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Phenetic analysis of the complex *Senna fabrisii—S. trichosepala* (Leguminosae, Caesalpiniodeae, *Aphyllae*) based on morphological characters and seed protein electrophoretic profiles

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Abstract

Multivariate and univariate analyses based on morphological characters and seed soluble protein electrophoretic profiles were carried out in order to understand the taxonomic status within the complex *S. fabrisii*-*S. trichosepala*. Twenty morphological characters from herbarium specimens were recorded and analyzed by ANOVA, principal component and cluster analysis. The seed protein electrophoretic analysis including cluster analysis was based on three populations: one of *S. fabrisii* and two of *S. trichosepala*. The results showed that both taxa differed only by three morphological features; in parallel, the CPA and CA demonstrate that no clearly separated group can be recognized. The seed protein electrophoretic profiles show a high degree of similarity. Based on these results, and due to the variability observed, the inclusion of *S. fabrisii* in the synonymy of *S. trichosepala* is proposed.

Key words: *Aphyllae*, morphometrical analyses, quantitative characters, *Senna*, taxonomic position

Introduction

Senna Miller (1754: 290) Series *Aphyllae* Bentham (1871: 542) occurs in southern Bolivia, from central to northwestern Argentina and in southeastern Paraguay. This series was previously considered under the genus *Cassia* Linnaeus (1753: 376), for which Burkart (1952) recognized five species; later Bravo (1978) described new taxa and distinguished 11 species. Irwin & Barneby (1982), based on morphological, vegetative and reproductive characters, divided *Cassia s.l.* into three genera: *Senna*, *Chamaecrista* Moench (1794: 272) and *Cassia* (*s. s.*). The series *Aphyllae* was included by Irwin & Barneby (1982) in *Senna* and they accepted the taxonomic proposal made by Bravo (1978). Robbiati *et al*. (2011) recognized seven species based on a new morphological evaluation of the diagnostic characters in the series. The differentiation of the species was based especially on habit, floral piece pubescence and divergence angle of branches. However, in many cases, the delimitation of species is difficult due to the absence of leaves and the similarity in many features especially of flower and pod, and has led to a conflicting circumscription with taxonomic confusion (Burkart 1952, Bravo 1978, Irwin & Barneby 1982, Robbiati *et al*. 2011).

Within the ser. *Aphyllae*, *S. fabrisii* (L. Bravo) H. S. Irwin & Barneby (1982: 570) and *S. trichosepala* (Chodat & Wilczek) H. S. Irwin & Barneby (1982: 570) form a complex characterized by the pubescence of the floral parts, especially the gynoecium and sepals; the habit; by having spiny branches; and by co-ocurrence (sympatry). These species inhabit the provinces of central and western Argentina: Catamarca, La Rioja, Mendoza, San Juan and San Luis. Bravo (1978) differentiated both species by calyx, ovary and pod pubescence and anther length of long abaxial stamens. On the other hand, in the same work she pointed out

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the occurrence of intermediate specimens between *S. fabrisii* and *S. trichosepala*. Robbiati *et al*. (2011) found variation between different populations of *S. fabrisii* and *S. trichosepala* particularly in the presence of calyx, ovary and pod pubescence, and suggested a reappraisal of the diagnostic characters used by Bravo (1978) for better species delimitation.

At this time there is no information related to the use of reserve seed protein in *Senna*. The seminal proteins have proved to be useful in providing complementary information to help clarify the classification at generic and infrageneric levels. (Vaughan *et al*. 1966, Przybylska *et al*. 1977, Aiken & Gardiner 1990, Pasha & Sen 1991, Lanham *et al*. 1994, Sanchez-Yelamo *et al*. 1995, Przybylska & Przybylska 1995, Lamarque & Fortunato 2003, Niknam *et al.* 2004, Lamarque *et al*. 2009). Furthermore, the patterns of the seed proteins are highly stable irrespective of environmental condition (Harborne & Turner 1984, Gepts 1990, Pedalino *et al*. 1992). There are considerable differences between similarity values for inter- versus intra-specific relationships. The similarity coefficient value between species is always less than 0.5, while intra-specific values vary between 0.75 and 1 (Potokina *et al*. 2000, Açik *et al*. 2004).

The aim of this work is to investigate the similarities between *S. fabrisii* and *S. trichosepala* to determine the taxonomic position of both taxa, through taxonomic study, morphometrical analyses using quantitative characters and seed protein electrophoretic profiles.

Material & Methods

Plant Material

Field studies were conducted between 2010 and 2012. Information regarding habitat and intra-population variation was recorded. Each population was collected as a bulk of seeds from at least three plants, with five fruits per plant. The material used for seed-protein analysis is listed in Table 3. Voucher specimens were deposited in the following herbaria: Instituto de Recursos Biológicos (INTA), Castelar, Buenos Aires (BAB) and Museo Botánico, Universidad Nacional de Córdoba (CORD), Argentina.

TABLE 1. Quantitative characters used for the phenetic analysis.

1	Anthers of long abaxial stamens length (mm)
2	Anthers of median stamens length (mm)
3	Asymmetric and lower petal length (mm)
4	Calyx pubescence (hair density cm^2)
5	Divergence angle of branches (degree)
6	Staminodial length (mm)
7	Longer filament of abaxial stamens length (mm)
8	Gynopodium length (mm)
9	Internodes length (mm)
10	Internodes width (mm)
11	Leaf length (mm)
12	Ovary length (mm)
13	Ovary pubescence (hair density cm^2)
14	Pedicel length (mm)
15	Peduncle length (mm)
16	Peduncle pubescence (hair density cm^2)
17	Plant height (m)
18	Long sepals inner length (mm)
19	Style length (mm)
20	Symmetric and upper petal length (mm)

Morphological characters

Thirty seven herbarium specimens (18 of *S. fabrisii* and 19 of *S. trichosepala*) were examined from: BAB, CORD, CTES, LIL, MA and SI. Eight vegetative and twelve reproductive characters (Table 1) were selected and measured based on the morphological differentiation between both taxa made by Burkart (1952), Bravo

(1978) and Irwin & Barneby (1982). Floral features were scored from one fully expanded and rehydrated flower and size measurements were made with an object micrometer using a binocular microscope Carl Zeiss 475003-9902. The dimensions of the internodes were taken from young branches, and the width was measured in the median part.

