

Paspalum schesslii (Poaceae, Paspaleae), a new species from Mato Grosso (Brazil) with an unusual base chromosome number

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Abstract *Paspalum schesslii*, a new species from the state of Mato Grosso in central-western Brazil, is described and illustrated. The new species is related to *P. malmeanum*, from central-western Brazil and eastern Bolivia, and *Paspalum eucomum*, from central Brazil. It comprises shorter plants with leaf blades and racemes shorter than those of the related species, and spikelets having obovate, deciduous upper florets. An unexpected chromosome number $2n = 12$ was found in specimens of *P. schesslii*; thus it differs from both *P. malmeanum*, which has $2n = 20$, and *P. eucomum*, for which $2n = 30$ and $2n = 32$ chromosome counts are here reported for the first time. The discovery of a new species having $2n = 12$, which often cohabits with diploid populations of the widespread related species, *P. stellatum*, is consistent with an hypothesis about the hybrid origin of the polyploid cytotypes of *P. stellatum* having $2n = 32$ and $2n = 52$ chromosomes. Moreover, such an hybrid origin involving

parental species with different base chromosome numbers ($x = 6$ and $x = 10$) could also explain the occurrence of 32 chromosomes in *P. eucomum*, potentially documenting a speciation mechanism that is otherwise unknown in the genus.

Keywords *Paspalum malmeanum* · *Paspalum eucomum* · *Paspalum stellatum* · Brazilian flora · Cytogenetics · Grass taxonomy

Introduction

With about 350 species (Chase 1929; Zuloaga and Morrone 2005), *Paspalum* L. is the largest genus in the subfamily Panicoideae. The species inhabit ecologically diverse areas along the American continent and show a remarkable diversity of growth forms (Rua and Gróttola 1997). The genus is particularly abundant in Brazil (Chase 1929; Pohl 1980) and its major center of diversity is located in the Cerrado region, a formation of tropical savannas comprising a large extension in central Brazil and minor inclusions in Bolivia and Paraguay.

Paspalum malmeanum Ekman, *P. eucomum* Nees ex Trin., and *P. stellatum* Humb. & Bonpl. ex Flüggé are three closely related species belonging to the subgenus *Ceresia*, one of four subgenera to which *Paspalum* is currently classified (Zuloaga and Morrone 2005). They are caespitose perennial plants that form dense bunches, with mostly basal and coarse foliage, involute blades, inflorescences composed of one or two racemes, and solitary spikelets densely covered with silky hairs.

Paspalum malmeanum was originally described from the state of Mato Grosso (Ekman 1911), in central-western Brazil, and is also found in the Chiquitania region, in

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eastern Bolivia (Killeen 1990; Zuloaga and Morrone 2005). *Paspalum eucomum* is distributed in Brazilian ‘cerrados’ and ‘campos’ from Mato Grosso and Goiás to Paraná (Valls and Oliveira 2013). Denham et al. (2002) and Zuloaga and Morrone (2005) differentiated *P. malmeanum* from *P. eucomum* by spikelet length (1.6–2 in *P. malmeanum* vs. 2.5–3.5 mm in *P. eucomum*). Moreover, Ekman (1911) pointed out some differences in spikelet indumentum and leaf anatomy. Nevertheless, specimens with intermediate spikelet sizes occur, so that identification of herbarium specimens is sometimes doubtful.

Paspalum stellatum has a wide distribution from Mexico to northeastern Argentina (Denham et al. 2002). It is distinguished from *P. malmeanum* and *P. eucomum* by having remarkably wider membranous rachises (4–10 vs. 1.2–2.5 mm wide) and upper florets shorter than (vs. as long as) the upper glume and lower lemma (Zuloaga and Morrone 2005). Intermediate morphologies between *P. stellatum* and *P. eucomum* have been observed in individuals from sympatric populations, which could not be undoubtedly ascribed to one species or the other (Bonasora et al. 2011b). Moreover, this species is phylogenetically related to *P. eucomum* and *P. malmeanum* (Scataglini et al. 2013).

Chromosome numbers and other cytological data provide useful taxonomic information, especially where species are morphologically similar and their distributions overlap (Stebbins 1971; Guerra 2012). Most species of *Paspalum* have a base chromosome number $x = 10$ (Burson 1975; Quarin 1992; Valls 2000; Honfi 2003), which is plesiomorphic for the genus (Rua et al. 2010; Scataglini et al. 2013). A few exceptions have been reported elsewhere (Mehra and Chaudhary 1973; Davidse and Pohl 1974; Selva 1976; Halappanarar and Chennaveeraiah 1981; Reeder 1984; Honfi et al. 1990; Pozzobon et al. 2000; Peñaloza et al. 2008; Silva 2013), some of them showing $x = 6$ (Quarin 1974; Burson 1975; Honfi et al. 1990; Pozzobon et al. 2000; Peñaloza et al. 2008). Multiple intraspecific chromosome numbers frequently occur in *Paspalum* as an outcome of different ploidy levels (Quarin 1992; Hojsgaard et al. 2009), but multiple basic numbers within a single species have only been mentioned for *P. stellatum*. Indeed, it is a unique case within the genus since reported chromosome numbers for this species include $2n = 20$ (Killeen 1990), $2n = 32$ (Honfi et al. 1990; Killeen 1990), and $2n = 52$ (Honfi et al. 1990). Regular meiotic behavior was reported for accessions with $2n = 20$ and $2n = 32$, with formation of 10 and 16 bivalents at diakinesis, respectively (Sader et al. 2008). On the other hand, a chromosome number of $2n = 20$ was reported for *P. malmeanum* (Killeen 1990; Sede et al. 2010), with 10 bivalents at early metaphase I (Sede et al. 2010). No cytological data are currently available for *P. eucomum*.

Multivariate analysis is largely used to differentiate related species on the basis of overall morphological similarity. In *Paspalum*, this approach was used to discriminate accessions of *P. distichum* L. and *P. vaginatum* Sw. (Echarte et al. 1992; Echarte and Clausen 1993; Guma et al. 1995), *P. polyphyllum* Nees ex Trin. and *P. bicilium* Mez (Silva 2013), and annual species in the group ‘Plicatula’ (Oliveira 2004). Thus, multivariate analysis would be a useful tool to summarize overall similarities and differences among populations within a species complex having fuzzy interspecific limits such as that of *P. malmeanum*–*P. eucomum*.

In recent years, new collections of *P. stellatum* and related species have been made in Brazil and Bolivia. Chromosome surveys and morphological observations on this material has thrown new light on the taxonomic and cytological relationships both within the *P. malmeanum*–*P. eucomum* complex, and between it and *P. stellatum*. The aims of this paper are (1) to report the occurrence of a new Brazilian species related to *P. malmeanum*–*P. eucomum*, with a novel chromosome number; (2) to compare the new species with *P. malmeanum* and *P. eucomum* and to discriminate the three entities within the complex morphologically using multivariate analysis; and (3) to discuss the taxonomic relationships between such entities and *P. stellatum* on the basis of morphological and cytological evidence.

Materials and methods

Plant material

Chromosome analyses and leaf anatomy studies were based on plants collected in the states of Mato Grosso, Goiás, and Paraná, Brazil, and the province of Ñuflo de Chávez, dept. Santa Cruz, Bolivia. Voucher specimens were deposited at BAA, CEN, UB, and USZ (Table 1). Cuttings or pieces of rhizomes were obtained in the field and then cultivated in pots in a greenhouse at the “Lucien Hauman” Botanical Garden of the Universidad de Buenos Aires. Morphological observations as well as additional anatomical observations were made in herbarium material deposited at BAA, CEN, MO, S, SI, UB, US, and USZ (Table 1). Geographical locations of all studied specimens are represented in Fig. 1.

