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Comparative study of the oospore morphology of two populations of a rare species *Chara baueri* A. Braun in Cedynia (Poland) and Batzlow (Germany)

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Abstract

Morphological features of oospores of *Chara baueri* A. Braun, one of the rarest charophyte species worldwide, were studied based on 100 oospores collected from a small and temporarily dried mid-field pond near Cedynia, Western Poland. This is the first Polish and fifth presently known locality of this species. For comparison 67 oospores from a German population (similar pond localized near Batzlow, Germany) were also measured. So far, data on morphology of *C. baueri* oospores as well as the species ecology are limited. The only more detailed study of oospores for this species was earlier performed on 15 oospores from Kazakhstan. Largest polar axis (*LPA*, length), largest equatorial diameter (*LED*, width), isopolarity index ($ISI = LPA/LED \times 100$), number of ridges, width of fossa, distance from apical pole to *LED* (*AND*) and anisipolarity index ($ANI = AND/LPA \times 100$) were measured. The comparative analysis revealed that the oospores from Poland are generally bigger and more prolate than the German ones. The differences for most of studied parameters were statistically significant. The finding is discussed in the context of habitat differentiation of both studied sites. Moreover, the results obtained of oospore measurements for both populations differs from most of the data known so far from the literature.

Keywords: *Chara baueri*, oospores, Characeae, charophytes

Introduction

Charophytes are multicellular organisms that occur in fresh and brackish water bodies throughout the world [1,2]. As macroscopic green algae they form neither tissues nor typical organs for vascular plants (Fig. 1). Their macroscopic, equisetum-like body is called a "thallus" and is attached to the bottom by delicate rhizomes. The main axis of charophyte thalli is composed of a number of nodes and internodes. The nodes are places where side-branches and whorls of branchlets are formed in numbers depending on the genus and species. Charophytes produce sexual propagules: oogonia (female) and antheridia (male) on the branchlets. Charophytes develop many other elements of morphology (e.g. bract cells, spine cells, stipulodes) that also vary among taxa.

Oospores are the mature oogonia (becoming dark after fertilisation), which are surrounded by a resistant wall made up of many layers (Fig. 2). The oospore wall ornamentation can be species specific, e.g. for genus *Nitella* [1], and can be helpful in distinguishing of the species [3-10]. Charophyte oospores can also be preserved in sediments and used as indicators of past conditions [11-13].

Chara baueri A. Braun (*Chara scoparia* Bauer ex Reichenbach) is one of the rarest species of the Characeae family worldwide. The only currently known sites of this species are localized in Kazakhstan and Germany [14-16].

A new locality of *Chara baueri* (the first known locality of this species in Poland) was found in August 2008, in a mid-field pond localized near Cedynia, mid-Western Poland [16]. Nowadays, *Chara baueri* occurs most numerously (3 sites) in Brandenburg (Germany), about 40 kilometers west of the Polish locality (Fig. 3).

The species is characterized by a stem with a triple cortex with distinct solitary spine-cells and ecorticate branchlets built by 3-4 segments with a specific coronula at the top of the final segment. Stipulodes are distributed in 1 tier, 1 per branchlet. All those features were well developed by individuals found in Polish population and clearly distinguishing *Chara baueri* from other *Chara* species [16]. The only species that could be mixed up in this case is *Chara braunii* Gmel., however, this is distinguished by being the only totally ecorticate *Chara* species in Poland.

It is also worth emphasizing that *Chara baueri* was amalgamated with *Chara muelleri* (described for population found in Australia) by Wood [17]. The oospores as well thalli of that taxon have been examined in a study by Casanova [6]. Still,

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current taxonomic approaches are not sufficient to confirm the conspecificity of these disjunct populations [7,15].

Resulting from the rarity of *Chara baueri*, the data on its ecology and habitat requirements are very limited [1,14-16]. To date, the only detailed study on morphological features of oospores of *Chara baueri* has been carried out by Hutorowicz [18] on the basis of 15 oospores, collected by Sviridenko in Kazakhstan, in 1995. Thus, the aim of this study was to characterize and compare the morphological features of oospores of *Chara baueri* from two new populations, regarding also their habitat.

