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A new polyploid species of *Mecardonia* (Gratioleae, Plantaginaceae) from South America

JULIÁN A. GREPPI[1], MARÍA DE LAS MERCEDES SOSA[2,3*] & MASSIMILIANO DEMATTEIS[2,3]

[1]*Instituto de Floricultura, Instituto Nacional de Tecnología Agropecuaria, De los Reseros y Las Cabañas s/nº, Hurlingham 1686, Buenos Aires. Argentina.*

[2]*Instituto de Botánica del Nordeste (UNNE-CONICET), Casilla de Correo 209, W3400CBL Corrientes, Argentina.*

[3]*Facultad de Ciencias Exactas y Naturales y Agrimensura, Universidad Nacional del Nordeste, Av. Libertad 5460, 3400, Corrientes, Argentina.*

**Author for correspondence. E-mail: mdlmsvg@yahoo.com.ar*

Abstract

Mecardonia reneeae, a new species from northeastern Argentina, is described and illustrated. This taxon has certain resemblance to *M. procumbens* var. *flagellaris*, but can be easily distinguished by the combination of life form, aspect of the plant, root and stem type, sepal width, fruit size, and chromosome number (2n=4x=44). A key to distinguish it from related species is also provided.

Introduction

Mecardonia Ruiz & Pavon (1794: 95) is a New World genus of the tribe Gratioleae (Plantaginaceae). It is characterized by annual or perennial herbs with erect or creeping habit, densely branched, mostly glabrous, glandular-punctate leaves, pedicels basally bibracteolate, yellow and white flowers, and anther thecae separated by an arm-like connective tissue (D'Arcy 1979). In the review by Rossow (1987), 10 species of *Mecardonia* distributed from the east coast of the United States to Argentinian Patagonia and Central Chile were considered. This number of species was modified by Souza (1997) and Souza & Giulietti (2009), who suggested seven species and established the center of diversification including Rio Grande do Sul (Brazil), Uruguay and northeastern Argentina. Recently, another species from Argentina was described: *M. kamogawae* Greppi & Hagiwara (2011: 44). However further studies are needed to clearly delimit the *M. procumbens* complex.

The chromosome number of *Mecardonia* species revealed that the genus has diploid and polyploid taxa (Sosa *et al*. 2016). *Mecardonia grandiflora* (Bentham 1836: 56) Pennell (1946: 87), *M. procumbens* (Miller 1768: 6) Small (1903: 1338) var. *procumbens* and *M. procumbens* var. *flagellaris* (Chamisso & Schlechtendal 1827: 575) Souza (1997: 186) are diploid (2n=22), whereas *M. kamogawae* is hexaploid (2n=66). Polyploidization has been recognized as an important process in plant evolution (Stebbins 1971, Grant 1989, Otto & Whitton 2000, Soltis *et al.* 2004). Polyploidy is a common phenomenon present in genera of Plantaginaceae, such as *Antirrhinum* Linnaeus (1753: 612), *Cymbalaria* Hill (1756: 113), *Chelone* Linnaeus (1753: 611), *Digitalis* Linnaeus (1753: 621), *Linaria* Miller (1754: 2), *Plantago* Linnaeus (1753: 112), *Stemodia* Linnaeus (1759: 1118), and *Veronica* Linnaeus (1753: 9) (Hair 1966, Subramanian & Pondmudi 1987, Sosa & Seijo 2002, Sosa *et al.* 2009, 2011, Wolfe *et al*. 2002, Vargas *et al*. 2004, Murray *et al*. 2010, Castro *et al.* 2012, Wong & Murray 2012, Ranjbar & Nouri 2015). Previous studies show that in *Stemodia*, diploids and polyploids are quite different in morphological and anatomical features (Sosa *et al.* 2012, Sosa & Dematteis 2014).

The results of a comparative morphological and cytological analysis in *Mecardonia*, allowed us to identify *Mecardonia reneeae* as a new species, which is here described. Additionally, an identification key including morphologically similar species is provided.

