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SHORT COMMUNICATION

First records of two planktonic Indo-Pacific diatoms: *Chaetoceros bacteriastroides* and *C. pseudosymmetricus* in the Adriatic Sea

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Summary Unusual occurrence of planktonic diatom species, *Chaetoceros bacteriastroides* and *Chaetoceros pseudosymmetricus*, was noticed in three different marine ecosystems of Adriatic Sea: the Krka Estuary and Telaščica Bay in the Central Adriatic, and in southern Adriatic offshore. From 2010 to 2015, these two *Chaetoceros* species were recorded in heterogeneous environmental conditions and in a very low abundances. Both species are regarded as very rare in world oceans, and consequently knowledge of their distribution and ecology is rather poor. Primarily described from tropical waters and showing Indo-Pacific distribution, *C. bacteriastroides* and *C. pseudosymmetricus* findings in Adriatic represent the northernmost records in world's oceans and seas. For *C. pseudosymmetricus* this is also the first occurrence in European seas. Areal expansion and introduction of new phytoplankton species in the Adriatic Sea might be related to different circulation regimes in the Ionian Sea and the concurrent rise in sea temperature in the Mediterranean in the last decade. Recent investigations have shown that entering currents, of either Atlantic/Western Mediterranean or Eastern Mediterranean origin, modify the composition of the plankton community in the Adriatic by bringing different newcomers.

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The genus *Chaetoceros* Ehrenberg (Bacillariophyta) is one of the most important diatom genera, with a great variety of species found worldwide (Malviya et al., 2016). In the Adriatic Sea the genus is ecologically very significant and represented with ca. 50 species (Viličić et al., 2002). Its identification is often problematic due to the large morphological variability within the species, the frequent occurrence of intermediate forms and the fact that some of the characters used for delineation can only be observed using electron microscopy (Bosak, 2013). In general, this genus is characterized by an extension of the valve named setae and the formation of chains that are joined in various structures associated mainly with the form and position of setae (Hasle and Syvertsen, 1997). One of their most conspicuous characteristics is the possession of four setae per cell in the majority of species (Round et al., 1990). In spite of these taxonomic characteristics *Chaetoceros bacteriastroides* Karsten has a larger number of setae making it morphologically different from other *Chaetoceros* species. *C. bacteriastroides* has been rarely recorded from the Indian Ocean (Karsten, 1907; Paul et al., 2007; Simonsen, 1974), the Pacific Ocean (Fryxell, 1978; Guilard and Kilham, 1977; Hernández-Becerril, 1993), Chinese seas (Ke et al., 2012; Liu, 2008) and the Tyrrhenian Sea (Sarno and Zingone, 2008). The chains are straight, usually long and robust. The cells are cylindrical, in girdle view appear rectangular with smooth corners and long perivalvar axis. Each cell contains numerous small chloroplasts. Each intercalary valve possesses six setae of which two are long and robust while four are shorter and very strongly spirally undulated (Hernández-Becerril, 1993). Another recorded diatom, *Chaetoceros pseudosymmetricus* Steemann-Nielsen is a very rare species, reported only three times from the Indian Ocean (Hernández-Becerril, 2000; Steemann-Nielsen, 1931; Thornington-Smith, 1970). The cells form straight and short chains of three to seven cells that are rectangular in girdle view and narrowly elliptical in valve view. The cells are delicate, and lacking chloroplasts within the setae, therefore belonging to the subgenus *Hyalochaete*. The intercalary setae emerge from the apices of the valves and fuse immediately to the sibling setae. After fusion, they become inflated for a short distance and then gradually taper to the tip (Hernández-Becerril, 2000).

Findings of *C. bacteriastroides* and *C. pseudosymmetricus* in the Adriatic Sea add to the scarce information on the geographical and ecological features of these rare diatoms. Also, these unusual reports for the whole Mediterranean contribute to the knowledge of phytoplankton biodiversity and checklists of European seas.

