Plant Syst Evol (2015) 301:2325–2339
DOI 10.1007/s00606-015-1231-0

ORIGINAL ARTICLE

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

Marisa G. Bonasora[1] · Marisa T. Pozzobon[2] · Ana I. Honfi[3] · Gabriel H. Rua[1]

Received: 3 October 2014 / Accepted: 26 June 2015 / Published online: 29 July 2015

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

Handling editor: Tony Verboom.

Electronic supplementary material The online version of this article (doi:10.1007/s00606-015-1231-0) contains supplementary material, which is available to authorized users.

✉ Marisa G. Bonasora
bonasora@agro.uba.ar

[1] Cátedra de Botánica Sistemática, Facultad de Agronomía, Universidad de Buenos Aires, Av. San Martín 4453, C1417DSE Buenos Aires, Argentina

[2] EMBRAPA Recursos Genéticos e Biotecnologia, Parque Estação Biológica, Final W-5 Norte, Caixa Postal 02372, Brasília, DF 70770-900, Brazil

[3] Programa de Estudios Florísticos y Genética Vegetal, Instituto de Biología Subtropical, UNaM-CONICET, Rivadavia 2370, N3300LDX Posadas, Argentina

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
P. eucomum	Brazil	GO, Alexania		16°04′56″S	48°30′25,8″W	*A. C. Allem* et al.	*2638*	CEN
P. eucomum	Brazil	DF, Brasília		15°46′47″S	47°55′47″W	*S. P. Almeida*	*1076*	CEN, UB
P. eucomum	**Brazil**	GO, Corumbá de Goiás		**15°55′25″S**	**48°48′31″W**	***W. R. Anderson***	***10,403***	**UB**
P. eucomum	Brazil	DF, São Sebastião	1195	15°54′32″S	47°41′25″W	*A. S. Rodrigues*	*310*	CEN
P. eucomum	Brazil	GO, Pirenópolis		15°51′09″S	48°57′33,1″W	*A. S. Rodrigues*	*120*	CEN
P. eucomum	**Brazil**	PR, Ponta Grossa	**807**	**25°12′01,2″S**	**50°04′08,5″W**	***A. Araújo***	***165***	**BAA**
P. eucomum	Brazil	DF, Brasília	1109	15°54′44″S	47°53′10″W	*R. G. Chacon*	*41*	CEN
P. eucomum	**Brazil**	MG, Serra do Cipó	**800**	**19°20′37″S**	**43°36′21″W**	***M. A. Chase***	***9097***	**MOBOT, BAA**
P. eucomum	**Brazil**	MT, Juscimeira		**16°20′00″S**	**55°00′00″W**	***M. A. Chase***	***11,962***	**MOBOT, NYBG**
P. eucomum	**Brazil**	DF, Sobradinho	**1075**	**15°44′01″S**	**47°44′10″W**	***C. A. S. Correia***	***212***	**UB**
P. eucomum	**Brazil**	DF, Brasília	**ca. 1000**	**15°36′00″S**	**47°42′00″W**	***J. A. da Silva***	***1***	**CEN**
P. eucomum	Brazil	GO, Alto Paraiso de Goias	1500	14°07′57″S	47°30′36″W	*J. A. da Silva*	*94*	CEN
P. eucomum	**Brazil**	DF, Brasília	**ca. 1000**	**15°36′00″S**	**47°42′00″W**	***J. A. da Silva***	***12_1***	**CEN**
P. eucomum	Brazil	DF, Brasília	ca. 1000	15°36′00″S	47°42′00″W	*J. A. da Silva*	*310_A*	CEN
P. eucomum	**Brazil**	DF, Brasília	**ca. 1000**	**15°36′00″S**	**47°42′00″W**	***J. A. da Silva* and *J. Fonseca Jr***	***105_1***	**CEN**
P. eucomum	Brazil	MG, Barbacena	1080	21°12′00″S	43°49′00″W	*G. Davidse* and *T. P. Ramamoorthy*	*10724*	UB, NYBG
P. eucomum	Brazil	DF, Brasília	ca. 1035	15°45′00″S	47°42′00″W	*J. O. De Jesus*	*3*	UB
P. eucomum	Brazil	GO, Luziânia		16°15′09″S	47°57′01″W	*J. C. Dianese*	*16*	UB
P. eucomum	Brazil	GO, Luziânia		16°15′09″S	47°57′01″W	*J. C. Dianese*	*25*	UB
P. eucomum	**Brazil**	GO, Luziânia		**16°15′09″S**	**47°57′01″W**	***J. C. Dianese***	***39***	**UB**
P. eucomum	**Brazil**	MG, Dores do Indaiá	**ca. 670**	**19°26′00″S**	**45°36′00″W**	***G. Eiten***	***A-275***	**UB**
P. eucomum	Brazil	DF, Brasília		15°48′00″S	47°52′00″W	*E. P. Eringer*	*10,162*	UB
