The subfamily Bambusoideae comprises three monophyletic tribes, Arundinarieae, Bambuseae and Olyreae. Here we report the gametic number and the chromosomal meiotic behavior of two species belonging to the herbaceous tribe Olyreae, *Olyra latifolia* and *Olyra humilis*. Accessions were collected in Misiones, at Northeastern Argentina. We report a new gametic number for *O. humilis*, \( n = 18 \), and we confirmed \( n = 11 \) for *O. latifolia*. Chromosomal features, like the basic and gametic chromosome number, are important in understanding the evolution of the Poaceae family, especially in delimiting clades and elucidate inter and intra-clades relationships, and therefore it is necessary to continue producing this type of data.

Key words: bamboos, cytogenetics, meiosis, ploidy, Poaceae

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**RESUMEN**

La subfamilia Bambusoideae comprende tres tribus monofiléticas, Arundinarieae, Bambuseae y Olyreae. Aquí reportamos el número gamético y el comportamiento meiótico de los cromosomas de dos especies pertenecientes a la tribu Olyreae de bambúes herbáceos, *Olyra latifolia* y *Olyra humilis*. Las introducciones se recolectaron en la provincia de Misiones, en el noreste argentino. Reportamos un nuevo número gamético para *O. humilis*, \( n = 18 \), y confirmamos \( n = 11 \) para *O. latifolia*. Los números cromosómicos básicos y gaméticos son importantes para comprender la evolución de la familia Poaceae, especialmente para delimitar sus clados y las relaciones existentes entre ellos, por lo que es necesario continuar produciendo este tipo de datos.

**Palabras clave:** bambúes, citogenética, meiosis, ploidía, Poaceae
INTRODUCTION

The subfamily of bamboo grasses (subfamily Bambusoideae) comprises 1,700 species belonging to 128 genera which are distributed worldwide (Clark et al., 2015; Zhang et al., 2020). Bambusoideae comprises three monophyletic tribes, Arundinarieae, Bambuseae and Olyreae; the first two are represented by woody bamboos, whereas Olyreae species are herbaceous without woody characteristic (Ruiz-Sanchez et al., 2021). Herbaceous bamboos have a wide distribution, inhabiting from sea level towards 2,200 m.a.s.l. (Clark et al., 2015). Olyreae species are native to the New World with one exception, i.e. Buergersiochloa that is a monotypic endemic genus of New Guinea (Oliveira et al., 2014; Clark et al., 2018).

The tribe Olyreae comprises 22 genera and 124 species, and occurs widely in the Neotropical region, from Mexico and the Caribbean to North Argentina, and Paraguay (Oliveira et al., 2014). Most genera in this tribe have species defined by vegetative characters. Generally, the spikelets exhibit little interspecific variation, with the exception of Olyra L., the most widespread and heterogeneous genus in the tribe (Judziewicz and Zuloaga, 1992). In addition, Olyra was defined as paraphyletic and polyphyletic, in order to explain diverse incongruences in phylogenetic trees based on plastid molecular markers (Judziewicz et al., 1999; Ruiz-Sanchez et al., 2019).

Olyra is the largest genus within the subtribe Olyrinae, with ca. 24 species. Probably this estimate will soon change since phylogenetic studies evidenced its non-monophyletic nature (Oliveira et al., 2014, 2020). Currently, four species of Olyra habit in Argentina, O. ciliatifolia Raddi, O. fasciculata Trin., O. humilis Nees and O. latifolia L. and the only two chromosome counts known for Argentinian specimens are from O. latifolia. Chromosomal and ploidy data are useful for clarifying phylogenetic relationships and tracing evolutionary trajectories (Honfi et al., 2021; Deanna et al., 2022). For this reason, this work aims to contribute with meiotic studies of O. latifolia and O. humilis, two significant herbaceous species of the tropical forest in Misiones.

MATERIAL AND METHODS

Table 1 shows a complete list of the studied accessions, including their geographic origin, voucher specimens, and herbaria. Herbarium acronyms follow Thiers (2017 onward). Voucher herbarium specimens were deposited at Universidad Nacional de Misiones Herbarium (MNES) and duplicates at the following herbaria: Instituto de Botánica del Nordeste (CTES), Instituto de Botánica Darwinion (SI) and Conservatoire et Jardin Botaniques de la Ville de Gèneve (G).