The Adriatic Sea is an elongated basin in the northern part of the Mediterranean, with three distinctive sections, the northern, central and southern parts where different water masses can be recognized (Viličić et al., 2002). Adriatic ecosystems are influenced by the regular exchange of water with the Eastern Mediterranean through the Strait of Otranto. Levantine Intermediate Water (LIW) and Ionian Surface Water (ISW) flow into the Adriatic along the sea's eastern border. The volume of this flow is greater in winter but, depending on climatic oscillations that occur from the Atlantic to the south-east Mediterranean, varies year-to-year (Grbec et al., 2002). Studies where previously unrecorded *C. bacteriastroides* and *C. pseudosymmetricus* were found have been made in three different marine ecosystems of the

Adriatic Sea: the Krka Estuary and Telašćica Bay in the Central Adriatic, and in the southern Adriatic offshore. Karstic river Krka is a salt-wedge, highly stratified estuary (Žutić and Legović, 1987), characterized by small tidal amplitudes and permanently brackish surface water (Svensen et al., 2007). Telašćica Bay is located about 32 nautical miles north-west from the Krka Estuary and it is a poorly investigated, semi-enclosed oligotrophic marine ecosystem that is formed by Dugi Otok island. Finally, the South Adriatic is the deepest and the widest part of the basin, characterized by the oligotrophic offshore and circular, 1243 m deep South Adriatic Pit.

The records of *C. bacteriastroides* and *C. pseudosymmetricus* in the Adriatic Sea are the results of 4 different research projects that were carried out from 2009 to 2016. Altogether, 24 research cruises were undertaken and in total 41 net samples (Krka Estuary and Telašćica Bay) and 272 bottle samples (South Adriatic) were analyzed. In the Krka Estuary (at 4 stations in June 2010) and Telašćica Bay (at 5 stations in March 2012) only net samples were collected by vertical hauling from 20 m to surface. Mesh plankton net (20 μm -pore-sized) samples were observed using an inverted Zeiss Axio Observer Z1 microscope and Mira II FE LMU scanning electron microscope (Bosak, 2013). Phytoplankton samples in South Adriatic were collected by 5-L Niskin bottles at 4 stations along the Dubrovnik-Bari transect monthly from February 2009 to February 2013 and at 12 stations from December 2015 to April 2016, both at standard oceanographic depths (0–200 m). Niskin samples were preserved in neutralized formaldehyde (2.5% final concentration) and observed using an Olympus IX-71 inverted microscope according to the Utermöhl method (Utermöhl, 1958). Subsamples (100 mL) were settled for 48 h in counting chambers (Hydro-Bios) before analyses. Observations were made over the entire area of the counting chamber base plate to obtain the most accurate estimation. The phytoplankton abundances are expressed as number of cells per liter (cells L^{-1}). Temperature and salinity profiles were taken by CTD multiparametric probe (SBE 911 plus, SeaBird Electronics Inc., USA). Identification of *C. bacteriastroides* and *C. pseudosymmetricus* was carried out following Fryxell (1978) and Hernández-Becerril (1993, 2000). In all examined samples from Krka Estuary, at Telašćica Bay and along the Dubrovnik-Bari transect *C. bacteriastroides* and *C. pseudosymmetricus* were encountered only in 7 cases (Fig. 1, Table 1). Particular records with locations and abundances as well as temperature and salinity conditions of *C. bacteriastroides* and *C. pseudosymmetricus* in Adriatic Sea are shown in Table 1. *C. bacteriastroides* (Fig. 2) was recorded for 6 times from 2010 to 2015, while *C. pseudosymmetricus* (Fig. 3) was recorded just once in 2015. Abundance of both species did not exceed $10^2 \text{ cells L}^{-1}$.

The migration of new planktonic species from tropical and subtropical areas to the temperate seas is a regular process that has changed the dynamic of Mediterranean and Adriatic biodiversity in the last decade (Batistić et al., 2014; Gómez, 2006; Halim, 1990; Pećarević et al., 2013; Zenetos et al., 2005). The opening of the Suez Canal slowly allowed some Indo-Pacific species to migrate north into the eastern Mediterranean while, at the other end of the basin, Atlantic water entering via the Straits of Gibraltar carries into the western Mediterranean species that are prevalently of (sub)

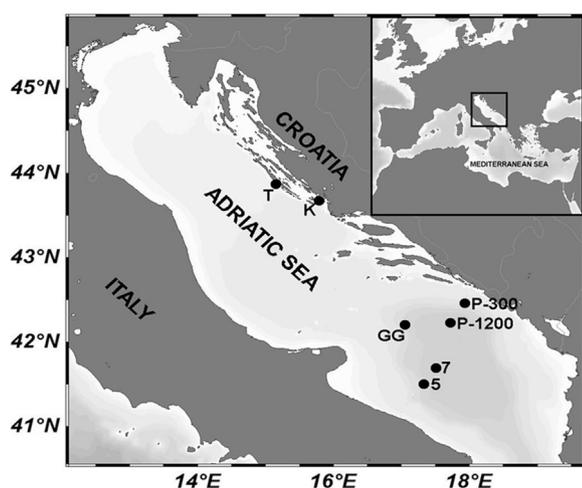


Figure 1 Map of the 7 station locations in the Adriatic Sea where *Chaetoceros bacteriastroides* and *C. pseudosymmetricus* have been recorded. Krka Estuary (K), Telaščica Bay (T) and Dubrovnik-Bari transect (GG, 5, 7, P-1200 and P-300).