Morphometric analyses

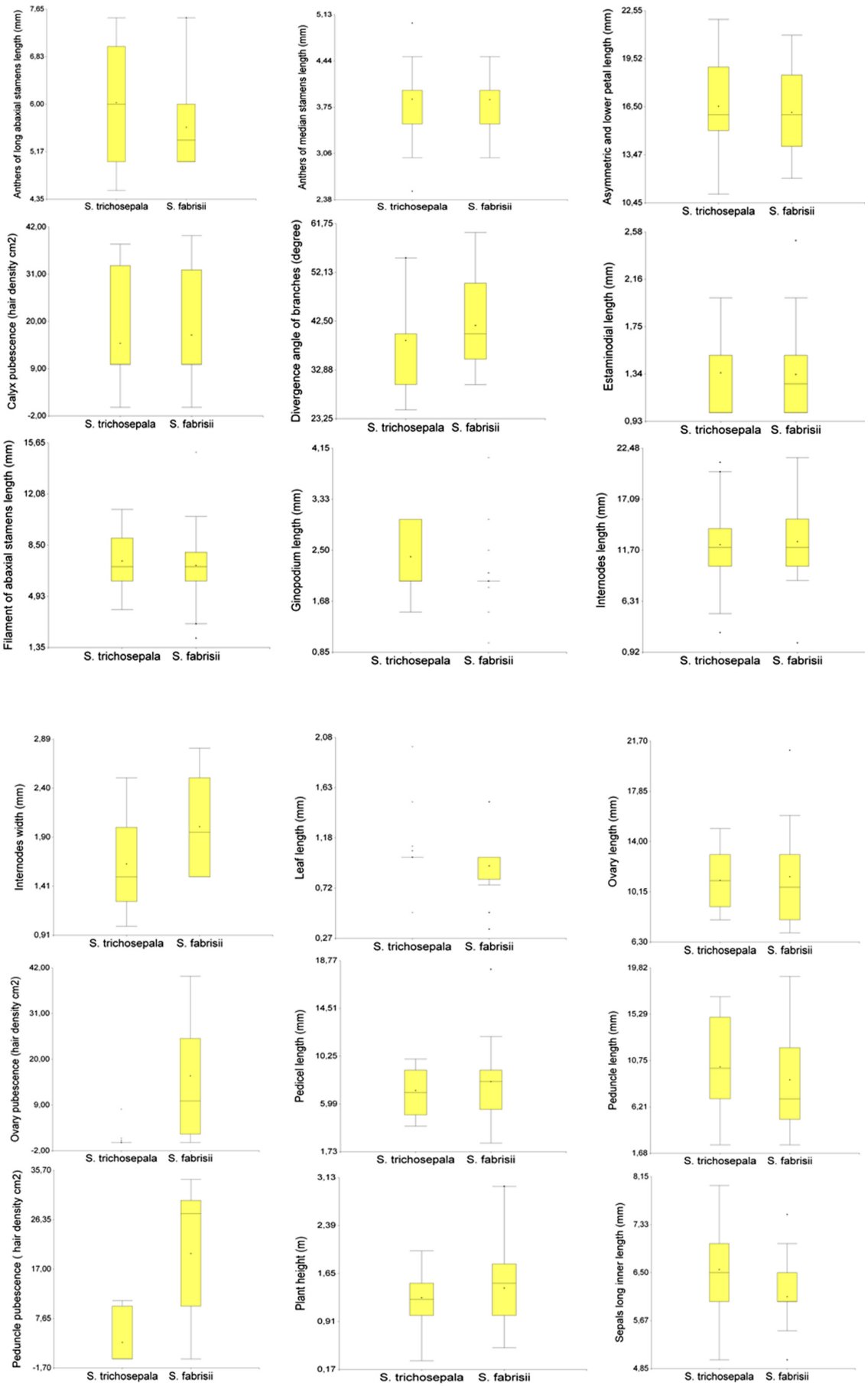
The statistical analyses were performed using INFOSTAT statistical program version 2012 (Group InfoStat). For the univariate analysis the average, standard deviation, coefficient of variation, minimum and maximum were recorded for each feature. To represent the variability of each character, box plots containing medians and quartiles were prepared. Characters that passed normality tests (the Kolmogorov-Smirnov D-test and the Shapiro-Wilk W-test) and the homoscedasticy test (Levene statistic) were subject to analysis of variance (ANOVA), and the significance was tested using Tukey's test with significant value of $p > 0.05$, and the Kruskal-Wallis test (K) was used for characters that failed the normality or homoscedasticy tests with significant value of $p > 0.05$. Prior to carrying out multivariate analyses, the data matrix itself was examined. A Spearman's Correlation was performed to identify pairs of characters with a high degree of correlation ($\rho > 0.6$). The principal components analysis was based on correlation matrix and the results were plotted in two-dimensional scatter plots. A dendrogram was constructed using the Unweighted Pair-Group Method using arithmetic averages UPGMA (Sneath & Sokal 1973). Each specimen was considered as an operational taxonomic unit (OTU). This analysis was performed on the initial data, which were standardized and used to compute a distance matrix based on Euclidian distance. The cophenetic correlation coefficient was calculated to determine the consistency between the data matrices and their resulting dendrogram.

Seed protein electrophoretic profiles

For protein extraction, 2.0 g of mature seeds was powdered using a mortar and pestle and homogenized with phosphate buffer, pH 7.4 for 1 h. The extract was centrifuged at 8917 rpm for 10 min and the protein in the supernatant precipitated with cold acetone, vacuum filtered and dried in desiccator. For electrophoresis, the protein was mixed with sample buffer (0.125 M Tris-HCl, pH= 6.8, 2% SDS, 0.5% β-mercaptoethanol, 0.1% bromophenol blue), denatured by boiling for 2 min in a water bath, cooled; and 30 µL of this mixture wasloaded in a gel slab, which was prepared as in Laemmli (1970). The loading gel was 3% acrylamide and the resolving gel was prepared with 10% acrylamide in a 3.5 M Tris-HCl pH 8.8 buffer with 10% SDS. The electrode buffer was Tris-glycine (6.0 g of Tris base, 28.8 g of glycine, 20 ml of 10% SDS in 2 L of water, pH 8.3). Electrophoresis was carried out at 20 mA for 2 h in a Dual vertical Mini-Gel Apparatus (Cole Parmer). Bio-Rad standard molecular weight proteins (Bio-Rad Co., Hercules, CA, USA): myosin (198.08 kDa), β-galactosidase (113.58 kDa), bovine serum albumin (96.36 kDa), ovalbumin (52.98 kDa), carbonic anhydrase (35.96 kDa), soybean trypsin inhibitor (28.49 kDa), lysozyme (18.53 kDa) and aprotinin (5.73 kDa) were used to estimate the weight of polypeptide bands.

After electrophoresis, gels were fixed with MeOH/acetic acid/H_2O (2:2:1) for 45 min and stained with Colloidal Brilliant Blue G- (Sigma-Aldrich, St. Louis, MO, USA).

Gels were scored for the presence (1) and absence (0) of specific bands and the value were used to compile a data matrix. A similarity index for all possible comparisons between samples was calculated by Jaccard coefficient. An UPGMA dendrogram was constructed based on the program NTSYS-pcnumerical taxonomy and multivariate analysis system, version 2. 2. (Rohlf, 2012) using the average method.



