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Anatomical variations of *Deschampsia antarctica* É. Desv. plants from distant Antarctic regions, *in vitro* culture, and in relations to *Deschampsia caespitosa* (L.) P. Beauv.

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Abstract: This paper presents a comparative study on the anatomy of the Antarctic hairgrass (*Deschampsia antarctica* É. Desv.) from natural populations of two distant maritime Antarctic regions: the Argentine Islands (Antarctic Peninsula region) and the Point Thomas oasis (King George Island, South Shetland Islands). Comparison of *D. antarctica* plants from natural populations of Argentine Islands region and plants originated from seeds of these populations cultivated *in vitro* also was made. Additionally anatomical features of *Deschampsia antarctica* were compared with ones for *D. caespitosa*. The results of our study do not provide enough evidence to assert more pronounced xerophytic anatomical features in *D. antarctica* plants from more harsh conditions of Argentine Islands region. Such features (both qualitative and quantitative) of *D. antarctica* mainly depend on local conditions, and not on the latitudinal or climatic gradient. In both regions it is possible to find individuals that represent different ecotypes which are adopted to open arid or more humid habitats. It has been shown that Antarctic hairgrass plants germinated from seeds and cultivated *in vitro* retain the qualitative anatomy features that are typical to plants from the initial natural populations. This is especially noticeable in the case of plants from Berthelot Island (BE1 study plots), which might indicate a genetic fixation and a manifested differentiation similar to DNA haplotypes or chromosomal forms. However, quantitative characteristics, in particular the epidermis parameters, are subject to changes due to the transfer to more favourable conditions. Also qualitative and quantitative difference of *D. antarctica* in contrast with *D. caespitosa* have been described. These differences could be useful for identifying these two species. Additionally the quantitative differences (such as the area of the epidermal cells and the

number and size of stomata on the adaxial surface) of Alaskan *D. caespitosa* grown from seeds were detected in contrast to the naturally grown plants of the same species from Ushuaia.

Key words: Antarctic, *Deschampsia antarctica*, leaf anatomy, *Deschampsia caespitosa*.

Introduction

The genus *Deschampsia* is particularly interesting due to adaptive species comprising this taxon. It includes a widespread polymorphic species – *Deschampsia caespitosa* (L.) P. Beauv. with a lot of forms and subspecies (http://www.theplantlist.org/tpl1.1/record/kew-407579), as well as one of the only two species of flowering plants yet found in Antarctica – *Deschampsia antarctica* É. Desv. for that only some ecotypes were described before (http://www.theplantlist.org/tpl1.1/record/kew-407539; Giełwanowska *et al*. 2005). *D. antarctica* can survive in the most extreme region on the Earth. It is assumed that *D. caespitosa* may also represent extremophiles. It was noted that this grass could survive on the plains of European continent during the Pleistocene glaciations (Szafer and Zarzycki, 1977). The precise evolutional and ecological reasons for presence of *D. antarctica* and the other flowering plant of the Antarctic – *Colobanthus quitensis* (Kunth) Bartl. are still poorly studied (Parnikoza *et al*. 2011a).

Recently a series of experimental works and reviews devoted to the adaptation of the flowering plants, especially anatomy features of the Antarctic species were published (see Chwedorzewska *et al*. 2008; Parnikoza *et al*. 2011a). In particular, the leaf anatomy together with the ultrastructure of leaf cell of *D. antarctica* got major attention. The researchers had pointed out fundamental xerophilic features and distinct characteristics of plants of different ecotypes. However, many of the above-mentioned research attempts mostly concerned *D. antarctica* in the area of South Shetland Islands of the maritime Antarctic (Giełwanowska 2005; Giełwanowska *et al*. 2005; Chwedorzewska *et al*. 2008). The molecular-genetic and cytogenetic studies published by Volkov *et al*. (2010), Navrotska *et al*. (2018) and Rabokon *et al*. (2019) suggest the existence of some variety of *D. antarctica* genotypes closer to the edge of the areal, which can happen in the process of the speciation. For *Arabidopsis thaliana* (L.) Heynh it was demonstrated that epigenetics contribute substantially to variation in plant growth, morphology, and plasticity, especially under stress conditions (Kooke *et al*. 2015). Part of the adaptation can be explained by the preexisting genetic variation in the populations. Recent studies have shown that new stable phenotypes can be generated through epigenetic modifications in few generations, contributing to adaptation (Thiebaut *et al*. 2019). Herrera and Bazaga (2010) shown that

adaptive specific of the *Viola cazorlensis* Gand. plants from different populations caused both genetic and epigenetic variation. Analysis in three allo-tetraploid sibling orchid species, which differ radically in their geographic and ecological context, revealed that ecological divergence of *Dactylorhiza* species is mostly due the epigenetic factors regulating gene expression in response to environmental stimulus (Paun *et al*. 2010). DNA methylation polymorphisms exist within and between *Laguncularia racemosa* (L.) C. F. Gaertn. natural populations (Lira-Medeiros *et al*. 2010) and it was a subject of studies done also for two different *D. antarctica* populations from King George Island (Chwedorzewska and Bednarek 2011). This variation can result in environmentally-induced phenotypic plasticity, which may be trans-generationally inherited. In our case, the anatomical characteristics of *D. antarctica* more close to southern range limits, such as the Argentine Islands region, might differ from the other regions of the Antarctic, not only by genetic variation, but also by their respective habitats, which promote phenotypic variation. In view of the recently published results which demonstrate a significant variation in terms of plant composition and soil conditions between the regions of South Shetland Islands and Argentine Islands (Parnikoza *et al*. 2011b; 2017), it would be essential to perform a comparison of Argentine Islands region plants with those from the King George Island, which are described as having milder environmental conditions.

Previously, to assess the variation of the anatomy of leaf blade, *D. antarctica* clones from the South Shetland Islands region were cultivated at different temperatures in the laboratory. As a result, strong variation was found in the anatomical characteristics of the leaf surface and in the leaf cross section, between plants growing in the field and plants *in vitro* growing at the higher temperature in the laboratory (Romero *et al*. 1999; Giełwanowska *et al*. 2005; Chwedorzewska *et al*. 2008). Romero *et al*. (1999) also showed more pronounced xerophytic features of leaves collected from plants growing in natural conditions compared with plants cultivated at 13°C. There is no other study on plants from more severe conditions than Argentine Islands region, as well as plants cultured *in vitro* from seeds of this region.

The principal differences of leaf anatomy of *D. antarctica* from other species of genus, especially from the most widespread and successful representative of this genus – *D. caespitosa*, require further and more detailed research.

The aim of this study was to identify the variability of anatomical characteristics of the *D. antarctica* according to the geographical region of distribution, as well as their variability due to transfer from natural populations conditions to the culture *in vitro* condition. Additionally, we aim to compare leaf anatomy of *D. antarctica* and *D. caespitosa*.

