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Generative reproduction of Antarctic grasses, the native species *Deschampsia antarctica* Desv. and the alien species *Poa annua* L.

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Abstract: The embryology of two species, *Deschampsia antarctica*, a native species, and *Poa annua*, an alien species in the Antarctic we studied. Flowering buds of plants growing in their natural habitats on King George Island and generative tissues of both plant species grown in a greenhouse were analyzed. Adaptations to autogamy and anemogamy were observed in the flower anatomy of both species. The microsporangia of the evaluated grasses produce a small number of three-celled pollen grains. Numerous pollen grains do not leave the microsporangium and germinate in the thecae. *Deschampsia antarctica* and *P. annua* plants harvested in Antarctica developed a particularly small number of microspores in pollen chambers. In *D. antarctica*, male gametophytes were produced at a faster rate: generative cells in pollen did not become detached from the wall of the pollen grain, they were not embedded in the cytoplasm of vegetative cells, and they divided into two sperm cells situated close to the wall. The monosporous Polygonum type of embryo sac development was observed in the studied species. The egg apparatus had typical polarization, and the filiform apparatus did not develop in synergids. Large antipodals with polyploidal nuclei were formed in the embryo sacs of *D. antarctica* and *P. annua*. *Poa annua* was characterized by numerous antipodal cells which formed antipodal tissue in the chalazal region of the embryo sac. Three distinct antipodals with atypical, lateral position in the vicinity of the egg apparatus were observed in *D. antarctica.* The diaspores of the investigated grass species were characterized by small size, low weight and species-specific primary and secondary sculpture of the testa and caryopsis coat.

Key words: Antarctic, hairgrass, annual bluegrass, chasmogamous flowers, cleistogamous flowers, embryo sac, diaspores.

Introduction

Plants have to be able to grow, develop and reproduce under local conditions to survive in their specific habitats, and the mechanism by which they reproduce in

time and space is an important adaptive strategy. In polar regions some flowering plants reproduce only vegetatively or generatively, whereas other species reproduce by both sexual and asexual methods (Edwards 1972; Convey 1996; Parnikoza *et al.* 2011). The extent to which a plant relies on a mixed reproduction system is species-specific, but it can also change over time and in response to environmental stressors to maximize reproductive success.

Vegetative reproduction in polar grasses. — The growing season is very short in the Antarctic geobotanical zone, and local plants are exposed to numerous stressors (Edwards 1972). Antarctic phanerogams propagate asexually to a different extent. *Deschampsia antarctica* spreads by producing new patches (Parnikoza *et al.* 2011) of apogeotropic shoots which are connected by a network of short runners (Giełwanowska *et al.* 2005). In Antarctica, *D. antarctica* forms dense turfs with a surface area of 1 to 100 m^2. Based on the observations made in the South Orkney Islands, Edwards (1972) noted that *D. antarctica* colonizes new territories mainly by vegetative propagation with the involvement of birds. Dominican gulls and south polar skuas use *D. antarctica* shoots to build nests, and they distribute parts of the plant across considerable distances. According to Parnikoza *et al.* (2011), vegetative fragments of *D. antarctica* and *Colobanthus quitensis* that have been separated from the parent plant and transported to a different location can put down roots, although this process is often inhibited by low soil moisture levels.

Generative reproduction of polar and Alpine plants. — Due to low temperatures and a short growing season in polar regions, the flowering stage may be prolonged to cover more than one summer, which implies that flower buds are produced in late summer and flowering occurs only in the following growing season. In light-sensitive Arctic and Alpine species, induction of flowering is a two-stage process. Flower buds are formed (primary induction) within a wide range of temperatures during a short day, whereas wintering flower buds are stimulated for growth (secondary induction) during a long day which often lasts 24 hours (Wagner *et al.* 2012).

Induction of flowering in Antarctic phanerogams was described by Holtom and Greene (1967). They observed that flowering is thermally induced in *D. antarctica* where inflorescences were formed only after 4 months of incubation at a constant temperature of 5°C and the 8:16 h light-dark cycle. No such correlations were reported in *C. quitensis* (Holtom and Greene 1967). In both species, the formation of flowers and inflorescences was promoted by a long day (20:4 h light-dark cycle), whereas constant low temperature (5°C) significantly delayed flowering and often inhibited the development of flowers and seeds. In the analyzed Antarctic species of vascular plants, the first flower buds generally appear in December, although significant variations in the time and intensity of flowering are observed both between and within localities (Holtom and Greene 1967).

Corner (1971) and Giełwanowska *et al.* (2005) pointed to significant differences in the number of generative shoots, flowers and inflorescences in Antarctic

phanerogams, subject to microhabitat conditions. Corner (1971) reported the presence of caryopses in the inflorescences of *D. antarctica* plants growing on the Argentine Islands. Caryopses were green and immature in wet habitats, but brown and mature in dry habitats. In the 2001–2002 season, the differences in the time during which Antarctic plants developed flower buds in various microhabitats on King George Island (South Shetland Islands) reached 6–7 weeks (Giełwanowska *et al.* 2005).

The effect of environmental factors on flowering and seed production in polar plants. — In West Antarctica, the growing season for spermatophytes lasts from November to March. The growing season may be shortened by even two months when adverse weather conditions, such as cloudy skies, frequent snowfall or lingering snow cover, limit light access. This is a critical time for reproduction and seed formation (Edwards 1974). Habitat conditions also play a crucial role in those processes. In dry and exposed localities, soil temperature on sunny summer days in Antarctica can reach even 30°C, whereas in wet habitats, it rarely exceeds 20°C (Edwards 1974). In cold and permanently wet localities, *D. antarctica* often fails to produce flowers, and if any flowers emerge, they are unlikely to produce viable seeds (Holtom and Greene 1967). Soil nutrient content also significantly influences flowering in Antarctic plants. In localities characterized by highly fertile substrates in the vicinity of sea animal habitats, *D. antarctica* flowers weakly but produces strong vegetative shoots (Smykla *et al*. 2007).

In the past few decades, global warming has contributed to an increase in biomass production in spermatophytes in Antarctica (Day *et al.* 2008) and has influenced their reproduction strategies. A clear rise in the intensity of sexual reproduction has been observed (Lewis-Smith 1994). Higher temperatures prolong the growing season, and plants have a more supportive environment for developing germ line cells and for sexual reproduction. Plants produce more viable seeds, some of which enrich soil seed banks (Wódkiewicz *et al.* 2013; Chwedorzewska *et al.* 2014). The number of seedlings and seedling survival rates increase, which gives rise to new localities and contributes to the spread of angiosperms across Antarctica (Lewis-Smith 1994; Day *et al.* 2008).