Material and methods

Material was collected from two separated populations: (***i***) near Cedynia, Western Poland (first locality of this species in Poland, found and studied in August 2008, [16]) and (***ii***) near Batzlow, Germany (found in 2006 [15]; Fig. 3). For both localities oospores and environmental data were collected in August 2008.

The studied sites of *Chara baueri* are shallow mid-field ponds. Individuals of *Chara baueri*, from which oospores were collected, occurred on mineral substratum at the depth of 15-20 cm, on a very insolated parts of habitat. Both sites differed as regards the vegetation structure. Pond near Cedynia was dominated by vascular plants (mainly *Ceratophyllum submersum* L.) and *Chara baueri* occurred there in small patches, whereas in pond near Batzlow no vascular vegetation was found and it was co-dominated by *Chara baueri* and *Nitella mucronata* (A. Braun) Miguel in equal proportions.

The oospores were collected from the living plants, from different parts of the thallus, choosing only mature oospores (dark colored or black). Morphological features of oospores were studied based on 100 oospores collected from Polish site near Cedynia. For comparison 67 oospores from a German population were measured. All material was stored and measured dry.

The largest polar axis (*LPA*, length) and the largest equatorial diameter (*LED*, width) were measured and then isopolarity index (*ISI* = *LPA*/*LED* × 100) was calculated (Fig. 3). The number of ridges, width of fossa, distance from apical pole to

