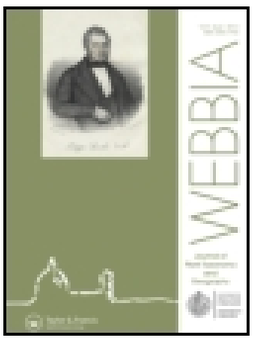

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Electrophoresis characterization of *Turnera sidoides* L. (Passifloraceae: Turneroideae) seed storage proteins and its systematic implications

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ABSTRACT

Twenty-four populations of *Turnera sidoides* were analysed, using seed storage protein fingerprinting techniques, including 19 populations of *Turnera* spp. and three of *Piriqueta* spp. for comparative purposes. The aim was to characterize the *T. sidoides* complex and to evaluate its profiles as a character to clarify its taxonomic position, as well as its evolutionary relationships within the genus *Turnera*. The present work is the first comparative study of the seed protein fingerprint in Turneroideae. The results proved that seed proteins are useful characters to discriminate between genus and species, as well as to characterize them. The finding of exclusive bands in *Turnera* and *Piriqueta* are evidence for the existence of genetic differences between genera, and support their taxonomic identity. Our results are in agreement with evolutionary tendencies of karyotype proposed for *Turnera*, and support the close relationships between species belonging to the same series, except *T. sidoides*, which should be singled out of *Leiocarpae*, supporting the proposal of its inclusion in an independent series.

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Evolutionary relationships; seed storage protein; sodium dodecyl sulphate–polyacrylamide gel electrophoresis; Turneroideae; *Turnera sidoides*

Introduction

Turneroideae (Pasifloraceae) comprises approximately 200 species distributed in 12 genera (Arbo 2007; Arbo and Espert 2009; Thulin et al. 2012): *Adenoa* Arbo, *Afroqueta* Thulin & Razafim, *Arboa* Thulin & Razafim, *Erblichia* Seeman, *Hyalocalyx* Rolfe, *Loewia* Urb., *Mathurina* Balf. f., *Piriqueta* Aubl., *Stapfiella* Gilg, *Streptopetalum* Hochst., *Tricliceras* Thonn. ex DC. and *Turnera* L. From these genera, seven are African, *Adenoa* is monotypic and endemic from Cuba; *Piriqueta* and *Turnera* represent 80% of the subfamily, and have representatives in America and África or Madagascar (Arbo 1977, 1979, 1987, 1995).

Turnera is one of the most important genera of Turneroideae in terms of specific abundance, with 141 species (Arbo et al. 2015) widely distributed in tropical and subtropical Americas, and with two African species (Arbo 1995, 1999). *Turnera* species are arranged into 11 series (Arbo 2008): *Annulares*, *Anomalae*, *Capitatae*, *Conciliatae*, *Leiocarpae*, *Microphyllae*, *Papilliferae*, *Salicifoliae*, *Sessilifoliae*, *Stenodictyae* and *Turnera*, which differ in the floral structure, the development and grade of adnation of the floral peduncle, the epicarp, and in seminal features such as shape, ornamentation, pubescence and development of chalaza.

Turnera sidoides L. is the only American species of the genus below 29°S. This complex of perennial rhizomatous herbs has a 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). The range of this species covers a wide diversity of habitats from mountain to sea level, and from open forests to grasslands (Arbo 1985, 2008; Solís Neffa 2000, 2010; Solís Neffa et al. 2004). Five subspecies have been recognized based on the variability of the leaf shape and the indumentum (Arbo 1985). Although this species was placed by Urban (1883) and Arbo (1985, 2008) in series *Leiocarpae*, it has morphological, anatomical and karyological features that are extremely different from those of the remaining species (Arbo 1985; González 2000; Solís Neffa and Fernández 2002). Moreover, some inconsistencies were detected in the position of *T. sidoides* in the phylogenies based on molecular (Truyens et al. 2005; Chafe 2009), morphological (Arbo and Espert 2009) and micromorphological (Arbo et al. 2015) data. Hence, the taxonomic position of *T. sidoides* in the genus as well as its evolutionary relationships still remain uncertain.

