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Morphological study of *Chaetoceros wighamii* Brightwell (Chaetocerotaceae, Bacillariophyta) from Lake Vrana, Croatia

Sunčica Bosak[1]*, Marija Gligora Udovič[1], Diana Sarno[2]

[1] University of Zagreb, Faculty of Science, Department of Biology, Rooseveltov trg 6, HR-10000 Zagreb, Croatia

[2] Stazione Zoologica Anton Dohrn, Villa Comunale, 80121 Napoli, Italy

Abstract – *Chaetoceros wighamii* Brightwell is a planktonic diatom species originally described from brackish waters. Since its original description, the species has been reported in a wide variety of habitats, ranging from freshwater to marine. Varying descriptions exist in the taxonomic literature and several taxa have been considered as synonyms, including freshwater species *Chaetoceros amanita*. In this study we provide morphological and ultrastructural information on a cultured strain isolated from freshwater sample collected in the Lake Vrana (Vransko jezero) in Croatia, in April 2011. The cells form short and robust chains with very narrow apertures, often partially occluded by silica membranes. Other distinctive features observable in light microscopy are the shape and orientation of the setae which are very long, straight and robust, diverging in various directions from the chain axis and the single parietal chloroplast extending from valve to valve. Distinct ultrastructural characteristics are the absence of processes either in intercalary or terminal valves and the ornamentation of the valve face with densely distributed ribs spreading from an irregular eccentric hyaline area without a clearly defined annulus. The outer surface of the terminal valve is ornamented with small spines and setae are composed of flat longitudinal filaments interconnected with short bars and ornamented with small spines tightly arranged around the setae. Our description agrees well with that reported for the freshwater morphotypes of *C. wighamii* (syn. *C. amanita*) and contributes for a reliable distinction of this intriguing taxon from similar morphotypes. This finding supports the interpretation of *Chaetoceros wighamii* as a freshwater/brackish species and represents the first report of a *Chaetoceros* species in lacustrine environment in Croatia and possibly in any Central European habitats.

Key words: *Chaetoceros*, diatom taxonomy, Lake Vrana, morphology

* Corresponding author, e-mail: suncica.bosak@biol.pmf.hr

Introduction

Chaetoceros Ehrenberg is one of the most species-rich genera among planktonic diatoms, including approximately 170 species (Vanlandingham 1968). This genus has been the subject of a number of taxonomical investigations due to its ecological relevance, extraordinary morphological diversity and complexity (Hustedt 1930, Rines and Hargraves 1988, Hernández-Becerril 1996, Jensen and Moestrup 1998). Although the species are primarily marine a few taxa occur at very low salinities in estuaries and freshwater lakes, e.g. the small, often solitary and lightly silicified species *Chaetoceros muelleri* Lemmermann or the more robust *C. elmorei* Boyer (Rushforth and Johansen 1986). The taxonomy and morphology of the most frequently occurring species *Chaetoceros muelleri* and similar non-marine taxa has been the subject of a revision in which two distinct varieties were recognized (Johansen and Rusforth 1985): the nominate variety *C. muelleri* var. *muelleri* and *C. muelleri* var. *subsalsum* (Lemmermann) Johansen et Rushforth.

Chaetoceros wighamii Brightwell was originally described (Brightwell 1856) shortly after the genus *Chaetoceros* was established (Ehrenberg 1844), with special attention to the distinctive spore morphology, and a quite vague illustration of the chain features. The description leaves no doubts on the continental nature of the type locality: *Chaetoceros wighamii* »occurred in a gathering made from a dirty ditch of brackish water at the back of a small public-house, called The Burney Arms, which is marked on the Ordnance maps« in a sample rich in brackish/freshwater diatom taxa such as *Campylodiscus clypeus* and one or more *Mastogloia* species (Brightwell 1856, p. 108).

The species *Chaetoceros amanita* Cleve Euler (Cleve-Euler 1915) was established at a later date based on the description of fossil resting spores, characterized by a spiny dome-shaped primary valve and a truncate secondary valve, which were similar to resting spores described by Brightwell for *C. wighamii*. Kaczmarska et al. (1985) provided the first detailed description of the vegetative forms of *C. amanita* from the Blue Lake Spring, Utah, USA, and discussed the similarity and the possible conspecificity of *C. amanita* and *C. wighamii*, that was already considered in previous studies (Kolbe and Krieger 1942, Cleve-Euler 1951).