In the late 1940s, only self-pollinating cleistogamous flowers were reported in the Antarctic species *D. antarctica* (Parodi 1949). In recent years, in addition to cleistogamous flowers with partially joined glumelles, chasmogamous flowers open for cross-pollination were also noted in *D. antarctica* (Giełwanowska *et al.* 2005). The thecae of chasmogamous flowers contained twice the number of pollen grains in comparison with cleistogamous flowers. Chasmogamous flowers of *D. antarctica* with separate glumelles were harvested on King George Island between December 2001 and March 2002 (Giełwanowska *et al.* 2005). It seems that in *D. antarctica,* cleistogamy can be described as cryocleistogamy because it is induced by low temperature and high humidity, similarly to Arctic grasses of the genus *Poa* (Levkowsky *et al.* 1981).

Materials and methods

Materials. — The experimental material comprised two species of flowering plants of the family Poaceae. They were: *Deschampsia antarctica* Desv., a grass species native to Antarctica, and *Poa annua* L., a species introduced to this polar region. Flower buds at different stages of development and mature diaspores were harvested in the area of the *Henryk Arctowski* Polish Antarctic Station (62°09.8´S and 58°28.5´W) during polar expeditions of 2009–2012 organized by the Department of Antarctic Biology of the Polish Academy of Sciences in Warsaw. Flower buds were chemically preserved upon harvest. Diaspores were chemically preserved or dried before transport. Diaspores of *P. annua* and *Deschampsia cespitosa* (L.) Beauv. plants harvested in the area of Olsztyn (53°77.9‘ N and 20°48.9‘ E) were used in selected experiments for comparative purposes.

Polar flowering plants grown in a greenhouse. — Polar flowering plants have been grown in the greenhouse of the Faculty of Biology and Biotechnology of the University of Warmia and Mazury in Olsztyn since 2002. The greenhouse collection was started with entire Caryophyllaceae and Poaceae plants and diaspores harvested during polar expeditions. Plants are grown at a temperature of 18–22°C in pots filled with a 2:1 mixture of horticultural soil and sand. Most plants produce flowers and viable seeds.

Micromorphological observations of diaspores under a light microscope (LM) and a scanning electron microscope (SEM). — Glumes and glumelles were removed, and the caryopses of the evaluated species were thoroughly cleaned. Before examination under a scanning electron microscope, diaspores were mounted on an aluminum holder and sprayed with gold powder in the JEOL JFC-1200 fine coater for 45 s. The microstructure of diaspore surfaces was observed under the JEOL JSM-5310LV scanning electron microscope at 15–20 kV. Images were registered digitally with the use of Thermo Scientific NSS Noran System 7 software. Diaspores were also viewed under the Nikon SMZ 1500 stereomicroscope, and digital images were acquired with the use of the Nikon NIS-Elements BR application.

Histological (LM) and ultrastructural analyses under a transmission electron microscope (TEM). — Flower buds at different stages of development and mature seeds of greenhouse-grown grasses and grasses harvested in Antarctica were used in anatomical and ultrastructural examinations. The experimental material was harvested on King George Island between November 2009 and March 2010 (summer season), and in the University greenhouse between 2010 and 2012. Flower buds (at harvest) and seeds (after 12 hours of imbibition) were fixed in 4% glutaraldehyde solution or a mixture of 4% formaldehyde and 1.25% glutaraldehyde in a phosphate buffer with pH 7.0–7.2 at a temperature of approximately 20°C. Secondary fixation was performed in 2.5% osmium tetroxide solution. Flower buds for the Periodic

acid-Schiff stain (PAS) were fixed in Carnoy's solution. The material was rinsed, dehydrated in a graded series of alcohols and acetone, and embedded in PolyBed epoxy resin. Semi-thin and ultra-thin sections were cut with the use of Diatome glass and diamond knives in the Ultracut R (Leica) ultramicrotome.

Semi-thin sections (1.5 μm in thickness) were stained with 1% toluidine blue, embedded in glycerin and examined under the Nikon Eclipse 80i fluorescence microscope. Images were registered with the use of the Nikon Digital Sight digital camera and NIS-Elements Advanced Research software. Ultra-thin sections (60–90 nm in thickness) were contrasted with a saturated aqueous solution of uranyl acetate and a saturated aqueous solution of lead citrate. Specimens were viewed under the JEOL 1400 transmission electron microscope at 80 kV. Electronograms were registered digitally with the use of the Olympus iTEM-TEM imaging system.

Measurement of diaspore biometric parameters. — One hundred manually separated and hulled caryopses were weighed on the Radwag MYA 3Y microscales (to the nearest 0.01 mg) in eight replications to determine 1000 seed weight. The result for every replication was multiplied by 10. Arithmetic means (X), standard deviations (SD) and coefficients of variation (V%) were calculated.

One hundred hulled and cleaned caryopses of each species were sampled to determine their geometric parameters. Glumes were removed from caryopses before measurement. Each caryopsis was measured to determine its length, width and slenderness (length-width ratio). Length and width were measured to the nearest 1 μm under the Nikon SMZ 1500 stereomicroscope with the use of the Nikon NIS-Elements BR image application. Arithmetic means (X), standard deviations (SD) and coefficients of variation (V%) were calculated for each parameter. The analyzed parameters of *D. antarctica* and *P. annua* diaspores are presented in Table 1.

Results

***Deschampsia antarctica* and *Poa annua* flowers**. — The generative organs of bisexual flowers of the analyzed grasses are covered by the palea and the lemma which form a reduced perianth (Figs 1c, 2c). In *D. antarctica* and *P. annua* flowers, the androecium is composed of three stamens with thecae, a centrally positioned pistil with a spherical ovary and extensive, two-segmented stigma as well as two lodicules at the base of the androecium (Figs 1d, lo, mi, ov, st, 2d, lo, mi, ov, st).

All flowers of greenhouse-grown grasses were chasmogamous, whereas the vast majority of the grasses harvested in Antarctica had cleistogamous flowers.

