Botanical Journal of the Linnean Society, 2017, **183**, 454–473. With 5 figures.

Phenotypic variation in arid and semi-arid zones of southern South America: the case of *Senna* series *Aphyllae* (Fabaceae, Caesalpinioideae)

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Received 27 April 2016; revised 5 September 2016; accepted for publication 7 November 2016

Senna series *Aphyllae* includes xeromorphic shrubs and subshrubs that occur in three different biogeographic subregions in arid and semi-arid habitats of southern South America. The series provides a good opportunity to understand better the relationship among geographical, climatic, and morphological variation in different taxa. Moreover, in this group the specific and varietal delimitation is still problematic due to the high morphological variation within and among taxa. Statistical analyses of climatic and morphological data and geographical distribution were used to understand the patterns of morphological variation among 394 individuals and to clarify the taxonomic delimitation of entities that belong to series *Aphyllae*. *Senna acanthoclada* and *S. nudicaulis* were segregated from each other and from the remaining taxa; the three recognized varieties of the *S. aphylla* complex were delimited; *S. spiniflora* was well-delimited and *S. crassiramea* and *S. rigidicaulis* were overlapping; *S. pachyrrhiza* was not differentiated from *S. aphylla*. Ecological niche modelling showed several areas of contact and a large overlap of suitable conditions for several species. The results of this work revealed that most morphological variability is associated with different environmental conditions. This phenotypic plasticity may be caused by the presence of different environments with climatic factors in the South American Transition Zone, Chaco, and northern region of Central Patagonia province.

ADDITIONAL KEYWORDS: ecological niche modelling – geographical distribution – multivariate analysis – taxonomic status.

INTRODUCTION

Plant morphology is a function of phenotypic changes in response to genetic variation within and among taxa, the biogeographic history of an individual species, geographical variation, and local climatic conditions (Scrivanti, Mestre & Anton, 2014). Several studies in plants growing under different climatic conditions and along elevational and latitudinal gradients showed that locally adapted phenotypes have a genetic background; however, variation in phenotype between individuals within species can arise from

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phenotypic plasticity (Clausen, Keck & Hiesey, 1948; Richards, Pennings & Donovan, 2005; Scheepens, Frei & Stöcklin, 2010; Scrivanti *et al.*, 2014). Geographical separation and/or morphological variation among individuals is also necessary for the formation of species and subspecies (Ellison *et al.*, 2004) and many plant species that grow in a range of different habitats have developed adaptive strategies suited to the particular habitats in which they occur (Coyne & Orr, 2004). According to Sultan (2000), phenotypic plasticity is the capacity of a given genotype to express different phenotypes in different environments.

However, it can still be commonly seen in the systematic literature that species boundaries are established mainly based on morphological traits. Nevertheless, Padial *et al.* (2010) predicted that 'taxonomy will no longer be a science restricted to the description of patterns but will be tightly linked to the study of processes generating diversity'. Several methods for testing species hypotheses and delineating species have been developed during the last years (e.g. Sites & Marshall, 2004; O'Meara, 2010; Fujita *et al.*, 2012; Gwynne, Balke & Meier, 2012; Leaché *et al.*, 2014; Kekkonen & Hebert, 2014). An integrative approach to taxonomy is necessary because the complexity of species biology requires that species boundaries be studied from multiple, complementary perspectives and collaboration between disciplines such as behavioural biology, ecology (biotic, abiotic, and geographical data), cytogenetics, comparative anatomy, phylogeography, and population genetics should become standard practice in taxonomy (Dayrat, 2005; Scrivanti *et al.*, 2014).

Fabaceae present a great phenotypic plasticity, which is expressed as different biological forms (e.g. trees, shrubs, herbs, and creepers) and by inhabitation of different climates, soils, and topography (Lewis *et al.*, 2005). Moreover, many works have demonstrated high phenotypic plasticity at specific and infraspecific levels in different genera of Fabaceae (e.g. Schlichting & Levin, 1986; Pappert, Hamrick & Donovan, 2000; Pohlman, Nicotra & Murray, 2005; Ceolin & Miotto, 2012; Santos *et al.*, 2015; Zhmud & Dorogina, 2015)

Representatives of *Senna* Mill. series *Aphyllae* (Benth.) H.S.Irwin & Barneby inhabit arid and semi-arid regions of southern Bolivia, central and north-western Argentina, and south-eastern Paraguay (Bentham, 1871). Based on cladistic biogeographic analyses of several plant and animal taxa, Morrone (2006) proposed a division of Latin America into areas, including the Monte and Prepuna provinces in the South American Transition Zone (SATZ), the Chaco Province in the Chaco subregion, and Central Patagonia in the Patagonian subregion. According to this, representatives of *Senna* series *Aphyllae* inhabit SATZ (Monte, Prepuna), the Chaco subregion (Chaco province), and the northern Patagonian subregion (Central Patagonia province). SATZ contains areas of biotic overlap, promoted by historical and ecological changes that allow the mixture of different biotic elements and the Chaco province harbours a remarkable biota (Cracraft & Prum, 1988; Crisci *et al.*, 1991; Abrahamovich, Díaz & Morrone, 2004; Morrone, 2006). In both environments, the interaction of different biotic and abiotic factors has promoted diversification, unusual ecological interactions, and the formation of new ecological niches (Guerrero *et al.*, 2013; Amarilla *et al.*, 2015; Ferreiro *et al.*, 2015). These zones deserve special attention because, unlike static lines, they represent areas of biotic interaction (Morrone, 2006). The few studies focused on phenotypic plasticity in plants that inhabit the semi-arid regions of the Southern Hemisphere have revealed that morphological variation in vegetative and reproductive characters may be correlated with environmental factors and clinal geographical distribution and in several cases that this phenotypic plasticity may give rise to problems in species delimitation (Chalcoff, Ezcurra & Aizen, 2008; Cosacov, Cocucci & Sérsic, 2012, 2014; Scrivanti *et al.*, 2014; Ferreiro *et al.*, 2015).

Senna series *Aphyllae* comprises xeromorphic shrubs and subshrubs with deeply penetrating woody roots. The leaves of adult branches are minute triangular or sublobate scales and the stem is junciform, green, and photosynthetic (Robbiati, Anton & Fortunato, 2011, Robbiati *et al.*, 2014a). The first comprehensive taxonomic studies in *Senna* series *Aphyllae* recognized 11 species, two subspecies, and two varieties (Bravo, 1978a, b, 1982; Irwin & Barneby, 1982; Bravo, Agulló & Palacios, 1986). Species differentiation in this series was based on the habit, pubescence of floral parts and stems, angle of branch divergence, and branch characteristics (Bravo, 1978a, b; Bravo *et al.*, 1986; Robbiati *et al.*, 2011).

Phylogenetic studies of *Senna* (Marazzi *et al.*, 2006) showed that the four species of series *Aphyllae* sampled in the analysis formed a monophyletic group. Recently, a series of taxonomic studies (Robbiati et al., 2011, 2013, 2014a; Robbiati, Anton & Fortunato, 2014b) based on morphological evaluation and seed protein profiles have led to a reinterpretation of taxonomy of series *Aphyllae*, with seven species and three varieties being recognized: *S. acanthoclada* (Griseb.) H.S.Irwin & Barneby (Fig. 1E), *S. aphylla* (Cav.) H.S.Irwin & Barneby var. *aphylla* (Fig. 1A), *S. aphylla* var. *divaricata* (Hieron.) Robbiati & Fortunato (Fig. 1B), *S. aphylla* var. *pendula* Robbiati & Fortunato (Fig. 1C), *S. crassiramea* (Benth.) H.S.Irwin & Barneby (Fig. 1F), *S. nudicaulis* (Burkart) H.S.Irwin & Barneby, *S. pachyrrhiza* (L. Bravo) H.S.Irwin & Barneby (Fig. 1D), *S. rigidicaulis* (L. Bravo) H.S.Irwin & Barneby (Fig. 1G), and *S. spiniflora* (Burkart) H.S.Irwin & Barneby (Fig. 1H). However, the relationships among these taxa are still unresolved. *Senna aphylla* and *S. pachyrrhiza* formed a complex characterized by the presence of unthickened

