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ORIGINAL PAPER

An update on the indigenous vascular flora of sub-Antarctic Marion Island: taxonomic changes, sequences for DNA barcode loci, and genome size data

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Abstract

The flora of sub-Antarctic Marion Island forms part of the unique South Indian Ocean Biogeographic Province, and is under threat from climate change and invasive species. Current information on the flora is necessary to rapidly identify and manage future changes. We conducted a literature search on the taxonomy of indigenous vascular plant species on Marion Island and found nomenclatural changes following taxonomic revisions for *Austroblechnum penna-marina* (Poir.) Gasper & V.A.O.Dittrich, *Carex dikei* (Nelmes) K.L.Wilson, *Leptinella plumosa* Hook.f., *Notogrammitis crassior* (Kirk) Parris, *Phlegmariurus saururus* (Lam.) B.Øllg., and *Polypogon magellanicus* (Lam.) Finot. Additionally, *Ranunculus moseleyi* Hook.f. was removed from our species checklist due to its long absence in floristic surveys, leaving 21 species in the indigenous vascular plant flora present on Marion Island. We also amplified and sequenced the universal plant barcoding loci *rbcL* and *matK* for 19 and 13 species, respectively, and found that ample interspecific genetic distance and minimal intraspecific genetic distance allowed for easy discrimination between species. Lastly, we obtained genome size estimates using flow cytometry for 12 species. Mean 2C genome size for species on Marion Island ranged from 0.44 to 21.44 pg, which is on the lower end of the known range for vascular plant species. We detected two distinct cytotypes in *Poa cookii* (Hook.f.) Hook.f. and one cytotype in all other species measured.

Keywords *C*-value · Flow cytometry · *matK* · Nomenclature · Prince Edward islands · *rbcL*

Introduction

The sub-Antarctic terrestrial bioregion comprises a small number of islands located within the vast Southern Ocean surrounding Antarctica. The flora on these islands is relatively species-poor, filtered by harsh environmental conditions including cool, wet, and windy climates, and geographic isolation which necessitates that colonizing species disperse long distances from the nearest species pools (Chown et al. 1998). Although some plant species that occur in the sub-Antarctic are widely distributed across multiple islands and even parts of South America, Australia, and New Zealand, several species have restricted distributions

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that contribute to making these floras unique, such as the South Indian Ocean Province endemics *Elaphoglossum randii* Alston & Schelpe, *Colobanthus kerguelensis* Hook.f., *Poa cookii* (Hook.f.) Hook.f., *Pringlea antiscorbutica* R.Br. ex Hook.f., and *Ranunculus moseleyi* Hook.f. (Greene and Walton 1975). The sub-Antarctic islands are among the most isolated landmasses on Earth and suffer relatively few direct human impacts compared to continental mainlands, but their ecosystems are still threatened by climate change (Pendlebury and Barnes-Keoghan 2007; Le Roux and McGeoch 2008; Bergstrom et al. 2015) and the introduction and establishment of invasive species (Frenot et al. 2001; Jansen van Vuuren and Chown 2007; Lee et al. 2007; Le Roux et al. 2013; Greve et al. 2017). With relatively simple and low-diversity communities, sub-Antarctic ecosystems may be especially vulnerable to invasion by non-indigenous species (Case 1990; Stachowicz et al. 1999; Lyons and Schwartz 2001), and with climate change moderating temperatures and wind patterns, the establishment and expansion in range of more species are expected to occur in the near-future (Ryan et al. 2003; Chown and Brooks 2019). Therefore, a thorough characterization of the present flora is necessary to rapidly identify and manage future changes.

Marion Island, a volcanic island approximately 290 km^2 in area and 450,000 years in age, is one of the sub-Antarctic islands. Together with Prince Edward Island, they comprise the Prince Edward Island (PEI) archipelago in the southern Indian Ocean (46.9° S, 37.8° E), approximately 1770 km southeast of South Africa, the nearest continental landmass (Fig. 1; Hänel and Chown 1998; McDougall et al. 2001). The PEI archipelago, along with the Crozet, Kerguelen, Heard, and McDonald islands, form the South Indian Ocean Biogeographic Province (Hänel and Chown 1998). The

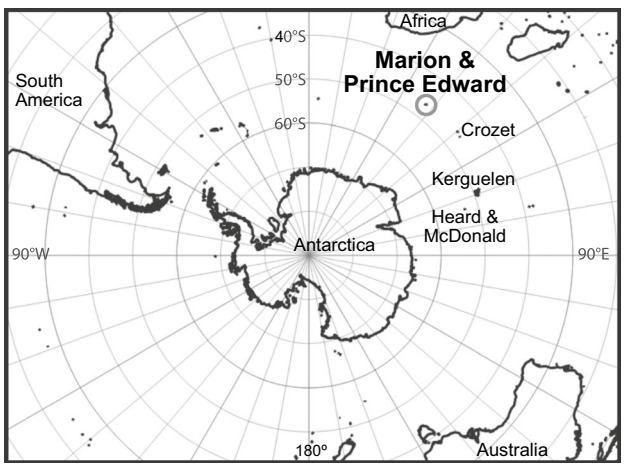

Fig. 1 Map indicating the location of Marion and Prince Edward islands, and other islands of the South Indian Ocean Biogeographic Province, in the Southern Ocean. World coastline data from Natural Earth (naturalearthdata.com)

vegetation of Marion Island consists of salt spray-tolerant species along the rugged coastline, marshy mire communities on the lowland coastal plains, closed fern-dominated carpets on lower slopes, and open fellfield communities near the upper elevational vegetation line (Huntley 1971; Smith and Mucina 2006). Plant collections during occasional visits to Marion Island have been made since the late nineteenth century, but the first extensive survey of the flora was only conducted in 1965–1966 as part of the South African Biological and Geological Expedition (Huntley 1971). Information on the flora of Marion Island has been revised as a result of this and continued annual scientific expeditions to the island (Greene and Greene 1963; Huntley 1971; Greene and Walton 1975; Hänel and Chown 1998), with the most recent species list for vascular plants compiled by Gremmen and Smith (2008) comprising 22 indigenous species and 21 introduced species.

