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Research paper

Overwintering and gyrogonite formation by the rare and endangered indicative macroalga *Lychnothamnus barbatus* (Meyen) Leonh. in eutrophic conditions

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ABSTRACT

Lychnothamnus barbatus (Meyen) Leonhardi is a rare, endangered charophyte species considered to be a reliable indicator of low water fertility, with its fossilized gyrogonites (calcified oospores) being applied as a biomarker of past oligotrophic conditions. This paper presents a case study from a lake in which *L. barbatus* occurs abundantly under eutrophic conditions (Lake Kuźnickie, central-western Poland). The species overwinters in this lake in the form of extensive meadows along a wide depth gradient and, in addition, produces fully developed gyrogonites.

The occurrence of *L. barbatus* in Lake Kuźnickie was investigated at 1 m depth intervals at nine permanent transects in November 2015 and in April 2016. Spring thalli were observed microscopically to determine whether *L. barbatus* overwintered (with new outgrowths on old thalli) or grew anew in spring (from oospores). The species occurred at almost 50% of the surveyed sites in both sampling periods. At most sites *L. barbatus* formed compact carpets in which it was the only species or co-occurred with the charophyte *Nitellopsis obtusa* or the angiosperm *Ceratophyllum demersum*. *L. barbatus* occurred along a wide depth gradient: 1.5–6.5 m deep in autumn and 2–6.5 m after the winter period. The species coverages in April 2016 were comparable to those observed in November 2015 (Mann-Whitney *U*-test, $P > 0.05$). The thalli collected in April were well preserved after the winter, with numerous spring outgrowths. In addition, fully developed gyrogonites were found in April 2016. This study indicates wider fertility tolerance of *L. barbatus* and its ability to overwinter under eutrophic conditions.

1. Introduction

Charophytes (stoneworts, Charophyta) are submerged macroscopic green algae, which include extant and fossil members of the order Charales. Charophyte body consists of delicate rhizoids, that anchor the aboveground equisetum-like thallus to the bottom sediments. These macroscopic algae are well distributed all over the world in various types of aquatic environments (e.g., Krause, 1997; Martin et al., 2003). However, charophytes are particularly abundant, often forming extensive underwater meadows, in clear, alkaline and nutrient-poor waters. Therefore, a decline of charophytes indicates increasing trophic state that is applied in water quality monitoring and the ecological status assessment (e.g., Hutchinson, 1975; Krause, 1981, 1997; Blindow, 1992; Ciecierska and Kolada, 2014).

Extant charophytes (Characeae family) are represented by 314 species gathered in six genera worldwide, out of which Lychnothamnus (Ruprecht) Leonhardi em. A. Braun is a monotypic genus (Wood and Imahori, 1965). The genus was well distributed in the past and represented by more than 20 species in Eurasia (Sanjuan and Martín-Closas, 2015; Soulié-Märsche, 2016 pers. communication). Today the genus is represented by the only extant species, *Lychnothamnus barbatus* (Meyen) Leonhardi (Wood and Imahori, 1965; Casanova et al., 2003a,b), although an attempt was made in China (Han, 1958; Jao and Lee, 1974) to describe another Lychnothamnus species, *L. longibracteatus*, probably being a variety or ecotype of *L. barbatus*.

L. barbatus is considered rare and in decline worldwide bringing a risk of the genus extinction (Casanova et al., 2003a,b; Chou et al., 2007; Sugier et al., 2010; Azzella and Abdelahad, 2011; Azzella, 2014; van de Weyer et al., 2016). *L. barbatus* has a status of an

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endangered species in many countries, being the first endangered non-vascular plant in Australia (as summarized by Sugier et al., 2010 and van de Weyer et al., 2016).

L. barbatus has been reported from a variety of freshwater habitats, mainly from lakes, and from different climates. Still, localities of this species were limited to some regions within a Euro-Australasian distribution, mainly to Central, Eastern and Southern Europe (Casanova et al., 2003a; Sugier et al., 2010). Between 2012 and 2014, new localities of *L. barbatus* were found in 13 lakes in Wisconsin, USA, to be the first discoveries of this species in America (Skawinski et al., 2015). These findings broaden the worldwide distribution range of the species and increase the number of known current localities to 54 (Pełechaty and Brzozowski, unpubl. data). It must be emphasized, however, that 62 localities of *L. barbatus* have disappeared since the end of the 19th century, probably because of increased recreational use and eutrophication of lakes (Casanova et al., 2003a; Sugier et al., 2010). Therefore, the species is considered a sensitive indicator of low fertility and high clarity of waters (Kolada, 2009; Pełechaty et al., 2009 and references therein). In addition, Karczmarz (1967) was of the opinion that only in nutrient-poor clear waters and at depths of 3 – 5(7) m is *L. barbatus* able to accomplish the full biological cycle. In eutrophic waters the species produces only immature oospores. It may, thus, be concluded that fully developed gyrogonites, calcified oospores commonly applied in palaeoreconstructions, may develop from oospores only in oligotrophic waters. On this basis, Soulié-Märsche and Martin-Closas (2003), Martin-Closas et al. (2006) and Mazzini et al. (2015) applied fossil *L. barbatus* gyrogonites as a biomarker of past oligotrophic conditions.

This paper presents a case study from a lake in which *L. barbatus* occurs abundantly under eutrophic conditions along a wide depth gradient. The study aim was to find out whether *L. barbatus* overwinters in this lake in the form of extensive meadows. In addition, we examined the plants microscopically to see if mature oospores (gyrogonites) were produced by *L. barbatus* in the unusual eutrophic conditions.