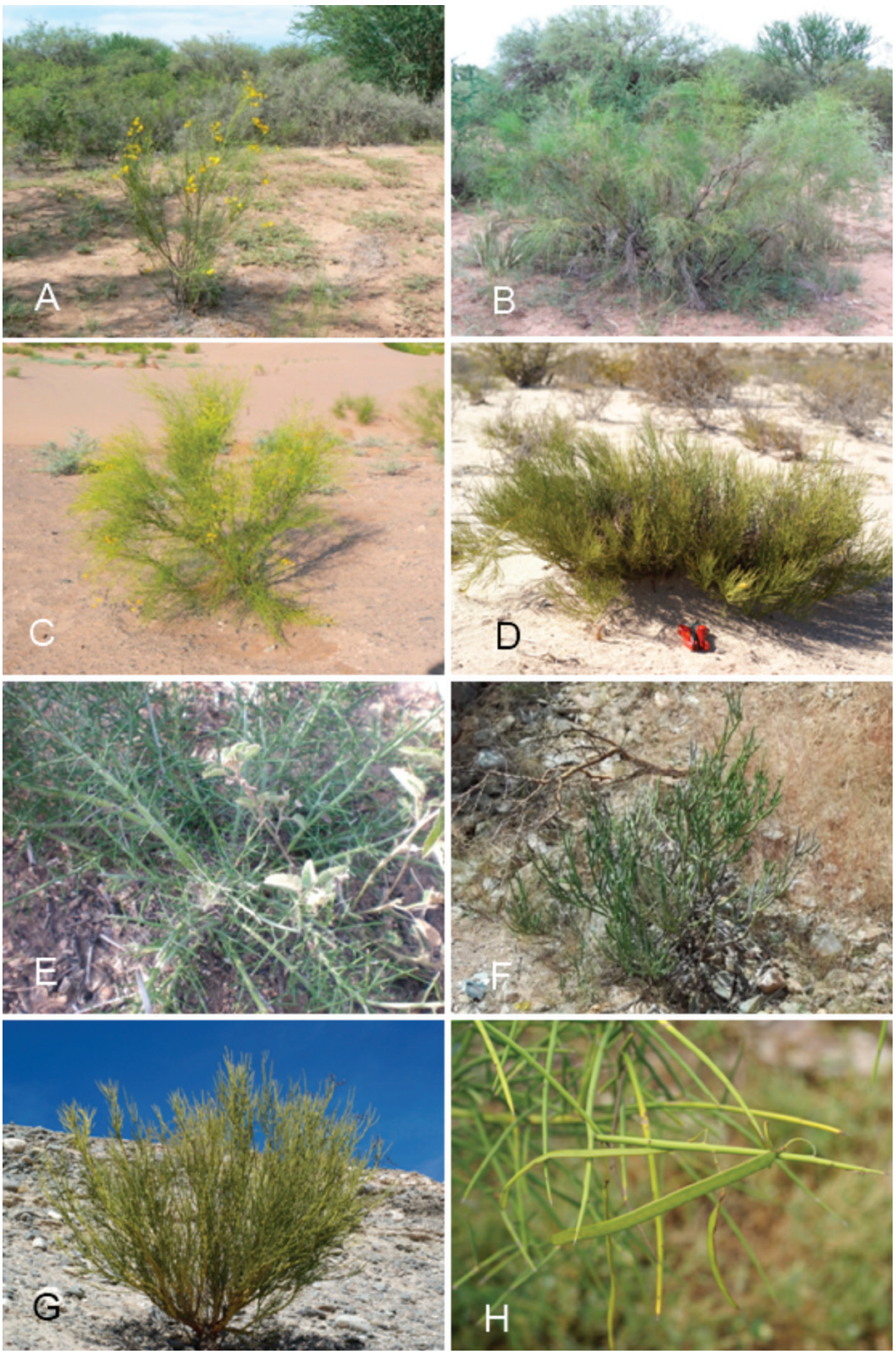

Figure 1. Growth form in *Senna* series *Aphyllae*. (A) *Senna aphylla* var. *aphylla*; (B) *S. aphylla* var. *pendula*; (C) *S. aphylla* var. *divaricata*; (D) *S. pachyrrhiza*; (E) *S. acanthoclada*; (F) *S. crassiramea*; (G) *S. rigidicaulis*; and (H) *S. spiniflora*.

branches, up to 4 mm in diameter (Robbiati *et al.*, 2011). Robbiati *et al.* (2014b) observed in *S. spiniflora* that in the northern portion of the distribution area many specimens had a pubescent calyx and stems, whereas southern specimens were glabrous and plants from the central area were variable. *Senna crassiramea* and *S. rigidicaulis* form a complex characterized by thickened and fastigiated branches (FB), differing only in the degree of thickening and fastigiation.

Based on these earlier studies, we asked if the morphological variation that gives rise to problems in species delimitation in *Senna* series *Aphyllae* could be influenced by environmental factors across its geographic distribution.

Moreover, in order to delimitate species in *Senna* series *Aphyllae* we combined phenetic and ecological approaches. The phenetic approach allows assessment of the degree of morphological similarities between members of a taxon and also helps in the evaluation of geographical patterns of morphological variation (Sokal & Crovello, 1970; Ghiselin, 1974; Sneath, 1976; Scrivanti *et al.*, 2014). On the other hand, the ecological approach permits evaluation of how abiotic factors influence the geographical distribution of a species (Graham *et al.*, 2004; Wiens & Graham, 2005; Rissler & Apodaca, 2007). Looking for congruence between different types of evidence is an efficient methodology for increasing the reliability of conclusions about species delimitation (Shaffer & Thomson, 2007; Wiens, 2007).

The aims of this work were (1) to evaluate if the morphological traits are geographically correlated; (2) to investigate if biologically relevant climatic data can reveal patterns of morphological variation in these species; (3) to reassess the morphological variation among and within taxa; and (4) to clarify the identity of closely related species and varieties. To meet these objectives, we carried out uni- and multivariate analyses to investigate patterns of morphological variation and its relationships with geographical and climatic gradients. Furthermore, we used ecological niche modelling (ENM) to understand the influence of environmental conditions on the geographical distribution of the study species.

MATERIAL AND METHODS

Morphological characters

Measurements were taken from 394 individuals (22 of *S. acanthoclada*, 44 of *S. aphylla* var. *aphylla*, 69 of *S. aphylla* var. *divaricata*, 116 of *S. aphylla* var. *pendula*, 11 of *S. pachyrrhiza*, 29 of *S. crassiramea*, three of *S. nudicaulis*, 26 of *S. rigidicaulis*, and 74 of *S. spiniflora*) in BAB, CORD, CTES, LIL, LP, MCNS, SI, and Z (herbarium abbreviations from http://sweetgum.nybg.org/ih/; Appendix S1). We observed that there is no drastic reduction in dimensions of morphological characters between fresh materials and herbarium specimens, and therefore morphometrical analysis was based on herbarium materials. Eight vegetative and 20 reproductive morphological characters selected based on morphological differentiation among taxa (Robbiati et al., 2011, 2013, 2014a, b) were used (Table 1), covering geographical range and morphological variation. Twenty-one characters were quantitative and seven were qualitative. Floral features were scored from one fully expanded and rehydrated flower and the dimensions of the internodes were measured at the widest part of the young branch. Size was measured using a binocular microscope Carl Zeiss 475003–9902 equipped with an ocular micrometer.

Multivariate analysis

To comply with the assumption of normality and homoscedasticity, anther length of the long abaxial stamen (AIF), anther length of the median stamen (AIN), gynopodium length (GL), leaf length (LL), ovary length (OL), staminodial length (ST), and style length (STL) were $\log^{-10}$ transformed. Variables were standardized before analyses. Multivariate analyses, including principal coordinates analysis (PCoA), cluster analysis (CA), and discriminant analysis (DA), were performed with the data sets using INFOSTAT 2012 (Di Rienzo *et al.*, 2012) (DA) and PAST (Hammer, Harper & Ryan, 2001) (PCoA, CA).

To perform PCoA, the Gower metric was used to create a dissimilarity matrix (Gower, 1971). Gower's index was used to analyze quantitative and qualitative variables because this index is sufficiently flexible to cope with nearly all forms of character coding (Gower, 1971). The Kaiser–Guttman criterion was used to select how many axes to retain and interpret.

CA was used to investigate the grouping pattern found in PCoA. This analysis involved a distance matrix based on Gower distance, which was subjected to the unweighted pair-group method, arithmetic average clustering algorithm (UPGMA; Sneath & Sokal, 1973). The cophenetic correlation coefficient was calculated to determine the consistency between the data matrices and the resulting dendrogram. DA was performed to investigate multivariate differentiation among *a priori* designated groups and to identify the most useful quantitative morphological traits for detecting differences.

Univariate analysis

Box-plots containing medians and quartiles were prepared for the morphological characters that were

Table 1. List of the characters used for the statistical analysis of *Senna* series *Aphyllae*

Character	Description
Anther length of long abaxial stamen (AIF)	mm
Anther length of median stamen (AIN)	mm
Asymmetric petal length (APL)	mm
Asymmetric petal width (APW)	mm
Fastigiate branches (FB)	1 = not fastigiate, 2 = slightly fastigiated, 3 = very fastigiate
Gynopodium length (GL)	mm
Height (H)	m
Internode length (IL)	mm
Internode width (IW)	mm
Leaf length (LL)	mm
Longest filament of abaxial stamens length (LFA)	mm
Longest sepal length (LLS)	mm
Longest sepal width (WLS)	mm
Ovary length (OL)	mm
Ovary pubescence (OP)	1 = glabrous, 2 = pubescent, 3 = densely pubescent
Pedicel length (PL)	mm
Peduncle length (PDL)	mm
Petal pubescence (PP)	1 = glabrous, 2 = pubescent
Sepal pubescence (SP)	1 = glabrous, 2 = pubescent, 3 = densely pubescent
Branch sinuosity (SB)	1 = not sinuous, 2 = slightly sinuous, 3 = sinuous
Smallest sepal length (LSS)	mm
Smallest sepal width (WSS)	mm
Staminodial length (ST)	mm
Stem pubescence (STU)	1 = glabrous, 2 = pubescent, 3 = densely pubescent
Style length (STL)	mm
Symmetric petal length (SPL)	mm
Symmetric petal width (SPW)	mm
Branch thickening (TB)	1 = not thickened, 2 = uniformly thickened, 3 = thickened in the central part

most discriminant and one-way analysis of variance (ANOVA) was carried out for the most discriminant characters for all taxa; significance was determined using Tukey's test with significant value of $P > 0.05$.

