ACTA BIOLOGICA CRACOVIENSIA Series Botanica 58/1: 19–28, 2016
DOI: 10.1515/abcsb-2016-0001

OVULE STRUCTURE OF SCOTCH THISTLE *ONOPORDUM ACANTHIUM* L. (CYNAREAE, ASTERACEAE)

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Received October 15, 2015; revision accepted December 18, 2015

Studies concerning the ultrastructure of the periendothelial zone integumentary cells of Asteraceae species are scarce. The aim was to check whether and/or what kinds of integument modifications occur in *Onopordum acanthium*. Ovule structure was investigated using light microscopy, scanning electron microscopy, transmission electron microscopy and histochemistry. For visualization of calcium oxalate crystals, the polarizing microscopy was used. The periendothelial zone of integument in *O. acanthium* is well developed and composed of mucilage cells near the integumentary tapetum and large, highly vacuolated cells at the chalaza and therefore they differ from other integumentary cells. The cells of this zone lack starch and protein bodies. Periendothelial zone cells do not have calcium oxalate crystals, in contrast to other integument cells. The disintegration of periendothelial zone cells was observed in a mature ovule. The general ovule structure of *O. acanthium* is similar to other members of the subfamily Carduoideae, although it is different to "Taraxacum", "Galinsoga" and "Ratibida" ovule types.

Keywords: ovule, integument, Asteraceae, calcium oxalate crystals

INTRODUCTION

The genus *Onopordum* includes about 60 species (Susanna and Garcia Jacas, 2007). In Poland *O. acanthium* L. was cultivated as a food plant in the past but now is used for its ornamental value in cottage gardens. This species has the status of archaeophyte in the Polish flora (Zając and Zając, 2014). Thistle species are invasive in both agricultural areas and disturbed places in warm climates, and this especially makes them a problem in the USA and Australia (Briese, 1989a, b). Thus, studies of the ovule and cypsela structure and also the germination of cypsela (e.g., Qaderi and Cavers, 2003) may help in determining how to effectively fight against this weed.

In the ovules of Asteraceae, part of the internal integument behind the endothelium and the region near the antipodal cells is called the periendothelial zone (Pandey et al., 1978). The cells of this zone have a specific differentiation that is different from other integumentary cells (Cooper and Brink, 1949; Engell and Petersen, 1977; Pandey et al., 1978; Musiał et al., 2013). Studies concerning the ultrastructure of the periendothelial zone integumentary cells are scarce and only a few taxa have been analyzed (Newcomb, 1973a; Figueiredo et al., 2006; Musiał et al., 2013; Kolczyk et al., 2014; Płachno et al., 2015). Recently, Płachno et al. (2015) showed that the periendothelial zone cells in *Taraxacum* are specialized mucilage cells. Thus, the thick cell wall material with a *Taraxacum* type of ovule such as in the genera *Solidago*, *Chondrilla* and *Bellis* probably also has a mucilage character (Płachno et al., 2015).

According the structure of periendothelial zone, three types of ovules were proposed by Kolczyk and co-authors (2014) in Asteraceae: "Taraxacum", "Galinsoga" and "Ratibida". However, the taxa that were examined in this paper belong only to the Asteroideae and Cichorioideae subfamilies. The ovule or fruit structure of Carduoideae mem-

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PL ISSN 0001-5296

bers was studied by several authors but mainly at the level of light microscopy (e.g., Dormer, 1961; Dittrich, 1968, 1970; Singh and Pandey, 1984; Pérez-García and Duran, 1987) or using SEM (Talukdar, 2013). Only Figueiredo et al. (2006) briefly described the special ovule tissues in *Cynara cardunculus* at the TEM level; however, there is much discussion about the interpretation of the development of this tissue (nucellar *versus* integumental) (Kolczyk et al., 2014).

The aim of this study was to check whether and/or what kinds of integument modifications occur in the *O. acanthium* member of subfamily Carduoideae.

MATERIALS AND METHODS

PLANT MATERIAL

Onopordum acanthium L. (Cynareae, Asteraceae) flowers were collected in the Botanic Garden of Jagiellonian University in Kraków during the summers of 2014 and 2015.

LIGHT AND ELECTRON MICROSCOPY STUDIES

The material was fixed in 2.5% buffered (0.1 M phosphate buffer, pH=7.4) glutaraldehyde, washed four times in the same buffer and dehydrated in a graded ethanol series for 15 min at each concentration and kept overnight in absolute ethanol. Later, the samples were infiltrated for 1 h each in 3:1, 1:1 and 1:3 (v/v) mixtures of absolute ethanol and Technovit and stored for 12 h in pure Technovit. The resin was polymerized by adding a hardener. The material was sectioned to 7 μm with a rotary microtome (Microm, Adamas Instrumenten), stained with 0.1% toluidine blue O (TBO) and mounted in Entellan synthetic resin (Merck).

The selected sections were stained with naphthol blue black (NBB) for total protein staining. The sections were immersed in 0.1% NBB in 7% acetic acid for 2 min and then destained in 7% acetic acid.

The periodic acid-Schiff (PAS) reaction was used to detect water insoluble polysaccharides with 1,2-glycol groups.

For the visualization of calcium oxalate crystals, the selected Technovit sections were analyzed using a Nikon Polarizing Microscope ECLIPSE E600 POL (in the Department of Mineralogy, Petrology and Geochemistry of Jagiellonian University in Kraków).

For the electron microscopy studies, ovaries or isolated ovules were fixed with 2.5% formaldehyde and 2.5% glutaraldehyde in a 0.05 M cacodylate buffer (pH=7.0) or 2.5% glutaraldehyde in a 0.1 M sodium phosphate buffer (pH=7.4). The material was postfixed in 1% OsO_4 in a cacodylate buffer for 2 h at room temperature, rinsed in the same buffer, dehydrated with acetone and embedded in an Epoxy Embedding Medium Kit (Fluka). Semithin sections were stained with methylene blue and examined using an Olympus BX60 microscope. Ultrathin sections were cut on a Leica Ultracut UCT Ultramicrotome. After contrasting with uranyl acetate and lead citrate, the sections were examined using a Hitachi H500 electron microscope at 75 kV (in the Department of Animal Histology and Embryology, University of Silesia).

