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Determination of stomatic density, index, and area as exposition biomarkers of pollution in *Deschampsia antárctica* Desv. (*Poaceae*)

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Abstract

Until not so long ago, Antarctica was considered to be a polar region practically pristine. The Antarctic Peninsula has the highest concentration of scientific stations from different countries. Anthropogenic activity has caused alterations in the Antarctic ecosystems directly affecting terrestrial vegetation. This fact requires the finding of biomarkers in native plants to estimate the effects of human impact. *Deschampsia antarctica* Desv. (Poaceae) is the unique native grass described so far for Antarctica and was used for multiple investigations. In this study, plants were collected on Carlini scientific station, 25 de Mayo (King George) Island, Potter Peninsula, South Shetland Islands. Thus, the main objective planned consists of the evaluation of leaf stomata-related parameters as pollution biomarkers. The results of the stomatic index (SI), density (SD), and area (SA) were shown at sites with different levels of human impact (close and far away from the scientific station). It was found that the correlation between SD and SI, on the adaxial side of the leaves, resulted in a good biomarker for estimating the degree of anthropogenic impact in each studied area.

Graphical abstract

Keywords *Deschampsia antarctica* · Stomata · Biomarker's pollution · Antarctica

Highlights

- *Deschampsia antarctica* leaves reflect anthropogenic impact.
- No differences in the structure or size of the stomatal pores on either side of the leaves were found
- Correlation between SD and SI on the adaxial side of the leaves could be a good biomarker.

Introduction

The Antarctic flora consists of mosses, lichens, and only two vascular plants: *Colobanthus quitensis* (Kunth) Bartl and *Deschampsia antarctica* due to the harsh climatic conditions in the area (Parnikoza et al., 2011).

If only the two vascular plant species are considered, *Deschampsia antarctica* Desv. (Poaceae) is the only native

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grass described so far for Antarctica, with a distribution mainly centered on the Antarctic Peninsula (Komárková et al., 1990; Convey, 1996; Montiel et al., 1999; Barcikowski et al., 2001, 2003; Bravo et al., 2001; Chwedorzewska et al., 2008; Vera, 2011; Casanova-Katny and Cavieres, 2012). This species is abundant in ice-free zones, areas that in general also coincide with the scientific stations ´ settlements, both permanent and temporary, where diverse foci of pollutionas solid waste, organics and non-organic pollutants, oil spills, traffic (foot or mechanized), trampling, aerosols, tourism, were among the most well established and known Antarctic anthropogenic impacts (Mishraa et al., (2004); Tin et al. 2009; Molina-Montenegro et al., (2019); Gao et al. 2021).

D. antarctica has been the subject of several studies on eco-physiological and genetic analyses, association with endophytes, pharmaceutical formulations, and climate change bioindicators (Bennett et al., 1982; Lewis Smith, 1994; Alberdi et al., 2004; Rosa et al., 2009; Parnikoza et al., 2011; Navrotska et al., 2014; Domaciuk et al., 2016; Gonzalez et al., 2016; Köhler et al., 2017; Malvicini et al., 2018; Zamarrón et al., 2019).

The effects or early signs of exposure to the contaminants can be estimated via biomarkers, which can be used for risk assessment from the molecular level down to the level of populations and communities (Ernst and Peterson, 1994; Sandermann HJr (2000); Pastor et al., 2003; Ferrat et al., 2003; Ratola et al., 2014; Mena Torres et al., 2017). Moreover, exposure to xenobiotics was reported to produce direct, measurable, and quantifiable effects on plants (Ellis, 1979; Meister and Bolhàr Nordenkampf, 2003). The responses of plants to stress are reflected in morphological variations and show insufficient adaptation to changing environmental conditions. Generally, these responses can be caused by natural processes: volcanic eruptions, floods, salt dispersion (Oosting, 1945; Collins, 1969; Grimoldi et al., 1998; Hotes et al., 2004; Pardos, 2004; Dale et al., 2005; Jiménez et al., 2013; Sakagami et al., 2020; Shao et al., 2020) or by anthropogenic activities (Bacci and Gaggi, 1987; Sandermann, 1992; García et al., 2006; Collins et al., 2011; Burden et al., 2020).

