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Stemodia diplohyptoides (Plantaginaceae, Gratiolae): a new diploid species from South America

MARÍA DE LAS MERCEDES SOSA & MASSIMILIANO DEMATTEIS
Instituto de Botánica del Nordeste (UNNE-CONICET), Casilla de Correo 209, W3400CBL Corrientes, Argentina. FACENA- UNNE, Av. Libertad 5460, 3400, Corrientes, Argentina.
E-mail: mdlmsvg@yahoo.com.ar; mdematteis@agr.unne.edu.ar

Abstract

Stemodia diplohyptoides (Plantaginaceae), a new species from Gratiolae tribe is described and illustrated. This taxon is found in northeastern Argentina, at the edge of the Parana River that surrounds the north and center of Misiones province (Argentina). It is a diploid that has been traditionally treated under *Stemodia hyptoides*, which is here re-circumscribed to include only autopolyploid plants (tetraploid and hexaploid). The diploid specimens may be distinguished from the polyploid *S. hyptoides* by several morphological features such as shape, pubescence and margin of the leaves, corolla length and corolla tube pubescence. In addition, scanning electron microphotographs of seeds, chromosomes numbers, a distribution map and a key to distinguish the related species are also provided. On the basis of morphological, cytological and geographical data, some evolutionary considerations are inferred.

Key words: Autopolyploid, Plantaginaceae, speciation, *Stemodia*

Introduction

The genus *Stemodia* Linnaeus (1759: 1118) comprises about fifty-six species distributed in America, Africa, Asia and Australia (Turner & Cowan 1993, 1994, Rahmanzadeh *et al.* 2005, Souza & Giulietti 2009). Thirty species have been recognized in the Americas, twenty of them growing in South America. In Argentina, nine species are distributed from the north to south of the Patagonia area, in Rio Negro province (Sosa 2012; Sosa & Dematteis 2013). *Stemodia* species are annual or perennial herbs, shrublets or small scrambling shrubs up to 3 m high with opposite subpinnately or pinnately veined leaves; flowers axillary, arranged along a terminal spiciform inflorescence; calyx with equal sepals, almost separate at the base, linear-lanceolate. The corollas are mostly lavender or purple-colored, zygomorphic, having well developed tube, anther thecae glabrous, divergent, with swollen connectives, or shortly stalked, styles 2–4 times longer than the stigmatic area; stigmatic area enlarged and usually curved, bifid; capsule mostly loculicidally 4-valvate, ovoid to orbicular; seeds, ellipsoid to subpyramidal (Turner & Cowan 1994).

Turner & Cowan (1994) indicated that some species restricted to the southern extreme part of South America exhibit a great morphological variation; this variability has been observed in the field and in some cases makes the correct identification difficult. Particular cases are *S. stricta* Chamisso & Schlechtendal (1828: 10), *S. hyptoides* Chamisso & Schlechtendal (1828: 8) and *S. lanceolata* Bentham (1846: 384) which are partially sympatric species distributed in northern Argentina. Moreover, the reports of different ploidy levels within some *Stemodia* species (Sosa & Seijo 2002, Sosa *et al.* 2009, 2011) suggest that the morphological variation found between and within species could be, at least in part, the result of the polyploidization process.

Stemodia hyptoides is currently recognized as a single taxonomic entity having diploid (2n=22), tetraploid (2n=44) and hexaploid (2n=66) populations (Sosa & Seijo, 2002; Sosa *et al.* 2009).

In many plants, examples of multiple cytotypes within a species have been shown to represent autopolyploids (or are presumed autopolyploids). However, only rarely an autopolyploid has been formally named and considered to represent a different species from its diploid progenitor (Judd *et al.* 2007). According to Soltis *et al.* (2007), it is clear that some cytotypes represent distinct evolutionary lineages that should be formally recognized as distinct species, providing names for them. In *Stemodia hyptoides*, the diploid and polyploid populations were considered up to the moment as a single taxonomic species.

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According to a previous analysis (Sosa *et al.* 2012), diploid populations differ considerably from polyploid populations. The original description of *Stemodia hyptoides* and their synonyms agree with the polyploid individuals, but the diploid plants remain unnamed. Therefore, they are described here as a different species, *Stemodia diplohyptoides*, the name following the convention outlined by Soltis et al. (2007) and previously used for diploid *Zea diploperennis* Iltis, Doebley & R. Guzmán (1979:186) and *Tolmiea diplomenziessi* Judd, Soltis & P.S. Soltis (2007:218). Furthermore, we provide scanning electron microphotographs of seeds, a distribution map and a key to distinguish the new from related species.

