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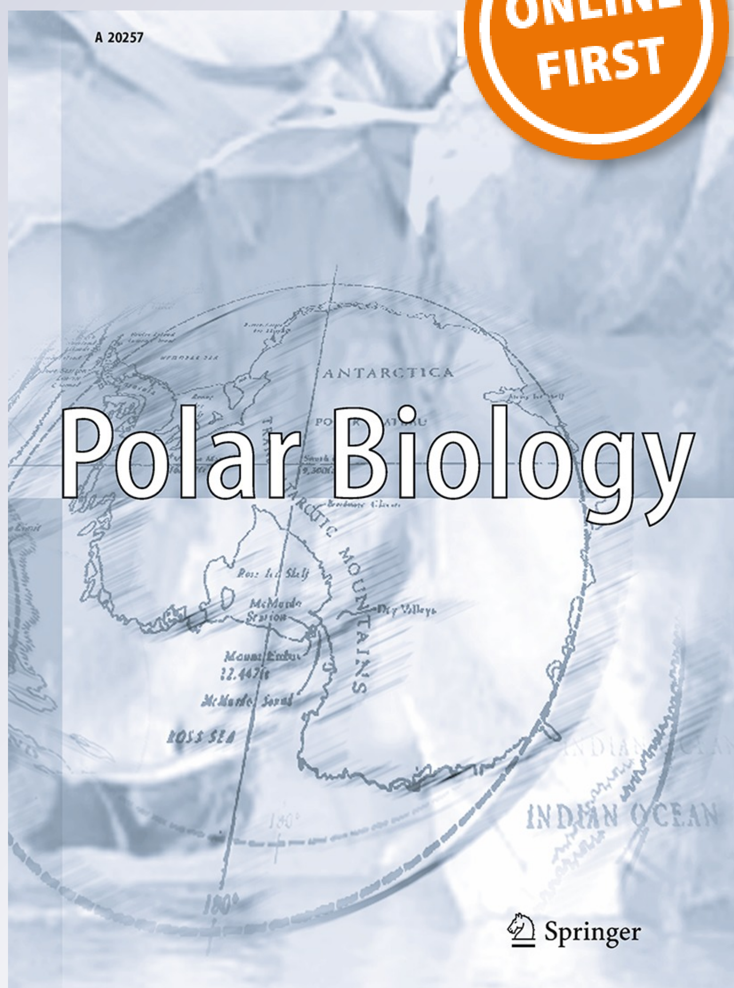

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ORIGINAL PAPER

Distribution of rDNA and polyploidy in *Deschampsia antarctica* E. Desv. in Antarctic and Patagonic populations

María Laura González[1] · Juan Domingo Urdampilleta[1] · Mariana Fasanella[2] · Andrea Cecilia Premoli[2] · Jorge Oscar Chiapella[1]

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Abstract Unlike the Arctic flora, with many flowering plant species offering opportunities to study evolutionary processes, the Antarctic flora offers only two. One of them is the Antarctic grass *Deschampsia antarctica* E. Desv., whose distribution spans from northern Patagonia (ca. 38°S) down to Alamode Island (ca. 68°S), in the west side of the Antarctic Peninsula. While some aspects of Antarctic plants have been extensively studied (e.g., anatomy, physiology, genetics), little is known about the related Patagonian populations. Particularly in cytogenetics, no single study has focused on continental populations and its relationships with the Antarctic plants. The combination of traditional fluorescent in situ hybridization (FISH) with a phylogenetic framework highlights the importance of cytogenetics in plant evolutionary studies, by allowing comparison of chromosome characters in phylogenetically related individuals. Most used characters for this purpose are the chromosome number, karyotype morphology and patterns of repetitive DNA. These were used to compare distant populations of *D. antarctica* in a phylogenetic framework, to obtain a first view of the cytogenetic structure of the species along its distribution. Patagonian populations have greater variability in the chromosomal and molecular characters, while Antarctic populations are very alike, hinting at a South American origin hypothesis. A polyploid population is reported for the first time, located on Central Patagonia populations, close to the northern limit of distribution range. Cytogenetic characteristics suggest that hybridization processes could have played an important role in the evolution of the genome of *D. antarctica*.

Keywords *Deschampsia antarctica* · Karyotype · FISH · Highly repetitive DNA · Polyploidy · Phylogeny

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Introduction

Deschampsia P. Beauv. (Poaceae, Poeae, Holcinae) (Soreng et al. 2015) is a monophyletic genus with about 30 species and a cosmopolitan distribution, occurring in humid soils of temperate and cold parts of the world, from sea level to ca. 4500 m. Some taxa are found in tropical regions, but restricted to the top of high mountains (Chiapella 2007; Chiapella and Zuloaga 2010). The Andes from southern South America have a high concentration of species of *Deschampsia*, including *D. antarctica* E. Desv. and the near cosmopolitan *D. cespitosa* (L.) P. Beauv., which is a common element in the north hemisphere. The Antarctic grass (*D. antarctica*) is a perennial plant distributed from scattered populations in northern Patagonia (ca. 40°S) to its southernmost known locality in the Alamode Island (68° 43′S), an island of the Terra Firma archipelago located off the Graham Land, at the west side of the Antarctic Peninsula (Komárková et al. 1985, 1990). It is one of the only two native vascular plants found in Antarctica; in comparison with Arctic regions of similar

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latitude with many taxa of different families, the Antarctic vascular flora paucity raises questions still not answered about its origin and evolution (Mosyakin et al. 2007; Parnikoza et al. 2007). The Antarctic grass has developed morphological and physiological adaptations to extreme climatic conditions (Vieira and Mantovani 1995; Alberdi et al. 2002; Parnikoza et al. 2007; Chwedorzewska et al. 2008), and it is therefore a valuable resource to identify genes associated with resistance to cold and freezing, light stress or photosynthetic capacity at low temperatures (Montiel et al. 1999; Ruhland and Day 2000; Bravo et al. 2001; Alberdi et al. 2002; Gidekel et al. 2003; John et al. 2009). Advances in this direction have so far produced transgenic plants of rice with enhanced cold tolerance (Byun et al. 2015).

Improving our knowledge of the cytogenetic of a species with a peculiar geographic distribution that is also a source of useful traits, allows to better understand its phylogenetic and evolutionary relationships and to foster crop improvement. Basic cytogenetic features necessary to achieve these aims are the chromosome number, karyotype morphology, pattern and distribution of heterochromatin and ribosomal DNA loci (Greilhuber and Speta 1976; Schwarzacher et al. 1980; Winterfeld and Röser 2007a, b). Most *Deschampsia* species have $2n = 26$ chromosomes, being $x = 13$ the most frequent basic number (Cardone et al. 2009). In *D. cespitosa*, Winterfeld and Röser (2007a) described the distribution pattern of heterochromatin and rDNA, with the presence of multiple sites of ribosomal DNA (18-5.8-26S and 5S rDNA) differing from other species of related Aveneae. *Deschampsia cespitosa* is the cytogenetically most studied species; $2n = 26$ and 52 are the most frequent cytotypes for this complex with large phenotypic plasticity (Tateoka 1955; Arohonka 1982; Davlianidze 1985; Hedberg 1986; Rothera and Davy 1986). Although related forms (*D. cespitosa s.l.*) with $2n = 41$; 49; 50 (Löve and Löve 1975; Engelskjøn 1979; Albers 1980) or $2n = 42$ (Sokolovskaya and Probatova 1975) have also been found, these unusual chromosome numbers still require corroboration. Harsh Arctic environmental conditions have been deemed as the driving forces to explain the abundance of hybrid and polyploid genomes in the Arctic (Stebbins 1950, Brochmann et al. 2004); Siberian taxa of *Deschampsia* lend some support to this hypothesis (Löve and Löve 1975; Petrovsky and Zhukova 1981; Chiapella and Probatova 2003).

The chromosome number reported for *D. antarctica* in Antarctica is $2n = 26$ ($x = 13$), with a karyotype composed of $10m + 6sm + 8st + 2t$ (Moore 1970; Cardone et al. 2009). Cells with irregular numbers of chromosomes were detected as a product of failure mitotic segregation (Cardone et al. 2009) or increased genome instability (Navrotska et al. 2014). Other genetic studies based on molecular markers (AFLP, RAPD, sequences of nuclear and plastid DNA) showed low genetic variability in Antarctic populations and a relative differentiation toward lower latitude populations (Holderegger et al. 2003; Van de Wouw et al. 2008). In these studies, the South American populations were absent or underrepresented; an understanding of the genetic structure of the species throughout its distribution range requires a wider sampling (Fasanella et al. in preparation).

Because genetic studies on this species have so far focused only in Antarctic populations, little is known about the South American populations and on the relationships between these two main groups. This study aims specifically (1) to describe basic karyotype features of South American populations; (2) to add new cytological data on more Antarctic populations; and (3) to compare chromosomal differences between the disjunct populations in a molecular phylogenetic framework. The incorporation of new chromosomal markers together with the inclusion of Patagonian populations will allow the assessment of the cytogenetic features of this species throughout its distribution range and elucidate the evolutionary history of Antarctic and South American populations.

Materials and methods

Plant material

We collected six populations of *D. antarctica*, two from Antarctica and four from Patagonia, during austral summer from 2012 to 2014 (Table 1; Fig. 1). Living plants were transported to the laboratory and kept in pots in culture chambers at 14 °C. The vouchers were included in the collection of the herbarium of the Botanical Museum of Córdoba (CORD).

Cytogenetic techniques

We used mitotic chromosomes preparations from root meristems. The roots were pretreated with 2 mM 8-hydroxyquinoline for 4–6 h at 14 °C and fixed in ethanol/acetic acid (3:1, v:v). The tissues were digested with pectinase enzyme solution (Novozymes) and squashed in 45 % acetic acid. Preparations were frozen in liquid nitrogen to remove the coverslip.

