Mediterranean Marine Science

Vol 20, No 3 (2019)

Benthic diatoms species richness at Dvuyakornaya Bay and other coastal sites of Crimea (the Black Sea) under various environments

ELENA L. NEVROVA, ALEXEI PETROV

doi: 10.12681/mms.20319

Research Article

Mediterranean Marine Science
Indexed in WoS (Web of Science, ISI Thomson) and SCOPUS
The journal is available on line at http://www.medit-mar-sc.net
DOI: http://dx.doi.org/10.12681/mms.20319

Benthic diatom species richness at Dvuyakornaya Bay and other coastal sites of Crimea (Black Sea) under various environmental conditions

Elena L. NEVROVA and Alexei N. PETROV

2, Nakhimov ave., 299011 Sevastopol, Russian Federation
Benthos Ecology Department, A.O. Kovalevsky Institute of Marine Biological Research RAS

Corresponding author: el_nevrova@mail.ru

Handling Editor: Lydia IGNATIADES

Received: 20 April 2019; Accepted: 27 May 2019; Published on line: 30 August 2019

Abstract

Initial research of the pristine waters of Dvuyakornaya Bay (South-eastern Crimea, Black Sea) revealed a high taxonomic richness in benthic diatoms (Bacillariophyta). A total of 304 species, 78 genera, 37 families, and 20 orders were identified. Among these, 68 novel species for the Black Sea were noted. The largest number of species was identified in the genera *Navicula* (41 species), followed by *Nitzschia* (29), *Amphora* (25), *Cocconeis* (20), *Diploneis* (16), *Lyrella, Fallacia*, and *Planothidium* (10 species each, respectively). An inter-regional comparative analysis of diatom species richness was performed using Bray-Curtis similarity coefficient and Venn diagrams. The diatom taxocene of Dvuyakornaya Bay (DB) was compared with Bacillariophyta flora from several Crimean coastal sites under different levels of anthropogenic impact, i.e. relatively pristine waters near Cape Fiolent (CF) and heavy polluted Sevastopol Bay (SB) and Balaklava Bay (BB). The highest species composition similarity (58%) was registered between intact biotopes (DB vs CF) and between heavily polluted areas (SB vs BB), despite the geographical remoteness of the compared areas as well as differences in their hydrological-hydrochemical conditions and bottom substrates patterns. The lowest diatom taxocene species similarity was observed between SB and CF (32.3%) and between SB and DB (36.5%). It was concluded that the content of technogenic pollutants (trace metals, PCBs, PAHs, and pesticides) in the bottom sediments, as well as the heterogeneity of microbiotopes, represent major factors influencing the species composition of benthic Bacillariophyta in the investigated coastal areas.

Keywords: Bacillariophyta; diatoms; taxocene; species richness; technogenic pollution; Black Sea.

Introduction

The study of the benthic diatom taxocene structure in various marine biotopes is highly relevant due to the key role of Bacillariophyta in coastal ecosystem functions. Evaluation of diatom species richness represents an important task for bioindication purposes and to determine species conservation priorities (Keck *et al*., 2016; Hafner *et al*., 2018; Nevrova *et al*., 2015; Stenger-Kovacs *et al*., 2014, 2016). With coastal marine ecosystems being under increasing anthropogenic impact, a primary task involves the assessment of taxonomical diversity and comparative evaluation of taxocene structure in biotopes with varying levels of pollution. These studies allow researchers to identify the peculiarities of diatom taxocene structures and create recommendations for biodiversity maintenance. Comparisons of diatom taxocene structures in pristine coastal habitats and anthropogenically perturbed biotopes facilitate the identification of various aspects of the formation and sustainability of Bacillariophyta diversity under changing environmental conditions (Heino *et al.,* 2007; Leira *et al.,* 2009; Nevrova, 2015; Petrov *et al.,* 2010; Stenger-Kovacs *et al.,* 2014, 2016).

In the Black Sea, studies of diatoms species richness in various environments is particularly important due to the intensive anthropogenic impact upon the marine environment and the necessity to apply the bioindication methods to assess the current state of nearshore waters (Nevrova *et al*., 2015; Petrov & Nevrova, 2004; Petrov *et al*., 2005). A comprehensive generalisation of the results obtained using both floristic studies and computative methods is required to research different aspects of microalgae species diversity, particularly the analysis of diatom taxocene structure in ecologically heterogeneous biotopes (Hafner *et al*., 2018; Heino *et al*., 2005, 2007; Petrov & Nevrova, 2007, 2014; Petrov *et al*., 2010; Stenger-Kovacs *et al*., 2016). The present work focuses on the taxonomic composition of benthic diatoms in Dvuyakornaya Bay (Eastern Crimea, Black Sea) – a pristine marine area that has not been previously investigated – and on a comparative analysis of species richness between the previously studied coastal locations of South-western Crimea that suffer from various levels of anthropogenic pollution.

Materials and Methods

Benthic diatom taxocenes of the Crimean coast eastwards from the Karadag State Reserve have not been previously investigated. Bacillariophyta species richness in Dvuyakornaya Bay (DB) (translation: "Two-anchored Bay") – which remains practically free of anthropogenic pollution – was studied for the first time in contrast to other locations of the Crimean coast (Fig. 1), such as the waters around Cape Fiolent (CF) (Nevrova, 2016), Balaklava Bay (BB) (Nevrova, 2014; Petrov *et al*., 2010), and SB (SB) (Nevrova, 2013; Petrov *et al*., 2005).

Dvuyakornaya Bay is an "open type" bay (S = 6.4 km^2) surrounded on the Northeast by Cape St. Ilya and from the South-west by Cape Kiik-Atlama. The steep coast consists of Jurassic conglomerates, clays, limestones, and siderite. Until the early 2000s, the bay was used as a military training area and was completely withdrawn from any municipal or recreational exploitation. As a result, the natural conditions of the area were preserved. Currently, the waterfront of this bay is being developed with yachting and recreation centres, which may adversely affect the ecological status of this area and the state of benthic communities in the coming years.

Sampling of bottom sediments in DB (sand with clay admixture) was performed in August 2008 at nine sampling sites (N 44°59'09'', E 35°23'05'') within a depth range of 2–10 m. Samples were taken by a diver in two replicates from the upper (2–4 cm) layer of soft bottom sediments using a meiobenthic tube with a surface area 16 cm^2. Sediment grain-size composition (as percentage of sandy, silty, and clay fractions) was determined by wet sieving and gravimetric sedimentation. Samples of bottom sediments were taken simultaneously from each site for the chemical analysis of inorganic and organic contaminants, and included measurements of 12 parameters: metals (Cu, Zn, Ni, Cr, Pb, Cd, and Hg), polychlorinated biphenyls (PCB, sum of four congeners), pesticides (4-4'-DDTs and metabolites), and the sum of polyaromatic hydrocarbons (PAHs). The content of silt+clay fractions and total organic carbon (C_{org}) of the sediments was also determined. Furthermore, chemical analysis of soft bottom sediments was conducted by colleagues from Institute of Colloid Chemistry and Water Chemistry NASU, Kiev (ICCWC) using previously described techniques (Burgess *et al*., 2009, 2011; Petrov *et al*., 2010).

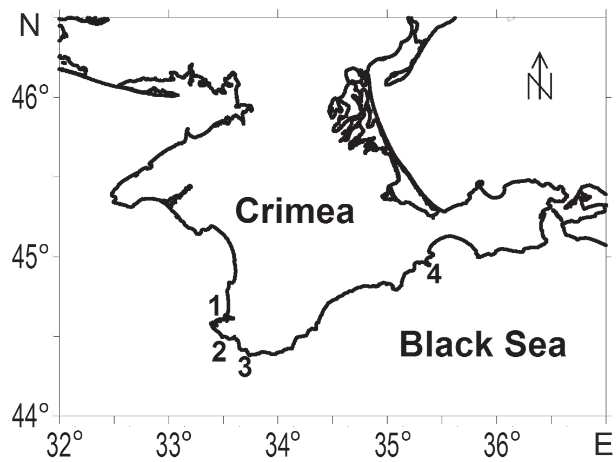

Fig. 1: Schematic map of Dvuyakornaya Bay (4) and other sampling sites along the coast of Crimea (Black Sea): 1-Sevastopol Bay, 2-Cape Fiolent, 3-Balaklava Bay.

Samples were initially treated in an ultrasonic bath for 20 min, then diatom valves were cleaned by HCl and H_2SO_4 with addition of $K_2Cr_2O_7$ (Proshkina-Lavrenko, 1974; Nevrova *et al*., 2015). Slides for light microscopy (LM) were created using Meltmount®. Sampling surveys and treatments were performed at the Institute of Marine Biological Research (IMBR). LM micrographs were taken using a Nikon Eclipse E600 with a PlanAPO 100× objective lens (Institute of Marine Sciences, University of Szczecin, Poland). Scanning electron microscopy (SEM) was performed using a Hitachi S-4500 (Goethe University, Frankfurt-am-Main, Germany). Slides for LM were stored in the collections of Dr. E. Nevrova (IMBR) and Prof. Dr. A. Witkowski (Institute of Marine Sciences, SZCZ). Stubs for SEM were deposited in the collection of Prof. Dr. H. Lange-Bertalot (Goethe University, FR). Valve measurements were made from digital images using ImageJ 1.4.3.67 (ImageJ, 2014).

For Bacillariophyta classification, the taxonomic system according to Round *et al*., (1990) with the latest update (Witkowski *et al*., 2000; Levkov, 2009 *etc*.) was used. Nomenclature taxa citation follows Fourtanier & Kociolek (1999, 2011) and the International Plant Names Index (2004).

