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45 Abstract

Kerguelen Islands harbor a unique and probably ancient flora with a high rate of endemism. 46 However, few is known on evolutionary history and characteristics of this flora. This concerns 47 in particular genome size and ploidy level variation, despite the evolutionary and ecological 48 significance of those traits. Here we report the first assessment of genome size, using flow 49 50 cytometry, for eight plant species of which two are endemics of Kerguelen Islands and four of 51 the South Indian Ocean Province. The 2C DNA value ranged from 1.08 pg for Pringlea antiscorbutica to 11.88 pg for Ranunculus biternatus. The chromosome numbers of 52 Colobanthus kerguelensis (2n = 80), Lyallia kerguelensis (2n = 96) and Poa kerguelensis (2n = 80)53 = 28), were also reported in this study for the first time. Overall, our data allowed to infer that 54 55 all Kerguelen studied species are polyploid (from tetra to octopolyploid). Intra-genus comparisons showed significant differences of 2C DNA values among Poa and among 56 Ranunculus species, despite their identical ploidy levels. In addition, our data highlight the 57 existence of an intraspecific variability of genome size for the two octoploid species 58 59 Colobanthus kerguelensis and Lyallia kerguelensis. Finally, our data also support the hypothesis according which polyploidy may have played a major role in the adaptation of 60 flowering plants to high latitudes, as it has been suggested for arctic species. 61

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Keywords 2C DNA value • Endemic species • Flow cytometry • Genome size • Ploidy level
• sub-Antarctic flora

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67 Introduction

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The sub-Antarctic islands (sensu Smith 1984; Van der Putten et al. 2010) host a depauperate 69 angiosperm flora of about 58 taxa. These islands were very early suggested to be important in 70 the floristic history of the Southern Hemisphere as possible stepping-stones for dispersal or 71 refuges for plant species during glacial times (e.g., Hooker 1847; Werth 1911; Hennion and 72 Walton 1997; Chown et al. 2001; Van der Putten et al. 2010). However, the evolutionary 73 74 histories of sub-Antarctic plant taxa have so far been little studied (Winkworth et al. 2015; Lehnebach et al. 2017). The sub-Antarctic plants are already feeling the impacts of 75 contemporary climate change (Le Roux et al. 2005; Frenot et al. 2006; Bergstrom et al. 2015). 76 This situation appeals urgently for deeper insights into biological and genetic characteristics 77 78 of native species that may contribute to their adaptation to this harsh environment and possibly to their resilience to climate change. 79

The Kerguelen Islands that appeared ~29 Ma ago (Nicolaysen et al. 2000), are one of 80 key sites for addressing both the history and future of the sub-Antarctic flora. Some species 81 82 may have been long present on the islands and survived Pliocene-Pleistocene climate change in situ (e.g., Wagstaff and Hennion 2007; Bartish et al. 2012) while others may be the result 83 of more recent, perhaps Holocene, colonisation (Lehnebach et al. 2017). While most previous 84 studies addressed morphological variability, ecology and ecophysiology of these species 85 86 (Hennion et al. 2012; Hermant et al. 2013; Labarrere et al. 2019), very little is known about the characteristics of their genome and especially their genome size. 87

Genome size (2C-value) or amount of DNA in a somatic unreplicated nucleus (Swift 88 1950; Greilhuber et al. 2005) is one of the most fundamental biological characters of living 89 organisms and it is frequently correlated with many biotic and abiotic parameters (Bennett 90 and Leitch 2005; Pustahija et al. 2013). Two processes are responsible for differences in 91 genome size and complexity. One is change in monoploid genome size 1Cx (DNA content of 92 a monoploid genome with chromosome base number x, according to Greilhuber et al. 2005) 93 94 due to variation in the copy number of genetic elements after duplication or deletion events. The other involves whole genome duplication or polyploidization. Knowledge on genome 95 size is useful in many disciplines such as ecology and phytogeography (Grime and Moworth 96 1982; Price and Johnston 1996; Pustahija et al. 2013), systematics and evolution (Cerbah et al. 97 98 1999; Niketic et al. 2013; Hajrudinovic et al. 2015), in biotechnology and agronomical sciences (Fyad-Lameche et al. 2016; Srisuwan et al. 2019) and also in biodiversity screening 99 (Bennett et al. 2000; Siljak-Yakovlev et al. 2010; Bou Dagher-Kharrat et al. 2013; Siljak-100 Yakovlev et al. 2019). 101

Among the different methods for estimating genome size, flow cytometry is now the most currently used (Marie and Brown 1993; Doležel et al. 2007; Pellicer and Leitch 2014; Bourge et al. 2018). This method is rapid, precise, easy for sample preparation and accurate for detection of small differences in DNA content (Karrat-Souissi et al. 2013; Pellicer and Leitch 2014). Despite its biological relevance, genome size has been estimated for only 3.1% of all angiosperms and 41% of gymnosperms (Pellicer et al. 2018).

108 The chromosome number is also one of relevant biodiversity characters (Guerra 2008)109 that has been considered in this work.

110 The present work mainly aims to study the interspecific variability of genome size, 111 chromosome number and ploidy levels of ten Kerguelen species including two strictly 112 endemic species (*Lyallia kerguelensis* and *Ranunculus moseleyi*) and four other species 113 endemic to the South Indian Ocean Province (*Colobanthus kerguelensis*, *Poa cookii*, *Poa* 114 *kerguelensis* and *Pringlea antiscorbutica*). At the same time, it is a first step in building a 115 genome size database for plants from the Kerguelen Islands.

