

Cytological studies in four species of *Mesosetum* (Arthropogoninae) reveal the lowest chromosome number among the Neotropical Poaceae

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Abstract Until recently, the basic chromosome number $x = 8$ was suggested for the *Mesosetum* genus, which was considered rare in the subtribe Arthropogoninae (Poaceae, Paspaleae). However, the number of accessions and species analysed is not large and the relation of chromosome numbers in this genus with others of the subtribe is uncertain. In this study, cytological and reproductive information in other species of *Mesosetum* was provided enabling a broad critical analysis of chromosomes evolution in the clade. Chromosome counting and meiotic behaviour profile were determined for six accessions of *Mesosetum* belonging to four different species. Mature embryo sac structure was determined to predict sexual or apomictic mode of reproduction. All accessions showed $2n = 8$ chromosomes, number recorded for first time in Neotropical Poaceae and Paniceae s. l. Chromosomes showed similar length and presence of one satellite pair in two perennial species. Meiosis was regular, with the formation of four bivalents whilst the embryo sacs were of *Polygonum* type. Although the basic chromosome number $x = 10$ appears to be plesiomorphic in Paspaleae tribe, current data indicate $x = 4$ as a possible additional number. These findings indicate that polyploidy could be involved in *Mesosetum* evolution and speciation.

Keywords Meiotic behaviour · Monocots · Reproduction · Grasses · Cytogenetic

Introduction

Most Poaceae species show broad chromosome size and number diversity (Schapova 2012), with basic numbers varying from $x = 2$ to $x = 18$ and somatic numbers, from $2n = 4$ to $2n = 263$ – 265 (De Wet 1987). This cytological diversity is believed to be related to aneuploidy, euploidy and hybridization processes during evolution of Poaceae species (Hilu 2004; Schapova 2012). However, for most tropical and subtropical species, current data available are as yet scarce (Honfi et al. 1991; Sede et al. 2010).

Although $x = 9$ and $x = 10$ are the predominant basic chromosome numbers in the subfamily Panicoideae, other basic numbers can also be found in this subfamily (Hilu 2004). Within the tribe Paniceae s. l., reports on chromosome numbers in *Mesosetum* Steud. suggest the basic number $x = 8$, which is rare in this tribe (Sede et al. 2010).

Mesosetum is a Neotropical genus comprising 25 species distributed from southern Mexico to northeast Argentina mainly in open habitats (Filgueiras 1989). Brazil harbours the centre of diversity of the genus with 21 species (Filgueiras 1989). In the last taxonomic revision by Filgueiras (1986), *Mesosetum* species are classified into five sections: *Bifaria* (Hack.) Chase; *Loliiformia* Swallen; *Mesosetum*; *Penicillata* Swallen and *Sclerochloae* Swallen. According to the phylogenetic tree proposed by Morrone et al. (2012), the genus *Mesosetum* is within the tribe Paspaleae ($x = 10$), subtribe Arthropogoninae. This subtribe includes heterogeneous genera considering morphological, anatomical and physiological features. Therefore, it is considered to belong to an “ambiguous clade”

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Table 1 Chromosome numbers in *Mesosetum* species, voucher number and collection locality

Species	Voucher	Count	Collection locality
<i>M. alatum</i> Filg.	RCO et al. 2765	$2n = 8$	Brazil, Mato Grosso, Água Boa, 14°18' 45"S, 52° 10' 05"W
<i>M. annuum</i> Swallen	ASS et al. 269	$2n = 8$	Brazil, Goiás, Flores de Goiás, 14° 24' 063"S, 47° 03' 0,29"W
<i>M. ansatum</i> (Trin.) Kuhl.	RCO et al. 2766	$2n = 8$	Brazil, Mato Grosso, Poconé, 16° 21' 04"S, 56° 29' 05"W
	RCO et al. 2773	$2n = 8$	Brazil, Mato Grosso, Poconé, 16° 20' 10"S, 56° 18' 06"W
	RCO and LR 2784	$2n = 8$	Brazil, Mato Grosso, Chapada dos Guimarães, Cachoeira do Reino
<i>M. longiaristatum</i> Filg.	RCO and CWF 2816	$2n = 8$	Brazil, Goiás, Teresina de Goiás, 13° 37' 44"S, 47° 12' 41"W