Microsporangium and male gametophyte. — Three differentiating theca layers were observed in the four-lobed anthers of the examined grasses. The outermost, monolayered epidermis was composed of elongated cells (Figs 1e, f, ep, 2e–g, ep). The cells of the endothecium, i.e. the subepidermal layer, initially re-

Table 1

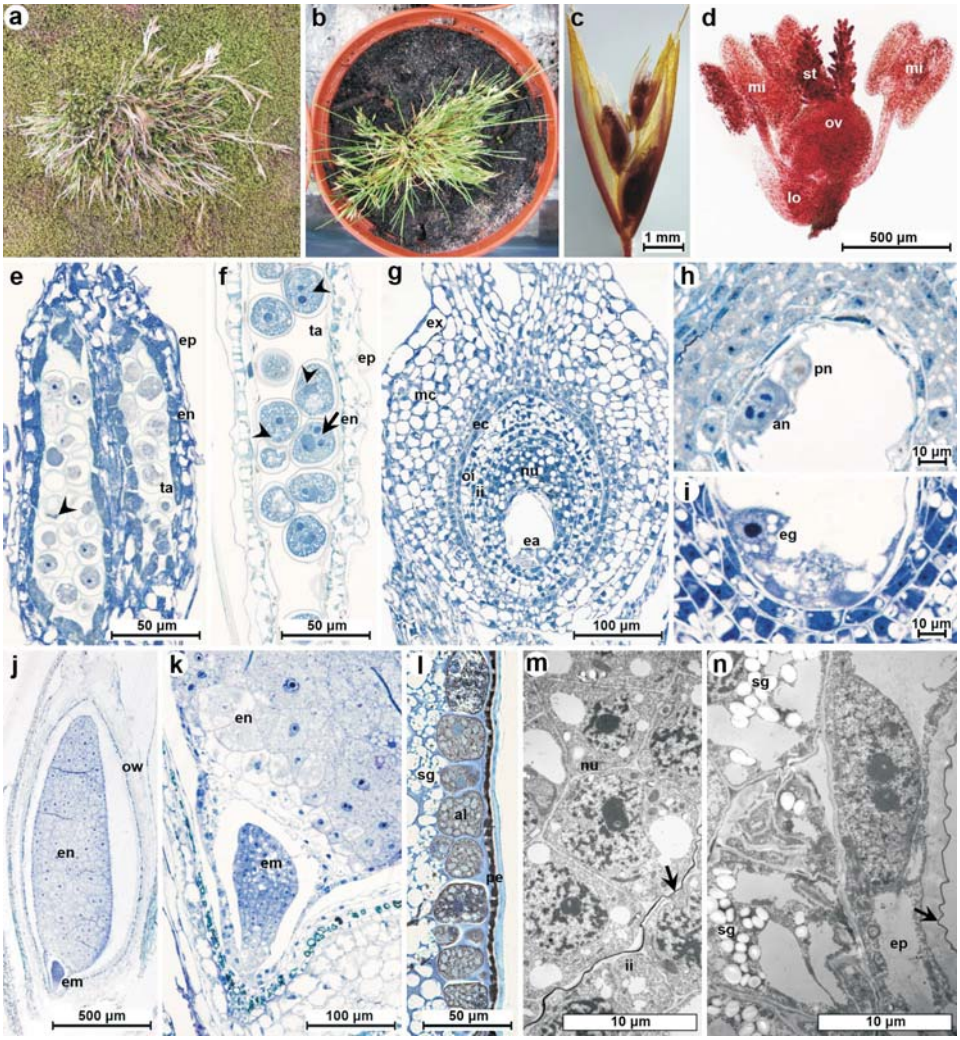
Arithmetic means (X), standard deviations (SD) and coefficients of variation (V%) for biometric parameters of caryopses in the analyzed Poaceae species. For *Deschampsia antarctica* and *Poa annua*, the presented data refer to caryopses produced during two growing seasons.

Species	Season	Index	1000 kernel weight [mg]	Length [mm]	Width [mm]	Slenderness
Deschampsia antarctica	2009/10	X	**224.3**	**1.771**	**0.506**	**3.52**
		SD	±7.1	±0.119	±0.047	±0.31
		V%	3.1	6.76	9.24	8.9
	2011/12	X	**226.4**	**1.628**	**0.480**	**3.41**
		SD	±10.2	±0.126	±0.051	±0.32
		V%	4.3	7.74	10.68	9.5
Poa annua Antarctica	2009/10	X	**314.5**	**1.440**	**0.721**	**2.01**
		SD	±3.6	±0.119	±0.073	±0.18
		V%	0.9	8.28	10.08	9.0
	2011/12	X	**321.5**	**1.404**	**0.578**	**2.46**
		SD	±8.6	±0.145	±0.062	±0.36
		V%	2.7	10.30	10.71	14.6
Poa annua Olsztyn	2010	X	**336.4**	**1.409**	**0.553**	**2.55**
		SD	±11.0	±0.112	±0.039	±0.24
		V%	3.3	7.98	6.97	9.4
Deschampsia cespitosa	2011	X	**311.4**	**1.625**	**0.574**	**2.84**
		SD	±8.2	±0.138	±0.047	±0.22
		V%	2.6	8.48	8.12	7.7

sembled parenchymal cells, but linear thickening was observed in inner adjacent and radial walls during the growth and development of microsporangia (Figs 1e, f, en, 2e–g, en). The secretory tapetum was the innermost layer of the microsporangium wall. In the final stage of tapetal differentiation and degeneration, tapetal residues in the form of fine, toluidine blue-stained granules were visible in thecae (Figs 1e, f, ta, 2e, ta).

Two daughter cells, including a large vegetative cell occupying most of the pollen grain and a smaller lens-shaped generative cell near the wall, were produced by mitotic division in polarized microspores of *D. antarctica*. During the development of pollen grains in *D. antarctica*, the generative cell remained attached to the wall of the pollen cell, where it underwent mitosis to produce two sperm cells (Fig. 1e, f, arrows, arrow head). In both, *D. antarctica* and *P. annua*, generative cells divided in closed microsporangia, and male gametophytes reached the three-celled stage before pollen discharge.