The banding patterns produced by seed storage protein electrophoresis provide taxonomically useful descriptors that are highly stable and free from environmental influence or seasonal fluctuations (Gepts 1990; Cooke 1995). They have been successfully used to clarify

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the taxonomy of species, genera and families (Johnson et al. 1967; Duvall and Biesboer 1989; Pasha and Sen 1991; Misset and Fontenelle 1992; Juan et al. 2007), and even of local varieties, as well as to analyse interspecific relationships (Ladizinsky and Hymowitz 1979; Panda et al. 1986; Ghafoor et al. 2002; Singh and Matta 2010, 2015; Swapan et al. 2015). Electrophoresis profiles of proteins were also used to analyse the genetic diversity within and among populations as well as in evolutionary studies of several plant groups (Burghardt et al. 2004: *Prosopis ferox* Griseb.; Sammour et al. 2007: *Lathyrus sativus* L.; Singh and Matta 2008, 2010, 2015: *Cucumis* L., *Citrullus lanatus* (Thunb.) Matsum. & Nakai, and *Cucurbita* L.; Kakaei and Kahrizi 2011: *Brassica napus* L.; Sinha et al. 2012: *Bauhinia* L.; Dudwadkar et al. 2015: Cucurbitaceae Juss.; Peddakasim et al. 2015: *Capsicum annuum* L.; Swapan et al. 2015: *Vigna* Savi, among others).

The aim of this study was to use seed storage protein fingerprints to characterize subspecies of *T. sidoides* and to evaluate its profiles as a chemical character to clarify the taxonomic position of this species complex, as well as its evolutionary relationships within the genus *Turnera*.

Material and methods

Material

Twenty-four populations of the five subspecies of *T. sidoides* were analysed. Also, and for comparative purposes, 19 populations belonging to nine species of *Turnera* and three species of *Piriqueta* were included in the analysis. Seed samples were obtained from natural populations as well as from living specimens growing in the greenhouse at the Instituto de Botánica del Nordeste (IBONE UNNE-CONICET, Corrientes, Argentina). Voucher specimens have been 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) (see Table 1 for details).

Protein profiling

Seed proteins (an average of 0.005 ± 0.001 g per seed and five seeds per population) were extracted in 60 µl of sample buffer of Tris–HCl (pH 6.8), 2% sodium dodecyl sulphate (SDS), 10% glycerol, 0.01% pyronine and 5% 2-mercaptoethanol). After shaking for 2 h at room temperature, the suspension was boiled for 2 min and centrifuged (5000 ***g***) for 5 min at room temperature. Total seed proteins were separated by the tricine–SDS–polyacrylamide gel electrophoresis (SDS–PAGE) method. The stacking gel (4%) and the separating gel (10%) were prepared according to Laemmli (1970). The buffer electrode used had 4% glycine, 3% Tris–HCl and 1% SDS on distilled water. The gels were loaded with 12 µl of the seed protein extracts (~0.001 g of seed weight). Electrophoresis was carried out at a constant 20 mA and free voltage for 3 h. Proteins were stained with a solution of 0.02% Coomassie Brilliant Blue R in 5% ethanol and 6% trichloroacetic acid for 24 h. Gels were stored in 20% glycerol solution after destaining in 10% acetic acid (Chrambach et al. 1967; Khan and Rubin 1975). SIGMA MW-SDS-70L was used as standards for electrophoresis.

The molecular weights of the dissociated polypeptides were determined based on the relative mobility (Rf) and those corresponding to marker with known molecular weights, i.e. bovine plasma albumin (66 kDa), chicken ovalbumin (45 kDa), rabbit glyceraldehyde-3-phosphate dehydrogenase (36 kDa), bovine carbonic anhydrase (29 kDa), bovine trypsinogen (24 kDa), trypsin inhibitor (20.1 kDa) and bovine α-lactalbumin (14.2 kDa). The relative mobility (Rf) of each observed band was estimated considering the relationship between the distance of migration of proteins from the seeding point and the distance of migration of the front marker run (Bromophenol Blue). A linear regression curve was generated from Rf values (x-axis) against the log of the known molecular weights (y-axis) of the marker. The curve obtained was used to calculate the molecular weights of the samples (Weber and Osborn 1969).

