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3
4 **Genome size and chromosome number of ten plant species from Kerguelen**
5 **Islands**

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24
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43
44

45 **Abstract**

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

62

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

65

66

67 **Introduction**

68

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

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

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

102 Among the different methods for estimating genome size, flow cytometry is now the most
103 currently used (Marie and Brown 1993; Doležel et al. 2007; Pellicer and Leitch 2014; Bourge
104 et al. 2018). This method is rapid, precise, easy for sample preparation and accurate for
105 detection of small differences in DNA content (Karrat-Souissi et al. 2013; Pellicer and Leitch
106 2014). Despite its biological relevance, genome size has been estimated for only 3.1% of all
107 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.

116

117 **Materials and methods**

118

119 **Plant material**

120

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

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

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

142

143 **Genome size estimation by flow cytometry**

144

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

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

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 (2n) sets of chromosomes, irrespective of ploidy level), was
174 calculated using the linear relationship between the fluorescent signals from stained nuclei of

175 the species and the internal standard. The total nuclear DNA content was calculated according
176 to the following formula:

177 $2C \text{ DNA sample (pg)} = (\text{Sample } 2C \text{ peak mean} / \text{Standard } 2C \text{ peak mean}) \times \text{Standard } 2C \text{ DNA}$
178 (pg)

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

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

191

192 **Chromosome number determination**

193

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

204

205 **Data analyses**

206

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

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

232

233 **Results**

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

236

237 **Variation of genome size among and within species**

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

245 For intra-genus comparisons, analyses of variance using rank values as well as
246 permutation tests showed significant differences of $2C$ values among *Poa* species ($F =$
247 167.64 , $df = (2;6)$, p -value < 0.0001 ; $H = 39.10$, p -value < 0.001 (permutation test) and
248 among *Ranunculus* species ($F = 9.64$, $df = (2;6)$, p -value = 0.013 ; $H = 12.82$, p -value = 0.016
249 (permutation test), despite identical ploidy levels within each genus.

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

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

260

261 **Chromosome number and estimation of ploidy levels**

262 The results of chromosome counts and ploidy levels of studied species are presented in Table
263 2 and Fig. 2. The high chromosome number and basic chromosome number of each genus
264 strongly supported the polyploid status of all investigated species. The chromosome numbers
265 of *Colobanthus kerguelensis* ($2n = 8x = 80$), *Lyallia kerguelensis* ($2n = 8x = 96$) and *Poa*
266 *kerguelensis* ($2n = 4x = 28$) are reported here for the first time, as well as for *Limosella*
267 *australis* ($2n = 6x = 60$) from Kerguelen.

268

269 **Discussion**

270 **Chromosome number and ploidy level**

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

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

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

290 Polyploidy or whole genome multiplication (WGM) is considered as one of major
291 evolutionary forces in angiosperm evolution (Solstis and Solstis 2009). Possibly all
292 angiosperms had at least one WGD during their evolutionary history (Van de Peer et al.
293 2017). In our panel all species are polyploid with different ploidy levels. *Colobanthus*
294 *kerguelensis* and *Lyallia kerguelensis* showed the highest ploidy level (octoploid), three
295 *Ranunculus* species and *Limosella australis* are hexaploids, and *Pringlea* and three *Poa*
296 species are tetraploids.

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

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

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

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

323

324 **Genome size variation among species**

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

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

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

346 For *Poa annua*, Bennett (1982) indicated almost the same value as in the present study,
347 $2C = 4.19 (\pm 0.06)$ pg. However, Frenot et al. (1999) reported a much smaller value of $2.95 (\pm$
348 $0.14)$ pg/ $2C$ for populations from French sub-Antarctic islands (Crozet and Kerguelen),

349 although the chromosome number ($2n = 28$) was identical to the one reported here. However,
350 the data obtained by these authors corresponded to relative DNA content because they used
351 DAPI (4',6-diamidino-2-phenylindole), fluorochrome specific for A-T bases, which, in
352 general, provides lower values than propidium iodide (DNA intercalating fluorochrome).

353 The value of relative 1C reported for *Pringlea antiscorbutica* (1C = 0.62 pg) by Kagale
354 et al. (2014) for material from Botanical Garden (Canberra, Australia) originally collected
355 from Heard Island, is very close to the mean value (2C = 1.08 pg) estimated on samples from
356 Kerguelen populations.