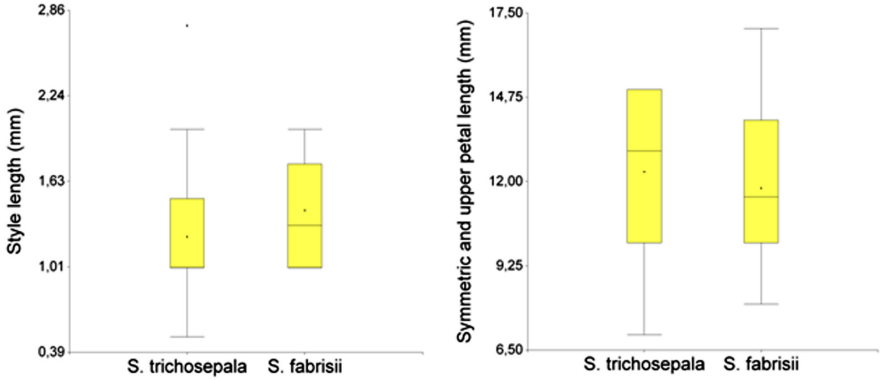


FIGURE 1. Box plots representing the variability of the quantitative characters in *S. fabrisii–S. trichosepala* complex. Points represent outliers.

Results

Univariate analysis

The average, median, standard deviation, coefficient of variation, minimum and maximum values of quantitative morphological characters are summarized (Tab. 2) and their variation is presented in box plots (Fig. 1). Of the 20 characters, 17 showed no significant difference between the means, including the length of the anthers of long abaxial stamens and density of calyx pubescence, referred as differential by Bravo (1978). Of the three variables that showed significant difference between the means, two were used to specific segregation: the pubescence of the ovary ($H=21.47$ $p<0.0001$) and the pubescence of the peduncle ($H=12.32$ $p<0.0002$), both characters considered relevant by Bravo (1978). The width of internodes ($H=4.46$ $p<0.0308$) also showed significant differences between means, even though this character was not cited by Bravo (1978) and Robbiati *et al*. (2011).

TABLE 2. Mean ± standard deviation, coefficient of variation and range (minimum–maximum) for the 20 characters used for phenetic analysis. Characters numbered according to Table 1.

Characters/taxa	*S. fabrisii*	*S. trichosepala*
1	M±Std 5.6±0.74 CV 13.28 Min.–Max. 5.0–7.5	M±Std 6.03±0.87 CV 14.5 Min.–Max. 4.5–7.5
2	M±Std 3.86±0.45 CV 11.59 Min.–Max 3.0–4.5	M±Std 3.87±0.52 CV 13.52 Min.–Max. 2.5–5.0
4	M±Std 16.14±2.83 17.52 Min.–Max. 12.0–21.0	M±Std 16.53±2.65 CV 16.06 Min.–Max. 11.0–22.0
4	M±Std 16.83±12.89 CV 76.6 Min.–Max. 0.0–40.0	M±Std 14.91±13.25 CV 88.9 Min.–Max. 0.0–38.0
5	M±Std 41.67±9.07 CV 21.78 Min.–Max. 30.0–60.0	M±Std 38.68±8.31 CV 21.47 Min.–Max. 25.0–55.0
6	M±Std 1.33±0.41 CV 30.84 Min.–Max. 1.0–2.5	M±Std 1.35±0.36 CV 26.77 Min.–Max. 1.0–2.0
7	M±Std 7.08±2.82 CV 39.88 Min.–Max. 2.0–15.0	M±Std 7.39±1.77 CV 23.91 Min.–Max. 4.0–11.0
8	M±Std 2.13±0.66 CV 31.01 Min.–Max. 1.0–4.0	M±Std 2.39±0.52 CV 21.54 Min.–Max. 1.5–3.0
9	M±Std 12.61 ±4.46 CV 35.34 Min.–Max. 1.9–21.5	M±Std 12.29±4.5 CV 37.02 Min.–Max. 3.0–21.0
10	M±Std 2.01±0.48 CV 23.96 Min.–Max. 1.5–2.8	M±Std 1.63±0.5 CV 30.67 Min.–Max. 1.0–2.5
11	M±Std 0.92±0.24 CV 25.85 Min.–Max. 0.35–1.5	M±Std 1.06±0.28 CV 26.79 Min.–Max. 0.5–2.0
12	M±Std 11.31±3.64 CV 32.22 Min.–Max. 7.0–21.0	M±Std 11.03±2.15 CV 19.5 Min.–Max. 8.0–15.0
13	M±Std 16.0±15.32 CV 95.75 Min.–Max. 0.0–40.0	M±Std 0.47±1.84 CV 387.79 Min.–Max. 0.0–8.0
14	M±Std 7.97±3.46 CV 43,37 Min.–Max. 2.5–18.0	M±Std 7.18±1.83 CV 25.54 Min.–Max. 4.0–10.00
15	M±Std 8.86±5.12 CV 57,76 Min.–Max. 2.5–19.0	M±Std 10.03±4.23 CV 42.2 Min.–Max. 2.5–17.0
16	M±Std 19.94±13.04 CV 65.36 Min.–Max. 0.0–34.0	M±Std 13±4.75 CV 151.83 Min.–Max. 0.00–11.00
17	M±Std 1.43±0.6 CV 41.9 Min.–Max. 0.5–3.0	M±Stt 1.27±0.4 CV 31.79 Min.–Max. 0.3–2.0
18	M±Std 6.08±0.69 CV 11.36 Min.–Max. 5.0–7.5	M±Std 6.55±0.9 CV 13.67 Min.–Max. 5.0–8.0
19	M±Std 1.41±0.39 CV 27.35 Min.–Max. 1.0–2.0	M±Std 1.22±0.51 CV 41.92 Min.–Max. 0.50–2.75
20	M±Std 11.78±2.51 CV 21.31 Min.–Max. 8.0–17.0	M±Std 12.32±2.52 CV 20.44 Min.–Max. 7.0–15.0

Principal component analysis

The two characters of length of symmetric and asymmetric petal presented strong correlation (ρ> 0.62), so the former was excluded from the principal components analysis. The first three principal components explain 0.42% (17.8, 12.9 and 11.3%, respectively) of the total variation (Tab. 4) and were projected on a two-dimensional plane (Figs. 2, 3). The cophenetic correlation (r=0,728), indicates good fit between the Euclidian distance among OTUs in the two dimensional plot. Loading on the first component was contributed mainly by the following characters: asymmetric and lower petal length, ovary length, anthers of median stamens length and peduncle length. Loading on the second component was contributed mainly by divergence angle from parent axis, internodes width, anthers of long abaxial stamens length, staminodial length and peduncle pubescence.

Loading on the third component was contributed mainly by internodes length, ovary pubescence, anthers of long abaxial stamens length, asymmetric and lower petal length, pedicel length and ovary length. Plotting on components 1, 2 and 3, no clearly separated group could be recognized, showing an overlap among *S. fabrisii* and *S. trichosepala*. Individuals that are further apart correspond to the specimens of *S. fabrisii* with more pubescence. In the dendrogram (cophenetic correlation, r=0.762) that illustrates phenetic relationships among species in the complex (Fig. 4), no clusters can be recognized.

