Polar Biol (2017) 40:2397–2409
https://doi.org/10.1007/s00300-017-2152-x

ORIGINAL PAPER

Phenotypic variability and genetic differentiation in continental and island populations of *Colobanthus quitensis* (Caryophyllaceae: Antarctic pearlwort)

Marely Cuba-Díaz[1] · Macarena Klagges[1] · Eduardo Fuentes-Lillo[1,2] · Cristian Cordero[1] · Daniela Acuña[1] · Génesis Opazo[1,3] · José M. Troncoso-Castro[4]

Received: 2 March 2016 / Revised: 20 June 2017 / Accepted: 23 June 2017 / Published online: 4 July 2017

Abstract *Colobanthus quitensis* (Antarctic pearlwort) is one of the only two native vascular plants to inhabit the extreme environmental conditions of Antarctica. *Colobanthus quitensis* has a wide geographic distribution, both in latitude and altitude, and always inhabits extreme environments. This makes it crucial for understanding environmental tolerance mechanisms, and a useful model for studies regarding genetic diversity and intraspecific morphology. Several morphological and molecular descriptors were applied to *C. quitensis* populations, constituting the first study of its kind in these species. We postulated that morphological variability is strongly linked to geographic distribution, and that this is manifested in external morphological characteristics and genetic structure. A large intra- and interpopulational morphological variability was verified. Both morphological variability and genetics made it possible to form two separate groups between continental and Antarctic island populations. The genetic diversity was high to moderate with the least amount of diversity towards the north. The genetic structure was high, and the gene flow between populations was low. The correlation between morphological, genetic, geographic and altitudinal distances permits the proposal of an isolation by distance model that can be used between populations with high Bio-geographical influence. Understanding what factors lead to local or colonization adaptation, and determining the morphological variations and genetic differentiation in populations of *C. quitensis,* is vital for the understanding of the evolutionary history that has contributed to the success of the establishment of this species in an environment as extreme as Antarctica. Additionally, this study demonstrates the usefulness of the combined use of morpho-physiological and molecular markers for variability and diversity studies.

Keywords Antarctic · Ecotypes · Genetic structure · Genetic variability · Morphological diversity · Antarctic pearlwort

Electronic supplementary material The online version of this article (doi:10.1007/s00300-017-2152-x) contains supplementary material, which is available to authorized users.

✉ Marely Cuba-Díaz
mcuba@udec.cl; mcubaster@gmail.com

[1] Laboratory de Biotecnología y Estudios Ambientales. Escuela de Ciencias y Tecnología, Universidad de Concepción, Campus Los Ángeles. Casilla 341, Juan Antonio Coloma, 0201 Los Ángeles, Chile

[2] Laboratorio de Invasiones Biológicas. Facultad de Ciencias Forestales, Universidad de Concepción, Victoria 631, Barrio Universitario, Concepción, Chile

[3] Laboratorio de Nanocelulosa y Biomateriales, Universidad de Chile, Beauchef 851, Piso 5, Santiago Centro, Chile

[4] Laboratorio de Palinología y Ecología Vegetal, Escuela de Ciencias y Tecnología, Universidad de Concepción, Campus Los Ángeles. Casilla 341, Juan Antonio Coloma, 0201 Los Ángeles, Chile

Introduction

Antarctica is one of the oldest ecosystems in the world. Human influence and exploration have been very limited due to its climate and geographic location (Convey 2010; Bokhorst et al. 2011). At present, only 0.34% of its territory is devoid of ice (Convey et al. 2009), which is why the terrestrial ecosystem is primarily developed in the coastal areas of the Antarctic Peninsula and the bordering islands (Convey 2010, 2013).

The prevailing environmental conditions, including strong winds, very low temperatures, low availability of nutrients, high UV radiation and marine aerosols, are obstacles for any plant species (Alberdi et al. 2002; Convey et al. 2014). These factors are considered to be the causes of the low diversity and simplicity of this extreme ecosystem, which contains only two native vascular plants, *Deschampsia Antarctica* Desv. (Poaceae) (common name: Antarctic hair grass) and *Colobanthus quitensis* (Kunth) Bartl. (Caryophillaceae) (common name: Antarctic pearlwort) (Moore 1970; Smith 2003; Convey 2011). Both species have been studied intensively in recent years, revealing that each possesses properties that enable it to survive in such adverse conditions (Xiong and Day 2001; Bascuñán-Godoy et al. 2006, 2010, 2012; Bravo et al. 2007; Cuba-Díaz 2011; Cuba-Díaz et al. 2011).

Colobanthus quitensis also presents a wide geographic range that extends from Mexico (17°N) to the north of the Antarctic Peninsula (68°S), and from 0 to 4200 m a.s.l. (Moore 1970; Smith 2003; Convey et al. 2011). In Chile, the species is located throughout the Andes, typically in bogs located at high altitudes (over 2500 m a.s.l.) in the north, but close to sea level in the polar latitudes (Moore 1970). This species grows in the form of mats or compact cushions, reaching several centimetres in diameter. It reaches a minimum height of 1.5 cm to a peak height of 2 cm, approximately. It is a self-fertilising species, with the capacity for asexual reproduction, and shows considerable morphological variability throughout its distribution (Moore 1970; Smith 2003; Gianoli et al. 2004).

This morphological variability can be influenced by several environmental factors, such as the degree of exposure, water availability, or other stressful abiotic conditions (Bradshaw and Hardwick 1989; Gianoli et al. 2004). It can also be influenced by its latitudinal and altitudinal distribution, since it is common to find different phenotypes in populations at different altitudes (Haider et al. 2011). This phenotypic diversity in natural populations has been seen to affect a large part of its characteristics, and may or may not be related to genetic changes (Hartl and Clark 1997). Regardless of the scale of the distribution of natural populations, the greatest degree of genetic differentiation is usually more associated with environmental factors, such as geographic barriers, availability of resources and/or isolation by distance, among others (Salinas et al. 2011; Westergaard et al. 2011). Knowledge of the genetic diversity of widely distributed species is important for their conservation, and genetic and phenotypic distinction. Generally, they show variation in the morphology and physiology as well as in the genetic structure of the species (Piña-Escutia et al. 2010; Ruiz et al. 2010; Westergaard et al. 2011).

In *C. quitensis*, the classification of two ecotypes has been established between the Antarctic populations (Arctowski Base, Admiralty Bay, King George Island/South Shetland) and Andean populations (Cerro la Parva/Metropolitan Region, Chile) through studies of adaptation to cold, morphological characteristics and internal transcribed spacer (ITS) sequences (Gianoli et al. 2004). The Antarctic ecotype grows in a sustained and stable temperature of −2 to 6 °C, usually with a low photosynthetic photon flux density (PPFD) (300–600 µmol Photon m^{-2} s^{-1}) (Xiong and Day 2001). Its life cycle is between December and March (Bascuñán-Godoy et al. 2010), and it is usually covered by snow from April to November (Zúñiga-Feest et al. 2009). The Andean ecotype grows under broad daily PPFD fluctuation (usually 2000 µmol Photon m^{-2} s^{-1}), and its life cycle is between October and April (Bascuñán-Godoy et al. 2010). This ecotypic differentiation was explained by Gianoli et al. (2004) as a response influenced by the extreme environment, mainly by stress due to freezing, which is a selective factor that acts on a timeline. Therefore, it can be assumed that the plant populations growing in cold environments are the object of genetic differentiation that enables them to adapt to local conditions (Körner, 2003). Moreover, in a morpho-physiological characterisation study of *C. quitensis* populations, Klagges et al. (2013) demonstrated a trend of differentiation between the island and continental populations; the variability and relations between geographically closer groups are attributed to the presence of geographic barriers between the study populations. It has been suggested that species that are widely distributed and in adverse conditions constitute excellent model systems for studying plant speciation and evolution (Archibald et al. 2006), as is the case with *C. quitensis,* with a wide geographic distribution and populations that have never before been studied. The study of morphological and genetic differentiation are key to understanding postglacial migration patterns, the effect of weather conditions on local adaptation of species, gene flow and genetic drift, and understanding how vicariance acts on the evolutionary processes of species (Convey et al. 2008). Several studies have analysed the morphological and genetic differentiation to understand these ecological processes, largely by long-lived species that have a wide distribution, including species of *Populus* sp., *Quercus* sp., species of the Cactaceae family and various Nothofagus types (Hamrick and Godt 1996; Kremer et al. 2002; Royer et al. 2008).