357 *Ranunculus biternatus* is spread across the whole circumpolar area (Lehnebach et al.
358 2017). Bennett et al. (1982) reported 2C = 14.50 pg for *R. biternatus* from South Georgia, a
359 sub-Antarctic island in the South Atlantic Ocean. The present value obtained for this species
360 was much smaller (2C = 11.88±0.23 pg). Variation in ploidy level cannot be invoked to
361 explain this result since the chromosome counts ($2n = 48$) are identical for both origins
362 (Bennett et al. 1982; Hennion and Couderc 1993; this work). This result could be explained
363 by the use of two different techniques of genome size assessment (Feulgen densitometry by
364 Bennett et al. 1982, and flow cytometry in our case). Alternatively, this result could reflect a
365 true difference in genome size between populations of this species in the two locations.

366 It should be noted that the *Ranunculus biternatus* lineage is sister to a clade of species
367 from Australia and New Zealand, with estimates indicating very recent spread to Crozet and
368 Kerguelen (Lehnebach et al. 2017). Therefore, populations from South Georgia, close to the
369 magellanics, may be only distantly related to the populations of the same species in
370 Kerguelen. A larger sampling effort of *R. biternatus* across its geographic range is required to
371 investigate genome size variability in this species.

372 Comparisons of genome size among the three *Ranunculus* species revealed that *R.*
373 *biternatus* displayed a significantly higher genome size than the two other *Ranunculus* species
374 (*R. pseudotrullifolius* and the endemic *R. moseleyi*) which did not differ one from each other.
375 This last result is in agreement with the hypothesis of a very recent divergence between these
376 last two species (Hennion et al. 1994; Lehnebach et al. 2017). Another explanation would be
377 that genome size of these two species might be the target of stabilizing selection, at least since
378 their divergence, which would maintain similar genome size values (Šmarda et al. 2010).
379 Contrarily, *R. biternatus* has been shown to belong to a clearly different evolutionary lineage
380 than *R. moseleyi* and *R. pseudotrullifolius* (Lehnebach et al. 2017). This is in agreement with a
381 significant difference in the genome size of these two taxa on one side and *R. biternatus* on
382 the other side.

383 *Colobanthus kerguelensis* showed 2C value of 2.41 (± 0.08) pg. This value is higher
384 than all values reported for the congeneric *C. quitensis*, a very wide-ranged species inhabiting
385 extreme environments in Antarctica and the Andes and rarer locations northwards (Cuba-Diaz
386 et al. 2017). Indeed, Bennett et al (1982) reported 2C = 1.4 pg for one population of *C.*
387 *quitensis* in South Georgia. Moreover, Cuba-Diaz et al. (2017) determined 2C = 1.95 in two
388 populations of *C. quitensis* (King George Islands in the maritime Antarctic and one location in
389 Patagonia) and 2C = 0.84 pg in one population from Araucania region, growing in a more
390 temperate zone and at high altitude (2575 m a.s.l.). The more than twofold difference in
391 genome size between the two populations of *C. quitensis* studied by Cuba-Diaz et al. (2017)
392 strongly suggests the existence of at least two ploidy levels in this species. Unfortunately, the

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

398

399 **Intraspecific variation of genome size at Kerguelen**

400

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

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

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

424

425 **Conclusion and perspectives**

426

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.
431 2017; Winkworth et al. 2015; Bartish et al. 2012; Van der Putten et al. 2010; Wagstaff and
432 Hennion 2007) are polyploids. This observation is in agreement with what has been observed
433 for species of high latitudes in the Northern Hemisphere but also in the Falkland Islands and
434 South Georgia in the sub-Antarctic. These data did not allow us to highlight a strong
435 variability in genome size at the intraspecific level except for *Lyallia kerguelensis* and
436 *Colobanthus kerguelensis*. Further analyses on a larger sample of populations are therefore

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

441

442 **Authors' contributions:**

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

450

451 **Compliance with ethical standards**

452

453 **Conflict of interest:** The authors declare that they have no conflict of interest