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117 Materials and methods

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119 Plant material

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Plant material from ten species were collected from natural populations in Kerguelen Islands 121 over 3 field campaigns between 2015 and 2019 (Table 1, Fig. 1). The studied species belong 122 to six families and six genera. Nine of them were native to Kerguelen Islands whereas Poa 123 annua was an introduced one (Table 1). Lyallia kerguelensis and Ranunculus moseleyi are 124 strictly endemic to Kerguelen (Hennion and Walton 1997; Lehnebach et al. 2017). 125 Colobanthus kerguelensis, Poa cookii, Poa kerguelensis and Pringlea antiscorbutica are 126 endemic to the South Indian Ocean Province (SIOP) that includes Kerguelen, Marion and 127 Prince Edward Islands, Crozet, and Heard (Smith 1984; Van der Putten et al. 2010). Except 128 for Limosella australis at least three populations per species were studied. 129

During the field work the plant material (leaves) was immediately dried and conservedin silica gel until use. Living plants were stored in zip-lock plastic bags then potted at

Kerguelen station. After a few weeks, living plants were shipped back to metropolitan France 132 and cultivated in a phytotron in CNRS-UMR 6553 ECOBIO, University of Rennes 1 (Ecolex 133 facility). Root tips for karyological analyses and leaves for genome size studies were sampled 134 from these plants in cultivation. Seeds of C. kerguelensis were collected from one population 135 in Pointe Suzanne in April 2017 and seeds of L. kerguelensis. from two populations in Ile 136 Australia in March 2019. Seeds were placed in paper bags, let dry a few days at room 137 temperature then stored in the presence of silica gel until use. The seeds of both species were 138 germinated in the lab at 20°C (Hennion and Walton 1997). Vouchers of the studied 139 populations are available in CNRS-UMR 6553 ECOBIO, University of Rennes 1, and in 140 CNRS-UMR 8079 ESE, University Paris-Saclay. 141

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143 Genome size estimation by flow cytometry

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The total nuclear DNA amount (2C-value) was assessed by flow cytometry according to 145 146 Bourge et al. (2018) using fresh or silica dried leaf samples and fresh leaves of one of four internal standards, in order to cover the range of variation of genome size: Solanum 147 lycopersicum L. 'Montfavet 63-5' (2C = 1.99 pg, Lepers-Andrzejewski et al. 2011) for very 148 small genomes, Petunia hybrida Vilm. 'PxPc6' (2C = 2.85 pg, Marie and Brown 1993) for 149 small genomes and Hordeum vulgare L. 'Sultan' (9.81 pg, Garnatje et al. 2004) for 150 intermediate genome size. A first genome size measurement was performed on fresh material 151 collected from seven species cultivated at Ecolex facility in CNRS-UMR 6553 ECOBIO, 152 153 University of Rennes 1 (Table 2). To test the reliability of genome size assessment on silica gel-dried material, parts of each fresh leaf sample were dried in silica gel before measurement 154 155 and the results of genome size measurements were compared in samples conditioned either way. 156

The leaves of both internal standard and target species were simultaneously chopped 157 using a razor blade in a sterile plastic Petri dish with 600 µl of cold Gif Nuclear-isolation 158 159 Buffer (GNB) which has been previously successfully tested on many dried samples (Razafinarivo et al. 2012), both on angiosperms and gymnosperms (Siljak-Yakovlev et al. 160 2019 and Farhat et al. 2019a respectively). This buffer contains 45 mM MgCl₂, 30 mM 161 sodium citrate and 60 mM MOPS acid pH 7.0, 1% PVP 10.000 and 10 mM sodium 162 metabisulfite (Na₂S₂O₅), a reducing agent less toxic than β -mercaptoethanol, and RNAse (2.5 163 U/ml). It was also complemented with 0.5% Triton X-100. The nuclei suspension was filtered 164 through (30 or 50 µm nylon) mesh. The nuclei were stained with 100 µg/ml propidium iodide 165 (PI), a specific DNA intercalating fluorochrome dye, and kept at least 5 min at 4°C. 166

167 DNA content of about 5000 stained nuclei was determined for each sample using the 168 cytometer CytoFLEX S with excitation 561 nm, 26 mW and emission through a 610/20 nm 169 band-pass filter (Beckman Coulter- Life Science United States). The samples for each species, 170 except for *Limosella australis*, comprised at least five individuals, measured separately. To 171 check the reproducibility of values, two replicates were performed for each individual.

172 CytExpert 2.3 software was used for histogram analyses. The total 2C DNA value 173 (DNA contents of the diploid (2*n*) sets of chromosomes, irrespective of ploidy level), was 174 calculated using the linear relationship between the fluorescent signals from stained nuclei of the species and the internal standard. The total nuclear DNA content was calculated accordingto the following formula:

177 2C DNA sample (pg) = (Sample 2C peak mean/Standard 2C peak mean)×Standard 2C DNA
178 (pg)

The symbol C corresponds to the holoploid nuclear genome size (the whole chromosome complement with chromosome number n in mitotic nuclear cycle), 1C DNA content presents one non-replicated holoploid genome with the chromosome number n and 2C DNA value corresponds to somatic chromosome number (2n), irrespective of ploidy level (Greilhuber et al. 2005).

