

Seed morphology and anatomy of *Hypericum majus* (A. Gray) Britton

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Abstract. *Hypericum majus* (A. Gray) Britton is an alien species for Europe. It originates from North America and, in recent years, has been spreading in Europe. Presently, this species is found in Germany, France, Italy, Bosnia and Herzegovina, and Belarus. In Poland, it was noted for the first time in 2017 in western part of the country. Thus far, no detailed morphology and anatomy of *H. majus* seeds based on light and electron microscopy studies has been presented. Considering the fact that seeds provide important taxonomical information and the discussed species has not been investigated in this respect, in this research, an attempt was made to broaden the present knowledge.

Investigations were conducted based on samples originating from three localities in Central Europe (Poland). The obtained results showed that *H. majus* seeds are very small – about 0.5 mm in length. In the SEM image, their surface is reticulate, typical of majority of species in this genus. Anatomical analysis showed that seed coat of matured seeds is very delicate, composed of two cell layers with strongly lignified walls.

Key words: carpology, greater Canada St. John's-wort, Hypericaceae, Poland, seed sculpture, seed coat

1. Introduction

The world flora of the genus *Hypericum* L. comprises, according to different sources, from about 370 to 520 species (Stevens 2007; Mabberley 2008; Nürk & Blattner 2010; Crockett & Robson 2011; The Plant List 2013; Hypericum online). In Europe, this genus is represented, depending on taxonomical approach, by 56-61 species (Mabberley 2008; Robson 2005), with 13 of these taxa observed in Poland to date (Mirek *et al.* 2002). The majority of species occurring in Poland are native taxa (*H. elegans* Stephan ex Willd., *H. hirsutum* L., *H. humifusum* L., *H. maculatum* Crantz, *H. montanum* L., *H. perforatum* L., *H. pulchrum* L., and *H. tetrapterum* Fr.) or ephemerophytes (*H. gymnanthum* Engelm. & A. Gray, *H. mutilum* L., and *H. veronense* Schrank). Furthermore, *H. calycinum* L. and *H. hooke-*

rianum Wight & Arn. were found in crops (Mirek *et al.* 2002; Jackowiak *et al.* 2013, 2017).

In the flora of Poland, a new, non-native species of *Hypericum*, which spreads also in many European countries, is *H. majus* (A. Gray) Britton (Nobis *et al.* 2018). This taxon originates from North America. It is a perennial plant that grows up to 0.7 m in height and develops one or several quadrangular stems. Leaf blades are lanceolate or elliptic (longitudinal) with entire margin. Inflorescence is apical and may consist of even 30 flowers. Flowers are stellate, 6-7 mm in diameter, petals golden-yellow (3.5-6.5 × 0.8-1.5 mm). Stamens 12-21, ovary 1.5-2.0 × 0.8-1.0 mm, ovoid to ellipsoid; styles 3; stigmas narrowly capitate (Hypericum online).

The floristic composition of *H. majus* phytocoenoses matches phytosociological preferences of this species in southern and western Europe (Bouchard 1954; Nezadal

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1984; Prince & Anielsbehere 2012; Milanović 2018). In Poland, optimal habitats of *H. majus* comprise periodically exposed pond bottoms, water body margins and periodically flooded former gravel pits. It forms dense phytocoenoses that hinder the development of small terophytes from the *Isoëto durieui-Juncetea bufonii* class. Furthermore, locally, this species poses a threat to phytocoenoses from the *Hydrocotylo-Baldellion* alliance (e.g., *Pilularietum globuliferae*). In overgrown, unused ponds, *H. majus* was also found in the communities representing the *Molinio-Arrhenatheretea*, *Phragmitetea australis* and *Scheuchzerio-Caricetea fuscae* classes (Nobis *et al.* 2018). In western Poland, *H. majus* was observed in several ponds occurring within three active or abandoned fish farms and in a periodic pond at the bottom of the abandoned gravel pit. In total, the species was found in 5 localities in western Poland (Nobis *et al.* 2018).