Fig. 1 Morphology and structure of generative organs of *Deschampsia antarctica* plants growing in the Antarctic (a, c–n) and in the greenhouse in Olsztyn (b). Semi-thin sections stained with toluidine blue. **a**. *Deschampsia antarctica* plants growing in moist, mosses carpet on King George Island. **b**. *Deschampsia antarctica* plants growing in a greenhouse in Olsztyn, immediately after the arrival of the Antarctic. **c**. Three florets spikelet of *D. antarctica* with developing hermaphroditing flowers shielded by large glumes. **d**. Generative organs of *D. antarctica* with lodicules (lo). Three stamina →



with short filaments and sparse microspores in anthers (mi). Thick-walled ovary (ov) with a two-segmented, feathery stigma (st). **e**, **f**. Longitudinal section of microsporangium with, two-celled (arrow heads) and three-celled (arrow) pollen grains; ep – epidermis, en – endothecium, ta – tapetum. **g**. Anatomy of the ovary with ovule. Wall of the ovary; exocarp (ex), mesocarp (me), endocarp (ec), and the ovule; outer integument (oi), inner integument (ii), nucellus (nu), with a differentiating embryo sac with egg apparatus (ea) in a longitudinal section of *D. antarctica* in a plane perpendicular to the micropylar-chalazal axis. **h**. Portion of the middle part of the embryo sac with polar nuclei (pn) and antipodal cells (an). Antipodal cells (an) with numerous large nuclei (with visible nucleoli) are positioned laterally, along the wall of the embryo sac. **i**. Portion of the micropylar part of the embryo sac with nucleus of an egg cell (eg) in the visible fragment of the egg apparatus. **j**–**l**. Developing caryopsis of *D. antarctica.* Embryo (em), endosperm (en) and ovary wall (ow). Endosperm with cells containing starch granules (sg), the aleurone layer (al), and the pericarp (pe). **m**. Cells of nucellus (nu) filled with dense cytoplasm and organelles. Arrow indicates cuticle on the surface of epidermal cells of inner integument (ii). **n**. Ovary wall with starch-filled (sg) cells. Visible cuticle (arrow) on the surface of epidermal cells (ep) of ovary wall.

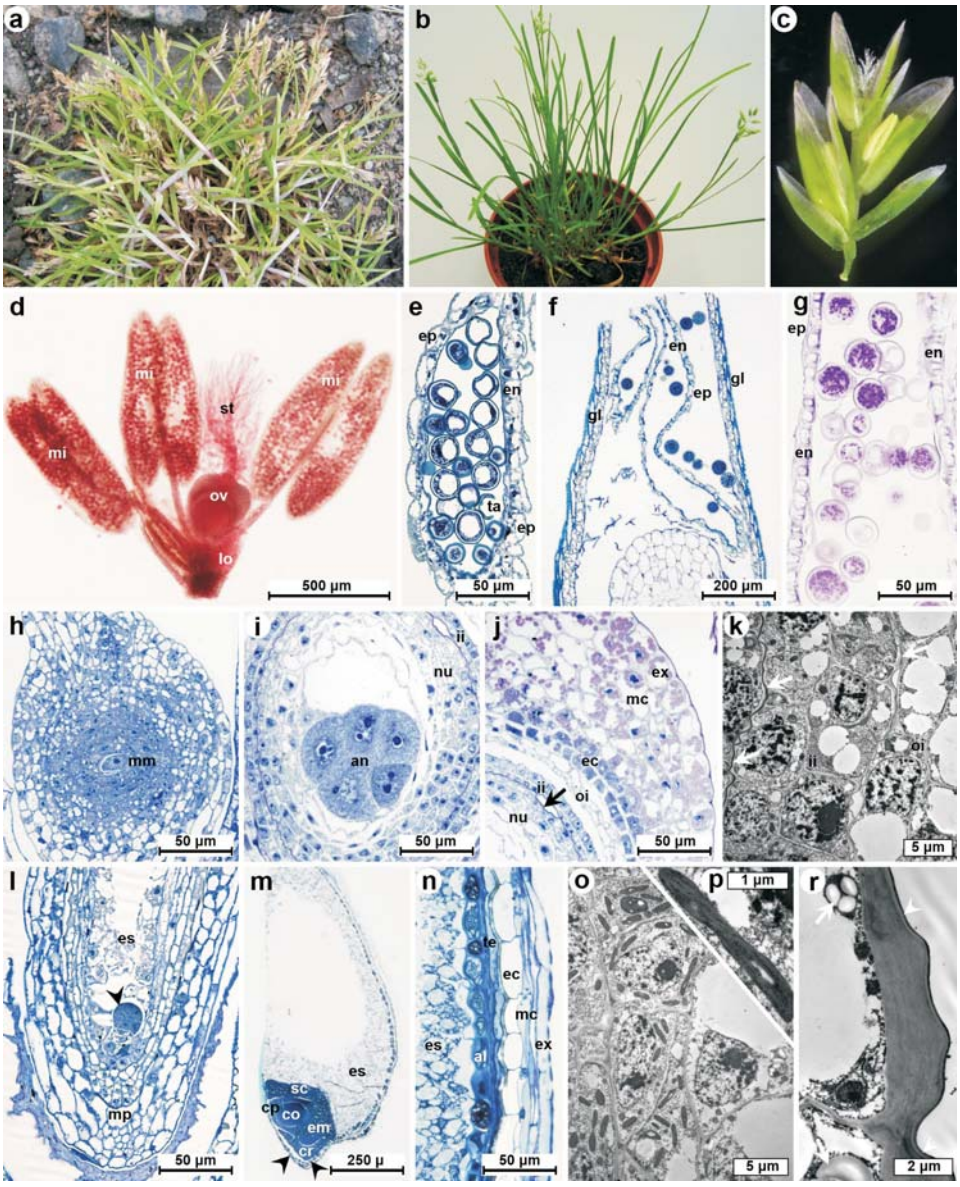
Large amounts of reserve substances, mostly starches as well as numerous small vacuoles with osmiophilic content, were accumulated in the developing pollen grains of the evaluated grasses (Figs 1e, f, 2g). The number of osmiophilic granules was particularly high in discharged pollen grains of *P. annua* (Fig. 2f). The thecae of Poaceae plants collected in Antarctica contained several dozen microspores and pollen grains in a single pollen chamber (Figs 1e, 2e). Pollen grains developed normally and synchronously, and deformed grains were sporadically observed. *Deschampsia antarctica* and *P. annua* had monoaperturate pollen grains.

Development of the ovary and ovules. — During the development of thick-walled, three-layered ovaries of *P. annua* and *D. antarctica* plants, large amounts of reserve substances, mostly starches, were accumulated in wall layers. The exocarp and mesocarp cells of *P. annua* (Fig. 2h–k, n–r, ex, mc) contained starch-filled plastids. Starch was not found in the epidermal cells of the ovary in *D. antarctica* harvested in Antarctica (Fig. 1g, ex, n, ep).