DNA sequencing data, which heretofore have not been generated for most plant species on sub-Antarctic islands, can be used to assess evolutionary relationships and to help identify specimens. The flora of Marion Island comprises few species, and for the most part, is relatively easy to identify based on morphology alone. However, the development of additional techniques to identify specimens, especially when morphology is unreliable, would help facilitate the study and management of the flora. DNA barcoding is a method in which sequences from standardized DNA regions are used to distinguish and identify specimens by comparison to a reference sequence library (Hebert et al. 2003; Kress et al. 2015). This approach can be especially useful for species-rich groups where taxonomic expertise is limited and for fragmented, small, or sterile specimens where identification based on morphology is difficult (Hollingsworth et al. 2016). For plants, the two-marker barcode comprising the plastid genes *rbcL* and *matK* has been found to be recoverable and informative for most groups (CBOL Plant Working Group 2009; Hollingsworth et al. 2011; Li et al. 2011), and reference sequence libraries have been generated for several large floras and taxonomic groups (Lahaye et al. 2008; Kress et al. 2009; Burgess et al. 2011). Reference sequences for the barcoding loci for the sub-Antarctic flora would help facilitate the rapid identification of species and may also be useful for ecological forensic applications where only fragmented or processed samples are available, such as in belowground root communities and in herbivore scat (Hollingsworth et al. 2016).

The extreme abiotic conditions of the sub-Antarctic may not only limit the richness of the vascular flora in the region, but could also affect genomic characteristics of the species that occur there. Genome size and ploidy level are variable traits among plant species that may be informative for evolutionary and ecological relationships (Ohri 1998). For instance, the flora of the northern polar Arctic region has a

disproportionately high number of polyploid lineages (Brochmann et al. 2004). It has been proposed that polyploidy, or whole-genome duplication, may facilitate adaptation to harsh environmental conditions (Grant 1981; te Beest et al. 2012) or may result from disturbance to the meiotic process due to low temperature stress (Bomblies et al. 2015). Polyploidy can have significant effects on cellular and genomic processes, as well as diversification and macroevolutionary processes (Otto 2007; te Beest et al. 2012; Scarpino et al. 2014; Soltis et al. 2014). Genomic patterns in the sub-Antarctic and Antarctic flora have been studied in a few areas (Bennett et al. 1982; González et al. 2016), but it is unknown if there are general trends that reflect those observed in the northern polar region (but see Rice et al. 2019). Additionally, many polyploids correspond to cryptic species that are morphologically similar to their diploid predecessors (Soltis et al. 2007; Husband et al. 2013), especially in autopolyploids (Soltis et al. 2007; Mairal et al. 2018). Thus, the possible presence of cryptic lineages makes the estimation of genome size and ploidy level especially relevant for unraveling evolutionary processes in sub-Antarctic ecosystems.

In this study, we give an update on the vascular flora of sub-Antarctic Marion Island and provide genetic resources to facilitate future research. First, we conducted a literature survey to find the most current taxonomy and nomenclature for the indigenous vascular plant species on the island, and note changes to the species present based on observations of experts working on the island. Second, we generated sequence data for the plant DNA barcoding loci *rbcL* and *matK* and evaluated recoverability and discriminatory power between taxa of these markers. Lastly, we provide new genome size estimates obtained from flow cytometry and compare measurements from Marion Island specimens with previously published genome size data for related taxa.

Materials and methods

Literature survey

A list of the indigenous vascular plant species of Marion Island was compiled from a recent checklist (Gremmen and Smith 2008) and unpublished observations and records of researchers who have worked extensively on the island. Species recorded as a single specimen on the island, namely *Ochetophila trinervis* (Gillies ex Hook.) Poepp. ex Endl. (Kalwij et al. 2019) and *Malus* sp. (M. Greve, personal communication), or with uncertain indigenous status were excluded from our list. Literature searches were conducted in Google Scholar (scholar.google.com) using each species name and "~phylogeny", "~taxonomy", or "~systematic" as the search terms (searched 11 August 2020). Search results and references cited therein were used to ascertain whether taxonomic and nomenclatural changes have been made since the publication of the previous checklist. We compared our findings with the status of names on Plants of the World Online (www.plantsoftheworldonline.org; accessed 11 August 2020).