ASS Anádría Stéphanie da Silva, CWF Christopher William Fagg, LR Luciana Rabellato, RCO Regina Célia de Oliveira



Fig. 1 Chromosome set in perennial species of *Mesosetum* with $2n = 8$ **a–c** *M. alatum*, RCO 2765; **a** mitotic metaphase with the arrows indicating the satellite chromosomes; **b** inflorescence segment

showing the rachis; **c** spikelet; **d–f** *M. ansatum*, RCO 2766; **d** mitotic metaphase with the arrows indicating the satellite chromosomes; **e** segment of inflorescence; **f** spikelet

according to Giussani et al. (2001). On the other hand, based on the analysis of one species (*M. chauseae*), Morrone et al. (2012) proposed that *Mesosetum* genus integrates a subclade together with *Keratochlaena* Morrone & Zuloaga and *Tatianyx* Zuloaga & Soderstr.

In *Mesosetum*, the chromosome number $2n = 16$ is recorded for three species: *M. loliiforme* (Hochst. ex Steud.) Chase, *M. chauseae* Luces and *M. pittieri* Hitchc. (Gould 1966; Gould and Soderstrom 1967; Pohl and

Davidse 1971; Davidse and Pohl 1972; Silva et al. 2012). However, Sede et al. (2010) determined for one *M. loliiforme* population the chromosome number $2n = 32$.

Reproductive data are poorly represented for the Neotropical Poaceae species. In some genera of the Paniceae s. l. tribe, such as *Panicum* L., *Paspalum* L. and *Urochloa* P.Beauv., diploid cytotypes commonly display the sexual mode of reproduction, whilst polyploids are preferentially apomictic (Quarín and Norrmann 1987;

