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Development of generative structures of polar Caryophyllaceae plants: the Arctic *Cerastium alpinum* and *Silene involucrata*, and the Antarctic *Colobanthus quitensis*

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Abstract: The embryology of three polar flowering plants of the family Caryophyllaceae was studied using the methods and techniques of the light, normal and fluorescence microscopes, and the electron microscopes, scanning and transmission. The analyzed species were *Colobanthus quitensis* of West Antarctic (King George Island, South Shetlands Islands) as well as *Cerastium alpinum* and *Silene involucrata* of the Arctic (Spitsbergen, Svalbard). In all evaluated species, flowering responses were adapted to the short Arctic and Australian summer, and adaptations to autogamy and anemogamy were also observed. The microsporangia of the analyzed plants produced small numbers of microspore mother cells that were differentiated into a dozen or dozens of trinucleate pollen grains. The majority of mature pollen grains remained inside microsporangia and germinated in the thecae. The monosporous Polygonum type (the most common type in angiosperms) of embryo sac development was observed in the studied species. The egg apparatus had an egg cell and two synergids with typical polarization. A well-developed filiform apparatus was differentiated in the micropylar end of the synergids. In mature diaspores of the analyzed plants of the family Caryophyllaceae, a large and peripherally located embryo was, in most part, adjacent to perisperm cells filled with reserve substances, whereas the radicle was surrounded by micropylar endosperm composed of a single layer of cells with thick, intensely stained cytoplasm, organelles and reserve substances. The testae of the analyzed plants were characterized by species-specific primary and secondary sculpture, and they contained large amounts of osmophilic material with varied density. Seeds of *C. quitensis*, *C. alpinum* and *S. involucrata* are very small, light and compact shaped.

Key words: Antarctic, Arctic, cleistogamous flowers, chasmogamous flowers, seeds, *Colobanthus quitensis*, *Cerastium alpinum*, *Silene involucrata*, pollen grains.

Introduction

Clonal growth and vegetative reproduction play a very important role in the propagation of plants inhabiting cold regions of the Earth, including the Arctic, Antarctica and high mountain ranges (Billings and Mooney 1968; Jónsdóttir *et al*. 1996). In clonally propagated plants, water and minerals can be obtained simultaneously from several sources, and nutrients are effectively managed by circulation inside and between ramets (Jónsdóttir *et al*. 1996). To date, no evidence has been found to confirm that *Colobanthus quitensis* (Kunth) Bartl., *Cerastium alpinum* L. and *Silene involucrata* (Cham. *et* Schltdl.) Bocquet, reproduce vegetatively, and the existing populations most probably developed from seeds (Edwards 1972; Parnikoza *et al*. 2011).

Generative reproduction in polar flowering plants. — Arctic vascular plants develop in a severe climate and produce a small number of mostly non-viable seeds (Billings and Mooney 1968; Bell and Bliss 1980; Phillip *et al*. 1990). The above is particularly observed in growing seasons with very low temperatures. According to Pirożnikow (1993), generative reproduction is successful in Arctic plants only in seasons with supportive weather, which occurs once every few years or even every decade. Similar observations were made in vascular plants native to Antarctica, *Colobanthus quitensis* and *Deschampsia antarctica* Desv. The above species produce numerous flowers and inflorescences every year, but they do not develop mature and fertile seeds regularly in every season (Holtom and Greene 1967; Edwards 1974; Convey 1996).

Bisexual flowers of vascular Antarctic plants are generally cleistogamous, but chasmogamous flowers are also reported. Chasmogamous flowers were produced in seasons with supportive weather conditions and in relatively warm, shielded and quiet microhabitats (Giełwanowska *et al*. 2005; Giełwanowska *et al*. 2011; Giełwanowska and Kellmann-Sopyła 2015). In the Antarctic *D. antarctica* and *C. quitensis*, cleistogamy probably fulfills the definition of cryocleistogamy because it is induced by low temperature and high humidity, which is also the case in Arctic grasses of the genus *Poa* (Levkovsky *et al*. 1981). Most Antarctic phanerogams produce cleistogamous flowers, and they are generally regarded as self-pollinating, although cross-pollination cannot be completely ruled out (Walton 1982; Parnikoza *et al*. 2011). According to Walton (1982), *C. quitensis* could be fertilized by wingless insects which abundantly colonized the flowers of the analyzed species on Signy Island. Many Arctic plants are self-pollinating, in particular in regions with extreme climate, but cross-fertilization with foreign pollen carried by wind and insects is also frequently observed (Bell and Bliss 1980).

According to Molau (1993), various generative reproduction strategies of Arctic and Alpine vascular plants are linked with their flowering phenology and the persistence of snow cover in the local habitat. The above author analyzed the reproductive traits of 137 Arctic and Subarctic-Alpine species and divided the flowering plants of the Arctic tundra into two categories. The first category comprises species that flower in spring and early summer and colonize habitats where snow cover melts relatively rapidly. Those plants reproduce by cross-fertilization and have a low ratio of the number of developed seeds to the number of ovules (Molau 1993). The second category covers species that flower in late summer and grow in habitats with persistent snow cover. Those plants are largely self-pollinating and highly fertile, but their seed development can be inhibited due to adverse weather in final stages of the growing season. Apomixis, a modified form of sexual reproduction where certain stages of the reproductive process are shortened, is often observed in this category of plants (Koltunow 1993). Accelerated reproduction can speed up seed development and maturation before the end of the short growing season in the Arctic (Hörland *et al*. 2011).

Materials and Methods

Plant material. — Three species of polar flowering plants of the family Caryophyllaceae were studied. Two species, *Cerastium alpinum* L. and *Silene involucrata*, were obtained from the Arctic region, whereas *Colobanthus quitensis* was harvested in Antarctica. Flower buds at different stages of development and mature diaspores were harvested in the area of the *H. Arctowski* Polish Antarctic Station (62°09.8'S and 58°28.5'W) and the *S. Siedlecki* Polish Polar Station in Hornsund, Spitsbergen (77°00'N and 15°33'E). The material was collected during Australian and Arctic summer 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 fixed upon harvest, and mature diaspores were preserved and dried before transport. Live plants were collected on the day of departure and transported to Poland in plastic containers. Diaspores of *Cerastium arvense* L. 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 plants of Caryophyllaceae 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. The collection is regularly refreshed by sowing, vegetative propagation and planting of specimens

transported from polar regions. Plants are grown at a temperature of 18–22°C in pots filled with a 2:1 mixture of hortisol and sand. Most plants produce flowers and viable seeds.