Leaves are the plant organs that best reflect the changes in environmental conditions (Mooney et al., 1991; Pedrol et al., 2000; Abbruzzese et al., 2009; Huang et al., 2011; Lázaro Nogal et al., 2015; Jumrani et al., 2017; Idris et al., 2018). In the presence of adverse conditions, most plants commonly respond with premature loss of leaves or modification of their color, which usually varies from pale green to yellow (chlorosis) or from yellowish to brown (necrosis) (Ernst WHO (2003)). Alterations in leaf morphology and leaf structures are reliable as stress' biomarkers (Pastor et al. 2003; Dimitrova and Yurukova 2005; Kardel et al. 2010; Komolafe et al. 2015; Idaszkin et al. 2019). Stomata are often used to detect the effects of environmental variations (Salas et al., 2001; Bruno et al., 2007; Rivera et al., 2013; Ganem et al., 2014; Naizaque et al., 2014).

Stomata are essential for the homeostasis of plants, and their number can vary among plant species (Evert, 2006; Ernst WHO (2003)). The opening and closing of stomata is primarily a mechanical function that depends on environmental factors and O2 requirements of the leaf (Meidner and Mansfield 1965; Lösh and Tenhunen 1981; Feller 2006; Lawson et al. 2014; Woolfenden et al. 2018). Stomatal development on the other hand, is genetically regulated and differ from species to species (Willmer, Fricker (1996); Bergmann et al., 2004; Casson and Gray, 2008; Casson and Hetherington, 2010; Araújo et al., 2011; Doheny-Adams et al., 2012; Zoulias et al., 2018; Wu et al., 2019).

Biotic parameters such as plant age, and abiotic parameters such as drought, UV-B radiation, presence of metals, or excess potassium, can modify the stomatic density SD (Ernst WHO (2003)). Several studies have described and characterized the stomata of *D. antarctica* (Romero et al., 1999; Barcikowski et al., 2003; Alberdi et al., 2004; Giełwanowska et al., 2005; Parnikoza et al., 2007).

The present work aimed to determine and evaluate the stomatal index (SI), their density (SD), and area (SA) as biomarkers of exposure to pollution in leaves of *D. antarctica*.

Material and methods

Plants of *Deschampsia antarctica* were collected during the southern summer of 2017 in and around the Argentine Carlini Research Station, Potter Peninsula, 25 de Mayo Island (King George), South Shetlands, Antarctica (Fig. 1A, B). For each sampled site, 15 individuals from 7 tussocks (with a small amount of substrate) were randomly chosen. The sampled sites were located in zones of high and low anthropogenic impact. Areas of high impact were the Supply Area (loading and unloading of fuel and supplies) (Fig. 1C), the lateral area adjacent to the Electric Power Station (Fig. 1D), and the area of Fuel Tanks (Fig. 1E). The sampling site with low impact was Peñón VII (62°15′9.82 "S, 58°40′23.05 "W) which is within an Antarctic Specially Protected Area (ASPA 132) an area considered free of anthropogenic activity (Fig. 1F). At the Argentinean laboratory of the Carlini Station, 2 leaves from each plant were taken and stained with Feulgen (Dopchiz and Poggio, 1999). To analyse the stomata structures, we photographed the leaves on both sides, using an Olympus® BX53 photomicroscope with a blue filter. In addition, we observed and classified the coloration of the leaves.

The other collected plants were transported to the Antarctic Institute laboratories (Buenos Aires, Argentina), in