- To check existing data and assign the status of newness for our reports, we used, apart 184 from bibliography for each species, existing genome size and chromosome number databases, 185 all of them accessed on January 25, 2020: Plant DNA C-values 186 database plant DNA (http://data.kew.org/cvalues), FLOWer, а flow cytometry database 187 188 (http://botany.natur.cuni.cz/flower/index.php), the Chromosome count database CCDB (http://ccdb.tau.ac.il/), the Index to Plant Chromosome Numbers (IPCN, www.tropicos.org/) 189 and the Brassicaceae: Chromosome number index (warwick_chrmno_data_xls). 190
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192 Chromosome number determination

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Root tips obtained from the potted plants cultivated in University of Rennes 1, CNRS-UMR 194 6553 ECOBIO, or by germination of seeds (case of Colobanthus kerguelensis and Lyallia 195 kerguelensis), were pretreated with 0.05% colchicine aqueous solution at room temperature 196 from 1 h 30 to 3 h or in 8-hydroxyquinoline 0.002 M during 2 - 3 h at 16°C and then fixed in 197 absolute ethanol and glacial acetic acid (3:1) for at least two days at 4 °C. Root tips were 198 hydrolyzed in 1 M HCl for 6 to 12 min at 60 °C, washed in distilled water at room 199 temperature, and stained in 1% orcein in 45% acetic acid for about 30 min. Root tip 200 meristems were squashed in a droplet of acetic carmine and observed under Zeiss Axiophot 201 microscopes. The best metaphase plates were photographed using CCD camera (RETIGA 202 203 2000R; Princeton Instruments, Evry, France).

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205 Data analyses

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207 Statistical analyses were carried out on the whole data set except for *Limosella australis* since only one sampled individual was available. Genome size variation was analyzed as described 208 in the following. For each species, non-parametric Fligner test was used to check for rank 209 variance homogeneity among populations. Non-parametric Kruskall-Wallis statistics were 210 used to test for significant variation of genome size among populations within species. In 211 addition, and because of small sample size, 10 000 random permutations of individuals among 212 population (within species) were also used to compute each p-value in order to confirm the 213 conclusion for the significance of the population effect of the Kruskall-Wallis statistics using 214 an independent test. For species showing a significant population effect, post-hoc non-215 216 parametric tests (Dunn, 1961) were used for population median pairwise comparisons. Finally, for the three Poa species and the three Ranunculus species, differences of genome 217

size among species within each genus were tested using two independent methods. Firstly, a 218 nested two-way analysis of variance was carried out on rank values of genome size to test for 219 comparisons among species and among populations as a potential dependence random factor 220 nested within species after testing for variance homogeneity using a Levene's test. The 221 population mean square was used as the error term to test for species difference. Adjusted p-222 223 values for multiple tests were computed according to Holm's method. Secondly, a non-224 parametric Kruskall-Wallis test was also carried out on genome size values to test for differences among populations (population factor nested within the species factor) as 225 described in Oron and Hoff (2006) and among species. Then, a Kruskall-Wallis test was 226 carried out on genome size values to test for significant differences among species. The p-227 228 value for the species effect was then computed using permutation of populations among species (1000 random permutations). Pairwise comparisons of median species differences 229 were carried out using a Dunn test (Dunn, 1961) using a Holm correction for multiple test 230 231 (Holm, 1979).

232

233 **Results**

The results on genome size assessment on fresh and dry materials did not show any significant differences. Therefore, only the results obtained on the dry material are presented.

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237 Variation of genome size among and within species

The genome size ranged from 2C = 1.08 pg for *Pringlea antiscorbutica* to 2C = 11.88 pg for *Ranunculus biternatus*. According to Leitch's et al. (1998) categories, the ten species displayed very small (2C < 2.8 pg; *Colobanthus kerguelensis*, *Limosella australis* and *Pringlea antiscorbutica*), small ($2.8 \le 2C < 7$; *Lyallia kerguelensis* and three *Poa* species) or intermediate ($7 \le 2C < 28$; three *Ranunculus* species) genome size (Table 2). No species with large ($28 \le 2C \le 75$) or very large (2C > 75 pg) genome size were found in the studied material.

For intra-genus comparisons, analyses of variance using rank values as well as permutation tests showed significant differences of 2C values among *Poa* species (F =167.64, df = (2;6), *p*-value < 0.0001; H = 39.10, *p*-value < 0.001 (permutation test) and among *Ranunculus* species (F = 9.64, df = (2;6), p-value = 0.013; H = 12.82, *p*-value = 0.016 (permutation test), despite identical ploidy levels within each genus.

In *Ranunculus* genus, post-hoc pairwise comparisons showed *R. biternatus* displayed a significantly higher genome size than the two other *Ranunculus* species. In *Poa* genus, post hoc pairwise comparisons between species were all highly significant.

For *Pringlea antiscorbutica*, the three *Poa* and the three *Ranunculus* species, no significant differences in 2C values among populations were observed. However, genome size varied significantly among populations of *Lyallia kerguelensis* (9.9% of population mean variation) and of *Colobanthus kerguelensis* (7.9% of population mean variation). For these two species, post-hoc pairwise comparisons showed that the population RBA10 for *C. kerguelensis* and the population MAC3 for *L. kerguelensis* displayed significantly smaller genomes than other populations from the same species.