Over the years, *C. wighamii* has been reported in a wide variety of habitats, ranging from freshwater (Sanchez Castillo et al. 1992) to marine (Cupp 1943, Snoeijs 1993, Jensen and Moestrup 1998, Haecky et al. 1998, Bérard-Therriault et al. 1999) and was considered as the valid synonym of different marine species, including *C. bottnicus* Cleve, *C. biconcavum* Gran, *C. caspicum* Ostenfeld (Hustedt 1930, Vanlandingham 1968, Hasle and Syvertsen 1997) and *C. fallax* Proschkina Lavrenko (Jensen and Moestrup 1998).

Sanchez Castillo et al. (1992) discussed the identity of *C. wighamii* in relation with the taxa traditionally associated to it, i.e. *C. amanita*, C. *bottnicus*, *C. biconcavum* and *C. capsicum.* The authors compared the original descriptions and material from different areas, including the type locality of *C. wighamii*, and recognized the existence of two different species: 1) *C. wighamii*, which corresponded to specimens from brackish/freshwater environments and has as the only valid synonym *C. amanita*, and 2) *C. bottnicus*, originally described from the Baltic Sea (Aurivillius 1896), which included most of the examined marine morphotypes. This view has been disregarded in some of the following studies (Hasle and Syvertsen 1997, Jensen and Moestrup 1998) or partially considered in other investigations where the name *C. wighamii* was retained but a possible misinterpretation was taken into account (Snoeijs 1993, Bérard-Therriault et al. 1999).

As a result of this complicated taxonomic history, *C. wighamii* has been often perceived as a highly variable species (Hustedt 1930, Cupp 1943, Rines and Hargraves 1988, Jensen and Moestrup 1998), whereas there are indications (Sanchez Castillo et al. 1992) that some of the morphological descriptions, synonyms and reports from different habitats, refer indeed to morphologically similar but yet distinct species. Possible misidentifications can be primarily attributed to the lack of strongly distinctive characters in the vegetative cell morphology reported by Brightwell (1856) and to the fact that the presence of the very typical resting spores was rarely used to support a positive identification of the species.

In this study we investigated the morphology of a strain of *C. wighamii* (syn. *C. amanita*) isolated from the freshwater Lake Vrana in Croatia using light (LM), scanning electron (SEM), and transmission electron (TEM) microscopy. The aim is to provide detailed information, especially on the ultrastructural features of the frustule, allowing for a better delineation of this intriguing taxon and its distinction from apparently similar morphotypes.

Material and methods

Study area

Situated in the central part of the eastern Adriatic Coast, Lake Vrana and its surroundings were declared a nature reserve on July 21, 1999. It is the largest Croatian lake (length 13.6 km and width between 1.4 and 3.4 km). The lake area is between 2980 ha and 3020 ha and extends parallel to the coast in a northwest-southeast direction, separated from the sea by an 800–2500 m wide limestone ridge (up to 113 m above sea level). The basin is a karst drainage system in the area of Ravni Kotari with a surface area of 49400 ha. It is a polymictic karstic cryptodepression connected to the Adriatic Sea by a narrow channel at its southern side. In the northwestern section the lake is between 0.5 and 1 m deep, and 4–6 m in the southeast. The flow of water in the lake is influenced by conduction waves caused by the south-eastern and north-westerly winds. The channel Prosika (length 800 m, width 4 m, depth 5–6 m) connects the lake with the Adriatic Sea. The channel is used for drainage and was constructed in 1895 but was subsequently expanded, deepened and regulated. The extensive floodplain around Lake Vrana is a natural marsh with high biodiversity.

The physico-chemical parameters presented in Table 1 were measured approximately bimonthly from February to October 2011 (N = 7) at a single station, situated in the central part of the lake (43°54’27’’N, 15°33’57’’E). The observed ranges of values agree well with the results of previous investigations (Gligora et al. 2007), indicating considerable influence of the Mediterranean climate conditions both on the nutrient levels and on temperature. During 2011 the salinity in the lake ranged between 2.4 and 6.5 psu, in April 2011, when the diatom strain was isolated in culture the salinity was 2.9 psu.