Climatic data and geographical distribution

The coordinates were obtained during field collection and from herbarium labels; when this information was not available, the herbarium specimens were geo-referenced using Google Earth 6.0 (http://www.google.com/earth/index.html). From each geo-referenced point, elevation and 19 bioclimatic variables were extracted from the WorldClim database (Hijmans *et al.*, 2005) with 5 arc-minutes resolution. We chose this level of resolution because most coordinate points were obtained from herbarium data. The environmental variables were extracted for each coordinate point using Diva-Gis 7.5 (Hijmans, Guarino & Mathur, 2012). To avoid over-estimation of climatic data and consequent misleading results, we reduced some data because of multicolinearity; for this, a Pearson's Correlation was performed to identify pairs of bioclimatic variables with a high degree of correlation ($r > 0.6$). We selected seven climatic variables (Bio1, Bio2, Bio3, Bio7, Bio12, Bio15, and Bio17) that were considered biologically meaningful and directly relevant to these species, plus elevation. Correlation tests were performed using INFOSTAT 2.0 (Di Rienzo *et al.*, 2012).

Geographical patterning of morphological variation was assessed by relating phenotypic distance matrices of morphological characters and geographical distance and elevational distance within each of the main groups resulting from the multivariate analyses using a Mantel test in PC-ORD (McCune & Mefford, 1995). Phenotypic dissimilarities were calculated as the Euclidean distances between each pair of specimens based on morphological data, whereas linear geographical distances between each pair of specimens were calculated using DIVA-GIS v.7.5. To estimate the proportion of morphological differentiation that could be associated with the seven bioclimatic variables selected, dissimilarity matrices were subjected to a Mantel test. Likewise, the relationship between geographical distance and each climatic variable was tested using a Mantel test, as described earlier. To examine whether characters exhibited clinal variation along elevational, latitudinal, and longitudinal gradients and across climatic variables, we conducted a Pearson's correlation analysis between each of the first PCA axes of the quantitative morphological variables and elevation, latitude, longitude, and the seven bioclimatic variables selected from the collection sites for each of the taxa, identified in the PCoA, and a correlation between geographical and climatic variables. Statistical significance was determined at $P < 0.05$. The data were standardized and analyzed using INFOSTAT 2.0 (Di Rienzo *et al.*, 2012). To evaluate the degree of association between the qualitative morphological variables with the climatic and geographical data, the Spearman (1904) correlation coefficient was calculated using INFOSTAT 2.0 (Di Rienzo *et al.*, 2012).

Ecological niche modelling

ENM has been employed to detect climatic differences in the habitats of closely related species. Three hundred and eighty-two occurrences (17 of *S. acanthoclada*, 232 of *S. aphylla*, 11 of *S. pachyrrhiza*, 27 of *S. crassiramea*, 25 of *S. rigidicaulis*, and 70 of *S. spiniflora*) were compiled using coordinate obtained for climatic analyses (Appendix S1). ENM models were run in MaxEnt using the following settings: convergence threshold = 10^{-5}, maximum iterations = 20 000, regularization multiplier = 1, replicates = 10, and replicates run type = cross-validation. MaxEnt uses only species presence data and performs well with small sample sizes (Hernandez *et al.*, 2006; Phillips, Anderson & Schapire, 2006). Models were then evaluated by the area under the receiver-operating characteristic curve (AUC) (Peterson, 2007; Lobo, Jiménez-Valverde & Real, 2008). AUC scores were first calculated using all records and then using 75% training vs. 25% testing data sets (Fielding & Bell, 1997). *Senna nudicaulis* was excluded from this analysis because only three samples were collected and there is no information available from other databases.

RESULTS

Morphometrical analyses

In the PCoA, the first three axes explained 41.75% of total variance (17.34, 16.96, and 7.45%, respectively) and were projected on a two-dimensional plane to observe the relationships among samples. The three first axes were retained. The plot of PCo1 vs. PCo2 (Fig. 2A) showed six groups: group I (*S. nudicaulis*), group II (*S. acanthoclada*), group III (*S. aphylla* var. *pendula*, *S. aphylla* var. *divaricata*, *S. pachyrrhiza*, and seven samples of *S. aphylla* var. *aphylla*), group IV (mostly composed of samples of *S. aphylla* var. *aphylla*), group V (*S. spiniflora*), and group VI (*S. crassiramea–S. rigidicaulis* complex). In group III, *S. pachyrrhiza* could not be distinguished from *S. aphylla* var. *divaricata* or *S. aphylla* var. *pendula*. In group VI, *S. crassiramea* and *S. rigidicaulis* were not clearly differentiated. The plot of PCo1 vs. PCo3 (Fig. 2B) displayed a similar clustering pattern between the taxa to that of PCo1 vs. PCo2, the main difference being that *S. spiniflora* and *S. crassiramea–S. rigidicaulis* complex were close to each, overlapping on one border, and one sample of *S. crassiramea* was gathered with the *S. spiniflora* group. The plot of PCo2 vs. PCo3 did not provide a clustering pattern.

The dendrogram (Fig. S1) obtained in CA (cophenetic correlation coefficient $r = 0.88$) showed a similar clustering pattern to that obtained in the PCoA; however, the samples of *S. crassiramea* and *S. rigidicaulis* in general were delimited, with only one sample of *S. rigidicaulis* being grouped with *S. crassiramea*, and three samples of *S. crassiramea* being grouped with *S. rigidicaulis*.

The DA classified individuals with 88.07% success in the classification matrix; the cross-validation values obtained from the discriminant function are shown in Table 2. All the studied specimens of *S. aphylla* var. *divaricata* showed a low rate of classification success (73.91%). The first and second canonical axes explained 71.7% of the total morphological variation (43.76 and 27.95%, respectively); these two axes were plotted (Fig. 3). In general, all taxa are overlapped in different degree by axis 1 and/or 2. Most samples of *S. spiniflora* were differentiated, but several samples overlapped with *S. aphylla* var. *divaricata*, *S. aphylla* var. *pendula*, and *S. crassiramea*. For *S. spiniflora* the best quantitative discriminant characters were internode length (IL) and smallest sepal length (LSS). *Senna crassiramea* showed a high level of morphological variation and its distribution in the plane overlapped with *S. aphylla* var. *pendula*, *S. rigidicaulis*, and *S. spiniflora*, internode width (IW) being the best discriminant character. *Senna rigidicaulis* showed a high level of morphological variation and was not well differentiated in the plane of *S. aphylla* var. *aphylla* and *S. crassiramea*; the best discriminant character was asymmetric petal width (APW).

In the *S. aphylla* complex, *S. pachyrrhiza* showed a classification error of 9.09%, with only one sample being classified as *S. aphylla* var. *divaricata*; nevertheless, in the biplot it was not differentiated from *S. aphylla* var. *divaricata* and the best discriminant characters were peduncle length (PDL) and smallest sepal width (WSS). *Senna aphylla* var. *aphylla* was partially differentiated since some samples overlapped with other members of the complex and with *S. rigidicaulis*; the discriminant characters were LSS and APW. Samples of *S. aphylla* var. *pendula* overlapped with most of the samples of *S. aphylla* var. *divaricata*; the best discriminant character for both taxa was asymmetric petal length (APL). *Senna acanthoclada* overlapped with *S. pachyrrhiza*, *S. aphylla* var. *divaricata*, and *S. aphylla* var. *pendula*; the best discriminant character was WSS. The samples of *S. nudicaulis* were not grouped and this taxon presented a high level of morphological variation. The variation of the discriminant characters is presented in box-plots (Fig. 4).

ANOVA showed that all characters that appeared discriminant in DA differed significantly between the taxa. Among the traits that have taxonomic value, IW of *S. crassiramea* and *S. rigidicaulis* is significantly wider than in the remaining taxa ($F = 111.6$, $P < 0.0001$). Asymmetric petal dimensions (APL and APW) differentiate ($F = 10.5$, $P < 0.0001$; $F = 68.3$,