In this work, different morphological markers were applied to determine the existence of phenotypic variations in five populations of *C. quitensis*: two of Antarctic island origin, one from the south of Punta Arenas, Chile and two from the Andes mountain range in southern and central Chile. Through the use of inter-simple sequence repeat (ISSR) molecular markers, the variability and genetic structure of three of these populations were characterised

and the correlation between the different markers and the geographic and altitudinal distances were established using Mantel's test, in order to determine which are the mechanisms underlying the differences between populations of *C. quitensis* across the latitudinal gradient presented by this species.

The goal is to show that the morphological variability previously described for *Colobanthus quitensis* is strongly connected to its geographic distribution, and that it is manifest in both external morphological characteristics and the genetic structure of the populations, which could explain its ability to adapt to the extreme environmental conditions in which it lives.

Materials and methods

Plant material

In this study, five populations of *Colobanthus quitensis* were analysed (Table 1), of which pPar, pC and pPA are the populations of continental Chile, and pA and pH are the island populations on King George Is. and Livingstone Is. in the South Shetland Islands, respectively. The individuals in each population were collected on site and kept in common garden conditions. They were reproduced vegetatively in plastic pots (19.7 × 12.8 × 9 cm) using a soil mixture of leaf:peat:pearlite (3:2:1), and were maintained at 14 + 1 °C in a growth chamber with a PPFD of 100–120 μmol photon m^{-2} s^{-1}, a photoperiod of 16/8 h light/dark and a relative humidity between 75 and 80% using manual irrigation. The plants were fertilised every two weeks with phostrogen (Solaris, Buckinghamshire, UK) using 0.2 g l^{-1}. The different populations were kept in separate chambers to prevent cross-breeding, and each individual analysed had been kept in the common garden for more than 6 months. Molecular analyses were made only with pPar, pPA and pA populations, due to the lack of adequate material from all populations.

Morphological and germination analyses

There were *210 plants* used from the different populations for the morphological analyses (Table 1), and 4 quantitative descriptors were used, corresponding to leaf width and length, main root length and crown diameter, which were measured with a calliper (0.5 mm resolution) (Mitutoyo, USA). From the leaf width and length data, the relation between these two parameters was established, and the leaf area was determined by taking *10 leaves* per population at random, digitising and analysing them with the public domain software image J v. 1.47 for Windows; these measurements *were taken in triplicate*. For the count and measurements of the stomatal anatomy, *10 leaves were taken* at random from each of the populations, *with 3 replicas* per population (*30 leaves in total*). Thin Sects. (100 μm) were cut from the middle sections of the leaves, and the tissue from the axial part was fixed with 10% glycerin and observed under a planachromatic trinocular optical microscope (Olympus CX31 RTSF). The stomatal density was determined by counting the number of stomata per unit of leaf surface, corresponding to 0.2 mm^2 according to the magnification used (40X). Stomatal width and length were measured using the software Micrometrics Premium 4 from images digitised with a camera (CCD Microimagi 5.1 megapixels) coupled to the microscope.

For the seed germination analysis, *all seeds were selected* and recorded per population, and a 24-h floatation test was performed at room temperature to eliminate any non-viable seeds (Suma and Srimathi 2014). Then, the seeds at the bottom were dried and conserved in sealed tubes at 4 °C for one week. Once this time had elapsed, they were sterilised by adding 70% ethanol to the tube for 3–5 s. After a spin-down, the ethanol was extracted and 5% sodium hypochlorite (Cloralex) was added. The tube was vortexed for 7 min, and after a spin-down, the supernatant was eliminated and washed three times with sterile distilled water under a laminar flow hood. Then, the seeds were

Table 1 Geographical location and sampling size details of *Colobanthus quitensis* (Antarctic pearlwort) populations studied

Populations	Location	Population code	Latitude/longitude	Altitude (m a.s.l.)	Sample size	
					Morphological	Molecular
La Parva	La Parva Hill	pPar	33°19′S; 70°16′W	3600	50	10
Conguillio	National Park Conguillio	pC	38°36′S; 71°36′W	2575	30	nd
La Marisma	Southern Punta Arenas	pPA	52°22′S; 70°58′W	1-3	50	10
Arctowski	King George Is., Antarctica	pA	62°09′S; 58°28′W	3-23	50	10
Hannah Point	Livingstone Is., Antarctica	pH	62°39′S; 60°37′W	10-200	30	nd
Total					210	30

Table 2 Morphological parameters assessed for each population of *Colabanthus quitensis* (Antarctic pearlwort)

	pPar	pC	pPA	pA	pH
Leaf length (mm)	15.81 ± 4.18^{c}	26.46 ± 3.38^{a}	21.25 ± 5.76^{b}	14.00 ± 1.98 ^{cd}	11.49 ± 1.16^{d}
Leaf width (mm)	1.12 ± 0.17^{b}	0.91 ± 0.06^{c}	1.10 ± 0.18^{b}	1.25 ± 0.12^{a}	1.11 ± 0.10^{b}
Relationship width/length of leaves	13.88 ± 1.89^{c}	28.97 ± 2.94^{a}	19.38 ± 4.01^{b}	11.20 ± 1.78^{d}	10.33 ± 1.27^{d}
Root length (mm)	48.69 ± 12.65^{b}	58.88 ± 4.42^{a}	52.33 ± 4.01^{ab}	39.87 ± 9.45^{c}	31.43 ± 14.4^{c}
Crown diameter (mm)	1.25 ± 0.24^{b}	1.63 ± 0.23^{a}	1.27 ± 0.30^{b}	1.38 ± 0.25^{b}	1.21 ± 0.14^{b}
Foliar area (cm^2)	0.26 ± 0.02^{a}	0.14 ± 0.05^{b}	0.25 ± 0.01^{a}	0.26 ± 0.03^{a}	0.11 ± 0.02^{b}
Stomatal density (number stomata mm^{-2})	105.19 ± 4.69^{c}	87.56 ± 5.33^{d}	145.21 ± 6.09^{b}	56.40 ± 2.13^{e}	176.24 ± 10.2^{a}
Relationship width/length stomata μm^{-2})	27.21 ± 3.98^{b}	30.21 ± 2.13^{b}	35.21 ± 1.21^{a}	22.31 ± 1.34^{c}	9.52 ± 1.67^{d}

Mean values ± standard error of each parameter are shown. Different letters mean significant differences $p < 0.05$

placed in Petri dishes in MS medium (Murashige and Skoog 1962) and maintained in a growth chamber at 22 ± 2 °C with a photoperiod of 16/8 h light/dark for 45 days. The percentage of viable seeds, the germination percentage and the average germination time (time at which 50% of the seeds germinated) were measured.

A *one-way ANOVA* was performed to analyse the morphological data shown in Table 2. To demonstrate significant differences among the analysed populations, *Scheffé's test* of multiple comparisons was used with a 95% confidence level. Euclidian distances (using population means) were measured to determine groupings among the morphological data and the study populations of *C. quitensis*. In order to analyse the germination-related data, a *one-way ANOVA* was performed, and to determine significant differences, *Tukey's multiple comparisons test* was performed with a confidence level of 95%. All analyses and graphics were done with the software *STATISTICA v. 6.0*.

Floral morphology

The floral structures were measured by taking *10 flowers* from each of the 5 study populations, and measuring the length of the flower stalk, capsule width and length, number of petals and sepals and number of seeds per capsule. A detrended correspondence analysis (DCA) and graphics were performed using the programme *Past 3*. This indirect ordination method was applied to determine groupings among the study populations and the measurements of the floral structures.

Molecular methods

In order to establish if there is a relationship between the expected morphological variability and the genetic variability between and within populations, a molecular analysis was performed using ISSR-PCR markers. The genomic DNA from *10 plants* from each population (Table 1; Fig. 1) was extracted from fresh leaf tissue using Plant DNAzol (Gibco-BRL), according to the manufacturer's instructions. The ISSR markers and the amplification conditions were done according to Cordero (2012). From the electrophoresis images where the amplified products were separated, a fingerprinting analysis was done, constructing a binary matrix and assigning to each amplified product the value of 1 and 0, depending on its presence or absence, respectively. Using the *software GenAlEx v. 6.1*, the bands of fragments amplified per primer, the percentage of polymorphic loci (*%P*), the number of alleles observed (Na), number of effective alleles (Kimura and Crow 1964), Shannon's index (Shannon and Weaver 1949) and Nei's genetic diversity (*h*) (Nei 1973) were calculated for each of the populations.