Chromosome analysis

Plants for cytogenetic analysis were transferred to clay pots, and root tips in active growth were collected from the bottom of the pot when they were 3–4 cm long by lifting the whole plant. Selected young root tips were pretreated with a

Table 1 Voucher specimens used for chromosome and morphological analyses and leaf anatomy studies, including species, origin, elevation, geographical coordinates, collector, collector number, and herbarium acronym

Species	Country	Locality	Elevation	Latitude	Longitude	Collectors	Coll. no.	Herbarium acronym
<i>P. eucomum</i>	Brazil	GO, Alexania		16°04'56''S	48°30'25,8''W	A. C. Allem et al.	2638	CEN
<i>P. eucomum</i>	Brazil	DF, Brasília		15°46'47''S	47°55'47''W	S. P. Almeida	1076	CEN, UB
<i>P. eucomum</i>	Brazil	GO, Corumbá de Goiás		15°55'25''S	48°48'31''W	W. R. Anderson	10,403	UB
<i>P. eucomum</i>	Brazil	DF, São Sebastião	1195	15°54'32''S	47°41'25''W	A. S. Rodrigues	310	CEN
<i>P. eucomum</i>	Brazil	GO, Pirenópolis		15°51'09''S	48°57'33,1''W	A. S. Rodrigues	120	CEN
<i>P. eucomum</i>	Brazil	PR, Ponta Grossa	807	25°12'01,2''S	50°04'08,5''W	A. Araújo	165	BAA
<i>P. eucomum</i>	Brazil	DF, Brasília	1109	15°54'44''S	47°53'10''W	R. G. Chacon	41	CEN
<i>P. eucomum</i>	Brazil	MG, Serra do Cipó	800	19°20'37''S	43°36'21''W	M. A. Chase	9097	MOBOT, BAA
<i>P. eucomum</i>	Brazil	MT, Juscimeira		16°20'00''S	55°00'00''W	M. A. Chase	11,962	MOBOT, NYBG
<i>P. eucomum</i>	Brazil	DF, Sobradinho	1075	15°44'01''S	47°44'10''W	C. A. S. Correia	212	UB
<i>P. eucomum</i>	Brazil	DF, Brasília	ca. 1000	15°36'00''S	47°42'00''W	J. A. da Silva	1	CEN
<i>P. eucomum</i>	Brazil	GO, Alto Paraiso de Goiás	1500	14°07'57''S	47°30'36''W	J. A. da Silva	94	CEN
<i>P. eucomum</i>	Brazil	DF, Brasília	ca. 1000	15°36'00''S	47°42'00''W	J. A. da Silva	12_1	CEN
<i>P. eucomum</i>	Brazil	DF, Brasília	ca. 1000	15°36'00''S	47°42'00''W	J. A. da Silva	310_A	CEN
<i>P. eucomum</i>	Brazil	DF, Brasília	ca. 1000	15°36'00''S	47°42'00''W	J. A. da Silva and J. Fonseca Jr	105_1	CEN
<i>P. eucomum</i>	Brazil	MG, Barbacena	1080	21°12'00''S	43°49'00''W	G. Davidse and T. P. Ramamoorthy	10724	UB, NYBG
<i>P. eucomum</i>	Brazil	DF, Brasília	ca. 1035	15°45'00''S	47°42'00''W	J. O. De Jesus	3	UB
<i>P. eucomum</i>	Brazil	GO, Luziânia		16°15'09''S	47°57'01''W	J. C. Dianese	16	UB
<i>P. eucomum</i>	Brazil	GO, Luziânia		16°15'09''S	47°57'01''W	J. C. Dianese	25	UB
<i>P. eucomum</i>	Brazil	GO, Luziânia		16°15'09''S	47°57'01''W	J. C. Dianese	39	UB
<i>P. eucomum</i>	Brazil	MG, Dolores do Indaiá	ca. 670	19°26'00''S	45°36'00''W	G. Eiten	A-275	UB
<i>P. eucomum</i>	Brazil	DF, Brasília		15°48'00''S	47°52'00''W	E. P. Eringer	10,162	UB
<i>P. eucomum</i>	Brazil	GO, Itaguara		15°48'43''S	49°34'10,9''W	A. F. M. Glaziou	22,555	G
<i>P. eucomum</i>	Brazil	MG, Uberaba		19°44'54''S	47°55'55''W	R. Goodland	3358	UB
<i>P. eucomum</i>	Brazil	SP, Botucatu	550	22°45'00''S	48°25'00''W	I. S. Gottsberger	2265	UB
<i>P. eucomum</i>	Brazil	DF, Brasília	1070	15°55'00'' S	48°01'00'' W	J. Guimarães Faria et al.	26	CEN
<i>P. eucomum</i>	Brazil	PR, Tibagi	900	24°30'33,8''S	50°24'49''W	G. Hatschbach et al.	59,101	MOBOT, UFMG, UEFS
<i>P. eucomum</i>	Brazil	DF, Brasília		15°57'00''S	47°56'00''W	H. L. Cesar	596	UB
<i>P. eucomum</i>	Brazil	MG, Riacho dos Machados	1100	16°24'12,4''S	43°01'18,6''W	H. S. Irwin et al.	23,169	UB, MOBOT, NYBG
<i>P. eucomum</i>	Brazil	MT, Juruena		10°17'23''S	58°12'00''W	J. G. Kuhlmann	1668	US
<i>P. eucomum</i>	Brazil	DF, Brasília	ca. 1032	15°45'00''S	47°51'00''W	N. Lima and Heringer	155	UB
<i>P. eucomum</i>	Brazil	MG, Jaboticatubas	ca. 734	19°21'30,7''S	43°52'24,6''W	L. B. Smith	6969	CEN
<i>P. eucomum</i>	Brazil	MG, Goiânia		16°41'18,8''S	49°16'00,4''W	A. Macedo	1818	MOBOT, BAA
<i>P. eucomum</i>	Brazil	DF, Brasília	1080	15°52'00''S	48°01'00''W	B. M. T. Walter	4846	CEN
<i>P. eucomum</i>	Brazil	DF, Brasília		15°43'53''S	47°55'36''W	C. R. Martins	378	UB
<i>P. eucomum</i>	Brazil	DF, Brasília		15°34'00''S	47°33'00''W	A. G. Moreira	s.n.	UB