The analyses of chromosome meiotic behavior were made according to Honfi et al. (2021) and Reutemann et al. (2021). Briefly, young inflorescences were fixed in 3:1 ethanol: acetic acid solution for at least 24 h at room temperature. Then, spikelets were transferred to 70% ethanol at 4ºC. Pollen mother cells (PMCs) were obtained from immature anthers and were stained with acetocarmine 2%. Permanent slides were made with Venetian Turpentine solution. At least 50 PMCs at diakinesis and/or metaphase I were analyzed for each accession of both species. Anaphase (I and II) and telophase (I and II) were analyzed to observe abnormal segregation. Microphotographs were taken with a Leica camera added to a Leica optical microscope.

Table 1. Chromosome gametic number (n) of Olyra species and studied accessions.

| Species       | n  | Provenance                                                                 |
|---------------|----|----------------------------------------------------------------------------|
| O. humilis Nees | 18 | Arg. Misiones, San Pedro, Parque Provincial Moconá. Honfi 500, 25/11/1993 (MNES, CTES, G). |
|               |    | Arg. Misiones, San Pedro, Parque Provincial Moconá. Honfi 511, 25/11/1993 (MNES, CTES, G). |
|               |    | Arg. Misiones, Cainguás, Campo Grande. Honfi 582, 19/2/1994 (MNES) |
|               |    | Arg. Misiones, Parque Provincial Moconá. Seijo 856, 29/11/1993 (MNES). |
|               |    | Arg. Misiones, Parque Provincial Moconá. Daviña 125, 18/12/1992 (MNES, CTES, SI) |
| O. latifolia L. | 11 | Arg. Misiones, Parque Provincial Moconá. Seijo 863, 29/11/1993 (MNES, CTES, G) |
RESULTS AND DISCUSSION

Chromosome meiotic behavior of six Olyra South American accessions was studied (Table 1). The two Olyra species studied in this work exhibited a persistent nucleolus, which was clearly distinguishable up to (and including) diakinesis in agreement with Nicora and Rugolo de Agrasar (1987) karyosistematic definition of Bambusoideae family.

Chromosome gametic number, ploidy levels and meiotic behavior for O. humilis are reported for the first time. Persistent nucleoli, sometimes four small ones, were present in PMCs at diakinesis (Figure 1A). The studied accessions showed a regular meiotic behavior with 18 bivalents at diakinesis and metaphase I (Figure 1B). Few PMCs exhibited 17 bivalents and 2 univalents. Chromosome segregation was normal without laggards. The gametic chromosome number of n=18 is a new haploid chromosome number for the genus. Alternatively, there is a new derived basic chromosome number x=18, or a polyploid based on x=9, which is an uncommon basic chromosome number for Olyra. Previous reports of a tetraploid cytotype with x=9 were originally described as Olyra (Davidse and Pohl, 1978). Currently, these accessions belong to Parodiodyra lateralis (Presl. ex Nees von Essenbeck) Soderstrom & Zuloaga (Soderstrom and Zuloaga, 1989). Therefore, there is no basic number in Olyra to explain the gametic number n=18 found here.

In Olyreae, chromosome data suggest that Olyra species have a descending aneuploid series in basic numbers that started from an ancestral and original x=12, and nowadays is composed by x=11, 10 and 7 (Table 2). However, several authors considered x=12 as a secondary ancestral basic number (Hunziker et al., 1982). At least two hypotheses can explain the chromosome number found in O. humilis. The first hypothesis is an allotetraploid origin based on x=9, with regular chromosome pairing in bivalents. In the second one, a new derived basic chromosome number of x=18 for Olyra results from an ascending aneuploid series. For example, a polyploid origin for n=18 found in Anomochloa marantoidaeae Brongn. (Anomochloaceae) was previously proposed, considering a primitive base number x=6 for bamboos and Poaceae in general (Hunziker et al., 1989). Judziewicz and Zuloaga (1992) suggested four morphological natural groups of species within Olyra, mainly based on the presence and distribution of trichomes on the female floret. The Glaberrima group of Olyra consists in species closely related to O. glaberrima Raddi, such as O. humilis, O. maranonensis Swallen and O. davideanae Judz. & Zuloaga (Judziewicz and Zuloaga, 1992). In addition, a close relationship between O. glaberrima and O. humilis is highly supported by recent phylogenetic analyses (Oliveira et al., 2014; 2020). Furthermore, Oliveira et al. (2014) corroborated the monophyly of Arberella with species of O. glaberrima group. Cytological studies in all members of Arberella and Glaberrima group can shed light in the new chromosome number found in O. humilis, especially if they are multiple of n=18 or x=9 or 18.