Hypericum species occurring in Poland are mainly perennials, rarely bushes or annuals (among others, Zarzycki *et al.* 2002; *Hypericum* online). Their seeds are of high diagnostic value in systematics and paleobotany (Robson 1981; Hageman 1987; Meseguer & Sanmartín 2012; Alonso *et al.* 2013). A particularly important feature is the sculpture of the seed coat and cell arrangement of its outer layer. However, only a relatively small number of *Hypericum* species have been studied so far in terms of seed morphology (Meseguer & Sanmartín 2012; Alonso *et al.* 2013; Szkudlarz & Celka 2016) and

information about the anatomical structure of seeds in this genus has been also very limited (Szabes & Morozowa 1991; Matzk *et al.* 2001; Szkudlarz 2014).

In Poland, all native *Hypericum* species were investigated in detail in terms of their seed morphology (Szkudlarz 2014; Szkudlarz & Celka 2016) and in case of *H. elegans* also anatomically (Szkudlarz 2014). A new species – *H. majus*, which spreads in western Poland, has not been studied yet in this regard in Europe. Thus, the aim of this work was an analysis and detailed description of morphological and anatomical structure of the seed coat in *H. majus*.

2. Material and methods

Seeds for the study were obtained from herbarium specimens representing populations of *H. majus* distributed in western Poland (Table 1, Fig. 1). These specimens are deposited in the Herbarium of Department of Plant Taxonomy of Adam Mickiewicz University in Poznań (POZ) (Fig. 2).

Morphological structure was investigated based on the dry seed analysis using a stereomicroscope and scanning electron microscope (SEM). For SEM observations, 5 seeds were sampled and coated with gold. All morphological measurements were made using a stereomicroscope with *NIS-Elements Microscope Imaging* Software. SEM images were used in analysis of quantitative traits of the seed coat. In this work, we

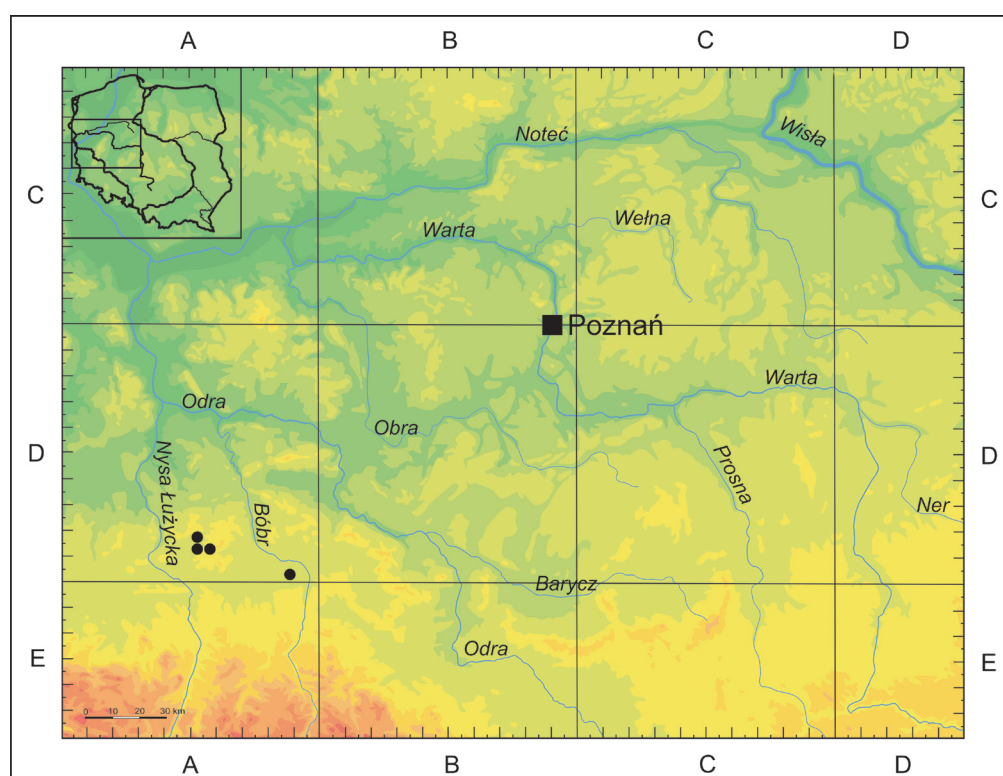


Fig. 1. Distribution of *Hypericum majus* in western Poland in the ATPOL grid squares of 5×5 km