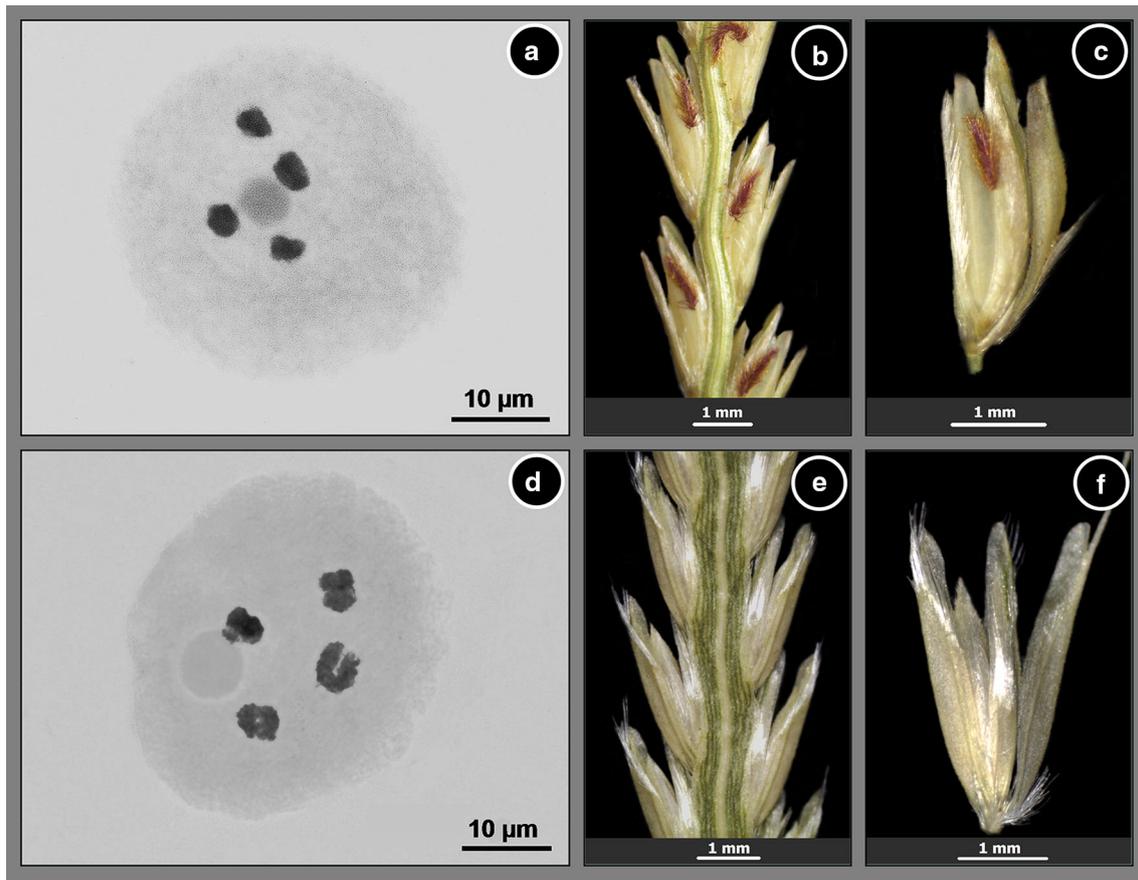


Fig. 2 Chromosome set in annual species of *Mesosetum* with $2n = 8$. **a–c** *M. annuum*, ASS 269; **a** diakinesis; **b** inflorescence segment showing the rachis; **c** spikelet; **d–f** *M. longiaristatum*, RCO 2816; **d** diakinesis; **e** inflorescence segment showing rachis; **f** spikelet

Table 2 Mitotic chromosome length (μm) in perennial *Mesosetum* species ($2n = 8$)

Cells analysed	<i>M. alatum</i> (RCO 2765)	<i>M. ansatum</i> (RCO 2766)
1	3.90–6.19	3.83–5.44
2	3.65–5.08	3.73–5.05
3	4.27–5.49	3.36–5.10
4	4.31–5.30	4.11–5.35
5	–	3.28–4.73
Min. and Max.	3.65–6.19	3.28–5.44

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Quarín and Burson 1991; Valle and Savidan 1996; Quarín et al. 2001; Hojsgaard et al. 2009; Ma et al. 2009; Hojsgaard et al. 2014; and others). For *Mesosetum*, these relationships are not yet clear. First, because the cytogenetic knowledge of *Mesosetum* is scarce and mostly restricted to chromosome number records and only *M. chaseae* had its embryo sac identified as of the *Polygonum* type (Silva et al. 2013), suggesting a sexual reproductive mode.

Considering the little knowledge on the cytogenetic and reproduction aspects of *Mesosetum* species, little information available on the basic chromosome number, and complexity of evolution in the group, this work provides further *Mesosetum* cytological aspects based on the analysis of six accessions of four different species, including data on chromosome number, meiotic behaviour and characterization of embryo sac to estimate the reproductive mode. These studies revealed a new and the lowest chromosome number in Paniceae s. l. tribe and for Neotropical Poaceae species, a valuable data to help understanding the position and evolution of *Mesosetum*.

Materials and methods

Plant material

Six accessions of *Mesosetum* Steud. included in four species: *M. alatum* Filg., *M. annuum* Swallen, *M. ansatum* (Trin.) Kuhlman and *M. longiaristatum* Filg. were analysed. Plants of these accessions were collected in the states of

Goiás and Mato Grosso, Brazil (Table 1). Plants were maintained in greenhouses at the Universidade de Brasília, Brasília-DF, Brazil (UnB) and Embrapa Genetic Resources and Biotechnology, Brasília-DF, Brazil. Vouchers were deposited in CEN and UB herbaria (acronyms in Thiers 2015).

Chromosome number, size and meiotic behaviour

Somatic chromosome number was determined following Pozzobon and Valls (1997), with minor modifications. Roots were pretreated with saturated α -bromonaphthalene aqueous solution for 2 h, at room temperature, then immersed in Carnoy fixative solution 3:1 (ethanol: acetic acid; v/v), and samples were then stored in 70 % ethanol aqueous solution at 4 °C. At least four metaphase cells from root meristem with well spread and clean chromosomes were observed after squashing into acetic carmine 2 % solution on a histological slide.

Meiotic behaviour was determined during microsporogenesis using at least 200 cells for each phase of meiosis (from diakinesis to tetrad). Samples were isolated from young inflorescences and immersed in the Carnoy fixative solution for 24 h at room temperature and stored in 70 % ethanol solution at 4 °C. Images capture and chromosome analyses were performed using Zeiss Axiophot microscope (Zeiss, Germany) and Axiovision Software (version 4.8.2).