Micromorphological observations of diaspores under a light microscope (LM) and a scanning electron microscope (SEM). — Whole mature and dried seeds of *Cerastium alpinum*, *Colobanthus quitensis* and *Silene involucrata*, harvested in the Arctic and Antarctica, and *Cerastium arvense* harvested in the Olsztyn area, were used in observations. Before examination under a scanning electron microscope, diaspores were mounted on an aluminium holder and sprayed with gold powder in the JEOL JFC-1200 fine coater. 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 plant species harvested in polar regions and grown in a greenhouse were used in anatomical and ultrastructural examinations. 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. Secondary fixation was performed in 2.5% osmium tetroxide solution. The material was dehydrated in a graded series of alcohols and acetone, saturated in PolyBed 812 epoxy resin and embedded in pure resin. After resin polymerization, 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 mounted on slides, 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 mounted on 300-mesh copper grids. They 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 seeds 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 seeds of each species were sampled to determine their geometric parameters. Each seed 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 species, date and place of harvest, and the analyzed parameters of the examined seeds are presented in Table 1.

Results

Development and morphology of generative structures in polar Caryophyllaceae plants. — In their natural habitats, the examined polar species of the family Caryophyllaceae – *Colobanthus quitensis* (Figs 1a, b), *Cerastium alpinum* and *Silene involucrata* – produce flowers that develop into dry fruit – seed capsules that split upon maturation. Greenhouse-grown specimens also produce flowers and fruit (Figs 1d–s). Unlike most Antarctic plants which produce cleistogamous flowers, greenhouse-grown *C. quitensis* had chasmogamous flowers. Similarly to individuals growing in their natural habitats, the flowers of *S. involucrata* plants, propagated from material harvested in the Arctic, were set on long pedicels (several centimeters in length). In successive stages of growth, *S. involucrata* produced flowers that were set directly on the shoot or on short pedicels (Figs 1o, r).

Small, bisexual flowers of *C. quitensis* grow individually at the top of modular shoots, on short pedicels in leaf corners. The perianth is undifferentiated, green (Figs 1d–f) and composed of unfused elements. A single pistil with a large, pear-shaped ovary and a short style (Fig. 1i) ends in five stigma. The androecium is usually composed of six stamens with thecae located at stigma level. Much larger bisexual flowers of *C. alpinum* and *S. involucrata* are differentiated at the top of the shoot (Figs 1 j–m, o) or in leaf corners, individually or in groups of two or three. They have varied perianths (Figs 1k–m, o–r) composed of five hairy calyx lobes and five white petals. In *C. alpinum*, calyx lobes are not fused (Fig. 1l), whereas the sepals of the calyx in *S. involucrata* are fused (Fig. 1p). In *C. alpinum* and *S. involucrata*, the androecium is composed of 10 stamens; a single pistil with a short style ends

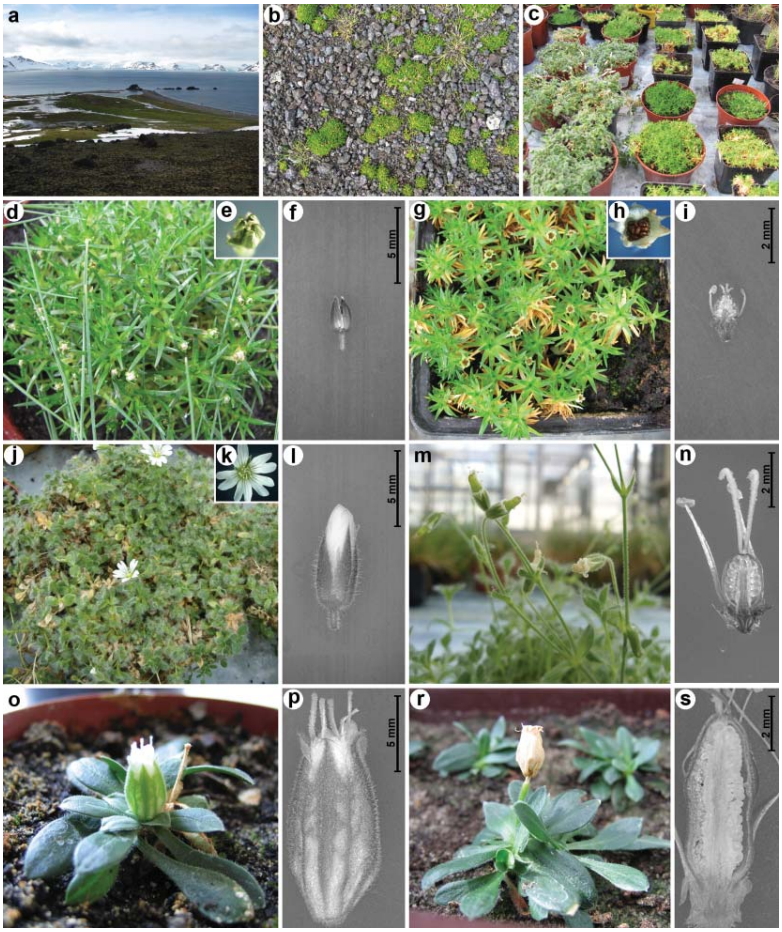

Fig. 1. Polish *H. Arctowski* Antarctic Station in the Admiralty Bay region (a) and morphology of analyzed species; *Colobanthus quitensis* plants growing in Antarctica (b), and in a greenhouse (c–i), *Cerastium alpinum* (c, j–n) and *Silene involucrata* plants growing in a greenhouse (o–s). **a** and **b.** In the Antarctic tundra *C. quitensis* plants are growing in a wet, fertile habitat at sea level (a) and in a several dozen meters from the Admiralty Bay (b). **c.** Plants of analyzed species grown in a greenhouse, at 18–20ºC. **d–f.** Developing bud of hermaphroditic flowers of *C. quitensis*. **g** and **h.** Brown mature seeds in open capsules of *C. quitensis*. **i.** Generative organs of *C. quitensis* flower with a large, pear-shaped ovary. Longitudinal section of the ovary with a centrally positioned placenta and ovules, two stamina (of the six most frequently differentiated stamina) and three stigmas (of the five most visible stigmas). **j** and **k.** Rapidly growing *C. alpinum* shoots with single pentamerous flowers. White corolla with five long petals and a deep groove in the perianth. **l.** Flower bud of *C. alpinum*. Densely hairy calyx sepals with a white and rolled corolla. **m.** Developing (green) capsules of *C. alpinum*, twice longer than the gamosepalous calyx with 10 sepals. **n.** Longitudinal section of the ovary of *C. alpinum* with numerous ovules a centrally (axially) positioned placenta, two stamina (of the five most frequently differentiated stamina) and two stigmas (of the five most visible stigmas). **o** and **p.** Developing flower of *S. involucrata* on a significantly shortened shoot (without pedicels). The plants were grown from seeds sown in a greenhouse in Olsztyn. **r.** Maturing capsule of *S. involucrata* on a short shoot. **s.** Longitudinal section of the ovary of *S. involucrata*. Highly numerous (white) ovules in an axially positioned placenta.