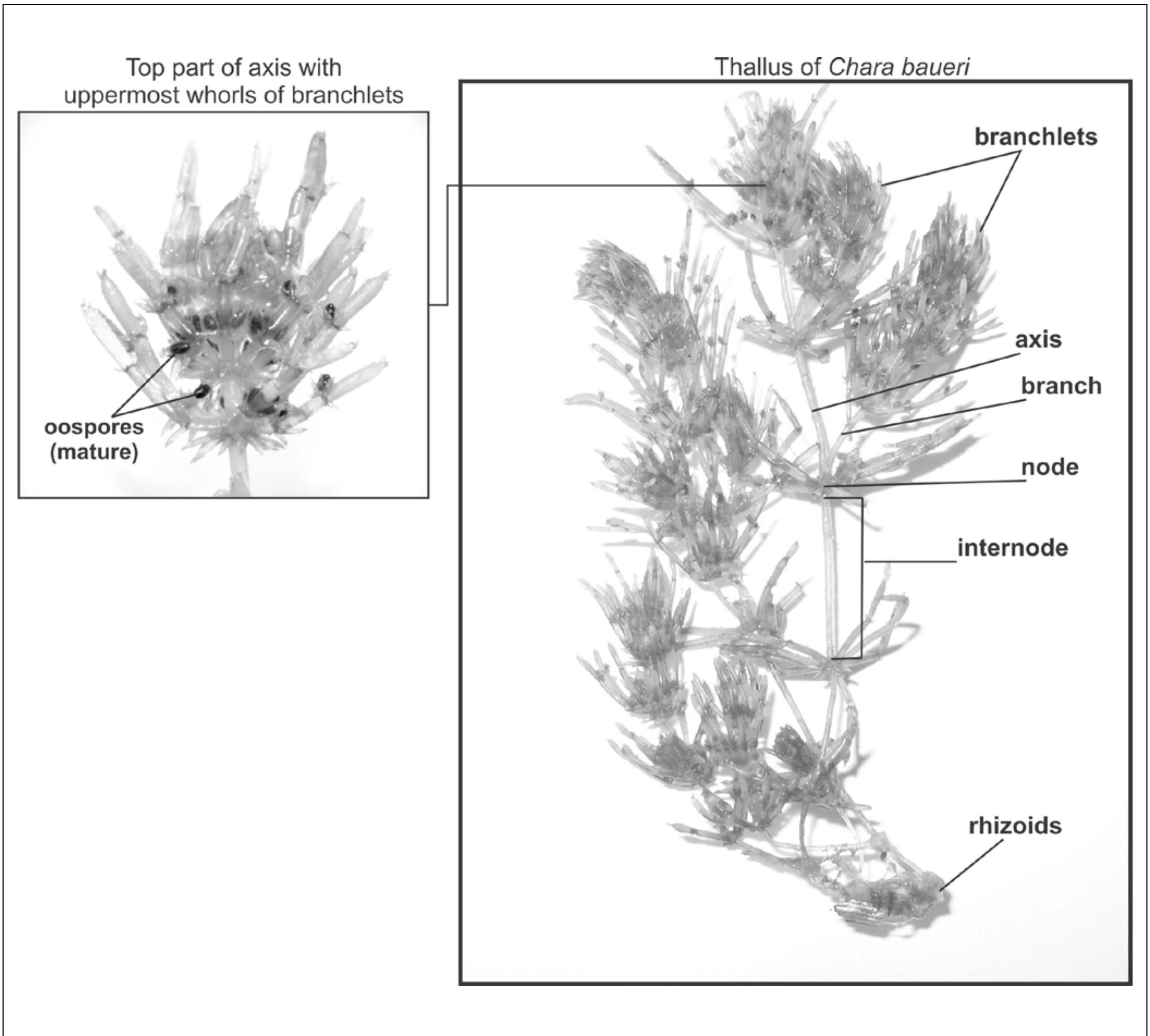

Fig. 1 Morphological structure of charophytes, main features.

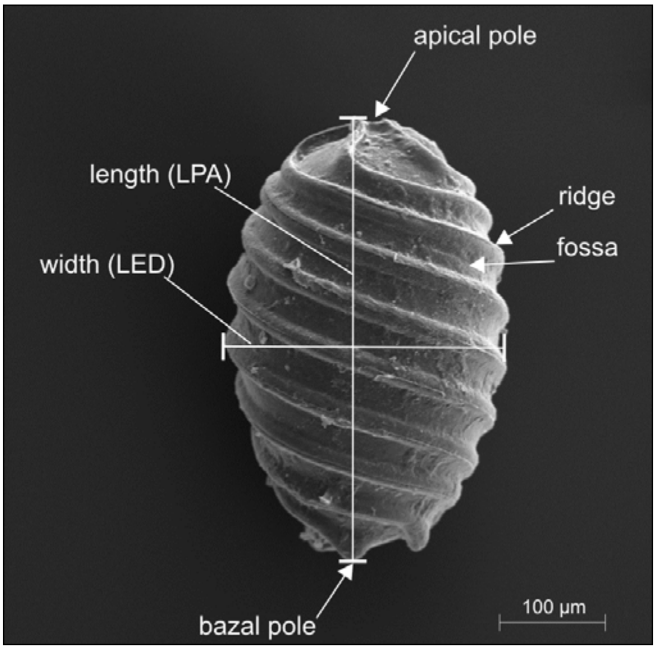

Fig. 2 Oospore morphology of *Chara baueri* (specimen from Polish site).

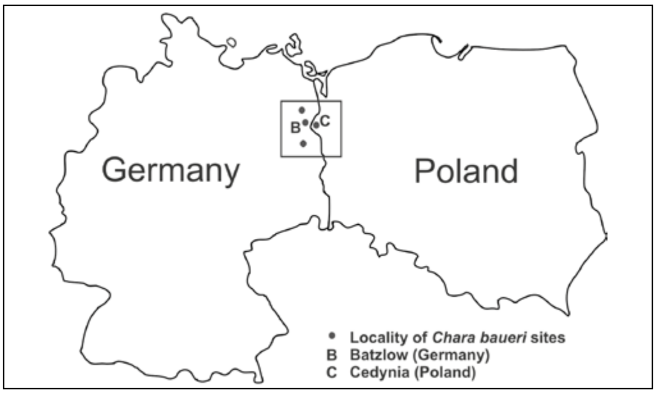

Fig. 3 Distribution map of *Chara baueri* sites.

LED (*AND*) and anisipolarity index ($ANI = AND/LPA \times 100$) were also examined according to Horn and Rantzien [19]. All measurements were performed using a stereoscopic microscope Olympus SZX 9. Additionally, the SEM photographs were taken at Electron and Conphocal Microscope Laboratory (Faculty of Biology, Adam Mickiewicz University, Poznań, Poland). Prior to the SEM images the standard cleaning procedure of careful calcium carbonate precipitation removing was used [20].

For both ponds basic physical-chemical parameters, reflecting habitat conditions, were determined. The measurements of conductivity, oxygen concentration, temperature and pH were performed at the central site of each pond, using the multifunctional Elmetron CX-742 field apparatus. Water samples for chemical analyses (total hardness, concentration of total phosphorus and total nitrogen) were collected from the surface layer and analysed according to commonly used analytical methods in accordance with the "Standard methods" [21].

