10.2478/v10067-008-0004-3

ANNALES
UNIVERSITATIS MARIAE CURIE-SKŁODOWSKA
LUBLIN — POLONIA
VOL. LXIII/1/4 SECTIO C 2008

EWA SZCZUKA[1], IRENA GIEŁWANOWSKA[2,3],
IRENA AGNIESZKA PIDEK[4], ALEKSANDRA SETA[1],
MARCIN DOMACIUK[1], WIESŁAW KOŁODZIEJSKI[3]

[1] Department of Plant Anatomy and Cytology, Maria Curie-Skłodowska University, Akademicka 19, 20-033 Lublin, Poland, eszczuka@biotop.umcs.lublin.pl
[2] Department of Plant Physiology and Biotechnology, University of Warmia and Mazury, Oczapowskiego 1A, 10-719 Olsztyn, Poland
[3] Department of Antarctic Biology, Polish Academy of Science, Ustrzycka 10/12, Warsaw, Poland
[4] Department of Physical Geography and Palaeogeography, Maria Curie-Skłodowska University, al. Kraśnicka 2 c/d, 20-718 Lublin, Poland

Pollen of the Antarctic plants *Colobanthus quitensis* and *Deschampsia antarctica* and its representation in moss polsters

Pyłek antarktycznych roślin *Colobanthus quitensis* i *Deschampsia antarctica* i jego reprezentacja w próbkach mchów

SUMMARY

Formation, structure, and representation in moss polsters of pollen grains of *Colobanthus quitensis* (Kunth) Bartl. and *Deschampsia antarctica* Desv. (the only two native flowering plants growing in Antarctica) were investigated by means of light microscopy. Microsporogenesis and male gametogenesis of both investigated plant species proceeded in the way typical of other angiosperms. *C. quitensis* forms spherical, two-nuclear pollen grains enveloped by the thick polyporate sporoderm. Pollen grains of *D. antarctica* are three-nuclear, and their sporoderm contains one aperture. Both investigated species form chasmogamic and cleistogamic flowers. In moss samples, collected according to Pollen Monitoring Programme method, the local element – Poaceae and *Colobanthus* prevailed. The Poaceae pollen type includes mostly *Deschampsia*, but other grains, such as *Poa annua,* which was reported to grow in the vicinity of H. Arctowski Station, cannot be excluded. Among long-distance elements, *Nothofagus* pollen prevailed. One to seven pollen grains of this tree were found in each sample, which had been transported by strong westerly winds from southern South America. All the examined spectra also contained numerous fungal spores and other unidentified (probably non-sporomorphous) elements.

STRESZCZENIE

Rozwój i budowa pyłku *Colobanthus quitensis* (Kunth) Bartl. i *Deschampsia antarctica* Desv. (jedyne, rodzime rośliny kwiatowe rosnące na Antarktydzie) oraz jego występowanie w próbkach mchów powierzchniowych były badane przy użyciu mikroskopu świetlnego.

U obydwu badanych roślin, mikrosporogeneza i męska gametogeneza przebiegały w sposób typowy dla innych okrytozalążkowych. *C. quitensis* formuje kuliste, dwujądrowe ziarna pyłku otoczone grubą wieloporową sporodermą. Ziarna pyłku *D. antarctica* są trójjądrowe, a ich sporoderma zawiera jeden otwór. Obydwa badane gatunki formują kwiaty chasmogamiczne i kleistogamiczne. W próbkach mchów, pobranych zgodnie z wymogami Pollen Monitoring Programme, przeważał pyłek lokalnych roślin – Poaceae i *Colobanthus*. Pyłek typu Poaceae zawiera głównie *Deschampsia*, ale także nie można wykluczyć obecności innych rodzajów traw, przypuszczalnie *Poa annua,* którą stwierdzono w pobliżu Stacji H. Arctowskiego. Wśród elementu pochodzącego z dalekiego transportu przeważał pyłek *Nothofagus*. W każdej próbce mchów stwierdzono od 1 do 7 ziaren pyłku tego taksonu. Były one transportowane przez silne zachodnie wiatry z południowej części Ameryki Południowej. Wszystkie zbadane spektra pyłkowe zawierały liczne zarodniki grzybów i inne niezidentyfikowane elementy, przypuszczalnie nie sporomorfy.