For conventional staining, the preparations were immersed in a 2 % solution of Giemsa and mounted in Entellan (Merck). For documentation of chromosomes, 10 metaphases were photographed per population, using an Olympus BX61 microscope coupled with monochrome camera and Cytovision software (Leica Biosystems). The chromosomes were organized and measured using

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Table 1 Populations of *Deschampsia antarctica* detailing the locality and the number of collection, the chromosome number ($2n$), the karyotype formula (KF) classifying chromosomes according to Guerra (1988) (where m metacentric, sm submetacentric, a acrocentric), the total chromosomal length (TCL) of the basic complement $x = 13$, average chromosomal size (ACS), the range (R) of chromosome size and asymmetry indexes A_1, A_2 indicating the group (a or b) which every population belong according to Tukey's test ($\alpha = 0{,}05$, *DMS* $A_1 = 0{,}02816$, *DMS* $A_2 = 0{,}02972$)

Population	Location	$2n$	KF	TCL	ACS	R	A_1	A_2	18-5.8-26S rDNA (Pos)	5S rDNA (Pos)	DAPI (Pos)
2772	Argentine Antarctic Sector, 25 de Mayo Island. Chiapella et al., 2772	26	$5m + 2sm + 6a$	56.62 ± 9.35	4.36 ± 0.72	6.56–3.13	0.567 (a)	0.206 (a)	2 (i,pc)	5 (i,st,t)	32 (pc,t)
2782	Argentine Antarctic Sector, Antarctic Peninsula. Chiapella et al., 2782	26	$5m + 2sm + 6a$	50.70 ± 5.71	3.90 ± 0.44	6.03–2.90	0.565 (a)	0.212 (a)	2 (i,pc)	5 (i,st,t)	20 (pc,t)
880	Santa Cruz, Argentina. Urdampilleta et al., 880	26	$5m + 2sm + 6a$	48.95 ± 4.92	3.77 ± 0.38	5.50–2.88	0.486 (b)	0.203 (a)	2 (t,pc)	5 (i,st,t)	24 (pc,t)
877	Santa Cruz, Argentina. Urdampilleta et al., 877	26	$5m + 2sm + 6a$	55.33 ± 7.18	4.26 ± 0.55	6.51–2.87	0.543 (a)	0.213 (a)	2 (i,pc)	5 (i,st,t)	38 (i,pc,t)
852	Chubut, Argentina. Urdampilleta et al., 852	26	$5m + 2sm + 6a$	51.04 ± 7.82	3.93 ± 0.60	5.67–2.85	0.502 (b)	0.190 (a)	1 (i)	5 (i,st,t)	22 (pc,t)
845	Chubut, Argentina. Urdampilleta et al., 845	52	$10m + 4sm + 12a$	44.36 ± 4.84	3.41 ± 0.38	5.75–2.23	0.505 (b)	0.242 (b)	3 (i,t,pc)	7 (i,st,t)	–

It details the number and position of 18-5.8-26S and 5S rDNA, and DAPI bands after FISH (*i* intercalary, *pc* pericentromeric, *st* subterminal, *t* terminal)

Photoshop CS4 software (Adobe Systems Inc.) and MicroMeasure v3.3 (Reeves 2001). Based on the measurements, idiograms were made organizing the chromosomes in groups according to their size and brachial index following the terminology of Guerra (1988) (metacentric: $r = 0$ to 1.49, submetacentric: $r = 1.5$ to 2.99 and acrocentric: $r > 3$). The asymmetry indices A_1 and A_2 (Romero Zarco 1986) were calculated and compared using ANOVA and Tukey's test on InfoStat (Di Rienzo et al. 2008).

We used fluorescence in situ hybridization (FISH) to detect regions of ribosomal DNA, following protocols by Schwarzacher and Heslop-Harrison (2000), with minor modifications. For sites 18-5.8-26S rDNA, a p*Ta*71 probe from the recombinant *E. coli* bacteria was used, labeled with biotin (BioNick, Invitrogen). To identify sites 5S rDNA, the DNA fragments used as probes were obtained by PCR using specific primers (Röser et al. 2001), labeled with digoxigenin (DIG Nick translation mix, Roche).

Cluster analysis

We constructed a data matrix of presence/absence of DAPI bands and rDNA sites among the homologous chromosomes from different diploid populations (Online Resource 1). The similarity was calculated with the Jaccard coefficient, and a cluster analysis was done by UPGMA with NTSYS 2.1 software (Rohlf 2000).

Alignment, Phylogenetic analysis and reconstruction of ancestral states

For the sequences of *D. antarctica,* DNA was isolated from approximately 0.5 g of dry material in silica gel following a modified CTAB method of Doyle and Doyle (1990) adding 0.0075 g of dithiothreitol (DTT) and 0.01 gr of polyvinylpyrrolidone (PVP) for each ml of buffer extraction before the 55 °C for 1-h incubation. We amplified the ITS region (ITS4-ITS5; White et al. 1990) and one noncoding regions of the chloroplast genome (rps16-trnK; Shaw et al. 2007). The PCR mix contained 1 µl of template DNA (10 ng), 0.5 U Taq DNA polymerase (Invitrogen), 2.5 µl 5X Taq buffer (Invitrogen), 0.25 mM of each dNTP and 0.3 µM of each primer in a total volume of 25 µl. Thermal cycling for PCR consisted of 35 cycles, each with 1-min denaturation at 94 °C, 1-min annealing 48–52 °C (depending on the primer pair used), 1-min extension at 72 °C and a final extension of 10 min. Amplification products were separated by electrophoresis on a 1 % agarose gel, stained with Sybr Safe (Invitrogen, Eugene, OR, USA) and visualized with UV transilluminator. PCR products were cleaned with exonuclease I (Fermentas, Burlington, ON, Canada) and shrimp alkaline phosphatase (USB, Cleveland, OH, USA). Cycle sequencing was

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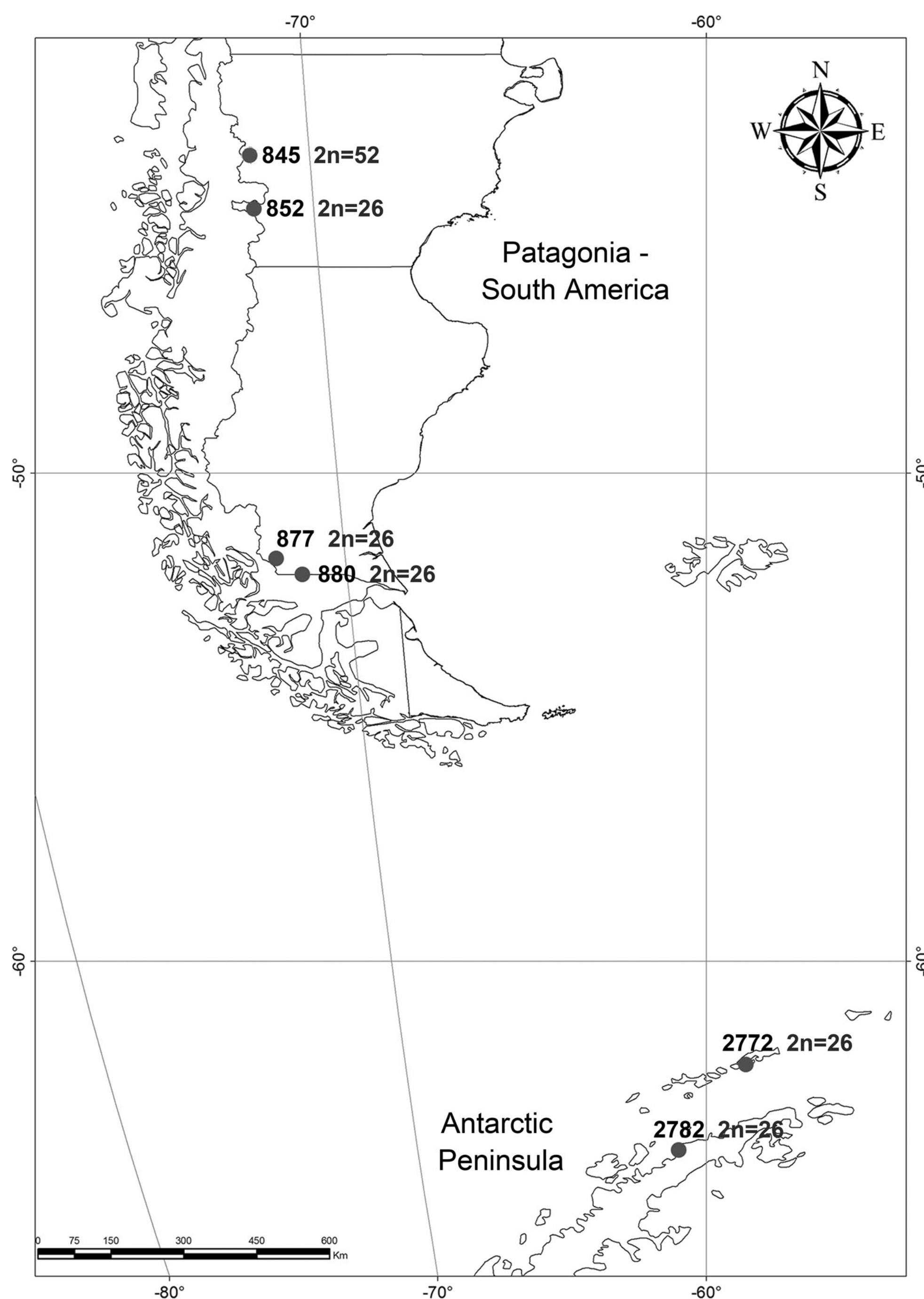

Fig. 1 Localities map of studied populations of *Deschampsia antarctica*. To the *left* of the population number is indicated de chromosome number

performed using Big Dye terminator chemistry (Applied Biosystems). Automated sequencing was conducted on an ABI Prism 3100 Avant (Applied Biosystem) of Laboratorio Ecotono and at Macrogen (Corea). All sequences were deposited in GenBank (Table 2).