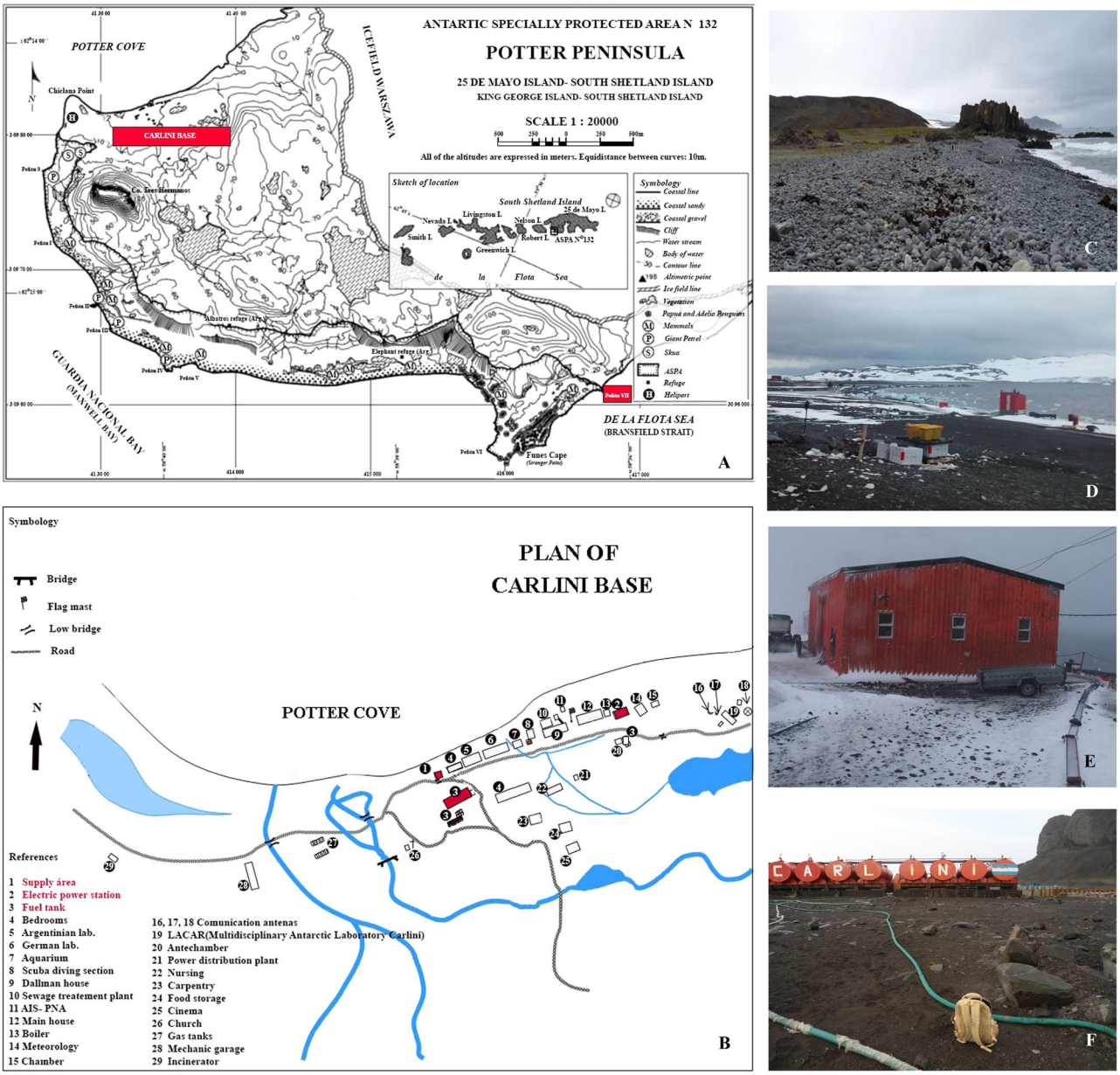

Fig. 1 Sampling sites at Potter Peninsula, 25 de Mayo Island (King George Island), South Shetland Islands, Antarctica. **A** Carlini Station.and Peñón VII (pristine zone) are highlighted in red **B** Location of the Carlini´s buildings. Locations of sampling sites are highlighted in red. **C** Peñón VII area. **D** Supply area. **E** Electric power station area. **F** Fuel tanks area

small containers to preserve the natural conditions of environmental humidity and environmental temperature. In the laboratories, a cold chamber to maintain the temperature conditions, with irrigation and photoperiod suitable for each time of the year, was conditioned.