For SEM, the material was fixed in the same way as for TEM and later hand-sectioned. Later, the samples, which had been dehydrated in ethanol as well as in an acetone series, were critical-point dried in liquid CO_2 and coated with gold using a JEOL-JFC 1100E sputter coater. The material was viewed under a HITACHI S-4700 microscope (Scanning Microscopy Laboratory of Biological and Geological Sciences, Jagiellonian University in Kraków).

RESULTS

O. acanthium has an anatropous, teninucellate, unitegmic ovule that is typical of Asteraceae. The mature ovule (at the organised female gametophyte stage) is large (about ~3838 μm long, ~1666 μm wide) and almost fillsthe ovary locule (Fig. 1a,b). In this stage the nucellus is absent. The funiculus is clearly visible but is not massive in comparison with the whole ovule (Fig. 1c). A single, well-developed vascular bundle enters the funiculus, passes through the chalaza and terminates in the apex of the integument near the micropyle (Fig. 1d). The tracheary elements in the vascular bundle are well developed.

The micropyle is closed; the micropylar canal is completely filled with the ovule transmitting tissue and its extracellular matrix (Fig. 2a,b). The cells of the ovule transmitting tissue are elongated in the direction of the embryo sac and rich in endoplasmic reticulum (not shown). The integument is thick (Fig. 3a). Its external epidermis consists of highly radially elongated cells. These cells are highly vacuolated and starch-less, but have electron-dense plastids (Fig. 3a–c). These cells are especially prominent at the micropyle pole (Fig. 1d). The next integument layer is a thick zone of small parenchyma cells. The innermost layer of the integument forms the endothelium. The inner region of the integument (periendothelial zone) is composed of large cells, which differ from the other integumentary cells (Fig. 3d). The cells of the periendothelial zone are starch-less in contrast to the other paren-

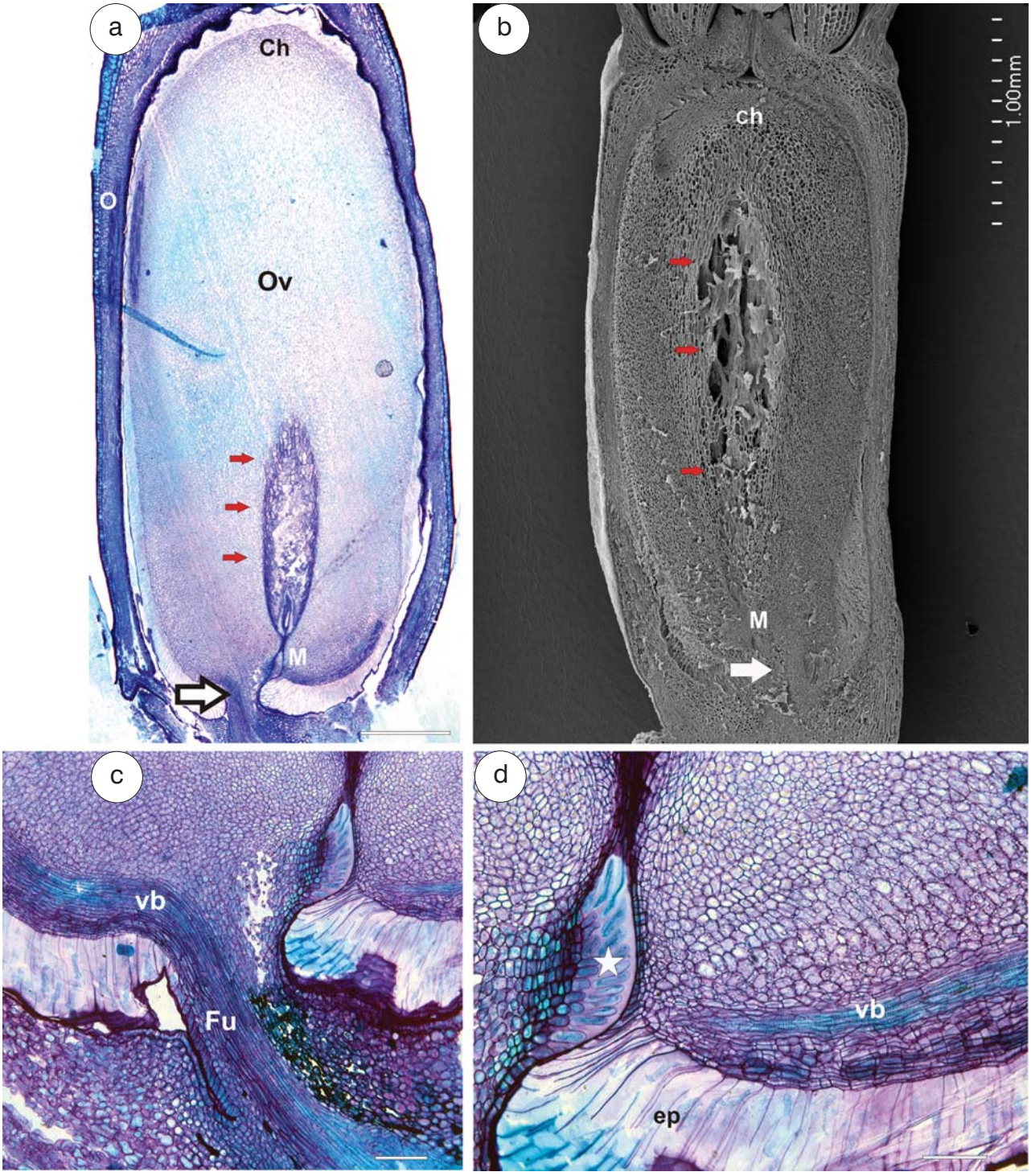

Fig. 1. General ovule structure in *Onopordum acanthium*. (**a**,**b**) Longitudinal section of the unilocular ovary (O) with the anatropous unitegmic ovule (Ov): chalaza (Ch), funiculus (white arrow), periendothelial zone (red arrows), micropyle (M); bar = 500 μm and bar = 1 mm, (**c**,**d**) Funiculus and micropyle structure: vascular bundle (vb), funiculus (Fu), ovule epidermis (ep), pollen transmitting tissue (star), bar = 100 μm and bar = 50 μm.