Strain isolation, culture condititons and morphological analyses

The water sample was collected at the surface (0.5 m depth) of the lake on April 26, 2011 and examined fresh upon the arrival at the laboratory, in a plastic Petri dish. The individual cell chains were isolated using a Pasteur micropipette and an inverted Olympus CKX41 light microscope (Olympus, Tokyo, Japan) equipped with bright-field optics and phase contrast. The chains were first placed in sterile 35-mm Falcon polystyrene tissue culture dish (model 353001 BD Labware, Le Pont de Claix, France) filled with ca. 2 mL F/2

Tab. 1. Physico-chemical variables in Lake Vrana from February to October 2011. MIN – minimum, MAX – maximum, AVG – average, STDEV – standard deviation. Number of sampling = 7. The values measured in April 2011 when the strain of *Chaetoceros wighamii* was isolated in culture are presented separately.

Variable	MIN	MAX	AVG	STDV	April 2011
Temperature (°C)	2.8	25.6	17.6	8.1	13.9
pH	7.9	9.0	8.5	0.4	7.98
Conductivity (μS cm^{-1})	3870	8760	5434	1797	4090
Total suspended solids (mg L^{-1})	3.6	14	8.6	3.8	14
Alkalinity m-value (mg $CaCO_3$ L^{-1})	66	196	121	61	196
Hardness (mg $CaCO_3$ L^{-1})	422	1242	706	279	689
O_2 (mg L^{-1})	7.8	12.9	9.5	1.8	9.1
Saturation O_2 (%)	83	119	97	13	88.3
NH_4^+ (mg L^{-1})	<0.010	0.129	0.047	0.041	0.031
NO_2^- (mg L^{-1})	0.002	0.016	0.007	0.005	0.016
NO_3^- (mg L^{-1})	0.031	1.980	0.722	0.790	1.340
Total inorganic N (mg L^{-1})	0.064	2.004	0.769	0.805	1.387
Organic N (mg L^{-1})	0.215	0.328	0.274	0.035	0.268
PO_4 (mg L^{-1})	0.008	0.099	0.034	0.039	0.008
Total P (mg L^{-1})	0.021	0.233	0.084	0.070	0.067

medium (Guillard 1975) and observed each day during the first week for growth and conditions of the cells. The culturing medium was prepared using Guillard's (F/2) marine water enrichment solution (Sigma-Aldrich) according to the manufacturer instructions. The water used for medium preparation was collected from the Lake Vrana and sterilized by filtration through 0.2-μm Nucleopore filters. When the cell density was high enough the established clonal culture was transferred from a dish to 70 mL Falcon polystyrene cell culture flasks (model 353082; BD Biosciences, Le Pont De Claix, France) filled with 30 – 40 mL of F/2 medium.

The strain designated PMF M1 was maintained in a growth culture chamber under cool white (40 W) fluorescent light (30 μmol photons m^{-2} s^{-1}) at a room temperature of 20 °C in a 16 h:8 h light:dark cycle and sub-cultured every 1–2 weeks. The material for morphological studies was obtained in the exponential growth phase within the first month after establishment of the culture.

For LM observations, a Zeiss Axiophot microscope (Carl Zeiss, Oberkochen, Germany), equipped with bright-field, phase contrast and Nomarski differential interference contrast (DIC) optics was used. Light micrographs were taken with a Zeiss AxioCam MRc digital camera and processed with an AxioVision 4.8.2 digital image processing software. The culture material was subjected to cleaning treatment in order to remove the organic matter from diatom frustules. The sample was first desalinized by rinsing with distilled water, treated with strong acids (1:1:4; sample:H_2SO_4:HNO_3), boiled for a few seconds and then washed again with distilled water. For SEM observations, the cleaned material was filtered and air dried on 3-μm Nucleopore polycarbonate filter (Nucleopore, Pleasanton,