P. eucomum	Brazil	GO, Itaguaru		15°48′43″S	49°34′10,9″W	*A. F. M. Glaziou*	*22,555*	G
P. eucomum	**Brazil**	MG, Uberaba		**19°44′54″S**	**47°55′55″W**	***R. Goodland***	***3358***	**UB**
P. eucomum	**Brazil**	SP, Botucatu	**550**	**22°45′00″S**	**48°25′00″W**	***I. S. Gottsberger***	***2265***	**UB**
P. eucomum	Brazil	DF, Brasília	1070	15°55′00″ S	48°01′00″ W	*J. Guimarães Faria* et al.	*26*	CEN
P. eucomum	Brazil	PR, Tibagi	900	24°30′33,8″S	50°24′49″W	*G. Hatschbach* et al.	*59,101*	MOBOT, UFMG, UEFS
P. eucomum	Brazil	DF, Brasília		15°57′00″S	47°56′00″W	*H. L. Cesar*	*596*	UB
P. eucomum	**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**
P. eucomum	**Brazil**	MT, Juruena		**10°17′23″S**	**58°12′00″W**	***J. G. Kuhlmann***	***1668***	**US**
P. eucomum	**Brazil**	DF, Brasília	**ca. 1032**	**15°45′00″S**	**47°51′00″W**	***N. Lima* and *Heringer***	***155***	**UB**
P. eucomum	Brazil	MG, Jaboticatubas	ca. 734	19°21′30,7″S	43°52′24,6″W	*L. B. Smith*	*6969*	CEN
P. eucomum	**Brazil**	MG, Goiânia		**16°41′18,8″S**	**49°16′00,4″W**	***A. Macedo***	***1818***	**MOBOT, BAA**
P. eucomum	Brazil	DF, Brasília	1080	15°52′00″S	48°01′00″W	*B. M. T. Walter*	*4846*	CEN
P. eucomum	Brazil	DF, Brasília		15°43′53″S	47°55′36″W	*C. R. Martins*	*378*	UB
P. eucomum	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
P. eucomum	Brazil	GO, Cocalzinho de Goiás	800	15°05′00″S	48°43′00″W	*R. C. Oliveira* and *J. B. Pereira*	*273*	CEN
P. eucomum	Brazil	MG, Itutinga	ca. 1082	21°17′53,1″S	44°39′28″W	*S. C. Pereira*	*5218*	UB
P. eucomum	Brazil	MG, Itutinga	ca. 1082	21°17′53,1″S	44°39′28″W	*S. C. Pereira*	*5247*	UB
P. eucomum	Brazil	MG, Pimenta	ca. 789	20°31′00″S	45°48′00″W	*S. C. Pereira*	*5272*	UB
P. eucomum	Brazil	MG, De Capitólio	ca. 790	20°36′00″S	46°03′00″W	*S. C. Pereira*	*5289*	UB
P. eucomum	Brazil	DF, Brasília	ca. 1120	15°54′44″S	47°00′46″W	*G. Pereira-Silva* and *A. B. Sampaio*	*3900*	CEN
P. eucomum	Brazil	DF, Brasília	1050	15°45′00″S	47°42′00″W	*D. Philcox* and *E. Oniski*	*4870*	UB
P. eucomum	**Brazil**	GO, Cristalina	**ca. 1240**	**16°45′00″S**	**47°40′00″W**	***C. E. B. Proença* and *R. S. Oliveira***	***1781***	**UB**
P. eucomum	Brazil	MT, Sete Lagoas		19°27′00″S	44°10′00″W	*R. C. F. C. de Carvalho*	*s.n.*	CEN
P. eucomum	Brazil	MG, Belo Horizonte	ca. 1200	19°58′00″S	43°54′00″W	*L. Roth*	*2420*	CEN
P. eucomum	Brazil	DF, Brasília	ca. 1115	15°47′00″S	47°53′00″W	*J. R. Santos* and *G. A. Moreira*	*29*	CEN
P. eucomum	**Brazil**	DF, Fercal	**870**	**15°32′15″S**	**47°50′06″W**	***G. H. Rua***	***908***	**CEN, BAA**
P. eucomum*	**Brazil**	PR, Ponta Grossa		**25°01′42,1″S**	**50°04′36,7″W**	***G. H. Rua***	***1124***	**BAA**
P. eucomum	Brazil	DF, Brasília	1115	15°53′25″S	48°00′28″W	*G. H. Rua* et al.	*879*	CEN, BAA
P. eucomum	Brazil	DF, Fercal	912	15°34′37″S	47°56′13″W	*G. H. Rua* et al.	*899*	CEN, BAA
P. eucomum*	**Brazil**	GO, Mineiros	**872**	**17°22′44,6″S**	**52°55′26,8″W**	***G. H. Rua* et al.**	***939***	**BAA, UB**
P. eucomum	Brazil	MT, General Carneiro	503	15°35′47,7″S	53°03′05″W	*G. H. Rua* et al.	*1009*	BAA, UB
P. eucomum	**Brazil**	DF, Brasília	**1054**	**15°44′07″S**	**47°55′39″W**	***G. H. Rua* et al.**	***837***	**CEN, BAA**
P. eucomum	**Brazil**	DF, Fercal	**832**	**15°32′39,2″S**	**47°50′05″W**	***G. H. Rua* et al.**	***1024***	**BAA, UB**
P. eucomum	Brazil					*H. L. Sello*	*s.n.*	UB