The sequences were edited and aligned with Mega 6 (Tamura et al. 2013) using the Muscle module (Edgar 2004). A phylogenetic analysis with ITS and *trn*K sequences was done. Sequences of *trn*K and ITS of *Festuca altissima* All., *Lolium perenne* L., *Avenella flexuosa* (L.) Drejer, *Vahlodea atropurpurea* (Wahlenb.) Fr. ex Hartm. and *D. cespitosa* included in the analysis were extracted from GenBank, and *F. altissima* and *L. perenne* were taken as outgroup (Table 2). The analysis was performed with a concatenated matrix of *trn*K and ITS, the gaps were treated as missing data and all characters as unordered. Maximum parsimony analysis (MP) was performed using Paup 4.0b10 (Swofford 2000) applying a bootstrap analysis (1000

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Table 2 Accession numbers of trnK and ITS sequences. *Deschampsia antarctica* sequences will be deposited in Genbank (gb 1-12)

Species	ITS accessions	trnK accessions
Festuca altissima	FM179404.1	JX871939.1
Lolium perenne	EF379073.1	NC009950.1
Avenella flexuosa	JQ972936.1	–
Vahlodea atropurpurea	AM041216.1	HQ114557.1
Deschampsia cespitosa	DQ539579.1	HQ114556.1
D. antarctica 2772	KU645365	KU645373
D. antarctica 2782	KU645366	KU645374
D. antarctica 880	KU645369	KU645371
D. antarctica 845	KU645367	KU645372
D. antarctica 852	KU645368	KU645375
D. antarctica 877	KU645370	KU645376

replicates) with heuristic search (10 replicates, TBR method). For the maximum likelihood analysis (ML), the best substitution model was chosen by jModelTest 2.1.4 (Darriba et al. 2012) using Akaike's information criterion (AIC). The analysis was also performed in PAUP 4.0b10 (Swofford 2000), with GTR + G model obtained in jModelTest, applying bootstrapping (1000 replicates) with heuristic search (10 replicates, TBR method). The ancestral state reconstruction was made on 9 chromosomal characters (Online Resource 1), using ML criterion under a Markov k-state one-parameter model (Mk1) with Mesquite 3.02 software (Maddison and Maddison 2015). Species data not analyzed in this paper were taken from the literature (Albers 1980; Harper et al. 2004; Winterfeld and Röser 2007a; Lideikyte et al. 2008).

Results

Karyotype structure

The chromosome number was verified for Antarctic populations, including populations of the Antarctic Peninsula (2782) and sub-Antarctic Islands (2772). Both Antarctic (2782 and 2772) and South American (880, 877 and 852) populations presented $2n = 26$ chromosomes, but a population from central Patagonia (Chubut Province, pop. 845) presented tetraploid cytotype with $2n = 52$ chromosomes (Table 1; Fig. 2).

Classical staining yielded the presence of an *m* chromosome pair significantly higher in size, as well as an sm chromosome pair with secondary constriction in the short arm in all populations. Note that the polyploid population, although it has chromosomes *m*, sm and *a* with similar proportions of diploid populations, has one pair of each of aforementioned markers (the largest *m* and the sm with satellite). In addition, this population has one *m* chromosome pair significantly smaller in size (Fig. 2).

The total length of the basic chromosomal complement $x = 13$ (TCL) ranged between 44.36 and 56.62 µm, with the lowest size observed in the polyploid population (845) and the largest in 2772 population. The chromosomes in *D. antarctica* were medium size, and the average chromosome size (ACS) varied between 3.41 and 4.36 µm (Table 1).

The karyotype of *D. antarctica* was relatively asymmetrical, displaying variation between chromosome morphology (intrachromosomal asymmetry, A_1 index) and chromosome size (interchromosomal asymmetry, A_2 index). Differences in the A_1 and A_2 indexes were significant ($p = 0{,}0001$ and 0.0042), and a range of values from 0.486–0.567 to 0.190–0.242 for A_1 and A_2, respectively (Table 1). For each index, two groups were determined according to the Tukey's test (Table 1; Fig. 3), but considering both indices of asymmetry, the Antarctic populations were very similar, while Patagonian populations showed relative variation in these characters. Particularly, the two Antarctic populations and the 877 population had the highest A_1 index, while the Patagonic polyploid population (845) had the highest A_2 (Fig. 3).

18-5.8-26S and 5S ribosomal DNA distribution

The pattern of ribosomal DNA distribution was relatively conserved for all studied populations of *D. antarctica*, with little variations (Fig. 4). The number of 18-5.8-26S rDNA loci ranged between 1 and 3. The locus belonging to sm chromosome with secondary constriction is localized in the intercalary region of the short arm in all studied populations except in the 880 population where it was mapped in terminal position (Fig. 4e). The second locus type is located on the short arm of *m* chromosome and presents a barely perceptible hybridization signal in populations 877 and 845 (Fig. 4c, d). This reduction in intensity of signal is marked in the population 852, in which the hybridization signal is completely lost (Fig. 4b). Finally, the third locus type is located in terminal position on the short arm of a sm chromosome and is observed only in the 845 population (Fig. 4d).

All diploid populations had 5 loci 5S rDNA, with differences in position for some of them. Populations 2772, 2782, 877 and 852 showed the same pattern of 5S rDNA. By contrast, population 880 differs with an extra region in subterminal position on the short arm of *m* chromosome (pair #5) and lacks a site located on the long arm of one *a* chromosome (pair #9), which was present in the remaining diploid populations (Fig. 4). In this population, 5S signal from chromosome pair #8 is noticeably more intense than the rest, so they were drawn differentially in

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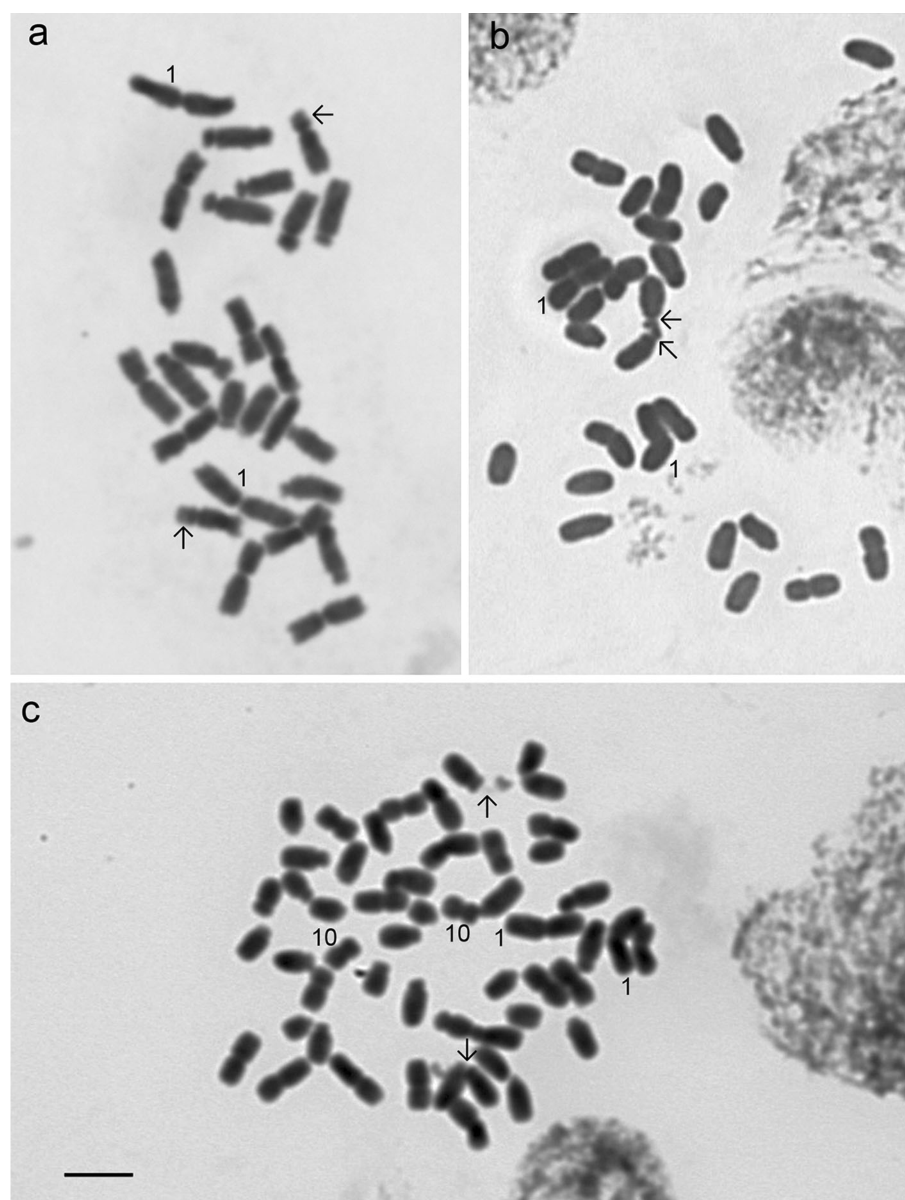

Fig. 2 Mitotic metaphase chromosomes of *Deschampsia antarctica* colored by the conventional technique (Giemsa): **a** 2772 Antarctic population ($2n = 26$), **b** Patagonian population 852 ($2n = 26$) and **c** Patagonian population 845 ($2n = 52$). *Arrows* indicate secondary constrictions of sm chromosomes, # *1* is the larger m pair, and # *10* is the shorter remarkable *m* pair in the population 845. All the same scale: 5 µm

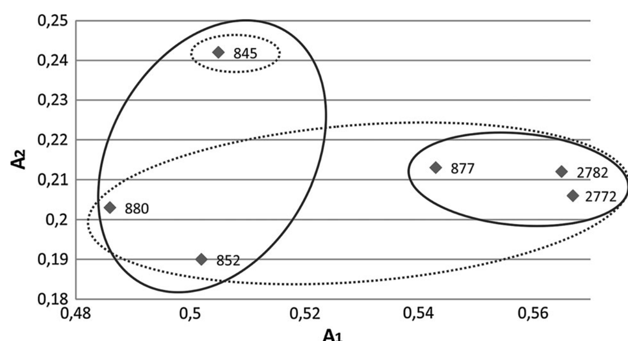

Fig. 3 *Scatter plots* of asymmetry indexes A_1 and A_2 for studied populations of *Deschampsia antarctica*. *Continuous lines* and *dashed lines* indicate groups significantly different for A_1 and A_2, respectively

the idiogram (Fig. 5). In polyploid population (845), 7 loci 5S rDNA hybridization signals were observed, located in the same types of chromosomes than diploid populations (Fig. 4d).

