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Anabaena poulseniana J. Boye Petersen – a species new to Polish flora

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Abstract

Anabaena poulseniana J. Boye Petersen Bot. Iceland 2, 1923 (Cyanobacteria), a cyanobacteria species new to Polish flora, was noted during a study of phytoplankton in one of Wrocław's clay pits. The species was noted in plankton samples among *Cylindrospermopsis raciborskii* (Woloszyńska) Seenayya et Subba Raju (Cyanobacteria) and *Mougeotia* sp. (Chlorophyta) filaments. The green algae created a bloom characterized by vegetation mats floating on the water.

The paper presents detailed characteristics for *Anabaena poulseniana*, and for the habitat and the ecological condition in the examined water reservoir during its presence. The paper also presents a comparison of morphological characteristics of *A. poulseniana* from Poland to the species from other geographical locations. The research will provide data on the morphology and ecology of the species. The species found during the study were documented in the form of original photos and illustrations.

INTRODUCTION

The progressing pollution of the water environment is connected above all with eutrophication, resulting in a surplus production of algae biomass, which manifests itself with a water bloom (Wilk-Woźniak 1998, Bucka & Wilk-Woźniak 2005, Burchardt & Pawlik-Skowrońska 2005, Imai et al. 2006). In water blooms, major elements include cyanobacteria from the Chroococcales order, such as *Microcystis aeruginosa* (Kützinger) Kützinger and *Woronichinia naegeliana* (Unger) Elenkin (Berg et al. 1987, Tarczyńska et al. 2001, Bucka & Wilk-Woźniak 2002, Oudra et al. 2002, Le Ai Nguyen et al. 2012, Zhang et al. 2012).

Recent research of eutrophicated lakes suggests, however, increasing occurrence of filamentous cyanobacteria such as *Plankthothrix*, *Aphanizomenon*, *Dolichospermum* (*Anabaena*) and *Cylindrospermopsis* (Krupa & Czernaś 2003, Stefaniak & Kokociński 2005, Błaszczuk et al. 2010, Fuentes et al. 2010, Ferrari et al. 2011, Kokociński et al. 2011, Werner et al. 2012). As a consequence, there are growing issues with the use of drinking water reservoirs and also with the decrease in quality of water in recreational basins, both natural and artificial water ecosystems (Codd 2000, Molica et al. 2005, Haande et al. 2007, Bláha et al. 2009, Duan et al. 2009).

Cyanobacteria and algae can influence the quality of water ecosystems in various ways. In shallow basins the rotting masses lead to anaerobic conditions at deeper levels due to the intense activity of microbes using large amounts of oxygen. This results in an oxygen deficit (Krzyżanek et al. 1993), impeded oxygen decomposition of organic matter and eradication of many oxygen sensitive organisms. Additionally, the growing decomposed substance leads to such basins becoming shallower (Kawecka &

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Eloranta 1994, Dojlido 1995, Bucka & Wilk-Woźniak 2002).

Moreover, some cyanobacteria produce toxic substances that are very dangerous for oxygen sensitive organisms (Jura & Krzanowska 1999, Romanowska-Duda et al. 2002, Mazur et al. 2003, Babica et al. 2006, Werner et al. 2012). These substances can be deadly to water animals (arthropoda, mollusca, fish), land animals (birds, cattle) (Bucka 1998, Bucka & Wilk-Woźniak 1999, Oudra et al. 2002, Pawlik-Skowrońska et al. 2004, Mankiewicz et al. 2005) and even humans (Barreto & Figueiredo 1996, Kuiper-Goodman et al. 1999, Carmichael 2001, Oudra et al. 2002, Bláha et al. 2009). The study of phytoplankton in one of Wrocław's clay pits revealed a new (for Polish flora) species: *Anabaena poulseniana* J. Boye Petersen. The species was discovered in plankton samples in the threads of *Cylindrospermopsis raciborskii* (Woloszyńska) Seenayya et Subba Raju and *Mougeotia* sp. green algae.

This paper presents detailed morphological characteristics of *A. poulseniana* and the habitat and ecological (physicochemical) conditions in the studied water basin. Additional comparison of the morphological characteristics of the taxon with specimens from other geographical locations will allow broader morphological and habitat characteristics of the species. The research is especially valuable because the systematic division of the genus is provisional and requires further study and, most of all, detailed descriptions and pictures of the forms found in many biotopes (Starmach 1966; Komárek & Anagnostidis 1989). The paper will attempt to provide some of this information.