in five stigma, and ovules are differentiated from a centrally located placenta (Figs 1i, 1n, 1s). The ovaries of *C. quitensis* and *C. alpinum* produce several to several dozen ovules, and the strongly elongated ovary of *S. involucrata* produces even hundreds of ovules.

Microsporangia, microspores and male gametophytes. — Four wall layers: epidermis, endothecium, intermediate layer and tapetum, as well as centrally positioned archesporial tissue whose cells undergo meiosis, are differentiated in the microsporangia of all three analyzed species of the family Caryophyllaceae. Male gametophytes differentiate from microspores in successive stages of development.

Elongated epidermal cells, which are parallel to the long axis of the microsporangium, constitute the outermost layer of the thecal wall in all analyzed species (Figs 2a, b, d, i, j, ep). The mechanical endothecium layer is located directly under the epidermis, and it is underlain with the intermediate layer. In early stages of thecal development, the endothecium was composed of parenchymal tissue, whereas characteristic thickening of the walls was observed in the final stage of differentiation when endothecium was transformed into mechanical tissue (Figs 2a, b, d, e, i, j, en).

The pollen chamber was lined with secretory tapetal cells which degenerated upon the maturation of pollen grains (Figs 2a, b, d, e, i, j, ta). In the mature and open thecae of *C. quitensis* and *Silene involucrata*, the only remnants of tapetal tissue were small granules intensely stained with toluidine blue (Figs 2a, b, i, j, arrowheads). In the open thecae of *C. alpinum* filled with germinating pollen grains, tapetal tissue was present in the form of cytoplasm fragments with numerous follicles (Figs 2e, ta). The presence of completely disorganized tapetal cells was observed in infrequent developing thecae of *C. alpinum* (Fig. 2d).

Nearly all *C. quitensis* microspores formed three-celled male gametophytes. *C. quitensis* plants harvested in Antarctica had a very small number of microspores and pollen grains per loculus (Fig. 2a).

The grain pollen sporoderm of the evaluated Caryophyllaceae plants featured two distinctive layers of exine and intine. Exine was thick and osmiophilic, whereas intine was thinner and bright. Exine was further divided into sculpted sexine and smooth nexine (Figs 2c, ex, in, se, me). Deposits of granular, osmiophilic material were observed in caverns formed by fused columellae in both *C. quitensis* and *C. alpinum* (Fig. 2c, arrowheads). All evaluated Caryophyllaceae plants had polyporate pollen grains whose apertures were covered with intine and nexine (Figs 2b, c, e, h, m, ap).

Mature and split microsporangia of *C. quitensis* contained trinucleate gametophytes where two sperm cells were located near the nucleus of a vegetative cell (Figs 2c, f, g, k, l). Pollen grains germinated on stigma cells (Fig. 2h) and inside pollen sacs (Figs 2i, j).