Data analyses

Selected protein bands were used for the construction of a distance matrix. Each protein band was considered as a qualitative character and coded as presence (1) or absence (0). The Jaccard's index (Jaccard 1908) was used to estimate similarity among the species. A dendrogram was produced from the similarity matrix using the unweighted pair group method with arithmetic average (UPGMA) using Statistix v.1.0 software.

Results

The SDS–PAGE profile of the *Turnera* and *Piriqueta* seed proteins studied showed 33 polypeptide bands. Their molecular masses ranged from 34.97 to 16.59 kDa. Two regions could be distinguished in gel slabs, each possessing a characteristic arrangement and number of bands (Figure 1).

Differences in the number and arrangement of bands were found among genera and species (Table 2). The largest number of bands (eight) was observed in *Piriqueta rosea* and *T. sidoides*, whereas the smaller (five) were found in *Turnera melochioides*, *Turnera diffusa* and *Turnera hermanioides*. In addition to the common bands between studied taxa, there were observed genus exclusive bands. In *Turnera*, 19 exclusive bands were observed and in *Piriqueta* seven were seen. At the same time, most species also presented exclusive bands, *T. sidoides* being the species with the greatest number (four: 34.97 D, 28.64 D, 22.38 D and 22.31 D). *Piriqueta viscosa* var. *viscosa* and *T. melochioides* were the only ones that did

Table 1. Details of studied taxa.

Taxa	Collector & collection number	Locality
Piriqueta		
P. grandifolia (Urb.) Arbo	Solís Neffa et al. 1558.	BOLIVIA. SANTA CRUZ: VELAZCO.
	Solís Neffa et al. 1987.	ARGENTINA. SALTA: MURILLO.
P. rosea (Cambess.) Urban	Demmateis 2907.	PARAGUAY.
P. viscosa Grises. v. viscosa	Solís Neffa et al. 1569.	BOLIVIA. SANTA CRUZ: VELAZCO.
	Solís Neffa et al. 1763.	BOLIVIA. SANTA CRUZ: VELAZCO.
Turnera		
Serie Leiocarpae		
T. hassleriana Urb.	Solís Neffa et al. 1912.	BOLIVIA. SANTA CRUZ: CHIQUITOS.
	Solís Neffa et al. 1246.	BOLIVIA. SANTA CRUZ: VELAZCO.
T. melochioides Cambess.	LP de Queiroz 13,612.	BRASIL. BAHÍA: MUCUGÉ.
T. pumilea L.	Solís Neffa et al. 1532.	BOLIVIA. SANTA CRUZ: ÑUFLO DE CHÁVEZ.
	Solís Neffa et al. 1692.	BOLIVIA. SANTA CRUZ: SANDOVAL.
T. sidoides subsp. *carnea* (Cambess.) Arbo	Solís Neffa et al.278.	URUGUAY. CERRO LARGO.
	Solís Neffa & Seijo 2091.	URUGUAY. COLONIA.
	Solís Neffa & Seijo 2010.	URUGUAY. SALTO.
	Solís Neffa & Seijo 2105.	URUGUAY. SAN JOSÉ.
T. sidoides subsp. *holosericea* (Urb.) Arbo	Solís Neffa & Seijo 2073.	URUGUAY. TACUAREMBÓ.
T. sidoides subsp. *pinnatifida* (Juss. ex Poir.) Arbo	Aguirre 454.	ARGENTINA. LA RIOJA.
	Krapovickas & Cristóbal 46,241.	ARGENTINA, TUCUMÁN: TRANCAS.
	Solís Neffa et al. 1025.	BOLIVIA. TARIJA: GRAN CHACO.
	Solís Neffa et al. 1505.	BOLIVIA. TARIJA: GRAN CHACO.
	Solís Neffa et. al 1511.	BOLIVIA. TARIJA: GRAN CHACO.
	Solís Neffa &2096.	URUGUAY. COLONIA.
	Solís Neffa & 2097.	URUGUAY. COLONIA.
	Solís Neffa & Seijo 2115.	URUGUAY. COLONIA.
	Solís Neffa & Seijo 2116.	URUGUAY. COLONIA.
	Solís Neffa & Seijo 2118.	URUGUAY. COLONIA.
	Solís Neffa et al. 2139.	URUGUAY. RÍO NEGRO.
	Solís Neffa & Seijo 2009.	URUGUAY. SALTO.
	Solís Neffa & Seijo 2134.	URUGUAY. SORIANO.
T. sidoides subsp. *sidoides*	Solís Neffa 407.	URUGUAY. MALDONADO.
	Solís Neffa & Speranza 2147.	URUGUAY. CANELONES.
	Solís Neffa & Speranza 2148.	URUGUAY. LAVALLEJA.
	Solís Neffa & Speranza 2150.	URUGUAY. LAVALLEJA.
	Solís Neffa & Speranza 2160.	URUGUAY. MALDONADO.
	Solís Neffa & Speranza 2180.	URUGUAY. ROCHA.
Serie Microphylae		
T. difusa Willd.	LP de Queiroz 13,678.	BRASIL. BAHÍA.
Serie Turnera		
Subserie Umbilicatae		
T. hermannioides Cambess.	LP de Queiroz 13,614.	BRASIL. BAHÍA, MUCUGÉ.
Subserie Turnera		
T. concinna Arbo	Solís Neffa et al. 1586.	BOLIVIA. SANTA CRUZ: SANDOVAL.
	Demmateis 2928.	PARAGUAY.
T. grandiflora (Urb.) Arbo	Solís Neffa et al. 1644.	BOLIVIA. SANTA CRUZ: SANDOVAL.
T. krapovickasii Arbo	Solís Neffa et al. 1975.	ARGENTINA. SALTA.
	Solís Neffa et al. 1973.	BOLIVIA. TARIJA: CORDILLERA.
T. orientalis (Urb.) Arbo	Solís Neffa et al. 1521.	BOLIVIA. BENI, TRINIDAD.
	Solís Neffa et al. 1541.	BOLIVIA. SANTA CRUZ: ÑUFLO DE CHÁVEZ.