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261 Chromosome number and estimation of ploidy levels

The results of chromosome counts and ploidy levels of studied species are presented in Table 2 and Fig. 2. The high chromosome number and basic chromosome number of each genus strongly supported the polyploid status of all investigated species. The chromosome numbers of *Colobanthus kerguelensis* (2n = 8x = 80), *Lyallia kerguelensis* (2n = 8x = 96) and *Poa kerguelensis* (2n = 4x = 28) are reported here for the first time, as well as for *Limosella australis* (2n = 6x = 60) from Kerguelen.

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269 **Discussion**

270 Chromosome number and ploidy level

In this study chromosome numbers and ploidy levels were reported for the first time for three
endemic species from Kerguelen or from the SIOP (*Lyallia kerguelensis*, *Poa kerguelensis*, *Colobanthus kerguelensis*) and for *Limosella australis* from Kerguelen.

Data relative to the ploidy level of species occurring in the sub-Antarctic region are yet limited except from the study of Bennett et al. (1982) concerning South Georgia. The Arctic region was shown to harbor a high occurrence of polyploid species (Brochmann et al. 2004). These authors also indicated that number and ploidy level strongly increased when going northwards in the Arctic region. Our data suggest that polyploidy may also be highly frequent in plant species living in high south latitudes.

We confirmed the tetraploid chromosome numbers (2n = 4x = 28) for *Poa annua* 280 previously reported in Kerguelen by Frenot et al. (1999). Only three reports on diploid (2n =281 14) in Slovakia, Australia and North India and two on hexaploid (2n = 6x = 42) P. annua 282 populations in China and Pakistan were indicated until now (Dhaliwal et al. 2018). In 283 numerus other areas across the world (at least 16 bibliographic references) this species occurs 284 285 as tetraploid in Kerguelen. Poa annua's introduction in Kerguelen is believed to be fairly recent, contemporary to first human visits on the island (first record in 1874 by Moseley; in 286 Frenot et al. 1999). Therefore, it appears that the chromosome number and the ploidy level of 287 *Poa annua* have not changed subsequently to its introduction. We confirmed the chromosome 288 number of 2n = 4x = 28 for *Poa cookii* (Hennion 1992). 289

Polyploidy or whole genome multiplication (WGM) is considered as one of major evolutionary forces in angiosperm evolution (Solstis and Solstis 2009). Possibly all angiosperms had at least one WGD during their evolutionary history (Van de Peer et al. 2017). In our panel all species are polyploid with different ploidy levels. *Colobanthus kerguelensis* and *Lyallia kerguelensis* showed the highest ploidy level (octoploid), three *Ranunculus* species and *Limosella australis* are hexaploids, and *Pringlea* and three *Poa* species are tetraploids.

In most reports so far *L. australis* presents the diploid chromosome number (2n = 20)except in New Zealand where it is hexaploid (2n = 60) as it was found in this study for the Kerguelen population (Blackburn 1939; Löve and Löve 1958; Hair and Beuzenberg 1960; Dawson 2000).

The case of *Pringlea antiscorbutica* is especially appealing. We confirmed the chromosome numbers (2n = 24) previously established for this species (Hamel 1951; Rollins and Rüdenberg 1971; Hennion and Couderc 1992; Kagale et al. 2014). This species is known to have diverged from a South American ancestor about 5 Mya ago (Bartish et al. 2012), but it

is not known whether it dispersed directly to the SIOP or whether Antarctica could be used as 305 a stepping-stone (Bartish et al. 2012). This species displayed the smallest chromosome 306 number 2n = 24, corresponding also to the smallest genome size (2C = 1.08 pg). In their study 307 on polyploid evolution using transcriptomic data in Brassicaceae, Kagale et al. (2014) 308 proposed that *Pringlea antiscorbutica* is a mesopolyploid (~10.22 Mya). Regarding the basic 309 310 chromosome numbers referenced in the Brassicaceae family (Kagale et al. 2014), Pringlea 311 could, at least, be considered as tetraploid with a basic chromosome number x = 6. Alternatively, it could be a hexaploid with x = 4, which is the smallest basic chromosome 312 number of Brassicaceae, according to Lysak et al. (2006). Interestingly, Kagale et al. (2014) 313 dated the few major polyploidy and lineage separation events they identified during 314 315 Brassicaceae evolution around epoch transitions characterized by prolonged unstable climatic conditions. These authors suggested that polyploidy might have increased tolerance of 316 Brassicaceae to these changes and facilitated species radiation. This might indeed be the case 317 for P. antiscorbutica which speciation estimated time is contemporary to climate cooling in 318 319 the southern landmasses and in Antarctica (Bartish et al. 2012).

We confirmed the chromosome numbers and ploidy levels (2n=6x=48) for the three *Ranunculus* species previously reported in Kerguelen by Hennion and Couderc (1992, 1993) and by Bennett et al. (1982) for *R. biternatus* from south Atlantic island South Georgia.

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324 Genome size variation among species

Data given in Table 2 are the first report on genome size for plant species growing in Kerguelen Islands except for *Poa annua* which was already measured from Crozet and Kerguelen by Frenot et al. (1999). The genome size was also already reported for *Ranunculus biternatus* but from South Georgia by Bennett et al. (1982).