Sampling, DNA extraction, PCR, and sequencing

Leaf samples from all indigenous vascular plant species on Marion Island were collected by experts in the flora of the island during trips to the island in 2016 and 2018 (Online Resource 1). Leaf samples were preserved in silica gel and later ground for DNA extraction using a modified CTAB protocol (Doyle and Doyle 1987). Two plastid loci used in DNA barcoding of plants were amplified by PCR following the PCR programme in Zietsman et al. (2009) besides the modifications below. The *rbcL* locus was amplified using the primers P1630 (rbcLa-F) and 1.2-*rbcL* (rbcLa-R) (Fofana et al. 1997; Levin et al. 2003) with an annealing temperature of 55 °C. The *matK* locus was amplified using the primers matK-472F (5′-CCCRTYCATCTGGAAATCTTGGTTC-3′) and matK-1248R (5′-GCTRTRATAATGAGAAAGATTTCTGC-3′) with an annealing temperature of 52 °C, and for recalcitrant samples of ferns, the fern-specific primers FERmatK-fEDR and FERmatK-rAGK (Kuo et al. 2011) were also tested with an annealing temperature of 50 °C. PCR products were purified before using as templates in sequencing reactions with the same primers as in PCR, and sequence reads were generated on an ABI 3730 Genetic Analyzer at the Stellenbosch University Central Analytical Facilities DNA Sequencing Unit (Stellenbosch, South Africa). Sequence chromatograms were checked, sequences were assembled into contigs, and consensus sequences of contigs were extracted using Geneious v9.1.6 (Biomatters, Auckland, New Zealand). Sample collection data and number of samples sequenced for each species can be found in Online Resource 1.

Genetic distance and phylogenetic analyses

For each locus, sequences were aligned in MAFFT (Katoh et al. 2002) using the Auto strategy and default settings. Pairwise percent identities were calculated in Geneious v9.1.6 (Biomatters, Auckland, New Zealand). In addition, pairwise genetic distances were calculated under the Kimura 2-parameter (K2P) model in PAUP* v4.0a166 (Swofford 2002).

For each alignment matrix, sequence ends were manually trimmed of sites with less than 25% coverage (Joly et al. 2007). For an outgroup for phylogenetic analyses, sequences from the bryophyte *Racomitrium lanuginosum* (Hedw.) Brid. were retrieved from GenBank (accession numbers: GU373444 and HG792577 for *rbcL* and

matK, respectively) and added to the respective alignment matrix. Phylogenetic analyses were performed under the maximum likelihood (ML) criterion in RAxML v8.2.10 (Stamatakis 2014). We conducted a search for the best-scoring ML tree and 100 rapid bootstraps using the GTR+gamma model of rate heterogeneity.

Flow cytometry for genome size

Flow cytometric analysis followed the chopping procedure of Galbraith et al. (1983) using Otto's buffers (Otto 1990; Doležel and Göhde 1995). Briefly, nuclei were released after chopping 0.5 cm^2 of dry leaf tissue of samples and 0.5 cm^2 of fresh leaf tissue of an internal standard with a razor blade in a Petri dish containing 0.5 mL of Otto I buffer (0.1 M citric acid, 0.5% Tween 20). Afterwards, the nuclear suspension was filtered using a 42-µm nylon mesh and stained with a solution containing 1 mL of Otto II buffer (0.4 M Na_2HPO_4_$12H_2O$), 4 mg mL^{-1} of DAPI, and 2 mg mL^{-1} of ß-mercaptoethanol (Castro et al. 2011). After 5 min of incubation, samples were analyzed in a Partec PA II flow cytometer (Partec GmbH, Munster, Germany). The fluorescence of at least 3000 nuclei per sample was analyzed using FlowMax v2.4 (Partec GmbH). We were unable to obtain fresh tissue for these analyses and dried samples generally provide lower quality readings, so as a quality standard, only histograms with a coefficient of variation (CV) below 3.5% were accepted. In all cases, we only accepted peaks that were clearly identifiable from the background noise. The DNA index was calculated for all samples by dividing the relative fluorescence of the G0/G1 peak of the target species by the relative fluorescence of the G0/G1 peak of the internal standard species.

We used *Bellis perennis* L. (internal reference standard with 2C = 3.38 pg; Schonswetter et al. 2007) as the internal standard to measure *Acaena magellanica* (Lam.) Vahl. We used *Solanum pseudocapsicum* L. (internal reference standard with 2C = 2.58 pg; Temsch et al. 2010) for *Callitriche antarctica* Engelm. ex Hegelm., *Carex dikei* (Nelmes) K.L.Wilson, *Colobanthus kerguelensis* Hook.f., and *Leptinella plumosa* Hook.f.. We used *Pisum sativum* L. cv. Ctirad (internal reference standard with 2C = 9.09 pg; Schonswetter et al. 2007) for *Austroblechnum penna-marina* (Poir.) Gasper & V.A.O.Dittrich, *Azorella selago* Hook.f., *Elaphoglossum randii* Alston & Schelpe, *Lycopodium magellanicum* (P.Beauv.) Sw., *Phlegmariurus saururus* (Lam.) B.Øllg., *Poa cookii* (Hook.f.) Hook.f., and *Polystichum marionense* Alston & Schelpe. The number of specimens measured and standard used for each species is provided in Table 2.

