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Preliminary analysis of the morphology and morphometry of *Chara globularis* Thuillier (Charophyta) oospores from Poland

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Abstract – The morphology and morphometry of *Chara globularis* oospores were studied in a population collected from Lake Wielkie Pocice (NW Poland) at two depths. The longest polar axis and the largest equatorial diameter of the oospores, number of ridges, and width of fossa at the equator, as well as the ISI (length:width ×100) and ANI (distance between the apical pole and the equator:width ×100) ratios, were measured and calculated. The wall structure was examined using scanning electron microscopy. Oospores were prolate and ovoidal or ellipsoidal in outline. The wall was granular with distinctly marked ridges. Oospores were 550-783 µm long and 283-433 µm wide. Values of the ISI index were between 146 to 245. There were 12-15 ridges on the oospore surface and the width of fossa varied from 33 to 58 µm. Variation coefficients were 5% for the number of ridges and 18% for the ANI index. Statistically significant differences in terms of length, ISI index and number of ridges between oospores from different depths were found. The largest and most prolate oospores occurred on individuals growing at a depth of 1.5 m. We hypothesize that this is related to increased light availability at that depth.

***Chara globularis* / Charophyta / depth gradient / morphological variability / morphometry / oospores / Poland**

Résumé – Observations préliminaires sur la morphologie et la morphométrie des oospores de *Chara globularis* Thuillier (Charophyta) en Pologne. La morphologie et morphométrie des oospores d'un peuplement de *Chara globularis* récolté à deux profondeurs dans le lac Wielke Plocice (Pologne NW) sont étudiées. La longueur de l'axe longitudinal majeur, le diamètre équatorial majeur, le nombre de crêtes spirales, la largeur de la fosse entre deux crêtes consécutives au niveau de l'équateur et les indices ISI (longueur/largeur × 100) et ANI (distance du pôle apical à l'équateur/largeur × 100) sont mesurés et calculés. La structure de la paroi est examinée au microscope électronique à balayage. Les oospores sont allongées et ovoïdes ou ellipsoïdales, la paroi granuleuse et les crêtes bien marquées. Les oospores mesurent 550-783 µm de long et 283-433 µm de large. Les valeurs de l'indice ISI sont comprises entre 146 et 245. Entre 12 et 15 crêtes spirales sont présentes sur la surface de l'oospore. La largeur de la fosse entre deux crêtes consécutives est comprise entre 33 et 58 µm. Les coefficients de variation sont de 5 % pour le nombre de crêtes et

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18 % pour l'indice ANI. Les différences observées en terme de longueur, indice ISI et nombre de crêtes entre les oospores provenant de profondeurs différentes sont statistiquement significatives. Les oospores les plus grandes et les plus allongées sont observées sur des individus croissant à une profondeur de 1,5 m. Ceci serait en relation avec une plus grande disponibilité de lumière à cette profondeur.

***Chara globularis* / Charophyta / gradient de profondeur / morphométrie / oospores / Pologne / variabilité morphologique**

INTRODUCTION

The stoneworts (Charophyta) are known for their high morphological variability. Species identification is based not only on the thallus morphology, but also on generative structures and perennating organs (oospores) which result from generative reproduction (Dąmbska, 1964; Wood & Imahori, 1965; Krause, 1997; Casanova, 2005). Because oospores are surrounded by a multi-layered wall characterised by high resistance to environmental factors (John & Moore, 1987; John *et al*., 1990; Casanova, 1991; 1997; Sakayama *et al*., 2005), they are able to survive well in sediments and can be used also for identification purposes in a diaspore bank (Haag, 1983; Casanova & Brock, 1990; de Winton *et al*., 2000), and feature prominently in paleobotanical investigations (Soulié-Märsche, 1994; Haas, 1999; Garcia & Chivas, 2004). Notable morphological differences between the oospores of some species have been documented (Krause, 1986; John *et al.,* 1990; Haas, 1994; Ray *et al*., 2001; de Winton *et al*., 2007), thus making it possible to use them for taxonomic identification purposes, while in others (Krause, 1986; John *et al.,* 1990; Haas, 1994; Ray *et al*., 2001; de Winton *et al*., 2007), oospores are extremely similar morphologically and therefore of little or no taxonomic usefulness.

Chara globularis is a morphologically highly variable species in terms of size, growth habit and structure, and a dozen or so forms have been distinguished within it (Migula, 1897; Dąmbska, 1964; Wood & Imahori, 1965). Its oospores are morphologically variable, which makes their identification difficult. A study of the interpopulation variability of *Chara globularis* oospores has shown considerable differences in the morphological features between populations (Boszke & Pełechaty, 2006). However, intrapopulation oospore variability has not been analysed so far in this species.