Materials and methods

This study was based on the morphological analysis of the type specimens and additional material deposited at BA, BAA, BAB, BM, CTES, G, G-BOIS, K, LIL and SI.

For morphological study, seeds were obtained from herbarium specimens deposited at CTES. An average of 10 mature seeds per sample were analyzed. The seeds were re-hydrated in boiling water and then analyzed under a Leica MZ6 stereoscopic microscope. The analyzed parameters were length, width and shape of the seed. The studied material was subjected to an increasing number of acetone and dried at critical point for obtaining photomicrography with a scanning electron microscope JEOL 5800 LV, which make possible to analyze the structure and sculpture of the seed coat the shape of the cells and the characteristics of the anticlinal and periclinal walls in surface view. The terminology used follows Barthlott (1981).

Material Examined: *Stemodia diplohyptoides*: ARGENTINA. Prov. Misiones: Eldorado, Puerto Eldorado, 20 November 2003, *Sosa & Keller 121* (CTES); San Martín, Puerto Rico. 12 April 2006, *Sosa & Rodríguez 251* (CTES). ***Stemodia hyptoides***: ARGENTINA. Prov. Corrientes: Saladas, San Lorenzo, 6 May 2001, *Sosa 45* (CTES); San Miguel, San Miguel, 5 March 2005, *Sosa 134* (CTES).

To examine mitotic chromosomes, living material was collected in pots for growing under greenhouse conditions. Root-tips were pre-treated with 8-hydroxyquinoline 0.002 M for about 3 h at room temperature, then fixed in 5:1 ethanol: lactic acid (Fernández, 1973) and kept in 70% ethanol at 4º C until analysis. Roots were stained following the Feulgen´s technique and meristems were macerated and squashed in a drop of 3 % acetic orcein. Permanent slides were prepared using Euparal as mounting media.

Material Examined: *Stemodia diplohyptoides:* ARGENTINA. Prov. Misiones: Parque Nacional Iguazú, 14 December 2004. *M. M. Sosa, R. O. Vanni & M. G. Lopez 123* (CTES). ***Stemodia hyptoides*:** ARGENTINA. Prov. Corrientes: San Roque, 29 April 2010. *M. M. Sosa 272* (CTES).

Results

Stemodia diplohyptoides Sosa & Dematteis, *spec. nov.* Figs. 1, 3, 4.

Stemodia hyptoides affinis sed numero chromosomatico diploideo, 2n=22 (non tetraploideo, 2n=44), foliis obovatis, 23–65 x 8–35 mm (non oblanceolatis, 40—90 x 10–45 mm), caulibus minoribus, corolla 8–10 mm longa (non 7.5 mm) differt.

Type:—ARGENTINA. Misiones: Parque Nacional Iguazú, 14 December 2004; *M. M. Sosa, R. O. Vanni & M. G. López 123* (holotype CTES, isotype MICH, CANB).

Perennial herbs, procumbent with ascending stems, 50–180 cm high. Stem 4-ribbed, 1.5–4 mm diameter at base, several axillary branches, basal internodes 14–45 mm long, with glandular and eglandular trichomes. Leaves opposite, narrowly elliptical, 20–60 x 8–35 mm, sessile, attenuate at base, basally clasping to auriculate, margin serrate, barely pubescent. Flowers arranged in bi-bracteate spiciform inflorescence, shortly pedicellate, pedicels 1 mm long, bracteole 5 mm. Sepals 5 all alike, 5 to 6 mm long, linear-lanceolate, glandular-pubescent. Corolla tubular of 8–10 mm long, inferior lip trilobate, pubescent in the throat with long hairs within, superior lip emarginate. Stamens 4, with short filaments (1.2–2 mm), and 2 long (4 mm); anthers separated by enlarged connective. Ovary ovoid, style straight, 4 mm, stigma laterally broadened. Capsule ovoid, 3–4 mm, 4-valvate. Seeds oblong-ovoid to ellipsoid with rounded margins, 340 x 150 µm, dark brown; exotesta reticulate with cells generally isodiametrical, hexagonal, anticlinal and periclinals