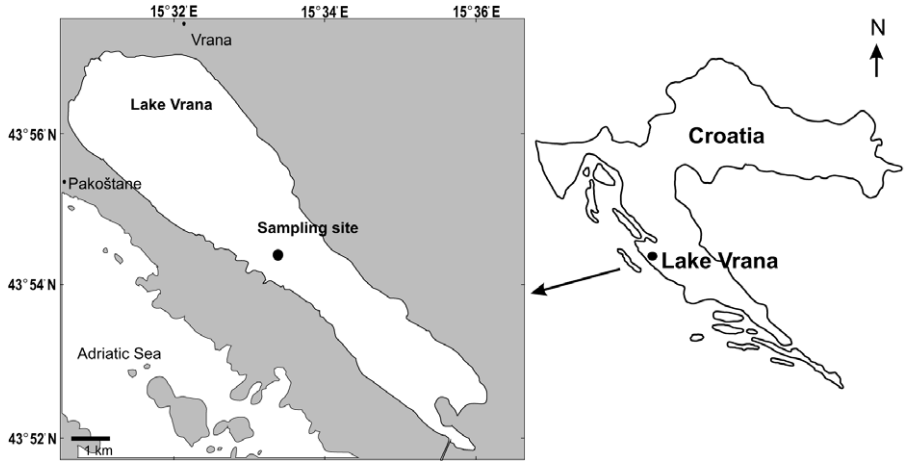

Fig. 1. Position of sampling site at Lake Vrana (Croatia).

CA), mounted on a stub, sputter coated with gold and examined in a JEOL JSM-6500F SEM (JEOL-USA Inc., Peabody, MA, USA). TEM observations were made by deposition of cleaned and rinsed material on Formvar/carbon coated 200-mesh nickel grids and examined in a LEO 912AB TEM (LEO, Oberkochen, Germany). Preserved material and TEM grids are available from the authors upon request.

The general diatom terminology used for the morphological descriptions follows proposals recommended by Anonymous (1975), Ross et al. (1979), Round et al. (1990), Hasle and Syvertsen (1997). The specific terminology for *Chaetoceros* genus follows Evensen and Hasle (1975), Rines and Hargraves (1988) and Kooistra et al. (2010).

Results

Chaetoceros wighamii Brightwell 1856: 108, pl. 7: figs 19–36

described as *Chaetoceros amanita* Cleve Euler in Kaczmarska et al. (1985), Rushforth and Johansen (1986), Rines and Hargraves (1988) and as *C. wighamii* in Sanchez Castillo et al. (1992).

Light microscopy observations

Chains are straight, robust and usually short, composed of 4–7 cells. Cells are elliptical in valve view and rectangular in girdle view with the pervalvar axis often shorter (8–15 μm) than the apical axis (14–26 μm). Each cell contains a single large chloroplast in the shape of a parietal plate extending from valve to valve (Figs. 2B–C). The valve face is flat, sometimes with a central inflation which is usually more visible on the terminal valves. No process was visible in either intercalary or terminal valves on > 30 observed chains. Valve mantle is low with a very slight constriction near the abvalvar margin. The girdle is usually equi-dimensional in height with the mantle. Valves of adjacent cells touch at the corners. Apertures are slit-shaped and very narrow, partially occluded by silica membranes which are sometimes visible in LM (not shown). Intercalary setae originate from the valve apices

and cross immediately at chain margin, without basal part (Figs. 2B–C). Setae lack chloroplasts. Intercalary setae are very long (up to 300 μm), straight, and stiff diverging in various directions, some perpendicular but more often they are oriented at a variable angle to the chain axis (Fig. 2A). In most cases sibling setae diverge from each other at an angle of 90° belonging to Brunel Group III (Fig. 2D), but in the same chain they can also diverge equally from the apical plane at an angle of 20–30° conforming to Brunel Group II. Terminal setae have the same morphology as intercalary ones but they are oriented more parallel to the chain axis, diverging in a broad U- or V-shaped curve towards the end of the chain (Fig. 2A).

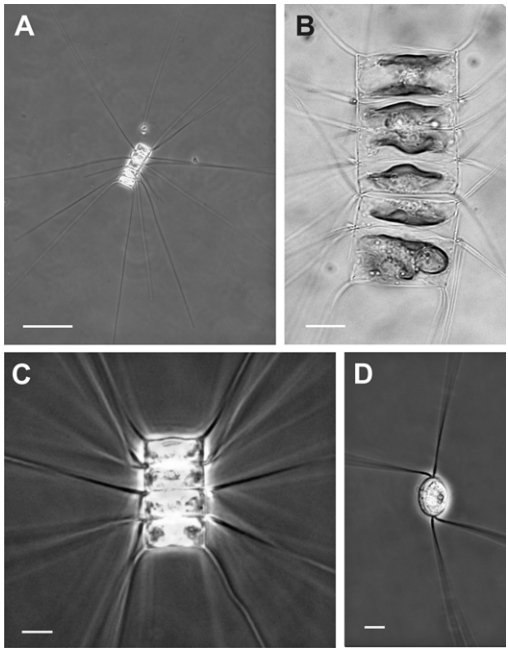

Fig. 2. Light micrographs of *Chaetoceros wighamii*, strain PMF M1. A) The complete chain showing orientation of the setae. B) Cells with one parietal chloroplast per cell extending from valve to valve. C) Four-celled chain. D) Sibling valves in valve view with setae diverging from each other at an angle of 90°, belonging to Brunel Group III. Scale bars: A = 50 μm; B–D = 10 μm.