A 11-fold variation was found between the lowest 2C value, 1.08 pg for Pringlea 329 antiscorbutica, and the highest 2C value, 11.88 pg for Ranunculus biternatus. However, seven 330 of these ten species belong to groups of very small (2C < 2.8 pg) or small ($2.8 \le 2C < 7$ pg) 331 genome size, according to Leitch et al. (1998) categories. The remaining three species 332 (*Ranunculus* ssp.) displayed an intermediate genome size ($7 \le 2C \le 28$). This finding supports 333 the idea that species living at high latitudes have, in general, a small genome (Bennett et al. 334 1982; Brochmann et al., 2004). This trend could be related to the need for plants living in 335 336 these regions to cope with short growing periods. Cell cycle length and growing periods are in general shorter in plants with small genome (Bennett 1972, 1987; Beaulieu et al. 2008). It has 337 also been shown that these characteristics are favoured in hostile environments, in particular, 338 under temperature, water and nutritional stresses (low concentrations of macronutrients) 339 (Petrov 2001; Šmarda et al. 2013; Guignard et al 2016; Pellicer et al. 2018). 340

By their large genome constraint hypothesis, Knight et al. (2005) designed that plants with large genomes are under-represented in extreme environments. Many example (Pustahija et al. 2013; Carta and Peruzzi 2015) even among aquatic plants (Hidalgo et al. 2015) illustrated this general trend. Results obtained in this study also supports this hypothesis in extreme climatic conditions which occur in the sub-Antarctic region.

For *Poa annua*, Bennett (1982) indicated almost the same value as in the present study, 2C = $4.19 (\pm 0.06)$ pg. However, Frenot et al. (1999) reported a much smaller value of 2.95 (\pm 0.14) pg/2C for populations from French sub-Antarctic islands (Crozet and Kerguelen),

- although the chromosome number (2n = 28) was identical to the one reported here. However, the data obtained by these authors corresponded to relative DNA content because they used DAPI (4',6-diamidino-2-phenylindole), fluorochrome specific for A-T bases, which, in general, provides lower values than propidium iodide (DNA intercalating fluorochrome).
- The value of relative 1C reported for *Pringlea antiscorbutica* (1C = 0.62 pg) by Kagale et al. (2014) for material from Botanical Garden (Canberra, Australia) originally collected from Heard Island, is very close to the mean value (2C = 1.08 pg) estimated on samples from Kerguelen populations.
- Ranunculus biternatus is spread across the whole circumpolar area (Lehnebach et al. 357 2017). Bennett et al. (1982) reported 2C = 14.50 pg for *R. biternatus* from South Georgia, a 358 359 sub-Antarctic island in the South Atlantic Ocean. The present value obtained for this species was much smaller ($2C = 11.88\pm0.23$ pg). Variation in ploidy level cannot be invoked to 360 explain this result since the chromosome counts (2n = 48) are identical for both origins 361 (Bennett et al. 1982; Hennion and Couderc 1993; this work). This result could be explained 362 363 by the use of two different techniques of genome size assessment (Feulgen densitometry by Bennett et al. 1982, and flow cytometry in our case). Alternatively, this result could reflect a 364 true difference in genome size between populations of this species in the two locations. 365
- It should be noted that the *Ranunculus biternatus* lineage is sister to a clade of species from Australia and New Zealand, with estimates indicating very recent spread to Crozet and Kerguelen (Lehnebach et al. 2017). Therefore, populations from South Georgia, close to the magellanics, may be only distantly related to the populations of the same species in Kerguelen. A larger sampling effort of *R. biternatus* across its geographic range is required to investigate genome size variability in this species.
- 372 Comparisons of genome size among the three *Ranunculus* species revealed that *R*. biternatus displayed a significantly higher genome size than the two other Ranunculus species 373 (*R. pseudotrullifolius* and the endemic *R. moseleyi*) which did not differ one from each other. 374 This last result is in agreement with the hypothesis of a very recent divergence between these 375 376 last two species (Hennion et al. 1994; Lehnebach et al. 2017). Another explanation would be that genome size of these two species might be the target of stabilizing selection, at least since 377 their divergence, which would maintain similar genome size values (Šmarda et al. 2010). 378 Contrarily, R. biternatus has been shown to belong to a clearly different evolutionary lineage 379 380 than R. moseleyi and R. pseudotrullifolius (Lehnebach et al. 2017). This is in agreement with a significant difference in the genome size of these two taxa on one side and R. biternatus on 381 the other side. 382
- Colobanthus kerguelensis showed 2C value of 2.41 (\pm 0.08) pg. This value is higher 383 than all values reported for the congeneric C. quitensis, a very wide-ranged species inhabiting 384 extreme environments in Antarctica and the Andes and rarer locations northwards (Cuba-Diaz 385 et al. 2017). Indeed, Bennett et al (1982) reported 2C = 1.4 pg for one population of C. 386 *quitensis* in South Georgia. Moreover, Cuba-Diaz et al. (2017) determined 2C = 1.95 in two 387 populations of C. quitensis (King George Islands in the maritime Antarctic and one location in 388 389 Patagonia) and 2C = 0.84 pg in one population from Araucania region, growing in a more temperate zone and at high altitude (2575 m a.s.l.). The more than twofold difference in 390 genome size between the two populations of C. quitensis studied by Cuba-Diaz et al. (2017) 391 strongly suggests the existence of at least two ploidy levels in this species. Unfortunately, the 392