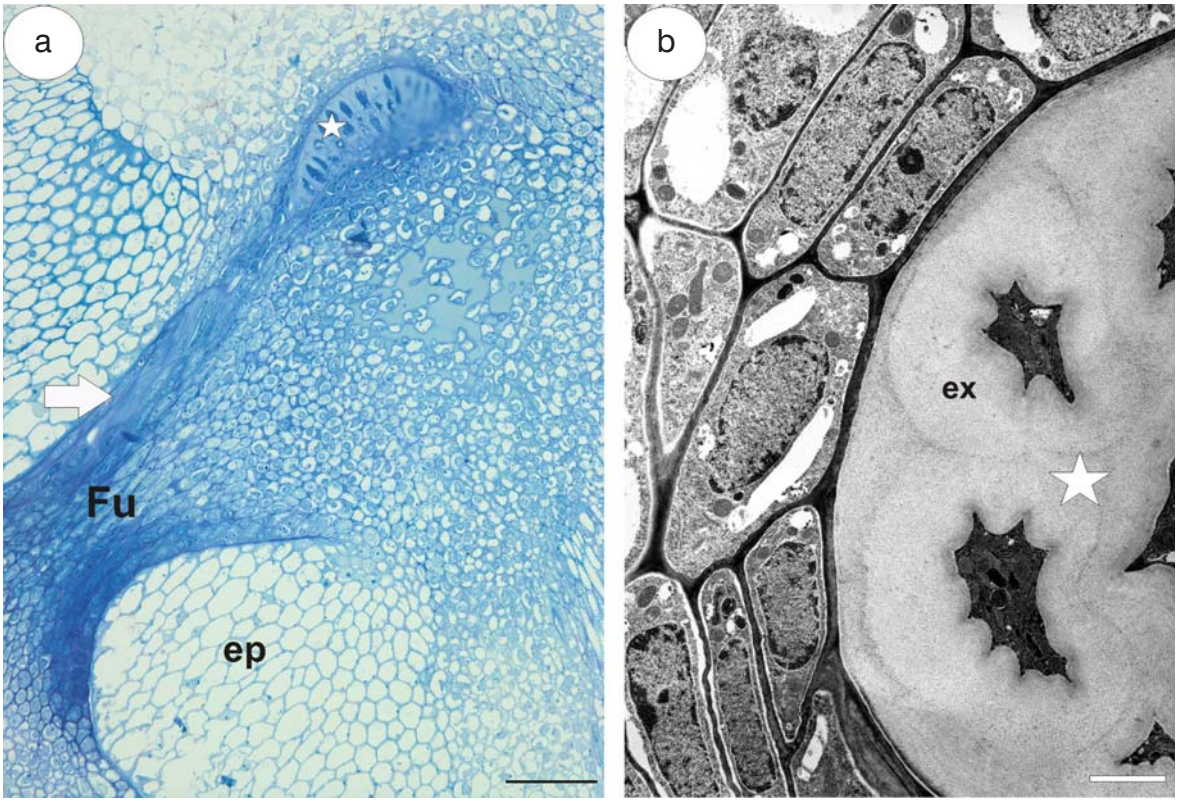

Fig. 2. Transmitting tissue structure. (**a**) Semi-thick section showing funicular transmitting tissue (arrow) and transmitting tissue in the micropyle (star), funiculus (Fu), ovule epidermis (ep), bar = 20 μm, (**b**) Electron micrograph showing micropylar transmitting tissue cells (star); extracellular matrix (ex), bar = 1.85 μm.

chyma of the integument and their cell walls are extremely swollen. The cell walls of the periendothelial zone cells react positively to the PAS test (Fig. 3e). Some disintegration of periendothelial zone cells was observed, which was also clearly visible in SEM (Fig. 3f).

There are differences in the case of the occurrence of calcium oxalate crystals (Fig. 4a). Most of the integumentary parenchyma cells have small rod-shaped calcium oxalate crystals. However, there is a ring of cells near the micropyle, which are especially rich in crystals (Fig. 4a). The periendothelial zone cells do not have calcium oxalate crystals or they have only a few (these could be artifacts of the preparations of the materials). There is a crystalliferous layer of the ovary wall (Fig. 3a and Fig. 4b). The cells of this layer have large prismatic crystals (Fig. 4c), and some of them are shaped like a fish-tail.

Staining for total proteins showed no protein bodies in the periendothelial zone cells. Protein bodies occur in the funiculus cells, transmitting tissue cells and crystalliferous layer of ovary (not shown).

ULTRASTRUCTURE OF PERIENDOTHELIAL ZONE CELLS

At the stage of mature embryo sac, some of the periendothelial zone cells near the integumental tapetum have reduced protoplasts and some of these have begun to degenerate (Fig. 5a,b). Loosely arranged material (mucilage) is deposited in the extraplasmatic space as a layer between the plasma membrane and the cell wall (Fig. 5c). Many strands of rough endoplasmic reticulum, dictyosomes and mitochondria are present in the cytoplasm of the periendothelial zone cells (Fig. 5c and Fig. 6a–c). The chalazal periendothelial zone cells are highly vacuolated. In these cells the mucilage accumulated in the cell wall (Fig. 6a–c).

DISCUSSION

The general structure of *O. acanthium* is similar to other members of Cynareae (e.g. Dittrich, 1968, 1970; Singh and Pandey, 1984; Figueiredo et al., 2006). The main character of the ovule of these taxa is the radially elongated epidermal cells (mem-

