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Diversity, occurrence, and habitats of the diatom genus *Bacteriastrum* (Bacillariophyta) in the northern Adriatic Sea, with the description of *B. jadrantum* sp. nov.

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Abstract

Ongoing phytoplankton monitoring programs over the last 12 years in the northern Adriatic Sea showed that the diatom genus *Bacteriastrum* is an important component of the pelagic diatom assemblage. The main goals of this study were to identify which *Bacteriastrum* species occur in the northern Adriatic Sea and describe their distributions. *Bacteriastrum comosum* and *B. hyalinum* f. *princeps* were rare, while *B. delicatulum*, *B. hyalinum*, and *B. mediterraneum* were frequently present, together with the newly proposed species *B. jadrantum*, described herein. This new species is weakly silicified and is mainly characterized by a large aperture between each cell of the filament. Cells are connected to each other by 8–12 setae, which cross at a distance equivalent to the diameters of 2–3 cells. There is no difference in the orientation of the setae on the two terminal cells, hence the species is placed in the section *Isomorpha*. The new species was recorded throughout the study area, in both coastal and offshore waters, with higher cell abundances in the vicinity of the Po River during the September–October period. The maximum cell abundance of *B. jadrantum* was 244.2×10^3 cells l⁻¹ in the surface waters at 44.75666667°N, 12.75°E in September 2006, sometimes representing up to 46% of the entire phytoplankton assemblage, as recorded in the surface waters at 45.06833333°N, 13.515°E in October 2008.

Keywords: *Bacteriastrum jadrantum*; diatom taxonomy; northern Adriatic Sea; phytoplankton.

Introduction

The Adriatic Sea is the northernmost part of the Mediterranean, which according to bathymetry and latitude is divided into a northern, middle, and southern part (Cushman-Roisin et al. 2001). It is a dilution basin with an overall cyclonic circulation, primarily created by the Po River that drives the southward flow of the western Adriatic current (Jeffries and Lee 2007). The northern Adriatic is shallow, with an average depth of 35 m (maximum 50 m), characterized by a marked west to east gradients of salinity, nutrients, chlorophyll concentrations, and phytoplankton abundances (Degobbi et al. 2000, Polimene et al. 2007, Mozetič et al. 2010), which are mainly controlled by wind-induced circulation patterns and discharges of the Po River (Zavatarelli et al. 2000). The western part of the northern Adriatic is under the direct influence of the Po River, while the eastern part is mostly influenced by the incoming oligotrophic eastern Adriatic current (Viličić et al. 2009a). A thermal stratification of the water column usually becomes evident in May, with breakdown occurring in October (Degobbi et al. 2000). The formation of anticyclonic gyres in the northern sector reduces the water exchange with the rest of the Adriatic Sea. During stable vertical stratification, characteristic for the late summer period, spreading of fresh (low-density) Po River water masses is evident in the majority of the northern Adriatic Sea. Under these conditions, river plume events can extend as far as to the Istrian coast (Bignami et al. 2007).

Phytoplankton blooms in the northern Adriatic Sea coincide with maximum discharges of the Po River and/or periods of low water column stability (Viličić et al. 2009a). Diatoms usually dominate the western coastal phytoplankton assemblages over most of the year, with maximum abundances in winter-spring and autumn (Bernardi Aubry et al. 2004, Godrijan et al. 2010), while flagellates generally prevail in open and more oligotrophic waters, and during the summer months (Totti et al. 2000). Viličić et al. (2002) regarded the diatom taxonomic composition of the Adriatic Sea as a *Chaetoceros-Rhizosolenia* (*Proboscia*) assemblage, similar to the one characterizing the eastern Mediterranean (Kimor 1983), and consisting of the following predominant genera: *Bacteriastrum* Shadbolt, *Cerataulina* H. Peragallo ex Schütt, *Chaetoceros* Ehrenberg, *Leptocylindrus* Cleve, *Proboscia* Sundström, *Pseudo-nitzschia* H. Peragallo, *Rhizosolenia* Brightwell, and *Thalassionema* Grunow ex Mereschkowsky.

According to VanLandingham (1968), slightly more than 30 taxa have been described so far in the genus *Bacteriastrum*. Most of these taxa form filament-like colonies, except for

a species recently described as solitary (Sarno et al. 1997). Filamentous colonies are formed through the connection of cells by long setae, usually more than two per valve, therefore placing the genus *Bacteriastrium* in the Chaetocerotaceae Ralfs (Evensen and Hasle 1975). The genus is divided into two sections based on the symmetry of the colony, which is determined by the orientation of the terminal setae: *Isomorpha* with isomorphic setae and *Sagittata* with dimorphic setae that can be differentiated into anterior and posterior (Pavillard 1925, Ikari 1927).

Setae of adjacent cells generally fuse at a certain distance from their points of origin on the valve surface and bifurcate afterwards. Further characteristics to distinguish species are the length of the fused part of setae and the orientation of the bifurcation, either in the perivalvar or valvar plane. Most defining features within the genus can be identified by light microscopy (LM). Only a few studies have included some electron microscopical observations, revealing further characteristics to delineate the genus (Sarno et al. 1997), such as the central process on the terminal valve, fine costae branching out from the annulus, and small scattered pores on the valve surface (Hasle and Syvertsen 1997). A further thorough revision of the various taxa is required to fully resolve the systematic position of this genus (Sarno et al. 1997, Kooistra et al. 2010).

Bacteriastrium has a worldwide distribution as a marine genus occurring in tropical and temperate regions (Hasle and Syvertsen 1997, Bérard-Therriault et al. 1999, Horner 2002, Hoppenrath et al. 2009). The genus is present over the whole Adriatic Sea, including the southern and mid sectors (Totti et al. 2000, Viličić et al. 2002, 2009a). Here, we present observations on the diversity and occurrence of the genus *Bacteriastrium* in the northern Adriatic Sea during the period 1998–2009. The main objectives of this study were to identify

species of the genus *Bacteriastrium* occurring in the northern Adriatic Sea and define their distribution, including the description of a new species, *Bacteriastrium jadrantum*, with comments on its habitat conditions and distribution in the northern Adriatic Sea.

Materials and methods

As part of a Croatian long-term monitoring program of phytoplankton assemblages in the northern Adriatic Sea, water and net samples were collected monthly at seven stations through the period 1998–2009 (Figure 1, black squares). From 2007 onward, additional stations were sampled monthly (Figure 1, black circles). Water samples for nutrient and phytoplankton analyses were taken with 5 l Niskin bottles at 0, 5, 10, 20, and 30 m depth, and at 2 m above the seabed. Net samples (53 μm mesh size) for cell culture and microscopical examination were vertically towed from 15 m depth to the surface. Temperature and salinity were measured using a conductivity, temperature, and depth probe (SBE 25 Sealogger CTD; Sea-Bird Electronics, Inc., Bellevue, WA, USA).