Table 1 continued

Species	Country	Locality	Elevation	Latitude	Longitude	Collectors	Coll. no.	Herbarium acronym
<i>P. eucomum</i>	Brazil	GO, Cocalzinho de Goiás	800	15°05'00"S	48°43'00"W	R. C. Oliveira and J. B. Pereira	273	CEN
<i>P. eucomum</i>	Brazil	MG, Itutinga	ca. 1082	21°17'53,1"S	44°39'28"W	S. C. Pereira	5218	UB
<i>P. eucomum</i>	Brazil	MG, Itutinga	ca. 1082	21°17'53,1"S	44°39'28"W	S. C. Pereira	5247	UB
<i>P. eucomum</i>	Brazil	MG, Pimenta	ca. 789	20°31'00"S	45°48'00"W	S. C. Pereira	5272	UB
<i>P. eucomum</i>	Brazil	MG, De Capitólio	ca. 790	20°36'00"S	46°03'00"W	S. C. Pereira	5289	UB
<i>P. eucomum</i>	Brazil	DF, Brasília	ca. 1120	15°54'44"S	47°00'46"W	G. Pereira-Silva and A. B. Sampaio	3900	CEN
<i>P. eucomum</i>	Brazil	DF, Brasília	1050	15°45'00"S	47°42'00"W	D. Philcox and E. Oniski	4870	UB
<i>P. eucomum</i>	Brazil	GO, Cristalina	ca. 1240	16°45'00"S	47°40'00"W	C. E. B. Proença and R. S. Oliveira	1781	UB
<i>P. eucomum</i>	Brazil	MT, Sete Lagoas		19°27'00"S	44°10'00"W	R. C. F. C. de Carvalho	s.n.	CEN
<i>P. eucomum</i>	Brazil	MG, Belo Horizonte	ca. 1200	19°58'00"S	43°54'00"W	L. Roth	2420	CEN
<i>P. eucomum</i>	Brazil	DF, Brasília	ca. 1115	15°47'00"S	47°53'00"W	J. R. Santos and G. A. Moreira	29	CEN
<i>P. eucomum</i>	Brazil	DF, Fercal	870	15°32'15"S	47°50'06"W	G. H. Rua	908	CEN, BAA
<i>P. eucomum</i> *	Brazil	PR, Ponta Grossa		25°01'42,1"S	50°04'36,7"W	G. H. Rua	1124	BAA
<i>P. eucomum</i>	Brazil	DF, Brasília	1115	15°53'25"S	48°00'28"W	G. H. Rua et al.	879	CEN, BAA
<i>P. eucomum</i>	Brazil	DF, Fercal	912	15°34'37"S	47°56'13"W	G. H. Rua et al.	899	CEN, BAA
<i>P. eucomum</i> *	Brazil	GO, Mineiros	872	17°22'44,6"S	52°55'26,8"W	G. H. Rua et al.	939	BAA, UB
<i>P. eucomum</i>	Brazil	MT, General Carneiro	503	15°35'47,7"S	53°03'05"W	G. H. Rua et al.	1009	BAA, UB
<i>P. eucomum</i>	Brazil	DF, Brasília	1054	15°44'07"S	47°55'39"W	G. H. Rua et al.	837	CEN, BAA
<i>P. eucomum</i>	Brazil	DF, Fercal	832	15°32'39,2"S	47°50'05"W	G. H. Rua et al.	1024	BAA, UB
<i>P. eucomum</i>	Brazil					H. L. Sello	s.n.	UB
<i>P. eucomum</i>	Brazil	MG				F. Sellow	s.n.	US
<i>P. eucomum</i>	Brazil	DF, Brasília	1080	15°50'23,4"S	47°55'21"W	F. Sellow	s.n.	K, HAL
<i>P. eucomum</i>	Brazil	DF, Brasília	1080	15°50'23,4"S	47°55'14,2"W	F. Sellow	s.n.	K
<i>P. eucomum</i>	Brazil					F. Sellow	s.n.	UB
<i>P. eucomum</i>	Brazil	DF, Brasília	1020	15°45'00"S	47°52'00"W	F. C. Silva et al.	427	CEN, MBM
<i>P. eucomum</i>	Brazil	TO, Mateiros	520	10°43'00"S	46°47'00"W	L. H. Soares e Silva et al.	952	UB
<i>P. eucomum</i>	Brazil	GO, Padre Bernardo	1020	15°33'00"S	48°15'00"W	R. F. Vieira et al.	721	CEN
<i>P. eucomum</i>	Brazil	MG, Paraopeba	900	19°24'24"S	44°19'39"W	Z. L. Wagner and P. L. Viana	9599	CEN, UFMG
<i>P. malmeanum</i>	Bolivia	SCZ, Velasco	250	14°36'09"S	60°51'00"W	E. Gutiérrez et al.	655	MO
<i>P. malmeanum</i>	Bolivia	SCZ, Ñuflo de Chávez	500	16°02'00"S	62°00'00"W	T. J. Killeen	898	MOBOT
<i>P. malmeanum</i>	Bolivia	SCZ, Ñuflo de Chávez	500	16°03'00"S	62°10'00"W	T. J. Killeen	2024	MOBOT
<i>P. malmeanum</i>	Bolivia	SCZ, Ñuflo de Chávez	500	16°00'00"S	62°00'00"W	T. J. Killeen	2076	MOBOT, US
<i>P. malmeanum</i>	Bolivia	SCZ, Ñuflo de Chávez	500	16°13'00"S	62°00'00"W	T. J. Killeen	2478	SI, US, MOBOT
<i>P. malmeanum</i>	Brazil	MT, Juruena		10°17'23"S	58°12'00"W	J. G. Kuhlmann	1667	US, JBRJ

Table 1 continued

Species	Country	Locality	Elevation	Latitude	Longitude	Collectors	Coll. no.	Herbarium acronym
<i>P. malmeanum</i>	Brazil	MG, Santa Ana da Chapada		15°26'00"S	55°45'00"W	<i>G. O. A. Malme</i>	<i>s.n.</i> (<i>isotype</i>)	MOBOT, US
<i>P. malmeanum</i>	Bolivia	SCZ, Velasco	150	14°36'00"S	60°50'34"W	<i>B. Mostacedo et al.</i>	1654	MO
<i>P. malmeanum</i> *	Bolivia	SCZ, Ñuflo de Chávez	483	16°11'05"S	62°00'54,4"W	<i>G. H. Rua</i>	1040	BAA
<i>P. malmeanum</i>	Bolivia	SCZ, Ñuflo de Chávez	481	16°09'57,8"S	62°01'32,6"W	<i>G. H. Rua</i>	1044	BAA
<i>P. malmeanum</i>	Bolivia	SCZ, Ñuflo de Chávez	478	16°00'47,7"S	62°01'08,5"W	<i>G. H. Rua</i>	1047	BAA
<i>P. malmeanum</i>	Bolivia	SCZ, Ñuflo de Chávez	477	16°00'51"S	62°01'06,3"W	<i>G. H. Rua</i>	1050	BAA
<i>P. malmeanum</i>	Bolivia	SCZ, Chiquitos	822	18°20'26"S	59°34'00"W	<i>J. R. T. Wood et al.</i>	24624	USZ
<i>P. malmeanum</i>	Bolivia	SCZ, Velasco	425	16°24'07"S	61°11'54,4"W	<i>J. R. T. Wood et al.</i>	24805	USZ
<i>P. schesslii</i> sp. nov.	Brazil	MT, Cuiabá		15°33'25,2"S	55°47'00"W	<i>G. H. Rua</i>	237	BAA
<i>P. schesslii</i> sp. nov.	Brazil	MT, Nossa Senhora do Livramento	135	16°06'33,6"S	56°19'54,2"W	<i>G. H. Rua et al.</i>	955	BAA, UB
<i>P. schesslii</i> sp. nov.*	Brazil	MT, Porto Estrela	212	15°39'57,8"S	57°16'22,2"W	<i>G. H. Rua et al.</i>	975	BAA, UB
<i>P. schesslii</i> sp. nov.	Brazil	MT, Porto Estrela	212	15°39'57,8"S	57°16'22,2"W	<i>G. H. Rua et al.</i>	981	BAA, UB
<i>P. schesslii</i> sp. nov.*	Brazil	MT, Cuiabá	192	15°27'49,6"S	56°03'02"W	<i>G. H. Rua et al.</i>	991	BAA, UB
<i>P. schesslii</i> sp. nov.	Brazil	MT, Nossa Senhora do Livramento	140	16°00'00"S	56°13'00"W	<i>M. Schessl</i>	3096	BAA

Specimens used for PCA are indicated in bold, whereas those used for cytology and leaf anatomy are marked by asterisks

saturated aqueous solution of 1-bromonaphthalene for 3 h at room temperature. Then they were fixed in 3:1 (absolute ethanol: glacial acetic acid) for 24 h and stored in 70 % ethanol at 4 °C. For the Feulgen's technique, the material was hydrolyzed with 1 N HCL at 60 °C for 10 min and stained with basic fuchsin for 1 h. Then, meristem cells were macerated in a drop of 2 % aceto-orcein and covered with a coverslip. To achieve good separation of cells, the coverslip was gently tapped several times, and then the slide was squashed. Coverslips were removed by freezing with CO₂, and permanent slides were made using DePeX or Euparal as mounting medium (Bowen 1956).

Young inflorescences of each accession were collected for meiotic studies, fixed in 3:1 for 24 h, transferred to 70 % ethanol, and stored at 4 °C until use. Microspores were prepared by staining with acetic carmine and squashing. About 30–70 meiocytes in different phases were observed, according to the availability of inflorescences, from which 15–27 meiocytes were selected to study the chromosome configurations at diakinesis or

metaphase I. Photographs were taken with a Zeiss Axio-plan optical microscope and a Zeiss AxioCam ERC 5S camera.