General distribution

The species was noted for the first time in 1914 in East Iceland in Mývatn water reservoirs, on stones and ashore, on *Cladophora* plants, and in the waters of the small Skútustaðir pond. *Anabaena poulseniana* occurred in straggling wisps rounded at the top, attached to the reservoir's bottom and supported by air-bubbles among other algae. These tufts were composed of Cyanobacteriaceae, especially *Anabaena poulseniana*, *Oscillatoria limosa* Agardh ex Gomont, *O. santa* Kützinger ex Gomont, *Tolypothrix* sp. and Diatomeae (Petersen 1923). The species was then found in Romania on *Sphagnum* (Tarnavski et al. 1956) and in Asia in freshwater reservoirs (Yakutia) (Komarenko & Vasileva 1975). It may be concluded from the literature data that this species was reported

in Europe and Asia, in standing waters, attached to the bottom or freely swimming objects, but, until now, it has not been reported in Poland.

In order to confirm the lack of occurrences of the studied taxon in Poland the following sources were consulted: Catalogue of Polish prokaryotic and eukaryotic algae (Siemińska & Wołowski 2003) and Polska Bibliografia Fykologiczna (Siemińska 1990, Siemińska & Pająk 1992). Additionally, a search was conducted in the catalogue of the Institute of Botany in the Polish Academy of Sciences in Kraków.

MATERIALS AND METHODS

The research was conducted from April to August 2008. The phytoplankton samples were gathered monthly, from April to August. The plankton samples were collected with a plankton net and then placed in 100 ml plastic containers. The study material was collected 0.5 m from the shore by casting and pulling the net along the shore for about 1.5 m at a depth of between 20 and 30 cm. Morphological observations were conducted with a Nikon Eclipse TE2000-S light digital microscope equipped with a Nikon DS-Fi1 camera. The taxon was digitally archived using the NIS image analysis program, which enables the images to be saved with a proper scale of objects. The identification was performed live and also on material preserved with 'etaform' preserver (3:1 alcohol, formalin). The taxonomy of algae is based on Hoek et al. (1995). Cyanobacteria and algae were identified according to the studies of Starmach (1966, 1972) and Komárek & Anagnostidis (1989, 1999, 2005). Additionally, the water from the studied reservoirs underwent physicochemical analysis, which was conducted in the Analytical Laboratory at Wrocław University of Environmental and Life Sciences. The samples for water analysis were gathered on two occasions, in April and August 2008.

RESULTS

New localities

The study was conducted in a water reservoir (51°8'22.38"N, 16°58'37.70"E) in the area of Kozanów housing estate (Fabryczna district) (Fig. 1). The examined water reservoir is artificial, has a clay-pit character and is kidney shaped. The area of the reservoir is 11.200 m² (reservoir and flood area); the depth does not exceed two metres. It is fed mainly by

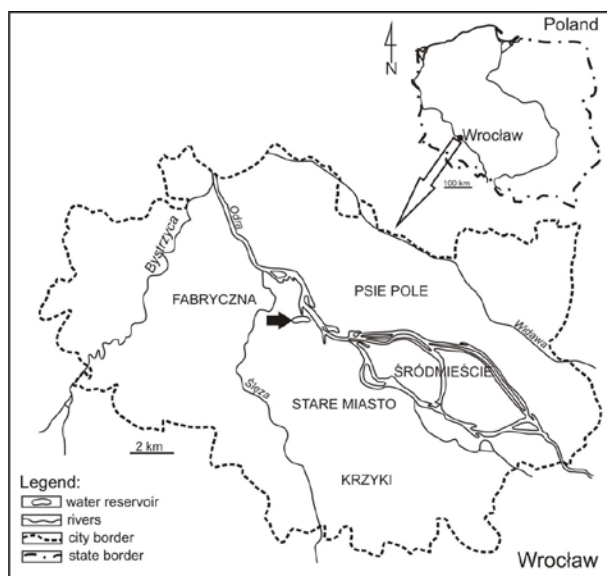


Fig. 1. Location of the studied water reservoir in Poland and Kozanów housing estate (Fabryczna district) in Wrocław; black arrow indicates the examined water reservoir