Materials and methods

Plant material. — For this study, six specimens of *Deschampsia antarctica* were collected from each three study plots that represent different ecological zones of Point Thomas oasis, King George Island, during the participation of Ukrainian scientist in Polish Antarctic Expedition in 2006 (Kozeretska *et al*. 2010), and six specimens from each three study plots of the distant Argentine Islands region (during 21-th Ukrainian Antarctic Expedition in 2017). Additionally, some morphometric data collected from Argentine Islands region in seasons 2010, 2014–2016 were used. As well, the *D. antarctica* cultivars, propagated *in vitro* from seeds of populations D4, D12 study plots from Galindez Island, and BE1 study plot from Berthelot Island (Argentine Islands region), were also studied (Table 1). Totally, we used six plants from each (D4, D12 and BE1) *in vitro* cultivars. Plants were grown on the B5 medium prepared manually in our lab (see Navrotska *et al*. 2018) in the light room under illumination of about 6500 lux, temperature of 18–20ºC, and humidity of 80%. For the experiment, each plant of the 70–75th passage was cloned into 3–5 plants and grown on the same medium for a month. *D. caespitosa* plant from the natural environment used in this study, we have collected in Ushuaia (marked as *D. caespitosa* Ush). We also used *D. caespitosa* exemplar grown from seeds collected in Alaska in July 2013 (marked as *D. caespitosa* Al) (see Table 1).

Morphometry and light microscopy. — For plants from nature populations the plant height (from base of plant to the inflorescence top), leaves length and flower count was prepared. For plants in culture, we measured only leave length.

For anatomical assessment, the middle part of the leaf blade (six plants, two leaves per plant from each site of the study) was taken. The material was fixated in FAA (formalin, acetate, and aldehyde) and poured over with gelatin using standard procedure (Romeis 1948). Cross-sections with a thickness of 10 microns were cut *via* a cryomicrotome and dyed with safranin. Additionally, the leaves were macerated for a detailed study of adaxial and abaxial epidermis. Images were captured *via* the Olympus System Microscope ModelBX41 and visualized in ImageJ software with subsequent measurements. The cellular area of the epidermis of the leaf blade was measured in between the veins, taking into account only the main cells of the elongated shape.

Statistical data were processed in Prism Graphpad 6 software. The variation was assessed *via* a multivariate analysis of variance (ANOVA) method with Tukey's adjustment. Photographic images were taken with the Olympus C5050 Zoom Digital Camera.

Table 1

Collection sites of *D. antarctica* and *D. caespitosa*.

Study plot, Species name	Collection site, coordinates	Short description
D4, *D. antarctica*	Galindez Island, Argentine Islands, maritime Antarctic, 65.248240°S, 64.237920°W	Penguin Point, coastal gull rock – Ship Rock, open exposed habitat, in zone of limited guano input from *Larus dominicanus* activity. Total vegetation cover (TVC) 1%: *D. antarctica* 1%, *Sanionia sp.* <1%, *Prasiola crispa* (Lightfoot) Kützing <1%, limpet shells deposits.
D12, *D. antarctica*	Galindez Island, Argentine Islands, maritime Antarctic, 65.247419°S, 64.252603°W	Stella Point, coastal gull rock – Gull Tower, combination of open habitat and places protected by stones. TVC 5–20%: *D. antarctica* 1–10%, bryophytes 4–10%, gravel and limpet shells deposits.
BE1, *D. antarctica*	Berthelot Island, Argentine Islands region, maritime Antarctic, 65.324000°S, 64.140600°W	Rock terrace on the northern side of the Berthelot Island, TVC 90%, *D. antarctica* 1%, *Polytrichum strictum* Menzies ex Brid., 44–80%, *Warnstorfia fontinaliopsis* (Müll.Hal.) Ochyra – 9–45%, Terrace near melting stream.
KG027, *D. antarctica*	Point Thomas oasis, King George Island, South Shetland Islands, maritime Antarctic 62.162467°S, 58.471117°W	North-eastern exposition with a slope inclination 5–10º, cut through a glacial stream. TVC 100%: *D antarctica*. – 50%; *C. quitensis* – 10%; bryophytes 40%
KG030, *D. antarctica*	Point Thomas oasis, King George Island, South Shetland Islands, maritime Antarctic 62.172480°S, 58.517993°W	Italian Valley, at the foot of the hill slope inclination 50º, northern exposition. Skuas' nests on top of the rock. TVC 100%: *D. antarctica* – 70%; *C. quitensis* – 28%; bryophytes – 2%.
KG016, *D. antarctica*	Point Thomas oasis, King George Island, South Shetland Islands, maritime Antarctic 62.169350°S, 58.464883°W	Zone of glacier retreat (in 1979 was under glacier), currently the north-eastern edge of Ecology Glacier (1–2 m asl., relief plain. TVC 56%: *D. antarctica* – 3%; *C. quitensis* – 3%, bryophytes – 50%.
D. caespitosa Ush, *D. caespitosa*	Ushuaia city, Terra del Fuego, Argentina, 54.819132°S, 68.320253°W	Plot in Ushuaia city near Bahia Encentrada, area impacted by peoples – dry grassland with patch of *D. caespitosa*, TVC 80%: *D. caespitosa* 20%, other plants – 60%
D. caespitosa Al, *D. caespitosa*	Dalton Highway, Alaska, USA, 65.634750°N, 149.034000°W	Plot on the edge of Dalton Highway on gravel, spruce forest (taiga) zone. TVC 90%: *D. caespitosa* 20%, other plants – 70%

Results

The leaf anatomy of *D. antarctica* plants from Point Thomas oasis and the Argentine Islands region. — *D. antarctica* growing in two distinct sites showed clear differences in particular morphological features. Plants from the investigated localities of the southern region of the Argentine Islands showed higher values in almost all studied morphometric parameters (Table 2). At the same time, variation in qualitative parameters of *D. antarctica* specimens from both studied regions of Antarctic was not considerable. Almost all plants (with the exception of KG030) were characterized by having an amphistomatous leaf. The leaf was covered with a single-layer epidermis with a thick cuticle (Fig. 1a).

Table 2

Morphometric parameters of the *Deschampsia antarctica* from natural population's plots (m ± SD, n = 20).

Study plot	Study date	Plant height, cm	Leaf length, cm	Flower count in an inflorescence
BE1	01.2010	5,6±0.4	7,8±0.9	25,9±5,1
D4	02.2014	3,9±0,7	3,0±0,9	12,3±8,2
D12	02.2014	5,5±1,3	4,1±1,5	17,6±6,6
D4	02.2015	4,3±0,5	3,6±0,9	26,7±7,4
D12	02.2015	–	2,5±0,6	–
D4	02.2016	3,7±0,7	2,4±0,7	23,8±13
D12	02.2016	4,6±1,2	4,1±1,5	24,6±13,7
KG027	12.2005	2,8±0.4	1,2±0.2	9.4±2.3
KG016	01.2006	2.7±0.15	2.0±0.09	13.3±0.30
KG030	01.2006	2.6±0.14	1.7±0.10	14.6±1.86

From the adaxial side, the main epidermis cells had elongated projections and straight lines. From the abaxial surface the cells had elongated projections and wavy contours (Fig. 1c). The epidermal cells of the leaves on the abaxial side of the plants from both studied regions were similar in cross-section: a rounded form with a thickened lignified outer cell wall. From the adaxial side, the epidermal cells were covered with a thinner cuticle, having predominantly an oval shape. There were square-shaped cells above the vascular bundle between the elongated epidermocytes. The vascular system of *D. antarctica* is represented predominantly by 3 collateral bundles, that are located in the central part of each midrib. A vascular bundle is surrounded by two sheath – one of which