Morphological character analysis

Ten morphological characters were explored and eight characters were selected to use in the multivariate analyses: upper glume length and width, lower lemma length and width, upper floret length and width, rachis length, and plant height. The two remaining characters, leaf blade length and rachis width, were not included because of the occurrence of many missing entries and non-informativeness, respectively.

Additionally, the upper floret form was quantitatively described using Elliptic Fourier Descriptors (EFD) following the procedure described by Zhang and Lu (2002) and implemented in the software package Shape 1.3 (Iwata and Ukai 2002). Images of the upper floret shapes were acquired from all specimens used for PCA, using a high-

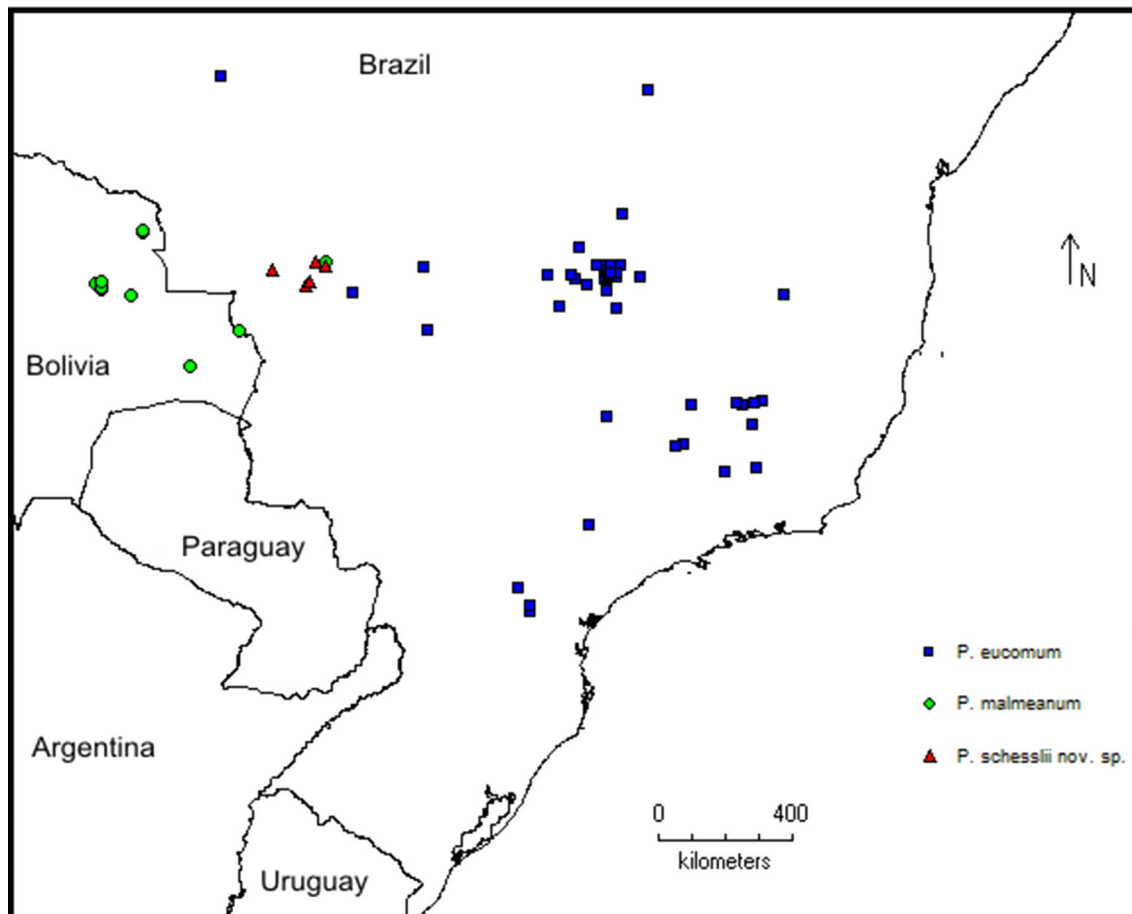


Fig. 1 Geographical locations of studied specimens *Paspalum eucomum* (squares), *P. malmeanum* (circles), and *P. schesslii* sp. nov. (triangles)

resolution scanner (800 dpi). The closed contour of each floret was then obtained as chain-coded data, from which the normalized coefficients of the elliptic Fourier descriptors (EFDs) were calculated. Using this procedure, the shape of each floret was approximated using the first 20 harmonics, and the information contained in the coefficients was summarized by performing a principal component analysis (PCA) based on a variance–covariance matrix (as implemented in the PrintComp program included in the Shape package). The first principal component (PC1) resulting from this analysis summarizes most of the information (87 %) about upper floret shape. Then, a morphological data matrix was assembled, which includes the eight characters previously mentioned together with two additional variables: the PC1 as a synthetic variable, and the area measured in pixels from the upper floret images. A PCA of the resulting data matrix was then conducted using the program InfoStat (Di Rienzo et al. 2008) and R version 3.2.0 (Development Core Team 2012) was used to summarize the available morphological information. Specimens with missing entries were discarded from the matrix during PCA.

Further, two descriptive characters were observed: number of racemes per inflorescence and occurrence of branching along the culms. Several morphological characters were also observed in the available cultivated material, although no statistical treatment of such data was carried out because the number of available samples was insufficient.

Leaf anatomy

Transverse sections were made from the middle portion of culm leaves. Fresh leaves were used when available; otherwise leaf portions were taken from herbarium material. Dried leaves were placed in boiling water to soften them until they become unfolded, then they were stored in ethanol 70 %. Fresh leaves were fixed in formalin–acetic–ethanol mixture (FAA) for 48 h and stored in ethanol 70 %. The fragments of leaves were pretreated with 10 % hydrofluoric acid to remove silica cells. After that, the material was dehydrated in an ethanol–alcohol series, embedded in paraffin, and sectioned on a rotatory microtome, 10- μ m thickness, according to standard methods.

Histological samples were stained with Safranin-Fast Green and mounted in Canada balsam (D'Ambrogio de Argüeso 1986; Zarlavsky 2014). Observations were made using a Zeiss Axioplan optical microscope and a Zeiss AxioCam ERc 5S camera. For anatomical descriptions, we followed the terminology developed by Ellis (1976).

Results

Chromosome analysis

The two studied accessions of the new species presented $2n = 12$ chromosomes in mitotic root tips cells (Fig. 2a). Meiotic behavior was normal with 6 bivalents at diakinesis in all studied pollen mother cells (PMCs) and regular chromosome segregation (Fig. 2b–d; Table 2). Meanwhile, all Bolivian accessions of *P. malmeanum* had a chromosome number $2n = 20$ and presented a normal meiosis with 10 bivalents in all analyzed PMCs (Fig. 3; Table 2).

Two accessions from Mato Grosso and Paraná (Brazil), morphologically corresponding to *P. eucomum*, had respectively $2n = 30$ (G. H. Rua et al. 939, Fig. 4a) and $2n = 32$ mitotic chromosomes. The accession G. H. Rua et al. 939 showed an irregular meiotic behavior (Table 2), as can be observed in the cells in Fig. 4b, c. Indeed, a meiotic configuration of 6I + 6II + 4III is shown in Fig. 4b, whereas in Fig. 4c unequal segregation with lag-gard chromosomes becomes evident. On the other hand, the accession G. H. Rua et al. 1124 showed regular meiotic behavior with formation of 16 bivalents at diakinesis and metaphase I (Fig. 4d–f).

Morphology

Observations of cultivated and herbarium material reveal qualitative differences between these groups: the new species usually had branched culms and inflorescences of two conjugated racemes, whereas *Paspalum malmeanum* had unbranched culms and inflorescences composed of (1)2–4(5) racemes. Culm branching varied among accessions of *P. eucomum*: culms were branched in the specimen G. H. Rua et al. 1124 and unbranched in G. H. Rua et al. 939.