All statistical computations (basic descriptive statistics, box-whiskers graphs and Mann-Whitney U-test) were done with the use of Statistica 8.1 software.

Results and discussion

Oospores of *Chara baueri* collected in Poland were prolate or perprolate (*ISI* index 156-291) and had an ovoid to ellipsoidal shape (*ANI* index 20-53.6). They were dark brown or black with prominent ridges and did not have the so called "shoulder" (indentation in the upper part of the oospore), a typical feature of some species (Fig. 2). The SEM analysis did not reveal any specific wall ornamentation details, hence it was not possible to describe it more precisely.

The measurements of oospores from Polish site revealed quite wide variability. The length was in range of 400-667 µm, averaging 514 ±41.9 µm, whereas width ranged from 183 to 300 µm, with an average of 262 ±25.7 µm (Tab. 1). There were 8-11 ridges on the oospore surface, most often 9. The mean width of fossa was 44.7 ±10.9 µm, as they can be from 25 to 66.7 µm. The coefficient of variation ranged from 8.2% for oospore length to 24.4% for width of fossa.

Tab. 1 Features of oospores of *Chara baueri*.

Feature	Mean	*SD*	Median	Min.	Max.	*V* (%)
Cedynia (Poland), *N* = 100						
ISI index	198	25.4	194	156	291	12.8
ANI index	35.9	7.3	34.5	20	53.6	20.3
LPA (µm)	514	41.9	500	400	667	8.2
LED (µm)	262	25.7	267	183	300	9.8
Number of ridges	9.2	0.8	9	8	11	8.7
Width of fossa (µm)	44.7	10.9	41.7	25	66.7	24.4
AND (µm)	184	40.5	183	100	316	22
Batzlow (Germany), *N* = 67						
ISI index	186	16.6	186	139	238	8.9
ANI index	38.4	7.1	37.9	24.1	60.7	18.5
LPA (µm)	479	33.9	483	417	550	7.1
LED (µm)	259	19.6	267	216	300	7.6
Number of ridges	9.7	0.6	10	8	11	6.2
Width of fossa (µm)	42.9	7.6	41.7	25	58.4	17.7
AND (µm)	184	32.4	184	117	283	17.6

AND – distance from apical pole to *LED*; *ANI* index – anisipolarity index $AND/LPA \times 100$; *ISI* index – isopolarity index $LPA/LED \times 100$; *LED* – largest equatorial diameter; *LPA* – largest polar axis; Max. – maximum; Min. – minimum; *SD* – standard deviation; *V* – variation coefficient.

The oospores from German site were 417-550 µm long, averaging 479 ±33.9 µm and 216 to 300 µm wide, with an average of 259 ±19.6 µm. Width of fossa ranged from 25 to 58.4 µm, with an average of 42.9 ±7.6 µm. The number of ridges was similar to those accounted for Polish (8-11 ridges on the oospore surface, with an average of 9.7 ±0.6) locality. The values of coefficient of variation were lower than for Polish oospores and ranged from 6.2% for number of ridges to 18.5% for *ANI* index.

To summarize, the oospores from Poland are bigger and more prolate than the German ones. In the case of oospores collected from the German site the variation coefficients were lower for each feature studied. In particular, the main