Two fully developed leaves (the 2nd and 3rd leaves) with less than 10 cm in length were selected from each plant. To estimate stomatal density (SD) and stomatal index (SI), impressions were taken from both leaf sides with transparent adhesive tape. Then, from each leaf, 25 fields *per* side were randomly and systematically analyzed. Stomata counting was performed on a Motic® BA310R optical microscope, using the 40X objective lens corresponding to a leaf area of 0.188 mm^2. Stomatal area (SA) was measured from photomicrographs taken with a Leica® DM 2500 photomicroscope. Fifty stoma pores were measured *per* leaf and, in each of them, the major axis (MA) and minor axis (ma) were determined. The ImageJ 1.51 k software (Rasband, 2017) was used to take the measurements. The SD was calculated as the number of stomata *per* leaf area.

The stomatal index (SI) was estimated, *per* leaf, as SI = (NS/ (NS + EC)) x 100, where NS = number of stomata and EC = number of other epidermal cells. The stomatal area (SA) was estimated as the ratio between MA and

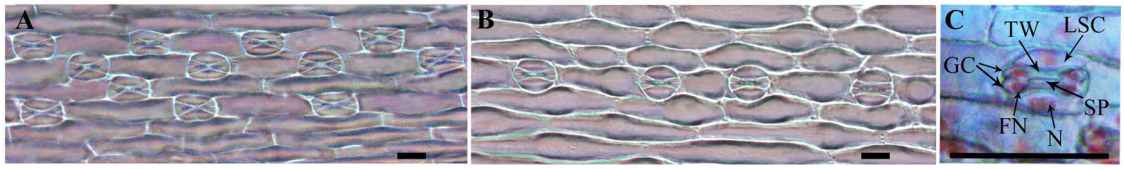

Fig. 2 The leaf of Deschampsia antarctica. General view: **A** Abaxial side. Section of the side with stomata. **B** Adaxial side. Face showing a lower number of stomata. **C** Detail of paracytic stoma. GC guard cells, LSC lateral subsidiary cell, N nucleus of the lateral subsidiary cell, FN filiform nucleus, SP stoma pore, TW thickened wall. Scale bar: **A** and **B** 20 µm, **C** 50 µm

Table 1 *Deschampsia antarctica:* stomatal density (SD) and stomatal index (SI) for each sampled site

Sampled area	SD (*per* 0.188 mm^2)		SI (%)		SA	
	AB	AD	AB	AD	AB	AD
Peñón VII (G)	57.88 ± 21.71 a(7264)	16.05 ± 8.79(1572)	16.34 ± 4.48	2.53 ± 1.76 a	0.08 ± 0.01	0.06 ± 0.02
Electric Power Station (Y)	60.35 ± 20.20 ab(6627)	20.20 ± 12.09(1391)	17.25 ± 3.82	1.51 ± 1.15 b	0.06 ± 0.01	0.09 ± 0.02
Supply Area (Lg)	66.50 ± 25.59 a(6750)	16.26 ± 10.87(1357)	18.25 ± 4.5	2.66 ± 1.75 a	0.05 ± 0.01	0.07 ± 0.01
Fuel Tanks (Y)	81.94 ± 27.82 b(4715)	11.62 ± 8.32(605)	18.77 ± 5.21	1.49 ± 0.85 b	0.06 ± 0.003	0.06 ± 0.01

Values are expressed as means ± standard error. The number of replicates (plants) per site is $n = 7$. The total numbers of observations are between brackets. Different letters means significate differences ($p < 0.05$)

AB abaxial side, *AD* adaxial side

ma (SA = MA/ma). Comparisons were made between the SD means of both faces among the four sites. The SI of the abaxial and adaxial faces was also calculated. For each leaf face, the SD, SI, and SA variables were correlated considering each sampling site.

Statistical analysis The results are presented as means ± standard error (SE). The differences in SD and SI between the faces of the leaf, and among the studied sites were performed using the Infostat program (Di Rienzo et al., 2020). Statistical significance was analysed by one-way analysis of variance (ANOVA) and Scheffe's method at 5% significance to compare differences between means (Sokal and Rohlf 1995). To assess the relationship between SD and SI in each face of the leaf, we carried out Pearson correlation tests by sampling site.

Results

The leaves of *Deschampsia antarctica* were found to be amphistomatic. The stomatal complex was paracytic (Prabhakar, 2004; Evert, 2006). The stoma was formed by a pair of guard cells with bulbous ends and filiform nuclei. The subsidiaries cells were shaped like a dome (Fig. 2). At each sampled site, no differences were found in the structure or size of the stomatal pores on either side of the leaves, nor were there particles observed obstructing the stomatal pore.