The diatom taxocene species similarities between DB and other biotopes of the Crimean coast (CF, SB, and BB) were estimated based on the Bray-Curtis similarity coefficient (for presence/absence data) using PRIMER v5.2 software (Clarke & Gorley, 2001). Computation the expected number of species (S_{exp}) that can be found under equal sampling efforts for all compared locations (i.e. in 8-9 samples) was conducted using an extrapolative model of the DIVERSE routine (PRIMER), and data were averaged over 1000 random permutations.

Moreover, in order to visualise regional differences in the species structure of benthic diatom assemblages, a Venn diagram analysis was also applied (Venn diagrams, 2019).

Results

Pollutants in bottom sediments of Dvuyakornaya Bay

The analysis of technogenic pollutants content in soft sediments of DB confirmed the high ecological quality of this area, which was previously closed to any civil activity (Table 1). Very low content of the trace metals (Cu, Zn, Ni, Cr, Pb, Cd, Ag, and Hg) was detected, and the main classes of organic pollutants (PCBs, PAHs, and pesticides) found did not exceed average levels for the soft bottoms of environmentally intact offshore areas of

Table 1. Median values and range (in brackets) of metals, organic contaminants, and elemental concentrations in surface sandy/muddy bottom sediments and the near-bottom water layer of Dvuyakornaya Bay and other coastal waters of Crimea (Black Sea).

Site Analyte	**Dvuyakornaya Bay**	**Cape Fiolent**	**Sevastopol Bay**	**Balaklava Bay**	**Background level for coastal zone***	**Background level for coastal zone****
			Metals ($\mu g \times g^{-1}$ dw)			
Cu	13,2 (12,5÷13,5)	22,8 (22,0÷23,5)	153 (58÷418)	142 (63÷390)	13	<20 (18÷30)
Zn	98,9 (96,8÷100,2)	124,4 (124,0÷125,3)	266 (107÷758)	202 (111÷431)	10,1	<50 (44÷94)
Ni	0,8 (0,4÷1,0)	5,5 (5,2÷5,8)	43 (31÷62)	39 (32÷50)	1,6	<40 (33÷67)
Cr	2,5 (2,2÷2,8)	0,5 (0,4÷0,8)	46 (26÷82)	33 (20÷72)	1,5	<45 (35÷84)
Pb	45,2 (43,5÷46,8)	21,8 (20,8÷22,8)	290 (86÷1362)	226 (87÷510)	9,7	<15 (10÷25)
Cd	0,022 (0,018÷0,028)	0,048 (0,044÷0,050)	0,95 (0,22÷6,25)	0,21 (0,11÷0,36)	0,15	<0,5 (0,1÷1,0)
Ag	0,06 (0,04÷0,08)	0,02 (0,015÷0,022)	0,50 (0,14÷2,30)	0,29 (0,14÷1,05)		
Hg	0,02 (0,017÷0,032)	0,02 (0,015÷0,030)	1,03 (0,04÷7,61)	0,96 (0,46÷1,42)	0,04	0,04
			Organic pollutants ($ng \times g^{-1}$ dw)			
Total PAHs	<5	<5	12854 (790÷41269)	4166 (1475÷15280)		
Total DDTs	0,93 (0,81÷1,05)	1,08 (0,73÷1,20)	168 (8÷2198)	30 (10÷64)	1,42	0,03÷0,1
Total PCBs	40 (32÷51)	16 (14÷20)	1897 (142÷12690)	594 (196÷4202)	4,5	50÷60
			Natural environmental parameters			
Water transparency, m	7,5 (3÷9)	8,6 (7÷10)	5,2 (3÷8)	5,4 (3÷7)		
Near bottom temperature, ^{0}C	22,4	21,3	16,8 (16,6÷17,4)	18,4 (17,3÷19,1)		
Salinity, psu	17,8	18,2	17,2	18,1		
Eh, mV	+237	+285	-296 (-152 ... -384)	-167 (-112 ...-320)		
O_2 in near bottom layer, $ml \times l^{-1}$	6,8	7,4	5,5 (4,8÷6,7)	6,5 (4,7÷8,2)		
C_{org} , %	0,5	0,5	6,0 (2,6÷11,4)	2,4 (1,6÷3,0)		
PO_4^{3-}	4,6***	2,2****	7,5 (2÷22)****	8,1 (0÷92)*****		
NO_3^-	7,2***	6,4****	27,6 (3÷86)****	15,7 (0÷57)*****		
NO_2^-	0,9***	0,7****	2,1 (0,4÷5,4)****	1,3 (0÷6)*****		
NH_4^+	<3***	<2****	12,5 (1,2÷33)****	8,9 (0÷40)*****		
Si	82***	65****	182 (44÷697)****	159 (30÷540)*****		

Note: *Samyshev *et al.,* 2014; **Mitropolsky *et al.,* 2006 ; ***Kovrigina *et al.,* 2009, ****Kovrigina *et al.,* 2016; *****Kovrigina *et al.,* 2010.

the Black Sea shelf (Mitropolsky *et al.*, 2006; Burgess *et al.*, 2009, 2011; Samyshev *et al.*, 2014). It has previously been shown that this set of pollutants can exert a strong influence on the quantitative development and spatial distribution of benthic diatoms (Petrov & Nevrova, 2004; Petrov *et al.*, 2005, 2010). According to the low concentrations of pollutants observed in the sediments (Table 1), DB is characterised as formally healthy with an undisturbed diatom taxocene species structure.

Taxonomic composition of diatoms of Dvuyakornaya Bay

As is known, the estimated diatom species richness usually depends on the sampling efforts (number of samples). The results of the prognostic modelling obtained for several nearshore Crimean locations have shown that the generalized relationship between the number of samples (X) and the number of observed species (Y) was reliably described by the log-equation $Y=79 \ln(X) + 35$ ($R^2 = 0.97$) (Petrov & Nevrova, 2012, 2013, 2014).

Based on this randomised curve, the number of species revealed in nine samples from DB represents 218 (73%) of the actual registered number (304). These results indicate that the actual diatom species richness of DB may significantly exceed the expected average level of richness, which can be revealed after the same sampling efforts throughout the coast of Crimea (Petrov & Nevrova, 2013). In total, 304 species (including 5 intraspecific taxa) belonging to 299 species, 78 genera, 37 families, 20 orders, and 3 classes of Bacillariophyta were identified. Among these, 68 novel Black Sea diatom flora taxa were revealed (Table 2). Moreover, eight species and taxonomic combinations which are described as new to science were also identified: *Lyrella abruptapontica* Nevrova, Witkowski, Kulikovskiy & Lange-Bertalot, *L. pontieuxini* Nevrova, Witkowski, Kulikovskiy & Lange-Bertalot, *L. pseudolyra* Nevrova, Witkowski, Kulikovskiy & Lange-Bertalot, *Navicula parapontica* Witkowski, Kulikovskiy, Nevrova & Lange-Bertalot, *N. pontica* Witkowski, Kulikovskiy, Nevrova & Lange-Bertalot (Nevrova *et al*., 2013; Witkowski *et al*., 2010). Additionally, four diatom species that were not registered in the Black Sea were also recorded: *Navicula glabriuscula var. elipsoidales* Proshk.-Lavr, *N. petrovii* Nevrova, Witkowski, Kociolek & Lange-Bertalot = Syn.: *N. scabriuscula* (Cleve et Grove) Mereschk. (Fig. 2: 40, Fig. 4: 29), *Toxonidea insignis* Donkin (Fig. 3: 26), and *Pinnularia trevelyana* (Donkin) Rabenh. (Fig. 3: 27) (Nevrova, 2015; Witkowski *et al*., 2014) (Table 2).

Representatives of four genera recently identified for Black Sea flora were found, including *Amicula* (Witkowski) Witkowski 2000, *Astartiella* Witkowski, Lange-Bertalot & Metzeltin 1998, *Chamaepinnularia* Lange-Bertalot & Krammer 1996, and *Cocconeiopsis* Witkowski, Lange-Bert & Metzeltin 2000 (Nevrova, 2015). Furthermore, the surveys also identified *Amicula specululum* (Witkowski) Witkowski (Fig. 4: 30), *Astartiella bahusiensis* (Grunow) Witkowski, Lange-Bertalot & Metzeltin (Fig. 2: 9; Fig. 4: 16), *Astartiella producta* Witkowski, Lange-Bertalot & Metzeltin (Fig. 4: 17), *Chamaepinnularia* sp. 1, *Cocconeiopsis breviata* (Hust.) Witkowski, Lange-Bertalot & Metzeltin (Fig. 2: 3), and *Cocconeiopsis pullus* (Hust.) Witkowski, Lange-Bertalot & Metzeltin (Fig. 4: 3). Several taxa were not identified at the species level but were included in the checklist since they have profound morphological differences from the registered species and were recorded on microphotographs. These

Table 2. Novel taxa for the Black Sea diatom flora in the Dvuyakornaya Bay area.
Designations: * newly discovered species for the Black Sea; ** recently described species; *** species not found in the Black Sea over the last 50 to 100 years of research; **** unidentified taxa that have profound differences to registered species.