Reproduction mode

Inflorescences with flowers at anthesis were collected from *M. ansatum* (RCO 2766) and immersed in a fixative solution containing 40 % formaldehyde, glacial acetic acid and 50 % ethanol, 5:5:90, (v/v), for 24 h at 4 °C and then stored in 70 % ethanol at 4 °C. Ovaries from mature hermaphrodite flowers were then isolated, dehydrated in an ethanol with crescent concentration series (80, 90, 95 and 100 %), treated with increasing proportions of the mixture xylol: methyl salicylate 3:1, 1:1 and 1:3, (v/v) and, finally, incubated in methyl salicylate as described by Araújo et al. (2004). The number and morphology of embryo sacs within each ovary were observed using Differential Interference Contrast (DIC) mode in a Zeiss Axiophot microscope. Images were captured using the Axiovision Software (version 4.8.2).

Results

Chromosome number $2n = 8$ was determined in all samples of the accessions analysed (Table 1; Figs. 1, 2). *M. alatum*, *M. ansatum* and *M. longiaristatum* belong to the section *Bifaria* whilst *M. annuum*, to the *Loliiformia*

section. The latter species and *M. longiaristatum* are annual species whilst the first two are perennials.

The plants of annual species (*M. annuum* and *M. longiaristatum*) did not survive under greenhouse conditions. In addition, their small root systems collected in the field did not provide sufficient samples for observation of the chromosome sizes at mitotic metaphase.

Chromosome lengths at mitotic metaphase of the perennials species *M. ansatum* and *M. alatum* varied from 3.28 to 5.44 and 3.65 to 6.19 μm , respectively. A single pair of satellite chromosomes was observed in each species and their lengths varied from 1.26 to 1.56 μm in *M. alatum* and about 0.57 μm in *M. ansatum* (Table 2; Fig. 1).

Meiotic behaviour along microsporogenesis was regular in all accessions analysed, with formation of four bivalents (Table 3; Fig. 3) and two predominant configurations: with 4 rings bivalents or 3 rings associated with 1 rod bivalents (Fig. 3a).

Meiotic irregularities such as asynchrony at meiosis II were observed in all species, although at low frequency (Table 3; Fig. 3i, k). Precocious chromosome ascension at metaphase I (Fig. 3c) and laggard chromosome at telophase I (Fig. 3f) were rarely observed and only in *M. ansatum*, whilst in *M. annuum* adherence at metaphase II was rare but present, and in *M. longiaristatum* bridges at anaphase I seldom observed. The meiotic index represented by the percentage of the presence of regular pollen tetrads (Love 1949) was around 90 % in all the analysed accessions, confirming their meiotic stability (Table 3).

Mature female gametophytes within 40 ovaries of *M. ansatum* (RCO 2766) were analysed to determine their structure. All ovaries showed one single embryo sac *Polygonum* type (Fig. 4). Besides the egg cell and two synergids, proliferating antipodal cells and two polar nuclei within the central cell could be observed in each embryo sac. The antipodal cells were observed at the chalazal pole in numbers varying from 4 to 15, although the majority showed 6 to 8 cells, with a polyhedral shape and voluminous appearance. Central cell cytoplasm is filled by vesicles, surrounded by a large vacuole, and the polar nuclei situated at the micropyle pole (Fig. 4a–c). This *Polygonum* type embryo sac structure with proliferating antipodals is common in sexually reproducing grasses (Koltunow 1993).

Discussion

The chromosome number $2n = 8$ registered for the accessions of *M. alatum*, *M. annuum*, *M. ansatum* and *M. longiaristatum* is new, and also the lowest chromosome number found for the genus, other Paniceae s. l. and species of Neotropical Poaceae. The dispersion of this

Table 3 Meiotic behaviour in *Mesosetum* species ($2n = 8$)

Species/accession	D + M I (%)	A I + T I (%)	P II + M II (%)	A II + T II (%)	I. M. (%)	Total cells
<i>M. annuum</i> ASS 269	408 (100.00)	410 (100.00)	410 (87.80)	400 (100.00)	243 (100.00)	1871
<i>M. ansatum</i> RCO 2766	526 (99.62)	471 (99.79)	480 (99.38)	407 (99.02)	215 (96.41)	2099
<i>M. longiaristatum</i> RCO 2816	442 (100.00)	433 (99.54)	426 (100.00)	425 (100.00)	220 (100.00)	1946

Percentages are regularity rates

D diakinesis, *M* metaphase I and II, *A* anaphase I and II, *T* telophase I and II, *P II* prophase II, *IM* Meiotic index

chromosome number in all accessions analysed, added to the occurrence of regular meiosis with four bivalents, provides strong evidence of this new basic chromosome number $x = 4$ in *Mesosetum* and in Paniceae s. l. tribe. Based on the angiosperm chromosome database (Goldblatt and Johnson 2015) and other authors (compiled at Table 4), the number $2n = 8$ chromosomes is rare in species of Poaceae, registered so far only for seven genera: six belonging to the tribe Poeae, and one to Andropogoneae (Table 4). All previous $2n = 8$ chromosome counts are related to species from the Old World and Oceania (Table 4), mostly found in Holarctic and Temperate Boreal regions.