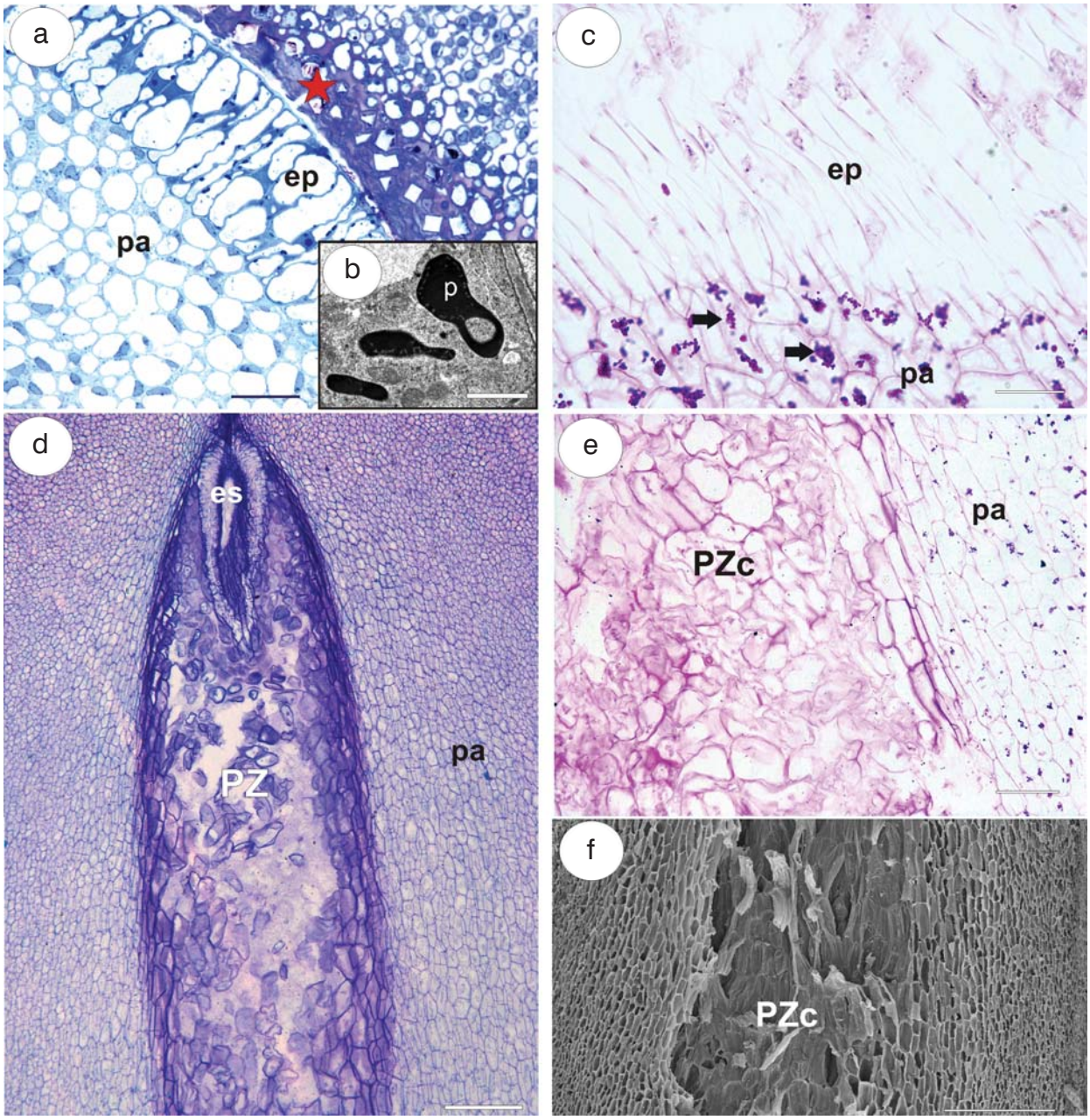

Fig. 3. Ovule structure and histo-chemistry. (**a**) Semithin section showing the ovule parenchyma (pa), epidermis (ep) and ovary wall with many calcium oxalate crystals (star), bar = 20 μm, (**b**) Electron micrograph showing electron-dense plastids in ovule epidermal cells, bar = 1.45 μm, (**c**) Semithin section showing the starch distribution (arrow) in the ovule parenchyma (pa) after PAS reaction, epidermis (ep), bar = 20 μm, (**d**) Section showing the progressive disintegration of the periendothelial zone (PZ). Embryo sac (es), ovule parenchyma (pa), bar = 100 μm, (**e**,**f**) Disintegration of the periendothelial zone cells (PZc) after PAS and in SEM, bar = 50 μm and bar = 200 μm.

bers of Carduinae and Centaureinae, Singh and Pandey, 1984). We observed electron-dense plastids in elongated epidermal cells of ovule, similar plastids were recorded in physiologically active cells, e.g., suspensor cells (Kozieradzka-Kiszkurno and Płachno, 2013). Understanding of their role needs further study but probably they may participate in the synthesis of lignins and soluble phenolics which occur in the coat of cypselas (Qaderi et al., 2002).

The structure of the pollen transmitting tissue in *O. acanthium* is similar to that of other members of the Asteraceae family: *Arnaldoa*,