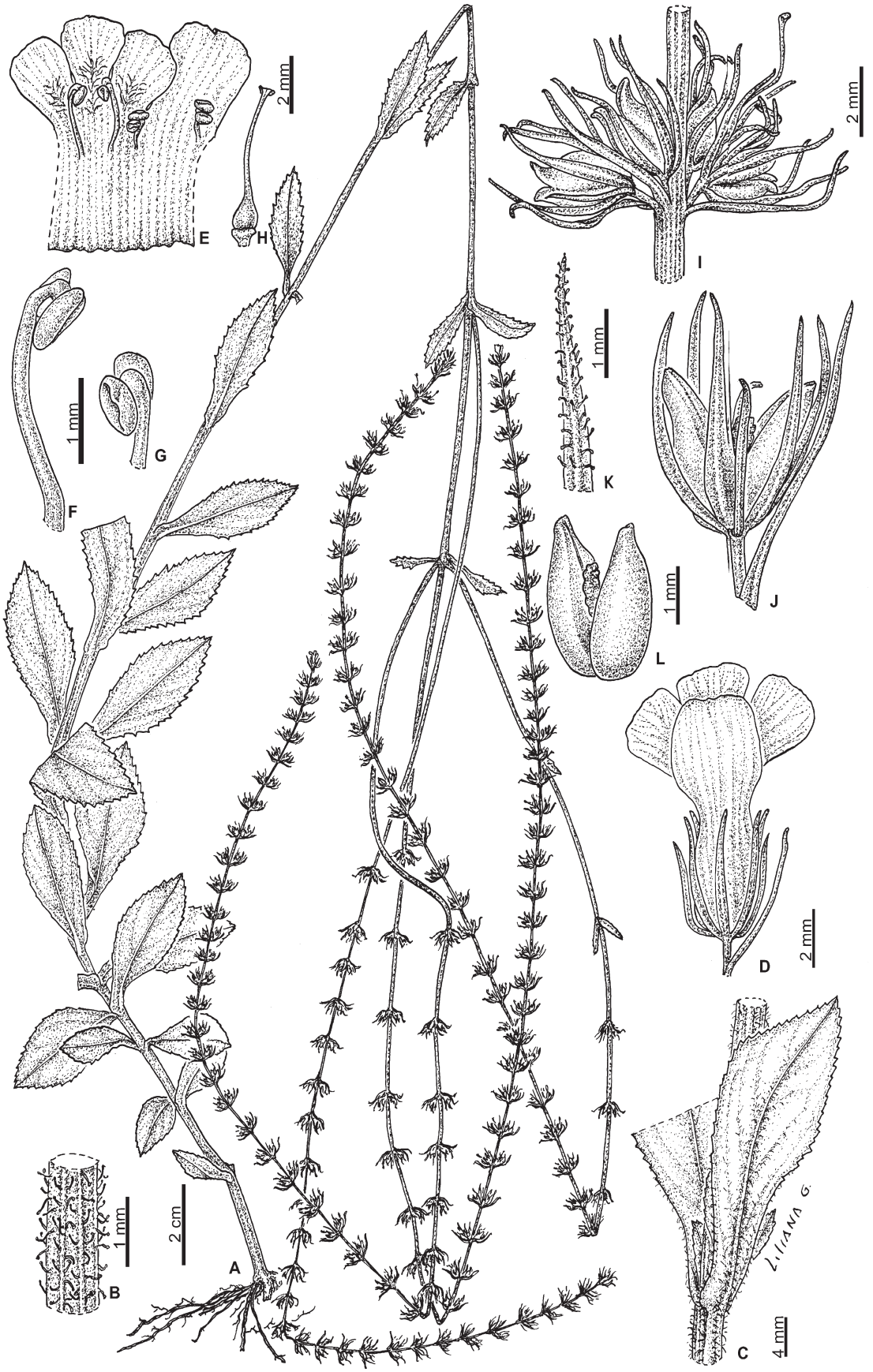

FIGURE 1. *Stemodia diplohyptoides*. A. Habit. B. Stem detail. C Detail of a stem. D. Flower. E. Corolla (inner view). F. Long stamen. G. Short stamen. H. Detail of ovary. I. Node with fruits. J. Fruit. K. Detail of sepal. L. Detail of fruit (A-L: Sosa *et al.* 123 CTES, illustrated by Mirtha Gómez).

FIGURE 2. Geographic distribution of *Stemodia diplohyptoides* (stars), *S. stricta* (circles) and *S. hyptoides* (squares) in southern South America.

walls straight, raised and thickened, with protuberances in the angles; secondary sculpture (fine relief of the cell wall) of micro-papillae (= "verrucose"). Ovules 98–133 µm long, stomata anomocytic of ± 30 µm long and with anticlinal walls of the upper epidermis of the leaf sinuate. 2n=22.