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Materials and methods

Field study and plant collection

Material of the new species was collected in the field; part of this material was prepared for herbarium collection, whereas some fruit and flower samples were stored for cytological and micromorphological studies. In addition, some plants were grown in greenhouses. Herbarium material is deposited at BAB, SI.

Cytological studies

Mitotic chromosome preparations were made from root meristems. The roots were pretreated in 0.002 M 8—hydroxyquinoline solution at room temperature for about 3 h, fixed in 5:1 absolute alcohol/lactic acid (Fernández 1973) and kept in 70% ethanol at 4º C until analysis. Roots tips were stained following Feulgen´s technique, and meristems were macerated and squashed in a drop of lactopropionic orcein (Dyer 1963). Permanent microscope slides were prepared in Euparal using the method of Bowen (1956) and examined and photographed under a Carl Zeiss microscope equipped with a Canon Power Shot A 640 digital camera.

Taxonomic treatment

Mecardonia reneeae Greppi & Sosa, *sp. nov.* (Figs. 1–2, Table 1)

Type:—ARGENTINA. Corrientes. Dep. Empedrado, Ruta Provincial 6 y arroyo Empedrado [27° 48'S, 58°30'W], 20 December 2005. *Greppi & Hagiwara 560* (holotype BAB!, isotype SI!).

Diagnosis:—*A Mecardonia procumbens var. flagellaris similis, sed habitu terophyto, caulibus erectis, radicibus gemmiferis, foliis serratis, petalis angustis, deltoideis et linearibus, fructus minore, chromosomatum numero tetraploideo 2n=44 (contra 2n=22).*

Terophyte, 15–17(–20) cm high. Roots gemiferous. Stems erect, quadrangular, glabrous, 1 mm diam., internodes 5–20 mm long. Leaves opposite, sessile, narrowly elliptic, 6–16(–20) × 2–5(–6) mm, margin serrate, base attenuate. Bracteoles 3–7 × 0.5–1.5 mm, linear, margin entire to denticulate. Pedicels 10–35(–40) mm long, erect, flexuous. Flowers one or two per node, 10–16 mm long, yellow with chestnut streaks in the throat. Calyx 5–7 mm long; sepals narrowly deltoid to linear, acute, dorsal sepal 5–7(–10) × 1–1.2 mm; two ventral sepals 5.5–7(–8) × 0.8–1(–3) mm; two lateral sepals 4–6(–8) × 0.3–0.6 mm. Corolla 8–14 × 6–8 mm, posterior lip 6–8 × 6–7 mm, entire, briefly apiculate, internally pubescent at base; middle lobe of frontal lip 6–7 × 7–8.5 mm; broadly obovate, glabrous; lateral lobes of equal size, sometimes obovate. Stamens 4, anterior filaments 5 mm long, posterior 4 mm long, all inserted at the base and reaching half of the corolla tube length. Ovary ellipsoid, 3 mm long; style 3–4 mm long. Capsule ellipsoid, light brown, 3–5 × 1.5–1.8 mm. Seeds, 0.4–0.5 × 0.3–0.4 mm, rough, blackish.

Distribution, habitat and phenology:—*Mecardonia reneeae* grows in open and humid areas, low clayey loam soils near watercourses, or border of streams in eastern Formosa, Chaco, northwest of Corrientes and northeast of Santa Fe in Argentina, within the Chaco phytogeographic province (Cabrera 1976). The species flowers from August to March.

***Etymology*:—**The specific epithet is dedicated to Renée H. Fortunato, Argentinean botanist dedicated to the study of Leguminosae.