Moreover, upper florets showed differences in shape, as reflected by the EFD analysis (not shown), and abscission. Indeed, upper florets of the new species were obovate and deciduous at maturity because of the formation of an

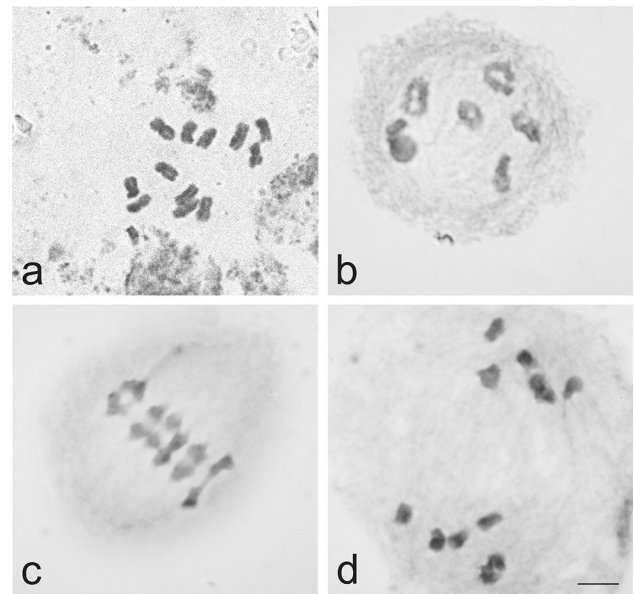


Fig. 2 *Paspalum schesslii* sp. nov., G. H. Rua et al. 975 **a** chromosomes at mitotic metaphase $2n = 12$, **b** microsporocyte in diakinesis with 6II, **c** microsporocyte in late metaphase I, **d** anaphase I. Scale bar 5 μ m

abscission layer at their basis, whereas those of *P. malmeanum* were elliptic and firmly attached to the rachilla. Upper florets of *P. eucomum* were highly variable in size and shape, although obovate deciduous upper florets were prevalent (Fig. 7).

The distribution of quantitative continuous characters was appraised using PCA and DA. Three groups were recognized by PCA, corresponding to *P. eucomum*, *P. malmeanum*, and the new species (Fig. 5). The first two PCs explained 62.4 and 18 % of the total variance. The characters mainly contributing to PC1 were related to spikelet measures, whereas PC2 was mainly determined by rachis length, plant height, and spikelet shape. *Paspalum malmeanum* and the new species were discriminated from *P. eucomum* by PC1, whereas PC2 was able to differentiate the new species from *P. malmeanum*. The specimens Irwin 23169 and Kuhlmann 1668 were placed by PCA in an intermediate position between the three groups, but they were assigned to *P. eucomum* by DA, which otherwise confirmed the results of PCA (not shown).

Individual variation ranges of most continuous characters overlapped for all three species, but they were wider for *P. eucomum* than for the other two species (Fig. 6; Online Resource 1). Thus, the three species can be only

Table 2 Chromosome counting and meiosis behavior of accessions of *Paspalum eucomum*, *P. malmeanum*, and *P. schesslii* sp. nov

Species/specimen	2n	No. CMP	Chromosome configurations			Range		
			I	II	III	I	II	III
<i>P. eucomum</i>								
<i>G. H. Rua</i> et al. 939	30	20	4.05	8.25	3.15	2–6	5–11	2–5
<i>G. H. Rua</i> et al. 1124	32	18		16				
<i>P. malmeanum</i>								
<i>G. H. Rua</i> 1040	20	27		10				
<i>P. schesslii</i> sp. nov.								
<i>G. H. Rua</i> et al. 975	12	24		6				
<i>G. H. Rua</i> et al. 991	12	15		6				

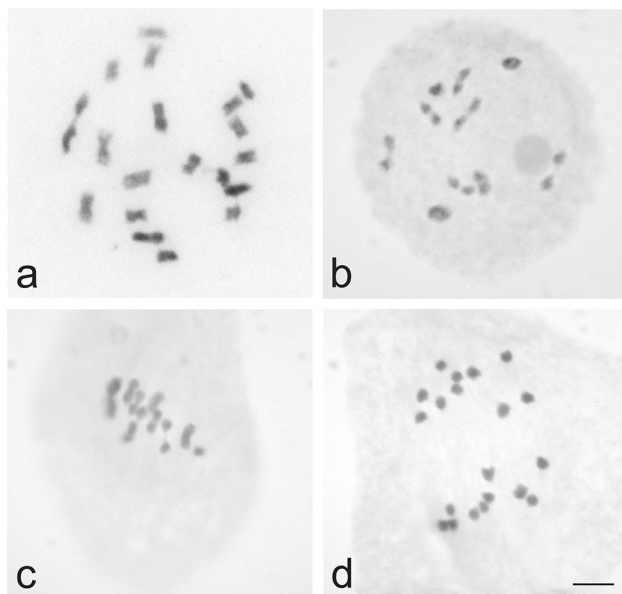


Fig. 3 *Paspalum malmeanum*, *G. H. Rua* 1040 **a** chromosomes at mitotic metaphase $2n = 20$, **b** microsporocyte in diakinesis with 10II, **c** metaphase I, **d** anaphase I. Scale bar 5 μ m

differentiated by combinations of continuous characters and it is a crucial issue to recognize *P. eucomum*, since this species is not easily diagnosable by qualitative differences.

Leaf anatomy

Anatomical features of leaf transverse sections were very similar in the new species (*G. H. Rua* et al. 991) and two accessions of *Paspalum eucomum* (*G. H. Rua* et al. 939 and *G. H. Rua* et al. 1124). The outline of the leaf blades in transverse section were expanded to open V-shaped, with a scarcely differentiated central keel and median vascular

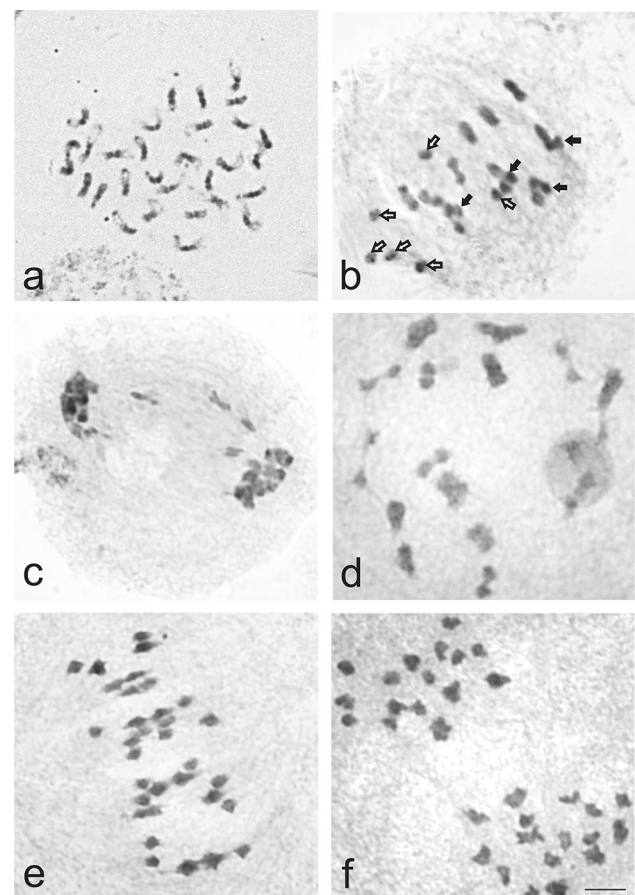


Fig. 4 *Paspalum eucomum*, *G. H. Rua* et al. 939 **a** chromosomes at mitotic metaphase $2n = 30$, **b** microsporocyte in metaphase I with formation of $6I + 6II + 4III$. White arrows indicated univalents and black arrows trivalents, **c** anaphase I with delayed segregation of some chromosomes. *P. eucomum*, *G. H. Rua* et al. 1124 **d** microsporocyte in diakinesis with 16II ($2n = 32$), **e** late metaphase I, **f** anaphase I. Scale bar 5 μ m