454

455

456 **References**

457

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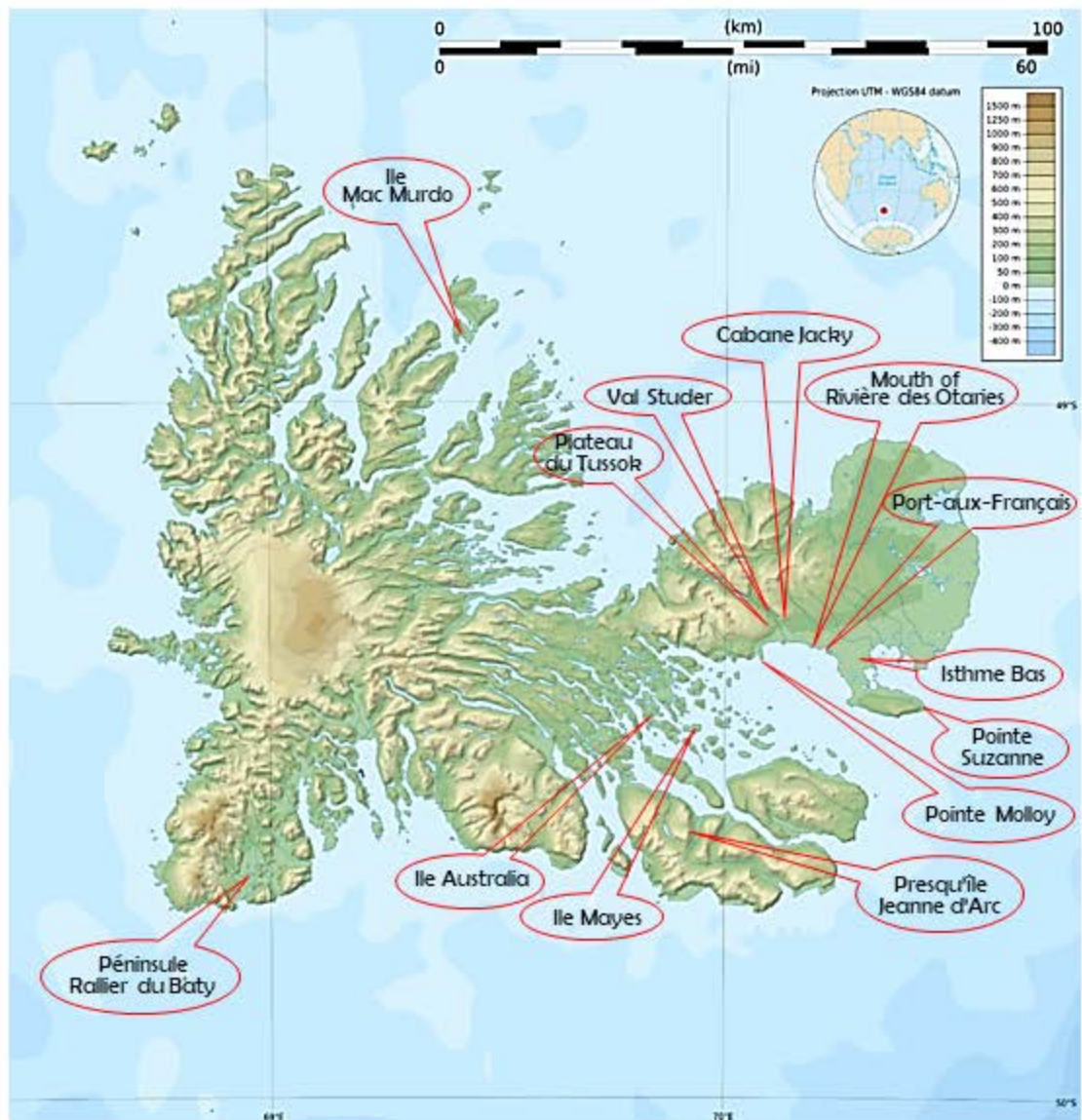
717 **Figure captions**