rainwater and water from surface run-off. The fluctuations in water level are rather small. The banks of the reservoir are differentiated from gentle to steep, and strengthened with wooden pickets. In the western part of the reservoir there is an island covered with Phragmites (Phragmitetea), which forms *Phragmites communis* Trin. and *Typha angustifolia* L. The bottom of the reservoir is densely overgrown with compact agglomerations of *Ceratophyllum demersum* Hild. The water surface is covered with *Lemna minor* L. and *Azolla* sp., which, during summer months, expand in huge quantities covering the whole water surface. Also, cotton-ball-like algae have formed as a result of the intense proliferation of green algae of *Mougeotia* genus, and numerous accompanying *Cylindrospermopsis raciborskii* are floating freely on the water surface in summer. *Anabaena poulсенiana* filaments were found in the filaments of *C. raciborskii*.

The physicochemical properties are shown in Table 1.

Detailed characteristics of *Anabaena poulсенiana* J. Boye Petersen (Figs 2-3)

Anabaena poulсенiana was found in the phytoplankton net in an artificial clay pit with standing water. This species was observed in increased numbers as sub-dominant in the plankton water bloom amongst other algae. These free-floating

Table 1

The physicochemical properties of the water reservoirs from which material was collected

Habitats:	Clay pit	
Year:	Samples for water analysis were gathered twice: in April and August 2008	
Variable	Mean	Range
Reaction (pH)	7.15	7.1-7.2
Conductivity ($\mu\text{S cm}^{-1}$)	926.00	887.00-965.00
Colour (mg Pt l^{-1})	91.50	43.00-140.00
Oxidation (COD_{Mn}) (mg l^{-1})	6.45	4.40-8.50
NO_2^- (mg l^{-1})	0.11	0.20-0.02
NO_3^- (mg l^{-1})	0.65	0.31-0.99
NH_4^+ (mg l^{-1})	0.125	0.102-0.103
Total nitrogen (mg l^{-1})	7.50	5.50-9.50
Organic nitrogen (mg l^{-1})	6.73	4.40-9.06
PO_4^{3-} (mg l^{-1})	0.12	0.02-0.22
Total phosphorus (mg l^{-1})	0.165	0.05-0.28
Organic phosphorus (mg l^{-1})	0.045	0.03-0.06
Fe^{2+} (mg l^{-1})	0.335	0.27-0.40
Na^+ (mg l^{-1})	68.10	47.40-88.80
K^+ (mg l^{-1})	28.34	18.49-38.20
Ca^{2+} (mg l^{-1})	85.65	74.80-96.50
Mg^{2+} (mg l^{-1})	22.32	19.35-25.30

tufts were composed of *C. raciborskii* and *Mougeotia* sp. as the dominant species and *A. poulсенiana* as the sub-dominant one. The observations were carried out mainly in summer and spring months (April – August) 2008.

Thalls *A. poulсенiana* – gelatinous, built of few tangled filaments.

Filaments straight or bent, pale blue-green (Figs 2 a-d; 3a, d-e), with colourless, unlamellated, clear and wide sheaths; the sheaths open at the ends. The filaments had a width of (8.30-) 8.80-10.20 (-13.30) μm .

Oval, square or widely cylindrical vegetative cells in trichomes, with length equal to width (4.0-) 4.5-5.5 (6.5) μm diameter or slightly longer at (3.33-) 4.0-4.20 (-4.40) μm long, (2.60) 3.20-3.70 (4.0) μm wide. Vegetative cells in trichomes do not touch; the distance between cells is (0.6-) 1.25-2.50 (-4.5) μm . Blunt conical apical cells (terminal cells) (Fig. 3f).

Rotund and slightly cylindrical heterocytes, often slightly elongated and narrowing at the ends, 6.0-8.0 (-9.0) μm long, 4.0-4.6 μm wide (Figs 2a-d; 3a-e).

Akinetes, cylindrical, 20.0-23.70 (-28) μm long, 6.50-8.0 μm wide, with blunt ends located individually far from heterocytes (Figs 3d-e).