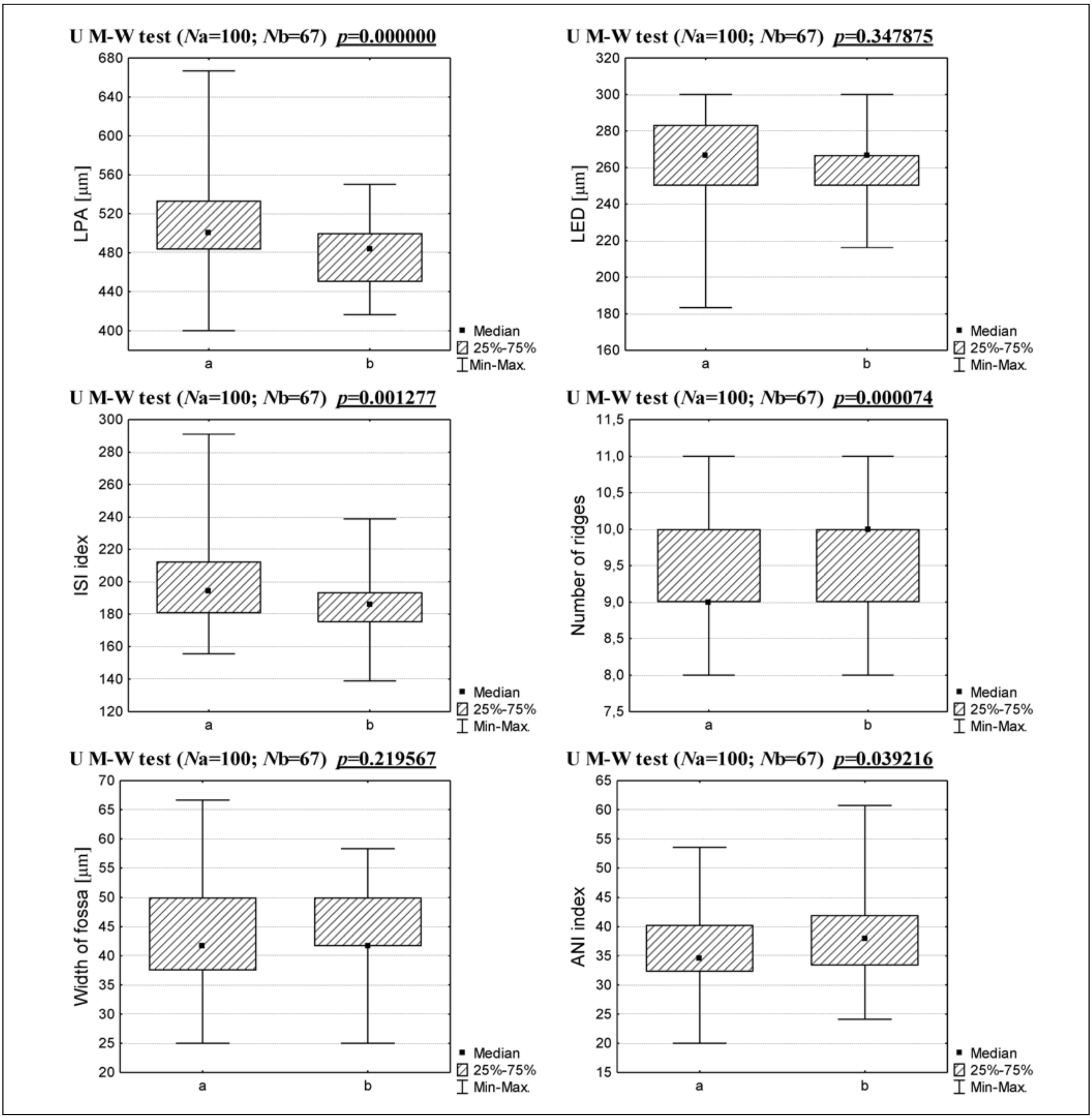

Fig. 4 Comparison of *Chara baueri* oospore features for: **a** Polish population; **b** German population.

size features had narrower ranges of values as compared to Polish population. Statistical analyses proved the differences for most of parameters are significant (Fig. 4). According to Mann-Whitney U-test only width of fossa and *LED* revealed no statistically sound differences ($p > 0.05$).

Looking for the reason of the assessed differentiation it should be stressed that the material for presented comparison was in both cases collected in a similar range of time and the sites (ponds) are situated just at a distance of few dozen kilometers. That preclude possible influence of the climate. Both sites differed as regards aforementioned structure of vegetation, that might indicate habitat differences (Tab. 2). However, the values of presented properties of water in most of cases did not reveal visible differences. The comparison of basic properties of water quality revealed that both ponds are highly eutrophic. Although data on the relationship between the morphometry of oospores and habitat conditions are lacking in worldwide literature, it can be presumed that this is just what may be one of main reasons of assessed differentiation of oospore characteristics. Moreover it should be stressed, that in this kind of small and shallow reservoirs also charophytes may influence on the habitat conditions. Such type of relations has been already described in the theory of alternative stable states wherein strong competitive interactions between phytoplankton and hydromacrophytes against habitat conditions background were stated with important contribution of charophytes [22-24]. It may be pointed out, e.g. by lower values of calcium in the pond near Batzlow, that was almost entirely overgrown by charophytes.