Results

Taxonomic changes

Twenty-one species of vascular plants occur on Marion Island with unambiguous indigenous status (Table 1). Six species have undergone nomenclatural changes since the most recent checklist of Gremmen and Smith (2008), mostly due to revisions in generic circumscriptions. These species are *Austroblechnum penna-marina* (Poir.) Gasper & V.A.O.Dittrich (= *Blechnum penna-marina* (Poir.) Kuhn) (Gasper et al. 2016, 2017), *Carex dikei* (Nelmes) K.L.Wilson (= *Uncinia dikei* Nelmes) (Global Carex Group 2015), *Leptinella plumosa* Hook. f. (= *Cotula plumosa* (Hook. f.) Hook. f.) (Lloyd and Webb 1987), *Notogrammitis crassior* (Kirk) Parris (= *Grammitis kerguelensis* Tard.) (Perrie and Parris 2012), *Phlegmariurus saururus* (Lam.) B.Øllg. (= *Lycopodium saururus* Lam.) (Øllgaard 2012), and *Polypogon magellanicus* (Lam.) Finot (= *Agrostis magellanica* Lam.) (Finot et al. 2013). In the case of *Carex dikei*, the species on Marion Island is also treated as distinct from the more widely distributed *Carex austrocompacta* K.L.Wilson (= *Uncinia compacta* R.Br.) (Global Carex Group 2015), which is supported by phylogenetic relationships inferred from nuclear ribosomal DNA sequence data (Starr 2001). We also checked all species names on Plants of the World Online. In almost all cases, this confirmed that the current species name listed in Table 1 is the accepted name. However, for *Austroblechnum penna-marina*, *Notogrammitis crassior*, and *Phlegmariurus saururus*, a different accepted name is given, likely because the more recent taxonomic studies we consulted were not considered by the online database. The species *Ranunculus moseleyi* Hook.f. had previously been included in the flora of Marion Island (Gremmen and Smith 2008) but is excluded from our list because it has not been recorded on Marion Island for more than 50 years and no voucher specimens of this species from Marion Island are known to exist (Huntley 1971; Lehnebach et al. 2017).

PCR and sequencing success

The locus *rbcL* was successfully amplified in 19 out of 21 vascular plant species using universal primers. The two species for which PCR was not successful, *Elaphoglossum randii* and *Polystichum marionense*, are both ferns in the family Dryopteridaceae. Sequence length ranged from 507 to 560 bp, and the trimmed alignment had a length of 546 bp. The locus *matK* was more difficult to amplify, being successful in only 13 out of 21 species tested. No

Table 1 Species of indigenous vascular plants on Marion Island (Prince Edward Island archipelago, South Africa), with taxonomic changes from the checklist of Gremmen and Smith (2008) highlighted

Current species name	Species name in Gremmen and Smith (2008)	Family
Acaena magellanica (Lam.) Vahl	*Acaena magellanica* (Lam.) Vahl = *Acaena adscendens* Vahl.	Rosaceae
Austroblechnum penna-marina (Poir.) Gasper & V.A.O.Dittrich	*Blechnum penna-marina* Kuhn	Blechnaceae
Azorella selago Hook.f.	*Azorella selago* Hook.f.	Apiaceae
Callitriche antarctica Engelm. ex Hegelm.	*Callitriche antarctica* Engelm.	Plantaginaceae
Carex dikei (Nelmes) K.L.Wilson	*Uncinia compacta* R.Br. = *Uncinia dikei* Nelmes	Cyperaceae
Colobanthus kerguelensis Hook.f.	*Colobanthus kerguelensis* Hook.f.	Caryophyllaceae
Crassula moschata G.Forst.	*Crassula moschata* G.Forst. = *Tillaea moschata* DC.	Crassulaceae
Elaphoglossum randii Alston & Schelpe	*Elaphoglossum randii* Alston & Schelpe	Dryopteridaceae
Hymenophyllum peltatum (Poir.) Desv.	*Hymenophyllum peltatum* (Poiret) Desv.	Hymenophyllaceae
Juncus scheuchzerioides Gaudich.	*Juncus scheuchzerioides* Gaud.	Juncaceae
Leptinella plumosa Hook.f.	*Cotula plumosa* Hook.f.	Asteraceae
Limosella australis R.Br.	*Limosella australis* R.Br.	Scrophulariaceae
Lycopodium magellanicum (P.Beauv.) Sw.	*Lycopodium magellanicum* Sw.	Lycopodiaceae
Montia fontana L.	*Montia fontana* L.	Montiaceae
Notogrammitis crassior (Kirk) Parris	*Grammitis poeppigiana* (Mett.) Pichi Serm. (= *Grammitis kerguelensis* Tard.)	Polypodiaceae
Phlegmariurus saururus (Lam.) B.Øllg.	*Lycopodium saururus* Lam.	Lycopodiaceae
Poa cookii (Hook.f.) Hook.f.	*Poa cookii* Hook.f.	Poaceae
Polypogon magellanicus (Lam.) Finot	*Agrostis magellanica* Lam.	Poaceae
Polystichum marionense Alston & Schelpe	*Polystichum marionense* Alston & Schelpe	Dryopteridaceae
Pringlea antiscorbutica R.Br. ex Hook.f.	*Pringlea antiscorbutica* R.Br.	Brassicaceae
Ranunculus biternatus Sm.	*Ranunculus biternatus* Sm.	Ranunculaceae

fern or lycophyte species were successfully amplified using universal primers or fern-specific primers. In addition, *matK* could not be amplified from *Crassula moschata* G.Forst. Sequence length for the *matK* locus ranged from 490 to 884 bp, and the trimmed alignment was 762 bp in length. All sequences are available on GenBank (accession numbers in Online Resource 1).

Species resolution

For the locus *rbcL*, interspecific pairwise genetic distance ranged from 1.53% between *Poa cookii* and *Polypogon magellanicus* to 28.33% between *Austroblechnum penna-marina* and *Leptinella plumosa* (Fig. 2, Online Resource 2). Interspecific pairwise identity ranged from 77.1% between *Austroblechnum penna-marina* and *Colobanthus kerguelensis* to 98.48% between *Poa cookii* and *Polypogon magellanicus*. Greater sequence divergence was observed for the locus *matK*. Interspecific pairwise genetic distance ranged from 6.47% between *Poa cookii* and *Polypogon magellanicus* to 45.65% between *Azorella selago* and *Poa cookii* (Fig. 2, Online Resource 3). Notably, the genetic distance in *matK* between several pairs of angiosperms was greater than the largest genetic distance in *rbcL* between a fern and an angiosperm. Interspecific pairwise identity ranged from 64.88% between *Poa cookii* and *Pringlea antiscorbutica* to 93.8% between *Poa cookii* and *Polypogon magellanicus*.