Electron microscopy observations

The valve is ornamented with very densely distributed anastomosing ribs spreading from an irregularly shaped hyaline area positioned slightly eccentrically on the valve face (Figs. 3B–C). The valve face is not perforated by poroids. The outer surface of the terminal valves is covered by minute spines (Fig. 3F). The process was not observed on ten intercalary and terminal valves examined with EM (Figs. 3A–E). In all valves the marginal ridge between the valve face and the valve mantle is ornamented with a high hyaline rim (Fig. 3E). In intercalary valves, the aperture between sibling cells is partially occluded by silica membranes appearing to project from the hyaline rim (Fig. 3A). The setae are circular in

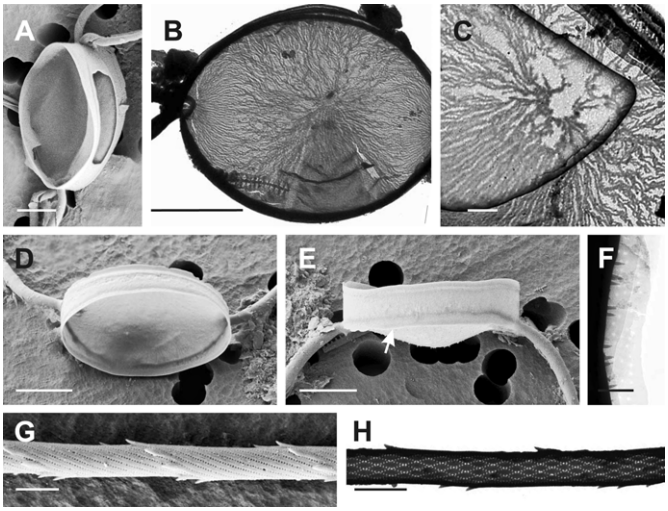

Fig. 3. SEM (A, D, E, G) and TEM (B, C, F, H) micrographs of *Chaetoceros wighamii*, strain PMF M1. A) Two sibling valves with aperture partially occluded by silica. B) Two overlapped sibling intercalary valves showing the valve pattern with anastomosing ribs. C) Detail of the valve with the irregularly shaped hyaline eccentric area. D) Internal view of the terminal valve. E) Terminal valve in girdle view showing the hyaline rim extending from the marginal ridge (arrow). F) Detail of the terminal valve with minute spines and the structure probably corresponding to the girdle band. G) Detail of a seta with spirally positioned spines. H) Detail of a seta. Scale bars: A, B, D, E = 5 μm; C, G, H = 1 μm; F = 0.5 μm.

cross-section, 0.6–0.8 μm in diameter, composed of flat, thick longitudinal filaments arranged in spiral pattern around the setae length and interconnected by very short transverse bars. The space between bars corresponds to a spiral row of minute poroids. The filaments are ornamented with spines (3 per 1 μm) arranged in a helicoidal pattern around the setae. (Figs. 3G–H).

Discussion

A combination of morphological and ultrastructural characters allows for the ascription of the morphotype from Lake Vrana to *C. wighamii* Brightwell (syn. *C. amanita*) *sensu* Sanchez Castillo et al. (1992). The robust colonies have very long, stiff and straight intercalary setae projecting in various directions from the chain axis and terminal setae oriented more parallel to the chain axis, diverging in a broad U or V- shaped curve towards the end of the chain. The apertures between sibling valves are partially occluded by a silica membrane which is sometimes visible in LM. The partial sealing of the apertures was considered peculiar for *C. wighamii* syn. C. *amanita* (Kaczmarska et al. 1985, Rushforth and Johansen 1986), but was not described in *C. wighamii* from Lake Chica (Sanchez Castillo et al. 1992) where apparently only terminal valves were observed by electron microscopy. A silica membrane partially occluding the apertures is not unique for *C. wighamii* as a similar structure (silica wall) has been observed in other very different species such as *C. decipiens* (Evensen and Hasle 1975) and *C. diversus* (Hernández-Becerril 1996).