DAPI banding pattern after FISH

The DAPI staining in FISH technique allowed recognition of strongly stained regions. These sites were observed more frequently on terminal chromosomal regions and rarely were detected on pericentromeric and intercalary bands. The most intense bands correspond to terminal regions and were mainly observed in *m* chromosomes (Fig. 4). DAPI

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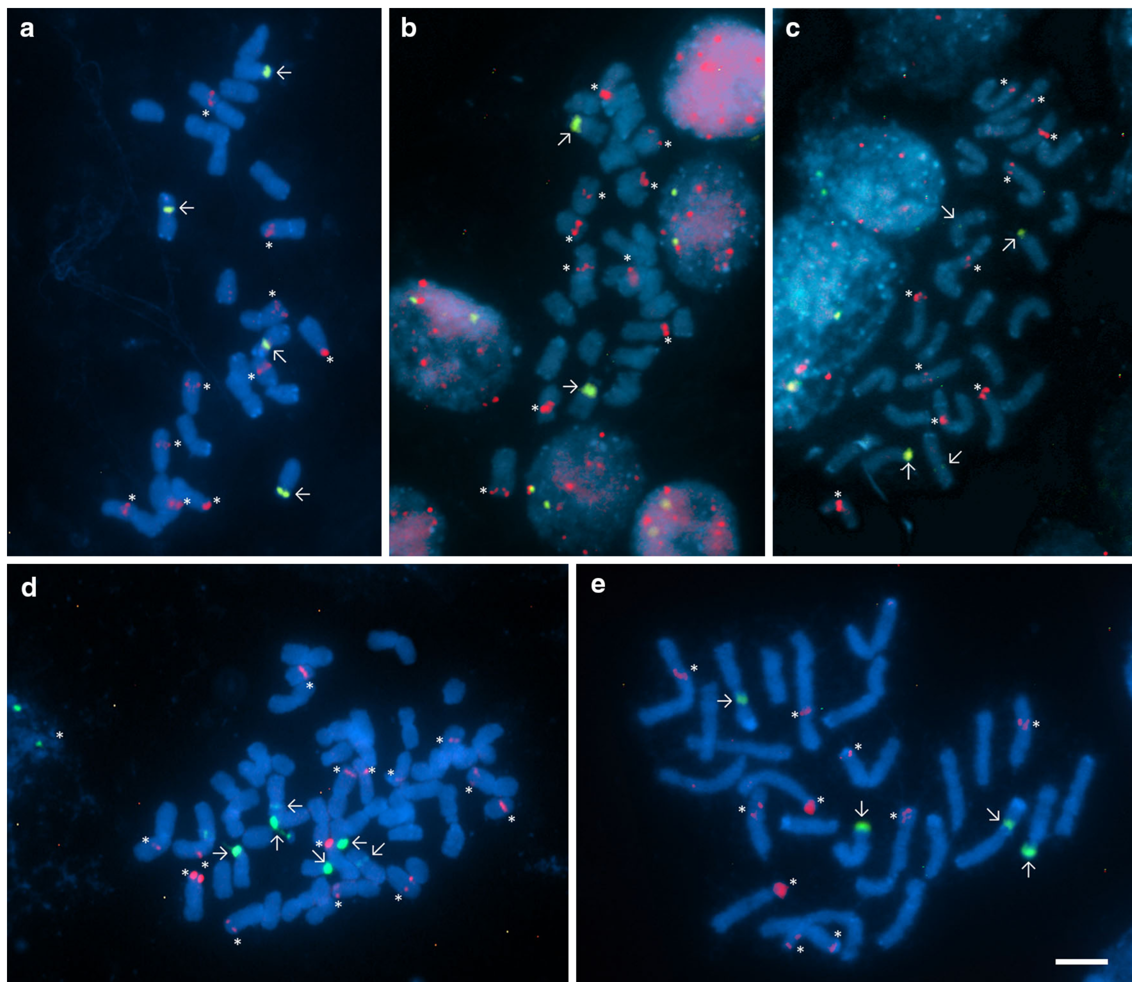

Fig. 4 FISH technique. *Arrows* indicate 18-5,8-26S rDNA signals (*green*), and *asterisks* indicate 5S rDNA signals (*red*) in *Deschampsia antarctica*. **a** 2772; **b** 852; **c** 877; **d** 845; **e** 880. *Scale* 5 µm. (Color figure online)

banding pattern and differences between diploid populations are detailed in the idiogram (Fig. 5). The population with highest number of DAPI bands was 877, while 2782 showed the least number.

Cluster analysis (UPGMA) with chromosome traits

For the cluster analysis, thirty-two presence/absence characters were used, of which twenty-four correspond to the pattern of DAPI bands and eight correspond to the rDNA pattern. Of the total, twenty-two were variable (Online Resource 1). Overall Antarctic populations yielded 60 % similarity with the Patagonian populations. In addition, Antarctic populations (2782 and 2772) are most similar to each other, showing a similarity of 74 %. The southernmost Patagonic 880 population was the most different from the rest (Fig. 6). This comparison could only be analyzed in diploid populations, due to the inability to recognize homologous chromosome pairs in the polyploid population.

Phylogenetic analysis and reconstruction of ancestral states

The matrix aligned and concatenated (ITS and *trn*K) presented 1348 total characters, of which 62 characters were informative. Consensus of 2831 MP trees (CI = 0.9193, HI = 0.0807, RI = 0.8850) and ML (−Ln = 2780.6) resulted in the same topology. *Deschampsia* is next to *Avenella* and *Vahlodea* and divided into two clades corresponding to two species: *D. cespitosa* and *D. antarctica*. Within the Antarctic clade, polyploid population 845 is clearly separated from diploid populations. This phylogenetic analysis indicates a close relationship between both Antarctic populations, together with populations 877 and

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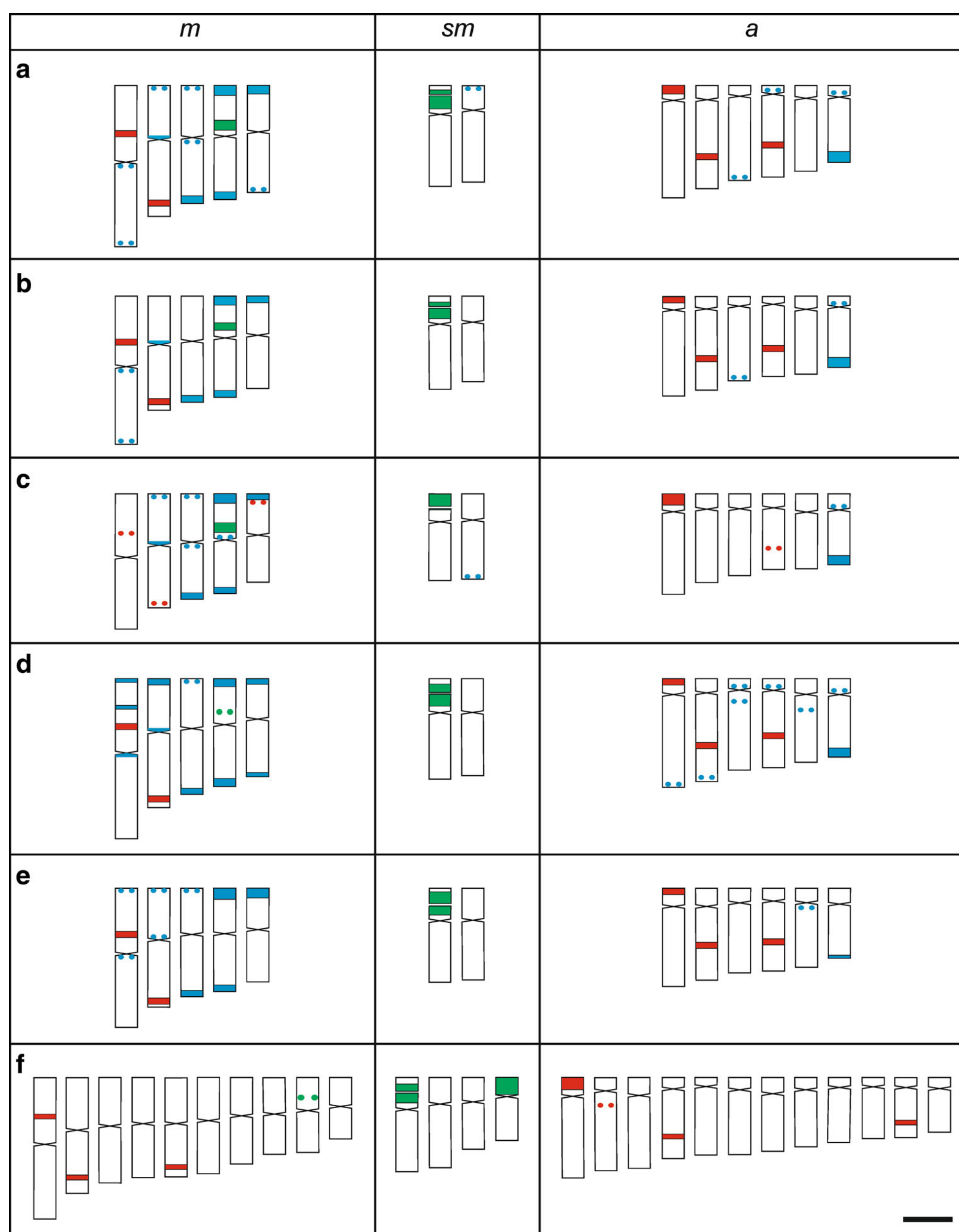

Fig. 5 Idiograms representing the haploid complement in populations of *Deschampsia antarctica*. **a** 2772 **b** 2782 **c** 880 **d** 877 **e** 852, **f** 845. Within each group (*m* metacentric, *sm* submetacentric, *a* acrocentric), chromosomes are arranged from highest to lowest length. *Green* represents the 18-5.8-26S rDNA regions, *red* 5S rDNA regions and *lightblue* the DAPI bands observed after FISH. The latter could not be identified in the polyploid population due to difficulty in recognizing the chromosome pairs. The *rectangles* represent strong signals, while *circles* represent signals of low intensity. *Scale* 2 μm. (Color figure online)

852, while southernmost South American population 880 was somehow distinct (Figs. 7 and 8).

The reconstruction of ancestral states to establish chromosomal characteristics for each clade showed that the ancestor of the genus *Deschampsia* has a high proportional likelihood (pl = 0.93) to present $x = 13$, in contrast to other genera of the tribe Poeae that were used as outgroup (Fig. 7a). Alternatively, the possibility that the ancestor of *Deschampia* consisted of a heteromorphic chromosome complement with chromosomes *m*, sm and *a* is also high (pl = 0.99) (Fig. 7b). Polyploidy in *D. antarctica* (population 845, $4x = 52$) is apparently a derived feature, since it is likely (pl = 0.74) that its ancestor was diploid (Fig. 7c).