718 **Fig. 1** Plant material collection sites in Kerguelen Islands

719 **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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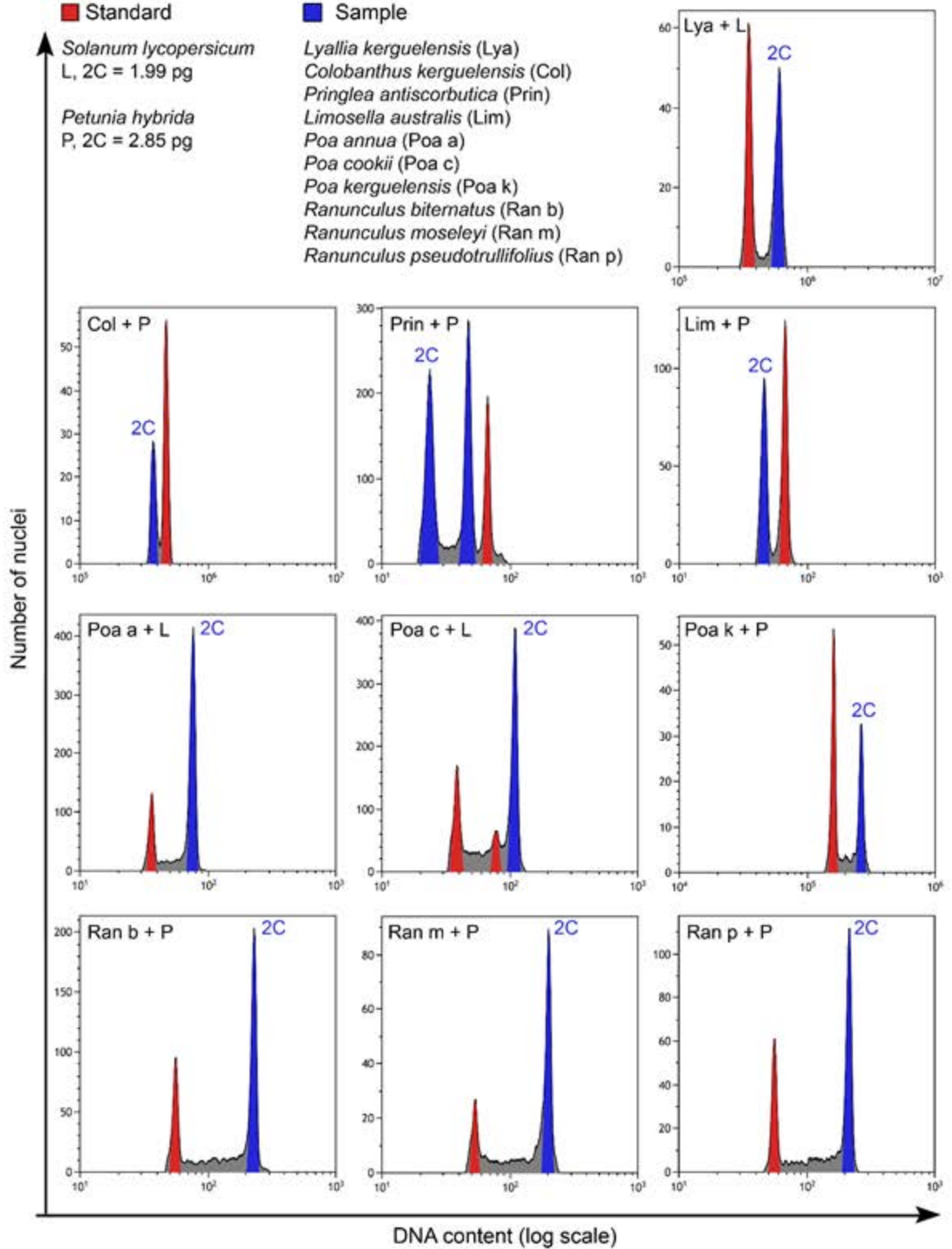
■ Standard

■ Sample

Solanum lycopersicum
L, 2C = 1.99 pg

Petunia hybrida
P, 2C = 2.85 pg

Lyallia kerguelensis (Lya)
Colobanthus kerguelensis (Col)
Pringlea antiscorbutica (Prin)
Limosella australis (Lim)
Poa annua (Poa a)
Poa cookii (Poa c)
Poa kerguelensis (Poa k)
Ranunculus biternatus (Ran b)
Ranunculus moseleyi (Ran m)
Ranunculus pseudotrullifolius (Ran p)