The binary matrix was used to estimate the number of polymorphic bands, the genetic structure index (Gst) (Nei and Li 1979) that provides information about the genetic divergence between populations. From the Gst value, the gene flow (Nm) was estimated, which represents the rate of effective migration, where N is the size of population and m the genetic migration by generations. The indices were calculated using the PopGene programme for dominant markers (Yeh et al. 1999). The distribution of genetic variability was determined with an analysis of molecular variance (*AMOVA*) (Excoffier et al. 2005) and principal components analysis (*PCA*), with all the loci detected using the *software GenAlEx v. 2.0.* The determination of Nei's genetic distance (Nei 1972) to establish the similarity between pairs of populations was performed using the *software TFPGA 3.1* (Miller 1997), and represented by a genetic distance dendrogram among populations using the *UPGMA method* (Sneath and Sokal 1973). Each node was supported by bootstrapping with 1000 permutations. From the genetic distance and morphological distance matrices, Mantel's test was applied (Mantel 1967) to associate these matrices with the geographic and altitudinal factors. This test was carried out through 1000 randomisations.

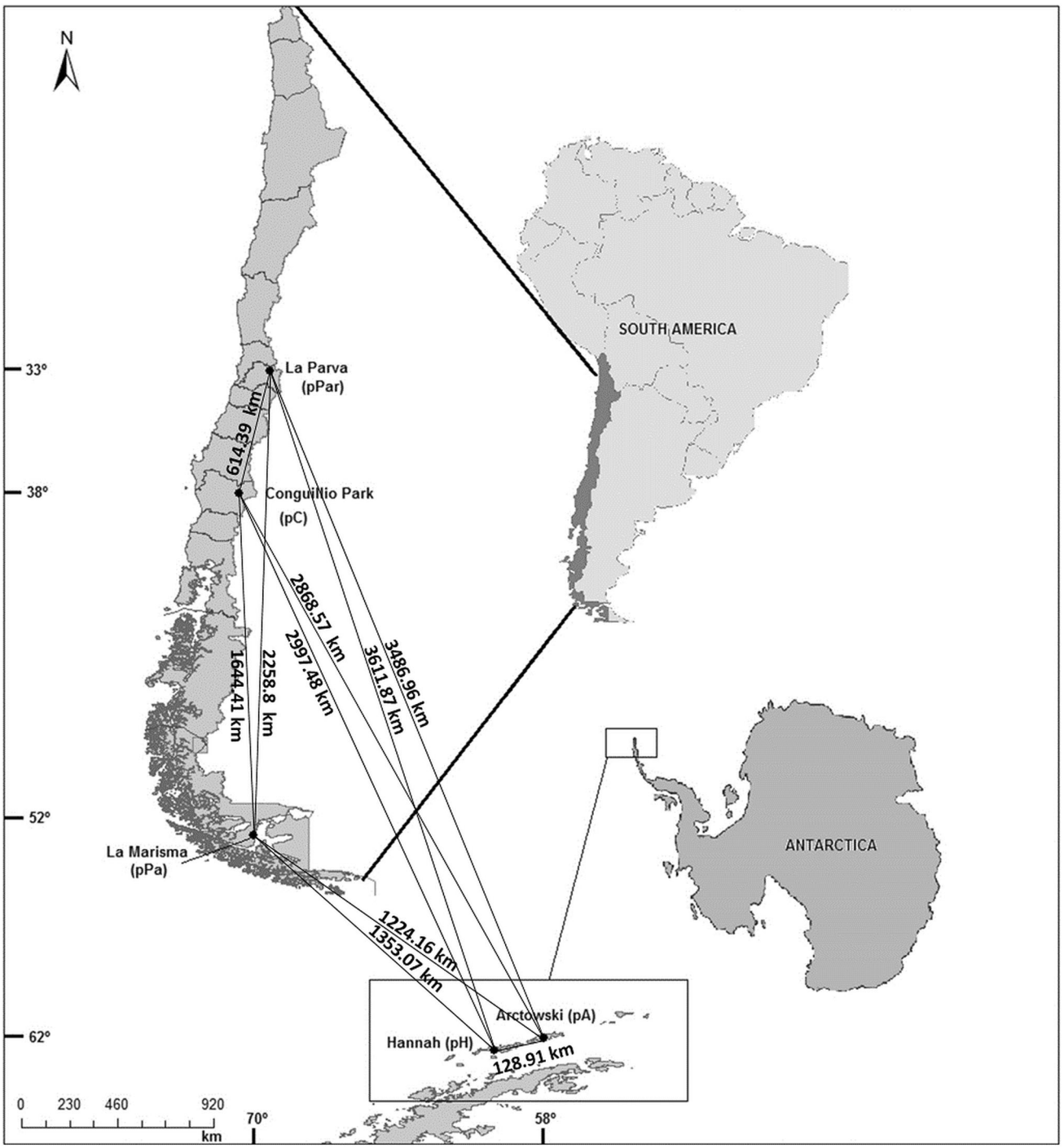

Fig. 1 Spatial distribution of *Colobanthus quitensis* (Antarctic pearlwort) populations analysed in this study. Geographic (km) distances are provided for each between-site link. For more details about this population's site, see Table 1

Results

Morphological variations

All the descriptors of the stomatal morphology and anatomy evaluated showed variability among the populations analysed. In general, the Conguillio population (pC) showed the highest mean values, except for leaf width, which also affected leaf area, which was lower than other populations in the stomatal parameters (Table 2). The lowest means were presented by the Hannah Point (pH) population, except in the stomatal density, where it showed the maximum density ($F_{(2.24)}$, 176.31 ± 10.2, $p = 0.001$, $n = 30$ stomata mm^{-2}) (Table 2). The Arctowski (pA), La

Table 3 Euclidian distance matrix between pairs of populations obtained from the morphological parameters measured in the five populations of *Colobanthus quitensis* (Antarctic pearlwort) studied

	pPar	pC	pPA	pA	pH
pPar	–	24.0	10.9	11.8	11.5
pC	24.0	–	13.5	34.2	33.8
pPA	10.9	13.5	–	21.6	21.1
pA	11.8	34.2	21.6	–	1.0
pH	11.5	33.8	21.1	1.0	–

Parva (pPar) and Marisma (pPA) populations showed intermediate values, but always following a south-north gradient where the southernmost populations were smaller and more variable.

The Euclidian distances between population pairs, measured from the average values of all the characteristics assessed (Table 3) established that pA and pH are closer to each other with a distance equal to 1.0. The most distant populations are pC and pA, with a distance equal to 34.2. The grouping of Euclidian distances among the populations and under the criterion of complete alignment corroborated these relations (Fig. 2). The first group was established between the populations to pPar and pPA, with a Euclidean distance estimated at 10.9. The second group contained pA and pH with a Euclidean distance estimated at 1.0 and separated from the first group by estimated distances from 11.5 between pPar and pH to 21.6 between pPA and pA. A third group contained pC, which also presented the greatest estimated Euclidean distances compared to the rest of the study populations (Table 3).

The DCA of the floral morphology of the five populations revealed a group between continental and island populations, with the latter being the most separated between them (Fig. 3). Although for these characteristics pC was part of the continental group, a sub-group was established where most of the individuals in this population

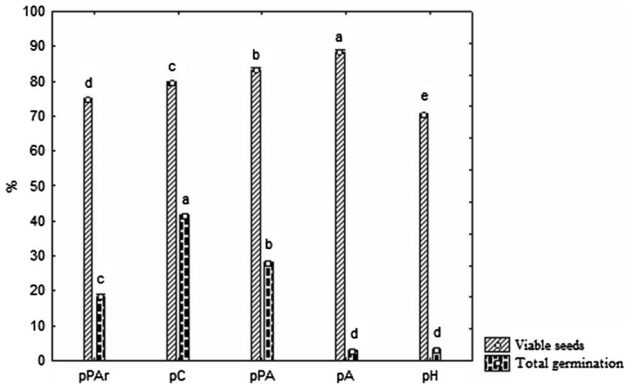

Fig. 3 DCA plot based on the floral characteristics in the five study populations of *Colobanthus quitensis* (Antarctic pearlwort) (For population names, see Table 1)

were grouped. The ordination of data was based on 26 segments with four iterations, with a total inertia value of 7.32 and an accumulated variance of 69.26%.