Samples for the determination of nitrate (NO_3^-), nitrite (NO_2^-), phosphate (PO_4^{3-}), and silicic acid [$\text{Si}(\text{OH})_4$] were measured using standard spectrophotometric methods (Parsons et al. 1984). Ammonium (NH_4^+) was analyzed with a modified indophenol method (Ivančić and Degobbis 1984). Samples (200 ml) for the identification and enumeration of phytoplankton cells were preserved in neutralized formaldehyde (2% final concentration) solution (Kemika d.d., Zagreb, Croatia) (Thronsen 1978). Phytoplankton cells were identified to the lowest possible taxonomic rank and enumerated using an inverted microscope (Zeiss Axiovert 200; Zeiss GmbH, Oberkochen, Germany)

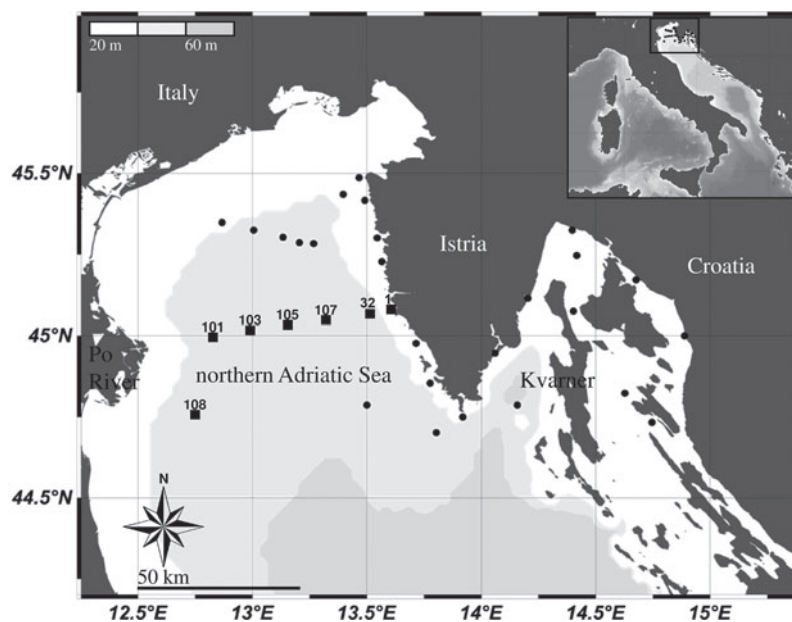


Figure 1 Map of the northern Adriatic Sea showing seven stations (black squares) of the monthly monitoring program (1998–2009) and additional stations (black circles) visited monthly after 2007.

equipped with phase contrast and differential interference contrast optics (Lund et al. 1958, Utermöhl 1958). Flagellates (<20 µm) were not identified at the species level but grouped in the following algal groups: cryptophytes, prymnesiophytes, prasinophytes, and chlorophytes. The main taxonomic references used to identify the phytoplankton were Hustedt (1930), Schiller (1937), Tomas (1997), Bérard-Therriault et al. (1999), and Horner (2002). Standard terminology of siliceous diatom structures followed Ross and Sims (1972) and von Stosch (1975), while specific terminology for the Chaetocerotaceae followed Evensen and Hasle (1975), Tomas (1997), and Rines and Theriot (2003). Drawings were made from light micrographs with AxioVision software V 4.8.1.0 (Zeiss GmbH).

Colonies of the new species *Bacteriastrum jadrantum* were manually isolated with a micropipette from a net sample collected at station 101 (44.998333°N, 12.830667°E) on October 29, 2009. Colonial cells were grown into monoclonal batch cultures in 100 ml f/2 medium (Guillard 1975) and incubated at 18°C and 75 µmol photons m⁻² s⁻¹ on 12:12 h light/dark photoperiod. Growing cells were subcultured every 3–4 weeks.

Net sample material was cleaned of organic matter by using 50% nitric acid at room temperature for 24 h followed by several rinses with distilled water to remove the acid. For transmission electron microscopical (TEM) examination, a drop of cleaned material was mounted on a 100-mesh copper grid covered with pioloform (Agar Scientific Ltd., Stansted, UK), air-dried, and observed with an FEI Tecnai TEM (FEI Co., Eindhoven, The Netherlands). For scanning electron microscopical (SEM) examination, the cleaned diatom material was collected on 0.22 µm cellulose filters (Millipore, Billerica, MA, USA). The filters were air-dried and mounted on aluminum stubs, gold coated with a sputter coater (S150A Sputter coater; Edwards Ltd., Crawley, UK), and observed with a Philips 515 SEM (FEI Co.).

For molecular analysis, a monoclonal culture of *B. jadrantum* was harvested by centrifugation of 2100 × g at 5000 rpm for 5 min (5417R, rotor F453011; Eppendorf AG, Hamburg, Germany). DNA was isolated with the Qiagen plant tissue kit (Qiagen GmbH, Hilden, Germany) according to the company's recommendations. The 606 base pair long hypervariable region of the 18S rRNA gene was amplified using the primers 5-ATTCCAGCTCCAATAGCG-3 and 5-GACTACGATGGTATCTAATC-3 according to Zimmermann et al. (2011) and sequenced on an ABI PRISM 3100 Avant Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) according to the company's recommendations. The resulting sequences from two runs for each direction were compared to exclude sequencing mistakes by majority rule (3:1). The resulting sequence was aligned into an alignment of near full-length 18S rDNA genes from seven *Chaetoceros* species and four other centric diatoms [*Attheya septentrionalis* (Østrup) Crawford, *Lithodesmium undulatum* Ehrenberg, *Ditylum brightwellii* (T. West) Grunow, and *Helicotheca tamesis* (Shrubssole) Ricard]. The alignment was based on the alignment of all publicly available full-length 18S rRNA gene sequences, including more than 1200 diatom sequences (SSURef_98_Silva_20_03_09_opt database; Pruesse et al. 2007). Sequences were manually aligned and compared using the ARB 5.1 software package (Ludwig et al. 2004). A neighbor-joining tree

was constructed using the integrated neighbor-joining algorithm, taking 1466 alignment positions into account. One thousand bootstraps were used for the 18S rRNA dataset.