In both, *P. annua* and *D. antarctica*, ovules were formed laterally on the inner surface of the ovary wall. In successive stages of development, ovules became curved, and most of them became campylotropous. In the analyzed grasses, ovules were composed of two differentiating integuments – the placenta-chalazal region and the nucellus with the developing embryo sac. The integuments surrounding the nucellus were composed of two cell layers. Outer integument cells were strongly vacuolized, whereas inner integument cells contained dense cytoplasm (Figs 1g, oi, ii, 2j, k, oi, ii). A thick cuticular layer separated the nucellus from the inner integument in both *P. annua* and *D. antarctica* (Figs 1m, arrow, 2j, k, arrows).

The monosporous Polygonum type of embryo sac development was observed in the studied grass species. The micropylar region of the embryo sac con-

Fig. 2. Morphology and structure of generative organs of *Poa annua* plants growing in the Antarctic (a, e–f, h–k, m–r) and in the greenhouse in Olsztyn (b–d, g, l). Semi-thin sections stained with toluidine blue and after PAS-reaction. **a**. *Poa annua* plants growing near the *H. Arctowski* Station on King George Island. **b**. *Poa annua* plants growing in a greenhouse, immediately after the arrival from the Antarctic. **c**. Three florets spikelet of *P. annua* plant growing in greenhouse with developing hermaphroditing, chasmogamic flowers shielded by large glumes. **d**. Generative organs of *P. annua*. Three stamina with short filaments and sparse microspores in anthers (mi). Thick-walled ovary (ov) with a two-segmented, feathery stigma (st). **e**, **f**. Pollen in a microsporangium in flower bud. Epidermal (ep) and endothecium (en) cells in anther walls. Pollen grains released from the theca and captured by glumelles (gl) in cleistogamic flower. **g**. Pollen with PAS-positive starch in theca in a developing flower bud. **h**. Megaspore mother cell (mm) in the center of a young, thin-walled ovule in ovary. **i**. Embryo sac in a thin-walled ovule. Antipodal cells (an) in the form of antipodal tissue in a mature embryo sac. Ovule sodiametric, vacuolated nucellus cells (nu) and inner integument (ii) in semi-thin longitudinal section. **j**. Anatomy of the ovary in a longitudinal section. The ovary wall contains numerous chloroplasts in exocarp (ex) and mesocarp (mc), and bilayered endocarp (ec). The outer (oi) and inner (ii) integument of the ovule and nucellus cells (nu). **k**. Ultrastructure of differentiated cells of the outer (oi) and inner (ii) integument and nucellus cells (nu) of the ovule of *P. annua*. **l**. Section of the central part of the ovule with a visible embryo (arrowhead) in the micropylar region (mp) of the ovule and micropylar endosperm (es) with visible cell nuclei. **m**. Longitudinal section of →



the caryopses with a differentiated embryo (em) and differentiating (maturing) endosperm (es). *P. annua* embryo (em) with a visible cotyledon (co) and a stem bud sheathed by the coleoptile (cp). Radicle sheathed by the coleorhiza (cr) visible at the oppositing embryonic pole. Visible scutellum (sc) between the embryo and endosperm. The embryonic region in the caryopsis is surrounded by a layer of endosperm cells (arrowheads) with aleurone bodies. **n** In the caryopsis coat are visible layer of epidermal (ex), mesocarp (mc), endocarp (ec) and testa cells (te) differentiated from ovule integuments. The aleurone layer (al) is composed of thick-walled, intensively stained cells with a visible layer of starch-bearing parenchyma underneath. **o–r**. Ultrastructure of the young ovary wall; vacuolated cells contain numerous chloroplasts with starch (arrows) and thick-walled epidermal cells with cuticle (arrowheads).

tained a differentiating egg apparatus composed of two synergids and an egg cell (Fig. 1g, h, i, ea, eg). The cytoplasm of central cell contained polar nuclei which were positioned in close proximity or were fused (Fig. 1h, pn). In *P. annua,* antipodal cells were observed in the chalazal region (Fig. 2i, an). Those large multinucleate cells were fitted together to form antipodal tissue. Their nuclei contained several nucleoli. In the antipodal regions of *D. antarctica,* several nuclei with numerous nucleoli were positioned laterally along the wall in the vicinity of the egg apparatus (Fig. 1h, an).