The analyses of the viability and germination capacity of the seeds collected from the different populations in common garden conditions revealed that there is no direct relationship between the seed viability and germination capacity (Fig. 4). pA showed the greatest viability percentage, and together with pH, showed the lowest germination percentage. The continental populations showed the highest germination percentages compared to the island ones (Fig. 4). Similarly, the germination time was directly related to the origin of the populations and their germination capacity, being 4 days for pC, 7 days for pPar and pPA and 28 days for both island populations, which obtained only 2.5% ($F_{(1.25)}$, $p = 0.00002$) and 2.9% ($F_{(1.25)}$, $p = 0.0009$) of germination in pA and pH, respectively.

Genetic variations

From 9 previously tested ISSR primers (Cordero 2012), in this work, 4 more informative primers were used for the

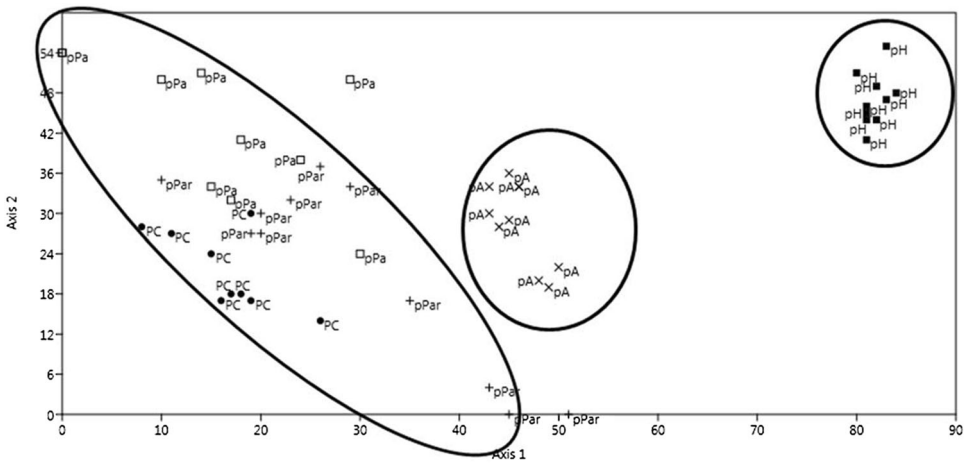

Fig. 2 Complete linkage dendrogram showing the *Colobanthus quitensis* (Antarctic pearlwort) population grouping from Euclidean distances of morphological characteristics (For population names, see Table 1)

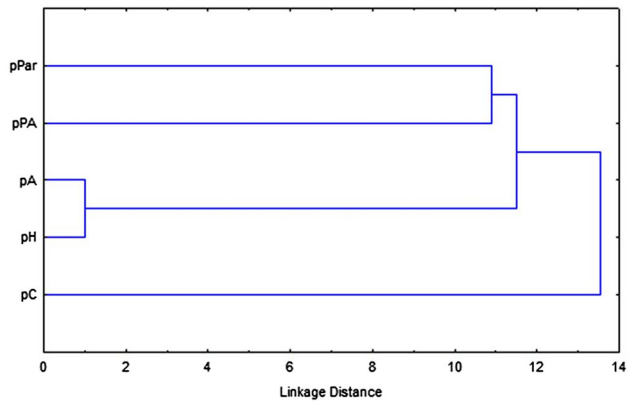

Fig. 4 Percentage of viable and germinating seeds in populations of *Colobanthus quitensis* (Antarctic pearlwort) (For population names, see Table 1)

Table 4 Genetic diversity parameters of three *Colobanthus quitensis* (Antarctic pearlwort) populations detected by all polymorphic fragments from the 4 most informative ISSR molecular markers

Population	Pa	PLn	*P* (%)	Na	Ne	*I*	*h*
pPar	5	39	52.00	1.293	1.371	0.299	0.205
pPA	4	44	58.67	1.387	1.393	0.330	0.224
pA	4	48	64.00	1.440	1.354	0.329	0.216

Pa Private alleles, *PLn* number of polymorphic loci, *P* percentage of polymorphic bands, *Na* number of different alleles per locus, *Ne* effective number of alleles per locus, *I* Shannon's information index, *h* Nei's genetic diversity

species, and a total of 75 clearly identifiable loci were obtained (Online Resource Table 1). Each primer amplified from 10 to 26 loci with a mean value of 18.75 fragments, which made it possible to determine a polymorphism range from 46.97 to 73.33% (Online Resource Table 1). The genetic variability analysis from the 75 loci detected private alleles in the three populations (Table 4), with pPar showing the highest number (5). This same population showed the lowest polymorphism percentage (52.0%), whereas pA showed the greatest percentage with a value of 64.0% (Table 4). The average number of effective alleles ranged between 1.354 in pA and 1.393 in pPar. The values from Shannon's genetic diversity index were similar for pA and pPA, with $I = 0.329$ and 0.330, respectively (Table 4). A similar relation among the populations was detected using Nei's genetic diversity analysis (1978), but with a

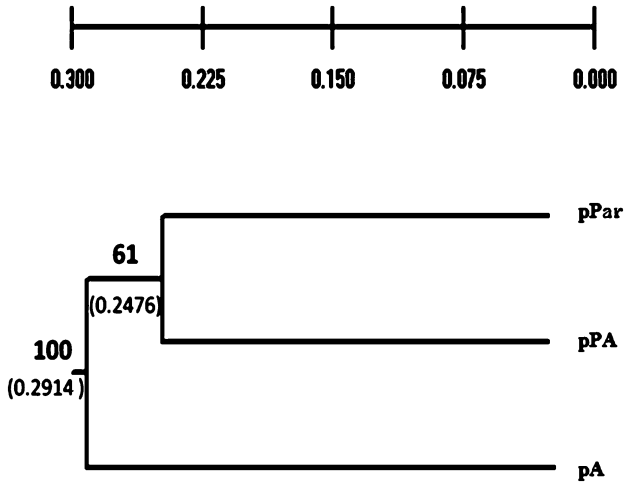

Fig. 5 Principal components analysis (PCA) in populations of *Colobanthus quitensis* (Antarctic pearlwort) using 75 polymorphic loci detected with ISSR markers. *Squares* pPA (La Marisma). *Triangles* pPar (La Parva). *Diamonds* pA (Arctowski)

slight variability among them: pPA had the highest average ($h = 0.224$), whereas pA and pPar showed averages of $h = 0.216$ and $h = 0.205$, respectively (Table 4).

To determine interpopulational structuring, the Gst was determined for dominant markers, analogous to the Wright fixation index (Fst) for codominant markers, which established a value of Gst = 0.3698, indicating a high genetic differentiation among populations. This was directly related to the low gene flow (Nm = 0.8521) among populations detected from the interpopulational Gst values.