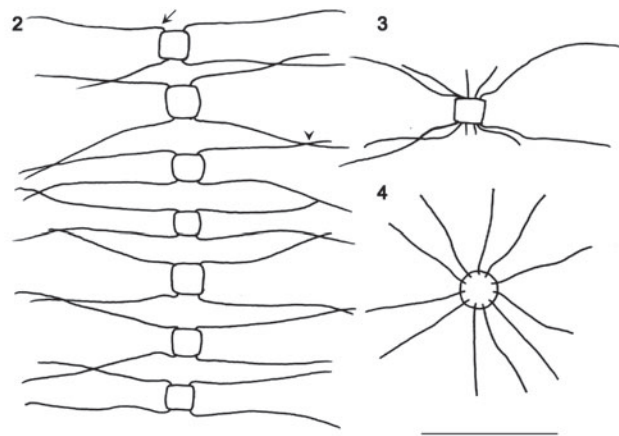
Only nutrient and biological data were log (1+x) transformed in order to reduce the impact of extremely high or low values on correlations between variables. The relationships between physicochemical and biological variables were performed using correlation-based principal component analysis (PCA) (Clarke and Warwick 2001). PCA is designed to reduce the complexity of the data by compression into fewer dimensions and to provide pattern recognition in the data, allowing a visual representation of similarity between variables. Variables used in the analyses were salinity (S), temperature (T), nutrient concentrations [NO₂, NO₃, NH₄⁺, PO₄³⁻, and Si(OH)₄], dissolved inorganic nitrogen (DIN) (calculated as the sum of NO₂, NO₃, NH₄⁺), and cell abundances of *Bacteriastrum* species. To express relationships between variables, nonparametric Spearman rank correlation was calculated. All graphical representations were performed with Systat 12.2.00 (Systat Software, Inc., Chicago, IL, USA), Statistica 10 (StatSoft, Inc., Tulsa, OK, USA), Primer (PRIMER-E Ltd., Luton, UK), Ocean Data View 4.3.5–2010 (Schlitzer, R., Bremenhaven, Germany), Grapher 8 and Surfer 9 (Golden Software, Inc., Golden, CO, USA).

Results

Species description

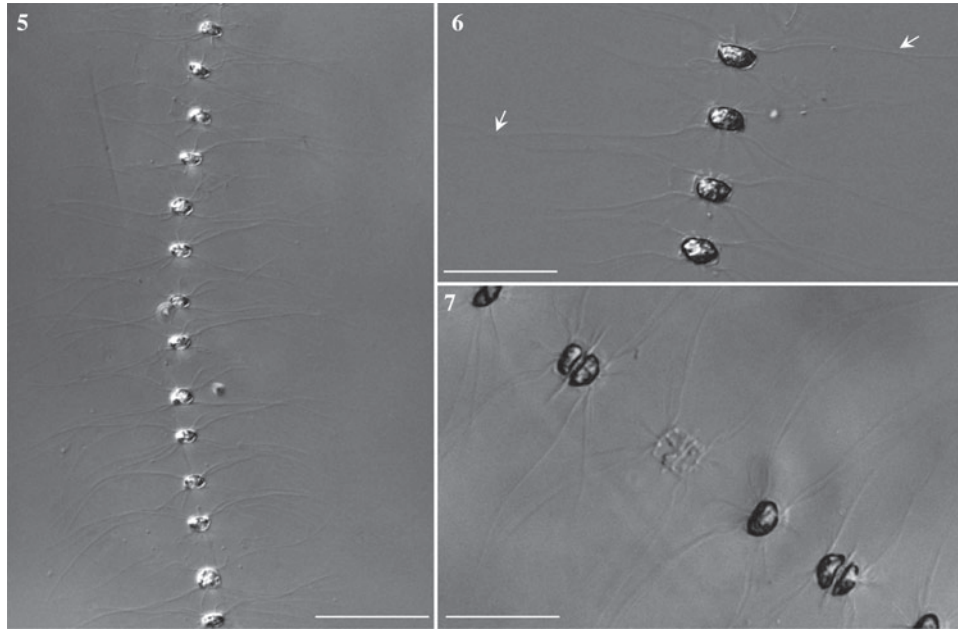
Bacteriastrum jadrantum Godrijan, Marić et Pfannkuchen sp. nov. (Figures 2–22)

Latin Description *Cellulae parum siliceae, cylindricae multis chloroplastis. Valva 12–19 µm in diametro, axis pervalvaris 6–16 µm. Frustula isovalvata. Parvus annulus*



Figures 2–4 Line drawings of *B. jadrantum* from the northern Adriatic Sea.

(2) Filament of seven cells in girdle view with only four visible setae per cell. Note: setae leave the cell first in the perivalvar direction and continue at right angles to the filament (arrow), then cross at one point at a distance equivalent to the diameters of two cells (arrowhead). (3) Cell in girdle view with several setae. (4) Cell in valve view with 11 setae. Scale bar=50 µm.



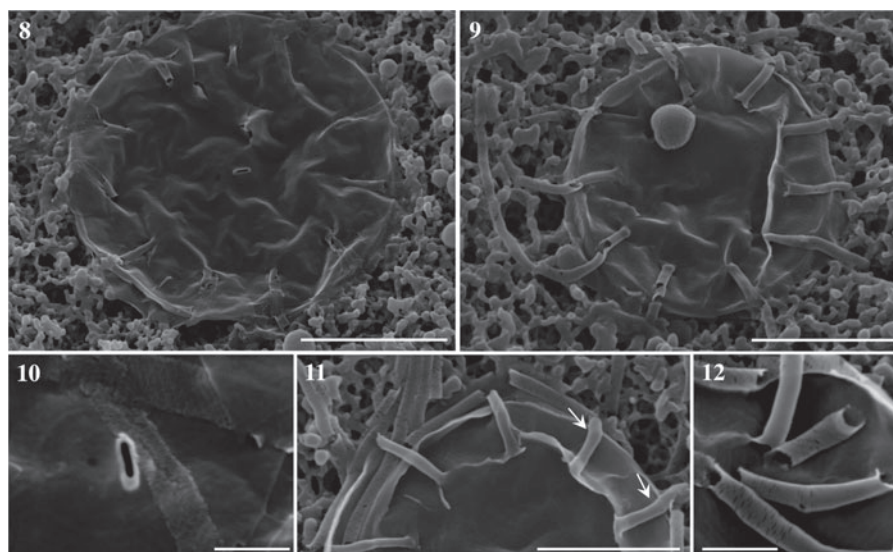
Figures 5–7 *B. jadrantum* from culture material (LM).