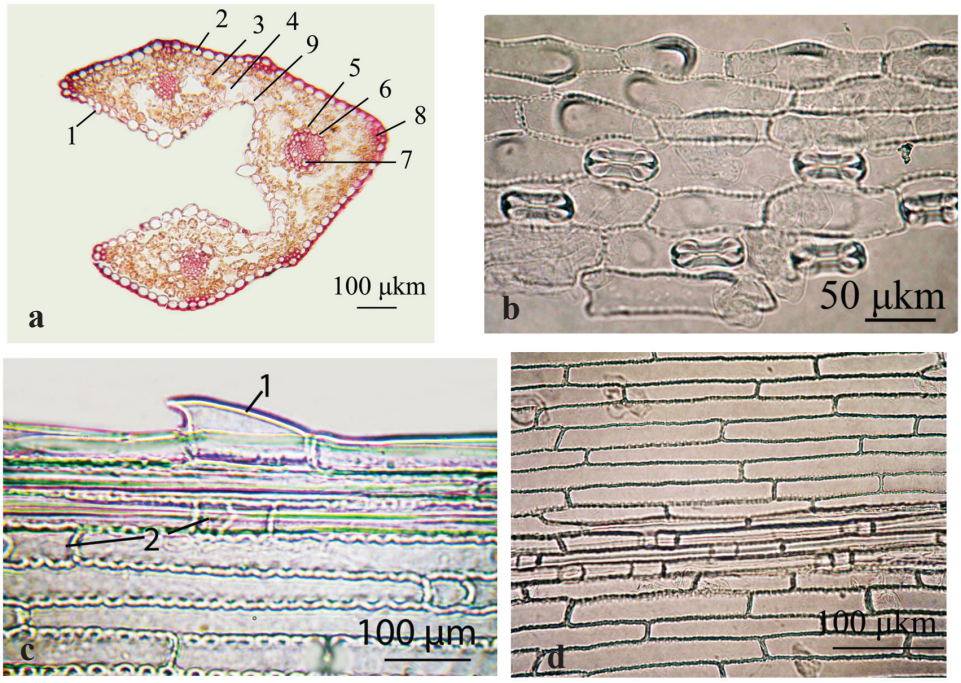

Fig. 1. Microphotographs showing: a) leaf cross-section of natural population *Deschampsia antarctica* plant from D12 study plot: 1 – adaxial epidermis; 2 – abaxial epidermis; 3 – mesophyllum; 4 – intercellular space; 5 – external lining of the vascular bundle; 6 – internal lining of the vascular lining; 7 – metaxylem; 8 – sclerenchyma; 9 – motor cells; b) leaf adaxial epidermis of natural population *D. antarctica* plant from KG016 study plot; c) leaf adaxial epidermis of *D. antarctica* from BE1 study plot; d) leaf abaxial epidermis of *D. antarctica* from KG030 study plot: 1 – simple trichome; 2 – small epidermal cells.

being internal lignified sclerenchymal one and the other – external parenchymal sheath with chloroplasts (Fig. 1a). The mechanical tissue was equally arranged by 3–5 sclerenchymal elements above the vascular bundle and over the motor cells from the abaxial side.

The parenchyma of *D. antarctica* from both studied regions could not be typified into palisade and spongy types. Instead it was represented by only a spongy type with larger or smaller intercellular spaces. The mesophylic cells were characterized by a varying shape, densely located on the abaxial side, whereas the adaxial surface contained large intercellular spaces, which contributes to more intense gas exchange. The leaf blade folded from the adaxial surface towards the inside due to the presence of large aquiferous motor cells in the inter-bundle space, that suggestively reduces the solar irradiation from this side of the leaf.

In addition, some qualitative anatomic differences were detected between the studied localities of *D. antarctica*. If in most cases the plants had symmetrical

3-rib leaves, the plants from BE1 region had both symmetrical 3-rib and asymmetric 4-rib leaves. In specimens from BE1 locality, there were found single-celled non-glandular pointy trichomes. There were also short, small epidermocytes of square shape, which were found not exclusively around the leaf veins (Fig. 1c). Plants from the Galindez Island had leaves with singular stomata on the abaxial surface, placed in one line at the edge of the leaf blade, while the central part of the leaf blade on the bottom side did not contain any stomata. Single papillae on the adaxial side (Fig. 1b) were identified in plants from the KG016 localization. The specimens from KG030 did not have stomata on the abaxial surface (Fig. 1d).

The quantitative analysis of leaf parameters of the specimens from natural *D. antarctica* populations of both regions is presented in Tables 3 and 4 and in Fig. 2. Among the natural populations *D. antarctica* plants, the largest stomata density, along with their larger size on the adaxial surface, was observed in specimens from the natural population of BE1 study plot (Table 3, Fig. 2a). Plants from other studied plots did not differ significantly from the others by these indicators. In wild-growing plants, the leaves with highest number of stomata on the abaxial side are identified in specimens from KG016 study plot (Fig. 2a), but the size of the stomata in those variants was not significantly different from that of the plants of other investigated plots. Only in plants from

Table 3

Morphometric parameters of *Deschampsia caespitosa* from natural environment of Ushuaia (*D. caespitosa* Ush) and *Deschampsia antarctica* from natural populations plots of the maritime Antarctic (m ± SD). Different letters indicate significant differences according to ANOVA analysis inside the parameters; the same letters indicate no difference at $P < 0.05$.

Study plot	Adaxial epidermis, n=50			Abaxial epidermis, n=50		
	Stoma length µm	Stoma width, µm	Epidermocyte area, µm^2	Stoma length, µm	Stoma width, µm	Epidermocyte area, µm^2
D. caespitosa Ush	51,5±4,2^{a}	29,8±3,5^{a}	5115±1748^{a}	–	–	4633±1630^{a}
BE1	45,2±3,5^{bc}	28,9±2,9^{a}	2747±890^{b}	40,7±2,4^{b}	29,5±3,7^{b}	2704±1018^{b}
D4	41,8±3,9^{de}	25,6±4,9^{bc}	2860±1027^{b}	41±3,1^{b}	28,9±3^{b}	2959±1074^{b}
D12	43,9±4,4^{cd}	26,2±3,2^{bc}	2910±1023^{b}	46,4±4,1^{a}	29±3^{b}	2694±1013^{b}
KG027	39,4±2,9^{e}	22,8±3,8^{d}	2424±706^{b}	40±4,5^{b}	34,6±2,7^{a}	2720±970^{b}
KG030	47±3,4^{b}	27,7±3,3^{ab}	2513±781^{b}	–	–	3112±990^{b}
KG016	42,4±3,5^{d}	25,5±3,6^{c}	2878±901^{b}	39,7±3,3^{b}	32,2±3,1^{a}	2866±1166^{b}

D12 study plot the length of stomata on the abaxial side was significantly higher (Table 3). Considering the area of epidermocytes, the variability in plants within each investigated plot was found greater than between the study plots. Plants from KG027 study plot had the lowest epidermis thickness on both adaxial and abaxial sides compared to other plots of the Point Thomas oasis (Fig. 2c,d). The maximum cell wall thickness of the adaxial epidermis is identified in plants from the Point Thomas oasis, KG016 study plot, as compared to all other plots of *D. antarctica*, suggesting more xerophytic growth conditions along with a large number of stomata on the abaxial side (Fig. 2b). The thickness of the epidermal outer cell wall on the abaxial side did not significantly vary between plants from different plots (Fig. 2f). Plants from KG016 plot had a significantly thicker leaf blade and a larger mesophilic cell area than others. The specimens of *D. antarctica* did not significantly differ by metaxylem vessels cross-section (where reduction of such cross-section suggests a xerophytic trait) and the cross-sectional area of the leaf (Table 4).