Coscinodiscophyceae	**Fragilariophyceae**
Actinoptychus senarius (Ehrenb.) Ehrenb.	*Delphineis surirella* (Ehrenb.) Andrews
Aulacoseira granulata (Ehrenb.) Simonsen	*Diatoma vulgare* Bory f. *breve* (Grunow) Bukht.
Auliscus sculptus (W. Sm.) Ralfs	*Diatoma vulgare* f. *lineare* (Grunow) Bukht.
Coscinodiscus radiatus Ehrenb.	*Fragilaria atomus* Hust.
Cyclostephanos dubius (Friske) Round	*Fragilaria pulchella* (Ralfs) Lange-Bert.
Cyclotella caspia Grunow	*Fragilaria vaucheriae* (Kütz.) Boey-Petersen
Cyclotella meneghiniana var. *kuetzingiana* (Thwaites) Playfair	*Grammatophora marina* (Lyngb.) Kütz.
Cyclotella ocellata Pant.	*Hyalosira delicatula* Kütz.
Cyclotella operculata (C. Agardh) Kütz.	*Licmophora gracilis* (Ehrenb.) Grunow
Cyclotella striata Grunow	*Opephora gunter-grassii* (Witkowski & Lange-Bert.) Sabbe & Vyverman*
Dimeregramma minor (W. Greg.) Ralfs	*Opephora marina* (W. Greg.) P. Petit
Dimeregramma sp.1	*Opephora minuta* (Cleve-Euler) Witkowski, Lange-Bert. & Metzeltin*

Continued

Table 2 Continued

Glyphodesmis distans (W. Greg.) Grunow	*Opephora mutabilis* (Grunow) Sabbe & Vyverman*
Hyalodiscus ambiguus (Grunow) Temp. & H. Perag.	*Opephora pacifica* (Grunow) P. Petit*
Hyalodiscus scoticus (Kütz.) Grunow	*Pteroncola hyalina* (Kütz.) Gusl.
Plagiogramma tenuissima Hust.*	*Tabularia gaillonii* (Bory) Bukht.
Puncticulata radiosa (Lemmerm.) Håk.	*Tabularia tabulata* (C. Agardh) P.J.M. Snoeijs
Stephanodiscus hantzschii Grunow	*Thalassionema nitzschioides* (Grunow) Mereschk.
Thalassiosira baltica (Grunow) Ostenfeld	*Toxarium undulatum* J.W. Bailey
Thalassiosira parva Proshk.-Lavr.	*Trachysphenia australis* (H.L. Smith) Cleve*
Thalassiosira parvula Makarova	*Ulnaria ulna* (Nitzsch.) Compère
Triceratium antediluvianum (Ehrenb.) Grunow	
Bacillariophyceae	
Achnanthes brevipes C. Agardh	*Hippodonta* sp.3
Achnanthes vistulana Witkowski*	*Hippodonta* sp.5
Achnanthes fimbriata (Grunow) R. Ross	*Hippodonta* sp.6
Achnanthes islandica Østrup*	*Hippodonta* sp.8
Achnanthes longipes C. Agardh	*Hippodonta* sp.D5
Achnanthes mercurii Witkowski, Metzeltin & Lange-Bert.*	*Hippodonta* 10 A 032
Achnanthes placentuloides (Gusl.) Witkowski & Lange-Bert.	*Lyrella abruptapontica* Nevrova, Witkowski, Kulikovskiy & Lange-Bert.**
Achnanthes sp.B06	*Lyrella aestimata* (Hust.) Nevrova, Kulikovskyi, Witkowski & Lange-Bert.*
Achnanthidium glyphos Riaux-Gob., Compère & Witkowski*	*Lyrella hennedyi* (W. Sm.) A. Stickle & D.G. Mann
Achnanthidium minutissimum (Kütz.) Czarn.	*Lyrella lyroides* (Hendey) D.G. Mann
Amicula specululum (Witkowski) Witkowski*	*Lyrella majuscula* (Hust.) Witkowski*
Amphora arcus W. Greg.	*Lyrella pontieuxini* Nevrova, Witkowski, Kulikovskiy & Lange-Bert.**
Amphora aspera P. Petit	*Lyrella pseudolyra* Nevrova, Witkowski, Kulikovskiy & Lange-Bert.**
Amphora jostesorum Witkowski, Metzeltin & Lange-Bert.*	*Lyrella rudiformis* (Hust.) Nevrova, Witkowski, Kulikovskiy & Lange-Bert.
Amphora eunotia Cleve	*Lyrella spectabilis* (W. Greg.) D.G. Mann
Amphora exilitata Giffen*	*Mastogloia cuneata* (Meister) Simonsen*
Amphora graeffeana Hendey	*Mastogloia exigua* Lewis
Amphora helenensis Giffen*	*Mastogloia paradoxa* Grunow
Amphora hyalina Kütz.	*Mastogloia pumila* (Cleve & Möller) Cleve
Amphora inconspicua Proshk.-Lavr.	*Mastogloia tenera* Hust.
Amphora marina W. Sm.	*Mastogloia* sp.3
Amphora obtusa W. Greg.	*Navicula aleksandrae* Lange-Bert., Witkowski, Bogaczewicz-Adamczak & Zgrundo*
Amphora ocellata Donkin	*Navicula arenaria* Donkin*
Amphora ostrearia Bréb.	*Navicula besarensis* Giffen*
Amphora ovalis (Kütz.) Kütz.	*Navicula cancellata* Donkin
Amphora parvula Proshk.-Lavr.	*Navicula bozenae* Lange-Bert., Witkowski, Bogaczewicz-Adamchak & Zgrundo*
Amphora pediculus (Kütz.) Grunow	*Navicula* cf. *fauta* Hust.*
Amphora proteus W. Greg.	*Navicula* cf. *lusoria* Giffen*
Amphora pusio Cleve*	*Navicula* cf. *mahoodii* Witkowski & Witon*
Amphora staurophora Jahlin-Dannfelt	*Navicula* cf. *opima* (Grunow) Grunow*
Amphora subacutiuscula Schoemann	*Navicula distans* (W. Sm.) Ralfs

Continued

Table 2 Continued

Amphora turgida W. Greg.	*Navicula erifuga* Lange-Bert.*
Amphora wisei (Salah) Simonsen	*Navicula flanatica* Grunow
Amphora sp.168-1W	*Navicula germanopolonica* Lange-Bert., Witkowski, Bogaczewicz-Adamchak & Zgrundo*
Amphora sp.2F	*Navicula glabriuscula* var. *elipsoidales* Proshk.-Lavr.***
Anorthoneis excentrica (Donkin) Grunow	*Navicula gregaria* Donkin
Astartiella bahusiensis (Grunow) Witkowski, Lange-Bert. & Metzeltin*	*Navicula northumbrica* Donkin*
Astartiella producta Witkowski, Lange-Bert. & Metzeltin*	*Navicula palpebralis* Bréb.
Astartiella sp. AW B362 1015*	*Navicula palpebralis* var. *angulosa* (W. Greg.) Van Heurck
Bacillaria paxillifera (O.F. Müll.) Hendey	*Navicula palpebralis* var. *semiplena* (W. Greg.) Cleve
Berkeleya rutilans (Trentep.) Grunow	*Navicula parapontica* Witkowski, Kulikovskiy, Nevrova & Lange-Bert.**
Berkeleya scopulorum (Bréb. & Kütz.) E.J. Cox	*Navicula perminuta* Grunow
Berkeleya sp.	*Navicula* cf. *perminuta* Grunow
Biremis ambigua (Cleve) D.G. Mann	*Navicula pontica* Witkowski, Kulikovskiy, Nevrova & Lange-Bert.
Biremis lucens (Hust.) Sabbe, Witkowski & Vyverman*	*Navicula radiosa* Kütz.
Biremis ridicula (Giffen) D.G. Mann*	*Navicula ramosissima* (C. Agardh) Cleve
Caloneis bacillum (Grunow) Cleve	*Navicula salinarum* Grunow
Caloneis densestriata (Proshk.-Lavr.) Gusl.	*Navicula salinicola* Hust.
Caloneis lancettula (Schulz) Lange-Bert. & Witkowski*	*Navicula* cf. *salinicola* Hust.
Caloneis liber (W. Sm.) Cleve	*Navicula petrovii* Nevrova, Witkowski, Kociolek & Lange-Bert.** (Syn.: *N. scabriuscula* (Cleve & Grove) Mereschk.***)
Caloneis schumanniana var. *biconstricta* (Grunow) Reichelt	*Navicula veneta* Kütz.
Caloneis sp.1	*Navicula viminoides* var. *cosmomarina* Lange-Bert., Witkowski, Bogaczewicz-Adamchak & Zgrundo*
Caloneis sp.2	*Navicula* sp.10
Campylodiscus thuretii Bréb.	*Navicula* sp.135/14
Chamaepinnularia sp.1	*Navicula* sp.146/2
Cocconeiopsis breviata (Hust.) Witkowski, Lange-Bert. & Metzeltin*	*Navicula* sp.2
Cocconeiopsis pullus (Hust.) Witkowski, Lange-Bert. & Metzeltin*	*Navicula* sp.3
Cocconeiopsis sp.1	*Navicula* sp.6
Cocconeis diminuta Pant.*	*Navicula* sp.9
Cocconeis clandestina A.W.F. Schmidt*	*Navicula* sp.B1
Cocconeis convexa Giffen*	*Navicula* sp.D1
Cocconeis discrepans A.W.F. Schmidt*	*Navicula* sp.D2
Cocconeis euglypta Ehrenb.	*Nitzschia acuminata* (W. Sm.) Grunow
Cocconeis guttata Hust. & Aleem*	*Nitzschia aequorea* Hust.*
Cocconeis pediculus Ehrenb.	*Nitzschia amphibia* Grunow
Cocconeis pelta A.W.F. Schmidt*	*Nitzschia angularis* var. *affinis* (Grunow) Grunow
Cocconeis peltoides Hust.*	*Nitzschia aurariae* Cholnoky*
Cocconeis placentula Ehrenb.	*Nitzschia capitellata* Hust.
Cocconeis pseudocostata O.E. Romero*	*Nitzschia coarctata* Grunow
Cocconeis pseudograta Hust.*	*Nitzschia* cf. *coarctata* Grunow
Cocconeis scutellum Ehrenb.	*Nitzschia compressa* (J.W. Bailey) Boyer
Cocconeis scutellum var. *parva* (Grunow) Cleve	*Nitzschia constricta* (Kütz.) Ralfs