In the genera *Zingeria* P.A. Smirn. and *Colpodium* Trin. (Poeae tribe), allopolyploidy is the hypothesis that explains the origin of $2n = 8$ chromosome number, originating from an ancestor with $2n = 4$, $x = 2$ (Kotseruba et al. 2003, 2005; Kim et al. 2009). This basic chromosome number $x = 2$ had its origin attributed to a fusion event from an ancestor with $x = 7$ that is the predominant basic number in Poeae (Cremonini et al. 2003; Hilu 2004; Cremonini 2005; Kim et al. 2009).

Nevertheless, the origin of $2n = 8$ within the genus *Mesosetum* is still uncertain. Based on morphological aspects, *Mesosetum* is divided into five sections (Chase 1911; Swallen 1937; Filgueiras 1989), and the section *Bifaria* was earlier considered by some authors, a segregated genus from *Mesosetum* (Kuntze 1898). Although there are morphological differences, the occurrence of $2n = 8$ chromosome number determined for species from sections *Bifaria* and *Loliiformia* reveals cytogenetic similarities between these sections.

Chromosome length (3.28–6.19 μm) herein observed at mitotic metaphase for *M. ansatum* and *M. alatum* from *Bifaria* section (both $2n = 8$) show similar lengths to those in *M. bifarium* (3.52–6.39 μm), also belonging to *Bifaria* section, but with $2n = 16$ (Ribeiro 2013). Furthermore, Ribeiro (2013) found tetravalent association at diakinesis in *M. bifarium* with $2n = 16$ chromosomes. Based on these data, it can be suggested that

chromosome number $2n = 16$ within *Bifaria* section (*M. bifarium* and *M. chaseae*, Ribeiro 2013 and Silva et al. 2012, respectively) must have originated from polyploidy in plants with $2n = 8$ chromosomes.

According to Sede et al. (2010), tetraploid species with bivalent pairing predominate in the Neotropical Paniceae. The results of the present study, combined with those of Silva et al. (2012) and Ribeiro (2013), confirm the occurrence of this pattern in *Mesosetum*. The low frequency of tetravalents observed in $2n = 16$ by Ribeiro (2013) substantiates the meiotic stability at the tetraploid level.

Whilst the suggestion of the polyploidy could be a plausible to explain the relationship between species with $2n = 8$ and $2n = 16$ chromosomes within the genus *Mesosetum*, the relationship between $2n = 8$ ($x = 4$) and the chromosome number of genera close to *Mesosetum* can be more complex and hardly explained by polyploidy events alone. According to the phylogenetic tree in Panicoideae and Paniceae s. l. established by Giussani et al. (2001) and Morrone et al. (2012), the basic chromosome number $x = 10$ is plesiomorphic in tribe Paspaleae and already registered in *Tatianyx* ($2n = 60$ and $x = 10$, Morrone et al. 1995), genus that integrates a subclade highly supported together with *Mesosetum* and *Keratochlaena* (x not determined). Cytological studies in more species and populations of these genera will be essential to clarify the relationship between $x = 4$ and $x = 10$.

Polyploidy in Paniceae species is usually linked to an apomictic mode of reproduction (Valle and Savidan 1996; Hojsgaard et al. 2009; Ma et al. 2009; and others). Herein, the embryo sac analyses in *M. ansatum* ($2n = 8$), as well as those in *M. chaseae* ($2n = 16$) (Silva et al. 2013), indicated the presence of a single *Polygonum* type of embryo sac, implying a sexual mode of reproduction. However, further morphological embryo sac analysis in a larger number of individuals from other populations of *Mesosetum* species is needed to confirm the prevalence of sexual reproduction mode.