Anatomical parameters in plants from natural populations and *in vitro* cultivars. — As it is generally accepted, a phenotype is the result of interaction of a genotype with the environment (see Baye *et al*. 2011). In this study, the variation of qualitative and quantitative anatomical characteristics of plants from

Table 4

Morphometric parameters of the leaves of of *Deschampsia caespitosa* from natural environment of Ushuaia (*D. caespitosa* Ush) and *Deschampsia antarctica* plants form natural population's plots. Different letters indicate significant differences according to ANOVA analysis inside the parameters; the same letters indicate no difference at $P < 0.05$.

Study plot	Width, µm	Area, µm ^{2}		
	Leaf blade	Mesophyll cells	Metaxylem vessels cross-section	Leaf cross-section
D. caespitosa Ush	383±74^{a}	693±228^{a}	192±73^{a}	695835±215515^{a}
BE1	168±36^{d}	241±71^{c}	57±20^{b}	131031±14178^{c}
D4	191±46^{cd}	424±94^{b}	38±22^{b}	148472±31772^{bc}
D12	194±47^{cd}	303±89^{c}	41±16^{b}	122294±9268^{c}
KG027	187±60^{cd}	298±123^{c}	12±8^{b}	126779±79858^{bc}
KG030	231±64^{bc}	476±151^{b}	68±33^{b}	210080±86145^{bc}
KG016	260±93^{b}	618±176^{a}	49±17^{b}	223455±100836^{b}

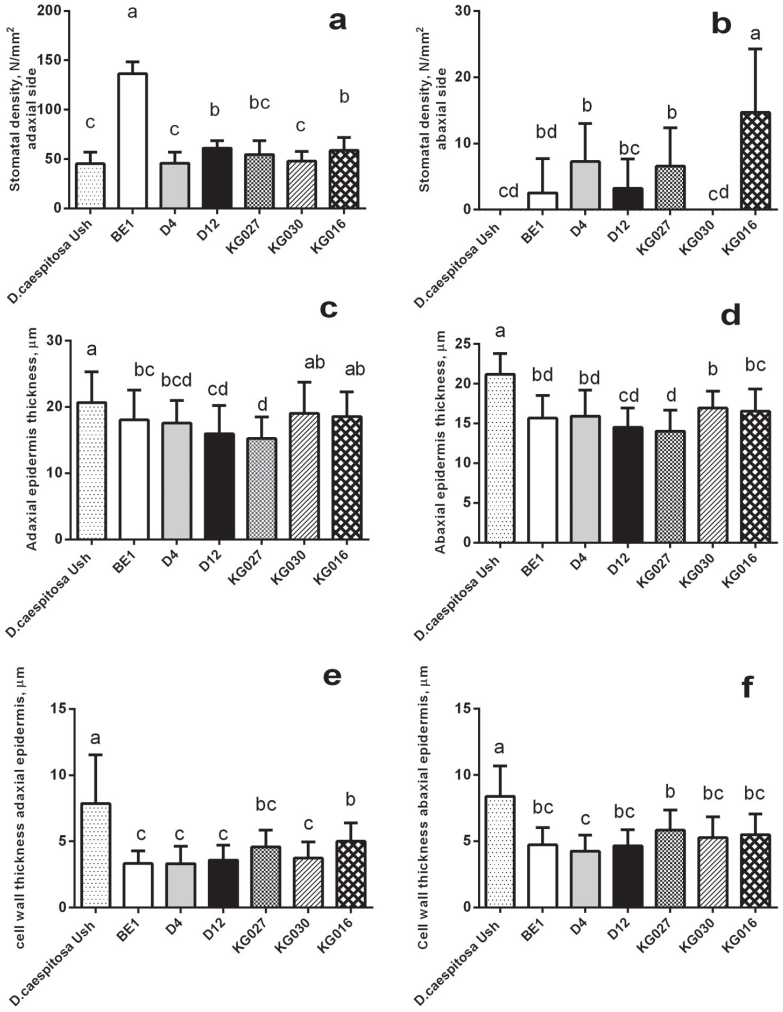

Fig. 2. Parameters of the leaf epidermis of *Deschampsia caespitosa* from Ushuaia (*D. caespitosa* Ush) and natural population of *D. antarctica* plants from two regions of maritime Antarctic. Different letters indicate significant differences according to ANOVA analysis inside the parameters; the same letters indicate no difference at $P < 0.05$.

natural populations and *in vitro* cultivars from seeds from natural populations was assessed to determine the extent to which the traits are established genetically.

The leaf length for *D. antarctica* cultivars from the seeds collected in the Argentine Islands are shown in Table 5. As it can be seen, there is a significant increase in leaf length in cultivars from seeds of D4 and D12 plots when compared with natural populations plants (Table 2). Whilst for the plants

Table 5

Leaf length of *Deschampsia antarctica in vitro* cultivars.

Cultivar	N studied		Mean ± SD (cm)
	plants	leaves	
BE1	10	71	8.2±2.6
D4	55	404	6.2±2.0
D12	59	372	8.1±3.8

originated from BE1 plot no significant change took place in leaf length after a transition into culture.

By qualitative, most plants in the culture did not differ from those from their original natural localities. It should be noted that the anatomical particularities found in the natural population plants from BE1 plot – 3–4 ribbed leaves and features of the epidermis – did completely remain in the cultivars *in vitro*. In contrast, we determined that plants from seeds of D4 and BE1 plots grown *in vitro* did not have stomata from the abaxial side as compared to their wild counterparts.

The results of the quantitative anatomical assessment of leaves of plants cultivated from seeds are presented in Tables 6, 7 and in Fig. 3. Similarly to the natural population plants, the *D. antarctica* cultivar from BE1 plot seeds had the largest stomata density from the adaxial side among all *in vitro* cultivars. By this parameter all cultivars had relatively lower values than their natural population counterparts (Fig. 2a and 3a). Regarding the parameters of the abaxial side of the leaf, the plants originated from D12 plot in case of nature population plants and cultivars did not differ by the stomata density (Figs 2b and 3b), but cultivars had significantly smaller stomata in comparison with the natural population plants.