Continued

Table 2 Continued

Cocconeis speciosa W. Greg.	*Nitzschia dissipata* (Kütz.) Grunow
Cocconeis stauroneiformis (Rabenh.) Okuno	*Nitzschia frustulum* (Kütz.) Grunow
Cocconeis cf. *stauroneiformis* (Rabenh.) Okuno	*Nitzschia granulata* Grunow
Cocconeis sp.1	*Nitzschia grossestriata* Hust.*
Cocconeis sp.A1	*Nitzschia inconspicua* Grunow
Cocconeis sp.5	*Nitzschia insignis* W. Greg.
Cymatopleura elliptica (Bréb.) W. Sm.	*Nitzschia lanceolata* var. *minima* Van Heurck
Cymatopleura solea var. *apiculata* (W. Sm.) Ralfs	*Nitzschia liebetruthii* Rabenh.
Cymbella angusta (W. Greg.) Gusl.	*Nitzschia* cf. *liebetruthii* Rabenh.
Cymbella cymbiformis C. Agardh	*Nitzschia lorenziana* Grunow
Cymbella excisa Kütz.	*Nitzschia pellucida* Grunow
Cymbella sp.4	*Nitzschia perindistincta* Cholnoky*
Cymbella sp.D1	*Nitzschia pusilla* (Kütz.) Grunow emend. Lange-Bert.
Denticula subtilis Grunow	*Nitzschia sigma* (Kütz.) W. Sm.
Dickieia resistans Witkowski, Lange-Bert. & Metzeltin	*Nitzschia spathulata* Bréb.
Dickieia subinflata (Grunow) D.G. Mann	*Nitzschia vidovichii* Grunow
Diploneis bombus (Ehrenb.) Cleve-Euler	*Nitzschia* sp.6
Diploneis chersonensis (Grunow) Cleve	*Nitzschia* sp.8
Diploneis coffaeiformis (A.W.F. Schmidt) Cleve*	*Oestrupia powellii* (Lewis) Heiden*
Diploneis crabro Ehrenb.	*Parlibellus hamulifer* (Grunow) E.J. Cox
Diploneis fusca (W. Greg.) Cleve	*Parlibellus* sp.4D
Diploneis incurvata (W. Greg.) Cleve	*Petrodictyon gemma* (Ehrenb.) D.G. Mann
Diploneis litoralis (Donkin) Cleve	*Petroneis humerosa* (Bréb.) A. Stickle & D.G. Mann
Diploneis notabilis (Grev.) Cleve	*Pinnularia cruciformis* (Donkin) Cleve
Diploneis notabilis var. *tenera* Proshk.-Lavr.	*Pinnularia quadratarea* (A.W.F. Schmidt) Cleve
Diploneis parca (A.W.F. Schmidt) Boyer	*Pinnularia trevelyana* (Donkin) Rabenh.***
Diploneis smithii (Bréb.) Cleve	*Pinnularia viridis* (Nitzschiae) Ehrenb.
Diploneis stroemii Hust.*	*Pinnularia* sp.2D
Diploneis suborbicularis (W. Greg.) Cleve	*Plagiotropis elegans* (W. Sm.) Grunow
Diploneis vacillans (A.W.F. Schmidt) Cleve	*Plagiotropis lepidoptera* (W. Greg.) Kuntze
Diploneis sp.1	*Planothidium deperditum* (Giffen) Witkowski, Lange-Bert. & Metzeltin*
Diploneis sp.2	*Planothidium delicatulum* (Kütz.) Round & Bukht.
Encyonopsis microcephala (Grunow) Krammer*	*Planothidium* cf. *delicatulum* (Kütz.) Round & Bukht.
Entomoneis paludosa (W. Sm.) Reimer	*Planothidium* cf. *delicatulum* f.1 (Kütz.) Round & Bukht.
Fallacia aequorea (Hust.) D.G. Mann*	*Planothidium dispar* (Cleve) Witkowski, Metzeltin & Lange-Bert.
Fallacia amphipleroides (Hust.) D.G. Mann*	*Planothidium lanceolatum* (Bréb.) Bukht.
Fallacia florinae (Moeller) Witkowski*	*Planothidium quarnerensis* (Grunow) Witkowski, Lange-Bert. & Metzeltin
Fallacia forcipata (Grev.) A. Stickle & D.G. Mann	*Planothidium* sp.1
Fallacia litoricola (Hust.) D.G. Mann*	*Planothidium* sp.2
Fallacia oculiformis (Hust.) D.G. Mann*	*Planothidium* sp.2F
Fallacia pygmaea (Kütz.) A. Stickle & D.G. Mann	*Pleurosigma aestuarii* (Bréb.) W. Sm.
Fallacia subforcipata (Hust.) D.G. Mann	*Pleurosigma angulatum* (Queckett) W. Sm.
Fallacia tenera (Hust.) D.G. Mann	*Pleurosigma elongatum* W. Sm.
Fallacia sp.7F	*Pleurosigma formosum* W. Sm.

Continued

Table 2 Continued

Fogedia finmarchica (Cleve & Grunow) Witkowski, Metzeltin & Lange-Bert.	*Pleurosigma rigidum* W. Sm.
Fogedia giffeniana (Foged) Witkowski, Lange-Bert., Metzeltin & Bafana*	*Psammodictyon panduriforme* var. *continua* (Grunow) P.J.M. Snoeijs*
Fogedia heterovalvata (Simonsen) Witkowski, Metzeltin & Lange-Bert.*	*Psammodictyon roridum* (Giffen) D.G. Mann*
Fogedia sp.2	*Psammodictyon rudum* (Cholnoky) D.G. Mann*
Gomphonema angustatum (Kütz.) Rabenh.	*Rhoicosphenia abbreviata* (C. Agardh) Lange-Bert.
Gomphonema sp.1	*Rhopalodia musculus* (Kütz.) O. Müll.
Gyrosigma fasciola (Ehrenb.) Cleve	*Sellaphora pupula* (Kütz.) D.G. Mann
Gyrosigma spenceri (Queckett) Griffith & Henfrey	*Seminavis* sp.1
Halamphora acutiuscula (Kütz.) Levkov	*Stauronella indubitabilis* Lange-Bert. & Genkal
Halamphora angularis (W. Greg.) Levkov	*Staurophora salina* (W. Sm.) Mereschk.
Halamphora coffeaeformis (C. Agardh) Levkov	*Surirella fastuosa* (Ehrenb.) Kütz.
Halamphora tenerrima (Aleem & Hust.) Levkov*	*Toxonidea insignis* Donkin
Hantzschia vivax (W. Sm.) Perag.	*Trachyneis aspera* (Ehrenb.) Cleve
Haslea subagnita (Proshk.-Lavr.) Makarova & Karajeva	
Hippodonta sp.2	

include *Gomphonema* sp. 1 (Fig. 3: 29), *Hippodonta* sp. 3 (Fig. 4: 7), *Hippodonta* sp. 5 (Fig. 2: 15, Fig. 4: 8), *Hippodonta* sp. 6 (Fig. 2: 16, Fig. 4: 9), *Hippodonta* sp. 8 (Fig. 4: 10), *Amphora* sp. 2F (Fig. 3: 25), *Astartiella* sp. AW B362 1015 (Fig. 2: 10), *Navicula* sp. D2 (Fig. 3: 11), *Navicula* sp.146/2 (Fig. 3: 10); *Diploneis* sp. 1 (Fig. 3: 1, Fig. 4: 20), *Diploneis* sp. 2 (Fig. 3: 3), *Navicula* sp. D1 (Fig. 4: 12), *Navicula* sp. 9 (Fig. 4: 13), and *Seminavis* sp.1 (Fig. 3: 28, Fig. 4: 25). Some of those species might be new to science, though further data collection is required to confirm or deny this notion.

Classes Coscinodiscophyceae and Fragilariophyceae are represented slightly in the taxocene, at 7.2 and 6.9% of the total species number, respectively. From Coscinodiscophyceae, 5 orders, 9 families, 14 genera, and 22 species were registered, while 6 orders, 6 families, 13 genera, and 20 species were found from Fragilariophyceae. Representatives from class Bacillariophyceae, which belong to 9 orders, 22 families, 51 genera, and 257 species (261 intraspecific taxa) were dominant (85.8%). Order Naviculales was the most abundant in the number of lower taxa, with 9 families, 25 genera, and 122 species observed. A lower richness was observed for the orders Achnanthales (3 families, 6 genera, and 44 species), Bacillariales (1 family, 5 genera, and 34 species), and Thalassiophysales (1 family, 2 genera, and 28 species). The highest species number was observed for the genera *Navicula* (41 species), followed by *Nitzschia* (29), *Amphora* (25), *Cocconeis* (20), *Diploneis* (16), *Lyrella*, *Fallacia*, and *Planothidium* (10 species each, respectively) (Appendix 1).

Similarity of species composition in benthic diatom taxocenes from different areas of the Crimean coast

The taxonomic composition of benthic diatoms in the aforementioned sites of the Crimean coast were represented by a total of 543 species, among which 44 species belong to class Coscinodiscophyceae, 42 to Fragilariophyceae, and 457 to Bacillariophyceae (Appendix 1). Species composition between the taxocene of DB and previously investigated areas of the Crimean coast were compared. However, total registered species richness in each sampling area could not be directly matched due to different sampling efforts (number of samles). Therefore, the expected number of species in each of the healthy and polluted comparison areas were evaluated for similar sampling effort (8-9 samples) (Table 3). Notably, a total of 32 samples were analysed for SB, whereas 16 were analysed for BB, and only 8 were analysed for CF.

Recalculation of the observed number of species for each sampling area based on cumulative randomised sequences of species demonstrated that, when considering a similar number of samples (8-9), 126-130 species were observed in SB, while 150-156 species were observed in BB. Thus, due to strong permanent pollution, the species richness in SB or in BB was nearly half of the value registered for each of the pristine areas based on identical sampling effort.

