTES). Vera, −33.1833 −57.4667, 69 asl, *Berro 1937* (MVFA). Dacá, −33.2558 −58.0192, 62 asl, *Berro 7767* (MVFA). Playa Agraciada, −33.8000 −58.2500, 51 asl, *del Puerto* et al. *8955* (MVFA).

Appendix 2

See Table 5.

Table 5 Bioclimatic variables analysed in the geographical area of each morphotype of *Turnera sidoides* subsp. *pinnatifida*

Climatic variable	Morphotype					*F* (ANOVA) *P*
	Andino	*Chaqueño*	*Mesopotamico*	*Pampeano*	*Serrano*	
BIO 1	17[a,b] ± 2	20[d] ± 2	19[c,d] ± 1	16[b,c] ± 1	15[a] ± 2	38.52**
	9–22	11–25	18–20	13–18	2–19	<0.0001
BIO 2	14[b] ± 2	14[a,b] ± 1	12[a] ± 0.5	11[a] ± 1	14[b] ± 1	5.34**
	11–17	11–16	11–12	8–14	12–15	0.0007
BIO 3	57[b] ± 6	50[a] ± 3	48[a] ± 0.81	46[a] ± 3	50[a] ± 3	20.31**
	48–72	45–60	46–49	40–58	45–55	<0.0001
BIO 4	364[a] ± 91	480[b] ± 63	468[b] ± 14	480[b] ± 43	496[b] ± 58	14.05**
	172–499	353–627	450–504	293–595	400–602	<0.0001
BIO 5	28[a] ± 3	34[b] ± 2	33[b] ± 0.5	30[b] ± 2	30[a] ± 2	45.48**
	19–34	23–37	31–33	25–32	26–34	<0.0001
BIO 6	4[a,b] ± 3	6[c] ± 3	8[c] ± 1	5[b,c] ± 2	2[a,b] ± 1	19.82**
	−4 to 9	−3 to 13	6–9	−2 to 8	−1 to 5	<0.0001
BIO 7	24 ± 3	27 ± 3	24 ± 1	24 ± 2	28 ± 2	2.37
	17–28	22–32	24–26	20–30	25–32	0.06

Table 5 continued

Climatic variable	Morphotype					F (ANOVA) P
	Andino	Chaqueño	Mesopotamico	Pampeano	Serrano	
BIO 8	$22^a \pm 3$	$25^d \pm 2$	$23^{b,c} \pm 1$	$21^c \pm 3$	$21^a \pm 2$	38.77**
	10–27	15–30	22–25	10–24	18–26	<0.0001
BIO 9	$13^b \pm 2$	$15^c \pm 3$	$14^{b,c} \pm 1$	$11^{a,b} \pm 3$	$9^a \pm 2$	23.98**
	5–18	6–22	12–15	8–21	6–13	<0.0001
BIO 10	$21^a \pm 3$	$26^c \pm 2$	$25^{b,c} \pm 0.5$	$22^b \pm 1$	$22^a \pm 2$	48.08**
	11–27	15–29	24–26	17–24	18–26	<0.0001
BIO 11	$13^b \pm 2$	$14^c \pm 3$	$14^{b,c} \pm 1$	$11^b \pm 1$	$9^a \pm 2$	23.59**
	5–17	6–20	12–15	8–12	6–13	<0.0001
BIO 12	$633^a \pm 173$	$701^a \pm 257$	$1175^b \pm 98$	$1008^b \pm 149$	$659^a \pm 90$	18.91**
	168–1044	154–1419	985–1367	777–1573	387–829	<0.0001
BIO 13	$140^b \pm 32$	$121^b \pm 27$	$145^b \pm 8$	$118^{a,b} \pm 29$	$100^a \pm 14$	2.84**
	48–205	40–175	132–167	79–273	54–133	0.03
BIO 14	$4^a \pm 5$	$11^a \pm 12$	$51^b \pm 10$	$55^c \pm 16$	$14^a \pm 8$	50.59**
	0–31	0–54	31–71	25–83	6–48	<0.0001
BIO 15	$99^c \pm 13$	$73^b \pm 18$	$31^a \pm 5$	$25^a \pm 13$	$60^b \pm 15$	68.50**
	48–127	34–125	23–46	7–68	19–77	<0.0001
BIO 16	373 ± 83	328 ± 76	384 ± 25	316 ± 82	275 ± 47	2.36
	125–537	105–497	316–437	221–779	129–381	0.06
BIO 17	$17^a \pm 16$	$40^a \pm 39$	$171^b \pm 35$	$178^c \pm 51$	$47^a \pm 29$	53.63**
	1–105	1–174	102–236	81–285	22–156	<0.0001
BIO 18 (mm)	339 ± 96	318 ± 72	332 ± 26	271 ± 34	269 ± 52	1.81
	125–523	103–475	268–401	132–328	116–381	0.13
BIO 19 (mm)	$18^a \pm 17$	$45^b \pm 39$	$171^c \pm 35$	$197^d \pm 103$	$47^{a,b} \pm 29$	61.01**
	3–106	3–174	102–239	81–732	22–156	<0.0001

Results of ANOVA for each variable are shown in the last column. Within a column, different letters indicate significant differences (Tukey post-hoc test for multiple comparisons of means, $P < 0.05$)

BIO 1, annual mean temperature (°C); BIO 2, mean monthly temperature range (°C); BIO 3, isothermality; BIO 4, temperature seasonality (°C); BIO 5, maximum temperature of warmest month (°C); BIO 6, minimum temperature of coldest month (°C); BIO 7, temperature annual range (°C); BIO 8, mean temperature of wettest quarter (°C); BIO 9, mean temperature of driest quarter (°C); BIO 10, mean temperature of warmest quarter (°C); BIO 11, mean temperature of coldest quarter (°C); BIO 12, annual precipitation (mm^3); BIO 13, precipitation of wettest month (mm^3); BIO 14, precipitation of driest month (mm^3); BIO 15, precipitation seasonality; BIO 16, precipitation of wettest quarter (mm^3); BIO 17, precipitation of driest quarter (mm^3); BIO 18, precipitation of warmest quarter (mm^3) and; BIO 19, precipitation of coldest quarter (mm^3)

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