Table 1. Origin of the examined seed samples of *Hypericum majus*

Locality	Geographic coordinates	Habitat	ATPOL	Data zbioru	Collector
Rościce village, Żarskie hills	51°36'24'' N 14°59'38'' E	alt. 160 m, in 3 ponds on a fishing farm	AD8503	2 August 2017	S. Rosadziński
Miłowice village, Żarskie hills	51°36'5,37'' N 15°3'18,26'' E	alt. 147 m, in an overgrowing pond	AD8504	3 August 2017	S. Rosadziński
near Bobrowice village, Dolnośląskie Forest	51°31'17'' N 15°31'30'' E	alt. 137 m, in an overgrowing pond	AD9804	18 August 2017	S. Rosadziński
near Boruszyn village, Żarskie hills	51°36'41,01'' N 14°58'23,50'' E	alt. 145 m, in an overgrowing pond	AD8503	16 November 2018	S. Rosadziński
near Żary city, Żarskie hills	51°37'43,04'' N 15°5'23,03'' E	alt. 157 m, in an abandoned gravel pit	AD8503	16 November 2018	S. Rosadziński

**Fig. 2.** Herbarium voucher of *Hypericum majus* from herbarium of the Department of Plant Taxonomy, Adam Mickiewicz University in Poznań (POZ)

followed terminology widely used in the studies on seed morphology (Robson 1981; Bojňanský & Fargašová 2007; Meseguer & Sanmartín 2012).

To investigate the seed anatomical structure, material was subjected to a microtechnique procedure. Seeds were placed in the mixture of 2% solutions of glutaraldehyde and paraformaldehyde, then, they were kept for 1h in vacuum chamber (0.2 atm) and, next, in fridge (4°C) overnight. Fixed seeds were three times rinsed in 0.05 M cacodylate buffer and dehydrated in the series of ethanol solutions with increasing concentration (5, 10, 30, 50, 70, 80, 90, 96 and 100%). To prepare microscopic slides, Technovit 7100 acrylic resin was used (Heraeus Kulzer GmbH, Wehrheim, Germany) as an embedding medium. Slices of 4 µm in thickness were stained with 0.05% solution of toluidine blue prepared in 1% sodium tetraborate. Additionally, seeds were stained with methylene blue-azure II and basic fuchsin prepared for the staining of epoxy-embedded tissue sections (Humphrey & Pitmann 1974). Slides were analysed under the AxioScopeA1 microscope.

Lignin detection in the walls of seed coat cells was conducted under the AxioScopeA1 microscope with equipped Colibri 4. Unstained microscopic slides were observed using excitation light of 485 nm. For this wavelength, autofluorescence of lignin shows up as green light emission within visible spectrum.

Photographic documentation of all slides was made using the AxioCam MRc5 digital camera (Carl Zeiss GmbH, Jena, Germany).

3. Results

3.1. Morphological structure

The studied seeds of *H. majus* are elongated, cylindrical, rounded at both ends with slightly marked beaks (Figs. 3-4). Their length is about 500 µm, while width about 200 µm. Seeds are slightly curved and



Fig. 4. *Hypericum majus*, seed (LM)



Fig. 5. *Hypericum majus*, seed (SEM), an abdominal view

there is a narrow, longitudinal scar after funiculus on the incurved side (Fig. 4-5). Under a light microscope, their surface is lustrous. Seeds are light brown (honey shade) in colour (Fig. 3). Under a SEM microscope, the surface is indistinctly reticulate, epidermal cells of the testa are polygonal (with 5-7 angles), distinctly wider than longer relative to the seed longitudinal axis (Fig. 6).

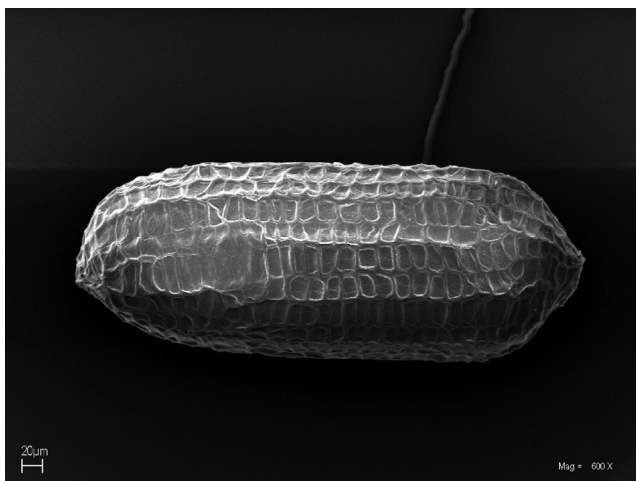


Fig. 3. *Hypericum majus*, seed (SEM)