Based on the Bray-Curtis similarity coefficient, the highest species similarity was revealed between the taxocenes of CF and DB (57%) – both of which represent environmentally healthy biotopes. A similarly high value of resemblance coefficient was observed between the taxocenes of heavily polluted SB and BB (Table 4). The lowest similarity was observed between the diatom taxocenes at SB and CF (32.3%) and between SB and DB (36.5%).

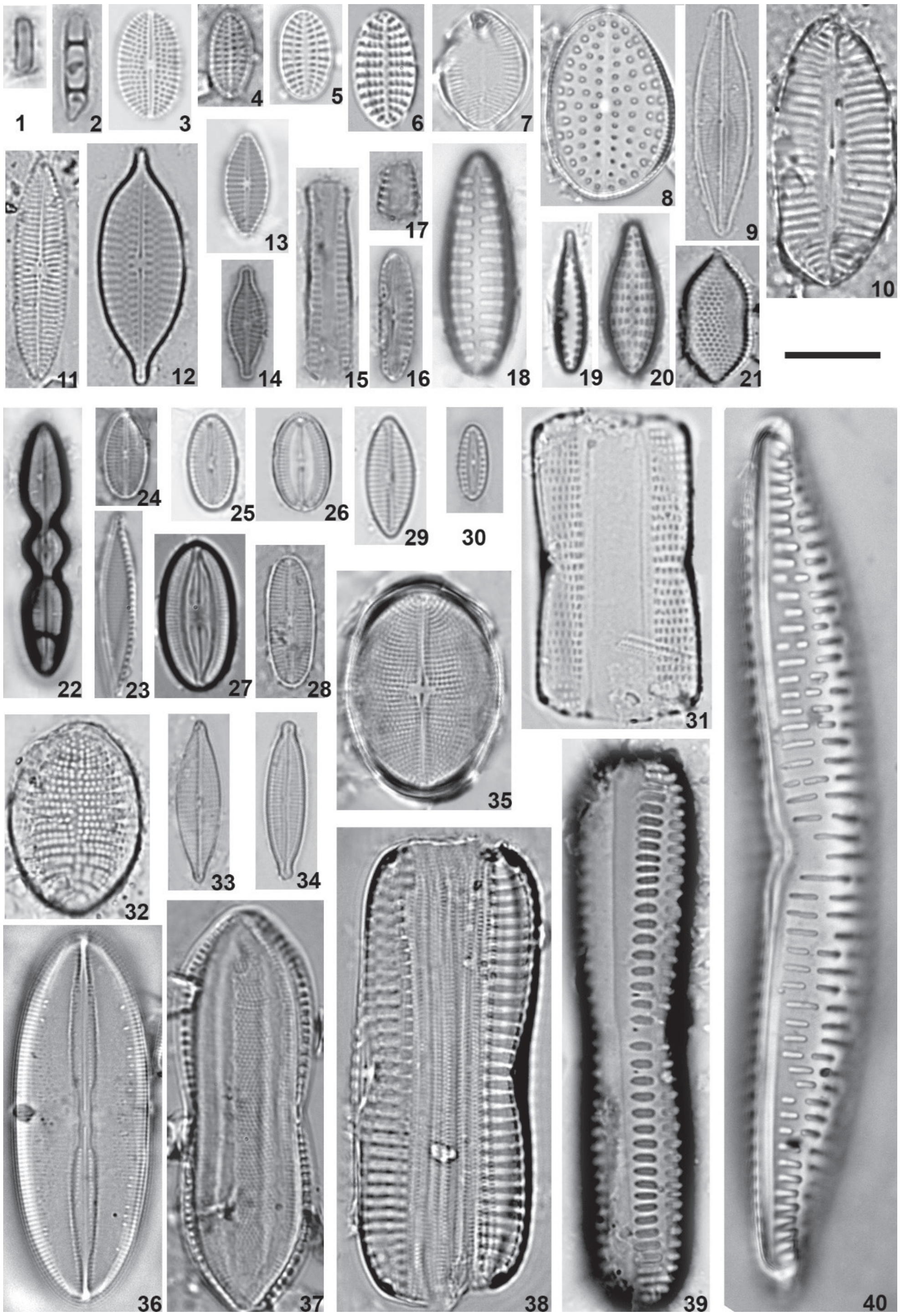

Fig. 2: New for the Black Sea diatom flora species found in Dvuyakornaya Bay (LM): 1 – *Achnanthes mercurii;* 2 – *Plagiogramma tenuissima*; 3 – *Cocconeiopsis breviata*; 4 – *Cocconeis discrepans*; 5 – *Cocconeis peltoides*; 6 – *Cocconeis pseudocostata*; 7 – *Cocconeis pseudograta*; 8 – *Cocconeis guttata*; 9 – *Astartiella bahusiensis*; 10 – *Astartiella* sp. AW B3621015; 11 – *Navicula arenaria*; 12 – *Fogedia giffeniana*; 13 – *Fogedia heterovalvata*; 14 – *Fogedia* sp.2; 15 – *Hippodonta* sp.5; 16 – *Hippodonta* sp.6; 17 – *Opephora gunter-grassii;* 18 – *Opephora pacifica;* 19 – *Opephora mutabilis*; 20 – *Trachysphenia australis*; 21 – *Psammodictyon panduriforme* var. *continua*; 22 – *Caloneis schumanniana* var. *biconstricta*; 23 – *Nitzschia aequorea*; 24 – *Fallacia aequorea;* 25 – *Fallacia amphipleroides*; 26 – *Fallacia florinae*; 27 – *Fallacia oculiformis*; 28 – *Fallacia litoricola;* 29 – *Navicula aleksandrae;* 30 – *Biremis lucens;* 31 – *Navicula northumbrica*; 32 – *Cocconeis clandestina*; 33 – *Navicula gregaria;* 34 – *Encyonopsis microcephala;* 35 – *Cocconeis convexa;* 36 – *Navicula glabriuscula* var. *elipsoidales;* 37 – *Psammodictyon roridum;* 38 – *Biremis ridicula*; 39 – *Oestrupia powellii*; 40 – *Navicula petrovii*. Scale bar 10 μm.

An analysis of species distribution between studied sites was performed using Venn diagrams (Venn diagrams, 2019) (Fig. 5). Despite their geographical remoteness, the highest number of species unique to each area was found in sites with the lowest degree of pollution – DB and CF – at which 98 and 96 species were observed, respectively. In contrast, the heavily polluted areas – SB and BB – the number of unique species was nearly two times less (47 and 37 species, respectively). A total of 79 common species were found in pristine sites, while

Table 3. Number of samples, total observed (S_{obs}) and expected (S_{exp}) values of benthic diatom species richness at four investigated coastal sites in Crimea. S_{exp} values were calculated for an equal number of samples (8-9).

Sampling site	Number of samples	S_{obs}	S_{exp}
Dvuyakornaya Bay	9	304	304
Cape Fiolent	8	290	290
Sevastopol Bay	32	186	126–130
Balaklava Bay	16	191	150–156

32 common species were observed in two polluted areas (SB and BB). Since only 47 species (out of 542) were recorded as common among all studied areas, it can be concluded that technogenic pollution could largely determine the diversity patterns of diatoms in coastal biotopes with similar environmental conditions (Sugie & Suzuki, 2017).

Discussion

The high number of benthic diatom species registered in the DB may due to this area not previously being subjected to anthropogenic disturbance. Levels of pollutants in this bay's soft bottom sediments were either close to the minimum analytical detection limits (for PAHs) or similar to average background levels registered for all offshore bottom areas of the Crimean coast (with the exception of Zn content) (Table 1) (Mitropolsky *et al*., 2006; Samyshev *et al*., 2014). Another possible cause of this high Bacillariophyta species richness is the variety of microbiotopes in DB (silty-clayed, rocky, gravel or sandy substrates with macrophyte thickets, and shell debris patches). The high landscape heterogeneity of the sea floor may determine conditions for the development of greater diatom species richness, including the occurrence of rare species, species sensitive to pollution, as well relics of Ponto-Caspian flora (e.g., *Navicula petrovii*; Witkowski *et al*., 2014) and invasive species. Notably, the latter could have been brought to the research area from other regions through ballast water or from phyto-periphyton on the hulls of vessels passing nearby DB to the ports of Crimea, Caucasus, and the Sea of Azov. The group of invasive species includes small-cell species, such as: *Achnanthidium glyphos*, previously known only from a typical habitat in the Indian Ocean (Riaux-Gobin *et al*., 2010); *Fogedia giffeniana,* found in the Gulf of Aden (Witkowski *et al*., 1997); *F. heterovalvata*, previously noted off the coast of the Baltic Sea and the Caribbean Islands (Witkowski *et al*., 1997); and *Navicula aleksandrae, N. germanopolonica, N. bozenae*, and *N. viminoides* var. *cosmomarina* (all described from Gdansk Bay, Baltic Sea; Lange-Bertalot *et al*., 2003). Among the large-cell diatoms, several species also are likely to be invading forms (e.g., *Diploneis coffaeiformis, D. stroemii, Mastogloia cuneata, Navicula arenaria, N. besarensis, N. northumbrica, N.* cf. *fauta, N.* cf. *lusoria, N.* cf. *mahoodii, N.* cf. *opima, Oestrupia powellii, Psammodictyon roridum, P. rudum*, and others). Although these species are widely distributed in oceans worldwide, they were recorded for the first time in the Black Sea flora of Bacillariophyta. However, it is possible to assume that several of the aforementioned common small-cell or rare large-cell species are autochthonous forms but were not previously found in the Black Sea due to insufficient research on benthic diatoms. The final conclusion regarding whether the novelty floristic finds of Bacillariophyta (68 species) are invading species can be made after additional taxonomic and molecular/genetic studies. Similar results suggesting high Bacillariophyta species richness (290 species) and a significant number of new floristic finds were also obtained from the ecologically intact area around CF, which is also characterised by a large variety of microbiotopes and very low technogenic pollutant content (heavy metals, chlororganic pesticides, and PAHs) in soft bottom sediments. A total of 68 species and 3 genera were previously recorded as being new to the Black Sea flora within the diatom taxocene of CF, while 3 species and 1 taxonomic combination were registered as new to science, and 4 species were not found in the Black Sea over the last 50 to 100 years of research (Nevrova, 2016). According to the Spearman's rank correlation analysis (Petrov *et al*., 2005, 2010), diatom species richness showed a significant correlation with most pollution-related variables (Table 1), while factors such as water temperature, salinity, pH, oxygen concentration, and nutrients in the

Table 4. The pairwise similarity (%) of benthic diatoms species composition in biotopes of the South-western and South-eastern coast of Crimea (using Bray-Curtis similarity coefficient).