not present exclusive bands. At an intraspecific level, most species had identical electrophoretic patterns both among and within populations; except for *T. sidoides* (populations Solís Neffa 2009, 2091, 2096, 2115, 2116, 2118, 2134, 2139, 2148, 2150, and 2160) and *Turnera concinna* that exhibit some polymorphism within populations. All subspecies of *T. sidoides* had similar electrophoretic patterns.

The results of Jaccard's similarity coefficient revealed coefficients between 0.57 and 0.00 (Table 3). The greatest similarity was observed between *Turnera hassleriana* and *T. melochioides*, and between *T. melochioides* and *Turnera pumilea*, whereas the smallest similarity was observed between *T. hermanioides* and *Piriqueta rosea*, and between *T. hermanioides* and *P. viscosa*. The UPGMA divided the species into two major clusters (Figure 2). The first cluster (group I) included all species of *Piriqueta*, whereas group II included all species of *Turnera*. Within group II, two subgroups (III and IV) were distinguished. Subgroup III was divided into three additional groups (IIIa, IIIb, IIIc). Group IIIa included *T. diffusa* (*Mycrophyllae* series) and the species belonging to *Leiocarpae* series, with the exception of *T. sidoides*, which formed an independent cluster (IIIc). Group IIIb included two species of series *Turnera*: *T. hermanioides* (subserie *Umbilicatae*) and *Turnera grandiflora* (subserie *Turnera*). The last group (group IV) comprised the remaining species from series *Turnera* (subseries *Turnera*).