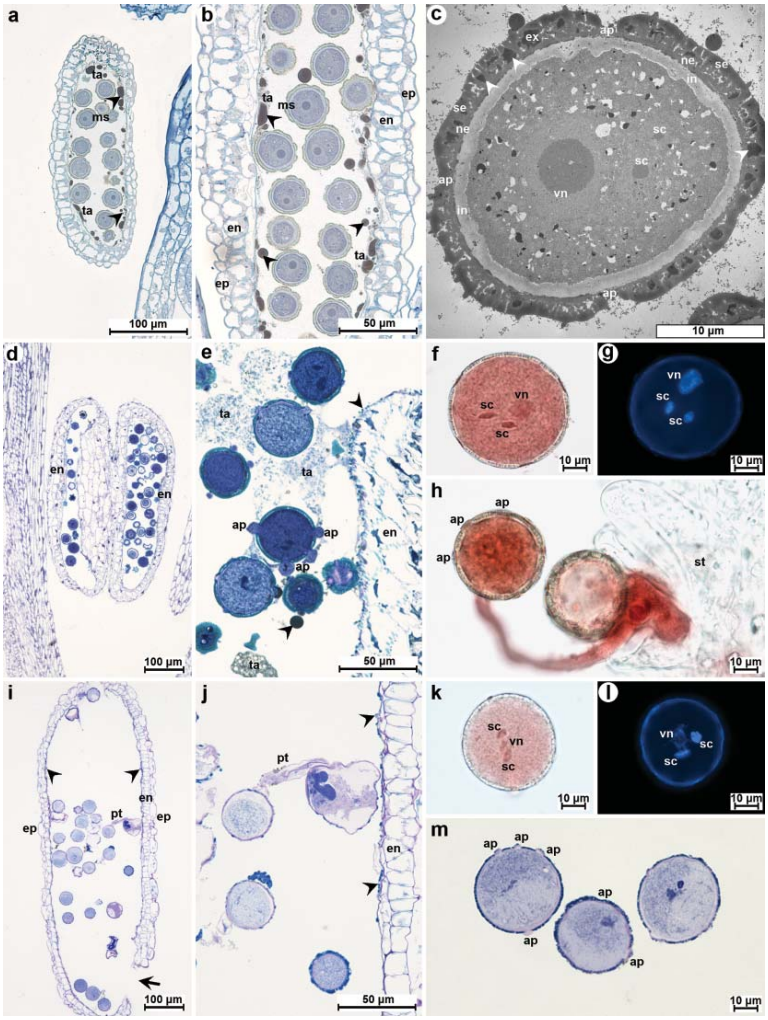

Fig. 2. Developmental stages of *Colobanthus quitensis* (a–c), *Cerastium alpinum* (d–h) and *Silene involucrata* (i–m) pollen grains in the anthers. **a** and **b.** Longitudinal sections of the microsporangium of an *C. quitensis* plant growing in Antarctica. Sparse microspores (ms) in the loculus surrounded by thick layer of tapetal tissue (ta, arrowheads), intermediate layers, endothecium (en) and epidermis (ep). Semithin sections stained with toluidine blue. **c.** Ultrastructure of the tricellular pollen grain of a *C. quitensis* plant growing in Antarctica. Vegetative nucleus (vn) and sperm cells (sc) of a male gametophyte of *C. quitensis* in the protoplast. Distinctive (osmiophilic) exine (ex) comprising the outer sexine (se), inner nexine (ne) and a weakly osmiophilic (polysaccharide) intine (in). Ultrathin section examined by TEM. **d** and **e.** Microspores in a bilocular anther of a greenhouse-grown *C. alpinum* plant. Monolayered endothecium (en) in the thecal wall facilitates thecal opening and pollen discharge. At the microspore stage, the tapetum is completely disorganized and comprises fragments of "foamy" cytoplasm and drops of osmiophilic material (arrowheads). Pollen grains covered with a thick sporoderm and numerous aperture sites (ap) develop synchronously. Necrotizing cells were observed sporadically. Semithin sections stained with toluidine blue. **f** and **g.** Tricellular mature pollen grains of *C. alpinum* plants

growing in a greenhouse. Vegetative nucleus (vn) and sperm cells (sc) of a male gametophyte in the protoplast. Material *in toto* stained with acetic Carmine (f) and with DAPI (g). **h.** Germinating pollen grains on the stigma (st) in *C. alpinum* plants growing in a greenhouse. Material *in toto* stained with acetic Carmine. **i** and **j.** Tricellular pollen grains in the anther of a greenhouse-grown *S. involucrata* plants. Anther with a broken wall at the stomium site (arrow). Germinating pollen grains inside the microsporangium. Pollen tube (pt) emerging through a distinctively larger aperture site of a polyaperturate pollen grain. Sheathed pollen grains on sporoderm surface. Thecal wall layers, epidermis (ep), endothecium (en), layer of parenchymal cells (intermediate) and tapetum residues (arrowheads). Semithin sections stained with toluidine blue. **k** and **l.** Tricellular mature pollen grains of *S. involucrata* plants growing in a greenhouse. Vegetative nucleus (vn) and sperm cells (sc) of a male gametophyte in the protoplast. Material *in toto* stained with acetic Carmine (k) and with DAPI (l). **m.** Polyaperturate (ap), mature pollen grains of *S. involucrata* plants growing in a greenhouse. Semithin sections stained with toluidine blue.

Morphology of ovules. — In the analyzed species of Caryophyllaceae plants, ovules with thick nucelli had two integuments, where the inner integument was differentiated before the outer integument (Figs 3a, b, e). The integuments grew simultaneously with the embryo sac, the wide ends of the inner integument were close to one another upon the maturation of the embryo sac, and they formed a micropylar canal in successive stages of development. Numerous organelles, mostly starch-filled chloroplasts, were visible during the development and maturation of seeds in all analyzed species. Cell vacuolization was accompanied by the accumulation of large and dark (osmiophilic) inclusions in the outer layer of the outer integument and the inner layer of the inner integument (Figs 3b, e, h).

Large nucelli contained dense cytoplasm and granulose cells with numerous organelles, variously shaped mitochondria and chloroplasts with dense, osmiophilic content. Upon the maturation of the embryo sac, the ovules of *C. quitensis* accumulated starch reserves, mostly in the chalazal region. After fertilization and during the development of the pre-embryo, starch reserves were also noted in lateral and micropylar segments of the ovule (Figs 3b, pe). Storage reserves were not accumulated in the nucelli of *C. alpinum* or *S. involucrata* in corresponding developmental stages (Figs 3e, h, i).

In the ovules of all examined Caryophyllaceae plants, megaspore tetrads were differentiated from meiotically dividing megasporocytes, and functional megaspores formed embryonic sacs of monosporous Polygonum type. The egg apparatus in *C. quitensis*, *C. alpinum* and *S. involucrata* was composed of an egg cell and two synergids with a distinctive and well-developed filiform apparatus. Polarization was observed in all three cells of the egg apparatus. In the ovule of *S. involucrata*, the filiform apparatus was differentiated at the top of synergids, and a large nucleus with a nucleolus were visible in the chalazal region of the egg cell (Figs 3a, e, i, ea, ec, sy). Synergid activity was clearly differentiated – one synergid accumulated reserve substances, and symptoms of degeneration were observed in the other (Fig. 3i, arrow, arrowhead).