	Sevastopol Bay	Cape Fiolent	Balaklava Bay
Sevastopol Bay (186 sp.)	*	*	*
Cape Fiolent (290 sp.)	32.3	*	*
Balaklava Bay (191 sp.)	57.8	39.1	*
Dvuyakornaya Bay (304 sp.)	36.5	57.1	42.6

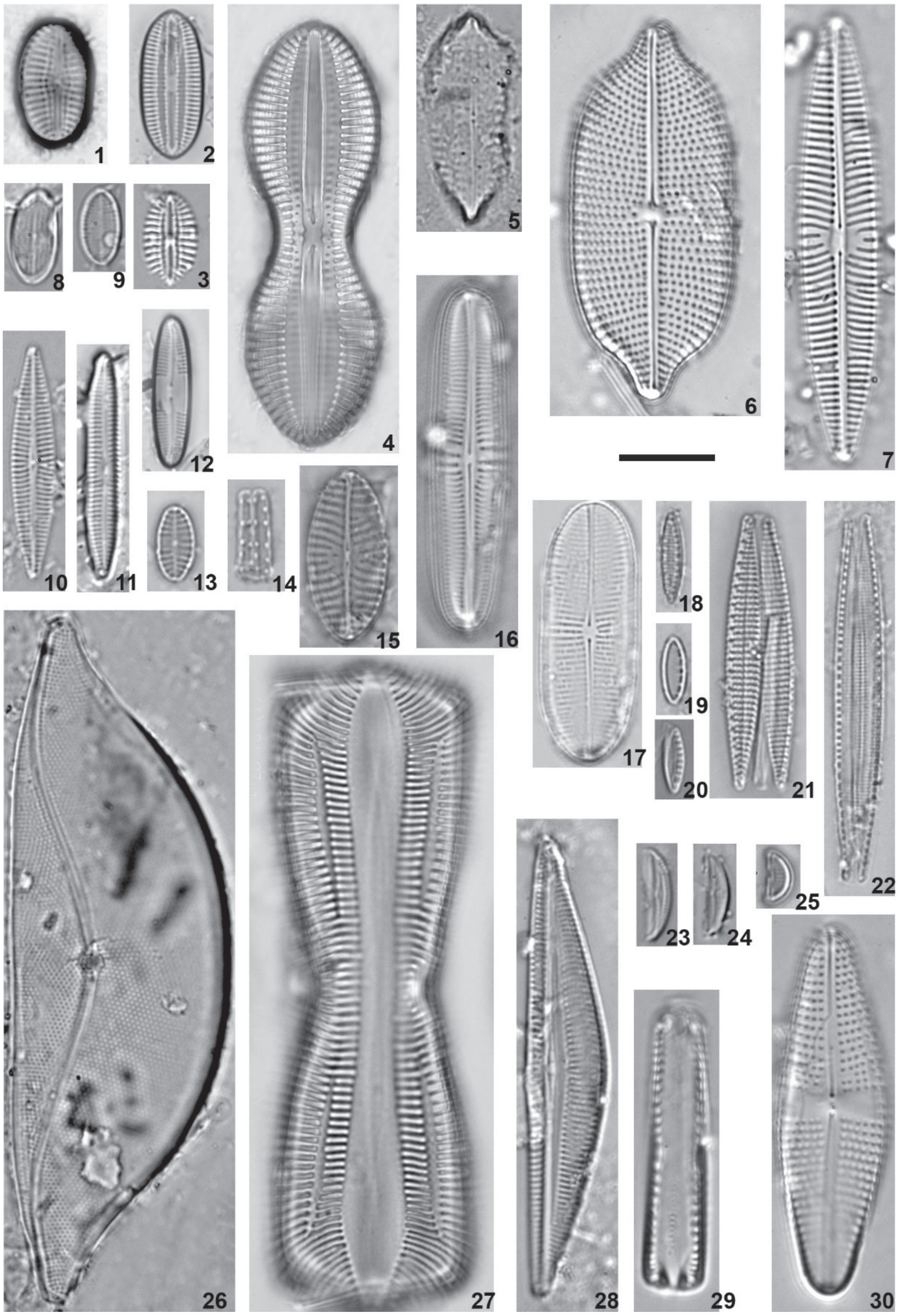

Fig. 3: New for the Black Sea diatom flora species found in Dvuyakornaya Bay (LM): 1 – *Diploneis sp.*1; 2 – *Diploneis parca;* 3 – *Diploneis sp.*2*;* 4 – *Diploneis stroemi*; 5 – *Navicula* cf. *opima;* 6 – *Navicula besarensis*; 7 – *Navicula* erifuga*;* 8 – *Cocconeis* sp.5W; 9 – *Cocconeis* sp.1; 10 – *Navicula* sp.146/2; 11 – *Navicula sp.*D2; 12 – *Caloneis lancettula*; 13 – *Planothidium deperditum*; 14 – *Denticula subtilis;* 15 – *Navicula* cf. *lusoria;* 16 – *Dickieia subinflata;* 17 – *Dickieia resistans;* 18 – *Nitzschia aurariae* (oblique light); 19 – *Nitzschia perindistincta*; 20 – *Nitzschia inconspicua;* 21 – *Nitzschia grossestriata;* 22 – *Nitzschia liebetruthii;* 23 – *Amphora helenensis;* 24 – *Amphora exilitata;* 25 – *Amphora sp.* 2F; 26 – *Toxonidea insignis;* 27 – *Pinnularia trevelyana*; 28 – *Seminavis* sp.1; 29 – *Gomphonema* sp.1; 30 – *Achnanthes islandica.* Scale bar 10 µm.

near-bottom water layer were only weakly correlated with the species diversity metrics.

Meanwhile, in SB – which has been exposed to intense long-term anthropogenic impact and is distinguished by a homogeneous silty-sandy bottom substrate covering up to 98% of the bay bottom area – a weakened oxygen regime with deeply negative redoxing conditions and high levels of C_{org} in sediment (Table 1) is characterised by reduced diatom taxocene species richness (186 species). Similarly, in BB, the bottom of which is nearly completely covered with silty deposits with an extremely high level of pollutants (Burgess *et al*., 2009), the diatom taxocene structure is also characterised by low species richness (191 species) similar to that of SB (Petrov *et*