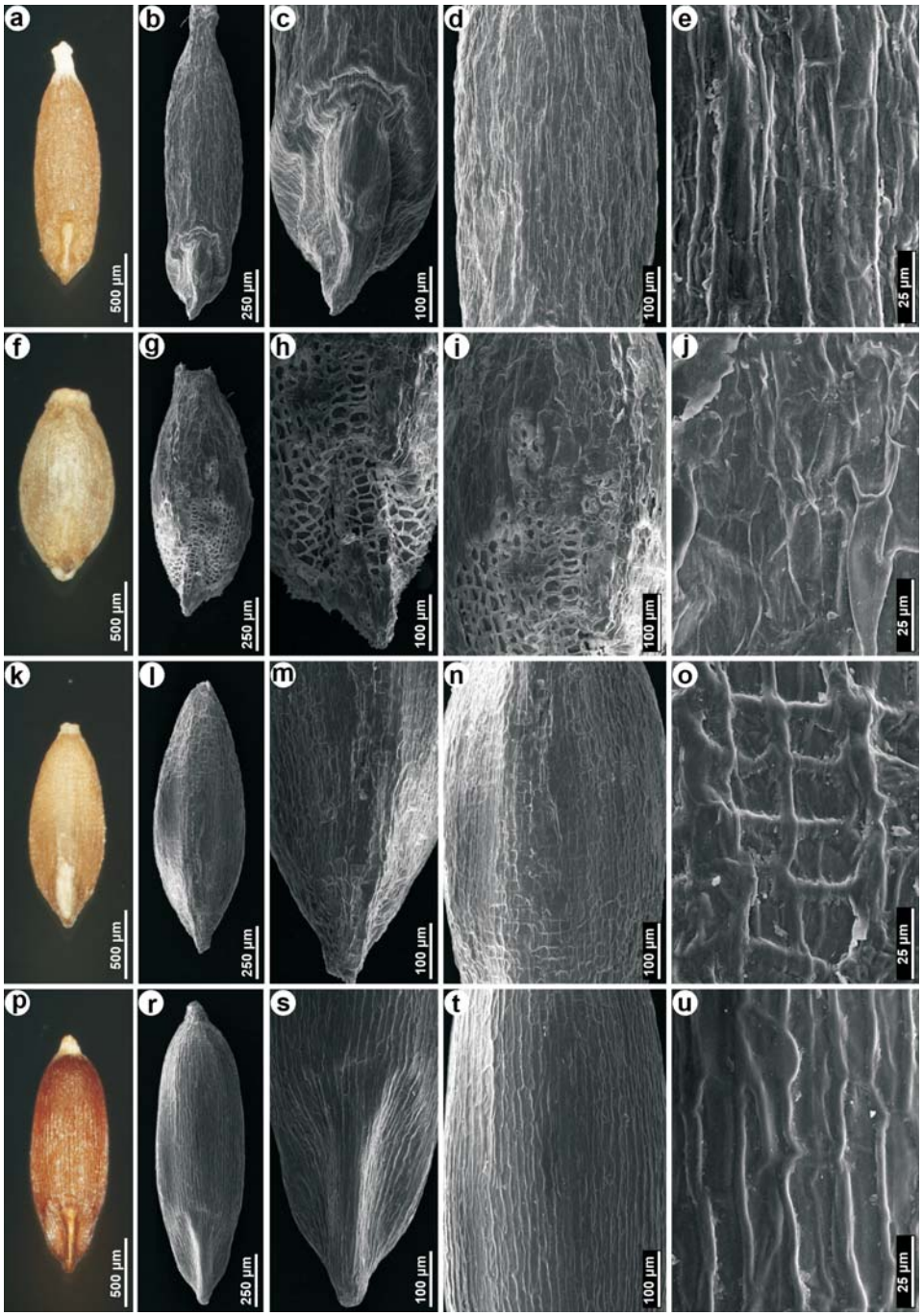
Caryopsis anatomy. — In the evaluated grasses, the mature embryo occupied a small part of the caryopsis, and it was positioned on the dorsal side of the caryopsis, at its base (Figs 1j, k, em, 2l, m, em, arrowhead, 3 a, f, k, p). The scutellum and embryonic axis with differentiated shoot and root sections were clearly visible in *D. antarctica* and *P. annua* embryos. Cotyledons and the stem bud were sheathed by the coleoptile at the tip of the shoot segment (Fig. 2m, em, sc, cp). The root segment was composed of a radicle surrounded by coleorhiza (Fig. 2m, cr).

The endosperm occupied the largest part of the seed in the mature caryopses of both polar grasses (Figs 1j, k, en, 2m, es). Nuclear endosperm development was observed in the ovules of *P. annua* containing young embryos. In initial stages of development, endosperm cells were multinucleate (Fig. 2l, es). Cell walls were developed gradually, beginning from the micropylar region and the proembryo (Fig. 2l, m, es). In successive stages of growth, the endosperm was differentiated into an outer single-celled aleurone layer and an inner multicellular starch-bearing layer (Figs 1k, l, al, sg, 2m, n, al, es).

In developing caryopses of the examined grasses, integuments and the pericarp fused to form the caryopsis coat. Testa cells were differentiated from degraded inner and outer integuments. In caryopses with fully developed embryos, the testa consisted of thin osmophilic bands, and on the outside, testa cells were adjacent to the pericarp (Figs 1l, pe, 2n, ex, mc, ec).

Large amounts of starch were observed in pericarp cells, but their starch content varied in different layers and regions of the ovary. In developing caryopses,

Fig. 3 Surface microstructure of caryopses of a *Deschampsia antarctica* plant growing in Antarctica (a–e), a *Poa annua* plant growing in Antarctica (f–j), a *P. annua* plant growing near Olsztyn (k–o), and a *Deschampsia cespitosa* plant growing in Olsztyn (p–u). **a**. Golden-yellow caryopsis coat of *D. antarctica*. **b–d**. Long (prosenchymatous) epidermal cells in the pericarp of *D. antarctica*. **e**. Minor folding of anticlinal walls and collapsed periclinal walls of long epidermal walls in the caryopsis. **f**. Grayish yellow caryopsis coat of a *P. annua* plant growing in Antarctica. **g**. Mesh-like sculpture of the caryopsis with a visible convex segment containing the embryo (micropylar region). **h**. Quadrilateral (mostly isodiametric) epidermal cells of the pericarp. **i**. Short quadrilateral epidermal cells of the caryopsis in the embryonic region and less regular, long epidermal cells in the remaining parts of the caryopsis. **j**. Irregular epidermal cells with mostly collapsed periclinal walls in the caryopsis of a *P. annua* plant growing in Antarctica. **k**. Yellow caryopsis coat of a plant growing near Olsztyn. **l–n**. Mesh-like surface formed by quadrilateral (mostly square) epidermal cells. **o**. Surface microstructure of epidermal cells in the caryopsis of a *P. annua* plant growing near Olsztyn. Regular, intact →



and non-collapsed anticlinal walls of quadrilateral epidermal cells and collapsed periclinal walls. **p**. Brown caryopsis surface in a *D. cespitosa* plant growing in Olsztyn. **r–s**. Smooth diaspore surface with somewhat collapsed fragments in the embryonic region (micropylar region) in *D. cespitosa*. **t–u**. Long epidermal cells with somewhat deformed periclinal walls.

exocarp and mesocarp cells grew in the direction of the longer axis, starch disappeared from certain regions of the pericarp, and the cells became flattened and crushed. In the mature caryopses of *D. antarctica*, flat pericarp cells were visible in the form of thin homogeneous layer stained with toluidine blue (Fig. 1l, pe).

Caryopsis size and 1000 kernel weight. — The caryopses of the evaluated grasses were small and did not exceed 2 mm in length. *Deschampsia* and *Poa* caryopses were similar in size, but they clearly differed in their slenderness ratio (Table 1). In the group of analyzed Poaceae species, the longest caryopses were produced by *D. antarctica* and *D. cespitosa*. Caryopsis length was determined at 1.771 mm and 1.628 mm in *D. antarctica* plants harvested in the 2009/2010 season and the 2011/2012 season, respectively, and at 1.625 mm in *D. cespitosa* plants. *D. cespitosa* caryopses were less slender than *D. antarctica* caryopses (Table 1). *Poa annua* caryopses harvested in Antarctica in the growing seasons of 2009/2010 and 2011/ 2012 were similar in length but somewhat different in width. Caryopses from the 2009/2010 season were wider and more oblong in shape than those produced in the 2011/2012 season. *Poa annua* caryopses collected in the Olsztyn area were similar in size to *P. annua* caryopses harvested in Antarctica in 2011/2012 (Table 1).