The greatest molecular variance detected was between individuals within the populations (59%), and only 41% between populations (Table 5), with a level of significance of $p < 0.01$. However, the variability between populations is considered moderate to high. The three populations of *C. quitensis* were placed in separated groups (Fig. 5). The variance of the first three axes presented values of 31.37, 26.65 and 20.28%, respectively, explaining as a whole 78.31% of the variation observed among the populations. Genetic differentiation is clear among all the populations without superposition between the individuals of the different populations, showing pA to be the farthest away, whereas pPA and pPar are closer together. The genetic distance values among populations (Nei 1972) show a shorter distance between pPA and pPar of 0.248, and a

Table 5 Analysis of molecular variance among and within the populations of *Colobanthus quitensis* (Antarctic pearlwort)

Source of variation	df	SS	MS	CV	% of the variation
Among populations	2	142.000	71.000	6.204	41
Within populations	27	242.000	8.963	8.963	59
Total	29	384.000		15.167	100

df Degrees of freedom, *SS* sum of squares, *MS* mean squares, *CV* coefficient of variance

Table 6 Nei's genetic distances (1972) between each pair of populations of *Colobanthus quitensis* (Antarctic pearlwort)

	pPar	pPA	pA
pPar	–	0.25	0.30
pPA	0.25	–	0.29
pA	0.30	0.29	–

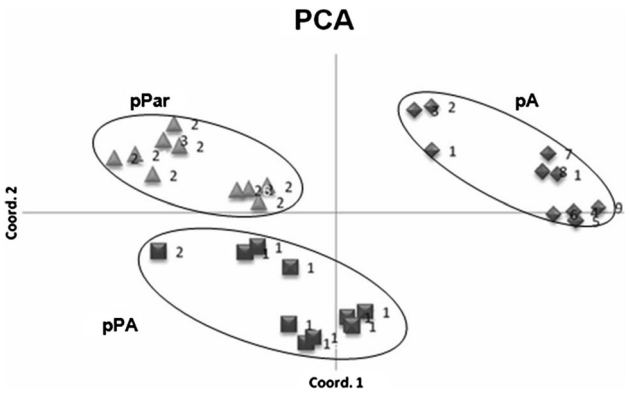

Fig. 6 Dendrogram constructed by means of a UPGMA analysis using Nei's genetic distances (1972). In the upper part of each node is the "bootstrap" value that supports it and underneath in parentheses are the genetic distances

maximum distance between pPar and pA of 0.295 (Table 6). The results obtained from the *UPGMA* dendrogram (Fig. 6), taken from the genetic distances between the population pairs (Table 5), are consistent with the results of the *PCA*, obtaining two groups with a bootstrap support greater than 60%. The first node grouped pPA and pPar with a genetic distance of 0.2476 and bootstrap support of 61%. The second node grouped the first node and pA with a genetic distance of 0.2914 and bootstrap support of 100% (Fig. 6).

Given the tendency to group populations both from the morphological characteristics and genetic differentiation determined from ISSR markers, Mantel's test (1967) was conducted, in which the Euclidian distance values of the morphological descriptors, the values of Nei's genetic distance and the geographic and altitudinal distances between the populations were included (Table 7). This test enabled us to establish correlations between the morphological characteristics and the genetic polymorphism detected, and to assess the degree of association of these with the variables of geographic and altitudinal distance. An association was found between the morphological and genetic distances, and between each of the factors to varying degrees. The greatest correlations were between the morphological distance and altitude ($r = 0.99$, very high), and between the morphological distance and the geographic distance ($r = 0.65$, high) (Table 7).

Table 7 Correlations between distances for morphological, genetic, geographic and altitudinal variables among populations of *Colobanthus quitensis* (Antarctic pearlwort) obtained using Mantel's test

Matrix 1	Matrix 2	R
Genetic Dist.	Altitudinal Dist.	0.35
Genetic Dist.	Morphological Dist.	0.40
Genetic Dist.	Geographic Dist.	0.50
Morphological Dist.	Geographic Dist.	0.65
Morphological Dist.	Altitudinal Dist.	0.99

Discussion

Morphological variation

All of the parameters of morphology, anatomy and germination detected a wide morphological variability among the different populations, with a certain tendency to form groupings between them. A clear separation between continental and island populations was maintained, but with a north–south gradient in terms of plant size. This is the first study that compares populations with a wide distribution, and that uses various variables that measure the morphological and genetic relationships that range from the species' own characteristics, to adaptive responses to the environment where each population develops. Klagges et al. (2013) reported similar results when comparing 11 morpho-physiological parameters within these populations. They observed the same trend of larger populations towards the northern end of the distribution. Moore (1970), studying the variations in the morphology of *C. quitensis* populations throughout their distribution, had already described a wide variability of the morphological characteristics of the different populations, demonstrating that the greatest variability was at the southern end of the species distribution. This variability was not expected, because it had been postulated that this species should have a north–south migration pattern. According to Moore (1970), these variations could be related to adaptations controlled by the environment, but they could also be genetically-based.

It has been observed in morphological and micro-morphological studies between pA and pPar that the latter presents the higher averages. Comparing these two populations in terms of their morphology in the presence of low temperatures in common garden conditions, Gianoli et al. (2004) found that pPar presented higher averages of leaf length and shape, and flower stalk and stem biomass than pA. The same trend was observed when 10 parameters of foliar micromorphology were compared in the same populations (Bascuñán-Godoy et al. 2010), showing that pPar presented the highest averages in all the characteristics

evaluated. In this study, pPar had lower average values than pPA and pC, which is not consistent with the north–south gradient observed, where larger plants were oriented towards the northern end of the distribution. This could be related to the stress conditions in the habitat, which are heavily influenced by humans due to the presence of pitches for winter sports, and may be placing selection pressure on the population (based on observations made directly on the field). It has been shown that genetic and morphological variability are negatively influenced by anthropogenic activity, propagule pressure and gene flow (Alexander and Edwards 2010; Haider et al. 2011; Westergaard et al. 2011).

In this work, only the 8 parameters of the morphology that showed the greatest variability among the populations were evaluated (Moore 1970; Cordero 2012; Klagges et al. 2013). Moore (1970) detected the greatest variability in characteristics related to leaf length, leaf length:width ratio, as well as the characteristics related to the floral morphology, such as the width of the floral capsule and the length of petals and sepals, which vary mainly due to influences of such external factors as drought, degree of exposure and pressure due to anthropogenic activity. However, it has been reported that stomatal number and morphology could be indicators of stress in populations of the same species with a wide distribution or with responses to temperature gradients (Jelling et al. 1983; Romero et al. 1999), or other types of abiotic stress. In this study, pH (Hannah) presented the greatest stomatal density, which is consistent with the literature, since pH is the study population found farthest south, and its habitat sees low temperatures all year round (the average temperature in the summer does not exceed 4 °C), it has low water and nutrient availability (Beyer et al. 2000) it is located on a steep slope on the side of a rocky cliff (Molina-Montenegro et al. 2012) and although several of these conditions are shared with pA, pH also has the disadvantage of being a smaller population than pA, which adds a higher abiotic pressure on the population. However, the pPA population also had a high stomatal density as well as larger stomata (Table 2) and is located in an estuary easily flooded by high tides in a narrow marsh with clayey sediments covered by herbaceous vegetation (Cuba-Díaz et al. 2013).In addition to the marine influence, it is also subject to anthropogenic pressure from being located along a highway, which, in our view, provides the greatest pressure on the population. In *D. antarctica*, the other vascular species native to Antarctica, greater stomatal density was observed in plants in natural conditions than in that of those acclimated for two years at 13 °C (Romero et al. 1999), which shows that stomatal density and size are closely related to environmental factors, such as temperature (Jelling et al. 1983).

According to Moore (1970), the characteristics that show greater variability are heavily influenced by the environment, where it has been identified that conditions such as water availability and degree of exposure can have a strong effect on them. This may explain the farthest pH group (Fig. 3), since generally this population presented the lowest averages, except for the number of seeds per capsule, where it presented the highest number (36 seeds). This last result can be explained on the basis of several studies into the reproductive capacity of this species in natural conditions. In Antarctica, it has been reported that although the plants bloom frequently, they do not produce mature seeds (Convey 1996), and despite an abundant seed bank being recorded, most are not viable or lose their germination capacity when favourable conditions to germinate cannot be found (McGraw and Day 1997; Ruhland and Day 2001; Kellman-Sopyla and Gielwanowska 2015).

The seed viability analysis and percentage of germination per population showed that the highest germination percentage and lower germination times appeared in the continental populations (Fig. 4). The pPar population, among the continental samples, demonstrated the lowest germination percentage, which is consistent with what was observed in analyses of the morphological characteristics (Table 1). Both island populations showed the longest germination times and the lowest germination percentages. This aligns with studies of seed banks and the reproductive properties of this species in Antarctica (Convey 1996; McGraw and Day 1997; Ruhland and Day 2001). pH presented the lowest percentage of viable seeds in spite of being the population with the highest average number of seeds per floral capsule (36 seeds). pA was the population that presented the greatest percentage of viable seeds (Fig. 4); however, it was the population with the smallest average number of seeds with just 16 per floral capsule. Recent studies have indicated that seeds of *C. quitensis* from Antarctica showed a high germination percentage, around 80%, and that germination occurred in temperatures ranging from 2 to 37 °C. Also, the germination time was determined solely by temperature, shortening at high temperatures and extending when temperatures were low. This determined that the optimum germination temperature is 18 °C (Kellman-Sopyla and Gielwanowska 2015). While Sanhueza et al. (2017) showed that seeds from plants grown at higher temperatures (11/5 °C) not only significantly shortened the germination time, but also had a high percentage of germination in a higher temperature range (10–25 °C) with respect to seeds from plants grown at low temperatures (5/2 °C).