Additional specimens examined:—ARGENTINA. Prov. Chaco: Dep. Bermejo, Las Palmas, 14 November 1983, *Fortunato et al. 607* (BAB); Dep. Primero de Mayo, Colonia Benítez, 10 June 1959, *Schulz 10437* (BAB). Prov. Corrientes: Dep. Capital, near to Ingenio Primer Correntino, 18 Km NE of Corrientes, 15 September 1982, *Schinini & Martínez-Crovetto 2636* (CTES); 5 Km E of Laguna Brava, 29 August 1970, *Krapovickas & Cristóbal 15836* (CTES); 10 Km E of Laguna Brava, route 5, 21 August 1970, *Krapovickas & Cristóbal 16099* (CTES); Parques de la Facultad de Ciencias Agrarias, 23 August 1974, *Quarín 2403* (CTES); Molina Punta, November 1978, *Arbo 1562* (CTES); Perichón, 8 September 1975, *Schinini 11601* (CTES); Dep. San Cosme, Paso de la Patria, 12 August 1967, *Krapovickas & Cristóbal 1302* (CTES); Desvío a Pto. González, 9 Km. near to Paso de la Patria, 10 September 1971, *Tressens et al. 168* (CTES); Dep. San Luis del Palmar, 10 Km SE of San Luis del Palmar, Route 6, 26 September 1973, *Quarín & Tressens 1349* (CTES). Prov. Formosa: Dep. Pilcomayo, Parque Nacional Pilcomayo, near to Puerto Soledad, 9 November 1991, *Fortunato et al. 2097* (BAB, SI). Prov**.** Santa Fe: Dep. Gral. Obligado, Villa Ana, 18 August 1973, *Quarín 1193* (CTES).