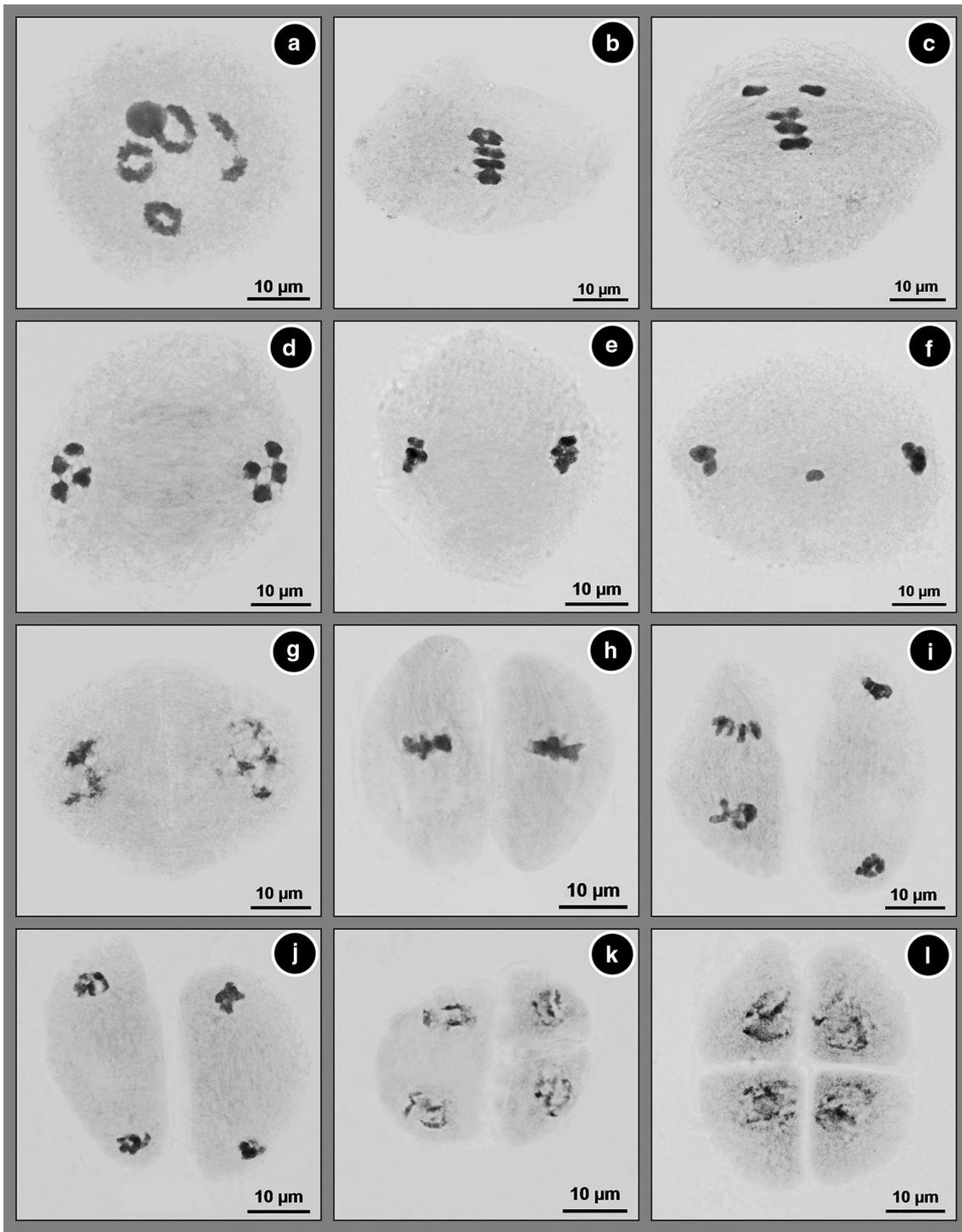


Fig. 3 Microsporogenesis in *Mesosetum ansatum*, RCO 2766. **a** Diakinesis; **b–c** metaphase I; **b** regular; **c** one pair with precocious ascension; **d** anaphase I; **e–f** telophase I; **e** regular; **f** with laggard;

g prophase II; **h** metaphase II; **i** anaphase II with asynchrony; **j** telophase II; **k–l** tetrad; **k** with asynchronous division; **l** regular