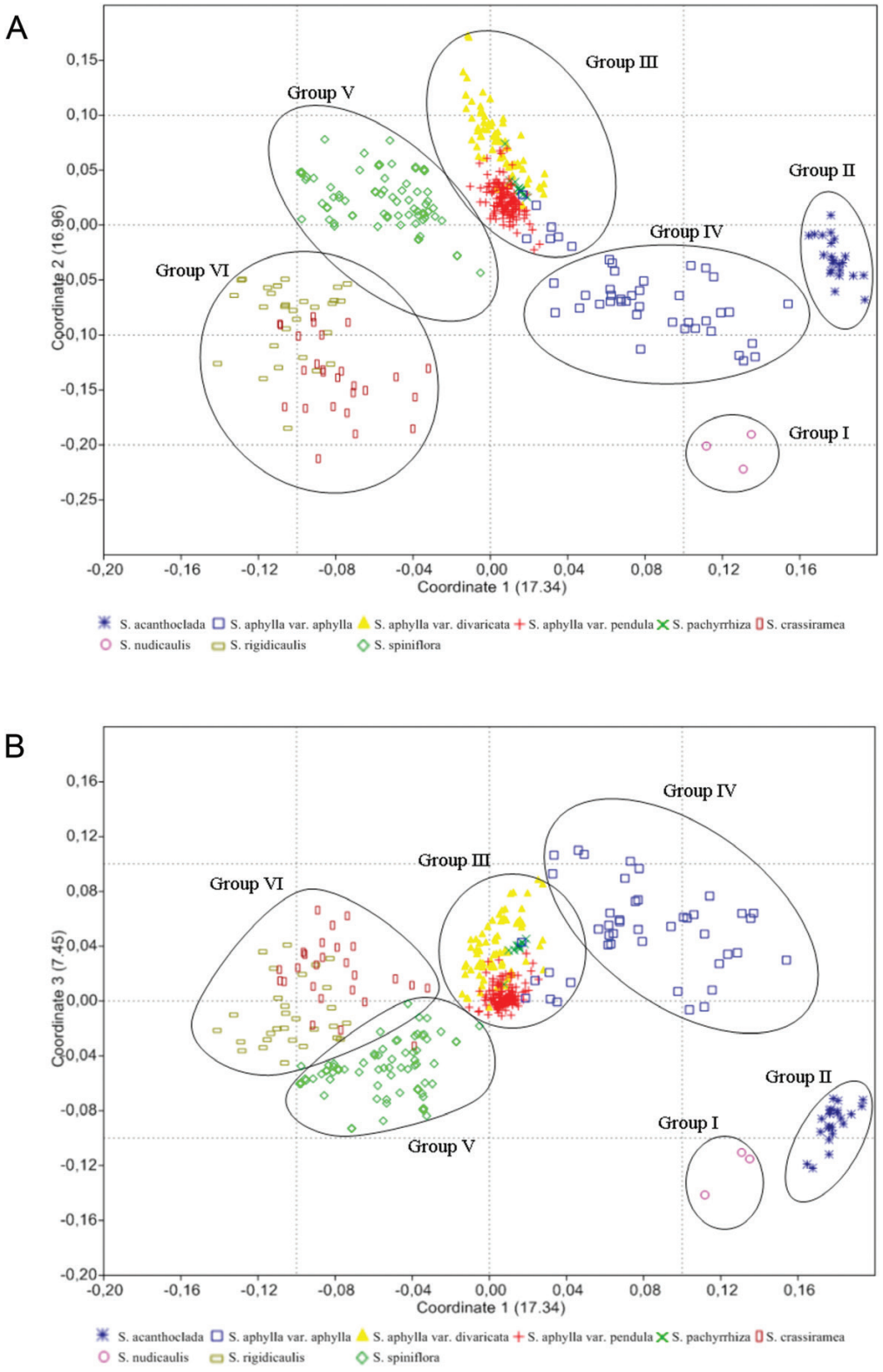

Figure 2. (A) Scatter plot of the first two coordinates from principal coordinate analysis (PCoA) based on 28 morphological traits of 394 specimens of *Senna* series *Aphyllae*. Six principal groups were formed. (B) Scatter plot of the first and third coordinates from PCoA. Six principal groups were formed. The morphological characters used in these analyses are listed in Table 1.

Table 2. Cross-validation test for discriminant analysis in *Senna* series *Aphyllae*

Taxon	SA	SAA	SAD	SAP	SCC	SN	SP	SR	SS	Total	Error (%)
SA	21	0	0	0	0	0	1	0	0	22	4.55
SAA	0	37	0	3	0	0	2	1	1	44	15.91
SAD	0	0	51	12	0	0	4	0	2	69	26.09
SAP	0	0	13	103	0	0	0	0	0	116	11.21
SCC	0	1	0	0	27	0	0	1	0	29	6.90
SN	0	0	0	0	0	3	0	0	0	3	0
SP	0	0	1	0	0	0	10	0	0	11	9.09
SR	0	0	0	0	1	0	0	25	0	26	3.85
SS	0	0	3	1	0	0	0	0	70	74	5.41
Total	21	38	68	119	28	3	17	27	73	394	11.93

Note: SA (*S. acanthoclada*), SAA (*S. aphylla* var. *aphylla*), SAD (*S. aphylla* var. *divaricata*), SAP (*S. aphylla* var. *pendula*), SCC (*S. crassiramea*), SN (*S. nudicaulis*), SP (*S. pachyrrhiza*), SR (*S. rigidicaulis*), and SS (*S. spiniflora*).

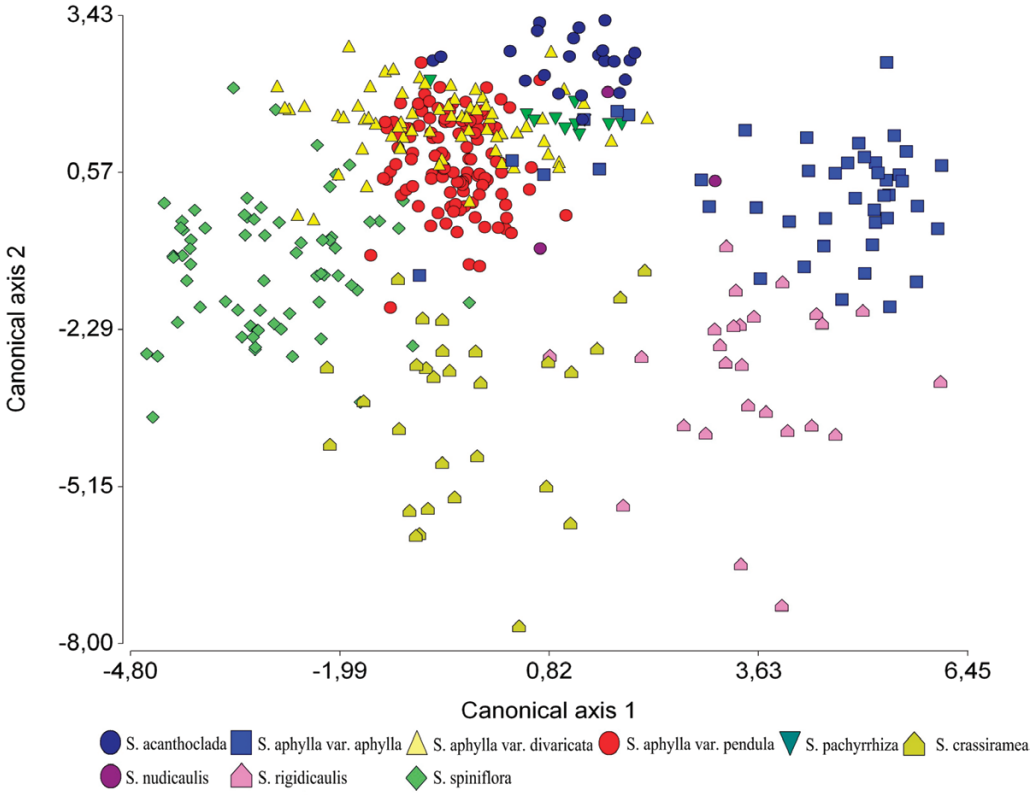

Figure 3. Scatterplot of the first two axes from discriminant analysis based on quantitative characters for *Senna* series *Aphyllae*. The morphological characters used in this analysis are listed in Table 1.

$P < 0.0001$, respectively) *S. aphylla* var. *aphylla* from the other varieties of *S. aphylla* varieties.

Climatic data and geographical distribution

The geographical distribution of the studied species is shown in Figure 5A–F. *Senna aphylla* was widely distributed (Fig. 5A) from north-western to south-eastern Monte, central-eastern Prepuna, and south-western Chaco provinces. *Senna aphylla* var. *aphylla* is present mainly in the western region in the Monte and Prepuna provinces; *S. aphylla* var. *divaricata* occurs mainly in the central-southern region of Monte province and *S. aphylla* var. *pendula* occurs mainly in the central region of Monte and Chaco provinces. However, the three varieties