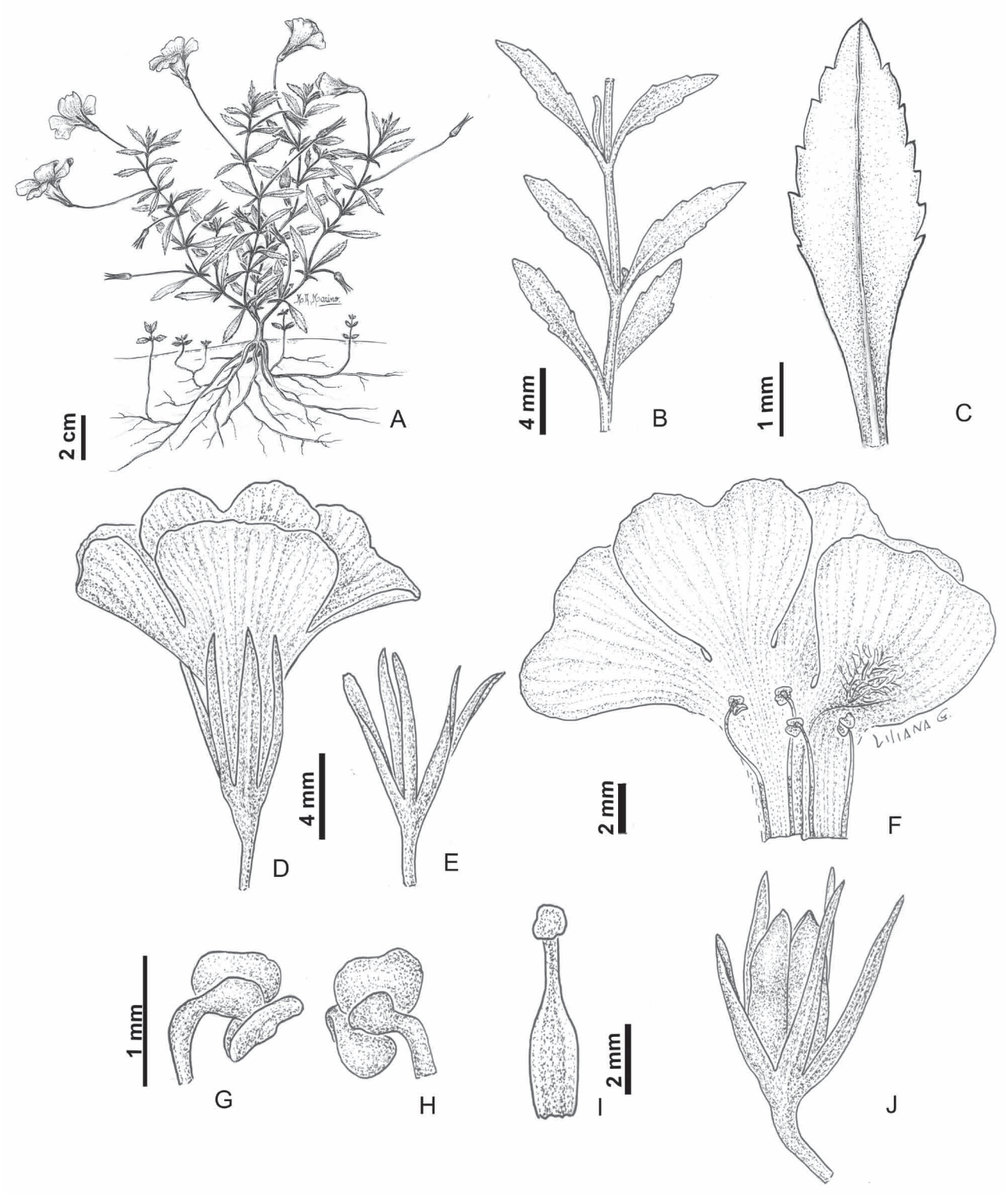

FIGURE 1. *Mecardonia reneeae*. A: Habit. B: Stem. C: Detail of the leaf. D: Flower in lateral view. E: Calyx. F: Dissected corolla. G: Anterior anther. H: Posterior anther. I: Gynoecium. J: Fruit (A-C: from *Greppi & Hagiwara 560*, BAB; illustrated by M. Marino; B–J: from *Schinini 11601*, CTES; illustrated by Mirtha L. Gómez).