In general, the variability in different characteristics of the vegetative and floral morphology, as well as the stomatal anatomy and germination capacity of the different *C. quitensis* populations, could be attributable to a

continuous selection process in which various characteristics can, as a whole, adapt to the prevailing environmental conditions in the habitat of the species (Moore 1970; Alexander and Edwards 2010; Haider et al. 2011). This could be closely linked to geographic fragmentation in their habitat and very little genetic exchange between populations (Manzur 2006; Westergaard et al. 2011). Our results show that this population variability, which can be considered geo-ecotypes, can be maintained even in common garden conditions, where the populations, although separated from each other to avoid crossover, are grown in the same environmental conditions.

Genetic variability

In order to demonstrate whether the morphological variability is attributable to genetic variability within the populations, the variability was examined in the ISSR regions using ISSR-PCR. In this study, only the pPar, pPA and pA populations could be evaluated due to a lack of plant material, but the goal of analysing the variability representing a north–south gradient was accomplished.

In a previous study with two populations of *C. quitensis* (pA and pPar), ITS markers did not detect significant differences between the sequences for ITS 1 and ITS 2 between the populations (Gianoli et al. 2004); it has been reported that ITS sequences are not variable in some cases. Archibald et al. (2006) suggest the use of ISSR to study populations of the same species with highly significant results that place ISSR above ITS. The detected polymorphism is coincident with the greater morphological variability found in the southernmost populations. The genetic diversity analysis, using AFLP, of island populations (pA and pH), a population from the Antarctic peninsula and a population of *C. quitensis* in Punta Arenas, revealed an increase in the genetic diversity from north to south (Acuña-Rodríguez et al. 2014). These results raise some questions, because they show that the populations with the least genetic diversity are the most recent from an evolutionary point of view (Allendorf et al. 2010). Seen in this light, it could be inferred that the populations farther north are the most recent, which suggests that the centre of origin for this species may be near Antarctica.

The genetic structure in this study was obtained on the basis of the genetic structure index (Gst) for dominant markers, an analogue to the Wright fixation index (Fst) for codominant markers, whose average value among *C. quitensis* populations was 0.3698, which indicates a high genetic differentiation among the three study populations (Hartl and Clark 1997). From this Gst value, the gene flow between populations was calculated, presenting an $Nm = 0.8521$, which indicates that the gene flow between the populations is low. These results are consistent with those obtained by Cordero (2012), who, when analysing populations of the same species, found a high genetic diversity between populations. Manzur (2006) established that the greatest genetic diversity between populations of the same species has a direct and positive association with the geographic distance of its distribution, as each population must face different environmental conditions to which it must adapt. In addition, Androsiuk et al. (2015) observed low genetic diversity and a moderate population differentiation in populations of *C. quitensis* near the Arctowski Base on King George Island using iBPS markers, linking these results to the geographic location of each population. With respect to the low gene flow, the result was expected because the three studied populations are very distant from one another, creating geographic barriers and a low seed dispersion. Gianoli et al. (2004) suggested that migratory birds such as *Muscisaxicola* ssp. possibly fulfil a seed dispersion role, but this has yet to be proven.

When observing a high genetic differentiation between the populations and a low gene flow of less than one individual per generation, a PCA analysis was performed of the 75 loci detected by ISSR-PCR (Fig. 5). It revealed genetic differentiation among all the populations. It also showed that the pA group is farther from the pPA and pPar groups, which are closer to one another. These results were corroborated when the genetic distances were calculated (Nei and Li 1979) between pairs of populations, obtaining a minimum distance of 0.248 between pPA and pPar and a maximum of 0.295 between pPar and pA (Table 6).

These results are consistent with the group obtained from Euclidean distances for the morphological characteristics, where the island populations are separate from the continental populations. Carlquist (1974) reported that island lineages tend to evolve quickly in a wide range of morphological forms, and occupy different areas within a geographically isolated region. Antarctica is a hostile ecosystem for plant development where, in addition to stress by freezing, there is a prevalence of other factors that limit plant growth, such as strong winds, sea spray, poor nutrient availability, and high UV radiation intensity. These factors could exert selection pressure on the species in terms of timeline, which together with geographic isolation (Körner 2003; Parnikoza et al. 2011), could explain the genetic differentiation of pA compared to the continental populations. Regardless of the presence of extreme environmental factors in the sites of the continental populations, the geographic and climatic isolation of Antarctica has a greater influence on population genetic variability, such as that shown in geographically closely related populations (Androsiuk et al. 2015). The pPar and pPA populations, although they are distant (more than 3080 km), may have greater homology between them, as the latitudinal differences are compensated by the altitudinal

differences (Gianoli et al. 2004). Future studies that include more populations with lower geographic distance and other markers are necessary to detect clearer parental and geo-climatic relationships.

The analysis of each of the variables: morphological, genetic, altitudinal and geographic distance, using the Mantel test, presented a very strong relationship between variations in the morphology and altitude (Table 7), which is consistent with variations in the phenotypic plasticity and genetic differentiation found in other species with a wide altitudinal distribution (Monty and Mahy 2009; Haider et al. 2011; Molina-Montenegro et al. 2011). DeWoody et al. (2015) indicated that factors such as altitude can be decisive in understanding the morphological and genetic differences, not so much due to geographical separation, but a mechanism of local adaptation to the particular climatic conditions where each population lives. The relationship between the genetic and morphological distances presented an average correlation (0.40), which shows a greater influence of the environment in terms of genetic variability on the variations observed in the morphology of the different populations, whereas the geographic distance between populations is the factor that has the strongest correlation with genetic diversity. With this relation, we can establish a model of isolation by distance between populations of *C. quitensis*.

The morphological differences found in the populations of *C. quitensis* may be related to the genetic differentiation found among populations. It is possible to infer that, generally, the degree of genetic differentiation found among populations implies that they can be considered as units with a certain evolutionary independence.

Conclusions and perspectives

This is the first study conducted on the relationship between morphological and genetic variability among different *C. quitensis* populations. The study and the results constitute an important contribution to the understanding of the relationship between morphological and genetic differentiation among populations, and sets guidelines for inquiry about the evolution of this species throughout its distribution, as well as the relationship that the different populations have with their habitats.

These results contribute to the understanding of the success of this species, which, along with *D. antarctica,* have adapted to extreme Antarctic conditions. We are currently inquiring about phylogeographic relationships among the different populations studied, and other new populations to investigate the presence of haplotypes, in order to analyse the genetic divergence between different geo-ecotypes (Wirtz et al. 2012), and to shed light on possible neutral genes that allow for evolutionary reconstruction (Poulin et al. 2014), and investigating possible patterns of postglacial colonization (Dewoody et al. 2015) for this species.

Acknowledgements This research was undertaken with financing from the Chilean Antarctic Institute through the INACH RT_03-09 and INACH RG_02-13 projects. We wish to thank the Chilean Antarctic Institute, the Chilean Air Force, Chilean Navy and Henryk Arctowski Polish Antarctic Station for the logistical support during the on-site activities. The authors would like to thank Dieter Piepenburg, Peter Convey, and the other two reviewers, for their thorough analysis that has led to a significant improvement in this work. Finally, we wish to thank the editors at International Journal Revisions for their contributions and comprehensive English language revision.

Compliance with ethical standards

Conflicts of interest The authors confirm no conflicts of interest.