Distribution and ecology:—*Stemodia diplohyptoides* has been found in the northeastern extreme of Argentina,

specifically in the north and center of Misiones province (Fig. 2). This species grows in marginal vegetation of Parana River, on sandbar next to the river, near streams, with modified soils. Following field observations, we hypothesize that *S. diplohyptoides* is adapted to ecological conditions that are different from the typical habitat of the other members of *Stemodia*, which prefer humid soils. Some specimens grow over rocky soils with permanent water, such as the terrace of the San Martin Island that receives water from the Iguazú falls. Other specimens were collected on rocky basalt and were found to have horizontal stems and rootlets at the nodes. We interpret the development of adventitious roots as adaptations to a soil poor in nutrients exposed to for leaching.

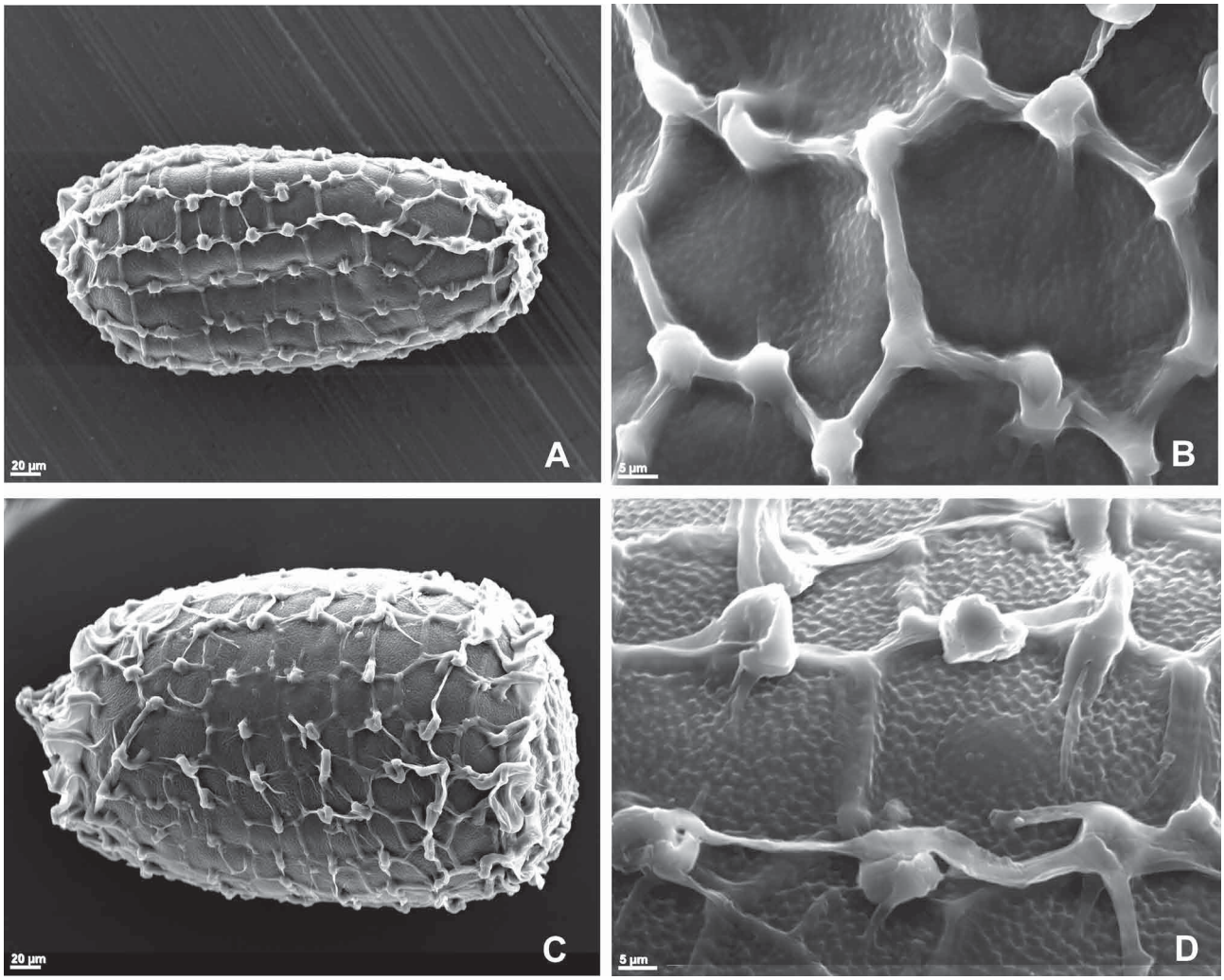

FIGURE 3. Seed morphology observations under SEM of *Stemodia.* A, B. Seed and detail of ornamentation on the seed surface of *S. diplohyptoides.* C, D. Seed and detail of ornamentation on the seed surface of *S. hyptoides.* (A-B: Sosa & Keller 121 CTES; C-D, Sosa 134 CTES).