P. eucomum	Brazil	MG				*F. Sellow*	*s.n.*	US
P. eucomum	Brazil	DF, Brasília	1080	15°50′23,4″S	47°55′21″W	*F. Sellow*	*s.n.*	K, HAL
P. eucomum	Brazil	DF, Brasília	1080	15°50′23,4″S	47°55′14,2″W	*F. Sellow*	*s.n.*	K
P. eucomum	Brazil					*F. Sellow*	*s.n.*	UB
P. eucomum	**Brazil**	DF, Brasília	**1020**	**15°45′00″S**	**47°52′00″W**	***F. C. Silva* et al.**	***427***	**CEN, MBM**
P. eucomum	Brazil	TO, Mateiros	520	10°43′00″S	46°47′00″W	*L. H. Soares e Silva* et al.	*952*	UB
P. eucomum	**Brazil**	GO, Padre Bernardo	**1020**	**15°33′00″S**	**48°15′00″W**	***R. F. Vieira* et al.**	***721***	**CEN**
P. eucomum	Brazil	MG, Paraopeba	900	19°24′24″S	44°19′39″W	*Z. L. Wagner* and *P. L. Viana*	*9599*	CEN, UFMG
P. malmeanum	Bolivia	SCZ, Velasco	250	14°36′09″S	60°51′00″W	*E. Gutiérrez* et al.	*655*	MO
P. malmeanum	**Bolivia**	SCZ, Ñuflo de Chávez	**500**	**16°02′00″S**	**62°00′00″W**	***T. J. Killeen***	***898***	**MOBOT**
P. malmeanum	**Bolivia**	SCZ, Ñuflo de Chávez	**500**	**16°03′00″S**	**62°10′00″W**	***T. J. Killeen***	***2024***	**MOBOT**
P. malmeanum	**Bolivia**	SCZ, Ñuflo de Chávez	**500**	**16°00′00″S**	**62°00′00″W**	***T. J. Killeen***	***2076***	**MOBOT, US**
P. malmeanum	**Bolivia**	SCZ, Ñuflo de Chávez	**500**	**16°13′00″S**	**62°00′00″W**	***T. J. Killeen***	***2478***	**SI, US, MOBOT**
P. malmeanum	**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
P. malmeanum	**Brazil**	MG, Santa Ana da Chapada		**15°26′00″S**	**55°45′00″W**	***G. O. A. Malme***	***s.n. (isotype)***	**MOBOT, US**
P. malmeanum	Bolivia	SCZ, Velasco	150	14°36′00″S	60°50′34″W	*B. Mostacedo* et al.	*1654*	MO
P. malmeanum*	**Bolivia**	SCZ, Ñuflo de Chávez	**483**	**16°11′05″S**	**62°00′54,4″W**	***G. H. Rua***	***1040***	**BAA**
P. malmeanum	**Bolivia**	SCZ, Ñuflo de Chávez	**481**	**16°09′57,8″S**	**62°01′32,6″W**	***G. H. Rua***	***1044***	**BAA**
P. malmeanum	**Bolivia**	SCZ, Ñuflo de Chávez	**478**	**16°00′47,7″S**	**62°01′08,5″W**	***G. H. Rua***	***1047***	**BAA**
P. malmeanum	**Bolivia**	SCZ, Ñuflo de Chávez	**477**	**16°00′51″S**	**62°01′06,3″W**	***G. H. Rua***	***1050***	**BAA**
P. malmeanum	Bolivia	SCZ, Chiquitos	822	18°20′26″S	59°34′00″W	*J. R. T. Wood* et al.	*24624*	USZ
P. malmeanum	Bolivia	SCZ, Velasco	425	16°24′07″S	61°11′54,4″W	*J. R. T. Wood* et al.	*24805*	USZ
***P. schesslii* sp. nov.**	**Brazil**	MT, Cuiabá		**15°33′25,2″S**	**55°47′00″W**	***G. H. Rua***	***237***	**BAA**
***P. schesslii* sp. nov.**	Brazil	MT, Nossa Senhora do Livramento	135	16°06′33,6″S	56°19′54,2″W	*G. H. Rua* et al.	*955*	BAA, UB
P. schesslii* sp. nov.	**Brazil**	MT, Porto Estrela	**212**	**15°39′57,8″S**	**57°16′22,2″W**	***G. H. Rua* et al.**	***975***	**BAA, UB**
***P. schesslii* sp. nov.**	**Brazil**	MT, Porto Estrela	**212**	**15°39′57,8″S**	**57°16′22,2″W**	***G. H. Rua* et al.**	***981***	**BAA, UB**
P. schesslii* sp. nov.	Brazil	MT, Cuiabá	192	15°27′49,6″S	56°03′02″W	*G. H. Rua* et al.	*991*	BAA, UB
***P. schesslii* sp. nov.**	**Brazil**	MT, Nossa Senhora do Livramento	**140**	**16°00′00″S**	**56°13′00″W**	***M. Schessl***	***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 fuchsine 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 CO_2, 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. Microsporocytes 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 Axioplan 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-