The common ancestor of *D. cespitosa* and *D. antarctica* has five 5S rDNA loci (pl = 0.88), which are maintained in both species (Fig. 7d). However in terms of 18-5.8-26S rDNA, *D. antarctica* shows a decrease from the ancestral state, with only two loci (pl = 0.79) (Fig. 7e). The population 845 presents a decrease in the amount of both loci 5S and 18-5.8-26S rDNA, while the population 852 presents a reduction only of the rDNA loci 18-5.8-26S (Fig. 7d, e).

Regarding the position of the rDNA loci, *D. antarctica* presents chromosome markers, absent in *D. cespitosa*: one *a* chromosome pair with 5S rDNA on the short arm and a sm pair with rDNA 18-5.8-26S in short arm (Fig. 7f, g). The terminal position of 18-5.8-26S rDNA loci on the sm chromosome pair is a derived feature that emerged in the

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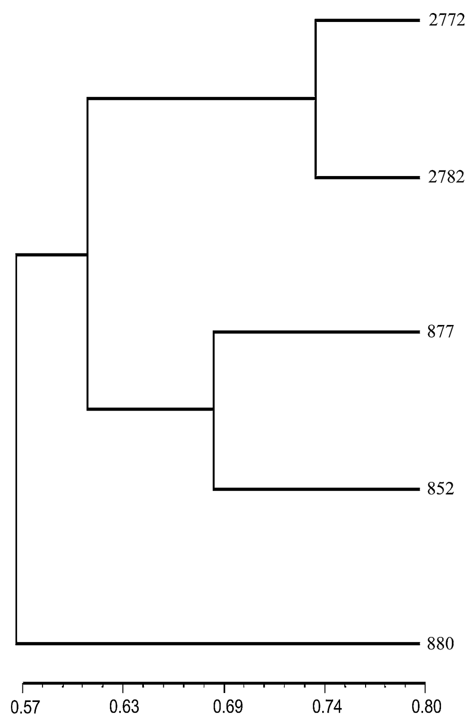

Fig. 6 Dendrogram of cluster analysis (UPGMA) of diploid populations of *Deschampsia antarctica*, made by rDNA data and DAPI bands. The horizontal axis represents the similarity coefficient of Jaccard

population 880 (Fig. 7g). Although the number of 5S rDNA regions is constant in diploid populations, there are variations in terms of their disposition in the genome. The population 880 presents the occurrence of a subterminal 5S rDNA loci on an *m* pair (Fig. 7h). The remaining diploid populations would present the gain of one *a* chromosomal pair with 5S rDNA on the long arm (Fig. 7i). Figure 8 summarizes the ancestral states reconstruction, showing the chromosomal characteristics specific to each clade.

Discussion

Chromosome number variations and karyotype features

Our results reveal a variation in ploidy level in *D. antarctica*, including diploid ($2n = 26$) and tetraploid ($2n = 52$) populations The chromosome numbers were known by counts from populations in the sub-Antarctic Islands (Moore 1970; Cardone et al. 2009; Navrotska et al. 2014). In this work, it was possible to analyze new populations of the Antarctic Peninsula and South America. The chromosome counts reported here confirm the basic number $x = 13$, which is shared by most species of the genus (Lawrence 1945; Kawano 1963; Albers 1980). Cells with 27 and 13 chromosomes were described for some Antarctic individuals from 25 de Mayo Island (King George Island), attributing this anomaly to failure during mitotic division (Cardone et al. 2009). Furthermore, chromosome numbers ranging from 13 to 39 in Darboux Island (west of Cape Perez; S65°23.707′, W64°12.905′) and Great Yalour Island (Wilhelm Archipelago; S65°14.039′, W64°9.761′) were related to genome instability in extreme environmental conditions (Navrotska et al. 2014). In contrast to unusual chromosome numbers found in some cells in other cytogenetic studies of Antarctic populations (Cardone et al. 2009; Navrotska et al. 2014), no irregularity was observed in this study in any of the populations analyzed. All cells had a complete chromosome complement and homologous pairs could be identified without problems, suggesting that meiotic failures are not common processes and need verification.

Polyploidy is an important driving force in plant evolution (Hunziker and Stebbins 1987; Otto and Whitton 2000; Wendel 2000) with a high incidence in Poaceae (Hilu 2004); several genomic duplication events were postulated in the evolution of the family (Soltis and Soltis 2009). Different polyploid cytotypes were reported for *D. cespitosa* complex in the northern hemisphere, where diploid $2n = 26$ and tetraploid $2n = 52$ populations are the most common (Albers 1980; Petrovsky and Zhukova 1981; Rothera and Davy 1986). The ecological and adaptive significance of the variation in ploidy level in *Deschampsia* is uncertain, but may be related to habitat differences: Diploid cytotype occurs mainly in stable environments (old growth forests), while tetraploids are common in modified environments, reflecting increased invasiveness (Rothera and Davy 1986). Variations of ploidy levels have been observed in other species of *Deschampsia,* mainly in several taxa (subspecies and varieties) of the *Deschampsia cespitosa* complex, including di-, tri- and tetraploids cytotypes (Albers 1980; Chiapella and Probatova 2003). Our study of *D. antarctica* discloses for the first time a polyploid complex distributed between Antarctica (only diploids) and midlatitudes of Patagonia (diploids and tetraploids). Triploid cytotypes were until now not found, while tetraploids were observed at lower latitude and higher altitude places within the distribution range studied. Our results do not support the hypothesis that polyploids would be better adapted to extreme weather conditions (Hagerup 1932; Brochmann et al. 2004) or areas with recent retraction of glaciers (Stebbins 1950), since at the high-latitude extreme of the distribution range of the species (i.e., Antarctica) only diploid cytotypes were found. In agreement with data on *D. cespitosa* (Rothera and Davy, 1986), the presence of polyploid populations of *D. antarctica* at one end of the distribution range may be related to differential ability of polyploids to colonize

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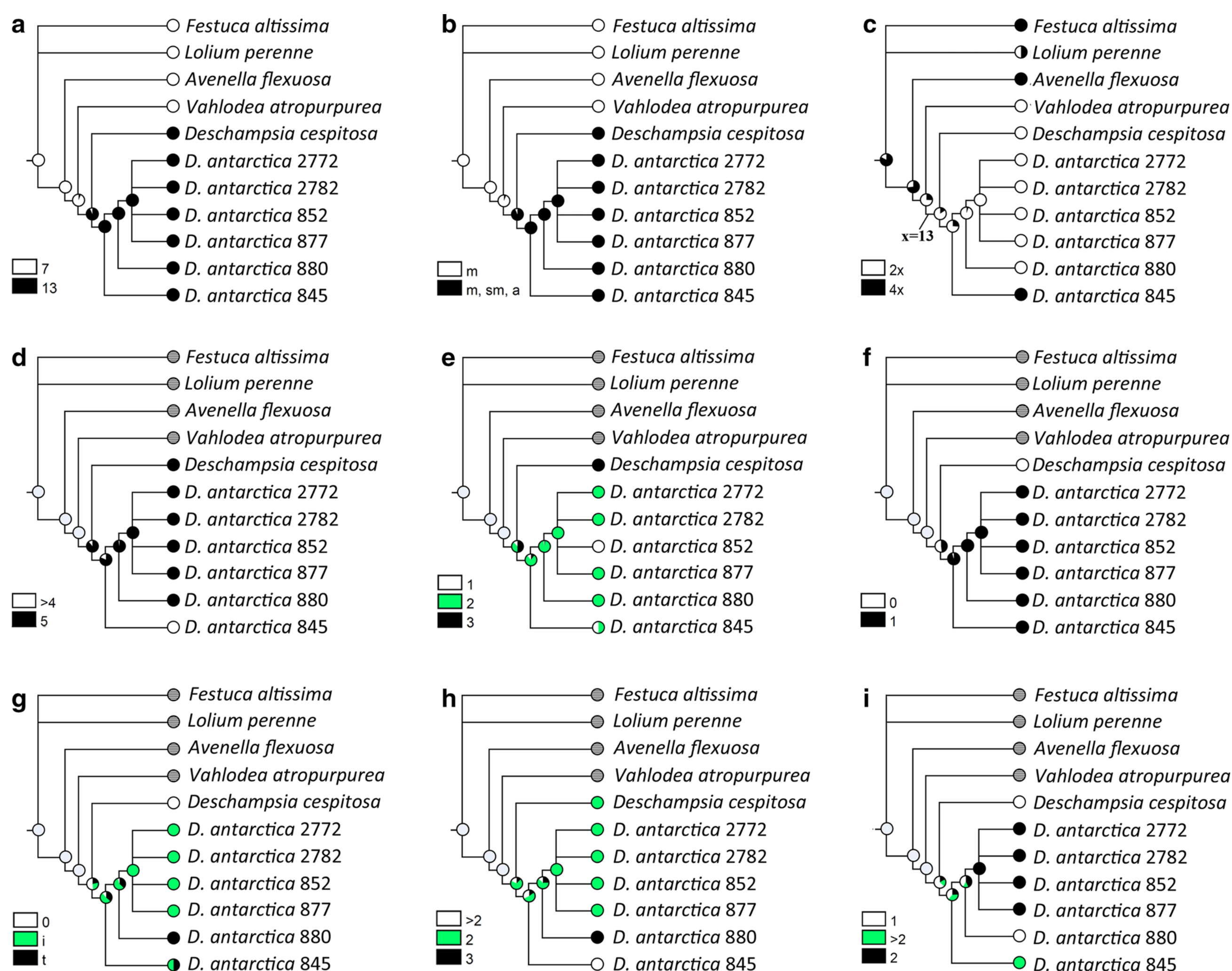

Fig. 7 Reconstruction of ancestral states in *Deschapsia antarctica* and related species. **a** Basic number; **b** chromosome complement; **c** ploidy level to the corresponding basic number; **d** number of 5S rDNA loci per basic genome $x = 13$; **e** number of 18-5.8-26S rDNA loci per basic genome $x = 13$; **f** presence of *a* pair with 5S rDNA loci on short arm; **g** presence and position of 18-5.8-26S rDNA loci on sm pair; **h** number of *m* pairs with 5S rDNA loci per basic genome $x = 13$; **i** number of *a* pairs with 5S rDNA loci on long arm per basic genome $x = 13$. In all trees, *gray*-colored taxa indicate no data

different environments. Polyploids have larger geographic ranges and/or occur in more habitats than diploids (Stebbins 1975; Levin 2002), and this is probably related to greater genetic and biochemical variability (Brochmann et al. 2004). However, historical factors might also have caused such distribution (see below) (Weiss-Schneeweiss et al. 2013). A greater sampling effort is necessary to verify whether the distribution pattern of polyploid cytotypes reflects the ecology and evolutionary history of the species.