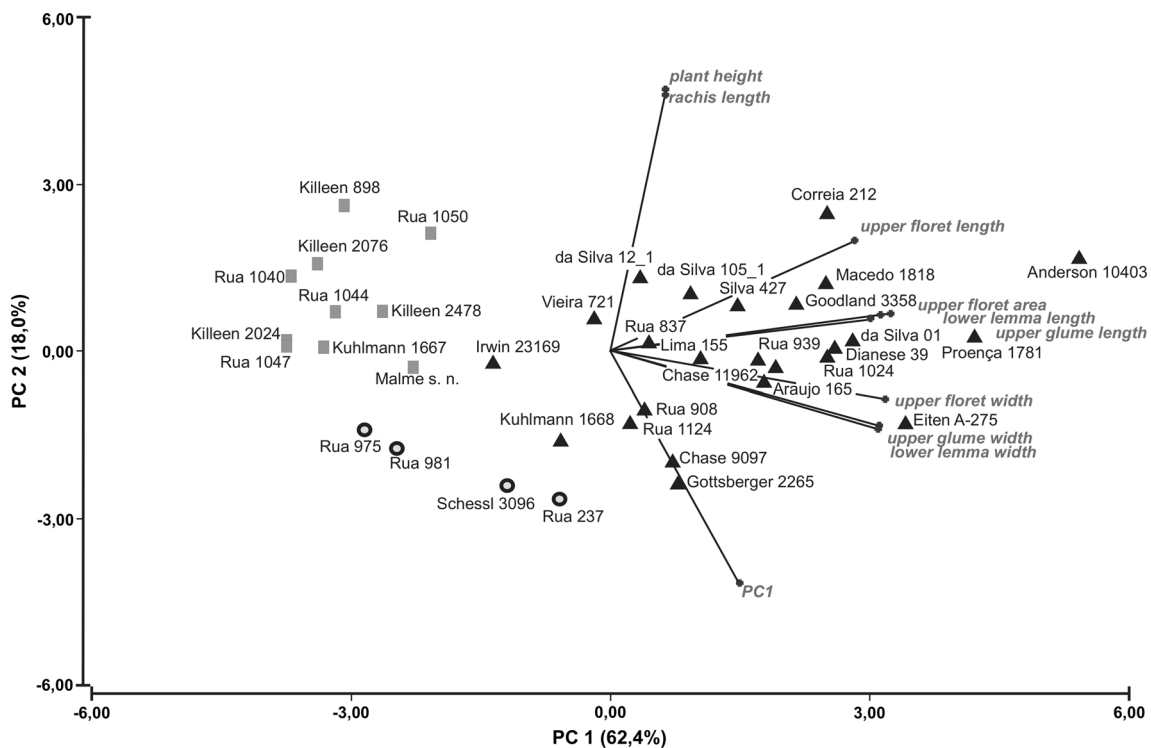


Fig. 5 Principal component analysis of morphological data; triangles *Paspalum eucomum*, squares *P. malmeanum*, circles *P. schesslii* sp. nov.

bundles associated to adaxial colorless parenchyma (Fig. 8a, c, d). Instead, *P. malmeanum* (*G. H. Rua 1040*) was distinguished by having an open U-shaped outline and first order vascular bundles with adaxial extensions of colorless parenchyma (Fig. 8b).

The adaxial surfaces presented deep furrows and rounded ribs associated to first and second order vascular bundles in *G. H. Rua 1040* (Fig. 8b), while in *G. H. Rua et al. 991* and *1124* furrows were less developed (Fig. 8a, d), and *G. H. Rua et al. 939* presented an irregular adaxial surface without conspicuous furrows and ribs (Fig. 8c). All studied specimens showed relatively smooth abaxial surfaces.

In all cases, the adaxial epidermis had elongated papillae arranged along the ribs. Macrohairs were present in both epidermises in *G. H. Rua et al. 991* and *G. H. Rua et al. 939* (Fig. 8a, c). Bulliform cells were fan-shaped, but in *G. H. Rua et al. 939* (Fig. 8c), they were poorly differentiated from the rest of the epidermal cells. Adaxial strands of sclerenchyma were associated to first and second vascular bundles, while in the abaxial surface, sclerenchyma was also present but it tended to form continuous hypodermal bunds (Fig. 8a, c, d). Additional observations of leaf anatomy were made in herbarium materials of the three species that showed no substantial intraspecific differences with the specimens described above.

Discussion

A new species from Brazil, related to *P. eucomum* and *P. malmeanum* is here described, which has a chromosome number unusual for the genus. Indeed, the chromosome counts of both studied accessions were $2n = 12$, i.e., a base chromosome number $x = 6$. Such base chromosome number differs from the plesiomorphic $x = 10$ (Rua et al. 2010; Scataglini et al. 2013), which is by far the most common in the genus (Quarin 1992; Zuloaga and Morrone 2005). A base chromosome number $x = 6$ has been confirmed only for *P. alnum* Chase (Quarin 1974; Burson 1975; Honfi et al. 1990; Pozzobon et al. 2000), and it probably also occurs in *P. filgueirasii* Morrone and Zuloaga ($2n = 24$) and *P. burmanii* Filg., Morrone and Zuloaga ($2n = 48$) (Peñaloza et al. 2008), although it has not yet been corroborated by meiotic studies. All these species seem to be neither morphological nor phylogenetically related to one another (unpublished data). Moreover, the new species is morphologically related to *P. malmeanum*, *P. eucomum*, and *P. stellatum*, which form a clade distinct from *P. alnum* (Scataglini et al. 2013) as well as from *P. filgueirasii* and *P. burmanii* (unpublished data). The origin of the base chromosome number $x = 6$ in *Paspalum* and the cytological relationships between species having $x = 6$ and $x = 10$ is unknown (Pitman et al. 1987), although fusion of entire chromosomes and