Keywords: *Deschampsia antarctica*, *Colobanthus quitensis*, morphology, anatomical features, flower structure, pollen structure, pollen deposition, South Shetland Islands, Antarctica

INTRODUCTION

Colobanthus quitensis (Kunth) Bartl. and *Deschampsia antarctica* Desv. are the only two native flowering plants growing in Antarctica, which is considered to be the most distant, the coldest, and the most inaccessible polar region of the Earth. The extremely severe climatic conditions of the Antarctic region induce scientists to investigate the possibility of plant growth and development in this inhospitable area. Both species are model plants for analysis of various adaptation mechanisms to abiotic stress factors acting in Antarctica (Levi-Smith 2003). Much less attention has been paid to the biology of flowering and reproduction of these species. Therefore, in this paper, we focus on the pollen of both Antarctic flowering plants and its representation in moss polsters.

MATERIAL AND METHODS

Material

Fresh buds of *Colobanthus quitensis* (Kunth) Bartl. (Caryophyllaceae) and *Deschampsia antarctica* Desv. (Poaceae) were collected from plants growing under natural conditions near H. Arctowski Antarctic Station on King George Island (the South Shetland Islands) (see map in Fig. 1) during the Antarctic summer, mainly in January 2004.

Light microscopy

Flower buds were fixed in 3.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.0) for 8 h at the room temperature. The fixed buds were washed in two changes of 0.1 M phosphate buffer and postfixed overnight in 2.5% OsO_4. The material was subsequently washed in buffer, dehydrated in a graded ethanol series, and transferred to mixtures with increasing ratios of Poly Bed 812 resin.

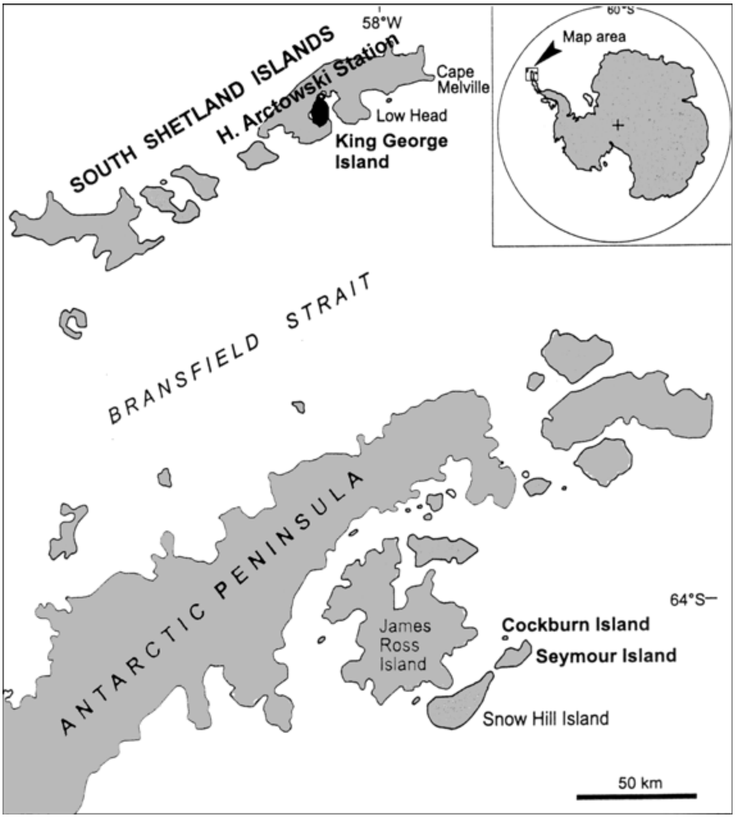

Fig. 1. Location map of King George Island in the South Shetland Islands Archipelago and Cockburn and Seymour Islands in the Antarctic Peninsula sector. Arrow on inset shows area of main map

Semi-thin sections (1–2 μm thick) were stained with toluidine blue and observed under the light microscope.

Moss-polsters 5 cm in diameter were collected from open tundra-like areas in the year 2002 at four sites where local Antarctic vegetation occurred. Sites no. 5 and 9 were located on a moraine (altitude10 m), site no. 10 – on Destiny Hill (altitude 25 m), site no. 12 – on Point Thomas (altitude of several m). At all the selected sites, *Colobanthus* and *Deschampsia* contributed in similar quantities to the composition of local vegetation.