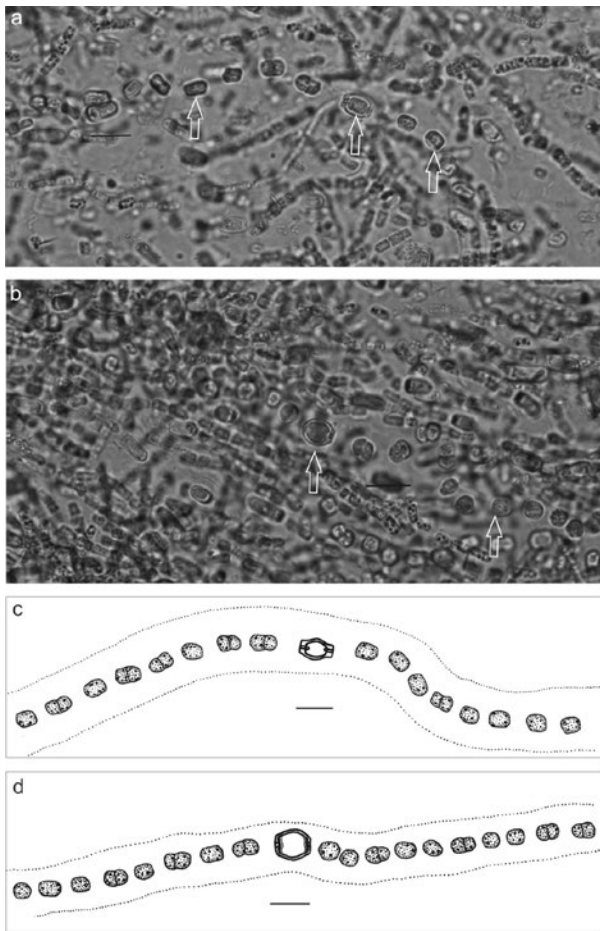


Fig. 2. *Anabaena poulseniana* Boye-Petersen: this study, **a-b** filaments of *A. poulseniana* between the filaments of *Cyndrospermopsis raciborskii* (Wołoszyńska) Seenayya et Subba Raju, arrows indicate vegetative cells and heterocysts, **c-d** details of filaments; scale bars 10 μm (according D. Richter)

Ecology

A periphytic species attached to underwater objects (e.g. stones) or water plants (*Cladophora*, *Sphagnum*) and also free floating between other filament species, forming floating vegetation mats in plankton (e.g. *Mougeotia* sp.).

DISCUSSION

Anabaena poulseniana J. B. Petersen belongs to the family Nostocaceae (Nostocales) (Komárek & Anagnostidis 1989) and is a haplotype of *Anabaena oscillatorioides* Bory (Guiry M.D. & Guiry G.M. 2013). Both species form similar gelatinous colonies attached to underwater objects or float freely in

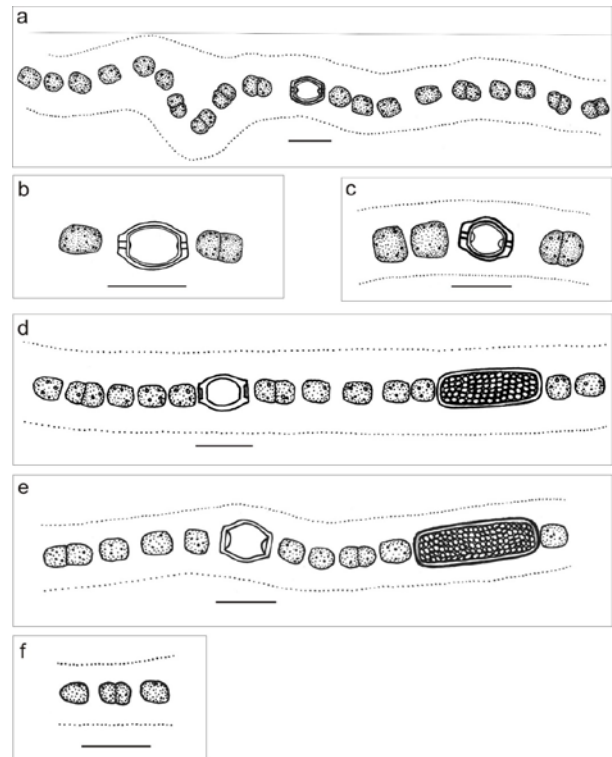


Fig. 3. *Anabaena poulseniana* Boye-Petersen, this study: **a** details of filament, **b-c** details of heterocysts, **d-e** filaments with akinetes, **f** apical cell, scale bars 10 μm (according to D. Richter)