For *rbcL*, only one haplotype was observed in each species, even where multiple samples of a species were sequenced, i.e., intraspecific genetic distance was zero in all such cases. However, for *matK*, two haplotypes were retrieved in different samples of *Montia fontana* L., *Poa cookii*, *Polypogon magellanicus*, and *Pringlea antiscorbutica*. In these cases, intraspecific genetic distance ranged from 0.14% in *Montia fontana* to 0.42% in *Poa cookii* (Fig. 2, Online Resource 3).

The phylogenetic trees for *rbcL* and *matK* showed substantial branch lengths, representing significant genetic divergences, separating species (Fig. 3). Monophyly of conspecific haplotypes of *Montia fontana*, *Poa cookii*, *Polypogon magellanicus*, and *Pringlea antiscorbutica* each had 100% bootstrap support in the phylogenetic tree for *matK* (Fig. 3a). In addition, the phylogenetic trees for *rbcL* and *matK* generally conformed to expected relationships based on the known land plant phylogeny (Soltis et al. 2011; PPG I 2016). The angiosperm clades Poaceae comprising *Poa cookii* and *Polypogon magellanicus*, Caryophyllales comprising *Colobanthus kerguelensis* and *Montia fontana*, and Lamiales comprising *Callitriche antarctica* and *Limosella australis* R.Br., and the fern eupolypod clade comprising

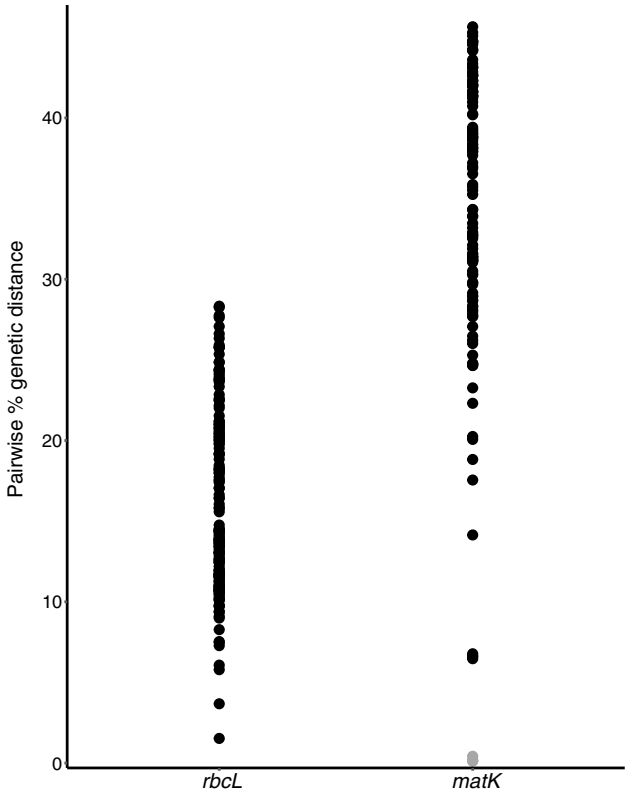

Fig. 2 Percent K2P genetic distances for pairs of indigenous vascular plant species on Marion Island for *rbcL* and *matK* loci. Black dots represent interspecific pairwise distances, and gray dots represent intraspecific pairwise distances

Austroblechnum penna-marina and *Notogrammitis crassior* (Kirk) Parris, as well as the major clades comprising all eudicots, all angiosperms, all ferns, and all euphyllophytes each had strong support in the *rbcL* phylogeny (Fig. 3b). The clades Poaceae, Caryophyllales, and Lamiales, as well as the campanulids comprising *Azorella selago* and *Leptinella plumosa* and the cyperids comprising *Carex dikei* and *Juncus scheuchzerioides* Gaudich., each received strong support in the *matK* phylogeny (Fig. 3a).

Genome size

We determined genome size for 69 samples representing twelve species using flow cytometry (Table 2, Fig. 4). Mean 2C genome size ranged from 0.44 pg in *Poa cookii* to 21.44 pg in *Elaphoglossum randii*. For most species, the variation in genome size among samples was low, with only one cytotype inferred in each species. However, this could be due to our limited sampling (1–4 samples) for most species. For *Poa cookii*, which we sampled more extensively (37 samples) due to known variation in genome size in other species of *Poa* (Mowforth and Grime 1989), there were two distinct clusters of values, with 34 samples having a genome size around 0.44 pg and three samples with a genome size around 0.89 pg, suggesting the presence of two cytotypes.