Although different moss species were contained in a single sample, special attention was paid to the exact diameter of a moss polster (5 cm) to allow future comparisons with the results obtained as part of the Pollen Monitoring Programme (PMP). This international project aims at quantifying pollen deposition in different plant communities throughout diverse regions by standardised methods. As a rule, Tauber-style pollen traps with an opening of 5 cm in diameter are used and, additionally, moss-polsters of the same diameter are collected next to the pollen trap (Hicks et al 1996, 1999). Research by Smith (1991), however, proved that Tauber traps were unsuitable for catching pollen and spores in the Antarctic region. Instead, mosses, which are very good catching media, were analysed to check pollen dispersal and deposition of the local flowering plants.

Laboratory treatment of moss samples

The moss samples were processed according to the standard PMP laboratory procedure (Hicks et al. 1999) including sieving, Erdtman's acetolysis, staining with fuchsine and mounting in glycerine. One tablet with *Lycopodium clavatum* spores (Stockmarr 1971) was added to each sample in order to calculate pollen influx values. The tablet contained 10679 spores of *Lycopodium clavatum*. The number of *Lycopodium* tablets was not greater due to a low frequency of sporomorphs. For the same reason, at least 4 slides were examined during microscopic analysis. The counting of pollen grains and spores continued until at least 70 *Lycopodium* spores were encountered. Pollen influx (PI) values expressed as the number of pollen grains of a given taxon per cm^2 were calculated according to the formula: (number of *Lycopodium* spores added/number of *Lycopodium* spores counted) x number of pollen grains counted

RESULTS

Colobanthus quitensis and *Deschampsia antarctica* are small plants which, in the investigated area (vicinity of H. Arctowski Antarctic Station on King George Island), usually grow together with mosses and lichens, forming flat, dense mats.

In natural conditions, *C. quitensis*, a hardy, perennial plant, forms cushions consisting of numerous multi-module individuals. The single, small, inconspicuous, and bisexual flowers of this species may terminate module shoot or may be axillary. The stamens (usually 5) and the pistil coated with five elements of the undifferentiated perianth develop in closed flower buds hidden between leaf blades. The number of perianth elements may vary, and there may be four to six of them. The perianth is formed by green sepals arranged in two verticils.

The stamen of *C. quitensis* consists of a short filament bearing two-loculed anther. The central part of the young microsporangium is occupied by archesporial tissue, and then by microsporocytes. After meiosis with simultaneous cytokinesis, tetrahedrally arranged microspores (tetrads of microspores) are formed from microsporocytes. The released microspores are enveloped by the sporoderm. After mitotic division of the microspores, two-nuclear pollen grains appear in the loculus. Single microsporangium in a transverse section is regular and circular in shape. At the stage when pollen grains are in the anther loculus, the wall of the microsporangium is built of three layers of cells: the external – epidermis, middle – endothecium, and the most internal – tapetum. Mature pollen grains of *C. quitensis* are rather spherical, two-nuclear, and enveloped by the thick polyporate sporoderm (Fig. 2). The separating callose wall between generative and vegetative cells is not formed. In closed flowers of *C. quitensis*, microsporangia dehisce releasing pollen grains, which reach the stigma surface and germinate (Fig. 4).

D. antarctica forms very small, bisexual flowers gathered into a spikelet. Usually the spikelet is built of two to four flowers. In the flowers, there develop three stamens with short filaments. A single anther consists of three or four microsporangia. The central part of the *D. antarctica* flower is occupied by a bipartite stigma. All flower elements are enclosed in the lemmas (Fig. 3). In the microsporangium, archesporial cells develop into microsporocytes which undergo microsporogenesis. As a result, tetrads of microspores are formed (though sometimes the number of microspores is lower or higher). The microspores are arranged isobilaterally, and in a transverse section of the microsporangium only one tetrad is visible. The sporoderm enveloping the microspore and, later, the pollen grain is relatively thin. Single microsporangium is circular in a transverse section. At the stage when microspores are in the loculus, the external layer (epidermis) envelops a single cell layer of endothecium, cellular tapetum, and microspores. Mature pollen grains of *D. antarctica* are spherical, three-nuclear, and their sporoderm contains one aperture.