The objective of this study is to provide a preliminary analysis of the morphological variability of *Chara globularis* oospores within a single population in relation to occurrence at two different depths, within the general context of oospore variability in changing environmental conditions. The overall future aim of our investigations is to better define the taxonomic diagnostic value of oospore morphology in the Charophyta.

MATERIAL AND METHODS

Lake Wielkie Płocice (N-W Poland) is a small, relatively shallow and eutrophic hardwater lake with alkaline pH (8.13), low conductivity (103.8 µS cm^{-1}) and calcium concentration of 24.7 mg dm^{-3}. The water is weakly coloured and rich in phosphorus (0.45 mg P dm^{-3}). The population of *Chara globularis* investigated here occupied a part of the littoral zone at depths between 1 and 3.5 m, and was composed of virtually monospecific patches with sporadically occurring *Chara contraria* and *C. rudis*. The substrate was mineral-organic, well hydrated (97.7%) and containing 8.7 mg calcium per g dry weight. Light availability varied along the depth gradient (relative intensity PAR = 18.7% at 1.5 m and 9.42% at 3 m).

Oospores from the *Chara globularis* population were collected for examination in July 2008 at depths of 1.5 and 3 m, from a total of 50 specimens (25 from each depth) from different parts of the thallus. Calcium capsules, if present, were removed mechanically. A total of 200 dry oospores (100 oospores from each depth) were analysed using an Olympus SZX 9 stereomicroscope. The longest polar axis (LPA, length) and the longest equatorial diameter (LED, width) of the oospores, the ISI index (LPA/LED × 100), the number of ridges and width of one fossa in the middle of the polar axis were measured using an eyepiece micrometer. The ANI index (AND/LPA × 100, with AND = distance from apical pole to LED) was also calculated (Horn af Rantzien, 1956). For scanning electron microscopy (SEM), oospores were air-dried, mounted on metal stubs, sputter-coated with Au and viewed at × 400-× 15000 magnifications in the Laboratory of Electron & Confocal Microscopy, Adam Mickiewicz University, Poznań, Poland.

The statistical significance of morphometric differences was evaluated using the Mann-Whitney U-test (two-tailed) using Statistica 7.1 PL software, owing to the fact that the analysed features did not follow a normal distribution. Spearman's rank correlation coefficient r_s was used to assess correlations between selected features, with DF = 198. The statistical significance of Spearman's rank correlation coefficients was evaluated using the t-test at $p < 0.05$ probability using Statistica 7.1 PL software.

RESULTS AND DISCUSSION

Chara globularis oospores had a granular wall with 1-μm granules densely and evenly distributed over the whole surface (Fig. 1). Oospores from the population under study were 550 to 783 μm long, 283 to 433 μm wide, with 12-15 ridges on the surface and fossae 33-58 μm wide, prolate and ovoidal or ellipsoidal in outline (ISI and ANI indices: 146-245 and 23-55, respectively), and black-dark brown in colour with distinctly marked ridges. By contrast, in the literature the range of oospore length of *Chara globularis* is 500-700 μm (Groves & Bullock-Webster, 1920; Dmbska, 1964; Haas, 1994), reaching 800 μm only exceptionally (Krause, 1997). Similarly, in the literature the range of oospore width is 350-450 μm (Groves & Bullock-Webster, 1920; Hollerbach & Krasavina, 1983; Krause, 1997). The range of the number of ridges, however, is 12-15 both in the present study and the available literature (Groves & Bullock-Webster, 1920; Hollerbach & Krasavina, 1983; Haas, 1994).

Oospores from different depths differed substantially in length, ISI index and number of ridges (Tables 1, 2). At a depth of 1.5m, oospores were 666.5 ± 46.4 μm long and 366.4 ± 29.8 μm wide. There were 13 ridges on average on each oospore. The fossa width ranged from 33 to 58 μm. Oospores from 3m depth were shorter but had a