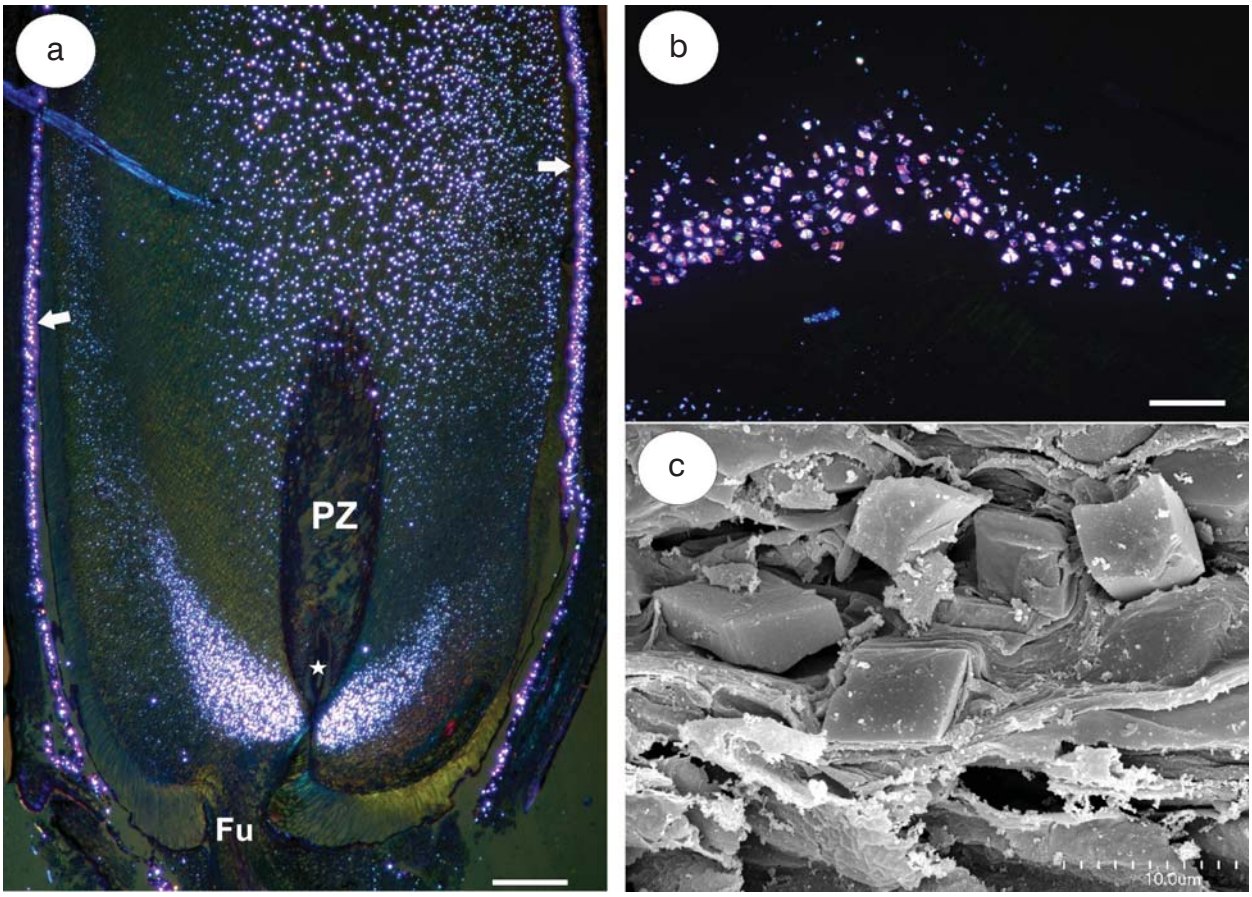

Fig. 4. Distribution of calcium oxalate crystals in the ovary and ovule of *Onopordum acanthium*. (**a**) Section through the ovary and ovule seen under a polarizing microscope: funiculus (Fu), periendothelial zone (PZ), crystalliferous layer of ovary wall (arrow), female gametophyte (star), bar = 375 μm, (**b**) The accumulation of crystals in the crystalliferous layer of the ovary wall, bar = 65 μm, (**c**) Calcium oxalate crystals from the crystalliferous layer of the ovary wall seen in SEM, bar = 10 μm.

Buphthalmum, *Cichorium*, *Helianthus*, and *Taraxacum* (Yan et al., 1991; Erbar and Leins, 2000; Erbar and Enghofer, 2001; Erbar 2003; Gotelli et al., 2010; Płachno et al., 2015a). Similar to *Helianthus* (Yan et al., 1991) and *Taraxacum* (Płachno et al., 2015a), the pollen transmitting tissue cells in *Onopordum* have a well-developed extracellular matrix and endoplasmic reticulum.

However, in the case of the periendothelial zone, the ovule of *Onopordum* is different from the ovule types of "Taraxacum", "Galinsoga", "Ratibida" (see Kolczyk et al., 2014). In *Taraxacum* (Taraxacum ovule type), the whole periendothelial zone consists of well-developed mucilage cells (Płachno et al., 2015), whereas in *Onopordum* well-developed mucilage cells occur mainly near the integumental tapetum. In the periendothelial zone cells in both taxa, numerous dictyosomes and well-developed ER have been observed. The latter character also occurs in *Solidago* spp. and *Galisoga quadriradiata* periendothelial zone cells (Kolczyk et al., 2014). The gelatinisation (= mucilage deposition) of the periendothelial zone was also described in *Youngia japonica* (Pandey et al., 1978). The periendothelial zone in *Cynara cardunculus* was interpreted by Figueiredo et al. (2006) as being hypostase. According to these authors, its cells have thick walls, an electron-dense cytoplasm with amorphous condensed material and are also highly vacuolated. In our opinion, the thick cell walls in Cynara may have a mucilage character; however, further studies are needed.

In the cyto-chemical studies, we observed a positive result of the PAS reaction in the periendothelial zone cells, which indicates that the deposited material is rich in water insoluble polysaccharides with 1,2-glycol groups. This is in agreement with our ultrastructure observations (mucilage