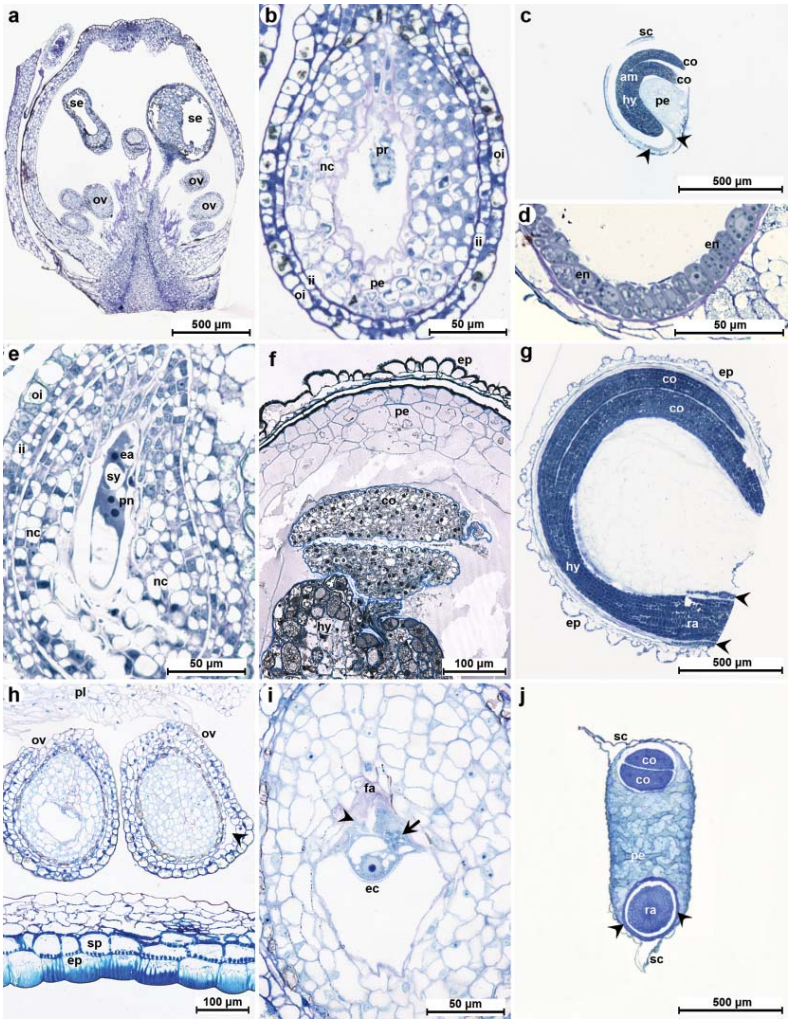

Fig. 3. Developing ovules and seeds in a flower buds of a *Colobanthus quitensis* (a–d), *Cerastium alpinum* (e–g) and *Silene involucrata* (h–j). Semithin sections stained with toluidine blue. **a.** Flower bud of a *C. quitensis* plant from Antarctica. Developing seeds (se) and less developed ovules (ov) in a longitudinal sections of the ovary. **b.** Developing proembryo (pr) and disorganization of protoplasts in nucellus cells (nc). Accumulation of lipid material in integument cells (in particular in the outer integument – oi). **c.** Anatomy of seed of a greenhouse-grown *C. quitensis* plant. Seed coat (sc) and a fragment of perispermium (pe), micropylar endosperm (arrowhead), embryonic shoot with an apical meristem (am), cotyledons (co) and a hypocotyl (hy). **d.** Part of the micropylar endosperm (en) in a *C. quitensis* seed. **e.** Anatomy of an ovule and the embryo sac of a greenhouse-grown *C. alpinum* plant. Cells of the egg apparatus (ea) in the micropylar region of an anatropous ovules. Synergids (sy) and polar nuclei (pn) with distinctive nucleoli in the central part of the embryo sac in the cytoplasm of a central cell. Differentiated layers of outer (oi) and inner (ii) integument cells and nucellus cells (nc). **f.** Part of *C. alpinum* seed cross-section along a plane perpendicular to the micropylar-chalazal axis. Visible testa with differentiated epidermal cells (ep), perisperm tissue (pe), embryonic cotyledons (co) and hypocotyls (hy).

g. *C. alpinum* seed cross-section along the shoot-root axis. Section of the root surrounded by micropylar endosperm (arrowheads). **h.** Anatomy of crassinucellate anatropous ovules (ov) of a greenhouse-grown *S. involucrata* plant. Ovules in the ovary chamber between placental tissue (pl) and the ovary wall, covered with epidermal (ep) and subepidermal (sp) cells with thickened walls. **i.** Egg cell (ec) with typical polarity; cell nucleus positioned at the chalasal region, and vacuoles – at the micropylar end of the egg cell. One synergid actively accumulates reserve material (arrows), and the other synergid shows clear symptoms of degeneration (arrowhead). Filiform apparatus (fa) of a synergid. **j.** Anatomy of seed of a *S. involucrata* plant growing in the Arctic. Seed cross-section along a plane perpendicular to the micropylar-chalazal axis. Seed coat (sc) with characteristic protrusions, perpendiculary incised, embryonic cotyledons (co), a radicle (ra) and micropylar endosperm (arrowheads) surrounding the radicle of a peripherally positioned embryo. Perisperm tissue (pe) is composed of cells filled with starch granules.

In the cytoplasm of the central cell in *C. alpinum*, polar nuclei (Fig. 3e, pn) and reserve substances identical to those found in the nucellus were visible in the vicinity of the egg apparatus (Fig. 3e, nu).

In the analyzed plants, ovules developed synchronously, but ovaries with developing seeds and less developed ovules were observed in selected flower buds of *C. quitensis* harvested in the Antarctic (Fig. 3a, ov, se).

Diaspore anatomy. — The seeds of the analyzed Caryophyllaceae species were composed of testa, perisperm (nutritive tissue), small amounts of endosperm and a curved, peripherally located embryo (Figs 3c, d, g, j). In mature diaspores, embryos had well developed organs: the apical meristem, two cotyledons, the hypocotyl-root axis and the radicle (Figs 3c, g, j, am, co, hy, ra). In mature seeds of the examined Caryophyllaceae, the radicle was surrounded by micropylar endosperm composed of a single layer of uniformly sized cells (Figs 3c, d, g, j, en, arrowheads). In *C. quitensis*, endosperm cells were filled with dense cytoplasm, organelles and osmiophilic droplets (Fig. 3d, en). The perisperm filled nearly 50% of the analyzed seeds. In mature seeds, perisperm cells contained mostly starch granules.

In the examined Caryophyllaceae species, testae were composed of modified cells of outer and inner integuments. The degree of cell aggregation and cell anatomy differed in various parts of the seed. In *C. quitensis*, two cell layers were visible in an area differentiating from the chalazal region (Figs 3b, ii, oi). Outer integument cells had thick and well-filled outer adjacent walls and much thinner inner adjacent and radial walls.

In *C. alpinum*, the seed coat was made up of two layers: the outer testa and the inner tegmen (Figs 3e–g, ii, oi, ep). Testa cells varied in shape. The outermost cells had thick outer adjacent and radial walls. Tegmen cells were flat, they had thin walls and were filled with osmiophilic material.

In *S. involucrata* seeds, the testa was composed of two cell layers. The thicker outer layer comprised isodiametric cells. Stratification of testa was observed in

certain regions of *S. involucrata* seeds (Figs 3h, j, sc, arrowhead). Large-cell protrusions were observed on the testa, perpendicular to the micropylar-chalazal axis at the level of the hypocotyl (Fig. 3j, sc).

Seed size and 1000 seed weight. — The analyzed Caryophyllaceae plants are characterized by very small seeds (Table 1). *Colobanthus quitensis* produces particularly small seeds, 0.6–0.7 mm in length and 0.4–0.5 mm in width. *C. quitensis* seeds harvested in two growing seasons in Antarctica had similar dimensions (Table 1). *Cerastium alpinum* and *Silene involucrata* produce somewhat larger seeds than *C. quitensis*. Average seed length and seed width reach 0.995 mm and 0.846, respectively, in *C. alpinum*, and 1.050 mm and 1.180 mm, respectively, in *S. involucrata*. The seeds produced by *Cerastium arvense* plants harvested in the Olsztyn area, characterized by an average length of 0.717 mm and an average width of 0.695 mm, were smaller than the seeds of the analyzed Caryophyllaceae species from the Arctic, but larger than the seeds of the Antarctic *C. quitensis*. The greatest differences in seed length and seed width, confirmed by the highest values of coefficients of variation, were observed in *S. involucrata* (Table 1).