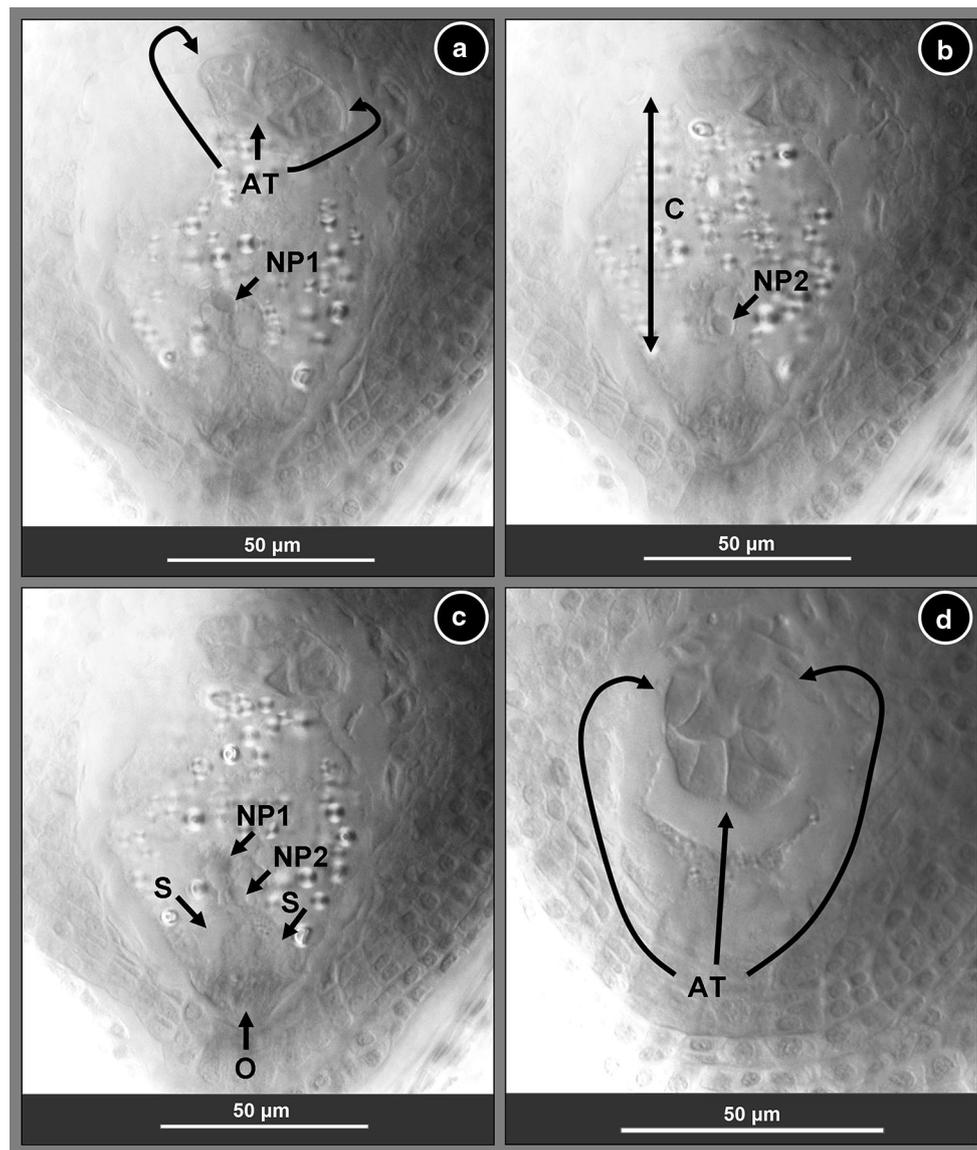


Fig. 4 Micrographs of cleared ovules of *Mesosetum ansatum*, RCO 2766. **a–c** Single embryo sac of *Polygonum* type in different focal planes; **d** aberrant embryo sac with 15 antipodals and no

distinguishing polar nuclei, synergids or egg cell. *AT* antipodals, *NP1* and *NP2* polar nuclei within the central cell (*C*), *S* synergids, *O* eggcell

Conclusions

The present work suggests a new basic chromosome number $x = 4$ in the genus *Mesosetum*, the tribe Paniceae s. l. and Neotropical grasses, being the lowest reported so far. The impact of this novelty for the Neotropical grasses highlights the strategic importance of consistent cytological characterization of plants, mostly those originating from remote areas of the Tropical America. Since the full *Mesosetum* species phylogeny based on molecular data has

not yet been determined, the origin of $2n = 8$ in species of the genus is poorly understood. Further studies with increased sampling within Arthropogoninae species and populations, use of molecular markers, associated with chromosome characterization (banding, full morphology and cytogenetic markers detection by fluorescence in situ hybridization) should be encouraged to improve the understanding on the origin and evolution of this very low and new chromosome number present in *Mesosetum* species.