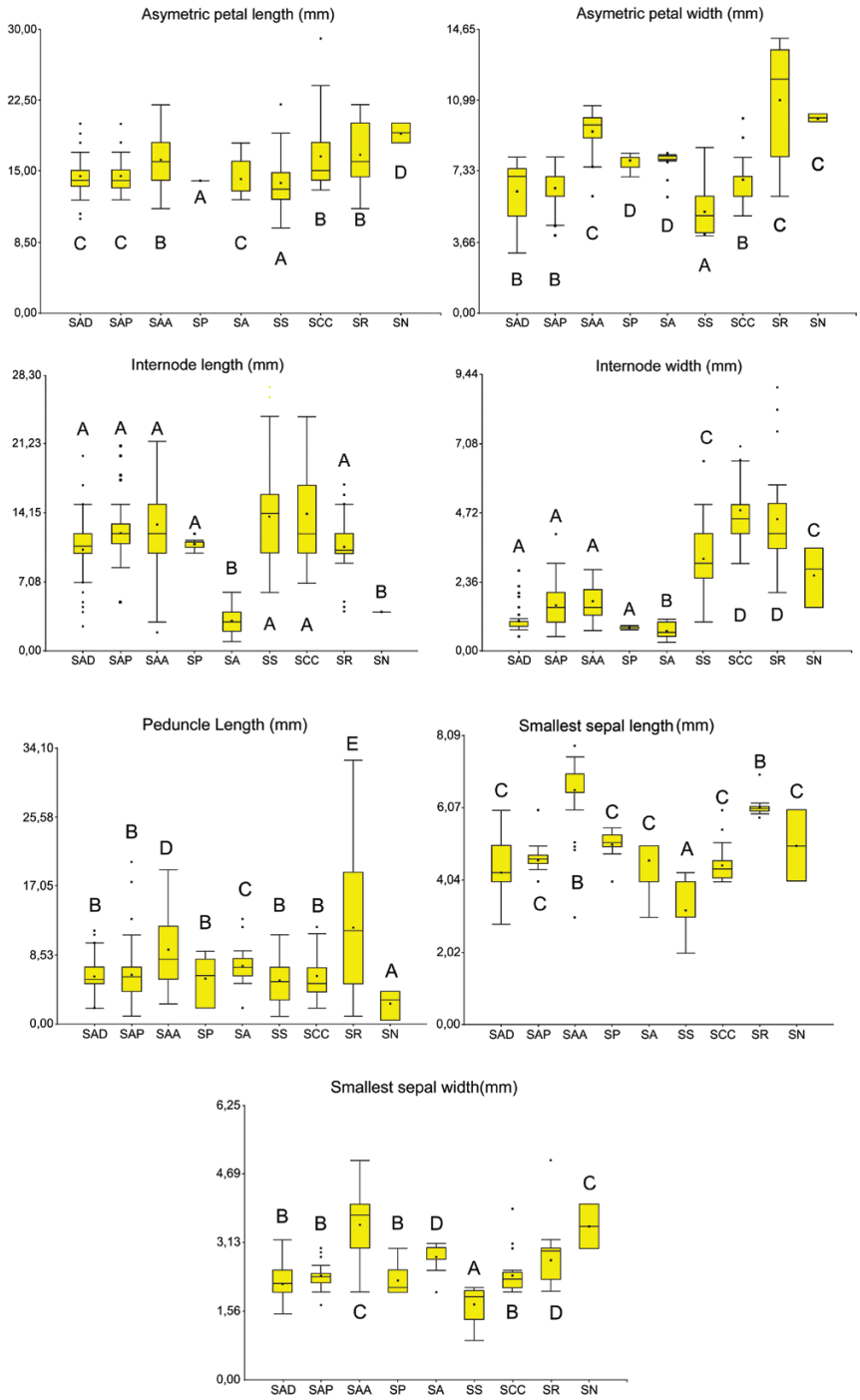

Figure 4. Box-plots representing the variability of discriminant characters in *Senna* series *Aphyllae*. The box represents the interquartile range; the upper horizontal line (bar) indicates the uppermost value; the lower horizontal line is the lowermost value; the circle within the box and the bar in the middle of the box represent the mean and the median, respectively. Points represent outliers. Different letters show significant differences between the means.

overlap in some areas. *Senna acanthoclada* occurs in Chaco province; *S. nudicaulis* in Patagonian province, in southern-central Argentina; *S. spiniflora* in Chaco province; *S. rigidicaulis* in the central-western area of the Monte and Prepuna provinces; and *S. crassiramea* in the northern-western area of the Prepuna province. The distribution of the last two entities overlaps.

The box-plots revealed that the following characters presented high levels of variation: APL for

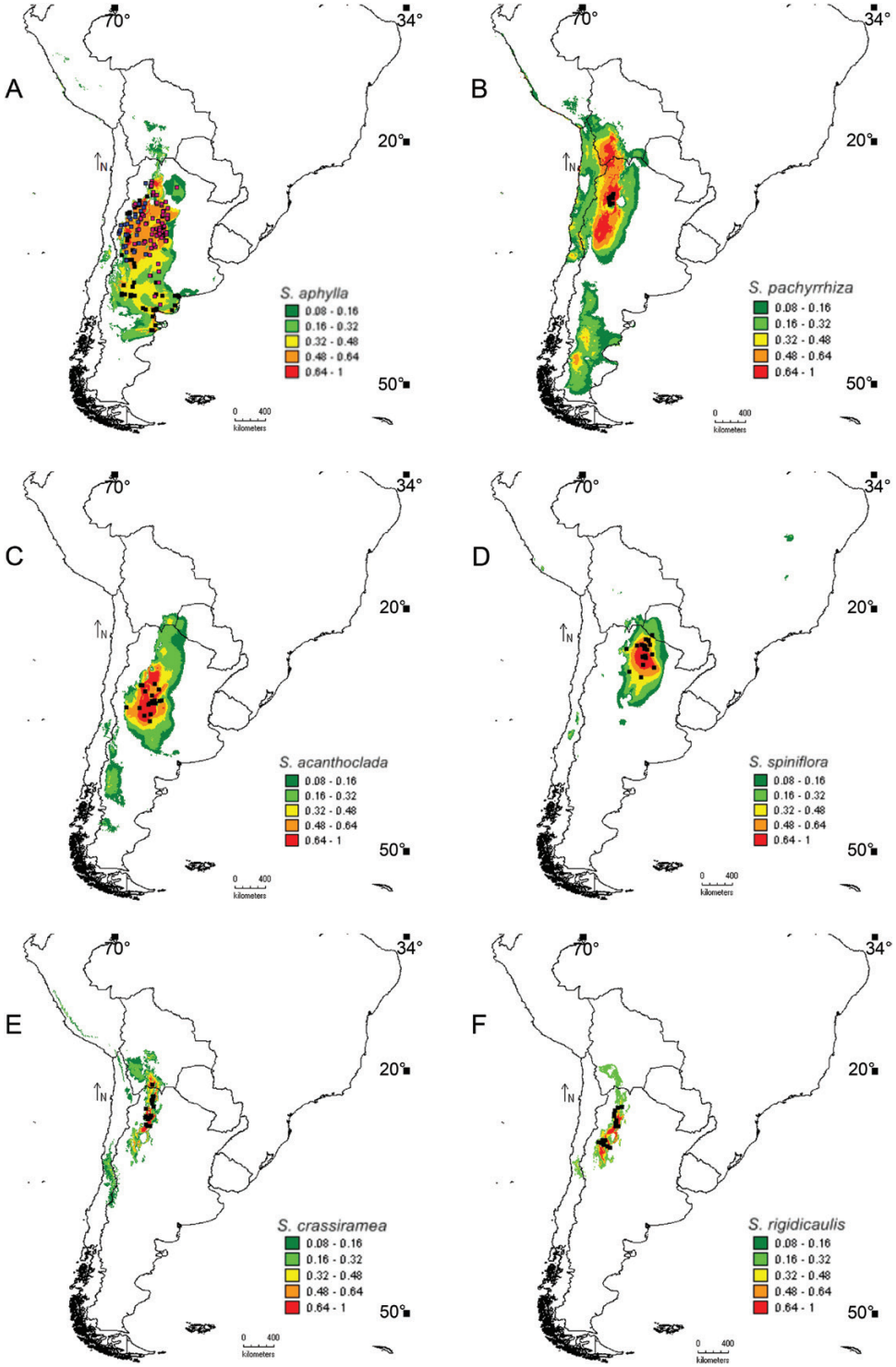

Figure 5. Predictive distribution models for six species of *Senna* series *Aphyllae*. Red colours indicate regions with a higher probability of species occurrence. Squares refer to point localities on which the models are based. (A) *S. aphylla*; (B) *S. pachyrrhiza*; (C) *S. acanthoclada*; (D) *S. spiniflora*; (E) *S. crassiramea*; and (F) *S. rigidicaulis*. For *S. aphylla* blue squares represent *S. aphylla* var. *aphylla* samples, black squares represent *S. aphylla* var. *divaricata* samples, and purple squares represent *S. aphylla* var. *pendula* samples.

S. aphylla var. *aphylla*, *S. crassiramea*, *S. spiniflora*, and *S. rigidicaulis*; APW for *S. aphylla* var. *divaricata*, *S. aphylla* var. *pendula*, *S. spiniflora*, and *S. rigidicaulis*; IL for *S. aphylla* var. *aphylla*, *S. spiniflora*, and *S. crassiramea*; IW for *S. crassiramea*, *S. nudicaulis*, *S. rigidicaulis*, and *S. spiniflora*; PDL for *S. aphylla* var. *aphylla*, *S. aphylla* var. *pendula*, *S. pachyrrhiza*, and *S. rigidicaulis*; LSS for *S. aphylla* var. *divaricata*, *S. acanthoclada*, *S. spiniflora*, and *S. nudicaulis*; and WSS for *S. aphylla* var. *aphylla*.

The Mantel test (Tables 3–6) revealed no association between the phenotypic distance matrix (MORPHO), geographical, elevational, and bioclimatic variables distance matrices for the major taxa. For *S. aphylla* var. *aphylla* and *S. rigidicaulis* there was an association with diurnal temperature range (Bio2). For *S. rigidicaulis* there was an association with temperature range (Bio7). For *S. aphylla* var. *pendula*, although small, there was an association with several bioclimatic variables. Geographical distances (DIST) presented an association with temperature and precipitation condition for all taxa, except *S. aphylla* var. *aphylla* and *S. pachyrrhiza*; elevational distance (ALT) showed an association with temperature and precipitation condition for all taxa, except *S. acanthoclada*, which only displayed correlation with Bio2; for *S. pachyrrhiza*, no association was revealed; association between DIST and ALT was observed in *S. aphylla* var. *divaricata*, *S. aphylla* var. *pendula*, *S. crassiramea*, *S. spiniflora*, and *S. rigidicaulis*.

A Pearson's correlation analysis was performed between each of the first PCA axes of the quantitative vegetative and reproductive morphological variables and the geographical and climatic variables. Several traits presented correlation with environment and geographical position. Correlation values are displayed in Tables S1–S8.