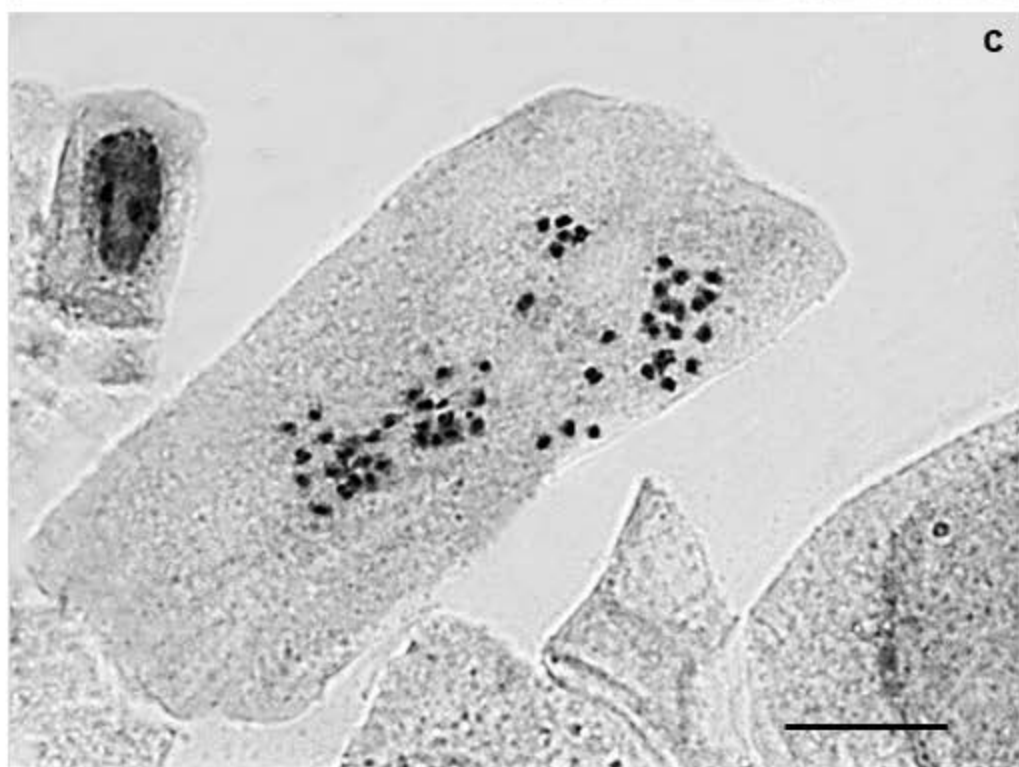
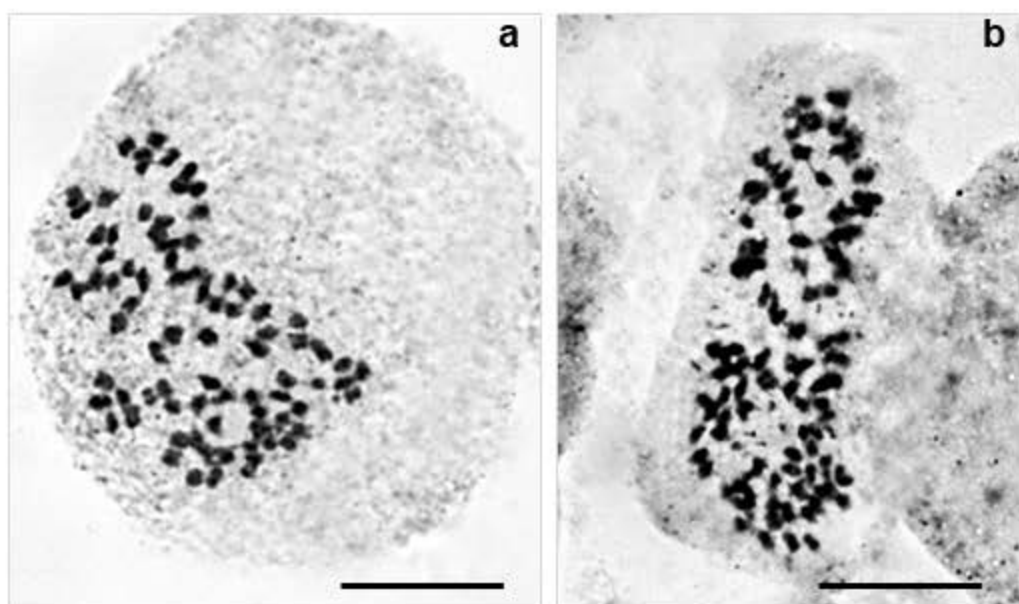