A rather distinctive feature in our material is the absence of any processes and central annulus in either intercalary or terminal valves. A process is present on terminal valves in most of the species in the subgenus *Hyalochaete*, and generally on every valve in the subgenus *Chaetoceros* (Evensen and Hasle 1975, Hernández-Becerril 1996). The lack of processes was recognized as a common feature in several unicellular inland taxa which are nowadays considered as synonyms of *C. muelleri* var. *subsalsum* (Lemmermann) Johansen & Rushforth (Reinke 1984, Johansen and Rusforth 1985) and was already reported in specimens from freshwater field material of *C. wighamii* (Kaczmarska et al. 1985 as *C. amanita*, Sanchez Castillo et al. 1992). Kaczmarska et al. (1985) were not able to confirm the presence of any process after examination of 200 valves but suggested that a rimoportula may be produced under some circumstances. Our observation in cultivated material, although based on a limited number of examined valves, constitutes a further evidence of the lack of the process in the freshwater specimens of *C. wighamii*.

The presence of the central process bearing a long external tube was illustrated in cultures of *C. wighamii* from the Baltic Sea (syn. C. *bottnicus*, *C. biconcavum*, *C. capsicum* and *C. fallax*, Jensen and Moestrup 1998). The projection is clearly visible in LM illustrations and sometimes is present in sibling cells, probably as a result of impending chain division with the process-bearing intercalary valves becoming terminal valves. The Baltic morphotype appears rather similar to the croatian morphotype as regarding the very variable orientation of the setae, but looks different for the more delicate aspect and the wider apertures of the chains. As noted by Snoeijs (1993), detailed studies on the species from the Baltic Sea are required to reveal if it corresponds to *C. wighamii* or to *C. bottnicus*, which was indeed considered as a synonym of *C. wighamii* by Jensen and Moestrup (1998).

The absence of a distinct annulus is not common among *Hyalochaete* species that generally have a thickened annulus surrounding the hyaline area from which the dichotomously branching ribs extend towards the valve margin (Evensen and Hasle 1975, Hernández-Becerril 1996). In *C. wighamii*, the valve face shows a peculiar ornamentation pattern with densely distributed ribs spreading from an irregularly shaped hyaline area. Kaczmarska et al. (1985) described a similar pattern as »composed of anastomosing areas of light silification« probably referring to the hyaline area between the ribs. The lack of a clearly defined annulus was also observed in the freshwater species *Chaetoceros muelleri* var. *subsalsum* (Johansen and Rushforth 1985) but the ribs are thicker and do not diverge in such a dense branching pattern as in *C. wighamii*. The presence of small spines or protrusions on the outer valve surface in *C. wighamii* was already noticed in previous studies (Kaczmarska et al. 1985 as *C. amanita*, Sanchez Castillo et al. 1992).

The seta ultrastructure has been considered as useful taxonomic character used in species delineation in *Chaetoceros* since the first EM studies were published (Evensen and Hasle 1975). This idea was recently developed further by Lee et al. (2014) who proposed the use of several setae characters as the identification criteria in the subgenus *Chaetoceros*, which includes species with thick and chloroplasts containing setae. *Chaetoceros wighamii* belongs to the subgenus *Hyalochaete* which comprises species possessing relatively thin setae without chloroplasts. In *C. wighamii*, the setae are composed of long longitudinal filaments, which are arranged in a spiral pattern around the seta axis and are connected by short transverse bars. The arrangement of longitudinal filaments and transverse bars results in a reticulate pattern when setae are observed in TEM (Kaczmarska et al. 1985). The filaments are adorned with somewhat strong small spines in a helicoidal arrangement. The