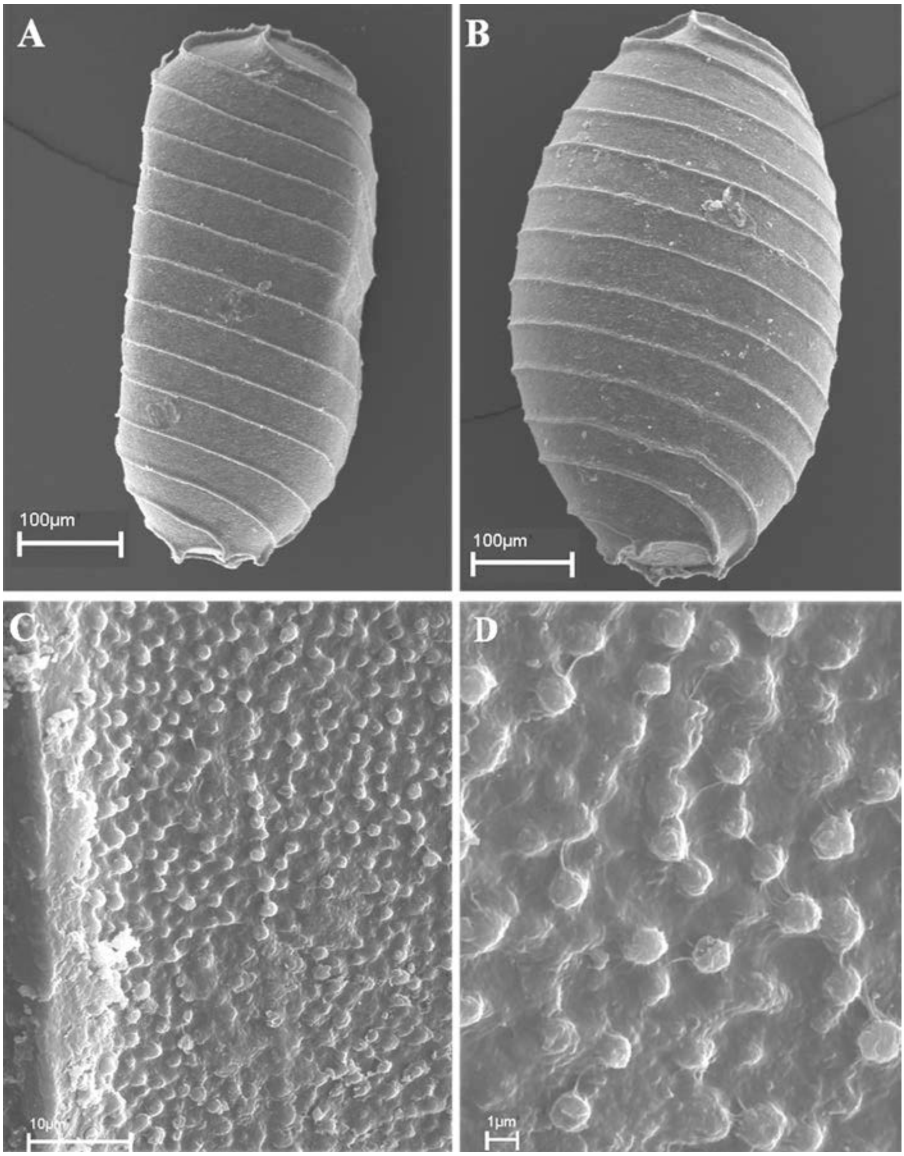

Fig. 1. *Chara globularis* oospores, SEM. **A.** Lateral view, specimen from 1.5m depth. **B.** Lateral view, specimen from 3m depth. **C, D.** Wall profile from the central part of the oospore at different magnifications.

similar width. Their average length and width were 638.3 ± 33.7 μm and 363.9 ± 24.3 μm, respectively. They were less prolate, which was also indicated by a slightly lower value of the ISI index (176 as compared to 183). Analogous to the 1.5m depth, the oospores at 3m depth had 13 ridges on average, with a slightly wider range (12-15 as opposed to 12-14). However, in terms of fossa width and oospore shape they did not differ significantly (Tables 1, 2).

Table 1. Features of oospores (A: 1.5 m depth, n = 100; B: 3 m depth, n = 100). Abbrevations: LPA – largest polar axis, LED - largest equatorial diameter, ISI index – isopolarity index LPAI/LED*100, AND - distance from apical pole to LED, ANI index - anisopolarity index AND/LPA*100, min. – minimum, max. – maximum, V – variation coefficient

A

Feature	*Mean*	*S. D.*	*Median*	*Min.*	*Max.*	*V %*
LPA [µm]	666.5	46.4	666.8	566.8	783.5	6.7
LED [µm]	366.4	29.8	366.7	283.4	433.4	8.1
ISI index	182.8	16.1	180.9	146.2	235.0	8.8
Number of ridges	13.4	0.6	13.0	12.0	15.0	4.5
Width of fossa [µm]	46.7	5.2	50.0	33.3	58.4	11.1
AND [µm]	261.9	50.1	250.1	166.7	366.7	19.1
ANI index	39.4	7.4	40.2	24.4	55.6	18.8