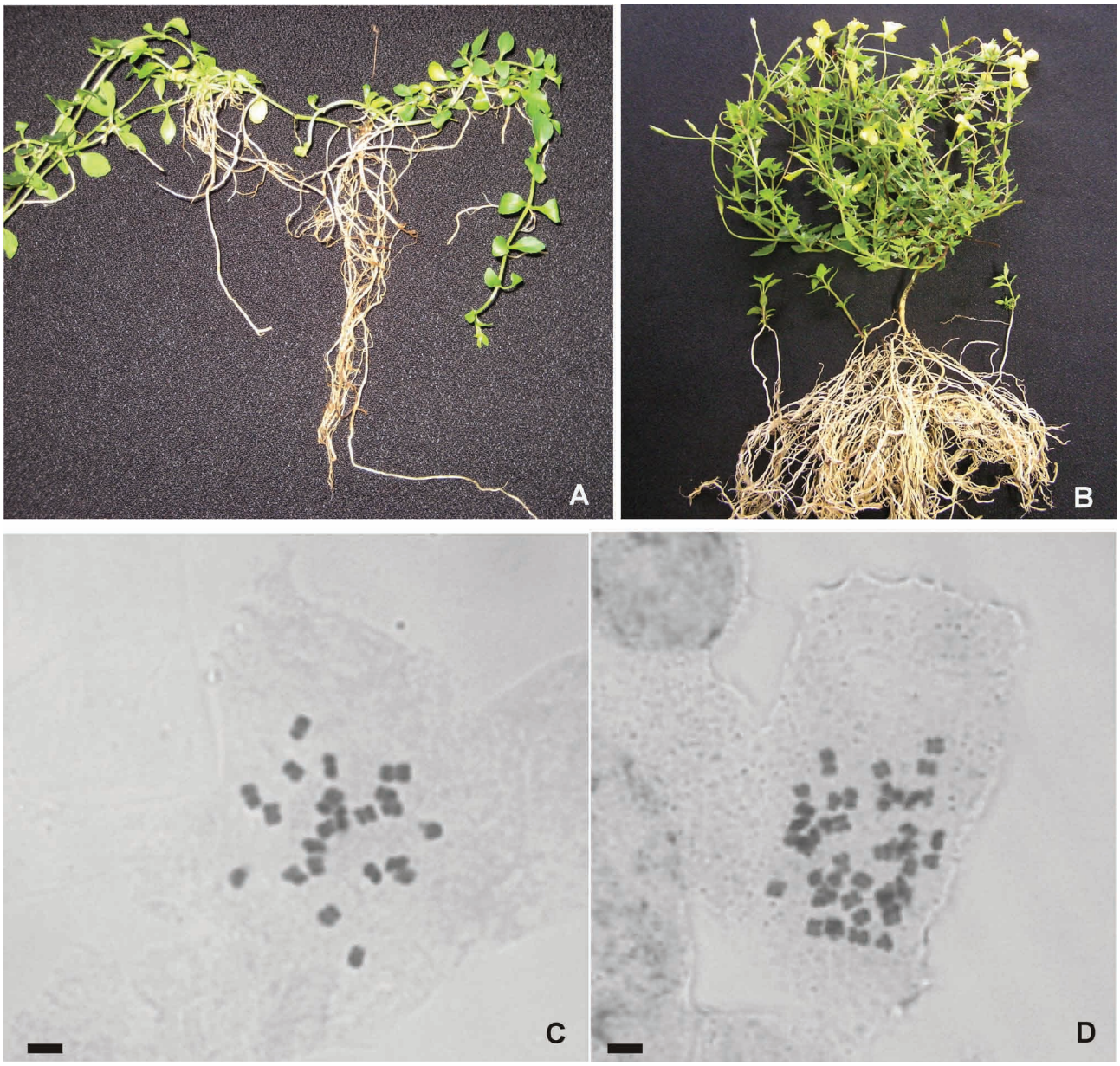

FIGURE 2. A, C: *Mecardonia procumbens* var. *flagellaris*. A. Habit, C. Metaphase image showing 22 chromosomes. B, D. *M. reneeae*. B. Habit. D. Metaphase image showing 44 chromosomes.

TABLE 1. Comparison of diagnostic characters between *Mecardonia procumbens* var. *flagellaris*, *M. reneeae* and *M. kamogawae*.

	M. procumbens var. flagellaris	***M. reneeae***	***M. kamogawae***
Life forms	camephyte decumbent	terophyte	geophyte
Stems	repens, radicant, stoloniferous	not radicant	not radicant
Root	not gemmiferous	gemmiferous	gemmiferous
Leaf margin	entire	serrate	serrate
Form leaf	linear to narrow elliptical	narrow elliptic	ovate to broadly elliptic
Consistency of the leaf	herbaceous	herbaceous	slightly succulent
Bracteoles (mm)	5–11 × 0.5–2	3–7 × 0.5–1.5	6–12 × 2–4
Dorsal sepal width (mm)	1.5–3	1–1.2	1.5–2
Capsule size (mm)	5–9 × 2–4	3–5 × 1.5–1.8	5–6 × 3–3.5
Chromosome numbers	2n=22	2n=44	2n=66

Discussion

We confirmed a chromosome number of 2n=4x=44 in *Mecardonia reneeae* (Fig. 2), as reported with the name *Mecardonia* sp. in Sosa *et al*. (2016). This taxon differs from other similar taxa that are diploid (2n=2x=22), such as *M. procumbens* var. *flagellaris*, or hexaploid, such as *M. kamogawae* (2n=2x=66).