The seed length to seed width ratio revealed that *C. quitensis* produced the most elongated seeds, whereas *S. involucrata* – the least slender seeds (Table 1). The average length to width ratio was somewhat higher in *C. alpinum* than in *C. arvense* seeds, but maximum and minimum values of the ratio were determined in the same range of 0.81 to 1.44 in both species.

C. quitensis seeds were characterized by the lowest weight and the smallest dimensions in the analyzed group of Caryophyllaceae plants. The 1000 seed weight of *C. quitensis* seeds did not differ significantly between batches of material harvested in growing seasons of 2009/2010 and 2011/2012, and it was determined at 50.8 mg and 53.3 mg, respectively. The 1000 seed weight of the remaining seeds was at least twice higher. *S. involucrata* produced the largest, but not the heaviest seeds (Table 1). The 1000 seed weight was highest in the Arctic species of *C. alpinum* (214.6 mg), and it was nearly half lower (115.5 mg) in *C. arvense*.

Shape and micromorphological parameters of seeds. — The seeds of the analyzed Caryophyllaceae plants were laterally flattened and kidney-shaped. *C. quitensis* produced triangular seeds which were narrower in the region of the radicle and wider near the cotyledons (Figs 4a–c). *C. alpinum* seeds were somewhat angular, sometimes spherical, whereas *C. arvense* seeds were generally more angular. Seeds produced by plants of the genus *Cerastium* were trapezoid in shape (Figs 4e, f, m, n).

Table 1

Arithmetic means (X), standard deviations (SD) and coefficients of variation (V%) for biometric parameters of seeds produced by the four examined Caryophyllaceae species. The data for *C. quitensis* refer to seeds harvested in two growing seasons.

Species	Season	Index	1000 seed weight [mg]	Length [mm]	Width [mm]	Slenderness
Colobanthus quitensis	2009/10	X SD V%	**50.8** ±1.7 3.3	**0.653** ±0.044 6.76	**0.481** ±0.036 7.46	**1.36** ±0.11 7.8
	2011/12	X SD V%	**53.3** ±2.3 4.3	**0.636** ±0.042 6.57	**0.485** ±0.032 7.46	**1.31** ±0.08 6.4
Cerastium alpinum	2012	X SD V%	**214.6** ±2.5 1.2	**0.995** ±0.103 10.40	**0.846** ±0.085 10.10	**1.18** ±0.11 9.3
Silene involucrata	2010	X SD V%	**171.6** ±3.1 1.8	**1.050** ±0.167 15.92	**1.180** ±0.168 14.26	**0.90** ±0.13 15.0
Cerastium arvense	2010	X SD V%	**115.5** ±2.1 1.9	**0.717** ±0.060 8.36	**0.695** ±0.063 9.11	**1.04** ±0.11 10.5

The shape of *S. involucrata* seeds ranged from kidney to angular. A wing-like protrusion (Figs 4i, j) composed of several, usually three to five convex epidermal cells, was observed on the dorsal side.

Mature seeds of all examined Caryophyllaceae species were brown. Under a scanning electron microscope, the surface of *C. quitensis* seeds was smooth and lustrous (Figs 4a–c), whereas the surface of seeds produced by plants of the genus *Cerastium* was strongly verrucous and matte in appearance (Figs 4e–g, m–o).

Testa epidermal cells of *C. quitensis* cells were elongated, with flat periclinal walls and folded and S-shaped anticlinal walls. Delicate and irregular secondary ornamentation, thickened along the edges, was observed on the surface of periclinal walls (Fig. 4d).

Epidermal cells of *S. involucrata* were characterized by collapsed and folded U- and V-shaped anticlinal walls as well as convex and partially collapsed periclinal walls. Fine, verrucous secondary sculptures were observed on the surface of periclinal walls (Figs 4k, l).

In seeds of the genus *Cerastium*, testa epidermal cells were star-shaped (Figs 4g, h, o, p). Periclinal walls in the epidermis of *C. alpinum* and *C. arvense* seeds were strongly convex, and anticlinal walls were collapsed, folded, U- and V-shaped (Figs 4h, p). Convex periclinal walls in strongly elongated cells