Table 1. Raw counts of different sporomorphs from four moss-polster samples

Taxon	sample 5		sample 9		sample 10		sample 12	
	No. of pollen grains	PI value	No. of pollen grains	PI value	No. of pollen grains	PI value	No. of pollen grains	PI value
LOCAL ELEMENT								
Colobanthus quitensis	101	451	6	27	1	6	9	58
Poaceae (mostly *Deschampsia*)	120	536	114	513	181	1108	109	707
LONG-DISTANCE TRANSPORT ELEMENT								
Nothofagus	5	22	4	18	7	43	1	6
Betula	1							
Rumex					1			
Urtica/Parietaria					2			
Cyperaceae					1			
Chenopodiaceae			1					
OTHER SPOROMORPHS and REMAINS OF UNRECOGNIZED ORIGIN								
Fungal spores	95		43		138		34	
Unknown spore type	87		29		88		6	
Unidentified (probably invertebrata remains)	66		36		16		20	
Indicator – *Lycopodium* spores counted	122		121		89		84	

The number of unidentified elements is also included. Pollen influx values were calculated for the two native Antarctic flowering plants and for *Nothofagus* – the most abundant long-distance element. Detailed pollen data are stored in the PMP Database at the University of Oulu (Finland).

In both species, *C. quitensis* and *D. antarctica*, apart from the cleistogamic (closed) flowers typically formed in unfavorable conditions, chasmogamic flowers were formed. In the case of the latter, the pollen grains were scatterred after anthesis in the vicinity of *D. antarctica* tussocks or *C. quitensis* tufts.

Thus, pollen dispersal and deposition of both plants can be traced in moss samples. In all the examined spectra, the frequency of sporomorphs was very low. The local element – Poaceae and *Colobanthus* prevailed (Table 1). The Poaceae pollen type includes mostly *Deschampsia*, but other grains, such as *Poa annua,* which was reported to grow in the vicinity of H.Arctowski Station, cannot be excluded. There was a significant difference in the number of *Colobanthus* pollen grains between the first sampling site (no. 5), where 101 grains were counted, and the remaining three sites (1–9 grains). Poaceae pollen, on the other hand, appeared in similar quantities in all the samples (Table 1). Among long-distance elements *Nothofagus* pollen prevailed (Fig. 6). One to seven pollen grains of this tree were found in each sample, which had been transported by strong westerly winds from southern South America.

The PI values for *Colobanthus* (Fig. 5) varied from 451 pollen grains/cm^2 at site no. 5 to 6 grains/cm^2 at site no. 10. The values for Poaceae ranged from 513 to 1108 grains/cm^2. All the examined spectra also contained numerous fungal spores and other unidentified (probably non-sporomorphous) elements.

DISCUSSION

Microsporogenesis of both investigated plant species proceeds in the way typical of other angiosperms. In *Colobanthus quitensis*, microsporogenesis ends with simultaneous cytokinesis, and in *Deschampsia antarctica* the division is successive. Simultaneous cytokinesis is characteristic of dicots and successive cytokinesis – of monocotyledones.

Similarly to microsporogenesis, male gametogenesis is typical of other angiosperms (for detailed description of both processes in flowering plants see Raghavan, 2000). Characteristically, in *C. quitensis*, the separating calose wall is not formed between generative and vegetative cells. In closed flowers of *C. quitensis* and *D. antarctica*, microsporangia dehisce releasing pollen grains, which reach the stigma surface and germinate.

Both investigated species form chasmogamic and cleistogamic flowers. The structure of both kinds of flowers indicates the possibility of cross-pollination as well as self-pollination (Szczuka et al 2006).