(5) Filament of 15 cells. (6) Colonial cells with crossing of setae (arrows) away from the filament. (7) Colonial cells dividing. Scale bars=100 µm (Figure 5); 50 µm (Figures 6, 7).

centralis, plus minusve circularis (1.0–2.5 µm in diametro) adest in utraque facie valvarum. Costae leves, radialiter dispositae (6 in 1 µm), oriundae ab annulo divisaeque dichotomiter in multos ramos minores attingentes valvae marginem. Cellula terminalis alia est: in media parte posterioris valvae rimoportula adest. Cellulae cum 8–12 longis setis, circiter 0.5 µm latis, circiter 80 µm longis, effectis ex costis helicae vel annulo similibus circiter 70 nm

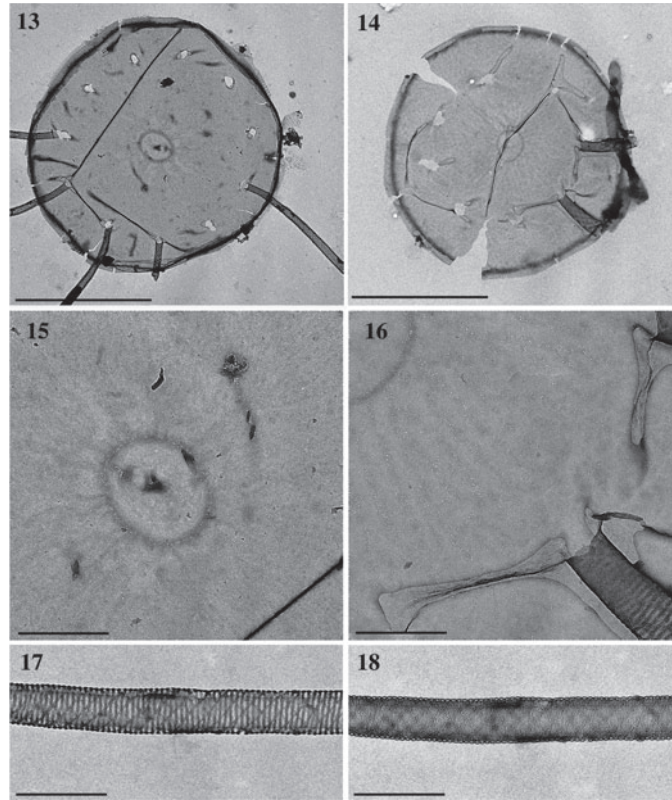
in diametro. Setarum bifurcatio non adest. Setae a cellula abeuntes primo in directione pervalvari, continuantes postea perpendiculares a plano valvari. Cellulae formantes longas catenas, setis inter se coniunctis intervallo 2–3 diametrorum cellulae. Sporae non observatae. Species marina planctonica.

Cells weakly silicified, cylindrical with numerous chloroplasts. Valve diameter 12–19 µm, pervalvar axis 6–16 µm



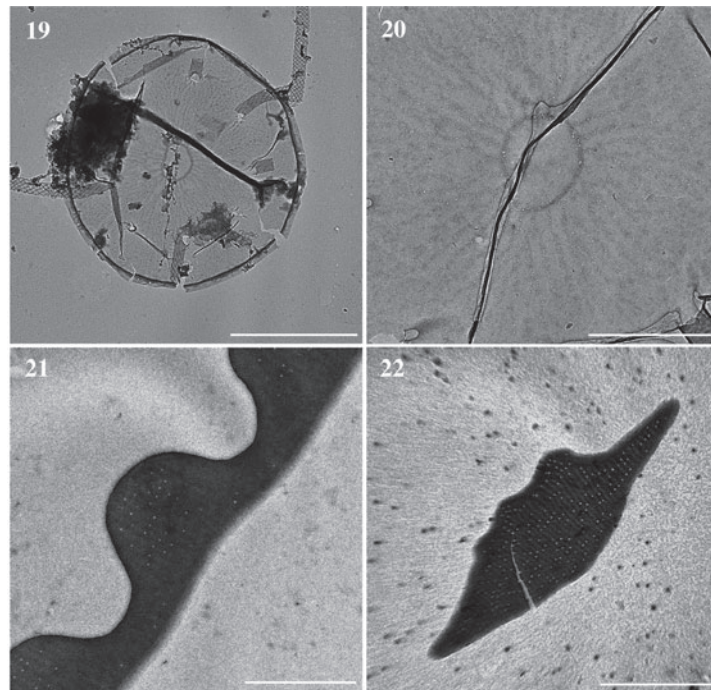
Figures 8–12 *B. jadrantum* from cleaned material (external views, SEM).

(8) Terminal cell with rimoportula at center. (9) Intercalary cell with 10 setae and no rimoportula. (10) Rimoportula within the central annulus of the terminal cell. (11) Insertion of setae on the valve; note the distinctive 90° angle of the setae (arrows). (12) Broken setae showing ring costae. Scale bars=5 µm (Figures 8, 9, 11); 1 µm (Figures 10, 12).



Figures 13–18 *B. jadrantum* from cleaned material (TEM).

(13) Valve showing a central annulus and 11 setae. (14) Valve with a central annulus. (15) Detail of the central annulus with radially arranged costae. (16) Radially arranged costae and basal part of a seta. (17, 18) Setae with helicoidal and ring-like costae. Scale bars=10 μm (Figures 13, 14); 2 μm (Figure 15); 1 μm (Figures 16–18).



Figures 19–22 *B. jadrantum* from cleaned material (TEM).

(19) Valve with a central annulus, radially arranged costae and two setae. (20) Valve of an intercalary cell without rimoportula. (21) Part of the copula. (22) Pleura. Scale bars=10 μm (Figure 19); 2 μm (Figures 20, 22); 1 μm (Figure 21).

long. Frustules isovalvate. A small, circular to oval central annulus (1.0–2.5 μm in diameter) is located in the centers of both valves. Radially arranged smooth ribs (6 in 1 μm) originating from the annulus and dichotomously subdivided into numerous minor ribs that reach the valve margin. Terminal cell different: rimoportula present in the central part of posterior valve. Cells with 8–12 long setae, with diameter of ca. 0.5 μm , length of ca. 80 μm , and composed of helicoidal and ring-like costae ca. 70 nm in diameter. No bifurcation of setae. Setae leave the cell first in the perivalvar direction, but then continue at right angles to the filaments. Cells form long chains through seta crossings at a distance equivalent to the diameter of 2–3 cells. Resting spores not observed. Marine plankton species.

Holotype Slide B 40 0040730, Botanischer Garten und Botanisches Museum Berlin, Germany.

Isotype Slide 007, Center for Marine Research slide collection, Institute Ruđer Bošković, Rovinj, Croatia.

Voucher material is also deposited under the collection number B 40 0040731, at the Botanischer Garten und Botanisches Museum, Berlin, Germany.

The partial 18S rRNA gene sequence (JF930145) has been registered with GenBank.

Type locality Station 101, northern Adriatic Sea (44.998333°N, 12.830667°E). Net sample from the upper 15 m water column collected by Jelena Godrijan on October 29, 2009.

Etymology The specific epithet refers to the type locality, Jadrán, in Croatian.