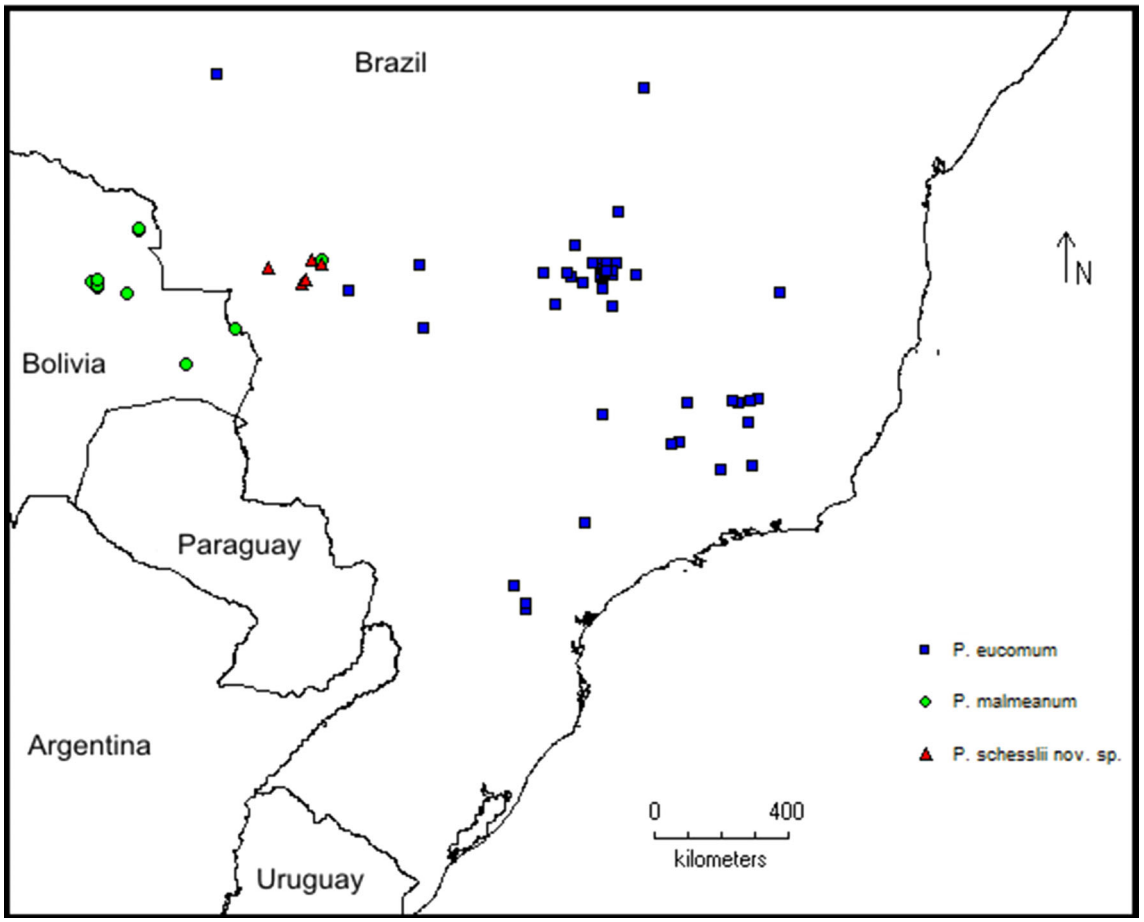


Fig. 1 Geographical locations of studied specimens *Paspalum eucomum* (*squares*), *P. malmeanum* (*circles*), and *P. schesslii* sp. nov. (*triangles*)

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-μm 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 laggard 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 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).

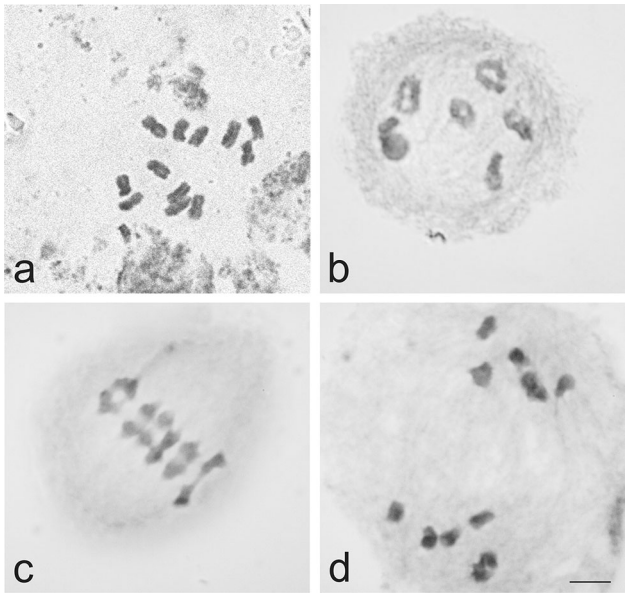


Fig. 2 *Paspalum schesslii* sp. nov., *G. H. Rua* et al. *975* **a** chromosomes at mitotic metaphase $2n = 12$, **b** microsporocyte in diakinesis with 6II, **c** microsporocyte in late metaphase I, **d** anaphase I. *Scale bar* 5 μm

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

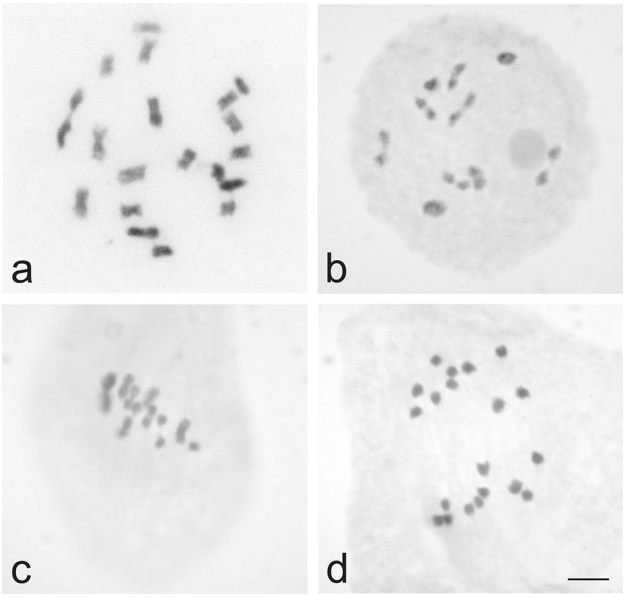


Fig. 3 *Paspalum malmeanum*, *G. H. Rua 1040* **a** chromosomes at mitotic metaphase $2n = 20$, **b** microsporocyte in diakinesis with 10II, **c** metaphase I, **d** anaphase I. *Scale bar* 5 μm