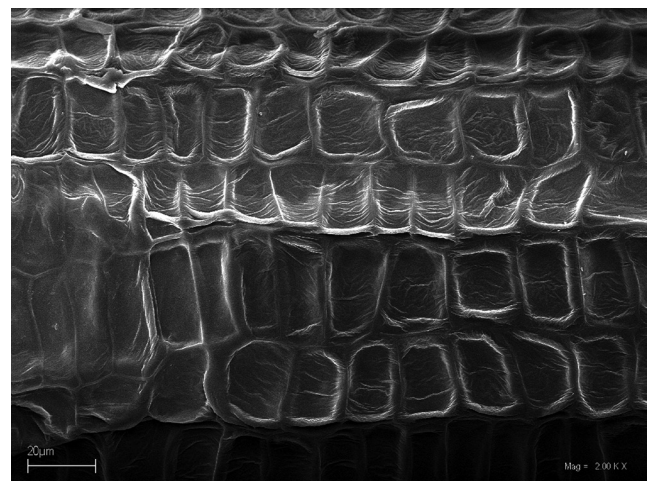


Fig. 6. *Hypericum majus*, surface of the seed coat (SEM)



Fig. 7. *Hypericum majus*, longitudinal section across a mature seed (LM) showing a fragment of the seed coat with the outer and inner layers and embryo cells in the area of the hypocotyl (H) and radicle (R)

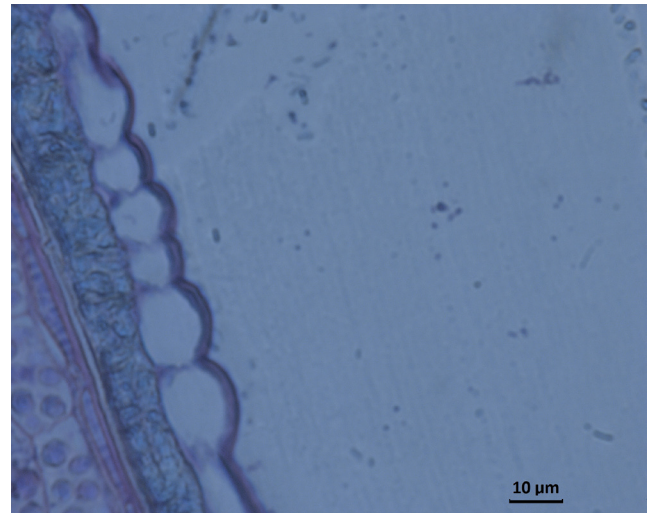


Fig. 8. *Hypericum majus*, fragment of the testa in the longitudinal section (LM), visible thickenings of the outer walls in the first cell layer

3.2. Anatomical structure

Two layers of cells can be usually distinguished in the coat structure of mature seeds, although in some places, the remains of additional, subepidermal layer may be visible (Fig. 7). The outer layer, originating from the epidermis of the testa, is composed of thin-wall cells, with a greater width than length, and with straight radial walls. The cell shape of this layer is shown in the SEM image (Fig. 6). In histological slides (longitudinal sections), the outer wall of these cells, convex on the outside, is distinctly thicker from radial walls and periclinal inner walls (Fig. 8). In mature dry seeds, the outer wall of the cells subsides, which results in characteristic reticulate surface.

The second layer of cells that make the seed coat of mature seeds has sclerenchymatous characteristics. It is

composed of isodiametric cells with highly thickened cell walls and numerous simple pits. Radial walls are strongly wavy (Figs. 9-10). The surface of these cells is visible after the removal of the outer layer. Analysis of histological slides under a fluorescent microscope showed that lignin is present in the cell walls of both layers (Fig. 11). The remaining layers of integument cells are subject to compression and do not contribute to the seed coat structure.