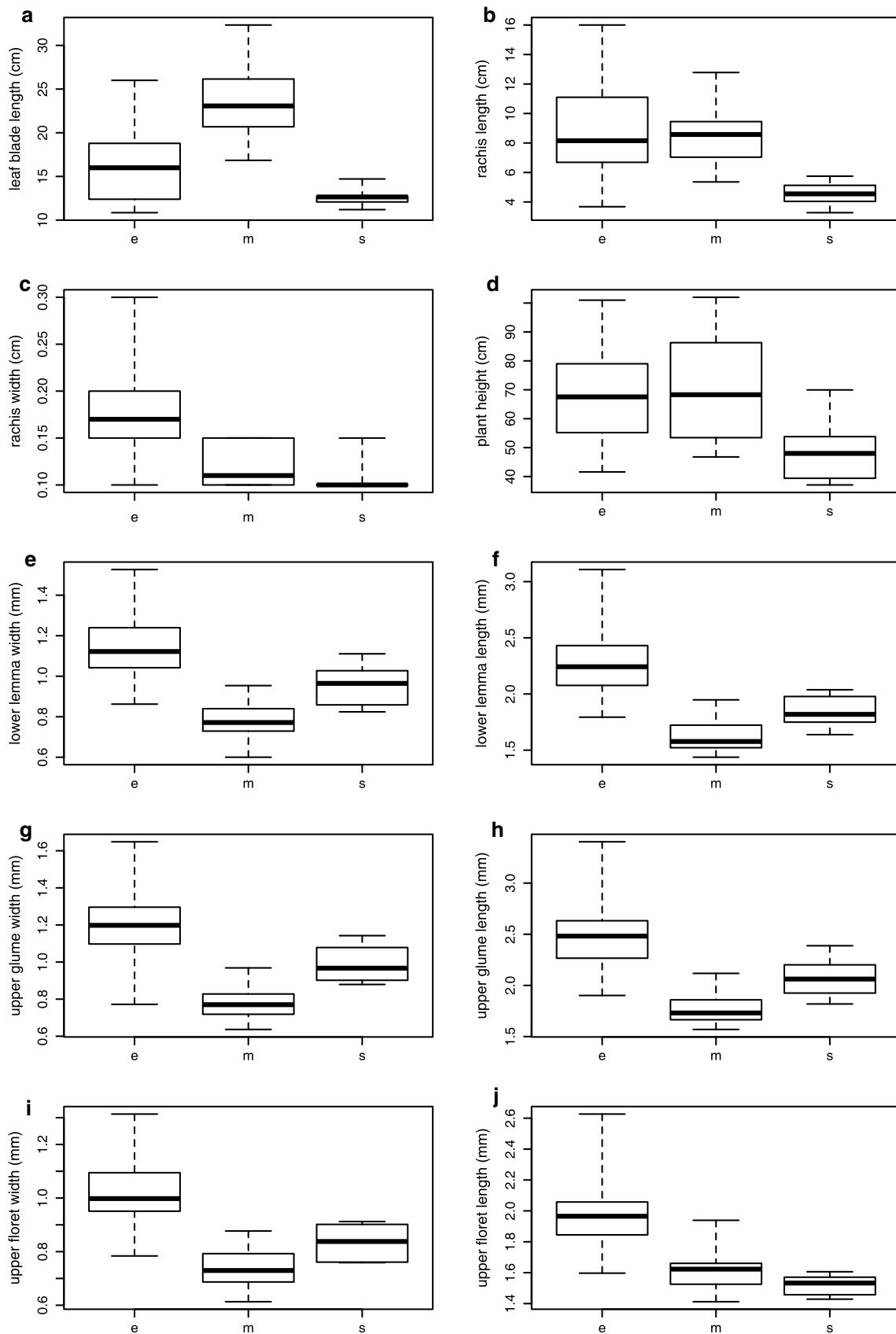


Fig. 6 Box and whisker plot of morphological characters, where the whiskers represent the min–max ranges, for *Paspalum eucomum* (e), *P. malmeanum* (m), and *P. schesslii* sp. nov. (s). **a** Leaf blade length, **b** rachis length, **c** rachis width, **d** plant height, **e** lower lemma width, **f** lower lemma length, **g** upper glume width, **h** upper glume length, **i** upper floret width, and **j** upper floret length

chromosome rearrangements have been suggested as plausible causes (Pitman et al. 1987; Scataglini et al. 2013).

In agreement with previous reports (Killeen 1990; Sede et al. 2010), the chromosome number $2n = 20$ ($x = 10$) is here confirmed for Bolivian accessions of *P. malmeanum*. Furthermore, chromosome counts for *P. eucomum* are here reported for the first time. The chromosome numbers $2n = 30$ and $2n = 32$ were found in the two available accessions of this species. Irregular meiosis was observed in the accession G. H. Rua et al. 939 ($2n = 30$) with formation of trivalents, suggesting that it is an autotriploid. It

could be presumed that it has asexual reproduction as do other autopolyploids of the genus (Quarin 1992), although this hypothesis should be corroborated by embryo sac cuttings and/or progeny studies.

Many species of *Paspalum* include both diploid and polyploid cytotypes. Diploid cytotypes are usually self-incompatible and sexual, whereas polyploid cytotypes are mostly apomictic, pseudogamous, and self-fertile (Quarin and Norrmann 1990; Quarin 1992; Valls 2000). Among the uncommon species having a base chromosome number $x = 6$, the only one for which both diploid and polyploid cytotypes are known is *P. alnum*. Indeed, diploids with $2n = 2x = 12$ chromosomes (sub *P. hexastachyum* Parodi, Quarin 1974) and autopolyploids with $2n = 4x = 24$ chromosomes (Quarin 1974; Burson 1975; Sader and Honfi 2007) have been reported. *Paspalum malmeanum* $2n = 20$ and the new species $2n = 12$ cannot be considered conspecific cytotypes as in the case of *P. alnum* and other *Paspalum* species having polyploid series, because both entities behave as diploids having different chromosome base numbers.

As far as the available cytological data show, morphological characters are associated with chromosome number and ploidy level. Indeed, the three groups discriminated by multivariate analysis, and also recognized by qualitative and quantitative discrete characters, respectively, include $2n = 12$ diploids (sp. nov.), $2n = 20$ diploids (*P. malmeanum*), and polyploids (*P. eucomum*). Summarizing, it becomes evident from our morphological and cytological evidence that the Brazilian entity with $2n = 12$ chromosomes corresponds to a new species, which is here formalized as *P. schesslii*.

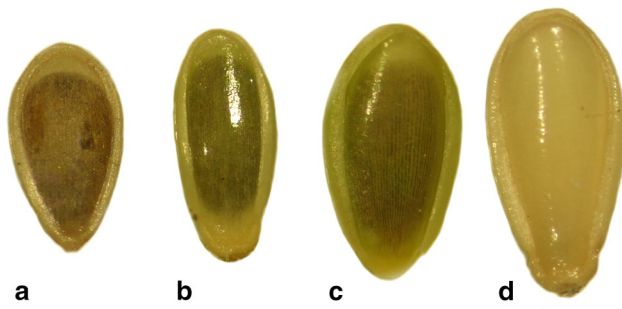
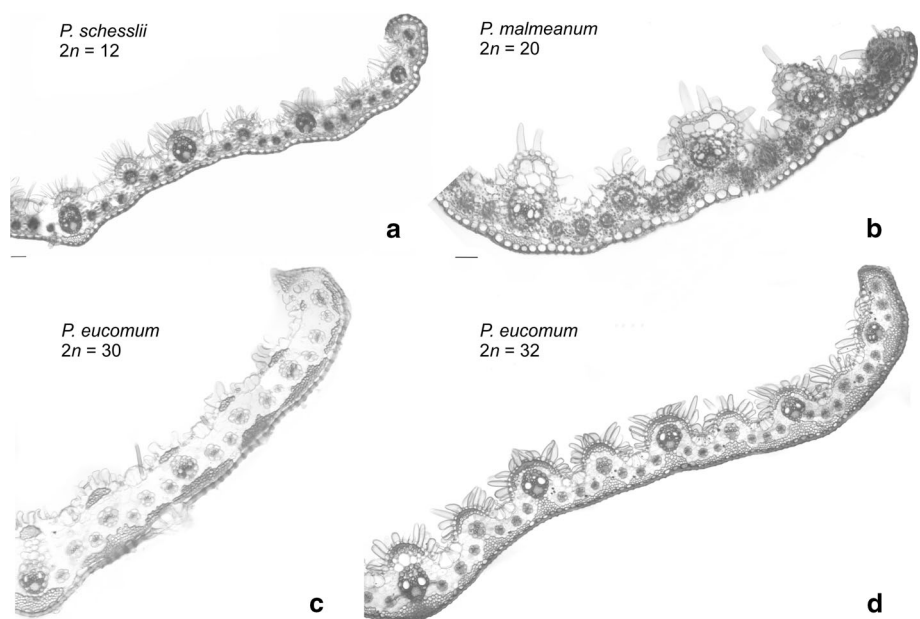


Fig. 7 Upper florets **a** *Paspalum schesslii* sp. nov., G. H. Rua et al. 991, **b** *P. malmeanum*, G. H. Rua 1040, **c** *P. eucomum*, G. H. Rua et al. 1124, **d** *P. eucomum*, G. H. Rua et al. 939. Scale bar 1 mm

Fig. 8 Leaf anatomy in transversal section **a** *Paspalum schesslii* sp. nov., G. H. Rua et al. 991, **b** *P. malmeanum*, G. H. Rua 1040, **c** *P. eucomum*, G. H. Rua et al. 939, **d** *P. eucomum*, G. H. Rua et al. 1124. Scale bar 50 μ m



It has been suggested that the polyploid cytotypes of *P. stellatum* with $2n = 32$ and $2n = 52$ chromosomes could have been originated through allopolyploidy, involving diploid *P. stellatum* ($x = 10$) and a chromosome donor with $x = 6$ (Sader et al. 2008; Bonasora et al. 2011a). The discovery of a new entity having $2n = 12$, which is sympatric to diploid populations of *P. stellatum* and morphologically related to this species, is consistent with this hypothesis, and its evolutionary implications will be further explored in an upcoming article. It would be pointed out the lack of large chromosomes in the mitotic cells of the cytotype $2n = 32$, similar to those of the new species ($2n = 12$). This fact, however, does not necessarily conflict with the allopolyploidy hypothesis, since allopolyploidization events can cause genome rearrangements in the genetic material, the outcome of which can differ from the expected if both parental genomes are barely added together (Vaio et al. 2007; Feldman et al. 2012; Tayalé and Parisod 2013).