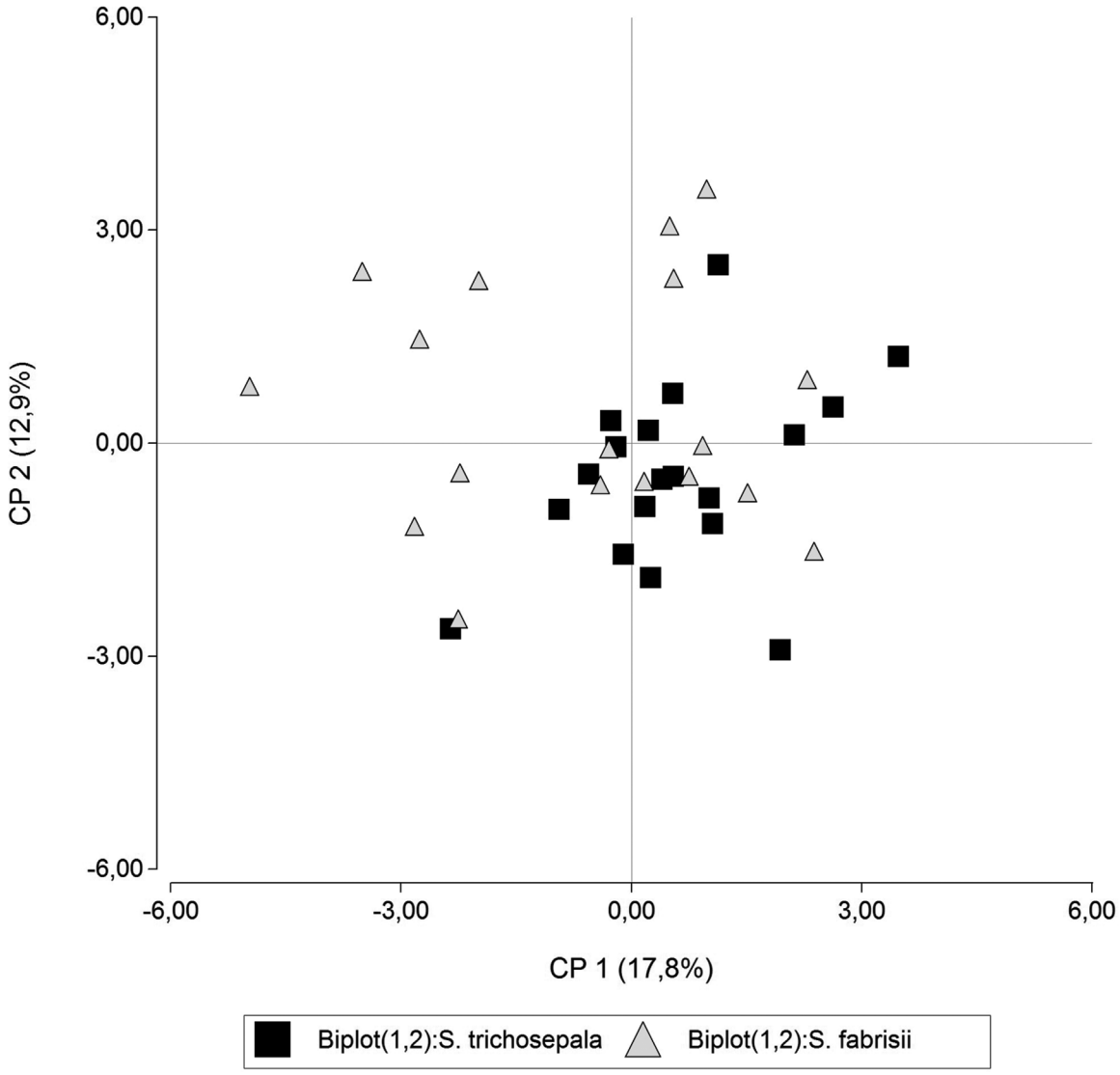


FIGURE 2. Principal component analysis (PCA) scatter plots of the first two components. The morphological characters used in this analysis are listed in Table 1.

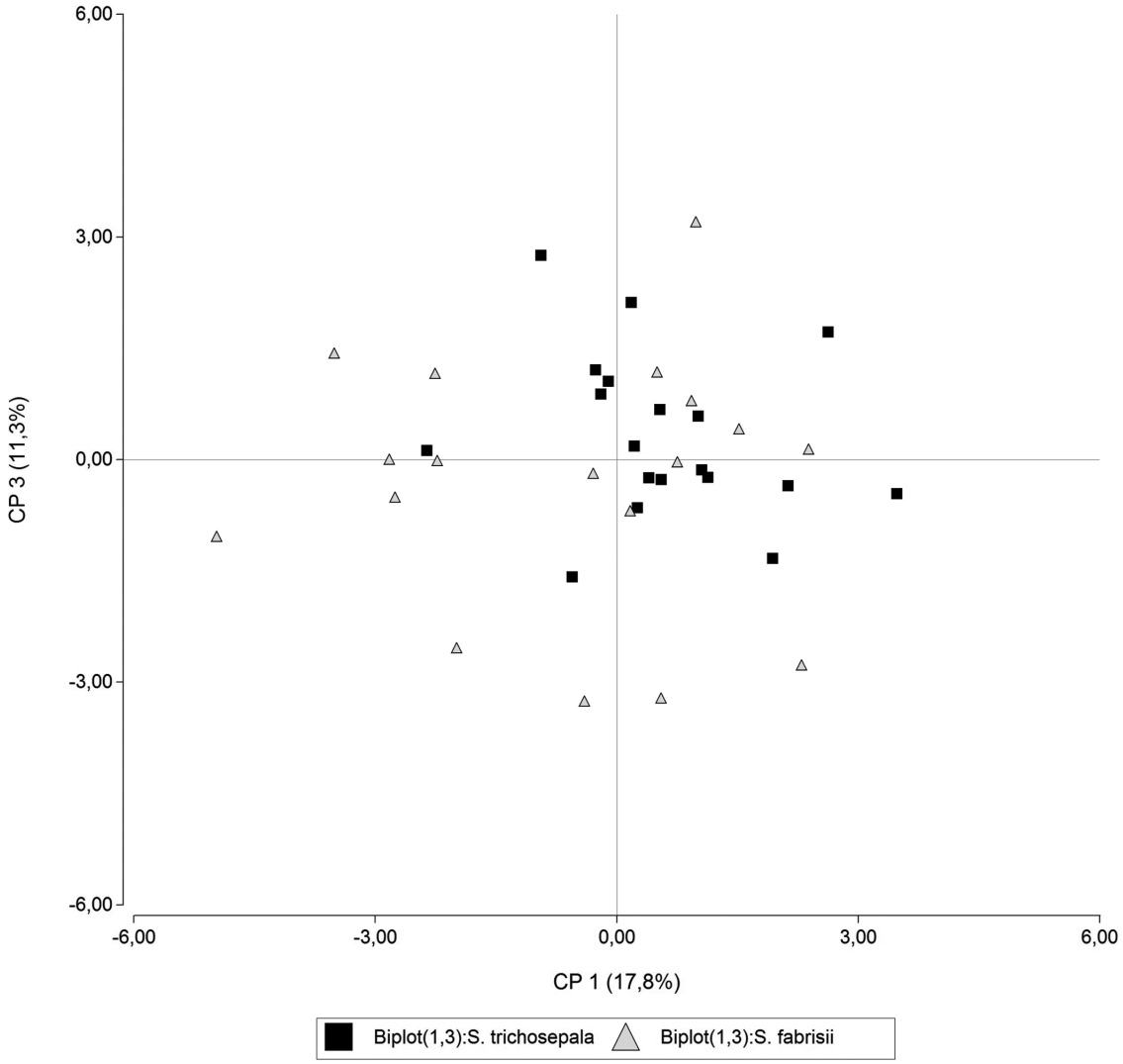


FIGURE 3. Scatter plots of the first and third components.