In vitro the variation of epidermocytes area was greater within cultivars than between the cultivars (Table 6). Epidermis thickness on both the adaxial and the abaxial sides of the cultivars from BE1 seeds were smaller than those of the natural population plants (Figs 2c–d and 3c–d). The thickness of the outer cell wall on the adaxial and abaxial surfaces was the highest in D12 cultivars among the all cultivated groups of *D. antarctica* cultivars and larger in comparison with natural population plants of this plot (Figs 2e–f and 3e–f). However, natural population plants of this plot did not differ from ones from other natural populations (Figs 2e–f and 3e–f). There was no significant difference found in the thickness of the leaf blade, its cross-sectional area, and the metaxylem vessels cross-section between the cultivars and their corresponding natural population plants (Table 7). The mesophyll cell size was notably lower in cultivars of D4 plot as well as tended to decrease in cultivars of different origin compared with natural population plants.

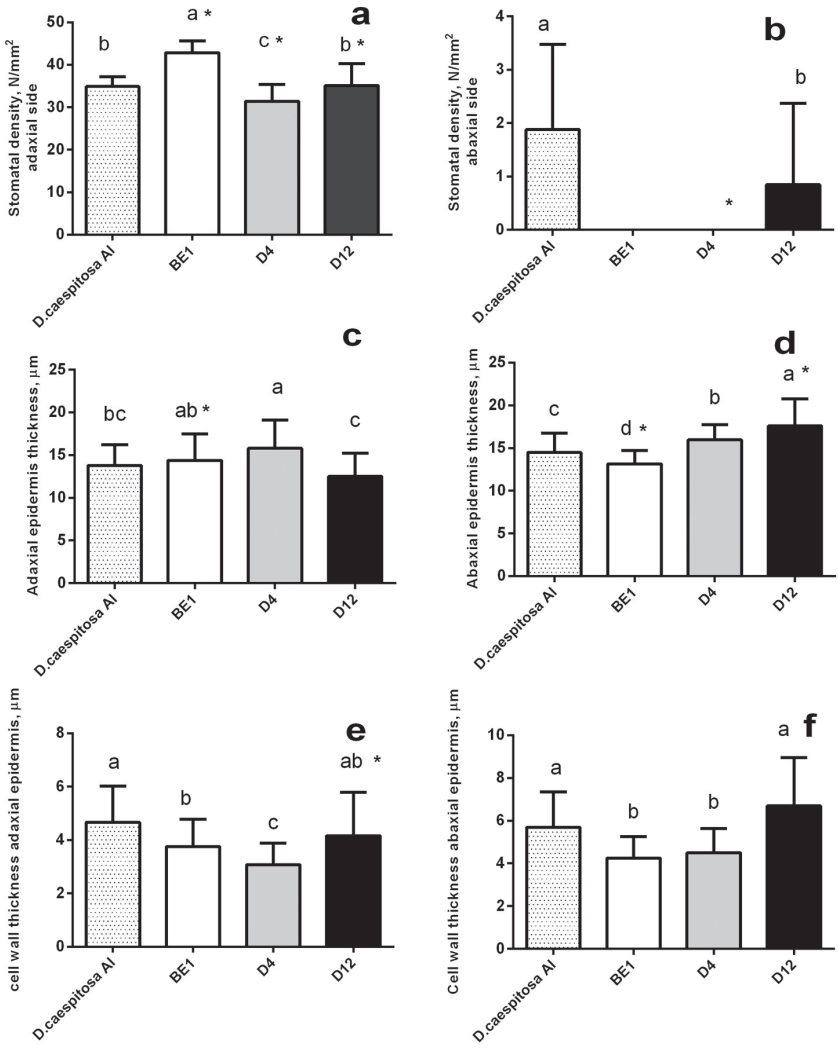

Fig. 3. Parameters of the leaf epidermis of *Deschampsia caespitosa* grown in the soil in laboratory from the wild-collected seeds from Alaska (*D. caespitosa* Al) and *D. antarctica* cultivars. * $P < 0.05$ in comparison with the wild plants. Different letters indicate significant differences according to ANOVA analysis inside the parameters; the same letters indicate no difference at $P < 0.05$.

Anatomical features of *D. caespitosa*. — The plants of *D. caespitosa* species from the natural environment of Ushuaia (*D. caespitosa* Ush) had the following qualitative characteristics: 7–9-rib leaves and hence, significantly larger leaf parameters in comparison with *D. antarctica* – in terms of thickness and the cross-sectional area (Table 4). Their leaves contained small duplex cuboid epidermis cells from the abaxial side along the entire leaf blade, as opposed to

Table 6

Morphometric parameters of the leaf epidermis of *Deschampsia antarctica in vitro* cultivars and *Deschampsia caespitosa* grown in the soil in laboratory from wild-collected seeds from Alaska (*Deschampsia caespitosa* Al).
* P < 0.05 in comparison with the wild plants.
Different letters indicate significant differences according to ANOVA analysis inside the parameters; the same letters indicate no difference at P < 0.05.

Cultivar	Adaxial epidermis, n=50			Abaxial epidermis, n=50		
	Stoma length, µm	Stoma width, µm	Epidermocyte area, µm^2	Stoma length, µm	Stoma width, µm	Epidermocyte area, µm^2
BE1	40.4±2.8^{*b}	20.8±2^{*b}	2290±1508^{a}	–	–	3318±1249^{b}
D4	43.2±3.5^{a}	21.4±2.9^{*ab}	2632±712^{a}	–	–	4114±1319^{*a}
D12	40.8±4.6^{*b}	20.7±2.6^{*b}	2488±667^{a}	40.6±3.2^{*b}	20.5±2.7^{*b}	3238±1207^{b}
D. caespitosa Al	44.3±3.3^{a}	22.1±2.4^{a}	2733±833^{a}	43.4±3.8^{a}	25.9±2.8^{a}	2998±908^{b}

Table 7

Morphometric parameters of leaves of *Deschampsia antarctica in vitro* cultivars and *Deschampsia caespitosa* grown in the soil in laboratory from wild-collected seeds from Alaska (*Deschampsia caespitosa* Al).
* P < 0.05 in comparison with the wild plants.
Different letters indicate significant differences according to ANOVA analysis inside the parameters; the same letters indicate no difference at P < 0.05

Cultivar	Width, µm	Area, µm^2		
	Leaf blade	Mesophyll cells	Metaxylem vessels cross-section	Leaf cross-section
BE1	170±38^{b}	185±63^{d}	44±14^{b}	127154±18473^{b}
D4	182±42^{b}	251±79^{*c}	80±32^{a}	101701±7949^{c}
D12	172±39^{b}	290±109^{b}	43±23^{b}	90691±23482^{bc}
D. caespitosa Al	293±20^{a}	364±87^{a}	87±30^{a}	528566±42366^{a}

having those only around the veins, as well as there were papillas detected in all epidermal cells from the adaxial side (Fig. 4c, d).