Since the basic karyotype features reported here are similar to those described by Cardone et al. (2009) for this species, and for other northern hemisphere taxa of *Deschampsia* (Kawano 1963; Albers 1980; García-Suárez et al. 1997; Winterfeld 2006), it is therefore assumed that the karyotype is relatively conserved in the genus. Among these conserved features are the presence of heteromorphic chromosomes, variable number of metacentric, submetacentric, and acrocentric chromosomes and a large metacentric pair. For the studied species, chromosomes are medium in size, with a range of variation relatively high, ranging from 2 to 6.5 μm between complement chromosomes, and a haploid chromosome size between 42 and 78 μm (Albers 1980; Winterfeld 2006; Cardone et al. 2009). The karyotype asymmetry is one of cytogenetic characteristics mostly used in systematics and evolution, by highlighting chromosomal evolution processes (Stebbins 1971; Paszko 2006). The A_1 and A_2 indexes (Romero Zarco 1986) of *D. antarctica* allowed detecting small variations, showing the close affinity between Antarctic populations, in addition to similarities

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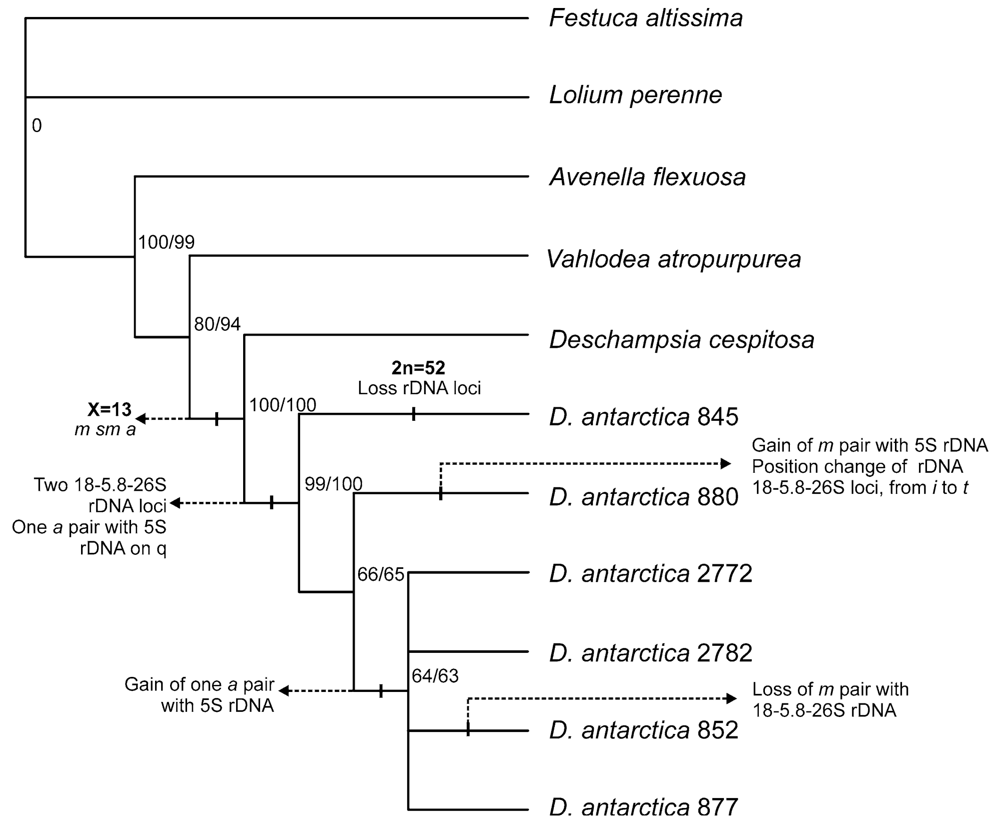

Fig. 8 Summary of ancestral reconstruction over phylogenetic tree of *Deschampsia antarctica* and related species. On branches, bootstrap is indicated for MP and ML to left and right, respectively. *Arrows* indicated the rise of determined chromosomes characteristics

detected between these and some Patagonian populations (see discussion below).

Patterns of repetitive DNA

The distribution pattern of ribosomal DNA in *Deschampsia* was studied in one population of *D. cespitosa* in Germany (Winterfeld and Röser 2007a). Our results provide new data of this characteristic in the genus, showing intraspecific variability that would be not resulting from large chromosomal rearrangements, but as of small inversions, transpositions or reinsertion of repetitive sequences (Raskina et al. 2008; Roa 2011). The distribution of rDNA sites of *D. antarctica* differs greatly from the pattern of *D. cespitosa*, and therefore, conservation of chromosome number and morphology does not necessarily imply stability in number and position of rDNA sites (Roa and Guerra 2012).

Regarding 18-5.8-26S rDNA, the loci of *m* chromosome tend to disappear in some populations of *D. antarctica*, as denoted by a marked reduction in the signal intensity. The loss of 18-5.8-26S rDNA sites could be associated with the loss of activity (Hasterok et al. 2006). The number of ribosomal cistrons and the formation of a secondary constriction in NOR apparently would be in direct relation to its transcriptional activity (Zurita et al. 1998). Therefore, it is possible to assume that the region with the greatest activity in *D. antarctica* is the secondary constriction of sm pair present in all populations studied. None of these markers was found in *D. cespitosa*, which in turn has three 18-5.8-26S rDNA loci: one on the short arm of an *a* pair and two in centromeric position of *m* chromosomes (Winterfeld and Röser 2007a).

The most frequent number of 5S rDNA loci in plants is one per karyotype (Roa 2011), while *Deschampsia* and related genera have multiple sites (Röser et al. 2001; Shelukhina et al. 2007; Winterfeld and Röser 2007a; Książczyk et al. 2010). The high number of these regions is probably related to the presence of transposable elements facilitating amplification and dispersion in the genome (Raskina et al. 2008; Roa 2011), which could led to the generation of the intraspecific variation observed. However, other mechanisms such as non-homologous recombination between regions with proximity during interphase could be related to the amplification and transposition of these regions of repetitive DNA (Schweizer and Loidl 1987). This mechanism would explain the appearance of equilocal regions in different chromosome pairs, such as those having intercalary 5S rDNA. *Deschampsia cespitosa* contains the same number of 5S rDNA loci. Some are present in *m* chromosomes, in similar positions as in *D.*

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antarctica, but they are also found in sm and *a* chromosomes, without similarity with the *D. antarctica* pattern (Winterfeld and Röser 2007a).

DAPI staining after FISH detected relatively constant among-population banding patterns. The DAPI bands showed heterochromatin rich in AT, which are often observed after in situ hybridization (Maluszynska and Heslop-Harrison 1993; Zoldos et al. 1999). However, in some taxa, staining with DAPI after FISH could reveal variations in the banding pattern (Barros e Silva and Guerra 2010). Most DAPI bands were terminally coinciding with observations in other *Deschampsia* species (García-Suárez et al. 1997; Winterfeld and Röser 2007b) and are probably a conserved feature of the genus. Unlike previous work on *D. cespitosa*, the presence of intercalated bands is rare. Additionally, pericentromeric bands were found while in other studies of genus were absent (García-Suárez et al. 1997; Winterfeld and Röser 2007b).

The features observed by FISH technique for populations with $2n = 26$ are summarized in the cluster analysis. When considering the pattern of rDNA and DAPI bands, the Antarctic populations were most similar to each other. This is consistent with previous observations of chromosomal asymmetry. On the other hand, the Patagonian diploid populations had lower similarity between themselves and with the Antarctic populations for the characters analyzed, which is also reflected in other karyotype aspects.

Cytogenetic characters in a phylogenetic framework

Overall the observed cytogenetic features agree with the information revealed by phylogeny, stressing the value of the combined use of both methods in studies of phylogeny and evolution (Urdampilleta et al. 2013). The basic number $x = 13$ and the heteromorphic chromosome complement observed for the *Deschampsia* clade (Fig. 8) suggest that the origin of the genus might be related to a hybridization event, from two different parental genomes $x = 7$: one composed of *m* chromosomes and other by *a* chromosomes (Albers 1980). The change from $x = 7$ to $x = 13$ would have been the result of the loss of a chromosome pair, from an ancestor $2n = 4x = 28$, giving rise to $2n = 26$ (Kawano 1963; Albers 1980; García-Suárez et al. 1997). Therefore, *Deschampsia* would be considered an amphitetraploid. The monophyly of distant and morphologically variable populations of *D. antarctica* is confirmed in the present analysis and is supported by chromosomal features.

Based on the reconstruction of ancestral states, it follows that the ploidy level of the population 845 ($2n = 4x = 52$) is a derived characteristic. DNA fragment loss is a common process in polyploids (Wendel 2000; Roa and Guerra 2012), and in this sense, the number of rDNA loci of that population probably resulted in the loss of rDNA sites. Polyploid population 845 is the least related to the remaining populations, forming a separate clade.

Low cytogenetic variability among diploid populations is consistent with reduced molecular variation. The 880 population presents derived condition in relation to the disposition of rDNA loci and forms a separate clade from the remaining diploid populations. The latter conform a group without resolution because of the low (or sometimes null) sequence variation of ITS and *tnrK*. This clade is characterized by the presence of an additional *a* pair with 5S rDNA on long arm, by basic genome $x = 13$ (Fig. 8).