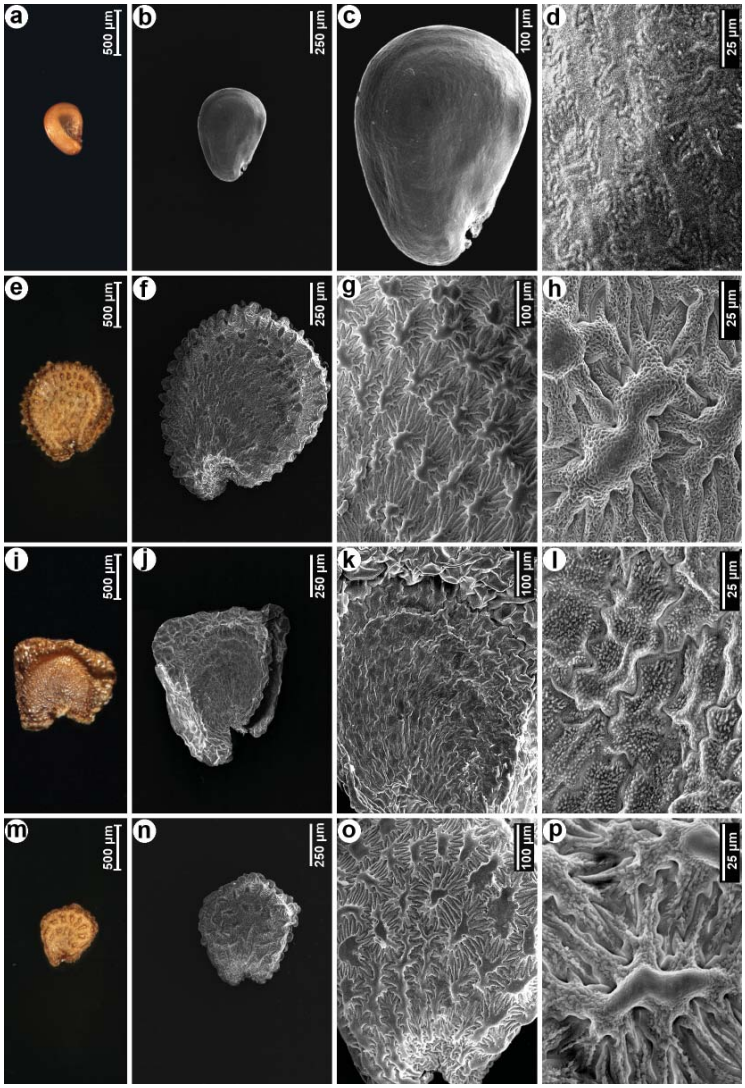

Fig. 4. Seed epidermis micromorphology of a *Colobanthus quitensis* plant growing in Antarctica (Figs 4. a–d), *Cerastium alpinum* (Figs 4. e–h) and *Silene involucrata* plants growing in the Arctic (Figs i–l), and *Cerastium arvense* plant growing near Olsztyn (Figs 4. m–p: a, e, i and m – images examined by LM; b–d, f–h, j–l and n–p – images examined by SEM. **a.** *Colobanthus quitensis* seed with distinctive contours of peripherally positioned embryo. **b.** and **c.** Smooth and glossy, without hairs or protrusions surface of a *C. quitensis* seed. **d.** Surface microstructure of a *C. quitensis* seed. Elongated testa epidermal cells with folded, S-shaped anticlinal walls, and periclinal walls covered with small, irregular nodes, with a clearly thickened secondary sculpture along the edges. **e.** *Cerastium alpinum* seed with distinctive contours of a peripherally positioned embryo. **f.–g.** Testa epidermal surface with distinctive sculpture of periclinal walls. **h.** Elongated testa epidermal cells with folded, U- and V- shaped collapsed anticlinal walls, secondary sculpture of convex periclinal walls covered with small nodules. **i.** Seed of a *S. involucrata* plant growing in the Arctic. Expanding testa produces a wing-like structure along ¾ of its circumference.

j.–k. Surface of the seed and epidermal cells with collapsed anticlinal walls. **l.** Epidermal cells with folded, U- and V-shaped collapsed anticlinal walls, sculpture of convex and collapsed periclinal walls covered with small nodules. **m.** Seed of a *C. arvense* plant growing near Olsztyn with clearly ornamented surface. **n.–o.** Regular distribution of epidermal cells with collapsed anticlinal walls, secondary sculpture of convex periclinal walls smooth or covered with small nodules.

formed a crest, and they were arranged in the shape of hills in cells with a more oval shape. Periclinal walls formed finger-like protrusions on the dorsal side of seeds. Epidermal periclinal walls in *C. alpinum* had verrucous surface (Fig. 4h). Epidermal periclinal walls in *C. arvense* seeds had partly verrucous sculpture, whereas the remaining, most convex regions had smooth secondary sculpture (Fig. 4p).

Discussion

Antarctica is characterized by significantly fewer species of flowering plants than the Arctic, and species diversity is both polar regions is much lower in comparison with other regions of the Earth. The key factors that inhibit the growth and development of flowering plants in polar regions are low temperature, low water availability and nutrient-poor soil (Billings 1987; Alberdi *et al*. 2002; Block *et al*. 2009). Other environmental hazards include high and largely unpredictable variability in temperature and moisture levels in local habitats (Bliss and Gold 1999; Jónsdóttir 2005; Convey 2012; Chwedorzewska *et al*. 2014; Giełwanowska *et al*. 2014, 2015; Giełwanowska and Kellmann-Sopyła 2015).

Polar vascular plants are characterized by a limited number of growth forms (Bliss 1962), which undoubtedly results from adaptation to extreme environmental conditions. The evaluated phanerogams, including the Antarctic *Colobanthus quitensis* and the Arctic *Cerastium alpinum* and *Silene involucrata*, as well as other representatives of polar fauna are small herbaceous plants with compact habit, producing numerous flowers and viable seeds.

The reproductive strategies of flowering plants native to polar regions have been widely discussed in the literature (Müller *et al*. 2011; Klimešová and Doležal 2012; Chwedorzewska *et al*. 2014), but the predominant form of reproduction in polar vascular plants has not yet been identified. The significance of vegetative reproduction in polar regions has been emphasized (Edwards 1974; Billings 1987; Pirożnikow 1993), but some authors noted that despite short and cold growing seasons that do not support generative reproduction, some polar vascular plants are capable of producing flowers and inflorescences that develop into fruit with viable seeds (Lewis Smith 1984; Convey 1996; Cooper *et al*. 2004; Alsos *et al*. 2013).

Morphology of generative structures in polar Caryophyllaceae plants

Development and anatomy of the male gametophyte. — In this study, microscopic analyses revealed that Caryophyllaceae produced flower buds with (in most cases) normally developed microsporangia, microspores and male gametophytes. Selected attributes of microsporangia and male germ line cells in Antarctic phanerogams growing in their native habitats were discussed in our previous studies (Giełwanowska *et al*. 2011).

Microscopic analyses of Arctic flowering plants indicate that similarly to the Antarctic species *Colobanthus quitensis*, pollen grains of *Cerastium alpinum* and *Silene involucrata* reach the trinucleate stage already before pollen dispersal. Some grains do not leave the theca and germinate in microsporangia, whereas in most angiosperms, the theca opens when pollen grains reach the stage of a two-celled male gametophyte, and their generative cells divide into two sperm cells only during the growth of the pollen tube. The production of trinucleate gametophytes in closed microsporangia of *Colobanthus quitensis* was observed in our earlier study (Giełwanowska *et al*. 2011). This strategy shortens the period between pollen release and successful fertilization, which minimizes the risk that pollen will be adversely influenced by external factors during its transport to the stigma. It was experimentally proven that three-celled pollen grains accumulate more mRNA and protein than two-celled gametophytes (Linskens 1988). Those compounds are accumulated during pollen maturation and are used up during germination and pollen tube growth. Trinucleate gametophytes which accumulate those substances germinate faster than binucleate gametophytes where mRNA and proteins are synthesized only during germination (Mascarenhas 1989).