The parenchyma in wild-growing plants of *D. caespitosa* Ush, like in case of *D. antarctica*, was represented predominantly by a spongy type with larger or smaller intercellular spaces. Mesophilic cells were of a varying shape, densely

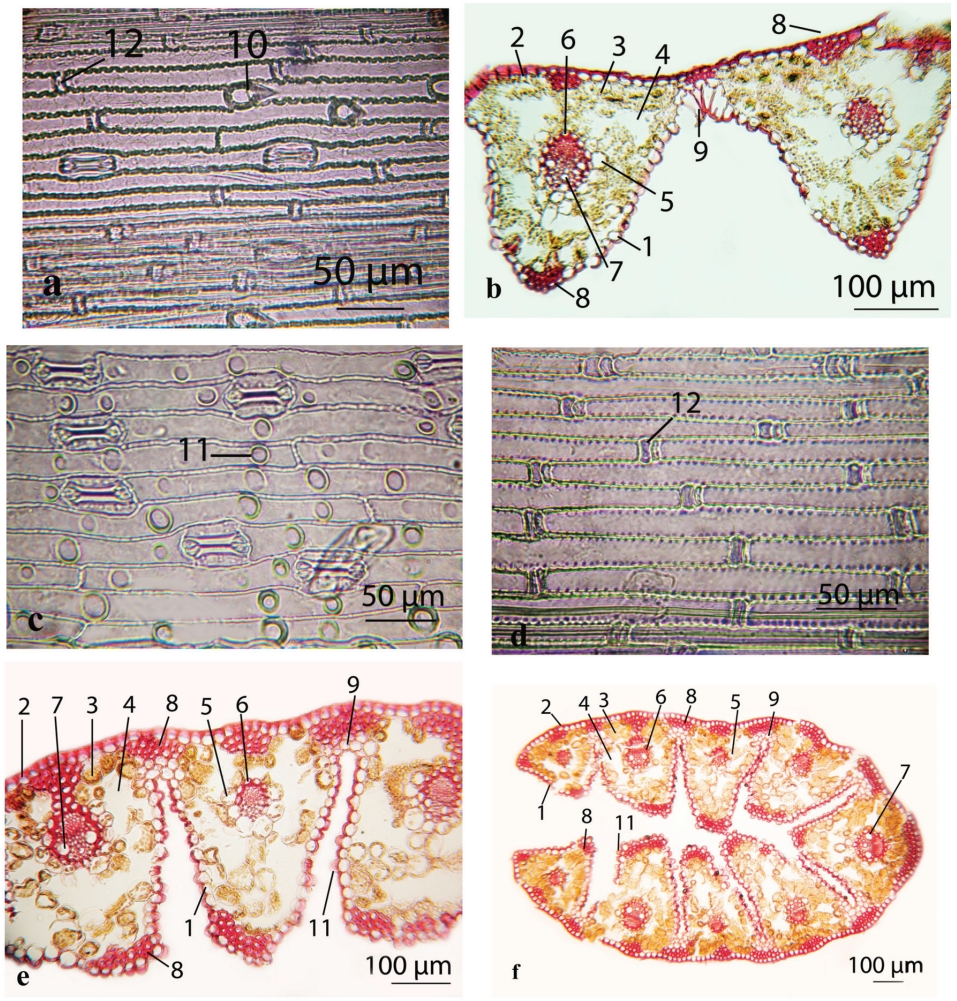

Fig. 4. Microphotographs of *Deschampsia caespitosa* Al: a) leaf abaxial epidermis, b) leaf cross-section; and *Deschampsia caespitosa* Ush: c) leaf adaxial epidermis, d) leaf abaxial epidermis, e–f) leaf cross-section: 1 – adaxial epidermis; 2 – abaxial epidermis; 3 – mesophyllum; 4 – intercellular space; 5 – external lining of the vascular bundle; 6 – internal lining of the vascular bundle; 7 – metaxylem; 8 – sclerenchyma; 9 – motor cells; 10 – simple trichome; 11 – papilla; 12 – small epidermal cells.

located on the abaxial side, and from the adaxial side there were large intercellular spaces. The leaf blade folded from the adaxial surface towards the inside due to the presence of large aquiferous motor cells in the inter-bundle space.

In *D. caespitosa* Ush, in comparison with *D. antarctica* from both Antarctic regions, there were found larger stomata from the adaxial side of the leaf (Table 3). In these plants stomata were absent on the abaxial side of the leaf.

Interestingly, the stomata in such place were present in the *in vitro* cultivated *D. caespitosa* from seeds from Alaska (*D. caespitosa* Al) and majority of the studied nature populations *D. antarctica* plants (Figs 2b and 3b). Larger epidermal cells on both leaf sides (Table 3) and larger motor cells between the edges of the leaf blade were found in wild-growing *D. caespitosa* Ush, which overall is also a xerophytic trait (Alvarez *et al*. 2008). In these plants from natural conditions, the clusters of up to 20 sclerenchymal elements localized in the same places from the abaxial side were detected similarly to the studied *D. antarctica*, as well as the clusters of up to 10 sclerenchymal elements on the edges of the adaxial side (Fig. 4e, f). In *D. caespitosa* Al that were cultivated in lab conditions, the vascular bundles were surrounded by internal sclerenchymal and external parenchymal sheath, the latter being U-shaped on adaxial side (Fig. 4b).

A significantly larger number of sclerenchymal fibers were found on both leaf sides of *D. caespitosa* Ush compared with *D. antarctica* from natural populations. *D. caespitosa* Ush had considerably larger, compared to natural populations of *D. antarctica*, values of all leaf morphometric parameters (Tables 3 and 4; Fig. 2). At the same time, the *D. caespitosa* Al propagated from seeds and cultivated in soil in laboratory had generally similar morphometric parameters as in *D. antarctica* natural population from D12 plot (Fig. 2 and 3).

A qualitative difference was also found between *D. caespitosa* Al and *D. caespitosa* Ush. That is the presence of single-celled non-glandular trichomes manifested on both sides of the leaf blade in case of *D. caespitosa* Al (Fig. 4a), and not just over the veins on the adaxial side like in case of *D. caespitosa* Ush.

Discussion

The results of our study do not provide enough ground-holding evidence to support the idea that *D. antarctica* plants from more severe growing conditions (*i.e*. 400 km to the south from Argentine Islands region) will tend to develop more xerophytic features in the leaf anatomy as compared with that in plants from more favorable conditions (as in Point Thomas oasis). Nonetheless, here we note as well that the morphometric parameters did vary greatly in response to the micro-habitat (Barcikowski *et al*. 2003; Kozeretska *et al*. 2010).