Discussion

The present work is the first compared study of the seed protein fingerprint in Turneroideae. The results proved

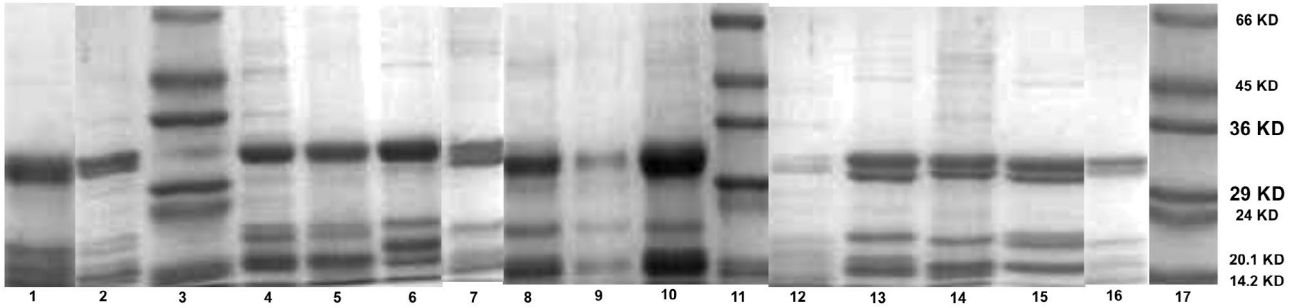

Figure 1. Lane 1, *Piriqueta grandiflora*. Lane 2, *Piriqueta viscosa*. Lane 3, standard molecular weights (MW in kilodaltons). Lane 4, *Turnera melochiodes*. Lane 5, *Turnera diffusa*. Lane 6, *Turnera pumilea*. Lane 7, *Turnera hassleriana*.Lanes 8–10, *Turnera sidoides*. Lane 11, MW. Lane 12, *Turnera hermannioides*. Lane 13, *Turnera grandiflora*. Lane 14, *Turnera krapovickasii*. Lane 15, *Turnera concinna*. Lane 16, *Turnera orientalis*. Lane 17, MW.