In O. latifolia, a gametic chromosome number of n=11 was found. Meiotic behavior was regular with the presence of a persistent nucleolus and 11 bivalents or 10 bivalents and two univalents in PMCs at diakinesis and metaphase I (Figure 2 A, B, C). Chromosomes segregated normally at anaphase I and microsporogenesis resulted in an isobilateral tetrad of microspores (Figure 2 D, E). Our data agree with previous chromosome counts reported in several works (Tateoka, 1962; Pohl and Davidse, 1971; Davidse and Pohl, 1972a, b; 1974; Olorde, 1975; Dujardin, 1978; Hunziker et al., 1989; Guo et al., 2019). Besides, a tetraploid cytotype was registered in Bahia, Brazil (Hunziker et al., 1982), Aragua and Bolivar, Venezuela (Davidse and Pohl, 1974; 1978) and Trinidad (Davidse and Pohl, 1972b). The basic chromosome number for this species is x=11 and the meiotic behavior indicates that the studied materials from Misiones are diploid accessions. O. latifolia is a robust herbaceous species widespread in the Neotropics, and the studied accessions are from the southernmost localities in South America. Diploids are the most frequent cytotype in Neotropics (America), and the only ploidy level found in Africa. Tetraploids are commonly detected in tropical areas (Table 2, Davidse and Pohl, 1974, 1978). Clearly, O. latifolia is a polymorphic complex with diploid and polyploid phenotypes. According to Davidse and Pohl (1972b), plants with very broad leaf blades, named O. cordifolia Kunth with 2n=ca. 30 (Gould and Soderstrom, 1967) are similar to tetraploid plants (n=22) of O. latifolia. Furthermore, in highly fertile diploid Mexican accessions of this species, meiotic unpaired chromosomes behaving as univalents or heterozygous translocation were also
Table 2. Records of chromosome numbers and ploidy level (x) of Olyra species.

| Species                  | Chromosome data | x   | Location                                                                 | References            |
|--------------------------|-----------------|-----|--------------------------------------------------------------------------|-----------------------|
| O. ciliatifolia Raddi    | n=11            | 2x  | Brazil: Rio de Janeiro                                                   | Hunziker et al., 1982|
| O. fasciculata Trin.     | 2n=14, n=7      | 2x  | Brazil: Rio de Janeiro, Guanabara, Estrada da Gávea. Brazil: Rio de Janeiro| Calderon and Soderstrom, 1973; Hunziker et al., 1982|
| O. humilis Nees          | n=18            | 4x  | Costa Rica: San José, La Cisica between Alto de San Juan and La Alfombra, highway 223, 11 mi. SW of San Isidro de General | This work             |
| O. latifolia L.          | 2n=22; n=11     | 2x  | Costa Rica: 30 km N of Caiitas; El Salvador: Santa Ana: 2 km SE of Metapin; Trinidad: 1 km W of Arirna; Venezuela: bolivar, 17 km W of the Rio Caura; Cote-d’Ivoire | Reeder, 1969          |
|                          |                 |     | Costa Rica: Cartago IICA, Turrialba; Guanacaste: 6 km S of La Cruz; 1km W of Cañas Dulces; Puntarenas; 5 km SW of Rincón de Osa; 4km NE of Escuela Santa Constanza; Mexico: Veracruz, near San Jose de Mata Clara; El Salvador: Chalatenango, near Chalatenango; Nicaragua: Zelaya, Lecus. | Pohl and Davidse, 1971|
|                          |                 |     | Costa Rica: Guanacaste: 30 km N of Caiitas; El Salvador: Santa Ana: 2 km SE of Metapin; Trinidad: 1 km W of Arirna; Venezuela: bolivar, 17 km W of the Rio Caura; Cote-d’Ivoire; Nigeria: University Campus, Ile-Ife; forest floor; Zaire: Kinshasa, Mont Ngafula, Lukaya Valley, 25 km S of Kinshasa; Bas-Zaire, Kasangulu, Kindongo, 15 km N of Kasangulu; Brazil: Bahia; Argentina: Misiones, Depto Iguazu, Cataratas del Iguazu; Argentina: Misiones, Depto Gral. M. Belgrano, 5 km SW of Deseado. | Davidse and Pohl, 1972a|
|                          |                 |     | Germplasm Bank of Wild Species in Kunming                               |                       |
|                          |                 |     | Brazil: Distrito Federal                                                | Guo et al., 2019      |
|                          | 2n=ca. 30       |     | Brazil: Distrito Federal                                                | This work             |
|                          | n=22            | 4x  | Venezuela: Aragua: Rancho Grande                                        | Gould and Soderstrom, 1967|
|                          | n=48            | 4x  | Colombia: Caqueta                                                      | Gould and Soderstrom, 1970|
| O. loretensis Mez        | 2n=22           | 2x  | Colombia. Amazonas                                                     | Gould and Soderstrom, 1970|
| O. obtusifolia Steud.    | 2n=23, 44       | 2x, 4x | Brazil: Para. Suriname: Zuid Rivier                                    | Gould and Soderstrom, 1967|
| O. taquara Swallen       | 2n=20           | 2x  | Brazil: Distrito Federal                                                | Gould and Soderstrom, 1967|