The 1000 kernel weight of the analyzed Poaceae species was determined at 200–350 mg. The lowest value of the examined parameter was observed in *D. antarctica*. The noted differences in 1000 kernel weight of *D. antarctica* and *P. annua* caryopses collected in 2009/2010 and 2011/2012 were not statistically significant. The heaviest caryopses were produced by *P. annua* plants growing in the Olsztyn area (Table 1).

Shape and micromorphological parameters of caryopses. — Mature caryopses of all analyzed grass species were elongated, spindle-shaped or ellipsoidal, and they differed in surface color. *D. antarctica* caryopses were golden-yellow (Fig. 3a), and *D. cespitosa* caryopses were brown (Fig. 3p). *Poa annua* caryopses from plants harvested in Antarctica were yellow-grey (Fig. 3f), and *P. annua* caryopses collected in the Olsztyn area were golden-yellow (Fig. 3k).

A groove characteristic of grass seeds was not visible on the ventral side of the analyzed caryopses. The contour of the embryo positioned along the ovary wall was visible on the dorsal side at the base of the examined caryopses (Fig. 3a–c, f–h, k–m, p–s).

Epidermal cells in the pericarp of *Deschampsia* caryopses grew in the direction of the long axis, their periclinal walls were somewhat collapsed, whereas their anticlinal walls were lightly folded in *D. cespitosa* and more folded in *D. antarctica* (Fig. 3d, e, t, u). *Deschampsia cespitosa* caryopses had a more homogeneous and smooth surface than *D. antarctica* caryopses. The caryopses of *P. annua* plants harvested in the Olsztyn area had a weaved texture. Epidermal cells had collapsed periclinal walls, anticlinal walls were not deformed, and a regular checked

pattern was observed on caryopsis surfaces (Fig. 3n, o). No such patterns were noted on the surface of *P. annua* caryopses from plants collected in Antarctica. Their periclinal walls were collapsed and unevenly folded (Fig. 3j). Regular, isodiametric and mostly quadrilateral cells were observed under the outer deformed cell layer (Fig. 3h, i).

Discussion

The analyzed Antarctic phanerogams are small herbaceous plants whose compact habit represents an adaptive strategy to the extreme environment of Antarctica (Convey 1996; Giełwanowska *et al.* 2014, 2015). Reproductive processes in polar vascular plants have been thoroughly researched (Müller *et al.* 2011), but the predominant form of plant reproduction in polar regions has not yet been identified. According to the literature, vegetative reproduction plays an important role in polar regions, but despite short and cold growing seasons that do not support generative reproduction, polar plants are capable of producing flowers and inflorescences with viable seeds (Lewis Smith 1984; Convey 1996; Cooper *et al.* 2004; Alsos *et al.* 2013).

Development and anatomy of the male gametophyte in polar plants. — The results of microscopic analyses performed in this study indicate that both *D. antarctica* and *P. annua* produce flower buds with normally developed microsporangia, microspores and male gametophytes. The development of microsporangia and male germ line cells in polar plants growing in Antarctica was discussed in our previous studies (Giełwanowska *et al.* 2005, 2011). We observed that pollen grains of *D. antarctica* and *P. annua* reached the three-celled stage before pollen discharge and that many grains did not leave the thecae and germinated inside microsporangia. In most angiosperms, pollen grains reach the stage of two-celled male gametophytes upon discharge, and generative cells divide into two sperm cells only during the growth of the pollen tube. Experimental results indicate that three-celled pollen grains accumulate more mRNA and proteins than two-celled gametophytes (Linskens 1988). Those compounds are accumulated during pollen maturation, and they are used up during germination and the growth of pollen tubes. Three-celled gametophytes germinate faster than two-celled gametophytes where mRNA and proteins are synthesized during germination (Mascarenhas 1989). The transport of male gametes to the embryo sac within a shorter time is a very important in extreme polar environments.

The thecae of grasses harvested in Antarctica contained a dozen to several dozen monoaperturate pollen grains, many of which did not leave microsporangia and germinated inside thecae. Plants of the family Poaceae generally contain more pollen grains, and a smaller number of pollen grains is probably indicative of self-pollination (autogamy) in cleistogamous flowers (Levkovsky *et al.* 1981) in

an unsupportive environment. At lower latitudes, species of the genus *Poa* generally produce chasmogamous flowers (Körner 1999) open for cross-pollination.

Structure of the embryo sac. — In an analysis of the embryo sacs of *D. antarctica* and *P. annua* plants, synergids and antipodals attracted particular attention. The filiform apparatus, through which the pollen tube penetrates the embryo sac in nearly all angiosperm species (Russell 1992), was not differentiated in the synergids of the evaluated grasses.

According to Kościńska-Pająk and Bednara (2006), the absence of a filiform apparatus in the synergids of diplosporous species *Chondrilla juncea* and *Taraxacum alatum* can be attributed to obligatory apomixis and autonomous development of the embryo and the endosperm. The above author emphasized that this hypothesis requires further research into the ultrastructure of the egg apparatus in apomictic plants with diplosporous and aposporous development of embryo sacs because very little is known about the ultrastructure of this part of the embryo sac, in particular in comparison with apomictic species.

Plants of the family Poaceae contain three to several hundred antipodals. In most species, the nuclei of antipodal cells can undergo polyploidy, and extensive protoplasts are used up by the endosperm and embryo. *Deschampsia antarctica* plants produce only three large antipodals with polyploid nuclei, which are atypically positioned on the side of the embryo sac in the proximity of egg apparatus cells. The above could indicate that antipodals have additional functions in *D. antarctica* plants.

In *Poa chaixii* and *Poa pratensis* (Yudakova 2009), embryos and additional embryo sacs are differentiated from antipodal cells. Seeds containing embryos of various origin and various degree of ploidy play an important role in those plants. According to the above authors, additional egg cells and embryo sacs enable the plant to select the optimal female gametophyte and gametes to increase its chances of survival in extreme habitats.

Disturbances in the development of thecae and ovules under the stress. — Numerous authors have demonstrated that low temperature is the major stressor during pollen development and fertilization (Kelly *et al.* 2010). Antarctic plants are exposed to low temperatures on a daily basis. Sudden temperature drops are observed even on sunny days, and temperatures can fluctuate by 20–30°C within hours or even minutes (Convey 1996). Disruptions in the development of male and female generative structures are rarely noted in Antarctic phanerogams, and they can probably be attributed to cold stress.