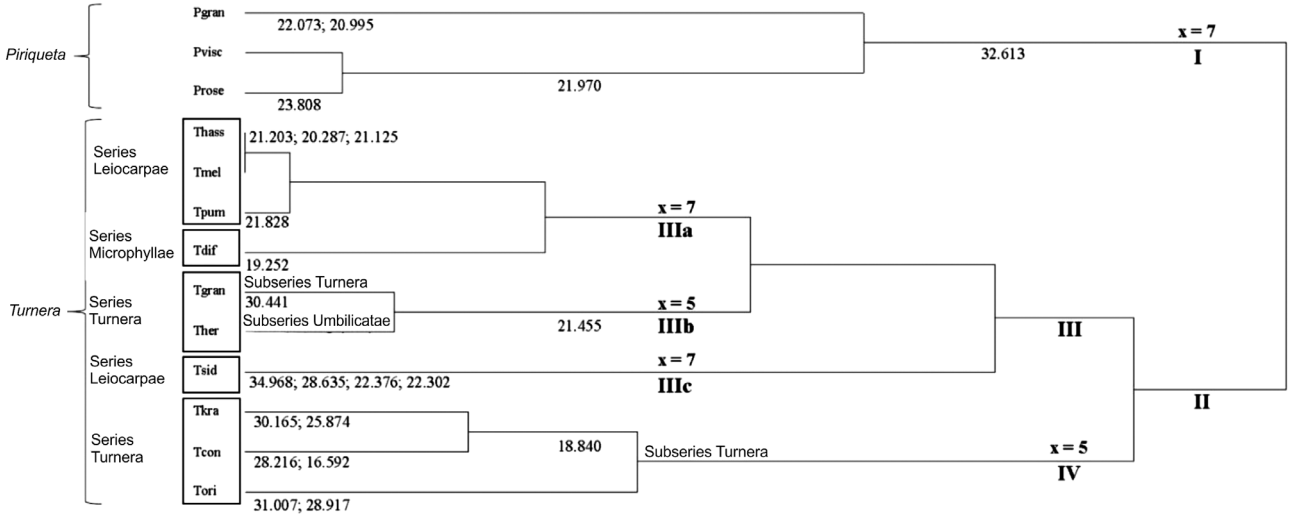

Figure 2. UPGMA dendrogram based on the patterns of seed storage protein of *Turnera* and *Piriqueta*. Pgran = *Piriqueta grandifolia*, Pvisc = *Piriqueta viscosa* v. *viscosa*, Prose = *Piriqueta rosea*, Thass = *Turnera hassleriana*, Tmel = *Turnera melochioides*, Tpum = *Turnera pumilea*, Tdif = *Turnera diffusa*, Tgran = *Turnera grandiflora*, Ther = *Turnera hermanioides*, Tsid = *Turnera sidoides*, Tkra = *Turnera krapovickasii*, Tcon = *Turnera concinna*, Tori = *Turnera orientalis*. Roman numerals indicate the main groups identified. The numbers correspond to the molecular weight of bands that characterized each species and/or species group. x=basic chromosome number.

Table 2. Occurrence of seed protein fractions in species of *Piriqueta* and *Turnera*.

PM(KD)	34.97	32.70	32.61	31.20	31.00	30.81	30.44	30.16	28.92	28.64	28.22	25.87	23.81	22.80	22.38	22.30
Piriqueta																
P. grandifolia	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0
P. rosea	0	1	1	1	0	0	0	0	0	0	0	0	1	1	0	0
P. viscosa v. viscosa	0	1	1	1	0	0	0	0	0	0	0	0	0	1	0	0
Turnera																
T. hassleriana	0	1	0	0	0	1	0	0	0	0	0	0	0	1	0	0
T. melochioides	0	1	0	0	0	1	0	0	0	0	0	0	0	1	0	0
T. pumilea	0	1	0	0	0	1	0	0	0	0	0	0	0	1	0	0
T. sidoides	1	1	0	0	0	1	0	0	0	1	0	0	0	0	1	1
T. diffusa	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0
T. hermanioides	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
T. grandiflora	0	1	0	0	0	1	1	0	0	0	0	0	0	0	0	0
T. krapovickasii	0	1	0	0	0	0	0	1	0	0	0	1	0	1	0	0
T. concinna	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0
T. orientalis	0	1	0	0	1	0	0	0	1	0	0	0	0	0	0	0

PM (KD)	22.07	21.97	21.83	21.45	21.20	21.12	20.99	20.70	20.29	19.90	19.25	19.00	18.84	18.40	18.00	16.90	16.59
Piriqueta																	
P. grandifolia	1	0	0	0	0	0	1	0	0	1	0	0	0	1	0	0	0
P. rosea	0	1	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0
P. viscosa v. viscosa	0	1	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0
Turnera																	
T. hassleriana	0	0	0	0	0	1	0	0	0	1	0	0	0	1	0	0	0
T. melochioides	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0
T. pumilea	0	0	1	0	0	0	0	0	0	1	0	0	0	0	1	0	0
T. sidoides	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0
T. diffusa	0	1	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0
T. hermanioides	0	0	0	1	0	0	0	0	0	1	0	0	0	0	1	1	0
T. grandiflora	0	0	0	1	0	0	0	1	0	0	0	0	0	0	1	1	0
T. krapovickasii	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	1	0
T. concinna	0	0	0	0	0	0	0	1	0	0	0	0	1	0	1	1	1
T. orientalis	0	0	0	0	0	0	0	1	0	0	0	0	0	1	1	1	0

Table 3. Matrix with Jaccard's similitude indexes among the taxa analysed.