same pattern was previously illustrated in *C. wighamii* (Kaczmarska et al. 1985 as *C. amanita*, Sanchez Castillo et al. 1992). Generally, *Hyalochaete* species possess setae with spines, with the exception of *C. vixvisibilis* (Hernández-Becerril et al. 2010), but their size and pattern shows differences among species (Hernández-Becerril 1996, Kooistra et al. 2010). Spines adorning setae in *C. wighamii* are tightly arranged around the axis and appear slightly longer and more silicified compared to the spines present in other species, as, for example, *Chaetoceros curvisetus* or *C. lauderi* (Kooistra et al. 2010, Evensen and Hasle 1975).The details on chloroplast number, shape and position have not been known previously in *Chaetoceros wighamii* syn *C. amanita*, as the previous observations were based mostly on cleaned material (Kaczmarska et al. 1985, Sanchez Castillo et al. 1992). According to our observations on live material, *Chaetoceros wighamii* has one chloroplast, which is plate-like or slightly lobed, situated parietally within the cell but extending from valve to valve, differently from, for example, *C. affinis* which has the single parietal plastid positioned around the girdle (Rines and Hargraves 1988).

The apical length size was considered by Cleve-Euler (1951) and later adopted by Kaczmarska et al. (1985) as one of the characters considered on which their specimens were designated as *C. amanita* and not as *C. wighamii*. The authors compared their measurements with the data on apical length reported for marine morphotypes named *C. wighamii* by Hustedt (1930) and Cupp (1943) which range between 7 and 18 µm, these are considerably smaller than the *C. wighamii* syn *C. amanita* range. However, the morphotype illustrated and described by Hustedt (1930) is very different from the original description of *C. wighamii*, with characters such as delicate chains, thin setae and cells with drawn up poles, which probably correspond to a different species with marine preferences. The cell apical length measured in our study ranged between 14 and 26 µm, which is comparable to the range reported in other studies of *C. wighamii* syn. *C. amanita*: 18–40 µm, 15–20 µm, 19–31 µm, (Kaczmarska et al. 1985, Rines and Hargraves 1988, Sanchez Castillo et al. 1992), respectively.

One of the most distinctive characters of *C. wighamii* is a resting spore with primary valve covered with spines and secondary valve shaped as a truncated cone (Brightwell 1856). Unfortunately, the resting spore has not been observed in this study. However, resting spores morphologically similar to those reported for *C. wighamii* by Brightwell (1856) and Sanchez Castillo et al. (1992) as well as by Kaczmarska et al. (1985) and Rines and Hargraves (1988) as *C. amanita,* have been frequently found in the sediments from the Lake Vrana (Fig. 4, I. Galović, personal communication). The distinct cone-shaped secondary

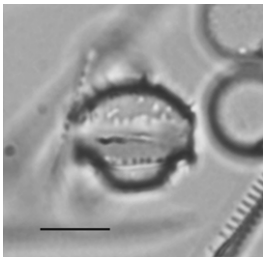

Fig. 4. The LM micrograph (photo by I. Galović) of a resting spore in Lake Vrana, having the spinose secondary valve distinctively shaped as a truncated cone considerably narrower in diameter than the primary valve. Scale bar = 10 µm.

valve is a feature observed in some other freshwater *Chaetoceros* species, e.g. *C. elmorei* and *C. muelleri,* which however generally form smooth spores (Rushforth and Johansen 1986).

The results of this study support the idea that the freshwater morphotypes of *C. wighamii* are distinct from the marine morphotypes. However, morphological and molecular studies on material from different geographical areas are required to definitely clarify if (1) *C. wighamii* is a very variable species which includes phenotypically different populations able to adapt to ecologically distinct habitats, or (2) different species have been identified over time as *C. wighamii.*

Future studies should also investigate phylogenetic relationships among different freshwater *Chaetoceros* species which share similar distinctive features such as absence of process and related spore morphology.

Conclusion

Our observations on morphology of *Chaetoceros wighamii* isolated from Lake Vrana, Croatia agree well with the description reported for the freshwater morphotypes of *C. wighamii* (syn. *C. amanita*) and contribute to a better distinction of this taxon from similar species. *C. wighamii* is a freshwater/brackish species whereas the taxonomical affiliation of the marine synonyms should be further investigated. Additional species-specific ultrastructural features are described, such as the particular valve ornamentation pattern and the position and shape of the chloroplast. Until now the morphotype corresponding to *Chaetoceros wighamii* (syn. *C. amanita*) including observations of both vegetative cells and resting spores has been reported from inland waters in USA (Kaczmarska et al. 1985), Southern (Sanchez Castillo et al. 1992) and Northern Europe (Brightwell 1856). This report represents the first report of this species from Croatia and from Central European habitats.

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