Pollen grains of the analyzed Caryophyllaceae species were polyporate and covered by thick sporoderm with species-specific sculpture. In pollen grains of *C. quitensis* and *C. alpinum*, sexine was composed of columellae and a tectum layer. Pollen grains produced by plant species of the family Caryophyllaceae are generally polyaperturate and characterized by thick sexine with a tectum layer (Bittrich 1993). In pollen grains produced by *C. quitensis* and *C. alpinum*, sexine accumulated large amounts of granular osmiophilic material. In *S. involucrata*, the sexine layer also featured crystal-like inclusions, probably proteins, intensely stained with toluidine blue, which could be both enzymes and glycoproteins that regulate pollen specificity in the progamic phase (Castro *et al*. 2013).

Anatomy of the embryo sac. — In an analysis of the embryo sacs of polar plants belonging to the family Caryophyllaceae, synergids attracted particular attention. The filiform apparatus, through which the pollen tube penetrates the embryo sac in nearly all angiosperm species, including the studied representatives of Caryophyllaceae, was differentiated in the synergids. In the Antarctic species

C. quitensis, the filiform apparatus is always differentiated in synergids, and it is always well developed in plants growing in their native habitats in the Antarctic and in a greenhouse (Giełwanowska *et al*. 2011).

Disruptions in the development of thecae and ovules. — Generative organs of flowering plants are particularly sensitive to cold. Low temperatures induce structural and functional changes in plant tissues, which could disrupt fertilization and lead to the degradation of developing seeds. Differences in cold sensitivity have not yet been determined between various stages of generative reproduction, but the existing research suggests that pollen development and fertilization are most susceptible to low temperature (Kelly *et al*. 2010).

During the growing season, Antarctic plants are exposed to low temperatures and sudden temperature drops of 20–30°C on a daily basis (Convey 2012). For this reason, the sporadically observed disruptions in the development of male and female generative structures can probably be attributed to cold and freezing stress.

Early degeneration of the tapetum was relatively frequently noted in the thecae of *C. quitensis* plants harvested in the Antarctic (Giełwanowska *et al*. 2011). 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. Microspores are particularly sensitive to cold stress in early stages of development, during or directly after release from tetrads (Nishiyama 1984). Over that period, the tapetum is fully developed, and it supplies microspores with nutrients and enzymes.

In most cases, ovules developed synchronously in the ovaries of the analyzed Caryophyllaceae plants, but in selected flower buds of *C. quitensis* from the Antarctic, ovaries also contained large, developing seeds as well as ovules which were several-fold smaller and less developed. Embryo sacs with differentiated cells, *i.e*. synergids, an egg cell and a central cell, were visible in ovules. The above could indicate that the female gametophyte developed in immature ovules, but it remains unknown whether those ovules continued to develop and produce seeds. Such ovules could be characterized by reduced vitality. The presence of ovules whose vitality was compromised by low temperature has been described in *Cicer arietinum* L. The above slowed down the growth of pollen tubes in pistil tissues and lowered fertilization success (Srinivasan *et al*. 1999). Only some ovules with low fertility can be successfully fertilized and produce viable seeds.

Development and anatomy of seeds. — The accumulation of electron-dense and variously osmophilic material in the protoplasts of epidermal cells of integuments and their adjacent outer walls was observed during ovule development in the studied Caryophyllaceae plants. The deposits on testa cell

walls lead to the formation of a hard seed coat which plays an important role in the seed life cycle, in particular after seeds have been separated from the mother plant. The material in exotesta and endotegmen cells in the seeds of Caryophyllaceae plants as well as the accumulated tannins protect seeds against pathogenic fungi (Bittrich 1993).

In the investigated Caryophyllaceae plants, the perisperm occupied a large part of the seeds. The perisperm develops from nucellar cells in the chalazal region, and it constitutes the main nutritive tissue in seeds produced by Caryophyllaceae plants and most plants of the order Caryophyllales. Starch was the major reserve substance in perisperm cells. The endosperm was composed of a single layer of cells only in the micropylar region where it surrounded the radicle. A large, peripherally located embryo occupied nearly one-half of the seed in all analyzed species of polar plants.

Seed morphology in polar plants of the family Caryophyllaceae as an adaptive strategy. — The size and shape of seeds are part of a plant's adaptation strategy to the local environment. All of the analyzed Caryophyllaceae species produce very small and light seeds. The seeds of *Cerastium arvense* plants growing in the Olsztyn area were similar in size to the seeds of the polar species of *C. alpinum*. Their length and width did not exceed 2 mm, and 1000 seed weight was very low in the range of 50–350 mg. 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 the Antarctic, *Colobanthus quitenis* and *Deschampsia antarctica* (McGraw and Day 1997; Ruhland and Day 2001), and one introduced species *Poa annua* (Wódkiewicz *et al*. 2013; Chwedorzewska *et al*. 2014; Kellmann-Sopyła and Giełwanowska 2015), are well represented in the Antarctic soil seed bank, 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 (McGraw and Vavrek 1989; 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.

In this study, the observations performed under a scanning electron microscope revealed similarities in the surface microstructure of Caryophyllaceae seeds. Despite the above, the size of epidermal cells, the shape of folds in anticlinal walls, the contour of periclinal walls and the degree of cuticular ornamentation were species-specific. Taxonomic units belonging to the family Caryophyllaceae generally differ significantly in the sculpture of their testae, a parameter that is used to identify and discriminate between species (Fawzi *et al*. 2010).

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. The only exception were *S*. *involucrata* seeds which developed wing-like structures made of a single layer of epidermal cells filled with air.

Zoochory seems to play a minor role in polar flowering plants due to the scarcity of fauna in polar regions, in particular in the Antarctic (Prestrud *et al*. 2004). According to Kerlick *et al*. (1986), animals rarely consume fine seeds weighing less than 3 mg, therefore zoochory is probably a relatively rare dispersion mechanism in the studied *C*. *alpinum*, *C*. *quitensis* and *S*. *involucrata*.

It remains unknown whether the diaspores of the analyzed Caryophyllaceae species can be effectively transported by water. The results of morphological and ecological studies of eight phanerogams growing on subantarctic Kerguelen Islands indicate that water plays an important role in seed dispersal (Hennion and Walton 1997). According to the above authors, seeds produced by *Colobanthus kerguelensis* Hook.f. are transported between Kerguelen Island by the sea, although this assumption has not been confirmed. A morphological analysis revealed that *C*. *kerguelensis* seeds are highly similar to *C*. *quitensis* seeds, which suggests that hydrochory could be an important dissemination mechanism in this species.

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