TABLE 3. Localities of the three populations used for the phenetic analysis bases on seed soluble protein profiles.

Code	Species	Collection number: BAB & CORD (herbarium acronym)	Locality
ST 1 Rodeo	S. trichosepala	Renée H. Fortunato 9934	ARGENTINA. San Juan: Iglesia, El Rodeo on road 150.
ST 2 Rodeo	S. trichosepala	Renée H. Fortunato 9934	ARGENTINA. San Juan: Iglesia, El Rodeo on road 150.
ST 3 Tucunuco	S. trichosepala	Renée H. Fortunato 9914	ARGENTINA. San Juan: Jáchal, 22 km southern Tucunuco, on road 40.
ST 4 Tucunuco	S. trichosepala	Renée H. Fortunato 9914	ARGENTINA. San Juan: Jáchal, 22 km southern Tucunuco on road 40.
ST 5 Tucunuco	S. trichosepala	Renée H. Fortunato 9914	ARGENTINA. San Juan: Jáchal, 22 km southern of Tucunuco on road 40.
SF 1Pismanta	S. fabrisii	Renée H. Fortunato 9939	ARGENTINA. San Juan: Iglesia: 2 km from Pismanta to Tucdum on road 150.
SF 2Pismanta	S. fabrisii	Renée H. Fortunato 9939	ARGENTINA. San Juan: Iglesia: 2 km from Pismanta to Tucdum on road 150.
SF 3Pismanta	S. fabrisii	Renée H. Fortunato 9939	ARGENTINA. San Juan: Iglesia: 2 km from Pismanta to Tucdumon road 150.

TABLE 4. Contributions of individual characters to the first three multivariate axes of the principal components analysis (PCA). Characters numbered according to Table 1.

Characters	PC1	PC2	PC3
1	0.2	0.33	0.32
2	0.32	0.24	-0.09
3	0.35	-0.08	-0.31
4	-0.21	-0.02	-0.11
5	-0.12	0.39	0.09
6	0.12	0.32	0.25
7	0.25	-0.17	-0.19
8	0.27	0.07	-0.29
9	0.13	-0.13	0.40
10	0.11	0.35	-0.01
11	0.20	-0.24	-0.11
12	0.33	-0.08	-0.17
13	-0.21	0.25	-0.34
14	0.22	0.18	0.30
15	0.29	0.05	-0.01
16	-0.27	0.28	-0.28
17	-0.20	-0.22	0.17
18	0.27	0.20	-0.02
19	-0.12	0.26	-0.28

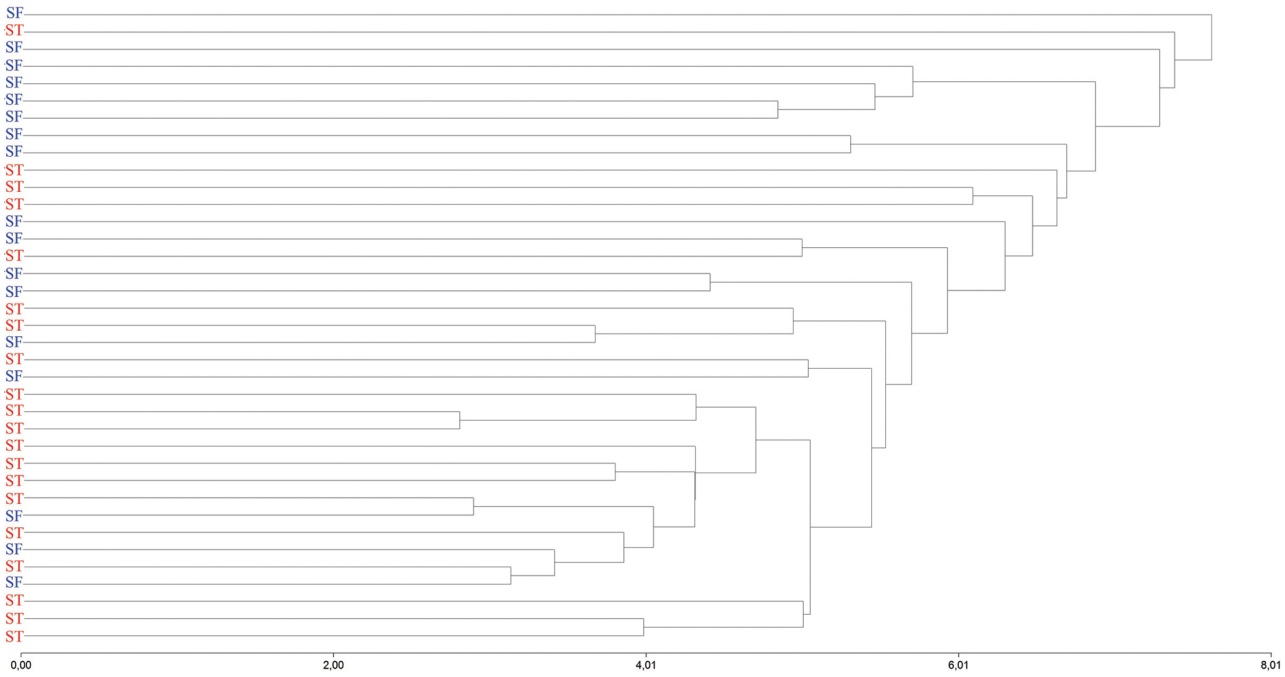


FIGURE 4. Dendrogram obtained from cluster analysis of the *S. fabrisi–S. trichosepala* complex using UPGMA based on morphological characters. (abbreviated as SF in blue (*S. fabrisii*) and ST in red (*S. trichosepala*).