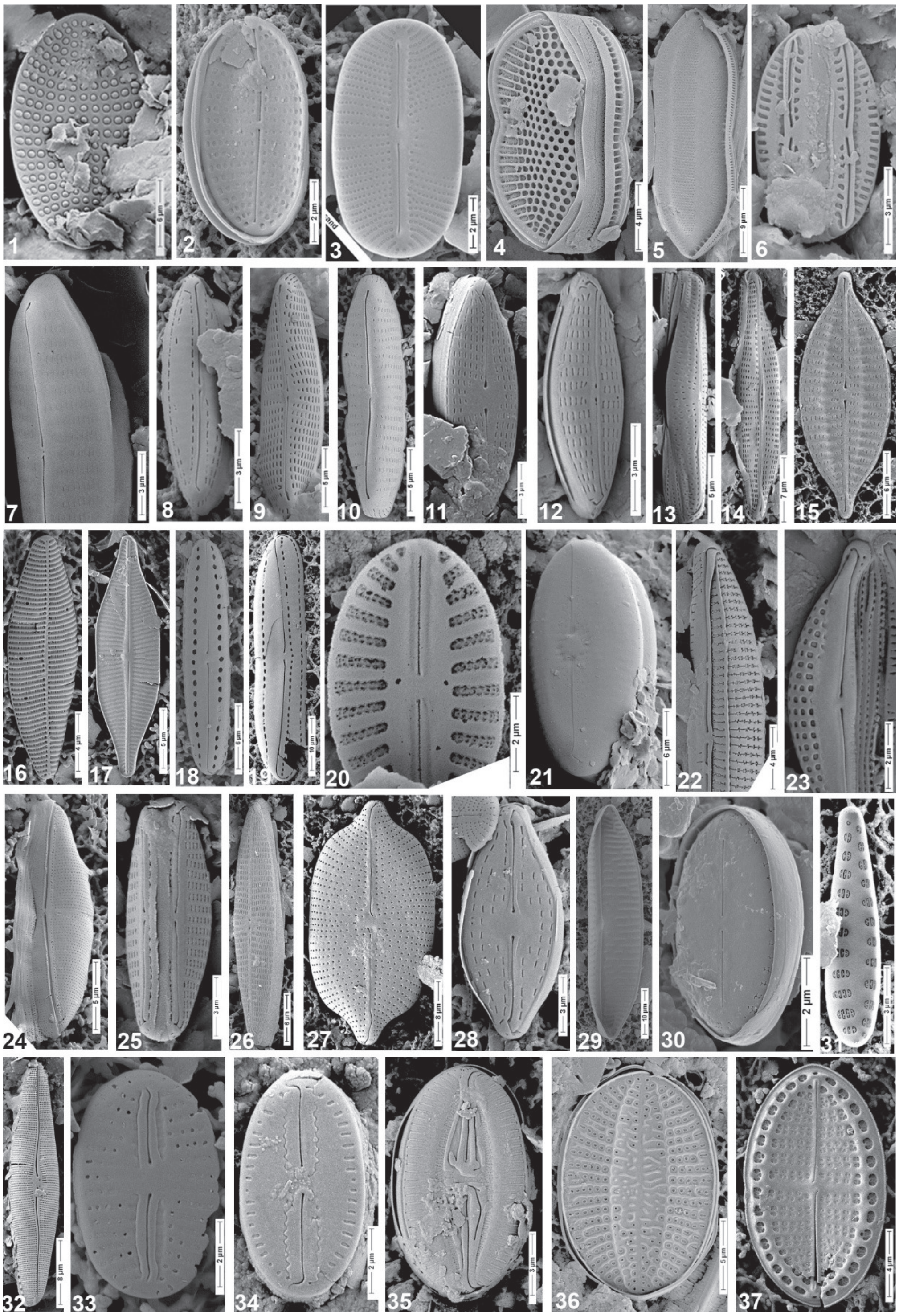

Fig. 4: New for the Black Sea diatom flora species found in Dvuyakornaya Bay (SEM): 1 – *Cocconeis guttata;* 2 – *Cocconeis diminuta;* 3 – *Cocconeiopsis pullus; 4 – Psammodictyon panduriforme* var. *continua*; 5 – *Psammodictyon roridum;* 6 – *Amphora helenensis;* 7 – *Hippodonta* sp.3; 8 – *Hippodonta* sp.5; 9 – *Hippodonta* sp.6; 10 – *Hippodonta* sp.8*;* 11 – *Navicula germanopolonica;* 12 – *Navicula* sp.D1; 13 – *Navicula* sp.9; 14 – *Fogedia* sp.2; 15 – *Fogedia giffeniana*; 16 – *Astartiella bahusiensis;* 17 – *Astartiella producta;* 18 – *Biremis lucens*; 19 – *Biremis ridicula*; 20 – *Diploneis* sp.1; 21 – *Diploneis coffaeiformis;* 21 – *Amphora pusio;*. 23 – *Halamphora tenerrima;* 24 – *Amphora jostesorum;* 25 – *Seminavis* sp.1; 26 – *Navicula parapontica*; 27 – *Navicula besarensis*; 28 – *Navicula viminoides var. cosmomarina;* 29 – *Navicula petrovii*; 30 – *Amicula specululum*; 31 – *Opephora minuta*; 32 – *Mastogloia cuneata*; 33 – *Fallacia aequorea*; 34 – *Fallacia florinae*; 35 – *Fallacia oculiformis*; 36 – *Cocconeis pelta*; 37 – *Cocconeis pseudocostata.* Scale bar indicated on each micrograph.

al., 2010). Such results are congruent with other studies stating that decreased species richness in diatom assemblages often occurred under reduced heterogeneity of microhabitats and anthropogenically stressed environments (Heino *et al*., 2007; Leira *et al*., 2009; Nevrova, 2014; Stenger-Kovacs *et al*., 2016).

The differences in diatom taxocene species structure at DB and other compared areas can be due to both exter-

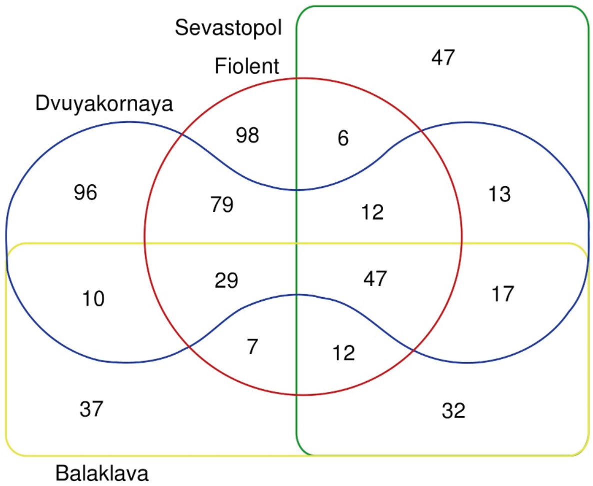

Fig. 5: Comparison of diatom species distribution between studied areas (using a Venn diagram).

nal (environmental) and internal factors that are primarily linked to the phylogenetic relationships of species within the taxonomic tree. Rather low values of species richness and a relatively high ratio of polyspecific branches in a taxonomic tree are often linked to microphytobenthos communities exposed to adverse effects such as anthropogenic pollution (Gottschalk & Kahlert, 2012; Heino *et al*., 2005, 2007; Petrov *et al*., 2010; Stenger-Kovacs *et al*., 2014, 2016). Meanwhile, high species richness and especially taxonomic richness above the species level (i.e., genera and higher) may indicate a considerable taxonomic alignment of the taxocene structure (i.e., proportional representation of taxons at different hierarchical levels of the taxonomic tree). Such features of the architectonics of the hierarchical tree of diatoms typically characterise taxocenes from ecologically healthy biotopes experiencing minimal adverse environmental effects (Leira *et al*., 2009; Nevrova *et al*., 2015; Rimet & Bouchez, 2012).

Other possible factors affecting diatom species richness include differences in water turbidity (and the related flow of photosynthetically active radiation (PAR)), oxygen content and redox conditions in the upper layer of bottom sediments, as well as nutrient levels (Kovrigina *et al*., 2009; 2010, 2016; Mitropolsky *et al*., 2006). In the present study, only C_{org} content and redox potential (Eh) values in the sediments of compared areas differed significantly (Table 1). Low C_{org} content and highly positive redox potential in the sediments of CF and DB likely influence the survival and occurrence of those low-tolerant diatom species that are absent in SB and BB.

Tests of statistical significance showed that values for nutrients, salinity, and PAR in the near-bottom layers of studied areas did not differ significantly. Results from comparing the levels of abiotic factors led to conclusion that microlandscape heterogeneity as well as the parameters of anthropogenic pollutants and redox conditions in the upper layer of sediments can represent key causes influencing the distinctness of benthic diatom species richness and the structure of taxocenes in biotopes with various environmental conditions.

As a result, in the pristine areas of DB and CF, high benthic Bacillariophyta species richness was revealed alongside a number of novel taxa to the Black Sea flora. In contrast, high levels of trace metal and organic pollutant accumulation in the bottom sediments of SB and BB exerted a negative influence on the species richness of diatom taxocene structure, though species tolerant to pollution or euribiontic forms prevailed.

Acknowledgements

This research was conducted by the Benthos Ecology Dept., IMBR RAS, within the framework of theme № 0828-2019-0002 (state registration № AAAA-A18-118020890074-2).

The authors would like to thank D.Sc. A.V. Terletskaya and colleagues (Institute of Colloid Chemistry and Water Chemistry of NASU, Kiev) for the results of their chemical analysis of sediment samples, Prof. D.Sc. A. Witkowski (University of Szczecin, Poland) for supervising and providing LM equipment, Prof. D.Sc. H. Lange-Bertalot and M. Ruppel (Frankfurt-am-Main, Germany) for their SEM microphotographs and taxonomic advice. The authors are also grateful to the two anonymous reviewers for their constructive and valuable comments that helped to improve the manuscript.

References

Burgess, R.M., Ho, K.T., Terletskaya, A.V., Milyukin, M.V., Demchenko, V.Y. *et al.,* 2009. Concentration and distribution of hydrophobic organic contaminants and metals in the estuaries of Ukraine. *Marine Pollution Bulletin*, 58 (8), 1103-1115.

Burgess, R.M., Portis, L.M., Ho, K.T., Konovets, I.M., Petrov, A.N. *et al.,* 2011. Distribution, magnitude and characterization of the toxicity of Ukrainian estuarine sediments. *Marine Pollution Bulletin*, 62 (11), 2442-2462.

Clarke K.R., Gorley R.N., 2001. PRIMER V5: User Manual/Tutorial, PRIMER-E, Plymouth, UK , 94 p.

Fourtanier, E., Kociolek, J.P., 1999. Catalogue of Diatom Genera. *Diatom Research,* 14 (1), 1-190.

Fourtanier, E., Kociolek, J.P., 2011. *Catalogue of Diatom Names.* California Academy of Sciences (Electronic resource). Comp. by E. Fourtanier, J.P. Kociolek. http://researcharchive.calacademy.org/research/diatoms/names/index.asp (Accessed 19 Sept. 2011)

Gottschalk, S., Kahlert, M., 2012. Shifts in taxonomical and guild composition of littoral diatom assemblages along environmental gradients. *Hydrobiologia*, 694 (1), 41-56.

Hafner, D., Car, A., Jasprica, N., Kapetanović, T., Dupčić Radić, I., 2018. Relationship between marine epilithic diatoms and environmental variables in oligotrophic bay, NE Mediterranean. *Mediterranean Marine Science*, 19 (2), 223-239.

Heino, J., Soininen, J., Lappalainen, J., Virtanen, R., 2005. The relationship between species richness and taxonomic distinctness in freshwater organisms. *Limnology and Oceanography*, 50 (3), 978-986.

Heino, J., Mykrä, H., Hämäläinen, H., Aroviita, J., Muotka, T., 2007. Responses of taxonomic distinctness and species diversity indices to anthropogenic impacts and natural environmental gradients in stream macroinvertebrates. *Freshwater Biology*, 52 (9), 1846-1861.

ImageJ, 2014. Image Processing and Analysis in Java. https://imagej.nih.gov/ij/download/

International Plant Names Index, 2004. Published on the Internet at: http://www.ipni.org; (Accessed 1 July 2018).