Even though both the Polish and German ponds are shallow and characterized by very similar habitat properties of water they differ as regards the size and water level fluctuations. The

Tab. 2 Physical-chemical properties of water bodies with Polish (pond near Cedynia) and German (pond near Batzlow) populations of *Chara baueri*.

Site	O_2 (mg/l)	Conductivity (µS/cm)	pH	Hardness (°dH)	Mg (mg/l)	Ca (mg/l)	P-PO_4 (mg/l)	TP (mg/l)	N-NH_4 (mg/l)	N-NO_3 (mg/l)	N-NO_2 (mg/l)	TN (mg/l)
Cedynia	6.14	611	8.01	12.9	14.9	71.8	0.63	1.04	1.31	0.47	b.d.	3.89
Batzlow	3.25	632	7.92	13.2	16.3	67.6	0.71	1.12	1.45	0.68	0.02	5.15

b.d. – below detection sensitivity.

whole pond near Batzlow is small, temporary ecosystem. By contrast, the pond near Cedynia is bigger, with a permanent, central deeper basin, that is surrounded (in wet years) by littoral part which is also more preferable for charophytes. So, in the same weather conditions, during dry period, the water level in the pond near Cedynia does not change so rapidly as in pond near Batzlow. Moreover, both ponds differ with respect to the vegetation structure. The pond near Cedynia was dominated by vascular plants, whereas pond in Batzlow was co-dominated by *Chara baueri* and *Nitella mucronata* with no vascular plants occurring. As was suggested by Casanova [7], in temporary habitats oospore size can vary less because there is strong selection pressure for germination success (which is also related to oospore size), and in permanent habitats there can be greater variation because there is more vegetative reproduction and less sexual reproduction.

Another factor that can influence the variation in oospore size for the investigated populations may be the local variability of weather fluctuations during the vegetation season. The field ponds, as very shallow ecosystems, localized in the open area are very susceptible to weather changes, especially to the torrential rains or strong winds. Such atmospheric conditions occur very often in a local scale, but at the same time it can diversify unstable ecosystems, even if the distance between individual ponds is not very long.

The obtained data shows not only the differences between studied populations. Comparing the results to the most of previously reported literature data [18,25-27], variation ranges of the *Chara baueri* oospore features in the studied populations were generally wider. It may be concluded that the oospores of both, the Polish and German population were in most cases bigger then in above cited literature. However, Hollerbach and Krassavina [27] postulated that the length may reach 720 µm, which is much more then was stated for samples from populations studied. On the other hand, maximal length found by Hutorowicz [18] was only 574 µm.

The number of ridges (8-11) also differed the investigated populations from the most of literature data, giving 8 ridges as the most typical for this species [13,25,26]. It is, however, consistent with the population from Kazakhstan, described by Hutorowicz [17].

What is crucial here, the presently known data about *Chara baueri*, show clearly that the species from different sites do not reveal significant differences as regards the species-typical morphometric features [1,14-16]. The reason of some discrepancies between the obtained results and the literature data regarding oospore features may results, e.g. from different number included in particular investigations, different parts of thalli from which the oospores were taken, the diversified maturity or different habitat properties.

With reference to the information mentioned in introduction that *Chara muelleri*, found and described for Australia was amalgamated with *Chara baueri* as the same species, it is worth to stress out that presented results, as well as literature data [13,18,25-27] differs clearly from the data given by Casanova [7]. That may strongly suggest, that the species found and described for Australia is not *Chara baueri*.

Even though this results may suggest ongoing speciation within *Chara baueri* species, such an event has not been documented yet. Despite considerable isolation of the presently known localities of *Chara baueri* sites (four in Europe and one in Kazakhstan), it is highly probable that they are dispersed at much bigger area between Europe and Central Asia. Limited knowledge about distribution of this species in all likelihood results from very peculiar (temporary) type of ecosystems occupied by this species [15,16].

The presented results widen the current knowledge about this extremely rare species. However, to know better the mechanisms of distribution and differentiation of *Chara baueri*, continuation of the investigations as well as additional analyses (especially the genetic ones) would also be desirable.

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