Morphologically, *Mecardonia reneeae* is similar to *M. kamogawae* in terms of the gemiferous root system (Fig. 2) but can be distinguished by the sepal width, consistence and color of leaves, bracteole length and fruit size. Furthermore, some materials of this new species were mistaken for *M. procumbens* var. *flagellari*s in the past. However, both species can be easily distinguished when studying living plants. *Mecardonia procumbens* var. *flagellaris* are plants with radicating stems, occasionally somewhat ascending at the ends; the stems and its branches grow up to 40 or 50 cm in length, spreading across the ground and producing adventitious roots; it does not have gemiferous roots; its appearance is not compact. In addition, the combination of erect and non-radicant stems and gemmiferous roots gives *M. reneeae* a compact appearance. The margin leaves of *M. procumbens* var. *flagellaris* are entire or sub-serrate and the sepals, mainly the dorsal sepal, are wider (1.5–3 mm *vs.* 1–1.2 mm in width), and the fruits are bigger (5–9 × 2–4 mm *vs.* 3–5 × 1.5–1.8 mm) than those of *M. reneeae*.

These species were also found to differ in their life form. During the winter season, when visiting different natural populations of *Mecardonia*, we observed that *M. procumbens var. flagellaris* maintains aerial shoots latent, whereas *M. reneeae* plants disappear completely and grows again when the new spring season approaches. According to the classification of Raunkiær (1934, modified by Ellenberg & Mueller-Dombois, 1966), *M. procumbens var. flagellaris* would behave as a camephyte species, whereas *M. reneeae* would be terophyte.

The results obtained from the intra- and inter-specific crosses in native *Mecardonia* species indicated that in the intraspecific crosses *M. reneeae* produced 100% of fruits with a high percentage of viable seeds. However, the interespecific crossses with *M .procumbens var. procumbens, M. procumbens var. flagellaris, M. kamogawae* and *M. grandiflora* produced no capsules. According to this result, *M. reneeae* is reproductively isolated from *M. procumbens var. procumbens, M. procumbens var. flagellaris, M. grandiflora* and *M. kamogawae*, and is therefore considered a valid species. The information of crosses is being processed for immediate publication.

There is also a distinction in the distribution among these three species. *Mecardonia reneeae* is restricted to northern Argentina; it grows in Chaco province (Cabrera & Willink 1973, Cabrera 1976), in low clayey loam soils near watercourses in areas near Paraná River. *Mecardonia kamogawae* is endemic to the center-east of Corrientes province; it grows in sandy soils, near Uruguay River. In contrast, *M. procumbens* var. *flagellaris* is widely distributed from Mato Grosso do Sul (Brazil) to Chubut (Argentina), including areas in Chile, Paraguay and Uruguay, and covering more phytogegraphic provinces. This pattern of cytotype distribution is characteristic of young polyploid complexes (Stebbins 1971). In these complexes, the geographic ranges of polyploid cytotypes are more restricted than those of diploids, but their ranges overlaps with those of the diploid species; thus, this overlap might contribute genomes to the tetraploid species, determining some degree of sympatry among these species.

In conclusion, *M. reneeae* seems to be closely related to *M. kamogawae* and in the past some materials of this new species were mistaken for *M. procumbens* var. *flagellari*s. We found that the combination of the morphological and chromosome characters, with geographical range information provides a satisfactory identification of nearly all specimens.

Key to M. reneeae and similar species

1. Stems prostrate, radicants, stoloniferous, sometimes with erect or ascending branches born from the prostrate stems. Root system not gemmiferous. Leaves entire. ..... *M. procumbens* var. *flagellaris*
- Stems erect, not radicants, gemmiferous root system. Leaves serrate. 2

2. Leaves herbaceous, narrowly elliptical, concolorous. Bracteoles 3–5 × 0.5–1.5 mm. Pedicels up to 35(–40) mm. Dorsal sepal 1–1.2 mm wide. Capsule 3–5 × 1.5–1.8 mm. ..... *M. reneeae*
- Leaves slightly succulent, ovate to broadly elliptical, discolorous. Bracteoles 6–12 × 2–4 mm. Pedicels up to 65 mm. Dorsal sepal 1.5–2 mm wide. Capsule 5–6 × 3–3.5 mm ..... *M. kamogawae*

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