Profiles of soluble proteins

The SDS-PAGE seed protein profiles of three samples of *S. fabrisii* from Pismanta and five samples of *S. trichosepala* from El Rodeo and Tucunuco are presented in Figure 5. The size of resolved polypeptides ranged between 200 and 5 kDa. Even though some differences in darkness and thickness of various bands were observed, *S. fabrisii* and *S. trichosepala* did not show significant qualitative variation in the SDS−PAGE profiles. All de samples show similarity in the distribution of the banding patterns, with bands that stand at 95, 65.7, 30, 19 and 5.7 kDa. The samples also have the same minor bands throughout the profile. The only distinctive feature of the seed protein banding profile was the 113 kDa band present in the samples of *S. trichosepala* from El Rodeo (indicated by a double-headed arrow in the figure).

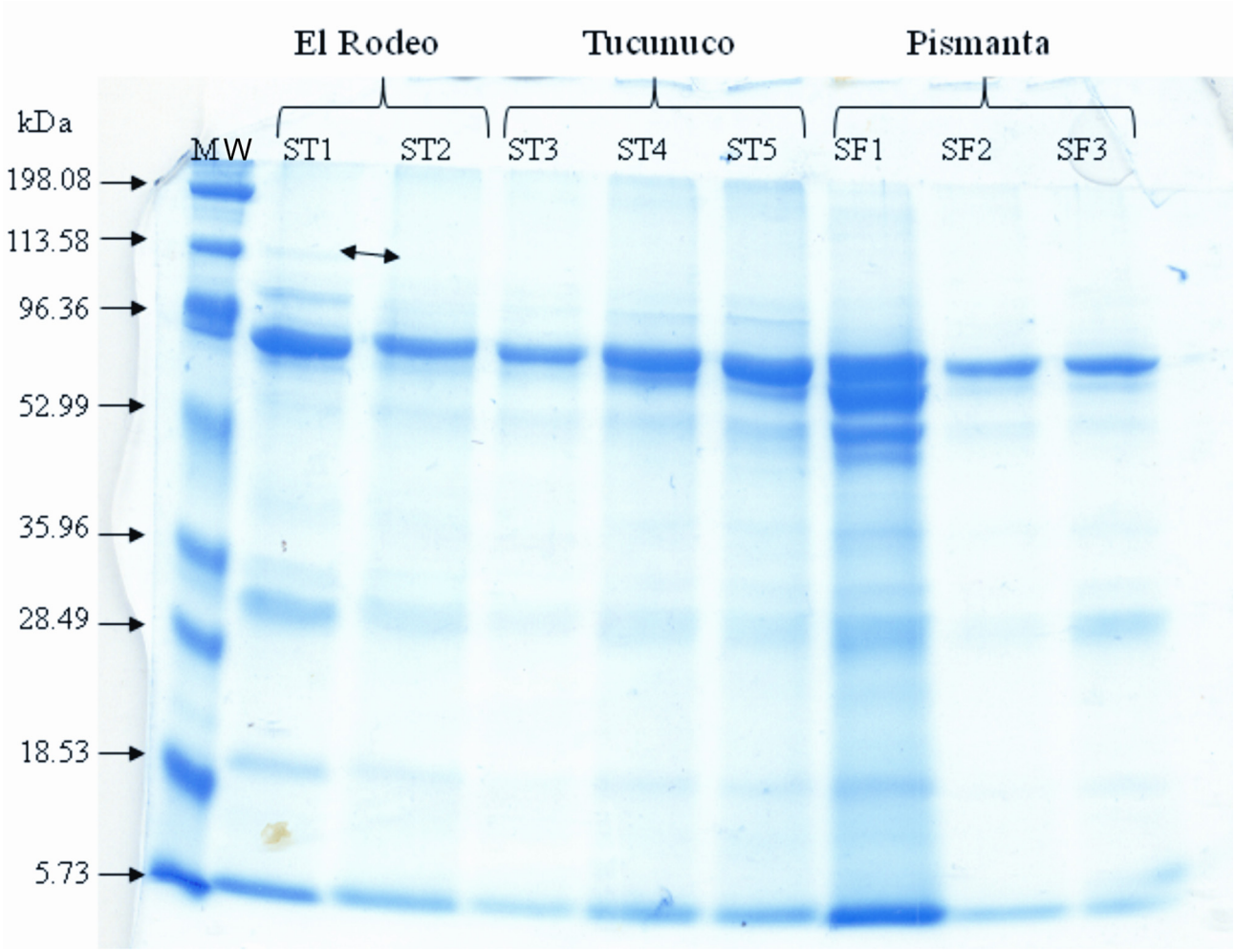


FIGURE 5. Gel showingthe SDS-PAGE seed protein profiles.

The dendrogram illustrating the relationships between the examined species, based on seed protein electrophoretic profiles are shown in Figure 6. The topology of this tree generally resembles that of the tree based on morphological characters (Fig. 4). The examined species were delimited in two clusters. The group A comprising the two samples of *S. trichosepala* from El Rodeo and the group B, including the samples of *S. fabrisii* from Pismanta and *S. trichosepala* from Tucunuco and the similarity value between the samples analyzed was 0.91.

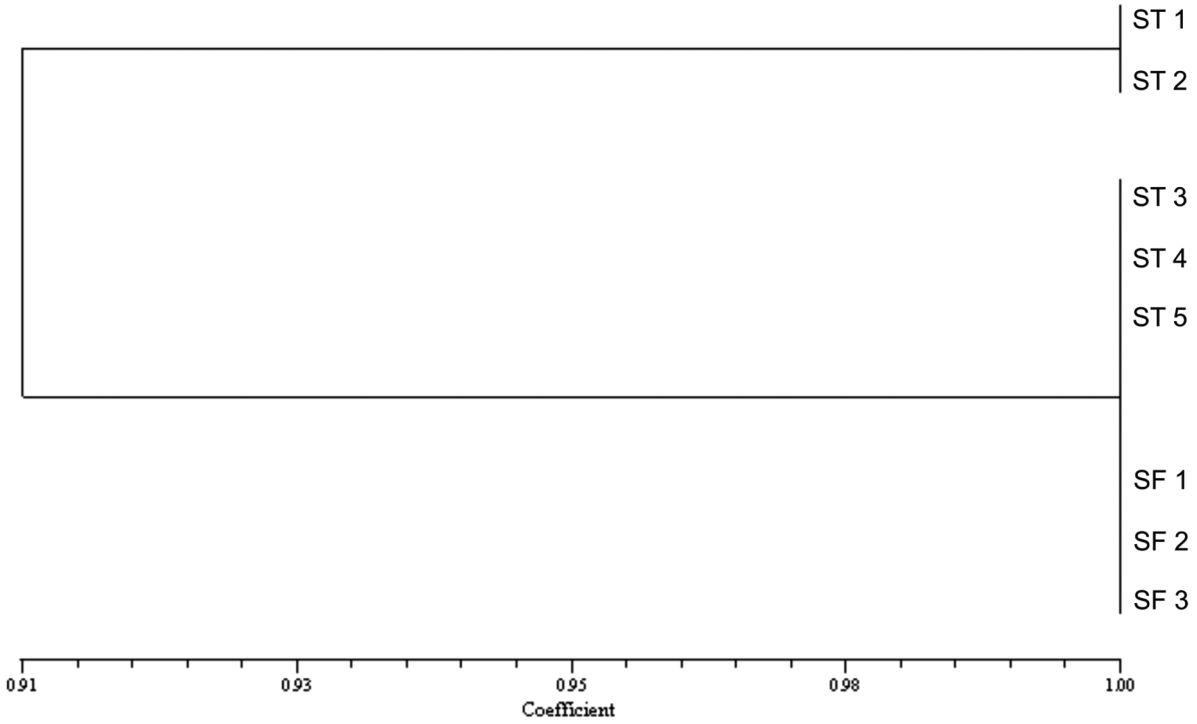


FIGURE 6. Dendrogram obtained from cluster analysis of the *S. fabrisii–S. trichosepala* complex using UPGMA based on soluble protein profile. Names of each OTU correspond to those recorded on herbarium sheets (abbreviated as SF (*S. fabrisii*) and ST (*S. trichosepala*) and the localities of occurrence of each taxon. The number attached with the abbreviations corresponds to the sample order.