Table 1 Origin of investigated populations with localities, GPS coordinates and year of collection

Species/Population	Geographical range	Locality	GPS coordinates	Campagne
<i>Colobanthus kerguelensis</i> Hook.f. (Caryophyllaceae)	endemic to SIOF			
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
<i>Limosella australis</i> 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
<i>Poa annua</i> L. (Poaceae)	wide, introduced			
AUS-N		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
<i>P. cookii</i> (Hook.f.) Hook.f.	endemic to SIOF			
AUS-N		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
<i>P. kerguelensis</i> (Hook.f.) Steud.	endemic to SIOF			
AUS-N		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
CAJ		Cabane Jacky	-49.3132; 70.1233	2016-17
<i>Pringlea antiscorbutica</i> R.Br. ex Hook.f. (Brassicaceae)	endemic to SIOF			
AUS-N		north of Ile Australia	-49.43763; 69.84476	2016-17

CAJ		Cabane Jacky	-49.3124; 70.1239	2016-17
TUS		Plateau du Tussok	n.a.	2016-17
<i>Ranunculus biternatus</i> Sm. (Ranunculaceae)	circumpolar 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
<i>R. moseleyi</i> 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
<i>R. pseudotrullifolius</i> Skottsbo.	Magellanic and Kerguelen			
AUS-N6		north of Ile Australia	-49.4497; 69.8642	2016-17
IB-1		Isthme-Bas mouth of Rivière des Otaries	-49.3849; 70.2752	2016-17
OTA-1			-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

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

Species/Population	2C DNA in pg ^a (range)	1C DNA in Mbp	N	S	2n (ploidy)	P-value §	Signif. level §
<i>Colobanthus kerguelensis</i>					80 (8x)		
IB	2.42 ^b (2.28-2.57)	1183	5	P			
CAJ	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	**
<i>Limosella australis</i>					60 (6x)		
PJDA3	1.91	934	1	P			
<i>Lyallia kerguelensis</i>					96 (8x)		
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	*
<i>Poa annua</i>					28 (4x)		
AUS-N	4.16 (4.12-4.21)	2034	6	H			
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
<i>Poa cookii</i>					28 (4x) ^d		
AUS-N	5.70 (5.45-5.89)	2787	5	H			
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
<i>Poa kerguelensis</i>					28 (4x)		
AUS-N	4.99 (4.97-5.15)	2440	5	H			
PAF	5.02 (4.96-5.14)	2454	5				

CAJ	4.86 (4.72-5.19)	2377	5			
Means (SD) for species	5.00 (0.14)	2445	15		0.470	NS
<i>Pringlea antiscorbutica</i> 24 (4x) ^d						
AUS-N	1.03 (1-1.07)	504	5	P		
CAJ	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
<i>Ranunculus biternatus</i> 48 (6x) ^d						
AUS-N	11.72 (11.7-11.74)	5731	5	T		
IB-13	11.87 (11.81-12.12)	5804	5			
IB-2	12.09 (11.51-12.38)	5912	4			
Means (SD) for species[‡]	11.88 (0.23)	5809	14		0.064	NS
<i>Ranunculus moseleyi</i> 48 (6x) ^d						
AUS-N5	11.37 (11.12-12.48)	5560	5	T		
IB-2	11.15 (10.89-12.11)	5452	5			
PAF-5	11.26 (10.93-11.46)	5506	5			
Means (SD) for species	11.37 (0.45)	5506	15		0.533	NS
<i>Ranunculus pseudotrullifolius</i> 48 (6x) ^d						
AUS-N6	11.34 (11.12-11.75)	5545	5	T	11.7	
IB-1	11.17 (10.72-11.82)	5462	5			
OTA-1	11.31 (11.08-12.74)	5530	5			
Means (SD) for species	11.45 (0.60)	5512	15		0.543	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.