Of the 17 quantitative floral characters studied, 15 (AIF, AIN, APL, APW, GL, LFA, LLS, OL, PDL, PL, SPL, SPW, STL, WLS, and WSS) were correlated with latitude (Lat), longitude (Long), elevation (Alt), and bioclimatic variables. All the quantitative vegetative characters considered (H, IL, IW, and LL) were correlated with Lat, Long, Alt, and bioclimatic variables. Alt and the seven bioclimatic variables

Table 3. Mantel tests of association among phenotypic distances, geographical distance, elevational distance, and climatic variables of specimens of *Senna acanthoclada* and *S. aphylla* var. *aphylla*

Matrix		*S. acanthoclada*		*S. aphylla* var. *aphylla*	
A	B	*R*	*P**	*R*	*P**
MORPHO	DIST	–0.03819	0.40	–0.0330	0.42
MORPHO	ALT	0.03312	0.36	0.06633	0.32
MORPHO	BIO1	–0.03665	0.29	0.04352	0.27
MORPHO	BIO2	–0.04840	0.45	0.15923	0.02
MORPHO	BIO3	0.1375	0.10	0.07767	0.15
MORPHO	BIO7	0.0987	0.19	0.02385	0.36
MORPHO	BIO12	0.05530	0.33	–0.00332	0.50
MORPHO	BIO15	0.04007	0.36	0.0383	0.27
MORPHO	BIO17	0.0927	0.21	–0.0065	0.48
DIST	ALT	0.0073	0.43	–0.0099	0.67
DIST	BIO1	0.3	0.0007	–0.0500	0.46
DIST	BIO2	0.4362	0.0005	–0.0853	0.19
DIST	BIO3	0.2557	0.0086	–0.0546	0.3203
DIST	BIO7	0.4884	0.0003	–0.0663	0.30
DIST	BIO12	0.4778	0.0002	–0.0252	0.47
DIST	BIO15	0.5354	0.0001	0.0157	0.26
DIST	BIO17	0.4882	0.0001	0.2173	0.04
ALT	BIO1	0.0950	0.13	0.6012	0.0001
ALT	BIO2	0.7613	0.0001	0.0058	0.42
ALT	BIO3	–0.0624	0.32	0.8530	0.0001
ALT	BIO7	–0.01696	0.47	0.7572	0.0001
ALT	BIO12	–0.01812	0.49	0.0002	0.40
ALT	BIO15	0.0213	0.37	0.34436	0.0004
ALT	BIO17	0.09502	0.13	0.0090	0.36

Note: *Probability that a random $Z <$ observed Z.

Table 4. Mantel tests of association among phenotypic distances, geographical distance, elevational distance, and climatic variables of specimens of *Senna aphylla* var. *divaricata* and *S. aphylla* var. *pendula*

Matrix		*S. aphylla* var. *divaricata*		*S. aphylla* var. *pendula*	
A	B	*R*	*P**	*R*	*P**
MORPHO	DIST	0.0047	0.45	–0.10	0.03
MORPHO	ALT	0.1	0.08	–0.02	0.40
MORPHO	BIO1	0.001	0.48	–0.03	0.30
MORPHO	BIO2	0.055	0.14	–0.04	0.28
MORPHO	BIO3	–0.07	0.155	–0.11	0.002
MORPHO	BIO7	–0.072	0.07	–0.09	0.03
MORPHO	BIO12	0.03	0.33	–0.07	0.06
MORPHO	BIO15	–0.04	0.22	–0.10	0.04
MORPHO	BIO17	–0.075	0.07	–0.07	0.03
DIST	ALT	0.67	0.0001	0.16	0.008
DIST	BIO1	0.23	0.0001	0.17	0.002
DIST	BIO2	0.39	0.0001	0.31	0.0001
DIST	BIO3	0.57	0.0001	0.22	0.0001
DIST	BIO7	0.29	0.0001	0.27	0.83
DIST	BIO12	0.15	0.003	0.29	0.0001
DIST	BIO15	0.89	0.0001	0.82	0.0001
DIST	BIO17	0.89	0.0001	0.67	0.0001
ALT	BIO1	0.30	0.0004	0.40	0.0001
ALT	BIO2	0.21	0.0002	0.17	0.0083
ALT	BIO3	0.79	0.0001	0.37	0.0001
ALT	BIO7	0.26	0.0001	0.02	0.26
ALT	BIO12	0.02	0.27	0.19	0.0006
ALT	BIO15	0.63	0.0001	0.35	0.0001
ALT	BIO17	0.52	0.0001	0.22	0.0001

Note: *Probability that a random $Z <$ observed Z.

considered were correlated with Lat and Long for several taxa.

The Spearman correlation test showed that *S. acanthoclada* had no qualitative character displaying correlation with elevation, latitude, longitude, or the seven bioclimatic variables selected. For *S. aphylla* var. *aphylla* Bio15 was significantly and positively correlated with sepal pubescence (SP) (r_S = 0.33, $P < 0.03$). For *S. aphylla* vars. *divaricata* and *pendula* and *S. pachyrrhiza* no qualitative character was correlated with elevation, latitude, longitude, or the seven bioclimatic variables selected. For *S. spiniflora*, Bio1 was significantly and positively correlated with stem pubescence (STU) (r_S = 0.38, $P < 0.0001$) and SP (r_S = 0.42, $P < 0.0001$); latitude was significantly and negatively correlated with SP (r_S = –0.42, $P < 0.001$) and STU (r_S = –0.28, $P < 0.01$). For *S. crassiramea*, Bio1 was significantly and negatively correlated with FB (r_S = –0.45, $P < 0.01$); Bio2 and Bio7 were significantly and positively correlated with FB (r_S = 0.36, $P < 0.05$ and (r_S = 0.33, $P < 0.05$ respectively) and elevation was significantly and positively correlated with FB (r_S = 0.44, $P < 0.02$). For *S. rigidicaulis* no qualitative character was correlated with elevation, latitude, longitude, or the seven bioclimatic variables selected.

Ecological niche modelling

The potential distributions of the species based on the Maxent algorithm are presented in Fig. 5A–E. The ENM results indicate that the models performed well (using all records and training-testing data with AUC > 0.95). The AUC for each group was better than random (i.e. model AUC values exceeded the 95th percentile of the null AUC distributions). For *S. acanthoclada* the projection of the distribution model was a fairly good representation of the extant geographical distribution and over-predicts the geographical distribution in the extreme north and south-east, where it has never been recorded. The variables Bio7 and Bio15 made the greatest contributions (Table S9). For *S. aphylla* the projection of the distribution model was a fairly good representation of the extant geographical

Table 5. Mantel tests of association among phenotypic distances, geographical distance, elevational distance, and climatic variables of specimens of *Senna pachyrrhiza* and *S. spiniflora*

Matrix		*S. pachyrrhiza*		*S. spiniflora*	
A	B	*R*	*P**	*R*	*P**
MORPHO	DIST	0.10	0.29	0.04	0.20
MORPHO	ALT	–0.02	0.50	–0.05	0.30
MORPHO	BIO1	0.10	0.30	–0.001	0.53
MORPHO	BIO2	0.01	0.36	–0.02	0.41
MORPHO	BIO3	0.02	0.42	0.03	0.26
MORPHO	BIO7	0.003	0.40	0.015	0.38
MORPHO	BIO12	–0.12	0.38	0.063	0.18
MORPHO	BIO15	–0.11	0.37	0.01	0.40
MORPHO	BIO17	–0.09	0.42	0.10	0.05
DIST	ALT	0.30	0.06	0.22	0.008
DIST	BIO1	0.61	0.002	0.77	0.0001
DIST	BIO2	0.61	0.002	0.39	0.0001
DIST	BIO3	0.38	0.02	0.71	0.0001
DIST	BIO7	0.83	0.83	0.58	0.0001
DIST	BIO12	0.63	0.006	0.38	0.0001
DIST	BIO15	0.63	0.005	0.36	0.0001
DIST	BIO17	0.74	0.0006	0.39	0.0001
ALT	BIO1	0.76	0.0004	0.46	0.0001
ALT	BIO2	–0.17	0.26	0.19	0.027
ALT	BIO3	0.88	0.0001	0.017	0.36
ALT	BIO7	0.34	0.066	0.009	0.37
ALT	BIO12	0.27	0.10	0.08	0.17
ALT	BIO15	0.11	0.29	0.71	0.0001
ALT	BIO17	0.18	0.15	0.37	0.0001

Note: *Probability that a random $Z <$ observed Z.

distribution and over-predicts the geographical distribution mainly in the northern extreme; the variables that most contributed with the model were Bio7 and Bio12 (Table S9). For *S. crassiramea* and *S. rigidicaulis* the distribution model showed similarity to the current distribution of the species and over-predicts the geographical distribution in the extreme north and south, including areas of central Bolivia and Chile, and for *S. crassiramea* in southern Peru, where it has never been recorded. The variables that contributed most to the model were elevation and Bio17 (Tables S9, S10). For *S. spiniflora* the distribution model showed great similarity to the current distribution of the species, with a slight over-prediction in central Chile and north-eastern Brazil; the variables that contributed most to the model were Bio7 and Bio15 (Table S10). For *S. pachyrrhiza*, an important over-prediction between projected and real distribution areas was found, covering a more extensive area of suitable habitats from northern Puna province to the Patagonia subregion (Andean Region); the variables that contributed most to the model were elevation and Bio7 (Table S10).