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

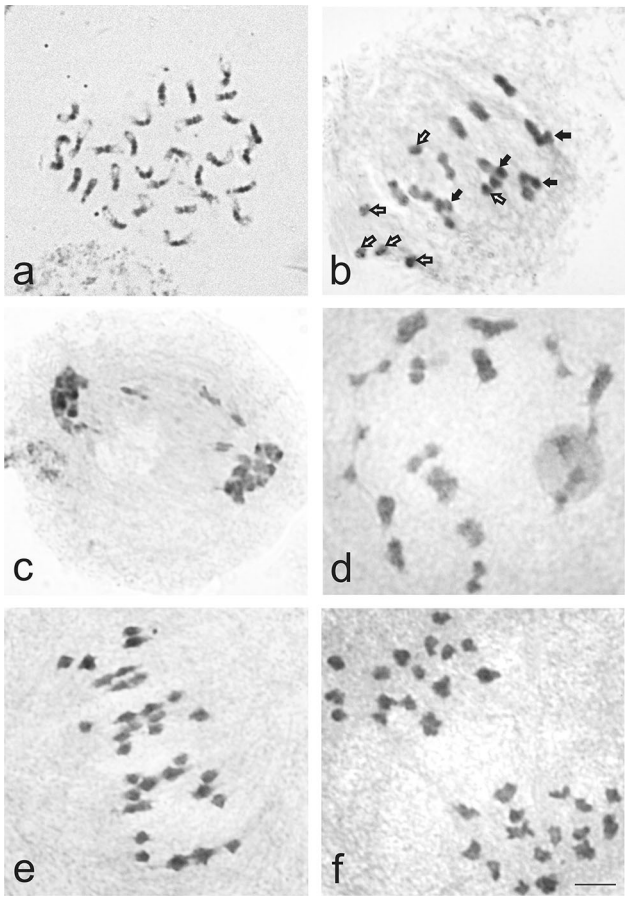


Fig. 4 *Paspalum eucomum*, *G. H. Rua* et al. *939* **a** chromosomes at mitotic metaphase $2n = 30$, **b** microsporocyte in metaphase I with formation of 6I + 6II + 4III. *White arrows* indicated univalents and *black arrows* trivalents, **c** anaphase I with delayed segregation of some chromosomes. *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 μm

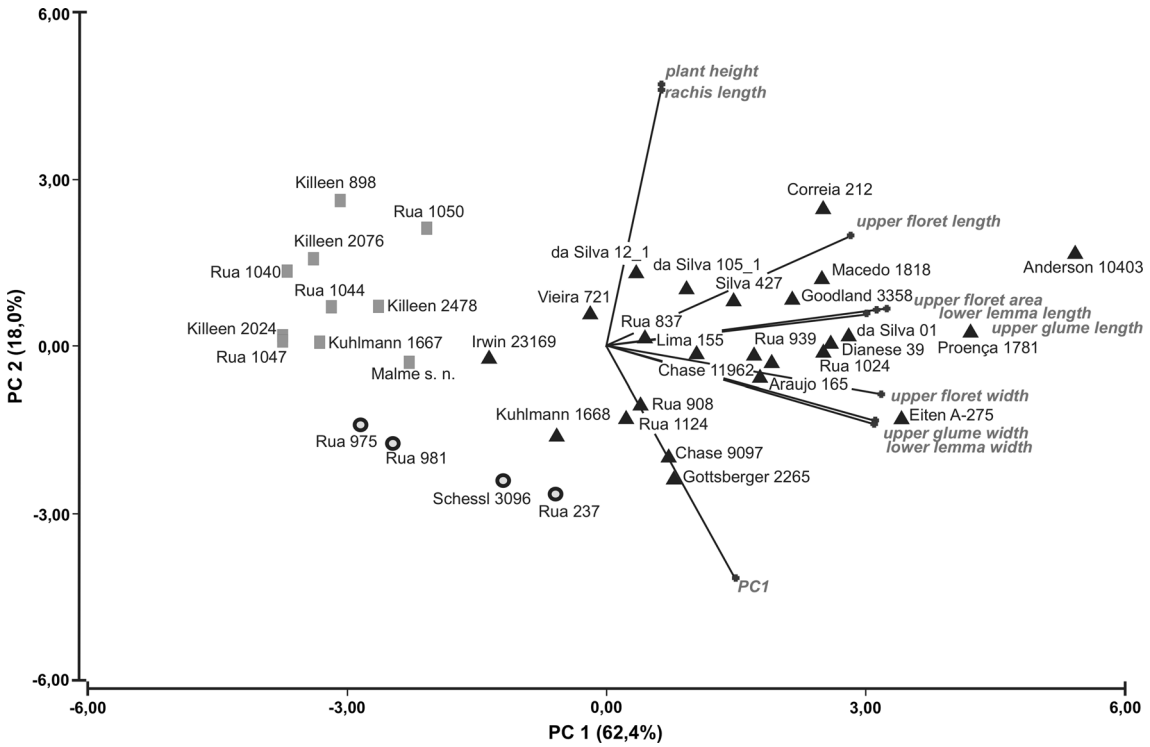


Fig. 5 Principal component analysis of morphological data; triangles *Paspalum eucomum*, squares *P. malmeanum*, circles *P. schesslii* sp. nov.