The very low pollen frequency in moss polsters was in accordance with low pollen production of both native plants and the cleistogamic mechanism of pollination. In spite of the fact that *Colobanthus quitensis* flowers do not release much pollen, at site no. 5 101 pollen grains (Fig. 5) were found in a moss-polster, which gave surprisingly high pollen influx values at this site (451 grains/ cm^2). The difference between this particular sample and the other three may have been caused by warmer conditions occured locally that enabled the development of chasmogamic instead of cleistogamic flowers. Pollen influx values of Poaceae were higher due to more abundant production and a more efficient mechanism of dispersal, probably, at least to some extent connected with the role of the wind. The characteristics of pollen spectra agree with the results obtained by van der Knaap and van Leeuven (1993) in the same region (vicinity of H. Arctowski Station). They found 13 thousand pollen grains of Poaceae, 226 grains of *Colobanthus* and 141 grains of *Nothofagus* in a moss sample (size15 x 20 cm) taken from a Penguin Ridge site. Both the Antarctic flowering plants were present among local vegetation. The large share of long-distance transported pollen in which *Nothofagus* prevailed was also stressed by Smith (1991) and Dorozhkina (2007 a, b). The latter author did not report any *Colobanthus* in her pollen spectra although local Poaceae were quite abundant among pollen types. The absence of *Colobanthus* seems strange as the moss-polsters were collected by Dorozhkina (2007 a,b) in King George Island.

Moss-polster sample taken by van der Knaap and van Leeuwen (1993) at Deception Island, where local population of *Colobanthus* was distant (ca. 1 km away from the sampling site), was very poor in pollen of both taxa (2 grains of *Colobanthus*, 15 of Poaceae), while *Nothofagus* was still the main component of the pollen spectrum together with a high number of unidentified spores. Most of the sporomorphs in this sample were long-distance transported (van der Knaap, van Leeuven 1993). *Nothofagus dombeyi* was also the dominating type in pollen diagrams from lake deposits of south-eastern Patagonia. This Andean element (where three species of *Nothofagus* were present) was reported to have been transported over thousands of kilometers towards Antarctica by strong winds (Haberzettl et al. 2005). As a rule, pollen of herbaceous plants is poorly dispersed and distributed and so, represents mainly strictly local vegetation, but in the Antarctics, even if the number of pollen grains of native plants is very small, still some of them may have originated outside the sampling point (van der Knaap, van Leeuven 1993).

REFERENCES

1. DOROZHKINA M. 2007 a. Results of monitoring annual pollen influx on the King George Island, Antarctica. [In:] Pollen Monitoring Programme. 6th International Meeting, 3rd–9th June 2007, Jurmala, Latvia. Volume of Abstracts: p.13.
2. DOROZHKINA M. 2007 b. Long-distance transported pollen and spores in the moss polsters from the King George Island, Antarctica. [In:] Pollen Monitoring Programme. 6th International Meeting, 3rd–9th June 2007, Jūrmala, Latvia. Volume of Abstracts: p.14.

3. Haberzettl T., Fey M., Lücke A., Maidana N., Mayr Ch., Ohlendorf Ch., Schabitz F., Schleser G. H., Wille M., Zolitscha B. 2005. Climatically induced lake level changes during the last two millenia as reflected in sediments of Laguna Potrok Aike, southern Patagonia (Santa Cruz, Argentina). Journal of Paleolimnology 33: 283–302.

4. Hicks S., Ammann B., Latalowa M., Pardoe H., Tinsley H. 1996. European Pollen Monitoring Programme. Project Description and Guidelines. Oulu Univ. Press, Oulu, Finland.

5. Hicks S., Tinsley H., Pardoe H., Cundill P. 1999. European Pollen Monitoring Programme. Supplement to the Guidelines. Oulu Univ. Press, Oulu, Finland.

6. Levis-Smith R. I. 2003. The enigma of *Colobanthus quitensis* and *Deschampsia antarctica* in Antarctica. [In:] Antarctic Biology in a Global Context. Huickes A. H. I., Gieskes W. W. C., Schorno R. L. M., van der Vies S. M. and Volff W. I. (eds.), Backham Publishers, Leiden, The Netherlands, 234–239.

7. Raghavan V. 2000. Developmental Biology of Flowering Plants. Springer–Verlag–New York, Inc. pp. 186–227.

8. Smith R.I. Lewis 1991. Exotic sporomorpha as indicators of potential immigrant colonists in Antarctica. Grana 30: 313–324.

9. Stockmarr J. 1971. Tablets with spores used in absolute pollen analysis. Pollen et Spores 13: 615–621.

10. Szczuka E., Giełwanowska I., Seta A., Domaciuk M. 2006. Pollen and pollination in the Antarctic flowering plant of *Colobanthus quitensis* (Kunth.) Bartl. Materials of XIXth International Congress on Sexual Plant Reproduction “From gametes to genes”. Budapest, Hungary, 11–15 July 2006: p. 221.