Discussion

Among the characters analyzed only the ovary and peduncle pubescence and the internode width showed significant differences. The first two characters were used by Bravo (1978) in the separation of *S. fabrisii* and *S. trichosepala*. The present study showed that the variability of these characters within *S. fabrisii* is in agreement with the occurrence of intermediate individuals pointed out by Bravo (1978) and the intra-population variability observed by Robbiati *et al*. (2011). Therefore, these characters must be considered of no value for specific delimitation. The length of the anthers of the long abaxial stamens did not show significant differences between species, even though Bravo (1978) used this character to separate *S. fabrisii* from *S. trichosepala* (longer in *S. fabrisii* and shorter in *S. trichosepala*). The density of calyx pubescence also was used by Bravo (1978) to differentiate both species. However, the value of this feature is not supported by the result of our analyses.

The CA and PCA analyses based on morphological characters display no clear delimitation between *S. fabrisii* and *S. trichosepala*, showing that no characters were useful in the segregation of the two taxa.

The electrophoretic analysis showed a high degree of correspondence between the samples studied, with more than 90% similarity in total soluble seed protein profiles. Under these results, both taxa do not have differences, at least at protein biochemical level, to be considered as two different species (Bravo, 1978) and are in agreement with their sympatric distribution: center, southern and western Argentina (Fig. 7).

In view of the data analyses, we conclude that *S. fabrisii* and *S. trichosepala* do not possess any significant morphological differences due to the continuous variation in the diagnostic characters, which simply represent a pattern of the phenotypic variation. Moreover the high degree of similarity in seed protein profiles does not allow the recognition of two different species. Therefore Bravo's proposal (1978) cannot be maintained and a new synonym is required.

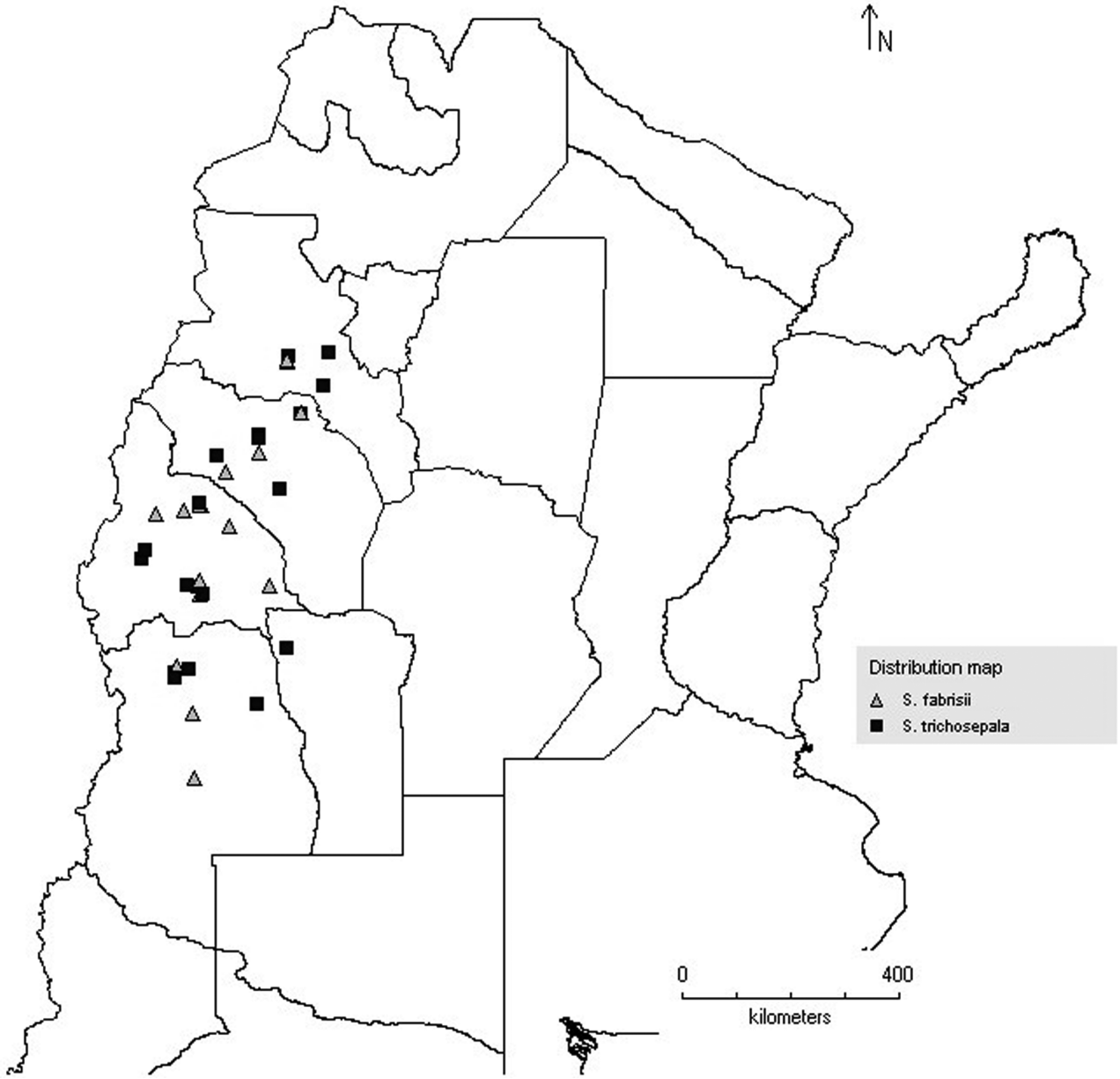


FIGURE 7. Distribution map of *S. fabrisii* and *S. trichosepala*.

Taxonomic treatment

Senna trichosepala (Chodat & Wilczek) H. S. Irwin & Barneby (1982: 570).

Cassia aphylla var. *trichosepala* Chodat & Wilczek (1902: 475). *Cassia trichosepala* (Chodat & Wilczek) Bravo (1978: 373). Type:—ARGENTINA. Mendoza: Dep. San Rafael, San Rafael, des les tables. Plantes des environs de St. Rafael et de la Vallée du Rio Atuel, *Wilczek 93* (holotype, US).