The ANOVA of SD showed significant differences among the sites studied for the abaxial side ($F = 4.896$, $p < 0.05$) but not for the adaxial side ($F = 2.612$, $p > 0.05$). On the other hand, the ANOVA of SI showed no significant differences for the abaxial side ($F = 1.519$, $p > 0.05$) but significant differences for the adaxial side ($F = 3.38$, $p < 0.05$). Mean SD of the abaxial side from leaves collected at Fuel tanks was significantly higher than at the Supply Area and Peñón VII, whereas no significant differences were detected between mean SD at the Fuel Tanks and Electric Power Station (Table 1). The adaxial side SD values as well as the both leaf sides SI and SA mean values did not show significant differences ($p > 0.05$) (Table 1). There were consistent positive correlations between SD and SI of the abaxial side at all sampling sites (Table 2). Correlations between SD and SI of the adaxial side were heterogenous among sampling sites; there was a strong positive correlation at the Peñón VII, moderate positive at the Supply Area, and very weak at both the Fuel Tanks and the Electric Power Station.

Green leaf cushions were observed at Peñón VII, while the cushions at all other sites studied were light green to yellowish (Table 1).

Discussion

Deschampsia antarctica leaves have paracytic stomata, a structure that is common in Poaceae (Abid et al., 2007; López and Devesa, 1991; Sanchez Anta et al., 1988; Dahlgren et al., 1985; Finot et al., 2006; Zarinkamar, 2006). Previous works on this species have shown that the leaves

Table 2 Pearson's correlation coefficients between the variables: Stomatic Density (SD) and Stomatic Index (SI) for each sampling site

	Peñón 7	Supply Area	Electric Power Station	Fuel Tanks
DE ab/ IE ab	$r = 0.7$, $p < 0.0001$	$r = 0.8$, $p < 0001$	$r = 0.7$, $p < 0.0001$	$r = 0.8$, $p < 0.0001$
DE ad/ IE ad	$r = 0.9$, $p < 0.0001$	$r = 0.5$, $p < 0.0043$	$r = 0.4$, $p < 0.001$	$r = 0.08$, $p < 0.00$

Consider the variables: Stomatal Density (SD) and Stomatal Index (SI) for each site. *ab* abaxial side, *ad* adaxial side. Only significant results were reported

of *D. antarctica* are susceptible to factors such as snow cover, soil type, and increased temperature (Gielwanowska, Szczuka (2005); Lewis Smith, 1994; Nuzhyna et al. 2019, 2021; Parnikoza et al., 2007, 2011; Romero et al., 1999). In all the studied sites, our observations showed that the stomata were distributed on both sides of the leaves (amphystomatic condition), with high numbers on the adaxial side as were observed by Romero et al. (1999) and Giełwanowska et al. (2005) in plants from different sites of the Antarctic Peninsula. However, in some sampled areas, plants showed leaves with stomata only present on the adaxial side (Barcikowski et al., 2003).

The results obtained in the Peñón VII plants (Table 2) differ from those observed by studies developed in other areas of the Antarctic Peninsula, for example, on Robert Island the SD number of the adaxial side was: 170.10 (number of stomata *per* mm^2) while in the abaxial side was: 382.50 (number of stomata per mm^2) (Alberdi et al. 2004); in the vicinity of the Polish Station Arctowski (Admiralty Bay, 25 de Mayo Island), the SD in the adaxial was: 5.56 (number of stomas per mm^2) (Barcikowski et al. 2003). These differences could be since populations and subpopulations are found in the Antarctic land, a particular scenery that has small slopes that can "*play a special role in the creation of microclimates*" (Parnikoza et al., 2011) giving rise to microhabitats (Chwedorzewska et al., 2008). In these microhabitats, leaves develop adaptations such as variation in epidermal cell size, cuticle thickness, or the number of stomata (Ruhland and Day, 2000; Barcikowski et al. 2001; Giełwanowska et al. 2005, Park et al. 2013). Therefore, it was considered that SD values should only be used as a reference for the studied sites. Morales Rodríguez et al. (2016) observed that the decrease in SD was related to the expansion of the leaf during its aging. Because Peñón VII is an area free of anthropogenic impact, leaves can age and expand showing a low SD. The plants located in the Carlini Station area were exposed to different types of stress (fuel, vapors, trampling) and, as a form of protection, their leaves were less expanded (Cornejo Toledo 2019, Giełwanowska et al. 2005).