References

Acuña-Rodríguez I, Osses R, Cortés-Vásquez J, Torres C, Molina-Montenegro MA (2014) Genetic diversity of *Colobanthus quitensis* across the Drake Passage. Plant Genet Resour 12:147–150. doi:10.1017/S1479262113000270

Alberdi M, Bravo LA, Gutierrez A, Gidekel M, Corcuera LJ (2002) Ecophysiology of Antarctic vascular plants. Physiol Plant 115:479–486. doi:10.1034/j.1399-3054.2002.1150401.x

Alexander JM, Edwards PJ (2010) Limits to the niche and range margins of alien species. Oikos 37:1377–1386. doi:10.1111/j.1600-0706.2009.17977.x

Allendorf FW, Hohenlohe PA, Luikart G (2010) Genomics and the future of conservation genetic. Nat Rev Genet 11:679–709. doi:10.1038/nrg2844

Androsiuk P, Chwedorzeswska K, Szandar K, Giełwanowska I (2015) Genetic variability of *Colobanthus quitensis* from King George Island (Antarctica). Pol Polar Res 36:281–295. doi:10.1515/popore-2015-0017

Archibald JK, Crawford DJ, Santos-Guerra A, Mort ME (2006) The utility of automated analysis of inter-simple sequence repeat (ISSR) loci for resolving relationships in the canary island species of tolpis. Am J Bot 93:1154–1162. doi:10.3732/ajb.93.8.1154

Bascuñán-Godoy L, Uribe E, Zúñiga-Feest A, Corcuera LJ, Bravo LA (2006) Low temperatura regulates sucrose-phosphate synthase activity in *Colobanthus quitensis* (Kunth) Bartl. By decreasing its sensitivity to Pi and increased activation by glucose-6-phosphate. Polar Biol 29:1011–1017. doi:10.1007/s00300-006-0144-3

Bascuñán-Godoy L, García-Plazaola J, Bravo LA, Corcuera LJ (2010) Leaf functional and micro-morphological photoprotective attributes in two ecotypes of *Colobanthus quitensis* from the Andes and Maritime Antarctic. Polar Biol 33:885–896. doi:10.1007/s00300-010-0765-4

Bascuñán-Godoy L, Sanhueza C, Cuba-Díaz M, Zúñiga GE, Corcuera LJ, Bravo LA (2012) Cold acclimation limits low temperature induced photoinhibition by promoting a higher photochemical quantum yield and a more effective PSII restoration in darkness in the Antarctic rather than the Andean ecotype of *Colobanthus*

quitensis Kunth Bartl (Cariophyllaceae). BMC Plant Biol 12:114. doi:10.1186/1471-2229-12-114

Beyer L, Bölter M, Seppelt RD (2000) Nutrient and thermal regime, microbial biomass and vegetation of Antarctic soils in the Windmill Islands Region of east Antarctica (Wilkes Land). Arct Antarct Alp Res 32:30–39. doi:10.2307/1552407

Bokhorst S, Huiskes AHL, Convey P, Sinclair BJ, Lebouvier M, Van de Vijver B, Wall DH (2011) Microclimate impacts of passive warming methods in Antarctica: implications for climate change studies. Polar Biol 34:1421–1435. doi:10.1007/s00300-011-0997-y

Bradshaw AD, Hardwick K (1989) Evolution and stress genotypic and phenotypic components. Biol J Linn Soc 37:137–155. doi:10.1111/j.1095-8312.1989.tb02099.x

Bravo LA, Saavedra-Mella FA, Vera F, Guerra A, Cavieres LA, Ivanov AL, Huner NP, Corcuera LJ (2007) Effect of cold acclimation on the photosynthetic performance of two ecotypes of *Colobanthus quitensis* (Kunth) Bartl. J Exp Bot 58:3581–3590

Carlquist S (1974) Island biology. Columbia University Press, New York. doi:10.5962/bhl.title.63768

Convey P (1996) Reproduction of Antarctic flowering plants. Antarct Sci 8:127–134. doi:10.1017/S0954102096000193

Convey P (2010) Terrestrial biodiversity in Antarctica: recent advances and future challenges. Polar Sci 4:135–147. doi:10.1016/j.polar.2010.03.003

Convey P (2011) Antarctic terrestrial biodiversity in a changing world. Polar Biol 34:1629–1641. doi:10.1007/s00300-011-1068-0

Convey P (2013) Antarctic ecosystems. Encyclopedia of biodiversity. Elsevier, San Diego. doi:10.1016/b978-0-12-384719-5.00264-1

Convey P, Gibson JAE, Hillenbrand CD, Hodgson DA, Pugh PJA, Smellie JL, Stevens ML (2008) Antarctic terrestrial life—challenging the history of the frozen continent? Biol Rev 83:103–117. doi:10.1111/j.1469-185x.2008.00034.x

Convey P, Bindschadler RA, Di Prisco G, Fahrbach E, Gutt J, Hodgson DA, Mayewski CP (2009) Antarctic climate change and the environment. Antarct Sci 21:541–563. doi:10.1017/s0954102009990642

Convey P, Hopkins DW, Roberts SJ, Tyler AN (2011) Global southern limit for flowering plants and moss peat accumulation. Polar Res 30:8929. doi:10.3402/polar.v30i0.8929

Convey P, Chown SL, Clarke A, Barnes DKA, Cummings V, Ducklow H, Frati F, Green TGA, Gordon S, Griffiths H, Howard-Williams C, Huiskes AHL, Laybourn-Parry J, Lyons B, McMinn A, Peck LS, Quesada A, Schiaparelli S, Wall D (2014) The spatial structure of Antarctic biodiversity. Ecol Monogr 84:203244. doi:10.1890/12-2216.1

Cordero C (2012) Caracterización y análisis de variabilidad moroflógica y genética en poblaciones de *Colobanthus quitensis* (KUNTH) Bartl. (Caryophylaceae) [Undergraduate thesis. Plant Biotechnology Eng.]. Universidad de Concepción, Chile

Cuba-Díaz M (2011) El clavelito antártico y los mecanismos que lo protegen del frio polar. Bol Antárt Chil 30:8–9

Cuba-Díaz M, Cid K, Navarrete A, Retamal C, Bravo LA (2011). Cold and photoperiod regulated expression of sucrose phosphate synthase (SPS) favors sucrose accumulation in *Colobanthus quitensis* during the Antarctic summer. In: Bravo LA, Leppe M (eds) VIII Reunión Chilena de Investigación Antártica Libro Resumen, pp 98–102. http://www.inach.cl/wp-content/uploads/2011/08. Accessed 30 Oct 2011

Cuba-Díaz M, Acuña D, Klagges M, Dollenz O, Cordero C (2013) *Colobanthus quitensis* de la Marisma, una nueva población para la colección genética de la especie. In: Leppe M, Molina-Montenegro M, González M, MacDonell S, Lavín P, Oses R, Gallardo J, Rivadeneira M, Arata J, Canales R (eds) Avances en Ciencia Antártica Latinoamericana. VII Congreso Latinoamericano de Ciencia Antártica, pp 436–439

Dewoody J, Trewin H, Taylor G (2015) Genetic and morphological differentiation in *Populus nigra* L.: isolation by colonization or isolation by adaptation? Mol Ecol 24:2641–2655. doi:10.1111/mec.13192

Excoffier L, Laval G, Schneider S (2005) Arlequin ver. 3.0: an integrated software package for population genetics data analysis. Evol Bioinform 1:47–50

Gianoli E, Zuñiga-Feest A, Reyes-Diaz M, Bravo LA, Corcuera LJ (2004) Ecotypic differentiation in morphology and cold resistance in populations of *Colobanthus quitensis* from the Andes of central Chile and the maritime Antarctic. Arct Antarct Alp Res 36:470–475. doi:10.1657/1523-0430(2004)036[0484:edimac]2.0.co;2

Haider S, Alexander J, Kueffer C (2011) Elevational distribution limits of non-native species: combining observational and experimental evidence. Plant Ecol Divers 4:363–371. doi:10.1080/17550874.2011.637973

Hamrick JL, Godt JW (1996) Effects of life history traits on genetic diversity in plant species. Philos Trans R Soc B 351:1291–1298. doi:10.1098/rstb.1996.0112

Hartl DL, Clark AG (1997) Principles of population genetics, 3rd edn. Sinauer Associates, Inc., Sunderland

Jelling AJ, Usher MB, Leech RM (1983) Variation in the chloroplast to cell area index in *Deschampsia antarctica* along a 16° latitudinal gradient. Brit Antarct Surv B 61:13–20