Moreover, a similar putative origin can be postulated for the cytotype $2n = 32$ of *P. eucomum*. Morphological similarities between *P. eucomum* $2n = 32$ and *P. schesslii* $2n = 12$ support this hypothesis, since the studied accessions share branched culms, a feature also mentioned by Denham et al. (2002), and a very similar leaf anatomy (Fig. 8a, d). More extensive collections and further cytological studies become necessary for a better understanding of the taxonomic and cytological status of *P. eucomum*. In fact, it seems to be a polyploid complex involving entities with different chromosome base numbers, what may explain its extraordinary morphological variability.

Taxonomic treatment

Paspalum schesslii Bonasora & G.H.Rua, **sp. nov.**—HOLOTYPE (designated here): Brazil, Mato Grosso, Mun. Porto Estrela, 15°39'57.8"S, 57°16'22.2"W, 212 m, 17 Apr 2011, G. H. Rua et al. 975 (BAA!; isotypes: UB!, US!) Fig. 9.

Etymology: The new species is dedicated to Michael Schessl, German researcher, devoted to study the vegetation of Pantanal and adjacent areas and collector of one of the paratypes.

Caespitose perennials. Tillers orthotropic, with a few basal striate, hirsute cataphylls followed by well-developed foliage leaves. Culms 40–55 cm tall, ca. 1.4 mm diam in the lowermost internodes, erect, branched; internodes 5–6, glabrous; nodes glabrous. Leaf sheaths up to 4 cm long, dorsally rounded, striate, pubescent at the base. Ligules ca. 0.2 mm long, membranous, truncate, hyaline, glabrous, pseudoligule ca. 0.4 mm. Blades 7–20 cm long, ca. 0.2 mm wide in flowering culms, linear, ribbon-like, ascending, flat, the base continuing with the leaf sheath, acuminate at apex, coarsely hirsute at the base of both margins,

otherwise glabrous; upper blades reduced. Peduncles 13–19 cm long, terete, glabrous, purplish. Inflorescences exserted; main axis truncate or ending in a short naked point; racemes 2, (1.2)2–7 cm long, conjugate, diverging; pulvini densely albo-pubescent; rachis of the racemes 0.1–0.15 mm wide, glabrous, narrowly winged, ending in a naked point, the middle portion greenish to purple, the wings purple; pedicels ca. 0.4 mm long, shortly hirsute, with radiate hairs at the apex. Spikelets solitary, imbricate in 2 series, dorsiventrally compressed. Lower glumes lacking. Upper glumes 1.8–2.4 mm long, ca. 0.8–1 mm wide, ovate, acute at apex, membranous, whitish, purple-tinged toward apex, 3-nerved, the lateral nerves submarginal, densely silky-pilose along the margins and on the external surface, except on the distal quarter that is glabrous, the marginal hairs up to 2 mm long. Lower lemmas similar to upper glumes, but the hairs shorter, up to 0.4 mm long. Upper florets 1.4–1.6 mm long, 0.7–0.9 mm wide, obovate, inverted-drop shaped, rounded at apex, plano-convex, crustaceous, glabrous, ivory, shiny, the upper lemma faintly nerved, barely stipitate (stipe up to 0.1 mm long), deciduous at maturity; lodicules 2, ca. 0.1 mm long; stamens 3, anthers ca. 1.3 mm long, purple-tinged; stigmas 2, purple, plumose. Caryopses 1.2 mm long, 0.7 mm, obovate, plano-convex, brownish; hilum elliptical, ca. 1/3 of the caryopsis length.

Paspalum schesslii differs from *P. eucomum* by its shorter stature [plants 40–55 cm vs. (40)50–100 cm tall], and by its shorter racemes [3–5 vs. (2)4–16 cm] and smaller upper florets (1.4–1.6 vs. 1.6–2.6 mm length). It differs from *P. malmeanum* by its shorter plants (40–55 vs. 60–100 cm), its branched culms (vs. unbranched), shorter leaf blades (11–15 vs. 17–32 cm), shorter racemes (3–5 cm vs. 6–12), barely stipitate (vs. sessile), deciduous (vs. firmly attached), obovate (vs. narrowly elliptic) upper florets, and a base Chromosome number $x = 6$ (vs. $x = 10$).

Chromosome number: $2n = 12$ (Fig. 2a).

Habitat: The new species inhabits wet areas on the edge of 'cerrado' vegetation, where it forms patches usually associated with *P. stellatum* (Fig. 10).

Distribution area: *Paspalum schesslii* has been only collected in the immediacy of Cuiabá, State of Mato Grosso, Brazil.

Additional specimens examined: Brazil, Mato Grosso, Mun. Cuiabá, Cuiabá a Chapada dos Guimarães, 15°33'25.2"S, 55°47'00"W, 225 m, 15 Mar 1996, G. H. Rua et al. 237 (BAA!); Mun. Cuiabá, 15°27'49.6"S, 56°03'01.9"W, 192 m, 18 Apr 2011, G. H. Rua et al. 991 (BAA!, UB!); Mun. Porto Estrela, 15°39'57.8"S, 57°16'22.2"W, 212 m, 17 Apr 2011, G. H. Rua et al. 981 (BAA!, UB!). Mun. Nossa Senhora do Livramento, 16°06'33.6"S, 56°19'54.2"W, 135 m, 16 Apr 2011, G. H. Rua et al. 955 (BAA!, UB!); Mun. Nossa Senhora do

Fig. 9 *Paspalum schesslii* sp. nov. **a** Habit (Scale bar 2 cm), **b** upper glume, **c** lower lemma, **d** upper floret, dorsal view, **e** upper floret, ventral view (Scale bar 1 mm). Drawn from the type specimen (G. H. Rua et al. 1975) by Natalia Gómiz



Fig. 10 Photographs of *Paspalum schesslii* sp. nov. in its habitat (Mun. Porto Estrela, Mato Grosso, Brazil), cohabiting with *P. stellatum* **a** patch of *P. schesslii* and *P. stellatum* in a wet area on the edge of ‘cerrado’ vegetation, **b** detail of inflorescences. *Black asterisks, P. schesslii* sp. nov. and *white asterisks, P. stellatum*



Livramento, about 25 km. north of Pirizal village, 16°00'00"S, 56°13'00"W, 140 m, 24 Mar 1993, *M. Schessl* 3096 (BAA!).

Key to *Paspalum schesslii* and related species

- 1a. Rachis remarkably winged, 4–10 mm wide.....
..... *P. stellatum*.
1b. Rachis inconspicuously winged, 1–3 mm wide....2.
2a. Upper florets narrowly elliptic, firmly attached to the
rachilla; racemes 1–5 *P. malmeanum*.
2b. Upper florets obovate, deciduous or not, racemes
2..... 3.
3a. Plants (40)50–100 cm high; racemes 4–16 cm
long; upper florets 1.6–2.6 mm long, deciduous or
not..... *P. eucomum*.
3b. Plants 40–55 cm high; racemes 3–5 cm long; upper
florets 1.4–1.6 mm long, deciduous at maturity
..... *P. schesslii*.

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Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

Informed consent For this type of study formal consent is not required.

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