We found that most *D. antarctica* plants from both studied regions had similar qualitative anatomical features. This suggests involvement of the same basic adaptation mechanisms in plants in their respective areas. For instance, in all plants we found and described three vascular bundles. This marker was the most stable in all sub-populations including the Admiralty Bay area (Barcikowski *et al*. 2003). The characteristics of mesophyll are substantial in this understanding as well, as its structure is known to be affected by high salinity and humidity (Giełwanowska 2005; Giełwanowska *et al*. 2005). It is suggested

that the irregular shape of mesophyll cells and large intercellular spaces promote gas exchange (Korner and Larcher 1988). Romero *et al*. (1999) showed the presence of both palisade and spongy mesophyll layers. In our study all variants had exclusively a spongy mesophyll. From the abaxial side, all specimens of *D. antarctica* had epidermal cells with wavy anticlinal walls. According to Ellis (1979), this feature helps to prevent damage to cell walls during draughts. *D. antarctica* individuals from both the Point Thomas oasis and the Argentine Islands region have a thickened cutin layer protecting plant from pathogens, as well as limiting loss of water through transpiration and also affecting the reception and further redistribution of sunlight (Baker 1982; Gunning and Steer 1996). We did not find any significant relationship between the folding patterns (*e.g*., creating V-shape leaves) and the environmental humidity, as described in the literature for *D. antarctica* (Chwedorzewska *et al*. 2008). However, we detected some qualitative differences, *e.g*. in plants KG016 (presence of papillae) and KG030 (absence of stomata from the abaxial side) from the area of Point Thomas oasis, as well as the anatomy of leaf blades in plants BE1: anatomical differences in the epidermis and four-rib leaves. It should be noted that in the previous study (Barcikowski *et al*. 2003) the stomata from abaxial side of the leaf were detected in only one sub-population from the relic ornithogenic soil from the Point Thomas oasis. In our study, they were found in all plants from the Argentine Islands and two study plots from Point Thomas oasis, and were absent only in KG030 plot. They were also typical for all plants from Robert Island (Romero *et al*. 1999). Regarding the number of leaf ribs, Giełwanowska *et al*. (2005) showed that in most of the Point Thomas oasis plants there were three-rib leaves, however some plants of the wet, fertile habitat were described as having 4–5 ribs if they were found in localities near the sea at the base of the Point Thomas Hills.

The studied *D. antarctica* specimens were similar in terms of quantitative parameters in the studied regions. Although, similar anatomical variations were found corresponding to changes in the environmental conditions in a similar way within each of the studied regions. In particular, we detected variation in the epidermal parameters on both sides of the leaf, as we suggest, in response to the environmental gradients in both studied regions (Kozeretska *et al*. 2010; Parnikoza *et al*. 2018). For instance, nature population plants from KG016 plot had a higher adaxial and abaxial stomatal density than most studied populations of the Point Thomas oasis. This could be attributed to them growing in the less favourable periglacial zone of Point Thomas oasis. Higher number of stomata, including their presence on both surfaces, facilitates a more intense transpiration, and hence, a more efficient water absorption under stress caused by low temperatures (Larcher 1995; Beering and Kelly 1996). Variations in the number of stomata were observed in plants from different locations of King George Island by Barcikowski *et al*. (2003). The maximum value in terms

of this parameter was observed at the abandoned elephant seal wallow and storm embankment. In our study, there were no significant differences found in epidermis thickness on both sides of leaves from the natural populations of *D. antarctica* from the two regions.

The plants in the periglacial zone of the Point Thomas oasis (KG016 plot) featured such biomarkers of xerophylization as thickening of the outer cell wall of the adaxial epidermis, increase in the mesophylic cell area and increase in the number of stomata from the abaxial side. Appearance of such biomarkers has beneficial effect on the survival in conditions of low temperatures and recurring droughts. The *D. antarctica* plants from KG016 and KG030 study plots had the largest cross sectional parameters of the leaf blade of all measured plants. The thickening of the leaf blade is a marker of xerophylization and, along with a higher quantity of stomata, promotes adaptation to the higher insolation typical to the Antarctic, and contributes to the diffusion of CO_2 in mesophyll cells on both sides of the leaf (Nobel and Walker 1985; Romero *et al*. 1999). We found no variation in metaxylem vessels cross-sections of natural populations of *D. antarctica* plants. It is suggested that the reduction in the vessel cross-section is the marker of adaptation to low temperature, as the water freezes more slowly in narrower vessels (Romero *et al*. 1999). In terms of the thickness of the leaf blade and the area of the vascular cross-section, wild-collected plants from the Robert Island, South Shetland Islands, Antarctic (Romero *et al*. 1999) were either similar or rather less xerophytic than the plants from the localizations that we studied (Table 4). The least xerophytic features of plants from that island are more common in those from the central part of the oasis (plot KG027).

Generally, adaptation to more xerophytic conditions in both regions is mainly attributed to variation in epidermal parameters (mainly adaxial ones). This completely corresponds to the previous observations (Romero *et al*. 1999; Giełwanowska *et al*. 2005). Therefore, positive xeromorphism is suggested by greater adaxial stomatal density in plants of the BE1 location compared with other studied areas of the Argentine Islands and Point Thomas oasis.

The inference of our results on the morphometry and anatomy in view of the previous study (Giełwanowska *et al*. 2005) on the determination of ecotypes in the Point Thomas area, suggests that the plants from KG016, KG030 and KG027 study plots fall under description of the ecotype from the exposed places. They had plant height of 2–3 cm, 3 ribs in leaf. The plants from the Argentine Islands region, despite the similarity of habitats on the exposed places, had more plant height (Table 2). The plants from BE1, in addition to the three-rib leaves had asymmetric four-rib leaves. These parameters bring plants from all 3 study plots of Argentine Islands region to the described by Giełwanowska *et al*. (2005) ecotype of the wet fertile habitat. This ecotype is characterized by inflorescence height of 6–7 cm, 4–6 ribs in leaf. We also note that our study plots are located in more extreme environment than described for this ecotype

by Giełwanowska *et al*. (2005). However, the approach to allocation of ecotypes of the Argentine Islands region is generally complicated by the limitations of ecologically homogeneous areas, which can determine the coexistence of various ecotypes within the smaller size area. This phenomenon might explain the variation in biomarkers within one study plot and needs to be further explored. One of the classic approaches to identify the genetic effects on the phenotype is the method of propagation of organisms of the same genotype under different environmental conditions (Baye *et al*. 2011). Hence, by revealing the nature of detected specificity we could more substantially analyse the variation of anatomical parameters in plants from the same plots of the Argentine Islands region growing in natural sites and *in vitro*.

We noted that the qualitative features of the leaf structure in the studied *D. antarctica* from different study plots did not change after the plants were transferred into *in vitro* culture. In particular it is interesting that the plants from Berthelot Island (BE1) plot have short squared-shape epidermal cells between the vascular bundles and single-celled non-glandular trichomes above the veins on both sides of the epidermis both in the natural populations and *in vitro* cultivars. Similar biomarkers (anatomy features) and their conservation after transition into culture have not been detected in the studies on the material collected in the South Shetland Islands (Romero 1999; Giełwanowska 2005; Giełwanowska *et al*. 2005). This may suggest the genetic expression of these biomarkers that is likely associated with the growth in colder regions (Korner and Larcher 1988; Romero *et al*. 1999). Xerophytic characteristics of the *D. antarctica* leaves, feature three-rib leaves in plants growing in greenhouse conditions and in the Antarctic tundra at 400 m from the sea (Giełwanowska 2005; Giełwanowska *et al*. 2005), whereas four- to five-rib leaves (close to those found in BE1) are more common in plants on the seafront in a humid environment at 30 m to the sea. Additionally this could be evidence of the beginning of the speciation process at the edge of natural range. Similar signs of differentiation were previously shown in the example of genetic variants distinct by ITS and β-tubulin genes (Volkov *et al*. 2010; Rabokon *et al*. 2018), as well as variation of chromosomal forms (Navrotska *et al*. 2017; 2018). At the same time, it should be noted that data on the effect of mixoploidy on the anatomical features of hairgrass is scarce (Nuzhyna *et al*. 2018), which probably suggests that there are no chromosomal patterns in the location of genes responsible for phenotypic manifestation of these traits.