In LM, cells of *B. jadrantum* are cylindrical in valve view and square to rectangular in girdle view (Table 1, Figures 2–4). Chains are long, sometimes with more than 20 cells (Figure 5) with distinct large apertures measuring 1–1.5 \times the diameter of the cells, but immediately following cell division (Figures 5–7). Setae leave their insertion point on the valve in a perivalvar direction, but abruptly curve and change direction at right angles to the chain (Figures 2, 11, arrows). Its basal part, which is the portion of the seta between its point of origin on the valve face and its point of fusion or crossing with its sibling seta, is 2–3 \times the cell diameter (Figure 2). Chains are formed by crossing of setae of two adjacent cells (Figures 2, arrowhead, 6, arrows).

In electron microscopy, the valves are weakly silicified and are characterized by a small, circular to oval central annulus, 1.0–2.5 μm in size (Figures 13, 14, 19, 20). Numerous, sometimes bifurcating, radially arranged rows (6 in 1 μm) of areolae extend from the central annulus to the valve margin (Figures 14, 16, 19, 20). A rimoportula is present in the central annulus of the terminal cells only (Figures 8, 10). The cingulum is composed of finely striated half-bands. Copulae have one straight and one undulating margin, while pleurae have pointed ends (Figures 21, 22). No specialized valvocopulae were observed.

There are 8–12 setae per valve (Figures 4, 9), each with an approximate diameter of 0.5 μm , positioned in a ring that lies about 2 μm inside the valve margin (Figures 4, 11–13). The setae are not branched and are more than 80 μm long. The setae are composed of helicoidal and ring-like costae approximately 70 nm in diameter (Figures 12, 17, 18). No spines were observed, and no differences were observed between the proximal, median, and distal parts of the setae.

Spores or resting stages were not observed. Single cells were noticed in the bottom layer of the water column immediately following the bloom, from October until November.

Neighbor-joining analysis of the partial 18S rDNA gene sequence consistently grouped *B. jadrantum* in a clade with *Attheya septentrionalis* and the genus *Chaetoceros* (Figure 23).

Habitats of *Bacteriastrium* species in the northern Adriatic Sea

During the 12-year sampling in the northern Adriatic Sea, the mean sea surface temperature varied from 11.6°C during December–February to 20.3°C during June–August, reaching a maximum value of 29.3°C in that same period (Table 2). Mean salinity values were rather constant throughout that same period and ranged from 37.3 to 37.9. Maximum salinity values in all periods were approximately 38.5 and these were recorded in bottom waters, whereas the lowest salinity values (minimum 28.7) were recorded closer to the Po River. All nutrients had highest mean concentrations recorded during December–February, while lowest mean concentrations for NO_2^- , NO_3^- , and PO_4^{3-} occurred during June–August. On average, PO_4^{3-} concentrations were generally much lower than DIN and SiOH_4 .

The northern Adriatic Sea phytoplankton had maximum average cell abundance during spring time (March–May) (Table 2). This period was largely dominated by flagellates, with a mean abundance of 646×10^3 cells l^{-1} , while diatoms recorded their highest average abundance during that same period, with a maximum of $15,155 \times 10^3$ cells l^{-1} recorded in April 1999 at station 101 (Table 2). Dinoflagellates were always in very low abundance, reaching a maximum during this same spring-time period, with 586×10^3 cells l^{-1} in May 2002 at station 107. The lowest phytoplankton abundance was recorded during the December–February period, with a mean value of 420×10^3 cells l^{-1} . During June–August, the abundance of phytoplankton was low, with a predominance of flagellates occurring at a density of 245×10^3 cells l^{-1} , while diatoms were dominant in September–November at a density of 237×10^3 cells l^{-1} .

The genus *Bacteriastrium* contributed 0.4% to the total phytoplankton assemblage and 0.9% to diatom abundance over the whole period investigated in the northern Adriatic Sea. The following species are considered as common, with their frequency of occurrence in parentheses: *B. delicatum* Cleve (6.4%), *B. mediterraneum* Pavillard (5.4%), *B. jadrantum* (4.4%), and *B. hyalinum* Lauder (2.6%), while *B. comosum* Pavillard and *B. hyalinum* f. *princeps* (Castracane) Ikari were rare, occurring at a frequency of <0.1% during the 12-year

Table 1 Morphological characteristics of *Bacteriastrium* species reported from the northern Adriatic Sea, including a comparison with *B. tenue*.

Taxon	Section <i>Isomorpha</i>			Section <i>Sagittata</i>			
	<i>B. jadranum</i>	<i>B. delicatulum</i>	<i>B. hyalinum</i>	<i>B. hyalinum</i> f. <i>princeps</i>	<i>B. comosum</i>	<i>B. mediterraneum</i>	<i>B. tenue</i>
Cell diameter (CD) (µm)	12–19	6–20	13–56	13–56	5–22	16–32	7–23
Cell shape in colony	Square-rectangular	Cylindrical	Square-rectangular	Square-rectangular	Cylindrical	Square-rectangular	Cylindrical
Number of setae	8–12	6–12	7–32	7–25	~8	~22	4–9
Aperture	1–1.5 CD	0.5–1 CD	Very small	Very small	0.5 CD	Barely discernible	2 CD
Basal part	2–3 CD	n.d.	n.d.	n.d.	n.d.	n.d.	Very small
Fused part of the setae	No fusion	2 CD	0.5–1 CD	0.5–1 CD	1–2 CD	1 CD	No fusion
Orientation of bifurcation	No bifurcation	Transverse to the chain axis	Parallel with chain axis	Parallel with chain axis	Transversal to the chain axis	Transverse to the chain axis	No bifurcation
Terminal setae	Directed toward the chain at both ends, not different from intercalary setae	Gently curved and directed toward the chain	Gradually curved and bent downwards, with spiral undulation	Gradually curved and bent downwards, with spiral undulation	Anterior – shorter than posterior, with undulations; posterior – campanuliform	Anterior – first directed posteriorly, then recurving outwards obliquely to chain; posterior – campanuliform	Anterior – curved downwards; posterior – campanuliform
Structure of setae	Helicoidal and ring-like costae	n.d.	n.d.	Coiled, two or three coils in the part of a seta continues after the basal part	n.d.	n.d.	n.d.
Central annulus (µm)	1.0–2.5	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Areolae in 1 µm	6	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Rimoportula	At center of the terminal valve	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Colony	Very long, slightly undulating	Long and straight	Long and straight	Long and straight	Straight	Long and straight	Short and straight
References	Cleve (1897), Pavillard (1924), Ikari (1927), Hustedt (1930)	Cleve (1897), Pavillard (1924), Ikari (1927), Hustedt (1930)	Lauder (1864), Pavillard (1916), Ikari (1927), Hustedt (1930), Drebes (1972)	Ikari (1927), Hustedt (1930)	Pavillard (1924), Ikari (1927), Hustedt (1930)	Pavillard (1916), Ikari (1927), Hustedt (1930)	Steenmann Nielsen (1931), Reinecke (1969)

n.d., not determined.