DISCUSSION

Traditionally, alpha taxonomic studies were focused on delimiting species based on morphological similarities and geographical distribution. Since the emergence of numerical taxonomy (Sneath & Sokal, 1973), multivariate and statistical analyses have become a powerful tool in delimiting species and assessing morphological variation and an increasing number of taxonomic works use complementary studies such as morphological, phylogenetic, cytogenetics, phylogeography, and population genetics as standard methodologies in taxonomy (Nordström & Hedrén, 2009; Akhavan *et al.*, 2015; Gale *et al.*, 2015; Ali *et al.*, 2016; Arbizu *et al.*, 2016; Banasiak *et al.*, 2016). Nevertheless, in the taxonomic literature there are few examples that deserve special attention with regard to morphological variation promoted by environmental factors and its taxonomic implications; these investigations demonstrated that phenotypic variation could hinder species delimitation (Ellison *et al.*, 2004; Ložiene, 2006; Cavallero *et al.*, 2011; Nicola, Johnson & Pozner, 2014; Scrivanti *et al.*, 2014; Lopez Laphitz, Ezcurra & Vidal-Russell, 2015).

In a group of species with restricted distributions, in which specialization to narrow and distinct climatic/environmental envelopes has been demonstrated to be the main force leading to speciation, the specialization of a newly discovered population to a climatic/environmental condition distinct from all known species in the group might be a suitable argument to advocate its species status (Padial *et al.*, 2010). Extreme environmental conditions might impose stabilizing selection on morphology, reducing or eliminating morphological change that can accompany speciation (Bickford *et al.*, 2007), and we believe that an integrative approach using analyses between morphological traits, environmental conditions where an organism occurs, and geographical data are necessary to propose species boundaries and detect diagnostic traits, considering a diagnostic character to be one that allows definition of a group and has no significant relationship with the climatic conditions and geographical distribution (elevation, latitude) (Scrivanti *et al.*, 2014).

In *Senna* series *Aphyllae*, the pubescence of stem and floral pieces plus the peculiarities of the branches were the most important traits considered in many taxonomic proposals (Bentham, 1871; Burkart, 1952; Bravo, 1978a; Irwin & Barneby, 1982). However, the results of the present work revealed that several characters considered relevant in the past to species differentiation show high variability at inter- and intraspecific levels. Moreover, this research shows that part of this morphological

Table 6. Mantel tests of association among phenotypic distances, geographical distance, elevational distance, and climatic variables of specimens of *Senna. crassiramea* and *S. rigidicaulis*

Matrix		*S. crassiramea*		*S. rigidicaulis*	
A	B	*R*	*P**	*R*	*P**
MORPHO	DIST	–0.05	0.24	0.073	0.13
MORPHO	ALT	–0.11	0.10	0.03	0.30
MORPHO	BIO1	–0.10	0.11	0.02	0.30
MORPHO	BIO2	0.02	0.32	0.27	0.0017
MORPHO	BIO3	–0.008	0.51	–0.082	0.21
MORPHO	BIO7	0.019	0.34	0.42	0.0001
MORPHO	BIO12	–0.17	0.048	–0.08	0.23
MORPHO	BIO15	–0.11	0.14	–0.03	0.42
MORPHO	BIO17	–0.11	0.20	–0.017	0.49
DIST	ALT	0.17	0.015	0.15	0.03
DIST	BIO1	0.18	0.13	0.09	0.11
DIST	BIO2	0.85	0.0001	0.28	0.001
DIST	BIO3	0.83	0.0001	0.27	0.002
DIST	BIO7	0.69	0.0001	0.54	0.0001
DIST	BIO12	–0.05	0.21	0.24	0.012
DIST	BIO15	0.15	0.018	0.36	0.0001
DIST	BIO17	–0.02	0.44	0.45	0.0001
ALT	BIO1	0.98	0.0001	0.96	0.0001
ALT	BIO2	0.30	0.0002	–0.08	0.15
ALT	BIO3	0.32	0.0008	0.65	0.0001
ALT	BIO7	0.22	0.002	0.25	0.002
ALT	BIO12	0.40	0.0007	0.04	0.22
ALT	BIO15	0.05	0.23	0.55	0.0003
ALT	BIO17	0.19	0.061	0.38	0.009

Note: *Probability that a random $Z <$ observed Z.

variation is influenced by climatic factors. Some works investigating correlation between environmental factors and morphological variation in arid and semi-arid regions have demonstrated that traits such as height, pubescence, and canopy architecture may be influenced by the environment (De Soyza *et al.*, 1997; Sandquist & Ehleringer, 2003; Scrivanti *et al.*, 2014).

The data allowed delimitation of *S. acanthoclada* and *S. nudicaulis* from the remaining species. For *S. acanthoclada*, WSS was discriminant, but cannot be considered of taxonomic value since it does not appear different in *S. rigidicaulis*. Among the characters correlated with environment, SPW was also correlated with longitude, and AIF and IL were correlated with latitude and elevation. These facts suggest that elevation and geographical distribution could be indirectly influencing phenotypic variation in these morphological traits. Correlation between morphological traits and elevation and geographical distribution has been reported in other plant groups (Morrison, 1984; Jonas & Geber, 1999; Milla, 2009; Milla & Reich, 2011; Scrivanti *et al.*, 2014).

In the *S. aphylla* complex, the multivariate analyses showed a continuum of variation among the three varieties proposed by Robbiati *et al.* (2014a). The poor differentiation between *S. aphylla* vars. *divaricata* and *pendula* shown in the discriminant analysis suggests that the differentiation between these two entities is mainly caused by qualitative characters such as branching patterns. Asymmetric petal dimensions for *S. aphylla* var. *aphylla* (APL and APW) are significantly greater than the remaining varieties of *S. aphylla.* However, given that variability present in the asymmetric petal dimensions, especially in APL, this character is not important for taxonomic differentiation (Robbiati *et al.*, 2014a).

In addition, the discriminant analyses revealed a lack of morphological difference between *S. pachyrrhiza* and *S. aphylla* var. *divaricata*. For all members of the *S. aphylla* complex, several qualitative and quantitative characters presented a correlation with geographical position and environmental condition. In the case of *S. aphylla* var. *aphylla*, among the five characters associated with environment, only SP was

previously consider to be of taxonomic value by Bravo (1978a) in separating *S. fabrissi* (L.Bravo) H.S.Irwin & Barneby and *S. trichosepala* (Chodat & Wilczek) H.S.Irwin & Barneby from *S. aphylla*. However, the present work revealed that a proportion of variation of this character depends on environment and did not reveal a geographical distribution pattern. These results are in agreement with the taxonomic proposal of Robbiati *et al.* (2013, 2014a), who considered *S. fabrissi* and *S. trichosepala* to be synonyms of *S. aphylla* var. *aphylla*. For *S. aphylla* var. *divaricata*, among characters that showed correlation with environments, APL displayed association with latitude and longitude. In addition, latitude was strongly correlated with elevation. The Mantel test showed a significant association between elevational distance and bioclimatic variables; these findings suggest the significant importance of elevation in petal size variability in this entity, which occurs between 0 and 2900 m a.s.l. Several works have demonstrated variation in flower size along elevational gradients (Jonas & Geber, 1999; Herrera, 2005; Zhigang *et al.*, 2006; Maad, Armbruster & Fenster, 2013)

The presence of decumbent stems, a massive xylopodium, terminal spinose branchlets, and a dwarf habit were the characters used to differentiate *S. pachyrrhiza* (Bravo, 1978a; Irwin & Barneby, 1982). Several of these morphological characters, such as pseudo-decumbent stem and dwarf habit, have been observed in the field by the authors in individuals of *S. aphylla* var. *divaricata* at high elevation in the Patagonian steppe in the central Patagonian province (*Bach 515* and *589*); furthermore for this taxon, plant height showed a negative correlation with elevation. Decrease in plant size as a response to high elevation is a well-known phenomenon (Galen, Shore & Deyoe, 1991; Coomes & Allen, 2007; Vitasse *et al.*, 2009; Scrivanti *et al.*, 2014). It results from a slower growth rate that may allow plants to use resources more efficiently under adverse climatic conditions (Grime, 1979, Bennington & McGraw, 1995). Moreover, the decrease in growth with increasing elevation may be interpreted as symptomatic of increasing environmental stress (Cordell *et al.*, 1998; Fabbro & Körner, 2004; Macek, Macková & de Bello, 2009; Jafari & Sheidai, 2011, Milla & Reich, 2011, Maad *et al.*, 2013).

The character branch sinuosity (BS), used as differential character for *S. aphylla* var. *divaricata*, has been observed in the field in several individuals of *S. pachyrrhiza*; this character did not show correlation with environmental condition. These findings suggest that BS may not represent a phenotypic response to environmental conditions and that it is genetically determined; therefore, it can be considered of taxonomic value. Given the morphological similarities found between *S. pachyrrhiza* and *S. aphylla* var. *divaricata* and the potential distribution of *S. pachyrrhiza* and the fact that plant height is correlated with elevation, this taxon may be considered a population of *S. aphylla* var. *divaricata*. Nevertheless, due to the restricted distribution of *S. pachyrrhiza* and to the lack of phylogenetic evidence to confirm that *S. aphylla* and *S. pachyrrhiza* form a monophyletic group, no taxonomic decision is taken; moreover, we cannot neglect the hypothesis of morphological convergence. For *S. aphylla* var. *pendula*, among the traits correlated with environmental variables, pedicel length (PL) was correlated with latitude and APW was correlated with altitude. Moreover, the Mantel test showed a significant association between geographical and elevational distance and the bioclimatic variables that were correlated with several morphological characters; these findings suggest the importance of elevation and geographical distribution in morphological variation. For *S. pachyrrhiza* only four quantitative reproductive characters showed correlation with temperature and precipitation, of which AIN and APL displayed a strong correlation with latitude. Nevertheless, the restricted distribution and the number of samples of this taxon could cause an underestimation of the real correlation.