	Species	1	2	3	4	5	6	7	8	9	10	11	12	13
1	*P. grandifolia*	1.000												
2	*P. rosea*	0.250	1.000											
3	*P. viscosa* var. viscosa	0.214	0.500	1.000										
4	*T. hassleriana*	0.188	0.167	0.182	1.000									
5	*T. melochioides*	0.125	0.182	0.333	0.571	1.000								
6	*T. pumilea*	0.111	0.154	0.200	0.5	0.571	1.000							
7	*T. sidoides*	0.071	0.067	0.167	0.167	0.182	0.273	1.000						
8	*T. diffusa*	0.250	0.182	0.200	0.375	0.429	0.375	0.182	1.000					
9	*T. hermanioides*	0.111	0.000	0.000	0.222	0.250	0.375	0.182	0.25	1.000				
10	*T. grandiflora*	0.091	0.071	0.167	0.182	0.333	0.300	0.250	0.200	0.500	1.000			
11	*T. krapovickasii*	0.077	0.154	0.25	0.182	0.333	0.182	0.071	0.091	0.091	0.273	1.000		
12	*T. concinna*	0.077	0.071	0.167	0.083	0.571	0.182	0.154	0.091	0.200	0.400	0.400	1.000	
13	*T. orientalis*	0.071	0.071	0.167	0.182	0.200	0.182	0.154	0.091	0.091	0.300	0.273	0.400	1.000

that this technique constitutes a valuable tool for the evaluation of taxonomic and evolutionary relations in this subfamily.

Seed storage protein profiling has been used in taxonomic studies and to analyse phylogenetic relationships among taxa (Singh and Matta 2010, 2015; Peddakasim et al. 2015), expecting that those more taxonomically related specimens show more similar electrophoretic patterns than those that are less related. Likewise, qualitative differences in electrophoretic patterns show the variability in protein structure due to genetic variability between taxa (Shechter and de Wet 1975). Our results showed that genera and species of Turneroideae are well defined based on the seed protein profile. The finding of exclusive bands in *Turnera* and *Piriqueta*, as well as the inclusion of species of both genera in different clusters, show the existence of genetic differences between genera, and support their taxonomic identity.

In *Turnera*, in agreement with morphological and molecular data (Truyens et al. 2005; Arbo and Espert 2009; Arbo et al. 2015), the dendrogram obtained from the seed protein profile grouped the species according to the infrageneric classification. An exception is the case of the *T. sidoides* complex, which, although it is included in series *Leiocarpae* (Urban 1883; Arbo 1985, 1987, 2008), forms an independent cluster from the remaining species of the series, as well as from species of other series. Unlike the other species of Leiocarpae, *T. sidoides* is one of the few species without foliar nectaries and is the only one with granulose fruits. It has a floral peduncle in basal flowers almost free or linked to the petiole and the pedicel is usually developed (Arbo 2008). Furthermore, its seed morphology differs from the remaining species of *Turnera* because, instead of being reticulate like those of the other species of the series, or striate as in the species of other series, it presents irregular crests arranged in lines (Arbo 1985; González 2000; Arbo et al. 2015). Moreover, in the phylogenetic analysis based on both morphological (Arbo and Espert 2009) and molecular (Truyens et al. 2005; Chafe 2009) data, the subspecies of *T. sidoides* formed a separate basal clade from Leiocarpae species. However, when seed micromorphology was included in the cladistics analyses *T. sidoides* fitted in a clade with species of series *Leiocarpae* and *Sessilifoliae*, and it is in a rather derived position (Arbo et al. 2015). The results obtained here support the position of *T. sidoides* on an independent series.

Turnera sidoides is also one of the species of the genus with the greatest geographic distribution and it presents great morphological variability (Arbo 1985; Solís Neffa 2010). Nevertheless, no large variations in the electrophoretic profile of seminal proteins between subspecies were detected, supporting their taxonomic identity and their inclusion as the same species.

In addition, the results obtained allow the characterization of the species from series *Turnera*. This series has the most complex and advanced floral structure in the family, and characterized by having the floral peduncle adnate to petiole and solitary epiphyllous flowers (Arbo and Espert 2009). It has 22 species, which are divided into two subseries, *Umbilicatae* and *Turnera*, based on their seminal characters. Additionally, from a cytogenetic analysis of interspecific hybrids, it has been suggested that species from series *Umbilicatae* are genetically isolated from species of series *Turnera* (Arbo and Fernández 1987) and that, in this latter, species with white/blue flowers are genetically isolated from those with yellow flowers, as no viable hybrids at diploid level were formed. On the basis of this background, species with white/blue flowers were assigned to genome 'C', and species of yellow flowers to genome 'A' (Fernández and Arbo 1989, 1990, 1993a, 1993b, 1996). These results were later sported by a phylogeny obtained from internal transcribed spacer sequences (Truyens et al. 2005), in which species with white/blue flowers formed a separate clade from those with yellow flowers. Our results showed that species from series *Turnera* are clearly differentiated into two groups taking into account the protein profile. One group includes one species from subseries *Turnera* with white/blue flowers (*T. grandiflora*) and *T. hermanioides* from subseries *Umbilicatae*. The other group included only species from subseries *Turnera* with yellow flowers.

