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PALAEOECOLOGICAL STUDY OF *DESMIDIOSPORA*-LIKE FUNGUS FROM POOR FEN IN NORTH-EASTERN POLAND

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INTRODUCTION

During mycological investigation carried out on Szeroki Bór mire complex (N-E Poland), strange fungal structures were found inhabiting living *Sphagnum spp.* leafs (Fig. 1). These structures were initially identified as belonging to a member of the genus *Desmidiospora* Thaxt. 1891. Interestingly, although this genus comprises 3 species (Index Fungorum), only the type species, *Desmidiospora myrmecophila* Thaxt. is an extant taxon, and the other two, namely *D. marginiconvoluta* Kalgutkar 1997 and *D. willoughbyi* (W.H. Bradley) D.L.E. Glass, D.D. Br. & Elsik 1987, are known only from fossil specimens of Eocene age. Moreover, an extant *D. myrmecophila* was described from mycelium infecting *Camponotus spp.* ants from USA (Thaxter, 1891), and the other two existing records of this species are also from *Camponotus spp* ants in USA (Clark and Prusso, 1986) and in Ghana, Africa (Evans and Samson, 1984).

No fungus of similar morphology was recorded in a list of taxa reported from peatlands compiled by Thormann (2007).

The morphology of the structures found in Poland resembles the most that in extinct *D. willoughbyi*, therefore the authors decided to further explore palynological literature in search of any similar records, and have found many reports from different geographic localities (Australia, Africa, Europe, USA; Venezuela) and of different age ranging from Eocene to Holocene (Bradley 1967; Hebda 1977; van Geel 1978; Germeraad 1979; Glass *et al.* 1986; Jarzen and Elsik 1986; Ramirez 2002; Garcia Massini and Jacobs 2011).

However, it appears that there is still a great confusion regarding the nature of these structures, and this confusion is reflected in various nomenclature attributed to them, e.g. "*Desmidiospora*" according to Hebda (1977), Hermann (2007), "*Entophlyctis lobata* " in van Geel (1978), "hyphopodia" in Jarzen and Elsik (1986), or "Microthyriaceous germlings" in Dilcher (1965) and Gaponoff (1984). It should be noted, that obviously because of the palynological character, none of the abovementioned studies attempted to carry out routine mycological investigation of the surface samples involving culturing trials.

Therefore, by focusing solely on the *Desmidiospora*-like structures, the aim of the present work was to elucidate the nature of these structures, by attempts to isolate them on standard synthetic media for culturing microfungi and direct observations in the fresh substrate. In addition, as peatlands are excellent ecosystems for any pelobiological research, we also aimed at investigatin the occurence of *Desmidiospora*-like structures during the history of the site in Szeroki Bór mire complex and to try to associate them with the main types of vegetation.

MATERIALS AND METHODS

The Szeroki Bór mire complex is located in the warmińsko-mazurskie voivodship, in the vivinity of Pisz town, in N-E Poland. It comprises ca. 30 small peatlands, mainly poor fens and forested bogs.

Two sample sites were designated, one on poor fen with a dense cover of *Sphagnum* spp. with *Carex limosa* L., *Eriophorum vaginatum* L., *Andromeda polifolia* L. and *Oxycoccus palustris* Pers., and the other on a small open area in the forested bog with a dense cover of *Sphagnum* spp. with *Oxycoccus palustris*, *Vaccinium uliginosum* L., *Andromeda polifolia* and in some places *Ledum palustre* L. and *Calluna vulgaris* (L.) Hull.

At each site, a peat core was taken using Russian peat borer, and subdivided into layers based on morphological characteristics of peat. Layers were wrapped in plastic sealed bags and transported to the laboratory. From each layer a ca. 5 cm^3 subsample was taken from the inside of the core to avoid contamination and mixed with water. No other preparation of sample was performed, as authors hoped to find fungal structures associated with plant material.

10 prep slides per layer were made, scanned under the light microscope (initial magnification x100, and x400 for confirmation of record) for the presence of *Desmidiospora*-like structures, and their frequency in the layer is described as rare (1-4 positive slides/10), frequent (5-7/10) or abundant (8-10/10).

Live *Sphagnum* moss and leaves of *Carex spp.*, *Eriophorum vaginatum*, *Andromeda polifolia*, *Vaccinium uliginosum*, *Oxycoccus palustris*, *Calluna vulgaris* and *Ledum palustre* were investigated directly for the presence of *Desmidiospora*-like structures by observation under dissecting microscope and subsequent preparation of slides.

Isolations were attempted by placing *Desmidiospora*-like structures (picked up from the *Sphagnum* leaves) on the plates with standard media (PDA and MEA agars) and plates with specially prepared agar with *Sphagnum* extract, and incubated in the room temperature (~22^{o}C).