In *D. antarctica*, premature degeneration of the tapetum was probably induced by a sudden temperature drop. Tapetal tissue protects and nourishes male germ line cells, and its degeneration products are used to rebuild the sporoderm or produce the pollen coat which facilitates pollination (Pacini *et al.* 1985). Microspores are particularly sensitive to cold stress in early stages of development, during or di-

rectly after release from tetrads. Over that period, the tapetum is fully developed, and it supplies microspores with nutrients and enzymes. In *Oryza sativa* serious damage to the tapetal layer, involving hypertrophy of tapetal cells and degradation of tapetal tissue in response to cold stress, was observed at the above stage (Nishiyama 1976). In the thecae of *D. antarctica* plants with prematurely degraded tapetum, microspores were live and contained protoplasts with cell nuclei, but their degradation could have taken place in successive stages of development. Degradation of atypically shaped pollen grains was also observed in several *P. annua* plants harvested in Antarctica.

Cytological changes indicative of disturbances in the development of female germ line cells were observed only sporadically in the analyzed ovules of *Deschampsia* and *Poa* plants harvested in Antarctica. They included degradation of egg apparatus cells in *D. antarctica,* which reduced the number of viable seeds.

Structure and development of diaspores of Poaceae plants. — In *D. antarctica* and *P. annua*, outer integument cells were vacuolized during embryo sac maturation, and they became degraded in successive stages of development. Inner integument cells had dense cytoplasm, and similarly to most Poaceae species, they were differentiated into testa cells (Evers and Millar 2002).

In *D. antarctica* and *P. annua* plants, significant amounts of starch were accumulated in developing pericarp cells, and starch reserves disappeared during caryopsis maturation. The transient accumulation of starch reserves in the pericarp is a characteristic feature of grasses. In the last stage of development, the degradation of starch is determined by amylase activity in pericarp cells (Goggin and Powles 2012). Pericarp cells, in particular mesocarp and exocarp cells, were elongated and flattened during the growth of *D. antarctica* and *P. annua* caryopses. When caryopses reached full maturity, pericarp cells were crushed, and together with testa cells, they formed a compact caryopsis coat.

The endosperm was the major component of *D. antarctica* and *P. annua* diaspores. In mature caryopses of most grasses, the endosperm is composed of dry, hard tissue. Selected Poaceae species have liquid or soft endosperm due to complete or partial absence of cell walls. A starch layer with numerous complex starch granules, which are most characteristic of the Poaceae family, was observed in the endosperm of mature caryopses of *D. antarctica* and *P. annua* plants (Sabelli and Larkins 2009). The aleurone layer was formed between the caryopsis coat and the starch layer. Similarly to corn and wheat (Evers and Millar 2002), the analyzed polar plants contained a single layer of thick-walled cells with large nuclei and dense and granular cytoplasm.

In the mature caryopses of *D. antarctica* and *P. annua*, the endosperm layer was clearly differentiated from the starch-bearing layer and the aleurone layer in the region between the small, laterally positioned embryo and the caryopsis coat. The above area, referred to as the embryo-surrounding region (ESR), is characterized by very high levels of metabolic activity, and it plays a crucial role in embry-

onic development (Cossegal *et al.* 2007). The analyzed grasses had very small embryos which occupied a small part of the seed.

Diaspore morphology in polar Poaceae plants as an adaptive strategy. — The caryopses of the analyzed grass species did not exceed 2 mm in length, and 1000 kernel weight was equally small in the range of 200 to 350 mg. The diaspores of wild growing *D. cespitosa* and *P. annua* plants in the Olsztyn area were similar in size to the diaspores of the same species collected in Antarctica.

The size and shape of diaspores are part of a plant's adaptation strategy to the local environment. According to Tilman (1988), seed size is correlated with the degree of competition for local resources. The cited author observed that fine seeds are produced in nutritionally deficient habitats where light access is not blocked by other plants. Polar regions are nutritionally deficient habitats with negligible competition, and local species produce large numbers of fine seeds, which seems to confirm the hypothesis proposed by Tilman (1988).

In numerous studies, the shape and size of seeds produced in extreme habitats was analyzed in view of their longevity and ability to create a soil seed bank. Thompson and Grime (1979) demonstrated that species contributing to soil seed banks produce very small seeds. A similar correlation was reported in 32 grass species in Great Britain (Leck *et al.* 1989). The seeds of species with a permanent soil seed bank were significantly smaller and more compact than the seeds of other species.

Two species of flowering plants native to Antarctica and one introduced species are well represented in the Antarctic soil seed bank (Wódkiewicz *et al.* 2013; Chwedorzewska *et al.* 2014), and they provide additional evidence that longevity and the ability to sustain a permanent soil seed bank are characteristic features of fine seeds. The above hypothesis was also confirmed in the Arctic region where more than 50% of local flora species produce permanent soil seed banks (Cooper *et al.* 2004). The formation of a permanent soil seed bank is a vital adaptive trait of flowering plants in polar regions which maximizes the reproductive success of plants in extreme, unstable habitats.

The observations performed under a scanning electron microscope revealed similarities in the microstructure of diaspore surfaces. Despite the above, the majority of microstructural traits, including the size of epidermal cells, shape of folds in anticlinal walls and the contour of periclinal walls, were species-specific.

The small size and low weight of diaspores in the analyzed Poaceae species indicate that the diaspores of polar plants are dispersed mainly by wind, although the analyzed caryopses were devoid of any structures that would facilitate this form of distribution. Glumes and glumelles play an important role in seed dispersal, and they can feature various structures, such as hairs or protrusions, that aid distribution by wind or animals. Short, hook-shaped protrusions were observed on the surface of glumes and awns in *D. antarctica*. The size of those structures suggests that they are not crucial for seed dispersal.

Edwards (1972) observed that birds use shoots of *D. antarctica* plants to build nests. The shoot segments carried by birds can contain panicles with caryopses which are thus distributed across significant distances. Fragments of *P. annua* plants can be used and dispersed in the same manner.

It remains unknown whether the diaspores of the analyzed Poaceae species can be effectively transported by water. The results of morphological and ecological studies of eight phanerogams growing on Kerguelen Islands (French Southern and Antarctic Lands) indicate that water plays an important role in seed dispersal (Hennion and Walton 1997). According to the above authors, seeds produced by other plant families, including Caryophyllaceae, are transported between Kerguelen Island by the sea, although this assumption has not been confirmed.

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