Keck, F., Rimet, F., Franc, A., Bouchez, A., 2016. Phylogenetic signal in diatom ecology: perspectives for aquatic ecosystems biomonitoring. *Ecological Applications*, 26 (3), 861-872.

Kovrigina, N.P., Troschenko, O.A., Shchyrov, S.V., 2009. Peculiarities of spatial distribution of hydrological and hydrochemical characteristics of Karadag near shore water area at the modern period (2005-2006). In: Karadag-2009: Collection of scientific papers dedicated to the *95th anniversary of Karadag Research station and 30th anniversary of the Karadag Natural Reserve of the NAS of Ukraine*. Gaevskaya A.V., Morozova A.L. (Eds.). Sevastopol: ECOSI-Gidrofizika, 446-461 (in Russian).

Kovrigina, N.P., Popov, M.A., Lisitskaya, E.V., Kuftarkova, E.A., Gubanov V.I., 2010. Complex monitoring of Balaklava Bay (Black Sea) in 2000-2007. *Marine Ecological Journal,* 9 (4), 62-75. (In Russian, Abstract in English).

Kovrigina, N.P., Troschenko, O.A., Subbotin, A.A., Bobko, N.I., Bogdanova, T.A. *et al.,* 2016. Hydrological and hydrochemical structures in musselfarm area according to long-term observations (Sevastopol coastal waters, the Black Sea). In: Proceed. Intern. Conf. dedicated to 145 anniversary of IBSS-IMBR, 3, 394-397. (In Russian, Abstract in English).

Lange-Bertalot, H., Witkowski, A., Bogaczewicz-Adamczak, B., Zgrundo, A., 2003. Rare and new small-celled taxa of *Navicula* s. str. in the Gulf of Gdansk and of its freshwater affluents. *Limnologica*, 33 (4), 258-270.

Leira, M., Chen, G., Dalton, C., Irvin, K., Taylor, D., 2009. Patterns in freshwater diatom taxonomic distinctness along an eutrophication gradient. *Freshwater Biology,* 54 (1), 1-14.

Levkov, Z. 2009. *Amphora sensu lato.* In: *Diatoms of Europe: Diatoms of the European Inland Waters and Comparable Habitats, vol. 5.* Lange-Bertalot, H. (Ed). A.R.G. Gantner Verlag K.G., Ruggell, 5-916.

Mitropolsky, O.Yu, Nasedkin, E.I, Osokina, N.P., 2006. *Ecogeochemistry of the Black Sea*. Kiev, 279 p. (In Ukrainian, Abstract in English).

Nevrova, E.L., 2013. Taxonomic diversity and structure of benthic diatom taxocene (Bacillariophyta) at Sevastopol Bay (the Black Sea). *Marine Ecological journal,* 3 (12), 55-67. (In Russian, Abstract in English).

Nevrova, E.L., 2014. Taxonomic diversity and environmental assessment of benthic diatoms at Balaklava Bay (South-Western Crimea, the Black Sea, Ukraine). *Algologia*, 24 (1), 47-66. (In Russian, Abstract in English).

Nevrova, E.L., 2015. *Benthic diatoms (Bacillariophyta) in the Black Sea: diversity and structure of taxocenes of varios biotopes. Extended Abstract of DSci Thesis (speciality 03.02.01 – botany and 03.02.10 – hydrobiology),* Moscow State University, Moscow, 46 pp. (In Russian.) https://dlib.rsl.ru/01005555099 (Accessed 13 November 2014).

Nevrova, E.L., 2016. The composition and structure of the benthic diatom taxocene (Bacillariophyta) near Cape Fiolent (the Crimea, the Black Sea). *Russian Journal of Marine Biology*, 42 (5), 392-401.

Nevrova, E., Witkowski, A., Kulikovskiy, M., Lange-Bertalot, H., Kociolek, J.P., 2013. A revision of the diatom genus *Lyrella* Karayeva (Bacillariophyta: Lyrellaceae) from the Black Sea, with descriptions of five new species. *Phytotaxa*, 83 (1), 1-38.

Nevrova, E.L., Snigirova, A.A., Petrov, A.N., Kovaleva, G.V., 2015. Guidelines for quality control of the Black Sea. Microphytobenthos. Gaevskaya, A.V. (Ed.). N. *Orianda, Simferopol, 175 pp.* (In Russian, Abstract in English).

Petrov, A.N., Nevrova, E.L., 2004. Comparative analysis of taxocene structure of benthic diatoms (Bacillariophyta) in regions with different level of technogenic pollution (the Black Sea, Crimea). *Marine Ecological Journal*. 3 (2), 72-83. (In Russian, Abstract in English).

Petrov, A.N., Nevrova, E.L., 2007. Database on Black Sea benthic diatoms (Bacillariophyta): its use for a comparative study of diversity pecularities under technogenic pollution impacts. p. 153-165. In: *Proceedings of Ocean Biodiversity Informatics: International Conference on Marine Biodiversity Data Management (Hamburg, Germany, 29 November-1 December 2004).* Vanden Berghe E., Appeltans W., Costello M.J., Pissierssens P. (Eds). Paris, UNESCO/IOC, VLIZ, BSH, IOC Workshop Report n. 22, VLIZ Special Publication n. 37.

Petrov, A.N., Nevrova, E.L., 2012. Evaluation of reproducibility and reliability of benthic diatoms species composition at coastal location of SW Crimea. Marine Ecological Journal, 11 (3), 79-88. (In Russian, Abstract in English).

Petrov, A.N, Nevrova, E.L., 2013. Prognostic Estimation of Species Richness of Benthic Bacillariophyta. *International Journal on Algae*, 15 (1), 5-25.

Petrov, A.N., Nevrova, E.L., 2014**.** Numerical analysis of the structure of benthic diatom assemblages in replicate samples (Crimea, the Black Sea). *Nova Hedwigia*, Beih. 143, 245-253.

Petrov, A.N., Nevrova, E.L., Malakhova, L.V., 2005. Multivariate analysis of benthic diatoms distribution across the space of the environmental factors gradient in Sevastopol Bay (the Black Sea, Crimea), *Marine Ecological Journal,* 4 (3), 65-77. (In Russian, Abstract in English).

Petrov, A., Nevrova, E., Terletskaya, A., Milyukin, M., Demchenko, V., 2010. Structure and taxonomic diversity of benthic diatoms assemblage in a polluted marine environment (Balaklava Bay, Black Sea). *Polish Botanical Journal,* 55 (1), 183-197.

Proshkina-Lavrenko, A.I., 1974. *Diatom Algae of USSR. (vol.1).* Nauka, Leningrad, 403 pp. (In Russian).

Riaux-Gobin, C., Witkowski, A., Compère, P., 2010. SEM survey and taxonomic position of small-sized Achnanthidium (Bacillariophyceae) from coral sands off Réunion Island (Western Indian Ocean). *Vie et milieu*, 60 (2), 157-172.

Rimet, F., Bouchez, A., 2012. Biomonitoring river diatoms: implications of taxonomic resolution. *Ecological Indicators*, 15, 92-99.

Round, F.E., Crawford, R.M., Mann, D.G., 1990. *The diatoms.*

Biology and morphology of the genera. Cambridge University press, Cambridge, 747 pp.

Samyshev, E.Z., Minkina, N.I., Orlova, I.G., 2014. Integral assessment of pollution in bottom sediments of coastal and open-sea areas of the Black sea. *Marine Ecological Journal*, 13 (4), 41-49. (In Russian, Abstract in English).

Stenger-Kovács, C., Tóth, L., Tóth, F., Hajnal. E., Padisák, J. 2014. Stream order-dependent diversity metrics of epilithic diatom assemblages. *Hydrobiologia,* 721, 67-75.

Stenger-Kovács, C., Hajnal, É., Lengyel, E., Buczkó, K., Padisák, J., 2016. A test of traditional diversity measures and taxonomic distinctness indices on benthic diatoms of soda pans in the Carpathian basin. *Ecological indicators*, 64, 1-8.

Sugie, K, Suzuki, K., 2017. Characterization of the synoptic-scale diversity, biogeography and size distribution of diatoms in the North Pacific. *Limnology and Oceanography*, 62, 884-897.

Venn Diagrams. (Electronic resource). (Accessed 12 April 2019) http://bioinformatics.psb. ugent.be/beg/tools/venn-diagrams

Witkowski, A., Metzeltin, D., Lange-Bertalot, H., Bafana, G., 1997. Fogedia gen. nov. (Bacillariophyceae), a new naviculoid genus from the marine littoral. *Nova Hedwigia.* 65 (1), 79-98.

Witkowski, A., Lange-Bertalot, H., Metzelin, D., 2000. *Diatom flora of marine coasts I. In: Iconographia diatomologica, vol. 7.* Lange-Bertalot, H. (Ed.). A.R.G. Gantner Verlag K.G., Ruggell, Liechtenstein. 1-925.

Witkowski, A., Kulikovskiy, M., Nevrova, E., Lange-Bertalot, H., Gogorev, R. 2010. The genus Navicula in ancient basins. I. Two novelties from the Black Sea. *Plant Ecology and Evolution*, 143 (3), 307-317.

Witkowski, A., Nevrova, E., Lange-Bertalot, H., Kociolek, J.P., 2014**.** *Navicula petrovii* sp. nov. (Bacillariophyceae), a naviculoid diatom with amphoroid symmetry and its relationship to *Navicula* sensu stricto and other naviculoid genera. *Nova Hedwigia.* 143, 469-484.

The following information is available for the article:
Appendix 1. Diatom species composition of Dvuyakornaya Bay, Cape Fiolent, Sevastopol Bay and Balaklava Bay (Crimea, the Black Sea) (Excel /on line).