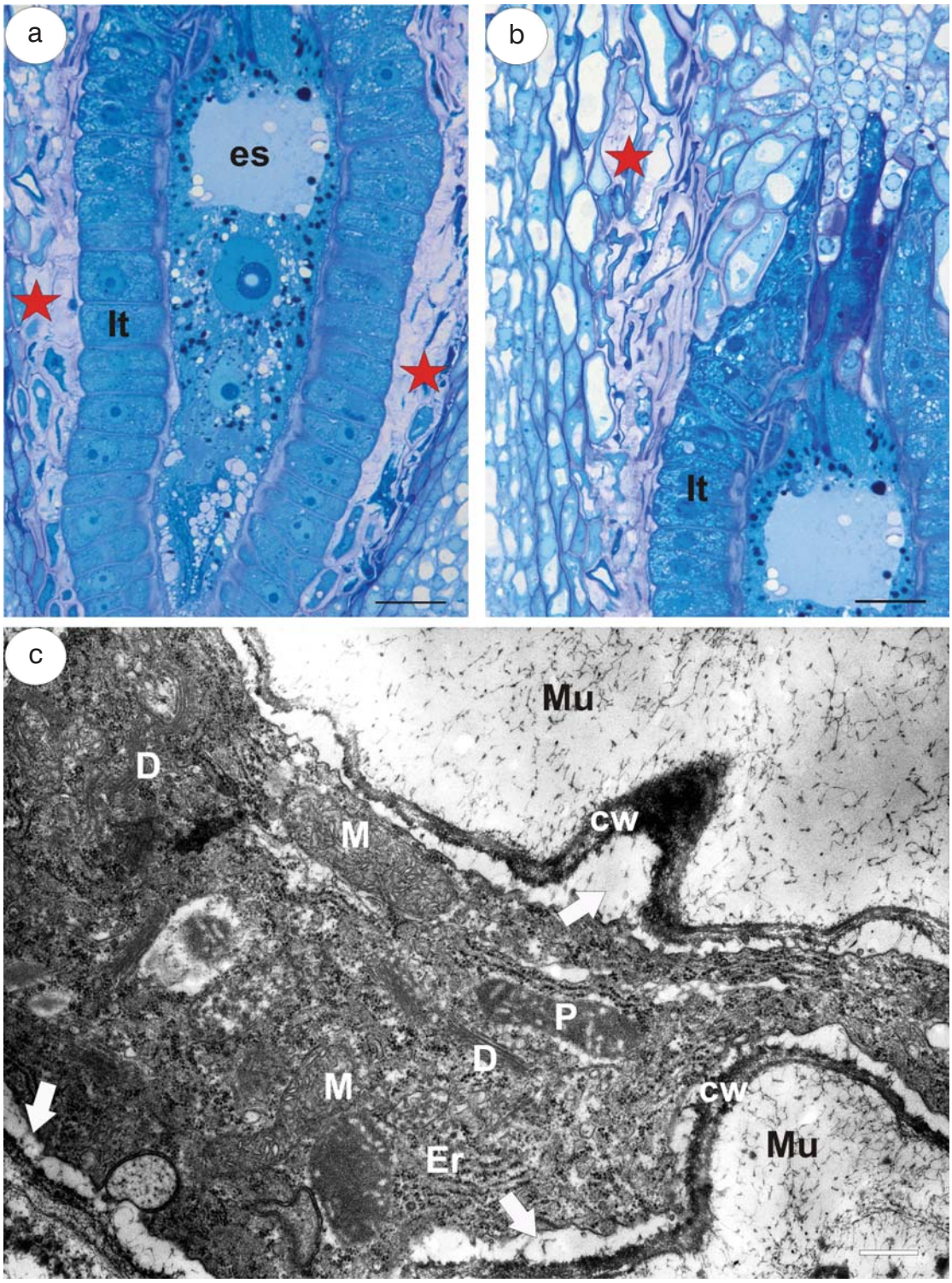

Fig. 5. Structure of the periendothelial zone. (**a**) Semithin section showing the periendothelial zone (star) near the micropylar and central parts of the embryo sac (es), integumental tapetum (It), bar = 20 μm, (**b**) Semithin section showing the periendothelial zone (star) near the chalazal part of the embryo sac, integumental tapetum (It), bar = 20 μm, (**c**) Electron micrograph showing the periendothelial zone cell ultrastructure; mitochondrion (M), endoplasmic reticulum (Er), dictyosome (D), cell wall (cw), plastid (P), mucilage (Mu), mucilage deposited in the extraplasmatic space (arrow), bar = 0.45 μm.