tropical affinity (Bianchi, 2007; Gómez et al., 2000; Por, 1990). Spreading of warm water species has been speeded up by the increase of sea temperature in the Mediterranean which enhances the survival of newcomers (Parravicini et al., 2015). The exact path of introduction of *C. bacteriastroides* and *C. pseudosymmetricus* into the Adriatic Sea is difficult to reconstruct. Regarding other introduced species in the Eastern Adriatic, possible vectors of introduction could be aquaculture activities and shipping (Pećarević et al., 2013). But most likely it might be related to the regular inflow of Levantine Intermediate Water (LIW) and Ionian Surface Water (ISW). The surface and subsurface temperature and salinity in the South Adriatic ($T > 14.5^{\circ}\text{C}$, $S > 38.5$) seem to be a signature of the cyclonic regime of circulation that brings warm and more saline Eastern Mediterranean Water (Batistić et al., 2012; Civitarese et al., 2010). However, both species have never been reported in the eastern Mediterranean but occurrence of *C. bacteriastroides* was noted in the Western Mediterranean, in Gulf of Naples (Southern Tyrrhenian Sea) in 2002 (Sarno and Zingone, 2008). Since then it has been found at the same site on other dates, but at relatively low abundances (Zingone, 2015). Remarkable is that occurrence of rarely reported *C. pseudosymmetricus* was observed for the first time in

European seas. Both species were defined tentatively as Indo-Pacific in origin (Hernández-Becerril, 1993, 2000) with scarce information about their ecology. Those findings in Adriatic represent the northernmost records in world's oceans and seas. Although rare, it is obvious that *C. bacteriastroides* found their ecological niche in the Adriatic Sea as it is recorded in different areas over a span of several years, being adaptive enough to survive. Thus, in the Adriatic it was recorded in different environmental conditions; from the estuarine waters with lower salinity to the South Adriatic offshore with a high salinity influence. However, if we compare the environmental conditions of warmer seas in which *C. bacteriastroides* was reported such as the Bay of Bengal ($T = 27\text{--}29^{\circ}\text{C}$, $S = 29\text{--}33$) and the China Sea ($T = 29\text{--}32^{\circ}\text{C}$, $S = 32\text{--}33$), this species in the Adriatic shows an expanded tolerance limits for temperature and salinity (Ke et al., 2012; Paul et al., 2007). It is certain that both newly recorded *Chaetoceros* in the Adriatic prefer oligotrophic conditions (Čalić et al., 2010; Šupraha et al., 2014; Viličić et al., 2002). For now it is still unknown whether the record of *C. pseudosymmetricus* is a temporary appearance in the Adriatic or will adapt and become a common taxon of the Mediterranean phytoplankton communities. As it was first described from below 50 m (Steedmann-Nielsen, 1931) and at 50 m in this study, *C. pseudosymmetricus* is considered as a more oceanic 'shade' form. Huge and sparsely studied area of the Mediterranean and the Adriatic open waters offers the possibility that those taxa have been being overlooked or misidentified in the past as is the case with numerous other phytoplankton species (Gómez, 2008; Hamsheer et al., 2013). However, for *C. bacteriastroides* and *C. pseudosymmetricus* misidentification is highly unlikely considering their peculiar morphology (Hernández-Becerril, 1993, 2000).

Recent records of these unusual *Chaetoceros* species in Adriatic Sea should be considered as an example of the expansion of thermophilic phytoplankton species. Although such sporadic events do hardly allow trend analyses they nevertheless provide useful information on environmental alteration and the global distribution of species. We can expect the spreading of warm-water phytoplankton species in the Mediterranean associated with sea warming in present day. The future regular studies are necessary to verify if new *Chaetoceros* species establish a healthy population in the Adriatic Sea. These findings will facilitate the identification of *C. bacteriastroides* and *C. pseudosymmetricus*, and possibly further records in Mediterranean.

Table 1 Spatio-temporal data of new *Chaetoceros* species in Adriatic Sea – abundances (A), temperature (T) and salinity (S).