Table 4 Species with $2n = 8$ in Poaceae recorded in literature

Species	Gam.	Sp.	Tribe ^a	References	Geographical distribution
<i>Airopsis tenella</i> (Cav.) Asch. & Graebn.	$n = 4$	$2n = 8$	Poeae	Fernandes and Queiros (1969), Albers (1975, 1980), Devesa et al. (1990b)	Genus: SW Europe and NW Africa (Watson and Dallwitz 2015); species: SW and SE Europe and N Africa (Clayton et al. 2015)
<i>Colpodium chionogeiton</i> (Pilg.) Tzvelev		$2n = 8$	Poeae	Hedberg and Hedberg (1994)	Genus: holartic and paleotropics—mountain (Watson and Dallwitz 2015); species: E Tropical Africa (Clayton et al. 2015)
<i>Colpodium hedbergii</i> (Melderis) Tzvelev		$2n = 8$	Poeae	Hedberg and Hedberg (1994)	Species: NE, E and S Tropical Africa (Clayton et al. 2015)
<i>Holcus gayanus</i> Boiss.	$n = 4$	$2n = 8$	Poeae	Devesa et al. (1990a), Caixinhas et al. (1991)	Genus: Europe and Asia (Watson and Dallwitz 2015); species: SW Europe (Clayton et al. 2015)
<i>Iseilema laxum</i> Hack.		$2n = 8$	Andropogoneae	Celarier and Paliwal (1957), Rao (1975)	Genus: Indomalaya and Australia region (Watson and Dallwitz 2015); species: India and Indo-China (Clayton et al. 2015)
<i>Milium vernale</i> M. Bieb.		$2n = 8$	Poeae	Tutin (1982), Bennett and Thomas (1991), Bennett et al. (1992)	Genus: Holartic, Temperate boreal region (Watson and Dallwitz 2015); species: Europe, N Africa and Temperate Asia (Clayton et al. 2015)
<i>Milium vernum</i> M. Bieb.		$2n = 8$	Poeae	Thomas (1984)	Species: Europe, N Africa and Temperate Asia (Clayton et al. 2015)
<i>Periballia laevis</i> (Brot.) Asch. & Graebn.		$2n = 8$	Poeae	Albers (1980)	Genus: Holartic; SE and SW Mediterranean; Europe; N and S Africa; Temperate Asia and Oceania: Australia (Clayton et al. 2015; Watson and Dallwitz 2015); Species: SW and E Europe, N Africa (Clayton et al. 2015)
syn. <i>Molineriella laevis</i> (Brot.) Rouy	$n = 4$		Poeae	Devesa et al. (1990b)	
<i>Periballia minuta</i> (L.) Asch. & Graebn.		$2n = 8$	Poeae	Albers (1980)	Species: SE and SW Europe, N and S Africa; Temperate Asia and Oceania: Australia (Clayton et al. 2015)
syn. <i>Molineriella minuta</i> subsp. <i>australis</i> (Paunero) Rivas Mart.	$n = 4$		Poeae	Devesa et al. (1990a)	
syn. <i>Molineriella minuta</i> subsp. <i>minuta</i> (L.) Rouy		$2n = 8$	Poeae	Devesa et al. (1990a)	
<i>Zingieria pisdica</i> (Boiss.) Tutin		$2n = 8$	Poeae	Kim et al. (2009)	Genus: Holartic, Euro-Siberia (Watson and Dallwitz 2015); species: SW Europe, W Temperate Asia and Caucasus (Clayton et al. 2015)
<i>Zingieria trichopoda</i> (Boiss.) P.A. Smirn.		$2n = 8$	Poeae	Sokolovskaya and Probatova (1979), Davlianidze (1985), Ghukasyan (2003, 2004) Kotseruba et al. (2003), Rodionov et al. (2006)	Species: Temperate Asia, W Caucasus and Arabia (Clayton et al. 2015)

Gam gametophytic, Sp sporophytic

^a USDA and ARS (2015)

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