The biomarkers of leaf epidermis, among other quantitative parameters, varied significantly in plants grown *in vitro* in contrast with nature populations plants. In general, the epidermocyte area did not vary in relation to the plants' growth conditions (in nature population conditions or *in vitro*). Only plants from D4 study plot had significantly smaller epidermal cells on the abaxial side in case of natural population, which might suggest more xerophytic conditions in case of this study plot (Tables 3 and 6). It was also recorded that the thickness of

the epidermis and its outer cell wall after transition to the culture may either decrease, as in plants of BE1 plot, and increase, as in plants of D12 plot (Figs 2c–f and 3c–f).

In plants from the all studied plots, there were significantly more stomata on both sides of the leaves in case of nature populations than in case of *in vitro* cultivars (Figs 2a,b and 3a,b). Indeed, the stomata density was higher in leaves from plants that grow in wild Antarctic than in plants cultivated in more favourable conditions of the laboratory (Romero *et al*. 1999). Increasing the number of stomata, including their presence on both surfaces, contributes to a more intense transpiration, and hence to a more efficient water absorption under water stress caused by low temperatures (Larcher 1995; Beerling and Kelly 1996), perhaps due to a higher saline viscosity. The highest adaxial stomata density in both cases (natural populations and *in vitro* cultivars) we describe in plants from BE1 plot, whereas it was slightly less in study plots D4, D12. The size of stomata, which also contributes to better transpiration, was higher in case of natural population plants of all studied plots, compared to cultivars grown *in vitro* (Tables 3 and 6). In case of cultivars originated from D4 there was a lack of stomata from the abaxial side of the leaf. The phenomenon of disappearing abaxial stomata is described for natural plants cultivated at 13°C (Romero *et al*. 1999).

Also between the natural populations and *in vitro* plants from BE1, D4 and D12 study plots did not significantly differ in the measured leaf parameters, although there was a tendency to decreasing thickness and cross-sectional area of leaves *in vitro,* which also seems quite logical due to more favourable conditions of the laboratory. The smallest values of the cross-sectional parameters and mesophyll cell area were also detected for plants of BE1 plot, both in nature populations and *in vitro* culture, which can further confirm conclusions about the genetic effect on the ecotype of plants from this localization. It should be noted that there was some variation in the leaf length value detected in plants of different cytotypes cultivated under standard conditions *in vitro* (Navrotska *et al*. 2018). However, a difference could not be established in analysis of the cross-sectional area of metaxylem of *in vitro* plants, as compared to plants from natural populations, although there was a certain tendency to logical increase of the cross-sectional area of conducting tissues in more favourable conditions.

Romero *et al*. (1999) obtained similar results in terms of the thickness of the leaf blade and the vascular metaxylem vessels cross section of plants from Robert Island in case of cultivars when compared to nature populations.

Cells of the external parenchymal sheath of vessels in the natural population's plants were somewhat more deformed as compared to those from *in vitro* cultivars; point out those conditions in natural habitat being more extreme.

A comparative analysis of the leaf anatomy of natural populations of *D. antarctica* from two regions of the maritime Antarctic and *D. caespitosa* Ush suggested the existence of several qualitative anatomical biomarkers that clearly

distinguish the two species. In particular, it is mainly about leaves in *D. antarctica* being three-rib, as opposed to 7 or 9-rib forms of *D. caespitosa* Ush leaves. There were also clear differences in the structure of epidermis. In naturally grown *D. caespitosa* Ush, there were small doubled cuboid epidermal cells from the abaxial side across all leaf blade surfaces, and not only around the veins like in *D. antarctica*. As well there were papillae present in all epidermal cells on the adaxial surface. The literature also describes that such biomarkers are typical for *D. antarctica* plants that grow in the wild and that they disappear when cultivated in a greenhouse under milder conditions (Romero *et al*. 1999). Instead, in our investigated *D. antarctica* from the two regions of Antarctic, such epidermal structures were absent both in natural population plants and *in vitro* cultivars. They were only detected in *D. antarctica* from the KG016 study plot on glacier's periphery. Papillae play an important adaptive role in low temperature conditions particularly due to the accumulation of carbohydrates (Larcher 1995).

The qualitative differences of *D. caespitosa* Al grown from seeds were detected in contrast to the naturally grown *D. caespitosa* Ush plants. That is the presence of single-celled non-glandular pointy trichomes that were found on both sides of the leaf blade, and not just over the veins of the adaxial side (Fig. 4a). Note that the rough surface of the leaves, which is in fact a primary identification marker of this species in Europe or North America, is not really recorded in the populations that we studied in the Ushuaia.

There were no stomata from the abaxial side in the investigated *D. caespitosa* Ush plant, whereas in the same species from the Alaskan seeds – *D. caespitosa* Al grown *in vitro* and in most of *D. antarctica* studied plots populations they were present.

The study also revealed differences in the quantitative parameters between *D. caespitosa* and *D. antarctica*. The wild *D. caespitosa* Ush has a much larger adaxial and abaxial epidermis thickness than *D. antarctica* in both natural population plants and *in vitro* cultivars. At the same time, for *D. caespitosa*, thickening of the outer epidermis cell wall, which is a xerophytic trait, prevents dehydration and is observed in various plant species at sites of intense insolation and low water availability (Schreiber *et al*. 2006; Lobo *et al*. 2013; Nuzhyna and Gaydarzhy, 2015; Kalashnyk *et al*. 2016). Bystrzejewska-Piotrowska and Urban (2009) found a high degree of adaptability of the photosynthetic apparatus in *D. antarctica* and the absence of such feature in *D. caespitosa* from a more moderate climate. The researchers explained this by a fact of a thicker epidermis in *D. caespitosa* plants, which is a prerequisite for lesser plasticity of the photosynthesis system under the influence of low temperatures.

A significantly larger number of sclerenchymal fibers are located on both sides in *D. caespitosa* Ush. The distribution of sclerenchyma has an ecological significance: the sclerenchyma is better developed in plants of arid environments,

while the one of tropical species is located in smaller groups associated with vascular bundles (Metcalfe 1960; Alvarez *et al*. 2005). It is also known that a larger number of sclerenchymal fibers is typical for leaves of greenhouse-grown plants, while the smaller one is usually observed in plants that grow in moist, saline Antarctic environment (Giełwanowska *et al*. 2005).

Interestingly, *D. caespitosa* Ush has significantly higher values of morphometric parameters as comparing with *D. antarctica*, while *D. caespitosa* Al has morphometric parameters generally similar to the Antarctic hairgrass. This suggests that the morphometric biomarkers respond stronger to the environmental conditions. Therefore, a closer similarity of *D. caespitosa* Al to *D. antarctica*, may imply the environmental conditions of Alaska and Antarctica being less favorable, as compared to the Ushuaia.

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