Species	Location	Station	Date	Sample	Depth [m]	A [cell L ⁻¹]	T [°C]	S
<i>Chaetoceros bacteriastroides</i>	43°41'N/15°49'E	Krka (K)	9.6.2010	Net	0–20	+ ^a	15.4–22	34.9–37.9
	43°50'N/15°12'E	Telaščica (T)	1.3.2012	Net	0–20	+ ^a	12–12.5	38.7–38.8
	42°13'N/17°42'E	P-300	12.1.2012	Bottle	100	140	14.3	38.7
	42°27'N/17°56'E	P-1200	12.1.2012	Bottle	75	180	14.7	38.7
	41°29'N/17°12'E	7	13.12.2015	Bottle	2	70	16.2	38.8
	42°58'N/17°04'E	GG	14.12.2015	Bottle	50	160	14.2	38.8
<i>Chaetoceros pseudosymmetricus</i>	41°19'N/17°07'E	5	13.12.2015	Bottle	50	80	14.2	38.8

^a +, present in net samples.

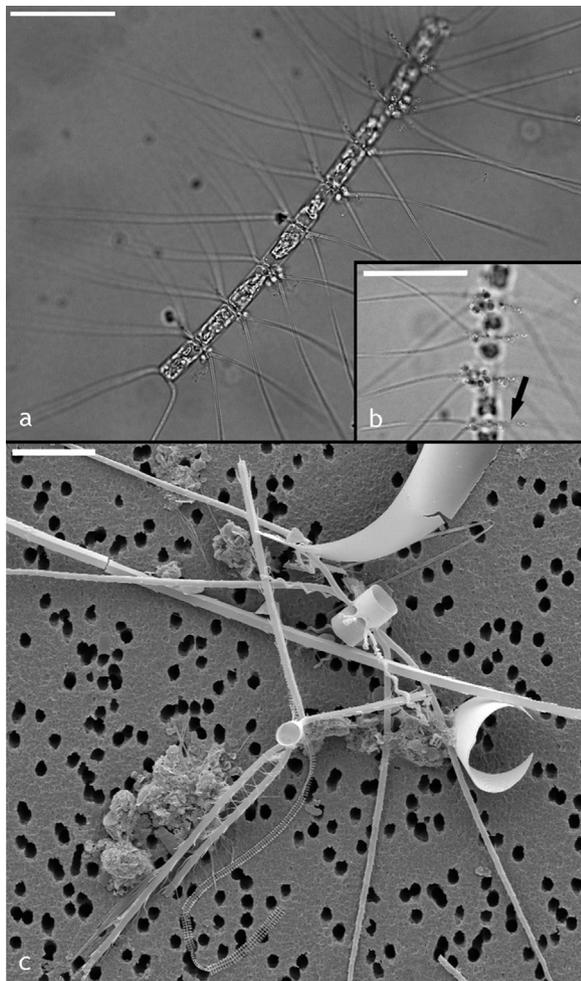


Figure 2 LM (a, b) and SEM (c) micrographs of *Chaetoceros bacteriastroides* from the South Adriatic (station P-1200) and Krka Estuary: Complete chain showing orientation of the setae (a); Detail of the intercalary valves and setae showing reduced spirally undulated setae (b); sibling valves in girdle view showing two pairs of reduced spirally undulated setae and two pairs of common intercalary setae (c). Scale bar = 50 μm (a, b) and 20 μm (c).

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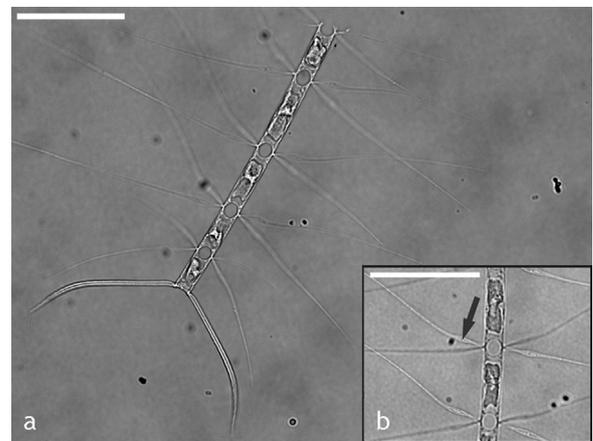


Figure 3 LM micrographs of *Chaetoceros pseudosymmetricus* from the South Adriatic (station 5): Terminal part of the chain with intercalary and terminal setae (a); detail of the intercalary valves and setae, showing inflation of intercalary setae (b). Scale bar = 50 μm .

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