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record of chromosome number in C. quitensis is limited to the data reported in Bennett et al. (1982) which is identical to the count obtained for C. kerguelensis in this study (2n = 80). 394 More detailed cytogenetic studies are needed in populations of C. kerguelensis across the 395 SIOP and C. quitensis across Antarctica and South America to get a more comprehensive 396 view of the variations in genome size in these two species. 397

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399 Intraspecific variation of genome size at Kerguelen

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Whether intraspecific genome size constancy is mainly due to balance between sequences 401 gain/loss events (Petrov 2002) or is the target of stabilizing selection (Waltari and Edwards 402 403 2002; Šmarda et al. 2010.) is still being debated. However, more recently, studies aiming at assessing the intraspecific genome size variability suggest it may have been widely 404 underestimated until a recent past (Vekemans et al. 1996; Šmarda and Bureš 2006; Suda et al. 405 2007; Siljak-Yakovlev et al. 2008; Karrat-Souissi et al. 2013; Abdeddaim-Boughanmi et al. 406 2019; Farhat et al. 2019b). 407

No significant variation of genome size was observed among Kerguelen populations 408 studied for Pringlea antiscorbutica, for the three Poa species and for the three Ranunculus 409 species. It is however possible that interpopulation variation exists in those species but was 410 411 not detected in this study due to the limited number of sampled populations.

In contrast, significant variation in genome size was observed among studied 412 populations in Lyallia kerguelensis and in Colobanthus kerguelensis. In L. kerguelensis the 413 population MAC3 (located in the north of Kerguelen) differed significantly from the other 414 415 two populations due to its smallest 2C DNA values (Table 2). In Colobanthus kerguelensis, the population of Rallier du Baty differed significantly from the other three populations. The 416 history of glacier extent at Kerguelen may explain this last result. Indeed, the south-west 417 region including Péninsule Rallier du Baty was partially isolated from the rest of the main 418 419 Kerguelen land during last millennia by several glaciers contributing to the Calotte Cook (Jomelli et al. 2018). Further investigations are needed to get better insights into the 420 geographic patterns of intraspecific genome size variation across Kerguelen Islands and its 421 link with the history of species dispersal and establishment under various climatic and 422 ecological conditions. 423

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Conclusion and perspectives 425

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427 The obtained results on genome size of several species from the native Kerguelen flora 428 contributed to fill the important lack of knowledge on their cytogenetic variability. In 429 addition, our results support the fact that a majority of species of this flora, having established 430 on Kerguelen across a period of several million years at least for some taxa (Lehnebach et al. 2017; Winkworth et al. 2015; Bartish et al. 2012; Van der Putten et al. 2010; Wagstaff and 431 Hennion 2007) are polyploids. This observation is in agreement with what has been observed 432 for species of high latitudes in the Northern Hemisphere but also in the Falkland Islands and 433 South Georgia in the sub-Antarctic. These data did not allow us to highlight a strong 434 variability in genome size at the intraspecific level except for Lyallia kerguelensis and 435 Colobanthus kerguelensis. Further analyses on a larger sample of populations are therefore 436

necessary to better quantify this variability and explore its relationship with the ecological and
spatial distribution of these species in Kerguelen. Comparison of polyploidy frequency
between Arctic and Antarctic floras is essential to better understand the role of polyploidy in
plant adaptation to such extreme environments.

442 Authors' contributions:

S.S-Y. and F.H. conceived the ideas. F.L., F.H. and T.R. collected the samples. S.S-Y., N.V.
and N.T. performed the flow cytometry measurement. F.H. cultivated the plants in the field
and in the laboratory in Rennes and F.H. and V.G. collected and treated the root samples for
karyology. S.S.Y. performed the chromosome counts. T.R. performed data analysis. S.S-Y.
wrote the manuscript with contributions from T.R., F.H. and N.T. All the authors read and
approved the manuscript. SS-Y, TR and FH revised the manuscript following comments from
the Editor and three reviewers.

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- 451 **Compliance with ethical standards**
- 453 **Conflict of interest:** The authors declare that they have no conflict of interest
- 454
- 455

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717 Figure captions

- 718 **Fig. 1** Plant material collection sites in Kerguelen Islands
- **Fig. 2** Metaphase chromosome plates: A. Colobanthus kerguelensis (2n = 80), B. Lyallia
- 720 *kerguelensis* (2n = 96), and C. *Limosella australis* (2n = 60). Bar = 10 µm
- 721 Fig. 3 Cytometric histograms for ten studied species
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DNA content (log scale)



Table 1 Origin of investigated populations with localities, GPS coordinates and year of collection