No data available for the following species: Olyra amapana Soderstrom & Zuloaga; O. bahiensis R. P. Oliveira & Longhi-Wagner; O. buchtianii Hackel; O. caudata Trin.; O. davisiana Judt. & Zuloaga; O. ecuadota Doell.; O. eformis Trin.; O. globersona Raddi; O. hoitumiana Soderstrom & Zuloaga; O. juruana Mez; O. latispicula Soderstrom & Zuloaga; O. longifolia Kunth; O. maranonensis Swallen; O. retrorsa Soderstrom & Zuloaga; O. standleyi Hitchc.; O. tamarquiensis Zuloaga; O. zuloagi Mez; O. wurdackii Swallen.
reported (Davidse and Pohl, 1972a). Extensive genetic system studies might be very helpful to understand the polymorphisms of O. latifolia complex across its range. 

Olyra latifolia and O. humilis have distinct positions in the phylogenetical topology in highly supported trees (Oliveira et al., 2014; 2020), and the basic and gametic chromosome numbers found here for both species agree with this result. Olyreae chromosome numbers are highly variable, and the tribe has the greatest variation in basic chromosome numbers and ploidy levels. In addition, polyploid complexes with 2x and 4x were described in O. latifolia. Intensive cytogenetical studies on Southamerican herbaceous bamboos are necessary to understand evolutionary trends in bambusoid subfamily, considering that all bamboos probably derived from herbaceous ancestors (Soderstrom and Calderon, 1974). 

Recently, the ploidy levels of bamboo grasses were reviewed by Zhou et al. (2017). As a rule, the chromosome number in temperate woody bamboo species remains constant with 2n=48, instead the chromosome number of paleotropical woody bamboos, was variable with 2n=70 ± 2, and a few species having 2n=64, 80, 96, 98, and 104 (Zhou et al., 2017 and references therein). The neotropical woody bamboos were also diverse but in a minor degree with 2n=40, 44 or 48 chromosomes (Zhou et al. 2017). A revision of chromosome data of herbaceous bamboo is not available yet. Olyra is the largest genus in Olyrae and has been poorly studied regarding its cytogenetics. Further cytological studies are needed to clarify the chromosome number evolution in this genus as a supporting contribution to understand phylogenetical and reproductive ongoing analyses.

Species of Olyrae are endangered due to the continuing loss of forests (Oliveira and Clark, 2009). Under the climatic change impact, is necessary to claim for conservation efforts of bamboo species because several species are endemic and poorly studied. Besides, we ignore if they are an important resource for other species as food, forage or grains in forest ecosystems. The conservation of natural populations in subtropical South America should be considered as the focus of future studies in this tribe.

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