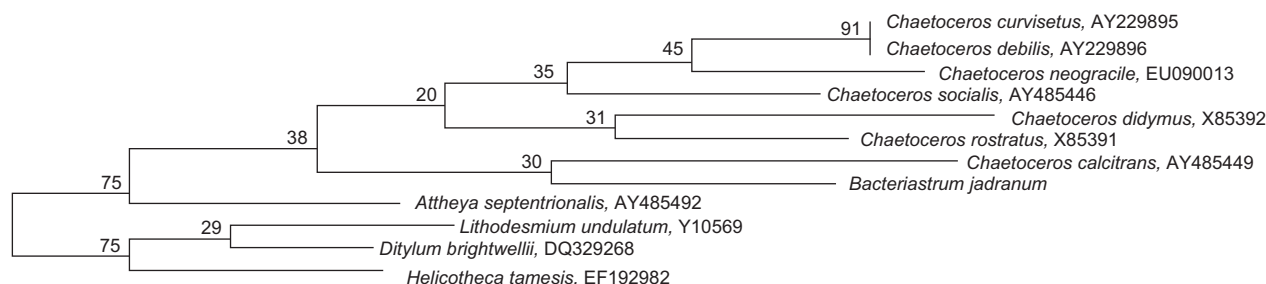


Figure 23 Phylogenetic tree of the class Mediophyceae, including *B. jadrantum*, inferred from neighbor-joining analysis of partial 18S rRNA. Numbers at nodes are bootstrap values.

Table 2 Summary statistics for environmental and biological variables measured in the northern Adriatic Sea during the 1998–2009 phytoplankton monitoring program.

	March–May Min (Mean±SD) Max	June–August Min (Mean±SD) Max	September–November Min (Mean±SD) Max	December–February Min (Mean±SD) Max
Temperature (°C)	7.7 (12.3±3.5) 20.2	11.6 (20.3±5.0) 29.3	11.5 (19.5±2.7) 25.0	5.2 (11.6±2.5) 18.2
Salinity	33.9 (37.6±1.2) 38.6	29.6 (37.4±1.2) 38.4	28.7 (37.3±1.0) 38.5	29.1 (37.9±1.1) 38.6
PO ₄ ³⁻ (μmol l ⁻¹)	d.l. (0.05±0.06) 0.30	d.l. (0.04±0.05) 0.30	d.l. (0.04±0.06) 0.54	d.l. (0.08±0.08) 0.59
NO ₃ ⁻ (μmol l ⁻¹)	0.13 (1.36±1.32) 5.40	0.05 (0.51±0.63) 5.53	d.l. (1.05±1.55) 17.4	0.36 (2.30±2.98) 28.5
NO ₂ ⁻ (μmol l ⁻¹)	0.02 (0.20±0.13) 0.54	d.l. (0.08±0.16) 2.09	0.01 (0.19±0.38) 3.57	0.10 (0.84±0.44) 2.52
NH ₄ ⁺ (μmol l ⁻¹)	0.02 (0.30±0.27) 1.59	d.l. (0.43±0.64) 5.54	d.l. (0.42±0.56) 4.47	0.01 (0.24±0.34) 3.74
DIN (μmol l ⁻¹)	0.31 (1.86±1.48) 5.89	0.17 (1.02±1.10) 6.91	0.16 (1.66±1.9) 18.5	0.83 (3.38±3.44) 32.8
Si(OH) ₄ (μmol l ⁻¹)	0.09 (2.24±2.05) 14.0	0.02 (3.42±4.43) 22.9	d.l. (3.00±4.66) 30.0	0.54 (4.79±3.93) 31.9
Diatoms (×10 ³ cells l ⁻¹)	<1 (280±1019) 15,155	<1 (179±428) 5202	1 (237±432) 7326	<1 (183±641) 7516
Dinoflagellates (×10 ³ cells l ⁻¹)	<1 (7±37) 586	<1 (5±13) 262	<1 (4±7) 111	<1 (2±2) 34
Flagellates (×10 ³ cells l ⁻¹)	14 (646±1172) 15,415	9 (245±412) 7944	9 (229±352) 3583	9 (237±333) 3008
Total phytoplankton (×10 ³ cells l ⁻¹)	21 (885±1571) 16,777	12 (426±662) 9689	14 (457±605) 8040	12 (420±749) 7649

SD, standard deviation; d.l., below detection limit.

sampling period. *Bacteriastrium delicatulum* was observed throughout the year (Figure 24A) and its highest cell abundances (maximum 119×10³ cells l⁻¹) were found in the deep water column during the June–August period (Figure 25A). *Bacteriastrium mediterraneum* was also observed throughout the year and its highest cell abundances (maximum 121×10³ cells l⁻¹) were recorded in surface waters during September–November (Figures 24B, 25B). *B. jadrantum*, which was first recognized in 2006, was present throughout the year, with the highest cell abundances (maximum 244×10³ cells l⁻¹) generally recorded in surface waters in September (Figures 24C, 25C). *Bacteriastrium hyalinum* was absent from the northern Adriatic Sea in February, March, and April (Figure 24D); its highest cell abundances (maximum 55×10³ cells l⁻¹) were recorded in surface waters in September (Figure 25D).

The first two PCA axes accounted for 41% of the cumulative variance. The first PCA axis was responsible for 28% of the cumulative variance in relation to environmental variables, including NO₂⁻, NO₃⁻, and DIN (Figure 26). The second PCA axis explained 13% of the variance in relation to temperature, salinity, and PO₄³⁻. *Bacteriastrium delicatulum* abundance was significantly negatively correlated

with NH₄⁺ (R=-0.12, p=0.000137, n=1022). *Bacteriastrium mediterraneum* abundance was significantly negatively correlated with salinity (R=-0.06, p=0.045859, n=1018), NO₃⁻ (R=-0.13, p=0.000268, n=1018), and DIN (R=-0.11, p=0.000003, n=1022) and positively correlated with temperature (R=0.14, p=0.000012, n=1022). Abundance of the new species *B. jadrantum* was significantly negatively correlated with salinity (R=-0.06, p=0.33119, n=1018) and positively correlated with NH₄⁺ (R=0.09, p=0.002956, n=1022). *Bacteriastrium hyalinum* abundance was significantly negatively correlated with temperature (R=-0.08, p=0.013921, n=1022) and positively correlated with NO₂⁻ (R=0.08, p=0.010760, n=1022).