2. Study lake

The study was carried out in Lake Kuźnickie (N 52°12′56.7″, E 16°05′31.0″, Fig. 1), a lake with extensively developed charophyte vegetation in which the study species, *L. barbatus*, has been known since its first discovery in 1978 (Gołdyn, 1983). Lake Kuźnickie is located 80 kilometers southwest of Poznań city and 12 km south of Nowy Tomyśl town (central-western Poland, Fig. 1), in the area of the Poznań Lake District. The lake surface area is 75.7 ha. It is a dimictic water body with the maximum and average depths of 13.2 m and 7 m, respectively (Fig. 1).The lake is used for recreational purposes and is managed by the Polish Angling Association (PZW, Polski Związek Wędkarski). Thus, angling is among the main forms of the lake's recreational usage. Bathing places, holiday cottages and leisure centers, located along the western shore of the lake, contribute to the human pressure the lake is subject to. The lake is located between Boruja and Kuźnia Zbąska villages. Although western part of the lake's catchment area is agricultural, forests predominate in the catchment basin, surrounding the lake from the north, east and south. Lake Kuźnickie is a moderately eutrophic water body (Dzieszko and Zwoliński, 2015). According to the Voivodship Inspectorate of Environment Protection in Poznań (VIEP, 2015, unpubl. data), yearly mean total phosphorus (TP) concentration was 49 $\mu g\,l^{-1}$ in 2015, while mean chlorophyll-*a* concentration and mean Secchi disc depth reached 5.4 $\mu g\,l^{-1}$ and 4.1 m, respectively. Waters of the study lake are rich in nitrogen (yearly mean total nitrogen (TN) concentration for 2015 was 976.5 $\mu g\,l^{-1}$). The waters are alkaline with calcium yearly mean

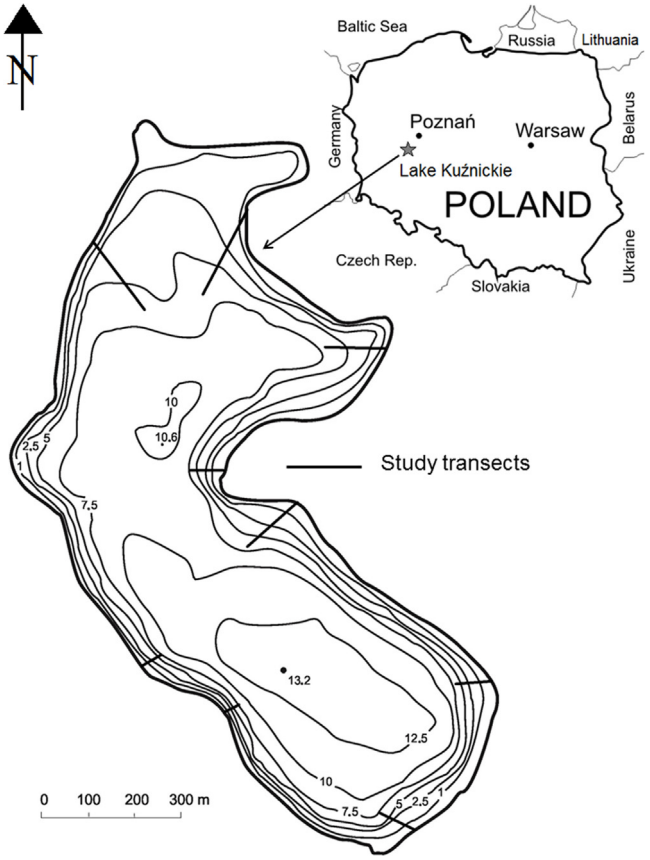

Fig. 1. Location of Lake Kuźnickie and distribution of study transects. Contour lines are in meters.

concentration of 37.1 mg l^{-1}, alkalinity of 2.49 mmol l^{-1} and pH of 8.1. However, solute content is rather low as evidenced by mean electrolytic conductivity of 223 $\mu S\,cm^{-1}$. VIEP monitoring revealed a significant share of charophytes in submerged vegetation in 2015. The phytolittoral area reached 24.72 ha, which constitutes 1/3 of the lake's area. The VIEP study also documented a recovery of *L. barbatus* after the species apparent disappearance caused by the lake stocking with grass carp (*Ctenopharyngodon idella*) and the related water quality deterioration in late 1980s (Gołdyn, 2016 pers. communication).

3. Methods

This study was carried out in November 2015 and in April 2016. Macrophytes were sampled by means of an anchor from the boat. Samples were taken along a depth gradient at nine permanent transects (Fig. 1). Between each subsequent meter of depth the plant species composition and cover were determined with the use of Braun-Blanquet's (1964) method. For statistical purposes the Braun-Blanquet scale (range from r to 5), was transformed into Van der Maarel (1979) scale (range from 1 to 9), as follows: r = 1, + = 2, 1 = 3, 2 = 5, 3 = 7, 4 = 8, 5 = 9. In the laboratory, the thalli collected in April 2016 were compared to those collected in November 2015 and observed with the use of stereomicroscope Olympus SZX9 to search for young side outgrowths. It allowed to distinguish between the thalli developed in the previous year from those newly grown in spring. Additionally, the thalli from spring samples were searched through for gyrogonites (calcified oospores).

In order to verify whether the species overwintered in Lake Kuźnickie we confronted the depth and coverage of *L. barbatus* at each site it occurred in autumn and, then, in spring. To do that the