Our results are also in agreement with the evolutionary tendencies of karyotypes proposed for *Turnera*. This genus is the most cytologically studied of Turneroideae. Karyological information is available for six of the eleven

series (Raman and Kesavan 1964; Hamel 1965; Barrett 1978; Barrett and Shore 1980; Arbo and Fernández 1983; Fernández 1987; Solís Neffa and Fernández 1993, 2001, 2002; Solís Neffa 1996). The basic number $x = 7$ is the most frequent, and it was found in series *Salicifolieae*, *Stenodictyae*, *Microphyllae* and *Leiocarpae*. The number $x = 13$ was found in series *Papilliferae* and $x = 5$ in series *Turnera*. Polyploidy had played an important role in the evolution of *Turnera* species and different ploidy levels, from 2× to 10×, were detected (Raman and Kesavan 1964; Barrett 1978; Arbo and Fernández 1983; Shore and Barrett 1985; Fernández 1987). The species with $x = 5$ and $x = 13$ were suggested to be derived from $x = 7$ species (Fernández 1987). Diploid changes would have been involved in the origin of $x = 5$; whereas basic number $x = 13$ would have resulted from the reduction in the chromosomal number by aneuploidy from a species with $2n = 4x = 28$ (Solís Neffa and Fernández 1993, 2000). On the dendrogram based on protein profiling, the species with $x = 7$, which have smaller chromosomes than species with $x = 5$ and $x = 13$, are included in the same cluster, excepting *T. sidoides*. From the cytological point of view, although *T. sidoides* shares the basic number, $x = 7$, with the other species of the series, its mean chromosome length is in accordance with the values observed among the species of series *Turnera* ($x = 5$). In addition, *T. sidoides* is the only species of the series that possesses a subtelocentric pair in its karyotype and shows an intermediate degree of karyotype asymmetry between species of the *Leiocarpae* and *Turnera* series (Solís Neffa and Fernández 1993, 2002). In agreement with cytological data, our results showed that *T. sidoides* forms an independent subgroup from species with $x = 7$.

Regarding species with x=5, it was possible to clearly distinguish two groups according to their protein profiles. One group included *T. grandiflora* (subseries *Turnera*) and *T. hermanioides* (subseries *Umbilicatae*), that despite being reproductively isolated, the great similarity in the protein profile detected between these species is in agreement with their karyotype features (Solís Neffa 1996). The other group included *T. concinna*, *T. krapovickasii* and *T. orientalis*. *Turnera concinna* is diploid, *T. krapovickasii* has diploid and autopolyploid cytotypes; whereas *T. orientalis* is allohexaploid (Fernández 1987; Lazaroff et al. 2016). Fernández and Arbo (1989) suggested that *T. concinna* would have derived from *T. krapovickasii*. Both species have a great similarity in their karyotypes (Solís Neffa and Fernández 1993). On the other hand, *T. concinna* is one of the putative parents of *T. orientalis*. The inclusion of these species in the same group, based on their protein profiles, supports this hypothesis.

In conclusion, seed proteins are useful characters to discriminate between genus and species. Our results also show the close relationships between species belonging to the same series, except *T. sidoides*, which should be singled out from the *Leiocarpae*. The profiles obtained provided valuable information that contributes to clarify the evolutionary and taxonomical relationships of *T. sidoides* complex in the genus *Turnera*.

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No potential conflict of interest was reported by the authors.

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