Species/Population	Geographical range	Locality	GPS coordinates	Campagne	
Colobanthus kerguelensis Hook.f. (Caryophyllaceae)	endemic to SIOP				
IB		Isthme-Bas	-49.3872; 70.27326	2016-17	
CAJ		Cabane Jacky	-49.3134; 70.1234	2016-17	
TUS		Plateau du Tussok	-49.2967; 70.04608	2016-17	
RBA-10		Péninsule Rallier du Baty	-49.6601; 68.9797	2017-18	
SUZ^*		Pointe Suzanne	n.a.	Avril 2017	
Limosella australis R.Br. (Scrophulariaceae)	wide, native				
PJDA3		Péninsule Jeanne d'Arc	-49.52387; 70.15679	2015-16	
<i>Lyallia kerguelensis</i> Hook.f. (Montiaceae)	strict endemics				
AUS-23		south of Ile Australia	-49.4646; 69.8769	2015-16	
MAY-2		Ile Mayes	-49.47404; 69.93306	2016-17	
MAC-3		Ile Mac Murdo	-48.89503; 69.41626	2017-18	
AUS-25*		south of Ile Australia	-49.46568; 69.87563	March 2019	
AUS-30 [*]		south of Ile Australia	-49.47672; 69.89263	March 2019	
Poa annua L. (Poaceae)	wide,				
AUS-N	milouuccu	north of Ile Australia	-49.43579; 69.84936	2016-17	
PAF*		Port-aux- Français	-49.3526; 70.2170	2016-17	
STU-7		Val Studer	n.a.	2015-16	
MOL		Pointe Molloy	-49.3523; 70.07608	2016-17	
P. cookii (Hook.f.) Hook.f.	endemic to				
AUS-N	SIOP	north of Ile Australia	-49.4387; 69.8496	2016-17	
MAC 4		Ile Mac Murdo	-48.8877; 69.4199	2017-18	
AUS-S		south of Ile Australia	-49.47223; 69.89118	2015-16	
P. kerguelensis (Hook.f.) Steud.	endemic to				
AUS-N	5101	north of Ile Australia	-49.43973; 69.85439	2016-17	
IB		Isthme-Bas	n.a.	2016-17	
PAF		Near to Port-aux-Français	-49.3227; 70.1768	2016-17	
САЈ		Cabane Jacky	-49.3132; 70.1233	2016-17	

Pringlea antiscorbutica <u>R.Br.</u> ex <u>Hook.f.</u> (Brassicaceae)

CAJ		Cabane Jacky	-49.3124; 70.1239	2016-17
TUS		Plateau du Tussok	n.a.	2016-17
Ranunculus biternatus Sm.	circumpolar			
(Ranunculaceae)	area			
AUS-N		north of Ile Australia	-49.43211; 69.83792	2016-17
IB-13		Isthme-Bas	-49.3818; 70.2752	2016-17
IB-2		Isthme-Bas	-49.3843; 70.2590	2016-17
R. moseleyi Hook.f.	strict endemic			
AUS-N5		north of Ile Australia	-49.4216; 69.8239	2016-17
IB-2		Isthme Bas	-49.3843; 70.2590	2016-17
PAF-5		Port-aux-Français	-49.3352; 70.2265	2016-17
R. pseudotrullifolius Skottsb.	Magellanic and			
AUS-N6	Kerguelen	north of Ile Australia	-49.4497; 69.8642	2016-17
IB-1		Isthme-Bas	-49.3849; 70.2752	2016-17
OTA-1		Otaries	-49.3456; 70.1654	2016-17

Site abbreviations: AUS: Ile Australia; CAJ: Cabane Jacky; IB: Isthme-Bas; MAY: Ile Mayes; MAC: Ile Mac Murdo; MOL: Pointe Molloy; OTA: mouth of Rivière des Otaries; PAF: Port-aux-Français; PJDA: Presqu'île Jeanne d'Arc; RBA: Péninsule Rallier du Baty. STU: Val Studer. SUZ: Pointe Suzanne; TUS: Plateau du Tussok. Name of site is followed by population number. SIOP: South Indian Ocean Province

* population used only for chromosome counting

Species/Population	2C DNA in pg ^a	1C DNA	N	S	2n (ploidy)	P-value	Signif.
	(range)	in Mbp				\$	level §
Colobanthus kerguelensis					80 (8 <i>x</i>)		
IB	2.42 ^b (2.28-2.57)	1183	5	Р			
САЈ	2.39 (2.36-2.48)	1169	5				
TUS	2.38 (2.34-2.44)	1164	5				
RBA-10	2.26 (2.21-2.34)	1105	6				
Means (SD) for species	2.41 (0.08) ^c	1178	21			0.009	**
Limosella australis					60 (6 <i>x</i>)		
PJDA3	1.91	934	1	Р			
Lyallia kerguelensis					96 (8 <i>x</i>)		
AUS-23	3.18 (3.08-3.20)	1555	5	S			
MAY-2	3.21 (3.14-3.42)	1569	5				
MAC-3	2.97 (2.75-3.17)	1452	5				
Means (SD) for species	3.13 (0.21)	1526	15			0.014	*
Poa annua					28 (4 <i>x</i>)		
AUS-N	4.16 (4.12-4.21)	2034	6	Н			
STU-7	4.16 (4.12-4.27)	2034	5				
MOL	4.26 (4.13-4.29)	2083	5				
Means (SD) for species	4.19 (0.06)	2049	16			0.146	NS
Poa cookii					28 $(4x)^{d}$		
AUS-N	5.70 (5.45-5.89)	2787	5	Η			
MAC-4	5.80 (5.30-5.87)	2836	5				
AUS-1	5.67 (5.60-5.96)	2772	4				
Means (SD) for species	5.72 (0.18)	2797	14			0.914	NS
Poa kerguelensis					28 (4 <i>x</i>)		
AUS-N	4.99 (4.97-5.15)	2440	5	Н			
PAF	5.02 (4.96-5.14)	2454	5				