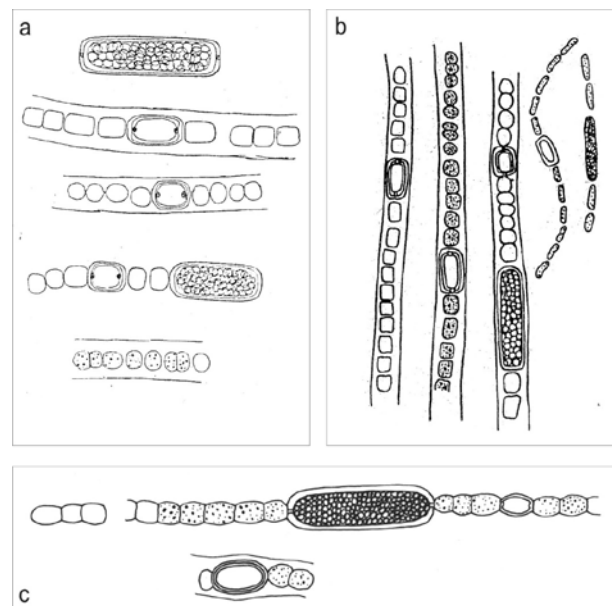


Fig. 4. *Anabaena poulseniana* J. Boye-Petersen: **a** – according to Boye-Petersen n. sp. 1923, **b** – according to Komarenko & Vasileva 1975, **c** – according to Tarnavski et al. 1956

Table 2

Morphological features of *Anabaena poulсенiana* J. Boye-Petersen

	Authors		
	Boye-Petersen, 1923 (from Petersen: Geitler 1925; Starmach 1966; Komarenko & Vasileva 1975)	Tarnavski et al. 1956	This study
Features			
Thallus	gelatinous	gelatinous	gelatinous
Attached	free floating in plankton or attached to stones and <i>Cladophora</i>	on <i>Sphagnum</i>	free floating in plankton, between other algae
Trichomes	straight or curved	straight or curved	straight or curved
Sheaths	clear	no data available	clear, wide, open at the ends
Vegetative cells			
shape	round or barrel shape, as long as wide or slightly longer than width	spherical, elliptical	oval, square or widely cylindrical, as long as wide or slightly longer than width
dimensions	4.0-4.5 μm diameter	4.0-4.8 μm diameter	(3.33-) 4.0-4.20 (-4.40) μm long, (2.60-) 3.20-3.70 (-4.0) μm wide or (4.0-) 4.5-5.5 (6.5) μm diameter
distance between cells in trichome	no data available	no data available	(0.6-) 1.25-2.50 (-4.5) μm
terminal cells shape	blunt conical	conical	blunt conical
Filaments			
width	no data available	no data available	(8.30-) 8.80-10.20 (-13.30) μm
Heterocytes			
shape	cylindrical, elliptical	cylindrical, elliptical	cylindrical, rotund, often slightly elongated and narrowing at the ends
length	11.0-17.6 μm	9.6 μm	6.0-8.0 (-9.0) μm
width	5.0-5.2 μm	4.8 μm	4.0-4.6 μm
Akinetes			
shape	cylindrical with dull edges, with a thin light-yellow trimming, often 2-4 together	cylindrical	cylindrical with dull edges, individual
length	15.0-44.0 μm	24.0-26.4 μm	20.0-23.70 (-28) μm
width	6.4-7.4 μm	8.4 with sheath or 4.8 without sheath μm	6.50-8.0 μm
Distribution	Europe, East Iceland, Mývatn, Skútustaðir; Asia, Yakutia	Europe, Romania, Poiana Stampii (Tinovel Mare)	Europe, West Poland (Wrocław)
pH	no data available	4.4-4.8	7.1-7.2
Figures	4a, b	4c	2-3

stagnant waters. They also resemble (in shape and size) the vegetative cells of the trichome. However, they differ in the width of the heterocytes (*A. poulсенiana* – 4.0-5.2 μm wide, *A. oscillatorioideis* – 8.0-10.0 μm wide), in the shape of the trichome's top cells (*A. poulсенiana* – blunt conical cells, *A. oscillatorioideis* – round cells), and in the position of the akinetes (*A. poulсенiana* – akinetes far from the

heterocytes, *A. oscillatorioideis* – akinetes on both sides of the heterocytes). The studied species also significantly differs from its haplotype in terms of the distances between the vegetative cells (Petersen 1923, Starmach 1966, Komárek & Anagnostidis 1989, this study).