RESULTS

Any attempts to cultivate this fungus failed, indicating that it may have some special requirements or may be an obligate parasite, being therefore unculturable or requiring specialised methods of cultivation.

The structures are generally circular, sometimes somehow elongated, 20-45 x 20 µm, disc shaped, with their margin deeply lobed but compact (Fig. 1). They are highly melanised with frequently visible light "hole" in the centre, possibly the region where a penetrating hypha or haustorium enters the underlying plant tissue.

The structures on *Sphagnum spp.* were found attached to the leaf surface and in some cases growing intracellularly, which was also reported by van Geel (1978).
The same structures were also found on the living leaves of *Andromeda polifolia* (upper and bottom side), *Oxycoccus palustris* (upper and bottom side) and *Calluna vulgaris*. On the upper side of *Vaccinium uliginosum* leaves similar structures were found, but always achieving maximum half size of the "normal" *Desmidiospora*-like structures, and more irregularly lobed, never compact as in "classic" types of *Desmidiospora*-like structures.
The structures were never found in connection with hyphae, although many melanised hyphae were seen in the vicinity on every plant species. In addition, on vascular plants' leaves, *Desmidiospora*-like structures were found among a dense net of superficial hyphae but with no evidence of connection between them.

Tab. 1. Peat core analyses from poor fen stand.

Depth (cm)	**Peat profile**	***Desmisiospora*-like structures**
0-23	Live *Sphagnum* moss	Present, abundant
23-90	*Sphagnum* peat; poorly decomposed	Present, abundant
90-150	*Sphagnum*, *Andromeda*, *Eriophorum* peat; poorly decomposed	Present, frequent
150-240	*Sphagnum*, *Andromeda*, *Eriophorum* peat; moderately decomposed	Present, abundant
240-280	*Sphagnum, Bryales*, *Carex* peat; poorly decomposed	Present, abundant
280-320	*Sphagnum, Bryales*, *Carex* peat; strongly decomposed	Present, abundant
320-355	*Bryales* peat; strongly decomposed	Present, rare
355-395	*Bryales*, *Carex* peat; moderately decomposed	Absent
395-425	*Bryales* peat; strongly decomposed	Absent
425-455	*Carex* peat; poorly decomposed	Present, rare
455-535	*Bryales*, *Eriophorum* gytia	Present, rare

Tab. 2. Peat core analyses from forested bog stand.

Depth (cm)	**Peat profile**	***Desmidiospora*-like structures**
0-20	Live Sphagnum moss	Present, abundant
20-80	Sphagnum peat with Eriophorum, Andromeda and Calluna; poorly decomposed	Present, abundant
80-180	Sphagnum peat with Eriophorum; moderately decomposed	Present, abundant
180-240	Sphagnum peat with Carex; moderately decomposed	Present, rare
240-440	Bryales peat with Carex; moderately decomposed	Present, rare
440-500	Detrital gitia with plant remains	Present, rare

No further development of *Desmidiospora*-like structures was evidenced (e.g. formation of stromata or ascomata), but several times assemblings of 3-4 specimens was seen – the fact reported also by van Geel (1978).

As seen from peat core analysis (Tab. 1, Tab. 2), *Desmidiospora*-like structures were found almost uniformly distributed and present in almost all layers in both examined sites.

The morphology of subfossil specimens was identical to the living ones (Fig. 2); similarly they were not found in association with hyphae. Interestingly, from these analyses they seem to be associated with *Sphagnum spp.*, for they were frequently found attached to their leaves and found in greater frequencies only with layers with dominance of *Sphagnum* remains.