Our results showed a clear trend towards an increase in SD on the abaxial side of the leaves from Peñon VII (pristine area) to fuel tanks (most impacted area) (Table 1). Increased stomata could lead to a loss of leaf protection by rendering leaves more susceptible to disease due to the pathogens´ entry as has been reported for *Solanum tuberosum* L. (Morales Rodríguez et al., 2016), *Datura innoxia* Mill, *Ligustrum lucidum* Aiton f. and *Quercus ilex* L. (Husen and Iqbal, 1999; Bruno et al., 2007; Fusaro et al., 2015). The morphological differences observed among the plant leaves from the different sites, would not be of genetic origin, since the populations and subpopulations had a low rate of genetic differentiation and were distinguished using specific molecular markers (Holderegger et al., 2003; Chwedorzewska et al., 2004, 2008; Rabokon et al., 2019). In summary, the variation in SD among the impacted sites would be a plastic response to stress conditions (Alberdi et al., 2004; Giełwanowska et al., 2005).

The increase in SD recorded in the plant leaves collected at the Supply Area was due to the effect of continuous trampling through periods of unloading food and fuel, during the summer months, a fact that occurred every year since the establishment of the Carlini (Jubany) Scientific Station in 1953. This effect was similar to that observed by Lewis Smith (1988) on Signy Island (South Orkney) in response to trampling by a natural biological agent, the Sea Lion *Arctocephalus gazella*. Moreover, Jägerbrand and Alatalo (2015) reported that trampling, even with low frequency, produces alterations in the ecosystem. In the present study, as a result of the trampling, the vegetation cover has disappeared in the supply area. Besides, the effect of the melting snow further erodes this transit area. Exposure to anthropogenic activity produced effects on plants that are reflected in altered SD and SI values, which depend on leaf age, plant type, and species, also (Pourkhabbaz et al., 2010; Kardel et al., 2010). Occasional fuel spills, which may occur mainly when the Scientific Station is restocked, would be directly responsible for the damage caused on the leaves. Therefore, an increase in SD, chlorosis and the formation of small mats was observed in the leaves, but without epidermal rupture or reduction in the size of the stomata, as was observed in other species such as *Sorghum bicolor* L. (Komolafe et al., 2015).

We did not find significant differences in SI among all the sampled sites so it would not be a good biomarker per se (Table 1). This parameter would be influenced by the incidence of sunlight during leaf development (Schoch et al., 1980) and, therefore, was not affected by anthropogenic impact. Although leaf size was not measured, the high positive correlation between SD and SI ($r = 0.92$, $p < 0.00$) recorded in Peñón VII, indicated that leaves were large in that area. Then, the decrease in correlation at the

impacted sites would be associated with a decrease in leaf size which would provide an adaptive advantage in *D. antarctica* by protecting its leaves from non-specific cell damage (Ferriol et al., 2004).

The present work showed that the correlation between SD and SI on the adaxial side of the leaves could be a good biomarker for the anthropogenic impact estimation. It was important to note that stomata did not experience morphological modifications in the studied sites of the present work, a fact that was, also, observed in other sites of the Antarctic Peninsula (Romero et al. 1999; Alberdi et al. 2004; Giełwanowska et al. 2005; Parnikoza et al. 2007; Nuzhyna et al. 2019). Thus, the developed methodology appeared good for applying wherever anthropogenically impacted areas.

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Compliance with ethical standards

Conflict of interest The authors declare no competing interests.

Ethics approval All applicable international, national, and institutional guidelines for sampling, care and experimental use of plants for the study were followed as established by the Article III, Annex II of the Madrid Protocol, Law 24.216 (Taking, Harmful Intrusion and Introduction of Species) within the framework of the projects evaluated by the Environment Office of the IAA and Dirección Nacional del Antártico (DNA).

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