Our analysis showed that *S. spiniflora* is morphologically well-differentiated from the remaining species. Here, we found that morphological traits such as stem and SP are correlated with environmental conditions and elevation and that elevation was strongly correlated with temperature and precipitation. Thus, the results support the taxonomic proposal to consider *S. chacöensis* (L.Bravo) H.S.Irwin & Barneby a synonym of *S. spiniflora* (Robbiati *et al.*, 2014b), suggesting that the variability in pubescence may be a response to temperature or water stress due to the high temperatures in the northern part of the distribution. Specifically, pubescence plays a direct role in energy and water balance by reducing both energy absorption and water loss, helping to reflect incident solar radiation and dissipate absorbed heat, thereby reducing leaf temperature and transpiration rates, as demonstrated for many other plant groups (Johnson, 1975; Ehleringer, Björkman & Mooney, 1976; Ehleringer & Mooney, 1978; Ehleringer, 1982; Ehleringer & Cook, 1990; Woodman & Fernandes, 1991; Pérez-Estrada, Cano-Santana & Oyama, 2000). Complementary studies of intra-and interspecific variation in pubescence have demonstrated correlations with climate that were consistent with water balance and a functional role (Ehleringer *et al.*, 1981, Sandquist & Ehleringer, 1997).

In general, multivariate analyses revealed a large morphological variation in the *S. crassiramea–S. rigidicaulis* complex. In this complex, phenotypic variation did not show a significant association with ALT and only three traits were correlated with ALT. These results suggest that variation in elevation in their small distribution areas is not an important factor in

morphological variation. Nevertheless, the environment may cause morphological variation, since six traits for *S. crassiramea* and seven for *S. rigidicaulis* were correlated with environment. The character FB was used to distinguish *S. crassiramea* and *S. rigidicaulis*, between each other and from the remaining taxa (Bravo, 1978a). Our results revealed that for *S. crassiramea* features were correlated with temperature and altitude. Others works have also shown that variation in branching architecture may be caused by environment (Neufeld *et al.*, 1988; De Soyza *et al.*, 1997). The trait IW has taxonomic importance for *S. crassiramea* and *S. rigidicaulis* (Bravo, 1978a; Robbiati *et al.*, 2011). This character was discriminant for both taxa, but only for *S. crassiramea* it was not associated with environmental variables. Given the inconsistent results of the different analyses for the *S. crassiramea–S. rigidicaulis* complex and the overlapping distributions, these entities cannot be clearly divided into well-defined species. Further studies are needed before making taxonomic decisions about their status.

The ENM analysis revealed that the variable temperature range was the one with greatest influence on the distribution of *S. acanthoclada*, *S. aphylla*, and *S. spiniflora*, suggesting that this variable may be biologically significant because it is associated with the beginning of the growing and flowering periods. On the other hand, for *S. pachyrrhiza*, elevation and Bio7 influenced the distribution patterns, suggesting that elevation in itself is not the most influential variable. According to this analysis, *S. pachyrrhiza* could occur in other regions; however, the orographic structure or physiological restriction could be hindering the establishment of this species in adjacent areas. The study of the geographical distribution revealed that the northern part of the distribution of *S. rigidicaulis* overlaps with the southern part of the distribution of *S. crassiramea*. In addition, ENM showed that elevation is the variable with the greatest influence on the distribution of *S. crassiramea* and *S. rigidicaulis*, suggesting that these taxa may have had physiological adaptations to high-mountain ecosystems.

CONCLUSIONS

The results of the present work provide further evidence that landscape heterogeneity with different environments in the SATZ, Chaco, and the northern part of Central Patagonia province could be promoting phenotypic variation in plant species. The results presented showed that of the 28 characters considered here, 16 reproductive and six vegetative characters show an association with environment; suggesting that a proportion of morphological variability in vegetative and reproductive characters, such as stem and SP, branch features, and plant height, which has caused problems in species identification among and within species, are environmentally based changes in the phenotype. Given that reproductive and vegetative characters do not show different patterns of variation, further studies are need to reveal if these traits show a degree of coupling and are responding to different evolutionary forces. According to our results, *S. acanthoclada*, *S. nudicaulis*, and *S. spiniflora* are three well-differentiated morphotypes with different geographical distributions: *S. nudicaulis* is endemic to south-central Argentina and without taxonomic conflict; *S. acanthoclada* is endemic to the southern Chaco province, and part of its morphological variability was correlated with environmental conditions; and *S. spiniflora* is endemic to the central Chaco province. In this last taxon, the variability in STU, which led to taxonomic problems in its recognition, is somewhat influenced by environmental conditions. *Senna aphylla s.l.* and *S. pachyrrhiza* formed a large complex scattered through a large geographical area and in different biogeographic regions. Part of the morphological variation in these entities was correlated with environment, but we do not propose the synonymy of *S. aphylla* var. *divaricata* and *S. pachyrrhiza* due to the lack of molecular evidence. Finally, *S. crassiramea* and *S. rigidicaulis* formed a complex characterized by the fastigiation of the branches. The affinities between these species are still unresolved and molecular evidence would be necessary to clarify their taxonomic status.

ACKNOWLEDGEMENTS

The authors are grateful to the curators of the herbaria cited for permission to study and/or loan specimens, to Marcelo Gritti (CONICET) for help with the preparation of the illustration and to Gustavo Bertone (CONICET) for the photograph of *S. acanthoclada*. The authors also thank the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) for the fellowship given to FOR and the Universidad Nacional de Córdoba (UNC) and the Estación Experimental Agropecuaria INTA Salta (EEA Salta) for providing facilities and work space. This work was supported by the following grants: Consejo Nacional de Investigaciones Científicas y Técnicas, Argentina (CONICET, PIP 112-200801-00323 and 11220080101557) and Instituto Nacional de Tecnología Agropecuaria (INTA).

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SUPPORTING INFORMATION

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

Appendix S1. Vouchers, specimens examined, and coordinates for *Senna* series *Aphyllae.*

Figure S1. Dendrogram based on 28 morphological traits of 394 specimens of *Senna* series *Aphyllae* using UPGMA clustering algorithm. Cophenetic correlation coefficient $r = 0.88$.

Table S1. Pearson correlation coefficient between the first PCA axes of the morphological and environmental variables of the collection localities of *Senna acanthoclada*. The horizontal line divides the reproductive and vegetative characters. Significant based on Tukey's test at significance level: $*P < 0.05$. Elevation (Al), Latitude (Lat), and Longitude (Long).

Table S2. Pearson correlation coefficient between the first PCA axes of the morphological and environmental variables of the collecting localities of *Senna aphylla* var. *aphylla*. The horizontal line divides the reproductive and vegetative characters. Significant based on Tukey's test at significance level: $*P < 0.05$. Elevation (Al), Latitude (Lat), and Longitude (Long).

Table S3. Pearson correlation coefficient between the first PCA axes of the morphological and environmental variables of the collection localities of *Senna aphylla* var. *divaricata*. The horizontal line divides the reproductive and vegetative characters. Significant based on Tukey's test at significance level: $*P < 0.05$. Elevation (Al), Latitude (Lat), and Longitude (Long).

Table S4. Pearson correlation coefficient between the first PCA axes of the morphological and environmental variables of the collection localities of *Senna aphylla* var. *pendula*. The horizontal line divides the reproductive and vegetative characters. Significant based on Tukey's test at significance level: $*P < 0.05$. Elevation (Al), Latitude (Lat), and Longitude (Long).

Table S5. Pearson correlation coefficient between the first PCA axes of the morphological and environmental variables of the collection localities of *Senna pachyrrhiza*. The horizontal line divides the reproductive and vegetative characters. Significant based on Tukey's test at significance level: $*P < 0.05$. Elevation (Al), Latitude (Lat), and Longitude (Long).

Table S6. Pearson correlation coefficient between the first PCA axes of the morphological and environmental variables of the collection localities of *Senna spiniflora*. The horizontal line divides the reproductive and vegetative characters. Significant based on Tukey's test at significance level: $*P < 0.05$. Elevation (Al), Latitude (Lat), and Longitude (Long).

Table S7. Pearson correlation coefficient between the first PCA axes of the morphological and environmental variables of the collection localities of *Senna crassiramea*. The horizontal line divides the reproductive and vegetative characters. Significant based on Tukey's test at significance level: $*P < 0.05$. Elevation (Al), Latitude (Lat), and Longitude (Long).

Table S8. Pearson correlation coefficient between the first PCA axes of the morphological and environmental variables of the collection localities of *S. rigicaulis*. The horizontal line divides the reproductive and vegetative characters. Significant based on Tukey's test at significance level: $*P < 0.05$. Elevation (Al), Latitude (Lat), and Longitude (Long).

Table S9. Analysis of variable contributions for *Senna aphylla*, *S. acanthoclada*, and *S. crassiramea*.

Table S10. Analysis of variable contributions for *Senna pachyrrhiza*, *S. rigidicaulis*, and *S. spiniflora*.