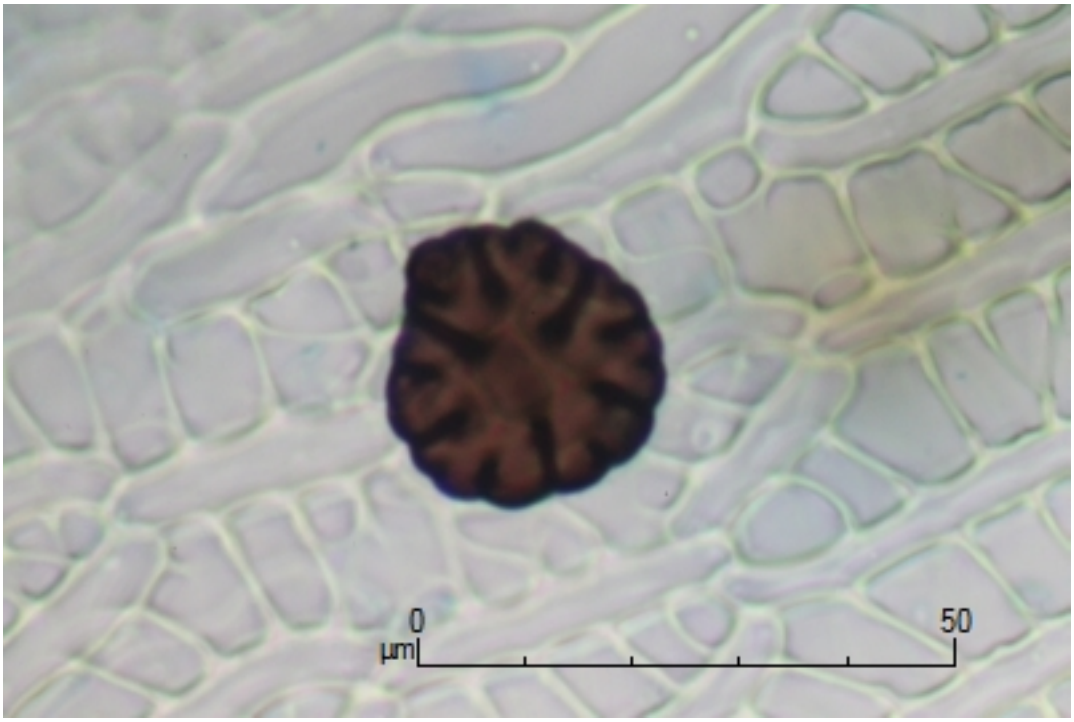

Fig. 1. The *Desmidiospora*-like structure on living *Sphagnum sp*. leaf.

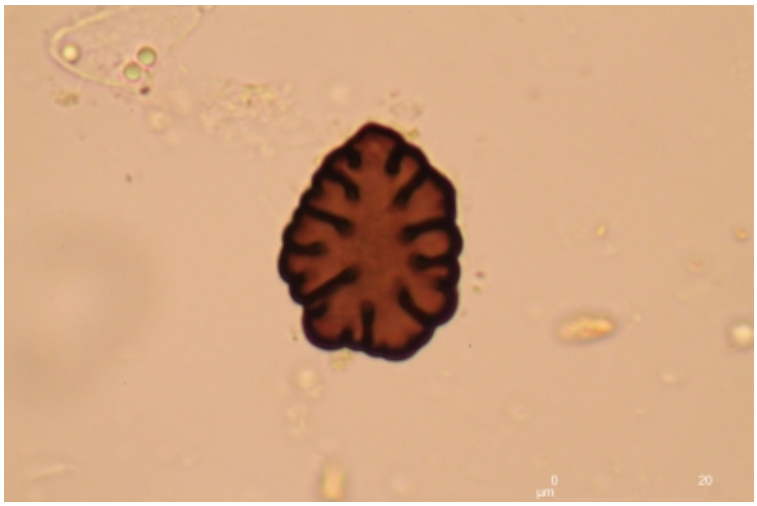

Fig. 2. The *Desmidiospora*-like structure from subfossil peat layer.

DISCUSSION

Although the cultivation attempts of *Desmidiospora*-like structures failed, and therefore no identification was possible, the authors are confident that these structures are produced by the fungus not belonging to the genus *Desmidiospora* as defined by Thaxter (1891). These structures, although bearing superficial resemblance to the conidia of *D. myrmecophila*, are probably infecting structures, the hyphopodia, and this presumption is strenghthen by the findings of these firmly attached to the leaf surface in every plant mentioned earlier.

What is more, when the descriptions and pictures of the other two (extinct) species of *Desmidiospora* are compared with the present and other reports, it seems clear that they represent the same type of structures, i.e. they were produced in the similar way but not as a result of conidiogenesis. Therefore, to avoid confusion and misinterpretation of data obtained from palynological research, these two species should be excluded from the extant genus *Desmidiospora* and placed in the separate form-genus, and this new form-genus should then encompass any similar fossil or subfossil structure.

The hypothesis that these structures are "Microthyriaceous germlings", i.e. they are early form stage in the development process of thyriothecium-type of ascomata seems, at least in a case of the Northern hemisphere specimens, unconvincing, for neither in this study, nor in the study of van Geel (1978), any further developmental stage was detected.

The host specificity of the *Desmidiospora*-like structures in the light of the present findings is unclear. In peat core analyses, they seem to be associated with *Sphagnum spp.*, and this was also stated by Hebda (1977). However, the findings on the leaves of vascular plants reported here but also reported by van Geel (1978), contradicts this assumption. Either we have a very common plant parasite occuring on peatlands with a broad host range, or we have several host-specific species, which produce morphologically similar infecting structures.
Yet to clarify this, much more sophisticated methods, e.g. single-spore DNA extraction, should be employed.

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