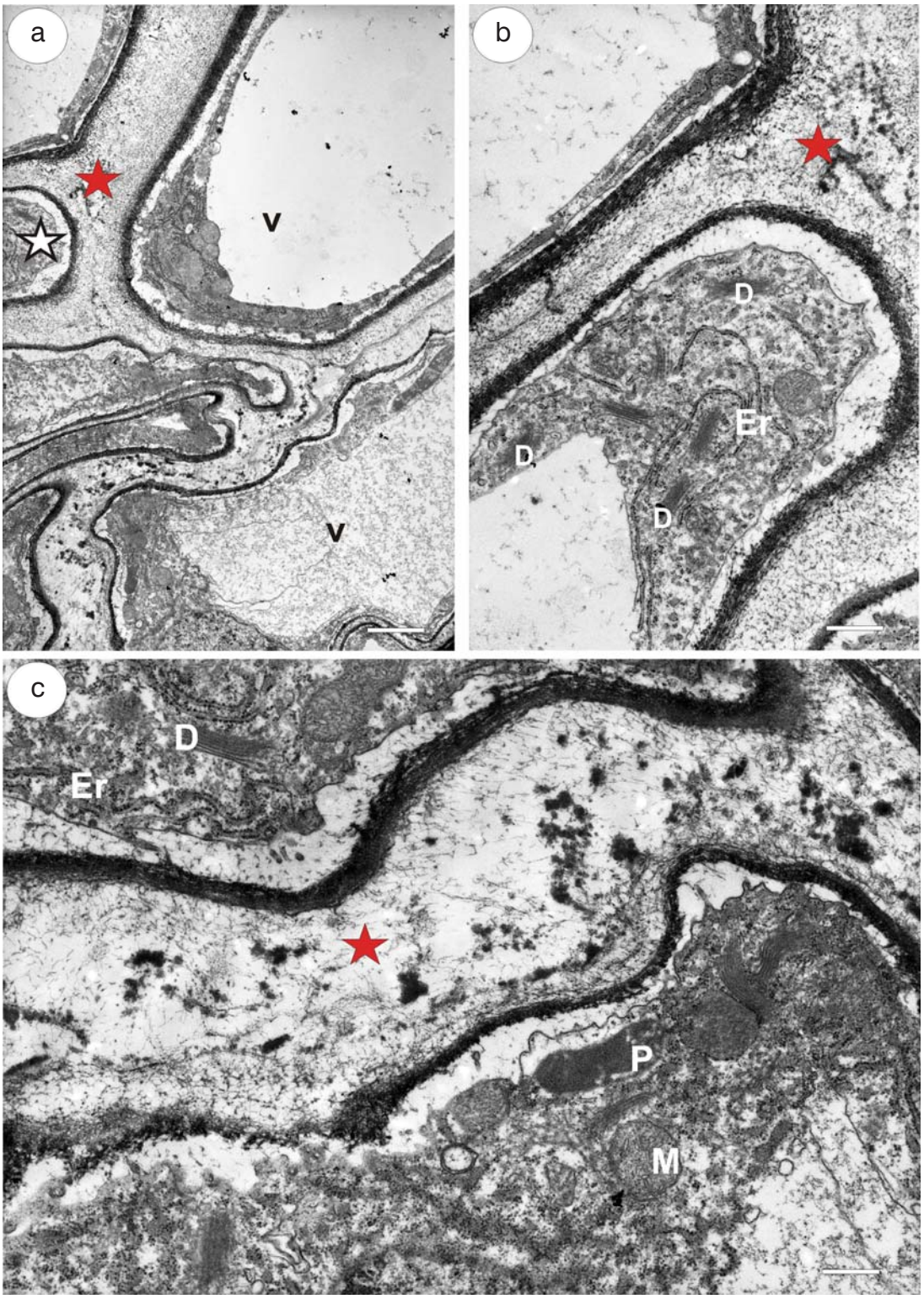

Fig. 6. Ultrastructure of the periendothelial zone cells. (**a**) Electron micrograph showing the chalazal periendothelial zone cells; vacuole (v), mucilage accumulation in the cell wall (red star), bar = 1.45 μm, (**b**) Enlarged part of the chalazal periendothelial zone cell (marked as white star on a); endoplasmic reticulum (Er), dictyosome (D), bar = 1 μm, (**c**) Mucilage accumulation in the cell wall (star) of the periendothelial zone cells; mitochondrion (M), endoplasmic reticulum (Er), dictyosome (D), plastid (P), bar = 0.6 μm.

deposition and/or the disintegration of the walls and the appearance of pectin material). Moreover, carbohydrate accumulation in the periendothelial zone cells was also observed in *Bellis* (Engell and Petersen, 1977), *Taraxacum* (Musiał et al., 2013) and *Chondrilla juncea* (Musiał and Kościńska-Pająk, 2013), which is connected with mucilage deposition in the periendothelial zone cells in the Taraxacum type of ovule (Płachno et al., 2015). According to Cooper and Brink (1949), accumulation of protein occurred in the periendothelial zone cells, although we did not detect it in the case of *Onopordum*.

According to Singh and Pandey (1984), the periendothelial zone in *Centaurea moschata* and *Saussurea marianum* (both belong to Cynareae) begins to disintegrate after fertilisation. In these species, as well as in *Carthamus oxyacantha*, the air space replaces the periendothelial zone during embryogenesis (Singh and Pandey, 1984). Apart from Cynareae, the degradation of periendothelial zone cells during embryogenesis has been recorded in several Asteraceae genera (Misra, 1972; Pandey et al., 1978), e.g., *Taraxacum* (Cooper and Brink, 1949), *Bellis* (Engell and Petersen, 1977) and *Helianthus* (Newcomb, 1973a, b). Our results obtained for *Onopordum* agree with this observation but also shed new light on how this process may occur. In the case of *Onopordum*, the intercellular spaces are formed by mucilagination of the cells and later by their degradation.

Studies of calcium oxalate (CaOx) crystals in Asteraceae have a long history as they began at the beginning of the 20th century and are still continuing today (e.g., Meric and Dane, 2004; Meric, 2008, 2009; Mukherjee and Nordenstam, 2010). The distribution and shape of calcium oxalate crystals in the ovaries of some members of Cardueae, including *Onopordum*, were described by Dormer (1961, 1962). However, in the case of *O. acanthium* and *O. illyricum*, Dormer (1961) only analyzed the ovary wall and described "crystalliferous tissues", which occur in the inner part of the ovary wall in this species. Our results agree with Dormer's observations. We also detected a crystalliferous layer of the ovary wall which contained numerous and large calcium oxalate crystals. However, we also provide new information about the distribution of calcium oxalate crystals in the ovule tissues. In contrast to *Onopordum* in *Youngia japonica*, in which the periendothelial zone gels, calcium oxalate crystals do not occur only in outer epidermis and endothelium (Pandey et al., 1978). We think that the lack of calcium oxalate crystals in the periendothelial zone cells of *Onopordum* may be connected with a specific function of this tissue and also with its short lifespan in comparison with other integumental tissues, especially due to the fact that different morphologies and distributions of calcium oxalate crystals may accommodate different rates of ion removal within and among plant tissues (Webb, 1999).

CONCLUSION

We showed some basic characters of *Onopordum* ovule although further ultrastructural studies should be done on both different stages of ovule development in *Onopordum* and on other members of Cynareae.

The disintegration of the periendothelial zone in *Onopordum* occurs as mucilagination of the cells and later by their degradation.

AUTHORS' CONTRIBUTION

All of the authors contributed to the conception and design, acquisition of data, analysis and interpretation of data, and drafting or critical revision of the paper.

The authors declare that there are no conflicts of interest.

ACKNOWLEDGEMENTS

This study was partially funded by the grants DS/MND/WBiNoZ/IB/7/2014 and DS/MND/WBiNoZ/IB/18/2015 for young scientists in the case of Jolanta Kolczyk, a PhD student. We would like to gratefully thank Professor Piotr Świątek (the Head of the Department of Animal Histology and Embryology, University of Silesia) for the use of the electron microscopy facilities and also Dr. Danuta Urbańska-Jasik for her technical assistance. We gratefully thank Professor Marek Michalik (Geology Institute, Jagiellonian University in Kraków) for the use of the polarizing microscope.

REFERENCES

Briese DT. 1989a. *A new biological control programme against thistles of the genus Onopordum in Australia*. Delfosse ES [ed.], Proc. VII Int. Symp. Biol. Contr. Weeds, 6–11 March 1988. Ist. Sper. Patol. Veg. (MAF), Rome, Italy.

Briese DT. 1989b. Natural enemies of carduine thistles in New South Wales. *Journal of the Australian Entomological Society* 28: 125–134.

Cooper DC, and Brink RA. 1949. The endosperm-embryo relationship in the autonomous apomict, *Taraxacum officinale*. *Botanical Gazette* 111: 139–152.

Dittrich M. 1968. Morphologische Untersuchungen an den Früchten der Subtribus Cardueae-Centaureinae (Compositae). *Willdenowia* 5: 67–107.

Dittrich M. 1970. Morphologische und anatomische Untersuchungen an Früchten der Carduinae (Compositae). I. Morphologischer Teil. *Candollea* 25: 45–67.

Dormer KJ. 1961. The crystals in the ovaries of certain Compositae. *Annals of Botany* 25: 241–254.

Dormer KJ. 1962. The taxonomic significance of crystal forms in *Centaurea*. *New Phytologist* 61: 32–35.