A. poulсенiana also resembles *Anabena laxa* (Rabenhorst) A. Bran and *Anabena inaequalis*

(Kützing) Bornet et Flahault, but the differences, as Petersen (1923) pointed out, are easy to spot during diagnosis.

The waters in which *A. poulseniana* was noted were characterized by acidic or weakly alkaline pH (Tarnavski et al. 1956, this study - Table 1); a broad pH tolerance might suggest the species' lack of preference as to acid reaction. According to total phosphorus content based on the trophic categories (Kawecka & Eloranta 1994), the basin belongs to the eutrophic group. Its high trophic is also confirmed by high $\text{NO}_3\text{-N}$, organic nitrogen, total phosphorus and colour indicator exceeding concentrations given by Zdanowski (1983), Dojlido (1995) and Elbanowska et al. (1999). Given the physicochemical parameters, it is safe to assume that the species prefers eutrophic basins. Additionally, the high amount of nitrates (decimal parts of mg l^{-1} or more) with concurrent rise in ammonia and nitrates is an indicator of pollution (Elbanowska et al. 1999). In the case of total phosphorus concentration, Zdanowski (1983) also suggests that concentrations above 0.1 mg P l^{-1} are characteristic of high trophic waters, which are often polluted.

The comparison of *Anabaena poulseniana* with species from other geographic regions (Figs 2-4, Table 2)

Our own research and literature suggest that the species forms gelatinous thalli in all locations. The colonies are built of straight or bent filaments with thick sheaths attached to the surface or floating freely (Petersen 1923, Geitler 1925, Tarnavski et al. 1956, Starmach 1966, Komarenko & Vasileva 1975, this study). The vegetation cells of the trichomes from the studied locations showed a diversity of shapes, being round, cylindrical (Petersen 1923), spherical, elliptical (Tarnavski et al. 1956), oval, square or widely cylindrical (this study), always with length similar to width or slightly longer.

In all the populations, the morphological structure suggests a significant similarity in phenotypical features. Only slight differences were observed in the sizes of heterocytes and akinetes. Studying various populations of the same taxon makes it possible to trace its morphological diversity. A detailed morphometric analysis of *A. poulseniana* gave us a broader perspective of the morphological diversity of the species and, consequently, contributed to our knowledge and facilitated future diagnosis.

CONCLUSIONS

The study presents detailed characteristics of the morphology, habitat and ecology of *Anabaena poulseniana*, a species new to Poland. The species has been noted in Europe (Romania, East Iceland and Poland) and in Asia (Yakutia) in stagnant waters, rich and with neutral or acid pH. It created gelatinous colonies. It occurred in straggling wisps attached to water basin bottoms or stones (between other cyanobacteria and algae) and on basin shores on *Cladophora* threads. It has also been found in floating bogs on *Sphagnum* and free floating in floating vegetation mats created by *Mougeotia* sp. (Petersen 1923; Tarnavski et al. 1956; Komarenko & Vasileva 1975; this study). The data will enable a better understanding of the biology of the species and provide additional information about the heterocyst species, which is especially important because there are no recent studies of the Nostocales group (Starmach 1966, Komárek & Anagnostidis 1989). Moreover, the comparison of the studied taxon with data from subject literature shall provide a full perspective of the morphological variety within the taxon.

The study of species forming water blooms is particularly significant because of the negative effects of large algae biomasses, which lead to ecological imbalance and rapid growth of water plants. Water blooms are most often caused by one or two species, which additionally contributes to a decrease in biological diversity (Bake et al. 2001, Bucka & Wilk-Woźniak 2002). Knowing the biology and ecology of water blooms would help monitor water reservoirs and, later on, assist in their protection.

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