Table 2 Genome size (2C DNA in pg and 1C DNA in Mbp), chromosome number and ploidy level of ten species from Kerguelen Islands

CAJ	4.86 (4.72-5.19)	2377	5				
Means (SD) for species	5.00 (0.14)	2445	15			0.470	NS
Pringlea antiscorbutica					$24(4x)^{d}$		
AUS-N	1.03 (1-1.07)	504	5	Р			
САЈ	1.08 (1.05-1.15)	528	5				
TUS	1.09 (0.97-1.2)	538	5				
Means (SD) for species	1.08 (0.06)	528	15			0.064	NS
Ranunculus biternatus					$48 (6x)^{\mathbf{d}}$		
AUS-N	11.72 (11.7-11.74)	5731	5	Т			
IB-13	11.87 (11.81-12.12)	5804	5				
IB-2	12.09 (11.51-12.38)	5912	4				
Moons (SD) for species f	11 88 (0 23)	5800	1/			0 064	NS
Means (SD) for species	11.00 (0.23)	3009	14			0.004	110
Ranunculus moseleyi	11.00 (0.23)	3009	14		48 (6 <i>x</i> ^{d}	0.004	110
Ranunculus moseleyi AUS-N5	11.37 (11.12-12.48)	5560	5	Т	48 (6 <i>x</i> ^{d}	0.004	110
Ranunculus moseleyi AUS-N5 IB-2	11.37 (11.12-12.48) 11.15 (10.89-12.11)	5560 5452	5	Т	48 (6 <i>x</i> ^{d}	0.004	
Ranunculus moseleyi AUS-N5 IB-2 PAF-5	$ \begin{array}{c} 11.37\\(11.12-12.48)\\11.15\\(10.89-12.11)\\11.26\\(10.93-11.46)\end{array} $	5560 5452 5506	5 5 5	Т	48 (6 <i>x</i> ^d	0.004	
Ranunculus moseleyi AUS-N5 IB-2 PAF-5 Means (SD) for species	11.37 (11.12-12.48) 11.15 (10.89-12.11) 11.26 (10.93-11.46) 11.37 (0.45)	5560 5452 5506 5506	5 5 5 15	Т	48 (6 <i>x</i> ^{d}	0.533	NS
Ranunculus moseleyi AUS-N5 IB-2 PAF-5 Means (SD) for species Ranunculus pseudotrullifolius	11.37 (11.12-12.48) 11.15 (10.89-12.11) 11.26 (10.93-11.46) 11.37 (0.45)	5560 5452 5506 5506	5 5 5 15	Т	48 (6x ^d 48 (6x) ^d	0.533	NS
Ranunculus moseleyi AUS-N5 IB-2 PAF-5 Means (SD) for species Ranunculus pseudotrullifolius AUS-N6	11.37 (11.12-12.48) 11.15 (10.89-12.11) 11.26 (10.93-11.46) 11.37 (0.45) 11.34 (11.12-11.75)	5560 5452 5506 5506 5545	5 5 5 15 5	Т	48 (6x ^d 48 (6x) ^d	0.533	NS
Ranunculus moseleyi AUS-N5 IB-2 PAF-5 Means (SD) for species Ranunculus pseudotrullifolius AUS-N6 IB-1	$ \begin{array}{c} 11.37 \\ (11.12-12.48) \\ 11.15 \\ (10.89-12.11) \\ 11.26 \\ (10.93-11.46) \\ 11.37 (0.45) \\ \end{array} $ $ \begin{array}{c} 11.34 \\ (11.12-11.75) \\ 11.17 \\ (10.72-11.82) \end{array} $	5560 5452 5506 5506 5545 5462	5 5 5 15 5 5	T	48 (6x ^d 48 (6x) ^d	0.533	NS
Ranunculus moseleyi AUS-N5 IB-2 PAF-5 Means (SD) for species Ranunculus pseudotrullifolius AUS-N6 IB-1 OTA-1	11.37 (11.12-12.48) 11.15 (10.89-12.11) 11.26 (10.93-11.46) 11.37 (0.45) 11.37 (0.45) 11.17 (10.72-11.82) 11.31 (11.08-12.74)	5560 5452 5506 5506 5545 5462 5530	5 5 5 15 5 5 5	Т	48 (6x ^d 48 (6x) ^d	0.533	NS

^a1pg=978 Mbp according to Doležel et al. (2003); ^bWithin each population, the median and range of variation (between brackets) are given; ^cFor each species the mean and the standard deviation was computed including all populations; ^dPrevious count (Hennion and Couderc 1992); N=Number of studied individuals per population; S=Standard: *Petunia* (P), *Hordeum* (H), *Solanum* (S) and *Triticum* (T); \$=p-value for the "population within species" factor effect; \$= significance level after Holm correction for multiple tests; **: 1%; *: 5%; NS: not significant.

Site abbreviations: AUS: île Australia (N-Nord; S-Sud); CAJ: Cabane Jacky; IB: Isthme Bas; MAC: île Mac Murdo; MAY: île Mayes; MOL: Pointe Molloy; OTA: mouth of Rivière des otaries; PAF: Port aux Français; PJDA: Presqu'île Jeanne d'Arc; RBA: Péninsule Rallier du Baty; STU: Val Studer; TUS: Plateau du Tussok. Name of site is followed by population number.