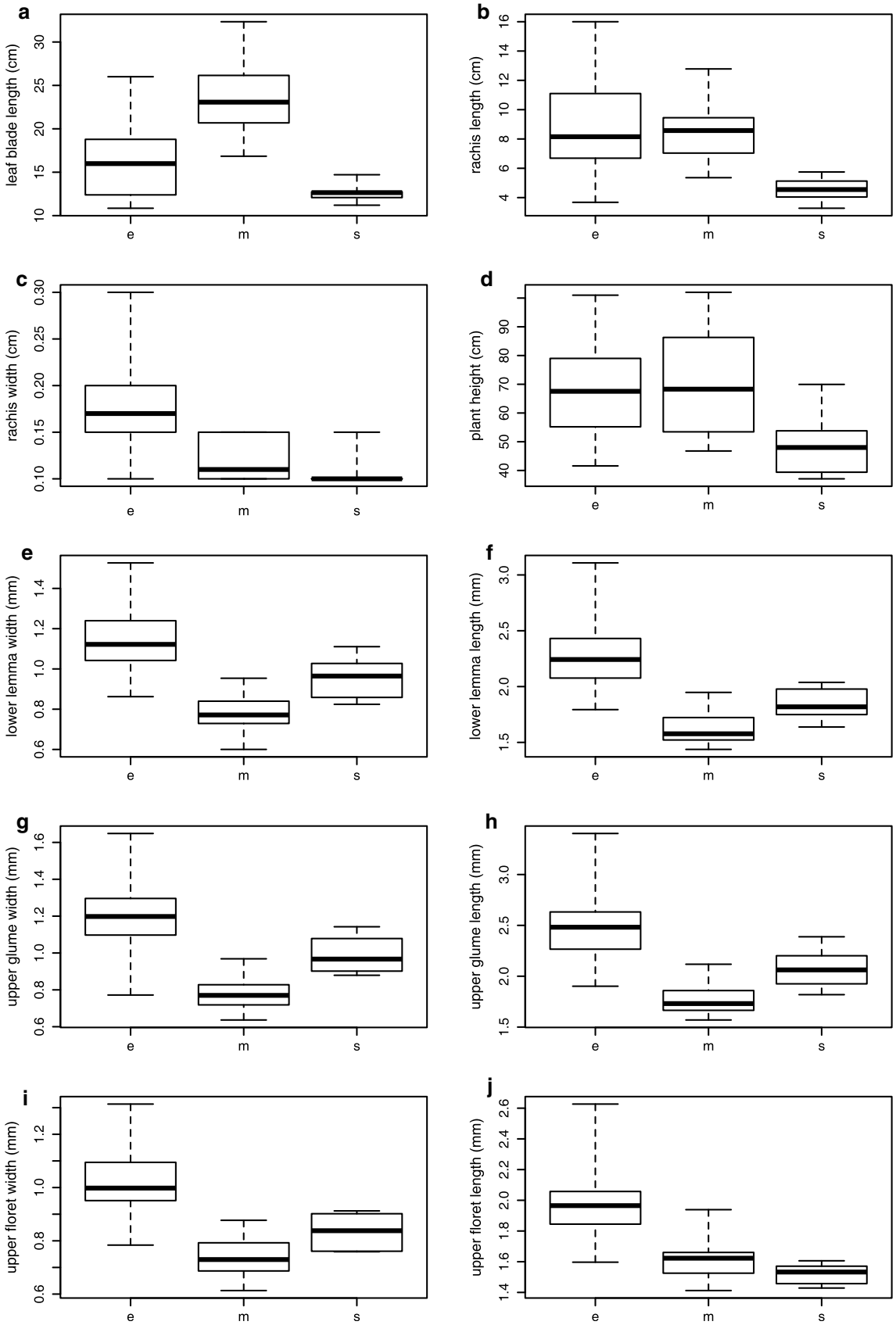
bundles associated to adaxial colorless parenchyma (Fig. 8a, c, d). Instead, *P. malmeanum* (*G. H. Rua 1040*) was distinguished by having an open U-shaped outline and first order vascular bundles with adaxial extensions of colorless parenchyma (Fig. 8b).

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. almum* 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. almum* (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


a
leaf blade length (cm)
10 15 20 25 30
e m s
b
rachis length (cm)
4 6 8 10 12 14 16
e m s
c
rachis width (cm)
0.10 0.15 0.20 0.25 0.30
e m s
d
plant height (cm)
40 50 60 70 80 90
e m s
e
lower lemma width (mm)
0.6 0.8 1.0 1.2 1.4
e m s
f
lower lemma length (mm)
1.5 2.0 2.5 3.0
e m s
g
upper glume width (mm)
0.6 0.8 1.0 1.2 1.4 1.6
e m s
h
upper glume length (mm)
1.5 2.0 2.5 3.0
e m s
i
upper floret width (mm)
0.6 0.8 1.0 1.2
e m s
j
upper floret length (mm)
1.4 1.6 1.8 2.0 2.2 2.4 2.6
e m s

◀**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 autoploids 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. almum*. 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. almum* 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*.

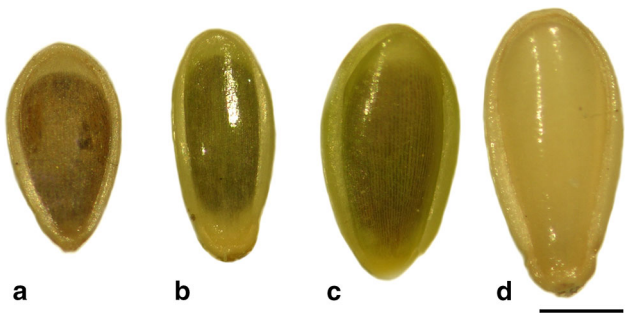


Fig. 7 Upper florets **a** *Paspalum schesslii* sp. nov., *G. H. Rua* et al. *991*, **b** *P. malmeanum, G. H. Rua 1040*, **c** *P. eucomum, G. H. Rua* et al. *1124*, **d** *P. eucomum, G. H. Rua* et al. *939*. *Scale bar* 1 mm