Discussion

Morphology of *Bacteriastrium jadrantum*

The most distinctive feature characterizing *B. jadrantum* is the cell arrangement within the filament. The aperture between the cells is greater in size than those previously described for other species (Ikari 1927, Tomas 1997). As the setae do not fuse or bifurcate, but rather cross one another at two to three

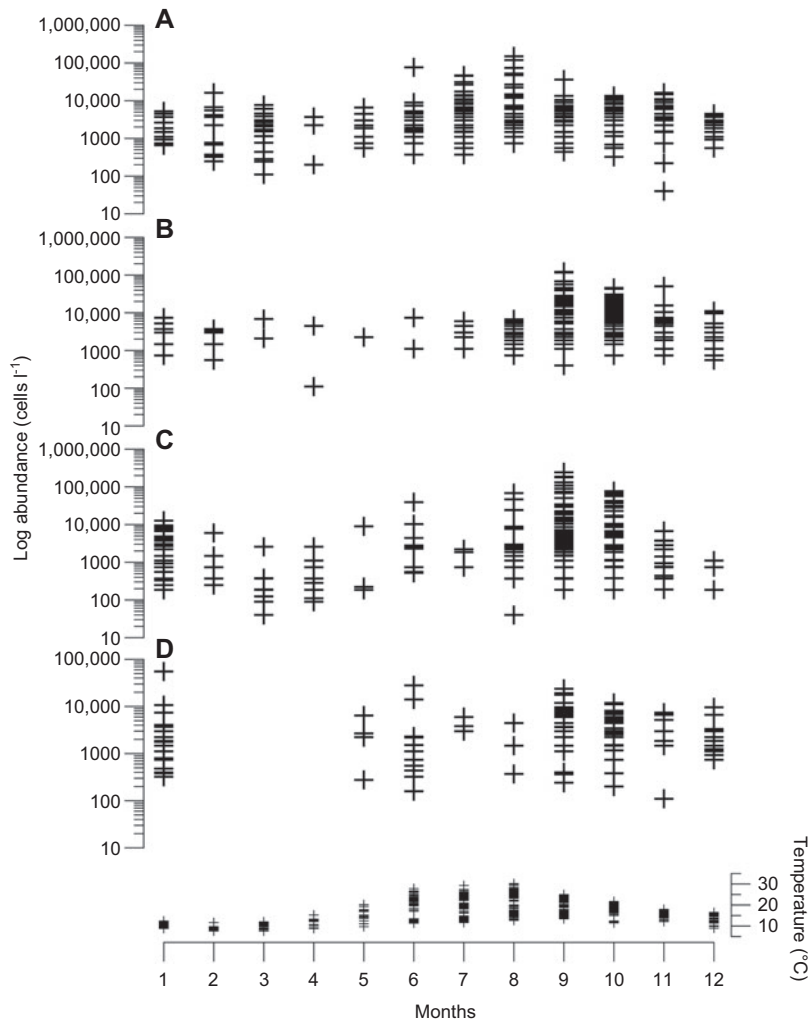


Figure 24 Monthly variation in cell abundance of (A) *B. delicatulum*, (B) *B. mediterraneum*, (C) *B. jadrantum*, and (D) *B. hyalinum*, and sea surface temperature in the northern Adriatic Sea between 1998 and 2009.

times the cell diameter from the filament axis, the cells are not closely packed within the filament and the apertures between them are substantial. These features are the main ones differentiating *B. jadrantum* from all other species reported in the northern Adriatic Sea until now (Table 1). The setae on both valves are morphologically identical in size and shape, which classifies *B. jadrantum* within the section *Isomorpha*. Its cell morphology shares some features with that of *B. tenue* Steemann Nielsen (Table 1) described from the Indian Ocean (Steemann Nielsen 1931). However, it differs from *B. tenue* by having a larger number of setae as well as in the morphology of the setae on the terminal cells; the terminal setae do not differ in *B. jadrantum*, but in *B. tenue*, the setae on the outer valve are curved (Reinecke 1969). The curvature of the setae allows differentiation between the anterior and posterior terminal cells, therefore classifying *B. tenue* in the section *Sagittata* (Sarno et al. 1997). In addition, cell morphology differs from *B. tenue*, which has elongate cylindrical cells (Steemann Nielsen 1931), while in *B. jadrantum*, cells are square-rectangular.

One of the features characteristic of *Bacteriastrium* species is the distinctive ultrastructure of the setae (Round et al. 1990, Tomas 1997). This feature has not been thoroughly investigated as electron microscopical investigations of this genus are scarce (Sarno et al. 1997). The setae in *B. jadrantum* are characterized by helicoidal and ring-like costae, and they lack both the spines and pores previously reported for *B. furcatum* Shadbolt and *B. parallelum* Sarno, Zingone et Marino (Sarno et al. 1997). The valve ornamentation is similar in all *Bacteriastrium* species investigated by electron microscopy, with a central annulus, bifurcating radial rows of areolae and a single rimoportula present in the central annulus of the terminal cells only (Fryxell 1978, Sarno et al. 1997).

Very few gene sequence analyses have been performed within the genus *Bacteriastrium*, except for *B. hyalinum*, for which there are partial 16S rDNA of the chloroplast (Rampen et al. 2009), partial internal transcribed spacer and 5.8S rDNA sequences (Moniz and Kaczmarek 2010), and partial 28S sequences [hypervariable regions D1–D4 of the large subunit (LSU) rRNA] (Kooistra et al. 2010). Phylogenetic 28S

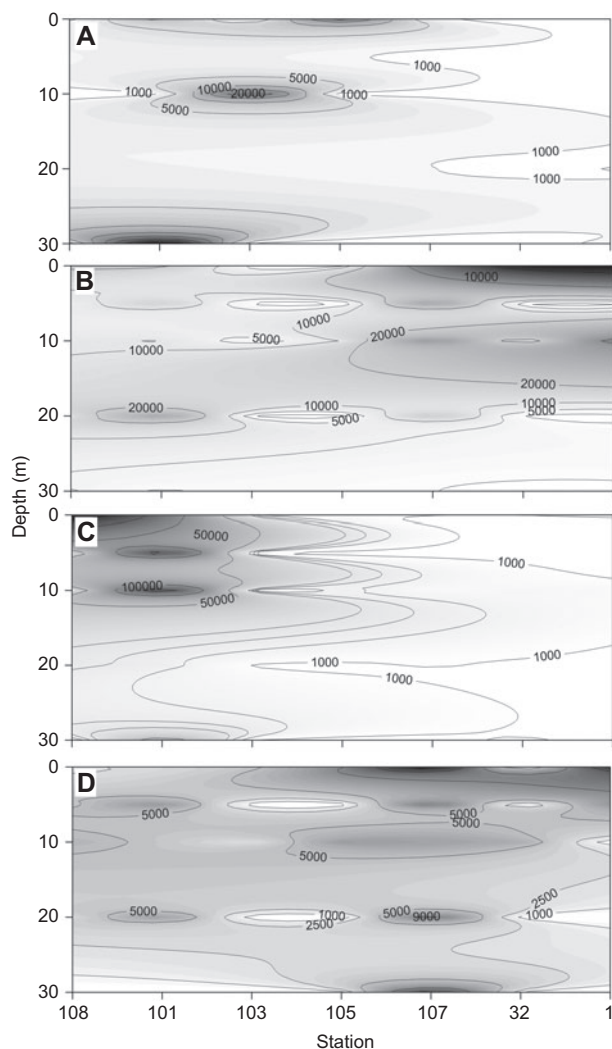


Figure 25 Spatial representation in the upper water column of the maximum abundances (cells l^{-1}) of the four most common *Bacteriastrium* species at selected dates during the phytoplankton monitoring program in the northern Adriatic Sea.