Discussion

Of the 22 species of indigenous vascular plants included in previous checklists of Marion Island's flora (Gremmen and Smith 2008), six species have undergone nomenclatural

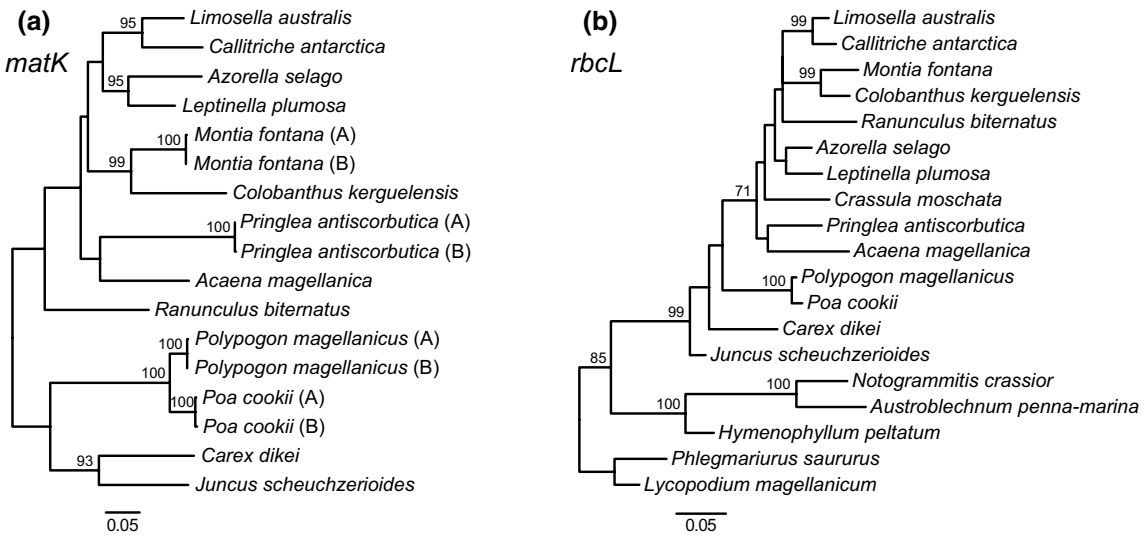

Fig. 3 Maximum likelihood phylogenetic trees inferred from **a** *matK* and **b** *rbcL* sequence data for indigenous vascular plant species on Marion Island. Bootstrap support values above 70% are shown at nodes. The bryophyte *Racomitrium lanuginosum* used as an outgroup is not shown

Table 2 Genome size measurements from flow cytometry for indigenous vascular plant species on Marion Island

Species	Mean 2C genome size (SE[a]) (pg)	Mean CV[b] (%)	N[c]	Standard[d]
Acaena magellanica	1.31 (0.023)	3.01	7	B.p.
Austroblechnum penna-marina	13.93 (0.368)	1.83	2	P.s.
Azorella selago	8.32 (0.063)	1.78	3	P.s.
Callitriche antarctica	2.99	2.62	1	S.p.
Carex dikei	2.89 (0.013)	1.50	3	S.p.
Colobanthus kerguelensis	0.82 (0.042)	2.66	3	S.p.
Elaphoglossum randii	21.44 (0.024)	1.34	3	P.s.
Leptinella plumosa	1.79 (0.020)	3.44	3	S.p.
Lycopodium magellanicum	5.37 (0.032)	2.34	2	P.s.
Phlegmariurus saururus	20.44 (0.115)	1.65	4	P.s.
Poa cookii (cytotype 1)	0.44 (0.001)	2.44	34	P.s.
Poa cookii (cytotype 2)	0.89 (0.012)	2.39	3	P.s.
Polystichum marionense	20.40	1.46	1	P.s.

[a]Standard error

[b]Coefficient of variation of individual peaks for single samples reflecting the quality of measurements

[c]Number of samples measured

[d]Internal reference standard used: B.p. = *Bellis perennis*, P.s. = *Pisum sativum*, S.p. = *Solanum pseudocapsicum*

changes due to altered understanding of genus and species limits, and one species, *Ranunculus moseleyi*, has been omitted from our list due to its absence in floristic surveys for more than five decades, leaving 21 species in our checklist. Sequences for DNA barcoding loci were generated for 19 species for *rbcL* and 13 species for *matK*. These sequences include the first ones published for *rbcL* for nine species (*Acaena magellanica*, *Carex dikei*, *Colobanthus kerguelensis*, *Crassula moschata*, *Juncus scheuchzerioides*, *Leptinella plumosa*, *Poa cookii*, *Pringlea antiscorbutica*, and *Ranunculus biternatus*) and the first sequences for *matK* for six species (*Acaena magellanica*, *Colobanthus kerguelensis*, *Juncus scheuchzerioides*, *Leptinella plumosa*, *Polypogon magellanicus*, and *Pringlea antiscorbutica*), based on a comparison of what was available on the GenBank sequence database (accessed 11 August 2020). Genome size was measured by flow cytometry for 12 species, including ten species for which this is the first report (*Austroblechnum penna-marina*, *Azorella selago*, *Carex dikei*, *Colobanthus kerguelensis*, *Elaphoglossum randii*, *Leptinella plumosa*, *Lycopodium magellanicum*, *Phlegmariurus saururus*, *Poa cookii*, and *Polystichum marionense*).

Taxonomy

Changes in our understanding of relationships among species and genera are reflected in changes to species names. In the Marion Island flora, phylogenetic evidence from DNA sequence data have resulted in revised generic placements for the species *Austroblechnum penna-marina* (Gasper et al. 2017), *Carex dikei* (Starr 2001; Starr et al. 2008), *Leptinella plumosa* (Himmelreich et al. 2012), *Notogrammitis crassior* (Perrie and Parris 2012; Sundue et al. 2014), and *Phlegmariurus saururus* (Field et al. 2016; Testo et al. 2018). Generic classification was also changed for the grass species *Polypogon magellanicus* based on morphological data (Finot et al. 2013), but its relationship with other *Polypogon* and *Agrostis* species should be evaluated with phylogenetic analyses using molecular data. Additionally, Holub (1991) suggested that the lycophyte species *Lycopodium magellanicum* be transferred to the segregate genus *Austrolycopodium*, but molecular data do not support this classification scheme (Wikstrom and Kenrick 2000; Burnard et al. 2016). For all species, samples from Marion Island should be included in future phylogenetic studies to confirm the relationship of Marion Island populations with populations in the rest of the species range.