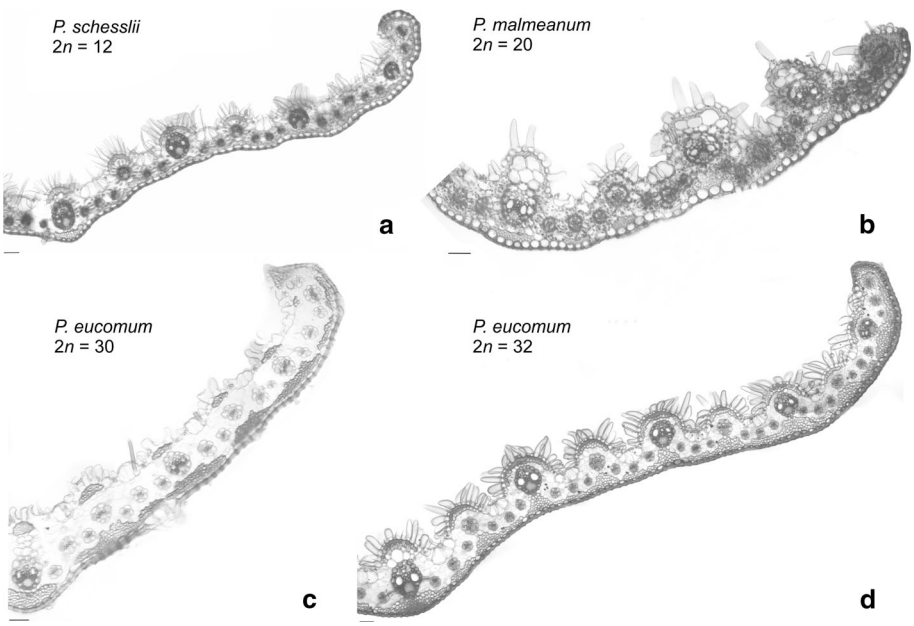


Fig. 8 Leaf anatomy in transversal section **a** *Paspalum schesslii* sp. nov., *G. H. Rua* et al. *991*, **b** *P. malmeanum*, *G. H. Rua 1040*, **c** *P. eucomum*, *G. H. Rua* et al. *939*, **d** *P. eucomum*, *G. H. Rua* et al. *1124*. *Scale bar* 50 µm

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 alloploidy 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. *975*) 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.*

Acknowledgments We are indebted to Anádria S. da Silva, Regina C. de Oliveira, and José F. M. Valls for field support in Brazil; Daniel Villarroel Segarra for kindly making available to us specimens from USZ; Arne Anderberg (S) for sending us some spikelets from one isotype of *P. malmeanum*; Susana Perelman for statistic support; Gabriela Zarlavsky, Liliana Fabbri, and Sandra Aliscioni for helpful advice in leaf anatomy procedures; the curators of BAA, CEN, MO, SI, UB, and US for making specimens available and/or providing herbarium facilities. This research was supported by grants BR/10RED/03 (Ministerio de Ciencia, Tecnología e Innovación Productiva—MINCYT, Argentina, and Coordenacão de Aperfeiçoamento de Pessoal de Nível Superior—CAPES, Brazil), UBACYT 20020090100194 (Universidad de Buenos Aires, Argentina), PICT 2011–2061 and PICT Bicentenario 1297 (Fondo para la Investigación Científica y Tecnológica, ANPCyT, Argentina). GHR and AIH are members of the ''Carrera del Investigador,'' CONICET, Argentina.

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.

References

Bonasora MG, Honfi AI, Pozzobon MT, Rua GH (2011a) Un nuevo citótipo de *Paspalum malmeanum* y su relación con los citótipos poliploides de *P. stellatum*. Bol Soc Argent Bot 46(Suppl.):51
Bonasora MG, Honfi AI, Rua GH (2011b) Citogeografía de *Paspalum stellatum* y *P. eucomum*. J Basic Appl Genet 41(Suppl.):117
Bowen CC (1956) Freezing by liquid carbon dioxide in making slides permanent. Biotech Histochem 31:87–90
Burson BL (1975) Cytology of some apomictic *Paspalum* species. Crop Sci 15:229–232
Chase A (1929) The North American species of *Paspalum*. Contrib US Natl Herb 28:1–310
D'Ambrogio de Argüeso A (1986) Manual de técnicas en histología vegetal. Hemisferio Sur, Buenos Aires
Davidse G, Pohl RW (1974) Chromosomes number, meiotic behavior, and notes on tropical American grasses (Gramineae). Canad J Bot 50:1441–1452
Denham S, Zuloaga F, Morrone O (2002) Systematic revision and phylogeny of *Paspalum* subgenus Ceresia (Poaceae: Panicoideae: Paniceae). Ann Missouri Bot Gard 89:337–399
Development Core Team (2012) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna. Avaliable at: http://cran.r-project.org
Di Rienzo JA, Casanoves F, Balzarini MG, Gonzalez L, Tablada M, Robledo CW (2008) InfoStat, versión 2008. Grupo InfoStat, FCA, Universidad Nacional de Córdoba, Córdoba. Available at: http://www.infostat.com.ar
Echarte AM, Clausen AM (1993) Afinidades morfológicas entre *Paspalum distichum* sensu lato y *P. vaginatum* (Poaceae). Bol Soc Argent Bot 29:143–152
Echarte AM, Clausen AM, Sala CA (1992) Números cromosómicos y variabilidad morfológica de *Paspalum distichum* (Poaceae) en la provincia de Buenos Aires (Argentina). Darwiniana 31:185–197
Ekman EL (1911) Neue brasilianische Gräser. Ark Bot 10:1–43
Ellis RP (1976) A procedure for standardizing comparative leaf anatomy in the Poaceae I. The leaf-blade as viewed in transverse section. Bothalia 12:65–109
Feldman M, Levy A, Chalhoub B, Kashkush K (2012) Genomic plasticity in polyploid wheat. In: Soltis PS, Soltis DE (eds) Polyploidy and genome evolution. Springer, Heidelberg, pp 109–135
Guerra M (2012) Citotaxonomy: the end of childhood. Pl Biosystems 146:703–710
Guma I, Echarte A, Clausen A (1995) Análisis multivariado de caracteres epidérmicos foliares de *Paspalum distichum* y *P. vaginatum* (Poaceae). Darwiniana 33:107–114
Halappanarar SP, Chennaveeraiah MS (1981) A report on the cytology of *Paspalum compactum* var. *fimbriatum*. Curr Sci 50:649–650
Hojsgaard DA, Honfi I, Rua GH, Daviña J (2009) Chromosome numbers and ploidy levels of *Paspalum* species from subtropical South America (Poaceae). Genet Resour Crop Evol 56:533–545
Honfi AI (2003) Citoembriología de poliploides impares en el género *Paspalum* (Panicoideae: Gramineae). PhD thesis, Universidad Nacional de Córdoba, Córdoba
Honfi AI, Quarin CL, Valls JFM (1990) Estudios cariológicos en gramíneas sudamericanas. Darwiniana 30:87–94
Iwata H, Ukai Y (2002) SHAPE: a computer program package for quantitative evaluation of biological shapes based on elliptic Fourier descriptors. J Heredity 93:384–385. Avaliable at: http://lbm.ab.a.u-tokyo.ac.jp/~iwata/shape/
Killeen T (1990) The grasses of Chiquitanía, Santa Cruz, Bolivia. Ann Missouri Bot Gard 77:125–201
Mehra PN, Chaudhary JD (1973) Gramineae. In: Löve Á (ed) IOPB chromosome number reports XLVI. Taxon 23:802–118
Oliveira RC (2004) O genero *Paspalum* L., grupo Plicatula (Poaceae: Paniceae) no Brasil. PhD thesis, Universidade Estadual de Campinas, São Paulo
Peñaloza APS, Côrtes AL, Pozzobon MT, Santos S, Rua GH, Valls JFM (2008) Unusual chromosome numbers in *Paspalum* L. (Poaceae: Paniceae) from Brazil. Genet Molec Res 7:399–406
Pitman M, Burson B, Bashaw E (1987) Phylogenetic relationships among *Paspalum* species with different base chromosome numbers. Bot Gaz 148:130–135
Pohl RW (1980) *Paspalum*. In: Burger W (ed) Flora costaricensis, Fieldiana Botany 4 (new series):400–459