(A) *B. delicatulum*, August 2000; (B) *B. mediterraneum*, September 2008; (C) *B. jadrantum*, September 2006; (D) *B. hyalinum*, September 2009.

(LSU) sequence analysis resulted in *B. hyalinum* being re-assigned within the genus *Chaetoceros* (Kooistra et al. 2010). Our neighbor-joining representation of 18S rRNA also placed the *B. jadrantum* in one clade with the genus *Chaetoceros* and *Attheya septentrionalis* (see Figure 23). However, the dataset of available 18S rRNA gene sequences for *Chaetoceros* and, especially for *Bacteriastrium* is very small, resulting in relatively low bootstrap values for neighbor-joining phylogenetic analyses within the genus *Chaetoceros*. We support a clear distinction between *Bacteriastrium* and *Chaetoceros* based on a series of morphological features, but these differences should also be reflected in a clear differentiation of the gene sequences (Rines and Theriot 2003, Kooistra et al. 2010). Therefore, a further clarification of the phylogenetic status

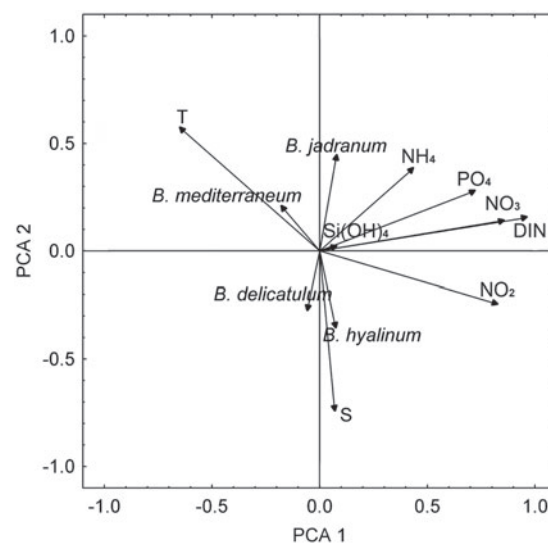


Figure 26 PCA plot of axes I and II showing the four common *Bacteriastrium* species in relation to environmental variables during the 1998–2009 phytoplankton monitoring program in the northern Adriatic Sea. S, salinity; T, temperature.

of the genus *Bacteriastrium* may be expected as more gene sequence analyses will become available for *Bacteriastrium* and *Chaetoceros*.

Habitats of *Bacteriastrium* in the northern Adriatic Sea

Conditions in the northern Adriatic Sea are controlled mainly by the Po River discharge, pycnocline dynamics, and wind-induced circulation patterns (Cushman-Roisin et al. 2001). The seasonal thermal cycle of the northern Adriatic Sea is typical of temperate latitude seas with winter vertical mixing of the water column and a seasonal thermocline formed during spring and summer (Zavatarelli et al. 2000). More recently, Mozetič et al. (2010) reported some oligotrophic trends in the northern Adriatic Sea as opposed to usual eutrophic conditions; these trends were attributed to a significantly lower Po River outflow in the 2000–2009 period and a predominance of an anti-cyclonic circulation, which were accompanied by a change toward higher salinity and lower nutrient budget of the entire northern Adriatic (Marić et al. 2012). The phytoplankton of the northern Adriatic Sea is mainly dominated by flagellates throughout most of the year, while diatoms have maximum abundances during the September–November period and also from March to May in the western part. Similarly, large diatom blooms have been reported to move from spring to autumn during the last decade (Viličić et al. 2009a, Mozetič et al. 2010), which is also demonstrated in this study with a predominance of diatoms over the flagellates only during September–November and paralleling the most commonly reported occurrences of the genus *Bacteriastrium*. The maximum cell abundance reported for the genus (244×10^3 cells l^{-1}) is greater than those reported for the oligotrophic southern Adriatic Sea (4×10^3 cells l^{-1}) (Viličić 1998), but similar to the

one from the middle, coastal part (224×10^3 cells l^{-1}) (Viličić et al. 2009b).

The genus *Bacteriastrium* comprises six taxa, including the new described species *B. jadrantum*, present in the northern Adriatic Sea, and two very rare taxa, *B. comosum* and *B. hyalinum* f. *princeps*. The common *B. delicatulum* occurred throughout the year without a clear seasonality, observations that are congruent with a recent report from Lim Bay in the northeastern Adriatic Sea (Bosak et al. 2009). During the cold months of the year when the sea surface temperatures were the lowest, *B. hyalinum* was the most abundant species in the northern Adriatic Sea, corroborating the report of Hendey (1964) that this species was the most common one in the North Sea. Furthermore, when studying its life cycle, Drebes (1972) showed that cells $<30 \mu\text{m}$ in diameter underwent sexual reproduction. Interestingly, *B. hyalinum* was absent in the northern Adriatic Sea from February to April, following high abundances in the winter time, which may be explained by a change in its life cycle during that period. When the sea surface temperatures were high in the northern Adriatic Sea, particularly from August to October, *B. mediterraneum* was very abundant in the surface waters, and this well-known species, along with *B. delicatulum*, also occurs throughout the whole Adriatic Sea (Viličić 1998, Viličić et al. 2009b, Totti et al. 2000). Among all the *Bacteriastrium* taxa present in the northern Adriatic Sea, *B. jadrantum* was the most abundant. When other diatoms were less abundant, *B. jadrantum* was still present at high cell abundances, strongly contributing to the total phytoplankton assemblage. *Bacteriastrium jadrantum* had the highest cell abundances during the strong event of the Po River plume in the northern Adriatic Sea in late summer. Since the recognition of this new species in our historical phytoplankton collection, we were able to track the first occurrence of *B. jadrantum* back to September 2001. It is therefore very possible that this species of *Bacteriastrium* may have been overlooked in the past.

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