Phenology:—flowering August to December.

Additional specimens examined: ARGENTINA. Prov. Misiones, Eldorado, Pto. Eldorado, 17 October 1977, *Cabrera et al. 28805* (CTES; SI); 8 September 1985, *Múlgura et al. 496* (BAB); Eldorado, Puerto Eldorado, 11 October 1999, *Keller 79* (CTES); 1 Km al S del puerto, 20 November 2003, *Sosa & Keller 121* (CTES); *Sosa & Keller 124* (CTES); 11 April 2006, *Sosa & Rodriguez 248* (CTES); Parque Schwelm, Balcón de las Guayabas, 26 March 2006, *Sosa & Rodriguez 247* (CTES); Iguazú, P. N. Iguazú, en cantera vieja, 14 February 2004, *Sosa et al. 131* (CTES), Isla San Martín, 8 August 1991, *Vanni et al.* 2827 (CTES); 31 March 1995, *Vanni et al. 3372* (CTES); 14 February 2004, *Sosa et al.* 133; 8 April 2008, *Múlgura de Romero et al. 4429* (CTES, SI); San Martín, Puerto Rico, 12 April 2006, *Sosa & Rodríguez 251* (CTES); Montecarlo, Puerto Piray, 12 April 2006, *Sosa & Rodríguez 249* (CTES); 13 November 1996, *Vanni et al. 3832* (CTES); Puerto Montecarlo, 17 August 1997, *Vanni et al. 4067* (CTES); 12 April 2006, *Sosa & Rodríguez 250* (CTES).

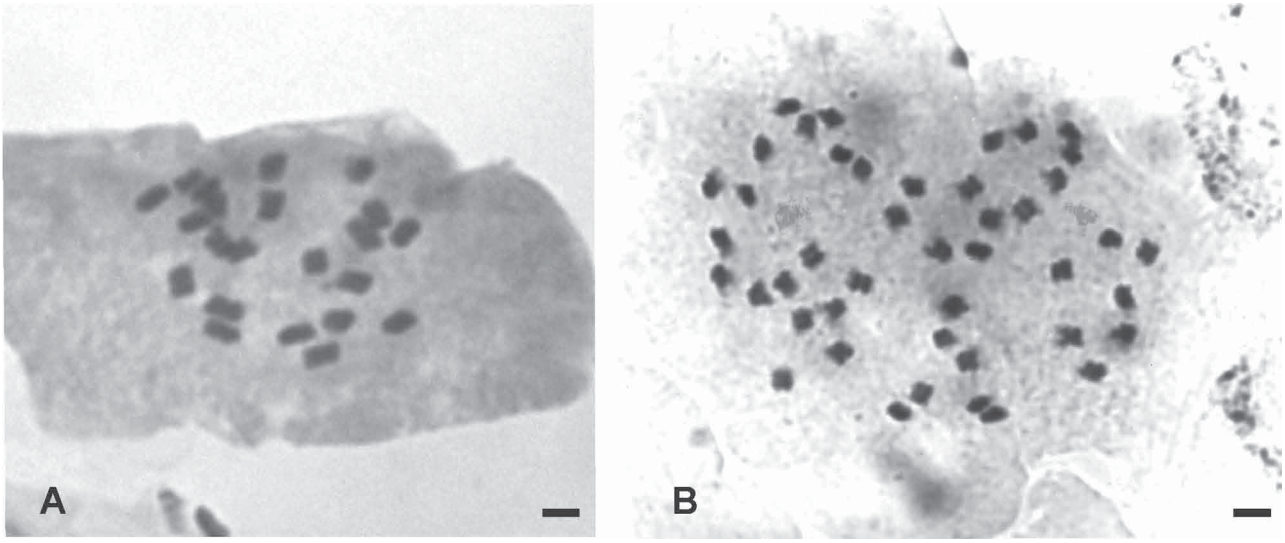

FIGURE 4. Mitotic chromosomes in species of *Stemodia*. A. *S. diplohyptoides,* 2n=2x=22. B. *S. hyptoides*, 2n=4x=44. Bar= 2 µm. (A, Sosa *et al.* 123 CTES; B, Sosa 272 CTES).