Almost an entire interior of *H. majus* seeds is occupied by an embryo. The embryo is straight, with well-developed cotyledons. The cotyledons constitute 1/5 of the embryo length. A highly elongated hypocotyl connects shoot and root poles. The entire embryo is surrounded by 2 layers of endosperm cells. Longitudinal sections of the embryo show 3 distinct areas of tissue differentiation. The first outer layer is protoderm, then

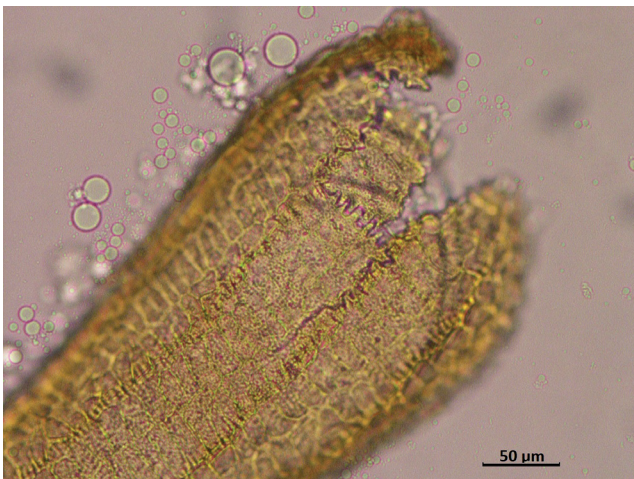


Fig. 9. *Hypericum majus*, surface view of the sclerenchyma layer (LM)

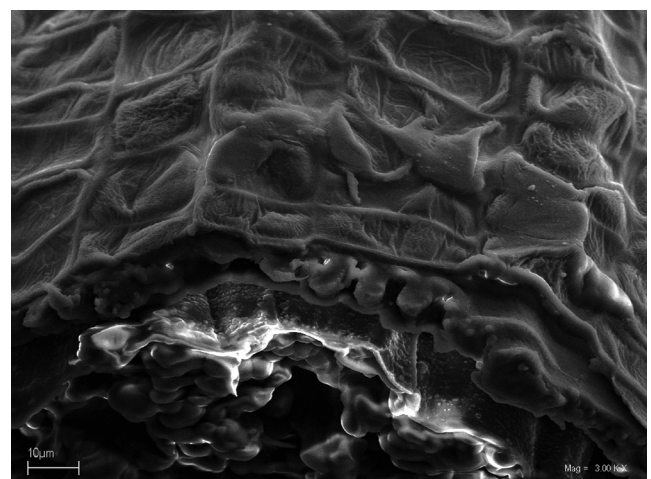


Fig. 10. *Hypericum majus*, the broken seed coat showing the wavy characteristics of walls in the sclerenchyma layer (SEM)

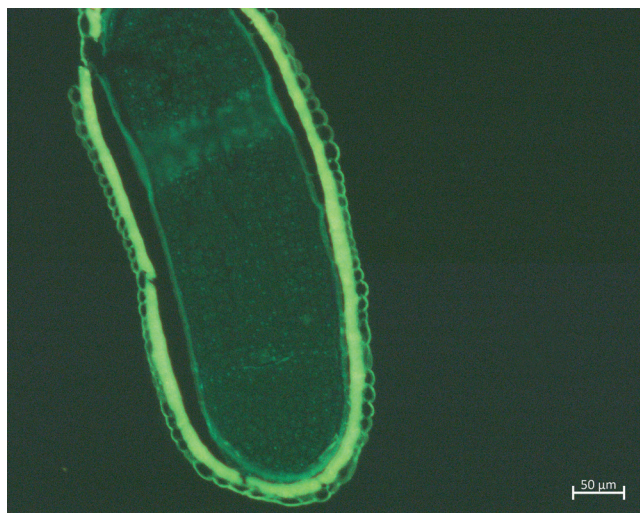


Fig. 11. *Hypericum majus*, longitudinal section (fluorescence microscope)