Pozzobon MT, Valls JFM, Santos S (2000) Contagens cromossômicas em espécies brasileiras de *Paspalum* L. (Gramineae). Acta Bot Brasil 14:151–162

Quarin CL (1974) Relaciones citotaxonómicas entre *Paspalum almum* Chase y *P. hexastachyum* Parodi (Gramineae). Bonplandia 3:115–127

Quarin CL (1992) The nature of apomixis and its origin in Panicoid grasses. Apomixis Newslett 5:7–15

Quarin CL, Norrmann GA (1990) Interspecific hybrids between five *Paspalum* species. Bot Gaz 151:366–369

Reeder JR (1984) Poaceae. In: Löve Á (ed) IOPB chromosome number reports LXXXII. Taxon 33:132–133

Rua GH, Gróttola CM (1997) Growth form models within the genus *Paspalum* L. (Poaceae, Paniceae). Flora 192:65–80

Rua GH, Speranza PR, Vaio M, Arakaki M (2010) A phylogenetic analysis of the genus *Paspalum* L. (Poaceae) based on cpDNA and morphology. Pl Syst Evol 288:227–243

Sader MA, Honfi AI (2007) Los cromosomas de *Paspalum almum* Chase. J Basic Appl Genet XVIII (Suppl.):114–115

Sader MA, Honfi AI, Hojsgaard DH, Daviña JR (2008) Análisis citogenético en poblaciones naturales de *Paspalum stellatum* Humb. & Bonpl. ex Flüggé (Poaceae) de Argentina y Paraguay. J Basic Appl Genet XIX(Suppl.):114–115

Scataglini MA, Zuloaga FO, Giussani LM, Denham SS, Morrone O (2013) Phylogeny of new world *Paspalum* (Poaceae, Panicoideae, Paspaleae) based on plastid and nuclear markers. Pl Syst Evol 300:1051–1070

Sede S, Escobar A, Morrone O, Zuloaga FO (2010) Chromosome studies in American Paniceae (Poaceae, Panicoideae). Ann Missouri Bot Gard 97:128–138

Selva SB (1976) Some preliminary cytological observations on a new basic number in *Paspalum convexum* (Gramineae). Canad J Bot 54:385–394

Silva AS (2013) Delimitação taxonômica e variabilidade genética de *Paspalum polyphyllum* Nees ex Trin. e *Paspalum bicilium* Mez (Poaceae, Paspaleae). Ms Sc thesis, Universidade de Brasília, Brasília

Stebbins GL (1971) Chromosomal evolution in higher plants. Edward Arnold, London

Tayalé A, Parisod C (2013) Natural pathways to polyploidy in plants and consequences for genome reorganization. Cytogenet Genome Res 140:79–96

Vaio M, Mazzella C, Porro V et al (2007) Nuclear DNA content in allopolyploid species and synthetic hybrids in the grass genus Paspalum. Pl Syst Evol 265:109–121

Valls JFM (2000) Impacto do conhecimento citogenético na taxonomia de *Paspalum* e *Axonopus*, Gramineae. In: Cavalcanti TB, Walter BMT (eds) Tópicos atuais em Botânica. SBB/Embrapa Recursos e Biotecnologia, Brasília, pp 57–60

Valls JFM, Oliveira RC (2013) *Paspalum eucomum*. Lista de espécies da flora do Brasil. http://www.reflora.jbrj.gov.br/jabot/floradobrasil. Accessed 25 Feb 2015

Zarlavsky GE (2014) Histología vegetal: técnicas simples y complejas. Sociedad Argentina de Botánica, Buenos Aires

Zhang D, Lu G (2002) Shape-based image retrieval using generic Fourier descriptor. Signal Process Image 17:825–848

Zuloaga FO, Morrone O (2005) Revisión de las especies de *Paspalum* para América del Sur austral: (Argentina, Bolivia, sur del Brasil, Chile, Paraguay y Uruguay). Monogr Syst Bot Missouri Bot Gard 102:1–297