B

Feature	*Mean*	*S. D.*	*Median*	*Min.*	*Max.*	*V %*
LPA [µm]	638.3	33.7	633.5	550.1	700.1	5.3
LED [µm]	363.9	24.3	366.7	300.1	416.8	6.7
ISI index	175.8	9.9	174.5	154.2	200.4	5.6
Number of ridges	13.0	0.62	13.0	12.0	14.0	4.6
Width of fossa [µm]	46.7	5.7	50.0	33.3	58.4	12.2
AND [µm]	251.7	43.2	250.1	150.0	350.1	17.2
ANI index	39.5	6.9	39.7	23.1	55.3	17.5

Table 2. Statistical assessment (U Mann Whitney test) of differences between oospore features

Feature	*U*	*p*
LPA	3209.00	0.000010
LED	4785.500	0.591983
ISI index	3640.000	0.000884
Number of ridges	3556.000	0.000046
Width of fossa	4924.000	0.836637
AND	4474.000	0.194501
ANI index	4986.500	0.973680

Positive correlations were found between the oospore length and fossa width (r = 0.56), the oospore length and width (r = 0.47), the width of oospore and fossa (r = 0.34) and the oospore length and number of ridges (r = 0.29). This is in agreement with Mandal & Ray (2004), who noticed that the most highly significant correlations are those between the oospore length and width, and the oospore length and fossa width. A negative correlation between the number of ridges and fossa width was also found (r = –0.36).

The intrapopulation variability of *Chara globularis* oospores observed in this study was almost as wide as the interpopulation variability reported by Boszke & Pełechaty (2006), who found the oospore length and width to be within the range of 483-733 µm and 300-467 µm, respectively, the number of ridges within the range of 9-14, and the fossa width within the range 33-58 µm. The fact that the oospores from the studied population were longer (and consequently larger) than those described elsewhere might depend, perhaps, from exceptionally favourable environmental conditions, especially at a depth of 1.5m.

Because the number of ridges is the least variable feature, it might be regarded, perhaps, as having a good taxonomic diagnostic value. Fossa width and oospore shape are more highly variable, analogous to *Chara tomentosa* (Hutorowicz, 2008) and *Chara rudis* (Boszke & Bociąg, 2008). However, it is impossible to perform a differential identification of *Chara globularis* oospores based on the number of ridges alone and, even when all the other morphological and morphometric features are considered as well the distinction between *Chara globularis* and *Chara delicatula* remains problematic. Haas (1994) suggested that, rather than being distinguished, the oospores of these species should be combined in one common group. *Chara delicatula* also has a similar wall structure morphology to *C. globularis* (Boszke, in preparation), i.e. a granular wall lacking a ribbon on the ridges, as noted for *C. globularis* also by Ray *et al.* (2001) and de Winton *et al.* (2007).

In the investigated population, relative PAR intensities at depths of 1.5m and 3m were 18.7% and 9.42%, respectively, and it could be hypothesised that this is the main causative agent of oospore variability. Light is known to be the limiting factor for stonewort growth (Hough *et al.*, 1989; Blindow, 1992; Steinman *et al.*, 1997; Kufel & Kufel, 2002), and unfavorable light conditions may reduce the formation of oospores (Corillion, 1975; Bonis & Grillas, 2002). Under such conditions, photosynthesis is limited and consequently the amount of starch produced and stored in oospores might be smaller. This might be a reason why the oospores from 3m depth were shorter than those at 1.5m depth.

Reduction of the oospores' degree of elongation (lower value of the ISI index) in response to unfavourable light conditions is not typical of stoneworts or universal among them. For instance, an inverse relationship was observed in *Chara rudis,* which showed increasingly prolate oospores along the depth gradient (Boszke & Bociąg, 2008). *C. globularis* oospores from ponds with turbid water were also characterised by a high value of the ISI index (Boszke & Pełechaty, 2006).

The present study supports the existence of a high degree of intrapopulation oospore variability, analogous to *Chara rudis* (Boszke & Bociąg, 2008). Such varaibility is thought to be typical of perennial species from permanent water bodies (Casanova, 1997), resulting perhaps from the lack of selection pressure in an environment where stonewort survical may be less dependent on oospores because vegetative reproduction takes place. However, the significant differences in the features of *C. globularis* oospores coming from two depths in the present study suggests that it is the diversity of selection pressure that shapes this variability. Szmeja (1994), whose theory was based on Grime (1979, 2002), noted that the type of selection pressure on macrophytes in lakes changed from type R (disturbance) to S (stress) with depth. It follows that, if a population occupies a wide range of depths, differences in the type of response to stress can be expected.

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