Despite the polytomy of diploids due to low molecular variation in DNA sequences, chromosomal variants detected highlight the value of accurate detection of repetitive DNA regions in the study of genome evolution. In addition, our results shed light on the hypothetical ancestral karyotype of *D. antarctica*, consisting of a diploid chromosome complement of $2n = 26$ chromosomes, with two 18-5.8-26S rDNA loci: one intercalar locus on the short arm of a sm pair and another pericentromeric locus on the short arm of a *m* pair; and five 5S rDNA loci: one on short arm of a *a* pair, one on long arm of another *a* pair and two on *m* pairs. The fifth loci position is uncertain. Both Antarctic populations such as the Patagonian number 877 population keep the ancestral states of *D. antarctica.*

Origin and chromosomal diversification in D. antarctica

The rDNA pattern in the polyploids does not represent an exact duplication of the diploid population genome, since only three have 18-5.8-26S rDNA loci and seven 5S rDNA loci, i.e., less than twice of the diploid. After formation, autopolyploids may develop a diploidization process, where the removal of DNA regions and heterochromatinization are important events in reducing unviable gametes (Dvořák and Appels 1982; Wendel 2000; Adams and Wendel 2005). This includes loss of rDNA sites (Wendel 2000; Winterfeld and Röser 2007a; Roa and Guerra 2012), a progressive process after the duplication which could indicate its age (Roa 2011). However, the decrease in rDNA regions could have been inherited from parental genomes, and therefore, it is not possible to disregard allopolyploidy, since for multiple ribosomal markers the presence of a single pair was observed, as well as for 18-5.8-26S rDNA loci. This, combined with the observation of unique chromosome pairs detected by conventional techniques, suggests a possible origin by hybridization of relatively distinct genomes of *D. antarctica* or other related species. Phylogenetic analysis could

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support such an origin, since this polyploid population forms a separate clade from the remaining diploid populations. Remarkably, population 845 is located close to an area considered a geographic barrier that prompted lineage divergence in other plant groups as Nothofagaceae, due to an ancient marine ingression (Premoli et al. 2012). Once the waters retreated, divergent diploid populations would have been again in secondary contact, originating the polyploid type. For a sound understanding of the origin and evolution of polyploids in *D. antarctica*, it is necessary to extend the study to other populations through new techniques of molecular cytogenetics and DNA sequence analysis, as well as the inclusion of other South American related species and more Antarctic populations in order to verify or discard the presence of polyploids.

Considering all chromosomal aspects of the studied populations of *D. antarctica*, the South American populations are more variable than the Antarctic. Molecular studies conducted using AFLP, RAPD, sequences of the chloroplast and nuclear ITS (Holderegger et al. 2003; Chwedorzewska et al. 2008; Van de Wouw et al. 2008; Andreev et al. 2010; Volkov et al. 2010) suggest that Antarctic populations generally have low genetic variation and that diversity increases at lower latitude (Holderegger et al. 2003; Van de Wouw et al. 2008). Until now, no genetic studies have included Patagonian and Antarctic populations; however, preliminary results from ITS sequences suggest that Patagonian populations of *D. antarctica* have more genetic variability that Antarctic populations (Fasanella et al. in preparation). The genomic variation of rDNA in marginal populations could be associated with geographic factors, being source of speciation processes such was proposed for other grasses (Raskina et al. 2004; Belyayev and Raskina 2013). However, the low genetic variation in Antarctica could be a result of a migration recent.

The distribution pattern of chromosomal variability supports the hypothesis that Antarctic populations of *D. antarctica* were originated from South American (Holderegger et al. 2003; Van de Wouw et al. 2008; Volkov et al. 2010); the moment of this dispersal is still uncertain. The arrival to Antarctica might have occurred before the formation of barriers such as the Drake Passage and the Antarctic Circumpolar Current (Parnikoza et al. 2007) some 30 million years ago (Barker et al. 2007); or the migration might be an even more recent event (Mosyakin et al. 2007). Despite the overall low genetic diversity found in Antarctica, Volkov et al. (2010) suggested that *D. antarctica* might have dispersed to Antarctica in several independent events from different populations. However, by comparing the cytogenetic and molecular variability between South American and Antarctic populations, it is easier to explain the low Antarctica variability if dispersal occurred from one or few populations. Antarctic homogeneity could be due to a small number of individual colonizers dispersing from South America, probably similar to the 877 population. The cytotypes (this paper) and haplotypes (Fasanella et al. in preparation) found in Antarctica are also present in South America, implying that no significant changes occurred since dispersal to Antarctica. In order to understand the mode and tempo of this rare dispersal event, a phylogeography of *D. antarctica*, including a good sampling throughout the whole distribution range, is needed.

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References

Adams KL, Wendel JF (2005) Polyploidy and genome evolution in plants. Curr Opin Plant Biol 8:135–141

Alberdi M, Bravo LA, Gutiérrez A, Gidekel M, Corcuera LJ (2002) Ecophysiology of Antarctic vascular plants. Physiol Plant 115:479–486

Albers F (1980) Vergleichende karyologie der Gräser subtriben Aristaveninae und Airinae [Poaceae Aveneae]. Plant Syst Evol 136:137–167

Andreev IO, Spiridonova EV, Kyryachenko SS, Parnikoza IY, Maidanyuk DN, Volkov RA, Kozeretskab IA, Kunakh VA (2010) Population-genetic analysis of *Deschampsia antarctica* from two regions of maritime antarctica. Moscow Univ Biol Sci Bull 65:208–210

Arohonka T (1982) Chromosome counts of vascular plants of the island Seili in Nauvo, southwestern Finland. Turun Yliopiston Julkaisuja, Sar A 2. Biol Geogr 3:1–12

Barker PF, Filippelli GM, Florindo F, Martin EE, Scher HD (2007) Onset and role of the Antarctic Circumpolar Current. Deep Sea Res Part 2 Top Stud Oceanogr 54:2388–2398

Barros e Silva AE, Guerra M (2010) The meaning of DAPI bands observed after C-banding and FISH procedures. Biotech Histochem 85:115–125

Belyayev A, Raskina O (2013) Chromosome evolution in marginal populations of *Aegilops speltoides*: causes and consequences. Ann Bot 111:531–538

Bravo LA, Ulloa N, Zuñiga GE, Casanova A, Corcuera LJ, Alberdi M (2001) Cold resistance in Antarctic angiosperms. Physiol Plant 111:55–65

Brochmann C, Brysting AK, Alsos IG, Borgen L, Grundt HH, Scheen AC, Elven R (2004) Polyploidy in arctic plants. Biol J Linn Soc 82:521–536

Byun MY, Lee J, Cui LH, Kang Y, Oh TK, Park H, Lee H, Kim WT (2015) Constitutive expression of DaCBF7, an Antarctic vascular plant *Deschampsia antarctica* CBF homolog, resulted in improved cold tolerance in transgenic rice plants. Plant Sci 236:61–74

Cardone S, Sawatani P, Rush P, García AM, Poggio L, Schrauf G (2009) Karyological studies in *Deschampsia antarctica* Desv. (Poaceae). Polar Biol 32:427–433

Chiapella J (2007) A molecular phylogenetic study of *Deschampsia* (Poaceae: Aveneae) inferred from nuclear ITS and plastid trnL

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sequence data: support for the recognition of *Avenella* and *Vahlodea*. Taxon 56:55–64

Chiapella J, Probatova N (2003) The *Deschampsia cespitosa* complex (Poaceae: Aveneae) with special reference to Russia. Bot J Linn Soc 142:213–228

Chiapella J, Zuloaga FO (2010) A Revision of *Deschampsia, Avenella,* and *Vahlodea* (Poaceae, Poeae, Airinae) in South America. Ann Mo Bot Gard 97:141–162

Chwedorzewska KJ, Giełwanowska I, Szczuka E, Bochenek A (2008) High anatomical and low genetic diversity in *Deschampsia antarctica* Desv. from King George Island, the Antarctic. Pol Polar Res 29:377–386

Darriba D, Taboada GL, Doallo R, Posada D (2012) jModelTest 2: more models, new heuristics and parallel computing. Nat Methods 9:772

Davlianidze MT (1985) Chromosome numbers in the representatives of the flora from Georgia. Bot Zhurnal 70:698–700 **(Moscow & Leningrad)**

Di Rienzo JA, Casanoves F, Balzarini MG, Gonzalez L, Tablada M, Robledo CW (2008) InfoStat, versión 2008. Grupo InfoStat, FCA, Universidad Nacional de Córdoba, Argentina

Doyle JJ, Doyle JL (1990) Isolation of plant DNA from fresh tissue. Focus 12:13–15

Dvořák J, Appels R (1982) Chromosome and nucleotide sequence differentiation in genomes of polyploid *Triticum* species. Theor Appl Genet 63:349–360

Edgar RC (2004) MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res 32:1792–1797

Engelskjøn T (1979) Chromosome numbers in vascular plants from Norway, including Svalbard. Opera Bot 52:1–38

García-Suárez R, Alonso-Blanco C, Fernandez-Carvajal M, Fernandez-Prieto J, Roca A, Giraldez R (1997) Diversity and systematics of *Deschampsia* sensu lato (Poaceae), inferred from karyotypes, protein electrophoresis, total genomic DNA hybridization and chloroplast DNA analysis. Plant Syst Evol 205:99–110

Gidekel M, Destefano-Beltran L, García P, Mujica L, Leal P, Cuba M, Fuentes L, Bravo LA, Corcuera LJ, Alberdi M (2003) Identification and characterization of three novel cold acclimation-responsive genes from the extremophile hair grass *Deschampsia antarctica* Desv. Extremophiles 7:459–469

Greilhuber J, Speta F (1976) C-banded karyotypes in the *Scilla hohenackeri* group, *S. persica*, and *Puschkinia* (Liliaceae). Plant Syst Evol 126:149–188

Guerra M (1988) Introdução à citogenética geral. Guanabara Koogan, Rio de Janeiro

Hagerup O (1932) Über Polyploidie in Beziehung zu Klima, Ökologie und Phylogenie. Hereditas 16:19–40

Harper JA, Thomas ID, Lovatt JA, Thomas HM (2004) Physical mapping of rDNA sites in possible diploid progenitors of polyploid *Festuca* species. Plant Syst Evol 245:163–168

Hasterok R, Wolny E, Hosiawa M, Kowalczyk M, Kulak-Ksiazczyk S, Ksiazczyk T, Heneen W, Maluszynska J (2006) Comparative analysis of rDNA distribution in chromosomes of various species of *Brassicaceae*. Ann Bot 97:205–216

Hedberg O (1986) On the manifestation of vivipary in *Deschampsia cespitosa* s. lat. Symb Bot Ups 27:183–192

Hilu KW (2004) Phylogenetics and chromosomal evolution in the Poaceae (grasses). Aust J Bot 52:13–22

Holderegger R, Stehlik I, Lewis Smith R, Abbott R (2003) Populations of Antarctic hairgrass (*Deschampsia antarctica*) show low genetic diversity. Arct Antarc Alp Res 35:214–217

Hunziker JH, Stebbins GL (1987) Chromosomal evolution in the Gramineae. In: Soderstrom TR, Hilu KW, Campbell CS, Barkworth ME (eds) Grass systematics and evolution. Smithsonian Institution Press, Washington, pp 179–188