Engell K, and Petersen GB. 1977. Integumentary and endothelial cells of *Bellis perennis*. *Botanisk Tidsskrift* 71: 237–244.

Erbar C. 2003. Pollen tube transmitting tissue: place of competition of male gametophytes. *International Journal of Plant Sciences* 164 (suppl): S265–S277.

Erbar C, and Enghofer J. 2001. Untersuchungen zum Reproduktionssystem der Wegwarte (*Cichorium intybus*, Asteraceae): Pollenportionierung, Narbenbelegung und Pollenschlauchkonkurrenz. *Botanische Jahrbücher fur Systematik, Pflanzengeschichte und Pflanzengeographie* 123: 179–208.

Erbar C, and Leins P. 2000. Some interesting features in the capitulum and flower of *Arnaldoa macbrideana* Ferreyra (Asteraceae, Barnadesioideae). *Botanische Jahrbücher fur Systematik, Pflanzengeschichte und Pflanzengeographie* 122: 517–537.

Figueiredo R, Duarte P, Pereira S, and Pissarra J. 2006. The embryo sac of *Cynara cardunculus*: ultrastructure of the development and localization of the aspartic proteinase cardosin B. *Sexual Plant Reproduction* 19: 93–101.

Gotelli M, Galati B, and Medan D. 2010. Structure of the stigma and style in sunflower (*Helianthus annuus* L.). *Biocell* 34: 133–138.

Kolczyk J, Stolarczyk P, and Płachno BJ. 2014. Comparative anatomy of ovules in *Galinsoga*, *Solidago* and *Ratibida* (Asteraceae). *Acta Biologica Cracoviensia Series Botanica* 56(2): 115–125.

Kozieradzka-Kiszkurno M, and Płachno BJ. 2013. Diversity of plastid morphology and structure along the micropyle-chalaza axis of different Crassulaceae. *Flora* 208: 128–137.

Meric C. 2008. Calcium oxalate crystals in *Conyza canadensis* (L.) Cronq. and *Conyza bonariensis* (L.) Cronq. (Asteraceae: Astereae). *Acta Biologica Szegediensis* 52: 295–299.

Meric C. 2009. Calcium oxalate crystals in some species of the Tribe Inuleae (Asteraceae). *Acta Biologica Cracoviensia Series Botanica* 51: 105–110.

Meric C, and Dane F. 2004. Calcium oxalate crystals in floral organs of *Helianthus annuus* L. and *H. tuberosus* L. (Asteraceae). *Acta Biologica Szegediensis* 48: 19–23.

Misra S. 1972. Floral morphology of the family Compositae. V. The seed coat and pericarp in Verbesina encelioides (Cav) Benth. and HK f Ex A Gray. *Journal of the Indian Botanical Society* 51: 332–341

Mukherjee SK, Nordenstam B. 2010. Distribution of calcium oxalate crystals in the cypselar walls in some members of the Compositae and their taxonomic significance. *Compositae News* 48: 63–88.

Musiał K, Płachno BJ, Świątek P, and Marciniuk J. 2013a. Anatomy of ovary and ovule in dandelions (*Taraxacum*, Asteraceae). *Protoplasma* 250: 715–722.

Musiał K, and Kościńska-Pająk M. 2013. Ovules anatomy of selected apomictic taxa from Asteraceae family. *Modern Phytomorphology* 3: 35–38.

Newcomb W. 1973a. The development of the embryo sac of sunflower *Helianthus annuus* before fertilization. *Canadian Journal of Botany* 51: 863–878.

Newcomb W. 1973b. The development of the embryo sac of sunflower *Helianthus annuus* after fertilization. *Canadian Journal of Botany* 51: 879–890.

Pandey AK, Singh R.P, and Chopra S. 1978. Development and structure of seeds and fruits in Compositae: Cichorieae. *Phytomorphology* 28: 198–206.

Pérez-García F, Duran JM. 1987. Fruit anatomy of *Onopordum nervosum* Boiss. (Asteraceae). *Phytomorphology* 37: 35–38.

Płachno BJ, Kurczyńska E, Świątek P. 2015. Integument cell differentiation in dandelions (*Taraxacum*, Asteraceae, Lactuceae) with special attention paid to plasmodesmata. *Protoplasma* DOI 10.1007/s00709-015-0894-2

Płachno BJ, Świątek P, Kozierdzka-Kiszkurno M, Majeský L, Marciniuk J, Stolarczyk P. 2015a. Are obligatory apomicts invested in the pollen tube transmitting tissue? Comparison of the micropyle ultrastructure between sexual and apomictic dandelions (Asteraceae, Lactuceae). *Protoplasma* 252: 1325–1333 doi:10.1007/s00709-015-0765-x

Qaderi MM, and Cavers PB. 2003. Effects of dry heat on the germinability and viability of Scotch thistle (*Onopordum acanthium*) cypselas: Interpopulation and interposition variation. *Canadian Journal of Botany* 81: 684–697.

Qaderi M, Cavers PB and Bernards MA. 2002. Seed bank dynamics of *Onopordum acanthum* emergence patterns and chemical attributes. *Journal of Ecology* 90: 672–683.

Singh RP, and Pandey AK. 1984. Development and structure of seeds and fruits in Compositae-Cynareae. *Phytomorphology* 34: 1–10.

Susanna A, and Garcia-Jacas N. 2007. Tribe Cardueae Cass. In: Kadereit JW, Jeffrey C, editors. The Families and Genera of Vascular Plants, Vol. 8. Berlin: Springer-Verlag, pp. 123–146.

Talukdar T. 2013. Cypselas diversity of the tribe Cardueae (Asteraceae) – an overview. LAP LAMBERT Academic Publishing. 74 p.

Webb MA. 1999. Cell-mediated crystallization of calcium oxalate in plants. *Plant Cell* 11: 751–761.

Yan H, Yang H, Jensen W. 1991. Ultrastructure of the micropyle and its relationship to pollen tube growth and synergid degeneration in sunflower. *Sexual Plant Reproduction* 4: 166–175.

Zając M, and Zając A. 2014. Survival problems of archaeophytes in the Polish flora. *Biodiversity: Research and Conservation* 35: 47–56.