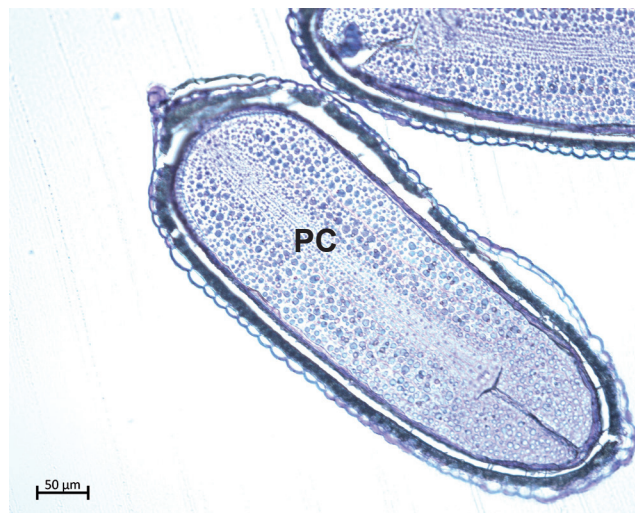


Fig. 12. *Hypericum majus*, longitudinal section across the mature seed (LM) showing two cotyledons in the embryo and the zone of procambial differentiation (PC)

procortex, while in the middle, in the embryo axis, there is a zone of procambial differentiation (Fig. 12). The cells of the hypocotyl and endosperm contain numerous deposits of reserve materials (Fig. 13).

4. Discussion

In Poland, *H. majus* is the third (besides *H. muticum* and *H. gymnanthum*) representative of the section *Trigynobrachys* (Y. Kimura) N. Robson, subsection *Knifa* (Adans.) N. Robson (Robson 1990; Hypericum online). *H. majus* seeds are small, even when compared to the seeds of other *Hypericum* species. The seed coat

of mature seeds is very delicate, and its structure is similar to the seed coat in *H. elegans* seeds (Szkudlarz 2014).

Many studies indicate that *Hypericum* seeds develop from anatropous, tenuinucellate ovules with two integuments (Netolitzky 1926; Corner 1976; Sokołowska 1983; Szabes & Morozova 1991). The outer integument is composed of two, while the inner integument of two or more cell layers (Corner 1976; Szabes & Morozova 1991). The previous studies showed that the seed coat of mature *H. majus* seeds consists of two cell layers. The outer, composed of thin-wall cells, and the inner – sclerenchymatous, composed of thick-wall cells.

Following other authors (Corner 1976; Szabes & Morozova 1991), it may be accepted that the outer layer originates from the epidermis of the testa, while other layers disappear during the seed maturation. Similarly, the sclerenchyma layer develops from the epidermis of the tegmen, while other layers disappear during the seed development. This layer is composed of cells with highly wavy radial walls, similarly like in other species of the genus (Netolitzky 1926; Corner 1976; Szkudlarz 2014). Additionally, in mature seeds, no deposits of any brown substances (tannins) have been observed in the epidermis of the testa, contrary to the reports of some authors (Ohlendorf 1907).

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Author Contributions

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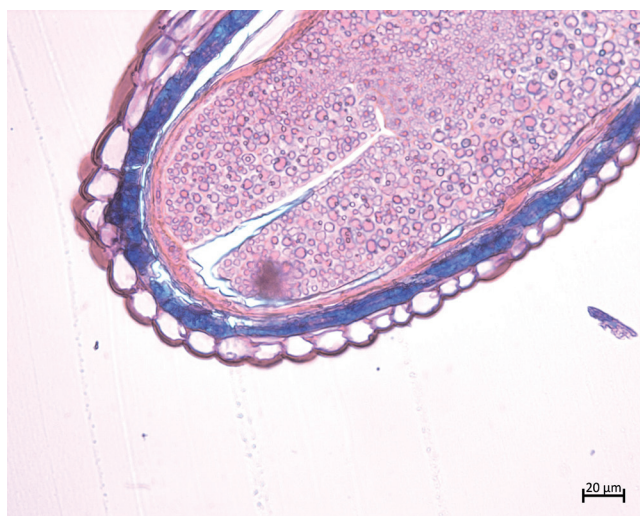


Fig. 13. *Hypericum majus*, longitudinal section across the mature seed (LM), the microscopic slide stained with the Humphrey and Pittman method (1974), visible numerous deposits of reserve materials in the embryo cells

Data analysis and interpretation: P. Szkudlarz, S. Rosadziński, M. K. Wojciechowicz
 Drafting the article: P. Szkudlarz, S. Rosadziński, M. K. Wojciechowicz, Z. Celka

Critical revision: P. Szkudlarz, M. K. Wojciechowicz, Z. Celka
 Final approval: P. Szkudlarz, Z. Celka

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