John UP, Polotnianka RM, Sivakumaran KA, Chew O, Mackin L, Kuiper MJ, Talbot JP, Nugent GD, Mautord J, Spangenberg GC (2009) Ice recrystallization inhibition proteins (IRIPs) and freeze tolerance in the cryophilic Antarctic hair grass *Deschampsia antarctica* E. Desv. Plant Cell Environ 32:336–348

Kawano S (1963) Cytogeography and evolution of the *Deschampsia cespitosa* complex. Can J Bot 41:719–742

Komárková V, Poncet S, Poncet J (1985) Two native Antarctic vascular plants, *Deschampsia antarctica* and *Colobanthus quitensis*: a new southernmost locality and other localities in the Antarctic Peninsula area. Arct Alp Res 17:401–416

Komárková V, Poncet S, Poncet J (1990) Additional and revisited localities of vascular plants *Deschampsia antarctica* Desv. and *Colobanthus quitensis* (Kunth) Bartl. in the Antarctic Peninsula area. Arct Alp Res 22:108–113

Książczyk T, Taciak M, Zwierzykowski Z (2010) Variability of ribosomal DNA sites in *Festuca pratensis*, *Lolium perenne*, and their intergeneric hybrids, revealed by FISH and GISH. J Appl Genet 51:449–460

Lawrence WE (1945) Some ecotypic relations of *Deschampsia cespitosa*. Am J Bot 32:298–314

Levin DA (2002) The role of chromosomal change in plant evolution. Oxford University Press, New York

Lideikytė L, Pašakinskienė I, Lemežienė N, Nekrošas S, Kanapeckas J (2008) FISH assessment of ribosomal DNA sites in the chromosome sets of *Lolium*, *Festuca* and *Festulolium*. Agriculture 95:116–124

Löve A, Löve D (1975) IOPB chromosome number reports. Taxon 24:504–507

Maddison WP, Maddison DR (2015) Mesquite: a modular system for evolutionary analysis. Version 3.03. http://mesquiteproject.org. Accessed 19 June 2015

Maluszynska J, Heslop-Harrison J (1993) Physical mapping of rDNA loci in *Brassica* species. Genome 36:774–781

Montiel P, Smith A, Keiller D (1999) Photosynthetic responses of selected Antarctic plants to solar radiation in the southern maritime Antarctic. Polar Res 18:229–235

Moore DM (1970) Studies in *Colobanthus quitensis* (Kunth) Bartl. and *Deschampsia antarctica* Desv. II. Taxonomy, distribution and relationships. Br Antarct Surv Bull 23:63–80

Mosyakin S, Bezusko L, Mosyakin A (2007) Origins of native vascular plants of Antarctica: comments from a historical phytogeography viewpoint. Cytol Genet 41:308–316

Navrotska DO, Twardovska MO, Andreev IO, Parnikoza IY, Betekhtin AA, Zahrychuk OM, Kunakh VA (2014) New forms of chromosome polymorphism in *Deschampsia antarctica* Desv. from the Argentine islands of the Maritime Antarctic region. Ukr Antarct J 13:185–191

Otto SP, Whitton J (2000) Polyploid incidence and evolution. Annu Rev Genet 34:401–437

Parnikoza IY, Maidanuk D, Kozeretska I (2007) Are *Deschampsia antarctica* Desv. and *Colobanthus quitensis* (Kunth) Bartl. Migratory Relicts? Cytol Genet 41:226–229

Paszko B (2006) A critical review and a new proposal of karyotype asymmetry indices. Plant Syst Evol 258:39–48

Petrovsky VV, Zhukova PG (1981) Chromosome numbers and taxonomy of some plant species of Wrangel Island. Bot Zhurnal 66:380–387 **(Moscow & Leningrad)**

Premoli AC, Mathiasen P, Acosta MC, Ramos VA (2012) Phylogeographically concordant chloroplast DNA divergence in sympatric *Nothofagus s.s.* How deep can it be? New Phytol 193:261–275

Raskina O, Belyayev A, Nevo E (2004) Quantum speciation in *Aegilops*: molecular cytogenetic evidence from rDNA cluster variability in natural populations. PNAS 101:14818–14823

Author's personal copy

Raskina O, Barber JC, Nevo E, Belyayev A (2008) Repetitive DNA and chromosomal rearrangements: Speciation-related events in plant genomes. Cytogenet Genome Res 120:351–357

Reeves A (2001) MicroMeasure: a new computer program for the collection and analysis of cytogenetic data. Genome 44:439–443

Roa F (2011) Análise da distribuição dos sítios de DNA ribossomal 5S e 45S em cariótipos de espécies vegetais. Tese de Doutorado, Programa de Pós-Graduação em Biologia Vegetal Universidade Federal de Pernambuco, Recife

Roa F, Guerra M (2012) Distribution of 45S rDNA sites in chromosomes of plants: structural and evolutionary implications. BMC Evol Biol 12:225

Rohlf FH (2000) NTSYS. Numerical taxonomy and multivariate analysis system. Version 2.1. New York

Romero Zarco C (1986) A new method for estimating karyotype asymmetry. Taxon 35:526–530

Röser M, Winterfeld G, Grebenstein B, Hemleben V (2001) Molecular diversity and physical mapping of 5S rDNA in wild and cultivated oat grasses (Poaceae: Aveneae). Mol Phylogenet Evol 21:198–217

Rothera S, Davy A (1986) Polyploidy and habitat differentiation in *Deschampsia cespitosa*. New Phytol 102:449–467

Ruhland CT, Day TA (2000) Effects of ultraviolet-B radiation on leaf elongation, production and phenylpropanoid concentrations of *Deschampsia antarctica* and *Colobanthus quitensis* in Antarctica. Physiol Plant 109:244–251

Schwarzacher T, Heslop-Harrison P (2000) Practical in situ hybridization. BIOS Scientific Publishers Ltd, Oxford

Schwarzacher T, Ambros P, Schweizer D (1980) Application of Giemsa banding to orchid karyotype analysis. Plant Syst Evol 134:293–297

Schweizer D, Loidl J (1987) A model for heterochromatin dispersion and the evolution of C-band patterns. In: Stahl A, Luciani JM, Vagner-Capodano AM (eds) Chromosomes today 9. Allen and Unwin, London, pp 61–74

Shaw J, Lickey EB, Schilling EE, Small RL (2007) Comparison of whole chloroplast genome sequences to choose noncoding regions for phylogenetic studies in angiosperms: the tortoise and the hare III. Am J Bot 94:275–288

Shelukhina OY, Badaeva E, Loskutov I, Pukhal'Sky V (2007) A comparative cytogenetic study of the tetraploid oat species with the A and C genomes: *Avena insularis*, *A. magna*, and *A. murphyi*. Russ J Genet 43:613–626

Sokolovskaya AP, Probatova NS (1975) Chromosome numbers of some grasses (Poaceae) of the USSR. Bot Zhurnal 60:667–678 **(Moscow & Leningrad)**

Soltis PS, Soltis DE (2009) The role of hybridization in plant speciation. Annu Rev Plant Biol 60:561–588

Soreng RJ, Peterson PM, Romaschenko K, Davidse G, Zuloaga FO, Judziewicz EJ, Filgueiras TS, Davis JI, Morrone O (2015) A worldwide phylogenetic classification of the Poaceae (Gramineae). J Syst Evol 53:117–137

Stebbins GL (1950) Variation and evolution in plants. Columbia University Press, New York

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

Stebbins GL (1975) The role of polyploid complexes in the evolution of North American grasslands. Taxon 24:91–106

Swofford DL (2000) PAUP* Phylogenetic Analysis Using Parsimony (* and Other Methods). Version, 4. Sinauer Associates Inc., Sunderland

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013) MEGA6: molecular evolutionary genetics analysis version 6.0. Mol Biol Evol 30:2725–2729

Tateoka T (1955) Karyotaxonomy in Poaceae III. Further studies of somatic chromosomes. Cytologia 20:296–306

Urdampilleta JD, Coulleri JP, Ferrucci MS, Forni-Martins ER (2013) Karyotype evolution and phylogenetic analyses in the genus *Cardiospermum* L. (Paullinieae, Sapindaceae). Plant Biol 15:868–881

van de Wouw M, van Dijk P, Huiskes AH (2008) Regional genetic diversity patterns in Antarctic hairgrass (*Deschampsia antarctica* Desv.). J Biogeogr 35:365–376

Vieira RC, Mantovani A (1995) Anatomia foliar de *Deschampsia antarctica* Desv. (Gramineae). Rev Bras Bot 18:207–220

Volkov R, Kozeretska I, Kyryachenko S, Andreev I, Maidanyuk D, Parnikoza IY, Kunakh V (2010) Molecular evolution and variability of ITS1–ITS2 in populations of *Deschampsia antarctica* from two regions of the maritime Antarctic. Polar Sci 4:469–478

Weiss-Schneeweiss H, Emadzade K, Jang TS, Schneeweiss GM (2013) Evolutionary consequences, constraints and potential of polyploidy in plants. Cytogenet Genome Res 140:137–150

Wendel JF (2000) Genome evolution in polyploids. Plant Mol Biol 42:225–249

White TJ, Bruns T, Lee SJWT, Taylor JW (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (eds) PCR protocols: a guide to methods and applications. Academic Press, New York, pp 315–322

Winterfeld G (2006) Molekularcytogenetische Untersuchungen an Hafergräsern (*Aveneae*) und Anderen Poaceae. Stapfia 86:1–170

Winterfeld G, Röser M (2007a) Disposition of ribosomal DNAs in the chromosomes of perennial oats (Poaceae: Aveneae). Bot J Linn Soc 155:193–210

Winterfeld G, Röser M (2007b) Chromosomal localization and evolution of satellite DNAs and heterochromatin in grasses (Poaceae), especially tribe Aveneae. Plant Syst Evol 264:75–100

Zoldos V, Papes D, Cerbah M, Panaud O, Besendorfer V, Siljak-Yakovlev S (1999) Molecular-cytogenetic studies of ribosomal genes and heterochromatin reveal conserved genome organization among 11 *Quercus* species. Theor Appl Genet 99:969–977

Zurita F, Jimenez R, Burgos M, de la Guardia R (1998) Sequential silver staining and in situ hybridization reveal a direct association between rDNA levels and the expression of homologous nucleolar organizing regions: a hypothesis for NOR structure and function. J Cell Sci 111:1433–1439