Kellman-Sopyla W, Giełwanowska I (2015) Germination capacity of five polar Caryophyllaceae and Poaceae species under different temperature conditions. Polar Biol 38:1753–1765. doi:10.1007/s00300-015-1740-x

Kimura M, Crow JF (1964) The number of alleles that can be maintained in a finite population. Genetics 49:725–738. doi:10.1016/0040-5809(71)90033-5

Klagges M, Cordero C, Cuba-Díaz M (2013) Las poblaciones de *Colobanthus quitensis* presentan diferenciaciones morfo-fisiológicas que podrían evidenciar la formación de ecotipo en su hábitat. In: Leppe M, Molina-Montenegro M, González M, MacDonell S, Lavín P, Oses R, Gallardo J, Rivadeneira M, Arata J, Canales R. eds. Avances en Ciencia Antártica Latinoamericana. VII Congreso Latinoamericano de Ciencia Antártica

Körner CH (2003) Alpine plant life. Functional plant ecology of high mountain ecosystems. Springer, Berlin

Kremer A, Kleinschmit J, Cottrell J, Cundall EP, Deans JD, Ducousso A, König AO, Lowe AJ, Munro RC, Petit RJ, Stephane BR (2002) Is there a correlation between chloroplastic and nuclear divergence, or what are the roles of history and selection on genetic diversity in European oaks? For Ecol Manag 156:75–87. doi:10.1016/s0378-1127(01)00635-1

Mantel N (1967) The detection of disease clustering and a generalized regression approach. Cancer Res 27:209–220

Manzur MI (2006) La diversidad genética, biodiversidad de Chile, patrimonio y desafíos. Comisión Nacional del Medio Ambiente de Chile

McGraw JB, Day TA (1997) Size and characteristics of a natural seed bank in Antarctic. Arct Antarct Alp Res 29:213–216. doi:10.2307/1552048

Miller MP (1997) Tools for population genetic analyses (TFPGA). Northern. Arizona University, Flagstaff. http://www.ccg.unam.mx/~vinuesa/tlem09/docs/TFPGADOC.PDF

Molina-Montenegro MA, Quiroz CL, Torres-Díaz C, Atala C (2011) Functional differences in response to drought in the invasive *Taraxacum officinale* from native and introduced alpine habitat ranges. Plant Ecol Div 4:37–44. doi:10.1080/17550874.2011.577459

Molina-Montenegro MA, Torres-Díaz C, Carrasco-Urra F, González-Silvestre LA, Gianoli E (2012) Phenotypic plasticity in two antarctic populations of *Colobanthus quitensis* (Caryophyllaceae) under a simulated global change scenario. Gayana Bot 69:152–160

Monty A, Mahy G (2009) Climal differentiation during invasion: *senecio inaequidens* (Asteraceae) along altitudinal gradients in Europe. Oecologia 159:305–315. doi:10.1007/s00442-008-1228-2

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

Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol Plant 15:473–497. doi:10.1111/j.1399-3054.1962.tb08052.x

Nei M (1972) Genetic distance between populations. Am Nat 106:283–292. doi:10.1086/282771

Nei M (1973) Analysis of gene diversity in subdivided populations. Proc Natl Acad Sci USA 70:3321–3323. doi:10.1073/pnas.70.12.3321

Nei M, Li WH (1979) Mathematical model for studying genetic variation in terms of restriction endonucleases. Proc Natl Acad Sci USA 76:5269–5273. doi:10.1073/pnas.76.10.5269

Parnikoza I, Kozeretska I, Kunakh V (2011) Vascular plants of the maritime Antarctic: origin and adaptation. Am J Plant Sci 2:381–395. doi:10.4236/ajps.2011.23044

Piña-Escutia JL, Vences-Contreras C, Gutiérrez-Martínez G, Vásquez-García M, Arzate-Fernández A (2010) Morphological and molecular characterization of nine botanical varieties of *Tigridia pavonia* (L.f) DC. Agrociencia 44:147–158

Poulin E, González-Wevar C, Díaz A, Gérard K, Hüne M (2014) Divergence between Antarctic and South American marine invertebrates: what molecular biology tells us about Scotia Arc geodynamics and the intensification of the Antarctic Circumpolar Current. Glob Plan Chang 123:392–399. doi:10.1016/j.gloplacha.2014.07.017

Romero M, Casanova A, Iturra G, Reyes A, Montenegro G, Alberdi M (1999) Leaf anatomy of *Deschampsia antarctica* (Poaceae) from the maritime Antarctic and its plastic response to changes in growth conditions. Rev Chil Hist Nat 72:411–425

Royer DL, McElwain JC, Adams JM, Wilf P (2008) Sensitivity of leaf size and shape to climate within *Acer rubrum* and *Quercus kelloggii*. New Phytol 179:808–817. doi:10.1111/j.1469-8137.2008.02496.x

Ruhland CT, Day TA (2001) Size and longevity of seed banks in Antarctica and the influence of ultraviolet-B radiation on survivorship, growth and pigment concentrations of *Colobanthus quitensis* seedlings. Environ Exp Bot 45:143–154. doi:10.1016/s0098-8472(00)00089-7

Ruiz E, Balboa K, Negritto M, Baeza C, Fuentes G, Briceño V (2010) Genetic and morphological variation and population structure in *Alstroemeria hookeri* subsp. *hookeri* (Alstroemeriaceae), endemic to Chile. Rev Chil Hist Nat 83:605–616. doi:10.4067/s0716-078x2010000400013

Salinas N, Armijos V, Jiménez P, Proaño K (2011) Caracterización y estudio de la diversidad genética del piñón (*Jatropha curcas*) mediantes el uso de marcadores moleculares. Ciencia 14:31–40

Sanhueza C, Vallejos V, Cavieres LA, Sáez P, Bravo LA, Corcuera LJ (2017) Growing temperature affects seed germination of the antarctic plant *Colobanthus quitensis* (Kunth) Bartl (Caryophyllaceae). Polar Biol 40:449–455. doi:10.1007/s00300-016-1972-4

Shannon CE, Weaver W (1949) The mathematical theory of communication. The University of Illinois Press, Urbana

Smith RIL (2003) The enigma of *Colobanthus quitensis* and *Deschampsia antartica.* In: Huiskes AHL, Gieskes WWC, Rozema J, Schoro RML, van der Vies SM, Wolff WJ (eds) Antarctic biology in a global context, pp 34–239

Sneath PHA, Sokal RR (1973) Numerical taxonomy: the principles and practice of numerical classification. W.H Freeman Company, San Francisco. doi:10.2307/2412767

Suma N, Srimathi P (2014) Influence of water flotation technique on seed and seedling quality characteristics of *Sesamum indicum*. IOSR J Agric Vet Sci 7:51–53. doi:10.9790/2380-07825153

Westergaard KB, Alsos IG, Popp M, Engelskjøn T, Flatberg KI, Brochmann C (2011) Glacial survival may matter after all: nunatak signatures in the rare European populations of two west-arctic species. Mol Ecol 20:376–393. doi:10.1111/j.1365-294x.2010.04928.x

Wirtz N, Printzen C, Thorsten Lumbsch H (2012) Using haplotype networks, estimation of gene flow and phenotypic characters to understand species delimitation in fungi of a predominantly Antarctic Usnea group (Ascomycota, Parmeliaceae). Org Divers Evol 12:17–37. doi:10.1007/s13127-011-0066-y

Xiong FS, Day TA (2001) Effect of solar ultraviolet-B radiation during springtime ozone depletion on photosynthesis and biomass production of antarctic vascular plants. Plant Physiol 125:738–751. doi:10.1104/pp.125.2.738

Yeh FC, Yang RC, Boyle T (1999) PopGene Microsoft windows-based freeware for population genetic analysis. University of Alberta, Edmonton. http://www.ualberta.ca/fyeh/popgene.download.html. Accessed 12 Jan 2014

Zúñiga-Feest A, Bascuñán-Godoy L, Reyes-Díaz M, Bravo LA, Corcuera LJ (2009) Is survival after ice encasement related with sugar distribution in organs of the Antarctic plants *Deschampsia antarctica* Desv. (Poaceae) and *Colobanthus quitensis* (Kunth) Bartl. (Caryophyllaceae). Polar Biol 32:583–591. doi:10.1007/s00300-008-0553-6