In the case of *Carex dikei*, not only was the generic classification changed from *Uncinia*, but the species on Marion Island was also segregated from the southern Australian species *Carex austrocompacta* (= *Uncinia compacta*) (Starr 2001; Global Carex Group 2015), making this species the only vascular plant endemic to Marion Island. Populations of what has been called *Uncinia compacta* on other sub-Antarctic islands, including Crozet and Kerguelen islands, where plants were initially described as *U. mosleyana* Boeck. (Greene and Walton 1975; Lord 2015), should be included in future studies to determine their relationship to the Marion Island and Australian species.

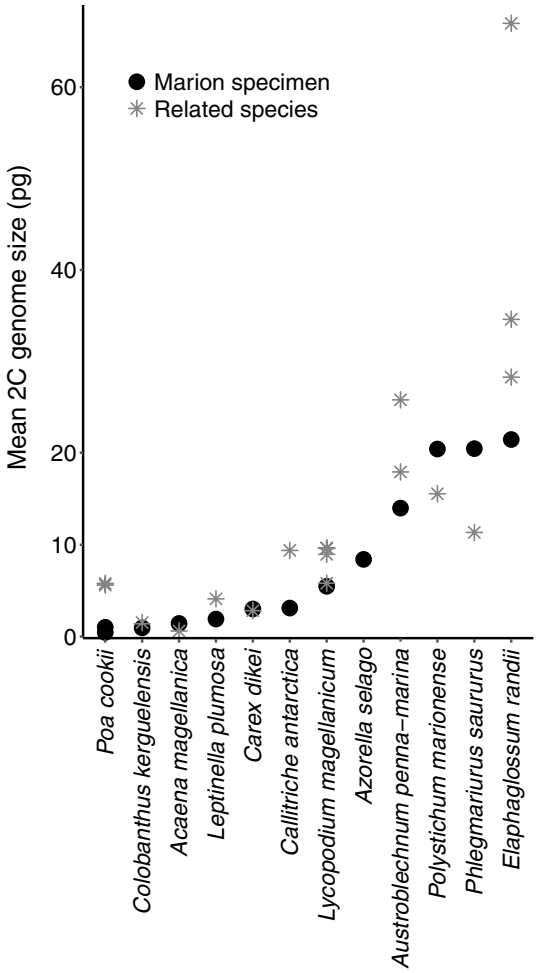

Fig. 4 Mean 2C genome size measurements from flow cytometry for indigenous vascular plant species on Marion Island and published genome size data for related species (see "Discussion" in text and Online Resource 4 for full list of related species and references)

Sequences for rbcL and matK

The locus *rbcL* was relatively easy to amplify, and sequences for 19 of 21 species of indigenous vascular plants on Marion Island are now available. The locus *matK* proved more difficult to amplify. We were still able to generate sequence data for *matK* in 13 of 14 indigenous angiosperm species, but amplification was not successful in any non-angiosperm. A published sequence for *matK* is also available for *Lycopodium magellanicum* (GenBank accession AM889722), which we were unable to sequence in this study, so in total *matK* sequences are available for 14 species of Marion Island's vascular flora. Both loci provided sufficient interspecific sequence divergence and minimal intraspecific genetic distance to allow for easy discrimination between indigenous species. The locus *matK* showed higher sequence variability, consistent with results from studies of larger floras, but the lack of universal primers and lower recovery rate, especially in non-angiosperms, remain an issue (CBOL Plant Working Group 2009; Kress et al. 2009; Hollingsworth et al. 2011; Li et al. 2011).

Besides use in species identification, DNA sequence data generated in this study can be utilized in phylogenetic studies of evolutionary relationships among taxa. Although our study did not focus on this topic for any species, our sequence data can be used in conjunction with other available or newly generated data to explore questions about evolutionary relationships and the biogeographic origin of species on Marion Island (e.g., Jansen van Vuuren and Chown 2007). For example, a BLAST search against the GenBank database of the *matK* sequence from Marion Island *Callitriche antarctica* showed that it is most similar to sequences from *C. antarctica* from New Zealand and the Falkland/Malvinas Islands (GenBank numbers: LC176826-LC176829) and then to *C. petriei* R.Mason from New Zealand (GenBank numbers: LC176851, LC176852), which supports the monophyly of *C. antarctica* specimens from across the sub-Antarctic and their close relationship to the New Zealand species (Ito et al. 2017).

Genome size

Genome size was successfully measured for 12 species using flow cytometry. The range of 2C values for Marion Island species, 0.44–21.44 pg, is on the lower end of the known range of 2C values for vascular plants, 0.13–304.4 pg (Pellicer et al. 2018; Leitch et al. 2019). This conforms to a pattern of smaller genome sizes in plants from colder environments, as also observed for sub-Antarctic South Georgia Island and the Antarctic continent, which may result from the higher fitness of individuals with lower DNA amounts where colder temperatures result in slower DNA replication (Bennett et al. 1982; Rayburn et al. 1985; but for evidence of opposing patterns, see Bennett 1976; Levin and Funderburg 1979; Murray et al. 2005).