11. Van der Knaap W. O., van Leeuwen J. F. N. 1993. A recent pollen diagram from Antarctica (King George Island, South Shetland Islands). Holocene 3,2: 169–173.

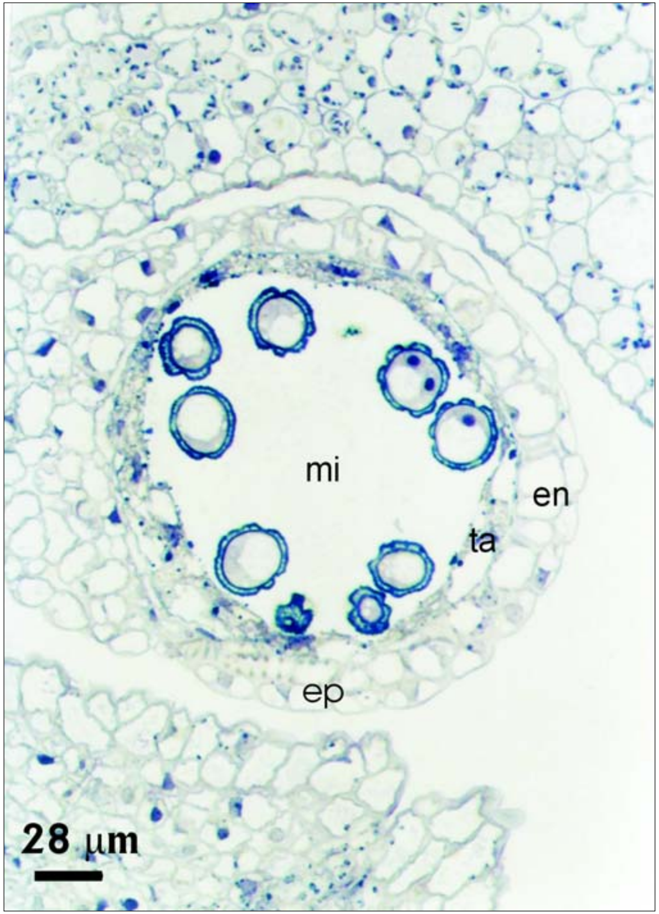

Fig. 2. A magnified microsporangium (mi) of *Colobanthus quitensis* with pollen grains in the loculus. A semi-thin, transverse section stained with toluidine blue. Visible epidermis (ep), endothecium (en) and disintegrated tapetum (ta)

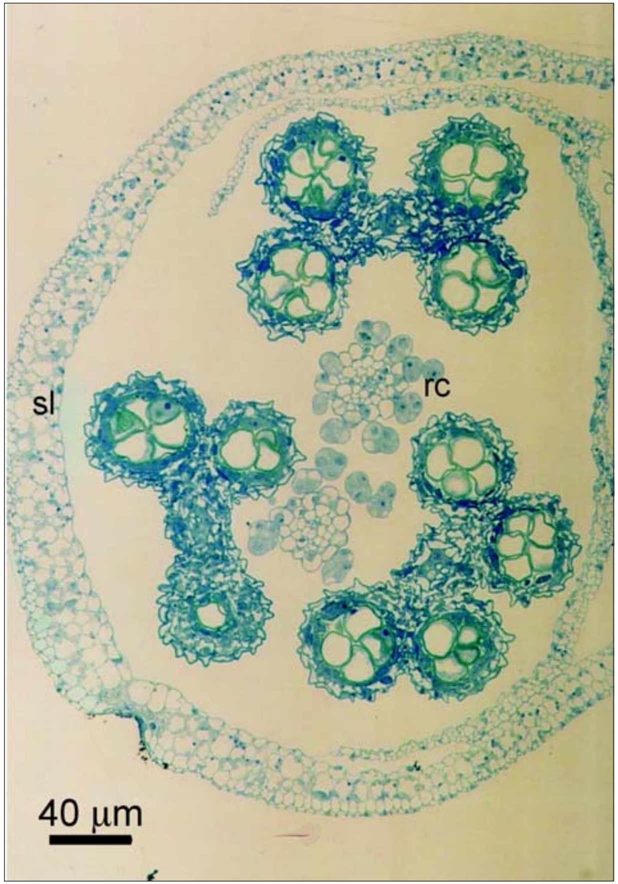

Fig. 3. Structure of a flower of *Deschampsia antarctica*. Stamina enclosed by the lemmas (sl). Three stamen heads with microsporangia are visible. In one head there are three microsporangia. In the center there is a bipartite stigma with receptive cells (rc). A semi-thin, transverse section stained with toluidine blue