Key to *S. diplohyptoides* and related species from southern South America

1. Corolla 12-16 mm long. Floral bracts mostly up to 2 times as long as the subtended flowers. Leaf blades lanceolate ..... ……. ……. ..... ……*S. lanceolata*
- Corolla 5-10 mm long. Floral bracts shorter than the subtended flowers. Leaf blades obovate to oblanceolate. 2
2. Herbs annual, 10-50 cm; leaves broad obovate, base clasping and somewhat flanged, leaf margin less serrate (≤ 20 teeth on each of side)……. ..... …….*S. stricta*
- Herbs perennial 50-200 cm; leaves narrow obovate to oblanceolate, base slightly auriculate; leaf margin more serrate (≥ 20 teeth on each of side)……..……. 3
3. Leaves 23–65 x 8–35 mm, narrowly obovate, barely pubescent. Corolla 8–10 mm long, ovules 98–133 µm long ..... ……..…. ..... ……..….*S. diplohyptoides*
- Leaves 40–90 x 10–45 mm, oblanceolate, notably hirsute pubescent. Corolla 7.5 mm long, ovules 107–151 µm long ..... ……. ..... ……. *S. hyptoides*

Discussion

We confirmed that the *Stemodia diplohyptoides* is diploid (2n=22), as reported in Sosa *et al.* (2009), while the related *S. hyptoides* has polyploid cytotypes with 2n=44 and 2n=66 (Sosa & Seijo, 2002, Sosa *et al.* 2009). The same chromosome number (2n=2x=22) is found in other related species of *Stemodia* such as *S. stricta* or *S. lanceolata* (Sosa *et al.* 2009), and as in those the chromosomes are small (1.7 µm).

In addition to morphological features, diploid and polyploid plants have quite different geographic distributions (Sosa *et al.* 2009). *Stemodia hyptoides* has a wide distribution throughout the east of Espinal and Chaco phytogeographic provinces, the northeast of the Pampean province of the Chaco domain and in southwest of the Paranaense province of the Amazonian phytogeographic domain (Cabrera 1971; Cabrera & Willink 1973). The polyploids are distributed throughout the area of distribution; whereas diploids were found only toward the northeast of the species range (in the Paranaense phytogeographical province). This pattern of cytotype distribution is characteristic of mature polyploid complexes (Stebbins 1971), where the geographic ranges of diploid cytotypes are more restricted. However, *S. diplohyptoides* is apparently reproductively isolated from *S. hyptoides* because triploid individuals have not yet been observed. Reproductive isolation is reinforced by the essentially allopatric ranges of the two species (Sosa *et al.* 2009). The populations of *S. diplohyptoides* could be considered a different evolutionary lineage which is clear from the lack of hybrid individuals.

Thieret (1954) described five types of seeds for the tribe Gratiolae. Ichaso (1978) reported new types of seeds as a granulate type that is found in *S. lanceolata*, *S. maritima* Linneus (1759:1118) and *S. stricta*. Also, this author included other *Stemodia* species within the reticulate-Bacopa type proposed by Thieret (1954), but they removed the generic epithet because this type included also other genera. According to Ichaso (1978), the tribe Gratiolae presents

a great variation of types, with the reticulate type predominant, and within the tribe, *Stemodia* was the single genus showing three types of seeds: granulate, reticulate and longitudinal-furrowed. The morphological analysis of the seeds of *S. diplohyptoides* and *S. hyptoides* is presented for the first time. These species have small seeds, all less than 1 mm long, very numerous (between 300 and 350 per capsule), with cells more or less irregularly reticulate. The shape of the seeds can also be ellipsoid to ovoid, with reticulate exotesta and salient edges. *Stemodia diplohyptoides* and *S. hyptoides* contain reticulate seeds, which is the most frequent type in Gratiolae tribe and the genus *Stemodia* (Ichaso, 1978). However, these species closely differ from related taxa such as *S. stricta* and *S. lanceolata*, and according to Ichaso (1978) contain granulate seeds.

The most significant morphological features that separate *Stemodia hyptoides* from the new species are leaf size, shape and pubescence; corolla length and pubescence. We consider that the characters given above, when used in conjunction with geographical range result in satisfactory identification of specimens. The morphological features of the polyploid specimens are subsumed under those of the type of *S. hyptoides*. However, the diploid specimens differ in several characters of the type material, and consequently should be considered as a different species.

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