Because fresh material was not available for Marion Island species, chromosome counts could not be performed to give exact chromosome numbers to compare with our genome size data. Nonetheless, comparisons between our measurements and published genome size and ploidy level data can be made; however, it must be kept in mind that extrapolations to ploidy level based on genome size can be imprecise since nuclear DNA content can be very variable even at constant chromosome numbers (Mowforth and Grime 1989). With this caveat, we note the following comparisons (Fig. 4, Online Resource 4). The mean 2C genome size of Marion Island *Acaena magellanica* was 1.31 pg, whereas the genome size for specimens of the same species from South Georgia was 0.6 pg (Bennett et al. 1982), suggesting that the Marion Island specimens have double the ploidy of the South

Georgia population, which were assumed to be diploid. Given this, Marion Island specimens are likely to be tetraploid. The mean 2C genome size of Marion Island *Austroblechnum penna-marina* was 13.93 pg, whereas that for specimens of the closely related *A. microphyllum* (Goldm.) Gasper & V.A.O.Dittrich (= *Blechnum microphyllum* (Goldm.) C.V.Morton) was 17.89 pg (Clark et al. 2016). The 2C genome size of Marion Island *Callitriche antarctica* was 2.99 pg, whereas that for octoploid specimens of the same species from South Georgia was 9.3 pg (Bennett et al. 1982), suggesting that the Marion Island specimens likely have a lower ploidy level. The mean 2C genome size of Marion Island *Carex dikei* was 2.89 pg, whereas that for specimens of the octoploid species *C. meridensis* (Steyerm.) J.R.Starr (= *Uncinia meridensis* Steyerm.) was 2.7 pg (Bennett et al. 1982), suggesting that the Marion Island specimens are likely also octoploid. The mean 2C genome size of Marion Island *Colobanthus kerguelensis* was 0.82 pg, whereas that for specimens of the tetraploid species *C. quitensis* (Kunth) Bartl. was 1.4 pg (Bennett et al. 1982), suggesting that the Marion Island specimens are likely diploid. The mean 2C genome size of Marion Island *Elaphoglossum randii* was 21.44 pg, whereas those for specimens of the diploid species *E. crinitum* (L.) Christ, *E. aubertii* (Desv.) T.Moore, and *E. hybridum* (Bory) Brack. were 28.26, 34.59, and 66.99 pg, respectively (Clark et al. 2016), suggesting that the Marion Island specimens are likely also diploid. The mean 2C genome size of Marion Island *Leptinella plumosa* was 1.79 pg, whereas that for specimens of the diploid species *Cotula coronopifolia* L. was 4 pg (Leitch et al. 2019), suggesting that the Marion Island specimens are likely also diploid. The mean 2C genome size of Marion Island *Lycopodium magellanicum* was 5.37 pg, whereas those for specimens of the diploid species *L. clavatum* L., *L. annotinum* L., *L. dendroideum* Michx., and *L. obscurum* L. were 5.71, 8.87, 9.52, and 9.58 pg, respectively (Bainard et al. 2011), suggesting that the Marion Island specimens are likely also diploid. The mean 2C genome size of Marion Island *Phlegmariurus saururus* was 20.44 pg, whereas that for specimens of the diploid species *Huperzia lucidula* (Michx.) Trevis. was 11.27 pg (Bainard et al. 2011). Two cytotypes were detected for the Marion Island specimens of *Poa cookii*, with mean 2C genome sizes of 0.44 and 0.89 pg, whereas specimens of the closely related tetraploid species *P. flabellata* (Lam.) Raspail and *P. ramosissima* Hook.f. were 5.5 and 5.69 pg, respectively (Bennett et al. 1982; Murray et al. 2005). The genome sizes of the Marion Island specimens are much smaller than that of the related species, and likely represent diploid and tetraploid cytotypes. The 2C genome size of Marion Island *Polystichum marionense* was 20.4 pg, whereas that for specimens of the diploid species *P. acrostichoides* (Michx.) Schott was 15.5 pg (Bainard et al. 2011), suggesting that the Marion Island specimens are likely also diploid. We did not find any published genome size data for close relatives of *Azorella selago* in the literature. Overall, we estimate that many of the vascular plant species on Marion Island are diploid, but more precise analyses of the proportions of diploid and polyploid species will have to await results from chromosome counts.

Conclusions

The islands of the sub-Antarctic region are some of the most isolated and least disturbed places on Earth. Therefore, they comprise one of the few regions where the indigenous flora can be characterized so fully, and where processes such as natural, as opposed to human-mediated, dispersal documented (e.g., Kalwij et al. 2019). Our updated checklist of the indigenous vascular flora of Marion Island reflects current understanding of taxonomic relationships and will allow for consistent communication about components of the sub-Antarctic flora across regions and studies. This information is key to understanding diversity patterns and drivers of diversity processes in the sub-Antarctic region as a whole (e.g., Chown et al. 1998; Greve et al. 2005; Shaw et al. 2010). At a local scale, the genetic information we present, namely sequence data for the universal plant barcoding loci *rbcL* and *matK* and genome size estimates, can facilitate the study and management of Marion Island's flora by providing additional resources for the recognition of species and cytotypes. Lastly, at a regional scale, DNA sequence data will enable broader phylogenetic studies into evolutionary and biogeographic patterns and processes across the sub-Antarctic region (e.g. Ito et al. 2017; Lehnebach et al. 2017).

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Data availability DNA sequence data generated in this study are available on GenBank.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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