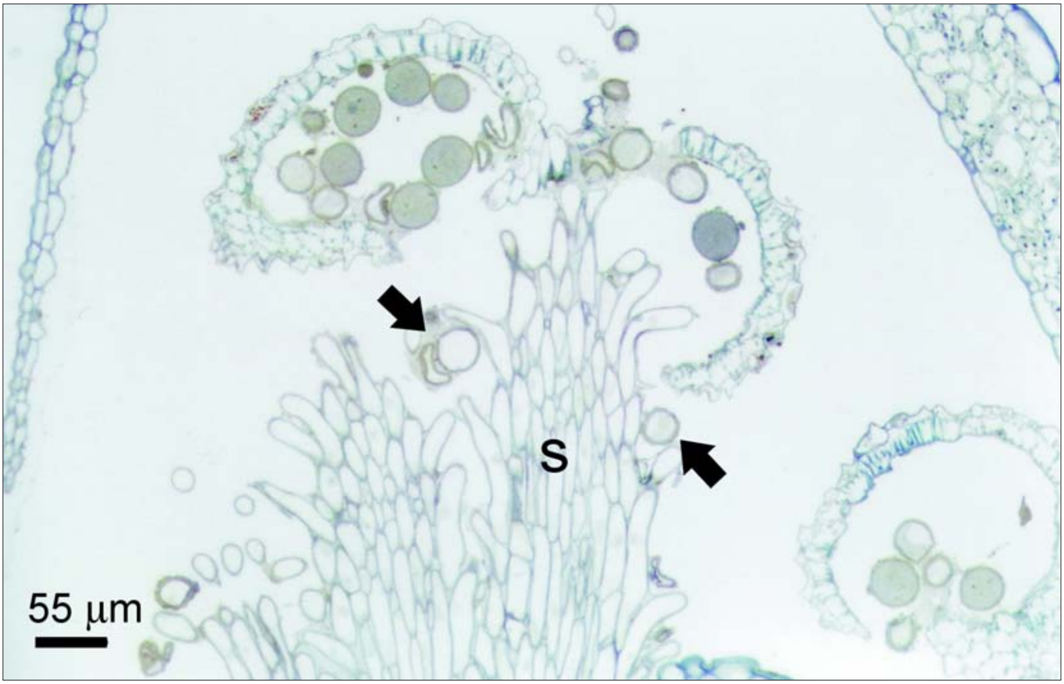

Fig. 4. A longitudinal section of a closed flower of *Colobanthus quitensis* with pollen grains in opened microsporangia or released from microsporangia. Pollen grains (arrows) on a dry, pulmose stigma (s). A semi-thin section stained with toluidine blue

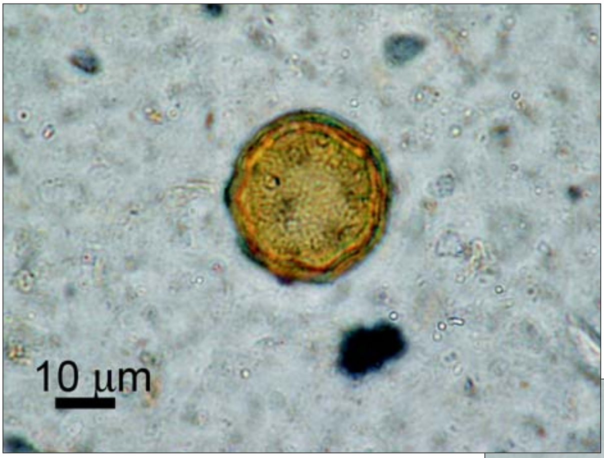

Fig. 5. A pollen grain of *Colobanthus quitensis* in a moss-polster

Fig. 6. *Nothofagus* pollen – the most abundant element of long-distance transport