Cassia fabrisii Bravo (1978: 377). *Senna fabrisii* (Bravo) H. S. Irwin & Barneby (1982: 370). Type:—ARGENTINA. San Juan: Dep. Iglesia, 10km de Pismanta hacia Rodeo, *R. Palacios & L. Bravo 593* (holotype, SI!; isotype BAFC!), *syn. nov.*

Shrubs 0.8−3.0 m tall; branches glabrous to silky at the base, hairs to ± 5 mm long, erect-ascending, divergence angle 30º−70º; leaf 0.5−1.0 mm long, 0.5 mm wide. Bracts 1.0−1.5 mm long, 0.25−0.75 mm wide; peduncle 2−19 mm long, 0.25−0.75 mm wide; pedicel 4−16 mm long, 0.25−0.75 mm wide, both the

peduncle and pedicel both glabrous to densely pubescent. Calyx glabrous to densely pubescent, the 2 smaller sepals 4−7 mm long, 2−4 mm wide and the 3 larger sepals 6−8 mm long, 4−6 mm wide. Petals 7−22 mm long, 4−12 mm wide. Androecium: 3 anthers of long abaxial stamens 4.0−6.5 mm long, and filament 1.5−11 mm long; 4 anthers of median stamens 3−4 mm long, and filament 1−2 mm long and 3 staminodials 1−2 mm long, and filament 1−3 mm long. Gynoecium 10−12 mm long, 0.75−1.0 mm wide, glabrous to densely pubescent. Pods 7.3−12.2 mm long, 4.0−5.5 mm wide, glabrous to sparsely pubescent. Seeds 3.5−4.5 mm long, 3.0−3.5 mm wide.

Distribution:—Catamarca, La Rioja, Mendoza, San Juan and San Luis provinces in the central and western area of Argentina. Inhabiting rocky and sandy soils.

Additional specimens examined:—ARGENTINA. Catamarca: Belén, pendiente de Belén, entre Andalgaláy Belén, 24 January 2005, *Barboza 1146* (CORD); La Rioja: Arauco, en el monte, 22 March 1944, *Hunziker 5101* (CORD); Chilecito, 7 April 1906, *Stuckert 15742* (CORD); *Hunziker 4282* (CORD). Entre San Miguel y Malingasta, February 1962, *Dawson 3384* (CTES); Independencia, Cueva del Chacho, entre Patquía y Los Colorados, 30 November 1961, *Cocucci 367* (CORD); 30 km al noroeste de Patquía, 5 February 1973, *Hunziker 9508* (CORD); Mendoza: Las Heras, Salangasta, 21 December 1991, *Lutz 106* (CTES); Uspallata, 20 December 1949, *Barkley 815* (LIL); 22 km al sur de Tucunuco, 30º 51´S 68º 37´W, 7 March 2011, *Fortunato 9914* (BAB); 12 km al este de Rodeo, sobre ruta nacional 150, 30º 12´S 69º 02´W, 20 January1997, *Fortunato 5564* (BAB); 25 de Mayo, 12 km al este de Encon, sobre ruta nacional 20, 32º 12´S 67º 40´W, 6 March 2011, *Fortunato 9913* (BAB).

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Appendix 1. Vouchersand specimens examined.

S. fabrisii (L. Bravo) H. S. Irwin & Barneby: ARGENTINA. Catamarca: Capital, 1879, *Schickendantz 36* (CORD); Belén, 1879, *Schickendantz 155* (CORD). La Rioja: Chilecito, 10 January 1906, *Kurtz1 3110* (CORD); Felipe Varela, Pagancillo, sobre la base del Cerro Rojado, cerca de Ojo de Agua, 20 January 1906, *Kurtz 13224* (CORD); Mendoza: Capital, n. d., *Piraldez 83* (BAB). San Rafael, Cuesta de los Teneros, sobre ruta provincial 144, 1934, *Molfino 59898* (BAB). 26 January 1977, *Crespo 1858* (BAB); San Juan: Albardón, Bañados de Salado, alrededor de las dunas, 13 October 1998, *Biurrum 5407* (CTES); Capital, n.d., *Hosseus1117* (CORD); Puesto de Los Chávez, cerca de Marayes Viejo, 16 February 1921, *Hosseus 2633* (CORD);Jáchal, de Rodeo a Tucdum, 31 December 1929, *Pérez Moreau 30* (CTES); Pismanta, 15 December 1957, *RuizLeal 18984* (SI); 10 km al norte de la entrada de Huaco, 15 Junuary 1975, *Palacios & Bravo 606* (SI); 2 km al sur de la entrada de Huaco, 15 January 1976, *Palacios & Bravo 605* (SI);Iglesia, 2 km de Pismanta a Tucdumsobre ruta 150, 7 March 2011, *Fortunato 9939* (BAB, CORD); Valle Fértil, entre Baldecito y Ischigualasto, 27 December 1997, *Biurrum 5000* (CTES); La Rioja: Arauco, November 1994, *Mazan 5101* (CORD).

S. trichosepala (Chodat & Wilczek) H. S. Irwin & Barneby: ARGENTINA. Catamarca: Andalgalá, 6 December 1915, *Jörgensen 70515* (LIL); Belén, Río de Belén, November 1887, *Schickendantz 93* (CORD); Pomán, Salar de Pipanaco, ruta provincial 46 y ruta provincial 62, 1 October 1997, *Alayón 12* (CTES); La Rioja: Arauco, entre Mazán y Aimogasta, 8 December 1954, *Hayward 2555* (LIL); Felipe Varela, Trancas, 16 January 1906, *Kurtz 13180* (CORD); Famatina, 15 February 1908, *Kurtz 15264* (CORD); 9 January 1949, *Krapovickas & Hunziker 5074* (CORD); Independencia, Guayapas, 17 November 1963, *Hayward 3162* (LIL); Mendoza: Capital, alrededor de Mendoza, December 1885, *Kurtz 3625* (CORD); Luján de Cuyo, Potrerillos, 22 February 2008, *Aedo 15445* (BAB); La Paz, Desaguadero, 14 February 1944, *Semper 71655* (CORD); San Juan: Capital, n. d., *Hosseus 1242*, *1146* (CORD); Calingasta, 5 km al norte de Villa Nueva, 31° 03,03´S 69° 27,95´W, 11 November 1995, *Leuenberger 4460* (CORD); Camino de Villa Nueva a Los Colorados, 17 January 2009, *Chiapella 2314* (CORD); Ullún, Hualilán, 8 December 1979, *Cabrera 31062* (SI); Iglesia: 2 km de Pismanta a Tucdum sobre ruta 150, 7 March 2011, *Fortunato 9938* (BAB, CORD); San Luis: Belgrano, entre Sierras de las Quijadas y La Calera, 23 December 2003, *Prina 2143* (CTES).